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# Developing Coarse-Grained Models for the Molecular Simulation of the Self Assembly of Skin Lipids

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# Outline

- Motivation and background
- Simulation details
  - Atomistic simulations
  - Coarse-grained (CG) model development
    - Reith, Pütz, Müller-Plathe (RPM) method
- Results
  - Free Fatty Acids (FFA)
  - Cholesterol (CHOL)
  - Water
  - Bilayer self-assembly
- Conclusions

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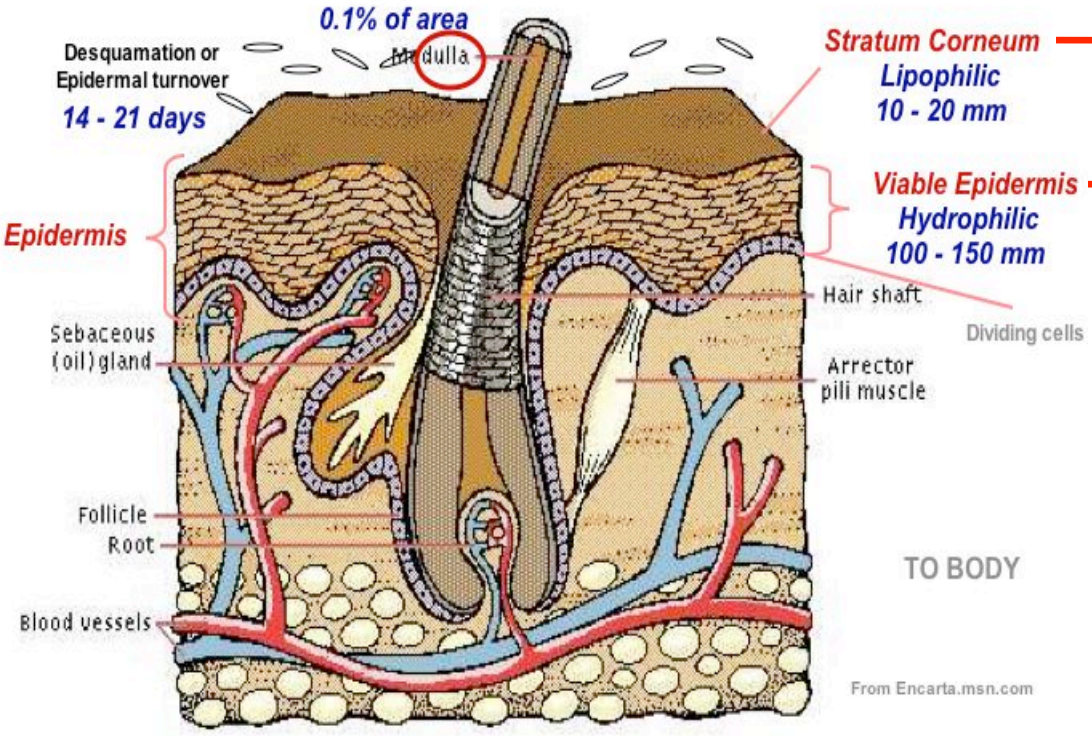
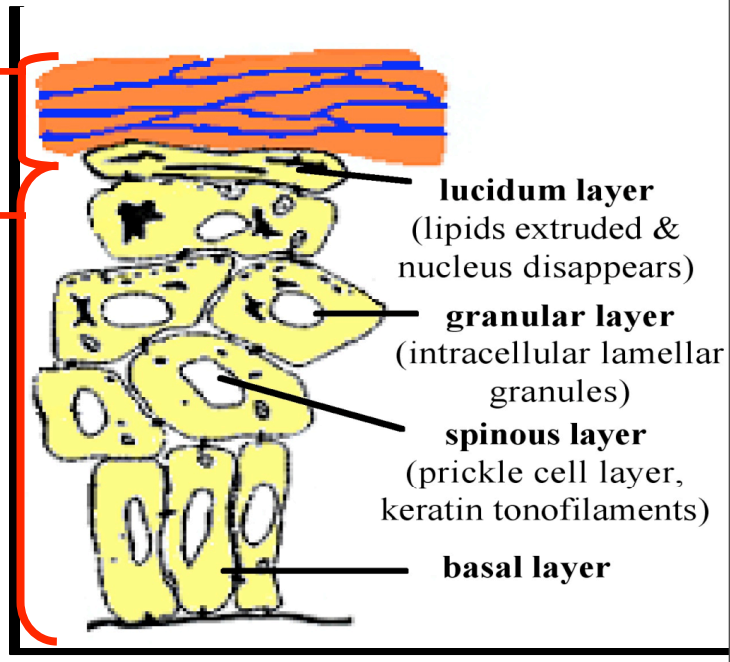
# Motivation

- Skin diseases are extremely common
  - More prevalent than obesity, hypertension and cancer
    - Atopic dermatitis alone afflicts more than 15 million people in the US with treatment costs exceeding $1 billion/year
- Diseased skin is often characterized by a reduced barrier function and an altered lipid composition and organization
  - Mounting evidence that an impaired skin barrier does have a significant, even life threatening, affect on systemic health
    - AD exhibits a strong concordance with asthma and hay fever
      - *50 - 75% of children with AD develop asthma*
- A clear understanding of how the lipids of the skin assemble is needed
  - A molecular basis for developing treatment strategies to restore barrier function in diseased skin
  - A molecular basis for breaching the barrier in a controlled manner to deliver drugs more effectively across the skin

# Structure of Human Skin




Cross-section of skin




Close-up of Epidermis

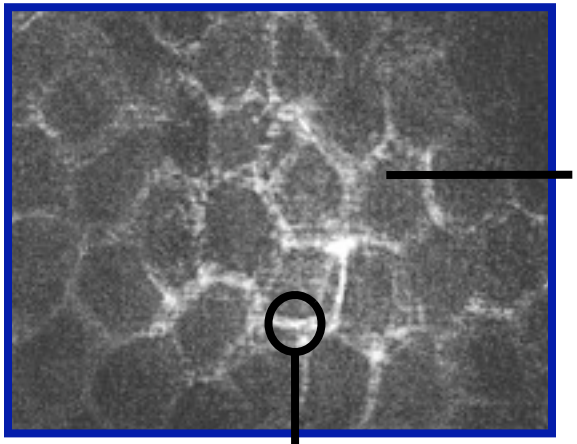
- Stratum corneum known to control barrier function of skin
  - Protects against foreign agents and disease entering the body
  - Water exiting the body

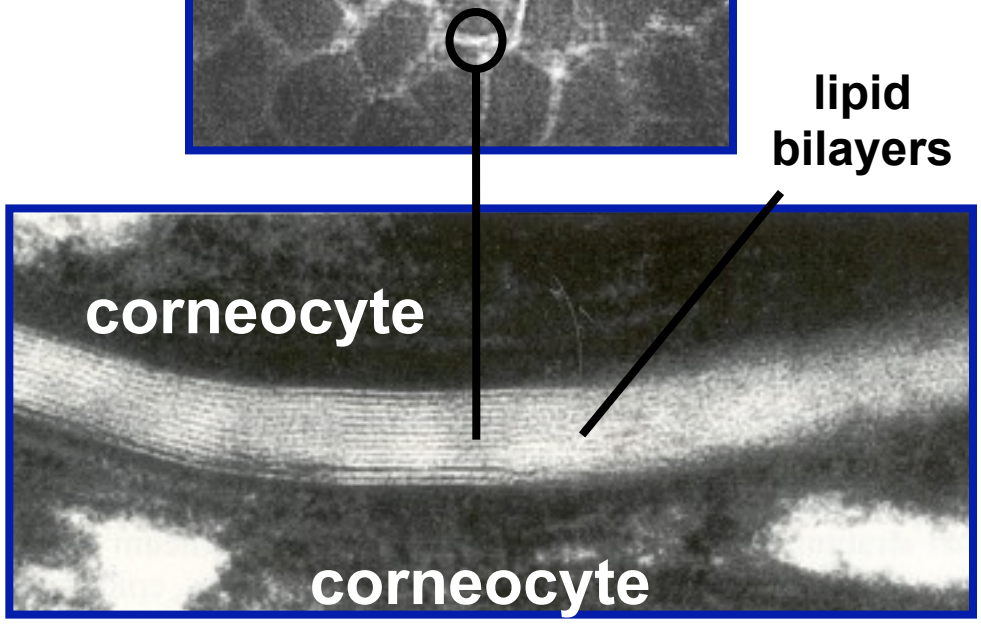
http://encarta.msn.com/media_461516297/Structure_of_the_Skin.html; Yardley HJ, Summerly R, *Pharm. and Therapeutics*, **13**, 357 (1981).

# Structure of Stratum Corneum

- Experimentally much is known about the nature of the SC
  - Consists of dead, essentially impermeable, flat, hexagonal, protein-enriched corneocytes
    - Organized in aligned sheets about 10 - 40 cells deep
    - Intercellular space surrounding corneocytes (~ 0.1 mm thick) is filled with highly organized lipid bilayers
      - *Control barrier function*

Confocal micrograph



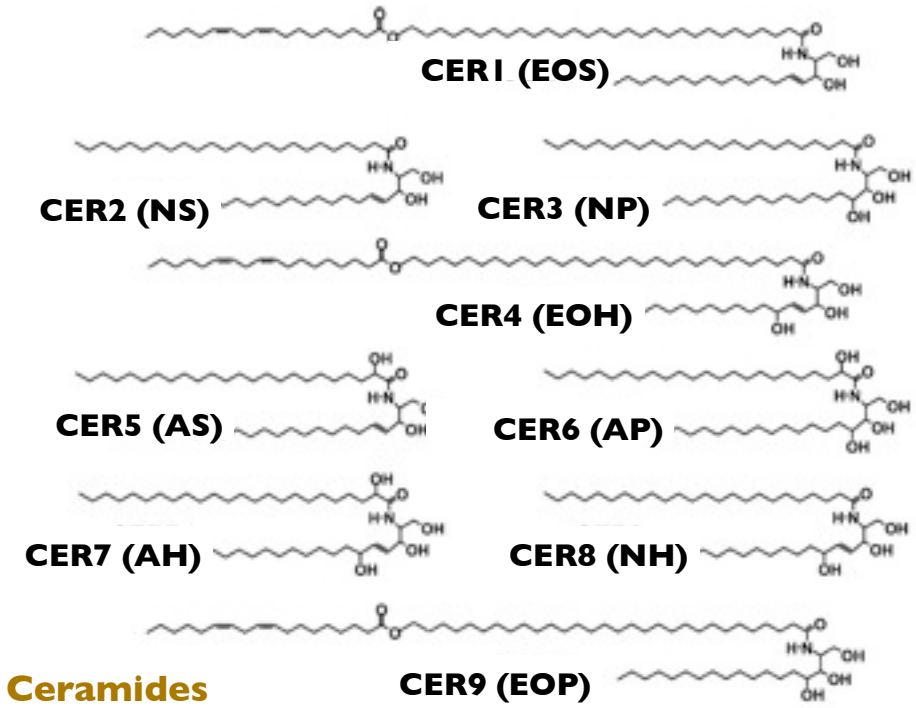

Transmission electron micrograph $RuO_4$ fixed

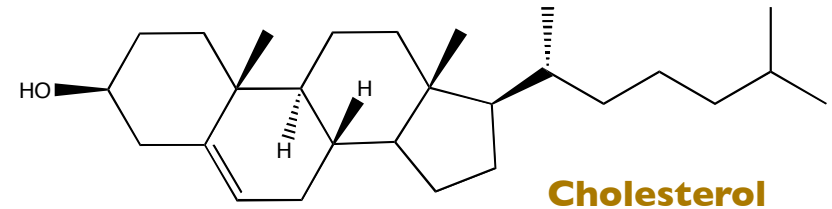
S. Mitragotri *et al*, *J Pharm Sci*, **84**, 697, 1996; D. C. Swartzendruber *et al*, *Cell Tissue Res*, **279**, 271, 1995

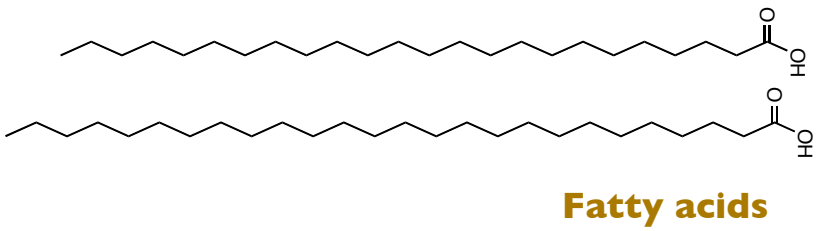
# Stratum Corneum Lipids

- Biologically unique lipid mixture
  - No phospholipids

| Component | Mole % |
|---|---|
| Ceramides | 20 |
| Cholesterol | 40 |
| Fatty acids | 40 |

