

# Adhesion forces between functionalized latex microspheres and protein-coated surfaces evaluated using colloid probe atomic force microscopy

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## Abstract

Proteins are important in bacterial adhesion, but interactions at molecular-scales between proteins and specific functional groups are not well understood. The adhesion forces between four proteins [bovine serum albumin (BSA), protein A, lysozyme, and poly-D-lysine] and COOH, NH<sub>2</sub> and OH-functionalized (latex) colloids were examined using colloid probe atomic force microscopy (AFM) as the function of colloid residence time ( $T$ ) and solution ionic strength (IS). For three of the proteins, OH-functionalized colloids produced higher adhesion forces to proteins (2.6–30.5 nN; IS = 1 mM,  $T$  = 10 s) than COOH- and NH<sub>2</sub>-functionalized colloids (1.6–6.8 nN). However, protein A produced the largest adhesion force ( $8.1 \pm 1.0$  nN,  $T$  = 10 s) with the COOH-functionalized colloid, demonstrating the importance of specific and unanticipated protein–functional group interactions. The NH<sub>2</sub>-functionalized colloid typically produced the lowest adhesion forces with all proteins, likely due to repulsive electrostatic forces and weak bonds for NH<sub>2</sub>–NH<sub>2</sub> interactions. The adhesion force ( $F$ ) between functionalized colloids and proteins consistently increased with residence time ( $T$ ), and data was well fitted by  $F = AT^n$ . The constant value of  $n = 0.21 \pm 0.07$  for all combinations of proteins and functionalized colloids indicated that water exclusion and protein rearrangement were the primary factors affecting adhesion over time. Adhesion forces decreased inversely with IS for all functional groups interacting with surface proteins, consistent with previous findings. These results demonstrate the importance of specific molecular-scale interactions between functional groups and proteins that will help us to better understand factors colloidal adhesion to surfaces.

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## 1. Introduction

Bacterial cell surfaces are composed of a complex mixture of proteins, phospholipids, polysaccharides, lipopolysaccharides (LPS), and other biopolymers. The presence and physicochemical properties of these biopolymers are known to be an important factor in bacterial adhesion to a surface in a variety of fields including bioremediation [1], membrane [2], biomaterial development [3], and marine biofouling [4,5]. Bacterial attachment to a solid surface is often modeled as a function of electrostatic, van der Waals, acid-base interactions and/or hydrophobic forces using extended DLVO (XDLVO) theory [6]. The primary limitation of the XDLVO theory is that it does not sufficiently incorpo-

rate contributions of specific biopolymers and functional groups on the bacterial surface during an adhesion event. Biopolymers on a bacterial cell surface can exhibit repulsive or attractive interactions with the surface [7]. These biopolymers contain a variety of different functional groups that can individually interact with the surface, and mediate the bioadhesion process [8,9].

Lipopolysaccharides (LPS) and proteins in the outer membrane of bacteria, and extracellular polymeric substances (EPS) on the surface of the bacteria, all have been found to influence bacterial adhesion [10–12]. The surface of *Escherichia coli* JM 109 contains 75% LPS and 25% proteins [13]. Measurements using atomic force microscopy (AFM) show that removal of the LPS from a bacterial surface decreases adhesion (between the cell and the AFM tip) by up to ~80% [14]. The amount of EPS has been correlated with bacterial adhesion to a surface, with EPS-rich strains generally demonstrating larger adhesion to a

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surface due to polymer–surface interactions [15]. Proteins on the outer surface of the bacteria, and on the adhering surface, are also known to be important to the initial attachment of bacteria to a surface. The expression of specific proteins, such as those used for iron reduction, can greatly increase the adhesion between a microbe and a surface [16]. The formation of specific bonds between proteins on the surface of living *E. coli* cells and a solid substrate can also be observed using AFM and confocal laser scanning microscopy [17]. In parallel-plate chamber experiments, extracellular proteins were shown to be released from the bacteria into solution, and then adsorbed onto the support surface. These proteins promoted initial bacterial adhesion events and the continued secretion of proteins during prolonged contact between the cell and surface further strengthened adhesion [18].

The interaction between proteins and surfaces can be examined in terms of adhesion forces using colloid probe AFM. Adhesion between protein-coated or bare colloids (latex and glass) and protein-coated glass surfaces varies as a function of ionic strength (IS), pH, loading force, and residence time [19,20]. The adhesion force between a colloid and a protein-coated surface increases with residence time ( $T$ ) and decreases with IS and pH. The increase in adhesion with time is a result of water exclusion, polymer rearrangement, and conformation changes in the proteins as well as the formation of hydrogen bonds [19,20]. Adhesion forces between proteins and surfaces are larger than those produced by a polysaccharide (dextran). While adhesion between proteins and surfaces can be measured, the specific molecules that promote increased or decreased adhesion forces have not been identified.

LPS, EPS, and proteins on bacterial cell surfaces can be viewed as polymers containing carboxylic, phosphoric, phosphodiester, amino, and hydroxyl groups [21]. Each of these functional groups can interact in different ways with a mineral surface or organic coatings on a surface. For example, the hydroxyl groups of LPS of gram-negative bacteria are thought to form hydrogen bonds with surface hydroxyl groups of  $\text{SiO}_2$  and surface-bound water, thereby dominating bacteria–surface interactions during adhesion [7]. The magnitude of the adhesion force of a functional group to a surface is related to the chemical nature of the binding site and the binding capacity. Titration of bacterial surfaces over a pH range of 4–10 suggests the presence (on the basis of  $\text{p}K_a$  values) of carboxylic (low  $\text{p}K_a$  values) and amine (high  $\text{p}K_a$  values) functional groups on the bacterial surface [21,22]. The binding capacity of these functional groups varies for different bacterial strains. It has been shown that the total capacity of binding sites of EPS on a bacterial surface can vary 20–30-fold [23], demonstrating the importance of specific components in the EPS for bacterial adhesion.

In order to better understand interactions between specific functional groups and organic-coated surfaces, we measured interaction forces between a functionalized colloid and different surfaces. The use of functionalized tips or colloids allows identification of specific interactions of chemical groups with other materials [24–26]. Colloid probes functionalized with different functional groups ( $\text{NH}_2$ ,  $\text{COOH}$ ,  $\text{OH}$ ), which are ubiqui-

itous on bacterial cell surface and proteins, were used to probe adhesion forces with bare and protein-coated surfaces. These forces were measured as a function of IS and residence time since these are two main factors influencing adhesion [27,28].

## 2. Material and methods

### 2.1. Functionalized colloids and surfaces

Three different functionalized polystyrene latex microspheres ( $\text{NH}_2$ ,  $\text{COOH}$ ,  $\text{OH}$ ) with a diameter of  $3.0 \pm 0.15 \mu\text{m}$  (Polysciences, PA) were mounted onto AFM tips to make colloid probes. Since the nature of the underlying colloid can affect the overall interaction force even when covered with a protein [19], a bare latex microsphere (latex) was used for comparison with functionalized colloids. Latex colloids were cleaned by rinsing three times with Milli-Q water (with centrifugation) prior to use. Micro cover glasses (12 Cir-1, VWR Scientific, West Chester, PA) were rigorously cleaned [29], rinsed with copious amount of ultrapure water (Milli-Q), and stored in ultrapure water at  $4^\circ\text{C}$  before being coated with proteins.

