# Fitting high resolution structures into low resolution EM maps


## Michael Rossmann

## Purdue University


### Fitting Processes

1. Map scaling
2. Symmetry constraints
3. Fitting criteria
   a. fit of atoms into density
   b. avoiding negative density
   c. steric hindrance, inter atomic clashes
   d. restraints imposed by known structural features
4. Combining different criteria
   a. normalization of each measurement
5. The search process
   a. rotational search
   b.multi-dimensional "climb" or least squares
6. Verification
   a. hand of map
   b. subunit contacts
7. Problems
   a. symmetry missmatches
   b. unknown structural components
   c. uninterpreted density

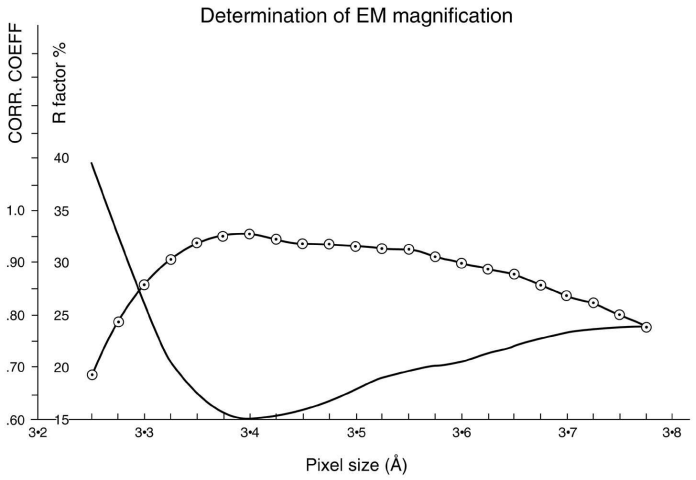
### Map Scaling

Minimize $\Sigma\ [\rho_1(x_1,y_1,z_1) - (a + b\ \rho_2(x_2,y_2,z_2))]^2$
where $\rho_1$ is the reference map (e.g. X-ray virus map)
and $\rho_2$ is the map of interest (e.g. virus plus ligand complex)
And $x_1 = x_2 + \delta x_2$, $y_1 = y_2 + \delta y_2$, $z_1 = z_2 + \delta z_2$
Requiring interpolation for determinin $\rho_2$

Or maximize the correlation C, where
$C = [\Sigma(<\rho_1> - \rho_1)(<\rho_2> - \rho_2)] / [\Sigma(<\rho_1> - \rho_1)^2].[\Sigma(<\rho_2> - \rho_2)^2]$

*Comparison of the PV1:CD155 EM map with the PV1 X-ray map:*

| Shell radius (Å) | 108 - 120 | 120 - 132 | 132 - 144 | 144 - 156 |
|---|---|---|---|---|
| Number of Pixels* | 3,918 | 22,203 | 17,914 | 3,424 |
| Correlation Coefficient† | 0.1881 | 0.2662 | 0.9105 | 0.9860 |




### Symmetry Constraints

Let the atomic positions of a model be given by $(X,Y,Z)$, or, in vector notation, by **X**, in an orthogonal coordinate system.
Let the origin of the model (defined by its center of mass) be at **S**.
Let the rotation matrix required to place the model into the "reference" EM density be $[E]$, Then

$$\mathbf{X'} = [E]\mathbf{X} + \mathbf{d},$$

where **X'** are the coordinates of the model atoms in the EM map and **d** is a translation vector.

Let **S'** be the approximate target position in the EM map for placement of the model's origin. Then

$$\mathbf{S'} = [E]\mathbf{S} + \mathbf{d}$$

and, hence,

$$\mathbf{d} = \mathbf{S'} - [E]\mathbf{S}$$

or

$$\mathbf{X'} = [E](\mathbf{X} - \mathbf{S}) + \mathbf{S'}\ .$$

Let the reference molecule be reproduced by $M$ "crystallographic" and T "NCS" symmetry operations given by $[R_m]$ ($m$ = 1, $M$ and t=1, $T$). Thus

