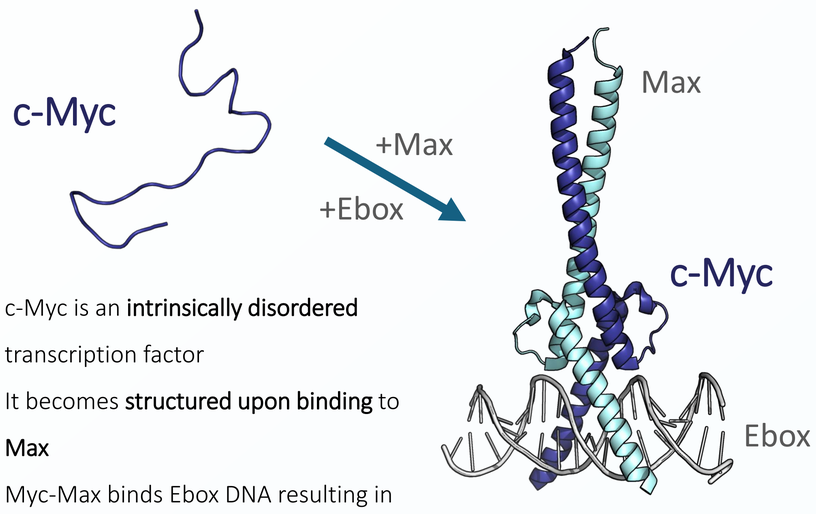
Conformational reprogramming of c-Myc via intracellular cyclisation

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c-Myc in Cancer



Structure of the c-Myc-Max heterodimer bound to Ebox DNA (PDB: 1NKP).[1]

- c-Myc is an **intrinsically disordered** transcription factor
- It becomes **structured upon binding** to **Max**
- Myc-Max binds Ebox DNA resulting in **transcriptional activation**[2]

Loss of regulation:

- c-Myc is oncogenic and **upregulated in 70% of cancers**
- Myc-Max binds weaker Ebox sequences
- Results in aberrant gene expression[3]

Targeting Myc:

- Key target for anti-cancer therapeutics
- No c-Myc antagonists have been clinically approved
- Considered '**undruggable**' due to its **lack of secondary structure** and its broad, shallow interaction with Max

Aims & Approach

Aim:

- To induce helicity in c-Myc using bis-alkylating agents, thereby **structurally stabilising an IDP**

Approach:

- Express and purify a series of double cysteine c-Myc mutants that have been **chemically stapled *in vivo***
- **Characterise** their interaction with both Max and Ebox DNA using circular dichroism (**CD**) and electromobility shift assays (**EMSA**)

Impact:

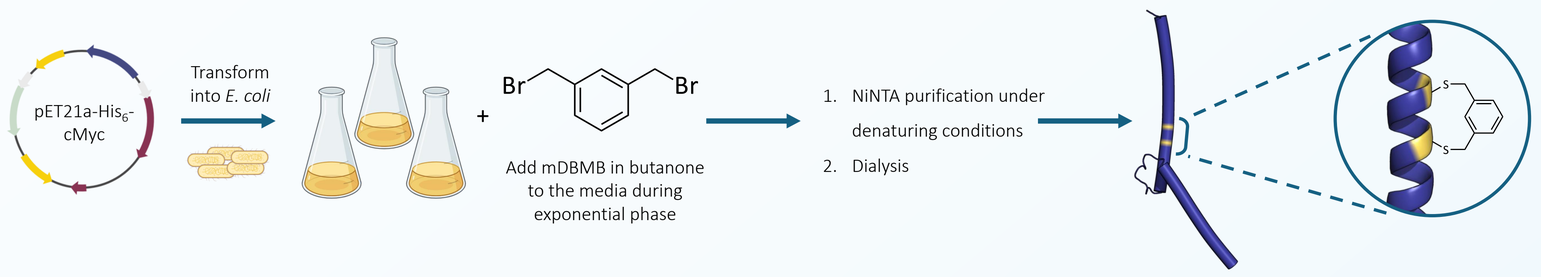
- Generating a structured c-Myc construct has the potential to **enable discovery of improved antagonists**
- Raises implications for structurally reprogramming other previously undruggable IDPs in a similar manner

