The dimeric ectodomain of the alkali-sensing insulin receptor–related receptor (ectoIRR) has a drop-like shape

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ABSTRACT

Insulin receptor–related receptor (IRR) is a receptor tyrosine kinase of the insulin receptor family and functions as an extracellular alkali sensor that controls metabolic alkalosis in the regulation of the acid–base balance. In the present work, we sought to analyze structural features of IRR by comparing them with those of the insulin receptor, which is its closest homolog, but does not respond to pH changes. Using small-angle X-ray scattering (SAXS) and atomic force microscopy (AFM), we investigated the overall conformation of the recombinant soluble IRR ectodomain (ectoIRR) at neutral and alkaline pH. In contrast to the well-known inverted U-shaped (or lambda-shape) conformation of the insulin receptor, the structural models reconstructed at different pH values revealed that the ectoIRR organization has a “drop-like” shape with a shorter distance between the fibronectin domains of the disulfide-linked dimer subunits within ectoIRR. We detected no large-scale pH-dependent conformational changes of ectoIRR in both SAXS and AFM experiments, an observation that agreed well with previous biochemical and functional analyses of IRR. Our findings indicate that ectoIRR’s sensing of alkaline conditions involves additional molecular mechanisms, for example, engagement of receptor juxtamembrane regions or the surrounding lipid environment.

Insulin receptor-related receptor (IRR), a receptor tyrosine kinase (RTK) of the insulin receptor family, attracts the attention of scientists due to its unusual properties. IRR gene was originally found in cloning experiments with the DNA of the insulin receptor (IR) as a probe (1). However, since this discovery in 1989, no endogenous ligands for IRR have been identified (2) and a physiological role of the IRR has remained uncertain for a long time. Recently, it has been determined that IRR can be activated merely by an increase in the extracellular pH and postulated that IRR is a regulator of the acid–base balance (3).

The activation of IRR by alkaline media has typical features of the ligand-receptor interaction (2,4,5). Since IR and insulin-like growth factor receptor (IGF-IR) cannot be activated by either alkali or acid, the IRR activation is quite specific. Upon alkali treatment, IRR shows a dose-dependent response with positive cooperativity that is quickly reversible when the media pH is neutralized. The IRR activation triggers intracellular signaling that starts with IRR autophosphorylation followed by
phosphorylation of intracellular signaling adapters IRS-1 and AKT-1. The IRR activation leads to intense blebbing and actin stress fiber rearrangement in transfected CHO cells. Actin cytoskeleton rearrangement was also observed in beta pancreatic MIN6 cells, where IRR is expressed endogenously (4,6,7). Finally, analyses of several chimeras of IR and IRR revealed the key importance of the IRR extracellular region in its pH sensitivity (5,6).

The IRR tissue expression profile distinguishes it from its relatives of the IR minifamily (3). Whereas IR and IGF-IR are essentially ubiquitous, detectable concentrations of IRR were only found in some specialized cells of kidney, pancreatic, stomach, ovary and nervous tissues. The physiological importance of IRR was also addressed by gene targeting. Unexpectedly, IRR knockout mice appeared to be quite healthy under normal conditions whereas IR and IGF-IR knockouts were not viable (8). Yet upon experimental alkali challenge, the IRR knockout mice showed defects in their renal function and behavioral abnormalities (9,10).

Despite the obvious functional differences between the IR family members, their structural features are similar. Unlike other RTKs that dimerize upon their ligand binding (a key step in their activation), IRR, IR and IGF-IR are expressed as disulfide-linked dimers of two subunits, αβ half-receptors, which derive from proteolytic cleavage but stay complexed due to covalent disulfide bonds. Thus, one can assume that transmembrane signaling by IRR and its homologs is not based on ligand-induced dimerization per se, but should involve allosteric structural changes within the existing receptor dimer (4). This kind of structural transition can occur at both global and local levels. In the extracellular part, the latter can possibly be limited by a local rotation of the domains due to flexible linkers between them (11).

The amino acid sequences of IRR, IR and IGF-IR are highly homologous with about 50% identity and 65% similarity. They also have identical domain structures. Their large N-terminal extracellular regions contain two leucine-rich repeat domains, named L1 and L2, joined by the cysteine-rich C-domain, and three C-terminal fibronectin type III repeats (FnIII-1, FnIII-2 and FnIII-3). The one transmembrane hydrophobic segment is followed by the C-terminal intracellular catalytic tyrosine kinase domain (12).

