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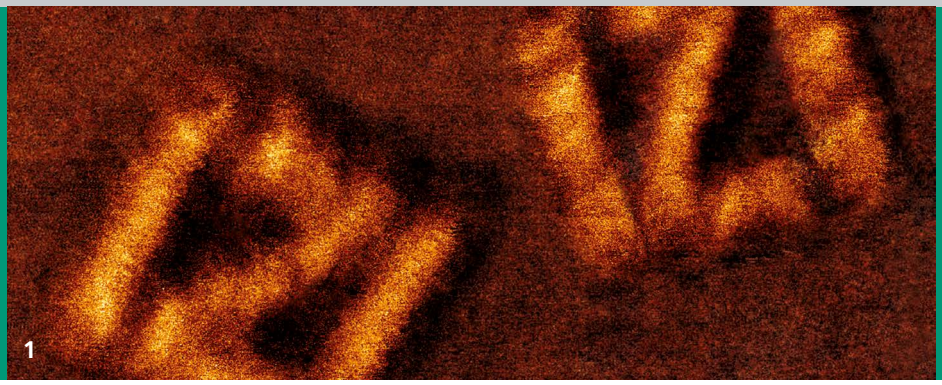

1 *Atomic Force Microscope image of immobilized proteins on a DNA origami molecular substrate, in the form of the Fraunhofer "IZI" monogram.*

ENHANCEMENT OF BIO-ACTIVE LIGANDS BY MULTIVALENT PRESENTATION ON NUCLEOTIDE SCAFFOLDS

The DNA Nanodevices group implements nucleotide-based tools for biomedical research with an eye towards biomedical translation. By re-envisioning DNA strands as construction material for nano-scale fabrication, these molecules and their characteristics are used to arrange and structure biomaterials with nanometer scale precision. This type of technology is applied to develop biosensors and nanocircuitry for biochips, in addition to being used to develop new procedures to specifically transport molecules in vivo and in vitro.

Using rational design principles, individual DNA strands can be assembled into precise nanostructures of nearly any shape or size. Functional bio-active ligands, such as peptides or glycans, can be attached to nearly any unique location or nanometer-precise arrangement on these nanostructures, with the spatial resolution of a single base pair (0.34 nanometers).

This is a key advantage due to the fact that many biologically relevant receptors are multimeric in nature and these fully addressable structures can be designed to mimic the spatial distance and orientation of their natural ligands. Taken together these features potentially allow the user to fine tune the affinity and degree of response the target exhibits when presented with a ligand mounted on these nanostructures. Likewise, it also has the ability to enhance the response from otherwise weak ligands, such as synthetic peptides, through the same principles of multivalency.

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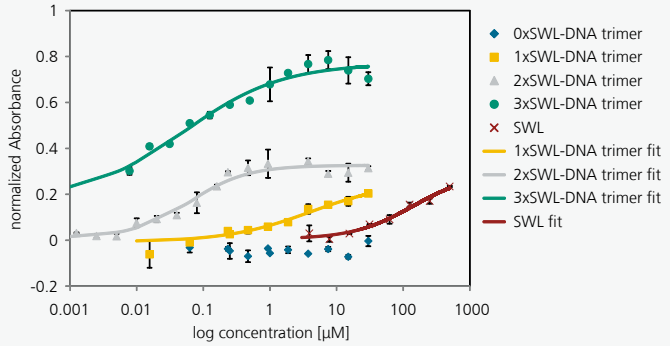
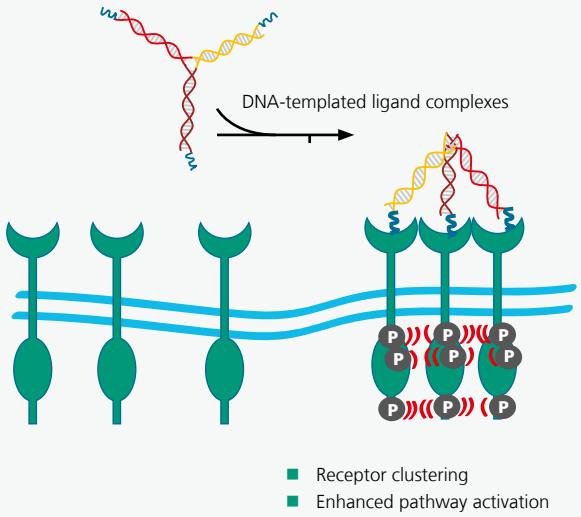


