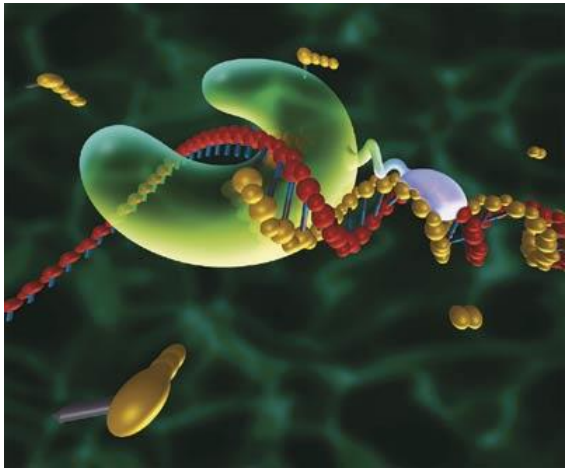
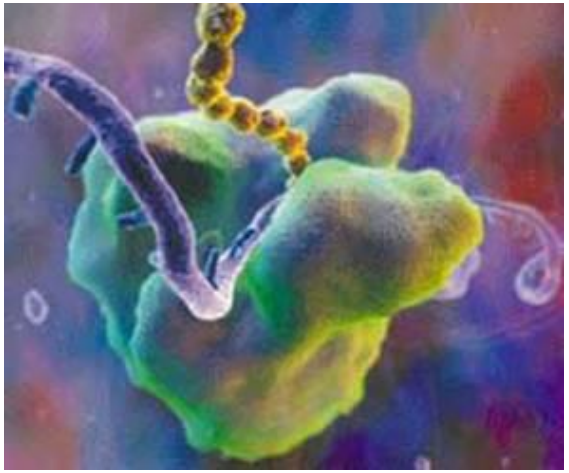
Macromolecular flexibility: The Zernike3D approach

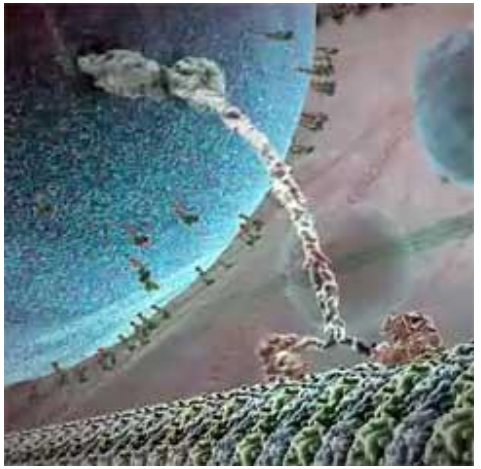
José-Maria Carazo

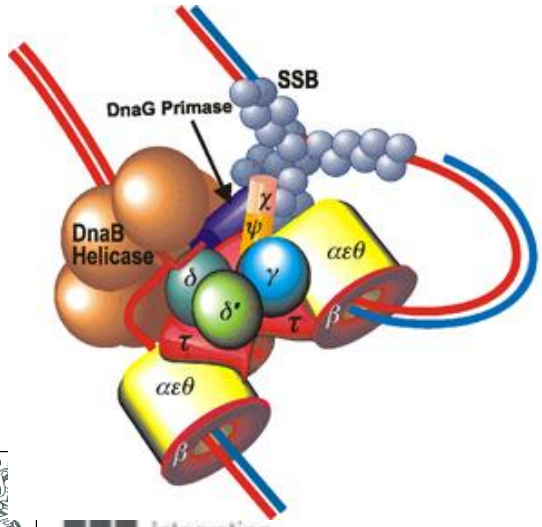
Biocomputing Unit, CNB-CSIC
INSTRUCT-ES. Instruct Image Processing Center, Madrid

