Antimicrobial polymer action: towards coarse graining

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Collaborators:

Experimental:
Kenichi Kuroda (University of Michigan)

Atomistic Simulations/FF development:
Ivaylo Ivanov (Georgia State University)
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Michael Klein (Temple University)

Coarse Graining:
Wataru Shinoda (AIST, Tsukuba, Japan)

Research Interests

Tools: Classical Molecular Dynamics, supplemented by force field development using (minimal) quantum chemistry tools, Coarse grain modeling

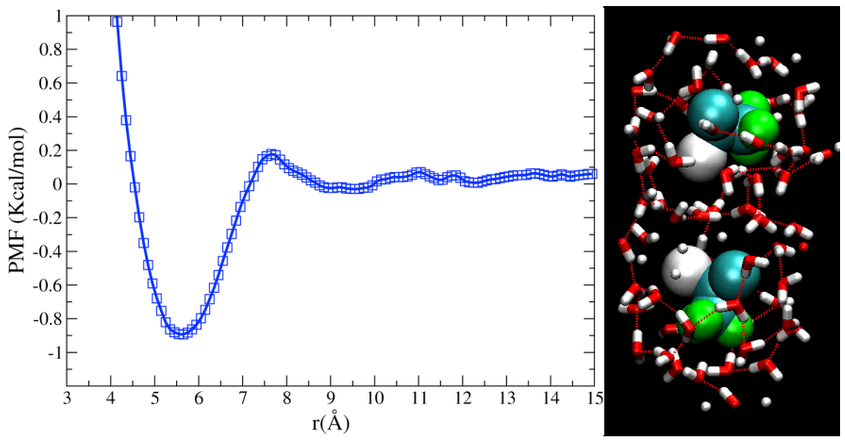
Systems: Polymers, Proteins, Lipid Membranes

Polymers:

- Self assembled monolayers
- Polyelectrolytes

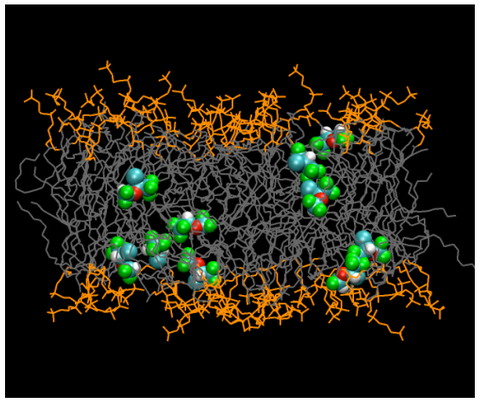
Proteins:

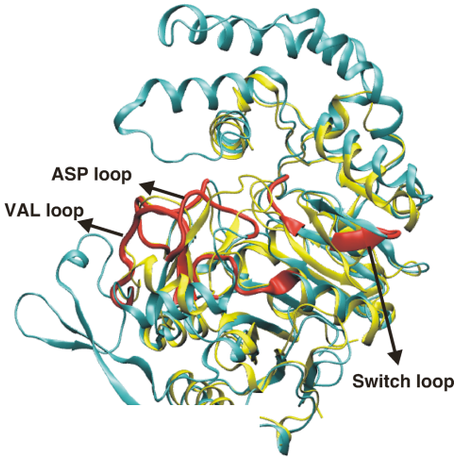
- Ion channels
- Thermal stability
- Protein-drug interactions

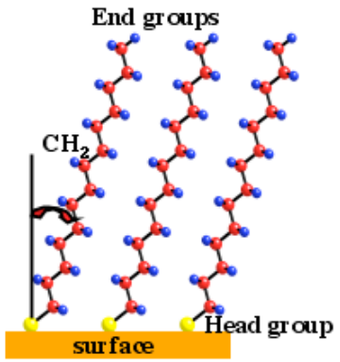


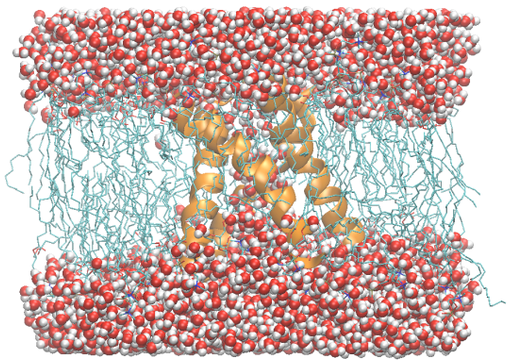
Lipid Membranes:

- Small molecule transport
- Antimicrobial peptides/polymers
- Rafts





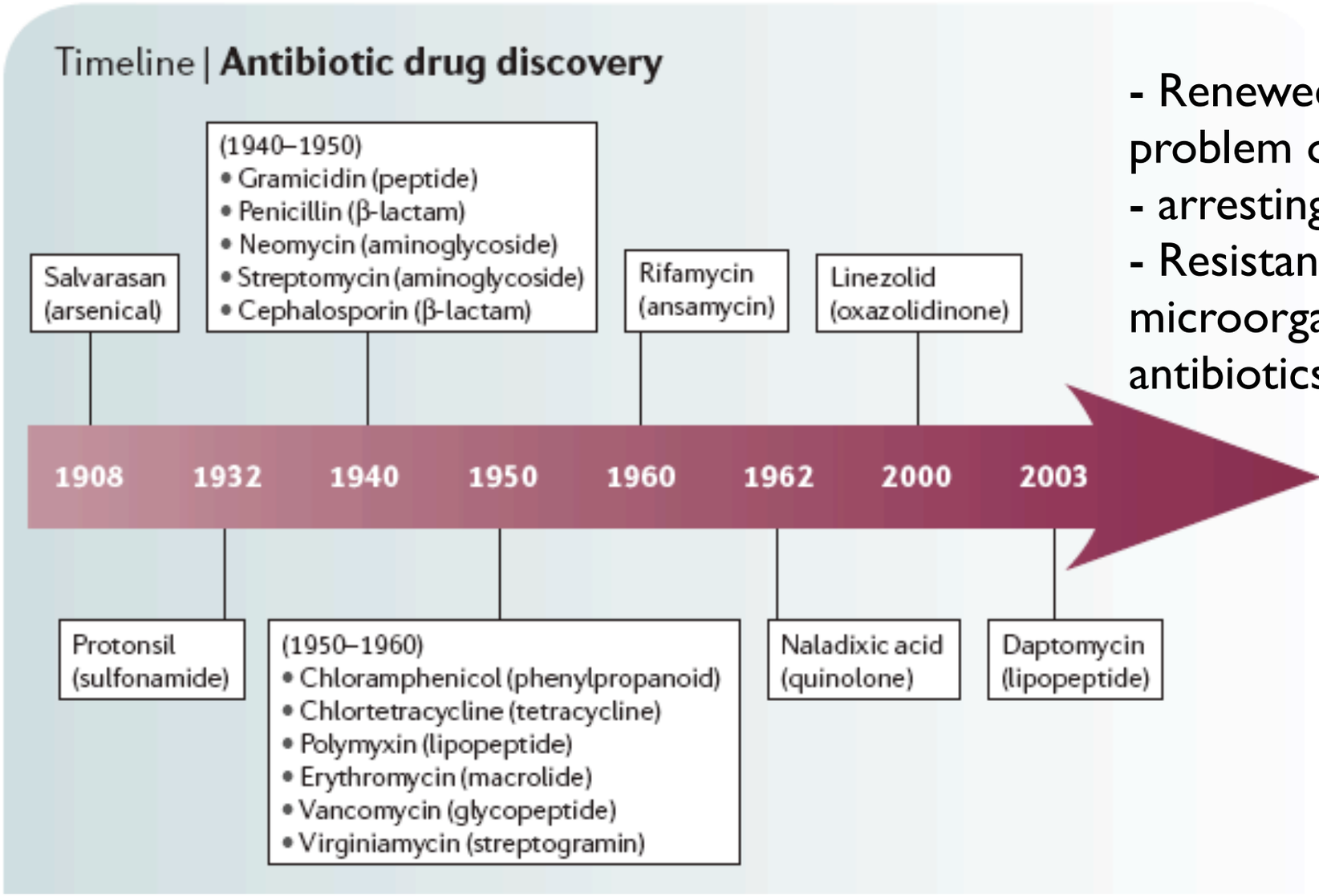




Outline

- Antibacterial mechanism
- All Atom simulations
- Coarse graining attempts

Antibiotics



- Renewed interest in antibiotics due to the problem of growing antibiotic resistance
- arresting cell growth
- Resistance is the continuous growth of microorganisms in the presence of antibiotics

antibiotics put selective pressure on microorganisms leading to “superbugs”

Nature Reviews Microbiology, 2007

Innate Immunity

Immunity

innate

(main form of immunity in less complex species including insects and plants)

adaptive

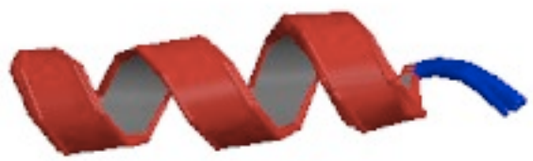
(evolutionary addition to the immune strategy of higher animals)

Innate immunity: highly effective set of mechanisms used by multicellular organisms to recognize and counter the constant threat of microbial infections

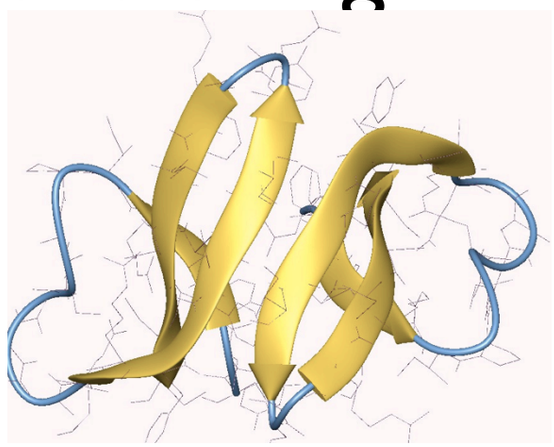
cationic host defense peptides:

- stimulated by bacterial signalling molecules
- an element of innate immunity in controlling infections

LL-37 (14-18 residues)

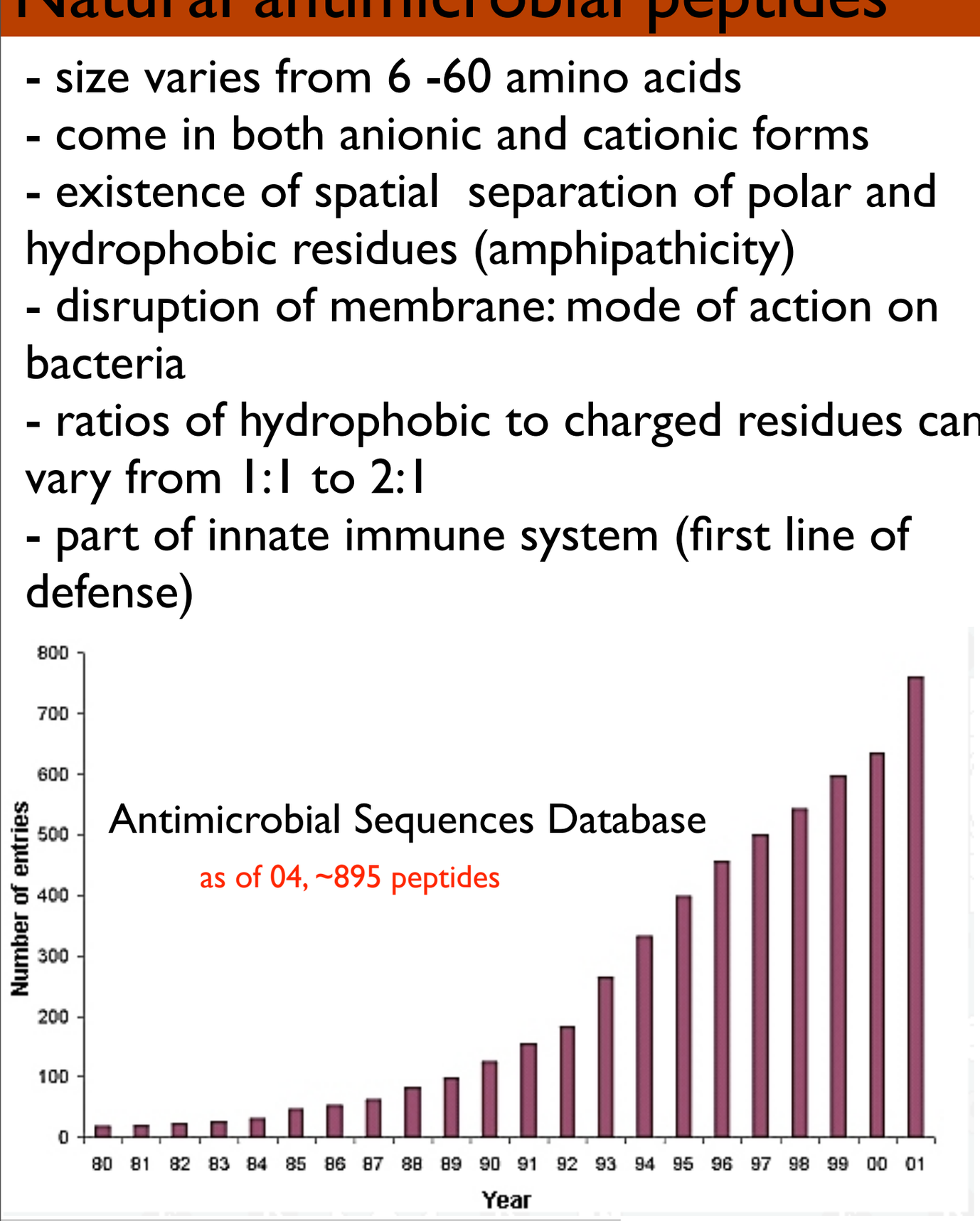


defensin (28-42 residues)

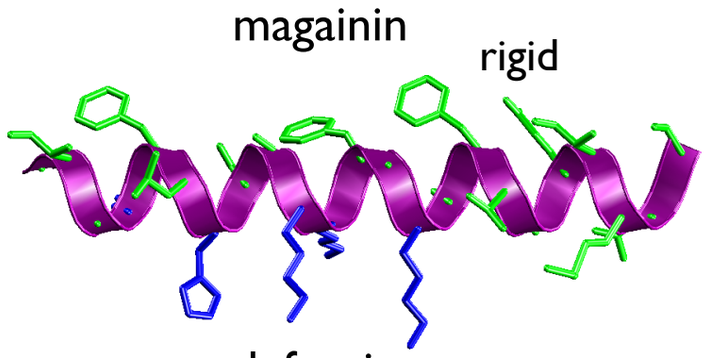


Natural antimicrobial peptides

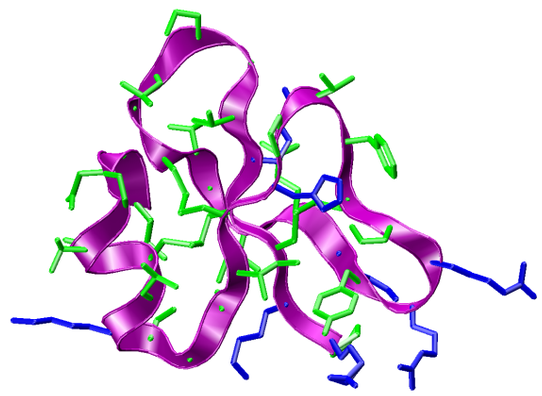
- size varies from 6 -60 amino acids
- come in both anionic and cationic forms
- existence of spatial separation of polar and hydrophobic residues (amphipathicity)
- disruption of membrane: mode of action on bacteria
- ratios of hydrophobic to charged residues can vary from 1:1 to 2:1
- part of innate immune system (first line of defense)



hydrophobic

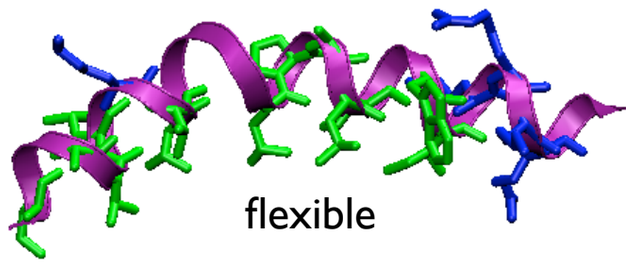


43%



37%

melittin



47%

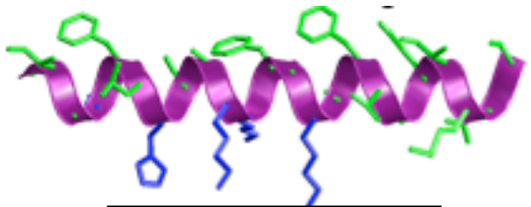
http://www.bbcm.univ.trieste.it/~tossi/pag5.htm

