



Protein Interactions with Synthetic Lipid Monolayers Studied by X-ray and Neutron Scattering Techniques

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Outline

I. Introduction - motivation, description of lipid/protein system

II. Results (2 diff. lipid monolayers)

- a. grazing incidence X-ray diffraction
- b. X-ray reflection
- c. neutron reflection - D₂O subphase
- d. neutron reflection - H₂O subphase

III. Summary and future work



Introduction

1. Motivation for studying protein - lipid membrane interactions *(membrane-associated, not integral membrane proteins)*

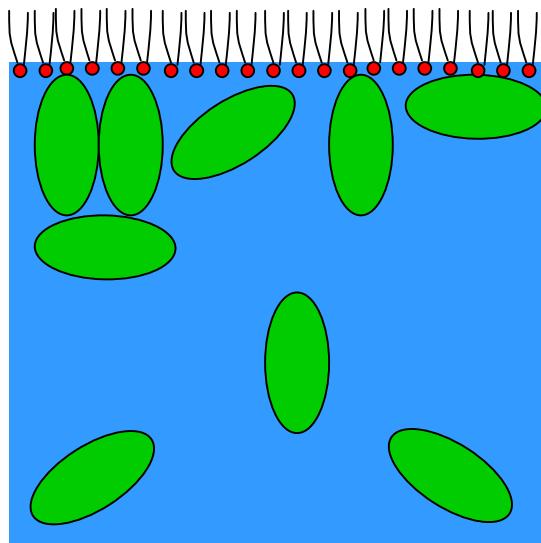
- a). control/direct the formation and growth of supramolecular structures (motor protein highways, protein complexes)
- b). mechanisms of toxin assault on cell membranes
- c). biosensors - binding modes determine chemical signals, dictate sensor response



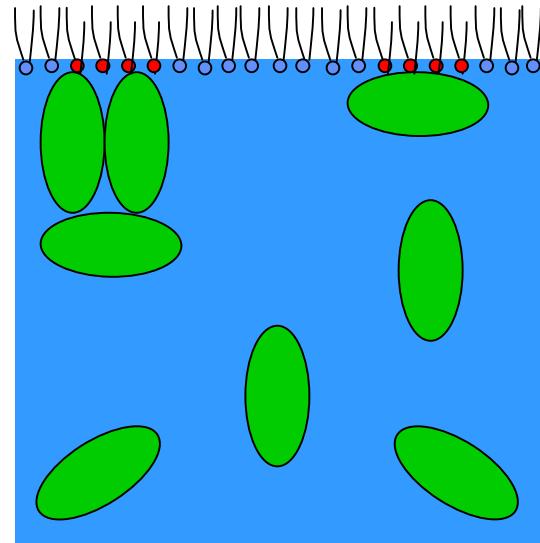
Introduction

2. Chemical system: Langmuir monolayers of metal-chelating lipids:

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



100% DSIDA layer forms 2-d
crystalline domains



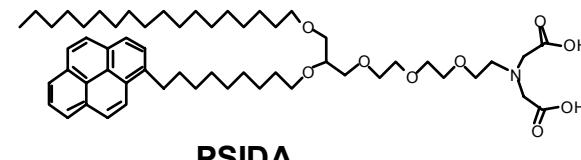
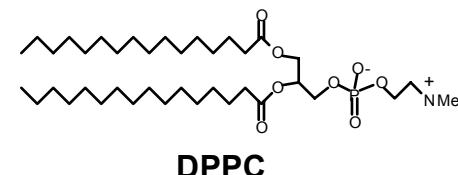
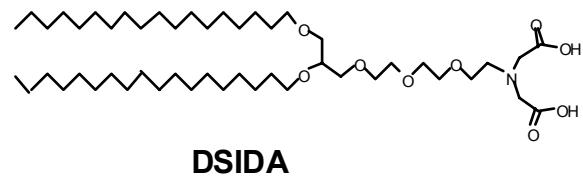
lipids segregate in-plane
to form domains

strong interaction between histidine units and chelated metal ions



Materials

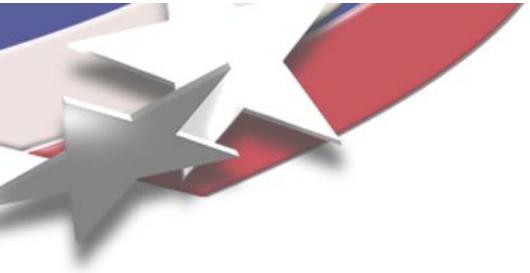
Lipids: 100% DSIDA, also 20% PSIDA/80% d-DPPC



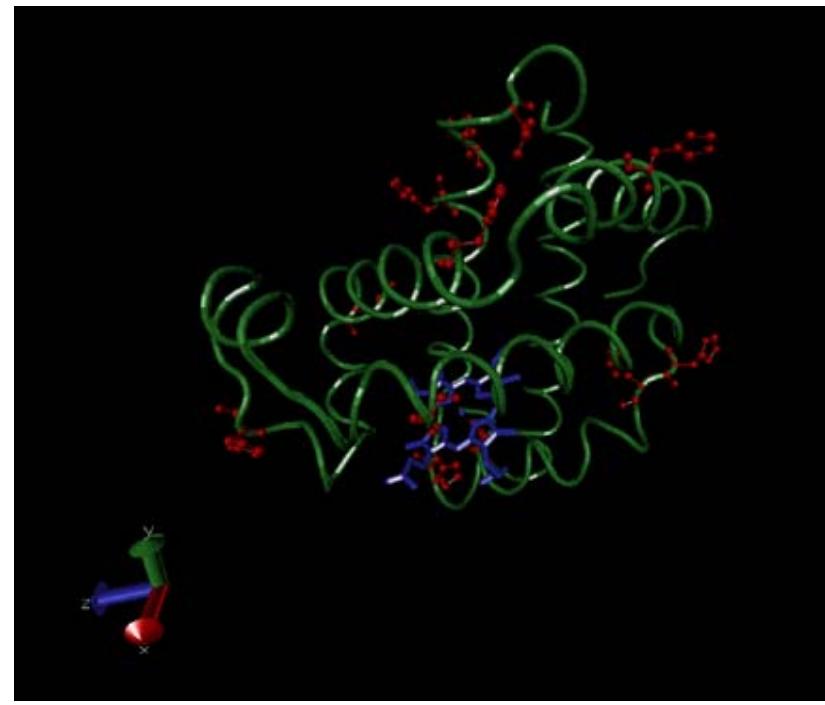
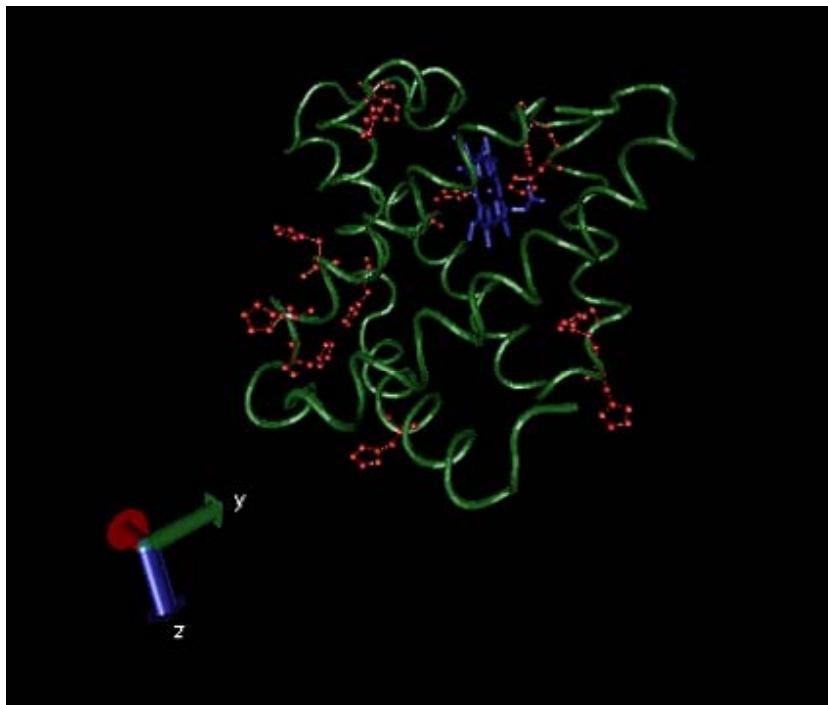
Metal ion: CuCl₂ (10 μM)

Protein: horse heart myoglobin (M_r = 17,641)
conc. = 10 μM

PO₄ buffer solution: pH = 7.4



Structure of Myoglobin



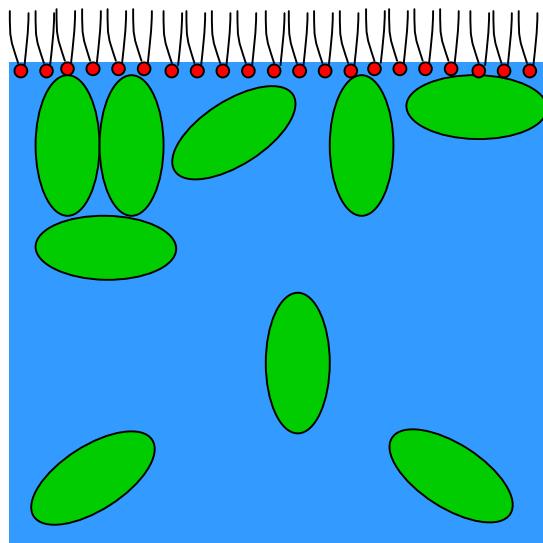
Unit Cell: dim[Å]: $a \text{ 64.84}$ $b \text{ 30.98}$ $c \text{ 34.92}$

Angles [°]: $\alpha \text{ 90.00}$ $\beta \text{ 106.02}$ $\gamma \text{ 90.00}$

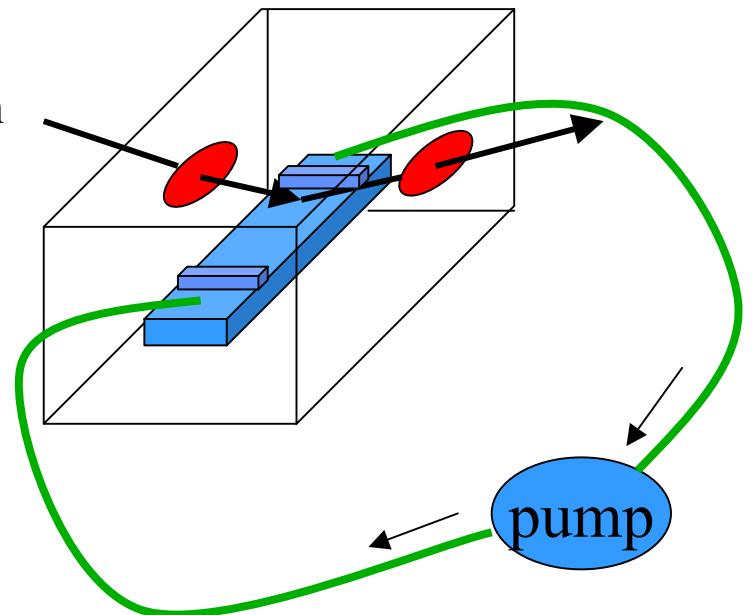
orientation of adsorbed protein will depend upon
the distribution of surface histidine residues



