Cell Engineering - Exercise Session 2

Please note that this exercise session is graded, and that each student will have to submit an individual report.

CAR-T cell therapy

In order to achieve full activation and proliferation, native T-cells rely on receiving three essential signals: Signal 1, mediated by T-cell receptors (TCRs); Signal 2, mediated by costimulatory receptors; and Signal 3, mediated by cytokine receptors. Each of these signals operates through distinct intracellular domains, as depicted in the left-hand diagram below.

Chimeric Antigen Receptors (CARs) offer a promising avenue in synthetic biology for enhancing T-cell functionality. CARs can incorporate one or a combination of the previously mentioned intracellular domains, as illustrated in the right-hand diagram below. However, the most effective CAR design incorporates all three types of domains, ensuring full T-cell activation upon recognition of tumor antigens.

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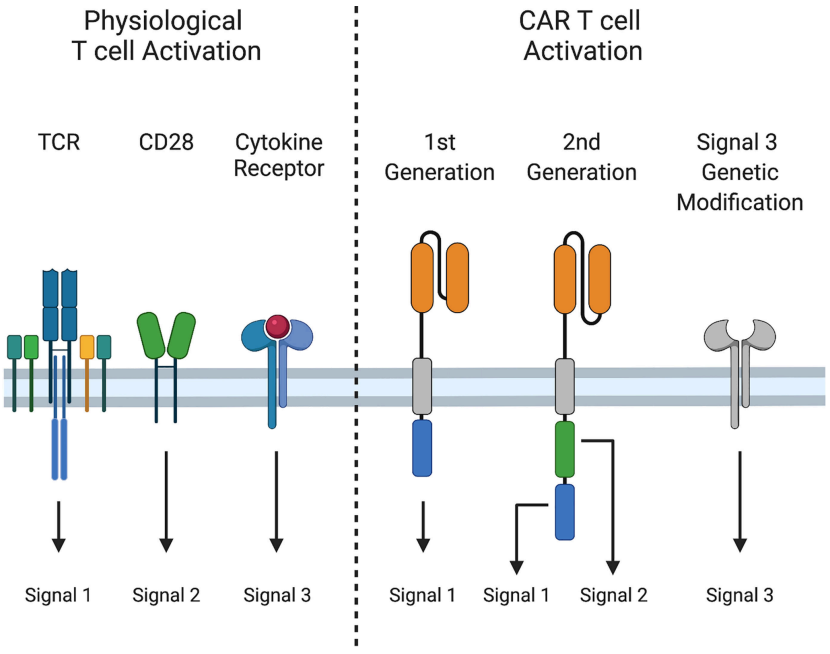
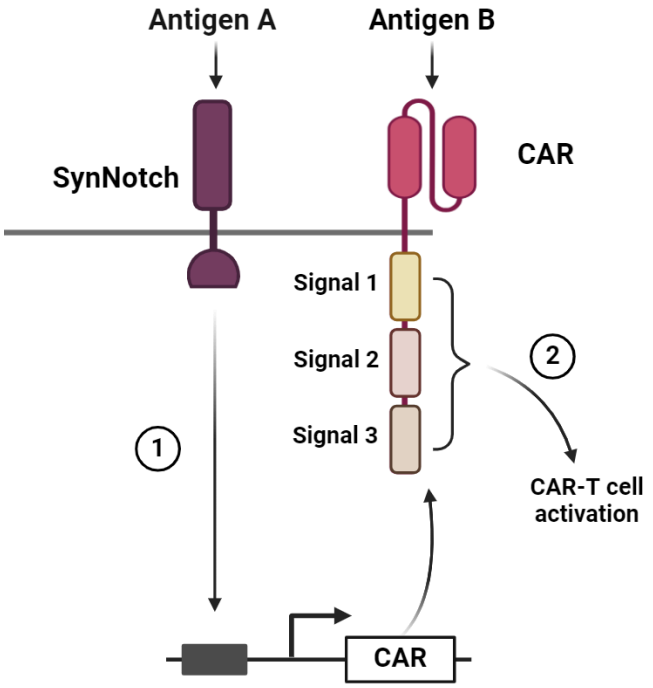


Please note that the figure referenced in this problem set, taken from https://doi.org/10.3389/fimmu.2021.684642, does not depict a Chimeric Antigen Receptor incorporating all three signals. However, it's important to acknowledge that CAR designs incorporating all three signals do exist.

A recent breakthrough has introduced an innovative approach based on AND logic gate mechanisms to regulate CAR T-cell activation. In this method, CAR expression is contingent upon the activation of another receptor (synNotch), offering precise control over T-cell response. The system works as follows (see figure below): (1) Antigen A activates a SynNotch receptor which then drives the expression of a chimeric antigen receptor. (2) Upon binding to antigen B, the CAR then activates the CAR T-cell through signal 1, 2 and 3 (all three signals are required for full and strong activation).



Question 1a)
A notable drawback of the circuit shown above is that the CAR T-cell response is rather slow. Can you think of another strategy to engineer AND logic gates that control T-cell activation with a more rapid and dynamic response?

- clearly mention what the slow step is
- please provide a clearly labeled schematic figure of your improved circuit and explain why it is more rapid than the original one.
- note: Multiple solutions are possible, just ensure that your designed circuit requires AND logic (requiring antigen A AND B) for full activation of the CAR T-cell. Assume that you can introduce constitutively expressed genes in your CAR-T cell, and that you can split and recombine domains as you like.

Question 1b) Do you foresee any potential problems/issues that might arise with your newly proposed strategy?