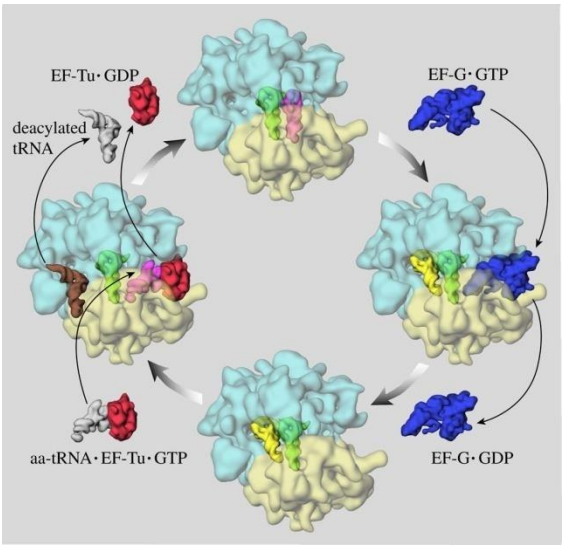
Life …. and atomic interactions

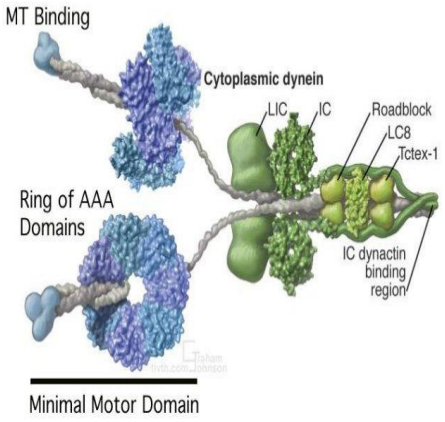












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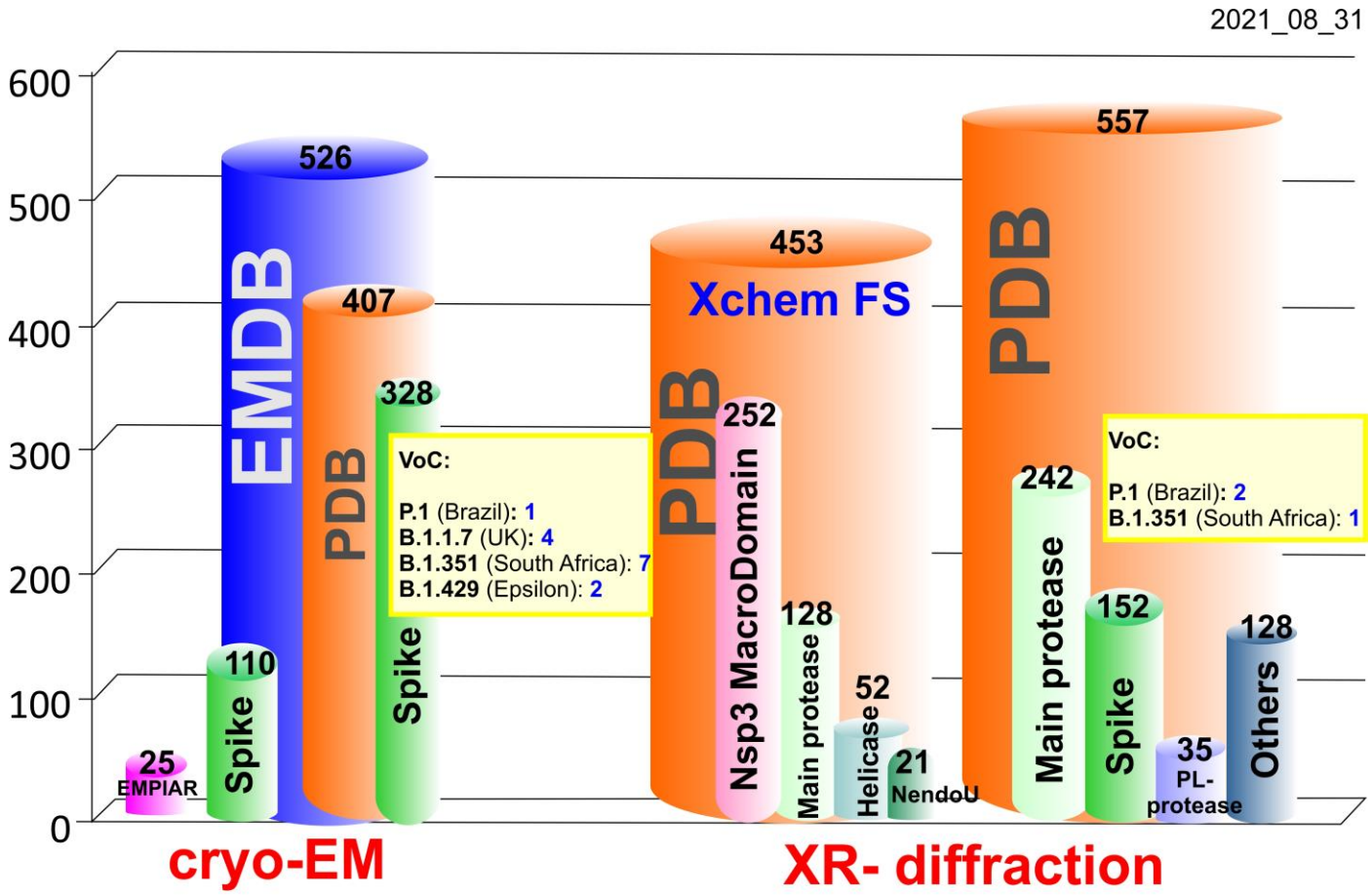
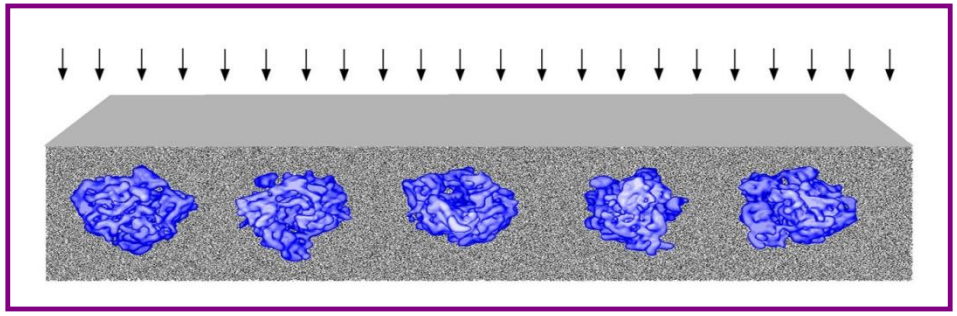
The role of cryo-EM: Statistics on SARS-CoV-2



Other experimental methods: NMR: 6 (PDB)
Neutron diffraction: 3 (PDB)

The cryo-EM SPA pledge

- **Trapped in ice, these molecules are close to their native state**
- **In 3D Electron Microscopy *individual molecules* are visualized**
- ***Therefore, flexible macromolecular machines in solution can be studied directly!***



