Isoform specific inhibition of human SUMO proteins using Affimer® binders

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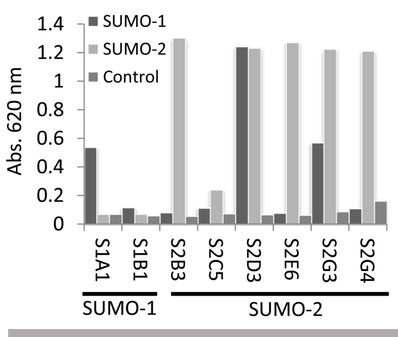
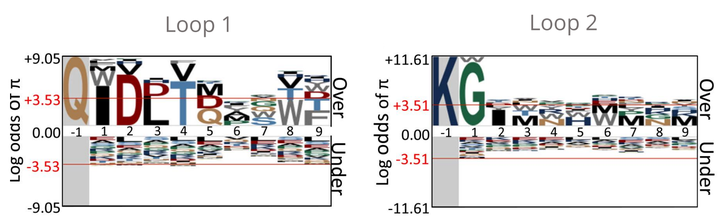
Introduction

Protein-protein interactions (PPIs) underpin many biological processes. Targeting them to understand their function, or to inform the development of therapeutics, represents an intriguing, yet difficult task. SUMOylation is a post translational modification that regulates numerous cellular pathways, and facilitates PPIs. Although new technologies are emerging to inhibit PPIs, the ability to generate isoform specific reagents capable of being expressed in cells represents a major challenge and a novel approach for understanding biological pathways. Using a recently developed artificial binding protein (Affimer) phage display library, we have isolated isoform-specific binders that inhibit SUMO-dependent PPIs. Crucially, they did not prevent SUMO conjugation either *in vitro* or in cell-based systems. Furthermore, by blocking SIM-dependent PPIs in cells we show that arsenic-induced PML-NB network formation is predominantly a SUMO-2-specific process. These novel inhibitors will enable the global investigation of isoform-specific SUMO/SIM interactions. This demonstrates the ability to target and study any PPI using this artificial binding protein technology.

The screen

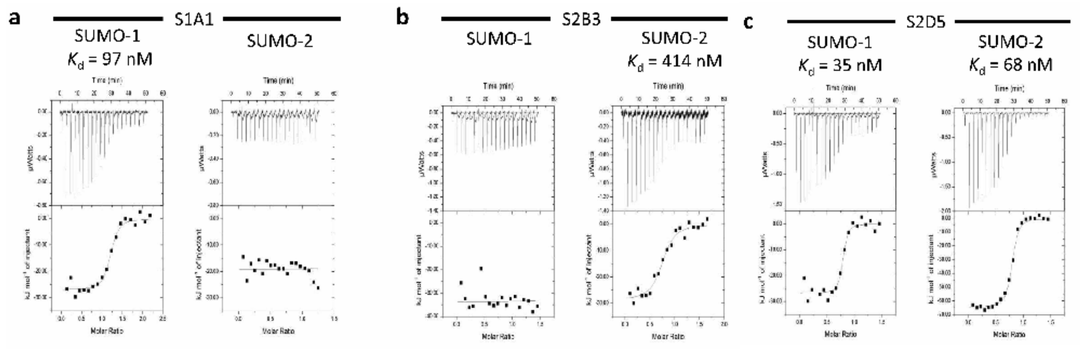
Human SUMO-1 and human SUMO-2 were screened using the Affimer libraries. After three panning rounds (taking two weeks), monoclonal reagents were checked for binding by phage ELISA. Specific reagents were isolated that bound to both isoforms.





