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# Field orientation of dipolar proteins in solution

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**Introduction.** Proteins are biological macromolecules that carry out the majority of cellular functions in all living organisms. The importance of proteins and our interest in them can in part be explained by their versatility. A multitude of chemical, physical and geometrical varieties can be created by combining the 20 standard amino acids into sequence-specific polymers, which has occurred naturally for nearly four billion years of evolution and yielded countless different proteins, and more recently through rational or AI-driven design of proteins with new properties and geometries.

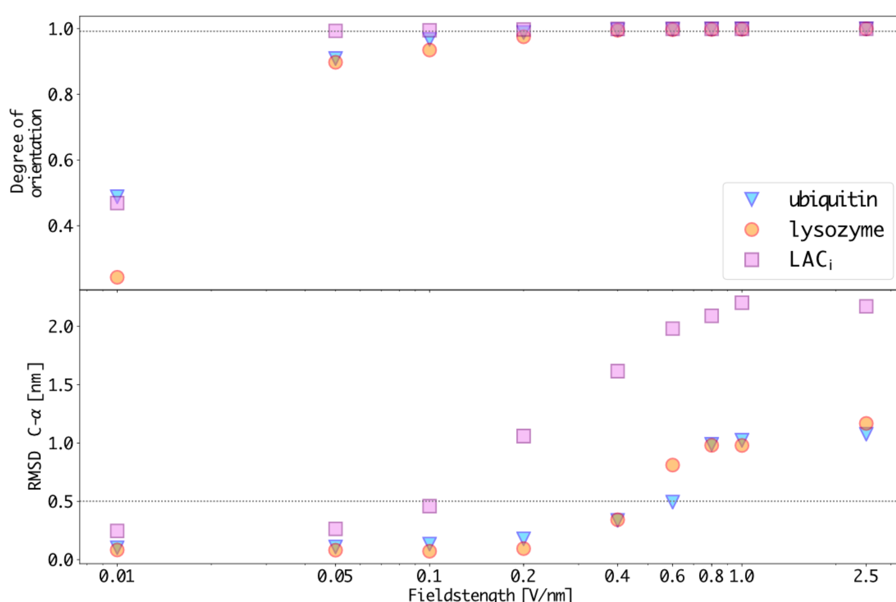


Figure 1. The degree of orientation for three proteins in a range of electric fields (top) and the root-mean-square deviations (RMSDs) of their backbones with respect to their starting structures (bottom).

Numerous techniques have been developed where external forces can be used to separate, probe, or manipulate proteins based on their physical characteristics. We have explored a new avenue to this end, and showed that strong electric fields can be utilised to control the orientation of proteins in the gas phase[1], that is, aerosolized proteins that travel through vacuum or a dilute gas. We have demonstrated how such orientational control has a clear benefit for structure determination using X-ray diffraction. While aerosolization and ionisation bring some advantages, the absence of solvent can be destructive for the protein structures. Therefore, we here aim to explore ***orientation of proteins with an external electric field in***

***solution***. This makes for a much more native-like environment and allows for combination with many common biophysical techniques. Our first application is to probe the structure of protein complexes with small angle X-ray scattering (SAXS), but we imagine that many other applications can exploit this phenomenon. For example, the efficiency of a chromophore depends on its relative angle to the polarisation of incoming light, and thus field orientation offers a means for controlling a protein's optical properties.

**Equilibrium distributions.** We have carried out molecular dynamics (MD) simulations of proteins in solution under the influence of an electric field. We find that the protein structures remain intact unless the field is too strong, while at the same time the field introduces a notable bias to the proteins' orientation (Figure 1). That augurs well for the successful field orientation of proteins, but our simulations are so far limited in time and size, precluding an exhaustive analysis of the interaction between proteins in solution and external electric fields. As such, we will seek ***analytical solutions for the distribution of orientations relative to the field direction***. Proteins in solution, unlike those in the gas phase, readily exchange energy with their surroundings. This allows us to build on a foundation of 150 years of statistical thermodynamics, with well-defined Boltzmann weights based on the interaction energy between the electric dipole of the protein and the electric field. These weights $w'$ can be expressed as $w' = e^{-U/k_BT}$, where $U$ is the potential energy of the electric dipole and the field ($U = \bar{\mu}\bar{E}$), $k_B$ is Boltzmann's constant and $T$ is the temperature. There is however also an angle-dependent degeneracy term that arises because the number orientations decreases as the dipole approaches a parallel or antiparallel arrangement with respect to the field, somewhat similar to the "gimbal lock" for euler rotations. The Boltzmann weights must thus be modulated with the degeneracies $g$, i.e., $w = e^{-gU/k_BT}$, where both $g$ and $U$ depend on the angle $\alpha$ between the field and the dipole vectors. $w$ can be seen as the distribution function of angles at equilibrium at temperature $T$ for a protein with a given dipole $\bar{\mu}$ in an electric field $\bar{E}$, and thus describes the expected distributions of angles in a macroscopic sample in an experiment. In order to predict the orientation of arbitrary proteins of any size (where large protein complexes often are the most interesting, but are also the most demanding to simulate with MD), ***we will use statistical mechanics to express the orientation distribution as a function of fundamental parameters of the proteins (dipole moments) and the experiment (field strength and temperature)***. From these distributions we will derive expectation values and measures of spread in order to identify suitable conditions for future experiments and predict the values of observables.

