THE JOURNAL OF PHYSICAL CHEMISTRY B
A JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Perspective

Protein–Polymer Interaction Characteristics Unique to Nanoscale Interfaces: A Perspective on Recent Insights

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Cite This: *J. Phys. Chem. B* 2021, 125, 6040–6057

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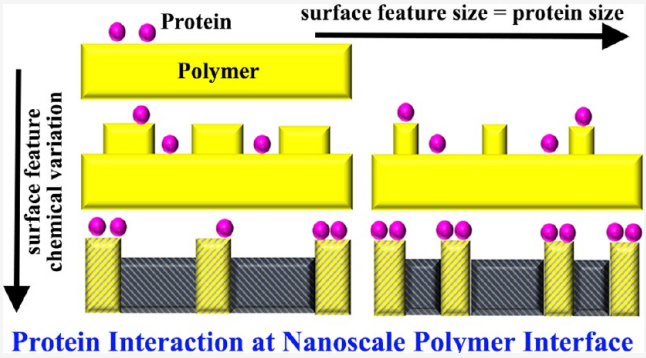
ABSTRACT: Protein interactions at polymer interfaces represent a complex but ubiquitous phenomenon that demands an entirely different focus of investigation than what has been attempted before. With the advancement of nanoscience and nanotechnology, the nature of polymer materials interfacing proteins has evolved to exhibit greater chemical intricacy and smaller physical dimensions. Existing knowledge built from studying the interaction of macroscopic, chemically alike surfaces with an ensemble of protein molecules cannot be simply carried over to nanoscale protein–polymer interactions. In this Perspective, novel protein interaction phenomena driven by the presence of nanoscale polymer interfaces are discussed. Being able to discern discrete protein interaction events via simple visualization was crucial to attaining the much needed, direct experimental evidence of protein–polymer interactions at the single biomolecule level. Spatial and temporal tracking of particular proteins at specific polymer interfaces was made possible by resolving individual proteins simultaneously with those polymer nanodomains responsible for the protein interactions. Therefore, such single biomolecule level approaches taken to examine protein–polymer interaction mark a big departure from the mainstream approaches of collecting indirectly observed, ensemble-averaged protein signals on chemically simple substrates. Spearheading research efforts so far has led to inspiring initial discoveries of protein interaction mechanisms and kinetics that are entirely unique to nanoscale polymer systems. They include protein self-assembly/packing characteristics, protein–polymer interaction mechanisms/kinetics, and various protein functionalities on polymer nanoconstructs. The promising beginning and future of nanoscale protein–polymer research endeavors are presented in this article.



I. INTRODUCTION

We encounter many examples of protein–polymer interfaces in everyday applications ranging from food processing and packaging, to health devices, to diagnostic tools, to medical products, to biomaterials, and to implant materials.[1−11] Elucidating the exact processes and degrees of interfacial protein behaviors on various polymers can be beneficial to the development of safer food packaging and human-aid products. Understanding the exact driving forces that result in certain protein–polymer interactions can be utilized to control protein arrangements on solid state bioarrays and biodevices for rapid and simultaneous diagnostics and detection.[12−19] Thorough understanding of protein interaction mechanisms and kinetics is also crucial to the development of biomaterials, implant materials, and tissue engineering platforms. The initial process of protein interaction with the underlying solid surface affects the subsequent cell growth and cellular response whose outcomes are central to the cell response[20−22] and the ultimate integration[2,4−7,9,10] of the materials.

With such importance and widespread impact on everyday products, proteins at polymer interfaces have remained an active subject of investigation for many decades.[1−10,23] However, the common and seemingly straightforward systems of protein–polymer interfaces have turned out to be highly complex and difficult to explain.[1,24] Moreover, polymers used in protein applications have evolved to serve multiple functions through intricate and complex interfaces. Recent advances in nanoscience for creating new and low dimensional polymer systems have fueled this transformation. Various top-down and bottom-up nanofabrication methods now enable a high level of spatial and chemical controls that are compactly presented in the form of nanoscale polymer interfaces. Hence, a growing number of biological and biomedical products contains protein–polymer interfaces that exhibit reduced dimensionality and increased chemical complexity as an essential component for their functions.

Received: January 25, 2021
Revised: May 3, 2021
Published: June 8, 2021

https://doi.org/10.1021/acs.jpcb.1c00684
J. Phys. Chem. B 2021, 125, 6040–6057

These changes underscore the importance of understanding the exact nature of protein interactions in nanoscale environments and identifying those unique protein interaction properties at polymer interfaces whose spatial and chemical variations of the interfacial periodicity are comparable to the size of individual proteins.[25−31] Nevertheless, our fundamental understanding of such nanoscale protein interactions is still very limited, and the research field of nanoscale protein−polymer interactions is at its early stage. More experimental, computational, and theoretical studies are yet to be forthcoming from the interdisciplinary areas of chemistry, biophysics, biology, and engineering.

II. FIRST STEP TOWARD UNDERSTANDING PROTEIN INTERACTIONS AT NANOSCALE POLYMER INTERFACES

It is worthwhile to note that important early steps have been made in bridging the crucial knowledge gap in nanoscale versus macro- or bulk-scale protein−polymer interactions, despite the field being at its infant stage. Research efforts have been made to reveal protein interaction characteristics specific to nanoscale polymer interfaces. Direct visualization of individual proteins and their unique adsorption behaviors at nanoscale, chemically alternating polymer surfaces were reported for the first time by Kumar et al. based on high resolution atomic force microscopy (AFM).[32] As the characteristic size of many proteins is on the nanometer scale, self-assembled block copolymer (BCP) nanodomains of polystyrene-*block*-poly(methyl methacrylate) (PS-*b*-PMMA) were employed in the study to stipulate nanoscale spatial and chemical constraints. Interaction behaviors of a model protein, immunoglobulin G (IgG), were subsequently examined under the confinements imposed by the nanoscale polymer interfaces. This seminal and other ensuing research efforts have led to key discoveries important for understanding protein interactions at nanoscale polymer interfaces that are distinctively different from their interactions with macro- and bulk-scale, chemically unvarying polymers.[26,32−39]

Protein adsorption onto solid surfaces, like the protein transfer process occurring at a liquid−solid interface between a bulk protein solution and a polymer surface, is an extensively documented process.[2,8−10,40−42] The transfer process is often explained by hydrophobic interactions between the protein and the solid surface, which ultimately brings an overall energetic advantage to the system via increased entropy due to the release of water molecules from the liquid−solid interface.[5,41,43−45] It is well-known that many proteins favor adsorption onto polymer surfaces, although the extent of the surface adsorption may differ depending on the degree of the interactions between given proteins and polymers.[24,41] It is also well-documented that proteins such as IgG, bovine and human serum albumins (BSA, HSA), horseradish peroxidase (HRP), fibronectin (Fn), and fibrinogen (Fg) all adsorb to a solid surface of PS and PMMA.[34,46−49] For example, when a solid surface of a PS homopolymer or PMMA homopolymer is dropped in a protein solution of IgG, both the PS and PMMA homopolymer surfaces are soon covered by IgG adsorbing from the solution.[34,39] However, this only occurs for a macroscale, chemically uniform polymer surface, and the proteins for the case of a nanostructured, chemically varying polymer surface were found to behave surprisingly different.

III. KEY DISCOVERIES IN NANOSCALE PROTEIN−POLYMER INTERACTIONS SO FAR

Figure 1 displays distinctively different protein behaviors exhibited when proteins interact with a macroscale, chemically uniform versus a nanoscale, chemically varying polymer surface. In the case of IgG on the nanoscale, chemically varying BCP of PS-*b*-PMMA, the protein−polymer interactions became highly selective of the polymer blocks to the degree that IgG molecules adsorb only on the PS nanodomains. The neighboring PMMA nanodomain regions of the BCP were left entirely free from any protein molecules, although the two polymer blocks of PS and PMMA exist spatially very close to each other. Figure 1A graphically summarizes the major findings from the early studies on nanoscale protein−polymer interactions.[32,34] IgG proteins

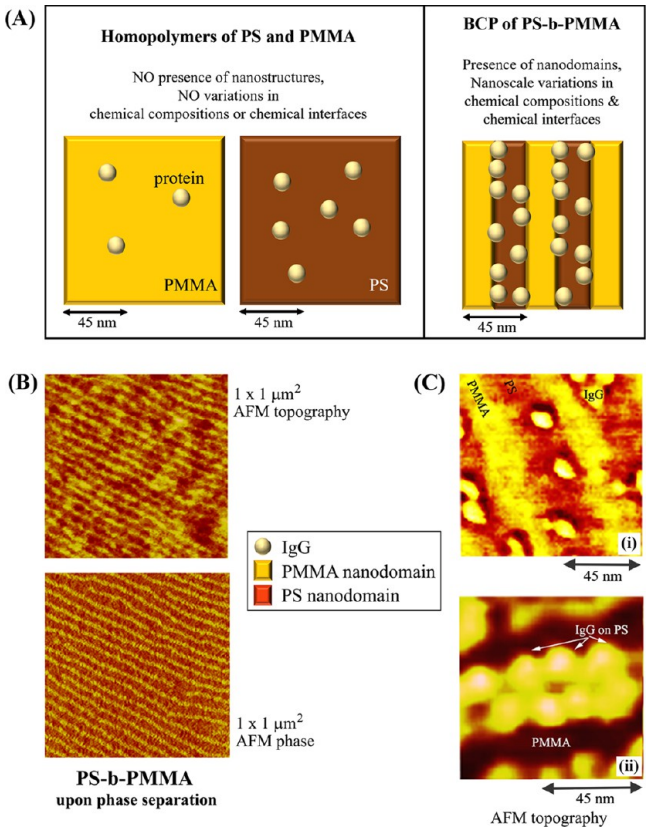


Figure 1. (A) The illustrations depict an example of distinctly different protein interaction behaviors observed on polymer surfaces that contain no nanostructures and no chemical variations (left, homopolymers of PS and PMMA) versus chemically varying nanostructures with nanoscale interfaces (right, BCP of PS-*b*-PMMA). The protein interaction behaviors exemplified here are those of IgG. (B and C) Single biomolecule level experimental evidence of the exclusive IgG interaction on the BCP depicted in (A) is provided. (B) AFM images of a clean PS-*b*-PMMA surface show alternating nanodomains of PS and PMMA with a repeat spacing of 45 nm. (C) AFM measurements after exposing the BCP surface in part B to varying concentrations of IgG protein solution reveal the exclusive interaction phenomenon of IgG with the PS nanodomain. Individual IgG molecules appear as spherical objects and are found solely on the PS nanodomain areas of the BCP, as seen in the AFM topography panels from a (i) lower and (ii) higher IgG concentration sample. Images in parts B and C are reproduced with permission from ref 32. Copyright 2005 American Chemical Society.

https://doi.org/10.1021/acs.jpcb.1c00684
J. Phys. Chem. B 2021, 125, 6040−6057

interact exclusively with the PS nanodomain regions on the BCP surface, which is different than the IgG behaviors on the PS or PMMA homopolymer depicted in the left panel. Unlike the protein behavior still showing interactions with the PMMA surface for the homopolymer case, no proteins adsorb on the PMMA nanodomain regions of the BCP. In addition, the interaction degrees of the protein are much less on the homopolymers of PS and PMMA relative to that on the BCP, leading to smaller amounts of proteins per unit area at the polymer interface.

