Taida: March 3, 2015

Membrane Biophysics & Soft Matter Physics

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The talk is about

- Proteins interacting with membranes.
- Physical process rather than chemical reactions.
- Functions of proteins in membranes are well defined, in fact by phase transitions.
- Unsolved problems.

Subject

- **Membrane-active Antibiotics**, often called antimicrobial peptides (AMPs). This talk is the story of how we found out how AMPs work.

  What is the significance of AMPs?

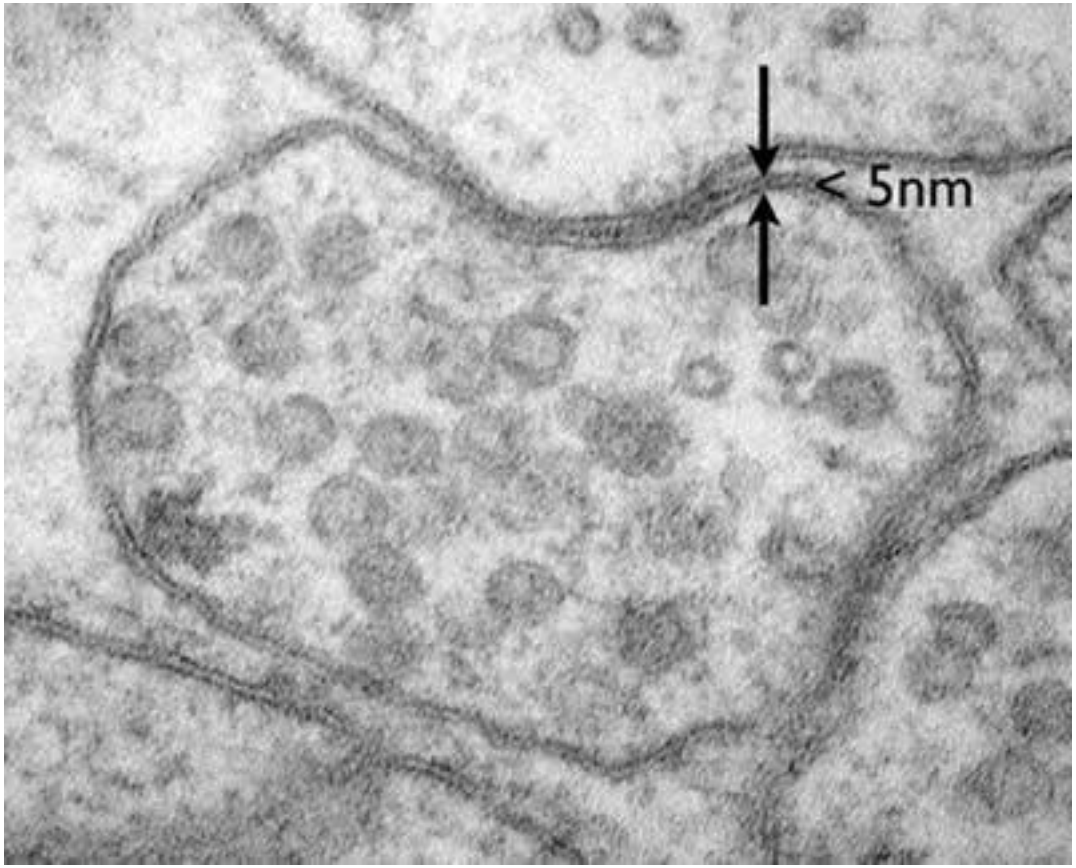
  What is the physics problem?

- **Unsolved membrane problems**

  Recently a 2nd kind of AMPs were discovered.

  Alzheimer's disease, mad cow disease, type II diabetes and other neurodegenerative diseases could also be membrane problems.

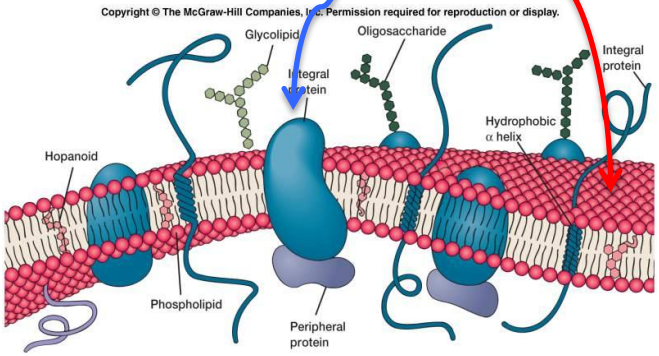
Cell Membranes



C. elegans

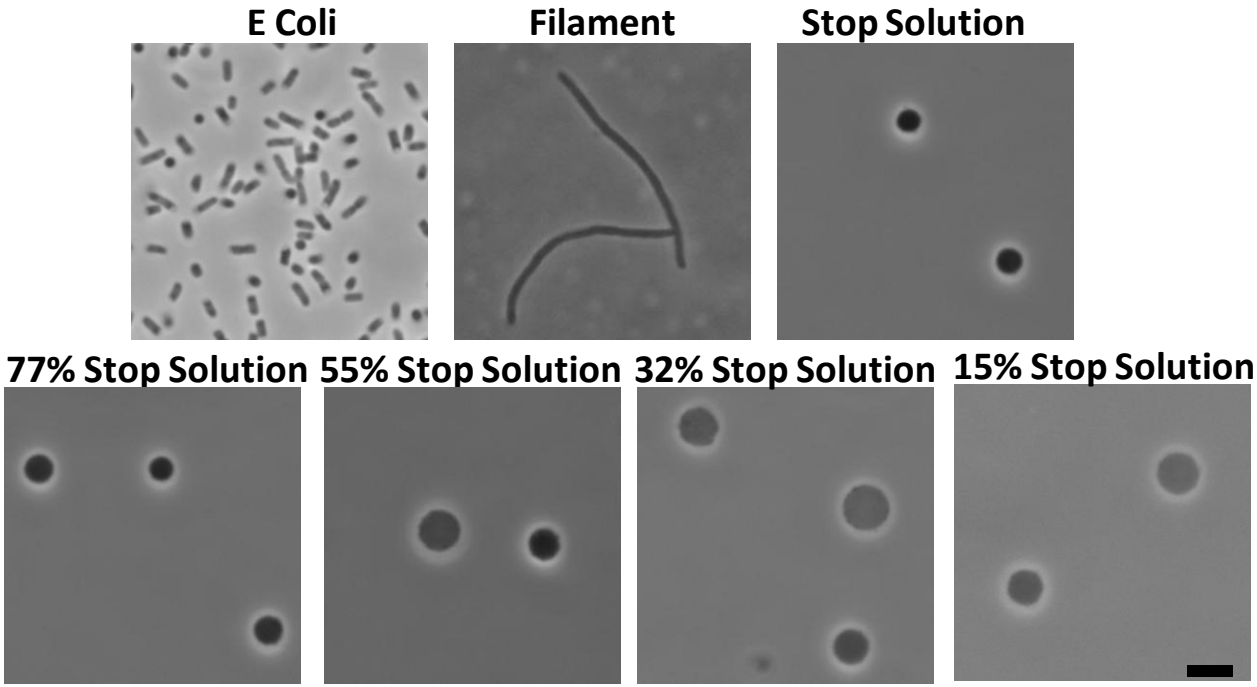
target of membrane-active antibiotics

target of conventional antibiotics

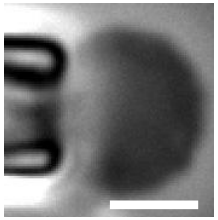


The red part is a lipid bilayer.

Bacterial membranes

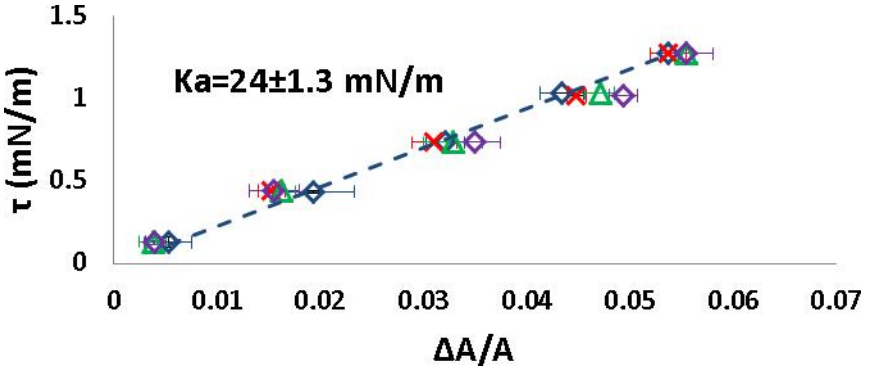


Cell membranes are not simple.

