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Modeling Pressure-Driven Transport of Proteins through a Nanochannel

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Abstract

Reducing the size of a nanofluidic channel not only creates new opportunities for high-precision manipulation of biological macromolecules, but also makes the performance of the entire nanofluidic system more susceptible to undesirable interactions between the transported biomolecules and the walls of the channel. In this manuscript, we report molecular dynamics simulations of a pressure-driven flow through a silica nanochannel that characterized, with atomic resolution, adsorption of a model protein to its surface. Although the simulated adsorption of the proteins was found to be nonspecific, it had a dramatic effect on the rate of the protein transport. To determine the relative strength of the protein–silica interactions in different adsorbed states, we simulated flow-induced desorption of the proteins from the silica surface. Our analysis of the protein conformations in the adsorbed states did not reveal any simple dependence of the adsorption strength on the size and composition of the protein–silica contact, suggesting that the heterogeneity of the silica surface may be a important factor.

I. Introduction

Transport of liquids through nanometer size structures is a ubiquitous process in modern biomedical engineering. The proposed applications of nanofluidics range from lab-on-a-chip technology [1], [2], epigenetic information analysis [3], [4], and biosensors [5], [6] to protein crystallization [7], drug delivery systems [8], and electronics [9], [10]. To transport biomolecules through nanoscale structures, hydraulic pressure gradients and/or electric fields are imposed across the device elements [1], [11]. Today, it is already possible to fabricate macroscopic length channels of a sub-ten nanometer cross-section [12], and assemble them into regular macroscopic arrays [13].

Under typical experimental conditions, pressure-driven flow of water through nanometer-size channels is laminar, as the Reynolds number of the flow is very low [11]. Under such conditions, the hydrodynamic equations of motion simplify considerably and, knowing the geometry of the channel, the properties of the flow can be easily determined [14]. The presence of proteins in the solution complicates the matter at high protein concentrations, because the protein–water mixture no longer behaves as a Newtonian fluid, and when the size of the channel becomes comparable to the size of the protein. In the latter case, the

interactions of the protein with the channel surface can dominate the protein transport and lead to spurious effects such as nonspecific adsorption, surface accumulation and eventually clogging of the nanochannel system [1].

In the simplest theoretical model of protein adsorption, a protein is assumed to bind to the surface with a certain probability so that the fractional coverage of the surface θ is described as a Langmuir isotherm $\theta = \alpha C / (1 + \alpha C)$, where C is the protein concentration and α is the Langmuir adsorption constant. The Langmuir constant effectively incorporates all the effects associated with the properties of the surface of the channel and the protein, and can be empirically associated with quantities like the total surface area of the protein, the surface charge, roughness, and hydrophobicity.

Ideally, one would want to predict the macroscopic behavior of the protein flow through a nanochannel taking into account the microscopic properties of the protein and the nanochannel material. Molecular dynamics (MD) is a computational method that can accurately predict the forces between biomolecules and inorganic surfaces in water if their atomic structures are known. The MD method has already been applied to simulate flow and sorption of simple solutes in nanochannels within a Lennard-Jones model [15]. All-atom MD simulations have characterized DNA transport through solid-state nanopores [16]–[18] and the conformations of proteins near silicon surfaces [19]. The binding of individual amino acids, histidine and phenylalanine, to a platinum surface has been studied in all-atom detail using classical MD parameterized through iterative *ab initio* quantum mechanics calculations [20]. Steered-MD (SMD) has been used to study the friction of a 15-mer polypeptide on hydrophobic and hydrophilic surfaces [21] as well as protein adsorption and desorption near a hydroxyapatite surface [22].

In this manuscript, we describe the first (to our knowledge) MD simulations of pressure-driven transport of proteins through a nanochannel. We begin with a description of our simulation methodology and then test the method by simulating pressure-driven transport of water. Next, we use a model protein to explore physical phenomena that governs protein transport through a nanochannel. For the silica channels studied in this manuscript, we find a strong tendency for the protein to spontaneously adsorb to the nanochannel surfaces. Hence, we investigate the statistical properties of the protein–silica contacts in the adsorbed states and the process of protein desorption induced by the flow.

