Controlling protein orientation using strong electric fields: perspectives for single particle imaging

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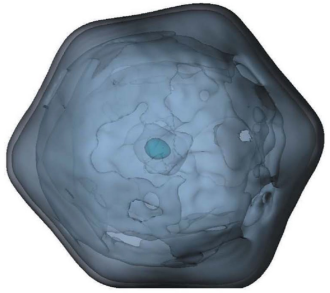
Outline

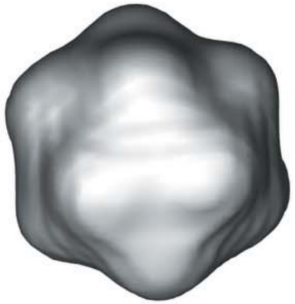
- **Single particle imaging**
- **Orientation of protein**
- **Computational approach**
- **Outlooks**

Melbourne virus

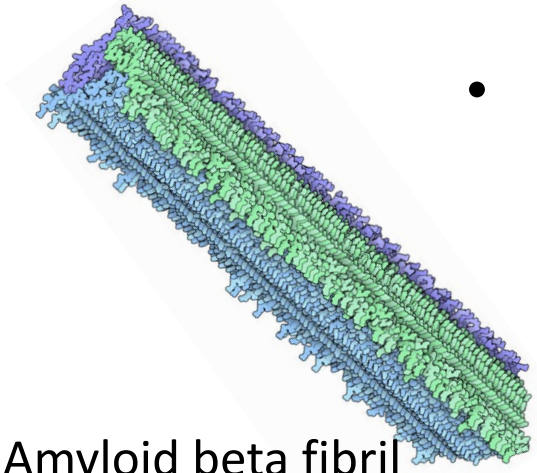


Lundholm *et al., IUCrJ* (2018)

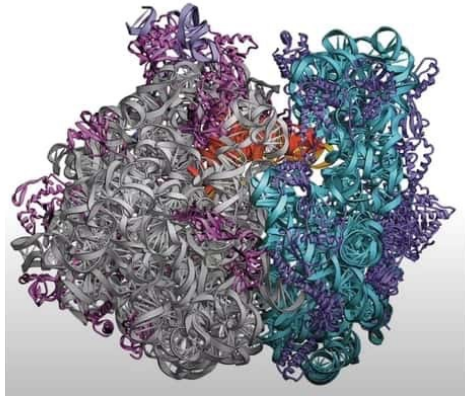
Bacteriophage



Rose, et al., *IUCrJ* (2018)



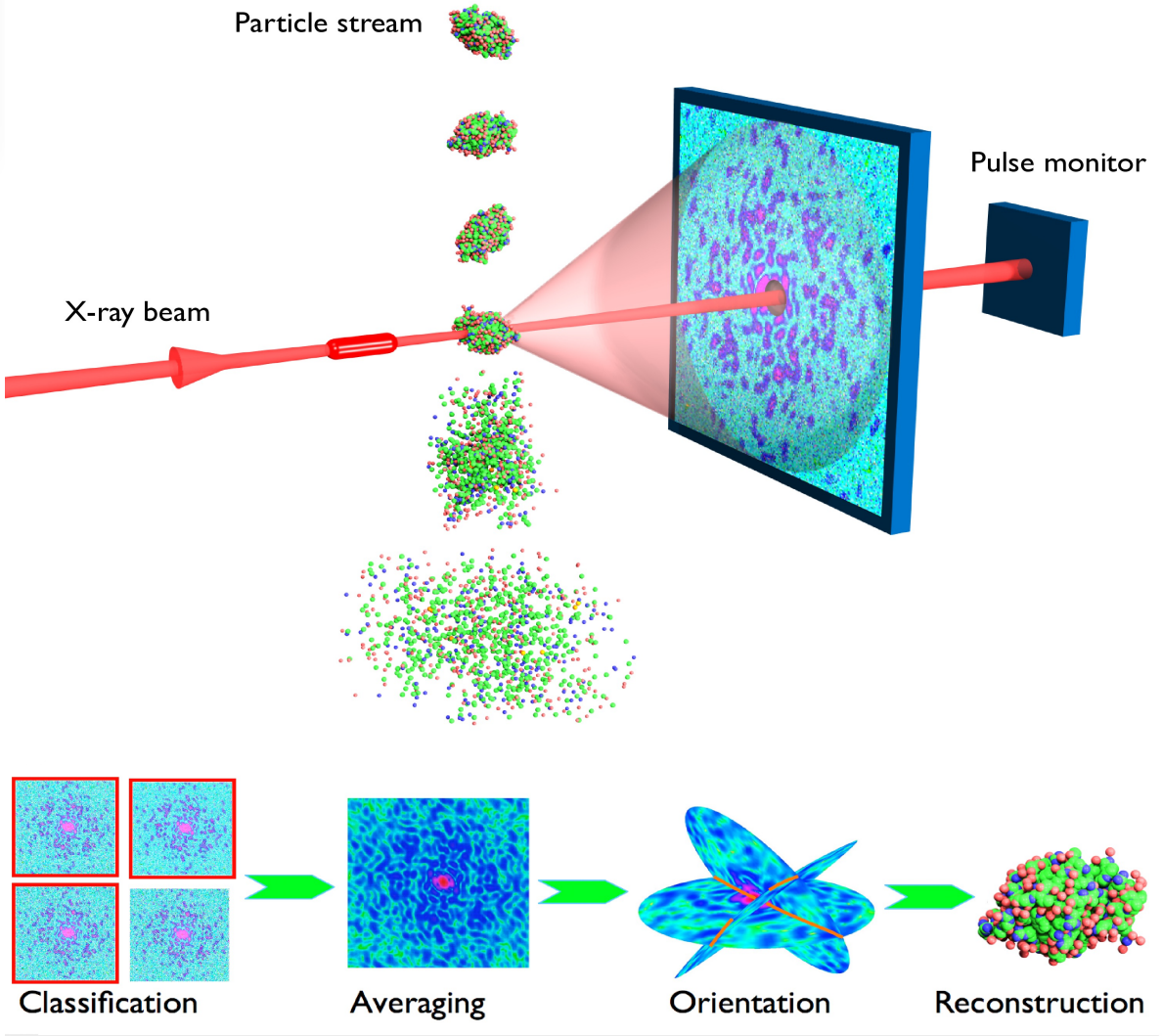
Amyloid beta fibril



Ribosomes

Human immunodeficiency virus

Single particle imaging (SPI)