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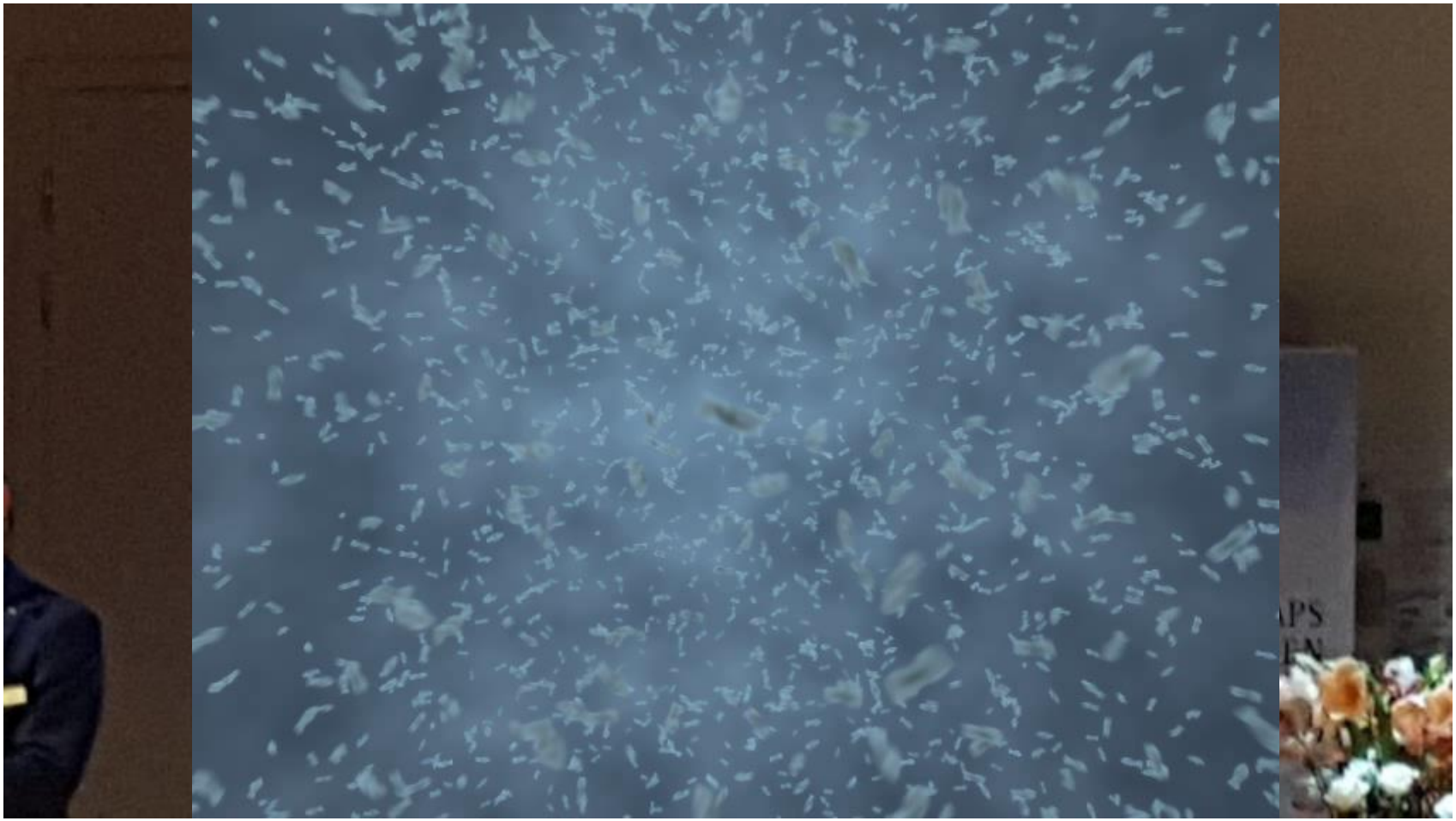
The principles of cryoEM



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Starting from “first principles”….

Nat Methods. 2007 Jan;4(1):27-9. doi: 10.1038/nmeth992. Epub 2006 Dec 10.

Disentangling conformational states of macromolecules in 3D-EM through likelihood optimization

Sjors H W Scheres [1], Haixiao Gao, Mikel Valle, Gabor T Herman, Paul P B Eggermont, Joachim Frank, Jose-Maria Carazo

“These probabilities calculations are based on the assumption that all Xi’s are projections of one of K underlying tree-dimensional objects ……..”

(So, continuous flexibility was not addressed)

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And where we are nowadays!

Estimating conformational landscapes from Cryo-EM particles by 3D Zernike polynomials

D. Herreros[1], R.R. Lederman[2], J.M. Krieger[1], A. Jiménez-Moreno[1], M. Martínez[1], D. Myška[3], D. Strelak[1,4], J. Filipovic[3], C.O.S. Sorzano*[1], and J.M.Carazo*[1]

Herreros et al., submitted

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Approximating deformation fields for the analysis of continuous heterogeneity of biological macromolecules by 3D Zernike polynomials

David Herreros,[a]* Roy R. Lederman,[b] James Krieger,[c] Amaya Jiménez-Moreno,[a] Marta Martínez,[a] David Myška,[d] David Strelak,[a,e] Jiri Filipovic,[d] Ivet Bahar,[c] Jose Maria Carazo[a] and Carlos Oscar S. Sanchez[a]

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Our aims:

- 1) Address continuous flexibility, not imposing discrete classes
  - But incorporating discrete classification information as well

- 2) Obtain reduced-dimensionality conformational landscapes, and analyze *interactively* (or by any sort of postprocessing) the flexibility they convey
  - We aim at subtle changes that may be masked by discrete classification
  - We also aim at simplifying the analysis process, minimizing decisions

- 3) Reduce map flexibility-induced blurring by "deforming back" images during the reconstruction process
  - This increases local resolution in flexible regions

How to study flexibility?

