

## Competitive adsorption–desorption of IgM monomers–dimers on silica and modified silica surfaces

Suvajyoti Guha<sup>a,b,1</sup>, Joshua Wayment<sup>b</sup>, Vinayak Rastogi<sup>b</sup>, Mingdong Li<sup>a,b</sup>, Michael J. Tarlov<sup>b</sup>, Michael R. Zachariah<sup>a,b,\*</sup>

<sup>a</sup> University of Maryland, College Park, MD 20742, United States

<sup>b</sup> National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, United States

### ARTICLE INFO

#### Article history:

Received 14 December 2012

Accepted 18 February 2013

Available online 26 March 2013

#### Keywords:

Electrospray

Differential mobility analysis

Protein

Competitive adsorption

Desorption

Bovine

Immunoglobulin

IgM

Silica surface

Gelatin

### ABSTRACT

Understanding competitive adsorption–desorption of proteins onto surfaces is an important area of research in food processing and biomedical engineering. Here, we demonstrate, how electrospray-differential mobility analysis that has been traditionally used for characterizing bionanoparticles, can be used for quantifying complex competitive adsorption–desorption of oligomeric proteins or multiprotein systems using monomers and dimers of IgM as a model example onto silica and modified silica surfaces. Using ES-DMA, we show that IgM dimers show a preference to stay adsorbed to different surfaces although monomers adsorb more easily and desorption rates of monomers and dimers of IgM are surface-type-dependent and are not significantly affected by shear. We anticipate that this demonstration will make ES-DMA a popular “label-free” method for studying multicomponent multi-oligomeric protein adsorption to different surfaces in the future.

Published by Elsevier Inc.

### 1. Introduction

Protein adsorption to surfaces is ubiquitous and is of great importance to the bio-pharma industry and food processing. Often times, to emulate the complexity of protein adsorption inside human plasma, the adsorption of multiple protein systems are studied onto different surfaces *ex-vivo*. For identifying different proteins from a multi-protein system it is common to use radio, fluorescent or gold labeling [1–5]. However, labeling may change the conformation stability of proteins, and affect their adsorption behavior or even promote aggregation in proteins [6–8]. To avoid the adverse effects of labeling, other “label-free” tools have been employed such as size exclusion chromatography [6,9], electrophoresis [10], ellipsometry [11–13], spectroscopy [14,15], surface plasmon resonance [16], quartz crystal microbalance [17],

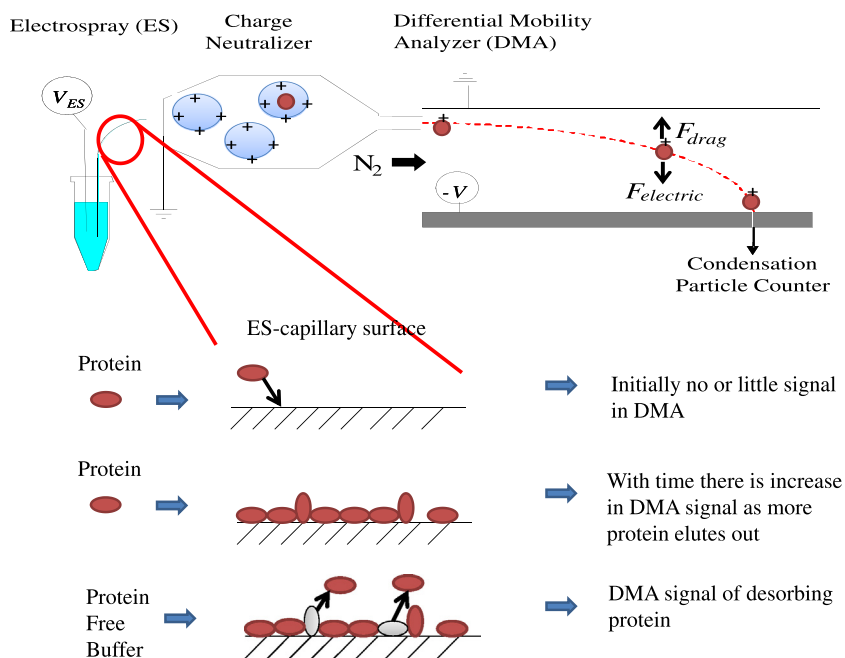
tensiometry [11], reflectometry [18], shear rheology [19], surface force apparatus [20] and imaging techniques [21,22]. However, there are only a few reports where these tools have been employed on oligomers (monomers, dimers, etc.) of the same protein [6,23,24]. In this article, we demonstrate through two proof of principle experiments how electrospray (ES) – differential mobility analysis (DMA), an atmospheric pressure based ion mobility method, can be used for quantifying adsorption–desorption of an oligomeric protein, IgM, onto different surfaces.

Traditionally, ES-DMA has been used for characterizing monomeric and oligomeric nanoparticles [25–27] and bionanoparticles such as proteins, viruses and nanoparticle–ligand conjugates [28]. The ES constitutes of a silica capillary through which analyte of interest in the liquid phase is eluted at shear rates of  $\approx 10^4 \text{ s}^{-1}$  [29] and then the analyte is aerosolized at room temperature by generating a fine mist of droplets at the end of the ES capillary by Coulombic repulsion. The DMA acts as a band pass filter and classifies particles by the balance of electric and drag force which is then counted to obtain mobility size distributions of the analyte [30] (Fig. 1). Such distributions can be obtained fairly quickly (e.g.  $\approx 75 \text{ s}$  are required for obtaining each size distribution analysis in this work). In prior work with monomeric proteins [29,31] we had seen that mobility distributions of proteins can be time

\* Corresponding author. Address: 2125 Glenn L. Martin Hall, Building 088, University of Maryland, College Park, MD 20742, United States. Fax: +1 301 314 9477.

E-mail address: [mrz@umd.edu](mailto:mrz@umd.edu) (M.R. Zachariah).

<sup>1</sup> Current address: Division of Solid and Fluid Mechanics, Center for Devices and Radiological Health, Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, United States.



**Fig. 1.** The electro-spray-differential mobility analyzer constitutes an electro-spray (ES) through which proteins (or other bionanoparticles) elute, are charge-reduced in the neutralizer, then classified in the differential mobility analyzer (DMA) and eventually counted by a condensation particle counter (not shown). Inside the ES capillary surface protein adsorption–desorption takes place that can be quantified by the DMA-CPC.

dependent and resulted from protein adsorption to the silica capillary walls. By correlating the known liquid phase concentration of the protein with the corresponding aerosol concentration obtained with ES-DMA we were able to quantify the amount of monomeric protein adsorbing onto or desorbing from the ES capillary surfaces [29]. A typical experiment for using ES-DMA for protein adsorption–desorption constitutes three stages as shown in Fig. 1. Protein is first passed through the ES capillary. If protein adsorption to the silica ES surface is favorable little or no protein exits the capillary to the DMA. However, as the surface becomes saturated with the protein, more protein elutes through the ES capillary, and is detected, eventually reaching a steady-state when the protein size distribution obtained with DMA becomes invariant with time. Finally, when the protein is replaced with a buffer solution, the signal of the desorbing proteins can be detected (Fig. 1). Using this methodology, we extend this approach to oligomeric proteins by studying the competitive adsorption and desorption of monomers and dimers of IgM on silica capillary surfaces or silica surface modified with gelatin.

