

Improving the Design of a Chimeric $(\beta\alpha)_8$ -Barrel with Rosetta

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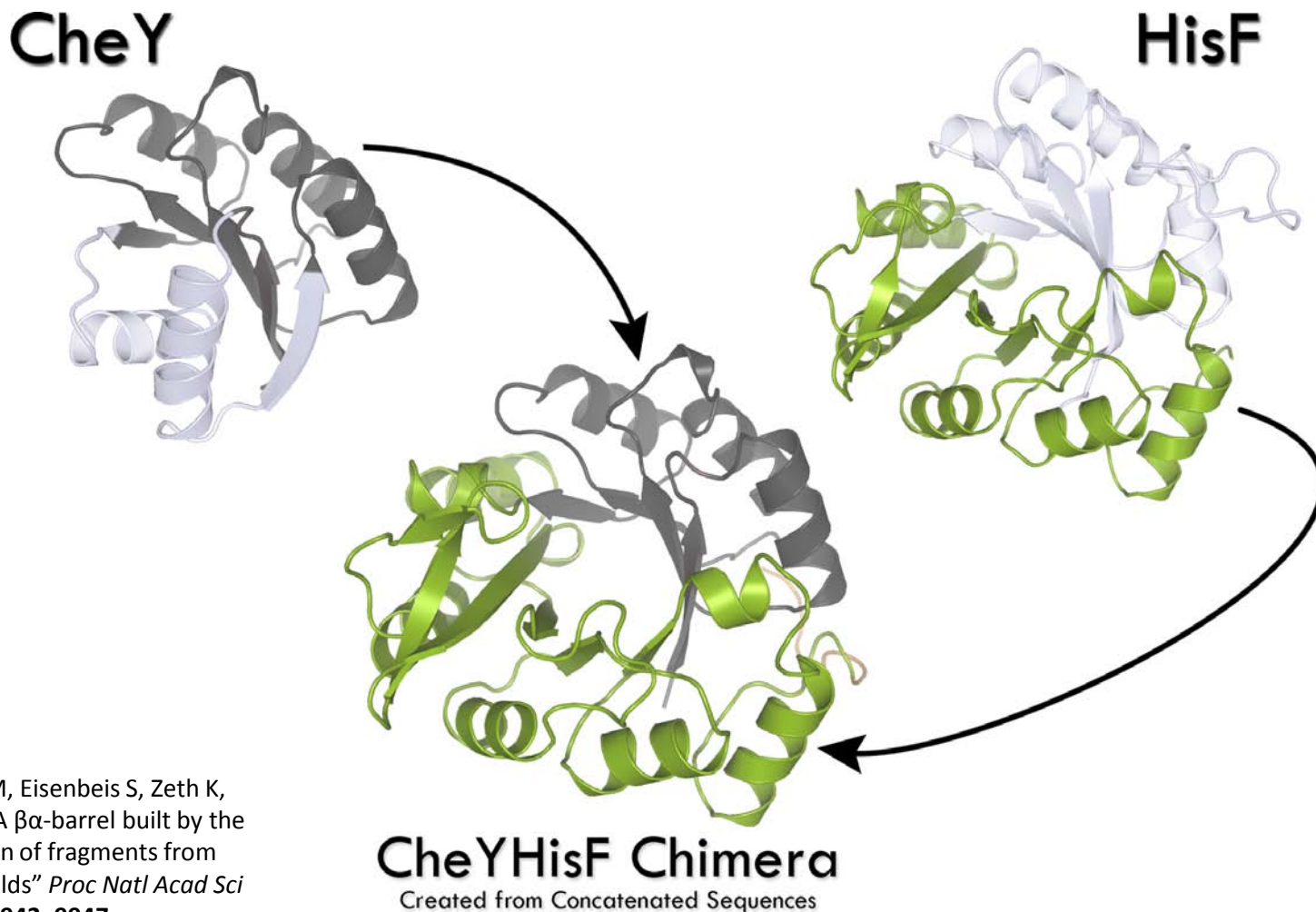
VANDERBILT
UNIVERSITY



MAX-PLANCK-GESELLSCHAFT

Protein Evolution Through Recombination

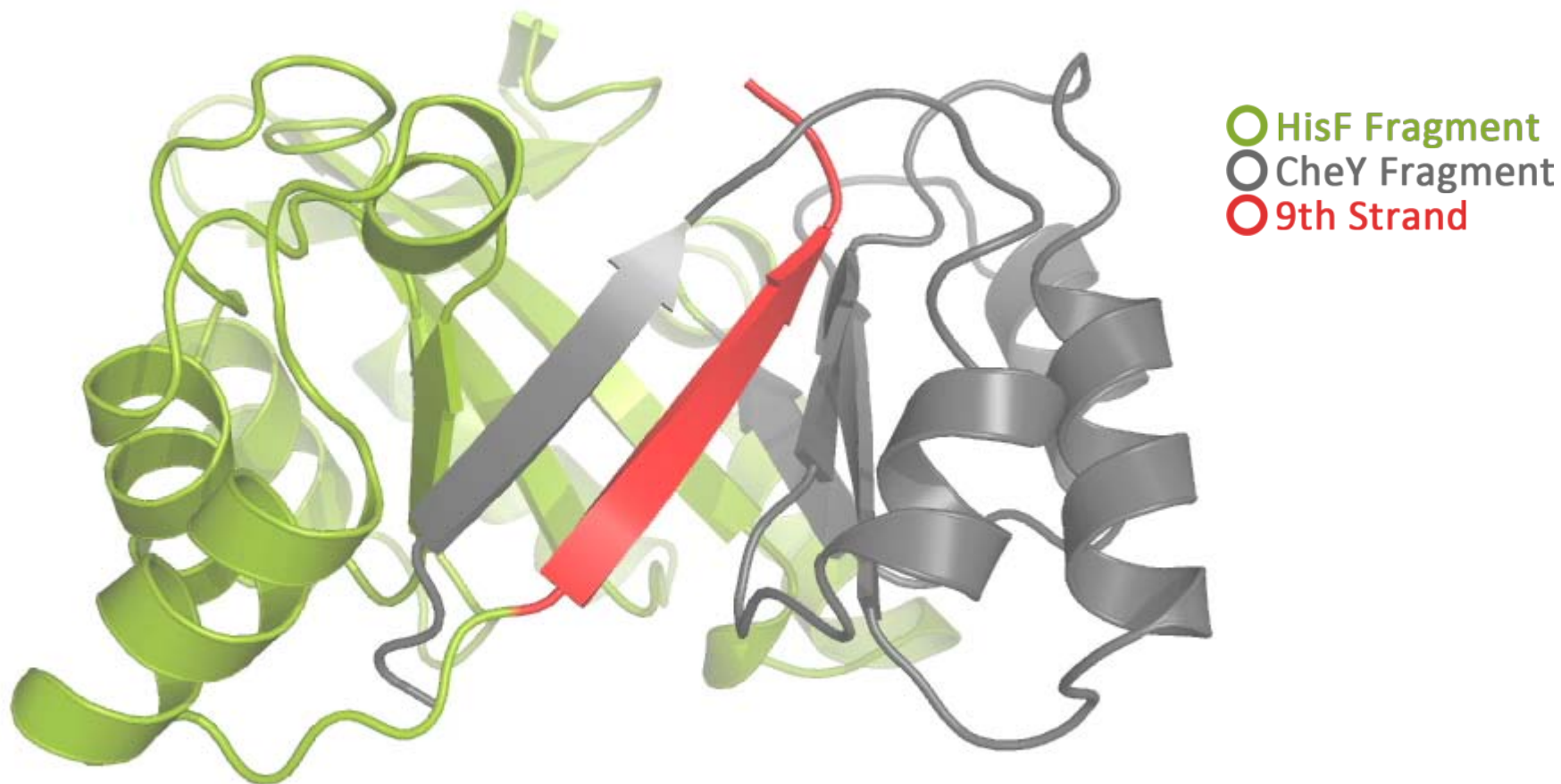
A viable evolutionary strategy?



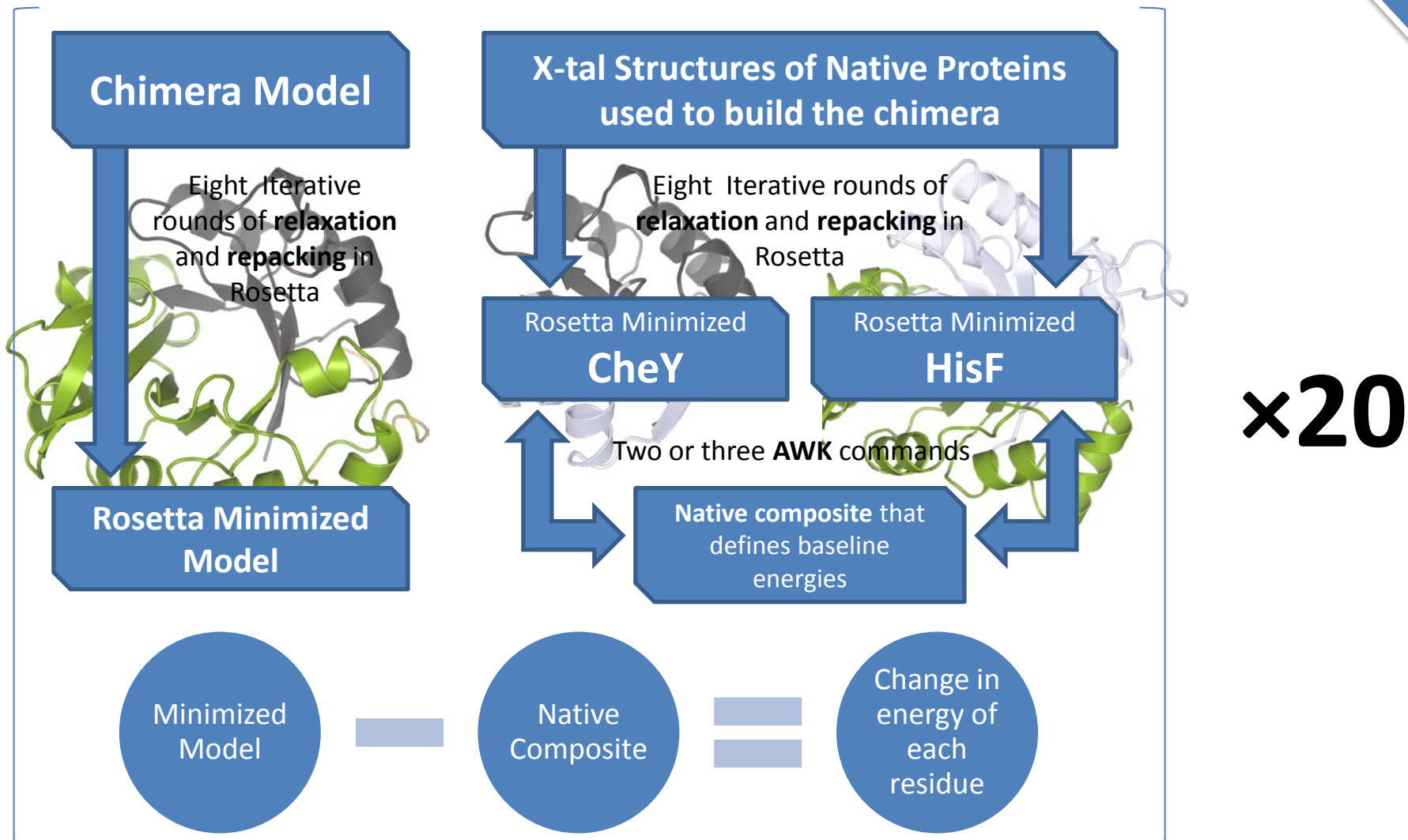
Bharat TAM, Eisenbeis S, Zeth K, Höcker B "A β -barrel built by the combination of fragments from different folds" *Proc Natl Acad Sci* 2008 105:9942–9947