### 2.2. Covalent bonding of proteins to glass surfaces

Four different proteins were covalently bonded to glass surfaces: bovine serum albumin (BSA), lysozyme from chicken egg white, protein A and poly-D-lysine (Sigma, St. Louis, MO). The isoelectric points of proteins, calculated using the Protein Calculator Program and the protein sequences (Scripps Research Institute), increased in the order: protein A  $\leq$  BSA  $<$  lysozyme  $<$  poly-D-lysine (Table 1; from Ref. [19]). BSA and protein A are negatively charged and lysozyme and poly-D-lysine are positively charged at pH 7.

Cover glasses were modified by bonding an amino-functional group to the glass, and then covalently bonding a protein to this amino group as previously described [19]. Briefly, the cleaned glass was soaked in 10 ml of a 10% solution of 3-aminopropyl trimethoxysilane (Fluka) in methanol for 15 min, and then rinsed with methanol followed by ultrapure water to remove excess silane. Proteins were covalently bonded to the silanized glass by first modifying them with 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDC, Pierce) and *N*-hydroxysulfosuccinimide (sulfo-NHS, Pierce). EDC (100 mM) was added into protein solution and reacted for 5 min to form an unstable intermediate, *O*-acylisourea, and then a sulfo-NHS solution in a phosphate buffer (40 mM) was added into the solution for another 5 min to form the stable intermediate with NHS groups. This protein–NHS solution was poured onto the amino-modified glass cover slip, and allowed to react overnight (with shaking at 50 rpm) to covalently bind the protein onto glass through formation of a CO/NH bond. The glass was rinsed with Milli-Q water to remove the unreacted protein-NHS before AFM experiments. Two glass surfaces lacking a protein coating were used as the controls. One was a cleaned and bare glass surface, and the other one was the amino-functionalized glass prepared using silane.

### 2.3. Zeta-potentials measurements

The zeta-potentials of latex colloids and protein-coated glass beads in phosphate buffer solution (PBS; IS = 1 or 100 mM, pH 7.3) were measured three times with 30 runs per analysis using a zeta-potential analyzer (Zetasizer Model Nano-ZS, Malvern Instruments, Worcestershire, UK).

### 2.4. Colloid probes

Colloid probes were prepared by gluing (DEVCON epoxy S-31/31345) a latex microsphere to a tipless silicon nitride cantilever (Veeco NanoProbe NP-OW) as previously described [30]. The spring constant of cantilevers (all taken from the same wafer) of each probe was determined using the thermal tuning method (Nanoscope V6.12r2), with values ranging from 0.056 to 0.072 N/m. After preparation, the colloid probe was stored in the refrigerator (4 °C) prior to use. Multiple probes were prepared for each type of colloid at the same time in order to obtain consistent responses of the probes between experiments. Each probe was inspected before an experiment using a microscope (Olympus IX70) to ensure probe integrity. Any broken or damaged probes were discarded and immediately replaced with a new probe prepared from the same batch. Each probe was tested for a consistent response to a clean glass surface prior to an experiment, and any probe that did not display behavior consistent with other probes from that batch was discarded.

### 2.5. AFM experiments

Adhesion force measurements were made using a Multimode AFM with a PicoForce and Nanoscope IIIa control systems (Veeco, Santa Barbara, CA) (Version 6.12r2). The piezoelectric scanner was calibrated using a standard topography reference grid (3  $\mu\text{m}$  pitch, Digital Instrument, Santa Barbara, CA) to ensure scanner accuracy. All force measurements were made in phosphate buffer solutions (IS = 1 or 100 mM; pH = 7.3) at scan rate 1 Hz and z scan size of 1  $\mu\text{m}$ . The loading force applied to each protein surface was fixed at ca. 5.4 nN by setting the trigger threshold of the cantilever at  $\sim 90$  nm depending on spring constant. The ramp delay with trigger mode in the software was set at different times producing different colloid residence times. Force measurements were performed at residence times of 0.001–100 s at four to five randomly selected locations over every sample studied. Force measurements taken at longer times (100–200 s) were unstable. Interaction force curves were shown to be reproducible for the same operating conditions by examining different locations on the surface. Large changes in the force curves in the case of protein-coated surfaces were assumed to indicate a location that did not contain any protein, and therefore other locations were selected. A consistent pattern of non-reproducible force curves was assumed to result from tip contamination, and therefore the probe was discarded. The adhesion force is the value measured at the point of maximum deflection during the colloid probe retraction from the surface. Ten force curves were obtained for each residence time at each location (1  $\mu\text{m} \times 1 \mu\text{m}$  area), with a total of ca. 50 force

curves used for each calculated average adhesion forces (error bars  $\pm$  S.D.).

### 2.6. Calculation of contact area

The contact radius and area of the interface region between a colloid and glass surface coated with protein was calculated using the JKR model which has been described elsewhere [19,31].

## 3. Results

### 3.1. Characteristics of proteins and colloids

Protein coatings on the glass surface varied in size, as indicated by the roughness of the coatings (Fig. 1). The roughness of the bare glass was  $R_q = 0.06 \pm 0.01$  nm, with the roughness of the protein-coated surfaces 6–25 times larger (Table 1). On the basis of the surface roughness, the maximum thickness of the protein coatings was calculated to decrease in the order poly-D-lysine > protein A > BSA > lysozyme. Except for protein A, the order of coating thickness follows that of the molecular weight of the proteins (Table 1). AFM images show the domain sizes of protein ranging from about 5 to 10 nm for BSA to 50 to 100 nm for poly-D-lysine. The amount of each protein bonded to a glass cover slip surface was: BSA, 51.7 mg/m<sup>2</sup>; lysozyme, 5.6 mg/m<sup>2</sup>; protein A, 47.1 mg/m<sup>2</sup>; and poly-D-lysine, 9.7 mg/m<sup>2</sup>.

The contact area of the interface region between a colloid and surface was estimated using JKR model as shown in Table 2. The contact area varied with the protein-coated surfaces and functionalized colloids. Colloids and bare glass produced the smallest contact area (500–600 nm<sup>2</sup>) while the poly-D-lysine coated surface produced largest contact area (2556 nm<sup>2</sup>, residence time of 0.001 s). The number of protein molecules interacting with the colloid varied for the different proteins. For example, based on the size and packing of the proteins on the surface, it was estimated that about 5–10 BSA molecules interacted with the colloid, while for poly-D-lysine it was only one to two molecules. Thus, the magnitude of the adhesion force varies among proteins just due to the number of contacts between protein molecules and the colloid. The contact radius for any one protein, however, varied over a small range as shown in Table 2.