The exclusive interaction between IgG and the PS nanodomain on the BCP is clearly revealed in the AFM images in Figure 1C. The BCP surface in Figure 1B is a clean PS-*b*-PMMA surface showing alternating nanodomains of PS and PMMA with a repeat spacing of 45 nm. The repeating PS and PMMA nanostructures are a result from the phase separation process of the two polymer blocks in the BCP. These are resolved as periodic nanostrips on the BCP template as shown in the AFM panels in Figure 1B. Considering the fact that the PS and PMMA nanodomains surface are only several tens of nanometers apart on the BCP, the exclusive interaction of the protein molecules and their complete partition to the PS block in Figure 1C is remarkable. The highly selective IgG interaction to PS on the BCP is induced by the nanoscale, chemically varying polymer surface. In addition to the highly discriminatory protein interaction and complete self-partition, an interesting phenomenon was revealed that emphasizes the important roles of chemical interfaces on the polymer surface in nanoscale protein−polymer interactions. It was discovered that, within the preferred PS nanodomains, IgG molecules favor the PS areas immediate to the chemical interfaces defined by neighboring PS and PMMA nanodomains.[34] This phenomenon was later explained to be associated with the inherently amphiphilic properties of proteins.[26,34,50,51] Figure 2 demonstrates such a preferential interaction behavior of IgG molecules with the chemical interfacial lines found at a nanometer and micrometer scale spacing on the surface of a BCP and a polymer blend sample, respectively. Figure 2A illustratively summarizes the preferred protein interactions at the chemical interfaces present on the BCP surface of PS-*b*-PMMA as well as on the polymer blend surface of PS/PMMA. The strong interaction preference of IgG molecules to the PS:PMMA chemical interfacial lines on a PS/PMMA blend surface is evidenced in the experimental data in Figure 2B. Figure 2B also shows that IgG surface density, i.e., the amount of proteins per given surface area, decreases exponentially as the distance away from a chemical interface increases. This trend indicates stronger interactions between IgG and surface sites near a chemical interface. Figure 2C displays IgG surface density as a function of separation between the two nearest PS:PMMA interfaces. The protein surface density is determined to be inversely proportional to the separation distance between two neighboring PS:PMMA interfaces. The smaller the distance is between two neighboring chemical interfaces on a polymer surface, the higher the protein interaction is. This results in a larger protein density, i.e., a greater number of proteins per unit surface area. Between the PS-*b*-PMMA BCP and the PS/PMMA blend cases depicted in Figure 2A, the separation distance between the two nearest PS:PMMA interfaces is much smaller for the BCP. Indeed, AFM investigations found that the IgG density on the BCP is higher than those on the blend sample.[34]

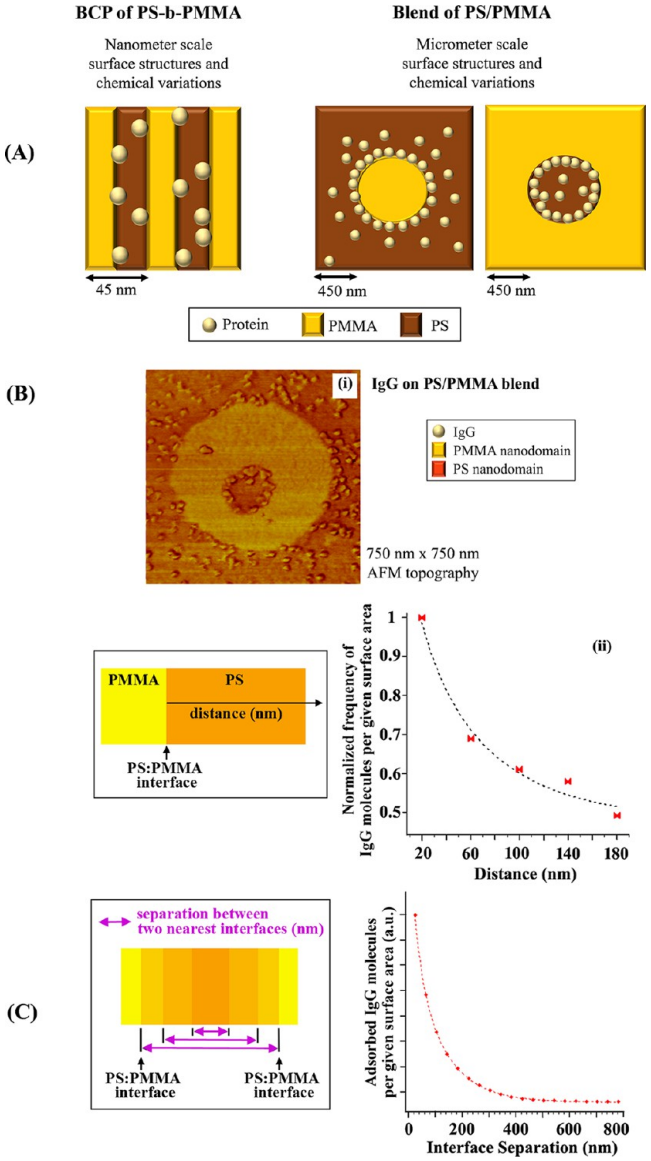


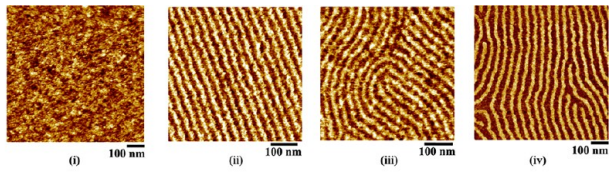
Figure 2. (A) The illustrations depict the phenomenon of preferred protein interactions at the chemical interfaces that are present with a nanometer scale spacing on the BCP of PS-*b*-PMMA and with a micrometer scale spacing on the polymer blend of PS/PMMA. The closer the surface site is to the chemical interface, the stronger the protein interacts with the polymer site. (B) Single biomolecule level experimental evidence for the phenomenon depicted in part A, i.e., protein interaction preference to chemical interfaces, is provided. The AFM topography in panel i displays the high interaction preference of IgG molecules to the chemical interfacial lines between PS and PMMA microdomains on a polymer blend sample of PS/PMMA. The scan size shown in the panel is 750 × 750 nm. The plot in panel ii corresponds to the normalized IgG surface density as a function of distance away from the PS:PMMA interface on the PS/PMMA blend. (C) IgG surface density is plotted as a function of separation between two nearest PS:PMMA interfaces. Images in parts B and C are reproduced with permission from ref 34. Copyright 2008 American Chemical Society.

The outer surfaces of proteins encompass amino acid moieties of varying charges and hydro-philicity/phobicity. BCPs upon phase separation, compared to polymers whose chemical compositions are the same over a macroscopic length scale with no chemical interfaces, display periodically

https://doi.org/10.1021/acs.jpcb.1c00684
J. Phys. Chem. B 2021, 125, 6040−6057

alternating, chemical interfaces due to the chemically distinctive polymer blocks in a given BCP. The more chemically rich environments near such nanoscale chemical interfaces can promote more favorable and stable interactions with the different amino acid groups on the surface of a protein.[26,34,50] Many proteins were indeed found to exist near the chemical interfacial lines on a BCP surface. For example, protein interaction was greatly favored with the PS area immediate to the PS:PMMA interfaces on either sides of a given PS nanodomain.[34,35,51] In fact, protein affinity to the interfacial regions, rather than to the center of a given PS nanodomain that is as far away as possible from the nonfavorable PMMA present on either sides of the PS, dominated protein−polymer interactions at low protein surface coverage until more proteins started to fill in, and protein−protein interactions in addition to protein−polymer interactions became important at higher surface coverage. Protein surface density, i.e., the number of protein molecules per a unit surface area, was found to decrease with increasing distance away from the chemical interface.[34] This trend is clearly seen in the data summarized in Figure 2. These findings indicate that the chemical interfacial regions periodically present at a nanoscale interval on BCP surfaces can be tailored to modify protein−polymer interactions and to control protein surface density, assembly, and packing behaviors for specific applications.[35,37] A later study by Xie et al. systematically investigated the extent to which protein-nanoscale polymer interactions can be controlled by the underlying polymer surface effect. As displayed in Figure 3, the work revealed that the highest protein surface density could be achieved when the BCP periodicity for the chemical interface was matched closest to the dimension of the protein.[37] As the length scale of the BCP's chemical interface was adjusted to be much larger or smaller than the dimension of the protein, protein−polymer interactions became weaker. In addition to the nanodomain periodicity, the effect of the nanodomain alignment on protein−polymer interactions was also assessed. It has been shown that protein−polymer interactions were the greatest when the BCP nanodomain was uniformly aligned with no topological defects and orientational variations. Figure 3 presents these experimental results which demonstrate the potential for altering protein behaviors at a solid interface by imposing different structural and chemical constraints through a nanoscale polymer surface. Parts A and B of Figure 3 show the different polymer surfaces of PS-r-PMMA, *dsa* PS-*b*-PMMA, *sm* PS-*b*-PMMA, and *com* PS-*b*-PMMA used for studying Fg interactions. These polymer templates were chosen to examine the effects of polymer surface-related parameters such as nanodomain alignment, nanodomain orientation, interface density, and nanodomain periodicity with respect to the size of Fg. As reported in Figure 3C, Fg interaction with the underlying polymer sample was found to be the greatest when the nanodomain periodicity (i.e., the distance between two nearest PS:PMMA interfaces) was commensurate with the size of the protein. The degree of Fg surface packing was also found to be the highest when the polymer nanodomains were uniformly aligned with no orientational variation. Overall, Fg surface density decreased with higher randomness in nanodomain orientation. This is seen in the cases of precisely aligned PS-*b*-PMMA (*dsa* PS-*b*-PMMA) versus randomly oriented PS-*b*-PMMA (*com* PS-*b*-PMMA). Protein surface density also decreased as the length scale of the nanotemplate associated with the chemical

(A)



(B)

Blank BCP nanotemplates			
Surface Type	Domain Alignment	Periodicity (nm)	Average surface roughness (nm), Root-mean-squared surface roughness (nm)
dsa PS-b-PMMA	Fully aligned	28	0.31 , 0.39
sm PS-b-PMMA	Random Fingerprint-like	25	0.27 , 0.33
com PS-b-PMMA	Random Fingerprint-like	45	0.29 , 0.36
PS-r-PMMA	N/A	N/A	0.21 , 0.26

(C)

Adsorbed Fg on the different BCP nanotemplates				
Polymer Template	Fg concentration (μg/mL)	Number of Fg/μm²	Density of Adsorbed Fg (mg/m²)	Surface Coverage (%)
dsa PS-b-PMMA	2.5	21.5 ± 4.8	0.011 ± 0.002	1.8 ± 0.4
	12.5	131 ± 18.7	0.066 ± 0.009	10.8 ± 1.5
	25	459 ± 74.0	0.23 ± 0.037	37.8 ± 6.1
sm PS-b-PMMA	2.5	14.6 ± 3.5	0.007 ± 0.002	1.2 ± 0.3
	12.5	141 ± 18.8	0.071 ± 0.009	11.6 ± 1.6
	25	349 ± 43.0	0.17 ± 0.022	28.8 ± 3.5
com PS-b-PMMA	5	80 ± 8.5	0.04 ± 0.0043	6.6 ± 0.7
	20	221 ± 19.4	0.11 ± 0.0097	18.2 ± 1.6
	50	360 ± 30.4	0.18 ± 0.015	29.7± 2.5
PS-r-PMMA	2.5	17 ± 4.0	0.0085 ± 0.002	1.4 ± 0.3
	12.5	73 ± 7.0	0.037 ± 0.0035	6.0 ± 0.5
	25	277 ± 19.4	0.14 ± 0.0097	22.9 ± 1.6

Figure 3. Controlling protein interaction behaviors through different structural and chemical constraints imposed by nanoscale polymer surfaces. (A and B) The different surfaces of block (b) and random (r) copolymers employed to study the effect of key surface parameters on protein interactions are shown in the AFM images. From the leftmost to the rightmost AFM panels, the surfaces correspond to (i) PS-r-PMMA, (ii) *dsa* PS-*b*-PMMA, (iii) *sm* PS-*b*-PMMA, and (iv) *com* PS-*b*-PMMA. The nanodomain periodicity on the BCP was adjusted to be much smaller than (i), smaller than (ii, iii), and comparable to (iv) the length of a model protein of fibrinogen (Fg). The nanodomain alignment was also adjusted to be randomly oriented (i, iii, iv) to fully aligned (ii). The chemical and physical descriptions for the polymer surfaces in part A are provided in part B. (C) Fg amounts determined on the different block and random copolymer samples specified in parts A and B are summarized in terms of protein surface density and protein surface coverage. For the reported Fg surface coverage, 100% is defined as the state in which the entire surface area of the underlying polymeric template is completely covered by the adsorbed Fg regardless of the nanodomain's chemical composition. All data reproduced with permission from ref 37. Copyright 2016 American Chemical Society.

interface was adjusted to be much larger or smaller than the dimension of the protein.

