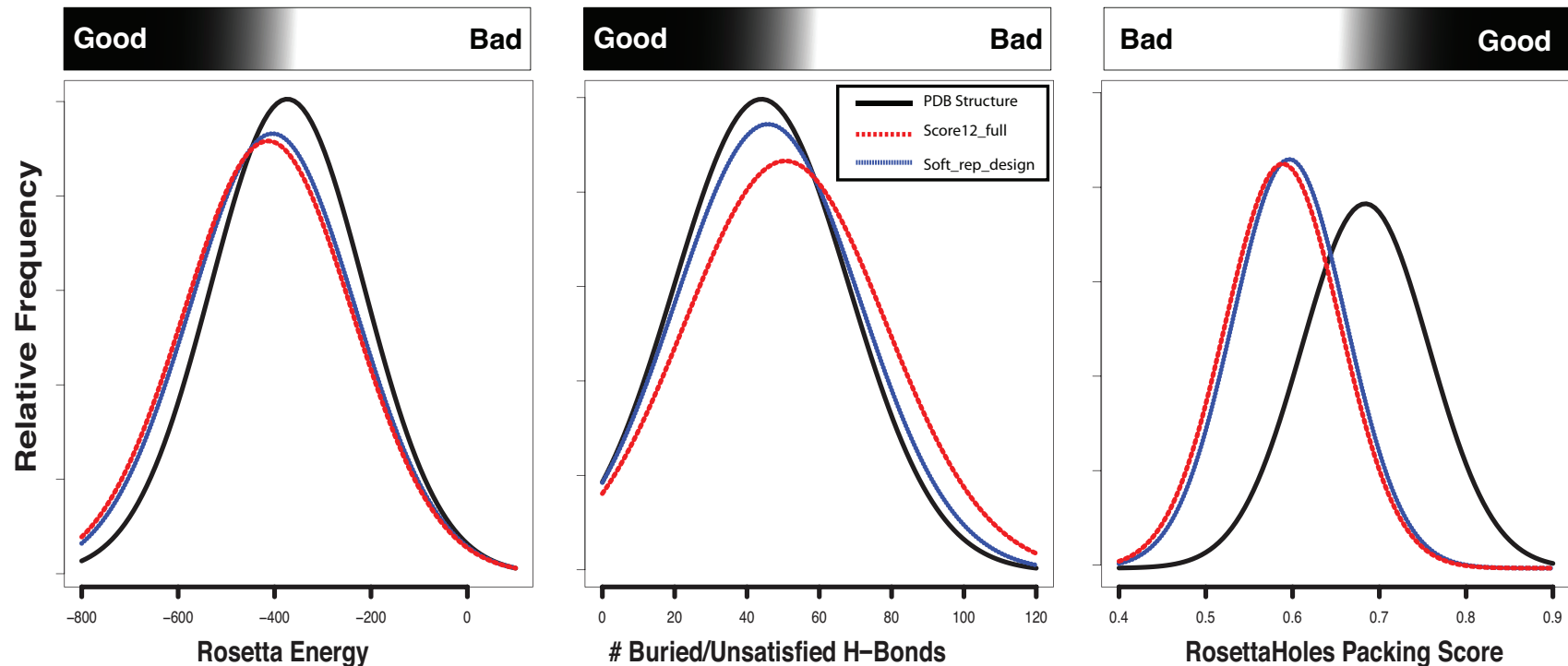# Automated Selection of Stabilizing Mutations by **V**oid **I**dentification and **P**acking

Benjamin Borgo

Havranek Lab @ Washington University

Fixed backbone design leads to models which differ from native structures

- Single iteration of fixed-bb design/relaxation **improves Rosetta energy**

- Number of buried **unsatisfied hydrogen bonds is worse** in designed proteins than crystal structures

- RosettaHoles **packing quality is greatly decreased** in designed proteins

How can we improve packing prior to experimental characterization?

- Strategy:
    - Use RosettaHoles to identify buried, unfilled voids
    - Reduce designable residue set to adjacent residues
    - Require a score function which will permit more clash (ie softer) but not ignore local steric complementarity
    - Fixed bb, but predictive of mutations which become favorable upon relaxation

