

Single Molecule Detection of Osteopontin in Nanopores using DNA Molecular Carriers

Micol Damiani

Prof. Shahid A. Khan , Prof Joshua B. Edel, Dr Aleksandar P. Ivanov

14th November 2019

Cholangiocarcinoma UK Annual Conference

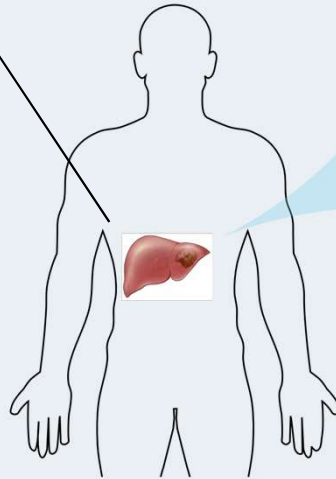
Jubilee Conference Centre – Nottingham

Cholangiocarcinoma & Osteopontin

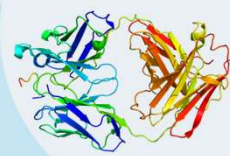
Cholangiocarcinoma

- **Symptoms** appear only at an **advanced stage**
- Tumour location limits imaging and diagnostic approaches

→ Need for reliable **diagnostic tools** for **early detection**



Osteopontin (OPN) 44 – 66 kDa

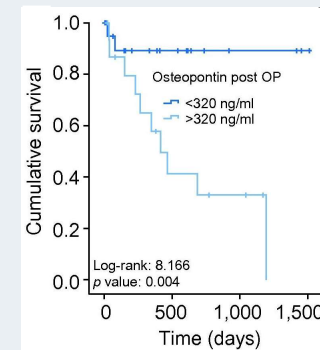
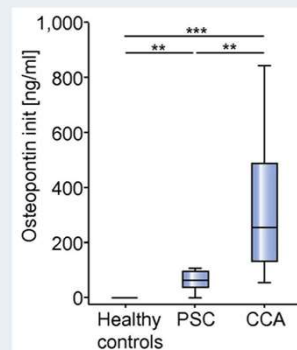


- **Overexpressed** in CCA

- Correlated to **disease aggressiveness**, lymph node metastasis, **tumour growth** and **patient survival**

