

Lecture 3:

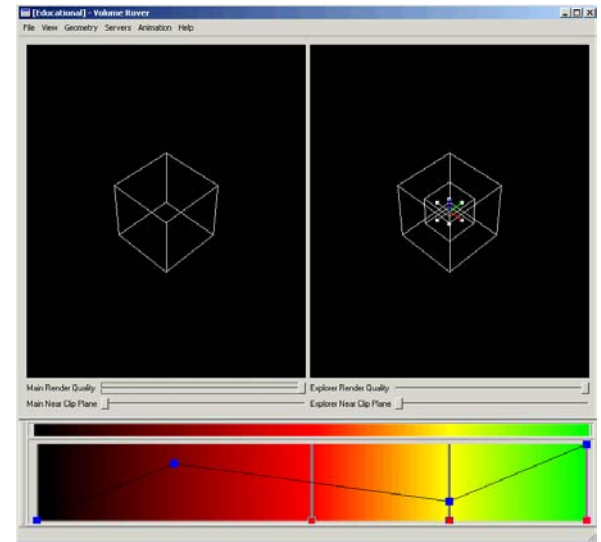
Geometric and Signal 3D Processing (and some Visualization)

Chandrajit Bajaj

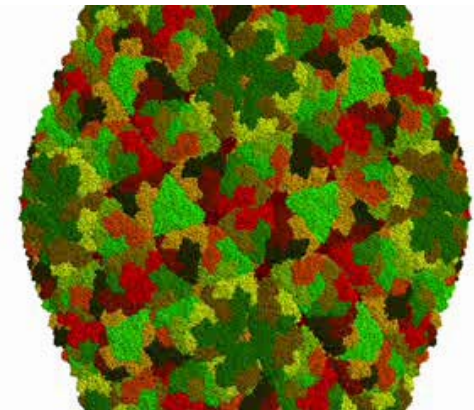


Algorithms & Tools

- **Structure elucidation:** filtering, contrast enhancement, segmentation, skeletonization, subunit identification
- **Structure Modeling:** finite element meshing, spline representations (A-spline, RBF representations) for structural fitting & complementary docking
- **Visualization:** multi-dimensional transfer functions, surface and volume texture rendering, wavelet compression, hierarchical representations, cluster based parallelism



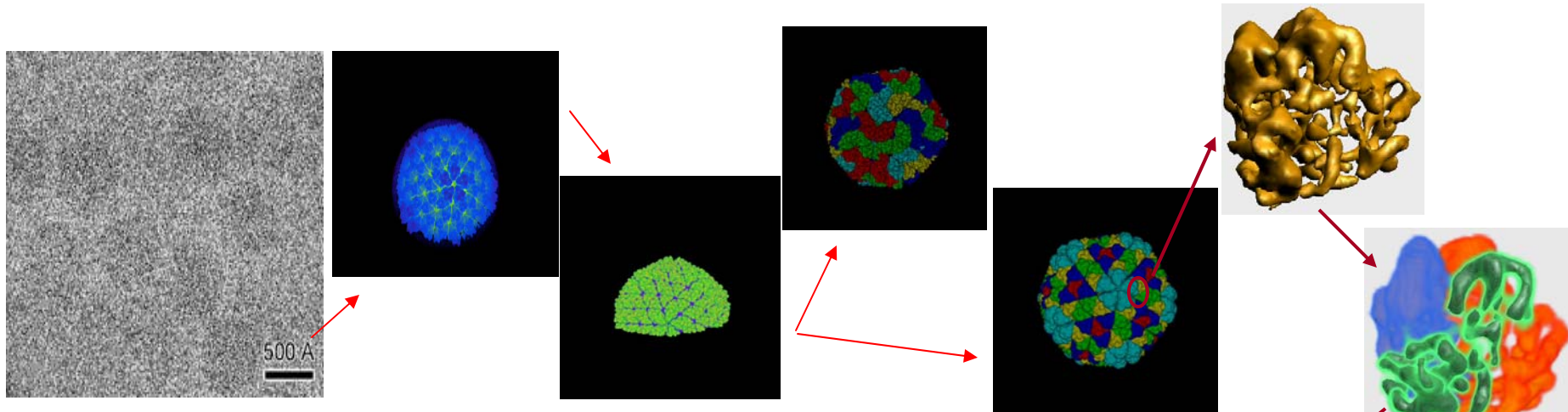
VolRover



TexMol

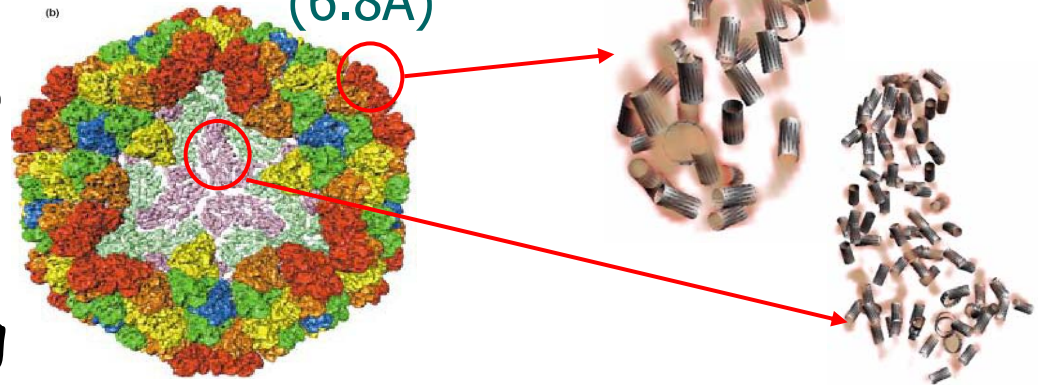


Sub-nanometer Structure Elucidation from 3D Cryo-EM



Cryo-EM → FFT based 3D
Reconstruction
→ Anisotropic and Vector
Diffusion Filtering →
Structure Segmentation
→ Quasi-Atomic Modeling
→ Visualization

Rice Dwarf Virus
(6.8Å)



**Sponsored by NSF-ITR, NIH



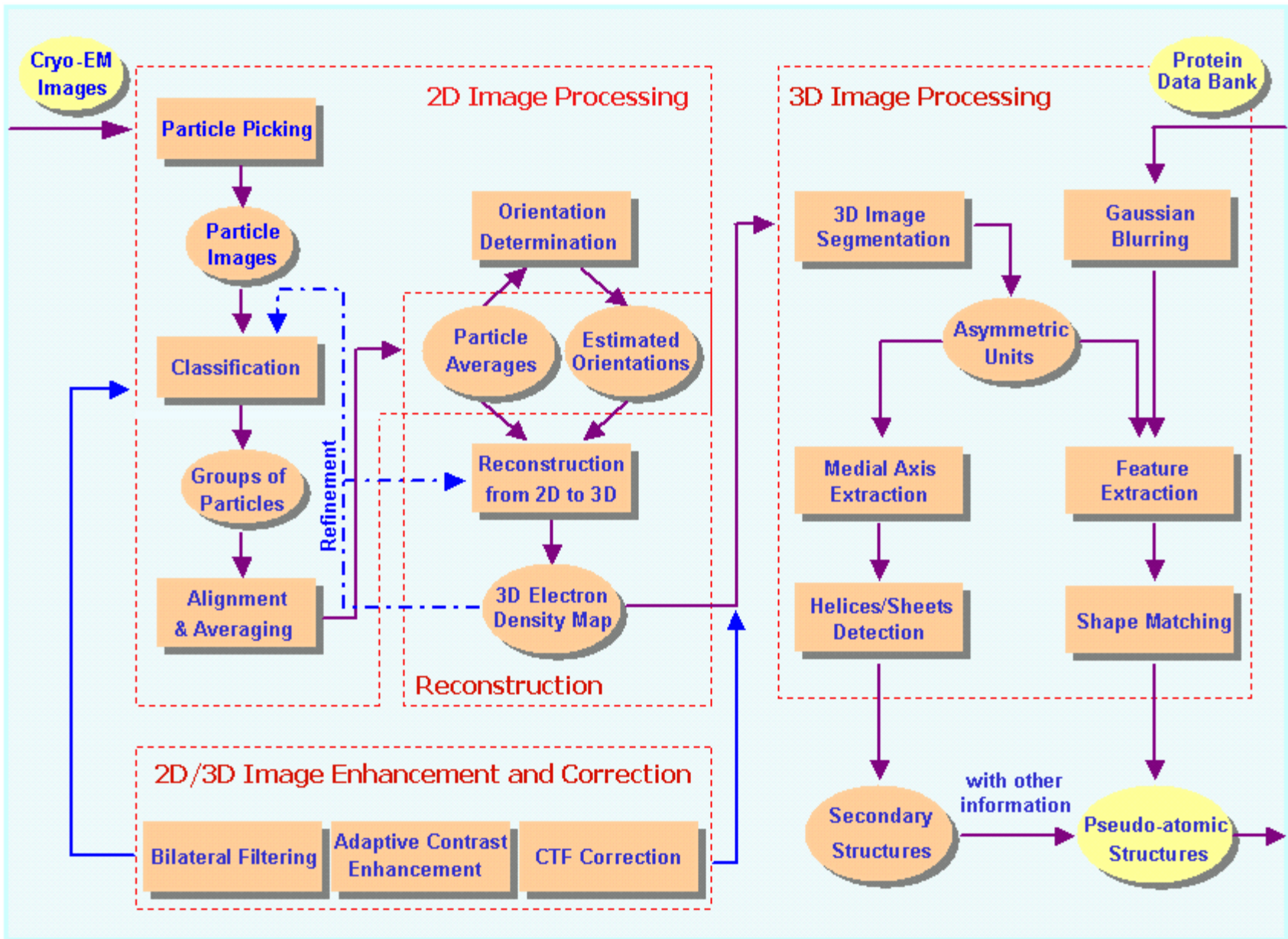
Center for Computational Visualization
Institute of Computational and Engineering Sciences
Department of Computer Sciences

(Collaborators: Wah Chiu, NCMi, Baylor
College of Medicine, Andrej Sali, UCSF)

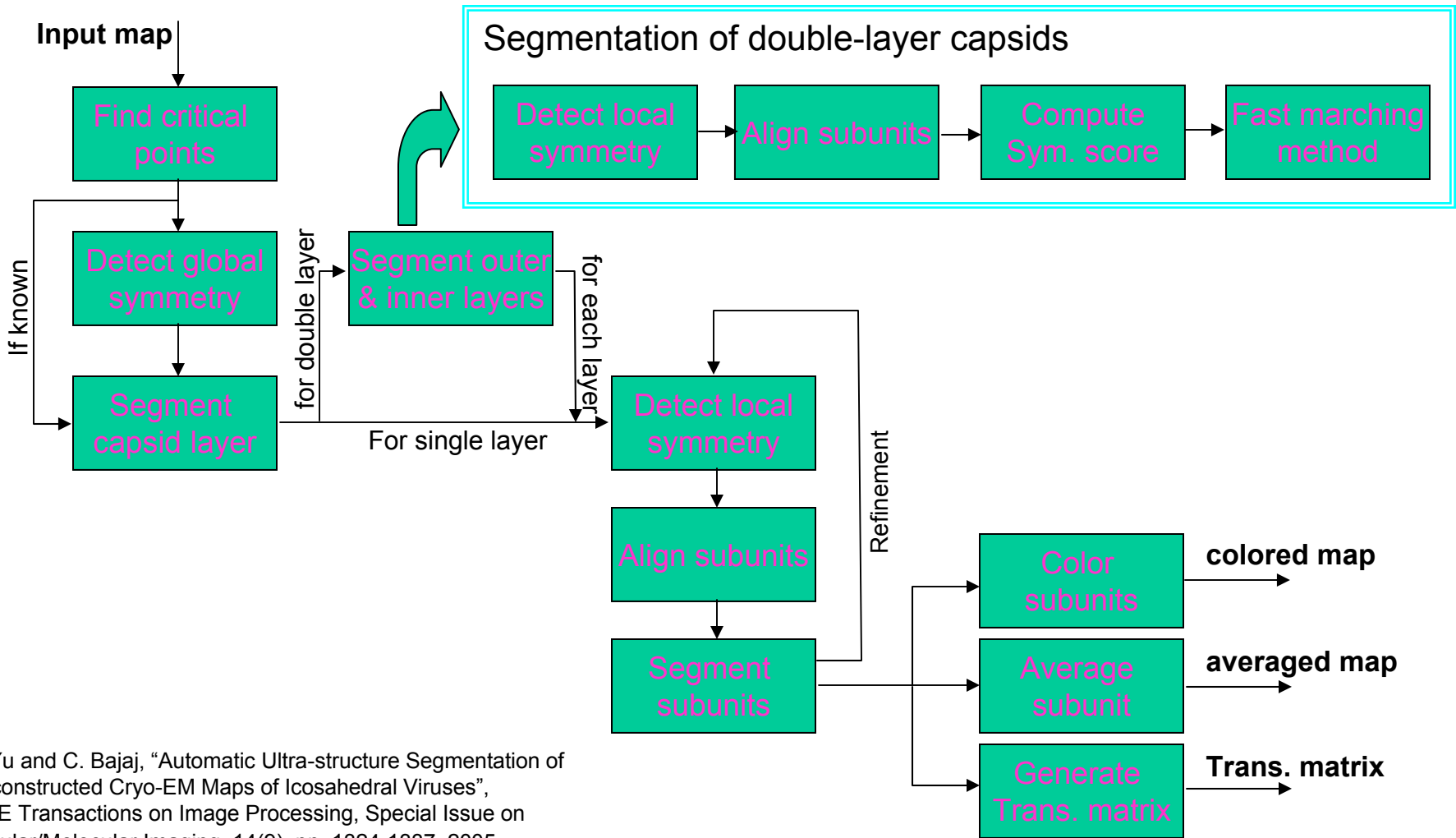
University of Texas at Austin

September 2007

A Structure Determination Pipeline for single particle cryo-EM



Structure Elucidation for Icosahedral Viruses



Z. Yu and C. Bajaj, "Automatic Ultra-structure Segmentation of Reconstructed Cryo-EM Maps of Icosahedral Viruses", IEEE Transactions on Image Processing, Special Issue on Cellular/Molecular Imaging, 14(9), pp. 1324-1337, 2005.



