SUPPLEMENTARY DATA

STRUCTURE AND MUTAGENESIS OF NEURAL CELL ADHESION MOLECULE DOMAINS: EVIDENCE FOR FLEXIBILITY IN THE PLACEMENT OF POLYSIALIC ACID ATTACHMENT SITES.

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Analytical Ultracentrifugation of purified Ig5-FN1-Ig5-FN1 purified from *Escherichia coli* was dialyzed in 50 mM Tris, pH 8.0, 250 mM NaCl. Sedimentation velocity experiments were performed at the Center for Structural Biology at University of Illinois at Chicago. Briefly, 400 µl of protein sample, ranging from 0.15 mg/ml to 1.5 mg/ml, was loaded into Epon double-sector centerpieces and centrifuged at 50,000 rpm in a Beckman ProteomeLab XL-1 centrifuge using an An-60 Ti rotor. Sedimentation was monitored by measuring absorbance at 280 nm. Buffer density and viscosity were determined using the program SEDNTERP (1) and data analyzed with the program SEDFIT (2).

Immunofluorescence analysis of NCAM localization-Cos-1 cells grown on 12-mm coverslips were transfected using 3 µl Lipofectin in 300 µl Opti-MEM 1, and 0.5 µg NCAM cDNA. After a 6 h incubation, 1 ml DMEM, 10% FBS, was added to each well. Sixteen hours later, cell media was removed and cells were washed twice with 1 ml PBS. One milliliter of -20°C methanol was used to permeabilize cells to visualize both internal and cell surface structures. The coverslips were then incubated with 1 ml immunofluorescence blocking buffer (5% normal goat serum in PBS). To evaluate localization, cells were incubated with anti-V5 tag antibody diluted 1:250 in blocking buffer, washed with PBS four times for 5 min, followed by incubation with fluorescence isothiocyanate-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) diluted 1:100 in blocking buffer. After further washing, coverslips were mounted on glass slides using mounting medium (15% (w/v) Vinol 205 polyvinyl alcohol, 33% (w/v) glycerol, 0.1% azide in PBS, pH 8.5). A Nikon Axiophot microscope equipped with epifluorescence illumination and a 60X oil immersion Plan Apochromat objective was used to visualize the cells and pictures taken with a SPOT RT color digital camera and processed with SPOT RT software version 3.5.1 (Diagnostic Instruments Inc, Sterling Heights, MI).

SUPPLEMENTAL FIGURE LEGENDS

FIGURE 1. **Analytical ultracentrifugation analysis of bacterially-expressed Ig5-FN1 indicates concentration-dependent dimer formation.** Sedimentation velocity analysis of purified Ig5-FN1 at a concentration of 0.15 mg/ml (blue) and 0.8 mg/ml (pink) using a 50 mM Tris, pH 8.0, 250 mM NaCl buffer.

FIGURE 2. **NCAM proteins containing engineered N-linked glycosylation sites localize to the Golgi and cell surface.** Cos-1 cells transiently expressing NCAM or various mutants were fixed with methanol and subcellular localization determined by indirect immunofluorescence microscopy using the anti-V5 epitope tag antibody. *Bar*, 10 µm.