Gaussian Overlap Energy

- Atoms treated as spherical Gaussian distributions

$$E_{goe} = \int_{-\infty}^{\infty} \rho(r_1, R_1)\rho(r_2, R_2)dr$$
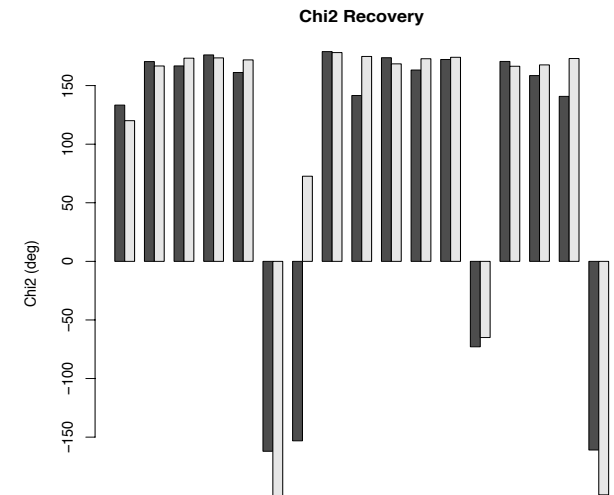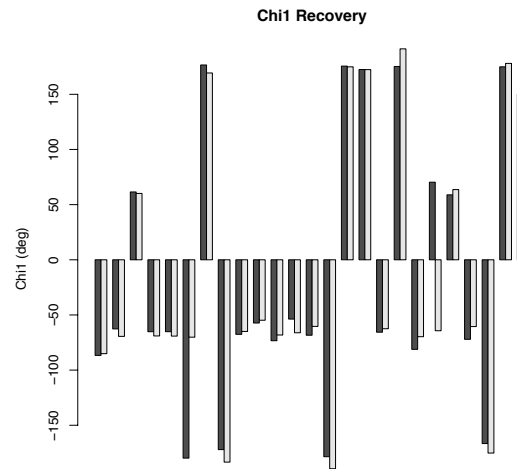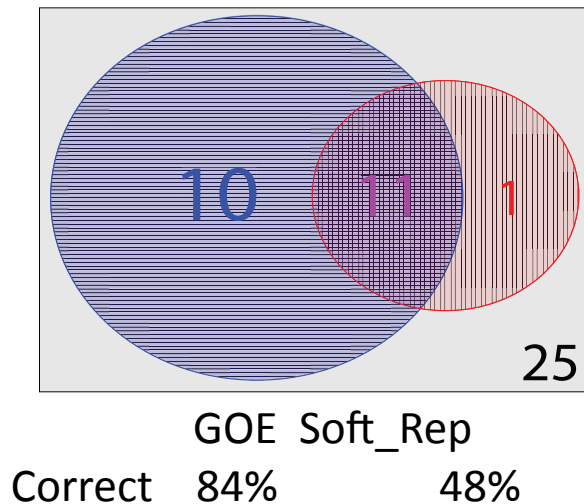
- Implemented as replacement for LJ rep in a minimal SF

$$E_{GOE} = w_1 E_{goe} - w_2 E_{LJ,atr} + w_3 E_{hSASA}$$

Computational Validation

Native Recovery

-- Is native aa favorable in mutant background?
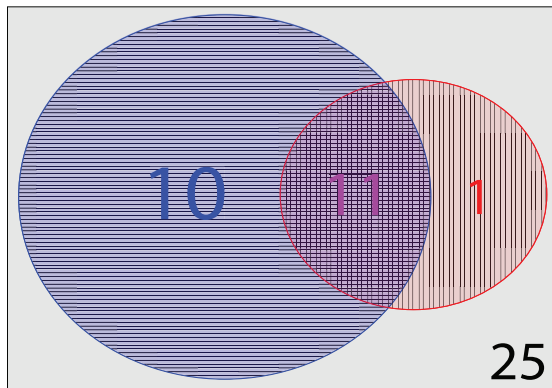
-- Is rotamer correct?

	GOE	Soft_Rep
Correct	84%	48%

Computational Validation

Native Recovery

-- Is native aa favorable in mutant background?

-- Is rotamer correct?

	GOE	Soft_Rep
Correct	84%	48%

Positive Test Set

-- Is known stable mutant favorable?
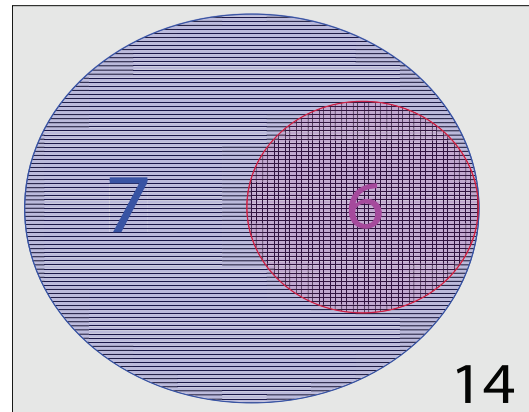
	GOE	Soft_Rep
Correct	93%	43%

Negative Test Set

-- Is known destabilizing, small->large mutant favorable?
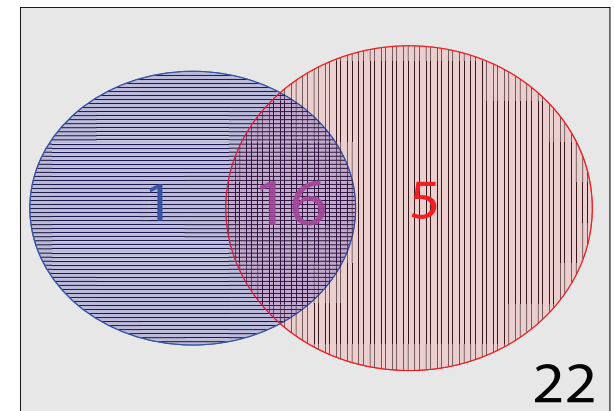
	GOE	Soft_Rep
Correct	77%	95%

RosettaVIP

Create and place cavity balls using RosettaHoles

Select mutatable residues utilizing user defined criteria

Accept or reject top design by comparison to previous best

Filter: User defined rejection criteria, repeat or terminate?

