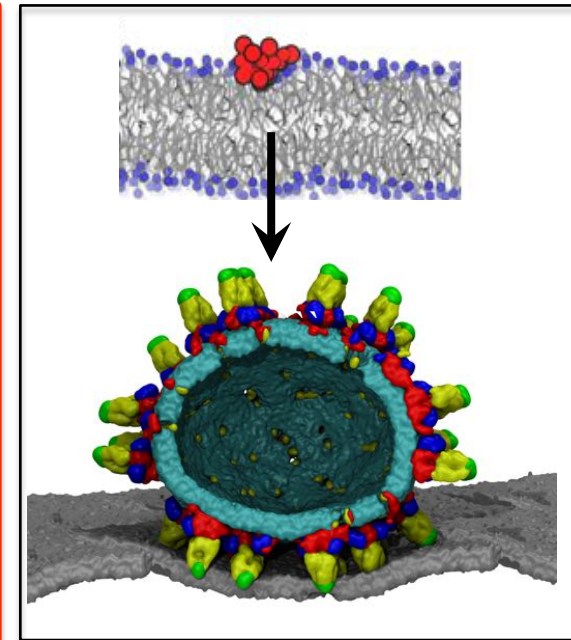
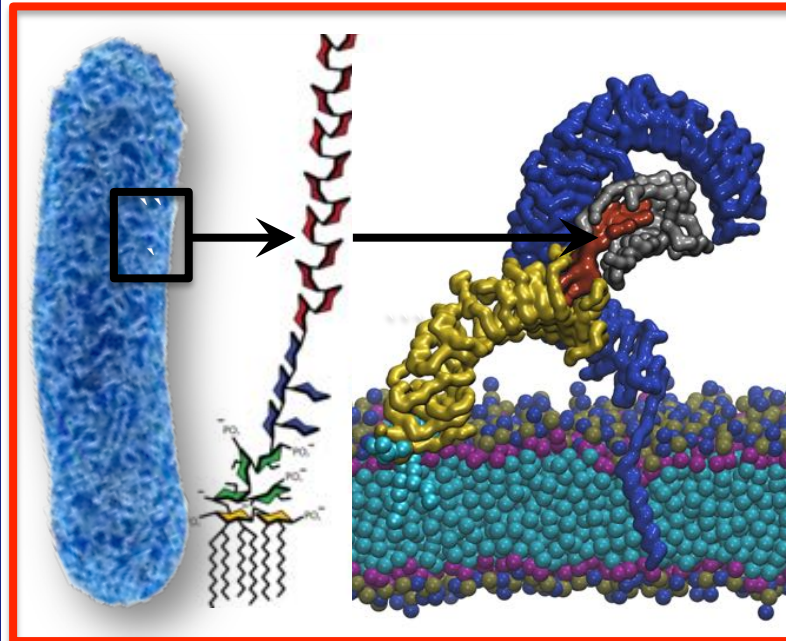


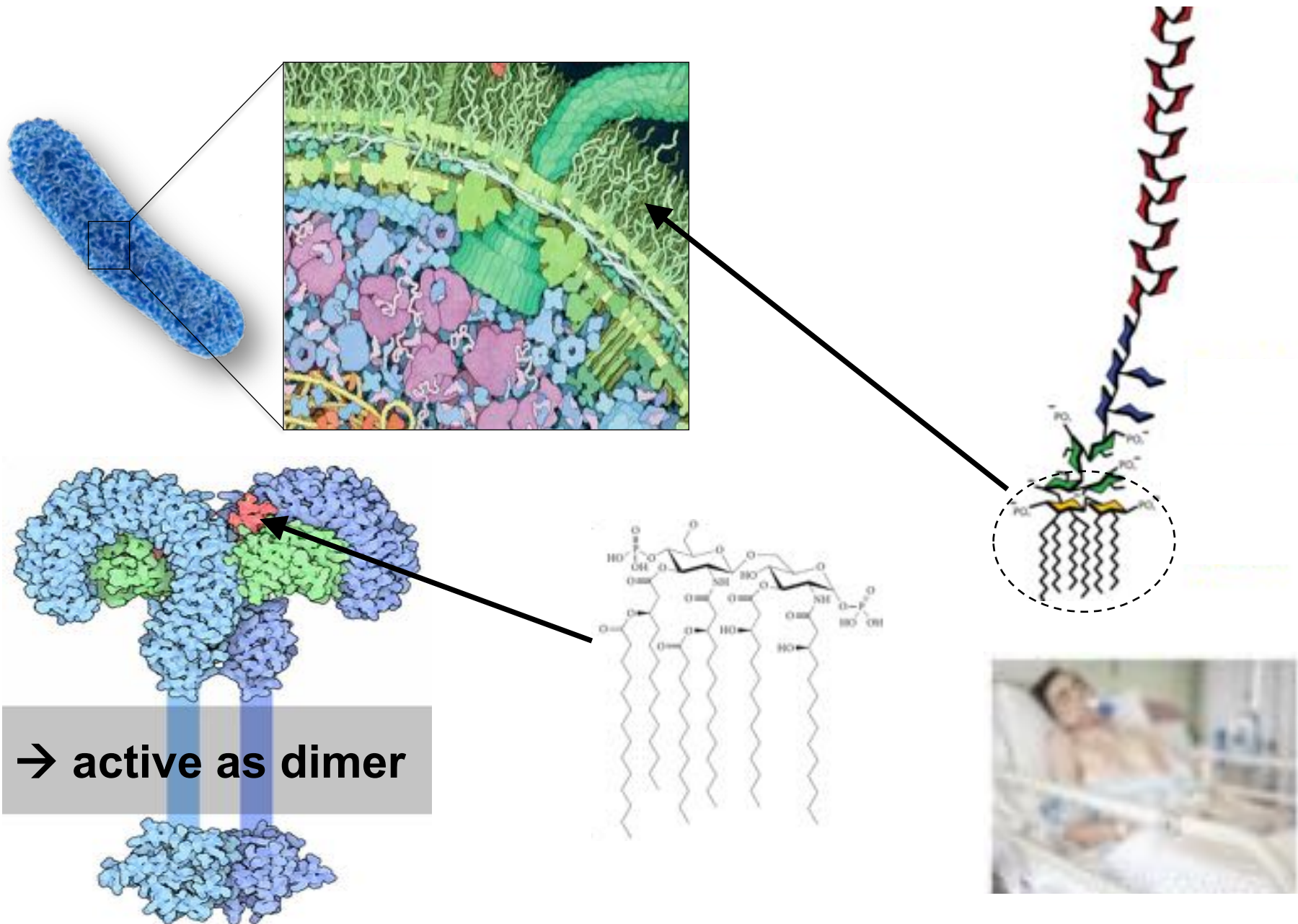
# Computational modelling of host–pathogen interactions: from atoms to systems

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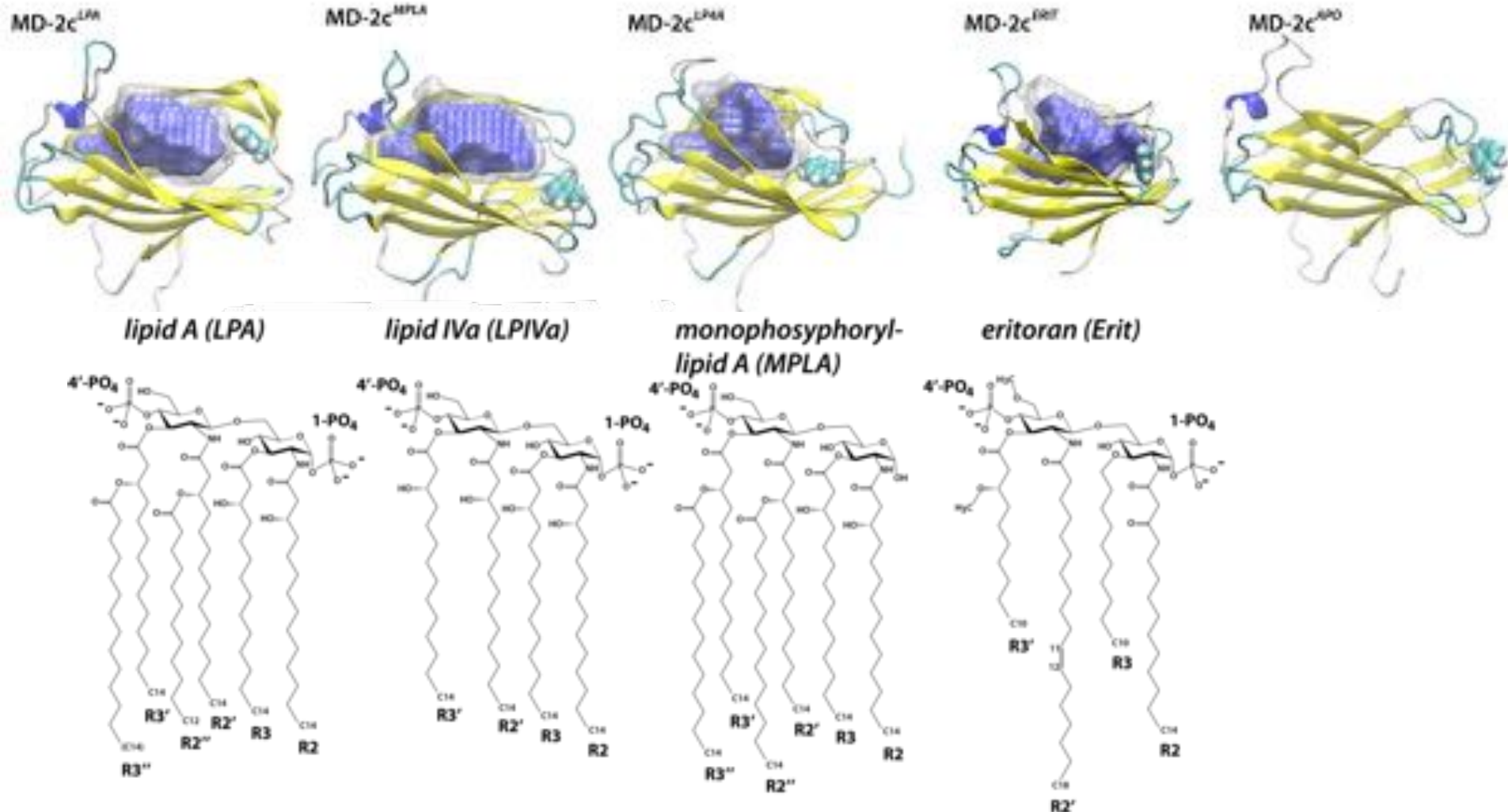
Peter J. Bond (BII)  
peterjb@bii.a-star.edu.sg



# Immune Recognition of Bacterial LPS



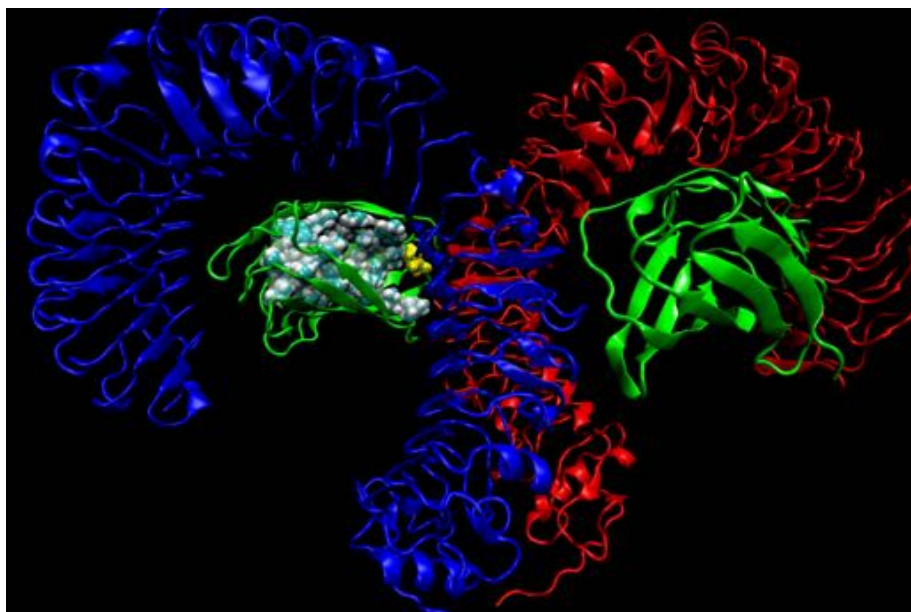
# MD-2 Co-Receptor “Gauges” LPS Structure



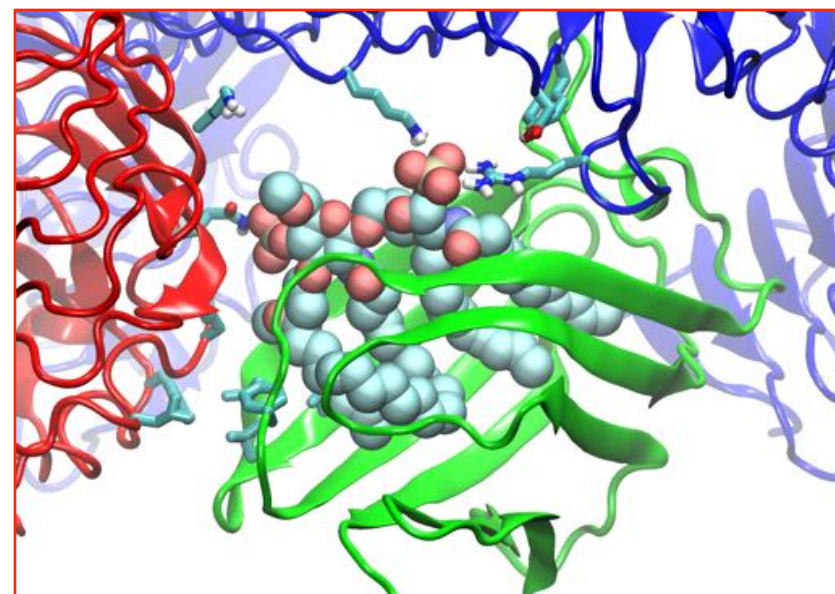
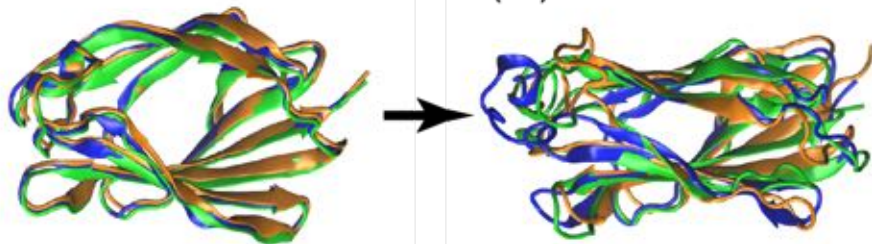
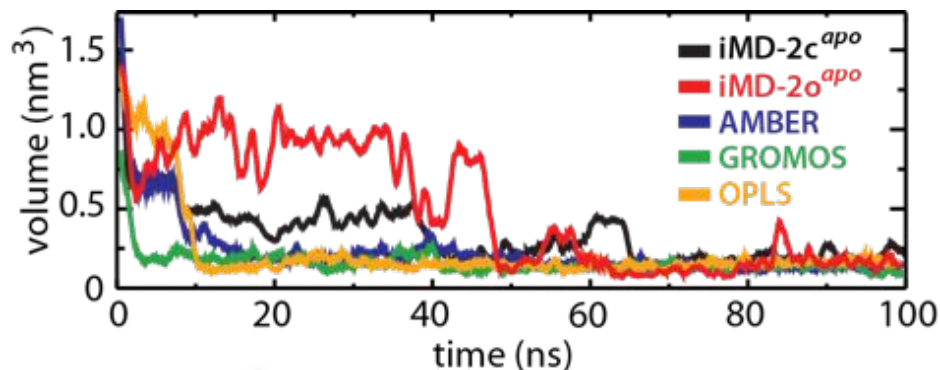
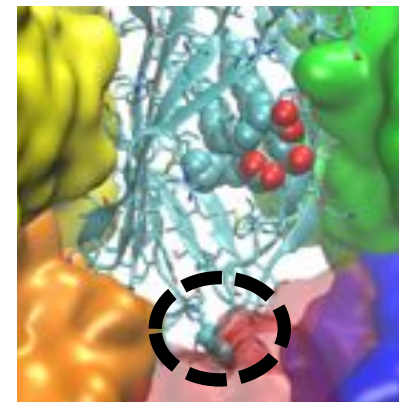
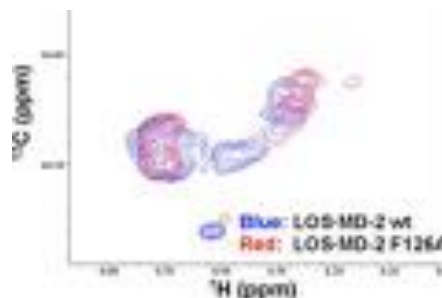
- Remarkably flexible cavity, assessed using trj\_cavity... *Paramo et al, JCTC, 2014.*
- “Clamshell motions” enable adaptation of cavity volume to different ligands.
- Size of hydrophobic component of lipids correlates (>98%) with MD-2 cavity volume.
- Paramo et al. J Biol Chem (2013) 288:36215-



