O-1
NMR in structural biology and structural genomics
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The present potentialities of nuclear magnetic resonance (NMR) spectroscopy in structural biology and in the newly emerging area of structural genomics will be reviewed and placed in perspective with the historical development of NMR in biological and biomedical research.

O-2
Regulation of Kv1.3 activity in human T lymphocytes: peptide blockers and molecular interactions
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Voltage-gated Kv1.3 potassium channels participate in the membrane potential control of T lymphocytes thereby regulating transmembrane signaling required for cell activation processes. The activity of these channels can be modulated by peptide blockers isolated from animal venoms. We were motivated to search for high affinity and high specificity blockers by their therapeutic potential as selective immunosuppressors in autoimmune diseases. Other factors influencing Kv1.3 activity could be interactions with protein kinases during T cell receptor (TCR/CD3) activation and/or the lipid composition in the cell membrane. The molecular environment for such regulation may be provided by the immunological synapse (IS). We set out experiments to describe the lateral distribution of Kv1.3 in the T cell membrane using electron microscopy, confocal laser scanning microscopy and fluorescence resonance energy transfer. We showed that the distribution of Kv1.3 in the T cell membrane is not random, Kv1.3 is in molecular proximity with the TCR/CD3 complex, and the channels are recruited into the IS formed between cytotoxic and target cells. These findings raise the possibility of a reciprocal regulation of the IS function and Kv1.3 activity during T cell activation.

O-4
Single molecule spectroscopy in situ: towards understanding the cell on a molecular level
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After years of exciting development of novel spectroscopic and microscopic techniques to investigate individual biomolecules, successful attempts have been made to enter the field of cell biology and study these functional elements in their native environment. Of particular importance are methods like single molecule imaging that allow to track large scale translocations, and confocal spectroscopy such as FCS that illuminates only a diffraction-limited confined spot and focuses on fast time scale fluctuations for analysis of molecular dynamics. In conjunction with two-photon excitation and spectrally resolved detection, FCS is a powerful means particularly for the study of molecular association or enzymatic turnovers. This technique raises strong hopes for quantitative in situ proteomics. Limitations and artefacts of FCS and other extremely sensitive techniques when applied to cells arise from the presence of cellular autofluorescence, but the most severe problem is often photobleaching of the labels during the observation, leading to wrongly decreased time scales of molecular dynamics. To overcome these limitations, smart excitation and detection schemes have to be applied, in conjunction to a selective data analysis. We applied FCS to a variety of cell-associated phenomena, among them protein-protein binding, enzymatic reactions, endocytosis, and gene delivery. To study processes on cell membranes, and to elucidate the delicate interplay between membrane proteins and the surrounding lipids, we devised cell-like model membrane systems mimicking the formation of membrane domains whose cellular counterparts are potentially active as recruitment platforms for signalling proteins.
O-5
On the nature of memory objects in the brain: a tentative view
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Our mind keeps a huge number of memories. We discuss here the number $M$ of neurons which must be implied in one primal memory object (the smell of a rose). We find that (in a storage area which is not genetically designed) spatial and connectivity requirements impose that $M$ be very small (of the order of 3). We then extend these considerations to associative memories (where the smell of a rose evokes the color of a rose).

O-6
Structural insights on the mechanism of membrane fusion catalysed by viral envelope proteins
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Enveloped viruses enter cells by protein-mediated membrane fusion, which is induced by a conformational change of the envelope glycoproteins present at the viral surface. This process results in the release of the viral genomic information into the cell. The best-characterized viral fusion protein is the influenza virus hemagglutinin, the current paradigm for all class I fusion proteins. This structural class also includes the fusion proteins of HIV and the SARS coronavirus. Alphaviruses and flaviviruses have a different class of fusion glycoproteins, class II, with an altogether different architecture compared to the class I proteins. The crystal structures of the pre-fusion form of E1 from the Semliki Forest alphavirus (SFV) and E from the tick-borne encephalitis flavivirus (TBE) showed that they are homologous. The crystal structures of their post-fusion trimeric form now show that during the fusion process the subunits fold back so that the fusion peptide loop and the transmembrane anchor are brought to the same end of a stable protein rod. This is similar to the case of class I fusion proteins, although the respective architectures are completely different. Furthermore, the structure of trimeric E1 from SFV revealed an intermediate in the fusion process, showing protein-protein and protein-lipid interactions that are essential for driving lipid merging.

O-7
Mapping molecular landscapes inside cells by cryoelectron tomography
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Electron Tomography (ET) is uniquely suited to obtain three-dimensional images of large pleomorphic structures, such as supramolecular assemblies, organelles, or whole cells. Technological advances have made it possible to develop automated data acquisition procedures. This, in turn, allowed to reduce the total electron dose to levels low enough for studying radiation-sensitive biological materials embedded in vitreous ice. As a result, we are now poised to combine the power of high-resolution 3-D imaging with the best possible preservation of specimens. High-resolution tomograms of organelles or cells contain vast amounts of information; essentially they are 3-D images of the cell’s entire proteome and they should ultimately enable us to map the spatial relationships of macromolecules in a cellular context. However, it is no trivial task to retrieve this information because of the poor signal-to-noise ratio of such tomograms and the crowded nature of cells. Denoising procedures can help to combat noise and to facilitate visualization, but advanced pattern recognition methods are needed for detecting and identifying with high fidelity specific macromolecules based on their structural signature. Provided that high- or medium-resolution structures of the molecules of interest are available, they can be used as templates for a systematic interrogation and interpretation of the tomograms.
Oral Presentations
– Proton Pumping Systems –

**O-8**
The mechanism of proton pumping by cytochrome c oxidase
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Cellular respiration involves electron transfer to oxygen through a series of membrane-bound protein complexes. The process maintains a transmembrane proton electrochemical gradient that is used, e.g., for the synthesis of ATP. In mitochondria and many bacteria the last enzyme complex in the electron transfer chain is cytochrome c oxidase, which catalyses the four-electron reduction of O₂ to H₂O using electrons delivered by a water-soluble donor, cytochrome c. The electron transfer through cytochrome c oxidase, accompanied by proton uptake to form H₂O drives pumping of four protons per reduced O₂ across the membrane. We use a combination of X-ray crystallography, site-directed mutagenesis and spectroscopic techniques to investigate the proton-pumping mechanism in cytochrome c oxidase at the molecular level.

**O-10**
Engineering electron-proton short-circuit prevention in cytochrome bc₁
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The photosynthetic reaction center and cytochrome bc₁ complexes are examples of bioenergetic proteins that rely on high and low potential redox chains for machine-like energy transduction that couples electron to transmembrane proton transfer. Each is vulnerable to energy wasting short-circuit reactions between the chains. The reaction centers avoid short-circuits by spending large amounts of redox free energy, while the more conspicuously reversible cytochrome bc₁ makes use of energetically modest and currently unproven mechanisms at the Q₀ site. Possibilities include redox-choirographed double-gating of a semiquinone intermediate or a genuinely concerted (within picoseconds or faster) double-electron transfer at the Q₀ site with no semiquinone involved at all. Deliberate induction of Q₀ site short circuits can clarify the energy conversion mechanism.

**O-9**
Structure-function relationship in the cytochrome bc₁ complex
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The cytochrome bc₁ complex (QCR) is a fundamental component of the energy conversion machinery of respiratory and photosynthetic electron transfer chains. This multisubunit, integral membrane protein complex catalyses electron transfer from ubiquinol to cytochrome c and couples it to the translocation of protons across the membrane. A combined approach of X-ray crystallography, biochemical analysis, site-directed mutagenesis and spectroscopy is used to obtain information about the molecular mechanism of QCR. X-ray crystallographic analysis of yeast QCR with bound substrate, substrate-analoga or inhibitor reveals details of the catalytic sites of the complex suggesting residues important for electron and proton transfer. Structural characterisation of the binding of a hydroxyquinone inhibitor supports the model that His181 of the Rieske protein and Glu272 of cytochrome b are direct ligands of ubiquinol in the enzyme-substrate complex. The structure provides experimental evidence for the rotational displacement of Glu272, which is required for the suggested proton transfer pathway. The role of selected amino acid residues for substrate binding, catalysis and proton transfer is studied by site-directed mutagenesis. The structural/functional characterisation of the variants will be presented and implications for the mechanistic model will be discussed.

**O-11**
The temperature dependence of the ultrafast electron transfer in cytochrome c oxidase
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Electron transfer (ET) within proteins occurs via chains of redox intermediates that favor directional and efficient electron delivery to an acceptor. Individual ET steps are energetically characterized by the electronic coupling Y, driving force ΔG, and reorganization energy λ. λ reflects the nuclear rearrangement of the redox partners and their environment associated with the reactions; λ≥700–1100 meV has been considered as a typical value for intra-protein ET. In non-photosynthetic systems, functional ET is difficult to assess directly. However, using femtosecond flash photolysis of the CO-poised membrane protein cytochrome c oxidase (CcO), the intrinsic rate of the low-ΔG electron injection from heme a into the heme a₃-Cu₅ active site was recently established at (1.4 ns)⁻¹. Here we determine the temperature dependence of both, the rate and ΔG of this reaction and establish that this reaction is activationless. Using the quantum mechanical form of non-adiabatic ET theory and common assumptions for the coupled vibrational modes, we deduce that λ<200 meV. It is demonstrated that the previously accepted value of 760 meV actually originates from the temperature dependence of Cu₅-CO bond breaking. We discuss that low ΔG, low λ reactions are common for efficiently channelling electrons through chains buried inside membrane proteins.
**O-12**

**Essential Arg of a subunit in FoF1-ATP synthase plays a key role in c-ring rotation**

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In a rotary motor F1,F1-ATP synthase (F0,F1), the ring of F0,F1 subunits (c-ring) rotates relative to F1,F1 as protons flow through channel(s) in F0,F1 and a carboxyl group in F1,F1. Previous reports have indicated that a conserved Arg in F0,F1 controls the proton transfer at the F0,F1c-ring interface. In fact, thermophilic F0,F1 with substitution of this Arg (aR169) to Glu, Ala, Val, Ile, Lys, Phe, or Trp lost proton-coupled ATP hydrolysis/synthesis activities, that is, no rotation occurred in these mutants. However, the mutants aR169E, aR169A and aR169W, but not other mutants, still mediated passive proton translocation. This proton translocation was completely blocked by the second mutation (cE56Q) of F1,F1. Then we generated a ‘rotation-impossible’ (c10–a)F1,F1 in which ten copies of F1,F1 in the c-ring and F0,F1 were all genetically fused as a single polypeptide. This (c10–a)F1,F1 had a native-like structure because activities appeared upon cleavage of c10/a linkage. We found that (c10–a)F1,F1 did not mediate passive proton translocation but (c10–a)F1,F1 with aR169A mutation did so though slowly. Thus, it appears that the large, delocalized positively-charged side chain of the conserved Arg in F0,F1 ensures proton-coupled c-ring rotation by preventing futile proton shortcut.

**O-13**

**Allosteric regulation of the Na+/H+ exchanger NHE-1 by cell membrane shape and composition**

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NHE-1 is an ubiquitously-expressed electroneutral mammalian Na+/H+ exchanger which maintains cytosolic pH and plays key roles in others cellular functions such as volume regulation, migration, proliferation and apoptosis. Its activation by intracellular acidification exhibits a cooperative behaviour for protons which increases upon growth factor stimulation. We have recently shown that this phenomenon can be described by a Monod-Wyman-Changeux model. In this mechanism, NHE-1 which is under a dimeric form, oscillates between a low and a high affinity conformation. Under resting conditions, the major form of NHE-1 is the low affinity exchanger. When the cytoplasm becomes acidic, the exchanger is converted into the high affinity form which actively extrudes protons from the cytoplasm. This new model was used for the quantitative characterization of NHE-1 response to different changes in membrane environment. The effects of membrane shape, tension and lipid composition on this allosteric transition will be presented. Implications for cell volume regulation will be discussed, in the light of functional sites possibly involved in this mechanism.

**O-14**

**The cytochrome b6f complex and a perplexing haem**

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In oxygenic photosynthesis, the cytochrome b6f complex transfers electron between two reaction centres, photosystem I and II. In so doing, it pumps protons across the membrane generating an electrochemical gradient of proton used for synthesis of ATP. The crystallographic structure of the chloroplastic (Stroebel et al. 2003, Nature 426:413) and cyanobacterial (Kurisu et al. 2003, Science 302:1009) complexes put the finishing touches to the structural description of the main participants to the photosynthetic chain of electron transfer providing an unique framework for the study of interacting complexes in the membrane. Large parts of the complex share homologies with the bacterial and mitochondrial complex bc1, suggesting similar mechanisms of proton and electron transfer for both complexes. However, intriguing features of cytochrome b6f raises several issues: The phytyl chain of a chlorophyll molecule reaches the quinone oxidising sire Qo, without an obvious function in a non light absorbing context. A beta-carotene molecule, but far from the chlorophyll, is also present. But the most unexpected feature is the presence of an additional haem in the quinone reducing site Qi. This haem, covalently bound to the protein, has no proteic axial ligand. This shed new lights on the controversial electron flow on the stromal side.
O-15

Fluorescence nanoscopy
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The resolution of a microscope using lenses and visible light has been limited by diffraction to ≈ 200 nm. We show that this barrier can be broken through reversible saturable optical (fluorescence) transitions (RESOLFT). First put forward as Stimulated Emission Depletion (STED) [1,3] and Ground State Depletion (GSD) microscopy [2, 3], RESOLFT concepts overcome diffraction by a saturated transition between two marker states, effected with a light featuring an intensity zero. Abbe’s equation is extended to \( \Delta x \approx \lambda / 2 n \sin(\alpha \sqrt{1 + 1/I_{sat}}) \) [4-6]. \( I_{sat} \) is the intensity required for saturating the transition and \( I \) is the one applied [4] \( \Delta x \) can be continuously decreased by increasing \( 1/I_{sat} \) [1-6]. We report on STED-microscopy with fluorescence spot sizes down to 16 nm, i.e. \( \sim \lambda/50 \) [6]. STED also allows fluorescence correlation spectroscopy with subdiffraction probing volumes [7]. Populating the marker’s triplet state [2,3] as well as the ‘switching’ between conformational states [4,5] in GFP-like proteins entail ultralow which should enable nanoscale resolution even with a lamp.

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[4] S. W. Hell Nature Biotech. 21, 1347 (2003).
[5] S. W. Hell Phys. Lett. A 326, 140 (2004).
[6] V. Westphal, S. W. Hell, PhysRevLett.(2005).
[7] L. Kastrup et al PhysRevLett (2005).

O-17

Single molecule imaging of glutamate receptor trafficking in and out synapses in neurons
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A central process in learning and memory formation is the modification of synaptic strength by changing the amount of glutamate receptors present at synapses. We will show that receptors exchange between synaptic and extrasynaptic spaces by lateral diffusion and that this might play an important role for the regulation of synaptic transmission. We applied single molecule tracking to follow glutamate receptor and lipid dynamics in living neurons during activity driven changes in synaptic transmission. We directly imaged AMPA and NMDA type glutamate receptor movements inside, at the periphery and outside synapses of live cultured hippocampal neurons using single-molecule fluorescence microscopy. In conditions of glutamate induced synaptic depression, receptor mobility increased inside synapses. Conversely, during the processes which lead to increased AMPARs numbers at synapses such as high frequency electrical stimulation, receptors are initially mobile in synapses and are then stabilized. Altogether, our results establish that synapses are specialized membrane microdomains whose components display a much higher dynamic than previously thought. This dynamic may explain the rapid changes in receptor composition observed during the processes of synaptic plasticity.

Borgdorff, A. and Choquet, D. (2002). Nature, 417, 649–653; Groc, L.,et al. (2004). Nat Neurosci 7, 695-696 ; Triller, A., and Choquet, D. (2005). Trends Neurosci 28, 133-139.

O-18

Secretory vesicle motions studied by total internal reflection fluorescence microscopy
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Total internal reflection fluorescence microscopy (TIRFM) is well adapted to the study of the motions of secretory vesicles located in the vicinity of the plasma membrane. Using this technique, we have monitored trajectories of fluorescently labeled subplasmalemmal vesicles in 3 dimensions. In order to better understand the interactions of the vesicles with their complex environment (cytoskeleton, membrane), we applied two different approaches: (i) we developed a software to dissect complex vesicle trajectories, (ii) we modified the microscope for dual-color observation in live cells. In approach (i), a given trajectory is divided into sub-trajectories, each exhibiting a distinct behavior (random, constrained or directed motion). These sub-trajectories are then quantitatively analyzed. This method allowed us to characterize precisely dynamics of subplasmalemmal vesicles. Using approach (ii), we directly visualized interactions between vesicles and cytoskeleton. Firstly, simultaneous labeling of microtubules and vesicles showed that a small fraction of vesicles move along microtubule tracks rapidly ( \( > 1 \mu m/s \) ) and over long distances ( \( > 5 \mu m \)). Secondly, simultaneous labeling of vesicles and actin filaments revealed that some vesicles are clearly attached to actin filaments.
O-19
Combined FRET and anisotropy measurements synchronized with patch clamping
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Fluorescence resonance energy transfer (FRET) is often regarded as a "spectroscopic ruler" to determine the distance of molecules in the range of nanometers. It is used for binding studies and conformational studies of proteins. Distance determination by FRET requires knowledge about the orientation of the fluorophores in addition to the amount of FRET occurring. Recently, it was suggested to use linearly polarized excitation and to acquire information about the polarization of the acceptor emission to get better information about the real amount of FRET occurring. However, in some cases there might be pitfalls with this method due to changes in the rotational mobility as well as high fluorophore concentration and bleaching both of which diminish apparent anisotropy.
To overcome these problems, we have developed a method to acquire additional information about orientation. With every excitation we toggle between donor and acceptor wavelength and acquire simultaneously donor and acceptor emission, both in parallel and perpendicular polarization direction. This setup allows us to measure the fluorescence anisotropy of the donor and acceptor when directly excited and the loss of acceptor anisotropy due to FRET occurring which was performed on a membrane anchor domain and a G-protein coupled receptor. Fluorescence measurements are synchronized with a patch clamp setup to gain information about conformation changes occurring on the voltage-gated, L-type calcium channel when switched between active and inactive states.

O-20
Ligand-induced monomer/dimer dynamics of the urokinase receptor in live cells by 2P-FLIM/FCS
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The urokinase receptor (uPAR) is a multifunctional receptor, which regulates cell adhesion, migration and proliferation in normal and pathological situations. It is currently unknown how the GPI-anchored uPAR transmits a variety of signals into the cell. We follow the effect of uPAR ligands on live HEK293 cells expressing functional fluorescent chimeras of uPAR, in which EGFP or spectral variants, were inserted between the GPI-anchor and the D3 receptor domain. By combining 2-photon FLIM and fluorescence fluctuation spectroscopy (FCS, PCH), we show that uPAR monomers and dimers are heterogeneously distributed in membrane microdomains. Dimers increase in the cell-to-cell contacts as well as in the presence of the amino terminal fragment of the ligand uPA. In contrast, PAI-1-dependent internalization of uPAR results in a reduction of uPAR dimers exposed in the plasma membrane. These results support the hypothesis that uPAR monomer/dimer interplay can be regulated by extracellular ligands such as uPA and PAI-1, introducing a novel concept that might contribute to understand the function of the receptor in signaling.

O-21
Single molecule imaging of nuclear transport in living cells and quantification of interactions
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We were able to clearly visualize single molecules inside cells and in living cells, using novel microscopy, Highly Inclined and Laminated Optical sheet (HILO) microscopy. GFP-tagged importin β, a carrier protein, and GFP-tagged cargo protein was examined during transport on the nuclear envelope. Image analysis of single nuclear pores showed that two point resolution of 70 nm was achieved. Kinetic parameters of the interactions between translocating molecules and nuclear pore complexes (NPCs) were obtained through quantitative analysis. Two types of binding site were found, weaker binding site, which gathers up to ~100 molecules/NPC and concentrates molecules locally, and stronger binding site in the absence of RanGTP, with an affinity that changes drastically upon translocation ability. Accessibility of importin β to the stronger binding site is critical for NPC translocation. In the presence of RanGTP, the stronger binding site disappears and the active site appears. Translation rates into the nucleus were obtained and correlated well with the retention time of single molecules. The correlation coefficient shows that the maximal binding was 8 (or possibly 16) molecules/NPC. Based on these findings, we propose a novel model of NPC translocation. Thus, single molecule imaging a powerful technique to quantify dynamics and kinetics of molecular interactions and to elucidate molecular mechanisms inside cells.

O-22
Fluorescence cross-correlation microscopy shows cytoskeleton-independent co-mobility of MHC I and II
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MHC class I and II glycoproteins are responsible for presenting antigens to T cells. We and others have shown previously that oligomerization of MHC I molecules increases the efficiency of antigen presentation. In addition to homoassociation of both MHC I and II molecules, their heteroassociation has also been demonstrated in B and T lymphocytes by fluorescence resonance energy transfer. In this study we used fluorescence correlation and cross-correlation microscopy to investigate the mobility and co-mobility of MHC I and II molecules in JY B lymphoma cells. In intact plasma membranes the diffusion coefficient of MHC I molecules was an order of magnitude lower than in blebs. Cross-correlation measurements on MHC I and II molecules labeled by Alexa 488- and Cy5-tagged mAbs resulted in positive cross-correlation amplitudes implying their co-mobility in intact membranes and in blebs. Our results suggest that interaction and aggregation of MHC I and II does not depend on the cytoskeleton.
**Oral Presentations**

-- Modelling Complex Systems --

**O-23**

Modelling collective behaviour

T. Vicsek

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Organisms and cells tend to exhibit spectacular kinds of collective behaviour including such well known examples as synchronization or flocking. When modelling such systems the standard methods of statistical physics are very useful and lead to simulations both agreeing with observations and allowing predictions. Here we consider a few selected phenomena ranging from the ordered motion of tissue cells to the propagation of waves and panic in crowds. In particular, when many keratocytes (skin cells from fish) are put in a Petri dish, interesting group motion patterns can be observed. On the other hand, people moving in the same environment also develop specific features of collective motion such as the formation of lanes, flocking or jamming at bottlenecks. We argue that these and the many other emerging behavioural patterns are universal and can be successfully interpreted by assuming realistic interactions between particles representing cells or humans.

* The above results have been obtained in collaboration with B. Adamcsek, A. Czirok, I. Farkas, D. Helbing and B.Szabo

**O-24**

Fluctuations, noise and survival: an outsider’s view on bacterial life

S. Leibler

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I will describe some recent experimental and theoretical efforts to understand fluctuations and noise on the microscopic level in individual cells. Molecular noise can be both resisted and harnessed by bacteria. Some lessons about survival will also be drawn.

**O-25**

Identifying structural details for protein-protein interactions

R. B. Russell

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Many experiments suggest that pairs of proteins are involved in physical interactions, though few give any insights as to the details of how they are mediated. In this talk, I will describe our approaches to predict structural details for protein-protein interactions in protein interaction networks, complexes and pathways. The approaches identify interactions involving large interfaces, such as those between two or more globular domains, in addition to those mediated by short peptides (e.g. SH3-polyproline). I will also describe predictions that we have validated details through experiments in the laboratory.