Sample point mutants for set of mutatables on fixed backbone

Relax and rescore top designs from fixed bb design

Iterate through set of all mutatable residues

Filter: User defined cutoff

Iterate through set of residues which pass filter

Experimental Validation

A Large Scale Test of Computational Protein Design: Folding and Stability of Nine Completely Redesigned Globular Proteins
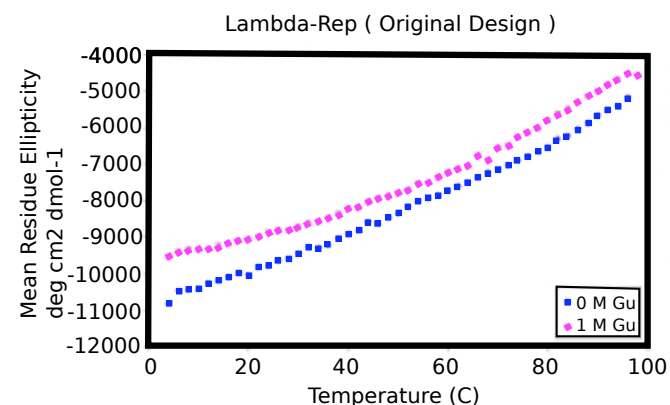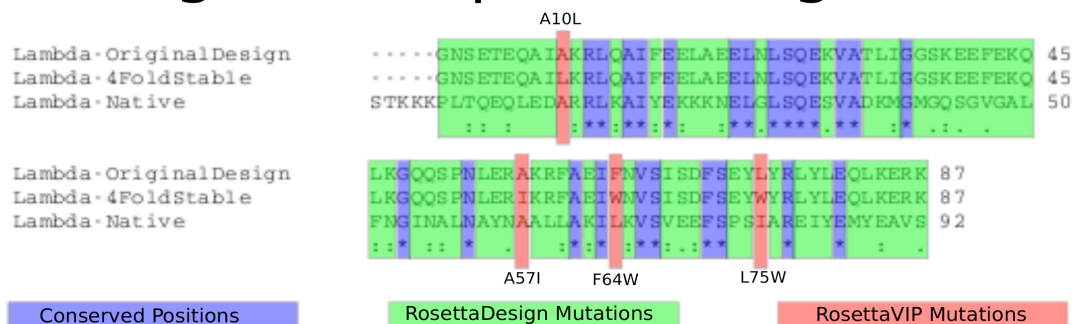
Gautam Dantas[1]†, Brian Kuhlman[1]†, David Callender[1]
Michelle Wong[1] and David Baker[1,2]*

- Full redesign of 9 small, globular proteins
- Experimental characterization ->
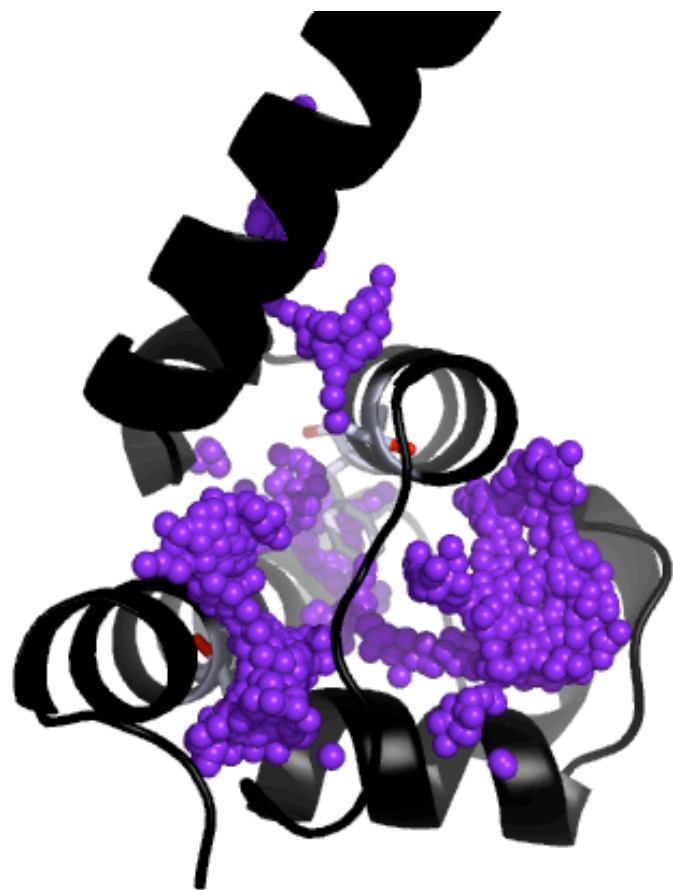 - Most stabilized wrt WT
  - Lambda Repressor

Experimental Validation: Design Recovery
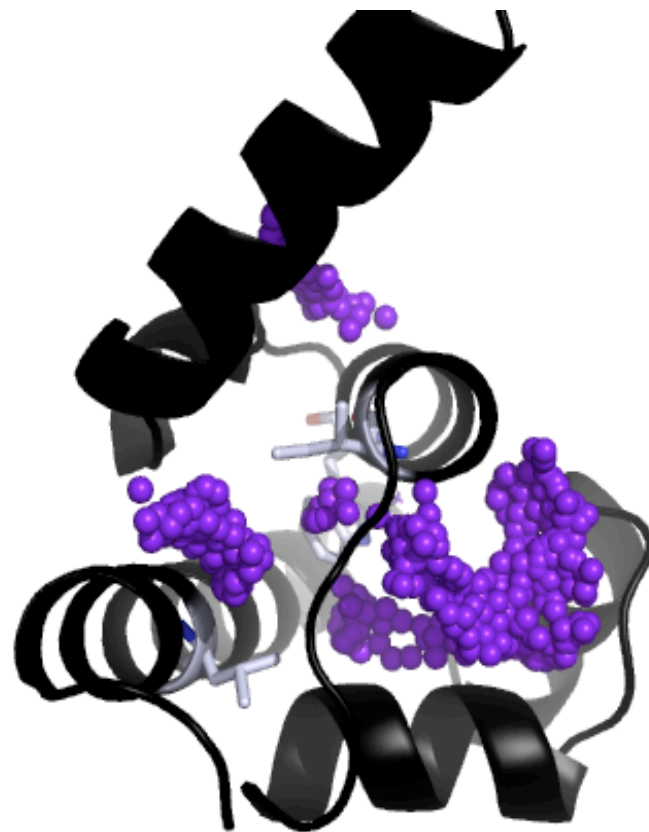
- ## Lambda Repressor *(Dantas et al.,JMB 2003)*

