August 5th, 2015

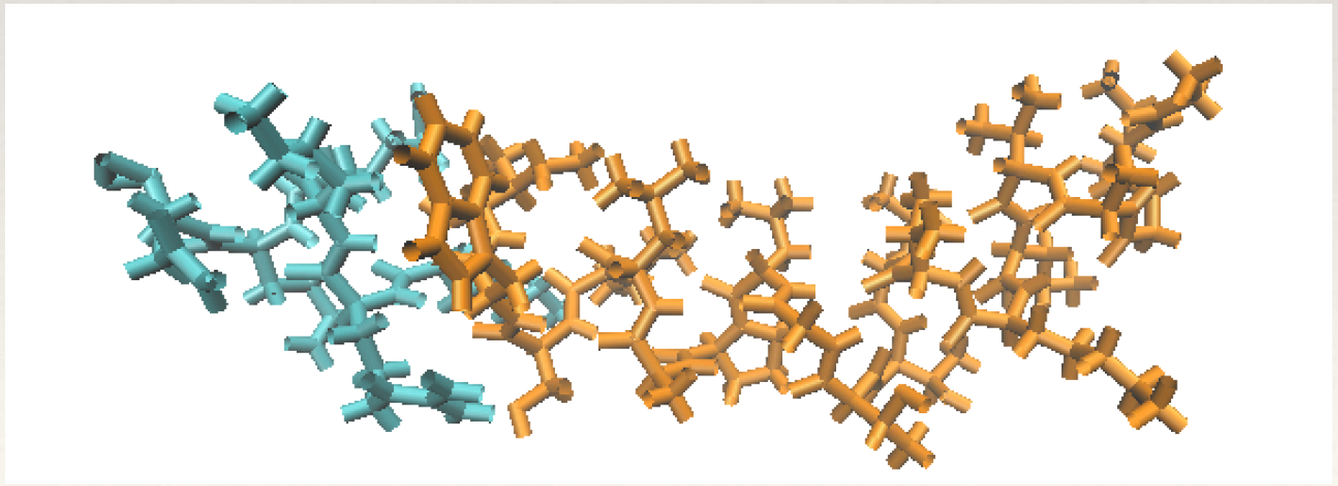
Molecular Dynamics(MD) Simulation of Melittin in a Dimyristoylphosphatidylcholine (DMPC) Bilayer Membrane

Speakers: XIE Zhiyao (Andy), JIANG Huanbo (Bobby)

Mentors: Dr. CHENG Xiaolin, Dr. TIAN Jianhui, Dr. WONG Kwai

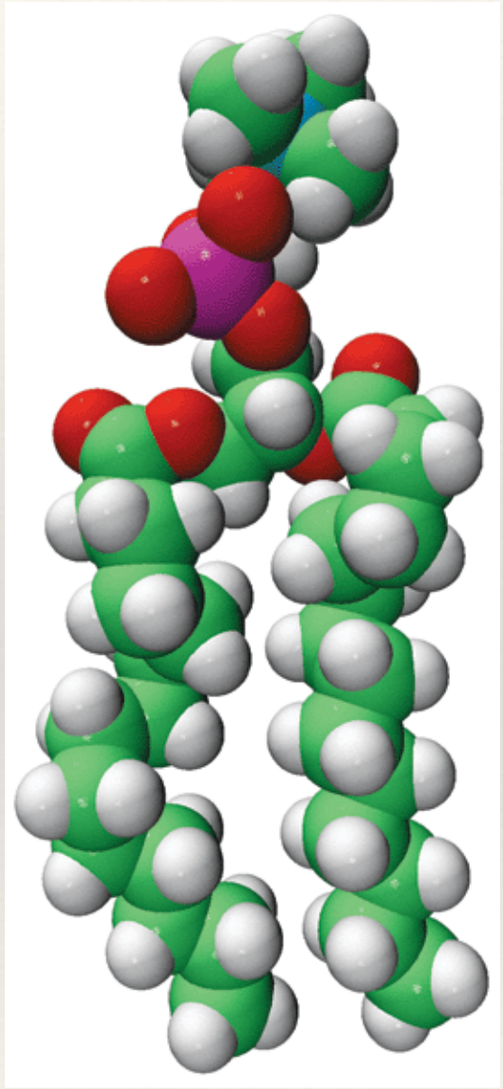
Melittin—amphiphilic property

- Melitin: Major protein component of the bee venom that has a pronounced effect on the lysis of membranes
- residues 1-20 (orange part) make up the amino-terminal region, which is predominantly hydrophobic
- carboxy-terminal region (residues 21-26, blue part) is hydrophilic and in contact with water



DMPC

- Dimyristoylphosphatidylcholine (DMPC) bilayer membrane
- The majority of phospholipids belong to the phosphatidylcholine (PC) or phosphatidylethanolamine (PE) families
- Lipid bilayers containing PC head groups express fewer functionalities, but they are central in maintaining membrane integrity

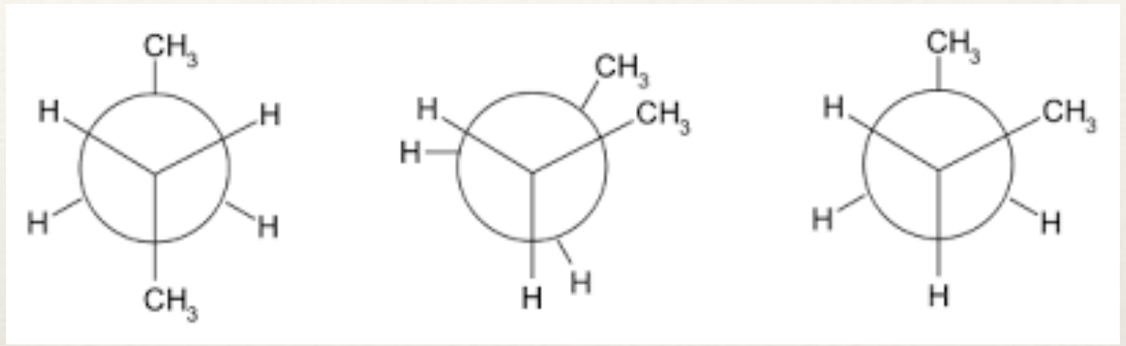


Phase transition

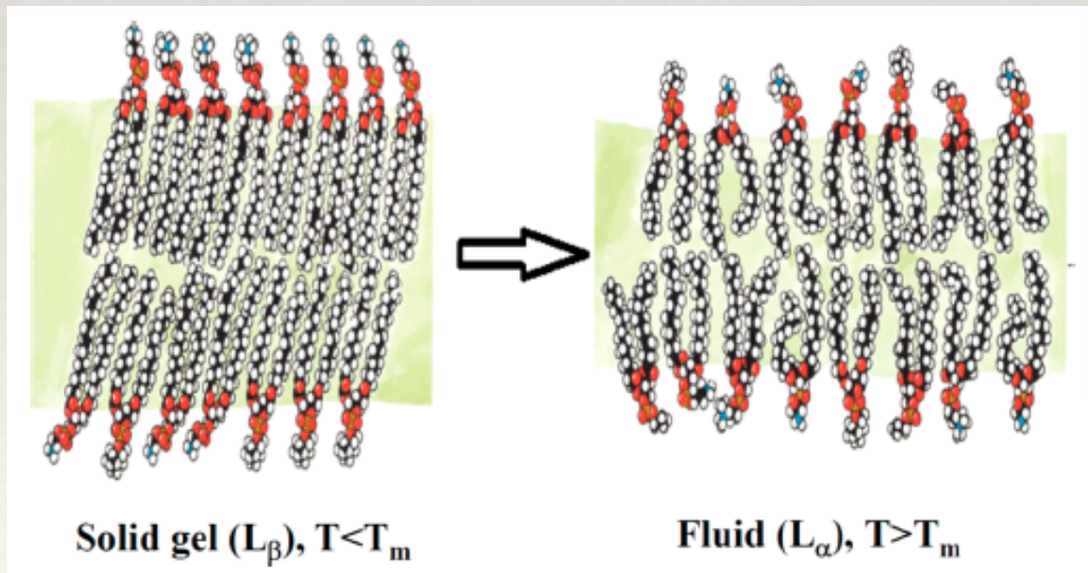
The phase transition temperature (Tm) of DMPC is about 296K.

At T > Tm, lipid membranes are in the liquid (fluid) phase. The two tails have a relatively higher fraction of gauche conformation and are more mobile and flexible.

When cooled to T < Tm, the hydrocarbon tails will have more trans conformations and be more ordered.

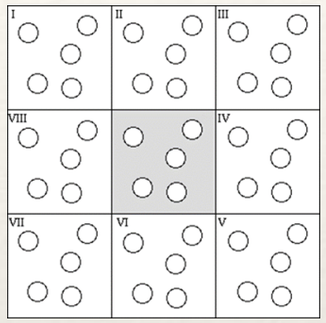


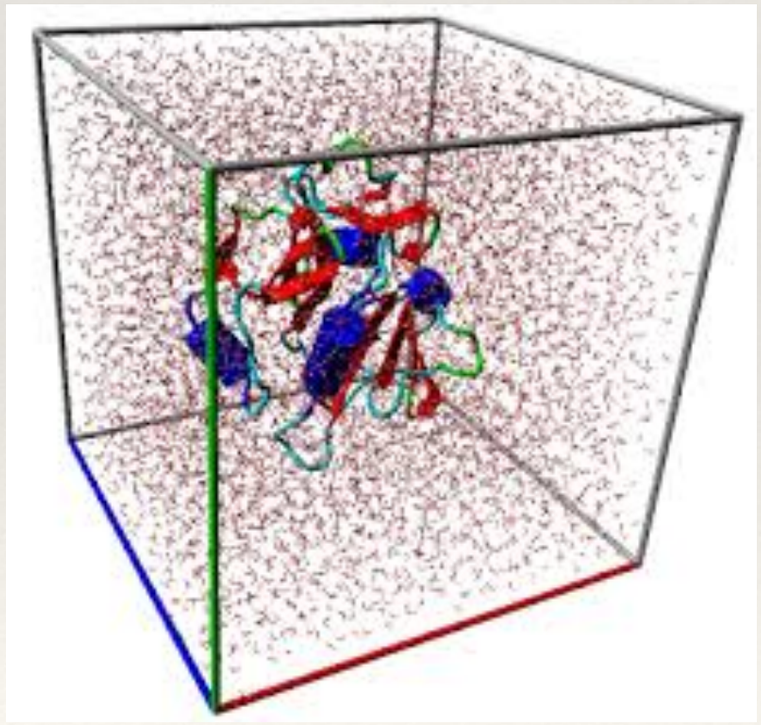
trans(left) and gauche(right) rotamers of butane



Molecular Dynamics (MD) Simulation -A Digital Laboratory

"In the real world, this could eventually mean that most chemical experiments are conducted inside the silicon of chips instead of the glassware of laboratories."





