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Magnetically controlled gravity for protein crystal growth

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Magnetically controlled gravity for protein crystal growth

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On earth, crystal growth from a supersaturated solution is accompanied by buoyancy-driven convection in the liquid, an effect often detrimental to crystal quality. For protein crystals a high quality is required for x-ray structure determination at high resolution, which is of great biotechnological and pharmacological importance. In order to avoid the adverse effects of convection, much effort has been put in examining the virtues of space-based microgravity for protein crystal growth. However, whether zero gravity is the ideal growth condition is still an open question.

It has been shown that gradient magnetic fields can influence convective flows in paramagnetic fluids and to apply the same approach to seemingly nonmagnetic proteins is appealing, since in fact all diamagnetic materials can be magnetically levitated. A number of experiments have been performed on protein crystal growth under levitation conditions. The criterion for damping convection during crystal growth is, however, quite different from that for levitation, because buoyancy-driven convection is suppressed in microgravity. Here the authors demonstrate that magnetic fields can be used to tune the effective gravity from 1 to −0.15 g during the growth of diamagnetic lysozyme crystals and that convection can be damped, stopped, and even reversed. The growth velocity is strongly reduced in simulated microgravity. This method provides a versatile and accessible way to realize an earth-based tunable gravity environment for crystal growth in which protein crystal quality may be optimized. © 2007 American Institute of Physics.

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camera. Variations in the concentration of the fluid result in local differences in the refractive index, which appear as intensity variations in the image. The sensitivity to concentration differences scales with the degree of being out of focus.\textsuperscript{15}

We used HEWL from Sigma-Aldrich (Lot No. 094K1454), which was dissolved and dialysed against a 0.05\textit{M} NaOAc/HOAc buffer solution of pH 4.5 at room temperature before use. Tetragonal lysozyme crystals were grown from a solution of 30 mg/ml HEWL, 0.685\textit{M} NaCl, and 0.05\textit{M} NaOAc/HOAc at pH 4.5 and 18 °C. Crystals were taken from the growth vessel and placed as a seed in the glass cuvette for the magnet experiments. The crystal was manipulated to be in the field of view of the insert, after which the solution was removed and the cuvette was placed in a refrigerator at 4 °C for 20 min. As a result, the crystal is attached to the glass wall of the cuvette. Finally, the cuvette was refilled with the same solution as used during growth and placed in the insert for experiments.

The condition for convection damping is determined by \( \alpha \) and \( \beta \) in Eq. (1). For HEWL \( \alpha \) is 0.303 kg m\textsuperscript{-3}/mg ml\textsuperscript{-1},\textsuperscript{16} and we have determined \( \beta \) to be \((-1.2\pm0.5) \times 10\textsuperscript{-9} \) ml/mg using a magnetic susceptibility balance. Inserting these values in Eq. (1) we expect that convection is damped at \( B_zB_z' \)

\( =-3100\pm1500 \text{T}^2/\text{m} \), which is significantly larger than the \(-1500 \text{T}^2/\text{m} \) needed for simple levitation of the bulk solution.\textsuperscript{9} We determined the actual gradient field at which the growth plume disappears by using shadowgraphy. Figure 1(e) shows a growing HEWL crystal at zero field gradient, and the convection plume is clearly visible as a white streak rising upward from the crystal. In the picture the crystal itself is blurred because for shadowgraphy out-of-focus images have to be taken. The growth plume disappears, and thus convection is suppressed at a gradient magnetic field of \(-4450\pm30 \text{T}^2/\text{m} \) [Fig. 1(g)]. The value falls within our estimate using \( \alpha \) and \( \beta \), but is much higher than previously expected,\textsuperscript{5,15} and requires the largest magnets currently available. In fact, this value for the gradient field accurately determines \( \beta \) as \((-0.84\pm0.06) \times 10\textsuperscript{-9} \) ml/mg.

This result unambiguously shows that gradient magnetic fields can create conditions on earth that mimic those in space-based microgravity. Most importantly, however, is the fact that by changing the magnetic field strength the effective gravity for convection can be continuously varied. If we define

\[
G_{\text{eff}} = 1 - \frac{\beta}{\alpha \mu_0 g B_zB_z'},
\]

\( G_{\text{eff}} \) is expressed in terms of the earth’s gravitational acceleration \( g \). By varying the magnetic field we are able to

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change $G_{\text{eff}}$ and as a result the convection is tuned from normal, with a growth plume upwards [Figs. 1(e) and 1(f)], via cancellation at $G_{\text{eff}}=0$ [Fig. 1(g)], to inverted with the growth plume downwards for negative values of $G_{\text{eff}}$ [Figs. 1(h) and 1(i)].

The range of field gradients at which convection is stopped is quite small, $\pm30$ T$^2$/m centered around $-4450$ T$^2$/m, which corresponds to $B=27$ T in the magnet we used. Decreasing (increasing) the magnetic field by only 0.1 T ($G_{\text{eff}}=0.005$) already results in appreciable convection and thus in upward (downward) growth plumes. This strong effect is caused by the steep dependence of the balance between convective flow and mass diffusion on $G_{\text{eff}}$, which is reflected by the thickness of the depletion zone. For example, Fig. 1(d) shows the theoretically calculated, and for NiSO$_4$ ·6H$_2$O experimentally demonstrated, dependence of the thickness of the depletion zone $d$ on gravity. Since $\Delta d \propto |G_{\text{eff}}|^{-1/4}$ it diverges near zero, which implies that the field gradient has to be set quite precisely. Such a strong dependence also puts constraints on the spatial variation of $G_{\text{eff}}$ within a magnet. From Eq. (2) we calculate $G_{\text{eff}}$ as function of the position around the crystal using the experimental field profile [inset of Fig. 2(b)], which shows that changes of $G_{\text{eff}}$ over the relevant region are within $\pm0.005$. Despite the precise condition on the required field gradient, milligravity rather than microgravity is sufficient to make convective transport slower than that due to diffusion and to dampen convection.

To show that the suppression of convection indeed affects crystal growth, we have measured the growth rate of two lysozyme crystals, one at $G_{\text{eff}}=1$ and one at 0, otherwise identical conditions (Fig. 3). Here the same imaging setup was used, but now with the crystal in focus to determine the position of its surface. The growth rate drops a factor of 15 from $30 \pm 2$ to $2 \pm 2$ $\mu$m/h when convection is stopped and the depletion zone is expanded, which is similar to results obtained under space-based microgravity.

In contrast with other methods to suppress convection such as gel growth and microfluidics, gradient magnetic fields offer a powerful way to tune the effective gravity during crystal growth under earth-based conditions, with far easier access, availability, and including in situ observation. Especially for protein crystal growth this possibility is very attractive, since the tunability will allow the optimization of the crystal quality by finding the right balance between mass transport towards the crystal and the incorporation rate of molecules at the crystal surface. The required gradient magnetic fields for suppression of convection are found to be in the 4000–5000 T$^2$/m range. Because density and susceptibility are closely related, we expect that this value is rather similar for most diamagnetic proteins. We foresee that our determination of the proper conditions for which convection is suppressed will trigger the design and construction of dedicated magnets that are capable of sustaining high field gradients for the several days that are needed to grow protein crystals.

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