http://aps.unmc.edu/AP/main.php

Antimicrobial mechanism

Antimicrobial peptide

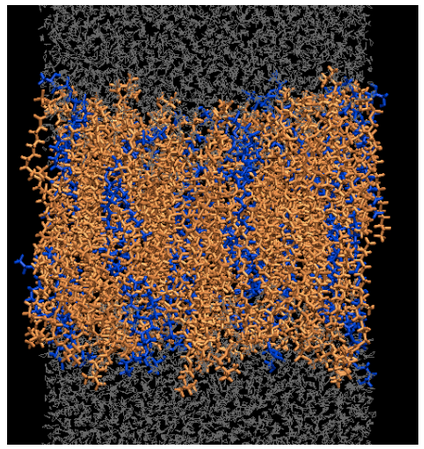
charged



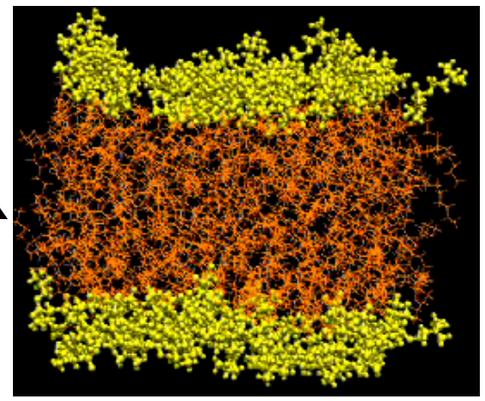
hydrophobic

Selectivity

+



bacterial membrane



plasma membrane

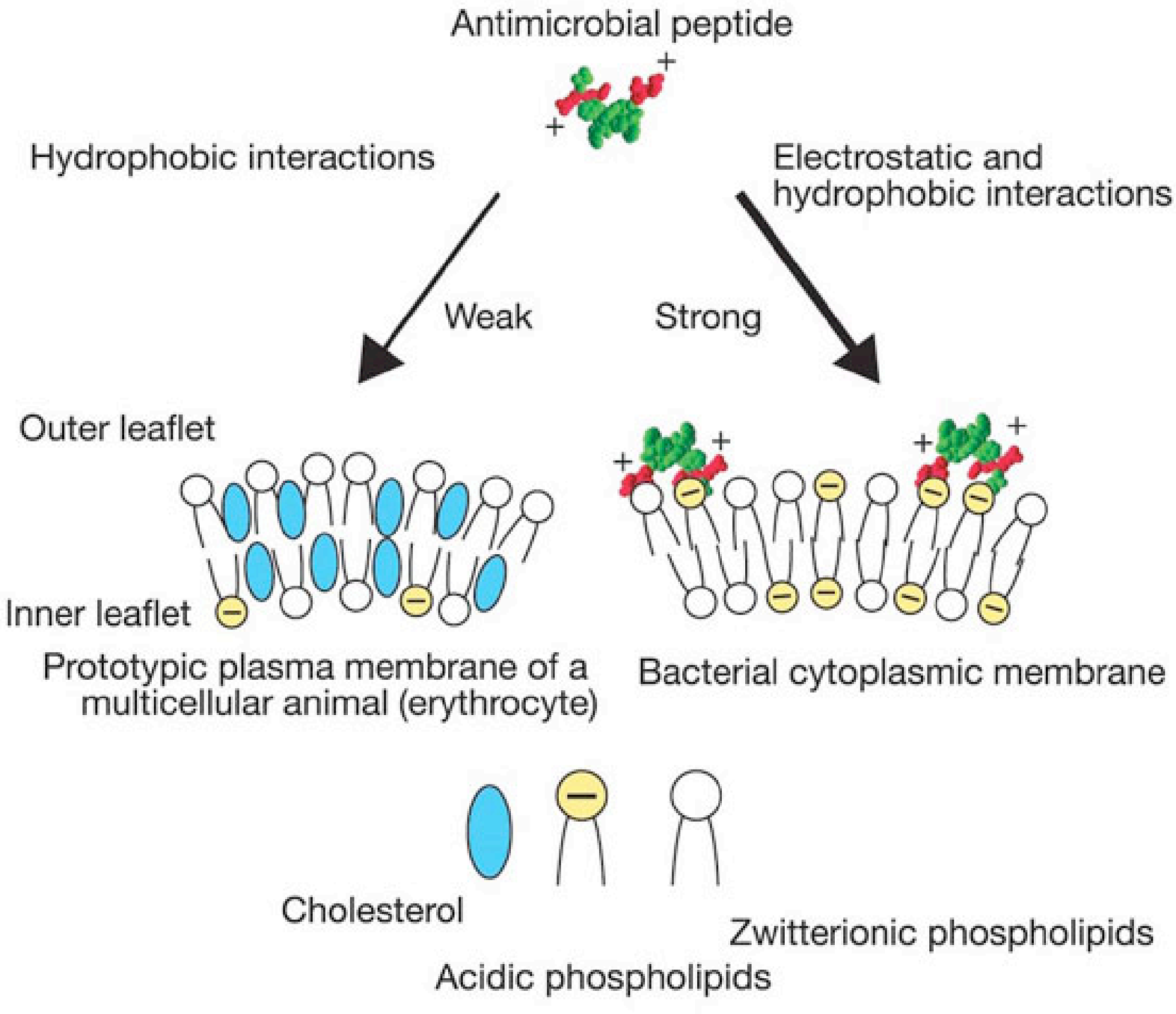
Disruption

Barrel-stave

Carpet

Toroidal

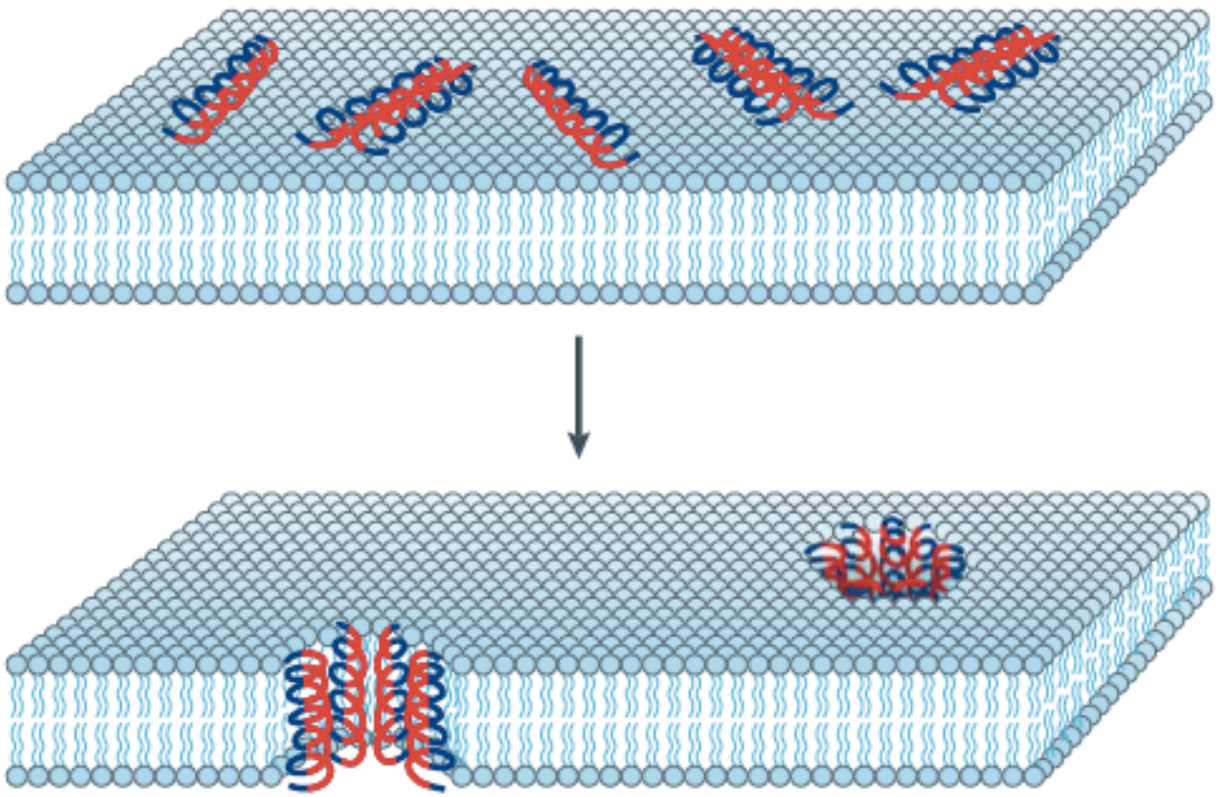
Antimicrobial mechanism: Selectivity



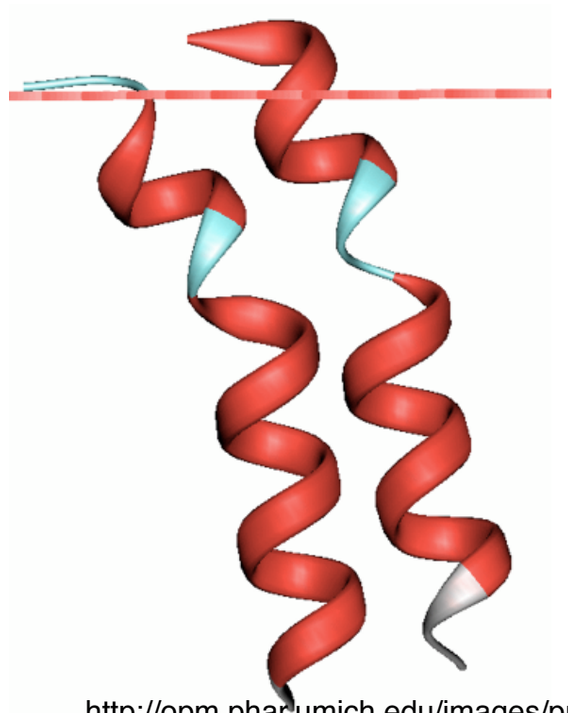
Bacterial membranes have 20-30% negatively charged lipids

Zasloff, Nature 2002

Disruption Mechanisms: Barrel Stave model



- Peptides aggregate
- hydrophobic parts (blue) aligns with the lipid core region
- hydrophilic parts (red) forms the interior of the pore

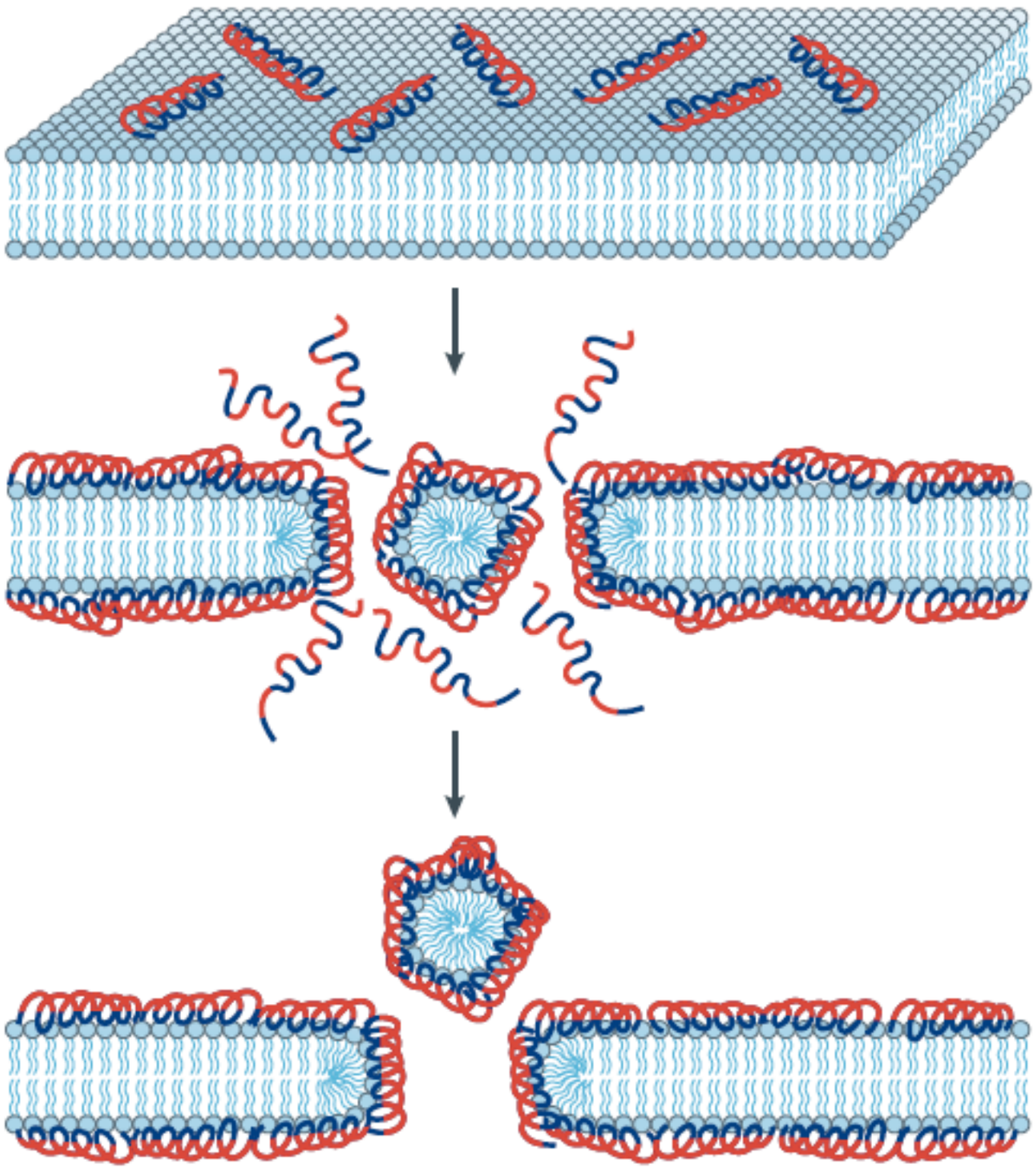


http://opm.phar.umich.edu/images/proteins/1amt.gif

Example: Alamethicin
- adopts helical configurations
- 3-11 helices
- inner diameter ~ 1.8nm
- changes in lipid composition can affect the number of peptides in the aggregate

Rocca et al, BBA, 1999
Yang et al, Biophys. J, 2002
Zasloff, nature, 2002
Brodgen, Nature Reviews Microbiology, 2005

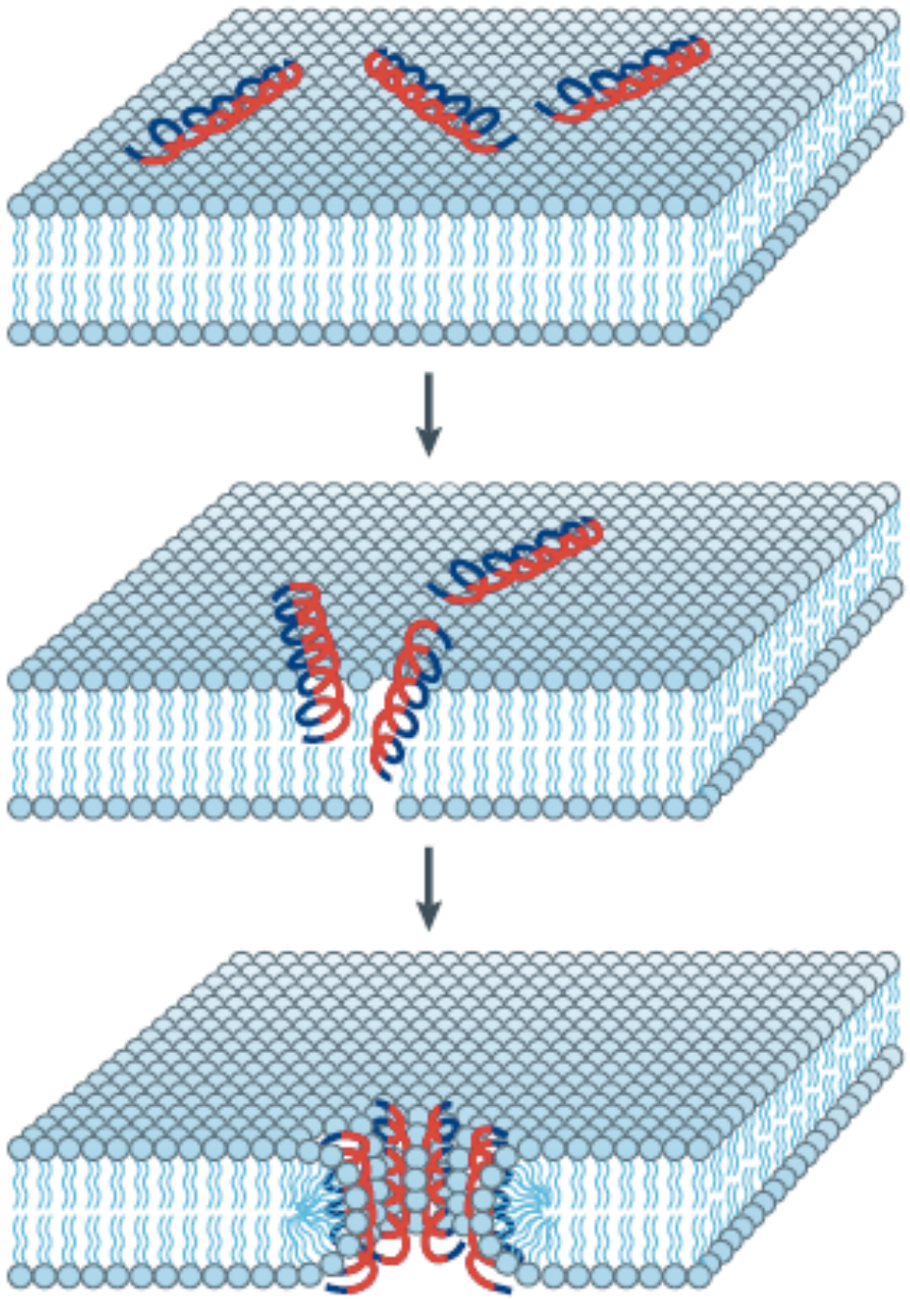
Disruption Mechanisms: Carpet model



- Peptides accumulates parallel to the bilayer
- electrostatic interactions with anionic sites of the bilayer
- at high peptide concentration, possible formation of micelles leading to lysis
- possible transient toroid like holes for further access