## 2. Experimental section

### 2.1. Sample preparation and capillary surface preparation

For electro-spraying 20 mmol/L ammonium acetate buffer solution was used for preparing bovine IgM solution (Sigma Aldrich, St. Louis, MO 978K4779) at a concentration of 0.1 mg/mL. This concentration was verified by using an ultraviolet–visible (UV–Vis) spectrometer (Lambda Bio 20, Perkin Elmer, Waltham, MA) by measuring the maximum absorbance at 280 nm. Further details about sample preparations are already available elsewhere [29,31].

It is important to note that the capillaries need to be properly cleaned prior to the experiments. To ensure this, six capillaries (obtained from TSI Inc, 24 cm long and 25  $\mu$ m inner diameter) were first treated with  $\sim 1.0$  M  $H_2SO_4$  for 20–30 min, and then rinsed with deionized (18 M $\Omega$ /cm) ultrapure water for at least 10 min. All six capillaries were then cleaned by electro-spraying with

20 mmol/L ammonium acetate buffer solutions for another 5–10 min. Three of the above capillaries were further passivated with  $\sim 1$  mg/mL gelatin (Knox, trade name: Gelatine, # 0-41000-03500-5) following a methodology described elsewhere [31].

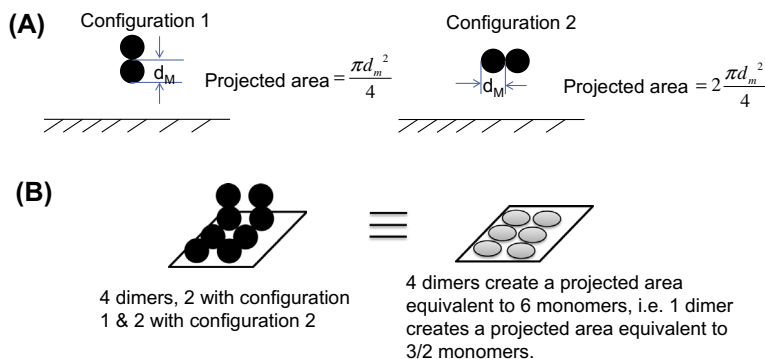
### 2.2. Electro-spray – differential mobility analysis operating conditions

An electro-spray (ES) source (TSI Inc., Shoreview, MN, #3480) was used for aerosolizing particles from the liquid phase. The ES-DMA was operated with a sheath flow rate of 10 L/min using nitrogen and an aerosol flow rate of  $\approx 1.5$  L/min using air. The liquid flow rate through the capillary was  $\approx 66$  nL/min. Particles passing through the DMA were then counted using a condensation particle counter (CPC). The CPC was operated at a high flow mode of 1.5 L/min. Size distributions of IgM were obtained by scanning from 13.5 nm to 24.5 nm obtained every 75 s. Time in our experiments is replaced with a dimensionless equivalent capillary volumes (which is defined by the product of time in minutes and capillary flow rate, divided by the total volume within the ES capillary [29]).

The experiment protocol followed in this work is in conjunction with our previous studies [29] (described in Fig. 1) and is as follows: IgM was electro-sprayed through a freshly prepared capillary (or capillary passivated with gelatin) until the mobility distribution became invariant of time (within experimental variability) for several capillary volumes (referred to as steady state henceforth). Finally the protein solution was replaced with buffer, which was electro-sprayed, such that any protein size distributions obtained subsequently are from desorbing proteins. Experiments with IgM on bare capillaries and gelatin passivated capillaries were repeated in triplicates, however, for clarity, only a single set of experiments are shown in Figs. 3 and 4.

### 2.3. Analytical ultracentrifugation operating conditions

Although ES-DMA is a popular tool for characterization it has rarely been used for quantification of oligomers. This is because



**Fig. 2.** (A) Two simplest possible configurations of dimers attaching to the surface of ES capillary. (B) Because configuration 1 takes less foot print area compared to configuration 2, hence this needs to be accounted for in our calculations. Assuming equal probability for configuration 1 and 2, dimers that can be accommodated occupy an area which is 3/2 times that of a monomer. Thus the right hand side of Eq. (8) is multiplied by a prefactor 3/2.

it is believed that different oligomers can provide different responses in ES-DMA much like ES-mass spectrometry. To ameliorate this concern, in our prior quantification studies with ES-DMA [27,32] we had validated our data with analytical ultracentrifugation (AUC) [33] which is regarded to be a gold standard for oligomer quantification. Similarly, in this work, we invoke a comparison of quantification of the IgM monomers and dimers obtained with ES-DMA and AUC. AUC measurements were made using Beckman–Coulter XL-1 Proteomelab (Brea, CA). The temperature of the centrifuge chamber was equilibrated to 20 °C. The reference cell was filled with a sample solution equivalent ammonium acetate buffer of matching molarity. 400  $\mu\text{L}$  volumes of the same sample as prepared for ES-DMA were spun at 30,000 RPM (3142.857 rad/s). Radial absorption measurements were recorded every 180 s at 280 nm wavelength with a radial position step size of 0.005 cm. The protein particles in the suspensions were subjected to a relative centrifugal force (rcf, relative to gravity) ranging from 62,384g to 72,245g, depending on their position with respect to the rotor center in the sector shaped cell. The radial scans were then analyzed using the Continuous Sedimentation Coefficient Distribution method in SEDFIT [33] fitting the baseline with a resolution of 200. Confidence level of  $1\sigma$  was used while fitting the AUC data using SEDFIT. The peak values in the  $c(S)$  versus ‘S’ distributions were then converted to molecular weight using SEDFIT, correspondingly exhibiting the existence of monomers, dimers and trimers.

Based on the size distributions obtained with ES-DMA and AUC (Appendix A) it is evident that bovine IgM constitutes of large proportions of monomers and dimers and that both techniques predict approximately the same amount of monomers and dimers. These results imply that for IgM monomers and dimers the response of ES-DMA is not oligomer dependent and thus IgM can serve as a model oligomeric protein for our studies. For simplicity, in all our subsequent analysis, we will neglect the larger oligomers of IgM.