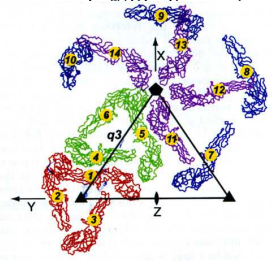
$$\mathbf{X''} = [R_{m,t}]\mathbf{X'}$$

And hence using

$$\mathbf{X'} = [E](\mathbf{X} - \mathbf{S}) + \mathbf{S'}$$

It follows that

$$\mathbf{X''} = [R_{m,t}]([E](\mathbf{X} - \mathbf{S}) + \mathbf{S'})$$




Sindbis Virus
$M$=60 icosahedral operators
$T$ = 4 quasi symmetry NCS operators

**Fitting criteria**

a. fit over N atoms into density

$$sumf = 100.\Sigma_{T} ( \Sigma_{N} \rho(X'') / TN\rho_{norm}$$

where $\rho_{norm}$ is either the maximum or rms density

b. The number of atoms (N′) in negative density, expressed as a %

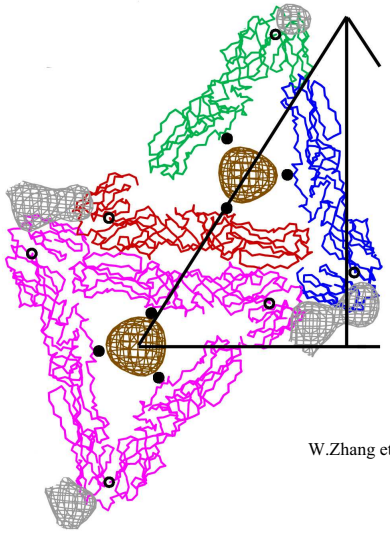
$$-den = 100.\Sigma_{T} (N') / TN$$

c. The number of atoms (N″) that approach atoms in another molecule to within 3.4A, expressed as a %.

$$clash = 100.\Sigma_{T} (N'') / TN$$

d. The average or rms distance between L specific fixed points ($R_i$) in the map and specific atoms on the molecule ($X''_i$) (e.g. Carbohydrate moities in the map and corresponding aas).

$$avgdist = \Sigma_{L} |(R_i - X''_i)| / L$$



Fitting the E1 protein of Sindbis virus : Using carbohydrate sites as restraints

W.Zhang et al, J.Virol, 2002, 76, 11645-11658

**Use of Restraints**

1. Minimizing the distance between recognizable features in the cryoEM map and the associated atomic Group of the molecule being fitted
2. Restraining the molecule being placed in a map to use a specific contact region to other parts of the structure
3. Keeping a short distance between the C-end of one domain and the N-end of the next, independently fitted, domain.

**Combining different criteria**

$$R_{crit} = \Sigma\, \omega_i s_i [(\nu_i - \langle\nu_i\rangle) / \sigma(\nu_i)] / \Sigma\omega_i$$

Where $\nu_i$ is the value of the ith criterion,

$\langle\nu_i\rangle$ is the standard deviation of $\nu_i$ taken over a set of randomly oriented molecular fits into the density,

$\omega_i$ is the weight (usually 1.0) to be placed on the given criterion and

$s_i$ is +1.0 if the criterion is to be maximized (e.g. *sumf*) or -1.0 if the criterion is to be minimized (e.g. *–den*, *clash* and *avgdist*)

Fitting the E1 protein of Sindbis virus. The top 25 best fit converge to only 4 different fits on refinement

a. Values of criteria

| Fit No | $R_{crit}$ | sumf | clash | -den | avgdist Å |
|---|---|---|---|---|---|
| 13 | 0.98 | 39.3 | 0.5 | 9.2 | 21.9 |
| 10 | 0.81 | 37.3 | 2.2 | 10.1 | 20.5 |
| 14 | 0.26 | 36.3 | 3.7 | 11.9 | 21.2 |
| 25 | -2.37 | 39.2 | 17.5 | 10.1 | 28.7 |

b. Criteria expressed as the number of **σ** above mean

| Fit No | $R_{crit}$ | sumf | clash | -den | avgdist |
|---|---|---|---|---|---|
| 13 | 0.98 | 2.38 | 0.19 | 1.52 | 0.93 |
| 10 | 0.81 | 1.40 | -1.35 | 1.18 | 1.48 |
| 14 | 0.26 | 0.48 | -2.78 | 0.42 | 1.22 |
| 25 | -2.37 | 2.32 | -23.10 | 1.15 | -1.67 |