- Classical Newtonian mechanics

  This is a simplification of what is actually going on

- Molecules in Solution

  proteins, RNA, and DNA are immersed in a sea of water molecules

- Periodic Boundary Conditions

  Simulate a segment of molecules in a larger solution by having repeatable regions

- Cutoff Methods

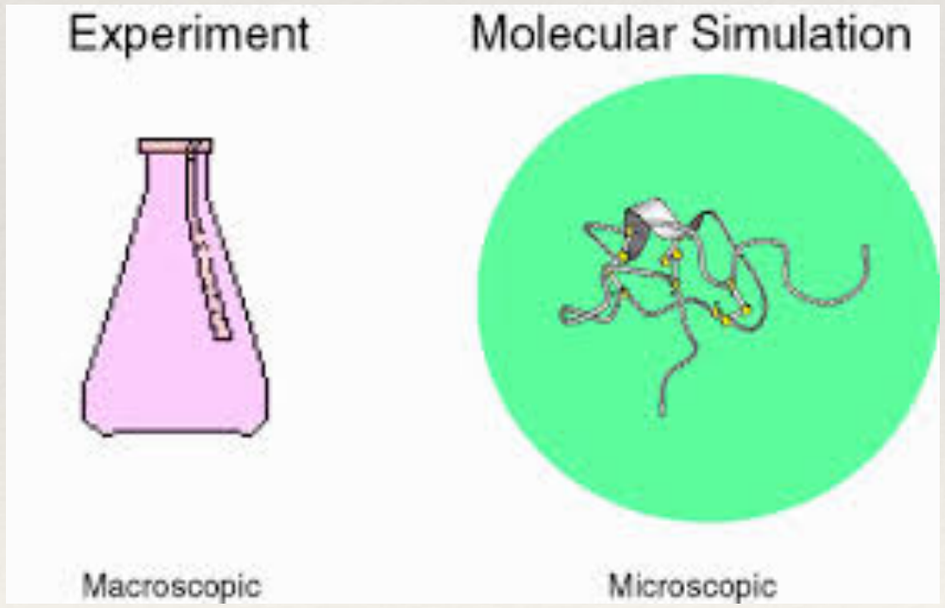
  Ignore atoms at large distances from each other

Why MD simulations

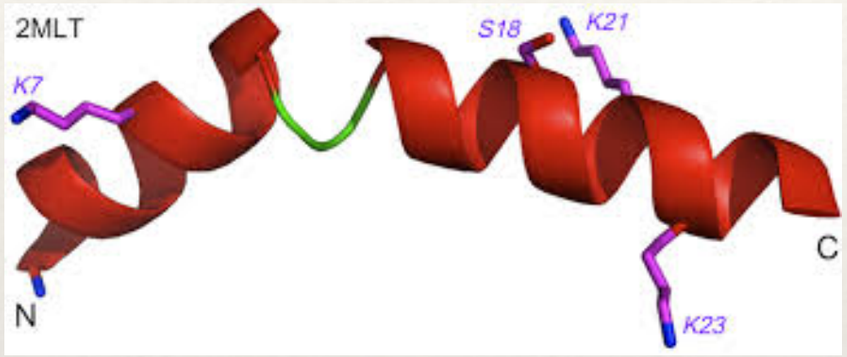
MD simulations allow prediction of properties for

- Novel materials which have not been synthesized
- Existing materials whose properties are difficult to measure or poorly understood
- Model validation

Other powerful techniques: x-ray crystallography, electron microscopy and nuclear magnetic resonance (NMR)



Why Melitin-DMPC model



- Specific lipid-protein interactions involved in the anchoring and stabilization of membrane-bound proteins are of central importance in a large number of fundamental processes occurring at the surface of the cell
- Understanding melittin-membrane interaction assumes greater significance due to the observation that melittin mimics the N-terminal of HIV-1 virulence factor Nef1-25

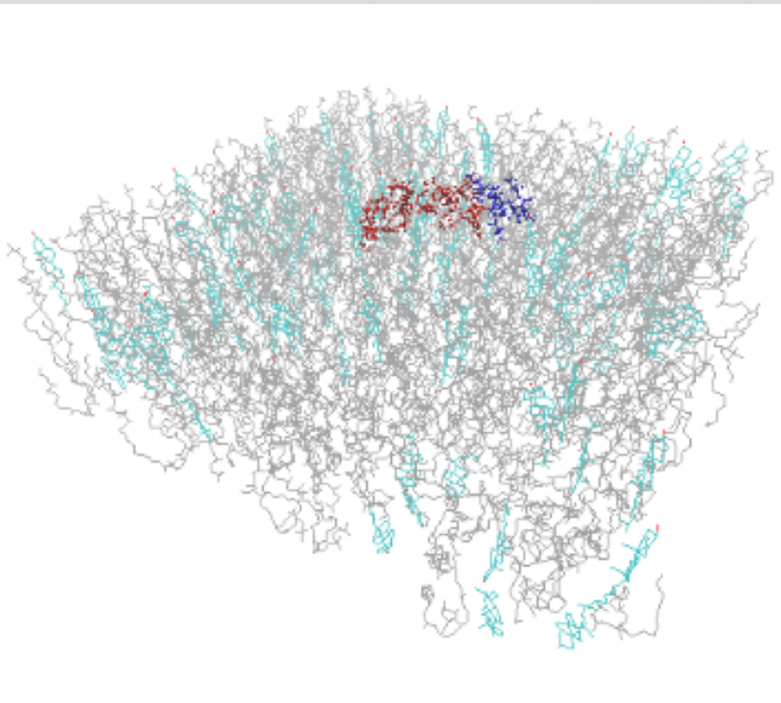
Proposed mechanisms for the lytic activity of melittin

- Melittin increases membrane permeability by partial penetration of the bilayer
- A canal structure by the aggregation of four transbilayer melittin molecules
- Aggregated melittin is involved in the solubilization of large lipid disks (leaving large holes in membrane)
- Binding of melittin to other membrane proteins is involved

Simulation systems

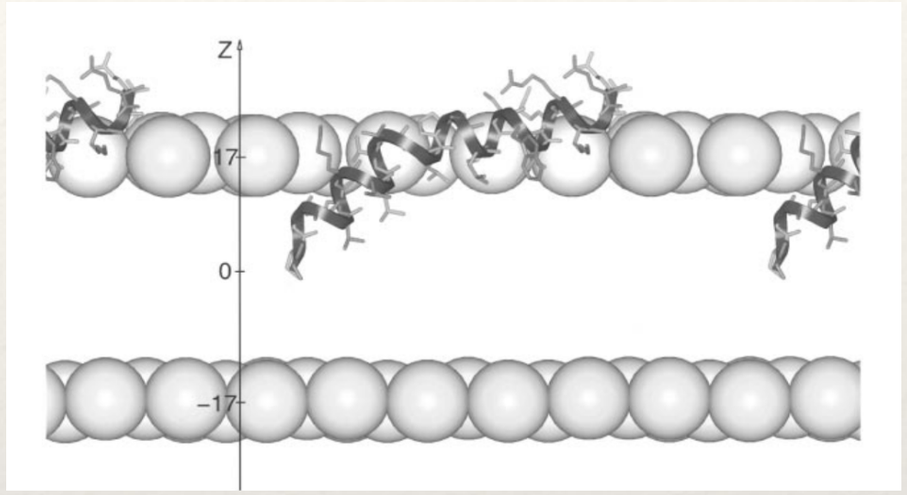
- 500 DMPC: 275K, 280K, … , 315K
- 500 DMPC + 1 Melittin: 275K, 280K, … , 315K
- 400 DMPC + 100 CHL: only 280K and 310K
- 400 DMPC + 100 CHL + 1 Melittin: only 280K and 310K

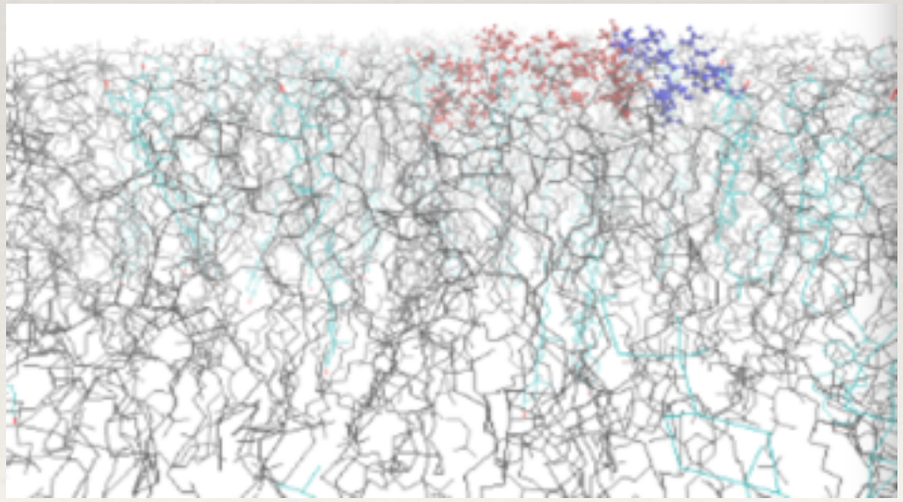
- Temperature scan for DMPC only system and DMPC+Melittin system for phase transition comparison.
- Observe the effects melittin and Cholesterol have on the dynamics of DMPC at both liquid and gel phases



Building our system

- Melittin is supposed to be deeply inserted into the upper layer of bilayer membrane
- Corresponding lipid molecules should be deleted to aviod heavy atoms overlap
- Hytrophilic residues contacting with water and hytrophobic residues in contact with lipid acyl chains
- Helix should still be oriented parallel to membrane-water interface





