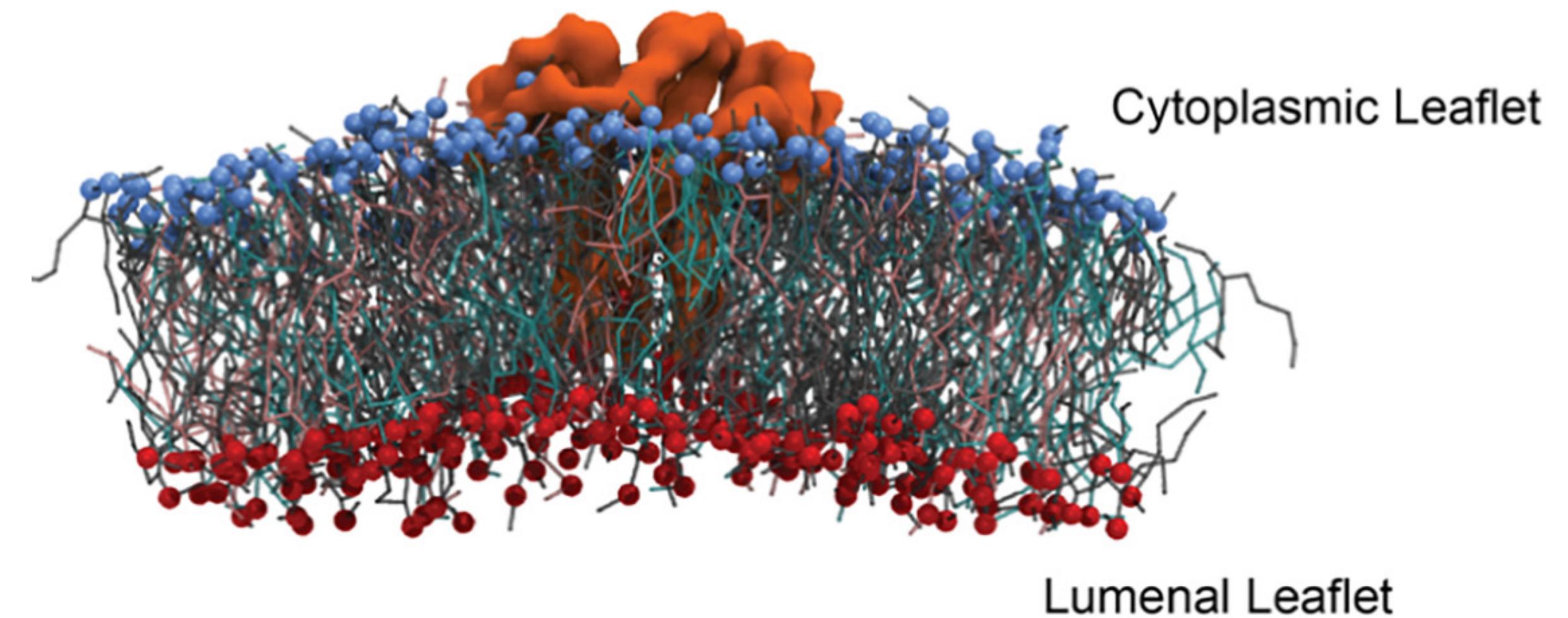
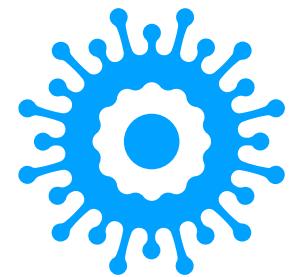


# Identifying how the E protein of SARS-CoV-2 bends unsaturated membranes in Coarse-Grained Molecular Dynamics simulations

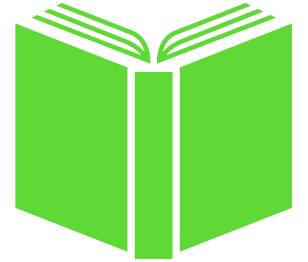
Jesse Sandberg & Grace Brannigan



# Agenda



E protein 101



Review of initial findings

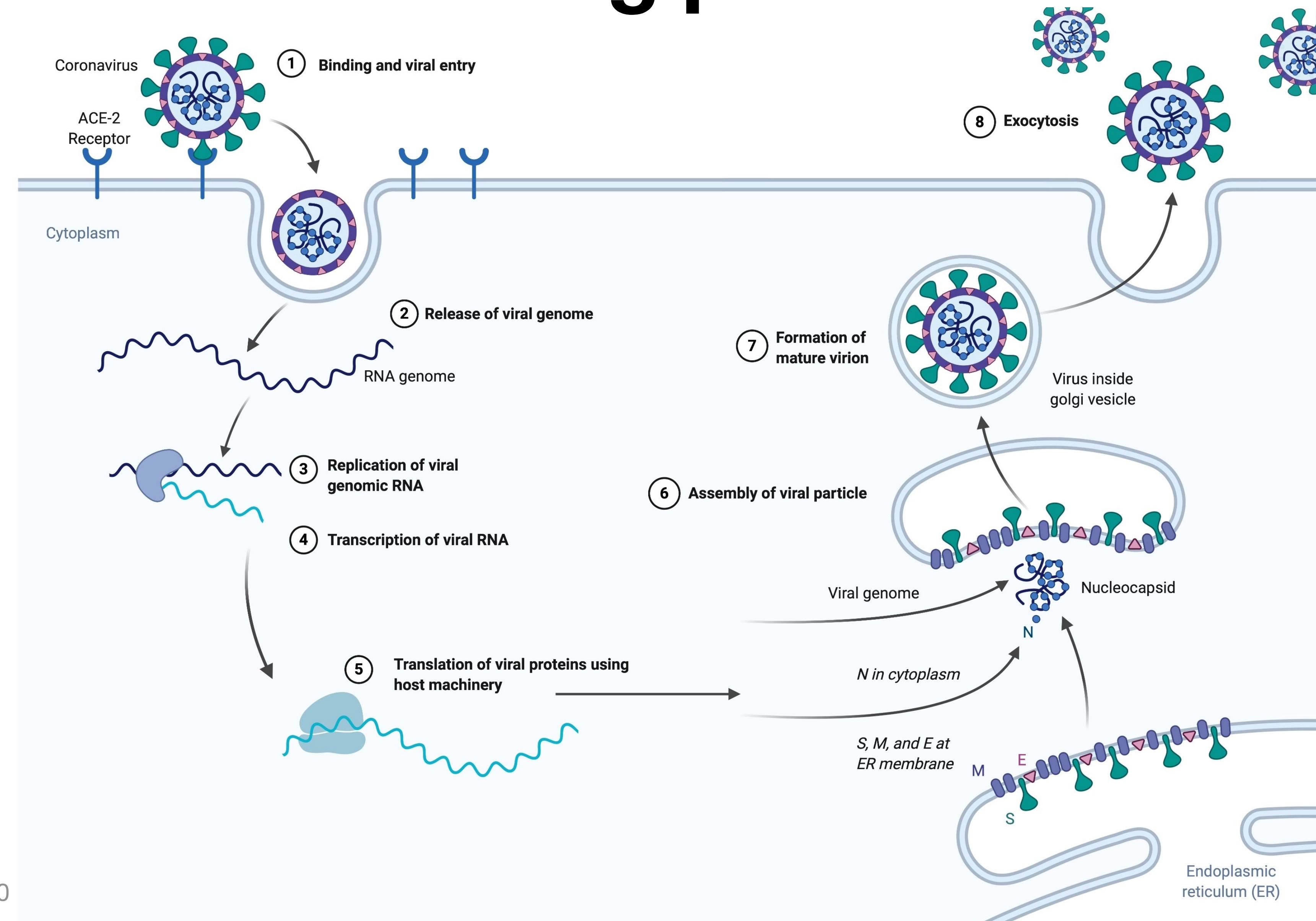


Bug (feature?) hunt

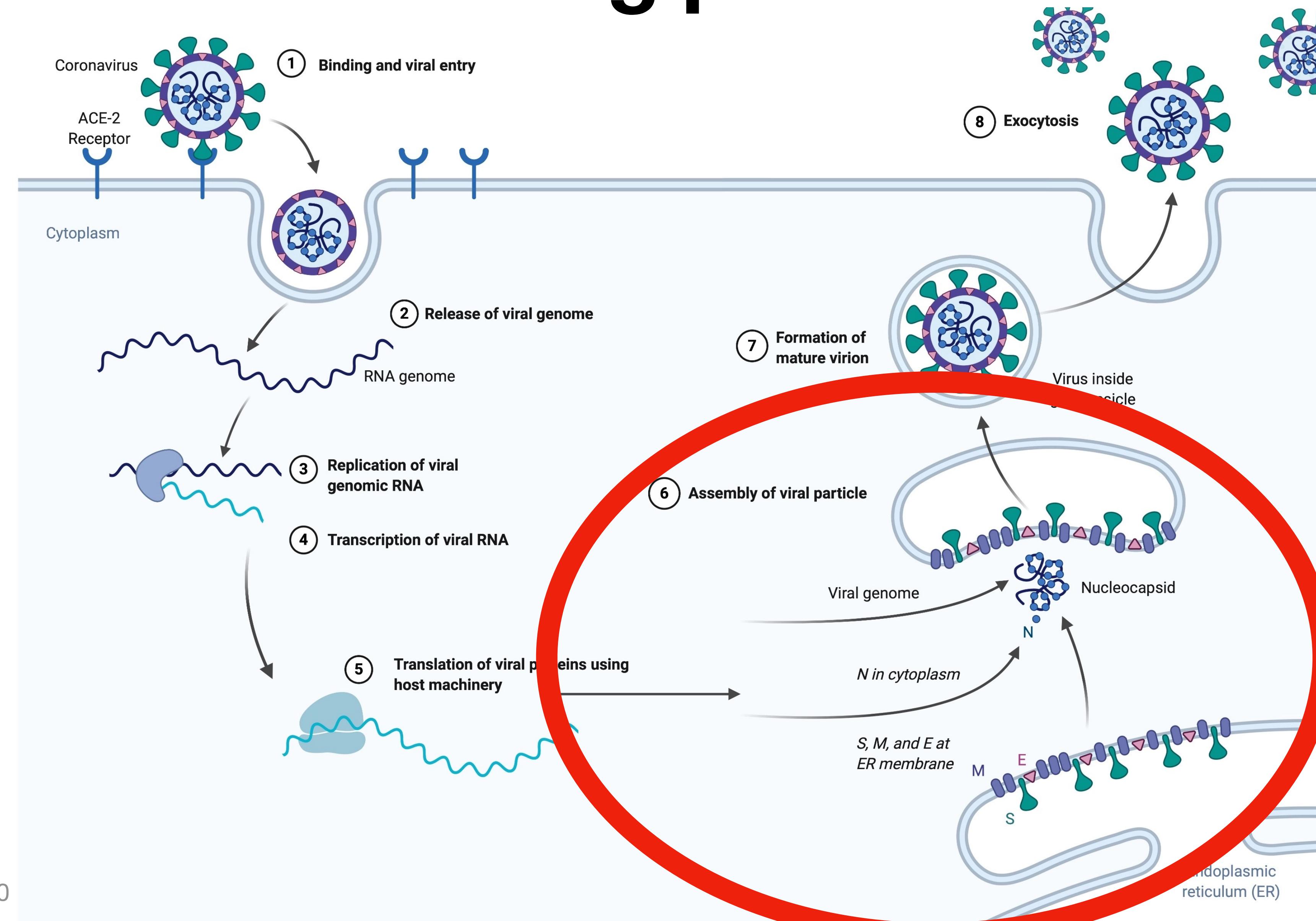


Conclusion

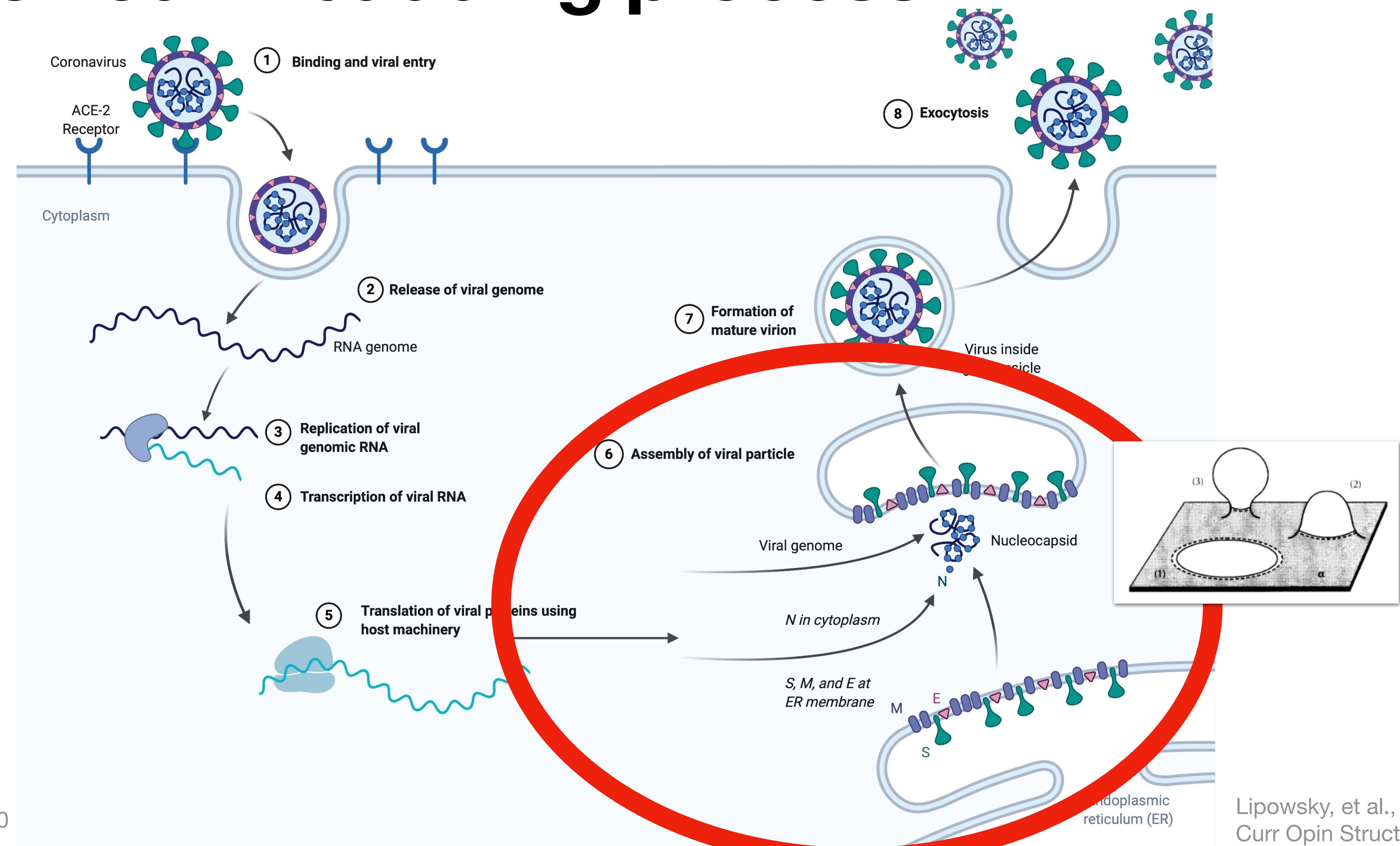
# E involved in budding process



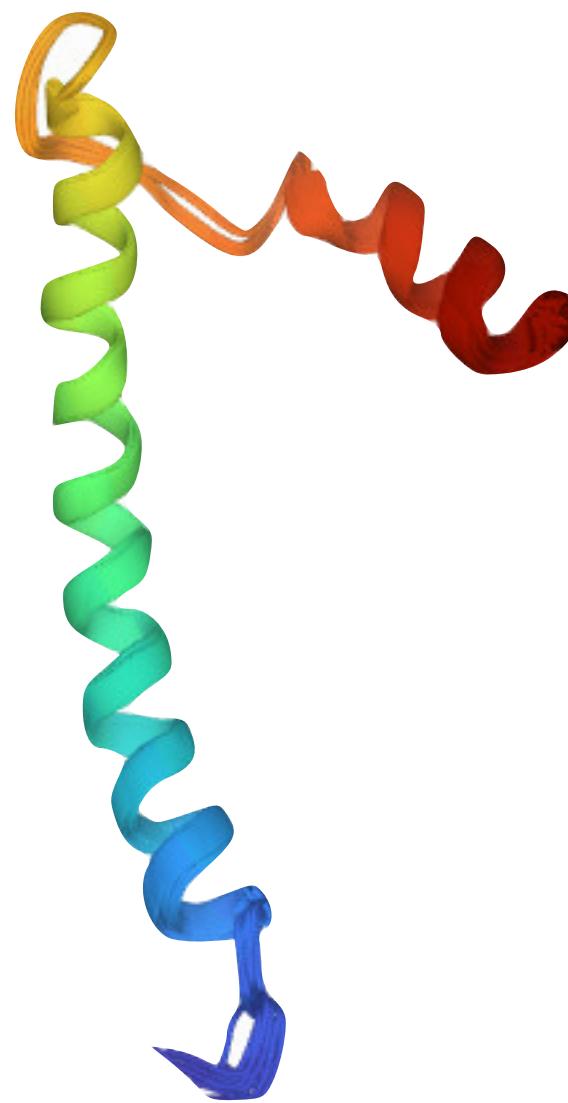
# E involved in budding process



# E involved in budding process

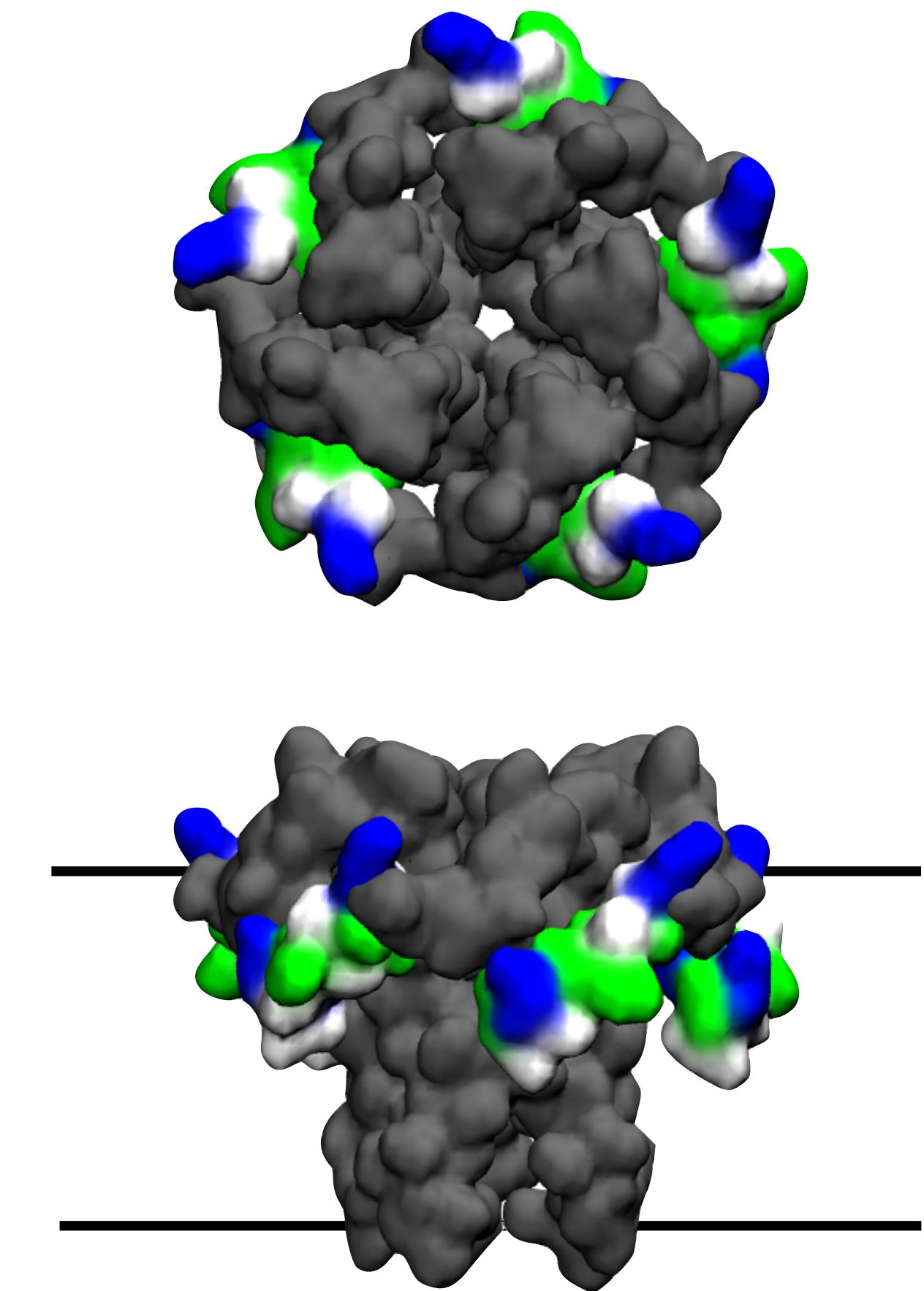
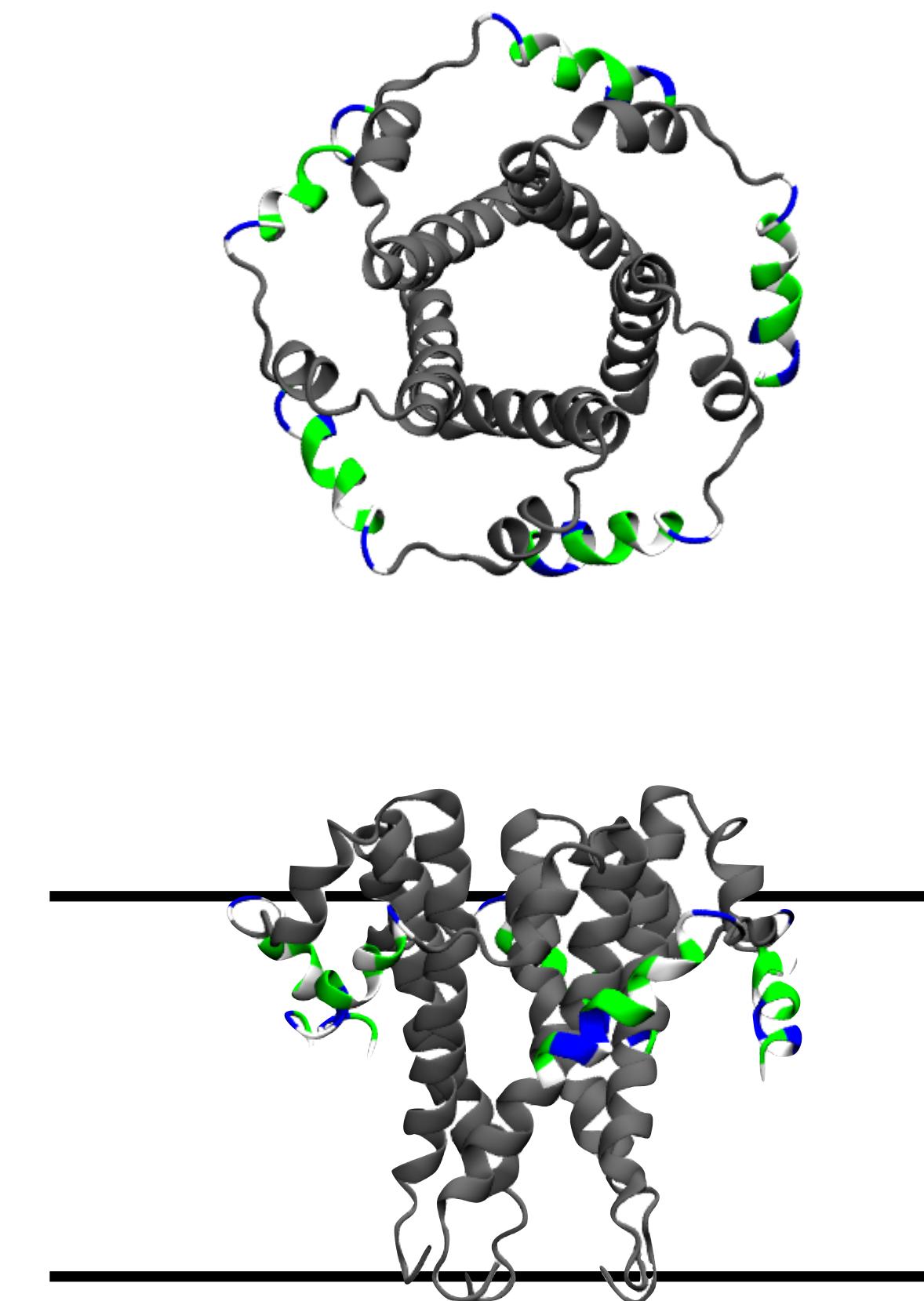
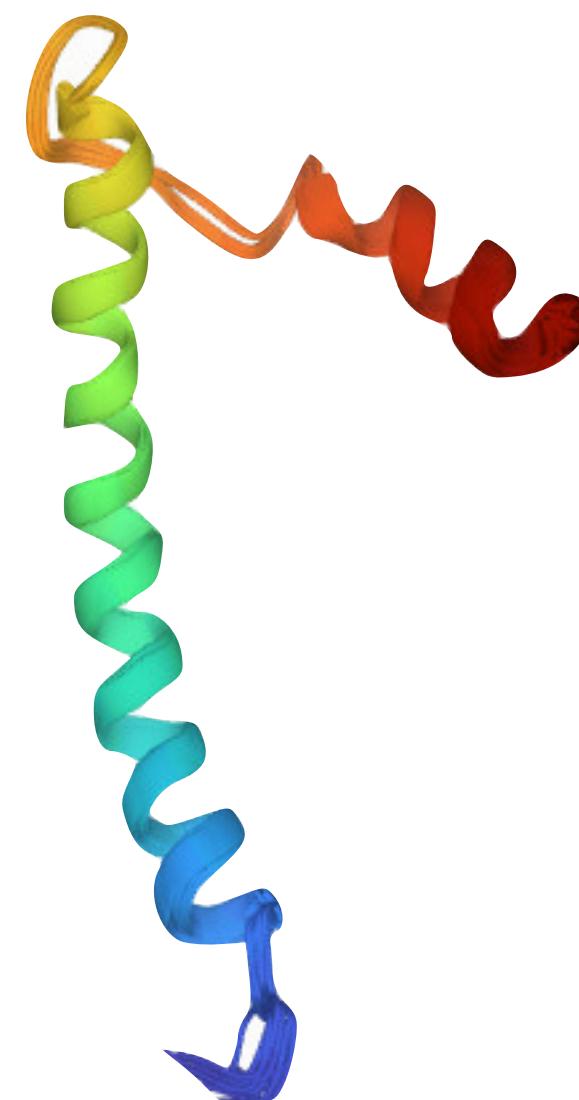


# E forms pentameric ion channel



PDB ID: 2MM4

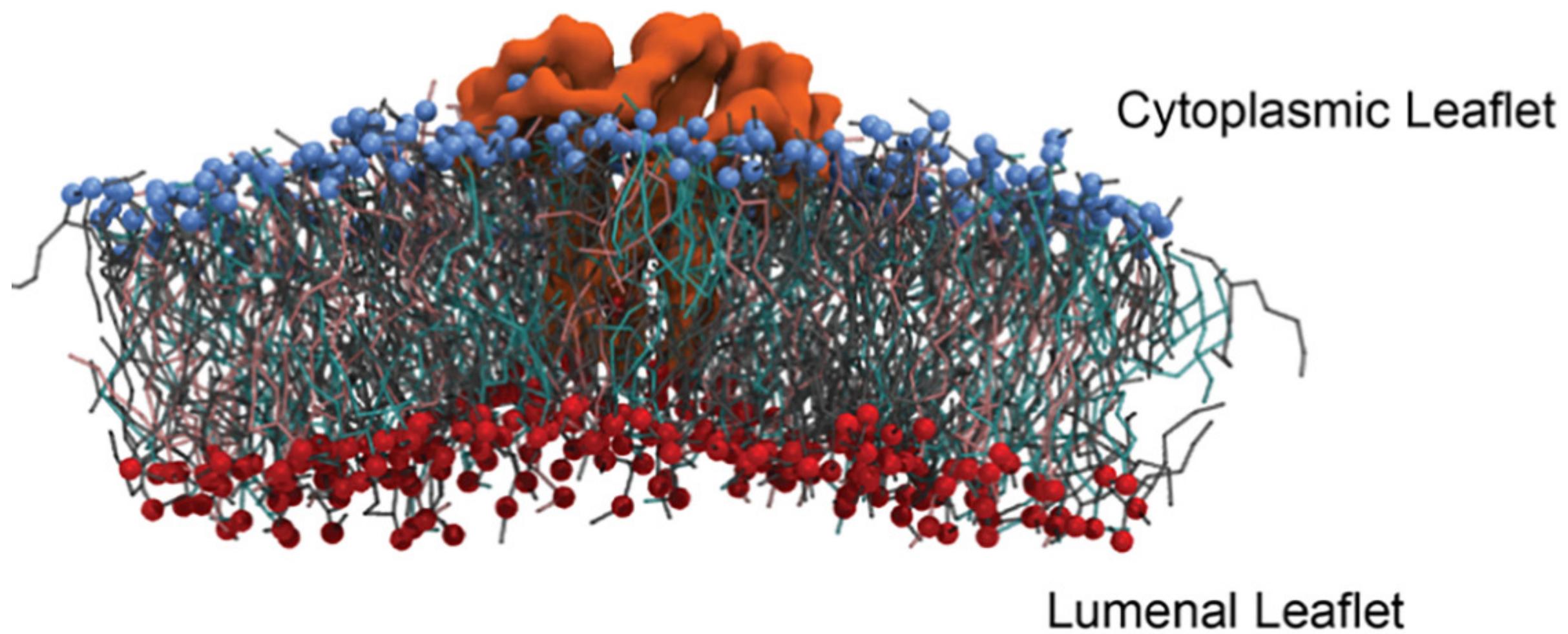
# E forms pentameric ion channel



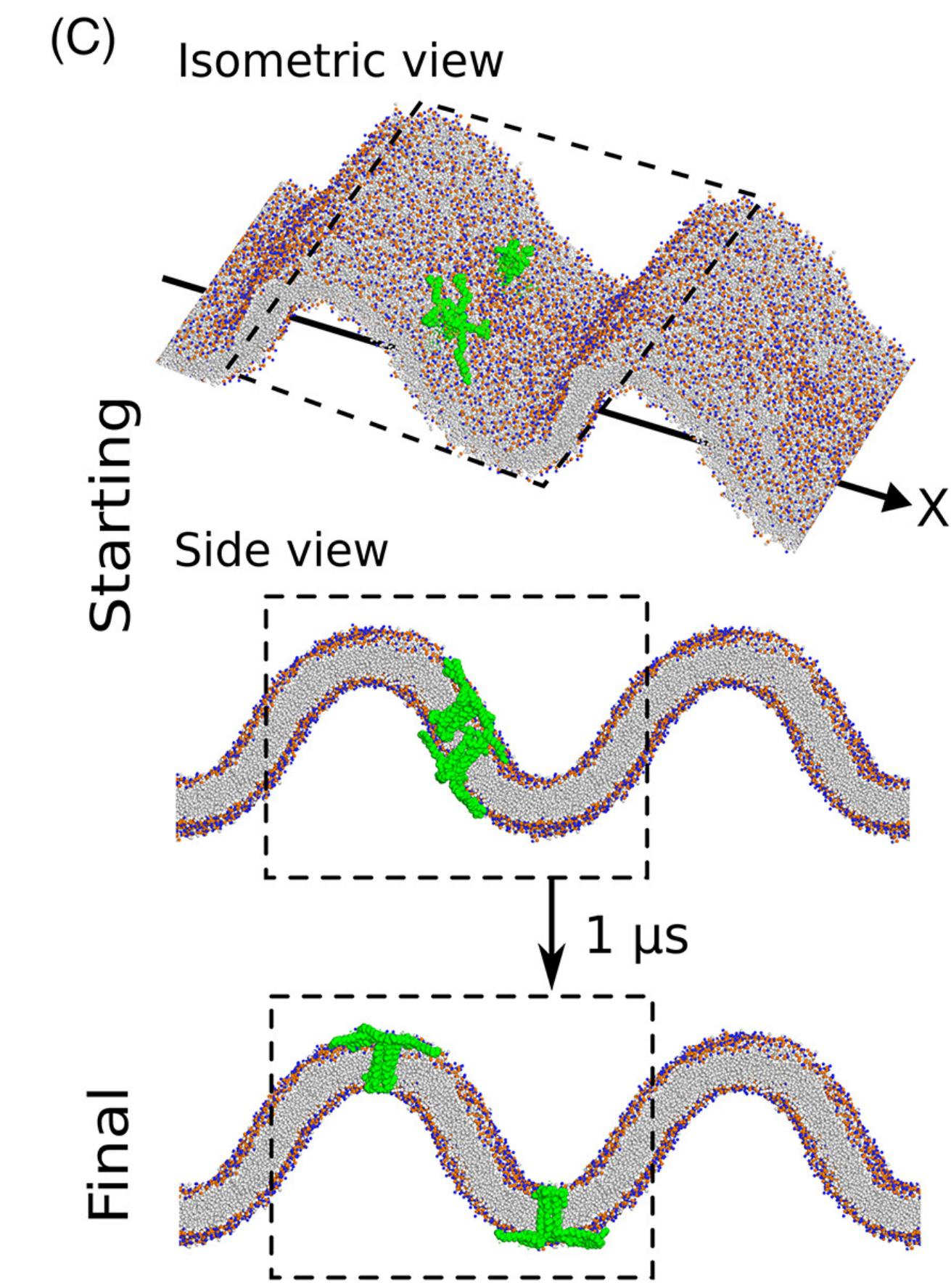
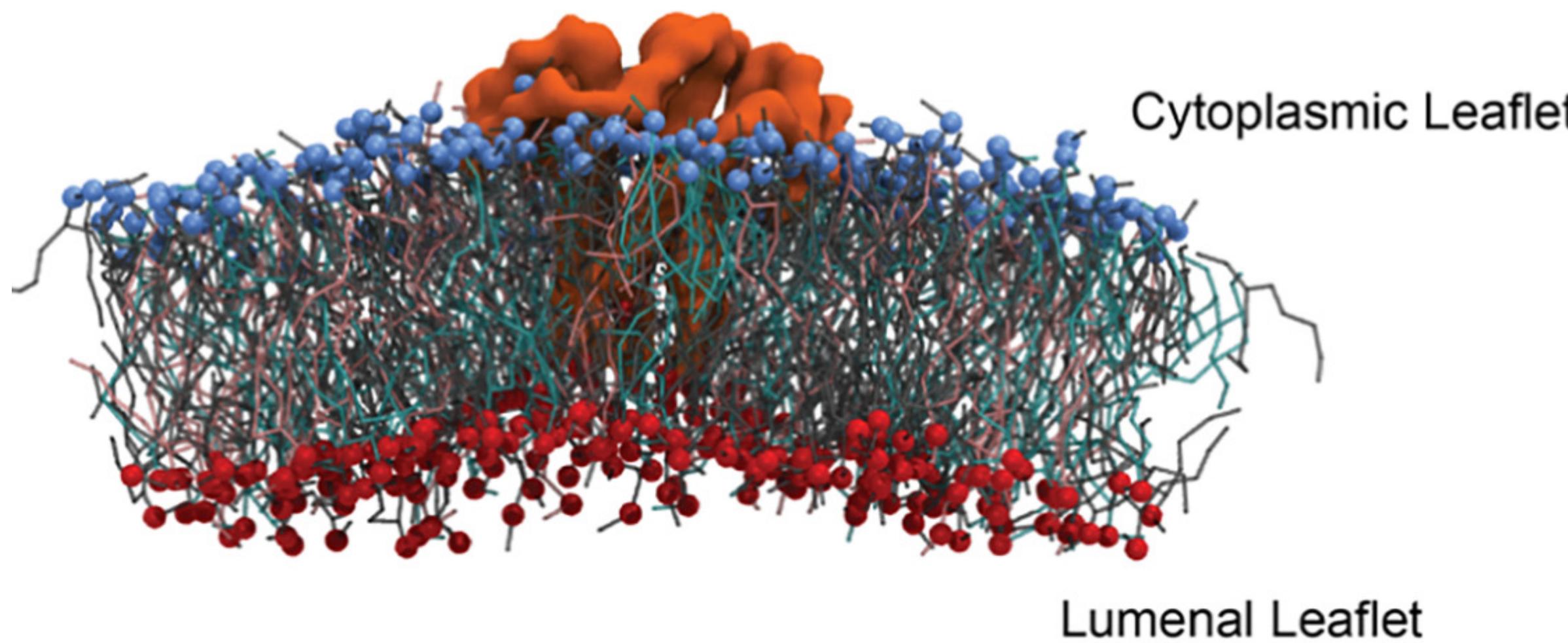
PDB ID: 2MM4