**The search process**

2. Explore all unique values of the three Eulerian angles that define the [E] rotation matrix, using fairly large angular intervals
   $0 \le \theta_1 < 2\pi$; $0 \le \theta_2 \le \pi$, $0 \le \theta_3 < 2\pi$
2. Rank according to sumf
3. Use results for determining the mean and standard deviation (σ) for each criterion required to calculate $R_{crit}$.
4. Refine the top n (e.g. 100) best fits by a six dimensional "climb" on $R_{crit}$, using fine angular and positional intervals.
5. Eliminate all but one of closely similar fits, leaving only distinctly different fits.

*Note: fitting more than one rigid body at a time can be done sequentially and refined by least squares*

**Refine using "Climb"**

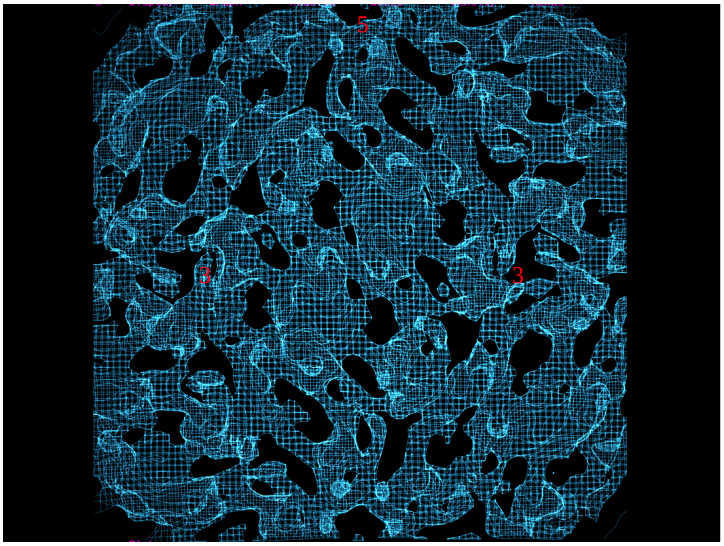
$R_{crit}$ values at end of climb

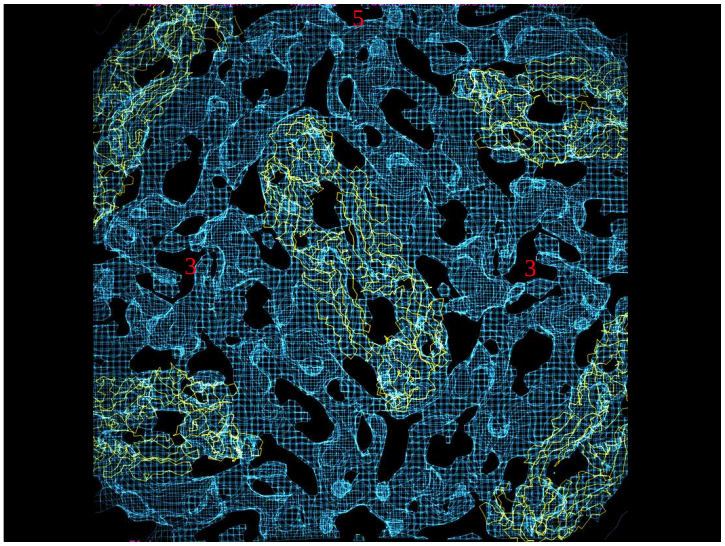
Refining the placement of the E1 glycoprotein
Into Sindbis virus cryoEM density