Zeta-potentials of silanized glass beads were smaller than those of the bare glass beads (1 and 100 mM IS solutions) due to the positively charged amino-functional groups on the bead surface (Fig. 2). The zeta-potentials of uncoated (bare) glass beads were larger than those of the glass beads coated with proteins in 1 or 100 mM IS solutions (Fig. 2), indicating proteins partially neutralize the negative charge on glass surface due to localized positive charges in the protein. Zeta-potentials decreased in the order: BSA  $\geq$  protein A  $\geq$  lysozyme  $\geq$  poly-D-lysine. This ordering was consistent with the calculated relative charges of the proteins at neutral pH (Table 1). In the 100 mM solution, the zeta-potentials for colloids coated with BSA, protein A and lysozyme were similar and therefore the nature of the proteins could not be distinguished based on overall charge at this ionic

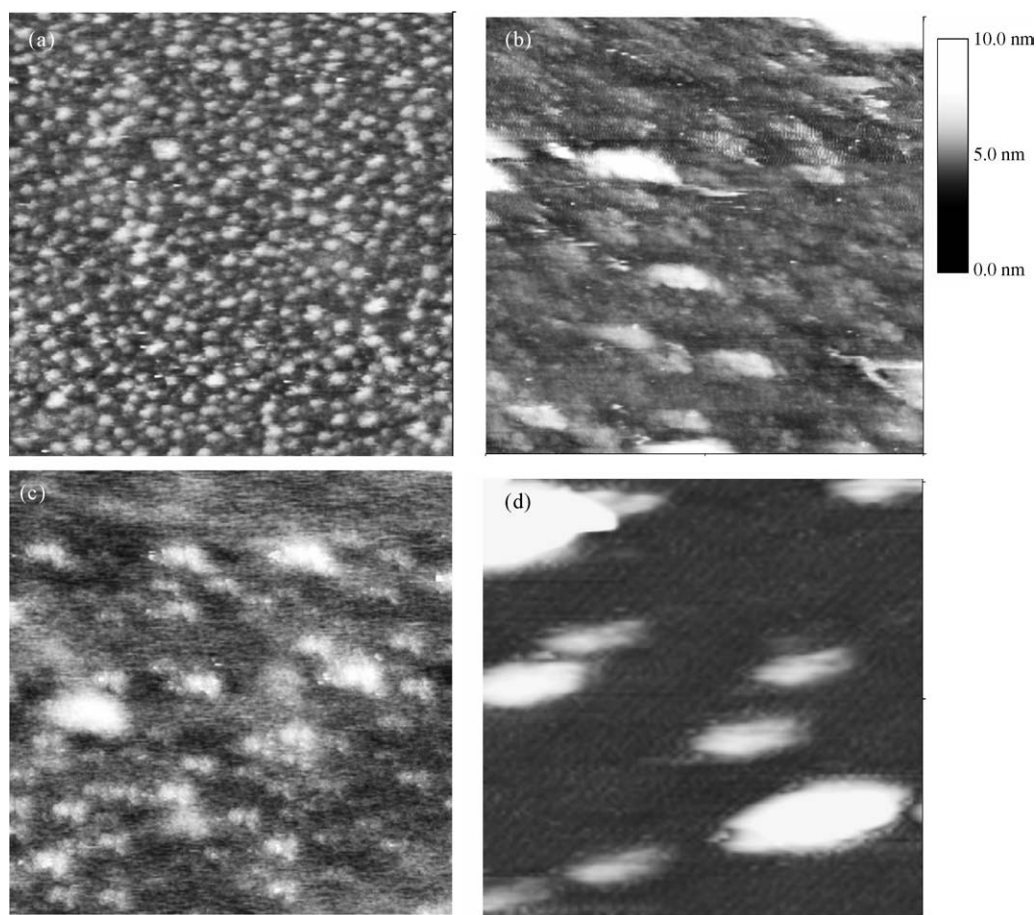


Fig. 1. AFM topographic images of proteins coated on the glass surface in 1 mM IS: (a) BSA, (b) protein A, (c) lysozyme, and (d) poly-D-lysine (size: 500 nm  $\times$  500 nm).

strength. Zeta-potentials for poly-D-lysine coated glass surfaces remained negative (ca.  $-10$  mV), indicating the heterogeneous distribution of poly-D-lysine on glass surface, as was directly observed in Fig. 1d.

The latex microspheres had the highest zeta-potentials with  $-139 \pm 8$  mV in 1 mM IS and  $-77 \pm 10$  mV in 100 mM IS (Fig. 2b). Functionalizing the microspheres decreased the overall zeta-potential of the latex microspheres in the order OH > COOH > NH<sub>2</sub>. This ordering showed that adding a neutrally charged functional group such as OH had little effect on the overall surface charge, while adding a charged functional group (e.g., COOH or NH<sub>2</sub>) decreased the overall surface charge. In all cases, colloid zeta-potentials were inversely related to IS, as expected [32].

### 3.2. Colloid adhesion to bare glass surfaces

The adhesion forces of functionalized latex microspheres to glass surfaces, measured using retraction force curves, increased with the residence time and IS (Fig. 3). The adhesion forces of functionalized microspheres to a bare glass surface decreased in the order: OH > COOH > NH<sub>2</sub>  $\geq$  latex in either the low or high IS solutions. For example, the adhesion forces for OH, COOH, NH<sub>2</sub>, and latex-functionalized microspheres at residence time of 10 s were  $2.9 \pm 0.7$ ,  $1.2 \pm 0.5$ ,  $0.4 \pm 0.2$ ,  $0.03 \pm 0.007$  nN in IS = 1 mM, but increased to  $3.3 \pm 0.9$ ,  $1.9 \pm 0.7$ ,  $0.7 \pm 0.6$ ,  $0.6 \pm 0.3$  nN in 100 mM solution, respectively. The higher adhesion forces observed between the OH-functionalized microsphere and glass surface likely resulted from the formation of

Table 1  
Properties of proteins and coatings

Protein or coating	Theoretically calculated			AFM measured	
	Molecular weight	Isoelectric point	Charge at pH 7.0	Roughness ( $R_q$ , nm)	Thickness (nm)
BSA	66k	6.17	-9.9	$0.53 \pm 0.10$	$5.1 \pm 0.5$
Protein A	55k	5.71	-6.8	$0.81 \pm 0.17$	$7.7 \pm 1.5$
Lysozyme	14k	9.04	+7.9	$0.36 \pm 0.09$	$3.5 \pm 1.0$
Poly-D-lysine	134k	12.9	$\sim +900$	$1.7 \pm 0.8$	$14.3 \pm 4.9$



Table 2  
Contact radius and area between a colloid and bare glass or protein-coated glass surfaces calculated using the JKR model

Surface	Colloid	Contact radius (nm)	Contact area (nm <sup>2</sup> )
Bare glass	Latex	12.9	523
	OH	14.5	656
	COOH	13.1	539
	NH <sub>2</sub>	13.6	584
BSA	Latex	14.1	622
	OH	13.0	534
	COOH	14.2	633
	NH <sub>2</sub>	13.7	591
Protein A	Latex	14.7	681
	OH	15.8	779
	COOH	21.2	1415
	NH <sub>2</sub>	15.2	726
Lysozyme	Latex	24.2	1846
	OH	27.1	2304
	COOH	15.1	718
	NH <sub>2</sub>	14.7	678
Poly-D-lysine	Latex	28.5	2556
	OH	26.5	2206
	COOH	21.2	1410
	NH <sub>2</sub>	18.8	1102

Data based on the AFM measurements at residence time 0.001 s.

relatively strong bonds between OH and SiO<sub>2</sub> groups, compared to those of COOH–SiO<sub>2</sub> or NH<sub>2</sub>–SiO<sub>2</sub>. Although the NH<sub>2</sub>-functional group is positively charged at neutral pH, NH<sub>2</sub>-functionalized colloids exhibited low adhesion forces to the negatively charged glass surface. This result indicated that adhesion force could not be predicted solely on the basis of the electrostatic charge, and that molecular-scale factors dominated in the adhesion event.