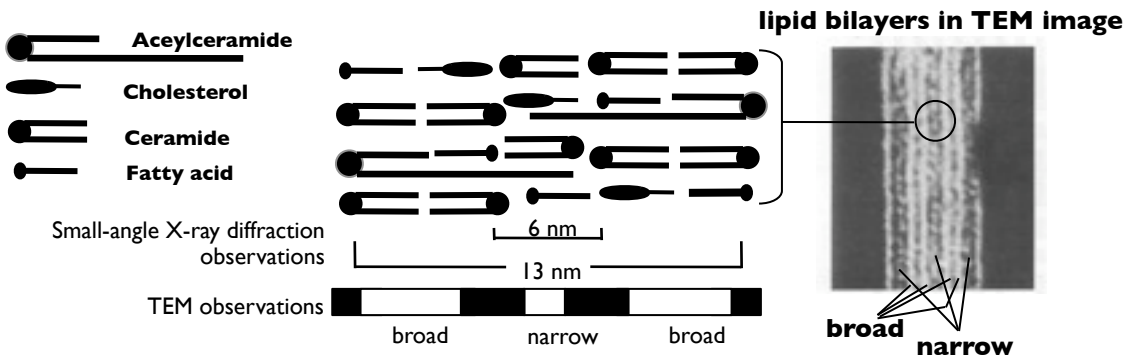


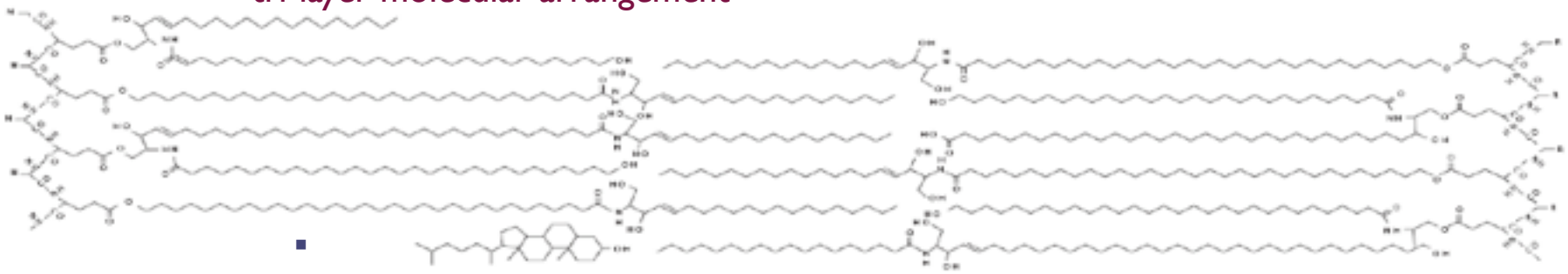



# Stratum Corneum Lipids

- Proposed organization of SC lipids
  - Sandwich model
    - Tri-layer model with ceramides in the hairpin configuration
      - *Repeat unit dimensions are consistent with experimental observations*



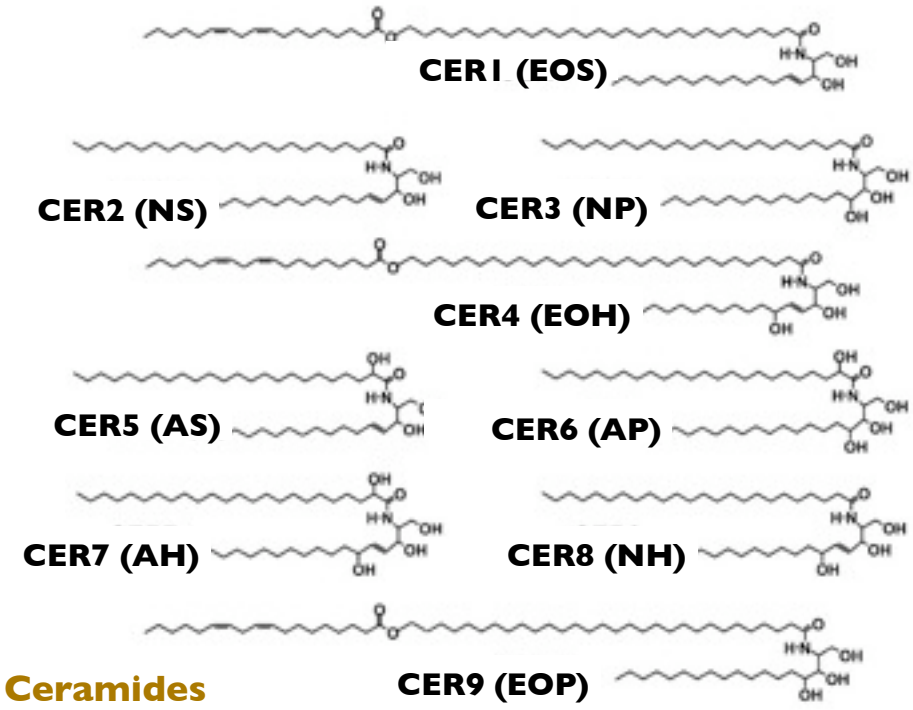

  - Stacked monolayer model
    - Tails of each ceramide are extended and inserted into adjacent layers resulting is a tri-layer molecular arrangement



Kuempel D, et al., *Biochimica Et. Biophy. Acta-Biom.*, **1372**, 135 (1998); Hill JR, et al., *Biochimica et. Biophy. Acta-Biom.*, **1616**, 121 (2003)

# Stratum Corneum Lipids

- Biologically unique lipid mixture
  - No phospholipids

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|---|---|
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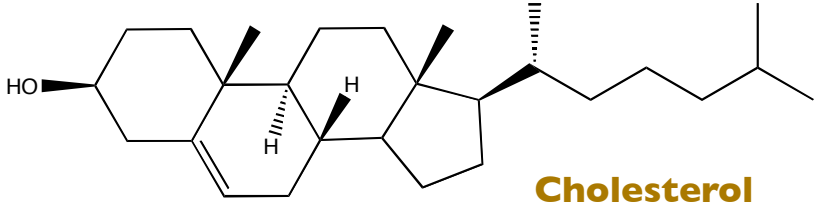
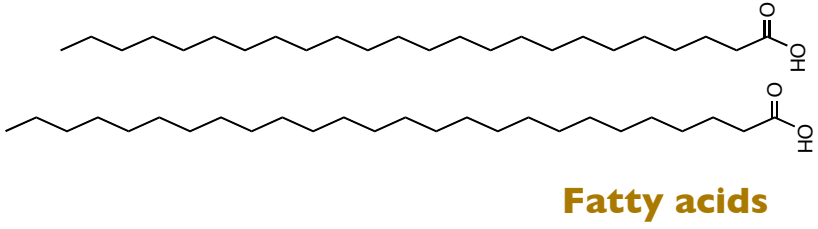
| Cholesterol | 40 |
| Fatty acids | 40 |




  - Lipids extracted from the SC exhibit the same phase behavior and lamellar structures outside the SC as observed in the SC
    - i.e., SC lipids self-assemble
    - Synthetic lipid mixtures containing as few as 6 synthetic CER, equimolar amounts of cholesterol and a 7 component mixture of FFA closely mimic the SC lipid organization to a large extent

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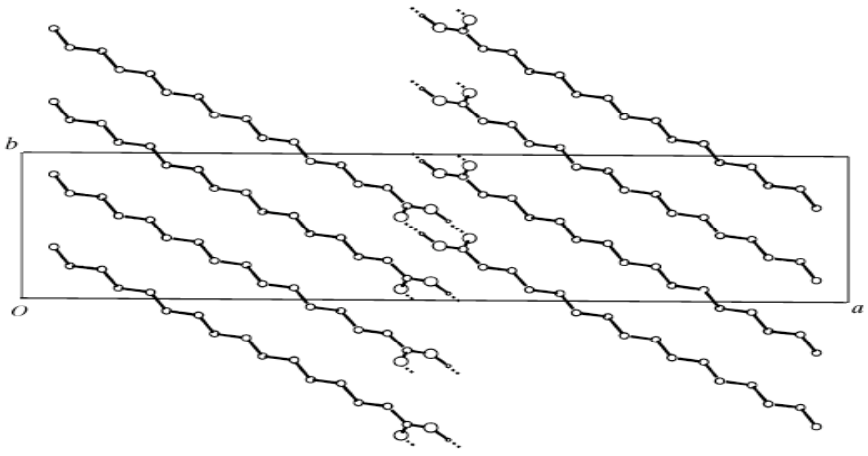
# Optimization of CG Model

- Perform and map atomistic MD simulations to CG level using center of mass to provide target radial distribution functions (RDF) for optimization
  - CHARMM all-atom atomistic force field
    - Simulations performed in LAMMPs and DL_POLY
  - Validate the atomistic force field via comparison to experiments
    - Verify crystal structures (density)
    - Melting points

Nielsen, S., C. Lopez, G. Srinivas, and M. Klein, *Journal of Physics-Condensed Matter* **16**, R481-R512 (2004)
Ayton, G. S., S. Izvekov, W. G. Noid, and G. A. Voth, *Computational Modeling of Membrane Bilayers,* **60**,181-225, (2008)
Bennun, SV; Hoopes, MI; Xing,CY; Faller, R., Chemistry and Physics of Lipids, **159** 59-66 (2009)

# Validation of FFA Atomistic Model

- Comparison of crystal structures for C16:0
  - Experimental*
    - Density (g/cc)
      - *1.027*
    - Crystal lengths (Å)
      - *a = 35.62*
      - *b = 4.9487*
      - *c = 9.406*
    - Crystal angles
      - $\alpha = 90.000^o$
      - $\beta = 90.000^o$
      - $\gamma = 90.447^o$
  - Atomistic
    - Density (g/cc)
      - *1.044*
    - Crystal lengths (Å)
      - *a =39.069 ± 0.223*
      - *b = 4.5682 ± 0.023*
      - *c = 9.1605 ± 0.053*
    - Crystal angles
      - $\alpha = 91.635^o \pm 0.577$
      - $\beta = 87.512^o \pm 0.314$
      - $\gamma = 87.416^o \pm 0.479$
  - Percentage difference
    - Density
      - *1.66%*
    - Crystal lengths
      - *a =9.68% ± 0.63*
      - *b = -7.69% ± 0.47*
      - *c = -2.61% ± 0.56*
    - Crystal angles
      - $\alpha = 1.82\% \pm 0.64$
      - $\beta = -2.76\% \pm 0.35$
      - $\gamma = -3.35\% \pm 0.53$



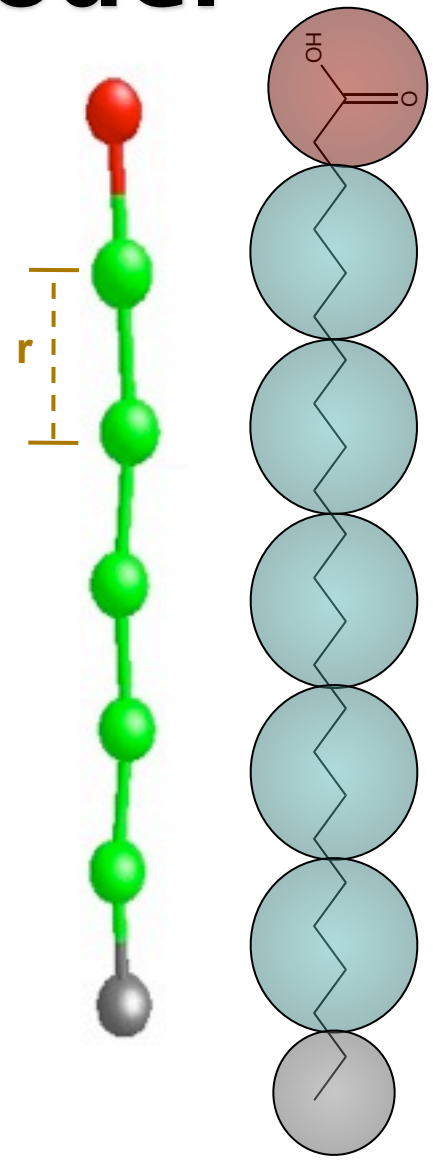

*Moreno E, et al., *Acta Crystal. Sect. C.*, **62**, O129 (2006).