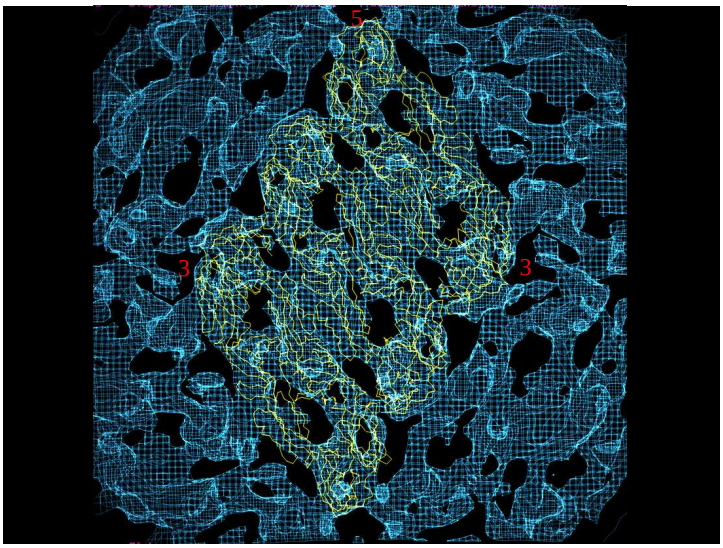
| param | ξ - Δξ | ξ | ξ + Δξ | ξ | Δξ |
|---|---|---|---|---|---|
| $\theta_1$ | 1.016 | 1.029 | 1.022 | 357.0 | 0.25 |
| $\theta_2$ | 1.008 | 1.029 | 1.026 | 40.5 | 0.25 |
| $\theta_3$ | 1.021 | 1.029 | 1.021 | 193.5 | 0.25 |
| x | 0.996 | 1.029 | 1.011 | 23.9 | 0.50 |
| y | 1.028 | 1.029 | 0.990 | 68.3 | 0.50 |
| z | 0.963 | 1.029 | 1.015 | 284.5 | 0.50 |

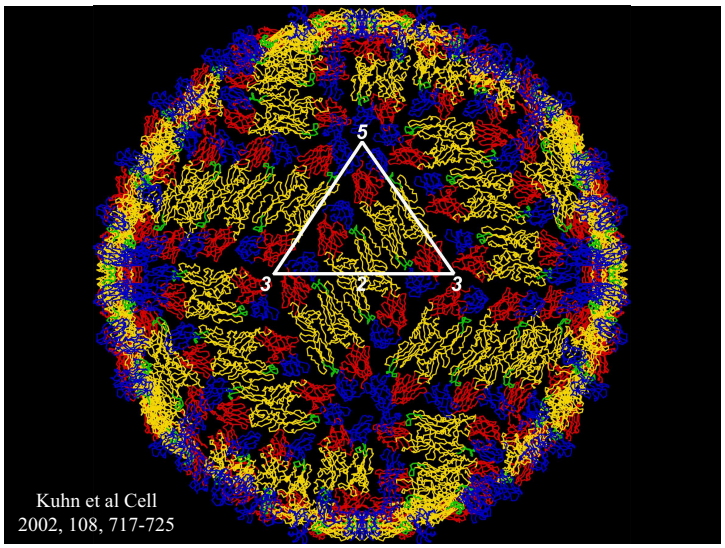
The E glycoprotein dimer of flaviviruses :
Sequential fitting into the mature dengue EM map

TBEV: F. Rey et al Nature, 1995, 375, 291-298
Dengue: Y. Modis et al PNAS, 2003, 100, 6986-6991
Y. Zhang et al, Structure 2004, 22, 2604-2613