→ OPN as a **biomarker**

→ Need for **OPN biosensor**



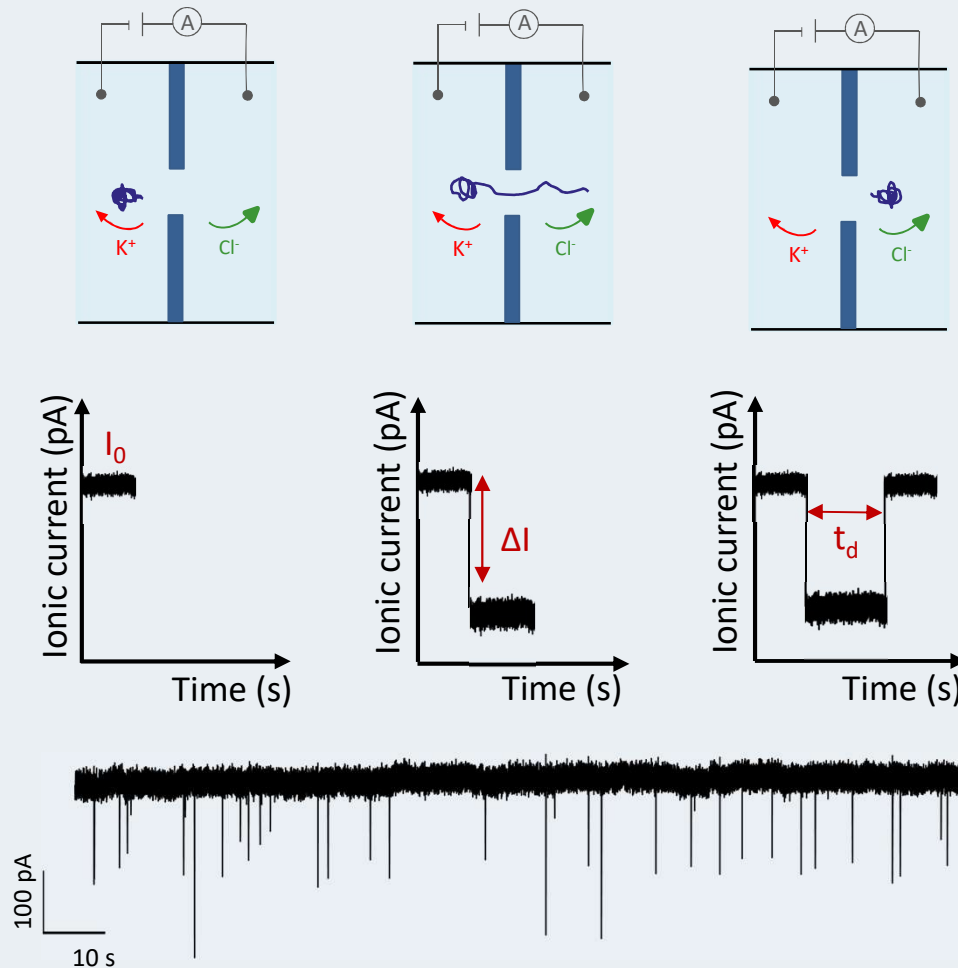
Limitations of Current Technologies

- **Limited sensitivity**
- Difficulty of probing low-abundant biomarkers in complex biofluids
- Need for **sample processing**:
 - Purification
 - Immobilisation
 - Labelling

→ Nanopore technology

- Single-molecule detection
- Label-free
- No sample processing
- Low reagent volumes

Nanopore Sensing

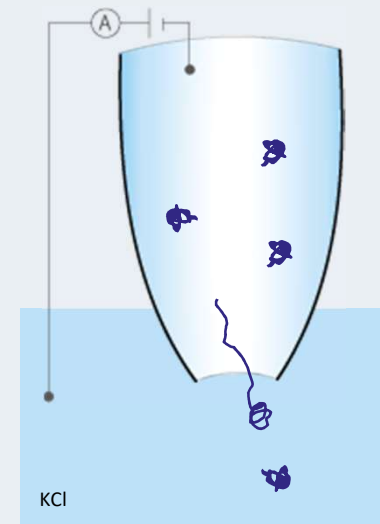
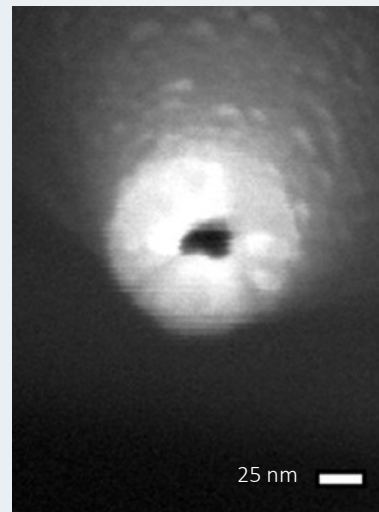
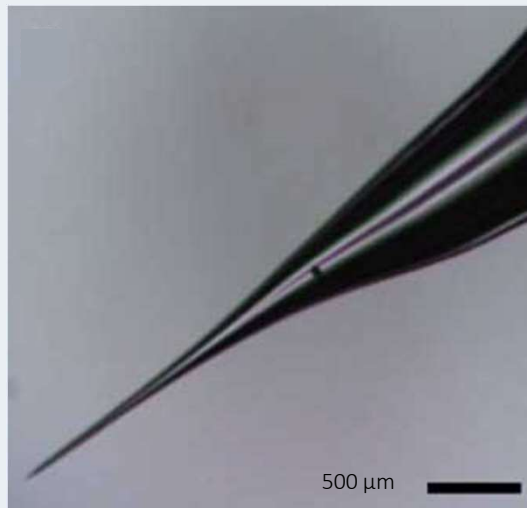


