Published in final edited form as:

Colloids Surf B Biointerfaces. 2013 December 1; 112: . doi:10.1016/j.colsurfb.2013.07.033.

Adsorption, structural alteration and elution of peptides at pendant PEO layers

Xiangming Wu, Matthew P. Ryder, Joseph McGuire, and Karl F. Schilke*

School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR 97331

Abstract

An experimentally based, quantitative understanding of the entrapment and function of small peptides within PEO brush layers does not currently exist. Earlier work provided a rationale for expecting that an ordered, compact peptide will enter the PEO phase more readily than a peptide of similar size that adopts a less ordered, less compact form, and that amphiphilicity will promote peptide retention within the hydrophobic region of the PEO brush. Here we more deliberately describe criteria for peptide integration and structural change within the PEO brush, and discuss the reversibility of peptide entrapment with changing solvent conditions. For this purpose, circular dichroism (CD) was used to record the adsorption and conformational changes of (amphiphilic) WLBU2 and (non-amphiphilic) polyarginine peptides at uncoated (hydrophobic) and PEO-coated silica nanoparticles. Peptide conformation was controlled between disordered and α -helical forms by varying the concentration of perchlorate ion. We show an initially more ordered (α -helical) structure promotes peptide adsorption into the PEO layer. Further, a partially helical peptide undergoes an increase in helicity after entry, likely due to concomitant loss of capacity for peptide-solvent hydrogen bonding. Peptide interaction with the PEO chains resulted in entrapment and conformational change that was irreversible to elution with changing solution conditions in the case of the amphiphilic peptide. In contrast, the adsorption and conformational change of the non-amphiphilic peptide was reversible. These results indicate that responsive drug delivery systems based on peptide-loaded PEO layers can be controlled by modulation of solution conditions and peptide amphiphilicity.

Keywords

peptide integration; PEO brush; WLBU2; cationic amphiphilic peptides (CAPs); polyarginine; circular dichroism (CD)

Introduction

In an earlier paper, we suggested the potential for surface coatings based on entrapment of bioactive agents into PEO brush layers for short-term medical device applications [1]. In particular, strategies featuring drug-loaded but otherwise nonfouling coatings for blood contact hold promise for enhancing the performance of medical devices, ranging from anti-

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*Corresponding Author: Gleeson Hall Rm. 102, Oregon State University, Corvallis, OR 97331 USA, karl.schilke@oregonstate.edu
FAX: 1-541-737-4600, Telephone: 1-541-737-7283.

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infective catheters to hemoperfusion modules with microscale flow features. Lampi et al. [1] used optical waveguide lightmode spectroscopy (OWLS) to describe the adsorption of poly-*L*-glutamic acid and the cationic amphiphilic peptide WLBU2 at polyethylene oxide (PEO) brush layers. Circular dichroism (CD) was also used to describe the structures of poly-*L*-lysine and WLBU2 at solid, hydrophobic surfaces, and in the PEO brush. The solution structure of each peptide was controlled between disordered and more ordered (α -helical) forms by varying the salt concentration in the peptide solutions. Although protein adsorption at sparse PEO brush layers is predicted and observed in practice [2-4], an experimentally based, quantitative understanding of the adsorption and function of small peptides at PEO brush layers does not currently exist. The results of our previous work [1] provide a rationale for expecting that a more ordered and compact (e.g. α -helical) peptide will enter the PEO phase more readily than a peptide of similar size that adopts a less ordered, less compact form. Furthermore, because a hydrophobic inner core is predicted to exist in PEO brushes [5], it is expected that amphiphilicity will promote the retention of peptides within this region of the PEO brush.

WLBU2 (RRWVRRVRRWVRRVVRVRRWVRR) is an engineered, 24-residue cationic amphiphilic peptide (CAP), with 13 positively charged arginine residues, and 11 nonpolar 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. Segregation of the positively-charged Arg and hydrophobic Val/Trp groups onto opposing faces of an α -helix confers the ability to disrupt bacterial cell membranes, even when immobilized [6-12]. While the hydrophobic residues in WLBU2 make it a highly amphiphilic peptide, poly-*L*-arginine (PLR) is chemically homogeneous and not amphiphilic, and thus serves as an excellent control for the effects of amphiphilicity on peptide adsorption and entrapment in PEO brush layers. When dissolved in water under neutral pH, polyarginine adopts a combination of random and extended (e.g. polyproline-II and 2.5₁ helix) structures, while WLBU2 shows little appreciable stable structure [1, 6-9, 13-17]. However, in the presence of perchlorate ion (ClO_4^-), both peptides will adopt a rigid α -helical structure [16].