# MD-2 $\beta$ -Cup is a Dynamic “Clamshell”

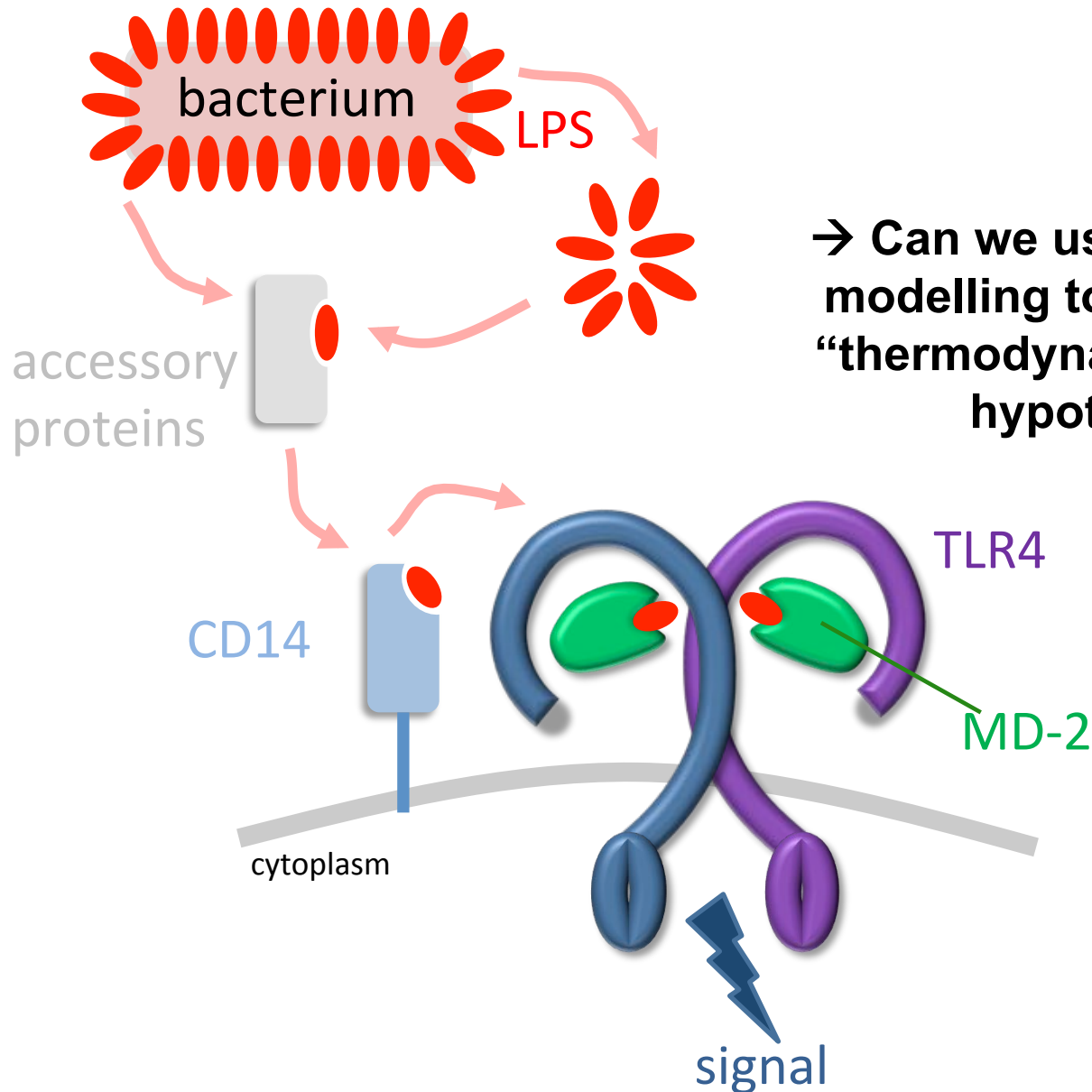


F126A mutant – LPS chemical shifts perturbed *J Biol Chem.* (2012) 287:16346-.



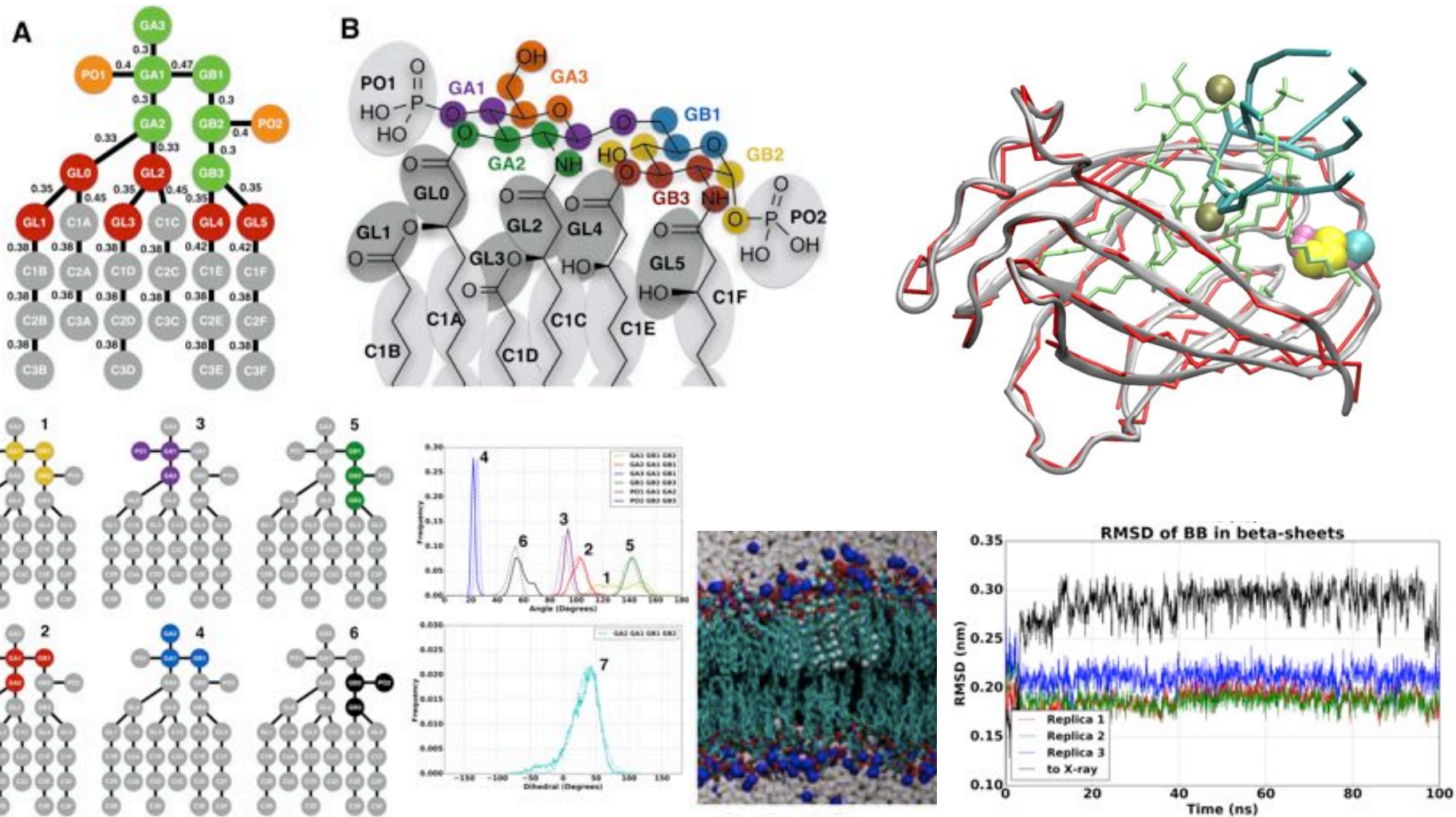
- Paramo et al. *J Biol Chem* (2013) 288:36215-
- Ortiz-Suarez & Bond, *Structure* (2016) 24:200-
- Paramo et al. *Scientific Reports* (2015) 5:17997
- Berglund et al. *Prog. Biophys. Mol. Biol.* (2015) 119:72-

# TLR4: Part of a “Funneled” System?



→ Can we use multiscale modelling to assess the “thermodynamic funnel” hypothesis

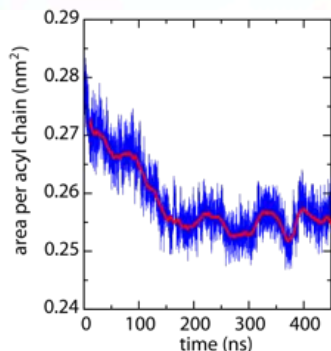
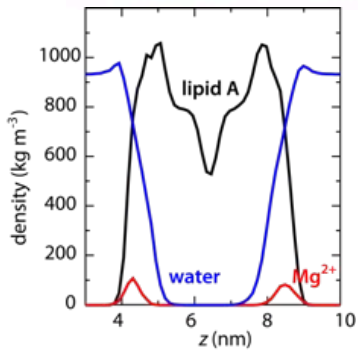
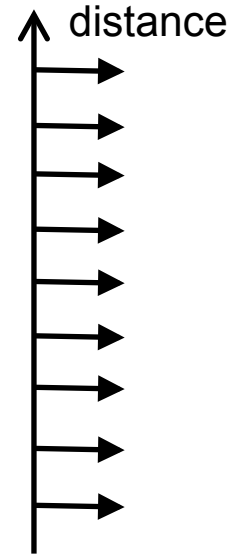
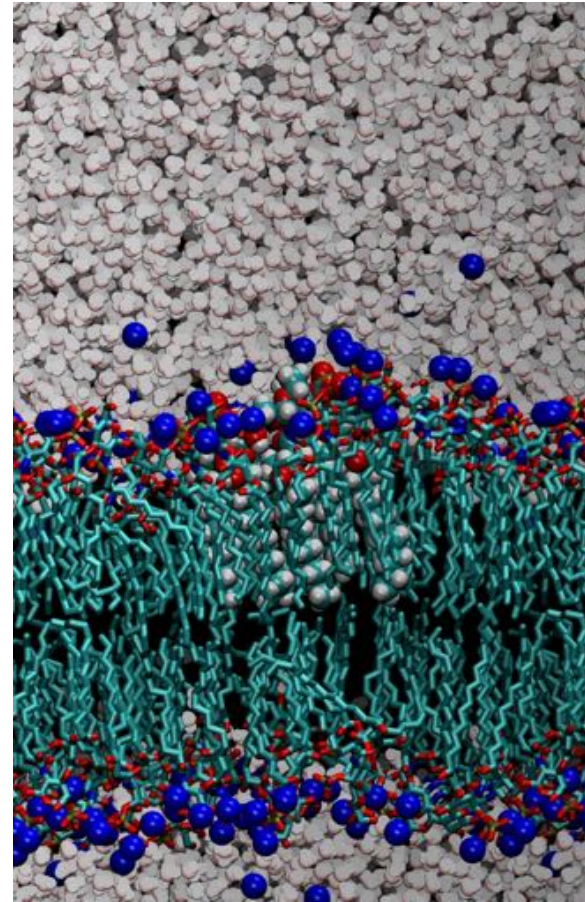
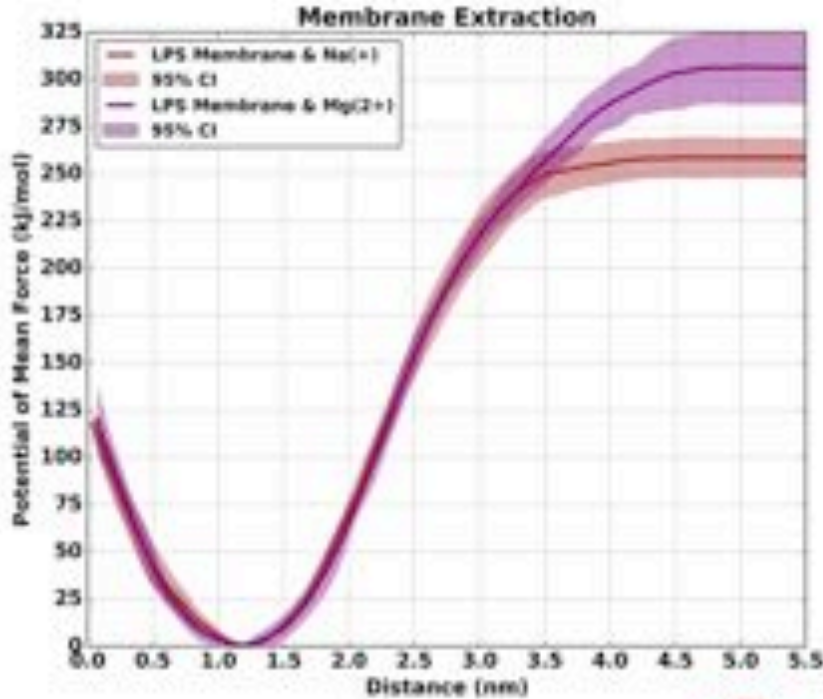
# Coarse-Grained Model for LPS & Receptors



- Iterative parameterization based on all-atom simulations.
- Membrane lipids (angles/dihedrals) & proteins (ENMs).