### 3. Data analysis

#### 3.1. Determination of coverage

In our previous work [29], we had developed the data analysis methods to quantify the coverage and desorption rate constants of monomeric proteins adsorbing to, and desorbing from ES capillary surfaces. In this study, the bovine IgM chosen is multiligomeric and hence the relevant equations need to be modified. To determine surface coverage, we define  $C_{sol}^M$  and  $C_{sol}^D$  as the measured monomer and dimer solution concentration respectively such that

the total concentration  $C_{tot}$  is given by (assuming higher order oligomers are negligible in concentration):

$$C_{sol}^M + 2C_{sol}^D = C_{tot} \quad (1)$$

$C_{tot}$  is determined by using UV–vis and  $C_{sol}^M$  and  $C_{sol}^D$  are determined by obtaining size distributions with the ES-DMA once steady state has been reached (Appendix A). As ES-DMA(-CPC) obtains concentration in the aerosol phase, we need to convert the aerosol to a liquid phase concentration. At any point of time  $t_i$  assuming  $C_{elu}^M$  is the amount of monomers eluting out of the capillary in the liquid phase we can write:

$$C_{elu}^M(t_i) = \alpha^M C_{gas}^M(t_i) \frac{Q_{cpc}}{Q_{capillary}} \quad (2)$$

where  $C_{gas}^M(t_i)$  is determined by charge correcting the CPC raw data and then integrated over the domain of interest and  $\alpha^M$  can be thought of as a “loss factor” that allows the conversion of ES-DMA results from the aerosol to liquid phase, and depends on transport losses, the protein, [29] and also accounts for day-to-day variation in sample preparation and the performance of the ES-DMA.  $Q_{cpc}$  and  $Q_{capillary}$  are the flow rates through the CPC and ES capillary, respectively. Note that at this point,  $\alpha^M$  and  $C_{elu}^M(t_i)$  are unknowns. A similar equation can be written for dimers where the loss factor would be denoted by  $\alpha^D$  and the aerosol phase dimer counts,  $C_{gas}^D(t_i)$ , and  $C_{elu}^D(t_i)$  for the eluting dimer concentration at any time  $t_i$ . The monomer counts and dimer counts of IgM are obtained by integrating from 13.5 nm to 17.6 nm and 17.8 nm to 23.5 nm, respectively, in the size distribution obtained by ES-DMA. The discussion on size distributions is made in Section 4.

Consistent with our previous work [29,31], assuming 100% recovery at steady state we can directly evaluate  $\alpha^M$  using Eq. (2), which relates the liquid phase concentration to our measured value steady state  $C_{elu,ss}^M$  by assuming:

$$C_{sol}^M = C_{elu,ss}^M \quad (3)$$

In the above equation  $C_{sol}^M$  is known from Eq. (1) at steady state. Using this procedure for monomers and repeating for dimers, the average value of  $\alpha^M$  and  $\alpha^D$  from all set of experiments were determined to be 9.6 and 4.6, respectively. This approximately 2-fold difference in the loss factors are because of the 2-fold difference in molecular weight of a monomer and dimer.

Further, knowing  $\alpha^M$  we can determine the coverage of the IgM monomer using the equation given below:

$$\sum_{i=0}^{i=ss} \Gamma^M(t_i) = \sum_{i=0}^{i=ss} \frac{(C_{sol}^M - C_{elu}^M(t_i))Q_{capillary}t_i}{\pi D_{capillary}L_{capillary}} \quad (4)$$

where  $D_{capillary}$  and  $L_{capillary}$  are the diameter and length of the capillary and  $\Gamma^M(t_i)$  is the coverage obtained in time  $t_i$ , with a similar equation written for dimers by replacing  $\Gamma^M(t_i)$  with  $\Gamma^D(t_i)$ ,  $C_{sol}^M$  with  $C_{sol}^D$  and  $C_{elu}^M(t_i)$  with  $C_{elu}^D(t_i)$  in Eq. (4). The lower limit in the summation  $i = 0$  represents the first quantifiable IgM monomers (=4 min for our operating conditions), and the upper limit of the summation  $i = ss$  represents when steady state is reached. This equation further needs to be multiplied by the molecular weight of the monomer for expressing coverage in mass per unit area. During desorption,  $C_{sol}^M = 0$  which then gives the amount of monomer desorbed as:

$$\sum_{i=ss}^{i=stop} \Gamma^M(t_i) = \sum_{i=ss}^{i=stop} \frac{C_{elu}^M(t_i) Q_{capillary} t_i}{\pi D_{capillary} L_{capillary}} \quad (5)$$

Here the lower limit  $i = ss$  represents when the desorption experiment was started (which also coincides with steady state), and the upper limit  $i = stop$  represents when the desorption data collection was stopped (which for our operating conditions was  $\approx 20$  min after desorption was started).

Further replacing  $\Gamma^M(t_i)$  with  $\Gamma^D(t_i)$  and  $C_{elu}^M$  with  $C_{elu}^D$  in Eq. (5), the amount of IgM dimer desorbed can be determined.

### 3.2. Estimation of fractional coverage

Another important parameter in protein adsorption–desorption studies, is the fractional coverage. Based on the dimensions of the monomers and the number of adsorbed monomers at any time, we can estimate the monomer fractional coverage ( $\theta_i^M$ ) as:

$$\theta_i^M = N_{surf,i}^M \frac{\frac{\pi d_m^2}{4}}{\pi D_{capillary} L_{capillary}} \quad (6)$$

Here  $d_m$  is the diameter of IgM monomer and  $N_{surf,i}^M$  is the number of monomers on the surface of the capillary after  $\sum \Gamma^M(t_i)$  amount of monomers have adsorbed and is given by:

$$N_{surf,i}^M = \sum \Gamma^M(t_i) \times \frac{N_{av}}{MW_{monomer}} \times \pi D_{capillary} L_{capillary} \quad (7)$$

where  $N_{av}$  is Avagadro's number and  $MW_{monomer}$  is the molecular weight of IgM monomers (=960 kDa). It should be pointed out that here we assume that the size of IgM monomer is equal to its mobility size although we understand that in liquid solutions dimensions of a protein may be different from the mobility determined by ES-DMA.

Following the same methodology the fractional dimer coverage ( $\theta_i^D$ ) can be obtained at any point of time. However, the size of the IgM dimer in the liquid phase during adsorption to the surface is not equal to the mobility size of the IgM dimer. This is because the mobility size of the dimer determined in the gas phase by ES-DMA is the average of the three projected areas along three principle axes and thus does not reflect the liquid phase size of the dimer. Also, note that the dimer can take two different orientations as shown in Fig. 2A which also needs to be accounted for while calculating the fractional coverage by using a prefactor (explanation provided in caption of Fig. 2) in the equation below:

$$\theta_i^D = \frac{3}{2} N_{surf,i}^D \frac{\frac{\pi d_m^2}{4}}{\pi D_{capillary} L_{capillary}} \quad (8)$$

where  $N_{surf,i}^D$  is the number of dimers on the surface and is given by:

$$N_{surf,i}^D = \sum \Gamma^D(t_i) \times \frac{N_{av}}{MW_{dimer}} \times \pi D_{capillary} L_{capillary} \quad (9)$$

when the amount of dimers adsorbed is  $\sum \Gamma^D(t_i)$  and molecular weight is  $MW_{dimer}$  (=1920 kDa).

### 3.3. Determination of rate of desorption

Based on the desorption data we can extract kinetic rate constants of desorption for IgM monomers and dimers. At steady state, the total monomers on the ES capillary surface  $N_{surf,ss}^M$  can be written as:

$$N_{surf,ss}^M = \Gamma_{ss}^M \times \frac{N_{av}}{MW_{monomer}} \times \pi D_{capillary} L_{capillary} \quad (10)$$

where  $\Gamma_{ss}^M$  is the monomer coverage at steady state (i.e.  $\sum \Gamma^M(t_i)$  is integrated up to steady state). Similarly the total number of dimers on the ES capillary surface can be calculated using:

$$N_{surf,ss}^D = \Gamma_{ss}^D \times \frac{N_{av}}{MW_{dimer}} \times \pi D_{capillary} L_{capillary} \quad (11)$$

where  $\Gamma_{ss}^D$  is the dimer coverage at steady state. It should be pointed out that the prefactor used in the previous section does not apply here as we are only interested in the total number of dimers at steady state which is independent of the orientation.