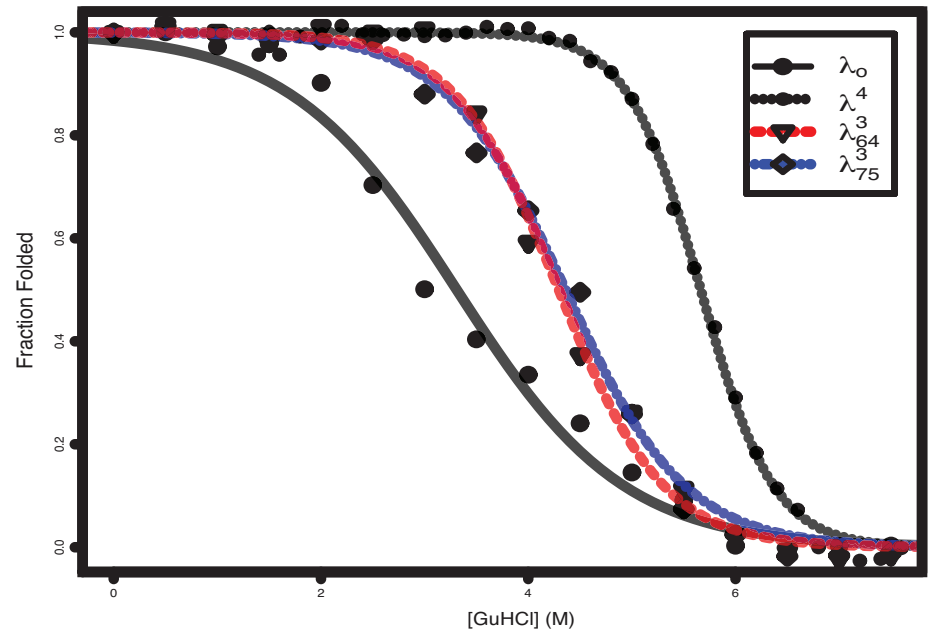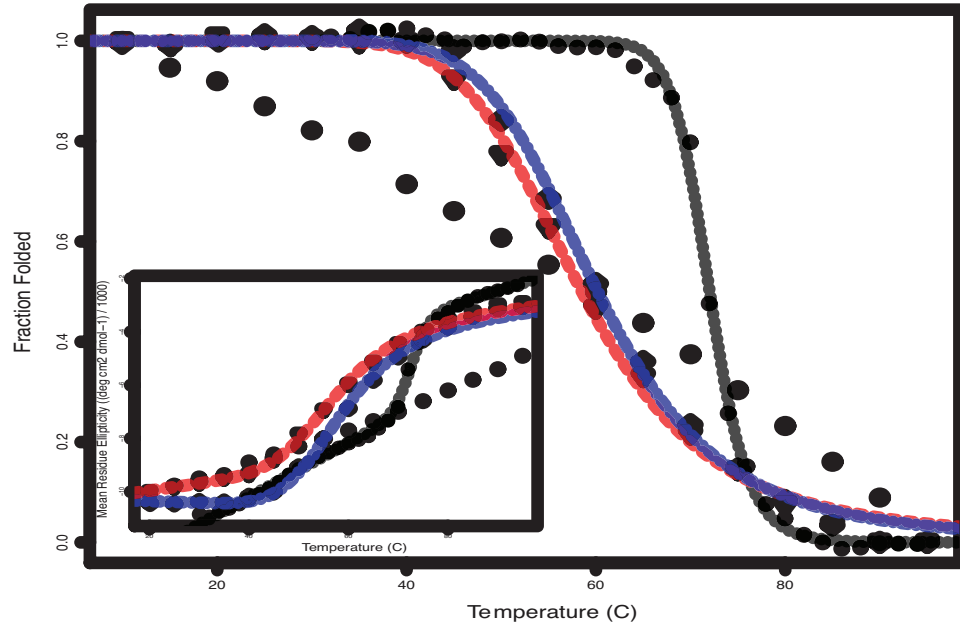# RosettaVIP Efficiently Rescues A Less than Perfect Design

- Packing: .53
- RosettaE: -216.7

- Packing: .71
- RosettaE: -221.4

Cooperativity is Recovered

- Cooperative folding is recovered
- Stability is increased over both original design and wild-type