II. Methods

A. General Molecular Dynamics Methods

All molecular dynamics (MD) simulations were performed using the program NAMD [23], a 1 fs integration time step, the particle mesh Ewald (PME) electrostatics [24], and periodic boundary conditions. Simulations in the NPT ensemble (constant number of particles N , pressure P and temperature T) were performed using a Langevin thermostat [25] and Nosé-Hoover Langevin piston pressure control [26] set at 295 K and 1 atm, respectively. The damping coefficient of the Langevin thermostat was 1 ps^{-1} , unless specified otherwise; the thermostat was applied only to the atoms of the nanochannel surface. A smooth (1.0–1.2 nm) cutoff was used to compute the van der Waals forces. External potentials were applied using grid-steered molecular dynamics (G-SMD) [27]. The nanochannel simulations were performed using the CHARMM [28] force field for water, ions and proteins and a custom forcefield for silica [29]. The charges of the silicon and oxygen atoms of silica were set to $0.9 e$ and $-0.45 e$, respectively, and the Lennard-Jones parameters were $r_{\text{Si}}^{\text{min}} = 0.4295 \text{ nm}$, $\epsilon_{\text{Si}} = 0.3 \text{ kcal/mol}$, $r_{\text{O}}^{\text{min}} = 0.35 \text{ nm}$, and $\epsilon_{\text{O}} = 0.15 \text{ kcal/mol}$. The silica membranes were generated using the BKS potential [30], [31] (see below).

B. Membrane Structure Generation

To produce the silica membrane used in this study, we created a $2.5 \times 2.5 \times 3.5 \text{ nm}^3$ block of crystalline silica containing 500 silicon and 1000 oxygen atoms by replicating a unit cell of SiO_2 . The resulting system was simulated for 20 ps at 7000 K using a $2.5 \times 2.5 \times 5.5 \text{ nm}^3$ periodic cell, which produced an amorphous silica membrane with two relaxed surfaces (normal to the z-axis). The silica was simulated with the BKS potential, a two-body potential proposed by Beest, Kramer and van Santen which has been shown to reproduce some properties of silica [30], [31]. As in Vollmayer et al. [31], the form of the BKS potential was modified at small distances to prevent spurious behavior at high temperature. The Coulomb portion of the BKS potential was computed using the PME method [24], while the van der Waals portion was smoothly shifted to zero at an interatom distance of 0.55 nm. During the annealing procedure, an external force was applied to prevent the atoms from evaporating into the vacuum region. The temperature was controlled by Langevin dynamics with a damping constant of 5 ps^{-1} . The resulting annealed silica structure was tiled in a four-by-four grid to create a $10.0 \times 10.0 \times 2.7 \text{ nm}^3$ silica membrane consisting of sixteen identical slabs of silica.

C. Protein

As a model protein for this study, we used toxin II of the scorpion *androctonus australis* hector [32] (PDB access code 1PTX). This rather small protein consists of 64 residues and has both α -helical and β -sheet secondary structure elements. The atomic coordinates of the protein were taken from its X-ray structure; the coordinates of all missing atoms were determined according to the CHARMM [28] protein topology file. To explore the stability of the protein in solution, a single copy of the protein was submerged in 10,000 TIP3P [33] water molecules with one chloride counterion to balance the positive charge of the protein—a system of 30,971 atoms. Following 2000 steps of energy minimization, the system's temperature was increased from 0 to 295 K within 25 ps by velocity rescaling. Following 0.5-ns equilibration with the protein backbone restrained to its X-ray coordinates, the system was equilibrated free of any restraints for 50 ns in the NPT ensemble.

D. Nanochannel

An atomic-scale model of a nanochannel was created from the silica membrane by imposing periodic boundary conditions that defined the spacing between the nanochannel walls (the channel's height) and made the channel effectively infinitely long and wide. For our test simulations of the water flow, the volume between the walls of the channel was filled with preequilibrated water molecules, which increased the total number of atoms in the system to 78,696.