**O-26**

Uncovering the overlapping community structure of complex networks in nature and society

I. Derenyi\(^1\), G. Palla\(^2\), I. Farkas\(^2\), T. Vicsek\(^1\)

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Many complex systems in nature and society can be described in terms of networks capturing the intricate web of connections among the units (proteins, genes, people, etc.) they are made of. A question of great current interest is how to interpret the global organization of such networks as the coexistence of their structural sub-units (communities) associated with more densely interconnected groups of nodes. Identifying these communities is crucial to the understanding of the structural and functional properties of networks. The existing methods used for large networks find separated communities, however, in most real networks the communities are believed to overlap with each other to a great extent. Here we introduce a new approach to determine the interwoven sets of overlapping communities of large real networks [1]. Among other examples, we demonstrate the power of our method by identifying the communities of proteins in the protein-protein interaction network of yeast, which then, for example, allows us to make predictions for yet unknown functions of some proteins.

[1] G. Palla, I. Derenyi, I. Farkas, and T. Vicsek, to appear in Nature.
O-27

Stochastic dynamics of coupled repressilators

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2Department of Computational Science and Engineering and Graduate
School of Information Science, Japan

Background/aim: Intrinsic noise in the gene expression is unavoidab-
le due to a small number nature of biomolecules in a cell. The sys-
tematic analyses of the stochastic dynamics, therefore, are most de-
sired to understand the design principle of the gene network. Here,
we theoretically examine collective motions of coupled genetic os-
cillators to examine how the noise affects the oscillatory behavior
of those circuits.

Model and Method: Repressilator is a network composed of three
genes in which protein synthesized by one gene represses the ex-
pression of the other gene cyclically. In usual experimental setup
multiple copies of plasmids are embedded in a cell, so that multiple
repressilators work in a coupled manner. We perform the stochastic
simulation of coupled repressilators with the Gillespie algorithm.

Results: Owing to the noisiness of dynamics, repressilators show
the coherent oscillatory behavior only in the limited range of the
rate of the gene switching. The range is enlarged and the oscillation
is stabilized when the number of coupled repressilators increases.
Each of those coupled repressilators, however, does not necessarily
oscillate in the same way but there is a fluctuation in the number
of repressilators which synchronize with the collective oscillation.
This fluctuation leads to the amplitude fluctuation in the number
of proteins to show the ordering-disordering transformation in the
phase-amplitude space.

O-28

Semiconductor chips with ion channels, nerve cells and brain tissue

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Electrical information processing in brains and computers relies on
different charge carriers, ions and electrons. We attempt to join the
two systems on a microscopic level. On the side of electronics,
we use simple silicon chips with transistors and capacitors and also
CMOS chips fabricated by very large scale integration. On the bio-
logical side, we distinguish ion channels, nerve cells and brain tis-
sue. We incorporated recombinant ion channels into cell-chip con-
tacts and characterized the signal transmission between electronics
and ionics. We connected individual nerve cells from snails and rats
to the chips and created small hybrid networks. Finally, tissue layers
from rat brain were used. With CMOS chips arrays of 128x128 sen-
sor transistors provided electrical maps of snail neurons, neuronal
networks and brain tissue.

O-29

Biophysical modelling of the heart

D. Noble
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Biological modelling of cells, organs and systems has reached a
very significant stage of development. Particularly at the cellular
level, there has been a long period of iteration between simula-
tion and experiment (Noble, 2002, Noble & Rudy, 2001). We have
therefore achieved the levels of detail and accuracy that are required
for the effective use of models in drug development. To be useful
in this way, biological models must reach down to the level of pro-
teins (receptors, transporters, enzymes etc), yet they must also re-
construct functionality right up to the levels of organs and systems.
This is now possible and three important developments have made
it so:
1. Relevant molecular and biophysical data on many proteins and
the genes that code for them is now available. This is particularly
true for ion transporters.
2. The complexity of the biological processes that can now be mod-
elled is such that valuable counter-intuitive predictions are emerg-
ing (Noble & Colatsky, 2000). Multiple target identification is also
possible.
3. Computer power has increased to meet the demands. Even very
complex cell models involving up to 100 different protein functions
can be run on single processor machines, while parallel computers
are now powerful enough to enable whole organ modelling to be
achieved (Crampin et al., 2004)
I will illustrate these points with reference to models of the heart.
**Oral Presentations**  
– Protein Reactivity and Dynamics –

**O-30**

Hydration structure changes around proteins at work

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Proteins fold and function in water exhibiting unusual physicochemical properties caused by hydrogen bonds between water molecules. Between water and proteins, interfacial structures so-called hydration structures exist. X-ray crystallography has great contribution to visualize the hydration structures of proteins. The tetrahedral geometry of hydrogen bonds of water molecules retain on the protein surface, and induce networks of hydrogen bonds over the entire surface of proteins. Thus, movements of their domains or segments in proteins at work require cooperative reorganization of hydration structures. Although it is still difficult to observe directly the reorganization accompanying protein motion, crystallographic analyses of multi-domain proteins provide structural information on the reorganization. Here I present a few examples of hydration structure changes cooperatively occurring in protein motions through crystal structure analyses and discuss the nature of water-protein interactions.

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**O-32**

Structural dynamics controls ligand binding in neuroglobin

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Neuroglobin (Ngb) is a small globular heme protein involved in the protection of neuronal tissue from ischemic damage. Its specific functional role, however, is not yet known. Ngb binds small gaseous ligands (O2, NO, CO), but its low expression level precludes a role as an oxygen storage protein. Ngb is also devoid of O2 and NO reductase activities. A possible function as an oxygen sensor has been suggested.

We have studied ligand binding in murine neuroglobin using time-resolved optical and infrared spectroscopies in combination with x-ray crystallography. In the absence of an exogenous ligand, the ferrous heme iron of Ngb is hexacoordinated by the proximal and distal histidines, His96 and His64. To bind an exogenous ligand, His64 dissociates from the sixth heme coordination, and the heme group slides deeper into the interior of the protein. Heme relocation is accompanied by a significant decrease of structural disorder, especially of the EF loop. This property may be related to the proposed sensor function. Further insights into the interplay between reactivity and structural changes have been gained by studies on Ngb mutants.

**O-33**

How elastic are biopolymers? Mechanical properties of proteins

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The viscoelastic properties of solid samples (crystals, amorphous films) of hen egg white lysozyme, bovine serum albumin, and sperm whale myoglobin were studied in the temperature range of 100-300°C at different hydration levels. Decreasing the temperature was shown to cause a steplike increase in the Young’s modulus of highly hydrated protein samples (with water content exceeding 0.3 g/g dry weight of protein) in the temperature range of 237-251°C, which we refer to as a mechanical glass transition.

Soaking the samples in 50% glycerol solution completely removed the steplike transition without significantly affecting the glass transition. The apparent activation energy determined from the frequency dependence of the glass-transition temperature was found to be 18 kcal/mol for wet lysozyme crystals. Lowering the humidity causes both the change of the Young’s modulus in response to the transition and the activation energy to decrease. The thermal expansion coefficient of amorphous protein films also indicates the glass transition at 150-170°C. The data presented suggest that the glass transition in hydrated samples is located in the surface layer of proteins and related to the immobilization of the protein groups and strongly bound water. The viscoelastic properties of globular proteins were found to display marked heterogeneity resulting both from its domain structure and enhanced mobility of surface layer.

**O-31**

Dominant features of protein internal dynamics

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Proteins are virtually degenerate systems adopting a large number of statistical states with closely similar structures and slightly different reaction rates for binding or releasing a ligand. The dissociation of the ligand triggers various dynamic processes involving the protein matrix and interfering with the further rebinding kinetics. This talk will review the dominant aspects of protein reaction dynamics as revealed by combining laser flash photolysis kinetic spectroscopy at low temperature and a "model free" analysis of the complex, polychromatic rebinding kinetics. Kinetic processes are identified and quantified directly from the data without the need for a priori assumptions or predefined kinetic schemes. In recent years, the characterization of kinetic processes has further progressed by using Xenon gas as a soft external perturbing agent that competes with the ligand for occupying hydrophobic protein cavities. These combined approaches permitted to disentangle the complex kinetics of ligand rebinding with hemoproteins such as an oxygen transporter (Myoglobin) or an enzymatic protein (Cytochrome P450cam). The emerging picture is that reaction dynamics are largely dominated by conformational relaxation and ligand migration, two general processes likely to occur in most proteins.
O-34
Fluorescence dynamics map reveals the pathway of protein amyloid fibril formation and structure
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Protein aggregation leading to amyloid fibrillation has drawn considerable attention in recent times due to its involvement in a variety of neuro-degenerative diseases. In this work, we report the first investigation of residue-specific fluorescence dynamics as a novel approach to characterize the formation of amyloid fibrils. Barstar, a model protein used extensively in folding studies, forms amyloid-type fibrils at low (≤3) pH and temperatures above 50°C. Seven single cysteine-containing mutant forms of barstar were made, and each was labeled with the thiol-active fluorescent probe IAEDANS. Additionally, Trp53 was also used as an anionic fluorescence probe. Picosecond time-resolved fluorescence was used to monitor rotational dynamics at these eight positions in the low pH A-form, and in amyloid fibrils. Based on this, we contruct a dynamic-amplitude map, which illustrates structural line points along the polypeptide. This map is further supported by measurements of solvent exposure, using dynamic quenching of by acrylamide. The map points out that the N-terminus is less involved in fibrillation when compared to the C-terminal region. Furthermore, it is shown that the transformation of the A-form into amyloid does not alter significantly the local structure at these positions, and hence, occurs directly.

O-35
Molecular dynamics simulations to study enzyme cold-adaptation: a family-centred point of view
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In recent years, there has been increased interest in the origin of enzyme adaptation to low temperature both for understanding the protein folding and designing of biocatalysts with enhanced activity and thermostability. The number of reports on enzymes from cold adapted organisms has increased significantly over the past years, and reveals that adaptative strategies varies among enzymes, which use different small selections of structural features for gaining increased molecular flexibility that in turn lead to increased catalytic efficiency and reduced stability.

Molecular flexibility is a parameter difficult to estimate by experimental methods, whereas molecular dynamics simulation of protein systems provides a suitable tool to evaluate flexibility and molecular properties of proteins and correlate them to protein structural and functional aspects. In the present contribution we report results obtained from several long molecular dynamics simulations of representative structures for mesophilic and psychrophilic enzymes at different temperatures, to explore the molecular basis of cold adaptation inside a specific enzymatic class. The molecular dynamics trajectories were compared and analyzed in terms of secondary structure contents, molecular flexibility, intramolecular interactions and protein-solvent interactions, unravelling putative structural and molecular determinants of thermostability and activity at low temperature for psychrophilic enzymes.

O-36
Narrowing the gap, a role for protein dynamics in biological quantum tunnelling
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We present an integrated approach that provides atomic level description of protein dynamics that impact on biological quantum tunnelling processes. The transfer of small particles such as electrons and hydrogen atoms is an essential part of a large proportion biochemical reactions and it is now widely accepted these processes occur by quantum tunneling. Catalysis of these reactions can occur by shortening of the tunnelling distance rather than decreasing the barrier height. Using two distinct protein systems each catalysing a distinct quantum tunnelling process, we will discuss the nature and scale of protein motions involved in narrowing the gap. The ubiquitous electron transferring flavoprotein (ETF) exhibits large-scale domain dynamics when complexed to redox partners that bring the redox cofactors within 14 Å, a distance small enough to allow fast electron transfer1–3. In contrast, the quinoprotein aromatic amine dehydrogenase (AADH) exhibits relatively small-scale motions along the reaction coordinate that can promote proton tunneling during the catalytic cycle4. In both cases, the relative decrease in required tunnelling distance due to protein motions is considerable, underpinning significant rate enhancement.

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Chromatin compaction at the mono- and trinucleosomal level
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Gene activity and silencing are related to temporary and local changes in chromatin compaction. Factors influencing this include the ionic environment, presence of linker histones, post-translational modification of the core histones and DNA methylation. We studied the effect of some such factors on reconstituted mono- and trinucleosomes by measuring linker DNA distances in solution by FRET and by observing in SFM the conformation of trinucleosomes attached to a surface. Monovalent ions lead to approach of the linker DNA arms in all observed cases. Binding of linker histones induces further approach of the linker DNAs and decreases internucleosome distances. Selective acetylation of core histones causes differential effects on the DNA geometry. DNA methylation is not unequivocally associated with changes in the local compaction.

Chromosome structure and gene regulation
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The genomic DNA of eukaryotes is tightly wrapped into chromosomes through a hierarchical series of folding steps. In the lowest level of compaction, short stretches of DNA are wrapped around histone protein spools, forming structures known as nucleosomes. The structure of the nucleosome occludes most of the wrapped DNA from interaction with the regulatory proteins and enzymes that must act on it. In this talk I will discuss dynamic properties inherent to nucleosomes that facilitate the invasion of nucleosomes by gene regulatory proteins, and I will describe studies testing these ideas in vivo. I will then discuss studies testing these ideas in vivo. I will then discuss studies testing these ideas in vivo. I will then discuss studies testing these ideas in vivo. I will then discuss studies testing these ideas in vivo. I will then discuss studies testing these ideas in vivo.
Oral Presentations
– From DNA to Chromatin –

O-41
Chromatin dynamics and structure studied at the single molecule level
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The DNA of all eukaryotes is arranged into a highly organized structure called chromatin whose fundamental unit is the nucleosome (NS). It is now established that chromatin is a dynamic structure that actively participates to gene regulation. Single molecule methods have already proved powerful in providing important information about chromatin structure and dynamics (1,2). Here, we describe new and complementary tools to study real time chromatin dynamics with excellent temporal and spatial resolution.

We first investigate quantitatively chromatin assembly in real time with fluorescence microscopy (2,3) and show that it strongly depends on histones modifications and on their chaperones (4). Then, we examine the mechanical response of nucleosome arrays under tension and torsion with magnetic tweezers (5). We demonstrate that NSs can undergo a structural transition under torsion in order to release rotational strain. A model, confirmed by bulk and single molecule experiments, is proposed to account for this process. Finally, the biological relevance of these results is discussed.

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O-42
Compaction of single-chain DNA by histone-inspired nanoparticles
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The complex of DNA with a cationic octamer of core histones is the elemental unit of chromatin which allows for a large-scale compaction while preserving gene activity. We elaborated a versatile experimental model of chromatin which consists of a single chain of long DNA that interacts with synthetic cationic nanoparticles of various sizes. The DNA/nanoparticle interaction was characterized by direct single-molecular observations in the bulk solution by fluorescence microscopy and the nanostructure of the DNA/nanoparticle complexes was resolved by transmission electron microscopy. We found that the single-chain DNA compaction by nanoparticles is stepwise and progressive. It proceeds through the formation of beads-on-a-string structures, the DNA chain wrapping around individual nanoparticles. We investigated the effect of particle size and salt concentration on compaction efficiency and mechanism of wrapping. Notably, the DNA compaction is optimal at a physiological salt concentration and complexed nanoparticles are overcharged by DNA.

O-43
DNA: not merely the secret of life
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Structural DNA nanotechnology uses the concept of reciprocal exchange between DNA double helices to produce branched DNA motifs, like Holliday junctions, or related structures, such as double crossover (DX), triple crossover (TX), parastic crossover (PX) and DNA parallelogram motifs. We have worked since the early 1980’s to combine DNA motifs, using sticky-ended cohesion, to produce specific structures. From branched junctions, we have constructed DNA stick-polyhedra, whose edges are double helices, and whose vertices are the branch points of DNA branched junctions. These include a cube, a truncated octahedron, and an irregular graph.

Nanorobotics are key to the success of nanotechnology. We have used two DX molecules to construct a DNA nanomechanical device by linking them with a segment that can be switched between left-handed Z-DNA with right-handed B-DNA. PX DNA has been used to produce a robust sequence-dependent device that changes states by varied hybridization topology. Recently, we have used this device to make a translational machine. A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed micron-sized 2-dimensional DNA arrays from DX, TX and two kinds of parallelogram motifs. We can produce specific designed patterns visible in the AFM from DX and TX molecules. We can change the patterns by changing the components. Recently, we have used robust triangular motifs to produce honeycomb-shaped arrays.
O-44

G-protein coupled receptors and their associated proteins

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During evolution, “molecular tinkering” of G-protein-coupled receptors (GPCRs) has proven to be greatly successful. This ‘tinkering’ has provided, from a single gene or a limited number of genes, more than 1000 GPCRs dedicated not only to cell-cell communication but also to the recognition of environmental signals, such as light, smell and taste. Once activated by their ligands, GPCRs catalyse the GDP-GTP replacement on a limited number of heterotrimeric G proteins that regulate second messenger production and ionic channel activity. GPCRs are also part of large protein complexes called ‘receptosomes’. The majority of ‘receptosome-associated’ proteins have been discovered step by step using the ‘two hybrid’ technology. We have described ‘in one step’ the ‘receptosomes’ associated with C-termini of some GPCRs using a proteomic approach. The C-termini of GPCRs were immobilized on affinity columns (GST fusion proteins or peptides) and ‘associated’ proteins were fished out from cell or tissue extracts. The proteins were identified after separation on 2D gels using mass spectrometry. ‘Receptosomes’ associated with PDZ ligands of the extreme C-termini of serotonin (5-HT) and glutamate receptors will be described. The functions of some proteins in signaling and organizing the synapse will be illustrated.

O-45

Kv channel remodeling in heart during late pregnancy

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We investigated changes in heart structure, function, and gene expression of known markers of pathological hypertrophy and cell stretching. In late pregnancy, hearts show eccentric hypertrophy, with normal left ventricular function and a reduction of systolic function. Pregnancy-related physiological heart hypertrophy does not induce expression changes of pathological molecular markers; it induced the remodeling of Kv4.3 channel and increased c-Src tyrosine kinase activity. Cardiac Kv4.3 channel gene expression was downregulated by ~3-5 fold, both at the mRNA and protein levels. Downregulation of Kv4.3 protein smaller peak outward K⁺ currents, longer action potential duration and longer QT. Peak outward K⁺ currents densities were significantly reduced from 48.9±6.7 pA/pF in NP to 29.5±2.7 pA/pF in LP mice. Downregulation of cardiac Kv4.3 gene expression was mimicked by estrogen treatment. c-Src activity increased by ~2 fold in late pregnancy without change in total c-Src protein levels. Increased c-Src activity was also observed in after E2 treatment. Our data reveal that pregnancy-related heart hypertrophy is associated with increased c-Src activity and downregulation of Kv4.3 expression controlled by estrogen. The fact that the downregulation of Kv4.3 expression in late pregnancy induced a longer QT interval provides a molecular explanation for the increase in QT interval and cardiac arrhythmogenesis in pregnancy.

O-46

Gating mechanism of KirBac3.1 based on the crystal structure of two gating intermediate forms

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Inwardly rectifying K⁺ (Kir) channels play a central role in a wide variety of physiological processes ranging from hormonal secretion, regulation of the heart’s activity to the re-uptake of K⁺ ions in the kidney. The selective nature of these channels is only half the reason as to why Kir channels can be involved in such diverse processes with gating, the mechanism of channel opening and closing, being the other half. The molecular structures of two crystal forms of the Kir channel from Magnetospirillum magnetotacticum (Kir-Bac3.1) have been determined. These structures provide a clearer understanding of the gating mechanism for this family of channels. They have revealed both Mg²⁺ and polyamines binding sites along the central ion conduction pathway, the molecules that are responsible for the rectifying properties of these channels. This along with an altered distribution of K⁺ ions present within the selectivity filters as compared to KcsA, C-terminal domain rotations, an increase in the diameter of the ion conduction pathway at the location of the membrane/cytoplasmic interface and a change in N- and C-terminal domain interactions provide evidence that these structures are intermediate structures between the closed and open states. A molecular mechanism of the gating process will be presented.

O-47

Fluorescence lifetime spectroscopy of KcsA reveals the existence of two gates in the permeation pore

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While it is generally agreed that the opening of the pore bundle crossing is a prerequisite for ion conduction, it still remains to be elucidated if the opening is also sufficient for pore conduction or if opening of a second gate near the selectivity filter is required. We addressed this problem by determining fluorescence lifetimes of KcsA as a function of pH labeled near C-terminus of TM2 right below the bundle crossing. The measurements were done with purified, labeled and reconstituted channels in lipid vesicles. The bound fluorophore showed two lifetimes, 3 ns and 1 ns, which were independent of pH. The relative amplitude of fast to slow lifetimes increased with lower pH. Since the pH dependence correlated well with the open probability of KcsA, we interpreted the two lifetimes as coming from two populations of channels either with closed (3 ns) or open (1 ns) bundle. The mutation A73E in the p-loop had a much higher open probability than wildtype KcsA, determined by Rh⁺-flux and in bilayer experiments. A73E showed the same fraction of fast lifetimes, indicating that the open probability of the bundle crossing does not change in this mutant. These results suggest the existence of a second gate near the selectivity filter, serial to the bundle crossing, which is predominantly closed in wildtype KcsA, but kept open in the mutants with high open probability.
Oral Presentations  
– Channels and Receptors –

O-48  
Modulation of Gj of Connexin36 (Cx36) Channels expressed in N2A cells: the role of CaM Kinase II  
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The gap junction protein Cx36 interconnects GABAAergic interneurons and other neuronal populations and also is found between pancreatic islet cells. In dual whole cell recordings from transfects of neuroblastoma (N2A) cells, we have found that junctional conductance (gj) of both C-terminally GFP-tagged and untagged Cx36 channels display the remarkable property of “run-up”, in which gj increases by ten-fold or more within minutes following cell break in with patch pipettes. In order to determine mechanisms underlying the run-up phenomenon, we have applied a variety of pharmacological tools and methods. KN-93, a selective inhibitor of CaM Kinase II (CaM KII) blocked the run-up and a similar effect was also observed when the cells were dialyzed with a peptide whose sequence overlaps the binding site for CaMKII of Cx36. Experiments with synthetic peptides overlapping the potential phosphorylation sites of Cx36 showed that phosphorylation of the S315 residue in the C-terminal (CT2), but not the S111 residue in the cytoplasmic loop (CL1) contribute to the “run up”. Other pharmacological inhibitors including colchicine and AMP-PNP also attenuated the “run up” implying that the run-up in the junctional currents observed in Cx36 transfectants may be due multiple pathways. Experiments are underway to determine whether these different pathways are parallel and synergistic or converge within a simple common signaling mechanism.

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O-49  
An atomistic simulation study of the nicotinic acetylcholine receptor transmembrane domain  
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The adult muscle-type nicotinic acetylcholine receptor (nAChR) is currently the most well-characterised member of the superfamly of ligand-gated ion channels. Recent electron microscopic studies (Unwin, J. Mol. Biol. 346(4):967-989) have elucidated the structure of the transmembrane, extracellular and part of the intracellular domains. Additionally, numerous experimental studies have demonstrated the influence of the lipid environment on its channel activity. We have performed molecular dynamics (MD) simulations on the transmembrane domain of the nAChR within a lipid bilayer in order to study its dynamics in a membrane environment, and to elucidate the nature of lipid-protein interactions at the atomic level. The evolution of the TM domain structure during the MD trajectory is described, with particular focus on the motions of the M2 helices and their influence on the dimensions of the pore. Correlations between M2 hinge-bending motions and fluctuations of the M2-M3 extracellular loops were observed, suggesting one possible mechanism by which conformational changes at the ligand binding domain may influence the dynamics of the pore-lining helices. Ion desolvation energies along the pore were estimated using a continuum solvent model and discussed with respect to the physical dimensions and polarity of specific regions within the channel. Additionally, we characterise the structural and dynamical properties of lipids in proximity to the protein.

