

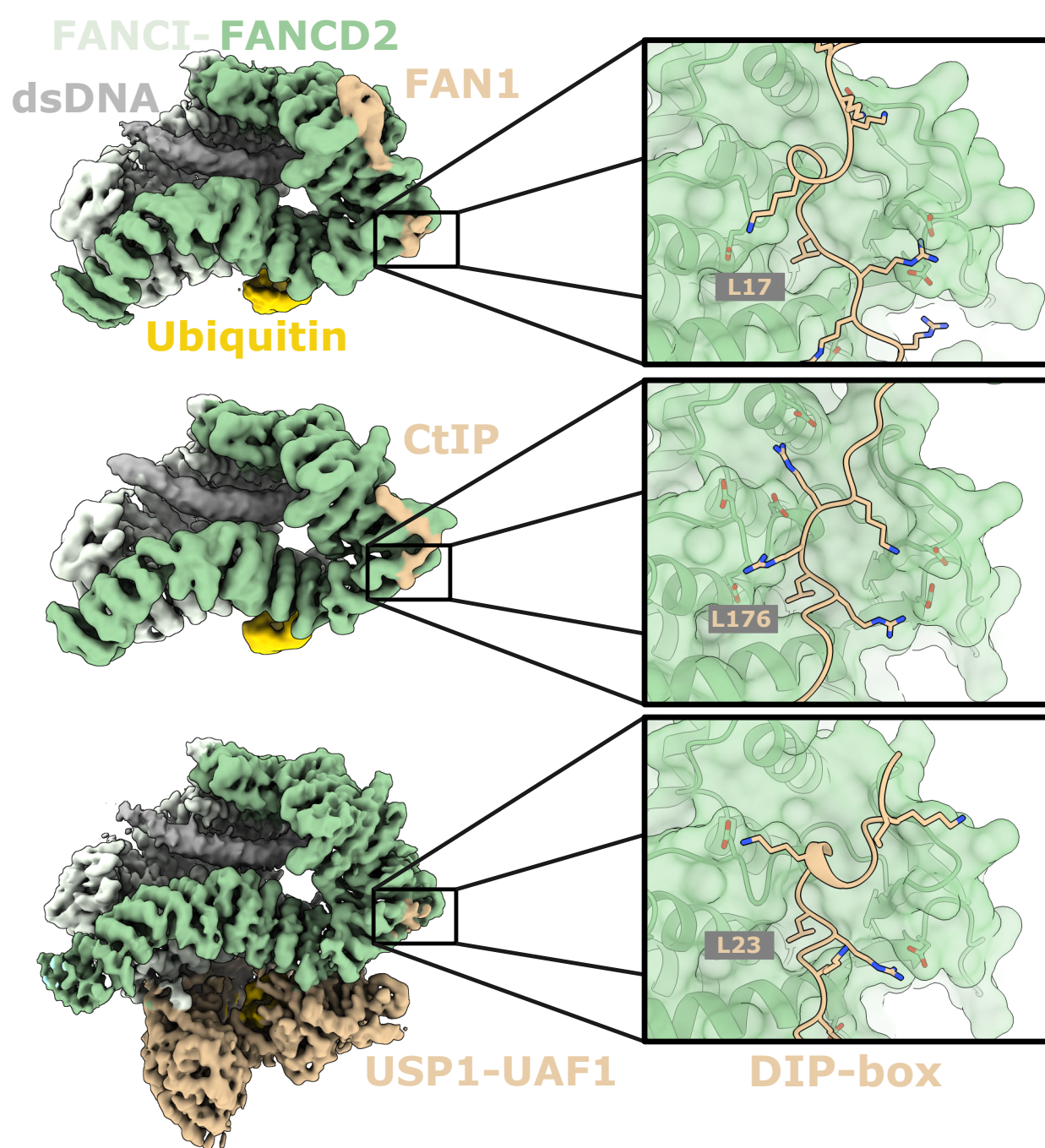
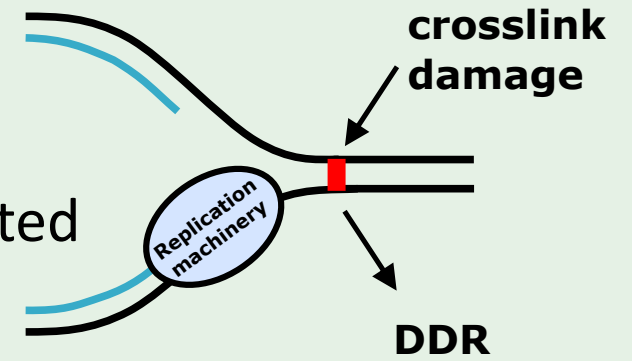
De novo designed binders as selective modulators of short linear motif interactions in DNA repair



