DESIGN OF DNA-PEPTIDE NANOSTRUCTURES AGAINST INTRACELLULAR TARGETS IN CANCER

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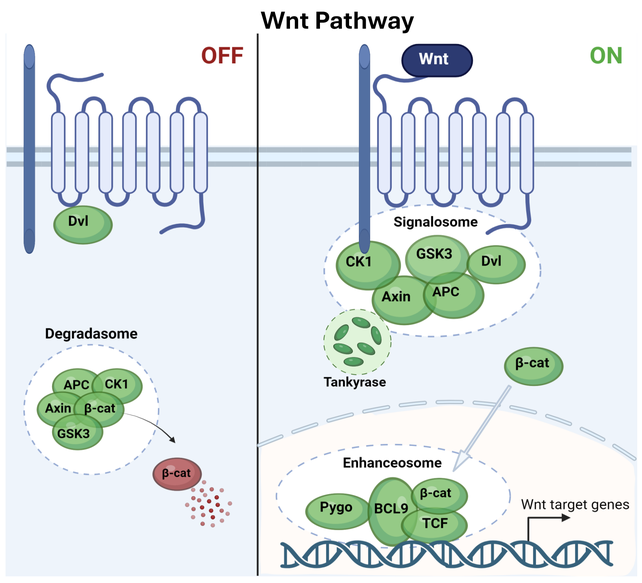
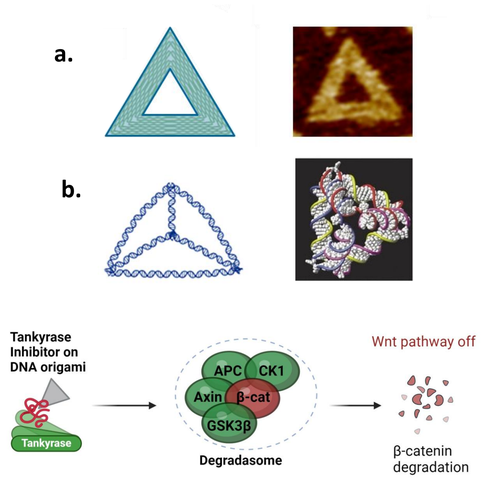
INTRODUCTION

We are developing a **modular platform of DNA nanostructures** functionalised with **peptide** therapeutics to inhibit hard-to-drug targets such as **tankyrases** (**TNKS**), which are upregulated in many cancer types. TNKS proteins are involved in the **Wnt signalling pathway**, which plays a major role in tissue homeostasis and regeneration, and carcinogenesis.

We are building on our previous work on inhibitors of TNKS [1,2] and addressing their activity and delivery to their target sites. We are using **DNA origami (**triangle and tetrahedron) as a drug delivery carrier, due to their ease-of-synthesis, water-solubility, biocompatibility, and biodegradability. DNA origami offers **exceptional design capability,** and their size and surface properties can be controlled. They can be easily customised to deliver a variety of **active molecules**, **fluorophores**, and to carry **aptamer** "anchors" that will enable attachment to specific targets.

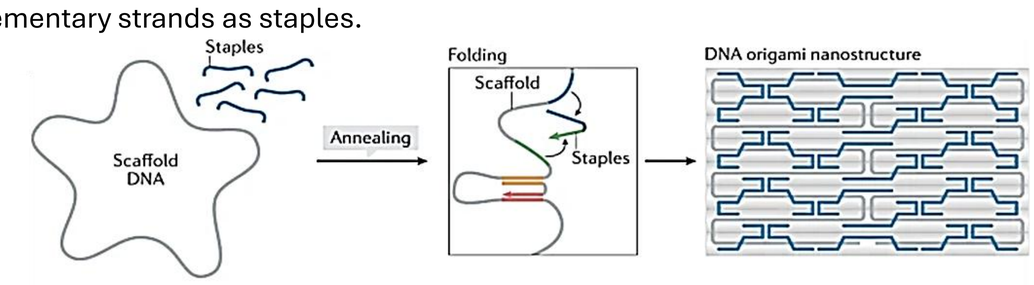


Nanostructures: 2D triangle (100 nm) and 3D tetrahedron (10 nm)

