Direct Evidence That Neural Cell Adhesion Molecule (NCAM) Polysialylation Increases Intermembrane Repulsion and Abrogates Adhesion*

Colin P. Johnson‡, Ichiro Fujimoto§, Urs Rutishauser¶, and Deborah E. Leckband†**

From the ‡Department of Chemistry and the §Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, the ¶Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana, Illinois, 61801, and the †Cell Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Molecular force measurements quantified the impact of polysialylation on the adhesive properties both of membrane-bound neural cell adhesion molecule (NCAM) and of other proteins on the same membrane. These results show quantitatively that NCAM polysialylation increases the range and magnitude of intermembrane repulsion. The repulsion is sufficient to overwhelm both homophilic NCAM and cadherin attraction at physiological ionic strength, and it abrogates the protein-mediated intermembrane adhesion. The steric repulsion is ionic strength dependent and decreases substantially at high monovalent salt concentrations with a concomitant increase in the intermembrane attraction. The magnitude of the repulsion also depends on the amount of polysialic acid (PSA) on the membranes, and the PSA-dependent attenuation of cadherin adhesion increases with increasing PSA-NCAM:cadherin ratios. These findings agree qualitatively with independent reports based on cell adhesion studies and reveal the likely molecular mechanism by which NCAM polysialylation regulates cell adhesion and intermembrane space.

Polysialic acid (PSA)1 is a long, linear α2,8-linked carbohydrate composed of N-acetyleneuraminic acid (Neu5Ac) residues (1). This carbohydrate is added post-translationally to the neural cell adhesion molecule (NCAM), which is responsible for a variety of functions, including axon pathfinding, synaptogenesis, and tissue formation in the central nervous system (2). The expression of the polysialylated form of NCAM (PSA-NCAM) peaks early in development and decreases with age. In some exceptions, such as the hippocampus, cells continue to express PSA-NCAM throughout the life of the organism. These regions of PSA expression are also associated with neural plasticity and the remodeling of neural connections (1, 2). Aberrant expression of PSA-NCAM is associated with tumor malignancy and metastasis, and the expression of PSA-NCAM has been detected in small cell carcinoma, neuroblastomas, and Wilms’ tumor (3).

Polysialic acid is thought to facilitate cell migration and plasticity by inhibiting cell adhesion to other cells and to the extracellular matrix, as a result of the large excluded volume of the polymer (4, 5). Electron microscopy images showed that PSA expression increased intercellular spacing by 10–15 nm (4). The latter could be because of the inactivation of adhesion proteins or to the increased inter-membrane repulsion resulting from the confinement of the carbohydrate chains. Light scattering studies demonstrated that NCAM polysialylation doubles the hydrodynamic radius of NCAM. However, the latter results were based on calculations, using light scattering data and the assumption that the rod-like proteins were spherical. While this indicates the approximate size of the protein, the hydrodynamic radius does not quantify the effect of the carbohydrates on NCAM-mediated adhesion. In one proposed mechanism, for example, the increased repulsive pressure between the membranes is hypothesized to push the cells apart (6). Such a shift in the force balance between cells from attractive to repulsive requires the increased intermembrane repulsion to be at least as large as the protein attraction at the membrane distance at which the proteins bind. For example, if NCAM bridges two membranes at a separation of 40 nm with an adhesion energy of −1000 kBT μm2, where k_BT is the Boltzmann constant and T is the temperature, then NCAM polysialylation would have to increase the repulsion at 40 nm by at least this amount, to disrupt the adhesive junction. Testing this, however, requires determining both the magnitudes of the intermembrane forces and their range.

The impact of ionic strength on the adhesion between cells expressing PSA-NCAM further supports the view that PSA acts by increasing the repulsion between cells. The hydrodynamic volume of polyelectrolytes decreases with increasing monovalent salt concentrations (7), and this would in turn reduce the repulsion between two membranes with surface-anchored chains. Consistent with this, an increase in the monovalent salt concentration from 0.15 to 0.5 M NaCl restored the adhesion between cells expressing PSA-NCAM (5).

Investigations of cell adhesion also suggested that the effects of PSA can be generalized to a diverse set of adhesion proteins, including NCAM, cadherin, L1, and integrins (8). Furthermore, this general abrogation of adhesion did not require NCAM domains beyond those minimally required for polysialylation. Recent structural studies utilizing both x-ray and neutron specular reflectivity show that the carbohydrate extends sig-

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significantly above the membrane surface. These findings are consistent with a mechanism in which PSA alters cell interactions by increasing the nonspecific, inter-membrane repulsion. This would offset protein attraction, alter cell adhesion, and hence shift the equilibrium membrane separation. However, there is no direct evidence linking intermembrane spacing and the inhibition of adhesion by PSA.

In this study, the surface force apparatus (SFA), and fluorescence correlation spectroscopy (FCS) were used to investigate the molecular mechanism by which polysialic acid abrogates NCAM and C-cadherin-mediated intermembrane adhesion. The SFA quantifies the distance dependence of the force between extended surfaces such as membranes (10, 11). These measurements are particularly relevant to gaining a mechanistic understanding of the biological activity of PSA. The configuration of grafted polyelectrolyte chains such as PSA, as well as the magnitude of the repulsive pressure they exert between membranes is a function of the chain density (7). In this case, single molecule measurements would not yield useful mechanistic information. The results reported here show that the increase in the intermembrane repulsion following NCAM polysialylation is sufficient to overwhelm both homophilic NCAM and cadherin attraction at physiological ionic strength. Some adhesion recovers at high monovalent salt concentrations, concomitant with a significant reduction in the PSA-dependent intermembrane repulsion. FCS provided further, independent evidence that the hydrodynamic radius of PSA exceeds that of the unmodified NCAM. Additional measurements with mixed monolayers of NCAM and C-cadherin show that the inhibition of trans adhesive interactions can be generalized to other cell adhesion molecules.

EXPERIMENTAL PROCEDURES

Materials—1,2-Di-tridecanoyl-sn-glycero-3-phosphocholine and 1,2-di-palmitoyl-sn-glycero-3-phosphoethanolamine were purchased from Avanti Polar Lipids (Alabaster, AL). 6-9-[2,3-Bis(dodecylxoy)propyl-(3,6,9-trioxanonyl-1-oxycarboxylamino)-2-[di(carboxymethyl)-amino]-aminohexanoic acid (NTA-TRIG-DLGE) was custom synthesized by Northern Lipids, Inc. (British Columbia, Canada). 1-Octadecanethiol, HEPES, sodium nitrate, nickel sulfate, and other high purity, inorganic salts were purchased from Aldrich. Rhodamine green was obtained from Molecular Probes (Eugene, OR). 125I was purchased from Pierce (Rockford, IL). Endonemaminidase-N (endo-N) was expressed and purified as described previously (12).

