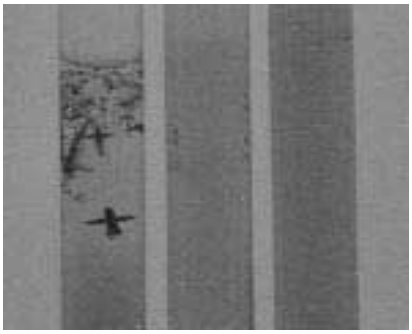
s8b.m5.p1 **Direct Visualization of an Impurity Depletion Zone**. A. A. Chernov[a], J. M. Garcia-Ruiz[b] and B. R. Thomas[a,c]. [a]*Universities Space Research Association, 4950 Corporate Dr., Suite 100, Huntsville, AL 35806,alex.chernov@msfc.nasa.gov* [b]*University of Granada, Instituto Andaluz de Ciencias de la Tierra, Laobratorio de Estuidios Cristalgrafico, Facultad de Ciencias, Fuentenueva s/n, 18002, Granada, Spain ,jmgruiz@goliat.ugr.es* [c]*Center for Microgravity Materials Research, University of Alabama in Huntsville, Huntsville, AL 35899. E-mail:bill.thomas@msfc.nasa.gov*
Keywords: methods crystallography, genesis, perfection of biomacromolecular.

When a crystal incorporates more impurity per unit of its volume than the impurity concentration in solution, the solution in vicinity of the growing crystal is depleted with respect to the impurity [1,2]. With a stagnant solution, e. g. in microgravity or gels, an impurity depletion zone expands as the crystal grows and results in greater purity in most of the outer portion of the crystal than in the core. Crystallization in gel provides an opportunity to mimic microgravity conditions and visualize the impurity depletion zone. Colorless, transparent apoferritin monomer (M ≅ 450 KDa) crystals were grown in the presence of red holoferritin dimer as a microheterogeneous impurity (M ≅ 900 KDa) within agarose gel by counterdiffusion with Cd^{2+} precipitant. Preferential trapping of dimers, (distribution coefficient $K = 4$ [1,2]) results in weaker red color around the monomer crystals grown in the left tube in the figure as compared to the control middle tube without monomer crystals. The left and the middle tubes contain colored ferritin dimers, the right tube contains colored trimers. The meniscus in the left tube separates the gel (below) and liquid solution containing Cd^{2+} (above). A similar arrangement, though without crystallizing monomer, is present in the middle and right tubes allowing diffusion of dimers and trimers. The area of weaker color intensity around crystals directly demonstrates overlapped impurity depletion zones.



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s8b.m5.p2 **Crystallization screening with electro phoretic gels.** J.M. García-Ruiz[1], A. Hernández-Hernández[1], J. López-Jaramillo[1], B. Thomas[2]. *1. Laboratorio de Estudios Cristalográficos, IACT-CSIC-Univ. Granada, Facultad de Ciencias (Campus Fuentenueva), 18002 Granada, Spain. 2.Center for Microgravity and Materials Research institute, Room D29, Univ. of Alabama, Huntsville, AL. 35806, U.S.A.*
Keywords: protein-crystallization, electrophoresis, gels.

Electrophoresis is the method currently used to separate biological macromolecules (nucleic acids and proteins) by molecular size, electric charge, and other physical properties[1]. In practical terms, the molecules under the effect of an electrical current, are forced to migrate across a layer of gel which works as a molecular sieve. The separated macromolecules form a series of bands which are perpendicular to the direction of the electric field. Two types of gels are currently used in electrophoresis: a) agarose, a linear polysaccharide extracted from sea weed that form thermal gels characterized by a very large pore size and therefore are used primarily to separate large molecules with a molecular mass greater than 200KDa and, b) polyacrylamide, a chemical gel which pore size can precisely controlled within a wide range. On the other hand, it is know that gels can be used as crystallization media not only for small molecules but also for large biological macromolecules[2,3,4].

Therefore, we envisaged a method to use the gel layers and cast resulting from an electrophoretic run straightforward as crystallization "reactors". We tested the above idea with: a) Two different gels, polyacrylamide and agarose, b) Two different electrophoresis techniques, native electrophoresis and isoelectric focusing, c) Three different proteins, lysozyme, thaumatin and ferritin, and d) Two different crystallization methods, direct mixing and counter-diffusion method.

We demonstrate that the gels resulting from different types of electrophoresis can be directly used for the screening of crystallization conditions of new proteins. The use of this method drastically reduces the waste of protein in further screening as they use a material (electrophoretic gels) that is currently trashed after their use in the previous and unavoidable process of purification. In addition, for those protein which have a strong tendency to denaturalize or to aggregate, this method is an advantage because the protein is crystallized just after its separation. In short, the corollary of this work is: don't trash your gels from electrophoresis, use them to search for the right crystallization conditions.

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