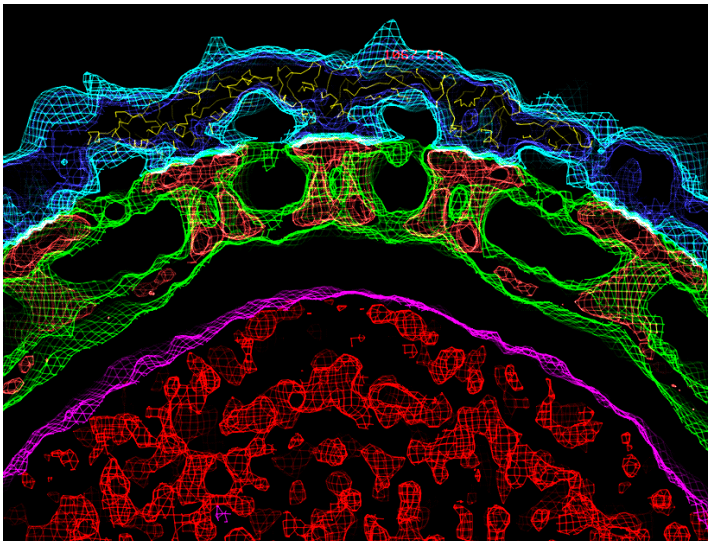


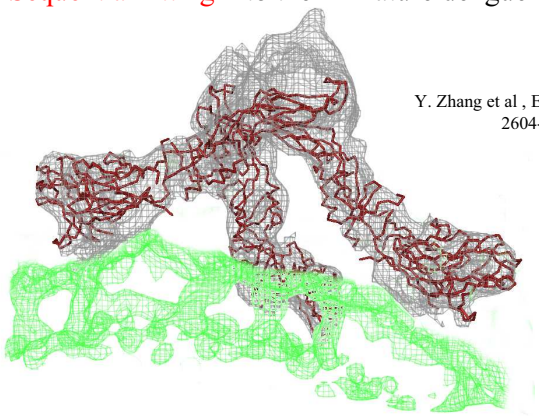




Kuhn et al Cell
2002, 108, 717-725



The E glcoprotein monomer of flaviviruses :
Sequential fitting into the immature dengue virus map



Y. Zhang et al , EMBO J 2003, 22, 2604-2613

Sequential fitting of E monomer into the immature Dengue cryoEM map

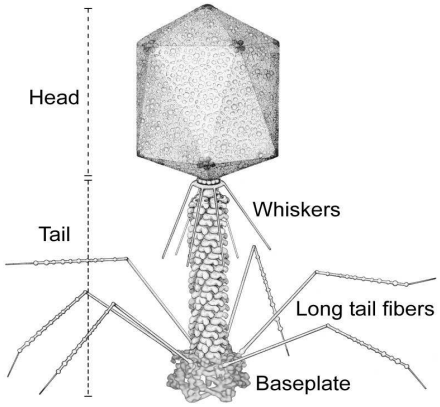
**Results are independent of order of fitting**

| MOL | | *sumf* DI | *sumf* DII | *sumf* DIII | x | y | z | θ1 | θ2 | θ3 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 1st | 50.8 | 55.8 | 42.3 | 32.0 | -7.7 | 220.9 | 15.0 | 61.0 | 349.2 |
| A | 2nd | 49.7 | 56.4 | 44.0 | 31.0 | -6.7 | 221.4 | 11.0 | 61.5 | 345.0 |
| A | 3rd | 50.9 | 56.0 | 40.5 | 31.5 | -6.7 | 220.4 | 10.8 | 61.5 | 355.2 |
| B | 1st | 48.4 | 57.6 | 42.9 | 72.1 | 8.2 | 210.6 | 38.0 | 64.5 | 162.5 |
| B | 2nd | 49.8 | 57.4 | 41.8 | 71.6 | 8.2 | 210.6 | 34.8 | 63.5 | 164.8 |
| B | 3rd | 49.7 | 57.4 | 41.9 | 72.1 | 7.7 | 210.6 | 37.5 | 64.0 | 163.5 |
| C | 1st | 48.9 | 54.7 | 42.1 | 10.5 | 48.3 | 217.0 | 19.8 | 58.0 | 240.2 |
| C | 2nd | 49.2 | 53.1 | 41.3 | 9.0 | 47.8 | 217.0 | 22.0 | 58.5 | 238.5 |
| C | 3rd | 49.5 | 54.9 | 42.8 | 10.0 | 48.3 | 217.0 | 18.2 | 57.0 | 241,8 |

## Validation

1. Is the hand consistent with each fitted protein?
2. Are distances between atoms in the interface reasonable?
3. Are the type of residues in the contact region appropriate? Look for:
   hydrophobic versus hydrophobic
   charge complimentarity
4. Have all the higher density regions been interpreted?
5. Do unexpected results make chemical sense?