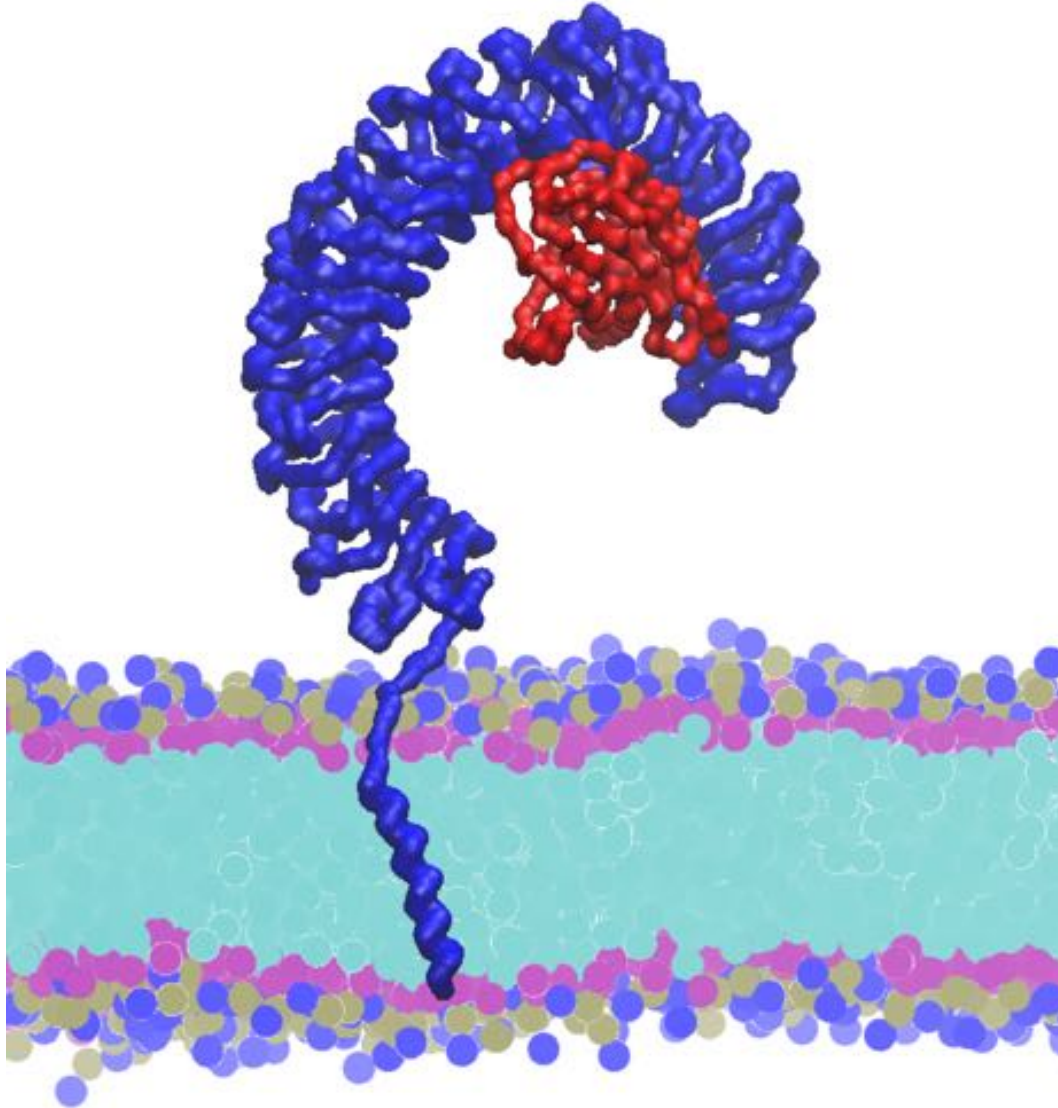


# Careful Validation of CG Lipid Model...



- Electron density profile + cation “cross-links”.
- AC  $0.255 \pm 0.004 \text{ nm}^2$  (X-ray diffraction =  $0.26 \text{ nm}^2$ .)
- $D_l \sim 4 \times 10^{-9}$  ( $2\text{-}3 \times 10^{-9} \text{ cm}^2\text{s}^{-1}$  from fluorescent labelling.)
- Free-energies of LPS affinity from atomistic simulations ( $250\text{-}300 \text{ kJ mol}^{-1}$ ) - *Scientific Reports* (2015) 5:17997

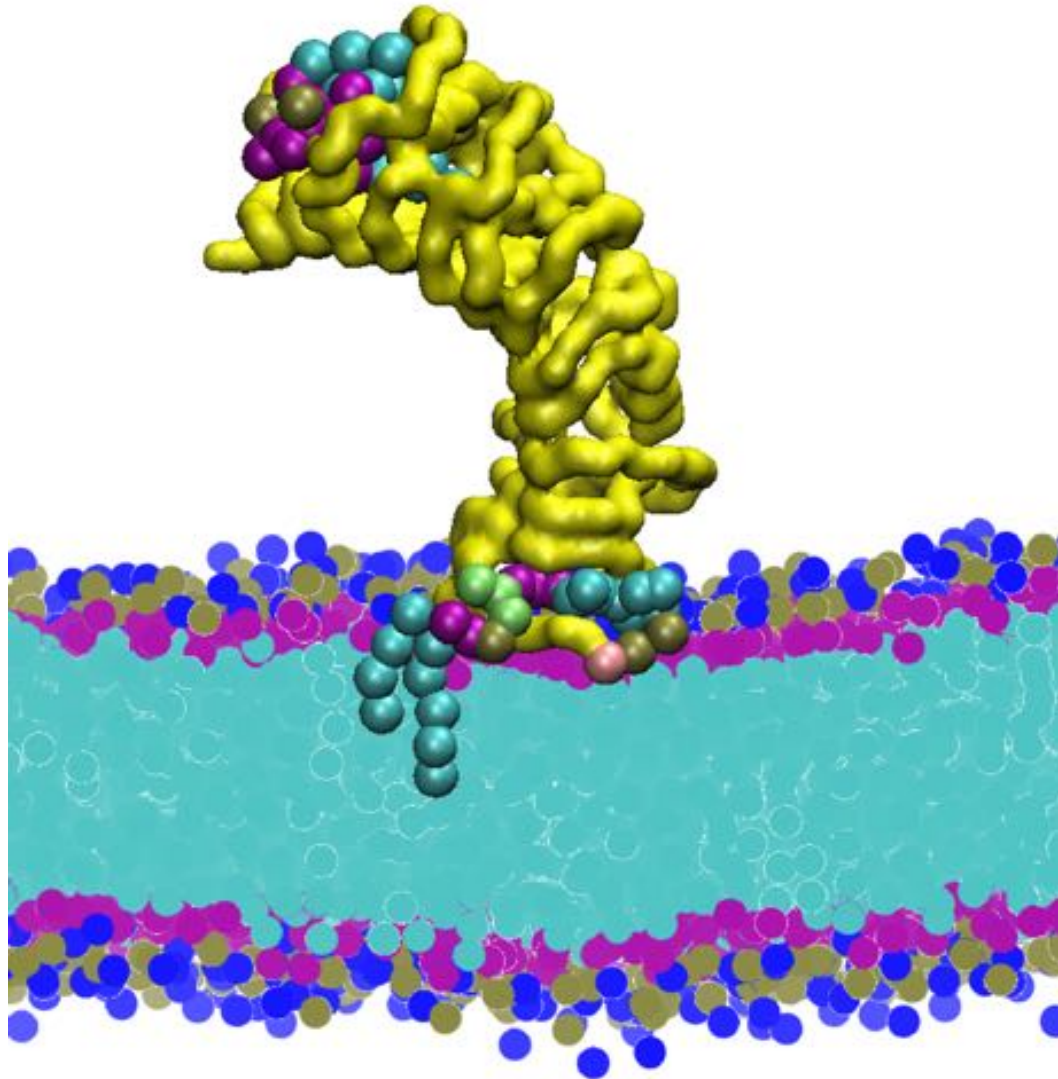
# TM TLR4/MD-2 – Coarse-Grained Model



- human TLR4 + MD-2 (based on X-ray structure)
- + modelled transmembrane helix (guided by CD experiments)
- + POPC membrane



# TM CD14 – Coarse-Grained Model

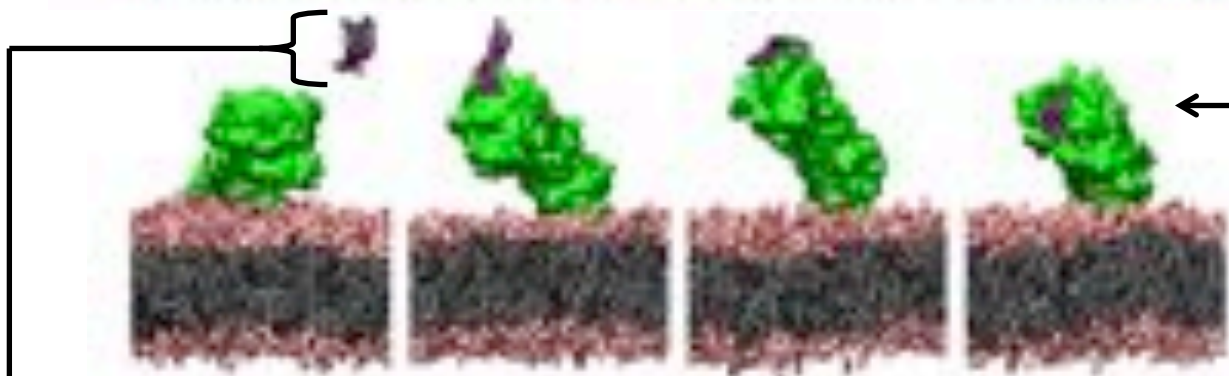


- human CD14  
(based on X-ray structure)
- + GPI anchor
- + LPS (based on atomistic assembly simulations)
- + POPC membrane



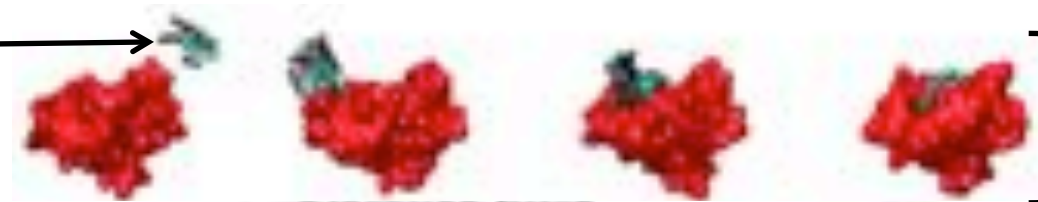
LPS

$\Delta\Delta G$   
+150  
kJ/mol



CD14•LPS

$\Delta\Delta G$   
-50  
kJ/mol



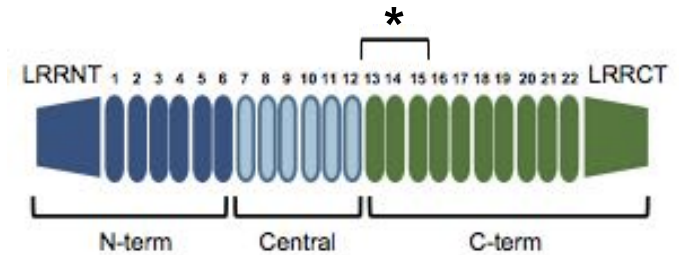
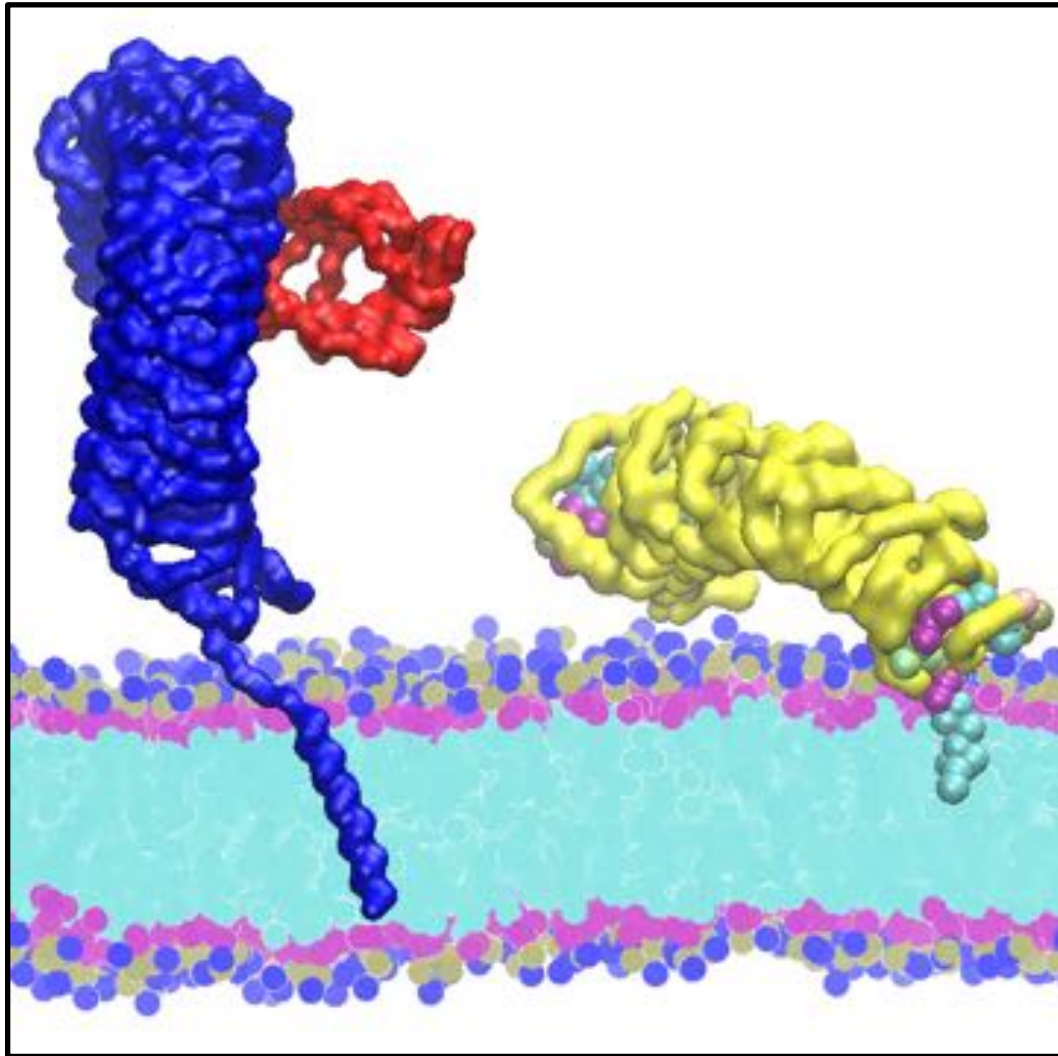
MD2•LPS

$\Delta\Delta G$   
-250  
kJ/mol

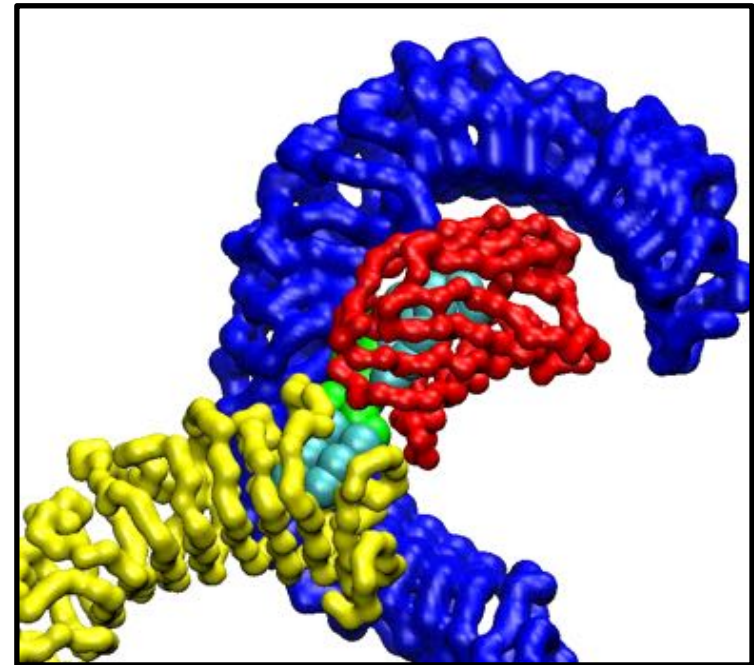


TLR4-  
MD2•LPS

# TLR4-MD-2 / CD14 Assembly & Lipid Exchange



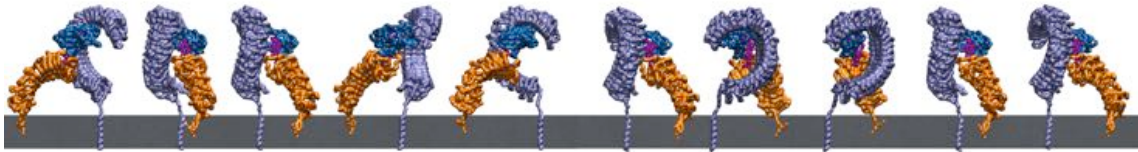
Ryu et al. 2017, Immunity 46, 1–13.



