HIV-1 gag protein insights into viral assembly

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Outline

Modeling protein in solution

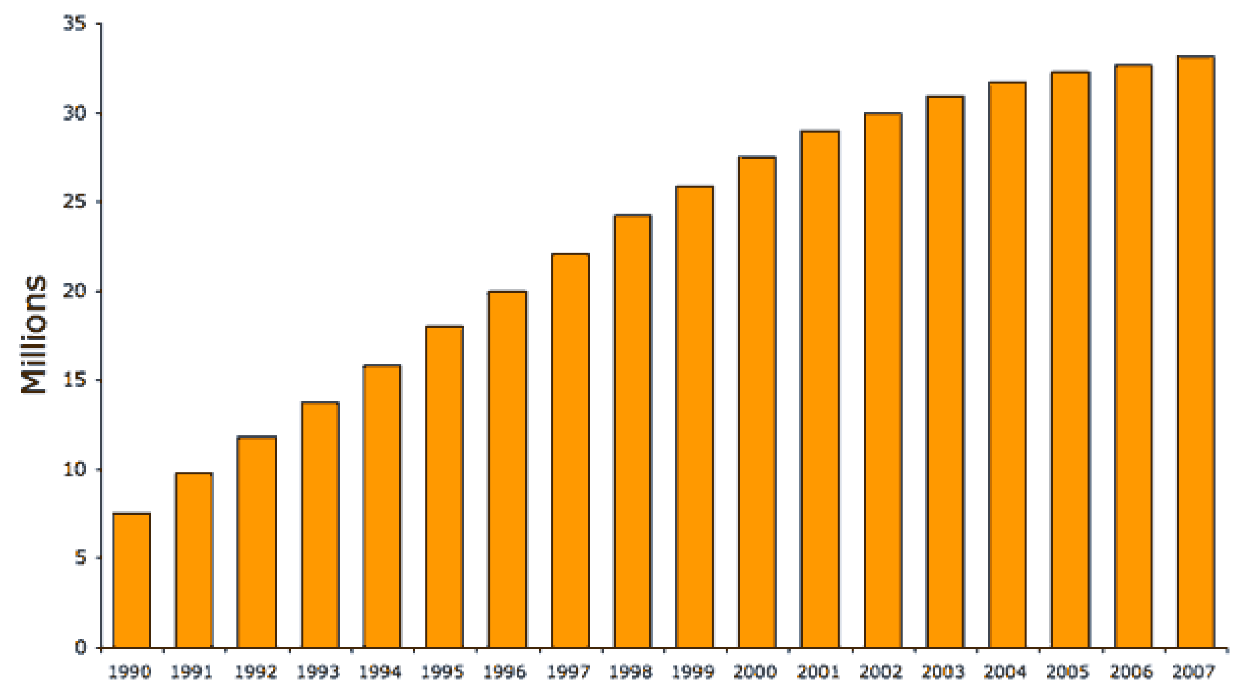
- SANS
- SASSIE

Model membrane formation

- Solid supported membranes
- Neutron reflectivity

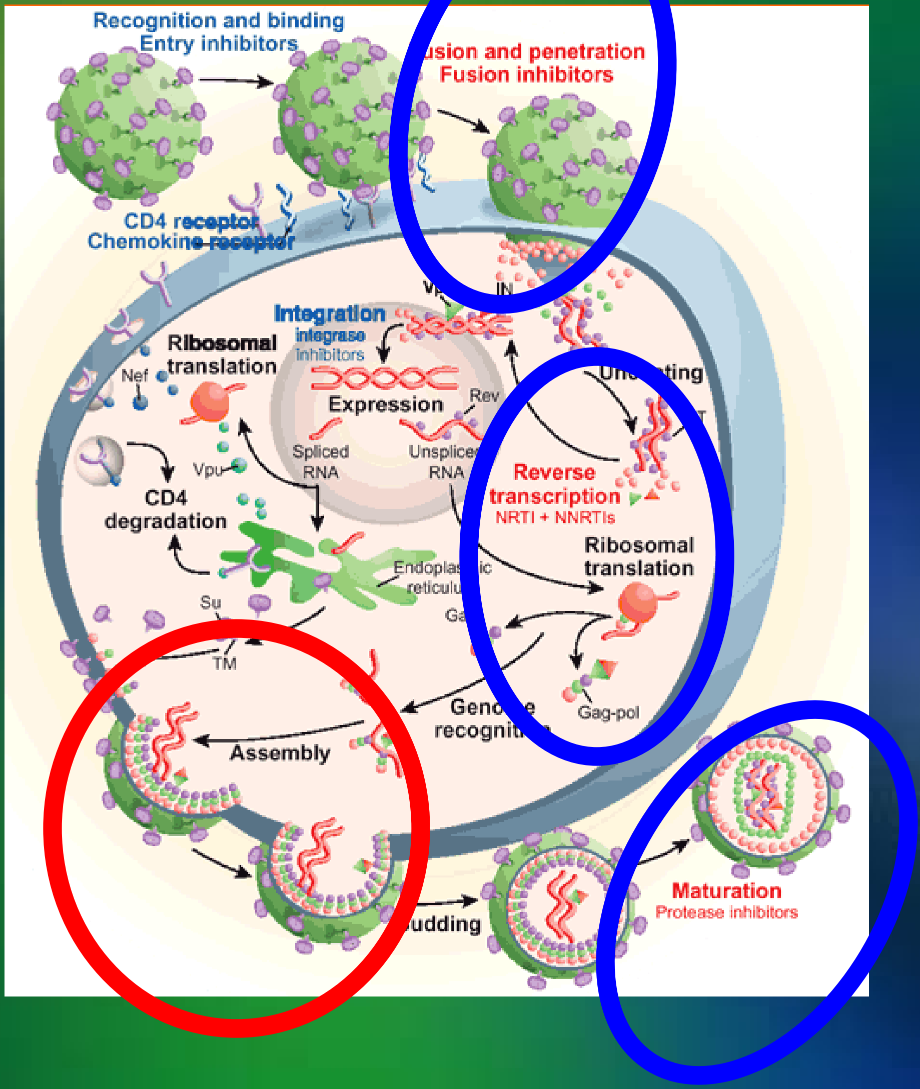
Human Immunodeficiency Virus

- Retrovirus (RNA based virus) which causes AIDS.
- Originated in Africa and detected in the United States in the early 80’s.
- 33.2 million people living with AIDS in 2007 and of those 2.1 million died