Protein Expression and Purification—The cDNA of chicken NCAM120, encoding the seven extracellular domains of NCAM, was truncated at the onset of the transmembrane segment and fused to a sequence encoding a hexahistidine tag and a 3-amino acid linker, as described (13). This sequence was cloned into a PEE14 expression vector (gift from B. Gumbiner), and the soluble protein was expressed in Chinese hamster ovary cells under methotrexate selection. The result-
ing protein consisted of the seven extracellular domains fused to a C-terminal polyhistidine tail (13). The polyhistidine tail allows both the purification of the protein and its immobilization on fluid NTA-TRIG-DLGE lipid monolayers (13). PSA-NCAM expression was achieved by co-transfecting Chinese hamster ovary cells with a plasmid encoding the NTA-TRIG-DLGE layer from the measured distance between the immobilized PSA-NCAM configuration on lipid bilayers in the di-
cation of the protein eluant with a Centricon YM-30 concentra-
tor (Millipore, Bedford, MA), the protein was further purified on a HiTrap Q anion exchange column (Amersham Biosciences). The PSA-NCAM fractions were collected and concentrated. Finally, the protein was purified to homogeneity by gel filtration (Sephacryl S2000, Amer-

sham Biosciences). The PSA-NCAM fractions were concentrated in a buffer containing 20 mM HEPES and 150 mM NaCl at pH 7.5. Optical densities at 280 nm and an extinction coefficient of 99,030 cm−1 M−1 were used to determine the NCAM concentration. The expression and purification of histidine-tagged NCAM and C-cadherin have been de-
scribed previously (13, 15). The extinction coefficient used to determine the C-cadherin concentration at 280 nm was 69,040 cm−1 M−1 (16).

Protein Monolayers—Force measurements were carried out with pro-
tains immobilized on opposing NCAM extracellular domains at 31 and 39 nm. c, the proposed binding configurations between proteins on oppo-
site, mixed monolayers of cadherin and NCAM, at membrane separa-
tions of 25, 31, and 40 nm.

FIG. 1. Protein configurations in direct force measurements. a, the immobilized PSA-NCAM configuration on lipid bilayers in the di-

test of the force measurements. The distance, D, refers to the distance be-
tween bilayers, and is determined by subtracting twice the thickness of the NTA-TRIG-DLGE layer from the measured distance between the 1,2-di-palmitoyl-sn-glycero-3-phosphoethanolamine layers on the oppo-
site surfaces: \( D = \frac{T_1}{2} - D_{NTA-TRIG-DLGE} \). b, the proposed binding configurations of opposing NCAM extracellular domains at 31 and 39 nm. c, the proposed binding configurations between proteins on oppo-
site, mixed monolayers of cadherin and NCAM, at membrane separa-
tions of 25, 31, and 40 nm.



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desired surface pressure at room temperature, and deposited onto the surface of a mica sheet (15, 16). The first lipid monolayer was gel phase 1,2-di-palmitoyl-sn-glycero-3-phosphoethanolamine, deposited onto the mica from the air-water interface at a density of 43 Å²/lipid. The second, outer fluid phase lipid layer, containing 75 mol % of NTA-TRIG-DLGE and 25 mol % of 1,2-ditridecanoyl-sn-glycero-3-phosphocholine was deposited at 36 mN/m, which corresponds to ~60 Å²/lipid. The supported bilayers were then incubated for 1 h at room temperature in a buffer containing 20 mM HEPES, 150 mM NaNO₃, 15 μM NiSO₄ (pH 7.7), and micromolar concentrations of the polyhistidine-tagged protein. The protein was adsorbed to the NTA lipid head group via the histidine tag. After 1 h, the lipid bilayers were washed with buffer to remove nonspecifically adsorbed protein, and the supported bilayers were then mounted in the surface force apparatus. For mixed protein monolayers, the supported bilayers were incubated either with C-cadherin and PSA-NCAM or with NCAM and C-cadherin at different molar ratios, to vary the PSA-NCAM:cadherin and NCAM:cadherin surface densities.

Force Measurements—The forces were measured between two protein monolayers as a function of the intermembrane distance, D, with a solid line through the data is a fit to the autocorrelation function (Equation 2). The fitted parameters are given in the text. For mixed protein monolayers, the supported bilayers were then incubated for 1 h at room temperature in a buffer containing 20 mM HEPES, 150 mM NaNO₃, 15 μM NiSO₄ (pH 7.7), and micromolar concentrations of the polyhistidine-tagged protein. The protein was adsorbed to the NTA lipid head group via the histidine tag. After 1 h, the lipid bilayers were washed with buffer to remove nonspecifically adsorbed protein, and the supported bilayers were then mounted in the surface force apparatus. For mixed protein monolayers, the supported bilayers were incubated either with C-cadherin and PSA-NCAM or with NCAM and C-cadherin at different molar ratios, to vary the PSA-NCAM:cadherin and NCAM:cadherin surface densities.

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From deflections in the spring supporting the lower disk, the force between the curved surfaces $F_{1}$, normalized by the radius, is determined as a function of the intersurface distance with a resolution of ±1 nN/m = mL/m² (10, 18). Using multiple beam interferometry, SFA measurements quantify the absolute distances between the surfaces with a resolution of ±0.1 nm (18). In this work, the distance, D, refers to the distance between the dehydrated surfaces of the bilayers (Fig. 1a) (13, 15–17). The radius $R$ is also quantified directly from the interference fringes (18).

An important aspect of this approach is that for geometries where the radius is significantly greater than the range of interaction, $R > D$, the total force between the macroscopic curved surfaces $F_{1}$ is directly related to the interaction energy between two equivalent flat surfaces $E_{1}$ by the Derjaguin approximation, $F_{1}/R = 2πE_{1}$ (19). Importantly, the curved geometry only introduces a $1/2R$ factor, which scales the magnitude of the force. The normalized force $F_{1}/2πR$ is therefore a direct measure of the intersurface potential. This well established result is described in several standard textbooks (20). The adhesion energy per area is related to the force to detach the surfaces, or the pull-off force, $F_{p}$, by the Johnson-Kendall-Roberts theory, according to $E_{1} = F_{p}^2 / \pi R^2$ (21).

**Quantification of Protein Surface Densities**—To determine the surface density of proteins immobilized on the NTA-TRIG-DLGE supported bilayers, we measured the amount of bound $^{125}$I-labeled protein, according to procedures described previously (22, 23). $^{125}$I measurements were conducted with pure NCAM and PSA-NCAM monolayers, as well as with mixed NCAM-cadherin and PSA-NCAM-cadherin monolayers at ~11 molar ratios. For the measurements with the mixed protein monolayers, one of the protein components was labeled with $^{125}$I, and both proteins were incubated with the supported bilayer. Thus, measurements with mixed monolayers were conducted with labeled NCAM and then with labeled cadherin.

