Michael G. Rossmann and Hybrid Methods: High Quality Interpretation of Low Resolution CryoEM Maps

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Why Was Michael Interested In Dengue Virus in Early 2000s?

- Enveloped, +RNA-genome containing flavivirus transmitted by *Aedes* mosquito
- Spectrum of symptoms: Mild fever to hemorrhagic shock and death
- Multiple outbreaks in the Americas in early 2000s:

2000- Ecuador, 23,000 cases

2000- Paraguay, 25,000 cases

2001- Peru, 23,000 cases

2002- Pan America, >1,000,000 cases

- Mature infectious form: Envelope (E) protein on surface
- E protein: Major target of neutralizing antibodies
- E protein dimer crystallized from TBEV (Felix Rey and Stephen Harrison, Nature 1995)
- No structure of mature infectious virus...







Structure of Dengue Virus: Implications for Flavivirus Organization, **First structure of infectious flavivirus** • Maturation, and Fusion

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- CryoEM, resolution=24Å
- Michael→interpretation

through the use of vaccination has proved to be elusive (Burke and Monath, 2001).

Electron micrographs showed that dengue virions are characterized by a relatively smooth surface, with a diameter of approximately 500 Å, and an electron-dense core surrounded by a lipid bilayer. In addition to the plus-sense RNA genome of \sim 10,700 nucleotides, there are three structural proteins that occur in stoichiometric amounts in the particle: core (C, 100 amino acids), membrane (M, 75 amino acids), and envelope (E, 495 amino acids). The atomic structure for the homologous E protein of TBEV has an elongated shape consisting of a central domain (I) that connects an Ig-like domain (III) to a dimerization domain (II) (Rey et al., 1995). Based on

CryoEM Map of Mature Dengue Virus @ 24Å Resolution

- Icosahedral reconstruction
- Smooth featureless golf-ball
- No spikes on surface \rightarrow
 - interpretation challenging



E Protein Distance from Viral Center?



At 24Å resolution, jumbled mess of a map, hard to interpret for everyone...



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Michael Pushes Technology Development



"Single Finger Fast Typing" Artist: Jianing "Kara" Fu Michael's last graduate student

Introducing EMfit



"Single Finger Fast Typing" Artist: Jianing "Kara" Fu Michael's last graduate student Journal of Structural Biology **136**, 190–200 (2001) doi:10.1006/jsbi.2002.4435, available online at http://www.idealibrary.com on IDELL®

Combining Electron Microscopic with X-Ray Crystallographic Structures

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Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392 Received November 2, 2001, and in revised form December 26, 2001; published online April 10, 2002

Principles of fitting crystallographic coordinates into EM map:

-Maximize number of atoms in high density regions
-Minimize atoms in low (or negative) density regions
-Minimize clashes between symmetry-related molecules

$$\mathsf{R}_{\mathsf{crit}} = \sum \omega_{\mathsf{i}} \, \mathsf{s}_{\mathsf{i}} \, \left[\frac{(\,\theta - \langle \theta \rangle)}{\sigma_{\mathsf{i}}(\theta)} \right] (\sum \omega_{\mathsf{i}})^{1/2}$$

 ω , weighting factor

s, sign factor (+1 for maximization; -1 for minimization)

- θ, parameter (+density, -density, clashes)
- σ , standard deviation of parameter (θ)

Interpreting the Dengue Virus Map

Dimer 2-fold axis || i2 axis Two search parameters: rotation & translation about i2 axis

i3

i5

i2

i3

| Table 1. Fit of TBEV E Dimers onto an Icosahedral 2-Fold Axis of the CryoEM Dengue Map | | | | | | | | |
|--|-----------------------|------------------|-----------------|---------|-----------------|------------------|--|--|
| (A) Using | g the TBEV Dimer as D | Determined Cryst | allographically | | | | | |
| Fit | Sumf | Clash | -Den | Theta 1 | Cent (x, y, z) | D1, D2, D3 | | |
| down+ | 53.0 | 0.0 | 29.0 | 24.5 | 0.0, 0.0, 232.5 | 48.6, 56.5, 49.4 | | |
| down- | 49.6 | 0.0 | 40.0 | 133.8 | 0.0, 0.0, 230.5 | 41.0, 52.1, 55.9 | | |
| up+ | 44.8 | 0.0 | 82.0 | 119.8 | 0.0, 0.0, 233.5 | 38.1, 48.9, 59.9 | | |
| up- | 40.2 | 0.0 | 116.0 | 30.8 | 0.0, 0.0, 229.5 | 43.3, 36.7, 43.1 | | |

i2

4 experiments: Reconstructed map ("+") Inverted map ("-") Dimer facing down Dimer facing down Dimer facing down Dimer facing down

Interpreting the Dengue Virus Map

Dimer 2-fold axis || i2 axis Two search parameters: rotation & translation about i2 axis

i3

i5

i2

i3



(A) Using the TBEV Dimer as Determined Crystallographically

| Fit | Sumf | Clash | -Den | Theta 1 | Cent (x, y, z) | D1, D2, D3 |
|-------|------|-------|-------|---------|-----------------|------------------|
| down+ | 53.0 | 0.0 | 29.0 | 24.5 | 0.0, 0.0, 232.5 | 48.6, 56.5, 49.4 |
| down- | 49.6 | 0.0 | 40.0 | 133.8 | 0.0, 0.0, 230.5 | 41.0, 52.1, 55.9 |
| up+ | 44.8 | 0.0 | 82.0 | 119.8 | 0.0, 0.0, 233.5 | 38.1, 48.9, 59.9 |
| up- | 40.2 | 0.0 | 116.0 | 30.8 | 0.0, 0.0, 229.5 | 43.3, 36.7, 43.1 |