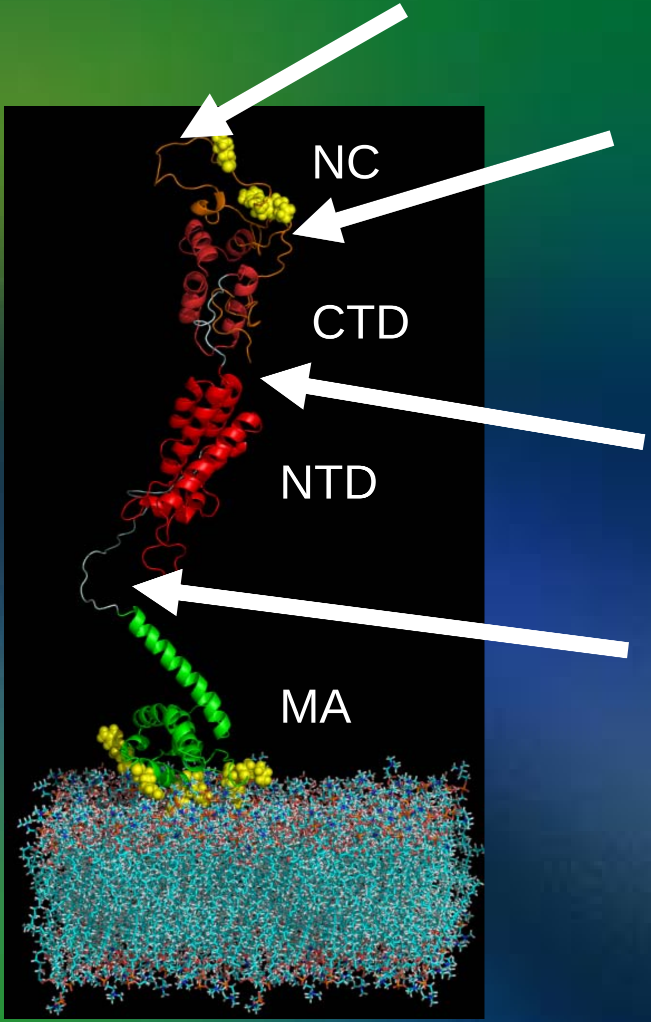
The Viral Life Cycle

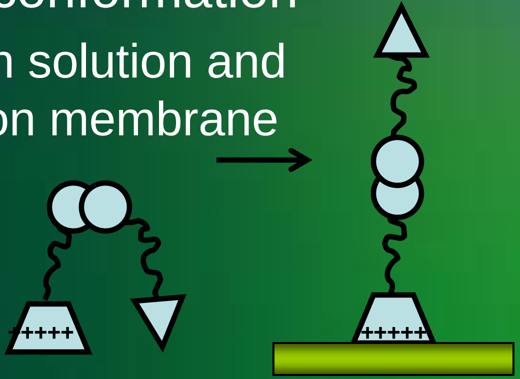
- Viral Life Cycle
- Therapeutics
- Why focus on Assembly?
- What do we know?



The Gag Protein

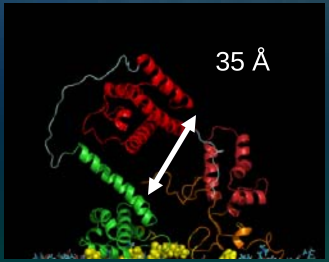
- Three structured domains
  - MA: matrix domain
  - CA: capsid domain
    - Ctd: c-terminal of capsid
    - Ntd: n-terminal of capsid
  - NC: neucleo-capsid domain
- Four Flexible linkers unstructured
  - Variability in conformation
    - Compact in solution and extended on membrane



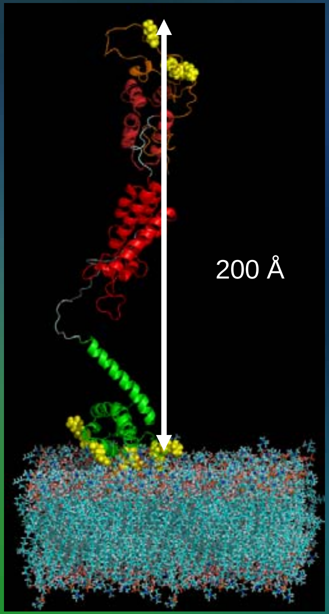


Methods of Studying Proteins

In Solution

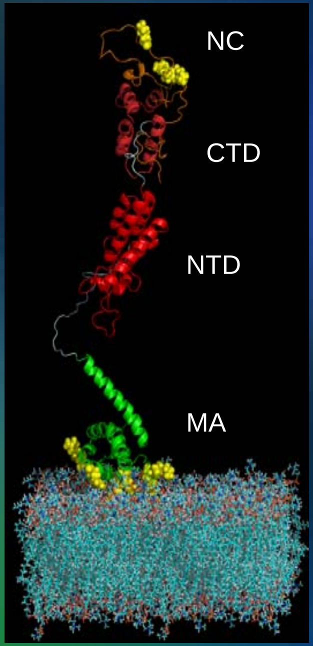


Model Membranes

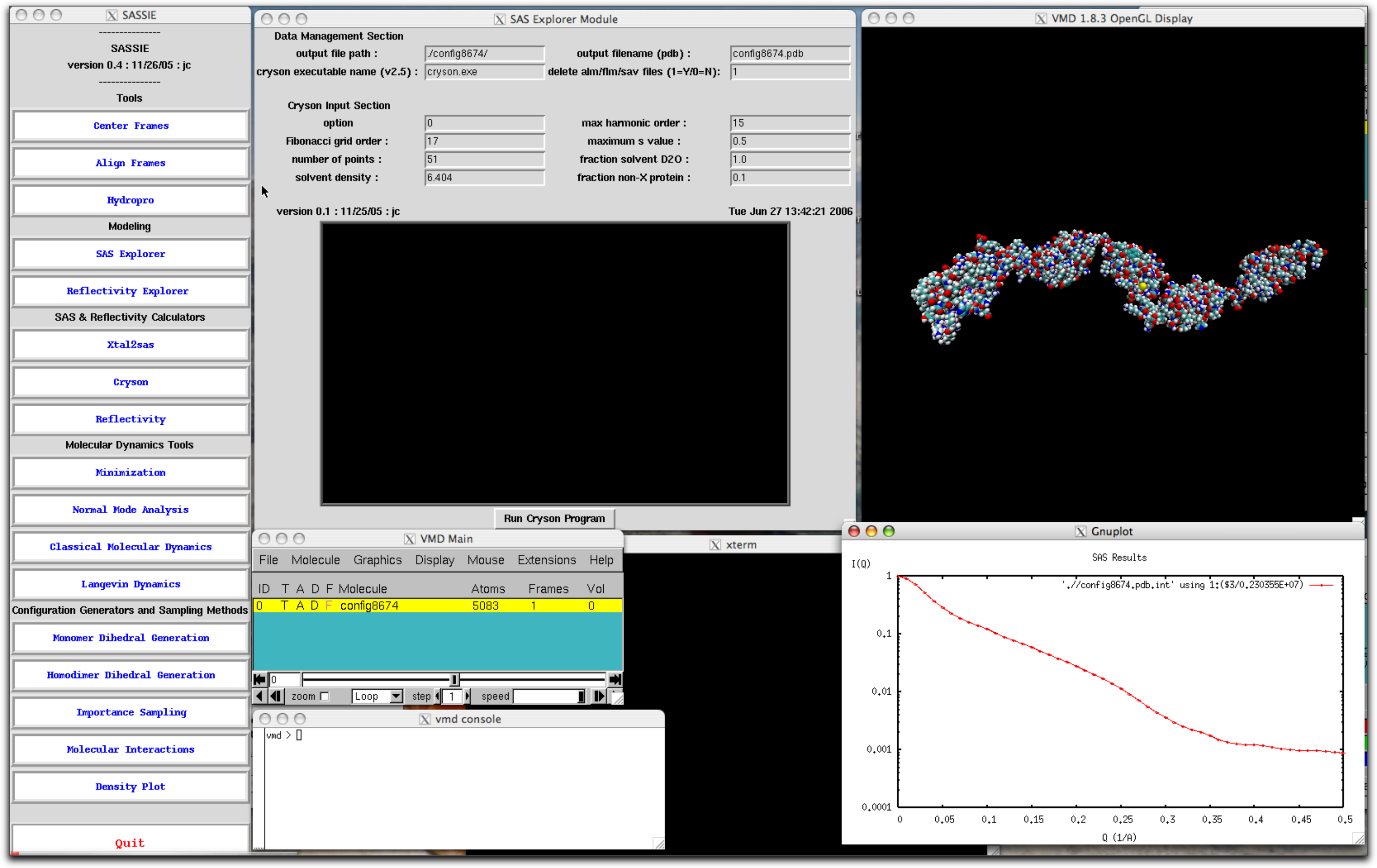


Modeling Gag In Solution

- SANS
- Computational model provide all possible conformations of flexible regions
- Compare SANS data to modeling (chi-squared)
- Characteristics about the protein:
  - Domain-domain interactions
- Regions of study:
  - NTD-CTD (capsid domain)
  - MA-NTD
  - NTD-CTD-NC
  - MA-NTD-CTD-NC (full protein)