In the investigation of protein−polymer interactions with fibrinogen (Fg), more intriguing and complex protein−polymer interaction behaviors have been unfolded at the nanoscale, chemically varying polymer interfaces. In contrast to a globular protein such as IgG, the elongated protein of Fg exhibits a high aspect ratio in the protein's width-to-length. For the case of Fg interacting with PS-*b*-PMMA, a more neutral affinity to the two polymer blocks in the BCP was observed, and the degree of Fg-BCP interaction was dependent on the concentration of the protein.[35] Although Fg displayed exclusive interactions only with PS nanodomains at higher protein surface coverage similar to what was observed from the globular proteins, Fg showed affinity to both PS and PMMA regions of the BCP at lower protein surface coverage. In this

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J. Phys. Chem. B 2021, 125, 6040−6057

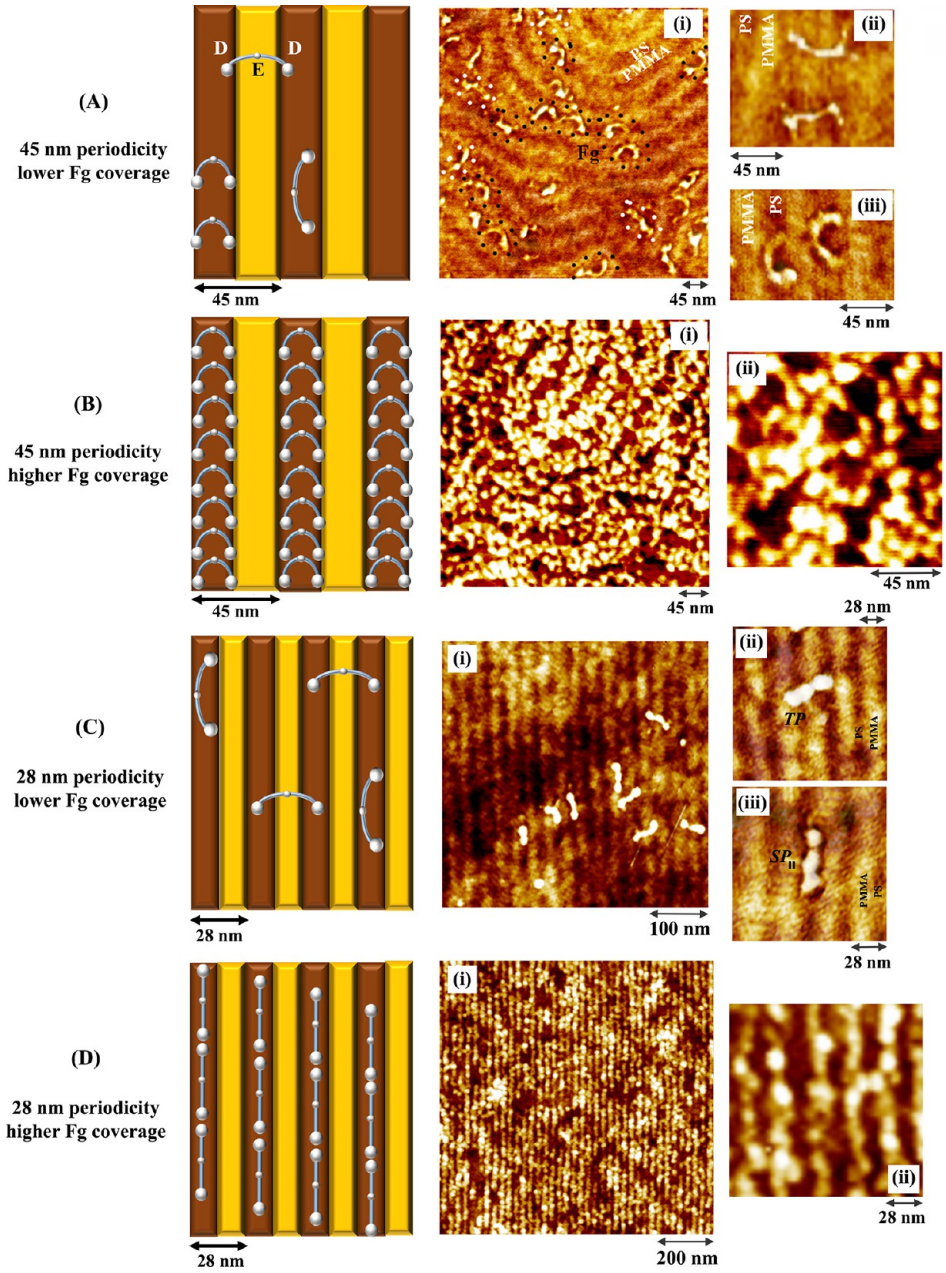

Figure 4. Controlling protein interaction behaviors through structural and chemical constraints imposed by nanoscale polymer surfaces. (A and B) AFM data of Fg upon its interaction with randomly oriented PS-*b*-PMMA nanodomains of 45 nm in periodicity are provided to show the unique phenomenon of Fg subdomain-specific protein−polymer interactions on the BCP. At low protein coverage shown in part A, Fg interaction is neutral to the two polymer blocks of PS and PMMA. At higher Fg coverage shown in part B, the adsorption and assembly of Fg are found to take place entirely on the PS nanodomains by orienting the protein backbone perpendicular to the long axis of the polymer nanodomains. This results in the "side-on" Fg packing geometry on the 45 nm BCP surface as shown in the (i) lower and (ii) higher magnification AFM panels AFM data in parts A and B reproduced with permission from ref 35. Copyright 2014 American Chemical Society. (C and D) AFM data corresponding to Fg on perfectly aligned PS-*b*-PMMA of 28 nm in periodicity are provided. (C) At low protein coverage displayed in panel i, Fg interaction is neutral to the two polymer blocks of PS and PMMA similar to the 45 nm BCP case. Fg molecules are found to exist both in (ii) TP and (iii) SP states. (D) At higher Fg coverage for the same 28 nm BCP surface, the adsorption and assembly of Fg molecules take place entirely on the PS nanodomains by orienting the protein backbone parallel to the long axis of the polymer nanodomains, forming the "end-on" Fg packing geometry on the 28 nm BCP surface as shown in the (i) lower and (ii) higher magnification AFM panels. AFM data in parts C and D reproduced with permission from ref 37. Copyright 2016 American Chemical Society.

neutral interaction coverage regimes, the different subunits of Fg (D, E, and αC subdomains) exhibited protein subunit-specific preferences to either PS or PMMA nanodomain areas. For instance, the D subdomain of Fg preferably interacted with PS, whereas PMMA was favored by the E subdomain. This coverage-dependent Fg interaction behaviors with the BCP was determined to be driven by the energetic interplay between electrostatic and hydrophobic interactions. Hence, Fg-BCP interactions were found to be drastically different from the predominantly hydrophobic interaction-driven globular protein interactions with the same BCP. Moreover, the fact that the length of Fg is commensurate with the periodicity of

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J. Phys. Chem. B 2021, 125, 6040−6057

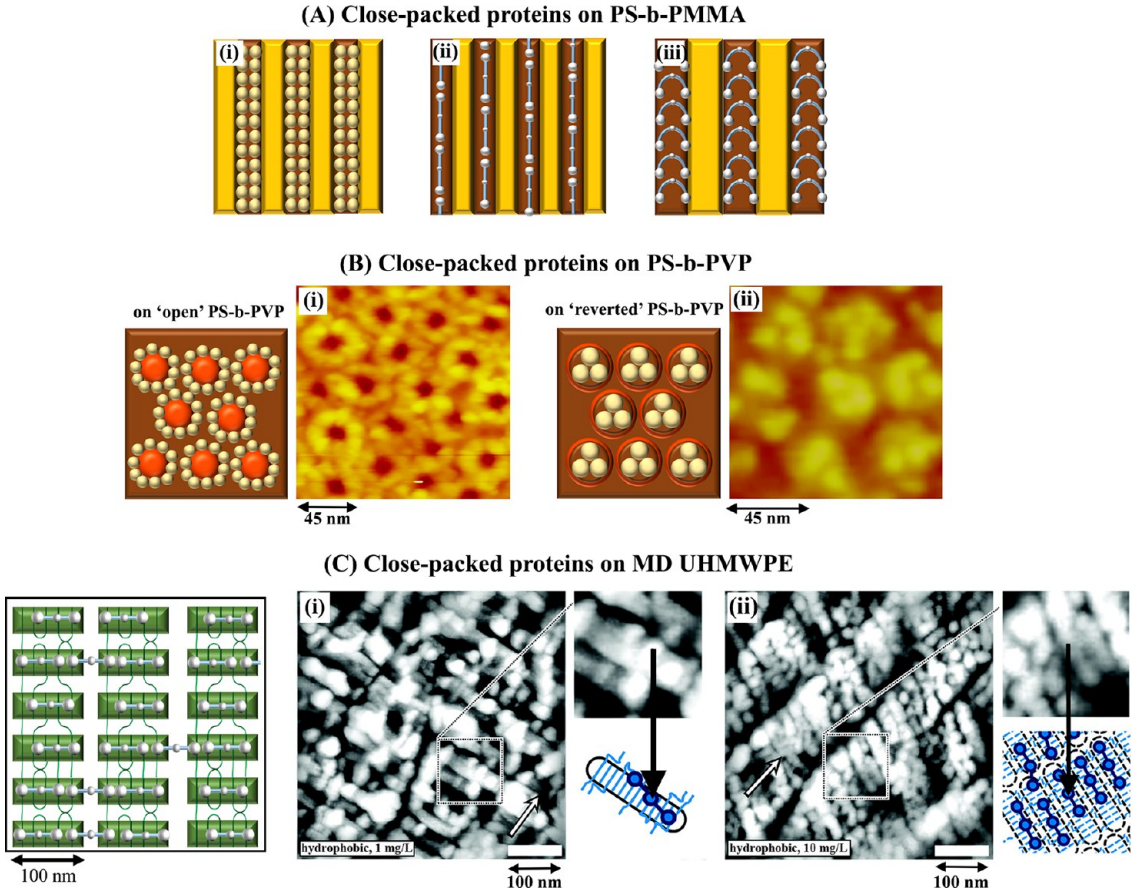

Figure 5. Examples of nanoscale protein−polymer interactions leading to highly organized and tight surface-packed protein self-assembly at various polymer interfaces. (A) The schematic representations display the close-packed protein assembly behaviors observed on the PS nanodomains of various PS-*b*-PMMA templates discussed earlier. The experimental evidence corresponding to each schematic depiction are provided in Figure 1 for panel i and Figure 4 for panels ii and iii. (B) The AFM topography panels show a highly ordered state of self-partitioned IgG proteins on the more favored PS regions of selective vapor-treated PS-*b*-PVP templates. (C) Well-packed Fg molecules observed on various melt-drawn ultrahigh molecular weight polyethylene (MD UHMWPE) surfaces are displayed. The AFM topography panels show Fg molecules organized on top of the crystalline lamellae polymer structures at (i) lower and (ii) higher protein coverage. Data in parts B and C are reproduced with permission from refs 33 and 52, respectively. Copyright 2007 and 2011 American Chemical Society.

the alternating PS and PMMA chemical interfaces on the BCP also played an important role in the coverage-dependent Fg interaction behaviors with the polymer.[37]