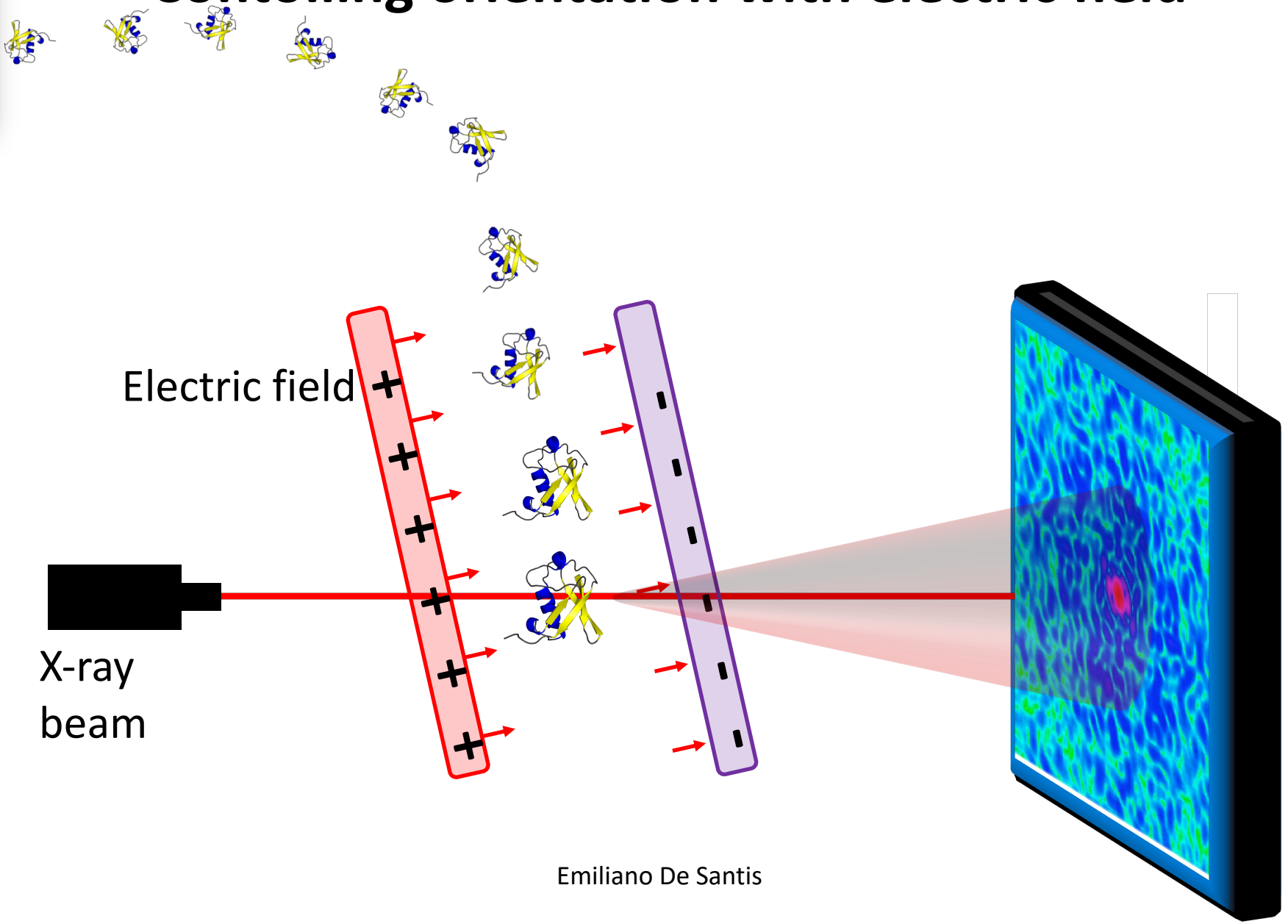


Gaffney and Chapman, *Science* (2007)

- Overcame the need of crystals
- No need for cryogenic cooling

- High radiation damage
- Thousands of diffractions needed
- Random orientation of each sample

Contolling orientation with electric field



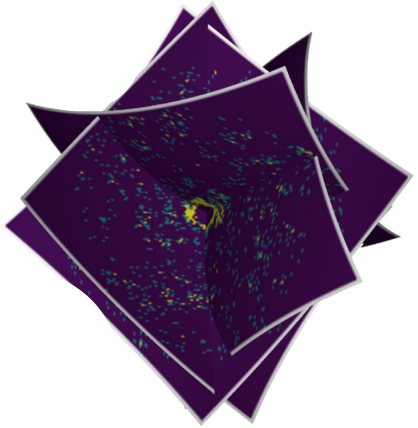
Computational apprach

Ab initio calculations

Soler, *et al*., J. Condens. Matter Phys (2002)

Classical MD simulations

Hess, *et al., J. Chem. Theory Comput***.** (2008)



Orientation recovery

Loh, et al.,*Phys. Rev. E* (2009)

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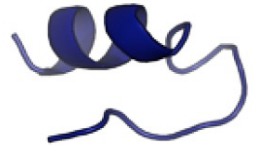
siesta

Ab initio calculations



Trp-cage^{+2}

$$F_{i,TOT}(E) = F(d_{CM}) + F(d_i) + F_{i,field}(E)$$

$$\langle |F(E_j)| \rangle = \frac{1}{N}\sum_i^N |F_i(E_j)| - |F_i(E=0)| \quad = \frac{1}{N}\sum_i^N |F_{i,field}(E)|.$$

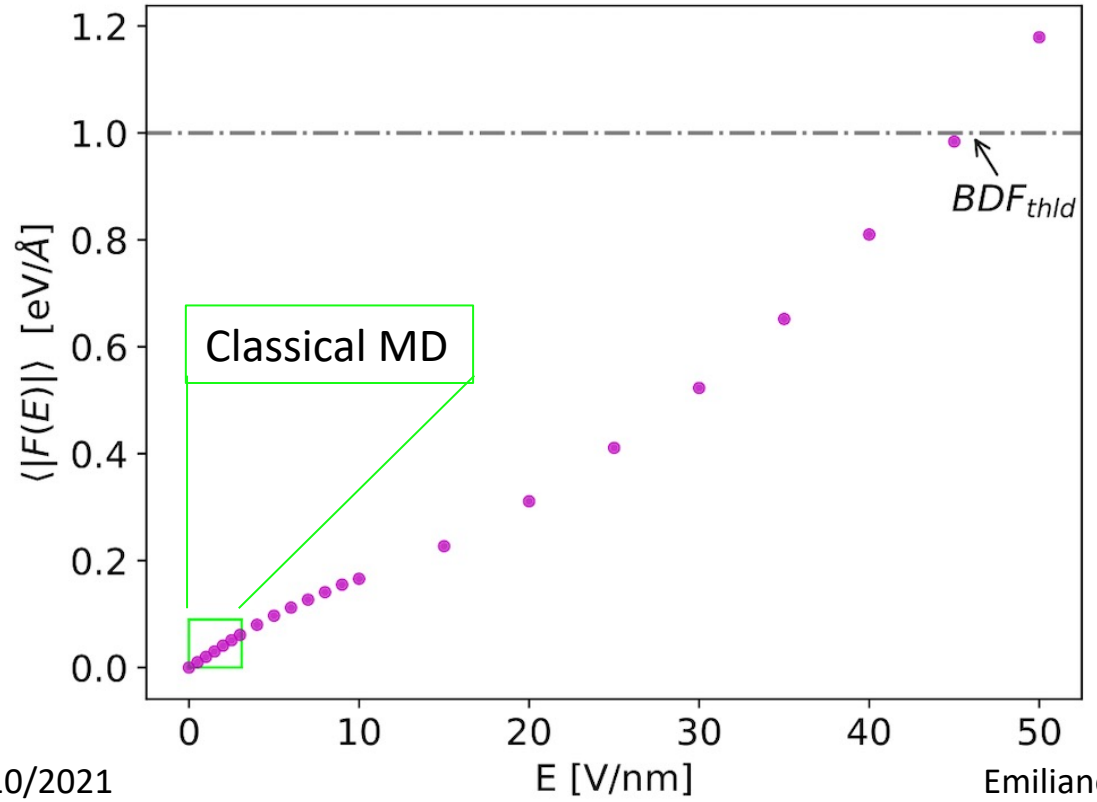


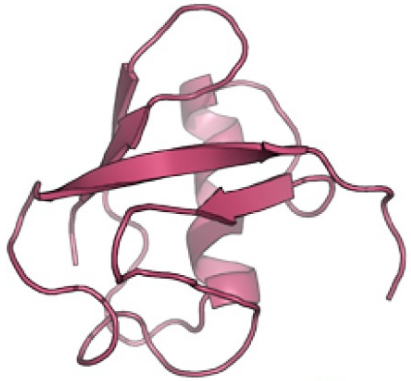
TABLE 1 Covalent and hydrogen bond forces at the equilibrium of particular relevance in proteins