The adhesion forces between the colloids and the silanized glass surfaces (amino-functionalized) were much larger than those for the bare glass surface (Fig. 4), indicating that the electrostatic forces and bonds formed between amino functional groups on the glass surface and functionalized colloids largely contributed to the adhesion forces. The adhesion forces decreased in the order of OH > COOH > latex > NH<sub>2</sub> in both

low and high IS solutions. The adhesion forces between the latex colloid and the silane glass were larger than those between NH<sub>2</sub> colloid and silane glass, but smaller than the forces of OH and COOH colloids. This result suggested that the electrostatic charge was not the predominated factor affecting the adhesion forces between amino-functionalized glass and OH or COOH colloids. The interaction forces between amino-functionalized glass surface and various functional groups can be expected to decrease in the order OH > COOH > NH<sub>2</sub>.

### 3.3. Colloids adhesion to BSA-coated glass surfaces

At short residence times ( $T < 0.1$  s) there was no significant difference ( $p > 0.05$ , ANOVA) of the adhesion forces measured for the different functionalized colloids to the BSA-coated surface. The adhesion force of all functionalized colloids with the BSA-coated surface increased over time, although the NH<sub>2</sub>-functionalized colloid increased only slightly after  $T = 1$  s (from  $1.3 \pm 0.3$  nN at  $T = 1$  s to  $2.2 \pm 0.2$  nN at  $T = 100$  s) (Fig. 5a). In contrast, the adhesion forces measured for the COOH and OH-functionalized colloids increased by factors of four and seven times at  $T = 100$  s, compared to the forces at  $T = 1$  s. At the longest residence time of  $T = 100$  s, the adhesion forces significantly decreased ( $p < 0.05$ , ANOVA) in the order OH > COOH > latex > NH<sub>2</sub> in both 1 and 100 mM solutions (Fig. 5). In all cases, the adhesion forces measured for colloids and BSA coated glass surfaces were inversely related to solution IS, consistent with previous results [20]. This change in adhesion is thought to be primarily due to conformational changes in the proteins caused by changes in IS. A high IS reduces the protein size (i.e. a salting out effect), decreasing the bonds available for adhesion to a surface, resulting in lower adhesion forces (Fig. 5) [20].

One concern with using the EDC/sulfo-NHS reaction to covalently bond COOH groups in the protein to the NH<sub>2</sub>-surface is that there is also a possibility of bonding COOH to NH<sub>2</sub> groups within the protein. Such intra-protein bonds, if extensively occurring, could lead to a different behavior of the modified protein than that of the unreacted protein. To determine if this bonding procedure affected the force curve results, protein

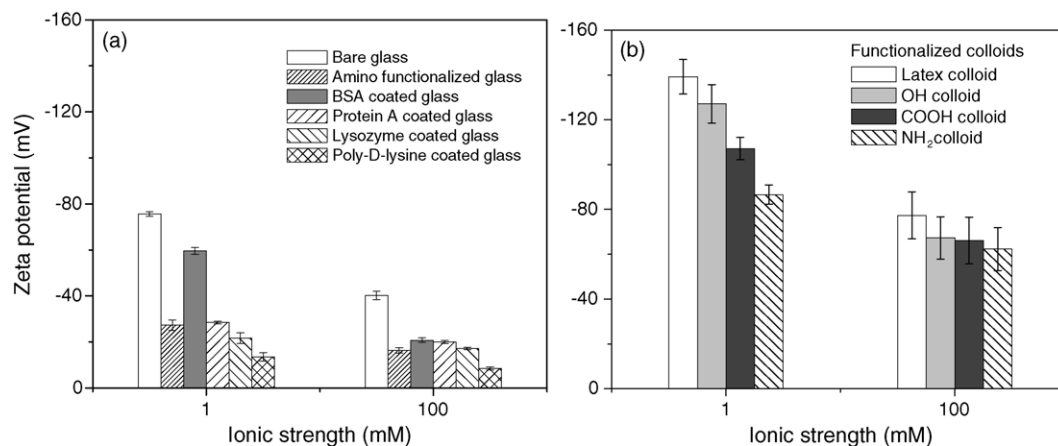


Fig. 2. Zeta-potentials of colloids in 1 and 100 mM IS solutions at pH 7.3: (a) glass beads coated with various proteins and (b) functionalized latex microspheres.

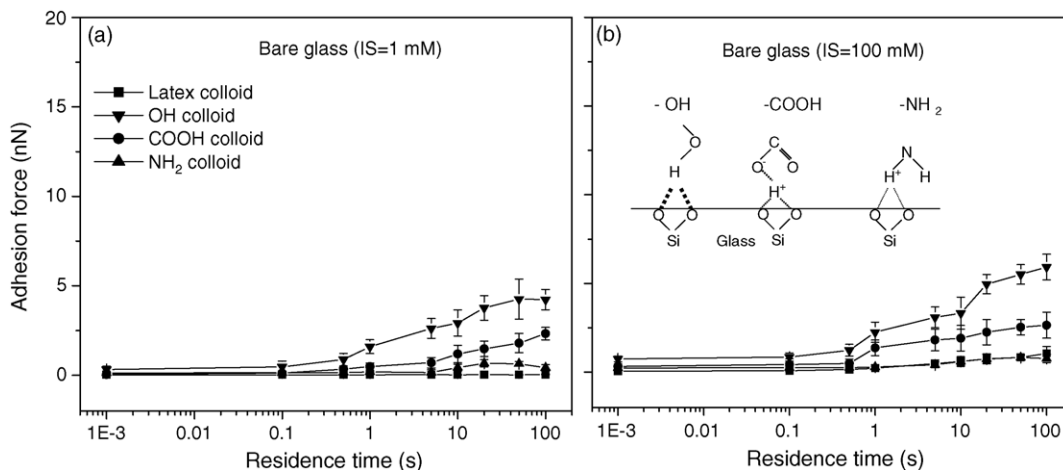


Fig. 3. Adhesion forces of colloid to bare glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.