Since it takes a finite amount of time to obtain a size distribution, we assume that the number of monomers,  $N_{des,g}^M(t_i)$ , that desorb as detected by the ES-DMA is constant over the scan time  $\Delta t$  (expressed in minutes if  $Q_{cpc}$  is expressed in minutes), and is obtained by integrating the area under the monomer peak. Then the number of monomers desorbing in liquid phase is  $N_{des,l}^M(t_i)$  at any time point  $t_i$ , such that

$$N_{des,l}^M(t_i) = N_{des,g}^M(t_i) Q_{cpc} \Delta t \alpha^M \quad (12)$$

Then at any time  $t_i$  the amount of monomers remaining on the surface  $N_{surf}^M(t_i)$  is given below,

$$N_{surf}^M(t_i) = N_{surf,ss}^M - N_{des,l}^M(t_i) \quad (13)$$

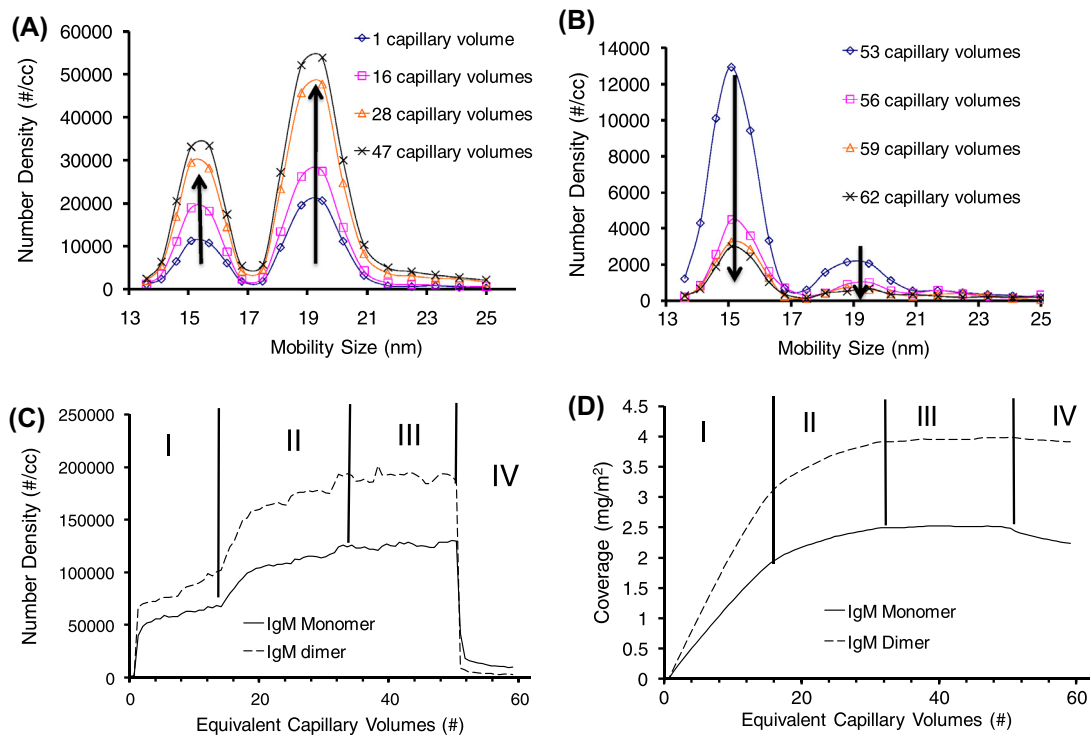
The desorption rate is the change of surface concentration with time which we assume to be a first order process and can then be integrated from time  $t = 0$  (when the buffer starts eluting and protein desorbing from the capillary reaches the CPC) to  $t = t_{stop}$  (when the experiment is stopped) to yield:

$$\log \left( \frac{N_{surf}^M(t_i)}{N_{surf,ss}^M} \right) = -K_{des}^M (t_{stop} - 0) \quad (14)$$

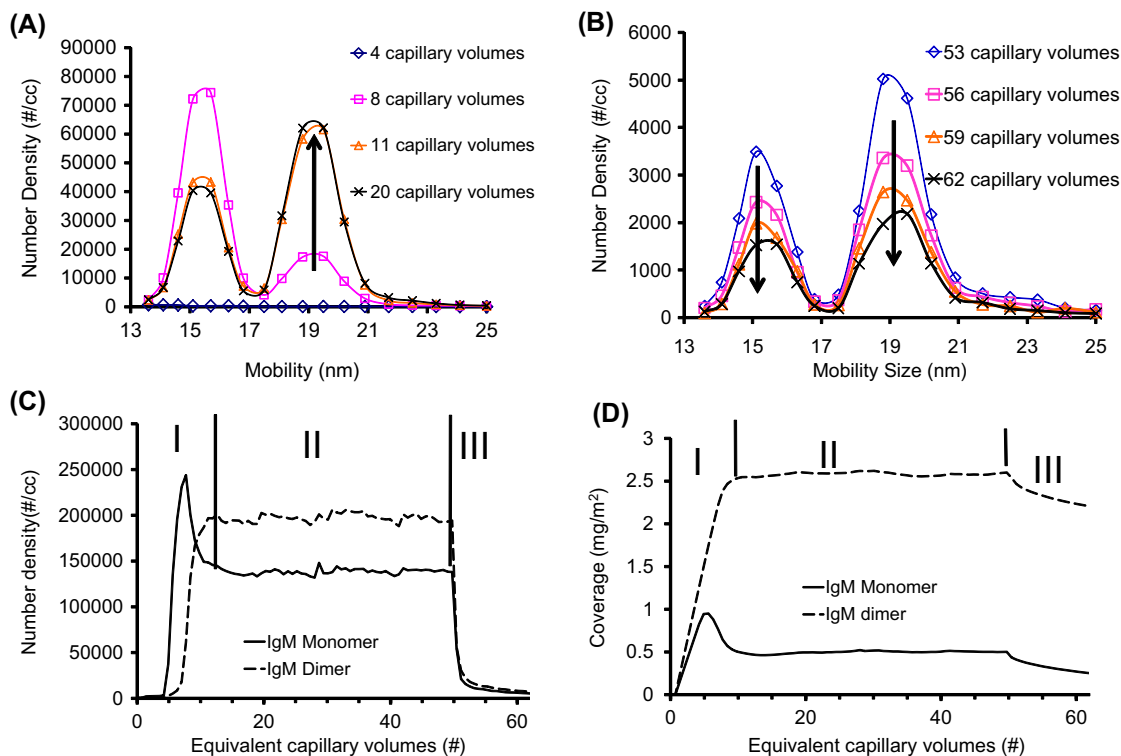
where  $K_{des}^M$  is the reaction rate constant for monomers. Following the same methodology, the rate constant for the dimers,  $K_{des}^D$  can be determined by replacing  $N_{des,g}^{M,t(i)}$  with  $N_{des,g}^D t(i)$ ,  $N_{des,l}^M(t_i)$  with  $N_{des,l}^D(t_i)$ ,  $N_{surf}^M(t_i)$  with  $N_{surf}^D(t_i)$  and  $N_{surf,ss}^M$  with  $N_{surf,ss}^D$  in Eqs. (12)–(14).