Examples: Ovispirin, cecropin

Disruption Mechanisms: Toroidal model



- attached peptides aggregate and induce lipids to bend continuously
-water core is lined with both hydrophilic parts of the peptide and lipid head groups
- peptides are always associated with lipid head groups (unlike barrel-stave model)

Examples: magainin, melittin

magainin induced toroidal pore have inner diameter of ~3-5 nm and each pore has 4-7 magainin monomers and ~90 lipid molecules

Biomimetic polymers: desitred properties

MIC: minimum inhibitory concentration

MIC was defined as the lowest polymer concentration to completely inhibit bacterial growth lower values preferred

HC_{50}: HC_{50} is the polymer concentration to achieve 50% hemolysis

higher values preferred

ment of peptidic analogues of AMPs as intravenously administered antibiotics.[1] Experimental studies *in vitro* have shown that bacteria cannot easily develop resistance to this class of compounds,[67] suggesting they might not suffer from some of the same problems of resistance encountered in other classes of antibiotics with more specific targets. Indeed, a compound designed by these principles successfully completed the first phase of human clinical trials as an iv antibiotic for treatment of multidrug-resistant *S. aureus*.[96]

ACCOUNTS of chemical research

De Novo Design of Antimicrobial Polymers, Foldamers, and Small Molecules: From Discovery to Practical Applications

GREGORY N. TEW,† RICHARD W. SCOTT,‡ MICHAEL L. KLEIN, AND WILLIAM F. DEGRADO*

2009

TABLE 1. Conformational Tuning of Properties of Antimicrobial Oligomers[a]

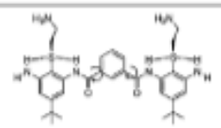
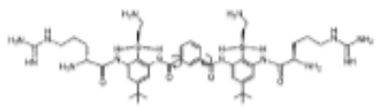
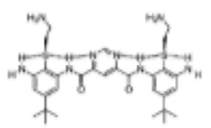
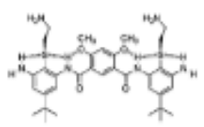
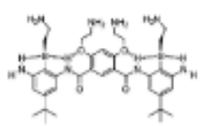
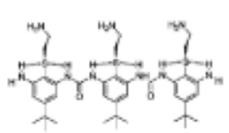
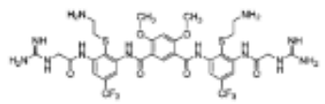
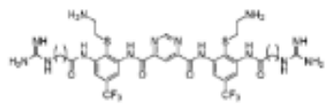
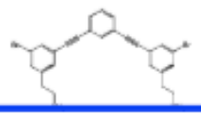
Compound	Structure	MIC (μM) *S. aureus*[b]	MIC (μM) *E. Coli* D31	HC_{50} (μM)[c]
2		50	12.5	14
3		7.8	3.9	445
4		0.80	0.80	18
5		0.87	0.87	145
6		0.66	5.3	593
7		0.70[e]	0.70	5
8		0.2	2.8	63-90
9		0.20	0.1	440
10		0.20	0.10	440

FIGURE 2 Facially amphiphilic structures of the SMAMPs; β-peptide **1** (left), pyrimidine arylamide oligomer **2** (middle), and phenylene ethynylene oligomer **3** (right).

Synthetic Mimics of Antimicrobial Peptides

Abhigyan Som[1], Satyavani Vemparala[2], Ivaylo Ivanov[3,4], Gregory N. Tew[1]

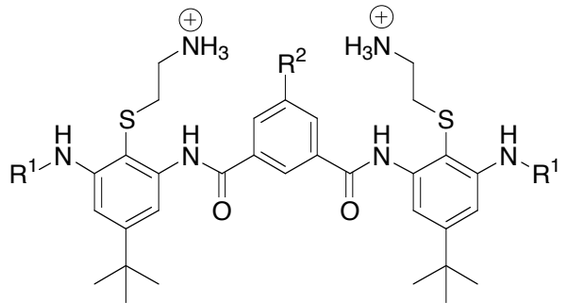
PeptideScience Volume 90 / Number 2 2008

SMAMP	MIC (Gram-positive) (μg/mL)	MIC (Gram-negative) (μg/mL)	HC_{50} (μg/mL)	Selectivity[a] (HC_{50}/MIC)
1[b]	—	9.0	135	15
1[c]	—	26.0	4680	180
2	0.8	0.8	14	17.5
3	0.2	0.1	88	880
MSI-78[d]	12.0	12.0	120	10

Biomimetic polymers

Facial amphiphilic

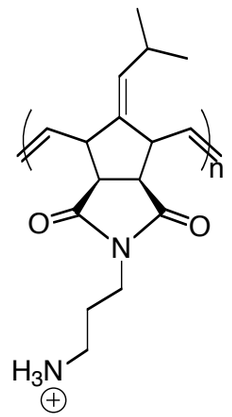
• Aryl amides:



• Tew, G. N., Liu D., et al. *PNAS*, **2002**, *99*, 5110

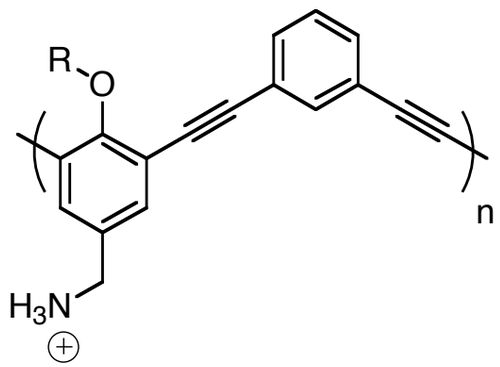
• Liu,D., et al. *Angew. Chem. Int. Ed.* **2004**, *43*, 1158

• Polynorbornenes



• Ilker, M. F., et al. *J. Am. Chem. Soc.* **2004**, *126*, 15870

• Poly(*m*-phenylene-ethynylene)



• Arnt, L., et al. *Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3860.

• Arnt, L.; Tew, G. N. *JACS*, **2002**, *124*, 7664

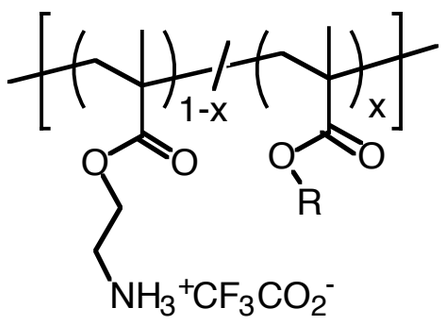
properties

• Rigid backbones

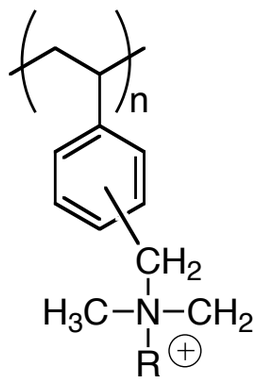
• Synthetically difficult to make

• Non-toxic to human cells

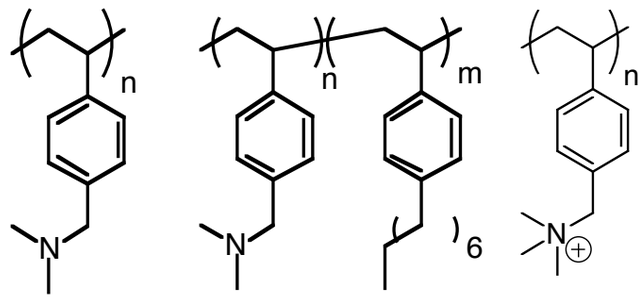
Flexible amphiphilic



Kuroda, K, DeGrado, W.F *JACS* **2005**, *127* 4128.



Ikeda, T., Tazuke, S., *Makromol. Chem. Rapid Commun.* **1983**, *4*, 459.



• Gelman, M. A.; Weisblum, B., Lynn, D. M.; Gellman, S. H. *Org. Lett.* **2004**, *6*, 557.

• Flexible backbones

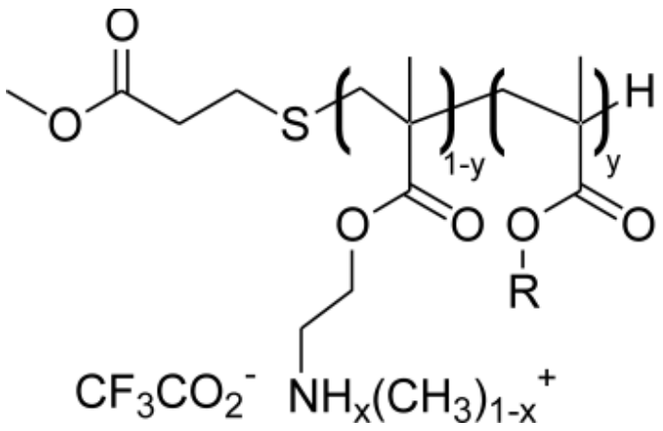
• Facile preparation

• Toxic to human cells

Biomimetic polymers: systems of interest

Design paradigms:

- amphiphilicity achieved through introduction of charged & hydrophobic groups
- rigid backbones allow pre-built facial amphiphilicity
- highly repetitive structures are thought beneficial and enhance activity
- aim is to get ***high selectivity*** and ***low toxicity***



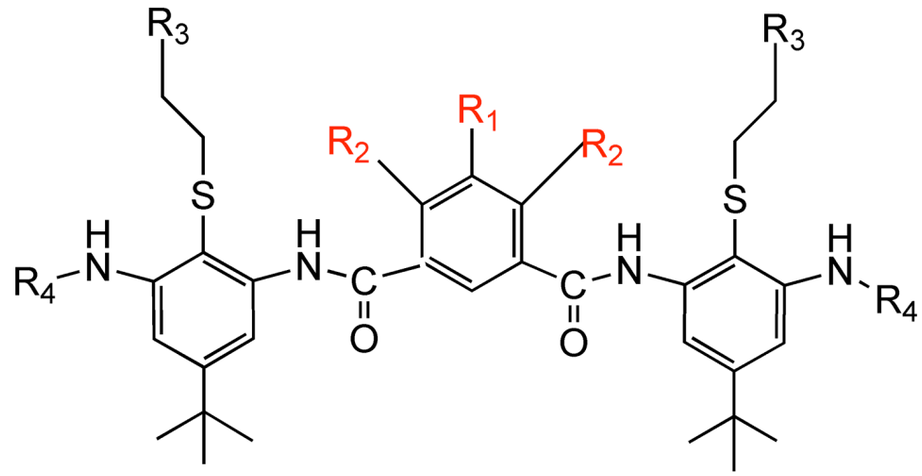
Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents

Kenichi Kuroda and William F. DeGrado*

4128 ■ J. AM. CHEM. SOC. 2005, *127*, 4128−4129

AIMS:

- is 'inbuilt amphiphilicity' a requirement for antimicrobial action?
- understand the interplay between hydrophilic & charged groups with respect to antimicrobial action



***De novo* design of biomimetic antimicrobial polymers**

Gregory N. Tew*†‡, Dahui Liu*‡, Bin Chen§, Robert J. Doerksen§, Justin Kaplan*, Patrick J. Carroll¶, Michael L. Klein§, and William F. DeGrado*¶

5110–5114 | PNAS | April 16, 2002 | vol. 99 | no. 8

AIMS:

- calculate appropriate backbone torsion potentials
- effect of substitutions (like R^1 & R^2) on the 'flexibility' of the molecule
- study interactions of arylamides with both zwittrionic & charged lipids

- understand aggregation and disruption mechanism
- phase space of charge & hydrophobicity

Computational approach

Single AMP interactions- AA simulations[1]

-Insertion mechanisms

-Free energy of binding

Aggregation- CG simulations

AM polymers[4,5]

Parameterization - DFT calculations[2,3]

-Effects of substituents on torsional barriers

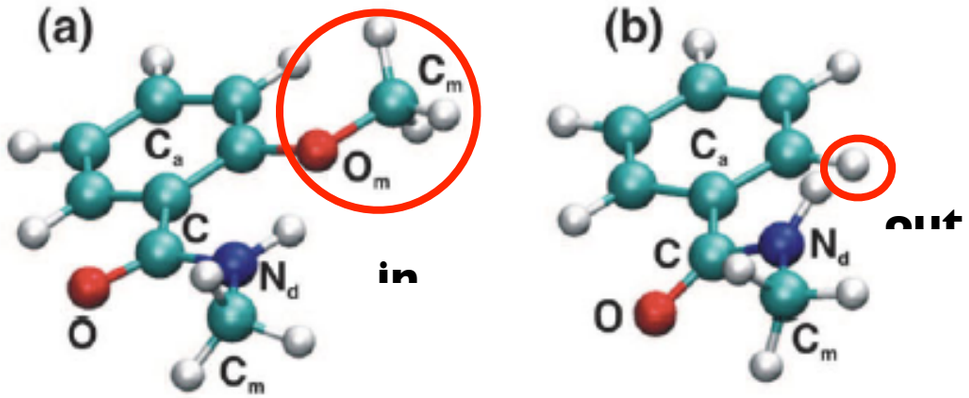
-Conformational rigidity

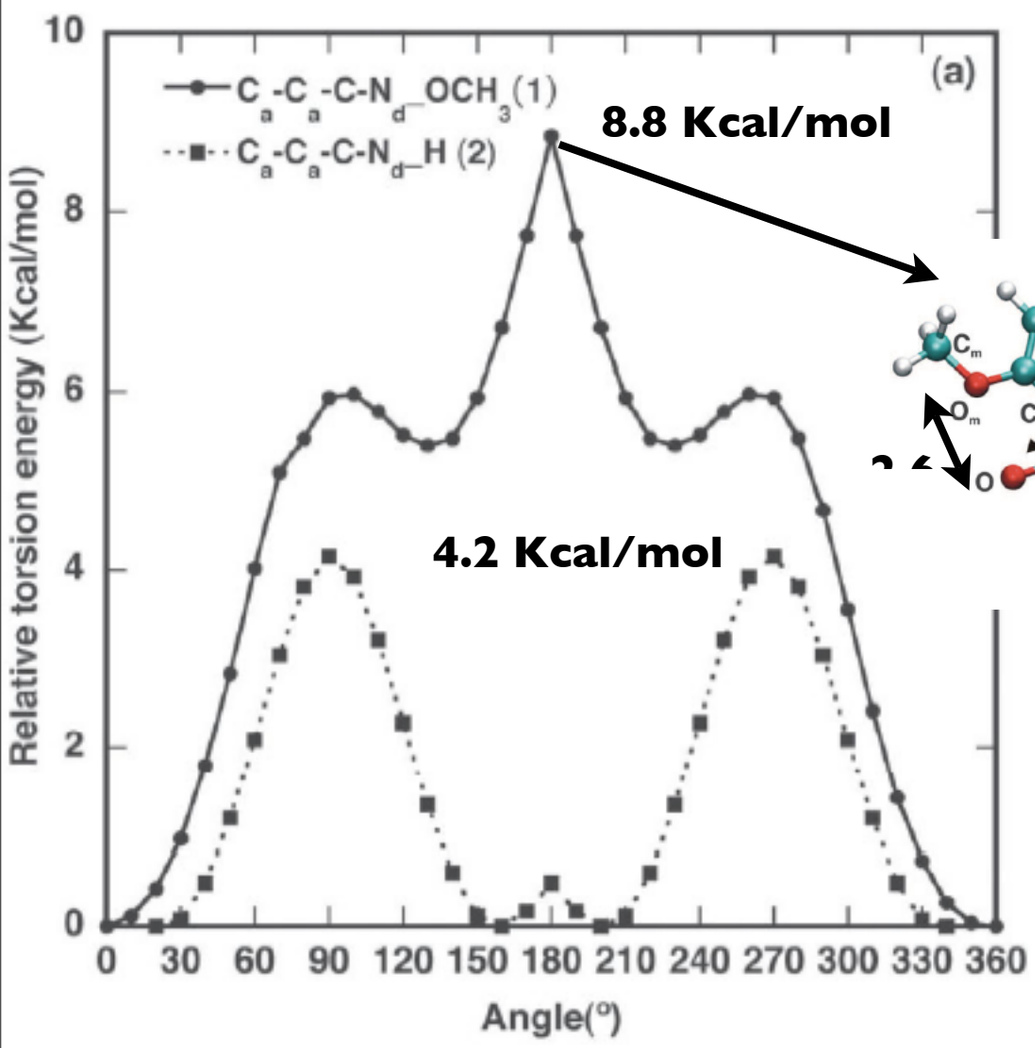
Lipids:

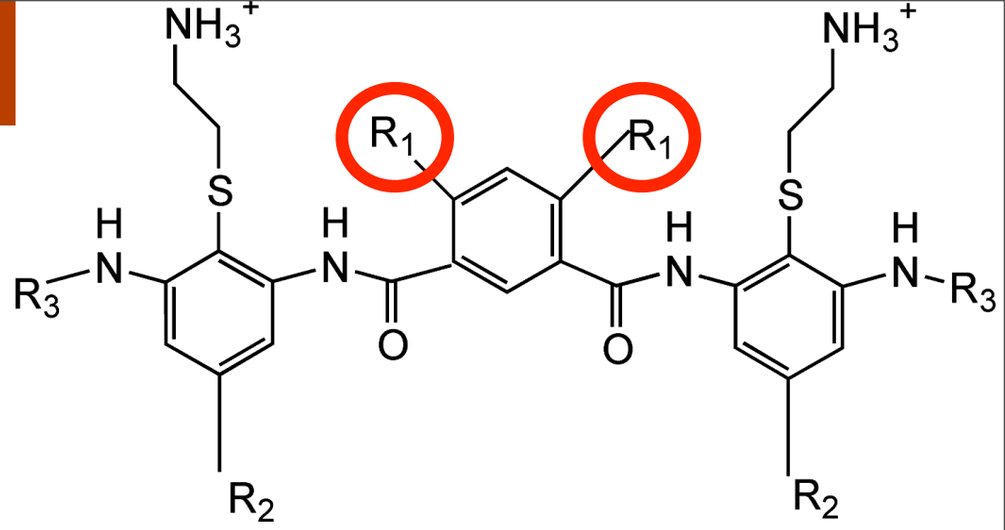
- AA zwittrionic/charged/mixed lipids
- United atom lipids[6]
- CG lipids[7]

1. JACS, 2006, 128, 1778-1779
2. J Comp Chem, 2006, 27, 693-700
3. J Phys Chem B, 2006, 110, 3517-3526
4. Peptide Science (Review), 2008, 90, 83-93
5. Acc. Chemical Res, 2009, ASAP article

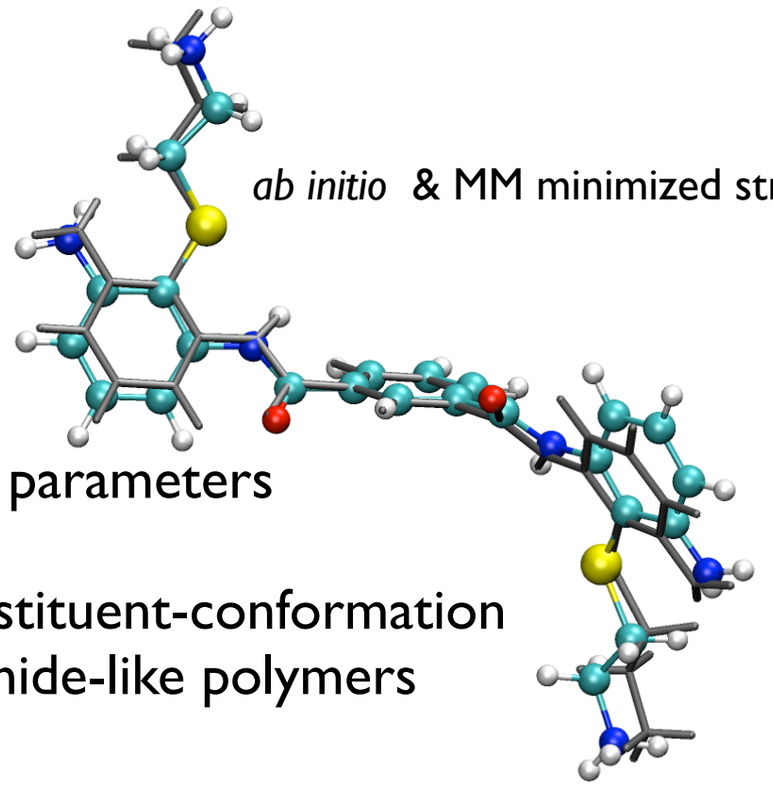
Arylamide polymers: Parameters







- Effect of methoxy group substitution on backbone flexibility
- intra molecular hydrogen bond (1.91 Å) between amide proton and ether oxygen



ab initio & MM minimized structures

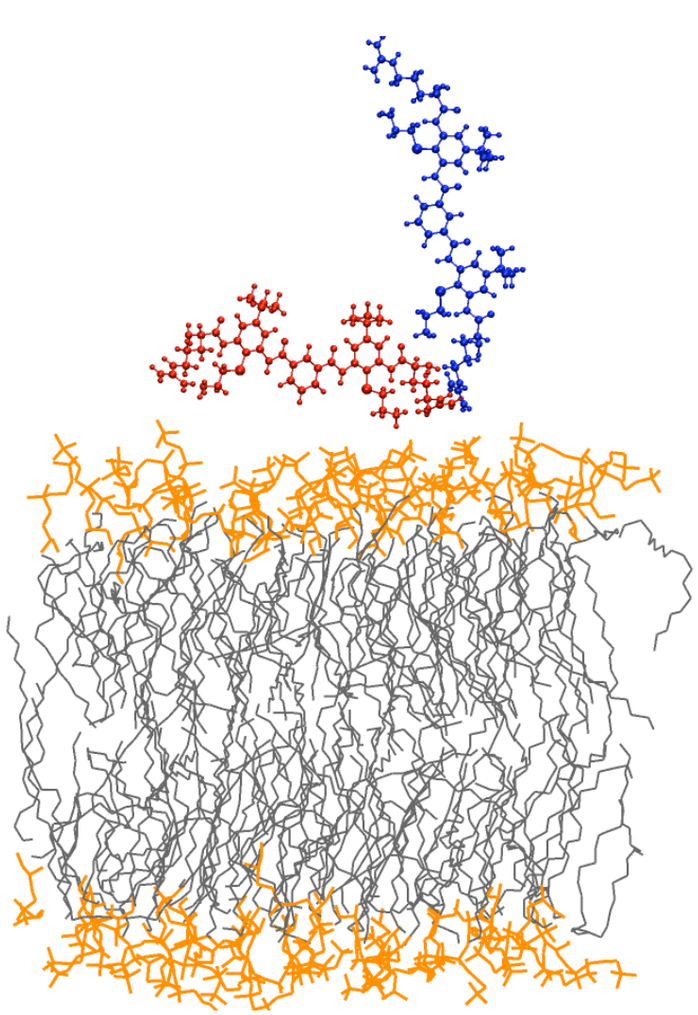
- new intra molecular parameters developed
- investigating the substituent-conformation relationships of arylamide-like polymers

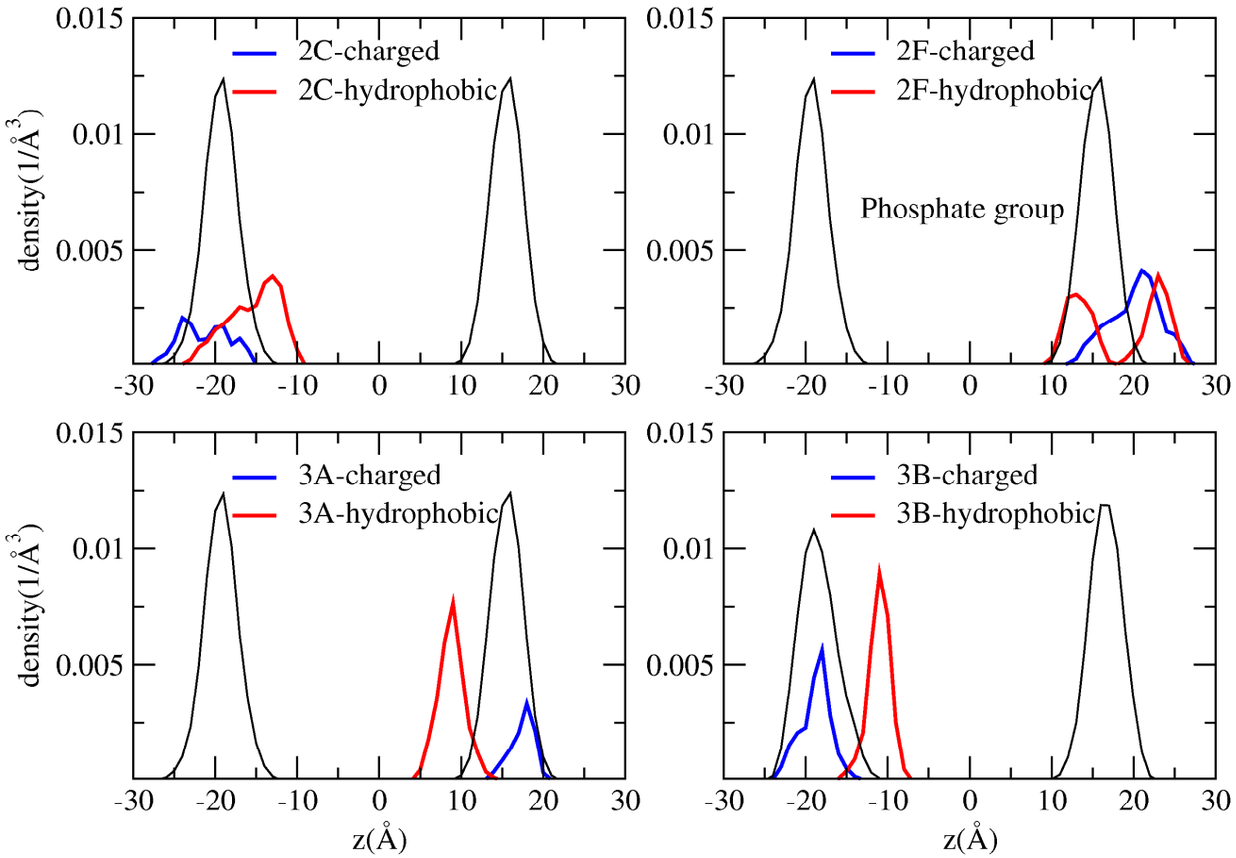
Ab Initio Calculations of Intramolecular Parameters for a Class of Arylamide Polymers

SATYAVANI VEMPARALA,[1] IVAYLO IVANOV,[2] VOJISLAVA POPHRISTIC,[3] KATRIN SPIEGEL,[1] MICHAEL L. KLEIN[1]

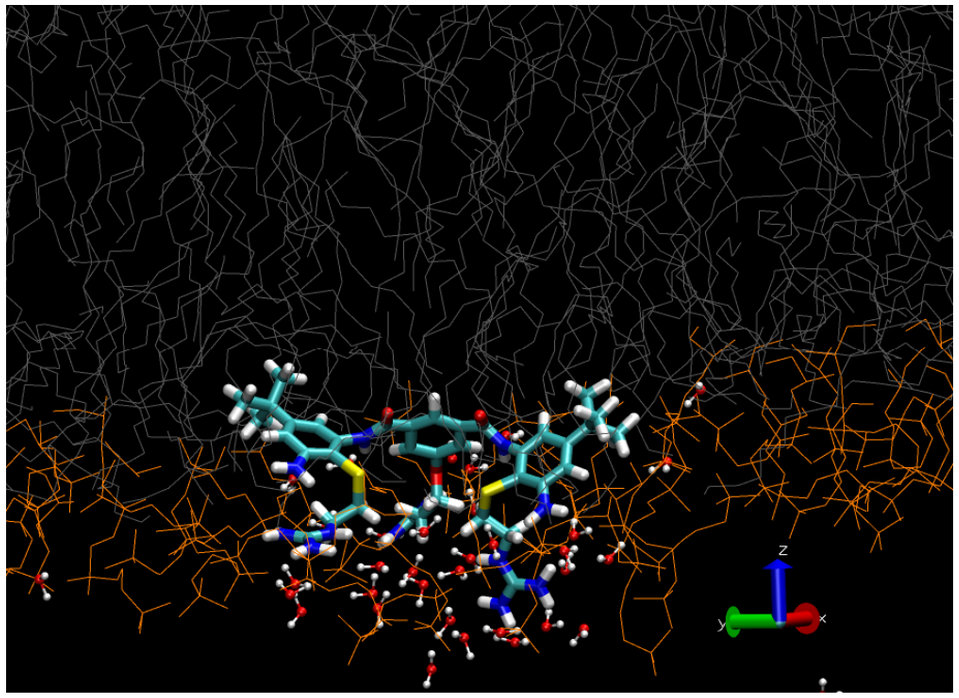
I. Comp. Chem. 2006.

Arylamide polymers: MD



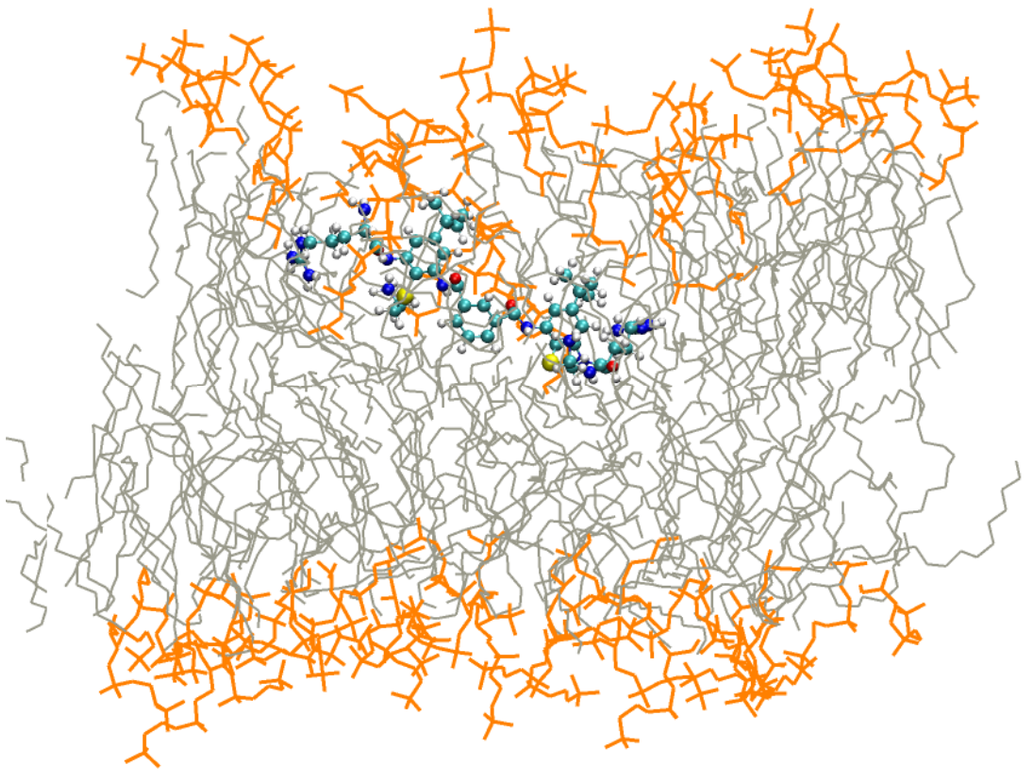


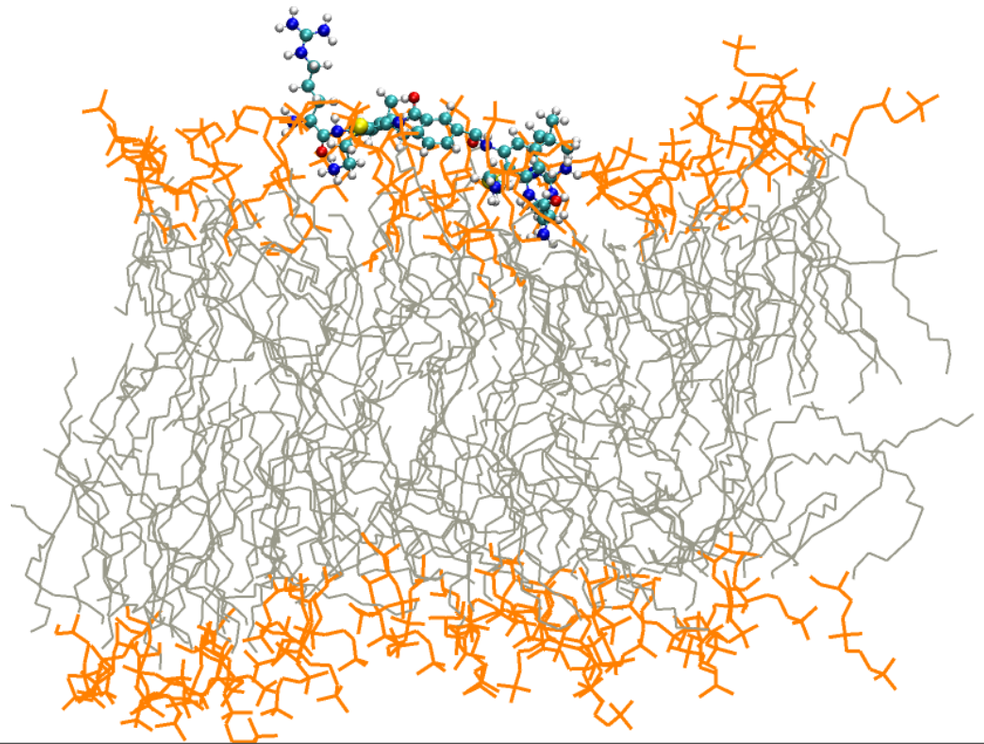
Simulation time: 8 ns (each of 4 arylamides)
Lipid Bilayer: DOPC
Temperature: 305K
NPT ensemble
PME electrostatics
Systems:
72 lipids
~18,000 atoms