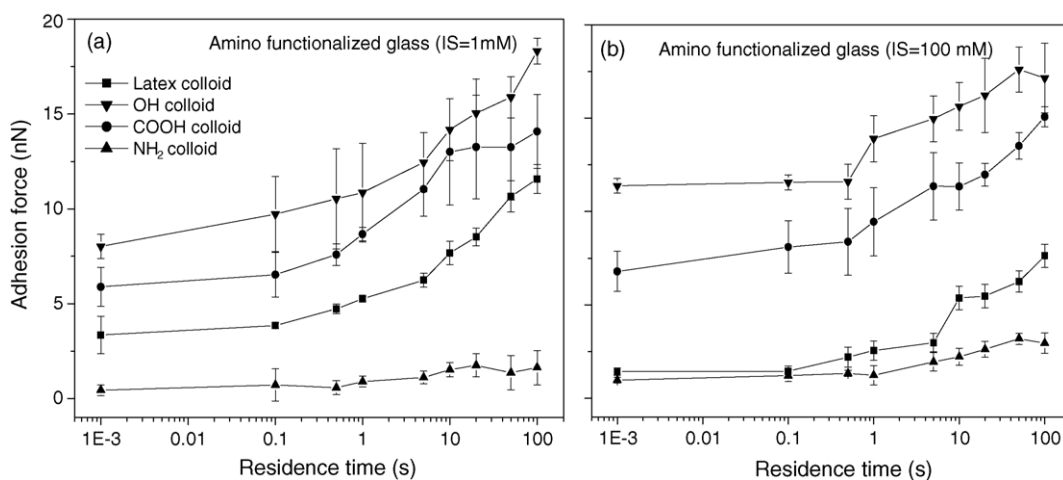


Fig. 4. Adhesion forces of colloid to amino functionalized glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.

was allowed to react overnight with the silanized glass surface in the absence of the EDC/sulfo-NHS chemicals. The magnitude of the adhesion forces, and the change in the adhesion force over time, was the same as that obtained using the covalently bonded

proteins (data not shown). This suggested that the protocol used to bind the protein to the surface did not adversely affect the protein relative to measurements of interest here. By using this binding procedure, it was possible to minimize the potential for

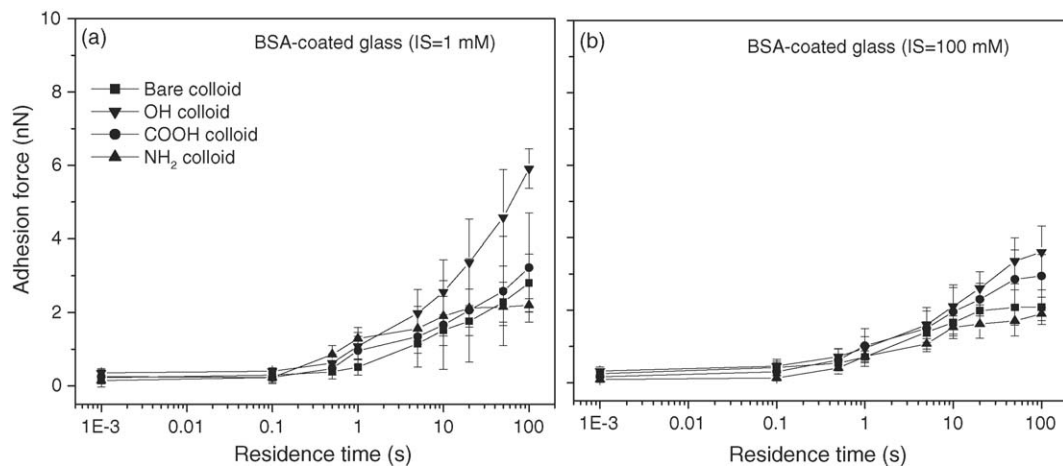


Fig. 5. Adhesion forces of colloid to BSA-coated glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.

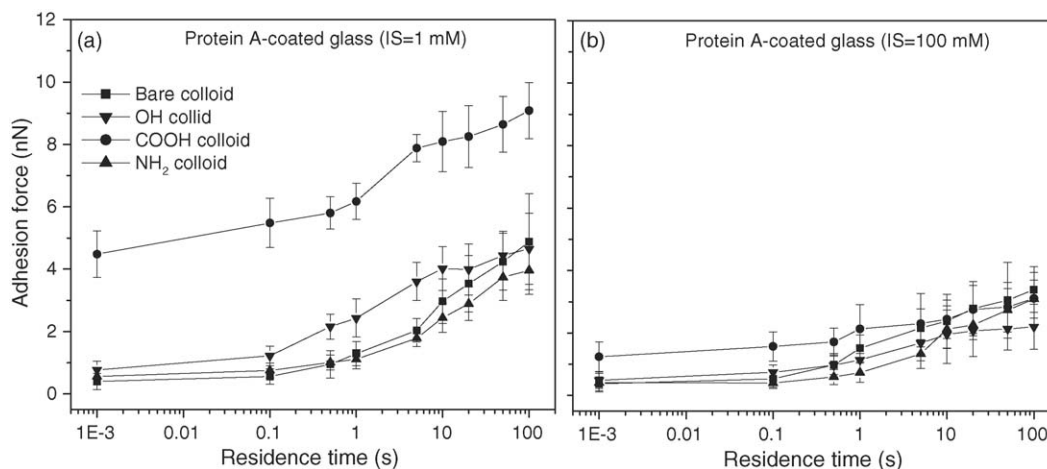


Fig. 6. Adhesion forces of colloid to protein A-coated glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.

protein desorption from the surface during force imaging, as well as contamination of the colloid tip.

### 3.4. Adhesion of colloids to protein A-coated glass surfaces

The adhesion forces between the functionalized colloids and protein A-coated glass surface did not exhibit the same trend with the different functional groups on the colloid, even though protein A and BSA are similarly negatively charged at neutral pH. For the protein A-coated surface, the adhesion decreased in the order  $\text{COOH} \gg \text{OH} > \text{latex} \geq \text{NH}_2$  (IS = 1 mM, ANOVA  $p$ -value < 0.05 at 0.001 s) (Fig. 6a). The adhesion forces for the COOH-functionalized colloid at IS = 1 mM were consistently larger than those measured for the other colloids. Even at the shortest residence time of  $T=0.001$  s, the force between the COOH colloid and protein A ( $4.5 \pm 0.8$  nN) was six times larger than values for the other types of colloids. After  $T=100$  s, this adhesion force ( $9.1 \pm 0.9$  nN) was still larger than that of the other colloids, although only by a factor of 2. At IS = 100 mM, all adhesion forces were smaller than those measured for IS = 1 mM (Fig. 6b).

The much larger adhesion force at short times observed for the COOH-functionalized colloid and the protein A surface can be better understood by examining approach curves. The approach force curve for COOH-functionalized colloid at IS = 1 mM demonstrated the presence of a large “jump-in” attractive force of  $0.7 \pm 0.3$  nN (Fig. 7). No “jump-in” attractive forces were observed with latex, OH, or  $\text{NH}_2$  colloids. This attractive force upon the colloid approach could arise from

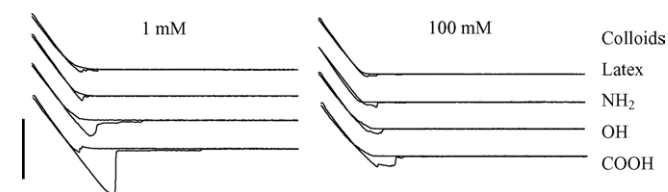


Fig. 7. Representative interaction force curves of protein A and various functionalized colloids at residence time 0.001 s (bar = 5.4 nN).

electrostatic interactions between the negatively charged COOH group and localized positive charges on the protein A surface. In 100 mM IS solution, the initial adhesion forces were all low, and there were no large “jump-in” attractive forces observed. However, a larger adhesion force was still measured for COOH colloid (Fig. 7). These results show the presence of specific protein–functional group interactions that were not expected based on overall surface electrostatic charge.