Simulation

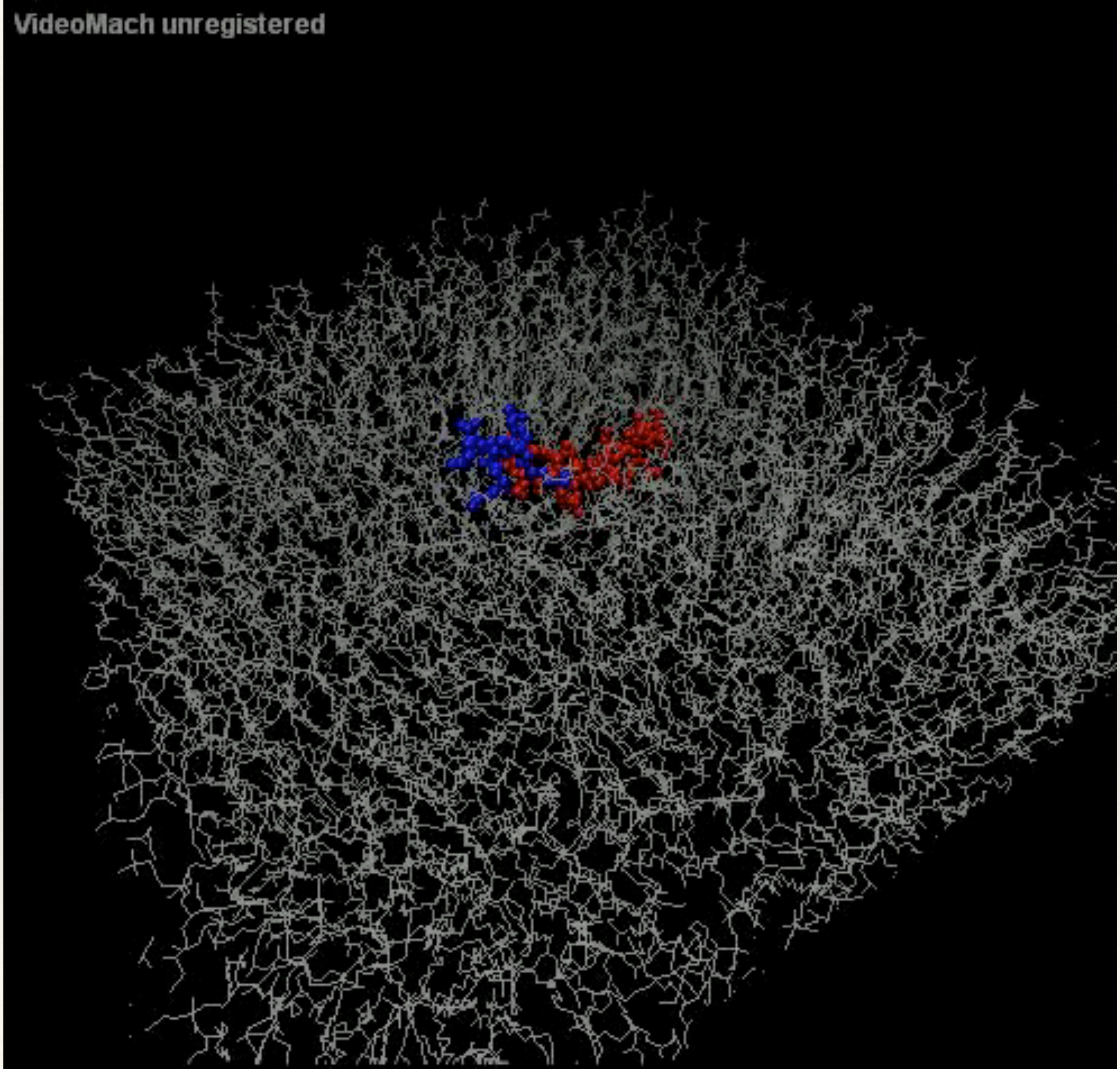
Software: Gromacs 5.0.1 double precision + mpi
Force Field: CHARMM 36

- Minimization (Relax the structure)

- Equilibration (Generate different temperatures here)

- Production (more than 60 ns)

Here is the trajectory of system with melittin at 315K



Cooperators' experimental results

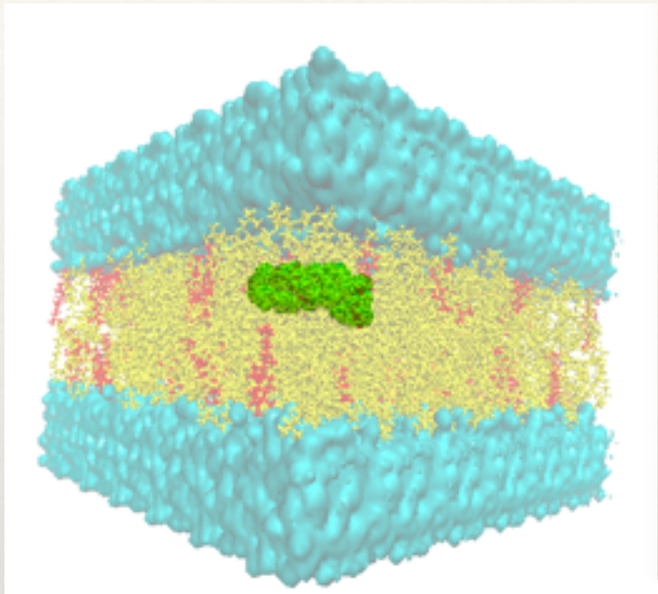
- At 7 °C and 37 °C, DMPC dynamics are very different from each other. Adding Melittin to DMPC, the dynamics are affected at both 7 °C and 37°C.

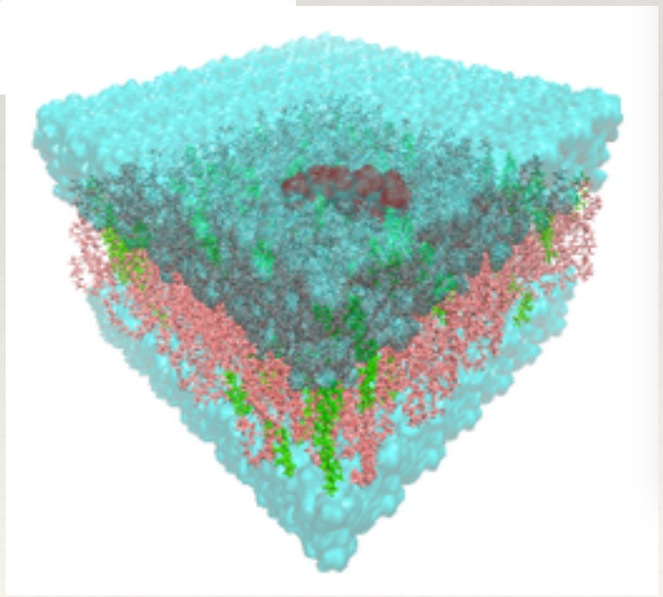
- At 7 °C and 37 °C, DMPC+CHL dynamics are different from each other. Adding Melittin to DMPC+CHL, the dynamics at liquid phase (37 °C) is not affected.

- Temperature scan for DMPC, there is phase transition. Adding Melittin to DMPC, there is no phase transition for temperature scan.

Results

- Area Per Lipid
- Order Parameter
- Gauche Conformation Fraction
- RMSF

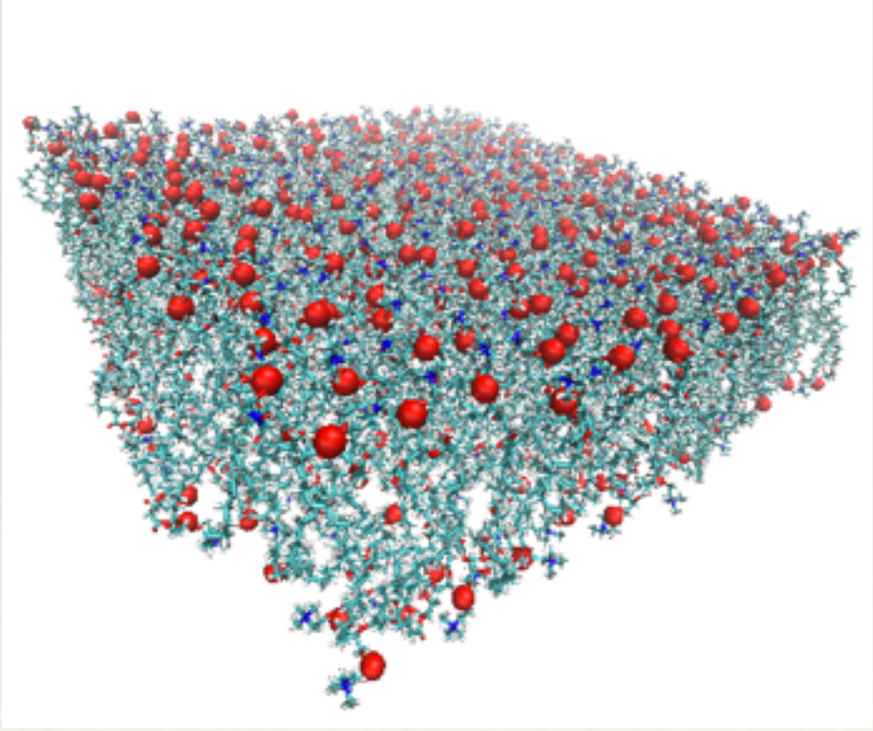




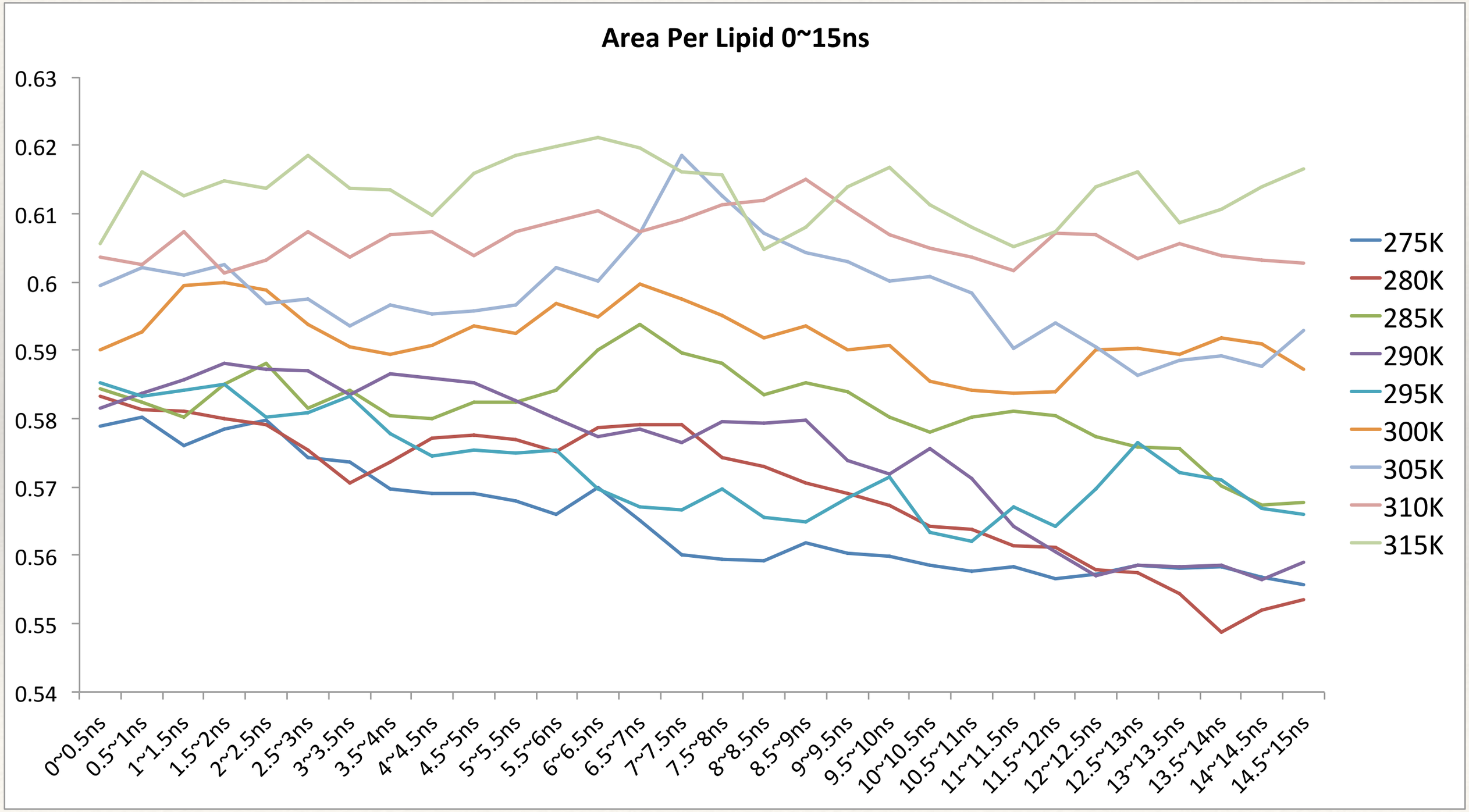
I. Area Per Lipid

Just as the name implies, Area Per Lipid is the average area each lipid possesses in the leaflet of DMPC bilayer. The equation below is how we calculated it.