PDB ID: 5X29

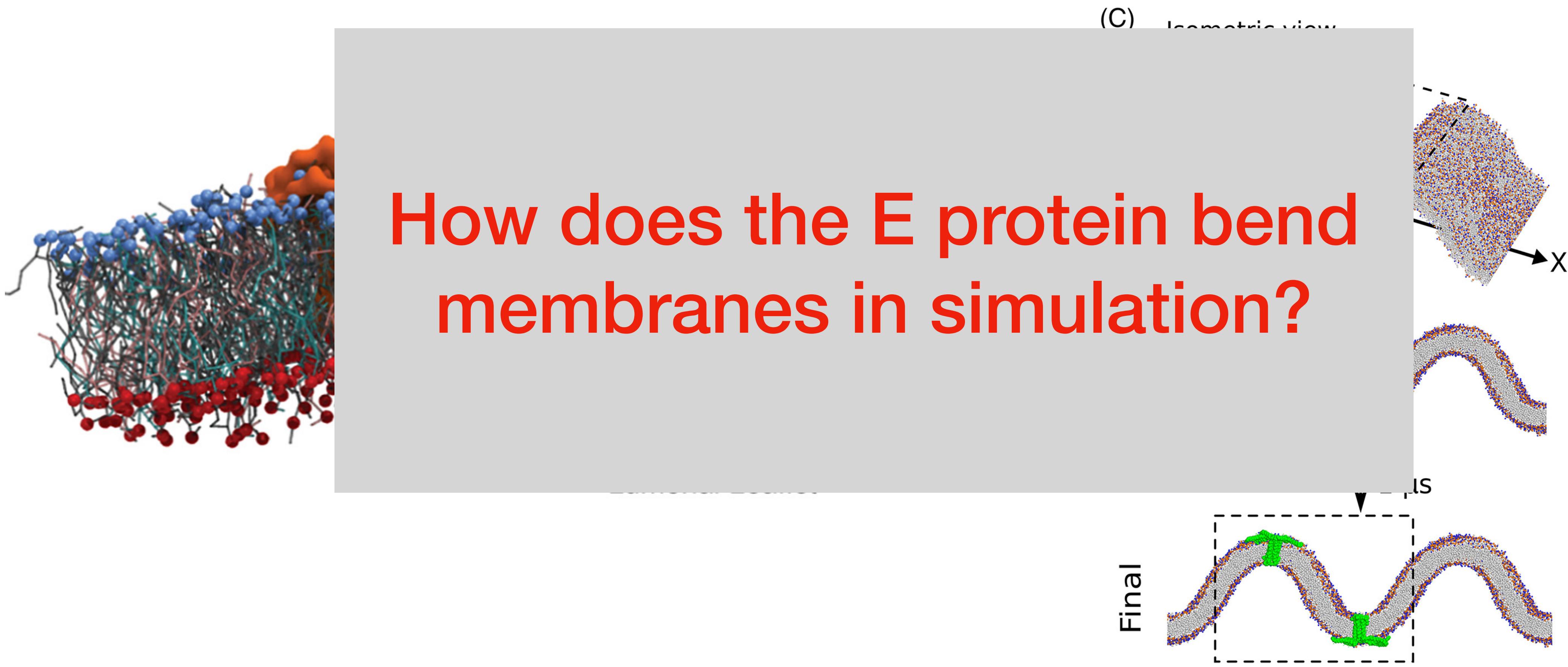
# E seems to be involved in generating or sensing membrane curvature



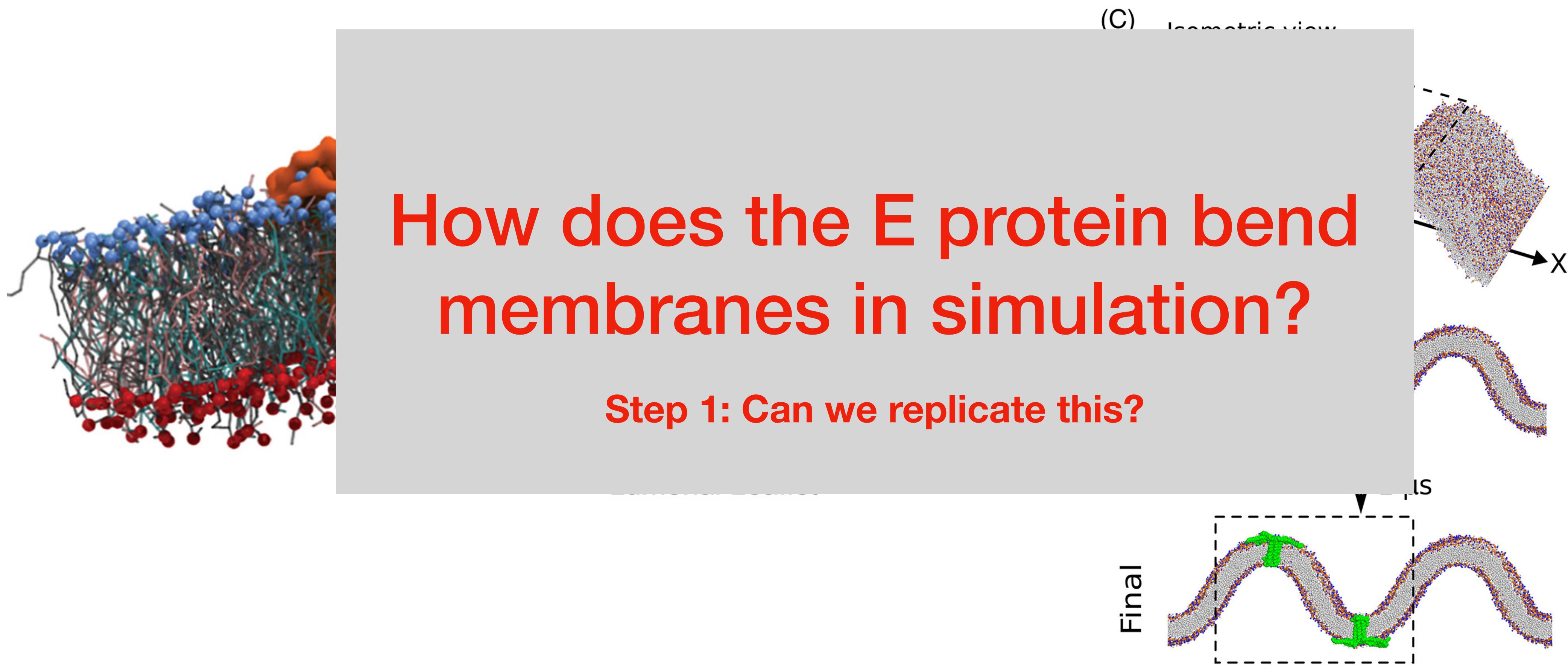
# E seems to be involved in generating or sensing membrane curvature



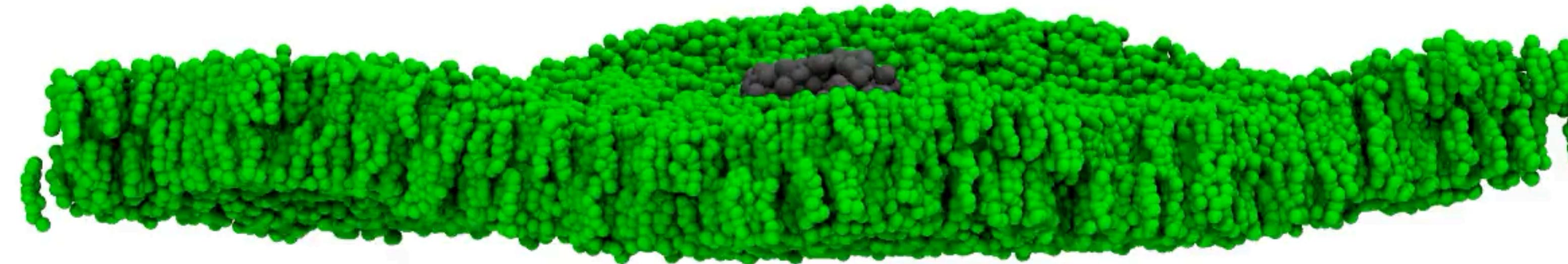
# E seems to be involved in generating or sensing membrane curvature



# E seems to be involved in generating or sensing membrane curvature

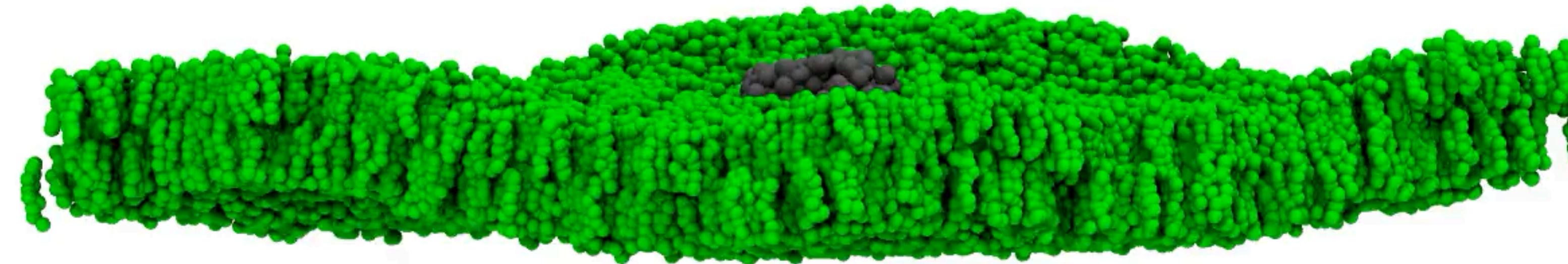


# E causes global bending in our simulations, too



Simulation box is 40x40 nm  
POPC and protein shown; water and ions hidden  
> 30 microseconds

# E causes global bending in our simulations, too

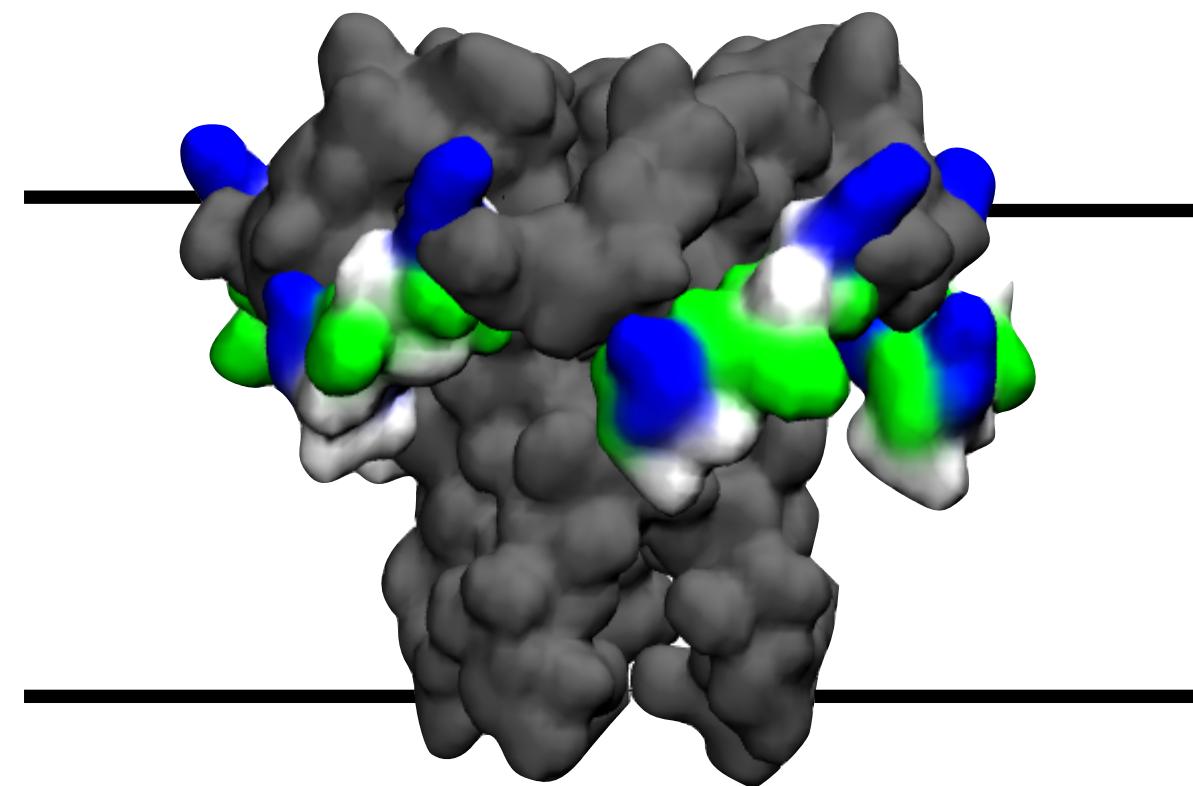


Simulation box is 40x40 nm  
POPC and protein shown; water and ions hidden  
> 30 microseconds

# Membrane thickness deformations near protein

## Observation:

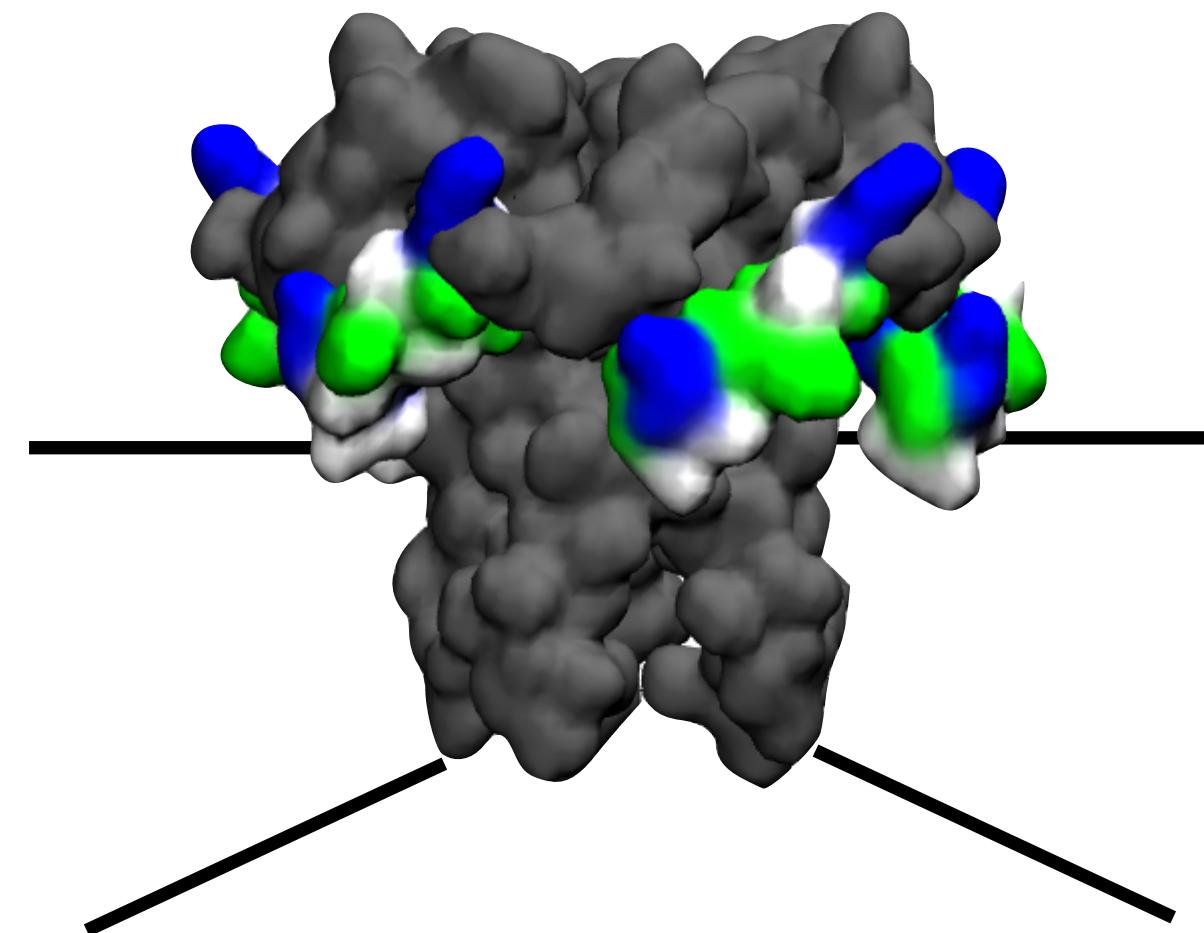
~Half of transmembrane domain  
**(TMD)** is not accessible to  
membrane



# Membrane thickness deformations near protein

## Observation:

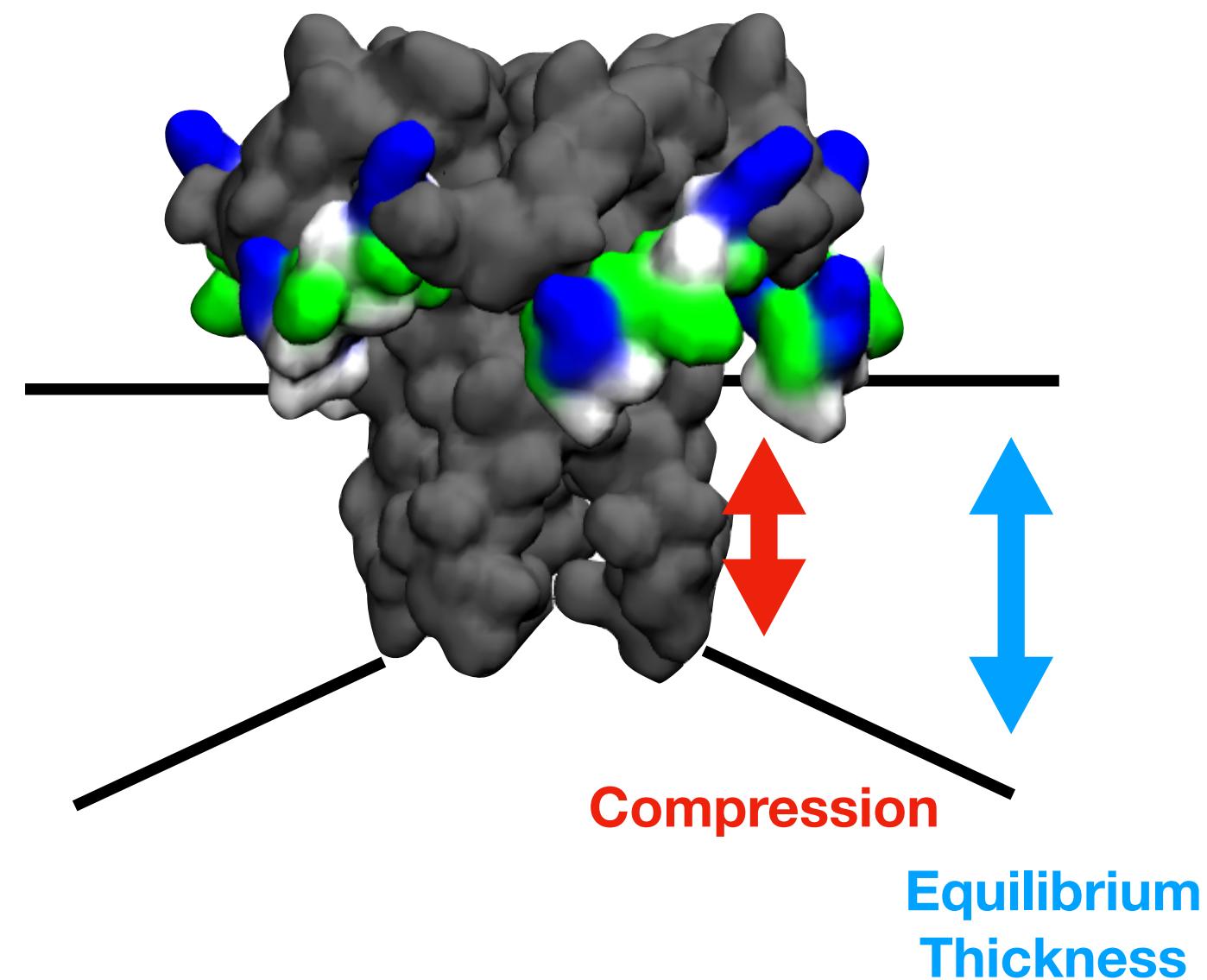
~Half of transmembrane domain  
**(TMD)** is not accessible to  
membrane



# Membrane thickness deformations near protein

## Observation:

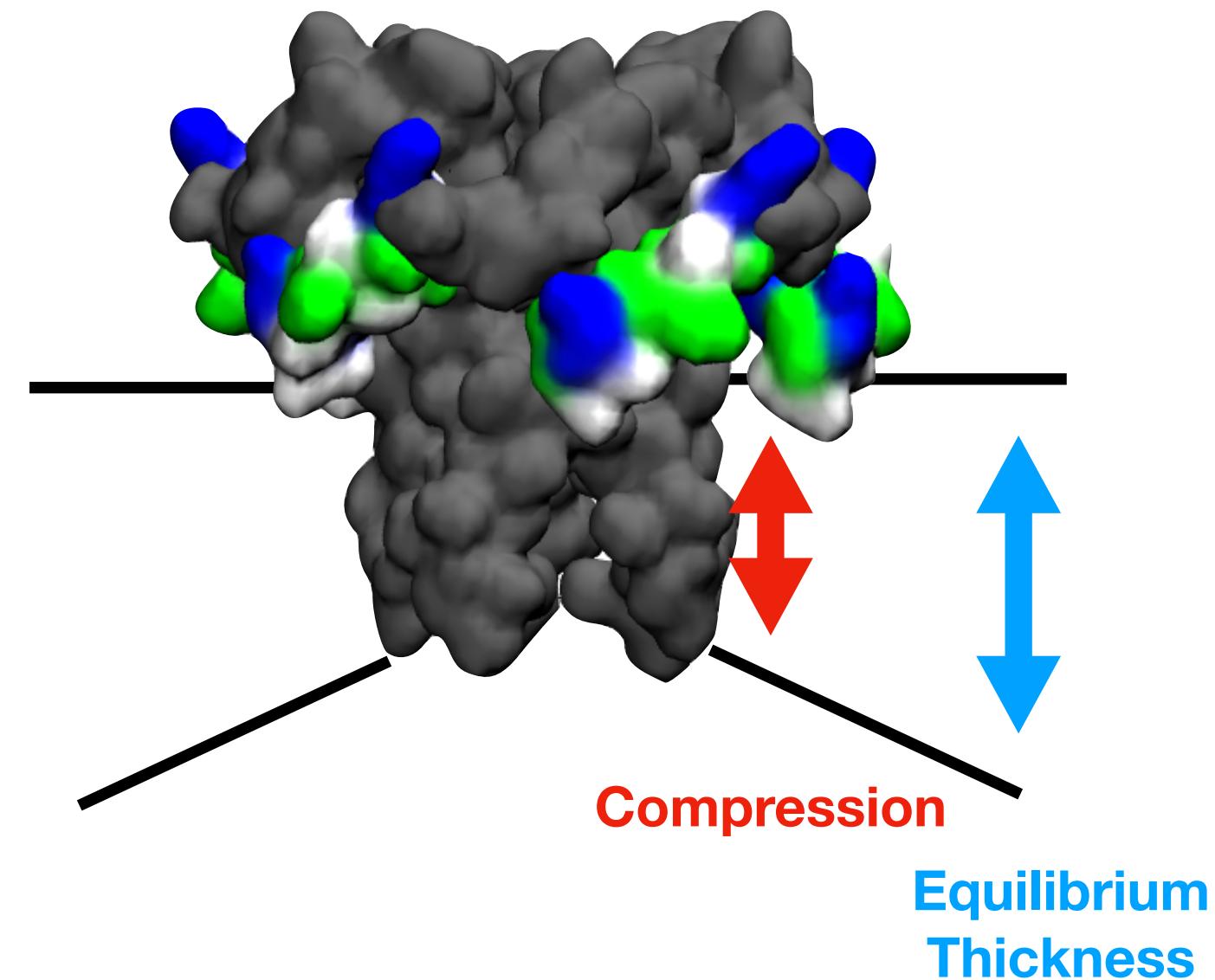
~Half of transmembrane domain  
**(TMD)** is not accessible to  
membrane



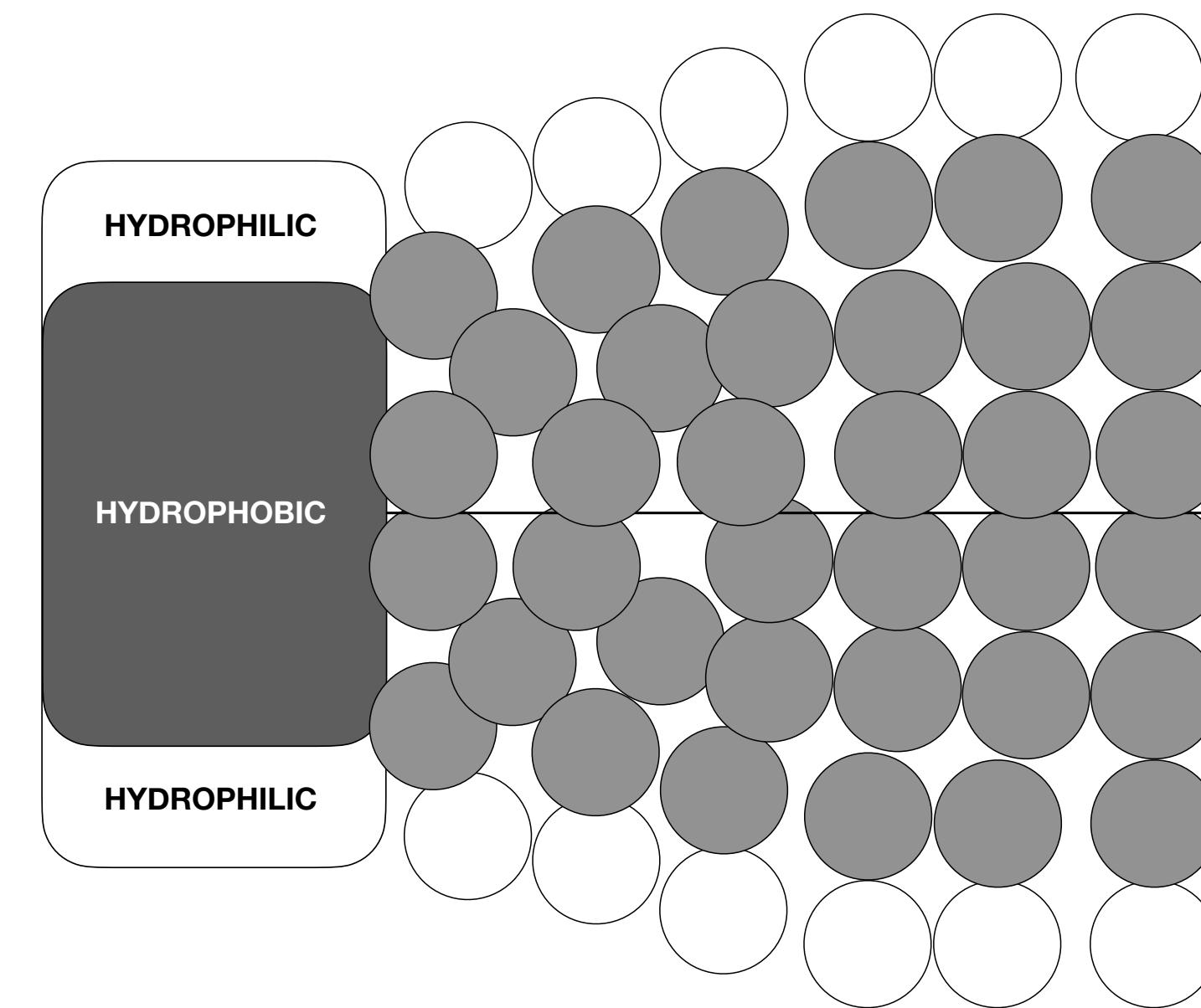
# Membrane thickness deformations near protein

## Observation:

~Half of transmembrane domain  
(TMD) is not accessible to  
membrane



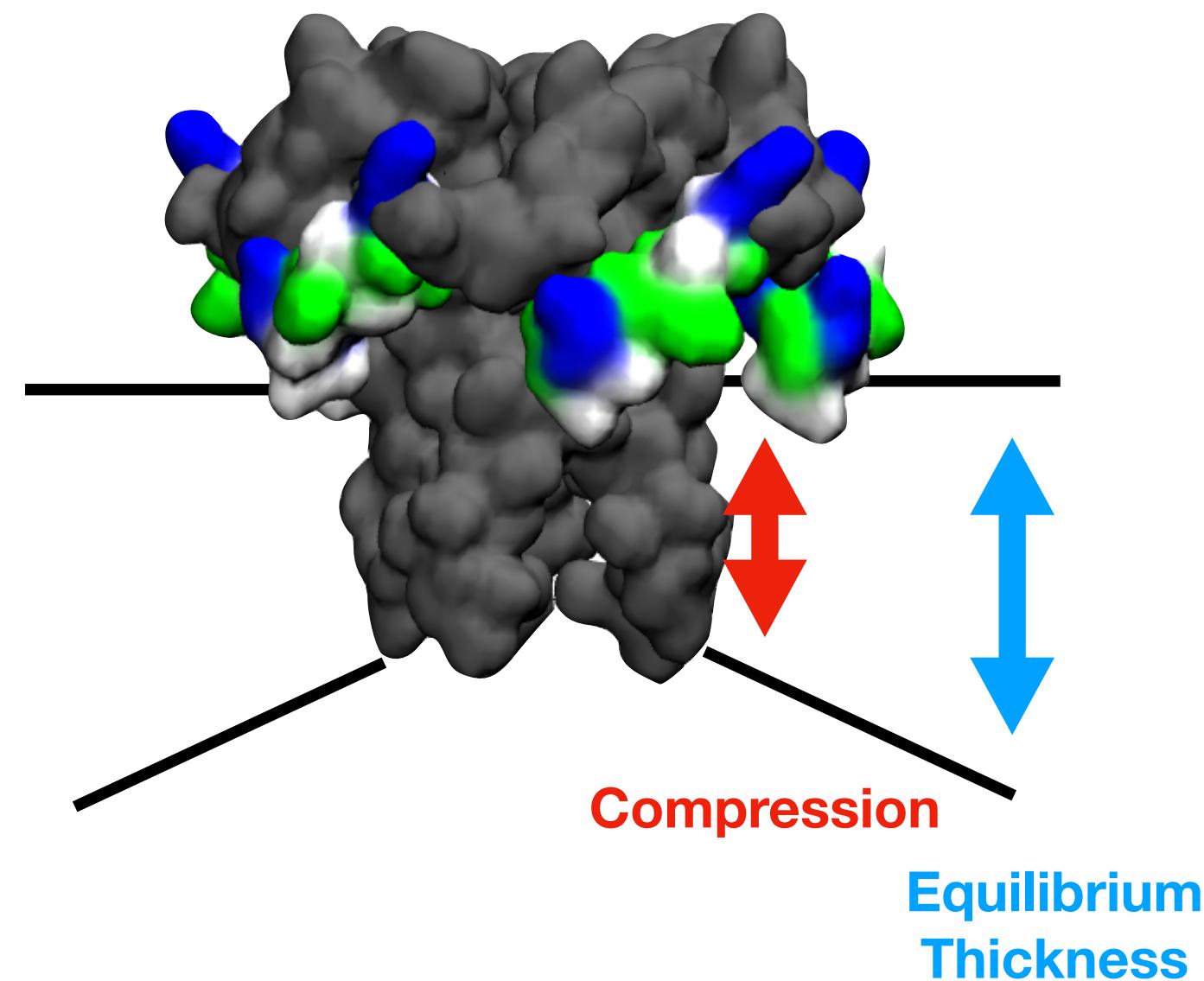
## Hydrophobic Mismatch



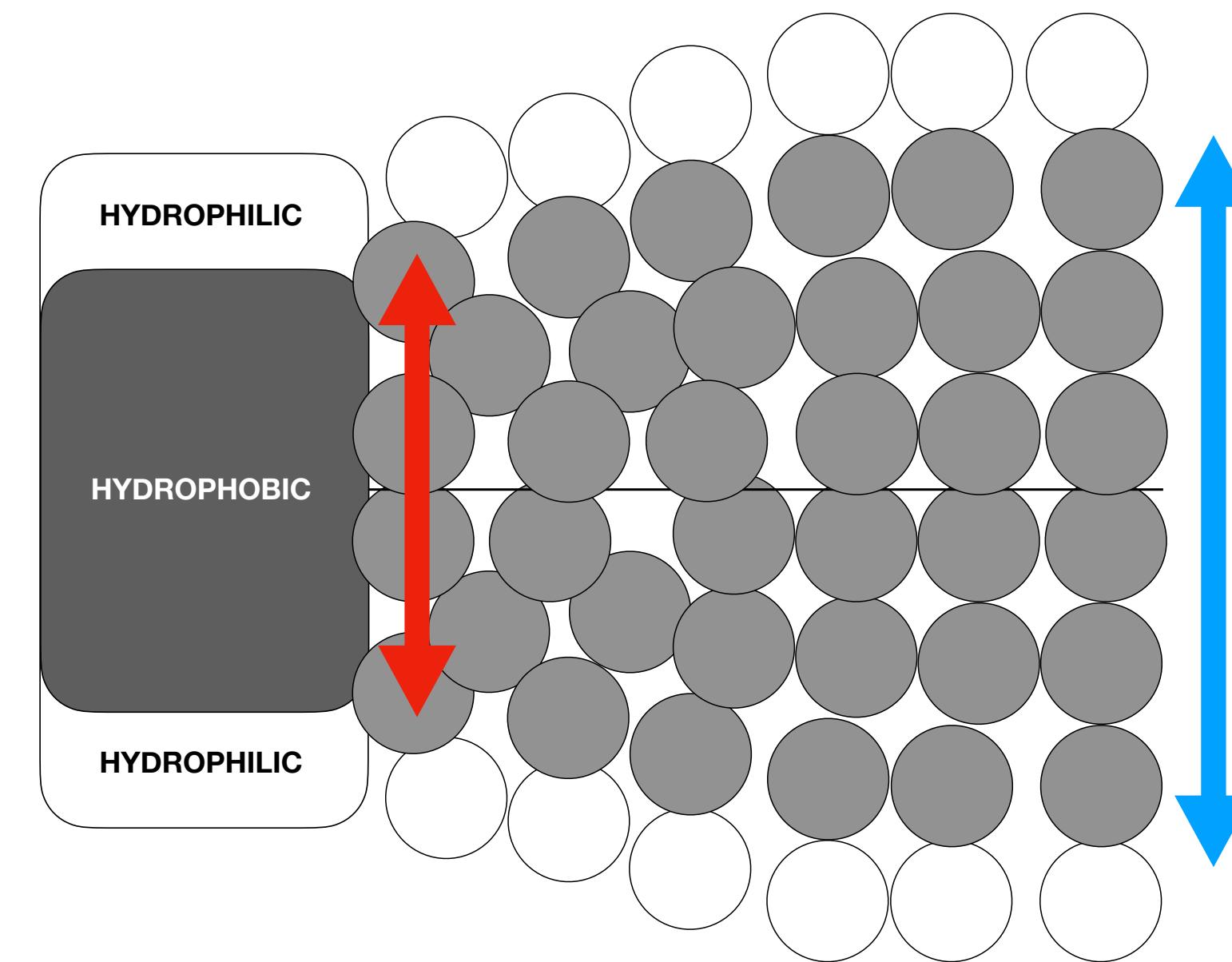
# Membrane thickness deformations near protein