Experimental Validation

JMB

Available online at www.sciencedirect.com

SCIENCE@DIRECT®

ELSEVIER

A Large Scale Test of Computational Protein Design: Folding and Stability of Nine Completely Redesigned Globular Proteins
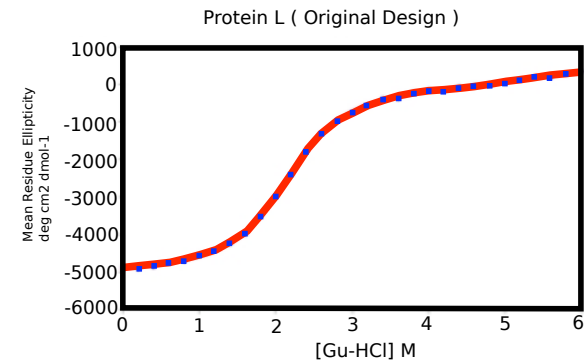
Gautam Dantas[1]†, Brian Kuhlman[1]†, David Callender[1]
Michelle Wong[1] and David Baker[1,2]*

- Full redesign of 9 small, globular proteins
- Experimental characterization ->
  - Most stabilized wrt WT

  - Lambda Repressor
  - Protein L

Experimental Validation: Design Improvement
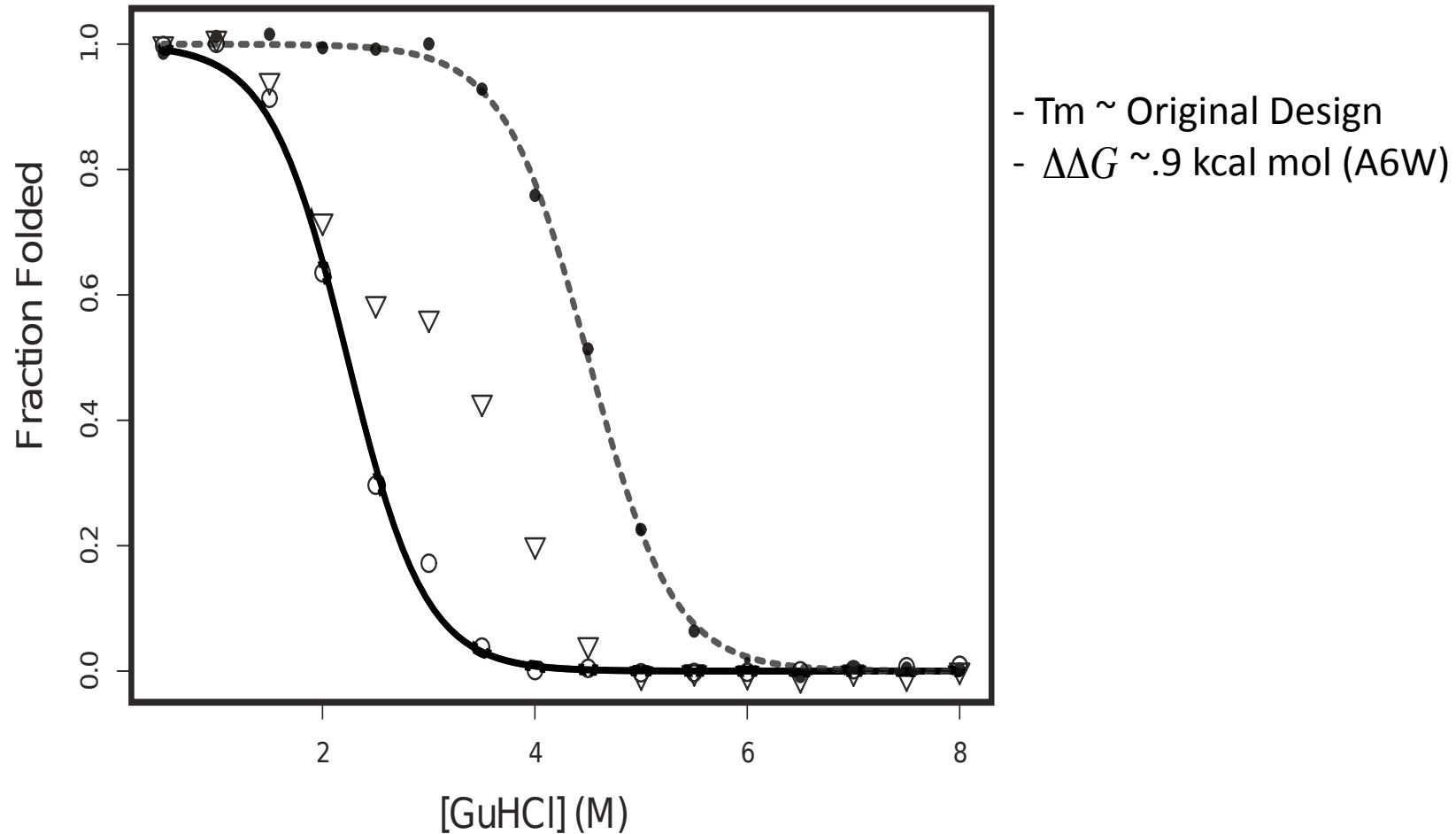
- ## Protein L *(Dantas et al.,JMB 2003)*

Designed Sequence Alignment

Conserved Positions | RosettaDesign Mutations | RosettaVIP Mutations

Protein L (Original Design)

- 42% Sequence Identity with native

Tm ~ 100 deg

Stability is increased, cooperativity maintained

- Tm ~ Original Design
- $\Delta\Delta G$ ~.9 kcal mol (A6W)