## 4. Results and discussion

Fig. 3A shows mobility size distributions of 0.10 mg/mL of IgM in 20 mmol/L ammonium acetate buffer at pH 7.0 as a function of capillary volumes while Fig. 3B shows mobility distributions for desorbing IgM when the protein solution is replaced with buffer after about 50 capillary volumes. Both Fig. 3A and B consists of two major peaks that can be identified as monomers and dimers of IgM based on an empirical correlation between mobility size and molecular weight [35]. In Fig. 3A, the monomers and dimers of IgM increase monotonically immediately after insertion of sample, and the mobility modes of the monomers and dimers stay constant at 15.6 nm and 19.2 nm throughout the experiment. The area under the monomer and dimer peaks from each mobility size distribution can be integrated (Fig. 3C) and the coverage of protein adsorbed onto ES capillary can be determined as outlined in Section 3.1. Using this procedure we show the temporal variation of the coverage in Fig. 3D. Domain I represents the initial phase of



**Fig. 3.** (A) Size distributions of monomers and dimers of IgM as a function of capillary volumes for a bare capillary when the capillary is flushed with the protein in 20 mmol ammonium acetate at pH 7.0 on a bare capillary surface. (B) Size distributions of monomers and dimers of IgM as a function of capillary volumes when the same bare capillary is flushed with 20 mmol ammonium acetate buffer at pH 7.0. (C) Integration of the monomer and dimer peaks obtained from each size distributions at each capillary volume as a function of equivalent capillary volumes. (D) Coverage of IgM monomers and dimers as a function of equivalent capillary volume.



**Fig. 4.** (A) Size distributions of monomers and dimers of IgM as a function of capillary volumes for a gelatin coated capillary when the capillary is flushed with the protein in 20 mmol ammonium acetate at pH 7.0. (B) Size distributions of monomers and dimers of IgM as a function of capillary volumes when the same gelatin coated capillary is flushed with 20 mmol ammonium acetate buffer at pH 7.0. (C) Integration of the monomer and dimer peaks obtained from size distributions at each capillary volume as a function of equivalent capillary volumes. (D) The coverage of monomer and dimers of IgM at the same conditions as a function of equivalent capillary volumes.

adsorption, followed by a step change represented by domain II (capillary volumes 15 onwards), and domain III which is the steady state (capillary volume 33 onwards) condition. Domain IV is when the protein is replaced with the buffer (capillary volume 52 onwards). The average initial rate of adsorption is  $0.11 \pm 0.02$  mg/m<sup>2</sup>/min and  $0.18 \pm 0.05$  mg/m<sup>2</sup>/min for monomers and dimers, respectively, i.e. dimers adsorb 1.6 times faster than monomers.

Qualitatively, based on the diffusion coefficients of the IgM monomers and dimers we can determine if the adsorption process is mass transfer limited or kinetically limited. As literature lacks diffusion coefficients of IgM monomers and dimers, we can ascertain these values indirectly by taking the following approach. The diffusion coefficient of IgG was previously determined to be  $4 \times 10^{-11}$  m<sup>2</sup>/s [36]. Further, as the diffusion coefficient correlates inversely with the one-third power of molecular weight [37], we can estimate the diffusion coefficient of IgM monomers as  $\sim 2.15 \times 10^{-11}$  m<sup>2</sup>/s and IgM dimers as  $\sim 1.71 \times 10^{-11}$  m<sup>2</sup>/s, respectively. Now, using the value of diffusion coefficient obtained above for IgM monomers, we can estimate a characteristic diffusion time of  $\approx 7.3$  s to traverse the radius of the capillary. This value is approximately a factor of 16 smaller than the capillary residence time, which in this regard is  $\approx 2$  min. This implies that the time dependent mobility distributions for IgM monomers cannot be ascribed to mass transfer effects, and must be associated with the intrinsic kinetics of adsorption. The same conclusions can be arrived at with IgM dimers. In prior work [29] we had seen that IgG would not elute through ES capillaries for several capillary volumes. As, we find IgM monomers and dimers elute from the beginning (capillary volume 1 in Fig. 3A) we may qualitatively infer that it is more difficult for IgM monomers and dimers to adsorb to silica compared to IgG. In the future, it would be interesting to perform similar studies at different temperatures to determine the adsorption barrier for IgM monomer and dimer adsorption to silica.

Using Eqs. (6)–(9) we can estimate the fractional coverage of the ES capillary surface at the end of domain I (Fig. 3D). About 56% of the total inner capillary surface is covered at the end of domain I with about 24% IgM monomers and 32% IgM dimers. We see a change in rate of adsorption at the end of domain I. Such a change in the adsorption rates have been reported for other proteins under submonolayeric conditions as well [29,34,38]. This experimentally observed reduction in the rate of adsorption at the end of domain I may imply conformation changes in the protein that may make adsorption of IgM monomers and dimers more difficult by a reduction in the area available for adsorption.

Using Eq. (4), the maximum average monomer and dimer coverage at steady state (end of domain II) are  $2.37 \pm 0.53$  mg/m<sup>2</sup> and  $4.24 \pm 0.74$  mg/m<sup>2</sup>, respectively. The literature on coverage of IgM adsorption to surfaces is sparse. Tengvall et al. determined human IgM adsorption to hydrophobic silica to be as much as 15 mg/m<sup>2</sup> [39] which implies multilayered adsorption. On the other hand, Lea et al. found submonolayer (34%) coverage of murine IgM onto mica surface using AFM [40]. These IgM samples were not as well characterized (w.r.t to monomer–dimer fractions) as our samples so it is not possible to ascertain the individual contributions of the monomers and dimers for the above cases. However, for our experiments on a silica surface, applying Eqs. (6) and (8), at the end of domain II, about 68% of the total inner capillary surface is covered (this is the fractional protein coverage) with 29% IgM monomers and 39% IgM dimers. Thus, our results are well within the broad range of values found in literature. The steady state in Fig. 3D (domain III) would seem to indicate a saturation of the surface, implying either our geometric arguments might be too simplistic (as proteins can change conformations upon adsorption) and that other lower surface density conformations may be present or that larger oligomers may have also adsorbed to the surface. To probe if it is because of larger oligomers would require us to

operate the ES-DMA at “stepping mode” [26], that would significantly increase the time required for obtaining a single size distribution from about 75 s to  $\approx 700$  s. This approach would have concomitantly reduced the kinetic resolution of our experiments, and hence this route was not adapted.