Construct Design

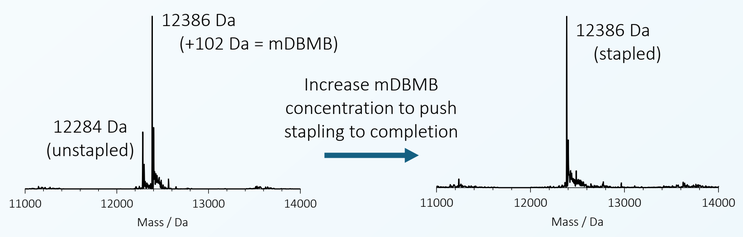
- Native c-terminal Cys mutated to Ser, creating **unstapled control construct**, MycSSA
- Generated a series of ***i* to *i+4* double cysteine mutants** at solvent-facing positions in c-Myc
- Sampled across the basic helix-loop-helix leucine zipper (bHLHZip) to assess the effect of staple position

```
Domain:        Loop       Helix 2            Leucine Zipper
Heptad:                 defgabcdefgab cdefgabcdefgabcdefgabcdefgabc def
>WT cMyc … PELENNEKAP KVVILKKATAYIL SVQAEEQKLISEEDLLRKRREQLKHKLEQ LRNSCA
>MycSSA … PELENNEKAP KVVILKKATAYIL SVQAEEQKLISEEDLLRKRREQLKHKLEQ LRNSSA
>MycH2  … PELENNEKAP KVCILKCATAYIL SVQAEEQKLISEEDLLRKRREQLKHKLEQ LRNSSA
>MycZ1  … PELENNEKAP KVVILKKATAYIC SVQCEEQKLISEEDLLRKRREQLKHKLEQ LRNSSA
>MycZ2  … PELENNEKAP KVVILKKATAYIL SVQAEEQKLISEECLLRCRREQLKHKLEQ LRNSSA
>MycZ3  … PELENNEKAP KVVILKKATAYIL SVQAEEQKLISEEDLLRKRREQLKHKLCQ LRCSSA
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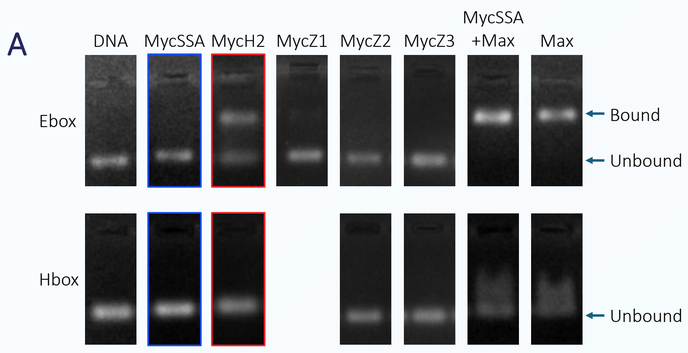
Expression & Purification



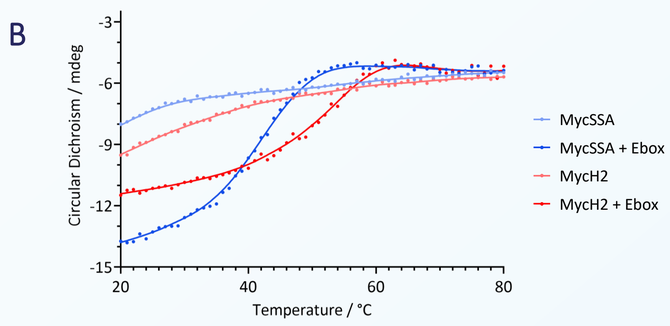
- The addition of mDBMB to the media during expression in *E. coli* results in the generation of stapled c-Myc constructs *in vivo*[4]
- This is despite c-Myc expression being insoluble, necessitating NiNTA purification under denaturing conditions
- Mass spectrometry analysis of each construct following dialysis demonstrates the +102 Da mass increase from mDBMB addition



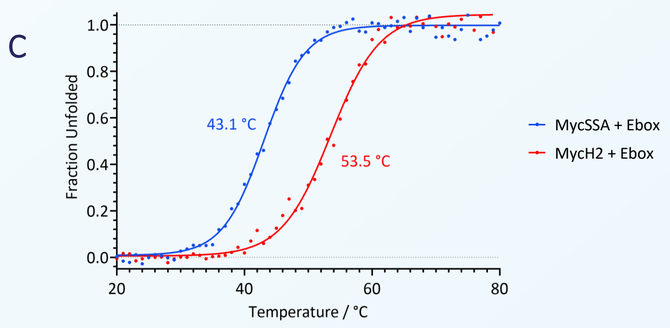
CD & EMSA Characterisation

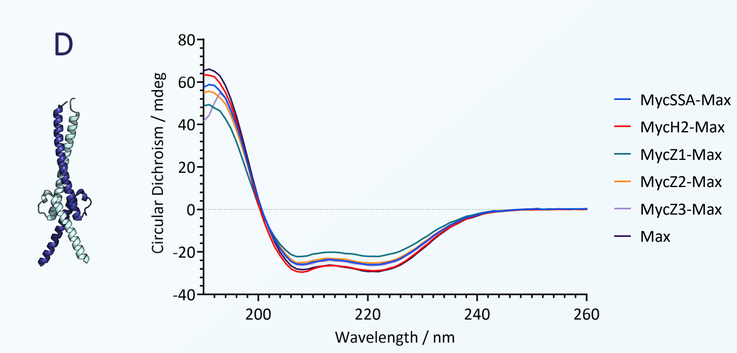


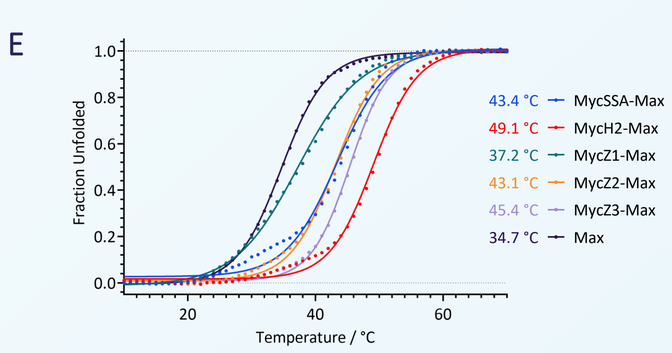
- EMSA data showed the expected shifts for the MycSSA-Max heterodimer and the Max homodimer in complex with Ebox DNA.
- However, a partial, **sequence specific shift** was **also observed for MycH2** in the **absence of Max** (Fig. A).

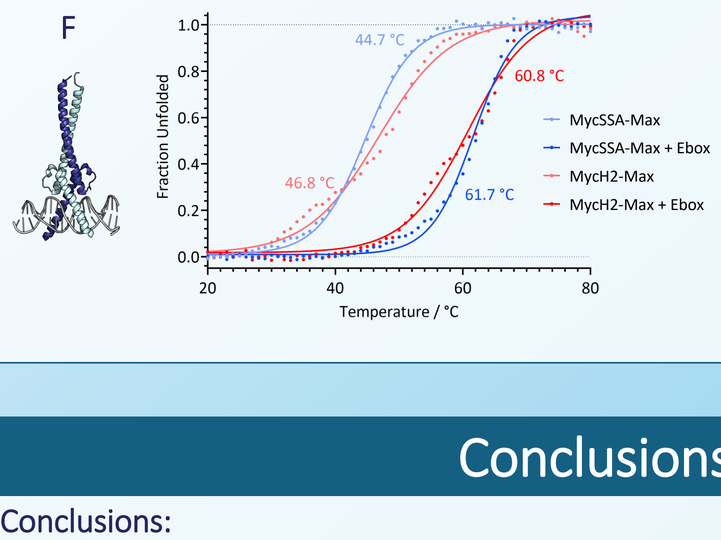


