**Self-Consistent Field Theory**

To obtain microscopic information of the protein brushes such as monomer density distributions, a polyelectrolyte continuous-space self-consistent field theory 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 charged block macromolecules immersed in a monovalent salt solution and grafted upon a non-interacting substrate. The theory considers the interplay between intrachain chain elasticity, short-range hydrophobicity, and electrostatics. By employing the standard self-consistent procedure, a series of continuous-space, self-consistent field equations can be used to report the morphology of protein brushes with a specified amino acid sequence. Each protein is mapped to the charged block macromolecular model by blocking neighboring residues based on their charges, which are calculated at the bulk pH using the Henderson---Hasselbalch equation. The generated protein density profiles describe the distribution of proteins in the direction normal to the substrate. 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 the Flory---Huggins parameter , which describes the short-range van der Waals interactions between the blocks and the solvent.

In the work, Phosphorylated serine and threonine residues are taken to Reported relative hydrophobicities (Monera 1995 J Pep Sci) are scaled such that , chosen for the best fit with experimental data.

**Results and Discussion**

**A screenshot of a computer screen

Description automatically generated**

*Pure brush*

SCFT-generated density distributions provide details on the morphology of the NF brushes.

Coarse-graining of filaments 🡪 number of blocks, detailed in Tables XX.

Height extracted from density distributions by 1e-05 to best approximate AFM probe touching brush

Both NFL and NFM exhibit classical brush morphologies. Due to the few numbers of phosphorylatable sites in both, the morphology is largely unaffected by phosphorylation. 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. \_\_\_.

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.

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.

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.