# Optimization of CG Model

- Parameterized using Reith, Pütz, and Müller-Plathe (RPM) method
  - Bonded Potential
    - Fit a bond lengths and angles to Gaussian distribution calculated from atomistic trajectory
      - *E.g., Bond distribution for lipid tail-tail interaction*
  - Numerical non-Bonded Potential

$$V_{j+1}(r) = V_j(r) + \delta kT \ln \frac{g_0(r)}{g_*(r)}$$

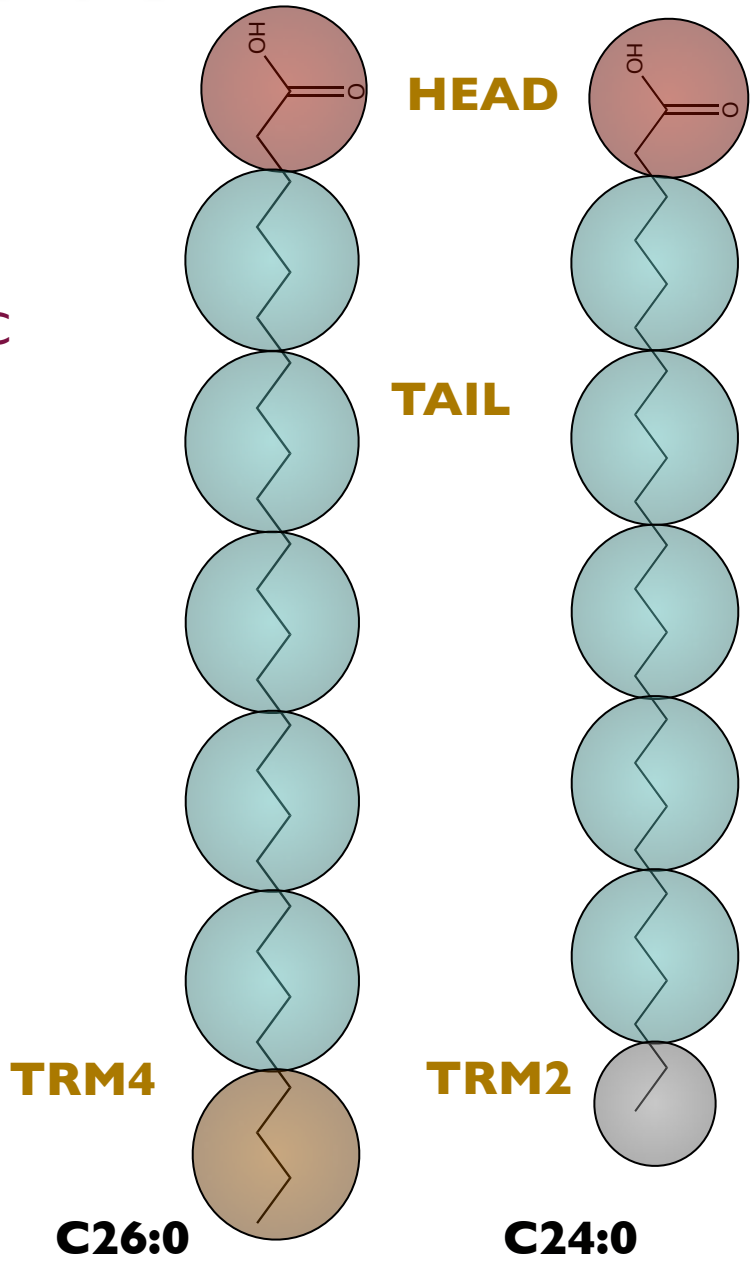
      - *i.e., if g0(r) > g*(r) the strength of attraction will decrease*
    - Repeat until termination criteria satisfied
      - *Negligible change in potential*
    - Damping factor stabilizes optimization in crystalline systems




Milano G, et. al., *J. Polymer Sci. B*, **43**, 871 (2005); Reith D, Pütz M, Müller-Plathe F, *J. Comp. Chem.*, **24**, 1624 (2003)

# Fatty Acids

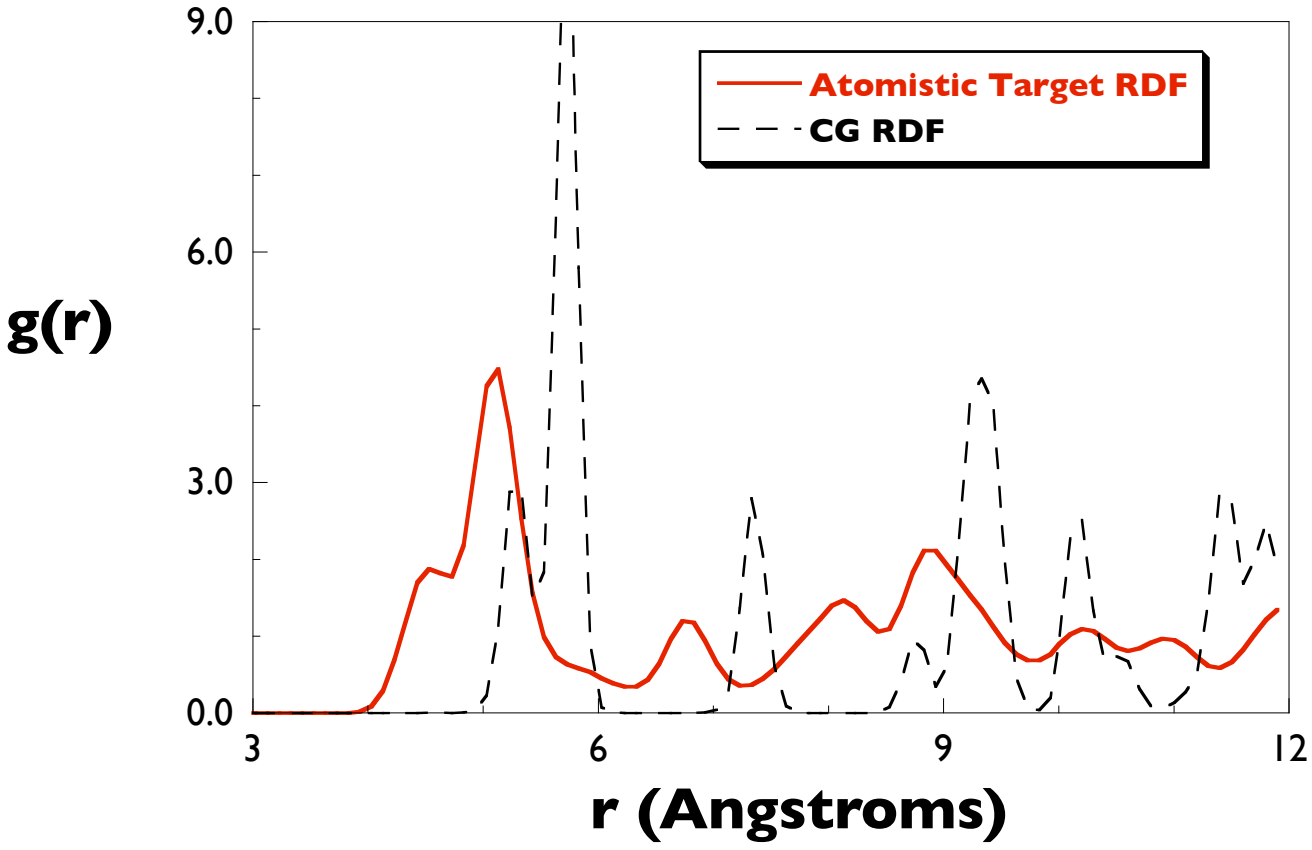
- Long carbon tail
  - 20-30 C's in length
    - C24:0 and C26:0 most prevalent in SC
    - Saturated tail
- Protonated acid head group
  - Softer interaction
- Key features of mapping
  - Simplicity
  - Transferability
    - Mapping
      - *Acid head group*
      - *4 carbons per tail bead*
      - *2 or 4 carbons per terminal bead*

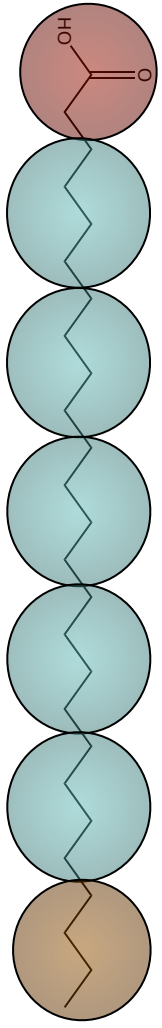



Hadley and McCabe, *Journal of Chemical Physics*, **132**, 134505 (2010).

# CG Force Field Parameterization

- Comparison of atomistic and coarse grained radial distribution functions for crystalline C26:0 simulation
  - Tail - tail RDF



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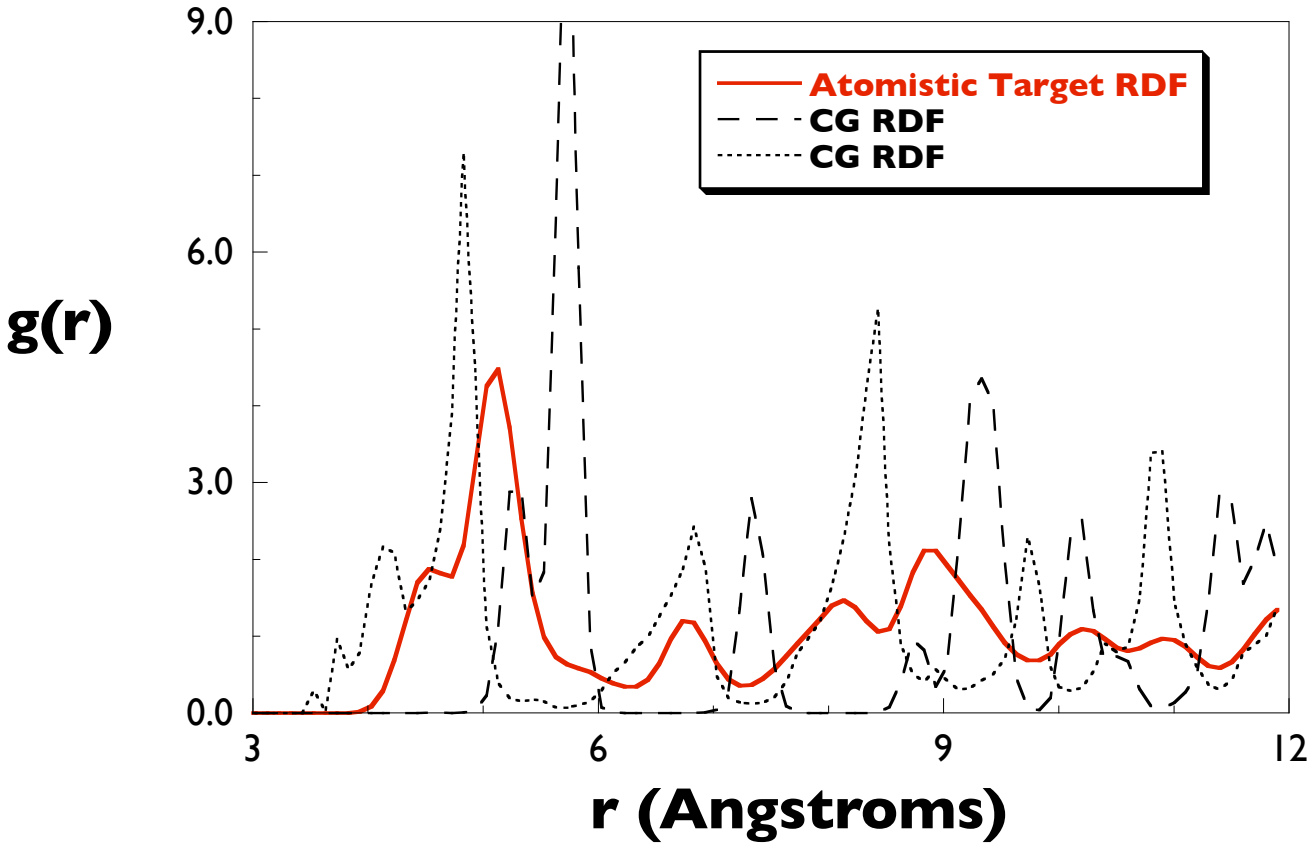


