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

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Immune Recognition of Bacterial LPS

→ active as dimer
• 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”


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).
• Electron density profile + cation “cross-links”.
• $AC \ 0.255 \pm 0.004 \ \text{nm}^2$ (X-ray diffraction = 0.26 nm$^2$.)
• $D_l \sim 4 \times 10^{-9}$ (2-3 $\times 10^{-9}$ cm$^2$s$^{-1}$ from fluorescent labelling.)
• Free-energies of LPS affinity from atomistic simulations (250-300 kJ mol$^{-1}$) - *Scientific Reports* (2015) 5:17997
• 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
CD14

\[ \Delta \Delta G^{\text{LPS}} = +150 \text{ kJ/mol} \]

\[ \Delta \Delta G^{\text{MD2}} = -50 \text{ kJ/mol} \]

\[ \Delta \Delta G^{\text{TLR4}} = -250 \text{ kJ/mol} \]
TLR4-MD-2 / CD14 Assembly & Lipid Exchange

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

LPS Multiscale Modelling: Now & Next


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

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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

I. Viral envelope membrane structure / dynamics

II. Nucleocapsid architecture / interactions

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

Marzinek et al. (2016) *Structure*, 24:1410-
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.

Huber et al. (2016). *Prog. Biophys. Mol. Biol.*
Comparing Theory & Experiment

90 E dimers + 90 M dimers

PC:PE:PS (6:3:1) lipid vesicle membrane

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

$t = 0$ ns: correlation = 0.37

~1 million coarse-grained particles (hundreds of ns required for equilibration).
Refinement of Envelope: Basis for Curvature

Integrative Modelling of Virion “Breathing”

- 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

- Emergence of protein / lipid pores – ion binding?

FRET - envelope expansion.
(Lim XX et al. Nat Commun. 2017 8:14339.)
Virion Dynamics: From “Breathing” to Fusion...

- Late endosomal membrane model - PC, PE, sterols, phosphatidylinositol 3-phosphate (PI3P) & bis(monoacylglycerol) 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

iterative testing of vesicle models

stem flexibility (HDX-MS)

fitting to cryoEM (26Å)

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
Membrane “Attack” by Virion Spikes

FP – lipid bilayer

$\Delta G \approx 3$ $G$

$\Delta G \approx 9$ $G$

PMF (kcal mol$^{-1}$)

Fusion peptide – membrane distance (nm)

PMF (kcal mol$^{-1}$)

Distance from the membrane (nm)
Spiky Virus Sculpts Host Membranes

cooporative FPs

Gui et al. J. Virology. 90:6948-
Multiscale Flavivirus Dynamics: Now & Next

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

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


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