X-ray and neutron grazing incidence scattering techniques



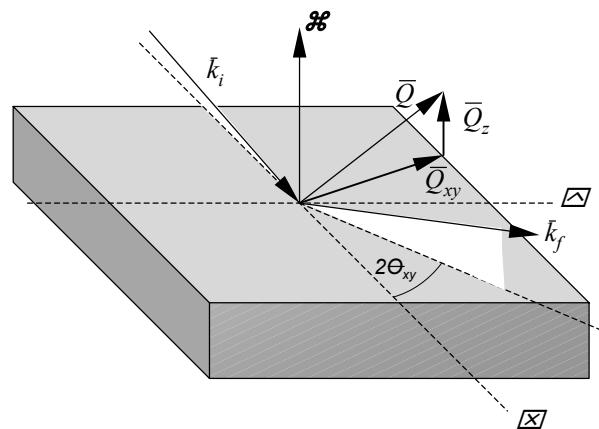
neutron or
X-ray beam



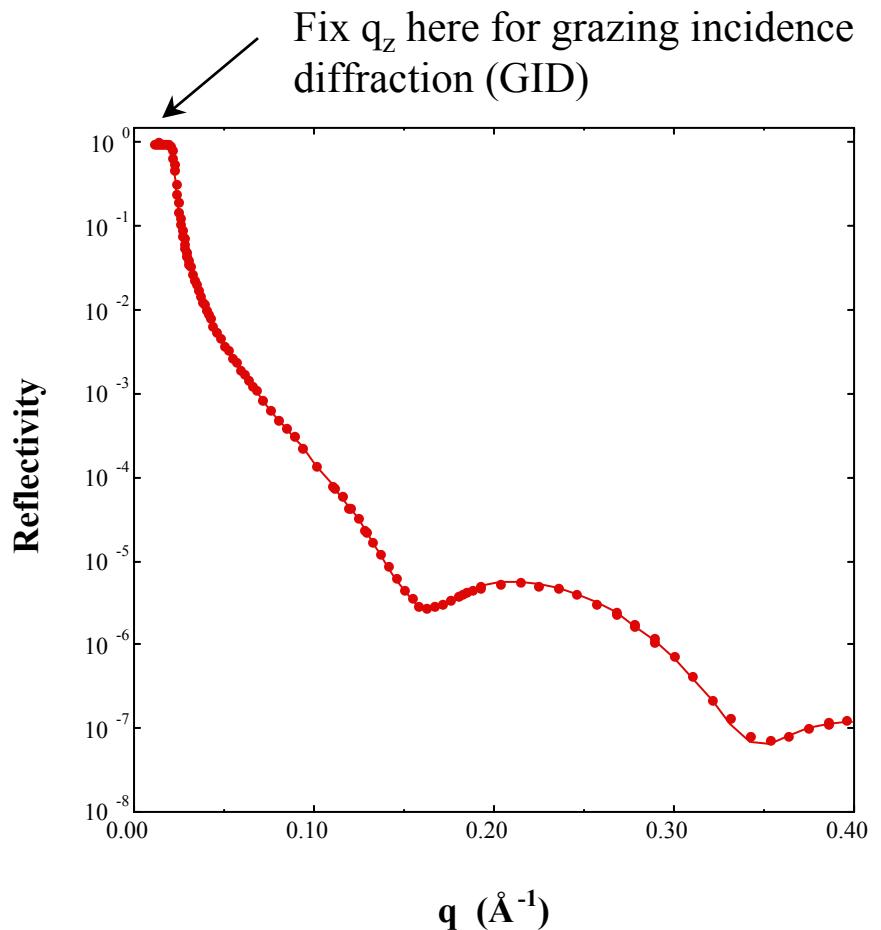
circulate copper and myoglobin into the subphase
underneath the lipid layer



X-ray and neutron grazing incidence scattering techniques



neutrons: reflection only
X-rays: reflection and diffraction

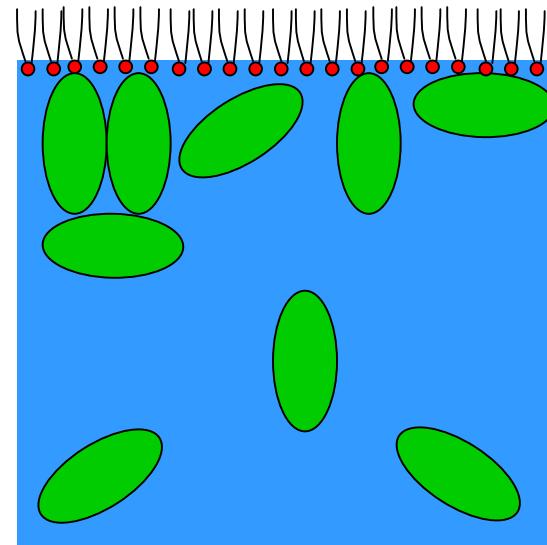




Results - 1st system

**myoglobin adsorbing to 100% DSIDA
film at 40 mN/m**

1. grazing incidence X-ray diffraction
2. X-ray reflection
3. neutron reflection - D₂O subphase
4. neutron reflection - H₂O subphase

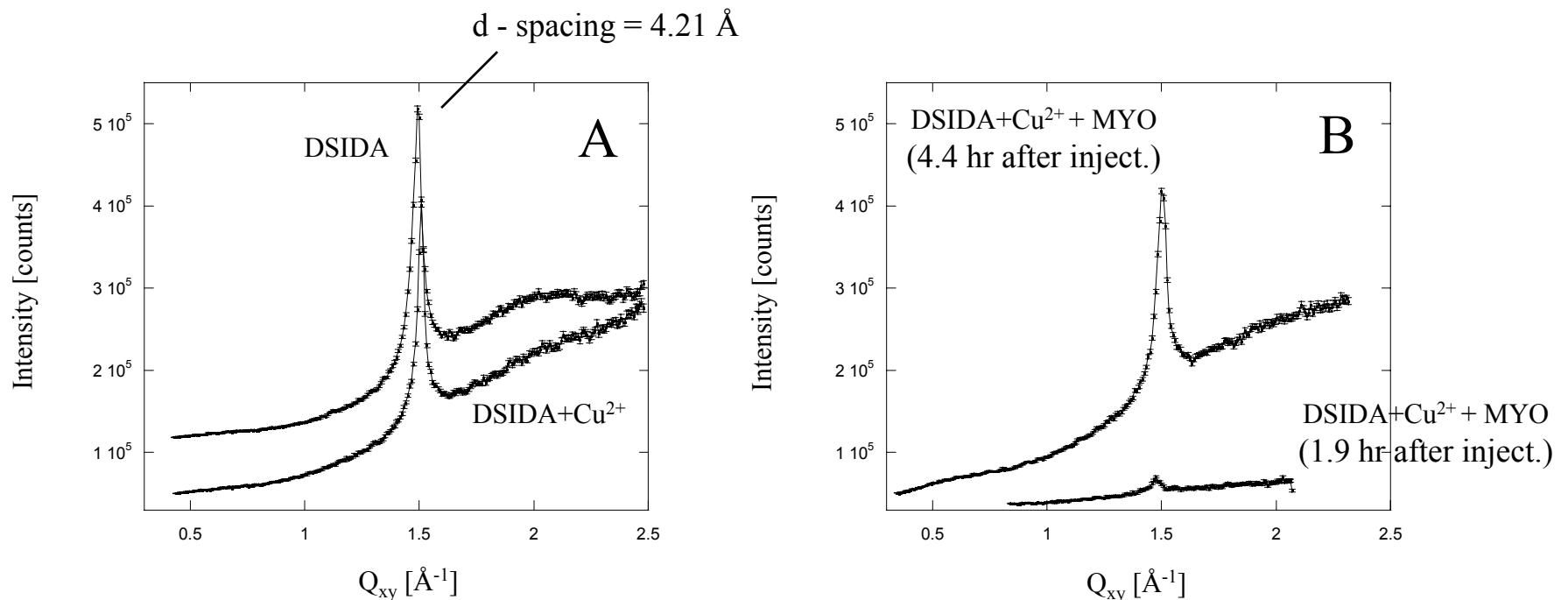


examine both the adsorbed layer of myoglobin and the response of the lipid layer



Expt #1. Grazing incidence X-ray diffraction

Diffraction peak due to hexagonal packing of lipid tails



lipid packing is disrupted upon injection of myoglobin, but
the crystalline structure gradually reforms!



Expt #1. Grazing incidence X-ray diffraction

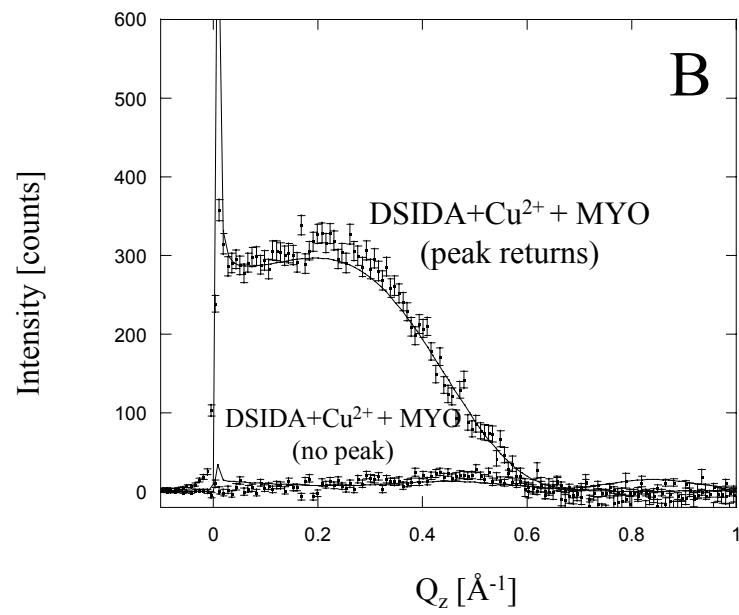
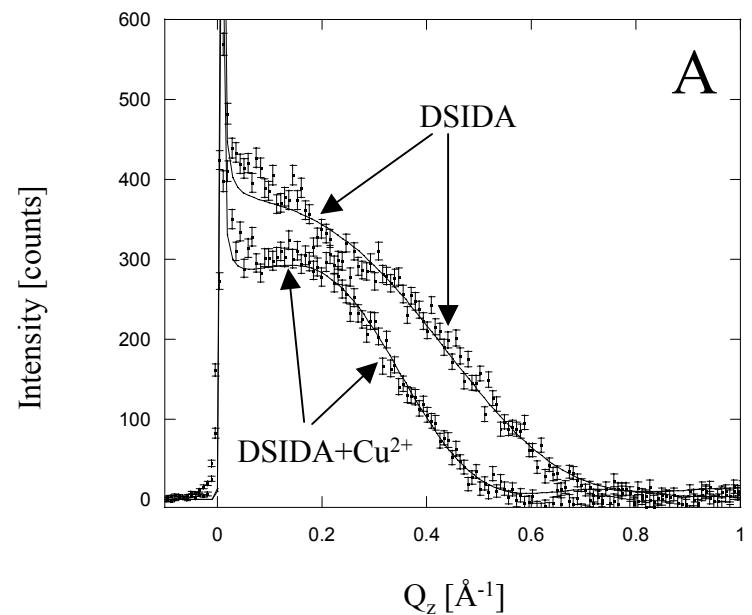
In-plane coherence length obtained from FWHM of peak

In-plane coherence length:

DSIDA only	120 Å
with copper	176 Å
with myo (4.4 hr)	100 Å



Expt #1. Grazing incidence X-ray diffraction



shape of Bragg rods gives further information about lipid structure



Expt #1. Grazing incidence X-ray diffraction

Out-of-plane coherence length and tilt angle obtained from Bragg rod shape:

Out-of-plane coherence length:

DSIDA only	12.3 +/- 0.4 Å
with copper	19.4 +/- 0.9 Å
with myo (4.4 hr)	17.8 +/- 1.1 Å

