Protein Interactions with Lipid Membranes by Neutron and X-ray Reflectivity and Grazing Incidence X-ray Diffraction

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Outline

1. Introduction to the methods
2. Model system (relevant to nanoscience)
3. Other systems - new opportunities with SNS

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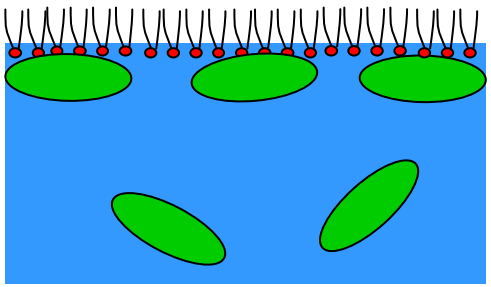
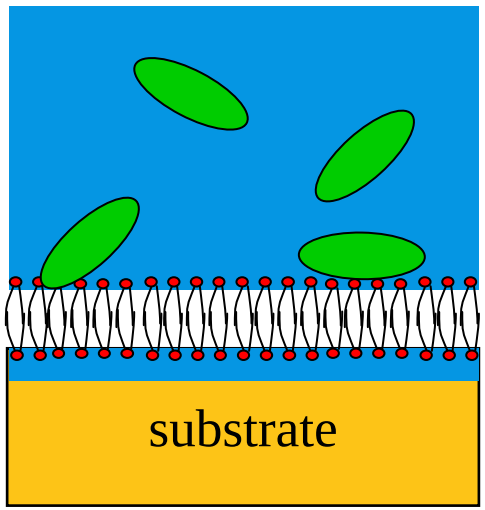
1. Introduction

Biomimetic membrane platforms used in scattering studies

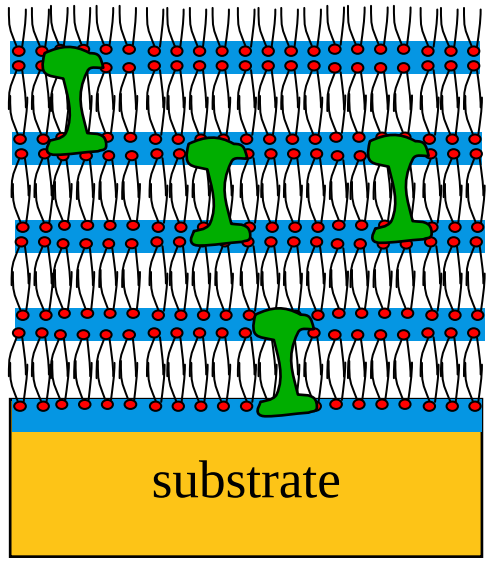
1. Langmuir monolayers



2. Supported bilayers



3. Hydrated stacks of bilayers



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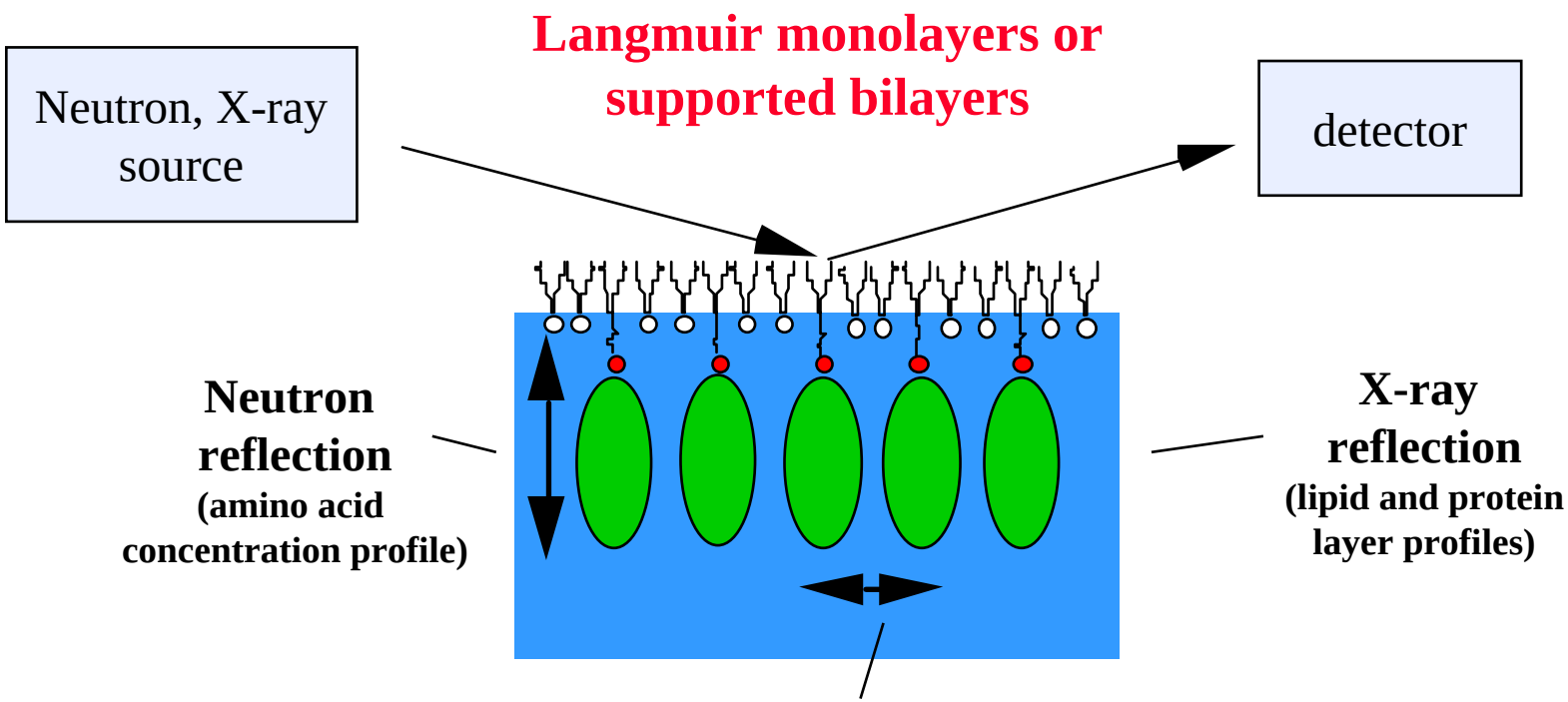
Introduction



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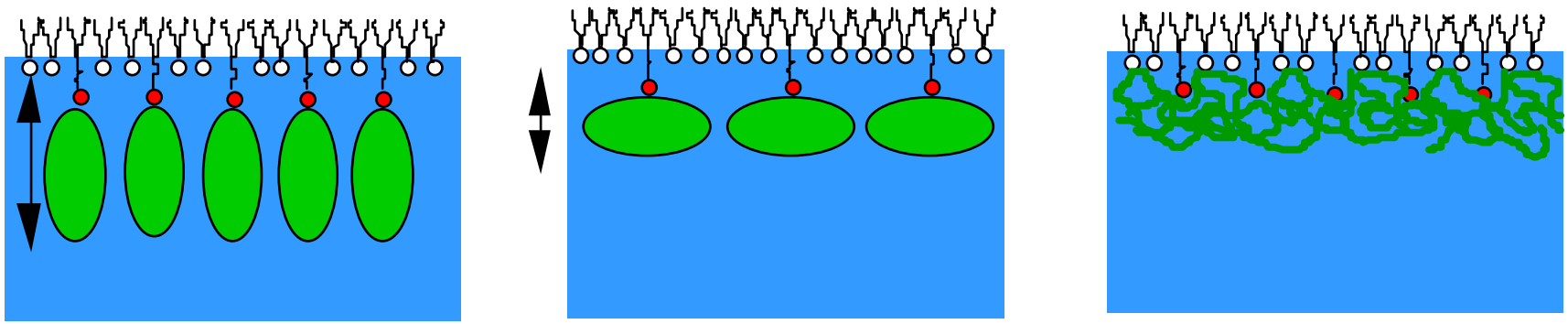
Neutron and X-ray reflection

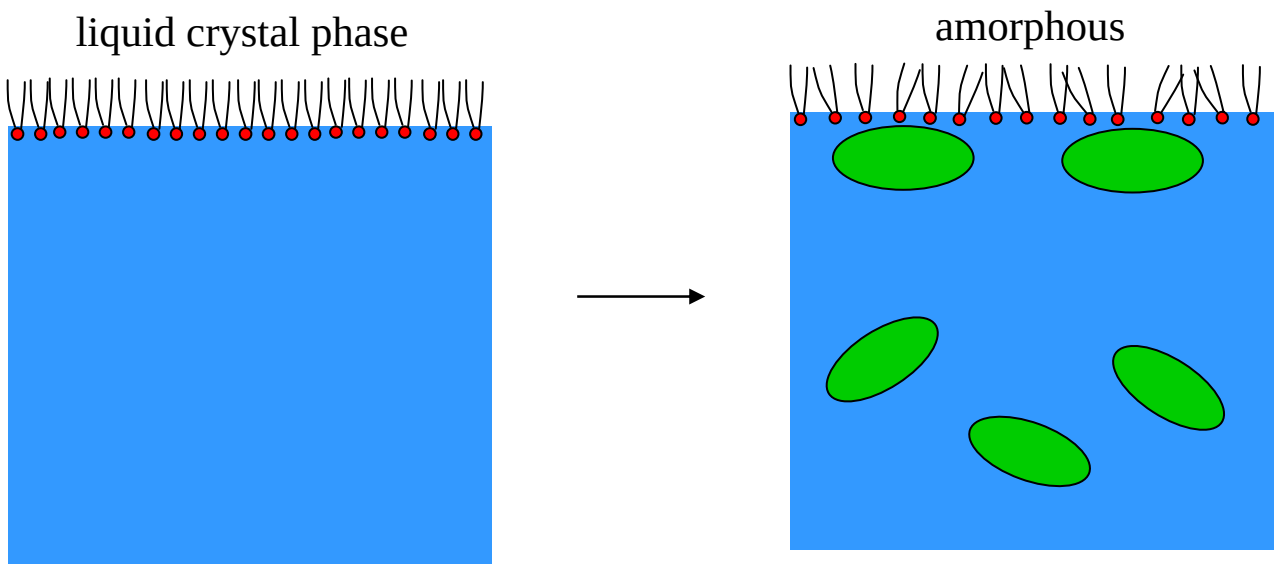
Probe amino acid segment profile

Insight into protein orientation and conformation



Grazing incidence X-ray diffraction

Probes in-plane correlations, such as crystallinity



When does protein binding impact lipid phase behavior?