In this paper, we more deliberately describe criteria for integration and structural changes of peptides within the PEO brush, and discuss the reversibility of peptide entrapment with changing solvent conditions for amphiphilic and non-amphiphilic motifs. For this purpose, circular dichroism (CD) was used to record the adsorption and conformational changes of PLR and WLBU2 at bare (hydrophobic) and PEO-coated silica nanoparticles. In order to elucidate the effect of structure on peptide interaction with the PEO brushes, the solution conformation of polyarginine and WLBU2 peptides was controlled between the disordered and α -helical forms by varying the concentration of perchlorate ion.

Materials and Methods

Peptides and materials

Lyophilized 30-residue average (4.7 kDa, PDI < 1.20) synthetic poly-*L*-arginine hydrochloride (PLR) was purchased from Alamanda Polymers (Huntsville, AL). PLR was dissolved at 5.0 mg/mL in HPLC water, and separated into 1.0 mL aliquots that were frozen and thawed prior to each experiment. The 5.0 mg/mL PLR stock was diluted to 0.2 mg/mL in HPLC water, or with 0.05 M or 0.5 M perchloric acid (HClO_4), to invoke either disordered or helical conformations, respectively. Similarly, lyophilized WLBU2 (3.4 kDa) was purchased from GenScript (Piscataway, NJ), and dissolved at 5 mg/mL in HPLC water and frozen in 1 mL aliquots. The WLBU2 stock solution was thawed prior to use, and diluted to 0.2 mg/mL in HPLC water, or with 0.2 M or 0.5 M HClO_4 . All peptides were

used as supplied, without further purification. Diluted peptide solutions were degassed for 40 min under vacuum immediately before use.

Self-assembled PEO brush layers were formed by suspension of hydrophobic silica nanoparticles (R816, Degussa, 190 m²/g, 10-12 nm) in Pluronic® F108 (BASF) in HPLC water for 10 h on a rotator. About 3.3 mg/m² of F108 are required for complete surface coverage [18]; a 5× excess of F108 over this amount was used to ensure good coverage of the silica nanoparticles. Uncoated and F108-coated nanoparticles were then incubated with PLR or WLBU2 at 0.2 mg/mL under different solvent conditions, for a desired period of time (2h to 7 d) at 20 °C. Nanoparticle concentrations (2 mg/mL and 10mg/mL) were selected based on previous OWLS results [1], and provided either sufficient surface area for complete adsorption of the peptide, or a 5× excess surface area for adsorption (to minimize peptide-peptide interactions).

Evaluation of peptide secondary structure

Peptide secondary structure in the presence or absence of nanoparticles was evaluated in triplicate by circular dichroism (CD) using a Jasco J-815 spectropolarimeter (Easton, MD) at 25 °C. The spectra from each of the three replicates for each sample were nearly identical, with only slight (~ 5%) differences in signal intensity; representative spectra are shown throughout. The instrument was calibrated using 0.6 mg/mL *D*(+)-camphorsulfonic acid. Spectra were recorded in a cylindrical cuvette (0.1 cm pathlength) from 185 to 260 nm in 0.5 nm increments, and 10 scans were averaged in order to increase the signal-to-noise ratio. The 0.2 mg/mL peptide samples prepared as outlined above were filtered (0.20 μm) prior to contact with nanoparticles and recording of CD spectra. Nanoparticles were rinsed by centrifugation (10,000 rpm, 20 min) and removal of the supernatant, after which the pellet was resuspended in water or HClO₄ of desired concentrations, and the process repeated a total of three times. All CD spectra were blanked against peptide-free solutions or NP suspensions.