Structure Elucidation 1(A)

- Adaptive contrast enhancement
- Bilateral filtering

$$h(x, \xi) = e^{-\frac{(x-\xi)^2}{2\sigma_d^2}} \cdot e^{-\frac{(f(x)-f(\xi))^2}{2\sigma_r^2}}$$

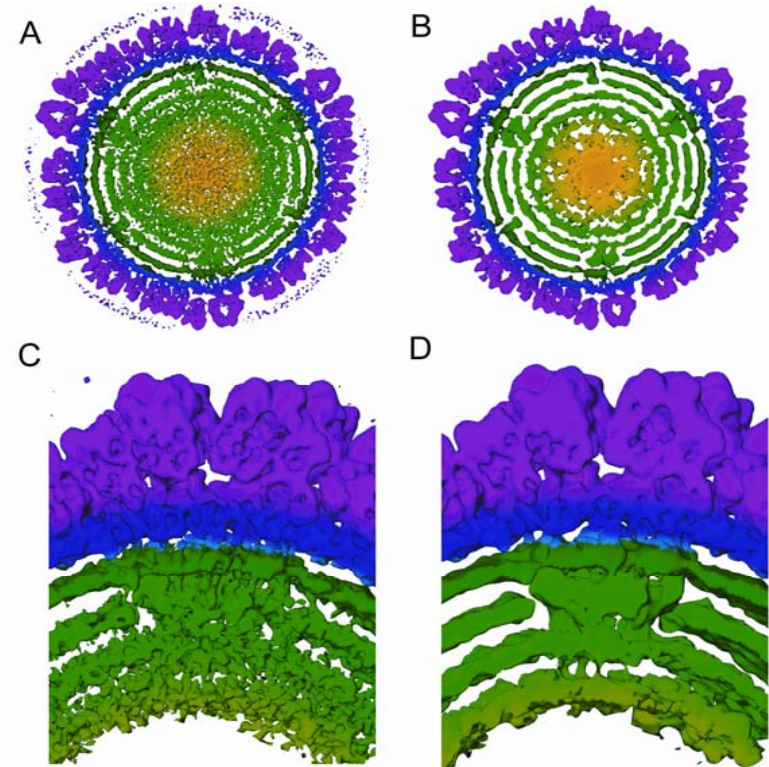
where σ_d and σ_r are parameters and $f(\cdot)$ is the image intensity value.

- Anisotropic diffusion filtering

$$\partial_t \phi - \text{div}(a(|\nabla \phi|) \nabla \phi) = 0$$

where \mathbf{a} stands for the diffusion tensor determined by local curvature estimation.

- Anisotropic gradient vector diffusion



W. Jiang, M. Baker, Q. Wu, C. Bajaj, W. Chiu, Journal of Structural Biology, 144, 5,(2003),114-122

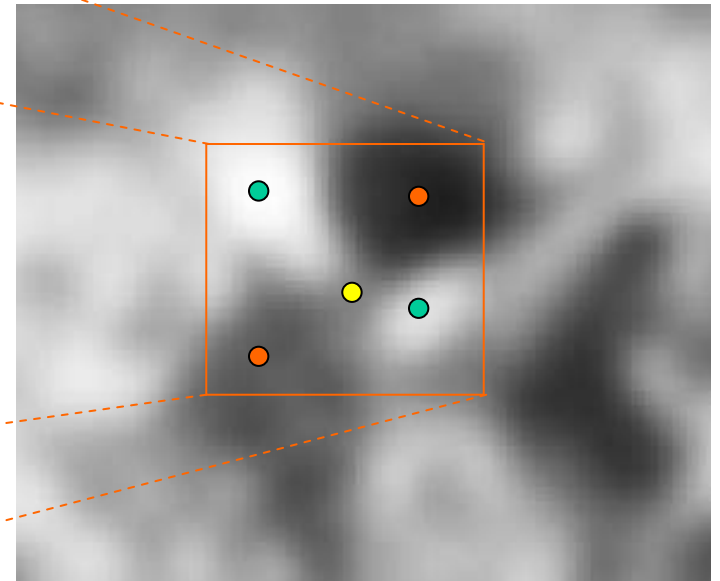
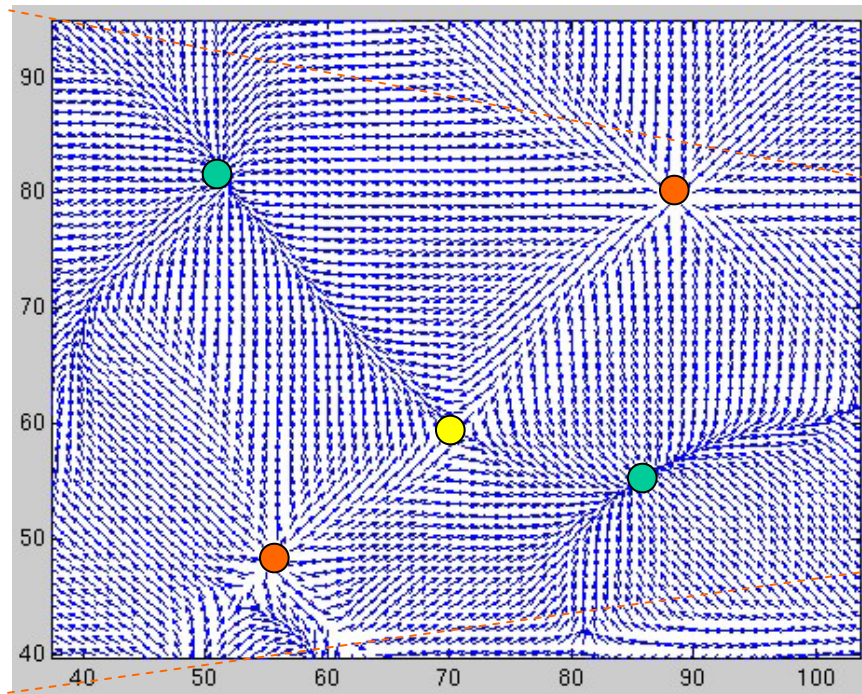
C. Bajaj, G. Xu, ACM Transactions on Graphics, (2003),22(1), 4 - 32.

Z. Yu & C. Bajaj, Proc. Int'l Conf. Image Processing, 2002. pp. 1001-1004.

Z. Yu & C. Bajaj, Proc. Int'l Conf. Computer Vision and Pattern Recognition, 2004. 415-420.



Compute Critical Points Using AGVD



● : minimum

● : maximum

● : saddle

(0)

(3)

(1, 2)



Anisotropic Gradient Vector Diffusion (AGVD)

Isotropic Diffusion (Xu *et al.*, 1998)

$$\begin{cases} \frac{\partial u}{\partial t} = \mu \nabla^2 u - (u - f_x)(f_x^2 + f_y^2) \\ \frac{\partial v}{\partial t} = \mu \nabla^2 v - (v - f_y)(f_x^2 + f_y^2) \end{cases}$$

Where:

$(u(t), v(t))$ stands for the evolving vector field;

μ is a constant;

f is the original image to be diffused;

$(f_x, f_y) = (u(0), v(0))$.

Anisotropic Diffusion (Yu & Bajaj ICPR'02)

$$\begin{cases} \frac{\partial u}{\partial t} = \mu \nabla (g(\alpha) \cdot \nabla u) - (u - f_x)(f_x^2 + f_y^2) \\ \frac{\partial v}{\partial t} = \mu \nabla (g(\alpha) \cdot \nabla v) - (v - f_y)(f_x^2 + f_y^2) \end{cases}$$

Where

$(u(t), v(t))$ stands for vector field;

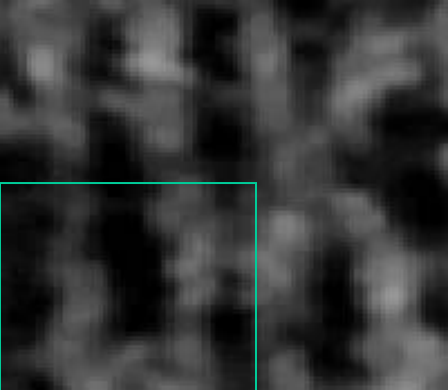
μ is a constant; $(f_x, f_y) = (u(0), v(0))$.

f is the original image to be diffused;

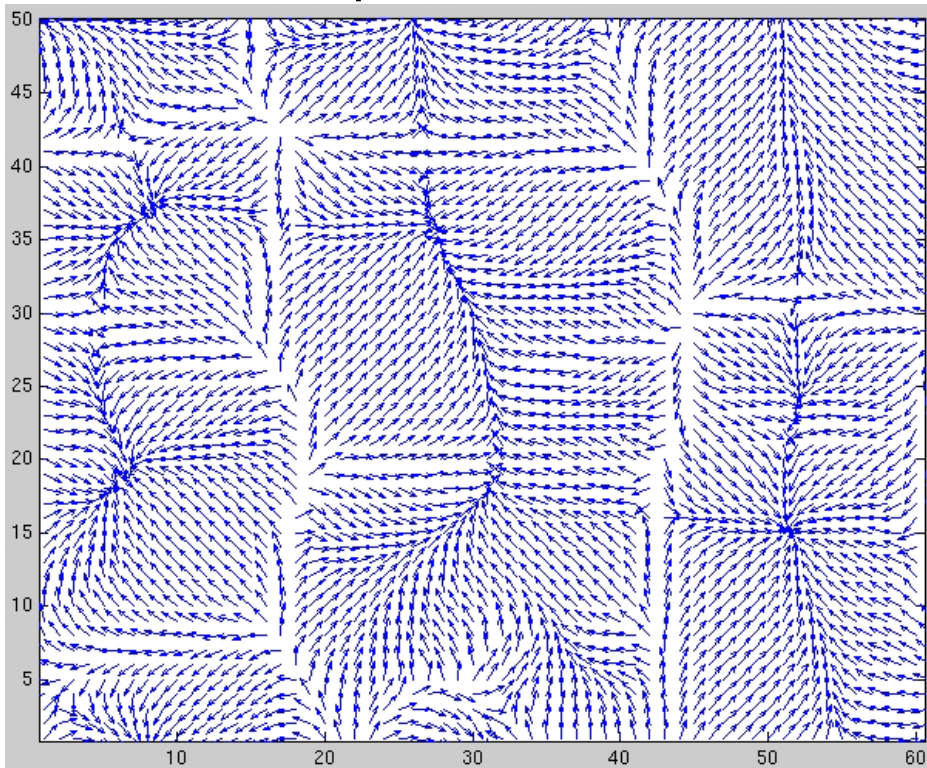
$g(\cdot)$ is the angle between two vectors