Nanopores

- Label-free high-throughput **single-molecule detection**
- **Working principle:** charged molecules driven through the pore by external bias
- **Translocation** of a molecule $\rightarrow$ ionic current temporarily modulated
- **Translocation parameters** $\rightarrow$ information about the analyte: size, shape, interactions

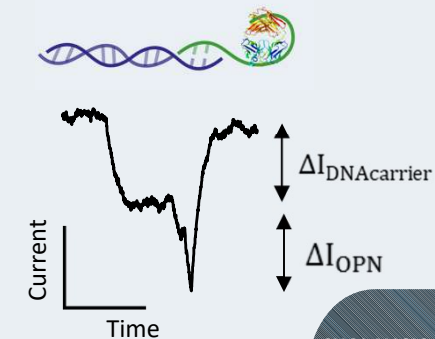
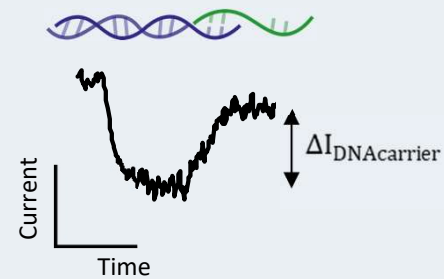
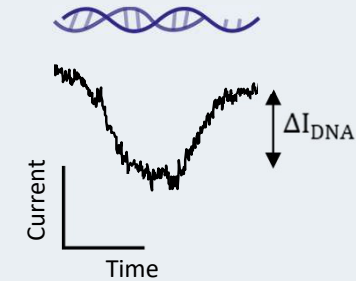
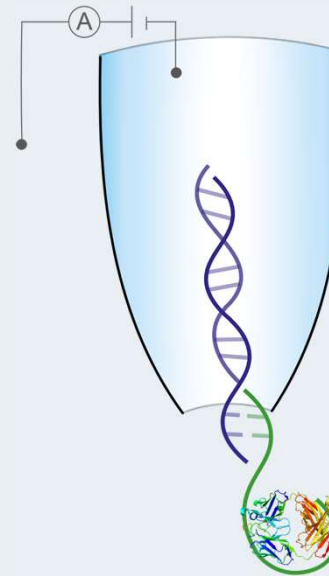
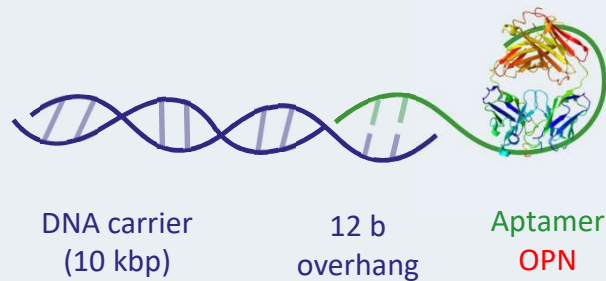
Nanopore Sensing

- Different types of nanopores exist (biological, solid state)
- **Quartz Nanopipettes:** cheap and easy to fabricate by laser-assisted pulling
- Pore size ~ 20 nm