Stabilization of F108 coatings on OWLS waveguides

SiO₂-coated OW2400c OWLS sensors were purchased from MicroVacuum (Budapest, Hungary). Sensors were cleaned using 3% aqueous SDS (30 min) followed by 10 min wash in 5:1:1 mixture of H₂O:HCl:H₂O₂ solution at 80 °C for 10 min. After cleaning, surfaces were rinsed with water, and dried under a stream of nitrogen. The sensor surfaces were then modified by vapor deposition of trichlorovinylsilane (TCVS, TCI America, Portland, OR). 200 μL of TCVS was evaporated at 20°C into a stream of dry nitrogen carrier gas, which was directed over the waveguide surfaces for 4 hrs. The silanized waveguides were then immersed in a solution of 5% w/v Pluronic® F108 in water, and were rotated in solution overnight. After incubation, samples were ⁶⁰Co irradiated with a ⁶⁰Co source to a total dose of 3 kGy to covalently the F108 to the surface [19, 20]. The irradiated waveguides were rinsed with water, dried with N₂, and stored desiccated under N₂ in the dark until used.

Measurement of the rate and extent of peptide adsorption

Peptide adsorption was measured with an OWLS 210 instrument (MicroVacuum, Budapest, Hungary). A Rheodyne manual sample injector was used to inject sample solutions through a flow loop (~ 4.0 mL) into the OWLS flow cell. Flow rates were maintained at 50 μL/min for 40 minutes of sample adsorption time, and solution temperature was kept at 20 °C by the internal TC heater/cooler unit. Incident angle scans were performed from -5° to 5° at a step size of 0.01°. Both peaks of each of the transverse electric and magnetic modes were measured to determine the relative refractive index of the surface adlayer. OWLS experiments began with a baseline of peptide-free water or perchloric acid, followed by injection of 0.1 mg/mL peptide in either water or perchloric acid, and a subsequent rinse

with either perchloric acid or water. A single OWLS experiment was performed for each adsorption/rinse condition. Reproducibility of OWLS measurements is high, typically with less than 5% variation in the plateau adsorbed mass at each adsorption/rinse step [21, 22].

Results and Discussion

Effect of perchlorate ions on structure of PLR

Polyarginine (PLR) exhibits a “disordered” (polyproline-II) structure in water under pH < 12, and an α -helical structure under pH > 12 [14]. However, pH could not be used in this study to influence peptide structure, as the silica nanoparticles used for CD would be hydrolyzed at basic pH [23]. Instead, perchlorate ions (ClO_4^-) were used to induce the α -helical conformation of PLR [16]. Circular dichroism spectra of PLR show it to be disordered in water, but the peptide becomes more helical with increased concentration of perchloric acid (Figure 1, left). This structural change is indicated by the change in the spectrum from a characteristic “random coil” to “ α -helix” form, as well as an increase in ellipticity at 222 nm [24-26]. Deconvolution of representative CD spectra with Dichroweb [27, 28] indicate that the helicity of PLR increases from approximately 2% in water to 31% in 0.05 M HClO_4 , and reaches 61% α -helix in 0.5 M HClO_4 .

Effect of perchlorate ions on structure of WLBU2

While WLBU2 is almost completely disordered in water [9], it exhibits a high α -helix content in HClO_4 , increasing from 3% in water to 15% in 0.2 M or 30% in 0.5 M HClO_4 , respectively (Figure 1, right). The lower α -helix content observed for WLBU2, when compared to PLR at equivalent HClO_4 concentrations, may be due to the lower arginine content (13 of 24 amino acids) of WLBU2. However, computed “absolute” helicity values are dependent upon the model implemented in the software, and should only be used for comparative purposes [24, 28].

HClO_4 concentrations above 0.5 M did not further increase the calculated helicity of either PLR or WLBU2 in an aqueous milieu (data not shown), although WLBU2 is reported to reach 81% α -helix in a membrane-mimetic solvent (20% trifluoroethanol) [9]. No conformational change was observed for either PLR or WLBU2 in aqueous solutions of PEO (data not shown), indicating that peptide conformation is largely unaffected by the presence of free PEO chains. This implies that any structural change observed in the presence of a PEO brush is due to the unusual environment of the brush layer, and cannot be attributed to individual PEO-peptide interactions.

