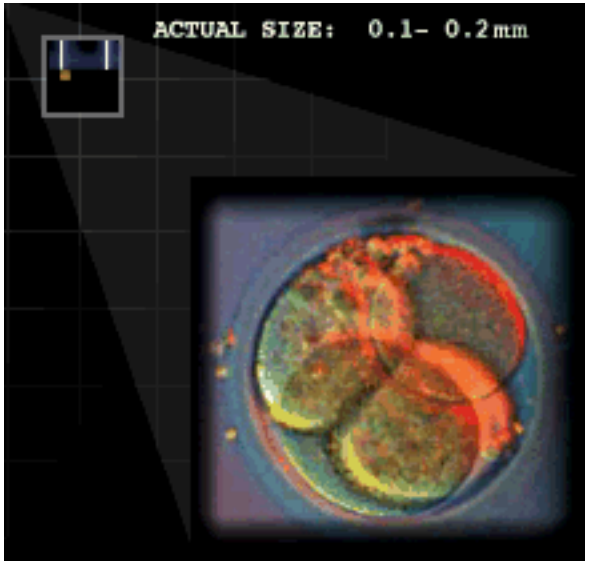
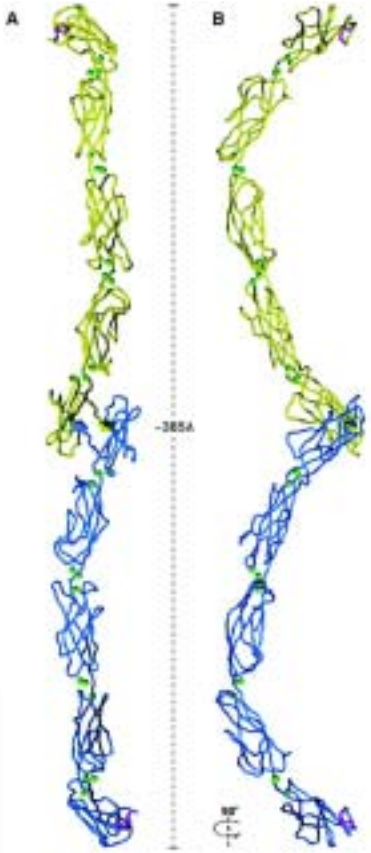
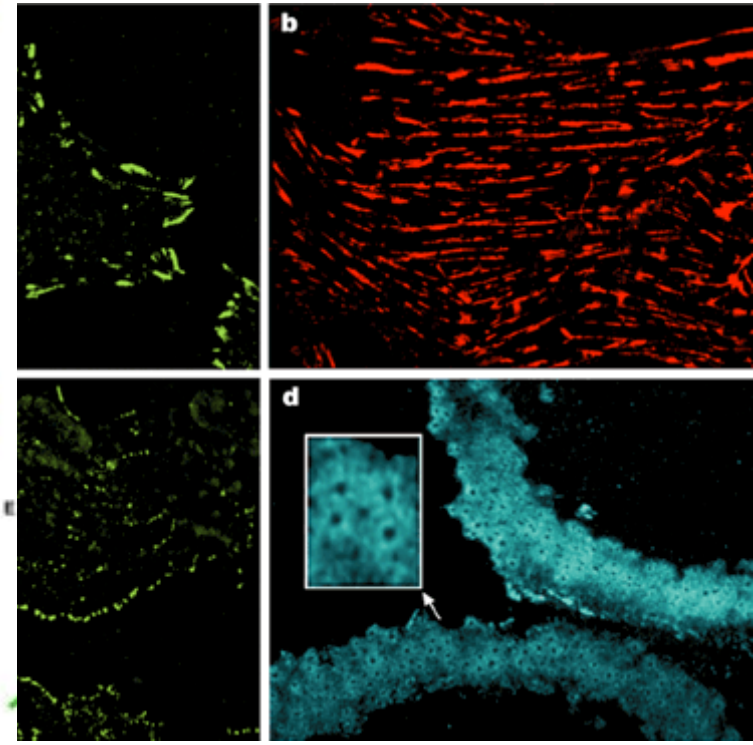
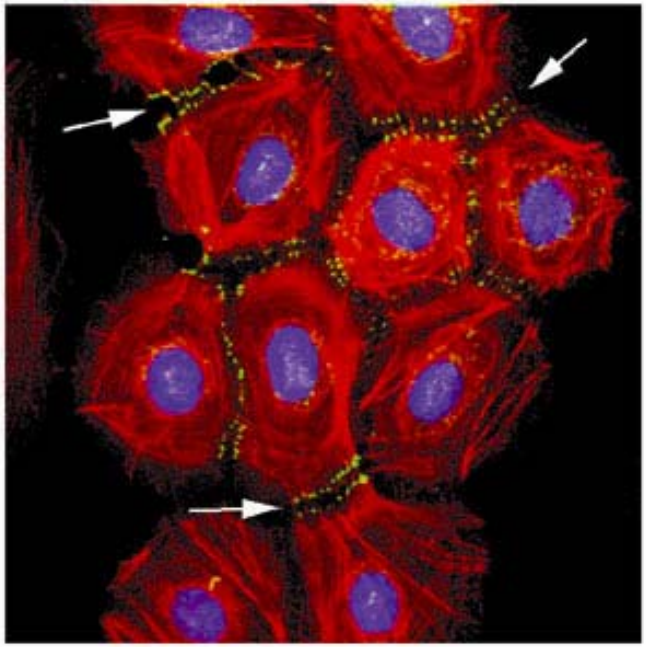
Molecular Design Rules of Biological Adhesion

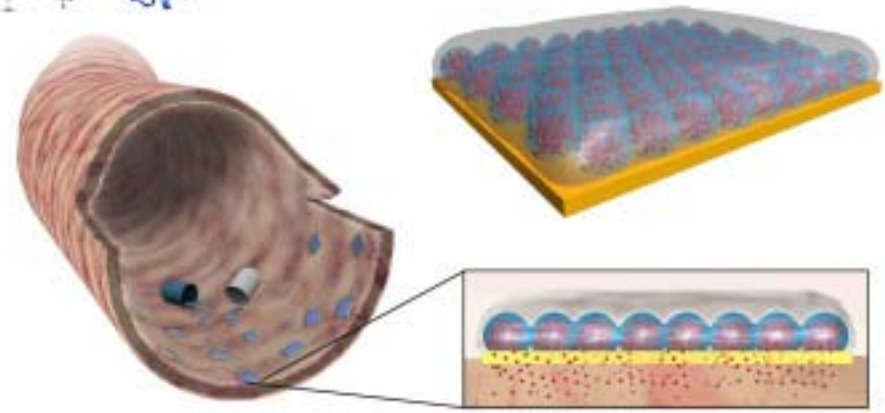
Deborah Leckband
Chemical and Biomolecular Engineering
Department of Chemistry
Center for Biophysics and Computational Biology
University of Illinois at Urbana-Champaign


