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# *Clarifying the role of carbohydrate and other additives in the preservation of protein during lyophilization: A new approach combining simulations and experiments*

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Freeze-drying, or lyophilization, is the most commonly used technique for the preservation of protein-based therapeutics. However, both the freezing and drying phases may result in loss of biological activity, and an appropriate formulation should be used to minimize undesired damage. Sugars, polyols and amino acids are generally employed as protectants, while buffers and surfactants are often added to control the pH, or prevent aggregation and surface-induced denaturation, respectively [1].

Several mechanisms have been proposed to explain the stabilizing action of these excipients, but knowledge of molecular-scale phenomena involved in cryo/lyoprotection is currently lacking, and the formulation design is mostly driven by previous experience. In the present work, a different approach will be proposed for the selection of formulation components, based on the application of all-atom molecular dynamics (MD) simulations.

The role of cryo- and lyoprotectants will be here addressed using this technique, for human growth homone (hGH) and lactate dehydrogenase (LDH) as model proteins (Fig. 1). The *in-silico* analysis suggests that differences exist among excipients, with the disaccharides being better than polyols, monosaccharides and amino acids both during freezing and in the dried state. Some molecular properties can also be identified, that correlate with the protective effect of stabilizers. For instance, a correlation is found to exist between the molecular volume of the excipient, and its degree of exclusion from the protein surface, which is at the basis of thermodynamic stabilization during freezing [2]. In this regard, it will also be shown that the protective effect of excipients may be related to their preferential exclusion from specific patches on the protein surface.

At the same time, a high hydrogen bonding propensity, resulting in the formation of a compact matrix, seems to be linked to the efficiency as a lyoprotectant [3]. The effect of common buffer species is also investigated, suggesting that pH control is not the only effect that buffers may have on protein stability. The role of surfactants will finally be studied, and simulation results will be presented that indicate an orientation-dependent mechanism of protein stabilization.

Overall, molecular dynamics will be shown to provide useful information about the molecular mechanisms of biopreservation, which are often not accessible by experimental techniques. However, due to the limited time and length scales accessible by molecular dynamics, experimental tests remain essential. A new approach is proposed, where simulations and experiments are conceived as complementary tools, in a joint effort to increase our knowledge about protein stability.

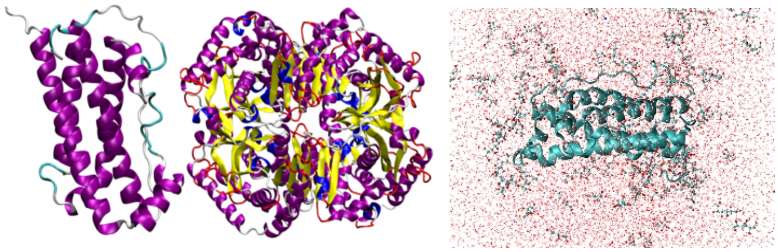

**Fig. 1** *Cartoon structures of hGH (left), LDH (center) and a typical MD simulation box (right).*

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2. Arakawa T, Timasheff SN, Stabilization of protein structure by sugars, Biochemistry 21 (1982) 6536-6544.
3. Arsiccio A, Pisano R, Water entrapment and structure ordering as protection mechanisms for protein structural preservation, J. Chem. Phys. 148 (2018) 055102.