Figure 4 further reveals interesting Fg-BCP interaction behaviors that are governed by the underlying BCP characteristics. The AFM data in parts A and B of Figure 4 correspond to Fg interaction with randomly oriented PS-*b*-PMMA nanodomains of 45 nm in periodicity (45 nm BCP). At the low Fg surface coverage shown in Figure 4A, Fg interaction is neutral to the two polymer blocks of PS and PMMA. As pointed out earlier, this is unlike the highly exclusive affinity of the globular proteins to the PS block. For those Fg circled in white dots in panel i of Figure 4A, a single Fg molecule adsorbed over both PS and PMMA nanodomains. This is shown magnified in panel ii of Figure 4A. For other Fg population indicated with black dots in panel i, the entire Fg molecule is located only on the PS nanodomain, as shown in panel iii. For those Fg in white circles, the case is termed as two-phase (TP) as the protein interaction involves the two polymer phases of PS and PMMA. In contrast, the adsorption state of those in black circles involves only a single polymer phase of PS. Thus, this case is termed as single-phase (SP). Among the SP population, SP parallel ($SP_{\parallel}$) and SP perpendicular ($SP_{\perp}$) further specify the orientation of the Fg backbone with respect to the long axis of the underlying PS nanodomain as being parallel and perpendicular, respectively. At the higher Fg coverage shown in Figure 4B, the adsorption and assembly of Fg are found to take place entirely on the PS nanodomains by orienting the protein backbone perpendicular to the long axis of the polymer nanodomains. This $SP_{\perp}$ assembly state of tightly space-packed Fg molecules leads to the "side-on" Fg packing geometry on the 45 nm BCP surface. This is shown in the (i) lower and (ii) higher magnification AFM panels of Figure 4B. In contrast, the AFM data in parts C and D of Figure 4 correspond to Fg interaction on the perfectly aligned PS-*b*-PMMA of 28 nm in periodicity (28 nm BCP). At low protein coverage displayed in panel i of Figure 4C, Fg interaction is neutral to the two polymer blocks of PS and PMMA of the 28 nm BCP. This is similar to the 45 nm BCP case, and Fg molecules are found to exist in both (ii) TP and (iii) SP states. However, no Fg exhibits its backbone perpendicular to the long axis of the PS nanodomain, and unlike those on 45 nm BCP, only the $SP_{\parallel}$ case is found among the SP population on the 28 nm BCP. This is due to the much smaller nanodomain periodicity relative to the size of Fg. At higher Fg coverage for the same 28 nm BCP surface, the adsorption and assembly of Fg molecules take place entirely on the PS nanodomains by orienting the protein backbone parallel

https://doi.org/10.1021/acs.jpcb.1c00684
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to the long axis of the polymer nanodomains, as displayed in Figure 4D. This $SP_{\parallel}$ assembly state leads to the "end-on" Fg packing geometry on the 28 nm BCP surface. The "end-on" assembly of Fg on the 28 nm BCP surface is clearly shown in the (i) lower and (ii) higher magnification AFM panels of Figure 4D.

Another intriguing aspect of protein interactions at nanoscale, chemically varying interfaces is that proteins tend to self-assemble into a highly organized and tight surface-packed manner over a large area of the polymer surface.[26,35,37,52] As the polymer surface contains chemically distinctive nanodomains, the ensuing protein interactions preferring different polymer nanodomains lead to nanoscale surface partitions of the proteins entirely based on self-assembly. This characteristic property of nanoscale size-matching protein−polymer interactions can be extremely beneficial to creating solid state protein nanoconstructs for use as protein nanoarrays and biomaterial platforms. Precise tuning of nanoscopic protein patterns for their size, shape, and alignment over a large surface area is not trivial to attain. Conventional methods to fabricate solid state protein nanoconstructs often involve slow, complex, and costly micro- and nanofabrication steps.[53−57] The use of external fields such as shear, electrical, magnetic, and electrochemical fields is also required in order to gain control over the surface organization processes of proteins on polymers.[58−63] However, these additional steps not only make the scale-up of the approaches difficult but also can interfere with the native property and function of the proteins. Considering these current limitations, the fact that precisely controlled, nanoscale protein−polymer constructs are straightforwardly and rapidly assembled by tuning protein−polymer interactions can be particularly attractive.

Densely packed, well-organized proteins of nanoscopic dimensions were first discovered on the BCP surfaces of PS-*b*-PMMA and PS-*b*-poly(4-vinylpyridine) (PS-*b*-P4VP).[26,35,37] A highly ordered state of nanoscale self-partitioned proteins on BCPs was yielded when the experimental conditions allowed a monolayer of protein molecules to cover all available BCP nanodomain areas consisting of the protein-favored PS block. The resulting surface organization of individual protein molecules mimicked atomic arrangements found in a close-packed, 2-dimensional (2D) crystal. However, the high level of protein ordering was not attained when a macroscopic, chemically uniform counterpart of the BCP was employed for the same protein interaction instead. These experimental observations once again pointed out the critical role of physical and chemical constraints in protein−polymer interactions, i.e., the effect of the reduced size scale of polymer domains and the presence of chemical differences on inducing highly ordered, high density proteins via self-assembly. Similar phenomena were later reported for Fg interactions with the nanocrystalline lamella features found in melt-drawn, ultrahigh molecular weight polyethylene (MD UHMWPE) and close-packed, needle-like crystals in MD isotactic polybutene-1 (iPB-1).[52,64] Figure 5 summarizes various examples found in the literature for highly organized and tight surface-packed protein self-assembly at nanoscale polymer interfaces. Figure 5A schematically depicts the 2D protein assembly and the resulting protein nanoarrays formed on different PS-*b*-PMMA templates that were presented earlier in Figures 1 and 4. In addition to these, additional cases of tight protein packing on the nanoscale structures of PS-*b*-PVP and UHMWPE are displayed in parts B and C of Figure 5. Figure 5B shows highly ordered, self-partitioned IgG proteins on the PS regions of different PS-*b*-PVP templates. Prior to protein interaction, the BCP templates were treated with a solvent vapor that is selective to one of the polymer blocks. Well-ordered, two-dimensional IgG nanoarrays were formed on (i) an ethanol-treated PS-*b*-PVP surface termed as an "open" template and (ii) subsequently toluene-treated PS-*b*-PVP surface termed as a "reverted" template in Figure 5B. The open hole structures in panel i were induced by the greater chemical affinity of ethanol to PVP over PS. The core and corona of the original PS-*b*-PVP micelles were formed from the PVP and PS blocks, respectively. Upon exposure to ethanol, open hole structures were developed on the PS-*b*-PVP surface due to opening of the PS corona to expose the ethanol-favored PVP core. Further treatment of the open PS-*b*-PVP surface to toluene, a poor solvent for PVP, reverted the PS chains back to its original position to cover the once hole areas of the PVP core, as shown in panel ii. IgG proteins deposited to these different PS-*b*-PVP surfaces all showed a highly ordered state of self-partitioned proteins on the more favored PS regions of the PS-*b*-PVP templates, Figure 5B. Figure 5C displays another example of well-assembled proteins on a polymer surface. In this system, the surface of MD UHMWPE presents nanoscopic topological features of crystalline lamellae onto which Fg molecules can organize. Well-packed Fg molecules formed on a polyethylene-based surface of MD UHMWPE are seen in the AFM data in Figure 5C.

IV. EXTENDED EFFORTS FOR UNDERSTANDING PROTEIN−POLYMER INTERACTIONS

Measurement methods typically used for protein−polymer interaction studies were largely limited to providing ensemble-averaged protein behaviors through analyzing indirect, rather than direct, signals from many proteins. Experimental tools relying on changes in resonance frequency,[43,65] infrared absorption frequency,[43,66,67] fluorescence intensity,[47,68−70] and refractive index[65,71,72] do not offer high enough spatial and chemical discernibility that can unambiguously and simultaneously resolve different nanoscale features of the protein−polymer interfaces at the single biomolecular level. Electron microscopy (EM), Cryo-EM, and X-ray diffraction (XRD) methods were also used in the past to investigate protein−polymer interactions, but they require special sample preparation procedures such as crystallization, labeling, conductive metal coating, and high vacuum/low temperature operation.[52,73−75] Hence, such techniques cannot be readily employed for real time monitoring or time lapse tracking of nanoscale biomolecular objects at polymer interfaces repeatedly over various sample treatments. These experimental difficulties may have been the main reason the unique protein interaction behaviors expressed at nanoscale, chemically varying polymer surfaces discussed above could not be resolved until the mid-2000s.

Since the pioneering work by Kumar et al.,[32] further advancements in protein−polymer characterization techniques have taken place for achieving sufficient spatial resolution and chemical discernibility. The additional experimental capabilities led to ensuing research endeavors that examined protein interaction behaviors with various polymers featuring nanoscale surface topology and/or chemical variations. The initial findings of protein−polymer interactions summarized in the previous section are further substantiated by studying extended protein−polymer systems. Figure 6 features examples of such

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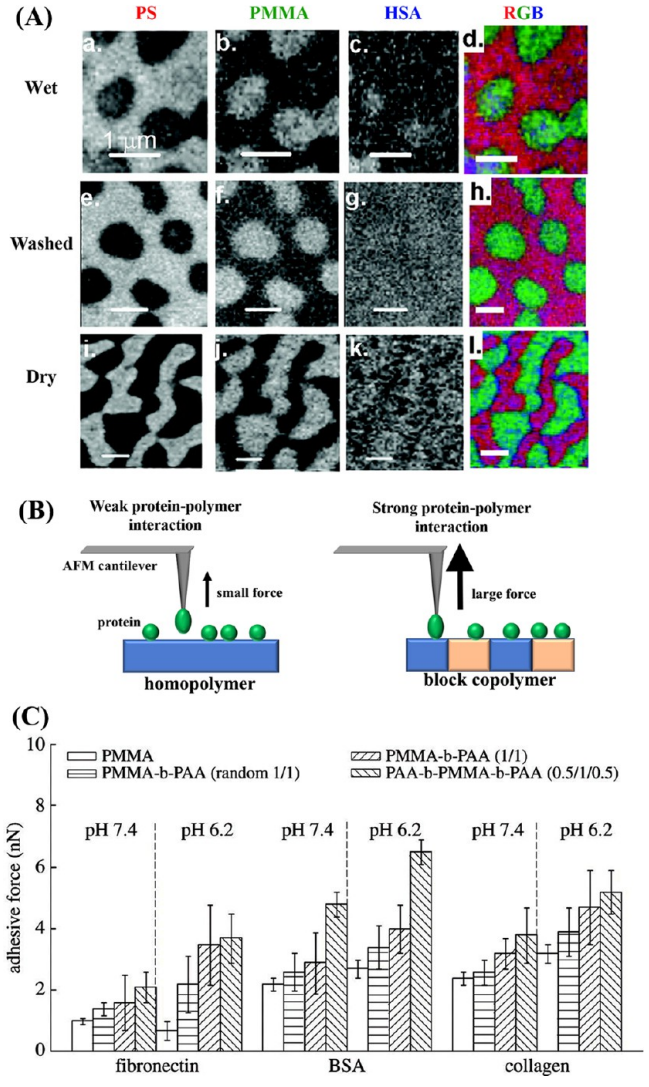

Figure 6. Mapping and quantifying protein interactions at nanoscale polymer interfaces with an extended set of measurement techniques. (A) Scanning transmission X-ray microscopy (STXM) images are displayed for HSA on three different PS/PMMA blend samples of (top row) fully hydrated, (middle row) washed, and (bottom row) dried. The PS (first column), PMMA (second column), HSA (third column), and all three combined (last column denoted as RGB) component maps in the image sequences are constructed using the C 1s STXM signal. The tendency of the protein interaction favoring the interfacial lines between PS and PMMA areas is clearly seen in the RGB composite map in the last column. (B) The schematic illustrations describe that forces between protein and polymer are greater for (right) the nanoscale, chemically varying BCPs relative to those on (left) the macroscale, chemically uniform homopolymers. (C) The adhesion force data charted in the bar graphs indicate that, relative to the PMMA homopolymer case, protein adhesion forces measured on the diblock and triblock BCPs of poly(methyl methacrylate)-*block*-poly(acrylic acid) (PMMA-*b*-PAA) and PAA-*b*-PMMA-*b*-PAA are several folds higher. Data in parts A and C are reproduced, respectively, with permission from ref 51 (copyright 2009 American Chemical Society) and from ref 83 (copyright 2010 The Royal Society).

research efforts put forth to better understand distinctive protein interaction properties at nanoscale polymer interfaces by capitalizing on an extended set of measurement techniques. A series of studies was conducted by Lau et al., Hitchcock et al., and Leung et al. to gain insight into interaction behaviors of IgG and BSA/HSA with the polymer surfaces of PS-*b*-PMMA and PS/PMMA blends.[51,76−78] In these works, combined measurement approaches were employed which included AFM in conjunction with surface plasmon resonance (SPR) as well as synchrotron-based X-ray photoemission electron microscopy (X-PEEM) along with scanning transmission X-ray microscopy (STXM). Figure 6A displays the STXM data of HSA proteins adsorbed on three different PS/PMMA blend samples. The RGB panels in the last column of the figure set clearly show the preferred adsorption of HSA (shown in blue) near the interfacial regions between the PS (red) and PMMA (green) areas on the blend samples. Other studies launched by Kumar et al., Parajuli et al., and Ibrahim et al. expanded the investigation of protein interaction behaviors at the PS-*b*-PMMA interfaces to include other proteins such as lysozyme, peroxidase, tyrosinase, ferritin, and fibronectin.[32−34,79−81] The findings from these works were consistent with the interaction preferences and trends as discussed in the earlier section for the globular proteins, i.e., exclusive interactions with the PS of the PS-*b*-PMMA and PS/PMMA blend surfaces and the tendency to favor the PS area adjacent to the chemical interfacial lines.

