Oriented immobilization of cadherin fragments on magnetic nanoparticles as novel magneto-mechanical cell actuators

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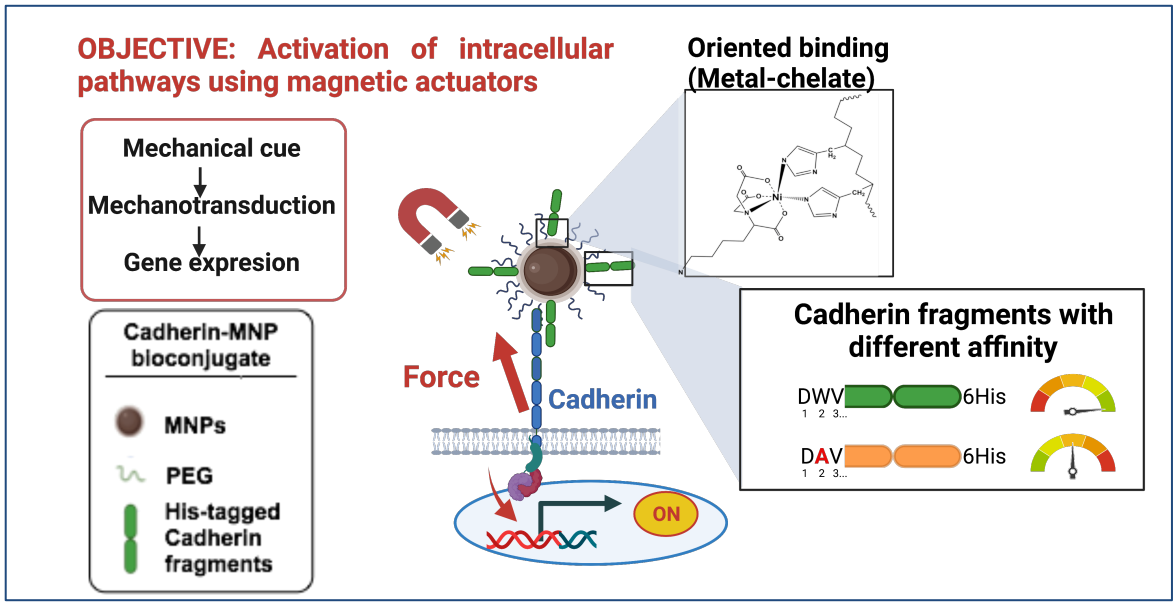
Magnetic nanoparticles (MNPs) can be used in a multitude of applications in the field of nanomedicine due to their unique characteristics. Among them, their ability to generate heat or tensile forces when manipulated by external magnetic fields are highly interesting. The forces generated by the remote stimulation of the MNPs can be harnessed to convert the MNPs in mechanostimulation platforms, able to exert forces directly on the cell membranes. By targeting receptors that convert mechanical stimuli into biochemical signals (mechanotransduction), MNPs could be used to activate intracellular pathways in a controlled way.[1] In this context, mechanotransduction can take place in the adherent junctions, which relies on the role of E-cadherin. In fact, E-cadherin mechanotransduction is critical to mediate collective epithelial remodelling that takes place during tissue repair. Functionalizing MNPs with cadherins would allow to attach the nanomaterials to the cadherins on the cell membrane in an orientation-dependent manner, prior to an external magnetic stimulation that could be used to activate intracellular pathways implicated in regeneration processes.

In the present work we generated different fragments of E-cadherin, composed of the first two extracellular domains, which are enough to establish stable homophilic interactions with the cadherins present on the cellular membrane. We used the *wild type* E-cadherin recombinant fragment, and two E-cadherin mutants generated by site directed mutagenesis, in order to control the binding affinity. The cadherin fragments were modified with a histidine tag (His-Tag) at the C-terminus to allow their oriented attachment *via* metal-chelate affinity to the MNPs. 15-nm iron oxide MNPs were grafted with polyethylene glycol (PEG) and functionalized with a nitrilotriacetic acid derivative (NTA), a molecule able to chelate metal ions like Ni^{2+} or Co^{2+}. Then, His-tagged cadherin fragments were bound in an oriented fashion to the MNPs, controlling at the same time the number of proteins/MNP. In order to use the MNPs as potential cellular mechanostimulators, besides controlling the number and orientation of cadherins over the MNPs surface, the strength of the union protein-MNP surface is another crucial step. Thus, we stabilized these links to reach a higher union strength, through two different strategies.

Finally, we immobilized the E-cadherin-MNPs on membrane of living cells that express E-cadherin. The selective binding of the MNPs functionalized with the *wild type* fragment on cells was assessed, while MNPs functionalized with E-cadherin mutants did not bind to them. This is the first step towards the selective activation of intracellular pathways linked to cadherins using MNPs.



References: 1-Del Sol-Fernández, S., et al. Magnetogenetics: remote activation of cellular functions triggered by magnetic switches. **Nanoscale**, 2022,**14**, 2091-2118

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