An investigation of the role of glycine residues in SasG domains

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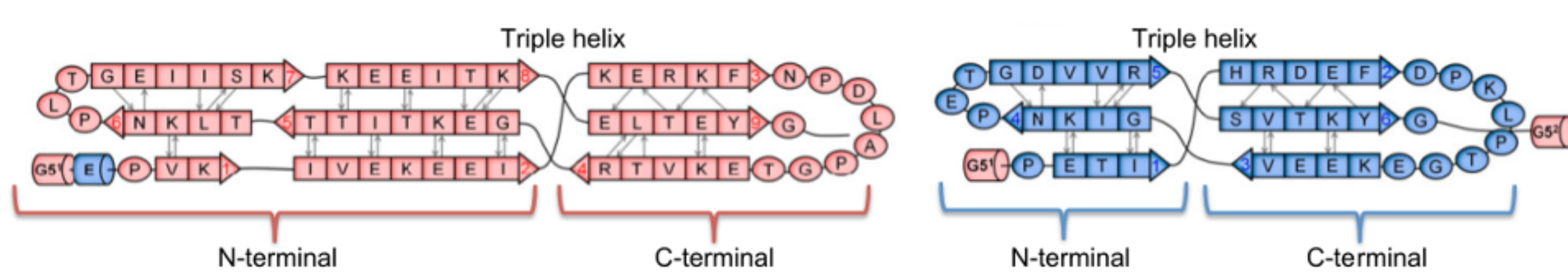
Introduction and Objectives

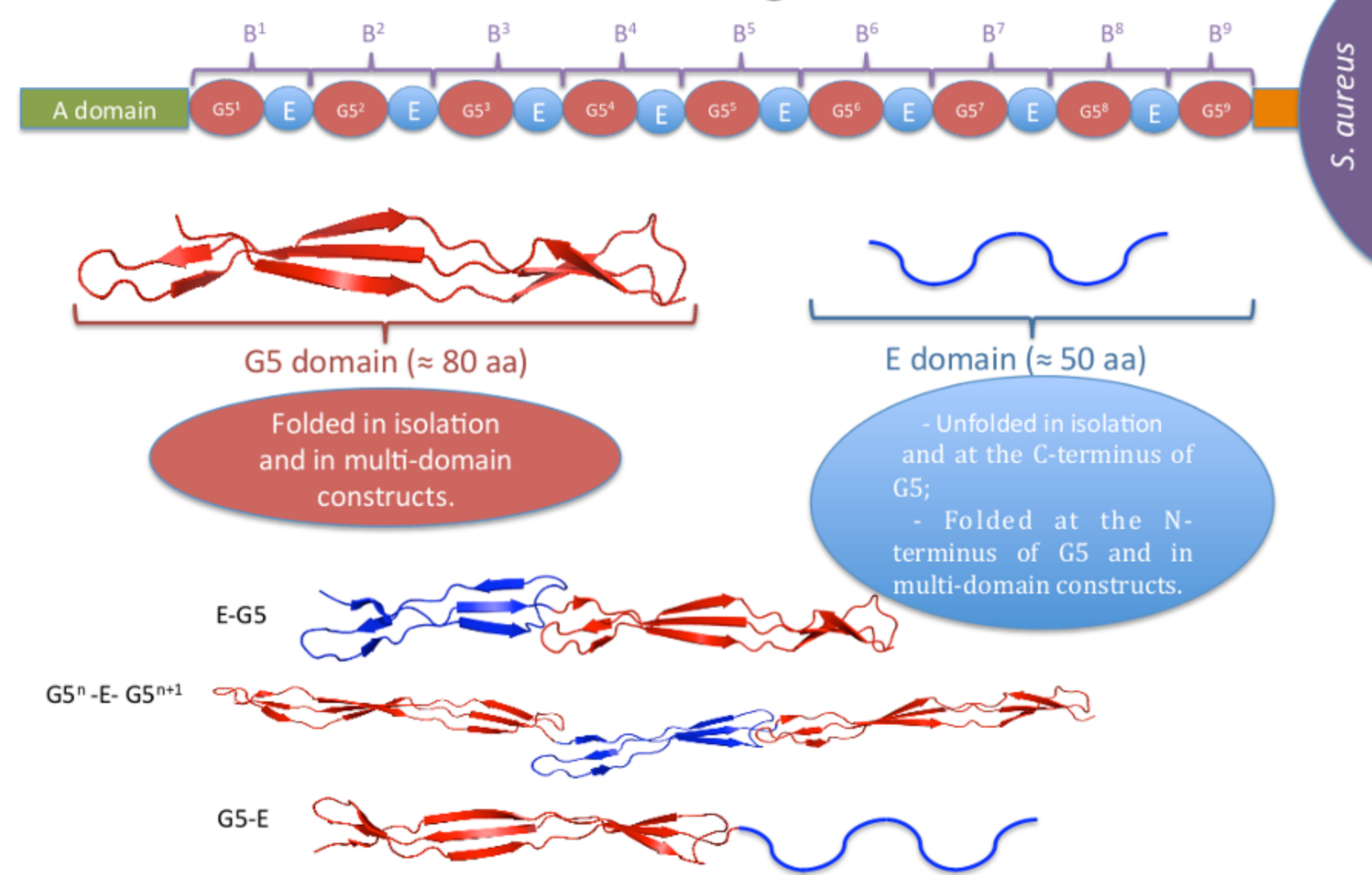
- SasG is a protein from *Staphylococcus aureus* that promotes cell-to-cell accumulation during biofilm formation.
- Due to their ability to form biofilms, staphylococci are the leading cause of infection associated with implanted medical devices (e.g. artificial heart valves, catheters).
- The protein B region is composed of tandemly arrayed 128 residue repeats. This sequence repeat is in fact comprised of two structurally related domains: G5 and E.
- Folded E and G5 domains are tightly connected in a head-to-tail fashion, resulting in a contiguous and elongated structure.