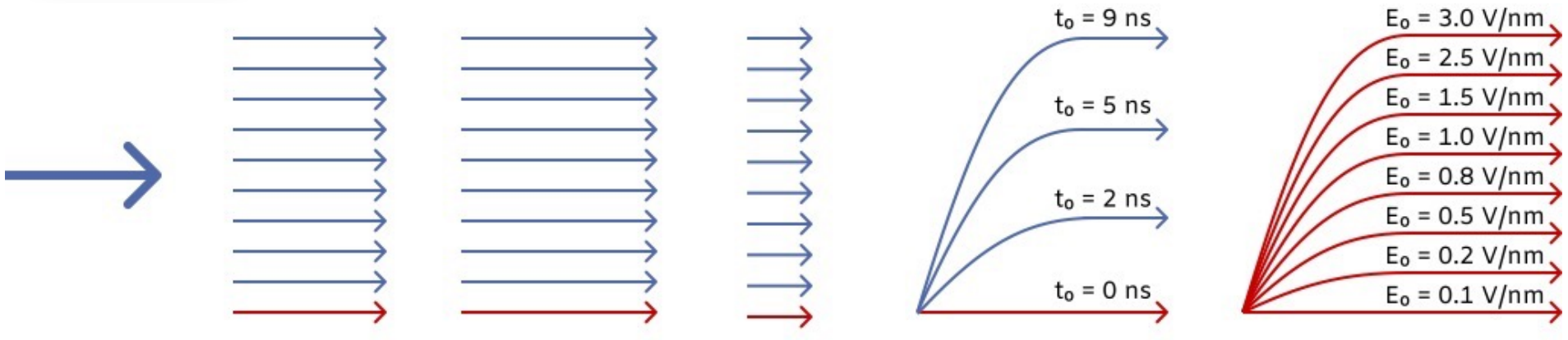
Covalent bonds (46)		Hydrogen bonds (47)	
Type	Force (eV/Å)	Type	Force (eV/Å)
C-N	~2.0	N-H···O	~0.08
C-C	~2.2	C-H···N	~0.12
C-S	~1.4	O-H···O	~0.10
C-O	~2.5	C-H···O	~0.20
S-S	~1.3	–	–

Force values are computed by dividing the tabulated energies by the tabulated equilibrium bond distances. Hydrogen bonds are indicated by ···.

Sinelnikova, EDS, *et al. Biophys J.* (2021)

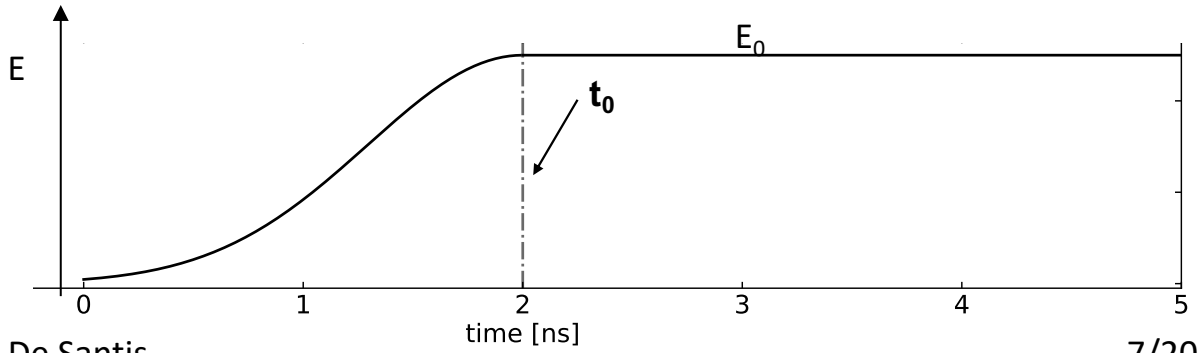
Classical MD simulations



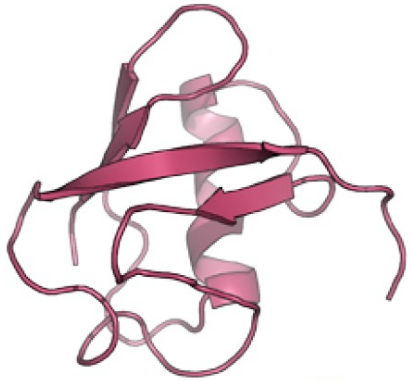


- Bulk simulations.
- 10 structures extraction;
- Water removal;
- Charge assignment.
- Energy minimisation;
- 10 ps at 300K;
- 10 ps at 300K;
- Dipole alignment.
- 4 electric field implementations.
- Simulations in presence of electric field.

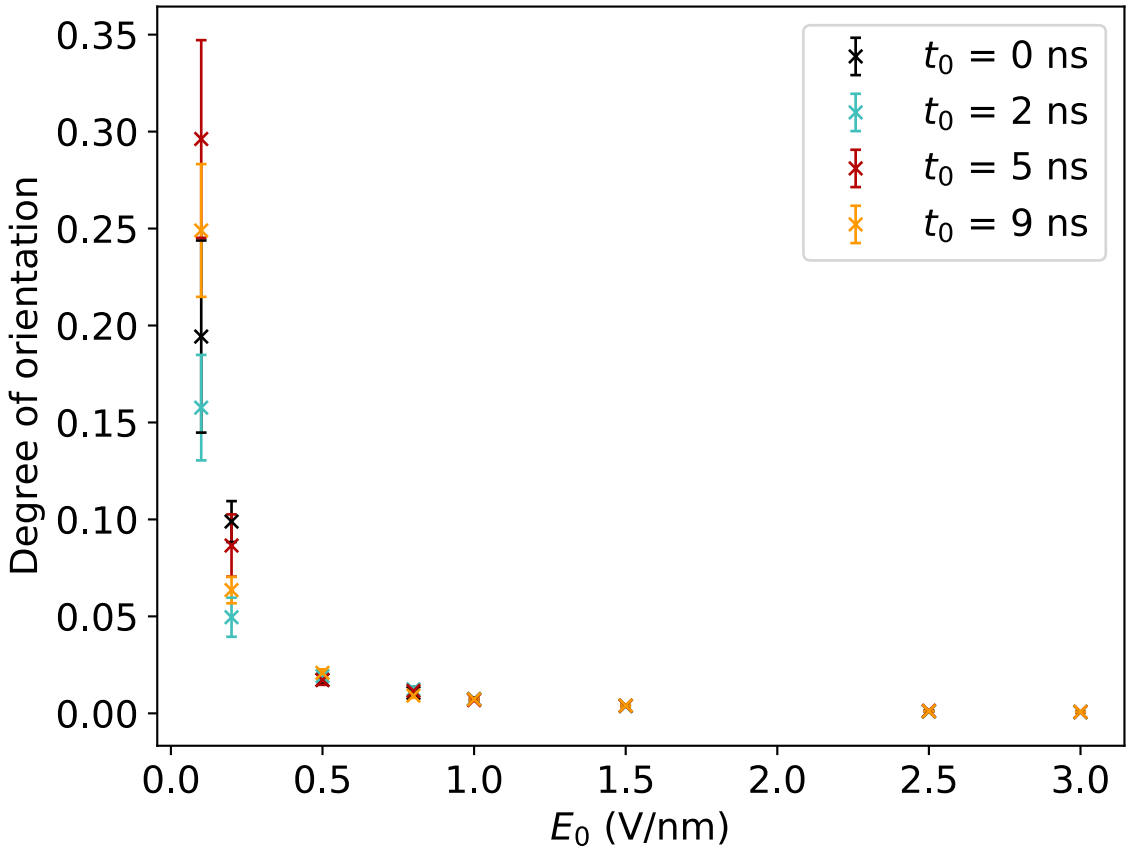
$$E(t) = E_0 \exp \frac{-(t-t_0)^2}{2\sigma^2} \times H(t_0 - t) + E_0 \times H(t - t_0)$$