No structural data are available for IRR whereas its homologs IR and IGF-IR have been thoroughly studied by X-ray crystallography, cryo-EM and SAXS (11,13-19). The analysis of the IR ectodomain co-crystallized with antibodies revealed its symmetrical lambda-shape structure with a head-to-tail complex of monomers (13). Major conformational changes of IR in the absence and presence of insulin were detected (20). It was shown that insulin binding to the dimeric receptor converts its ectodomain from the lambda-shape conformation to the T-shaped one (20). Single particles cryo-EM investigations of the IR ectodomain-insulin complex described the structure of the complex with higher resolution (4-7 Å) revealed conformational changes upon ligand binding and presented a number of conformations, the shape of which differed from those shown previously (11,20). As of now, the mechanism of IRR homologs activation remains somewhat contradictory.

Although IR family members have highly related structures, there must exist fundamental differences that underlie their ligand specificities, in particular, the pH sensitivity of IRR. The quantitative analysis of IRR/IR chimeras activation by alkali showed the key importance of the IRR ectodomain in IRR with the primary role of L1C, FnIII-2 and FnIII-3 domains (6,21). As no structural information for IRR was available, the results of these studies were interpreted on the basis of the structure of the IR ectodomain obtained by crystallography (18).

This study is devoted to the structural analysis of the IRR ectodomain by two complementary methods of structural biology: small-angle X-ray scattering and atomic force microscopy (AFM). SAXS is a particularly useful tool for investigating the solution states of proteins that includes modeling the quaternary structure of multidomain proteins (22-27). It was also used to analyze structural changes of IR and IGF-IR ectodomains induced by ligand binding with the conclusion that structural movements within ectodomain upon ligand binding are small and limited to local rotation of domains (11). Another structural metod, AFM, can provide direct real space information about the size and shape of particles and electrochemical properties of a macromolecule based on its binding with surfaces. Complementarity between SAXS and AFM allows to cross-validate models thereby increasing the reliability and confidence in the results (28,29). On the basis of SAXS and AFM analyses of the IRR ectodomain we propose its asymmetrical drop-like model.

**Results**
Analysis of scattering curves

To obtain structural information, samples of recombinant ectoIRR were analyzed by SAXS. Since IRR is an alkali sensor the measurements were made in parallel at pH 7.0 and pH 9.0. The processed experimental scattering patterns from ectoIRR are displayed in Figure 1. Concentration dependent effects on the measured scattering intensities were not observed for samples spanning 0.5 to 3 mg/ml. Figure 1 shows scattering curves from 3 mg/ml protein solution.

The scattering profiles of ectoIRR, measured at pH 7.0 and 9.0 do not show dramatic differences and almost coincide when the data are scaled relative to each other (Figure 1a, curves 1 & 4, and Figure SI 2). Their similarity is emphasized by the superposition of the bell-shaped Kratky plots, which indicates that the protein is mostly folded (30), and by the corresponding p(r) distance distribution functions (Figure 1, inset), which show little difference in the distribution of internal vector lengths or the respective D_{max} of either sample (ca. 19 nm; Figure 1c). The slope of the Guinier plots is also similar (Figure 1b), yielding very close R_g of ectoIRR at pH 7.0 (R_g = 5.30 nm) and pH 9.0 (R_g = 5.25 nm). The experimental MM estimates obtained from the data are consistent with the MM calculated from the electrophoresis of ectoIRR (Figure SI 3) and indicate that ectoIRR forms a dimer in solution. A summary of the experimental structural parameters (R_g, D_{max}, V_p, MM_{obs}, MM_{Porod}, and MM_{Bayesian}) extracted from the SAXS data are reported in Table 1.

As revealed by the SAXS data analysis, the integral structural characteristics of the protein in solution remain the same despite changes in pH. These results are in a some degree similar to the data obtained in the work of Whitten, et al. (11). The authors suggest that structural changes of the insulin receptor (IR) and the homologous Type 1 insulin-like growth factor receptor (IGF-1R) induced by ligand binding are small and possibly limited to local rotation of domains.