To quantify the surface densities of immobilized NCAM, PSA-NCAM, and cadherin prepared from solutions of two proteins at molar ratios other than 1:1, we quantified the fluorescence intensities on the supported membranes. By fluorescently labeling one of the proteins and measuring the fluorescence intensity from monolayers prepared from the different protein mixtures, we determined the relative change in the surface density from the measured changes in the fluorescence. The surface densities of proteins adsorbed from 3:1 NCAM-cadherin and 1:3 PSA-NCAM-cadherin were then scaled by the change in fluorescent intensity relative to protein monolayers adsorbed from equimolar protein solutions. For example, a 3-fold increase in the fluorescence intensity from labeled NCAM would signal a 3-fold greater NCAM density. The rhodamine green (RG) dye used does not self-quench, and the measured intensities were below the saturation limit of the detector. Therefore, the intensity is assumed to scale linearly with the protein coverage.

Because the fluorescence measurements quantify relative differences in fluorescence intensities, we calibrated the fluorescence intensity against the amount of $^{125}$I-labeled protein immobilized under identical conditions. For example, if the surface density of NCAM adsorbed from a 1:1 NCAM:cadherin solution is $2.0 \pm 0.6 \times 10^{10}/\mu$m², then a 2-fold increase in the fluorescence intensity would correspond to a surface density of $4 \times 10^{10}/\mu$m².

The proteins were fluorescently tagged with rhodamine green carboxylic acid, succinimidyl ester (Molecular Probes, Eugene, OR), according to the manufacturer’s instructions. The molar ratio of dye to protein was kept at a stoichiometric ratio of 3:1 dye:protein. This yielded a labeling ratio of 1.1 for NCAM, 0.9 for PSA-NCAM, and 1.8 for cadherin.

Fluorescence intensity images were taken using a ×100 UPLANFI oil objective (Olympus, Tokyo, Japan), on a BX60 optical microscope from Olympus (Hamburg, Germany), which was interfaced to a digital camera (Diagnostics Instruments, Sterling, MI). For these measurements, a supported bilayer displaying a mixed protein monolayer, prepared as described above, was placed in a fluid cell and mounted on the microscope stage. The fluorescence intensity was determined by averaging the intensities from a minimum of 10 images taken from different regions of the supported bilayer.

**FCS**—The diffusion coefficients of the macromolecules in solution were quantified by FCS. FCS quantifies fluctuations in the fluorescence intensity, as labeled molecules diffuse through a small excitation volume. The analysis of the fluorescent signal using an autocorrelation function (Equation 2) yields the diffusion coefficient of the macromolecule according to Ref. 24,

$$G(\tau) = \frac{〈F(\tau)F(\tau + \tau)〉}{〈F(t)〉^2} = \frac{〈F(\tau)F(\tau + \tau)〉}{〈F(t)〉^2}$$  (Eq. 2)

**Table 1**

| Proteins | Molar ratio of NCAM-cadherin in solution | Measured surface density $10^9/\mu$m² | Immobilized NCAM-cadherin ratio |
|----------|----------------------------------------|--------------------------------------|-------------------------------|
| NCAM⁺   |                                        | 2.4 ± 0.4                            |                               |
| Cadherin⁺|                                        | 1.9 ± 0.5                            |                               |
| NCAM:cadherin⁺ | 1:1                                | 2.0 ± 0.24 ± 0.7  | 1:1.2                         |
| NCAM:cadherin⁺ | 3:1                                | 3.3 ± 0.61 ± 0.5 | 3:1                          |
| PSA-NCAM⁺ |                                    | 4.2 ± 0.5                            |                               |
| PSA-NCAM:cadherin⁺ | 1:1                                | 1.0 ± 0.25 ± 0.6 | 1:2.5                         |
| PSA-NCAM:cadherin⁺ | 1:3                                | 0.7 ± 0.26 ± 0.6 | 1:3.7                         |
| PSA-NCAM:cadherin⁺ | 1:8                                | 0.34 ± 0.128 ± 0.3 | 1:8.2                         |

⁺ Quantified with isotopically labeled protein.
⁺ Quantified by fluorescence imaging.
where $G(\tau)$ is the autocorrelation amplitude and $\delta(t)$ is the fluctuation in the number of molecules in the excitation volume at time $t$ (25, 26). If applied to the translational diffusion of point-like particles, Equation 2 can be expressed as the following:

$$G(\tau) = \frac{1}{2a} \left( 1 + \frac{8d^2}{w^2} \right) \left( 1 + \frac{8d^2}{z^2} \right)^{-1/2}$$  \tag{3}$$

Here, $w$ and $z$ are the radii of the beam in the $xy$ and $xz$ planes, respectively, $N$ is the number of proteins, and $d$ is the diffusion coefficient of the protein. We can thus accurately quantify differences in the hydrodynamic radii of NCAM and PSA-NCAM from their relative diffusion coefficients.

NCAM and PSA-NCAM were fluorescently labeled with RG as described above. The protein was separated from excess dye by NTA affinity chromatography as described above, and was stored at 4 °C until use. The instrumentation for the measurement and the software for the analysis of FCS data are at the Laboratory for Fluorescence Dynamics at the University of Illinois at Urbana-Champaign (24, 26). To excite the sample, a mode-locked Ti:sapphire laser (Mira 900, Coherent, Palo Alto, CA) pumped by an intracavity doubled Nd:YVO4 vanadate laser (Verdi, Coherent Inc., Santa Clara, CA) was used as a photon source. Photon counts were collected using an Avalanche photodiode detector (model SPCM-AQ-151, EG&G) and the output was directed toward a data acquisition card. A Zeiss Axiovert 135 TV microscope (Thornwood, NY) was used with an oil immersion objective (NA = 1.4). The PSA-NCAM and NCAM concentrations used in the FCS experiments were $\sim 10^{-8} \text{M}$. Data were analyzed to determine the diffusion coefficient by fitting the experimental results to an autocorrelation function that assumes a Gaussian-Lorentzian intensity profile (25, 26). The Gaussian-Lorentzian function depends on the experimental setup and therefore must be calibrated. A solution of $1 \times 10^{-8} \text{M}$ fluorescein in 50 mM Tris was used both for calibration and as a standard for determining the size of the beam waist.