## Observation:

~Half of transmembrane domain  
(TMD) is not accessible to  
membrane

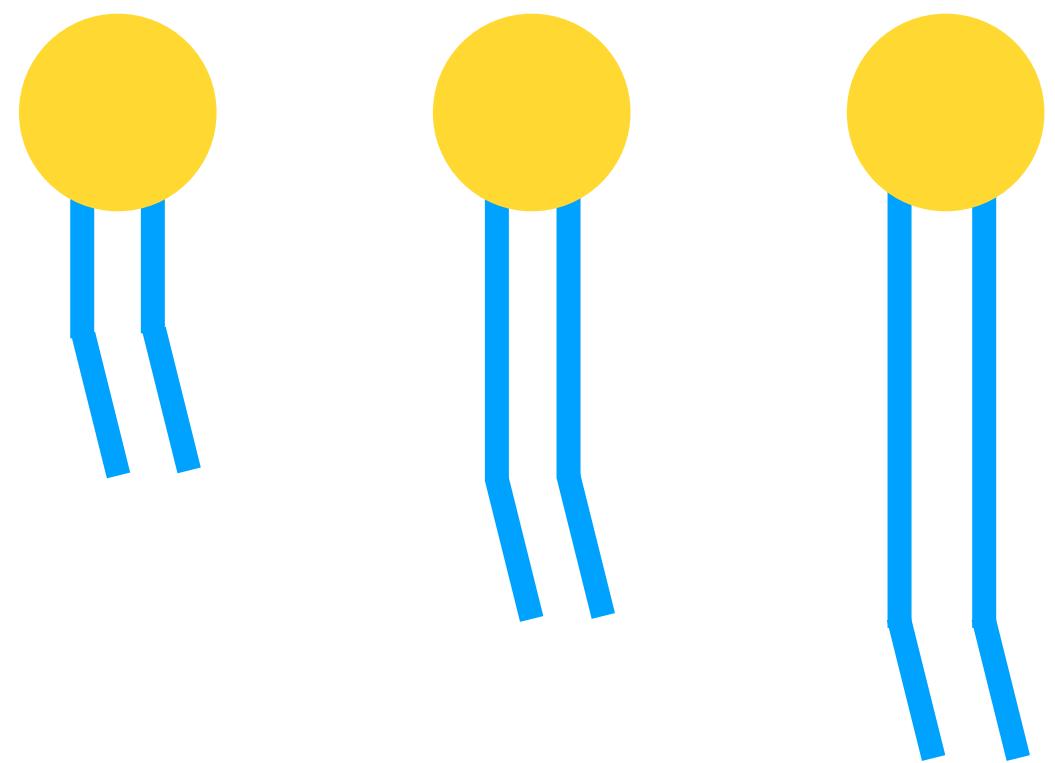


## Hydrophobic Mismatch



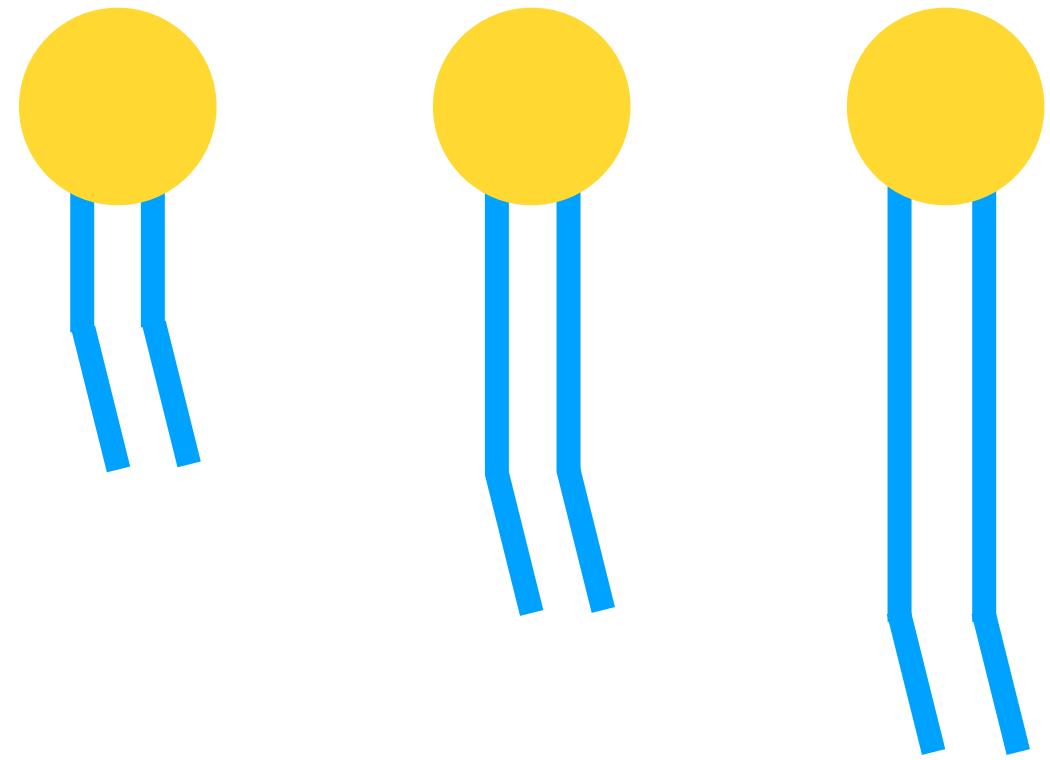
# Initial Approach

**Approach 1:**  
**Embed E protein in membranes of  
varying thickness**



# Initial Approach

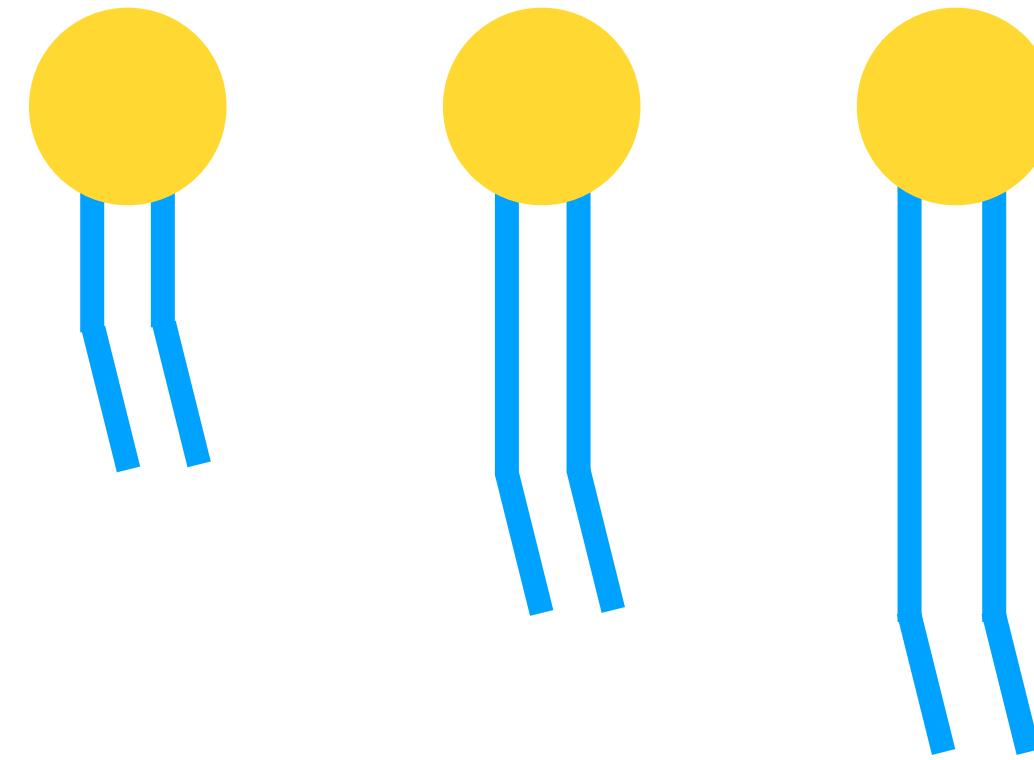
**Approach 1:**  
**Embed E protein in membranes of  
varying thickness**



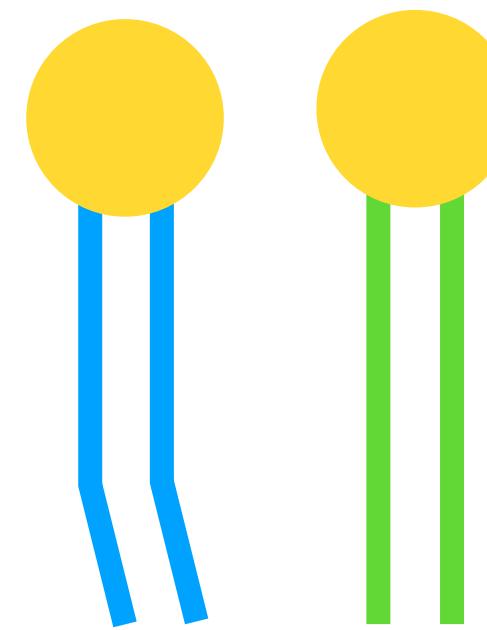
**Expectation 1:**  
**Clear thickness dependence in  
the bending signal**

# Initial Approach

**Approach 1:**  
Embed E protein in membranes of  
varying **thickness**



**Approach 2:**  
Embed E protein in membranes of  
varying **flexibility**

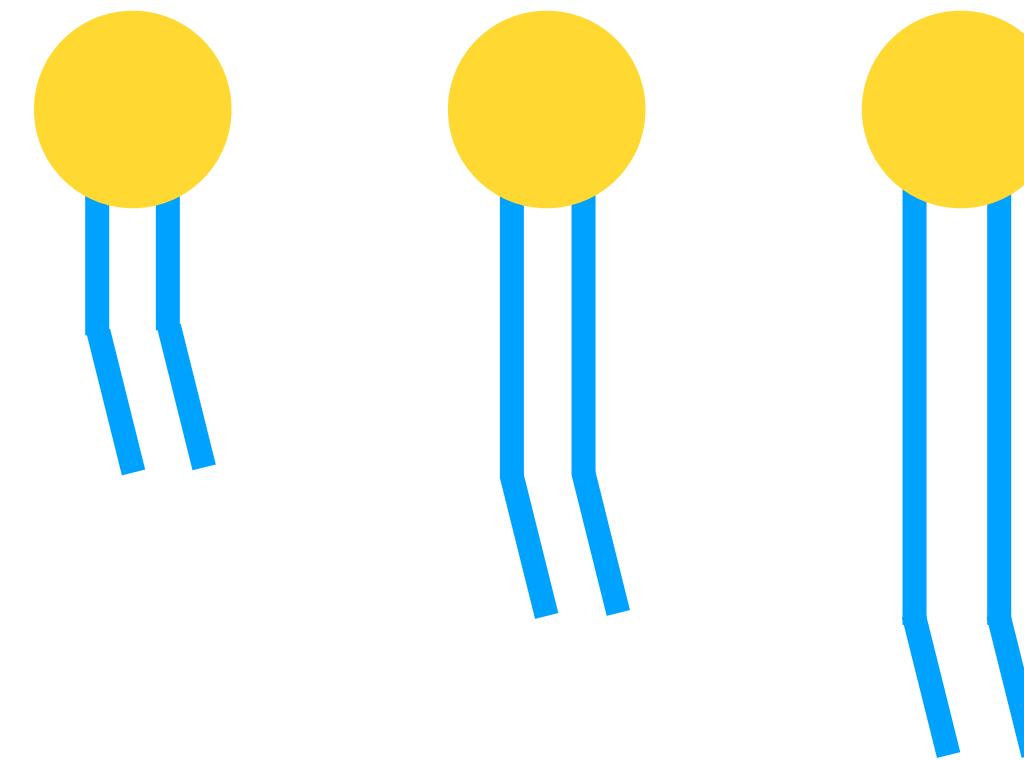


**Expectation 1:**  
Clear thickness dependence in  
the bending signal

# Initial Approach

## Approach 1:

Embed E protein in membranes of varying **thickness**

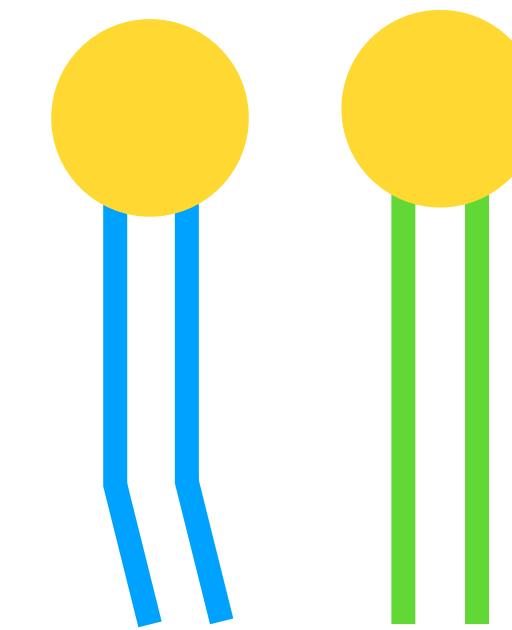


## Expectation 1:

Clear thickness dependence in the bending signal

## Approach 2:

Embed E protein in membranes of varying **flexibility**

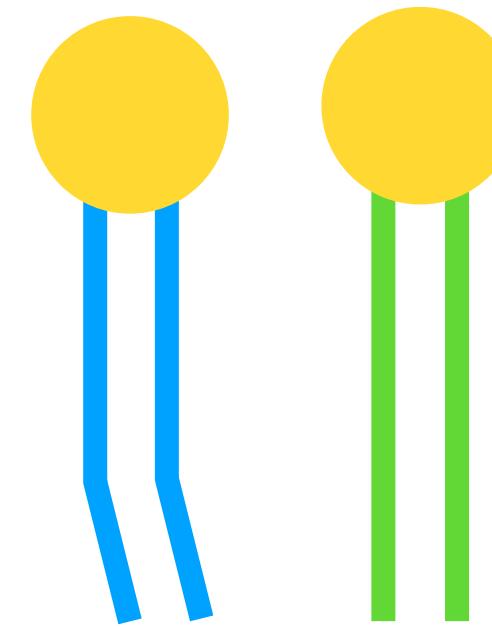


## Expectation 2:

Saturated membranes are less flexible, so will heal over longer distances



**Approach 2:**  
**Embed E protein in membranes of**  
**varying flexibility**

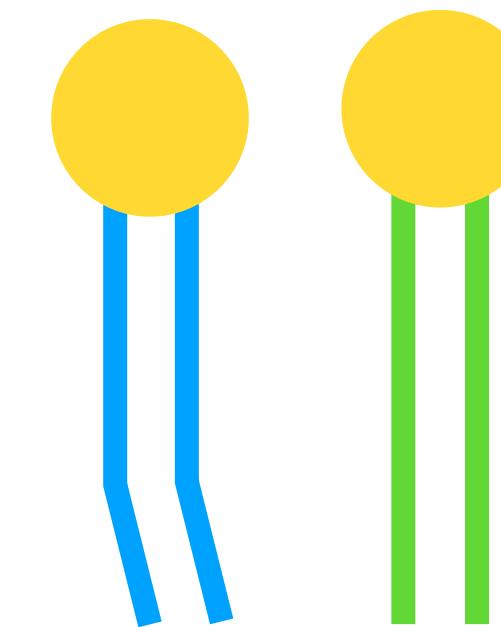


**Expectation 2:**  
**Saturated membranes are less**  
**flexible, so will heal over longer**  
**distances**



$$F_{\text{symmetric}} \propto k_c H^2$$

**Approach 2:**  
**Embed E protein in membranes of varying flexibility**



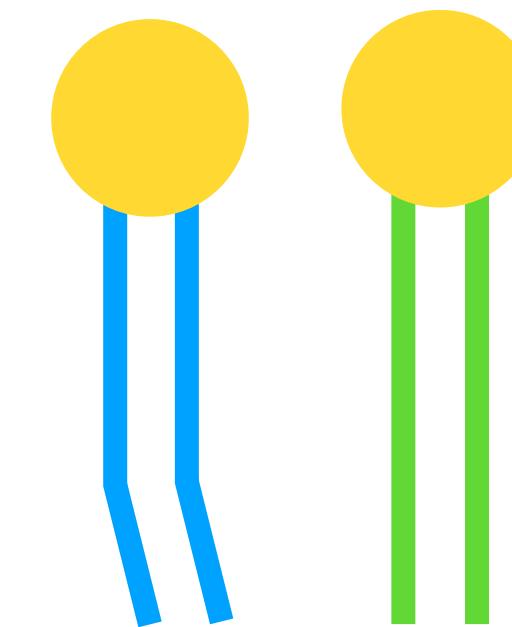
**Expectation 2:**  
**Saturated membranes are less flexible, so will heal over longer distances**



$$F_{\text{symmetric}} \propto k_c H^2$$

Bending Modulus      Curvature

**Approach 2:**  
**Embed E protein in membranes of varying flexibility**

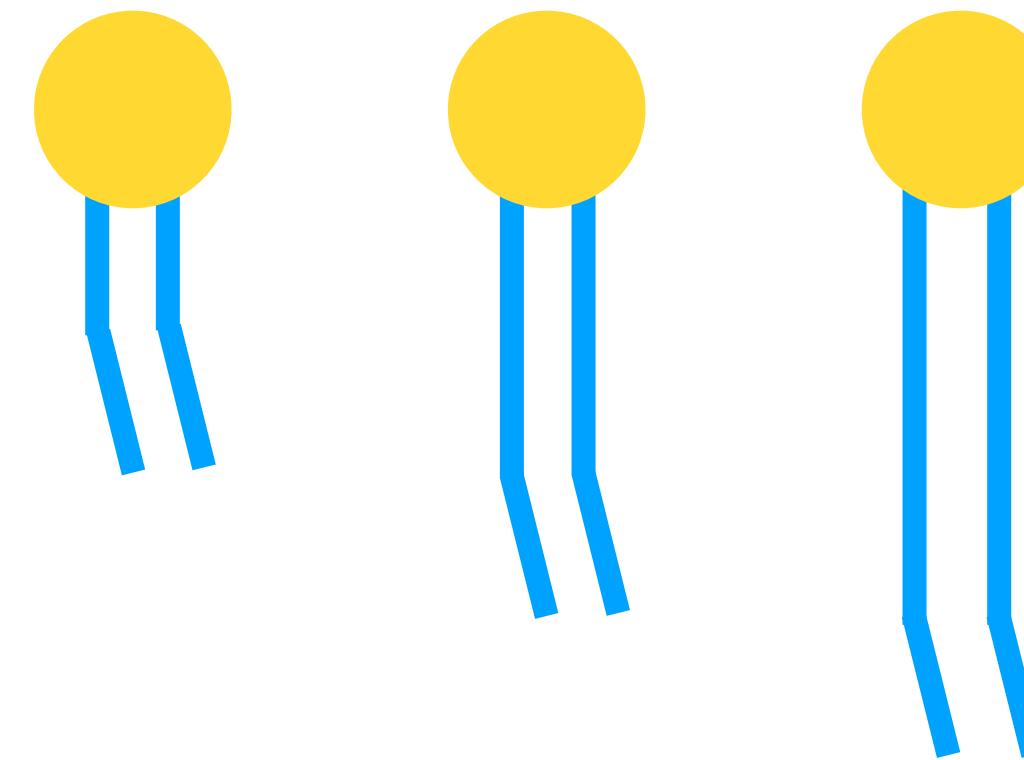


**Expectation 2:**  
**Saturated membranes are less flexible, so will heal over longer distances**

# Initial Approach

## Approach 1:

Embed E protein in membranes of varying **thickness**

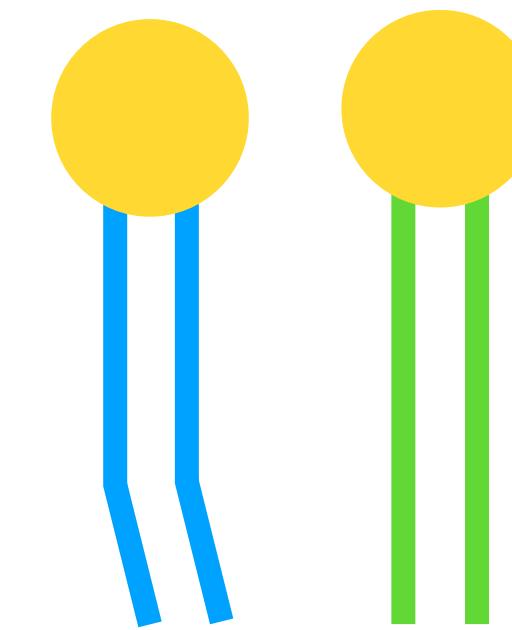


## Expectation 1:

Clear thickness dependence in the bending signal

## Approach 2:

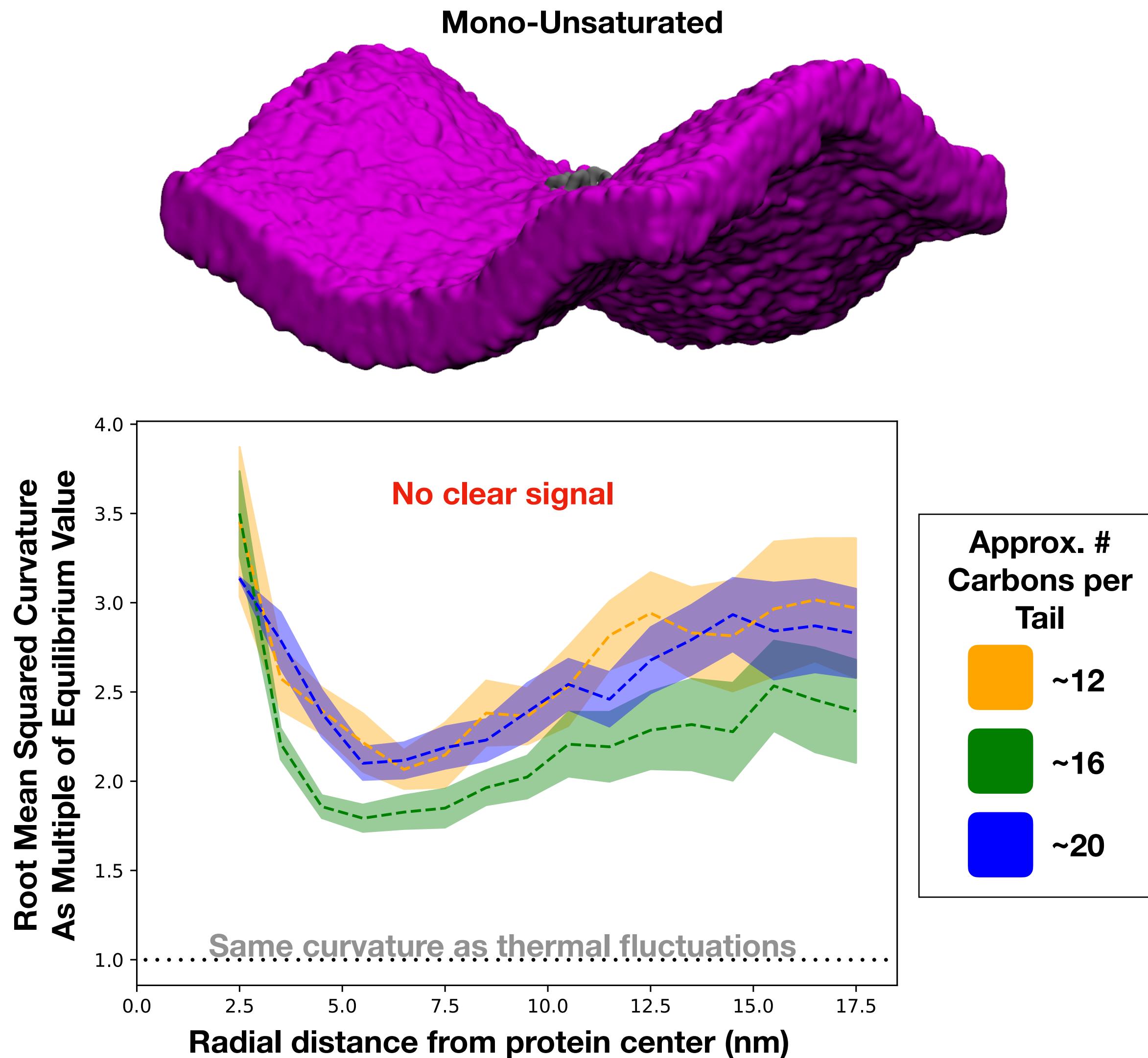
Embed E protein in membranes of varying **flexibility**



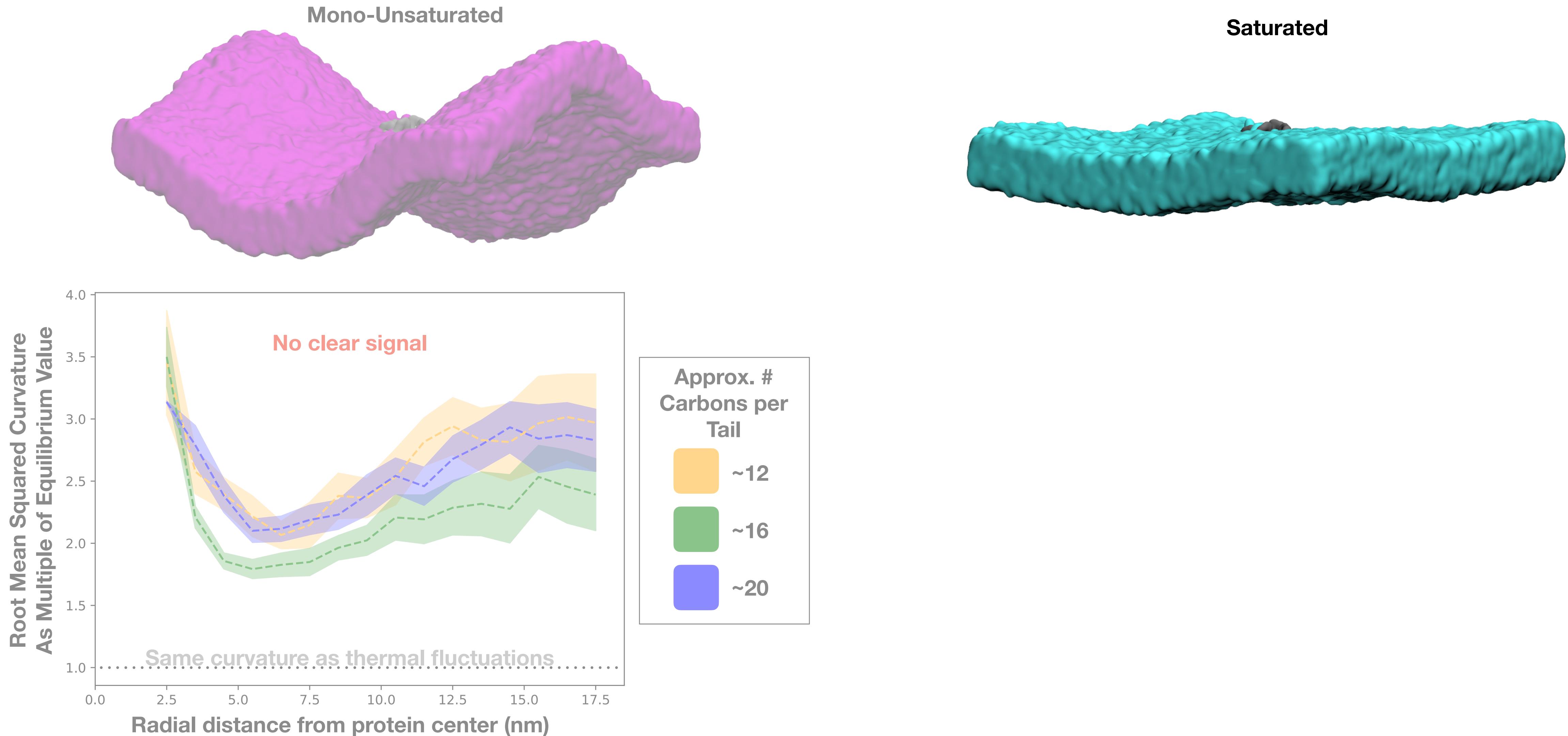
## Expectation 2:

Saturated membranes are less flexible, so will heal over longer distances

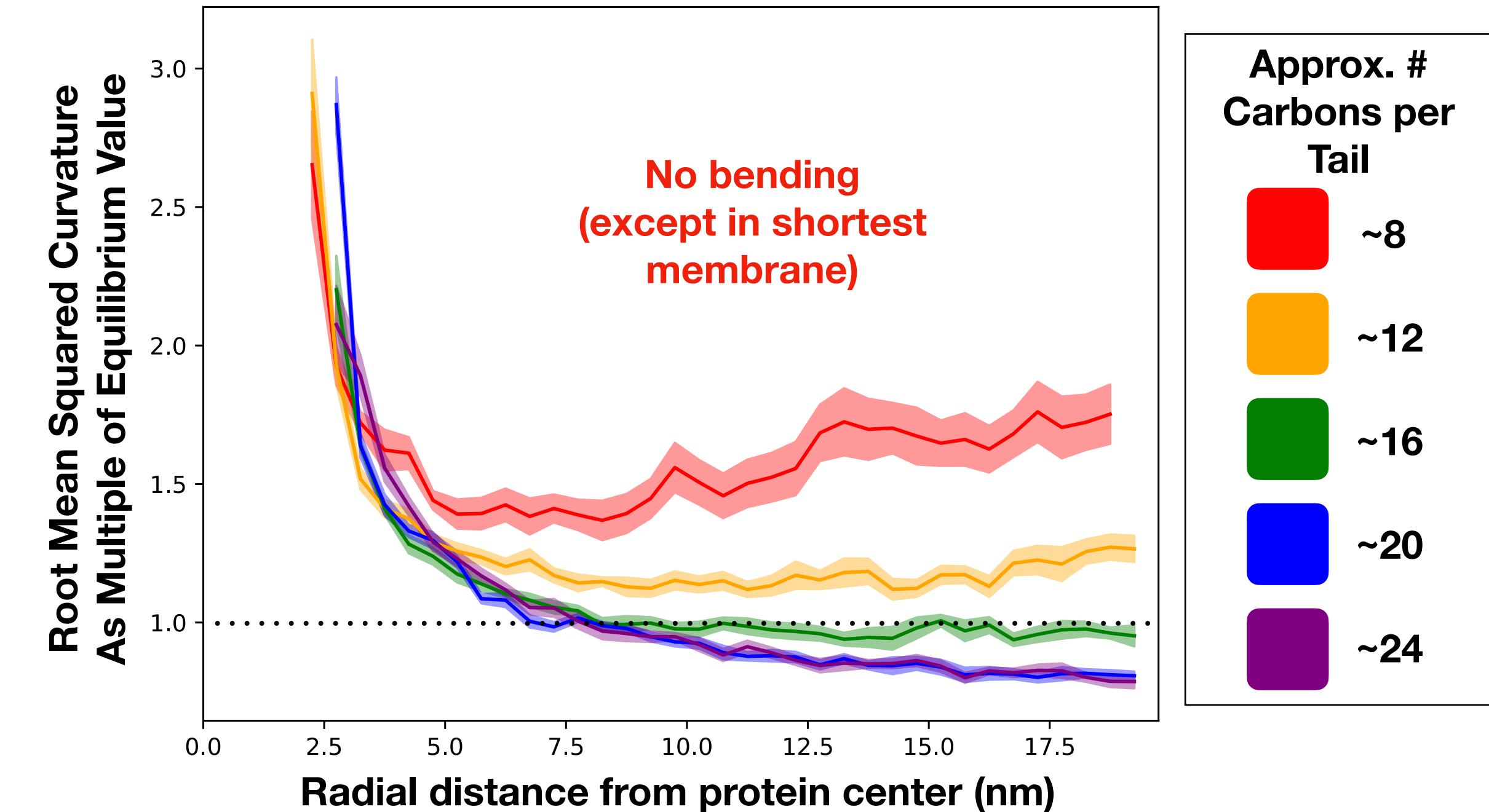
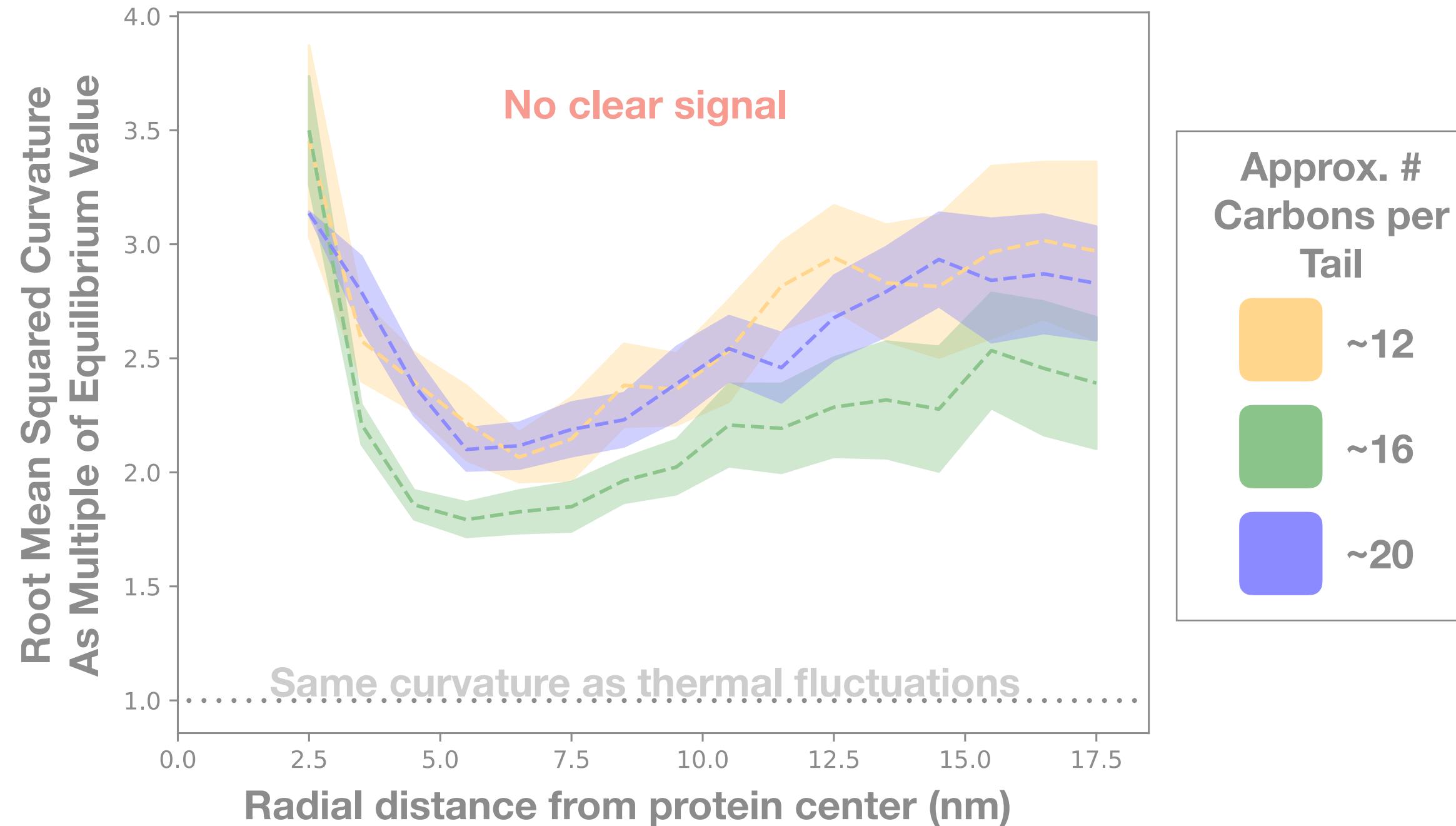
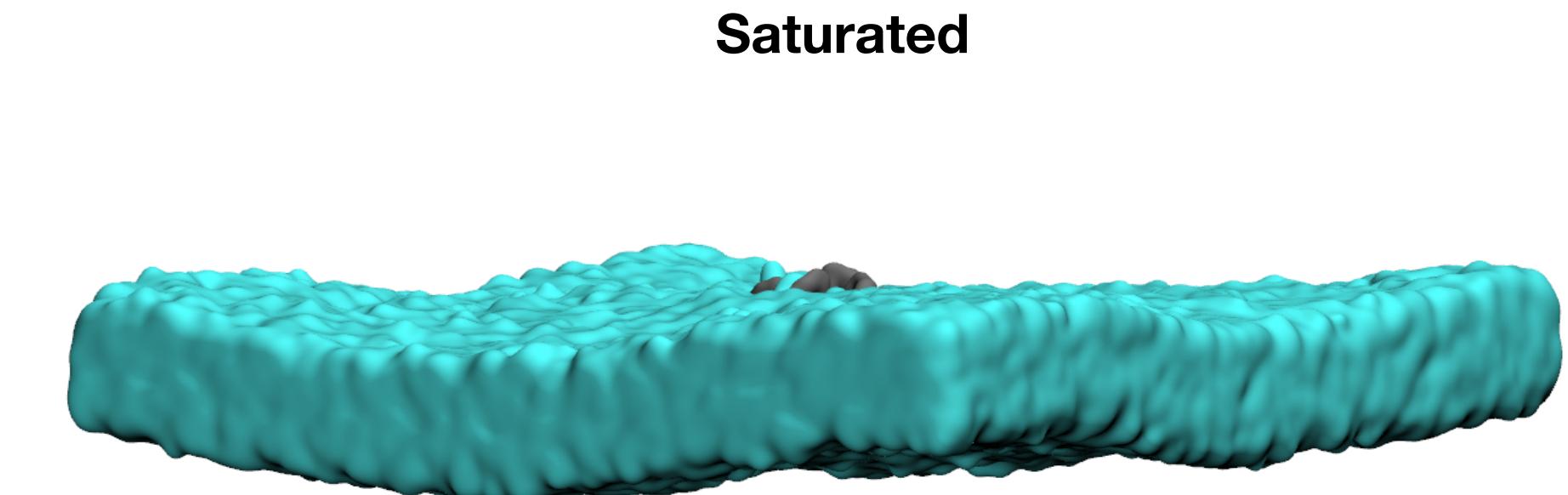
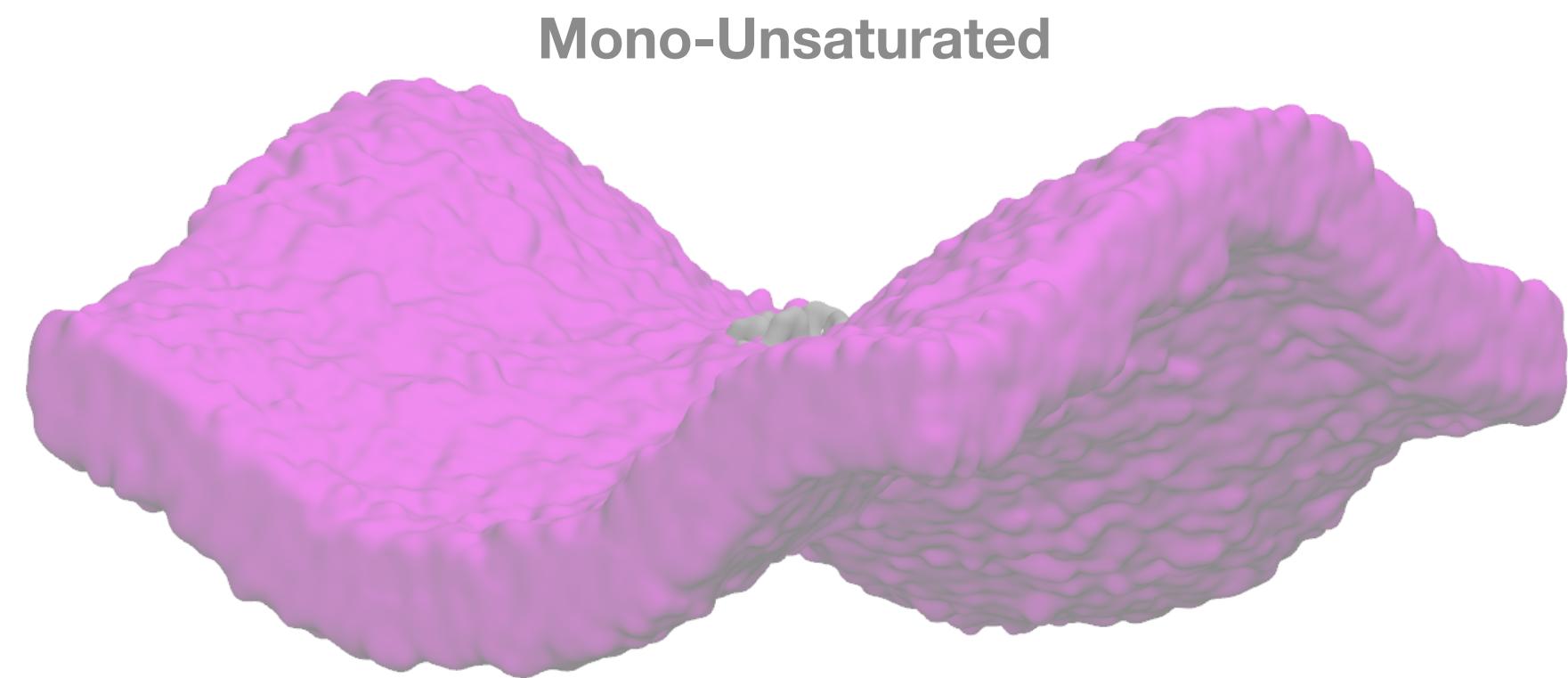
# Expectations confounded