Typical ab initio shape reconstructions of the ectoIRR dimer at both pHs are presented in Figure 1, d & e. At the given resolution, the ectodomain shape does not change significantly with a pH increase (Figure SI 2, b); the normalized spatial discrepancy NSD in this case is 0.50. The second-order symmetry was not used for DAMMIN shape reconstruction to obtain a model independent of artificially introduced symmetry, since the use of any restrictions dramatically narrows the area of resulting models. Moreover, due to flexibility of the links between domains the protein can achieve slightly different coexisting conformations in solution. Thus, ab initio reconstruction without symmetry more adequately reflects an average structure of the sample. Nevertheless, since the macromolecule of the protein is a dimer consisting of two identical monomers, P2 symmetry was also used for the shape reconstruction. However, a comparison of the obtained low-resolution models demonstrates their overall similarity. On the other hand, reconstructions with P2 symmetry yield systematically worse fits to the experimental data through multiple runs of the program, while scattering patterns computed from the ab initio models obtained by DAMMIN without symmetry yield good fits to the experimental data: $\chi^2 = 1.3$ for pH 7.0 and 1.4 for pH 9.0 (Figure 1 a).

Therefore, on this stage of the SAXS data analysis one can conclude that the overall low-resolution shape of the ectoIRR does not change at the alkalization. Given the availability of flexible links between protein domains, it should be assumed that pH changing leads to some local changes that occur at the level of rotation of the individual domains. This assumption is supported by small but detectable differences in the structural characteristics of the protein (Figure 1 & Table 1). In order to obtain more detailed structural organization of the ectoIRR in solution, hybrid modeling was performed using CORAL. The available high-resolution X-ray crystal structure of the insulin receptor ectodomain (derived from the PDB entry: 4ZXB) as the closest IRR homolog was split into individual subdomains. The splitting was done between the head and tail domains such that the first subdomain contained 1–465 amino acids and the second contained amino acids 472–910. Amino acids 466–471 constitutes the flexible linker, as this loop contains only several contacts with the rest of the monomer structure (Figure 2). Usage of only two intact domains per polypeptide chain was chosen in order to keep the number of free parameters low with a reasonable number of degrees of freedom to avoid overfitting.

First, the modeling has been performed for pH 9.0. The resulting model yields good fit to the experimental data with $\chi^2 = 1.27$ (Figure 3 a). Given the similarity of the SAXS experimental data at pH 7.0 and pH 9.0, further CORAL modeling (against pH 7.0 data) was performed starting from the rigid body model obtained for pH 9.0. This refinement also yielded a good fit with $\chi^2 = 1.30$ (Figure 3 b). Structural comparison of these two CORAL models performed by the program SUPCOMB ones again demonstrated
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their overall similarity with normalized spatial discrepancy equal 0.97 (Figure 3 c). When comparing CORAL models to the corresponding DAMMIN shapes NSDs were found to be 1.3 for both models at pH 7.0 and pH 9.0 (Figure 3 d & e).

Thus, the hybrid modeling has demonstrated that the ectoIRR dimers achieve similar low resolution shapes at two different pH values. It should be noted that due to the flexibility of inter-domain links and the coexistence of slightly different protein conformations in solution, the resulting structures are averaged and correspond to the most probable constitution of the protein under given conditions. The overall structural characteristics for the CORAL shapes of the ectoIRR dimer calculated by the program CRY SOL from their atomic coordinates almost coincide: \( R_g = 5.3 \text{ nm} \) & 5.4 nm, envelope diameter \( D = 21.9 \text{ nm} \) & 21.8 nm and average electron density \( \rho = 0.5205 \) & 0.5204, respectively. These parameters also correlate well with those obtained from the experimental small-angle scattering data (Table 1).

An attempt has also been made to obtain a symmetrical conformation by imposing the two-fold symmetry axis between the ectoIRR dimer subunits, such that for each atom with the coordinates \((X, Y, Z)\) in the first monomer a symmetry mate in the second monomer has the coordinates \((-X, -Y, Z)\). Using the P2 symmetry, as in the case of ab initio shape reconstruction, the constraint, however, yielded worse fits to the experimental data with the \( \chi^2 = 1.6 \) (Figure SI 4).