Classical Algorithms

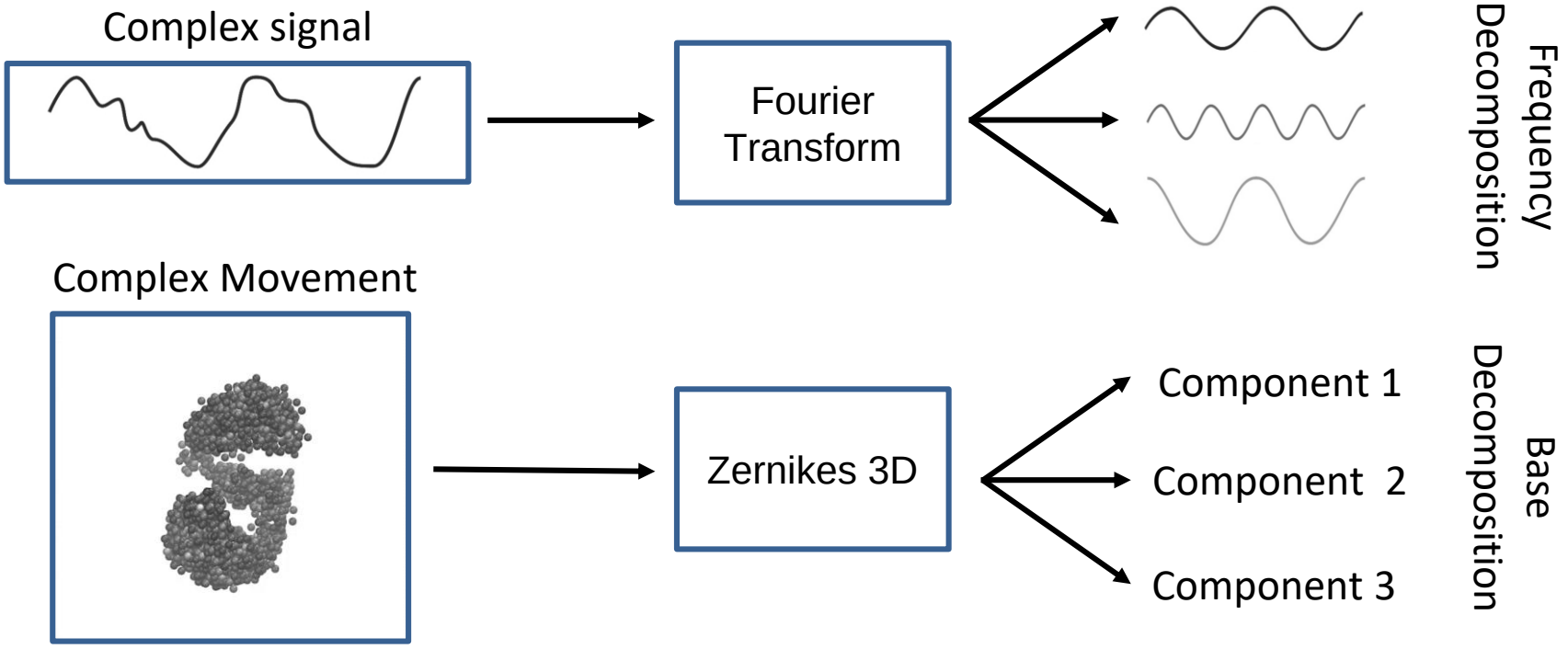
- Normal Mode Analysis
- Molecular Dynamics
- Principal Components Analysis

New Algorithms

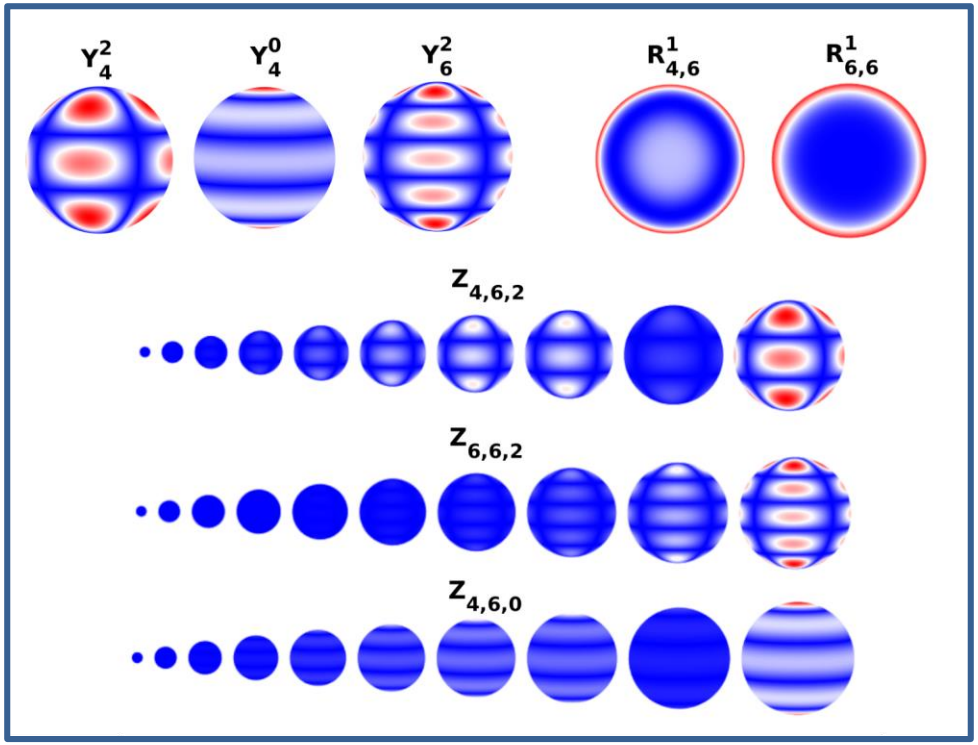
- REFINED Manifold embedding
- Normal Mode Analysis"Plus"
- CryoDRGN
- Gaussian Mixtures
- Relion (CCPEM-Relion)
- CrioSpark BioXriv on elastic deformations
- **Zernikes 3D**

"Hot topic!!!!!"

Zernikes3D: A completely different approach. Projecting onto a 3D base



Mathematical principles: How the basis look



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Mathematical Basis: Expressing the deformation field ONLY

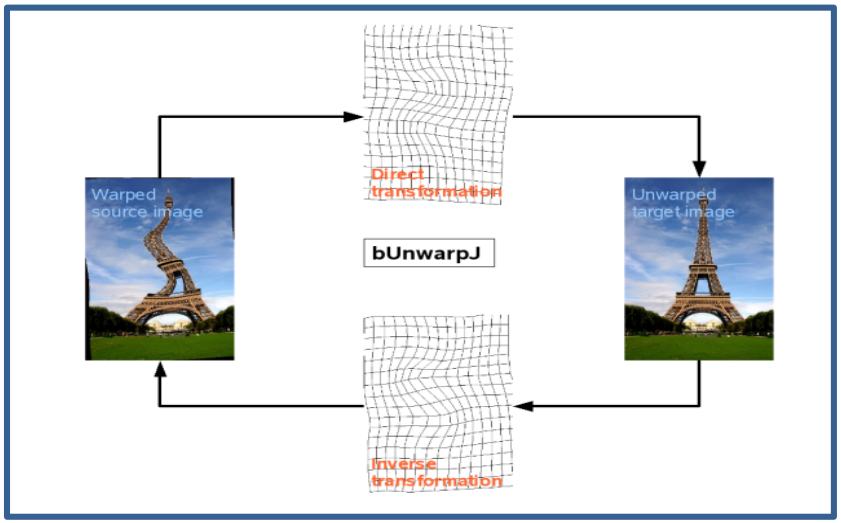
$$\min_{g_L} \int \left| V_1(\vec{r}) - V_2\left(\vec{r} + \vec{g}_L(\vec{r})\right) \right| d\vec{r}$$

$$g_L(\vec{r}) = \sum_l \sum_n \sum_m \vec{c}_{N_1,N_2,m} Z_{l,n,m}(r, \theta, \phi)$$

