

Developing Probes to Target c-Myc i-Motif DNA?



@SRosonovski

Summer S. Rosonovski, Christopher J. Morris, & Zoë A. E. Waller

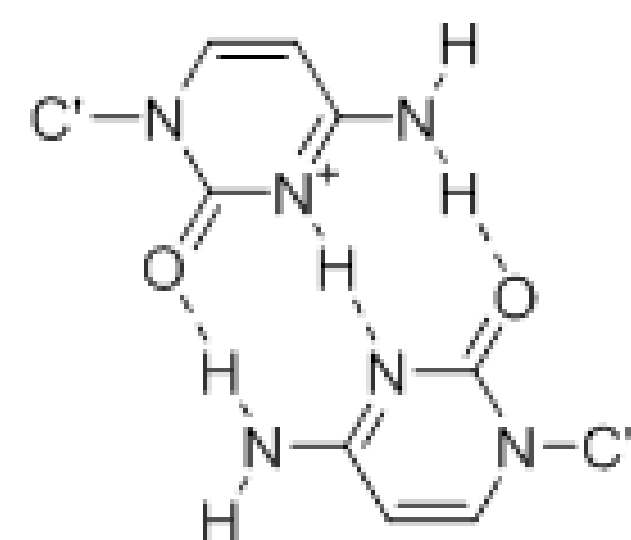
School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ



s.rosonovski@uea.ac.uk

What is i-Motif DNA?

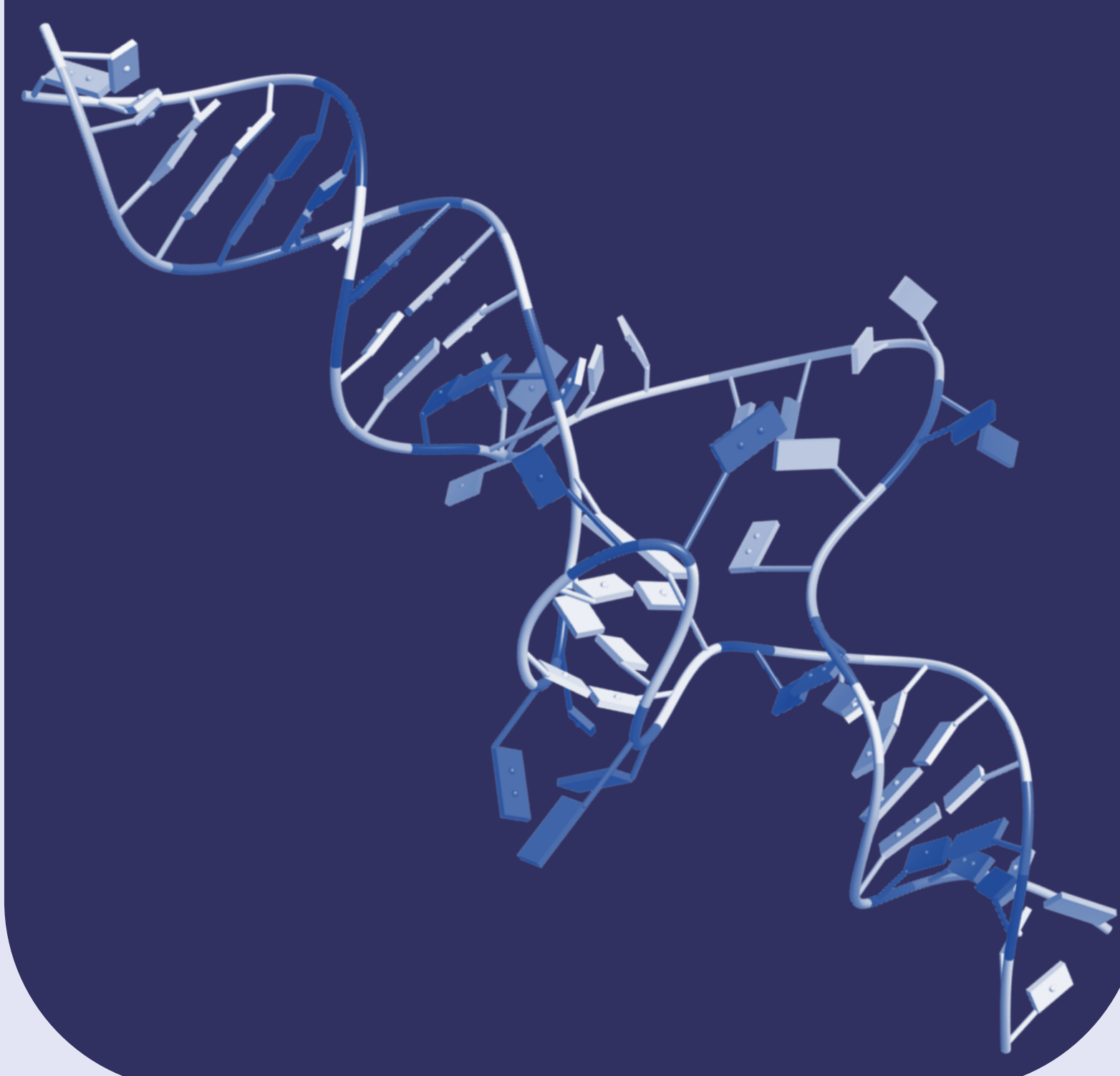
- Formed by C-rich sequences
- Two parallel duplexes intercalated in an anti-parallel orientation
- Stabilised by hemiprotonated C-C⁺ base pairs