Classical MD simulations

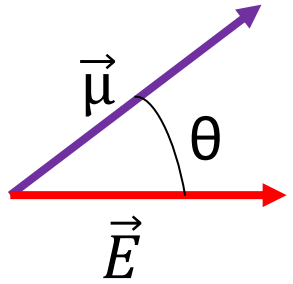


ubiquitin^{+7}

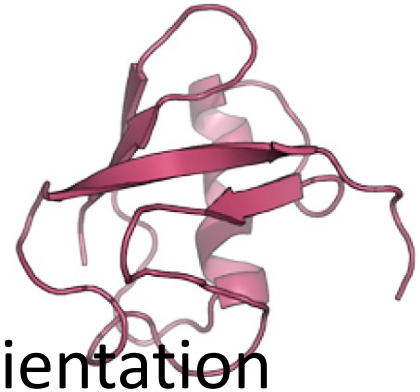


Degree of orientation

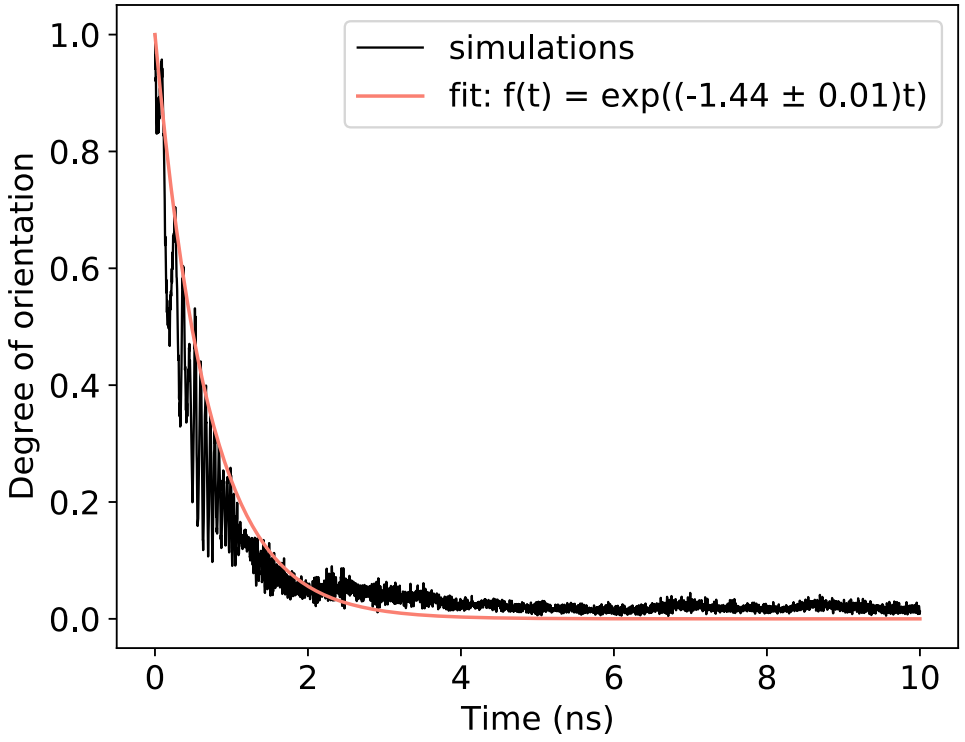
$$\Theta = 1 - \cos(\theta)$$



Classical MD simulations

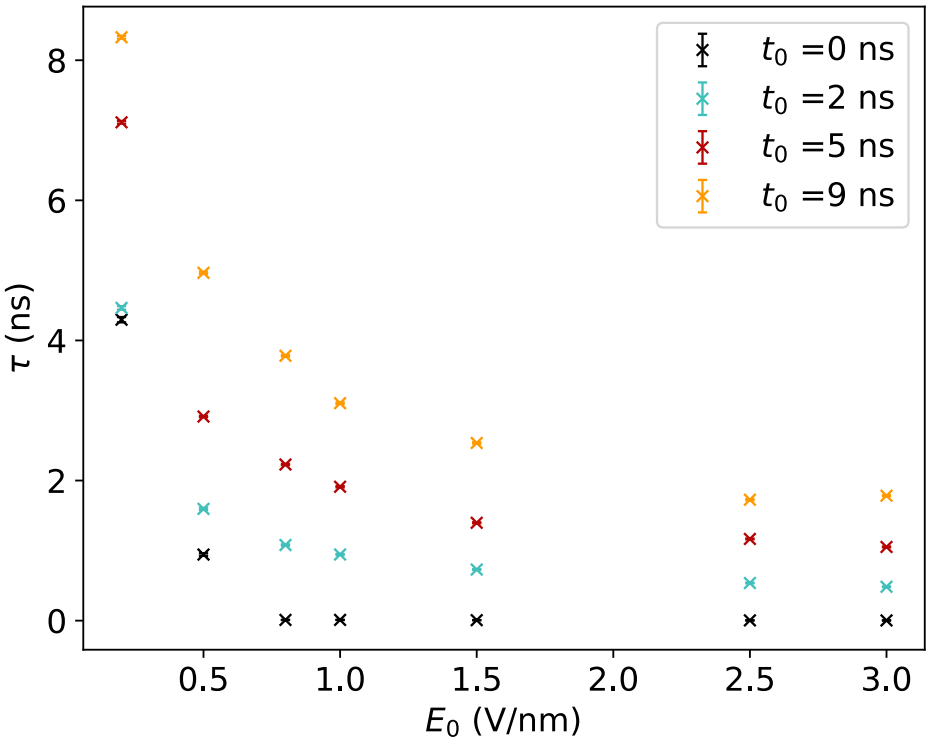


Speed of orientation

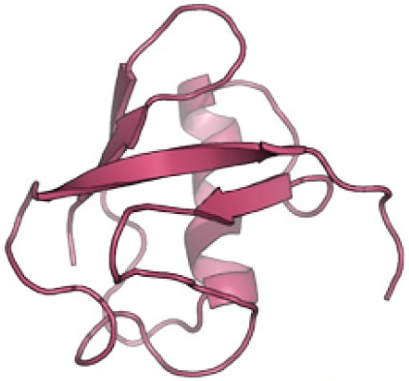


Time of orientation

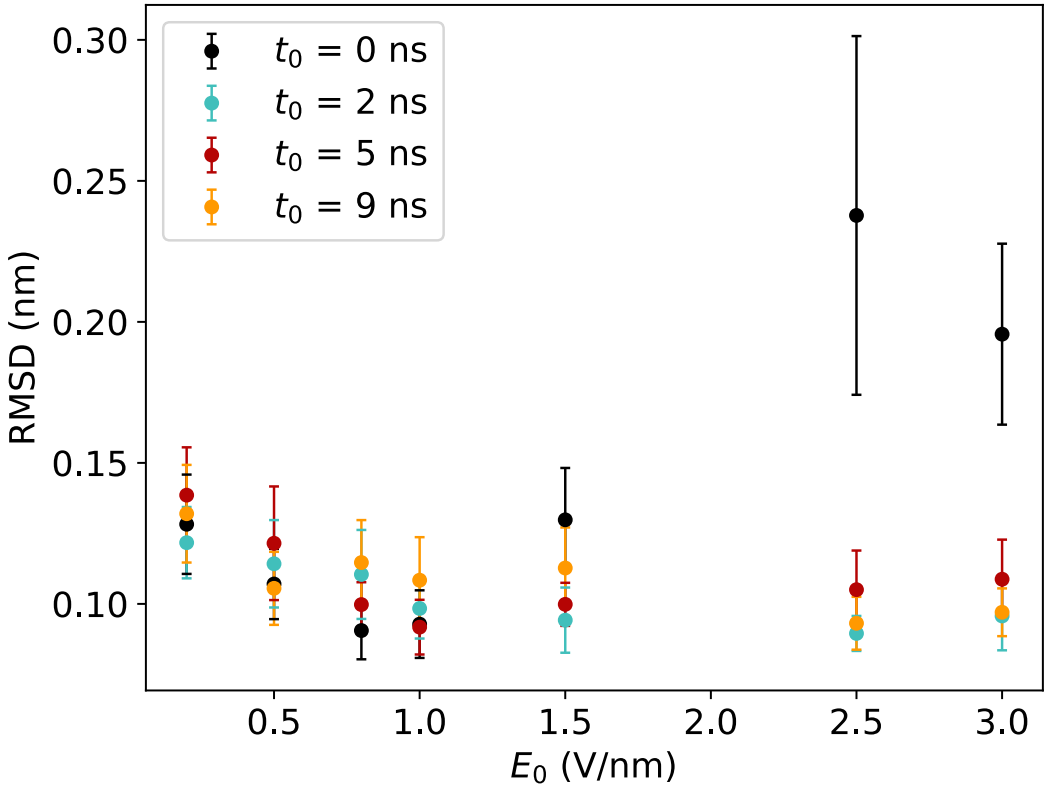