Ligand	EC_{50} [µM] (mean ± standard deviation)
SWL	153.8287 ± 115.3869
ephrin-A1	0.0027 ± 0.0005
1xSWL-DNA trimer	2.7427 ± 1.9837
2xSWL-DNA trimer	0.0572 ± 0.0113
3xSWL-DNA trimer	0.0190 ± 0.0046

2



3

Technology

- Coupling small biologically active ligands to DNA scaffolds
- Enhanced bio-availability and bio-activity of DNA-ligand complexes
- Amenable to multivalent binding
- Defined molecular stoichiometry
- Defined position and distance between ligands
- DNA structures can be made rigid or flexible and of varying sizes

Applications

- Stimulation of dimeric / trimeric extracellular receptors
- Steric blocking or inhibition of receptors
- Neutralization of pathogen adhesion to host cells
- Diagnostic of binding events and affinity
- Can be used to capture and separate ligand targets

Pinpointed stimulation of EphA2 receptors via DNAtemplated oligovalence

When these molecules are ligands that bind to specific targets, their spatial arrangement can be controlled according to the desired target's geometry. This results in optimized binding and / or signaling interactions.

In this project, the efficacy of SWL, an ephrin-mimicking peptide that binds specifically to EphrinA2 (EphA2) receptors, was enhanced by a factor of nearly four orders of magnitude by presenting three of these peptides on small DNA nanostructures in an oligovalent manner.

Ephrin signaling pathways are critical in the development and progression of many types of cancer, and are potential targets in cancer diagnosis, imaging and treatment. Here, the impact of SWL valency on binding affinity, phosphorylation (a key player for activation) and the regulation of phenotype prostate cancer cells that express EphA2 was quantitatively demonstrated. DNA structures with three SWL peptides significantly enhanced EphA2 phosphorylation by 8000-fold. Furthermore, the pinpointed interaction of these constructs showed an enhanced impact on the retraction of cells compared to one of EphA2's natural ligands – ephrin-A1.

These results demonstrated that simple DNA structures can be used to greatly enhance the potency of otherwise weak signaling peptides, using principles of a nanometer-scale oligovalent arrangement.

Citations

Möser C, Lorenz JS, Sajfutdinow M, Smith DM. **Pinpointed Stimulation of EphA2 Receptors via DNA-Templated Oligovalence.** Int J Mol Sci. 2018 Nov 6;19(11). pii: E3482. doi: 10.3390/ijms19113482.

Lorenz JS, Schnauß J, Glaser M, Sajfutdinow M, Schuldt C, Käs JA, Smith DM. **Synthetic Transient Crosslinks Program the Mechanics of Soft, Biopolymer-Based Materials.** Adv Mater. 2018 Mar;30(13):e1706092. doi: 10.1002/adma.201706092. Epub 2018 Feb 15.

PCT/EP2018/063841 **"Nanostructure with a nucleic acid scaffold and virus-binding peptide"**

DE 102019110314.2 **"Biological and synthetic molecules inhibiting respitory syncytial virus infection"**

2 *Receptor clusters are formed by the oligovalent, ligand-coupled DNA nanostructures binding to cell surface receptors. This leads to clustering-induced effects like phosphorylation, and subsequently enhanced activation of intracellular signaling pathways.*

3 *This type of forced clustering by oligovalent DNA-templated ligands leads to significant enhancement of activation compared to monovalent ligands. Here, trivalent presentation of an EphA2-activating peptide enhances its effect by a factor of ~7500 compared to the monovalent peptide.*