O-50  
Ca-activated potassium channels drive agonist-stimulated NO synthesis in human endothelial cells  
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The production of nitric oxide (NO) by the vascular endothelium affects hormone-induced vasodilation, platelet adhesion and angiogenesis. Pharmacologic studies have demonstrated that agonist-stimulated vasodilation can be largely prevented by apamin and charybdotoxin (ChTx), selective inhibitors of small and intermediate conductance, Ca-activated K+ channels (SKCa and IKCa channels, respectively). Both types of channels have been recently identified in vascular tissue, however, the cellular mechanism(s) by which they contribute to vasorelaxation remains controversial. We have hypothesized that activation of endothelial SKCa and IKCa channels by Ca-mobilizing stimuli serves as a critical step in the cellular pathway leading to NO production. Experimentally, agonist-induced changes in membrane potential or SKCa and IKCa channel currents were performed in single patch clamped HUVECs loaded with either DAF-FM or Fluo-3 to monitor NO synthesis or cytosolic [Ca2+]i, respectively. Selectively inhibition of SKCa and IKCa channels by apamin and ChTx prevented NO synthesis, membrane hyperpolarization, and increased cytosolic [Ca2+]i resonance to either histamine or ATP. Similar effects were produced by 80 mM external K+ or removing external Ca2+. However, agonist-stimulated NO synthesis was unaffected by 50 µM Ba2+ and 100 µM ouabain. These data suggest a mechanistic model in which endothelial SKCa and IKCa channels ‘drive’ NO production in response to Ca2+ mobilizing agonists via membrane hyperpolarization.

O-51  
Store-operated Ca2+ entry is regulated by mitochondria in acinar cells of rat submandibular salivary gland  
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In non-excitable cells the major route for Ca2+ influx is through store-operated Ca2+ channels in the plasma membrane that are activated by emptying of intracellular Ca2+ stores following the stimulation. However, little is known about the downstream consequences of depletion of internal Ca2+ stores in submandibular cells. We have studied the extent of the cytosolic Ca2+ increase (Ca2+increase) from 3 µM thapsigargin (Tg) in cells preloaded with fura-2/AM. In the absence of external Ca2+, Tg triggered small transient Ca2+ release from the internal stores with the amplitude 51 ± 14 nM; in the presence of external Ca2+ the Ca2+ signal was more sustained with the amplitude 112 ± 11 nM. We found that mitochondria (Mit) are required to support Ca2+ entry in the acinar cells. In these experiments, the stores were depleted by exposing cells to Tg for 20 min in Ca2+-free solution and then to CCCP (10 µM) for further 15 min. The rate of Ca2+ entry and amplitude of [Ca2+]i rise, measured following readdmission of external Ca2+, were significantly lower when Mit had been depolarized. Thus, the emptying of intracellular Ca2+ stores in acinar cells activates the sustained store-operated Ca2+ entry and Mit may have a crucial physiological role in regulating store-operated influx.

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O-52
How hydrogenases respond to inhibitors, including CO, O₂, NO, and other small ligands
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Hydrogenases catalyse redox transitions of the smallest known substrates (2H⁺/H₂). They are metalloenzymes, widespread throughout the microbial world, which contain Fe, Ni and unusual exogenous ligands like CO and CN. Due to the very nature of the steps that are involved – H₂ binding, H-H bond cleavage, hydride coordination, electron transfer, and proton transfer, the catalytic action of these enzymes is vulnerable to competing reactions that block and even destroy the fragile active sites. Indeed, chemists who synthesise and study small analogue molecules that are based on or inspired by these centres know only too well that they must be protected from O₂ and even water. We are conducting research into the reactions of hydrogenases from different sources and extracting information that is not only mechanistically revealing but is also relevant for developing future hydrogen-cycling technologies. Our approach is to attach the enzyme molecules to the surface of a rotating carbon electrode and use the electrode potential to control catalysis and initiate interconversions between states, all under strictly controlled conditions in a sealed cell. These investigations complement and enrich the studies carried out by biologists, spectroscopists and crystallographers. This lecture will highlight recent examples of our efforts to help solve problems in hydrogenase research and will look ahead to how these catalysts may be exploited in energy technologies.

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O-54
Structural differences between the ready and unready oxidized states of [NiFe]-hydrogenases
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[NiFe]-hydrogenases catalyze the reversible oxidation of molecular hydrogen. When exposed to molecular oxygen, two major inactive states of these enzymes, called Ni-B (ready) and NiA/SU (unready), are generated that are distinguishable by their very different activation kinetics. The structural basis for this difference is not currently understood because of lack of relevant crystallographic data. We have recently determined the crystal structure of the ready Ni-B state of Desulfovibrio fructosovorans [NiFe]-hydrogenase and show it to have a putative α-hydroxy Ni-Fe bridging ligand at the active site (1). We have also improved the X-ray crystallographic refinement of putative unready Ni-A/Ni-SU states that display a more elongated electron density for the bridging ligand suggesting that it is a diatomic species. Consequently, the slow activation kinetics of the Ni-A state, compared to the rapid activation of the Ni-B state, is most likely due to the presence of chemically different ligands in the two oxidized species. Taken together, our results and very recent electrochemical studies (F. Armstrong talk) suggest that the diatomic ligand could be hydro-peroxide.

O-53
Gating of proton and water transfer in cytochrome c oxidase
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The membrane-bound enzyme cytochrome c oxidase is responsible for cell respiration in aerobic organisms, and conserves free energy from O₂ reduction into an electrochemical proton gradient by coupling the redox reaction to proton pumping across the membrane. O₂ reduction produces water at the bimetallic heme a₃Cu₅ active site next to a hydrophobic cavity deep within the membrane. Water molecules in this cavity have been suggested to play an important role in the proton-pumping mechanism. Molecular dynamics simulations will be reported suggesting that the conserved arginine-heme a₃Δ-propionate ion pair provides a gate, which exhibits reversible thermal opening that is governed by the enzyme’s redox state and the water molecules in the cavity. An important role of this gate in the proton pumping mechanism is supported by site-directed mutagenesis experiments. Transport of the product water out of the enzyme must be strictly controlled to prevent proton leaks that could compromise the proton-pumping function. Exit of product water is observed via the same arginine-propionate gate, which provides an explanation for the observed extraordinary spatial specificity of water expulsion from the enzyme.

O-55
Investigation into the kinetics of the direct electron transfer to a multi-centered membrane protein
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By immobilising a bacterial Cytochrome c Oxidase-mutant from Rhodobacter sphaeroides in a lipid architecture [1], it is possible to orient this protein with its natural electron pathway entrance towards the electrode surface. Potential-dependent SERRS in comparison with resonance raman spectra of CoO₃ in solution shows that the redox site structures of the surface confined enzyme are preserved. During the immobilisation [2]. Attempts are made to use the potential dependent behavior of specific marker bands in the finger-print region, to observe the kinetics of the electron transfer inside the protein including the catalytic centre. Complementary to raman spectroscopy, electrochemical measurements such as scan-dependent cyclic voltammetry (CV), differential pulse voltammetry (DPV) and voltammetry at the rotating disc electrode (RDE) are performed and analyzed in detail. Trum pet plots are used to calculate the kinetic rate constants of electron transfer to the first redox site of the multi-centred protein [3]. Differential pulse voltammetry shows catalytic O₂ reduction [4] and discharge of H⁺ transported actively across the protein [B]. Binding of CN⁻ to the O₂-reduction site of CoO₃ results in the complete inhibition of the H⁺ pumping.

[1] Giess, F; Friedrich, M.G. et al.; Biophys. J. 87, (2004), 3213
[2] Friedrich, M.G. et al.; Chem. Commun (2004) 2376
[3] Armstrong, F.A. et al.; Biochemistry. 2003, 8653-8662.
Cytochrome c oxidase couples the reduction of O₂ to water with the translocation of protons across the mitochondrial or bacterial membrane. The mechanism of electron coupled proton transfer is however still poorly understood. The P₄₅⁰-intermediate of the catalytic cycle is an o xoferriyl-state whose generation requires one additional electron, which cannot be provided by the two metal centres. The missing electron is suggested to be donated to this binuclear site by a tyrosine residue that forms a radical species, and which can be detected in the P₄₅⁰ and P₄ intermediates. One possibility to produce these intermediates artificially is the addition of H₂O₂ and a tyrosine radical species has been observed using EPR [1]. From activity measurements as well as optical and EPR experiments on several variants the origin of the radical species was found to be Y167 [2]. This result is surprising because Y167 is not part of the active site, where H₂O₂ reacts with the enzyme. Upon inspection of the active site it becomes evident that W272 could be the actual donor of the missing electron, which can then be replenished from Y167 (in the case of the reaction with H₂O₂) or from the Y280-H276 cross link in the natural cycle. The difference between the reaction with H₂O₂ and the natural cycle might be that H₂O₂ carries two electrons plus a Cytb₃ radical that is essential for enzymatic activity [1]. We have studied the mouse R2 protein with several spectroscopic methods (e.g., Raman, HF EPR at 285 GHz [2], ENDOR and MCD [1]). We therefore still poorly understood. The P₄₅⁰-intermediate of the catalytic cycle is an o xoferriyl-state whose generation requires one additional electron, which cannot be provided by the two metal centres. The missing electron is suggested to be donated to this binuclear site by a tyrosine residue that forms a radical species, and which can be detected in the P₄₅⁰ and P₄ intermediate. One possibility to produce these intermediates artificially is the addition of H₂O₂ and a tyrosine radical species has been observed using EPR [1]. From activity measurements as well as optical and EPR experiments on several variants the origin of the radical species was found to be Y167 [2]. This result is surprising because Y167 is not part of the active site, where H₂O₂ reacts with the enzyme. Upon inspection of the active site it becomes evident that W272 could be the actual donor of the missing electron, which can then be replenished from Y167 (in the case of the reaction with H₂O₂) or from the Y280-H276 cross link in the natural cycle. The difference between the reaction with H₂O₂ and the natural cycle might be that H₂O₂ carries two electrons plus two protons into the active site whereas in the natural cycle only one proton enters the active site.

[1] MacMillan, F. et al. (1999) Biochemistry. 38, 9179
[2] Budiman, K. et al. (2004) Biochemistry 43, 11709

A novel cluster and spectroscopic studies of the radical iron site in mouse ribonucleotide reductase

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Ribonucleotide reductase (RNR) is the enzyme that converts ribonucleotides to their corresponding deoxyribonucleotides. The R2 protein reacts with ferrous iron and dioxygen to generate a tyrosyl radical that is essential for enzymatic activity [1]. We have studied the mouse R2 protein with several spectroscopic methods e.g., Raman, HF EPR at 285 GHz [2], ENDOR and MCD [1]. We find cooperative binding of Fe(II) (or Co(II)) and hydrogen binding to tyrosyl radical [1, 2]. Here we present structures of mouse R2 soaked in iron [3]. We now observe a biologically unusual new type of tri-nuclear metal ion cluster, clearly different from a tri-nuclear metal ion cluster recently observed in an R2 protein from Corynebacterium ammoniagenes. In methanol soaked mouse R2, the tri-nuclear cluster is located ~10 Å from the di-nuclear iron cluster and is attached to the protein by a surprising and novel type of coordination. The cluster is ~15 Å away from the electron/radical transport chain in mouse R2, and therefore probably not involved in radical transfer. It is not yet clear whether the observed tri-nuclear cluster is an artefact from the soaking conditions, or if it has some biological relevance. [1] Kolberg, Strand, Graff, Andersson Biochem. Biophys. Acta 2004, 1699, 1-34 [2] Anderson et al. J. Biol. Inorg. Chem. 2003, 8, 235-247; [3] Strand et al. J. Biol. Chem. 2004, 279, 46794-46801. Norwegian Cancer Soc.
**Oral Presentations**

**– Single Molecule Biophysics –**

**O-60**

**In-vitro and in-vivo single molecule molecular rulers**

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Advances in single molecule studies of: (1) protein folding; (2) initiation of transcription by e-coli RNA polymerase; (3) targeting and detection of individual proteins in live cells using peptide-coated quantum dots; and (4) their application to the study of lipid rafts in membranes will be reviewed.

**O-61**

**Single-molecule and single-particle imaging in solution and in live cells**

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Understanding the molecular mechanisms of complex biological processes is one of the major goals in modern biology. As molecular and cell biology get increasingly quantitative, a comprehensive understanding of biological processes at the molecular level is becoming more readily accessible. However, roadblocks still exist, among which is the challenge that we face in characterizing the complex dynamics of biological processes. The existence of multiple kinetic paths and transient intermediate states often makes these processes difficult to dissect, as individual steps of a multi-step process are typically not synchronized among molecules. To tackle this problem, we are exploring optical imaging techniques to monitor, in real-time, the behavior of individual biological molecules and complexes, in vitro and in live cells.

In this talk, I will report our recent progress in the following two areas. (1) Molecular mechanisms of viral infection: our single-virus tracking experiments allow us to visualize the viral infection process in real-time, dissect individual stages of the viral entry pathway, and obtain a better understanding of the molecular mechanisms governing the influenza infection. (2) Structural dynamics of RNA and ribonucleoprotein enzymes: our single-molecule studies provide new insights into the molecular mechanisms governing RNA structural dynamics, and the effects of proteins on the structural dynamics of RNA enzymes.

**O-62**

**Title not communicated**

V. Croquette
École normale supérieure, Paris, France

**O-63**

**A dual DNA molecule experiment reveals details of H-NS mediated bridging of DNA**

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Bacterial chromatin is organized and compacted at least in part by interaction with nucleoid-associated proteins. We have shown (with Scanning Force Microscopy) that one of these proteins, H-NS, compacts DNA by the formation of bridges (links) between adjacent DNA duplexes. This property is exploited not only for providing a means of compaction, but is also essential to the regulatory role of H-NS in transcription. DNA bridging directly explains the recognition of curved DNA and transcriptional repression by H-NS.

We have developed an experimental setup that features quadruple optical tweezers which allows the simultaneous and independent manipulation of two DNA molecules. Using this setup, we demonstrate the formation of H-NS mediated physical links between two DNA molecules. Disassembly can be induced by applying an external force to unzip or shear the bridged complexes. Applying a shear force results in the gradual disruption of bridged tracts, whereas the application of an unzipping force leads to the disruption of such an area in a stepwise manner. Qualitative and quantitative analysis of the steps and forces involved leads us to propose a refined model for the compaction and the organisation of DNA by H-NS.
O-64
Single molecule multistep FRET: revealing complex molecular interactions
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During recent years, fluorescence resonance energy transfer (FRET) has become a key method for the analysis of molecular interactions with exquisite sensitivity down to the single molecular level. As nearly every major process in a cell is carried out by assemblies of multiple dynamically interacting molecules it is of great interest to extend single molecule FRET-technologies to the study of three and more chromophores. We show the applicability of single molecule multistep FRET for the design of DNA based molecular photonic wires including FRET over five chromophores. To make more complicated energy transfer in complex 3D biomolecular interactions analysable, a general approach based on alternate 3 color laser excitation making use of new acousto-optical devices to rapidly switch laser lines and optimize detection efficiency is presented. Besides enabling absolute distance determination of three chromophores and colocalization of chromophores beyond the FRET-range, correlative movements of different segments within a molecule can be unravelled.

O-66
Dynamics of single GABAAR in nerve growth cone : from molecular interactions to axonal guidance
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During axonal growth, the distal part of the axon – the growth cone (GC) – is able to detect spatial gradients of guidance cues, such as GABA, netrins or BDNF, leading to accurate axonal pathfinding and formation of a functional network. Although the signal transduction pathways leading to GC steering have been widely investigated, the remarkable sensitivity of GCs in gradient sensing remains mostly unexplained. We address this issue by studying membrane dynamics of single quantum dot-tagged GABA receptors (GABA₄R) in GCs of spinal neurons. In addition to Brownian motion, we measured directed movements for 80% of the receptors. A statistical analysis of trajectories combined with pharmacological treatments by nocodazol and taxol revealed that these motions were due to transient interactions with microtubules. To investigate the functional role of these interactions, we analyzed the dynamics of GABA₄Rs in the presence of an external GABA gradient. We observed a redistribution of receptors in the direction of the source, which is specific of GABA₄R activation and is microtubule-and calcium-dependent. These observations lead us to propose a model for guidance signal amplification based on the autocatalytic spatial redistribution of receptors through interactions with microtubules.

O-65
Light generated and light driven micromachines for single particle manipulation
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Photopolymerisation offers a relatively simple method to produce microscopic particles of arbitrary shape that are practical to expand the possibilities of optical manipulation. There are different ways to influence the orientation of non-spherical objects in the optical traps. For example, propeller shaped micrometer sized rotors are rotated in optical tweezers, while flat objects are oriented in traps formed by linearly polarized light. The force and torque exerted by these devices falls in the range of those occurring in biology. Consequently, such elements and the possibilities opened up by their use would find numerous applications in biology, both in fundamental science and biotechnology (lab-on-a-chip devices) by realising new types of manipulation. Photopolymerisation is also a practical method to create microchannels, microvessels, as well as optical waveguides. By the combination of these elements, complex, fully integrated systems are built where the manipulation and observation of single molecules, cells or other biological objects of up to micrometer size can be realised in a practical manner.

We demonstrate the function of the different elements and show how they can be applied in single particle experiments.

O-67
Dynamical high-accuracy observations of individual membrane protein molecules using X-rays
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Recently, we succeeded in vitro time-resolved (~ms) x-ray observations of picometer-scale slow Brownian motions of individual membrane protein molecules in aqueous solutions. Diffracted X-ray Tracking can monitor dynamics of the individual molecules or specific sites in individual single protein molecules. Now, we observed Brownian motions and momentarily structural change of individual single Bacteriorhodopsin (BR) molecules, which is a light-driven proton pumping found in the purple membrane, in the light irradiation (560nm). We have consequently confirmed Brownian motions, and that the average size of the momentarily structural changes by light irradiation in 35th residue of BR was 76±48.2 pm.
In conclusion, the TREK channels are polymodal K⁺ residues. Down-modulation of TREK-1 is mediated by receptor-critical for channel activity and is controlled by a cluster of cationic physical stimuli such as temperature (TRPV1/M8) or the binding physiological membrane potentials, which are caused by various compounds (mustard, garlic), renal Ca²⁺/Mg²⁺ handling and blood pressure regulation.

TRP channels were considered as voltage independent channels. It is now shown that some TRP channels are voltage dependent. They use dramatic shifts of their voltage dependence towards physiological membrane potentials, which are caused by various physical stimuli such as temperature (TRPV1/M8) or the binding of ligands (TRPV1/V3/M8/M4) including Ca²⁺ (TRPM4/S).

Modifications in a putative S4 voltage sensor and in the C-terminus induce shifts in the voltage dependence. It will be shown that small changes in Gibb’s energy induce large shifts in TRP channel’s voltage dependence which is pronounced by their small gating charge, z (~0.7 for TRPs, 4-13 for classical voltage dependent channels). The small charge of TRP channels might be an important evolutionary structural prequise for the gating diversity of TRP channels.

O-68
TRP channels as unique cellular sensors
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TRP (Transient Receptor Potential) channels form a novel cation channel family consisting of nearly 30 mammalian members. TRP channels are universal biological sensors that detect changes in the cellular environment. TRP channels gate in response to cold or hot temperatures, natural chemical compounds (menthol, camphor, “hot pepper”), mechanical stimuli, or changes in the composition of the lipid bilayer. TRP channels are crucially involved in physiological processes, e.g. taste perception, thermosensation, pain perception, mechanosensation, sound detection, perception of pungent compounds (mustard, garlic), renal Ca²⁺/Mg²⁺ handling and blood pressure regulation.

TRP channels were considered as voltage independent channels. It is now shown that some TRP channels are voltage dependent. They use dramatic shifts of their voltage dependence towards physiological membrane potentials, which are caused by various physical stimuli such as temperature (TRPV1/M8) or the binding of ligands (TRPV1/V3/M8/M4) including Ca²⁺ (TRPM4/S).

Modifications in a putative S4 voltage sensor and in the C-terminus induce shifts in the voltage dependence. It will be shown that small changes in Gibb’s energy induce large shifts in TRP channel’s voltage dependence which is pronounced by their small gating charge, z (~0.7 for TRPs, 4-13 for classical voltage dependent channels). The small charge of TRP channels might be an important evolutionary structural prequise for the gating diversity of TRP channels.

O-69
Molecular mechanism of mechanosensory transduction in bacteria
B. Martinac
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Bacteria possess multiple adaptation mechanisms enabling them to grow in a wide range of external osmolarities. To survive they require “emergency valves” for release of osmotic stress. Mechanosensitive (MS) ion channels constitute mechanism that prevents excessive water inflow and buildup of excessive turgor pressure in bacteria. These channels have large conductance and mostly lack ionic specificity which allows them to serve as osmosensors that regulate the cellular turgor. They act as mechanoelectrical switches, which open in response to cell membrane deformations caused by osmotic forces under hypotonic conditions. Without this response, the bacteria lyse. Bacterial MS channels as well as MS channels of other prokaryotes are gated by bilayer deformation forces, which indicates that lipid-protein interactions are crucial for the channel function. Mechanism of mechanotransduction in bacterial MS channels is defined by both local and global asymmetries in the transbilayer tension profile and/or bilayer curvature at the lipid protein interface. The demonstration of bilayer-controlled functional properties of bacterial mechano-transducer channels emphasizes that bilayer is much more than a neutral solvent by actively modulating the specificity and fidelity of signaling by membrane proteins.

[Supported by Australian Research Council.]

O-70
Sensing pressure with K_{2P} channels
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The 15 human K_{2P} channel subunits are made of four transmembrane segments and two P domains in tandem. Functional homo- and hetero-dimers with different biophysical and pharmacological properties have been demonstrated.

The K_{2P} channel TREK-1 is predominantly expressed in the nervous system. TREK-1 is activated by membrane stretch as well as cell swelling. Moreover, intracellular acidosis strongly sensitzes TREK-1 to membrane stretch, leading to channel opening at atospheric pressure. TREK-1 is reversibly opened by polyunsaturated fatty acids, including arachidonic acid (AA). Additionally, TREK-1 channel activity is reversibly stimulated by volatile general anesthetics, including halothane.

Mutagenesis studies have demonstrated that the cystolic carboxy terminal domain of TREK-1 plays a key role in TREK-1 gating. Protonation of a key residue in this region, E306, leads to channel activation. Interaction of the cytosolic carboxy terminal domain of TREK-1 with the inner leaflet phospholipids, including PIP2, is critical for channel activity and is controlled by a cluster of cationic residues. Down-modulation of TREK-1 is mediated by receptor-coupled protein kinase A (PKA) phosphorylation of residue S333. In conclusion, the TREK channels are polymodal K⁺ channels that integrate multiple physical and chemical stimuli.

O-71
Functional tethered membranes: a sensing platform for ion channels
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Tethered lipid bilayer membranes have been proven to be a powerful architecture for the study of membrane protein in an artificial, but quasi-natural environment. Based on a lipid bilayer membrane, which is coupled via a spacer group to a solid substrate, they provide not only excellent stability but possess also the necessary conditions for functional incorporation of membrane proteins, especially fluidity and high electrical resistance.

We could synthesize several lipids, which allow the construction of membrane architecture both on gold as well on silicon surfaces and provide good electrical sealing properties. We could functionally incorporate a variety of membrane proteins, e.g. ion channels and shuttle proteins.