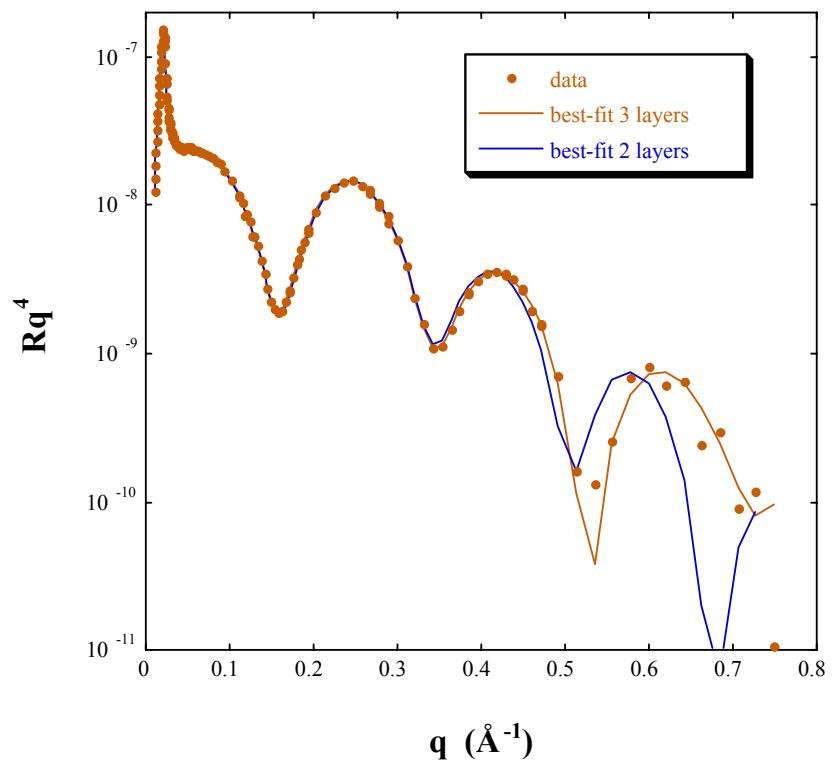
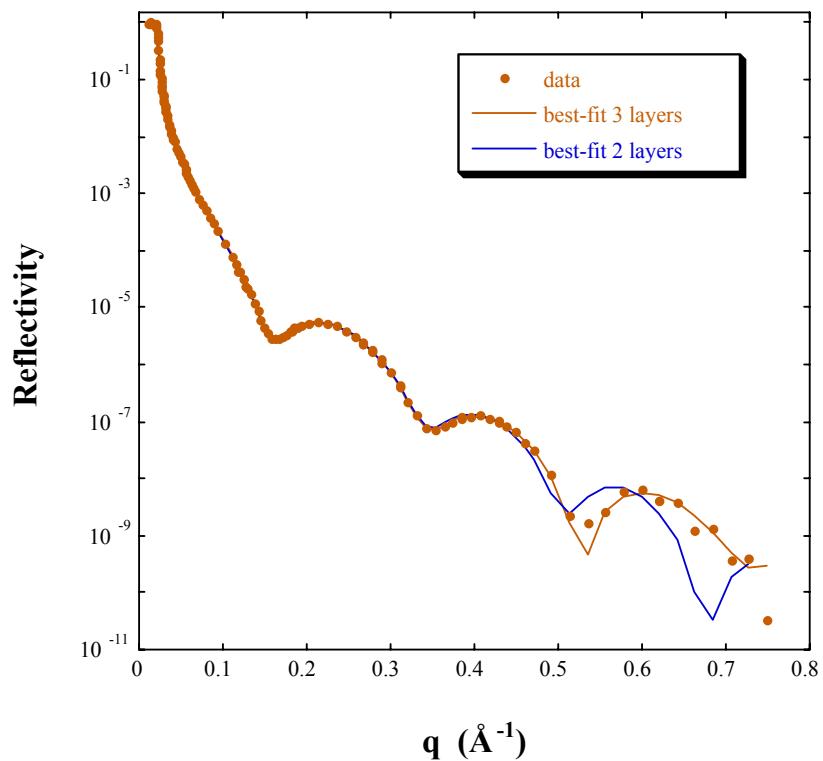
Tilt angle:

DSIDA only	12.4 +/- 0.2°
with copper	10.5 +/- 0.2°
with myo (4.4 hr)	12.9 +/- 0.1°



Expt #2. X-ray reflection

DSIDA on H₂O, 40 mN/m

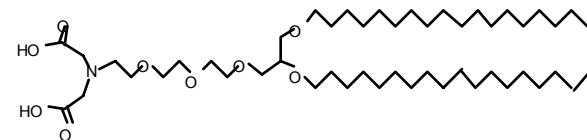
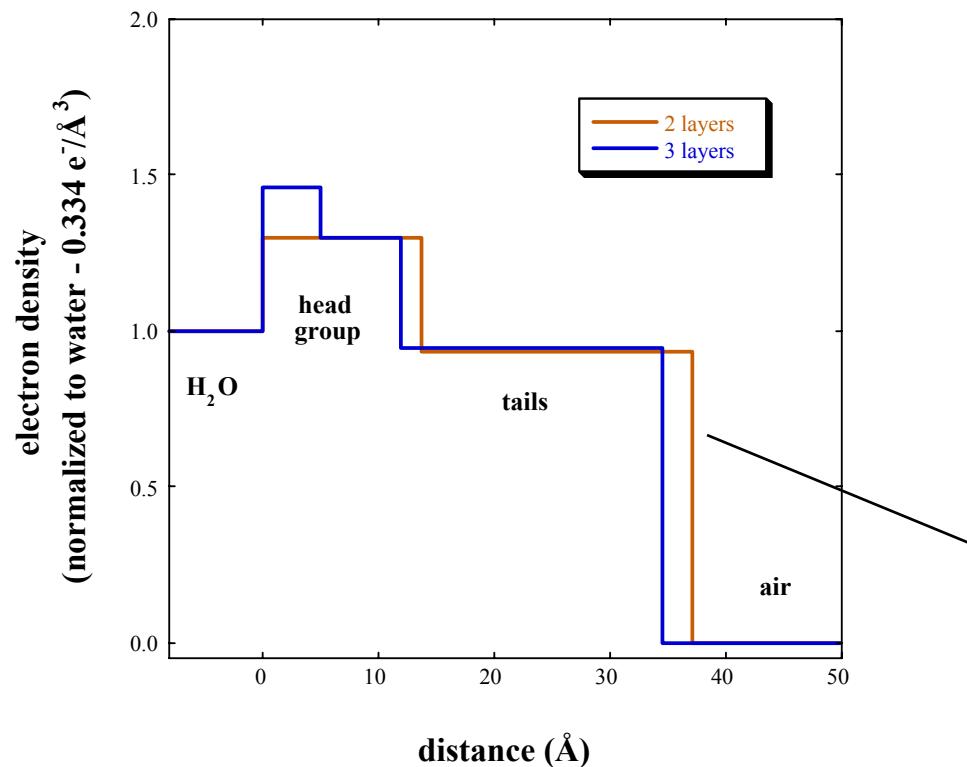


three layers are required to describe the lipid monolayer



Expt #2. X-ray reflection

electron density profile for DSIDA on PO_4 buffer ($\text{pH} = 7.4$, $T = 20^\circ\text{C}$)

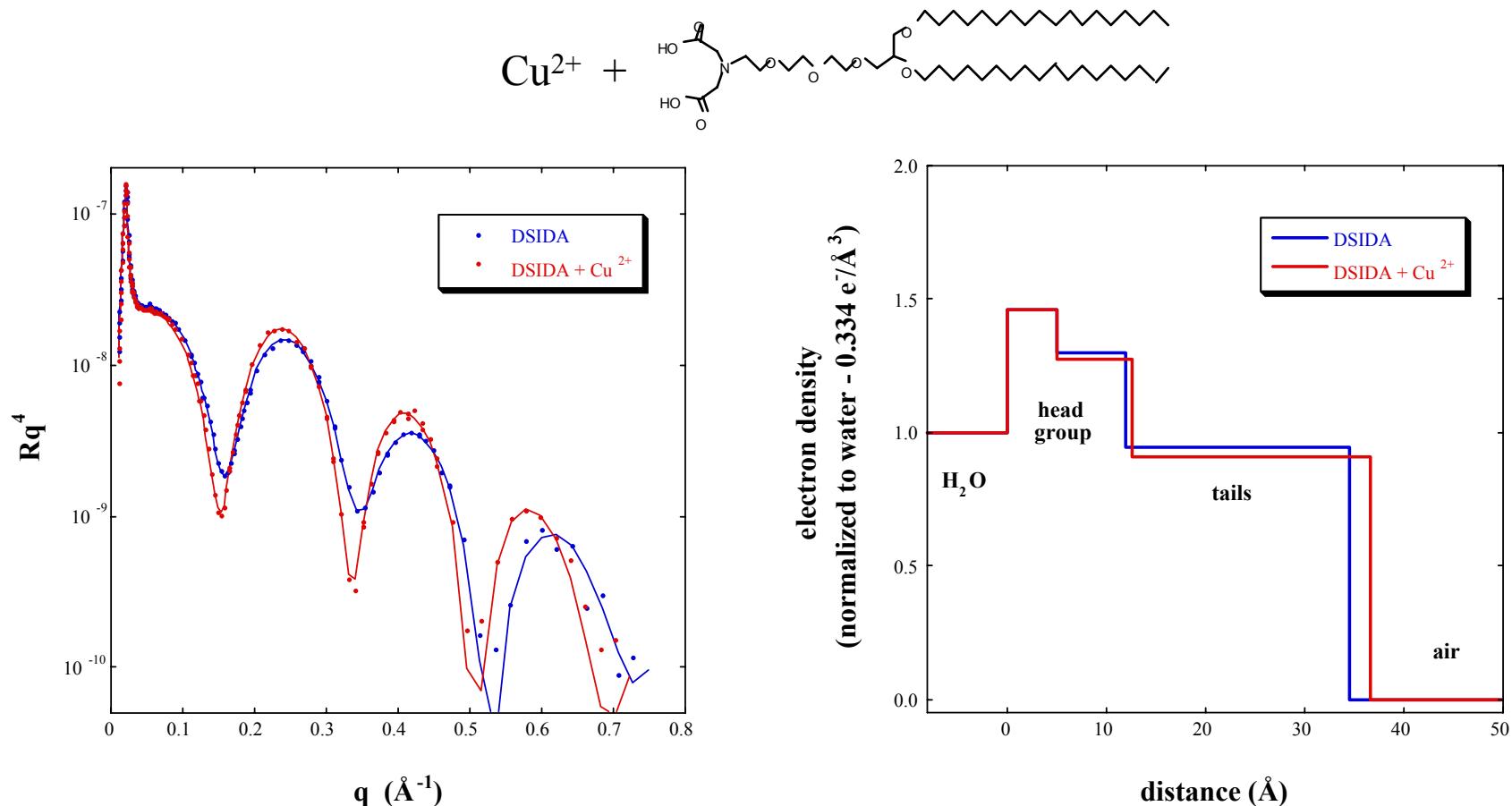


DSIDA

tail layer constrained
by area/molecule
and # of electrons

three layers are required to describe the lipid monolayer

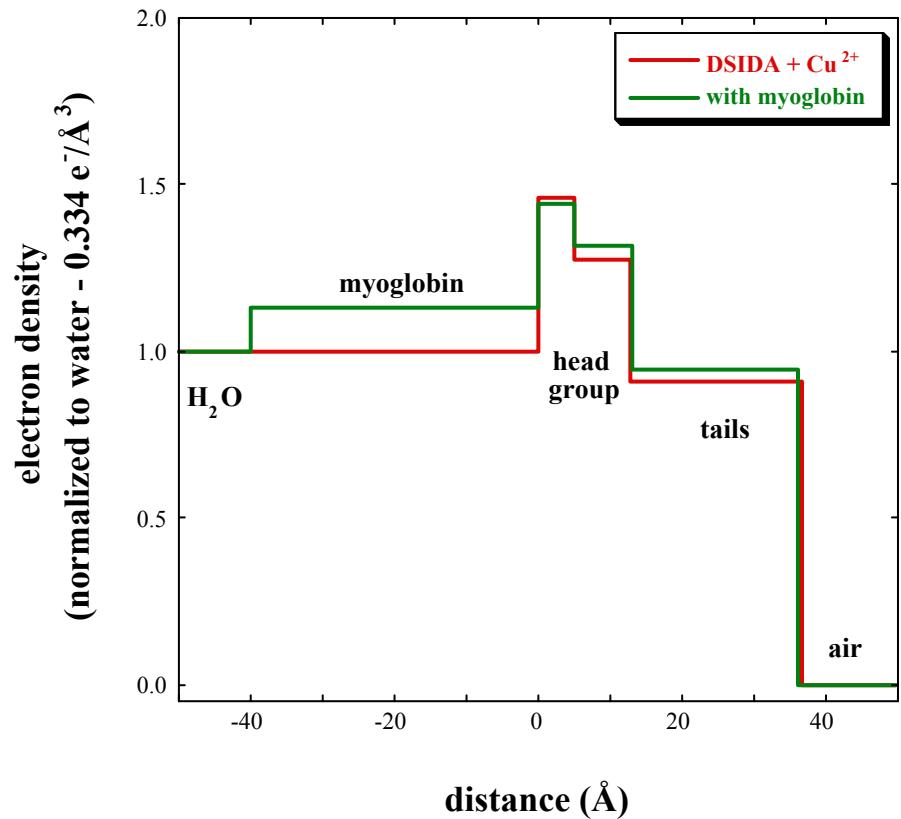
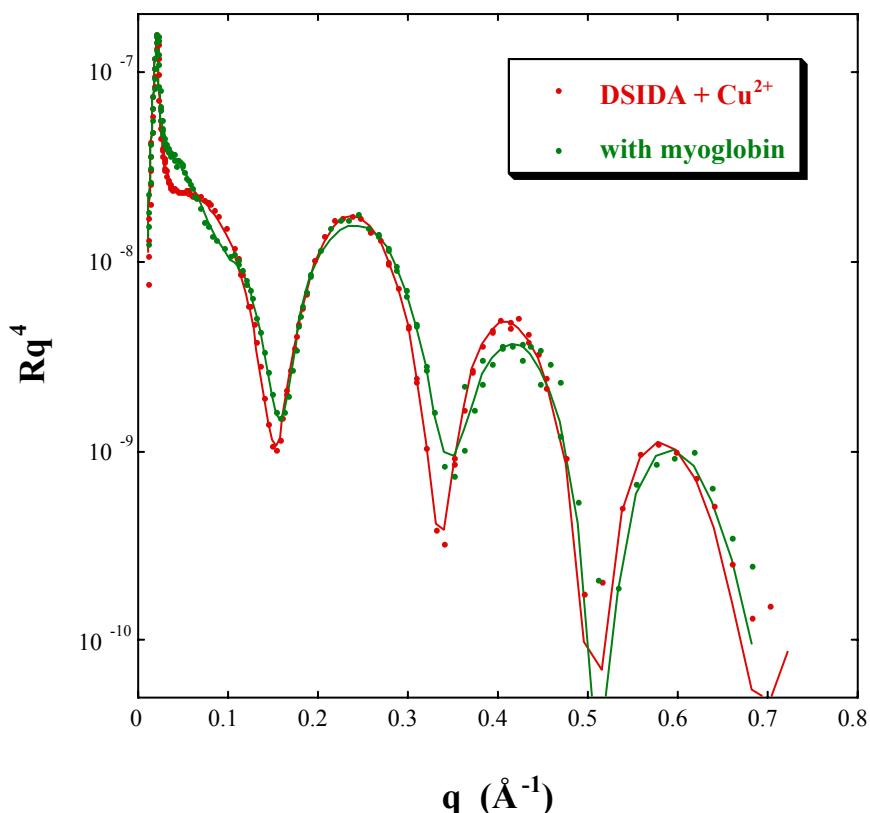
Expt #2. X-ray reflection



adsorption of Cu^{2+} causes a reduction in the tilt angle,
consistent with Bragg rod analysis

