Prediction of protein adsorption isotherms

Molecular dynamics simulations to predict protein adsorption isotherms

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In Short

- Characterization of protein adsorption on Q Sepharose FF
- Determination of the parameters of the Steric Mass Action isotherm with MD simulations
- Atomistic resolution molecular dynamics (MD) simulations for a deeper understanding of the protein adsorbent-interaction
- Free energy calculation for the determining of the equilibrium constants of protein adsorption

A growing part of the pharmaceutical products are biomolecules. In 2021 about 31.4 % of the revenue in the pharmaceutical industry were made with biomolecules [1]. A majority of these biomolecules were proteins for example vaccines or hormones [2]. The down streaming processing takes up to 80 % of the product cost. Therefore, the enhancement of these processes has a lot of potential. The most used purification process type is ion-exchange chromatography. In our project, the adsorption of proteins on such media will be investigated.

This project is a close collaboration of the Institute of Thermals Separation Processes and the Institute of Process Systems Engineering at the Hamburg University of Technology (TUHH). The project is funded by the 'Deutsche Forschungsgemeinschaft' (DFG, Project-ID: 461481804) [3]. The main goal of this project is the characterization of protein adsorption and the prediction of protein isotherms with molecular dynamics (MD) simulations. The MD simulations will be performed in the Institute of Thermal Separation Processes, whereas experimental investigations will be conducted in the Institute of Process Systems Engineering. This project is a continuation of work previously done within both involved groups [4]. A close collaboration between experimental and simulative work will be ensured since the involved research assistant will be involved in both fields.

The MD simulations will be performed for a deeper understanding of the interactions between proteins with the surrounding media and the adsorbent. Recently, we have published a proof of concept study to show how to calculate protein adsorption isotherms with MD simulations. This was done with a coarse-grained force field and one conclusion was that simulations on the atomistic scale would be beneficial. All atom models contain a large number of interacting atoms and as such parallel calculations are required.

Within this project the parameters for the Steric Mass Action Model [5] will be determined, which is a physically reasoned protein adsorption model. Two parameters can be determined with relatively low effort [5,6]. However, to calculate the third parameter, the equilibrium constant, computationally demanding free energy calculations are needed. Such equilibrium constants have been successfully determined by our group in a previous study [4].

The target of this project is not only to determine adsorption isotherms but also to get a deep understanding of the influences on protein adsorption, which will help to design more effective and therefore more cost-efficient processes. Especially, the influence of the adsorbent is not fully understood to this point.

Even though the ion-exchange chromatography is a standard in pharmaceutical protein downstream processing, a deep understanding of the process has not been reached up to now. For the process design numerous experiments have to be carried out and the demand of ion-exchange media is large to ensure effective separation.

As a model system for our investigations we will use bovine hemoglobin and bovine serum albumin. These proteins are widely used for protein adsorption investigations and differ significantly in their adsorption behavior, while having nearly the same size and are therefore ideal for the research of different adsorption effects. Q Sepharose FF will be used as the model adsorbent. It has an highly linked agarose matrix with a quaternary amine group as ligand. It is a strong anion exchanger, which is widely used in the research and in the industry. Recently, we have developed a method [4], which is able to determine a whole protein adsorption isotherm with MD simulations. As a starting point for the method we use the Steric Mass Action (SMA) isotherm [5]

$$c_p = \frac{q_p}{K} \cdot \left(\frac{c_s}{\Lambda - (\sigma + \nu_p) \cdot q_p} \right)^{\nu_p}$$

with c_p and c_s being the protein and the salt concentration in the bulk phase.

q_p stands for the protein concentration on the adsorbent, Λ for the total ionic capacity of the adsorbent, ν for the ligands which are in contact with the protein and σ the ligands which are blocked (sterically hindered) by one protein to interact with another protein. The total ionic capacity has to be

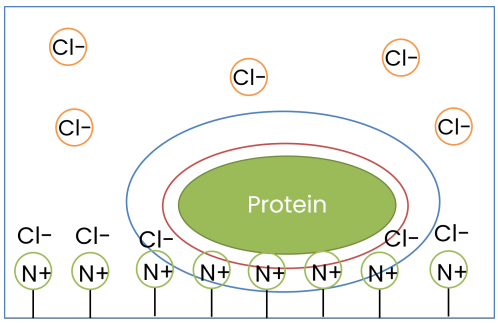

Figure 1: *Visualization of the methods used for the determination of the number of ligands in contact with the protein in red and the number of ligands that are sterically hindered in blue. In both cases ligands with a certain distance to the protein are considered in contact with the protein (red bubble) or sterically hindered by the protein to interact with another protein (blue bubble). The difference between the methods is the defined distance to the protein.*

determined experimentally, whereas ν and σ can be determined by the distance to the protein. Fig. 1 shows the adsorbed protein on the adsorbent represented by the ligands at the bottom. The red bubble serves as a visualization of the determination of the ligands which are in contact with the protein. Any ligand which is touched by the red bubble is considered in contact with the protein. Similar, the blue bubble serves as the visualization for the sterically hindered ligands.

The main difficulty in this approach however, is the determination of K, which is the equilibrium constant of the salt ions and the proteins. It is possible to calculate the equilibrium constant for the proteins through free energy calculations [4]. A proof of concept has already been done and has shown reasonable results for a protein adsorption isotherm [4]. However, it was concluded that the used coarse-grained force field lacks the desired accuracy and that the sampling of protein orientation was not sufficient. In this project we will use all-atom force fields and a new technique to ensure sufficient sampling. Therefore, a large number of individual MD simulations are needed, whereby each has about 216 thousand atoms, this is only possible with the high performance computing at the NHR.

In the current project a new free energy approach has been developed to describe the orientation of the protein on the adsorbent surface in the adsorbed state. It has been shown that the protein is more likely to bind in orientations in which it has more contact with the adsorbent surface. The project also includes experimental work which is needed for the determination of the total ionic capacity. Furthermore, different influences on the protein adsorption are investigated for example the influence of different salts, pH-value, temperature and salt concentration. Moreover, experimental data were obtained, which is needed for the validation and refinement of the methods.

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https://www.tuhh.de/tuhh-thermische-verfahrenstechnik

More Information

[1] T. Daubenfeld, B. Geis, L. Gros *Chemie Ingenieur Technik* **93**, 1-9 (2021). doi: 10.1002/cite.202100047

[2] R.A. Rader, *BioProcess International* **11**, 7 (2013).

[3] DFG - Entwicklung und experimentelle Validierung einer molekulardynamischen Simulationsmethode zur Vorausberechnung von Adsorptionsisothermen von Proteinen https://gepris.dfg.de/gepris/projekt/461481804

[4] S. Jakobtorweihen, J. Heuer, T. Waluga, *J. Chromatography A* **1620**, 460940 (2020). doi:10.1016/j.chroma.2020.460940

[5] C.A. Brooks, S.M. Cramer, *AIChE Journal* **38**, 1969-1978 (1992). doi:10.1002/aic.690381212

[6] J. Liang, G. Fieg, F.J. Keil, S. Jakobtorweihen, *Industrial Engineering Chemistry Research* **51**, 16049-16058 (2012). doi:10.1021/ie301407b

[7] J. Liang, G. Fieg, S. Jakobtorweihen, *Industrial Engineering Chemistry Research* **54**, 2794-2802 (2015). doi:10.1021/ie504374x

Project Partners

Institute of Process Systems Engineering, http://www.tuhh.de/psi

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