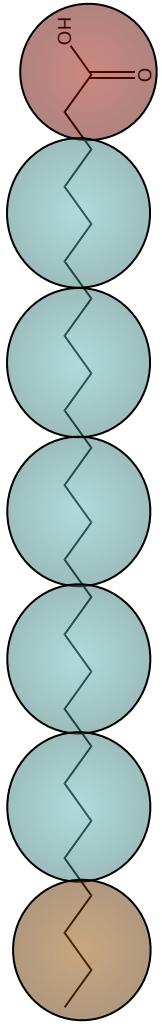

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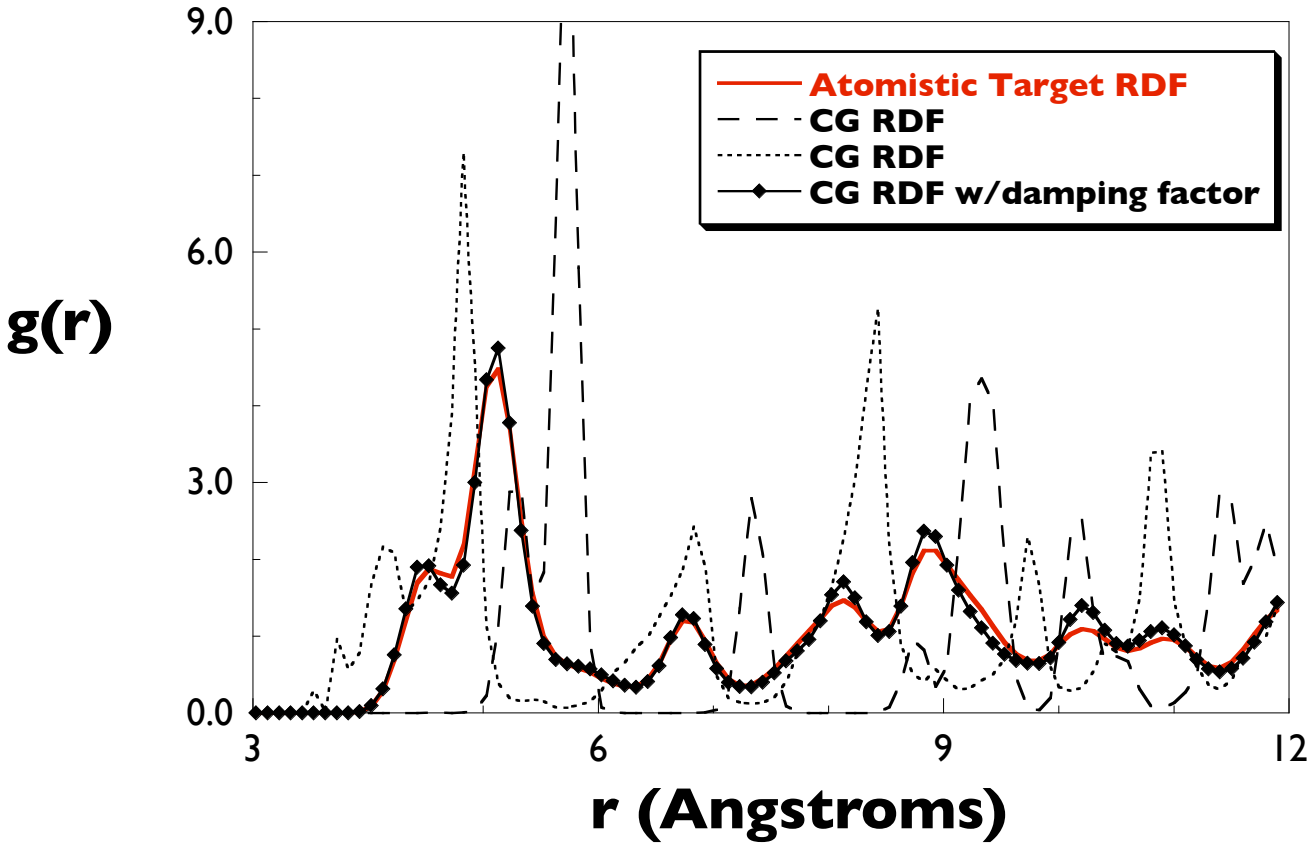


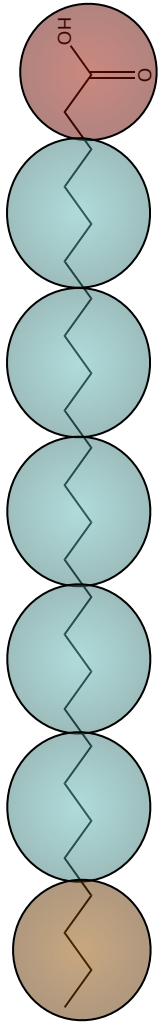

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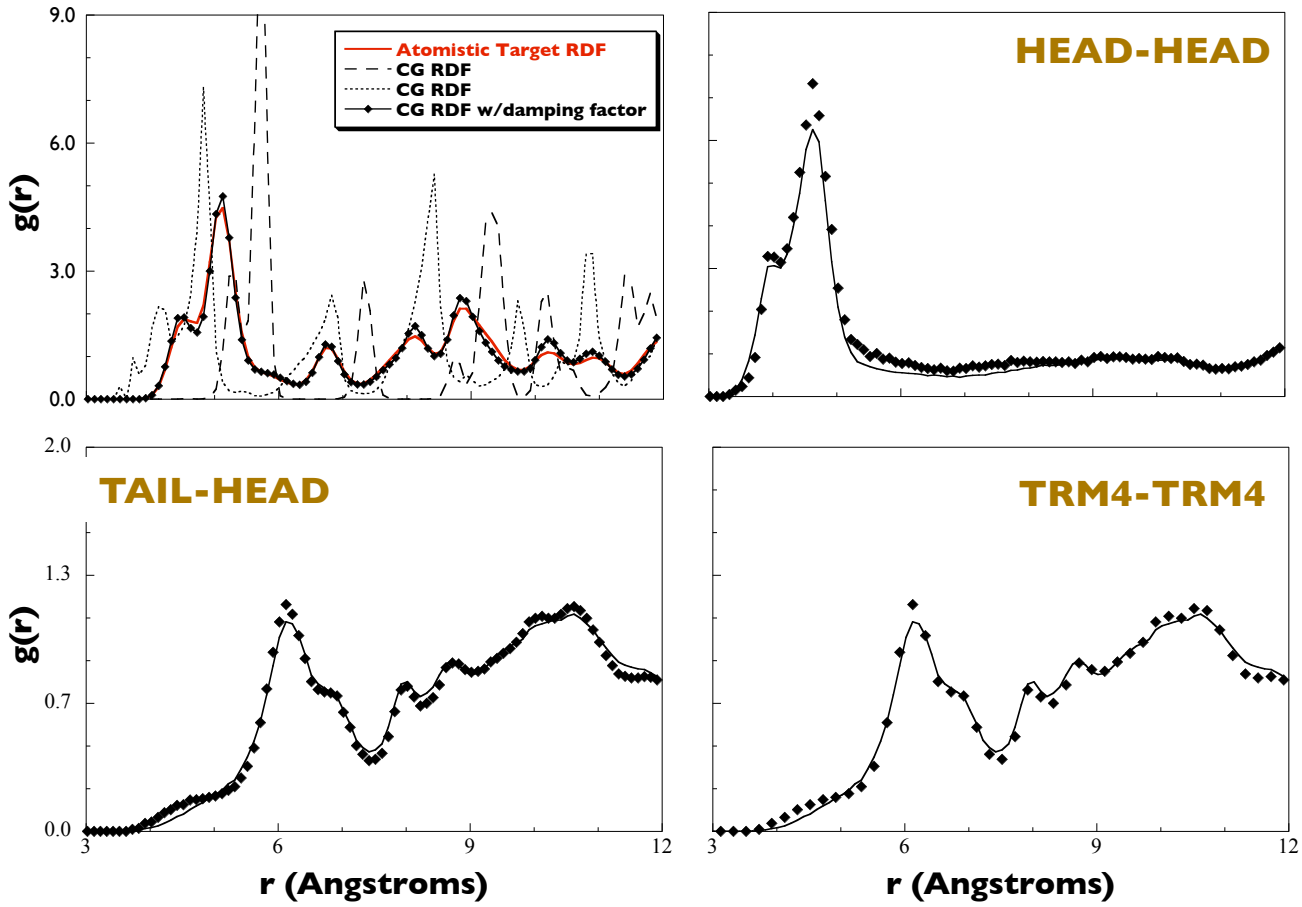
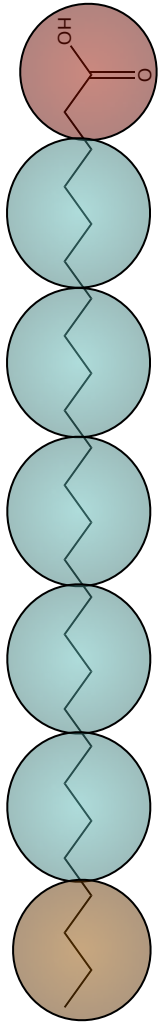


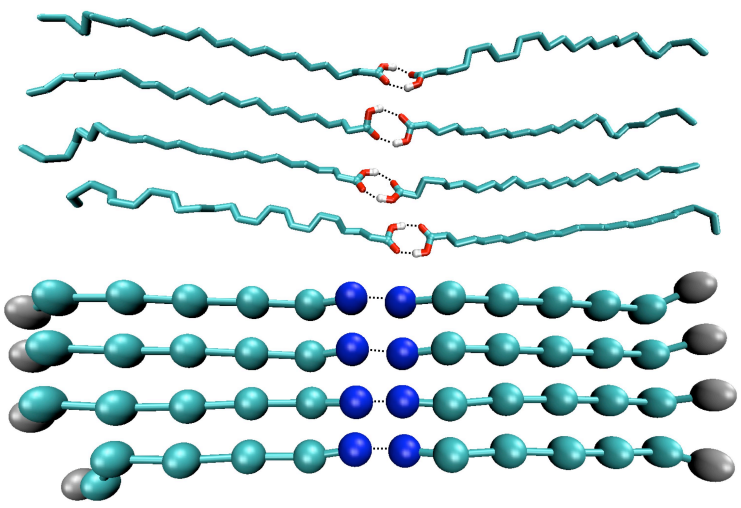
# Validation of CG FFA Model

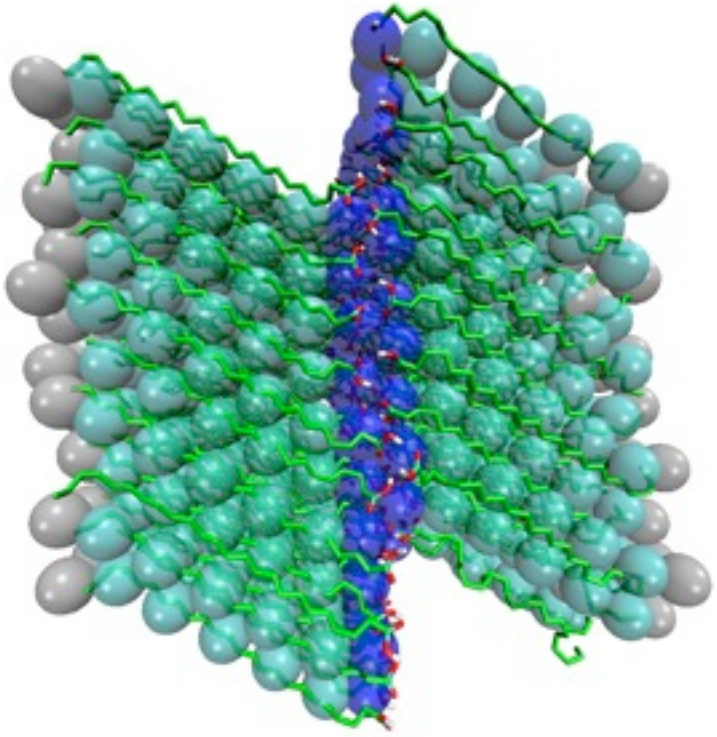
- Comparison of crystal structures for C24:0
  - Extrapolated*
    - Density (g/cc)
      - *1.004*
    - Crystal lengths (Å)
      - *a = 52.401*
      - *b = 4.9487*
      - *c = 9.406*
    - Crystal angles
      - $\alpha = 90.000^o$
      - $\beta = 90.000^o$
      - $\gamma = 90.447^o$
  - Atomistic
    - Density (g/cc)
      - *1.002*
    - Crystal lengths (Å)
      - *a =52.790 ± 0.178*
      - *b = 4.8738 ± 0.015*
      - *c = 9.517 ± 0.036*
    - Crystal angles
      - $\alpha = 88.842^o \pm 0.537$
      - $\beta = 89.690^o \pm 0.278$
      - $\gamma = 87.225^o \pm 0.731$
  - Coarse-Grained
    - Density (g/cc)
      - *1.044*
    - Crystal lengths (Å)
      - *a =55.930 ± 1.875*
      - *b = 4.6469 ± 0.282*
      - *c = 9.087 ± 0.168*
    - Crystal angles
      - $\alpha = 90.414^o \pm 1.271$
      - $\beta = 89.694^o \pm 1.453$
      - $\gamma = 88.153^o \pm 6.859$

*Moreno E, et al., *Acta Crystal. Sect. C.*, **62**, O129 (2006).

