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Structural attributes affecting peptide entrapment in PEO brush layers

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Abstract

A more quantitative understanding of peptide loading and release from polyethylene oxide (PEO) brush layers will provide direction for development of new strategies for drug storage and delivery. In this work we recorded selected effects of peptide structure and amphiphilicity on adsorption into PEO brush layers based on covalently stabilized Pluronic®F 108. Optical waveguide lightmode spectroscopy and circular dichroism measurements were used to characterize the adsorption of poly-L-glutamic acid, poly-L-lysine, and the cationic amphiphilic peptide WLBU2, to the brush layers. The structure of WLBU2 as well as that of the similarly-sized homopolymers was controlled between disordered and more ordered (helical) forms by varying solution conditions. Adsorption kinetic patterns were interpreted with reference to a simple model for protein adsorption, in order to evaluate rate constants for peptide adsorption and desorption from loosely and tightly bound states. While more ordered peptide structure apparently promoted faster adsorption and elution rates, resistance to elution while in the PEO layer was dependent on peptide amphiphilicity. The results presented here are compelling evidence of the potential to create anti-fouling surface coatings capable of storing and delivering therapeutics.

Keywords

PEO brush layer; Peptide structure; Peptide entrapment; Peptide adsorption kinetics; OWLS; WLBU2

1. Introduction

The adsorption of the antimicrobial peptide nisin and various aspects of its behavior at PEO-coated surfaces have been examined through ellipsometry [1], circular dichroism and assays of antibacterial activity [2], zeta potential [3], and TOF-SIMS [4]. In this paper we complete the first step in describing the effects of peptide structure and amphiphilicity on adsorption into PEO brush layers. Optical waveguide lightmode spectroscopy (OWLS) was used to record the adsorption of poly-L-glutamic acid, poly-L-lysine, and the cationic amphiphilic peptide WLBU2 to PEO brush layers based on covalently stabilized Pluronic®F108. The structure of the homopolymers as well as that of WLBU2 was controlled between disordered and more ordered (helical) forms by varying solution conditions (Fig. 1).

Poly-L-glutamic acid (PLG) and poly-L-lysine (PLL) are homopolymers of amino acids, with regular structure that can extend the full length of the peptide [5]. As with natural

proteins, the solution pH, ionic strength, polarity, and peptide concentration can be modulated to adjust the structural conformation from compact α -helix to a variety of “disordered” states [6,7]. WLBU2 is an engineered, cationic amphiphilic peptide (CAP) with 13 positively-charged arginine residues, and 11 hydrophobic valine or tryptophan residues. It shows substantial promise for clinical applications, due to its wide spectrum antimicrobial activity against both Gram-negative and Gram-positive bacteria under physiological conditions [8–11]. Segregation of the positively-charged and hydrophobic groups onto opposing faces of an α -helix confers the ability to disrupt bacterial cell membranes, killing the bacteria. Moreover, in addition to its high, broad-spectrum potency in blood, it has been shown to retain its antimicrobial activity when immobilized by a number of methods at solid surfaces [12–15].

While it shows no appreciable stable structure in water, WLBU2 exhibits high α -helix content in membrane-mimetic solvents (e.g., 81% α -helix in 30% trifluoroethanol in phosphate buffer)[10]. Finally, while WLBU2 is highly amphiphilic, neither PLG nor PLL is amphiphilic. Thus, WLBU2 serves both as a good control for the effects of amphiphilicity on peptide integration into brush layers, and as a clinically-relevant application for this research. There is great potential for biocompatible surface coatings based on entrapment of bioactive agents, for relatively short-term medical device applications (e.g., anti-infective coatings for acute hemodialysis catheters) as well as blood treatment applications featuring high surface-to-volume ratio, high flow rate, extracorporeal microfluidic devices. Any of a variety of microfluidic device architectures (e.g., microchannels, micropost arrays) can accommodate the high blood flow rates necessary for applications such as sepsis treatment, or the chemical processing of blood for transfusions. Strategies featuring drug-loaded but otherwise nonfouling coatings for blood contact hold promise for enhancing the performance of such devices.