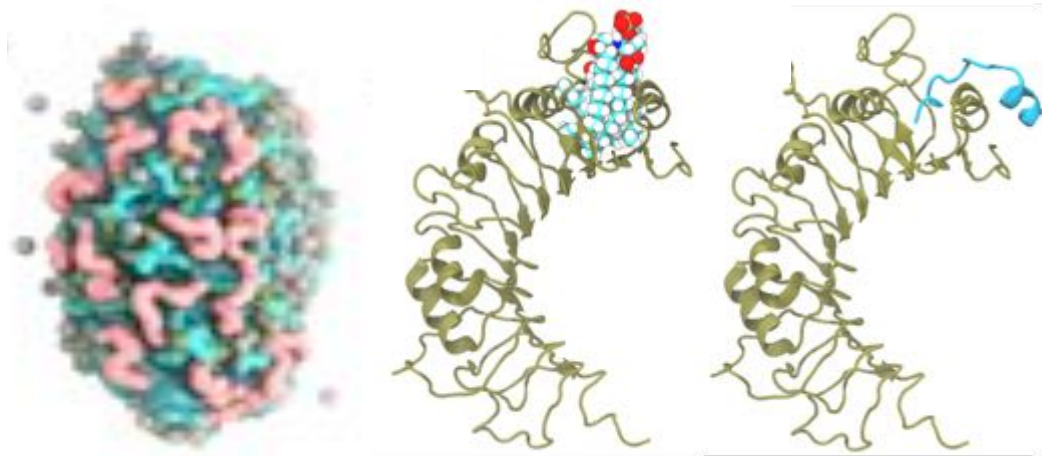
- Multiple replicas map interaction interface & dynamics.
- Assembled “productive” states seed lipid transfer sims.



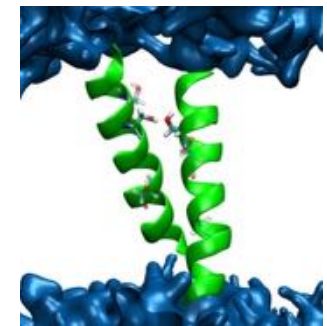
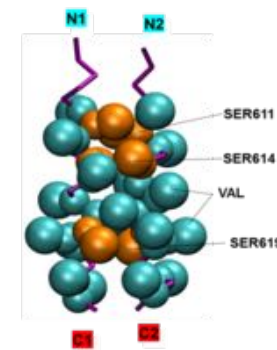
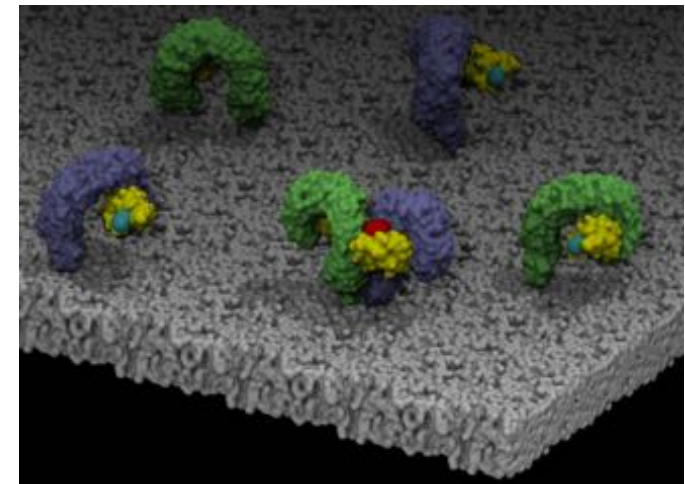
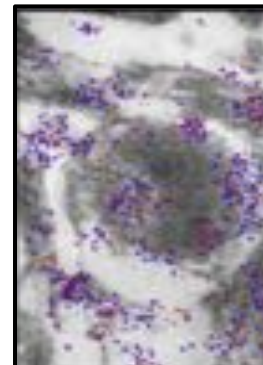
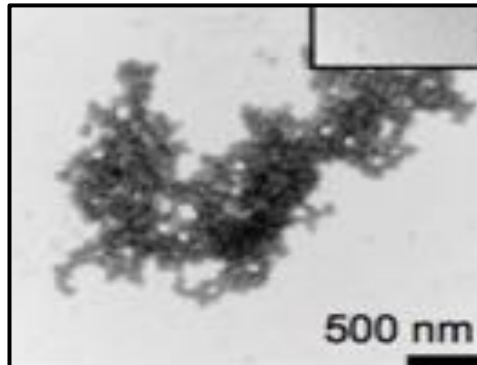
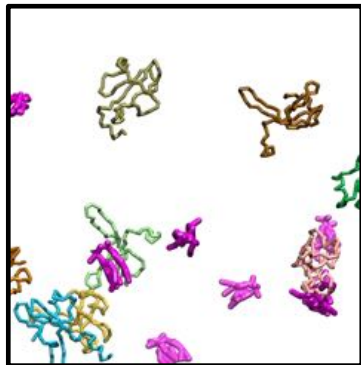
# LPS Multiscale Modelling: Now & Next



- LPS/TLR4 relay – from atoms to systems via multiscale simulation, integrative modelling, and experimental calibration. (Huber et al. 2017, under review).



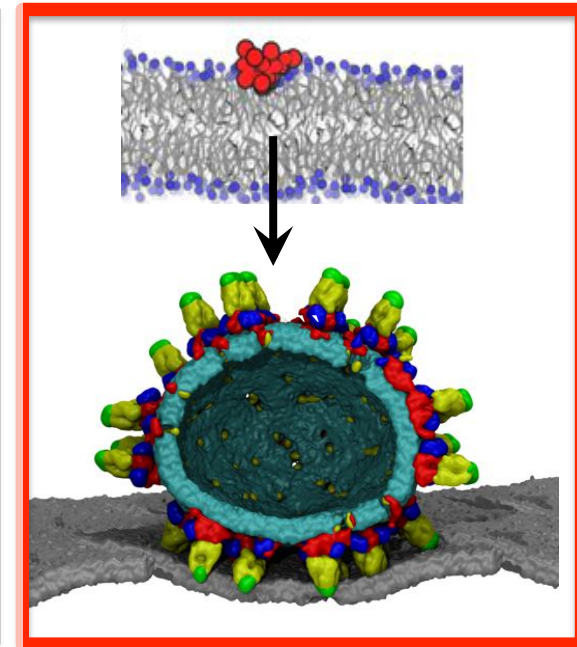
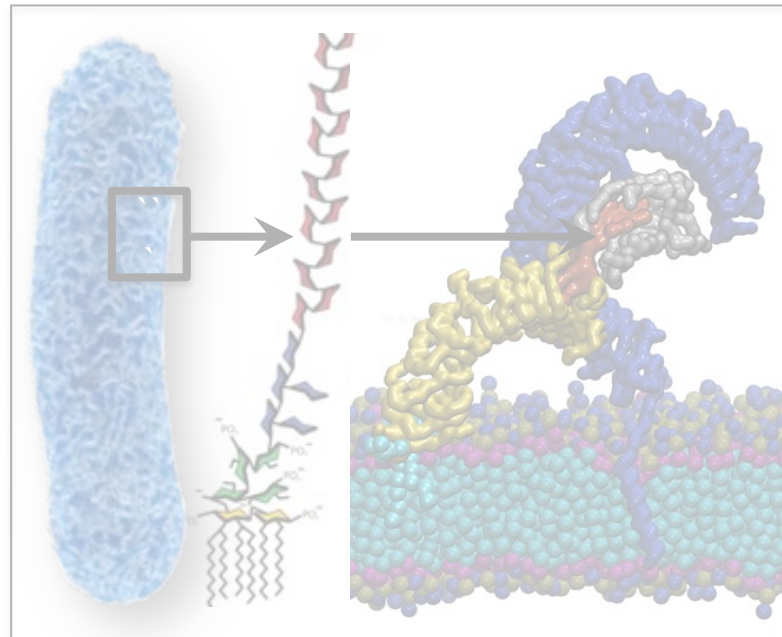
- Anti-bacterial/anti-LPS/anti-CD14: thrombin fragments in wound healing.
- Schmidtchen (LKCMed, Lund Uni), Huber, et al. 2017, PNAS.



- TLR TM domains (& peptidomimetics). Hubert Yin (U. Colorado at Boulder), Kargas, Marzinek, Holdbrook et al, 2017, BBA Biomembranes.

# Computational modelling of host–pathogen interactions: from atoms to systems

Peter J. Bond (BII)  
peterjb@bii.a-star.edu.sg

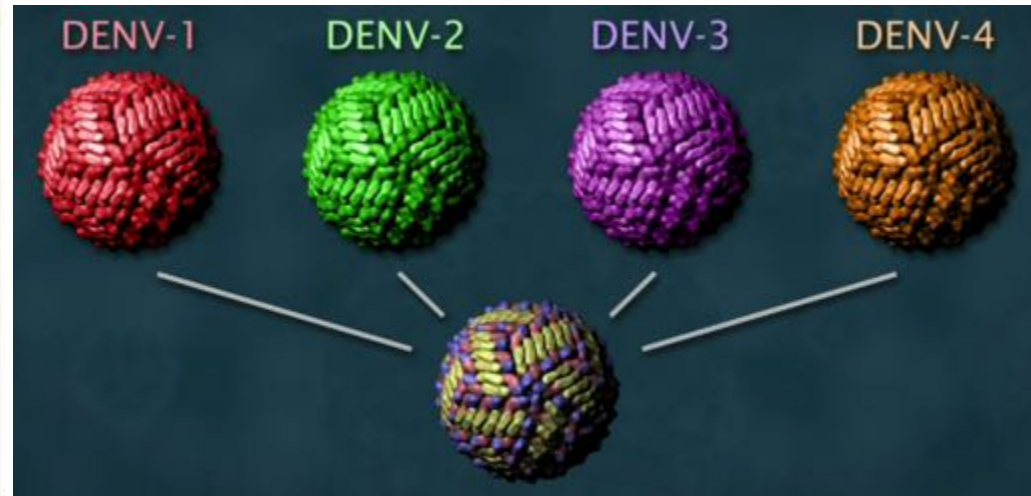




# Dengue Danger: Local & Worldwide



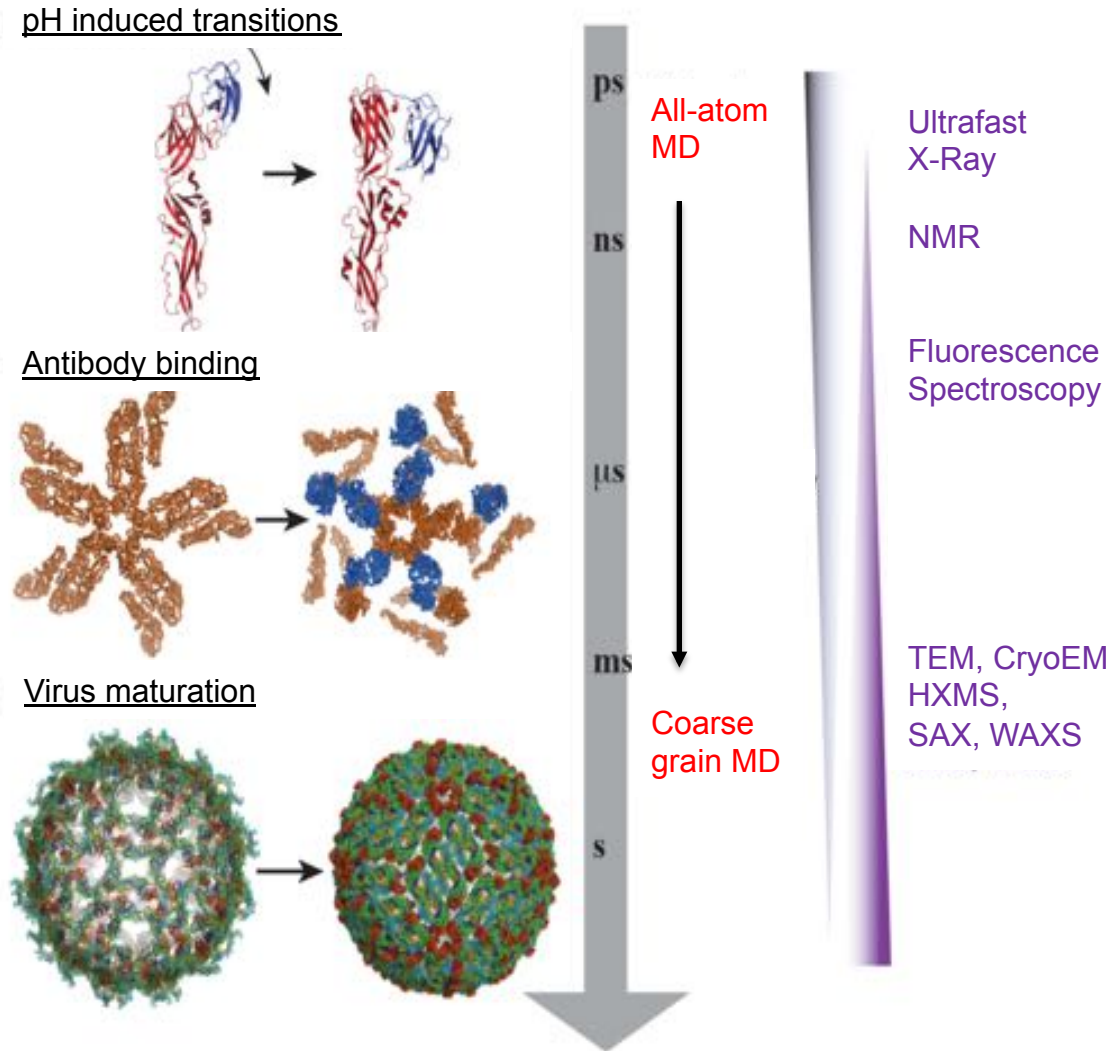
- ~400m cases per year
- Mosquito borne (*A. aegypti*)
- Different serotypes....
- Vaccines <100% effective