Expt #2. X-ray reflection

3 .7 hrs after injecting myoglobin

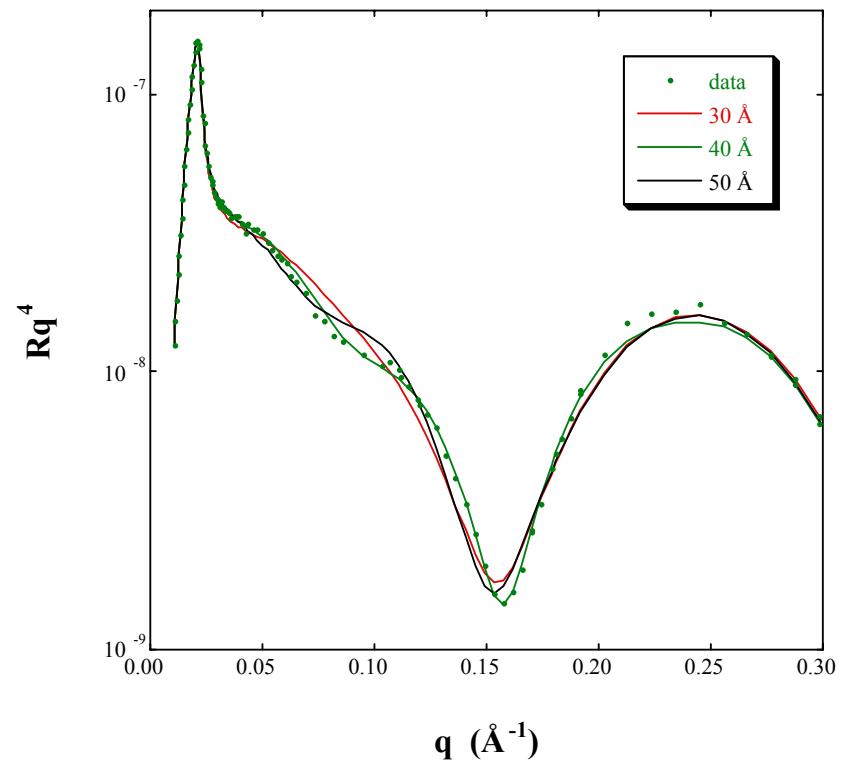
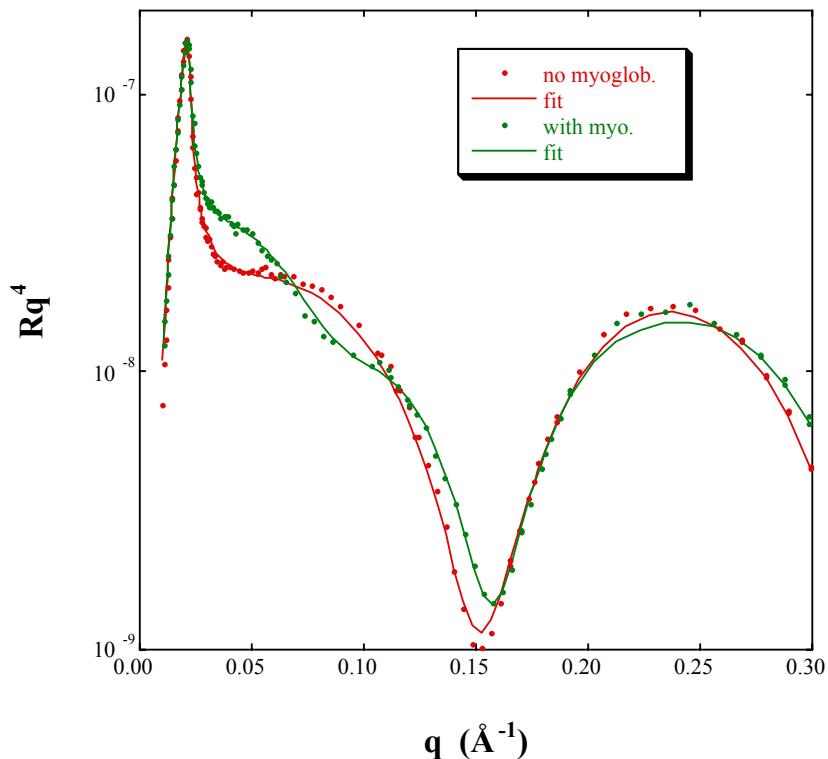


protein layer can be observed! thickness = 40 \AA , vol. fract. = 0.31



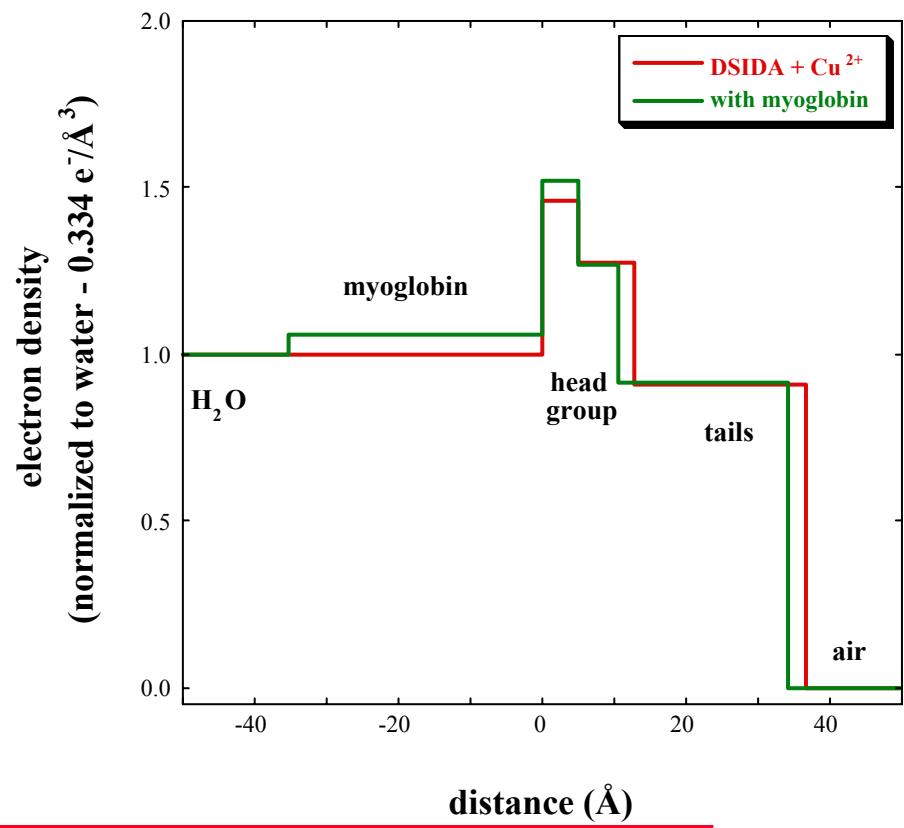
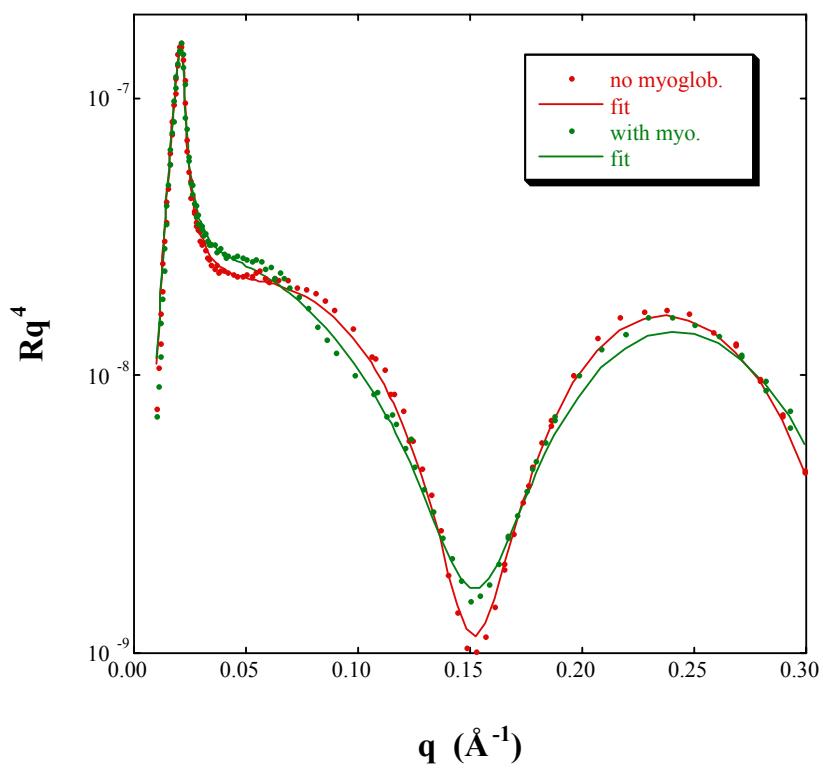
#2. X-ray reflection

How accurate is the thickness obtained? ($\pm 5 \text{ \AA}$)