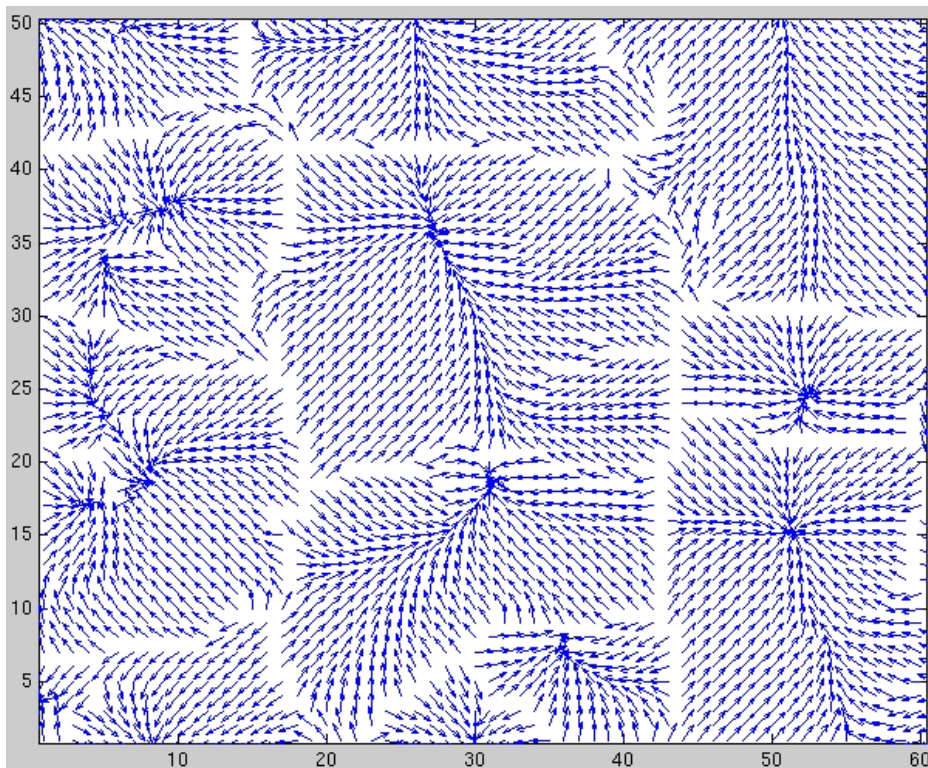
GVD v.s. AGVD



Isotropic diffusion

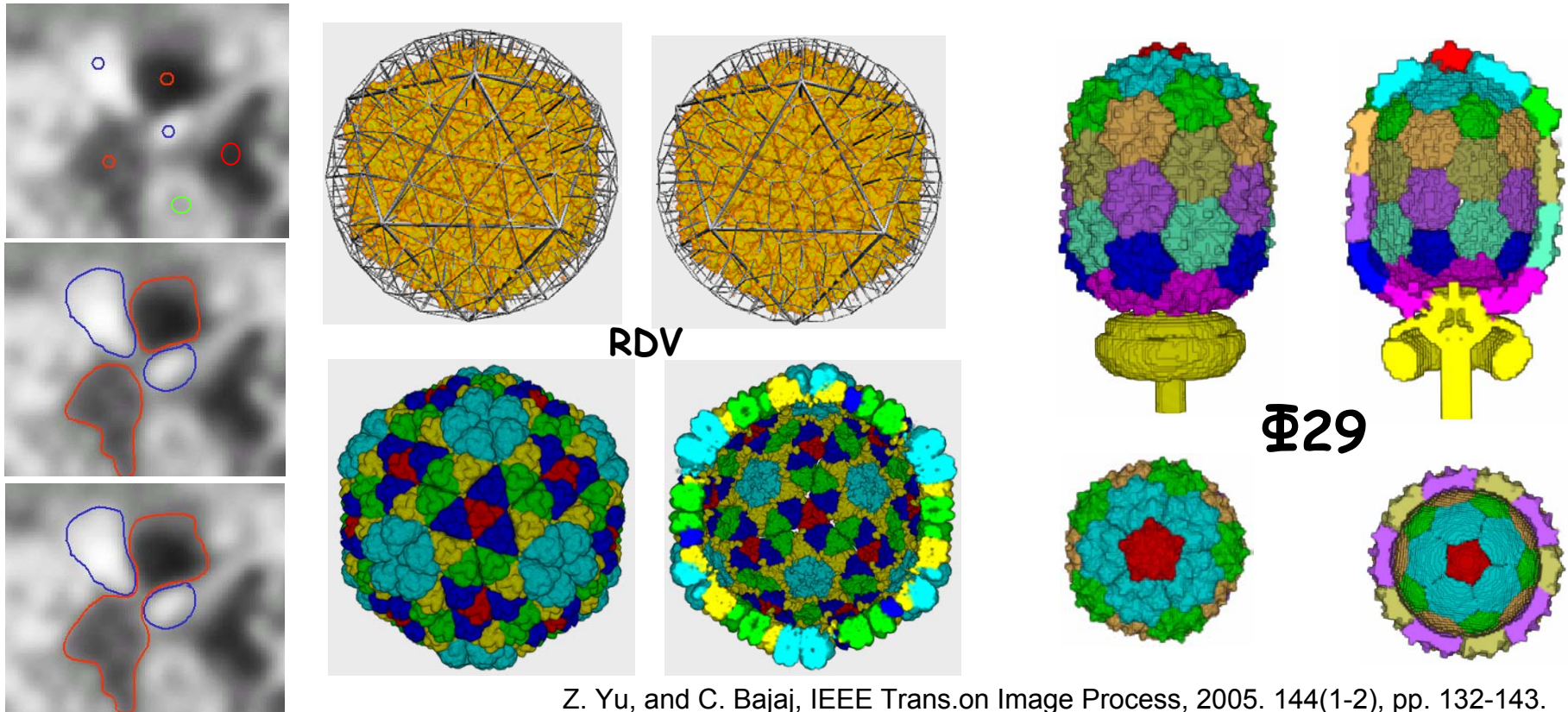


Anisotropic diffusion



Structure Elucidation 1(B)

- Multi-seed Fast Marching Method
 - Classify map **critical points** as seeds based on local symmetry.
 - Each seed initializes a contour, with its group's membership.
 - Contours march simultaneously. Contours with same membership are merged, while contours with different membership stop each other.

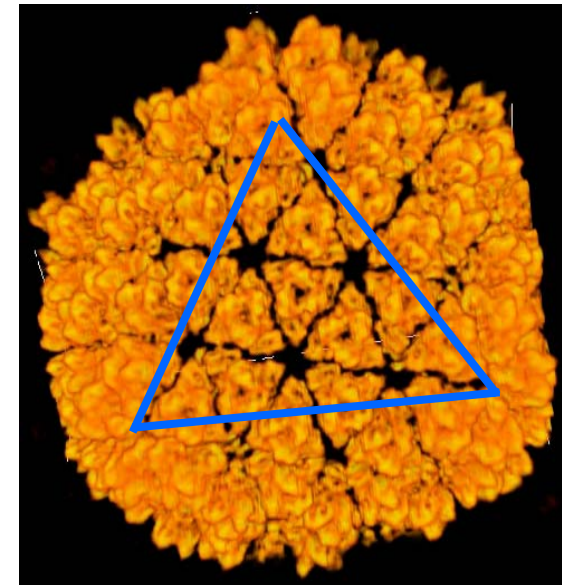
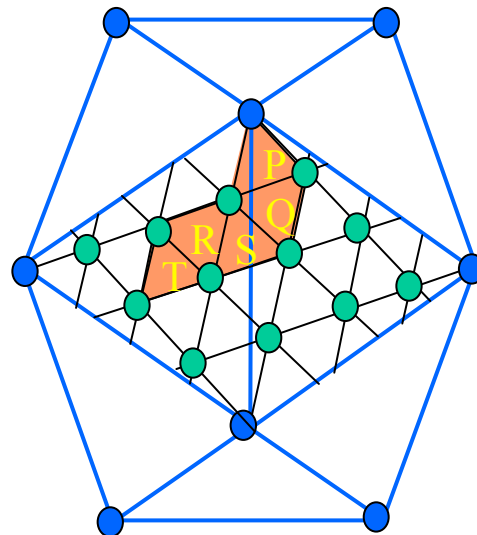
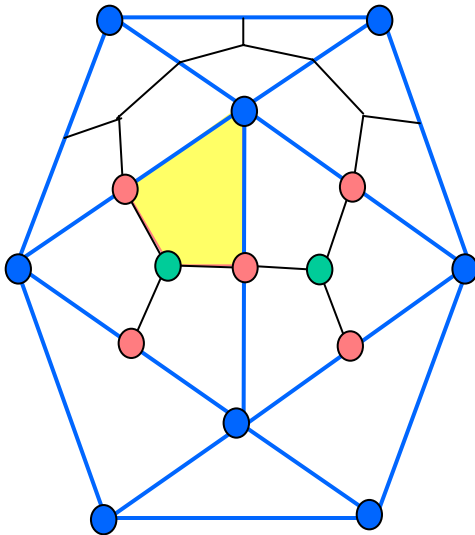


Z. Yu, and C. Bajaj, IEEE Trans.on Image Process, 2005. 144(1-2), pp. 132-143.



Global and Local Symmetry

- Automatic structure unit identification in a 3D Map



- Two-fold vertices
- Three-fold vertices
- Five-fold vertices

Example: RDV



Symmetry Detection: Correlation Search

$$C(\theta, \varphi) = \sum_{\vec{r} \in V} f(\vec{r}) f(R_{(\theta, \varphi, 2\pi/5)} \cdot \vec{r})$$

- **Algorithm:** *detect 5-fold rotation symmetry*

- Compute the scoring function

- For every angular bin B_j , compute θ_j, φ_j {

- For every critical point C_i {

$$\vec{r}_k(C_i, B_j) = R_{(\theta_j, \varphi_j, 2k\pi/5)} \cdot C_i, \quad k = 0, 1, 2, 3, 4$$

$$Dev(C_i, B_j) = \frac{1}{5} \sum_{k=0}^4 (f(\vec{r}_k) - \bar{f}) \}$$

$$SF(B_j) = \frac{1}{p} \sum_{i=0}^p Dev(C_i, B_j) \}$$

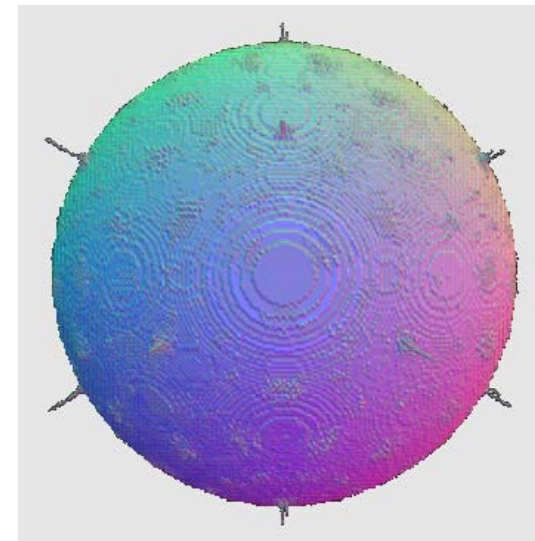
- Locate the symmetry axes

- The 12 peaks

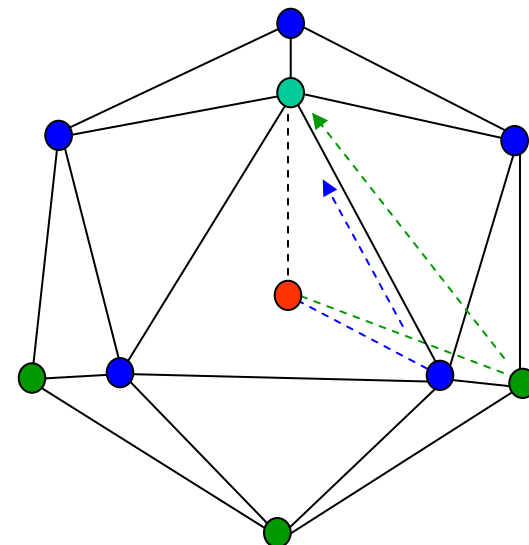
- Refine the symmetry axes

- In order to locate a perfect icosahedron

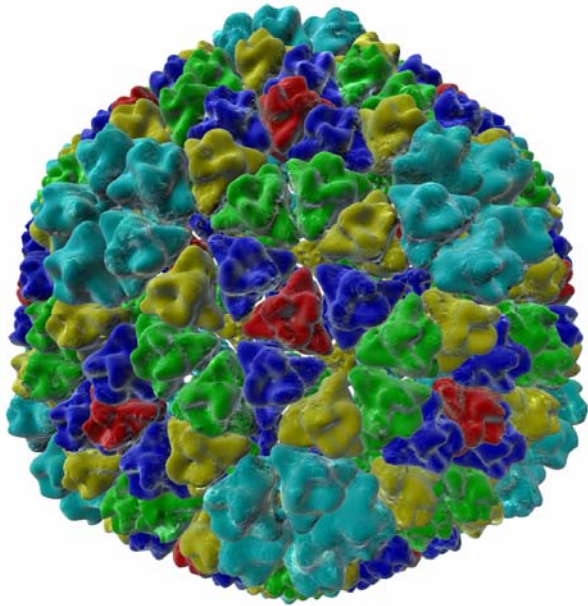
(rotate the axes by $0^0, 63.43^0, 116.57^0, 180^0$)