Crystal Structure Reveals 9th Beta Strand

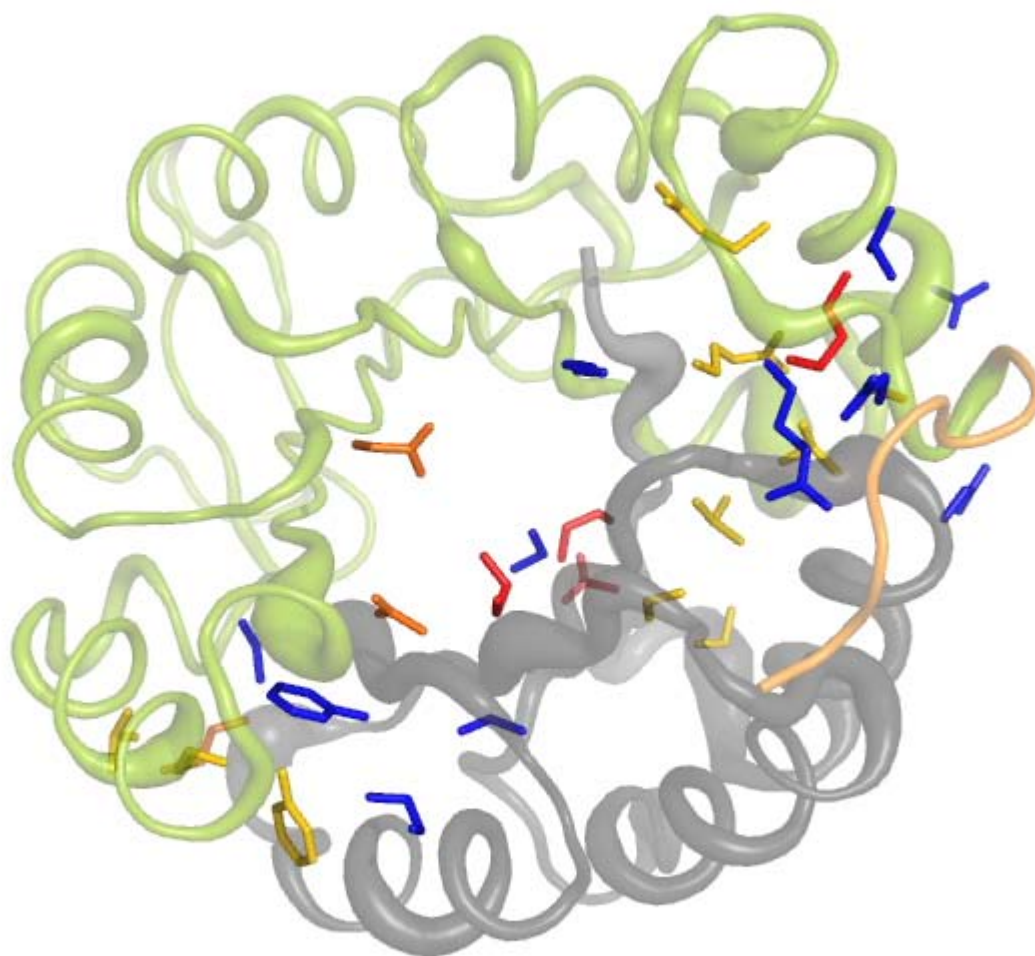
...formed from an attached histidine purification tag



Energy Comparison with Wildtype Fragments In Rosetta

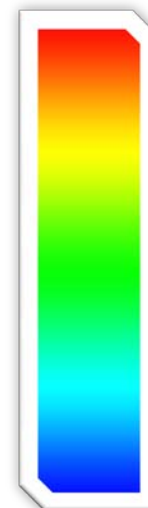


Energy Comparison with Wildtype Fragments Reveals Stress Along the Interfaces



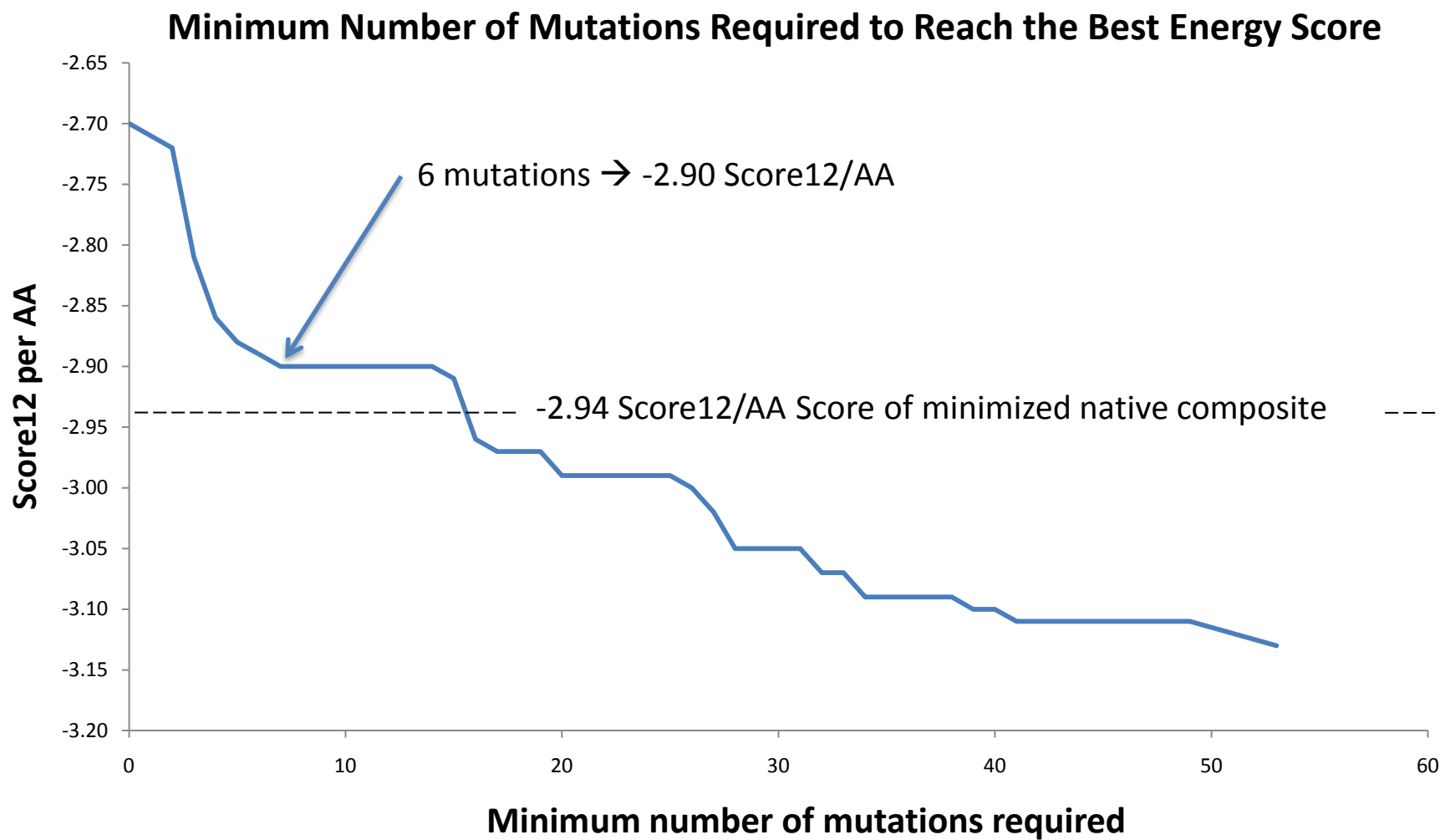
○ HisF Fragment
○ CheY Fragment

Increase in Energy
(Approx. $\Delta 6$ Score12 Units)



Decrease in Energy
(Approx. $\Delta -2$ Score12 Units)

Rosetta Predicts Six Mutations Are Necessary to Reach Wild Type Energy



Rosetta-suggested mutations stabilize the fold, reducing clash along the interfaces of the two fragments