In addition, AFM force spectroscopy (FS) based research efforts have been made in order to quantify adhesion forces resulting from protein−polymer interactions.[82−93] AFM FS approaches to investigate protein−polymer interactions may employ different measurement configurations as those involving a protein-coated AFM probe and a bare polymer surface, a bare AFM probe, and a protein-coated polymer surface, as well as a polymer-coated AFM probe and a protein-coated surface. Regardless of the configuration used, previous AFM FS studies to measure the adhesion forces of model proteins such as BSA, Fg, lysozyme, collagen, and myoglobin to various polymer surfaces concluded that the presence of hydrophobic as well as electrostatic interactions between proteins and polymers results in larger adhesion forces than hydrophilic interactions.[83−95] In these studies, protein adhesion forces for hydrophobic and electrostatic interactions were reported to be within the range of 2−7 nN, whereas hydrophilic adhesion forces ranged in the 0.1−0.3 nN range.[83−95] Hydrophobic and electrostatic interactions with larger adhesion forces were described as rigid interactions from strongly bound proteins at a polymer interface.[89] In comparison, hydrophilic interactions were considered as softer interactions due to more weakly bound proteins.

Despite these efforts to quantitatively determine adhesion forces, AFM FS investigations in the past have been largely focused on protein interactions with macroscopic scale, chemically uniform polymer surfaces. Only a small subset of the studies has reported protein adhesion forces on polymer surfaces that contain nanoscopic surface features with chemically different domains.[83,90,96] Representative data provided in Figure 6C signify noteworthy endeavors in this direction.[83] In this AFM FS study, Palacio et al. compared protein adhesion forces on a homopolymer versus BCP surface.[83] The adhesion force data in Figure 6C indicate that, relative to the case of a PMMA homopolymer, protein adhesion forces are several folds higher on the diblock and triblock BCPs of poly(methyl methacrylate)-*block*-poly(acrylic acid) (PMMA-*b*-PAA) and PAA-*b*-PMMA-*b*-PAA, respectively. The stronger protein interactions with the BCPs relative to homopolymers were reported to be persistent independent of the type of protein

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(fibronectin, BSA, collagen) and pH (6.2, 7.4) used in the study. The outcomes of the AFM FS measurements confirm the protein−polymer interaction trend discussed earlier i.e., the interaction forces of protein−polymer increase for a case of a BCP relative to that of a homopolymer made from the same polymer blocks in the BCP. Figure 6B schematically depicts larger protein adhesion forces expected on a nanoscale, chemically varying BCP (right) relative to those on a macroscale, chemically uniform, homopolymer surface (left).

Research endeavors to assess the effect of nanoscale protein−polymer interactions on protein functionality have also been attempted.[37,79,80] Figure 7 displays representative

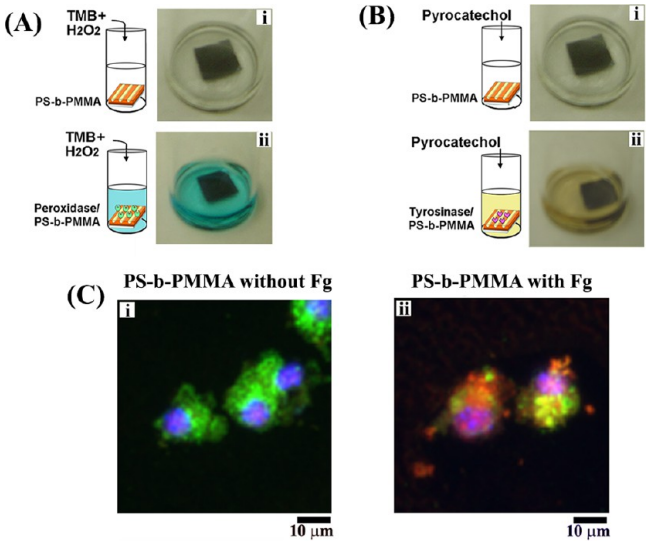

Figure 7. Evaluation of protein functionality upon immobilization onto polymer interfaces. (A and B) Enzymatic activities were assessed using the model proteins of (A) horseradish peroxidase (HRP) and (B) mushroom tyrosinase (MT) upon the enzyme adsorption to the BCP surface of PS-*b*-PMMA. Enzyme assays were carried out on (i) the control substrate of neat BCP and (ii) the test substrate of enzyme-bound BCP by introducing pertinent reagents for the enzyme reactions. Digital images of the assay solutions for (A) HRP and (B) MT are shown. The distinctive solution colors of blue and yellow corresponding to the catalytic reaction of HRP and MT, respectively, were produced only on the test substrates shown in panel ii. (C) The Fg functionality upon the protein adsorption to the BCP surface of PS-*b*-PMMA was assessed for microglial cell activation. Primary mouse microglial cells grown on (i) the control construct made from neat BCP and (ii) the test construct of Fg-covered BCP were further immunostained to assess the degree of cell activation. The resulting fluorescence signals by targeting the cell nuclei with 4,6-diamidino-2-phenylindole (DAPI, blue), inducible nitric oxide synthase (iNOS, red), and *β*-integrin marker of microglia (CD11b, green) are shown in panels i and ii for the control and test constructs. The fluorescence data confirmed that Fg functionality in activating the microglial cells was preserved even after protein immobilization to the BCP surface. Image data in parts A and B and part C are reproduced with permission from ref 80 (copyright 2007 American Chemical Society) and ref 37 (copyright 2016 American Chemical Society), respectively.

data from such studies of quantitatively or qualitatively assessing proteins' native functionalities after protein immobilization on nanoscale polymer surfaces and interfaces. Unlike the free motion state encountered in solution, the native property and functionality of proteins may become restricted after protein−polymer interactions, especially for those proteins transferred to solid state polymer surfaces. In the latter scenario, protein conformations may be compromised and, hence, downstream reactions relying on particular binding sites in proteins may be affected. Quantitative comparison of protein activity between a free solution versus a polymer surface-bound state requires accurate information on the exact number of protein molecules bound on a surface, which is not trivial to attain. The unique quantification capability discussed earlier that was available through the direct visualization of proteins on polymer surfaces as well as size-matching of proteins to polymer nanodomains finally permitted accurate quantification of the protein molecules adsorbed on a BCP surface and activity comparison between a surface-bound versus a free-solution state for the same number of protein molecules. It was reported that a high percentage of enzyme molecules such as peroxidase and tyrosinase molecules maintained their activity, up to roughly 85% and 78% of its free-state activity after being transferred to the BCP surface of PS-*b*-PMMA and PS-*b*-P4VP, respectively.[33,80] In another study, the well-known functionality of Fg in activating microglial cells was evaluated after Fg was transferred to a polymer surface. By comparing the activation degree of microglial cells cultured on a BCP construct with and without Fg, it was confirmed that the protein's functionality in microglial cell activation was retained even after the protein assembly on the BCP surface.[37] These results signify promising signs for creating and utilizing novel nanoscale protein−polymer constructs with functional, self-assembled proteins on the solid surface.

The possibility of controlling proteins via their interactions at the solid interface was also demonstrated by computer simulation studies. Molecular dynamics (MD) simulations carried out by Hung et al. showed the role of amphiphilic amino acids in facilitating the adsorption of cytochrome C (Cyt C) onto different mixed-composition surfaces of heterogeneous chemical segments.[97] In a different MD study carried out by the same group, the most energetically favorable conformation of lysozyme (Lyz) was determined for each model surface with varying chemical heterogeneity. The model surfaces ranged from the pure surfaces of 1-octanethiol (OT) and 6-mercapto-1-hexanol (MH) to the mixed surfaces of OT:MH in different ratios.[50] In another all-atom MD simulation work by Penna et al. on a protein of EAS hydrophobin, the authors reported that the effect on protein adsorption is the greatest for the surfaces presenting both nanoscale chemical structures and surface roughness relative to those surfaces presenting either none of the surface factors or just one of the two surface factors.[98] It is not trivial to run all-atom level computer simulation for protein−polymer interactions, especially in the context of whole protein interactions at nanoscale polymer interfaces, a topic of focus in the current paper. In addition to the difficulty in simulating the chemical heterogeneity and nanoscale topology of polymer interfaces, additional challenges exist due to the sheer size of whole proteins and large polymers, and the number of chemical moieties that need to be considered for simulation efforts. Due to these difficulties, not many atomistic simulation studies are currently available for protein−polymer interactions. Despite this, the simulation findings so far emphasize the critical role that nanoscale polymer interface plays and reiterate the significance of the surface constraints in protein interactions, i.e., distinct chemical features organized at a length scale comparable to the individual protein dimension in nanoscale protein−polymer interactions. As protein orientation on a solid

https://doi.org/10.1021/acs.jpcb.1c00684
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surface and interface is an integral part of protein functionality for its interaction with other proteins and cells, the simulation results also underscore that the structural and chemical parameters of a polymer interface can be effectively used to control the ultimate biological consequences of protein−polymer constructs. Figure 8 exemplifies the important roles that surface nanostructures combined with distinct chemical compositions can play in nanoscale protein−polymer interactions, especially when the surface features are organized at a length scale comparable to the individual protein dimension. Figure 8A schematically depicts different polymer surfaces leading to different protein orientations due to dominant interactions between a particular protein subdomain and a given polymer surface. Figure 8B displays MD simulation results of lysozyme (Lyz) binding to eight different surfaces terminated with varying ratios of 1-octanethiol (OT) and 6-mercapto-1-hexanol (MH) groups. It is clear from the simulation outcomes that the structural and chemical factors of an underlying surface can dictate the protein orientation upon its binding to the solid interface.

