Doing more with less: low mass, affinity measurement using variable-length nanotethers

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Summary

Nanotether Discovery Science (NDS) is a technology spin-out from Cardiff University, UK. The Company is in the process of developing its patented nanotether technology for applications in high throughput screening and compound profiling.

Nanotether technology utilises variable length DNA molecules as tethers for binding biomolecules in close proximity, thereby reducing the volume of proteins and ligands. Effectively, a nano-scale 'reaction chamber' is produced around each interaction pair, which enables very high density multiplex chips to be printed.

Protein targets are tethered to the end of one arm of a Y-shaped molecule, with the ligand attached to the second arm, hence increasing the effective concentration. The ligand can be a small molecule, peptide or another protein, thereby enabling a broad range of targets, including protein-protein binding and epigenetics.

Nanotether Advantages

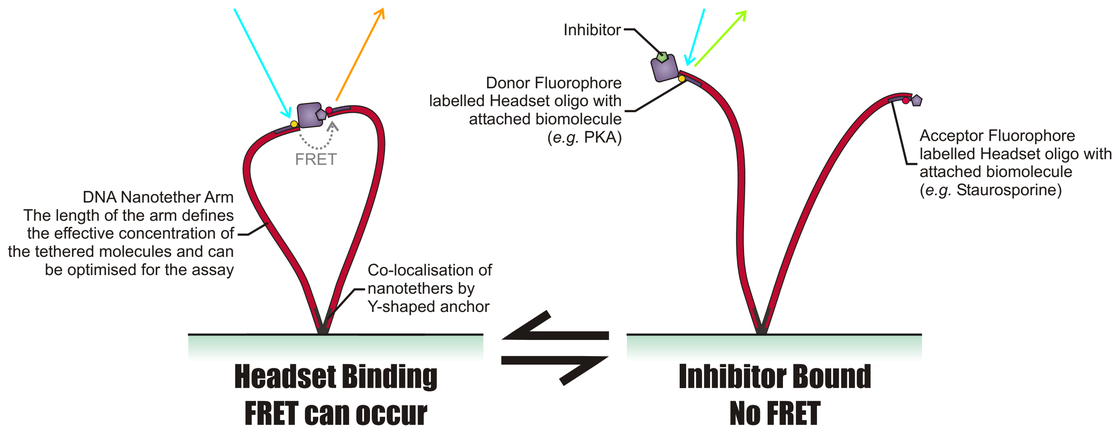
- Flexible assay system
- Multiple target classes, including low affinities
- Highly parallel affinity measurements
- Kinetic measurements
- High sensitivity
- Significant reduction in reagents

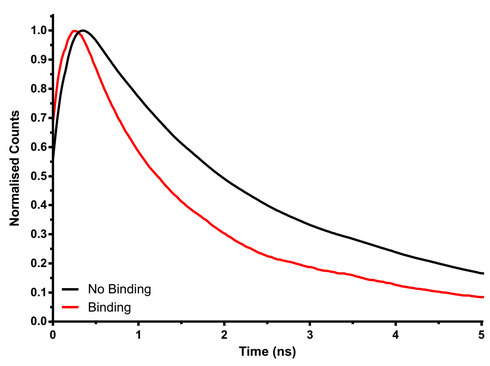
Nanotether Applications

The nanotether technology is being developed for applications including:

- Multiplexed high throughput and secondary screening
- Compound profiling
- Measurement of protein-protein interactions
- Measurement of protein-small molecule interactions
- Epigenetics

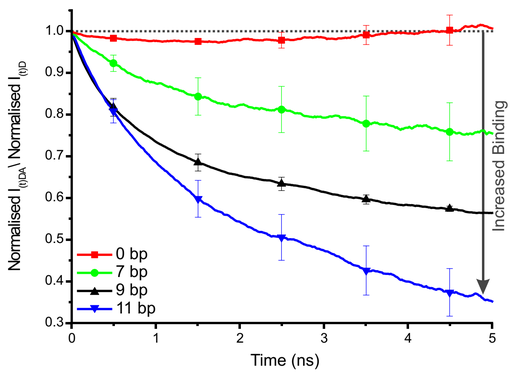
Nanotether Technology





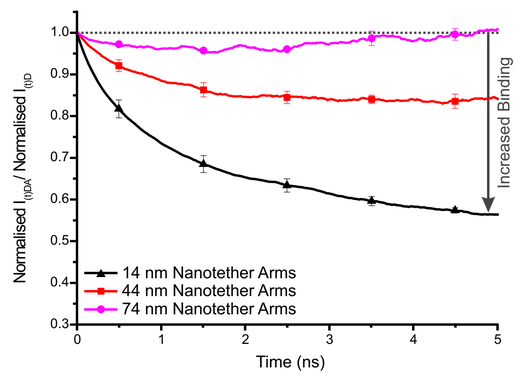
The difference between the bound and unbound states of the nanotether can be clearly seen in the donor fluorphore lifetime data and easily converted to more useful measurements such as the fraction of headsets undergoing binding.