# Expectations confounded



# Expectations confounded



# **Review + Additional Evidence**

# Review + Additional Evidence

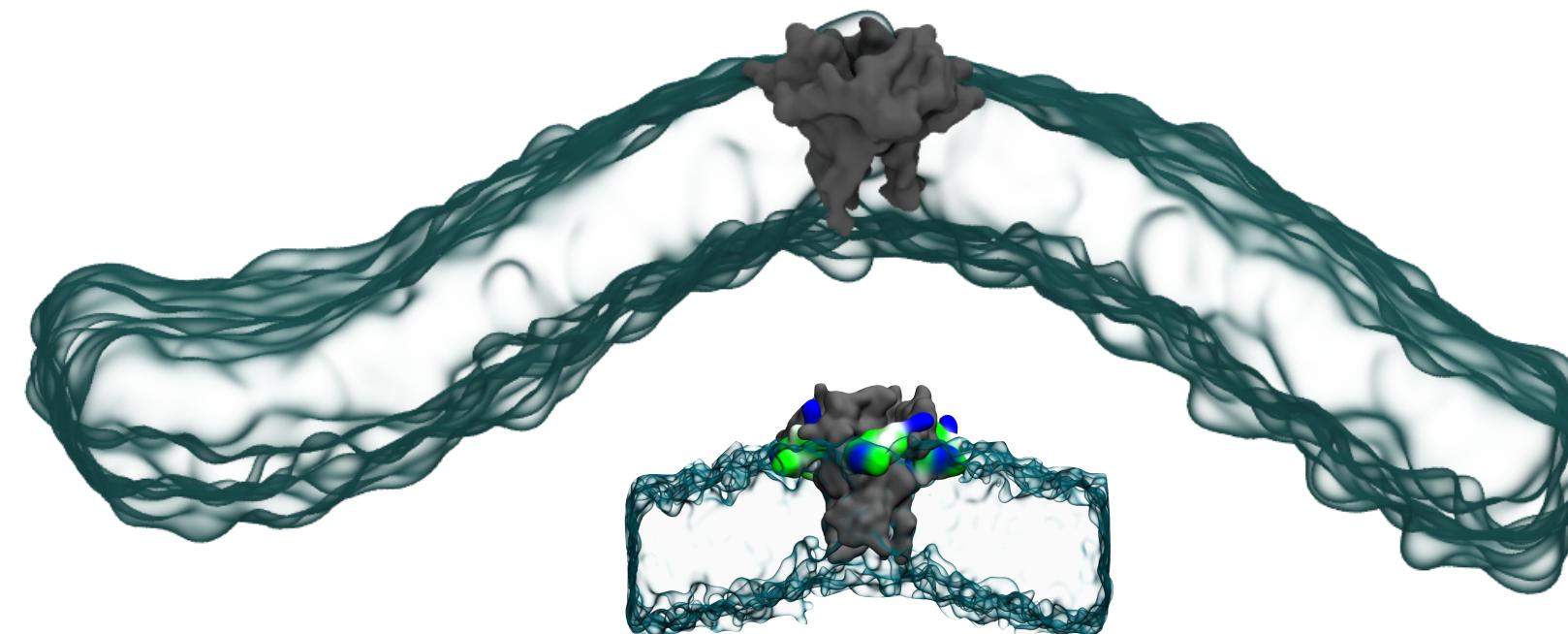
- No clear thickness dependence in bending signal

# Review + Additional Evidence

- No clear thickness dependence in bending signal
- Saturated membranes heal quicker than unsaturated ones

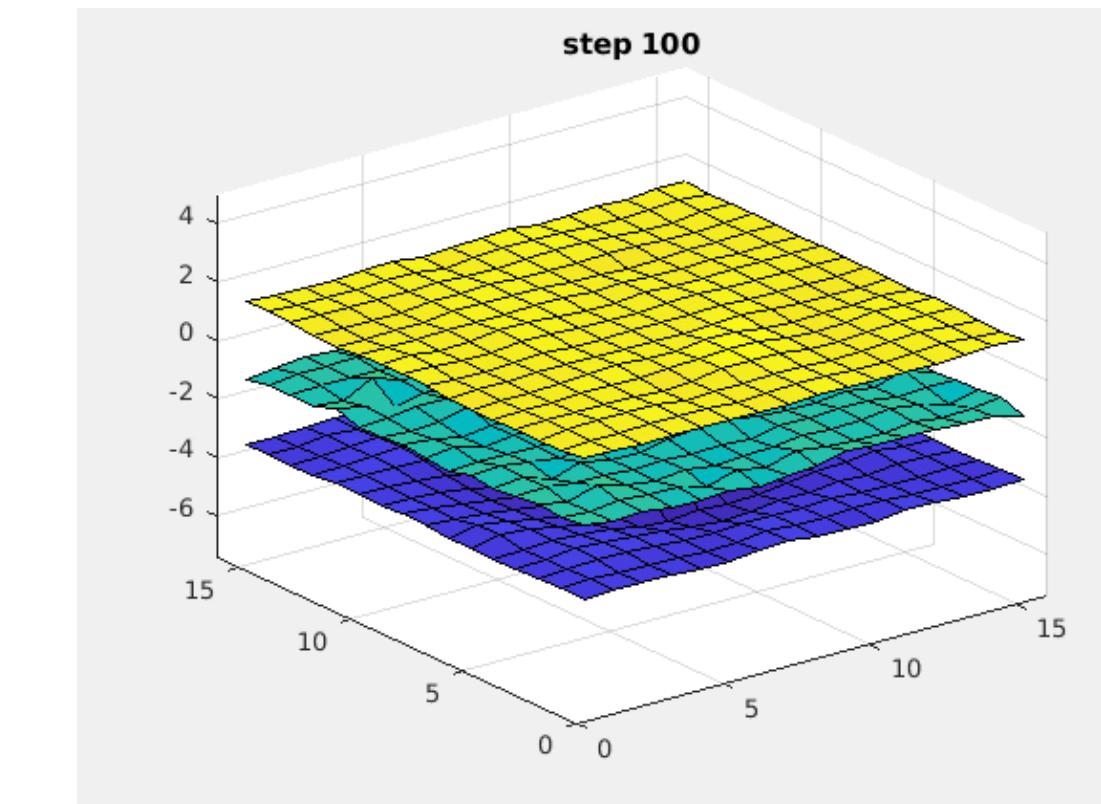
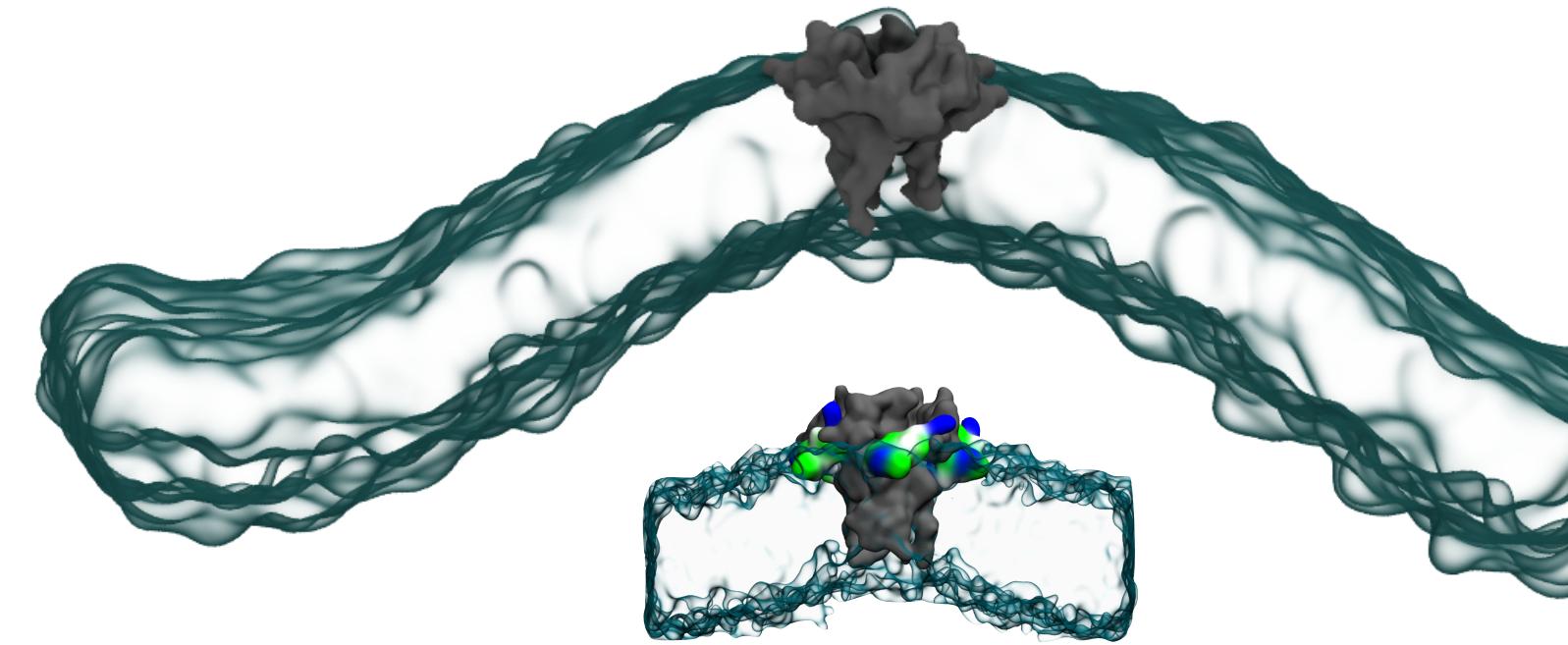
# Review + Additional Evidence

- No clear thickness dependence in bending signal
- Saturated membranes heal quicker than unsaturated ones
- Bending increases with system size



# Review + Additional Evidence

- No clear thickness dependence in bending signal
- Saturated membranes heal quicker than unsaturated ones
- Bending increases with system size
- Bending not replicable using continuum model / Monte Carlo simulations

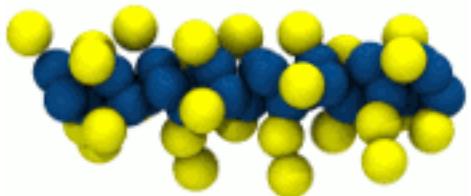
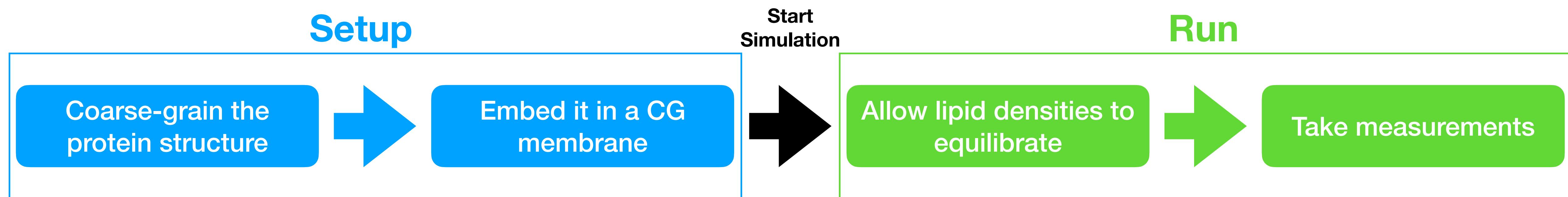


ME

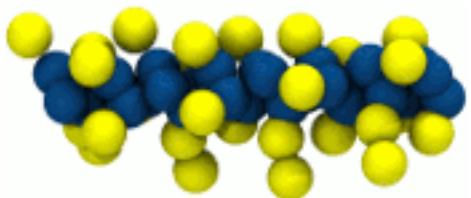
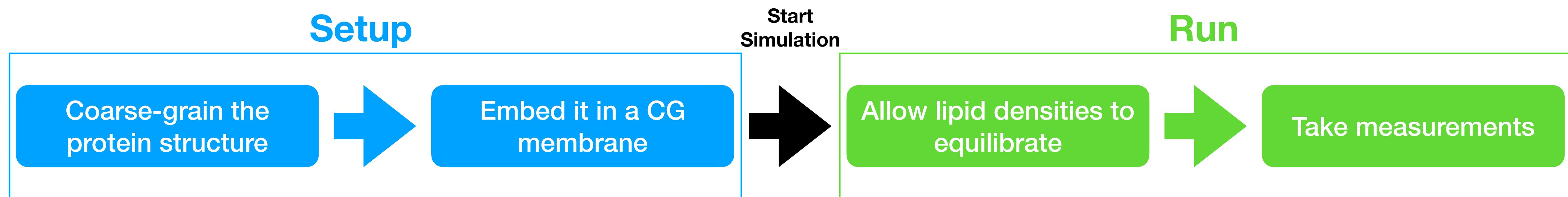
MY RESULTS



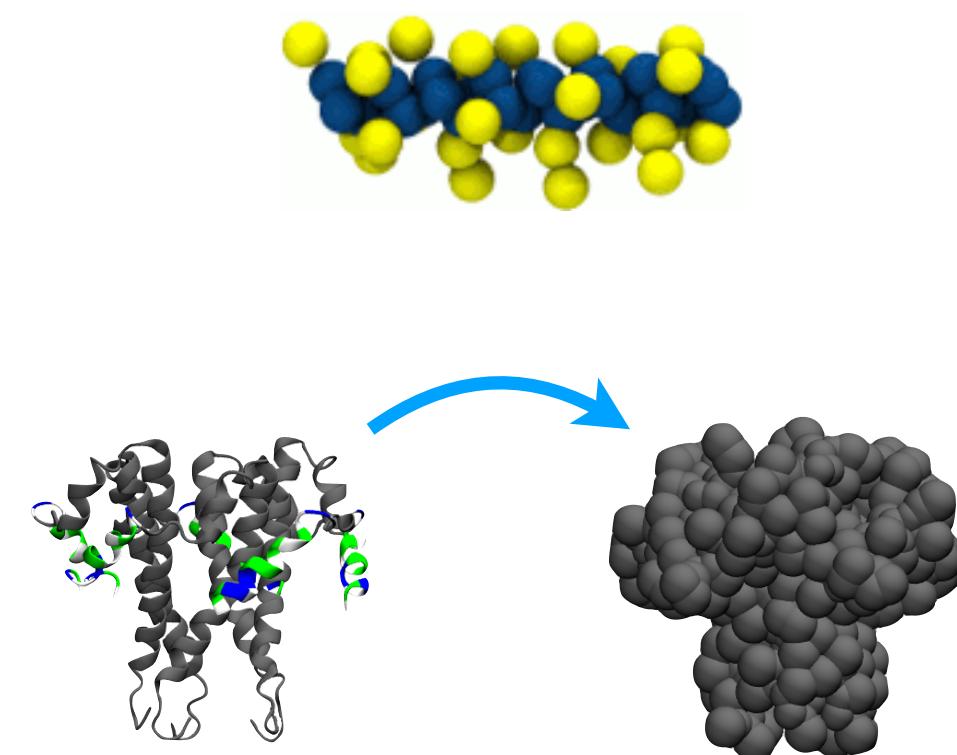
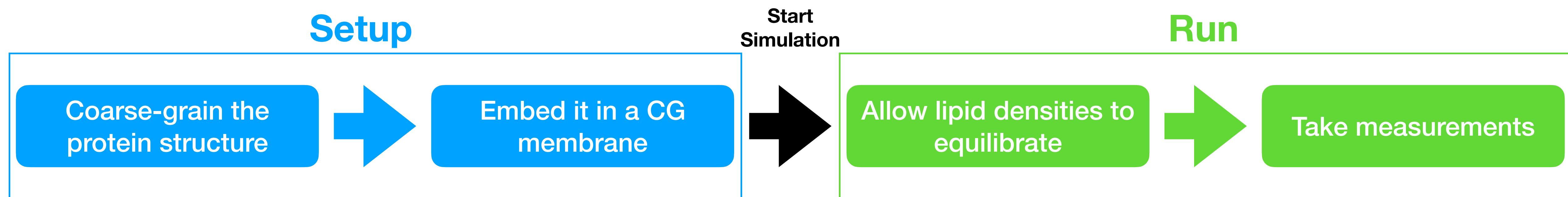
# CG-MD Workflow Map



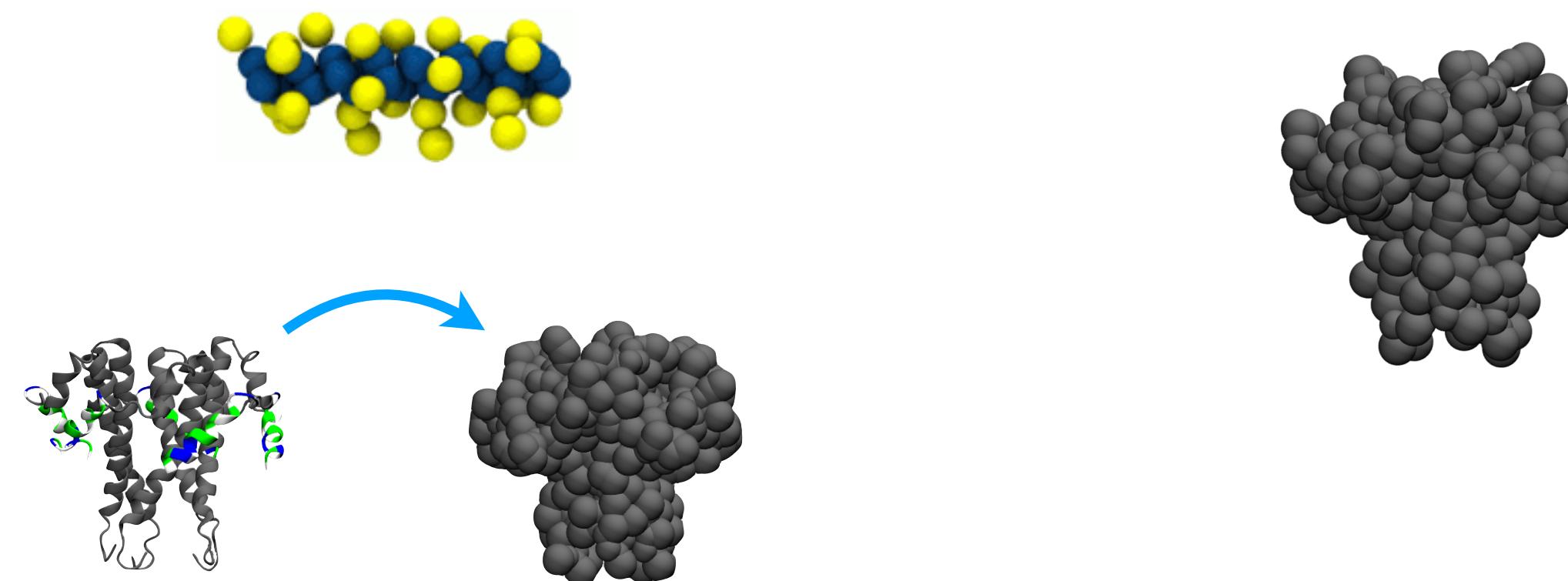
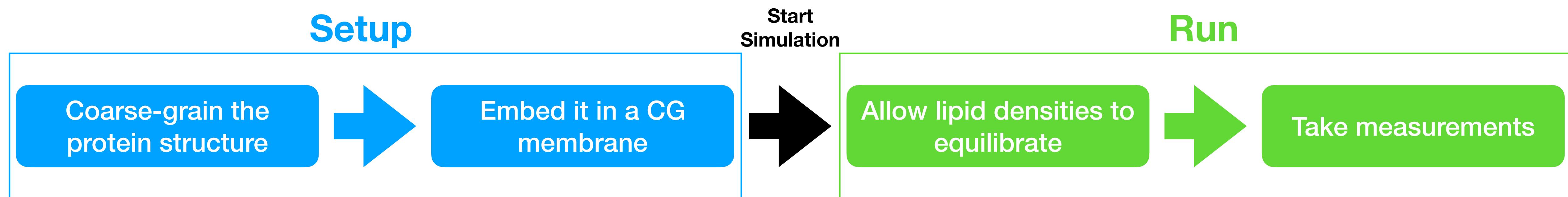
# CG-MD Workflow Map



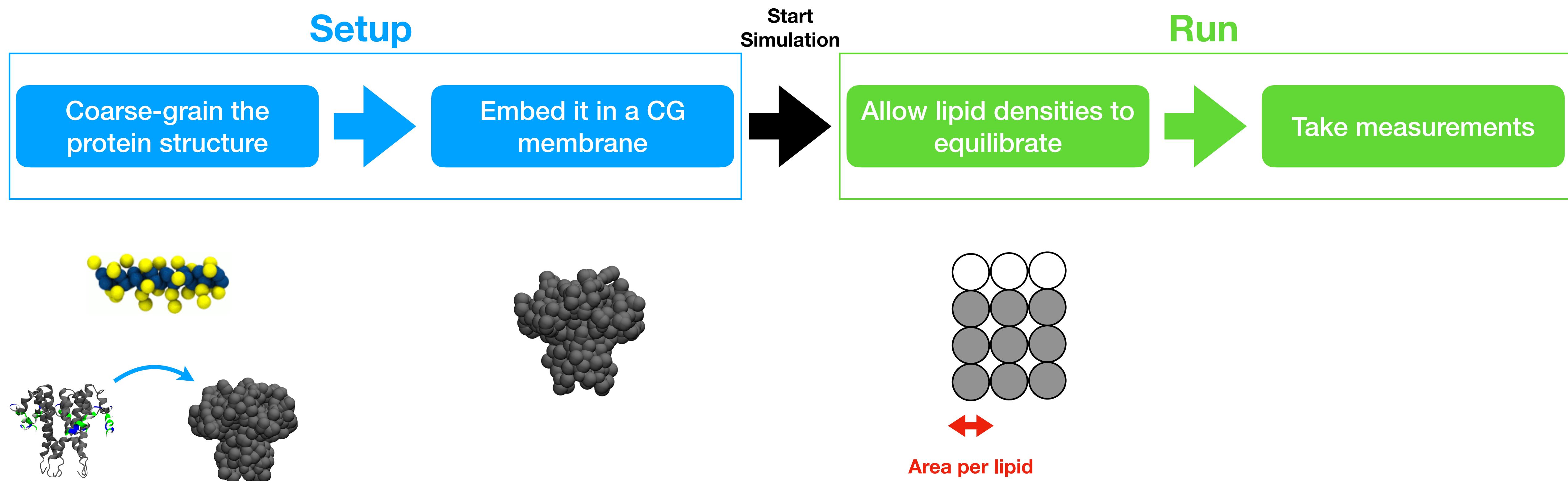
# CG-MD Workflow Map



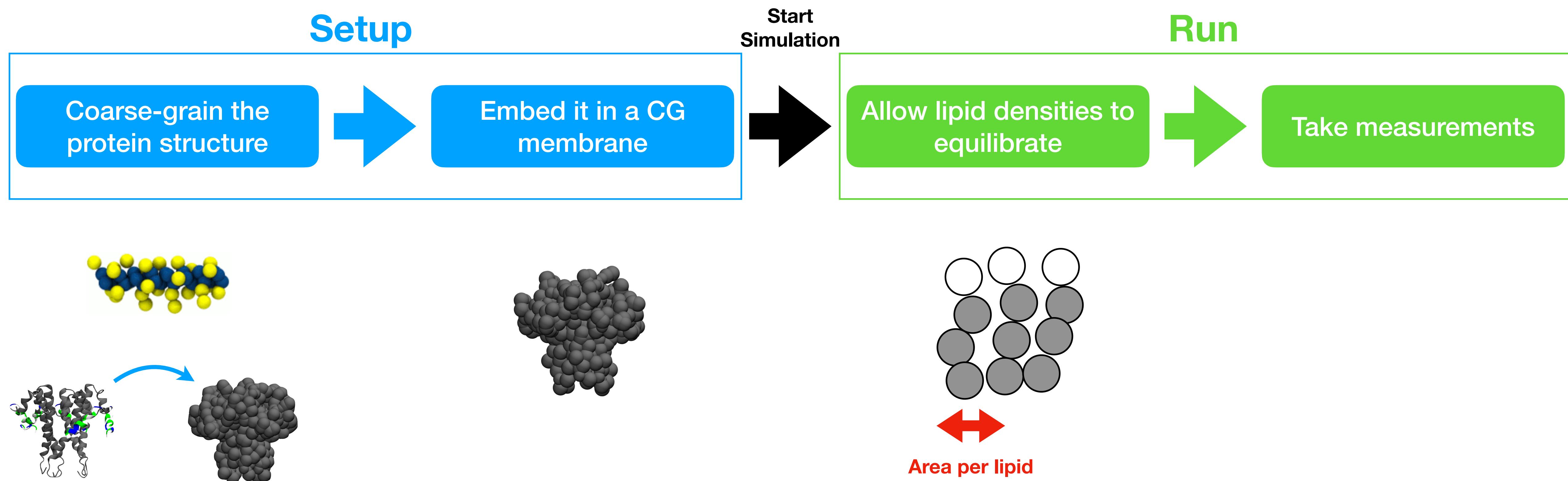
# CG-MD Workflow Map



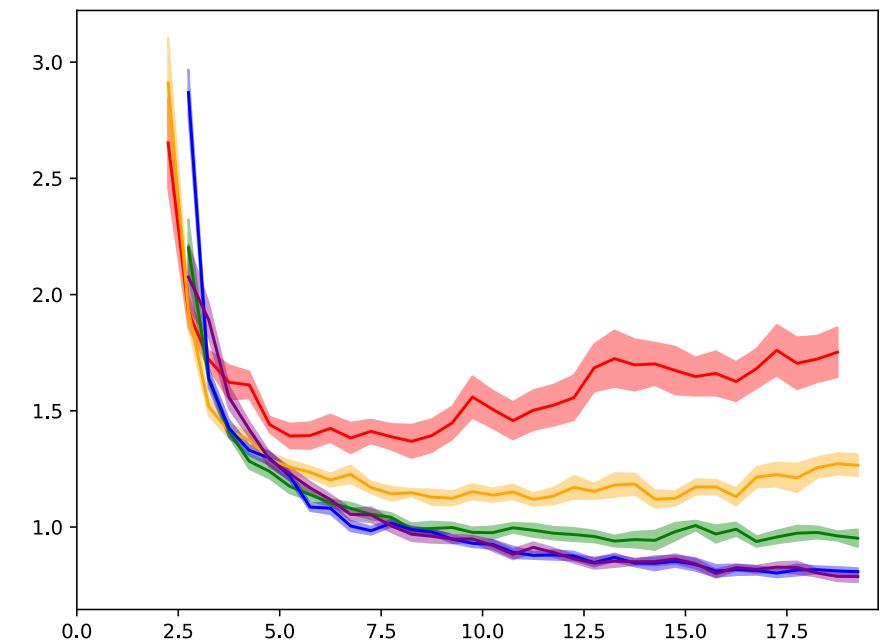
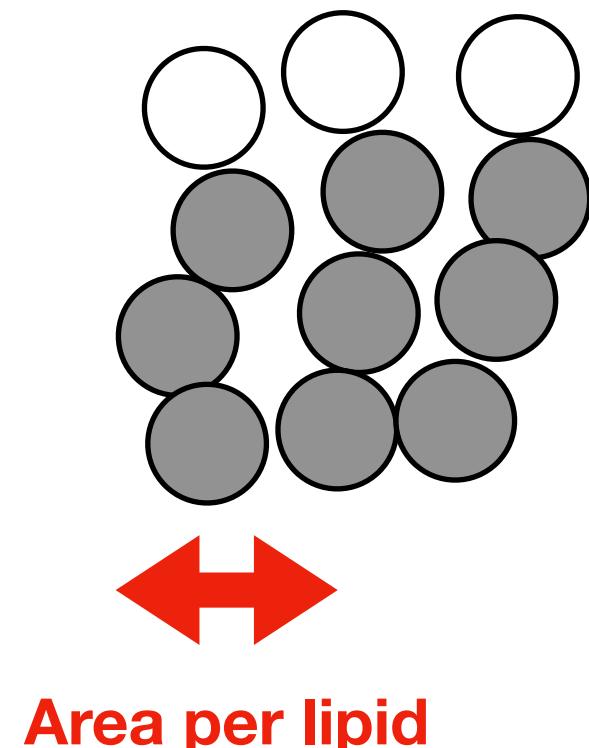
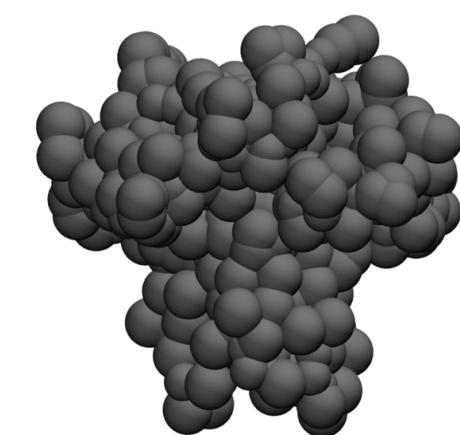
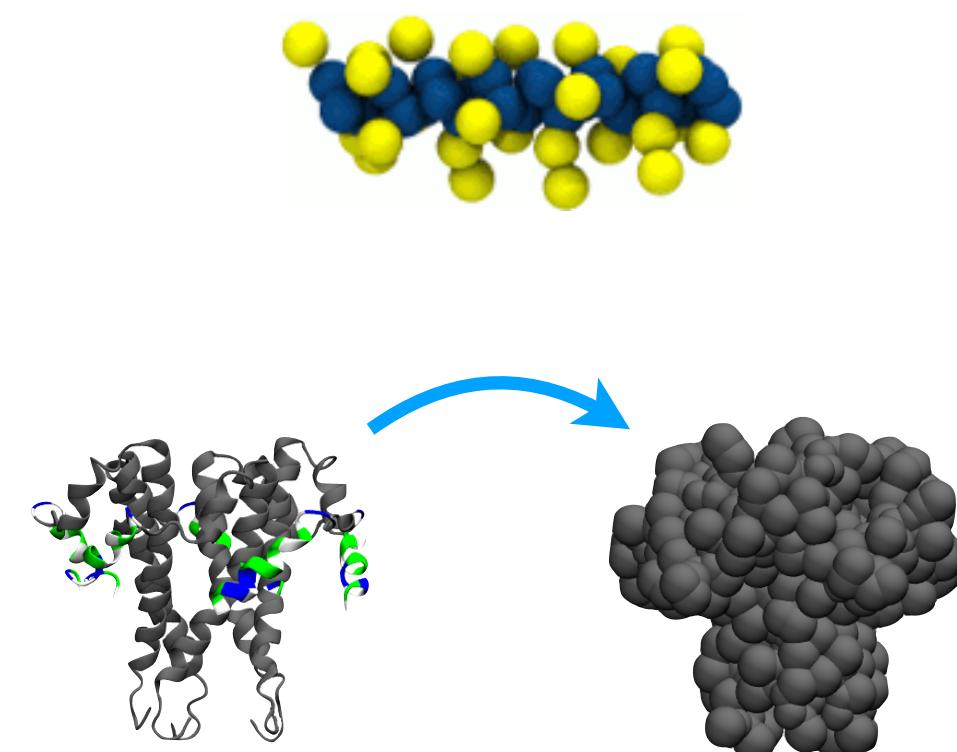
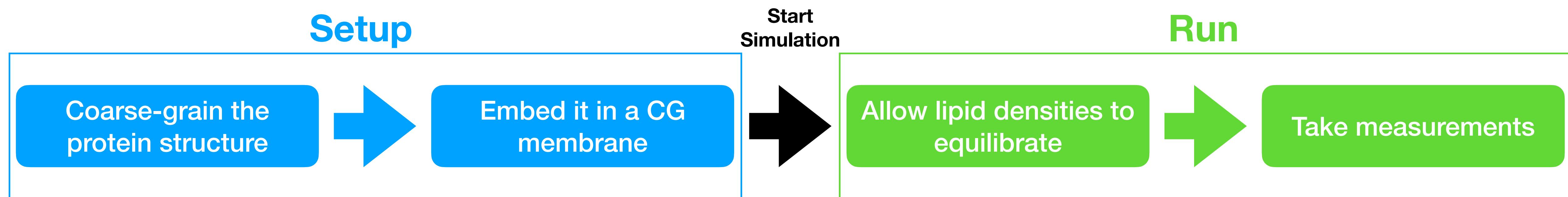
# CG-MD Workflow Map