RESULTS

Fluorescence Correlation Spectroscopy—To quantify the relative hydrodynamic dimensions of NCAM and PSA-NCAM, measurements were conducted using FCS. FCS measurements were performed with (RG)/PSA-NCAM or (RG)/NCAM. Nonlinear least squares fits of the data to Equation 2 (Fig. 2) yields an average diffusion coefficient of $26 \pm 4 \mu \text{m}^2/\text{s}$ for PSA-NCAM and $56 \pm 3 \mu \text{m}^2/\text{s}$ for the unmodified NCAM. Assuming a spherical shape, we estimated the hydrodynamic radius $a$ using the Stokes-Einstein relation $a = k_BT/6 \pi \mu a$. Here $a$ is the translational diffusion coefficient, $k_B$ is the Boltzman constant, $\mu$ is the fluid viscosity, and $a$ is the hydrodynamic radius. Using this relationship and the determined diffusion coefficients, the calculated ratio of the two hydrodynamic radii is $a_{\text{PSA-NCAM}}/a_{\text{NCAM}} = 2.1 \pm 0.3$. PSA modification thus doubles the hydrodynamic radius of NCAM in 150 mM NaCl. Previous light scattering studies of PSA-NCAM extracted from neural cells gave a ratio of 1.5 (5).

Protein Density Determinations—The absolute surface densities of NCAM, PSA-NCAM, and cadherin were determined with isotopically labeled proteins. The surface density of $^{125}$I-labeled His$_6$NCAM on membranes comprising 75 mol % NTA-TRIG-DLGE and 25 mol % 1,2-di-tridecanoyl-sn-glycero-3-

Repel at $D < 48 \text{ nm}$. Upon separation, the proteins adhere at 31 (open circles) and 39 nm (open squares) (taken from Ref. 13). The out arrows show the forces at which the bonds yielded and jumped out of adhesive contact. The vertical dashed line indicates the position of the steep steric repulsive force. The error in the measured force is the size of the symbols. $b$, force profiles measured during the approach of PSA-NCAM monolayers (filled squares). The onset of the repulsion occurs at $\sim 48 \text{ nm}$ and then increases more steeply at 26 nm. The steric force between NCAM monolayers (filled circles) is shown for comparison. $c$, force-distance profiles between PSA-NCAM monolayers during approach (filled circles) and separation (open circles). Upon separation, there is some hysteresis, but no adhesion. $d$, force-distance curves between PSA-NCAM treated with endo-N during approach (filled circles) and separation (open circles). Upon separation, the proteins adhere at both 31 and 39 nm (out arrows).
phosphocholine was 2.4 ± 0.4 × 10^4 NCAM/μm^2. These results are summarized in Table I. In studies performed with [125I]-labeled His<sub>6</sub>PSA-NCAM, the measured surface density was somewhat higher at 4.2 ± 0.7 × 10^4 PSA-NCAM/μm^2. In mixed monolayers adsorbed from a 1:1 solution of NCAM:cadherin, the NCAM surface density was 2 ± 0.6 × 10^4 NCAM/μm^2, whereas the cadherin surface density was 2.4 ± 0.7 × 10^4 cadherin/μm^2 (Table I). 125I measurements of monolayers adsorbed from a 1:1 PSA-NCAM:cadherin mixture yielded surface densities of 1 ± 0.5 × 10^4 PSA-NCAM/μm^2 and 2.5 ± 0.6 × 10^4 cadherin/μm^2. Thus, in a solution of equal protein concentrations, the ratios of the adsorbed proteins differ somewhat from those in solution.

Fluorescence intensities were used to quantify the protein coverage at ratios other than 1:1. A comparison of the average intensities from (RG)NCAM on monolayers adsorbed from (RG)NCAM:cadherin solutions shows a 68 ± 3% increase in the fluorescence intensity when the solution composition was changed from 1:1 to 3:1 NCAM:cadherin. Scaling the intensity by the NCAM surface density determined from radiolabeling measurements (Table I), we calculate an NCAM density in the 3:1 monolayer of 3.3 ± 0.6 NCAM/μm^2. The measurement was repeated with labeled cadherin. In this case the average fluorescence intensity decreased by 59 ± 3% when the relative amount of cadherin in solution decreased from 50 to 25 mol %.

Using the known cadherin density on monolayers prepared from the 1:1 mixture, the calculated cadherin surface density is 1.1 ± 0.5 cadherin/μm^2 (Table I).

Fluorescence measurements with 1:1 and 1:3 (RG)/PSA-NCAM:cadherin ratios show that the intensity decreased 34% when (RG)/PSA-NCAM was adsorbed at the lower concentration (Table I). The calculated surface density on the resulting monolayer was 0.7 ± 0.5 PSA-NCAM/μm^2. The fluorescence intensity changed little when comparing the intensities of labeled cadherin adsorbed from solutions with 1:1 and 1:3 cadherin:PSA-NCAM ratios. The surface density increased by only 2%. The results are summarized in Table I.

**PSA Modification Inhibits trans NCAM Adhesion**—Previous direct force measurements of the homophilic NCAM adhesion revealed that NCAM binds homophilically through two separate, spatially distinct binding configurations (13). As seen in Fig. 3a, at bilayer distances greater than 48 nm, there is no force between the NCAM monolayers. However, the onset of the repulsive force (F_R > 0) is approximately D < 48 nm. As the distance, D, decreases further, the steric repulsion between proteins increases, until a steep repulsive wall is reached at D < 22 nm.

Upon separating the bilayers, the force curve drops below the advancing force curve, because of the attractive force between the proteins. At the force minimum, which corresponds to the maximum gradient in the intersurface potential, adhesive failure occurs, and the two surfaces jump out of contact. The minimum is at D = 31 ± 1 nm, which corresponds to full, antiparallel overlap between the Ig1–5 segments of opposing NCAM ectodomains (Fig. 1b) (13). The adhesion is −0.3 ± 0.05 mN/m (Table II). Using the JKR theory (20, 21), we calculate an adhesion energy per area of 1.6 × 10^4 k_BT/μm^2 at this NCAM density. A second bound state is also detected at D = 39 ± 1 nm, when the surfaces are brought to distances that allowed only partial protein overlap. This bound state is mediated by the outer two Ig domains of NCAM (Fig. 1b) (13). The adhesive strength at 39 nm is −0.15 ± 0.07 mN/m (Table II), which corresponds to 7.5 × 10^4 k_BT/μm^2.

In force measurements between identical PSA-NCAM layers at a surface density of 4.2 ± 0.7 × 10^4 PSA-NCAM/μm^2, the addition of the PSA substantially increases the magnitude of the intersurface repulsion. Fig. 3b compares the distance dependence of the repulsive force between approaching PSA-NCAM monolayers with that measured between bare NCAM. With PSA-NCAM, the initial onset of the intersurface repulsion is at D < 48 ± 2 nm. The magnitude of the repulsion increases with decreasing separation, and then increases steeply at D < 28 ± 1 nm. The repulsive force is larger than between bare NCAM at all distances, and the steep increase in the repulsion is shifted out to 28 nm (Fig. 3b). For example, at 30 nm, the repulsive force between PSA-NCAM monolayers is 3 mN/m compared with 1 mN/m between NCAM monolayers at the same distance. Using the Derjaguin approximation (19), this difference corresponds to an increase in the intersurface repulsive energy per area by ΔE_r = ΔF_r/2πR = −0.32 mJ/m^2 or 8 × 10^4 k_BT/μm^2. This is five times the magnitude of the NCAM adhesion energy at this same distance.