$$Area\ Per\ Lipid = \frac{Area\ of\ the\ laeflet}{Lipid\ number\ pear\ leaflet} = \frac{X \cdot Y}{250}$$



Area Per Lipid

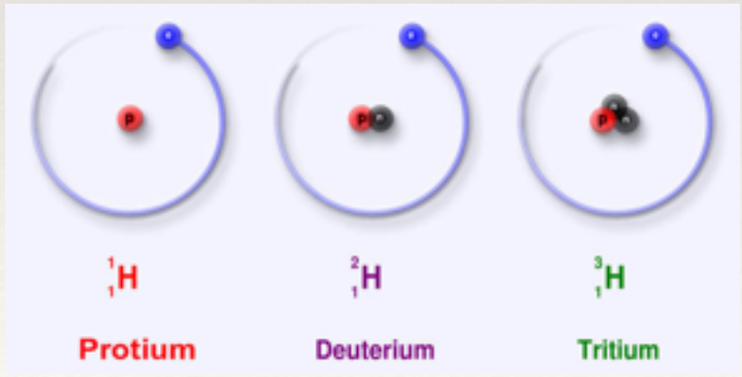


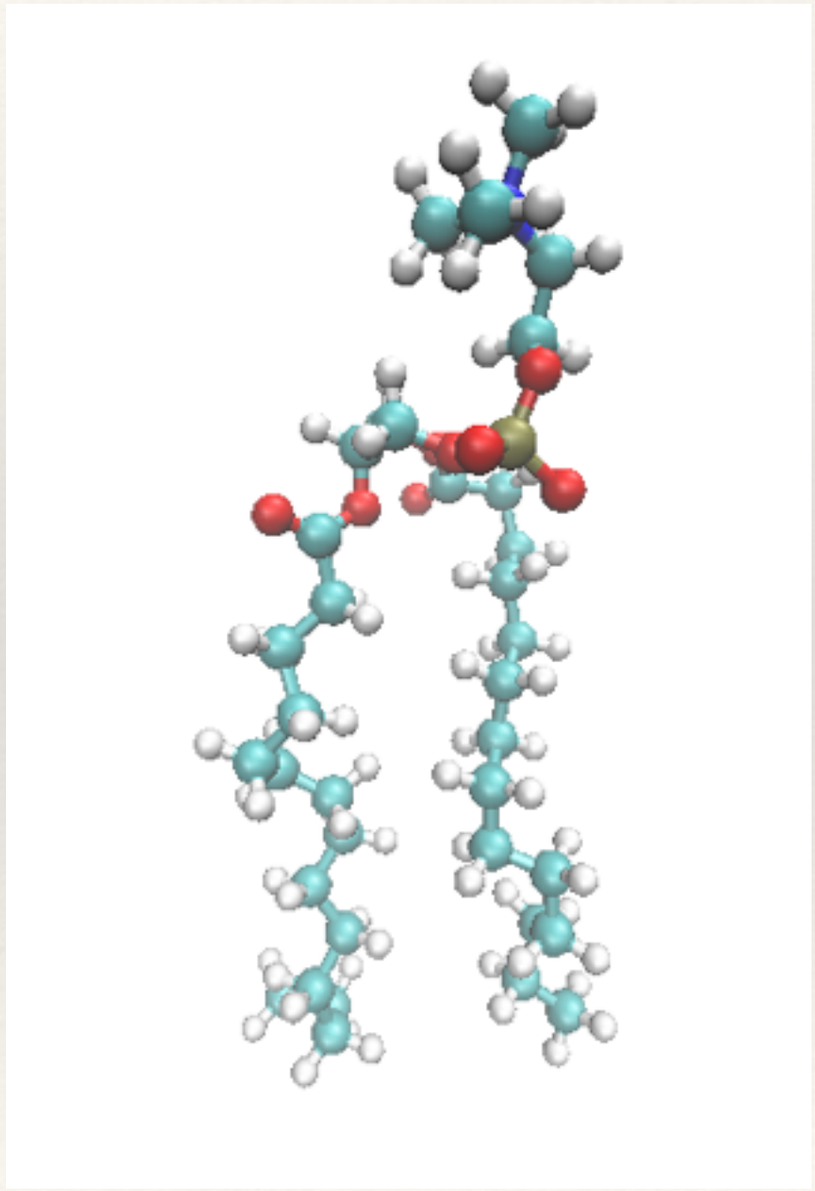
II. Order Parameter

Lipid order parameter is a measure of the orientational alignment of the C–D (carbon-deuterium) bond and is defined as

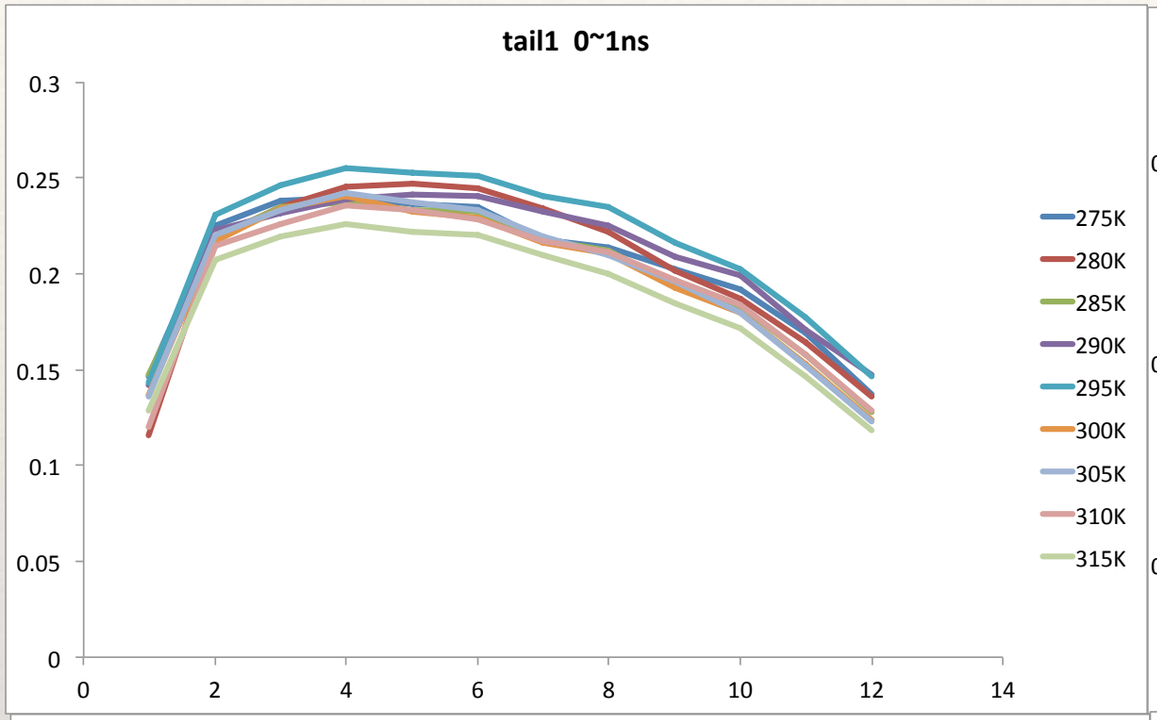
$$S = \left\langle \frac{3\cos^2\Theta - 1}{2} \right\rangle$$

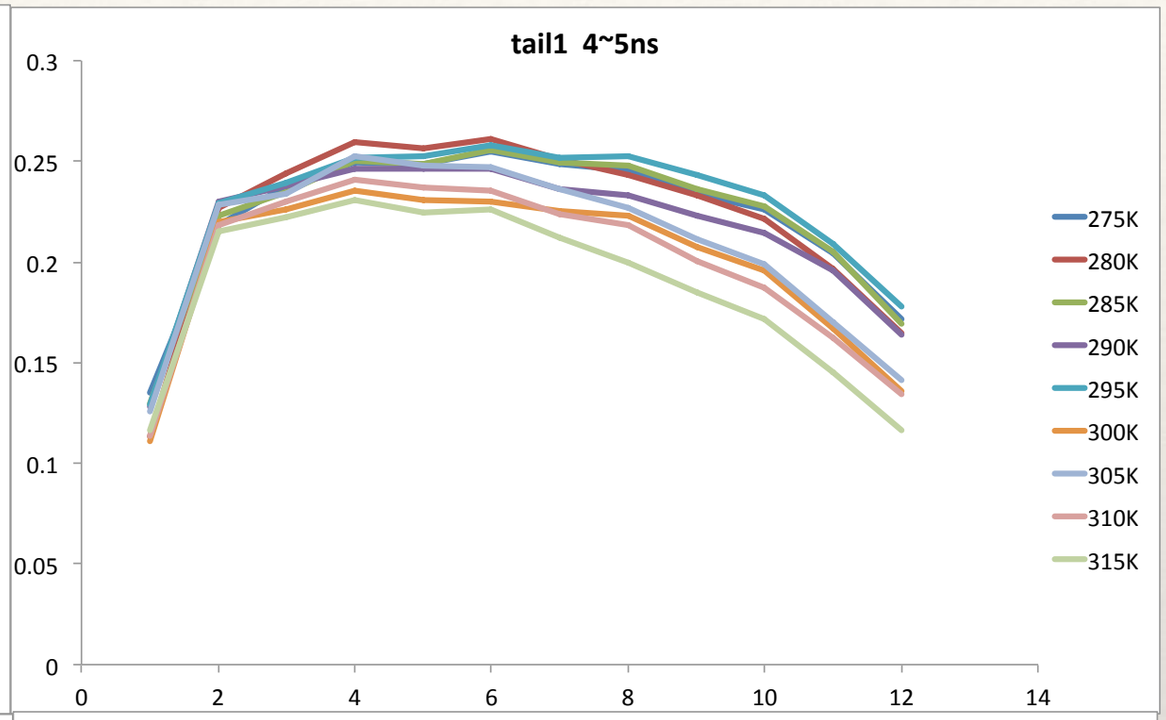
where Θ is the instantaneous angle between the director of the C-D bond and the bilayer normal. By observing the order parameter of the two tails of DMPC, we can have a general idea of how the DMPCs are aligned in the DMPC bilayer.