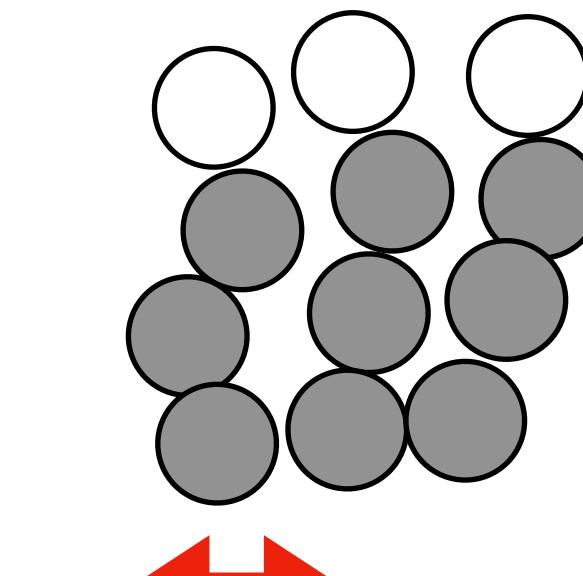
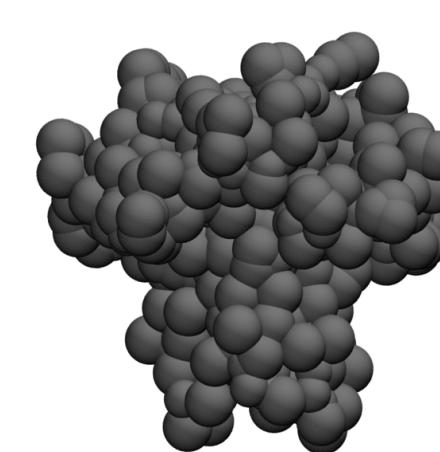
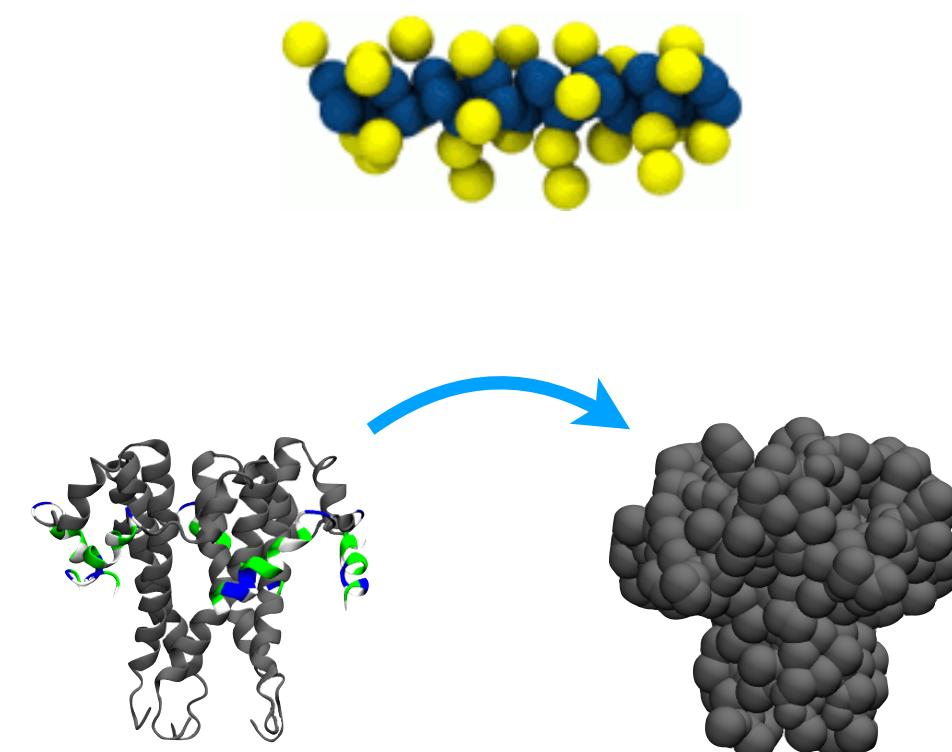
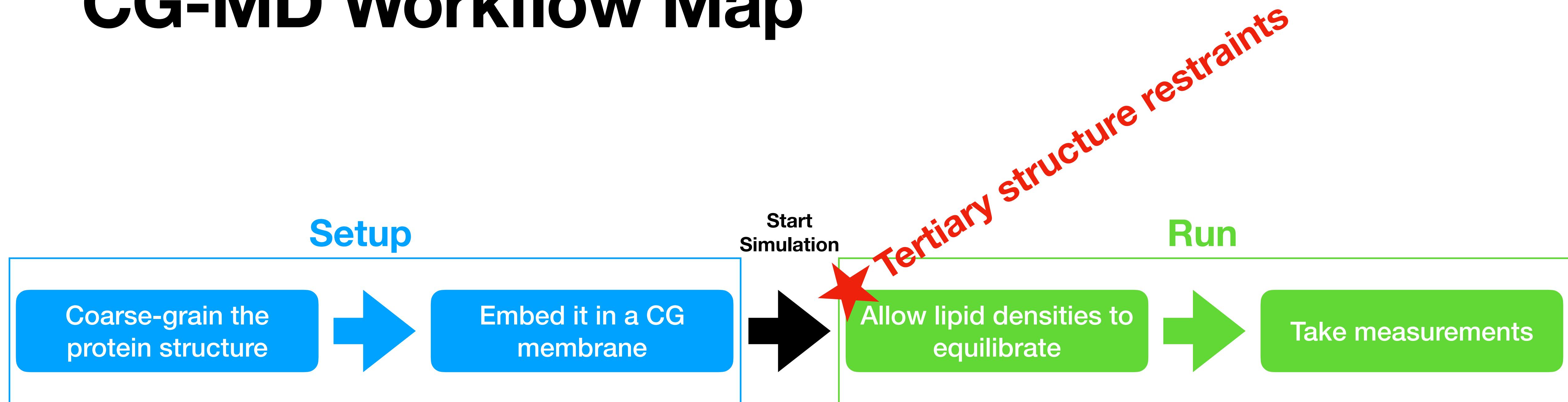
# CG-MD Workflow Map



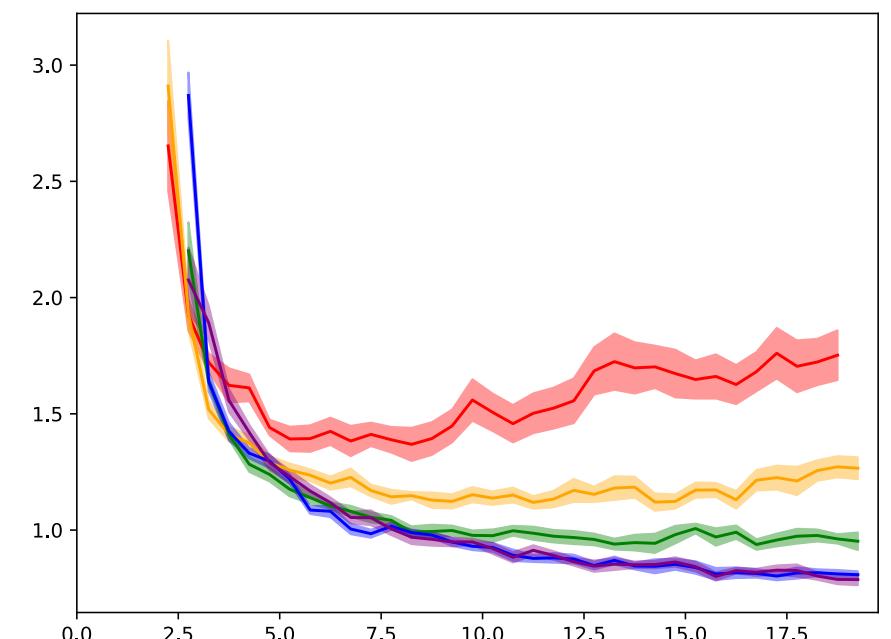
# CG-MD Workflow Map



# CG-MD Workflow Map



Area per lipid



# Tertiary structure restraint types in CG-MD

**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates

**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

# Tertiary structure restraint types in CG-MD

**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates



**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

# Tertiary structure restraint types in CG-MD

**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates

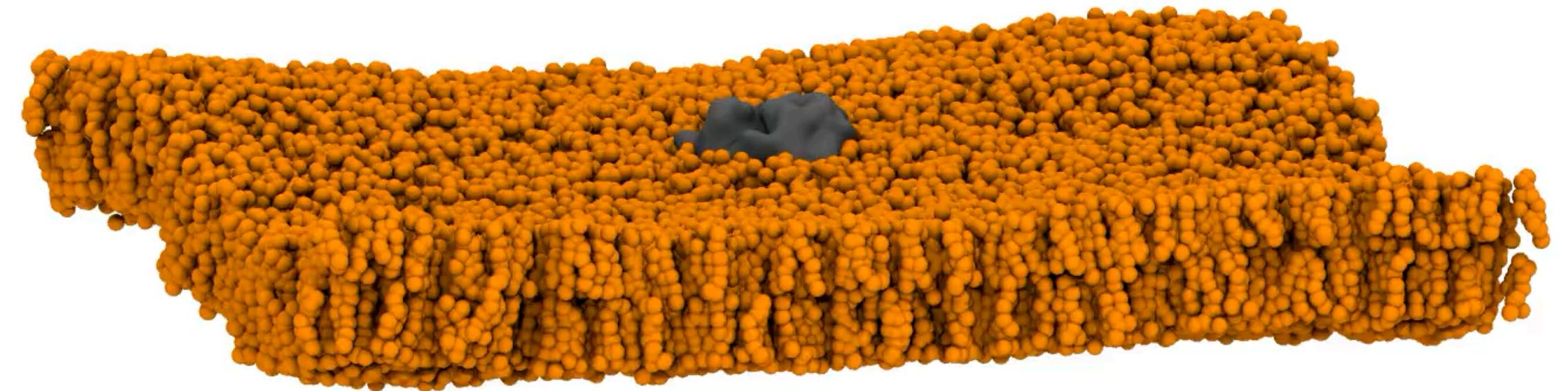


**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

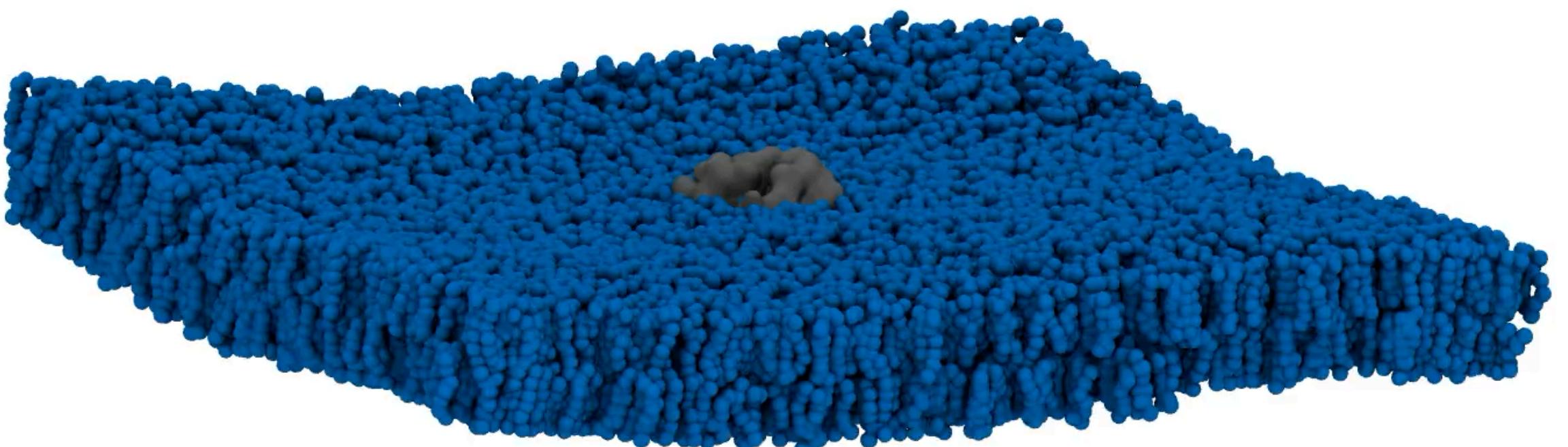


# First clue: Different restraint / Different result

**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates

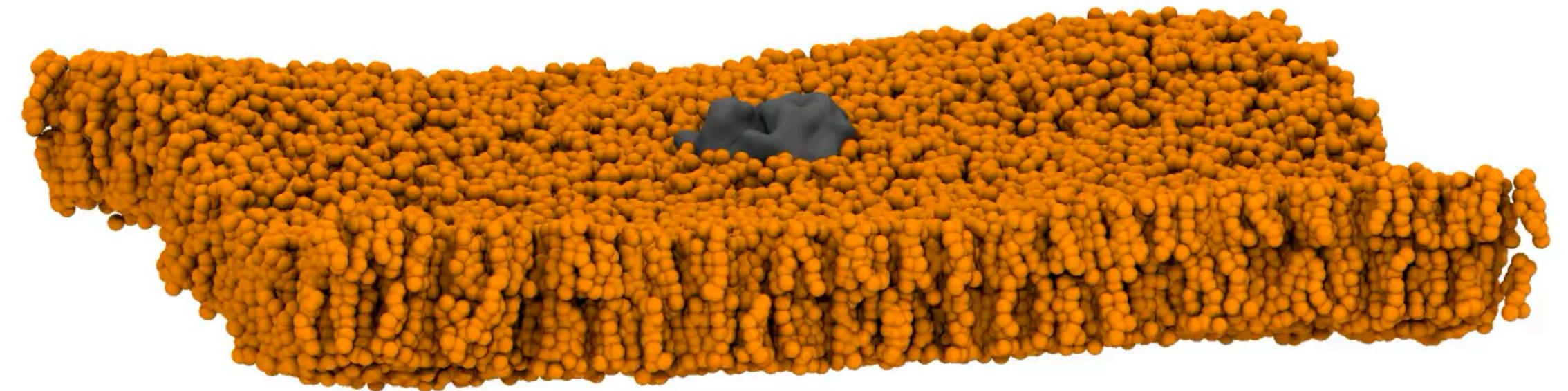


**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

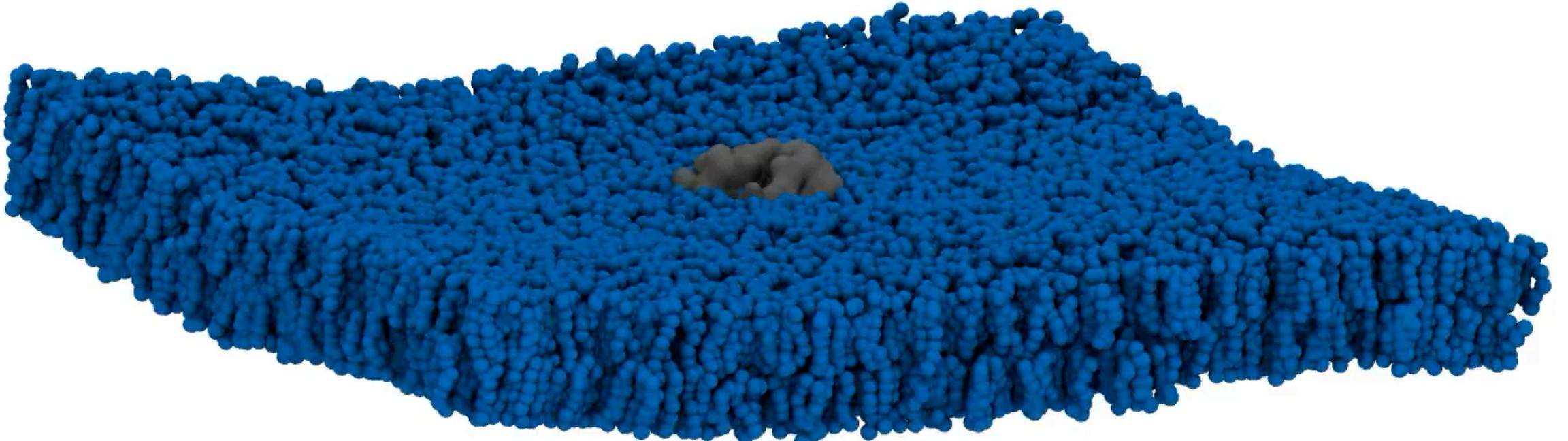


# First clue: Different restraint / Different result

**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates

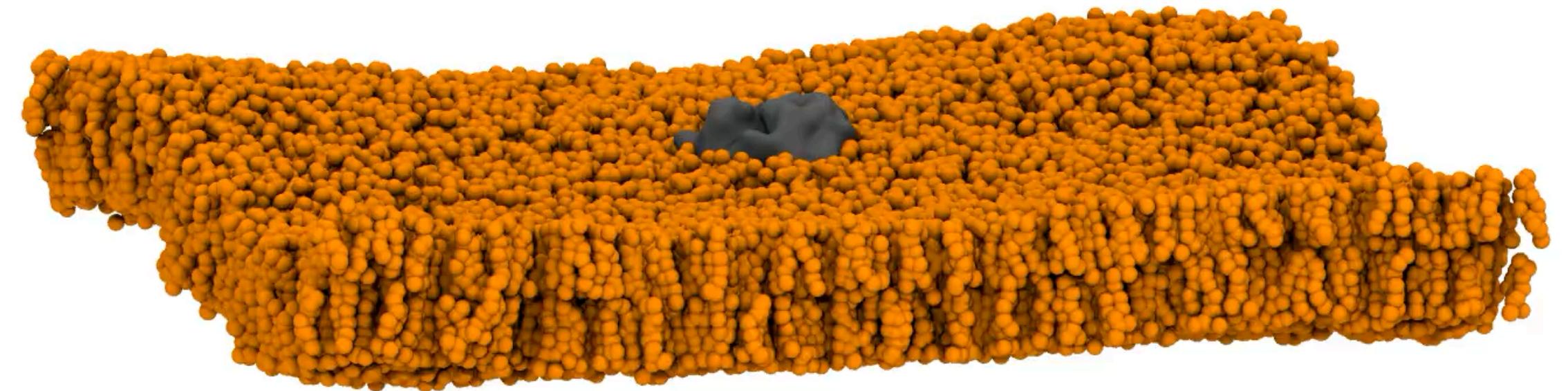


**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

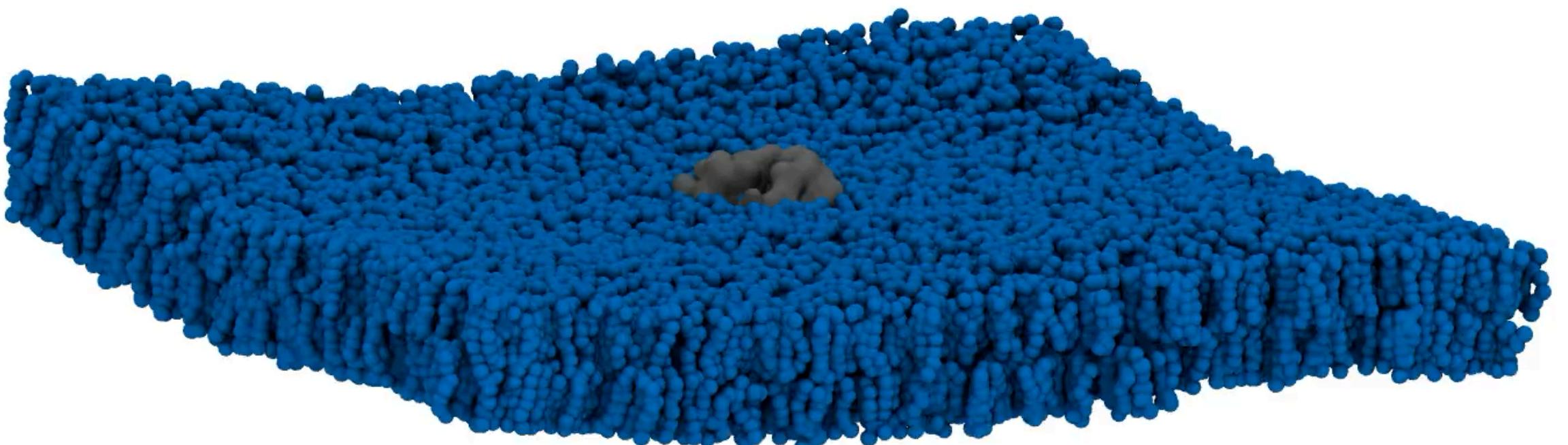


# First clue: Different restraint / Different result

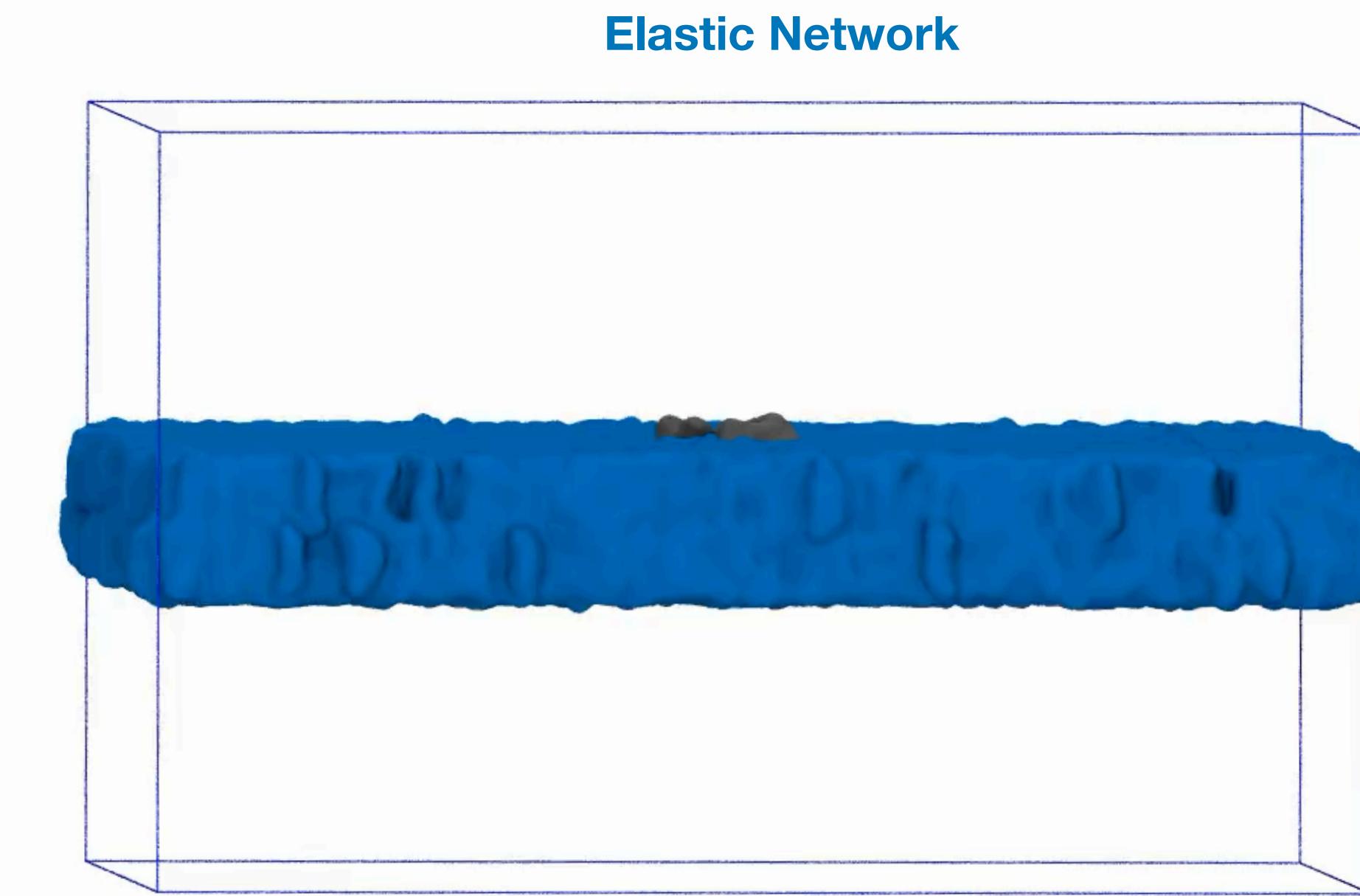
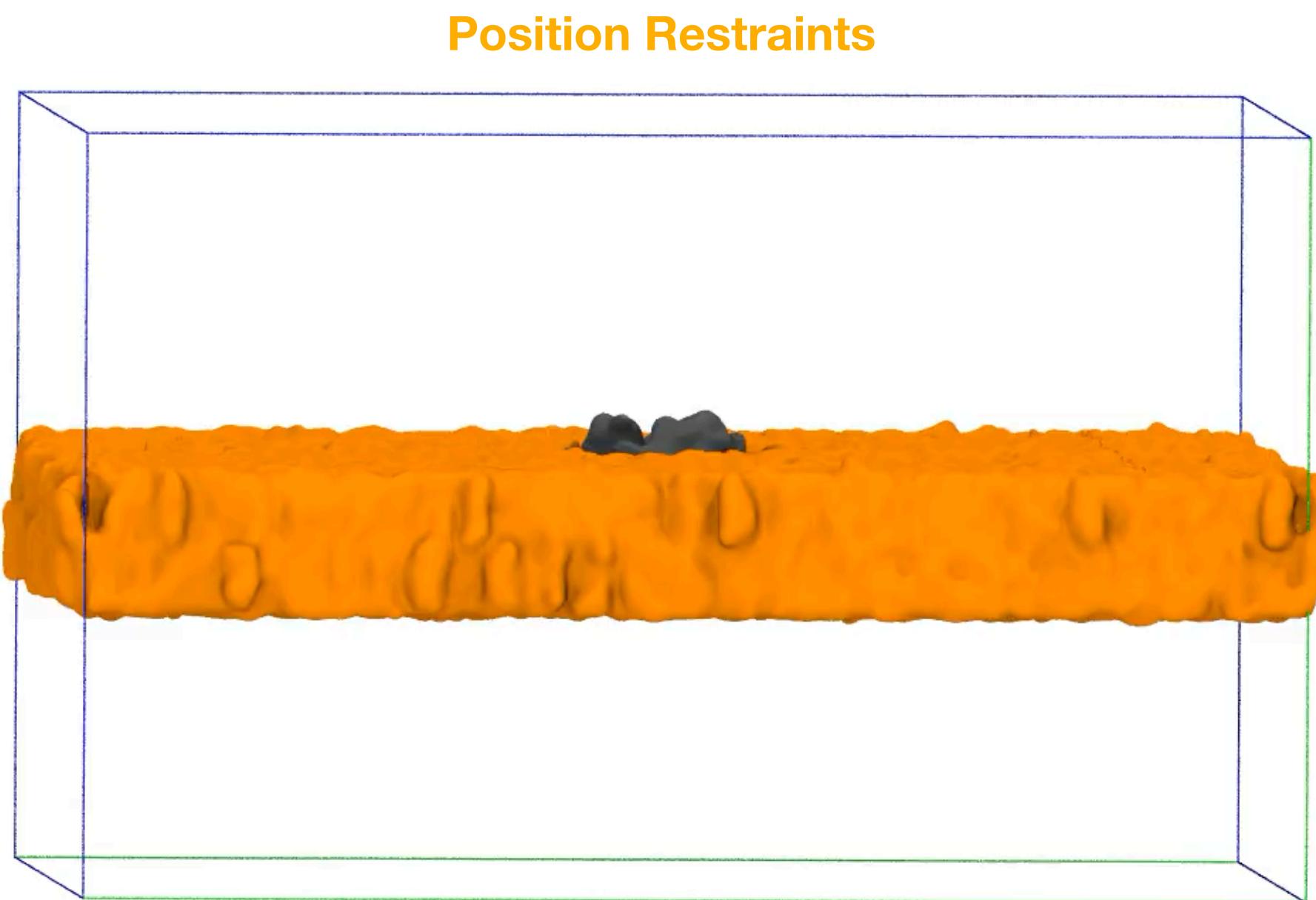
**Position restraints:** Each backbone bead is “bonded” to its starting position in XYZ coordinates



**Elastic Network** restraints: Each backbone bead is “bonded” to its nearest neighbors

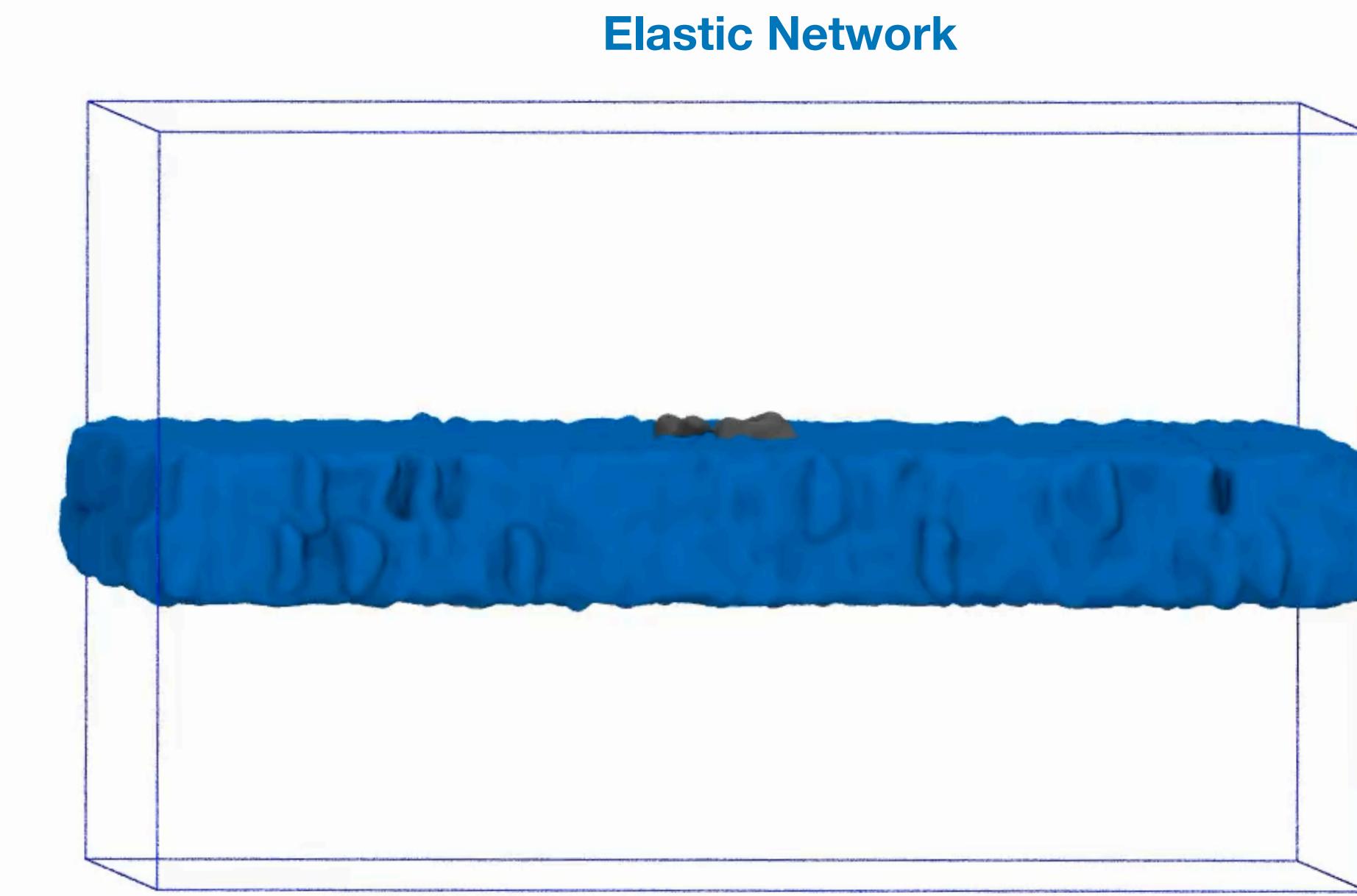
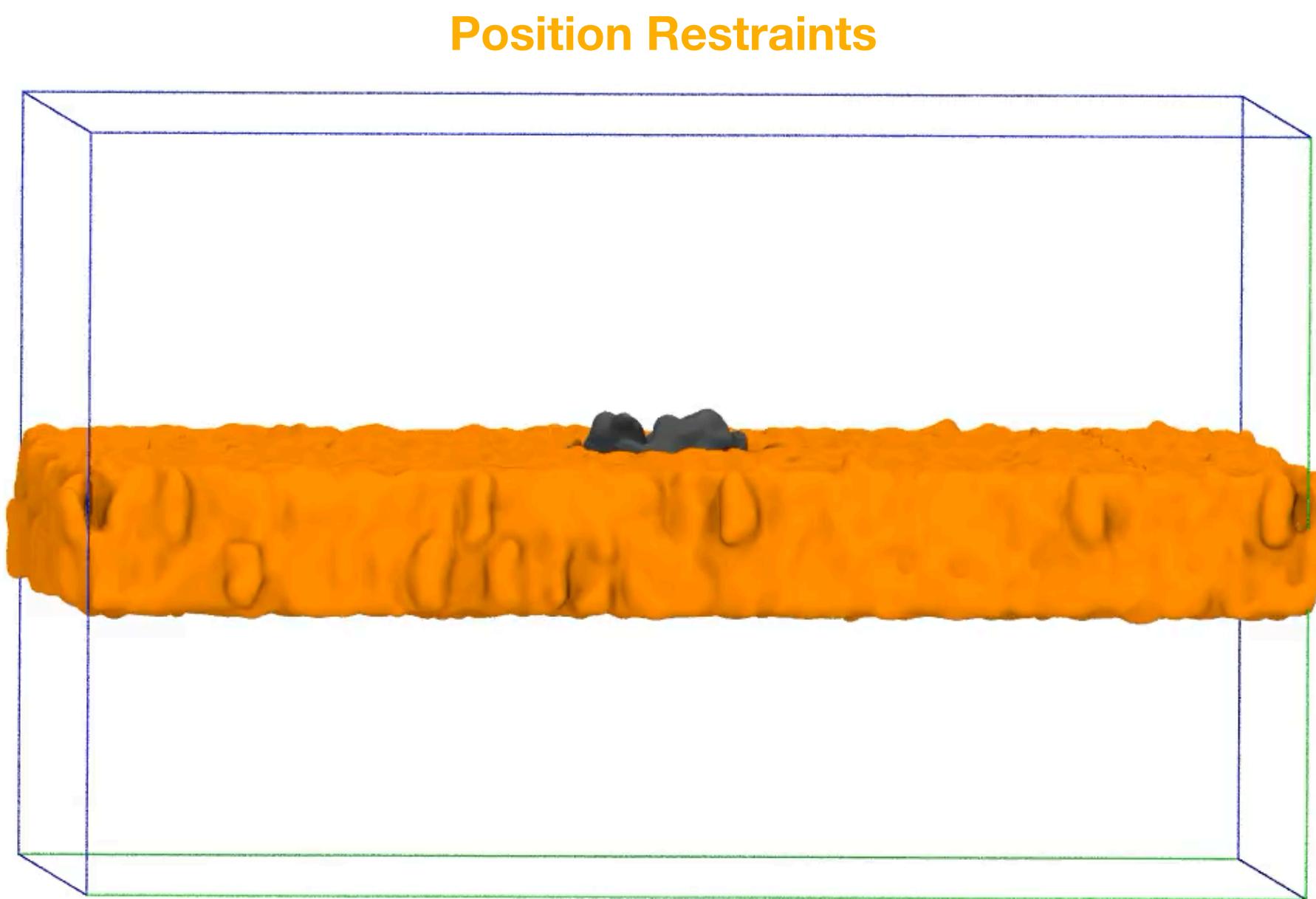