ACTUAL SIZE: 0.1- 0.2mm

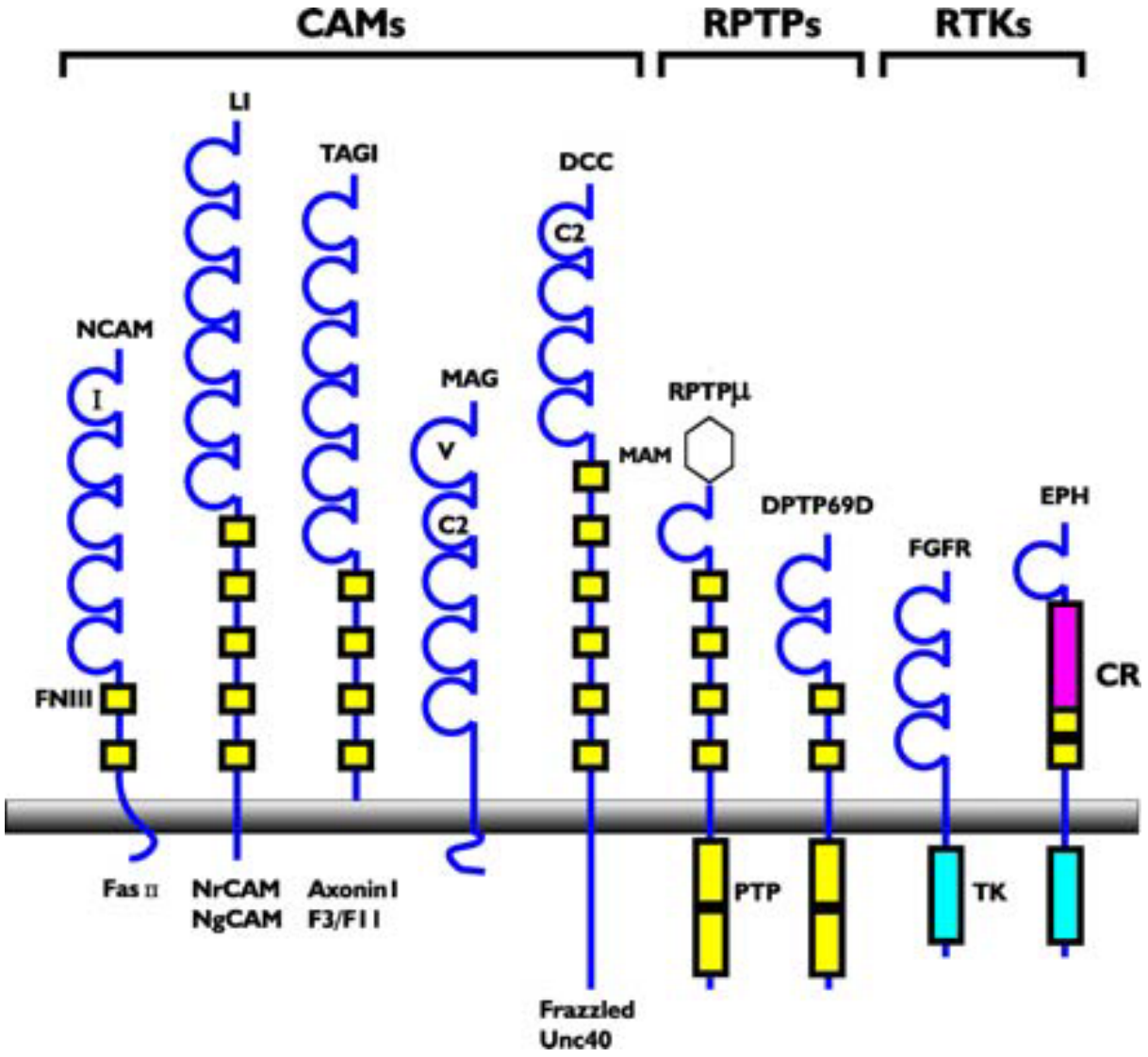

A
B

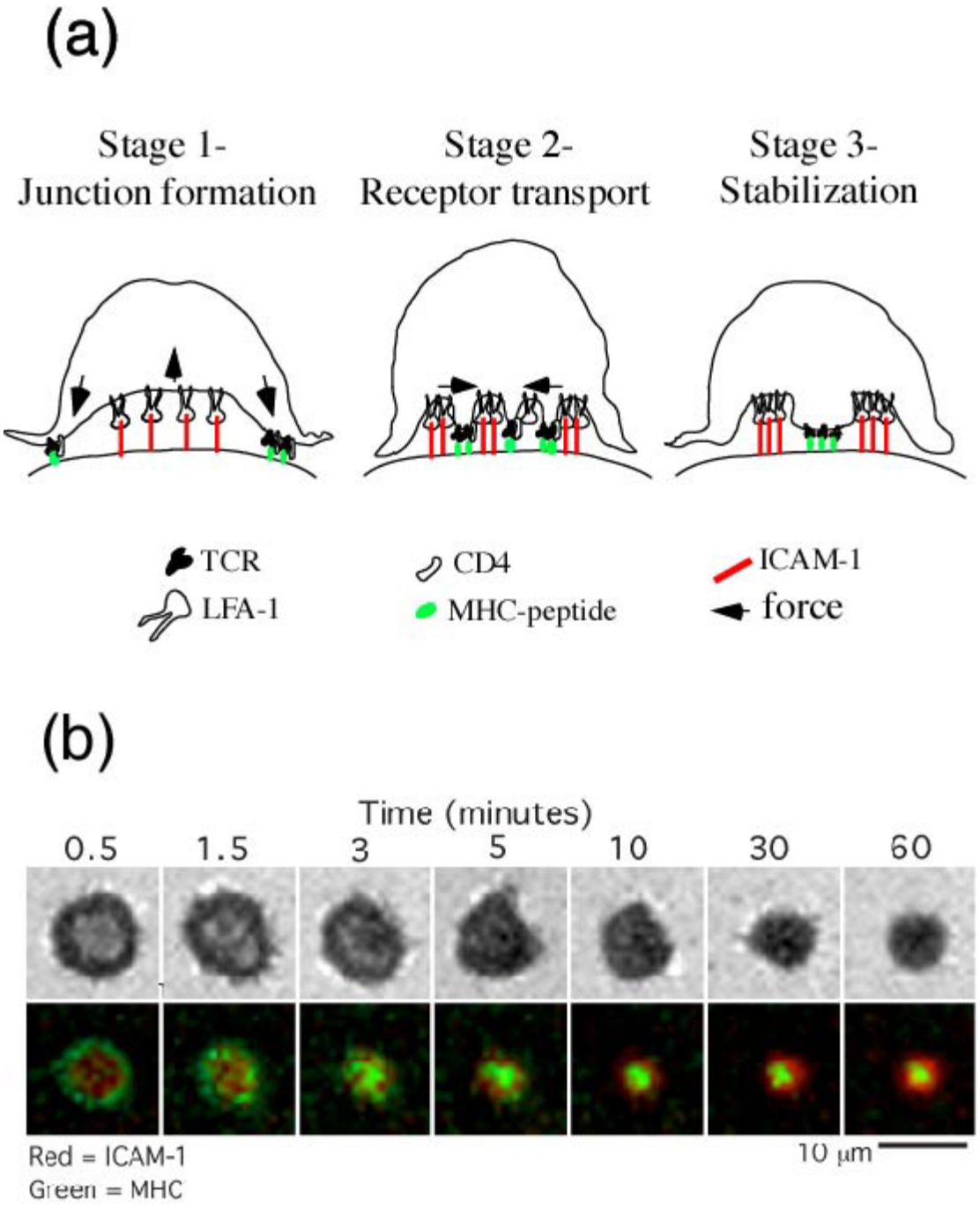

b
d





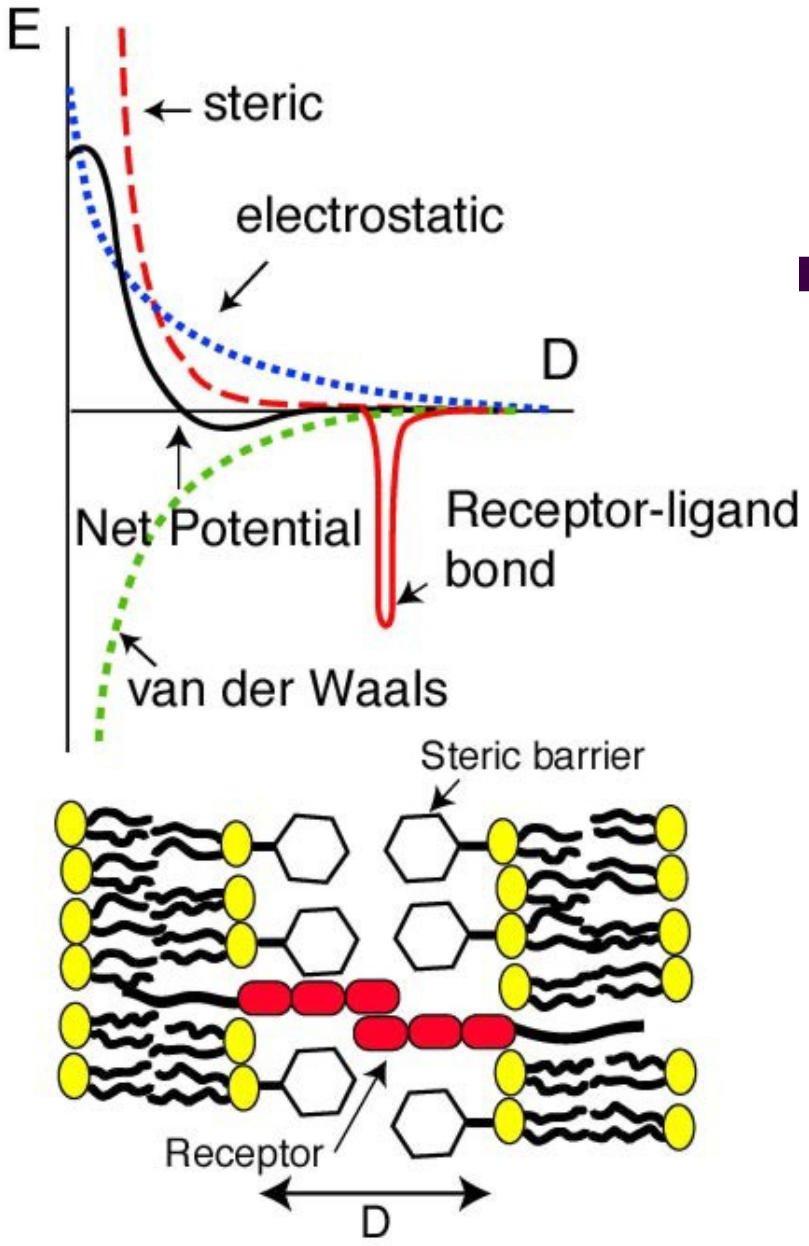
Many Adhesion Protein Structures Exhibit Multiple Tandem Repeats





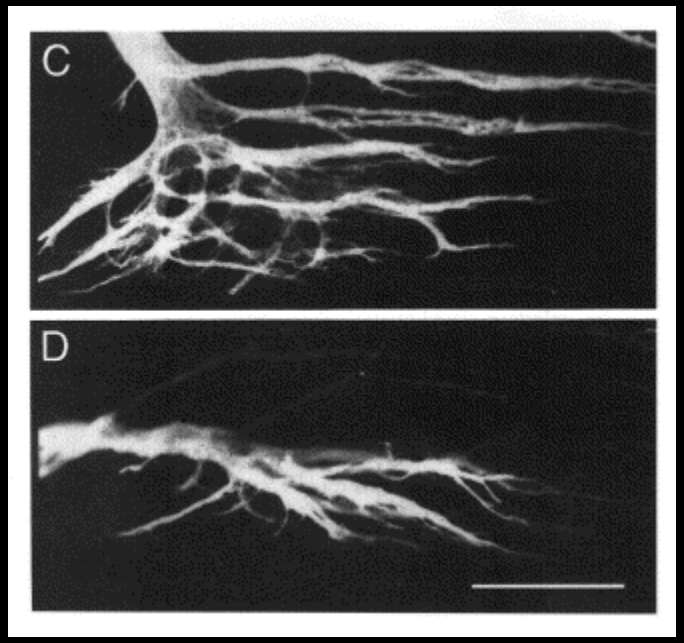
- Adhesion complex dimensions regulate intercellular space
- Protein size segregation drives the assembly and organization of adhesive junctions

Intercellular Interactions



- Intermembrane potentials are largely repulsive.
- How do proteins facilitate intercellular adhesion?
- How is adhesion regulated?

Neural Cell Adhesion Molecule (NCAM)

