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Orientation of monoclonal antibodies in ion-exchange chromatography: A predictive quantitative structure–activity relationship modeling approach

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ABSTRACT

Chromatographic separation of biopharmaceuticals in general and monoclonal antibodies (mAbs) specifically is the bottleneck in terms of cost and throughput in preparative purification. Still, generalized platform processes are used, neglecting molecule specific characteristics, defining protein-resin interaction terms. Currently used *in silico* modeling approaches do not consider the orientation of the molecule towards the chromatographic resins as a result of the structural features on an atomic level. This paper describes a quantitative structure–activity relationship (QSAR) approach to model the orientation of mAbs on ion exchange chromatographic matrices as a function of property distribution and mobile phase characteristics. 6 mAbs were used to build a predictive QSAR model and to investigate the preferred binding orientations and resulting surface shielding on resins. Thereby different dominating orientations, caused by composition of F_{ab} fragments of the mAbs, could be identified. The presented methodology is suitable to gain extended insight in molecule orientation on chromatographic resins and to tailor purification strategies based on molecule structure.

1. Introduction

Ion-exchange chromatography is a major unit operation in biomolecule separation processes, with anion and cation-exchange chromatography used in monoclonal antibody (mAb) purification. mAb purification processes are often based on platform processes, with the promise of less development efforts, therefore reducing the time-to-market and early stage material consumption [1]. Nevertheless, standardized processes compromise on efficiency as they restrict the design space and rely on unchanged molecule properties within the application space. With the growing number of mAb in development for biopharmaceutical and bioanalytical applications, optimized and scalable processes, which work outside the box of predefined process sequences and materials, are needed [2]. This can be achieved by the use of *in silico* approaches in process development. The application of semi-empirical models to describe chromatographic separation spread wide in the last decades due to increasing fundamental understanding of process mechanistics and adsorption mechanisms. Today, a variety of modeling techniques can be applied to describe the influence of different parameters on ion-exchange chromatography, including mobile-phase composition, resin types, and protein characteristics to different extent [3–5]. These models require the determination of protein and adsorbent specific parameters, and therefore can not be applied to predict the behavior of new molecules *ab initio*. Physical adsorption is affected by multiple factors as van der Waals forces, electrostatic interaction, or hydrophobic effects, which are defined by molecular properties, chromatographic material and mobile phase composition as pH and ionic strength. Due to their size and structure, mAbs show different characteristics for their subunits. The isoelectric point (IEP), which defines the pH value at which the net charge of the molecule is zero, is generally higher for F_{ab} fragments than for the complete antibody. In consequence, the IEP of F_c fragments is smaller, compared to the full molecule [6–8]. At the IEP, the F_{ab} fragments will therefore carry a positive charge, while the F_c fragment is negatively charged.

The dipole moment of antibodies was found to point from the F_c to the $(F_{ab})_2$ fragments which leads to an "end-on"/F_c binding orientation on positively charged surfaces. A "head-on"/F_{ab} orientation was observed for negatively charged surfaces. Further, at

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low electrostatic forces, where van der Waals interactions dominate, mAb showed a lying flat orientation on surfaces [9]. A shift in electrostatic potential of a mAb can therefore alter the binding orientation on surfaces as chromatographic resins. For example, a pH change from pH 4 to 8 was shown to change the preferred binding orientation of IgG from a "head-on" to an "end-on" orientation [10]. Binding orientation is of interest in development of assays, which use passive binding of IgG onto hydrophobic surfaces as used in enzyme-linked immunosorbent assays (ELISA). Here, an "end-on"/F_c binding would increase capture efficiency. In antibody purification, binding orientation defines key parameter as affinity coefficients and ligand shielding, as lying flat orientations block more space on an adsorbent surface. A higher variance in affinity is expected for mAb binding in a "head-on"/F_{ab} orientation, compared to an interaction with the F_c fragment.

The orientation of molecules on charged surfaces is subject of investigation via different approaches, balancing the need of information with complexity of the simulation and corresponding computational efforts, which are presented by the size of IgG.

Monte Carlo simulations were used to describe protein-surface interactions in various studies for smaller molecules with varying degree of model abstraction [11,12]. Juffer et al. used explicit atom simulations to improve orientation matching for model proteins [13]. The united-residue model, in which each amino acid is represented as a sphere centered at the α-carbon position, allowed for analysis of the effect of amino acid composition on antibody orientation [14,9].