2. Materials and methods

2.1. Peptide preparation

Lyophilized 20-residue average, 3.0 kDa molecular weight poly (L-glutamic acid sodium salt, PLG) was purchased from Alamanda Polymers (Huntsville, AL), and was used as supplied. PLG was dissolved at 1.0 mg/mL in HPLC water, and separated into 1.0 mL aliquots that were frozen and thawed prior to each experiment. The 1.0 mg/mL PLG (pI ~ 2.5) was diluted to 0.10 mg/mL in either 1.0 N HCl (EMD Chemicals) or a dilute HCl aqueous solution, pH 4.7, to invoke either helical or disordered conformations, respectively. Similarly, a 4.2 kDa 21-residue poly-L-lysine hydrobromide (PLL, Almanda Polymers) was dissolved at 1.0 mg/mL in HPLC water, and separated into 1.0 mL aliquots that were frozen and then thawed prior to each experiment. The 1.0 mg/mL PLL (pI ~ 11.8) solution was diluted to 0.10 mg/mL in either 1.0 N NaOH or HPLC water to invoke either helical or disordered conformations, respectively. Lyophilized WLBU2 (GenScript, Piscataway, NJ) was dissolved at 5.0 mg/mL in HPLC water and frozen in 200 μ L aliquots. Prior to use, the WLBU2 stock solution was thawed and diluted to 0.10 mg/mL in HPLC water, 10 mM sodium phosphate with 150 mM NaCl, pH 7.4 (PBS), or alternatively, with PBS with additional salt. All 0.10 mg/mL peptide solutions were degassed for 40 min under vacuum immediately before use.

2.2. Preparation of OWLS sensor chips

OWLS waveguide sensor chips with a ~10 nm surface silica thin film coating (MicroVacuum Ltd.) were cleaned by immersing in chromosulfuric acid (ACROS Organics) for 10 min at room temperature, rinsed with HPLC water, and dried with nitrogen gas. After cleaning, the sensors were immersed in molecular sieve-dried absolute ethanol, and dried

with a stream of nitrogen to remove bulk moisture. Silanization was performed by chemical vapor deposition at room temperature [16]. For this purpose sensors were placed in a sealed reaction chamber with the waveguide surface facing up. Argon gas was used for 20 min to purge the system, creating an environment devoid of moisture. 0.20 mL of trichlorovinylsilane (TCVS, TCI America) was then injected into the system, vaporized, and delivered to the waveguide surfaces using the argon carrier gas. After 1 h of vapor deposition, a second 0.20 mL aliquot of TCVS was injected into the system and allowed to react for 1 h. The waveguides were then removed and cured at 150 °C for 1 h.

Self-assembled F108 brush layers were formed on the silanized waveguide surfaces by incubating overnight with 5% Pluronic® F108 (BASF) in HEPES (Gibco BRL), pH 7.4. The brush layers were then gamma irradiated at 0.3 Mrad to covalently attach the F108 to the surface. The PEO functionalized sensors were dried with nitrogen and stored until use in the dark to prevent oxidation of the vinyl moieties. PEO brush layers with good steric-repulsive function are expected to form at chain densities greater than about 0.2 chains/nm² [17]. Adoption of a brush configuration by the methods used here was evident by their excellent fibrinogen repulsion as we have described elsewhere [18]. Hydrophobic control sensors were prepared as described above, but in the absence of F108.

2.3. Measurement of peptide adsorption kinetics

Buffer solutions were prepared to match the composition of the 0.10 mg/mL peptide solutions and degassed for 5 h. The buffer for helical PLG was prepared at 0.9 N HCl to account for dissolving the peptide in HPLC water prior to diluting to 0.10 mg/mL in 1.0 N HCl. Based on similar reasoning, the solution used for the disordered form of PLG was prepared by adding 10% HPLC water to the HCl solution originally used to dissolve the peptide. The buffer for helical PLL was prepared at 0.9 N NaOH, and HPLC water was used for experiments with the disordered form. The buffers for WLBU2 testing were 10 mM sodium phosphate including 150 mM NaCl, pH 7.4 (PBS), or PBS with salt added to 400 or 600 mM NaCl.

Waveguide sensors were equilibrated overnight in the appropriate buffer and the refractive index of the HCl solutions were calculated using linear interpolation of tabulated values [19]. At the start of each experiment, the sensor was allowed to equilibrate within the system for 40–60 min. OWLS Relative Intensity Mode (RIM) with buffer introduced at 50.0 µL/min (OWLS™ 210-SIS) was used. When the baseline slopes were on the order of 1.0×10^{-9} , about 4 mL of 0.10 mg/mL peptide solution was introduced to the system and adsorption to the sensor was allowed to occur for 30 min. Adsorption was followed by a 30 min rinse where peptide-free buffer was introduced. The adsorption-rinse cycle was then repeated. Each cyclic, adsorption–elution experiment was performed at least twice on each type of surface. At the conclusion of each test, the flow cell was cleaned with HPLC water and 0.1 N HCl.