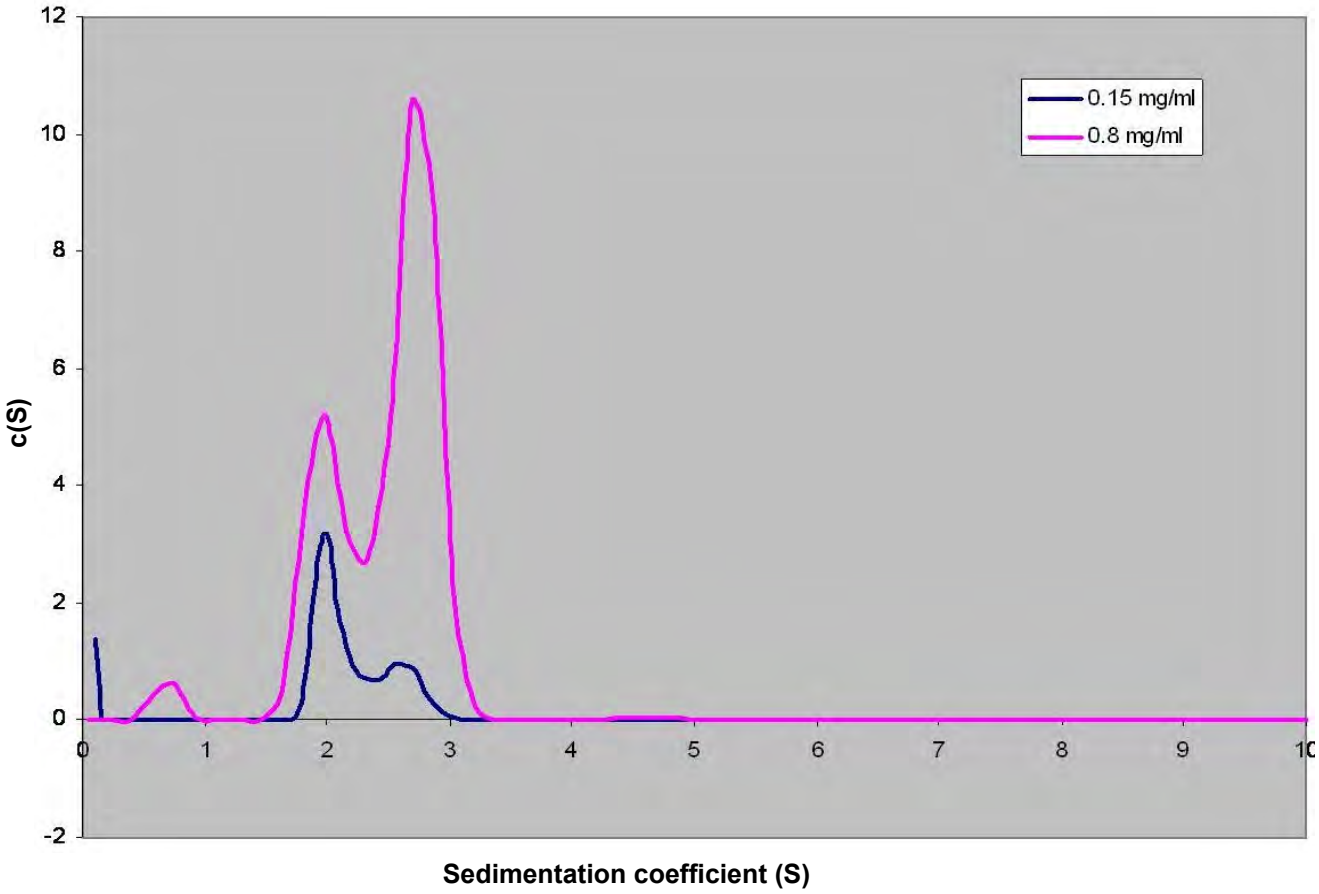
SUPPLEMENTAL REFERENCES

1. Hayes, D., Laue, T., and Philo J. http://www.rasmb.bbri.org
2. Schuck, P. Biophys. J. (2000) **78**, 1606-1619

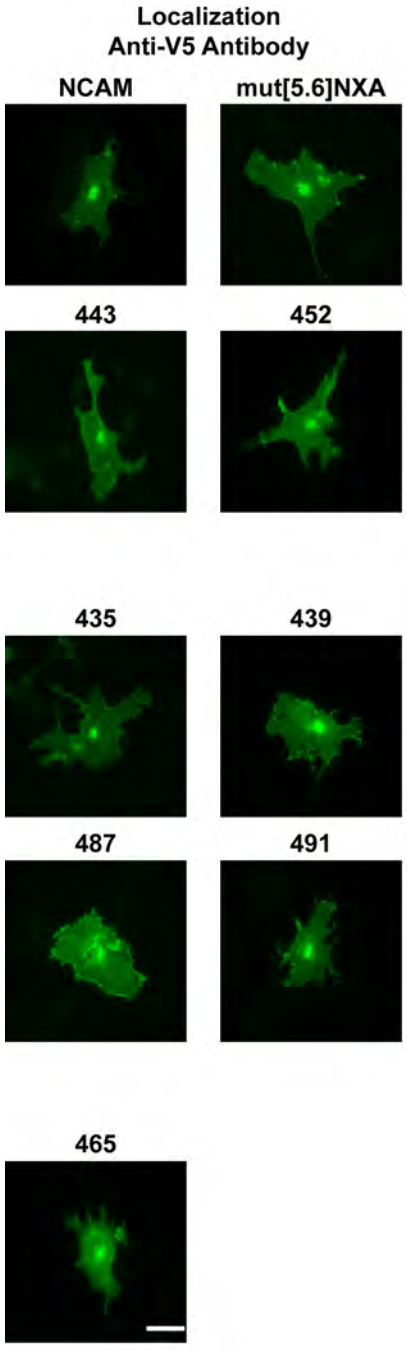
Supplemental Table 1: List of primers used for site-directed mutagenesis of NCAM and Ig5-FN1

