# Growth of Protein Seed Crystals with High-Strength Hydrogels

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X-ray protein crystallography is a valuable tool for gaining the three-dimensional (3D) structure of proteins at the atomic level. The 3D structures of proteins offer important information for the structure-function relationship and the structure-based drug design. However, X-ray crystallographers rarely use hydrogen atoms and hydration water molecules to obtain an in-depth understanding of the protein 3D structure. Neutron crystallography provides insights into protein structure protonation details. One important purpose of neutron macromolecule crystallography is to obtain extremely large protein crystals to obtain an analyzable diffraction pattern from the available neutron beam. So far, investigators have developed several growth techniques for large protein crystals, including macroseeding, the slow-cooling method, the floating-and-stirring technique, the large-scale hanging drop method, and top-seeded solution growth. However, several obstacles remain with these methods. For example, with the macroseeding technique that is often employed to grow protein single crystals, we must pay scrupulous attention when handling the seed crystal. A seed crystal is introduced into a pre-equilibrated protein solution, and this cycle is repeated several times. However, it is often difficult to use a seed crystal because protein crystals are usually very small and fragile. To overcome this difficulty, we used hydrogels [1]. We previously developed a new method for growing protein crystals in high-strength hydrogels [2, 3]. Our study demonstrated that the high-strength hydrogels increase the mechanical stability of the protein crystals while considerably reducing osmotic shock, in part because incorporating hydrogel fibers into the crystal during growth significantly strengthens the crystal. Here we report the novel combinational technique of seeding and reinforcing hydrogel-grown protein crystals for neutron crystallography [4].

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