# Box fluctuations abnormally damped in position restraint system



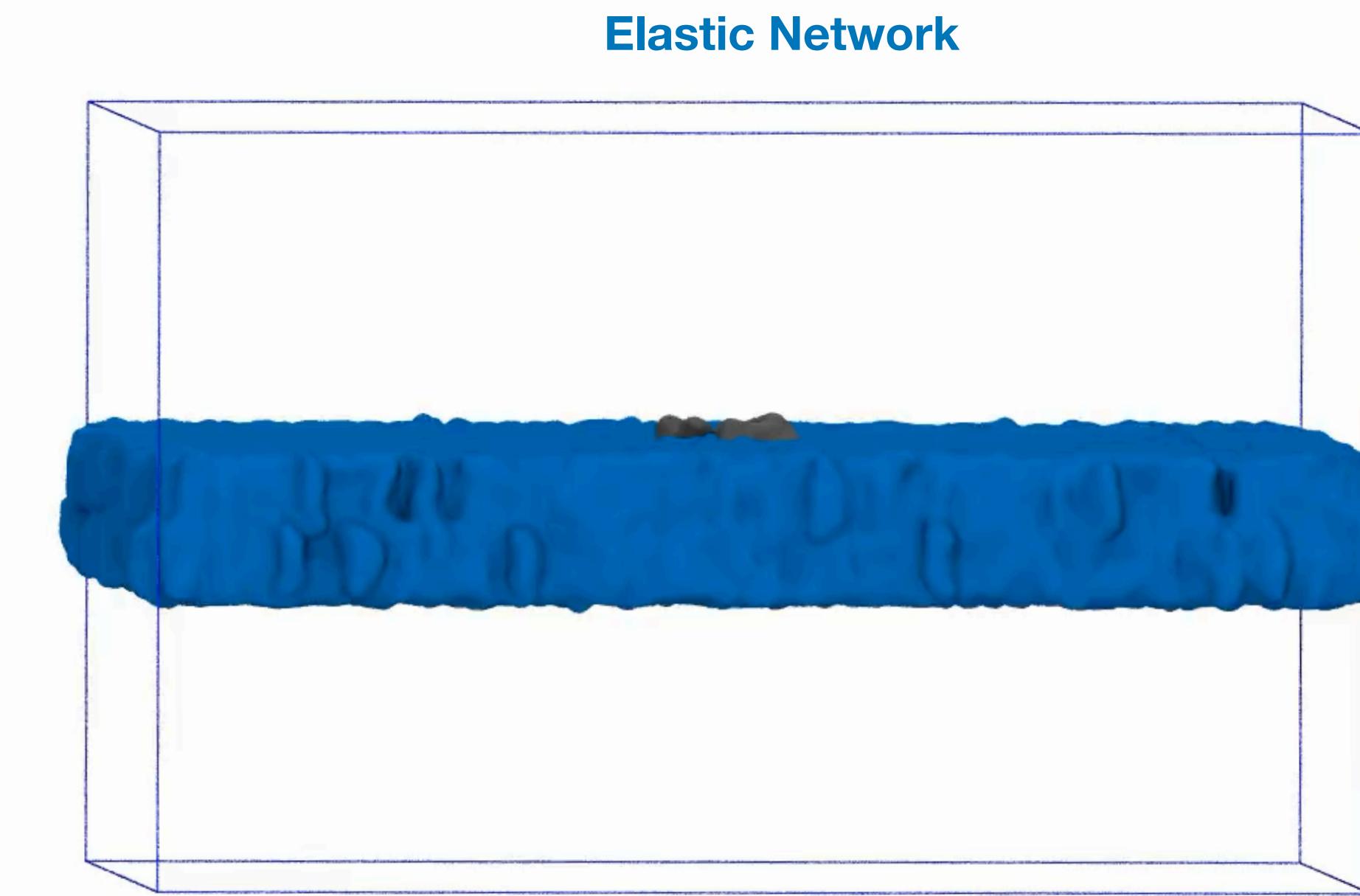
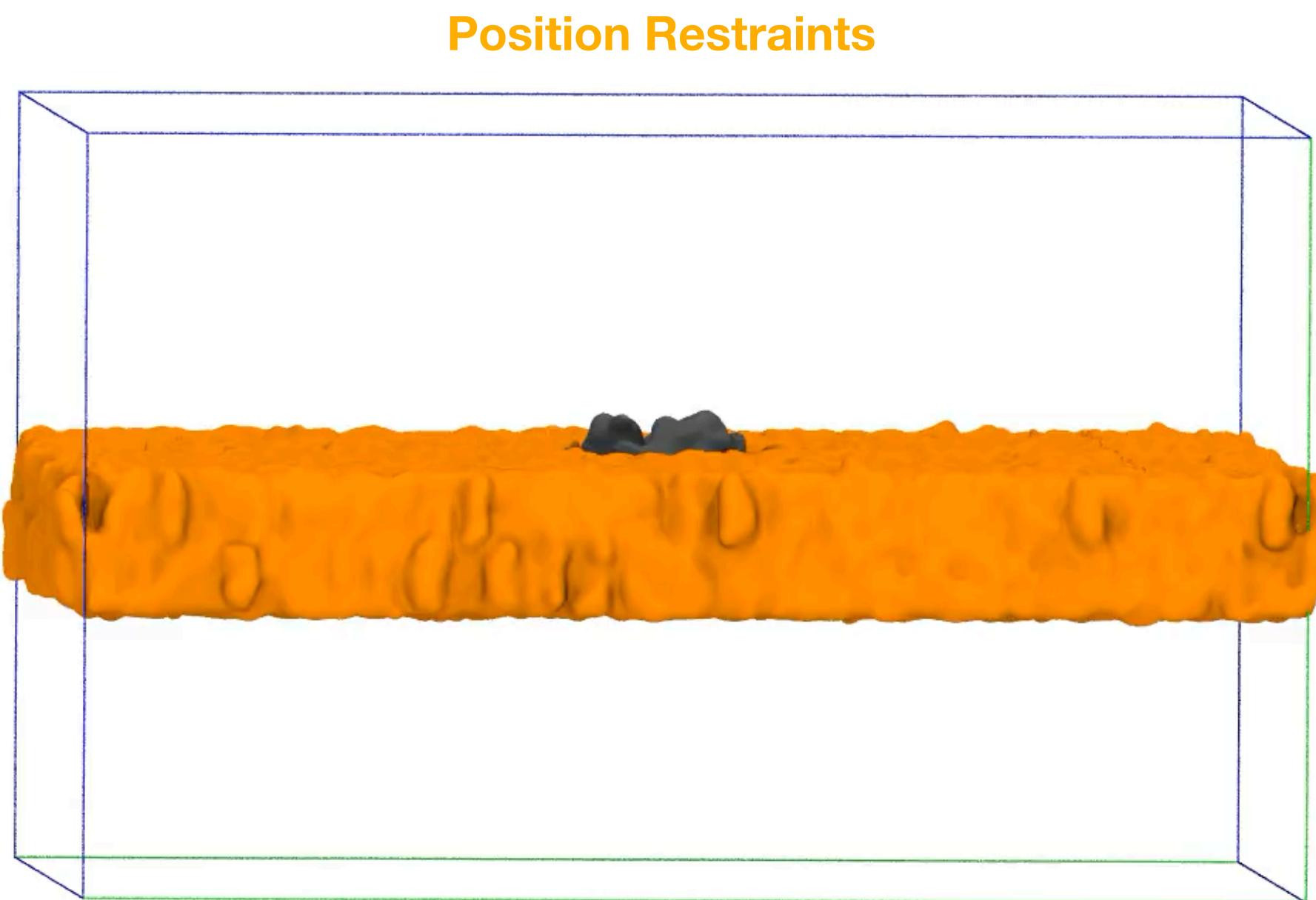
Initial Box Size  
Actual Box Size

# Box fluctuations abnormally damped in position restraint system



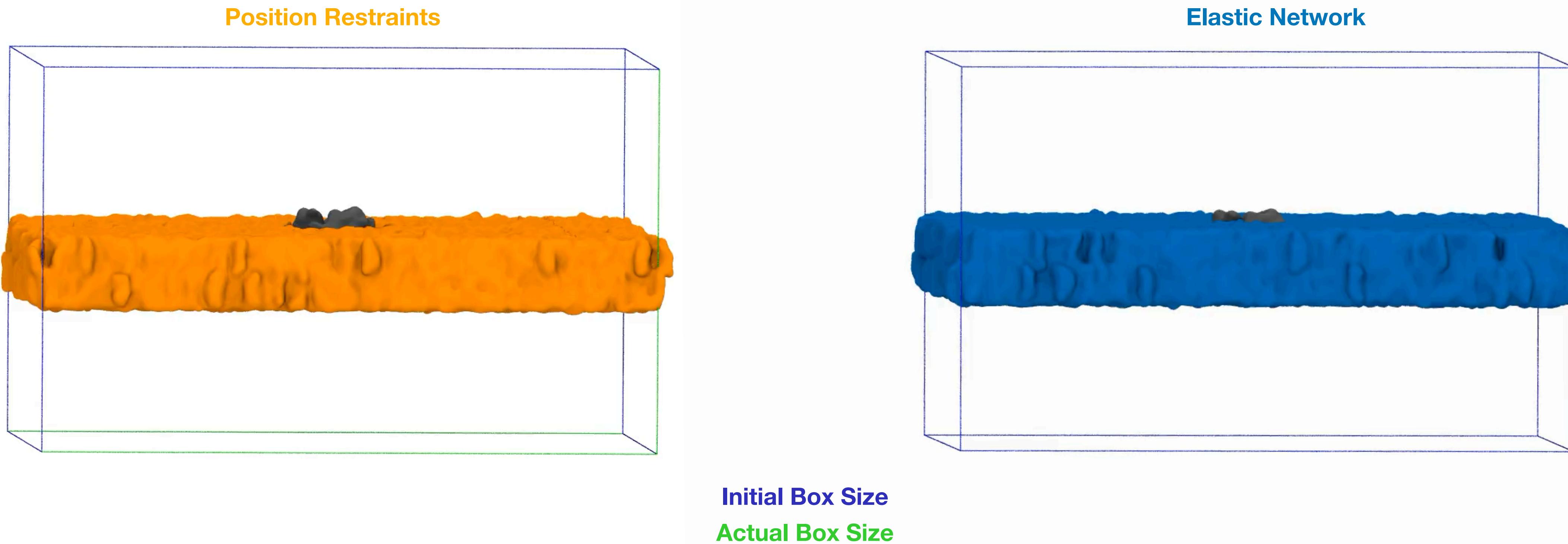
Initial Box Size  
Actual Box Size

# Box fluctuations abnormally damped in position restraint system



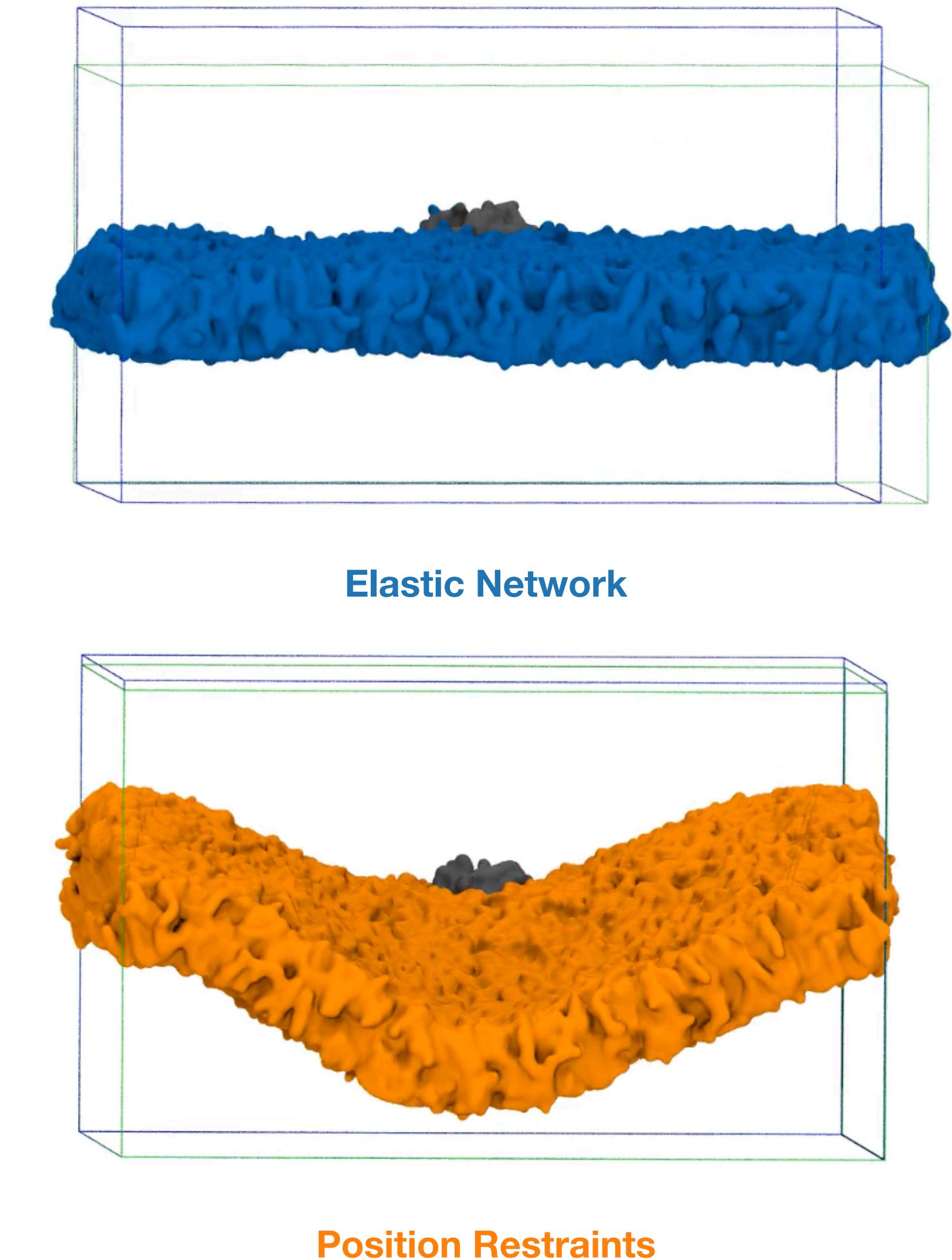
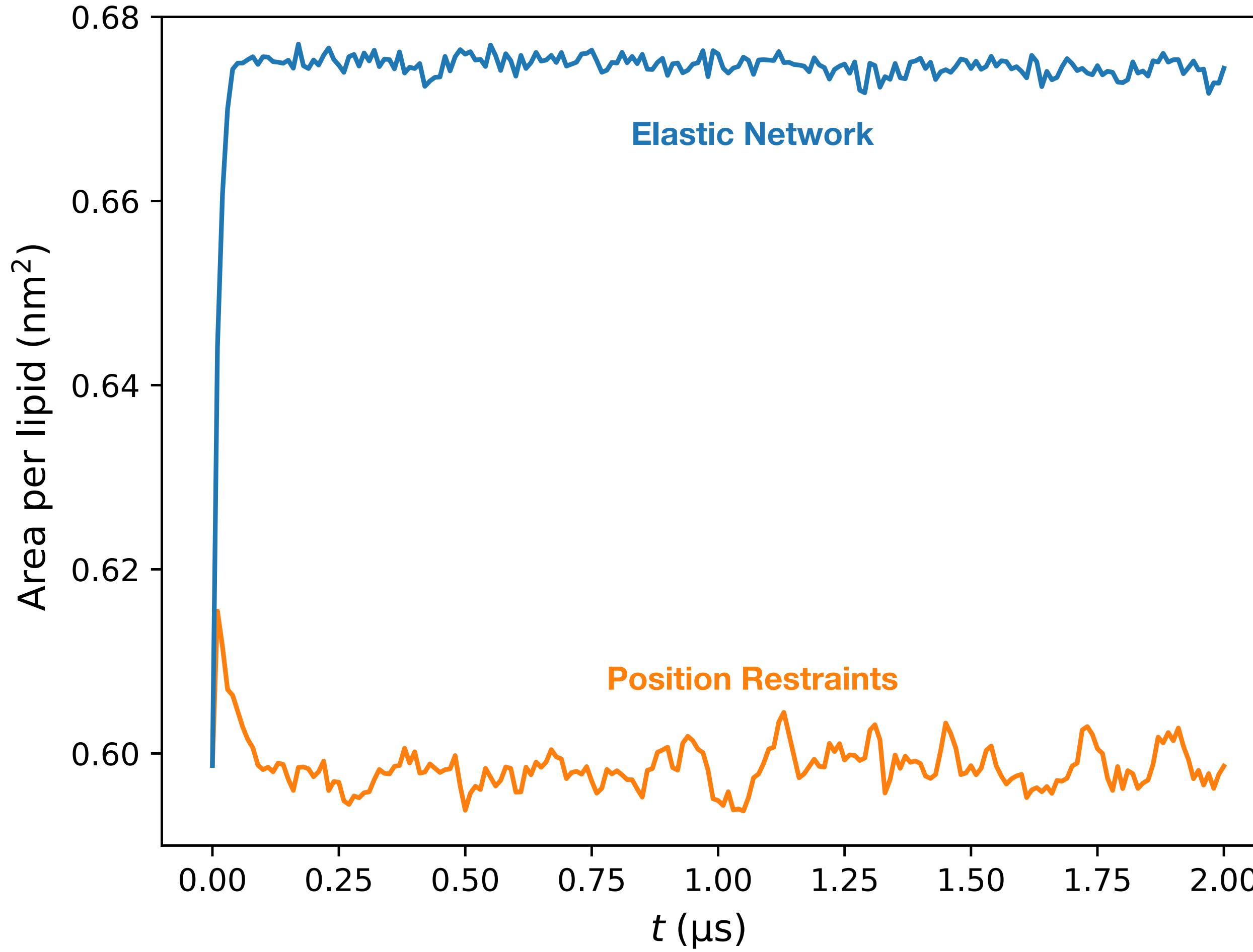
Initial Box Size  
Actual Box Size

# Box fluctuations abnormally damped in position restraint system

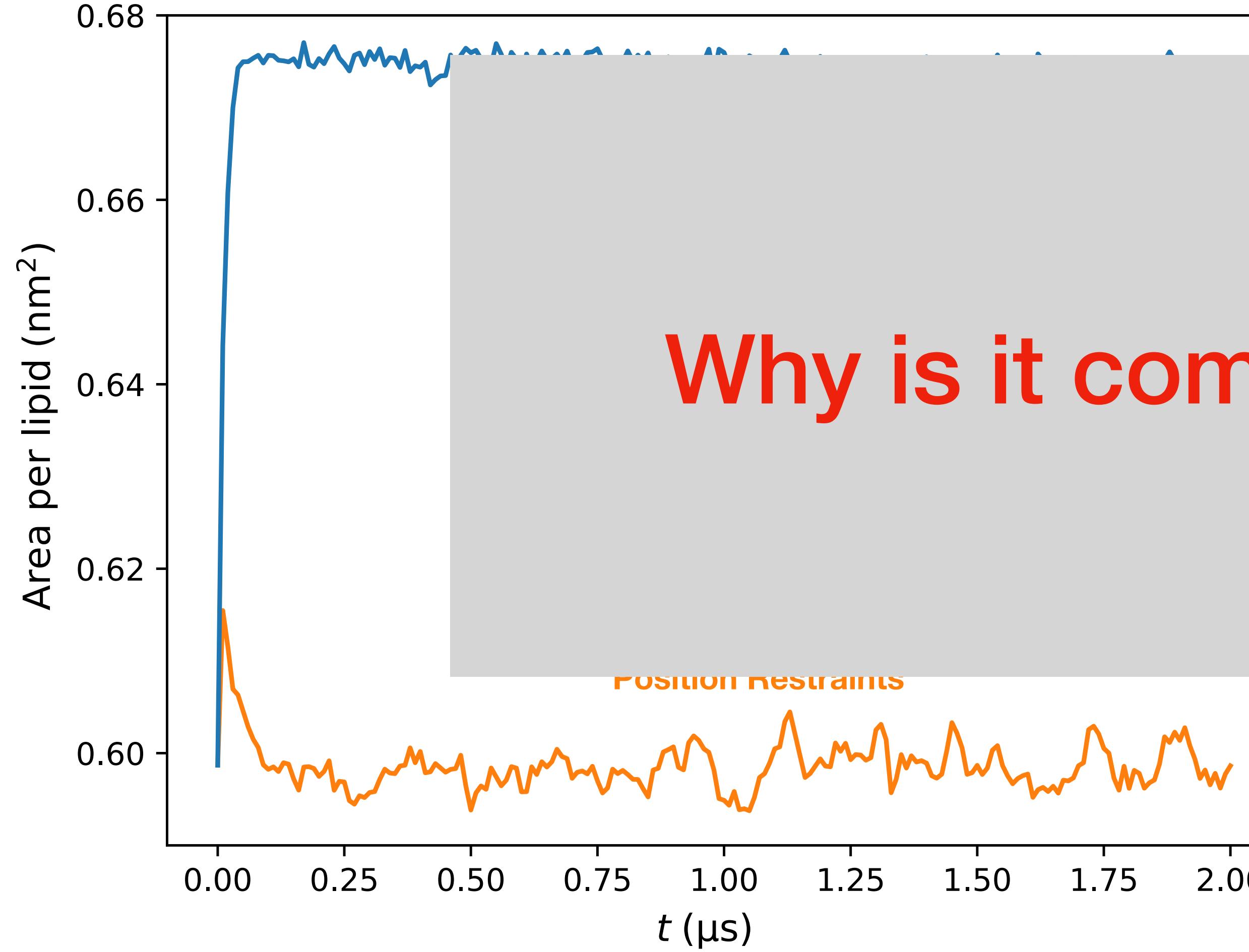


The box isn't fluctuating the way it is supposed to in our systems that use position restraints!

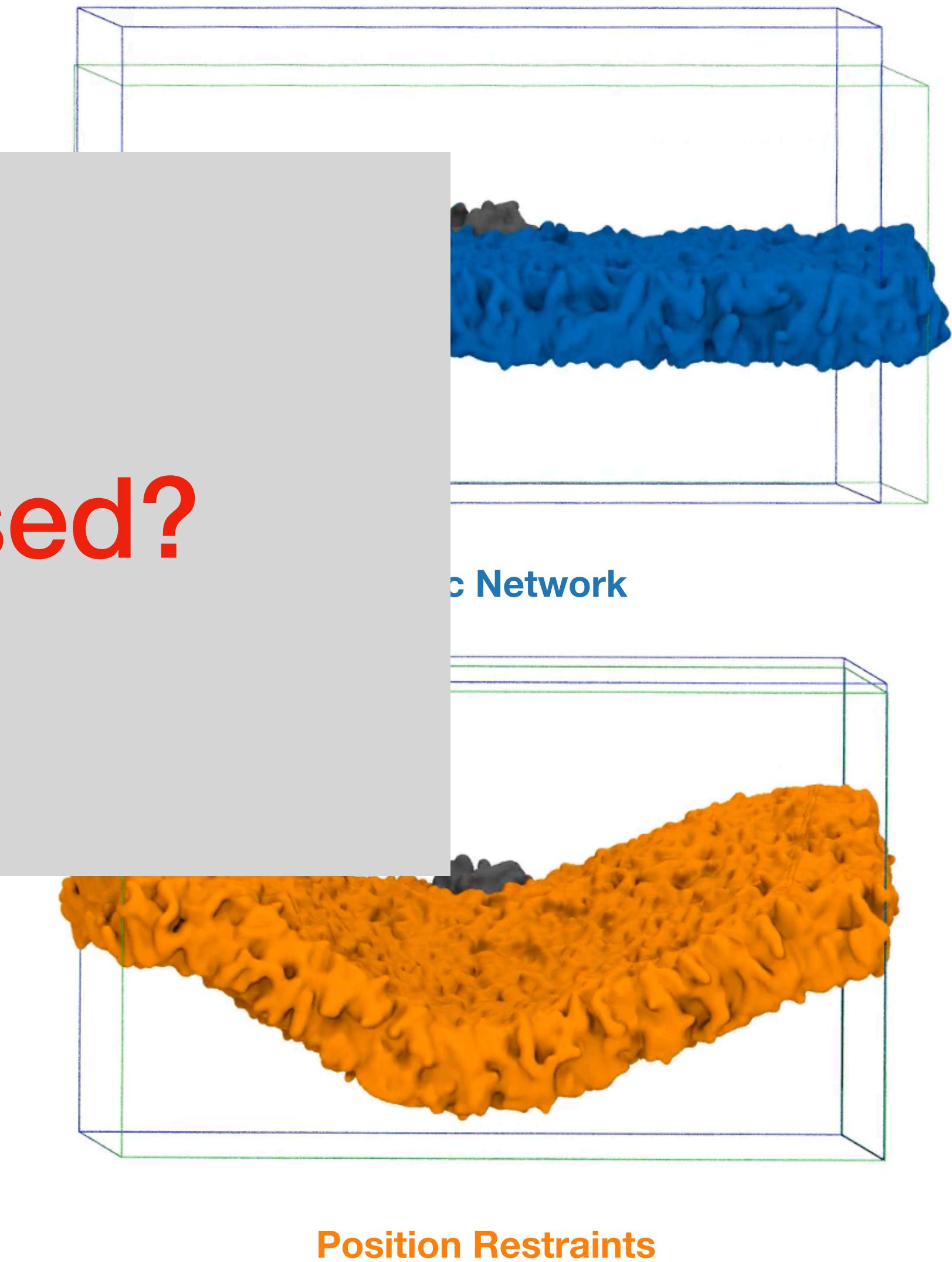
# The membrane is bending because it is compressed



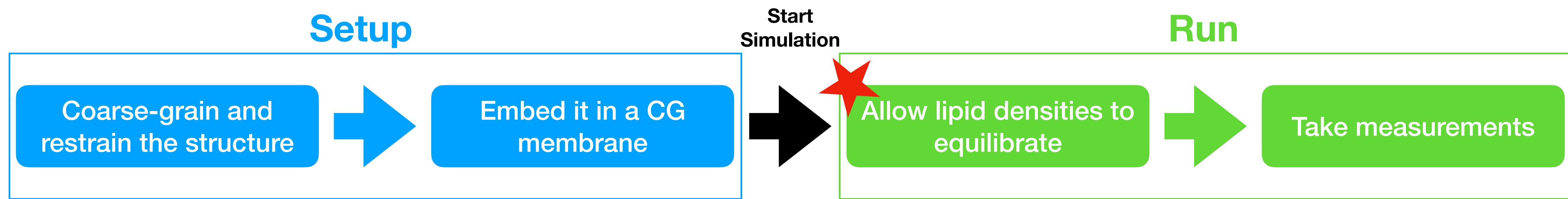
# The membrane is bending because it is compressed



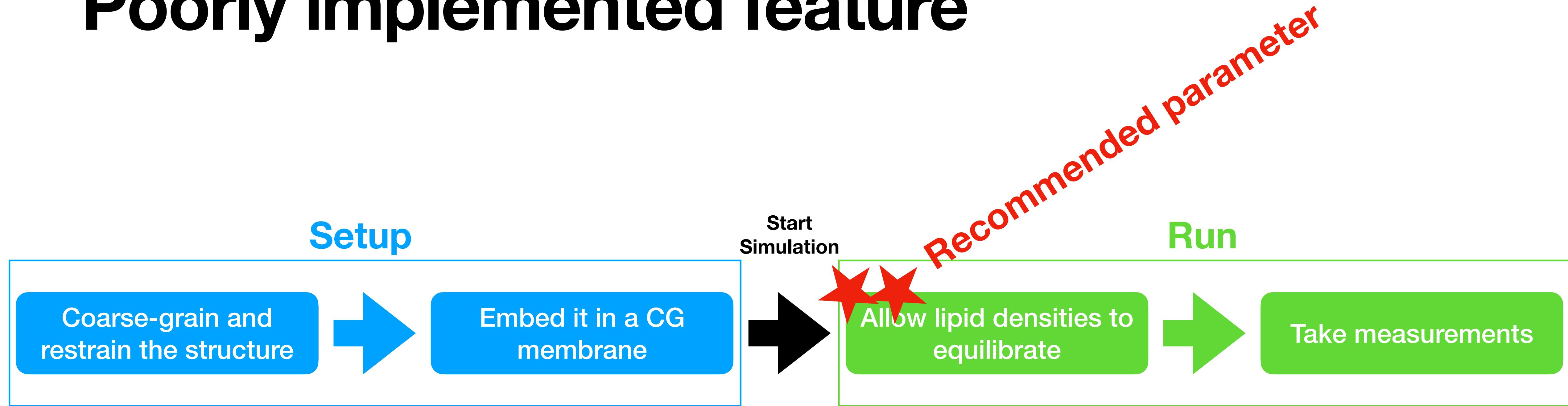
Why is it compressed?