#2. X-ray reflection

0.9 hrs after injecting myoglobin



much less adsorbed protein: thickn. and vol. fract. uncertain

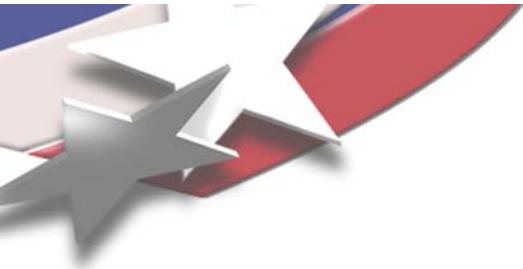


#1 and 2. GIXD and X-ray reflection

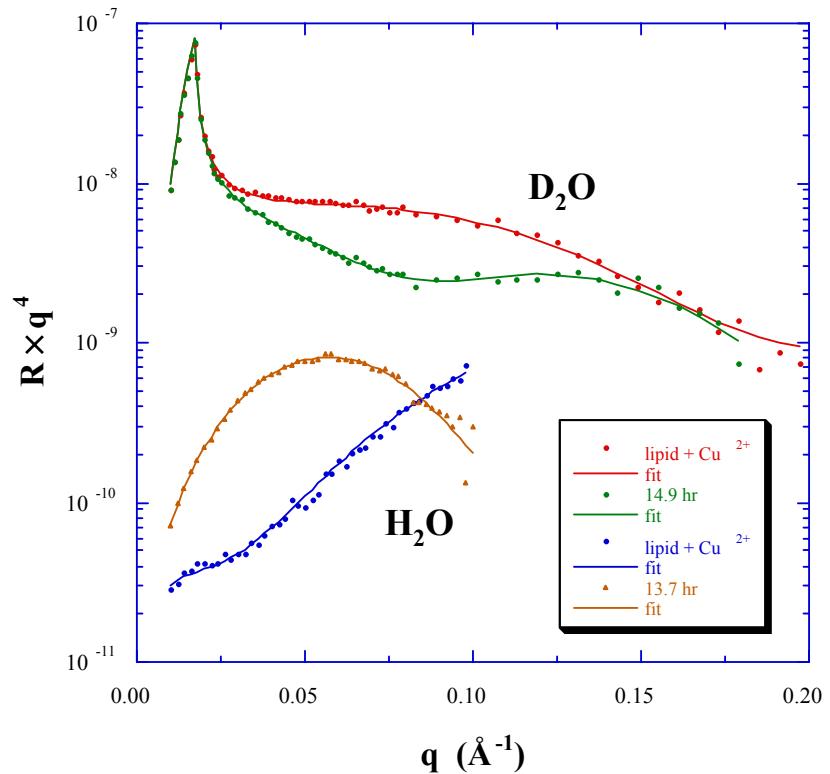
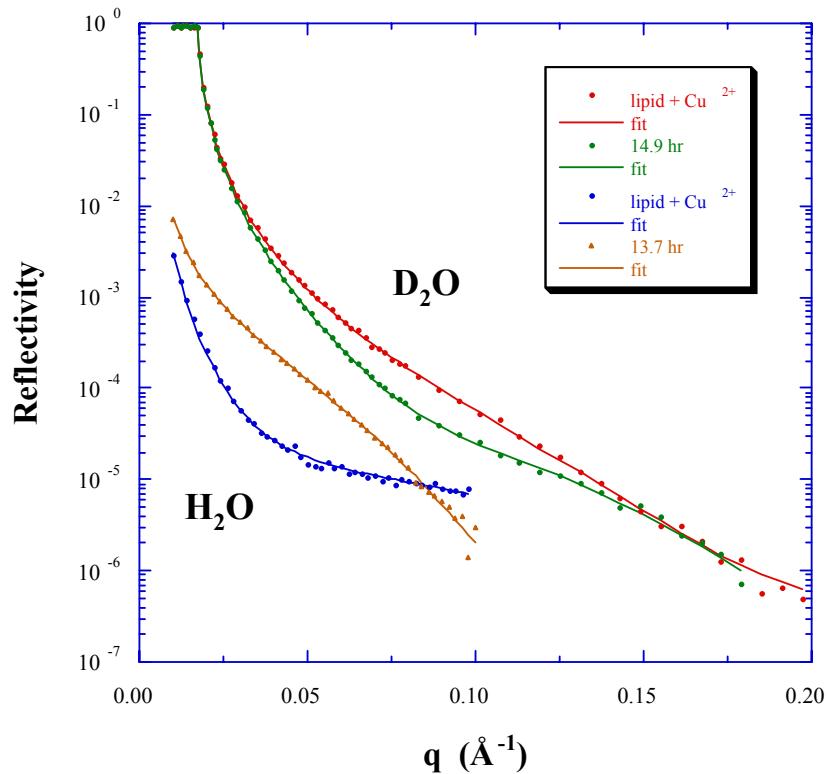
SUMMARY:

Adsorbed amount of protein is small when the diffraction peak disappears!

Reappearance of diffraction peak coincides with high adsorbed amount of protein

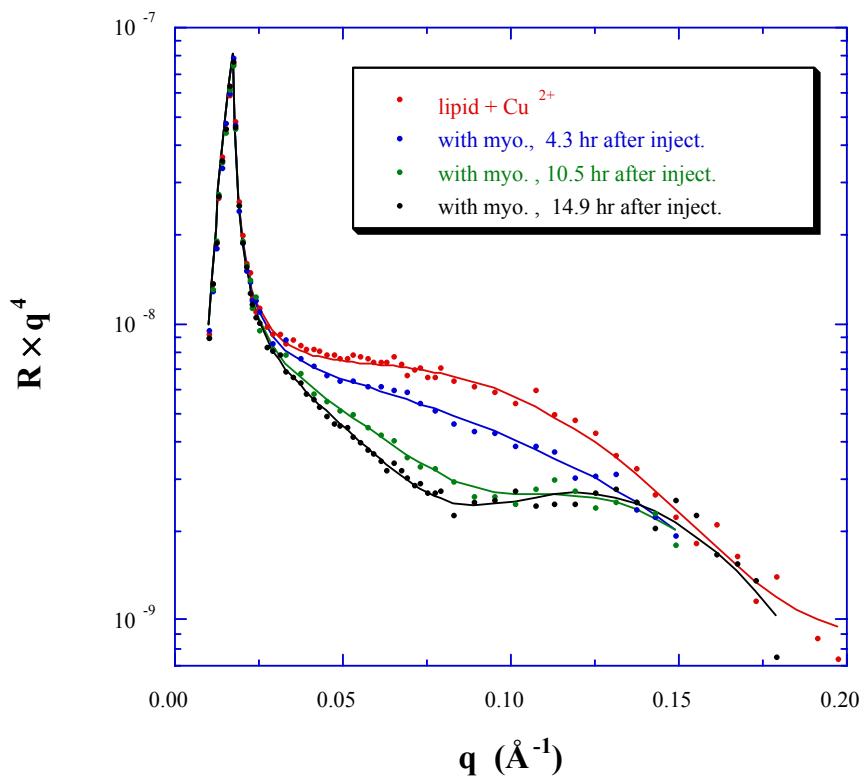


Expt #3 and 4. Neutron reflection



neutron reflection has much greater sensitivity to the protein!

Expt #3. Neutron reflection - D₂O subphase



Protein layer

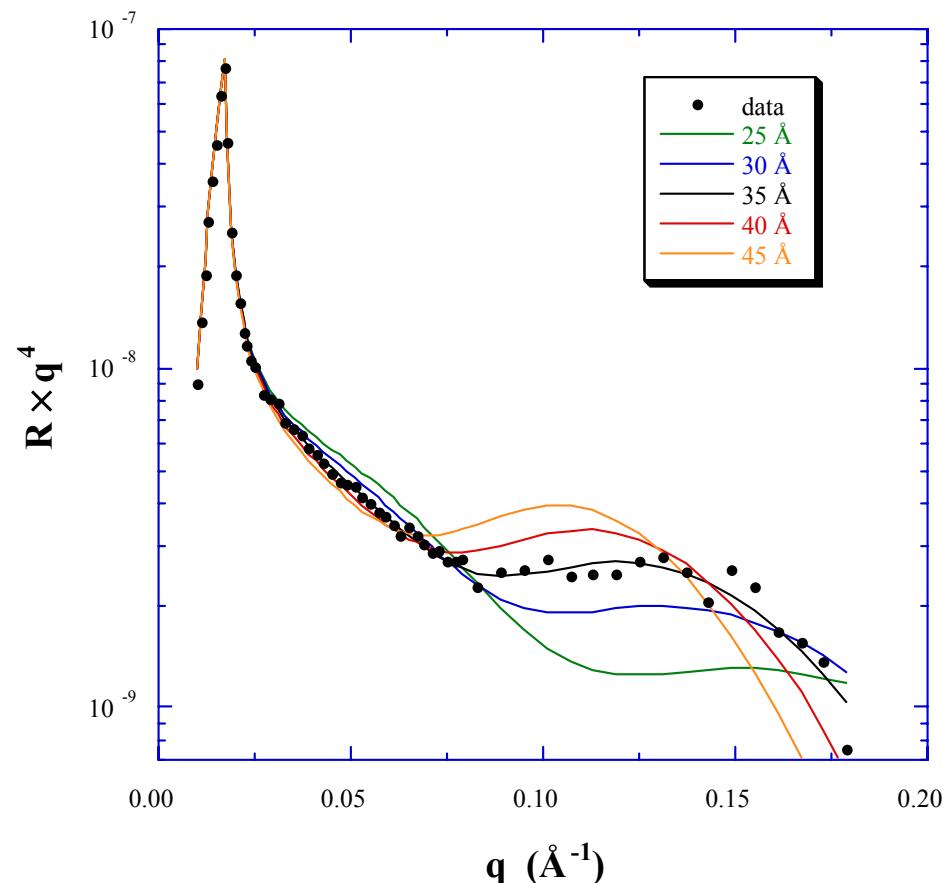
	thickness	ϕ
4.3 hr:	$30 \pm 5 \text{ \AA}$	0.18
10.5 hr:	$30 \pm 3 \text{ \AA}$	0.55
14.9 hr:	$35 \pm 2 \text{ \AA}$	0.55

protein layer reaches quasi-equilibrium at ~ 15 hrs



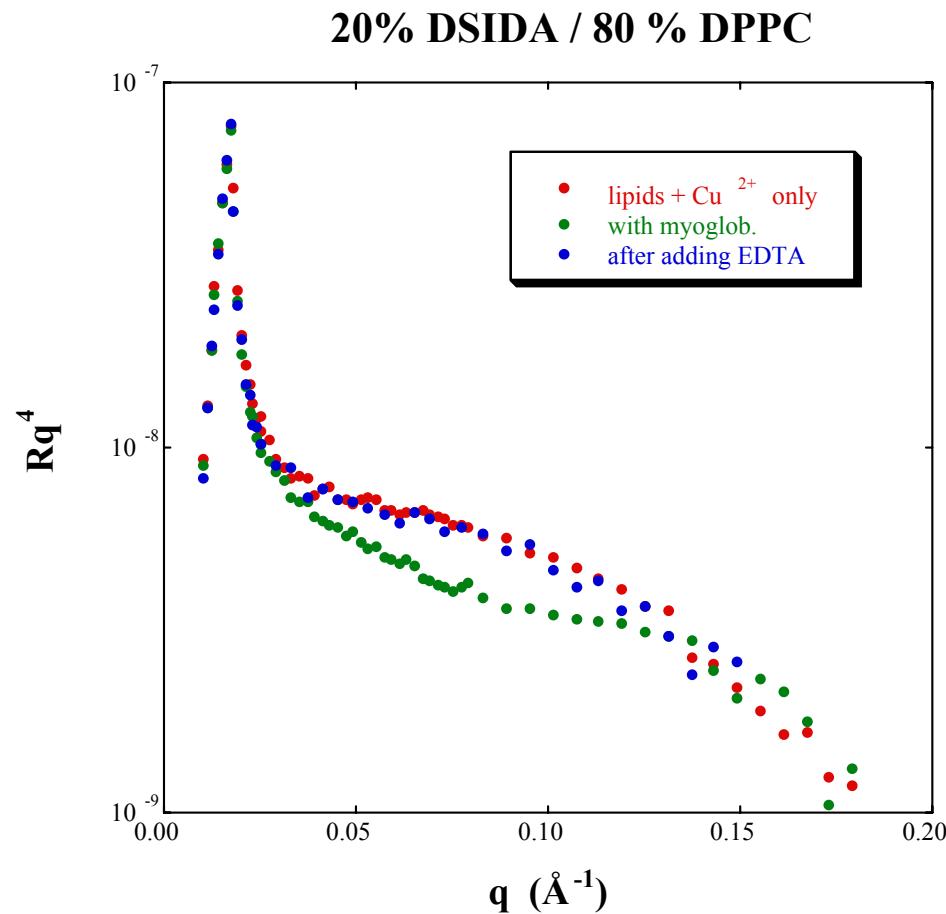
Expt #3. Neutron reflection - D₂O subphase