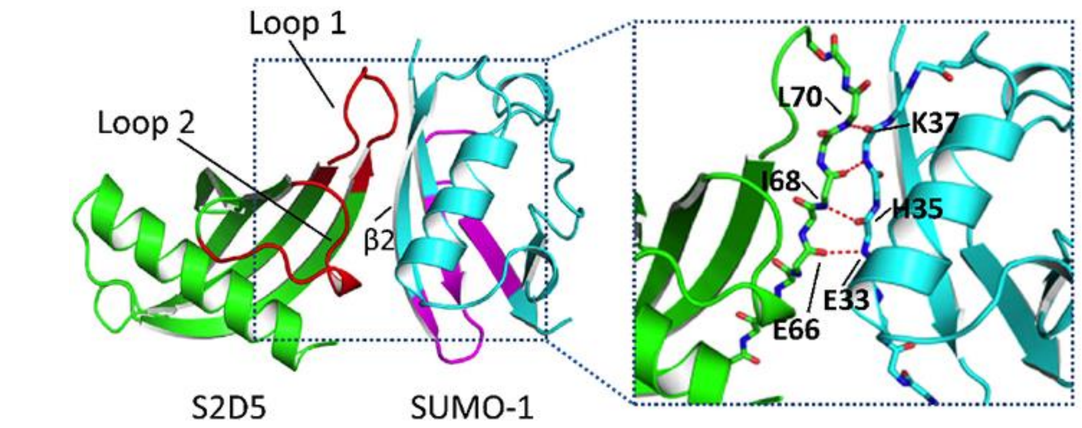
Sequence analysis of many binders showed a SUMO interacting motif in the first loop region.

Characterising SUMO binding Affimers by ITC

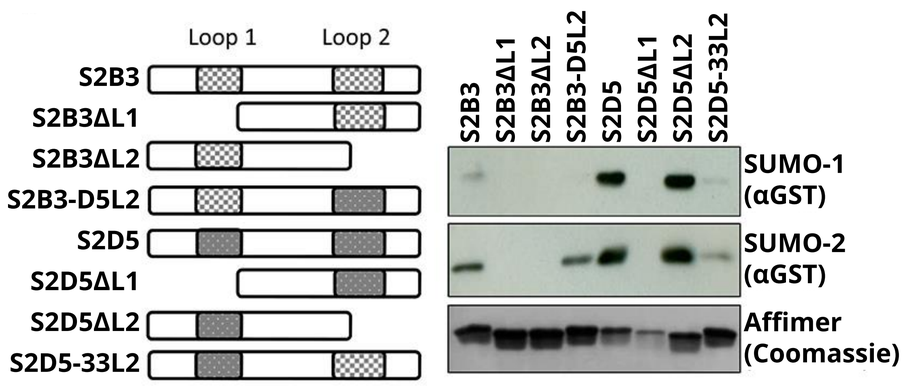


ITC was used to confirm the specificity of unique Affimers identified using phage ELISA.

Crystallisation and loop shuffling

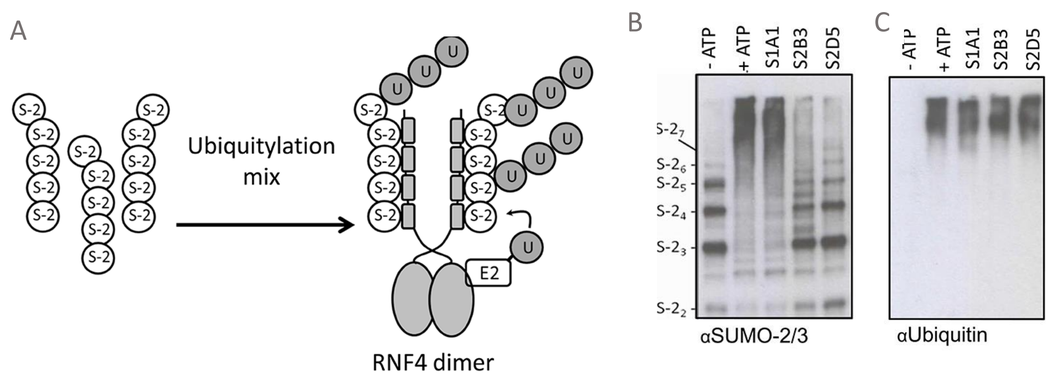


SUMO-Affimer crystal complexes have been solved and analysed to examine the interactions in binding.