Using Eq. (5), the desorbed average amounts of monomer and dimer per unit area are calculated to be  $0.60 \pm 0.30$  mg/m<sup>2</sup> and  $0.09 \pm 0.02$  mg/m<sup>2</sup>, respectively, i.e. 25% of monomers desorb while only 2% of dimers desorb, implying less dimers come off the surface when flushed with buffer. Prior desorption studies using different proteins and different instruments, suggest that it is common for 10–50% of proteins to desorb from the surface [29,31,34,41]. Thus our findings are consistent with prior work. The lower desorption of IgM dimer could be attributed to the larger number of binding sites for IgM dimer compared to its monomer. The lower desorption rate of IgM dimers can also be quantified based on a data analysis developed in Section 3.3 and is discussed later in this work. Further, the mobility size of the IgM monomers and dimers are unchanged during adsorption and desorption implying either (a) the tertiary structure change of the monomers and dimers upon adsorption is not significant, or (b) that both the monomers and dimers upon desorption relax back to their respective native tertiary structures or (c) the changes in their tertiary structures upon adsorption and then subsequent desorption are beyond the resolution of the ES-DMA which in this regard is 0.3 nm [26,42].

In a previous work, we had found that gelatin passivated silica capillaries prevent adsorption of IgG effectively [31]. However, upon electrospraying IgM through such a gelatin passivated surface we found significant adsorption, and the adsorption–desorption pattern was also significantly different from a bare surface as discussed here. Firstly, we observe no monomer or dimer of IgM elution for  $\approx 4$  capillary volumes (Fig. 4A), implying that in contrast to IgM adsorption to bare silica, IgM has a greater affinity towards gelatin. This is followed by a sudden increase in monomer intensity (Fig. 4A) that also corresponds to the appearance of dimers. The monomer intensity then decreases while the dimer intensity continues to monotonically increase till both reach a steady state (at  $\approx 11$  capillary volumes) significantly quicker than our previous case with the bare capillary. During desorption, as shown in Fig. 4B, the concentration of dimers desorbing is higher than that of monomers, and indeed higher than dimers desorbing from IgM adsorbed to bare silica. Fig. 4C shows the integrated concentrations divided into three domains: I where we initially see no protein eluting, followed by preferential expulsion of monomers, domain II (11 capillary volume onwards) where both monomer and dimers reach a steady state, and domain III (53 capillary volume onwards) when the protein is replaced with buffer. The increase in the monomer signal early on does not show any concomitant temporal variation in the dimer adsorption in Fig. 4C. Thus one may qualitatively argue that the dimers are not expelling the monomers. It maybe that higher molecular weight species such as trimers, tetramers and pentamers that are present in small quantities (see Fig. A1 in Appendix) may be responsible for the displacement of the monomers. As already discussed, accurate quantification of these larger aggregates would have required increased scan times in the ES-DMA which would have resulted in more infrequent data collection. To avoid this, the larger aggregates were not quantified. Nevertheless, the expelling of the monomers observed is consistent with literature and is referred to as the “Vroman effect” after the seminal work by Vroman et al. in 1970s showed that low molecular weight proteins would adsorb to a surface first but would subsequently be displaced by high molecular weight proteins [5,43,44]. It is not clear to us as to why we did not see such an adsorption pattern during the adsorption of IgM to bare silica. Qualitatively, this may be because of differing affinity

**Table 1**

Desorption rate constants for IgM monomers and dimers from silica and gelatin modified silica surface.

	Desorption rate constant for IgM monomers (min <sup>-1</sup> )	Desorption rate constant for IgM dimers (min <sup>-1</sup> )
Silica	0.0057 ± 0.0029	0.0006 ± 0.0001
Gelatin modified Silica	0.0189 ± 0.0053	0.0043 ± 0.0021

of IgM to bare silica and to gelatin surfaces and thus implies that Vroman effect is surface dependent.

The maximum IgM monomer and dimer coverages are determined to be  $0.42 \pm 0.02$  mg/m<sup>2</sup> and  $2.35 \pm 0.22$  mg/m<sup>2</sup> respectively, which is about a factor of five (for monomer) and two (for dimer) smaller than on a bare capillary, which based on our prior geometric arguments (Eqs. (6)–(9)) implies that about 6% and 21% of the ES capillary surface is covered with IgM monomers and dimers, respectively. The amount of IgM monomers and dimers desorbed are  $0.29 \pm 0.02$  mg/m<sup>2</sup> and  $0.38 \pm 0.02$  mg/m<sup>2</sup>, respectively, which corresponds to 69% of monomers and 16% of dimers that had adsorbed. Thus we find that IgM adsorption on a gelatin surface is significantly lower compared to a bare silica surface and desorption of both monomers and dimers is significantly increased.

It is evident from the desorption data from both bare silica and gelatin passivated silica surface (Figs. 3D and 4D) that dimers have a lower propensity to desorb from the silica surface compared to gelatin surface. This can further be quantified if we were to assume desorption to be a first order process and use the monomer and dimer concentration decay as a function of time during the buffer flush (Eqs. (10)–(14)). These values are shown in Table 1 below. These values are well within the wide range of desorption rate constants ( $\approx 10^{-4}$  min<sup>-1</sup> to  $\approx 1$  min<sup>-1</sup>) found in the literature at stagnant and low shear rate conditions [29,45–47]. There are three important inferences: (a) the monomer desorbs faster than the dimer for both surfaces, (b) the propensity for both the monomers and dimers of IgM to stay adsorbed on the gelatin-passivated surface is significantly lower and (c) as these rate constants are consistent with rate constants obtained at stagnant and low shear conditions [33,45–47], this may imply that the high shear inside ES capillaries does not influence the rate of desorption for IgM. However, as shear rate constants obtained with different techniques may vary significantly, such results should be interpreted with caution and more studies will be required in future using other independent techniques at high shear to validate our findings.

There appear to be two major similarities for IgM adsorption to bare silica and gelatin coated silica: (a) The amount of dimers adsorbed in both the cases is higher than monomers as seen in Figs. 3D and 4D. This is not surprising since the dimer would have significantly higher surface area available for adsorption and (b) the monomer and dimer mobilities sizes obtained during desorption are invariant of the two surfaces which may either imply that the two surfaces do not cause a significant change in the tertiary structures of the protein at least within the uncertainty of the instrument which in this regard is about  $\approx 0.3$  nm [42], or that once the proteins desorb from the surfaces they return to their native state.

## 5. Conclusions

In summary we showed through two “proof of principle” experiments that ES-DMA can be used as a “label-free” tool for studying competitive adsorption–desorption of oligomers of the same protein onto different surfaces. For two different surfaces we found that IgM monomers adsorb slower than dimers and desorb more

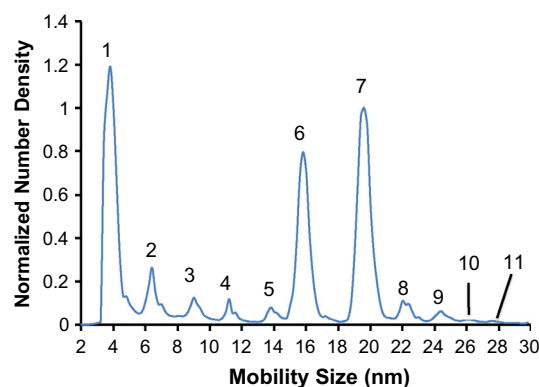
easily. Gelatin passivation can reduce monomer and dimer adsorption of IgM and change adsorption patterns significantly. In the future there are at least two directions in which ES-DMA can be used w.r.t protein adsorption–desorption: (a) adsorption of complex systems constituting of multiple proteins with each protein having multi-oligomeric intrinsic aggregates (as long as the sizes of these proteins and their oligomers are different) onto silica and modified silica surfaces and (b) proteins desorbing from the ES capillaries can be resuspended into the liquid phase either after the ES or after the DMA to further characterize them with other biophysical methods (such as Fourier transform infra-red spectroscopy or circular dichroism) to study how adsorption and then eventual desorption changes the primary and secondary structures of the different proteins inside ES capillaries. We anticipate that ES-DMA has the potential to bring in new answers to the realm of adsorption of biopolymers to solid–liquid interfaces.