Following a 2000-step minimization, the system was equilibrated for 2.5 ns in the NPT ensemble with the SiO_2 atoms harmonically restrained to the coordinates obtained at the end of the annealing simulation. In the silica membrane, harmonic bonds of the same strength (6950 pN/nm spring constant) were applied between the nearest neighbors. After equilibration, the volume inside the nanochannel unit cell had dimensions of $9.9 \times 9.9 \times 5.5 \text{ nm}^3$; the water density was 1.01 g/cm^3 . The boundary of the silica membrane was defined as the surface where the number density of silica was five times that of the water.

To study the transport of proteins through nanochannels, we created three systems (A, B and C of 83,542, 83,398 and 83,377 atoms, respectively) by placing water, two copies of the protein and two chloride counterions inside each of three copies of the silica nanochannel. In each system, the proteins were placed in a random orientation with respect to the surface of the channel, representing a unique initial condition; the minimum surface-to-protein distance was 0.55 nm in system A and 1.15 nm in systems B and C. After 2000 steps of

minimization, the systems were equilibrated in the NPT ensemble for 0.3 (system A) or 0.6 (systems B and C) ns, with the SiO₂ atoms restrained to their coordinates at the end of the NPT equilibration of the water-only channel system. The latter restraints had spring constants of 13900 pN/nm; nearest neighbor atoms of the silica membrane were connected by harmonic bonds of 1390 pN/nm. Restraining the silica to the final coordinates of the NPT equilibration, rather than to the annealed structure, reduced the periodicity of the channel surface introduced by the tiling method used to create the systems. The change in the spring constants from the water-channel simulation reflects an optimization of the dielectric response of the membrane [34], changing the dielectric constant from 5.5 to 4.0 (± 0.2). After equilibration, the volume inside the channel unit cell had dimensions of $10.0 \times 10.0 \times 5.8$ nm³.

E. Simulation of the Flow

To induce water flow through a nanochannel, a constant pressure difference was created by applying a lateral force to all the water molecules in the system. As detailed in Zhu et al. [35], applying a lateral force, F , to N atoms results in a pressure difference across the system of

$$\Delta P = N \cdot F / A, \quad (1)$$

where A is the area perpendicular to the applied force.

III. Results and Discussion

A. Pressure-Driven Transport of Water Through a Nanochannel

To test our computational method, we simulated the flow of water through a nanochannel at five different pressure gradients. Each simulation lasted from 10 to 25 ns. Fig. 2 summarizes the results of these simulations. First, we determined that the mass flux of water through the nanochannel scales linearly with the applied pressure gradient within the entire range of pressure gradients (spanning three orders of magnitude) tested, Fig. 2 a.

The water flux was computed by measuring the change in the collective coordinate of all the water oxygens in the nanochannel [36], divided by the channel's cross section area A_{channel} :

$$Flux = \frac{I}{A_{\text{channel}}} \sum_{i=0}^{N_{\text{water}}} \frac{\Delta x_i}{L_{\text{channel}}}, \quad (2)$$

where L_{channel} is the length of the channel in the direction of the flow.

Next, we investigated the dependence of the water velocity on the distance from the surface of the channel. In Fig. 2 b, we plot the dimensionless average water velocity (scaled with its density and normalized by the total flux) versus the distance from the surface of the channel normalized by the channel width. The simulated dimensionless flow profiles at five pressure gradients overlap, indicating a linear scaling of the flow profile with the pressure gradient. The water velocity profiles were calculated through the following process. First, the velocity of each water oxygen along the direction of the pressure gradient was computed from its displacement over a 10-ps interval. Next, these velocities were grouped into bins spaced by 0.2 nm along the height of the channel. Water molecules that moved between the bins were given fractional counts for the respective bins. Finally, the velocities were averaged over each bin and the last 9 to 19 ns of each simulation.