Adsorption of disordered PLR and WLBU2

Both WLBU2 and PLR show substantially disordered structure when dissolved in water (Figure 1). Our previous OWLS and CD experiments showed that disordered poly-*L*-lysine (PLL) and WLBU2 have little affinity for F108-coated surfaces [1]. Here, we applied CD to the evaluation of PLR and WLBU2 structure in the presence and absence of uncoated (hydrophobic) and F108-coated nanoparticles. Spectra recorded for disordered PLR in the presence of uncoated and F108-coated nanoparticles are quite similar (Figure 2). The CD signal is greatly decreased after washing the bare or coated nanoparticles one time with water. This result indicates that the disordered peptides do not interact strongly with the nanoparticles, and are easily eluted from bare or F108-coated surfaces. Similar behavior has been observed for disordered PLL at hydrophobic and F108-coated OWLS sensors [1]. Presumably, the large solution volume of the swollen, “disordered” peptide prevents penetration and integration into the PEO brush. Slightly more PLR was retained on the bare nanoparticles, presumably through electrostatic interactions between the positively charged guanidinium groups and negatively charged uncoated nanoparticle surface. However, this

interaction with the bare surface is apparently too weak to cause any substantial conformational changes in the PLR [29].

As with PLR, the “disordered” conformation of WLBU2 in water is similar and independent of the presence or absence of F108-coated nanoparticles. The loss of CD signal indicates that the peptide was almost completely removed after washing with water (Figure 3).

However, a substantial conformational difference was observed for WLBU2 in water and in suspension with bare, hydrophobic nanoparticles. Unlike PLR, WLBU2 is amphiphilic and its hydrophobic groups have great affinity for the uncoated hydrophobic surface. We speculate that initial adsorption of the hydrophobic side-chains (which are ordered along one face of the helical form of the amphipathic peptide) favors the formation of a partially α -helical structure on the surface. The CD signal is only partially reduced by washing with water, indicating that a large population of adsorbed and non-elutable peptides remains on the nanoparticles. Figure 3 also suggests that the wash preferentially removes loosely-bound “disordered” peptides, as the remaining adsorbed peptides produce a weak yet characteristic α -helical spectrum.

Adsorption of α -helical PLR and WLBU2

As discussed above, both PLR and WLBU2 are substantially α -helical (61% and 30%) in 0.5M HClO₄. The α -helix conformation of PLR in 0.5M HClO₄ was mostly independent of the presence of uncoated hydrophobic nanoparticles (Figure 4, left). However, the helicity of PLR increased slightly, from 61% to 82%, in the presence of F108-coated nanoparticles. This phenomena is more obvious in Figure 4 (right), in which PLR of a lower helicity (31% in 0.05 M HClO₄) was added to suspensions of uncoated and F108-coated nanoparticles. Again, no conformational change occurred in the presence of the uncoated nanoparticles, but an increase in helicity (from 31% to 49%) was observed in the presence of the F108-coated nanoparticles. These results suggest that interactions between the peptide and the hydrophobic core of the PEO brush, in which a peptide with a small amount of initial α -helix conformation becomes more helical as a result of contact with the brush. In contrast, a completely disordered peptide is completely excluded from the brush and undergoes no conformational change.

Perchlorate ions stabilize a peptide's α -helical structure by competing with water molecules which would normally solvate the peptide, causing a loss of hydration and promoting the intra-peptide hydrogen-binding characteristic of the α -helix conformation [30]. Theoretical and experimental evidence suggests that a hydrophobic region that is favorable for protein adsorption exists in the interior of a PEO brush [4, 5, 31]. A similar effect is expected when a partially dehydrated, helical peptide penetrates into the hydrophobic inner region of the PEO brush, promoting the peptide's further dehydration and increasing its helicity. Similar conformational changes in response to the hydrophobic cell membrane are thought to be responsible for PLR's cell-penetrating and cytotoxic properties [32].

