A team of researchers from the University of Brussels, the Eindhoven University of Technology, Structural Biology Unit CIC bioGUNE of Bizkaia and the Institute des Sciences de la Terre in Grenoble, have uncovered the molecular details of polymorph selection of macromolecule crystals.

Since long crystals have been essential for solving the three-dimensional structures of proteins, which is crucial for understanding the molecular mechanisms of life itself. In more recent times these crystals are also used as pharmaceutical delivery agents, being insulin the best-known example: the injection of a suspension of insulin microcrystals will slowly dissolve and ensure a steady supply over time to the system. Unfortunately, crystallizing macromolecules can be excruciatingly difficult. This in part is because we still do not understand how a crystal is "born". Any crystal will grow from a crystalline seed, i.e. nucleus, which forms by the spontaneous grouping of molecules in solution that adopt a regular structure in three-dimensions. How molecules realize this feat has remained largely a mystery. To add some more complexity, a single molecule system can also organize itself in different structures, called polymorphs. These polymorphs usually have different physical properties, which can have a profound effect on the final characteristics of the formed material. For example ice and cacao crystal polymorphs will dictate the quality of our much-loved ice cream and chocolate. In the case of protein crystals, polymorphs are important because they will have, among others, different diffraction capacities (crucial for structure determination) and dissolution rates (important for drug delivery). At present, controlling the crystallization process to obtain the polymorph of choice is still challenging because the mechanism underlying polymorph selection are still unclear.

By using cryo-transmission electron microscopy this interdisciplinary team managed to catch the "birth" of protein crystals with molecular resolution and uncovered a hierarchical self-assembly process that involves three subsequent stages of self-assembly at ever increasing length scales. This unexpected complexity for the nucleation pathway proved to be far more intricate than what was previously assumed. These observations are the first in their kind and provide a new way to gain information on the self-assembly processes of macromolecules into larger structures. But, the team went one step further, and mapped the nucleation pathways of multiple polymorphs. They showed that polymorph selection is dictated by the architecture of the smallest possible fragments formed at early time-points. Once such structures are formed, the faith of the system is decided. By analyzing and understanding the differences in structure of the various nuclei, the authors developed strategies to guide the polymorph selection process. This was achieved by tuning, through mutagenesis, the different modes of interaction that exist between the molecules, and thus "pushing" the outcome of the nucleation process in one direction or another.

In a nutshell, these findings greatly advance our fundamental understanding of nucleation and polymorph selection. These insights are not only relevant for macromolecules but can also be translated to other substances that form crystals (such as pharmaceutical compounds). Moreover, the experimental methodology develop for this studies opens a new avenue to follow protein self-assembly processes that are implicated in a range of pathological disorders, such as liquid-liquid phase separation in eye cataract formation or the formation of amyloid fibers associated with a range of neurological disorders.