**AFM analysis and comparison with the SAXS results**

The SAXS data were further verified by atomic force microscopy analysis (Figure 4). AFM experiments of ectoIRR adsorbed on the surface of atomically flat mica revealed the shapes of the homodimer similar to those observed by SAXS, without significant differences under conditions of pH 7.0 and pH 9.0. Figure 4 shows the typical structures observed by AFM overlapped with the SAXS models. The average sizes of the fitted homodimers are listed in Table 2. Thus, using AFM we were able to detect the ectoIRR conformation essentially similar to that was observed by SAXS at both pH 7.0 and pH 9.0.

**Discussion**

The present work is the first reconstruction of the 3D structural model of the IRR extracellular ectodomain in solution at both neutral and alkaline pH by means of small-angle X-ray scattering and atomic force microscopy. The drop-like structures obtained by ab initio reconstruction of the ectoIRR dimer under pH 7.0 and pH 9.0 in solution did not differ significantly, both SAXS and AFM gave the same integral structural (Table 1 and 2).

More detailed structural study of ectoIRR by the hybrid modeling with usage of the available high-resolution X-ray crystal structure of the insulin receptor ectodomain, demonstrated that the ectoIRR dimer can get asymmetric drop-like conformation, quite different from the lambda shape of the IR ectodomain (Figure 5). It was interesting to compare the experimental SAXS curve of ectoIRR and a theoretically calculated scattering curve based on the published three-dimensional structure of the insulin receptor ectodomain, which shows a lambda-shaped symmetrical head-to-tail complex of two disulfide-linked monomers (18) (derived from the PDB entry: 4ZXB). The comparison of the experimental and computed data confirmed that the solution organization of ectoIRR and the crystal structure of the IR ectodomain stabilized by complexing with antibodies are different (Figure SI 5).

As reported recently, it is possible that two identical insulin receptor or EGFR dimer subunits can be complexed symmetrically or asymmetrically (20,31,32). It is tempting to speculate that the asymmetrical shape of ectoIRR may reflect activation-induced conformational changes of the full-size IRR that involve a relative shift of the transmembrane segments with consequent repositioning of the catalytic domains resulting in the receptor autophosphorylation. In a recent cryo-EM study of the IR ectodomain complexed with insulin and artificially connected juxtamembrane C-termini by a leucine zipper, an asymmetrical dimeric structure was described (33). Remarkably, this structure of the IR ectodomain bound to one insulin molecule (33) resembles the shape of ectoIRR in solution. It is also worth noting that the proposed structural organization of ectoIRR is different from the major T-shaped dimer conformation (labeled as 1T) of IR with close proximity of fibronectin domains from different monomers, which was demonstrated by electron microscopy after insulin binding (20). However, it is similar to an alternative T-shaped dimer conformation of the full-length IR after its reconstitution into nanodiscs (20). This shape was indicated as II-shaped conformation, which represents an asymmetric arrangement of the monomers with close positions of opposite fibronectin domains.
Another key feature of the found ectoIRR conformation is a small distance between the fibronectin domains of the two subunits (Figure 5a). This structural feature agrees with the mutant analysis of ectoIRR indicating that FnIII-2 with FnIII-3 domains are most critical for IRR activity (5). Swapping FnIII-2 and FnIII-3 domains or all FnIII domains of IRR with the IR led to significant glycosylation of these chimeras and completely abolished the IRR pH sensing property. Partial removal of glycosylation by point mutagenesis in IRR/IR chimeras resulted in a partial restoration of IRR pH-sensing activity (34) suggesting that a larger carbohydrate coat of FnIII domains provides a steric hindrance to the IRR activation.

The published mutagenesis data also revealed the importance of five conserved amino acid residues (6) of the L1C domains. In the lambda model, these residues are exposed to the aqueous phase whereas in the drop-like asymmetrical model five of them are located at the contact surface between L1C and fibronectin domains within the tethered subunits of the ectoIRR dimer (Figure 6) that better explains their role in the IRR function and identifies L1C domains of the tethered subunit together with the fibronectin domain of both subunits as the pH sensing mechanism with one or two centers of activation.