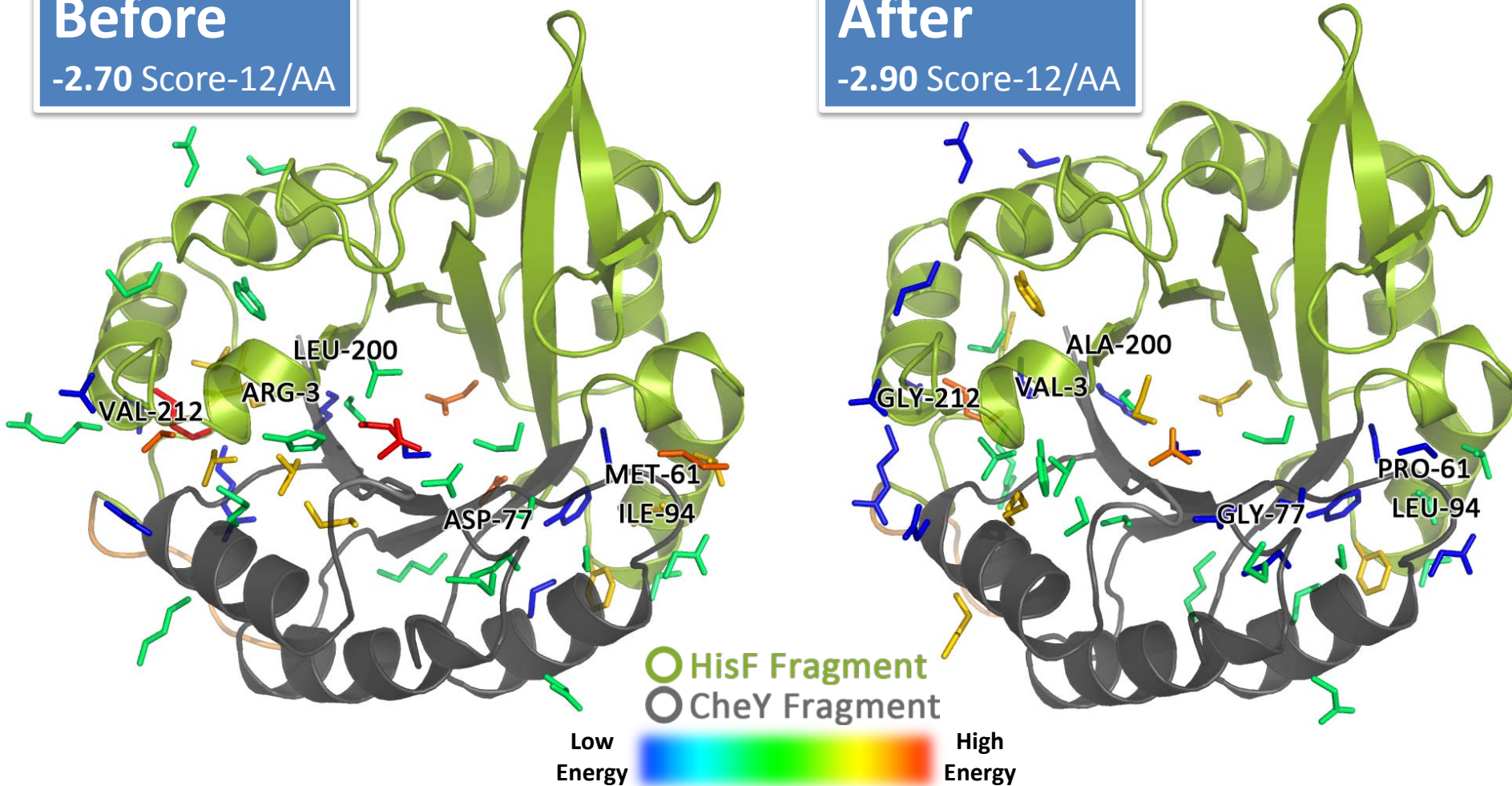
+ 6 Mutations

Before

-2.70 Score-12/AA

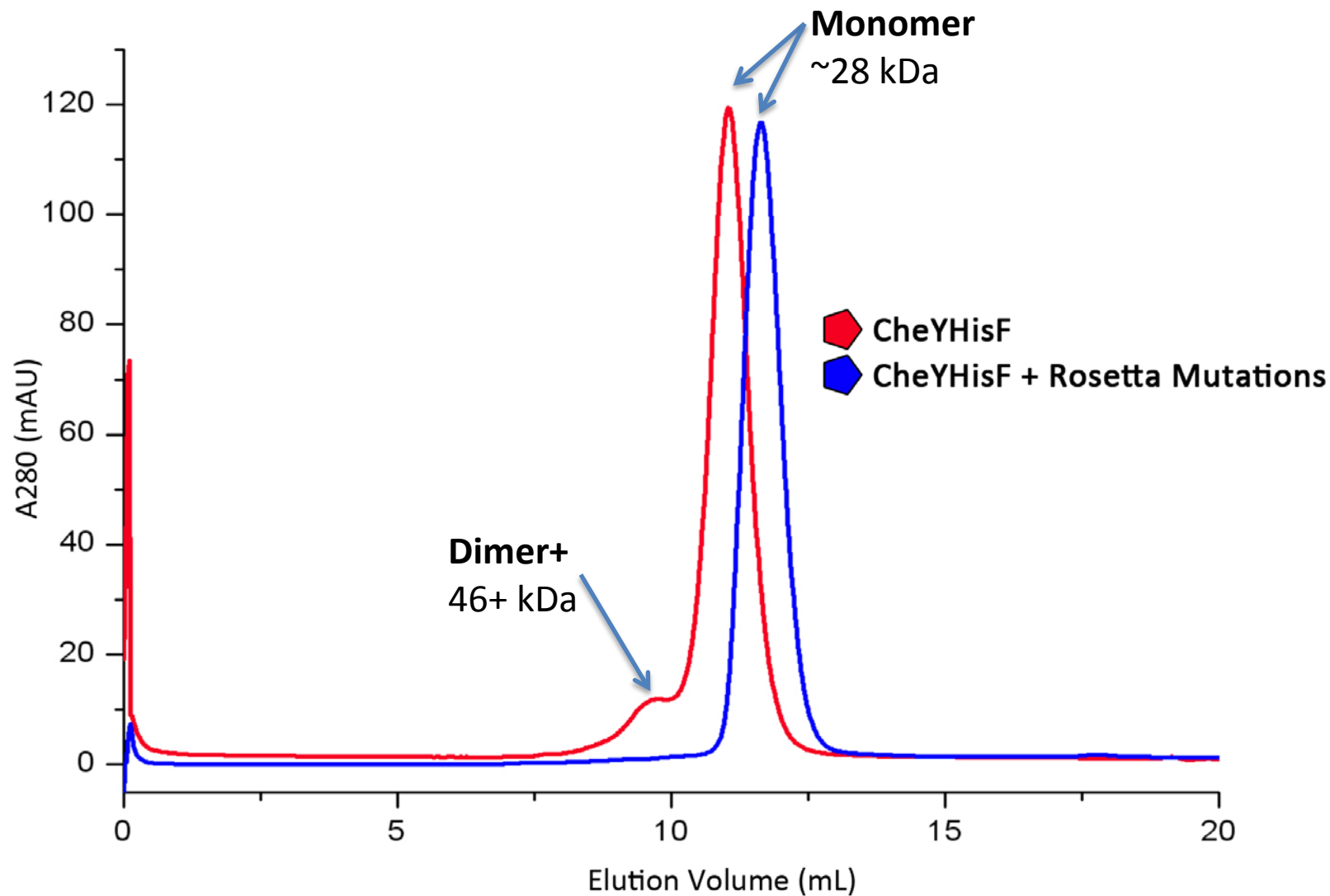
After

-2.90 Score-12/AA

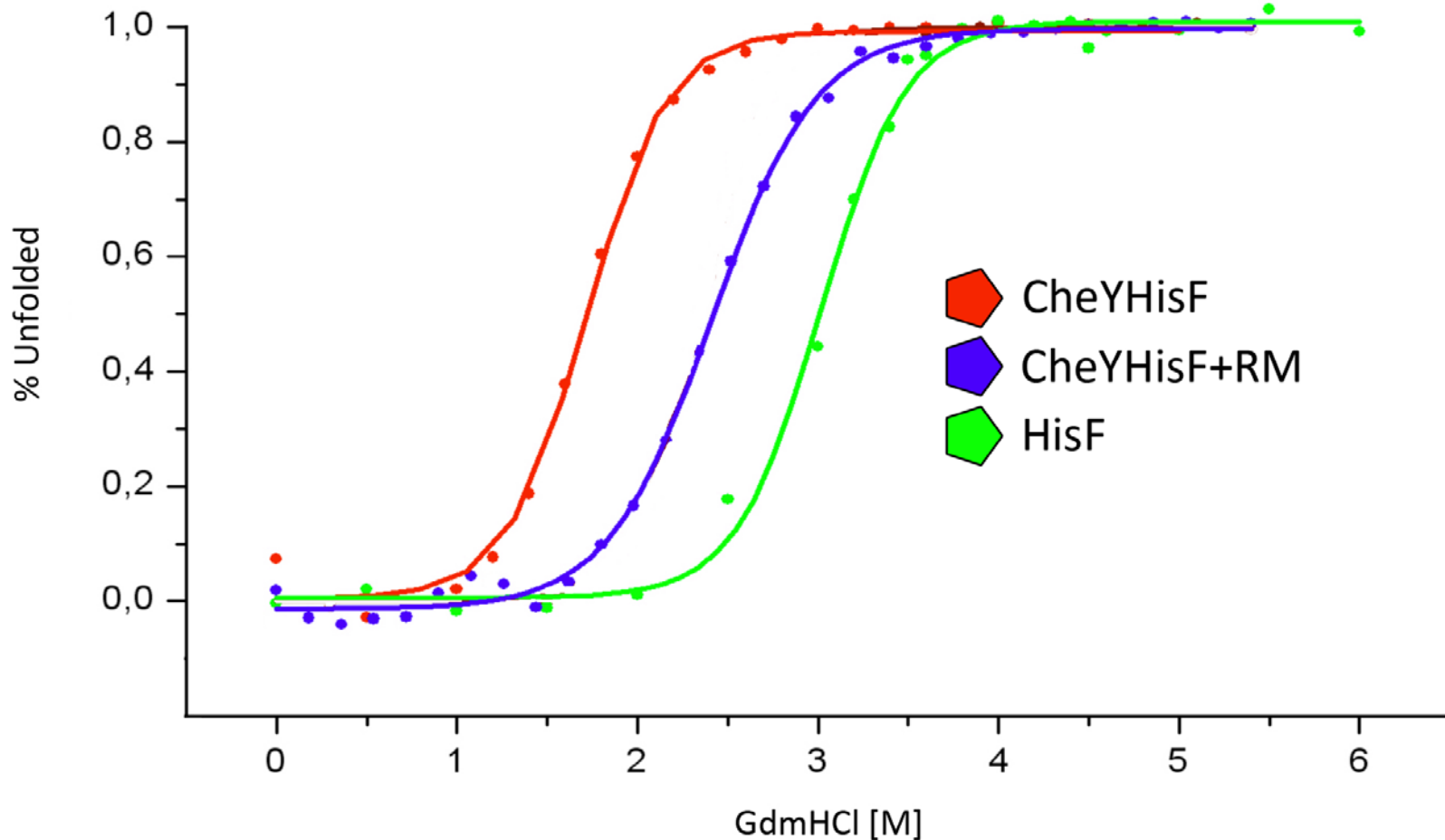


Native: -2.94 Score-12/AA

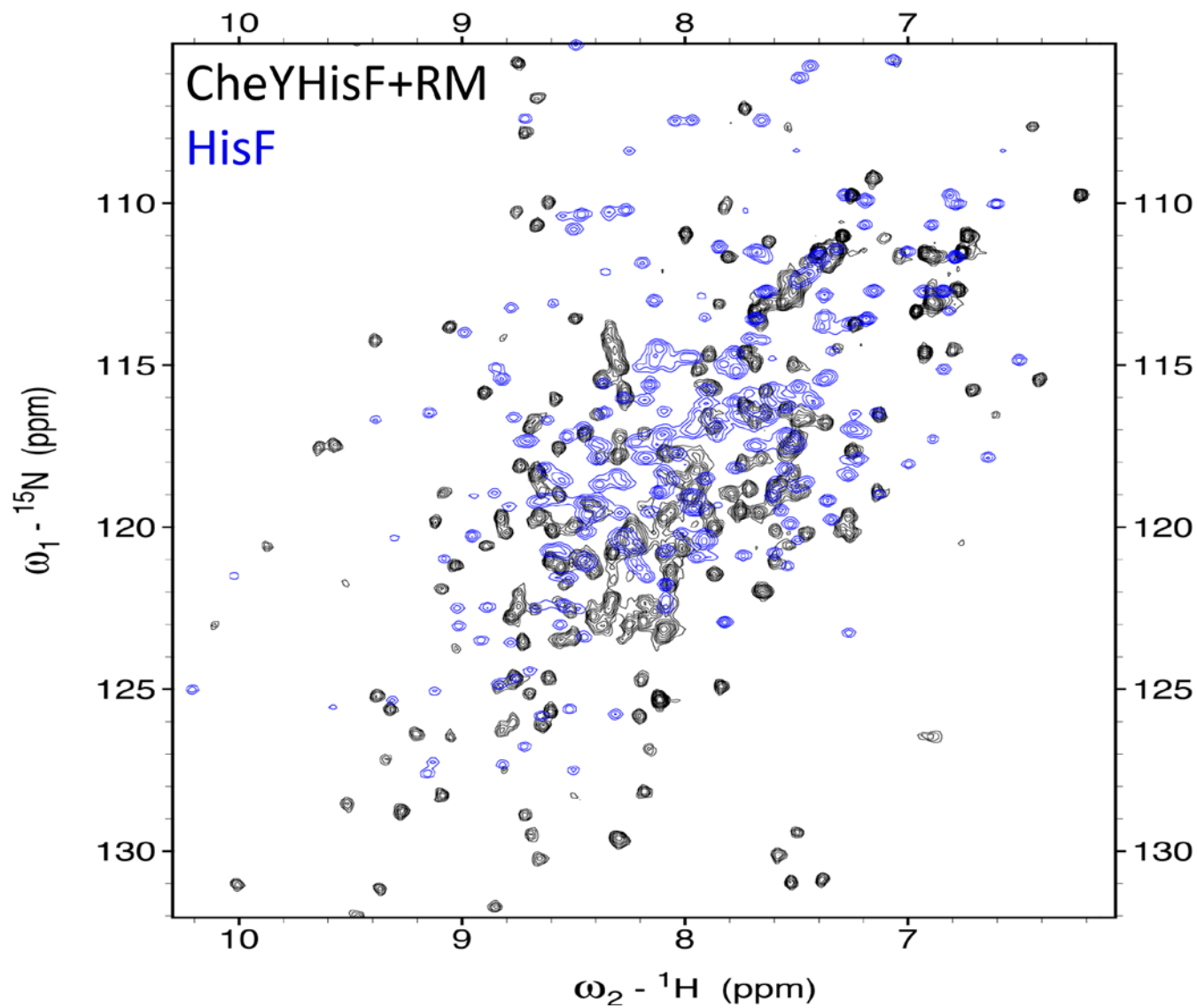
Size Exclusion Chromatography (SEC) indicates the mutated chimera is less prone to oligomerization



Comparison of Unfolding by Chemical Denaturation Indicates that Rosetta Mutations Stabilize the Fold



HSQC of HisF and CheYHisF with the Rosetta Mutations Shows Similar Dispersion and Ordered Structure

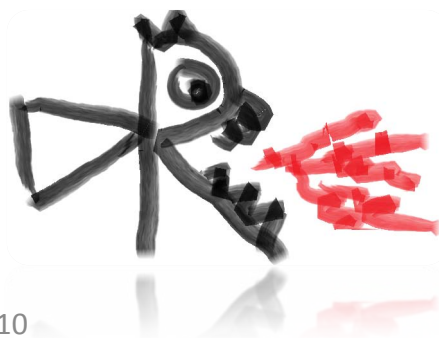


Conclusions

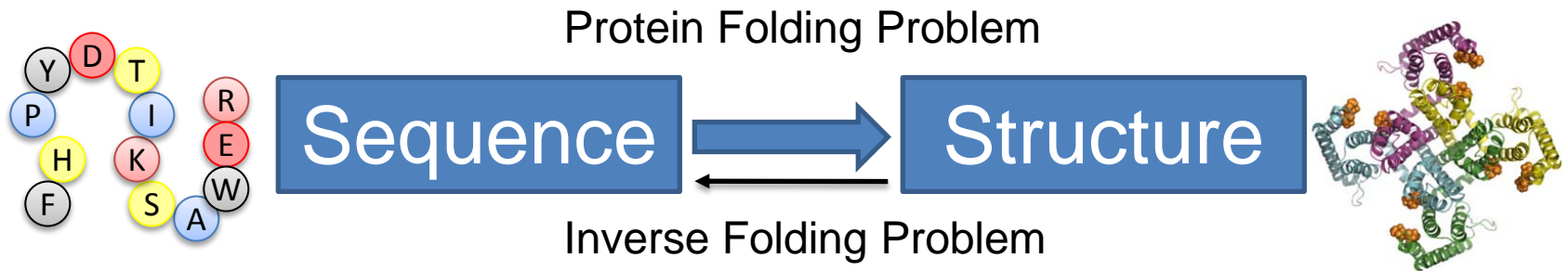
- *Lego* style fragment combination is a viable strategy for building larger proteins
- Using existing structures to build new models allows for trivial ddG calculations to find potential problems in the design BEFORE you get to the wetlab.
