Novel Vapour Diffusion Method

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We present a new method for the crystallization of biological macromolecules, combining advantages of the vapour diffusion method with advantages of the micro-batch method.

The classic vapour-diffusion, either in its hanging-drop or sitting-drop configuration is still the preferred method by many crystallographers. This is mainly due to its inherent dynamics and the final endpoint of the diffusion. The new method uses a combination of two oils. As in the micro batch method, the total system is protected from evaporation by paraffin oil (first phase). However, the protein/precipitant mixture (fourth phase) is able to equilibrate with a reservoir solution (third phase) via a second layer of oil (second phase), present under the upper layer of paraffin oil. The second oil is not miscible either with the paraffin oil, nor does it interact and influence the properties of both phases three and four. Procedures for identifying suitable oils for the second phase and their influence on the crystallization dynamics are presented.

The system can be set-up in several ways. In one approach, the protein/precipitant and the reservoir can be applied under the paraffin oil, resulting in a classic micro-batch experiment. Successively, vapour diffusion can be turned on by the application of the second oil, allowing the diffusion of water molecules from the protein droplet to the reservoir solution. Since the system is directly protected from evaporation by the paraffin oil, smaller volumes of protein solution can be applied without any hassle. A suitable micro-plate for the novel method is presented.

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