To quantify the adhesion between the PSA-NCAM monolayers, the discs were brought to membrane separations D < 31 nm and 31 nm < D < 39 nm, before separating the proteins. At these distances, we would detect any residual NCAM binding (cf. Fig. 3a). There was no measured adhesion upon separation (Fig. 3c), although there was some hysteresis in the receding curve near 31 nm (Table II).

It is important to point out that, to abolish trans NCAM adhesion merely by opposing the NCAM attraction with a nonspecific repulsive force, the repulsion would have to increase by at least 0.3 mN/m at 31 nm (cf. Fig. 3a and Table II), or −0.6 mN/m if we take into account the higher PSA-NCAM surface density (Table I). With PSA-NCAM, the repulsion at 30 nm increased from −1 mN/m between bare NCAM to −3 mN/m. This difference of 2 mN/m, which is because of the osmotic repulsion between the carbohydrate chains, is more than sufficient to overwhelm the NCAM-mediated attraction and abrogate adhesion, without invoking the disruption of in-plane interactions.

The pretreatment of PSA-NCAM with endo-N before immobilizing the protein established that the increased repulsion and consequent inhibition of adhesion is because of the carbohydrate. Endo-N removes polysialic acid by cleaving PSA randomly along the carbohydrate chain (27). Because endo-N requires a minimum of 5–8 sialic acid residues for cleavage, even after complete digestion, a small glycan core remains (27, 28).

Fig. 3d shows the force measurements conducted with PSA-NCAM after endo-N treatment. These force curves are similar to those measured with unmodified NCAM (cf. Fig. 3a). Specifically, the magnitude of the repulsion was significantly

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**Table II**

| Protein                | [NaNO<sub>3</sub>] | Minimum | Adhesion | Minimum | Adhesion |
|------------------------|--------------------|---------|----------|---------|----------|
|                       | μM | nm | mN/m | nm | mN/m |
| NCAM<sup>a</sup>      | 150 | 31 ± 1 | −0.3 ± 0.05 | 39 ± 1 | −0.15 ± 0.07 |
| PSA-NCAM              | 150 | Hysteresis | None | None | None |
| Endo-N-treated PSA-NCAM | 150 | 30 ± 1 | −0.15 ± 0.07 | 39 ± 2 | −0.12 ± 0.05 |
| PSA-NCAM              | 20 | None | None | None | None |
| PSA-NCAM              | 1000 | 32 ± 2 | −0.29 ± 0.07 | Hysteresis | None |

<sup>a</sup> From Ref. 13.
smaller, and the stiff steric repulsion at 28 nm shifted inward to ~23 nm. Perhaps the most significant feature is the recovery of NCAM adhesion at both 31 ± 1 and 39 ± 1 nm. We note that the magnitude of adhesion at 31 nm is slightly lower than between non-sialylated NCAM ectodomains (13). The adhesion at 31 nm was −0.3 ± 0.05 and −0.15 ± 0.07 mN/m, for bare and endo-N-treated NCAM, respectively (Table II). There was not a statistically significant difference between the bound states at 39 nm (Table II). SDS-PAGE showed that the molecular weight of the treated PSA-NCAM was the same as the unmodified protein, within error, indicating essentially complete PSA removal. The reduced adhesion at 31 nm is therefore attributed to the inability of endo-N remove the glycan core (27, 28).

PSA is anionic, and theory predicts that the dimensions of polyelectrolytes in good solvent decrease when the charges are screened by electrolyte (7). The dependence of the extension of surface-anchored polymers, and hence the range of the osmotic repulsion between grafted polyelectrolytes, scales roughly with $C_s^{−1/3}$, where $C_s$ is the concentration of monovalent electrolyte (7). Increasing the ionic strength would therefore reduce the hydrodynamic radius of PSA and correspondingly reduce the range and magnitude of the intermembrane repulsion. If the repulsive and attractive forces between the NCAM ectodomains are additive, then the intermembrane adhesion will increase with the decreasing repulsion.

Measuring the forces between PSA-NCAM monolayers in 1 M NaNO₃ tested the relationship between the PSA-dependent repulsion, the abrogation of adhesion, and the ionic strength. Fig. 4a compares the steric repulsion between PSA-NCAM bathed in 1 M electrolyte relative to the repulsive force measured with 150 mM NaNO₃. In 1 M NaNO₃ both the range and magnitude of the repulsion are lower, and the steep repulsive wall shifted inward from 28 ± 1 to 25 ± 1 nm. Additionally, during the separation of the proteins (Fig. 4b), the NCAM-dependent adhesion reappeared at 31 ± 1 nm, with a magnitude of −0.29 ± 0.07 mN/m (Table II). This reduction in the repulsion, coupled with the recovery in the adhesion at high ionic strength, further supports the view that PSA functions by increasing the nonspecific intermembrane repulsion (1, 6, 8).

Unlike the studies with endo-N-treated PSA-NCAM, the second adhesive bond at 39 nm was not detected in 1 M NaNO₃ (Table II). Thus, although the excluded volume of the carbohydrate is much smaller in 1 M NaNO₃, the polymer still contributes a large enough repulsion (~0.2 mN/m) at 39 nm to overwhelm the second weaker bond.

Additional studies were also carried out with 20 mM NaNO₃ in the bathing medium (Fig. 4c). Under these conditions, the range of the repulsion shifted outward to $D \sim 60$ nm, and the position of the steep repulsion increased to ~30 nm. The overall magnitude of the repulsive force is also greater at the lower ionic strength. Additionally, in contrast to measurements with 150 mM NaNO₃ (Fig. 3c), there was no hysteresis in the receding curves (Table II), that is, the curves measured during approach and separation overlap.

**Adhesion Mediated by Mixed Monolayers of NCAM and Cadherin**—The organization of intercellular space is often governed by more than one type of adhesion molecule, and NCAM polysialylation influences the adhesive function of other proteins on cell membranes (8). Force measurements between membranes displaying mixed monolayers of PSA-NCAM and C-cadherin were carried out to investigate the molecular basis of the impact of PSA on the function of cadherin. Experiments were also conducted with mixed monolayers of NCAM and cadherin, for comparison.

