Project 1. Improving cancer immunotherapies with powerful immune cells expressing engineered membrane proteins

Cell engineering technologies have made tremendous progress in recent years and are becoming central for synthetic biology applications. For example, engineered cells with customized signaling responses to disease-associated molecules provide promising and powerful new therapeutic agents for cancer immunotherapy, regenerative medicine and autoimmune disorders. In cancer immunotherapies, immune cells are engineered to express chimeric synthetic receptors coupling the recognition of a tumor associated antigen to the activation of critical immune cell functions. While effective for certain cancer types, most current approaches lack sustained anti-tumor responses due to immune inhibitory signals at the tumor site.

Our lab is developing and applying novel computational-experimental protein design approaches for improving the anti-tumor response of engineered immune cells. Specifically, we seek to design membrane receptors recognizing immune inhibitory molecules and redirect these signals into activating/proliferative responses inside the immune cell. The technology has been tested on a simple proof of concept (Feng et al., *Nat Chem Biol* 2017; Young et al., *under review*) and can now be applied to several classes of signaling receptors that are critical regulators of immune cell functions.

Specifically, the project will involve some aspects of computational protein modeling and design using the techniques developed in the lab complemented by the directed evolution of desired protein functions in mammalian cells. Engineered proteins will be validated using in vitro and cell-based assays reporting selective protein cellular functions and then tested in human immune cells for promoting tumor cell killing and enhanced function. The most promising candidates of the engineered cells expressing the designed proteins will be tested in mouse xenograft models in collaboration with laboratories at the CHUV/UniL (e.g. Caroline Arber, George Coukos) before potential translation to the clinic. Marrying empirical and computational protein engineering approaches has the unique potential to reprogram a broad spectrum of signaling functions for engineering powerful therapeutic cells with novel and sustained anti-tumor responses.

Project 2. Interrogate genome sequences with protein modeling for precision personalized cancer medicine.

Whole human genome sequencing has become less expensive and more efficient, allowing new approaches using personal genome information to help diagnose, treat, and even prevent human diseases for which genetic variations can be risk factors or causative. However, the evaluation of the potential pathogenicity of individual variations and the mechanistic relationships between pathogenic variations and their physiological consequences is partly limited by the lack of reliable predictions of their effects on the protein structure, dynamics, function, and systems-level interactions between proteins. To address this limitation in a proof of concept study, we applied a new physical model of protein allostery and sequence/structure alphabet of protein stability developed in our laboratory (Chen et al., *PNAS* 2012, Feng et al., *Nat Chem Biol* 2016, Chen et al., *under review*, Keri et al., *in prep*) to predict the effects of retinitis pigmentosa related mutations...
in the visual G protein coupled receptor rhodopsin on receptor signaling properties. Other specific molecular and cellular consequences of these mutations had been previously identified and classified but correlate poorly with clinical phenotypes. Remarkably, a large majority of the mutations displayed a strong correlation between predicted changes in receptor activity and acuteness (i.e. age) of early nighttime and daytime vision loss, suggesting that receptor activity may constitute a novel strong predictor of the disease severity at early stages (Chen et al., in prep).

We will expand our investigations to an ensemble of G protein coupled receptors (GPCR) that have recently been found implicated in the development and progression of a wide array of cancers and cancer types. So far, studies have limited their scope to only a few receptors and a single cancer type. To gain a more holistic understanding of GPCR involvement in cancer, we will combine sequence bioinformatics and high-throughput structure modeling approaches to predict the structure and signaling effects of clinical mutations from The Cancer Genome Atlas (TCGA) across 280 receptors. We hypothesize that, given a sufficiently large data set, we will identify conserved clusters of mutations within the GPCR structure fold with similar effects on receptor signaling as well as correlations between mutations and specific signaling pathways or conserved functions in cancer progression. The study will then be extended to several other protein classes involved in the progression of diverse cancer types.

Specifically, the project will involve a large diversity of bioinformatics and protein structure modeling approaches. As novel blind predictions of disease-associated mutations on receptor functions will be achieved, the project will also involve the experimental validation of the predictions using in vitro and cell-based assays reporting receptor cellular functions. This latter part of the project could also be performed in collaboration with experimentalists in the laboratory or with external collaborators. These studies will shed light on common mechanisms of cancer progression, and provide a rational basis for future personalized cancer diagnoses, risk stratifications and treatments.

**Project 3. Expanding the universe of protein functions by computational protein modeling and design.**

Our lab is developing and applying novel computational protein design approaches for engineering classes of proteins with new functions for cell engineering, synthetic biology and therapeutic applications.

Our laboratory is part of RosettaCommons, a collaborative network of academic laboratories which develop the software platform Rosetta for macromolecular modeling and design. The specific project involves the extension of the software Rosetta for the modeling and design of membrane proteins and their interactions with drug and lipid molecules that regulate their functions. Ultimately, we aim at developing a versatile tool for the academic and industry communities to leverage the design of novel potent and selective molecules that can modulate the function of important membrane receptors in normal physiology and disease. Additionally, the methods will provide a universal platform for designing proteins with precise and novel functions to improve cell engineering approaches (e.g. cancer immunotherapy). Protein modeling and engineering projects in collaboration with experimentalists in our lab are also possible. Candidates should have strong programming skills in C/C++ and python, and have a good background in computational biomolecular modeling or bioinformatics.