CHEMICAL ENGINEERING
UNIVERSITY of WASHINGTON

SEMINAR

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Programmable Engineering Circuits for Smart Therapeutics

Monday August 14th
Lecture 4:00-5:00 p.m. | Physics/Astronomy Auditorium (PAA) A110
Reception 5:00-6:00 p.m. | Benson Hall Lobby

Bio

Alexander Vlahos is currently a Human Frontier Science Program Fellow in the laboratory of Dr. Xiaojing Gao at Stanford University. He applies principles in synthetic biology and protein engineering to develop tools for programming intercellular signalling. Previously, he completed his PhD in Biomedical Engineering with Dr. Michael Sefton at the University of Toronto, where he used principles in tissue engineering, regenerative medicine, and biomaterials to develop platforms to improve vascularization of the subcutaneous space for islet transplantation. His goal is to converge his background in synthetic biology, systems biology, and tissue engineering to mechanistically study and manipulate multicellular systems to improve the long-term engraftment of therapeutic cells.

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Abstract

One of the primary goals of tissue engineering is to develop functional tissues that can integrate with the host to replace damaged organs. Various tissue engineering approaches have been developed delivering therapeutic cells within biomaterials and scaffolds to improve graft survival and function. Despite these advances, a critical challenge remains in balancing vascularization with immune protection. Although systemic immunosuppression can prevent acute rejection, due to the detrimental off-target effects there has been a shift towards developing strategies for local immune modulation. These strategies include delivering therapeutic cells within biomaterials, alongside anti-inflammatory drugs, or with other endogenous cells. However, like systemic immunosuppression, they fail to promote long-term engraftment due to their inability to dynamically respond to the immune system or a finite reservoir of immune-modulating biologics.

In comparison, the field of synthetic biology is uniquely suited to provide the means to dynamically respond to the immune system by using engineered cells that can respond to combinatorial environmental inputs, interrogate natural systems, and produce controlled therapeutic responses. Creating programmable behaviours using protein based circuits have advantages such as fast operation, compact delivery, and robust context-independent performance compared to traditional transcriptional circuits. Previously, we described a generalizable platform called RELEASE (**R**etained **E**ndoplasmic C**lea**vable **Se**cretion) which enabled intercellular signalling through the removal of ER-retention motifs compatible with pre existing protein-based circuits (**Fig. 1A**). RELEASE enabled basic Boolean logic operations such as AND/OR, however, to achieve functional completeness it lacked the capabilities to implement NOT gate logic. Furthermore, to program complex expression patterns such as logic operations or quantitative processing, multiple orthogonal proteases are required, which may preclude their use in viral vectors with limited packaging capacities.

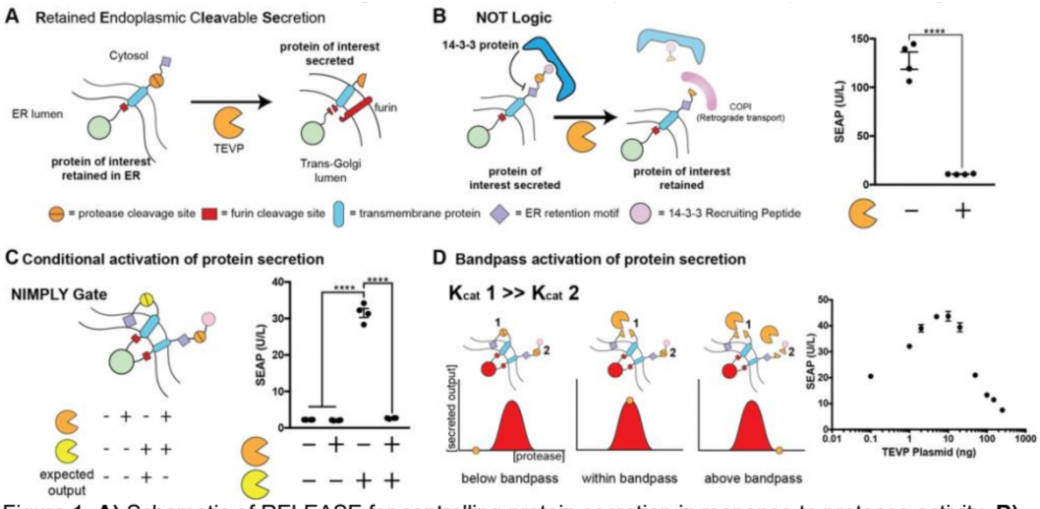

Figure 1. **A)** Schematic of RELEASE for controlling protein secretion in response to protease activity. **B)** Recruitment of 14-3-3 proteins via small peptides antagonizes the activity of the ER retention motif, resulting in constitutive protein secretion. Removal of the peptide via protease activation restores ER retention activity, implementing NOT gate logic. **C**) Conditional activation of protein secretion with multiple outputs. **D)** By creating a variant with two protease cut sites of different cutting efficiencies (K_{cat}), we can implement bandpass activation of protein secretion. Protein secretion will only occur when the concentration of the input is within a specific range.

My current work focuses on expanding the programmable capabilities of RELEASE by harnessing additional protein-protein interactions with endogenous proteins such as 14-3-3 protein to control protein secretion. Using a small peptide, we can control the recruitment of 14-3-3 proteins to antagonize the retention capabilities of RELEASE by blocking interactions with the native retrograde transport machinery of the conventional secretory pathway. By placing a protease cut site upstream of the 14-3-3 recruiting peptide we could manipulate this interaction to implement NOT gate logic control of protein secretion and achieve functional completeness (**Fig. 1B**). We created a suite of RELEASE variants, known as Compact-RELEASE to implement every type of Boolean logic control of protein secretion (**i.e. NIMPLY – Fig. 1C**). Furthermore, Compact-RELEASE can be modified to enable quantitative processing and control of protein secretion, such as bandpass activation (**Fig. 1D**). Pilot studies are underway to subcutaneously deliver engineered cells to control the local concentration of secreted proteins using small-molecule activators, *in vivo*. Compact-RELEASE expands the programmable capabilities of synthetic protein circuits without requiring additional processing proteases, enabling their delivery using pre-existing viral vector methods with limited packaging capabilities, such as adeno-associated viruses or adenoviruses.

Collectively, my work has helped create programmable synthetic protein circuits as "smart therapeutics", which enable engineered cells to interface with complex biological systems. As almost all cells communicate via intercellular signals, using synthetic protein circuits as the engine to model complex multicellular systems and determine key signals to bias therapeutic responses, my lab will be at the frontiers of synthetic biology and tissue engineering. **This work has significant implications for improving human health in critical areas including regenerative medicine, cancer, and autoimmune diseases.** Moreover, due to the plug-and-play nature of synthetic protein circuits, and the complexity of the immune system, this work will also be an asset for basic research into complex biological networks.