Fig. 5a shows the force-distance profiles between mixed pro-
NCAM and cadherin at a surface density ratio of 1:1.2. The bathing medium contained 150 mM NaNO₃. The vertical dashed line indicates the position of the steep steric repulsion between the membranes. Filled circles show the forces during approach. Upon separation (open circles), the proteins adhere at 25, 31, and 40 nm. Bond failure is indicated by the out arrows. b, normalized force profiles between PSA-NCAM:cadherin monolayers at surface density ratios of 1:2.5 and 1:8 (c). The bathing medium contained 150 mM NaNO₃. The filled circles give the forces measured during approach, and the open circles correspond to separation. d, normalized force profiles between PSA-NCAM:cadherin mixed monolayers (1:2.5), in a buffer containing 1 M NaNO₃. Filled circles show forces during approach and open symbols to separation. The out arrows indicate the jump out from adhesive contact at D = 31 and D = 40 nm.

FIG. 5. Forces between mixed monolayers of cadherin and NCAM. a, normalized force profile between mixed monolayers of NCAM and cadherin at a surface density ratio of 1:1.2. The bathing medium contained 150 mM NaNO₃. The vertical dashed line indicates the position of the steep steric repulsion between the membranes. Filled circles show the forces during approach. Upon separation (open circles), the proteins adhere at 25, 31, and 40 nm. Bond failure is indicated by the out arrows. b, normalized force profiles between PSA-NCAM:cadherin monolayers at surface density ratios of 1:2.5 and 1:8 (c). The bathing medium contained 150 mM NaNO₃. The filled circles give the forces measured during approach, and the open circles correspond to separation. d, normalized force profiles between PSA-NCAM:cadherin mixed monolayers (1:2.5), in a buffer containing 1 M NaNO₃. Filled circles show forces during approach and open symbols to separation. The out arrows indicate the jump out from adhesive contact at D = 31 and D = 40 nm.

PSA Inhibition of Cell Adhesion

To test for adhesion at other intermembrane spacings, retreating force profiles were measured from distances D > 25 nm, where the ectodomains of both proteins only partially overlap. Using this method, which is described extensively in earlier publications (13, 15, 16), a second, weaker adhesive minimum was detected at 30 ± 1 nm, with a strength of −0.35 ± 0.05 mN/m (Table III). At this intermembrane distance, both NCAM and cadherin adhere (Fig. 1c) (13, 15). Retreating force profiles measured at distances D > 31 nm detected a third adhesive interaction at D = 41 ± 1 nm (Table III). This distance is, within error, the same as the positions of the outer bound states of both cadherin (D = 40 ± 1 nm) and NCAM ectodomains (D = 39 ± 1 nm) (Fig. 1c). The adhesion at 41 nm is therefore attributed to the combined contributions of both proteins. The magnitude of the adhesion was −0.21 ± 0.06 mN/m. No other adhesive interactions were detected.

The dependence of the adhesion on the protein densities was determined in a second set of force measurements with an NCAM:cadherin ratio of 3:1 (Table I). As in Fig. 5a, the advancing profile in this case differed from those of either NCAM or cadherin (not shown). The retreating force profile similarly detected three bound states at the same three distances. However, in this case, the adhesion at 25 nm decreased from −0.7 ± 0.05 to −0.35 ± 0.07 mN/m, because of the reduced cadherin density (Table III). The adhesion at 30 ± 1 and 41 ± 1 nm changed only slightly (Table III), because the reduction in cadherin-mediated adhesion is offset by the increased NCAM density, and hence by the larger number of NCAM bonds.

PSA-NCAM Inhibits Cadherin Adhesion—Direct force measurements between monolayers of PSA-NCAM and C-cadherin at a 1:2.5 molar ratio are shown in Fig. 5b. The bathing medium contained 150 mM NaNO₃. The range and magnitude of the repulsion are much greater than between the NCAM:cadherin monolayers (Fig. 5a). The onset of repulsion occurs at D < 50 ± 2 nm, and the steep repulsive force is at D = 27 ± 1 nm. There is also no adhesion at any distance (Table III). The magnitude of the force at 25, 30, and 40 nm is 3.69 ± 0.06, 1.22 ± 0.08, and 0.43 ± 0.08 mN/m, respectively. At all three distances, the repulsive force exceeds the combined attractive forces of both cadherin and NCAM (Table II), and is sufficient to abrogate the protein-mediated adhesion.

If the loss of adhesion is because of the additive effect of medium contained 150 mM NaNO₃. The vertical dashed line indicates the position of the steep steric repulsion between the membranes. Filled circles show the forces during approach. Upon separation (open circles), the proteins adhere at 25, 31, and 40 nm. Bond failure is indicated by the out arrows. b, normalized force profiles between PSA-NCAM:cadherin monolayers at surface density ratios of 1:2.5 and 1:8 (c). The bathing medium contained 150 mM NaNO₃. The filled circles give the forces measured during approach, and the open circles correspond to separation. d, normalized force profiles between PSA-NCAM:cadherin mixed monolayers (1:2.5), in a buffer containing 1 M NaNO₃. Filled circles show forces during approach and open symbols to separation. The out arrows indicate the jump out from adhesive contact at D = 31 and D = 40 nm.
steric repulsion and protein attraction, then lower PSA-NCAM densities should correspondingly reduce the magnitude of the repulsion and increase the attraction. Force measurements with monolayers containing a 1:3.7 PSA-NCAM:cadherin ratio tested this. Decreasing the PSA-NCAM surface density decreased the repulsive force, as expected, but the magnitude of the remaining repulsion still exceeded the protein attractive forces, and there was no adhesion (not shown). There was hysteresis between the advancing and receding force curves, and we attribute this to some protein binding. However, between monolayers adsorbed from a 1:8 PSA-NCAM:cadherin solution, the range and magnitude of the repulsion were lower, and the protein monolayers adhered at \( D = 31 \pm 1 \) and \( 41 \pm 1 \) nm (Fig. 5c). The magnitude of adhesion at \( D = 31 \) nm was \(-0.29 \pm 0.08\) mN/m, and the magnitude of the adhesion at \( D = 41 \) nm was \(-0.13 \pm 0.06\) mN/m. There was no adhesion at distances \( D < 31 \) nm (Table III).

To assess the relationship between ionic strength and PSA in controlling cadherin adhesion, force measurements were conducted with solutions containing \( 1 \) mM \( \text{NaNO}_3 \) (Fig. 5d). In comparison to the measurements with mixed PSA-NCAM:cadherin monolayers in 150 mM \( \text{NaNO}_3 \) (Fig. 5b), the range and magnitude of the repulsive force are reduced (Fig. 5d). Specifically, the onset of the repulsion shifted inward from 50 to 42 ± 1 nm, and the steep repulsive force shifted inward from 27 ± 1 to 20 ± 1 nm.

Upon separation, we measured two adhesive interactions: namely, \(-0.31 \pm 0.08\) mN/m at 31 ± 1 nm and \(-0.2 \pm 0.07\) mN/m at 41 ± 2 nm (Table III). There was no adhesion at 25 nm. Control measurements with NCAM and cadherin in 1 mM \( \text{NaNO}_3 \) showed that high monovalent salt concentrations do not affect either protein.

