Artificial actin crosslinkers for control of cell motion

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Actin is a crucial cytoskeletal element that exhibits unique properties. In cellular systems actin structures are usually cross-linked, which leads to a variety of emerging structures accompanied by an increased mechanical complexity. Cells employ multiple cross-linkers with very different properties. Some of these cross-linkers mainly form bundled structures while others mainly form networks. Controlled studies of the entire phase space, however, were infeasible since they were restricted to naturally occurring cross-linkers. These proteins, such as alpha-actinin and fascin, cannot be controllably varied since they differ in many parameters such as the binding affinity, cross-linker length, and the number of binding domains. To examine the effect of crosslinkers on functional cells and to determine what crosslinkers do in cells, we engineered artificial DNA-based actin-crosslinkers. This novel approach allows the investigation of the impact of one distinct parameter such as the binding strength, cross-linker length, or valency on overall network properties in a decoupled manner, and potentially, opens a door for new therapeutic approaches. These artificial constructs constist of covalently conjugated actin-binding peptides on each site of a double-stranded DNA spacer [Fig. 1A]. To vary the binding strength, we chose LifeAct as a weak and Phalloidin as a strong F-actin binder. Both binders were attached to the DNA hybrid using a copper-free Click Chemistry approach [1].
We used bulk shear rheology to investigate the mechanical properties of these cross-linked actin networks. These studies showed that both artificial crosslinker-types stiffen actin networks *in vitro*. Interestingly, we were able to reproduce the mechanical features of actin networks cross-linked by fascin as well as alpha-actinin by using our artificial complexes [2]. Performing 2D migration assays, we saw a delayed migration of both HeLa and PC3 cells [Fig.1 A, B], which were transfected with the actin-binding construct, indicating that we are somehow changing the actin dynamics. Cells which were transfected with just double-stranded DNA or buffer did not show a delayed migration.

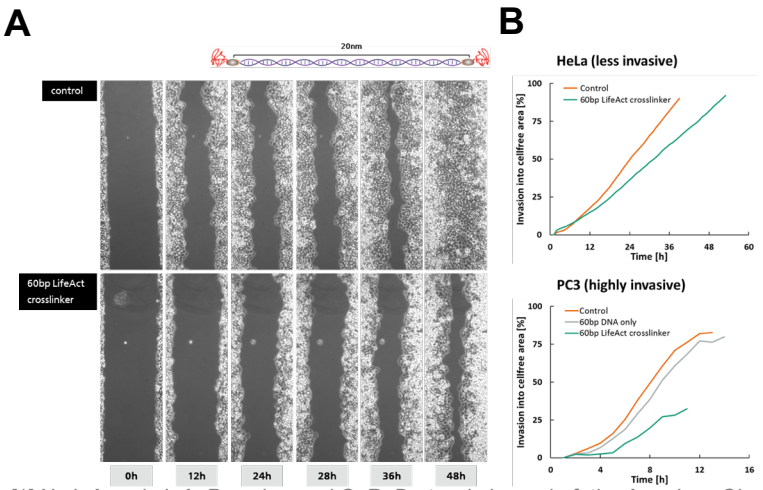

Fig. 1: 2D Migration assay. (A) HeLa cells were transfected w/ and w/o 150nM artificial 60bp LifeAct crosslinker and observed over 48h. (B) Analysis of the invasion of HeLa and PC3 cells into the cell-free area over time using the ImageJ plugin "MiToBo". Cells transfected with actin-binding crosslinkers showed a delay in migration.

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