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

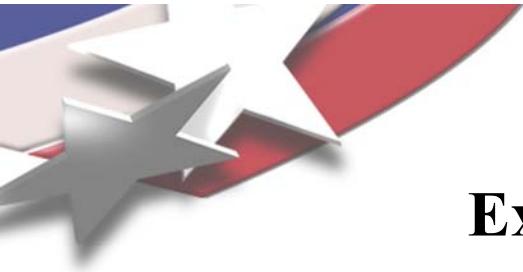




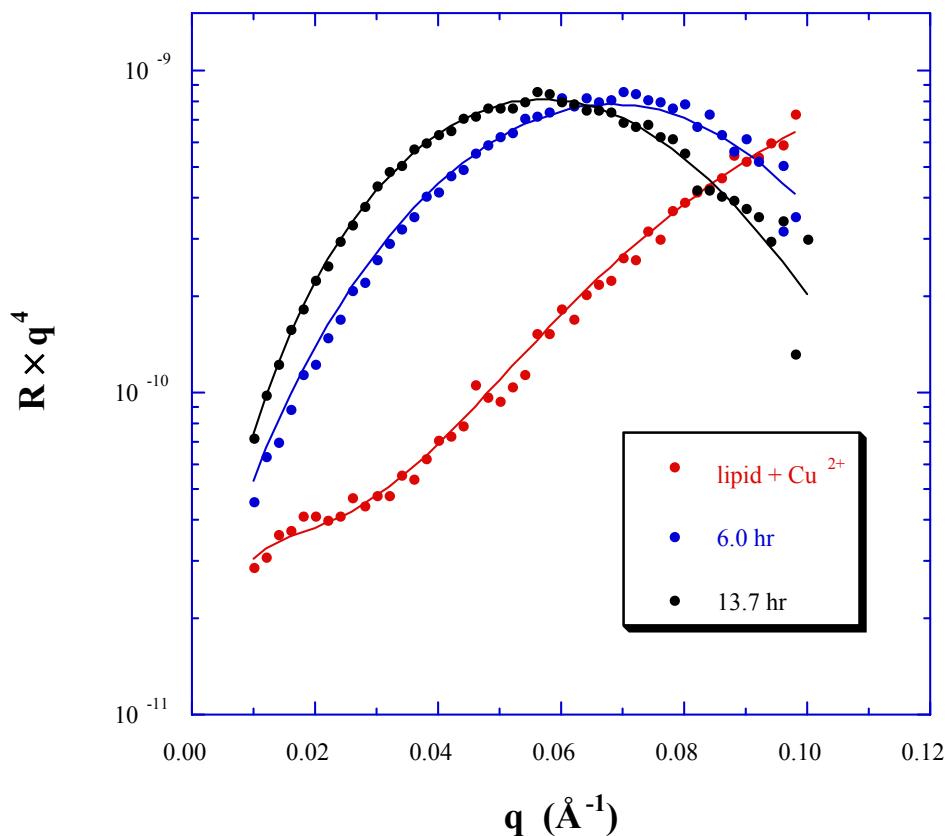
Control experiment



Adsorbed myoglob. removed upon addition of EDTA



Expt #4. Neutron reflection - H₂O subphase



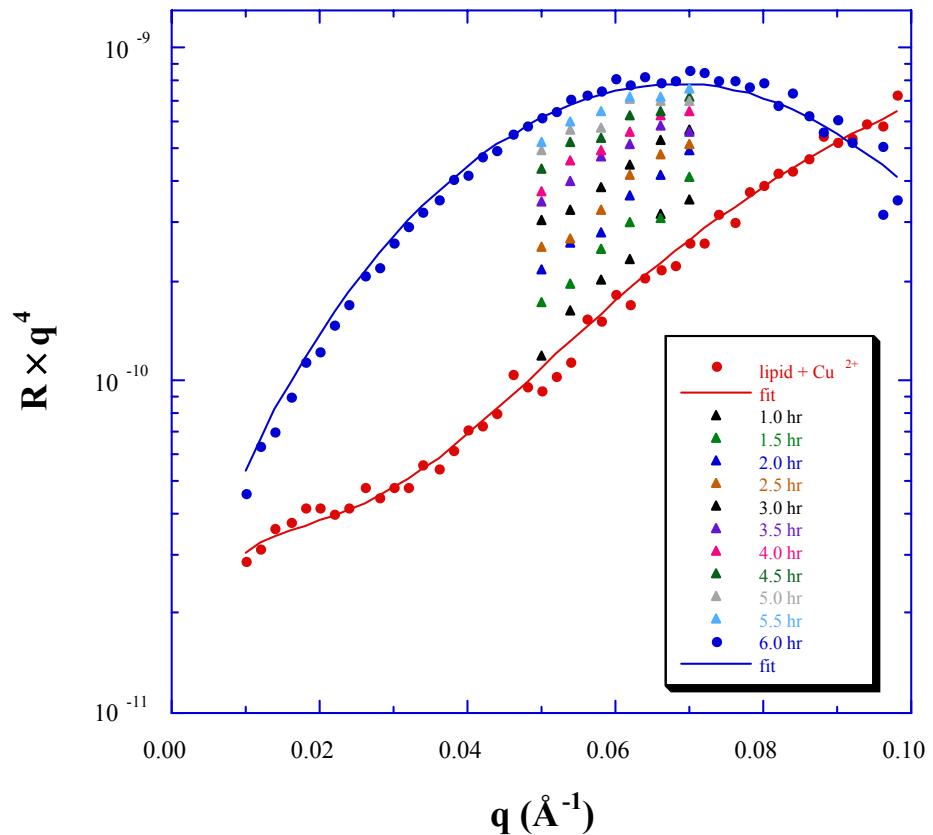
Protein layer

	<u>Thickness</u>	ϕ
6.0 hr:	37 +/- 3 Å	0.38
13.7 hr:	40 +/- 2 Å	0.55

protein layer reaches quasi-equilibrium at ~ 14 hrs

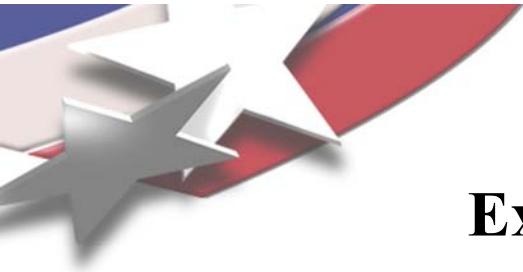


Expt #4. Neutron reflection - H₂O subphase

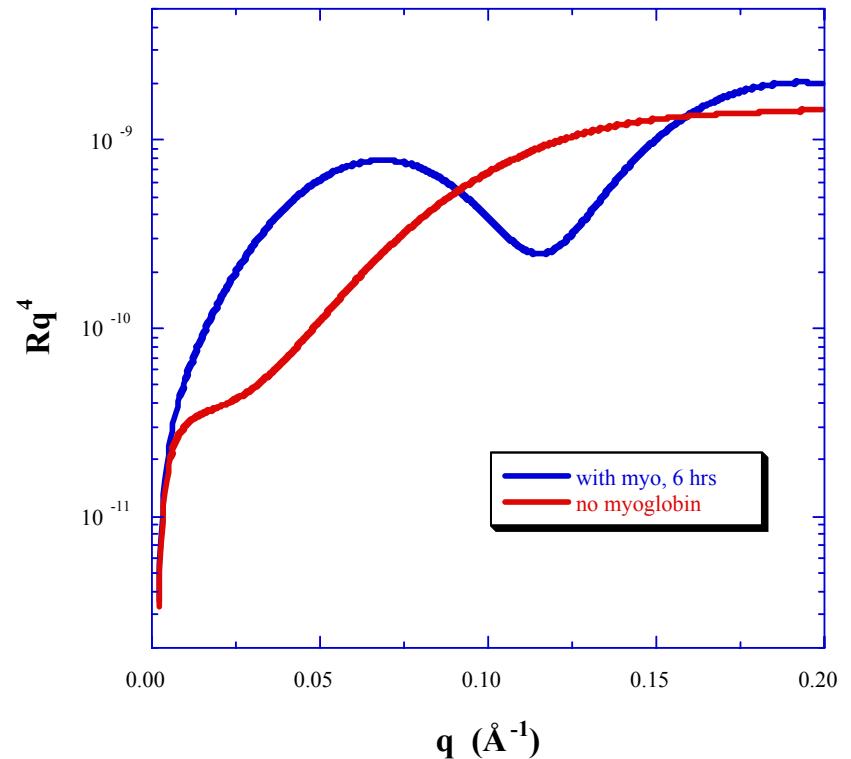
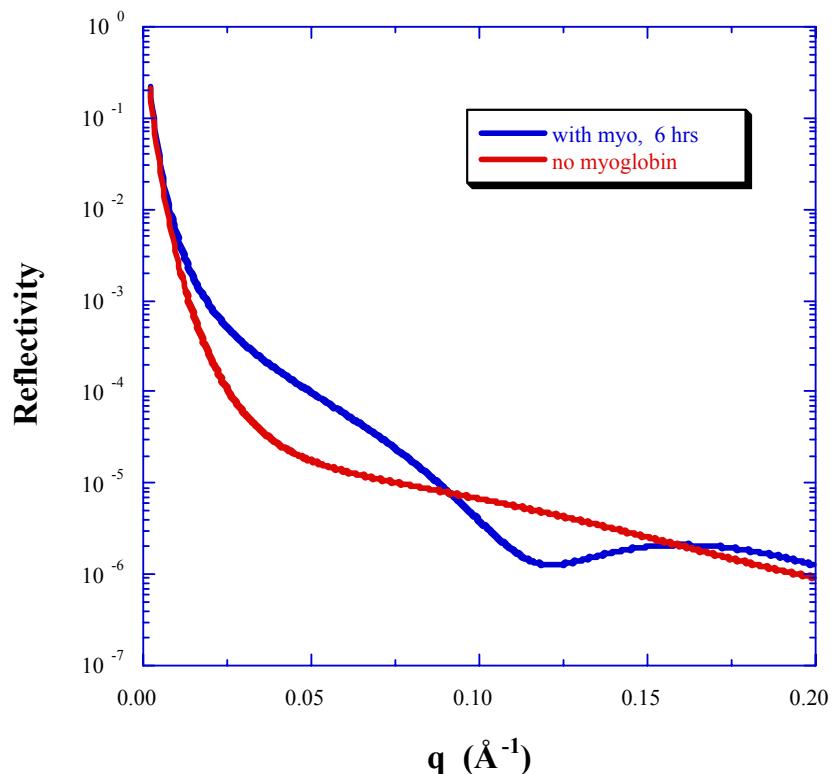


low adsorbed amount
(\ll monolayer)
at 2.0 hr when diffraction
peak vanished!

can't get the details of the protein conformation at early times with current sources - need SNS!



Expt #4. Neutron reflection - H₂O subphase

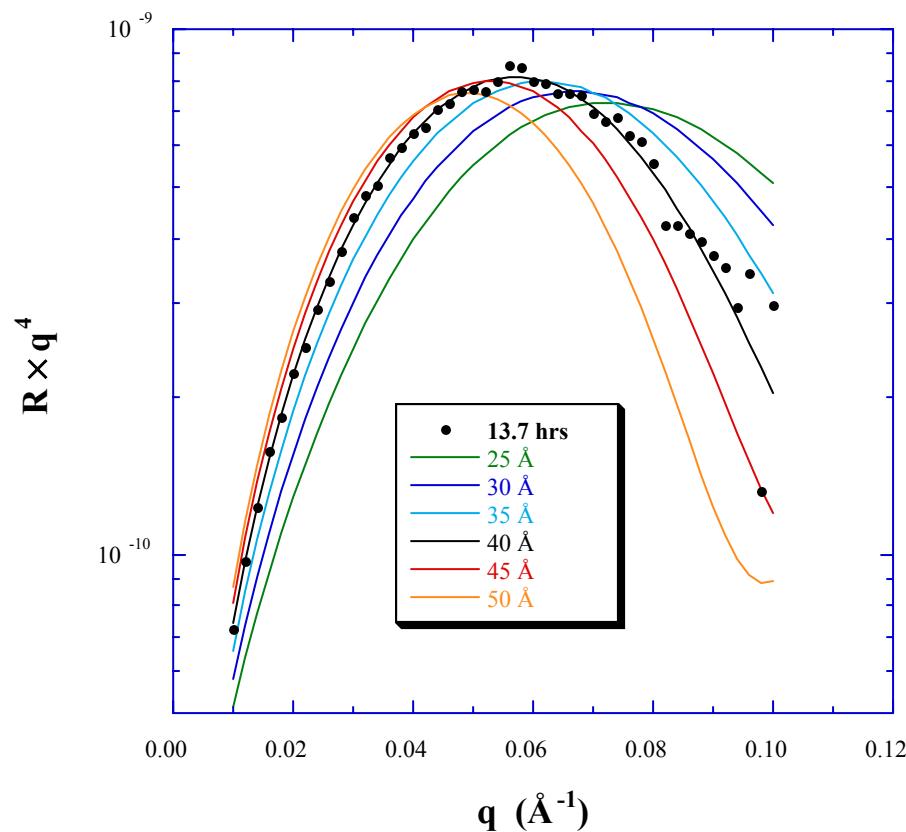


need to get to $q = 0.2$ (refl. = $1\text{e-}6$) in 15 minutes!



Expt #4. Neutron reflection - H₂O subphase

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





Summary

short time (< 2 hr)

- low adsorbed myo. seg. density, thick./orientation unknown
- myo./lipid interaction leads to near complete loss of crystallinity within the lipid layer

long time (> 4 hrs.)