SASSIE



SASSIE
version 0.4 : 11/26/05 : jc

Data Management Section
output file path : ./config8674/
output filename (pdb) : config8674.pdb
cryson executable name (v2.5) : cryson.exe
delete alm/flm/sav files (1=Y/0=N): 1

Cryson Input Section
option : 0
max harmonic order : 15
Fibonacci grid order : 17
maximum s value : 0.5
number of points : 51
fraction solvent D2O : 1.0
solvent density : 6.404
fraction non-X protein : 0.1

version 0.1 : 11/25/05 : jc
Tue Jun 27 13:42:21 2006

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
0	T	A	D	F	config8674	5083	1	0

```
vmd >
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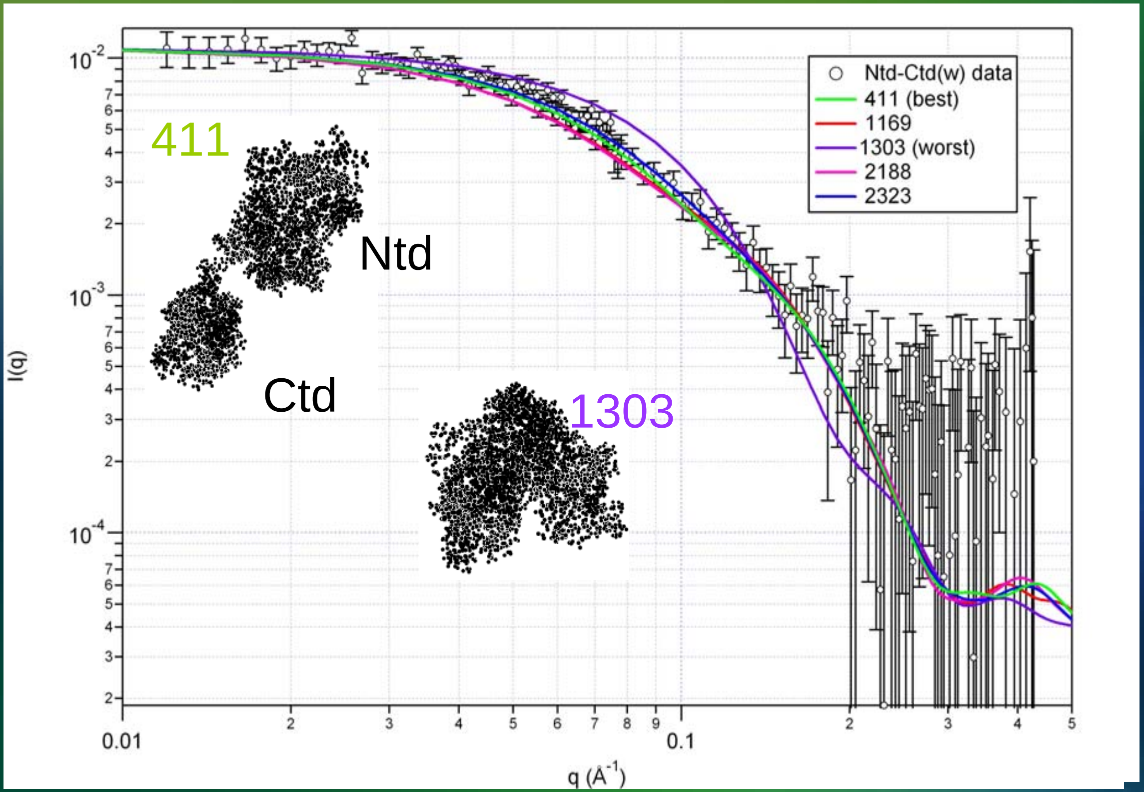
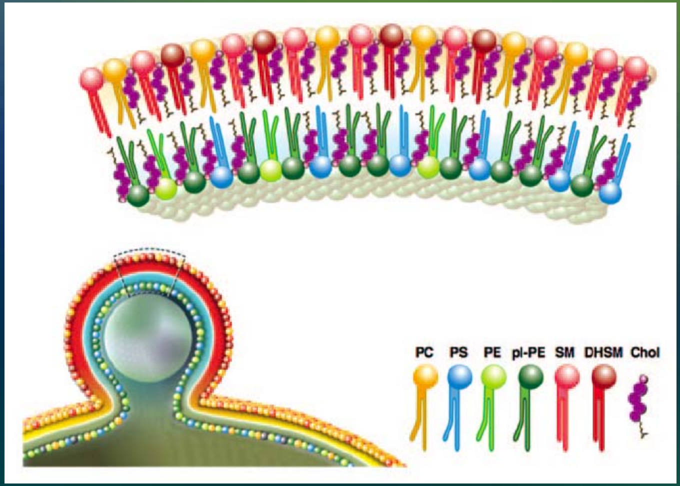
Modeling Ntd-Ctd



Gag-membrane interaction