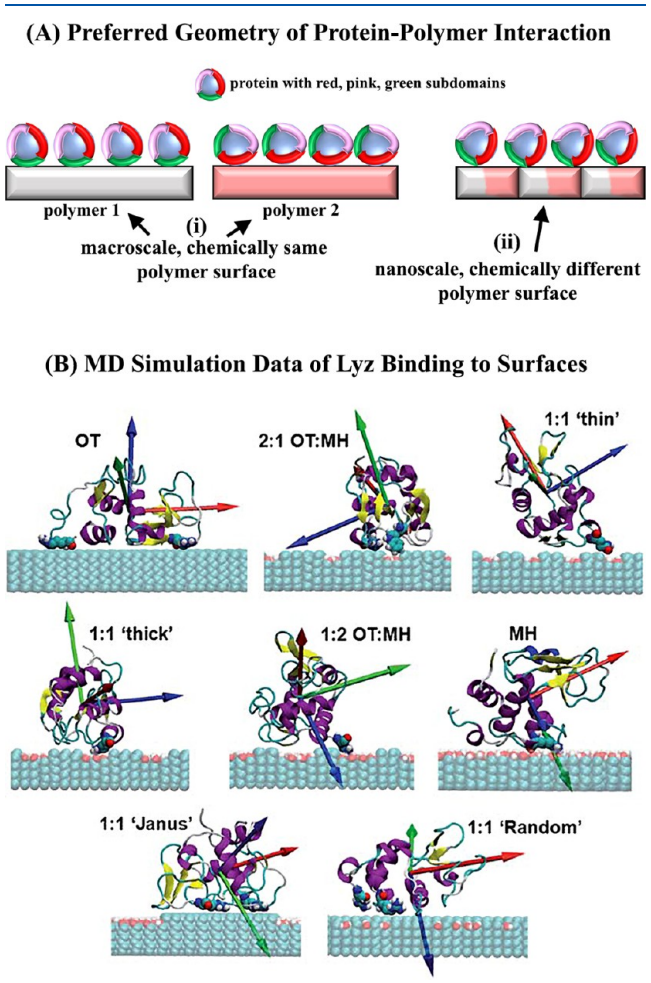

Figure 8. Nanostructures of distinct chemical compositions organized at a length scale comparable to individual protein dimensions in nanoscale protein−polymer interactions. (A) The cartoon depiction illustrates a potential scenario in which a dominant protein−polymer interaction geometry is determined by the underlying polymer. As an example, simplified scenarios are shown for the cases of macroscale, chemically same polymer surfaces (i) and a nanoscale, chemically different polymer surface (ii). In all cases, the protein displays a polymer surface-specific orientation at the solid interface. (B) A series of MD simulation data is provided for 8 different surface cases of Lyz binding. The different surfaces are terminated with varying ratios of 1-octanethiol (OT) and 6-mercapto-1-hexanol (MH) groups. The different surface termination groups are shown as blue and red spheres for the OT- and MH-containing areas, respectively. The Lyz configuration in each panel displays the MD simulation result for the most energetically favorable protein binding orientation on the given surface. The three principal axes of Lyz are indicated as red, blue, and green vectors in the order of longest to shortest axis of the protein. Data in part B reproduced with permission from ref 50. Copyright 2013 Royal Society of Chemistry.

V. UNDERSTANDING PROTEIN−NANOSCALE POLYMER INTERACTIONS IN MORE COMPLEX PROTEIN SYSTEMS

Very little is understood about competitive and/or cooperative interaction processes of multicomponent proteins in general, let alone for those polymer systems of nanoscale topology and chemical variability. Hence, concerted research efforts in this area are especially needed. Present knowledge on single component protein models cannot adequately explain more complex, multicomponent protein−polymer interactions frequently encountered in real life settings. For effectively bridging this knowledge gap, one of the most critical undertakings is recognized as acquiring experimental data at single biomolecule level that can unambiguously reveal time-dependent, multiprotein interaction processes between the protein themselves as well as between the protein and polymer interface.[8,9,39,41]

To this end, initial research efforts have been launched by Song et al. and Xie et al. for elucidating the nature of competitive interactions between two different protein components at nanoscale polymer interfaces.[36,38] In the work of examining simultaneously competing BSA and Fg interactions with a BCP at the single protein level, time-dependent characteristics of the competitive protein interactions were found to be significantly different on a nanoscale, chemically varying BCP surface when compared to the behaviors of the same two proteins on a macroscopic, chemically homogeneous surface.[36] The extent to which the initially adsorbed protein component of BSA resisting their displacement by the later arriving protein of Fg was observed to be much greater on the BCP surface of PS-*b*-PMMA relative to that on PS homopolymer. Parts A and B of Figure 9 display the experimental results that reveal the time-dependent behaviors of the dual component system of BSA and Fg during their simultaneous and competitive interactions with the nanoscale BCP versus PS homopolymer surfaces. A significant delay in time was recorded for the protein exchange process of the two proteins on the BCP. The temporal trend in the protein exchange process on the nanoscale versus macroscopic scale polymer surface is clearly seen in the colored bar graphs presented in Figure 9B. The study marks the first direct, single biomolecule level, experimental proof showing that nanoscale, chemically varying surfaces can promote stronger and stable interaction environments for already adsorbed proteins, leading to better resistance to other protein adsorption from the surroundings and/or displacement by them. The nanostructures and chemically varying interfaces present on BCP permit stronger protein−polymer interaction environments not only during the initial protein interaction stage at the liquid−solid interface but also after the protein is transferred to the polymer surface. Figure 9C schematically

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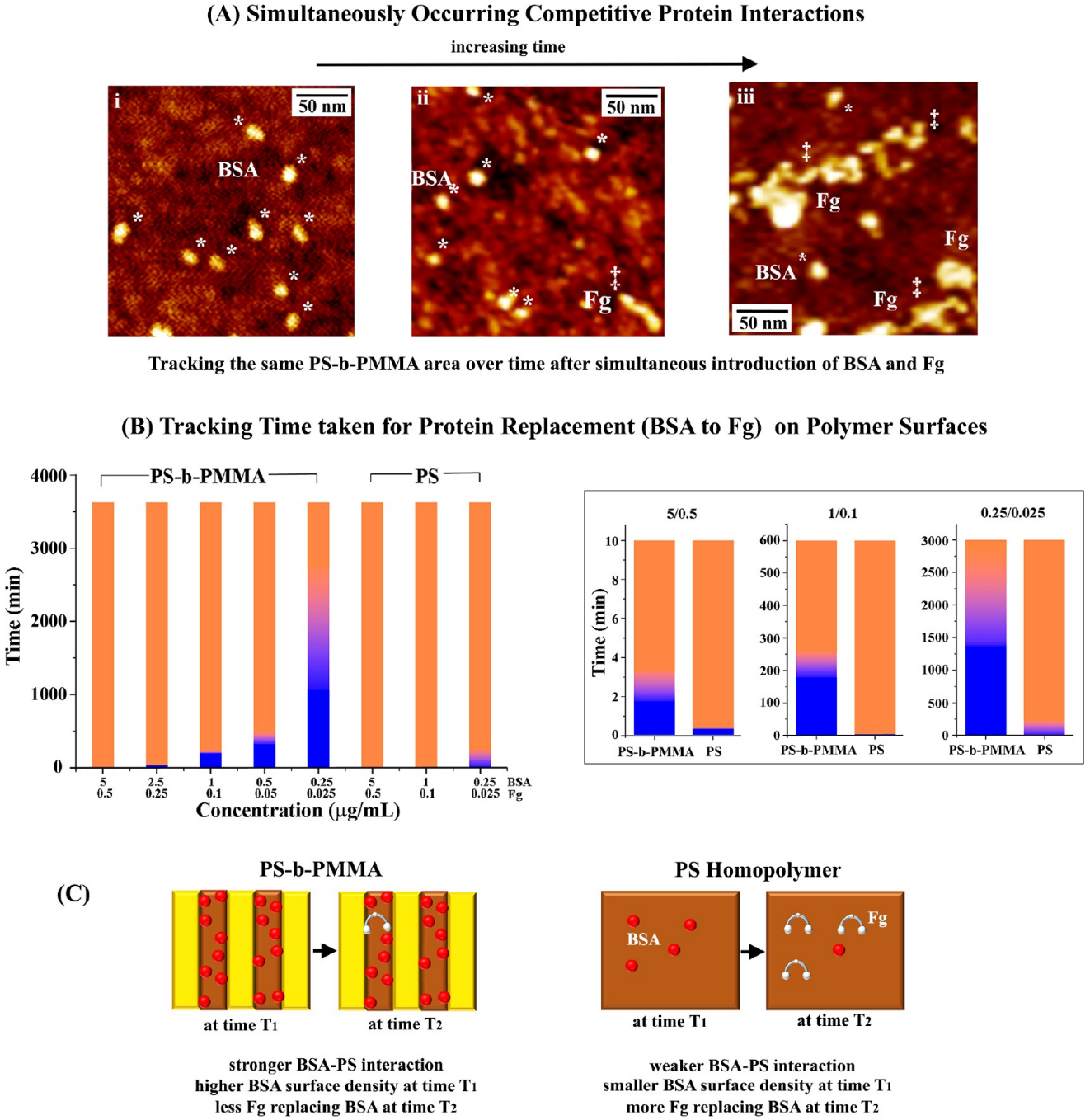

Figure 9. Simultaneously occurring competitive protein interactions with a nanoscale polymer surface. (A) Representative AFM images shown in the three panels i–iii display different stages of BSA (sample protein marked with * in the panel) and Fg (‡) adsorption over time when the two proteins were simultaneously introduced to a polymer surface. The surface was initially dominated by BSA but, with time, BSA was slowly replaced by Fg. (B) Competitive adsorption experiments of BSA and Fg were carried out on the BCP surface of PS-*b*-PMMA as well as on the homopolymer surface of PS. The timelines associated with mostly BSA, BSA being replaced by Fg, and predominantly Fg on the polymer surface were determined for the protein mixtures containing different concentrations of BSA and Fg as specified above each set of the bar graphs. The colored bar graphs display the outcomes of surface residence times associated with BSA-dominant (blue), BSA to Fg replacement (gradient purple), and Fg-dominant (orange) stages on the surfaces of BCP and PS homopolymer. The time windows of BSA to Fg turnover are more clearly shown in the zoomed-in graphs inside the black box. (C) The illustrations depict and summarize the simultaneous adsorption cases of BSA and Fg onto (left) the nanoscale, chemically varying PS-*b*-PMMA versus (right) the macroscale, chemically uniform PS homopolymer surface. Data images in parts A and B reproduced with permission from ref 36. Copyright 2016 Royal Chemical Society.

summarizes key findings from the study of the simultaneously occurring, competitive protein interactions with a nanoscale polymer surface.[36] Once initially bound to PS at time T_1, the interaction between BSA and PS is stronger on the BCP surface compared to the BSA-PS interaction on the PS homopolymer surface. For a given area of PS, the surface density of BSA is also higher on the BCP relative to the homopolymer case. The strong BSA-PS interaction on the BCP, in turn, leads to prolonged residence time of BSA on the BCP and higher tendency to resist desorption from the surface