MUTANT	TEMPLATE	PRIMER	PRIMER SEQUENCE
Ig5-FN1 Asn[5.6]Gln	Ig5-FN1	N449Q	5'-GCCAAGCTCCCAGTACAGCAATATCAAG-3'
			5'-CTTGATATTGCTGTACTGGGAGCTTGGC-3'
	Ig5-FN1 Asn[5]Gln	N478Q	5'-GGGAACTACCAATGTACTGCAGTGAAC-3'
			5'-GTTCACTGCAGTACATTGGTAGTTCCC-3'
Ig5-FN1 Asn[4.5.6]Gln	Ig5-FN1 Asn[5.6]Gln	N423Q	5'-GGAACCAGGTGCAAATCACCTGCGAG-3'
			5'-CTCGCAGGTGATTTGCACCTGGTTCC-3'
mut[5.6]NXA	NCAM	S451A	5'-CAAGCTCCAATTACGCCAATATCAAGATC-3'
			5'-GATCTTGATATTGGCGTAATTGGAGCTTG-3'
	NCAM Asn[5]NXA	T480A	5'-GGGAACTACAACTGTGCTGCAGTGAACCGC-3'
			5'-GCGGTTCACTGCAGCACAGTTGTAGTTCCC-3'
sNCAM mut4	sNCAM	T425A	5'-GAACCAGGTGAACATCGCCTGCGAGGTATTTG-3'
sIg5-FN1 mut4	sIg5-FN1		5'-CAAATACCTCGCAGGCGATGTTCACCTGGTTC-3'
mut[5.6]NXA E465N	mut[5.6]NXA	E465N	5'-CTGCCAGCTATCTGAACGTGACCCCAGACTC-3'
			5'-GAGTCTGGGGTCACGTTCAGATAGCTGGCAG'-3'
mut[5.6]NXA Q443LL-NLS	mut[5.6]NXA	Q443LL-NLS	5'-CATGGTTTCGGGATGGCAACCTGTCGCCAAGCTCCAATTACG-3'
			5'-CGTAATTGGAGCTTGGCGACAGGTTGCCATCCCGAAACCATG-3'
mut[5.6]NXA N452IK-NIS	mut[5.6]NXA	N452IK-NIS	5'-CAATTACGCCAATATCTCGATCTACAACACCCCC-3'
			5'-GGGGGTGTTGTAGATCGAGATATTGGCGTAATTG-3'
mut[5.6]NXA F439RD-NRS	mut[5.6]NXA	F439RD-NRS	5'-GTGCCACGATCTCATGGAATCGGTCTGGCCAGCTGCTGCCAAG-3'
			5'-CTTGGCAGCAGCTGGCCAGACCGATTCCATGAGATCGTGGCAC-3'
mut[5.6]NXA Q487N	mut[5.6]NXA	Q487N	5'-GTGAACCGCATTGGGAACGAGTCCTTGGAATTC-3'
			5'-GAATTCCAAGGACTCGTTCCCAATGCGGTTCAC-3'
mut[5.6]NXA E491FI-NFT	mut[5.6]NXA	E491FI-NFT	5'-GGGCAGGAGTCCTTGAACTTCACCCTTGTTCAAGCAGAC-3'
			5'-GTCTGCTTGAACAAGGGTGAAGTTCAAGGACTCCTGCCC-3'

Interference Fits



Supplemental Figure 1



Supplemental Figure 2