## Appendix A. Quantifying proportion of IgM monomers and dimers using ES-DMA and AUC

Using ES-DMA, IgM monomers and dimers have been characterized before [35]. We can also use ES-DMA for quantification of the IgM monomers and dimers. One of the artifacts of the ES that hampers quantification is, the probability of two monomers getting encapsulated in the same ES droplet to produce a “droplet induced” dimer that is not characteristic to the solution. Such an artifact is a strong function of the solution concentration and droplet size, with increasing concentration or droplet size enhancing the artifact [27]. Thus to ameliorate this artifact a relatively low concentration of IgM ( $6.25 \times 10^{13}$  particles/mL if only existing as monomers, equivalent to 100 µg/mL) was electrosprayed. Then through a statistical model of the probability of finding two monomers within an electrospray droplet [27] we can determine the extent of the dimer formation by this artifact and is given by:

$$\frac{\text{Dimer}}{\text{Monomer}} = \frac{C_{\text{solution}} \times V_d}{2} \quad (\text{Equation A1})$$

where  $C_{\text{solution}}$  is the concentration of particles in solution and  $V_d$  is the volume of the electrospray droplet and determined to be  $\approx 130$  nm [27]. For the concentration of IgM used in this study, which in this regard is 100 µg/mL, the dimer to monomer ratio comes out to be 0.04 which implies that if all particles were monomers, then we would see only 4% dimers because of “droplet induced aggregation”. A detailed derivation and outline of this approach is presented elsewhere [27]. Further, if the solution has



**Fig. A1.** Size distribution of 0.10 mg/mL of IgM suspended in 20 mmol/L ammonium acetate buffer at pH 7.0 obtained using ES-DMA. All peaks are identified using an empirical correlation developed by Bacher et al. that relates mobility size with molecular weight of proteins. The Y-axis has been normalized with respect to the dimer peak.

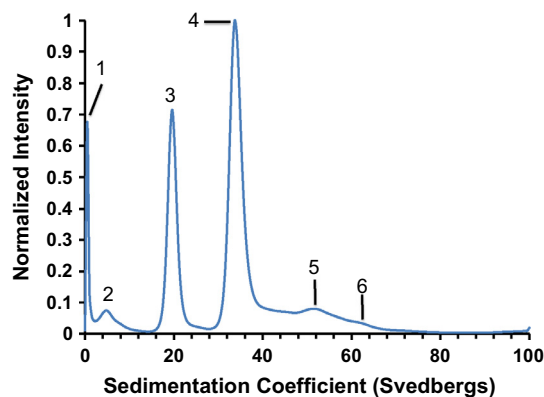


Fig. A2. Size distribution of  $\approx 0.1$  mg/mL of IgM obtained using AUC at pH 7.0. The Y-axis has been normalized with respect to the dimer peak.

intrinsic monomers and dimers, then for the same concentration of 100  $\mu\text{g/mL}$ , the amount of monomers in solution would be further reduced, thus further suppressing the ES artifact.

Fig. A1 shows the size distribution of 100  $\mu\text{g/mL}$  of IgM in 20 mmol/L ammonium acetate buffer at pH 7.0 obtained at steady state. All protein oligomers can be identified based on a well-established empirical correlation between mobility size and molecular weight [35]. During the ES droplet drying process the nonvolatile stabilizer salts dry up and appear in the size distribution at sizes smaller than 5 nm (assigned peak 1 in Fig. A1). Peak 2, 3 and 4 in Fig. A1 are probably protein fragments, IgG monomers and IgG dimers respectively. Since IgM is a pentamer of IgG, it could be that the IgG monomers and dimers may come off from IgM. After the droplet generation, during the neutralization process, most particles come out with single positive, negative and neutral charges [28]. However, depending on the efficiency of the neutralizer, a small fraction of doubly charged IgM monomers are also generated and these are assigned peak 5 in Fig. A1. Theoretically, there should also be doubly charged IgM dimers in the distribution but they probably overlap with the monomers and hence cannot be seen in Fig. A1. The rest of the peaks, 6–11 are, respectively, monomers, dimers, trimers, tetramers, pentamers and hexamers of IgM. It is evident from Fig. A1 that there is significant amount of dimers thus ruling out any “droplet induced aggregation”, or in other words, these dimers are intrinsic to the solution. The size distribution data has been normalized with respect to the dimer peak. In this particular study, unlike all experiments in the main text, the size distribution was obtained from 2 nm to 30 nm using a step size of 0.2 nm that allows a better resolution of all the individual peaks [26], although it increases the data acquisition time to  $\approx 23$  min. The relative proportions of the trimers, tetramers, pentamers and hexamers combined are much less (<5%) compared to monomers and dimers and thus their effects have been neglected throughout the study in the main text. By integrating the area under the monomer and dimer peaks, the dimer to monomer proportion was determined to be  $\approx 1.5$ .

To further validate the existence of monomers and dimers in solution and quantify them, the IgM sample was analyzed using AUC. In an AUC, the protein suspension is subjected to centrifugal force and as a result protein particles sediment and segregate over time. During the sedimentation process the UV–vis absorbance measurements were done at regular time and radial intervals in a sector shaped cell. The sedimentation properties of each species and size distribution of the entire population was then obtained by analysis of the absorbance data using SEDFIT software [33]. Fig. A2 shows size distribution obtained with AUC. Like ES-DMA it shows two primary peaks at 20 Svedbergs and 35 Svedbergs (labeled 3 and 4), thus qualitatively suggesting that there are two primary

species in solution. Assuming reasonable values for protein density (1.3 g/mL), solvent density (1.0 g/mL) and solvent viscosity (1.09 cP) these two peaks can be identified as monomers and dimers of IgM respectively. Besides the two peaks with the maximum intensity, it also shows some protein fragments (at <1 Svedbergs, label 1), IgG peak at  $\sim 7$  Svedbergs (label 2) and some larger aggregates (>50 Svedbergs, label 5 and 6) even though the larger aggregates are not well resolved compared to ES-DMA (Fig. A1). The integration of the area under the IgM monomer and dimer peaks obtained with AUC yields a dimer to monomer proportion of  $\approx 1.5$  thus implying the quantification of the monomers and dimers of IgM obtained with ES-DMA is accurate. Based on this observation, it is going to be assumed in the main text that  $C_{sol}^M = 0.04$  mg/mL for IgM monomers and  $C_{sol}^D = 0.03$  mg/mL for IgM dimers so that the total protein concentration = 0.10 mg/mL. As mentioned in the main text, for simplicity in data analysis, we neglect the larger aggregates.