- Chimeric proteins have limitless potential

Acknowledgements

- Jens Meiler
- Birte Höcker
- Simone Eisenbeis
- Kristian Kaufmann
- Meiler Lab
- Höcker Group



Protein Design - Can we build larger proteins by combining fragments of smaller ones?



- **Inverse Folding Problem**

- *De novo* design is difficult: assume a total of 100 conformations for all 20 natural occurring amino acids side chains in a 100 amino acid protein → 10^{200} possible conformations!
- Instead of starting from scratch, can we build larger proteins by **combining fragments** of smaller ones?

Outline

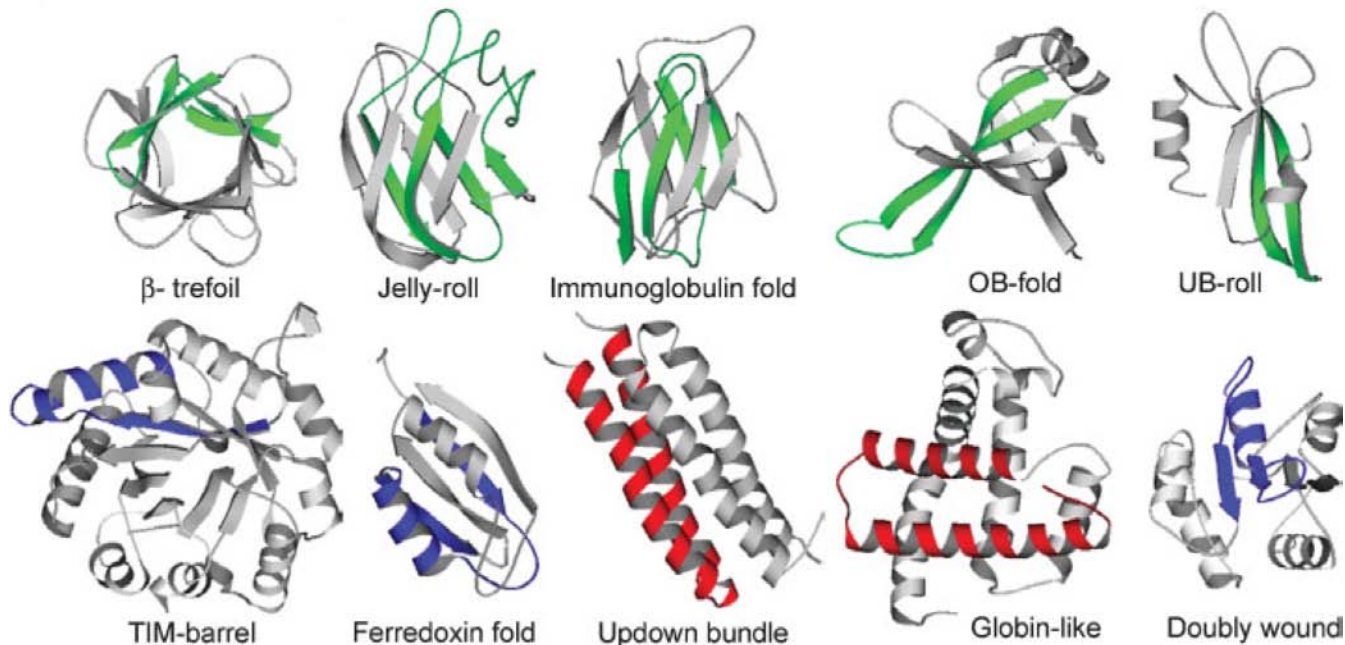
- Background
- Redesign of the Chimera with Rosetta
- Experimental Validation
- Implications for Protein Design

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Chimeras capitalize on nature's hypothesized pathway to larger proteins by using modular assembly