4

i2

4 experiments: Reconstructed map ("+") Inverted map ("-") Dimer facing down Dimer facing up Dimer facing down Dimer facing up

The Icosahedral E Protein Dimer



Coordinates and cavities

The Remainder of the Asymmetric Unit



The Remainder of the Asymmetric Unit



The Remainder of the Asymmetric Unit

q2 axis

Orientation

of q2 axis

| Table 2. Fitting the Second Dimer into the CryoEM Map after All Pixels Occupied by the First Dimer Were Set to Zero Fit Sumf Clash -Den Theta 1 Cent (x, y, z) D1, D2, D3 D4, D5 | | / | | | | | | | |
|---|------------|------------|---------------------|----------------|---------------|-----------------|--|--------------------------------------|--------------------------------------|
| Fit Sumf Clash – Den Theta 1 Cent (x, y, z) D1, D2, D3 D4, D5 | | Tabl | e 2. Fitting the Se | cond Dimer in | to the CryoEl | M Map after All | Pixels Occupied by the F | irst Dimer Were Set to | Zero |
| | | Fit | Sumf | Clash | -Den | Theta 1 | Cent (<i>x</i> , <i>y</i> , <i>z</i>) | D1, D2, D3 | D4, D5, D6 |
| Rotation & down+ 48.3 20.0 57.0 204.5 48.8, -33.5, 221.6 44.2, 52.3, 51.8 51.3, 4 down- 43.8 2026.0 105.0 345.2 87.5, -25.2, 210.8 39.1, 47.3, 43.1 49.2, 4 | Rotation & | dow dow | n+ 48.3 n- 43.8 | 20.0 2026.0 | 57.0 105.0 | 204.5 345.2 | 48.8, -33.5, 221.6 87.5, -25.2, 210.8 | 44.2, 52.3, 51.8 39.1, 47.3, 43.1 | 51.3, 47.3, 40.9 49.2, 47.1, 30.8 |

• Radial quasi-2-fold axis ("q2")

- Dimer 2-fold axis || q2
- Three parameters refined:
 - Translation
 - Rotation
 - q2 axis orientation

A Raft is Discovered...



...By a Sailor



Structure of Dengue Virus by Michael Rossmann



Specific domain organization of E protein: <u>Domain I</u>: i3 axis <u>Domain II</u>: i2 axis <u>Domain III</u>: i5 axis

Confirmed by high resolution structures of Dengue, Zika, TBEV, KUNV, WNV



MGR: Deep understanding of symmetry, truly believed in viral icosahedral symmetry→guiding principle of his career

So Much For Michael's Principles (of Icosahedral Symmetry)



Flaviviruses have imperfect icosahedral symmetry

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Edited by Ian A. Wilson, The Scripps Research Institute, La Jolla, CA, and approved September 27, 2018 (received for review May 30, 2018)

Flaviviruses assemble initially in an immature, noninfectious state and undergo extensive conformational rearrangements to generate mature virus. Previous cryo-electron microscopy (cryo-EM) structural studies of flaviviruses assumed icosahedral symmetry and showed the concentric organization of the external glycoprotein shell, the lipid membrane, and the internal nucleocapsid core. We show here that when icosahedral symmetry constraints were excluded in calculating the crvo-EM reconstruction of an immature flavivirus, the nucleocapsid core was positioned asymmetrically with respect to the glycoprotein shell. The core was positioned closer to the lipid membrane at the proximal pole, and at the distal pole, the outer glycoprotein spikes and inner membrane leaflet were either perturbed or missing. In contrast, in the asymmetric reconstruction of a mature flavivirus, the core was positioned concentric with the glycoprotein shell. The deviations from icosahedral symmetry demonstrated that the core and glycoproteins have varied interactions, which likely promotes viral assembly and budding.

flaviviruses | cryo-electron microscopy | assembly | budding | asymmetry

С

Results

Cryo-EM movies of immature and mature KUNV were collected on an FEI Titan Krios equipped with a K2 direct electron detector. Immature and mature KUNV showed characteristic spiky and smooth particles, respectively (SI Appendix, Fig. S1 A and B). An asymmetric reconstruction (Materials and Methods) of immature KUNV was performed with the program RELION 2.1 (25), starting with an icosahedral reconstruction (Fig. 1 A and B) as an initial model. The asymmetric reconstruction was refined to ~20 Å resolution (Fig. 1C and SI Appendix, Fig. S2). The position of the majority of the glycoprotein trimers in the asymmetric reconstruction of the immature virus correspond to their position in the icosahedral reconstruction, demonstrating that the glycoprotein shell has approximate icosahedral symmetry. In addition, the position of the transmembrane densities of these glycoproteins in the asymmetric reconstruction is essentially the same as in the icosahedral reconstruction.

However, unlike the icosahedral reconstruction in which the nucleocapsid core density is situated concentric with the glycoprotein shell, in the asymmetric reconstruction, the nucleocapsid