**Relative Contributions of Cadherin and NCAM**—To demonstrate that the adhesive interactions at 25, 30, and 40 nm are because of the combined effects of both proteins, the cadherin was inactivated by decreasing the calcium concentration to 50 \( \mu \text{M} \) (9, 29). Cadherin adhesion involving the outer N-terminal domain is abolished at this calcium concentration (9). Between cadherin monolayers \( (1.9 \times 10^4 \text{ cadherin/}\mu \text{m}^2) \) in 50 \( \mu \text{M} \) \( \text{Ca(NO}_3\text{)}_2 \), there was no adhesion at 32 and 40 nm. However, the proteins still bound, albeit more weakly, at 25 nm, with an adhesion of \(-0.3 \pm 0.1\) mN/m (Table III).

Between mixed NCAM:cadherin (1:2.5) monolayers in a buffered solution containing 50 \( \mu \text{M} \) calcium, the adhesion at 31 nm was significantly reduced to \(-0.15 \pm 0.08\) mN/m (Table III). In the absence of cadherin adhesion, we attribute this to binding between NCAM Ig1–5 domains (cf. Fig. 1b) (13). The adhesion at 40 nm also decreased to \(-0.1 \pm 0.06\) mN/m, and is similarly attributed solely to homophilic NCAM binding. In similar measurements between mixed PSA-NCAM:cadherin (1:2.5) monolayers in 1 mM \( \text{NaNO}_3 \) and 50 \( \mu \text{M} \) \( \text{Ca(NO}_3\text{)}_2 \), the hysteresis at \(-25\) nm was absent. The adhesion at \( D = 30 \) nm decreased to \(-0.1 \pm 0.06\) mN/m, and the only evidence for binding at \( 40 \pm 1 \) nm was the hysteresis in the receding curve. These findings confirm that, at the higher calcium concentrations, both cadherin and NCAM contribute to the adhesion measured at 31 and 40 nm.

A control measurement of the force profile between PSA-NCAM in 3 mM calcium tested whether the divalent calcium altered the PSA properties. The \( \text{Ca(NO}_3\text{)}_2 \) salt did not significantly alter the ionic strength, because \( \text{NaNO}_3 \) is the dominant electrolyte. The calcium had no effect on the force curves.

**DISCUSSION**

The force measurements described here provide direct support for the proposed "push-pull" mechanism for the inhibition of NCAM-mediated adhesion by PSA (6). This model postulates that the additive contribution of attractive protein forces and repulsive steric forces determines the equilibrium separation between cells. For PSA to block NCAM adhesion by increasing the nonspecific repulsive force between cells, the force balance requires that the magnitude of the additional repulsion exceed the NCAM attraction at both membrane separations at which NCAM binds. These measurements show quantitatively that the post-translational modification of NCAM substantially increases the magnitude of the repulsive pressure between membranes, sufficient to abrogate both NCAM- and cadherin-mediated adhesion. The increased excluded volume of PSA-modified NCAM was confirmed by both FCS and direct force measurements, but the latter directly demonstrated the functional impact of this modification on protein-mediated intermembrane adhesion. Furthermore, PSA reportedly has a general effect on several adhesion proteins, including cadherin. For PSA to similarly influence other adhesion molecules, the magnitude of the steric repulsion would also have to be large enough to abrogate, for example, cadherin binding at all three cadherin binding distances. The quantified magnitude of the steric repulsion at 25, 31, and 40 nm, the cadherin binding distances, again bears this out. Even with a 3-fold excess of cadherin, the PSA-associated repulsion exceeded the cadherin attraction, and abolished all adhesion.

The incomplete recovery of NCAM adhesion at 31 nm following endo-N digestion raises the question of the efficiency of the PSA removal. However, it is important to consider that the effects of glycosylation can be local, because of short-ranged steric repulsion between proteins, or long-ranged due to polymer repulsion between membranes. Because the NCAM adhesion at 31 nm requires full Ig1–5 overlap, the bulky glycan remaining on Ig5 would likely impede this association by short-ranged steric repulsion. However, the short-ranged force would not affect the outer bond, and this is the observed behavior. Nevertheless, if this attenuation is because of long-ranged repulsion from uncleaved chains, then polyelectrolyte theory estimates the reduction in PSA chain lengths that would decrease the measured repulsion from 2 to \(-0.15\) mN/m. For

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**TABLE III**

| Proteins          | [NaNO₃][Ca(NO₃)₂] | 1st Adhesive minimum | 2nd Adhesive minimum | 3rd Adhesive minimum | Adhesion   |
|-------------------|-------------------|----------------------|----------------------|----------------------|------------|
|                   | nm                | mN/m                 | nm                   | mN/m                 | nm         |
| Cadherin \(^a\)   | 150 mm, 3 mm      | 26 ± 1               | -2 ± 1               | 32 ± 1               | 40 ± 1     | -0.25 ± 0.1 |
| NCAM:cadherin (1:1.2) | 150 mm, 3 mm  | 25 ± 1               | -0.7 ± 0.05          | 30 ± 1               | 41 ± 1     | -0.21 ± 0.06 |
| NCAM:cadherin (3:1) | 150 mm, 3 mm      | 26 ± 1               | -0.35 ± 0.07         | 30 ± 1               | 41 ± 1     | -0.18 ± 0.05 |
| PSA-NCAM:cadherin (1:1.25) | 150 mm, 3 mm | None                 | None                 | None                 | None       | None         |
| PSA-NCAM: cadherin (1:3) | 150 mm, 3 mm | Hysteresis          | None                 | None                 | None       | None         |
| PSA-NCAM:cadherin (1:1.25) | 1 m, 3 mm    | Hysteresis          | 31 ± 1               | -0.31 ± 0.08         | 42 ± 1     | -0.2 ± 0.07  |

\(^a\) From Ref. 15.
monodisperse chains at a uniform grafting density in polyelectrolyte, the repulsion scales with the square of the degree of polymerization $N^2$ (7), so that,
\[ F_1 / F_2 = (N_1 / N_2)^2 \]  
(Eq. 4)

where $F_1$ and $F_2$ are the magnitudes of the repulsive forces at 31 nm before and after PSA cleavage, respectively. The average degree of PSA polymerization $N_1$ is estimated to be greater than 55 (14) and SDS-PAGE suggests that it could be as much as 200.\(^3\) If we assume 100, then the degree of polymerization of the shortened chains would be,
\[ N_2 = 100 \sqrt{0.15 / 2} = 27 \]  
(Eq. 5)

Although this is a very rough estimation, the endo-N-treated PSA-NCAM does not exhibit a molecular weight range consistent with this amount of residual PSA. This suggests that the reduced adhesion is most likely because of local steric repulsion by the residual glycan core.