Brownian dynamics simulation methods or molecular dynamic (MD) simulations, representing single atoms in the molecular structure and with mobile phase described as continuous dielectric media were established for small molecules, such as peptides [15]. This approach was used to investigate interaction energies and orientation of biomolecules in cation-exchange chromatography by Dismer et al. [16–18]. They used an atomistic representation of molecule and the interacting surface including bound ligands. This approach was later extended to complex proteins (i.e. including non-standard residues) and anion-exchange chromatography by Lang et al. [19,20].

Due to molecule size and an exponential increase in computational cost with number of atoms in simulation, this approach is still prohibitive for application to mAbs in all-atom representation and corresponding adsorber surface. A recently introduced approach to QSAR descriptor calculation, capturing steric hindrance in protein-surface interaction as well as electrostatic potential shielding by the mobile phase showed good predictivity regarding mobile phase properties and molecule structures [21]. This approach is extended to describe the binding orientation of mAb on ion-exchange chromatographic resins.

2. Materials and methods

Monolconal antibody structures and samples were provided by Lonza Biologics PLC. To provide a diverse data source for model generation further model proteins were included in lab and *in silico* experiments.

2.1. Structure preparation

Model proteins were selected based on isoelectric point to ensure binding on cation-exchange resin at chosen conditions and availability of structural information. Corresponding RCSB entries were selected based on most complete structure and high resolution. Table 1 lists the chosen proteins, corresponding UniProt IDs and PDB IDs for structural information used for descriptor calculation.

Table 1
Proteins used in QSAR model generation and evaluation with their corresponding UniProt ID, PDB ID, and theoretical pIs as calculated according to Bjellqvist et al. [27,28] selected for descriptor calculation.

Name, origin	UNIProt ID	PDB	pI
Lysozyme C, hen egg	P00698	1LYZ	9.32
Chymotrypsinogen A, bovine	P00766	2CGA	3.52
α-chymotrypsin, bovine	P00766	1YPH	6.09
Cytochrome C, equine	P00004	1HRC	9.59
Cytochrome C, bovine	P00125	2B4Z	6.50
Ribonuclease A, bovine	P61823	1FS3	8.64
Phospholipase A2, bovine	P00593	1BP2	6.10
Myoglobin, equine	P68082	2V1F	7.36

Structure preparation was done with the simulation software YASARA [22], which is capable of parameterizing non-standard residues (e.g. bound calcium or a heme-group) via the integrated AutoSmiles algorithm [23–25].

Loaded structures were cleaned from water and substitutes, a simulation box around the molecular structure was defined, and the corresponding pH value was set. The structure was protonated according to set pH value.

To correct the covalent geometry, an energy minimization was performed with the AMBER99 force field [26], using a 7.86 Å cut-off. After an initial steepest descent minimization, a simulated annealing simulation was performed to obtain an optimized structure. Partial charges of molecule atoms were derived based on the AMBER99 force field and saved along with molecule information in pqr file format, which captures atomic charges and radii information.

2.2. Descriptor calculation

To represent the orientation of a biomolecule towards an adsorbent surface, three types of descriptors were calculated.

Projection plane. To capture the shielding of electrostatic potential and steric hindrance, 120 theoretical planes were positioned around the molecule in an equidistant manner with a set distance of 5 Å towards the molecule surface. The plane-molecule distance was selected according to [18,19,21]. Electrostatic potential was then projected onto the plane. Descriptor values were calculated based on plane values.

Surface patch. To capture orientation sensitive interaction potential of heterogeneous molecules and short range hydrophic interactions, surface patches were calculated for hydrophobic and electrostatic properties. Surface patch sizes and orientation were calculated according to [21].

From the 120 different orientations calculated, the orientation with the highest electrostatic potential density as projected on a plane was selected to be included in descriptor calculation. Further, the orientation with highest hydrophobic potential based on a solvent accessible surface area (SASA) patch were included in QSAR model calculation, as an alternative orientation driven by hydrophobic interactions for high ionic strength conditions of the mobile phase.

Full surface. The complete surface of the molecule was considered in descriptor calculation for a non-orientation sensitive approach for electrostatic and hydrophobic properties.

The descriptors calculated for the three different projection types and for hydrophobicity and electrostatic potential are listed in Table 2. In total, 120 different descriptors were considered per experimental condition.

A detailed description of electrostatic potential and hydrophobicity property calculation and their projection towards molecule surface and projection planes including applied shielding factors is given in [21].