$$\tau = \frac{\ln(10)}{k}$$



Classical MD simulations



RMSD(τ)



!!!!!Orientation before destruction!!!!!!

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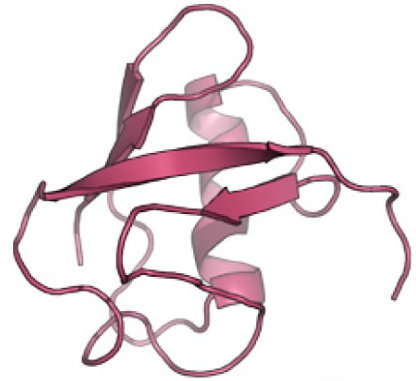
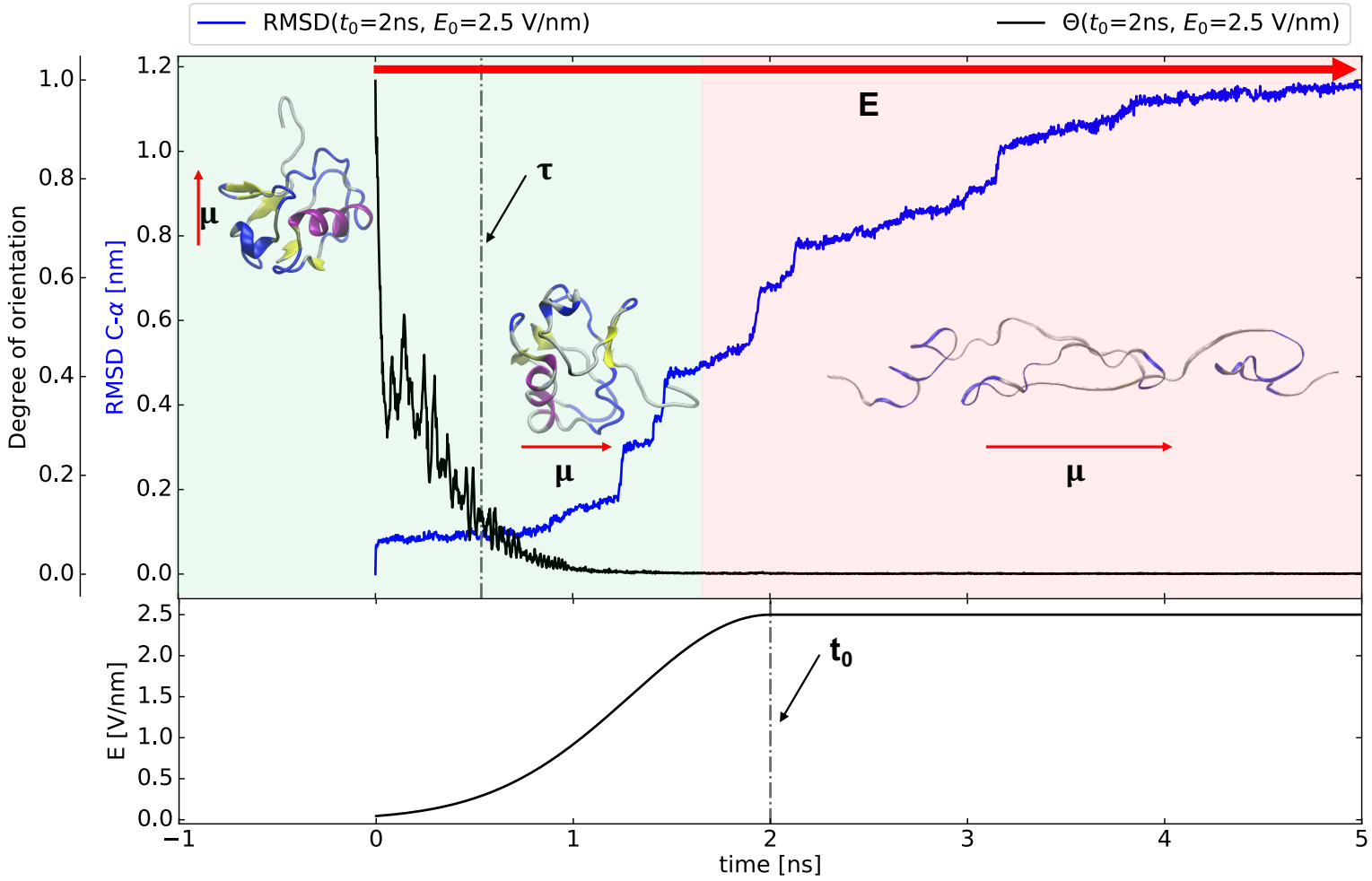
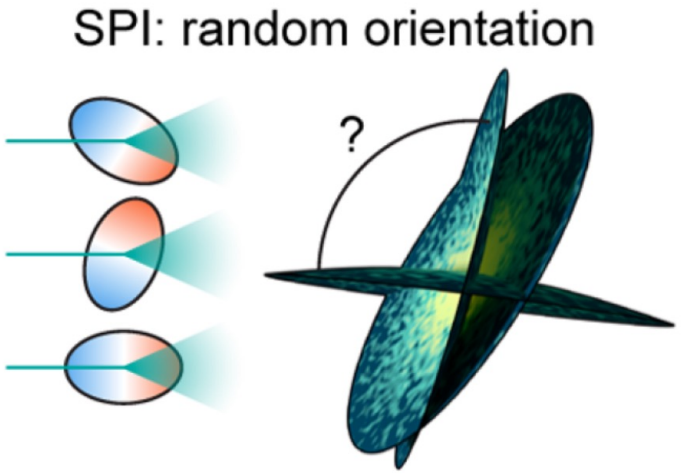
Classical MD simulations

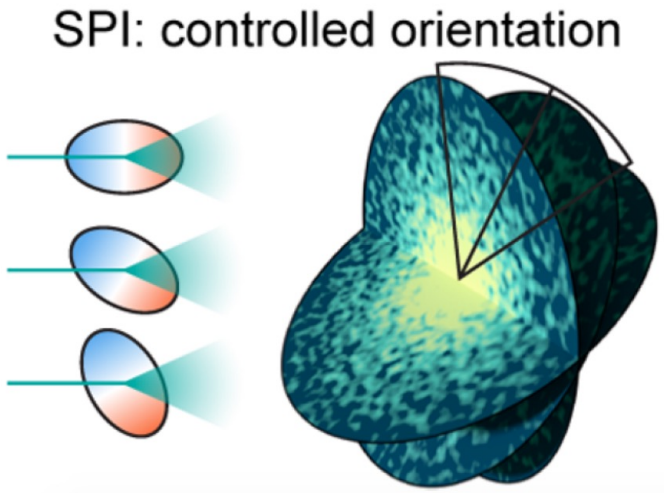
GROMACS
FAST. FLEXIBLE. FREE.