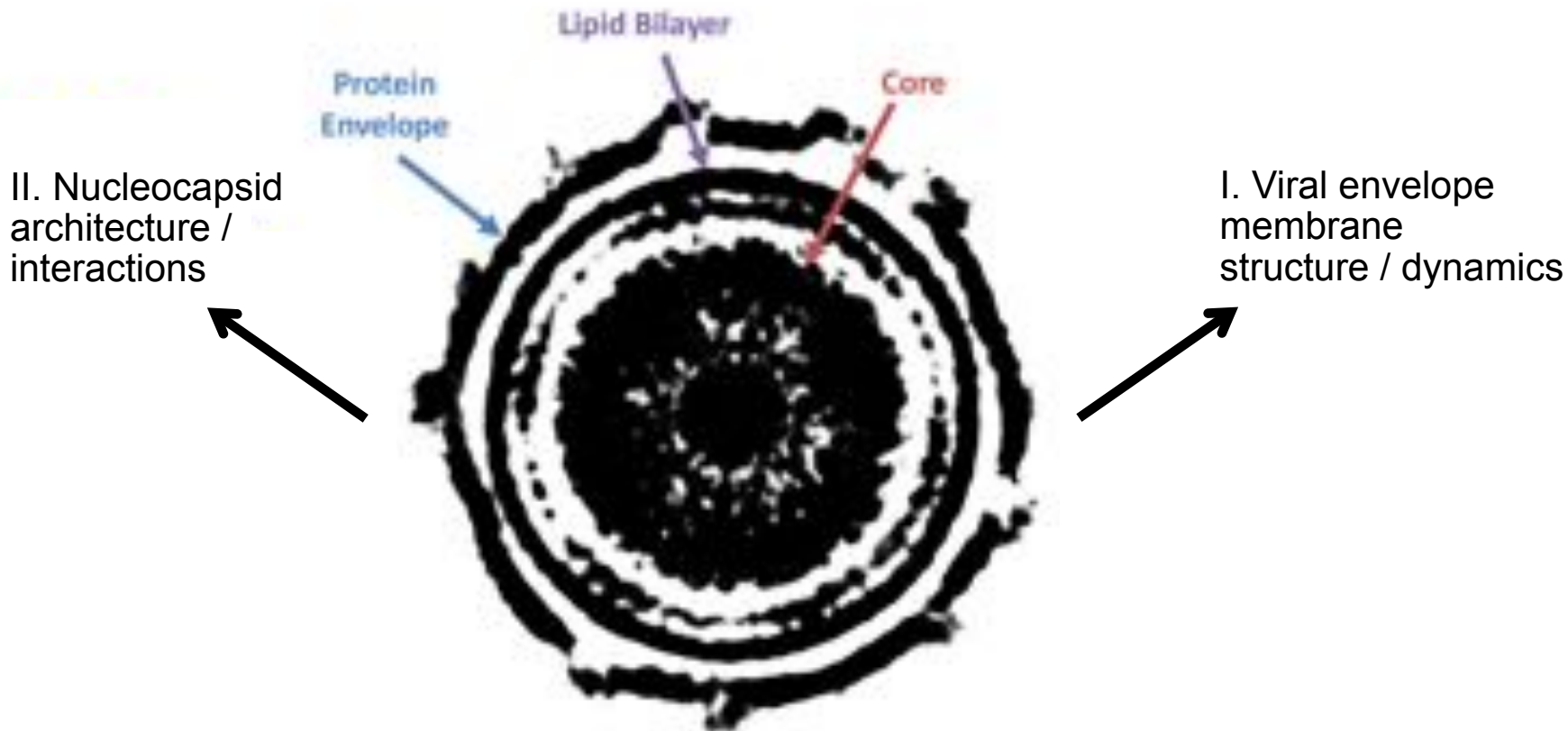


# Multiscale Dynamics of Dengue (DENV)

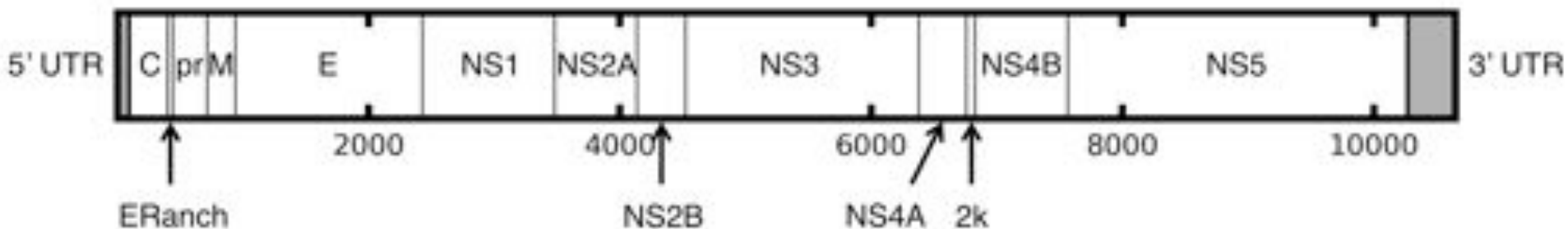
- “An integrated computational and experimental platform to study multi-scale dynamics”.
- SGD\$19M MoE Tier 3 project involving ~15 PI’s.
- NUS, NTU, Duke-NUS, & BII.
- Integration of experimental data:
  - CryoEM
  - H/D exchange
  - SAXS
  - NMR
  - Fluorescence
- Multiscale computational platform:
  - (BII, A\*STAR).



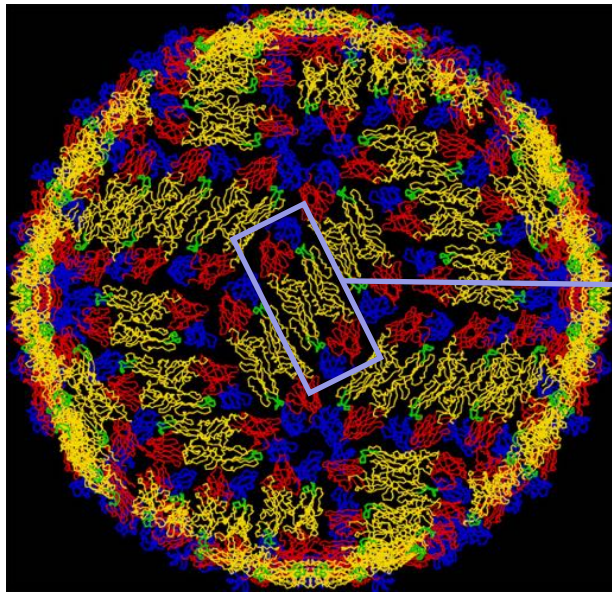
# Dengue (Flavivirus) Architecture



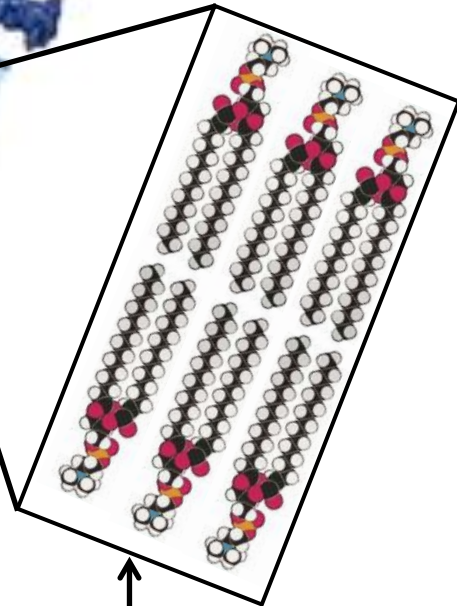
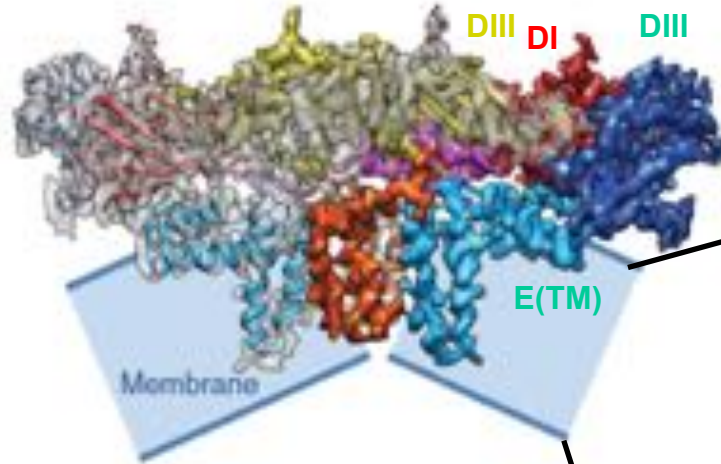
Cryo-EM map of Dengue 2 (37°C)  
(Fibriansah et al. (2013) *J Virol* 87, 7585)



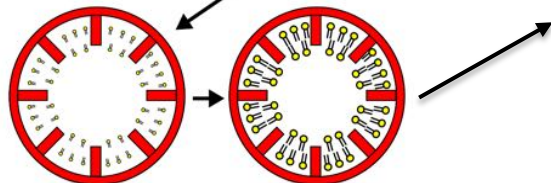
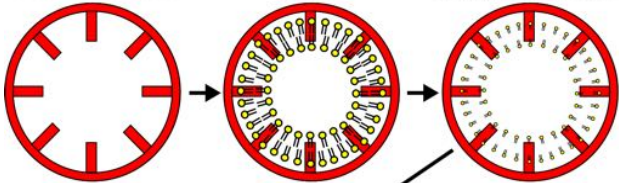
# I. Modelling Viral Envelope Structure & Dynamics



DENV-2, cryoEM, PDB: 3J27



Dengue coat with TM domains      Build spherical vesicle      Shrink lipids along principal axes



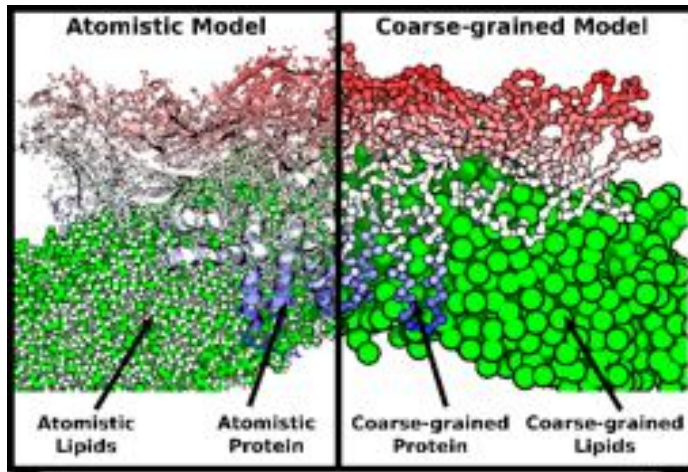
Delete lipids overlapping with protein      Minimize structure with position restraints on protein



PC:PE:PS, 6:3:1 ratio (lipidomics)

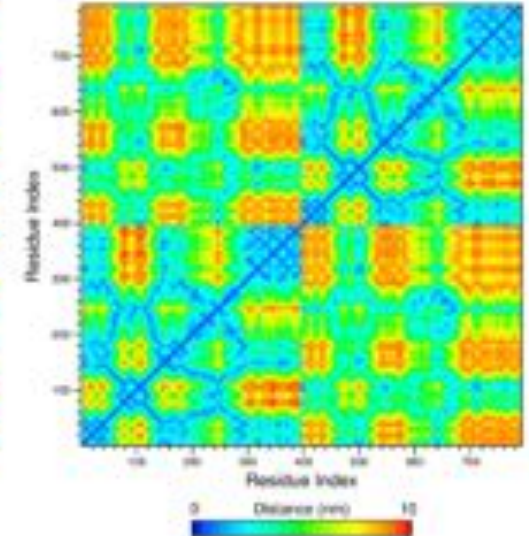
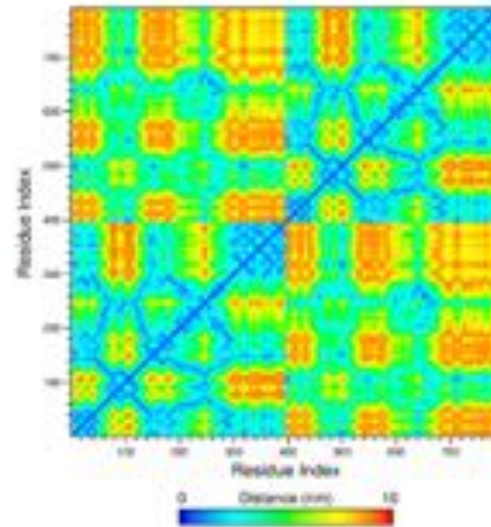
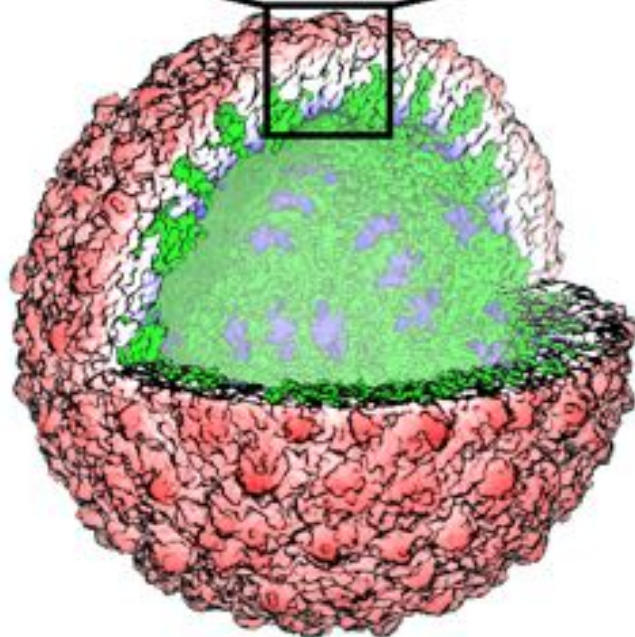
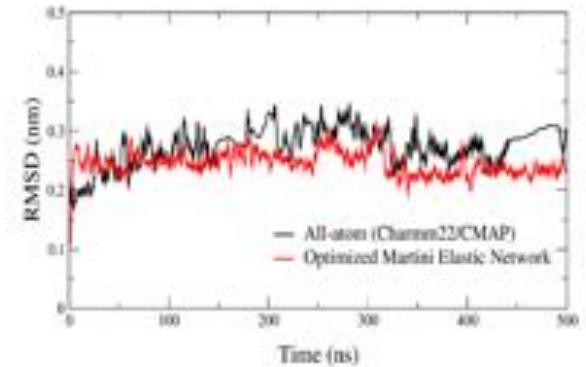