### 3.5. Colloid adhesion to lysozyme-coated glass surfaces

Adhesion forces measured during functionalized colloid retraction for the lysozyme surface increased with residence time as observed for the other protein-coated surfaces (Fig. 8). At  $T=50$  s, adhesion forces decreased in the order:  $\text{OH} > \text{latex} \gg \text{COOH} \geq \text{NH}_2$  either in 1 or 100 mM IS solutions (Fig. 8). The most notable difference for the lysozyme-coated surface, compared to the other protein-coated surfaces, were the high initial adhesion forces for the OH-functionalized and bare latex colloid. This high adhesion force was not expected based on consideration of overall electrostatic charges of the different surfaces.

The approach curves can be used to explain the strong adhesion force at short times observed for the OH-functionalized and bare latex microspheres. Approach force curves for the lysozyme surfaces demonstrated the presence of a “jump-in” during OH colloid approach at IS = 1 and 100 mM, but not for either the COOH or  $\text{NH}_2$  colloids (Fig. 9). The magnitudes of the jump-in forces were  $1.0 \pm 0.3$  and  $1.9 \pm 0.5$  nN for the bare latex and the OH-functionalized colloids, respectively. These large attractive forces likely arise from electrostatic interactions between the negatively charged colloids and positively charged lysozyme surface. The bare latex and OH-functionalized colloids had more negative zeta-potentials than the carboxyl- and amino-functionalized colloids (Fig. 2).

In high IS solution (100 mM), the “jump-in” attractive forces in approaching curves decreased due to the compressed double electric layers when the latex and hydroxyl-functionalized colloids approached to the lysozyme surface (Fig. 9). The measured

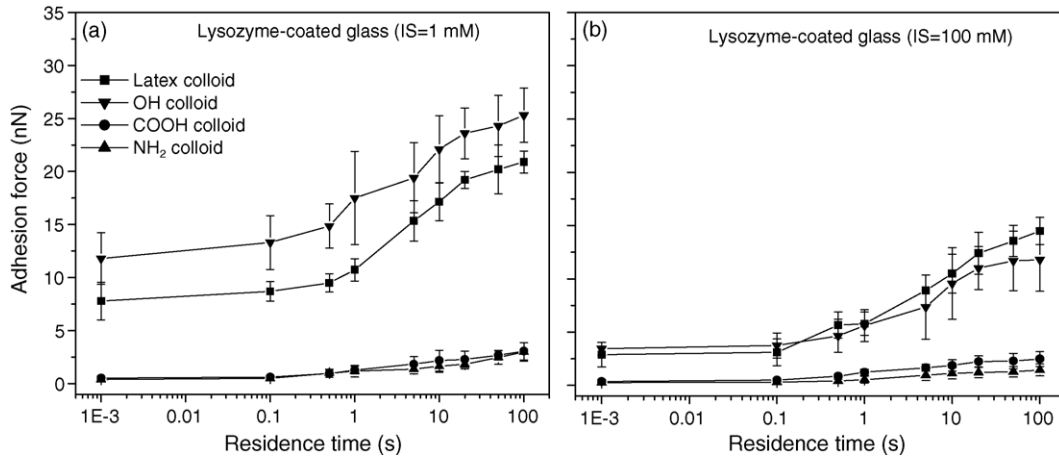


Fig. 8. Adhesion forces of colloid to lysozyme-coated glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.

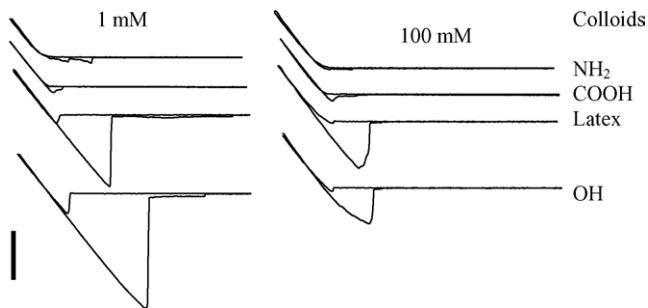


Fig. 9. Representative interaction force curves of lysozyme and various functionalized colloids at residence time 0.001 s (bar = 5.4 nN).

adhesion forces decreased in all cases, similar to previous results (Fig. 8b).

### 3.6. Colloid adhesion to poly-D-lysine-coated glass surfaces

Very large adhesion forces were measured in retraction force curves for poly-D-lysine surfaces compared to those for BSA, protein A and lysozyme (Fig. 10). For example, the adhesion

force with the OH-functionalized colloid with the poly-D-lysine surface was  $27.7 \pm 1.4$  nN ( $T = 5$  s), while the forces measured for the other proteins ranged from 2.0 to 19.4 nN. The adhesion forces for the colloids to poly-D-lysine decreased in the order: latex > OH > COOH > NH<sub>2</sub> in either 1 or 100 mM IS solutions (Fig. 10). This ordering of adhesion forces is consistent with the order of zeta-potentials of the colloids (Fig. 2b) indicating that the electrostatic forces were predominant in the interactions of these functionalized colloids with the poly-D-lysine surface.

Large “jump-in” forces were also observed for the poly-D-lysine-coated surface, similar to that observed for the lysozyme-coated surface, but only for the bare latex and OH-functionalized colloids (Fig. 11). The average “jump-in” attractive forces were  $0.9 \pm 0.6$  nN for a latex colloid and  $1.5 \pm 0.3$  nN for the hydroxyl-functionalized colloid. In 100 mM IS solution, the “jump-in” attractive forces decreased to  $0.3 \pm 0.2$  nN for the bare latex colloid, and  $0.4 \pm 0.2$  nN for the OH-functionalized colloid (Fig. 11). The “jump-in” attractive forces likely resulted from two approaching surfaces having opposite electrical charge since poly-D-lysine is positively charged and colloids (latex, OH) were highly negatively charged.