G5 domain: named after its five conserved Glycine residues, also identified in the **E** domain.

G5 and **E** domains have a similar topology:



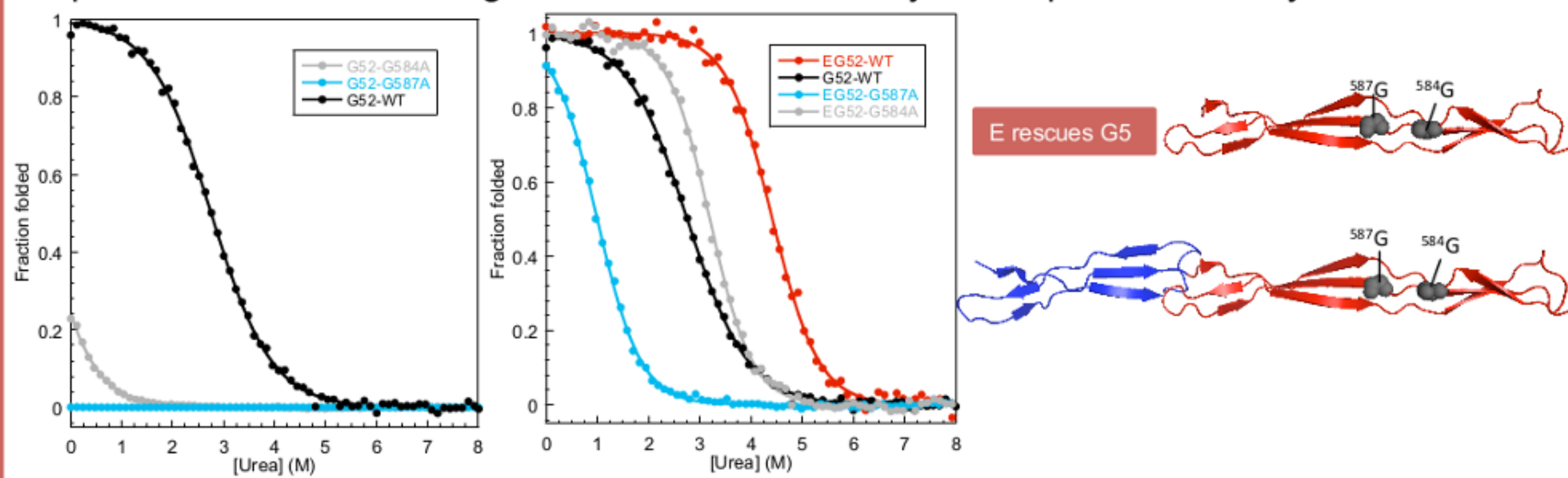
Domains are structurally similar but differ in stability.



Main question: What is the role of conserved glycine residues in SasG E and G5 domains?