## References

- [1] M. Holmberg, X.L. Hou, *Langmuir* 25 (2009) 2081–2089.
- [2] M. Holmberg, X.L. Hou, *Langmuir* 26 (2010) 938–942.
- [3] W.G. Pitt, K. Park, S.L. Cooper, *J. Colloid Interface Sci.* 111 (1986) 343–362.
- [4] P. Somar, Z. Rice, C.A. Siedlecki, *Micron* 39 (2008) 832–842.
- [5] P. Wojciechowski, P. Tenhove, J.L. Brash, *J. Colloid Interface Sci.* 111 (1986) 455–465.
- [6] H.G.W. Lensen, D. Bargeman, P. Bergveld, C.A. Smolders, J. Feijen, *J. Colloid Interface Sci.* 99 (1984) 1–8.
- [7] J.K. Elhorst, F.M. Olthuis, D. Bargeman, C.A. Smolders, J. Feijen, *Int. J. Artif. Organs* 1 (1978) 288–292.
- [8] W.H. Grant, L.E. Smith, R.R. Stromberg, *J. Biomed. Mat. Res.* 11 (1977) 33–38.
- [9] K. Kandori, M. Mukai, A. Yasukawa, T. Ishikawa, *Langmuir* 16 (2000) 2301–2305.
- [10] T. Arai, W. Norde, *J. Colloid Interface Sci.* 51 (1990) 17–28.
- [11] V.S. Alahverdijeva, D.O. Grigoriev, V.B. Fainerman, E.V. Aksenenko, R. Miller, H. Mohwald, *J. Phys. Chem. B* 112 (2008) 2136–2143.
- [12] C.G. Golander, E. Kiss, *J. Colloid Interface Sci.* 121 (1988) 240–253.
- [13] B.W. Morrissey, *Annals New York Acad. Sci.* 283 (1977) 50–64.
- [14] K.K. Chittur, *Biomaterials* 19 (1998) 357–369.
- [15] B.O. Leung, A.P. Hitchcock, J.L. Brash, A. Scholl, A. Doran, P. Henklein, J. Overhage, K. Hilpert, J.D. Hale, R.E.W. Hancock, *Biointerphases* 3 (2008) FB27–FB35.
- [16] R.J. Green, M.C. Davies, C.J. Roberts, S.J.B. Tendler, *Biomaterials* 20 (1999) 385–391.
- [17] K. Glasmaster, C. Larsson, F. Hook, B. Kasemo, *J. Colloid Interface Sci.* 246 (2002) 40–47.
- [18] B.K. Lok, Y.L. Cheng, C.R. Robertson, *J. Colloid Interface Sci.* 91 (1983) 104–116.
- [19] F.S. Ariola, A. Krishnan, E.A. Vogler, *Biomaterials* 27 (2006) 3404–3412.
- [20] P.M. Claesson, T. Arnebrant, B. Bergenstahl, T. Nylander, *J. Colloid Interface Sci.* 130 (1989) 457–466.
- [21] E. Servoli, D. Maniglio, M.R. Aguilar, A. Motta, J.S. Roman, L.A. Belfiore, C. Migliarese, *Macromol. Biosci.* 8 (2008) 1126–1134.
- [22] P.Q. Ying, Y. Yu, G. Jin, Z.L. Tao, *Colloids Surf. B-Biointerphases* 32 (2003) 1–10.
- [23] M. Okubo, I. Azume, Y. Yamamoto, *Colloid Polymer Sci.* 268 (1990) 598–603.
- [24] R.L.J. Zsom, *J. Colloid Interface Sci.* 111 (1986) 434–445.
- [25] I.W. Lenggoro, Y. Bin, K. Okuyama, J.F. de la Mora, *Langmuir* 18 (2002) 4584–4591.
- [26] D.H. Tsai, R.A. Zangmeister, L.F. Pease, M.J. Tarlov, M.R. Zachariah, *Langmuir* 24 (2008) 8483–8490.
- [27] M.D. Li, S. Guha, R.A. Zangmeister, M.J. Tarlov, M.R. Zachariah, *Langmuir* 27 (2011) 14732–14739.
- [28] S. Guha, M.D. Li, M.J. Tarlov, M.R. Zachariah, *Tr. Biotech.* 30 (2012) 291–300.
- [29] S. Guha, J.R. Wayment, M. Li, M.J. Tarlov, M.R. Zachariah, *Langmuir* 27 (2011) 13008–13014.
- [30] E.O. Knutson, K.T. Whitby, *J. Aerosol Sci.* (1975) 443–451.
- [31] S. Guha, J.R. Wayment, M.D. Li, M.J. Tarlov, M.R. Zachariah, *J. Colloid Interface Sci.* 377 (2012) 476–484.
- [32] S. Guha, J.R. Wayment, M.J. Tarlov, M.R. Zachariah, *J. Pharm. Sci.* 101 (2012) 1985–1994.
- [33] P. Schuck, *Biophys. J.* 78 (2000) 1606–1619.
- [34] J.S. Buijs, P.A.W. vandenBerg, J.W.T. Lichtenbelt, W. Norde, J. Lyklema, *J. Colloid Interface Sci.* 178 (1996) 594–605.
- [35] G. Bacher, W.W. Szymanski, S.L. Kaufman, P. Zollner, D. Blass, G. Allmaier, *J. Mass. Spec.* 36 (2001) 1038–1052.
- [36] C.F. Lu, A. Nadarajah, K.K. Chittur, *J. Colloid Interface Sci.* 168 (1994) 152–161.
- [37] M.E. Young, P.A. Carroad, R.A. Bell, *Biotech. Bioeng.* 22 (1980) 947–955.
- [38] J.S. Buijs, J.W.T. Lichtenbelt, W. Norde, J. Lyklema, *Colloids Surfaces B-Biointerphases* (1995) 11–23.
- [39] P. Tengvall, A. Askendal, I. Lundstrom, *Colloids Surfaces B-Biointerphases* 20 (2001) 51–62.



- [40] A.S. Lea, A. Pungor, V. Hlady, J. Andrade, J. Herron, E.W. Voss, in: *Manipulation of Proteins on Mica by Atomic Force Microscopy*, 1992, pp. 68–73.
- [41] C. Calonder, Y. Tie, P.R.V. Tassel, *Proc. Natl. Acad. Sci.* 98 (2001) 10664–10669.
- [42] S. Guha, L.F. Pease III, K.A. Brorson, M.J. Tarlov, M.R. Zachariah, *J. Virol. Methods* 178 (2011) 201–208.
- [43] L. Vroman, A.L. Adams, *Thromb. Diathesis Haemorrhagica* 18 (1967) 510.
- [44] L. Vroman, A.L. Adams, *Thromb. Diathesis Haemorrhagica* 19 (1968) 604.
- [45] V. Ball, A. Bentaleb, J. Hemmerle, J.C. Voegel, P. Schaaf, *Langmuir* 12 (1996) 1614–1621.
- [46] J.A. Nye, J.T. Groves, *Langmuir* 24 (2008) 4145–4149.
- [47] R.J. Wegner, A.W. Wark, H.J. Lee, E. Codner, T. Saeki, S. Fang, R.M. Corn, *Anal. Chem.* 76 (2004) 5677–5684.