Stability of peptides at nanoparticle surfaces

The data presented thus far suggest that an increase in α -helix conformation is associated with integration of the peptides into F108 brushes. If so, these peptides should be more resistant to elution than would peptides which were conformationally changed but merely loosely-bound or unassociated with the brush. Uncoated and F108-coated nanoparticles were incubated with α -helical PLR in 0.5 M HClO₄ solution, and then washed twice with 0.5 M HClO₄ (maintaining conditions which promote α -helix structure). The partial decrease in CD signal after each wash with 0.5 M HClO₄ (Figure 5) is consistent with some loss of peptide with each rinse. However, the residual CD signal after washing indicates that

considerable α -helical peptide remained on both surfaces after rinsing them with HClO_4 . Importantly, the spectra are nearly identical in the presence or absence of F108, suggesting that the interactions of the helical peptide with the hydrophobic surface are closely mimicked by the apolar conditions which are expected to exist within the F108 brush [4, 5].

In contrast, however, when the nanoparticle suspensions were contacted with α -helical PLR in HClO_4 and then washed with water, the peptide was nearly completely eluted from both F108-coated and uncoated surfaces (Figure 5), although a small residual signal suggests some ordered helical form for the remaining peptides. Remarkably similar behavior was observed for nanoparticles contacted with “disordered” peptides in water (Figure 2). The bulk concentration of perchlorate ion would be greatly reduced during washing, thus eliminating the helix-stabilizing microenvironment and allowing the peptide to resume a “disordered” conformation. Taken together, these results suggest that elution of peptides from the F108 brush is at once governed and controllable by bulk solution conditions.

Optical waveguide lightmode spectroscopy (OWLS)

OWLS experiments were carried out to verify that α -helical peptides are stably adsorbed on F108-coated surfaces, and their rate of elution is primarily determined by solution conditions (Figure 6). One obvious criticism of the CD experiments is that the F108 is not covalently linked to the nanoparticle surface, and thus some results might be interpreted as competitive displacement of the triblocks by the peptides (especially the inherently amphiphilic WLBU2). Although no obvious desorption of triblocks by nisin (a CAP of similar size to WLBU2) was observed in previous work [18, 33], we investigated peptide adsorption at immobilized F108 brushes using TCVS-modified OWLS waveguides on which we covalently immobilized F108 using γ -irradiation [18, 19]. Results with polyarginine in water (Figure 6) are entirely consistent with those in this work: contact of the F108-coated waveguide with disordered PLR resulted in negligible adsorption, while ordered peptide adsorbed strongly. As suggested above, the adsorbed α -helical peptide was relatively resistant to elution under helix-promoting solution conditions, but was quickly and completely desorbed when eluted with water (which favors the “disordered” form of the peptide, Figure 5).

Changes in peptide structure in F108 brushes

WLBU2 in 0.5 M HClO_4 (initially 30% helical) achieves considerable α -helix content (39%) after adsorption onto a bare hydrophobic surface (Figure 7, left) and into a PEO layer (43% helical). The increase in helicity is more obvious in Figure 7 (right), when WLBU2 is in 0.2 M HClO_4 (initially 15% α -helical), its helicity increases to 43% after entrapment into the PEO layer. Importantly, regardless of the initial helicity, the final α -helix content of the adsorbed WLBU2 is the same (43%) after adsorption into a PEO layer. This is different from the behavior of PLR (Figure 4), suggesting a conformational change due to the strong interaction between hydrophobic groups on the peptide and the hydrophobic inner region of PEO layer. Figure 7 also suggests that the interaction between WLBU2 and the PEO brush, while strong, allows for good molecular flexibility, since the final helicity of partially-ordered WLBU2 in the PEO brush is greater than on the bare surface (Figure 7, right).

As previously described with PLR, uncoated and F108-coated nanoparticles were incubated with α -helical WLBU2 in HClO_4 solution, and then washed with HClO_4 or water. In all cases, a small fraction of peptide was removed by washing (Figure 8). However, while changing the solvent from the helix-promoting HClO_4 to water (which favors a “disordered” conformation) resulted in nearly complete loss of PLR (see Figure 5 and Figure 6), solution changes had little effect on the intensity or shape of CD spectra of adsorbed WLBU2 (Figure 8). It is reasonable to expect that amphiphilicity is the cause of this retention of WLBU2 (but