**Experimental Procedures**

**Production of recombinant IRR ectodomain**

The IRR ectodomain expression construct was obtained by inserting a fragment of cDNA coding for the first 918 amino acids of human IRR protein fused with c-myc tag to pEE6.HCMV expression vector for GS expression system (Lonza). CHO-K1 cells were transfected by this plasmid and stable expression clones of CHO-K1 was obtained. The cells were grown in serum free medium GibriC-1-CHO (PanEco), the medium was collected and purified by anion-exchange, gel filtration and hydrophobic chromatography as described (35). The protein identity was verified by SDS-PAGE and Western blotting with antibodies against ectoIRR and C-myc as described (36). Purity was controlled by high performance gel filtration on Superose 6 Increase 10/300 GL column (Figures SI 1).

**Scattering experiments and data analysis**

Synchrotron SAXS measurements were performed at the European Molecular Biology Laboratory (EMBL) on the EMBL-P12 BioSAXS beam line at the PETRAIII storage ring (DESY, Hamburg) equipped with a robotic sample changer and a 2D photon counting pixel X-ray detector Pilatus 2M (DECTRIS, Switzerland). The scattering intensity, I(s), was recorded in the range of the momentum transfer 0.08 < s < 3.5 nm⁻¹, where s = (4πsinθ)/λ, 2θ is the scattering angle, and λ = 0.124 nm, the X-ray wavelength (37). The measurements were carried out in either 150 mM NaCl, 20 mM Tris, pH 7.0, or 150 mM NaCl, 20 mM Tris, pH 9.0 buffer at 10°C using continuous sample flow operation over a total exposure time of 1 second, collected as 20 x 50 ms individual frames to monitor for potential radiation damage (no radiation effects were detected (38)). The data were corrected for the solvent scattering and processed using standard procedures (39). To account for the interparticle interactions, solutions of ectoIRR at concentrations of 0.5 and 3 mg/ml were measured.

The values of the total forward scattering at zero angle, I(0), and radii of gyration, Rg, were calculated from the experimental SAXS profiles using the Guinier approximation, which is valid in the range of (sRg) approximately < 1.3 (27,39):

\[
I_{\exp}(s) = I(0) \exp(-s^2R_g^2/3). \quad (1)
\]

These parameters and the maximum particle dimension, Dmax, were also computed from the probable distance distribution function, p(r), using program GNOM (40):

\[
p(r) = \int_0^\infty 4\pi r^2 I(s) \sin(sr)ds. \quad (2)
\]

The molecular masses (MM) of each sample were calculated from the SAXS data using the value of I(0) combined with protein concentration relative to a bovine serum albumin standard (41) as well as from the concentration-independent excluded Porod volume (MM_{Porod}) (42). The latter was determined given that the empirical ratio between the Porod volume (V_p) and MM of a protein is approximately 1.65 (25). A Bayesian inference approach was also applied affording an accuracy above that of the individual methods, and reports MM (MM_{Bayesian}) estimates together with a credibility interval (43).

The low-resolution shapes of ectodomain IRR were reconstructed *ab initio* using the program DAMMIN (44) that generates 3D dummy atom (bead) model spatial representations of a particle. Starting from a random assembly, the program utilizes a simulated annealing (SA) algorithm to build models fitting the experimental data I_{exp}(s) that minimizes the discrepancy...
\[ \chi^2 = \frac{1}{N - I} \sum_j \left( \frac{I_{\text{exp}}(s_j) - cI_{\text{calc}}(s_j)}{\sigma(s_j)} \right)^2, \]  

(3)

i.e., the reduced \( \chi^2 \) test, where \( N \) is the number of experimental points, \( c \) is a scaling factor and \( I_{\text{calc}}(s_j) \) and \( \sigma(s_j) \) are the calculated intensity from the model and the experimental error on the intensities at the momentum transfer \( s_j \), respectively.

Hybrid rigid-body modeling was performed by program CORAL (24), which refines the spatial positions of domains with known high-resolution structures combined with linkers connecting the domains that are represented as chains of dummy amino acids. The program searches for the optimal orientations of the domains and the possible conformation(s) of the linkers by minimizing the fit to the experimental SAXS data using the reduced \( \chi^2 \) test. The high-resolution structures used for each of the domains were obtained from the Protein databank (PDB) accession code PDB entry 4ZXB (the first subdomain contained 1–465 amino acids and the second one contained 472–910, while amino acids 466–471 constituted the flexible linker). The modeling was performed using P1 and P2 symmetry. Program CRY SOL v. 3.0 (24,45) was used to calculate the theoretical scattering from the atomic coordinates of the insulin receptor crystal structure (PDB entry: 4ZXB) and from those of its domains to select ensembles of conformations whose combined-weighted scattering best fit the experimental data.