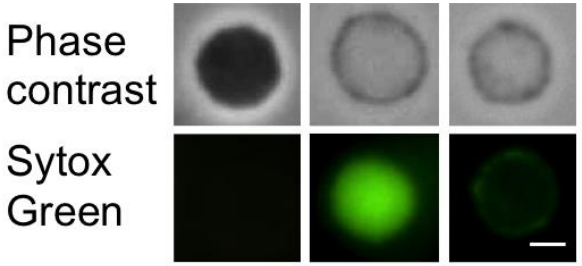


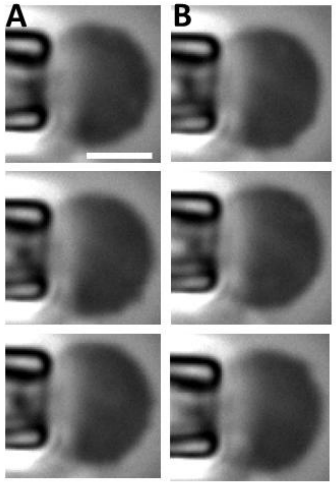
Bar=2.5um

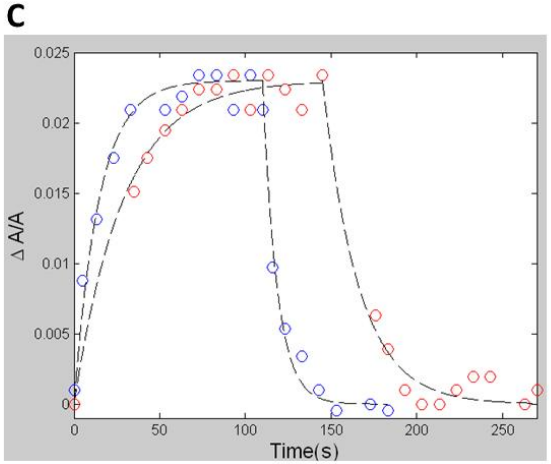
Live cell

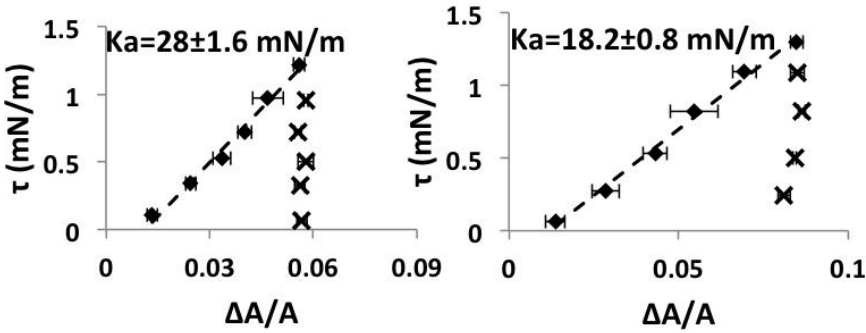


Dead cell



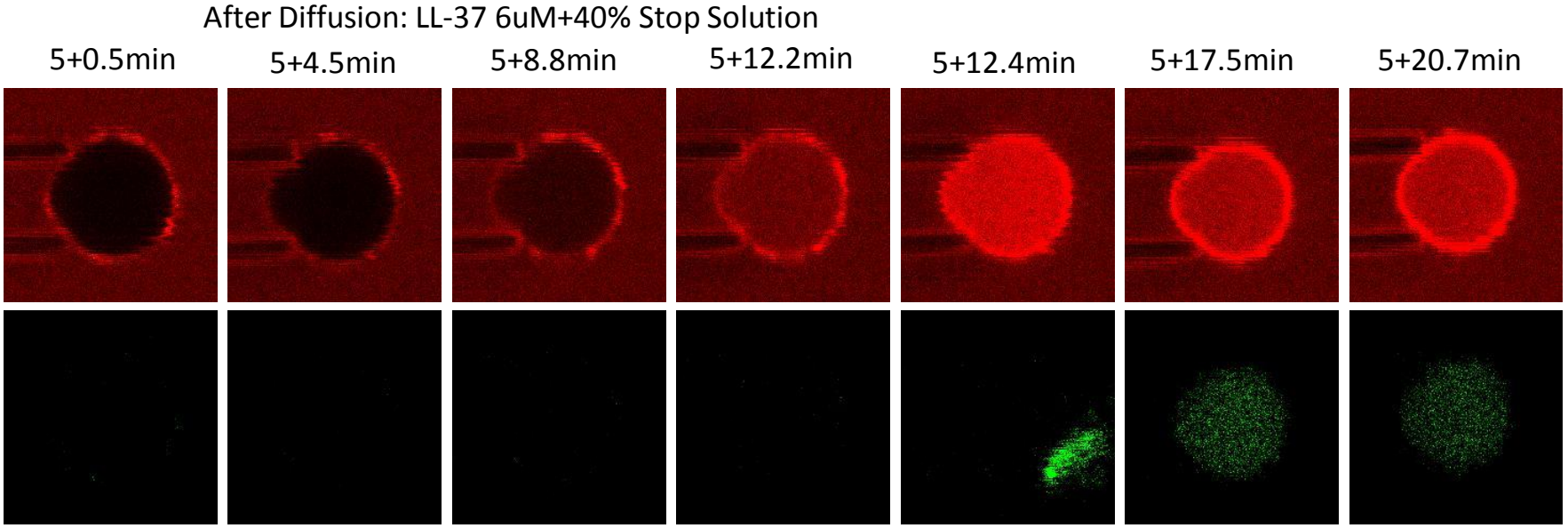






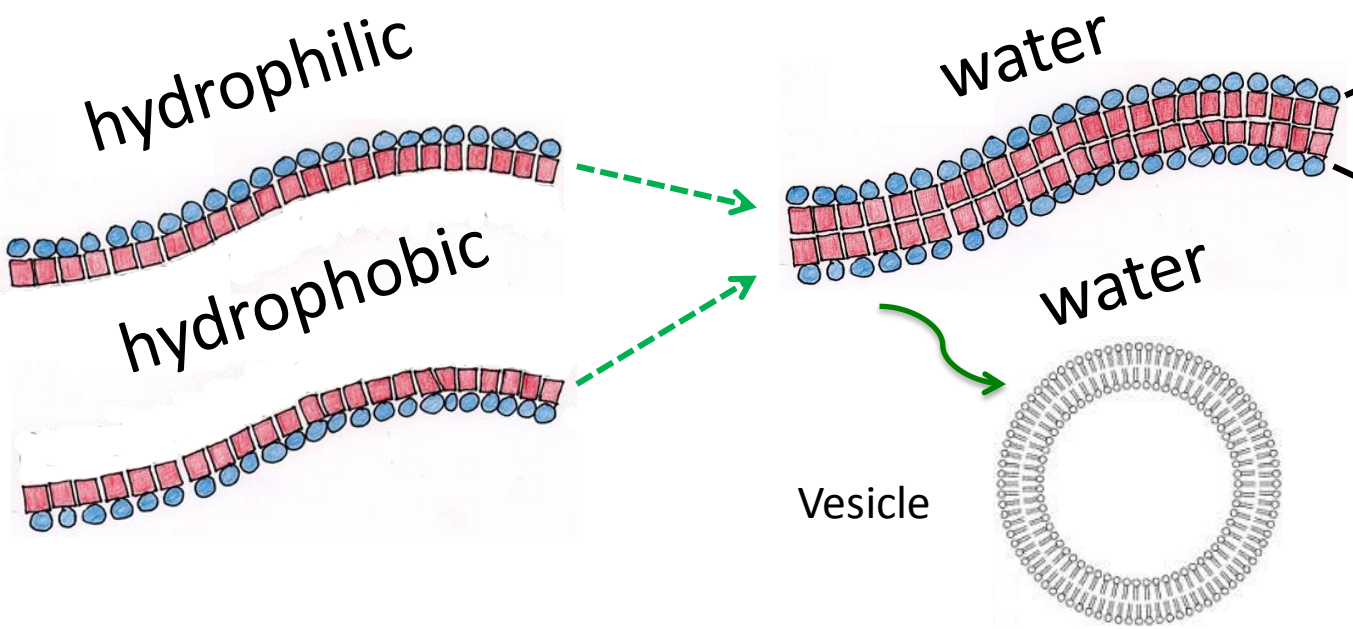
Biophys J. 107, 2082 (2014)

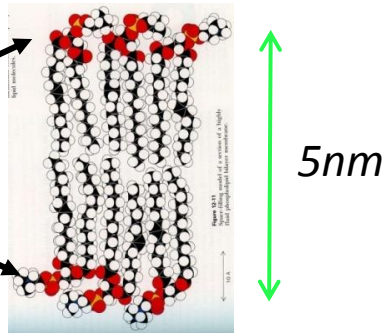
It is very difficult to know what happens when an antibiotic attacks a bacteria except that it kills.



Model membranes and AMP

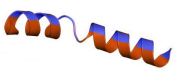
This is the problem:



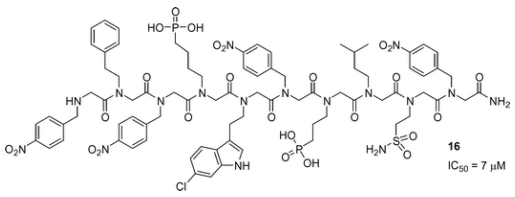


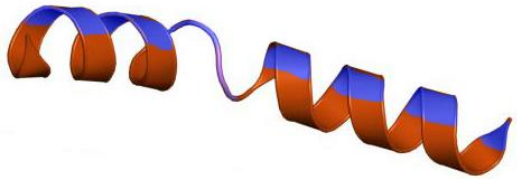
Soft!

1 nm X 2-3 nm

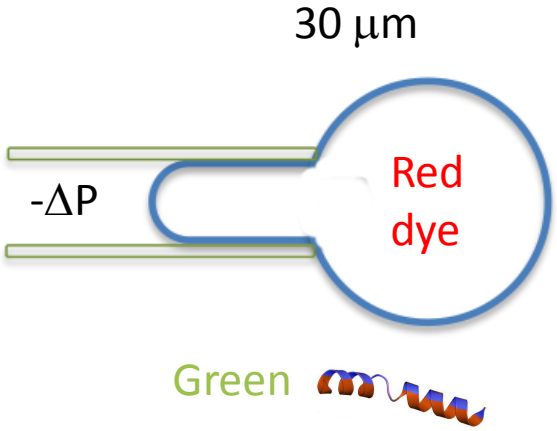


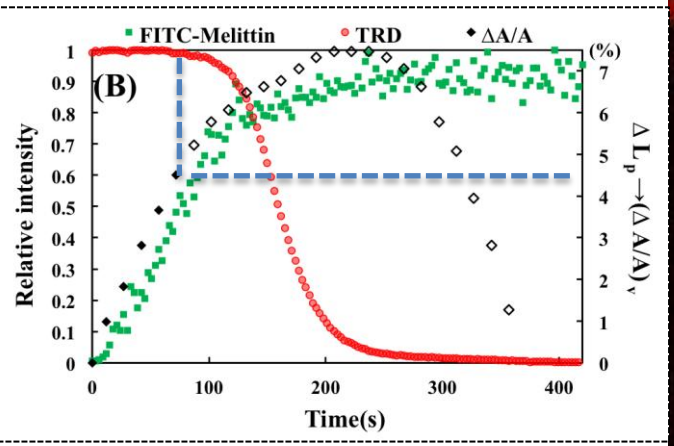
A typical antibiotics or AMP (melittin)

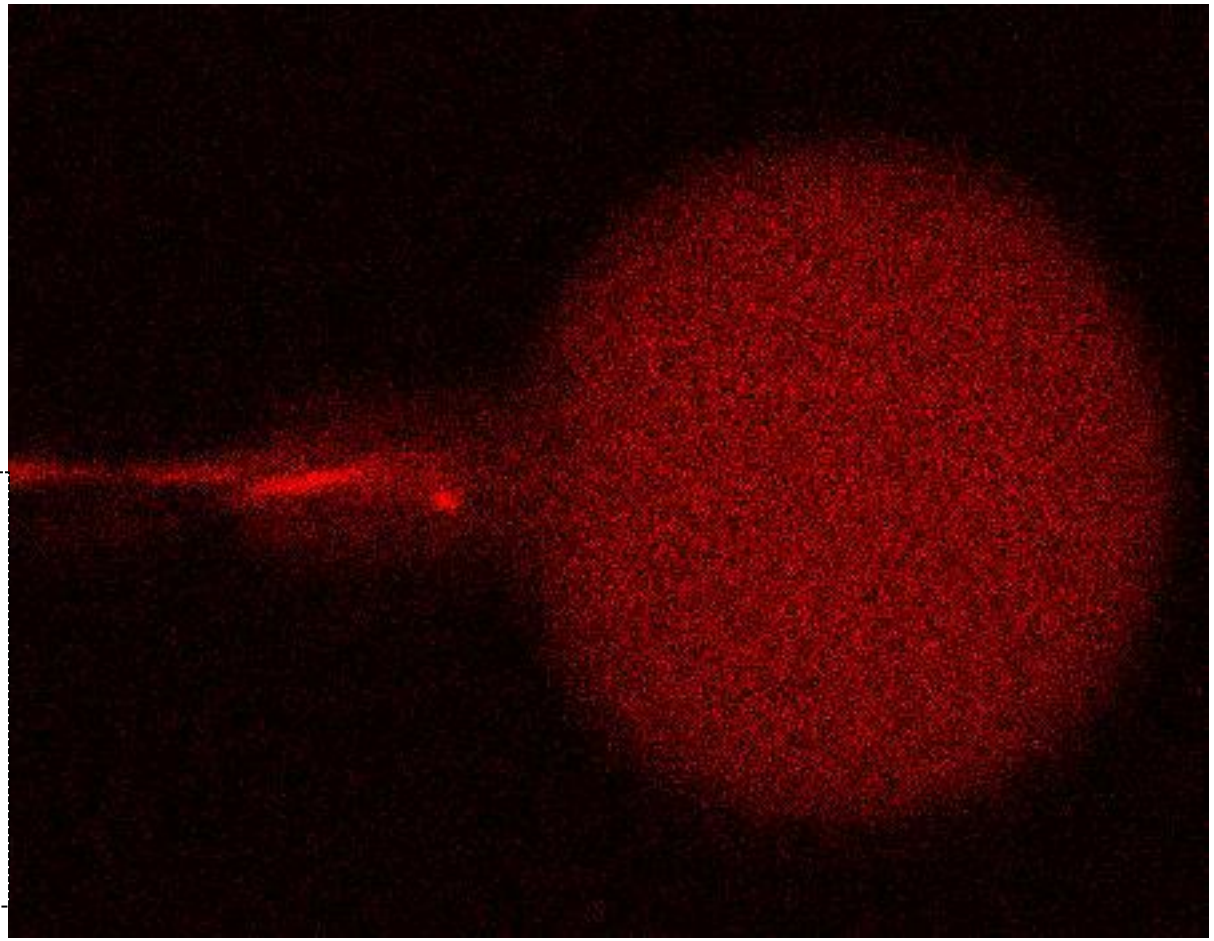




Model membrane attacked by membrane-active antibiotics

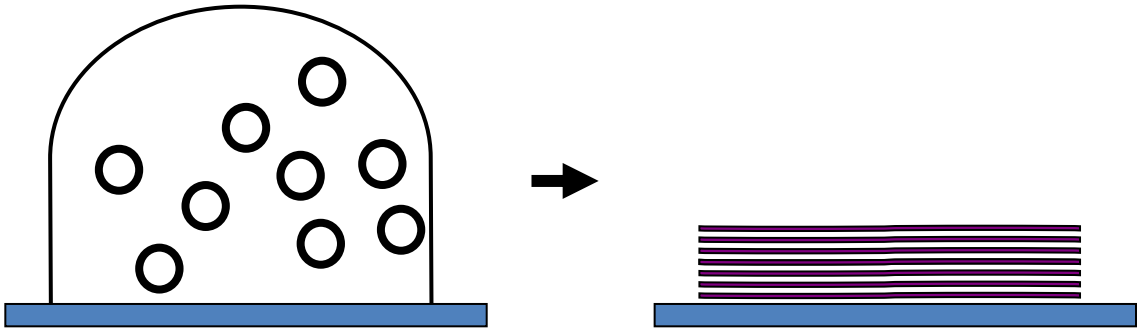






PNAS 110, 14243 (2013)

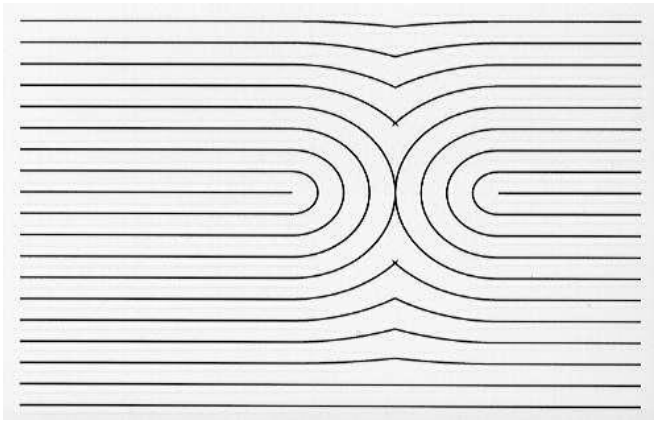
Parallel Multilayers of Membranes



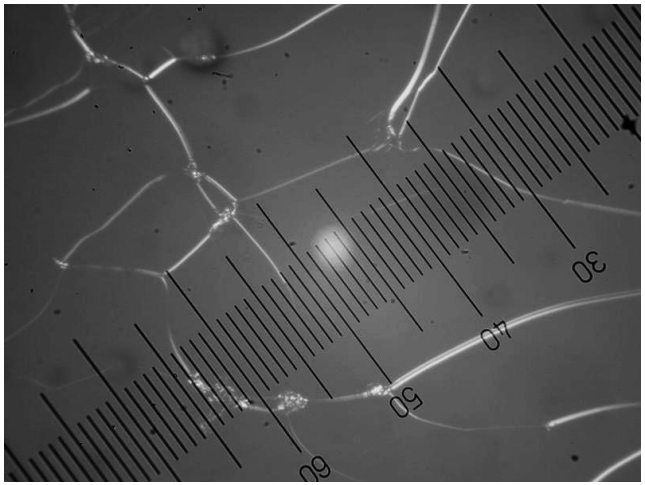
Liposomes (vesicles)

