Structural Regulation of a Neurofilament-inspired Intrinsically Disordered Protein Brush by Multisite Phosphorylation

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Figure 1. Enzymatic phosphorylation induces swelling of the IDP brush, which can be reversed by alkaline phosphatase (ALP).

Intrinsically disordered proteins (IDPs) play central roles in numerous cellular processes. While IDP structure and function are often regulated by multisite phosphorylation, the biophysical mechanisms linking these post-translational modifications to IDP structure remain elusive. For example, the intrinsically disordered C-terminal sidearm domain of the neurofilament heavy subunit (NFH-SA) forms a dense brush along axonal NF backbones and is subject to extensive serine phosphorylation. Yet, biophysical insight into the relationship between phosphorylation and structure has been limited by the lack of paradigms in which NF brush conformational responses can be measured in the setting of controlled phosphorylation. Here, we approach this question by immobilizing a recombinant NFH-SA (rNFH-SA) as IDP brushes onto glass, and controllably phosphorylating the sequence \textit{in situ} with mitogen-activated protein kinase 1 (ERK2) pre-activated by mitogen-activated protein kinase kinase (MKK). We then monitor brush height changes using atomic force microscopy, which shows that phosphorylation induces significant brush swelling to an extent that strongly depends upon pH and ionic strength, consistent with a mechanism in which phosphorylation regulates brush structure through local electrostatic interactions. Further consistent with this mechanism, the phosphorylated rNFH-SA brush may be dramatically condensed with micromolar concentrations of divalent cations. Phosphorylation-induced height changes are qualitatively reversible via alkaline phosphatase-mediated dephosphorylation. Our study demonstrates that multisite phosphorylation controls NFH-SA structure through modulation of chain electrostatics and points to a general strategy for engineering IDP-based interfaces that can be reversibly and dynamically modulated by enzymes.