- Evidence suggests regulate gene expression
- Potential to be powerful therapeutic targets

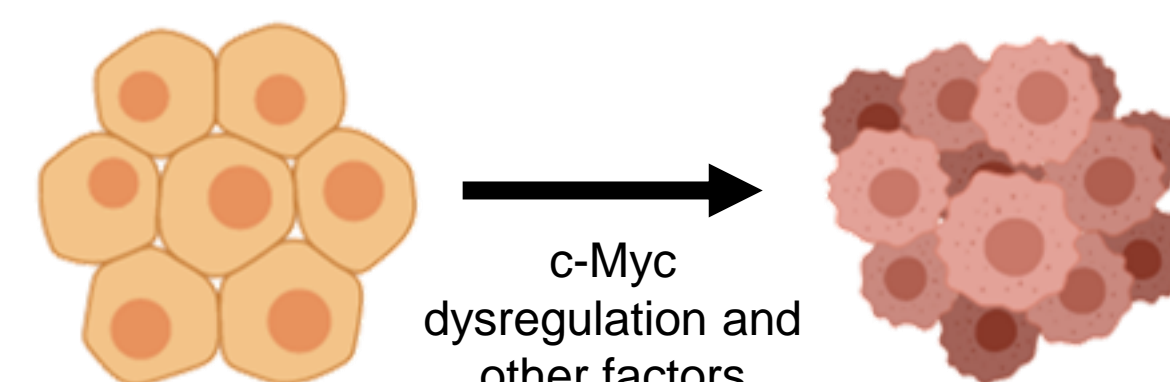
Aims

- Identify c-Myc i-motif binding peptides
- Identify c-Myc i-motif small molecules



Why Target the c-Myc i-Motif?