Multilayers (smectics)

side view

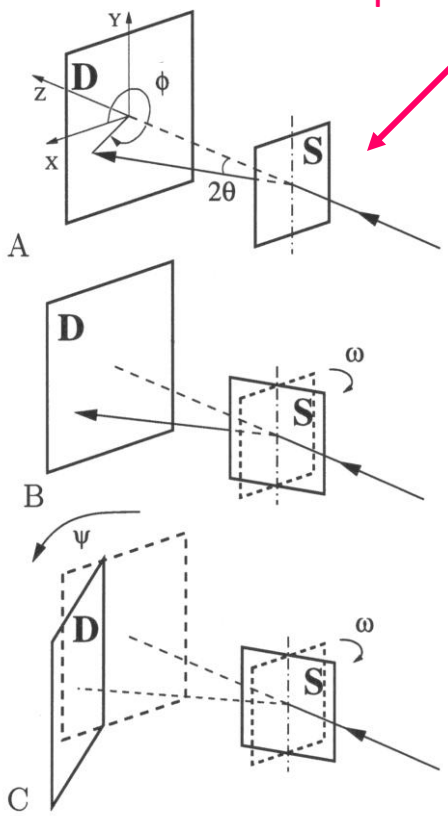


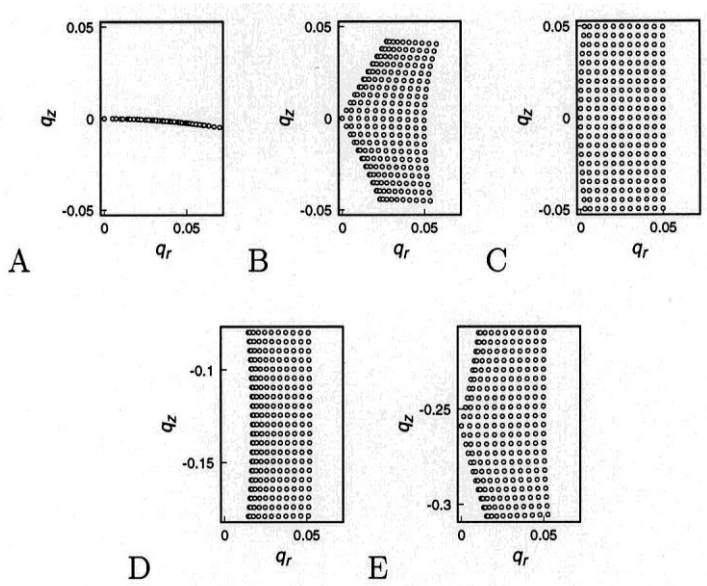
top view



Neutron scattering

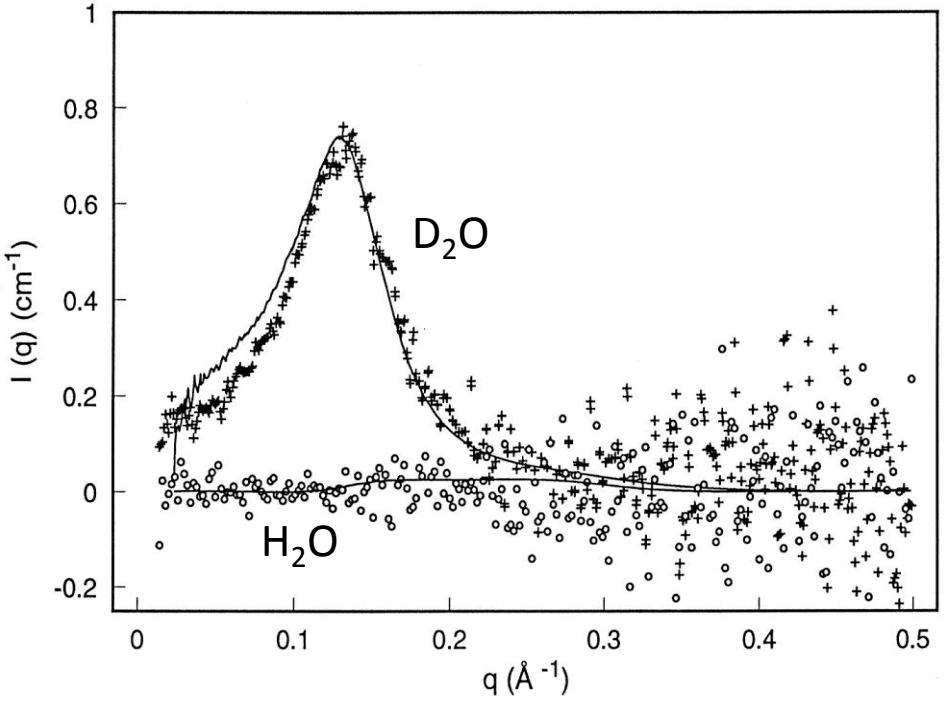
in-plane scattering



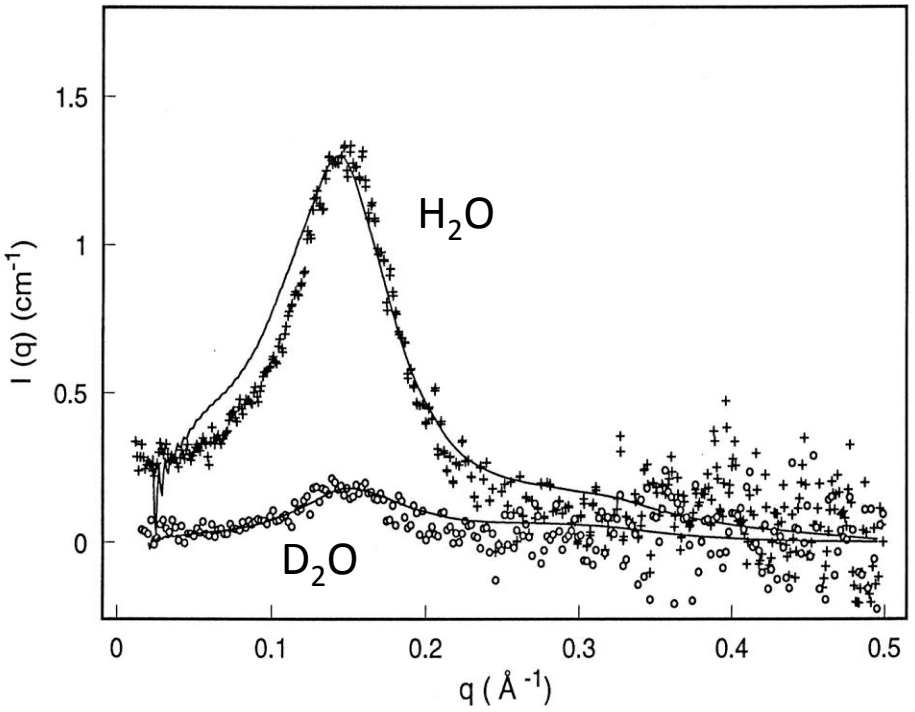


Using D_2O to show the water pores

Natural lipid with D_2O or H_2O



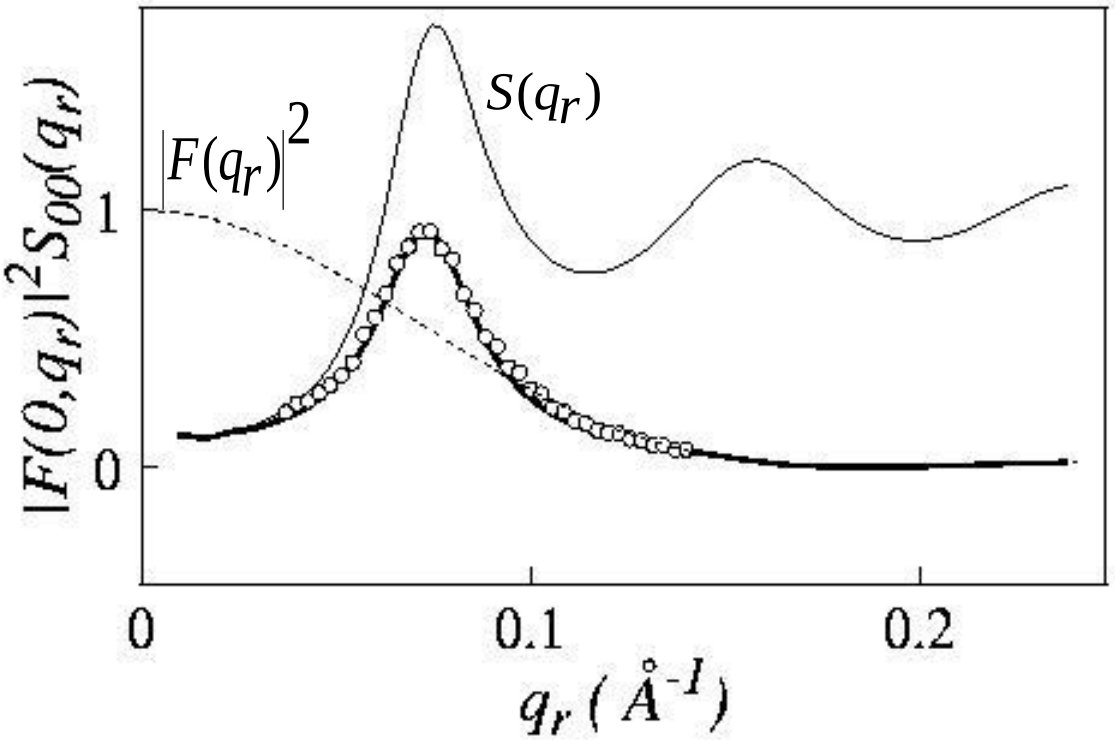
perdeuterated lipid with H_2O or D_2O



Biophys. J. 70,, 2659 (1996)

Analysis of neutron scattering from fluid membranes

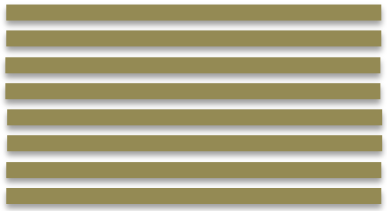
$$I = |F(q_r)|^2 S(q_r)$$



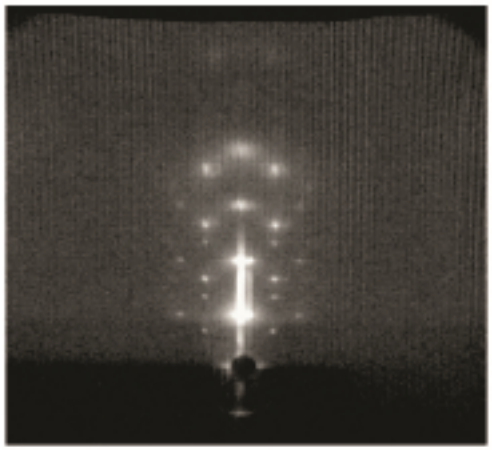
Pore size ~4.4 nm diameter

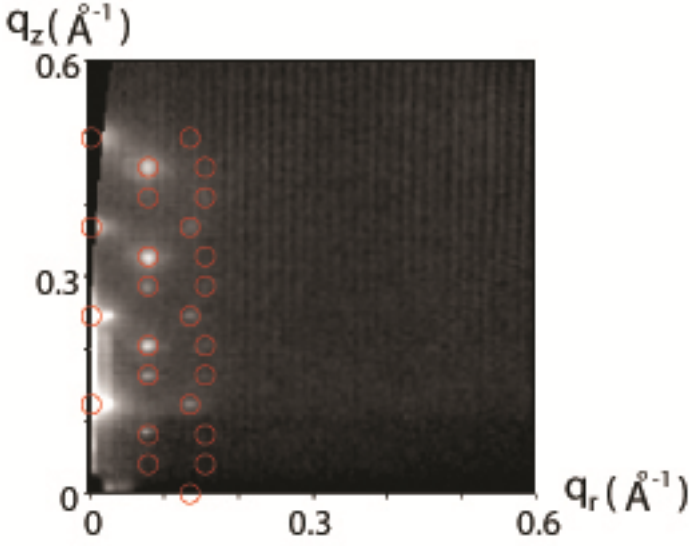
4-7 melittin in the pore

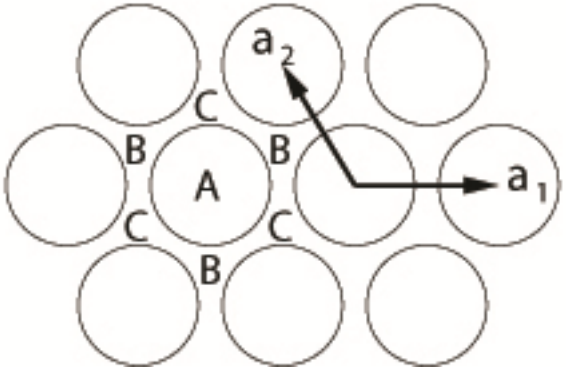
Phase transition by dehydration



dehydration



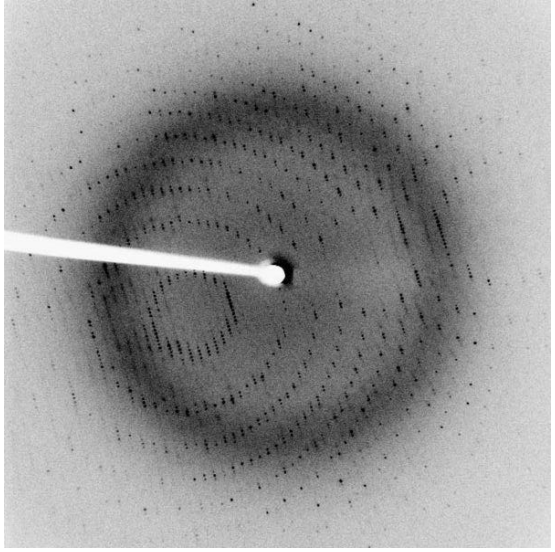




Biophys. J. 79, 2002 (2000)

