SRP Diabetes Postdoctoral Fellowship Program 2023

Project title: Mapping the biodistribution of multivalent insulin variants in zebrafish and in mice

Project outline

Background

Insulin receptors (IRs) form nanoclusters at the cell membrane. However, the functional significance of the nanoscale spatial organization of insulin receptors is largely unknown.

We have previously developed DNA-origami based strategies to control the the spatial distribution of ligands with nanoscale precision and regulate signalling mediated by Ephrin receptors in cancer cells (Shaw et al., 2014 and Verheyen et al., 2020) and PD-1 receptor in T-cells (Fang et al., 2021). Here, nanoclusters of insulin molecules are engineered to bind and control the activation levels of insulin receptors.

Objectives

In this project we will map the biodistribution and tissue specific effects of different multivalent insulin variants in wild type and diabetes models in zebrafish and mice.

Methodology

DNA origami is a nanofabrication technology that uses DNA self-assembly to drive the precise formation of 3D nanostructures (Rothemund, 2006). A long single strand of DNA, called the scaffold, is combined with hundreds of synthetic short DNA sequences, the staples, each of which with regions of complementarity to different segments of the scaffold. Hybridization between each staple and the scaffold brings these different segments in the scaffold into proximity, thereby folding it in a sequence-defined fashion. Staple strands and the scaffold self-assemble into a 3D nano-object with user-defined shape and dimensions. In DNA origami the position of every staple in the structure is uniquely and precisely specified. Selected staples can be designed to protrude from the structure at certain points, forming a pattern of single stranded oligonucleotides available for hybridization with complementary oligonucleotides conjugated to any molecule of interest. Building on our previous work, DNA origami nanoclusters are here used to probe the roles of spatial organization in insulin receptor mediated signalling.

Work plan

The postdoctoral researcher will map the biodistribution of variants of insulin-DNA-origami nanostructures with different valencies in zebrafish over time using light sheet microscopy. These analyses will be complemented with a characterization of the cell types that are targeted by the nanostructures, through FACS sorting of labelled cells followed by single cell sequencing. This strategy will not only allow us to identify the cell types that are targeted by the nanostructures but will also provide insights into cell-type specific insulin receptor-mediated transcriptional responses. These experiments will provide a set of insulin-DNA-origami nanostructures selected for further studies in mice, performed in collaboration with Dr. Li Ye, Scripps Research, USA (Nudell et all, Nature Methods 2022).

Shaw, A[*], Lundin, V[*], Petrova, K, Fordos, F, Benson, E, Al-Amin, A, Herland, A, Blokzijl, A, Hogberg, B[#], and **Teixeira, Al.**[#] Spatial control of membrane receptor function using ligand nano-callipers. **Nature Methods**. 2014; 11:841-6.

Fang, T, Alvelid, J, Spratt, J, Ambrosetti, E, Testa, I, and **Teixeira, AI**. Spatial Regulation of T-Cell Signaling by Programmed Death-Ligand 1 on Wireframe DNA Origami Flat Sheets. **ACS Nano**. 2021 Feb 8;15(2), 3441-3452.

Verheyen, T*, Fang, T*, Lindenhofer, D, Akopyan, K, Lindqvist, A, Högberg, B, **Teixeira, AI**. Spatial organization-dependent EphA2 transcriptional responses revealed by ligand nanocalipers. **Nucleic Acids Research**. 2020 Jun 4;48(10), 5777-5787.

Nudell V, Wang Y*, Pang Z, Kanim W, Lal N, Huang Min, Shaabani N, Teijaro J, Maximov A, and Ye L. HYBRiD: Hydrogel reinforced DISCO for clearing mammalian bodies. **Nature Methods.** 2022; 19:479-485.

Relevance of project for diabetes

Our previous work suggests that insulin multivalency can modulate insulin receptor activation, independently of insulin concentration. This project will determine how multivalent forms of insulin target and activate insulin mediated signalling in different tissues. This work can form the basis for the future development of insulin variants with tailored tissue targeting patterns.

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