not PLR) at F108-coated surfaces following a solvent change. While WLBU2 present in the hydrophilic outer region of PEO layer might be removed by rinsing with peptide-free buffer, an amphiphilic peptide WLBU2 which is entrapped in the hydrophobic inner region should show greater resistance to elution than would a non-amphiphilic peptide PLR. Moreover, the WLBU2 entrapped in the brush maintains its α -helical structure, even when the surrounding solvent has been changed from HClO₄ to water (Figure 8), while entrapped PLR undergoes a helix-coil transition in response to changes in solution conditions (Figure 5). Unlike the disordered peptides (Figures Figure 2 Figure 3), which do not interact with the interior of the brush, the spectra of PLR and WLBU2 on F108-coated and uncoated hydrophobic nanoparticles (Figures Figure 5 and Figure 7) are quite similar. This is consistent with partial helix formation in the peptides, which is presumably induced by either the hydrophobic environment at the particle surface or in the inner region of the brush.

In summary, an initially more ordered (α -helical) structure promotes the adsorption of a peptide into the PEO layer. A partially helical peptide undergoes an increase in helicity, probably due to the loss of peptide-solvent H-bonding in the apolar region within the brush [16, 32]. An amphiphilic peptide (e.g. WLBU2) is expected to have a much stronger interaction with the hydrophobic inner region of the PEO layer than a non-amphiphilic one (e.g. PLR). This interaction results in entrapment and conformational change of the amphiphilic peptide that is irreversible with respect to elution. In contrast, the adsorption and conformational change of non-amphiphilic peptides are reversible, making such peptides highly elutable because of their weak interaction with the brush.

Conclusions

The results reported here direct us to expect that some minimal degree of structural order (α -helix) is necessary for peptide entry into the PEO layer, and that peptide location within the hydrophobic inner region of the PEO brush may result in an increase in α -helix content. Once the non-amphiphilic peptide polyarginine (PLR) was entrapped among the PEO chains of the F108 brush, we found it to be partially elutable as long as the same helix-stabilizing solvent used during the adsorption step was used for elution. However, in contact with water (which favors its disordered, non-adsorbable conformation), the adsorbed PLR was entirely elutable. In contrast, the amphiphilic peptide WLBU2 was highly resistant to elution in all cases, even upon contact with a solvent which promotes its disordered form. Previously, we suggested that the well-known helix-coil transition of homopolyamino acids (e.g. PLL or PLR) might be used to reversibly anchor peptides or their conjugates within a PEO brush as a novel drug-delivery strategy. It appears, however, that the property of amphiphilicity (such as exhibited by WLBU2) is required to control peptide desorption from a PEO brush when the bulk solution conditions are changed.

This work provides direction for development of responsive drug delivery systems based on modulation of solvent conditions and bioactive peptide structure within PEO brush layers. Previous studies indicate that cationic peptides entrapped in PEO brushes are protected from competitive elution by large blood proteins (e.g. fibrinogen) [34, 35]. In addition, our recent work indicates that peptide-loaded brushes retain their ability to repel fibrinogen (unpublished data). Therefore, the presence of a PEO layer at the surface of medical devices could stabilize entrapped therapeutic peptides against competitive desorption by blood proteins, as well as provide desirable non-fouling characteristics to the device. Entrapment of therapeutic peptides may also support novel drug delivery strategies (e.g., PEO-coated nanoparticle carriers) that can potentially overcome barriers to oral delivery of peptide drugs [36]. Work currently underway in our laboratory toward these ends features the sequential and competitive adsorption behavior of peptide and peptide-protein mixtures at pendant PEO brush layers, and will contribute to the subject of future reports.

Acknowledgments

The authors thank Dr. Kerry McPhail of the OSU College of Pharmacy for use of her CD instrument, and Dr. Victor Hsu and Dr. Elisar Babar for valuable discussion on interpretation of the CD spectra. This work was supported in part by the National Institute of Biomedical Imaging and Bioengineering (NIBIB, grant no. R01EB011567). The content is solely the responsibility of the authors and does not necessarily represent the official views of NIBIB or the National Institutes of Health.