2.4. Peptide structural evaluation

Hydrophobic silica nanoparticles (Product R816, Degussa, 190 m²/g, 10–12 nm), were coated with F108 by suspension in HPLC water for 10 h on a rotator. The amount of F108 used for this purpose was sufficient to cover the surface presented by the silica nanoparticles in suspension (about 3.3 mg/m²) [2]. F108-coated silica nanoparticles were then incubated with PLL or WLBU2 at 0.20 mg/mL for a desired period of time (2 h to 7 days) at room temperature. The amount of nanoparticles (10 mg/mL) selected was based on OWLS results and provided 5 times more surface area than would be required for complete adsorption.

Peptide structure in the presence or absence of nanoparticles was evaluated by circular dichroism (CD) using a Jasco J-815 spectropolarimeter (0.1 cm path length, cylindrical cuvette) at 25 °C. The instrument was calibrated using 0.6 mg/mL D(+) – camphorsulfonic acid. Spectra were recorded from 260 to 195 nm in 0.5 nm increments and digitally stored. In each case 10 scans were recorded and averaged in order to increase the signal-to-noise ratio. The 0.10 mg/mL (without nanoparticles) and 0.20 mg/mL (with nanoparticles) peptide samples prepared as outlined above were filtered (PVDF 0.20 μ m filter) prior to recording CD spectra. CD spectra were recorded along with peptide-free reference samples in each case in order to subtract background signals and ensure the measurement of peptide properties only. Peptide-nanoparticle suspensions were then washed by centrifugation (10,000 rpm, 20 min), and resuspended in water. CD spectra were then recorded again, under the same conditions outlined above. All experiments were performed in triplicate.

3. Results and discussion

3.1. Adsorption of WLBU2

Fig. 2 shows representative results for adsorption from a solution of WLBU2 dissolved in PBS at uncoated and PEO-coated surfaces. The peptide adsorbed with high affinity to the uncoated surface and showed substantial resistance to elution, owing to its highly cationic, amphiphilic character. ATCVS-treated silica surface has a negative zeta potential but is otherwise hydrophobic [3]. The high level of adsorption at the uncoated surface may be indicative of multilayer adsorption, presumably involving electrostatic association with the surface and hydrophobic association between adsorbed peptide layers. Adsorption of WLBU2 was also evident at the PEO layer, however in much lower amounts as compared to the uncoated surface. While an appreciable fraction of the amount present at the end of a 30 min adsorption cycle was elutable, an elution plateau attained after each cycle indicates the presence of a peptide population entrapped in a fashion irreversible to elution by peptide-free buffer.

While WLBU2 is disordered in water, it exhibits a high α -helix content in membrane-mimetic solvents (e.g. 30% trifluoroethanol). Such solvents were not used in this study, as the pendant PEO configuration present in water and aqueous buffers would be significantly compromised. However, in aqueous solution it is fair to expect that the presence of added salt may shield the charged groups on the peptide that prohibit adoption of secondary structure. In fact the CD spectra of Fig. 3 show WLBU2 to be disordered in water, but increasingly helical in PBS with the addition of salt [20,21]. The amount of α -helical structure in a peptide can be calculated as proportional to the molar ellipticity at 222 or 208 nm [22–24]. A greater magnitude of ellipticity at either wavelength indicates greater α -helical structure. The representative spectra of Fig. 3 indicate that salt increases the fraction of helical structure in WLBU2, being 9.3, 15.8, 16.2, or 17.8% α -helix when dissolved in water, PBS, PBS + 250 mM NaCl, or PBS + 450 mM NaCl, respectively. In summary the peptide structure tends to be more ordered as the amount of salt in the aqueous solvent is increased.

Fig. 4 shows representative results for WLBU2 adsorption from pure water in comparison to WLBU2 adsorption from PBS at PEO-coated surfaces. Adsorption was slower and less extensive in water than in PBS, but the elution plateaus were similar. Slower adsorption in water is consistent with the peptide having no stable secondary structure in that solvent. The peptide is small enough to become entrapped independent of its secondary structure, but its entry into the PEO layer is apparently facilitated by adoption of a more ordered conformation.