RosettaVIP Efficiently Improves A Neutral Design
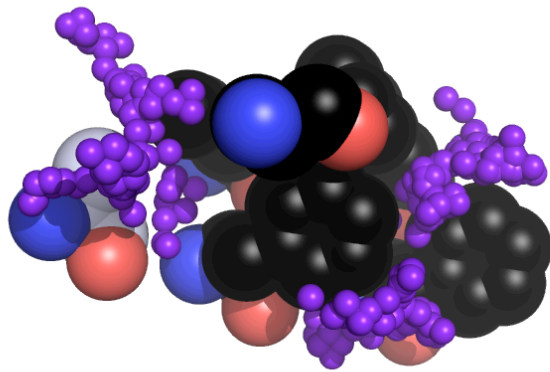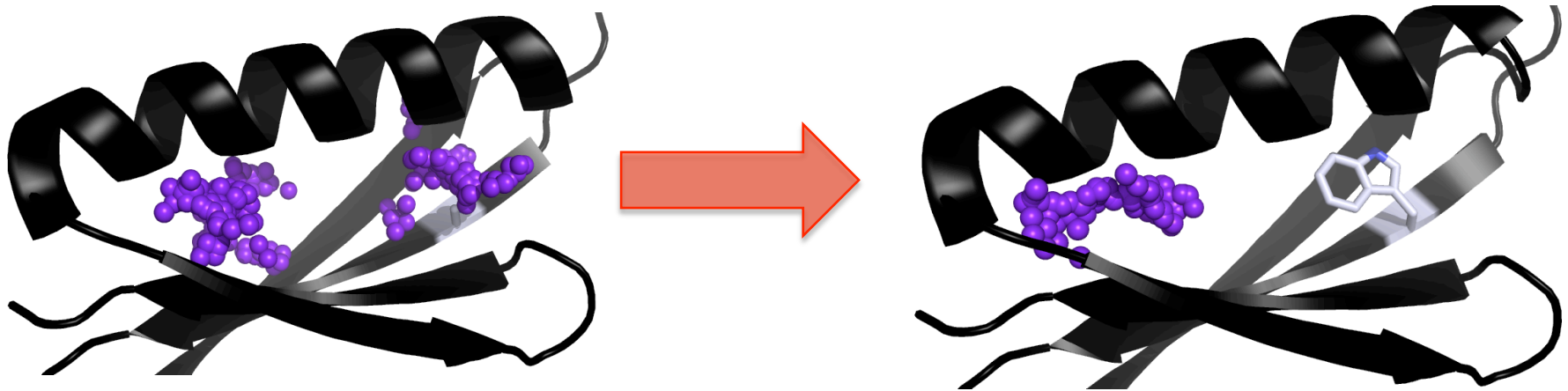
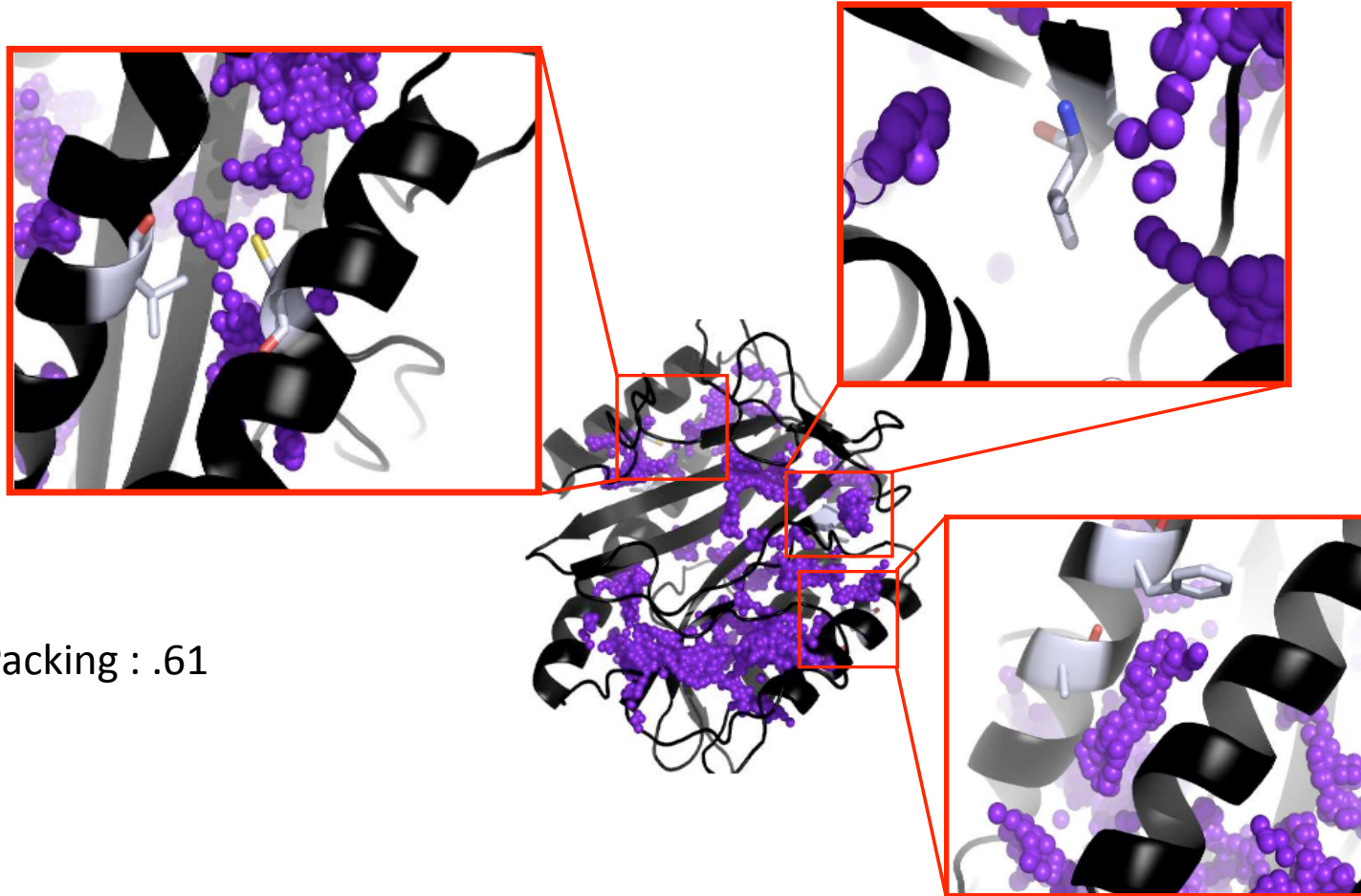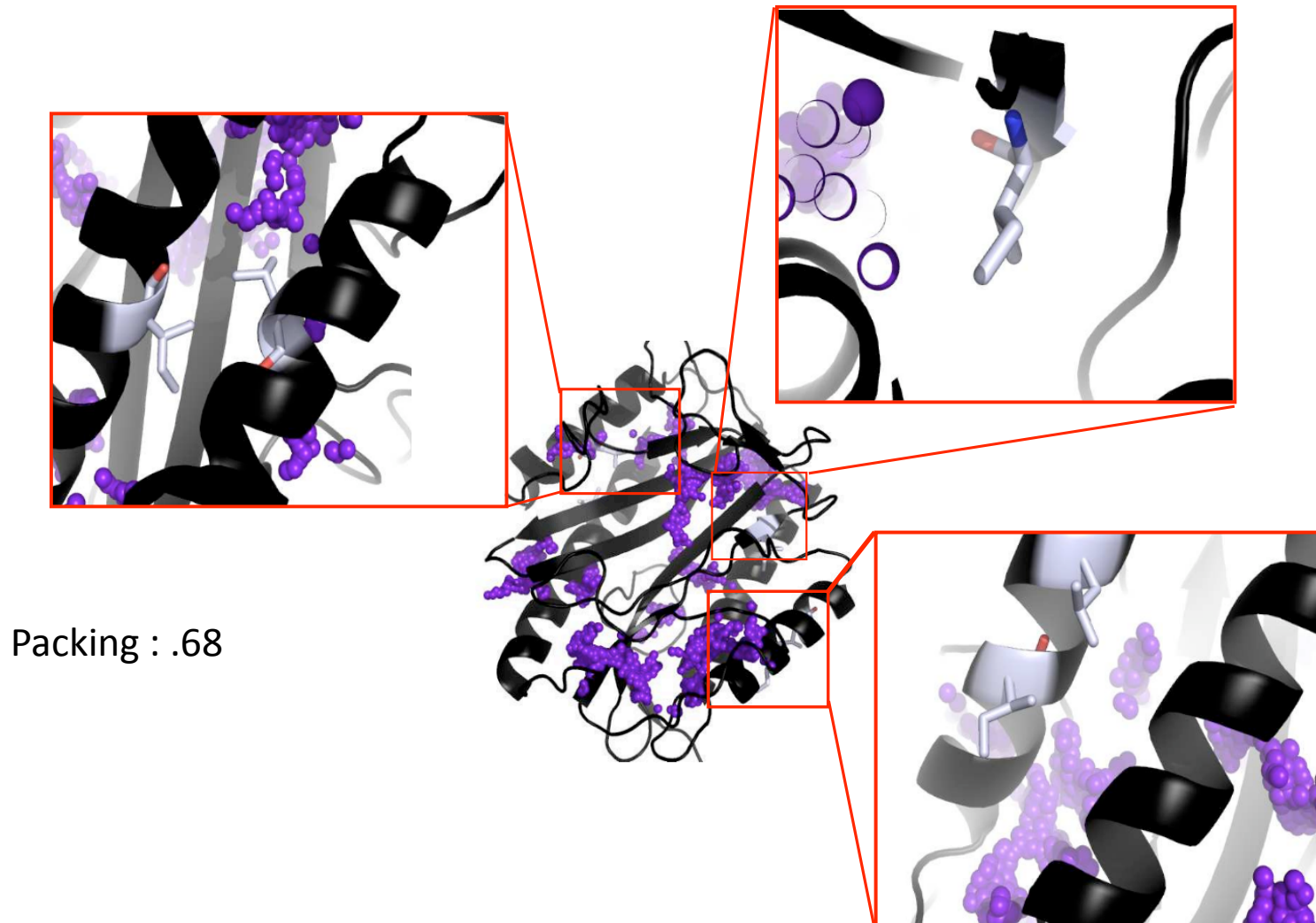Packing: .58
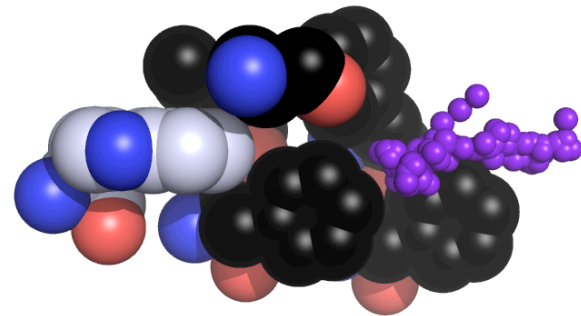
Packing: .66