# Validation of CG FFA Model

- Comparison of crystal structures for C24:0
  - Hydrogen-bonding network is contained within the same cross-sectional plane as in the atomistic crystal
    - Angle between the plane and the acid tails is reproduced
      - *37.28° ± 2.76 (atomistic) and 36.97° ± 3.51 (CG)*
    - Average distance between hydrogen-bonded HEAD beads retained
      - *5.563 Å (atomistic) and 5.113 Å (CG)*

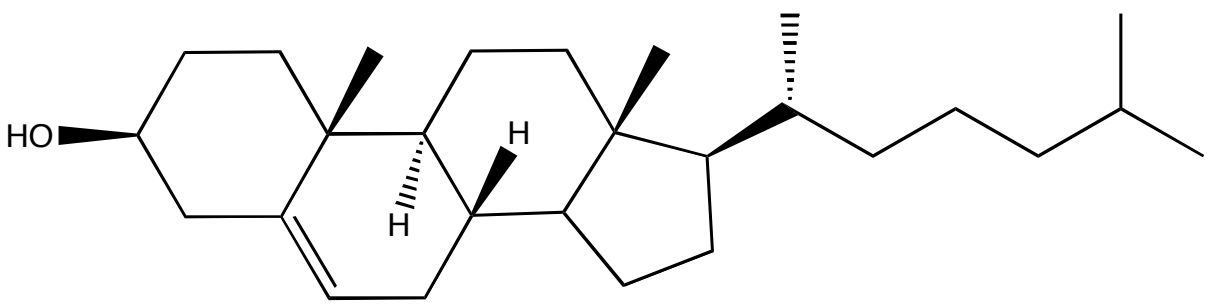




Hadley and McCabe, *Journal of Chemical Physics*, **132**, 134505 (2010).

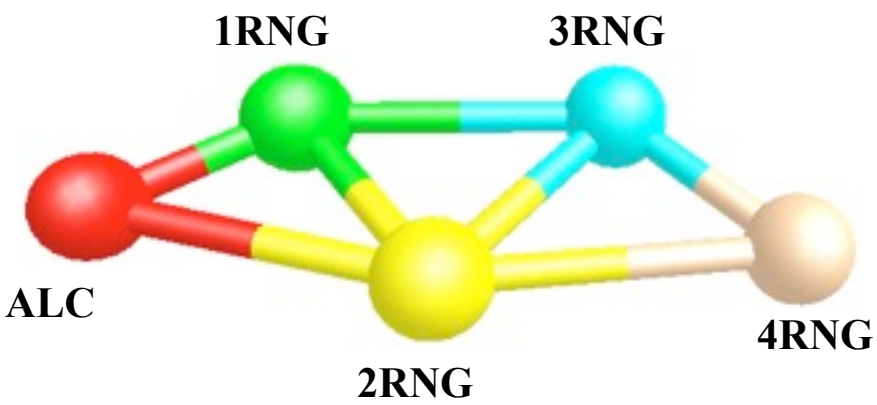
# Cholesterol Structural Features

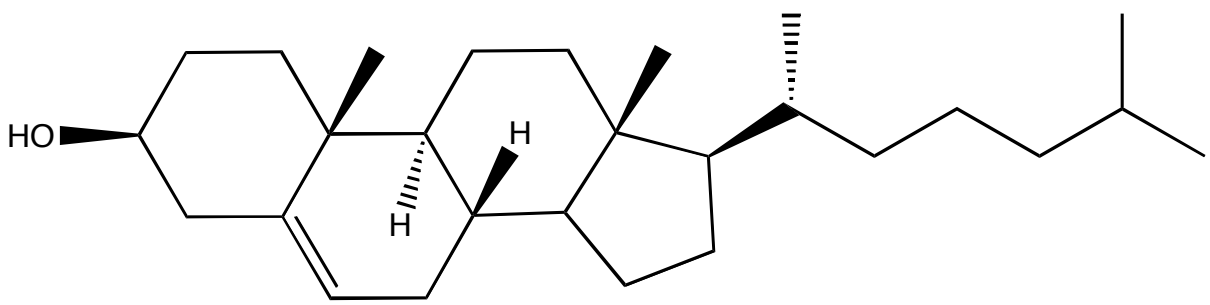
- Rigid regions and flexible regions
  - Flexible tail
  - Rigid multi-ring structure
    - Rough top face
      - *Chiral methyl groups*
    - Smooth bottom face
  - Amphiphilic
    - Hydrophobic and hydrophilic regions
      - *Hydrophilic alcohol group*



# Cholesterol CG Mapping

- Retains important features of atomistic molecule
  - Multi-ring structure



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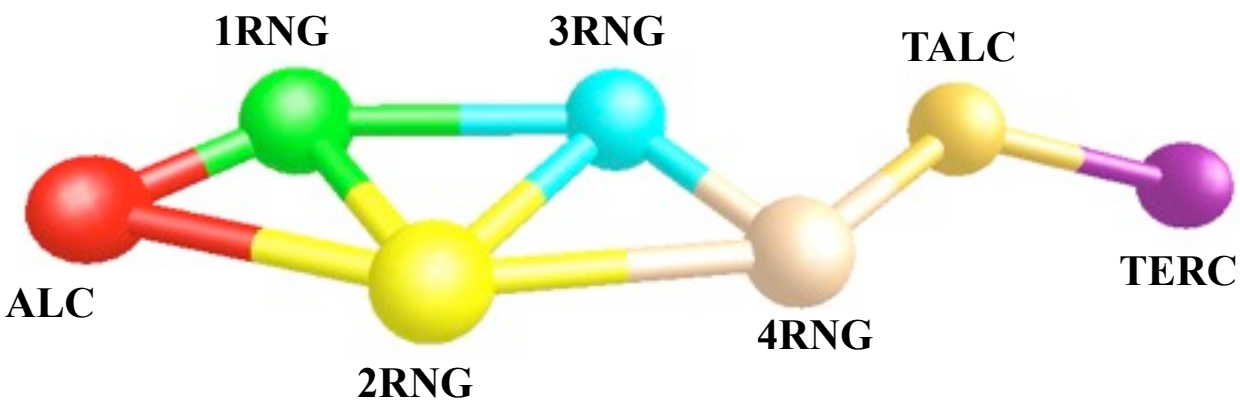


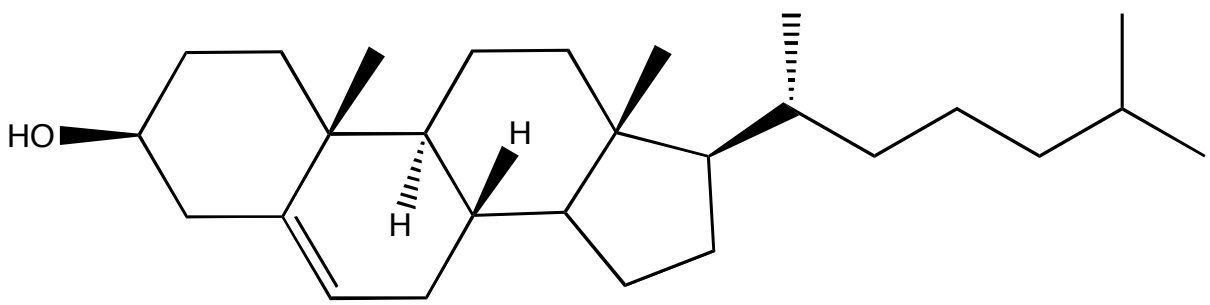

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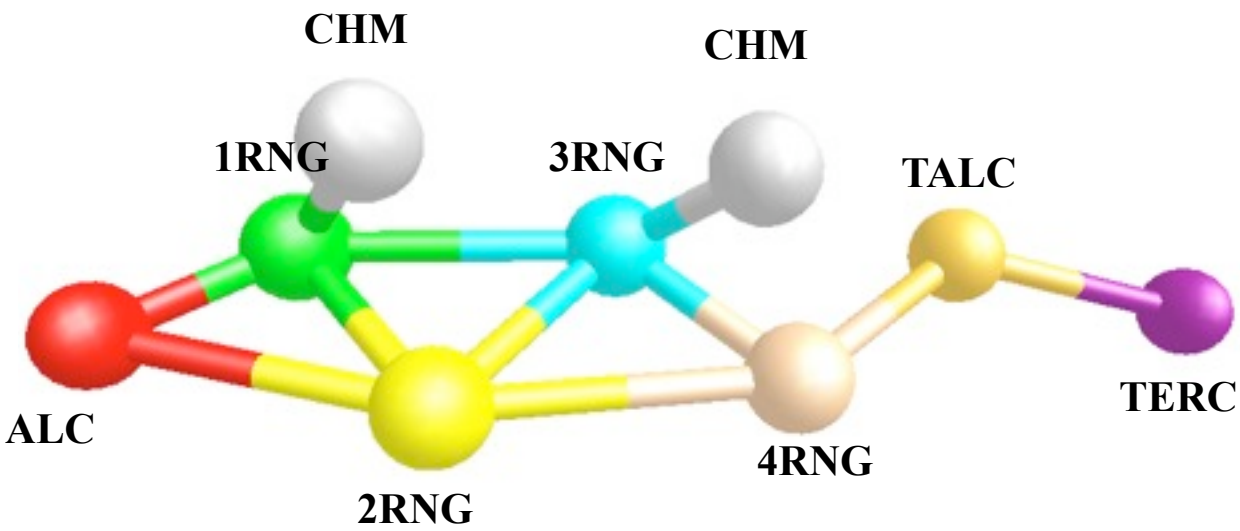
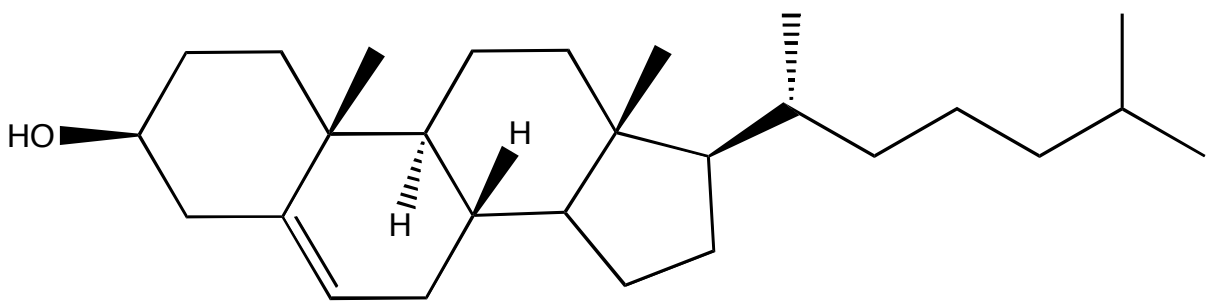
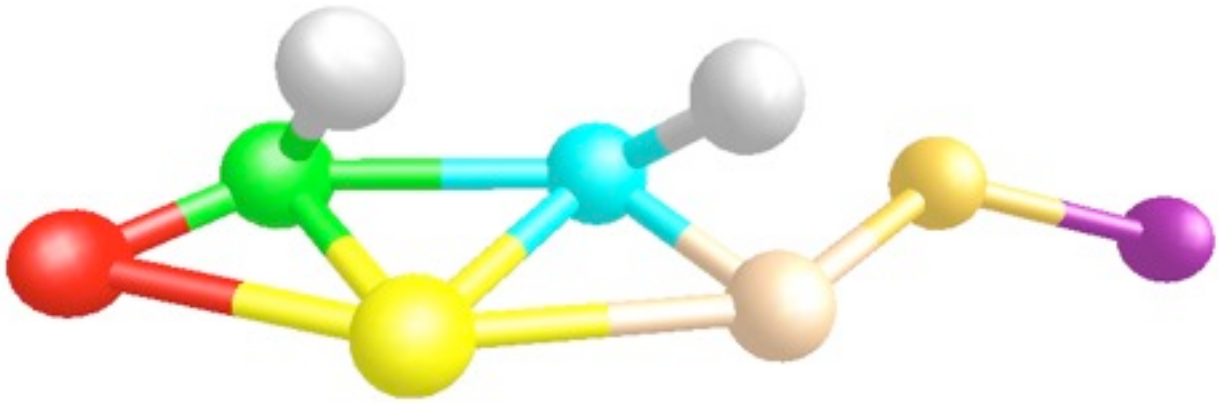