3.2. Adsorption of PLG and PLL

The carboxylic acid side-chains of PLG are protonated ($-\text{COOH}$) and neutral at low pH, but become deprotonated ($-\text{COO}^-$) and negatively charged at higher pH. CD studies of its secondary structure in aqueous NaCl show a sharp transition in conformation from α -helix to random coil as the solution pH is increased [5,25]. This loss of orderly structure at higher pH was attributed to electrostatic repulsion between the negatively-charged (unprotonated) carboxylic side-chains. Cations from salts (e.g. Ca^{2+} or Na^+) may also shield the net negative charge of unprotonated PLG, causing similar conformational changes.

Unprotonated (disordered) PLG in dilute HCl (pH 4.7) was found to adsorb to both the uncoated surface and the PEO brush layer, albeit in small amounts (Fig. 5). Being at once negatively charged and non-amphiphilic, PLG would be expected to show little affinity for the uncoated surface. The flat elution plateaus recorded at that surface (Fig. 5, left panel) indicate a population of peptide that is irreversibly bound, presumably localized at defects (e.g., asperities, other physical heterogeneities) on the surface. While in a disordered form, as observed for WLBU2 in water, PLG is apparently small enough to enter the PEO layer (Fig. 5, right panel). But the absence of an elution plateau suggests the adsorbed peptide is entirely elutable.

The representative results of Fig. 5 also indicate that, with comparable amounts of adsorption recorded at each surface, peptide adsorption to a PEO brush was still distinctly different than that observed at the surface not coated with PEO. Visual inspection of the elution patterns indicates that there is no irreversibly bound peptide at the PEO layer. This suggests the peptide is (reversibly) located within the PEO brush itself, and not associating with the underlying surface. If peptide was associating with the underlying surface, we would expect an elution plateau similar to that seen at the uncoated surface (Fig. 5, left panel). Moreover, there is an obvious history dependence on adsorption recorded with the uncoated surface that is not apparent with the PEO layer. In particular, with adsorption to the uncoated surface, the initial adsorption rate during the second adsorption step is substantially greater than that observed at the same surface coverage during the first step. This behavior was not observed at the PEO layer, suggesting again that peptide association with the underlying surface does not play a significant role in adsorption in that case.

No appreciable adsorption to either the uncoated surface or the PEO brush layer was recorded by OWLS for the protonated, helical (ordered) form of PLG. Similarly, no evidence of PLL adsorption in either form was detected by OWLS. While it is entirely reasonable to expect insignificant adsorption by the homopolymers, we applied CD to the evaluation of PLL structure in the presence and absence of uncoated and F108-coated nanoparticles. The representative results shown in Fig. 6 (top panel) indicate that spectra recorded for PLL are similar, independent of the presence of F108-coated nanoparticles. Moreover, after the F108-coated nanoparticles (suspended in PLL solution) are washed with water, Fig. 6 (top panel) indicates that peptide was substantially removed from the sample. These results are consistent with no obvious location of PLL within the PEO brush. On the other hand, Fig. 6 (bottom panel) shows that relatively little peptide is removed upon washing a suspension of WLBU2 and F108-coated particles. This result is indicative of peptide entrapment and is consistent with the outcome of OWLS detection of WLBU2 adsorption and elution from PBS (Fig. 4). These spectra also show WLBU2 gains considerable α -helix content after entrapment within the F108 layer from PBS (increasing from 15.8 to 24.9% α -helix after entrapment). Induction of α -helix in this way is owing to the hydrophobicity of the PEO layer (below).

Contrary to OWLS detection of WLBU2 adsorption from HPLC water (Fig. 4), CD spectra provided no corroborating evidence of WLBU2 entrapment from this solvent by F108-

coated nanoparticles (data not shown). In particular, spectra recorded for the disordered form of WLBU2 in HPLC water with and without F108-coated nanoparticles were similar, and after washing with water the peptide was substantially removed from suspension with the coated nanoparticles. This finding suggests some degree of structural order may be necessary for entry into the brush. We have recently completed a comprehensive CD investigation of this possibility, which will be described in a separate report.