The simulated water profiles could be accurately described as a Poiseuille flow through an infinite slit [14]:

$$U_p(x) = \frac{\Delta p}{8\eta L} (h^2 - 4x^2), \quad (3)$$

where η is the viscosity, $\Delta p/L$ is the pressure gradient, h is the channel height and x is the position in the channel, such that $x = \pm \frac{h}{2}$ at the channel's surfaces. The dimensionless profile of the Poiseuille flow is shown in Fig. 2 b as a dashed line. To compute the theoretical curve, we used the simulated viscosity of our water model (TIP3P) at 298 K [37]. Some deviations from the theoretical predictions were observed near the surface of the channel, where the breakdown of the continuum description could be expected. Considerable deviations of the flow profile from the master curve were observed in the simulation at the smallest pressure gradient (0.70 bar/nm). The time scale of this simulation (20 ns) was too short for the flow velocity to attain a stable, symmetric profile, although the total water flux already attained a value consistent with the linear pressure–flux dependence, Fig. 2 a. Even though our temperature control applied only the silica membrane, the temperature of the entire system remained within 1.2% of that observed in the absence of the flow.

B. Spontaneous Adsorption of Proteins onto Channel Surfaces

Once we had tested our protocol for simulating a pressure-induced flow through a nanochannel, we applied this method to study the transport of proteins. In our first simulation, the two proteins were initially positioned near the surface with a minimum surface-to-protein distance of 0.55 nm (system A). For this system, we performed two simulations applying different pressure gradients, 0.69 bar/nm and 3.5 bar/nm, which lasted 97 and 86 ns, respectively. The proteins became bound to the surface of the nanochannel within 10 and 32 ns from the beginning of the simulations at 0.69 and 3.5 bar/nm pressure gradients, respectively. As depicted in Fig. 1, the proteins twist and roll as they flow through the nanochannel, adopting many conformations before eventually binding to the surface.

To determine the influence of the initial conditions on the protein orientation in the adsorbed state, we simulated two additional systems (B and C) that had the proteins positioned further away (1.15 nm) from the surface. These simulations were performed applying a pressure gradient of 0.69 bar/nm for 62 (system B) and 57 (system C) ns. Thus, from the four simulations with two proteins each, we have acquired eight independent protein trajectories in total. The protein motion through the channel is characterized in Fig. 3 a, which shows the closest distance between the protein's α -carbon atoms and the silica surface. The graph indicates that all proteins eventually bind to the surface. To determine if the proteins can tumble or slide while being in close proximity to the surface, we plotted in Fig. 3 b the distance each protein traveled along the direction of the flow. The figure indicates that regardless of the initial conformation and the applied pressure gradient, the distance travelled by a protein along the channel reaches a constant value, meaning that all proteins eventually bind to the silica surface and become immobile. Fig. 3 a and b also show that some proteins do not bind to the surface immediately after they first encounter the surface. Rather, they roll and slide along the surface for some time before becoming immobile.

C. Conformations of Adsorbed Proteins

Visual inspection of the obtained trajectories revealed that the proteins bound to the surface of the nanochannel in different conformations. To determine if there was any underlying order, we inspected the amino acid makeup of the protein parts that made the contact with the silica membrane. We defined an amino acid to be in contact with the silica surface if any of its non-hydrogen atoms were located within 5 Å (center to center) of any silica atom, and say it is bound if it is in contact for more than 75% of the time the protein was bound.

Table I and Fig. 4 summarize the results of our analysis. Thus, in eight binding events, different sets of amino acids were found to contact the silica surface. Furthermore, the contacts were found to contain clusters of neighboring residues, indicating that, in addition to any specific interactions between silica and particular types of amino acids, the three-dimensional structure of the protein plays a major role in determining which amino acids bind the surface. The protein–silica contacts were found to involve from 6 to 13 (± 1) protein residues and be from 4.0 to 6.5 nm² in area with the standard deviation ranging from 0.4 to 1.1 nm². The binding site, residues bound more than 75% of the time, contained all types of amino acids—acidic, basic, polar, nonpolar, aromatic; each binding site contained at least one charged residue.