By variation of the anchor group, we have a generic model for all kinds of substrates. Modification of the spacer architecture allows the adaptation of the system to different membrane proteins. We will give an overview about the systems obtained so far. Experimental characterization is made by divers surface analytical tools such as SPR, QCM, FRAP and impedance spectroscopy. Our construction kit offers now the possibility to combine directly microelectronic read-out systems with a biological compound. We will present a device concept, where ion channels, embedded in a tethered membrane are used as sensing elements of a new type of bio-electronic sensor.
Oral Presentations

Sensing with Ion Channels

O-72

A novel µ-conotoxin potently blocks TTX-R sodium channels in rat dorsal root ganglion neurons

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Mu-conotoxin SIIIA, a novel blocker of tetrodotoxin-resistant (TTX-R) voltage-gated sodium channels (VGSCs) has been identified from the fish-hunting cone snail, Conus striatus, via cDNA cloning. By means of the whole-cell patch clamp technique, the present study is to examine the effects of mu-conotoxin SIIIA on neuronal sodium currents. The experiments are performed in 127 acutely isolated dorsal root ganglia (DRG) neurons (<25um) from the Sprague-Dawley male rats, which express both subtypes of sodium currents. After aether anaesthesia, the L4-L6 DRGs were picked out and dissociated into cells by enzyme. mu-conotoxin SIIIA has no significant effect on TTX-Sensitive sodium current, but produces an irreversible, concentration and time-dependent inhibition of TTX-R sodium current amplitude, but doesn’t affect the voltage range of channel activation, suggesting a frequency-dependent blocking effect.

In the behavioral test, intraplantar injection of µ-conotoxin SIIIA (6 ug) significantly slows the thermal hyperalgesia development and increases the paw withdrawal latency to noxious heating in the carrageenan-induced inflammatory rat, which suggests that TTX-R sodium channels expressing in the peripheral afferent fibers innervating the receptive field are blocked by µ-conotoxin SIIIA. The present results indicate that mu-conotoxin SIIIA is a novel potent inhibitor of TTX-R sodium channel.

O-73

Modulation of the channel activity of Vpu from HIV-1

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Vpu is a viral ion channel protein encoded by HIV-1 to enhance the rate of virus particle release from an infected host cell by altering the electrochemical potentials across host lipid membranes. The 81 amino acid monomers have a single transmembrane α helix, which oligomerises within the host’s sub cellular membranes forming bundles which enable ion flux. The transmembrane (TM) domain has been synthesised by continuous-flow solid phase peptide synthesis and the full-length wild type protein has been expressed in recombinant E. coli. Conductance measurements have been obtained by bilayer recording of Vpu reconstituted into membranes of a variety of different compositions and the inhibition of ion permeation by the amphipathic ligand HMA has been studied. The binding of HMA and a putative inhibitor resembling the TM domain of the TASK channel has been simulated using a computer docking approach.

O-74

Mechanopharmacology of ion channels: biophysics and disease

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All cells have mechanosensitive ion channels (MSCs), and hence are likely to be involved in pathologies, most of which have yet to be defined. Some examples of relevant pathologies may include arrhythmias generated by mechanical stress, atrophy of muscle in muscular dystrophy, hyperactive bladder incontinence, and stimulation of glioma growth.

There are two types of MSCs: those that sense the activating force from attached proteins such as collagen and tubulin: these are the specialized receptors, and those that sense the force from stress in the lipid bilayer. The latter are found in all cells from E. Coli to humans.

There is only one known specific drug for mechanosensitive ion channels, a 4kD peptide called gsmtx4 derived from tarantula venom. It acts as a gating modifier, shifting the gating curve so as to require higher tension for activation. The peptide has a charged (pentavalent face) and hydrophobic face favoring binding to the lipid/water interface. The charge on the peptide can also reduce the local concentration of permeant cations. The mirror image molecule, the D form of GsMTx4, has a similar activity suggesting there is no chiral binding site. As a drug the D form has the advantage of not being digestible.

GsMTx4 has the ability to block fibrillation induced by inflation of the atria, reduce the Ca²⁺ influx into dystrophic muscle, inhibit stretch sensitive channel in bladder muscle, and to inhibit stress induced ET-1 secretion from astrocytes.
The 'roll and lock' mechanism of force generation in muscle and its blocking by Pi analogues

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It is widely believed that muscle force is produced by a tilt of the light chain domain (lever arm) of a myosin head with respect to its motor domain that remains firmly bound to actin. Our time-resolved X-ray diffraction experiments with permeabilized muscle fibres suggest a more complex sequence of force-generating events in muscle. The main feature of the model we propose is a 'roll and lock' force-generating transition of myosin heads bound to actin non-stereo-specifically (weakly) to a stereo-specifically (strongly) bound state. Only then the lever arm can tilt. The fraction of strongly bound heads can be monitored on the diffraction pattern by the intensity of the 1st actin layer line, A1. It increases synchronously with tension after a temperature jump or during partial tension recovery after a step shortening. The 'roll and lock' transition in permeabilized muscle fibres can be blocked by some analogues of inorganic phosphate so that the heads cannot produce active tension and do not contribute to A1 although provide significant muscle stiffness and rotate in response to step stretches of muscle fibres.

The rate-limiting step for skeletal muscle contraction is temperature dependent

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Previous studies showed that the rate constant of ATP-induced actomyosin dissociation (K1k1.2) and the rate constant for ADP release (kAD) from pure mammalian myosin isoforms were correlated with the unloaded shortening velocity (V0) of single skinned muscle fibres containing the corresponding isoforms. However, the different ionic strengths used in these assays made the comparison of rapid kinetic and unloaded shortening velocity data complicated.

To overcome this problem we used in this work myosin fragments (S1 and HMM), which are soluble in low salt buffers. The fragments were prepared from pure fast myosin isoforms isolated from single muscle fibres. We measured the K1k1.2 and kAD values with flash-photolysis methods under the experimental conditions used to measure V0 (12°C and 200 mM ionic strength). At 12°C kAD was faster than K1k1.2 and too fast to determine V0. The K1k1.2 values were of the right magnitude to be the rate-limiting step for V0 at this temperature. The temperature dependence of K1k1.2 and kAD revealed that K1k1.2 increased significantly with temperature with Q10 of 2.52, while the value of kAD did not increase (Q10 of 1.01). Therefore increasing temperature decreased the relative contribution of K1k1.2 in determining V0 whereas increased the contribution of kAD. Extrapolation of K1k1.2 and kAD to physiologic temperatures suggested that at these temperatures kAD becomes rate-limiting for V0.

Linear dichroism, applied to structural analysis of protein fibres, membrane proteins and DNA

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Understanding the organisation of molecules in naturally occurring ordered arrays (eg membranes, protein fibres and DNA strands) is of great importance in understanding their biological function. Unfortunately few biophysical techniques provide detailed structural information in these non-crystalline systems. Ultra-violet, and visible linear dichroism (LD) has the ability to provide such information and may hold the key to understanding molecular mechanisms of such fundamental biological processes as amyloid fibre formation, and membrane protein folding. We have developed LD apparatus that has allowed us to study the structures of number of systems including protein fibres (Alzheimer’s fibres, actin, tubulin and the bacterial cell division protein FtsZ). Data from these studies has provided novel information of the orientation of these proteins within the fibre as well as showing the details of conformational changes that occur during fibre function. We have also expanded the technique to encompass studies of the conformation of membrane proteins in lipid vesicles as well as proteins binding to and drugs binding to DNA. Taken together this work provides an exciting glimpse of the importance of LD in the future biophysical studies.
O-79
Mechanism of myosin-V processivity
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To investigate the kinetic basis of myosin-V processivity, we have compared the kinetics of product dissociation of myosin-V-HMM and S1 using the substrate 7-diethylaminocoumarin-3-carboxylic (deac)ATP. This novel fluorescent ATP derivative provides a much better signal/noise ratio than the conventionally used 2’-deoxy3’-mannoATP (mdATP). Double-mixing stopped-flow fluorescence was used to measure deacADP dissociation from actomyosin-V-S1-deacADP-Pi and from actoHMM-ADP-Pi, using ATP or ADP chase. With the ADP chase, the decrease in fluorescence was fit to a single exponential for myosin-V-S1 and a double exponential for the HMM construct with the second component ~1000 fold slower. With an ATP chase, the fluorescence signal is single exponential for both S1 and HMM. ADP (unlabeled) dissociation occurs in a single step from acto-S1-ADP-Pi and in two steps from actoHMM-ADP-Pi. The later has two observed rate constants, one similar to acto-S1-ADP-Pi and a second slower process. Our solution kinetic data provide a very accurate comparison of the kinetics of product dissociation from actomyosin-V-S1-ADP-Pi and actomyosin-V-HMM-ADP-Pi. The data support a model for the processive movement of the myosin-V molecule in which slow dissociation of ADP from the lead head produces directionality of movement and provides a mechanism to reduce the possibility of simultaneous dissociation of both heads from actin. Although similar mechanisms had been proposed by others these data provides the first compelling evidence to support the model.

O-80
Depolarization-induced slow calcium transients stimulate transcription of genes in skeletal muscle cells
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Inositol 1,4,5-trisphosphate (IP3) mediated slow calcium signals evoked by hormones and electrical stimulation of skeletal myotubes have been shown to be involved in regulation of gene expression. Using semi-quantitative RT-PCR, we have shown that K+ depolarization of rat myotubes and C2C12 cell line induces a transient increase in IL-6 mRNA level, which peaks at 3-4 h, and is independent of extracellular calcium. Inhibitors that suppress slow calcium signals, such as U73122 (phospholipase C inhibitor), 2-APB (inhibitor of IP3 induced signals) and xestospongin-C (IP3 receptor blocker), decreased activation of IL-6 gene expression. Ryanodine, which inhibits the fast calcium transient had no effect on IL-6 induction. Depolarization of myotubes transfected with a luciferase reporter gene containing nucleotides –651 to +1 of the human IL-6 promoter, induced a two fold increase on IL-6 promoter activity. Increased luciferase expression was abolished in the presence of either 2-APB or U73122 whereas remained unaffected after ryanodine treatment. Site directed mutagenesis of the parental construct allowed us to identify AP-1 and NF-kB sequences as cis regulatory elements responsible for the upregulation of IL-6 expression in transfected myotubes. Our results provide evidence for the involvement of IP3 mediated calcium signals on IL-6 transcription in skeletal muscle cells and further support it regulatory role in gene expression. Financed by FONDAP # 15010006

O-81
The distribution and conformation of myosin heads in shortening muscle studied with X-ray interference
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In an isometrically contracting muscle fibre the interference between the two arrays of myosin heads in each sarcomere splits the M3 X-ray reflection from the 14.5 nm axial repeat of the heads into two closely spaced peaks. The ratio of the intensity of the high angle peak to that of the low angle peak (RM3) is ca 0.8. Due to the bipolar arrangement of the myosin heads in the two halves of the thick filament, the interference distance reduces when actin-bound heads execute the stroke that drives the actin filament towards the centre of the sarcomere and RM3 reduces accordingly. The X-ray interference method was used to investigate the conformation of myosin heads during steady isotonic shortening at different velocities obtained by imposing force steps ranging between 0.875 and 0.125 T0 on the isometric contraction. The results indicate that (1) the number of working myosin heads reduces and their axial distribution increases with the reduction of load; (2) actin-myosin interactions are briefer near unloaded shortening velocity. Supported by MIUR (Italy), MRC (UK), NIH (USA).
Dynamic organization and function of cell lipids
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The various intracellular membranes have different protein and lipid compositions. In view of the rapid traffic between these membranes via vesicles, there must be selectivity in transport. The basis for the selective transport of lipids is thought to reside in aggregation of sphingolipids and cholesterol into microdomains or “rafts”. These rafts would then be expected to play a role in protein sorting as well. We have recently localized the sphingomyelin synthase to the Golgi and intend to use our tools to study the effects of manipulating sphingolipid synthesis in the Golgi on protein sorting, as we did in yeast. Little is known on the intracellular localization of the glycosphingolipids. The first glycolipids, glucosylceramide and galactosylceramide, are synthesized on the cytosolic surface of Golgi cisternae and on the luminal aspect of the ER membrane, respectively. We are now setting up methods to study the intracellular localization of glycolipids, and methods to study how membrane lipids are translocated across cellular membranes. Previous studies have suggested a role for distinct families of P-type ATPases and ABC-transporters in this process. Finally, we have been working for years on the mechanism by which glycosphingolipids are needed for pigmentation in melanocytes. We now have evidence on what glucosylceramide does.

Holthuis, J.C. and Levine, T.P. (2005) Nat. Rev. Mol. Cell Biol. 6, 209-220; van Meer, G., and Sprong, H. (2004) Curr. Opin. Cell Biol. 16, 373-378

Lipid segregation and plasma membrane interactions related to IgE-receptor signaling
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The complex role of plasma membrane structure in orchestrating receptor-mediated signal transduction is addressed in collaborative studies investigating how antigen crosslinking of IgE-receptors initiates signaling pathways leading to multiple cellular responses. Segregation of liquid ordered regions from disordered regions of the plasma membrane provides protection from transmembrane phosphatases and thereby a mechanism for crosslinking-dependent phosphorylation of IgE-receptors by active Lyn kinase. Fluorescence correlation spectroscopy reveals that antigen crosslinking reduces the diffusion of receptors and also Lyn with intermittent interactions occurring between these two components. Spatial definition of crosslinked IgE-receptors on the micron scale with patterned lipid bilayers enables visualization of interacting components with spatial and temporal resolution. Lyn activity precedes detectable, actin-dependent concentration of Lyn and other components associated with lipid rafts; outer and inner leaflet raft components are uncoupled. Electron spin resonance allows the phase-like behavior of plasma membranes to be examined in purified preparations and living cells and reveals distinct ordered and disordered regions. Lipid phase separations in membranes can be examined with fluorescent probes; separations that are altered by crosslinking a membrane component are observed.

Can lipid domains control membrane permeabilization and DNA uptake in cells submitted to electric field?
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We visualized the early events of membrane permeabilization and DNA transfer by electric field. A fast exchange of small molecules takes place across the membrane. In the case of DNA transfer, a complex process is present involving a key step of association of DNA with the membrane. This results in the formation of 0.2 µm membrane domains where DNA is trapped. This data is consistent with a multi step model of DNA transfer across the membrane and suggest the existence of competent membrane area for DNA transfer (1). The purpose of this work is to better understand the molecular state of the cell membrane where plasmid interacts and therefore to characterize the domains of the membrane/DNA interaction. Our hypothesis is that lipid rafts may control DNA electrotransfer. For that purpose, CHO cells have been depleted in cholesterol by using cyclohexim. Incubations of cells with 5mM of cyclohexim, 15 min at 37°C, resulted in decreasing their cholesterol by 40%. Under these conditions, cell viability is preserved. Membrane permeabilization to electric pulses does not significantly change. DNA membrane interaction is not affected. Gene expression dramatically decreases tenfold. These results show a correlation between plasma membrane cholesterol content and DNA expression. They suggest the involvement of cholesterol depletion on nucleic acid traffic and the resulting protein expression.

Golzio, Teissié and Rols. PNAS 99: 1292-7 (2002)

Physical characterization of membrane microdomains
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The molecular interactions within a fluid lipid bilayer provide a set of fundamental mechanisms for formation of lipid domains and laterally differentiated areas within the bilayer. A fluid membrane should therefore be considered a liquid structured in space and time and whose lateral structure most appropriately is characterized by the correlation length ξ. Nano-meter scale formation of fluid domains in lipid bilayer membranes will described in terms of ξ in the case of (1) lipid mixtures, (2) lipid-cholesterol mixtures, and (3) lipid-integral-membrane protein mixtures.
O-86
Do domains form in asymmetric membranes? A fluorescence and atomic force microscopic study
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The concept of lipid rafts (cholesterol-rich domains) is generally accepted for eukaryotic cells, however, not much is known about the formation of domains in asymmetric membranes lacking cholesterol and the mutual interaction of the two monolayers. We studied the outer membrane (OM) of Gram-negative bacteria which is extremely asymmetric with respect to its lipid composition: The lipid matrix of the inner leaflet is composed of a phospholipid mixture (PL), whereas the outer leaflet is composed solely of lipopolysaccharides (LPS). In lipid monolayers of natural LPS prepared on an aqueous subphase the formation of domains could be observed, thus, a formation of domains in the OM seems likely. We focused on the determination of domains formed in asymmetric planar bilayers prepared by the Montal-Mueller technique using fluorescent microscopy. Furthermore, we imaged asymmetric solid supported bilayers prepared by the Langmuir-Blodgett technique using combined fluorescent and atomic force microscopy. We found that domains, which are clearly visible in LPS monolayers, were not found in asymmetric bilayers prepared on the side of LPS and on the other of PL, indicating a strong interaction between the two monolayers.

O-87
Nanoscale clusters of GPI-anchored proteins in living cell membranes: implications for the existence of rafts
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Cholesterol and sphingolipid-enriched “rafts” have been proposed as platforms for the sorting of specific membrane components including glycosyl-phosphatidylinositol-(GPI) anchored proteins, but both their existence and physical properties have been controversial. We have investigated the size of lipid-dependent organization of GPI-anchored proteins in live cells, using homo and hetero-FRET-based experiments, combined with theoretical modeling [1]. These studies reveal an unexpected organization wherein cell surface GPI-anchored proteins are present as monomers and a smaller fraction (20%-40%) as nanoscale (<5 nm) cholesterol-sensitive clusters. These clusters are composed of at most four molecules and accommodate diverse GPI-anchored protein species. In conjunction with an analysis of the statistical distribution of the clusters, these observations suggest an active mechanism for lipid-dependent clustering of GPI-anchored proteins. These studies support a picture wherein larger scale rafts are induced from pre-existing small-scale lipid dependent structures, actively maintained by cellular processes [2].

[1] P. Sharma, R. Varma, R.C. Sarasij, Ira, K. Gousset, G. Krishnamoorthy, M. Rao and S. Mayor, Cell 116 (2004) 577-89.
[2] S. Mayor and M. Rao, Traffic 5 (2004) 231-40.

O-88
Digital signal transduction? Investigations by single-molecule observations
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Using single molecule techniques, the movement, localization, and activation reactions of single signaling molecules have been investigated in living cells. The major finding by using single molecule techniques is that the activation period for each individual signaling molecule is often shorter than a second, namely, activation of single signaling molecules occurs like a short pulse although signaling molecules collectively exhibit activation lasting over a minute, a time course which is the same as that detected biochemically. Therefore, it is concluded that the cellular signaling adopts digital or frequency-modulated signal transduction mechanism in the sense that each signaling event or the elementary step for each signal transduction step takes place like a transient pulse-like on-signal. Such a pulse-like activation is likely based on transient cooperative formation and disassembly of the signaling complex.
O-90
Morphogenetic processes may have their origin in the shape behavior of vesicular objects
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Vesicular objects are characterized by a thin membranous envelope separating the inner and outer aqueous media. Their shape behavior involves universal properties that do not depend on the compositional details of the envelope. These properties are postulated to form the basis of various morphogenetic processes. In particular, they might have guided the evolution of the latter at times when biological systems or their precursors still lacked the present-day compositional and structural complexity. Different specific cases are examined to corroborate these principles. First we describe the parameters that define vesicle shapes, and show how at attaining certain of their values vesicles transform, either continuously or discontinuously, into shapes with different symmetries. The attainment of asymmetric shapes is considered as a possible physical basis for the establishment of cellular polarity. Then the mechanical properties of unicellular sheets are revealed that allow the shape transformation from spherical blastula into the cup-shaped gastrula to run in a continuous manner. Lastly, the analysis of morphological aspects of vesicle self-reproduction shows that this process can only occur under a condition that relates certain vesicle and environmental parameters. This result suggests for growing vesicle populations to be under the control of a selectivity mechanism.

O-91
Stem cell biophysics
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Anchorage-dependent cells generally exert tractions on their substrates and thereby respond to substrate elasticity. Mesenchymal stem cells (MSCs) are not only anchorage-dependent but are also directed in their lineage by local tissue elasticity. We studied MSC differentiation on gels of different elasticity under identical growth conditions. On substrates with a Young’s modulus (E) near that of striated muscle, MSCs exhibit a myoblast morphology and express Myogenic Differentiation Factor 1 (MyoD1). On far stiffer substrates, MSCs have a polygonal shape resembling osteoblasts and express the osteogenic marker Core Binding Factor α1 (CBFα1). On soft substrates, MSCs adopt neuronal, branched morphologies and co-localize neurofilament heavy chain (NFH) with F-actin. MSCs express myosin II and inhibition with blebbistatin blocks differentiation, implicating myosin-based contractility in how MSC feel their microenvironment and differentiate in response to it.

O-92
Measuring the adhesion and mechanical properties of individual cells using atomic force microscopy
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For the last decade people have been using the AFM to measure colloidal interactions. This is achieved by attaching a particle onto an AFM cantilever and measuring that particles interaction, either with a second particle, or more usually a flat surface made of a similar material to that of particle. Initially DLVO forces were measured, quickly followed by steric and hydrophobic interactions. The AFM has been used also in the field of biology, largely as an imaging technique, but also to measure the strength of specific interactions such as antibody-antigen interactions. In this presentation I shall describe a method whereby single living biological cells, namely yeast, erythrocytes and fibroblasts can be attached to AFM cantilevers, and their interactions with various surfaces have been determined. The technique enables the adhesion of cells to surfaces to be studied and results for a range of surfaces will be presented. One problem encountered during this work, which has proven to yield interesting mechanical details about the cells was that during an experiment the cells deform. By comparing data of the interactions of the cells with a surface and the bare cantilever with the surface, it is possible to estimate the deformation of the cell. It is found that the deformation is well described by Hertz theory of elasticity, enabling us to estimate the Young’s modulus of the cells: yeast 0.65 +/- 0.15 MPa; erythrocytes 0.25 +/- 0.1 MPa; and fibroblasts 20 +/- 10 kPa.
Mimicking cadherin-mediated cell-cell adhesion

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Cell adhesion is considered to play an important role in many biological functions such as the regulation of organ and tissue development during embryogenesis, and the maintenance of normal tissue structure in the adult organism. The specificity of cell-adhesion is controlled by genetic expression of receptors at the cell surface, called cell adhesion molecules (CAMs). One of the major families of CAMs is the cadherins that initiate intercellular junctions by homophilic ligation of their extracellular domains in the presence of calcium. The aim of this work is the creation of a biomimetic system, which permits to understand mechanisms by which a defined number of cadherin molecules coordinate cell-cell adhesion with dynamic changes in the cytoskeleton. To address these questions, we have developed patterned surfaces, based on the block copolymer micelle nanolithography, where geometrical well-defined gold dots are functionalized by a chimera of the cadherin ectodomains fused to the Fc region of human IgG1. Using an AFM, we measure the strength of the interactions between the cadherins onto the nanodots and the cadherins at the cell surface. First, to vary the cadherin distance allows us to apply nanopatterned substrates as a kind of "nanoruler" to measure important length scales in cadherin molecules. Second, we control the number of binding sites offered to the cell and this allows us to probe potential cooperative effects in cell adhesion phenomena through controlled cadherin clustering by clear chemical and physical means.

Neutrophil spreading: from touchdown to first steps

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Time resolved dynamics of spreading of human neutrophils following activation was observed by Reflection Interference Contrast Microscopy (RICM). Images were analysed to identify simultaneously the changes in the overall cell shape and the zones of close contact with the substrate and these were followed over time. We show that in case of neutrophils, cell spreading is anisotropic and directional from the very beginning resulting in a translation of the cell centre in case of neutrophils, cell spreading is anisotropic and directional. The instantaneous velocities of cell membrane segments are calculated. Spreading is found to be a result not of monotonous and calculated to changes in the adhesion state and/or dynamical state of the cell. The curve describing the spreading area of the cell as a function of time can be fitted piecewise as a series of power law functions. All cells exhibit an initial slow spread regime followed by a fast spread regime, each characterised by a different exponent of the power law function. The different spreading regimes are related to changes in the adhesion state and/or dynamical state of the cell. The instantaneous velocities of cell membrane segments are calculated. Spreading is found to be a result not of monotonous and directional movement of the membrane but a series of apparently random fluctuations.