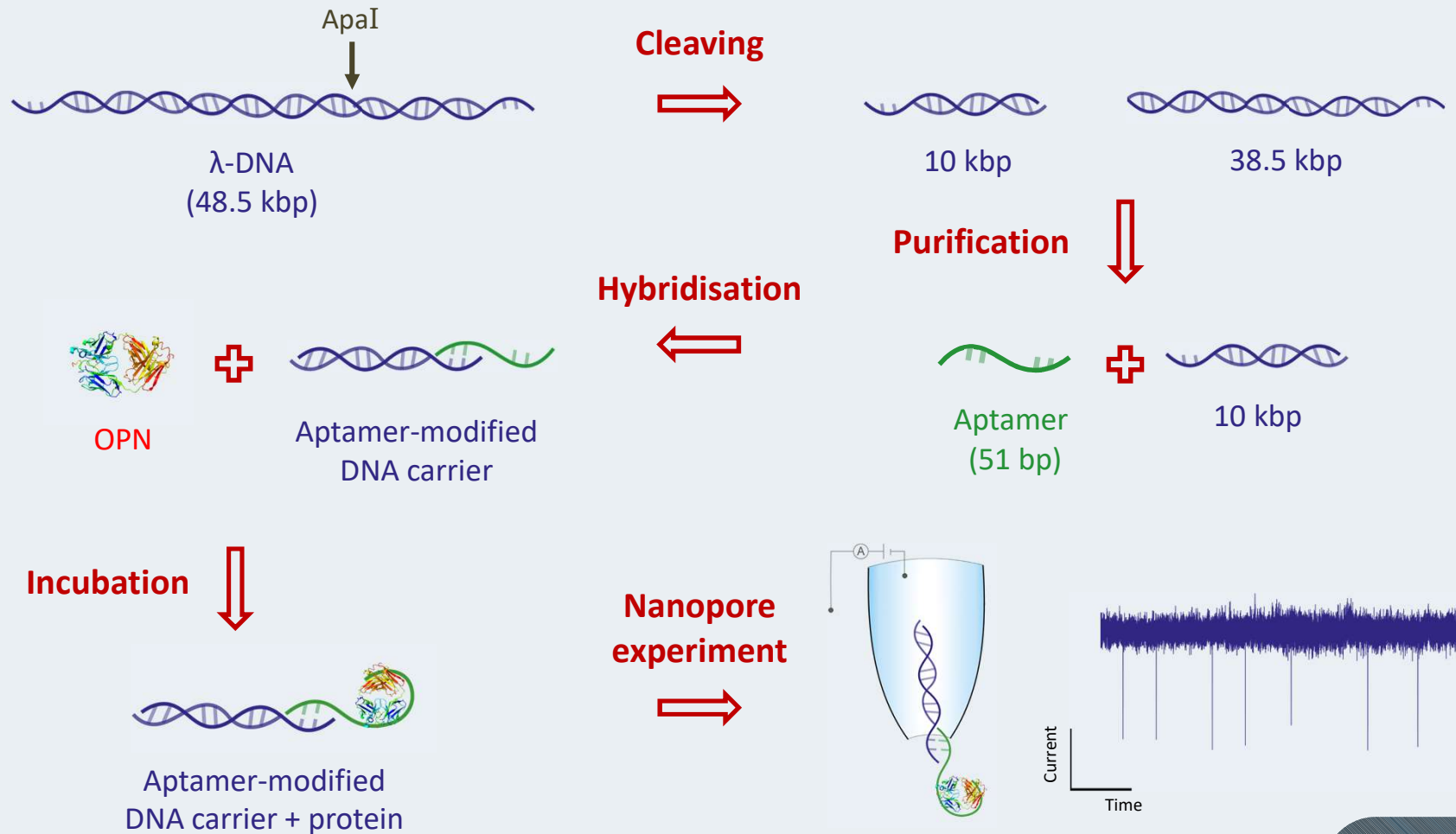
The Project: DNA Molecular Carrier to Detect Osteopontin

- Quartz Nanopipettes
- DNA Carrier:
 - 10 kbp dsDNA
 - ssDNA aptamer specific for OPN