Martin Rennie
School of Molecular Biosciences



DNA repair background: Genome integrity relies on the repair of DNA damage that can arise due to intrinsic sources (e.g. metabolism) and extrinsic sources (e.g. chemotherapy). DNA damage response (DDR) pathways require the coordinated action of numerous proteins to manipulate the DNA and repair the damage.

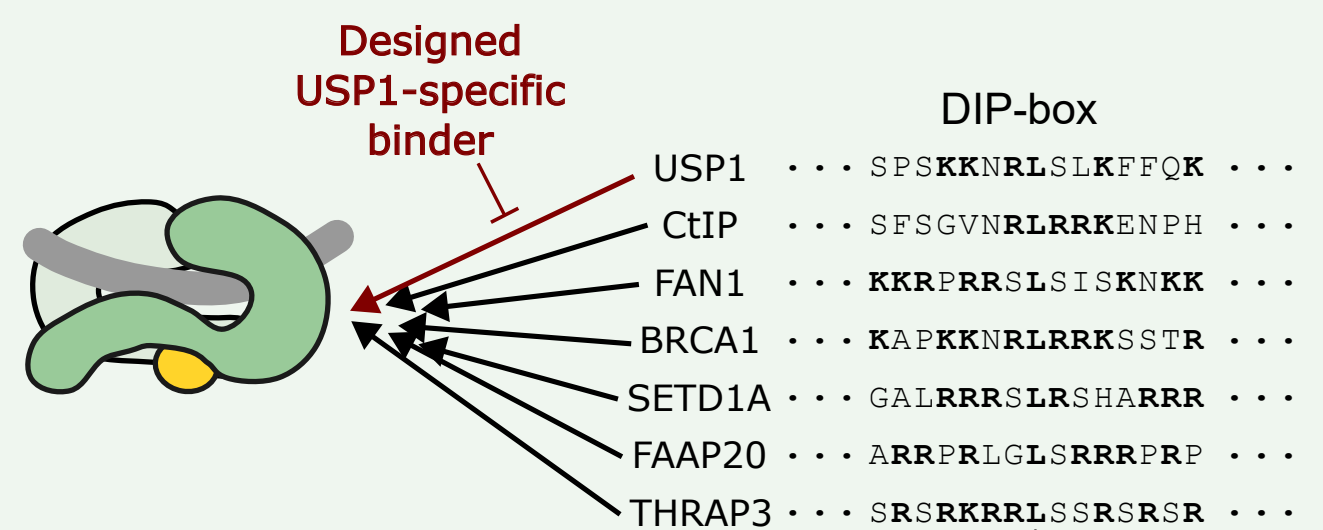


CryoEM reconstructions of the FANCI-FANCD2 DNA clamp with three DIP-box proteins^{2,3}.

FANCD2 is a hub protein in DNA repair:

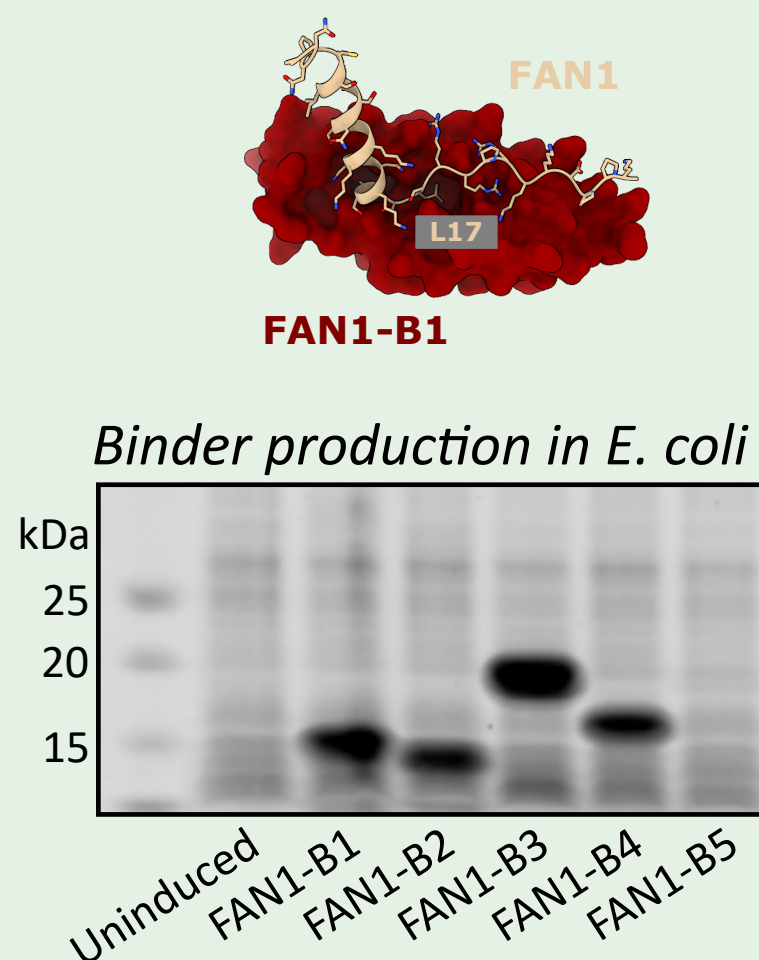
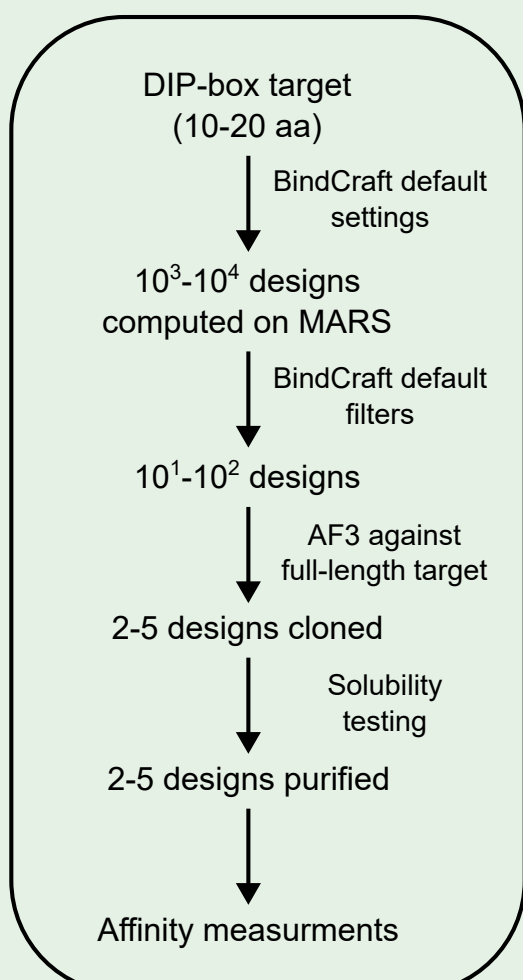
FANCI-FANCD2 is a DNA clamp involved in the repair of DNA interstrand crosslinks¹. Mono-ubiquitination of FANCD2 is critical for this role. We have recently shown that FANCD2 is a hub for binding of a short linear motif (SLiM), that we term a D2-interacting motif or DIP-box².

Binders as SLiM probes: To understand the roles of each DIP-box, I am developing binders to selectively recognise each DIP-box thereby sterically blocking the interaction with FANCD2.