References

1. Lampi MC, Wu X, Schilke KF, McGuire J. *Colloids Surf B Biointerfaces*. 2013; 106:79–85. [PubMed: 23434695]
2. Bosker WTE, Iakovlev PA, Norde W, Cohen Stuart MA. *J Colloid Interface Sci*. 2005; 286:496–503. [PubMed: 15897063]
3. Fang F, Satulovsky J, Szleifer I. *Biophys J*. 2005; 89:1516–1533. [PubMed: 15994887]
4. Sheth SR, Leckband D. *Proc Natl Acad Sci U S A*. 1997; 94:8399–8404. [PubMed: 9237988]
5. Lee H, Kim DH, Witte KN, Ohn K, Choi J, Akgun B, Satija S, Won YY. *J Phys Chem B*. 2012; 116:7367–7378. [PubMed: 22616550]
6. Costa F, Carvalho IF, Montelaro RC, Gomes P, Martins MCL. *Acta Biomater*. 2011; 7:1431–1440. [PubMed: 21056701]
7. Deslouches B, Gonzalez IA, DeAlmeida D, Islam K, Steele C, Montelaro RC, Mietzner TA. *J Antimicrob Chemother*. 2007; 60:669–672. [PubMed: 17623696]
8. Deslouches B, Islam K, Craigo JK, Paranjape SM, Montelaro RC, Mietzner TA. *Antimicrob Agents Chemother*. 2005; 49:3208–3216. [PubMed: 16048927]
9. Deslouches B, Phadke SM, Lazarevic V, Cascio M, Islam K, Montelaro RC, Mietzner TA. *Antimicrob Agents Chemother*. 2005; 49:316–322. [PubMed: 15616311]
10. Gonzalez IA, Wong XX, De Almeida D, Yurko R, Watkins S, Islam K, Montelaro RC, El-Ghannam A, Mietzner TA. *Nat Precedings*. 2008
11. Onaizi SA, Leong SSJ. *Biotechnol Adv*. 2011; 29:67–74. [PubMed: 20817088]
12. Skinner MC, Kiselev AO, Isaacs CE, Mietzner TA, Montelaro RC, Lampe MF. *Antimicrob Agents Chemother*. 2010; 54:627–636. [PubMed: 20008784]
13. Adzhubei AA, Sternberg MJE, Makarov AA. *J Mol Biol*. in press.
14. Bochicchio B, Tamburro AM. *Chirality*. 2002; 14:782–792. [PubMed: 12395395]
15. Mikhonin AV, Myshakina NS, Bykov SV, Asher SA. *J Am Chem Soc*. 2005; 127:7712–7720. [PubMed: 15913361]
16. Rifkind JM. *Biopolymers*. 1969; 8:685–688. [PubMed: 5362531]
17. Tiffany ML, Krimm S. *Biopolymers*. 1969; 8:347–359.
18. Tai Y-C, McGuire J, Neff JA. *J Colloid Interface Sci*. 2008; 322:104–111. [PubMed: 18377919]
19. McPherson TB, Shim HS, Park K. *J Biomed Mater Res*. 1997; 38:289–302. [PubMed: 9421750]
20. Tseng Y-C, McPherson T, Yuan CS, Park K. *Biomaterials*. 1995; 16:963–972. [PubMed: 8580258]
21. Höök F, Vörös J, Rodahl M, Kurrat R, Böni P, Ramsden JJ, Textor M, Spencer ND, Tengvall P, Gold J, Kasemo B. *Colloids Surf B Biointerfaces*. 2002; 24:155–170.
22. Calonder C, Tie Y, Van Tassel PR. *Proc Natl Acad Sci U S A*. 2001; 98:10664–10669. [PubMed: 11535805]
23. Salmio H, Brühwiler D. *The Journal of Physical Chemistry C*. 2006; 111:923–929.
24. Greenfield NJ. *Anal Biochem*. 1996; 235:1–10. [PubMed: 8850540]
25. Greenfield NJ, Fasman GD. *Biochemistry (Mosc)*. 1969; 8:4108–4116.
26. Woody, RW. Circular dichroism and the conformational analysis of biomolecules. In: Fasman, GD., editor. *Theory of circular dichroism of proteins*. Plenum Press; New York: 1996. p. 25-30.
27. Whitmore L, Wallace BA. *Nucleic Acids Res*. 2004; 32:W668–W673. [PubMed: 15215473]
28. Whitmore L, Wallace BA. *Biopolymers*. 2008; 89:392–400. [PubMed: 17896349]
29. Puddu V, Perry CC. *ACS Nano*. 2012; 6:6356–6363. [PubMed: 22725630]