- Viral Membrane

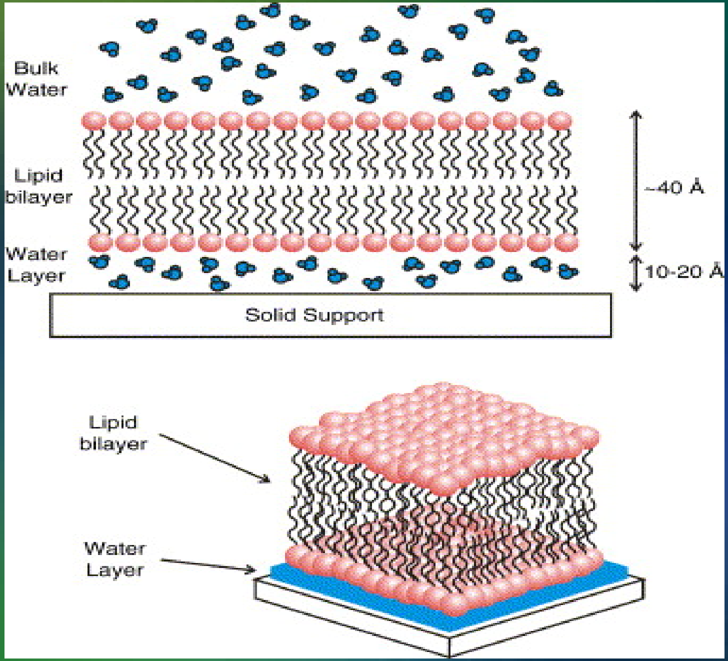


-multi-lipid system

-Asymmetric bi-layer

  -Protein binds to charged leaflet

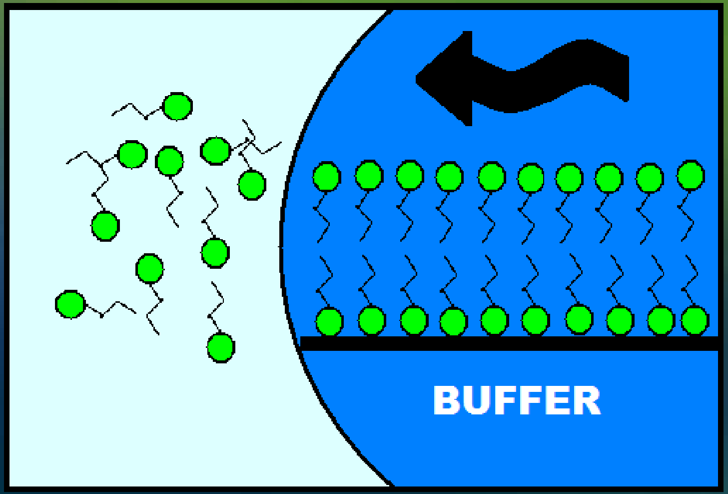
- Model Membrane



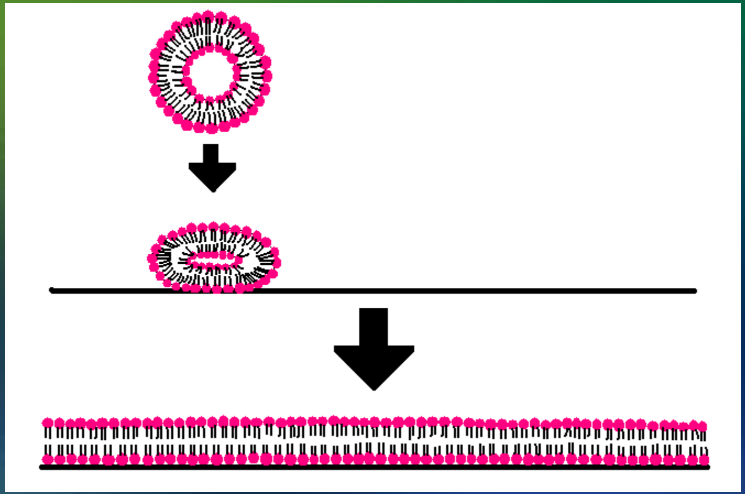
-Complete bi-layer

-Charged lipid

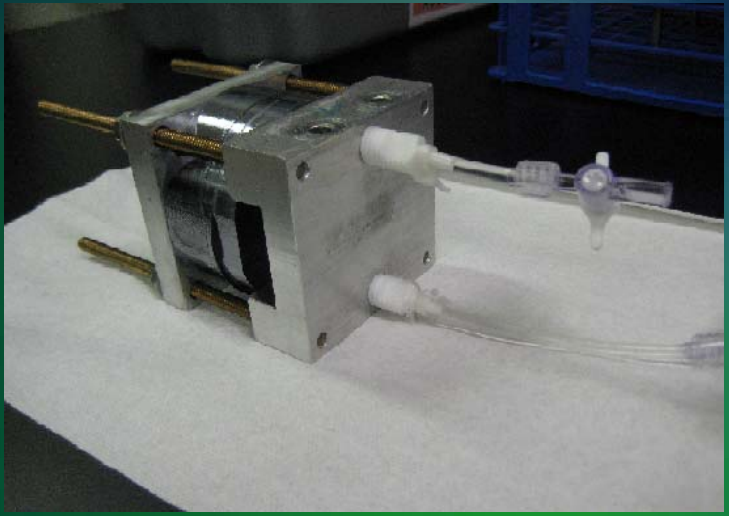
Two Methods of Membrane Formation



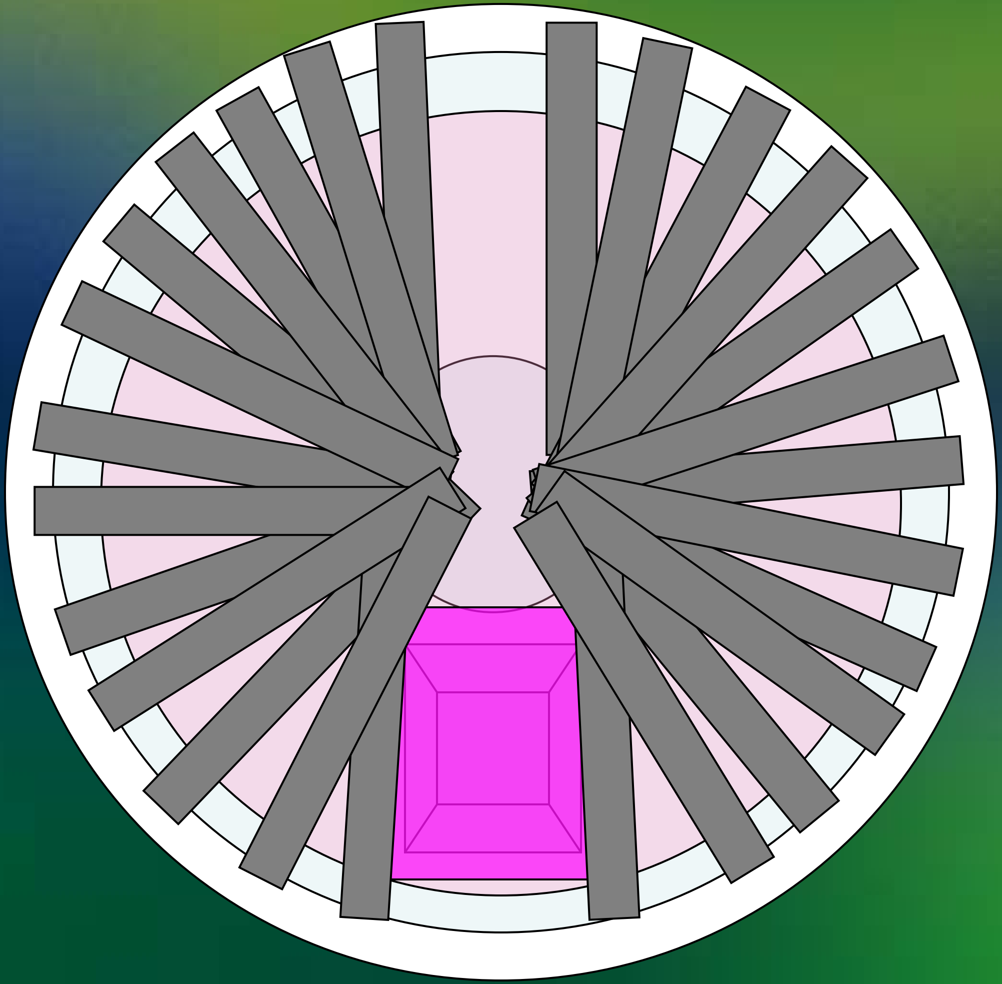
Rapid Solvent Exchange

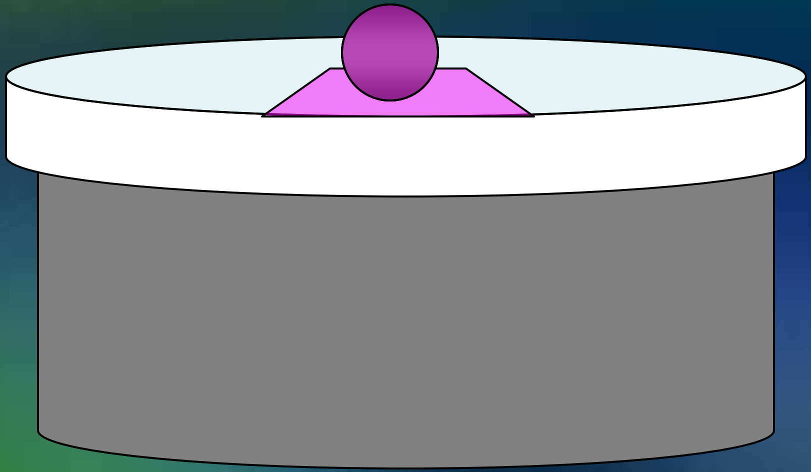


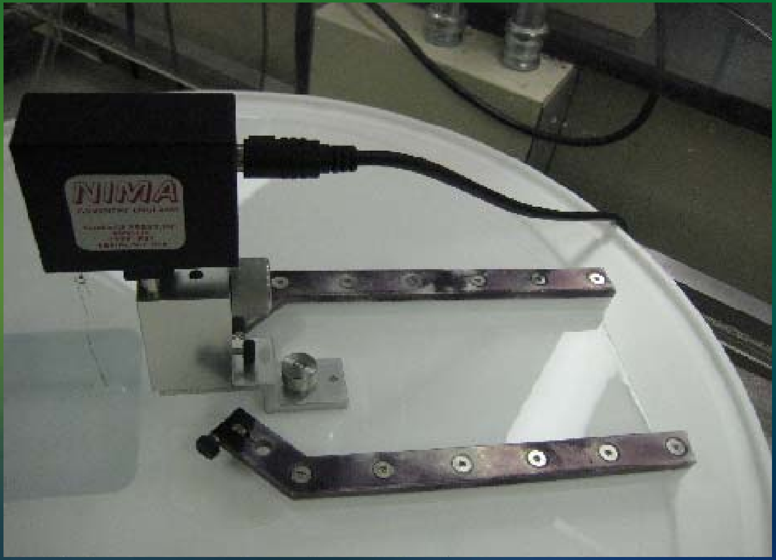
Vesicle Fusion

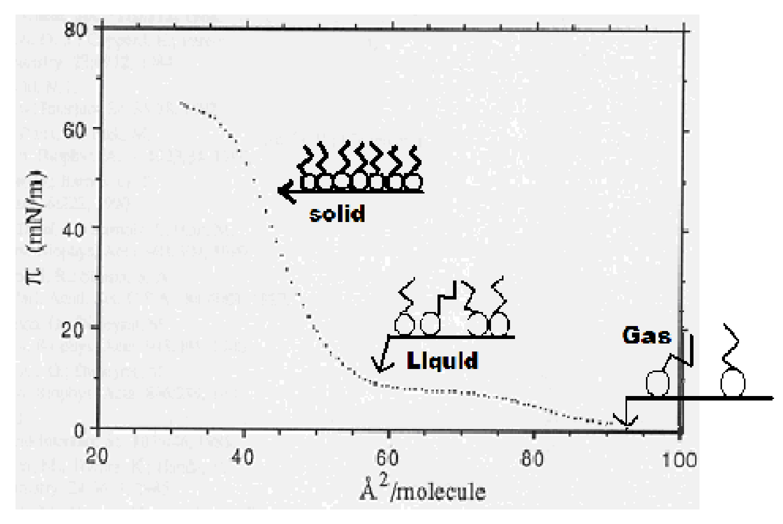


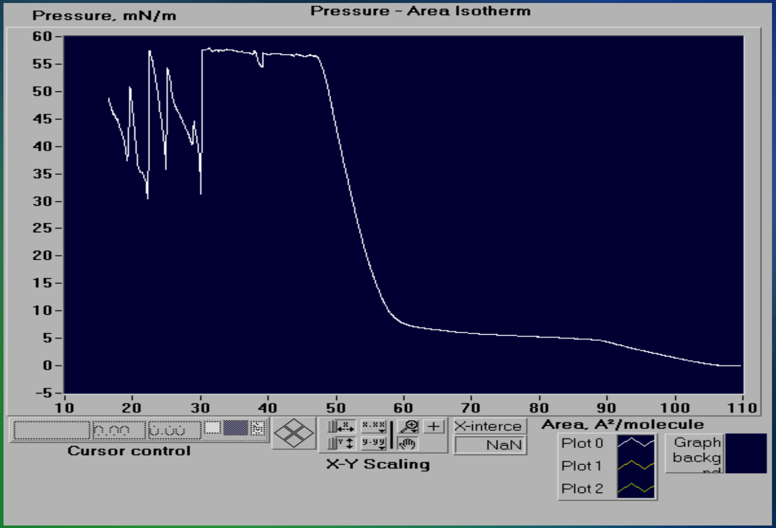
Langmuir Blodgettry!



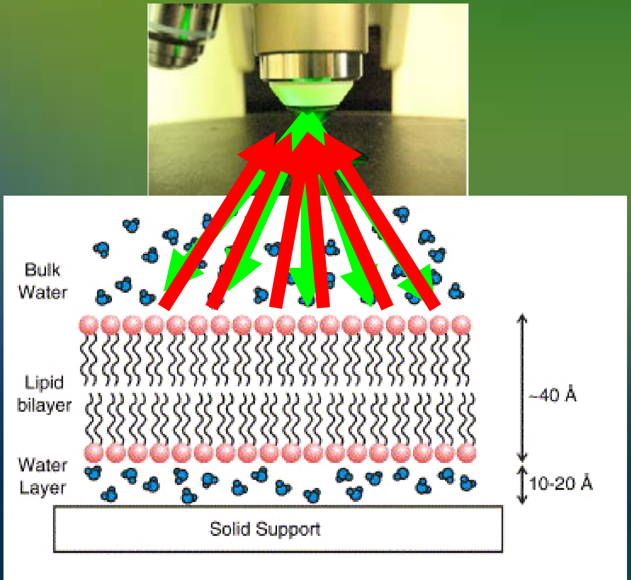


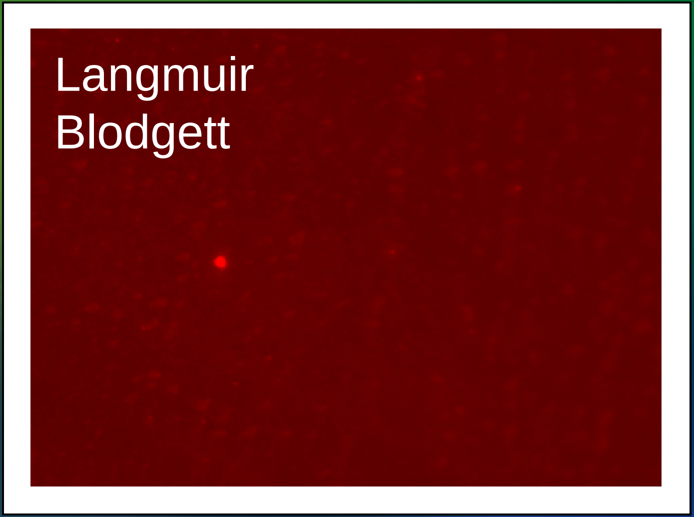

NIMA

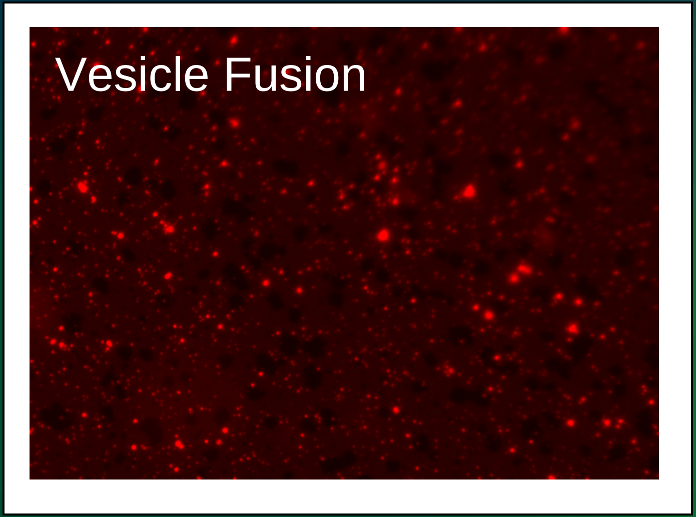

π (mN/m)