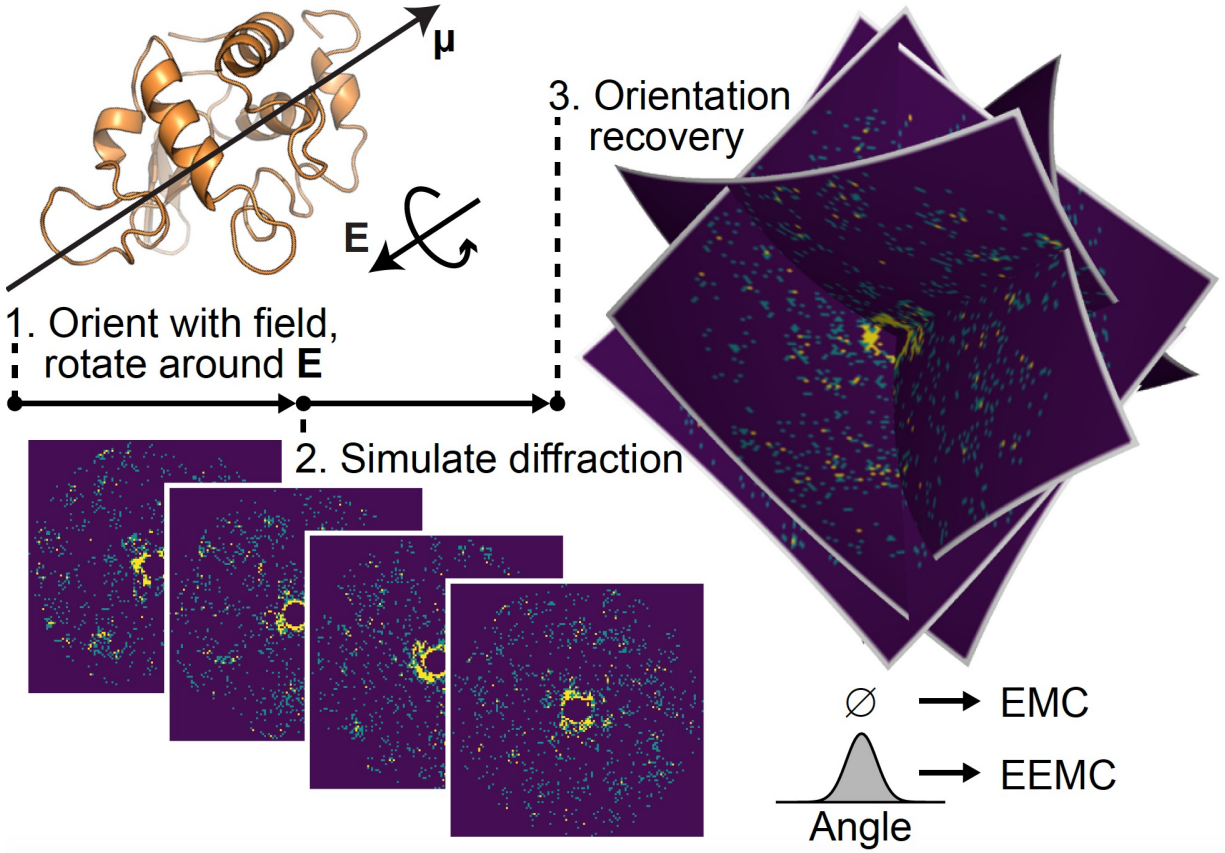




Orientation recovery







Marklund, *et al., Phys. Chem. Lett.* (2017)

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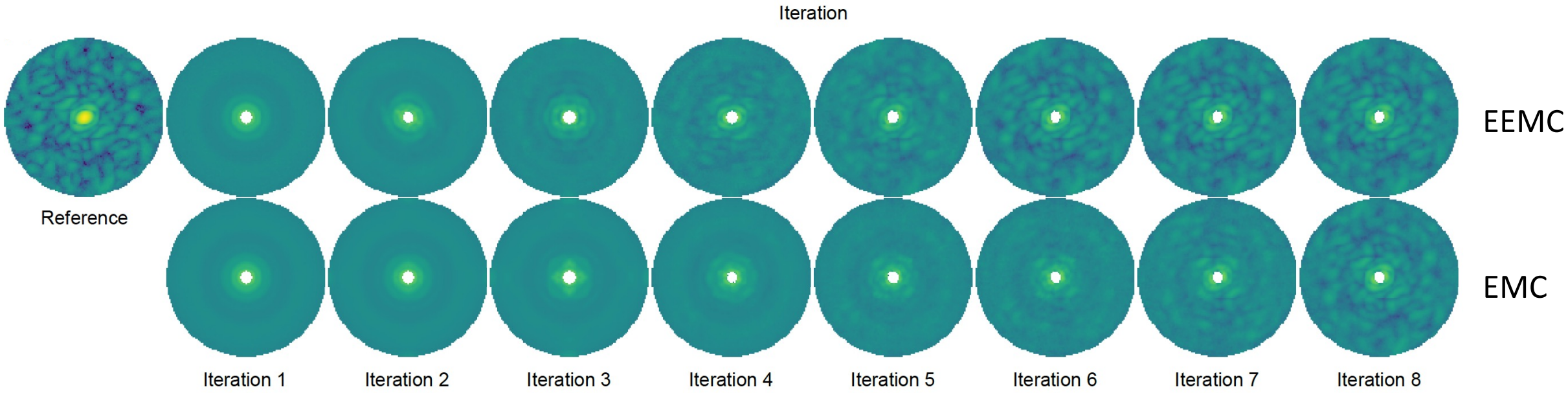
Orientation recovery