In Fig. 4, we have calculated the fraction of the binding sites' surface area contributed by individual amino acids and compared that to the amino acid content of the protein surface in bulk water. The plot indicates that residues with bulky side chains are more likely to bind silica than residues with smaller side chains. This is most likely simply due to the fact that side chains with more atoms have more opportunities to be near the silica surface than side chains with fewer atoms. Apart from that observation, we could not determine any definite pattern to how the proteins bind. The binding did not appear to involve any particular set of residues, nor was there a preference for certain residue types to bind to the surface more frequently than the other. Thus, we concluded that the protein binding observed in our simulations was nonspecific.

D. Flow-Induced Desorption of Proteins

To determine the relative binding strength of each protein conformation, we attempted to remove the proteins from the surface by gradually ramping up the pressure gradient. We first increased the pressure gradients in the simulations of systems A, B and C from 0.69 to 3.45 bar/nm, the same pressure gradient we used in the second simulation of system A. The conformations of the two proteins in each systems appeared to be stable within the 10 ns simulation at the new pressure gradient: neither unbinding nor rolling was observed. Subsequently, we increased the pressure gradients (and hence the shear force of the flow on the protein) in 3.45 bar/nm increments, running the simulation at each pressure until the proteins were desorbed or shown to be stably bound for at least 10 ns. If the protein rolled on the surface and again became bound, the simulation was run until the protein was stable for 10 ns in the new conformation. From these simulations, we have calculated the mass flux of the protein–water mixture in the nanochannel as a function of the pressure gradient and plotted the result in Fig. 2 a, alongside the data for the pure water system. As in the case of the pure water system, the protein solution's mass flux was found to increase linearly with the pressure gradient, but with a slightly smaller slope, presumably because of the higher effective viscosity of the water-protein mixture.

The protein desorption process is characterized in Fig. 5, which is analogous to Fig. 3. The protein displacement in the direction normal to the surface of the channel is plotted in Fig. 5 a, which shows the closest distance between any α -carbon atom of the protein and the silica surface. The protein displacement in the direction of the flow is plotted in Fig. 5 b. The trajectories terminate when the proteins unbind. Fig. 5 reveals that protein desorption occurs in a wide range of pressure gradients: from 6.9 to 27.6 bar/nm. One protein failed to desorb in a 117-ns simulation where the pressure gradient was increased up to 38.0 bar/nm.

As the pressure gradients were increased, the proteins were observed to change their conformations presumably because of the increased shear stress. Thus over the course of the simulations, the center of mass of the bound proteins moved, on average, 0.3 nm closer to the silica surface, whereas the number of amino acid chains in contact with the surface and the contact area increased by 20–60 and 11–37 %, respectively. The change in the

conformation of the protein that failed to unbind, B1, was much larger, 53 and 225 %, respectively. Thus, it appears that the increasing shear forces of the flow press bound proteins toward the surface. In contrast, conformations adopted by proteins after they were observed to slide or roll on the surface and subsequently rebind showed little or no change with increased shear stress.

Table II lists the properties of the protein–silica contacts in the last stable conformation observed before the proteins unbind. Proteins that unbind and rebind are shown multiple times. The protein that did not unbind (B1) is shown as a reference. Table II does not reveal any simple correlation between the number of protein residues bound to the silica surface, their composition and contact area with their ability to maintain a bound conformation at high pressure gradients. These results reinforce the conclusion that the proteins bind nonspecifically to the silica surface. While we have investigated the residues of the protein involved in binding, our analysis did not include the atomic-scale heterogeneity of the silica surface. Each protein was bound to a different location on the silica surface, suggesting that the variations in protein binding sites and binding strengths may be, at least partially, attributed to variations in atomic-scale properties of the silica, such as surface roughness and number of dangling bonds [29].

