Electro-assisted crystallization cell for biological macromolecules

Patent Application: **MX/a/2015/011982**

Status: patent pending

Abstract: The present invention relates to the field of biomacromolecular crystallography, methods and devices used for the nucleation and growth of crystals of biological macromolecules. Unlike existing devices in the prior art, the electro-assisted crystallization cell described in this invention has a different conception, since said crystallization cell, is a novel design based on plastic material (polyethylene, PET).

Background

The stages in molecule crystallization are mainly three: nucleation, growth of the crystal and end of growth. The ideal scenario would involve controlling and separating all these phenomena to obtain just a few nuclei that generate crystal of the desired sizes. During years, control of the nucleation of macromolecular biological systems has been a bottleneck in biological and biomedical research; for example, even the production of larger crystals for structural X-ray studies has been complicated.

There exist several techniques that allow to produce crystals through conventional and nonconventional methods. In this sense, nonconventional methods employing electric or magnetic fields have opened new routes in the study of the growth behavior of crystals of biological macromolecules. Every cell up to the introduction of this invention has employed Platinum wire electrodes that may unfortunately electrolyze water. The first cell that used electro assisted crystallization in which electric or magnetic fields were used was published in 2004; however, it was until 2011 that the use of transparent ITO electrodes was introduced to avoid any issues with water electrolysis. One issue with the technique was however, the separation of crystalline samples from the interior of the cell for X-Ray structural studies. In 2013 an alternative to this technique was published for the crystallization of proteins on vapor-phase hanging droplets. The fundamental difference between 2013's cell and the previous ones was that the former uses glass mounted ITO electrodes.

ITO, in contrast with glass is an inorganic material which is hard, fragile and sometimes transparent. This material contains silica and sodium or calcium carbonate in a tertiary composition with indium, Tin and Oxygen in variable proportions. Its character as a ceramic material or as an alloy depends on the oxygen content. ITO is transparent in thin films and is one of the most used transparent conducing oxides due to its electrical conductivity and its optical properties. ITO has been widely used for the deposition on transparent screens or displays such as Liquid Crystal Displays (LCDs), Organic Light Emitting Diodes (OLEDs), solar cells, etc.

Description

The invention describes a cell for electro-assisted crystallization manufactured with **Indium Tin Oxide (ITO)** electrodes mounted over plastic (**ITO over polyethylene**). These electrodes were employed in the nucleation and growth of crystals of macromolecules such as proteins, nucleic acids polysaccharides and macromolecular complexes of combinations of these structures. With the proposed cell in this invention, the nucleation process and the crystal growth (from these biological macromolecules) may be **controlled**.

Stage of research

The research group has described the manufacturing process for the cell and tested the methodology with positive results, obtaining good crystals of some proteins such as lysozyme.

Application market

The market and the industry concerning this invention is broad and varied such as the agricultural and food industry, the cosmetic industry, the pharmaceutical industry, ceramics and even art. Some examples are:

- Target drug design
- Protein/drug crystallization
- Crystallization of compounds for small molecules
- Crystallization kinetics
- Manufacturing of intelligent materials
- Food preserving
- Cosmetics
- Construction materials
- Superlight alloys
- Crystallization devices
- Diffraction experiments

Advantages

The electro-assisted crystallization cell presented in this invention is manufactured with ITO electrodes mounted on plastic (polyethylene) and presents several advantages compared to its predecessor:

1. It allows the configuration of the crystallization of biological macromolecules that proceeds inside the cell with hanging-droplet methods within an electric field. This electro-assisted process occurs *in situ*.
2. It facilitates the separation of the obtained crystals by cutting a small window on the opposite side from where the crystallization occurs. The crystals can be easily recovered by cutting the cell with a conventional blade.
3. In cases where biological macromolecules contain too much structural water, or in cases where the sample are very sensible upon extraction from the cell, ITO electrodes allow to collect X-Ray crystals *in situ* because they are transparent to this radiation.
4. This cell design allows to have an influence on the crystallization process, controlling the growth of crystals, overcoming the difficult task of separating the nucleation and crystal growth processes. With this technique, it is possible to obtain just a few nuclei with adequate dimensions, and thus solving deficiencies of existing technologies.
5. The cell allows advantageously to observe the experiments with a conventional microscope and even record the experiments in real time.
6. The electro-assisted crystallization cell can be employed at various temperatures.
7. Its configuration may be modified for batch crystallization.

Thanks to these advantages it is possible to perform high resolution structural experiments without extracting the sample from the cell where it was grown.

To summarize, this invention allows:

- ✓ *In-situ* crystallization
- ✓ Easy recovery of the crystals
- ✓ Controlling the crystallization technique
- ✓ Observing crystals with conventional microscope
- ✓ Wide variety of temperatures

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