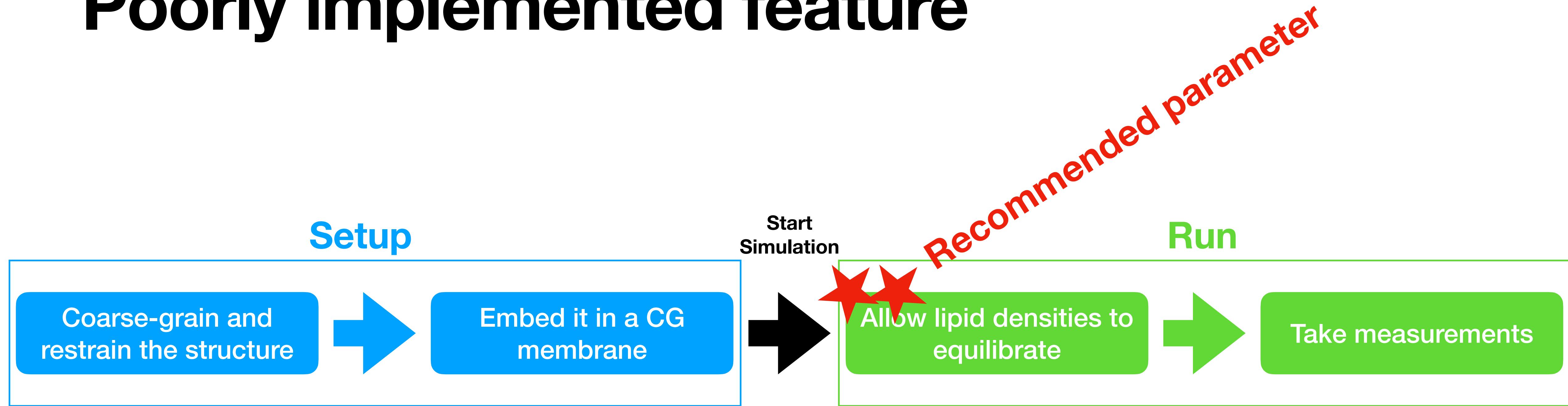
# Poorly implemented feature



# Poorly implemented feature

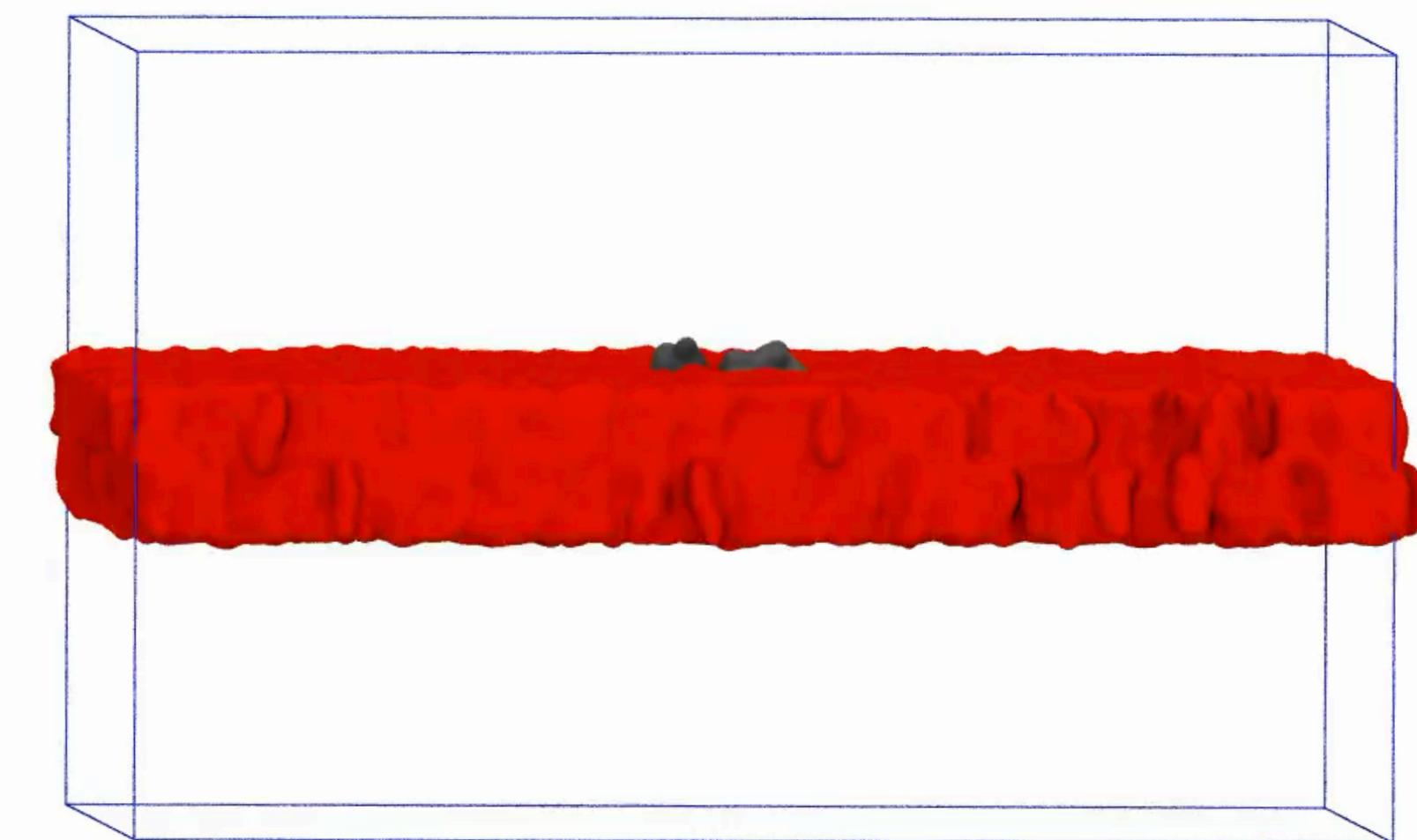
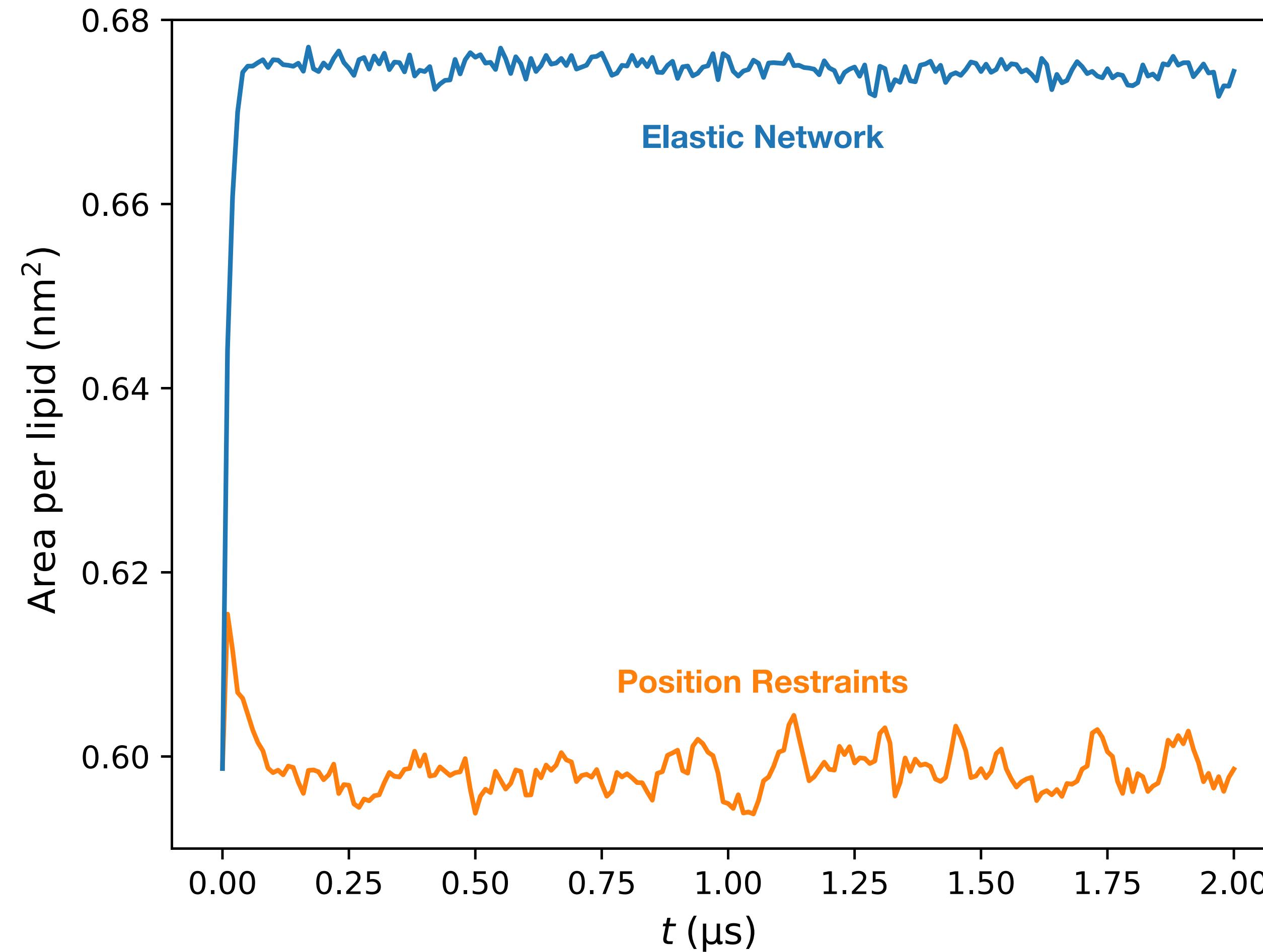


# Poorly implemented feature



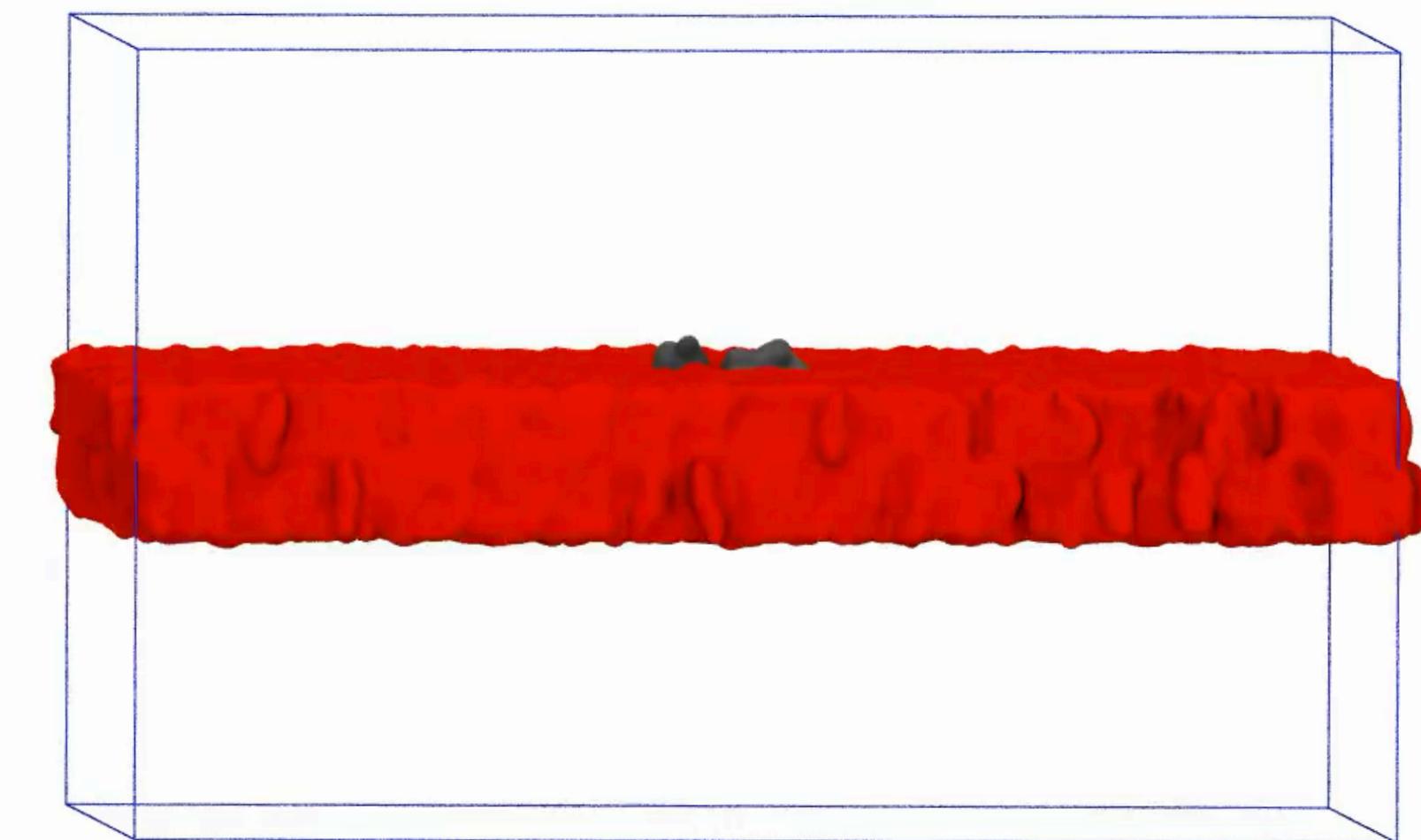
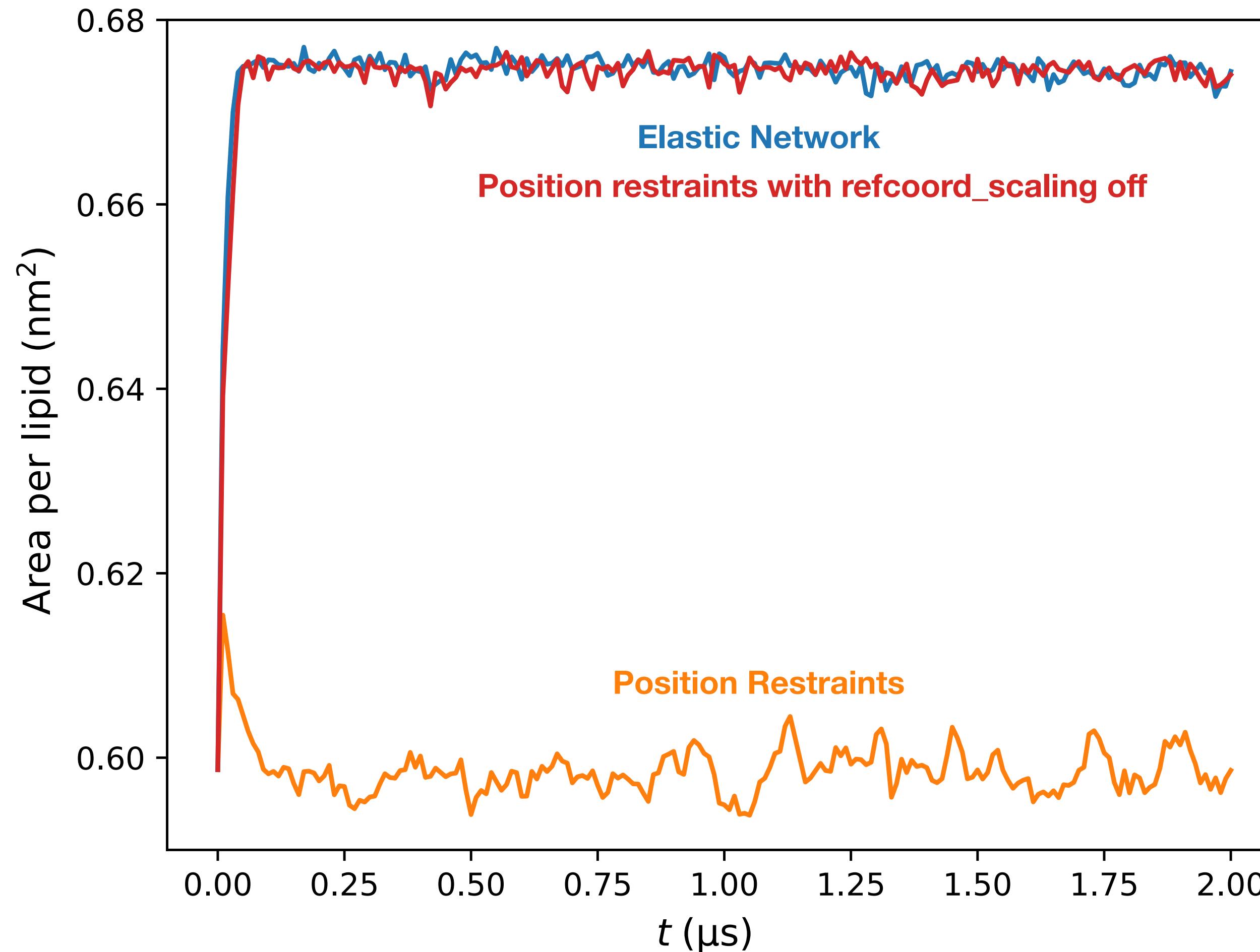
**Feature designed to speed up  
equilibration actually breaks  
simulation barostat**

# Turning the feature off fixes the bending



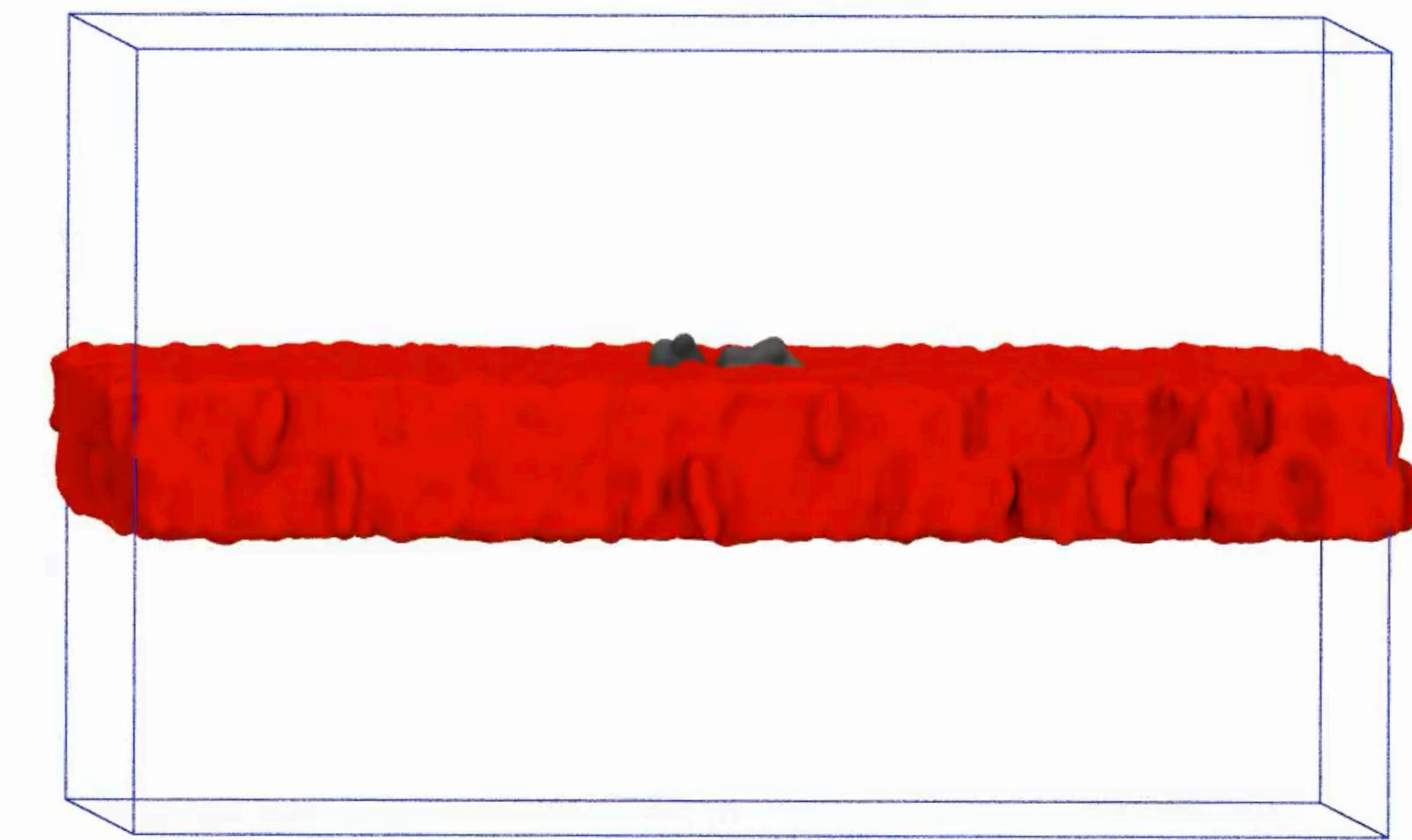
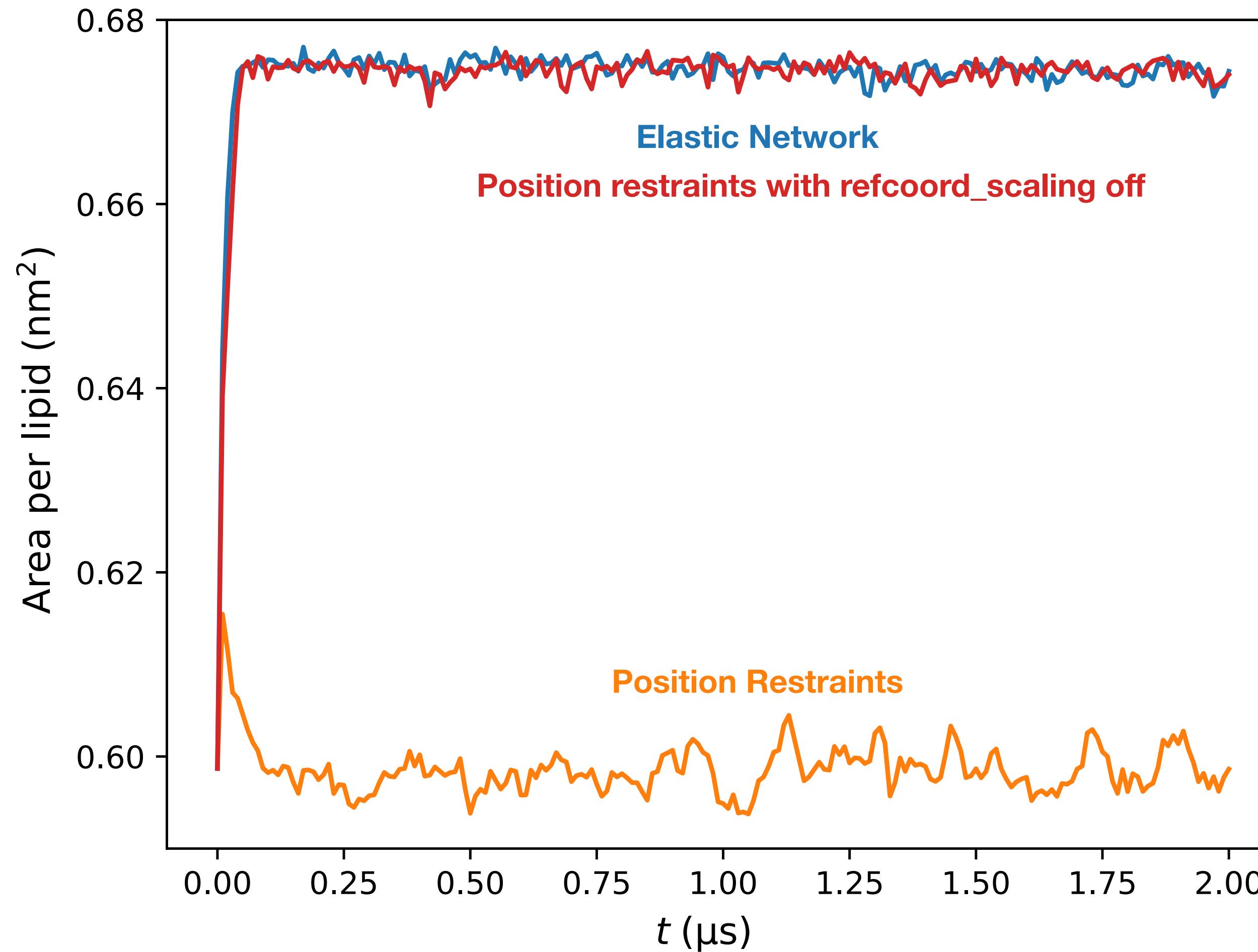
Position restraints with `refcoord_scaling` off

# Turning the feature off fixes the bending



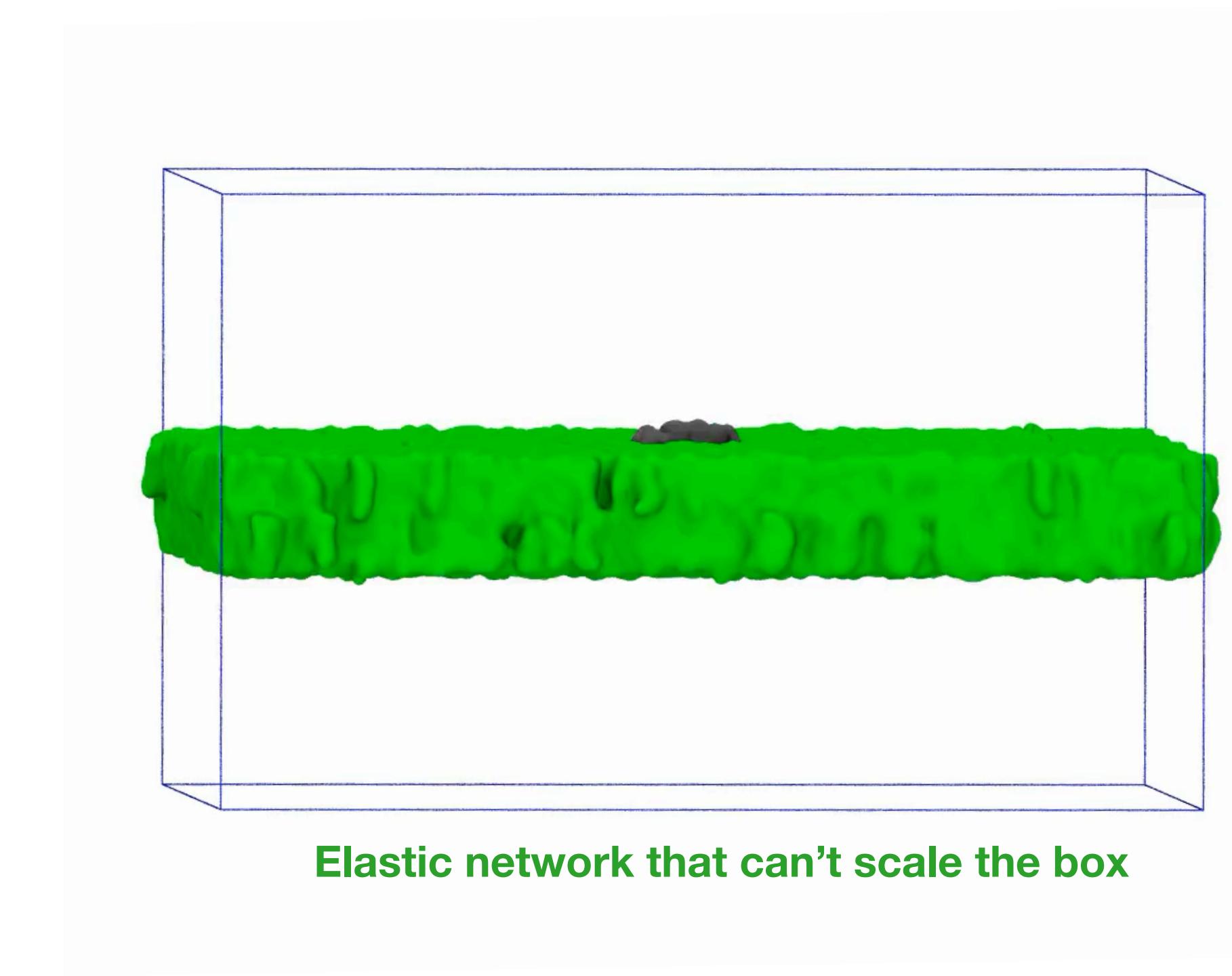
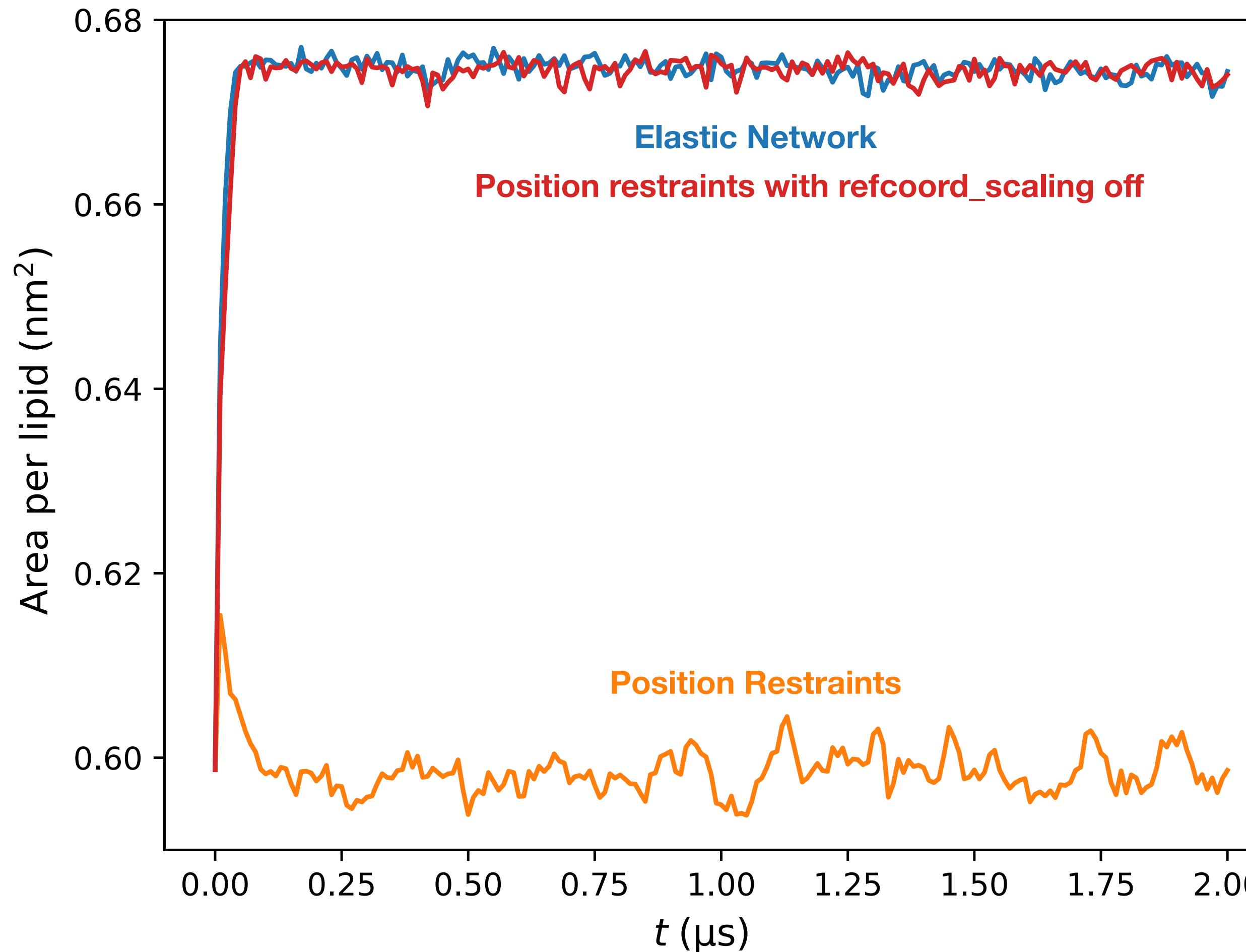
Position restraints with `refcoord_scaling` off

# Turning the feature off fixes the bending

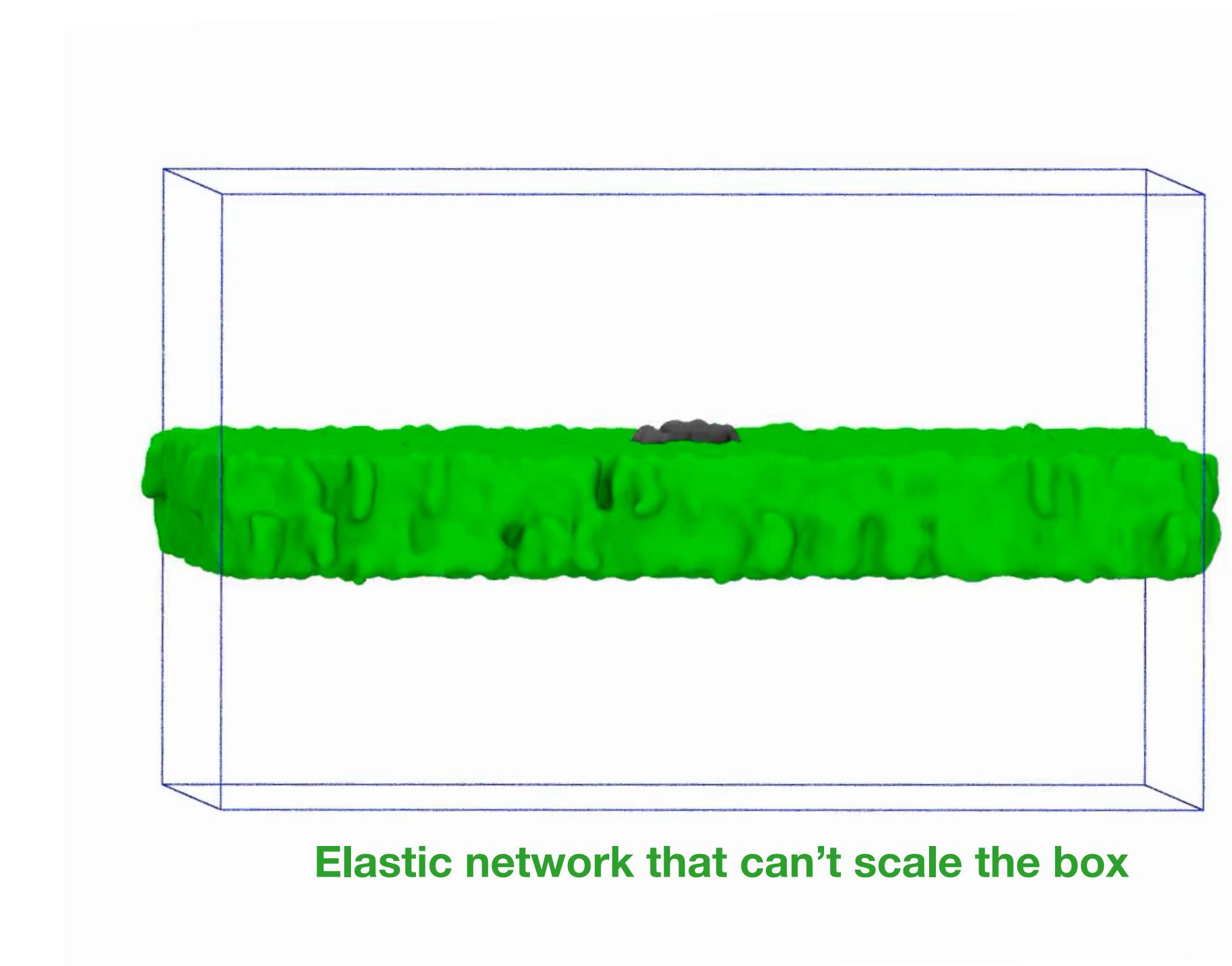
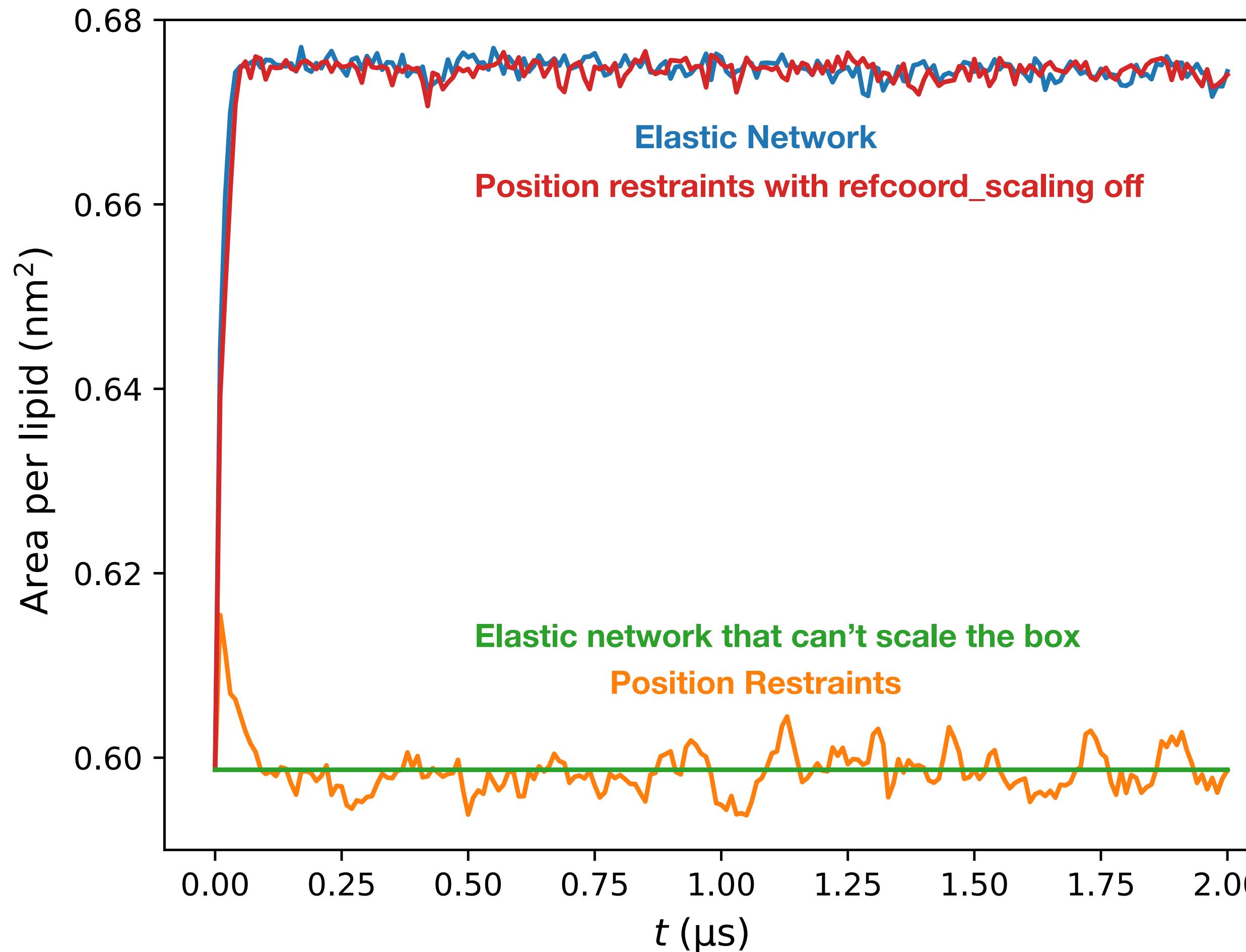