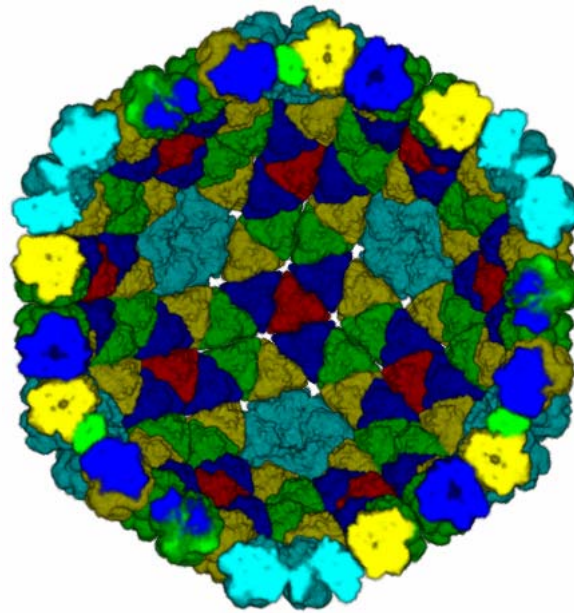
Inverted and normalized SF(B_j)



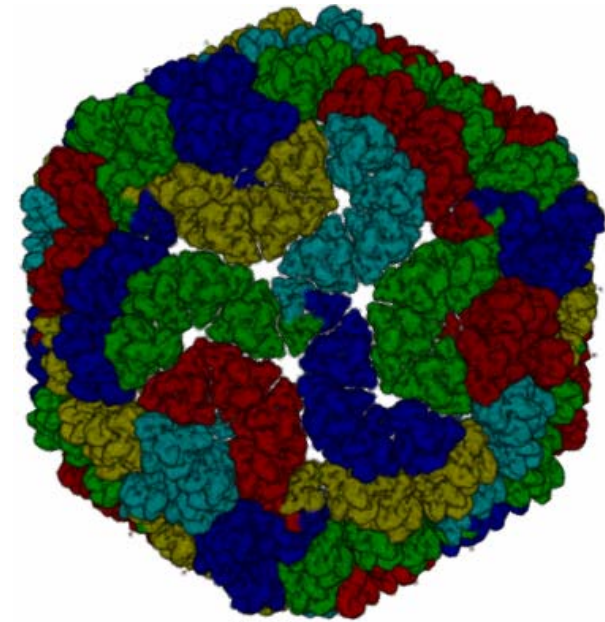
Structure Elucidation Results: RDV (Bakeoff)



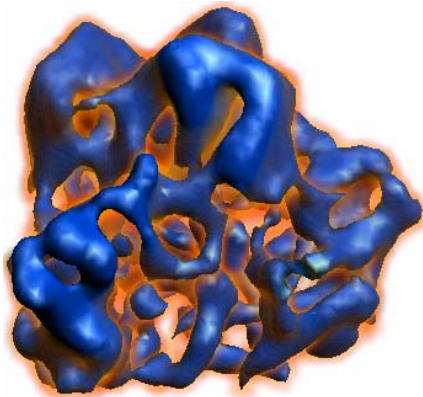
surface rendering (outside)



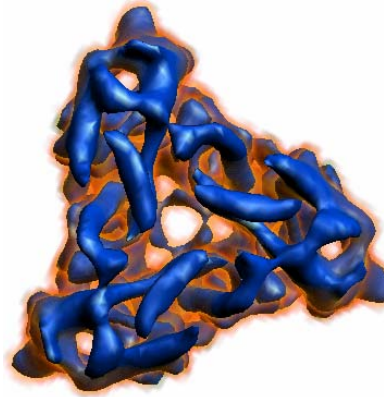
volume rendering (inside)



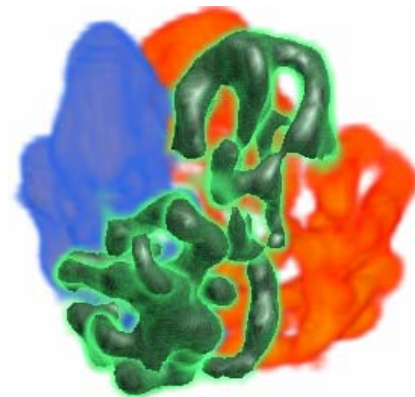
volume rendering (asymmetric unit)



averaged trimer (side)



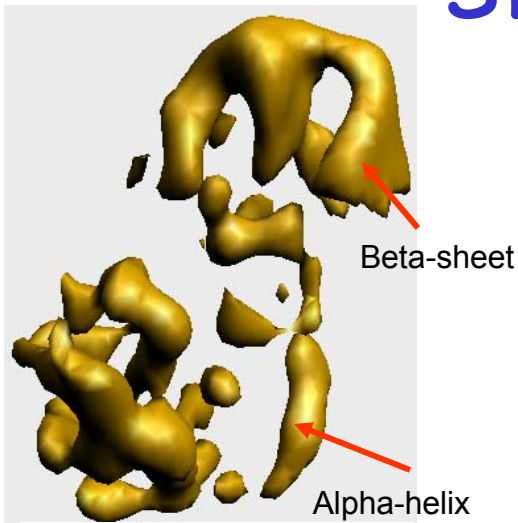
averaged trimer (bottom)



segmented monomers

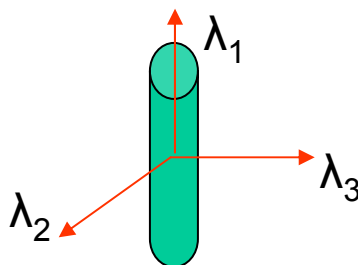
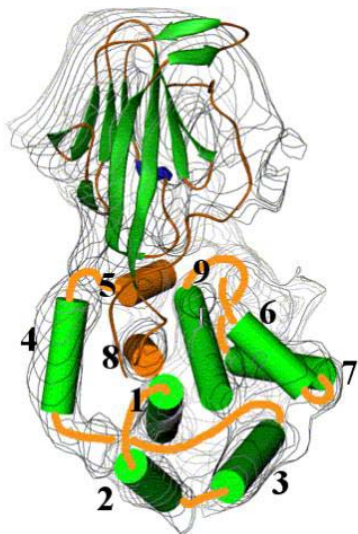


Structure Elucidation 1(C): Secondary Structure Identification



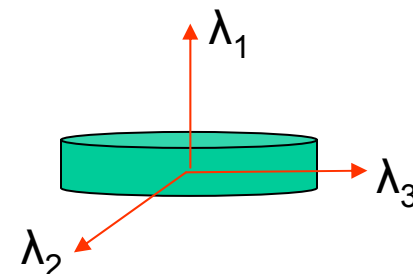
$$G_{\sigma} * \begin{pmatrix} I_x^2 & I_x I_y & I_x I_z \\ I_x I_y & I_y^2 & I_y I_z \\ I_x I_z & I_y I_z & I_z^2 \end{pmatrix}$$

The eigenvectors of the local structure tensor give the principal directions of the local features:



Line structure (alpha-helix)

$$\lambda_2 \approx \lambda_3 \gg \lambda_1 \approx 0$$

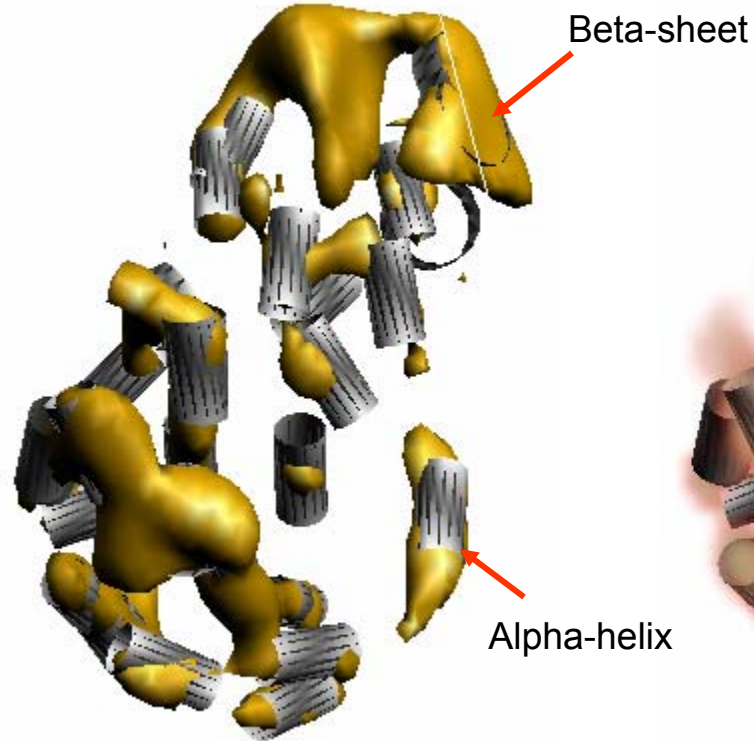
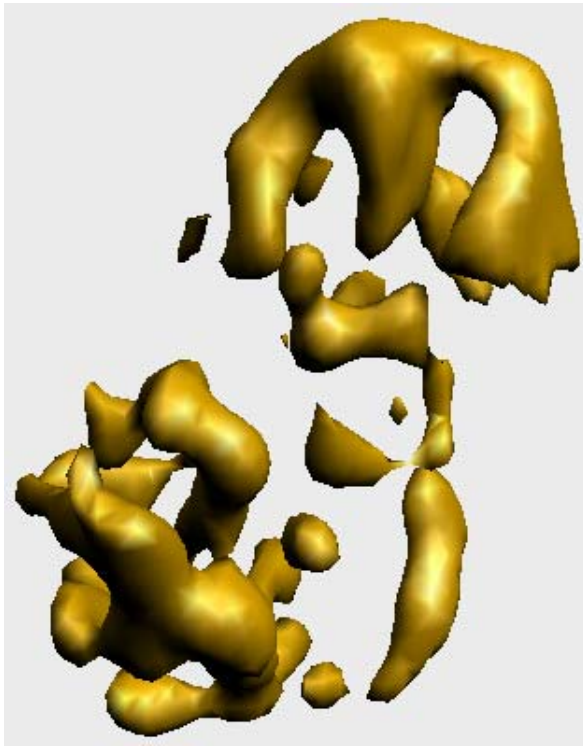


plane structure (beta-sheet)

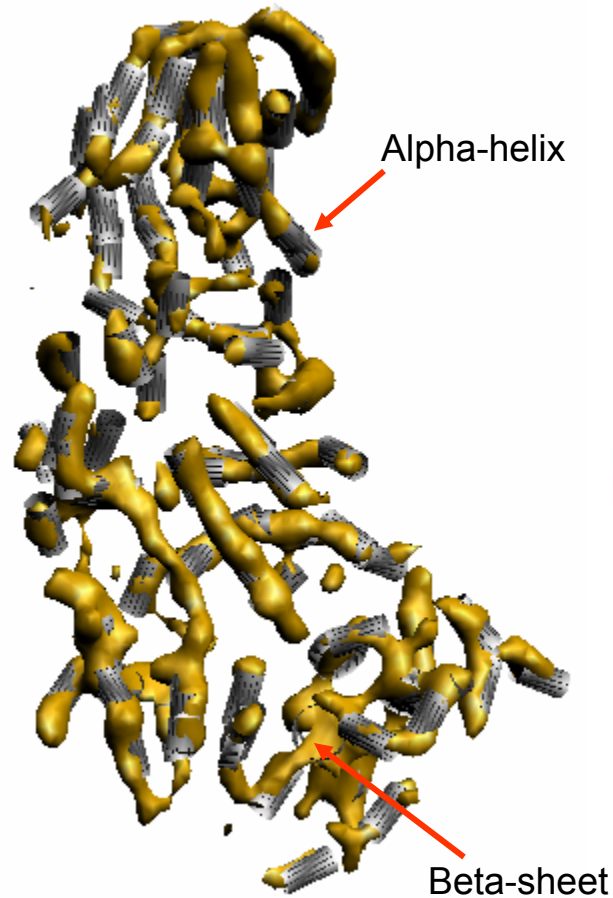
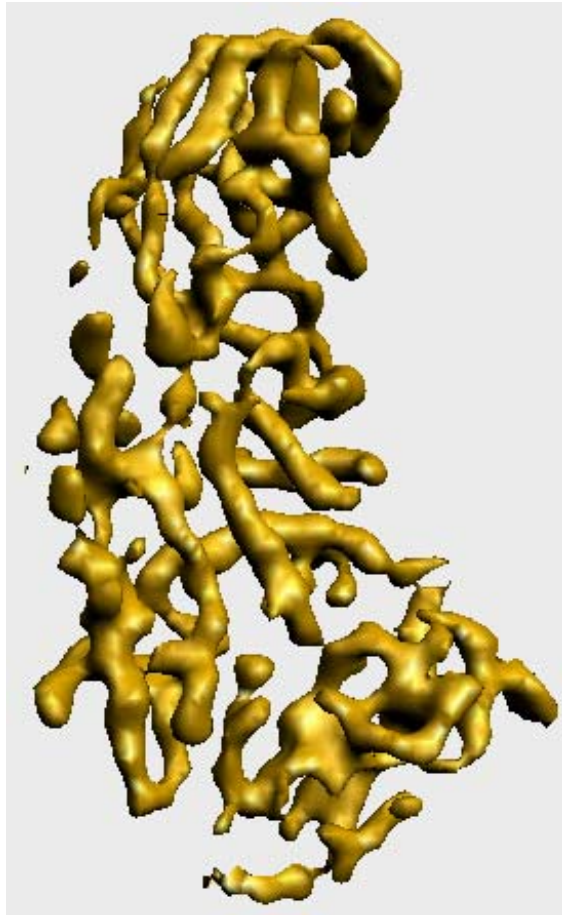
$$\lambda_1 \gg \lambda_2 \approx \lambda_3 \approx 0$$