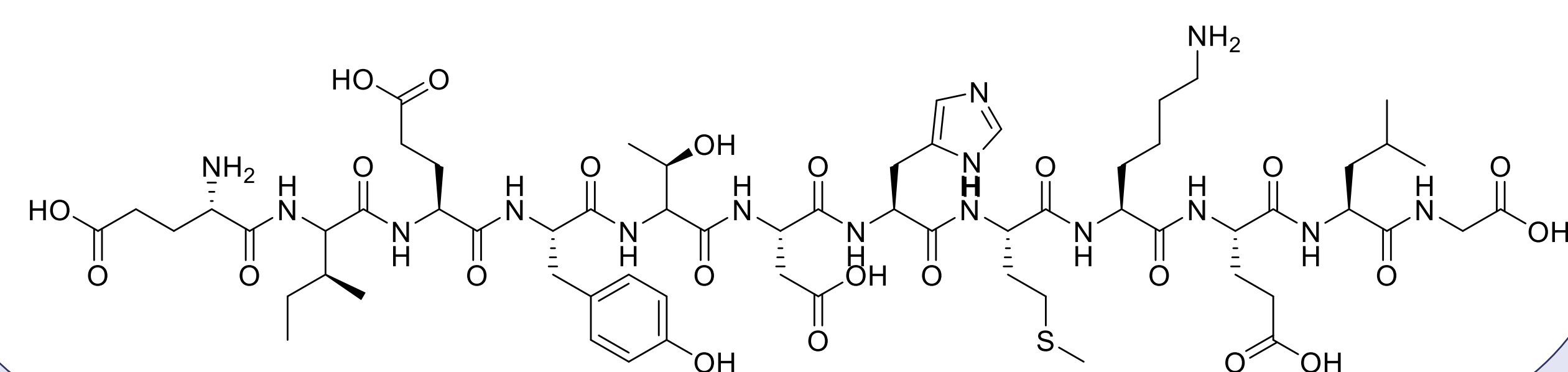
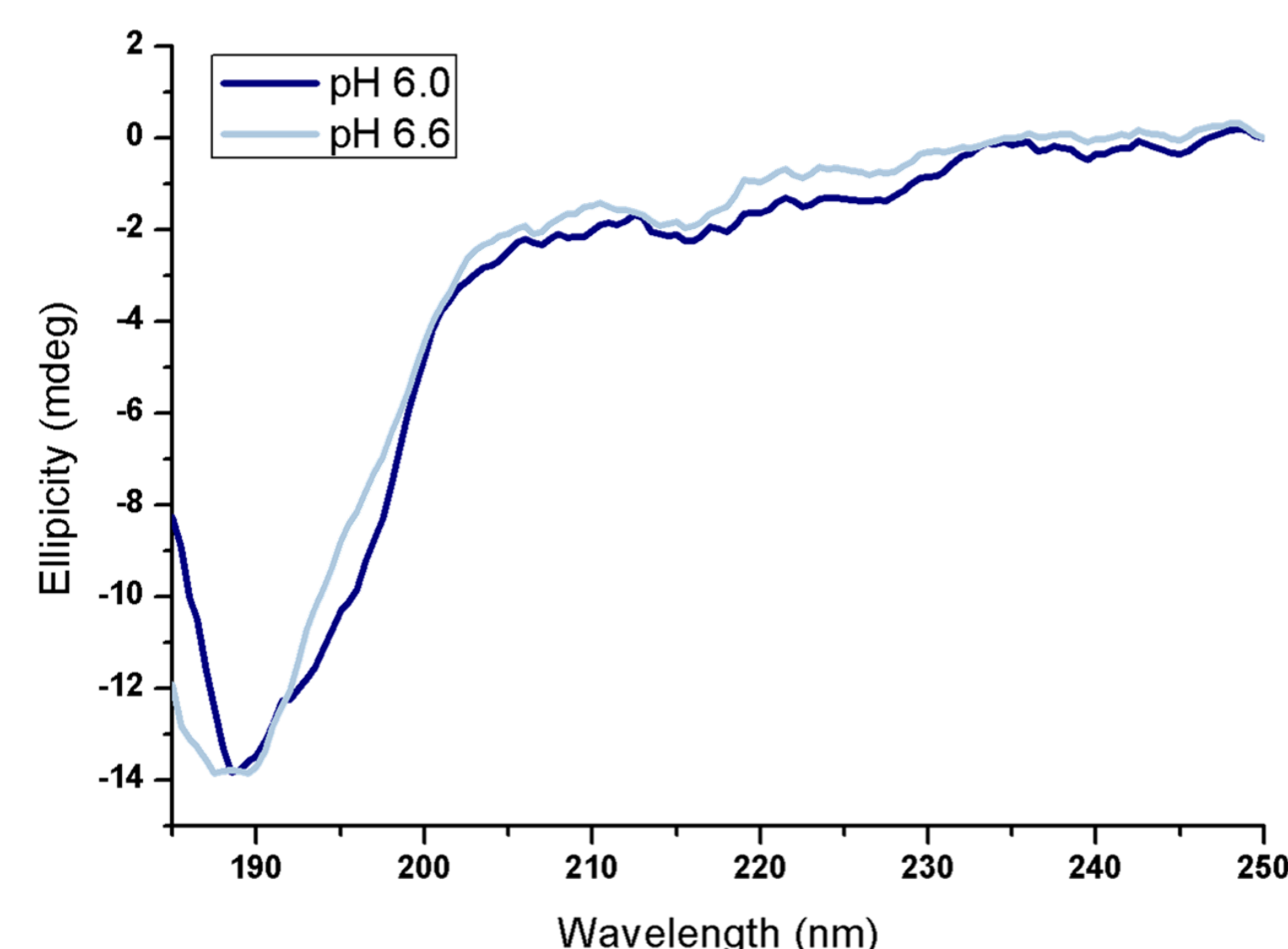
- c-Myc is a master regulator controlling many aspects of cell growth and metabolism
- Expression is tightly controlled by multiple mechanisms
- Frequently dysregulated in cancer but challenging to target