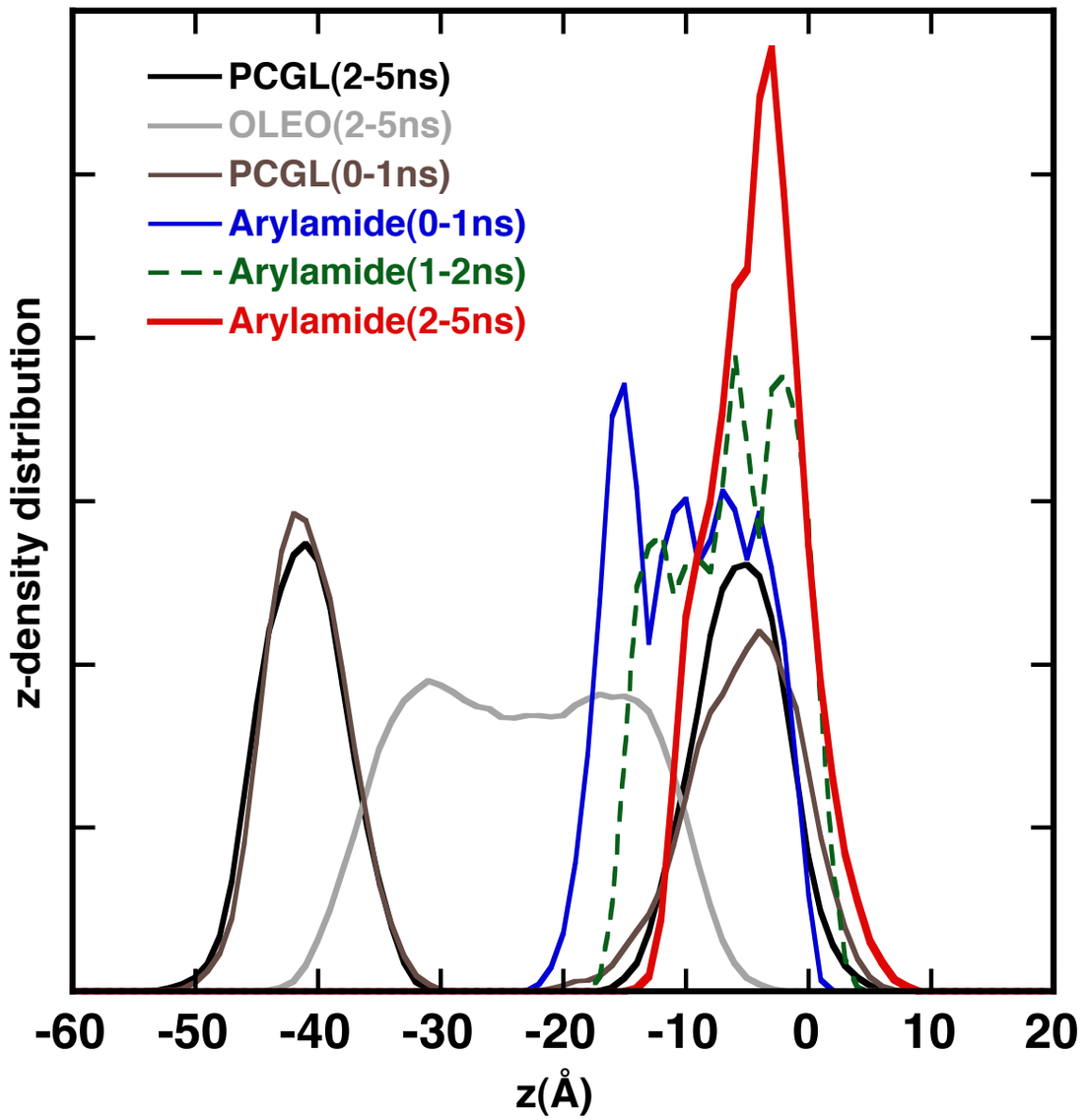


Snorkeling of guanidine/amine side chains enables them to make polar interactions with complex lipid-head-water region. The hydrophobic side chains are located well into the lipid tail region

Arylamide polymers: MD

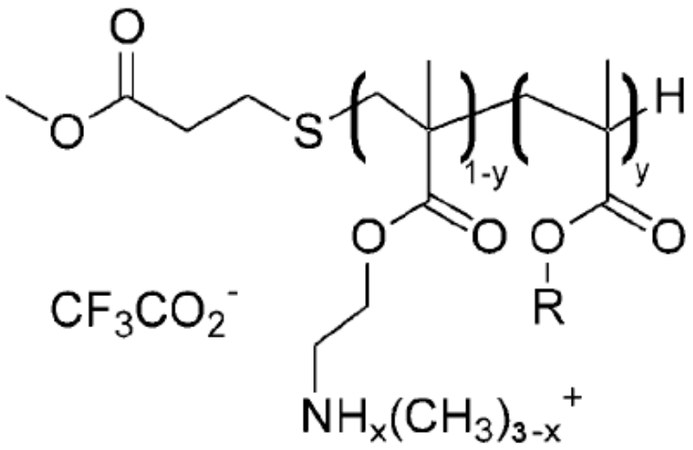




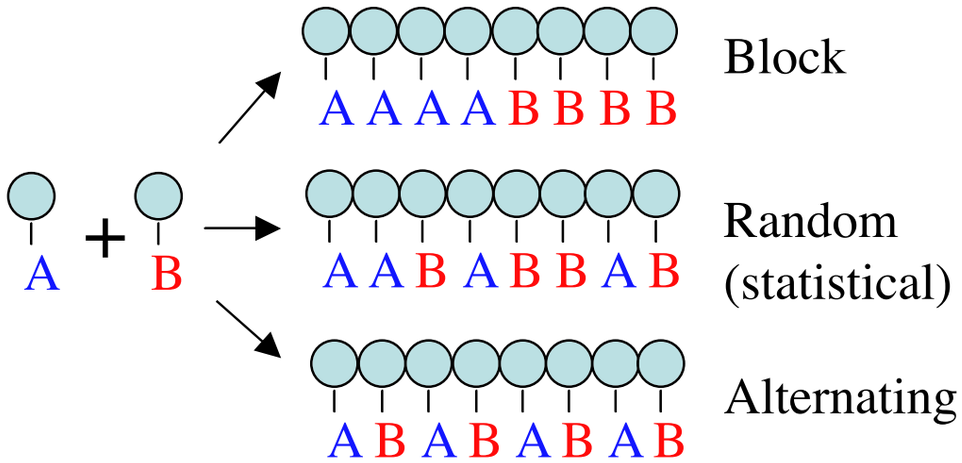


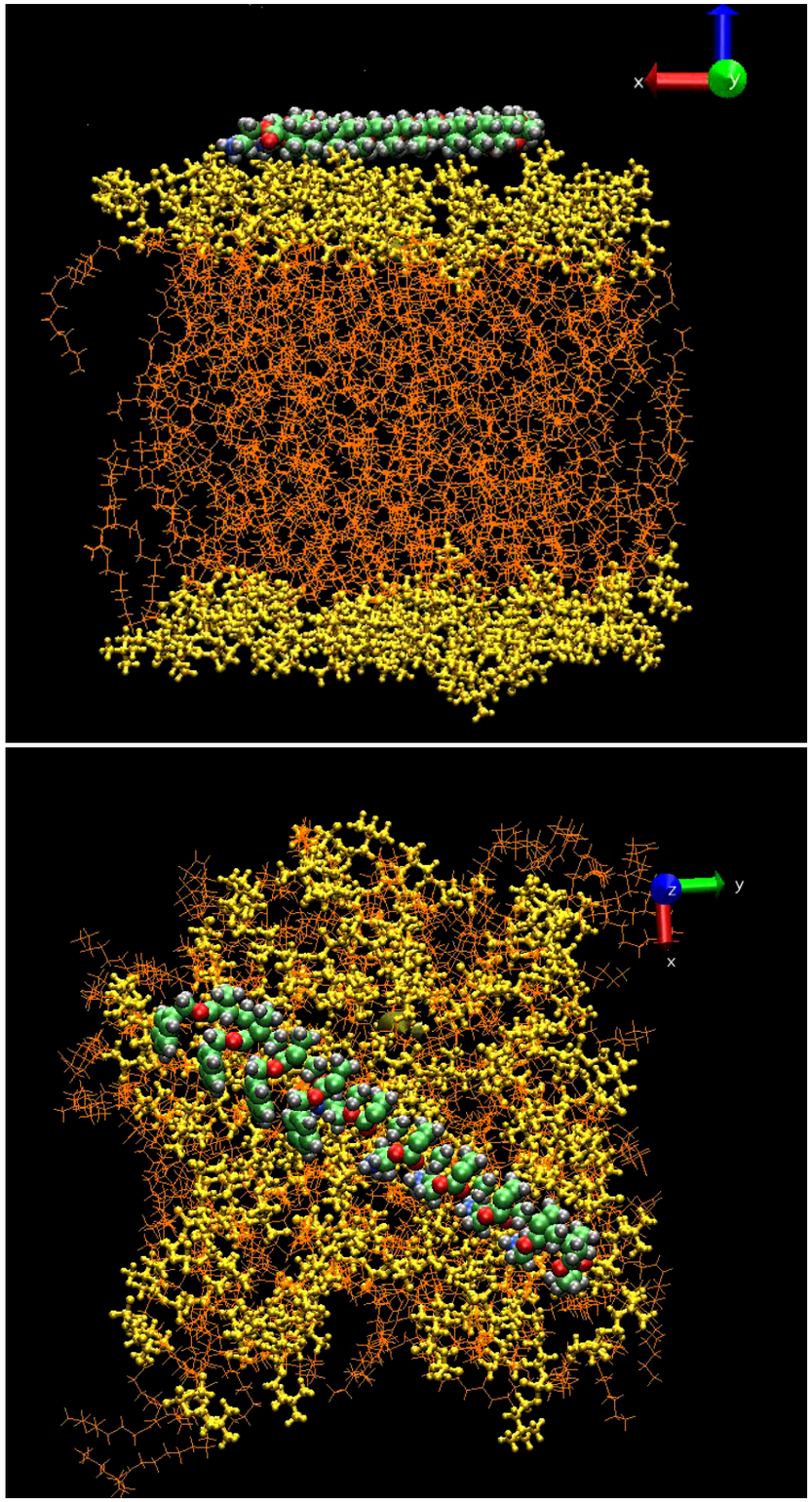
A coarse steered molecular dynamics simulation has been done to get different starting points for further MD. Shown in the right is a starting point where the arylamide was just below the head group region. On relaxing and running the system for 5ns more, the most optimal postion for the arylamides is in the head group

System setup: random copolymers



R= ethyl; butyl; hexyl or benzyl
y= hydrophobic fraction
x= 0 - 3





Simulation time: 7.5 - 9.5 ns(each of 20 polymer systems)
Lipid Bilayer: DOPC
Temperature: 303K
NPT ensemble
PME electrostatics
Systems:
72 or 85 lipids
19000 to 24000 atoms

20-12-8-ben-nch0-block

20-12-8-ben-nch0-alt

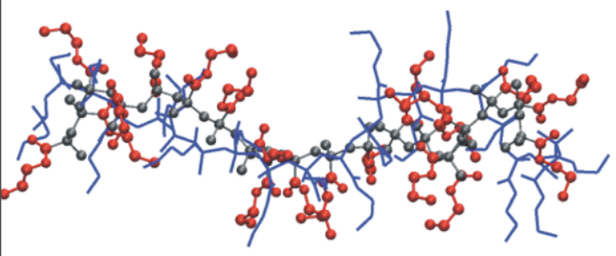
Characterization of Nonbiological Antimicrobial Polymers in Aqueous Solution and at Water−Lipid Interfaces from All-Atom Molecular Dynamics

Ivaylo Ivanov,*,† Satyavani Vemparala,‡ Vojislava Pophristic,§ Kenichi Kuroda,‡,∥ William F. DeGrado,∥ J. Andrew McCammon,† and Michael L. Klein‡

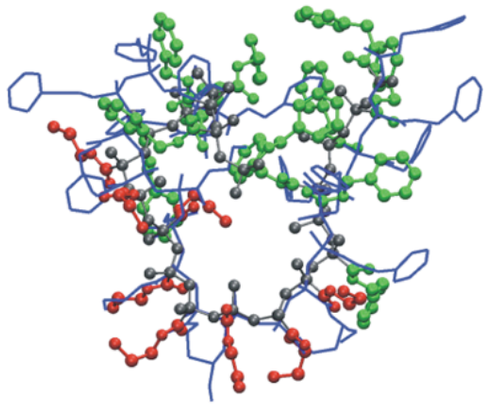
1778 ■ J. AM. CHEM. SOC. 2006, *128*, 1778−1779