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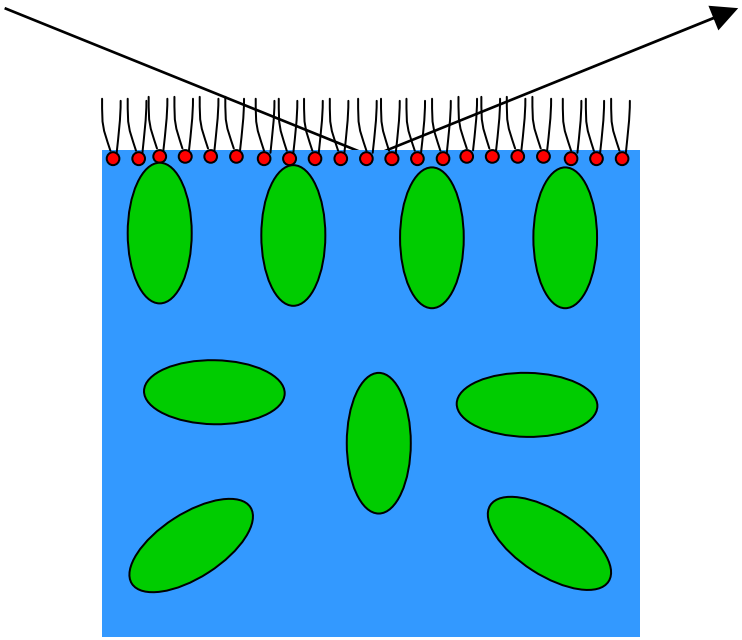
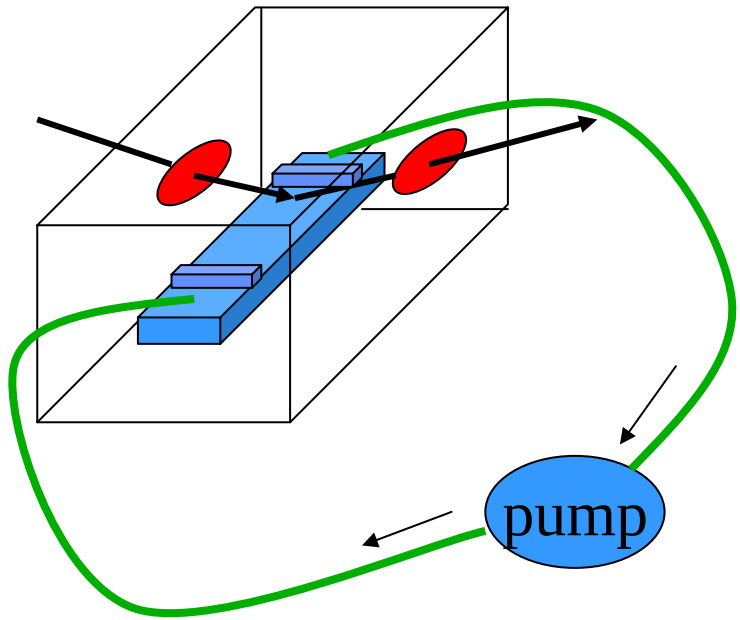
protein adsorption to lipid monolayers



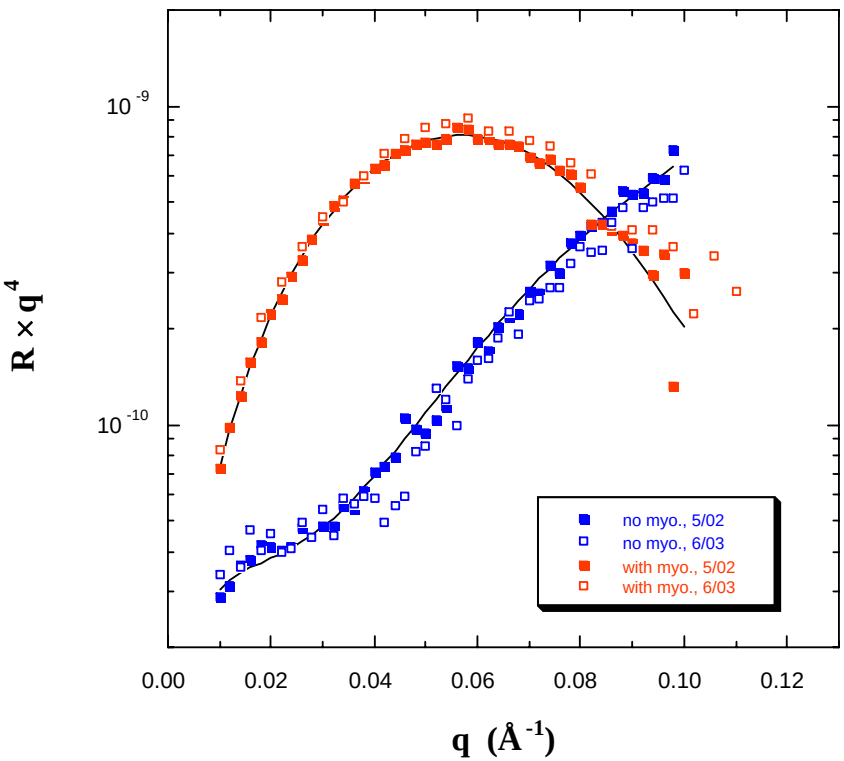
neutron or
X-ray beam



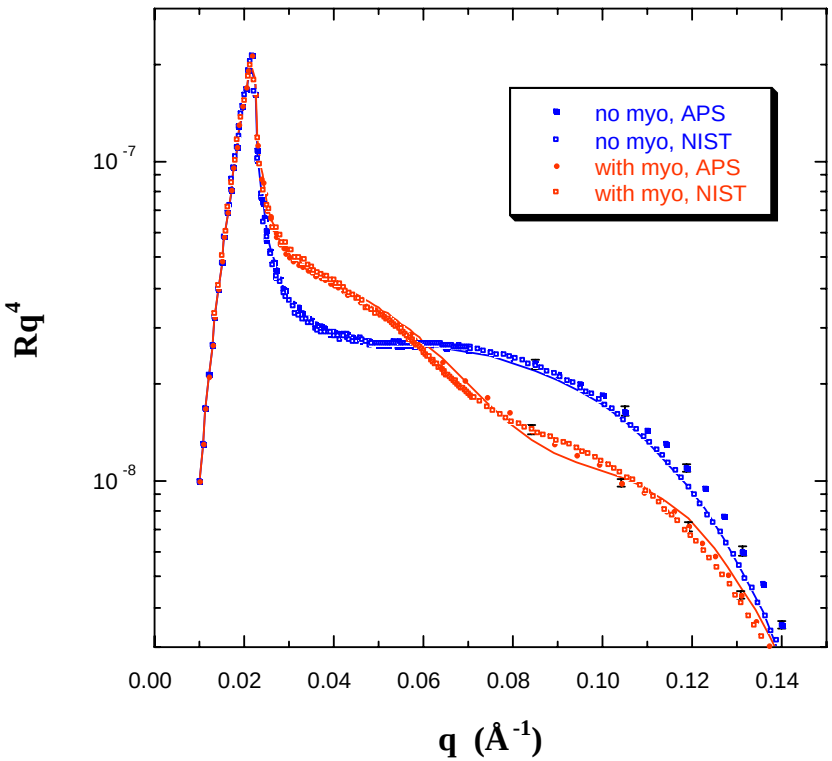
Alter conditions in the subphase underneath the lipid layer
(pH, composition, protein conc., etc)

More sensitivity to the protein with neutron reflection

neutron refl. (H_2O subphase)



X-ray refl.



$\phi = 0.55 +/- 0.05, \quad \rho_{myo.} = 1.43\ g/cm^3$

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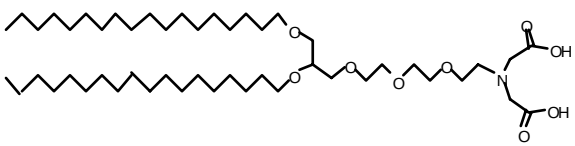
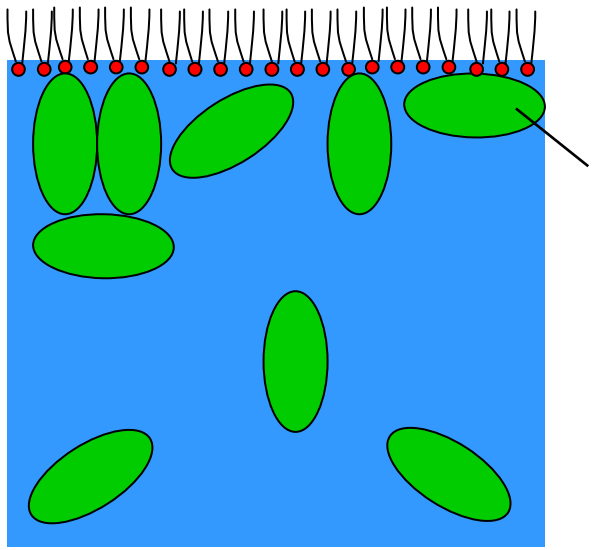
Model system: Proteins and peptides adsorbing to monolayers of metal-chelating lipids

1. Shnek, Pack, Sasaki, Arnold *Langmuir* (1994), 10, 2382.
2. Ng, Pack, Sasaki, Arnold *Langmuir* (1995), 11, 4048.



DSIDA

● = Cu^{2+}, Ni^{2+}, Zn^{2+}



protein

specific interaction between histidines and chelated metal ions,
Cu^{2+} (8.4 kT) > Ni^{2+} (7.0 kT) > Zn^{2+}

