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Functional Modification of Silica through Enhanced Adsorption of Elastin-Like Polypeptide Block Copolymers

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Abstract

A powerful tool for controlling interfacial properties and molecular architecture relies on the tailored adsorption of stimuli-responsive block copolymers onto surfaces. Here, we use computational and experimental approaches to investigate the adsorption behavior of thermally-responsive polypeptide block copolymers (elastin-like polypeptides, ELPs) onto silica surfaces, and to explore the effects of surface affinity and micellization on the adsorption kinetics and the resultant polypeptide layers. We demonstrate that genetic incorporation of a silica-binding peptide (silaffin R5) results in enhanced adsorption of these block copolymers onto silica surfaces as measured by quartz crystal microbalance and ellipsometry. We find that the silaffin peptide can also direct micelle adsorption, leading to close-packed micellar arrangements that are distinct from the sparse, patchy arrangements observed for ELP micelles lacking a silaffin tag, as evidenced by atomic force microscopy measurements. These experimental findings are consistent with results of dissipative particle dynamics simulations. Wettability measurements suggest that surface immobilization hampers the temperature-dependent conformational change of ELP micelles, while adsorbed ELP unimers (i.e., un-micellized block copolymers) retain their thermally-responsive property at interfaces. These observations provide guidance on the use of ELP block copolymers as building blocks for fabricating smart surfaces and interfaces with programmable architecture and functionality.

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Supporting Information: Full sequences of ELP block copolymers, characterization of purified ELP block copolymers using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), detailed information on the dissipative particle dynamics (DPD) simulation, an elemental composition analysis of the ELP modified surfaces by X-ray photoelectron spectroscopy, and a quantitative analysis of the kinetics of adsorption of ELP block copolymers.

Graphical Abstract

Keywords

Block copolymers; elastin-like polypeptides (ELPs); silaffin R5 peptides; micelle adsorption; thermally responsive surfaces

INTRODUCTION

Block copolymers, defined as macromolecules with two or more different sequences of varying monomer composition, can self-assemble from solution into supramolecular structures such as micelles,¹ vesicles,² cylinders,³ and lamellae.⁴ Terminal attachment of block copolymers onto a solid surface constitutes an interface that is of particular interest. It has been previously reported that a terminal anchor group in the corona of micelles can greatly enhance the adsorption of synthetic block copolymers,⁵ resulting in a stable layer that is less susceptible to desorption or displacement as compared to block copolymers lacking the anchor group.^{5–7} In addition, the end-grafting of block copolymers onto surfaces provides a means to control orientation and conformation to expose desired functional groups, which facilitates applications ranging from colloidal stabilization⁸ and nanofabrication⁹ to bioactive surface fabrication.^{10–12} Furthermore, coating surfaces with block copolymers that respond to external stimuli (e.g., pH,^{13,14} ionic strength,¹⁵ light,^{16,17} and temperature^{18–20}) enables the development of innovative drug delivery systems^{21–25} and substrates for tissue engineering.^{26,27}

While a broad range of synthetic, stimuli-responsive block copolymers are available,^{22,28–31} genetically engineered polypeptide block copolymers provide unique and attractive material design features. For example, genetically encoded synthesis provides precise control over block composition, molecular weight and chain length,^{32–34} which is difficult to obtain using synthetic polymerization approaches. In addition, functional peptide sequences or other small bioactive moieties can easily be incorporated into engineered polypeptides at the gene level, further extending their functionality.^{35–39} Surface-grafted, stimuli-responsive polypeptides have been exploited in immunoassays,⁴⁰ microcantilever sensors,⁴¹ and molecular switches.^{42,43} Surprisingly, however, few stimuli-responsive surfaces that incorporate block copolypeptides have been fabricated.⁴⁴