Adopted structures

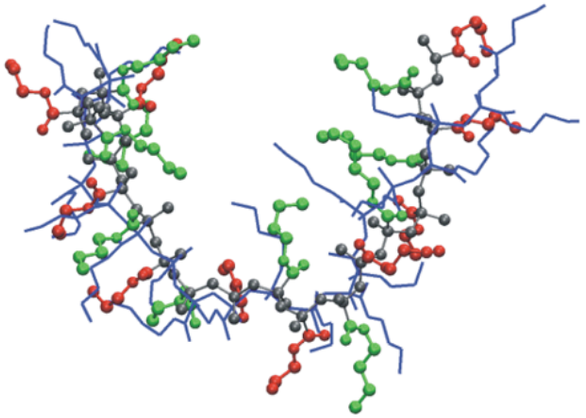
(a) 20-0-20-but-nch0



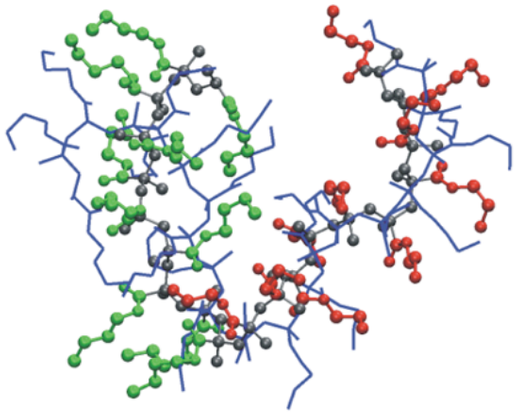
(b) 20-12-8-ben-nch0-block

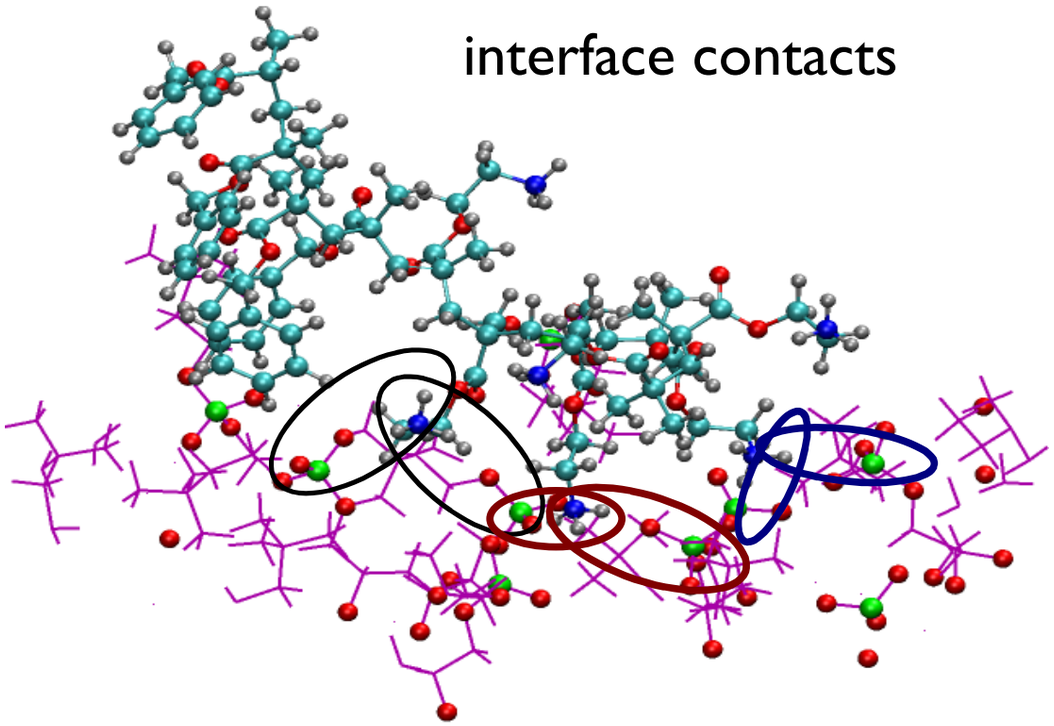


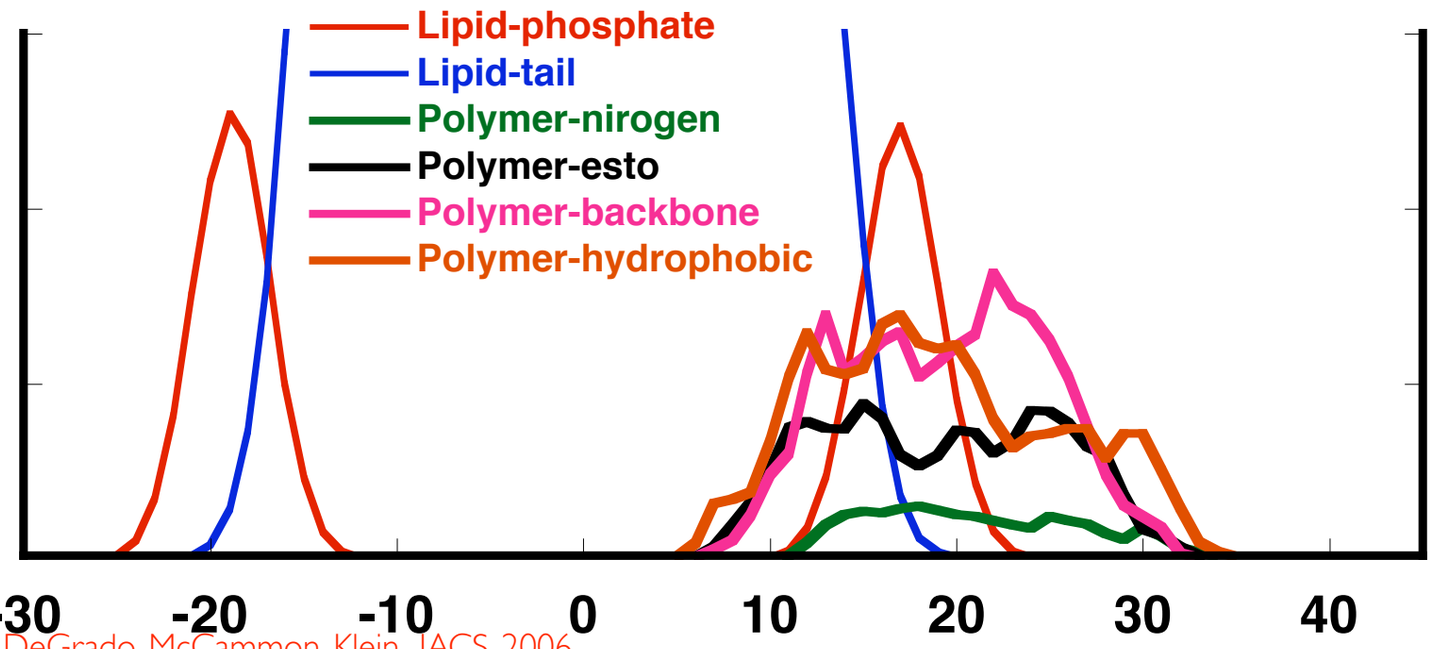
(c) 20-10-10-but-nch0-alt



(d) 20-10-10-but-nch0-block

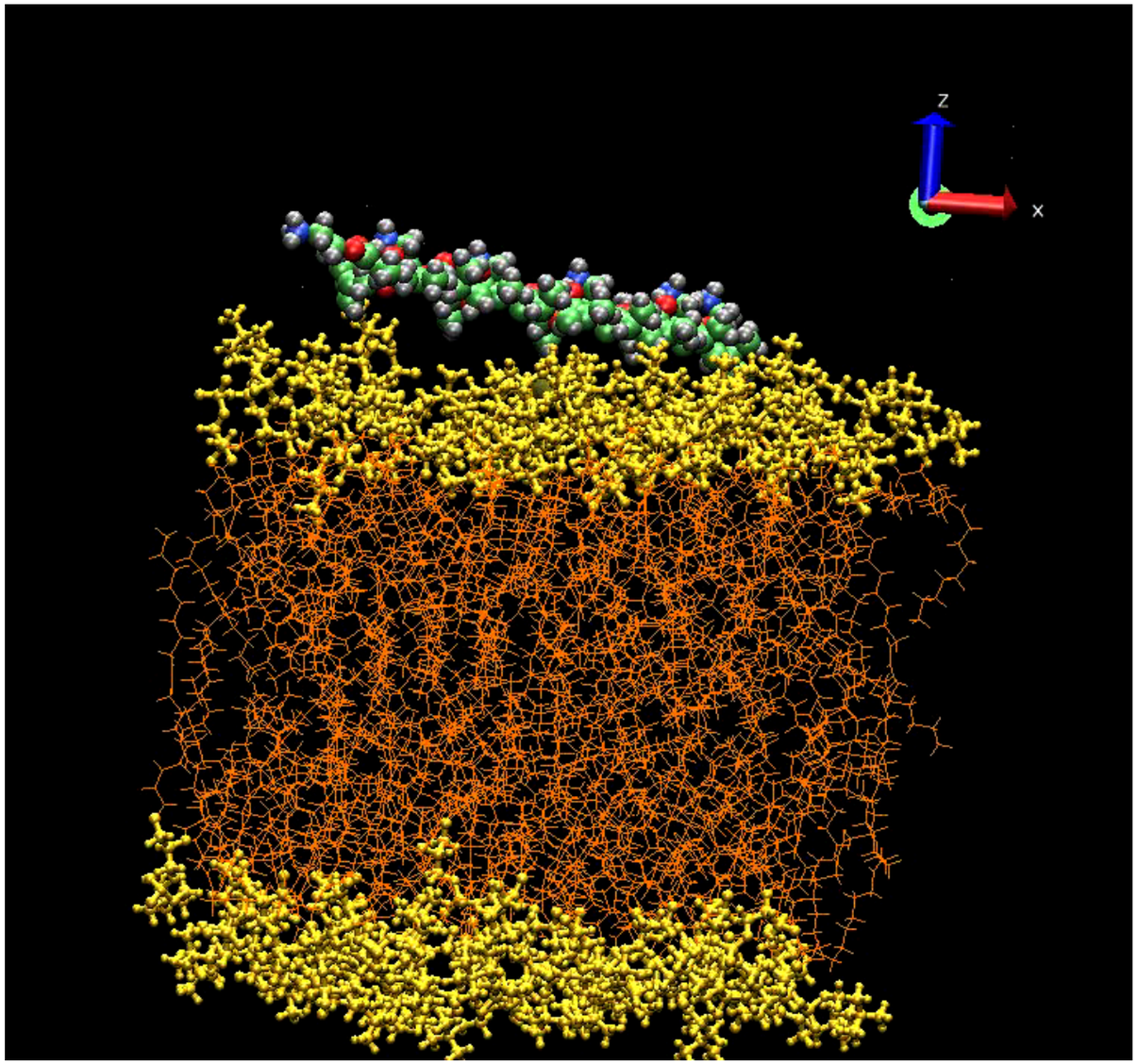


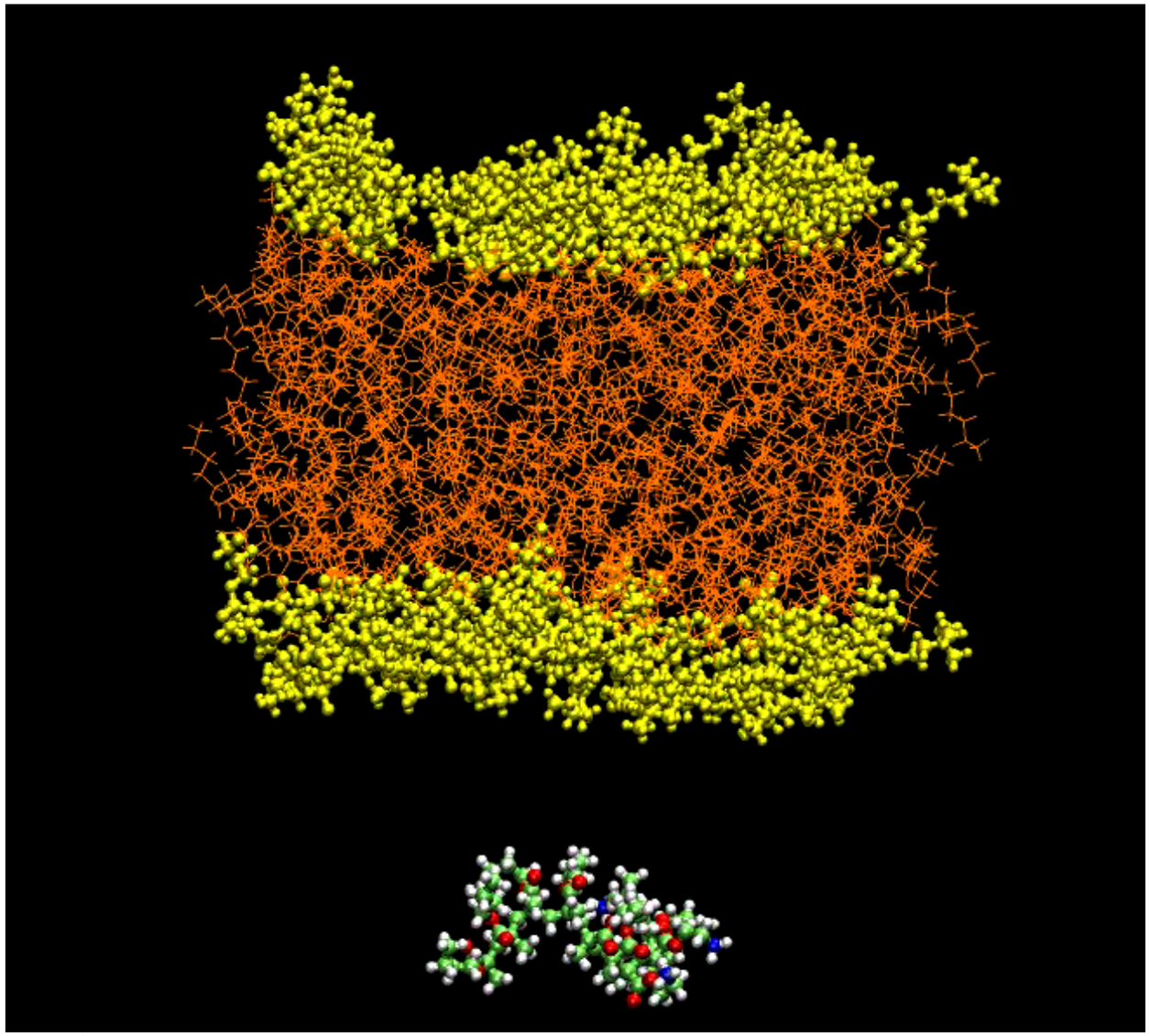




Ivanov, Vemparala, Pophristic, Kuroda, DeGrado, McCammon, Klein JACS, 2006

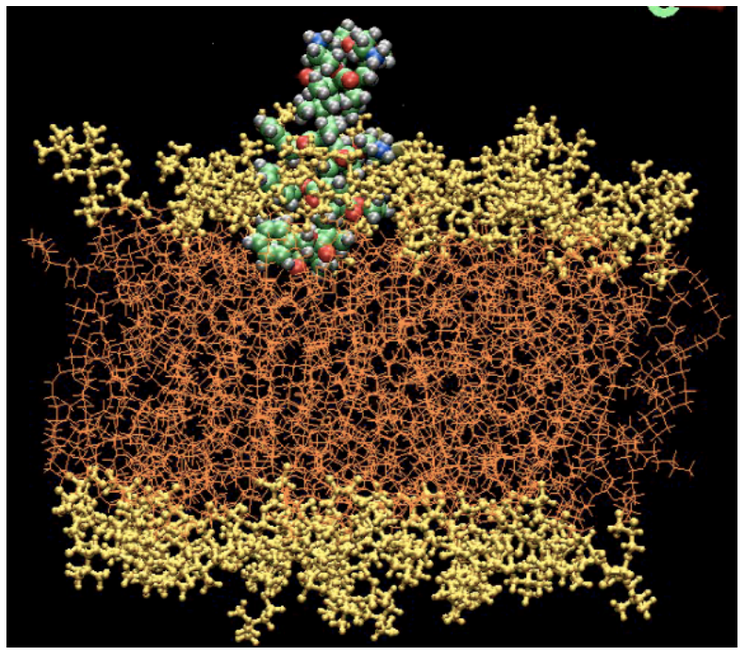
Membrane insertion



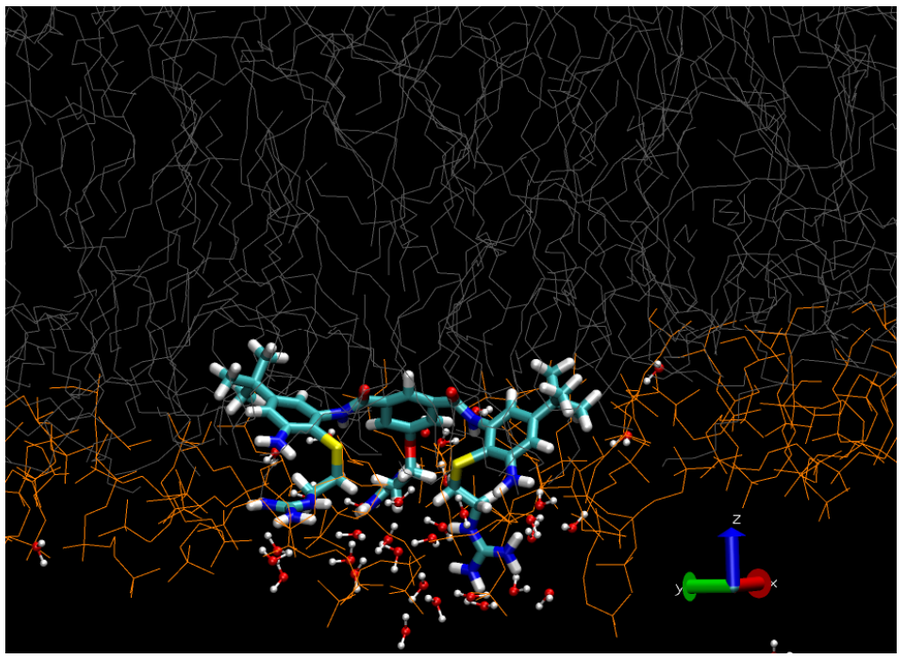


- More hydrophobic sequences undergo partial insertion
- Insertion: parallel to membrane normal
- amine groups play role in initial ‘docking’ to the membrane
- sequences with only charged groups remained in solution phase
- primary driving force: hydrophobic effect
- upon insertion, hydrophobic groups form favorable interactions with lipid tails
- dynamic flexibility allows polymer to adopt structures
- *structural properties depend on composition; hydrophobicity is primary determinant of activity and charged amine groups are important for selectivity*

