EFFECTS OF MUTATIONS ON THERMOSTABILITY OF CELLULOSOME PROTEINS: A COARSE-GRAIN SIMULATION STUDY

ŁUKASZ MIODUSZEWSKI[1] AND MATEUSZ CHWASTYK[2]

[1]*Faculty of Mathematics and Natural Sciences, School of Science, Cardinal Stefan Wyszyński University, Warsaw, Poland*

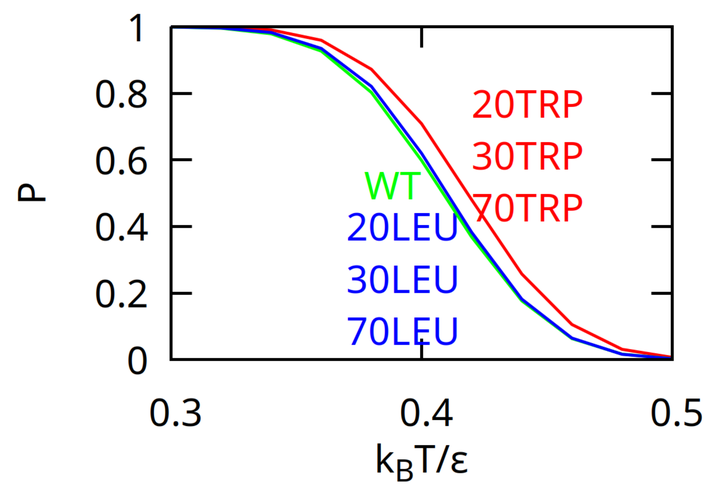
[2]*Institute of Physics, Polish Academy of Sciences, Warsaw, Poland*

e-mail: chwastyk@ifpan.edu.pl

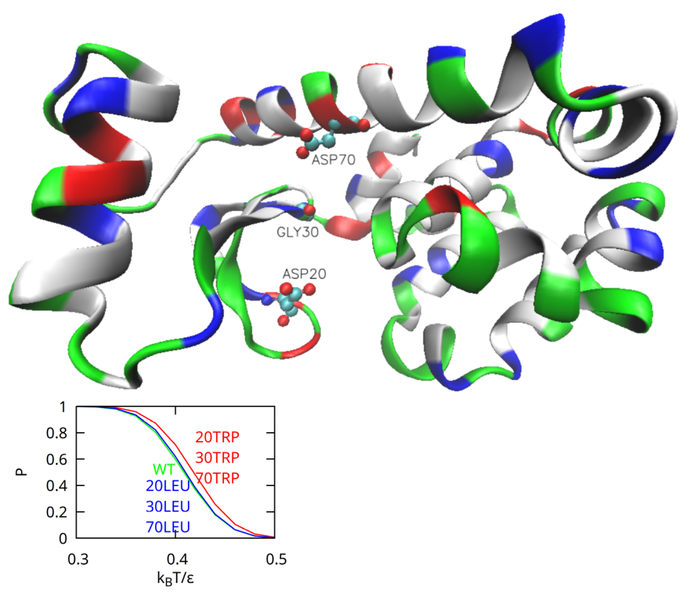
ABSTRACT

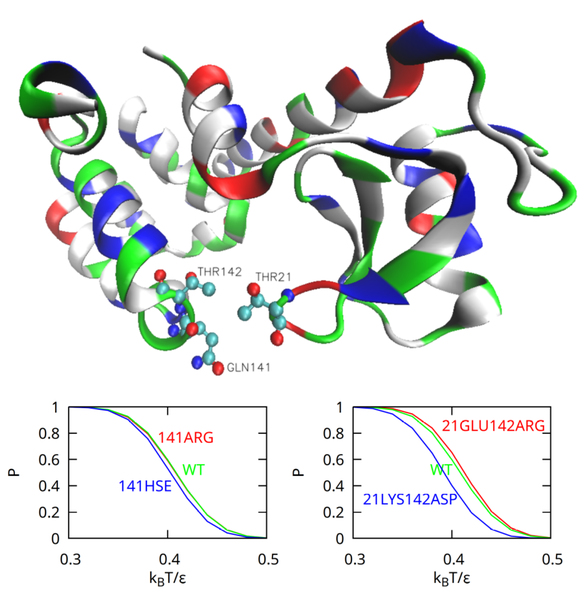
We use a combination of coarse-grained and all-atom simulations to assess the effect of a set of single-point and multi-point mutations on dockerin, a highly mechanostable module from scaffoldin CipA from *Clostridium thermocellum*. Our method was successfully applied to cohesin, another module from this scaffoldin. We use all-atom simulations to predict structural shifts with respect to the native protein and construct a contact map. We then analyze the mutants using a coarse-grained model based on that map. We examine transitions in contacts between residues and find that alterations between chemically similar amino acids lead to large changes in thermostability, which is measured by determining the probability of protein folding for a range of temperatures. We have identified mutations that may lead to a substantial increase in the thermostability by making systematic substitutions into alanine, whereas substitutions into isoleucine lead to decreased thermostability. We use experimental data for lysozyme as a benchmark, achieving promising results, however the uncertainty in our method is considerable.

