HIGH-THROUGHPUT SYNTHESIS AND SCREENING OF OPTICAL PROBES FOR ALTERNATIVE DNA STRUCTURES IN CELLS

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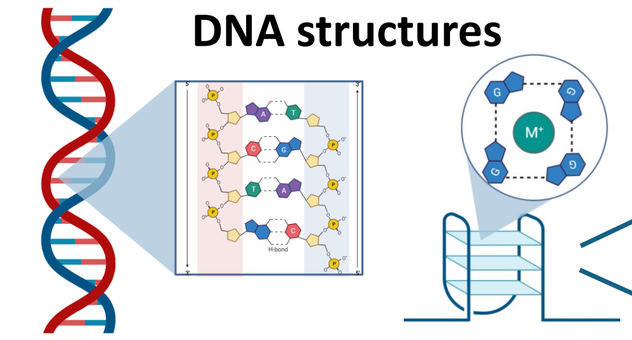
IMPERIAL

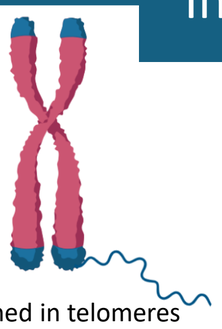
Introduction

DNA structures

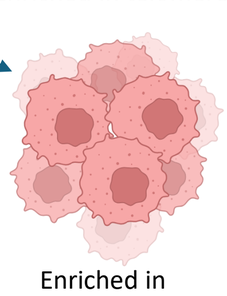


Regular **Duplex DNA**

G-quadruplex **(G4) DNA**



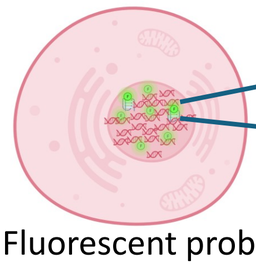
Enriched in telomeres

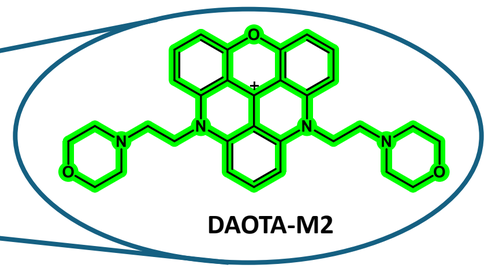


Enriched in cancer-causing genes

While the double helix (**duplex**) is the most well-known structure of DNA; it is not the only form DNA can take. One of the most stable alternative structures is G-quadruplex (**G4**) DNA.[1] G4s are interesting biological targets because they have shown to be related to DNA replication, cell maintenance and cancer.