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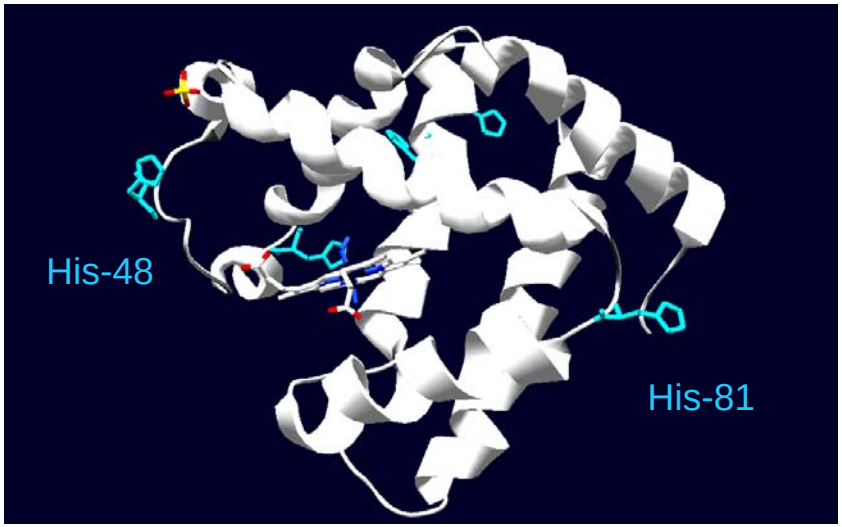
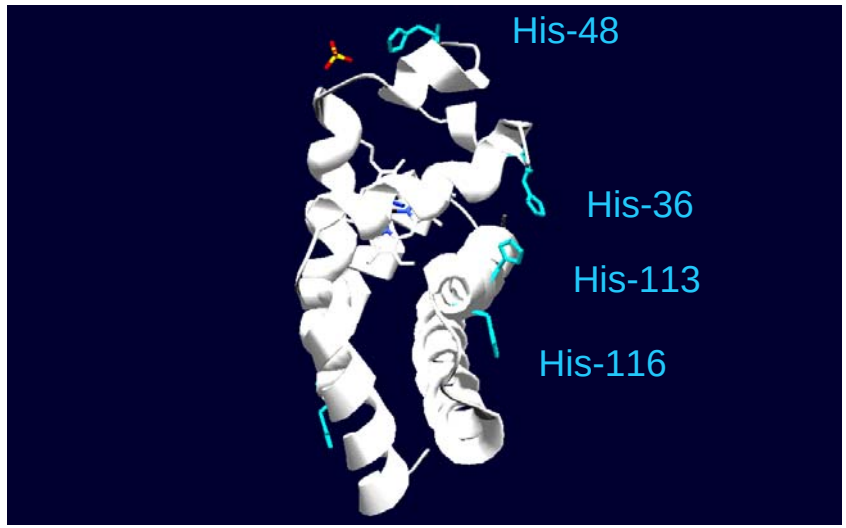
Myoglobin





Dimensions [Å]: *44 x 44 x 25*

11 histidines, 5 exposed on surface

orientation of adsorbed protein will depend upon which histidines bind

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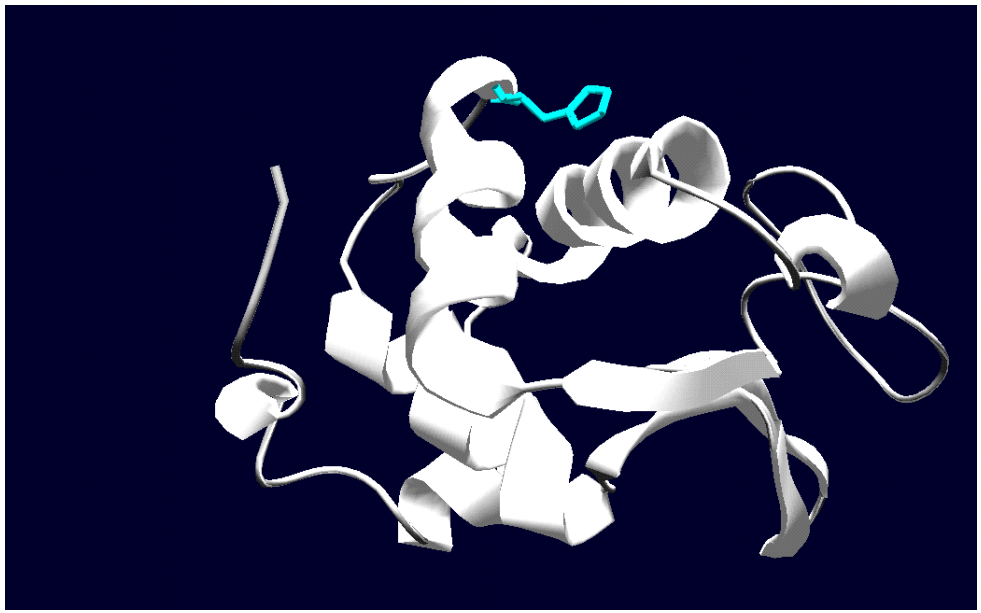
Structure of lysozyme



Dimensions [Å]: *44 x 28 x 26*

1 histidine, exposed on surface

Only one orientation expected upon binding

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Introduction

Biophysics questions addressed in this study:

- importance of single versus multiple-site binding

- orientation and reversibility

- conformational changes of proteins upon adsorption

- effect of protein binding on lipid phase behavior

Learn how to manipulate proteins at synthetic surfaces

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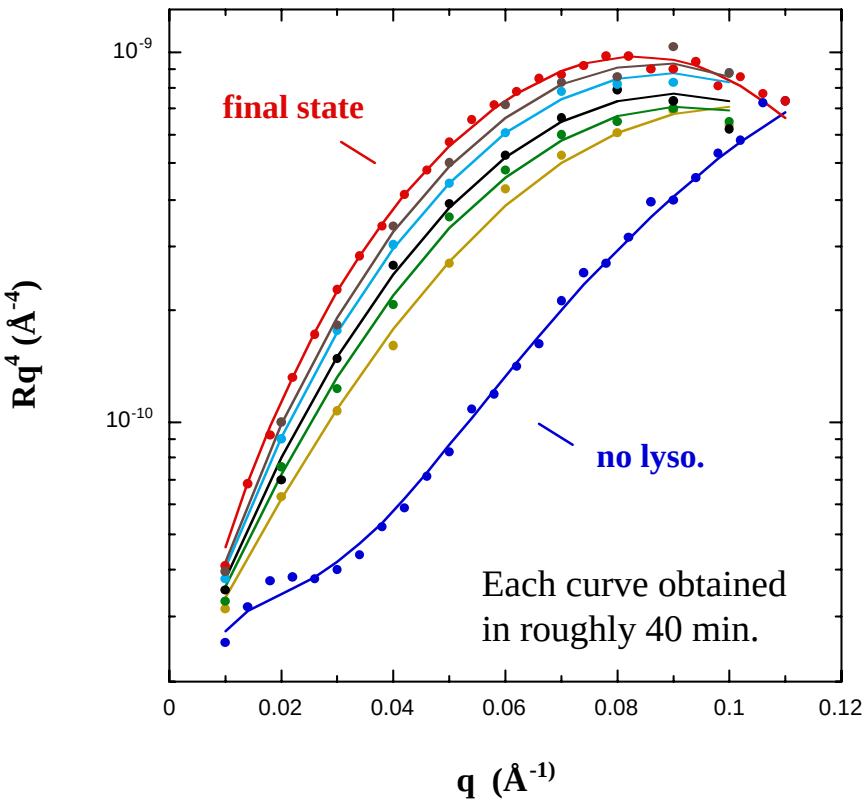
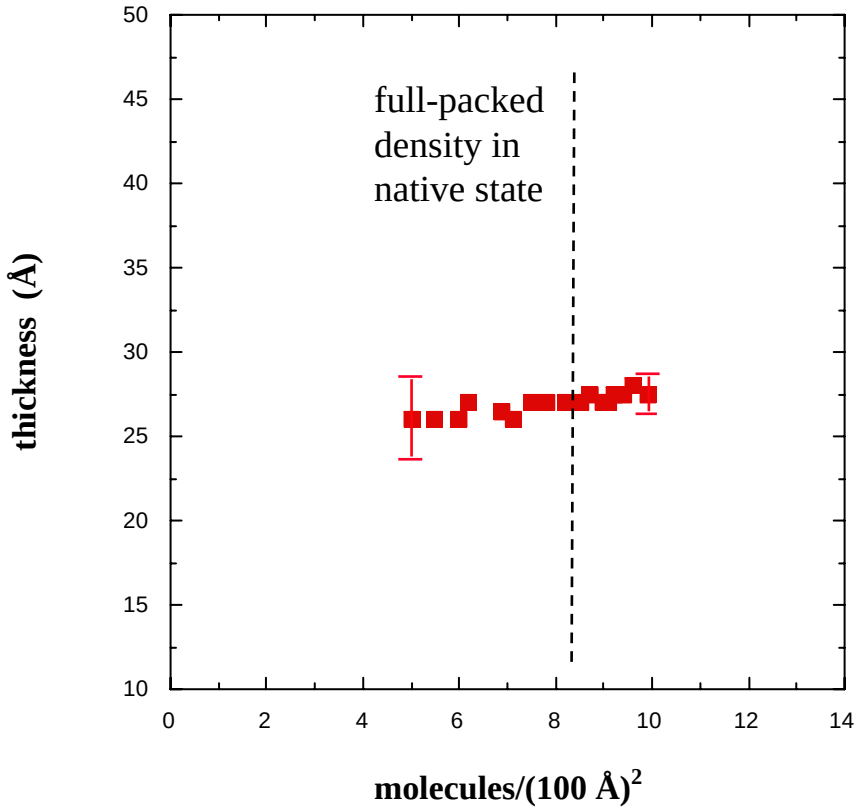
Neutron reflection - Lysozyme

time dependence



Dimensions [Å]: *44 x 28 x 26*



Little change in layer thickness with coverage

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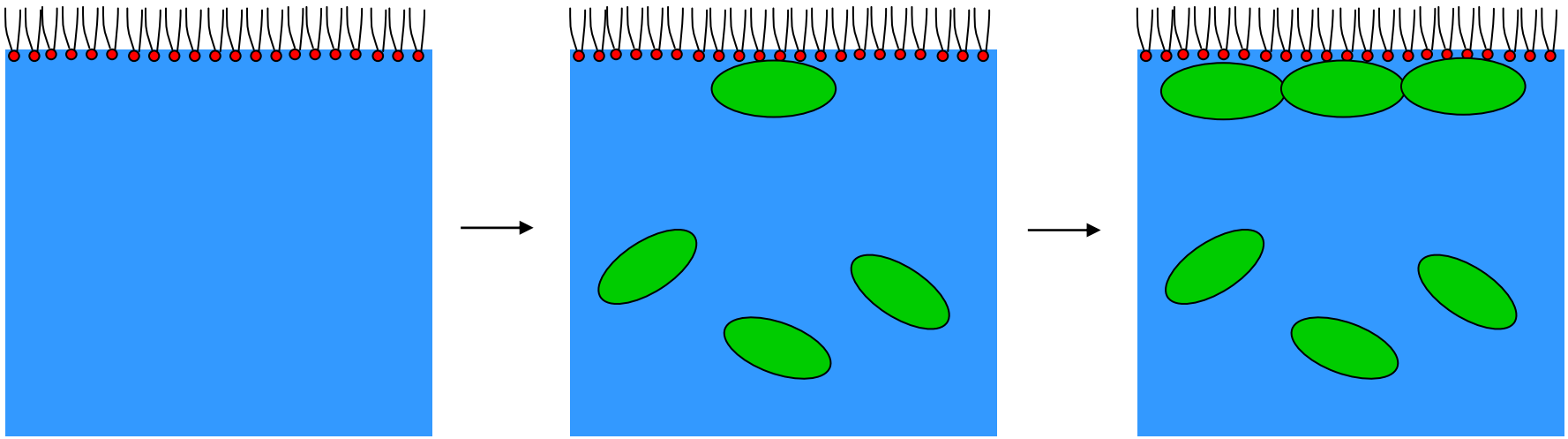
Lysozyme