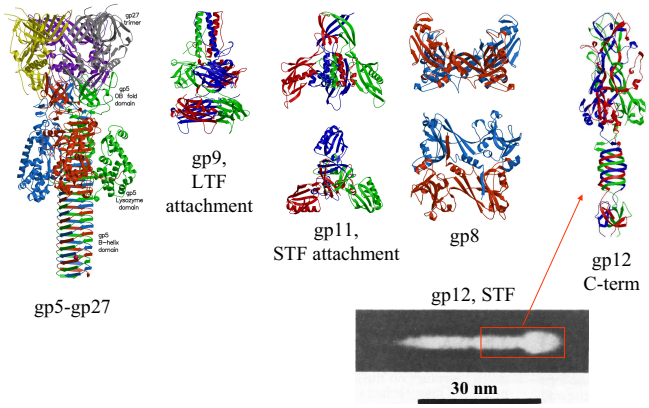
**Validation: Consistent hand verification of the cryoEM map using T4 phage baseplate proteins**




Hexagonal conformation (tube-baseplates)

- Initial model – hexagonal prism connected to a tube
- Sixfold symmetry
- 945 particles used in the reconstruction
- Defoci 1.5 – 3.5 µm
- 12 Å resolution

## Some crystal structures of the baseplate proteins

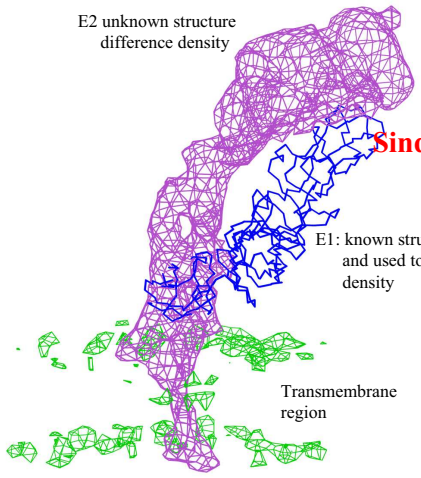




Kostyuchenko et al, Nat. Struct. Biol. 2003, **10**:688-693

## T4 Hand determination: Un-normalized correlation coefficients

| Baseplate protein | Correct hand | Incorrect hand |
|---|---|---|
| gp8 | 1.1 | 0.7 |
| gp11 | 0.9 | 0.7 |
| gp10 | 1.2 | 0.7 |
| gp12 | 1.1 | 1.0 |

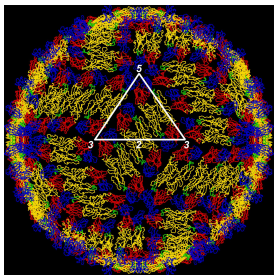



## Validation : Interactions of the E1 and E2 Sindbis virus glycoproteins

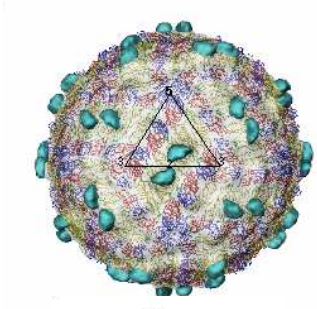
## Validation: Has all of the significant density been interpreted?

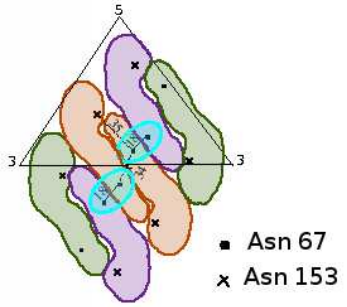
**Original analysis of Dengue Virus Map at 26Å resolution**

| height | ratio1 | ratio2 |
|---|---|---|
| -7 | | 147.0 |
| -6 | | 59.6 |
| -5 | 129.8 | 21.7 |
| -4 | 38.9 | 12.5 |
| -3 | 17.9 | 4.9 |
| -2 | 11.6 | 3.5 |
| -1 | 6.6 | 1.9 |
| 0 | 5.1 | 2.7 |
| 1 | 3.4 | 0.8 |
| 2 | 2.7 | 0.5 |
| 3 | 2.4 | 0.3 |
| 4 | 1.8 | 0.2 |
| 5 | 2.0 | 0.1 |
| 6 | 1.8 | 0.1 |
| 7 | 1.9 | 0.0 |
| 8 | 0.9 | 0.0 |
| 9 | 2.0 | 0.0 |
| 10 | 2.3 | 0.0 |