Cells are similarly followed when they complete their spreading phase and start to migrate. RICM data is correlated to traction forces exerted by the cells.

The use of impedance spectroscopy to monitor barrier integrity and cellular differentiation

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Electrical impedance spectroscopy was used to monitor the development of a maturing differentiated state of mono-layers of cells during culture. The cells utilised in this study were the endothelial-like cell line ECV304 and the epithelial colonic cell line Caco-2. The cells were grown on a permeable support membrane in mono-culture and following co-culture with the astrocytic cell line 1321NI. Electrical impedance measurements were made applying a transversal alternating current through the culture using four pairs of internal electrodes over a frequency range (1300-1900 kHz). Measurements of Trans-cellular Electrical Resistance were used to monitor the presence of tight junction’s barrier integrity. The data present here permit the assessment of the ability of electrical impedance spectroscopy to monitor the formation of tight junction integrity as an indicator of cellular differentiation. Results show a good correlation of impedance measurements with culture growth and indicate sensitivity to the detection of the increasing tight junction integrity/complexity and offer a non-destructive method for monitoring differentiation in barrier culture systems.
**O-97**

**Combining advanced force and optical microscopy techniques for biophysics research**

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The range of biophysical applications for atomic force microscopy (AFM) continues to grow, as the advantages of high resolution imaging in physiological environments are combined with measuring and manipulating the structures under investigation. A key factor is the ability to combine AFM with advanced optical techniques, such as phase contrast, DIC and epifluorescent, TIRF or confocal imaging. This enables correlation of the high resolution structural information with specific labelling of active molecules. The BioCell allows full environmental control of samples on coverslips in liquid, for maximal optical resolution, single molecule imaging and spectroscopy, and cell studies.

The CellHesion development kit has been specially designed to extend the capabilities of AFM in cell binding and recognition assays, giving reproducible and quantitative analysis of cell-cell and cell-substrate binding forces. Single cells within a culture can be selected, attached to a flexible cantilever and subsequently allowed to adhere to a second, specific cell or region of substrate. Simultaneous information from advanced optical techniques gives insight into many additional cellular processes that occur on binding, such as changes to actin structure, calcium flushes, distribution of labelled proteins, or morphological changes.

[1] Poole et al. FEBS Letters, 565: 53-58 (2004)  
[2] Poole and Müller British J. Cancer 92:1499-1505 (2005)

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**O-98**

**Characterization of protein hydration: a new approach to crystallization and folding problems**

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The vital biological functions of proteins in the cell are based on their intact native structure. Stabilized by water, the structure of proteins is highly dependent on the equilibrium with its environment (hydration state) which can be significantly influenced by external factors such as temperature, ionic strength or drugs. Disturbance of this sensitive equilibrium often leads to misfolding and aggregation. The recent development of molecular acoustics opened up new application possibilities including high resolution observation of hydration changes in proteins. Characterization and monitoring of protein hydration has been used as an efficient approach for the structural and thermodynamic analysis of proteins. Thereby, molecular acoustics assisted the finding of optimal crystallization conditions.

Here we demonstrate the sensitivity of molecular acoustics by monitoring protein folding and stability. Thermal denaturation and renaturation of several proteins were characterized using temperature scans and revealed significant differences in general folding behaviour, the number of folding intermediates, general thermal stability and renaturation efficiency. Thus, molecular acoustics proved to be an efficient analytical approach to characterize the structural behaviour and stability of various proteins under native conditions.
O-99

The DNA packaging machinery of complex ds DNA viruses
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The assembly pathway of complex double-stranded (ds) DNA bacteriophages leads to the assembly of a viral prohead, followed by the packaging of the DNA. The connector, a homo-dodecameramic structure that plays a fundamental role in the first steps of the prohead assembly and in the packaging of DNA, is found in a unique vertex of the prohead and is associated with other viral ATPases, called terminases, building the portal vertex. Electron microscopy studies have revealed that connectors from different viral systems are ring shaped, with 12-fold symmetry, and a channel along the longitudinal axis of the particle. The three-dimensional structure of the connectors of bacteriophages Ø29 and T7 have been solved at 8 Å resolution using cryo-electron microscopy from purified recombinant connectors. The general morphology of Ø29 and T7 connectors reveals many structural similarities, including the presence of a conspicuous α-β-α-β-α motif that builds the narrow side of the DNA translocating channel. The structure the proheads of these two viruses, each one containing the specific connector, has been solved by cryo-electron microscopy revealing the topology of the components of the DNA translocating machinery.

O-100

Structural studies of functional complexes in solution by X-ray and neutron small-angle scattering
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Small-angle scattering of X-rays and neutrons (SAS) is a fundamental tool in the study of biological macromolecules. SAS allows one to study the low resolution structure of native particles in nearly physiological solutions and to analyse structural changes in response to variations in external conditions. The method is applicable to a broad range of sizes, from individual macromolecules to multi-domain proteins and large macromolecular assemblies. Recent progress in instrumentation and data analysis significantly enhances resolution and reliability of structural models provided by the technique and makes SAS a useful complementary tool to high resolution methods, especially powerful in the analysis of functional complexes.

Advanced methods to analyze X-ray and neutron scattering data from solutions of biological macromolecules will be presented including ab initio low resolution shape and domain structure determination; modeling of quaternary structure of functional complexes by rigid body refinement; the use of specific deuteriation combined with contrast variation in neutron scattering to construct detailed structural models of multi-component complexes; quantitative analysis of equilibrium mixtures of oligomeric proteins and complexes. Practical applications of the methods will be illustrated by recent examples.

O-101

Dual mode of binding in type I cohesin-dockerin complexes
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The utilization of organized supramolecular assemblies to exploit the synergistic interactions afforded by close proximity is an emerging theme in cellular biology. Anaerobic bacteria harness a multi-protein complex, termed the “cellulosome”, for efficient degradation of the plant-cell wall. This megaDalton catalytic machine organizes an enzymatic consortium upon a multifaceted molecular scaffold (termed CipA) whose Type I “cohesin” domains interact with corresponding Type I “dockerin” domains of the enzymes. The crystal structure of the type I cohesin-dockerin complex show that the beta-sheet cohesin domain interacts predominantly with one of the helices of the dockerin. Significantly, internal sequence duplication within the dockerin is manifested in a near-perfect internal 2-fold symmetry suggesting that both “halves” of the dockerin may interact with cohesins in a similar manner. The structure of type I complexes whose dockerin residues Ser-45 Thr-46 that dominate the hydrogen bonding network with the cohesin at the C-terminal helix where mutated revealed binding through the first half of the dockerin. Together the data confirms that a dual mode of binding orchestrates the formation of type I cohesin-dockerin complexes.

O-102

The nDsbD-SS-CcmG complex from E.coli: structural basis for DsbD-dependent cytochrome c maturation
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Escherichia coli DsbD transports two electrons from cytoplasmic thioredoxin to substrate proteins (DsbC, DsbG, and CcmG) in the periplasm. DsbD is composed of a N-terminal periplasmic domain (nDsbD), a C-terminal periplasmic domain and a central transmembrane domain. Each of these domains possesses two cysteines, which are required for the electron transport. Fast (3.9x10^5 M^-1s^-1) and direct disulfide exchange occurs between nDsbD and CcmG, a highly specific disulfide reductase essential for cytochrome c maturation. We solved the crystal structure of the disulfide-linked complex between nDsbD and the soluble part of CcmG at 1.94 Å resolution. In contrast to the other two known complexes of nDsbD with target proteins, the N-terminal segment of nDsbD contributes to specific recognition of CcmG. This and other features, such as the possibility of using an additional interaction surface (like in the disulfide-linked complex with DsbC), constitute the structural basis for the adaptability of nDsbD to different protein substrates.
Protein flexibility and entropy on the edge of binding

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Protein recognition puts systems of many thousand interacting atoms on the edge between two conformational states – free and bound. Structure fluctuations may have a profound impact on this process. Yet, flexibility remains until today the weak point in our understanding of protein-protein binding.

We selected a set of 17 protein complexes for which the three-dimensional structures of both free components and the complex were available and performed molecular dynamics simulations in explicit water for each of these 31 systems. Most uncomplexed binding sites proved more flexible than the remaining surface and lost conformational freedom upon complex formation. However, contrary to common expectation, binding did usually not restrict the overall motion of proteins. We calculated the change in conformational entropy from longer simulations on 7 complexes (21 systems) using a new method based on quasiharmonic analysis. Two small complexes and an antibody-antigen system exhibited a significant loss (up to 157±41 cal/(mol K)) whereas three larger complexes showed increased (by up to 43±23 cal/(mol K)) or unchanged conformational entropy.

Our results blend in with a unified model of flexible protein recognition [Grünberg et al. (2004) Structure 12, 2125-36] that reconciles the ideas of induced fit and conformer selection. Structure dynamics should influence both the speed of recognition and the stability of protein-protein complexes.

Citrate-dependent and heparan sulfate-mediated cell surface retention of cobra cardiotoxin A3

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Anionic citrate is a major component of venom, but the role of venom citrate in toxicity is poorly understood other than its inhibitory effect on the cation-dependent action of venom toxins. By immobilizing Chinese hamster ovary (CHO) cells in microcapillary tubes and heparin on sensor chips, we demonstrated that heparan sulfate (HS)-mediated cell retention of the major cardiotoxin (CTX) from the Taiwan cobra, CTX A3, near membrane surfaces is citrate dependent. X-ray determination of a CTX A3-heparin hexasaccharide complex structure at resolution 2.4 Å revealed a molecular mechanism for toxin retention in which heparin induced conformational changes of CTX A3 lead to citrate-mediated dimerization. A citrate ion bound to Lys23 and Lys31 near the tip of loop II stabilizes hydrophobic contact of the CTX A3 homodimer at the functionally important loop I and loop II regions. Additionally, the heparin hexasaccharide interacts with five CTX A3 molecules in the crystal structure, providing another mechanism whereby the toxin establishes a complex network of interactions that result in a strong interaction with cell surfaces presenting heparin. Our results suggest a novel role for venom citrate in biological activity and reveal a structural model that explains cell retention of cobra CTX A3 through HS-CTX interaction.

Structure of the transcription apparatus by electron microscopy

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Gene expression is mainly modulated at the transcription level and determines the execution of all normal cellular processes as well as the evolution towards pathologic situations. Transcription initiation controls the recruitment of RNA polymerase II to the gene promoter and requires the concerted action of 7 general transcription factors to form the pre-initiation complex. The transcription factor TFIIH is involved at an early step in the process since it recognizes the gene promoter and nucleates the assembly of the pre-initiation complex. In TFIIH the TATA Box Binding factor (TBP) associates with 14 subunits (TAFs) to form a one mega dalton complex. TFIIH and the transcription/repair factor TFIIH are required at a later step and support the opening of the transcription bubble and promoter clearance.

These molecular assemblies integrate multiple regulatory or catalytic activities and are constantly reorganized during the reaction cycle. The determination of the structure and conformational changes of these molecular machines thus represents a major challenge for structural biology. We promote a multi-disciplinary approach including structural genomics to identify crystallisation targets, X-ray crystallography and NMR to solve atomic structures and cryo electron microscopy to determine structural models of the whole complex. The integration of structural information at various levels of resolution will yield useful models to explain the mode of action of these complexes.
Mechanically induced titin kinase activation studied by force probe molecular dynamics simulations

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The conversion of mechanical stress into a biochemical signal in a muscle cell requires a force sensor. Titin kinase, the catalytic domain of the elastic muscle protein titin, has been suggested as a candidate. Its activation requires major conformational changes resulting in the exposure of its active site. Here, force-probe molecular dynamics simulations were used to obtain insight into the tension-induced activation mechanism. We find evidence for a sequential mechanically induced opening of the catalytic site without complete domain unfolding. Our results suggest the rupture of two terminal beta-sheets as the primary unfolding steps. The low force resistance of the C-terminal relative to the N-terminal beta-sheet is attributed to their different geometry. A subsequent rearrangement of the autoinhibitory tail is seen to lead to the exposure of the active site, as is required for titin kinase activity. These results support the hypothesis of titin kinase as a force sensor.

Large scale dynamics of immunoglobulin G

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Dynamical effects in diffusion-driven encounters between antibodies and foreign agents represent a problem of broad relevance to physics and biology. Based on a mechanical model parameterized directly upon results from single-molecule experiments, we investigate a typical antigen-antibody reaction chain. We demonstrate that the role of dynamics in the encounter process may be easily described within a simple, intuitive theoretical framework, that we formulate analytically. This enables us to show that the inner dynamics of antibody molecules results in a cooperative behavior of their individual sub-units. Our results constitute the first application of the DEER method, a thoroughly general integrated strategy to simulate large-scale functional dynamics of proteins, based on structural information extracted from single-molecule experiments.
O-111
Parallel superpleated beta-structure as a fold for amyloid fibrils
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In several neurodegenerative diseases, including Alzheimer’s, Huntington’s diseases and prion diseases, proteins that are normally soluble polymerize into amyloid fibrils that correlate with the disease state. Despite much effort, no high resolution structure has yet been determined for any amyloid fibril. Recently, we proposed a fold called the parallel superpleated β-structure as the structural basis for filaments of the yeast prion protein Ure2p and several other amyloids (1, 2). In this fold, β-strands zig-zag in a planar serpentine arrangement and serpentinae stack axially, in register, generating parallel β-sheets with a small left-handed twist. The filament is stabilized by packing of apolar side-chains and by H-bonded ladders of polar residues. This molecular model emerged after a survey of experimental data on Ure2p. We have also found that a shorter three-stranded serpentine design is consistent with experimental data on human amylin protiflowerls whereby two or three protiflowerls coil into fibrils (2). We further envisage that amyloid fibrils of huntingtin, Sup35p, α-synuclein have similar architectures (1). These observations have potential for the identification of amyloidogenic protein sequences and the discovery of therapeutic agents for the treatment of amyloid diseases.

1. Kajava, A.V., Baxa, U., Wickner, R.B. and Steven, A.C. (2004) Proc Natl Acad Sci USA 101: 7885
2. Kajava, A.V., Aebi, U. and Steven, A.C. (2005) J. Mol. Biol. (in press)

O-113
Insights into the GB1 fold domain swapping mechanism by molecular dynamics simulations
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We used Designerr, a fully automatic procedure for predicting the amino acid sequences compatible with a given target structure based on the CHARMM package, to investigate how specific mutations in the B1 domain of protein G, known to trigger dimerization, would behave when forced to fit in the wild type fold.

From the above work, we proposed a new mechanism that could possibly be the cause behind dimerization, differently from the initially proposed model of clashes between V39 and A34F (Byeon et al., 2003). In our model, A34F would clash with W43 by forcing it to adopt a less favorable rotamer, in addition to a more unfavorable long range interaction between A34F and V54.

This model, together with several analyses done for Molecular Dynamics (MD) simulations of 5 ns for the wild type structure and the dimeric sequence in the wild type fold at 300 K, showed that the second hairpin undergoes considerable conformational rearrangements compared to the control simulation, which could be an indication of the intrinsic fluctuations created by the mutations that eventually lead to the swapping phenomenon.

Reference: Byeon II, Louis JM and Gronenborn AM. J. Mol. Biol. (2003) 333, 141-152.

O-114
Elastic lever-arm model for myosin V
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We present a model for processive motion of myosin V. We describe the dimeric molecule as two identical heads, connected with elastic lever arms. We show in a quantitative fashion why the head leads only binds to actin after the power stroke in the trail head and why it undergoes its power stroke after the release of the trail head. This provides an explanation for the coordinated hand-over-hand motion [1]. We calculate the distribution of step sizes for different lever arm lengths. The best agreement with observed (EM) distributions is achieved when fluctuations in the actin helix are taken into account. We also show how processivity studies could help determining the kinetic rates of an individual head.

[1] A. Vilfan, Elastic lever-arm model for myosin V, Biophys. J. 88, in press (2005), doi:10.1529/biophysj.104.046763
**O-115**

The dark side of protein folding: high pressure studies with amyloidogenic proteins

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The formation of amyloid aggregates is the hallmark of the amyloidogenic diseases. Transthyretin (TTR) is involved in senile systemic amyloidosis (wt) and familial amyloidotic polyneuropathy (variants). Through the use of high hydrostatic pressure (HHP), we compare the stability among wt TTR, two disease-associated mutations (V30M and L55P) and a trans- suppressor mutation (T119M). Our data show that the amyloidogenic conformation, easily populated in the disease-associated variant L55P, can be induced after decompression, rendering the wt protein highly amyloidogenic. After decompression, the recovered wt structure has weaker subunit interactions (looser tetramer called T4 upd) and its stability is similar to L55P. The observed sequence of stability was: L55P < V30M < wt << T119M. After decompression at 37°C and pH 5.6, TTR (wt and variants) undergoes aggregation very rapidly (∼ 30 min). This HHP protocol has allowed us to screen anti-amyloidogenic compounds. Although all amyloid fibrils exhibit a common architecture (cross beta-sheet topology), we decided to investigate whether they display differences in stability that might be correlated with their primary sequences. Indeed, this was the case for the fibrils composed by TTR or by alpha-synuclein, involved in Parkinson disease. We have also shown that the fibrils and the protofibrils have different stabilities. The relevance of these differences in stability to the pathogenesis of these diseases will be discussed.

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**O-116**

Crystal structures of SARS coronavirus proteins

Z. Raa

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Since the 2003 SARS outbreak, we have elucidated a number of important crystal structures of SARS coronavirus proteins. The first structure of a SARS protein, the SARS coronavirus main protease (Mpro or 3CLpro), and its complex with an inhibitor were determined in 2003. The 33.8-kDa SARS-CoV Mpro is pivotal in mediating viral replication and transcription and is therefore an important therapeutic target. The SARS Mpro structure was used to design inhibitors effective against the four coronavirus groups. The structures of SARS Mpro and TGEV Mpro in complex with the above inhibitors provide an important structural basis for rational drug design. The second structure we determined is the SARS-CoV membrane fusion protein. The coronavirus spike (S) protein is characterized by two heptad repeat regions, HR1 and HR2, that form a fusion-active conformation. From the crystal structure, we propose a molecular mechanism for coronavirus membrane fusion and subsequent viral entry. This work provides a new avenue for the design of anti-SARS therapeutics via strategies aimed at inhibiting viral entry by blocking hairpin formation. A third structure was recently solved. The complex between two non-structural proteins reveals exciting new functional insights into the SARS coronavirus.

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**O-117**

Kir2.1 potassium channel measurements with a 4Pi-microscope: Imaging, Deconvolution, Quantification

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Strong inwardly rectifying potassium channels of the Kir2.x family play a central role in the regulation of cellular excitability in the brain and in the heart. Mutations in Kir2.1 cause Andersen’s Syndrome, a hereditary disease characterised by periodic paralysis, cardiac arrhythmia and dysmorphic features. The 4Pi microscopy technique allows a significant improvement of the axial resolution of light optical measurements by the use of interference of wavefronts focused by two opposing lenses compared to confocal microscopy. Due to technical difficulties that have been resolved recently, 4Pi microscopy has to date rarely been applied for the study of mammalian cells. We have for the first time successfully used 4Pi microscopy to measure characteristics of Kir2.1 ion channel clustering in the cell membrane of mammalian HEK293 cells at a spatial resolution of 100 nm in all three axes. We show that Kir2.1 channels are concentrated in large clusters that are distributed homogenously over the cell membrane. The clusters have a rather cylindrical shape with the long axis along the optical axis of the measurements. The size of a single cluster could be measured. In summary, we have shown that 4Pi microscopy can be applied to study the morphology of cell membrane structures at nanoscopic resolution. The measurements are done with the commercial 4Pi-microscope constructed by Leica-microsystems, Mannheim, Germany.

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**O-118**

Interaction between model membranes and the anti-apoptotic domain BH4

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Cell regulation via programmed cell death is an essential part of life, enabling the body to control its cell population. If this tight regulation fails, pathological cells can escape their fate thereby causing lethal diseases. The origin of these diseases seems to be a serious distortion of the interplay between pro- and anti-apoptotic factors. Pro-apoptotic proteins such as Bax have a similar overall structure except for the BH4 domain. We therefore synthesized the BH4 domain of the Bcl-2 protein [Khemtemourian, Sani et al., J. Pep. Science, (2005) in press] to investigate the nature of its interactions with membrane models. Using circular dichroism, the peptide is shown to adopt a helical structure in the TFE and is disordered in water. Addition of vesicles (MLVs) leads to a transitional helix conformation that finally results in a β-sheet conformation. Interestingly, when BH4 is pre-incorporated in membranes, it already adopts a β-sheet structure. Using acyl chain perdeuterated DMPC, 2H NMR revealed cholesterol-like action of the pre-incorporated peptide on MLVs. External addition of BH4 seems to adopt an intermediate state between free and membrane-inserted state. This effect could give a new insight of the anti-apoptotic role of the protein Bcl-2.
O-119

Ion channels and disease: cellular and biophysical properties of P/Q-type calcium channels in SCA6

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The molecular basis of neuronal dysfunction in spinocerebellar ataxia type 6 (SCA6) is poorly understood. SCA6 is a trinucleotide repeat disorder that affects the neuronal voltage dependent P/Q-type calcium channel (CaV2.1). The disorder may arise due to altered calcium channel function (channelopathy), due to the cellular consequences of the polyglutamine expansion at the channel C-terminus (glutaminopathy) or by a combination of both mechanisms. Here we transfected cells with either with CAG11 (WT); CAG23, CAG72 (SCA6); or EGFP and compared to untransfected controls. Channels were expressed in non-excitable HEK293 cells and/or in cultured cerebellar granule neurons (CGN) from P/Q-type channel KO mice. We will present data on the biophysical properties of WT and SCA6 P/Q-type channels. In particular, the ability of SCA6 channels to interact with β4 subunits was explored in HEK293 cells. β4 subunits interact with the α subunit C-terminal region near the mutation locus in SCA6. Calcium current kinetics, calcium- and voltage-dependent inactivation; and calcium-dependent facilitation were quantified in SCA6 and WT channels. Second, to determine whether calcium channel activity affects neuronal survival in SCA6, we used genomic DNA degradation to quantify the calcium channel influence in the degree of programmed cell-death in CGN. Supported by AHA 0335142N.

O-120

Lipidic membranes are potential ”catalysts” in ligand activity of the pentapeptide neokyotorphin

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NKT is a multifunctional pentapeptide, involved in biological functions as diverse as analgesia, antihistaminic regulation and proliferation stimulus of tumour cells. The interaction of NKT with cell membranes is potentially important to all these multiple processes, since receptor-mediated processes were proposed. Sargent and Schwzyer proposed in their ”membrane catalysis” model that ligands would interact with membrane lipids in order to adopt the necessary conformation for cell receptors. We have used fluorescence techniques to study in-depth location, orientation and extent of incorporation of NKT in model systems of membranes. The roles of lipid charge, membrane phase and sterol presence were investigated. The phenolic ring of tyrosine is located in a shallow position in membranes. The extent of partition decreases in gel crystalline membranes relative to liquid crystalline membranes. Addition of cholesterol causes a reorientation of the tyrosine ring in the interface of lipidic bilayers. Lipidic membranes meet all the conditions to be potential ”catalysts” in the ligand activity of the multifunctional pentapeptide NKT because they modulate the exposure and orientation of the phenolic ring, which is most likely involved in docking to receptors.