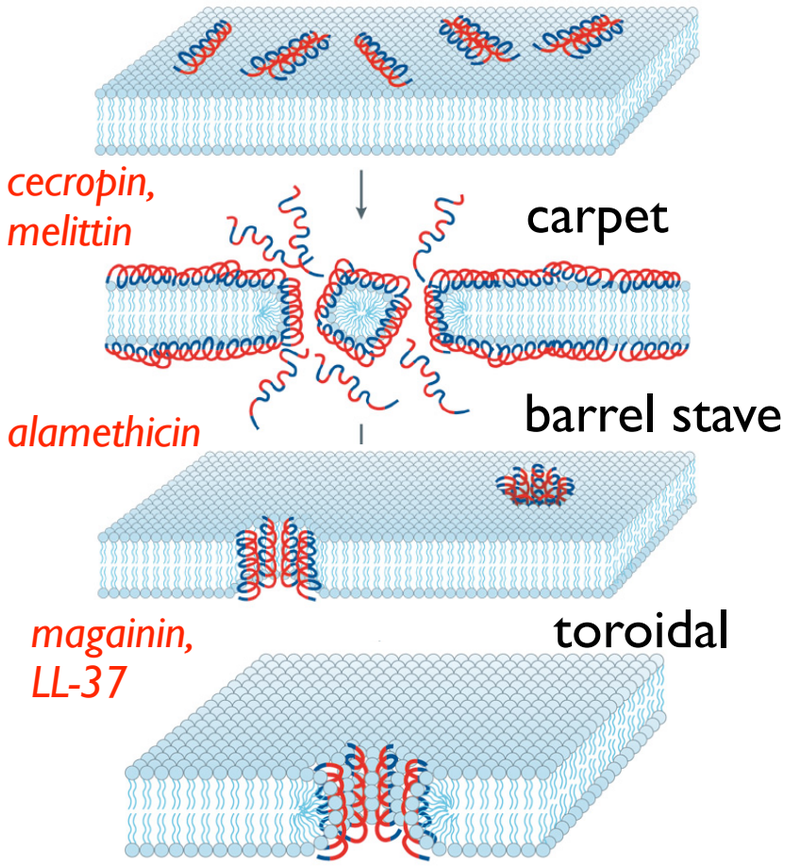
Summary:antimicrobial polymers



Barrel stave?



Carpet mode?



- The composition, molecular weight and hydrophobicity of short co-polymers can be modulated to achieve structural diversity, which is crucial in controlling the antimicrobial activity.

- The primary driving force leading to insertion is confirmed to be the hydrophobic interaction with the membrane.

- Appropriate torsion backbone potentials have been developed for arylamide foldamers

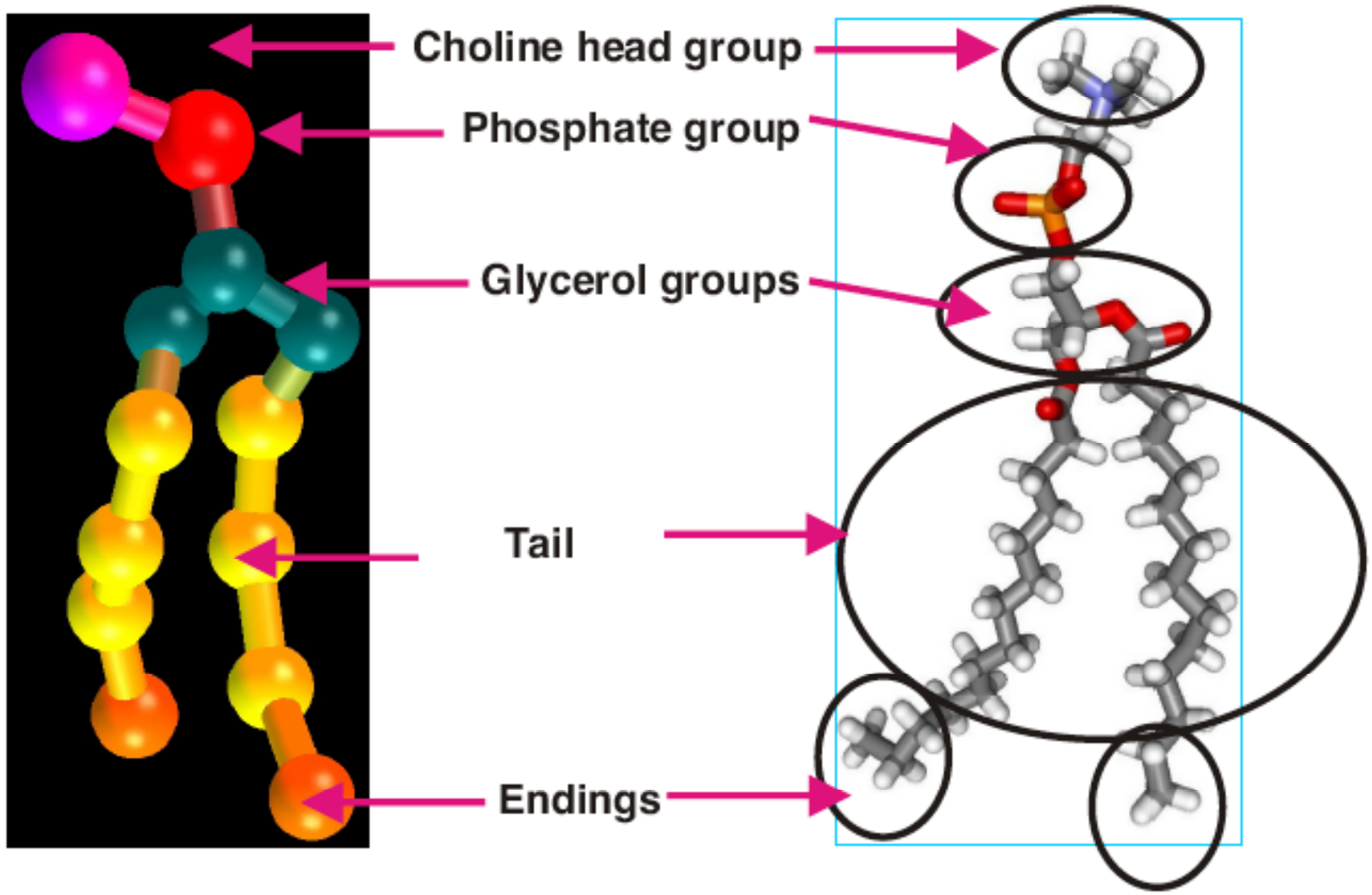
-Our results indicate sensitive dependence of the overall shape on the sequence, suggesting that experimentally observed changes in activity can be correlated with particular sequences, providing an avenue for rational design.

To understand more clearly the interplay of charged & hydrophobic groups and antimicrobial activity
To study antimicrobial activity in the context of aggregation

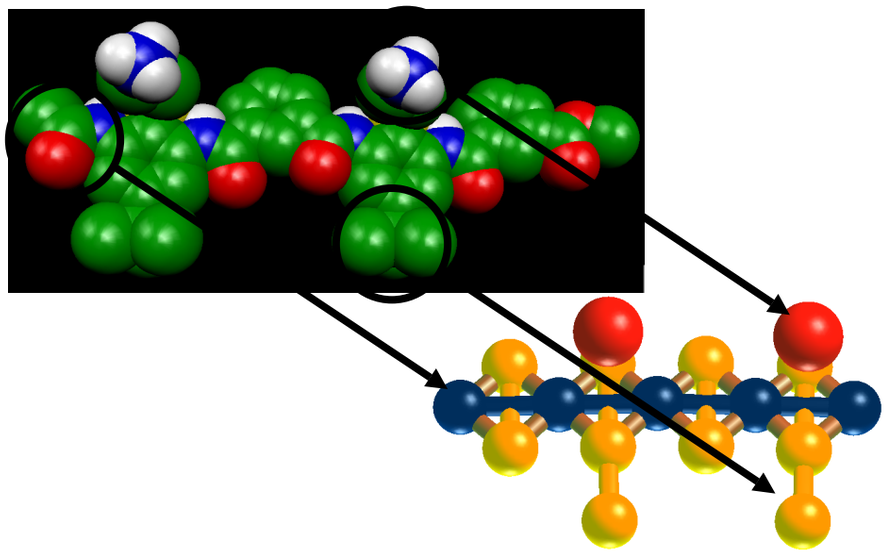
coarse grain simulations

Coarse grained simulations?

Lipids:



AM polymers



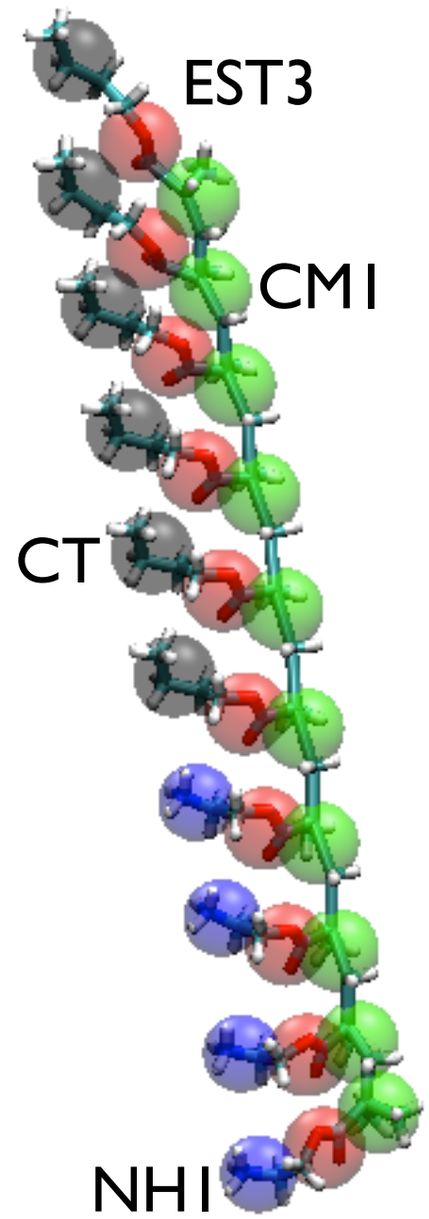
CG model: functional forms

$$U_{\text{intra}} = \sum^{\text{Bond}} k_b(r - r_0)^2 + \sum^{\text{Angle}} k_a(\theta - \theta_0)^2$$

$$U_{\text{bond}}(r) \propto -k_B T \ln[P(r)/r^2]$$

$$U_{\text{angle}}(\theta) \propto -k_B T \ln[P(\theta)/\sin\theta]$$

$P(r), P(\theta)$ = AA probability distributions



$$U_{\text{LJ9-6}}(r) = \frac{27}{4}\varepsilon\left\{\left(\frac{\sigma}{r}\right)^9 - \left(\frac{\sigma}{r}\right)^6\right\},$$ pairs involving water

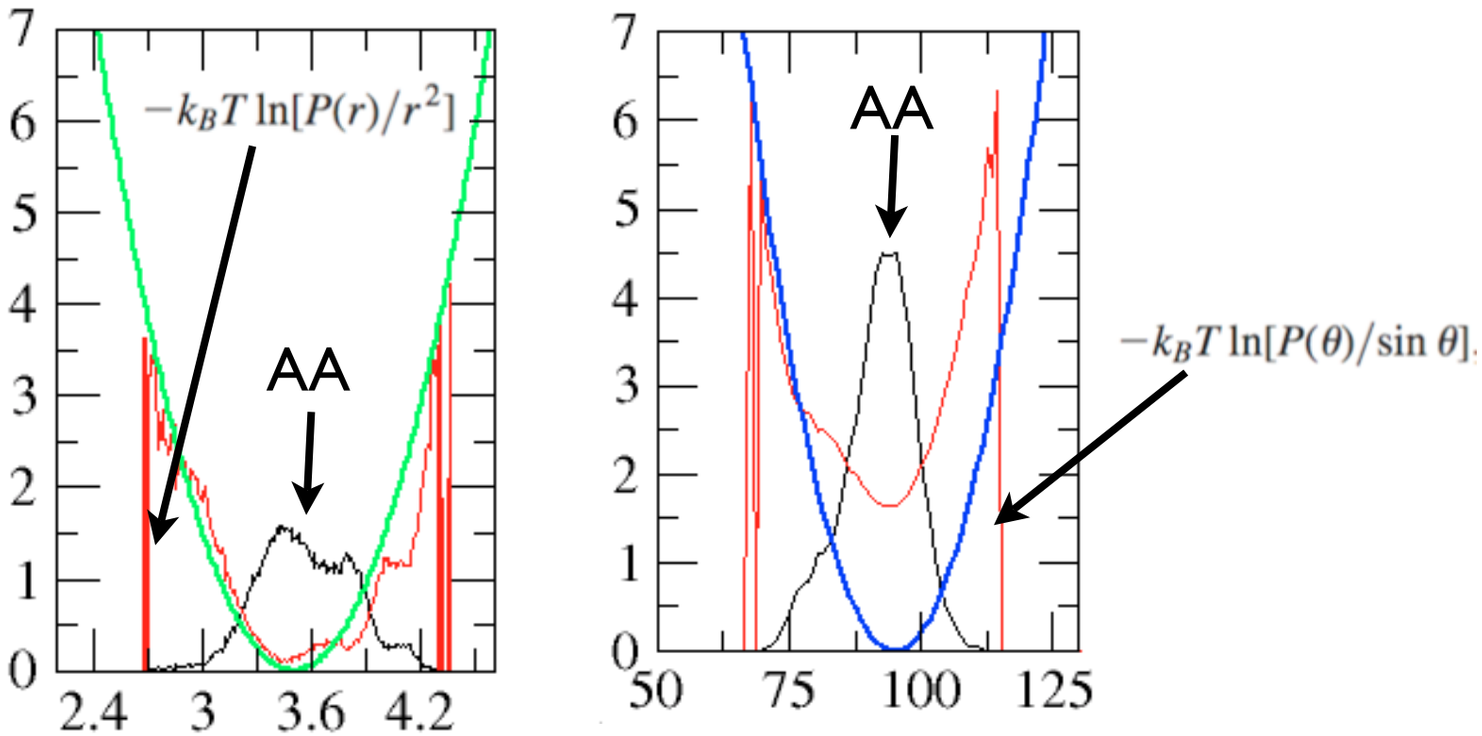
$$U_{\text{LJ12-4}}(r) = \frac{3\sqrt{3}}{2}\varepsilon\left\{\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^4\right\}.$$ all other pairs

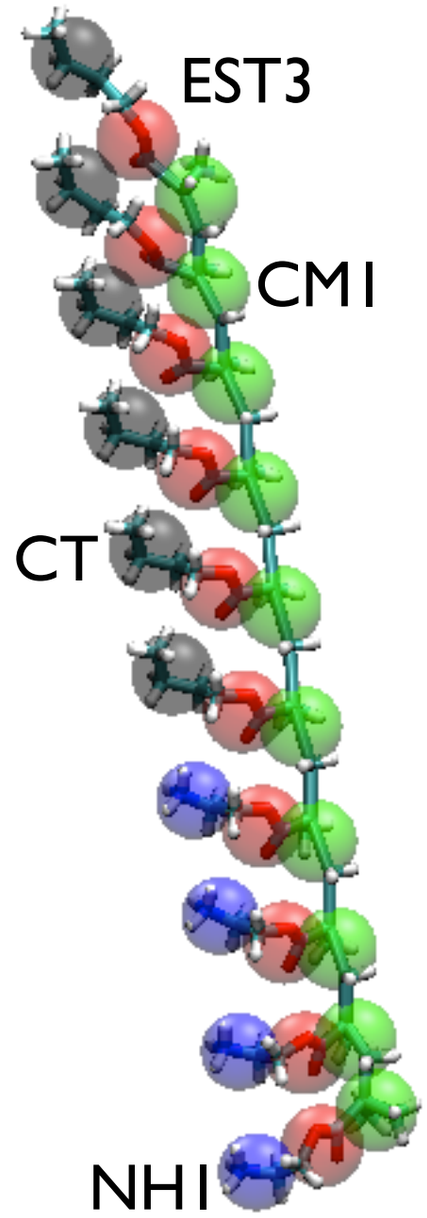
Shinoda, W., DeVane, R. and Klein, M. L.(2007)'Multi-property fitting and parameterization of a coarse grained model for aqueous surfactants',Molecular Simulation,33:1,27 — 36