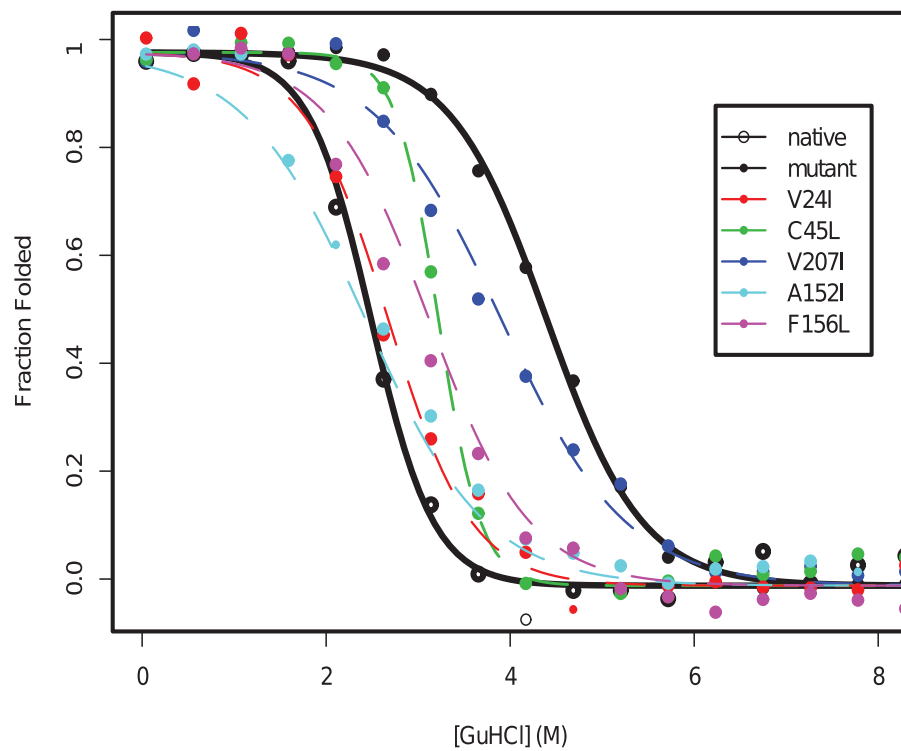# Experimental Validation: Stabilization of a mesophilic enzyme