Diffraction by molecular crystalline

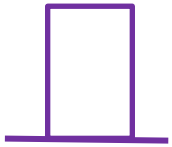
$$I \sim |F(q)|^2 S(q)$$

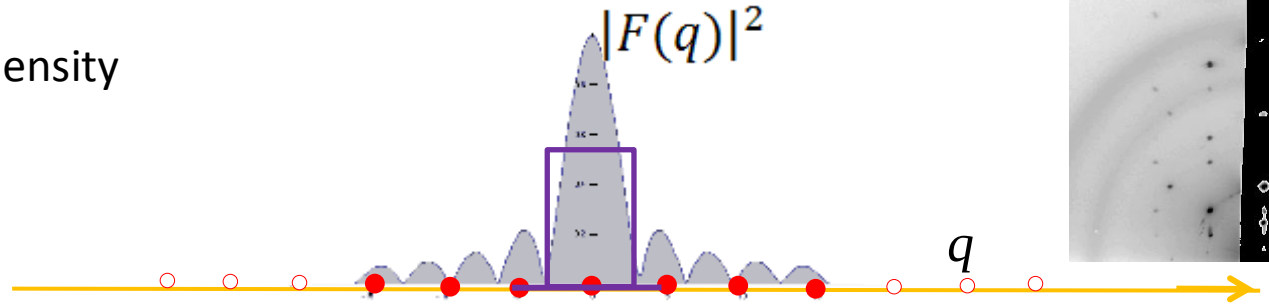


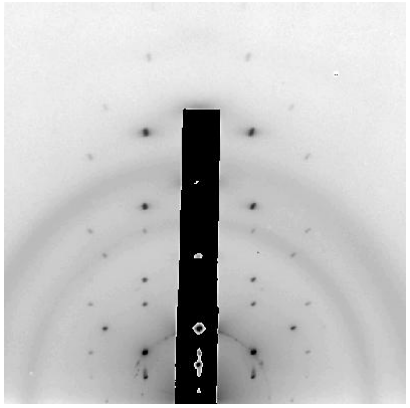
Diffraction by soft matter crystals

Same $S(q)$, but $F(q) = \sum_i e^{iq \cdot r_i} \rightarrow \int \rho(r) e^{iq \cdot r} \, d^3r$

Ex. 1D constant density
In the unit cell







Anomalous Diffraction for centrosymmetric structures

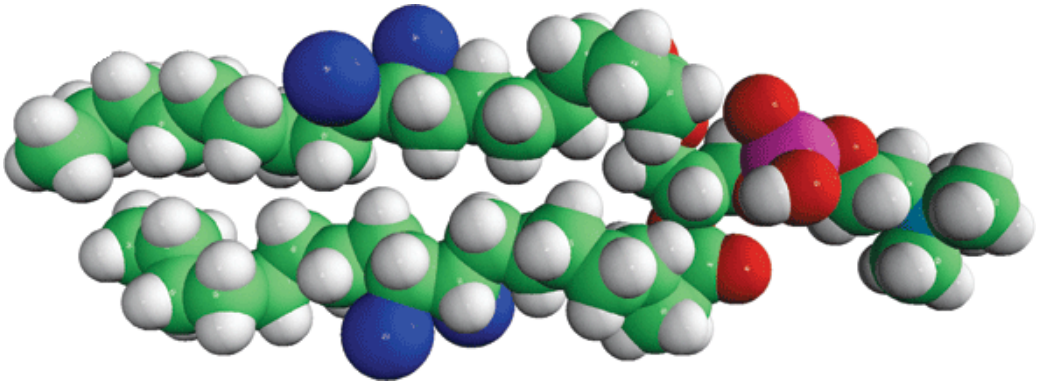
$$F_\lambda(\boldsymbol{q}) = \sum_j f_j^n \exp(i\boldsymbol{q}\cdot\boldsymbol{r}_j) + \sum_k (f^n + f'+if'')\exp(i\boldsymbol{q}\cdot\boldsymbol{r}_k)$$

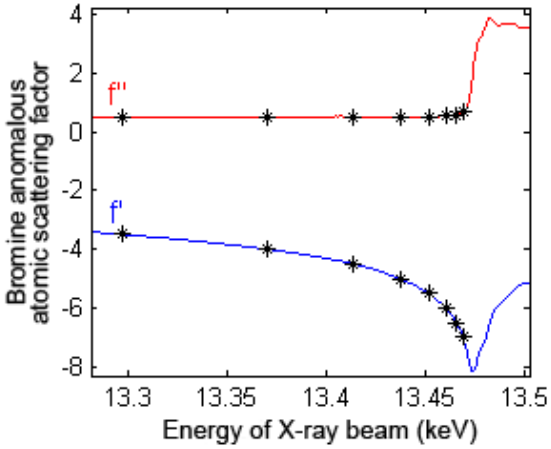
$$= F_o + \frac{f'+if''}{f^n} F_2$$

$$|F_\lambda|^2 = \left(F_0 + \frac{f'_\lambda}{f^n} F_2\right)^2 + \left(\frac{f''_\lambda}{f^n}\right)^2 F_2^2$$

Multiwavelength Anomalous Diffraction (MAD) Method

di18:0(9,10Br)PC





$$f_\lambda'' \sim 0.1 f_\lambda'$$

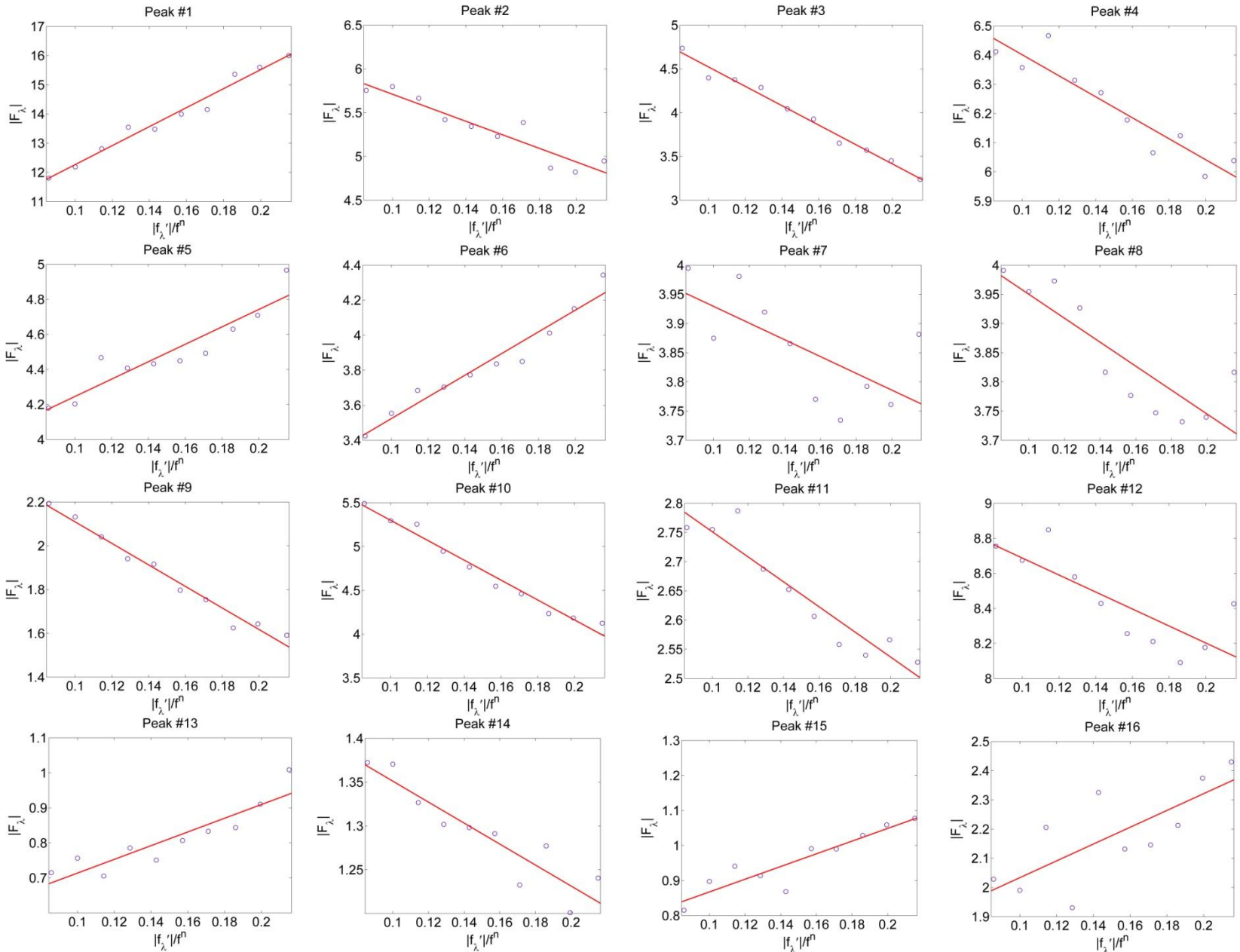
$$|F_\lambda|^2 = \left(F_0 + \frac{f_\lambda'}{f^n} F_2\right)^2 + \left(\frac{f_\lambda''}{f^n}\right)^2 F_2^2$$

$$|F_\lambda| \approx \pm\left(F_0 + \frac{f_\lambda'}{f^n} F_2\right)$$

JACS 128, 1340 (2006)