The genetically engineered elastin-like polypeptides (ELPs) used in this study are a class of thermally-responsive biopolymers that consist of a pentapeptide repeat motif (VPGXG), where the guest residue X is any amino acid except proline. A hallmark property of ELPs is their lower critical solution temperature (LCST) phase behavior in water; i.e., ELPs at a given concentration in aqueous solvent, phase separate to form protein-rich coacervates

above their cloud point transition temperature (T_c).^{32,45} Furthermore, ELP block copolymers comprised of dissimilar ELP sequences with decoupled LCST phase transition behaviors can form micellar structures above their critical micellization temperature (CMT).^{21,46–49} The genetically encoded synthesis of ELPs enables the construction of polypeptide block copolymers with complex block architectures and functional domains.⁵⁰ These ELP block copolymers have the ability to self-assemble into predictable structures, together with a pre-defined, molecularly encoded bioactivity. Thus, they have been used for drug delivery,^{23,51–53} tissue engineering,²⁷ and biomineralization applications.⁵⁴ Furthermore, the uniformity of the ELP block copolymers with respect to chain length and composition,^{55,56} facilitates the investigation of block copolymer adsorption onto surfaces. However, the adsorption of block co-polypeptides, and specifically, ELP block copolymers onto substrates remains largely unexplored.

Several studies have investigated the adsorption of synthetic block copolymers at the solid/liquid interface.^{6,7,57–63} In general, the molecular structure of the block copolymer plays an important role in the adsorption process because it can affect adsorption kinetics and structural characteristics of the adsorbed layer (e.g., molecular architecture, topography). We focus this study on understanding the adsorption behavior of ELP block copolymers with respect to their supramolecular structures (i.e., unassociated block copolymers, herein referred to as unimers vs. micellar assemblies). We also explore how genetic incorporation of a material-binding peptide into the hydrophilic block can affect the attachment of block copolymers onto surfaces. Several material-binding peptides with an affinity for certain substrates (e.g., silica, gold, zinc) have been identified^{64–69} and fused to proteins to facilitate protein surface immobilization.^{70,71} We hypothesized that an end-terminal silaffin peptide tag^{72,73} would enable enhanced attachment of ELP block copolymers onto silica surfaces. The amino acid sequences and homogeneous (i.e., in solution) phase transition behavior for the ELP block copolymers without and with the silaffin tag, referred to here as ELP_{BC} and silaffin-ELP_{BC}, respectively, are depicted in Figures 1(a–d).

In this study, we investigated how an affinity tag and micellization influence the adsorption behavior of ELP block copolymers onto silica surfaces, leading to distinct architecture, morphology and functionality of the adsorbate layers. Schematics of the structures inferred from experimental results on ELP_{BC} and silaffin-ELP_{BC} modified silica surfaces are shown in Figures 1(e–h) for adsorption below and above the CMT of the parent and silaffin-functionalized ELP_{BC}. Below the CMT, the adsorption of unimers results in a homogeneous surface coverage (Figs. 1e, g), and silaffin-ELP_{BC} unimers adsorbed more with a higher surface coverage onto surfaces than ELP_{BC} unimers. Above the CMT, we find that ELP_{BC} micelles without a silaffin binding tag, form “patchy” surfaces with a lower surface coverage (Fig. 1f). In contrast, adsorbed silaffin-ELP_{BC} micelles are densely packed and evenly dispersed on surfaces with a high surface coverage due to the high binding affinity and positive charge of the silaffin tag (Fig. 1h).

MATERIALS AND METHODS

ELP expression, purification and characterization

The ELP block copolymers (ELP_{BC}) used here have an *N*-terminal leader sequence (MGCGWP) that provides a unique cysteine for conjugation of fluorophores or other imaging agents, followed by a hydrophobic block that is 60 pentapeptides in length with valine as the guest residue, and a hydrophilic block that is 60 pentapeptides in length with alanine and glycine at a 1:1 ratio as the guest residue, where the guest residue is the residue X in the VPGXG pentapeptide repeat unit of the ELP. The silaffin R5 peptide is encoded at the *C*-terminus of the ELP_{BC}, and these constructs are referred to as silaffin-ELP_{BC}. The genes for silaffin-ELP_{BC} and ELP_{BC}, available from a previous study,⁵⁴ were expressed in BL21(DE3) *E. coli* and purified by inverse transition cycling, as described previously.³⁸ The purified ELPs were characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE; BioRad, Inc). A representative gel image is shown in Figure S1 in the Supplementary Information. To characterize the aqueous LCST phase behavior of the ELPs, the optical density at 350 nm (OD₃₅₀) of a 40 μM solution of ELP_{BC} and silaffin-ELP_{BC} in phosphate-buffered saline (1X PBS with 7.4 pH: 3 mM Na₂HPO₄; 1 mM KH₂PO₄; 155 mM NaCl) were measured as a function of solution temperature. Samples were heated between 25°C and 75°C at 1 °C/min in a Cary 300 UV-visible spectrophotometer equipped with a multicell thermoelectric temperature controller (Varian Instruments, Walnut Creek, Ca).