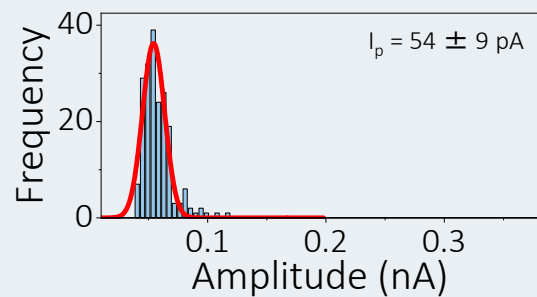
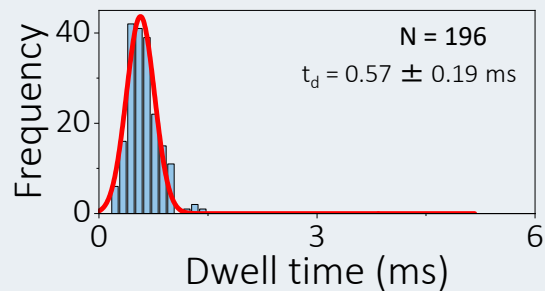
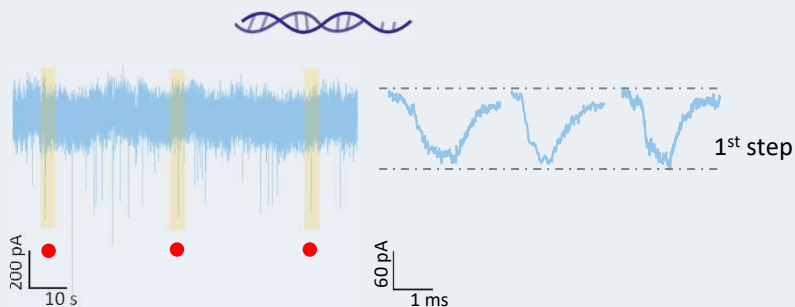
- **Aptamers** → **specific detection** of target
- Negative DNA backbone → **efficient transport**
- **How?**
 - By analysing the **shape of the translocation event**

Protocol: DNA Carrier Preparation Steps

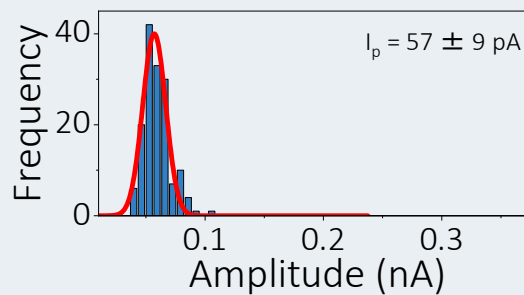
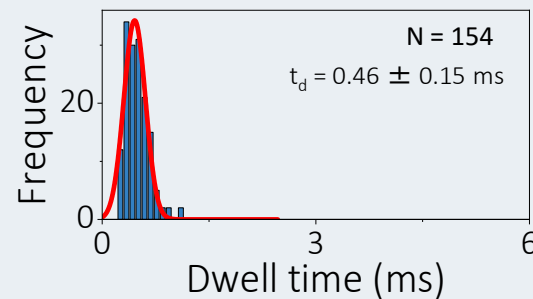
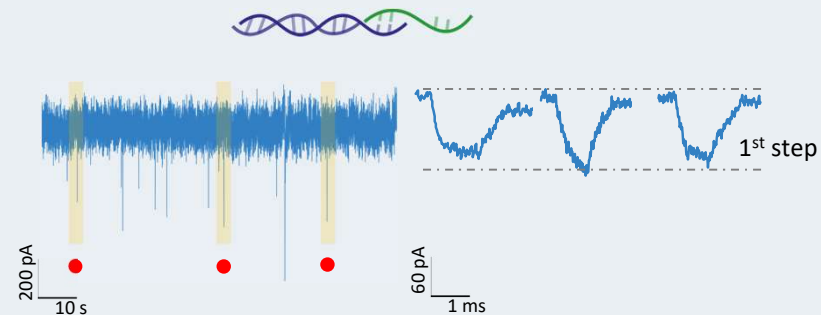