one binding site - one expected orientation



Reflectivity data consistent with side-on orientation

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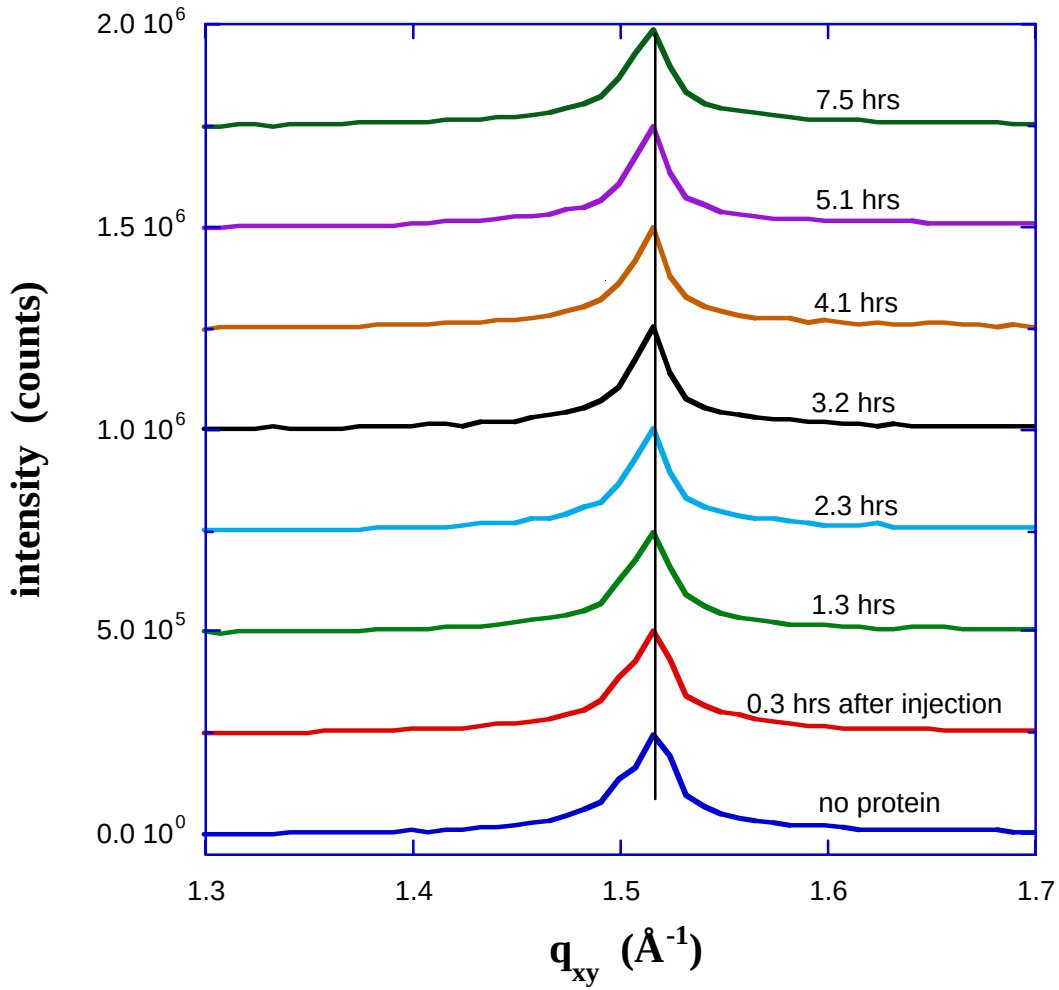
Grazing incidence X-ray diffraction

lysozyme (constant Π = 35 mN/m)



Lysozyme binding does not alter crystalline phase

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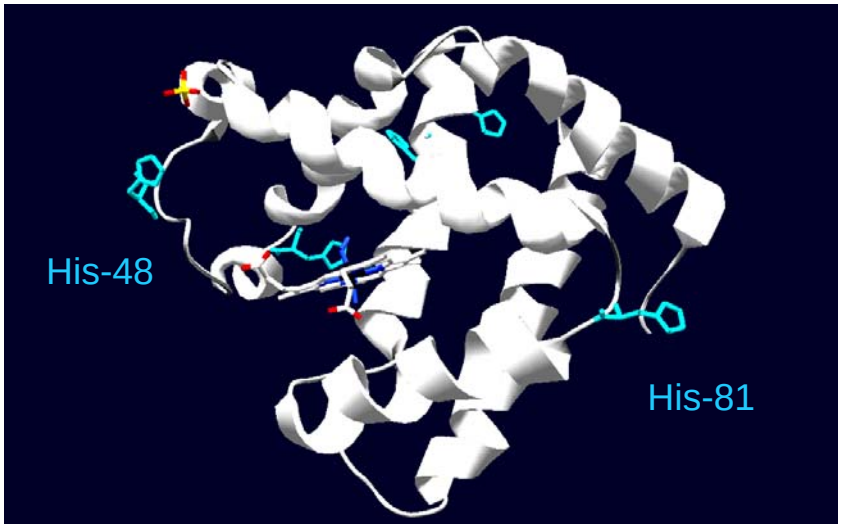
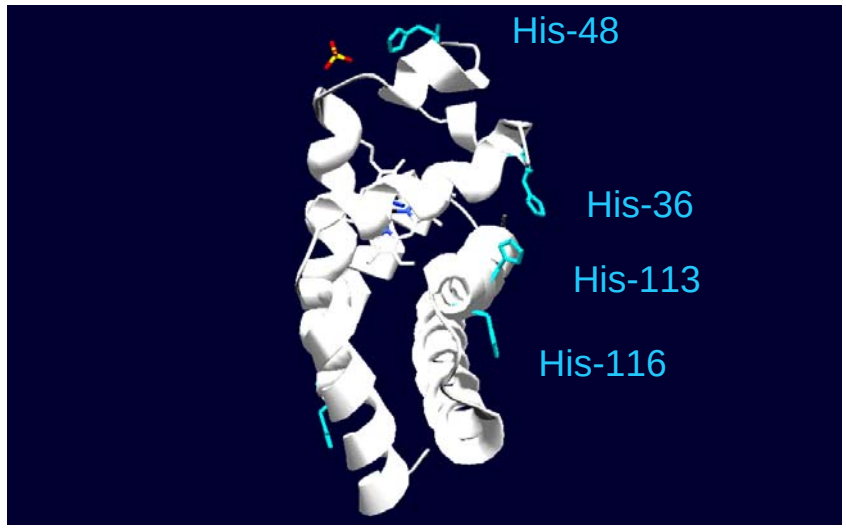
Langmuir **2005**, 21, 6815
Langmuir **2004**, 20, 2819

Myoglobin





Dimensions [Å]: *44 x 44 x 25*

11 histidines, 5 exposed on surface

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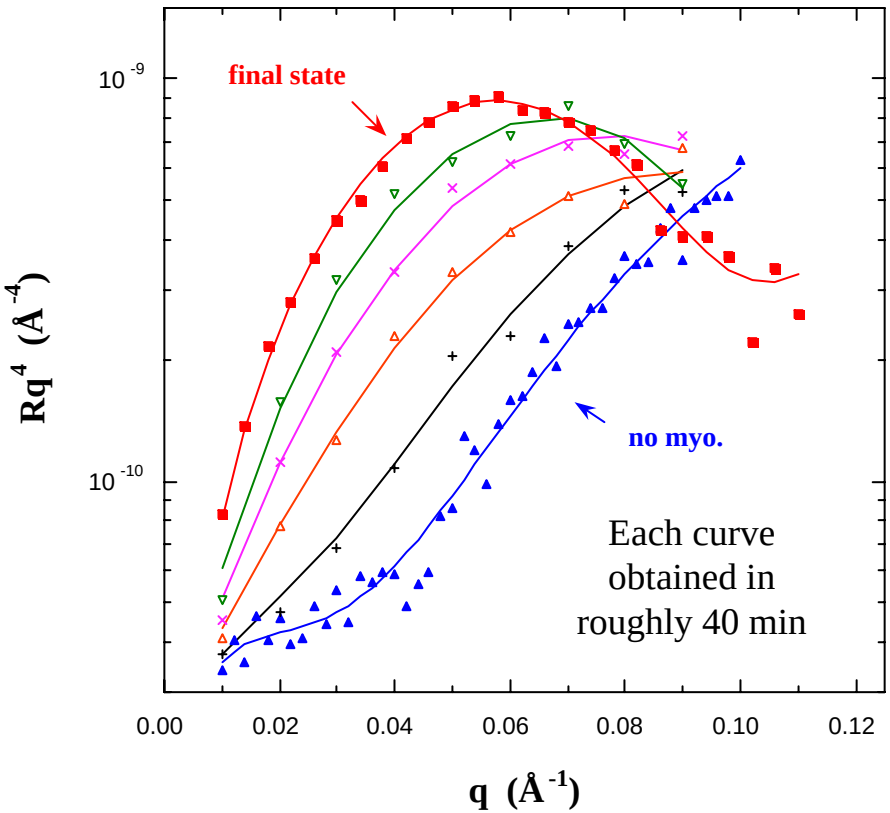
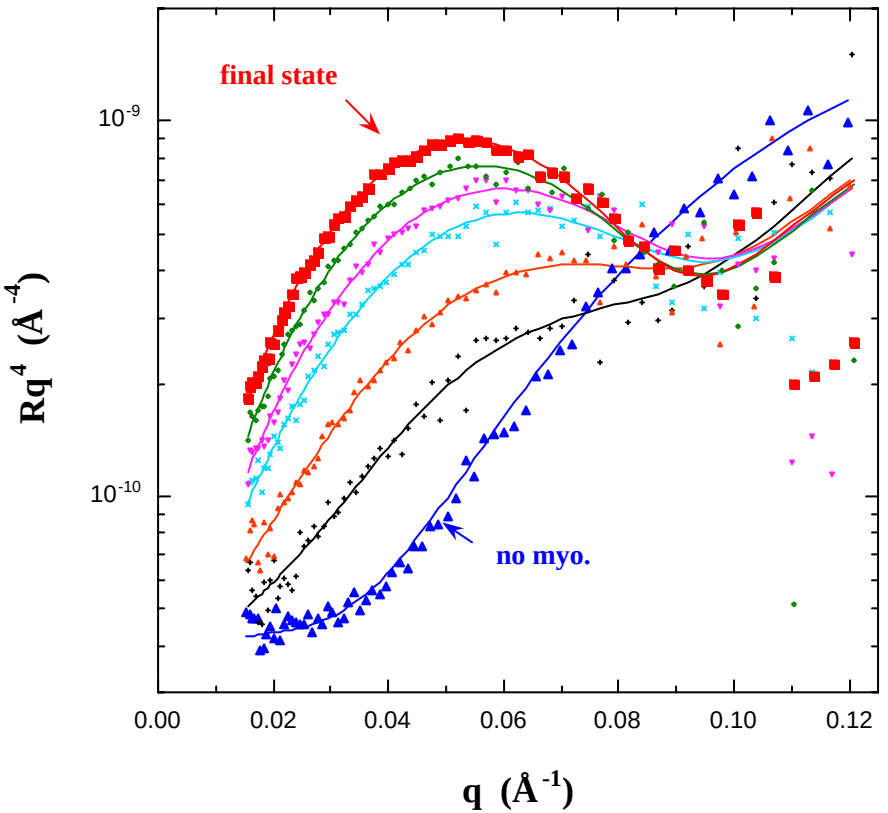
Neutron reflection (time dependence)

