Freeze Drying of Therapeutic Proteins: A Simulation Approach to Optimize Formulation and Process Conditions

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Protein-based pharmaceuticals are playing an increasingly important role in the treatment of a wide number of human diseases. This occurs because they are highly effective and have fewer side effects compared to other therapeutics. For instance, the global sales of biopharmaceutical medicines were as high as US$ 237 thousand million in 2018 and are estimated to be valued at US$ 389 thousand million in 2024. However, a problem which is intrinsic to proteins is their high instability, and their tendency to lose therapeutic activity. The development of technologies capable of preserving their three-dimensional structure, and therefore biological potency, is therefore of utmost importance.

In this context, freeze-drying, or lyophilization, is a commonly used method for preparing solid protein-based pharmaceuticals. Despite this, both the freezing and the drying steps may result in undesired stresses for the active ingredient, and therefore potential loss of activity. In the worst-case scenario, an immune response may even be generated, which may result in serious consequences for the patient. For this reason, a suitable formulation and a well-designed process should be selected to minimize denaturation phenomena.

However, the choice of the formulation is at present mainly based on experience, lacking real knowledge of the molecular-scale phenomena involved. Also, the selection of optimal process conditions is often extremely time-consuming and non-systematic, resulting in poor control strategy. The present work aims to address these two problems, using an *in silico* approach.

After a brief introduction, where the state of the art and objectives of the work are discussed, the use of molecular dynamics to clarify the molecular mechanisms of protein stabilization and guide the choice of excipients will be investigated. The outline of a typical simulation is illustrated in Fig. 1.

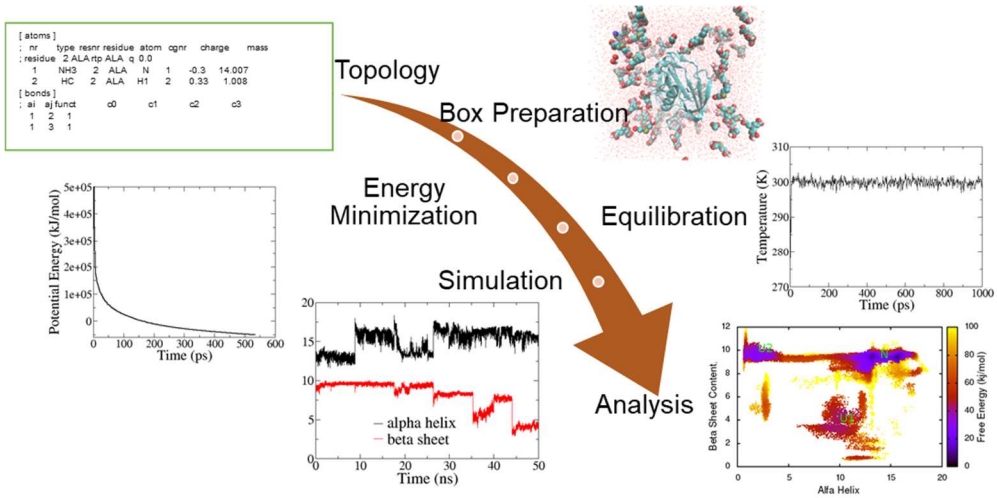

Fig. 1: *Outline of a typical molecular dynamics simulation.*

Attention will be first focused on commonly used cryo- and lyoprotectants, with the aim to provide a better understanding of their stabilizing action and identify molecular properties responsible for their effectiveness. The effect of buffers in common pharmaceutical formulations, and their role in modifying protein-excipients interaction, will also be addressed.

Ample coverage will then be given to the role of surfaces and surfactants, employing in this case also advanced molecular dynamics techniques, such as umbrella sampling and metadynamics. The mechanisms of protein denaturation induced by the air-, ice- and silica-water surfaces will be studied, and a possible explanation for the role of surfactants in these phenomena will be proposed (see Fig. 2).