- The regulation element NHE III₁ controls 80-90% c-Myc transcription
- NHE III₁ forms noncanonical DNA structures that likely regulate transcription

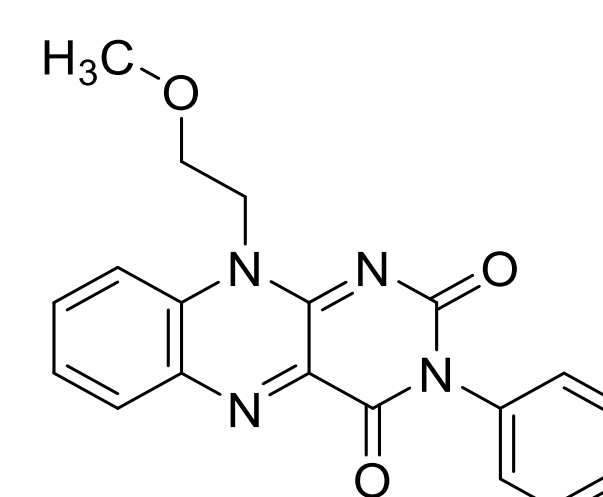
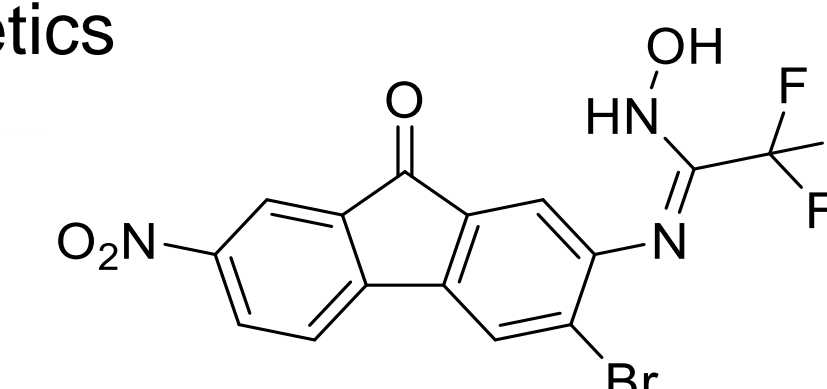
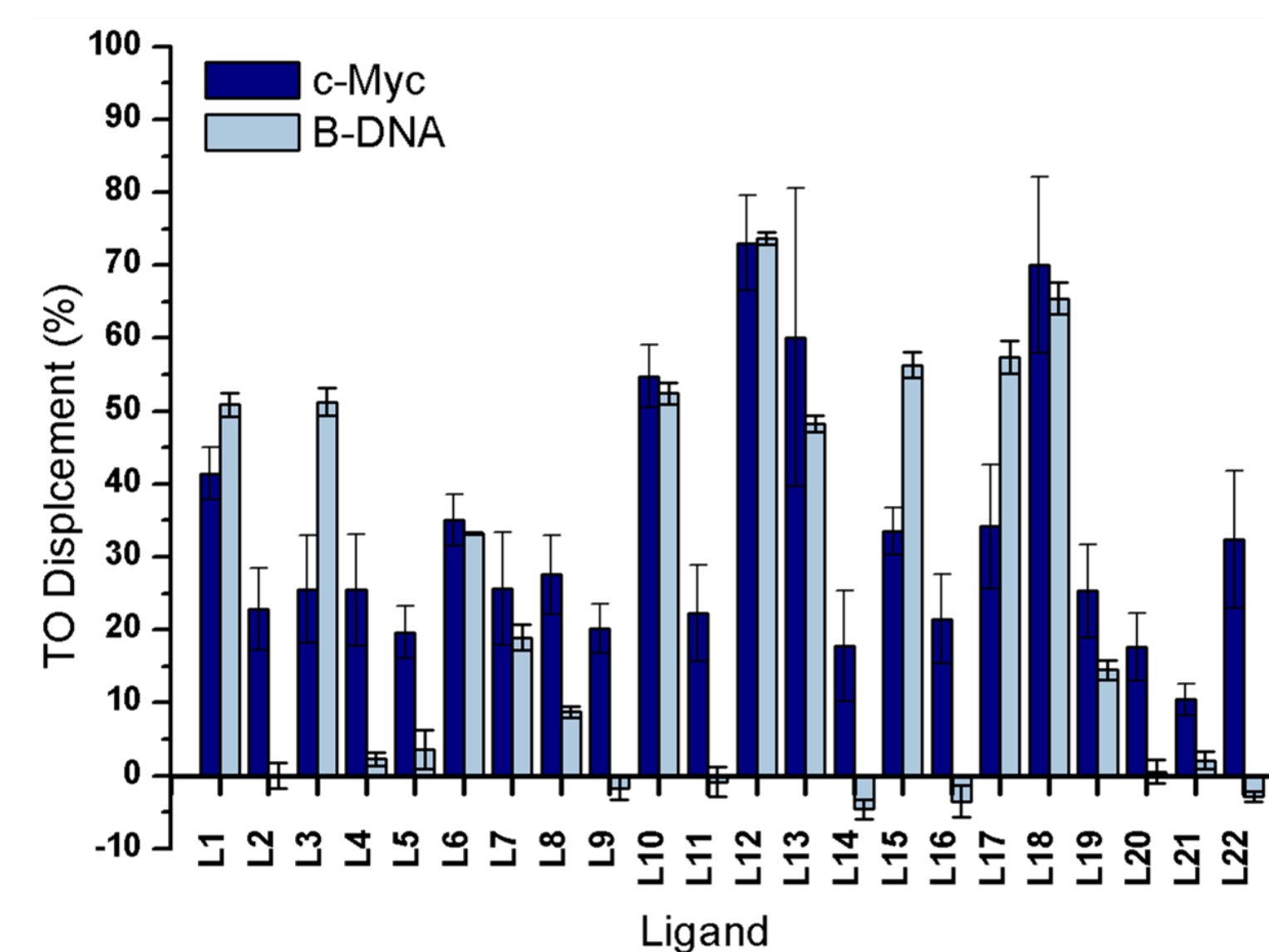
Phage Display Results

- 1x10⁹ 12-mer peptides and 1x10⁹ 7-mer cyclic peptides were screened
- Five 12-mer peptides identified as binders
- No 7-mer cyclic peptides were identified
- 80% of sequences Pep5, suggesting it is the best peptide probe
- Circular dichroism determined Pep5 is a random coil