- lipid layer regains 2-D crystallinity after ~ 3-4 hrs
- protein has formed a dense packed monolayer under DSID □ A
quasi-equilibrium after ~ 13 hrs.
thick = 40 Å, seg. dens. = 0.55, **adsorbs side-on**
no multilayers



Some Interesting Questions

1. Why/how is the crystallinity disrupted and why does it reform?
Would same effect occur without Cu²⁺ ions or for DPPC?

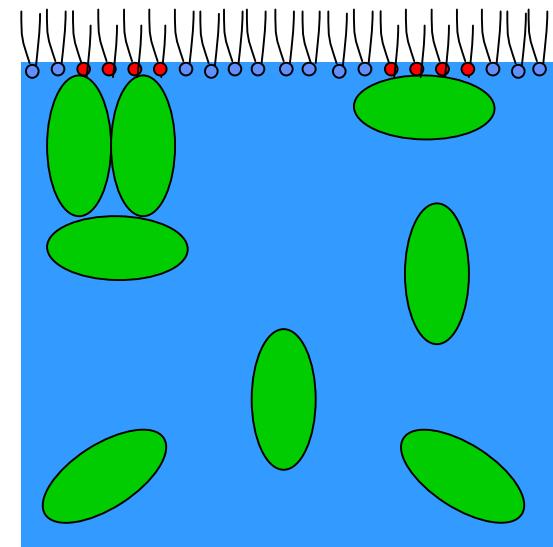
2. What is the protein layer like that initially interacts with DSIDA and disrupts the crystallinity?
-How can all crystallinity be disrupted by only a low density of protein?
-Is there a precursor adsorbed layer of myoglobin with other than the native conformation?



Results - 2nd system

**Myoglobin adsorbing to 20%/80%
PSIDA/DPPC film at 35 mN/m**

1. grazing incidence X-ray diffraction
2. X-ray reflection
3. neutron reflection - D₂O subphase

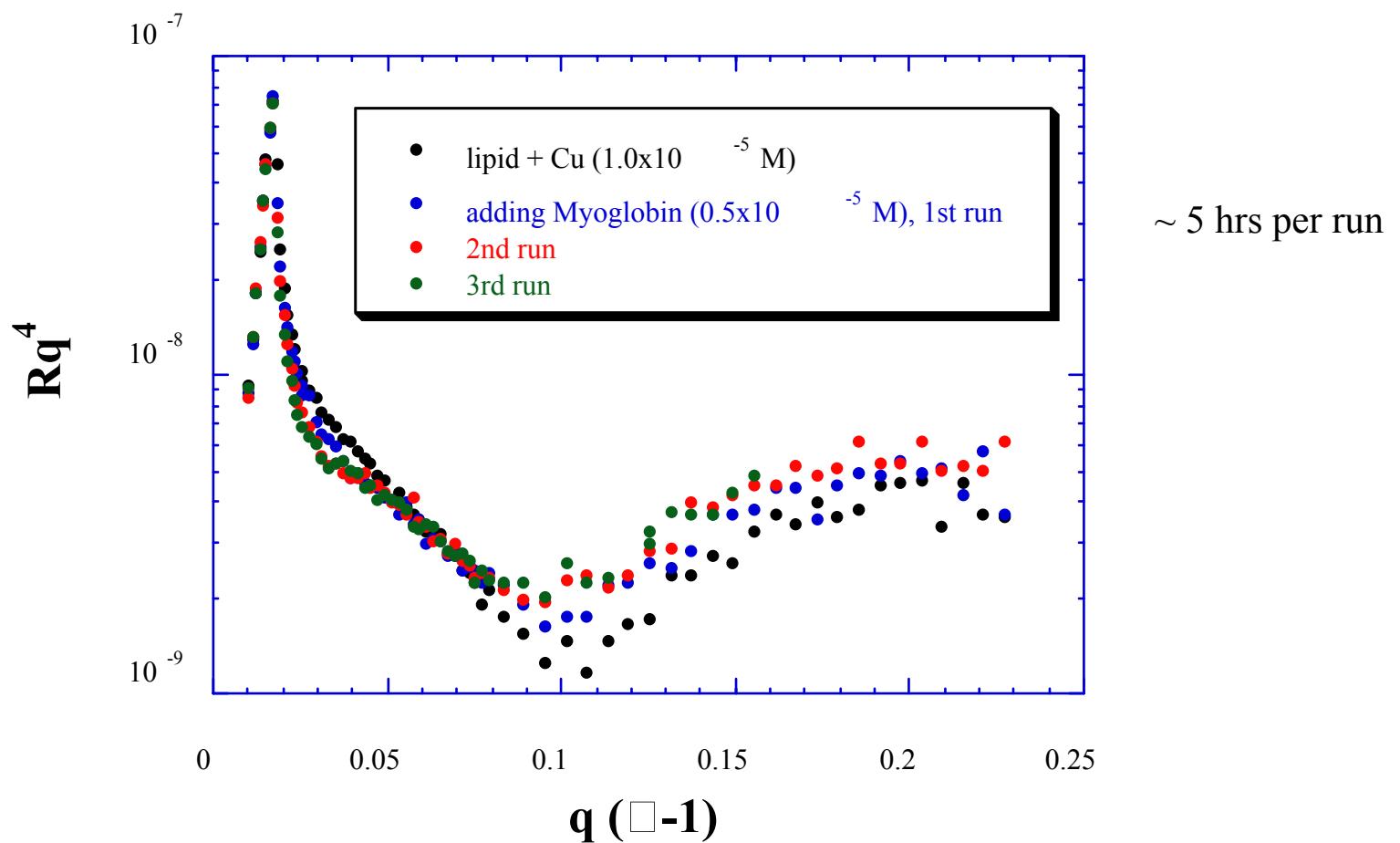


lipids segregate in-plane
to form domains

**Examine both the adsorbed layer of myoglobin and the
response of the lipid layer**

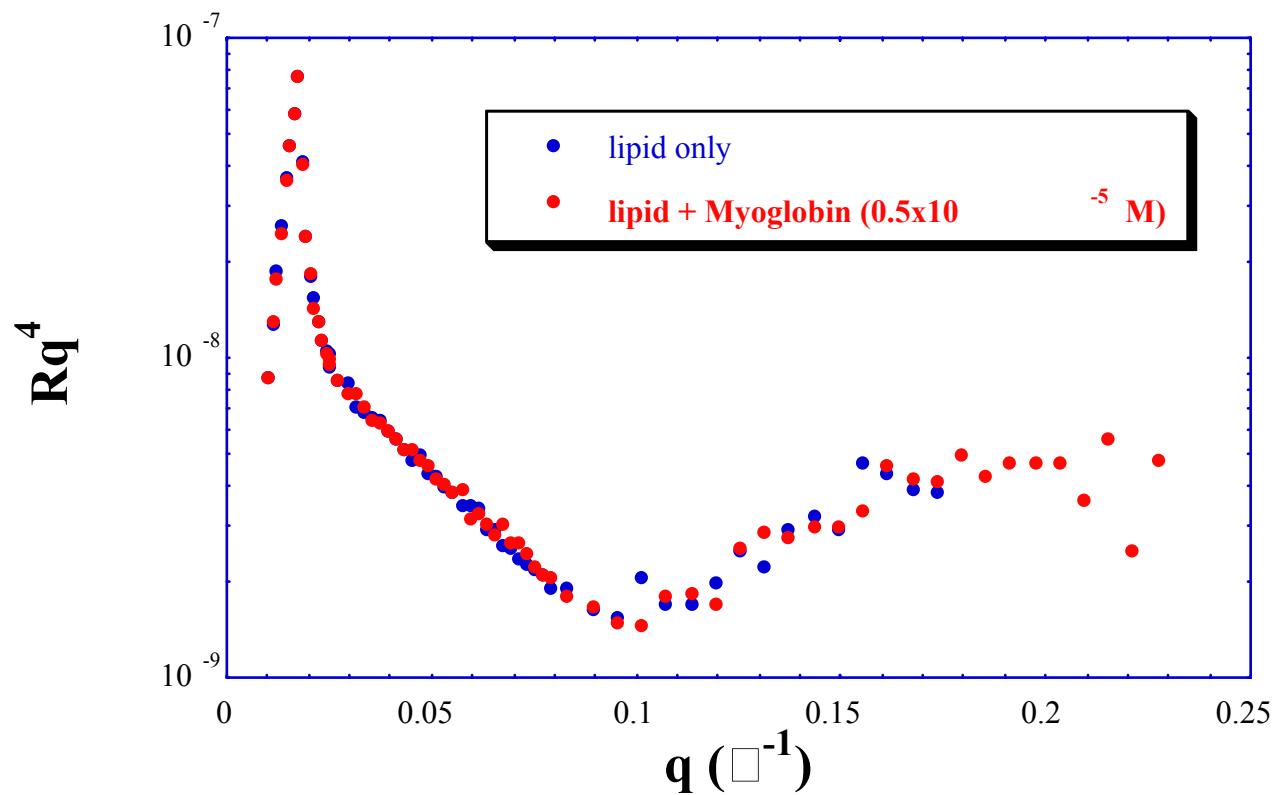


20/80 PSIDA/DPPC: time dependence





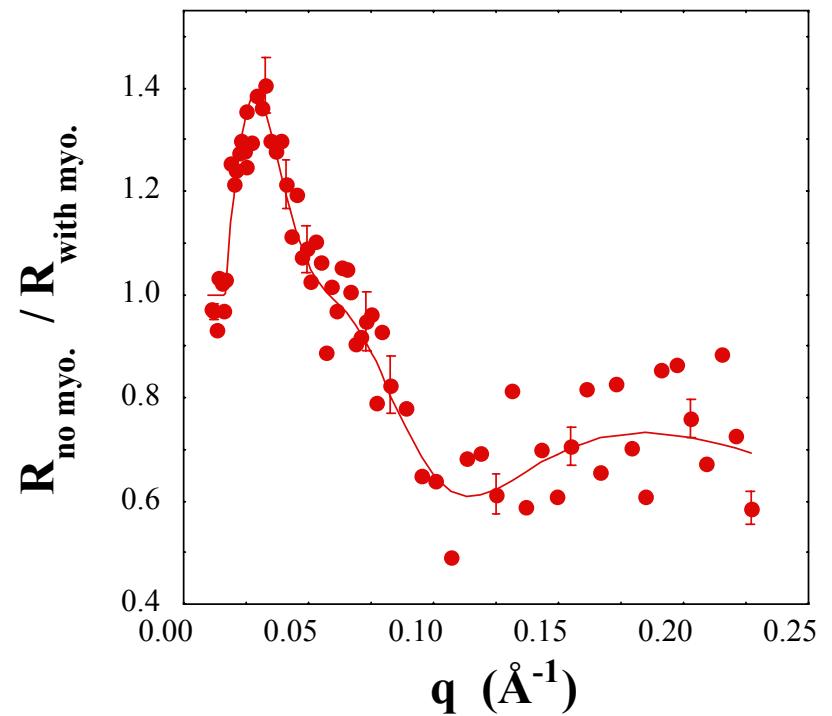
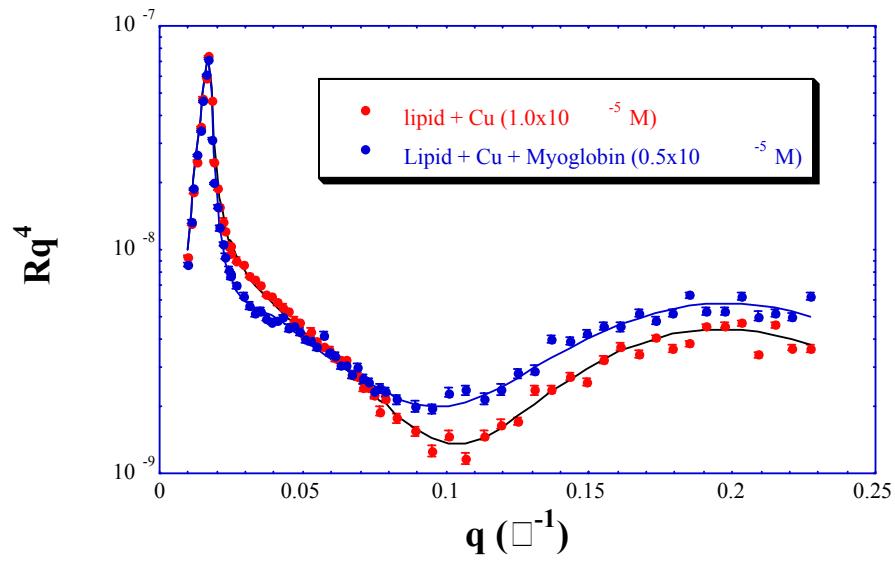
Control experiment