Monomeric Unit of Outer Capsid of RDV

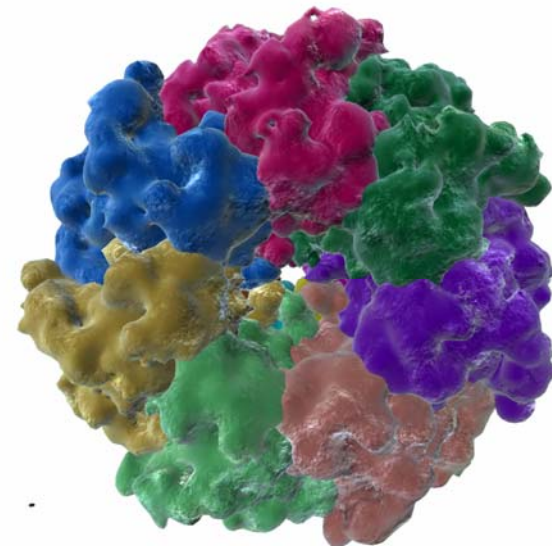
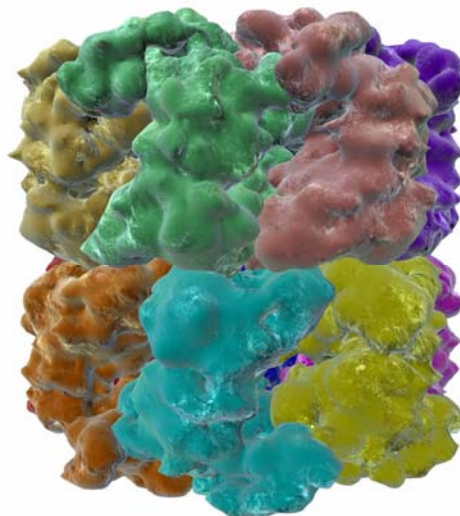
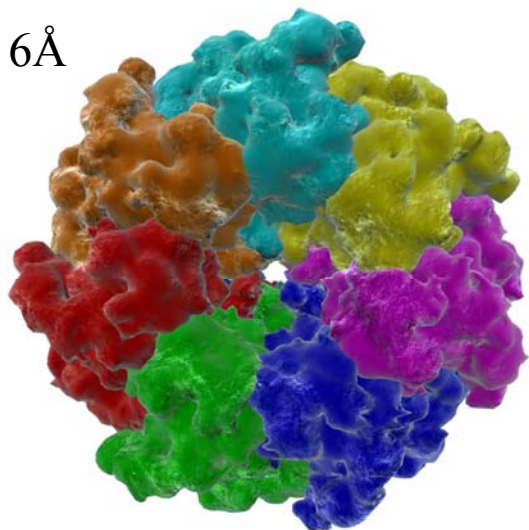


Monomeric Unit of Inner Capsid of RDV

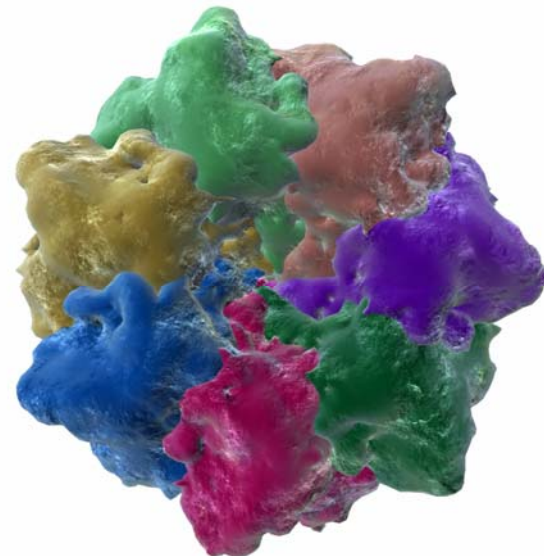
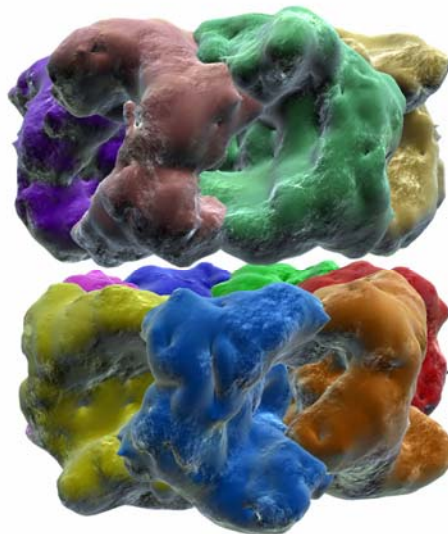
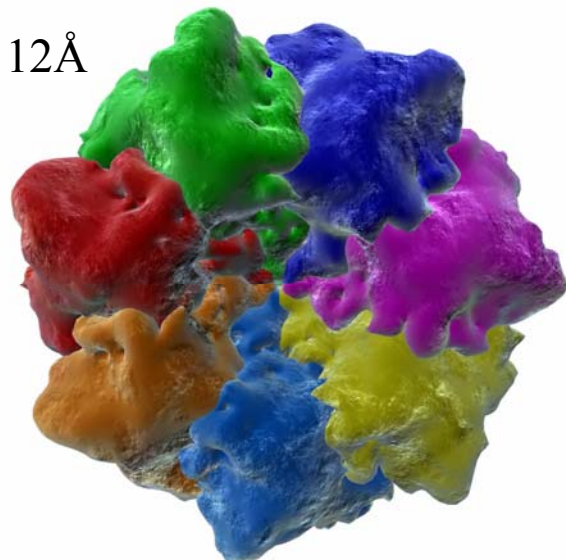