Position restraints with `refcoord_scaling` off

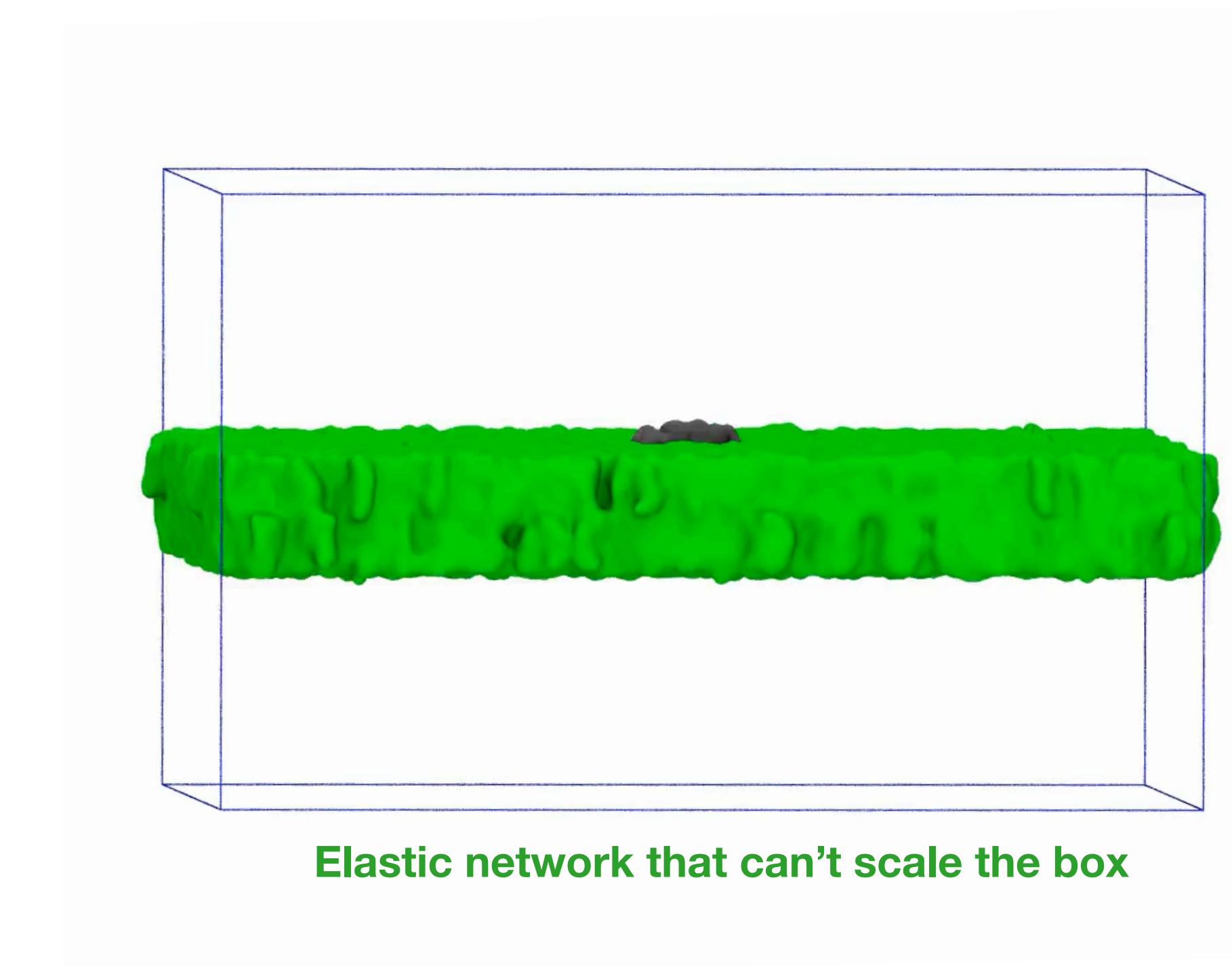
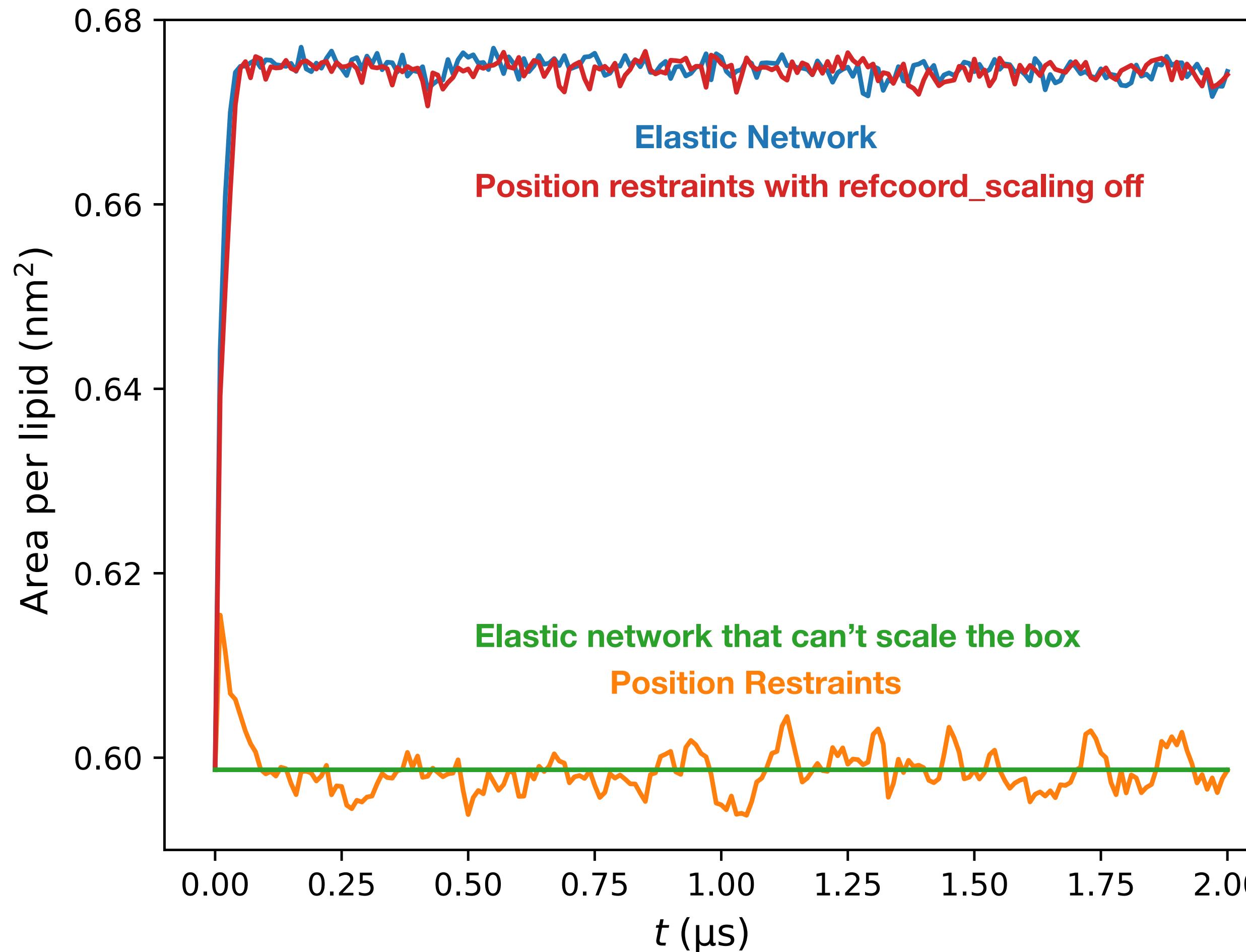
# Preventing box scaling causes bending



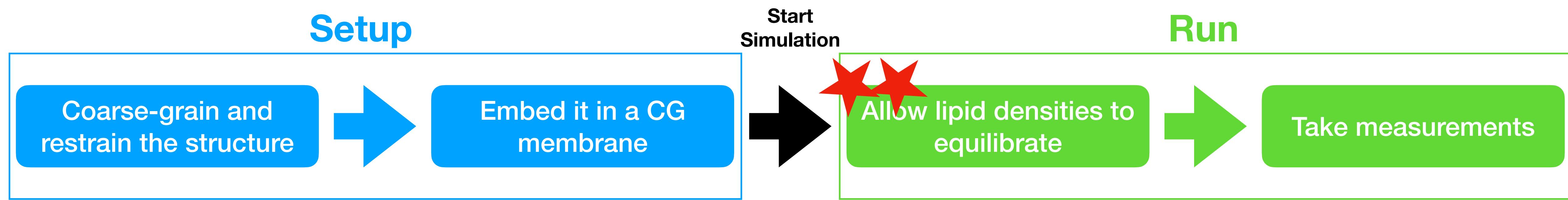
# Preventing box scaling causes bending



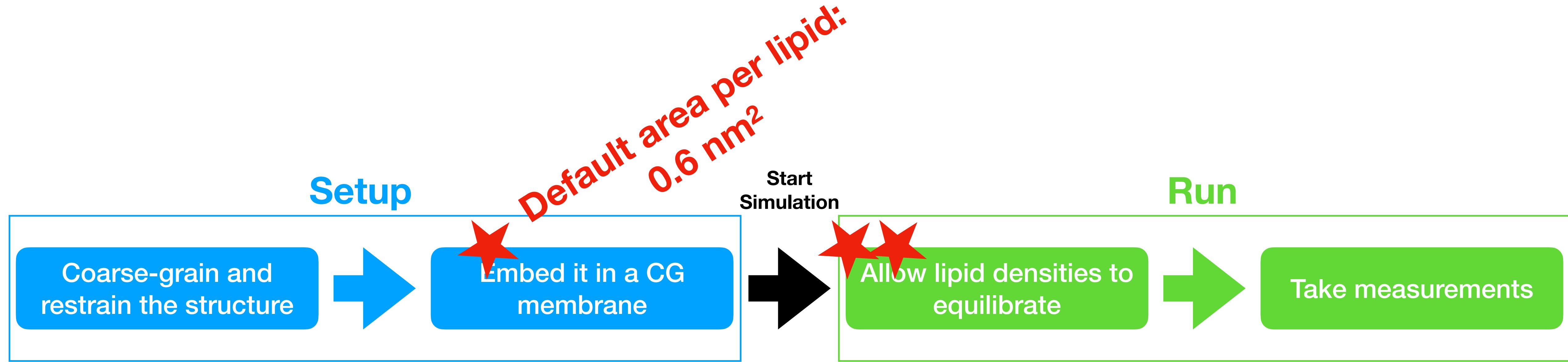
# Preventing box scaling causes bending



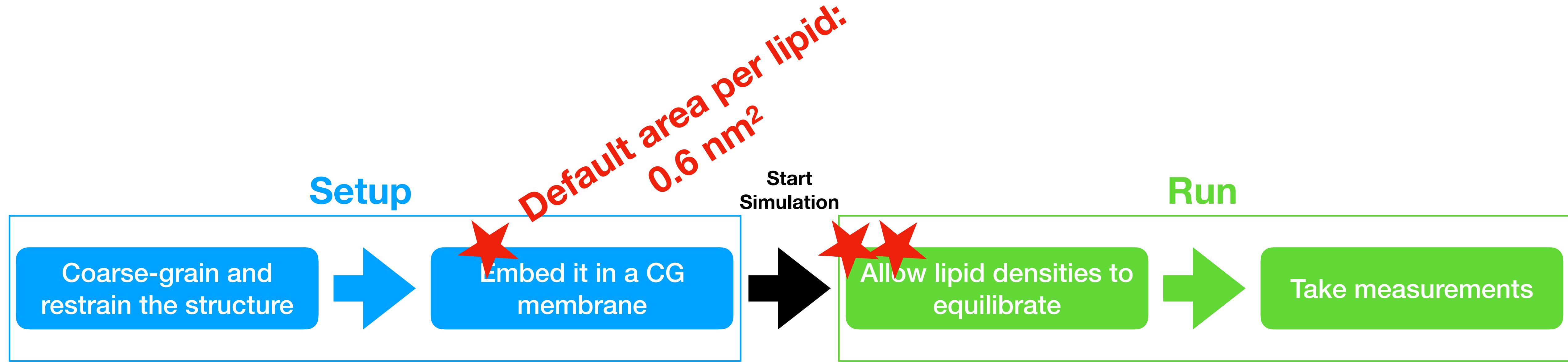
# Why did only unsaturated systems bend?



# Why did only unsaturated systems bend?

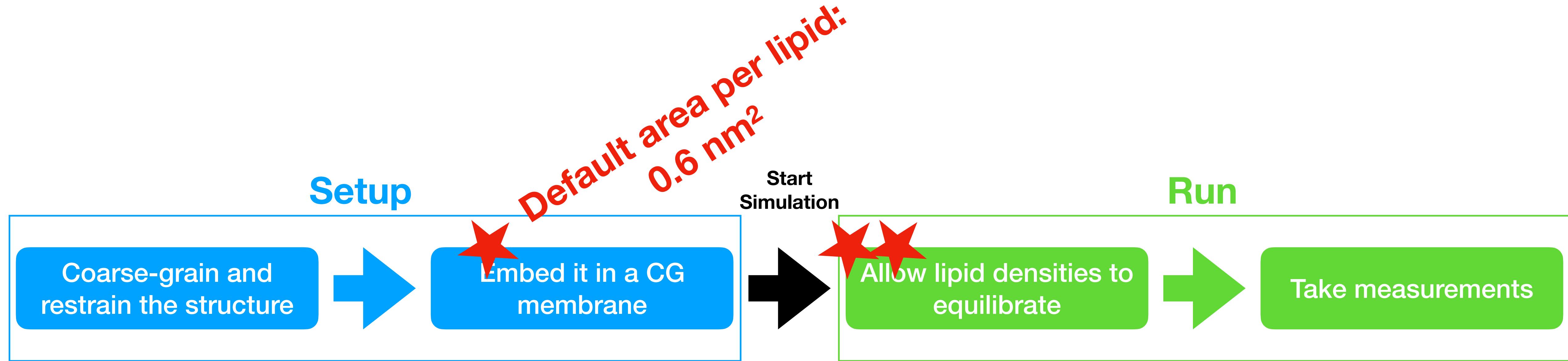


# Why did only unsaturated systems bend?

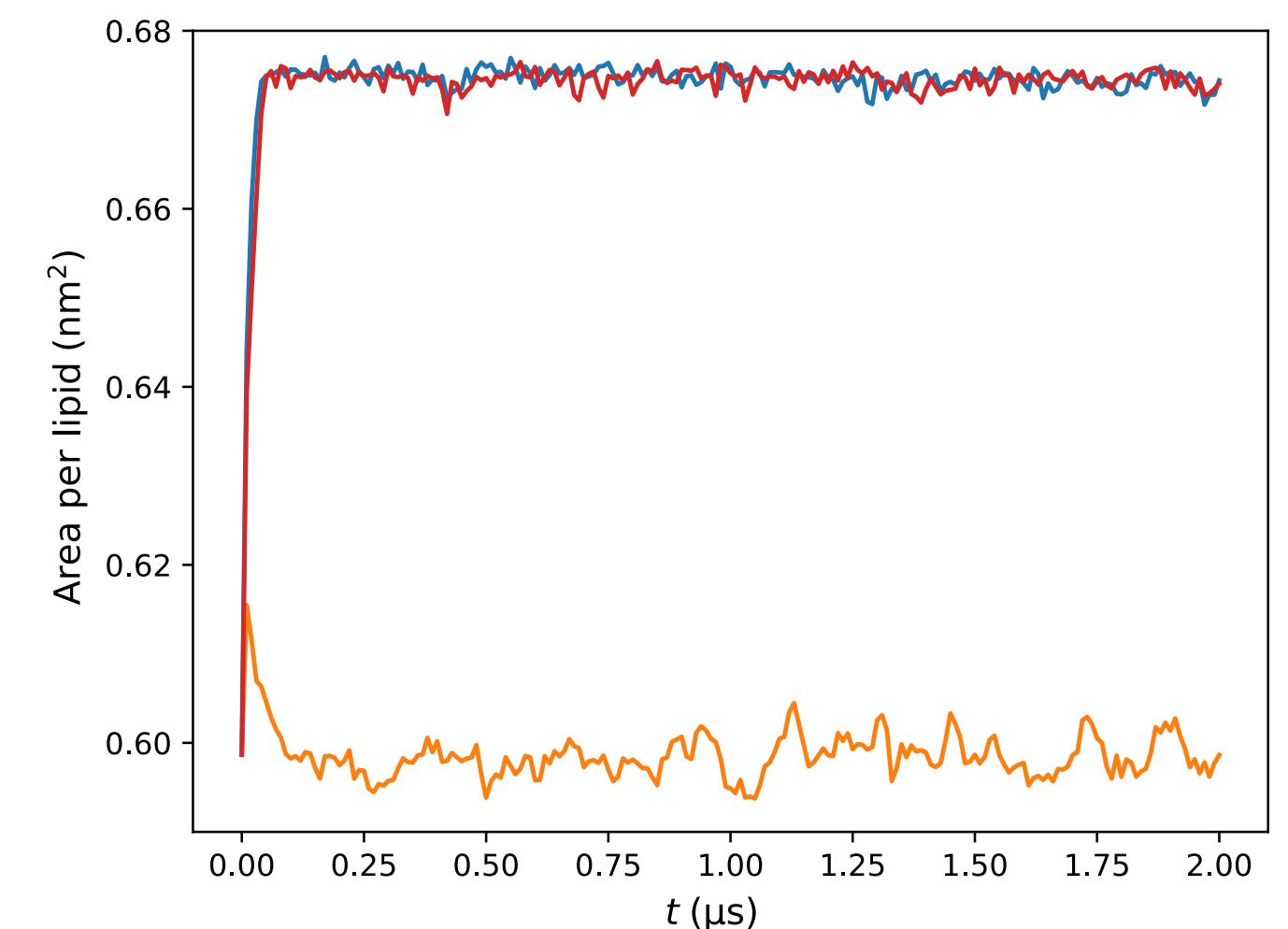


0.6 nm<sup>2</sup> is a good approx. for  
saturated lipids;  
unsaturated lipids have larger  
equilibrium areas

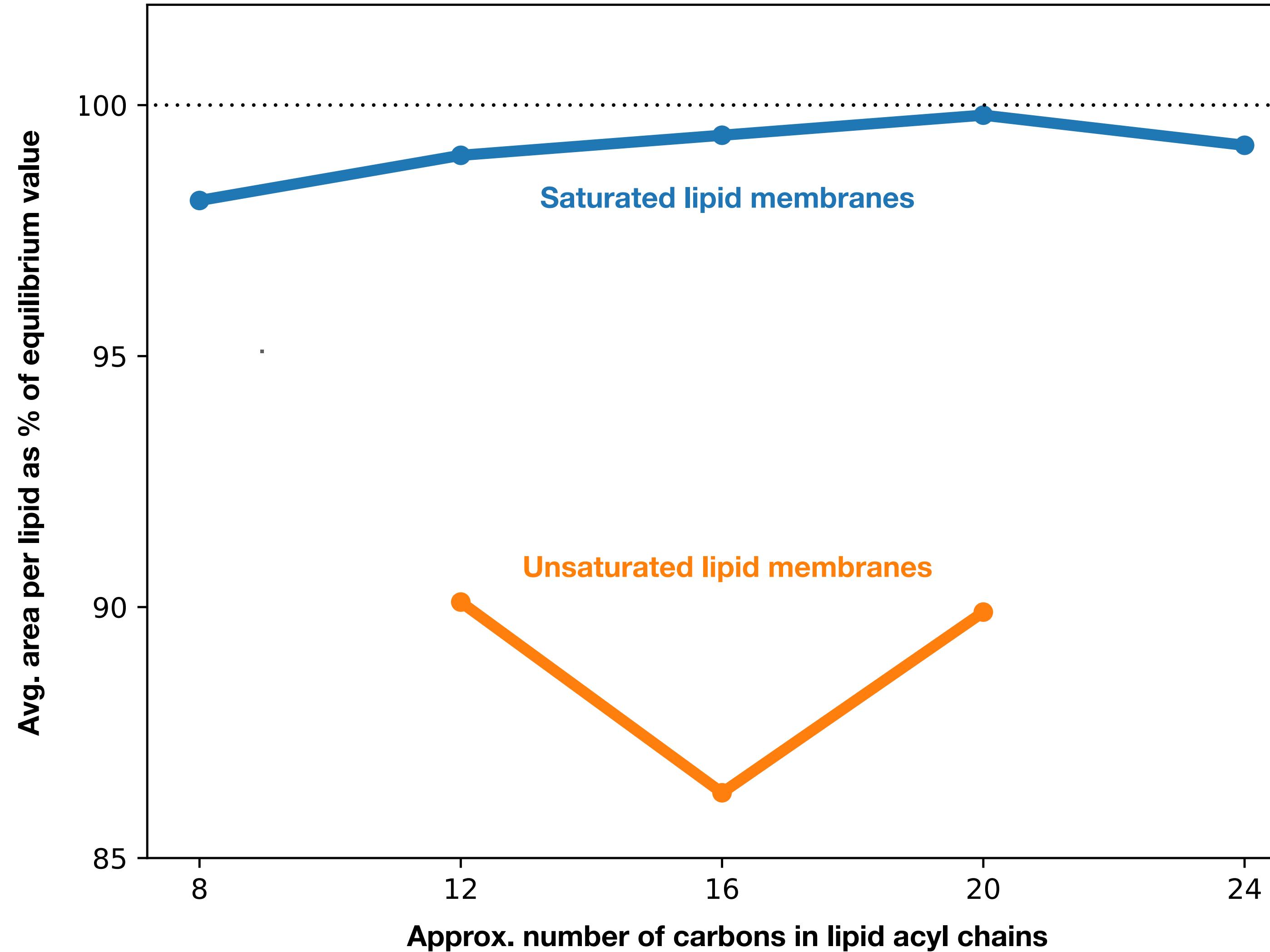
# Why did only unsaturated systems bend?



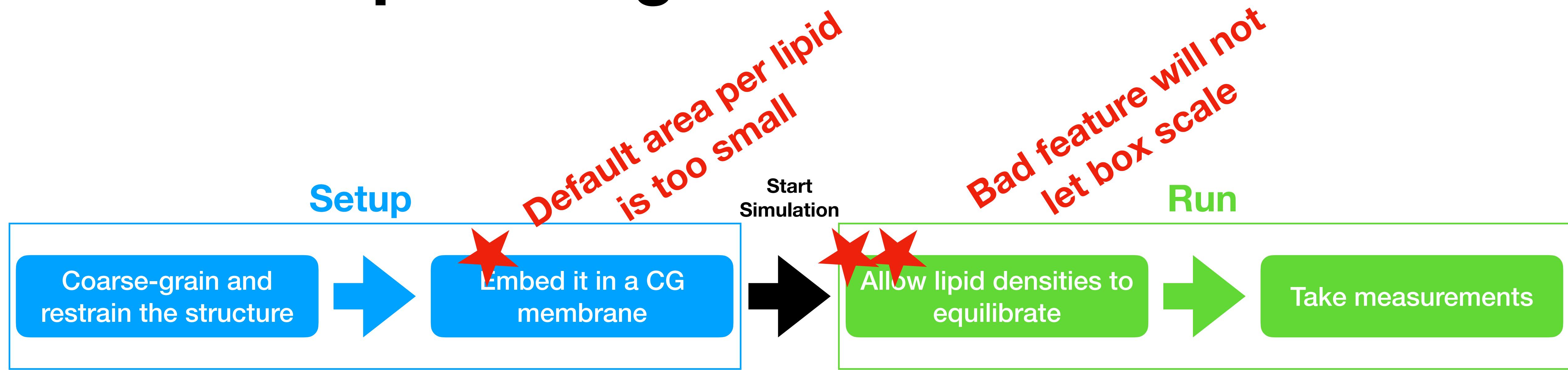
0.6 nm<sup>2</sup> is a good approx. for saturated lipids;  
unsaturated lipids have larger equilibrium areas



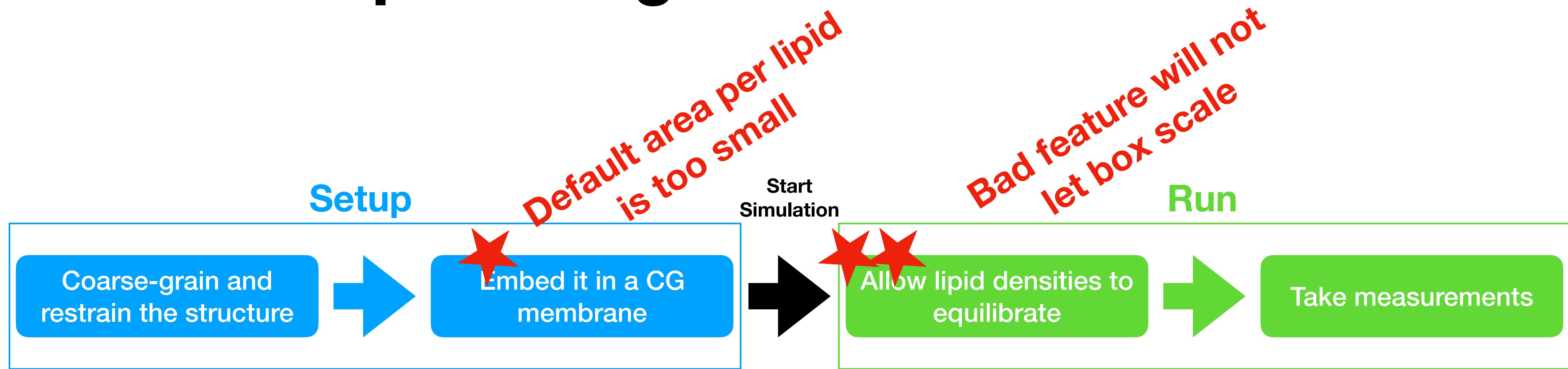
# Unsaturated lipids started out compressed AND the box could not scale



# Two compounding issues



# Two compounding issues

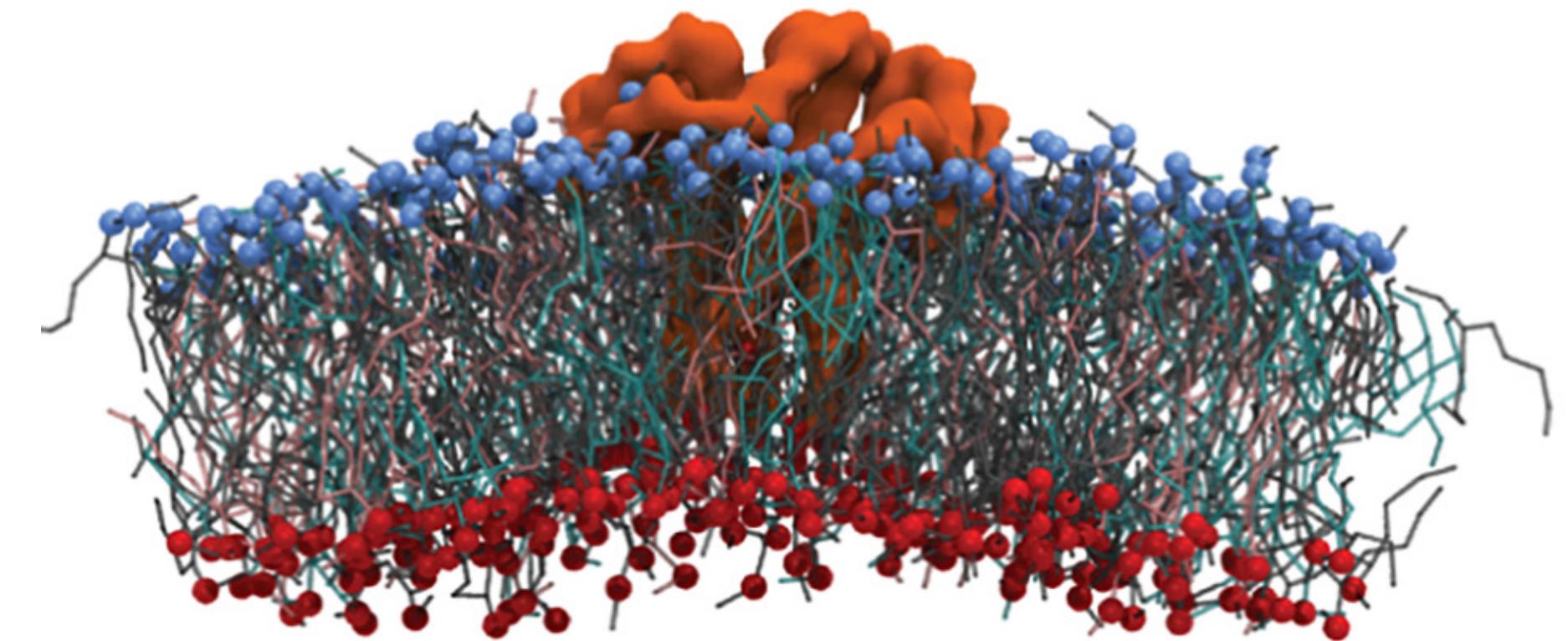


**Either of these problems would have been surmountable on their own, but both together caused a massive problem**

# **Summary**

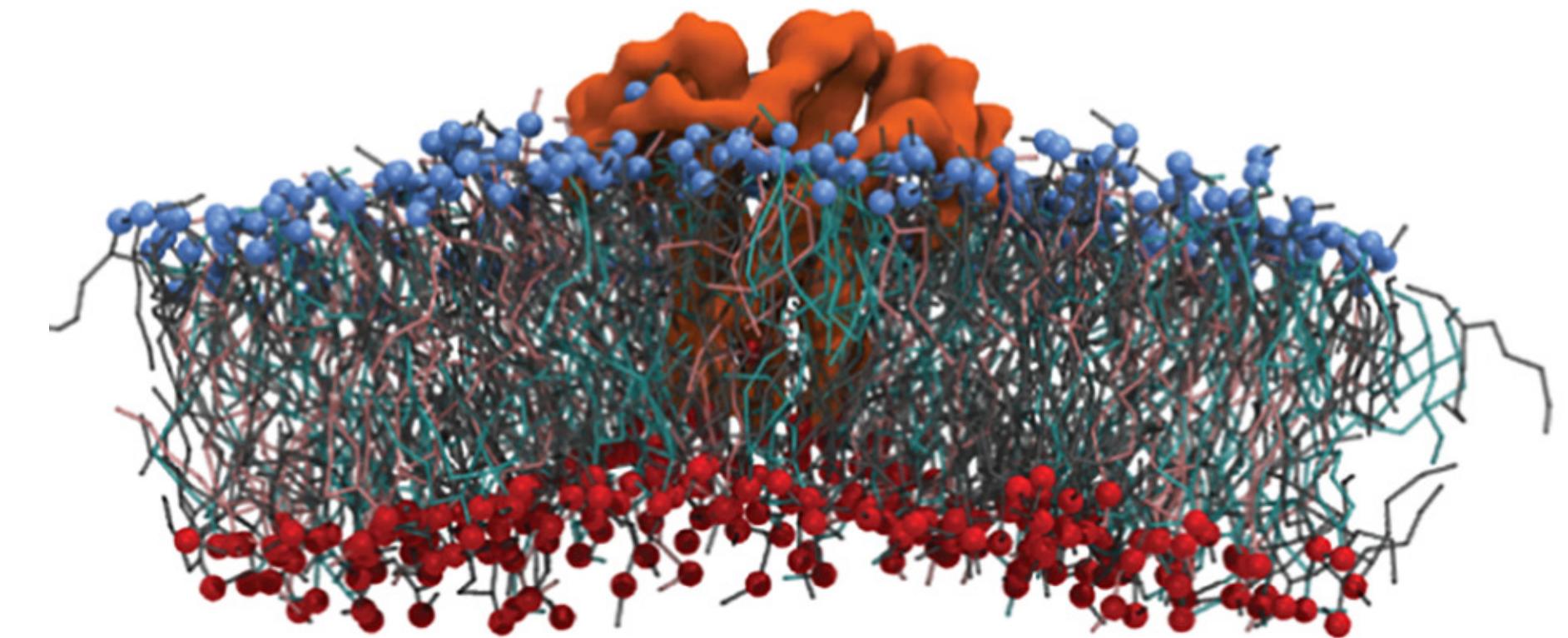
# Summary

- We found a bug that affects MD simulations that use position restraints
  - Some groups have been affected by this bug and published
  - Many other groups have been affected and then *not* published



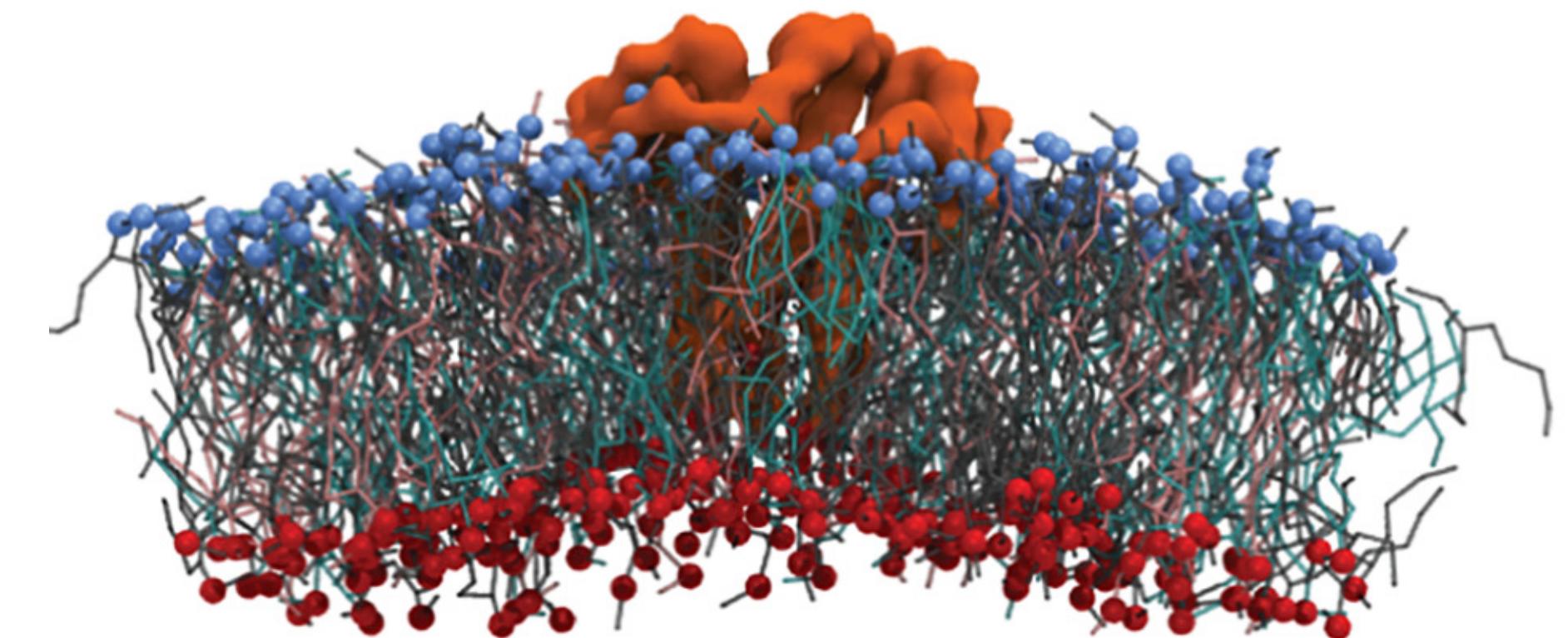
# Summary

- We found a bug that affects MD simulations that use position restraints
  - Some groups have been affected by this bug and published
  - Many other groups have been affected and then *not* published
- The E protein does have interesting features
  - Asymmetric thickness deformations
  - Diffuses to and/or stabilizes point of maximal curvature



# Summary

- We found a bug that affects MD simulations that use position restraints
  - Some groups have been affected by this bug and published
  - Many other groups have been affected and then *not* published
- The E protein does have interesting features
  - Asymmetric thickness deformations
  - Diffuses to and/or stabilizes point of maximal curvature
- We do not have much evidence for induction of curvature / membrane bending at the moment



# **Next steps / other projects**

# Next steps / other projects

- This project:
  - Analyze membrane response to E protein in elastic network simulations
  - Quantify local thickness deformations / leaflet asymmetry
  - Publish our findings
  - File a bug report

# Next steps / other projects

- This project:
  - Analyze membrane response to E protein in elastic network simulations
  - Quantify local thickness deformations / leaflet asymmetry
  - Publish our findings
  - File a bug report
- Other projects:
  - Density-threshold affinity paper accepted!
  - Measuring bending modulus from vesicle fluctuations (Hi, Josh!)
  - Identifying binding sites for PUFAs on the nAChR

# Thank You!

## Brannigan Lab Members

**Dr. Grace Brannigan**  
**Ezry Santiago-McRae**  
**Connor Pitman**  
**Lindsey Riggs**  
**Jahmal Ennis**  
**Regina Salzer**  
**Ryan Lamb**

## Lab Alumni

Noureen Abdelrahman  
Mariadelia Arguello-Acuna  
Dr. Sruthi Murlidaran  
Dr. Ruchi Lohia  
Dr. Liam Sharp  
Anushriya Subedy

## Brannigan Lab Collaborators

Dr. Tom Joseph  
Dr. Jerome Henin  
Dr. Matt Hansen

## Special Thanks To:

NSF  
CAREERS  
Rutgers OARC  
Galen Collier  
Udi Zelzion  
Tom Skipper