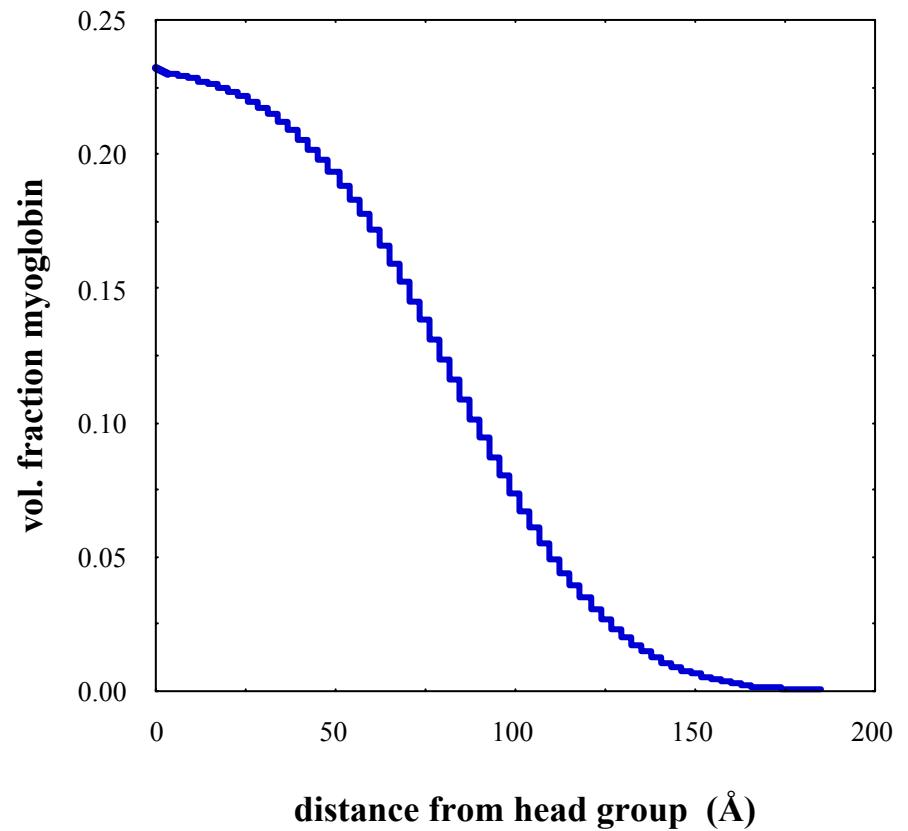
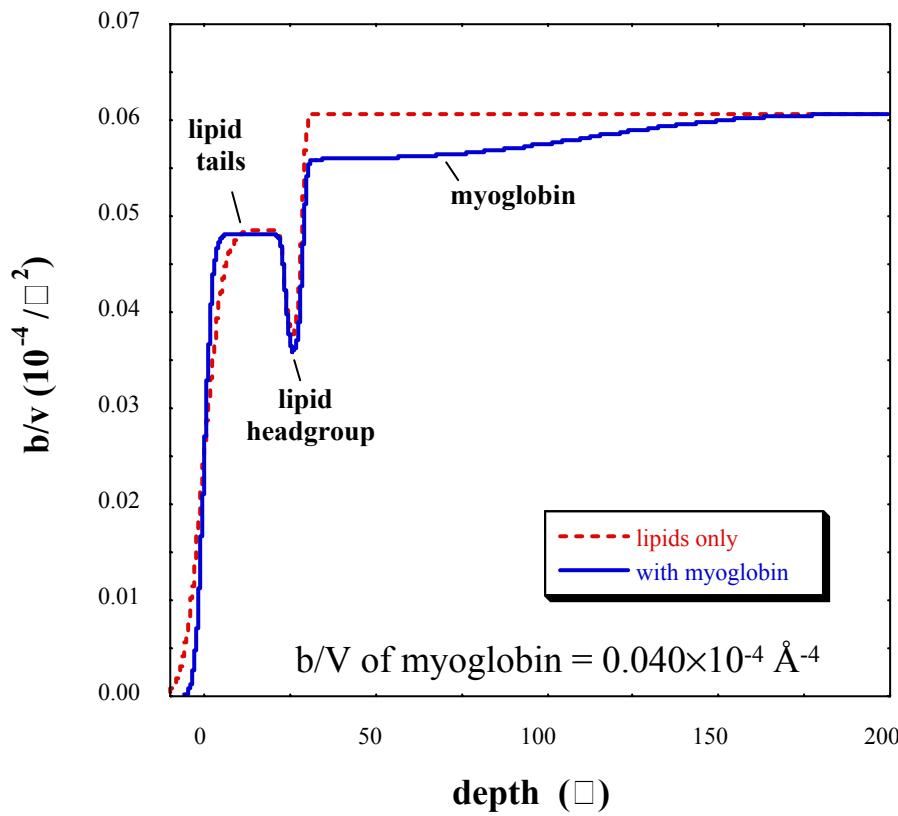
No myoglobin is detected in the absence of Cu^{2+}



Final equilibrium state

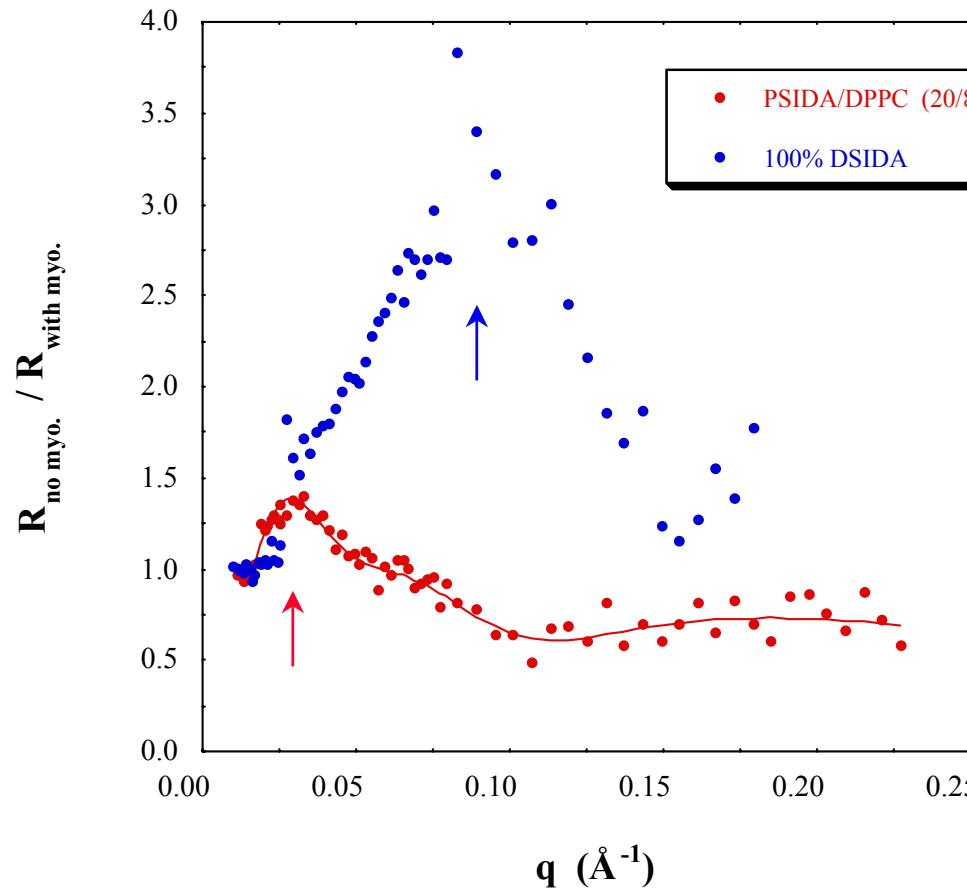


Fitting results



The maximum segment volume fraction is lower than in the crystalline unit cell. The thickness is larger than the maximum crystalline unit cell dimension.

Final equilibrium state



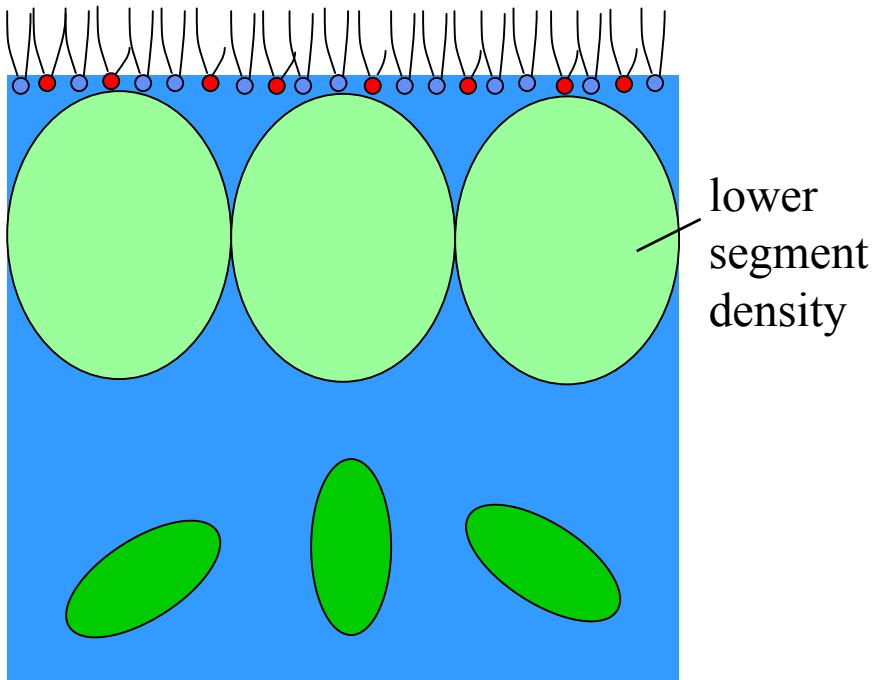
peak positions
indicate much
diff. thicknesses:

PSIDA/DPPC - 87 \AA
DSIDA - 40 \AA

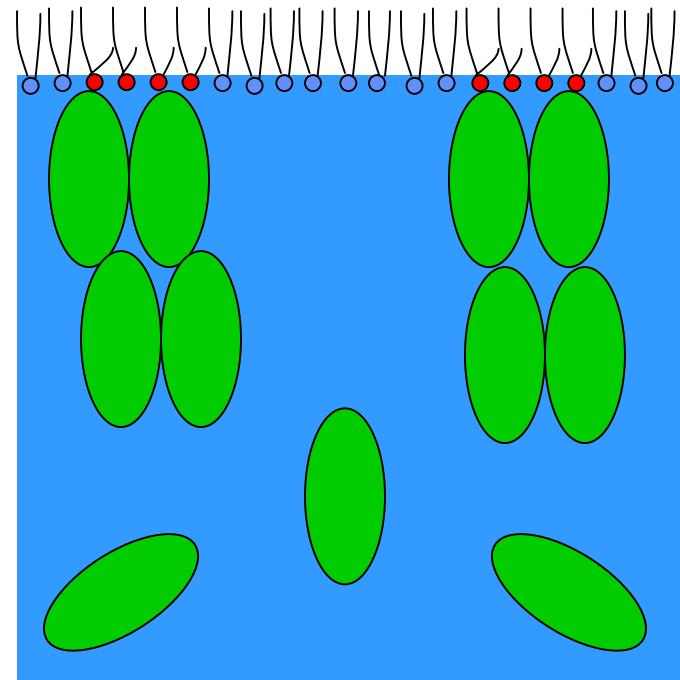
the adsorbed protein layers are very different in these two cases



Possible interpretations



Lipids dispersed, protein unfolds
to some extent upon adsorption



In-plane lipid domains,
multilayers of protein



Conclusions

1. **Protein adsorption to lipid films (conformation and dynamics) can be studied by these techniques**
(many other examples, need more neutron flux to get the whole picture)

2. **With X-rays, can follow and correlate lipid response along with protein adsorption**

3. **Myoglobin adsorbs to DSIDA film in a side-on conformation at quasi-equilibrium**

4. **The adsorbed layers are very different for 100% DSIDA and the 20%/80% PSIDA/DPPC mixture.**



Future Work

Lipid mixtures

(crystalline-amorphous, crystalline-crystalline, amorphous-amorphous)

Different metal ion

(earlier data suggests that protein conformation is different with Ni^{2+} rather than Cu^{2+})

Different or modified proteins

Toxins (pertussis toxin, B oligomer; tetanus toxin, C-fragment)

interacting with Langmuir monolayers, supported lipid bilayers