FID Results

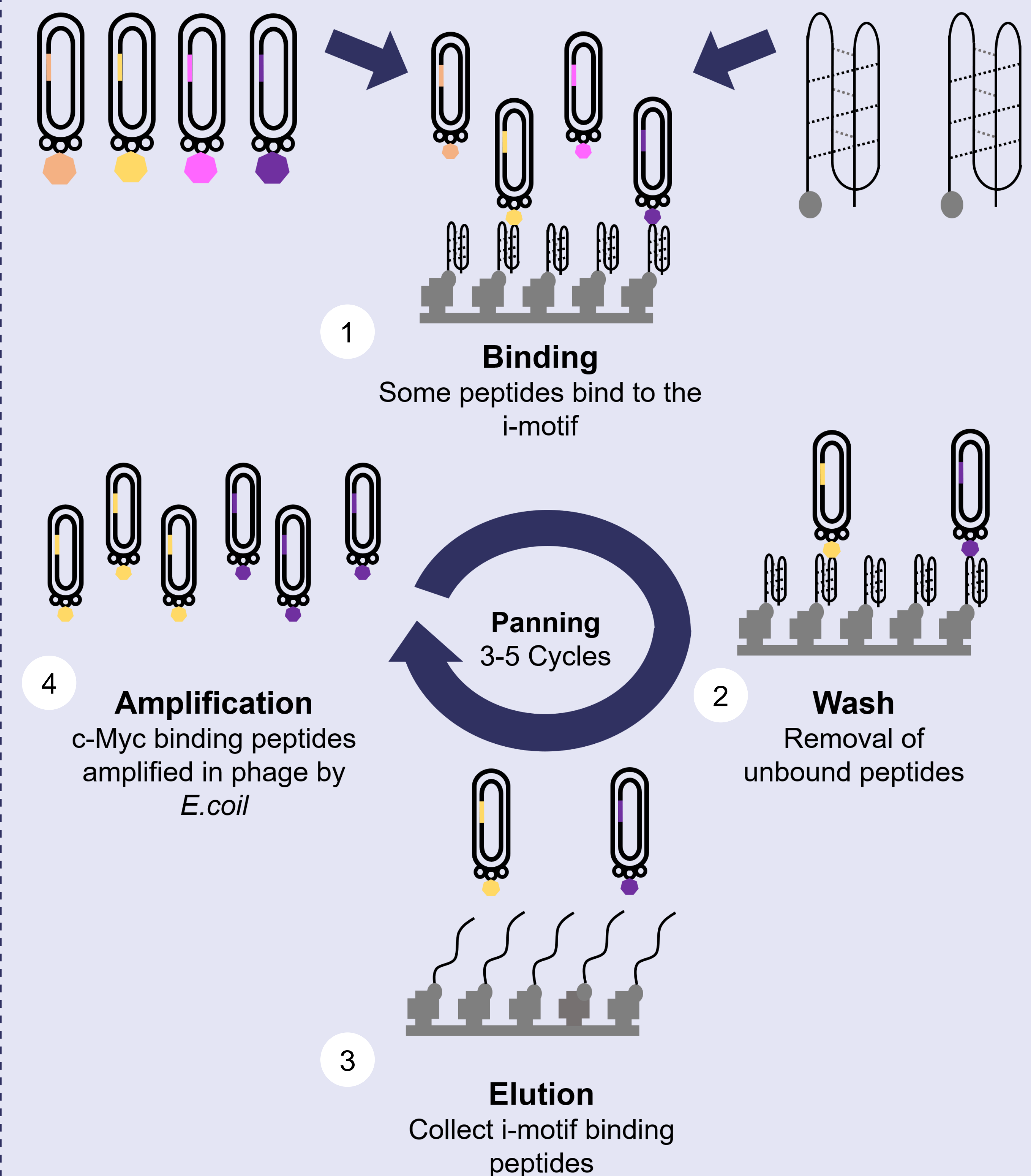
- 1595 NCI compounds screened
- 22 compounds initially identified as binders
- 11 of these bound B-DNA and a further 8 were deemed poor probes
- Identified three promising c-Myc i-motif small molecules
- Next Steps: determine binding affinity and kinetics