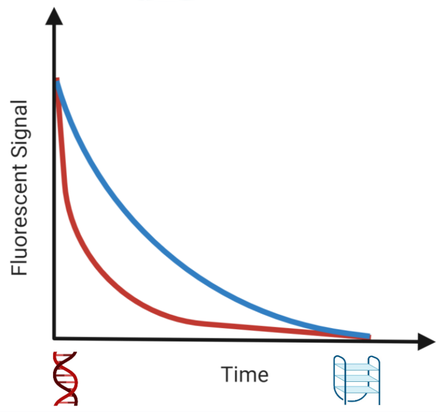
Visualising G4s





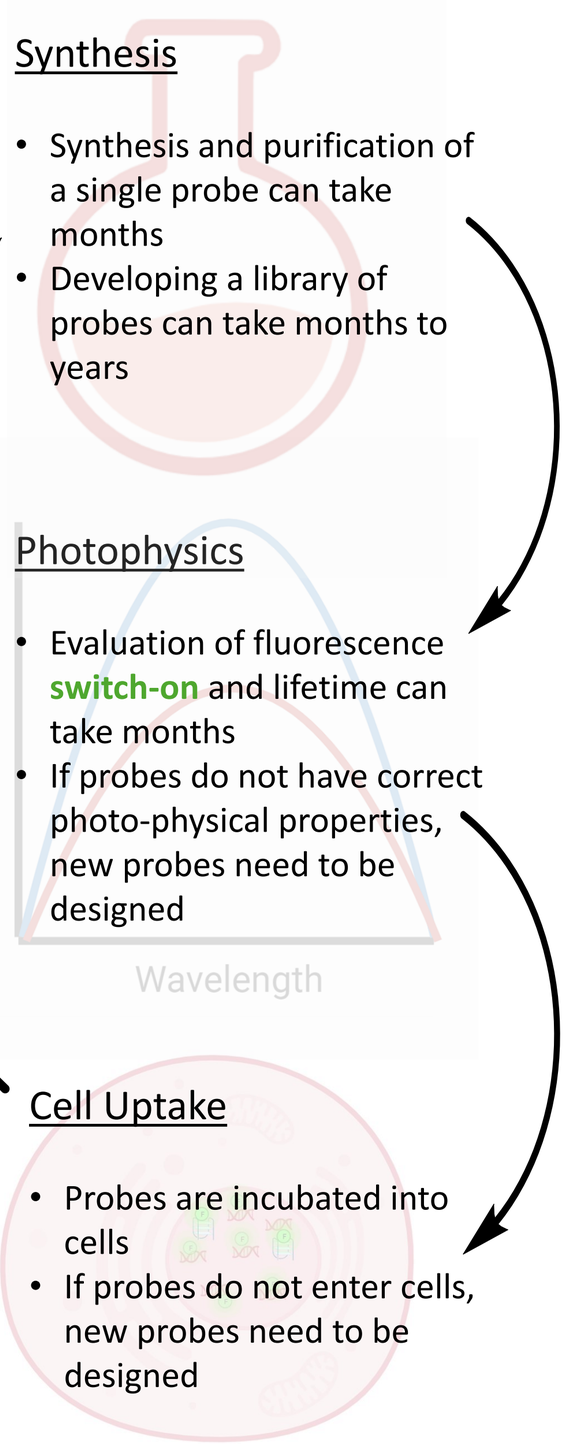
Fluorescent probes predominantly use **switch-on (brightness)** to differentiate between DNA topologies, however in live cells it can be tricky to detect G4s because they are so rare.

Our group uses fluorescence lifetime, which monitors how fast the signal decays rather than how bright it is, to differentiate between DNA topologies, which allows to uncover even very rare events.[2]



Results and Discussion

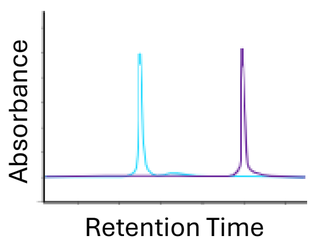
A. Traditional methods

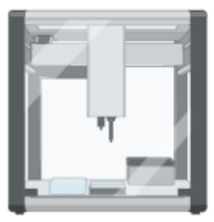


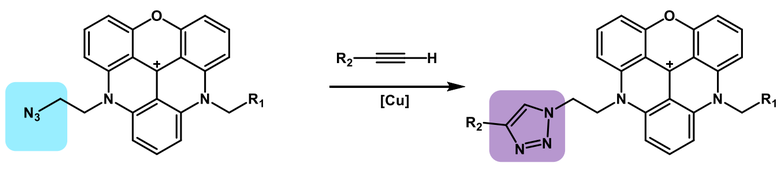
Can take YEARS

1. Synthesis

- Uses "Click" Chemistry
- Requires no purification, monitored via LC-MS
- 32 reactions at the same time as it takes for 1 using traditional methods
- **21/32 successful hits**

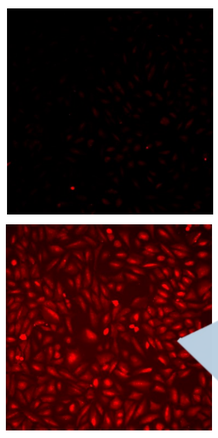


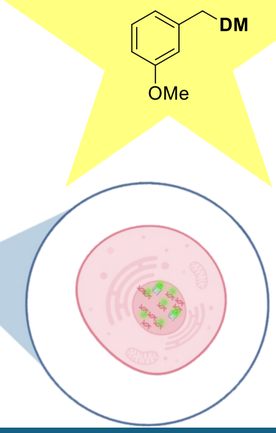




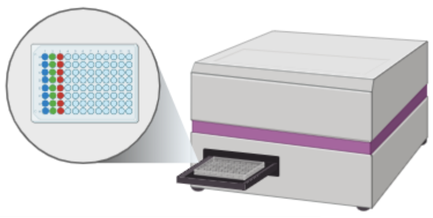
3. Cell Uptake

- Probes were incubated in bone cancer cells to monitor uptake
- Images were collected using an automated microscope
- Cell uptake was measured within 1 hour, the brighter the image, the more uptake of the probe
- **2/32 successful hits**