Results: Carrier Characterisation

Carrier

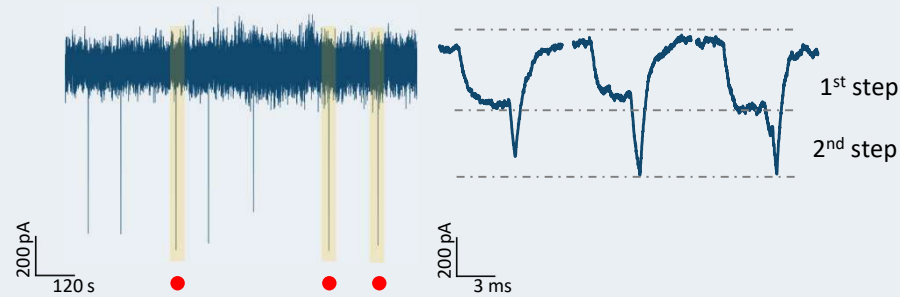


Aptamer-modified Carrier



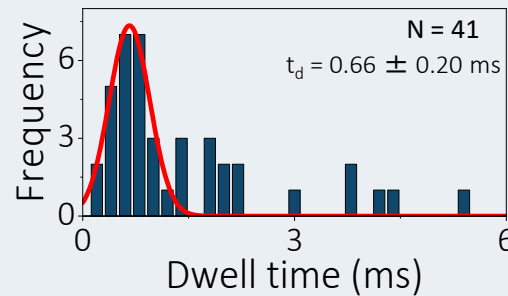
Results: Osteopontin Detection

Aptamer-modified Carrier + OPN

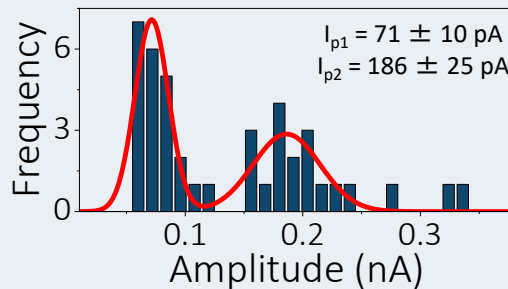


Clear sub-peak in
25% of translocation
signals

Increase in the duration
of the translocation



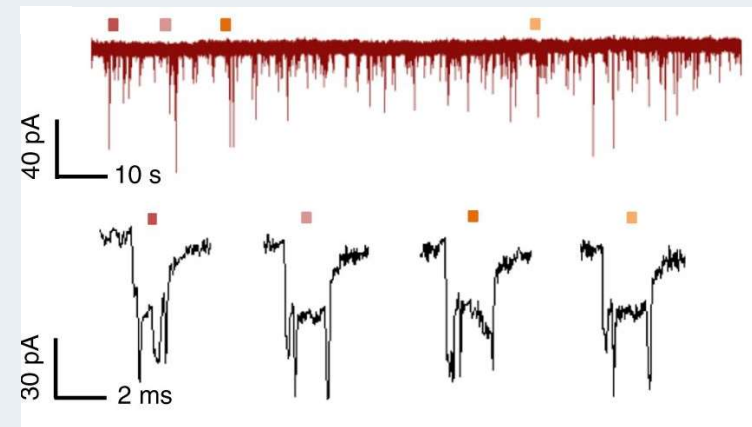
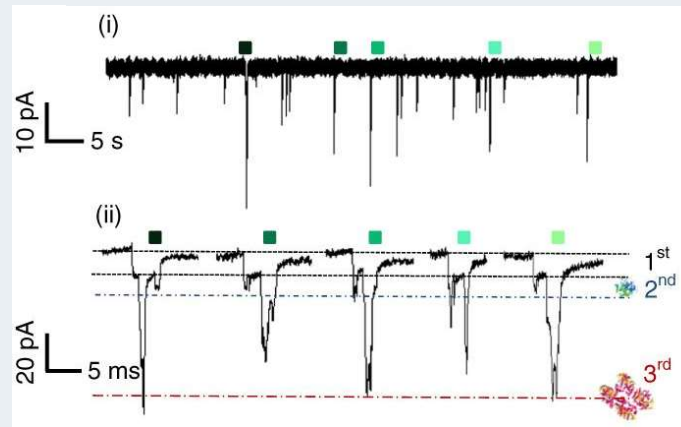
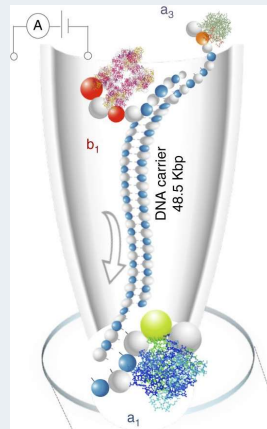
Increase in the signal
amplitude