Structure Elucidation Results: GroEL

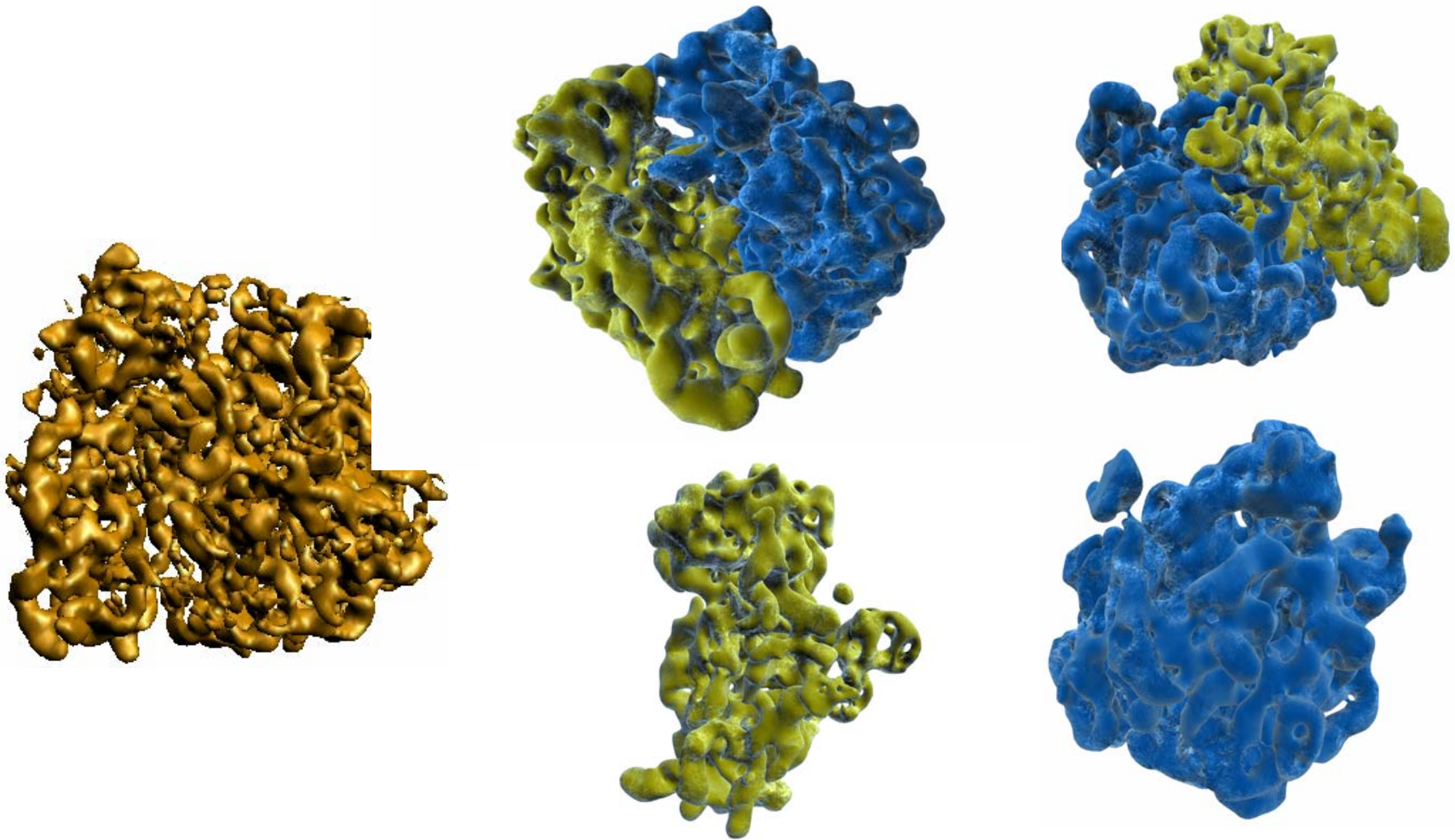
6Å



12Å



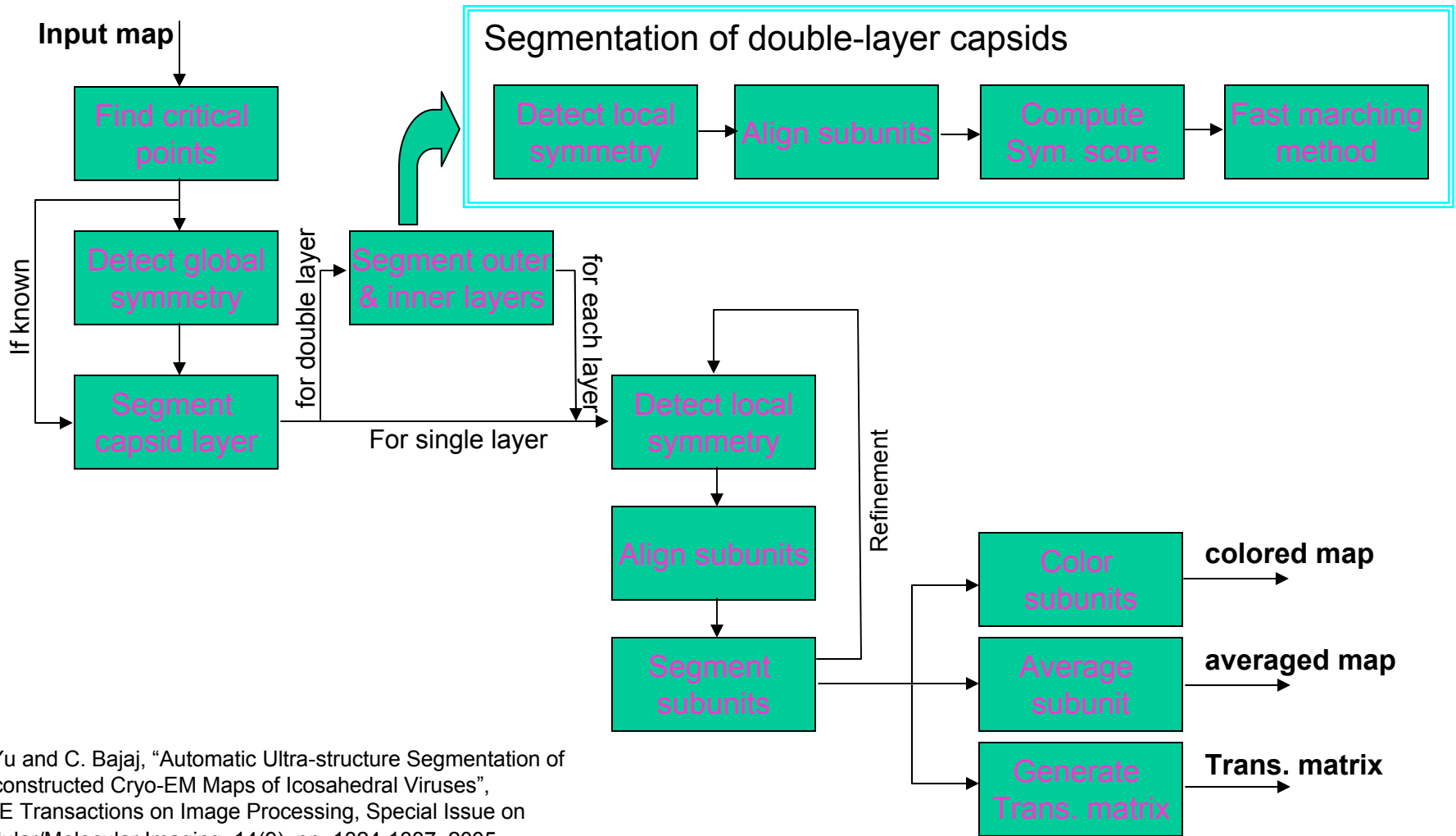
Segmentation Results: Ribosome (Bakeoff)



70S ribosome from *E. coli* complex. 70S-tRNA^{fMet}-MF-tRNA^{Phe}. Data courtesy: EBI & J.Frank



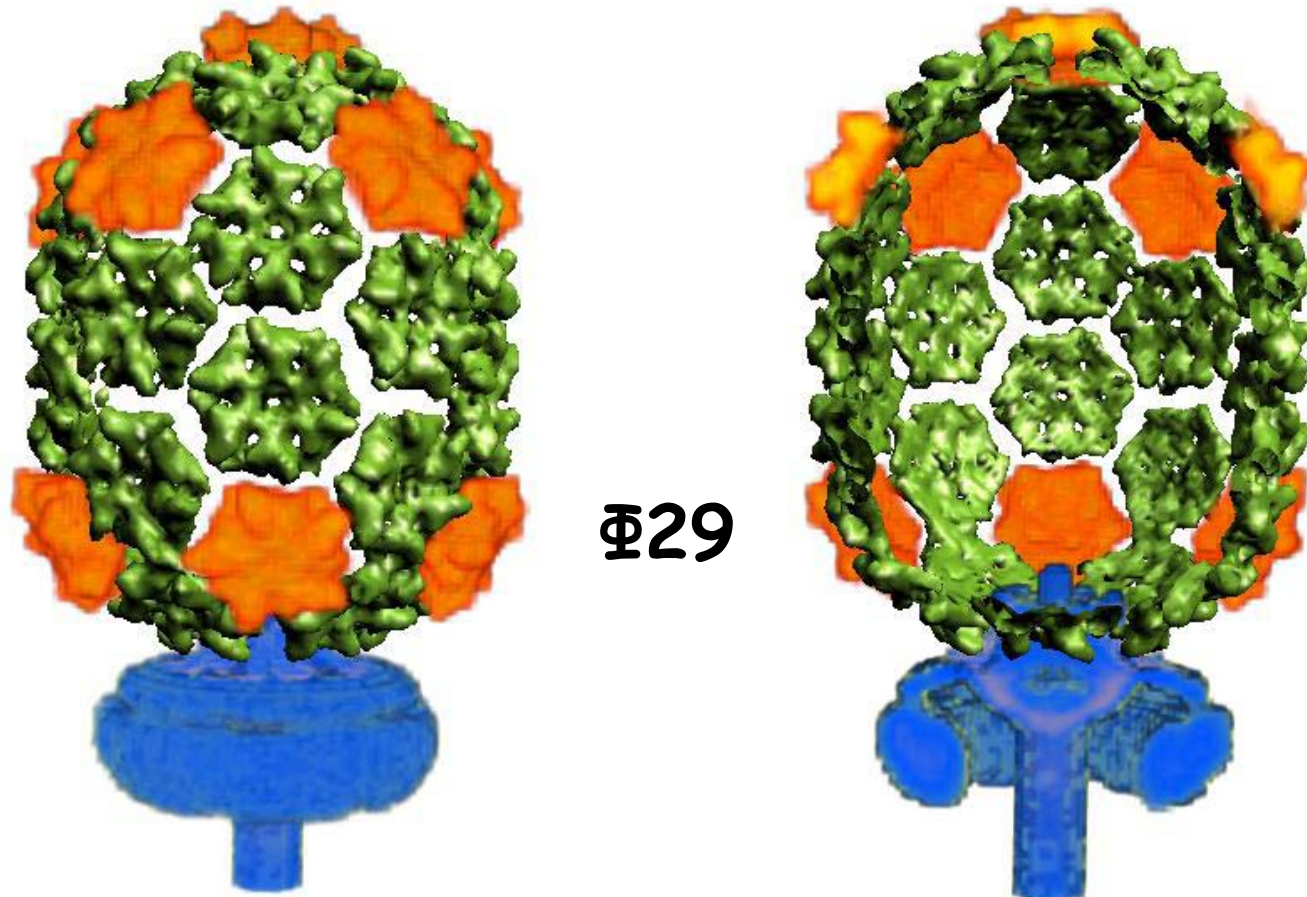
Structure Elucidation for Symmetric Capsid Viruses



Z. Yu and C. Bajaj, "Automatic Ultra-structure Segmentation of Reconstructed Cryo-EM Maps of Icosahedral Viruses", IEEE Transactions on Image Processing, Special Issue on Cellular/Molecular Imaging, 14(9), pp. 1324-1337, 2005.



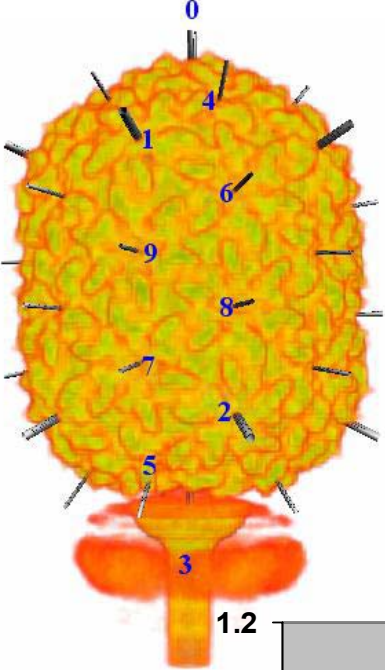
Subunit alignment (1): averaging



The above two pictures (left: outer; right: inner) show the averaged capsid layer, calculated from one 5-fold subunit (orange) and one 6-fold subunit (green). The tail structure (blue) is augmented after the averaging.



Structure Elucidation 1(C): Subunit Alignment

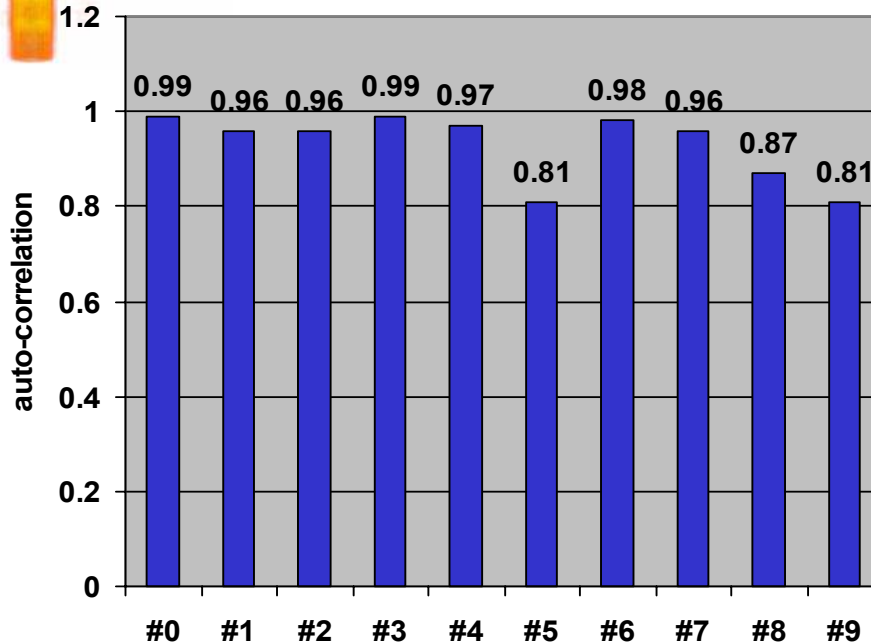


- Symmetry score

- Cross-correlation

	#0	#1	#2	#3
#0	1	0.95	0.95	0.34
#1	0.95	1	0.96	0.31
#2	0.95	0.96	1	0.31
#3	0.35	0.31	0.32	1

$\Phi 29$



	#4	#5	#6	#7	#8	#9
#4	1	0.79	0.95	0.94	0.87	0.88
#5	0.79	1	0.79	0.78	0.77	0.79
#6	0.95	0.79	1	0.96	0.88	0.88
#7	0.94	0.78	0.96	1	0.89	0.88
#8	0.87	0.77	0.88	0.89	1	0.94
#9	0.88	0.79	0.88	0.88	0.94	1

segmented subunit

Center for Computational Visualization

Institute of Computational and Engineering Sciences

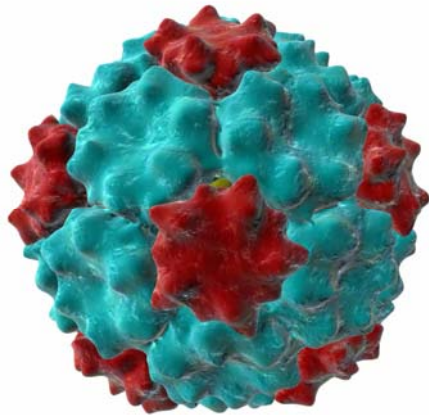
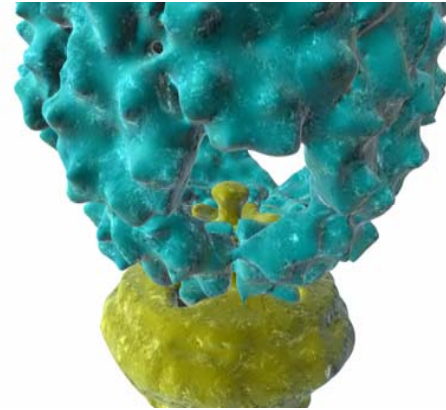
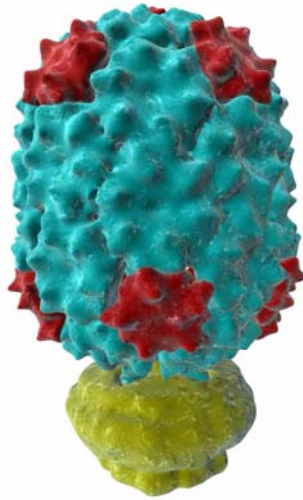
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University of Texas at Austin