Packing : .61

RosettaVIP improves packing away from active site

Packing : .68

Stability Significantly Increased
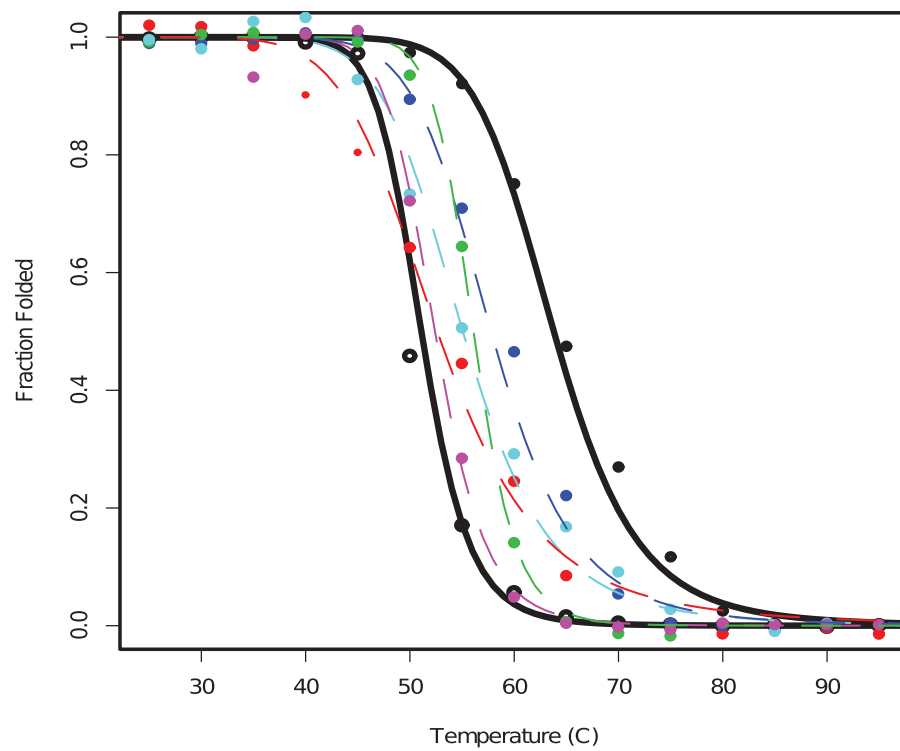
Conclusions

- Results from automated designed of proteins exhibit notable packing defects

- RosettaVIP identifies and fixes some of these defects

- Fixing defects which negatively impact RosettaHoles packing scores significantly improves the stability of designed proteins