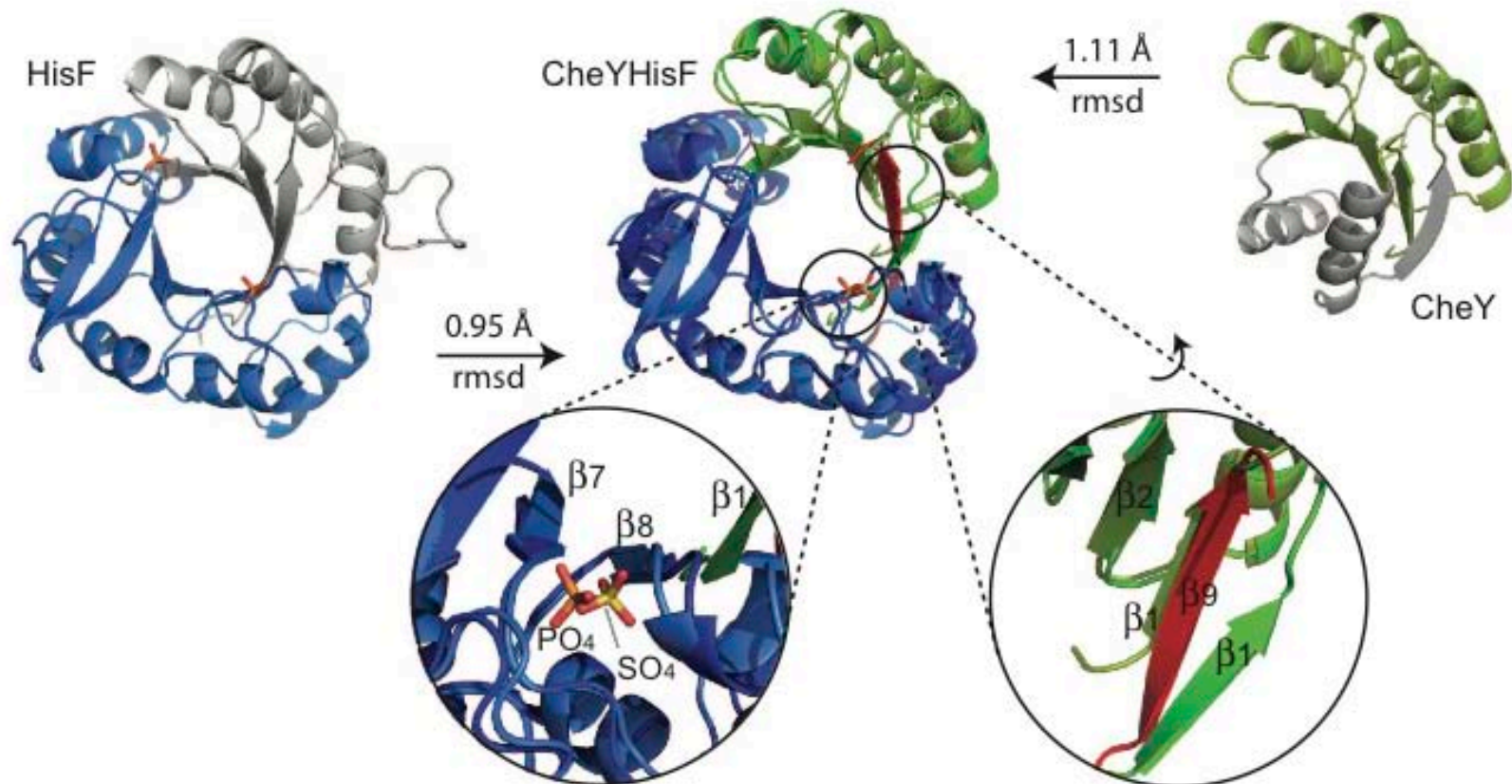
- Many proteins in nature share fragments that are similar in structure and sequence



- Modular assembly protocol can be applied to the design of new structures

Soeding J, Lupas AN (2003) "More than the sum of their parts: On the evolution of proteins from peptides." *BioEssays* 25:837–846.

Crystal structure reveals an extra beta strand



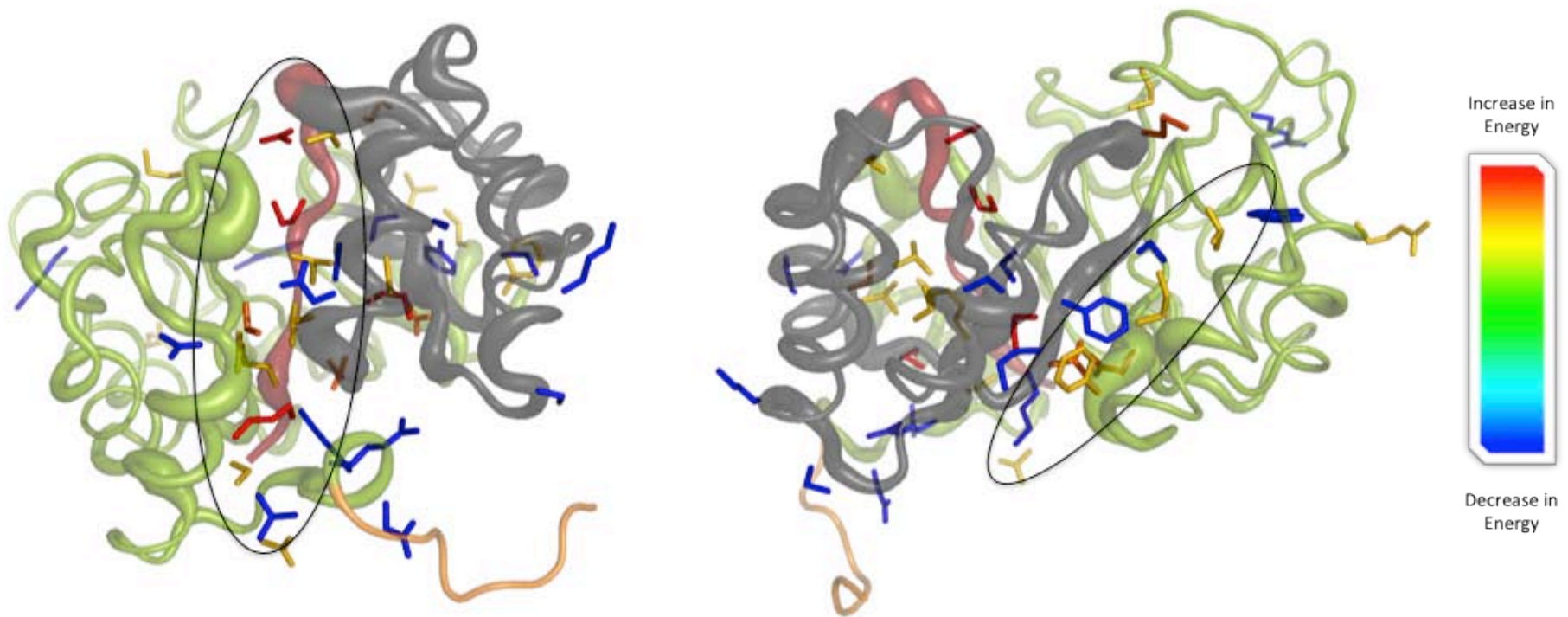
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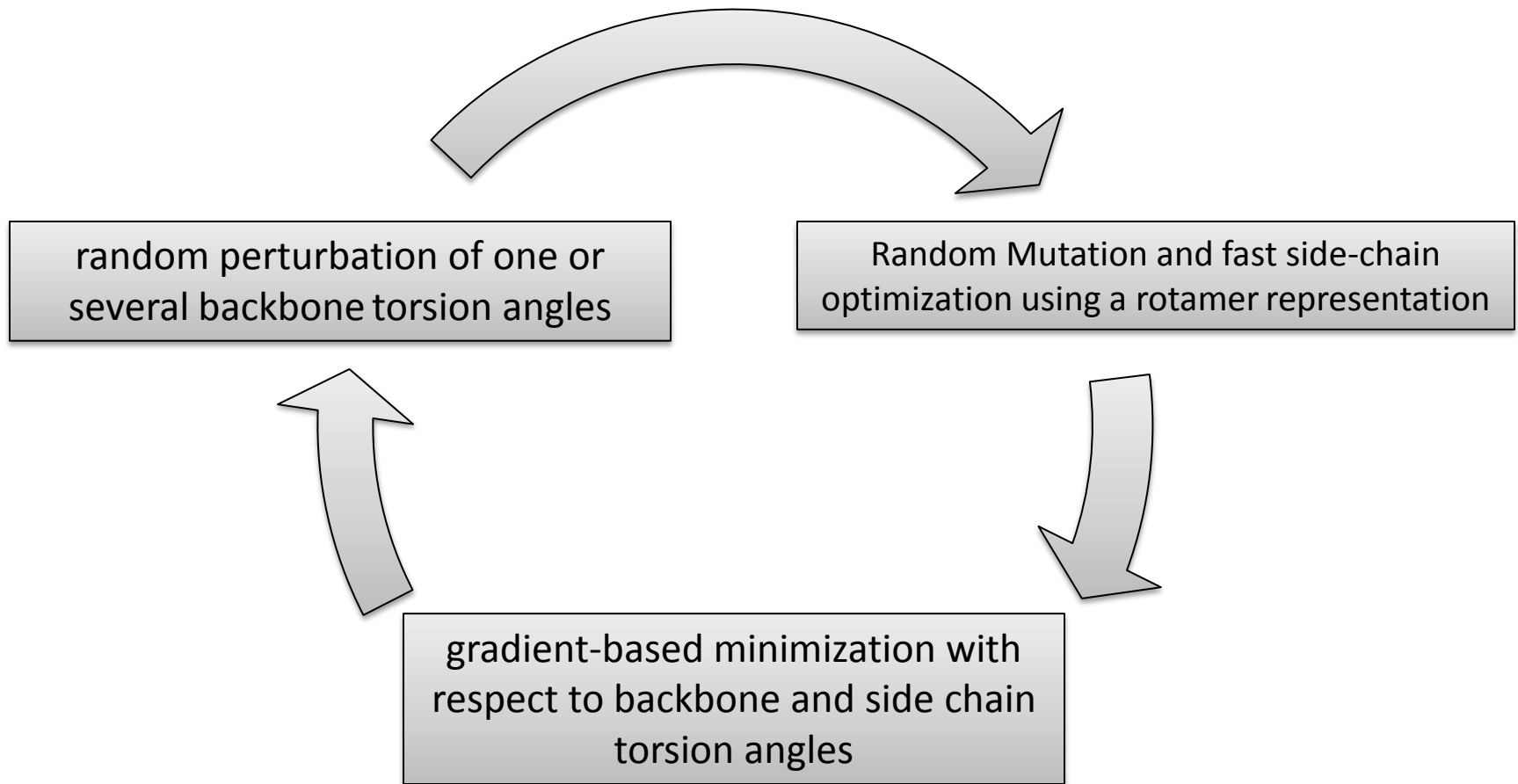
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Locating Clash in the Chimera