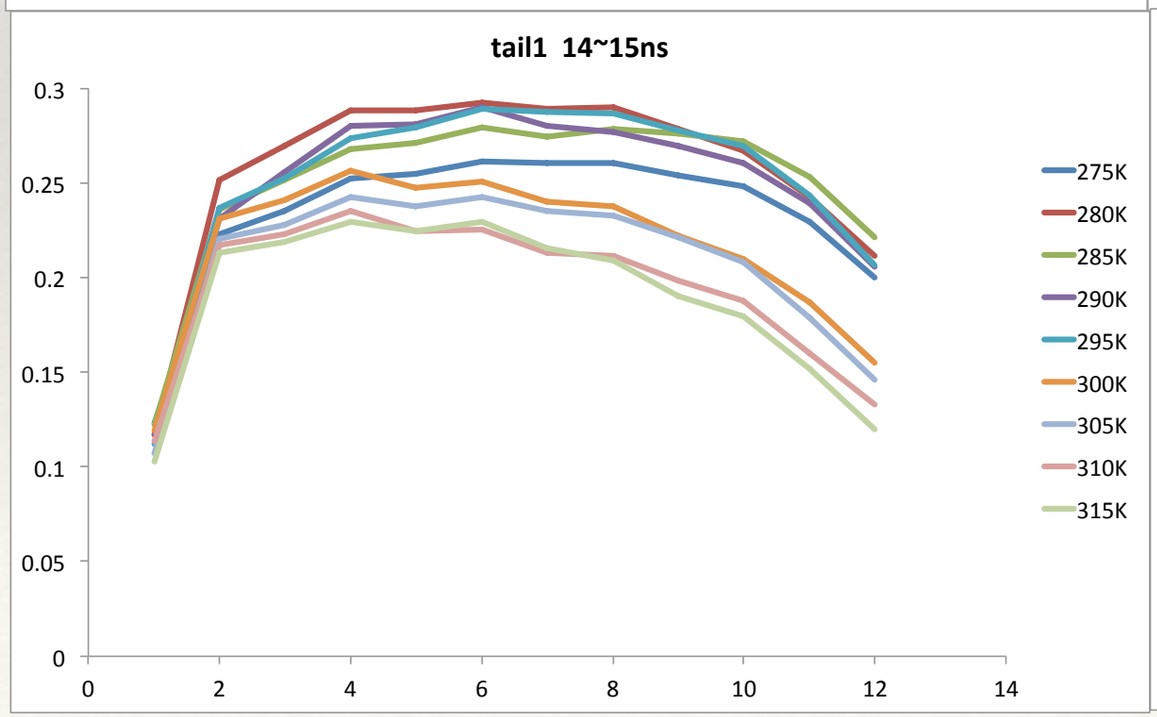


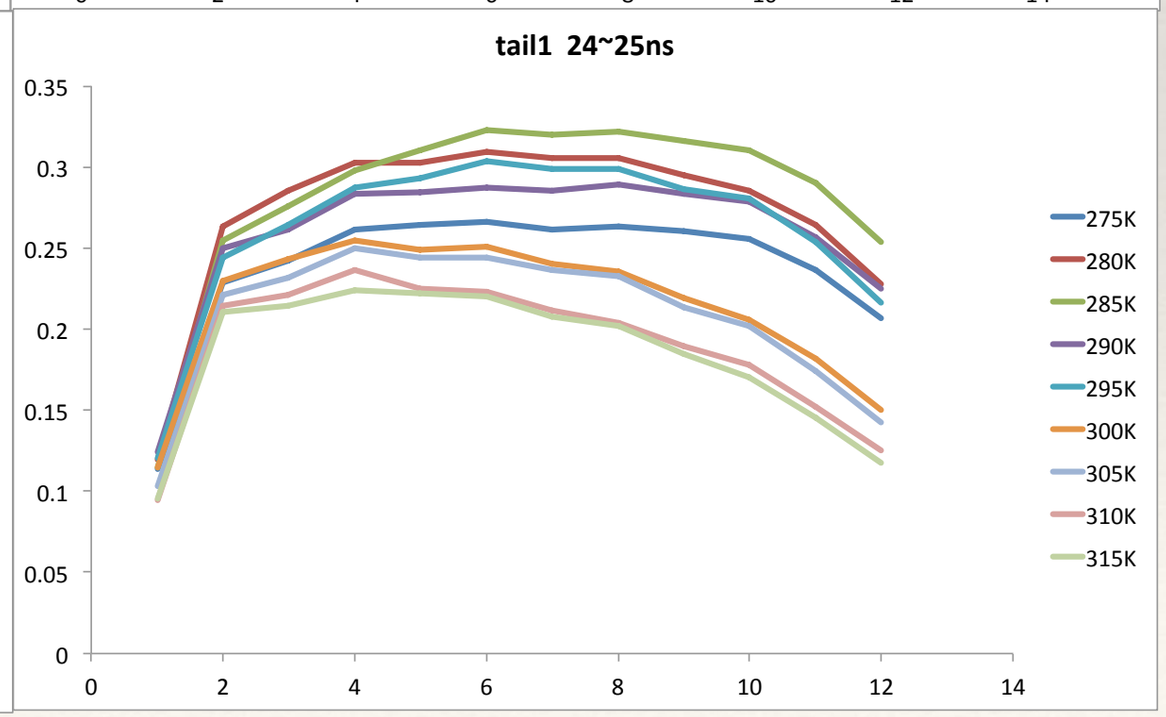


Order Parameter - tail 1

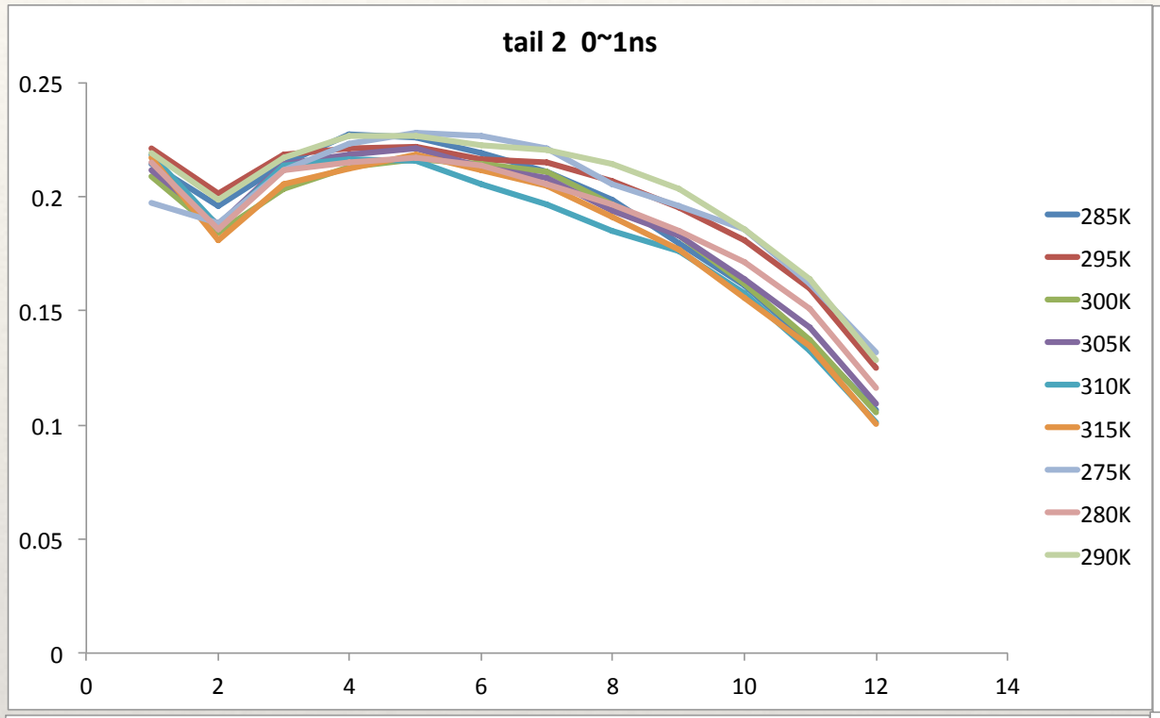


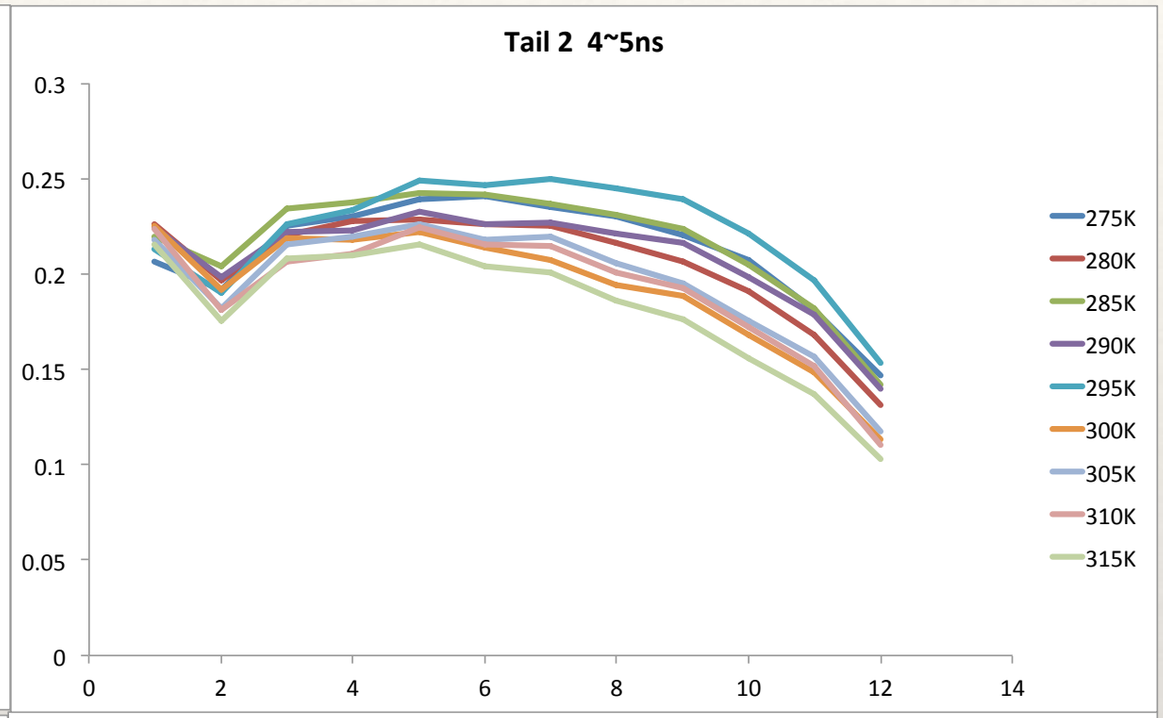


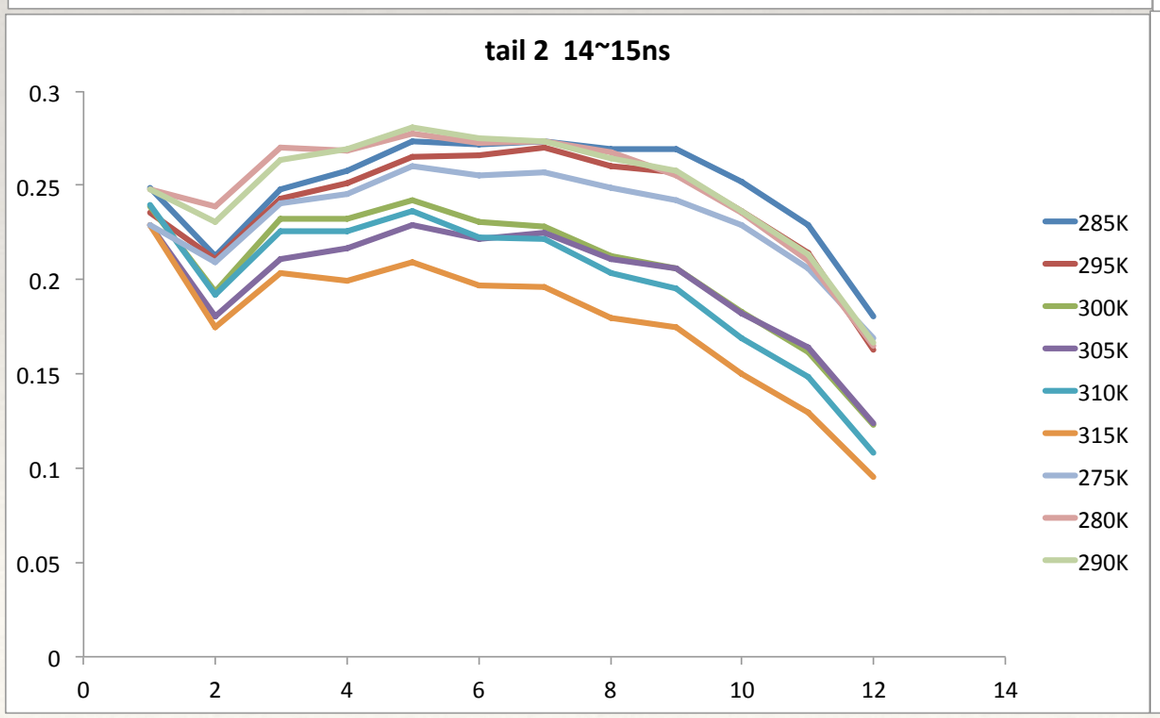


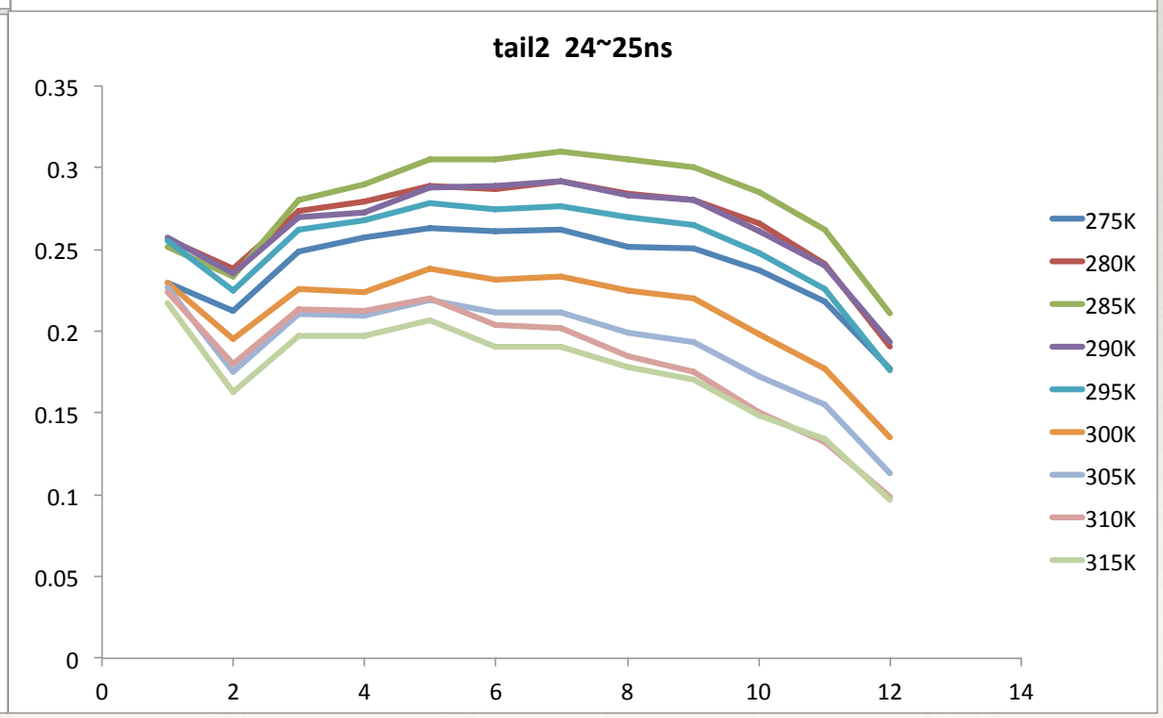


Order Parameter - tail 2





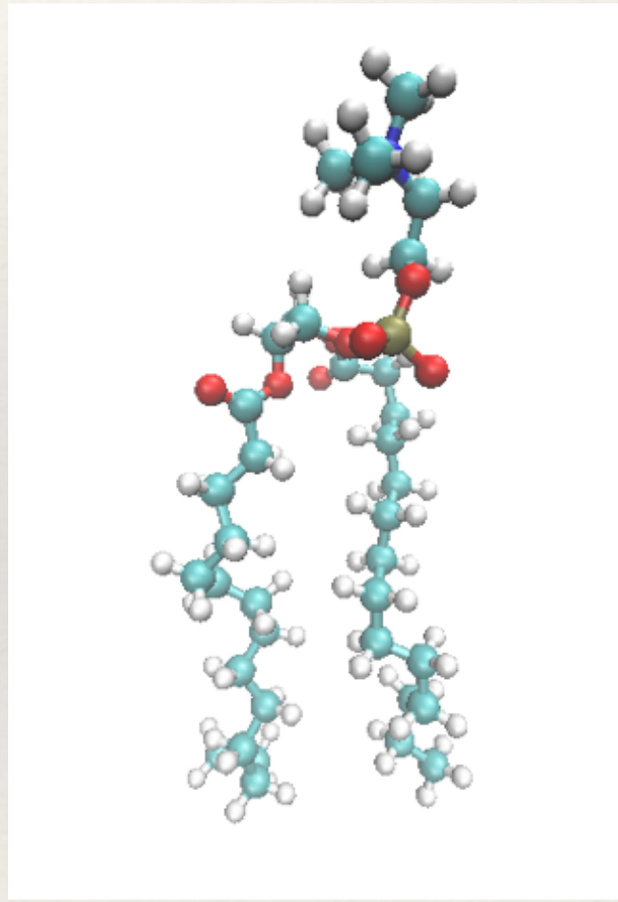


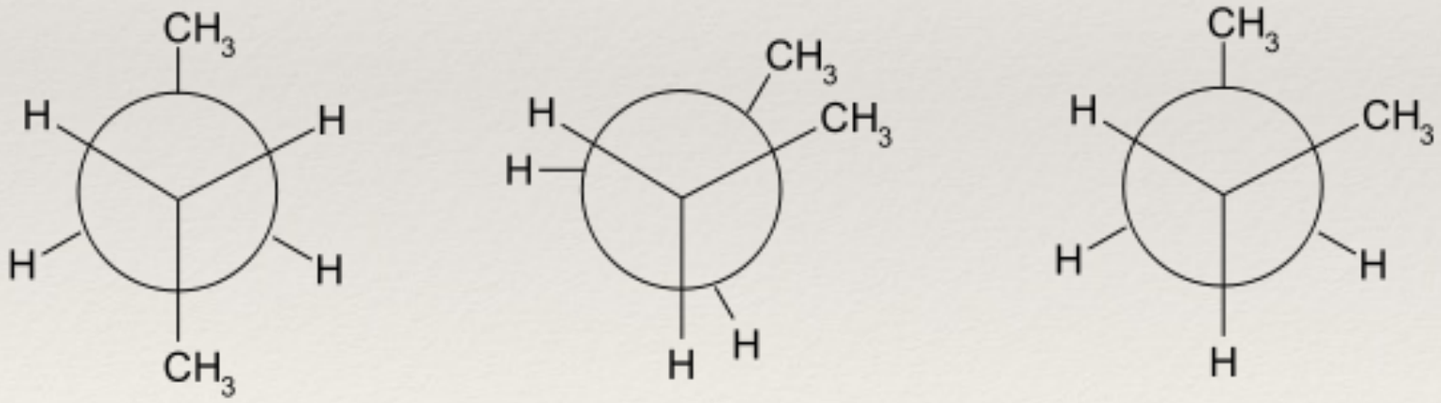


III. Gauche Conformation Fraction

In stereochemistry, The term “gauche” refers to conformational isomers(conformers) where two vicinal groups are separated by a 60° torsion angle.

Figure below shows the trans(left) and gauche(right) rotamers of butane. The two methyl groups can be in an anti-bonding relationship, or offset at sixty degree dihedral angles.