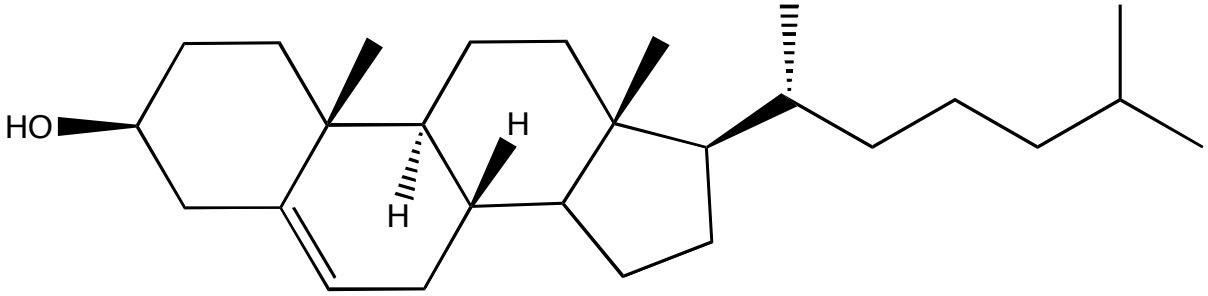




- Multi-ring structure
- Flexible tail
- Chiral methyls (CHM) mapped as separate beads to represent the "rough" face

# Cholesterol CG Mapping

- Retains important features of atomistic molecule
  - 8 different bead types
    - 36 interactions to be optimized in a pure cholesterol system





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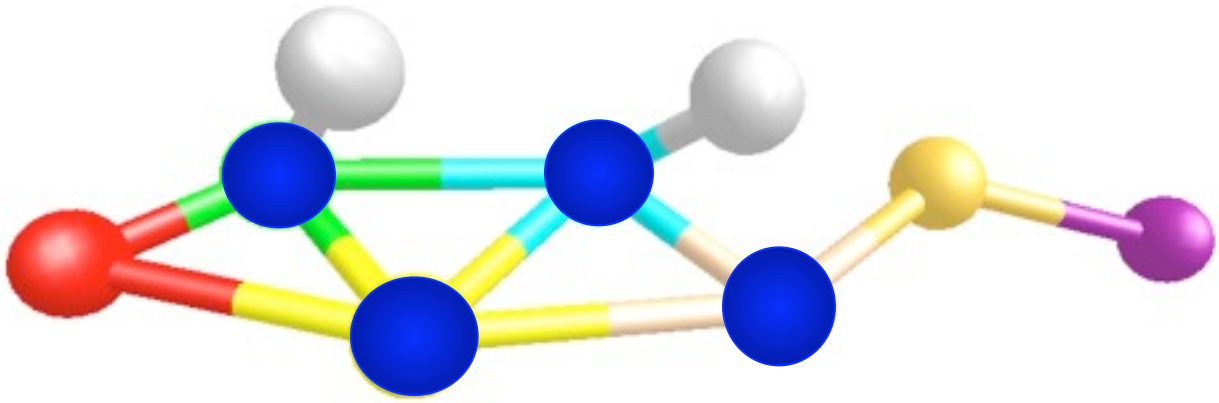
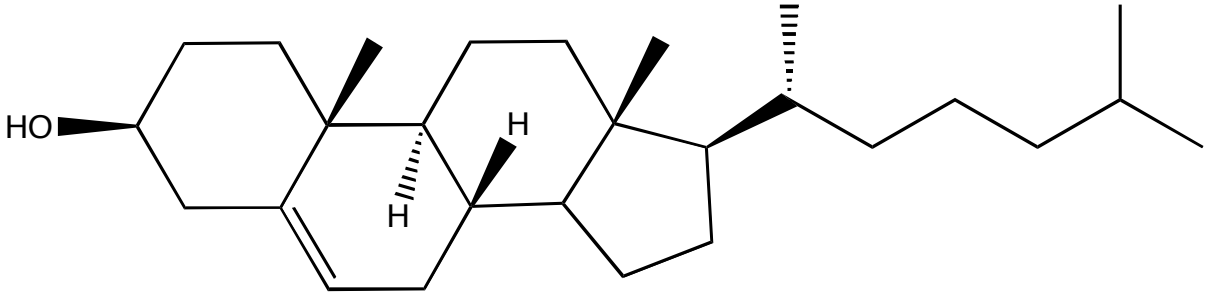





- Homogenized model
  - Replace 4 different ring beads with one kind of ring bead
    - 5 different bead types and 15 interactions to optimize

# Validation of CG Force Field

- Crystal structure parameters for cholesterol

- Experimental
  - Density (g/cc)
    - *1.021*
  - Crystal Lengths (Å)
    - *a = 14.172*
    - *b = 34.209*
    - *c = 10.481*
  - Crystal Angles
    - $\alpha = 94.64^o$
    - $\beta = 90.67^o$
    - $\gamma = 96.32^o$

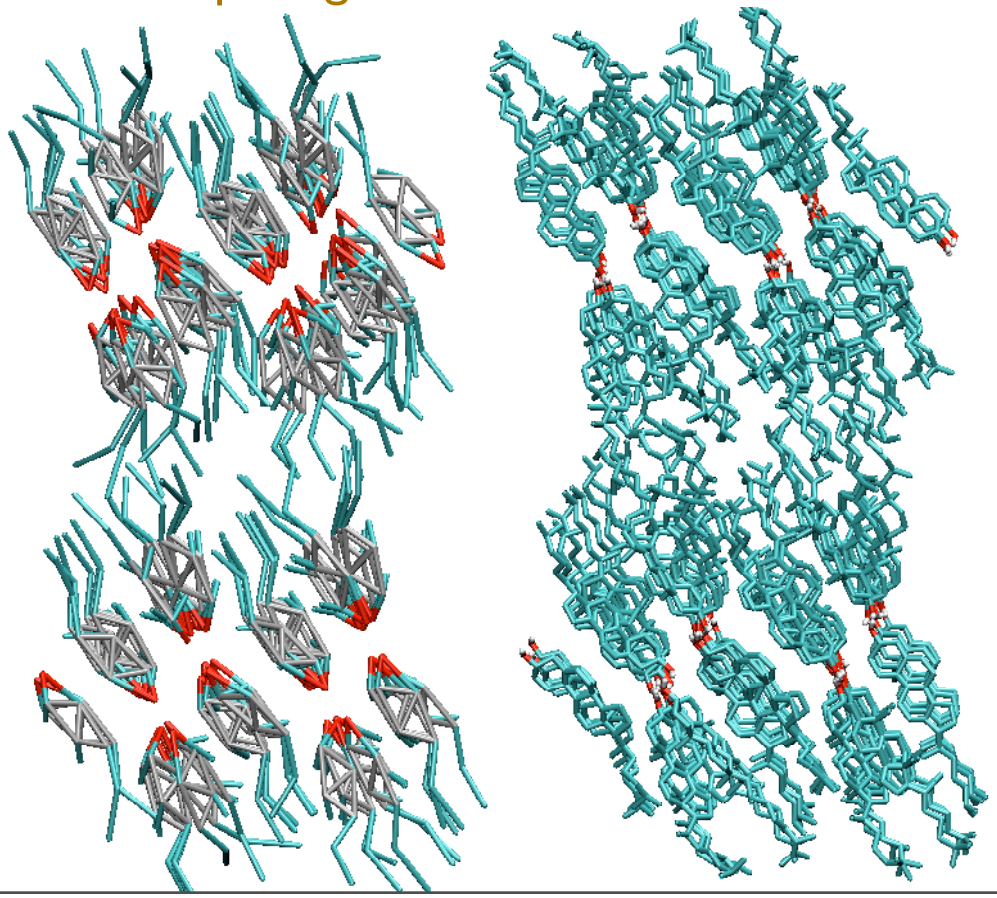
- Full CG
  - Density (g/cc)
    - *1.007*
  - Crystal Lengths (Å)
    - *a = 14.133 ± 0.226*
    - *b = 34.315 ± 0.763*
    - *c = 10.748 ± 0.077*
  - Crystal Angles
    - $\alpha = 94.40^o \pm 1.65$
    - $\beta = 89.82^o \pm 0.83$
    - $\gamma = 99.24^o \pm 5.60$

- Homogenized CG
  - Density (g/cc)
    - *1.03*
  - Crystal Lengths (Å)
    - *a = 13.949 ± 0.062*
    - *b = 33.823 ± 0.598*
    - *c = 10.749 ± 0.086*
  - Crystal Angles
    - $\alpha = 97.51^o \pm 3.72$
    - $\beta = 89.99^o \pm 0.99$
    - $\gamma = 94.93^o \pm 3.29$

Shieh HS, et al., *Acta Cryst. Sect. B* **37**, 1538 (1981); Cournia, Z., J. C. Smith, and G. M. Ullmann. *Journal of Computational Chemistry* **26**:1383 (2005).

# Validation of CG Force Field

- With the full CG model the overall shape of the crystal is retained
  - Spacing between the molecules and their orientations are in good agreement with that seen atomistically
  - The molecules maintain sheets as observed in the atomistic simulations with similar spacing between the sheets



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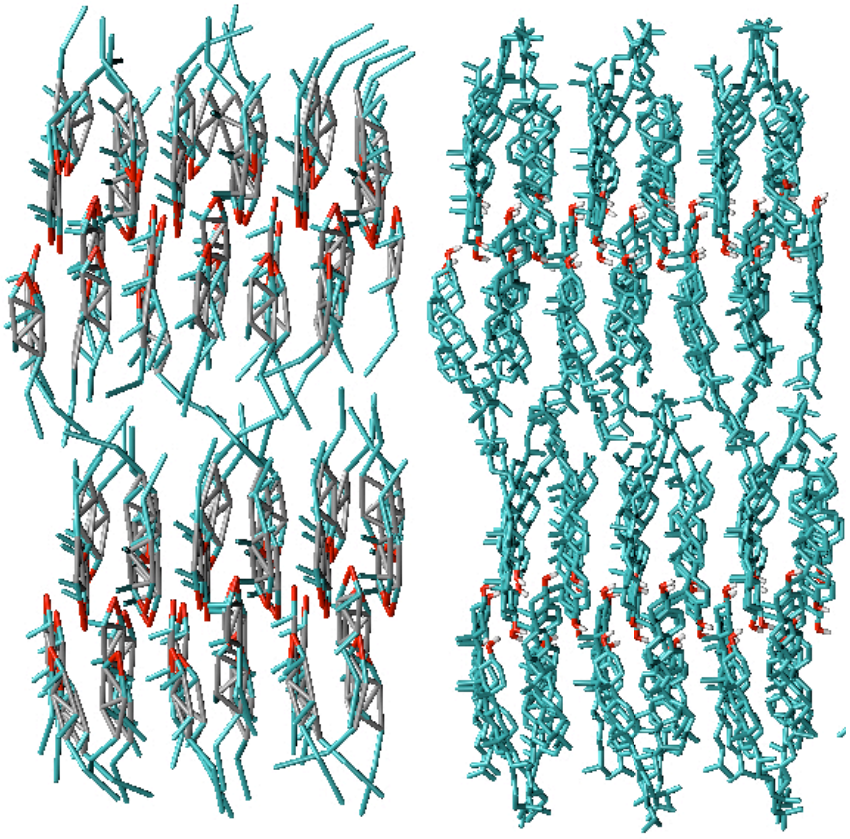
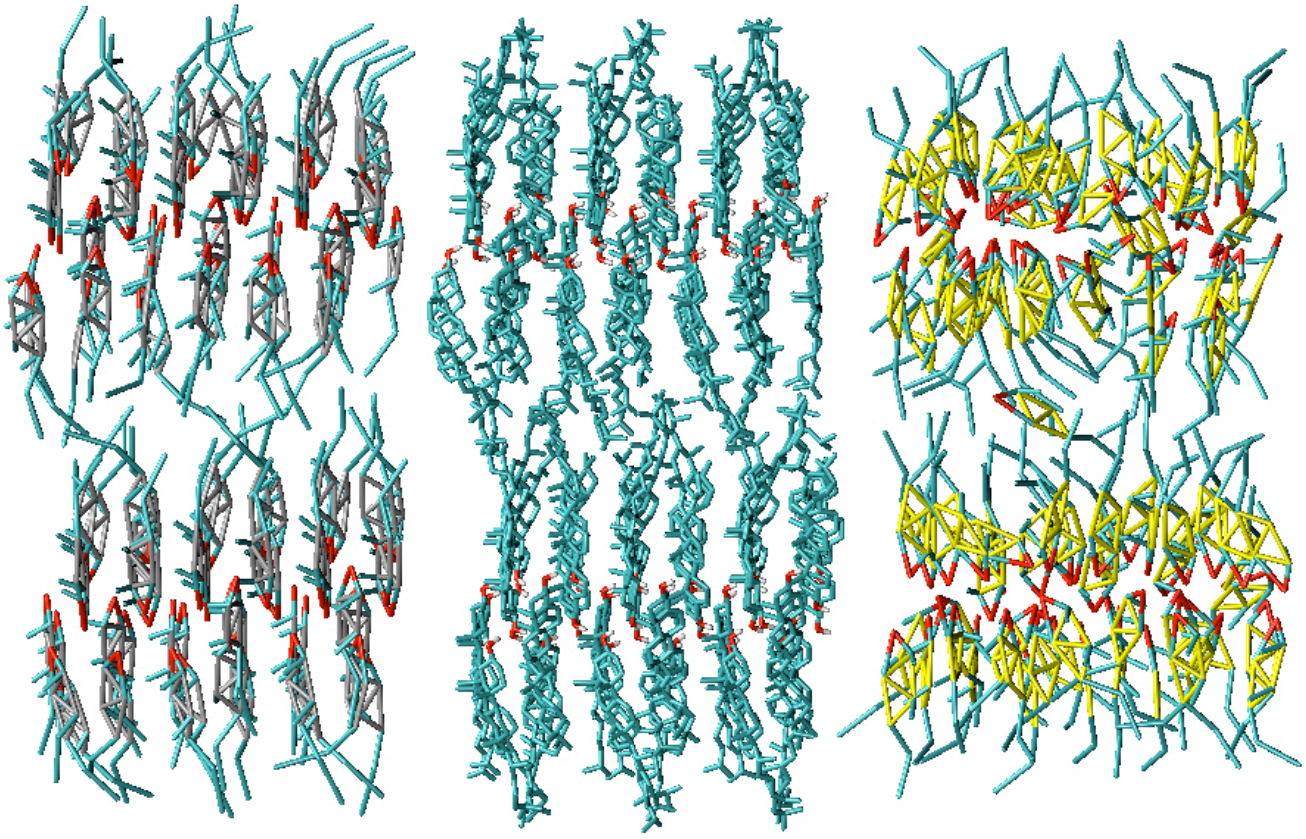