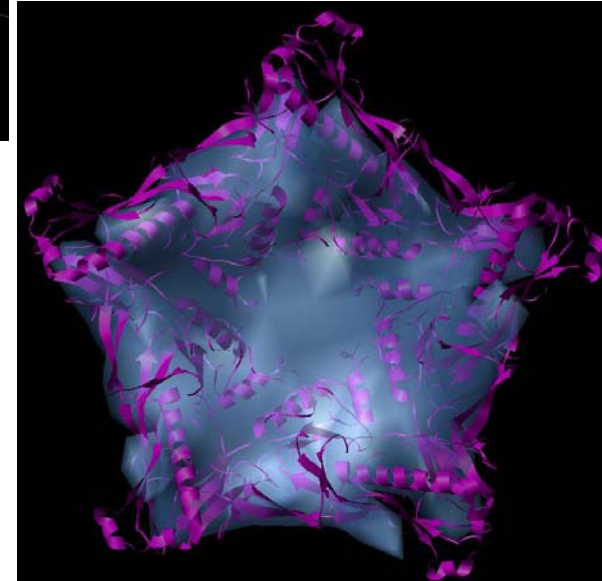
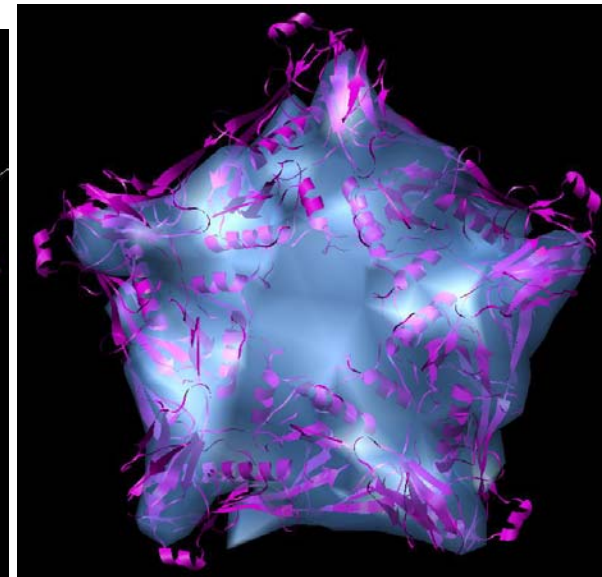
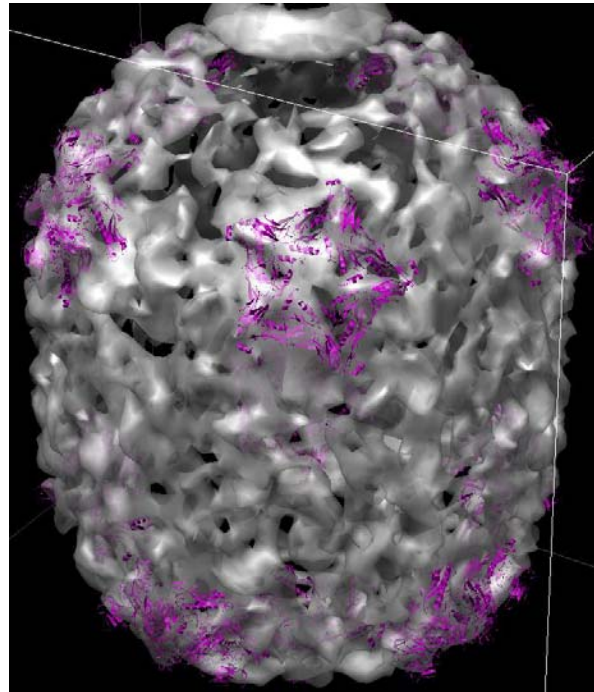
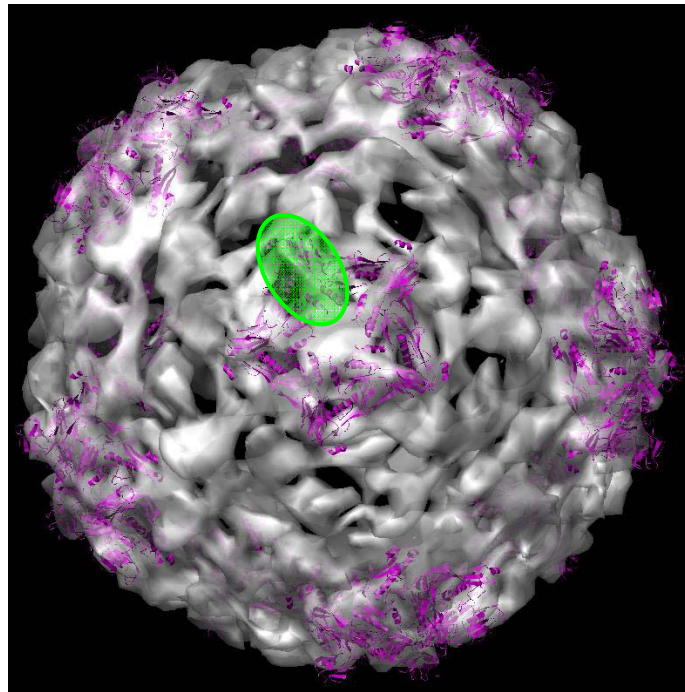
September 2007



Structure Elucidation Results: $\Phi 29$



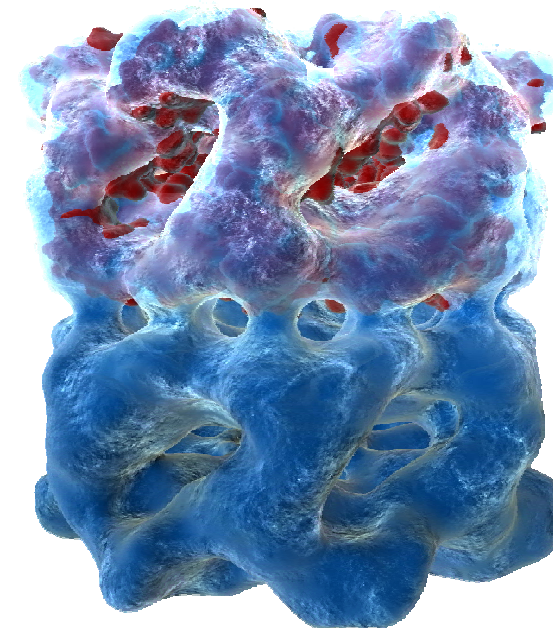
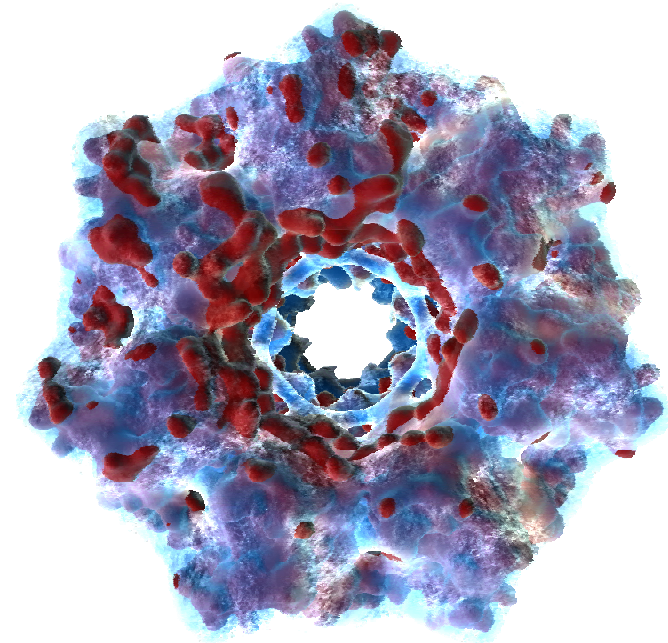
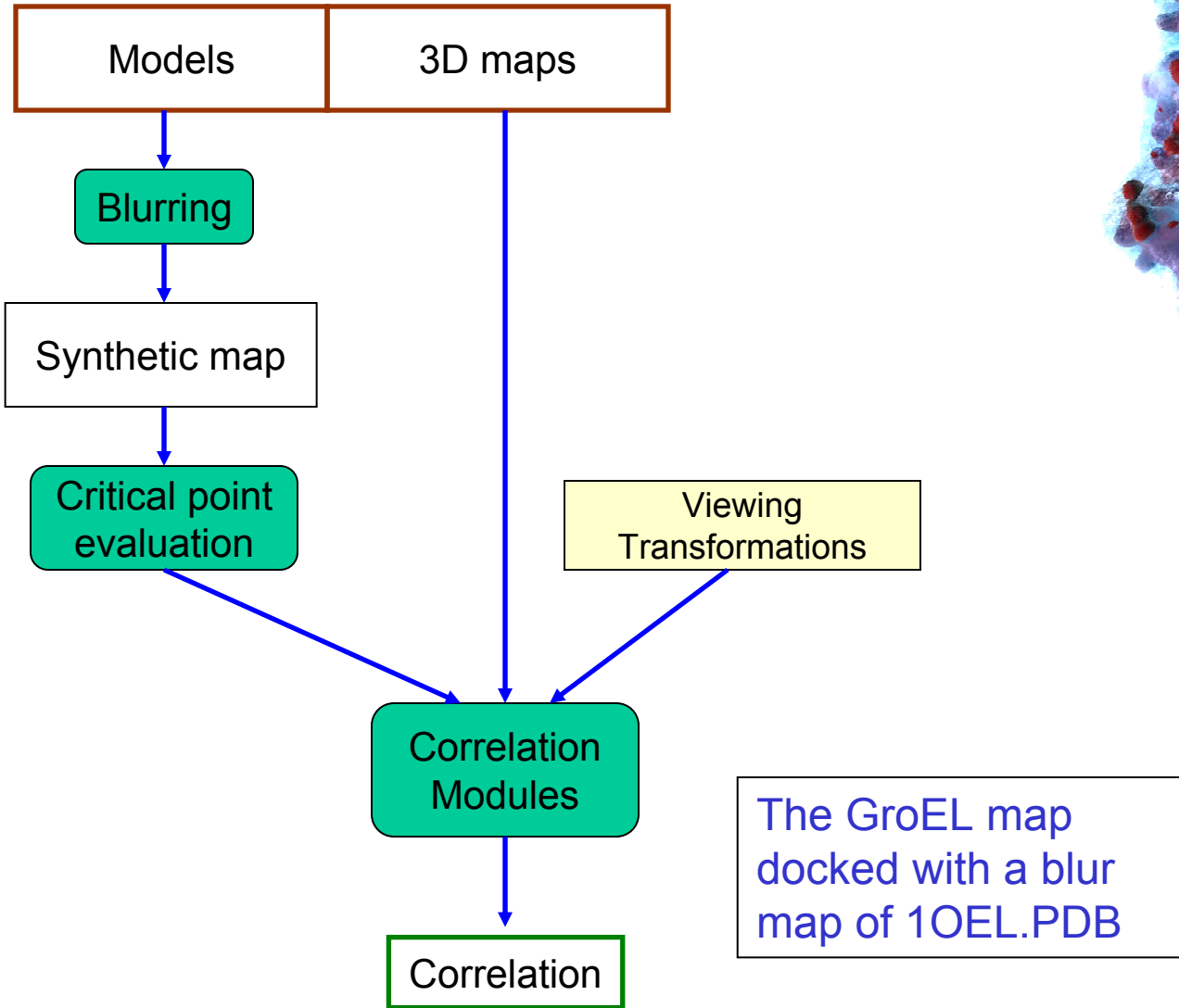
Subunit alignment (2): Fitting



The PDB structure of one monomer is matched & fit into the cryo-EM map (as shaded in green in the left figure). Then all the quasi-symmetric 5-fold subunits are computationally fit with the PDB structure using the transform matrices obtained in subunit alignment. Similar procedure can be applied to all 6-fold subunits.