O-121

Interplay of molecular and biophysical mechanisms in tumor invasion

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Tumor invasion requires the acquisition of migratory capacity by the constituent cells, established through by the competition of cell-cell and cell-matrix interactions. To study the contribution of these factors, adhesive and invasive properties of nine human brain tumors were investigated, using molecular and biophysical approaches. The biomolecular analysis entailed to measuring the expression level of N-cadherin (predominant cell adhesion molecule (channelopathy), due to the cellular consequences of the polyglutamine expansion at the channel C-terminus (glutaminopathy) or by a combination of both mechanisms. Here we transfected cells with either with CAG11 (WT); CAG23, CAG72 (SCA6); or EGFP and compared to untransfected controls. Channels were expressed in non-excitable HEK293 cells and/or in cultured cerebellar granule neurons (CGN) from P/Q-type channel KO mice. We will present data on the biophysical properties of WT and SCA6 P/Q-type channels. In particular, the ability of SCA6 channels to interact with β4 subunits was explored in HEK293 cells. β4 subunits interact with the α subunit C-terminal region near the mutation locus in SCA6. Calcium current kinetics, calcium- and voltage-dependent inactivation; and calcium-dependent facilitation were quantified in SCA6 and WT channels. Second, to determine whether calcium channel activity affects neuronal survival in SCA6, we used genomic DNA degradation to quantify the calcium channel influence in the degree of programmed cell-death in CGN. Supported by AHA 0335142N.

O-122

Clinical proteomics: characterization and structural studies of new proteins from human body fluids and tissues

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Clinical applications of proteomics involve the use of proteomic technologies at the bedside. By comparing the proteins present in diseased samples with those present in normal samples, it is possible to identify changes in expression of proteins that may potentially be related to organ toxicity. Several structures of a new family of regulatory proteins secreted from the mammary glands during involution that are christened as signalling proteins (SPX-40) from six species have been determined. The structures of these proteins revealed a topology with βαααα domain having the triose-phosphate isomerase (TIM) barrel in the core and a small α + β domain. These structures are similar to the structures of chitinase and chitinase like proteins. However, these new proteins are unable to bind the carbohydrates but display a unique surface that was found to be involved in protein-protein interactions. A number of peptides with complementary structures to this novel surface binding site of SPX-40 proteins were synthesized and were found binding to these proteins with affinity constants ranging up to 10⁻⁸ M. The structures of the complexes formed between SPX-40 proteins and designed peptides have also been determined. These structures revealed the positions of peptides close to Trp 191, Asp 186 and Tryp 78 and are held through several hydrogen bonds and hydrophobic interactions. These are new findings and have far reaching implications.
O-123

Combining NMR and SAXS data for the resolution of the 3D structure of the complex Akt : TCL1

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Akt plays a central role in the regulation of cellular antiapoptosis and, thus, is a core intra-cellular survival factor underlying various human neoplastic diseases. We have previously demonstrated that TCL1 (a protooncogene underlying human T cell polylymphocytic leukemia) interacts with Akt and functions as an Akt kinase co-activator. TCL1 co-activate Akt by binding to its pleckstrin homology domain (PHD). Here, we combined NMR methods and small angle X-ray scattering to determine the affinities, molecular interfaces and low resolution structure of the complex formed between Akt-PHD and TCL1. We show that TCL1 target Akt-PHD at a site which has not yet been observed in PHD-protein interactions. Located opposite the phospholipid binding pocket and distal from known protein-protein interaction sites on PH domains, the binding of dimeric TCL1 proteins to this site would allow to cross-link two Akt molecules at the cellular membrane in a pre-activated conformation without disrupting certain PH-ligand interactions. Thus this interaction strengthens membrane association, promotes autophosphorylation and hinders desactivation of Akt.

O-125

Quadrupolar ions spin-spin relaxation used for the cellular apoptosis survey

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The importance of alkali cations to the normal functioning of cells is well known, as well as the changes induced by certain cellular pathological states to alkali dynamics or compartmentalisation. What makes sodium and potassium very attractive from the NMR point of view is the possibility of distinguishing, completely non-invasive, ion pools where the quadrupolar interaction is averaged (extreme narrowing condition) from bound ion pools where the quadrupolar interaction is effective (bound ions), via relaxation measurements. The singular value decomposition of the relaxation multiexponential curves gives an objective set of parameters, the singular values, capable of characterising the distinguishable sodium and potassium pools and their intrinsic evolution, as well as the influences between adjacent pools in physical contact. Pathological state is accompanied by an important increase of bound pool population due to significant changes of the cellular membrane structure. Intermediate exchange process, between bound and free ions pools was put into evidence and eventually used for characterising the pathology.

O-126

Use of 1H NMR of bile to distinguish benign from malignant biliary strictures

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Cholangiocarcinoma accounts for 10% of all primary liver cancers and is the leading cause of mortality in primary sclerosing cholangiitis. A method to find patients at risk for malignant development and for early diagnosis of cholangiocarcinoma is needed. We describe here the use of 1H NMR of bile for the diagnosis of cholangiocarcinoma. Bile specimens from thirty patients were studied using magnetic resonance spectroscopy at 360 MHz. Eight patients with normal cholangiograms were used as controls. The spectra were analyzed both conventionally (peak intensity/area ratio) and by a statistical classification method. Bile spectra from cholangiocarcinoma patients differ in several aspects. Using the statistical classification strategy it was possible to distinguish cholangiocarcinoma from the benign condition with 100% accuracy. Choline, deoxycholic acid, acetocetate, glutamine and cholesterol contribute to this classification. There were no statistically significant differences between the normal controls and the primary sclerosing cholangitis cases in the analysis of any of the resonances. Cholangiocarcinoma can be distinguished from benign conditions using this simple procedure. Primary sclerosing cholangitis did not differ from the normal controls. 1H NMR of bile has diagnostic potential in liver and biliary diseases. This potential will be confirmed by the study of 100 more specimens.
Magnetic resonance spectroscopy and spectroscopic imaging of the human brain: clinical applications to tumors and stroke

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Proton localized magnetic resonance spectroscopy (MRS) is a method of noninvasive exploration of human neurochemistry based on the magnetic resonance phenomenon. This exploration of brain metabolism, performed without any injection, detects neuronal, glial, and membrane markers. MRS of the brain tends to be a "metabolic biopsy", but unique applications for brain MRS are (1) quantitating the oxidative state of the brain and defining neuronal suffering, (2) assessing and mapping neuron damage, (3) evaluating membrane alterations and demyelination, (4) characterizing glial activation or gliosis, (5) identifying macrophagic invasion and/or hypoxia, (6) detecting modification in the metabolism of macromolecules (lipids, proteins), and (7) pinpointing anomalies in the metabolism of glial and neuronal aminoacids.

Brain MRS (single voxel spectroscopy and chemical shift imaging) can be performed routinely after conventional MRI, without moving the patient, as a valuable metabolic and functional complement to the anatomical evaluation of the cerebral status of patients. Examples of clinical applications will be presented in the diagnosis of brain tumors, follow-up of tumor patient response to therapy, and characterization of stroke.
The melting points of the membranes and thus lateral pressure created by the surface-adsorbed proteins can lower the hydration threshold can be understood on the basis of molecular crowding (e.g. in cytochrome c binding to charged membranes). Crowding and association reactions taking place in the crowded medium and those of the same reactions taking place in dilute solution. We summarize theoretical predictions and recent experimental observations from our and other laboratories confirming the predictions, in some cases quantitatively.

### O-128

**Effects of macromolecular crowding on protein associations and stability: predictions and observations**

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Biological fluid media contain a significant volume fraction (5 - 40%) of macromolecules and are referred to as ‘crowded’. The volume excluded by one macromolecule to others of comparable or larger size in a crowded solution is predicted to result in substantial changes in the chemical potential and diffusional mobility of all macromolecular species, dilute as well as concentrated, within these solutions. These changes are manifested as substantial differences between the rates and equilibria of macromolecular isomerization and association reactions taking place in the crowded medium and those of the same reactions taking place in dilute solution. We summarize theoretical predictions and recent experimental observations from our and other laboratories confirming the predictions, in some cases quantitatively.

### O-129

**Protein folding by the effects of macromolecular crowding**

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Unfolded states of ribonuclease A were used to investigate the effects of macromolecular crowding on macromolecular compactness and protein folding. The extent of protein folding and compactness were measured by circular dichroism spectroscopy, fluorescence correlation spectroscopy (FCS), and NMR spectroscopy in the presence of polyethylene glycol (PEG) or Ficoll as the crowding agent. The unfolded state of RNase A in a 2.4 M urea solution at pH 3.0 became native in conformation and compactness by the addition of 35% PEG 20000 or Ficoll 70. In addition, the effects of macromolecular crowding on inert macromolecule compactness were investigated by FCS using fluorescence-labeled PEG as a test macromolecule. The size of Fluorescence-labeled PEG decreased remarkably with an increase in the concentration of PEG 20000 or Ficoll 70. These results show that macromolecules are favored compact conformations in the presence of high concentration of macromolecules and indicate the importance of a crowded environment for the folding and stabilization of globular proteins. Furthermore, the magnitude of the effects on macromolecular crowding was investigated by using smaller size background molecules (PEG200). RNase A and Fluorescence-labeled PEG did not become compact by the addition of PEG 200. The effect of the chemical potential on the compaction of a test molecule in relation to the relative sizes of the test and background molecules is also discussed.

### O-130

**Molecular crowding on membrane surfaces: Adsorption and insertion of proteins**

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Proteins adsorb to or insert into the lipid membranes of biological cells. Typically about 50% of the mass of biomembranes consists of proteins. The adsorption of proteins is often described in terms of a Langmuir isotherm, relating the binding affinity to the ratio of free and occupied surface area. For the random arrangement on continuous surfaces, however, the available free surface area is typically significantly less than the total area minus the occupied area, and the Langmuir isotherm is far from describing binding accurately. The proteins adsorbed to membranes may be considered as a twodimensional gas exerting a density dependent lateral pressure. We show here that this pressure may be quite significant. It influences not only the adsorption itself but also in-plane protein aggregation and reaction equilibria as well as surface induced protein denaturation (e.g. in cytochrome c binding to charged membranes). Crowding may also lead to the insertion of proteins into the membranes. The formation of some oligomeric peptide pores and its concentration threshold can be understood on the basis of molecular crowding on membrane surfaces. Also, many biological membranes display melting transitions close to physiological temperatures. The lateral pressure created by the surface-adsorbed proteins can lower the melting points of the membranes and thus influence all physical properties related to chain melting, e.g. domain formation and the elastic constants.

### O-131

**Monte Carlo simulation of enzymatic reactions in 2D and 3D crowded media**

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The fractal-like approach for the enzyme kinetics considers that the rate coefficients for the diffusion controlled reactions are not constants but they are depending on time. We apply fractal kinetics approach using a Monte Carlo simulation for studying Michaelis Menten enzymatic kinetics in a 2D and 3D lattices with obstacles following Berry’s algorithm. We apply this algorithm for situations more physically realistic in the cellular media, as different degrees of mobility and different sizes for big molecules and obstacles. These simulations suggest that in crowded media we always deal with a fractal-like kinetics, but with different types of fractality.
Effect of macromolecular crowding on the associations and assembly of the bacterial cell division FtsZ protein

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The GTP-mediated assembly and disassembly of FtsZ, a bacterial GTPase homolog of tubulin, are thought to be essential for the formation of the dynamic septal ring during cell division. We have investigated the excluded volume effects on FtsZ oligomerization and assembly in highly crowded solutions resembling the bacterial interior. FtsZ in its GDP-bound state oligomerizes in solution by means of a magnesium-linked non-cooperative process that is unfavoured by ionic strength and moderately enhanced by crowding [1-3]. In the presence of GTP, FtsZ forms a variety of polymers depending upon solution conditions [3,4]. We have found that high concentrations of unrelated macromolecules favour the cooperative assembly of FtsZ to form bidimensional ribbons that are dynamic and disassemble upon GTP consumption [3]. These ribbons, when compared with the FtsZ filaments observed in vitro under dilute solutions, show a retard in the GTPase activity, a reduced rate of GTP hydrolysis and the GTP exchange within the polymers. Because these ribbons tend to form spontaneously in crowded solutions, we propose that in the bacterial cytoplasm these structures may form part of the FtsZ ring at division, and therefore would be more physiologically relevant than the FtsZ polymers formed in vitro under dilute solutions.

1. Rivas et al. (2000) JBC 275:11740
2. Rivas et al. (2001) PNAS-USA 98:3150
3. González et al. (2003) JBC 278:37664
4. González et al. (2005) PNAS-USA 102:1895

Neutron scattering study of nucleic acids dynamics in cellula

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Neutron scattering experiments have been performed on whole natural bacterial cells and established the biological significance of dynamics on a macromolecular level (Tehei and al, 2004). Two parameters were measured: mean square displacement, which corresponds to macromolecular flexibility that is essential to the biological function, and resilience, which corresponds to an effective force constant associated to the structure stability (Zaccai, 2000). Both parameters referred to the average contribution of all macromolecules inside cells and reflected how average dynamics had adapted to the bacterial physiological temperature. Neutron scattering cross-sections are significantly different for H and 2H(D) and selective H-D labelling can be used to examine dynamics in different parts of a cell. In the present work, dynamics of Escherichia coli RNA components were investigated in vivo, by selective H-labelling of nucleic acids in D-cells. Analytical centrifugation and mass spectrometry were used to control H-isotope level in the RNA and proteins.

MCE and CE results for chemical modifications vs mutational in determining valence

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Capillary electrophoresis (CE) has been used to compare the mobility of charge ladders of T4 lysozyme produced in two ways: by chemical modification (acylation of lysine residues) and by mutation. Valence determinations made solely from assumed differences in net charge (the “charge ladder method”) are compared with values calculated from mobility using simple Debye-Hückel theory, the later requiring the use of the hydrodynamic radius. Sedimentation velocity studies were done to compare the hydrodynamic radius of the charge ladder to that of each individual mutant. The radius was also calculated from the charge ladder method for comparison. Furthermore, charge was measured by membrane confined electrophoresis (MCE) under the same conditions and found to be in excellent agreement with CE results. There has been some question as to whether the use of a single radius for all species is valid in valence calculations derived from the charge ladder method as well as whether charge regularization might limit its utility. Results given here suggest that, accurate charge values can be obtained using a single radius and that charge regularization is minimal in the range.

Effect of macromolecular crowding on the associations and assembly of the bacterial cell division FtsZ protein

O-134

O-133

Neutron scattering study of nucleic acids dynamics in cellula

O-132

MCE and CE results for chemical modifications vs mutational in determining valence

O-132

MCE and CE results for chemical modifications vs mutational in determining valence

O-133

Neutron scattering study of nucleic acids dynamics in cellula
**O-135**

Single-molecule protein folding

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Fluorescence spectroscopy on the single-molecule level can provide a unique real-time view of biomolecular dynamics. A particularly rich example is provided by the protein folding reaction. We have recently developed a method, based on trapping of single molecules within surface-tethered lipid vesicles, which allowed us to obtain for the first time folding and unfolding trajectories of individual protein molecules [1]. Our method involves measuring fluorescence resonance energy transfer to probe conformational changes of vesicle-trapped molecules in real time. Our study of the protein adenylate kinase exposed dramatic heterogeneity in folding/unfolding pathways [2]. Surprisingly, some motions on the energy surface of the protein could be time-resolved as very slow drifts from one state to another. In contrast, studies of a small cold shock protein confirmed two-state folding behavior even on the single molecule level, presenting much more homogenous dynamics [3]. Further characterization of single molecule trajectories aims at measuring the time scales of molecular motions at various points on the energy landscape, with a particular eye on the transition state, which cannot be directly probed in ensemble measurements.

References:
[1] Boukobza, E. et al. (2001) *J Phys Chem B* 105, 12165-12170.
[2] Rhoades, E. et al. (2003) *PNAS* 100, 3197-3202.
[3] Rhoades, E. et al. (2004) *J Am Chem Soc* 126, 14686-7.

**O-136**

The use of repeat-proteins to experimentally determine protein folding energy landscapes

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Energy landscapes and “folding funnels” have been provided as illustrations of the complex process of protein folding, but have been difficult to connect to experimental observations because partly folded structures, which form the coordinates of landscape illustrations, are not populated. To provide an experimental connection to landscape theory, we have initiated folding studies of repeat-proteins. We find these modular proteins to be highly cooperative in their folding. However, unlike globular proteins, we find repeat-proteins to be tolerant to deletion, duplication, and insertion, providing access to the internal distribution of stability. From a deletion series in an ankyrin domain from the Notch receptor, we have demonstrated that cooperativity comes from strong nearest-neighbor interfacial interactions among repeats. Moreover, this deletion series experimentally defines the energy landscape at an unprecedented resolution. This equilibrium landscape suggests an early barrier to folding, and also suggests a low energy channel by which kinetic folding might occur. We tested the kinetic relevance of these features, and demonstrate that the low energy equilibrium channels do carry the bulk of the kinetic flux. To further demonstrate this, we show that by stabilizing one end of this linear protein, thus rescultping the equilibrium landscape, the transition state is repositioned to follow the new low-energy path, illustrating that folding kinetics is thermodynamically controlled.

**O-137**

*Streptococcus pyogenes* bacteriophage hyaluronate lyase: its unusual structural characteristics

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Hyaluronidase enzymes produced by Streptococci are thought to aid in the spread of the organism in host tissue. The *Streptococcus pyogenes* bacteriophage 10403 contain gene *hylP2* that encode for the protein hyaluronidase in this organism. We have cloned and overexpressed this gene in *E. coli* and purified to homogeneity its respective protein product. The recombinant *hylP2* proteins exist as homo-trimer of molecular masses of about 109 kDa under physiological conditions. Detailed structural studies showed that the *hylP2* protein contains two main structural domains: the N-terminal domain and the C-terminal domain. The folded N- and C-terminal domains had a tendency to associate. The N-terminal domain seems to have an open flexible conformation whereas the C-terminal domain has a compact conformation. The C-terminal domain of *hylP2* protein under physiological conditions was found to be stabilized as an enzymatically active trimer. The studies clearly demonstrate that the N-terminal domain of *hylP2* protein plays no role in the stabilization of protein trimer or in the functional activity.

**O-138**

Folding of parallel beta-sheet proteins associated with bacterial pathogenesis

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Many virulence factors from gram-negative bacteria are autotransporter proteins. The final step of autotransporter secretion is C-terminal threading through the outer membrane (OM), followed by folding. This process requires neither ATP hydrolysis nor a proton gradient. Pertactin, an autotransporter from *Bordetella pertussis* and the largest β-helix structure solved to date, folds much more slowly than expected based on size and native state topology, yet folding intermediates are not aggregation-prone. Equilibrium denaturation results in the formation of a partially folded structure, a stable core comprising the C-terminal half of the protein. Examination of the pertactin crystal structure does not reveal the origin of the enhanced C-terminal stability. Yet sequence analysis reveals that, despite size and sequence diversity, all autotransporters are predicted to fold into parallel β-helices, suggesting this structure may be important for secretion. For example, slow folding *in vivo* could prevent premature folding of in the periplasm prior to the assembly of the OM porin. Moreover, extra stability in the C-terminal rungs of the β-helix may serve as a template for the formation of the native protein during secretion, and formation of the growing template may contribute to the energy-independent translocation mechanism. Coupled with the sequence analysis, these results suggest a general mechanism for autotransporter secretion.
O-139
Unraveling the physical origin of the structure of fully denatured ubiquitin
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The structure and dynamics characterization of non-native states of proteins is crucial for understanding the mechanism of protein folding. Recently many experimental studies have shown variations of conformational propensity and flexibility along the backbone chain of fully denatured proteins. It has been supposed that areas of residual structure may serve as initiation sites of protein folding. However, the physical origin of these variations is still unclear. We analyze the structure of fully urea-denatured ubiquitin. The experimental verification of conformational propensities of protein backbone is obtained through structurally dependent NMR parameters. Although the secondary structure of ubiquitin under strong denatured conditions is not detectable and no correlation with the native overall topology is found, the variations of NMR parameters along the backbone follow the secondary structure elements of its native state. We show that these variations are in accord with the recently developed electrostatic screening model of denatured proteins (1). In this model, the backbone conformations of residues in unfolded protein are determined by local backbone electrostatic interactions and their screening by backbone solvation.

1. F. Avbelj & R.L. Baldwin, Proc. Natl. Acad. Sci. U.S.A. 100, (2003) 5742-5747.

O-140
Direct observation of mini-protein folding using fluorescence correlation spectroscopy
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The "Trp-cage" motif represents the smallest and one of the fastest folding mini-proteins known to date. The globular fold is characterized by a hydrophobic core burying a single tryptophan (Trp) residue. Here, we report on the direct observation of Trp-cage folding kinetics using fluorescence correlation spectroscopy (FCS). Our method is based on the selective fluorescence quenching of oxazine dyes by Trp which becomes efficient only upon contact formation between the dye and the indole moiety of Trp. By site-specifically labeling the dye to Trp-cage, temporal fluorescence fluctuations of the dye-peptide conjugate, caused by intramolecular contact formation between dye and Trp, directly report on conformational dynamics and folding transitions of the peptide chain. In order to measure fluorescence fluctuations directly in solution we used FCS on a confocal fluorescence microscope setup. FCS allows us to reveal conformational dynamics with nanosecond time-resolution, under thermodynamic equilibrium conditions, and in highly dilute solutions (i.e. at molar sample concentrations).

Our method confirms microsecond folding kinetics of the Trp-cage motif, previously estimated with non-equilibrium temperature-jump techniques. We further investigated stability and folding rates under denaturing conditions and at various temperatures, giving further insight into structural transitions during the folding process.

O-141
Study on multiple unfolding trajectories of GB1 by molecular dynamics simulations under the physical property space
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The 9 independent thermal unfolding simulations of GB1 have been performed. 12 physical property parameters of protein structure were chosen to construct a 12-dimension physical property space. Then the 12-dimension property space was reduced to 3-dimensions principle component property space. Under the property space, the unfolding pathway ensemble of GB1 was obtained. The pathway ensemble likes a funnel that was gradually emanative from the native state ensemble to the unfolded state ensemble. The unfolding trajectories have the similar variable trend during the native state and the transition state ensemble. During the unfolded state, the 9 unfolding trajectories were divided into two types that one includes only one trajectory and the other include 8 trajectories. The first type of unfolded state was a discontinuity step distribution model, which is not random distribution. The second type of unfolded state was a near ellipsoid distribution model and a near random. There were substantial overlaps of unfolded state, indicating that thermal unfolded state consists of a confined set of property values that makes the number of unfolded state of protein to be much smaller than that was believed before.

O-142
Theoretical studies of pressure effects on folding/unfolding of proteins
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Proteins denature at high hydrostatic pressures, implying that the unfolded proteins in aqueous solution have lower volume than the folded state. A model that explains pressure unfolding requires water to penetrate the protein interior and disrupt the protein hydrophobic core. I will explore the energetics of water penetration and the effect of pressure on hydrophobic interactions. I will also describe molecular simulations of the reversible folding/unfolding equilibrium as a function of density and temperature of solvated peptides that can form alpha helices (the AK peptide) and beta hairpins (the C terminal domain of protein G). I will characterize the structural, thermodynamic and hydration changes as a function of temperature and pressure. To study protein folding equilibrium thermodynamically we use an extension of the replica exchange molecular dynamics (REMD) method that allows for density and temperature Monte Carlo exchange moves. We studied multiple thermodynamic states, covering a density range from 0.96 g cm$^{-3}$ to 1.14 g cm$^{-3}$, and a temperature range from 300 to over 500 K.
**O-143**

Structure and mechanism of the lactose permease of Escherichia coli

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Membrane transport proteins that transduce free energy stored in electrochemical ion gradients into a concentration gradient are a major class of membrane proteins. We have reported the crystal structure of the Escherichia coli lactose permease, an intensively studied member of the Major Facilitator Superfamily of transporters. The crystal structure of the lactose homologue, β-D-galactopyranosyl-1-thio-β-D-galactopyranoside (TDG), reveals the sugar-binding site in the inward-facing conformation of the transporter. The structure positions residues that play major roles in substrate recognition and proton translocation are identified. A possible mechanism for lactose/proton symport mechanisms based on the structure and features from new form crystals will be discussed.