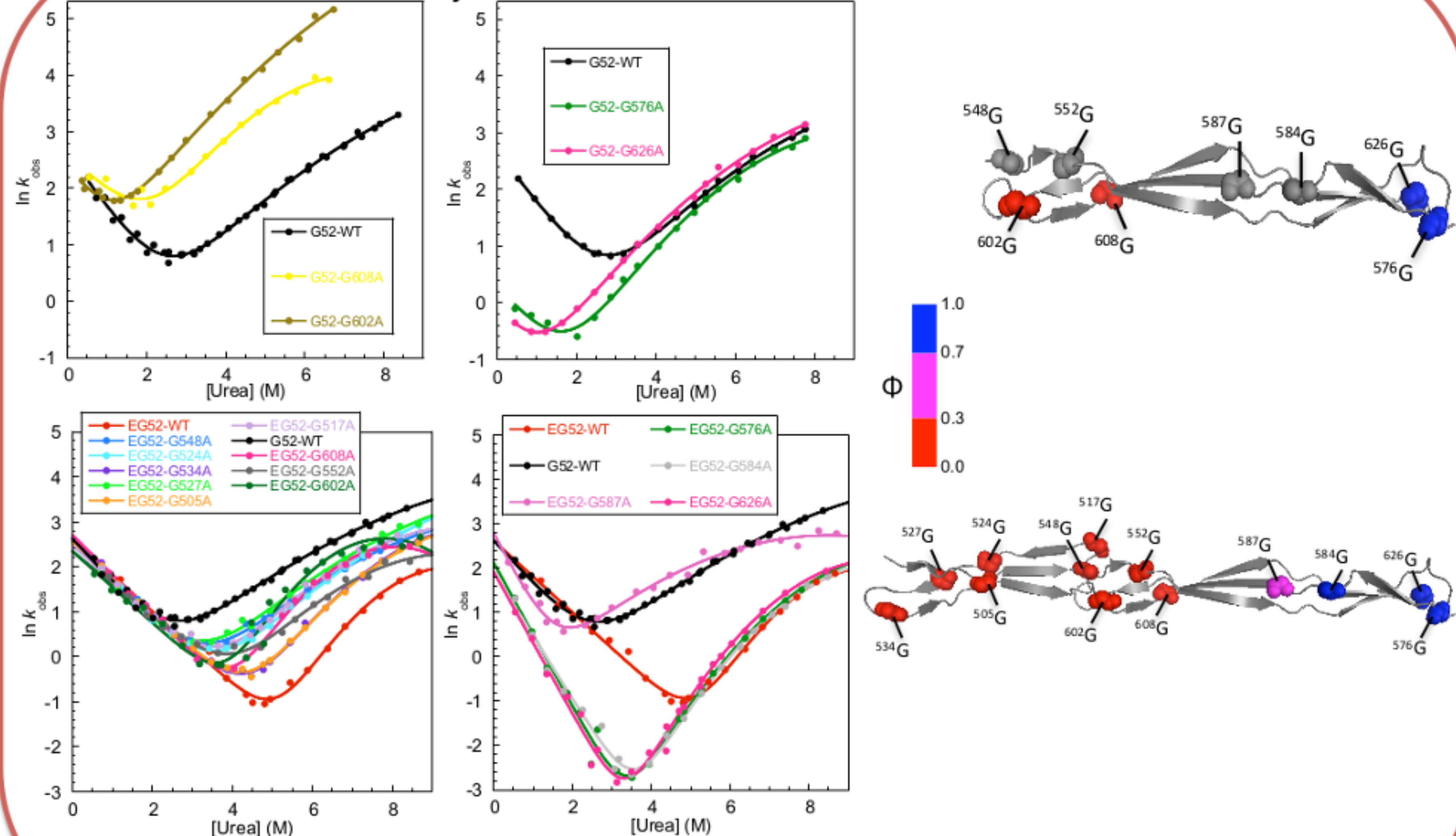
Results and Discussion

- Equilibrium studies: the two mutations located in the triple helical region (G584A, G587A) were disruptive to the structure of G5[2] in isolation. Surprisingly, the same mutations in the context of N-terminal E domain (which is unstructured in isolation), give equilibrium curves showing a considerable recovery of the protein stability.



- The identified stability differences between mutated G5[2] and EG5[2] suggest that there is a significant free energy contribution that comes from the E-G5 interface. This interface contributes more to the stability of EG5[2] than the domains themselves.