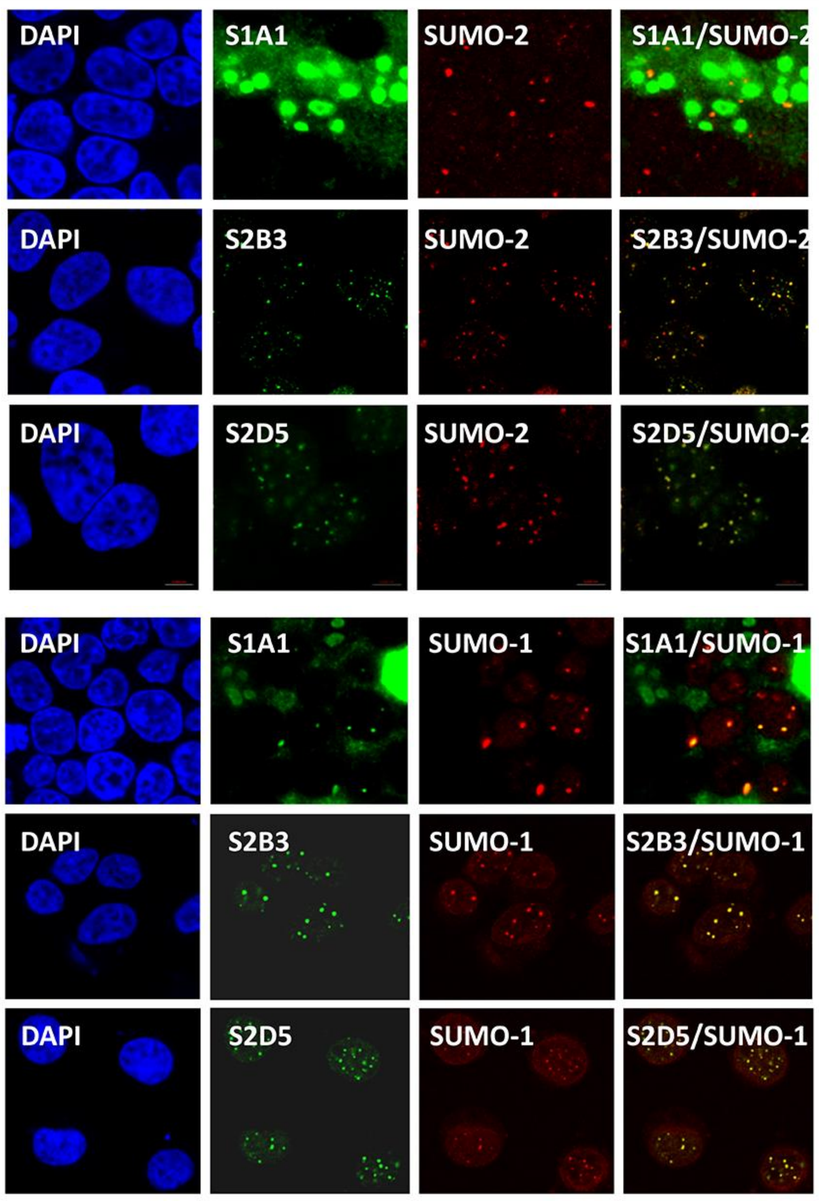
The loops of different Affimers were removed or swapped to determine which loops were responsible for binding. Results confirmed co-crystal structures.

SUMO2 Affimers inhibit SUMO in *in vitro* assays



The Affimers were used in an *in vitro* assay to assess their ability to inhibit SUMO mediated protein-protein interactions (A). The two SUMO-2 specific Affimers inhibited ubiquitination of polySUMO, while the SUMO-1 binder had no effect as expected (B). None of the Affimers inhibited RNF4 in an autoubiquitination assay in which RNF4 ubiquitinated itself in a SUMO independent manner (C).

Co-localisation of Affimer clones with SUMO1 & 2



SUMO-Affimers co-localise with SUMO proteins in arsenic treated cells demonstrating the ability to express Affimers in cells to study protein-protein interactions.

Summary

An Affimer is a small, highly stable, single domain protein scaffold free of PTMs and disulphide bridges. Specific Affimers can be generated to target proteins or small-molecules using phage display. Several phage libraries have been constructed.
We have demonstrated the utility and speed of development of the Affimer platform to generate binders to Human SUMO-1 and SUMO-2, the first truly novel isoform-specific SUMO/SIM-dependent competitive inhibitors. These reagents provide a unique opportunity to probe the fundamental aspects of SUMO-dependent PPIs in a global fashion, and how these PPIs regulate cellular processes in response to specific stimuli (such as heat shock, oxidative stress or the DNA damage response).

References

Stadler *et al*. (2011) Structure-function studies of an engineered scaffold protein derived from Stefin A. II: Development and applications of the SQT variant. PEDS 24(9) 751-63.
Tiede *et al*. (2014) Adhiron: a stable and versatile peptide display scaffold for molecular recognition applications. PEDS 27(5) 145-155.