DNA nanostructures as multivalent carrier for peptides

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A major benefit of DNA nanostructures is the possibility to functionalize the single DNA strands on their 3´ and 5´ends via different chemical reactions. This enables the precise arrangement of molecules such as peptides in a specific distance. We utilize a copper-free click chemistry approach to modify small DNA dendrimer structures as well as dsDNA with different biologically-active peptides. By doing so, we want to create a construct that enables many and thus strong reversible multivalent interactions of the ligands/peptides on their target acceptors.

In detail, we study not only the anchorage but also the activation of EphrinA2 receptors (EphA2) upon multivalent presentation of EphA2-binding peptides. EphA2 receptors are overexpressed in many types of cancer (breast, pancreatic, ovarian, prostate and lung cancer) whereas tumor suppressor properties are reported when their signaling ability is activated by ephrin ligands. Dimerization, activation and signaling are accompanied by changes in the cytoskeleton which we hope to measure using real-time deformability cytometry (RT-DC) [1]. Our idea is to attach those ephrin-binding peptides to DNA nanostructures in order to provide optimal binding to the receptors, to promote dimerization and thus stronger receptor activation. Initial results confirm cytoskeletal changes (Fig. 1A).

Moreover, we examine the spatially-dependent multivalent display of hemagglutinin (HA)-binding peptides (PeB) attached to simple DNA dendrimer structures which consist of only three DNA sequences. Those peptides are able to block receptors on the outer surface of the influenza virus, thus inhibiting it from entering cells when displayed in a multivalent manner. A common assay to check for virus-cell binding is the hemagglutination inhibition (HAI) assay. First results show an improvement of the peptide-decorated DNA constructs in comparison to the monomeric peptide (Fig. 1B).

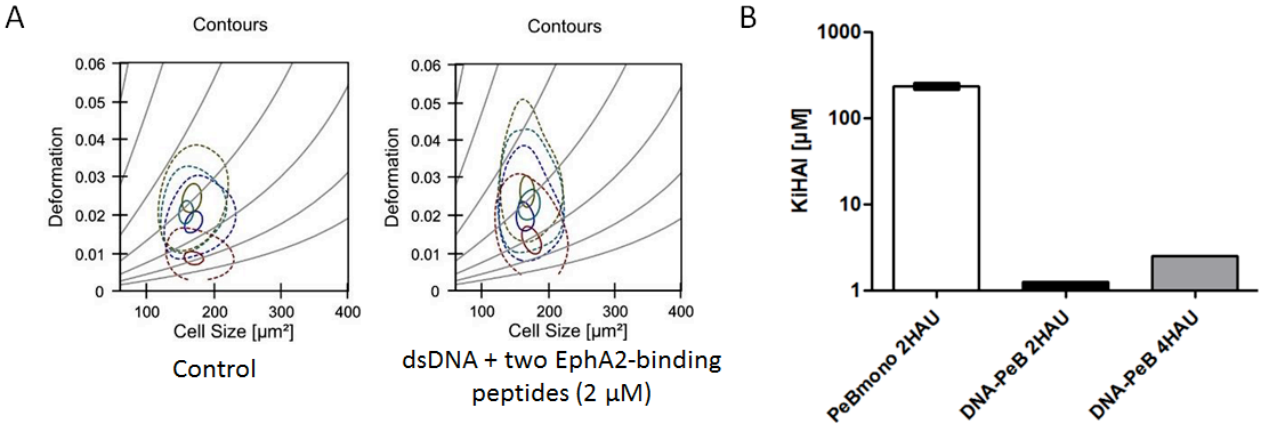

Figure 1: A) RT-DC measurements using PC3 cells. Shape changes were measured using different flow rates (0.16 µl/s, 0.24 µl/s, 0.36 µl/s) and before deformation. **B)** HAI assay to determine the inhibitor concentration (K_i^{HAI}) that is necessary to prevent binding of viruses to red blood cells. Comparison between peptide only (PeBmono) and conjugated to DNA (DNA-PeB); HAU = hemagglutination units.

[1] O. Otto, P. Rosendahl, A. Mietke, S. Golfier, C. Herold, D. Klaue, S. Girardo, S. Pagliara, A. Ekpenyong, A. Jacobi, M. Wobus, N. Töpfner, U. F. Keyser, J. Mansfeld, E. Fischer-Friedrich, and J. Guck, "Real-time deformability cytometry: on-the-fly cell mechanical phenotyping.," Nat. Methods, vol. 12, no. 3, Feb. 2015.