CG model

Bonded Parameters:

- structural properties (pair distribution functions from AA-simulations)





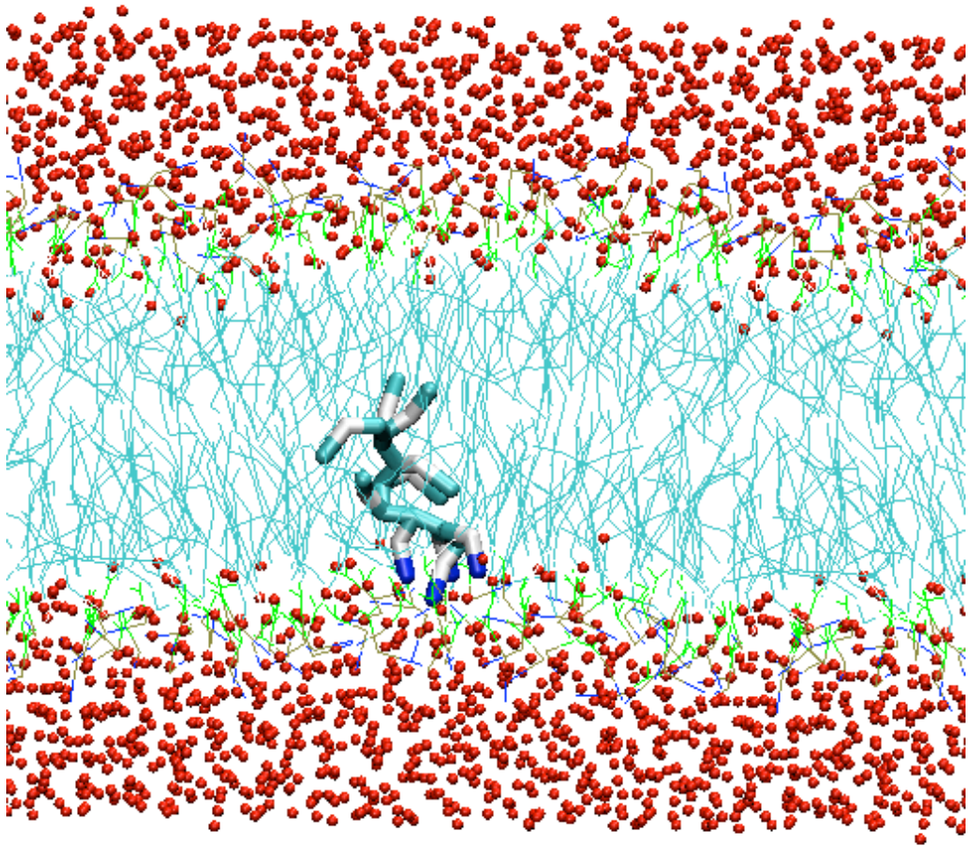
Non-bonded Parameters:

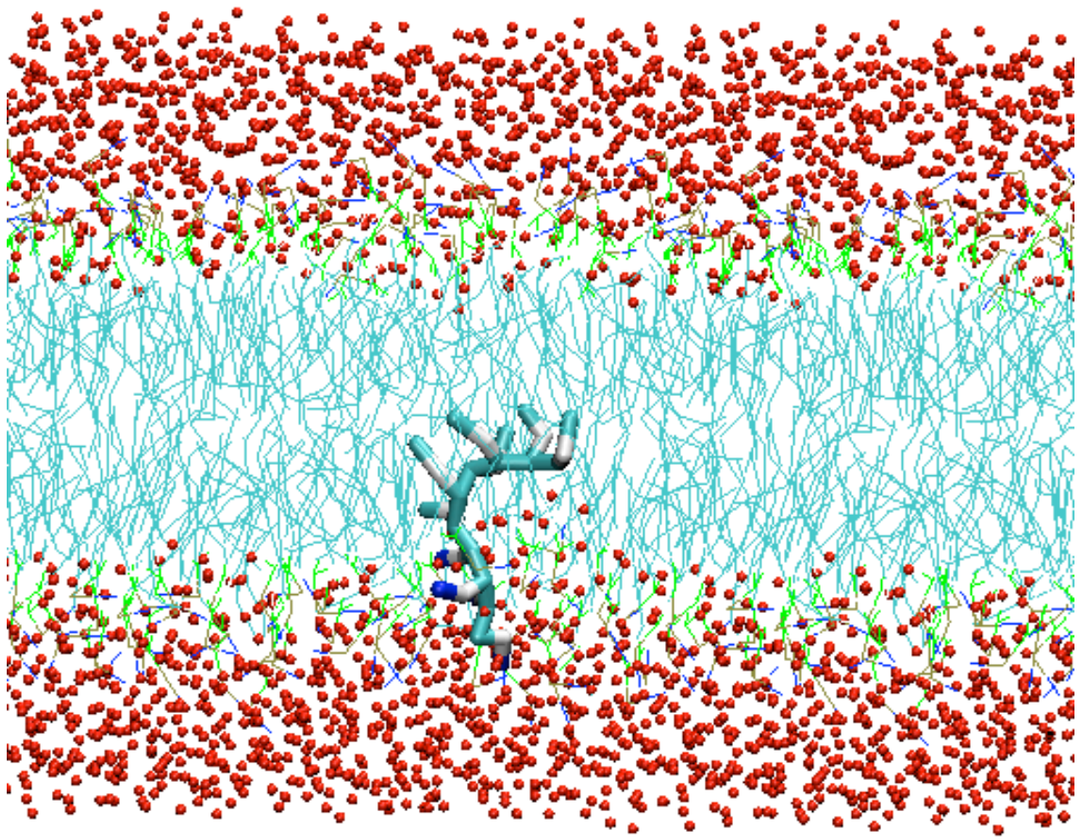
- reproduce surface tension & density (if experimental numbers available)
- hydration free energy (X-water interaction)
- structural properties (pair distribution functions from AA-simulations)

Initial parameters from Shinoda *et al.*,

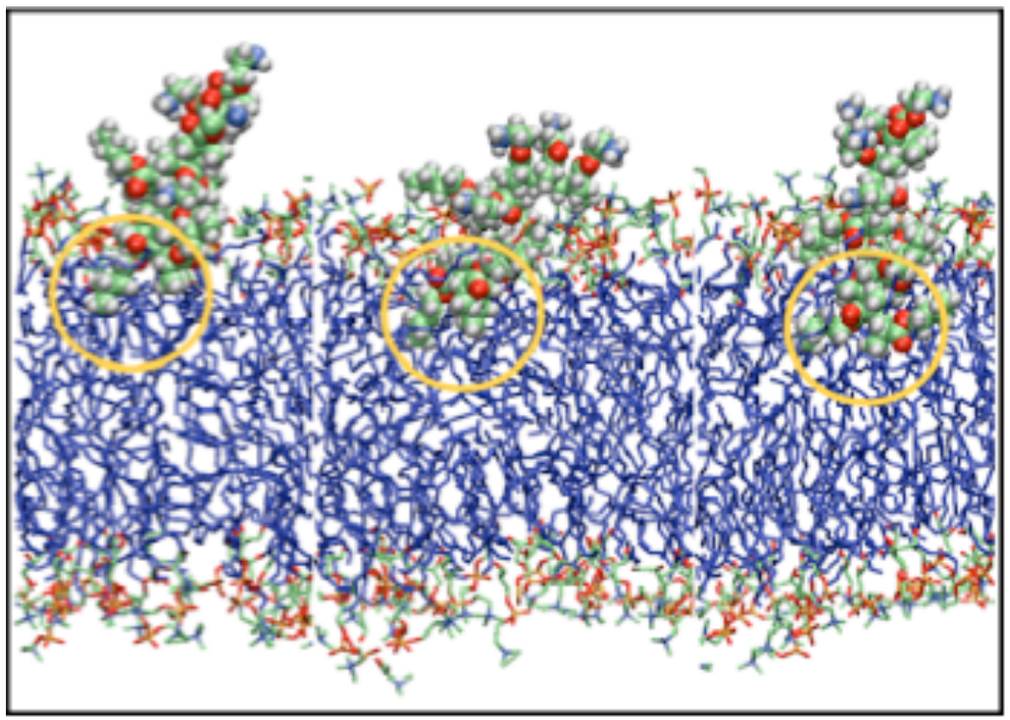
Shinoda, W., DeVane, R. and Klein, M. L.(2007)'Multi-property fitting and parameterization of a coarse grained model for aqueous surfactants',Molecular Simulation,33:1,27 — 36

AMP in lipid (single)

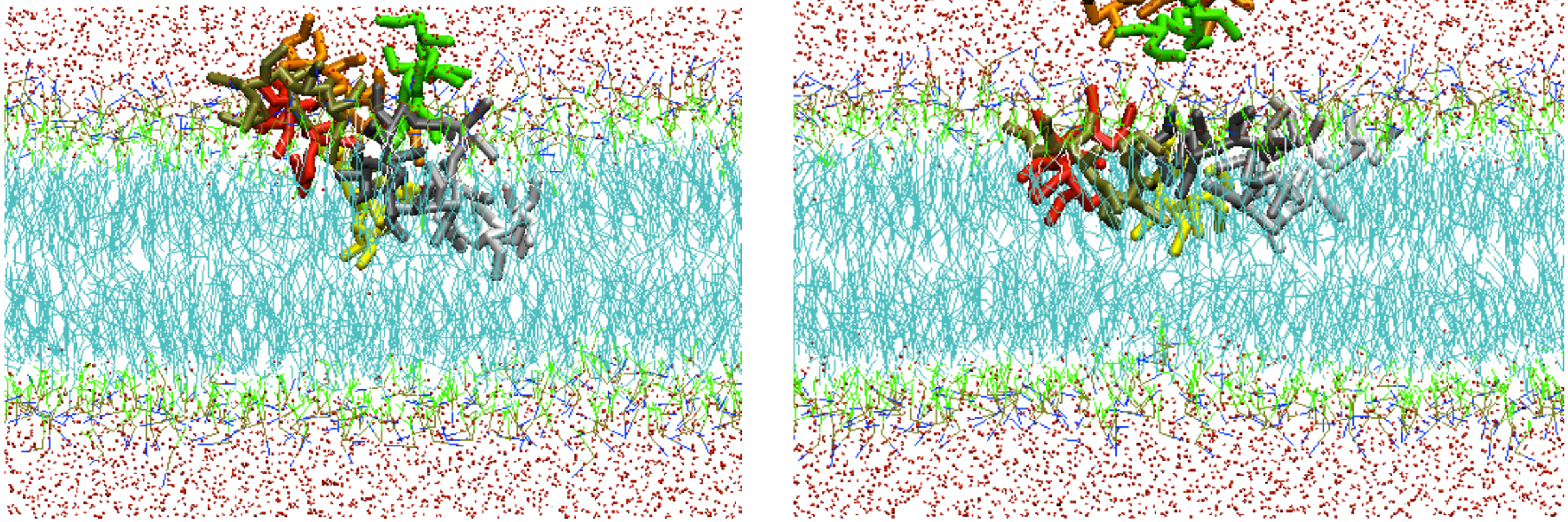




CG sites: ~ 3400
atoms in AA: ~25000



AMP in lipid (aggregate)



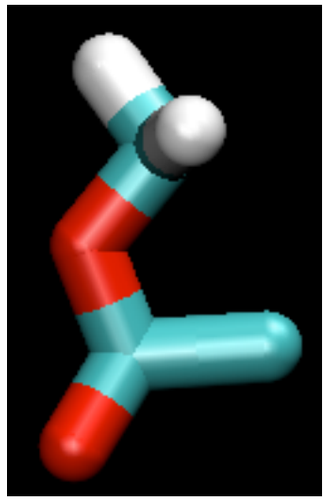
10 ns

CG lipids: 512
CG waters: 5000
CG AMP: 8

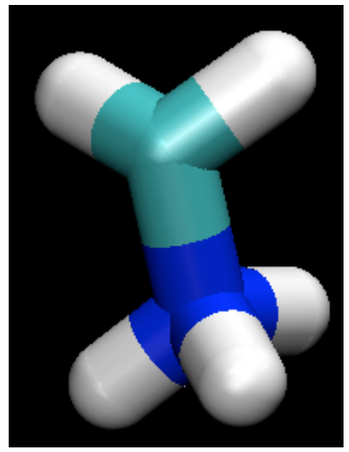
Ongoing.....

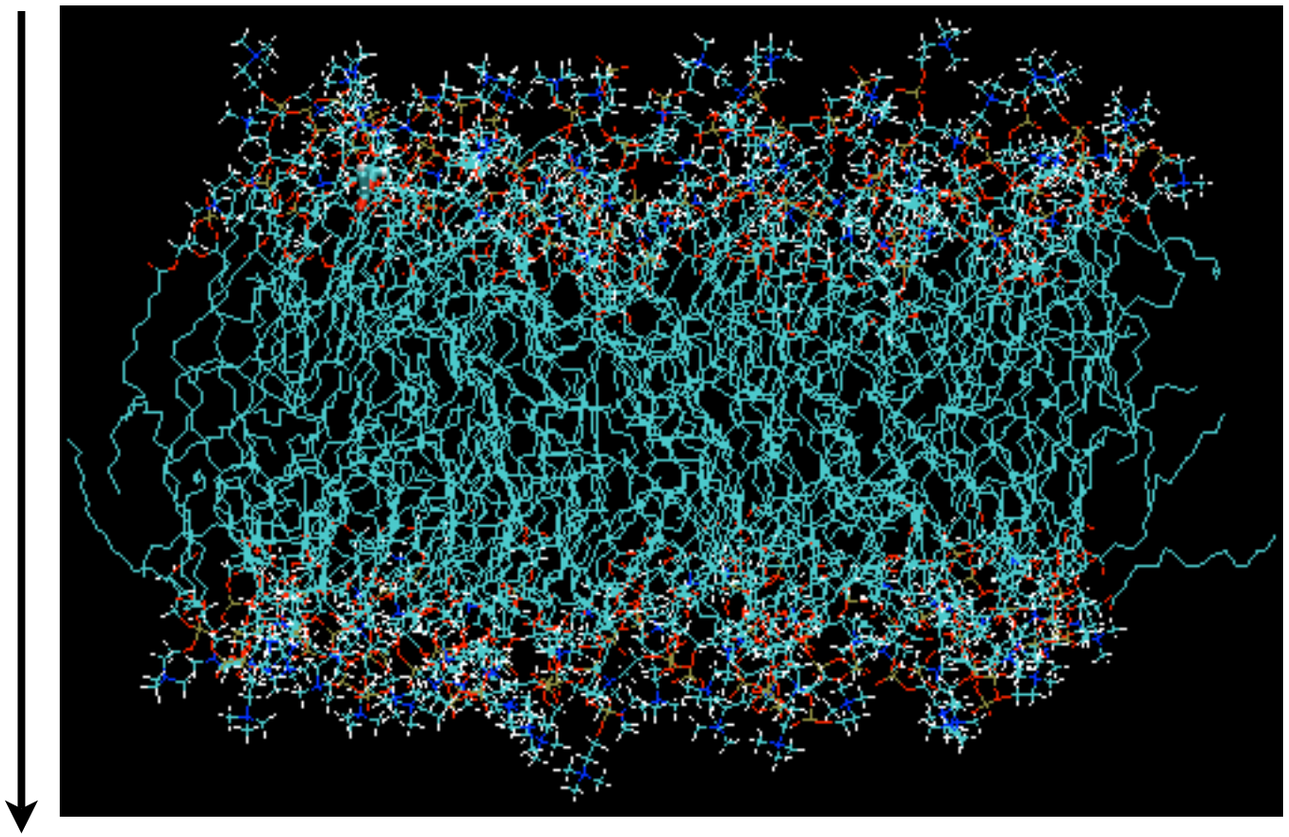
further refinement:
NB parameters based on PMF

EST3



NH31





Computational approach

Single AMP interactions- AA simulations[1]

-Insertion mechanisms

-Free energy of binding

Aggregation- CG simulations

AM polymers[4,5]

Parameterization - DFT calculations[2,3]

-Effects of substituents on torsional barriers

-Conformational rigidity

Lipids:

- AA zwittrionic/charged/mixed lipids
- United atom lipids[6]
- CG lipids[7]

1. JACS, 2006, 128, 1778-1779
2. J Comp Chem, 2006, 27, 693-700
3. J Phys Chem B, 2006, 110, 3517-3526
4. Peptide Science (Review), 2008, 90, 83-93
5. Acc. Chemical Res, 2009, ASAP article

Thank You