Purpose of the tool: to find a per-point displacement (deformation) that minimizes the distances between between two images

Parameters

- L and N → Maximum degree of the basis (movement frequency)
- R_{max} → Maximum radius of the sphere (enclosing the molecule)



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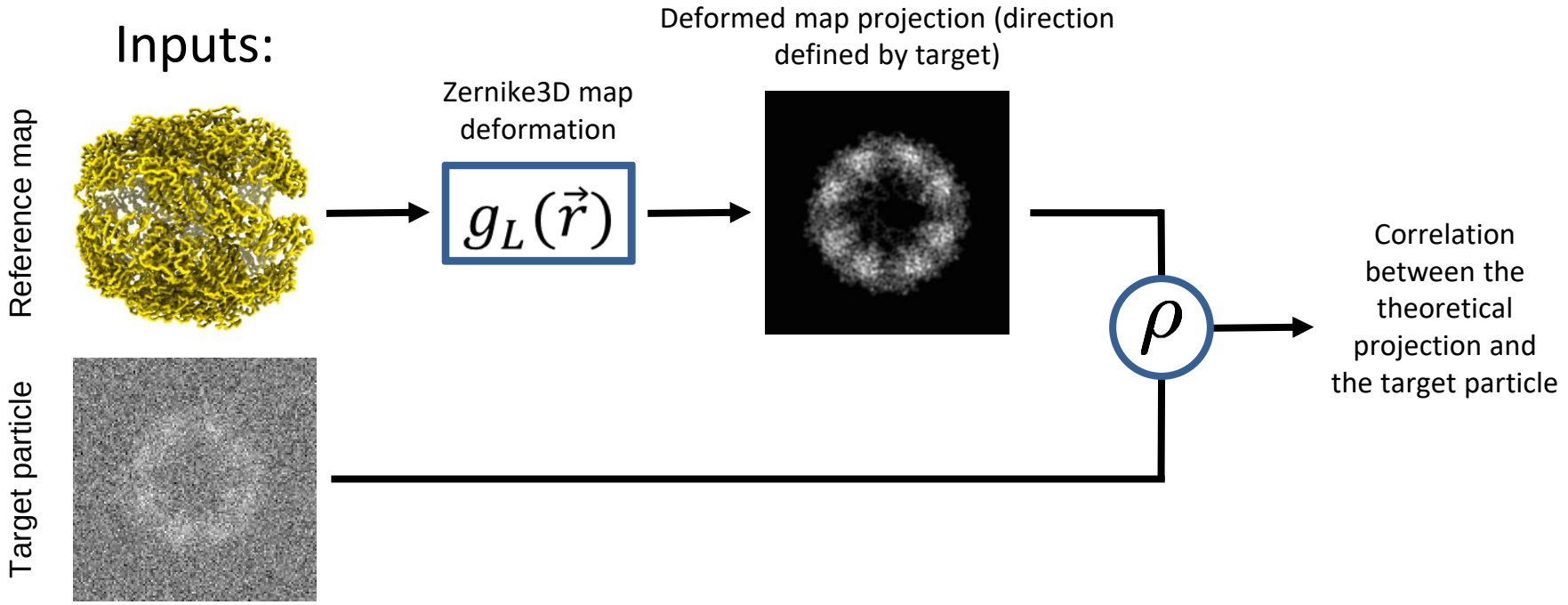
Starting from 2D cryoEM images, model the 3D deformation field



Purpose of the tool: to find a per-point displacement (deformation) that minimizes the distances between between **two images (+ volume)**

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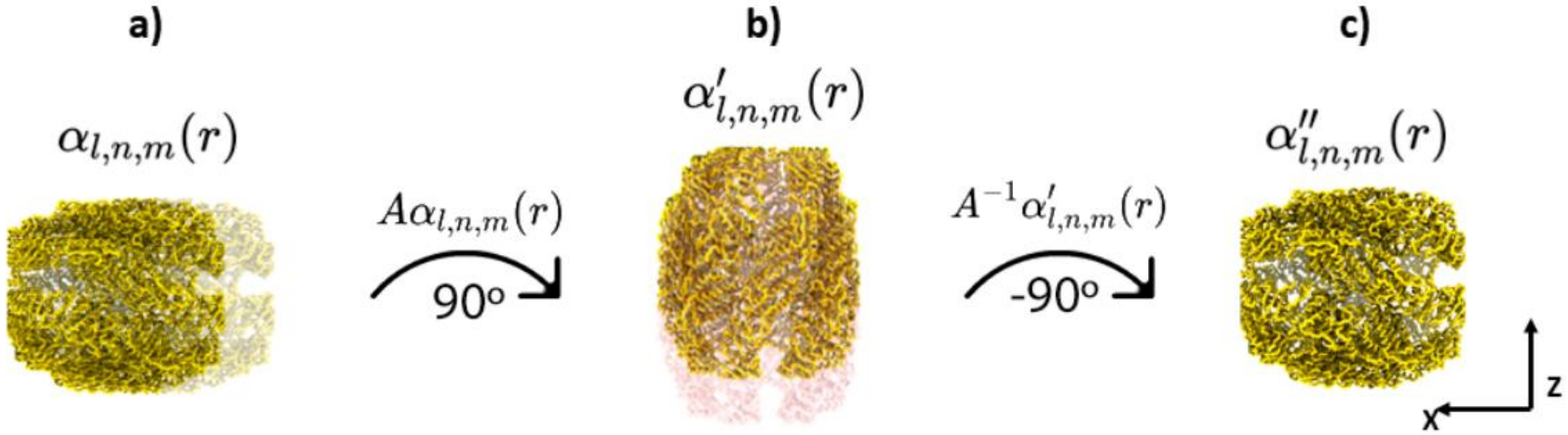
Formally addressing the different information content along projection directions



$$\overrightarrow{g_l}(r'_i) = \overrightarrow{g_l}(Rr_i) = R\overrightarrow{g_l}(r_i)$$

$$\overrightarrow{g_l}(r_i) = R^{-1}\overrightarrow{g_l}(Rr_i) = \sum_l \sum_n \sum_m R^{-1} c_{l,n,m} Z_{l,n,m}(Rr_i)$$

