ABSTRACT

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Thesis title:

Molecular Dynamics Simulations to Enhance Stability in Biopharmaceutical Formulations - A Focus on Cyclodextrins and Interfaces

Summary and brief description of the obtained results

In recent years, the interest towards biopharmaceutical products has increased, posing new challenges in formulation and product design, development and manufacturing. While biopharmaceutical products present many appealing advantages, their production requires a higher standard of quality and process control. In fact, these substances (among which proteins) may be subject to a variety of stressing conditions throughout the manufacturing process and subsequent storage, possibly leading to their degradation and/or loss of biological activity. These harmful and undesired phenomena may have different environmental causes, such as high and/or low temperatures, pH shifts, presence of interfaces, viscous stresses, presence of destabilizing solutes, chemical reactions and microbial activity.

Since it is not always possible to eliminate such environmental stresses and/or to rule out the onset of any harmful phenomena, it is therefore necessary to design and develop an appropriate formulation. However, formulation design and development are complex and time-consuming processes, as they usually require extensive experimental campaigns performed in different conditions, with a variety of stabilizers. To speed up this crucial stage of product's development, it is possible to rely on the aid of computational techniques. In fact, it is possible to simulate formulations at the molecular scale, enabling a deeper and, possibly, unprecedented understanding of the molecular phenomena affecting biopharmaceutical products' stability. Among the above mentioned computational techniques, molecular dynamics (MD) allows to simulate molecular systems.

Cyclodextrins (CDs) are cyclic oligosaccharides, made up by several repeating units, which can be functionalized to confer them new and, potentially improved, physicochemical, pharmacological and toxicological properties. CDs can also

interact and form complexes with various chemical compounds or moieties, thanks to their hydrophobic cavity. These properties make CDs appealing excipient candidates, thus they were selected to be investigated in a variety of physicochemical conditions, as well as in different types of formulations.

Initially, the impact of different CDs on the stability of the granulocyte colony-stimulating factor (GCSF) was investigated both at the air-water and at ice-water interfaces, revealing a crucial role played by the functionalization of the CDs. Specifically, the stabilizing action exerted by 2-hydroxypropyl-β-cyclodextrin (HPβCD) at the air-water interface on GCSF was attributed to its functional group, as its native counterpart was not able to contain the conformational changes caused by this environment.

Then, a physically accurate and realistic model for HPβCD was developed, in order to investigate its interactions with amino acids. MD simulations revealed a preferential mechanism of interaction involving the hydrophobic cavity of the CDs and the peptide backbone of the amino acids.

Successively, the properties of HPβCD were also investigated in a co-solvent formulation, namely in combination with tert-butanol (TBA), and compared with other common excipients such as mannitol and sucrose, in presence of a model protein, i.e., myoglobin. The detrimental effect of TBA as a co-solvent for myoglobin's formulations was attributed to its accumulation in proximity of the protein, negating the stabilizing effect of the excipients.

Lastly, since explicit MD simulations of biopharmaceutical formulations often imply considerable computational costs, a fast and simple approach to predict the effect of cosolutes/excipients on proteins' stability was developed. Such predictions were obtained by using inexpensive thermodynamics- and statistical mechanics-based models, combined with MD trajectories, drastically reducing the computational costs. These predictions were hence tested against the results of enhanced sampling simulations, revealing the strengths and the shortcomings of this framework.