B6. *In silico* DEVELOPMENT OF A scFv ANTAGONISTIC TO α4β1 INTEGRIN.

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INTRODUCTION Integrins are heterodimeric glycoprotein receptors located in celular membrane. These proteins are involved in various physiological processes, mediating signaling pathways, which coordinate cellular functions. The α4β1 integrin is found in the immune system cells and they take part of leukocyte migration during inflammatory processes, such as encephalomyelitis, Chron's disease, multiple sclerosis and others diseases. Single Chain Fragment Variable (scFv) are the smallest shape of antibodies. They conserve the variable regions of light and heavy chains, therefore preserving their specificity. Currently, the only monoclonal antibody commercially available, which interacts with α4β1 integrins is Natalizumab. However, this antibody is not specific just for α4β1, it also interacts with α4β7.

OBJECTIVE The aim of this project is to build a scFv specific to α4β1 integrin *in silico*.

METHODOLOGY A search of monoclonal antibodies, which recognize subunits α4 or β1 from integrins, was performed against the database Integrity. From the selected sequences, the comparative modelling of tridimensional structures of the light and heavy antibodies' chains was done. In addition, the evaluation of the structures was performed on the SAVES and Molprobity servers. Two types of scFv were made using the modeled chains. The first one uses a short peptide linker, GGGGS, to connect the light and heavy chains. The second one uses a long linker made of three repetitions of the first linker. A molecular docking of all scFv and the α4β1 integrin was done through Haddock server. The best clusters generated were submitted to Robetta Alanine Scanning for hotspot identification. From these results, mutations in strategic residues interacting with α4β1 were done. A new docking using the modified scFv and α4β1 and α5β1 integrins was performed. The best scFv was selected and submitted to docking with α4β7. Thus, new mutations were done to ensure the antibody specifity for α4β7 comparing to the others integrins.

RESULTS Three sequences were obtained from Integrity under 257898, 670484 and 725144 codes. The modeled chains obtained satisfactory values on Molprobity and SAVES evaluation. All modifieds scFvs showed a better Haddock Score for α4β1 integrin docking comparing to the originals antibodies. However, the best scFv was the modified 257898 one, with a short linker due to better Haddock score, Cluster size, and RMSD values. After the new mutations, this scFv also presented the best Haddock Score for α4β1 comparing to α5β1 and α471.

CONCLUSION A scFv specific to α4β1 integrins was obtained. In addition, it was able to better distinguish among α4β1, α5β1 and α4β7 integrins. As perspective, scFv molecular dynamics will be performed to analyze its stability and mode of interaction. This method is preliminarily used to select better proteins in a group of generated mutants, following the *in vitro* evaluation.

KEYWORDS antibodies, integrins, α4β1.