80
60
40
20
0
20
40
60
80
100
Å²/molecule
solid
Liquid
Gas


Pressure, mN/m
Pressure - Area Isotherm
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
10
20
30
40
50
60
70
80
90
100
110
Area, A²/molecule
X-interce
NaN
Cursor control
X-Y Scaling
Plot 0
Plot 1
Plot 2
Graph backg

Microscopy of pure DPPC membranes

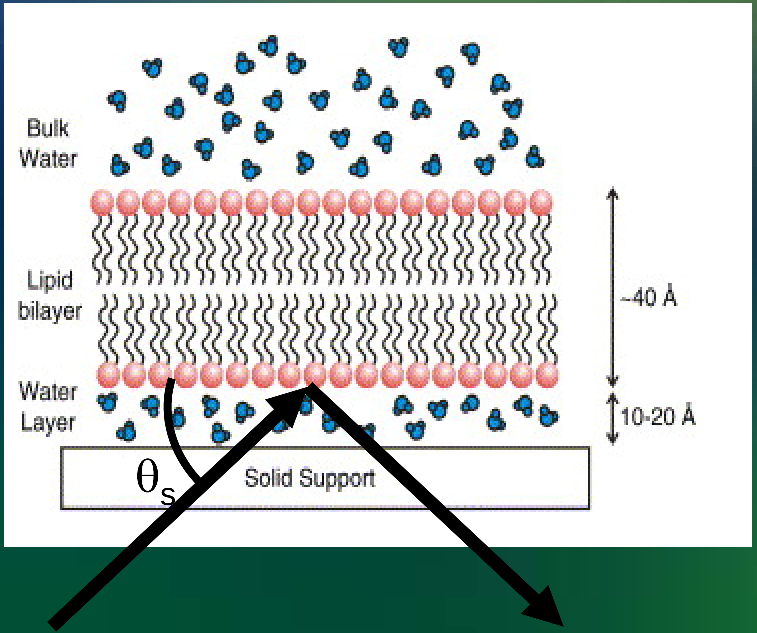


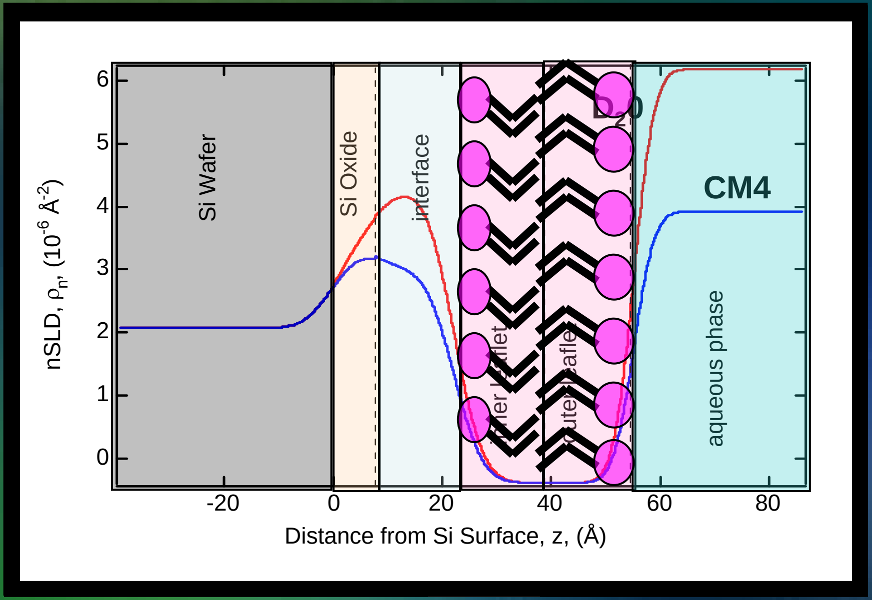

Langmuir
Blodgett


Vesicle Fusion


Rapid Solvent
Exchange

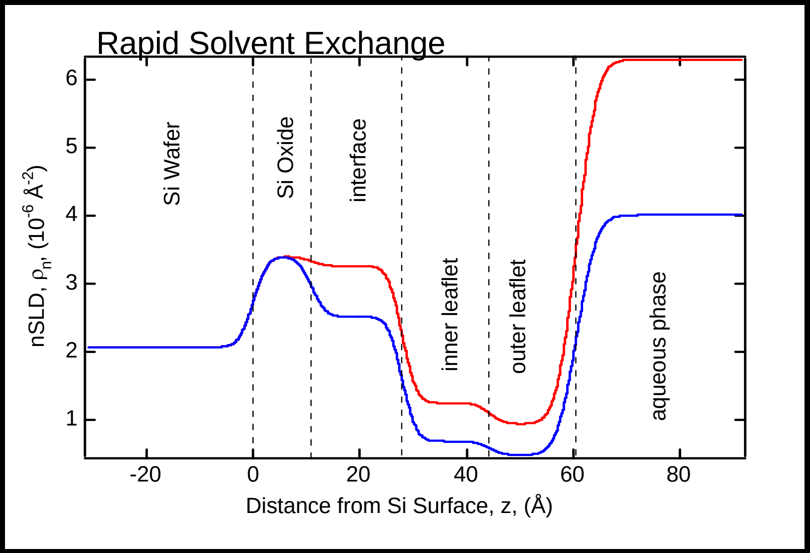
Advanced Neutron diffractometer and reflectometer (ANDR)





$Q = (4\pi/\lambda) \sin\theta_s$

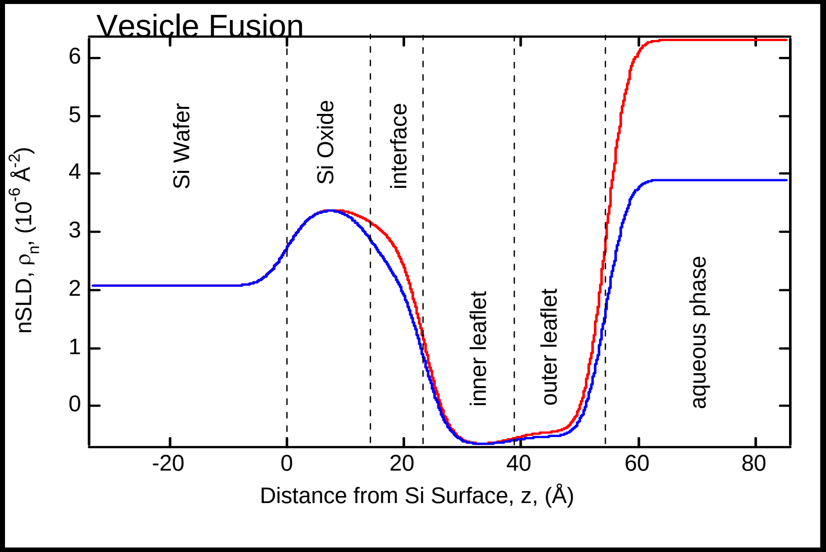
Summary of pure DPPC Results

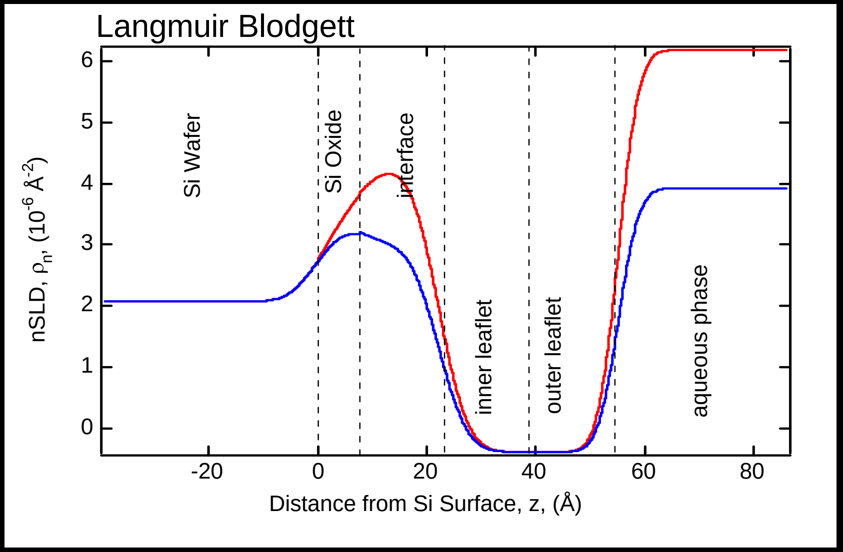


🟥 D_2O

🟦 CM4

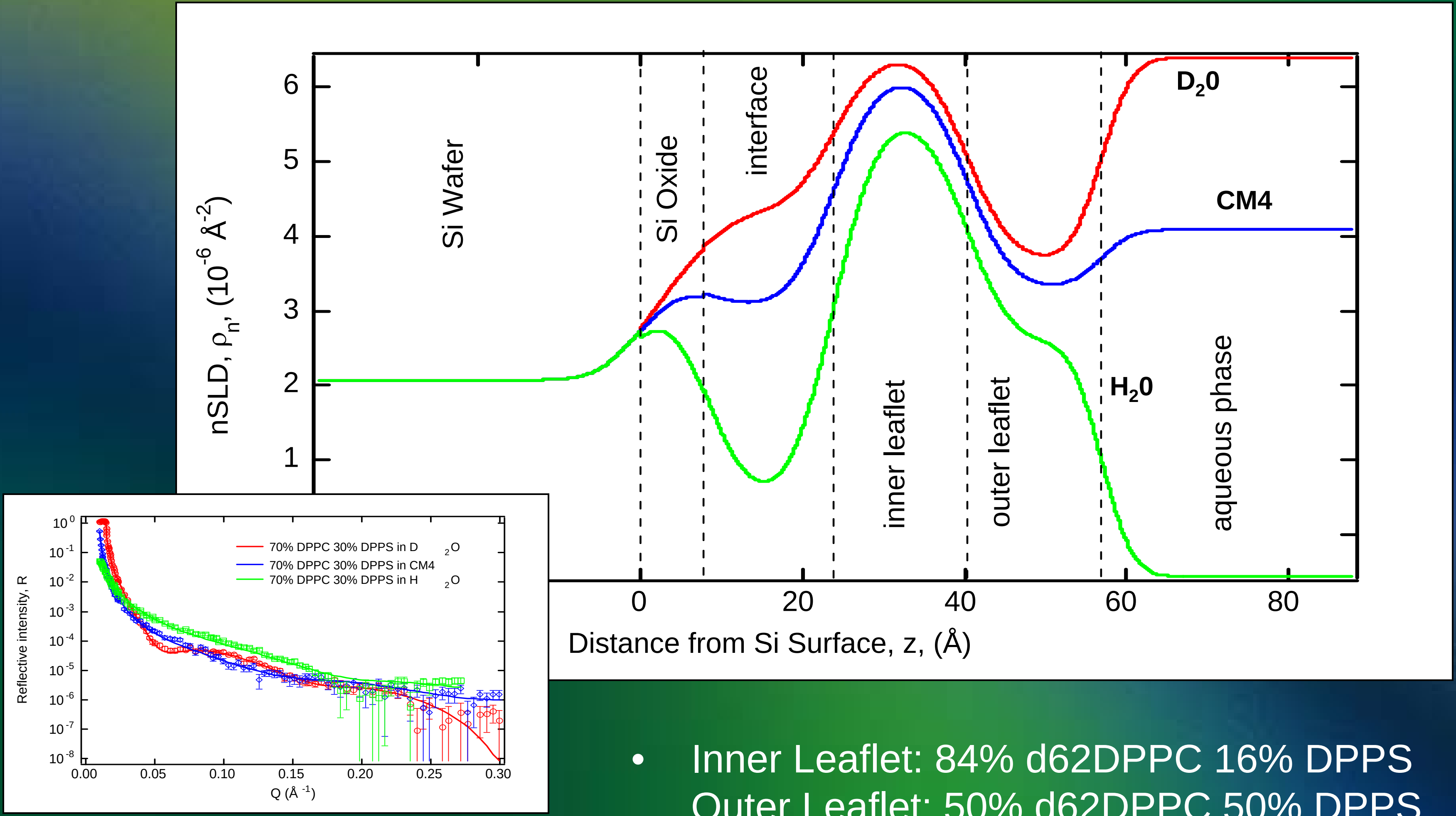
