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Biomimetic ligands for IgG purification

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Objectives

While antibody-based biotherapeutics can already be produced at the g/L scale, purification processes still require optimization and improvement towards less costly, more specific and selective methods. Affinity chromatography based on Protein A from *Staphylococcus aureus* (SpA) is still the most widespread and accepted methodology for monoclonal antibodies (mAbs) purification. However, the cost of biological receptors, their stability and adsorbent leakage raised concerns on their standard application. *de novo* rational designed ligands based on the triazine-scaffold, namely the artificial protein A biomimetic ligand 22/8 has been explored over the last 15 years for the purification of several biomolecules and its recognition and binding mechanism was computationally rationalized here.

Methodology

Molecular docking protocols allied to molecular dynamics simulations are important tools to predict accurately ligand poses and to evaluate the main interactions with their target proteins. Then, a blind docking, covering entirely each IgG fragment, was setup around the receptor's centre of mass using the AutoDock 4.2 docking tool package. Docking results were compared with structural-based knowledge, as well as validated through the use of the non-binding ligand 0/0 to human IgG, as a negative control. Independent MD simulations were carried out by using the GROMACS 4.5 software, running in parallel on the Sun Grid Engine (SGE) high performance computer cluster.

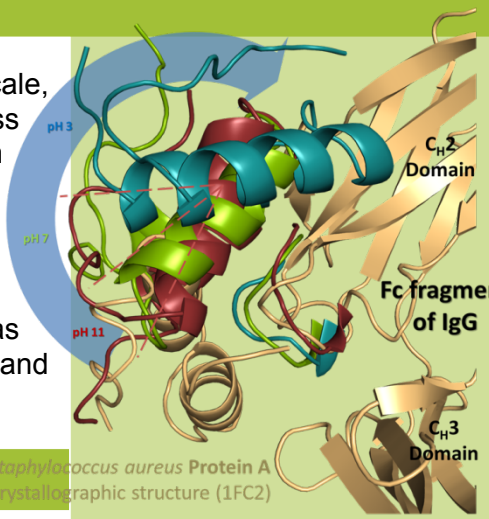

Figure 1: Interactions between the Fc fragment of IgG and the SpA domain simulated at different pH conditions through a docking and molecular dynamics computational approach.

Expected Results

The biomimetic ligand 22/8 binds preferentially to the Fc fragment of IgG through the same non-covalently bond interactions found in the crystallographic structure of the natural complex with the fragment B of SpA. Alternative specific binding sites at the hinge between V_H and C_H1 domains of Fab fragment were theoretically predicted, with a much lower affinity constant. The main interactions between ligand 22/8 and the IgG fragments found at pH 7 were weaker at pH 3 and pH 11 and in these conditions the ligand start losing tight contact with the binding site, corroborating the experimental evidence for protein elution from the chromatographic adsorbents at these pH conditions [1].

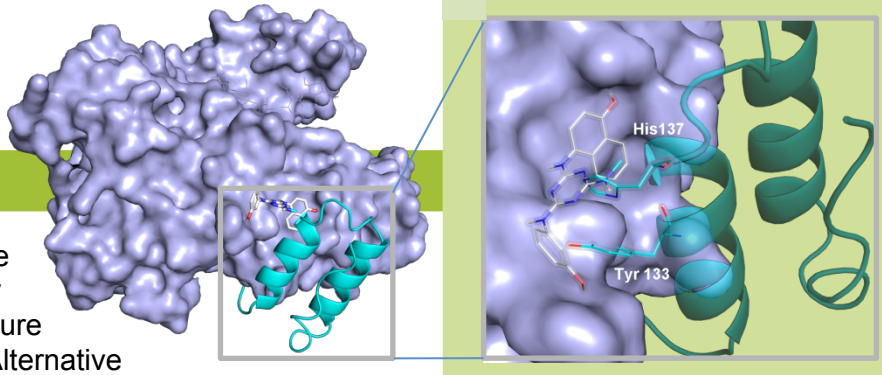

Figure 2: Docking result of the protein A biomimetic ligand 22/8 bound to the human Fc fragment of immunoglobulin G (IgG), and superimposed to the native binding site of the fragment B of SpA (PDB ID 1FC2).

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[1] Branco, *et al.* J. Chromatogr A, 1244 (2012), 106-115.