Invited Lecture

Protein nucleation kinetics and polymorphic formation: the practical consequences of external influencers

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Macromolecular crystallization occurs in a complex scenario in which several variables such as the solution pH, the precipitant nature, the presence of additives or the temperature, among others, play an essential role. After finding the right crystallization conditions, efforts are focused either on controlling crystal quality to get the best possible diffraction data for the 3D structural models determination, or to control nucleation and crystal size in order to meet pharma and/or industrial applications requirements. In this sense, hydrogel media have been used to mimic on earth the reduced convective flow regime occurring in microgravity environments, often improving crystal quality [1], but also playing a key role in controlling nucleation and crystal growth [2]. The use of hydrogels has also allowed the observation of unexpected polymorphs co-existing with the most favoured ones [3], a behaviour also observed in experiments where nucleation was induced by *ad-hoc* modified surfaces [4].

The formation of polymorphs is an ubiquitous phenomenon dictated by different molecular interactions/assemblies during nucleation. The formation, persistence and observation of each particular polymorph depends on the combined effect of crystallization kinetics and thermodynamics [5]. Therefore, a molecule -a protein in this particular case - can generate multiple distinct assemblies during the nucleation process selecting the outcomes but that allows the existence of other possibilities although observable only under particular conditions.

In this talk we will summarize our findings on the role of hydrogels to control protein nucleation and will revise three examples for the obtention of new unexpected polymorphs supporting the hypothesis of their presence as subcritical aggregates during the nucleation step. Their prevalence and co-existence with more regular polymorphs point to very slight energetic differences and demonstrate the difficulty to predict the precipitation behaviour in systems in which weak and ultra-weak interactions may play a significant role [6].

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