Protein crystallization in hydrogels, current status and future prospect

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Although crystallization in hydrogels is not a frequent practice in bio-crystallography, the benefits of using a polymeric media for the crystallization of macromolecules are multiple **-** i.e. prevents convection and crystal sedimentation, acts as impurity filter, etc. - and have been proven to produce crystals of higher quality (1). The used of hydrogels also avoids the manipulation of the crystals and therefore the osmotic and mechanical stress exerted on the crystals during handling preserving the integrity of the macromolecule crystals even when exposed to organic solvents.

There are many types of hydrogels (agarose, silica, PEGs base hydrogel, sephadex, etc) that are compatible with protein crystallization. Among them, agarose is probably the most widely tested and can be used at concentrations below its critical gelling point, thus facilitating its mix with the protein solution. Hydrogel gel media should also be taken into account not only for improving crystal quality but also to exert control over the nucleation and growth processes. Even more, gel-grown protein crystals have been recently shown to be excellent candidates to produce crystals of bigger size for neutron diffraction studies using crystals grown in agarose as seeds (2) or even be used as media for the continuous delivery of nano/micro-metre size crystals needed for serial femtosecond crystallography. It is therefore expected that protein crystals grown *in situ* in hydrogel will be used in XFEL experiments to minimize sample consumption (3). In this work we will revise the current tends in the use of hydrogels focusing on our most recent results obtained with dipeptides-based hydrogels for the production of high quality protein crystal (4) and the formation of new polymorphs (5). The preservation of crystal integrity will be explained on the basis of the incorporation of the gel matrix within the crystals. The amount of incorporated hydrogel is quantified using thermo-gravimetrical analysis (TGA) of silica grown crystals.

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