30. Ascietto EK, General IJ, Xiong K, Asher SA, Madura JD. *Biophys J*. 2010; 98:186–196. [PubMed: 20338840]
31. Halperin A. *Langmuir*. 1999; 15:2525–2533.
32. Takechi Y, Mizuguchi C, Tanaka M, Kawakami T, Aimoto S, Okamura E, Saito H. *Chem Lett*. 2012; 41:1374–1376.
33. Tai Y-C, Joshi P, McGuire J, Neff JA. *J Colloid Interface Sci*. 2008; 322:112–118. [PubMed: 18359037]
34. Ryder MP, Schilke KF, Auxier JA, McGuire J, Neff JA. *J Colloid Interface Sci*. 2010; 350:194–199. [PubMed: 20619847]
35. Schilke KF, McGuire J. *J Colloid Interface Sci*. 2011; 358:14–24. [PubMed: 21440897]
36. De Jong WH, Borm PJ. *Int J Nanomed*. 2008; 3:133.

Abbreviations

CAP	cationic amphiphilic peptide
CD	circular dichroism
NP	nanoparticle
OWLS	optical waveguide lightmode spectroscopy
PEO	polyethylene oxide
PLR	poly- <i>L</i> -arginine
PLL	poly- <i>L</i> -lysine

Research Highlights

- α -Helical peptides penetrate PEO brushes, while “disordered” conformations do not.
- Peptide α -helicity increases in the hydrophobic inner region within a PEO brush.
- The elutability of non-amphiphilic peptides depends on bulk solution conditions.
- Amphiphilic peptides are resistant to elution, regardless of solution conditions.
- Structure-based peptide-PEO associations may enable novel drug delivery strategies.

Figure 1.

CD spectra of PLR (left) in water, 0.05 M HClO₄, 0.5 M HClO₄ and WLBU2 (right) in water, 0.2 M HClO₄, 0.5 M HClO₄. Characteristic spectra for peptides in “disordered” (random coil) and α -helix conformations are labeled.

Figure 2.
CD spectra of PLR in water, and in suspension with uncoated and F108-coated nanoparticles before and after washing.

Figure 3.
CD spectra of WLBUE in water, and in suspension with uncoated and F108-coated nanoparticles before and after washing.

Figure 4.

CD spectra of: (left) PLR in 0.5 M HClO_4 , and in suspension with uncoated and F108-coated nanoparticles, (right) PLR in 0.05 M HClO_4 , and in suspension with uncoated and F108-coated nanoparticles.

Figure 5.

CD spectra of: PLR in HClO_4 , and in suspension with F108-coated (left) and uncoated (right) nanoparticles before and after washing with HClO_4 or water.

Figure 6.

Adsorption and elution profiles of PLR on an OWLS waveguide coated with immobilized F108. Baseline was achieved using HPLC H₂O or 0.5M perchloric acid, followed by adsorption of 0.1 mg/mL PLR in H₂O or HClO₄, and then elution with H₂O or HClO₄. Little PLR adsorption was observed in water (), suggesting that aqueous (disordered) PLR does not integrate into the F108 brush layer. -Helical PLR adsorbed substantially from HClO₄, but was nearly completely removed from the brush by rinsing with water (—). In contrast, PLR adsorbed from and rinsed with HClO₄ (—) was only partially eluted, suggesting stable integration of the peptide in the brush.

Figure 7.

CD spectra of: (left) WLBu2 in 0.5 M HClO₄, and in suspension with uncoated and F108-coated nanoparticles, (right) WLBu2 in 0.2 M HClO₄, and in suspension with uncoated and F108-coated nanoparticles.

Figure 8.

CD spectra of: WLBu2 in HClO_4 , and in suspension with F108-coated (left) and uncoated (right) nanoparticles before and after washing with HClO_4 or water.