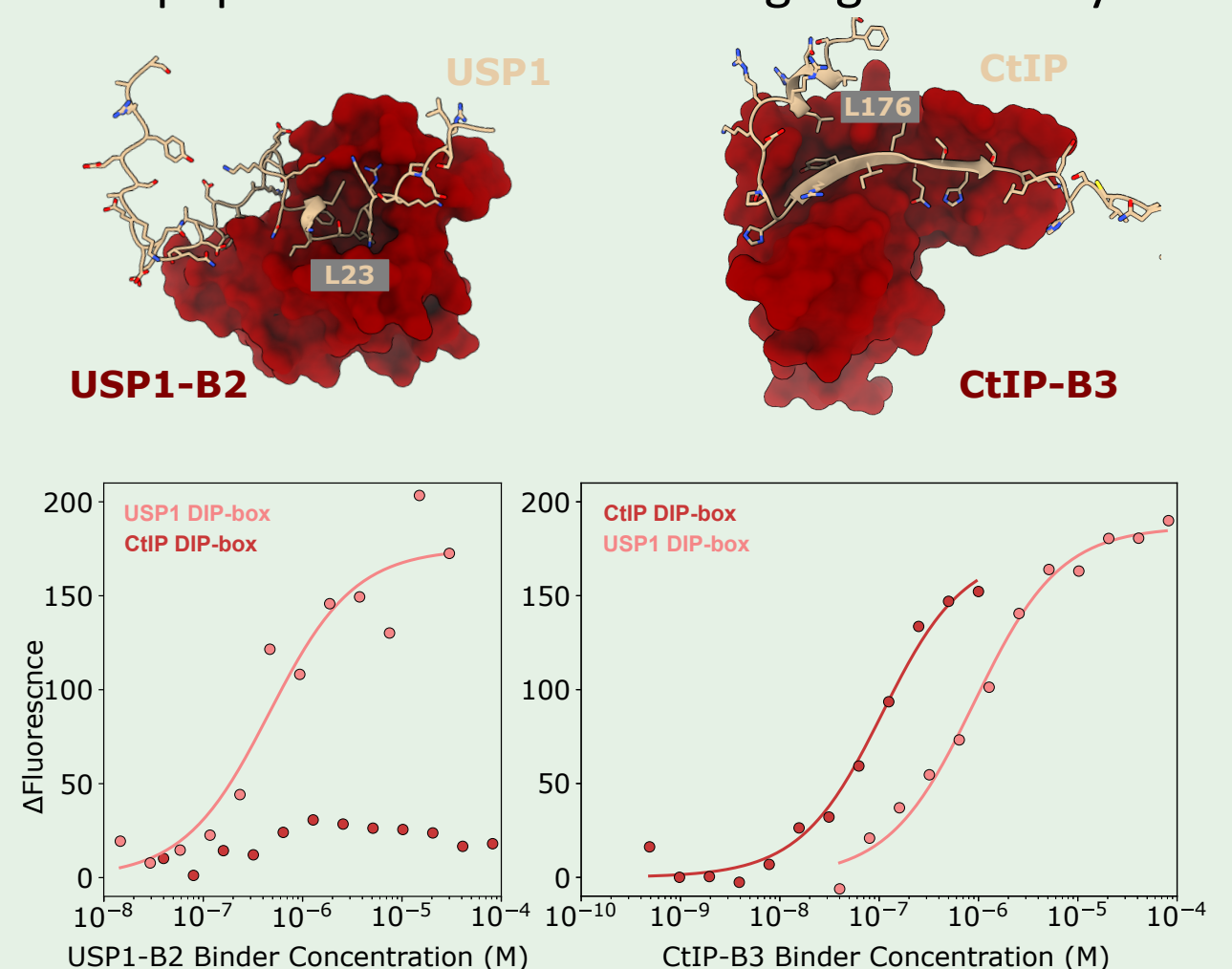


BindCraft designed binders against SLiMS:

Using BindCraft⁴ and models of the FANCD2-bound DIP-boxes, I have generated designs with ipTM>0.75 (AlphaFold3).



DIP-box Binding Assay: Purified binders titrated into fluorescently labelled DIP-box peptides indicate encouraging selectivity.



References

- ML Rennie, K Lemonidis, C Arkinson, V Chaugule, M Clarke, J Streetley, L Spagnolo, H Walden (2020). Differential functions of FANCI and FANCD2 ubiquitination stabilize ID2 complex on DNA. *EMBO Reports*, 21, e50133.
- Z Cao, GR Briola, C Ionita, J Streetley, H Walden, ML Rennie (2026). Discovery of a FANCD2-interacting protein motif (DIP-box) linking DNA Damage Response processes. *bioRxiv*
- ML Rennie, C Arkinson, V Chaugule, H Walden (2022). Cryo-EM reveals a mechanism of USP1 inhibition through a cryptic binding site. *Sci Adv* 8, eabq6353.
- Pacesa, M., et al. (2025). One-shot design of functional protein binders with BindCraft. *Nature*. 646, 483-492.