

HOW BIOLOGY USES POLYMERS TO REGULATE ADHESION IN VIVOD.Leckband^{1,2}, C.Johnson¹, C.Perrin-Tricaud³, I.Fujimoto³ & U.Rutishauser³¹*Dept. of Chemical and Biomolecular Engineering.*²*Dept. of Chemistry, University of Illinois, Urbana, IL USA.*³*Celular Biology and Biophysics, Memorial Sloan Kettering Cancer Center, NY, NY USA.*

INTRODUCTION: The neural cell adhesion molecule (NCAM) is one of the most abundant cell adhesion proteins in the central nervous system. This protein mediates both cell adhesion and cell-cell signaling via homophilic interactions with identical proteins on opposing cells (Figure 1).¹ It is also involved in neuronal development and plasticity.

The extracellular regions of NCAM consist of five tandemly arranged immunoglobulin domains (IgSF) followed by two C-terminal fibronectin type III (Fn III) domains (Figure 1).¹ The functional significance of these different domains is a subject of some controversy.

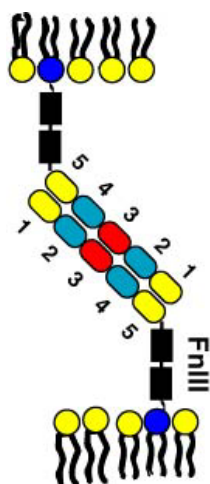


Fig. 1: Proposed mechanism of homophilic NCAM adhesion.

In addition, a unique post-translational modification (PTM) of the protein involving the addition of polysialic acid, (PSA, a long linear, negatively charged carbohydrate polymer) changes the protein function from an adhesion promoter to an adhesion inhibitor.^{2,3} This post-translational on domain 5 has significant biological consequences. It increases neuronal plasticity and the measured separation distances between cells.^{2,3} However, the physical mechanism by which the attachment of this carbohydrate chain alters the adhesive function of NCAM is still unknown.

This study describes direct force vs. distance measurements of the interactions between NCAM. From these measurements, we determined the mechanism by which NCAM mediates intermembrane adhesion. In addition, we quantified the effect of PSA on the range and magnitude of the intermembrane repulsion, and determine the physical mechanism by which PSA regulates NCAM adhesion.

METHODS: The surface force apparatus⁴ was used to measure the normalized force between oriented monolayers of NCAM extracellular segments. The NCAM monolayers were self-assembled onto supported planar lipid bilayers. The bilayers contained lipids with headgroups modified with NTA (nitrilo triacetic acid) moieties (NTA-DLGE). The NCAM was engineered with a C-terminal hexahistidine tag at the position in the sequence where the extracellular domain of the wild type protein begins to thread through the membrane. These engineered proteins bound to the NTA-lipids via their histidine tags. Surface plasmon resonance and X-ray reflectivity measurements were used to characterize the self-assembly of the protein monolayers.

RESULTS: The surface force apparatus⁴ was used to quantify the ranges and magnitudes of the attractive and repulsive forces between identical NCAM monolayers with and without the polysialic acid modification. With the unmodified form of NCAM, we demonstrated that the protein forms two bound states at two, different membrane spanning distances. Each of these two states involves different domains of the protein. These data reconcile prior apparently contradictory findings regarding the mechanism of NCAM adhesion and the domains involved in adhesion. Measurements with mutants lacking different protein domains further identified the key modules participating in these adhesive interactions.

Measurements between monolayers of the post-translationally modified form of NCAM show that the addition of the linear carbohydrate chain to domain 5 increases the magnitude of the repulsive force between the membranes. Importantly, the range and magnitude of the increased repulsion is sufficient to abrogate binding via both of the binding alignments measured with the unmodified protein. The enzymatic removal of PSA recovered both binding interactions between the proteins and restored NCAM-mediated intermembrane adhesion. These data suggested that PSA alters cell-cell adhesion by increasing the electrosteric repulsion between cell membranes over a distance that interferes with binding between NCAMs as well as other adhesion proteins on the cell surface.

The molecular origin of the PSA-dependent reduction in adhesion was confirmed by measurements performed at different ionic strengths. Theory predicts that the excluded volume of polyelectrolytes will decrease with the ionic

strength. If the PSA merely impeded binding by nonspecific electrosteric repulsion, then reducing the excluded volume of the polymer should correspondingly increase the intermembrane attraction. Consistent with this, increasing the ionic strength reduced the magnitude of the intersurface repulsion, and the intermembrane attraction increased correspondingly. Conversely, decreasing the ionic strength increased both the magnitude and range of the inter-surface repulsive force. The range and magnitude of the repulsion at physiological ionic strengths suggest that this polymer may influence not only NCAM binding, but also the adhesive interactions of other cell surface adhesion proteins.

DISCUSSION & CONCLUSIONS: These measurements show that NCAM mediates cell adhesion via a complex mechanism involving multiple protein domains. Importantly, we further show that the regulation of NCAM-mediated intermembrane adhesion by the post-translational addition of polysialic acid is due to the

corresponding increase in the nonspecific electrosteric repulsion between the membranes. Importantly, our findings directly demonstrate the use of surface attached hydrophilic, waters soluble polymers to regulate of cell-cell interactions *in vivo*.

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