Dissipative particle dynamics (DPD) simulation of peptide adsorption

All simulations were performed using dissipative particle dynamics (DPD) via LAMMPS.⁷⁴ DPD is a coarse-grained simulation technique in which one DPD bead represents a group of atoms or a volume of fluid.^{75,76} Details regarding the simulations are provided in the Supplementary Information. Briefly, the silica substrates were modelled as dense layers of DPD beads, and ELP block copolymers were coarse-grained into DPD polymer beads connected with harmonic spring forces. Each ELP block copolymer consists of a 10-bead relatively hydrophobic block representing the VPGVG block, and a 10-bead relatively hydrophilic block representing the VPGXG block, where X is alanine and glycine at a 1:1 ratio. The silaffin-ELP_{BC} block model had the same parameters as ELP_{BC} block with one extra self-repulsive and hydrophilic bead representing silaffin. The DPD parameters for charged silaffin were chosen following our work on implicit solvent ionic strength model.⁷⁷ The repulsive parameters for the interactions between various types of DPD beads that represent the affinity between ELPs, water, and the walls are listed in Table S1. In the first step of the DPD simulation, the self-assembly of diblock ELPs (with/without silaffin) was simulated starting from a random dispersion of 440 ELP chains in aqueous solution with a periodic boundary condition in three directions. The simulation set-up of the self-assembly of diblock ELPs (with/without silaffin) and parameters were chosen to reasonably guarantee that the most stable micellar structures would be found. The micelles obtained were characterized in terms of micellar size and aggregation number (Fig. S2 and Table S2). In the second step of the simulations, a number of micelles, which were formed in the first step and consisted of 1320 amphiphilic ELP chains, were placed in the middle of the simulation box with a periodic boundary condition applied in the *x* and *y* directions. Double walls were

added at the top and bottom in the z direction (Fig. S3), which were modelled by creating two dense layers of frozen DPD beads that represent several wall atoms and arranged on a lattice (following the work of Li and Cao^{78,79}). Simulations were conducted with a cubic simulation box of size $40 \times 40 \times 40 r_c^3$, where r_c is the DPD unit of length. The total number of ELPs and water beads in the system was 192,000. In the DPD model, the properties of the system were expressed using dimensionless quantities in units of the length cutoff (r_c), the energy scale ($k_B T$), and the bead mass (m_0). (The length cutoff defines the extent of the interaction range. All of the interaction forces between DPD beads act within the cut-off radius.) Therefore, the unit of time (τ) is $\tau = \sqrt{r_c^2 m_0 / k_B T}$. Time evolution of the system was calculated by the Verlet algorithm with a time step $\Delta t = 0.05 \tau$. The total bead number density in the simulation system is $\rho = 3 r_c^{-3}$. We ran the simulations for 8.0×10^6 time steps to attain thermodynamic equilibrium.

Preparation of peptide-modified silica surfaces

Silicon wafers were obtained from University Wafer Inc. (Boston, MA). The wafer surfaces were cleaned in a 1:3 (v/v) solution of 30% H_2O_2 : 98% H_2SO_4 (piranha etch) for 5 h. *(CAUTION: Piranha solution reacts violently with organic compounds and should be handled very carefully!)* After rinsing in deionized water and drying under a stream of nitrogen, the silicon wafers were placed into a 40 μ M silaffin-ELP_{BC} or ELP_{BC} solution in PBS buffer at a specified temperature (25°C or 50°C). The silicon wafers were incubated in the ELP solutions for at least 24 h, and then rinsed with deionized water prior to X-ray photoelectron spectroscopy (XPS) analysis, atomic force microscopy (AFM) measurements and contact angle measurements.

Atomic force microscopy (AFM)

The morphology of the adsorbed ELPs was characterized by tapping mode AFM (Multimode 8, Bruker, Santa Barbara, CA). AFM images were obtained in deionized water using triangular Si_3N_4 cantilevers (Bruker, Scanasyt-Fluid) with a nominal spring constant of 0.7 Nm^{-1} . For experiments performed at 50°C, a thermal controller (Bruker, Santa Barbara, CA) was used to heat the sample.