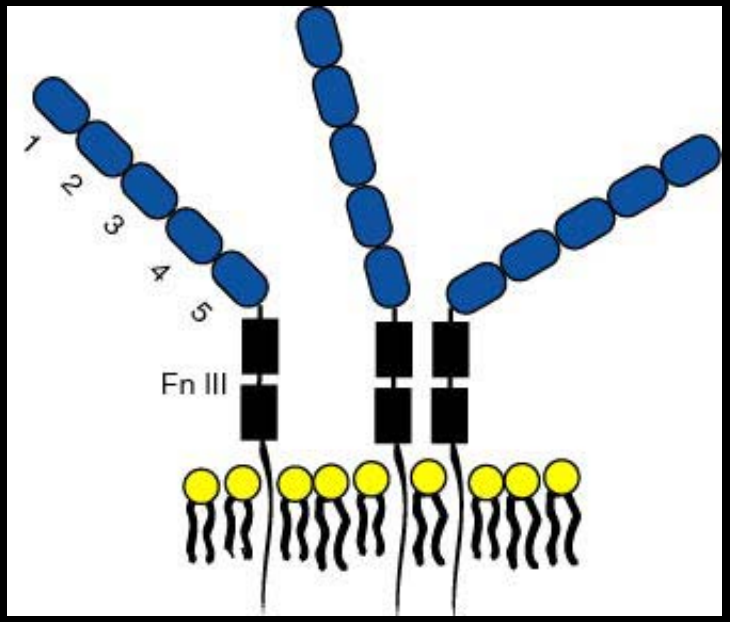


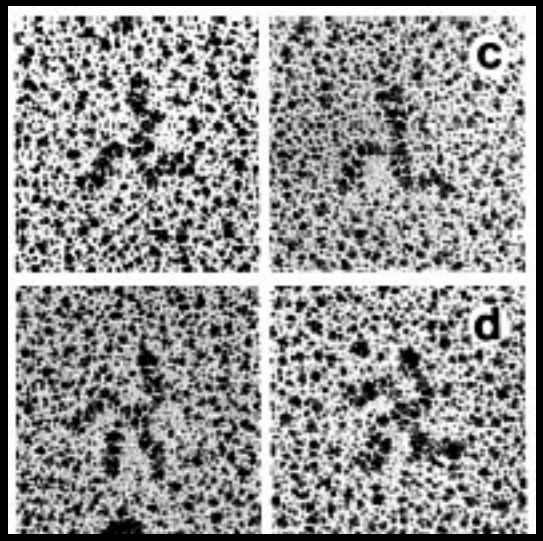
Landmesser et al. (1990) Neuron, 4, 655

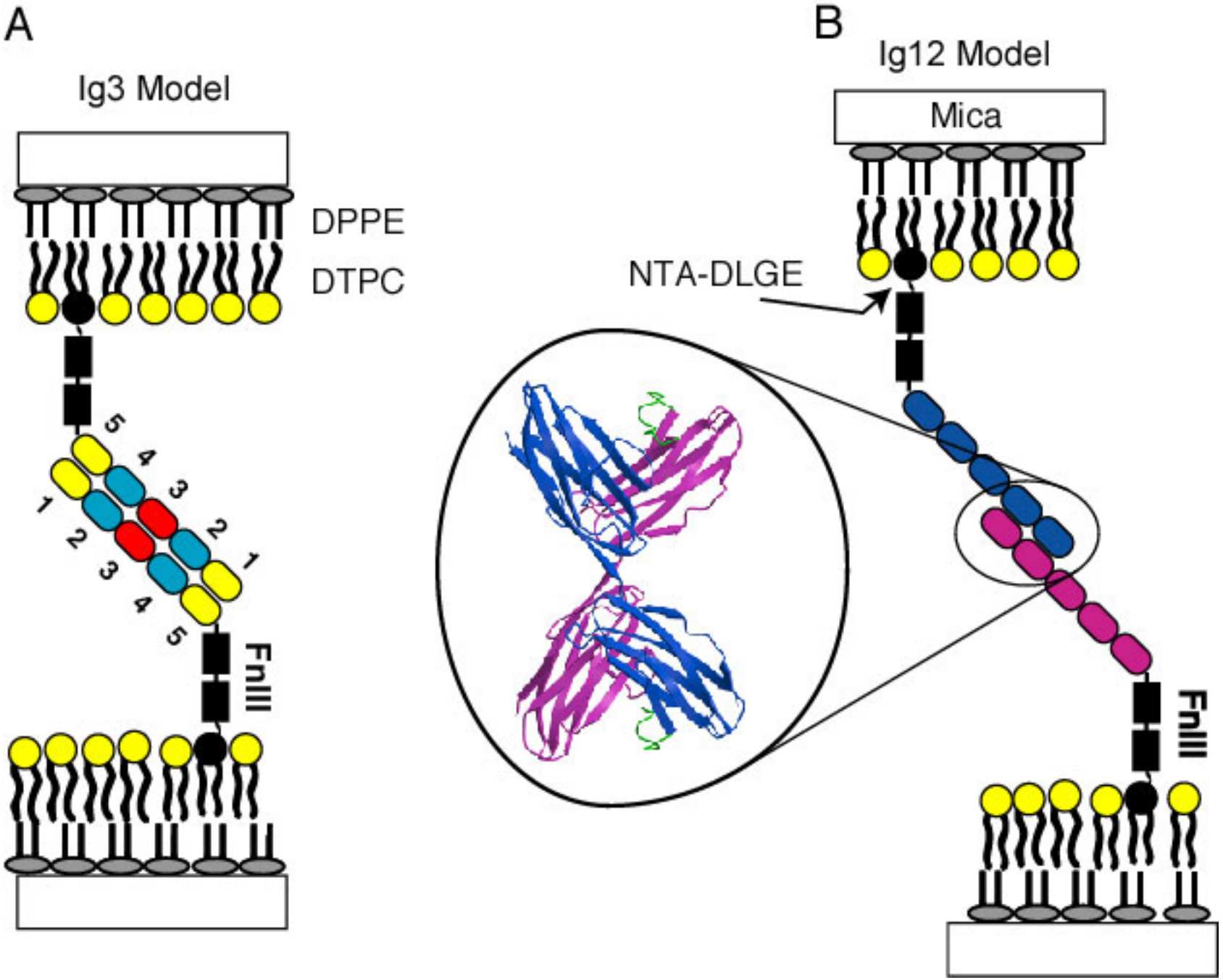
- NCAM regulates cell adhesion and signaling in the developing nervous system
- Post-translational modification alters NCAM function

Structural Features of NCAM

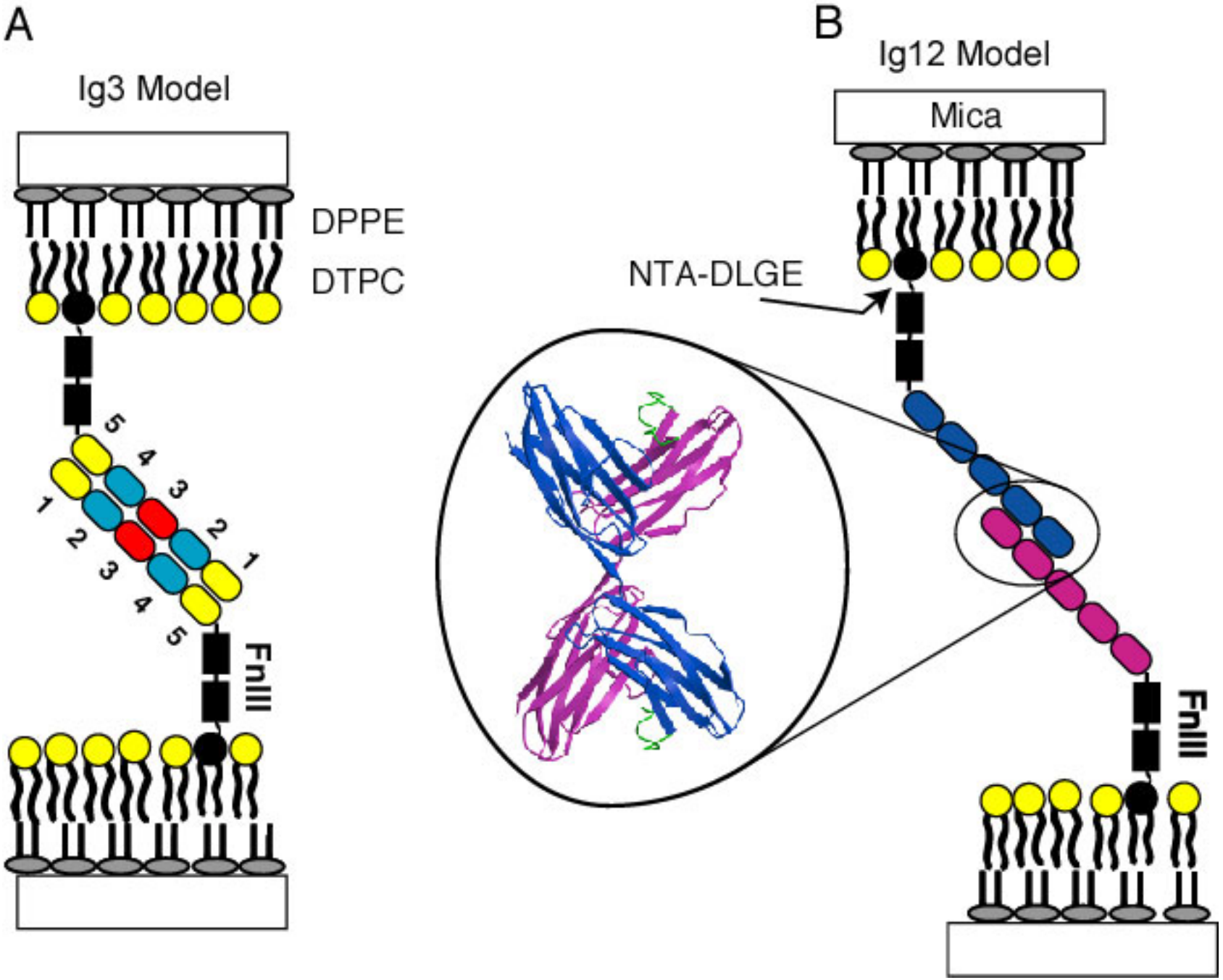
- NCAM consists of seven tandemly arranged domains
- A flexible hinge generates a bend angle of 98°±45°
- NCAM binds to NCAM proteins on adjacent cells



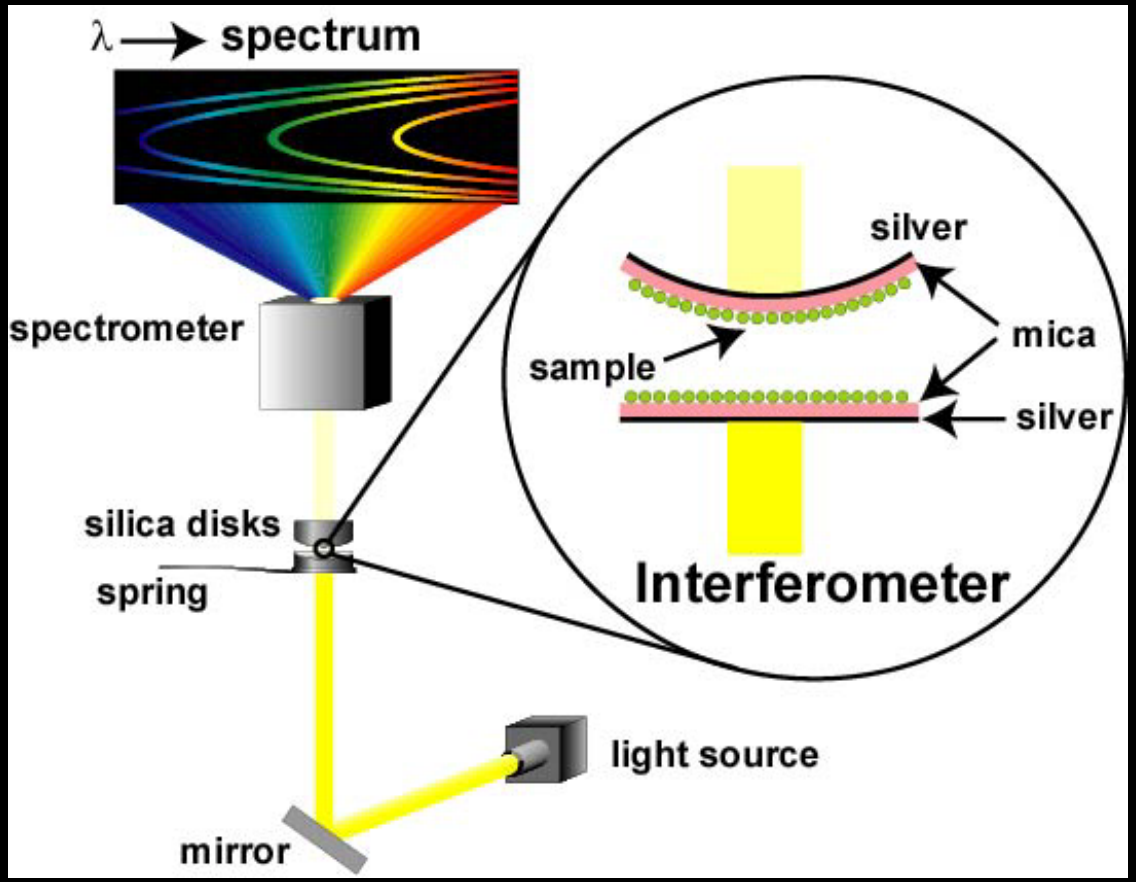



A
Ig3 Model
DPPE
DTPC
5
4
3
2
1
1
2
3
4
5
FnIII
B
Ig12 Model
Mica
NTA-DLGE
FnIII

Do Any of These Structures Represent the Adhesive Configuration?