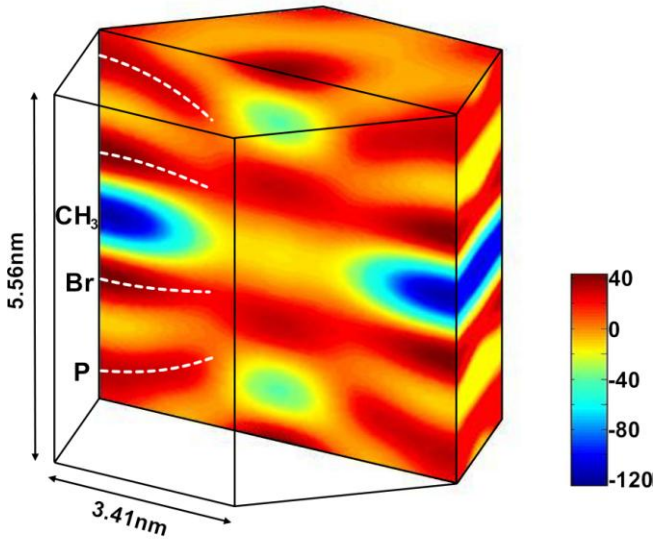
Solving F_0 and F_2

$$|F_\lambda| \approx \pm\left(F_0 + \frac{f'_\lambda}{f^n} F_2\right)$$

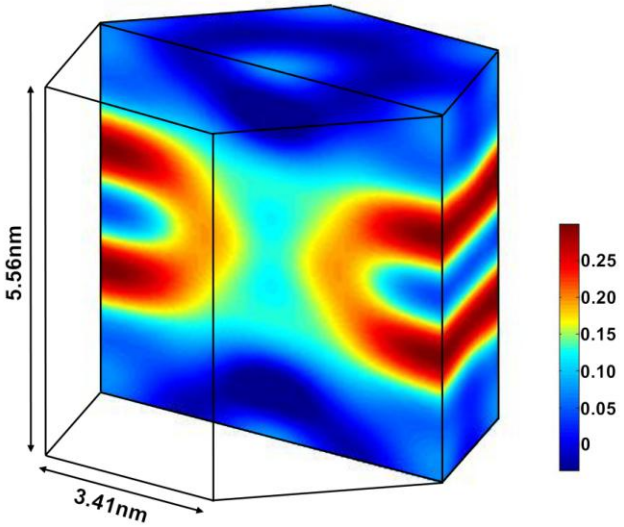


F_0 and F_2

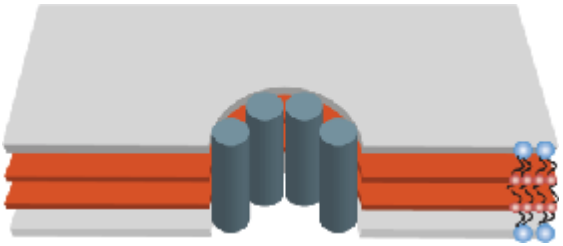
Complete electron density

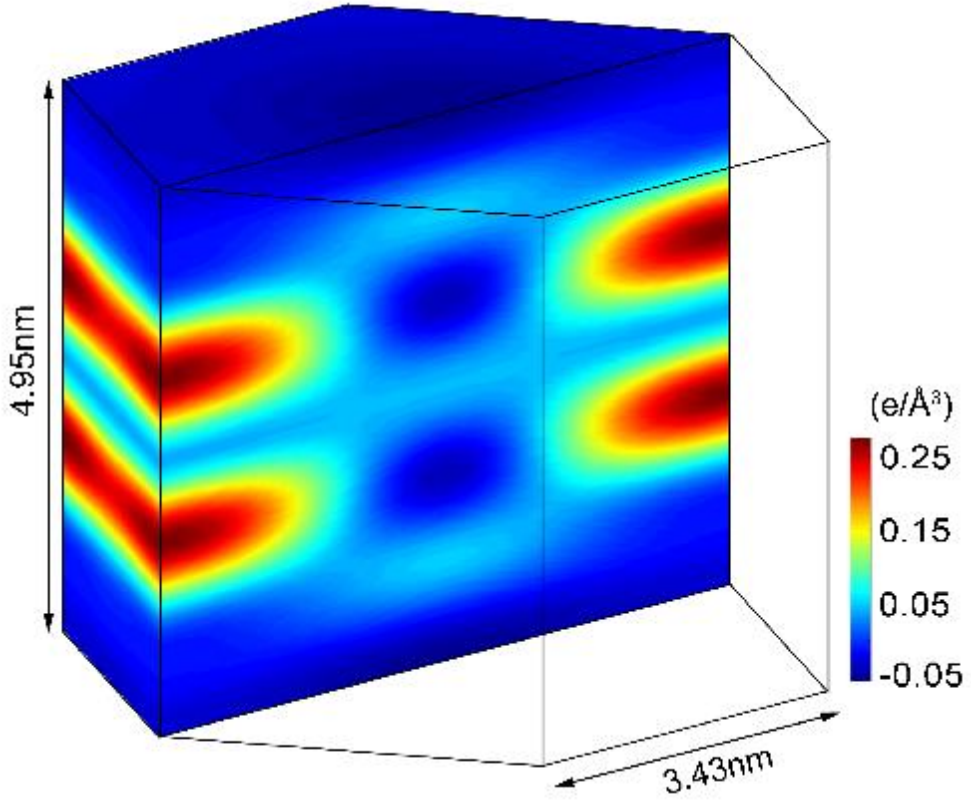


Label only electron density



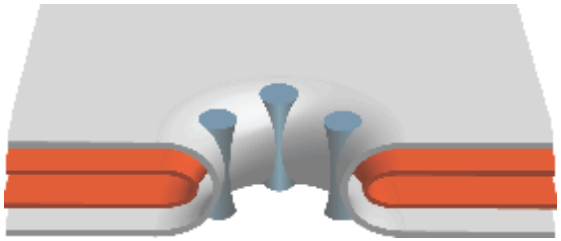
Only one AMP forms pores of the (barrel-stave model)

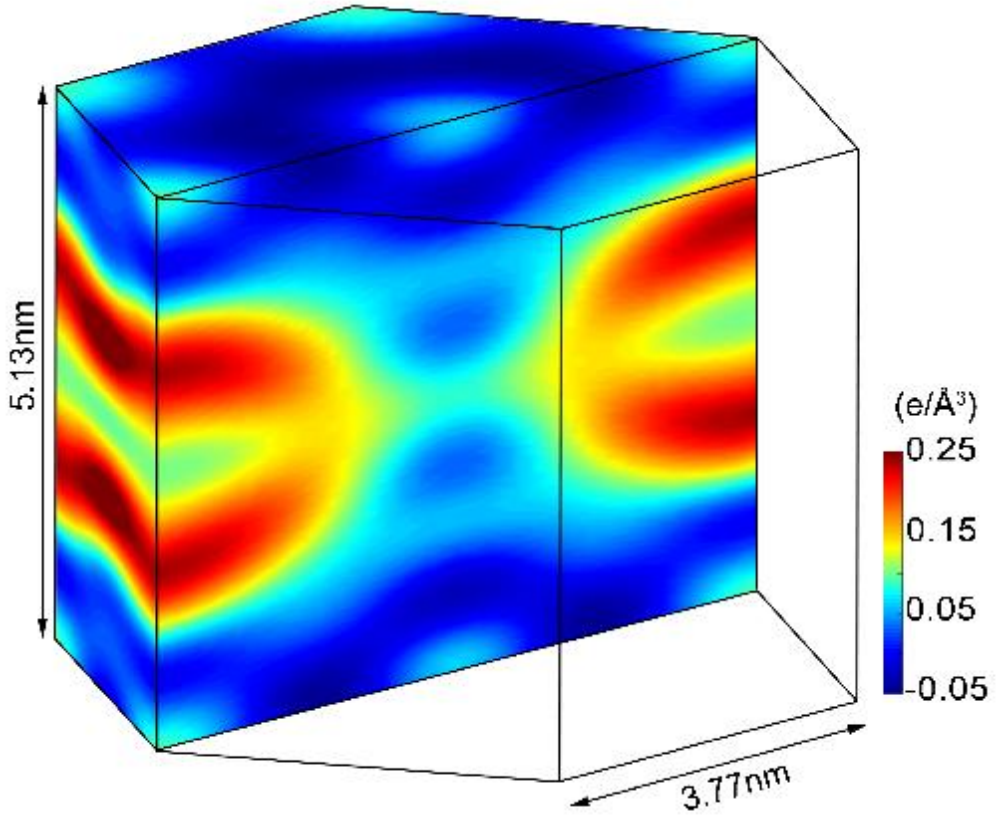




PNAS 105, 17379 (2008)

All AMPs except one form pores of the (toroidal model)





A topological question!

PNAS 105, 17379 (2008)
PNAS 110, 14243 (2013)

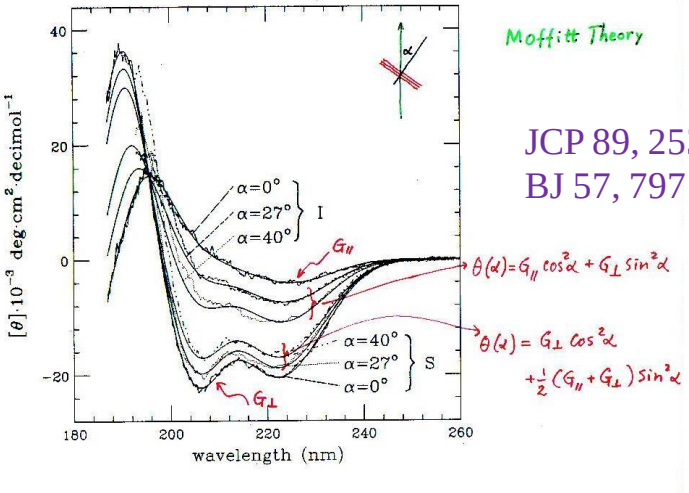
Physics of pore formation in membranes

- But why do the antibiotics make pores when $\Delta A/A$ exceeds ~4%?
- Note that making pores when $\Delta A/A$ exceeds ~4% represents a concentration on-off switch.
- All biological on-off switches are by concentrations!

The biggest problem in membrane biophysics is: How to detect the physical state of proteins in membranes?

Method 1: Oriented circular dichroism

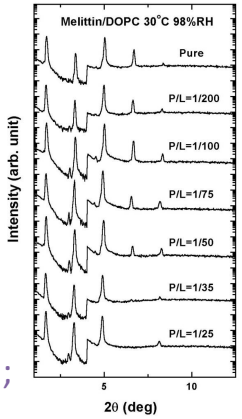
We measured the orientation of helices as a function of antibiotic concentration.



JCP 89, 2531 (1988)
BJ 57, 797 (1990)

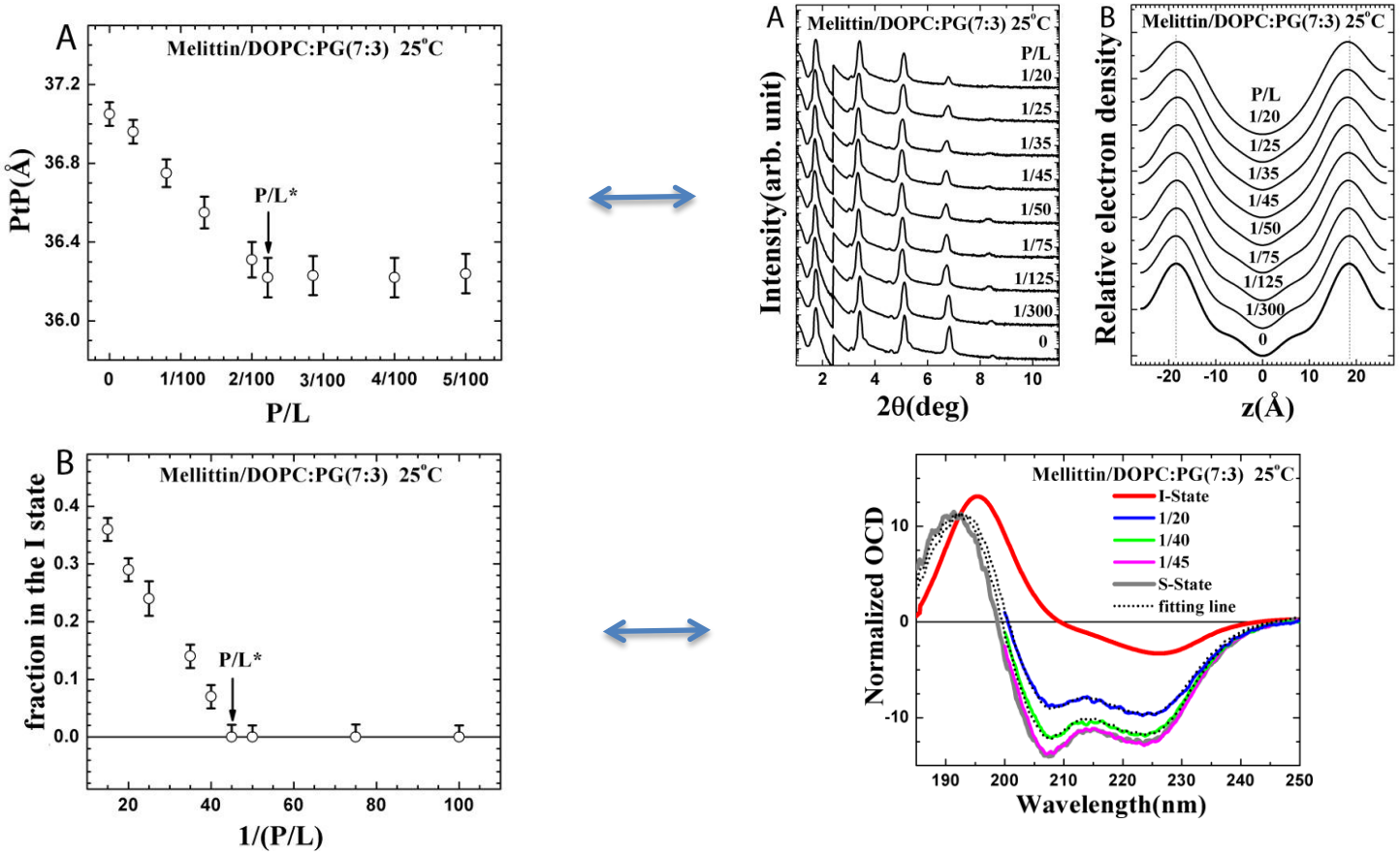
Method 2: Lamellar diffraction

We measured the membrane thickness as a function of antibiotic concentration.



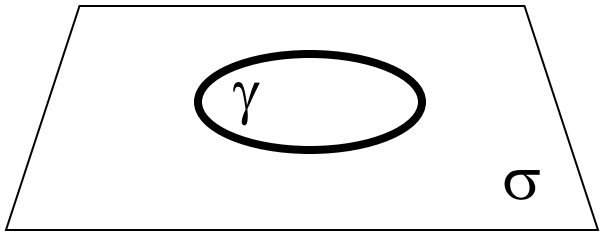
BJ 68, 2361 (1995)
Biochemistry 34, 16764 (1995);
BJ 84, 3751 (2003)

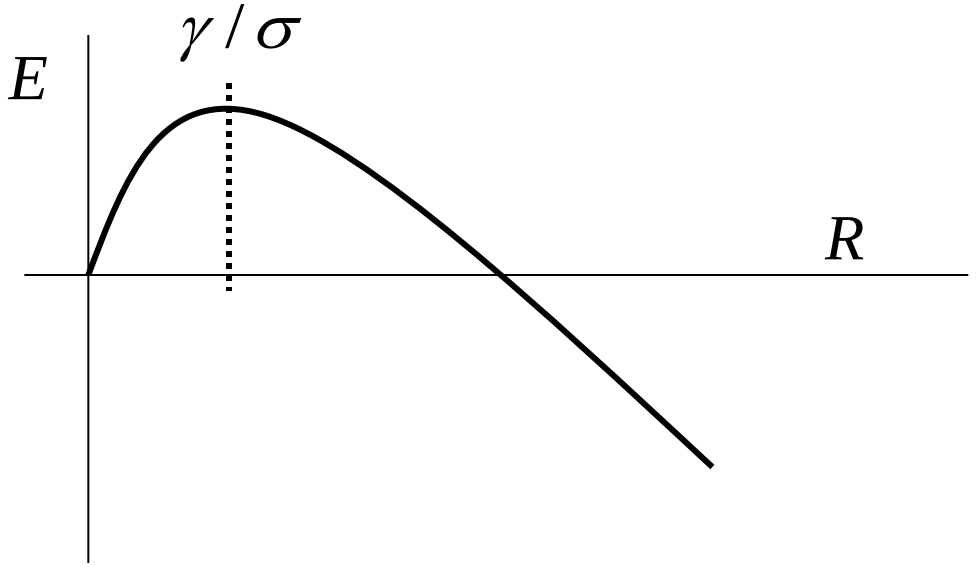
We detected a critical concentration.



Physics of pore formation in a thin layer

$$E_R^o = 2\pi R\gamma - \pi R^2\sigma$$





Litster, Phys. Rev. Lett. A35, 193 (1975)

Taupin et al. Biochemistry 14, 4771 (1975)

Phase transition as a function of P/L.