IV. Conclusion

In this study, we have modeled the transport of proteins through silica nanochannels, and investigated the modes by which they bind to the silica surface. Reducing the strength of protein–surface interactions and/or designing surfaces to bind specific types of proteins is a task of outstanding importance. Through the use of combinatorial phage display techniques, researchers have been able to design short peptides, which bind specifically to particular inorganic surfaces [39]–[42]. These peptides can be included in a given protein to give it a moiety that will bind specifically to inorganic surfaces such as silica [43]. In contrast to such specialized proteins, we find that the binding of our model protein to silica is nonspecific, but is by no means trivial. Indeed, we have shown that nonspecific adhesion of proteins to inorganic surfaces can be of very high affinity and thus can have a dramatic effect on protein transport. We believe that the nonspecific binding of proteins to surfaces will be one of the most challenging obstacles to overcome in the next generation of nanofluidic devices, and our results serve to reinforce this belief.

Ideally, one would want to predict the adsorption constant of a given protein simply from the knowledge of its atomic structure and material properties of the nanochannel. In order to design surfaces which minimize such nonspecific binding, we need to better understand the mechanisms by which individual amino acids bind to inorganic surfaces. By combining such information with the knowledge of the atomic structure of the protein, the binding affinity of the entire protein could be described in statistical terms, which would have numerous applications in nanobiotechnology [44]–[47]. The availability of the atomic-scale models of the inorganic surfaces in aqueous environment would be critical to the success of such an approach, as their heterogeneous nature could only then be accurately taken into account. Our future studies will focus on detailing how such heterogeneity in silica surfaces impacts the binding of proteins and small molecules.

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Fig. 1. Spontaneous adsorption of a protein to the surface of a nanochannel. The protein is shown in cartoon representation; the silica nanochannel is shown as a gray molecule surface. Water and counterions are not shown. The snapshots represent a typical MD simulation of protein flow through a nanochannel. The water velocity profile is shown schematically as dashed lines and arrows.

Fig. 2.

MD simulations of water flow through a nanochannel. a) Mass flux versus pressure gradient in the water-only (black) and protein-water (gray) systems. The inset shows an enlarged view of the main plot at small pressure gradients. b) Simulated water velocity profile. The dimensionless average velocity of the water flow is plotted versus the relative distance from the nanochannel walls. The normalized velocity profiles are shown for five simulations at different pressure gradients; the symbols are defined in panel a. The theoretical Poiseuille flow profile is shown as a dashed line. The simulated absolute flow velocities at the center of the nanochannel were 0.39, 2.4, 4.6, 24, and 48 nm/ns for the pressure gradients of 0.70, 3.5, 7.0, 35 and 70 bar/nm, respectively.

Fig. 3.

Spontaneous adsorption of proteins to the nanochannel surface. (a) The shortest distance between the channel surface and any α -carbon atom of the protein. (b) The distance travelled by a protein in the channel in the direction of the flow. The center of mass of the protein is plotted relative to its initial position in the channel. The data for the two proteins of system A were obtained at two pressure gradients, shown in blue (0.69 bar/nm) and red (3.45 bar/nm); the data for the proteins in systems B and C were obtained at 0.69 bar/nm and are shown in black. Only the first 65 ns of the simulations of system A are shown.

Fig. 4.

Amino acid content of protein–nanochannel contacts. (a) Fractional area of the solvent accessible surface of the protein in bulk water associated with each amino acid type. The solvent-accessible area was calculated using VMD [38] and a solvent probe radius of 1.4 Å. The values given were obtained by averaging over an ensemble of instantaneous conformations observed within the last 30 ns of a 50 ns trajectory sampled every 5 ps. (b) Fractional area of the protein–silica contact associated with each amino acid type. The contact area was defined as a solvent-accessible area of a silica membrane located within 5 Å of the protein. The data shown were obtained by averaging over ensembles of conformations observed in all bound states, weighted with the respective average area of the contact and sampled every 5 ps. The sum of the fractional area over all amino acid types is greater than 1 because the same silica atoms can be in contact with more than one amino acid. The protein studied does not contain Met residues.

Fig. 5.