X-ray photoelectron spectroscopy (XPS)

The elemental composition of the surfaces was determined with a Kratos Analytical Axis Ultra X-ray photoelectron spectrometer equipped with a monochromatic Al K α source. Survey scans were acquired with a pass energy of 160 eV and a resolution of 1.0 eV. All XPS data were analyzed using CasaXPS software (Casa Software Ltd., UK). All binding energies were referenced to the main hydrocarbon peak designated as 285.0 eV.

Contact angle goniometry

A contact angle goniometer (Model 100; Rame-Hart Instrument, Co.) was used for measurement of contact angles of captive air bubbles in water. The sample was placed inside an environmental chamber filled with deionized water that was heated by circulating water from the temperature-controlled water bath. A thermocouple inside the chamber was used to measure the temperature. The samples were equilibrated at a specified temperature for 20

min before measurement. Contact angle values reported are the average of at least six replicates.

Quartz crystal microbalance (QCM)

A Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden) was used to investigate the adsorption behavior of the ELPs. Prior to measurement, silicon dioxide coated sensor crystals (50 nm) (QX 303, Västra Frölunda, Sweden) were immersed in 2% sodium dodecyl sulfate (SDS) for 2 h, followed by rinsing with deionized water and exposing to ultraviolet (UV)/ozone for 10 min. The crystals were then thoroughly rinsed with deionized water, and dried under a stream of nitrogen gas. For each measurement, PBS buffer was first loaded into the flow module until a stable baseline was reached. An ELP solution at a concentration of 40 μM was then injected into the flow module at a constant flow rate of 50 $\mu\text{L}/\text{min}$, followed by a PBS wash to remove loosely-bound ELP. Changes in the crystal frequency and dissipation were continuously monitored using the Q-Soft software (Q-Sense AB). The frequency (f) and dissipation (D) values measured at the third overtone were used for analysis.

Ellipsometry

The thickness of each ELP adlayer on silicon wafer surfaces was measured in air using a spectroscopic ellipsometer (M-88; J.A. Woollam Co., Lincoln, NE). Spectroscopic scans were acquired over a wavelength range of 400–700 nm at a 65° angle of incidence. Ellipsometric data were analyzed using WVASE32 software by fitting 3-layer model composed of a silicon layer at the bottom, a thin silicon dioxide layer and a polymer layer on the top. The thickness of the polymer film was obtained using a Cauchy layer model with fixed constants ($A_n = 1.45$, $B_n = 0.01$, and $C_n = 0$).⁸⁰ The nominal thickness of the ELP adlayer was determined from the average of at least six thickness measurements taken per sample.

RESULTS AND DISCUSSION

Study of polypeptide block copolymer adsorption by dissipative particle dynamics

We used dissipative particle dynamics (DPD) simulations⁷⁶ to model the adsorption of ELP micelles onto surfaces and to predict the architecture and morphology of the resulting micellar layers. DPD is a meso-scale simulation technique that has been used to study and predict the phase behavior and properties of copolymer aggregates as well as complex self-assembled structures such as multicomponent micelles and vesicles.^{77,81–83} DPD has also been applied to explore the interactions between biopolymeric components and nanostructures.⁸⁴ One of the most appealing features of DPD is that it can predict experimental results at reasonable scales of size and time. Herein, the silica substrates were modeled as dense layers of DPD beads, and the ELP_{BC} were coarse-grained into DPD polymer beads connected by spring forces (see details in Supplementary Information). To be consistent with our experimental procedure, the silaffin-ELP_{BC} and ELP_{BC} were self-assembled in aqueous solution first. A number of pre-formed micelles (1320 chains in total) were then placed into the simulation box such that the geometric center of each micelle was equidistant from the box boundaries in the z -direction. The box was subsequently filled with