To assess the convergence of the models obtained in individual simulated annealing runs and to average the models a normalized spatial discrepancy NSD (46) is employed. This approach reveals a dissimilarity measure between individual models containing in general case different number of scatterers, e.g. two \textit{ab initio} models or a bead and rigid body models. Superposition of distinct models is done by program SUPCOMB (46). The averaging of \textit{ab initio} models is performed by the program DAMAVER (47). First, the most typical model (having the lowest average NSD to all the others) is selected and used as a reference. After the alignment of all the models with the reference one, the entire collection of beads is remapped onto a densely packed hexagonal grid, which is filtered to yield an average model with the volume equal to the averaged volume of the input models.

**Atomic Force Microscopy**

Atomic Force Microscopy of ectoIRR was carried out on the Multimode Nanoscope IV setup (Veeco Digital Instruments, USA) equipped with J-type scanner and electrochemical fluid cell. Scanning was performed in the tapping mode with the aid of SiN\(_3\) cantilevers with nominal spring constant of 0.06 N/m (type SNL, Bruker, USA) and the tip radius of approximately 2 nm. All experiments were conducted in the working buffer solution (150 mM NaCl, 20 mM Tris) with the corresponding pH value of 7.0 or 9.0. Samples were prepared by placing 200 μl droplets of the protein solution with the bulk concentration of 7.5 μg/ml on the surface of freshly cleaved mica. After 45 minutes of incubation, the sample was rinsed 5 times with the working buffer solution to remove any unbound protein. The sample was then placed into the AFM cell filled with the corresponding buffer solution for scanning. All images were processed using WSxM software (48). Dimensions of ectoIRR were obtained as the maximal size of the cross-section profile in the corresponding plane and corrected in the lateral plane by the tip radius of the cantilever.

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**Author Contributions**

Designed the research: E.V.S., O.V.B., D.I.S. Performed the experiments: E.V.S., L.A.D., A.A.M., A.S.G., M.V.P., N.A.L, O.V.B., C.MJ. Analyzed the data and made theoretical models: E.V.S., M.V.P., A.G.P., I.E.D., E.V.B., C.M.J., D.I.S. O.V.B. Contributed reagents/materials/analysis tools: A.G.P. All authors wrote and read the manuscript.
Additional Information
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### Table 1. SAXS derived overall structural parameters of the ectoIRR dimer in solution.

Notations: $R_g$, radius of gyration from Guinier; $V_P$, Porod volume estimate; $D_{\text{max}}$, maximum particle dimension; $M_{\text{aa}}$, calculated molecular mass from the amino acid sequence without the contribution of glycosylation of the ectoIRR dimer; $M_{\text{Porod}}$, molecular mass from Porod volume, $M_{\text{Bayesian}}$, molecular mass by the Bayesian inference approach. The associated errors for $R_g$ represent standard deviations directly evaluated by the error propagation from the experimental data. For $D_{\text{max}}$, the deviations were assessed by repetitive runs with varied analysis parameters.

| Sample           | $R_g$, nm | $D_{\text{max}}$, nm | $V_P$, nm$^3$ | $M_{\text{aa}}$, kDa | $M_{\text{Porod}}$, kDa | $M_{\text{Bayesian}}$, kDa |
|------------------|-----------|-----------------------|---------------|----------------------|-------------------------|---------------------------|
| ectoIRR, pH 7.0  | 5.30±0.02 | 19.5±0.3              | 460±20        | 201.5                | 280±15                  | 243 ($\text{credibility interval: 221-373}$) |
| ectoIRR, pH 9.0  | 5.25±0.02 | 19.0±0.2              | 455±20        | 201.5                | 270±15                  | 243 ($\text{credibility interval: 221-373}$) |
Table 2. AFM derived spatial dimensions of the ectoIRR dimer absorbed on the mica surface.