Flow-induced desorption of proteins bound to nanochannel surfaces. (a) The shortest distance between the channel surface and any α -carbon atom of the protein. (b) The distance travelled by a protein in the channel in the direction of the flow. The center of mass of the protein is plotted relative to its initial position in the channel. During desorption, proteins were observed to roll along the channel surface and occasionally rebind before entering bulk water. The data are colored according to the pressure gradient.

TABLE I

Properties of the protein–silica contacts for the two proteins from each of the four simulations.

System-Protein	VP (bar/nm)	# Res. in contact	Bound residues	Surface area (nm ²)
A-1	0.69	8.0 ± 1.4	Asp9 Asp53 His54 Arg56 Cys63 His64	4.0 ± 0.4
A-2	0.69	6.0 ± 1.4	Vall Asp53 Pro60 His64	4.9 ± 0.7
A-1	3.5	8.5 ± 1.4	Asp9 Val10 Asn11 Lys30 His54 Arg56 His64	5.8 ± 1.1
A-2	3.5	13.0 ± 1.1	Arg18 Asn19 Glu32 Ser33 Tyr35 Cys36 Gln37 Trp38 Ala39 Tyr49 Lys50 Arg62 His64	6.5 ± 0.5
B-1	0.69	6.4 ± 1.2	Asp9 Lys28 Lys30 His54 Arg56	4.3 ± 0.4
B-2	0.69	8.7 ± 1.3	Ala39 Ser40 Pro41 Tyr42 Gly43 Arg62 His64	4.5 ± 0.6
C-1	0.69	8.9 ± 1.4	Asp53 Arg56 Lys58 Pro60 Arg62	5.3 ± 0.8
C-2	0.69	9.6 ± 1.2	Asp3 Glu32 Tyr35 Trp38 Ala39 Tyr49 Lys50	5.1 ± 0.4

TABLE II

Properties of the protein–silica contacts at the highest pressure gradients (∇P) for which the proteins remained bound to the surface. Proteins listed more than once were observed to unbind and rebind.

System-Protein	∇P (bar/nm)	# Residues in contact	Bound residues	Surface Area (nm ²)
A-2	3.5	6.5 ± 1.3	Val1 Asp53 Pro60 Cys63 His64	4.9 ± 0.4
B-2	3.5	8.7 ± 1.3	Ala39 Ser40 Pro41 Tyr42 Gly43 Asn44 Arg62	4.4 ± 0.6
C-1	6.9	9 ± 2	Asn11 Asp53 His54 Arg56 Pro60 Arg62	6.2 ± 1.0
A-2 [*]	10.4	12.0 ± 0.8	Arg18 Asn19 Ala20 Asn23 Glu32 Ser33 Tyr35 Ala39 Ser40 Pro41 Tyr42 Lys50	5.4 ± 0.3
C-1 [*]	10.4	6.5 ± 1.5	Asp9 Lys30 His54	4.6 ± 0.7
A-1	13.8	9 ± 2	Asp9 Val10 Glu24 Lys28 Lys30 Arg56 Arg62 Cys63 His64	4.3 ± 1.3
B-2 [*]	13.8	13.8 ± 1.6	Asp9 Val10 Phe15 Gly17 Arg18 Tyr21 Ala39 Ser40 Pro41 Arg62 His64	7.7 ± 0.7
C-2	17.3	11.3 ± 1.1	Asp3 Glu32 Tyr35 Trp38 Ala39 Tyr47 Tyr49 Lys50 Arg62 His64	6.6 ± 0.5
B-2 [*]	24.2	9.8 ± 0.9	Asp8 Asp9 Val10 Arg18 Tyr21 Glu24 Lys28 Arg56 Arg62	5.0 ± 0.4
B-1 [†]	27.6	13.0 ± 0.5	Val1 Lys2 Asp8 Asp9 Val10 Asn11 Asp53 His54 Arg56 Thr57 Lys58 Arg62 His64	6.6 ± 0.2

^{*} Conformations due to rebinding.

[†] This protein did not unbind.