

## BIO.16 - Anti-CD19 CAR-T engineering through Molecular Dynamics Simulation

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**Introduction:** Chimeric Antigen Receptors (CARs) are designed to be inserted in the effector immune cells (T cells) membrane, conferring specificity to determined tumor cells. These receptors consist of three portions: an ectodomain, a transmembrane domain and an endodomain. The latter two are related to signal transduction and cytotoxic response. The ectodomain is generally formed by a single chain fragment variable (scFv) that recognizes a receptor on tumor cells, being responsible for the affinity and specificity to its antigen. Molecular Dynamics (MD) was used to study the scFv-antigen interface to understand how this interaction affects the CAR action. The antigen CD19 is a B cell receptor with no significant homology to other known correlated proteins, so it is a perfect biomarker for lymphoma diagnosis and then for CAR-mediated immunotherapy.

**Objective:** To build the scFv of an anti-CD19 antibody, to analyze through MD simulation the complex scFv-CD19 structural stability in water and to determine energetic components involved in the formation of the scFv-CD19 interface.

**Methodology:** The anti-CD19 scFv was built from crystallographic data (PDB 6AL5) containing its VL and VH domains structures and from a built Whitlow linker (GSTSGSGKPGSGEGSTKG) that connects these two antibody portions. The scFv was submitted to MD for 300 ns at CHARMM36 force field with 25205 TIP3P water model, 310 K and physiological concentration of 0.15 M (74 Na<sup>+</sup> and 73 Cl<sup>-</sup>). Then, the scFv-CD19 complex (CD19 also obtained from PDB 6AL5) was simulated for 300 ns at same MD parameters. The structural stability was determined by Root Mean Square Deviation (RMSD). The Intermolecular Interaction Potential (IIP), which is nonbonded Coulomb and Lennard-Jones interactions, between scFv and CD19 residues was measured along the simulation. The Gromacs package 5.1.3 was used during equilibration, molecular trajectory acquisition and analysis.

**Results:** The simulation time was enough to stabilize the scFv and CD19 structures in water. The scFv achieves stability in water after 200 ns with a RMSD of  $0.2 \pm 0,02$  nm, the VL and VH domains are stable since the beginning of the simulation with the same RMSD of  $0.09 \pm 0,01$  nm. As expected, the Whitlow linker RMSD is higher than VL and VH domains, due to its majority composition of glycine and serine flexible residues. The CD19 structure achieves stability after 100 ns with a RMSD of  $0.44 \pm 0,04$  nm.

**Conclusion:** A stable scFv-CD19 complex was obtained, allowing us to identify the CD19 binding site and how structural and energetic components are involved in scFv-CD19 interaction. Therefore, it is expected to obtain an improved anti-CD19 CAR-T cell, that will be tested *in vitro* and *in vivo* further on.

**Keywords:** CAR-T; CD19; Molecular Dynamics