water DPD beads at a density of $\rho=3r_c^{-3}$. The equilibrium snapshots of the simulation box from each simulation are shown in Figure S3. A micelle was defined as “adsorbed” when at least 10% of beads in the micelle were within a distance of $2r_c$ from surface beads.⁸⁴ Figures 2a, and 2b show equilibrium snapshots of silica surfaces with adsorbed ELP_{BC} micelles and silaffin-ELP_{BC} micelles. The simulation results predict that, in contrast to ELP_{BC} micelles which adsorb at a relatively low density and form patchy micellar clumps, silaffin-ELP_{BC} micelles form densely packed layers on the silica surfaces. The difference in adsorption behavior of ELP_{BC} micelles and silaffin-ELP_{BC} micelles on silica surfaces was further revealed by estimating the number of ELP chains adsorbed on the surface with the size of $40r_c \times 40r_c$ and the thickness of the ELP layers. The simulation predicts that a larger amount of silaffin-ELP_{BC} micelles will adsorb onto silica surfaces compared to that of ELP_{BC} micelles (Fig. 2c). In addition, silaffin-ELP_{BC} will form thicker layers than the ELP_{BC} (Fig. 2d). The silaffin tag also promoted adsorption stability, which was reflected by the smoother adsorption profiles of silaffin-ELP_{BC} which showed smaller fluctuations. We attribute the larger fluctuations seen in the adsorption profiles for ELP_{BC} to the frequent desorption of micelles from the surface. Together, these simulation data suggest that incorporation of an end-terminal silaffin tag in ELP block copolypeptides results in stronger micelle-surface attraction, leading to dense packing and more even distribution of silaffin-ELP_{BC} micelles on surfaces compared to the parent ELP_{BC} micelles.

Characterization of adsorbed polypeptide block copolymers

We used a combination of experimental techniques to characterize the surface coverage, morphology and thermoresponsive properties of polypeptide modified surfaces. We first incubated silicon wafers in ELP_{BC} or silaffin-ELP_{BC} solutions for 24 h below and above their CMT (i.e., at 25°C and 50°C). As the ELP block copolypeptides have a critical micelle concentration (CMC) $< 10 \mu\text{M}$,⁴⁶ polypeptide solutions at a concentration of $40 \mu\text{M}$ were used to ensure formation of stable micelles above the CMT ($\sim 40^\circ\text{C}$). The morphology of the adsorbed layers was characterized by tapping mode atomic force microscopy (AFM) at 25°C or 50°C (Fig. 3). At 25°C, the substrate surfaces were homogeneously covered by adsorbed polypeptides (Figs. 3a and 3c) interspersed by large, local aggregates, which is consistent with a previous study on ELP coated surfaces.⁸⁵ Silaffin-ELP_{BC} covered surfaces showed a larger number of these aggregates compared with surfaces covered with ELP_{BC} unimers. Closer inspection of Figure 3c shows that the polypeptide adsorbates appear to be slightly larger than in the case of adsorbed unimers (Fig. 3a), which could be due to a tendency of silaffin-ELP_{BC} to form somewhat larger clusters. For ELP_{BC} at 50°C, large aggregates (possibly micellar aggregates) formed, and their adsorption led to a heterogeneous surface morphology with patches of aggregates interspersed on bare silica surface (Fig. 3b). This phenomenon has also been observed for synthetic block copolymer micelles,^{61,86} where the adsorption of micelles leads to a “patchy” surface coverage. By contrast, silaffin-ELP_{BC} micelles at 50°C formed more uniform layers that likely consisted of intact micelles directly adsorbed and densely packed on the surface, again interspersed with some larger aggregates (Fig. 3d). The diameter of the adsorbed silaffin-ELP_{BC} micelles is $\sim 40\text{--}60 \text{ nm}$, which is consistent with the micellar diameter in solution, measured by dynamic light scattering (DLS).⁵⁴

An elemental composition analysis of the surfaces by X-ray photoelectron spectroscopy (XPS, Fig. S4) shows a significantly higher silicon content for ELP_{BC} micelle modified surfaces compared to silaffin-ELP_{BC} micelle modified surfaces, and is consistent with the observed “patchy” adsorption behavior of ELP_{BC} micelles. Furthermore, we suggest that the positively charged, silaffin-functionalized polypeptides at the periphery of the micelle corona may induce sufficiently strong repulsive interactions between micelles to inhibit their aggregation, and may lead to the observed, uniform nature of the ELP micelle layer. These results also agree with the predictions from DPD simulations, and are consistent with previously reported observations of adsorbed cationic diblock copolymer micelles⁶⁰ and polyelectrolyte micelles,⁸⁷ where charges on the corona strongly affect the spacing of the micelles on surfaces. In summary, our results demonstrate that below the CMT, adsorption of block copolypeptides leads to uniform adsorbed layers largely of ELP unimers, while above the CMT, direct adsorption of micelles and their aggregates (in case of ELP_{BC}) onto surfaces occurs. Furthermore, conferring material binding affinities and charges to the corona of the micelles increases their binding density and homogeneous distribution on a surface.