B. High-throughput platform

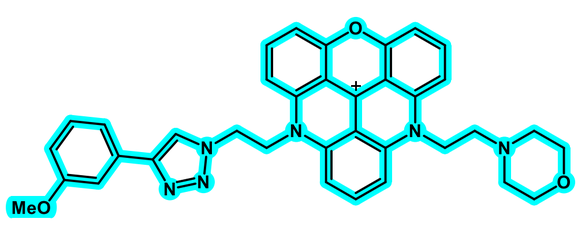


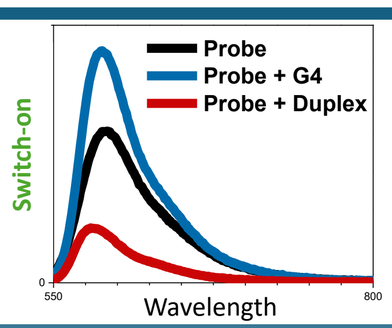
2. Photophysics

- Probes were mixed with duplex DNA and G4s in 96-well-plate
- Using a plate reader, the switch-on and fluorescence lifetime could be measured for each combination within 1 day to see if there was any difference of the probe with duplex vs G4 DNA
- **9/32 successful hits**

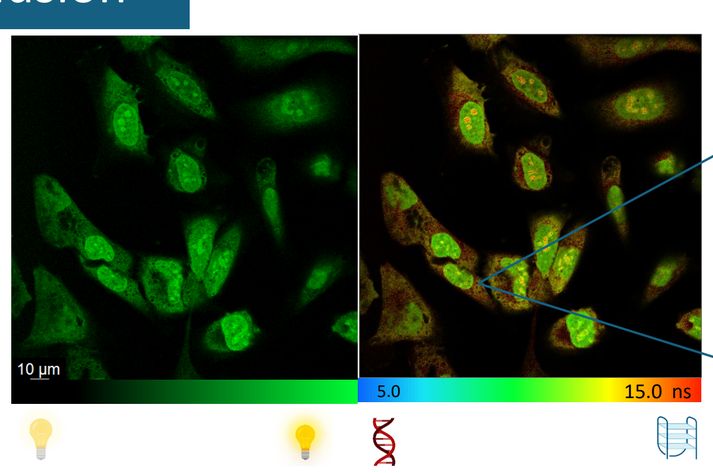
3 DAYS from synthesis to optimal probe

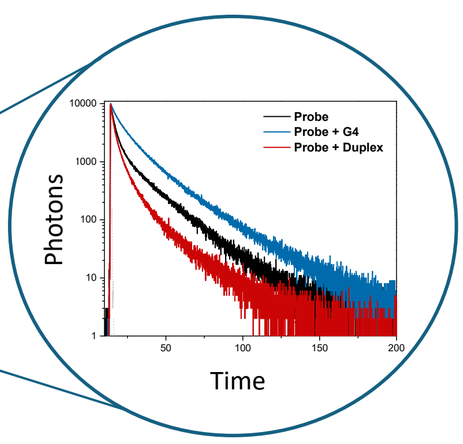
Conclusion





- Created platform for the fast discovery and selection of optimal G4 probe (potential pathway is **3 DAYS** rather than **YEARS**)!
- Probes capable of differentiating between G4 and duplex DNA via fluorescence lifetime in live cells.





Each pixel is a fluorescence lifetime decay

References

1. J. Spiegel, S. Adhikari and S. Balasubramanian, *Trends. Chem.*, 2020, **2**, 123–136.
2. P. A. Summers, B. W. Lewis, J. Gonzalez-Garcia, R. M. Porreca, A. H. M. Lim, P. Cadinu, N. Martin-Pintado, D. J. Mann, J. B. Edel, J. B. Vannier, M. K. Kuimova and R. Vilar, *Nat. Commun.*, 2021, **12**, 1–11.