A
Ig3 Model
DPPE
DTPC
5
4
3
2
1
1
2
3
4
5
FnIII
B
Ig12 Model
Mica
NTA-DLGE
FnIII

The Surface Force Apparatus Quantifies the Energy-Distance Profiles Between Two Surfaces



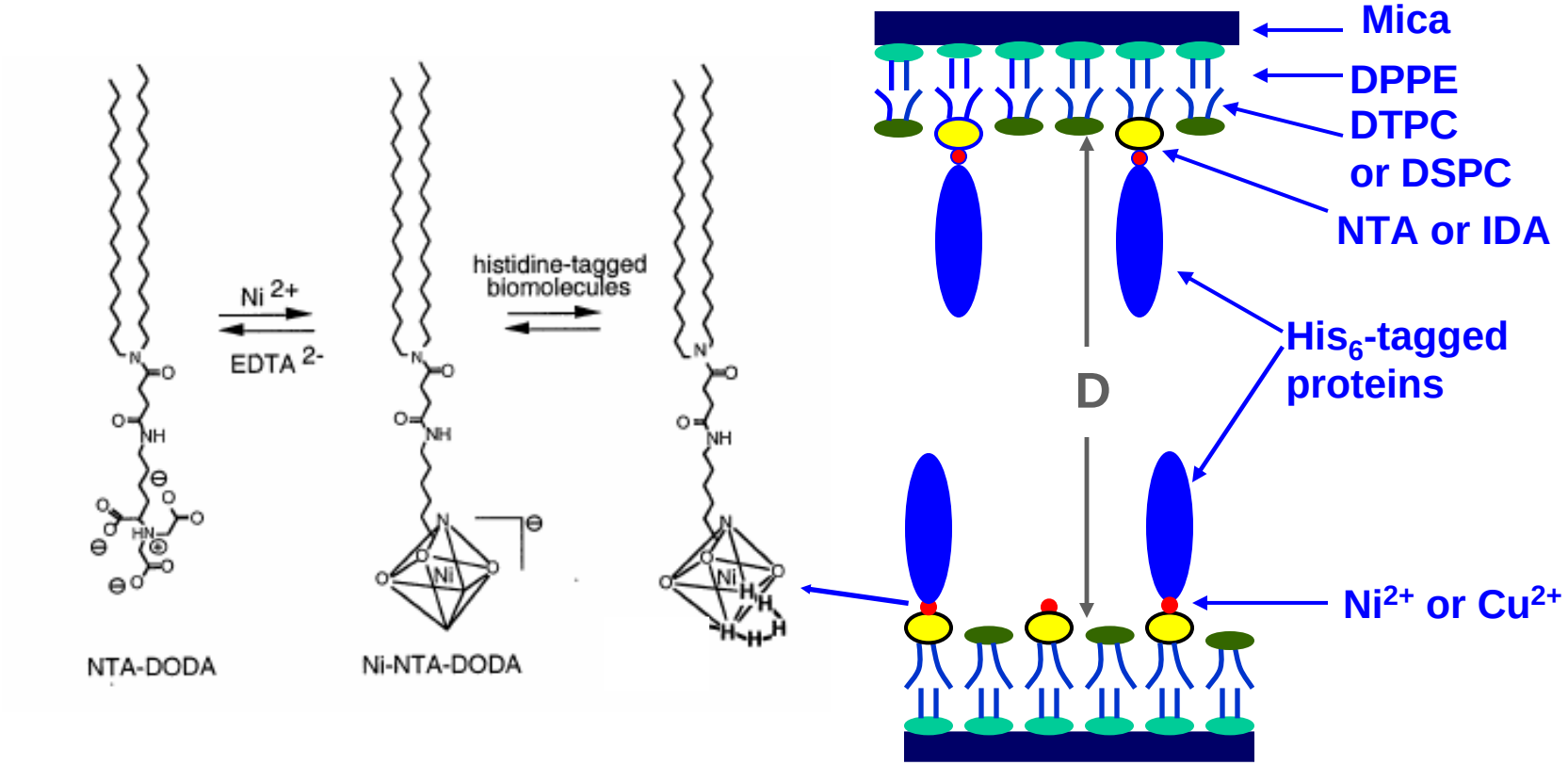
Bond energies of order kT

Absolute distances within ±1Å

Tabor and Winterton, Proc. R. Soc. A, **312**, 435 (1969)

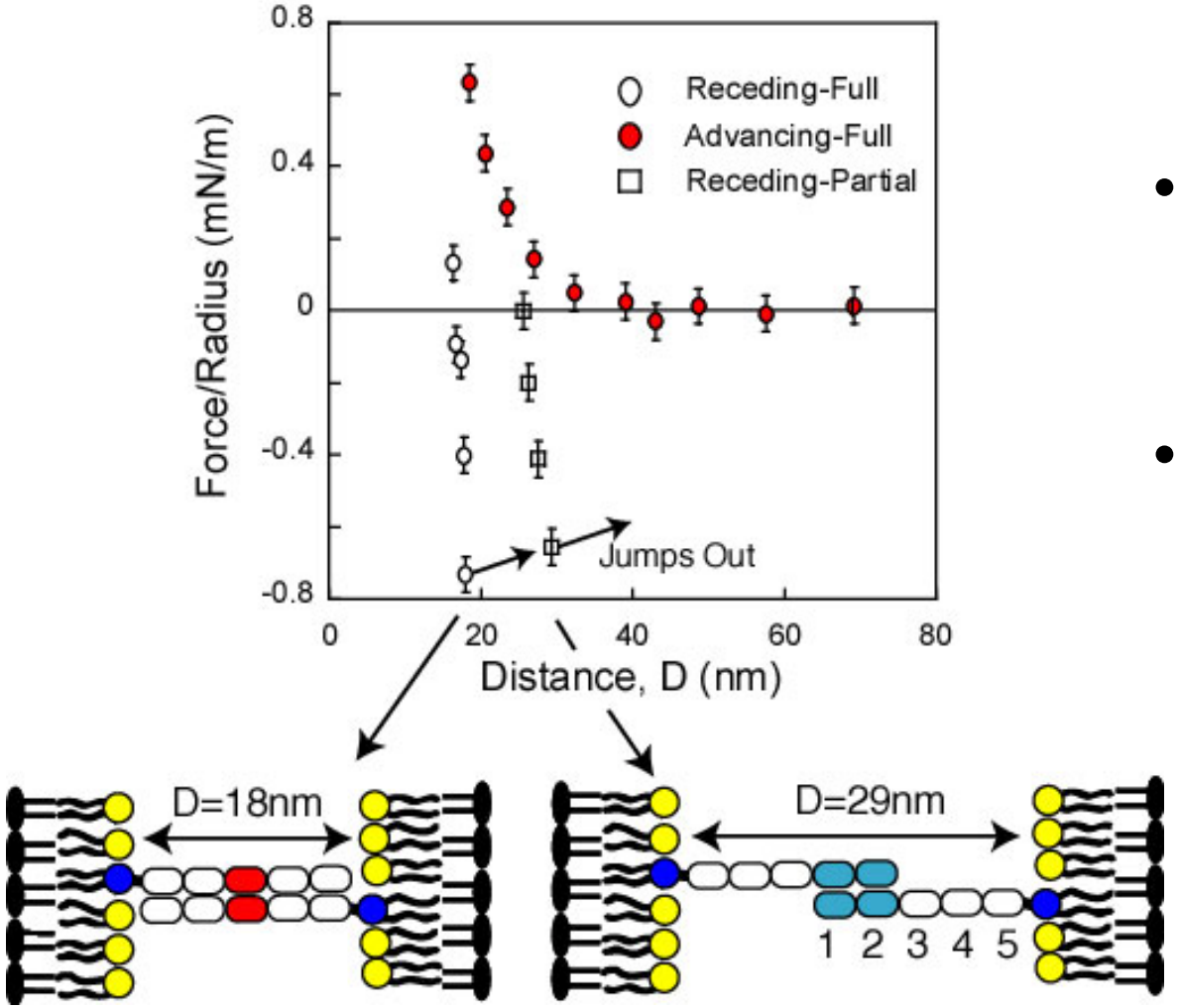
Israelachvili and Tabor, Nature, **236**, 106 (1972)

Building Cell Membrane Mimics

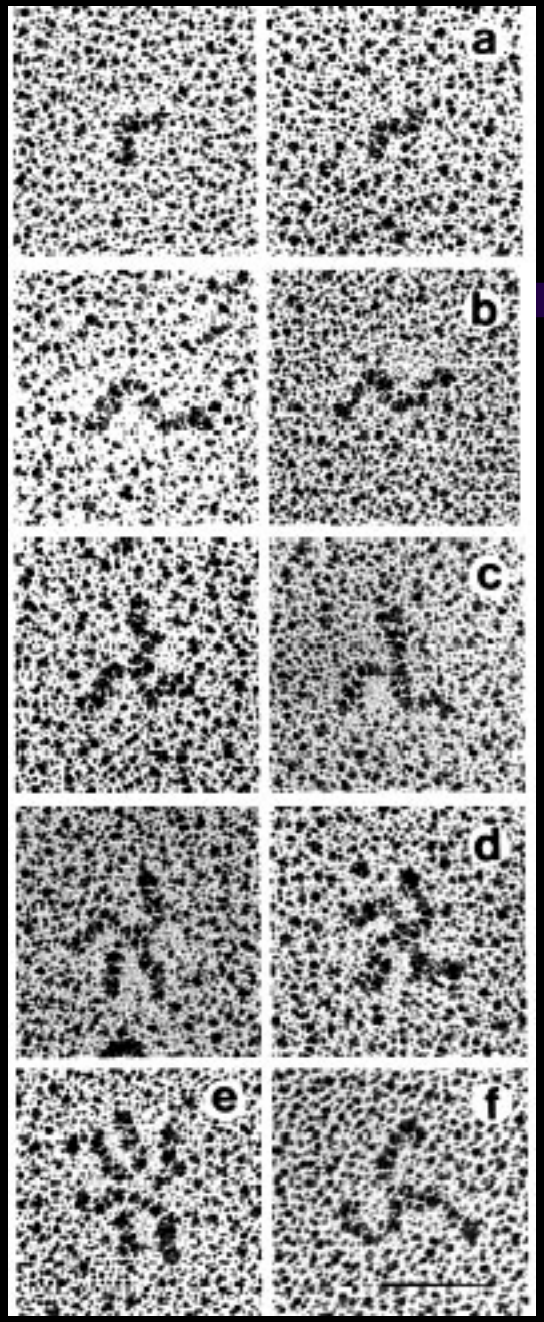


Dietrich et al (1995) PNAS 92:9014

Ig1-5 Forms Two Bound States



- Trans Ig12 Bond
- Trans Ig3 bond

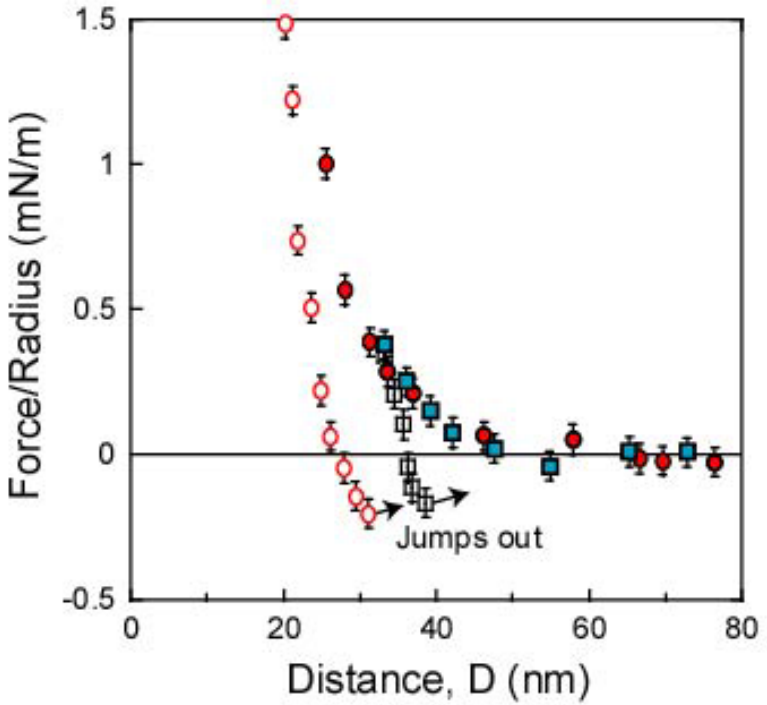


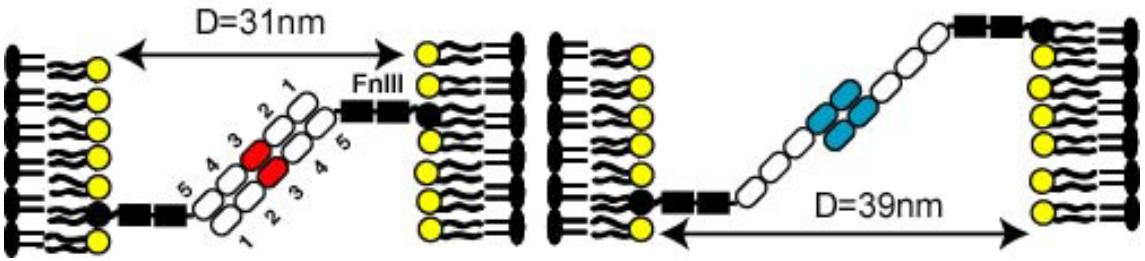
Electron Microscopy

Ig5-Fn III interdomain linker:

Chicken QADTPSSPSIDRVEPYSSTARV
Human QADTPSSPSIDQVEPYSSTAQV
Mouse QADTPSSPSIDRVEPYSSTAQV

Full Length NCAM Forms Two Bound States

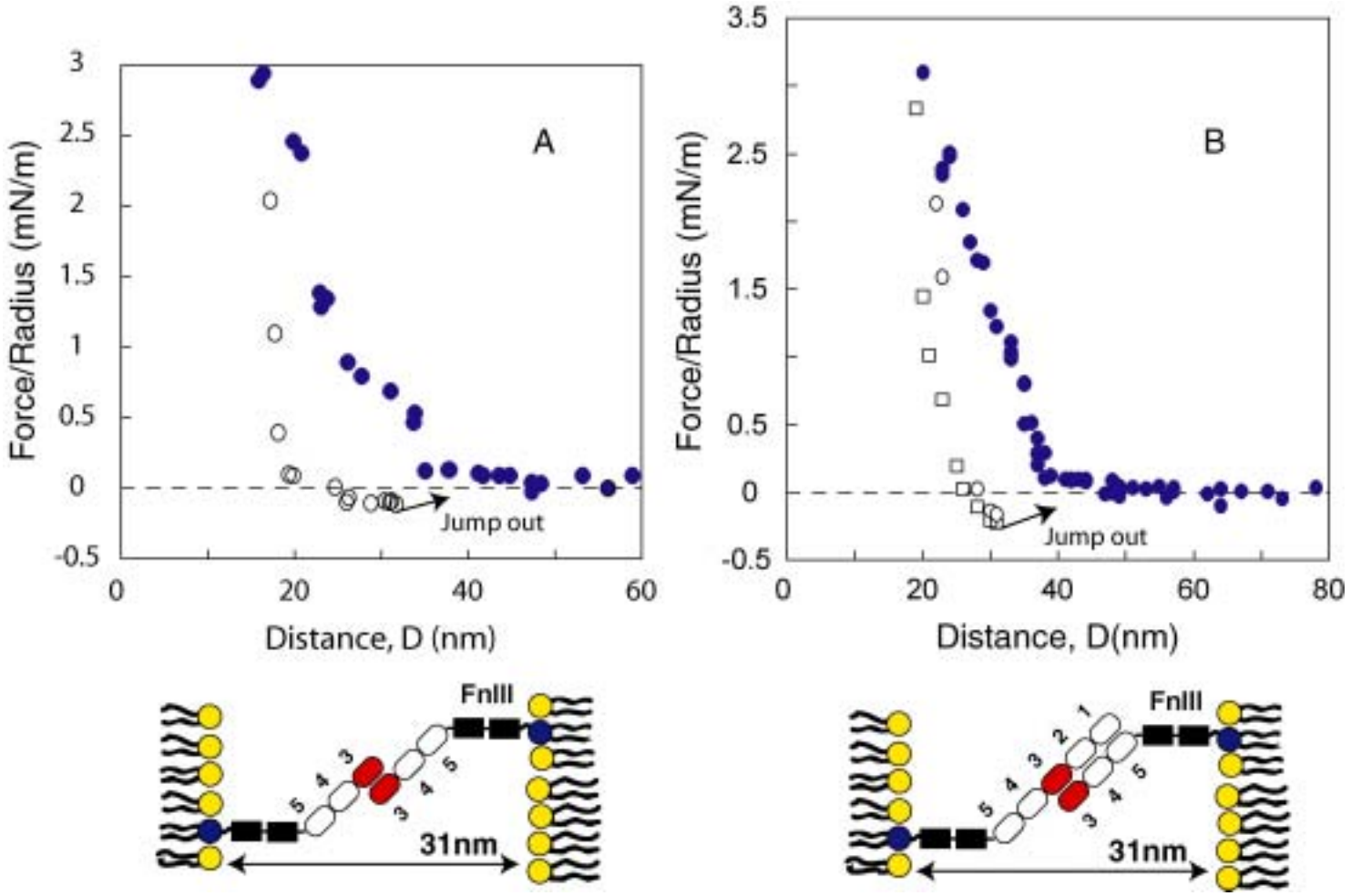




- Range of repulsion is consistent with a bent NCAM configuration.
- Hinge is likely at the Ig5-Fn III junction.

Which Domains Mediate Adhesion?

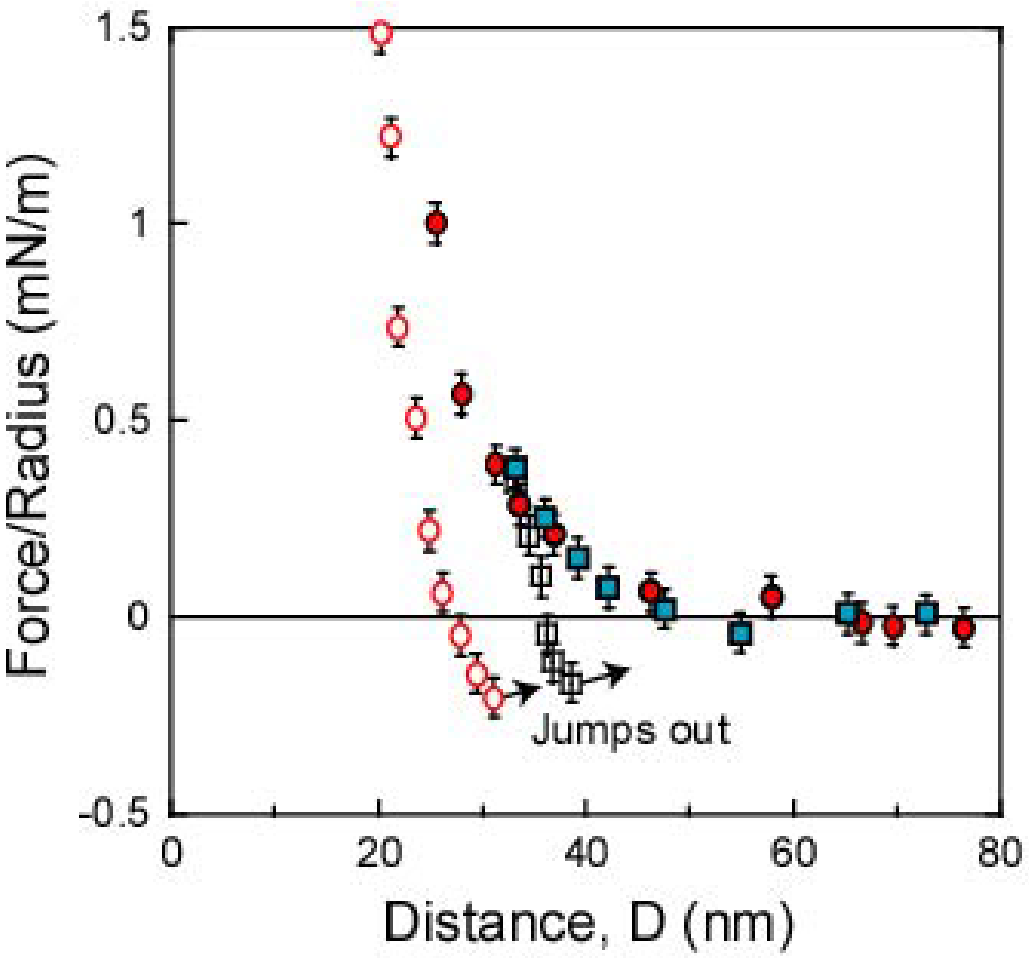
Removing Ig12 Removes only the Outer Trans Bond