# Multiscale Modelling of the Dengue Envelope



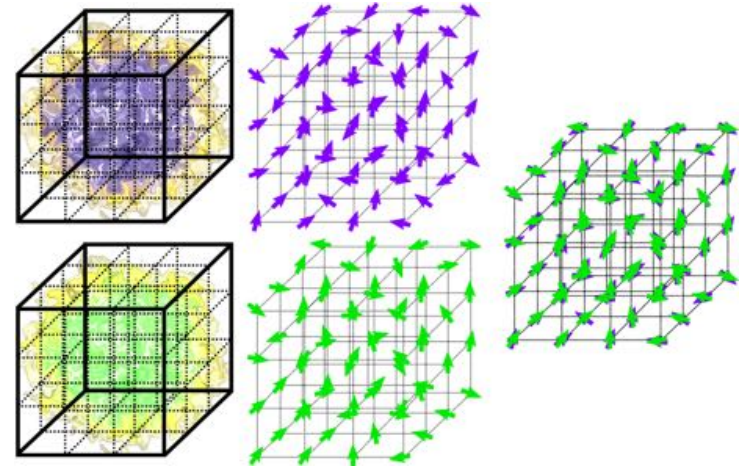
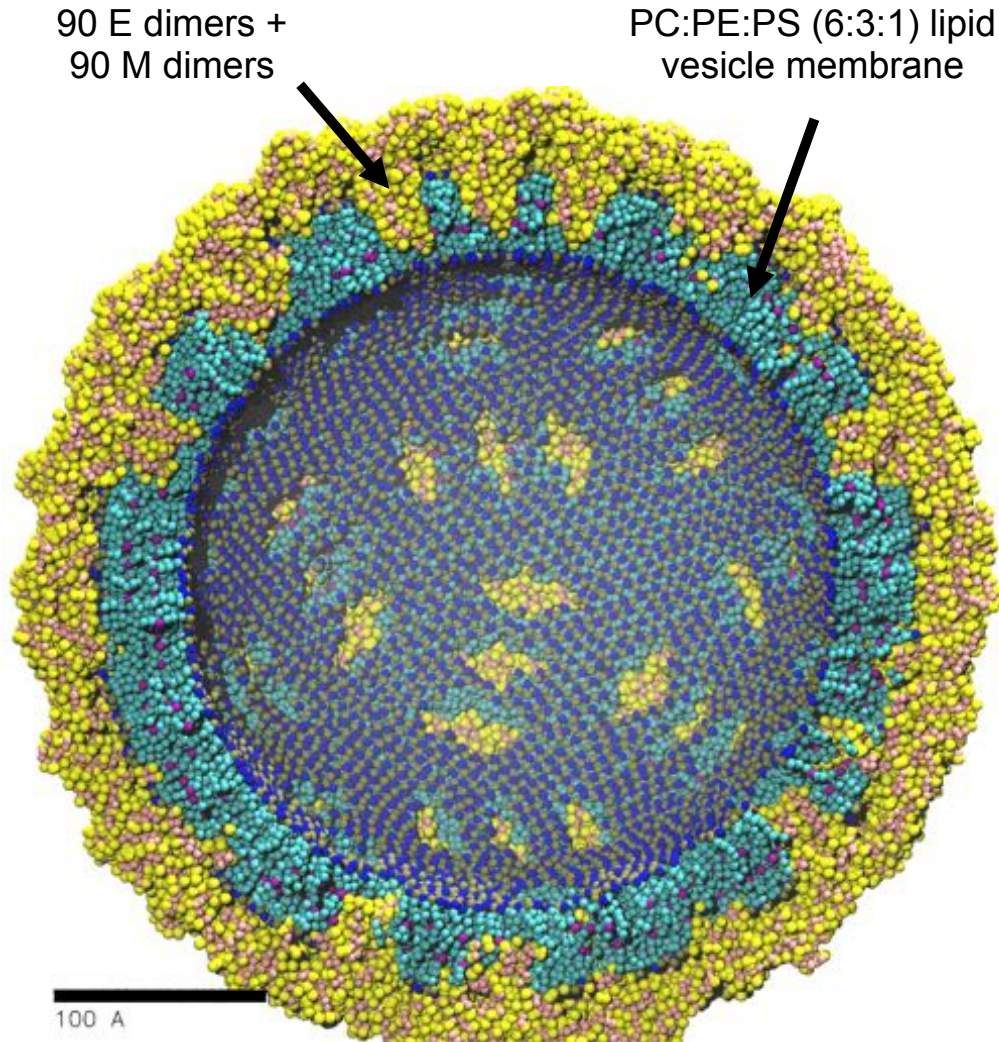
Coarse grained model based - Martini. ~4:1 mapping; particles mimic polarity / charge / H-bonding.

CG elastic network model for envelope protein tuned to atomistic MD simulations.

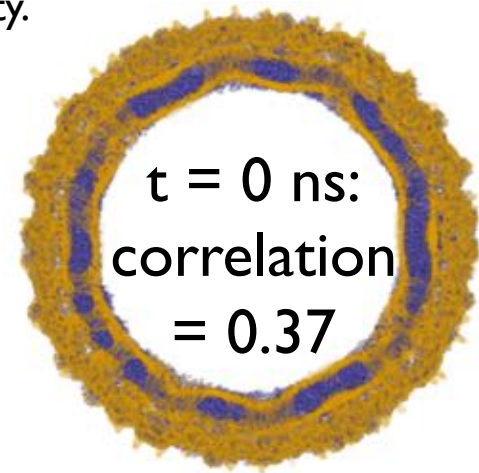




# Comparing Theory & Experiment



Correlation between aligned electron-density grid maps, calculated as mean cosine similarity.



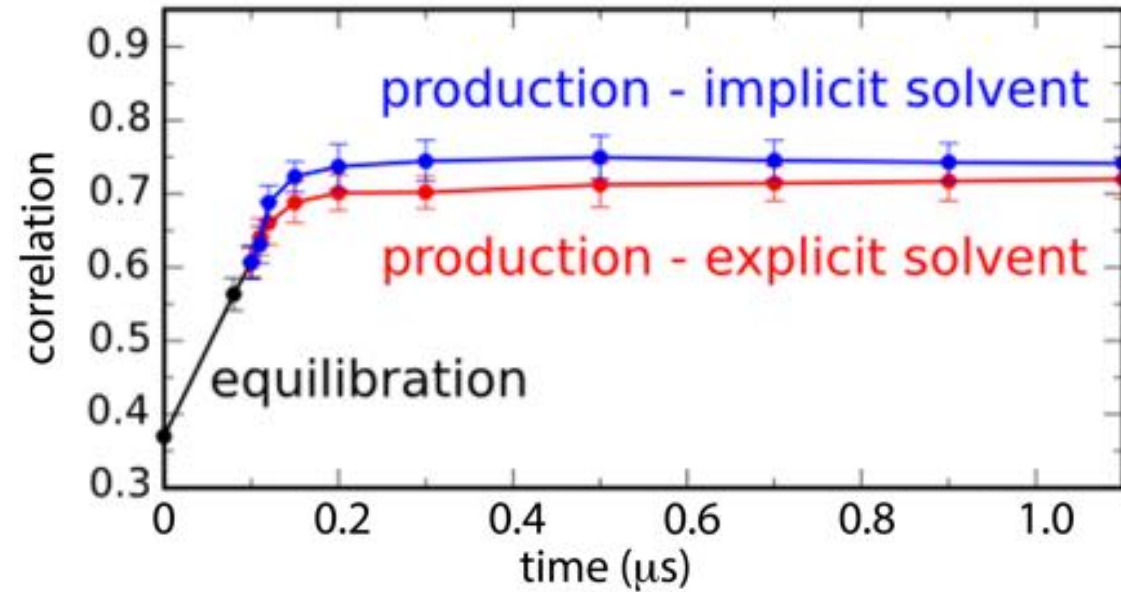
~1 million coarse-grained particles  
(*hundreds of ns required for equilibration*).

UCSF CHIMERA

an Extensible Molecular Modeling System

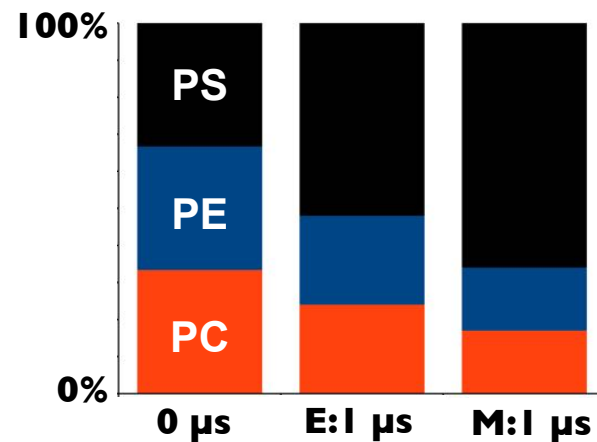


# Refinement of Envelope: Basis for Curvature

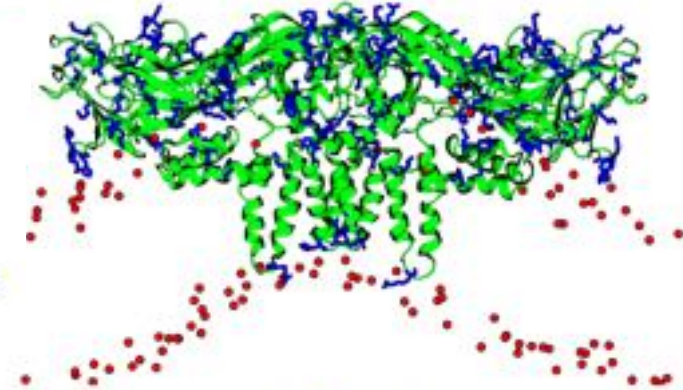
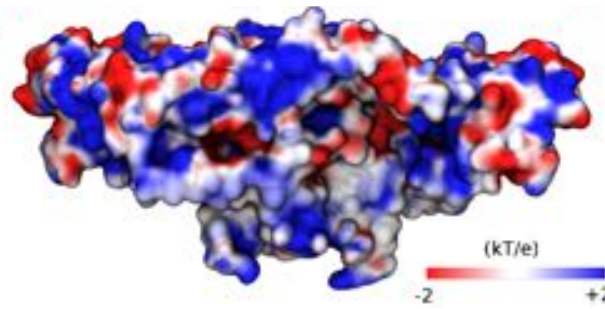


1  $\mu\text{s}$  vs cryo-EM map

Marzinek et al. Pushing the Envelope: Dengue Viral Membrane Coaxed into Shape by Molecular Simulations. (2016) *Structure*, 24:1410-1420.



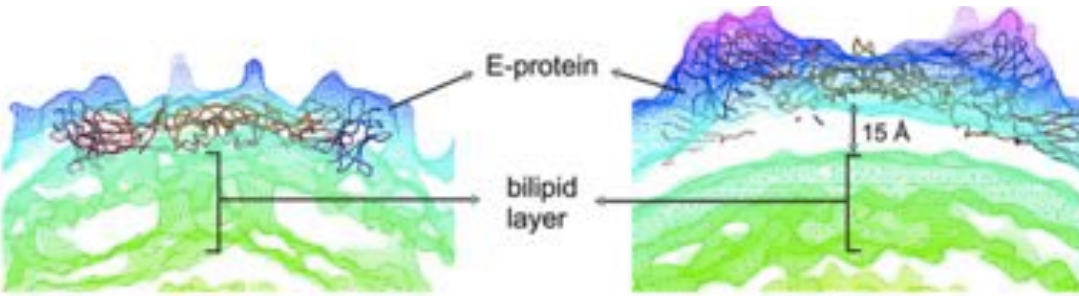
PC:PE:PS (6:3:1) enrichment



TM & curvature require anionic lipid



# Integrative Modelling of Virion “Breathing”



Fibriansah G et al. *J Virol* (2013) 87:7585-

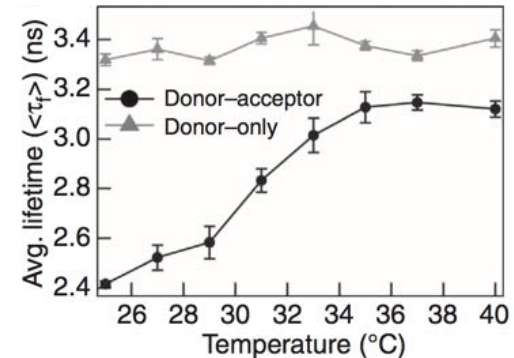
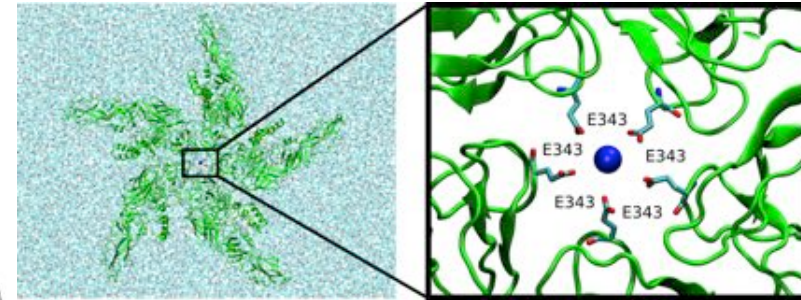
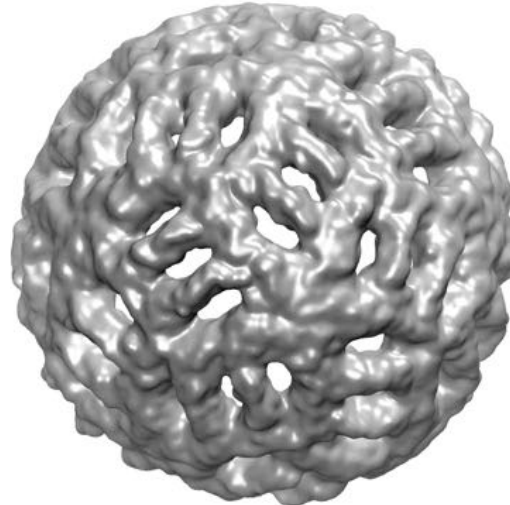
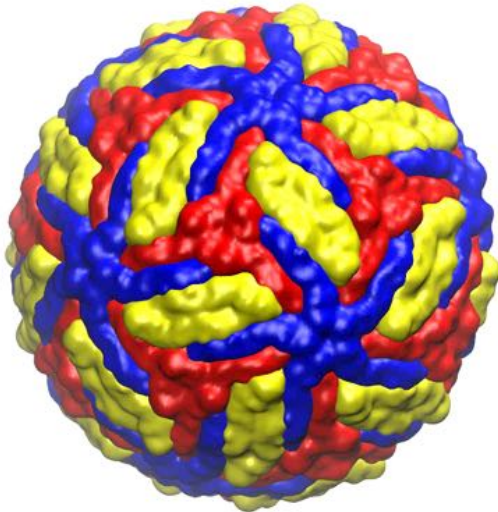
- 13.7 Å cryoEM: no TM/stem/lipid.
- MD-flexible fitting (MDFF) – external potential from cryo-EM density map defined on grid of all  $R$  particle coords.
- Mass-weighted ( $\omega_j$ ) & scaled ( $w_j$  – e.g. exclude TM) force proportional to gradient of density map applied:

$$E_{total} = E_{MD} + E_{MAP} + E_{SS}$$

$$U_{MAP}(R) = \sum_j \omega_j V_{MAP}(r_j)$$

$$F_j^{MAP} = -\nabla U_{MAP}(R) = -w_j \frac{\partial V_{MAP}(r_j)}{\partial r_j}$$