Convergence vs iterations



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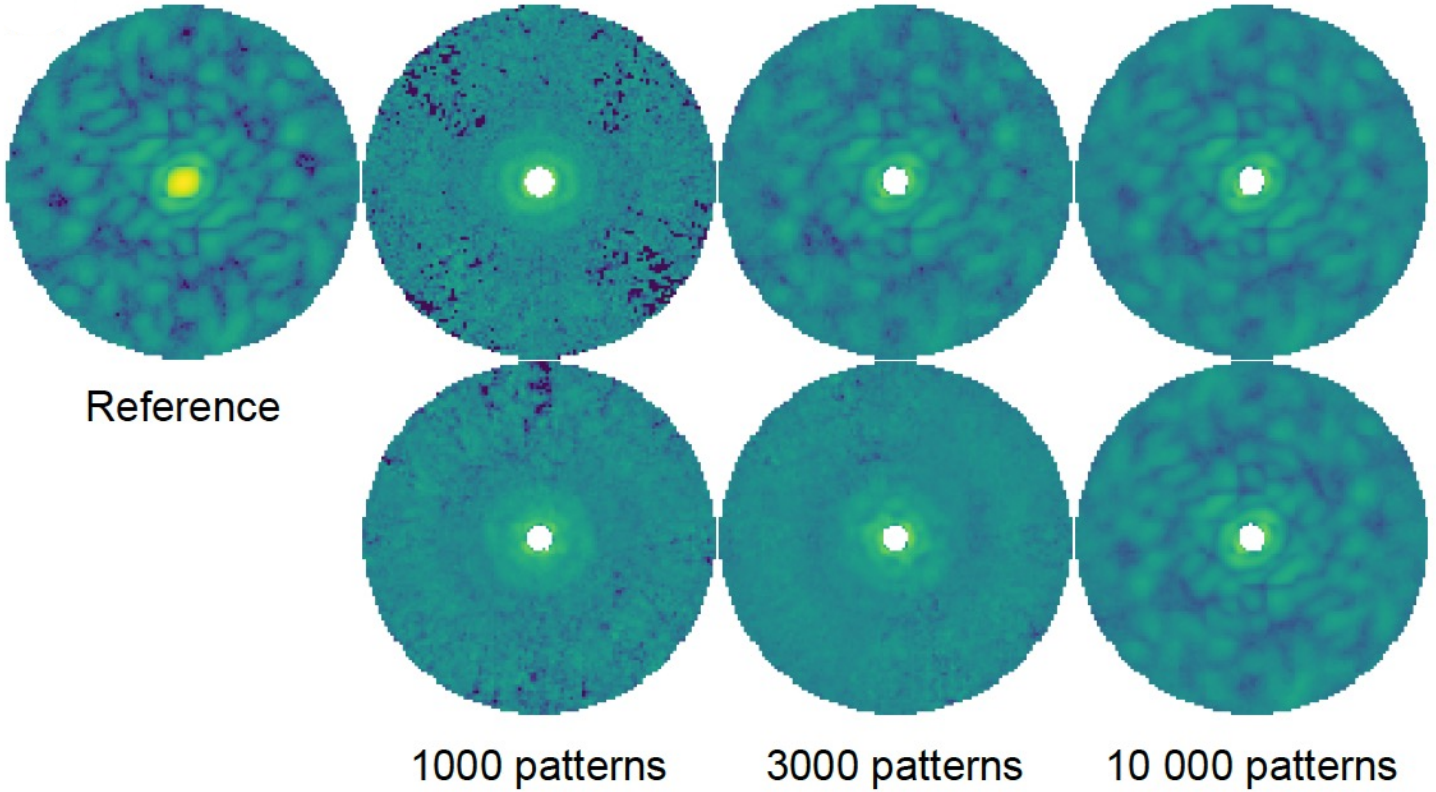
Orientation recovery

Number of patterns



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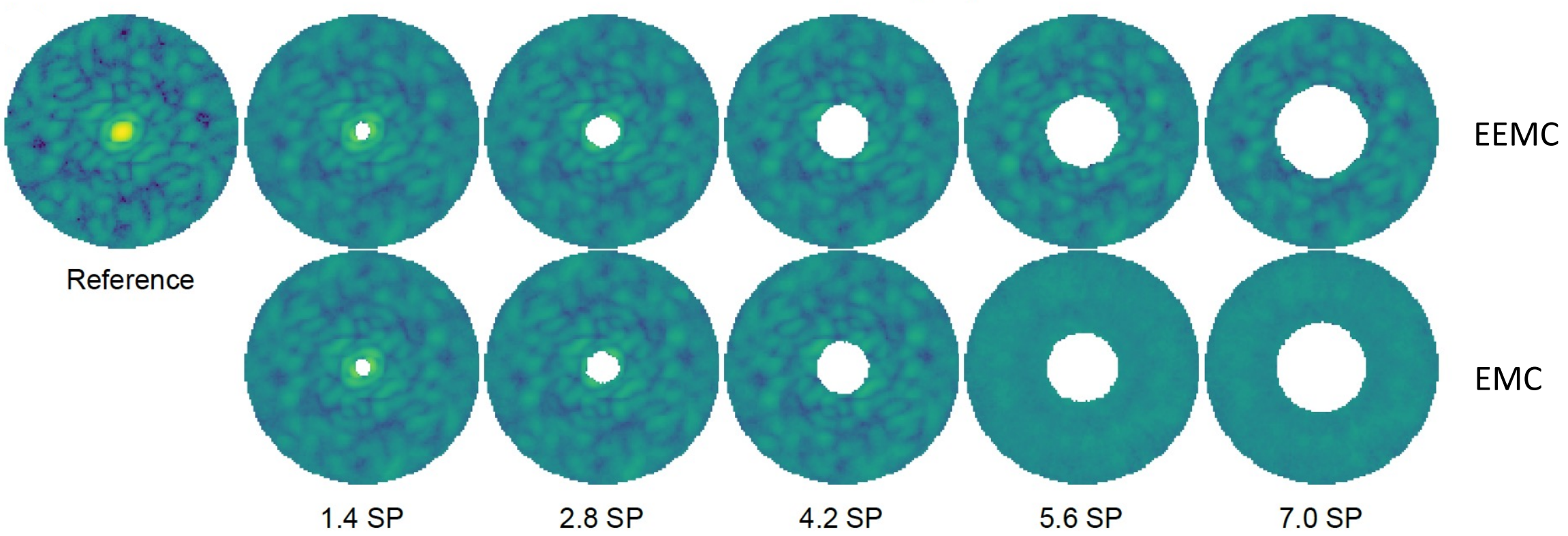
Orientation recovery

Detector masking



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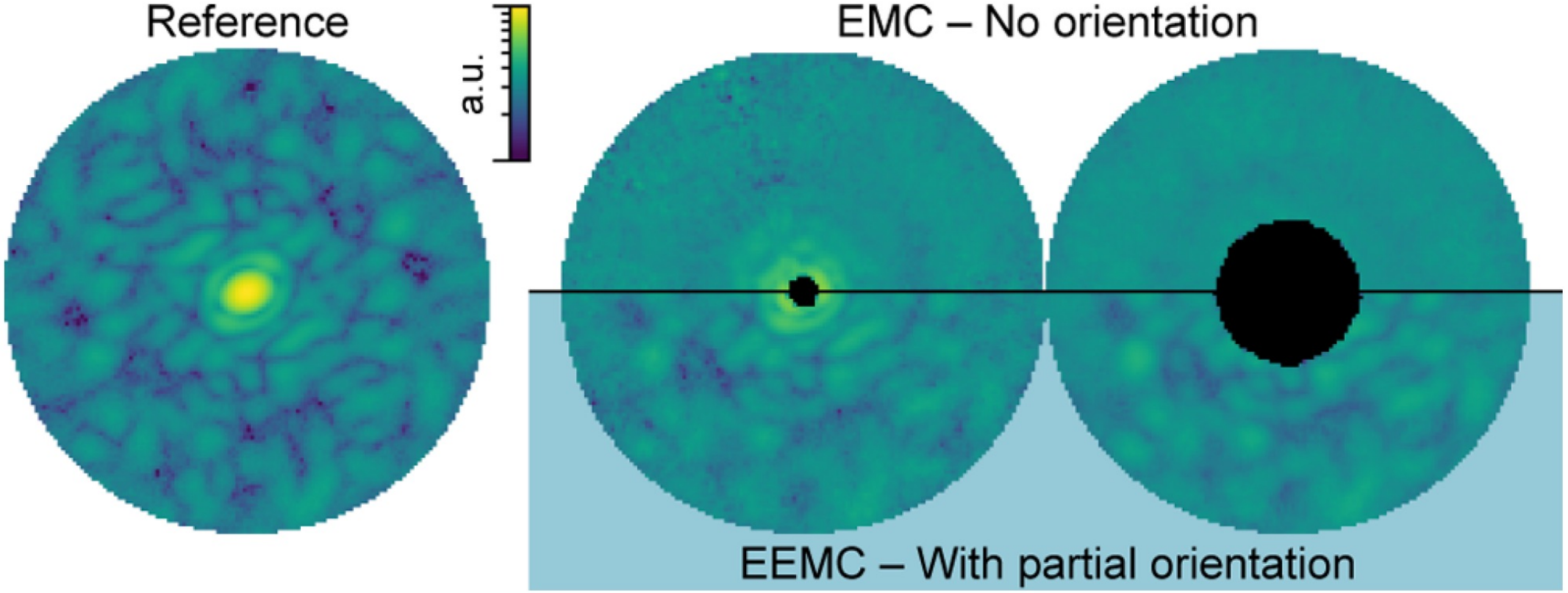
Orientation recovery