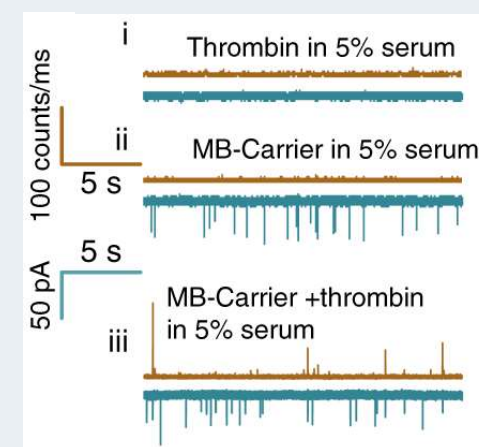
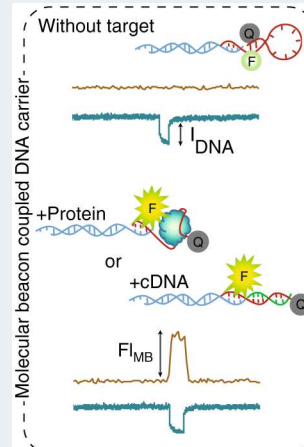
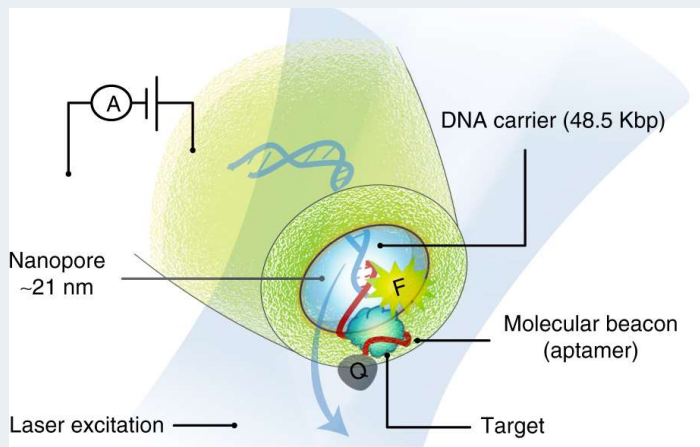
→ Successful detection of
osteopontin

Previous Results with DNA Molecular Carriers

Multiplexed detection in human serum



Pairing electrical and optical detection



Conclusions

- **Fabrication** of a reliable and reproducible **DNA molecular carrier**
- Preliminary results that confirm the **successful binding** of OPN and DNA carrier and **successful detection** of the complex

Future Work

- Additional optimisation of **aptamer binding** conditions and stability
- Repeat DNA carrier experiments with **different ratios of DNA:OPN**
- Add more tumour biomarkers to the detection (**multiplexed sensor**)
- Test the optimised sensors on patient's samples

THANK YOU!!



Prof Shahid Khan



Prof Joshua Edel



Dr Aleksandar Ivanov



- The Institute of Chemical Biology at Imperial College London
- The Virdee family