- Kinetics: Φ-value analysis



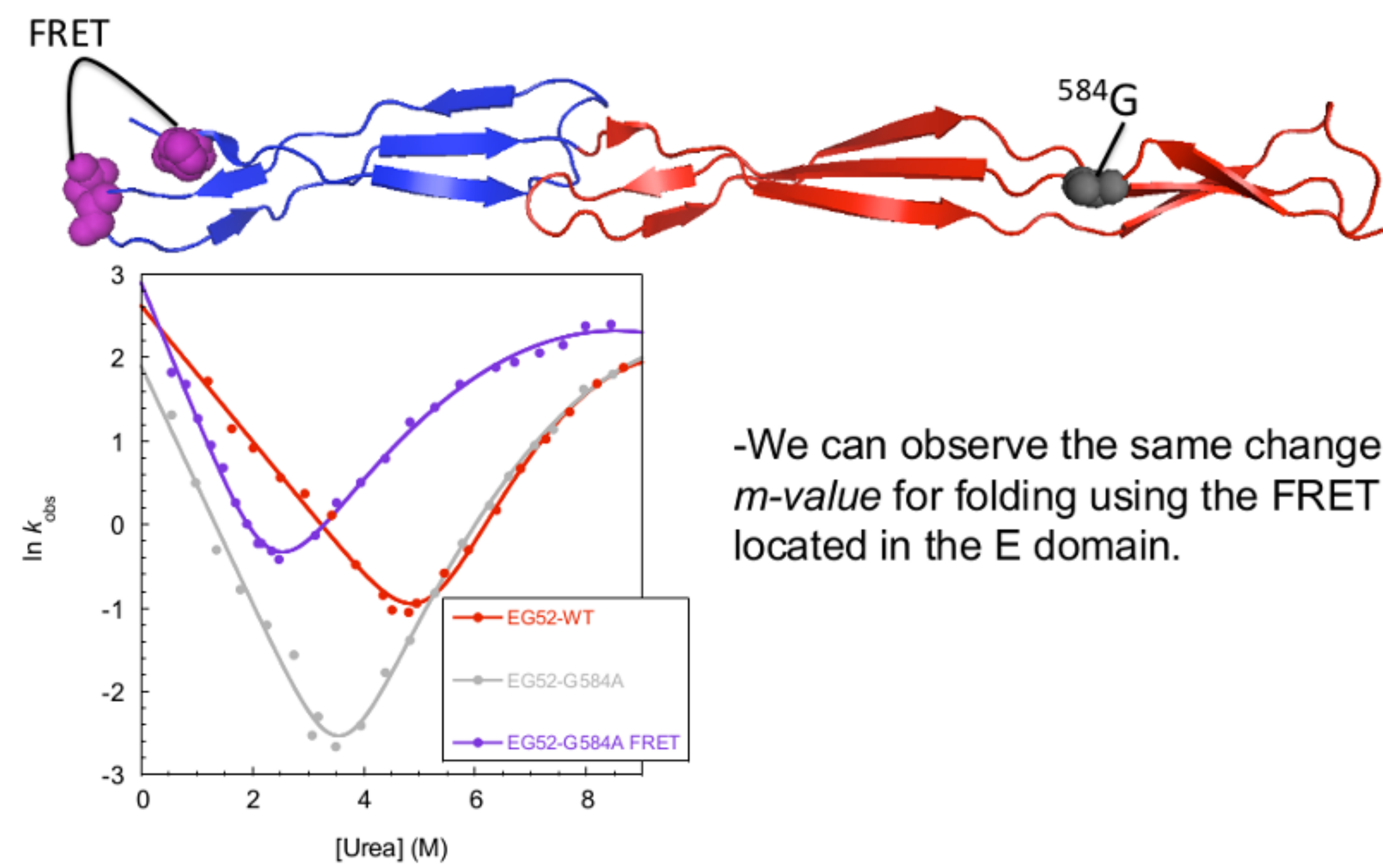
- The kinetics analysis show a change in the *m*-value (slope) in the folding arm of the chevron plot for the mutants: G576A, G584A, G587A and G626A.