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Conclusions

- It is possible to orient a protein with EF
- We found a window of electric field strengths that allows orientation without destroying the protein structure
- There is a substantial benefit of the pre-orientation on the 3D reconstruction

Mass Spectrometry for Single-Particle Imaging of Dipole Oriented Protein Complexes

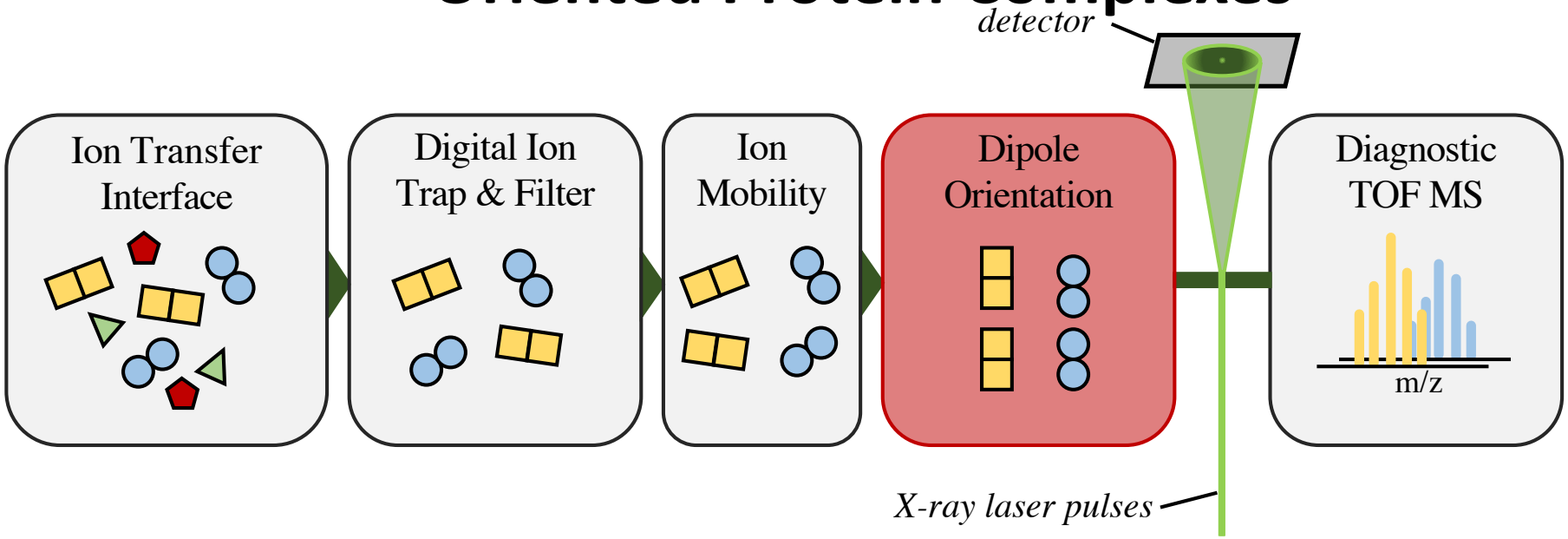

Image taken from M. Brodmerkel half-time PhD thesis

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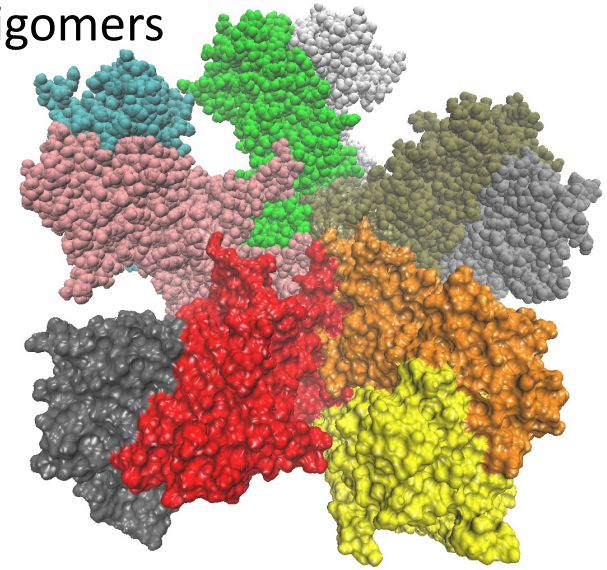
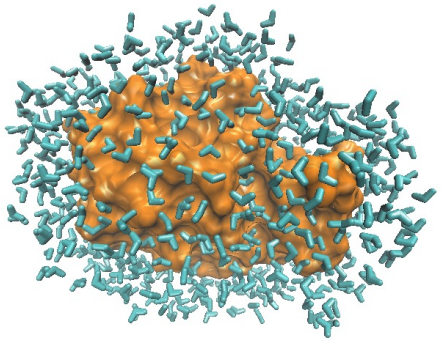
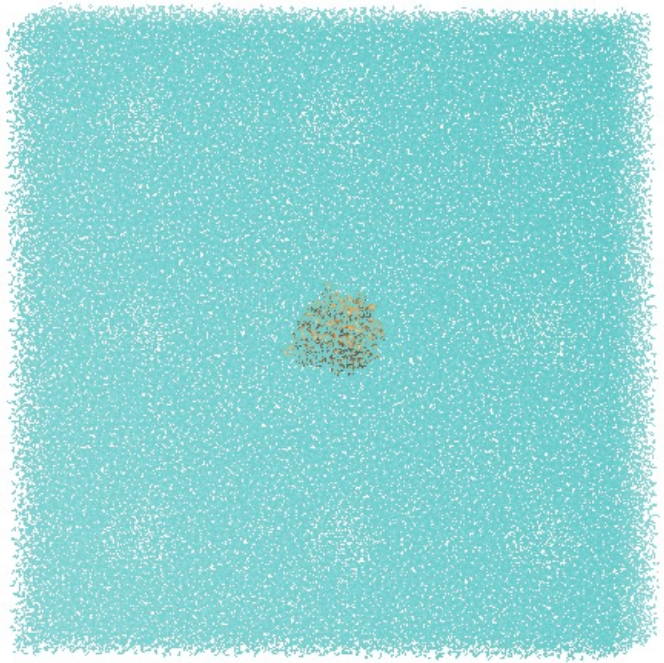
In progress…

Orientation of virus capsid oligomers





Orientation of proteins incapsulated in water layers



Orientation of proteins in solution

Acknowledgments

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-Thomas Mandl
-Harald Agelii

MS SPIDOC

Carl Triggers foundation

Molecular Dynamics & Structure group

Thank you for your kind attention!!!!