**Self-Consistent Field Theory**

To obtain microscopic information on the protein brushes, a polyelectrolyte self-consistent field theory (SCFT) is employed. While the key process is outlined here, the details have been reported previously (Yokokura 2024 Biomacromolecules). In a semicanonical ensemble, the proteins comprising the brush are modeled as multiblock charged macromolecules immersed in a monovalent salt solution and grafted upon a non-interacting substrate. The theory considers the interplay between intrachain elasticity, short-range hydrophobicity, and electrostatic interactions. Each constituent protein is treated as a Gaussian chain with number of segments with volume and Kuhn length . After following the standard procedure (refs 48—52 Yokokura BM) of decoupling the interacting system and using the mean-field, saddle point approximation, a series of continuous-space, self-consistent field equations are obtained. Assuming the protein brush to be homogeneous in the directions parallel to the grafting surface, the generated protein density profiles describe the distribution of proteins in the direction normal to the substrate. Amino acid sequences can be mapped to the charged block macromolecular model by blocking neighboring residues according to their charges, which are calculated at the bulk pH using the Henderson---Hasselbalch equation. The charge density of each block is thus the average of its constituent amino acids. The density distribution of Block is related to the overall density by for number of total blocks per amino acid sequence. The hydrophobicity of Block is manifested by its Flory---Huggins parameter , which describes the short-range van der Waals interactions between the block and the solvent.

In this work, pure brushes composed of NFL, NFM, NFH, and NFHD2 sidearms were modeled using the same grafting densities as measured experimentally (0.0365 nm−2, 0.0278 nm−2, 0.0301 nm−2, and 0.01854 nm−2, respectively). Reported relative hydrophobicities (Monera 1995 J Pep Sci) were scaled such that and the charges of phosphoserine and phosphothreonine were set at , both chosen for the best fit with experimental data. For simplicity, the same Kuhn length nm and segment volume nm3 were chosen based on best fit with experimental data for all proteins except phosphorylated NFH, where nm3 to account for the addition of the bulky phosphate groups. The bulk pH was taken to be 7.44. For simplicity, the solvent was chosen to be pure water and the relative dielectric constant of the system was assumed to be uniform at the value of water, . The temperature was set at K.

**Results and Discussion**

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*Pure brush*

SCFT is used to predict experimentally inaccessible, structural information underlying the measured brush heights. First, as shown in Figs. XX, NFL, NFM, and NFH are each mapped to the multiblock charged macromolecular model, yielding distributions of and 7 number of blocks, respectively. Due to the few number of modifiable sites in NFL and NFM, their comprising blocks are similar after phosphorylation. On the other hand, the number of blocks needed to model phosphorylated NFH decreases to 4, where Block 3 contains most of the KsP sites. Details on the coarse-grained blocks for each protein can be found in Sec. XX of the SI. To best approximate those experimentally measured by AFM, brush heights were extracted from the density distributions by choosing a threshold density of . After choosing appropriate model parameters such as and, the height response to changing ionic strength predicted by the SCFT are in good agreement with those measured experimentally, as shown in the insets of Figs. XX.

Protein density distributions generated from the theory can help to explain the morphological response of the NF brushes. NFL sidearms form brushes whose densities are mostly concentrated close to the substrate (Fig. XX) due to their neutrally charged grafted ends comprising Block 1. On the other hand, the brush regions from nm are predominantly formed by the highly charged Blocks 2, 3, and 4 of NFL, which collapse toward the surface as ionic strength is increased. As shown in Fig. XX, NFM sidearms similarly form dense regions near the substrate due to an uncharged Block 1. Additionally, a shoulder in the distribution forms at nm across varying ionic strengths. Block density distributions of NFM (Fig. XX) indicate that the shoulder in the overall density distribution is caused by its bimodal charge distribution, where the constituent chains organize into two, stacked brushes. Block 3 is constrained by the grafting point and possesses a high charge fraction; to distribute the charge of the remaining residues—especially the highly charged Block 7—a subpopulation of the NFM sidearms is expelled far into the solution. As the electrostatic screening is increased, the fraction of NFM proteins in the outer layer remains significant, even at 150 mM. Neither NFL nor NFM brush morphologies change significantly after phosphorylation due to the minimal change in their charge distributions.

As shown in Fig. XX, brushes comprised of NFH sidearms are nearly charge-neutral, resulting in fully condensed morphologies whose heights are insensitive to ionic strength. In contrast to NFL and NFM sidearms, NFH sidearms are strongly impacted by phosphorylation, with the average charge decreasing from to . Similar to NFM brushes, the high charge density of a block close to the grafting surface (Block 2) causes a portion of the phosphorylated NFH brushes to be expelled into diffuse outer layers at low ionic strengths ( mM). However, in contrast to NFM brushes, the outer layer collapses to form a condensed layer far from the substrate at intermediate ionic strengths (i.e., nm at mM). This layer gradually collapses to the surface as the ionic strength is further increased. Notably, the multilayered morphologies for NFM and phosphorylated NFH are similar to those predicted for NF brushes by the Scheutsjen-Fleer model (Zhulina 2007 Biophys J; Zhulina 2010 Biophys J), a lattice-constrained SCFT. However, the results of the current work are extracted from experimental results and are evidence of the role of the amino acid sequences intrinsic to each of the constituent proteins within NFs on the brush morphologies.

We note that there are discrepancies between the experimentally measured heights and those predicted by the theory, particularly at low ionic strengths. The systematic height discrepancies at low ionic strengths could be attributed to the simplified treatment of a uniform dielectric environment, as discussed in a previous work (Yokokura 2024 BM). Additionally, despite being different polymers, the same coarse-grained Kuhn lengths and segment volumes were used for all proteins in this work. Finally, the mismatch in height profile for phosphorylated NFH at intermediate ionic strengths could be from not considering the deprotonation of the monobasic phosphate group in phosphoserine and phosphothreonine, where the pKa is 5.6 and 5.9 (Xie 2005 Anal Biochem), respectively. For polyacids at low salt concentrations, the local hydrogen ion concentration is higher than the bulk (Zhulina 1995 Macromolecules) resulting in fewer ionized groups in brushes compared to free amino acids. As the ionic strength is increased, the salt ions displace the local hydrogen ions, eventually approaching the bulk pH.

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*NFH Del2*

Deleting subsegments of amino acid sequences is straightforwardly treated by the SCFT. As shown by the protein density distributions of NFHD2, the deletion of the negatively charged block causes the constituent chains to expand (Fig. XX). While this effect cannot be directly contributed to the change in net charge ( to as a result of the deletion), the deletion of the constrained, negatively charged subsegment eliminates the local attractive interactions between itself and the subsequent, positively charged region comprised of unphosphorylated KsP repeats. In phosphorylated NFH, the KsP repeats are negatively charged; thus, the deletion of the negatively charged block results in a systematic decrease in brush heights. As shown in Fig. XX, as a result of the reduced intrabrush repulsion, the outer condensed layers formed at intermediate ionic strengths are no longer favorable.