- A T_m could not be determined for MycSSA or MycH2 by CD (Fig. B) - either alone or with Hbox (data not shown).
- **Transitions were observed** for both MycSSA and MycH2 in the **presence of Ebox DNA** (Fig. C), with a significant **10°C rightward shift** for the **stapled construct**.









- All five Myc-Max heterodimers adopt a typical **alpha helical, coiled coil structure** (Fig. D) and show **higher melting temperatures** compared to the Max homodimer (Fig. E).
- A **~15°C rightward shift** was seen upon **addition of Ebox** DNA (Fig. F), corroborating the EMSA observations.
- Collectively, this shows that **stapling c-Myc has not adversely affected its ability to bind Max and Ebox**.

Conclusions & Future Work

Conclusions:

- Bis-alkylating agents can be added to media during expression in E. coli to generate stapled proteins *in vivo*.
- EMSA and CD data shows that stapling in helix two uniquely enables Ebox DNA binding in the absence of Max.
- Gain of function does not recapitulate the coiled coil conformation of Myc-Max, instead adopting a non-native yet functional conformation.
- Stapling does not perturb native heterodimerisation or ternary complex formation.

Future Work:

- Structurally characterise the interaction of stapled MycH2 with Ebox.
- Investigate the potential benefits of screening against a stapled c-Myc construct to elucidate more potent inhibitors.

Selected References:

1. Nair S. K. and Burley S. K. *Cell*, **2003**, *112* (2), 193-205, DOI: 0.1016/s0092-8674(02)01284-9
2. Whitfield J. R. and Soucek L. *Nat. Rev. Drug Discov.* **2025**, *24* (6), 445-457, DOI: 10.1038/s41573-025-01143-2
3. Madden S. K., Dantas de Araujo A., Gerhardt M., Fairlie D. P. and Mason J. M. *Mol. Cancer*, **2021**, *20* (1), DOI: 10.1186/s12943-020-01291-6
4. Brennan A., Vance K. W. and Mason J. M. *Cell Chem. Biol.* **2026**, *33* (3), 409-423, DOI: 10.1016/j.chembiol.2026.02.002