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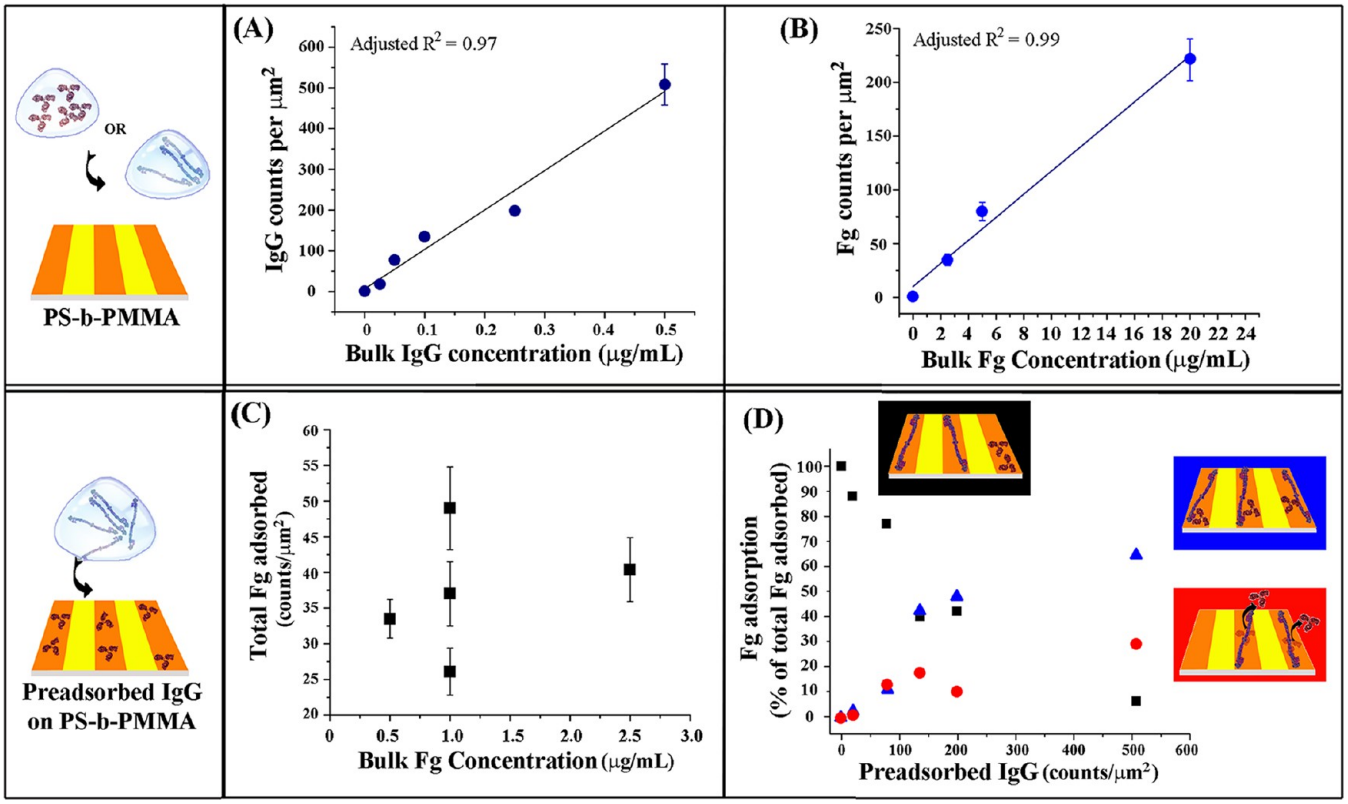

Figure 10. Sequentially occurring competitive protein interactions at a nanoscale polymer interface. Protein interaction behaviors found on (A and B) neat versus (C and D) pretreated PS-*b*-PMMA surfaces are summarized. (A and B) The plot of protein surface density versus protein concentration was obtained by examining the adsorption of a single component protein of (A) IgG and (B) Fg onto a clean BCP substrate. (C and D) The reported data correspond to the interaction behavior of Fg in a sequentially occurring, competitive adsorption case. In parts C and D, Fg was introduced as a subsequent stage adsorber to the BCP surface already covered with IgG from protein adsorption in a prior stage. The data in part C displays Fg surface density versus Fg concentration. The plot in part D shows the occurrence frequencies of Fg as a function of preadsorbed IgG counts on the BCP surface following the prior deposition step. The black, blue, and red data points shown in part D correspond to the different adsorption pathways of distal Fg adsorption, proximal Fg adsorption, and Fg replacing IgG, respectively. Images reproduced with permission from ref 38. Copyright 2018 Royal Society of Chemistry.

and replacement by Fg. Hence, at the same time point of T_2, a smaller percentage of BSA is replaced by Fg on the BCP surface relative to the case on the PS homopolymer.

An equally intriguing competitive protein interaction behaviors were reported in a combined experimental and computer simulation study of a multicomponent protein system in which protein interactions with polymer occurred in a sequential manner.[38] In this work of sequential competitive protein interactions, individual proteins of IgG and Fg were tracked on the same BCP surface locations over time between a different series of protein and buffer solution treatments. The single protein-level tracking measurements enabled unambiguous determination of key competitive protein−polymer interaction mechanisms that could not be resolved before. For instance, valuable information such as dominant competitive adsorption pathways, occurrence frequency associated with specific pathways, protein mobility on surface, protein self-association tendency, and directionality in protein exchange were provided for nanoscale protein−polymer interactions. Another important finding pertains to novel protein−polymer interaction phenomena that stemmed from the altered interactions of a neat BCP surface of PS-*b*-PMMA versus a prior stage protein-covered BCP surface. Unlike the case of protein interaction with a neat BCP surface in the very first stage of a sequential interaction series, the interaction characteristics between a prior stage protein-covered BCP surface with a subsequent stage protein were significantly changed. The surface density of the subsequent stage protein was no longer dependent on the solution concentration of the subsequent stage protein, but instead, it became a function of the amount of the prior stage protein adsorbed on the BCP surface.[38] Figure 10 summarizes the experimental outcomes revealed from the single biomolecule level study of sequentially occurring, competitive protein interactions with the nanoscale PS-*b*-PMMA surface. The plots of protein surface density versus protein concentration in parts A and B of Figure 10 correspond to the adsorption of a single component protein solution of IgG (A) and Fg (B) onto a clean BCP substrate. In contrast, the plots in parts C and D of Figure 10 are from Fg as a later stage adsorber to the BCP whose surface was already covered with IgG from a prior adsorption stage. Figure 10C demonstrates that Fg surface density is no longer dependent on the solution concentration of the Fg protein in this sequentially occurring, competitive protein adsorption. These findings are unlike the interaction cases of single component proteins to a neat BCP surface in parts A and B. Rather, the surface density of Fg (subsequent stage protein) is linearly dependent on the amount of IgG (prior stage protein) on the BCP surface, as evidenced in Figure 10D.

These experimental findings were further substantiated by Monte Carlo (MC) simulations designed to model competitive

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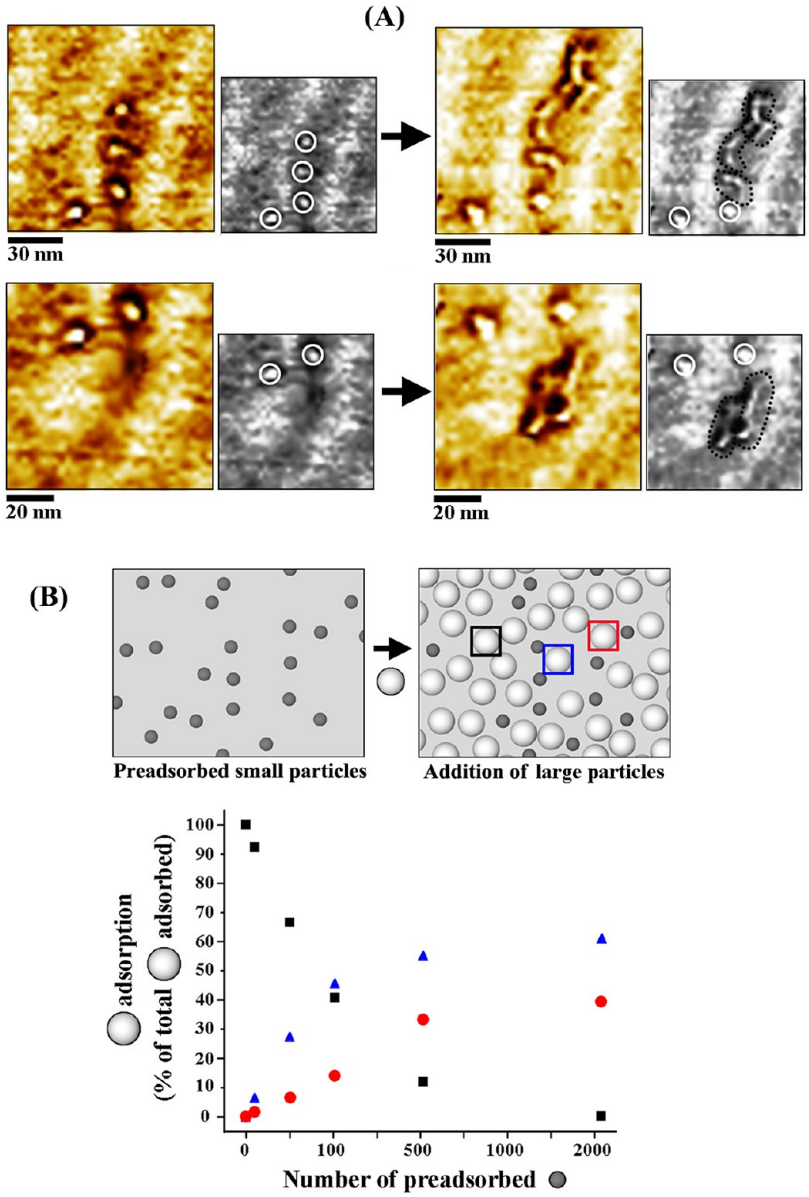

Figure 11. Revealing sequentially occurring competitive protein interaction pathways on nanoscale polymer surfaces through experiments and computer simulations. (A) Distinct adsorption events were tracked by AFM at the single protein level for multicomponent protein deposition onto the BCP of PS-*b*-PMMA. AFM measurements were conducted from an identical surface location. Representative imaging data are shown side by side for direct comparison of the dynamic events occurring after (left) IgG introduction and (right) subsequent Fg introduction at a later time point. (B) MC simulations were performed for the adsorption of large particles (modeling for the later arriving protein adsorber of Fg) onto a 2D box preadsorbed with small proteins (the initially adsorbed protein of IgG). Occurrence frequencies for the different adsorption events of the large particles were plotted as a function of the number of small particles preadsorbed on the 2D box. The simulation results are displayed for the cases of large particles adsorbing far from small particles (black, distal adsorption), close to preadsorbed small particles (blue, proximal adsorption), and by substituting a preadsorbed small particle (red, protein replacement). The three different particle adsorption pathways represent the cases of distal Fg adsorption, proximal Fg adsorption, and Fg replacing IgG, respectively. The Fg occurrence frequency graphs obtained from the MD simulations belonging to the three adsorption pathways provided direct means to compare with the experimental data in Figure 10. The MD results are consistent with the main experimental findings discussed earlier. Images reproduced with permission from ref 38. Copyright 2018 Royal Society of Chemistry.

interactions between two different particles sequentially added to a 2D box. The initial adsorption, desorption, and replacement events of the two particle types showing different interaction energetics to the 2D box were simulated for varying scenarios of sequential particle treatments to mimic the experimental conditions of sequentially introduced IgG and then Fg on the BCP. The MC simulation outcomes confirmed the major trends that were observed experimentally in Figure 10 such as the dominant competitive adsorption pathways, the occurrence frequency associated with specific pathways, and the fact that subsequent stage protein interaction becomes solely dependent on the surface coverage of the preadsorbed proteins from the prior step. This effort by Xie et al. represents the first endeavor to elucidate multicomponent, competitively interacting, nanoscale protein−polymer systems via combined experimental and computer simulation approaches.[38] Figure 11 introduces these exemplar means to ascertain sequentially occurring, competitive protein interaction pathways on a solid

