BIO_16 - *In silico* design of therapeutic single domain antibodies for asthma

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Introduction: Asthma is a chronic disease, still without a cure, and a globally significant public health problem, mainly due to continuous increase in cases. Treatment is costly due to immunosuppressive drugs mainly based on corticosteroids and beta-2 adrenergic receptor agonists. However, because of refractory cases, monoclonal antibodies usage proposed to inhibit specific molecules has become an attractive therapeutic method with promising results although expensive. The use of single domain antibodies (sdAbs), as well as camelid heavy- chain antibody variable domain (VHH), are cutting-edge biotechnological tools since they preserve the inhibition potential, specificity, sensitivity, and high-affinity but with a lower production cost and immunogenicity.

Objectives: The purpose of the study is to design a model *in silico* of stable and specific sdAb against a pivotal pro-inflammatory cytokine involved in the allergic asthma process.

Methodology: The structure of a variable heavy domain from a monoclonal antibody against this crucial pro- inflammatory cytokine, with known therapeutic effects against asthma, was used as a template to *in silico* build different sdAbs. Using the "camelization" approach to increase VHs solubility and stability, three specifics mutated sdAbs against this cytokine were designed. Molecular dynamics simulations of these antibodies and the wild-type VH isolated or associated with the cytokine were performed to study their predicted interaction, stability, and solubility.

Results: The results for the sdAbs:cytokine complexes show that the proposed antibodies interact in a stable and long-lasting way, in addition to a broad contribution from the CDRs, that provide the interaction specificity. All mutants had higher binding free energy and hydrogen bonding scores than the wild-type, suggesting better- predicted affinity. Additionally, the chosen mutations in the sdAbs improved the stability in the mutated region and decreased the time for structure stabilization on dynamics approach. Furthermore, the predicted solubility of the mutants was higher than the wild type and similar to previously soluble nanobodies produced by our group.

Conclusion: These results suggest that the proposed *in silico* mutations may improve the stability and solubility of these sdAbs. In addition, these mutations did not decrease the antibody ability to have a stable and long-lasting interaction with the cytokine and increased the predicted affinity, possibly contributing to the inhibitory and therapeutic effects. Nonetheless, further studies are still needed to confirm the *in silico* results and analyze possible side effects.

Keywords: Asthma, single domain antibodies, camelization, biopharmaceutical, molecular dynamics