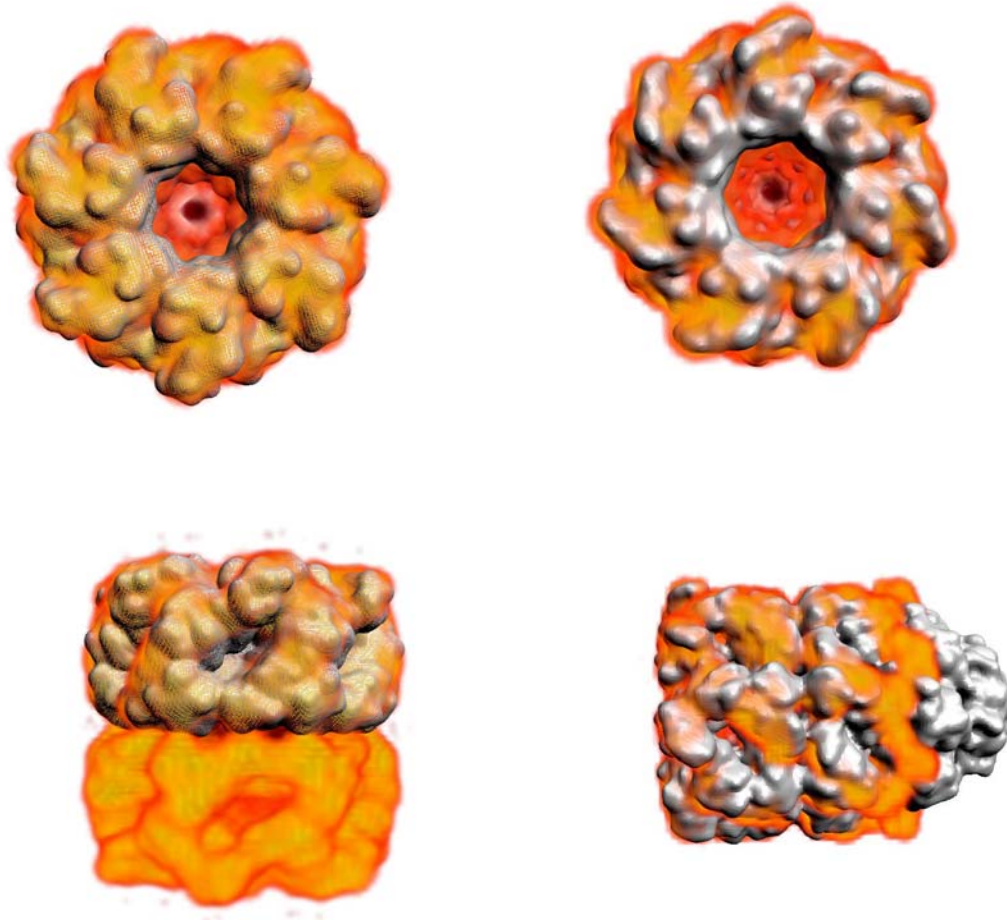
“Interactive” Fitting



The GroEL map docked with a blur map of 1OEL.PDB



Gro-EL: X-ray structures docked in Cryo-EM

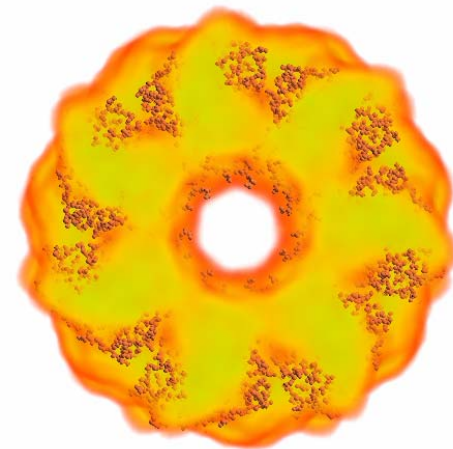
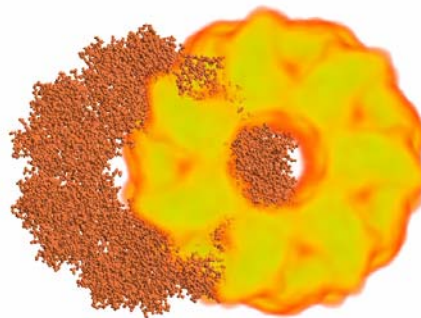
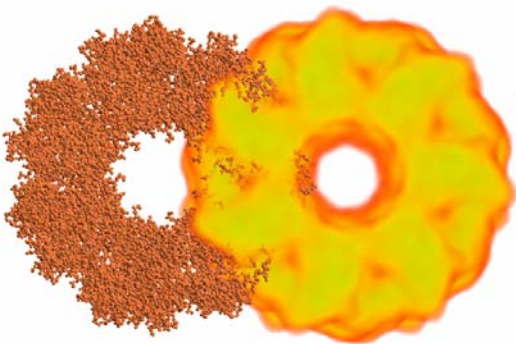


Interactive Correlation Analysis

C = 0.2235

C = 0.269

C = 0.593

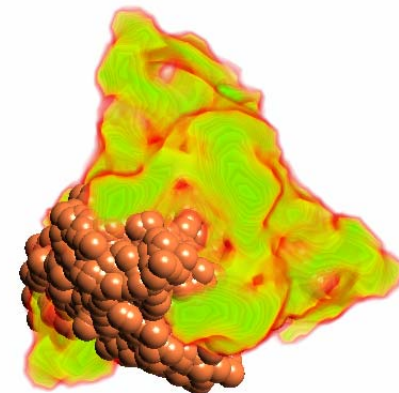
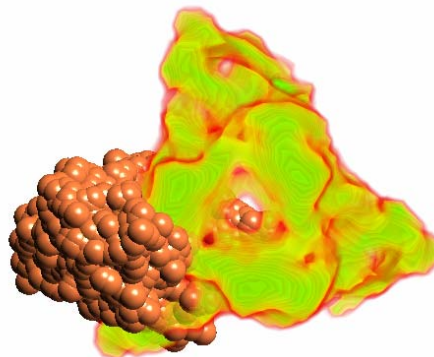
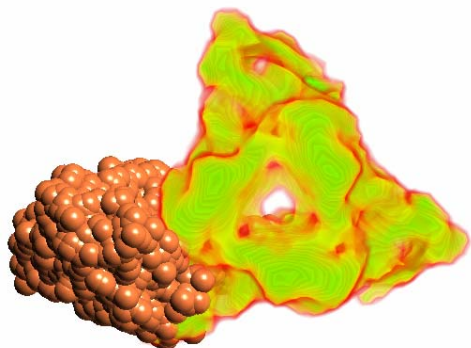


12A GroEL map and 1OEL.pdb

C = 0.208

C = 0.387

C = 0.542



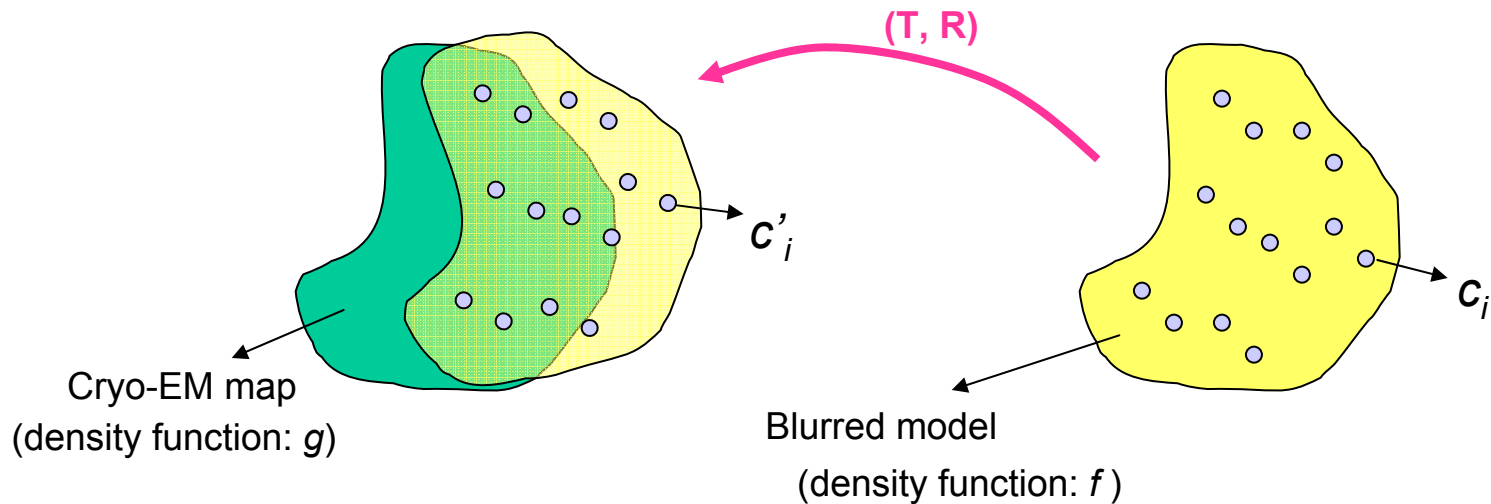
6.8A RDV map and 1UF2.pdb



Approximate Correlation Analysis

$$\text{score} = 1 - \frac{\sum_{i=1}^N |f(c_i) - g(c'_i)|}{\sum_{i=1}^N \max(f(c_i), g(c'_i))}$$

Where f is the normalized density function of the blurred crystal structure;
 g is the normalized density function of the cryo-EM map;
 c_i , $i=1,2,\dots,N$, are the critical points of the blurred crystal structure;
 c'_i , $i=1,2,\dots,N$, are the transformations of the critical points.



Blurring I

- For a molecule with M atoms, we can define a 3D electron density map as

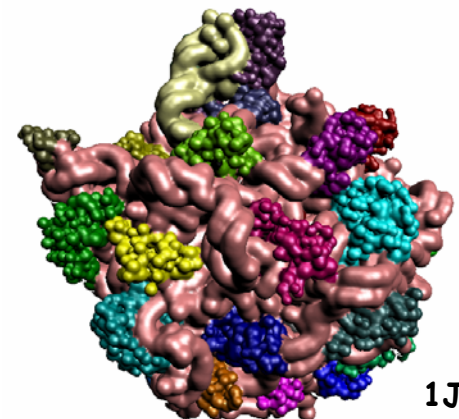
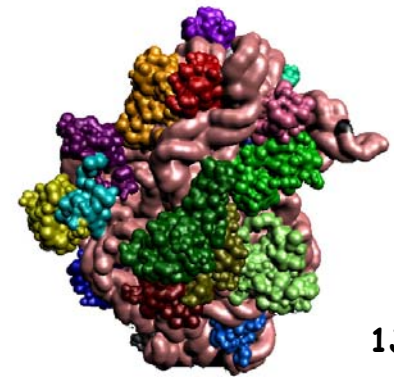
$$f_{elec_dens}(\vec{\mathbf{x}}) = \sum_{i=1}^M G_i(\vec{\mathbf{x}}) \quad \mathbf{x} \in \mathbf{R}^3$$

- For quadratic decay kernels, $A_i = e^{-d}$:

$$f_{elec_dens}(\mathbf{x}) = \sum_{i=1}^M A_i e^{-\frac{d}{r^2} \mathbf{x}^2} \delta(c_i)$$

- For linear decay kernels, $A_i = e^{-d r_i}$:

$$f_{elec_dens}(\mathbf{x}) = \sum_{i=1}^M A_i e^{-d |\mathbf{x}|} \delta(c_i)$$



Atomic Shape Parameters

$$G_i(\mathbf{x}) = e^{-\frac{d}{r_i^2}((\mathbf{x}-\mathbf{x}_{ci})^2 - r_i^2)}$$

$$G_i(\mathbf{x}) = e^{-d(|\mathbf{x}-\mathbf{x}_{ci}|-r_i)}$$

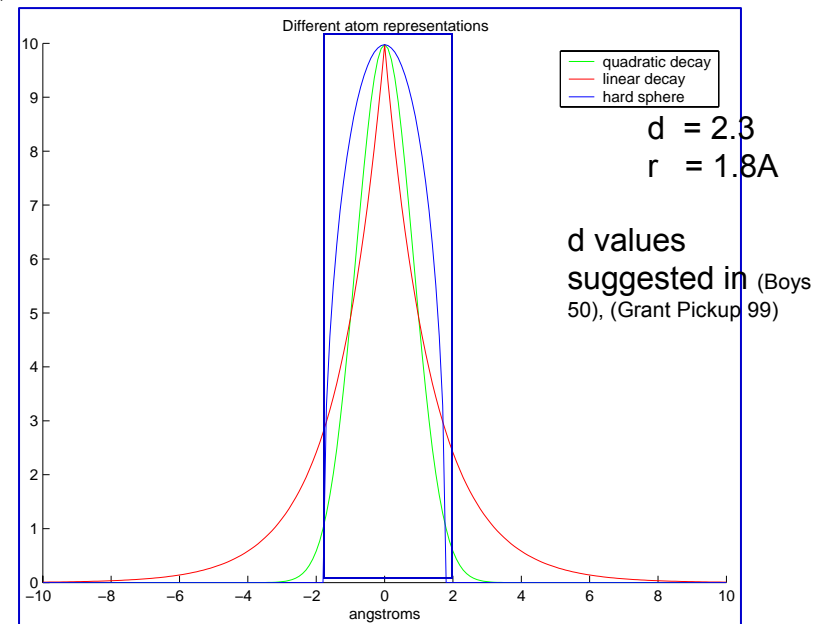
- Isotropic Quadratic Kernel

- Isotropic Linear Kernel

➤ where

- The decay d controls the shape of the Gaussian function.
- The van der Waals radius is r_i
- The center of the atom is \mathbf{x}_c .

- Anisotropic Kernels



Blurring II

- For quadratic decay kernels, $A_i = e^d$:

$$f_{elec_dens}(\mathbf{x}) = \sum_{i=1}^M A_i e^{-\frac{d}{r^2} \mathbf{x}^2} \delta(c_i)$$

- For linear decay kernels, $A_i = e^{d r_i}$:

$$f_{elec_dens}(\mathbf{x}) = \sum_{i=1}^M A_i e^{-d|\mathbf{x}|} \delta(c_i)$$

- For above kernels G :

$$f_{elec_dens}(\mathbf{x}) = G \otimes \sum_{i=1}^M A_i \delta(c_i)$$