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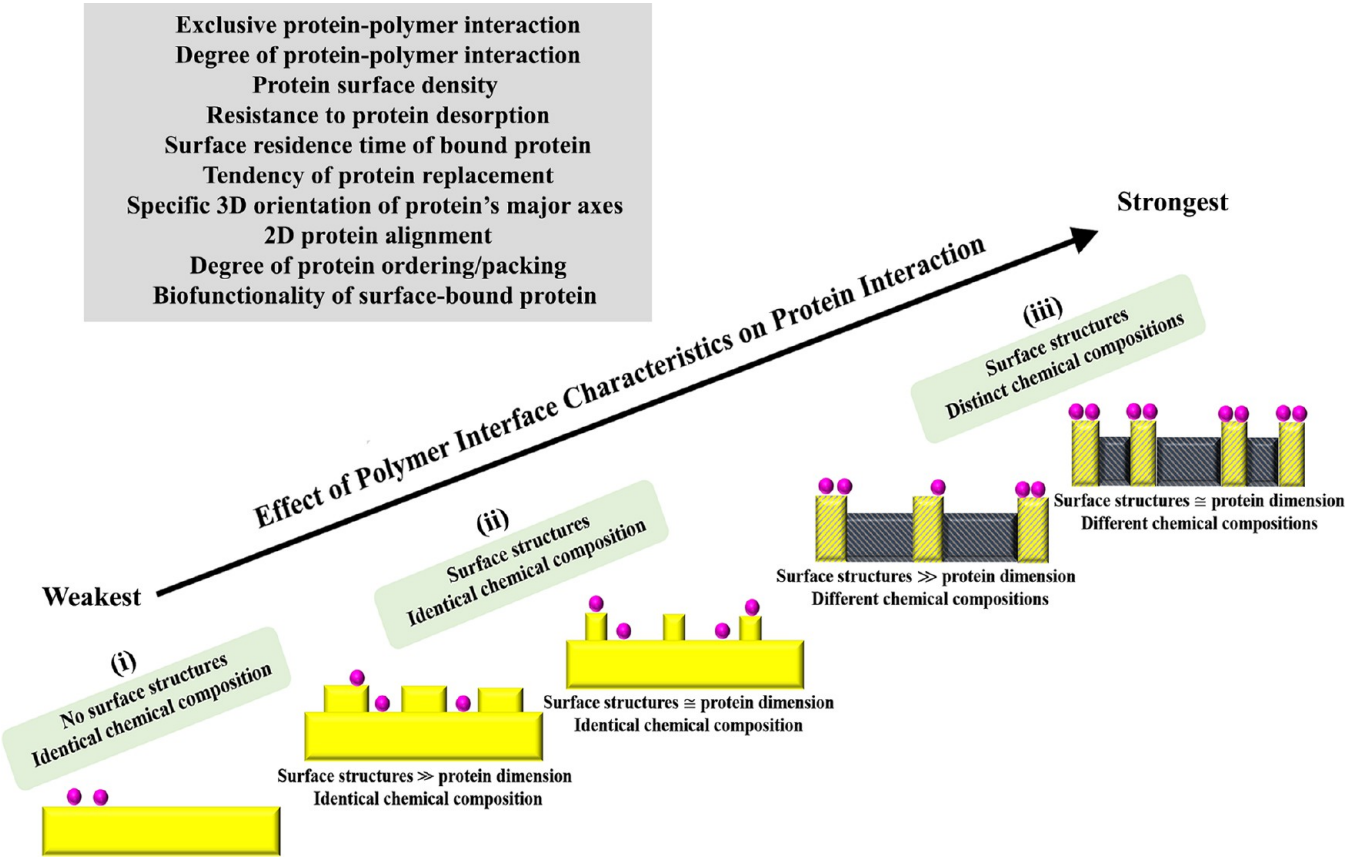

Figure 12. Schematic illustrations are displayed as a summary of previous research results from single biomolecule-level investigations of protein interactions at nanoscale interfaces. Polymer surfaces discussed in this paper are categorized in three groups of (i) having no surface structures along with no chemical variation, (ii) having an identical chemical composition on the polymer surface while exhibiting surface structures (left) much larger than and (right) comparable to individual protein dimensions, and (iii) having distinctively different chemical compositions on the polymer surface while exhibiting surface structures (left) much larger than and (right) comparable to individual protein dimensions. The effects of these polymer interfacial properties on protein interactions are summarized in the order of weakest to strongest for the key protein−polymer interaction characteristics that are specified in the gray box on the top.

surface, and the computer modeling results for the sequential interactions of large and small particles in a 2D box are displayed.

VI. SUMMARIZING REMARKS AND PERSPECTIVES

Spearheading research efforts made so far to understand protein interaction properties and behaviors at nanoscale, chemically varying polymer interfaces were summarized. Overcoming a crucial measurement hurdle, that had limited the protein−polymer investigations in the past to ensemble-averaged proteins interacting with macroscale and chemically unvarying polymers, made the initial research steps possible toward elucidating the mechanisms and kinetics specific to nanoscale protein−polymer interactions. More advanced experimental tools have since been demonstrated to offer individual protein tracking even at structurally and chemically intricate polymer interfaces with a spatial resolution on the order of nanometers. Owing to this, temporally and spatially resolved experimental data that were much needed for advancing the research field of protein−polymer interactions have begun to emerge at the single biomolecule level. Access to direct experimental proof for particular protein interaction pathways and kinetics that could only be speculated upon in the past due to the lack of single biomolecule level experimental data has propelled the research field toward building thorough and comprehensive knowledge on nanoscale protein−polymer interactions. As covered in this article, the groundbreaking efforts in this research field have demonstrated that the nature and degree of protein−polymer interactions are greatly affected by the interfacial environment, i.e., nanoscale versus macroscale surface structure, uniform versus varying chemistry on the polymer surface, and local versus global protein−polymer interaction. In particular, it is important to recognize that polymer interfaces, whose defining feature dimensions and associated chemical partitions on the surface are comparable to the size of individual proteins, induce the most significant effect on protein−polymer interactions. This, in turn, led to highly distinctive protein interaction behaviors that are not observed at macroscale and/or chemically uniform interfaces as detailed in this article. Figure 12 schematically illustrates the important roles of polymer interfaces in protein interactions that have been identified in the previous, single biomolecule-level investigations of nanoscale protein−polymer interactions. Key protein−polymer interaction characteristics as those specifically listed in the gray box in Figure 12 have been reported to be directly influenced by the structural and chemical properties of the polymer interfaces. The impacts from the early exploratory studies on protein interactions at nanoscale polymer interfaces are expected to reach beyond advancing fundamental understanding of protein−polymer

https://doi.org/10.1021/acs.jpcb.1c00684
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interaction mechanisms and kinetics. The research accomplishments made so far signify important foundations on which a set of new guiding principles can be built upon in the future that can be broadly applicable for the development of next-generation biomaterials, biosensors, bioimplants, and *in vitro* bioscaffolds.

It is critical that steady research efforts continue in the future to identify protein−polymer interaction characteristics at nanostructured, chemically intricate interfaces and to ultimately achieve control over the spatial, kinetic, and functional properties of various protein components on polymers. In the continuing efforts, it is worthwhile to note that the crucial lessons from the early groundbreaking studies underscore the need for truly nanoscale, individual biomolecule level, mechanistic and kinetic investigations of protein−polymer interactions. More experimental research efforts, inspired by the initial works on nanoscale protein−polymer interactions discussed in this article, are highly warranted. More studies need to be conducted to collect direct and unaltered signals from proteins that will provide the much needed, single biomolecular level experimental evidence of protein−protein or protein−surface interactions induced by nanosized interfaces. In particular, research efforts to examine the dynamic and multicomponent protein interaction properties at nanoscale polymer interfaces are required to better address the more complex protein interaction situations commonly encountered in practical settings. More endeavors in the future should also be made to ascertain time-dependent multicomponent protein interaction mechanisms and kinetics on nanoscale polymer surfaces. To best capitalize on newly identified, unique protein interaction properties on nanoscale polymer surfaces, future research should also systematically examine the effect of nanoscale polymer interfaces on the protein structure−function relationship. The initial works in this area have demonstrated that the physical and chemical characteristics of a given polymer interface can result in substantial changes in a protein's 2D orientation as well as 3D conformation, which consequently affects the ultimate functionality of the proteins. Hence, future efforts in this regard will be able to provide valuable insights into advancing the biomedical application field. In addition, future research endeavors are warranted to determine the roles of nanoscale polymer interfaces in time-dependent protein stability and functionality in biologically relevant settings and to reach the full application potential of nanoscale protein−polymer constructs. The anticipated outcomes will eventually be able to promote the tailoring of highly miniaturized protein−polymer constructs with time-programmed biological functionalities through controlling the mediating layers of proteins at polymer interfaces.

Hand in hand with the experimental efforts for direct experimental proof at the single biomolecule level, more theoretical and computer simulation efforts are needed in the future. For establishing the validity of empirical guidelines and/or developing optimal protein−polymer nanomaterials for desired applications, future endeavors in computer simulation should be able to examine multilength scales associated with protein−polymer interactions ranging all the way from nanometer to bulk scales. The experimental parameter space for protein−polymer systems is inherently very large since there exists a sundry of proteins and polymers with widely varying chemical and biological properties. Although it is essential to experimentally interrogate such wide-ranging protein−polymer systems to obtain broadly applicable knowledge on protein−polymer interactions, the large parameter space makes it either extremely difficult or sometimes impossible to do so. Future theoretical and computer simulation endeavors will thus be critical in this aspect. Entirely new mechanisms and kinetics of protein interactions specific to nanoscale polymer interfaces may finally emerge from forthcoming theoretical and simulation efforts. Figure 13 illustrates various research areas that still need to be explored in depth in order to gain thorough understanding of protein interactions at nanoscale polymer interfaces at the single biomolecule level. Additionally, related focus areas for future research are included in Figure 13 for attaining multifaceted and multilength scale knowledge whose fundamental principles for protein−polymer interactions can be valuable in effectively guiding the development of next-generation protein−polymer materials for their wide-reaching applications.

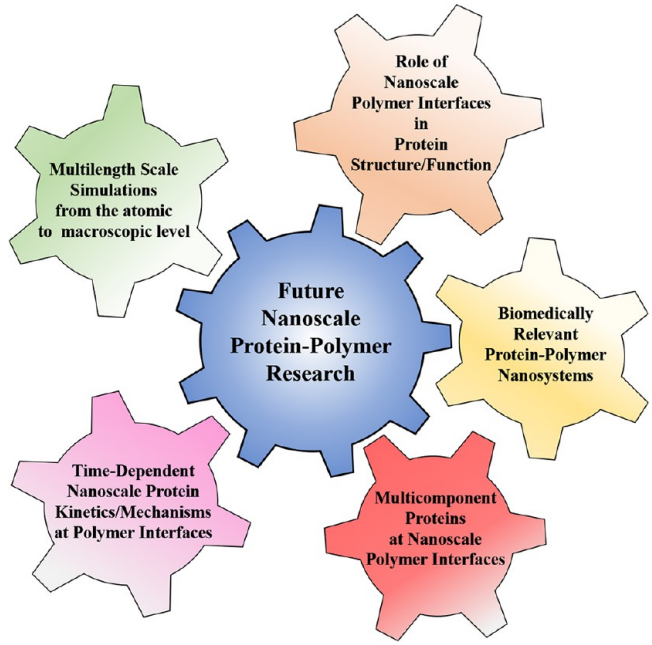

Figure 13. Future research areas to gain multifaceted insights on protein−polymer interactions at nanoscale interfaces at the fundamental and application level.

The anticipated knowledge from such multifaceted experimental, theoretical, and computer simulation approaches will ultimately bring about innovative protein nanopatterning and 2D/3D nanopartitioning strategies based on self-assembly. New capabilities based on the bottom-up self-assembly strategies may be realized in the future that will indeed enable precise mechanistic and kinetic control over the amounts, spatial registry, packing degree, orientation, and alignment of proteins at polymer interfaces in a rapid, simple, and inexpensive manner. Current technologies to produce and characterize protein−polymer constructs rely heavily on top-down methods whose fabrication steps can be slow, costly, complicated, and often done without sufficient single biomolecular level information on protein interactions. As such, present approaches to rationally design smaller and well-tailored protein−polymer constructs using top-down methods have been met with various challenges. Gaining comprehensive understanding of nanoscale protein−polymer interactions and

https://doi.org/10.1021/acs.jpcb.1c00684
J. Phys. Chem. B 2021, 125, 6040−6057

tuning their interactions through self-assembly will revolutionize the research and development fields of biomaterials, implant materials, and solid state protein arrays. Attaining more extensive knowledge on nanoscale protein−polymer interactions in the future may also prove that self-assembly can be exploited to control not only the static but also time-dependent biological functions of proteins. The prospect of being able to custom-program time-dependent protein functionalities into protein−polymer constructs via self-assembly, even for those systems consisting of multicomponent proteins at nanostructured and complex chemical interfaces, is noteworthy in leading toward transformative advancements to the future of the different protein−polymer application areas.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

D.H.C. and J.H. acknowledge financial support on this work by the National Science Foundation (NSF Award No. CHE1903857) from the Macromolecular, Supramolecular, and Nanochemistry Program under the Division of Chemistry.

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https://doi.org/10.1021/acs.jpcb.1c00684
J. Phys. Chem. B 2021, 125, 6040−6057