Energy Comparison with Wildtype Fragments Reveals Stress Along the Interfaces



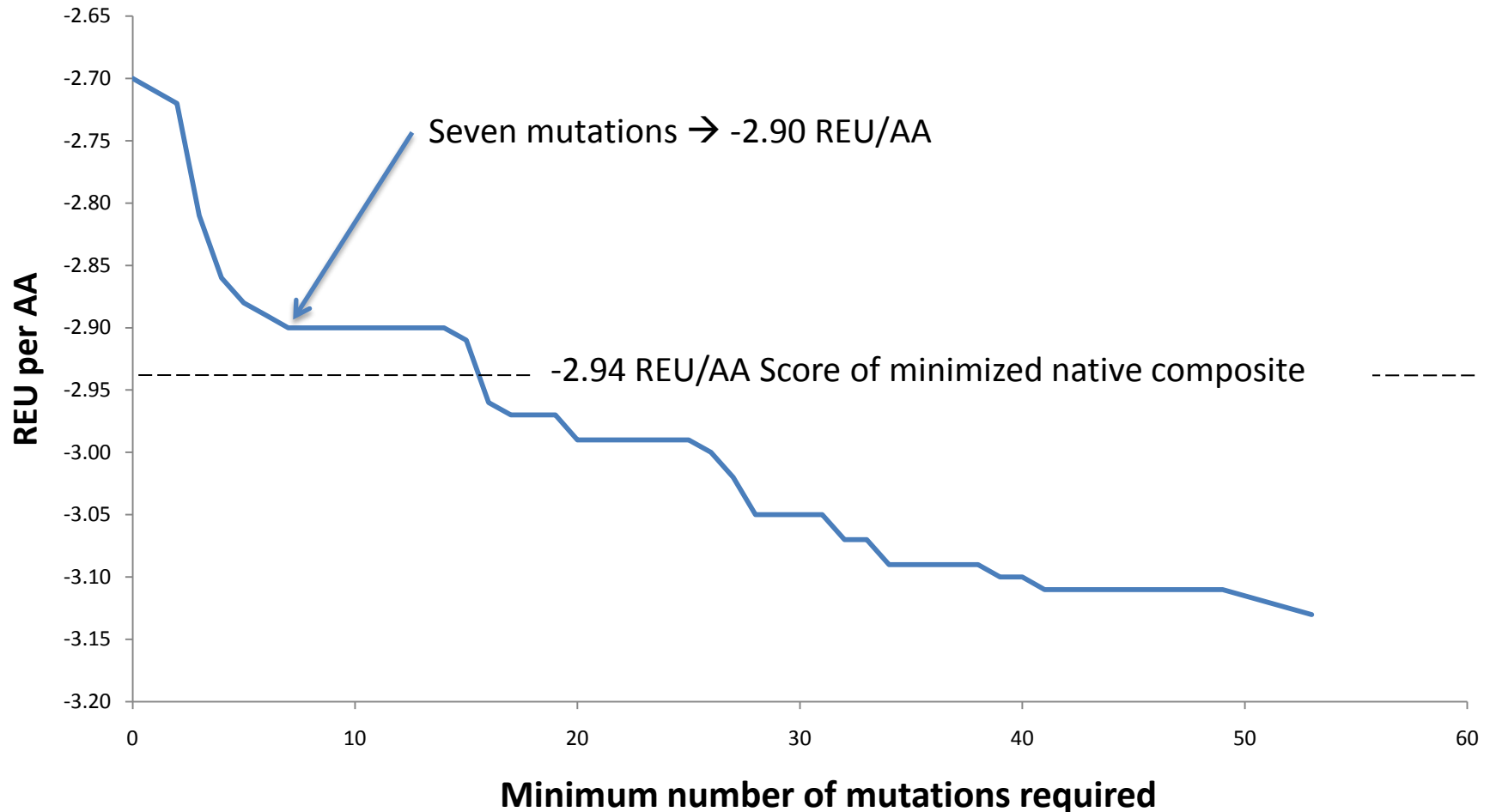
Redesigning the Chimera in Rosetta



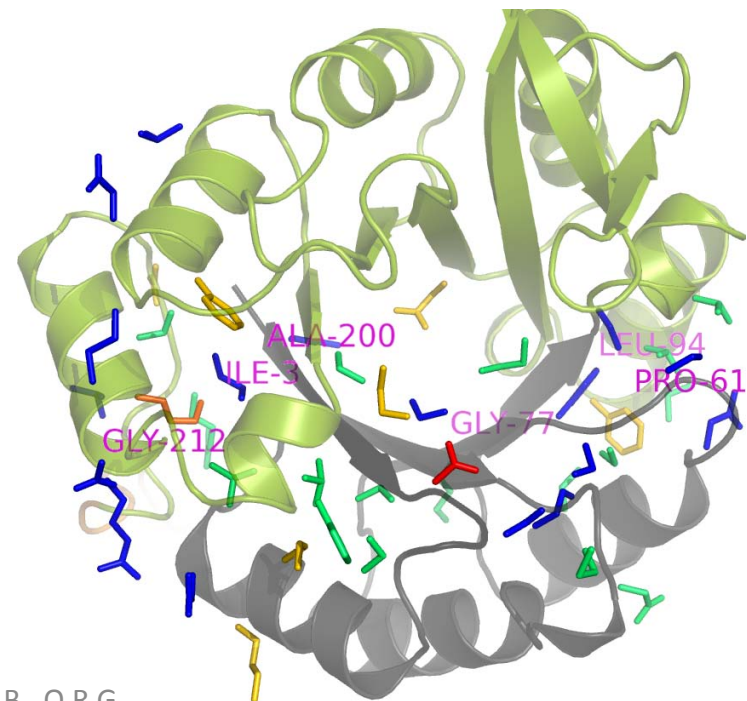
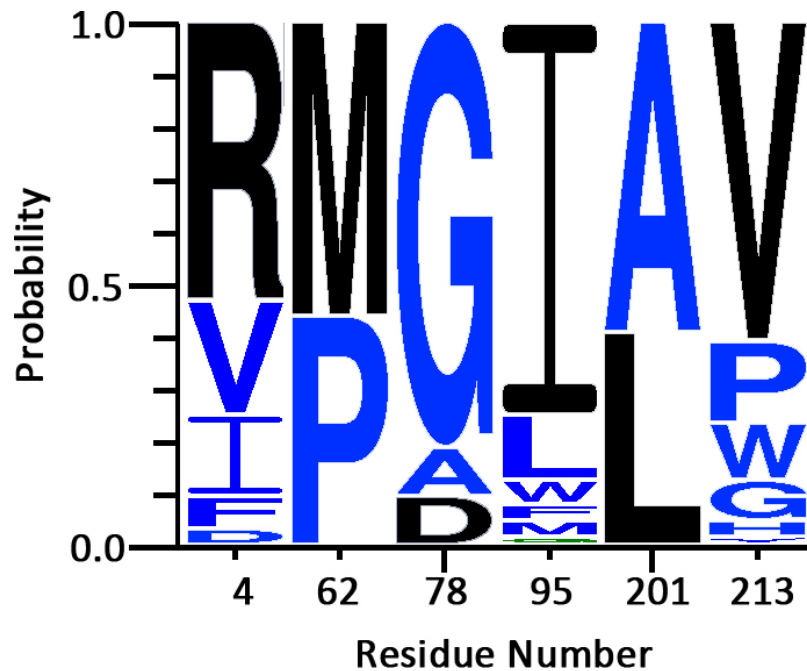
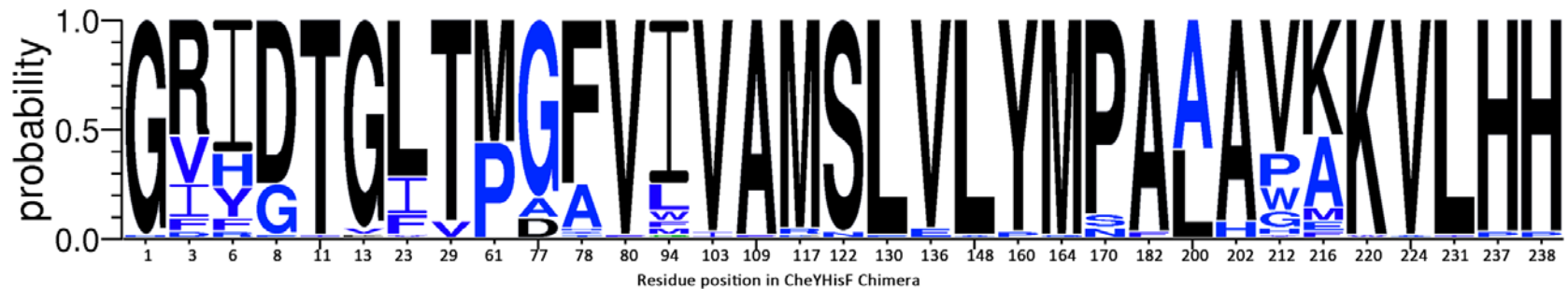
Redesigning the Chimera in Rosetta