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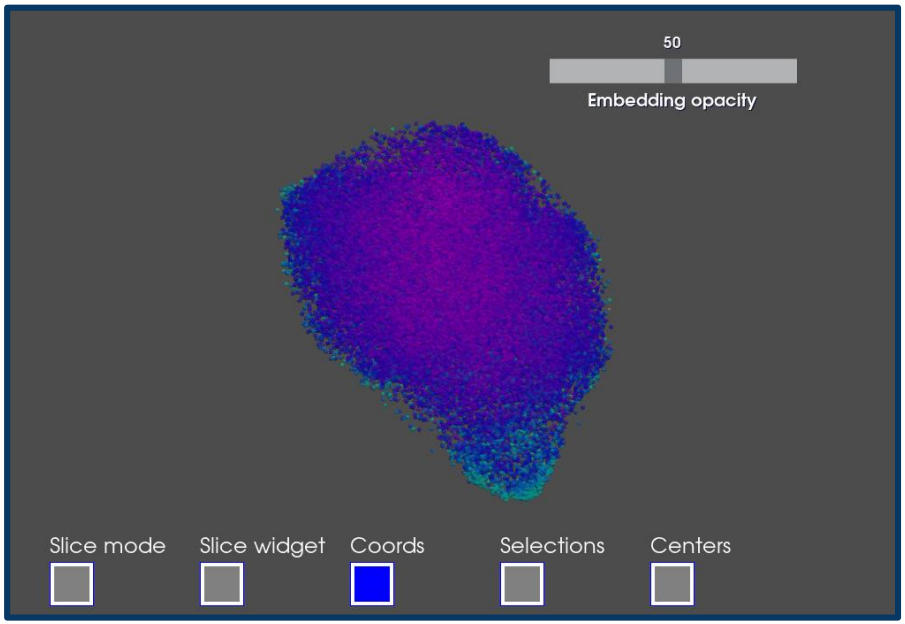
Zernike3D coefficient embedding

Low-dimensional representation of the coefficient space defined by a set of CryoEM particles

EMPIAR-10080

- Representative dataset of the Pf80S rotation state
- Two well differentiated areas found in the embedding
  - Unrotated (reference) state in purple
  - Rotated state in green



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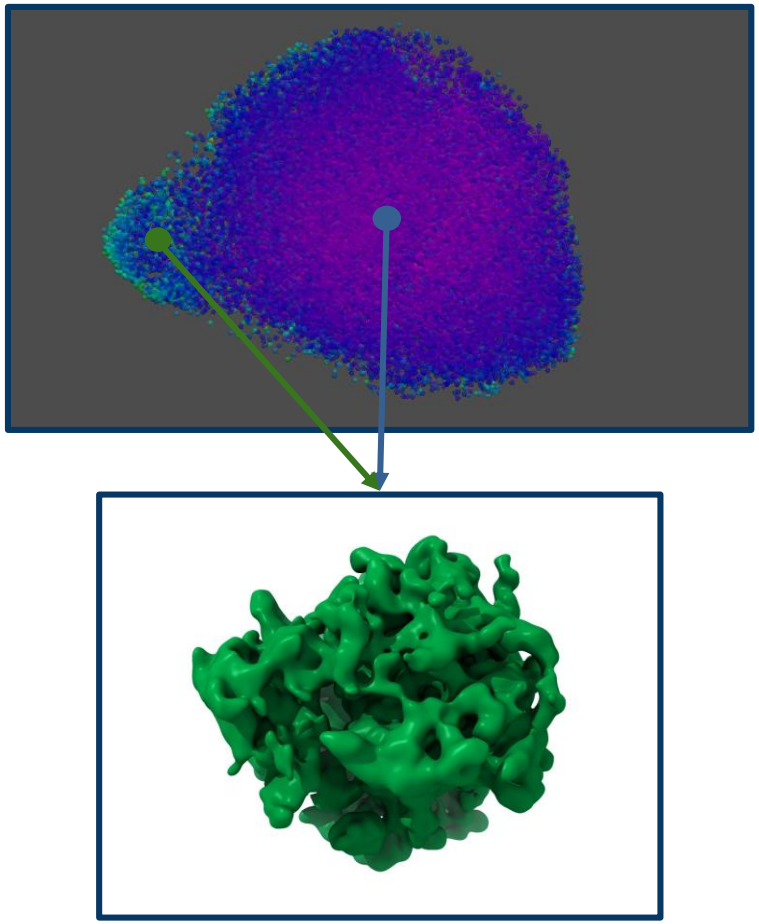

