Engineering protein-protein interactions with higher mechanical binding strength

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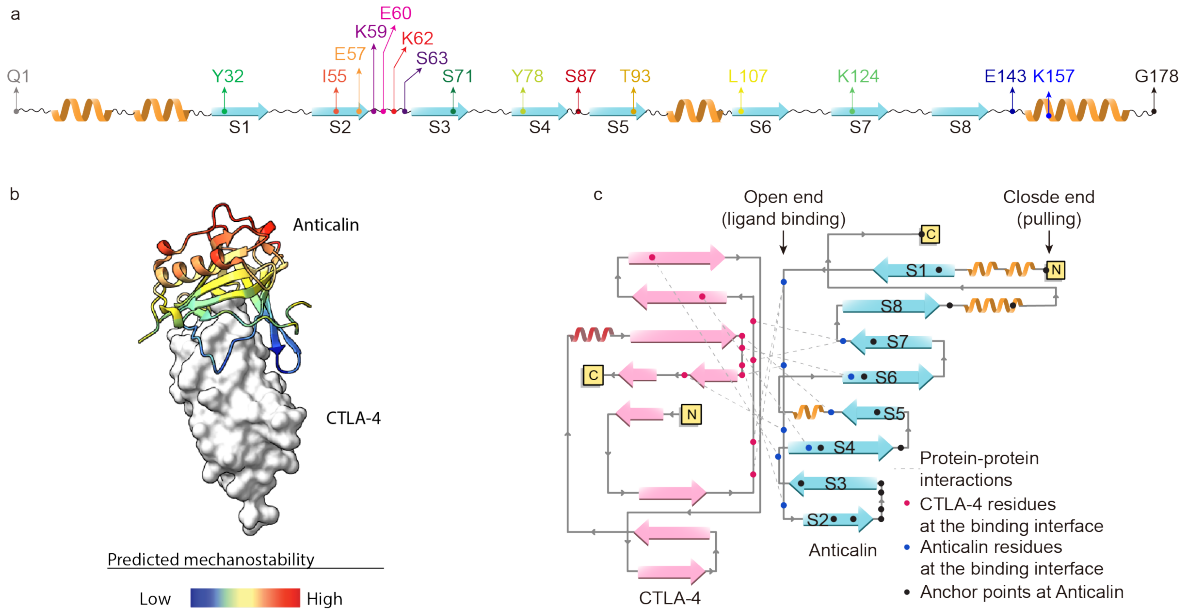
Protein-protein interactions underlie many biological processes and are essential for cellular signalling and communication, immune responses, homeostasis and regulation, cell adhesion and development, pharmacology and drug development, etc. Achieving high binding strength is essential for efficient delivery of molecular cargo to targets in the in vivo mechanical environment. However, it is still challenging to modulate the mechanical strength of protein-protein interactions under mechanical stress. The mutation of amino acid residues involved in protein interface often simultaneously destroys the high thermodynamic affinity. Here, we investigate how altering the surface immobilization residues (i.e. anchor point) within a protein-binding scaffold impacts the mechano-stability and adhesion performance of the bound complex when exposed to shear force. We studied a non-antibody scaffold (Anticalin) engineered with bio-orthogonal clickable amino acids at various anchor points and quantified the mechanical stability of Anticalin:(CTLA-4) complex under different molecular loading geometries using single-molecule AFM force spectroscopy and bead-based adhesion assays. [1, 2] Multi-regression analysis of the physicochemical properties of the anchor points revealed that the distance between the anchor point on Anticalin and the center of mass of CTLA-4 was a key determinant of mechanical stability, providing new insights into the role of protein structural features in generating mechanical resistance in protein-protein interactions. This method does not require mutation of amino acid residues at the interaction interface between proteins, which means it will not destroy the thermodynamic stability of the protein binding complex. It provides an inspiring idea for large-scale rational design, regulation and screening of protein-protein interactions with regulated mechanical strengths when exposed to shear stress in fluidic environments.



[1] C. Rudd, *Nature Reviews Immunology*, **2008**, *8.2*, 153-160.
[2] D. Schönfeld, et al., *PNAS*, **2009**, *106.20*, 8198-8203.