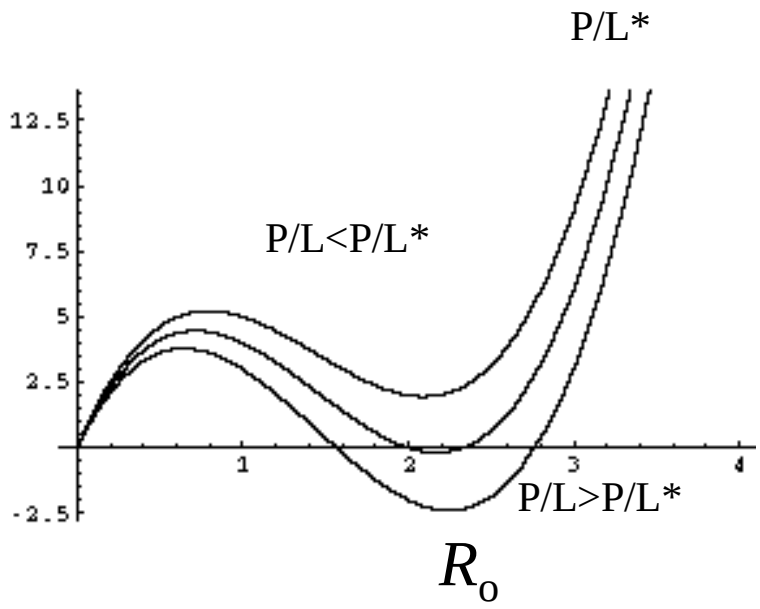
$$E_R^o = 2\pi R\gamma - \pi R^2\sigma$$

$$\sigma_o = K_a(A_P / A_L)(P / L)$$

(for P/L>P/L*)

$$\sigma = K_a(A_P / A_L)(P - P_I) / L$$

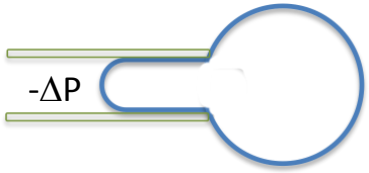
$$P_I = N_p \Gamma_\ell 2\pi R$$

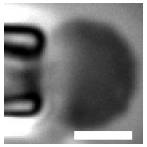


$$E_R = 2\pi R\gamma - \pi R^2\sigma_o + (4/3)\pi^2 R^3\sigma_o(N_p / P)\Gamma_\ell$$

Phys. Rev. Lett. 92, 198304 (2004)

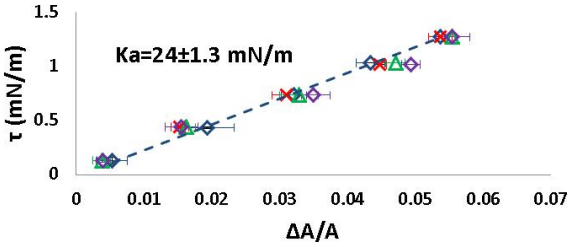
With this understanding, we can now go back to the case of live cells.



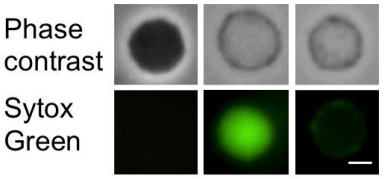


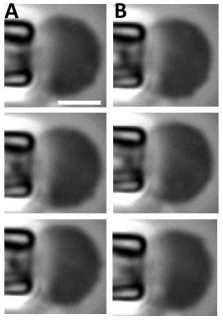
Bar=2.5um

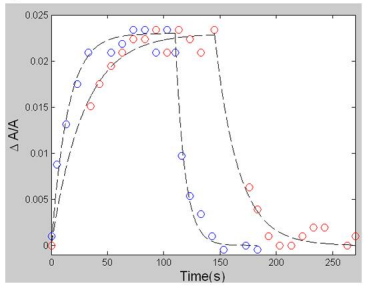
Live cell

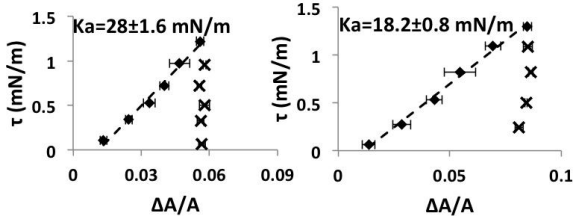


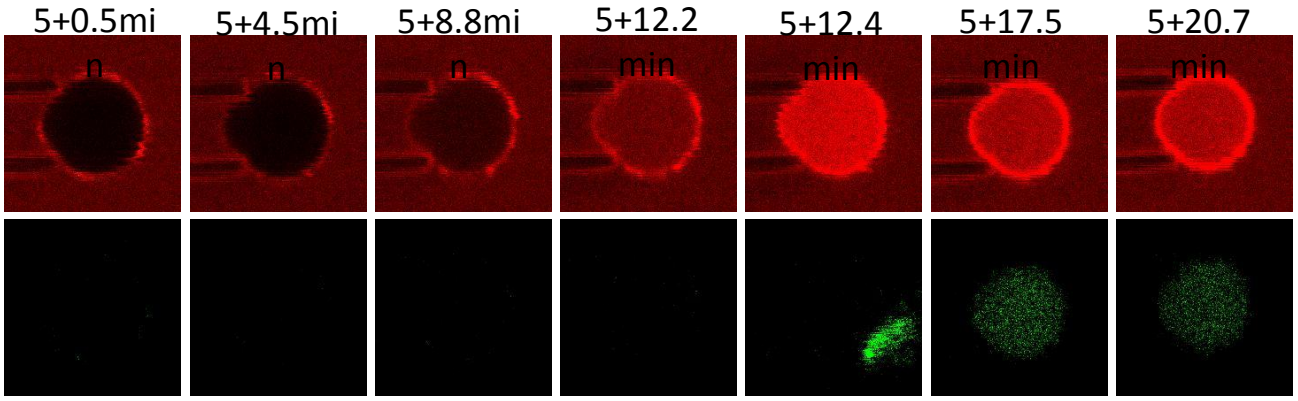
Dead cell







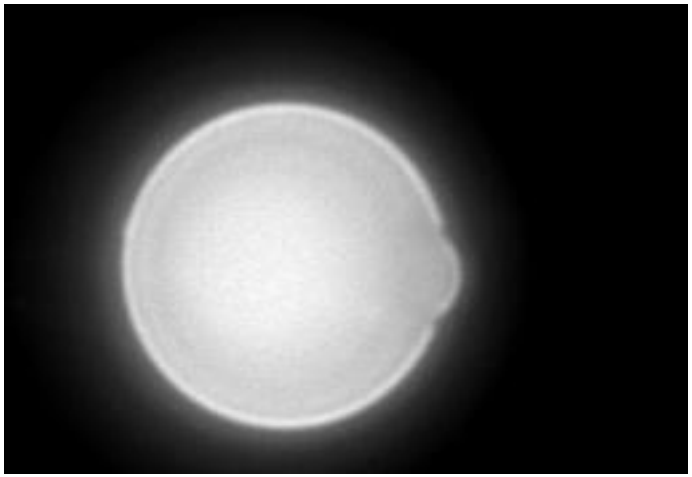


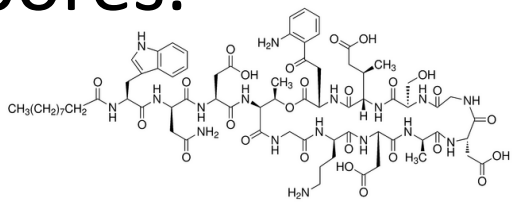


of pore formation story

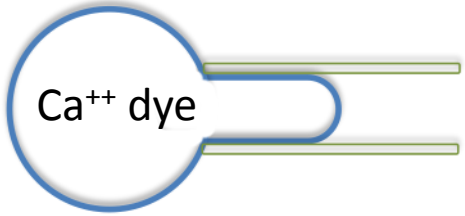
Although the pore-forming antibiotics have not yet been approved as drugs.

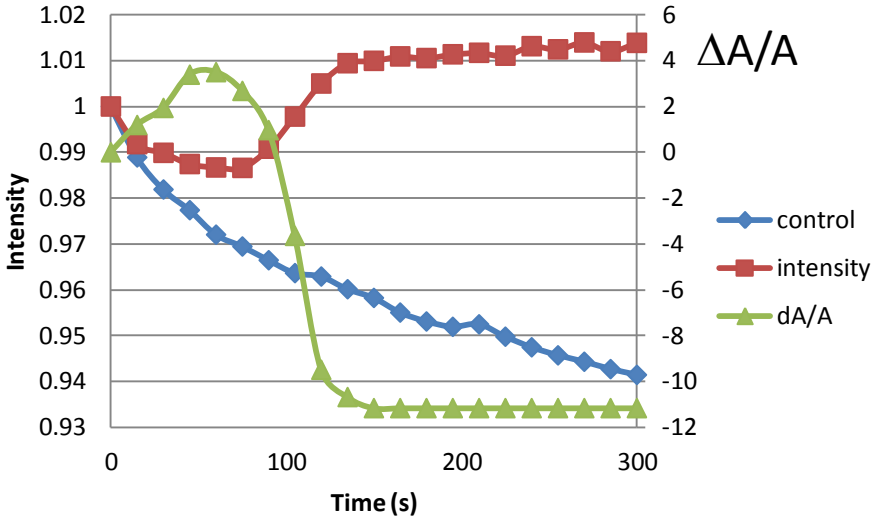
The 2nd kind of membrane-acting antibiotics (daptomycin) do not make pores.



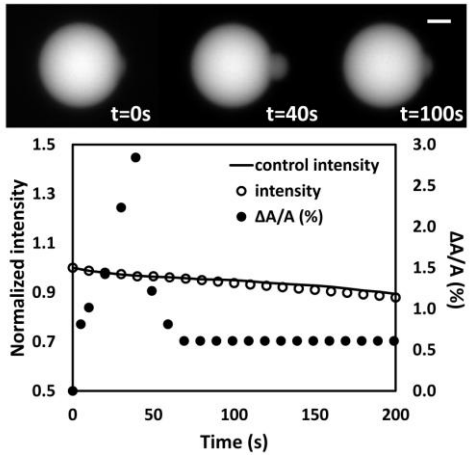


Daptomycin,
Ca^{++}





No leakage



Biochemistry 53, 5384 (2014)

Amyloidoses
(one of the most important medical problems)

Alzheimer's disease

Type II diabetes

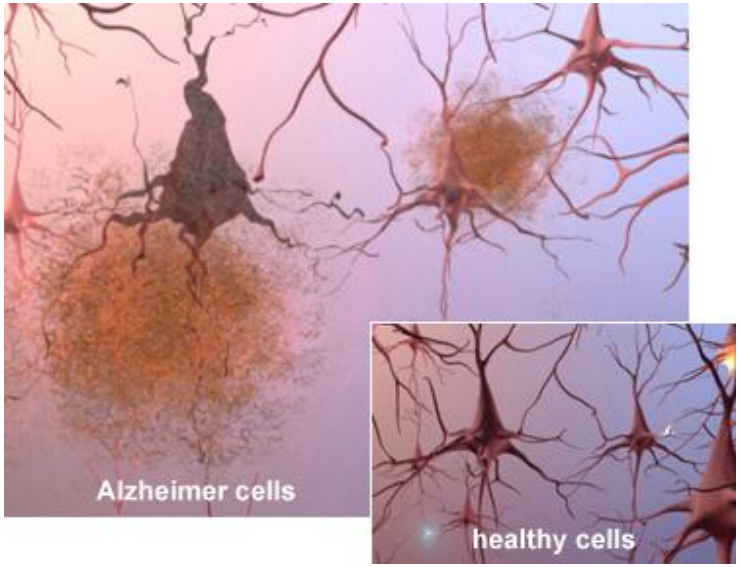
Mad Cow disease

Parkinson's diesease

other ~20 amyloidoses

Common characteristics of amyloidoses

- Each disease is strongly correlated with a protein.
- The disease is associated with the presence of protein plagues.
- But the fibrils and plagues do not harm cells.



Characteristics II (somewhat controversial)

- Protein-membrane interactions turn the proteins into the plaques. During this interaction something happens to the cell membranes. But how?

We need experimental tools to study proteins in membranes.