Rosetta Predicts Seven Mutations Are Necessary to Reach WildType Energy

Minimum Number of Mutations Required to Reach the Best Energy Score

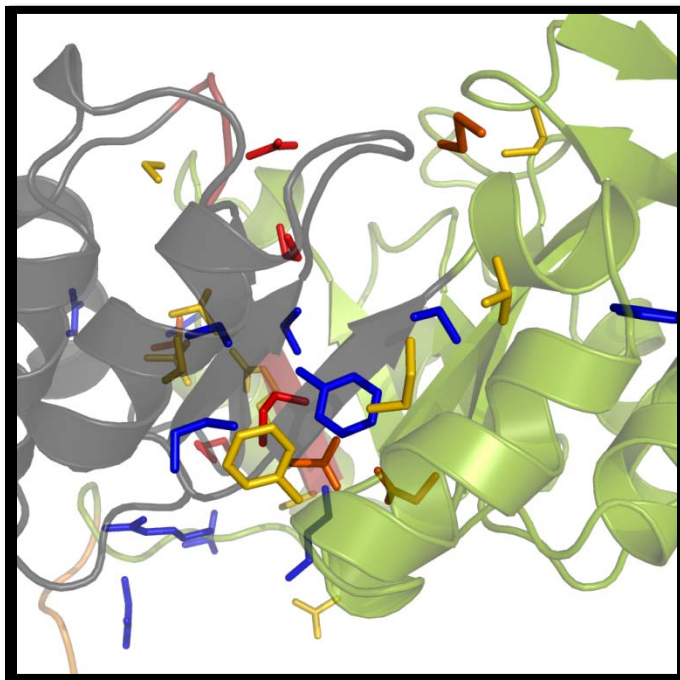


Sequence comparison reveals commonalities in the best ranked designs



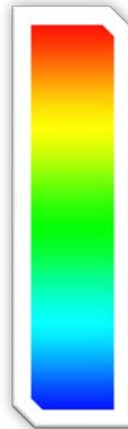
Mutation Analysis

- Significant improvement along the first and second interfaces

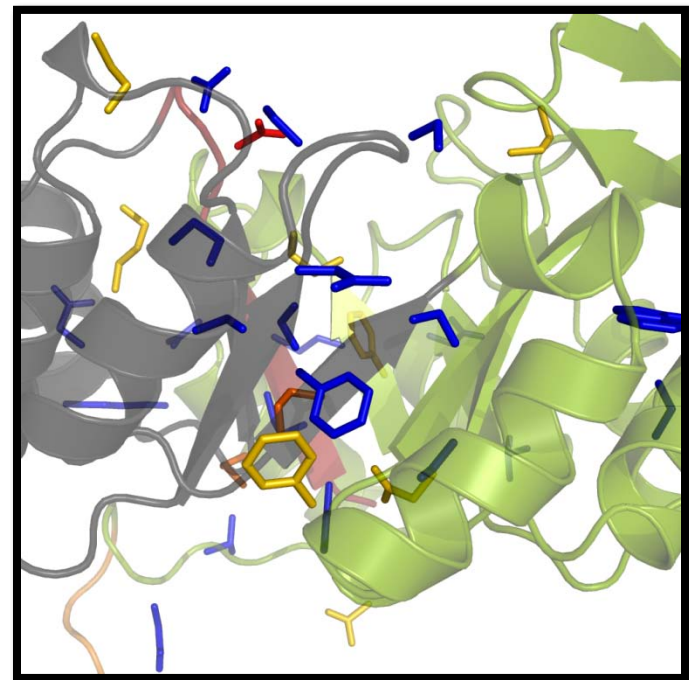


Before: -2.70 reu/aa

Increase in
Energy



Decrease in
Energy

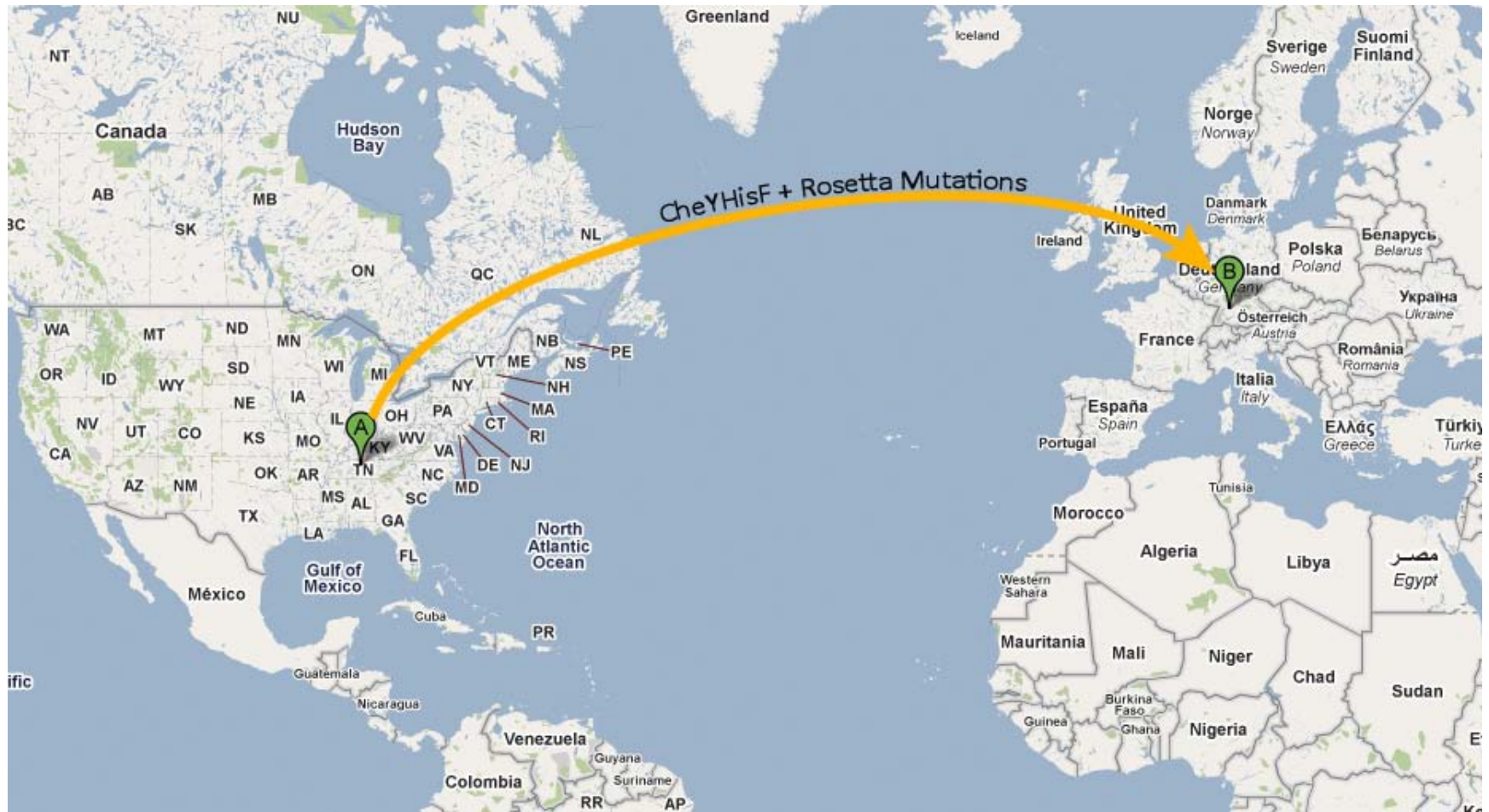


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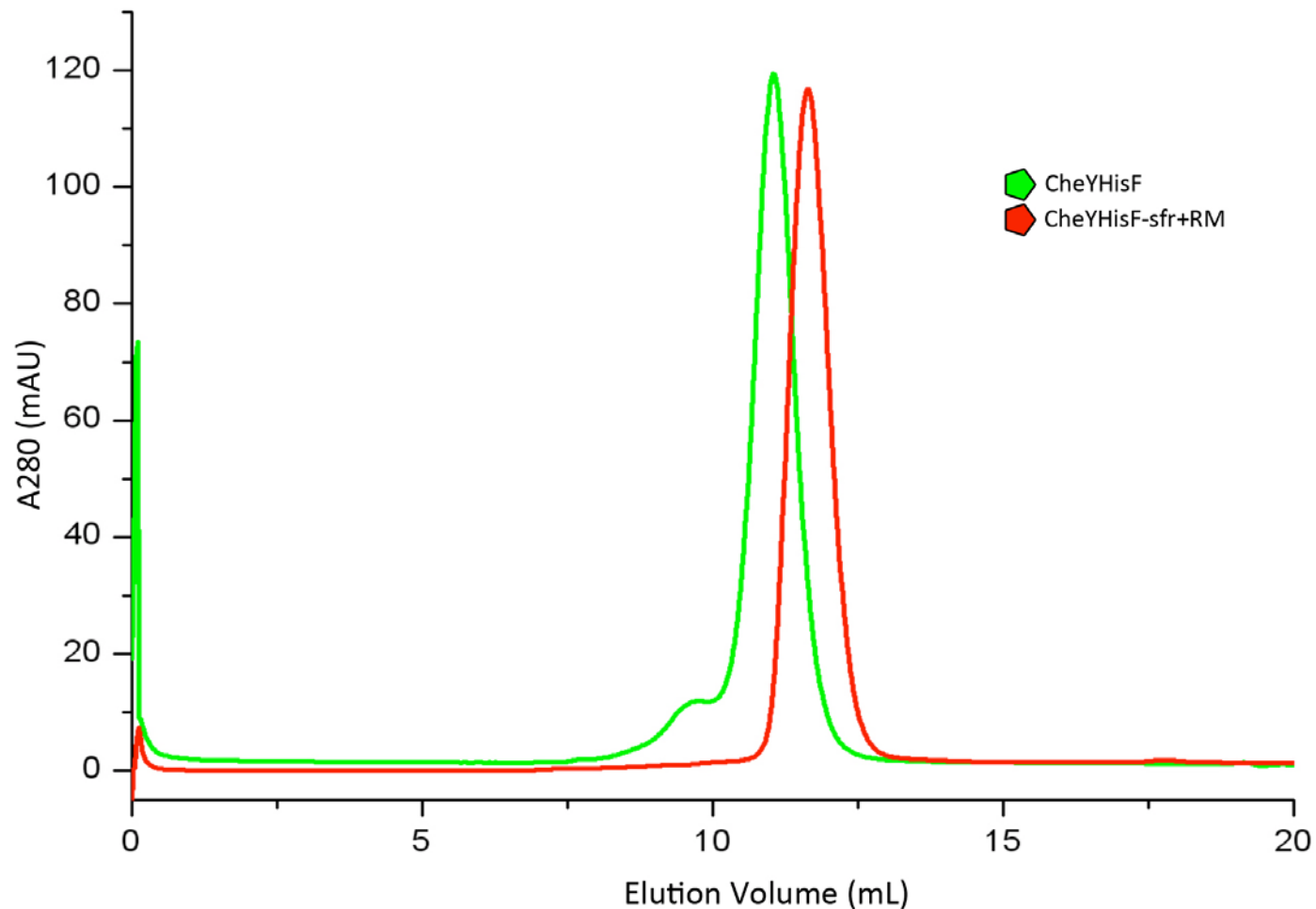
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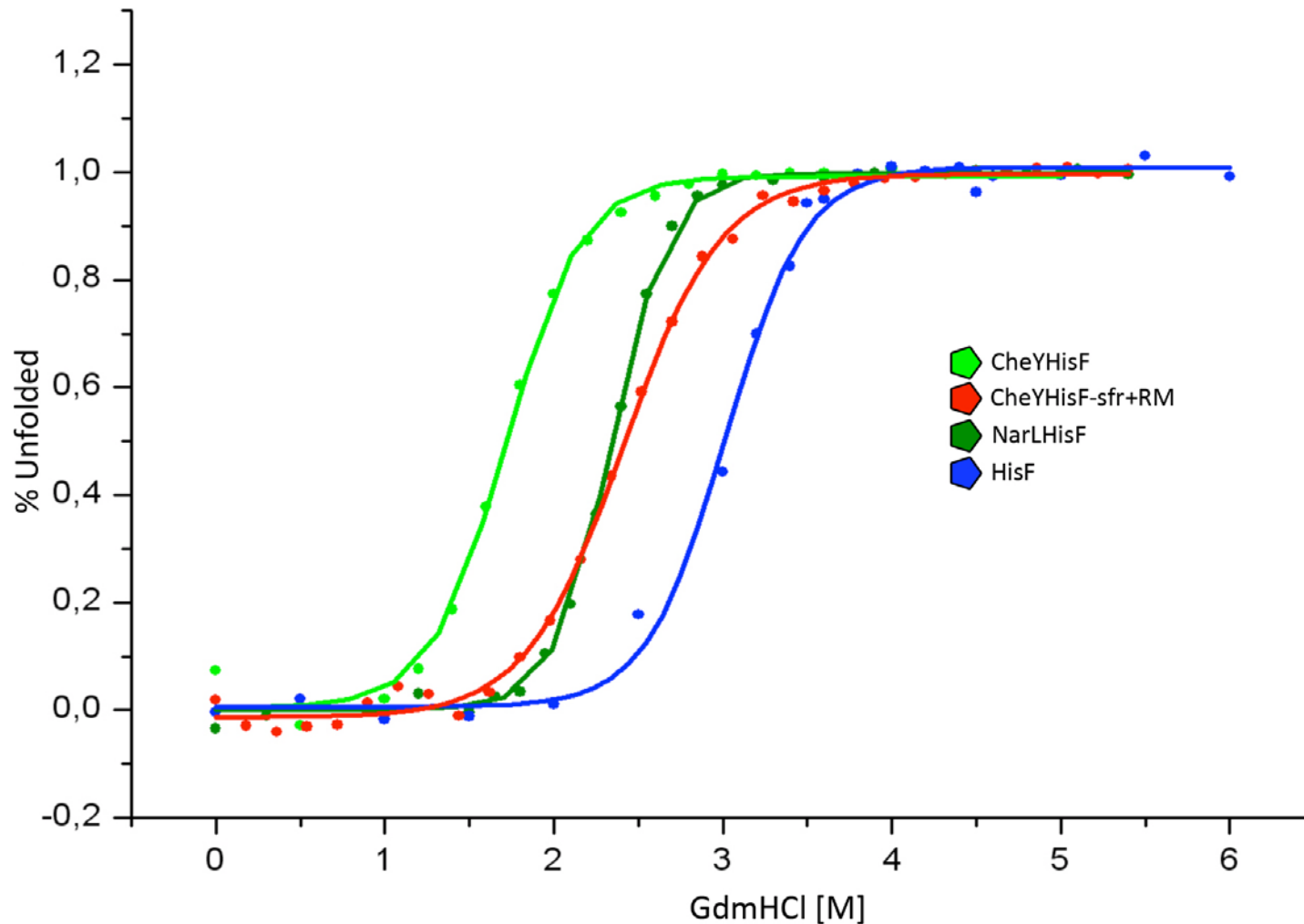
The Chimera with the Rosetta Mutations



Size Exclusion Chromatography (SEC) Shows New Chimera is Less Prone to Oligomerization

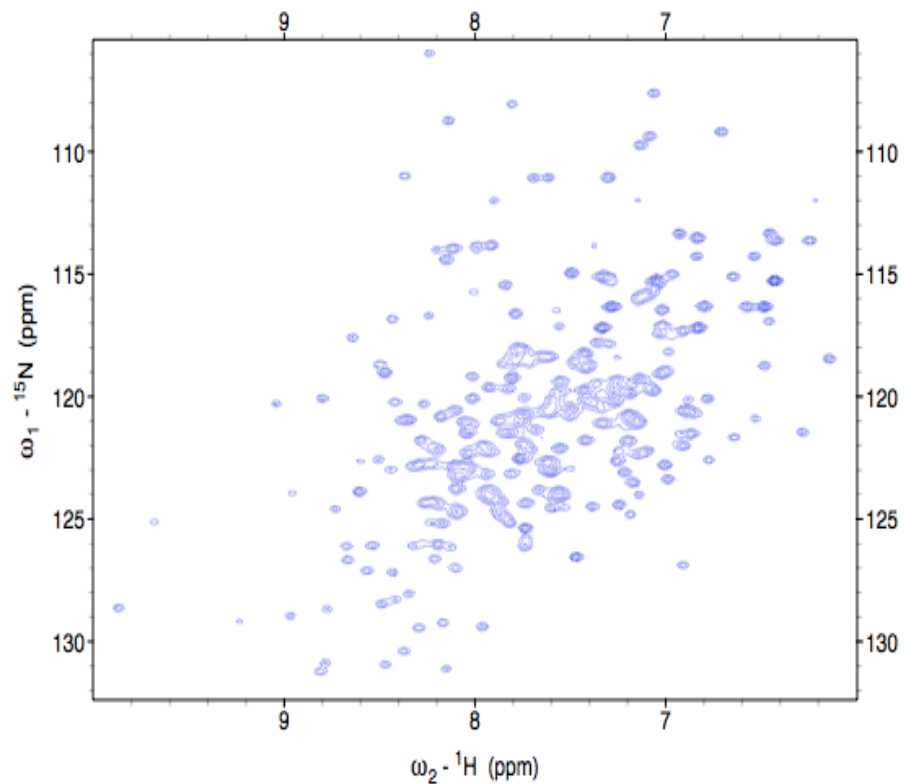