# Validation of CG Force Field

- With the homogenized CG model the molecules rotate along the axis of the molecule
  - The molecules can arrange themselves within the crystal structure in multiple ways that produce the same RDFs, but represent different molecular arrangements within the unit cell
    - Hydrogen-bonding between ALC beads is retained in the homogenized model



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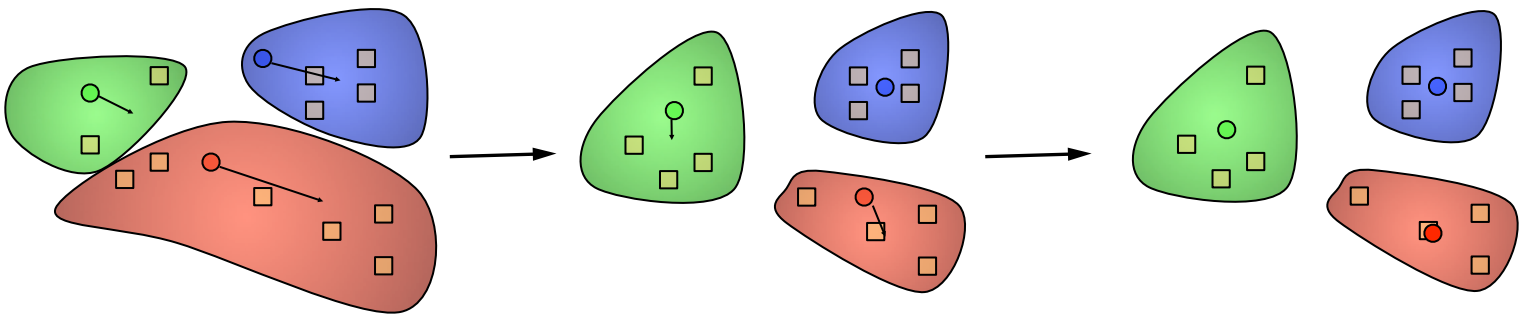
# CG Water Models

- Single site
  - Non-bonded interactions fitted using center of mass (COM) CG methods
    - Reith, Pütz, and Müller-Plathe (RPM) method
    - Force-matching (Voth, et al.)

- Multiple waters mapped to single beads
  - E.g., Marrink, et al., J. Phys. Chem. B 750 (2004)
    - Four waters to one bead
      - *Coordination number = 4.35*
    - Based on matching phase behavior
  - Incompatible with COM-based methods

# Novel CG Water Model

- K-means algorithm used to map waters to CG beads
  - Determine number of water beads
    - i.e., number of waters per CG bead
  - Find initial position for water beads
    - Determine which bead each water is closest to
    - Calculate center of mass of waters within each bead
    - Move the bead's position to the center of mass
  - Repeat until position of the bead remains constant

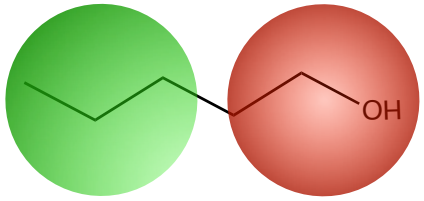


- Benefits of using K-means algorithm
  - Compatible with COM-based CG methods
  - Capable of mapping multiple waters to single beads

Hadley and McCabe, *Journal of Physical Chemistry B*, **114**, 4590–4599 (2010).

# CG Water Model

- Determine optimum degree of coarse-graining for water
  - Studied pure water and mixtures of representative solutes
    - 1-pentanol
    - Hexadecanoic acid as a representative lipid




- Map all atomistic simulations to CG level
  - Apply K-means algorithm to map water to CG beads
    - Study several H2OX models
      - *X = 1, 3 -6, 8 and 9*
- Scoring function based on combination of speed and accuracy

$$S_{fxn} = \frac{Speed\ scale}{RDF * \%Diff(\rho_{pure})}$$

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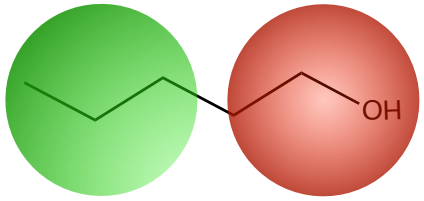


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- Scoring function based on combination of speed and accuracy

| Waters/bead | Speed scale | RDF merit function | RDF scale | Density of pure water | % difference in density | Scoring function |
|---|---|---|---|---|---|---|
| atomistic | 1.00 | 1.88E-03 | 1.00 | 0.9983 | 0.0% | n/a |
| 1 | 11.92 | 1.86E-02 | 9.85 | 0.9741 | -2.4% | 49.87 |
| 3 | 34.14 | 3.38E-03 | 1.79 | 0.9343 | -6.4% | 296.61 |
| 4 | 52.95 | 7.75E-03 | 4.11 | 0.9996 | 0.1% | 763.92 |
| 5 | 66.50 | 2.35E-02 | 12.45 | 0.9815 | -1.7% | 316.78 |
| 6 | 76.32 | 5.00E-02 | 26.52 | 1.0942 | 9.6% | 29.97 |
| 8 | 85.99 | 5.18E-02 | 27.48 | 1.0190 | 2.1% | 150.98 |
| 9 | 94.18 | 5.35E-02 | 28.39 | 0.9412 | 5.7% | 57.96 |

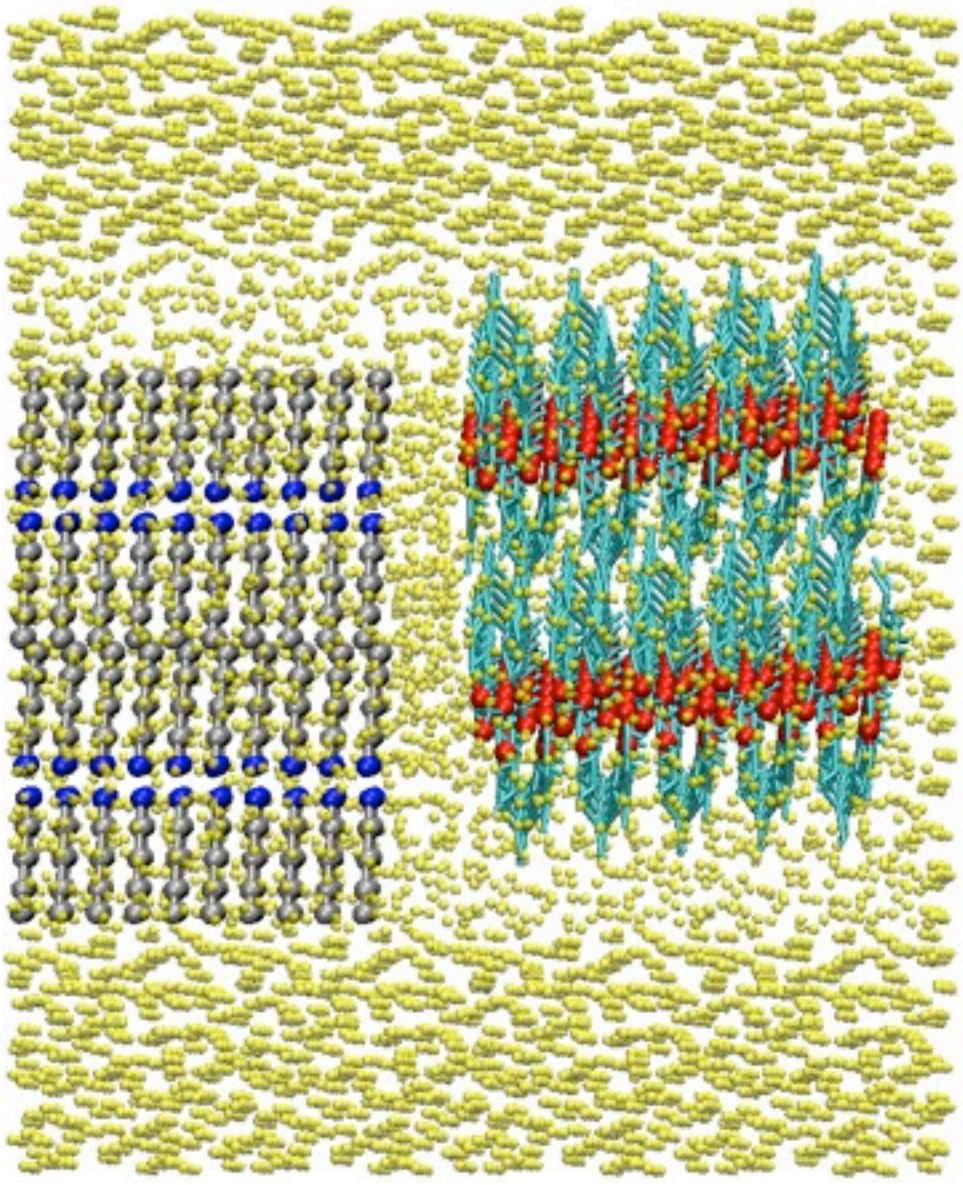
# FFA/CHOL/Water Bilayer System

- Study based on experimental work of Lafleur and coworkers on phase behavior of hydrated mixtures of C16:0 and CHOL
  - Below 50˚C separate crystalline domains observed
    - At 50 mol % CHOL a bilayer forms with a coexisting fluid phase on heating
    - CHOL was shown to modify the behavior of the C16:0 by lowering its melting point compared to the pure state
      - *At lower temperatures FFA crystals are more stable than bilayer*
      - *At higher temperatures FFA is fluid enough to mix with CHOL*
  - Symbiotic relationship
    - Fatty acid acts as a source of hydrophobic shielding for CHOL
    - CHOL acts as a rigidifier of FFA tails preventing formation of liquid phase