Acknowledgement

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Tzu-Lin Sun

Collaborators:

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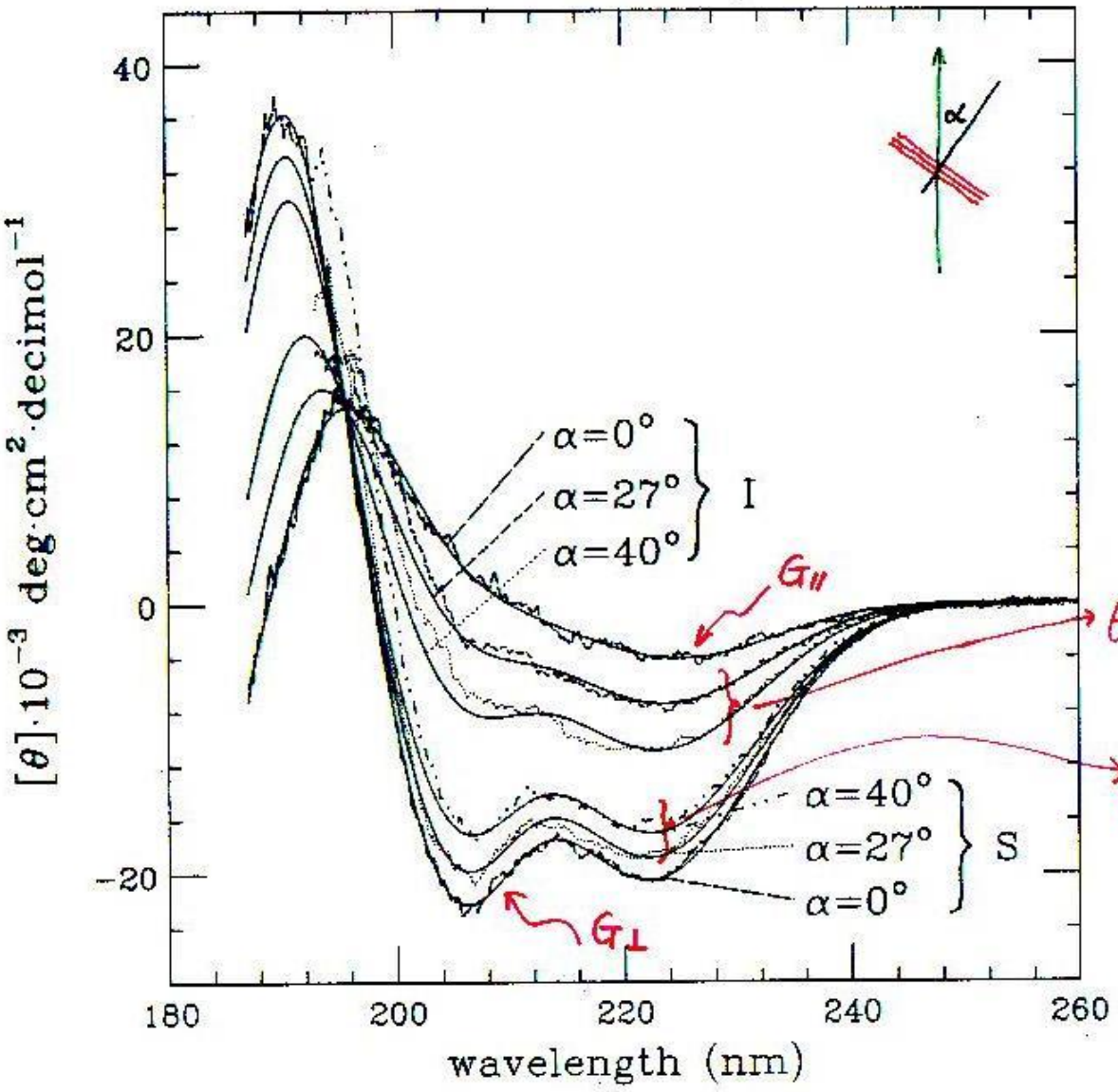
Shuo Qian (ORNL)

Ming-Tao Lee (NSRRC)

Wei-Chin Hung (Mil.Acad.tw)

SUPPORTED by:

Method of Oriented Circular Dichroism



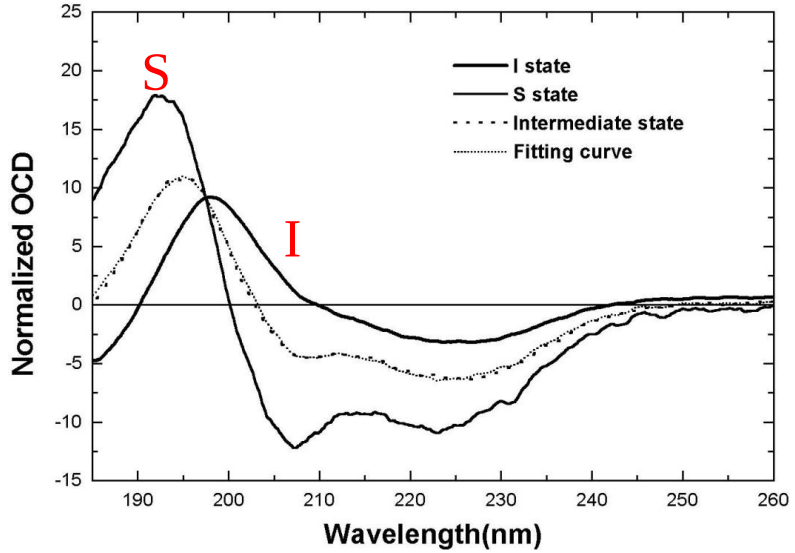
Moffitt Theory

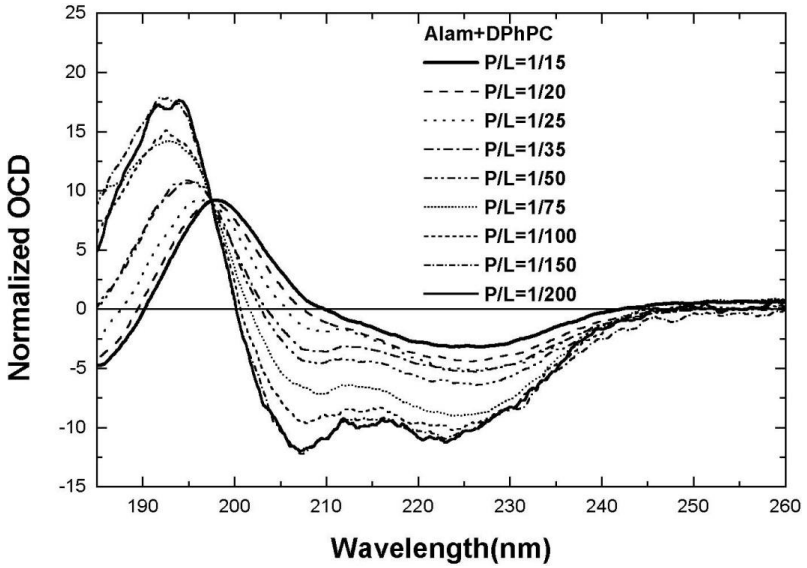
$\theta(\alpha) = G_{\parallel}\cos^2\alpha + G_{\perp}\sin^2\alpha$

$\theta(\alpha) = G_{\perp}\cos^2\alpha + \frac{1}{2}(G_{\parallel} + G_{\perp})\sin^2\alpha$

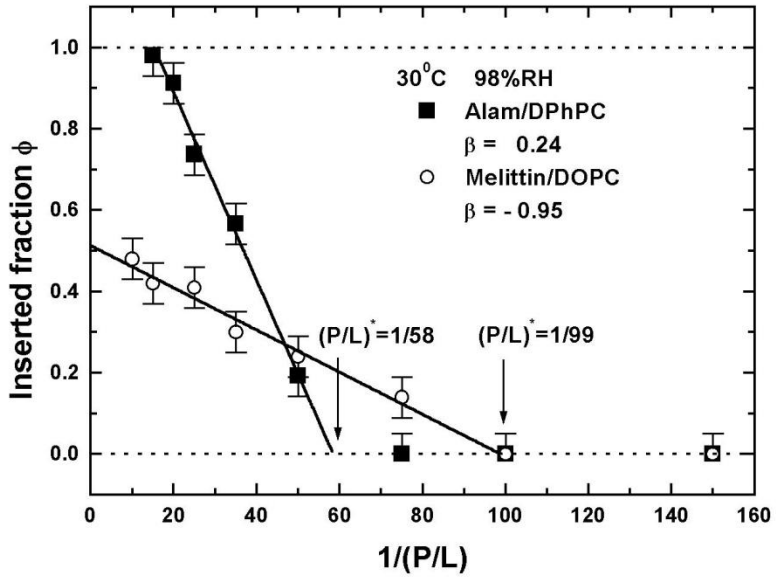
JCP 89, 2531 (1988)
BJ 57, 797 (1990)

Above a critical concentration (P/L), Peptide orientation changes with (P/L)*



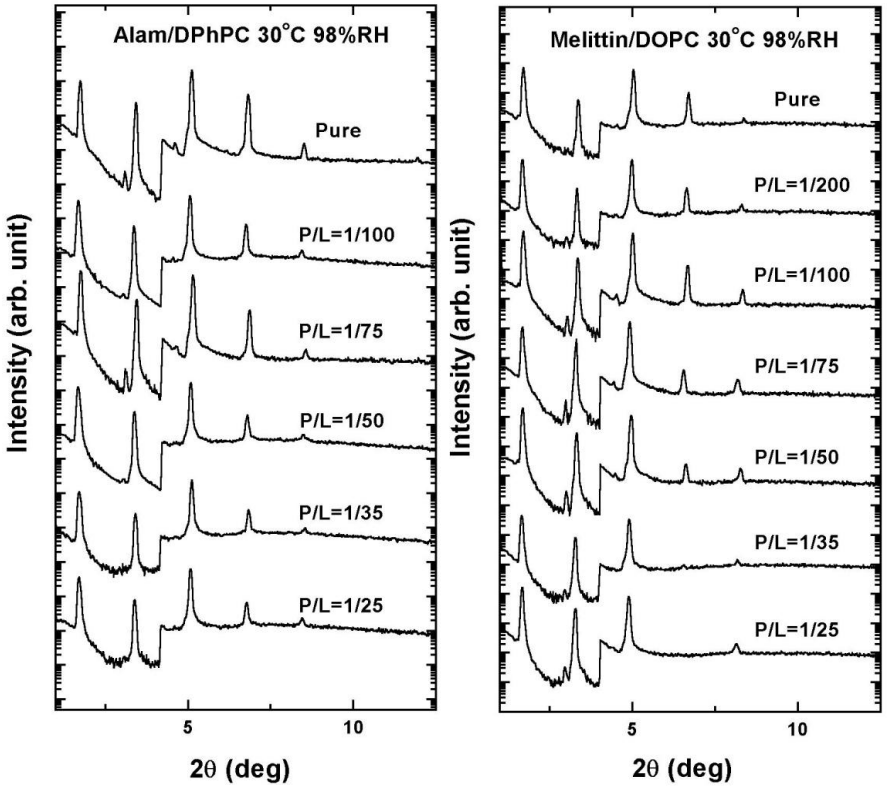


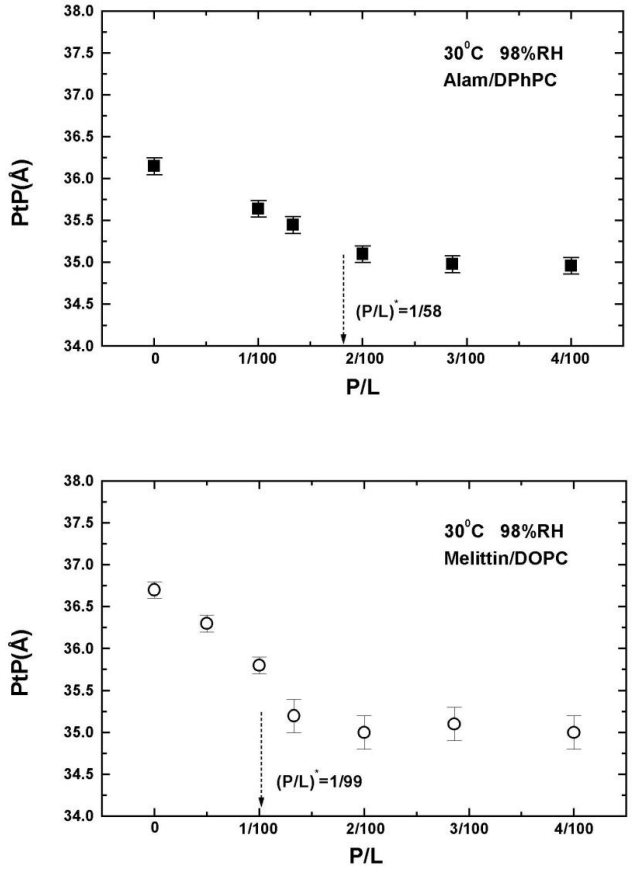
All pore-forming peptides studied showed critical orientation transitions.



BJ 82, 908 (2002)

Membrane Thinning Effect





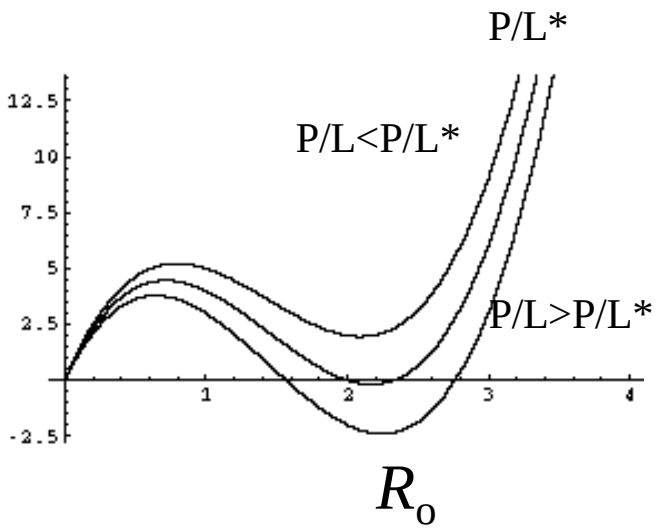
Membrane thinning and peptide orientation change have the same critical concentration.