|         | pH 7.0                        | pH 9.0                        |
|---------|-------------------------------|-------------------------------|
| **Height** | 3.0 ± 0.6 nm (SD, n = 28)   | 3.2 ± 0.3 nm (SD, n = 9)     |
| **Length**  | 20 ± 3 nm (SD, n = 28)        | 20 ± 2 nm (SD, n = 9)        |
| **Width**   | 9 ± 3 nm (SD, n = 28)         | 10 ± 2 nm (SD, n = 9)        |
Figure 1. Experimental SAXS curves of ectoIRR and corresponding structural analysis.

a) Experimental SAXS curve at pH 7.0 (1); scattering patterns computed from the *ab initio* model DAMMIN at pH 7.0 (2); the transformed from $p(r)$ and extrapolated to zero scattering angle intensity at pH 7.0 (3); experimental SAXS curve at pH 9.0 (4); scattering patterns computed from the *ab initio* model DAMMIN at pH 9.0 (5); the transformed from $p(r)$ and extrapolated to zero scattering angle intensity at pH 9.0 (6). The experimental curves for pH 7.0 (curve 1) and pH 9.0 (curve 4) together with corresponding fits are shifted along the ordinate by one logarithmic unit for better visualization. Unshifted curves are presented in Figure SI 2. The sample concentration was 3 mg/ml at both pH values. Inset: distance distribution functions $p(r)$ computed by GNOM at pH 7.0 (1) and at pH 9.0 (2).

b) The Guinier plot at pH 7.0 (1) and at pH 9.0 (2); Guinier fits are presented by black lines. c) Kratky plots at pH 7.0 (1) and at pH 9.0 (2). Typical structural models reconstructed by *ab initio* modeling program DAMMIN: d) DAMMIM shape reconstruction performed for the sample at pH 7.0 and e) DAMMIN shape reconstruction performed for the sample at pH 9.0. P1 symmetry was used in both cases. The models d) and e) are shown in two different orientations.
**Figure 2.** High-resolution X-ray crystal structure of the homodimer of the insulin receptor ectodomain (a) with flexible linkers (red) between the head and tail domains (b) that allowed hybrid modeling performed by program CORAL.
Figure 3. Rigid body modeling of the ectoIRR dimer structure: a) – modeling at pH 7.0; b) – modeling at pH 9.0; experimental scattering curves (1); scattering patterns computed from the CORAL models (2). Insets: structural organizations of the ectoIRR dimer in solution obtained by CORAL. Red circles mark obtained configuration of the amino acids 466–471 as a dummy residues chain to fit the computed scattering patterns. c) – comparison of the CORAL models obtained for pH 7.0 (red) and pH 9.0 (green); NSD = 0.97. d) & e) – comparison of the CORAL models to the corresponding DAMMIN shapes at pH 7.0 and pH 9.0, respectively; NSD = 1.3. P1 symmetry was used for all shape reconstructions.
Figure 4. Representative AFM topology images of the ectoIRR dimer absorbed on the mica surface from water solution (150 mM NaCl, 20 mM Tris) at (a) pH 7.0 and (b) pH 9.0. The full z-scale (height) is 6.0 nm in both images. The spatial structure of the ectoIRR dimer obtained by CORAL modeling is overlapped with the AFM topology images by the most suitable rotation.
Figure 5. Structural organization of the drop-like conformation of the ectoIRR in solution obtained by CORAL (a), and a comparison it with the lambda shape of the IR ectodomain (b).
Figure 6. Distribution of the five amino acid residues playing a key role in alkali sensing (6) within the drop-like conformation of the asymmetric ectoIRR dimer (a) (highlighted by green and red in the extended and tethered subunits of the dimer, respectively) and within tethered dimer subunit zoomed on (b) and (c) with some rotation. The subdomain names of the extended and tethered subunits of the dimer are highlighted by green and pink, respectively.
The dimeric ectodomain of the alkali-sensing insulin receptor–related receptor (ectoIRR) has a drop-like shape
Eleonora V. Shtykova, Maxim V. Petoukhov, Andrey A. Mozhaev, Igor E Deyev, Liubov A. Dadinova, Nikita A. Loshkarev, Alexander S. Goryashchenko, Eduard V. Bocharov, Cy M. Jeffries, Dmitri I. Svergun, Oleg V. Batishchev and Alexander G Petrenko

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