Cu^{2+} ions



Ni^{2+} ions



Results are different for Cu^{2+} and Ni^{2+} ions

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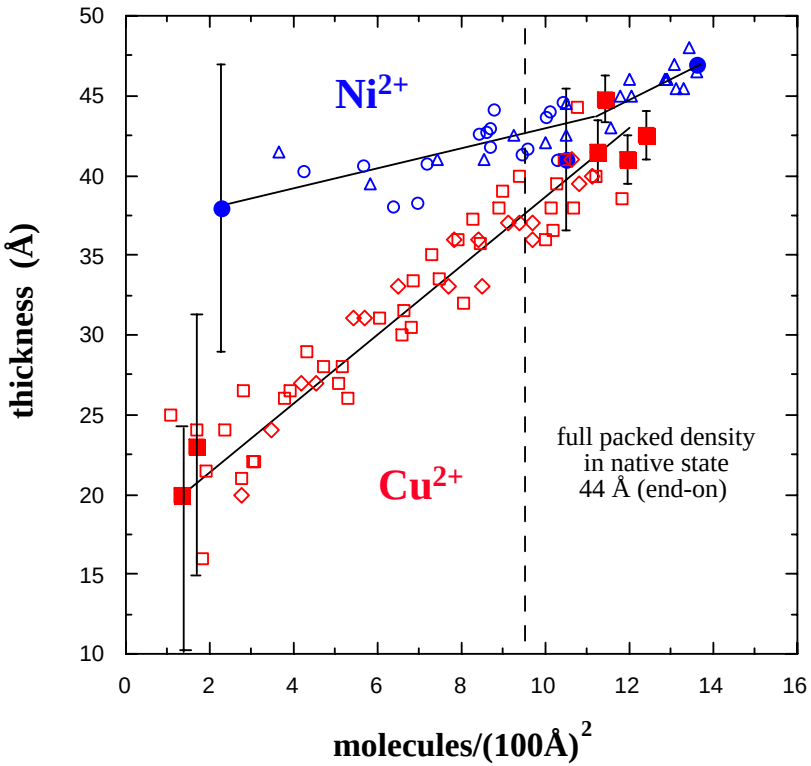
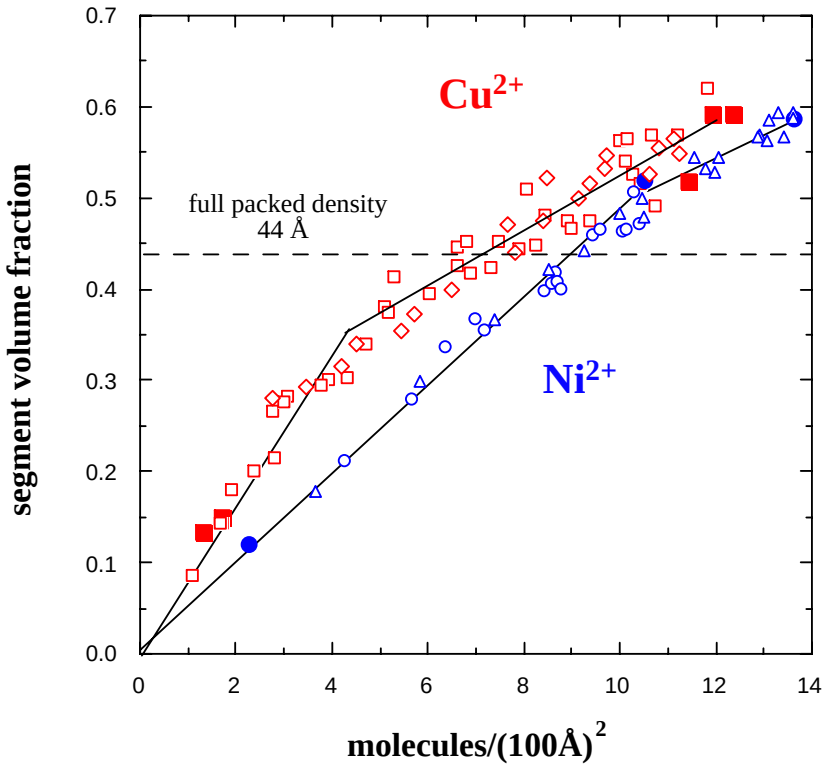
Langmuir **2005**, 21, 6815
Langmuir **2004**, 20, 2819

Summary

myoglobin dimensions [Å]: *44 x 44 x 25*





Isolated chains adsorb in a much thinner layer with Cu^{2+} than with Ni^{2+}

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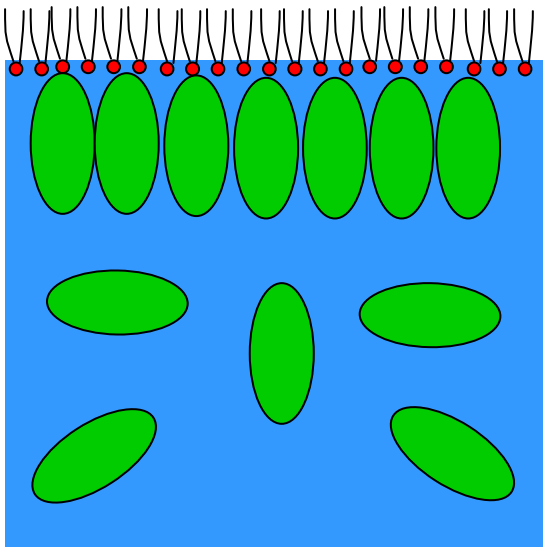
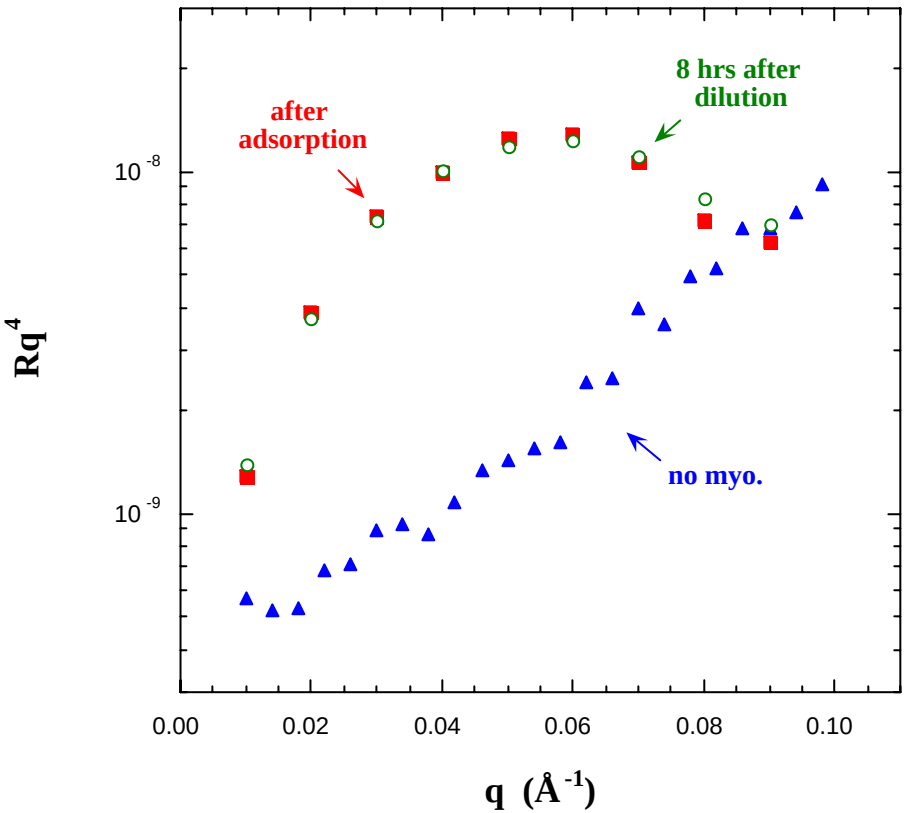
Adsorption is irreversible on experimental timescale!



diluted from
50 μM to 1.4 μM



Irreversible even at low coverage (for Cu(II) and Ni(II))