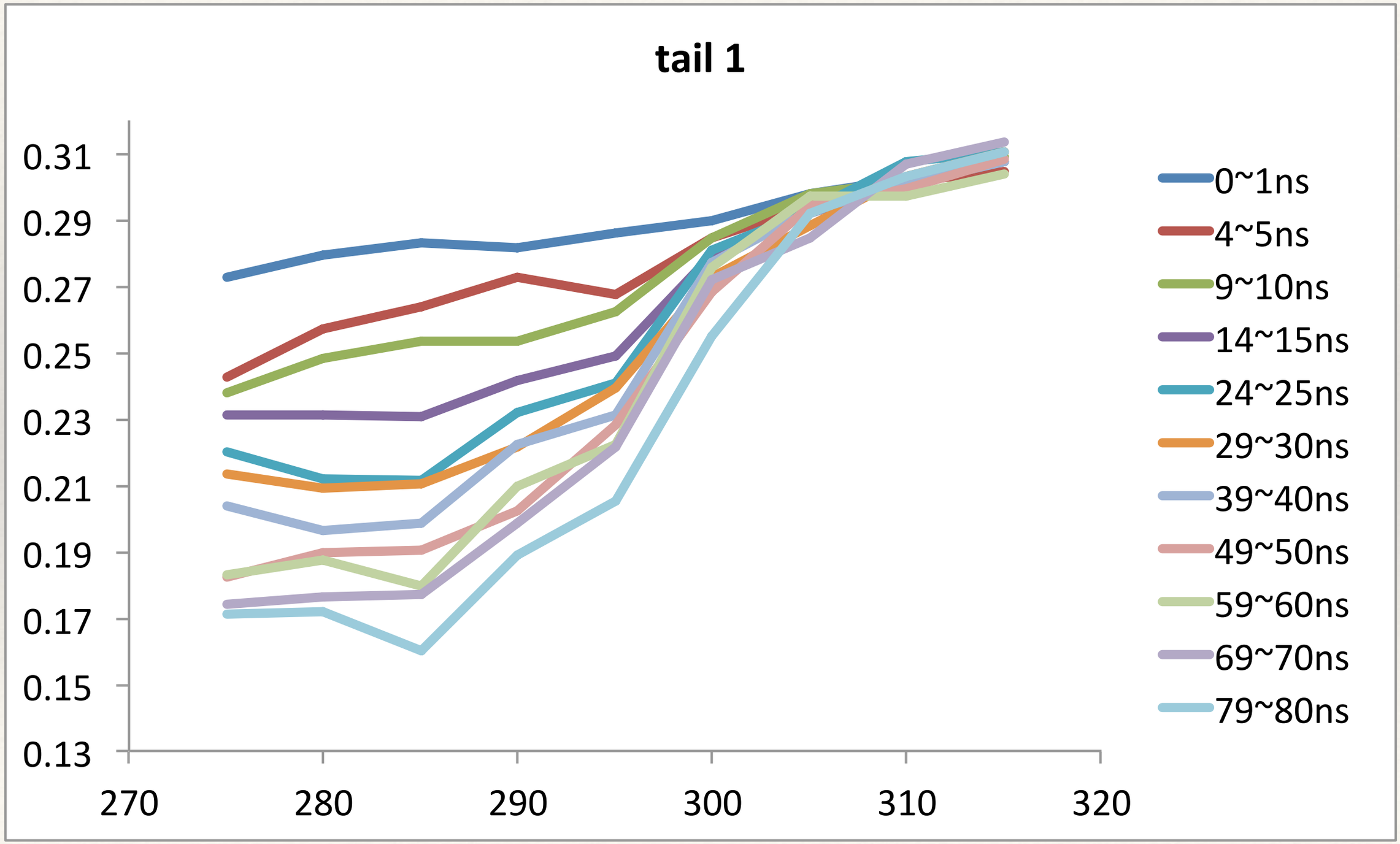




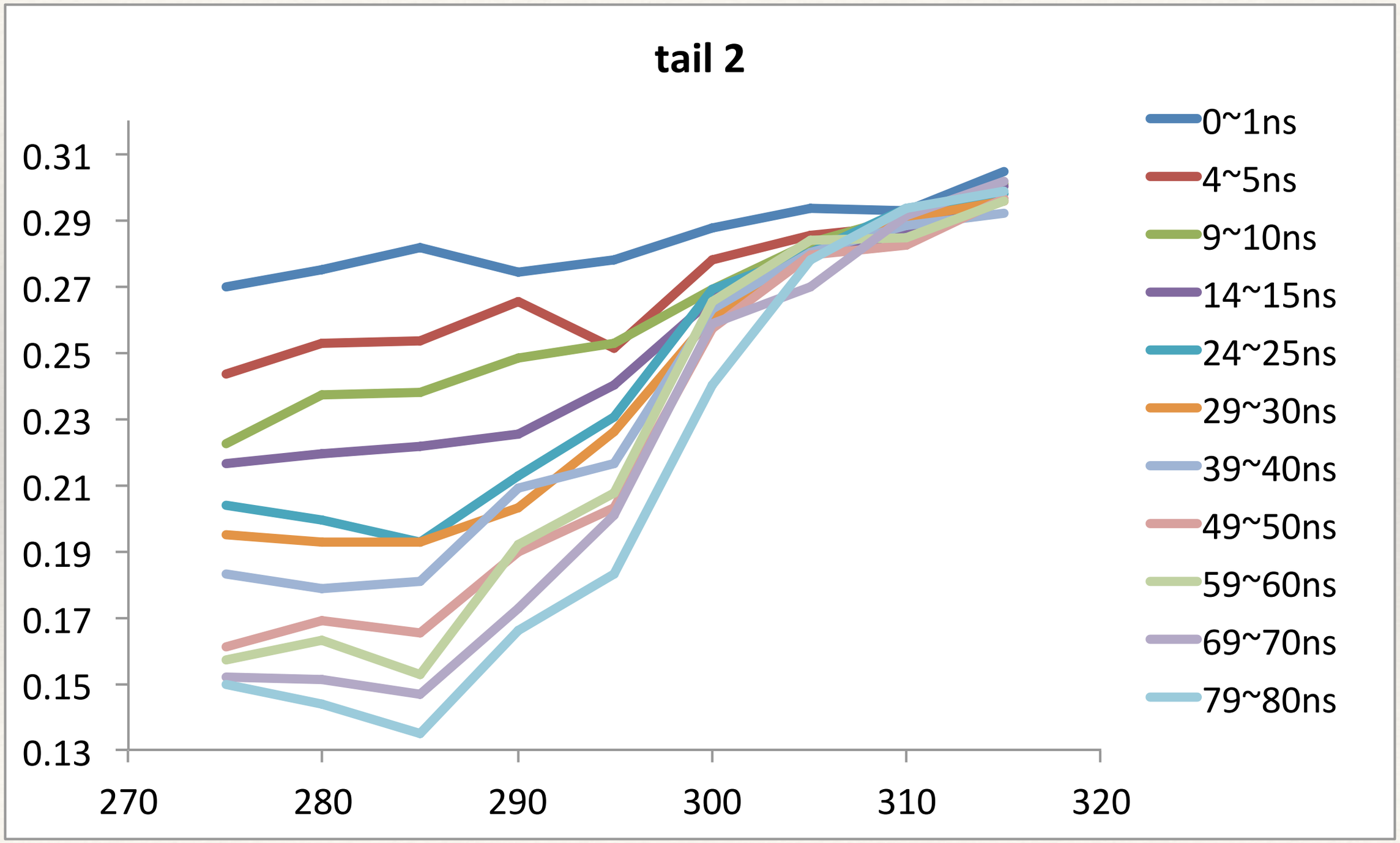
Trans

Gauche

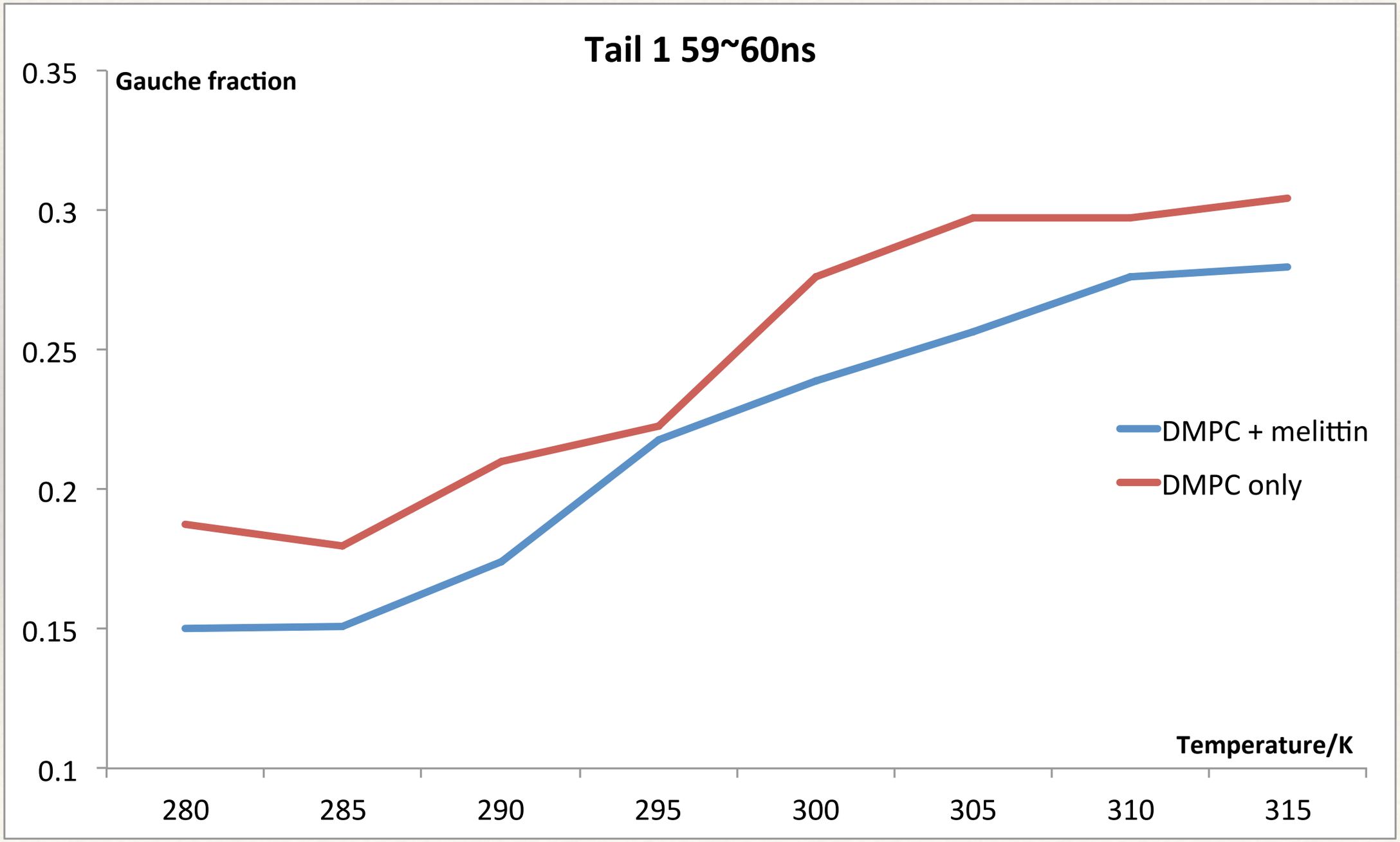
Gauche Conformation Fraction



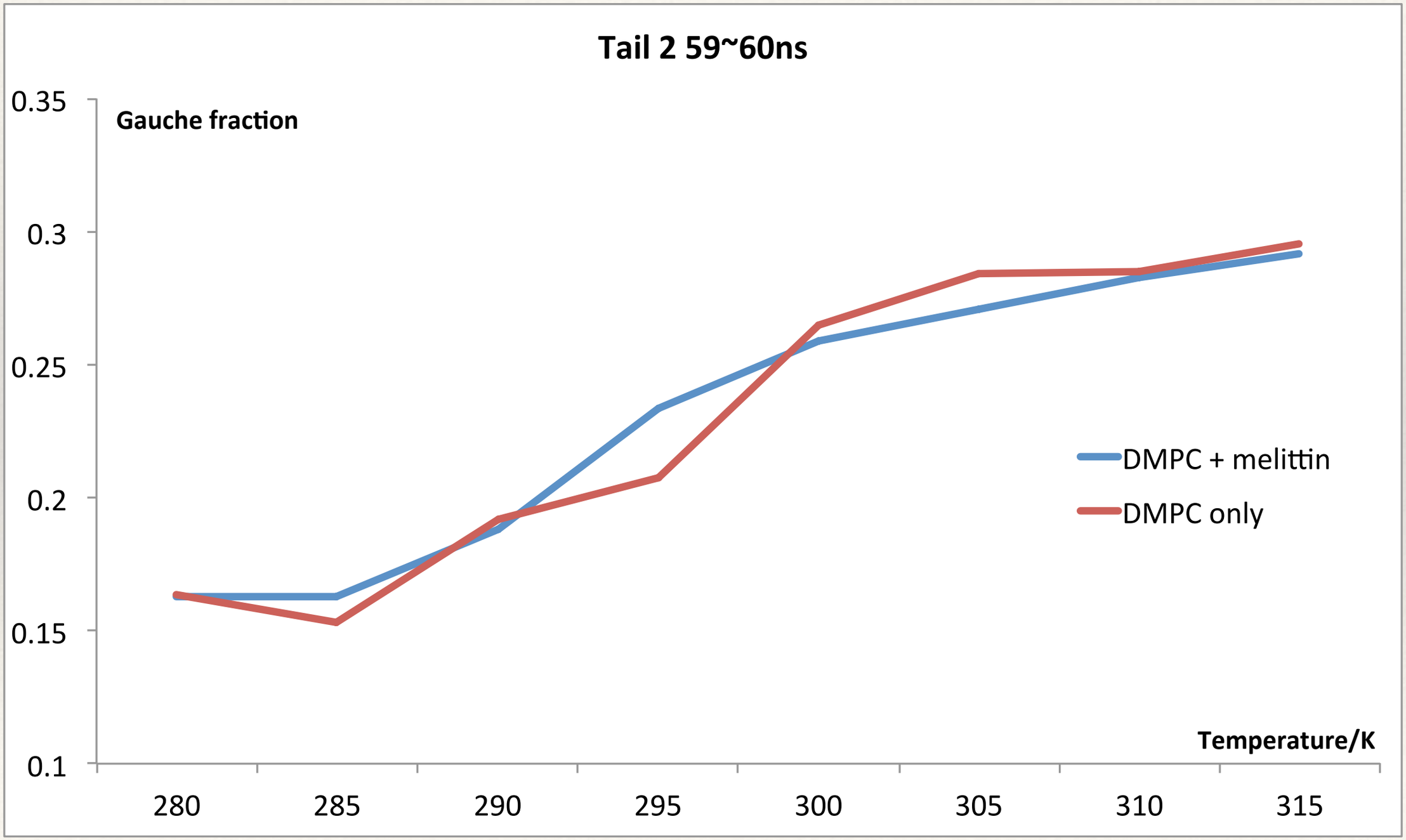
Gauche Conformation Fraction



Gauche Conformation Fraction



Gauche Conformation Fraction

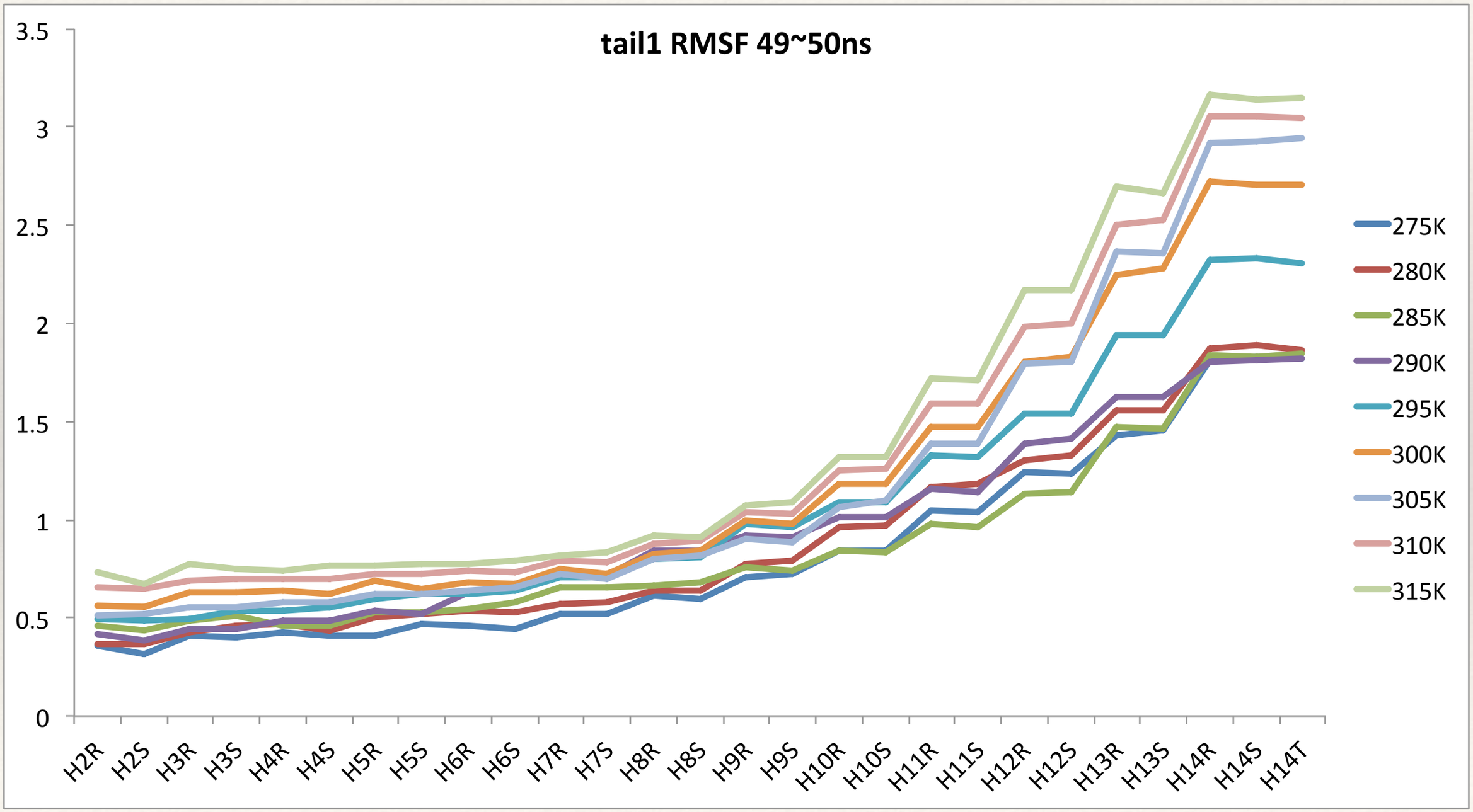


IV. RMSF

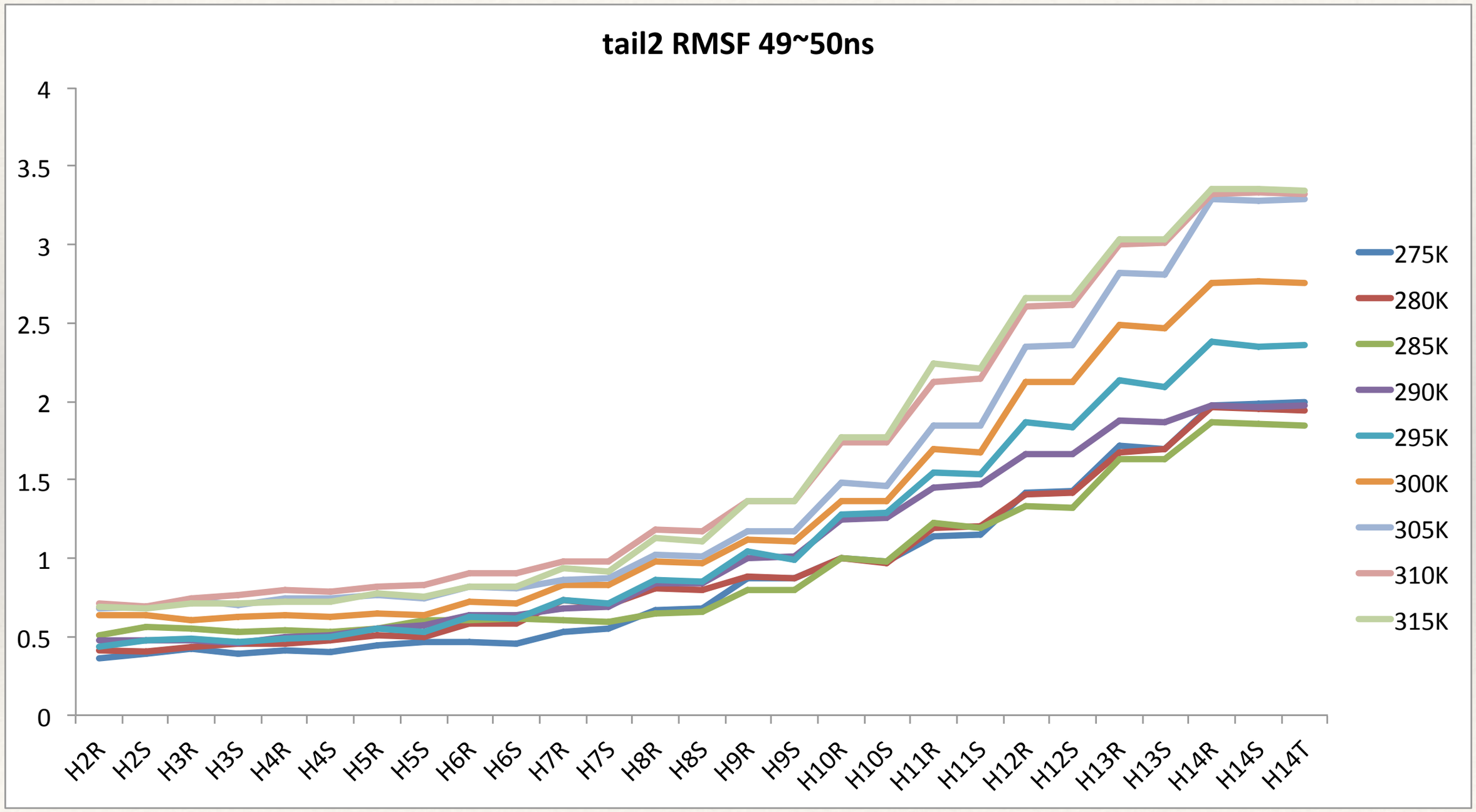
RMSF stands for root mean square fluctuation, which shows the mobility of atoms. The RMSF of all the hydrogen atoms attached to the carbon in the two tails are calculated. From the head to the tail, RMSF significantly increases, which is consistent with the model experimentalists used. A big gap around 295K appears, which represents the phase transition.

$$MSF = \sqrt{\frac{1}{T}\sum_{t_j=1}^{T}(x_i(t_j) - \tilde{x}_i)^2}$$

RMSF


tail1 RMSF 49~50ns
3.5
3
2.5
2
1.5
1
0.5
0
H2R
H2S
H3R
H3S
H4R
H4S
H5R
H5S
H6R
H6S
H7R
H7S
H8R
H8S
H9R
H9S
H10R
H10S
H11R
H11S
H12R
H12S
H13R
H13S
H14R
H14S
H14T
275K
280K
285K
290K
295K
300K
305K
310K
315K

RMSF


tail2 RMSF 49~50ns
4
3.5
3
2.5
2
1.5
1
0.5
0
H2R
H2S
H3R
H3S
H4R
H4S
H5R
H5S
H6R
H6S
H7R
H7S
H8R
H8S
H9R
H9S
H10R
H10S
H11R
H11S
H12R
H12S
H13R
H13S
H14R
H14S
H14T
275K
280K
285K
290K
295K
300K
305K
310K
315K

Thanks for your attention!

Q & A