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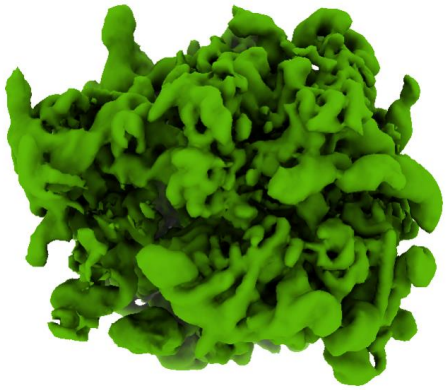
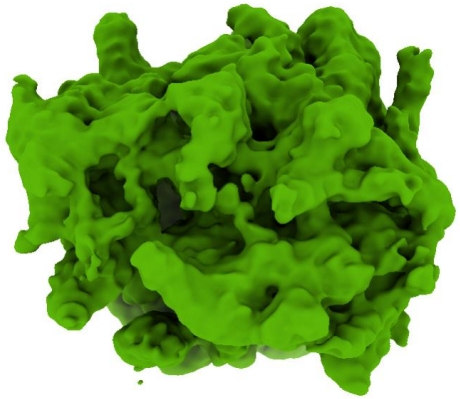
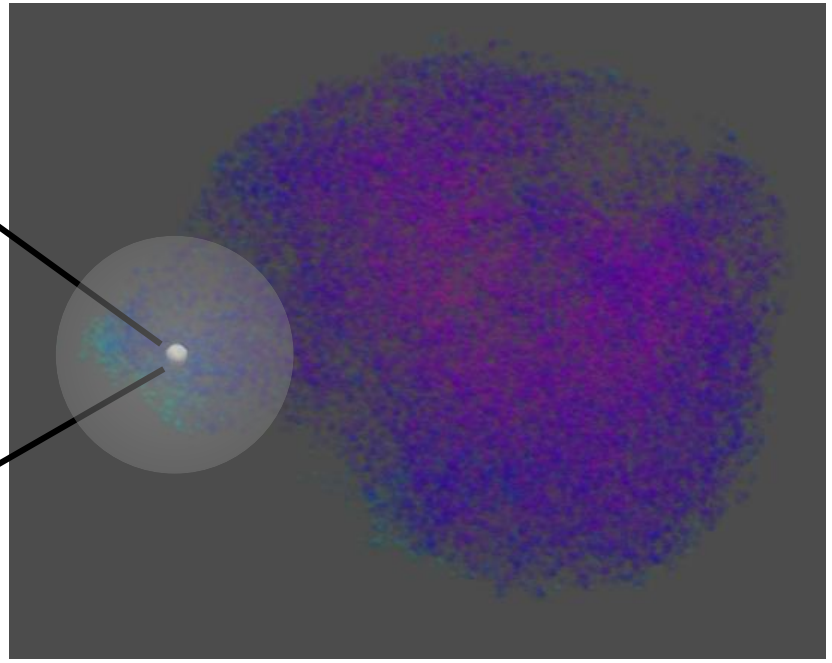
Zernikes3D representations are very accurate

Zernike3D



Refinement





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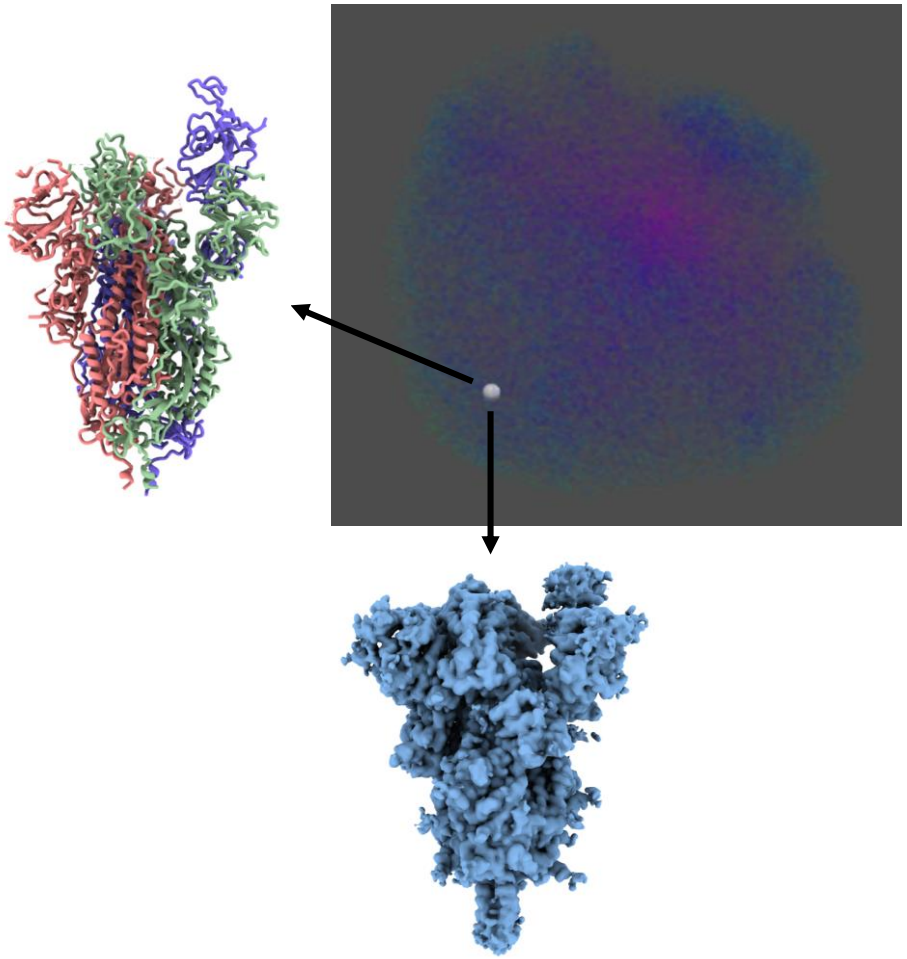
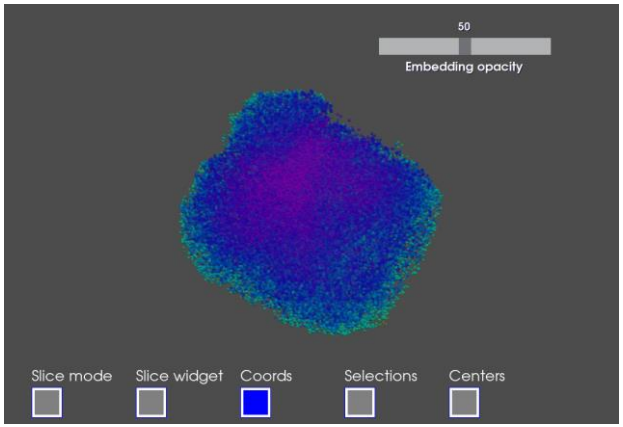
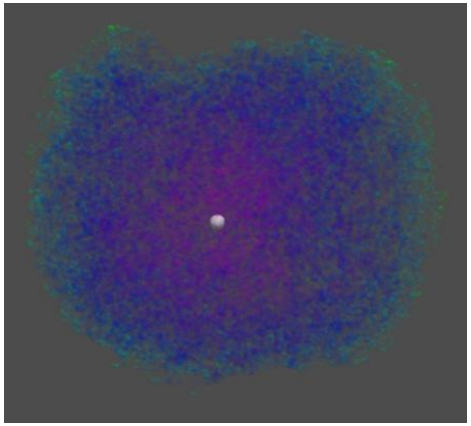
Working with maps and models