It is reasonable to expect based on Figs. 4–6 that any peptide retention in the PEO layer is owing to its amphiphilicity. Theoretical and experimental evidence suggests that below the hydrophilic outer region of a PEO brush there exists a hydrophobic region that is favorable for protein adsorption [26,27]. In particular, using surface force measurements, Sheth and Leckband [26,27] provided direct evidence for the formation of strong attractive forces between PEO and protein (streptavidin). Forces were repulsive on approach, but became attractive when the proteins were pressed into the PEO layer. They rationalized this in relation to the competitive interactions between solvent as well as proteins for the chain segments, and to the ability of PEO to adopt higher order intrachain structures. Lee et al. [28] recently demonstrated that PEO chains are not hydrophilic when they are arranged in the polymer brush configuration. They suggested that, at the high PEO chain concentrations consistent with brush formation, the specific configuration of the polymer that enables the hydrophilic interaction with water may become disrupted, rendering the polymer less soluble (or even insoluble) in water. Others had in fact predicted theoretically [29,30] and shown experimentally [31] that beyond some threshold PEO chain density the PEO brush would “collapse” owing to this effect. While increasing chain density within a brush layer might eventually favor lateral compression, Lee et al. concluded that the widely observed, steric-repulsive character of PEO brushes is retained because the hydrophobicity of the brush (which favors compression) is not sufficient to overcome the opposing force of the chain conformational entropy (which resists collapse) [28].

We have used OWLS to record changes in adsorbed mass during cyclic adsorption-elution experiments with the cationic, amphiphilic peptide nisin, at uncoated and PEO-coated surfaces [18]. PEO layers in that work were prepared by radiolytic grafting of F108 as well as Pluronic® surfactant F68 to silanized waveguides, producing long- or short-chain PEO layers (141 vs. 80 EO units), respectively. As recorded here with WLBU2, nisin adsorption to the uncoated surface showed history dependence while nisin adsorption to the F108-coated surface did not show history dependence. While nisin entry into the F68 brush was observed during the adsorption step, it was completely eluted upon introduction of peptide-free buffer, indicating that the peptide did not associate with the underlying surface. The lack of stable surface contact in that case suggested nisin entrapment within (the longer PEO chains of) F108 involved its location within the hydrophobic inner region, without contacting the underlying surface. In summary, while peptide adsorption in a fashion resistant to elution (entrapment) within the PEO brush was not detected with OWLS or CD in the case of the homopolymers, entrapment was evident in the case of WLBU2. Thus we conclude that stable entrapment of a peptide in PEO is explainable by its association with the inner, more hydrophobic region of the PEO brush, while the lack of retention of a peptide can be explained by its inability to take part in such associations.

3.3. Comparison to a model for protein adsorption

It is instructive to interpret the adsorption-elution patterns recorded with OWLS at F108-coated surfaces with reference to a simple model for protein adsorption and desorption [32]. When a peptide solution is introduced to the PEO layer, the change in adsorbed amount as a

function of time, $d\Gamma/dt$, may be written as $d\Gamma/dt = k_a c_b \Phi - \sum_i k_{d,i} \Gamma_i$, where $k_a c_b$ is the intrinsic

adsorption rate, Φ is the cavity function, $k_{d,i}$ is the desorption rate constant for the peptide adsorbed in each state i , and Γ_i is the amount of peptide adsorbed in the state i . These adsorption “states” represent different peptide conformations, etc., that result in different resistances to elution. The cavity function is defined as the fraction of the surface on which the center of an incoming molecule could adsorb without overlapping a previously adsorbed molecule [1,32–35]. During elution, when the contacting peptide solution is replaced by a peptide-free solution, peptide concentration in the bulk goes to zero, and a net desorption

from the surface occurs, such that
$$\frac{d\Gamma}{dt} = - \sum_i k_{d,i} \Gamma_i$$

Estimation of the rate constants from experimental data is straightforward based on the above. The lumped adsorption rate constant ($k_a c_b$) was obtained by using the data of Figs. 4 and 5b to generate plots of $d\Gamma/dt$ vs. Γ . Linear regression of the early kinetics and extrapolation to $\Gamma = 0$ approaches a condition where desorption does not occur and $\Phi = 1$, such that the “y-intercept” is $k_a c_b$, and $d\Gamma/dt|_{\Gamma=0} = k_a c_b$.

The desorption rate constants were estimated using the elution profiles, assuming the existence of two desorbable states, one that is less tightly bound (state 1) and one that is more tightly bound (state 2), and one irreversibly bound state (state 3). The values of k_{d1} and k_{d2} were found using the slopes of a plot of $d\Gamma/dt$ vs. Γ during the first rinse cycle. From this plot, two distinct linear regions could be identified, with k_{d1} characterizing the slope of the line at high surface coverages and k_{d2} characterizing the slope of the line at lower surface coverages.

