Method for electrostatically aligning proteins in solution

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Introduction:
Determining how proteins change shape (move/conform) is a new, active area of research. Presently we use terahertz time-domain spectroscopy (THz-TDS) to measure the vibrations of proteins and the water molecules that surround them. However, we need to separate these two signals in order to study the proteins’ motions. Water molecules are distributed around proteins nearly uniformly, so if we could align the proteins in one direction, we could distinguish their contribution from that of the water. Essentially, alignment creates a contrast which we can use to separate the signals!

We can align proteins in two ways.
1) **Protein crystals** are regular, repeated arrangements of proteins, which naturally keep the proteins aligned. Optical measurements on such crystals first definitively measured protein vibrations. While many proteins can be crystallized, this extra step in sample preparation can be time consuming and difficult. Furthermore, the forces in a crystal constrain the natural motions of proteins, causing them to deviate from the free motion of proteins in cells.
2) **Electrostatic alignment** allows us to exploit the strong net electric dipole moment that most proteins naturally possess to align proteins in solution. Similar to a compass in a magnetic field, proteins with a strong net dipole moment will align themselves along the direction of an electric field, which we can provide. The surrounding water molecules will not align in any particular fashion, and we can distinguish the protein’s signal with very low noise.

Methods:
We align proteins using a microfluidic cell fabricated in UB’s clean room facility. The designs for this cell were first described by Deepu George in his recent doctoral thesis; we have made slight modifications, but the core of the design remains the same and is shown in figure 2. Fabricating this cell is a multi-step process, involving:
1) Making the electrode pattern using Shipley 1818 photoresist (a UV-light sensitive chemical)
2) Evaporating Al (aluminum) onto the patterned wafer
3) Removing excess Al through a lift-off technique
4) Applying a thin coating of polyamide to protect the electrodes from electrochemical reactions
5) Applying the thicker SU-8 photoresist and patterning the side-wall structure
6) Attaching the top plate and drilling inlet/outlet holes.

After filling the cell with our protein solution we apply a high-frequency (225kHz) AC electric field to the electrodes; alignment is almost instantaneous. We can then conduct our measurements.

Impact:
Protein motions are related to their function in biological systems. Therefore, a better understanding of these motions could lead to more effective treatments for a wide range of medical conditions, as well as a better understanding of how our bodies work at the cellular level.

Characterization of the Cell:
In order to rigorously show the usefulness of this device, we must determine:

1) **That the cell achieves alignment**: Proteins are too small to see through standard microscopes; we want to be able to see alignment occur when we apply a bias to our cell. Dielectric rods are ideal for this, but they must be less than 100 micrometers long in order to fit between the electrodes. As they cannot be purchased this small, our rods were made in-house using the photoresist SU-8, and after fine-tuning some size/bias parameters we witnessed alignment of these rods in our cell (see figure 1.)

2) **The cell’s optical sensitivity to alignment of standard systems**: Liquid crystals have well-known optical properties and have previously been aligned using fluid cells with large (~20nm) electrode spacing. As such we decided to attempt alignment of the liquid crystal E7 with our prototype cell; initial data is shown in figure 3. The phase and absorption shifts apparent when we change the orientation of E7 is in line with known measurements of E7’s optical anisotropy.

Results and Continuing Work:
We have demonstrated the alignment capabilities of this cell with SU-8 micros, as shown in figure 1b. We discovered that a polyamide coating is a required feature for the cell, as without it the electrodes rapidly destroy themselves in an electrochemical reaction. We have also found that a polarization effect is introduced when the aluminum electrodes are thicker than about 500 Angstroms; we must either fabricate cells with this parameter in mind, or take this effect into consideration during data analysis. Our recent study of the liquid crystal E7 makes us confident that our cell is more than capable of aligning proteins in solution. We now intend to study the protein lysozyme in solution, as its conformational modes (motions) are well known and useful for comparison.

References:

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