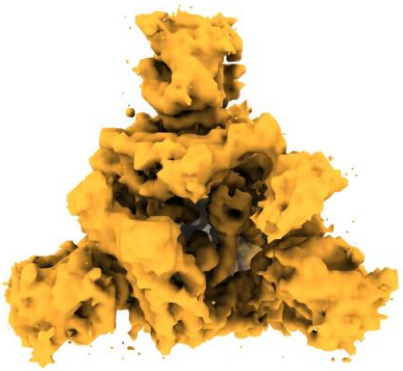
Zernike3D coefficients can be applied simultaneously to CryoEM maps and structures



Focused heterogeneity analysis







Flexibility correction: ZART reconstruction

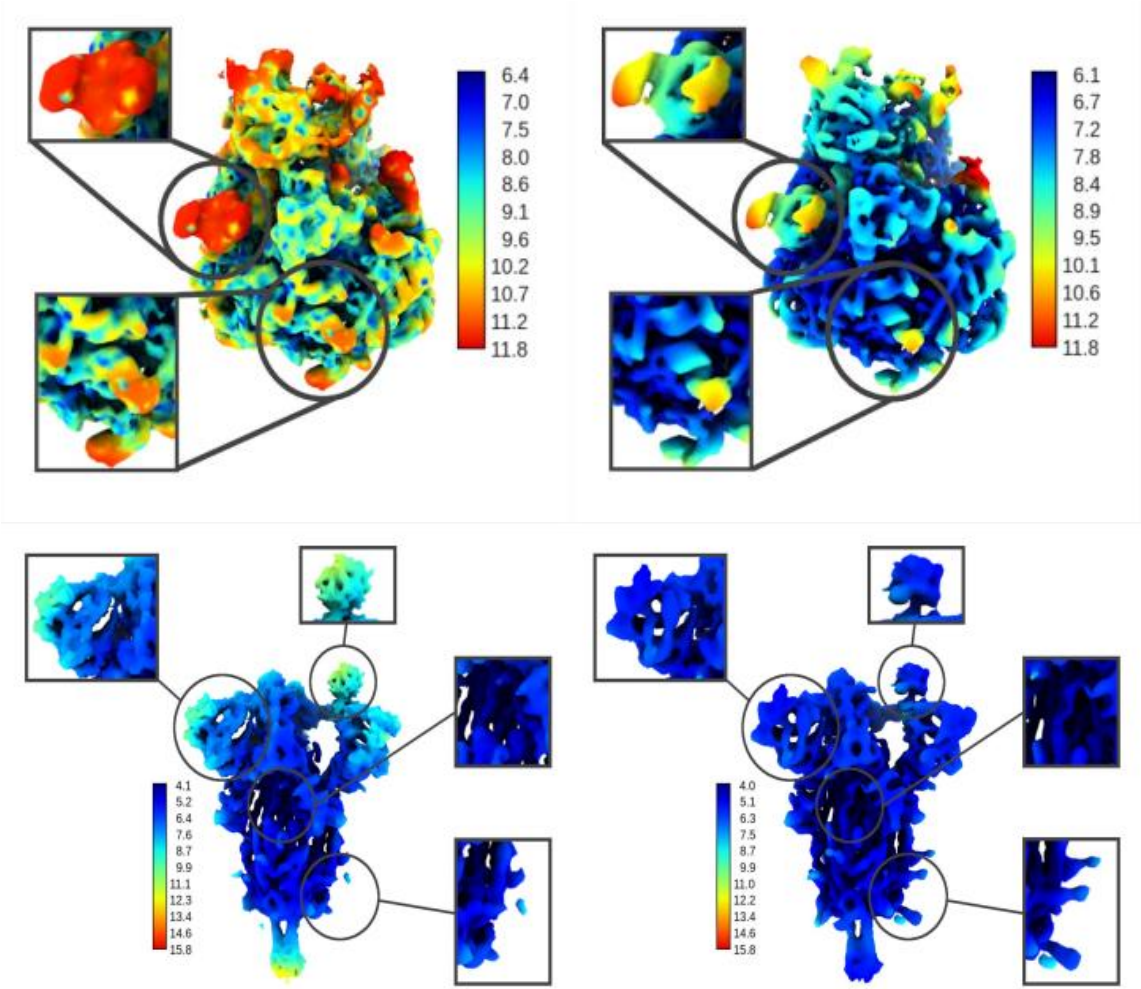
- We have develop a new ART based reconstruction algorithm that incorporates the per-particle deformation field information of the Zernikes3D coefficients in the reconstruction process
  - Improves resolvability of moving areas in the maps by decreasing the motion blurring

ART $\longrightarrow$ $$V(r)^{k+1} = V(r)^{k} + \lambda(P_H(V(r)) - I(P_H(r)))$$

$\downarrow$

ZART $\longrightarrow$ $$V(r)^{k+1} = V(r)^{k} + \lambda(P_H(V(r + g_L)) - I(P_H(r)))$$

“Undoing” flexibility: ZART reconstruction



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In a nutshell

- 1) We do obtain continuous macromolecules deformation fields
- 2) We get very informative low dimensional representations of these deformation fields
- 3) We can “Undo” deformations and increase local resolution
- 4) We are opening a new way to interface with Molecular Dynamics

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