ELECTROKINETICALLY ACTUATED PROTEIN CRYSTALLIZATION

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ABSTRACT
Currently, x-ray crystallography is the primary experimental tool employed to understand how proteins fold and assemble into well-defined structures. These diffraction methods critically depend on the ability to produce high-purity crystal samples, but conventional crystallization processes are inherently slow and challenging to control. Here we explore a fundamentally different electrokinetically-actuated approach to overcome these limitations. Instead of the conventional method involving increasing protein concentration throughout the entire bulk volume using evaporation, we employ electrokinetics to increase protein concentration to achieve supersaturated conditions favorable for crystallization within a very small localized zone inside a microchannel network.

KEYWORDS: Protein Crystallization, Electrokinetics, Electrophoresis

INTRODUCTION
Proteins and protein-based biomarkers are playing an increasingly critical role in screening and detection of disease, identification of therapeutic targets, and monitoring of treatment efficacy. But the ability to elucidate molecular-level information is hindered by the slow trial-and-error nature of conventional characterization approaches. X-ray diffraction is the most widely used experimental tool capable of providing these molecular-scale structural details. Diffraction methods critically depend on the ability to produce high-purity crystal samples for analysis. But current crystallization approaches, such as vapor diffusion, suffer from a number of drawbacks including the time required to run a series of trials (days to weeks) and a limited supply of often expensive reagents. These factors combine to create an incredibly inefficient methodology that is highly empirical and requires hundreds or thousands of trials in order to zero-in on optimal conditions.

Microfluidic methods have proven to be a key enabling technology to address some of these barriers by increasing throughput via massive parallelization and by eliminating the need to harvest and manipulate the crystals via in-situ x-ray diffraction analysis [1]. But in the majority of these formats, the fundamental mechanism by which the crystallization process is executed remains identical to the macroscale, with the exception that the size of the “container” encapsulating the crystallization solution is greatly reduced. Timescales of hours to days are generally required.

THEORY
Protein crystallization generally starts from a protein solution of suitably high concentration to which reagents that reduce protein solubility (precipitants) are added. Once the solubility limit of the protein is exceeded, the solution becomes supersaturated and metastable, and crystals may start to grow given favorable conditions. Conventional methods for protein crystallization, such as vapor diffusion, increase protein concentration throughout the entire bulk volume and it takes days to weeks to reach the desired protein concentration.

Instead of increasing protein concentration throughout the bulk, we would like to determine if microfabricated electrode arrays can be used to increase protein concentration to extremely high levels within a very thin layer (< 5 µm) at the electrode surface. In this way, supersaturated conditions favorable for crystallization can be quickly established within this enriched region. Our approach employs arrays of individually addressable microfabricated electrodes positioned along the floor of a microchannel with the ability to sequentially concentrate a protein sample and focus it into a narrow zone (Fig. 1). This technique transports charged biomolecules between active electrodes upon application of a small potential difference, and is capable of achieving orders of magnitude concentration increases within a small device footprint [2,3]. After injecting a biomolecule-laden sample into a microchannel, the concentration process begins by applying a 1-2 V DC potential between a pair of electrodes in the array. The biomolecules (protein) present in solution between electrodes, being intrinsically polyanionic, migrate toward the appropriate electrode in response to the electric field and become trapped or ‘captured’ there.

EXPERIMENTAL
We demonstrated the feasibility of this approach using hen egg white lysozyme as a model protein (Fig. 2). Experiments were performed using a commercial crystallization kit to prepare a protein solution in a crystallization buffer containing 30% w / v polyethylene glycol monomethyl ether 5,000, 1.0 M sodium chloride, 50 mM sodium acetate trihydrate pH 4.5. A freshly prepared solution was injected into a microfluidic channel with gold electrodes and the loading ports were sealed to prevent evaporation. A 2 V potential was then applied between electrodes in the array. Within 1 min, formation of a dense halo of compacted protein around the outer perimeter of the cathode was observed. The voltage was then switched off to release the compacted protein zone back into the bulk solution.
Figure 1. Microelectrode arrays enable capture and label-free detection of charged biomolecules. (a) Microdevice design incorporating a glass microchannel bonded on top of a microelectrode array on a silicon substrate. (b) Compaction of fluorescently labeled DNA at the electrode under a low 1–2 V potential. (c) The same arrangement enables detection of unlabeled DNA under white light due to high surface reflectivity. (d–g) The surface reflectivity can be reversibly switched on, off, and moved between neighboring electrodes. Similar reflectivity is observed with proteins like lysozyme at (h) pH 8 and (i) pH 11; and (j) PSA at pH 7. (k) Small molecule cyclodextran HS-β-CD: Heptakis (6-O-sulfo)-β-cyclodextran (MW = 1,849 Da).

Scale: electrodes are 50 μm wide horizontally.

Figure 2. Preliminary results demonstrating feasibility of forming lysozyme single crystals by electrokinetic actuation. Within one minute after application of a 2 V potential between electrodes, a dense ultra-compacted protein layer becomes evident surrounding the capture electrode. The densely packed proteins within this “halo” are then released when the voltage is switched off, and the resulting densely compacted front returns to equilibrium ionic conditions while remaining in a supersaturated state for nucleation of crystallization. Large single crystals are formed within 60–90 min. (bars = 50 μm).
RESULTS AND DISCUSSION
In the absence of the electric field, large single protein crystals appeared within 60–90 min. A parallel negative control experiment in which the electric field was not applied did not produce crystals after several days. These results can be explained by considering the interplay between electrophoretic transport of the protein macromolecules and small-molecule ionic species present in the solution. When the potential is applied, the small-molecule species are likely to exhibit a higher mobility, resulting in rapid establishment of a dense cloud around the electrodes. In this stage, crystallization is suppressed due to this non-equilibrium ionic environment and/or conformational changes in the protein near the electrode surface. When the potential is switched off, the compacted protein halo begins to disperse and re-enters more favorable ionic conditions. The protein concentration within this front remains high enough to lie within the supersaturated regime, enabling it to act as a trigger for crystallization, thereby explaining the formation of crystals at some distance away from the cathode. The electric field is not applied during the crystallization process itself, it only serves as an initial pre-concentration step.

CONCLUSION
This instrumentation has the potential to reduce the timescales required for protein crystal formation by several orders of magnitude while simultaneously expanding the range of conditions that can be screened via x-ray structural analysis.

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REFERENCES

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