It is important to point out that this PSA-dependent repulsion is a general property of the polymer, and is independent of the identities of other proteins on the membrane. For this reason, the repulsive pressure between the PSA chains on adjacent membranes will have a general effect on the ability of other proteins to support intermembrane adhesion at membrane separations less than 45 nm, the measured range of the steric repulsion.

The ionic strength dependence of the range and magnitude of the PSA-mediated intermembrane repulsion provides further direct evidence that PSA impacts cell adhesion primarily through its large excluded volume. These data show directly that increasing the ionic strength decreases the steric dimensions of PSA. This in turn reduces the intermembrane repulsion, and restores the intercellular adhesion.

The repulsion between grafted brushes also scales with the grafting density of the chains (7). The influence of PSA on homophilic cadherin adhesion similarly depends on the ratio of PSA-NCAM to cadherin on the membranes; and hence on the balance between the steric repulsion and cadherin attraction. Again, this is a graded response, with the magnitude of cadherin adhesion increasing with decreasing PSA-NCAM coverage and with the corresponding decrease in the intermembrane repulsion. At the 1:2.5 PSA-NCAM:cadherin ratio, the cadherin-mediated adhesion, which was distinguished by binding at 25 nm, was abolished. At a 1:3.7 protein ratio, any residual attraction was only apparent as hysteresis. Cadherin adhesion finally reemerged at a ratio of ~1:8. This correlation between the PSA-NCAM density, the magnitude of the repulsion, and the adhesion further supports the hypothesis that the impact of PSA is directly linked to the range and magnitude of the associated nonspecific, intermembrane repulsion.

These findings show quantitatively that the magnitude of PSA-dependent repulsion correlates directly with the decreased trans homophilic adhesion by NCAM and cadherin. It is important to point out that the polymer occupies a three-dimensional volume. Although there are no data showing that PSA affects any cis interactions, known or otherwise, the steric barrier generated by the excluded volume could also affect other, lateral NCAM interactions. This issue will be addressed in future studies.

**REFERENCES**

1. Bruses, J. L., and Rutishauser, U. (2001) Biochimie 83, 635–643
2. Walsh, F. S., and Doherty, P. (1997) Annu. Rev. Dec. Biol. 13, 425–456
3. Tanaka, R., Otake, Y., Nakagawa, T., Kawano, Y., Miyahara, R., Li, M., Yanagihara, K., Nakayama, J., Fujimoto, I., Ikenaka, K., and Wada, H. (2000) Cancer Res. 60, 3072–3080
4. Yang, P., Yin, X., and Rutishauser, U. (1992) J. Cell Biol. 116, 1487–1496
5. Yang, P., Major, D., Yin, X., and Rutishauser, U. (1994) J. Biol. Chem. 269, 23039–23044
6. Rutishauser, U. (1996) Curr. Opin. Cell Biol. 8, 679–684
7. Pincus, P. (1991) Macromolecules 24, 2912–2919
8. Fujimoto, I., Bruses, J. L., and Rutishauser, U. (2001) J. Biol. Chem. 276, 31745–31751
9. Pertz, O., Bozie, A., Koch, W., Auser, C., Brancaccio, A., and Engel, J. (1999) EMBO J. 18, 1738–1747
10. Israelachvili, J. (1992) J. Surf. Sci. Rep. 14, 110–159
11. Leckband, D. (2000) Annu. Rev. Biophys. Biomol. Struct. 29, 1–25
12. Prosser, R. A., Rutishauser, U., Ungers, G., Fedorkova, L., and Glass, J. D. (2003) J. Neurosci. 15, 652–658
13. Johnson, C. P., Fujimoto, I., Perrin-Tricaud, C., Rutishauser, U., and Leckband, D. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6983–6988
14. Livingston, B. D., Jacobs, J. L., Glick, M. C., and Troy, F. A. (1988) J. Biol. Chem. 263, 9443–9448
15. Sivasankar, S., Gumbiner, B. M., and Leckband, D. (2001) Biophys. J. 80, 1758–1768
16. Zhu, B., Chappuis-Flament, S., Wang, E., Jensen, I. E., Gumbiner, B. M., and Leckband, D. (2003) Biophys. J. 84, 4033–4042
17. Marra, J., and Israelachvili, J. (1985) Biochemistry 24, 4608–4618
18. Israelachvili, J. (1973) J. Coll. Int. Sci. 44, 259–272
19. Derjaguin, B. V. (1934) Kolloid Z. 98, 155–164
20. Israelachvili, J. (1991) Intermolecular and Surface Forces, Academic Press, New York
21. Johnson, K. L., Kendall, K., and Roberts, A. D. (1971) Proc. R. Soc. A 324, 301–313
22. Yeung, C., and Leckband, D. (1997) Langmuir 13, 6746–6754
23. Yeung, C., Purves, T. Koss, A., and Sligar, S. (1999) Langmuir 15, 6829–6836
24. Muller, J. D., Chen, Y., and Gratton, E. (2003) Methods Enzymol. 361, 69–92
25. Cheng, Y., Muller, J. D., Berland, K. M., and Gratton, E. (1999) Methods 19, 254–259
26. Sanchez, S., Chen, Y., Muller, J. D., Gratton, E., and Hazlett, T. L. (2001) 40, 6903–6911
27. Hallenbeck, P. C., Vinr, E. R., Yu, F., Bassler, L., and Troy, F. A. (1987) J. Biol. Chem. 262, 3553–3561
28. Finne, J., and Makela, P. H. (1985) J. Biol. Chem. 260, 1265–1270
29. Koch, A. W., Pokutta, S., Lustig, A., and Engel, J. (1997) Biochemistry 36, 7677–7675

\(^3\) C. P. Johnson, I. Fujimoto, U. Rutishauser, and D. E. Leckband, unpublished observations.
Direct Evidence That Neural Cell Adhesion Molecule (NCAM) Polysialylation Increases Intermembrane Repulsion and Abrogates Adhesion
Colin P. Johnson, Ichiro Fujimoto, Urs Rutishauser and Deborah E. Leckband

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Direct evidence that neural cell adhesion molecule (NCAM) polysialylation increases intermembrane repulsion and abrogates adhesion.

Colin P. Johnson, Ichiro Fujimoto, Urs Rutishauser, and Deborah E. Leckband

Page 137: The grant number listed in the footnote at the bottom of the page was misprinted. The correct citation should read as follows: “This work was supported by National Institutes of Health Grant 1RO1 GM63536.”

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Regulation of zipper-interacting protein kinase activity in vitro and in vivo by multisite phosphorylation.

Paul R. Graves, Karen M. Winkfield, and Timothy A. J. Haystead

Page 9373, under “Acknowledgments”: The following was inadvertently omitted from this section: A cDNA clone expressing the full-length sequence of human ZIP kinase was very kindly provided by Dr. Hiroshi Hosoya (Department of Biological Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima, Japan).

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