	Surface Coverage (Inner / Outer)	Thickness per leaflet (Å)
Vesicle Fusion	100% / 97%	15.6
Langmuir-Blodgett	100% / 100%	16.5
Rapid Solvent Exchange	76% / 79%	16.3





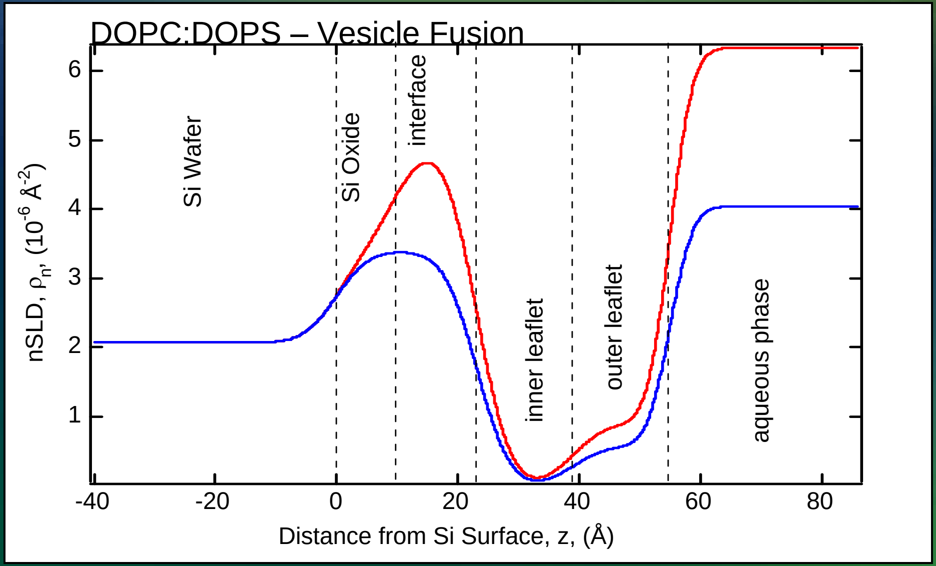
Mixed Lipid Systems

Langmuir Blodgett



- Inner Leaflet: 84% d62DPPC 16% DPPS
  Outer Leaflet: 50% d62DPPC 50% DPPS

Mixed Lipid System Results



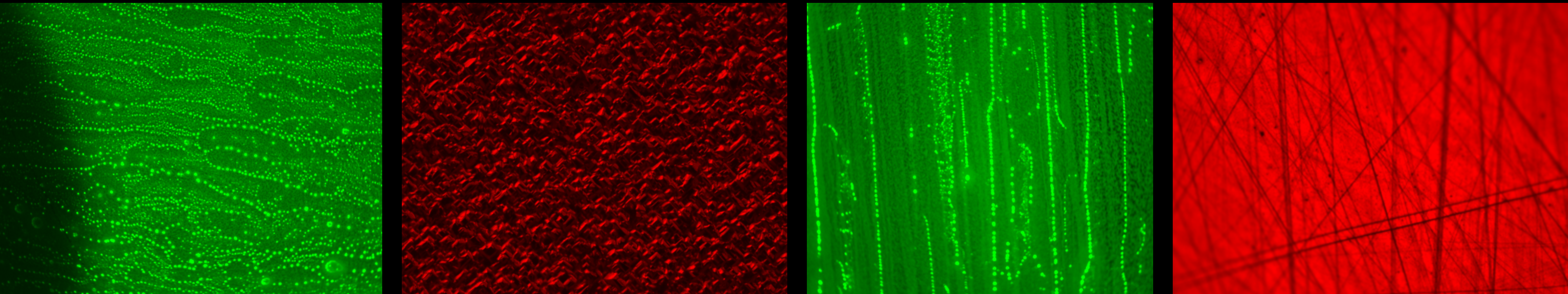
	Surface Coverage (Inner / Outer)	Thickness per leaflet (Å)
LB (70:30 DPPC:DPPS)	88% / 84%	16.5
VF (70:30 DPPC:DPPS)	8% / 4%	-------
RSE (70:30 DPPC:DPPS)	30% / 18%	-------
VF (80:20 DOPC:DOPS)	100% / 86%	15.9

Conclusions

- Successfully deposited neutral lipids on silica wafers.
- Negatively charged lipids interact unfavorably with the silica oxide layer making deposition more difficult.