We further explored the wettability of these polypeptide layers using contact angle measurements. Although the interfacial phase transition behavior of surface-grafted ELP homopolymers has been previously reported,⁸⁸ little is known regarding the thermally-responsive properties of adsorbed ELP block copolymers. Samples were prepared by incubating silica substrates in a 40 μ M silaffin-ELP_{BC} or ELP_{BC} solution for 24 h below and above their CMT (i.e., at 25°C and 50°C), followed by rinsing with deionized water. We then used the captive-air-bubble-in-water method to measure the contact angle of polypeptide-modified surfaces as a function of temperature. As a comparison, temperature-dependent turbidity profiles for ELP block copolymer solutions were measured by UV-visible spectrophotometry. The solid lines in Figure 4 represent the contact angles of the adsorbed ELP block copolymers as a function of temperature, while the red, dashed lines represent the optical density measurements of the same ELP block copolymers in solution. As the temperature is increased for ELP unimer-modified surfaces, two abrupt changes in the contact angle were observed, which may be due to the sequential collapse of the two ELP blocks, consistent with the thermal transition behavior of the ELP unimers in solution. The silaffin-ELP_{BC} unimer modified surfaces demonstrated more distinct temperature-dependent changes in contact angles compared to parent ELP_{BC} unimer modified surfaces, which may be due to a lower amount of ELP_{BC} unimers adsorbed on surfaces in the absence of the silaffin binding tag (see below). These results suggest that the unimer modified surfaces have switchable wettability in response to temperature. No changes in contact angle were observed as temperature was increased for micelle modified surfaces. We are aware that the wetting of rough, heterogeneous surfaces is a complex function of chemical interactions between surface and fluid components as well as topographical parameters (e.g., roughness, surface coverage)^{89–91} and so unambiguous interpretation of the observed wettability for all samples is difficult here. Nonetheless, our contact angle measurements demonstrate that the conformation of ELP block copolymers upon adsorption (i.e., unimers *vs.* micelles) can substantially affect the thermally-responsive properties of the adsorbed polypeptide layers.

Kinetics of adsorption of ELP block copolymers onto silica surfaces

To investigate the effects of the silaffin tag and micellization on the adsorption kinetics of ELP block copolymers, we adsorbed ELP_{BC} and silaffin-ELP_{BC} (both at 40 μ M) onto SiO₂ surfaces below and above the CMT (i.e., 25°C, 50°C). A quartz crystal microbalance with energy dissipation (QCM-D) was used to follow the polypeptide adsorption process. QCM-D is a surface-sensitive technique that has been widely used to monitor the kinetics and conformation of macromolecular binding to materials that can be coated onto a quartz crystal.^{92,93} The magnitude of the change in the frequency of the mechanical oscillator is proportional to the amount of mass coupled on the quartz crystal surfaces.^{94–96} Figure 5 shows the frequency change (Δf) and the corresponding dissipation change (ΔD) for the adsorption of ELP_{BC} and silaffin-ELP_{BC} to silica-coated quartz crystals at both 25°C and 50°C. In both cases, i.e., below and above the CMT, the adsorption of silaffin-ELP_{BC} resulted in a faster decrease in the frequency changes compared to that for ELP_{BC}, indicating a higher initial adsorption rate for silaffin-ELP_{BC}. Additionally, silaffin-ELP_{BC} reached a larger $|\Delta f|$ relative to that of ELP_{BC}, suggesting that silaffin-ELP_{BC} adsorbed to a greater extent to the silica surfaces than ELP_{BC}.

Figure 5 also demonstrates that attachment of ELP micelles onto surfaces results in a larger change of frequency as compared to that of ELP unimers, which suggests that a larger amount of ELPs adsorbed from the micelle-containing solution. This observation agrees with previous adsorption studies of diblock copolymers onto octadecylsilane surfaces.^{97,98} A coverage of 90% of the saturation value was reached within 5 min for adsorption from micellar solutions. With increasing surface coverage, the adsorption rate decreased rapidly. Furthermore, a quantitative analysis of the kinetics of adsorption indicates that micellization and the silaffin tag enhanced the binding affinity of ELP block copolymers to silica surfaces, thus resulting in a higher surface coverage (see Figure S5, Table S3). The QCM data thus reflect the trends seen in our simulation results and further confirm the effects of the silaffin tag and micellization on the kinetics and coverage of ELP block copolymer adsorption process.