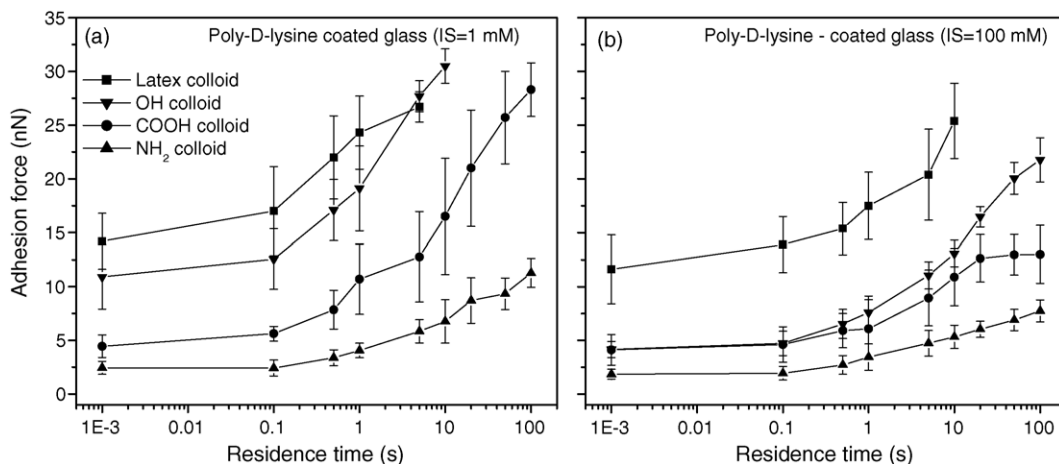


Fig. 10. Adhesion forces of colloid to poly-D-lysine-coated glass surfaces as the function of residence time: (a) 1 mM IS and (b) 100 mM IS.



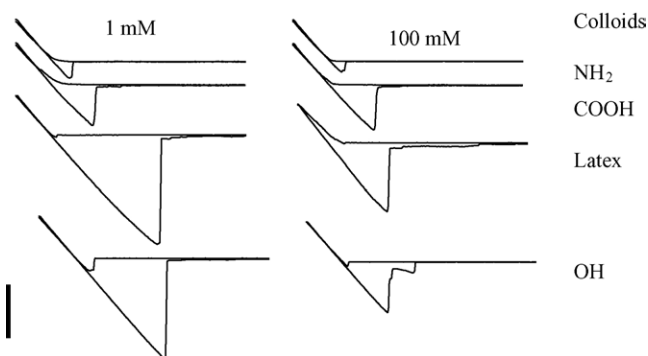


Fig. 11. Representative interaction force curves of poly-D-lysine and various functionalized colloids at residence time 0.001 s (bar = 5.4 nN).

The partial coverage of poly-D-lysine on the glass surface (Fig. 2) was apparent when obtaining forces curves as several locations selected on the surface indicated low adhesion forces that were more indicative of the silane group than the poly-D-lysine. A comparison of these low adhesion sites with those for silane showed good agreement as the silanized glass produced lower adhesion forces than poly-D-lysine. For example, the adhesion forces for silane glass with colloids were  $14.2 \pm 2.6$  nN for OH,  $13.0 \pm 2.8$  nN for COOH, and  $1.5 \pm 0.6$  nN for NH<sub>2</sub> at a residence time 10 s in 1 mM IS (Fig. 4), while the corresponding adhesion forces of the colloid to poly-D-lysine were  $30.5 \pm 1.6$ ,  $16.5 \pm 5.4$ , and  $6.8 \pm 2.0$  nN, respectively. Therefore, these low adhesion sites were not included in the poly-D-lysine analysis. The large adhesion forces observed between OH/poly-D-lysine or COOH/poly-D-lysine indicated the importance of bonding between NH<sub>2</sub> in the protein and OH or COOH groups.

### 3.7. Relationship between adhesion force and residence time

In all cases, the adhesion forces of colloids to protein-coated surfaces consistently increased with residence time. The adhesion forces can be correlated to residence time using a simple empirical model [20] as

$$F = AT^n \quad (1)$$

where  $F$  is the adhesion force and  $A$  and  $n$  are empirical coefficients determined from log–log plots. The results using this model, shown in Table 3, indicate that the parameter  $n$  was nearly constant for various systems and conditions, with an average value of  $n = 0.21 \pm 0.07$ . The magnitude of  $A$  varied for the different sample systems and experimental conditions. In general, the higher adhesion forces produced larger values of  $A$ . The  $A$  values were the largest for the poly-D-lysine and smallest for BSA. The increase in adhesion force reflects the rearrangement of proteins and water exclusion which contribute to increased adhesion over time.

## 4. Discussion

The measured adhesion forces between three of the four proteins (BSA, lysozyme, poly-D-lysine) and functionalized col-

loids were consistently larger for the OH functional group than the COOH or NH<sub>2</sub> groups, while the NH<sub>2</sub>-functional group tended to exhibit the smallest adhesion force. These results are surprising given the relative charges of these different groups and proteins. At neutral pH, the COOH, NH<sub>2</sub> and OH groups are negatively, positively, and neutrally charged. Thus, we expected a stronger attraction between the NH<sub>2</sub> and negatively charge proteins (BSA and protein A), and the smallest adhesion between the COOH groups and the negatively charge proteins. The unexpected outcome of the adhesion measurements between the functionalized colloid and the surface must be a result of molecular-scale interactions between these functional groups and localized groups in the protein. The four different proteins were attached to the glass by covalent bonds between the COOH group in the protein and NH<sub>2</sub> on the functionalized glass surface. This method of bonding could have exposed relatively more NH<sub>2</sub> groups in the protein than COOH groups to the water phase, making the surface of the proteins exposed to the colloids predominantly NH<sub>2</sub>. van der Vegte and Hadziioannou [33] calculated that bonding forces between NH<sub>2</sub> groups would decrease in the order of OH/NH<sub>2</sub> > COOH/NH<sub>2</sub> > NH<sub>2</sub>/NH<sub>2</sub>, based on JKR theory. Their calculated results are general agreement with the trend in adhesion forces measured here for these functional groups, suggesting the importance of the NH<sub>2</sub> groups in bound proteins in adhesion measurements.

Adhesion measurements with the functionalized colloids demonstrated the importance of electrostatic interactions for the tests with the positively charged proteins (lysozyme and poly-D-lysine). The approach curves for these two proteins demonstrated “jump-in” attractive forces with the highly negatively charged bare latex microsphere and the neutrally charged OH-functionalized microsphere. The latex microsphere is a “soft” microparticle with the heterogeneous surface chemistry distribution [34]. The OH group likely had little effect on the charge distribution of the colloid as shown by the high zeta-potential compared to the COOH- and NH<sub>2</sub>-functionalized microspheres (Fig. 2). This high negative charge explains the high attraction of the latex and OH-functionalized colloids to the lysozyme (Fig. 9) and poly-D-lysine (Fig. 11) containing highly positively charged functional groups. The adhesion of the poly-D-lysine to the different functionalized colloids is also reflected in the values of the coefficient  $A$  in the model, with these values of  $A$  changing in the same order as the zeta-potential of the different functional groups (Table 3). The large adhesion forces between poly-D-lysine and the different functional groups were a direct result of the substantially higher content of NH<sub>2</sub> groups in poly-D-lysine than in the other proteins. A high concentration of NH<sub>2</sub> groups in a protein produces a high positive surface charge, and increase the bonding forces of the OH/NH<sub>2</sub> and COOH/NH<sub>2</sub> groups. This effect of the NH<sub>2</sub> groups is easily seen in the force curve results shown in Fig. 4 between the different functional groups and silane glass surface.