SIMULATION PROTOCOL

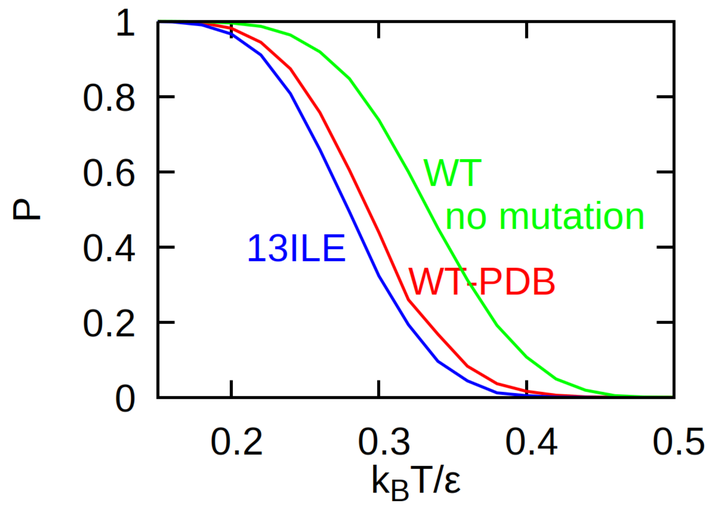


LYSOZYME MUTATIONS (CONTROL). 1LYD.PDB

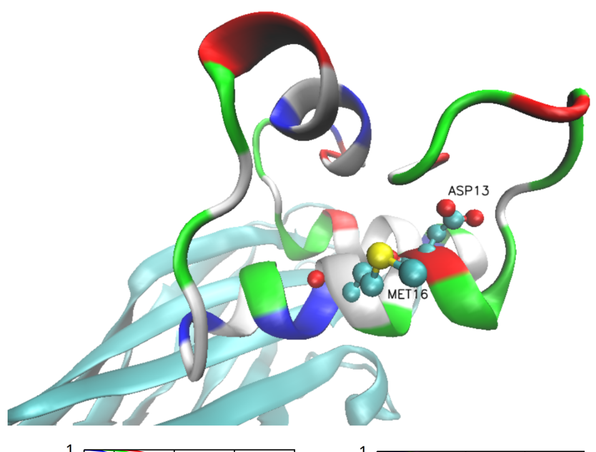


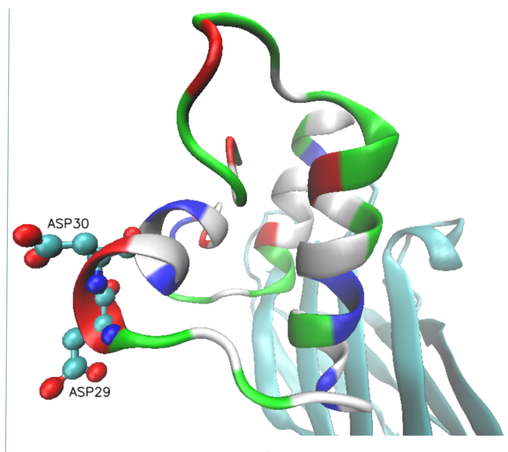


WILD TYPE THERMOSTABILITY



DOCKERIN MUTATIONS (RESULTS). 1OHZ.PDB (CHAIN B)





CONCLUSIONS

- The results for lysozyme correlate with theory as long as neutral pH is used in the experiment - it is a factor hard to recreate in the simulation.
- Difference between isoleucine (lower thermostability) and alanine (higher thermostability) is clearly visible in the results.
- The change of a charged residue into isoleucine may change hydrophobicity too much, but alanine is a promising candidate (glycine may not suitable due to its increased flexibility).
- We have identified mutations that would lead to a substantial increase in thermostability of dockerin.
- Thermostability of the wild type protein depends on the simulation preparation protocol, leading to large uncertainty in the results.
- Our theoretical method seems to be a valid approximation for a rapid screening of the effects of mutations in the mechanical and thermal stabilities of proteins.

REFERENCES

[1] M. Chwastyk, A. M. Vera, A. Galera-Prat, M. Gunnoo, D. Thompson, M. Carrión-Vázquez, and M. Cieplak, Nonlocal effects of point mutations on the stability of a protein module., *Journal of Chemical Physics* **147**, 105101 (2017)

[2] Ł. Mioduszewski, J. Bednarz, M. Chwastyk, and M. Cieplak, Contact-based molecular dynamics of structured and disordered proteins in a coarse-grained model: Fixed contacts, switchable contacts and those described by pseudo-improper-dihedral angles, *Computer Physics Communications* **284**, 108611 (2023)

[3] X. Zhang et al, Enhancement of protein stability by the combination of point mutations in T4 lysozyme is additive, *Protein Engineering, Design and Selection* **8**, 10, 1017–1022 (1995)