QCM-D measurements can also determine the magnitude of energy dissipation (D) of polypeptide layers. The binding of ELP block copolymers induced dissipation changes, suggesting that the peptide layers are viscoelastic. The adsorption of ELP_{BC} micelles resulted in the largest increase in the dissipation energy, which is likely due to the fact that ELP_{BC} micelles formed localized micellar aggregates on the surface (as shown in AFM images). In contrast, silaffin-ELP_{BC} micelles were more tightly-packed on the silica surfaces and thus cause less dissipation. The specific dissipation (defined as $D/\Delta f$) was calculated to compare the relative viscoelastic properties of the adsorbed layers. This value is a measurement of the rigidity of adsorbed layers normalized for adsorbed amounts on the surface and is a commonly used measure in QCM-D studies that reflects the viscoelastic properties of the adsorbed layer.^{99–103} The specific dissipation for silaffin-ELP_{BC} unimer, ELP_{BC} micelle and unimer modified surfaces were $18.5 \times 10^{-8} \text{ Hz}^{-1}$, $13.9 \times 10^{-8} \text{ Hz}^{-1}$, and $11.9 \times 10^{-8} \text{ Hz}^{-1}$, respectively. These values are consistent with reported specific dissipation values for soft ELP layers ($\sim 10\text{--}15 \times 10^{-8} \text{ Hz}^{-1}$).¹⁰⁴ Because large specific dissipation values are commonly associated with extended, flexible conformations of individual

biomolecules with a high water content¹⁰² or loose surface binding,^{96,103} these specific dissipation values suggest that ELP unimers may form soft and hydrated layers and ELP_{BC} micelles may bind loosely on the surfaces. However, silaffin-ELP_{BC} micelle coated surfaces showed a smaller value of specific dissipation ($3.6 \times 10^{-8} \text{ Hz}^{-1}$), suggesting that these micelles adhered strongly to the substrate surface and formed more compact adsorbed layers. These results are consistent with our AFM data and further confirmed the strong attraction between silaffin-ELP_{BC} micelles and the silica surface.

We also used ellipsometry to measure the dependence of dry film thickness on adsorption time. Figure 6 shows that the adsorption of ELP block copolymers proceeded at an initially high rate followed by a decrease in rate at longer times as the surface was gradually saturated. Additionally, silaffin-ELP_{BC} exhibited a higher initial adsorption rate and reached saturation faster than the ELP_{BC}, which agrees with the trends observed in QCM measurements and DPD simulations. The polypeptide layers formed by silaffin-ELP_{BC} were thicker than those formed by ELP_{BC} at both 25°C and 50°C. Taken together, these data suggest that ELP block copolymers with a silaffin tag exhibit a higher binding affinity to silica surfaces. This finding is consistent with a previous study on material binding peptides, where, for example, incorporation of ZnO-binding peptide into biomolecules enhanced the binding of biomolecules to ZnO particle surfaces.⁶⁶

As expected, for both ELP_{BC} and silaffin-ELP_{BC}, ELP micelles formed thicker layers than unimers. This is likely due to the tendency of amphiphilic ELPs to self-aggregate in the micelles adsorbed to surfaces as confirmed by AFM and QCM. We also observed higher standard deviations for the thickness of ELP micelle modified surfaces, indicating a rough surface morphology, which is in accordance with AFM data. Together, QCM and ellipsometry data demonstrate that micellization and the silaffin tag enhanced the adsorption of ELP diblock copolymers onto silica surfaces, leading to thicker polypeptide layers.