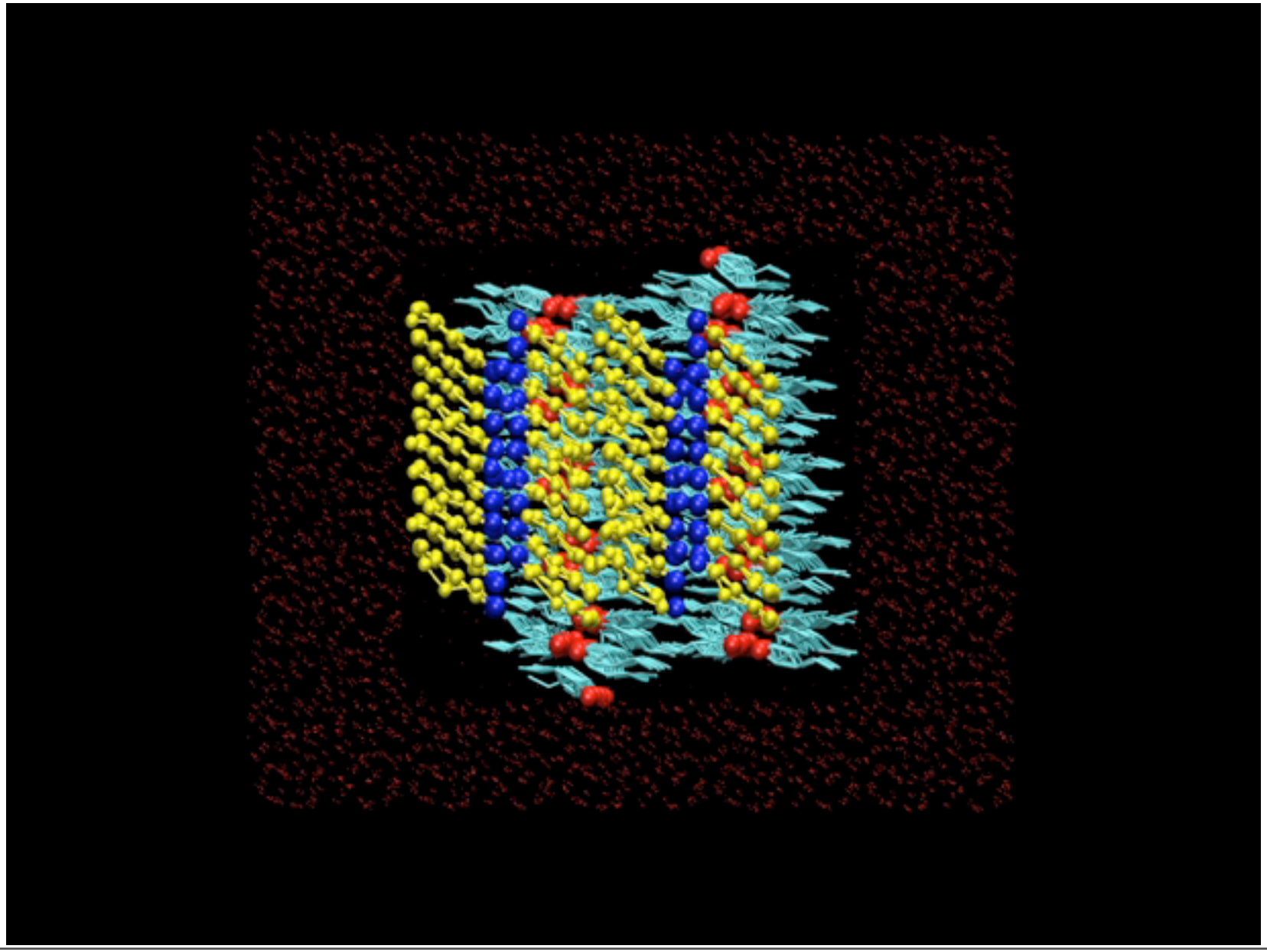
Ouimet, J.; Croft, S.; Pare, C.; Katsaras, J.; Lafleur, M. *Langmuir* 2003, **19**, 1089

# FFA/CHOL/Water Bilayer

- CG system
  - 400 C16:0
  - 400 CHOL
  - $10^4$ water beads
- Atomistic system
  - Pre-assembled bilayer
  - 144 C16:0
  - 144 CHOL
  - 6000 waters



# FFA/CHOL/Water Bilayer



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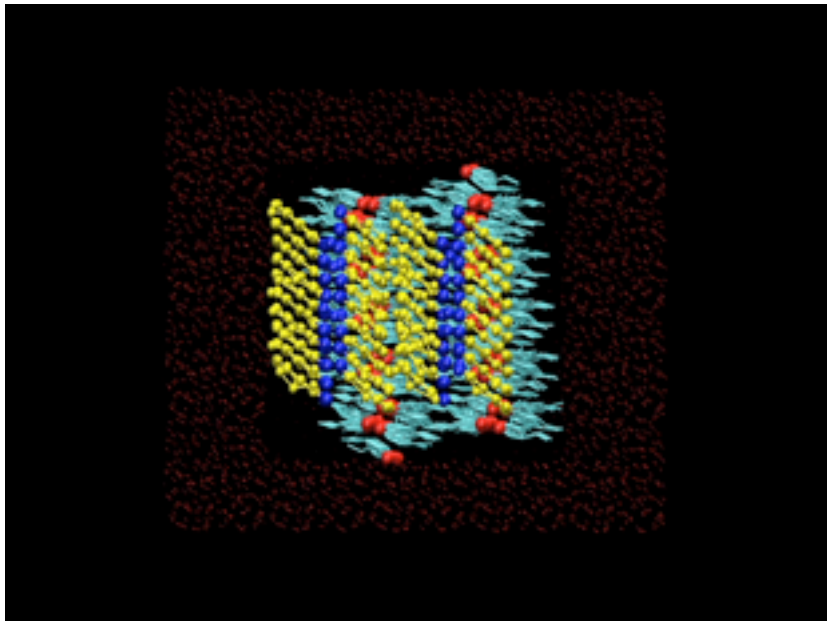
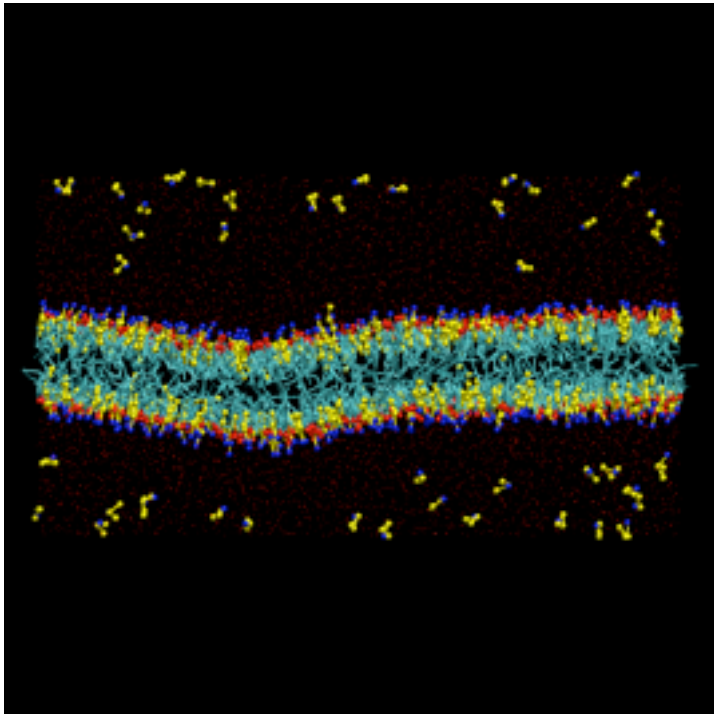


- Integration of both lipids
  - Lipid aggregation initiated by hydrophobic interactions
  - Bilayer structure induced by hydrophilic interactions
- Average bilayer height 30 Å and area/lipid 34 $Å^2$
  - Compare well to experimental and atomistic values
    - Bilayer height ~32 Å
    - Average area/lipid - 33 $Å^2$

# C12:0 Bilayer Destabilization

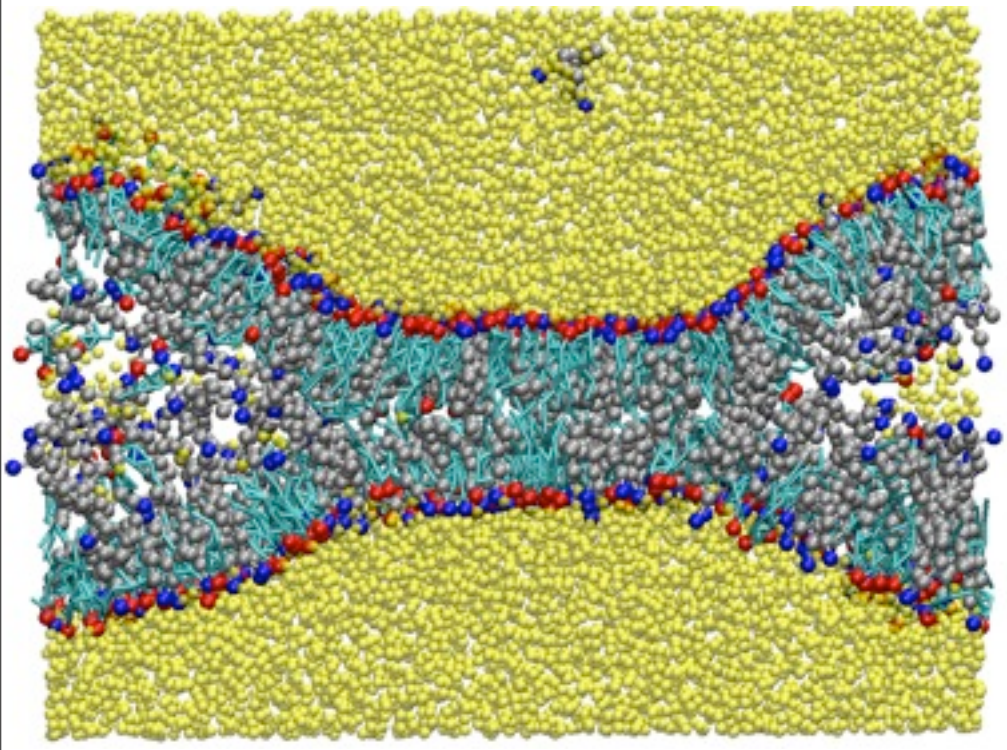
- Experimentally chain lengths between 14-18 carbons required for bilayer formation
  - Hydrophobic match maximizes VDW contacts



  - C12:0 fatty acid more soluble in water phase
    - Micelles cause buckling in bilayer

# C24:0 Bilayer Destabilization

- Longer tails create gaps between leaflets



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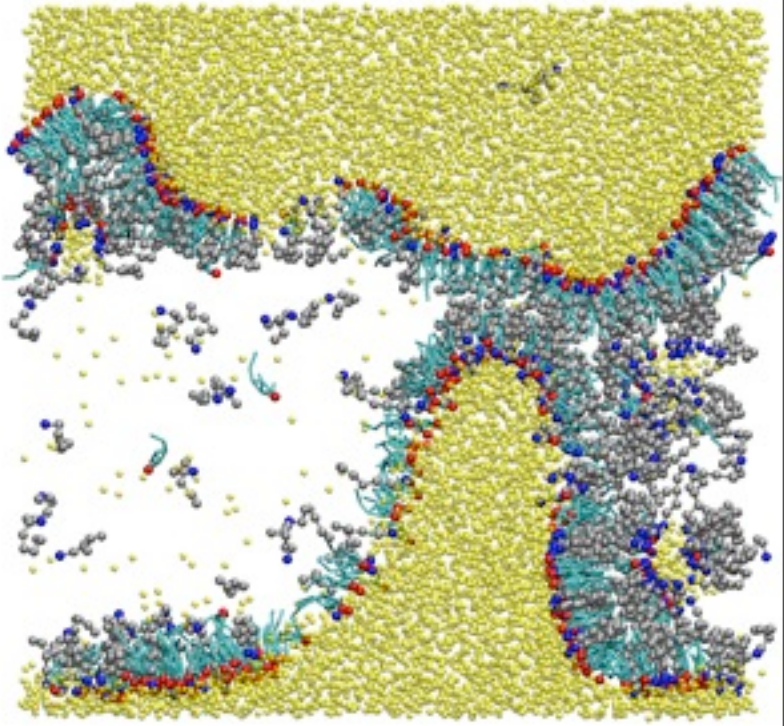

- Water enters bilayer between leaflets
  - Cause leaflets to "unzip"

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# Conclusions

- Robust and transferable CG models developed
  - Crystal structures can be reproduced
    - Requires damping factor
  - K-means algorithm maps multiple waters to CG beads
    - 4-water bead has ideal degree of coarse-graining
      - *Speed and accuracy*
- Models capable of self-assembly
  - C16:0/CHOL/water system
    - Predicted structure agrees well with experimental bilayer
    - Can predict destabilization behavior
      - *C12:0 forms spherical micelles which buckle the bilayer*
      - *C24:0 allows for water pockets to "unzip" bilayer leaflets*

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NIAMS
National Institute of Arthritis and Musculoskeletal and Skin Diseases