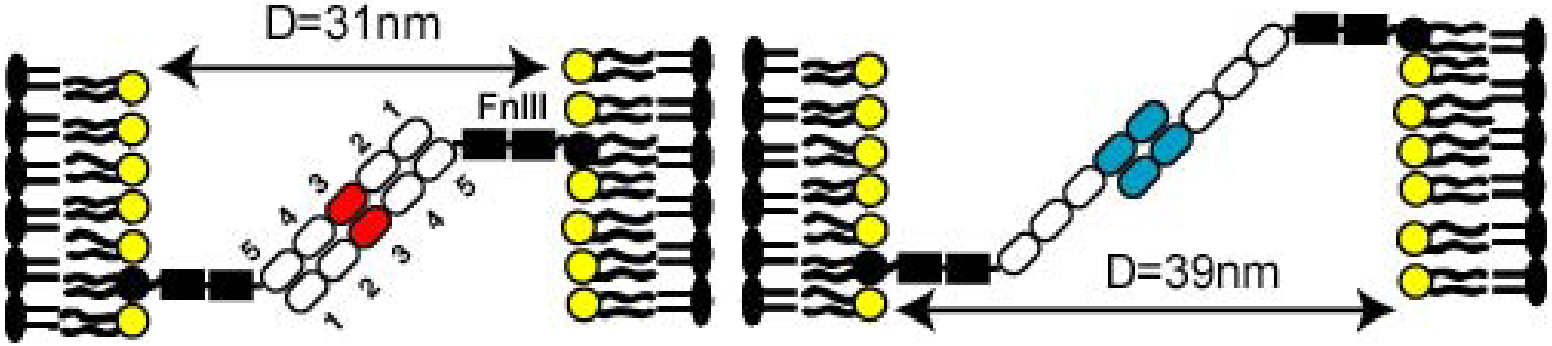


SPR Analysis Supports Two-State Binding Model

K_d (x 10^7) Between NCAM Fragments

	NCAM	ΔIg3	ΔIg12
NCAM	4±1	3.4±0.4	1.1±0.3
ΔIg3		1.0±0.6	-----
ΔIg12			1.7±0.3

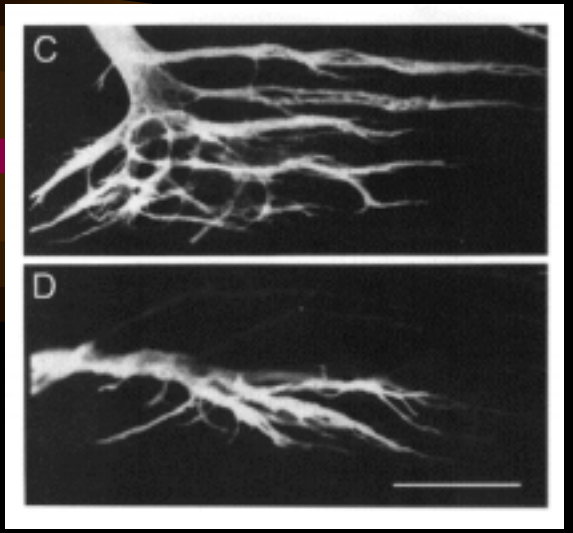

Force/Radius (mN/m)
1.5
1
0.5
0
-0.5
0
20
40
60
80
Distance, D (nm)
Jumps out

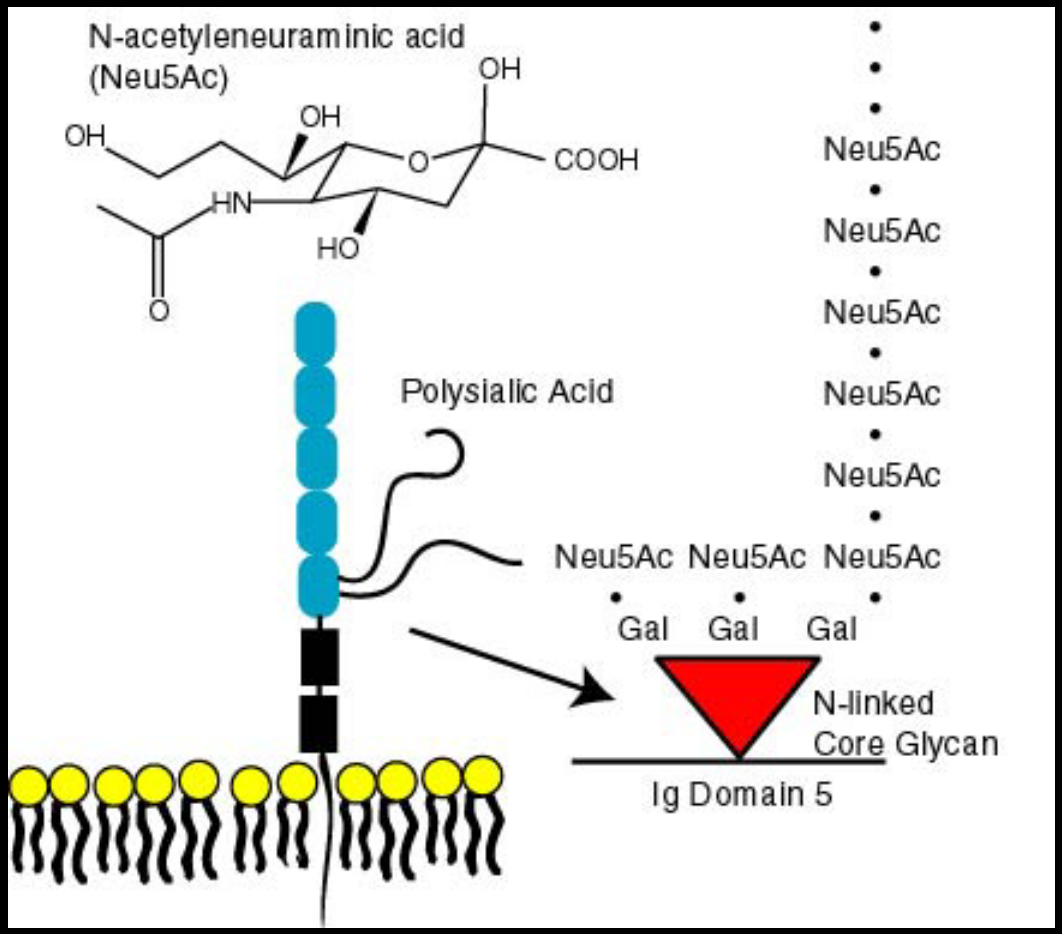

D=31nm
FnIII
D=39nm

Conclusions I:

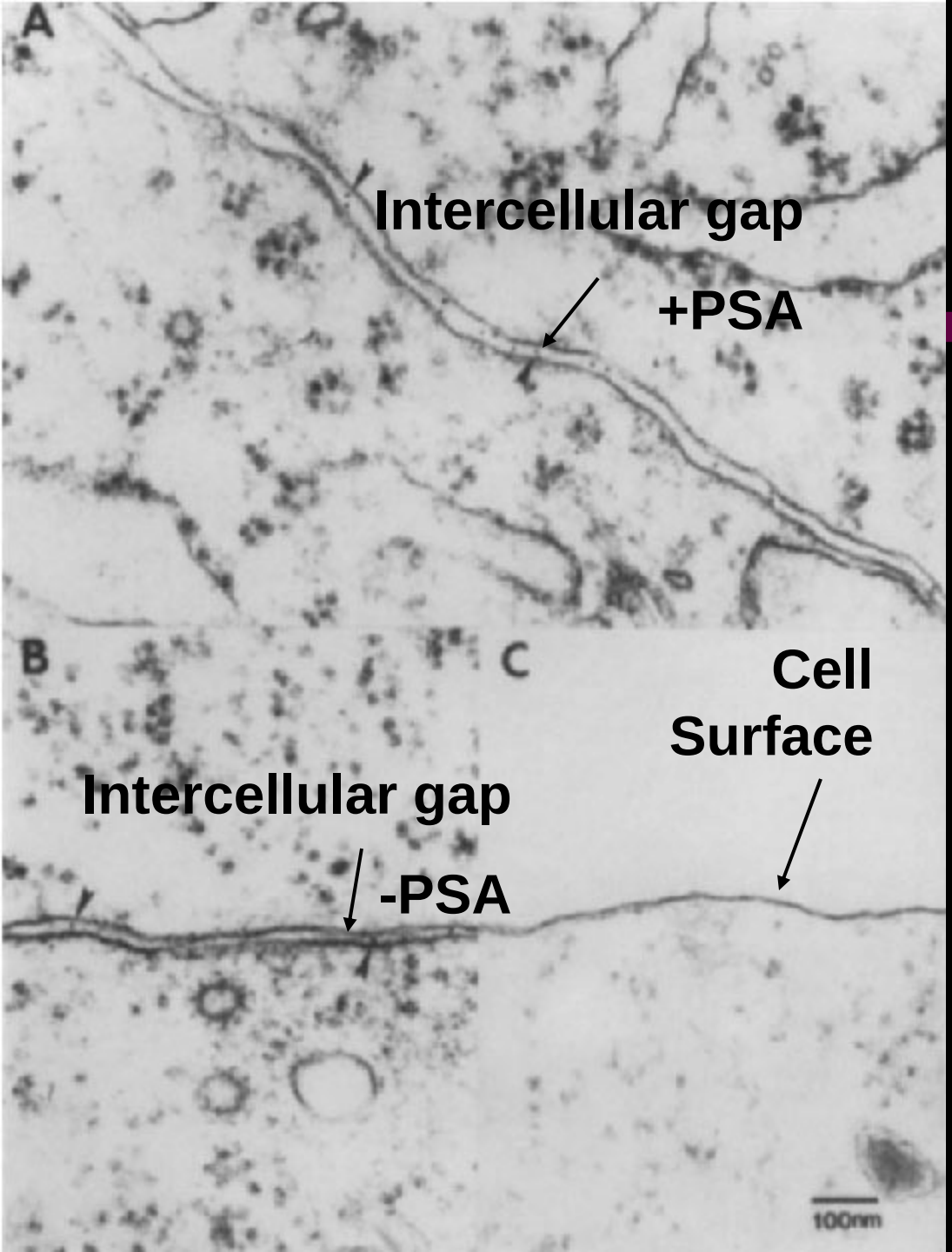
- NCAM forms two bound states
- Both Ig12 and Ig3 domains mediate *trans* adhesion
- Force measurements discriminated between different models

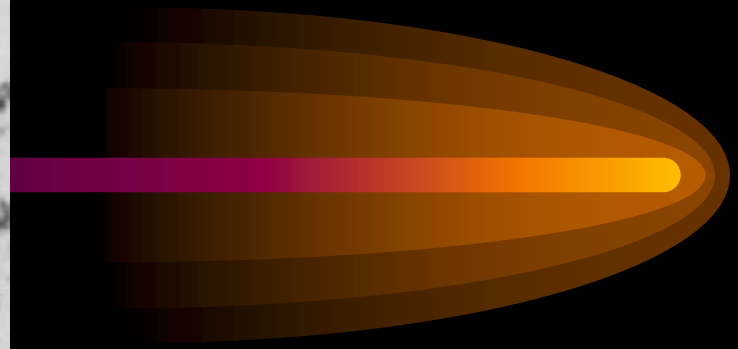
Polysialic Acid Modification Alters NCAM Function





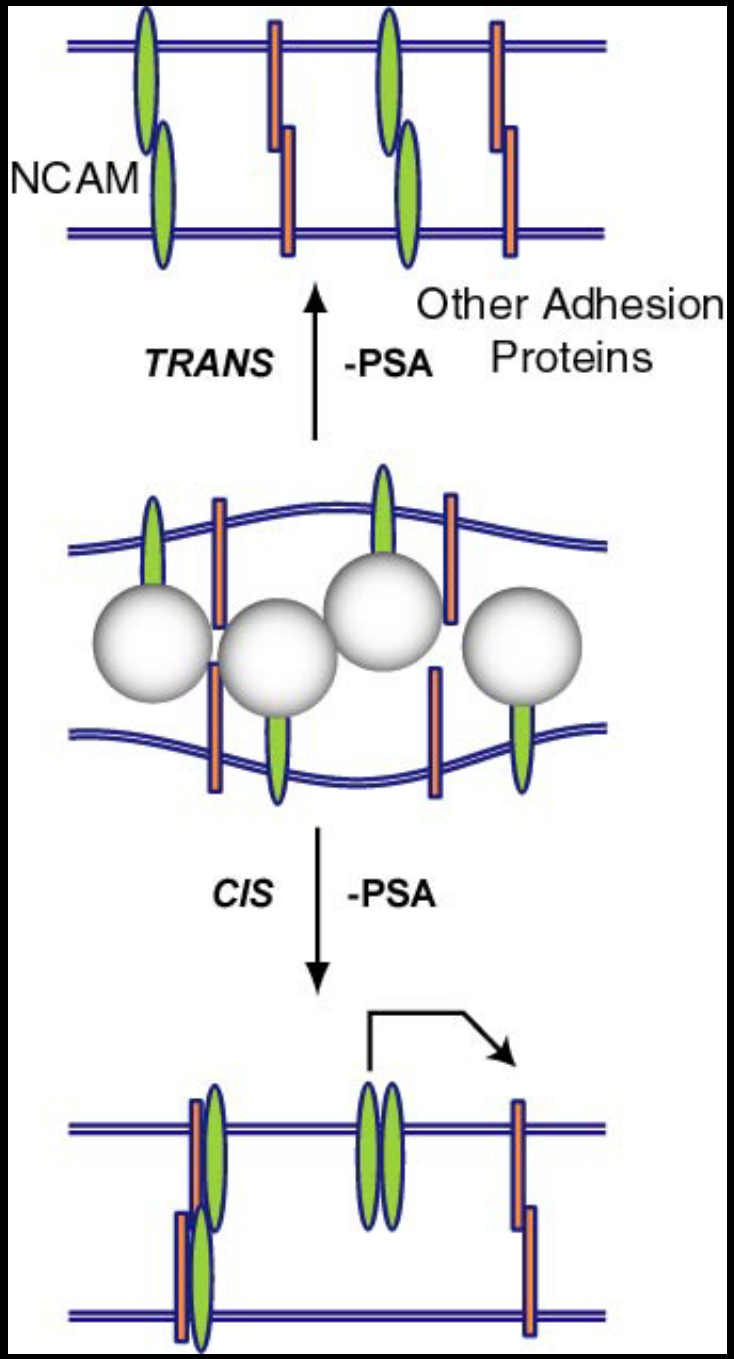
- PSA increases neural plasticity
- PSA expression is linked to tumor progression