CONCLUSIONS

We have systematically studied the adsorption of thermally-responsive ELP block copolymers to develop an understanding of the effects of surface affinity and micellization on the adsorption kinetics and equilibrium, as well as the morphology and functionality of the adsorbed layers. Compared to the parent ELP_{BC}, silaffin-ELP_{BC} demonstrated a higher adsorption rate and formed thicker layers with a greater surface coverage, indicating the silaffin peptide enhanced binding of ELP block copolymers to silica surfaces. In addition, silaffin-ELP_{BC} micelles were tightly packed and evenly dispersed on the surfaces, while adsorption of ELP_{BC} micelles led to a “patchy” surface coverage. These results suggest that incorporation of material-binding peptides can affect the distribution of micelles and the architecture of adsorbed micellar layers. Furthermore, the micellization of block copolypeptides also profoundly affects the adsorption process and properties of the adsorbed layers. In particular, micelles adsorbed faster and formed thicker layers with a rougher morphology and a higher surface coverage compared to unimers. Additionally, ELP micelles were no longer thermally-responsive after surface immobilization, while unimers retained their temperature-responsive property at the interfaces. This study thus demonstrates the enhanced adsorption of ELP block copolymers onto target substrates by incorporating end-

terminal peptide sequences tailored to interact with these surfaces. It also provides insight into the design of smart surfaces based on ELP block copolymers, with controlled structure-architecture-function relationship.

ELP block copolymer based systems hold promise as model systems for further, systematic investigation of block co-polypeptide adsorption onto surfaces. Genetically designing ELP blocks, with an assortment of amino acid guest residues and various chain lengths, can direct their supramolecular self-assembly into distinct structures, such as spherical and cylindrical micelles,^{46,105,106} vesicles,²⁴ and microemulsions.¹⁰⁷ In addition, functional segments (e.g., material-binding,¹⁰⁸ crosslinking,^{27,109} cleavage,¹¹⁰ and biomolecule/cell recognition sequences^{111–115}) can be inserted at precise locations and spacing within the ELP blocks, offering virtually limitless variations in the properties of the final assemblies. This will enable mechanistic studies of the role each block/functional segment plays in their self-assembly process, as well as how self-assembled structures affect the kinetics and thermodynamics of the adsorption process of block copolypeptides. This will also provide state-of-the-art surface engineering approaches to fabricate bioactive surfaces with desired architecture and functionality for a variety of applications, such as cell culture, biosensing, diagnostics and drug delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Nominal amino acid sequences for (a) ELP_{BC} and (b) silaffin-ELP_{BC}. Full sequences are provided in Supplementary Information. (c, d) Schematic of the temperature-triggered micellization of ELP block copolymers without (i.e., parent) and with silaffin. As the temperature is raised above the CMT (~40°C under the conditions of this study), the more hydrophobic (cyan) blocks undergo hydrophobic aggregation resulting in formation of micelles. Schematic of parent ELP_{BC} unimer (e) and micelle (f) coated surfaces, and silaffin-ELP_{BC} unimer (g) and micelle (h) modified surfaces.

Figure 2. Representative, final snapshots of silica surfaces with adsorbed (a) ELP_{BC} micelles and (b) silaffin-ELP_{BC} micelles. The cyan, magenta, yellow and grey beads represent hydrophobic block, hydrophilic block, silaffin, and silica, respectively. Time evolution of (c) the number of adsorbed chains on silica surfaces and of (d) the estimated thickness of the adsorbed layers.

Figure 3. AFM tapping mode height images in deionized water of (a) an ELP_{BC} unimer modified surface at 25°C, (b) an ELP_{BC} micelle modified surface at 50°C, (c) a silaffin-ELP_{BC} unimer modified surface at 25°C, and (d) a silaffin-ELP_{BC} micelle modified surface at 50°C.

Figure 4. Contact angles of captive-air-bubbles in water measured as a function of temperature for (a) an ELP_{BC} unimer modified surface, (b) an ELP_{BC} micelle modified surface, (c) a silaffin-ELP_{BC} unimer modified surface, and (d) a silaffin-ELP_{BC} micelle modified surface. The contact angles reflect the mean values $\pm$ standard deviation of six independent measurements. The red, dashed lines represent the turbidity profiles of 40 μ M ELP_{BC} solution (a, b) and silaffin-ELP_{BC} solution (c, d) in PBS as a function of temperature.

Figure 5.

Quartz crystal microbalance (QCM) measurements of the time course of (a) frequency shift (Δf), and (b) dissipation shift (ΔD), for the binding of ELP_{BC} and silaffin-ELP_{BC} (40 μ M) to silica surfaces at 25°C and 50°C. The arrows indicate the time when the surface was rinsed with PBS buffer.

Figure 6.

Ellipsometric thickness of ELP block copolymer adlayers on SiO₂ in the dry state as a function of adsorption time. In each case the solution concentration of ELP was maintained at 40 μ M.