**Dynamic behaviour.** Maintaining strong fields for extended times in experiments can be challenging, and can in principle prove destructive for the protein structures. As such it is important that we charter the dynamic behaviour of the protein at the onset of a strong field. Here, ***we will numerically simulate the rotational diffusion of a dipolar protein in an electric field***, where we will use the rotational diffusion coefficient of the protein as part of the input, which will be estimated using empirical models or alternatively estimated from atomistic MD simulations. This will give us the characteristic time scale for a protein's orientational response to an applied electric field, which will be important for designing electric field pulse profiles in future experiments.

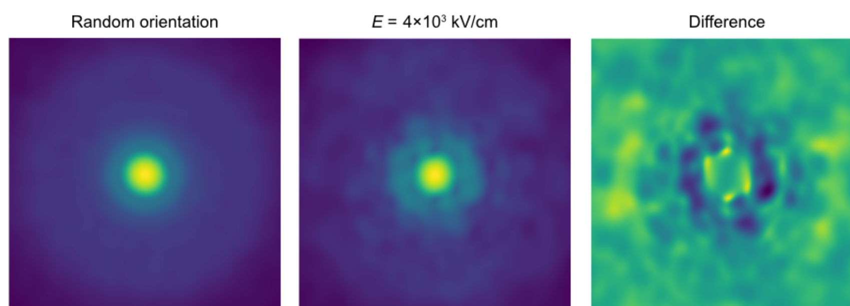


**Figure 2.** Simulated SAXS diffraction for randomly oriented and field oriented proteins.

**SAXS for oriented proteins.** In conventional SAXS, the pattern is isotropic because the particles in the solution are randomly oriented, and the pattern is integrated over all angles to make a one-dimensional (1D) curve with intensity vs distance from the centre of the detector. For oriented particles however, the scattering pattern can be expected to hold more information about the structure of the particle[2], since angle-dependent differences are no longer averaged out, as illustrated in Figure 2. In order to prepare for such experiments we must use the expected distribution of orientations based on the dipole and the field, and calculate how it affects the relevant features of the scattering pattern. The diffraction from a protein is given by the 3D fourier transform of its electron density, and the diffraction from an ensemble of particle is the incoherent addition of the diffraction from all particles. This relates the diffraction patterns to the structures of oriented proteins in solution, enabling us to interpret the former to inform about the latter. We have chosen SAXS because even with poor data we should be able to get the radius of gyration ($R_g$) of the protein from the low-resolution part of the scattering pattern. For perfectly oriented proteins, this means that the intensity in vertical and horizontal direction of the pattern give the $R_g$s along and around the protein's dipole moment. As such, the scattering pattern holds the information about the aspect ratio of the protein even for poor scattering data, giving a worst-case scenario for how well we can probe the structure using this technique.

**A marriage of disciplines.** The project is truly cross-disciplinary, combining biology, physical chemistry and biophysics, which are held together with a mathematical framework of statistical mechanics and Fourier transforms. Using mathematical derivations and numerical simulations, the research will forward the physical chemistry of proteins under external forces, as well as biophysical methods for probing the structures and properties of proteins. The basic and applied life sciences will be able to capitalise on this, which leads to medical advances. The project will be carried out in the environments provided by CIM and the Uppsala Biophysics Network.

# References

(1) Marklund, E. G.; Ekeberg, T.; Moog, M.; Benesch, J. L. P.; Caleman, C. Controlling Protein Orientation in Vacuum Using Electric Fields. *J. Phys. Chem. Lett.* **2017**, *8* (18), 4540–4544. https://doi.org/10.1021/acs.jpclett.7b02005.
(2) Fritscher, C.; Hüsing, N.; Bernstorff, S.; Brandhuber, D.; Koch, T.; Seidler, S.; Lichtenegger, H. C. In Situ SAXS Study on Cationic and Non-Ionic Surfactant Liquid Crystals Using Synchrotron Radiation. *J. Synchrotron Radiat.* **2005**, *12* (6), 717–720.

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