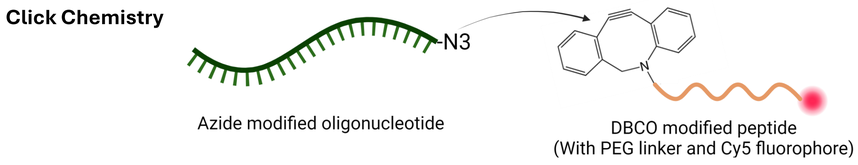


METHODOLOGY

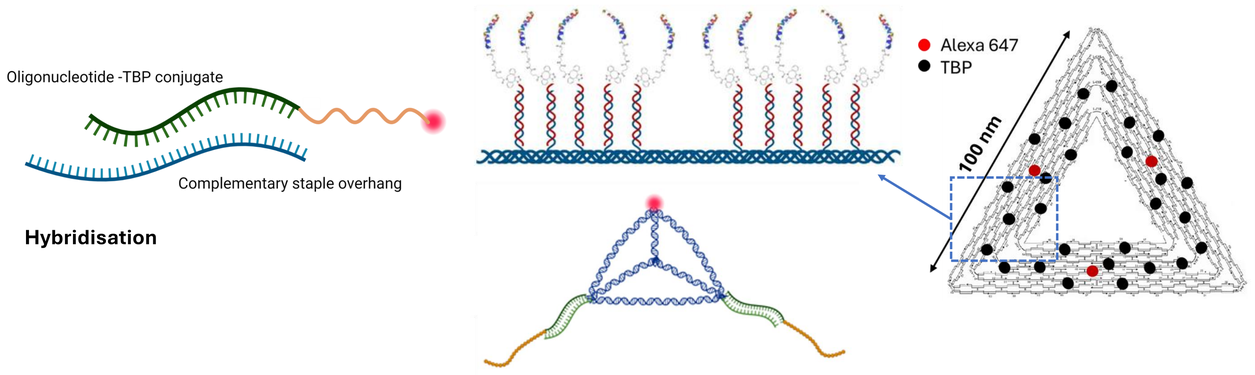
DNA origami is made by folding a long single strand of DNA into a specific shape using short complementary strands as staples.



The tankyrase binding peptide (**TBP**) is conjugated to an oligonucleotide via azide/DBCO based click-chemistry, and the unreacted peptide and oligonucleotides are removed via HPLC.

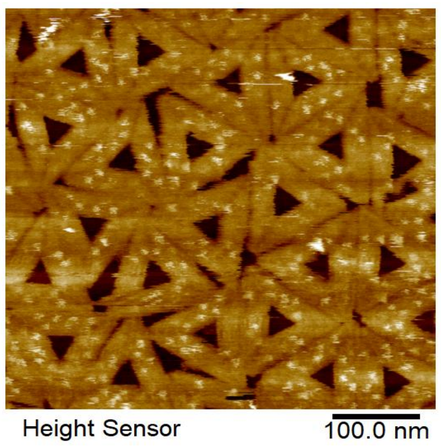


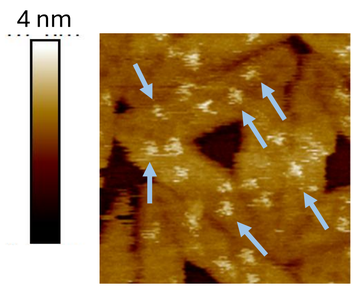
The peptide/oligonucleotide conjugate is hybridised on complementary DNA staples on the nanostructures ("sticky ends") on **predesigned** modification sites (28 modification sites per triangle and 2 per tetrahedron).