“smooth” vs “bumpy” virion



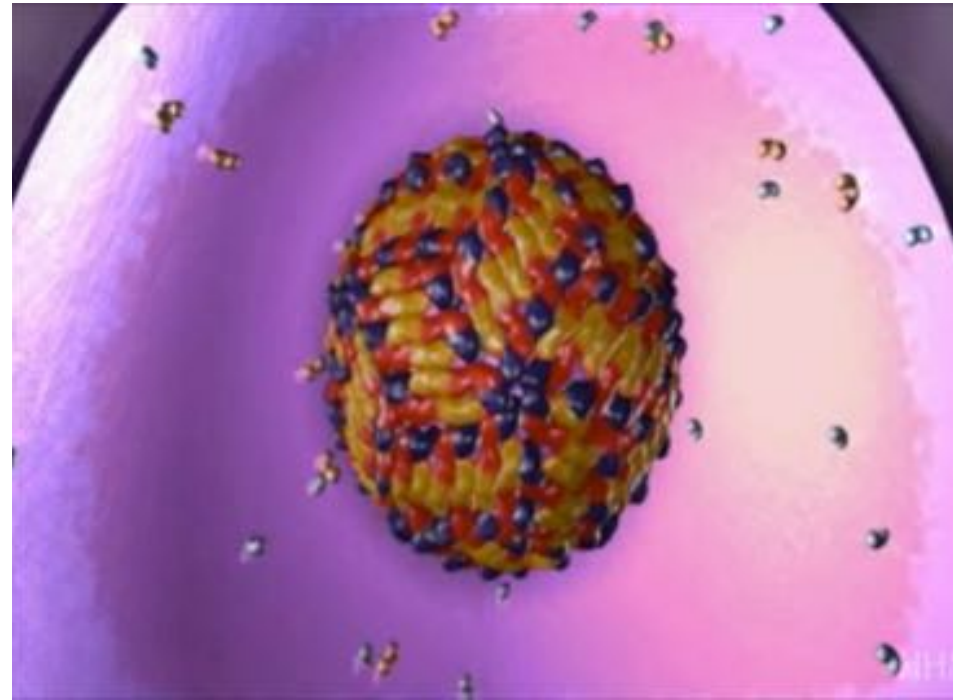
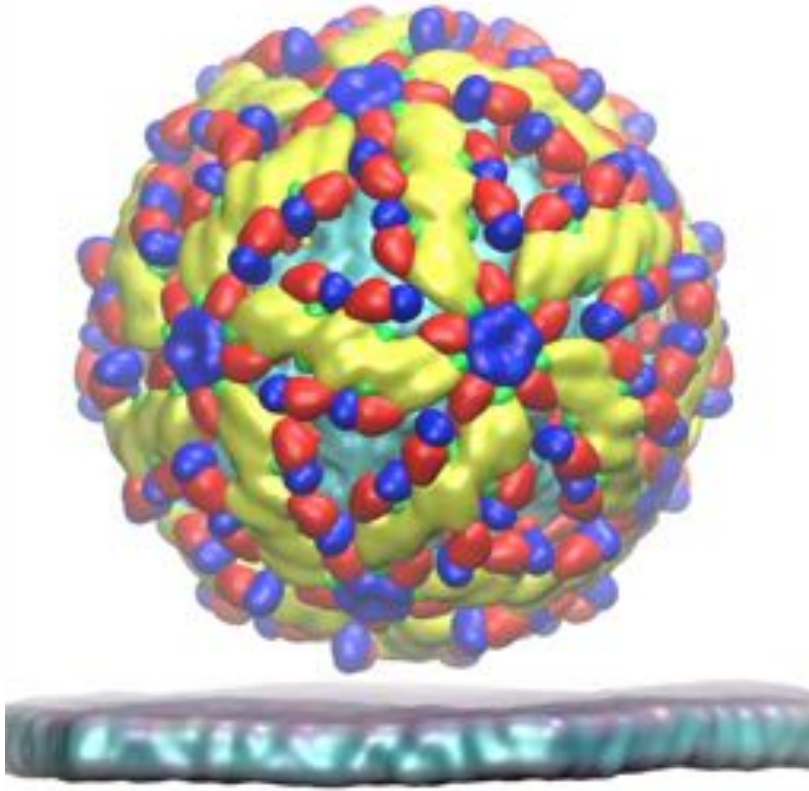
FRET - envelope expansion.

(Lim XX et al. *Nat Commun.* 2017 8:14339.)

- Emergence of protein / lipid pores – ion binding?

# Virion Dynamics: From “Breathing” to Fusion...

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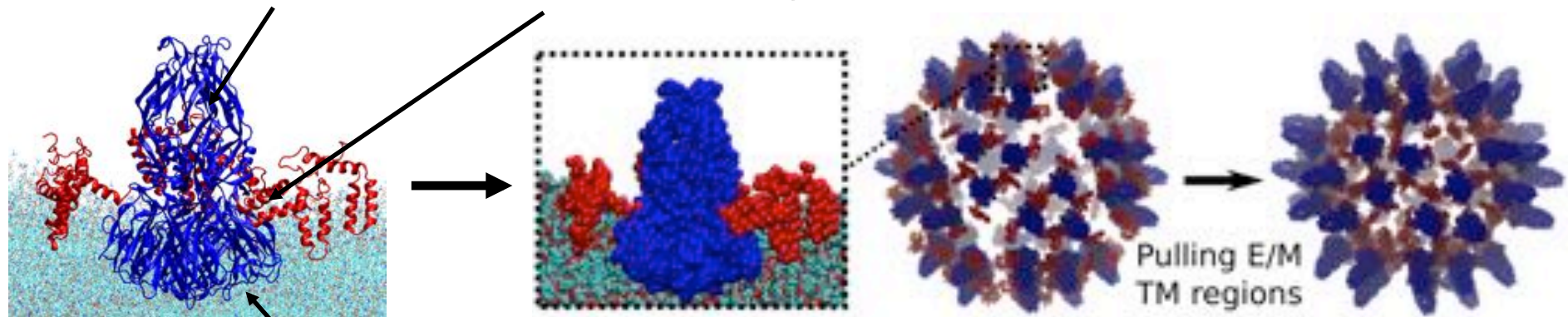
Dengue Virus Life Cycle | HHMI's BioInteractive, 2010

- Late endosomal membrane model - PC, PE, sterols, phosphatidylinositol 3-phosphate (PI3P) & bis(monoacylglycero) phosphate (BMP).
- No significant fusion for expanded virion - particle is “robust”.
- Need for major conformational changes induced by pH drop...



# Integrative Model of a “Spiky” Virion

ectodomain (X-ray) stem+TM (cryoEM)



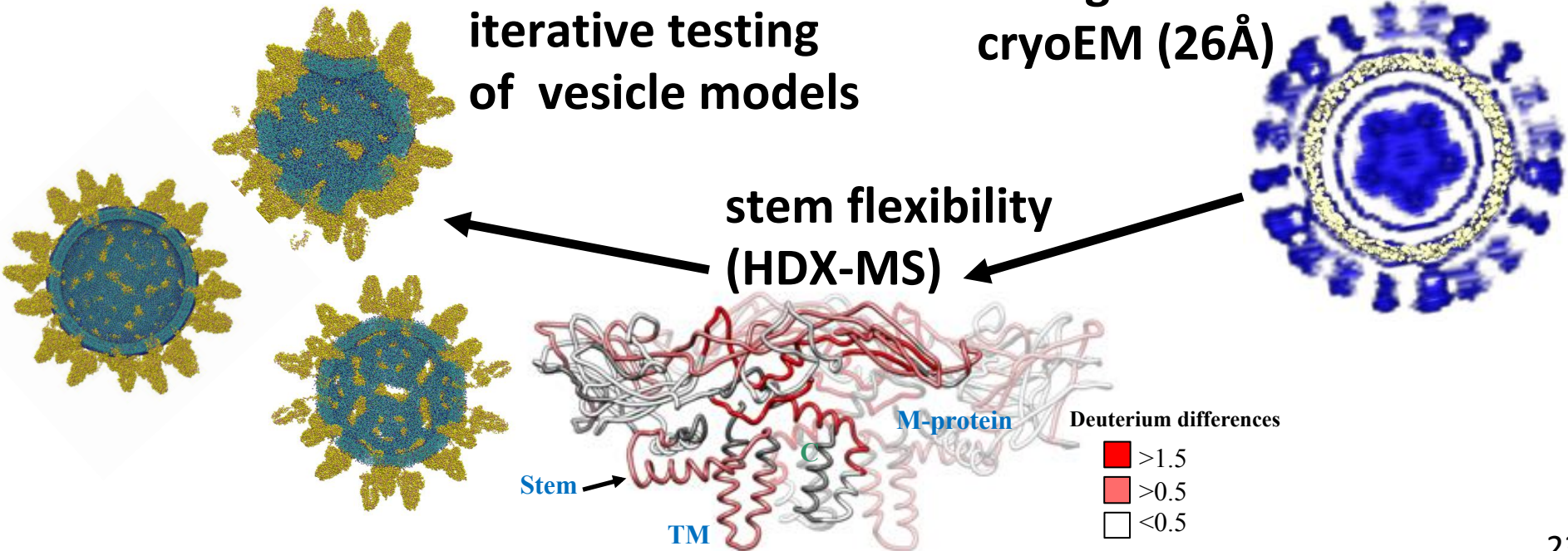
viral membrane

Pulling E/M  
TM regions

fitting to  
cryoEM (26Å)

iterative testing  
of vesicle models

stem flexibility  
(HDX-MS)

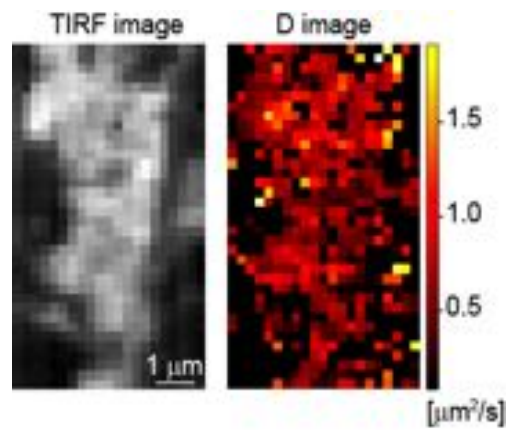
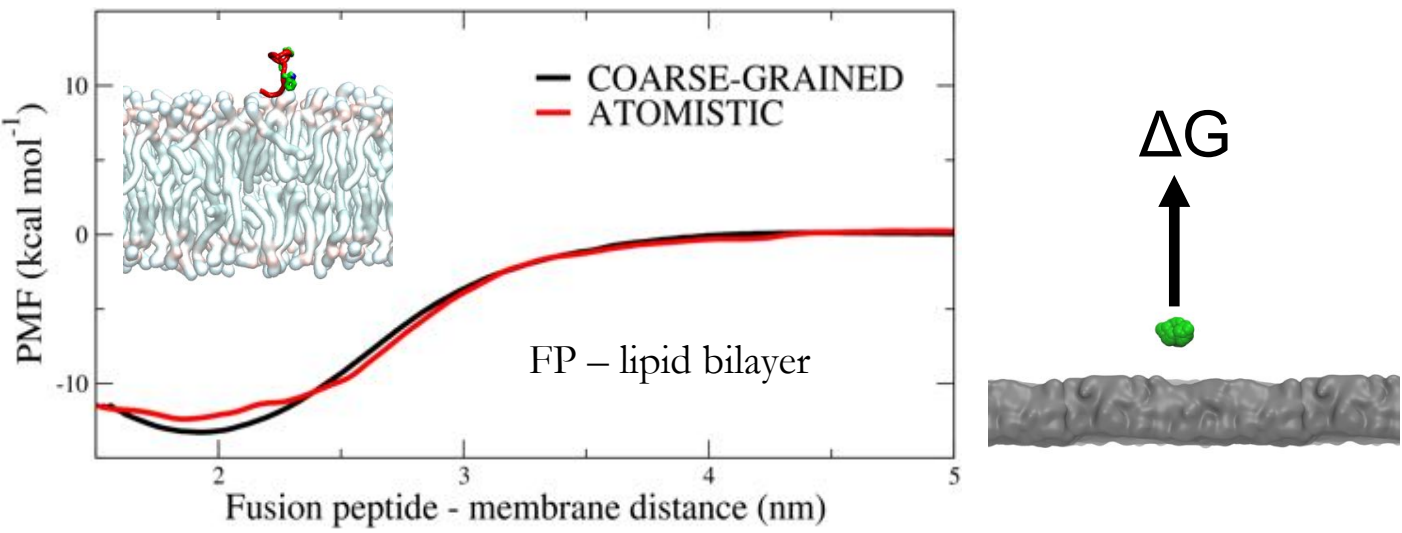