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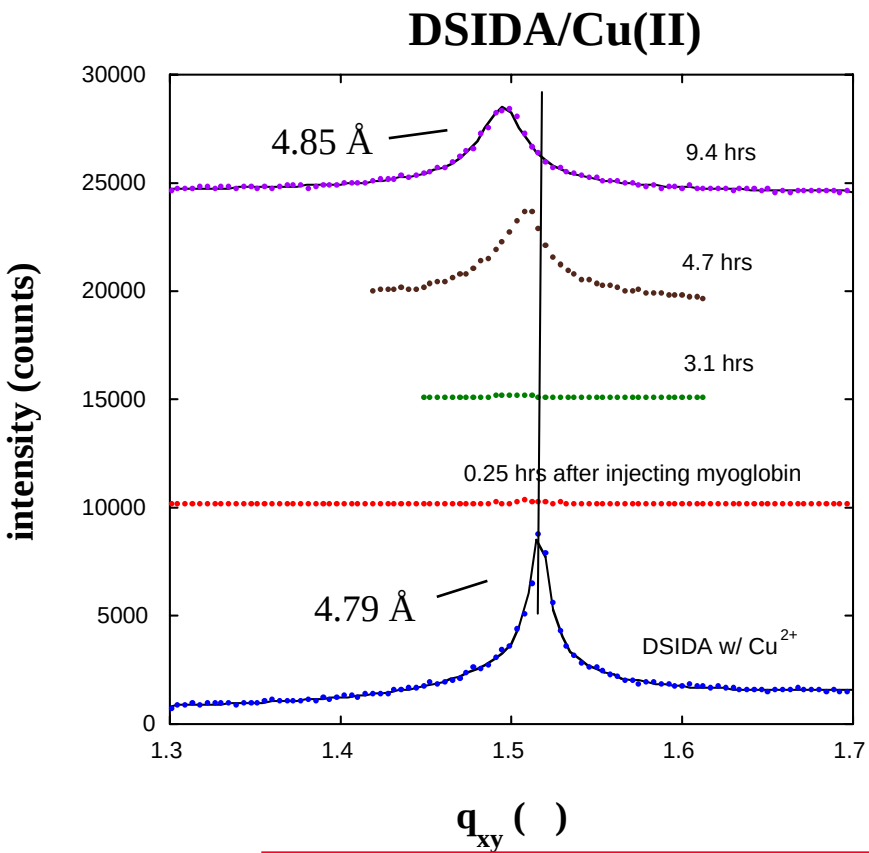
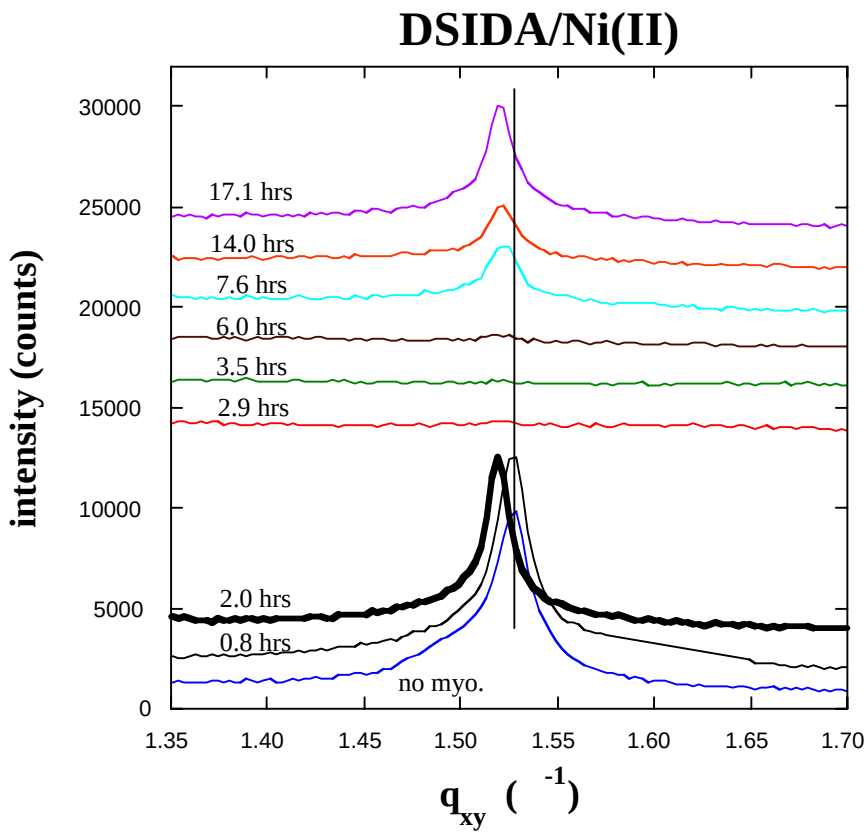
Interpretation

Some unfolding or denaturation occurs upon adsorption of myoglobin to Cu(II)-DSIDA

Adsorption alters lipid phase behavior

myoglobin (constant Π = 40 mN/m)





Peak shift suggests insertion of segments into lipid layer

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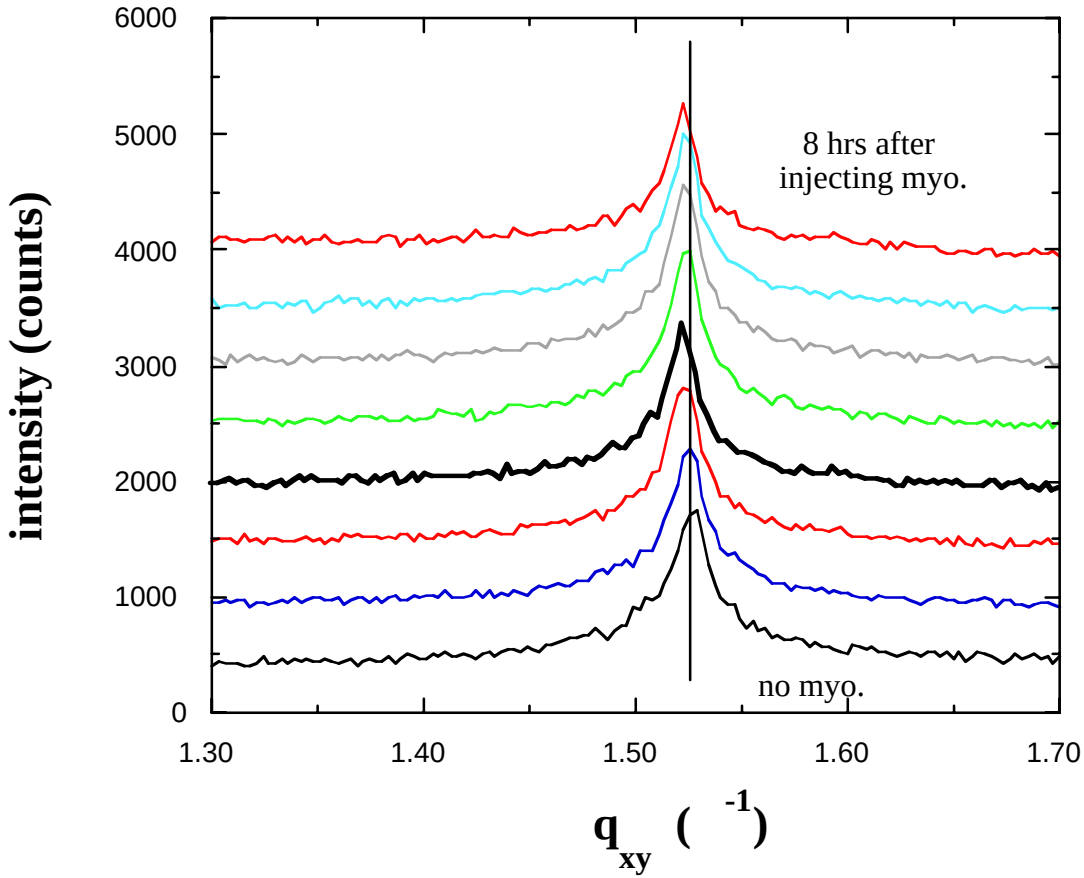
Myo. adsorption to DSIDA/Zn^{2+}

(constant Π = 40 mN/m)



Little or no effect on lipid phase state

(strong protein adsorption)

specific interaction between histidines and chelated metal ions,

Cu^{2+} (8.4 kT) > Ni^{2+} (7.0 kT) > Zn^{2+}

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Conclusions

Multiple site binding:

-causes unfolding of myoglobin

greater extent for stronger interaction
may lead to segmental insertion

-perturbs lipid packing

greater extent for stronger interaction

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Future work

Probe stability of specific protein folds/structures

Probe dynamics within lipid membranes:

vary surface pressure
add cholesterol, fluid phase lipids, etc
vary protein characteristics

Probe effect of protein binding on lipid phase state for other systems

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Future work possible with SNS?

Denaturation of proteins on hydrophobic surfaces

Conformational changes of bound proteins

Dynamic assembly of protein complexes

Location of small molecules within ion channels, lipid bilayers, integral membrane proteins

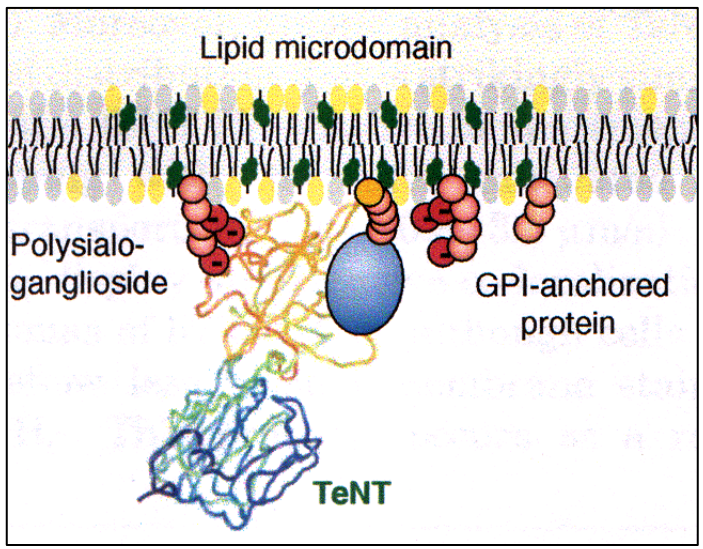
Orientation of bound proteins using crystal structure

Botulinum, tetanus, and diphtheria toxin assault on cell membranes

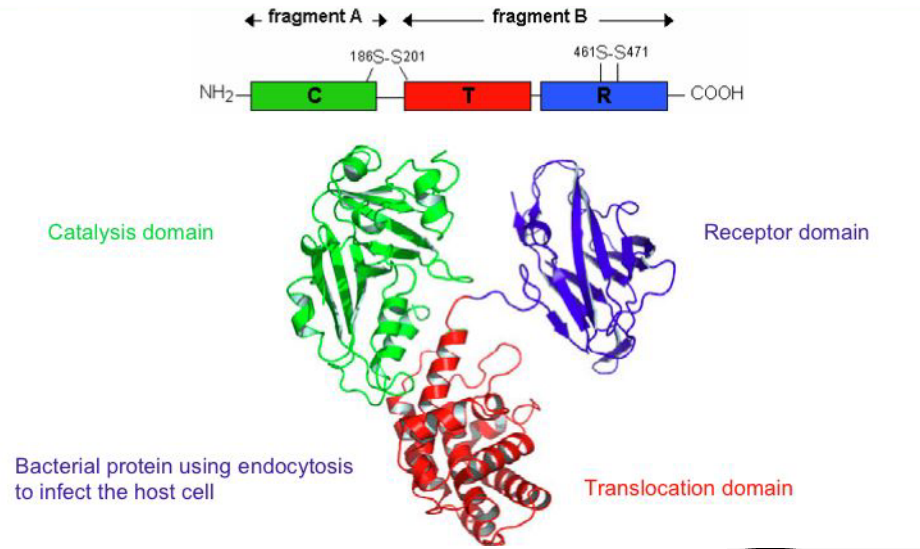
study recognition and permeation processes:

-binding orientation
-conformational changes associated with change in pH
-effect of receptor conc.

tetanus



diphtheria



From: Lalli et al, Trends in Microbiol, 2003

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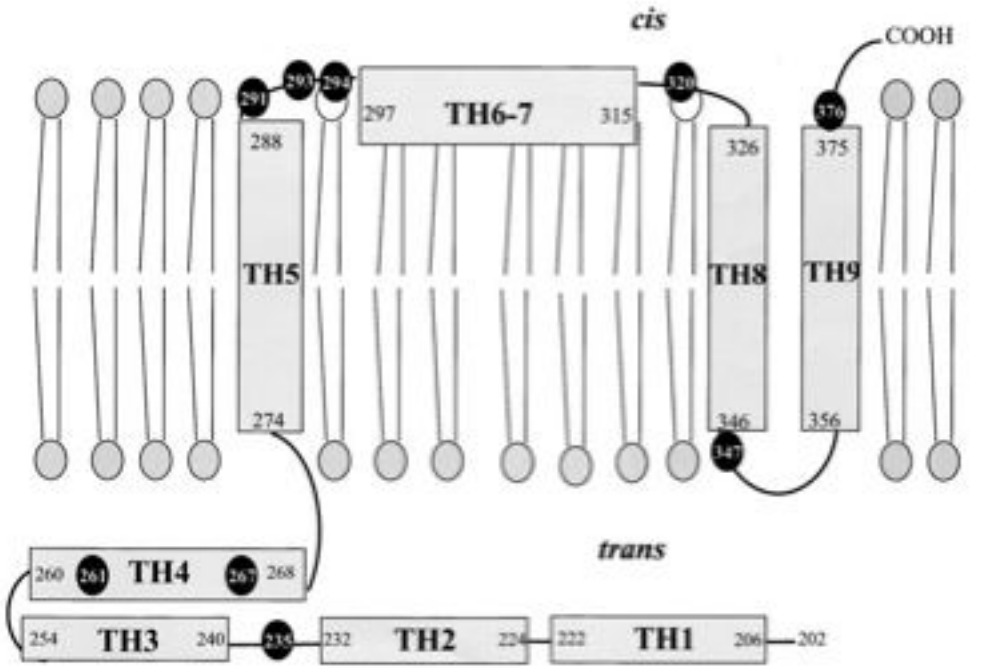
Botulinum, tetanus, and diphtheria toxin assault on cell membranes

Diphtheria - one model of “open-pore” structure



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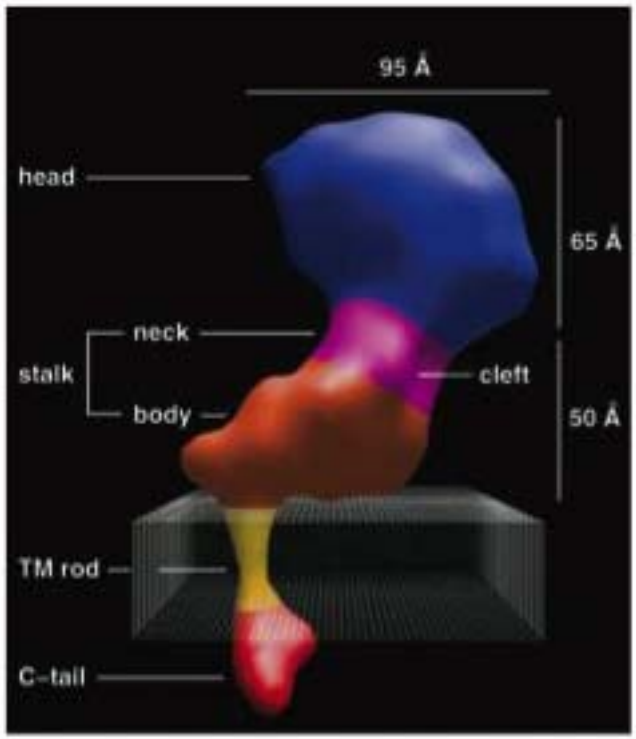
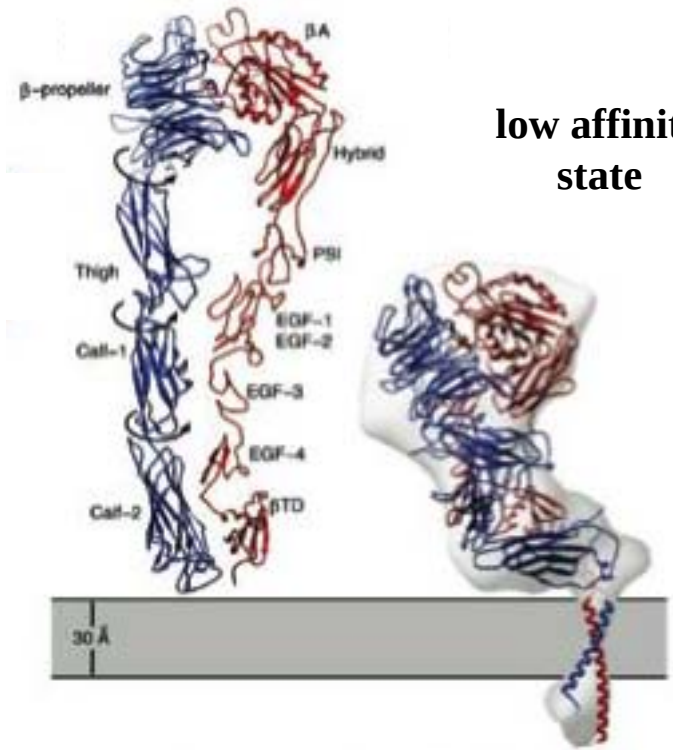
human platelet integrin $\alpha_{IIb}\beta_3$

from: Adair and Yeager, PNAS 2002, 99, 14059



high affinity state



low affinity state

Conformational changes induced by:

- **ligand binding**
- **changes in divalent cation coordination in MIDAS**

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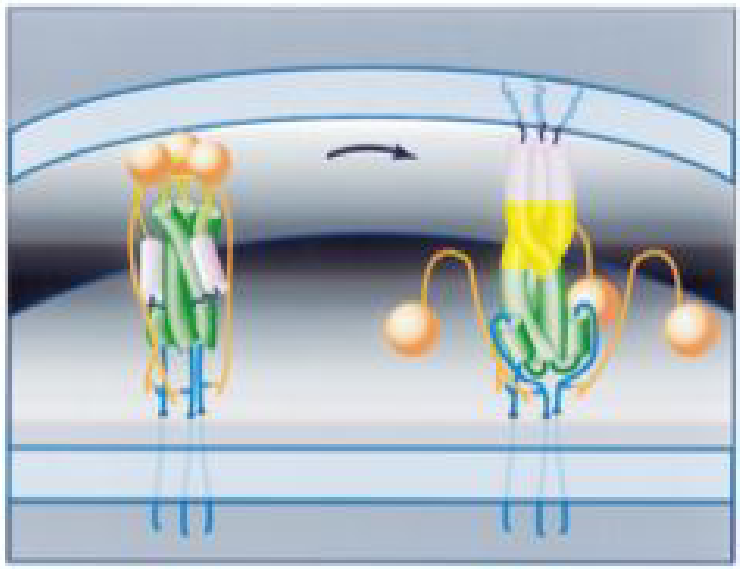
Influenza hemagglutinin

from: Carr et al., PNAS 1997, 94, 14306

“Influenza hemagglutinin is spring-loaded by a metastable native conformation”



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SNAREs

from Tochio et al Science 2001, 293, 698

Conformational changes are key to auto-inhibitory regulation

C-terminal transmembrane anchoring domain
SNARE motif (parallel α-helical bundles)
H_{abc} N-terminal domain (intramolecular chaperone?)

H_{abc} domain interacts with the SNARE motif to generate a closed form

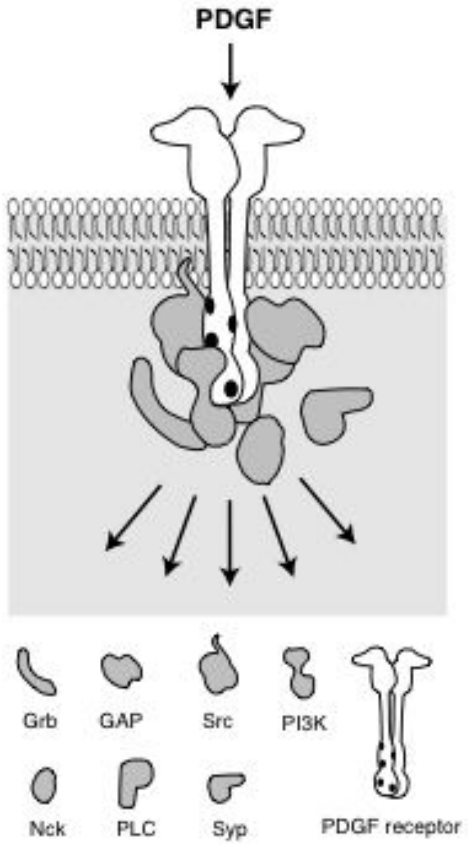
Induced conformational changes (open-activated or closed - inactivated) appear to influence:

-the specificity of SNARE pairing
-the kinetics of SNARE complex formation

Conformational change induced by: nSec1 binding, pH increase 7 - 8.8

Protein complexes

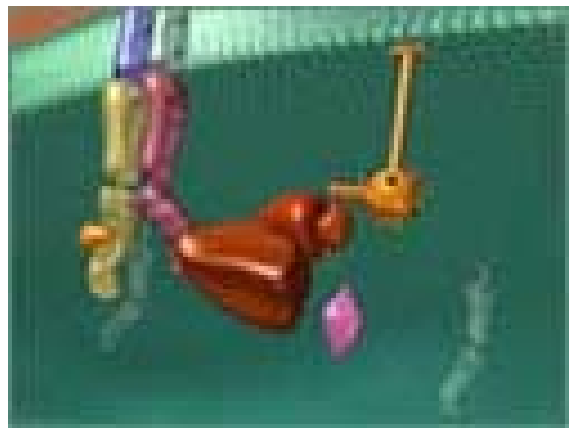
Platelet-derived growth factor receptor



D. Bray
Ann. Rev.
Biophys Biomol.
Struct., 1998, 59

How many proteins are bound at any time?
To what extent do they interact with each other?

