**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 constituent proteins 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 with similar charges, which are calculated at the bulk pH using the Henderson---Hasselbalch equation. The charge density of each block is denoted . The density distribution of Block is related to the overall density by for number of total blocks. The hydrophobicities of each block are manifested by their 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 uniform at the value of water, . The temperature was set at K.

**Results and Discussion**

**A screenshot of a computer screen

Description automatically generated**

*Pure brush*

As shown in Figs. XX, NFL, NFM, and NFH were mapped to the multiblock charged macromolecular model, yielding distributions of 4, 9, and 7 number of blocks, respectively. Due to the few number of modifiable sites in NFL and NFM, the 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 . Overall, the heights 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 with much of the density concentrated close to the substrate (Fig. XX) due to their neutrally charged grafted ends comprising Block 1. On the other hand, the brush region from nm is predominantly formed by the highly charged Blocks 2,3, and 4 of NFL, which collapses toward the surface as ionic strength is increased. As shown in Fig. XX, NFM sidearms similarly form a dense region near the substrate due to an uncharged Block 1. Additionally, a shoulder in the distribution forms at nm across varying ionic strengths. The density distributions of the blocks comprising NFM (Fig. XX) indicate that the shoulder is caused by its bimodal charge distribution, where a subpopulation of the constituent chains is expelled far into the solution to lower the local charge distribution.

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 can be attributed to electrostatic correlations: whereas the \_\_\_ . Additionally, the same coarse-grained b, v values were used for all proteins in this work. \_\_\_. *Effect of pKa on ionic strength/alpha?* The height profile of NFH does not match at intermediate ionic strengths due to the pKa of the phosphate being phosphoserine 5.6, phosphothreonine 5.9 (Xie 2005 Anal Biochem). As the ionic strength is increased from the salt-free case, the salt ions displace the local hydrogen concentration, resulting in a lower charge fraction than used for the quenched case. At high salt concentrations, the screening effect nullifies the overestimation in charge fraction.

\_\_\_ Draft \_\_\_

NFH is strongly impacted by phosphorylation, where the [X, XX] segment of the protein increases in charge density significantly. This increase caused a portion of the brush to be expelled into a diffuse outer layer at low ionic strengths. As the electrostatic screening is increased, the brush morphology changes such that a condensed layer is formed at \_\_ nm, much like the flower morphologies previously predicted for NFH by SJ-SCF (Zhulina 2007 Biophysical Journal). The density distributions of the comprising blocks indicate \_\_ as the conformation of the constituent proteins.

A screenshot of a computer

Description automatically generated

*NFH Del2*

Deletion of segments is straightforwardly treated by SCFT, providing physical reasoning for the trends measured experimentally.

As shown by the polymer density profiles of NFHD2, the deletion of the Block 2 results in an increase in local charge density, providing a local attraction with the positively charged Block 3. In pNFHD2, the deletion of Block 2 results in a systematic decrease in height due to the decrease in local charge density. While the accessed morphologies remain the same as the unmodified pNFH, the decrease in charge density causes the flower morphology to appear at lower ionic strengths.