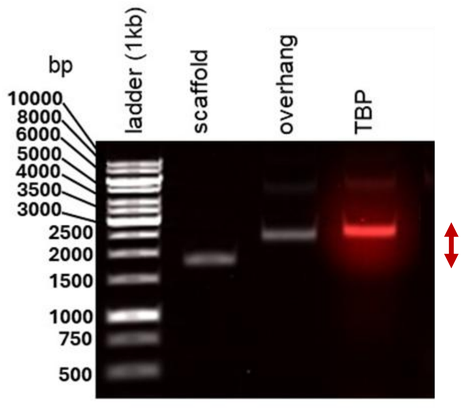


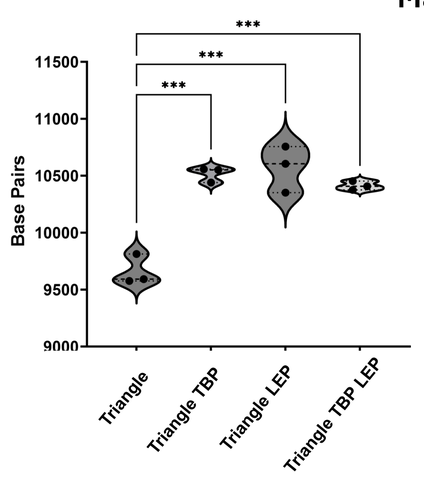
RESULTS

The nanostructures are efficiently functionalised with peptides and bind the protein-target TNKS