Epidermal growth factor receptor



Conformational changes upon phosphorylation?
Timing and sequence of events?

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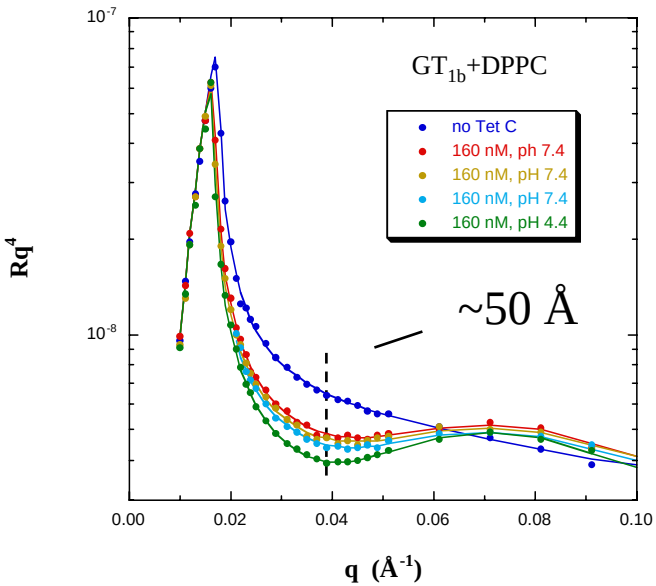
Orientation of bound proteins using crystal structure

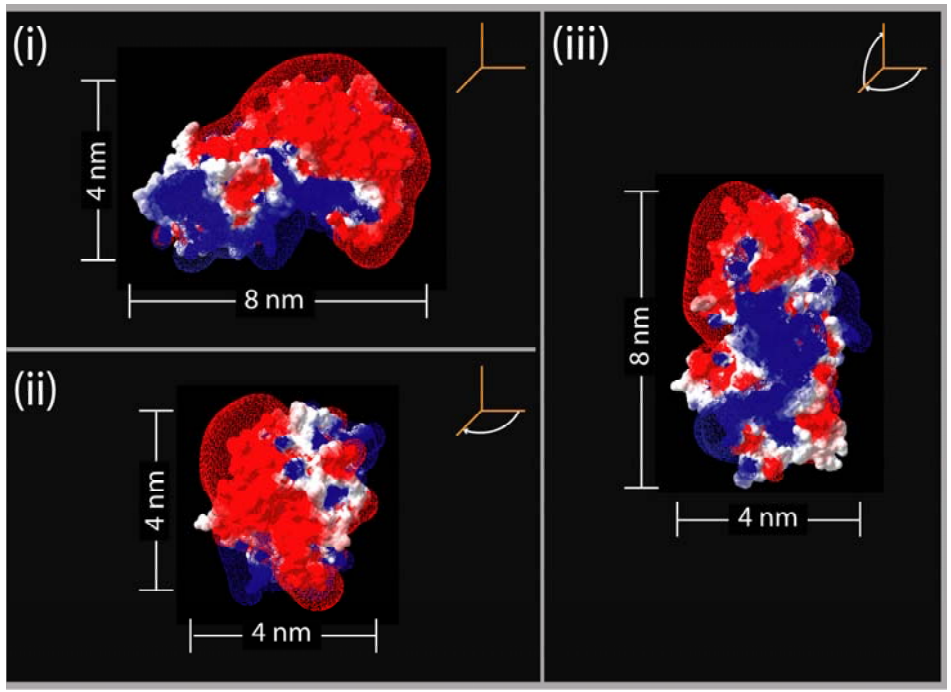
Multiple possible ganglioside binding sites

tetanus, Hc-fragment



~ 10% coverage

Data to higher q required for this method!
(Schlossman et al Biophys. J. 2005 89, 1861)



C fragment is 47 kD with a large negatively charged area (red) opposing the high positive charge area (blue) of the ganglioside receptor site

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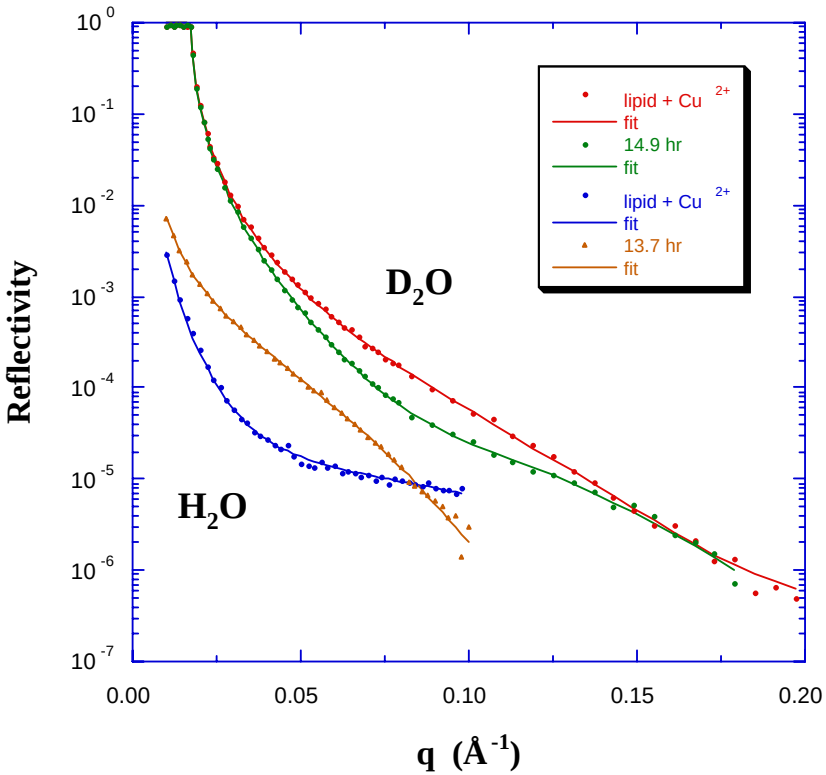
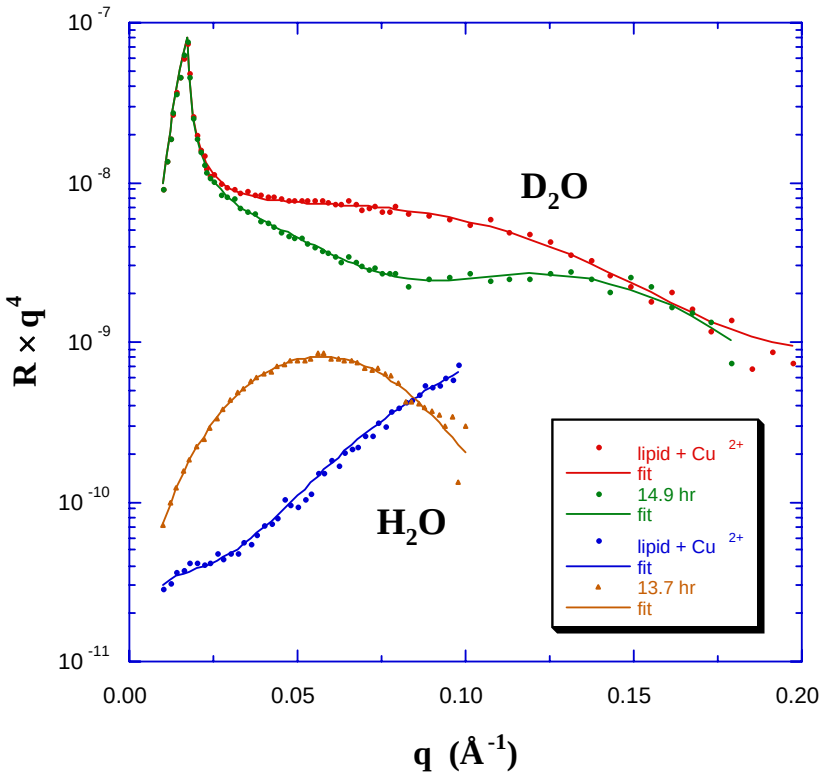
Neutron reflection





Greater sensitivity to the protein in H_2O!

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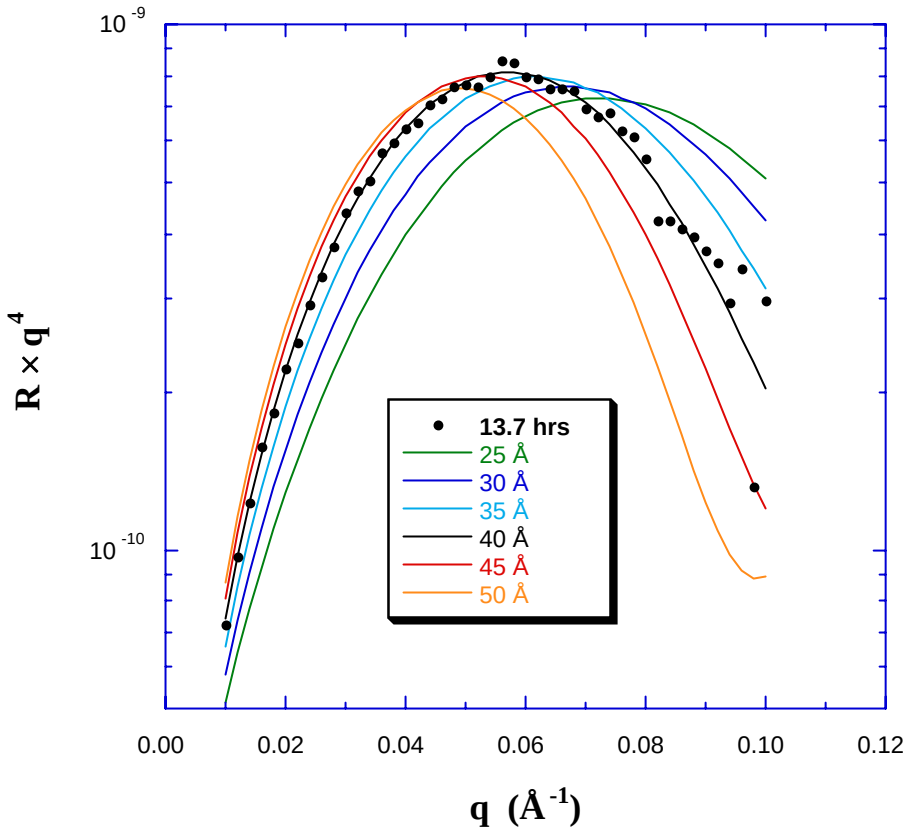
Neutron reflection - H_2O subphase: Error bars

How accurate is the thickness obtained? (+/- 2 Å)



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