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
Single particle imaging (SPI)

- Overcame the need of crystals
- No need for cryogenic cooling
- High radiation damage
- Thousands of diffractions needed
- Random orientation of each sample

Controlling orientation with electric field

Electric field

X-ray beam
Computational approach

**siesta**

*Ab initio* calculations


**GROMACS**

Classical MD simulations


Orientation recovery

\[ F_i,\text{TOT}(E) = F(d_{CM}) + F(d_i) + F_{i,\text{field}}(E) \]

\[ \langle |F(E_j)| \rangle = \frac{1}{N} \sum_{i}^{N} |F_i(E_j)| - |F_i(E = 0)| = \frac{1}{N} \sum_{i}^{N} |F_{i,\text{field}}(E)|. \]

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

<table>
<thead>
<tr>
<th>Covalent bonds (46)</th>
<th>Hydrogen bonds (47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Force (eV/\AA)</td>
</tr>
<tr>
<td>C-N</td>
<td>~2.0</td>
</tr>
<tr>
<td>C-C</td>
<td>~2.2</td>
</tr>
<tr>
<td>C-S</td>
<td>~1.4</td>
</tr>
<tr>
<td>C-O</td>
<td>~2.5</td>
</tr>
<tr>
<td>S-S</td>
<td>~1.3</td>
</tr>
</tbody>
</table>

Force values are computed by dividing the tabulated energies by the tabulated equilibrium bond distances. Hydrogen bonds are indicated by \( \cdots \).
Classical MD simulations

- Bulk simulations.
- 10 structures extraction;
- Water removal;
- Charge assignment.
- 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

Degree of orientation

\[ \Theta = 1 - \cos(\theta) \]
Classical MD simulations

Speed of orientation

Time of orientation

\[ \tau = \frac{\ln(10)}{k} \]

- simulations
- fit: \( f(t) = \exp((-1.44 \pm 0.01)t) \)
Classical MD simulations

RMSD(τ)

!!!!!Orientation before destruction!!!!!!
Classical MD simulations

![Diagram of MD simulation results](image)

- **RMSD** ($t_0=2\text{ns}, E_0=2.5\text{ V/nm}$)
- **θ** ($t_0=2\text{ns}, E_0=2.5\text{ V/nm}$)

- Degree of orientation
- RMSD $\alpha$ [nm]

- E [V/nm]

- Time [ns]

- Ubiquitin$^7$
Orientation recovery

Orientation recovery
Convergence vs iterations
Orientation recovery

Number of patterns

Reference

1000 patterns  3000 patterns  10 000 patterns

EEMC

EMC
Orientation recovery
Detector masking

Reference

EEMC

EMC

1.4 SP
2.8 SP
4.2 SP
5.6 SP
7.0 SP
Orientation recovery
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

X-ray laser pulses

Diagnostic TOF MS

m/z

Ion Transfer Interface

Digital Ion Trap & Filter

Ion Mobility

Dipole Orientation

Image taken from M. Brodmerkel half-time PhD thesis
Orientation of proteins encapsulated in water layers

Orientation of virus capsid oligomers

Orientation of proteins in solution

In progress...
Thank you for your kind attention!!!!

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