BJ 68, 2361 (1995); Biochemistry 34, 16764 (1995); BJ 84, 3751 (2003)

Peptide-induced pores are stable.

$$E_R = c_1 R - c_2 R^2 + c_3 R^3$$

$$R_o = (c_2/3c_3) + \sqrt{(c_2/3c_3)^2 - (c_1/3c_3)}$$



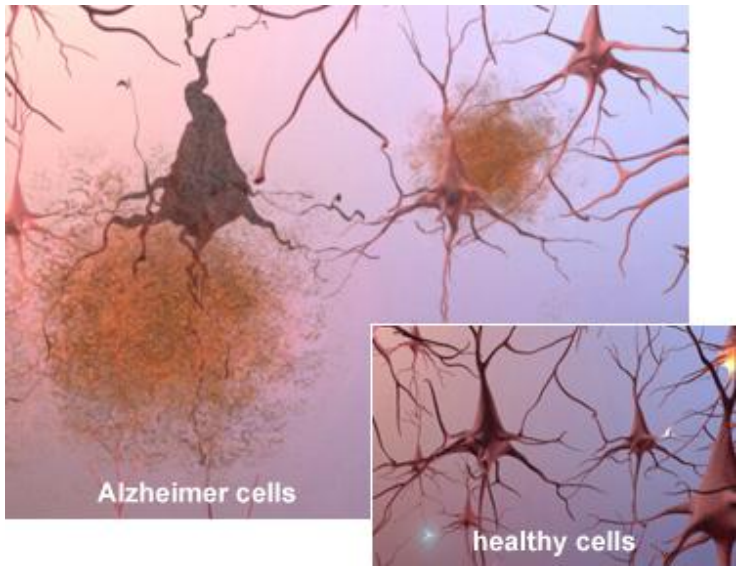
$c_1 = 2\pi\gamma$ decreases with P/L.

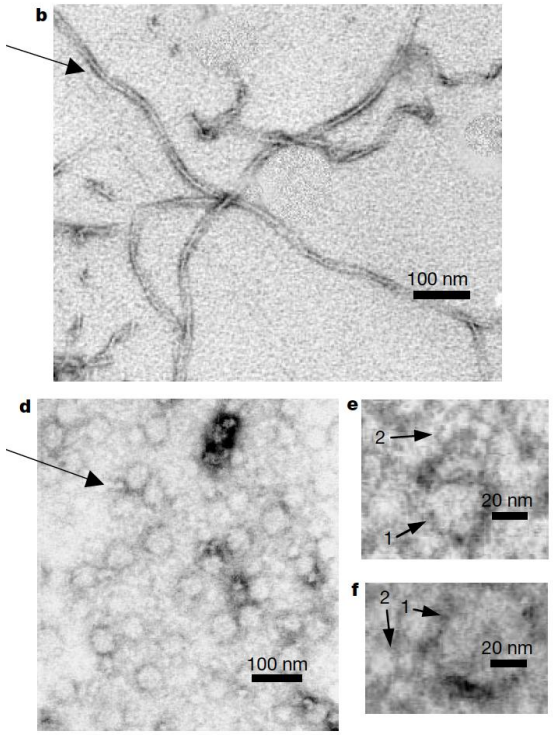
$$c_2 / c_3 = 3(P/L) \Big/ \left\lfloor 4\pi (N_p / L) \Gamma_\ell \right\rfloor = 3.1nm$$

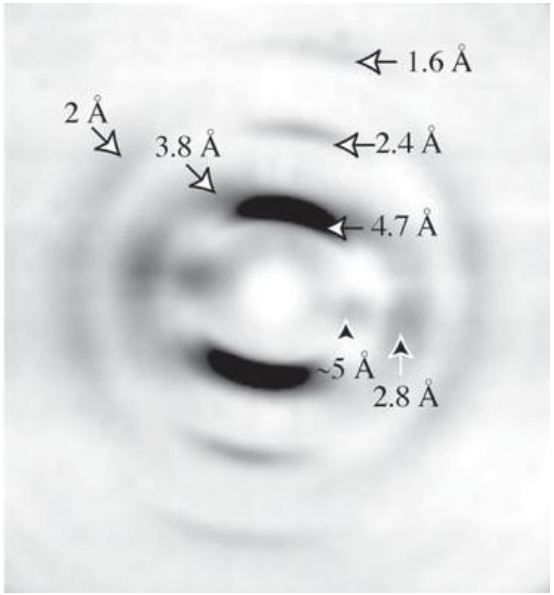
R_o~1-2nm

Phys. Rev. Lett. 92, 198304 (2004)

The diseases are each associated with the presence of plagues (fibrils) of one particular protein that misfolds.





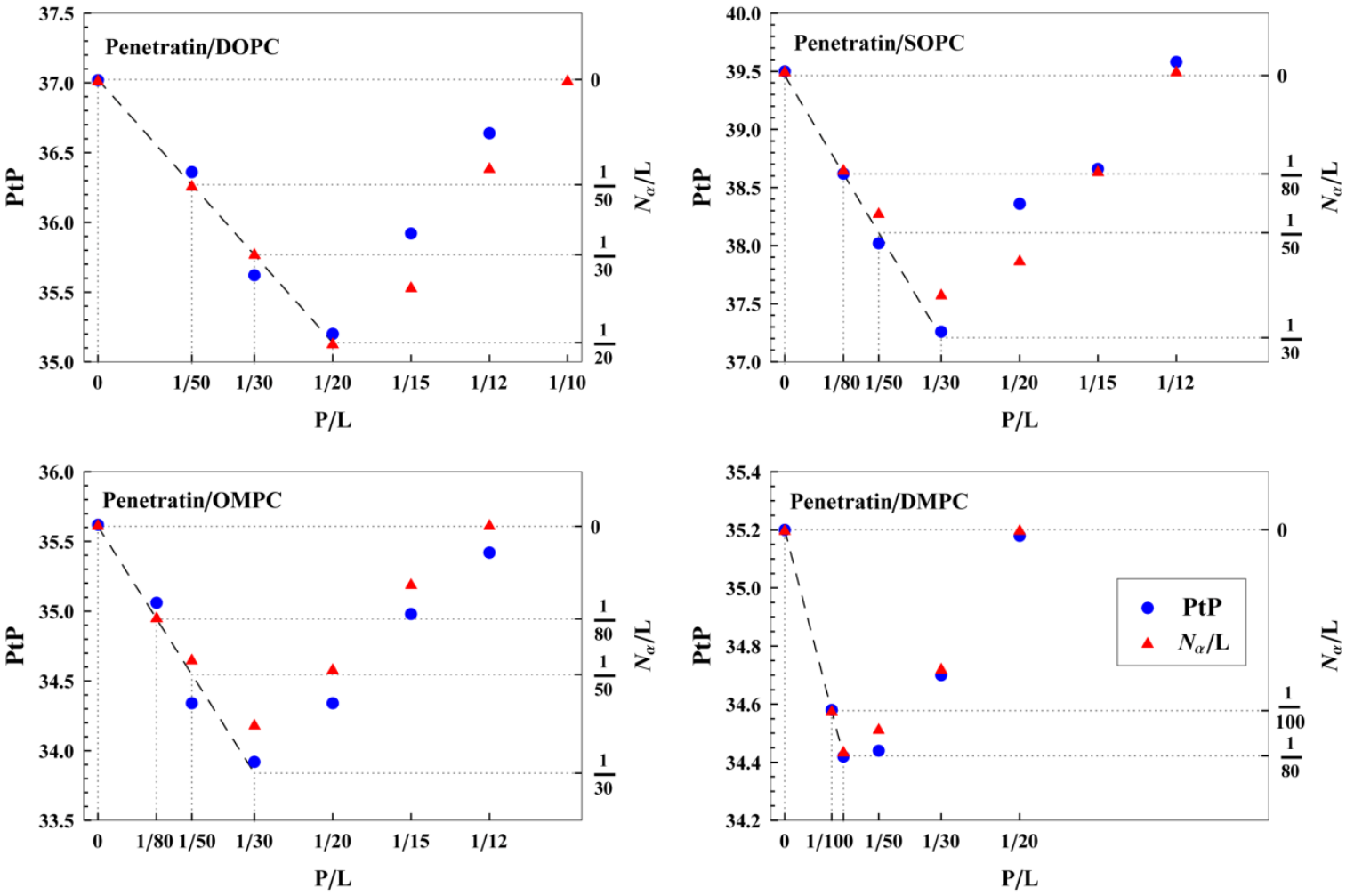


The disease is strongly correlated with the protein.

β-cells co-secret insulin and amylin (an amyloid protein). Human amylin and rat amylin differ by a few amino acids. Human has diabetes; rat has not. But if the rat gene is modified to human gene, rat develops diabetes.

Mad cow disease (bovine spongiform encephalopathy) can be transmitted to human beings by eating the animal protein.

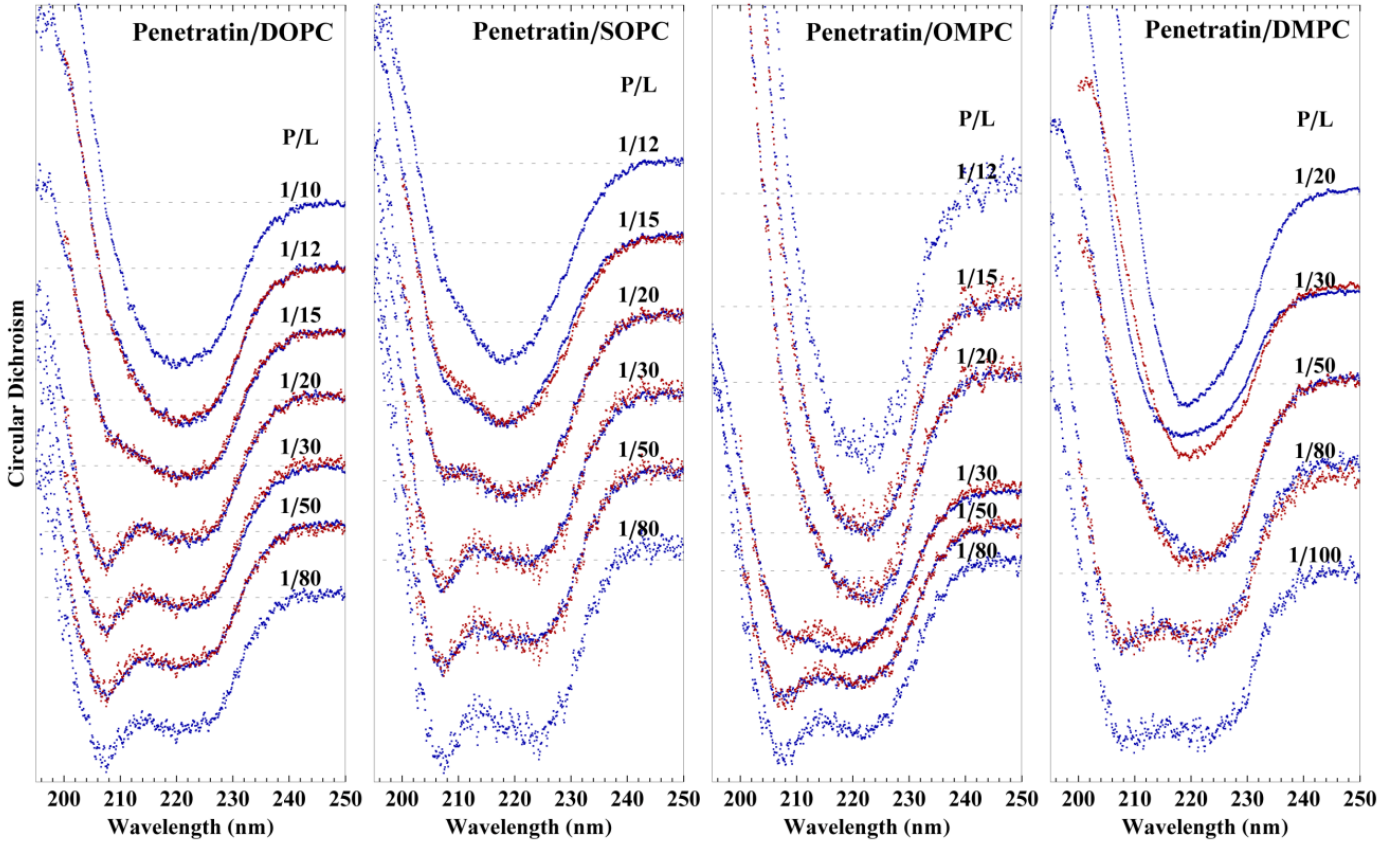
Penetratin binds to the membrane and comes out.



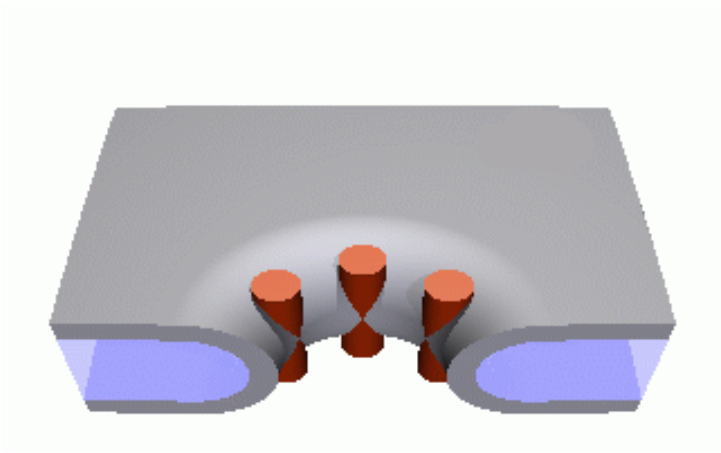
Biophys. J. 98, 2236 (2010)

Penetratin in membranes

Peptide donformation change: CD vs. P/L



A topological question.



$$H = \iint dA[\frac{\kappa}{2}(c_1 + c_2 - c_o)^2 + \bar{\kappa}c_1c_2]$$

Helfrich (1973)

Gauss-Bonnet Theorem (for a closed surface)

$$\iint dA c_1 c_2 = 4\pi(n_c - n_h)$$