- Langmuir Blodgett gave evidence of asymmetric bi-layers (inner leaflet has to be neutral).
- Developed consistent protocol for LB.
- SASSIE modeling indicates partial extension of linker regions.
- Helped de-bug SASSIE.

Outlook

- Study protein association to model membranes
  - Co-factors responsible for extension
- Characterize complex lipid systems
- Explore dimerization of the gag protein using SASSIE.



Acknowledgements

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NCNR
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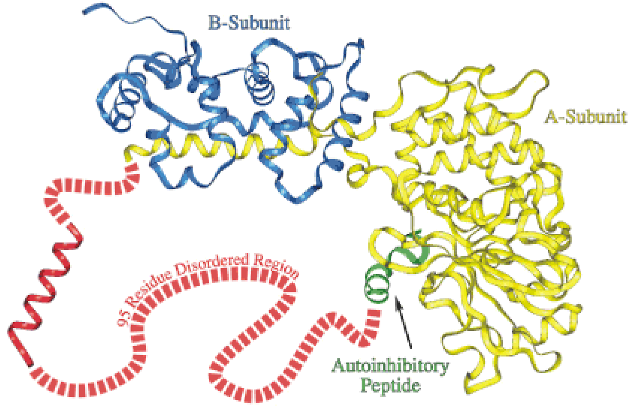
UMD

Intrinsically Disordered Proteins

A Definition:
Proteins or regions of proteins that fail to form specific 3-D structure under physiologic conditions *in vitro*.