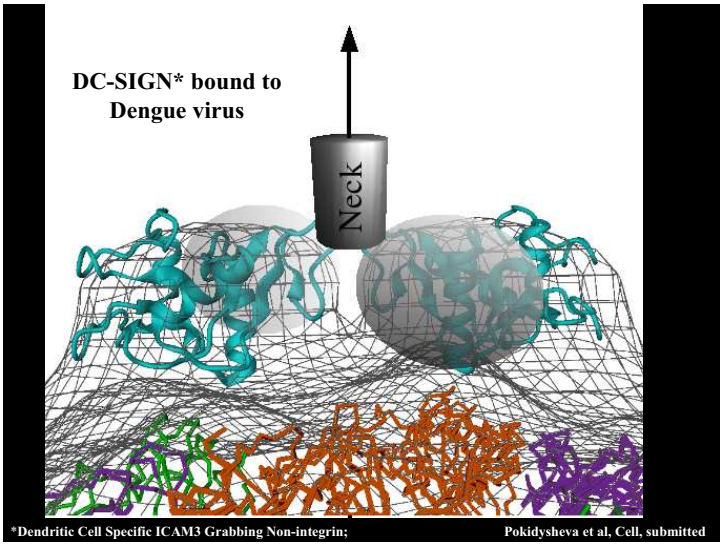
**Ratio=unused/used pixels** (between radii 230 & 250Å)
**Ratio1**: after fitting dimer on i2
**Ratio2** : after fitting dimer on i2 and q2



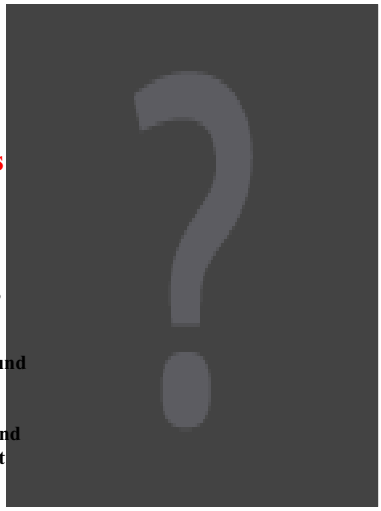



## Validation: Chemical Reasonableness

**Receptor recognition by Dengue virus**




## Other problems:
## 1. Symmetry missmatches
## 2. Envelope of proteins whose structure is unknown



**1. T4 phage 5-fold head symmetry, 6-fold tail symmetry**

**2. Yellow are the HOC molecules found by using a HOC$^-$ mutant**

**3. White are the SOC molecules found by using a HOC$^-$ SOC$^-$ mutant**

Fokine et al, PNAS, 2004, **101**:6003-6008\

## Relevant references

Gao et al, Structure, 2005, **13**, 401-406.
Hansen et al. Biophysics J., 2005, **88**, 818-827.
Navaza et al, Acta Cryst 2002, **D58**, 1820-1825.
Roseman et al, Acat Cryst 2000, **D56**, 1332-1340.
Rossmann et al, J. Struct Biol. 2001, **136**, 190-200.
Volkmann et al, J. Sruct Biol. 1999, **125**, 176-184.
Wriggers et al., Structure 2001, **9**, 779-788,
Wriggers et al, J. Struct Biol 1999, 125, 185-189.

## Acknowledgements

**T4**
Petr Leiman, Victor Kostyuchenko, Paul Chipman, Shuji Kanamaru, Mark van Raaij, Andrei Fukin, Fumio Arisaka, V. Rao, Vadim Mesynanzhinov, Anthoni Battisti

**Dengue Virus**
Wei Zhang, Ying Zhang, Suchetana Mukhopadhyay, Elena Pokidysheva, Glenn Gregorio, Shee-Mei Lok, Carol Bator-Kelly, Anthoni Battisti, Paul Chipman, Tim Baker, Wayne Hendrickson, Jim Strauss, Richard Kuhn

**Sindbis Virus**
Wei Zhang, Suchetana Mukhopadhyay, Sergei Strelkov, Tim Baker, Richard Kuhn

**Polio Virus**
Yongning He, Steffen Mueller, Carol Bator-Kelly, Valorie Bowman, Paul Chipman, Eckard Wimmer, Richard Kuhn

**Program development**
Chuan (River) Xiao, Ricardo Bernal