**O-144**

Role of tryptophan residues in the functional conformation of an ion channel

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The linear peptide gramicidin forms prototypical ion channels specific for monovalent cations and has been extensively used to study the organization, dynamics and function of membrane spanning channels. The role of tryptophan residues is a key issue in ion permeation by gramicidin (Becker et al. (1991) Biochemistry 30: 8830-8839). We have monitored the organization and dynamics of gramicidin tryptophans in various types of microheterogeneous molecular assemblies using wavelength-selective fluorescence and other approaches (Rawat et al. (2004) Biophys. J. 87: 831-843; Kelkar and Chattopadhyay (2005) Biophys. J. 88: 1070-1080). Taken together, these results provide comprehensive information on the dynamics of the functionally important tryptophan residues of gramicidin. Experiments using synthetic analogues of gramicidin containing single tryptophan residues further help to delineate the crucial role of tryptophan in maintaining the ion conducting conformation of gramicidin.

**O-145**

ADP/ATP transport in mitochondria: crystal structure of the bovine mitochondrial carrier

E. Pebay-Peyroula1, H. Nury1, G. Brandolin2, V. Trézeguet3, E. Pebay-Peyroula and G. Brandolin (2004) Acta cryst. D 50: 877-886; 30:

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Specific transport through the inner mitochondrial membrane is achieved by carriers which form a large transport family, MCF for mitochondrial carrier family. The exchange of ADP and ATP is of particular significance, as human beings daily consume their own weight of ATP. Regeneration of ATP in mitochondria is achieved by a membrane protein, the ADP/ATP carrier able to import ADP and export ATP. The structure solved at a resolution of 2.2 Å by X-ray crystallography in complex with an inhibitor, carboxyatractysloside, consists of six α-helices. At the surface oriented towards the mitochondrial inter membrane space, the protein has a deep depression. In combination with earlier biochemical results, our structure suggests that nucleotides bind to the bottom of the cavity. In the middle of the cavity, four highly conserved residues constitute the access to the bottom, possibly forming a selectivity filter. Several biochemical and biophysical data, evidenced the functional unit to be a dimer. A second crystal form revealed favorable protein-protein interactions mediated by cardiolipins. In light with these observations the role of a putative dimer will be discussed.

E. Pebay-Peyroula et al. (2003) Nature. C. Dahout-Gonzalez et al. (2003) Acta cryst. D. E. Pebay-Peyroula and G. Brandolin (2004) Current Opinion in Structural Biology.

**O-146**

EPR studies of the flip-flop phenomenon in unsymmetrical bolaamphiphile medium-sized vesicles

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Bipolar lipids found in archaeobacterial membranes, generally termed bolaamphiphiles, induce increased stability in membranes exposed to environments such as acidic conditions, high temperatures, high salt concentrations and/or absence of oxygen. We have synthesized a spin labeled unsymmetrical bolaamphiphile that self-organises in water solutions in multilamellar vesicles and shows slow flip-flop phenomenon in comparison to conventional liposomes. Generally, the flip-flop from the exovesicular to the endovesicular membrane surface is a relatively slow process, which is due to the high energy barrier in transferring the polar amphiphilic heads through the lipophilic membrane. It can be involved in membrane transport mechanisms and in facilitating the transport, cells have evolved to use various supramolecular strategies. The half-life of the flip-flop is estimated to more than twelve hours. We are now modulating the flip-flop rate by incorporating chemical modifications such as addition of cyclopentanes, double or triple bonds into the bridging chain of the molecule, in order to control the membrane transport via the flip-flop mechanism.
**Oral Presentations**  
-- Regulation of Membrane Transport --

**O-147**
Oriental and conformational changes in transmembrane domains of membrane proteins
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Multidrug transporter proteins promote the active transmembrane efflux of drugs thereby decreasing their accumulation in the intracellular medium. Crystallisation of these resistance proteins is a way to gain deeper insight into the resistance mechanism. To overcome difficulties related to crystallization of membrane proteins, new experimental approaches have been developed to gain information on the structural changes involved in drug transport. We examine here and illustrate with examples how infrared and fluorescence spectroscopy can provide new insights into the structure and orientation of the membrane domains of transporters in particular and how ligand-protein interaction can affect the structure and orientation of transmembrane domains. We will also illustrate how such methods opens new possibilities for the detection of conformational changes that are transmitted from the cytosolic domains to the transmembrane domains and vice-versa. How lipid micro-domains modify the transmembrane structural parameters will be discussed through a few examples.

**O-149**
Molecular dynamics studies of a bacterial ATP-binding cassette transporter
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ATP-Binding Cassette (ABC) Transporters form an important superfamilly of membrane proteins which couple ATP hydrolysis to the active transport of diverse compounds across the cell membrane. Their biomedical relevance is highlighted in examples such as multidrug resistance to antibacterial and anticancer agents, and cystic fibrosis. The availability of crystal structures of three complete bacterial ABC transporters provides an opportunity to study structure-function relationships at the atomic level. In this work, we carry out multi-nanosecond Molecular Dynamics simulations of the Vitamin B12 importer from *E. coli* (BtuCD), with both the complete multimeric transporter embedded in a phospholipid bilayer and the soluble subunits in a membrane-free environment, in an attempt to elucidate some of the conformational changes which arise during the transport event. ATP-bound and ATP-free structures are used to investigate the effect of nucleotide on the system. A range of analytical techniques have been applied to assess the dynamic behaviour of the protein during the simulations, which includes measurements of: conformational drift, residue flexibility, transmembrane domain (TMD) movement, concerted protein motions, nucleotide-binding and translocation pathway changes.

**O-148**
Interaction of various types of amphipols with the Ca2+-ATPase from sarcoplasmic reticulum
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Amphipols are amphipathic polymers designed to replace or supplement detergents in membrane protein solution studies. For the study of the Ca2+-ATPase from sarcoplasmic reticulum, previous experiments have revealed both advantages and disadvantages to the use of a polyacrylate-based amphipol, A8-35. These issues have been reinvestigated using four different amphipols. Size exclusion chromatography showed that, although A8-35 aggregates in the presence of millimolar concentrations of calcium -an effect that probably accounts for most of the aggregation of ATPase/A8-35 complexes observed in our previous work-, aggregation can be avoided by resorting to a sulfonated version of A8-35. We also found that all amphipols tested slowed down the rate of calcium dissociation from its binding sites and reduced ATPase activity, while protecting the solubilized protein against denaturation. This suggests that association with the polymer may damp the protein’s dynamics, perhaps due to the multipoint attachment of the polymer to its hydrophobic transmembrane surface. Such a “Gulliver” effect could contribute both to the protection of membrane proteins against denaturation and to the reversible inhibition of SERCA1a.

**O-150**
Electrophysiological characterization of mitochondrial uncoupling protein 2
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2 Department of Membrane Transport Biophysics, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
Electrophysiological characterization of the vast number of annotated channel and transport proteins in the postgenomic era would be greatly facilitated by the introduction of rapid and robust methods for the functional incorporation of membrane proteins into defined lipid bilayers. We present an automated method for reconstitution of membrane proteins into lipid bilayer membranes, that substantially reduces both the reconstitution time and the amount of protein required. We have applied this well-defined system to the characterization of a novel mitochondrial uncoupling protein, UCP2 and demonstrated that UCP2 exhibits protonophoric function exclusively in the presence of fatty acids, similar to that previously shown for its homologue UCP1. The membrane conductance was proportional to the concentration of the reconstituted UCP2 in presence of oleic acid or eicosatrienoic acid, and was inhibited by ATP.
O-151
Photoreceptors and photosignalling processes in microorganisms
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Many freely motile unicellular microorganisms can perceive the quantity and quality of light in the environment by transforming the absorption of a photon into a signal recognizable and exploitable by the cell. Thanks to this photosensory capability, spatial and temporal variations in the external light field elicit modifications of movement patterns. Among the key issues in these studies are: a) the localization of the photoreceptor and the determination of spatial and spectral maps in living cells; b) the identification of the primary molecular events which trigger the photosensory transduction chain. Two series of studies, currently under development in our Laboratory and related to photolyase, will be reported: the use of two-photon confocal microscopy to face the first problem in intact cells and the use of steady state and ultrafast optical spectroscopies to investigate chromophore protein interactions. Spectral and spatial maps of B. japonicum show the presence of the photoreceptor pigment also in the cilia, thus suggesting a direct influence of the photoinduced reactions taking place in the cilia on cell motion modulation. The excited-state kinetics of the chromoprotein in the picosecond regime significantly differs from that of the isolated chromophore. A strong and very fast biexponential decay was observed between 640 and 750 nm, which seems to sign a specific deactivation channel that follows a still hypothetical sub-picosecond intermolecular electron transfer.

O-152
Crystal structures of major light-harvesting complex (LHC-II) from Spinach and Cucumber
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Light harvesting is the primary process in photosynthesis. In green plants, the function of harvesting solar energy is fulfilled by a series of light-harvesting complexes in the thylakoid membrane of chloroplasts. LHC-II, the most abundant integral membrane protein in chloroplasts, exists as a trimmer and binds half of the thylakoid chlorophyll molecules. Besides the light-harvesting function, LHC-II has also been shown to function in the non-radiative dissipation of excess excitation energy formed under high-light conditions. It has a crucial role in minimizing the damaging effects of excess light by operating this photoprotective mechanism as light intensity becomes increasingly saturating. Moreover, LHC-II also takes part in regulating the distribution of excitation energy to photosystems II and I.

The structures of LHC-II from Spinach and Cucumber have been determined at higher resolution using X-ray crystallography in our laboratory. In our model, we provide the basis for investigating quantitatively the underlying mechanism of the light-harvesting process and its adjustment in LHC-II. We also reveal for the first time an elegant arrangement of membrane proteins in the isosahedral proteoliposome assembly. The structural comparison of LHC-II between spinach and cucumber was performed and the photoprotective mechanism of LHC-II was discussed.

O-153
Electron transfer and proton dynamics in DNA photolyase: hopping along the FAD-Trp-Trp-Trp chain
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DNA photolyase is a flavoprotein that catalyzes light-driven repair of major UV-induced DNA lesions (pyrimidine dimers). The photorepair reaction is presumably initiated by electron transfer from the excited state of the fully reduced FAD cofactor to the pyrimidine dimer. Another photoreaction of photolyase, named photocatalytic, serves to re-establish the fully reduced form of the FAD cofactor once it has become oxidized to its semi-reduced form, the neutral radical FADH that is typically found in isolated photolyase. The presentation will focus on the reaction mechanism of photocatalytic in E. coli photolyase. Upon excitation, FADH oxidizes the tryptophan residue W306 that is ~15 Å apart and close to the surface of the protein so that it is readily re-reduced by extrinsic reductants. Evidence will be presented that
(1) oxidation of W306 occurs in two kinetically well separated steps: electron abstraction in <10 ns and proton release to bulk water in ~200 ns.
(2) Electron transfer from W306 to photoexcited FADH is a three-step hopping process that involves two more tryptophan residues, W382 and W359, that bridge the distance between the flavin and W306.
Part of the work has been reviewed recently by Martin Byrdin, Valère Sartor, André P.M. Eker, Marten H. Vos, Corinne Aubert, Klaus Brettel & Paul Mathis in Biochim. Biophys. Acta 1655, 64-70 (2004).

O-154
Structures of intermediates, functional relaxations and kinetics from time-resolved X-ray data
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Recent developments [Schmidt et al., 2003, Biophys. J. 84 2112; Schmidt et al., 2004, PNAS 101 4799] allow the determination of the structures of intermediates from time-resolved X-ray crystallographic data. In parallel, functional relaxations and chemical kinetic mechanisms can be investigated. Experiments on three proteins are reported. In the L29W mutant of Myoglobin initial protein relaxations extend into the ns time-regime although the heme itself has completely relaxed even at the fastest times [Schmidt et al., 2005, PNAS, submitted]. Early relaxations are interpreted in the light of existing investigations on protein dynamics [Parak, 2003, Rep. Prog. Phys. 66 103]. From 1 ns to 1.3 ms the CO is uniformly absent from the heme iron and accumulates at the proximal Xe1 site. Final relaxation occurs in concert with the CO rebounding. The detailed analysis allows the determination of the structure of a short lived species. An almost complete description of the photocycle of the Photooactivc Yellow Protein becomes possible by using time-resolved crystallography on the wild-type and mutant E46Q [Ihee et al., 2005, PNAS, in press; Rajagopal et al., 2005, Structure 13, 55]. Relaxations were analyzed by singular value decomposition. 4 relaxation times, each, were determined. By fitting kinetic mechanisms the structures of photocycle intermediates were identified and refined. The SVD based analysis will pave the way to a general application of time-resolved macromolecular crystallography to other proteins and enzymes.
Protochlorophyllide oxidoreductase takes an abnormal reaction pathway below the glass transition

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Motions through the energy landscape of proteins lead to biological function. At temperatures below a dynamical transition (150-250 K), the activity of some proteins cease. In this work, we describe an enzyme that, instead, engages into a non-productive pathway below 160K. Protochlorophyllide oxidoreductase (POR) catalyzes the reduction of protochlorophyllide (PChlide) into chlorophyllide (Chlide), a key step in chlorophyll biosynthesis. POR is one of the two enzymes known to require light for catalysis.

When illuminated with gentle light at 165 K, the complex of T. elongatus POR with Pchlide and NADPH transforms into a non-fluorescent intermediate. Upon warming, several fluorescent intermediates develop, and at 290K Chlide is released. When illuminated at temperatures below 155K, POR behaves differently. If gentle light is used, the reaction can not start. Instead, if a blue laser source is used, the initial complex disappears, like at 165K. However, upon warming, a new intermediate develops that fluoresces at 694nm and leads to a dead-end product.

By using fluorescence microspectrophotometry, we have measured the solvent glass transition temperature of the system to be 158K. The solvent glass transition, possibly controlling a POR dynamical transition, may be the determinant that switches the enzyme reaction pathway from a non productive to a productive one. The non-productive pathway results from a two-photons absorption mechanism, whereas the productive pathway is a one-photon mechanism.
O-157

FMRI illustrated - The delineation of low order visual areas in the healthy subject

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Developed for more than two decades now, magnetic resonance imaging (MRI) has become the “golden standard” for diagnostic – tomographic - imaging of any organ within the human body. Besides other specificities, the major strength of the technique lies in the possibility it provides to modulate image contrasts. Various instrumental timings determine the relative sensitivity of the technique to certain physiological parameters such as the volume density of the water molecules in the tissue, certain relaxation times of the water protons, tissue velocity, diffusion properties of the water molecules, and many others. During the last decade, MRI has furthermore developed as the method of choice for studying brain function. MRI mapping of brain function relies upon the possibility to render the MRI signal sensitive to the blood oxygenation. Oxygenation of the blood in the capillary bed and downstream may vary locally in the brain, as a result of a mismatch between the relative functional increases in cerebral perfusion and oxygen consumption by the neuronal populations involved in activation. The possibility to map brain function by means of MRI has triggered numerous applications, at the level of the clinic as well as in the fields of neurosciences and cognition. In this talk, the principles underlying MRI mapping of brain function will be recalled. The delineation of the low order visual areas in the healthy individual will be presented as an example.

O-159

Cell-silicon synapse: vesicle exocytosis monitored by field-effect transistors

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We cultured bovine chromaffin cells on an array of electrolyte-oxide-silicon field-effect transistors (EOS FET) and monitored granule secretion. By stimulation with barium chloride, vesicles are released into the narrow sheet of electrolyte between the chip surface and the plasma membrane. The interaction of released protons with the silicon dioxide surface of the chip alters the threshold voltage of the transistor and gives rise to a measurable signal. Simultaneously performed measurements with a carbon fibre showed a correlation of the transistor signals and amperometric current traces. We conclude that the transistors are able to monitor exocytosis on a single vesicle level.

To elucidate the role of protons, we destroyed the proton gradient across the vesicle membrane by nigericin and valinomycin. As a result a massive reduction of the transistor signals was induced, whereas there was only little change of the amperometric records. We conclude that released protons are responsible for the detection of vesicles with transistors.

The individual transistor records of vesicle exocytosis can be explained by combining the dynamics of the exocytotic event with the diffusion in the cell-chip junction.

Transistor recording of exocytosis does not depend on the electrochemistry of transmitters. As many kinds of exocytotic vesicles contain a large amount of buffered protons it can be applied to numerous kinds of exocytotic events, independent on the nature of the transmitter.

O-158

Immunofluorescence microscopy and flow cytometry in live animals

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Detection and quantification of specific cell populations in the circulation are typically accomplished by taking blood samples and analyzing them with a flow cytometer. Recently we have developed an in vivo flow cytometer that performs real-time detection and cell counting in the circulation of live animals without the need to draw blood samples. Fluorescently labeled cells can be tracked in the same animal over extended periods of time, allowing direct assessment of the number of circulating cells (e.g. cancer cells and immune cells) during the course of disease progression and in response to therapeutic intervention. The interaction of circulating cells with local tissue microenvironment can furthermore be imaged by in vivo confocal and multiphoton microscopy. In particular, molecular signals expressed on the endothelial cells of blood vessels are recognized by the circulating cells during their transit. These cellular “traffic signals” can be visualized using the new method of in vivo immunofluorescence microscopy.

O-160

In vivo subcellular structures recognized with phase

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Phase contrast transmission electron microscopy has been developed to enable a high contrast and a high resolution observation for unstained ice-embedded samples. To enhance the image contrast, two methodologies have already been developed; i) scattering contrast for stained samples with small aperture diaphragms and ii) defocus contrast for unstained or stained samples with deep defocusing. The former prevails in histochemical sciences and the latter is popular in electron crystallography. Both methods, however, have a common drawback that the contrast is only improved by impairing the image quality. This drawback can be removed with use of the phase contrast method using phase plates, which has traditionally been used in visible light microscopy. Due to the severe obstacle of the charging of phase plates, however, the idea has not yet been materialized. We have solved the phase-plate charging problem. An experiment 300kV with TEM for a whole cell from cyanobacterium unstained and ice-embedded fulfilled the expectation. Only weak and vague contrast was obtained for the conventional image of the cell even with a very deep defocus. Contrarily a high-contrast image has appeared for phase contrast images, where various fine structures are clearly recognized. This may be a first example to observe nanometer scale structures in details in the intact cell. Other examples including intact state intravesicular structures will be shown.
O-161

Refractive effects in Coherent Anti-stokes Raman Scattering (CARS) microscopy
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Coherent anti-Stokes Raman scattering microscopy (CARS) is a new approach for chemical imaging of molecular systems within cells and tissues, with high sensitivity, high spatial resolution, and three dimensional sectioning capabilities, without using fluorophores that are prone to photobleaching. This technique permits to map selectively molecular species, by using vibrational properties of their chemical bounds. The Epi detected (E-CARS) and forward detected (F-CARS) intensities depends on the shape, the size of the sample, as well as the index of the solvent. In this presentation, after introducing the CARS microscopy technique, we show the first CARS studies of the refractive effect of the sample, comparing the E-CARS and F-CARS signals for different diameters of polystyrene beads, in different refractive index solvents. We present several simulations, comparing forward-detected and backward-detected signals in different sized polystyrene beads, embedded in different index solvents, and we show that, the backward-reflected F-CARS dominates the experimentally epi-detected signals. Furthermore, we demonstrate experimentally and theoretically that the maxima of forward and epi-detected signals are generated at different positions along the Z axis in the sample. We finally discuss how index mismatch in cells can alter CARS images.

O-162

Evaluation of tumor response of breast cancer patients by diffusion weighted MRI
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Diffusion weighted MR imaging (DWI) measures the diffusion of water molecules in tissues and is quantified by apparent diffusion coefficient (ADC). DWI can be used to differentiate tumors from normal tissue and also can be used to monitor the response of tumor to chemotherapy.

Thirteen healthy volunteers and twelve patients were recruited for the study. DW images were obtained prior to therapy (n=10) and after three cycles of therapy (n=3). The mean ADC value of tumors (0.83 x 10^{-3} ± 0.05 mm²/s) was significantly less (p < 0.05) compared to the normal tissue (1.80 x 10^{-3} ± 0.2 mm²/s). Decrease in ADC in tumor is due to an increase in the cellularity which restricts the diffusion of water molecules. In patients receiving neo-adjuvant chemotherapy, the ADC values were higher (1.36 x 10^{-3} ± 0.86 mm²/s) and were closer to that of the normal tissue (p <0.05), indicating response of the tumor to chemotherapy.

The post-therapy increase in ADC is due to the cell damage caused by the therapeutic agents which increases the fractional volume of the interstitial space, causing an increase in the mobility of water. The study showed that DWI can be used non-invasively to assess the response of breast cancer patients to neo-adjuvant chemotherapy.
O-163
The case for case studies in biophysics education
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Best practices in education increasingly involve modes of instruction that go beyond the traditional “sage on the stage” model of formal lecture to include more student-centered and inquiry-based methods. Although students in higher education are usually pre-selected for success in the traditional lecturing/listening mode of instruction, students of biophysics would nonetheless be well served by incorporating some of these best practices into the biophysics classroom.

Case studies provide an opportunity to incorporate inquiry-based methods into biophysics education at both the undergraduate and graduate levels. Case studies, long used in graduate schools of business, present technical or scientific problems embedded within a specific human context, often illustrated by a simple story line involving identifiable characters. The story line, which can be historical or purely fictitious, forms the starting point for independent investigation or problem solving by the student either individually or as part of a group. The solution, which may build from independent inquiry, literature research, and group discussion among other modes of investigation, builds on and goes beyond material presented in class or in readings. The interaction of student inquiry with classroom content encourages higher level thinking while also teaching the process as well as the product of science.

The use of case studies will be discussed in detail and illustrated with a specific case study appropriate for an introductory biophysics class.

O-165
e-learning biophysics to biochemists
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A huge amount of web-sites has been dedicated to Biophysics and associated sciences, like biophysical chemistry, structural biology, microscopy ... Most if not all aspects of biophysics are freely accessible on WWW. But the accumulation of encyclopedic knowledge is not sufficient to constitute a learning activity.

During the last ten years, new concepts have appeared describing and modelizing various learning interactions, and most learning management systems (LMS) are at least partially compliant for actual standards. LMS also currently help tracking and scoring student activities. Many commercial or free authoring tools are able to help learning designers to illustrate scientific knowledge with animation, video, simulation, and 3D presentation. To day, the technical difficulties are on the point of being fixed. The biophysica community could decide to build and manage a large network of scientific content repositories sharing their resources and giving any teacher in the world the opportunity to choose elements for his course among all individual contributions.

In opposition to some current fears, teachers continue to have a key role in e-learning. He must select pertinent resources, enlighten what is important at a given time and for a given student level. The evaluation questions have to be adapted to the academic and professional assessments. These points will be discussed about e-learning biophysics to biochemists.