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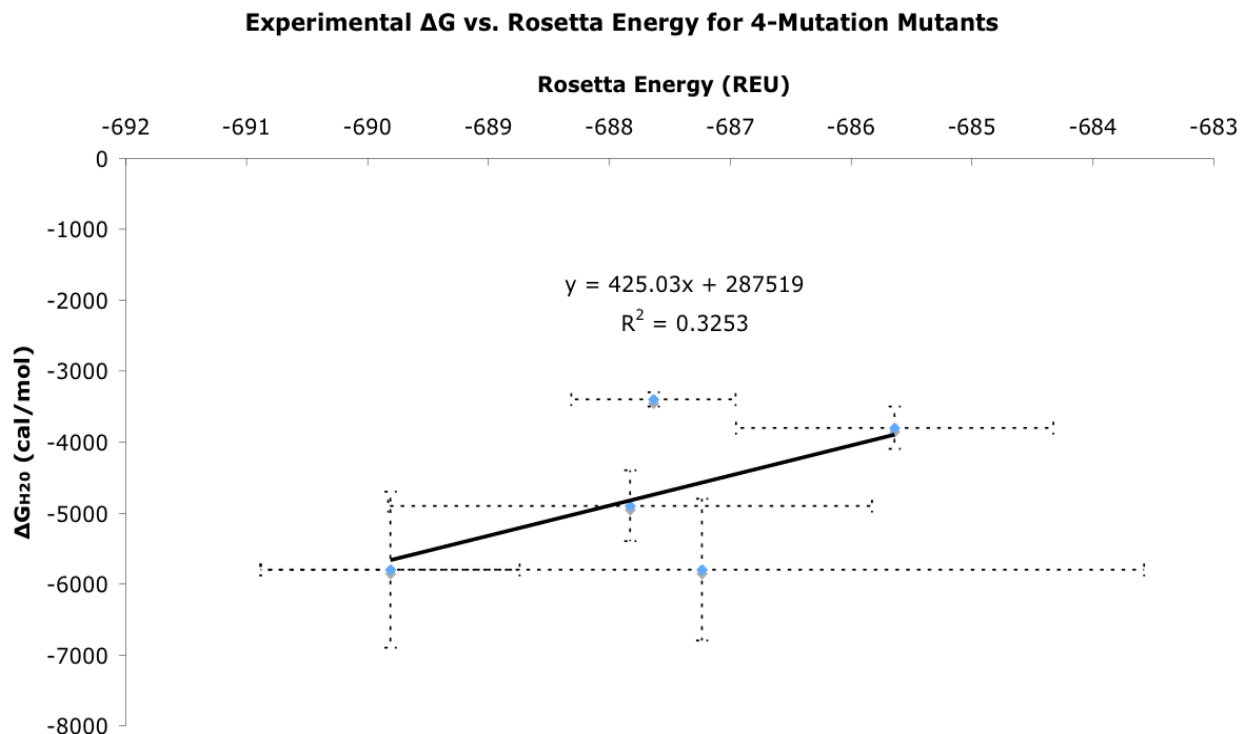
HisF



CheYHisF+RM

Incremental Addition of Mutations shows Theoretical Pathway for Cloning in the wetlab

Comparison of Experimentally and Computationally Obtained ΔG Values Shows very Successful Prediction/Ranking for Specific Mutants



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Implications for Protein Design

- Concatenation of protein subunits combined with a computational protocol to redesign interfaces could allow for rapid creation of larger design proteins
 - Higher probability of achieving the predicted fold
 - Retain active sites of the respective protein subunits

Acknowledgements

- Jens Meiler
- Birte Hoecker
- Simone Eisenbeis
- Kristian Kaufmann



Experimental ΔG rankings vs. Rosetta Energies

- Comparison of experimentally and computationally obtained ΔG values shows very successful prediction/ranking for specific mutants.