The amount of peptide adsorbed in each state at the onset of rinsing, Γ_1 , Γ_2 , and Γ_3 , is also easily determined from the same plot of $d\Gamma/dt$ vs. Γ . The value of Γ at the intercept of the two lines was taken as equal to $\Gamma_2 + \Gamma_3$, i.e. the surface concentration after all peptide in state 1 had been eluted. Γ_1 was thus determined by a simple mass balance. The value of Γ corresponding to the “x-intercept” of the second linear region (i.e., of slope k_{d2}) was taken as Γ_3 and Γ_2 was then also determined by a mass balance. (If the plot of $d\Gamma/dt$ vs. Γ during elution showed only a single linear region, then Γ_2 was set to zero.) The kinetic parameters and surface coverages in each state for peptide adsorption to PEO (as recorded by OWLS) are summarized in Table 1.

As discussed by Calonder et al. [33], in order to compare independent OWLS data sets in a quantitative way, it is vital to use the same waveguiding surface each time. Without repositioning the waveguide in a given OWLS experiment, the reproducibility between any two adsorption experiments (separated by an *in situ* cleaning step) is very high. However, in order to avoid artifacts introduced by incomplete washing of the PEO brush layers used here, independent experiments were performed with a new, repositioned waveguide each time. Thus the parameters in Table 1 simply offer a quantitative representation of the adsorption and elution trends revealed by Figs. 4 and 5, as opposed to absolute rate constants and surface coverages. Consistent with earlier visual inspection of peptide adsorption at the F108-coated surfaces, this analysis shows that the intrinsic adsorption rate constant is greater for the more ordered form of WLBU2 than for the disordered WLBU2 or the disordered PLG. In addition, the desorption rate constants are lower for the amphiphilic WLBU2 than for the non-amphiphilic PLG. The amphiphilic WLBU2 also showed a substantially greater proportion of irreversibly entrapped peptide than did the non-amphiphilic PLG.

4. Conclusions

We have seen that, for a peptide of a size allowing adsorption to a PEO layer, the structure and amphiphilic character of the peptide will affect its adsorption affinity. In particular, the

results reported here direct us to expect that a more ordered, compact peptide will enter the PEO phase more readily than a peptide of similar size that adopts a less ordered, less compact form. We also expect amphiphilicity will promote peptide retention, by association with the inner hydrophobic region of the PEO layer. An experimentally based, quantitative understanding of the adsorption and function of peptides at otherwise protein-repellent PEO layers does not currently exist, and these results provide a rationale for hypotheses to drive further discovery and understanding in this important area.

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Fig. 1. Poly-L-glutamic acid and poly-L-lysine will adopt compact α -helical forms or disordered, highly-charged conformations depending on solution conditions. WLBU2 is an amphiphilic cationic peptide that is α -helical in membrane mimetic solvents and disordered in aqueous solution.

Fig. 2. Cyclic adsorption and elution of WLBU2 at (left) an uncoated, silanized, γ -irradiated surface and (right) a PEO layer. The initial rate of adsorption in the second cycle is shifted back for illustrative purposes, to allow comparison of adsorption rates at equal surface coverages in each cycle.

Fig. 3. CD spectra of WLBU2 in water, PBS, PBS + 250 mM NaCl, and PBS + 450 mM NaCl.

Fig. 4. Adsorption of WLBU2 from water at a PEO layer (gray symbols). WLBU2 adsorption from PBS at a PEO layer (black symbols) is replotted from Fig. 2 for comparison.

Fig. 5. Cyclic adsorption and elution of poly-L-glutamic acid (dilute HCl, pH 4.7) at (left) an uncoated, silanized, γ -irradiated surface and (right) a PEO layer. The initial rate of adsorption in the second cycle is shifted back for illustrative purposes, to allow comparison of adsorption rates at equal surface coverages in each cycle.

Fig. 6. CD spectra of: (top) poly-L-lysine in water, and in suspension with F108-coated nanoparticles before and after washing, and (bottom) WLB2 in PBS, and in suspension with F108-coated nanoparticles before and after washing.

Table 1

Adsorption and desorption kinetic parameters, and surface coverages in each state estimated for peptide adsorption to PEO.

Parameter	WLBU2 in water (disordered)	WLBU2 in PBS (more ordered)	Poly-L-glutamic acid (disordered)
$k_a c_b$ (ng (cm ² min) ⁻¹)	0.11	1.0	0.16
k_{d1} (min ⁻¹)	0.0054	0.0069	0.012
k_{d2} (min ⁻¹)	–	–	0.00061
Γ_1 (ng/cm ²)	8.0	25	11
Γ_2 (ng/cm ²)	–	–	18
Γ_3 (ng/cm ²)	16	18	0.62