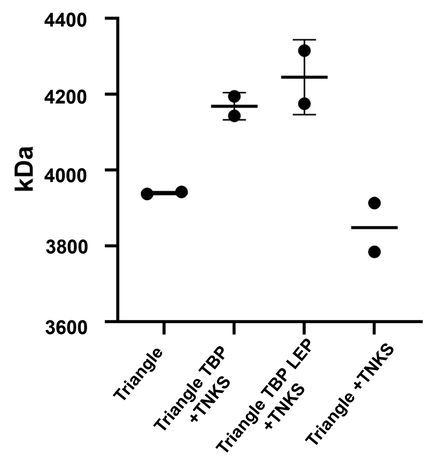




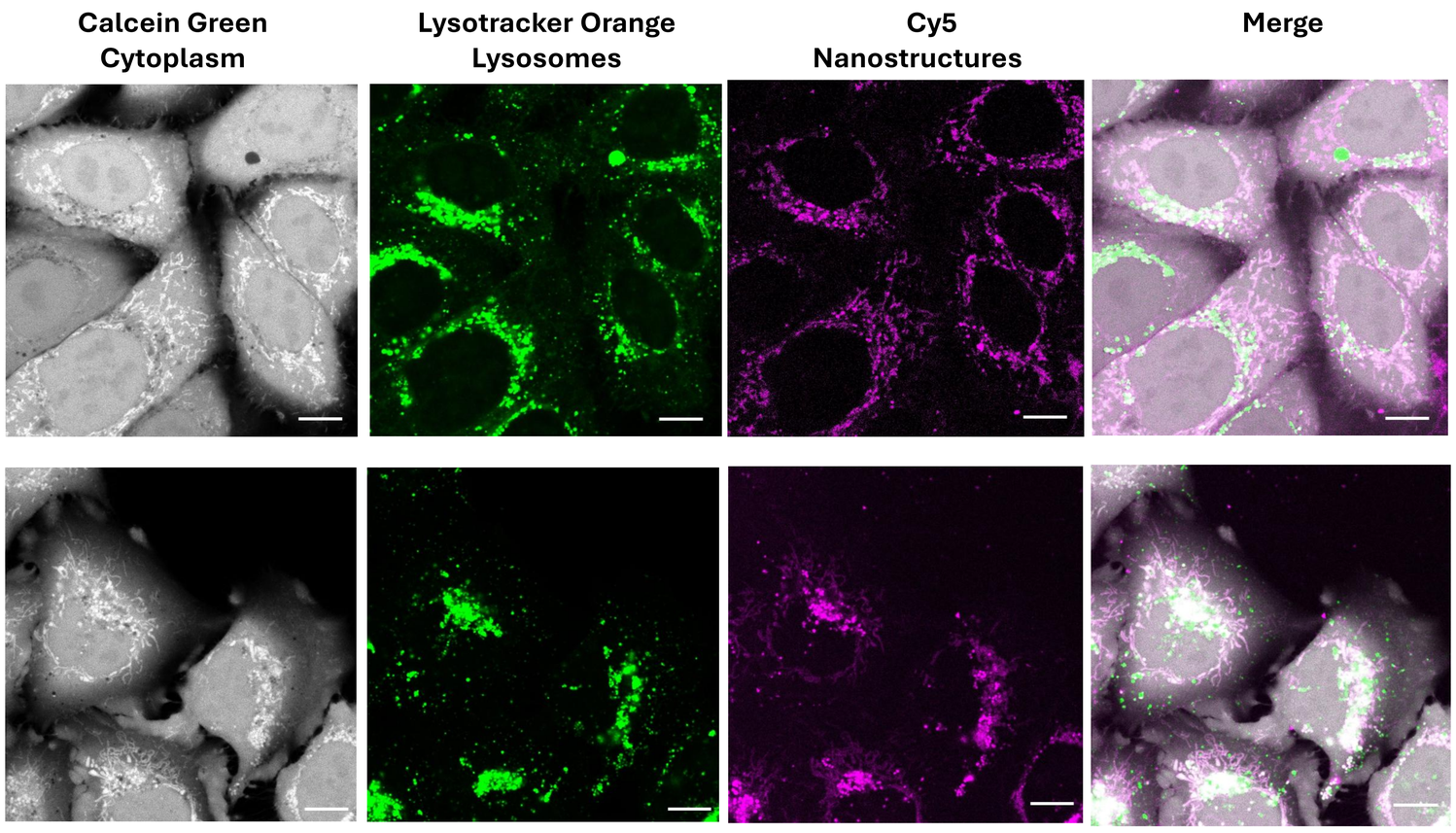


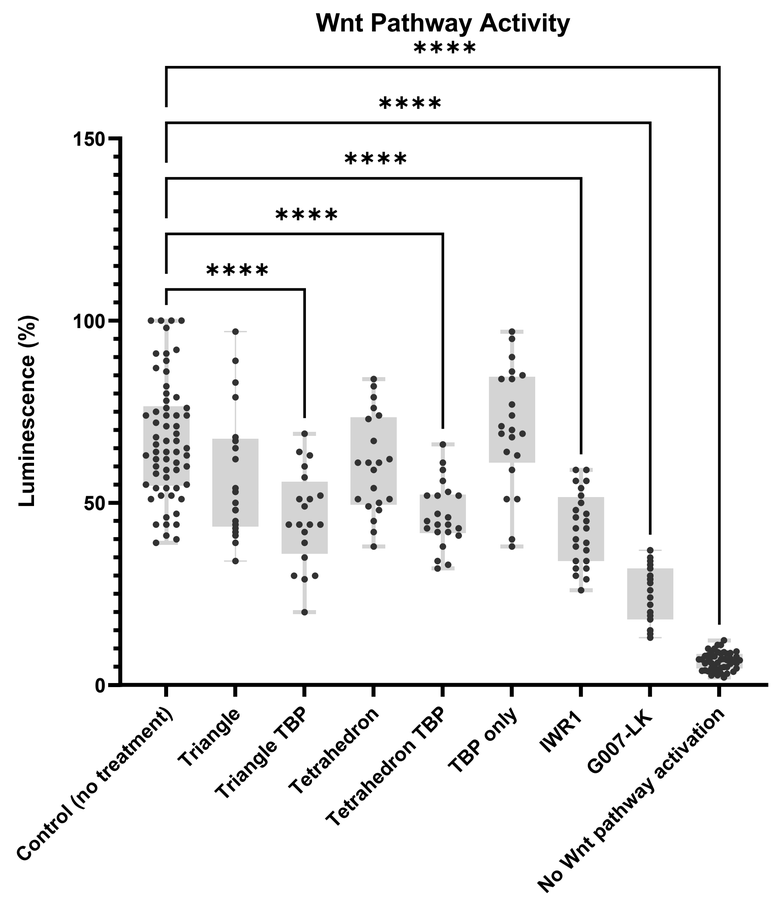


Mass Photometry



Both non-functionalised and peptide-conjugated DNA nanostructures are internalised by HeLa, HEK192, and SW480 cells upon incubation. Wnt pathway suppression is only observed with the functionalised nanostructures.





FUTURE PERSPECTIVES

- Further investigate the mechanism of nanostructure endocytosis (clathrin-mediated, caveolae) and distribution between early and late endosomes, and lysosomes.
- Assess how the properties (e.g. size, geometry, lipid encapsulation) of the nanostructures affect their sub-cellular localisation. Anchoring the nanostructures to certain receptors (transferrin) may promote receptor-mediated endocytosis.
- Use the nanostructures for targeted protein degradation by recruiting the ubiquitin/proteasome system.

REFERENCES

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