PSA Regulates Intercellular Spacing

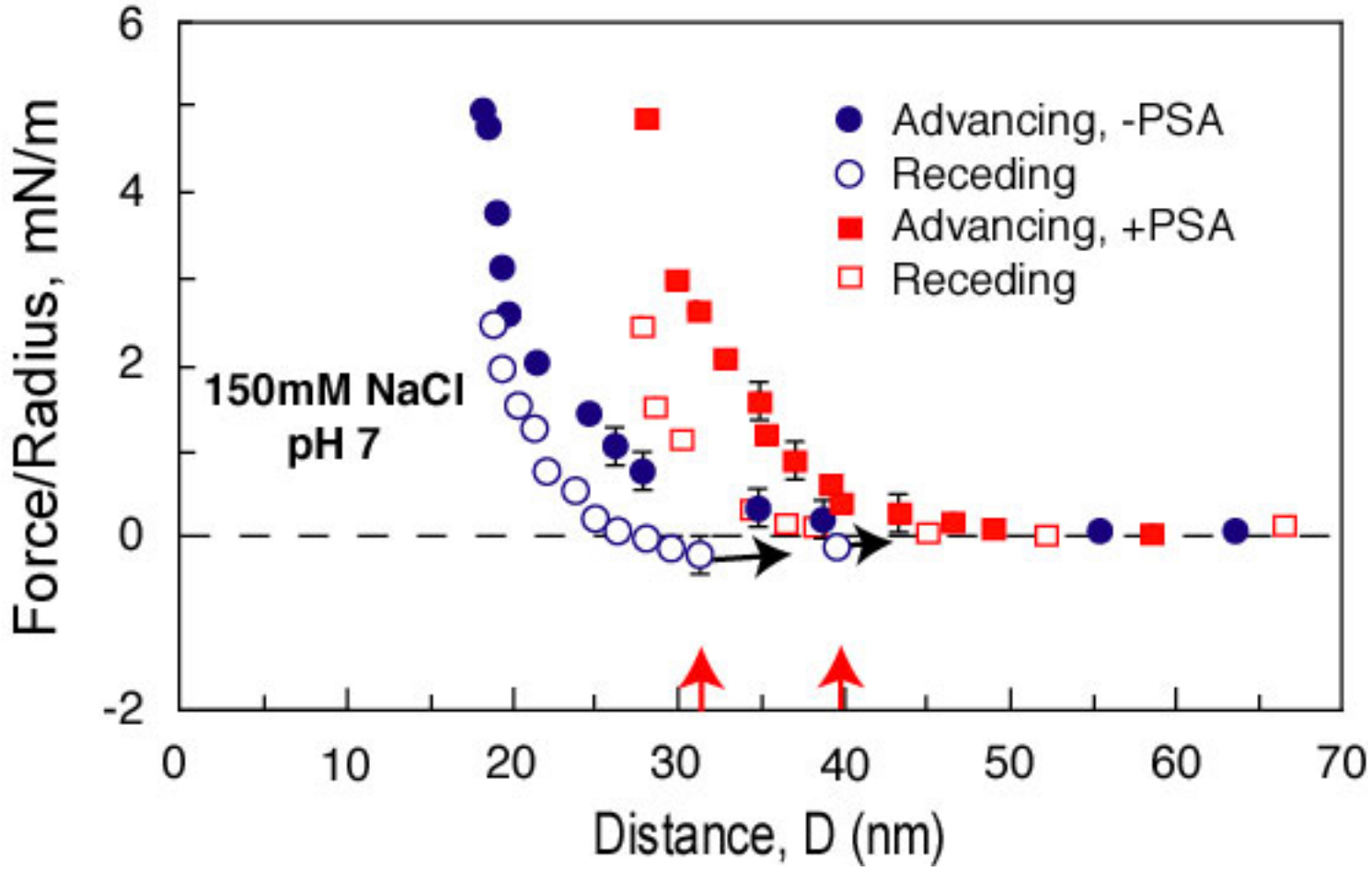
Yang et al. (1992) J. Cell. Biol., **116**, 1487-1496

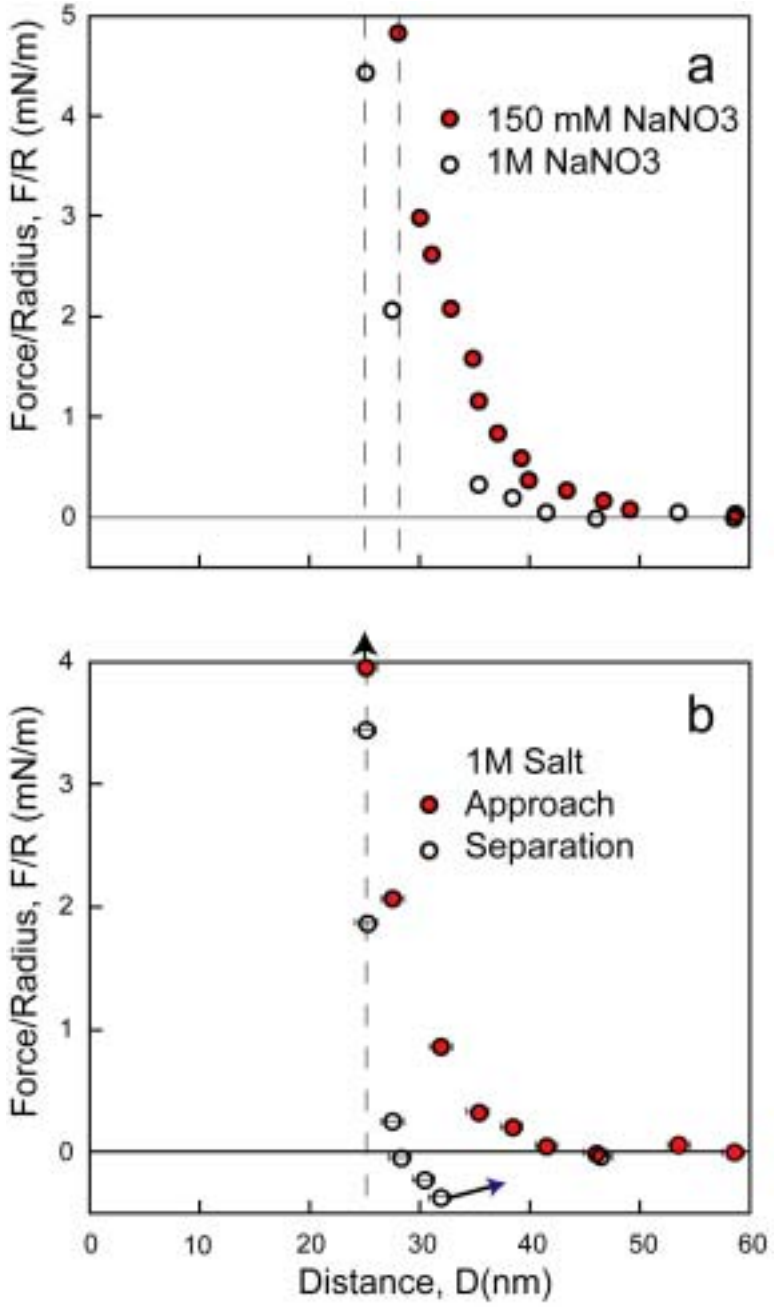


PSA Affects the Adhesive Function of Several Proteins, In Addition to NCAM

- PSA could increase nonspecific repulsion between membranes
- PSA may increase repulsion between proteins on the same membrane

PSA Abolishes Both the Inner and Outer Adhesive NCAM Bonds



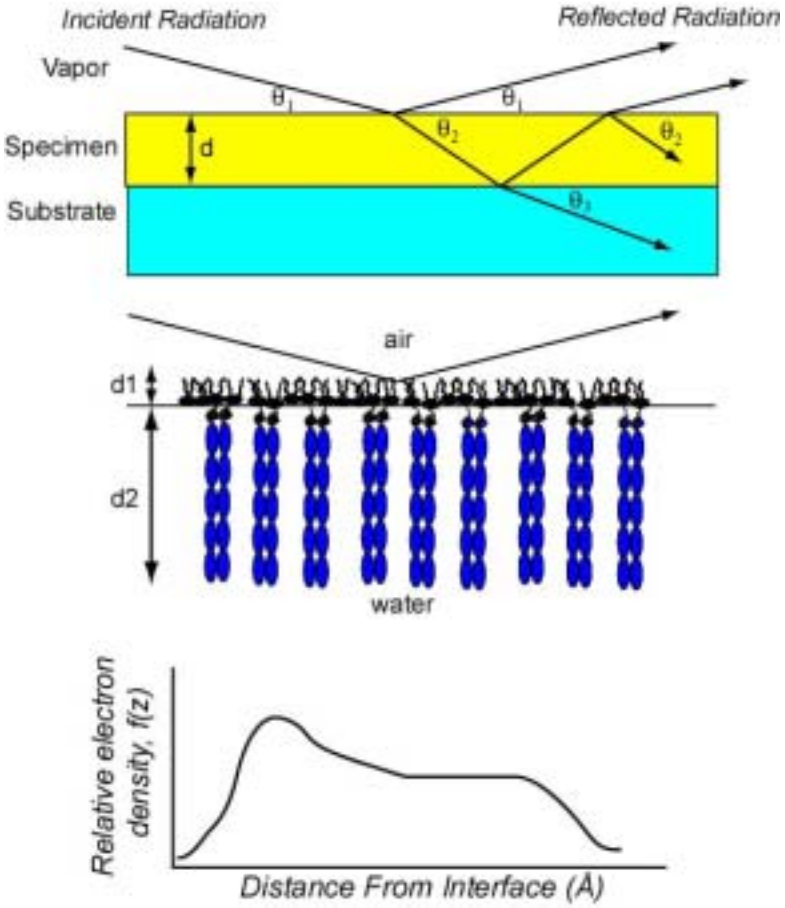


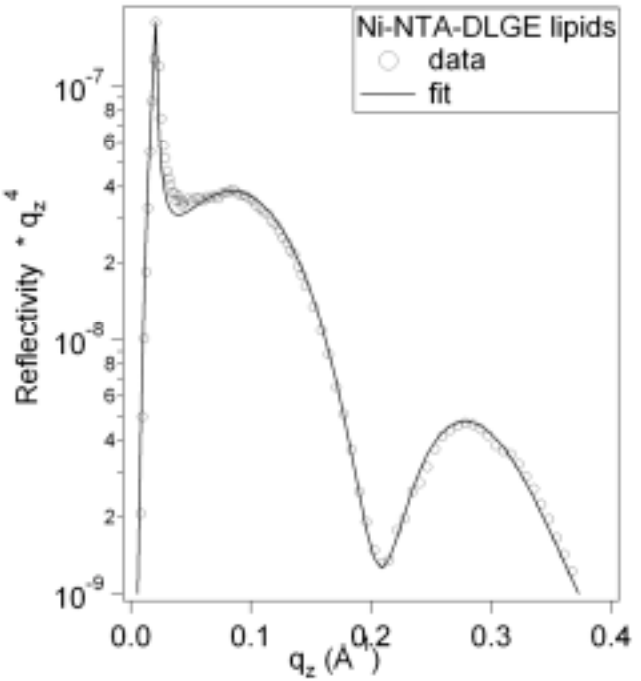
- Range of the steric repulsion dependends on the ionic strength