Deuterium differences

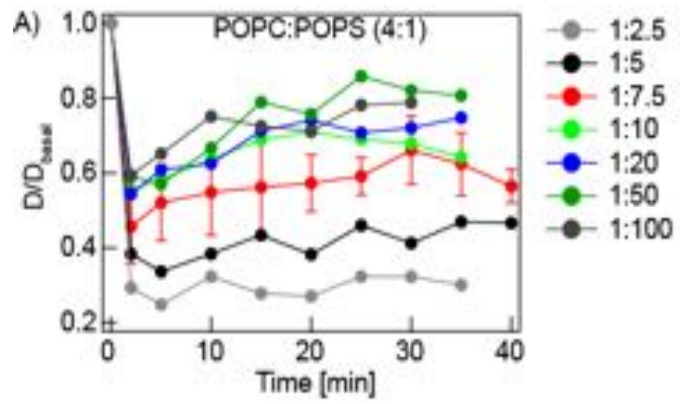
- >1.5
- >0.5
- <0.5



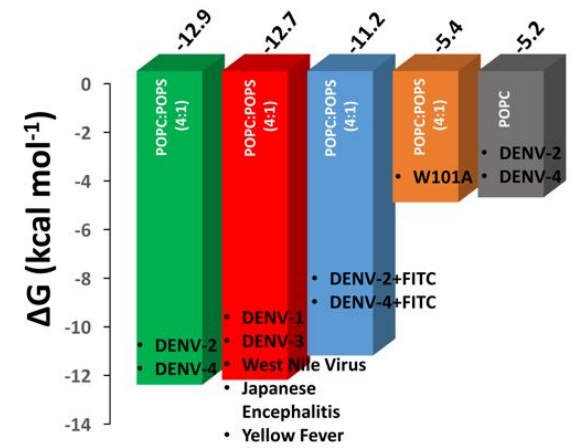
# Membrane “Attack” by Virion Spikes



FITC-labelled peptide diffusion



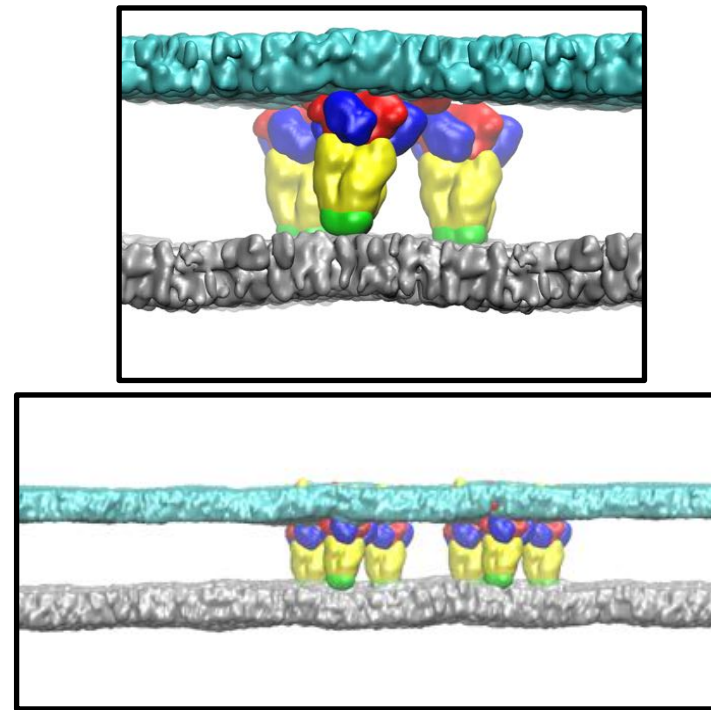
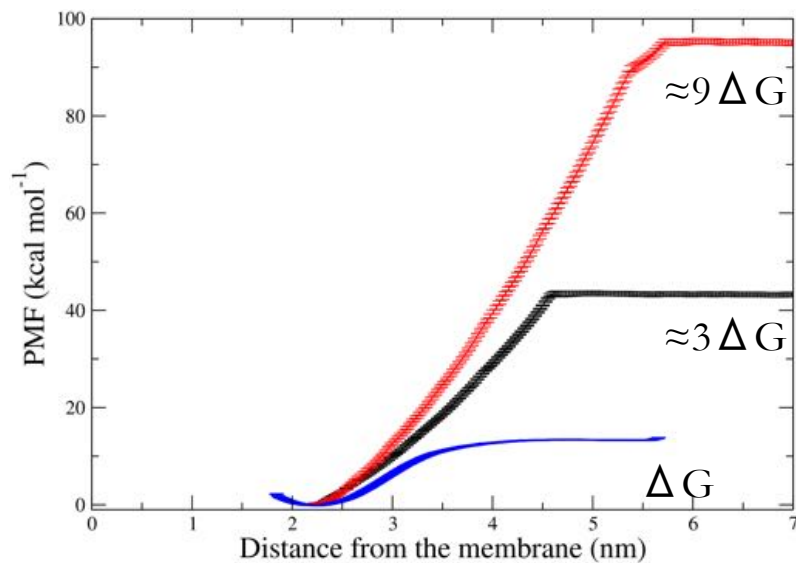
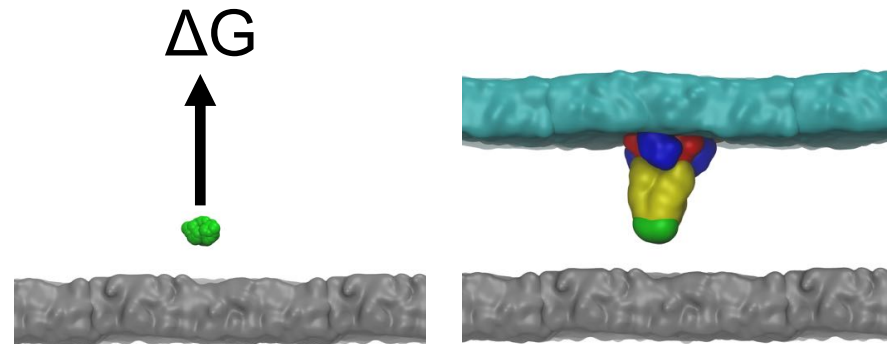
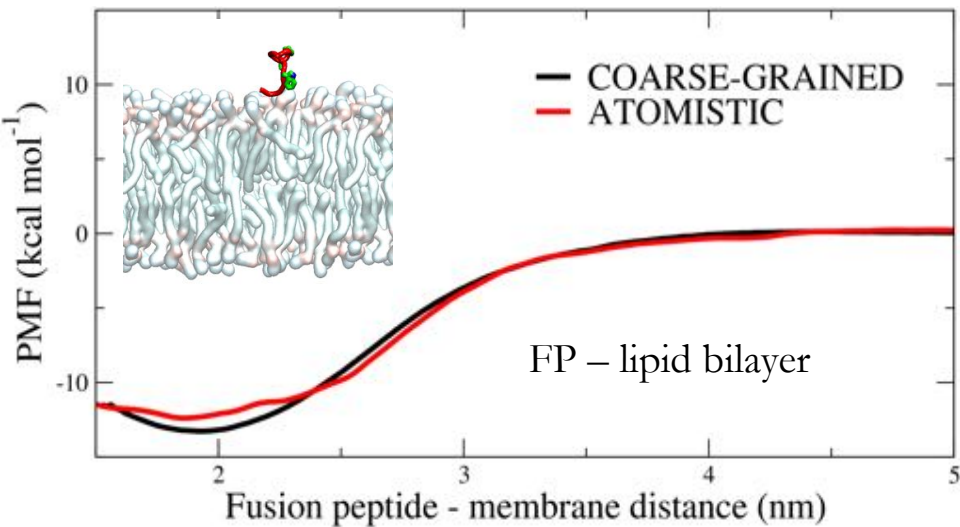
Rhodamine-labelled lipid diffusion



2017. Marzinek et al. *Submitted*

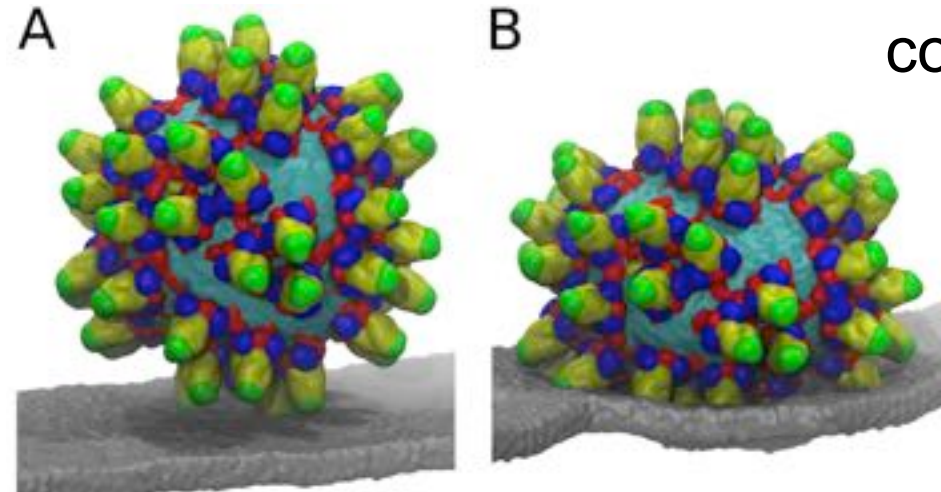
2016 Marzinek et al, *Sci Rep.* 5, 19160

# Membrane “Attack” by Virion Spikes

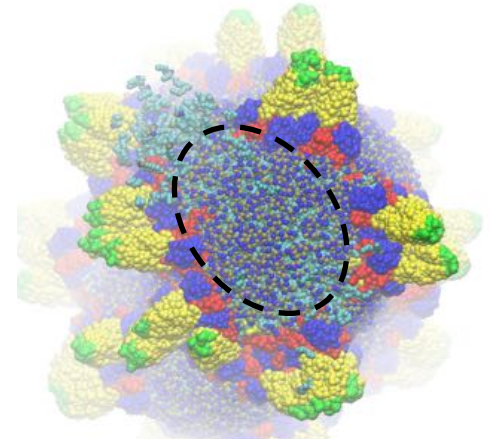
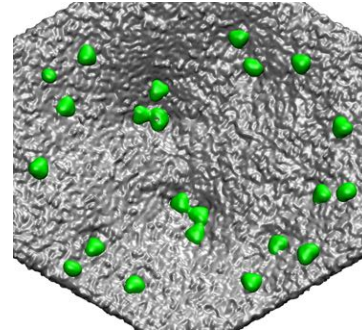




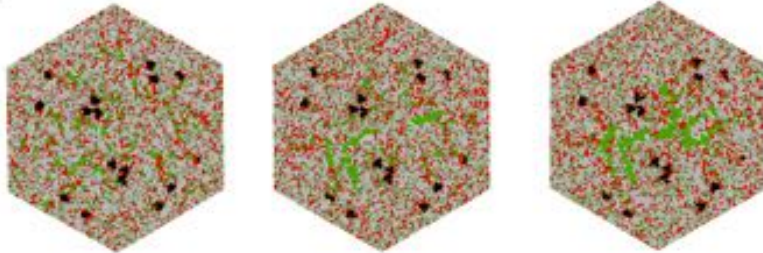
# Spiky Virus Sculpts Host Membranes



cooperative FPs



Equilibration

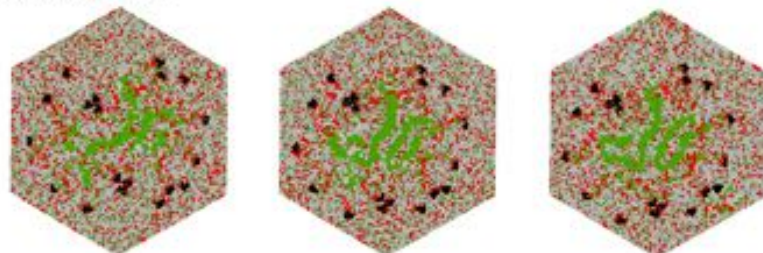


0  $\mu$ s

0.20  $\mu$ s

0.90  $\mu$ s

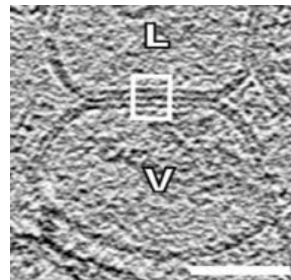
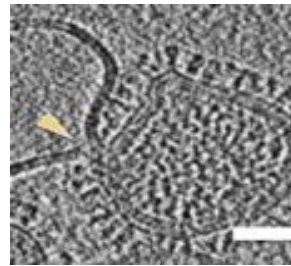
Production run



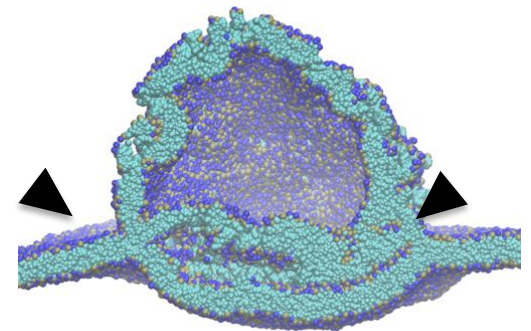
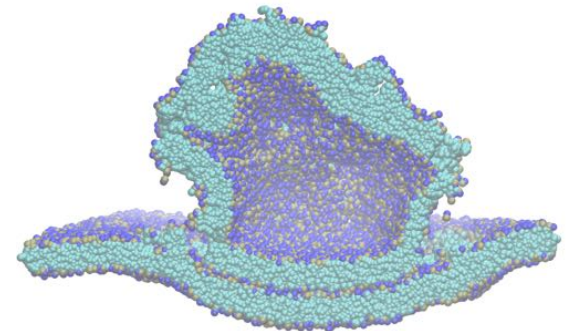
1.35  $\mu$ s

2.70  $\mu$ s

4.50  $\mu$ s



Gui et al. *J. Virology*.  
90:6948-



■ Fusion peptide   
 ■ Cholesterol   
 ■ Anionic lipids (BMP+PI3P)   
 ■ Zwitterionic lipids (POPC+POPE)

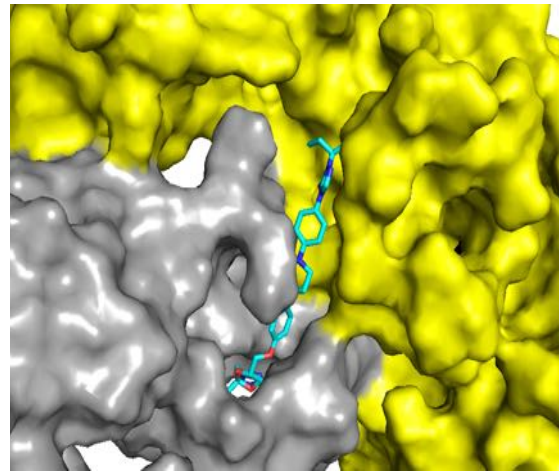


# Multiscale Flavivirus Dynamics: Now & Next

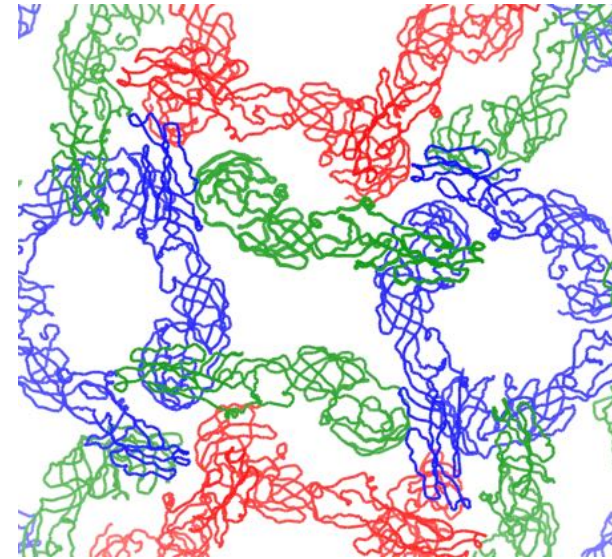
- General approach to enveloped viruses → dynamics & fusion.
- Multiscale integrative modelling of nucleocapsid.
- Future: therapeutics, antibodies...



Capsid inhibition... Cf. Faustino et al. **Understanding Dengue Virus Capsid Protein Disordered N-Terminus and pep14-23-Based Inhibition.** '15 *ACS Chemical Biology* 10:517-526.



Screening for cryptic pockets, antivirals (Soni et al., *submitted*, 2017).



**Structural basis for antibody-induced dengue maturation / infectivity.** (Wirawan et al. *Submitted*, 2017).

# Acknowledgements

Jim Warwicker  
Bob Ford  
Martin Ulmschneider  
Olivera Francetic  
Tom Piggot  
Syma Khalid

“LPS Network”  
Artur Schmidtchen  
& Jitka Petrlova  
Graeme Lancaster  
Clare Bryant  
Sebastian Hiller  
Hubert Yin  
LKC Medicine & Lund  
BakerIDI Melbourne  
Uni. Cambridge  
Uni. Basel / EMBL  
Uni. Colorado

Computing  
BII  
ACRC  
NSCC

## SGD\$19M, MoE Tier 3 grant.

- Singapore-wide project involving 15 PI's, hosted by NUS (Paul Matsudaira).
- **Chandra Verma (BII)**
- **Sheemei Lok (Duke-NUS)**
- **Thorsten Wohland (NUS)**
- **Ganesh Anand (NUS)**
- **Gerhard Gruber (NTU)**



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of Singapore



**NANYANG**  
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**Aishwary Shivgan**

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