O-164
The challenges of biophysics education
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The teaching of Biophysics poses different problems ranging from the general strategies to be used to the definition of the subject itself. Furthermore, in promoting Biophysics in developing countries, one additionally faces the lack of resources, both human and material. It is often considered that sending students to learn and work in developed countries and organizing visits of qualified scientist and teachers to developing countries will soon improve the education level and promote the activity in those places. Although the later stimulate some young student to enter a Biophysics career, the former may produce, as it often happens, a brain drain on the region.

In the paper we present an approach mainly based on horizontal cooperation within a given region complemented with the support of developed countries in the above mentioned activities. Moreover, we propose Regional Post-graduate Programs in Biophysics using mainly local resources combined with student exchange within and outside the region oriented to activities that can be done in their home countries. This strategy may contribute to improve the level of Biophysics in the different regions and retain the young students that will act synergistically to close the gap between developed and developing countries.

O-166
Education and capacity building in biophysics for developing countries
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As a result of the meeting initiated by the IUPAB Task Force on Education and Capacity Building in Asian and African countries held on 21-25 February 2003 in Roorkee, India, several proposals were made to promote the Biophysics education for students in less developed countries in Africa, Latin America, Eastern Europe and Asia. A major proposal is to establish a system of international education leading to MS and Ph.D. degrees, which aims to give the prospective students the possibility to study courses in Biophysics regardless of their geographical occupation and financial situation through internet and workshops, followed by the choice of Ph.D. programs at the host counties. While this effort is being continued, several other proposals are taking action. For example, an international workshop was held successfully for young scientists in developing Asian countries in Osaka in January 2004, organized by an international initiative and collaboration of Japanese scientists in the field of biological NMR, financially supported by Japan Society for the Promotion of Science. Various efforts are being made in Japan in capacity building in biophysics at the level of graduate and postdoctoral education for students from Asian, East European and African countries including China, Korea, India, Nepal, Mongolia, Philippines, Sudan and Poland.
O-167
High-throughput structural biology and drug discovery: opportunities and challenges
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Knowledge of 3-D structures of protein targets now emerging from genomic data has the potential to accelerate greatly drug discovery, but technical challenges and time constraints have traditionally limited their use to lead optimization. Their application is now extended into new approaches for lead discovery (Blundell et al. (2002) Nature 407, 1029-1034). However, recent analyses have identified a large number in which one component involves a flexible or unstructured region of the polypeptide chain (Pellegrini et al. (2002) Nature 420, 287-293). We suggest that such proteins forming interactions with a ligand that comprises a continuous region of flexible peptide may be more druggable targets.

O-169
Dynamics- and structure-based drug design targeted at the hepatitis C virus
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Interactions between a biological macromolecule and a small molecule ligand involve intricate recognition events which are strongly influenced by both structure and dynamics factors. Given that the ligand and receptor must adapt to each other to form a strong complex, detailed knowledge of these factors would certainly aid drug design efforts. This presentation will describe two examples of rational design efforts, which employ novel dynamics- and structure-based design strategies, to target the essential enzymes of the hepatitis C virus (HCV), NS3 protease and NS5B polymerase. The strategies include (1) the facile detection of binding of early leads to the NS3 and NS5B enzymes, (2) the identification of important ligand substituents that directly contact the receptor pockets, and (3) the determination of free and protein-bound structure and dynamics features of these compounds. Moreover, an emphasis will be made on the rational use of such data for medicinal chemistry purposes. Central to this is an on-going effort to qualitatively elucidate, and exploit, the binding modes/roles of the substrates of ligands using a combination of data from NMR spectroscopy, structure-activity relationships, computational chemistry, and X-ray crystallography.

O-168
Cell imaging by secondary ion mass spectrometry (SIMS): basic principles and biological applications
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Ion microscopy offers remarkable possibilities because it can apply with great sensitivity to the totality of the elements (and their isotopes). This technique is based on the mass spectrometry of secondary ions (SIMS) extracted from the surface of a solid sample under the impact of a beam of primary ions. This secondary beam is guided to the entrance of a double focusing magnetic sector. Secondary ions are sorted in energy in the electrostatic sector before undergoing specific deviation by the magnetic field according to their m/z ratio while keeping the topological information of their origin. Numerous applications have emerged in such diverse fields as surface analysis, materials science, geochemistry and electronics. However, in spite of application in biology, over more than 30 years ago, ion microscopy has been for a long time considered only as a marginal method in the life sciences, due mainly to poor lateral and mass resolution. Many technological and conceptual improvements led to significant progress in both lateral resolving power and mass resolution. The last high-resolution dynamic SIMS equipment will be briefly described. It has a lateral resolution of less than 50nm with primary Cs+ ion, the ability to detect simultaneously 5 different ions from the same micro volume and a very good transmission even at high mass resolution. The decisive capability of high resolution dynamic SIMS imaging in biology will be illustrated with the most recent examples of utilization.

O-170
Understanding the maturation process and inhibitor design of SARS-CoV 3CLpro
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Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel human coronavirus. The viral maturation requires a main protease (3CLpro) to cleave the virus-encoded polyproteins. We report here that the 3CLpro containing N- and/or C-terminal additional in-frame sequences underwent autoactivation to cleave the tags and yielded the mature protease in vitro. The 3-D structure of the C145A mutant protease shows that the active site of one protease of the dimeric protease is bound with the C-terminal six amino acids of the protease in another asymmetric unit, suggesting a possible mechanism for maturation. The tagged C145A mutant protein served as a substrate for the wild-type protease and the N-terminus was first digested (55-fold faster) followed by the C-terminal cleavage as shown by the SDS-PAGE analysis. The analysis of the quaternary structures for the tagged and mature proteases by analytical ultracentrifuge experiments reveals the remarkably tighter dimer formation for the mature enzyme than for the mutant (C145A) containing the N-terminal or the C-terminal 10 extra amino acids. Taken together, the study here provides insights to the design of our new structure-based inhibitors.
O-171
Interplay between polymerized liposomes physicochemical properties and lipid composition and citotoxicity
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This study was aimed at investigating whether there is an interplay between diacetylenic polymerized liposomes physicochemical properties and lipid composition affecting citotoxicity in vitro. Unsaturated 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine with saturated 1,2-dimiristoyl-sn-glycero-3-phosphocholine in molar ratio 1:1, were combined to give a chemically modified membrane by UV-polymerization. Biophysical characterization was carried out determining the hydrophobic factor and hydrodynamic radius. Cytotoxicity was evaluated through haemolytic capacity on bovine red blood cells and indirectly by capacity of induction of lipid peroxidation on microsomes or mitochondrial membranes. The haemolysis percentage in presence of DC8,9PC/DMPC is less than that induce by polymers used in dentistry. The data obtain suggests that the polymerized lipids can not induce lipid peroxidation on natural membranes. The polymerized diacetylenic liposomes showed less interaction with serum proteins than non polymerized and lower citotoxicity as compared with natural lipids. Also cell viability was determined in cell line NIH3T3 after exposure to lipids systems under study.

The hydrophobic factor showed further augmentation for polymerized liposomes and is discussed in relation to in vitro stability. The above results suggest that polymerized and non-polymerized liposomes would serve as an effective delivery vehicle.

O-172
Encapsulation of clone vector DNA by cationic diblock copolymer vesicles for gene delivery
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We will discuss the design, control, and structural characterization of cationic copolymer vesicles loaded with DNA. These vesicles serve as a model system for diverse applications such as gene delivery, micro-arraying techniques and packaging of DNA in congested states. Encapsulation of DNA was achieved with a single emulsion technique. For this purpose, an aqueous pUC18 or pEGFP-N1 plasmid solution is emulsified in an organic solvent and stabilized by an amphiphilic diblock copolymer. The neutral block of the copolymer forms an interfacial brush, whereas the cationic block complexes with DNA. A subsequent change of the quality of the organic solvent results in a collapse of the brush and the formation of a capsule. The capsules are subsequently dispersed in aqueous medium to form vesicles and stabilized with an osmotic agent in the external phase. Inside the vesicles, the DNA is compacted in a liquid-crystalline fashion as shown by the appearance of birefringent textures under crossed polarisers and the increase in fluorescence of labeled DNA. The compaction efficiency and the size distribution of the vesicles were determined by light and electron microscopy, respectively, and the integrity of the DNA after encapsulation and subsequent release was confirmed by gel electrophoresis. We demonstrate the gene transfer ability of this new carrier system by the transfection of encapsulated pEGFP plasmid into HeLa cancer cells.

O-173
Docking-molecular dynamics studies on the peroxidase site of prostaglandin endoperoxide H2 synthase
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Prostaglandin endoperoxide H2 synthases-1 and 2 (PGHS-1 and 2) catalyze the first step in the biosynthesis of prostaglandins, prostacyclins and thromboxanes. Arachidonic acid is transformed into prostaglandin G2 (PGG2) at the cyclooxygenase site of the enzyme and the 15-hydroperoxide oxygen-oxygen bond of PGG2 is subsequently cleaved by reaction with haem at the distinct peroxidase site (POX) to produce prostaglandin H2 (PGH2). Herein we present a plausible productive conformation obtained by docking calculations for the binding of PGG2 to the POX site of PGHS-1. The enzyme-substrate complex stability was verified by a 500-ps molecular dynamics simulation. Structural analysis unveils the requirements for enzyme-substrate recognition and binding: The PGG2 15-hydroperoxide group is in the proximity of the haem iron and participates in a hydrogen bond network with the invariant His207 and Gln203 and a water molecule, whereas the carboxylate group establishes salt bridges with the remote lysines 215 and 222.
O-174
Mechanisms of Self-assembly and switching of the bacterial flagellum
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The bacterial flagellum is made of a rotary motor and a long helical filament by means of which bacteria swim. The flagellar motor rotates at around 300 Hz and drives the rapid rotation of each flagellum to propel cell movements. The long helical filament, a tubular structure with a diameter of about 20 nm, is made of a single protein flagellin. The filament switches between left- and right-handed helical forms in response to the twisting force produced by reversal of the motor rotation, allowing bacteria to alternate their swimming pattern between “run” and “tumble” for taxis. A short, highly curved segment called hook connects the motor and the helical propeller, and its bending flexibility makes it work as a universal joint. The flagellum is constructed by self-assembly of component proteins translocated from the cytoplasm through its narrow central channel to the distal end, where different cap complexes help efficient self-assembly of proteins. The protein export is driven by the flagellar type III protein export system. We have solved some parts of these structures by X-ray crystallography, fiber diffraction and electron cryomicroscopy. All these structures present interesting implications for the function of the flagellum, demonstrating the importance of dual nature of protein molecules, flexibility and precision.

O-175
Dynamic polymorphism of actin subunits in the filament
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Movement is fundamental for cell function. Actin plays a key role there but its function in the filamentous form is not well understood. We used single-molecule FRET to directly observe dynamic behaviors of individual subunits within actin filaments. The results showed that the actin subunit in a filament existed in two major conformational states at slow equilibrium and the equilibrium was shifted to one state during interaction with myosin in the presence of ATP and another during inactivated by chemical crosslink. Thus, the actin filament allosterically switches between the active and inactive conformational states. Furthermore, the results suggest that the actin shows hysteretic or memory effect. Such two-state allosteric behavior and memory effect of actin should be important for the dynamic and flexible regulation of the actin-mediated motility. Thus, actin plays a positive role in cell motility.

O-176
Control of actin assembly in cell motility
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Living cells change shape and move in response to environmental signals. These motile processes play a pivotal role in morphogenesis, migration of embryonic and metastatic cells, angiogenesis, synaptogenesis, immune response and interaction of the host cells with pathogens. They are generated by polarized, spatially directed actin assembly in filaments organized in specific structures. It is the treadmilling (dissipative turnover) of actin filaments, regulated by specific proteins, which is responsible for force and directional movement. Two protein machineries are responsible for spatially directed initiation of actin filaments. They operate with different mechanisms and in distinct processes, 1) the WASP-Arp2/3 system is at the origin of the formation of a branched filament array responsible for protrusion of lamellipodium ; 2) formins, in association with profilin, catalyze the rapid processive assembly of non-branched actin filaments arranged in parallel bundles, in the cytokinetic ring and in adhesive structures. We have combined a biochemical and a biomimetic approach to understand the molecular mechanisms of these auto-organized processes. We have reconstituted the sustained actin-based movement of a N-WASP- or formin-functionalized particle in a biochemically controlled medium, which enables measurements of force production in correlation with structure and motility, and we can derive informations on the molecular mechanism of movement by single molecule measurements.

O-177
Optical trap with fast programmable feedback loop to study rotary molecular motors
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An optical trap with back-focal plane detection and fast programmable feedback has been developed for the study of rotary molecular motors. A helium-neon laser (632 nm) is used for position detection and a solid state fibre laser (1064 nm, 3W CW) forms the trap. Acousto-optic deflectors (AODs) controlled by a digital signalling processing board are used to activate programmable feedback loops with flexible control options and speeds up to 8 kHz. Several modes of feedback are demonstrated, controlling both bead position (x,y) and angle (r, θ). Polystyrene beads or bead pairs can be held at set (x,y) or θ, and the set-point can be changed while the program is running. For example, feedback can be used to move a bead or a bead pair in a circle. Results of using the system to study the bacterial flagellar motor are presented.
**O-178**

Cooperative extraction of membrane nanotubes by molecular motors

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In eukaryotic cells, nanotubes represent a substantial fraction of transport intermediates between organelles. They are extracted from membranes by molecular motors walking along microtubules. We previously showed that kinesins fixed on giant unilamellar vesicles in contact with microtubules are sufficient to form nanotubes in vitro. Motors were attached to the membrane through beads, thus facilitating cooperative effects. Koster et al. [Koster et al. (2003) PNAS USA] proposed that motors could dynamically cluster at the tip of tubes when they are individually attached to the membrane. We demonstrate, in a recently designed experimental system, the existence of an accumulation of motors allowing tube extraction. We determine the motor density along a tube by using fluorescence intensity measurements. We also perform a theoretical analysis describing the dynamics of motors and tube growth. The only adjustable parameter is the motor binding rate onto microtubules, which we measure to be 4.7 +/- 2.4 s⁻¹. In addition, we quantitatively determine, for a given membrane tension, the existence of a threshold in motor density on the vesicle above which nanotubes can be formed. We find that the number of motors pulling a tube can range from four at threshold to a few tens away from it. The threshold in motor density (or in membrane tension at constant motor density) could be important for the understanding of membrane traffic regulation in cells.

**O-179**

Single kinesin motor proteins walking through the searchlight

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The dimeric motor protein kinesin steps by a hand-over-hand mechanism. This means that the centre of mass moves with 8 nm steps, while the two motor domains, the one after the other, move 16 nm to the next binding site on the microtubule. The molecular details of what happens during a step are not fully understood, partly because of lack of time resolution in wide-field, single-molecule fluorescence experiments. We set out to develop an approach to study the motility of kinesin with a time resolution below a millisecond (a single step takes on the order of 10 milliseconds). This approach allows us to look into the mechanochemistry and coupling of the two kinesin motor domains while they are stepping. Our method is based on confocal microscopy and we study the fluorescent properties of single labeled motors while they walk through the confocal laser spot. We present the experimental details of our approach and show our results on human kinesin constructs that are specifically labeled in the tail. We show that our approach enables us to study the mechanism of kinesin with a much higher time resolution than what was achieved before with single-molecule fluorescence experiments.

**O-180**

Fluorescence Imaging with One Nanometer Accuracy (FIONA): Application to molecular motors

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We have achieved 1.5 nm resolution using fluorescence imaging, approximately 300 times better than the diffraction limit of conventional light. Recently we have been able to increase the time resolution to 1 msec, from a previous value of 500 msec. Using this increased time-resolution, we have looked at molecular motors inside living cells. We have been able to see individual cargos being moved by individual kinesin and dynein, two important motors. We find that both kinesin and dynein move cargo 8 nm per ATP (the universal food of the cell), in opposite directions in a cell. Amazingly, these two molecular motors do not engage in a tug-of-war, but appear to be cooperative, giving the particle extra speed.
**Oral Presentations**

– The RNA World –

**O-181**

X-ray Crystal Structures of the E. coli 70S ribosome at 3.5 Å resolution

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We are using x-ray crystallography to probe the structural basis for the many aspects of protein biosynthesis that require the intact ribosome. Our goal is to make an atomic-resolution “movie” of a ribosome in the process of making a protein. We have obtained crystals of the entire E. coli ribosome that diffract x-rays to a resolution of 3.1-3.2 Å. Thus, we now have the means to determine the first atomic-resolution structure of the intact ribosome, the first frame of the movie. Moreover, we are using these crystals to probe in atomic detail the effects of antibiotics on the full ribosome and mutations in the ribosome that lead to antibiotic resistance or perturb key steps in translation. We are now refining ribosome structural models at a resolution of 3.5 Å, the results of which will be presented.

**O-182**

Substrate selection and catalysis by the ribosome

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Protein synthesis in the cell is performed on ribosomes, large ribonucleoprotein particles that in bacteria consist of three RNA molecules and over 50 proteins. Ribosomal protein synthesis entails the rapid polymerization of amino acids from aminoacyl-tRNAs which are delivered to the ribosome in a complex with elongation factor Tu (EF-Tu) and GTP. The ribosome recognizes aminoacyl-tRNA through shape discrimination of the codon-anticodon duplex and regulates the rates of GTP hydrolysis by EF-Tu and aa-tRNA accommodation in the A site by an induced fit mechanism. Peptide bond formation is the main enzymatic activity of the ribosome. The catalytic site of the ribosome consists of RNA, indicating that the ribosome is a ribozyme. Recent results of biochemical, biophysical, and structural studies, including kinetic measurements, ribosome crystallography, single molecule FRET measurements, and cryo-electron microscopy, suggest the mechanism of ribosome function in substrate selection and catalysis of peptide bond formation.

**O-183**

Hijacking the ribosome: translation initiation by a hepatitis C viral RNA

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RNA molecules have been discovered at the heart of several aspects of gene expression, from protein biosynthesis by the ribosome to viral translation initiation to the targeting of new proteins to the correct intracellular locale. Understanding how these RNA-mediated processes work will illuminate central aspects of modern cell biology and also provide important clues to the possibly fundamental role of RNA in the evolution of life. I will describe our research over the past ten years to discover the structural basis for RNA function, highlighting recent discoveries about hepatitis C viral RNA hijacking of human ribosomes.

**O-184**

Modified nucleosides and across the anticodon loop interactions in tRNA

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In several interesting tRNA molecules, the (34\textsuperscript{th}) and (37\textsuperscript{th}) nucleosides are hyper modified. As an example, unique hypermodified nucleosides mcm\textsuperscript{4}c\textsuperscript{2}U\textsubscript{34} and ms\textsuperscript{2}A\textsubscript{37} are crucial in human tRNA\textsubscript{A\textsubscript{183}}, which acts as a primer in HIV replication. Modified nucleosides may facilitate or hinder across the loop interactions. Large substituents in 34\textsuperscript{th} and 37\textsuperscript{th} modified nucleosides if oriented suitably may also interact with each other. Across the loop interactions may lead to unconventional anticodon loop structures also affecting flexibility of the anticodon loop. This may restrict or enlarge synonymous codon choice and decoding during protein biosynthesis. Except for tRNA\textsubscript{Asn} (with interacting Q\textsubscript{34} and P\textsubscript{A}\textsubscript{37}), our studies show conventional ‘open’ loop structure - free of across the loop interactions, for a number of interesting tRNA anticodon loops with diverse hyper modified nucleosides at both of these locations. Molecular dynamics simulations of hydrated anticodon arm of tRNA\textsubscript{Asn} show persisting interaction involving the diol group of Q\textsubscript{34} and carbonyl group of ureido linkage in P\textsubscript{A}\textsubscript{37}. Additionally, the Hoogsteen edge of 37\textsuperscript{th} adenine base participates in hydrogen bonding with Watson-Crick edge of 34\textsuperscript{th} base and thus contributes to unique loop structure of tRNA\textsubscript{Asn}. Resulting suboptimal Q-C base pairing leads to unbiased reading of U or C as the third codon letter. Absence of queuosine modification, Q\textsubscript{34} happens to be also associated with uncontrolled rapid proliferation of cells and malignant growth.
O-185

Modeling the long range entropy of RNA:
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Non-coding RNA appears to make up a large part of the human genome. A reliable RNA structure prediction program is needed to understand the structure of this non-coding RNA.

We recently developed a new way to model the long range entropy in RNA and applied it to RNA secondary structure prediction. In some of instances, the new approach is able to achieve far better predictions than the state of the art secondary structure programs even given exactly the same parameters. Predictions using this method tend to show distributions that are funnel shaped. A new and important parameter in these calculations is the persistence length (a measure of the correlation and flexibility of the RNA).

This new approach has now been extended to prediction of pseudoknots. The method is a heuristic wherein the hierarchical folding hypothesis is used to find the pseudoknots as the RNA secondary structure is folding, and corrections to that secondary structure are made to accommodate the pseudoknot. It is able to do these searches in roughly N^4 time. The model is consistent with the hierarchical hypothesis and it is possible to estimate RNA folding times that are of the correct order of magnitude using this model.

With further adaptations to account for the size, shape and variability of amino acid residues (hydrophobicity etc.), the model also appears to be transferable to protein folding problems.

O-186

HIV-1 NC-facilitated TAR RNA/DNA annealing is initiated through a loop-loop kissing interaction
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Annaling of the TAR DNA hairpin to a complementary TAR RNA hairpin, resulting in the formation of an extended duplex, is an essential step in the minus-strand transfer process of HIV-1 reverse transcription. In this work, we use gel-mobility-shift analysis to follow the kinetics of this reaction in the absence or presence of HIV-1 NC prepared by solid-phase peptide synthesis. To elucidate the reaction pathway, we use either the complete 59-nt TAR hairpins or truncated 27- to 32-nt minihelices (mini-TAR) derived from the top part (i.e. hairpin loop) of TAR. Assays were also carried out with mutant TAR constructs. The annealing kinetics were studied systematically as a function of DNA concentration and temperature.

We show that the annealing initiates through a weak loop-loop kissing interaction, followed by a much slower conversion step, which results in formation of the extended duplex. NC facilitates both reaction steps, resulting in the overall 10^4-fold and 10^6-fold rate enhancement for mini-TAR and TAR annealing, respectively. We show that the kissing step is facilitated by the NC-induced nucleic acid aggregation, which is more pronounced for the longer TAR hairpins. At the same time, the conversion steps in TAR and mini-TAR appear to be very similar and are similarly facilitated by NC 10-100-fold. The later effect relays on the ability of NC to destabilize nucleic acid duplexes, and is equivalent to destabilization of a few base pairs required for the conversion initiation.

O-187

The catalytic mechanism of the hairpin ribozyme
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The hairpin ribozyme catalyses phospho-transesterification reactions. The RNA folds by loop-loop interaction to create the local environment in which catalysis can proceed. Using single-molecule FRET experiments we can observe individual hairpin ribozyme molecules as they undergo multiple cycles of cleavage and ligation, and measure the rates of the internal reactions, free of uncertainties in the contributions of docking and substrate dissociation processes. On average, the cleaved ribozyme undergoes several docking-undocking events before a ligation reaction occurs. On the basis of these experiments, we have explored the role of the nucleobases G8 and A38 in the catalysis. Both cleavage and ligation reactions are pH dependent, corresponding to the titration of a group with pK_a = 6.2. We have used a novel ribonucleoside in which these bases are replaced by imidazole to investigate the role of acid-base catalysis in these ribozymes, observing significant rates of cleavage and ligation.

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