A PERSPECTIVE ON COLD PROCESSING OF PROTEINS
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Temperature is one of the most critical variables influencing the stability of biological systems and therefore a major concern for the production and commercialization of biopharmaceutical products. The general observation and practical rule is that refrigeration (including freezing) extends significantly the preservation of biological products.

Decreasing temperature is generally expected to decrease degradation kinetics, however, the complexity of proteins and the multiple phenomena that are triggered by cooling, often brings unanticipated results. For example, when a solution is freezing, the growth of the ice crystals causes all the solutes concentrate, typically by one or two orders of magnitude, potentiating interactions such as crystallization or protein aggregation. Buffer salts may precipitate, causing pH shifts, osmotic pressure and ionic force increase, protein molecules unfold (by cold), ice interfacial area extends, oxygen and other gases saturate forming air interfaces (bubbles) and dehydration is generalized. The mechanistic contribution of each of these variables cannot be clearly deconvoluted and it is also not easily correlated with product quality because of essentially three factors: (1) the stochastic nature of phase nucleation (either ice or dissolved substances), (2) the spatial anisotropy that is generated by freezing (on local composition, interfacial area, etc.), (3) and the amplification of the previous by limited (or deficient) process control (e.g. heat transfer). In general this setting contributes to inconsistent thermal history from container to container (which can be bags, vials, bottles or large steel vessels). This is somewhat tolerable when molecules are robust, but can trigger frustrating hurdles for sensitive proteins. In this work we approach the “cold” challenge by identifying cornerstones related to the preservation of protein molecules and by selecting some examples that can provide a mechanistic interpretation for the contribution of important process variables.