Change in the *m*-value

Possible explanation: alternative folding pathway

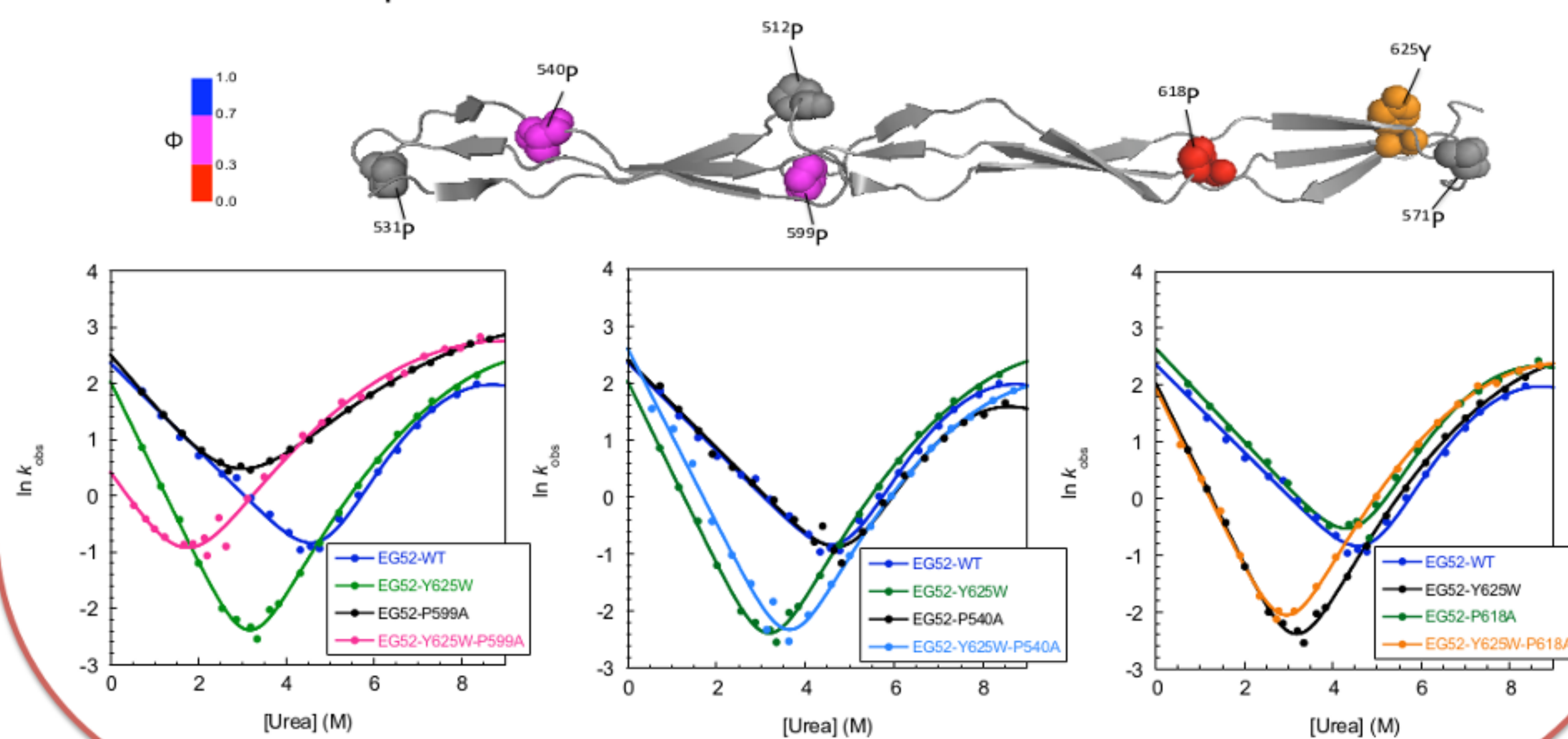
Hypothesis: folding via E-G5 interface

- Kinetics: FRET pair monitoring folding of E in the context of EG5[2]-G584A



-We can observe the same change in the *m-value* for folding using the FRET probes located in the E domain.

- Kinetics: selected proline mutations in the context of EG5[2]-Y625W



References

- Gruszka, D. T. *et al.* (2012) *PNAS*, **109**(17), E1011-E1018
- Corrigan R. M. *et al*, (2007) *Microbiology* **153**, 2435-2446
- Harris L. G. *et al.* (2006) *Injury* **37**, S3-S14
- Fersht A. R *et al.* (2004) *PNAS*, **101**(21), 7976-7981

Acknowledgements

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Conclusions

The study of Gly-Ala mutations in SasG reveals new insights into the stability and protein folding mechanism. Mutations in the E domain reveal that the folding of the G5 domain is the rate-limiting step in the folding of EG5 constructs. G5 domain in isolation starts to form its structure via the C-terminal region and mutations in its triple helical region are disruptive to the structure.

In the EG5[2] construct, mutants in the triple helix of G5[2] show a recovery in stability suggesting that the E-G5 interface must be key for its stability. There is a change in the folding *m*-value for these mutants and those located at the C-terminus of G5[2]. Hence, we identified an alternative folding pathway for EG5[2], in which the E-G5 interface is formed first.