- Decrease in the repulsion corresponds with an increase in adhesion

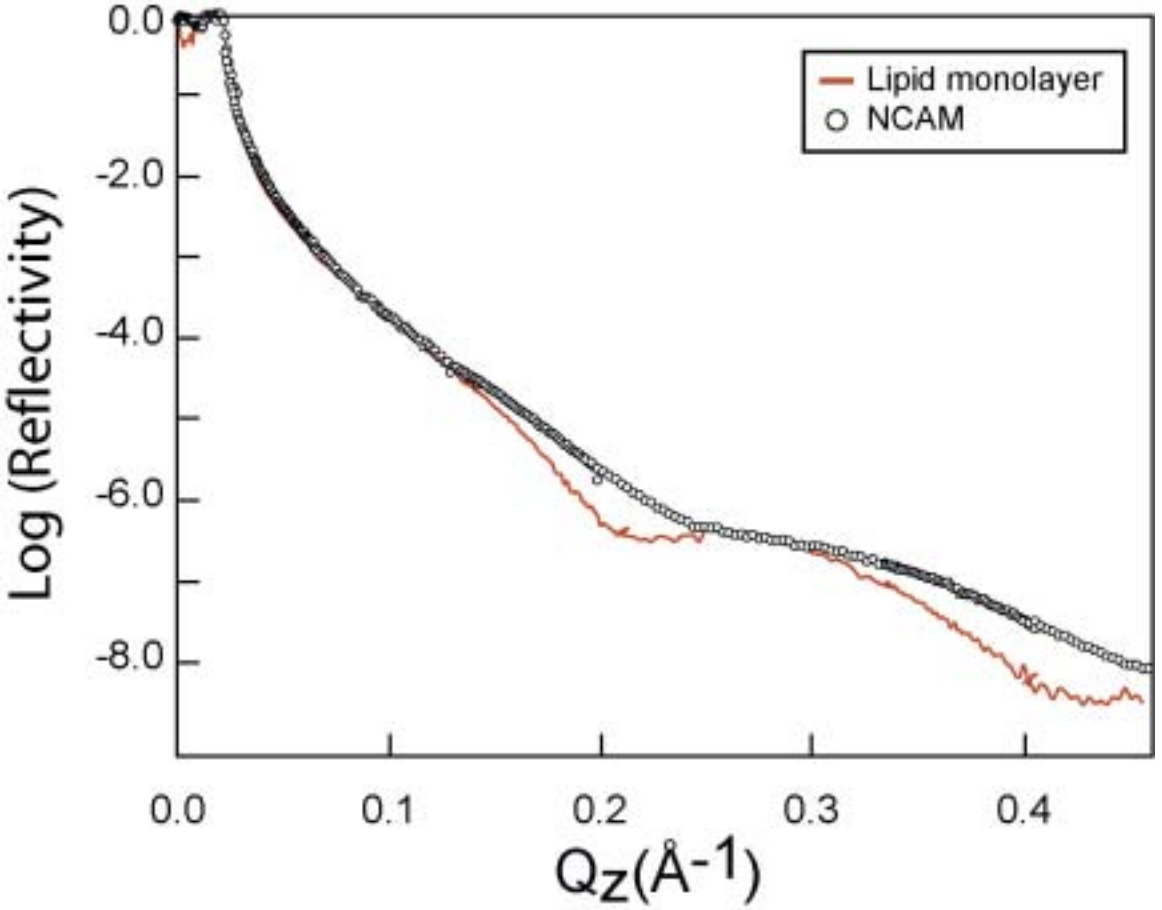
How Does the Polymer Extension Compare with the NCAM Dimensions?

Structural Studies of Adhesion Protein Assemblies

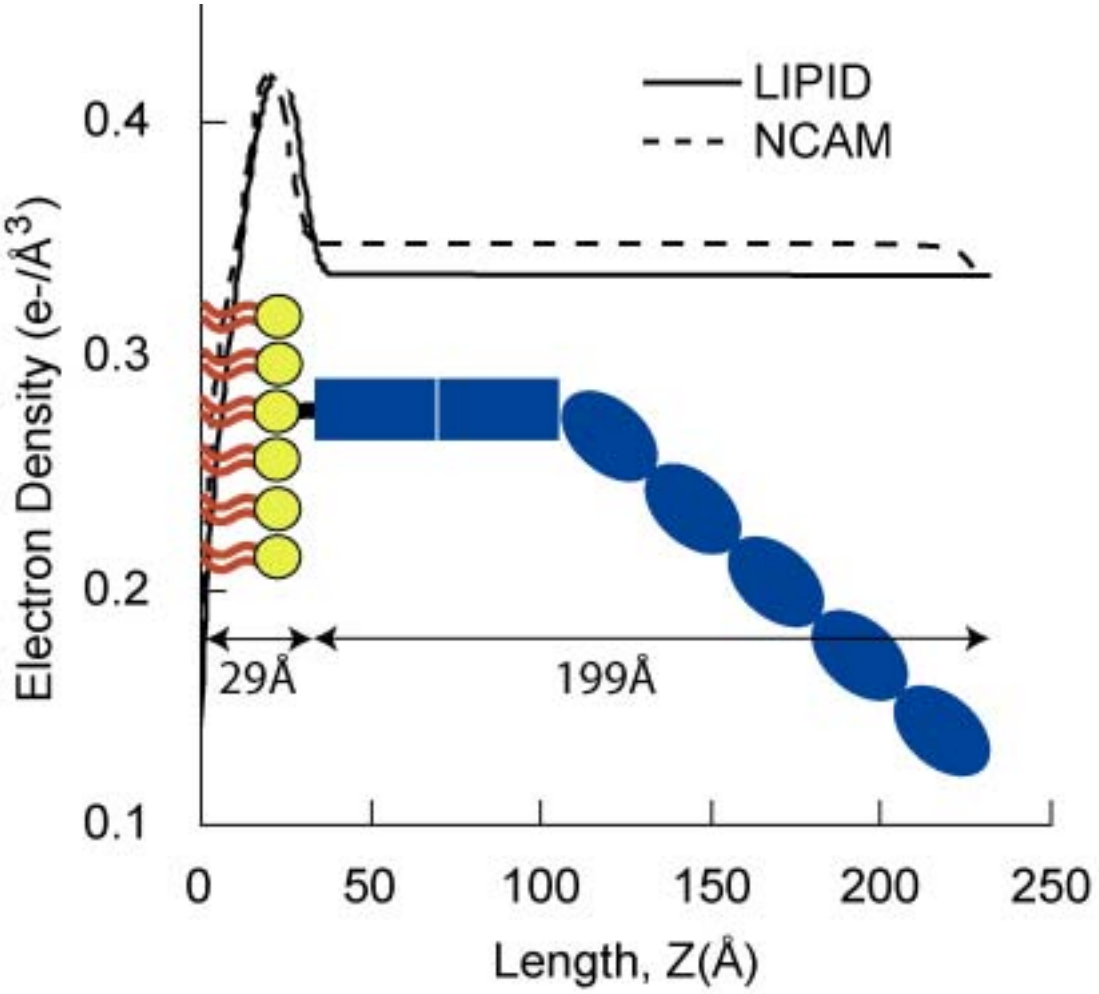


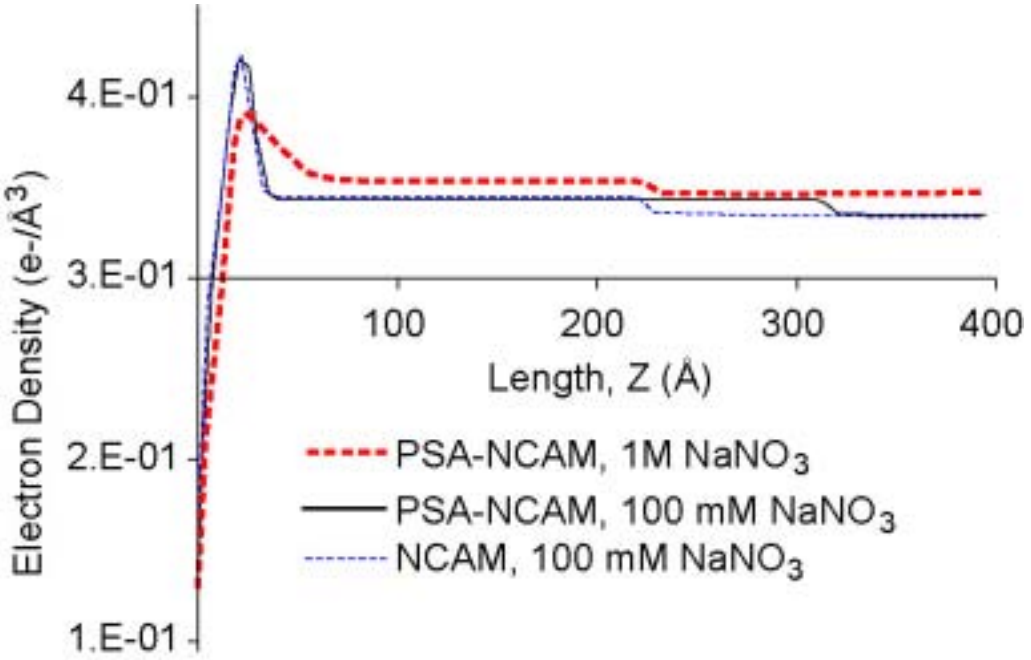


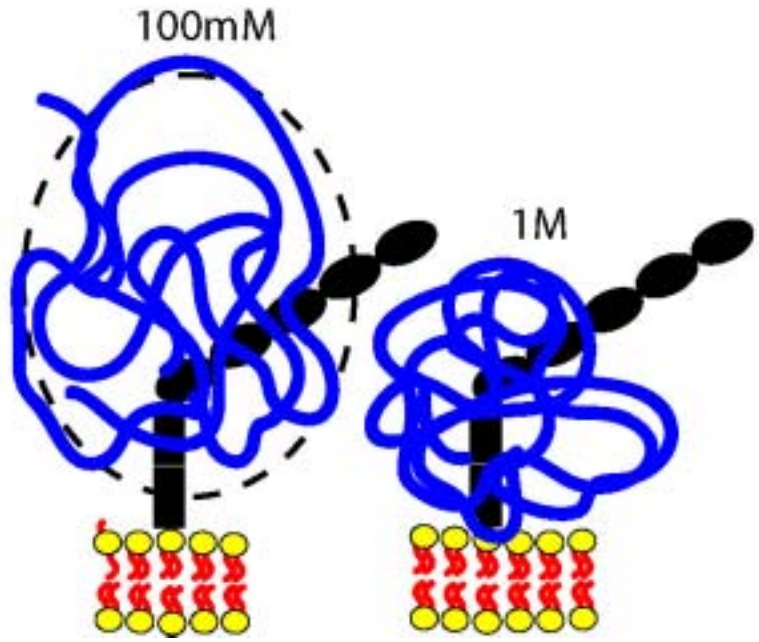
X-Ray Reflectivity Profile of NCAM Monolayer



Model Interpretation of the NCAM Reflectivity Profile







The PSA-NCAM Thickness is Ionic Strength Dependent

Conclusions II:

- PSA increases the range and magnitude of the *nonspecific* intermembrane repulsion
- Reflectivity measurements show that PSA extends beyond the adhesive protein domains
- Steric repulsion model validated

Conclusions

Distance matters!

*Force measurements reveal relationships between protein architecture and function**

*Leckband and Israelachvili (2001)

Quart. Rev. Biophys., **34**, 105-267

Acknowledgements

University of Illinois

- Colin Johnson

ESRF-Grenoble (ID10)

- Jean Francois Legrand
- Oleg Konovalov

ILL-Grenoble

- Giovanna Fragneto

Support $$

- NIH RO1 GM33986

Sloan Kettering Cancer Institute

- Claire Perrin-Tricaud
- Ichiro Fujimoto
- Urs Rutishauser*