Nanotether Assays



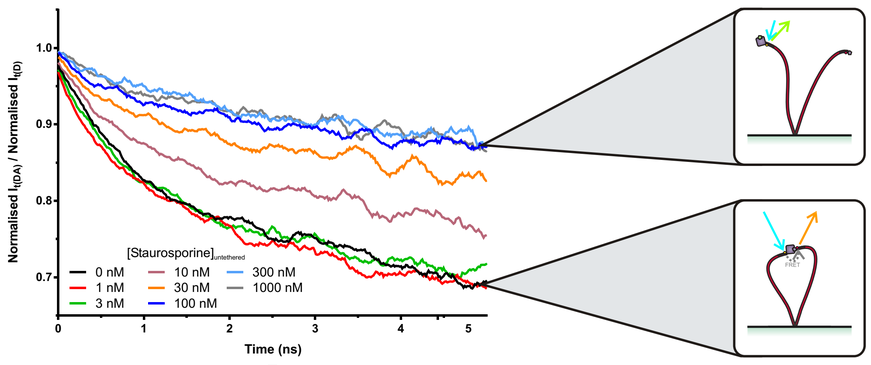
The binding of complementary DNA sequences provides a simple system to study nanotether behaviour. Shorter DNA overlaps have lower affinities for each other. When attached to the nanotether, lower levels of binding are observed with the shorter overlaps compared to the longer ones.

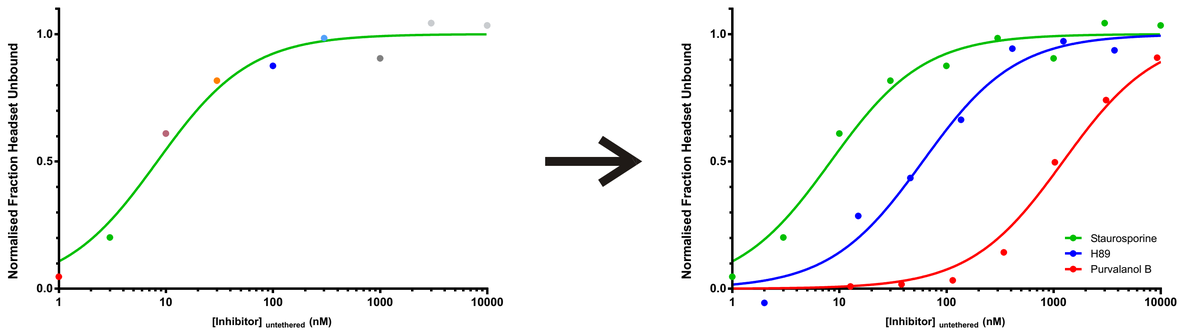
Increasing the length of the nanotether arms increases the volume which can be explored by the tethered molecule. This reduces the effective concentration of the tethered molecule and thus reduces the amount of binding measured.



Competitive Inhibitor Assays

In this example assay the catalytic subunit of PKA has been attached to the end of one nanotether arm and Staurosporine (an ATP-competitive inhibitor) to the end of the other arm. Various concentrations of test compounds can be introduced into the system to form a competitive binding assay.

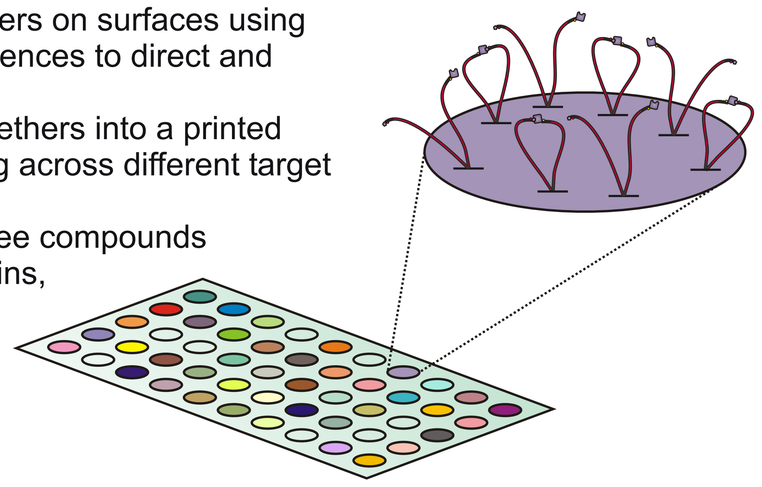




Staurosporine: IC_{50} = 10 nM (95%CI 8 - 13 nM, n = 9); H89: IC_{50} = 0.11 µM (95%CI 0.06 - 0.19 µM, n = 5); Purvalanol B: IC_{50} = 0.83 µM (95%CI 0.71 - 0.98 µM, n = 7)
Only representative data from a single spot (replicate) is shown on the graphs.

Current & Future Developments

- Reduction of protein requirements to allow headset production through *in vitro* translation (IVT)
- Self-assembly of nanotethers on surfaces using complementary DNA sequences to direct and immobilise headsets
- Fabricating spots of nanotethers into a printed array, allowing multiplexing across different target classes
- Use of fluidics to deliver free compounds to nanotether bound proteins, allowing both further multiplexing and measurement of binding kinetics



References

[1] Perrins, R.D., Orchard C., Zavodszky M., Kasry A., Nikolaev N., Harwood A., Borri P. & Dale T. (2011) Analytical Chemistry **83**, 8900–8905
[2] Kasry A., Borri P., Davies P.R., Harwood A., Thomas N., Lofas S. & Dale T. (2009) ACS Appl. Mater. Interfaces **1**, 1793 - 1798
[3] Advances Wales (2013) **68**, 13
[4] Patent Application: PCT/GB2006/004208