DNA nanotechnology involves utilizing DNA molecules to design and synthesize higher order nucleic acid nanostructures for several biotechnological uses. Nanopores can be self-assembled from a variety of biological building blocks including proteins, peptides, synthetic organic compounds and more recently DNA. One way to create these synthetic pores is by DNA origami. The basic principle of DNA origami being that a long DNA scaffold strand is folded with shorter DNA staple strands to yield predefined 2D or 3D folded architectures. The folding of these DNA nanostructures rely heavily on the cooperative self-assembly process of single stranded DNA sequences.

**Figure 1:** The rationally designed large membrane-spanning DNA nanopore, NP. (a) The pore is composed of squarely arranged DNA duplexes, which are illustrated as blue and orange cylinders. The latter carry cholesterol lipid anchors for membrane insertion. Protein trypsin (green) can pass via the pore from cis to the trans side of the membrane. (b) Top-down and side views of the nanopore. (c) Cross-sectional side view illustrating the geometry of the pore lumen with annotated dimensions. (T. Diederichs et al, 2019)

In this project, we will create biomimetic versions of membrane pores to advance synthetic biology, cell biological research and biotechnology. In line with the above mentioned goals, we will use AFM to visualise the topography of individual DNA nanopores immobilised on solid substrates. Additionally, we will strive to observe the morphology of these DNA transporters tagged with biotin on streptavidin monolayer on mica.

**Figure 2:** Design, shape, and dimensions of a DNA origami porin. (A) Envisioned positioning of the funnel-shaped DNA porin (red) in the lipid membrane (yellow), roughly drawn to scale. (B) Design (side and top views) and dimensions of the DNA porin with 19 cholesterol tags (orange). (C) AFM images confirming the correct assembly of the DNA origami porin. (K. Göpfrich et al, 2016)

The outcome will be structurally characterising these nanopores with respect to size and height distributions as well as confirming the accuracy of successful structural assembly via gel electrophoresis. Single molecule force spectroscopy will also be used to study binding and/or rebinding events between single stranded DNA receptors positioned within the pore lumen and their complementary strands coupled to the AFM tip.

**Tasks performed by the student:**

- AFM imaging: Using AFM to structurally characterise DNA nanopores.
- Tip-chemistry: Functionalization of AFM cantilever tips using short known DNA strands.
- Force spectroscopy: Using AFM to measure interaction forces between DNA strands on the tip and complementary strands placed within the pores.
- Data evaluation and extraction of interaction force, and chemical rate constants.

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