

AGAROSE, THE GEL TO TAILOR YOUR PROTEIN CRYSTALS

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Abstract: The growth of protein crystals in gel has proved to date to be the cheapest means to produce protein crystals of high quality similar to those obtained under microgravity conditions (Gavira et al., 2020; Robert & Lefauchaux, 1988; Snell & Helliwell, 2005). Gels create a stable environment for crystals to grow in convection-free conditions avoiding sedimentation and the formation of aggregates and increasing crystals uniformity. The use of agarose has allowed progress in the limitations of crystal size and quality and even to obtain protein crystals inaccessible by other techniques (Sica et al., 1994).

In this work we have exploited the nucleation inducing ability of agarose gels in diffusion-dominated environments. Crystal size was successfully tuned in a wide range of agarose, protein and precipitant concentrations. The impact of gel content on crystal size resulted to be independent of the specific protein, allowing the mathematical prediction of crystals size and pointing out the exclusivity of the physical interactions between the gel and the protein to explain the observed behaviour. The versatility of the technique and the fine-tuning of the nucleation flux was demonstrated by crystallizing five different model proteins using two different techniques, batch and counter-diffusion. In addition, the potential of agarose to be used as a growth and delivery medium for serial crystallography applications has been proven by preparing unidimensional micro-crystals slurries in 0.1 % (w/v) gel.

Key words: protein crystallization, gel, nucleation, batch, counter-diffusion.

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