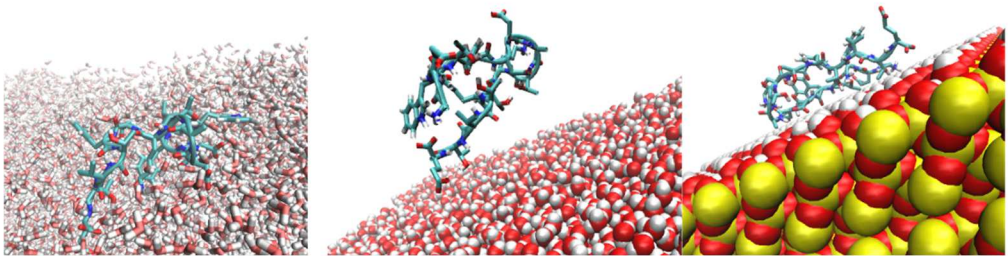

Fig. 2: *A model peptide adsorbed at the air-water (left), ice-water (center), or silica-water (right) interface.*

In particular, an orientation-based mechanism of stabilization will be observed, where the protein is stabilized when surrounded by the hydrophilic heads of the surfactant. Attention will be particularly focused on the ice-water surface, because it plays a central role in the freezing step of the lyophilization process. It will be shown how the ice interface may perturb the secondary and tertiary structure of a protein by enhancing the mechanisms of cold denaturation.

Since the extent of the ice-water interface seems to be of utmost importance for the protein stability, some experimental work will also be presented, where the effect of the freezing protocol on protein stability is studied. Both conventional and controlled nucleation will be considered, for the case of human growth hormone and factor VIII as model proteins. It will be shown that the use of controlled nucleation is beneficial for cycle efficiency, product homogeneity and reconstitution time. However, the effect on protein stability strongly depends on the system being considered.

Finally, a modelling approach based on energy and mass balance equations will be proposed to build the design space for the freezing process. The design space is a tool in the framework of the Quality by Design (QbD) concept that can be used to guide the selection of optimal operating conditions. The freezing step of freeze drying is considered in this Thesis because it determines product morphology, and therefore has an impact on cycle duration, thermal stress for the product being dried and protein activity (see Fig. 3). It will be shown that it is possible to predict the ice crystal size obtained as a result of freezing, as well as its effects on primary drying and protein stability. The proposed approach will be tested upon experimental data, and it will be demonstrated that it could help to improve both cycle efficiency and product quality.

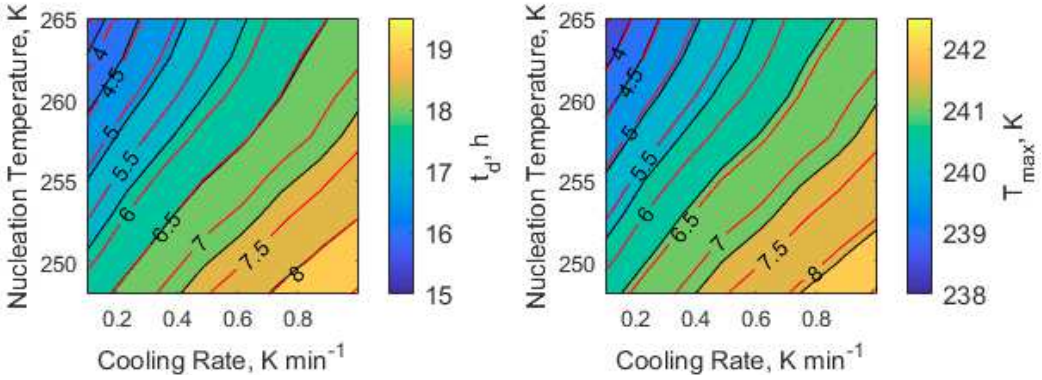

Fig. 3: *Design space showing the effect of freezing conditions (cooling rate and nucleation temperature) on drying time (t_d) and maximum product temperature during drying (T_{max}).*

Overall, the whole work has the aim to prove that modelling can help lyoprofessionals in the selection of optimal freeze-drying conditions for protein-based therapeutics. It will be shown that simulations and experiments provide information at different levels. A model needs experimental data to be tuned and validated, but it can afterwards output information that cannot easily, or quickly, be accessed by current experimental techniques. Simulations and experiments are therefore not alternative, but complementary. The combination of these approaches may therefore be extremely beneficial, boosting up both process and formulation development.