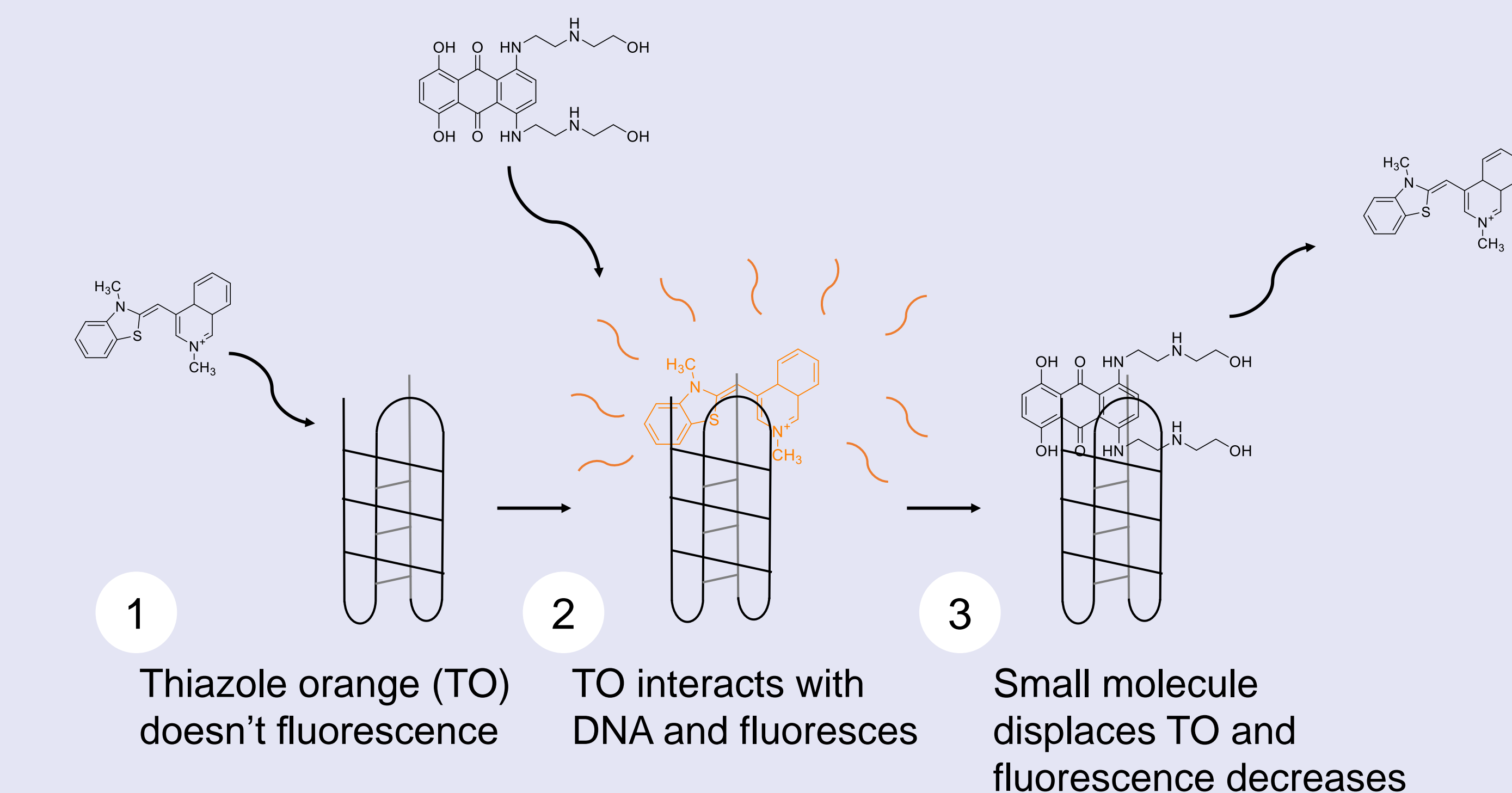
Phage Display: Identifying Peptides

M13 Phage Library
Peptides displayed on phage surface

Biotinylated i-Motif DNA
Biotin will bind to the streptavidin plate



Fluorescence Indicator Assay (FID): Identifying Small Molecules



References

- Gehring, K., Leroy, J.-L. & Guéron, M. *Nature* **363**, 561–565 (1993).
- Zeraati, M., Langley, D. B., Schofield, P., Moye, A. L., Rouet, R., Hughes, W. E., ... Christ, D. *Nat. Chem.* **10**, 631–637 (2018).
- Amato, J., Iaccarino, N., Randazzo, A., Novellino, E. & Pagano, B. *ChemMedChem* **9**, 2026–2030 (2014).
- González, V. & Hurley, L. H. *Annu. Rev. Pharmacol. Toxicol.* **50**, 111–129 (2010).