from Le Gall et al., J. Biomolecular Struct. and Dynamics **24**, 325 (2007)

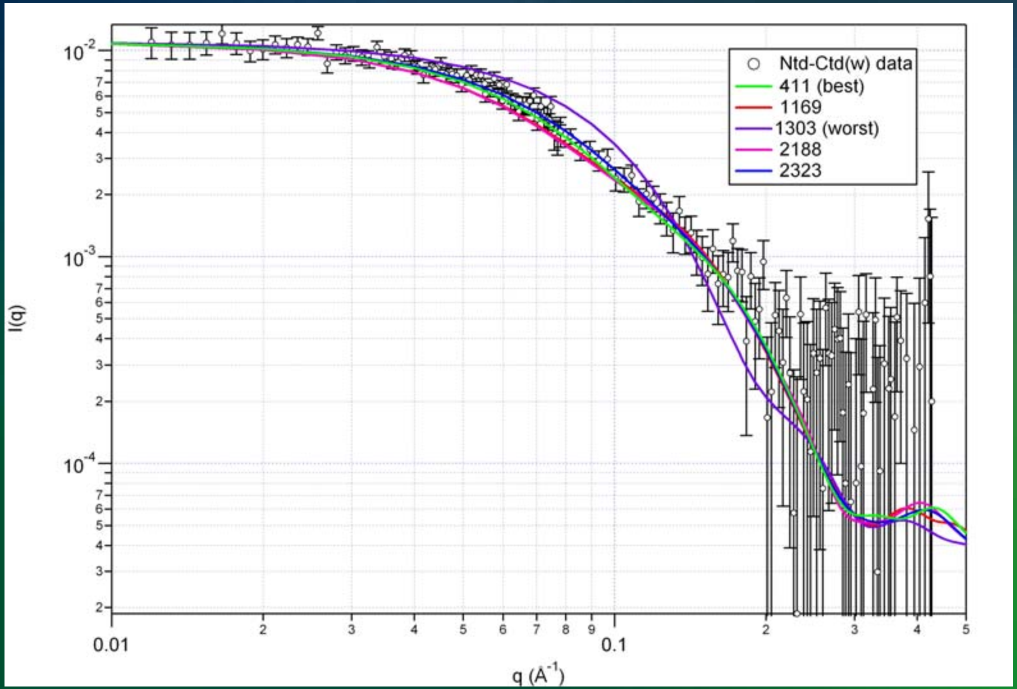


(Image adapted from: Kissinger CR, et al. 1995. "Crystal structures of human calcineurin and the human FKBP12-FK506-calcineurin complex." Nature 378:641-4.)

There are a lot of them!
~30 % of proteome has proteins with sequences of disordered segments of ≥ 50 amino acids

Standard Methods are unable to resolve flexible domains
- X-ray
- NMR

Ntd-Ctd Run 1



I(q)
q (A^{-1})
10^{-2}
10^{-3}
10^{-4}
0.01
0.1
2
3
4
5
6
7
8
9
Ntd-Ctd(w) data
411 (best)
1169
1303 (worst)
2188
2323

Ntd-Ctd Run 1

Structure Number	Rg (Å)	χ^2
411 (best)	22.90	0.25
904	22.67	0.38
1169	25.05	0.41
1303 (worst)	17.27	3.59
2045	22.88	0.40