The protein-coated surfaces consistently produced an increase in the adhesion force between the colloid and the surface over time (0–100 s). Using an empirical model to correlate residence time and adhesion data, the model constant,  $n$ , found

Table 3  
Fitting parameters for the model used to relate adhesion forces ( $F$ ) to the residence time ( $T$ )

Protein	Colloid	IS (mM)	$A$	$n$	$R^2$	
BSA	Latex	1	$0.66 \pm 0.05$	$0.32 \pm 0.02$	0.99	
		100	$0.89 \pm 0.09$	$0.21 \pm 0.03$	0.93	
	OH	1	$0.99 \pm 0.07$	$0.39 \pm 0.02$	0.99	
		100	$1.06 \pm 0.08$	$0.28 \pm 0.02$	0.98	
	COOH	1	$0.92 \pm 0.06$	$0.30 \pm 0.02$	0.99	
		100	$0.75 \pm 0.08$	$0.32 \pm 0.03$	0.97	
	NH <sub>2</sub>	1	$1.05 \pm 0.11$	$0.21 \pm 0.04$	0.92	
		100	$0.67 \pm 0.09$	$0.27 \pm 0.04$	0.93	
	Protein A	Latex	1	$1.41 \pm 0.10$	$0.28 \pm 0.02$	0.98
			100	$1.40 \pm 0.09$	$0.20 \pm 0.02$	0.97
OH		1	$2.45 \pm 0.14$	$0.17 \pm 0.02$	0.96	
		100	$1.22 \pm 0.06$	$0.16 \pm 0.02$	0.96	
COOH		1	$6.62 \pm 0.14$	$0.07 \pm 0.001$	0.95	
		100	$2.02 \pm 0.05$	$0.09 \pm 0.006$	0.97	
NH <sub>2</sub>		1	$1.31 \pm 0.09$	$0.25 \pm 0.02$	0.98	
		100	$0.94 \pm 0.10$	$0.27 \pm 0.03$	0.96	
Lysozyme		Latex	1	$12.29 \pm 0.65$	$0.13 \pm 0.02$	0.91
			100	$6.40 \pm 0.36$	$0.20 \pm 0.02$	0.97
	OH	1	$17.52 \pm 0.47$	$0.08 \pm 0.008$	0.95	
		100	$6.15 \pm 0.08$	$0.16 \pm 0.02$	0.93	
	COOH	1	$1.28 \pm 0.06$	$0.19 \pm 0.01$	0.98	
		100	$1.13 \pm 0.08$	$0.20 \pm 0.02$	0.96	
	NH <sub>2</sub>	1	$1.02 \pm 0.06$	$0.22 \pm 0.02$	0.98	
		100	$0.63 \pm 0.05$	$0.19 \pm 0.02$	0.94	
	Poly-D-lysine	Latex	1	$23.26 \pm 0.78$	N/A	0.93
			100	$17.36 \pm 0.57$	N/A	0.92
OH		1	$20.64 \pm 1.11$	$0.16 \pm 0.03$	0.92	
		100	$8.18 \pm 0.49$	$0.22 \pm 0.02$	0.98	
COOH		1	$10.02 \pm 0.61$	$0.23 \pm 0.02$	0.98	
		100	$7.15 \pm 0.48$	$0.17 \pm 0.03$	0.91	
NH <sub>2</sub>		1	$4.33 \pm 0.29$	$0.21 \pm 0.02$	0.97	
		100	$3.50 \pm 0.17$	$0.17 \pm 0.01$	0.97	
Total average (mean $\pm$ S.D.)				$0.21 \pm 0.07$		

N/A: not applicable.

here was constant ( $n = 0.21 \pm 0.07$ ) for most cases. This value of  $n$  compares favorably to  $n = 0.29 \pm 0.06$  previously obtained for bare latex microspheres and protein-coated glass and latex microspheres interaction with protein-coated surfaces [20]. The consistent value of  $n$  supports previous findings that water exclusion and protein rearrangement were the two main factors producing an increase of adhesion forces over time. As a colloid is forced into contact with a surface, water is pushed out from between these surfaces. The change in water content changes the orientation of charged portions of the molecule with itself and the two surfaces. Over the relatively longer periods of residence time ( $\sim 5$  to 100 s versus  $< 1$  s), the protein molecules rearrange, bridge, and then bind to the opposing surface until all bonds reach the lowest energy state for the imposed condi-

tion. Withdrawing the colloid from the surface after this polymer rearrangement therefore requires a greater force relative to that initially needed.

The parameter  $A$  in the model reflects the magnitude of the interaction force during the initial contact time. These forces are determined by the protein molecular structure, surface electrostatic charges, and solution chemistry. The  $A$  values observed were the largest for the poly-D-lysine and smallest for BSA (Table 3). This is consistent with the fact that poly-D-lysine has the highest concentration of positively charged groups among these proteins. Thus, the interaction of negatively charged functional colloids and positively groups within the poly-D-lysine produced the largest adhesion forces. Values of  $A$  are also affected by the protein structure. The different tertiary structures

of the proteins can affect the number and accessibility of interaction sites possible between different functional groups and the colloid. IS will alter the conformation of the protein. A high IS solution caused protein folding and compression, resulting in low *A* values [20].

#### 4.1. Relevance to bacterial adhesion

Bacterial adhesion and desorption to a surface are the residence-time-dependent processes [28,35,36]. The time dependence of this process can be regarded as the result of the interaction and attraction of bacterial exopolymers (e.g., proteins, lipopolysaccharides, and other biopolymers) to the surface, and subsequent formation of weak and strong chemical bonds of the polymers with the surface. The bond between biopolymers and a surface may strengthen or weaken over time, leading to adsorption and desorption rates that vary between bacterial strains [36]. The functional groups examined here (OH, COOH, and NH<sub>2</sub>) are ubiquitous in the biopolymers on the bacterial cell surface. The current work with these functionalized groups highlights the importance of both the specific functional group as well as residence time on the adhesion force between polymers and a surface. We infer from our studies that the same effects observed here with these functional groups over time occur with polymers on the surfaces of bacteria. It has been found, for example, that adhesion force between an AFM tip and a bacterial surface increases over time [28], consistent with our expectations based on results from the current study. Taken together, these observations demonstrate the importance of specific molecular-scale interactions between functional groups and surfaces conditioned with proteins and other polymers, and help us to better understand factors that affect bacterial adhesion.

## 5. Conclusions

The OH-functionalized latex colloid produced the higher adhesion forces than COOH or NH<sub>2</sub>-functionalized colloids to three of the four proteins (BSA, lysozyme, poly-D-lysine). The NH<sub>2</sub>-functionalized colloid exhibited the smallest adhesion force. The adhesion forces between functionalized colloids and protein-coated surfaces increased with residence time due to protein rearrangement and water exclusion over time (<100 s). Adhesion forces decreased inversely with IS in all cases, likely as a result of conformational and size changes of the proteins in response to solution IS.

These AFM measurements demonstrate the importance of molecular-scale interactions between proteins and specific functional groups on surfaces. Studying these specific interactions will help us to better understand the interactions between bacterial cell surfaces, which are composed of a complex mixture of proteins, polysaccharides and other biopolymers, and surfaces which are often pre-conditioned by organic matter in water. A better understanding of interactions of functional groups and biopolymers at the molecule scale will improve the understanding the factors that influence microbial adhesion, leading to improved methods to control adhesion.

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