LES DIABLERETS, MAY 2025

Enzymes as viscoelastic catalytic machines

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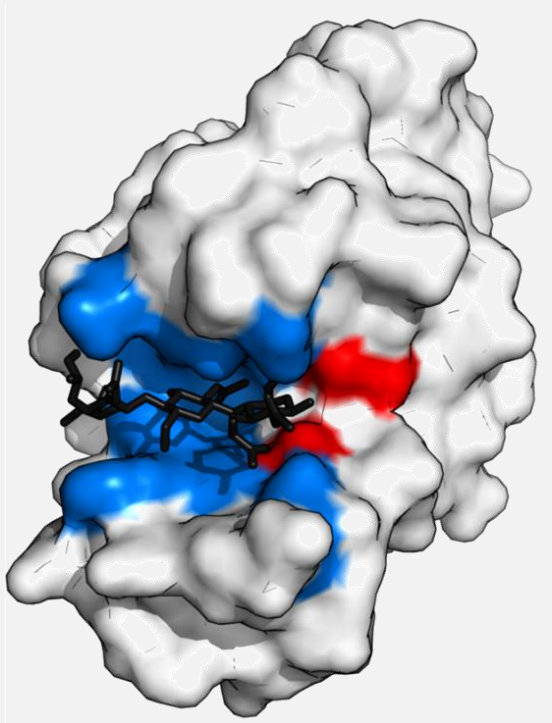
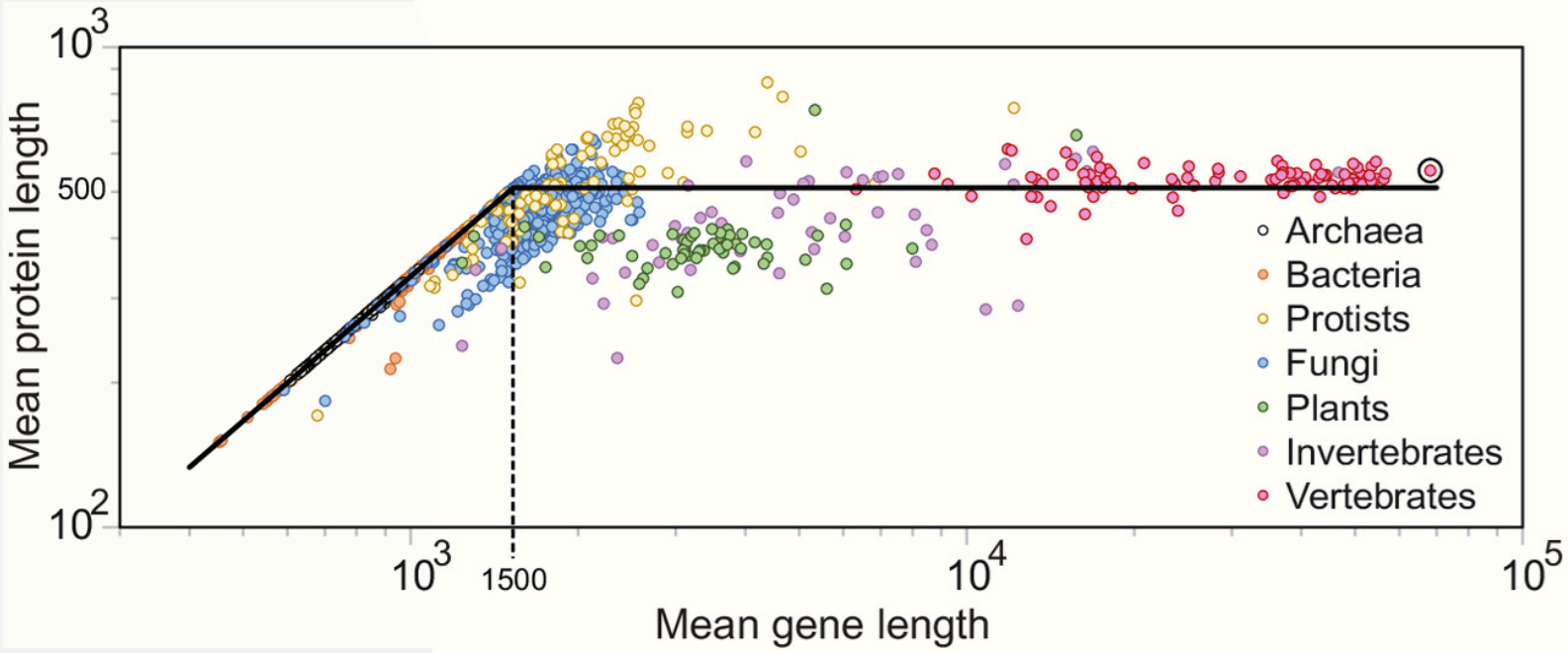
Y Peleg, T Unger, S Albeck, Y Fridmann-Sirkis,
S Lushchekina, JL Sussman (Weizmann)
J-P Eckmann, J Rougemont (Geneva)
R Renault (Qnergy), G Zocchi (UCLA)
JM McBride, MM Siek (UNIST)

Please ask questions during the talk

UNIST
ULSAN NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY
2007

Why proteins are so BIG?

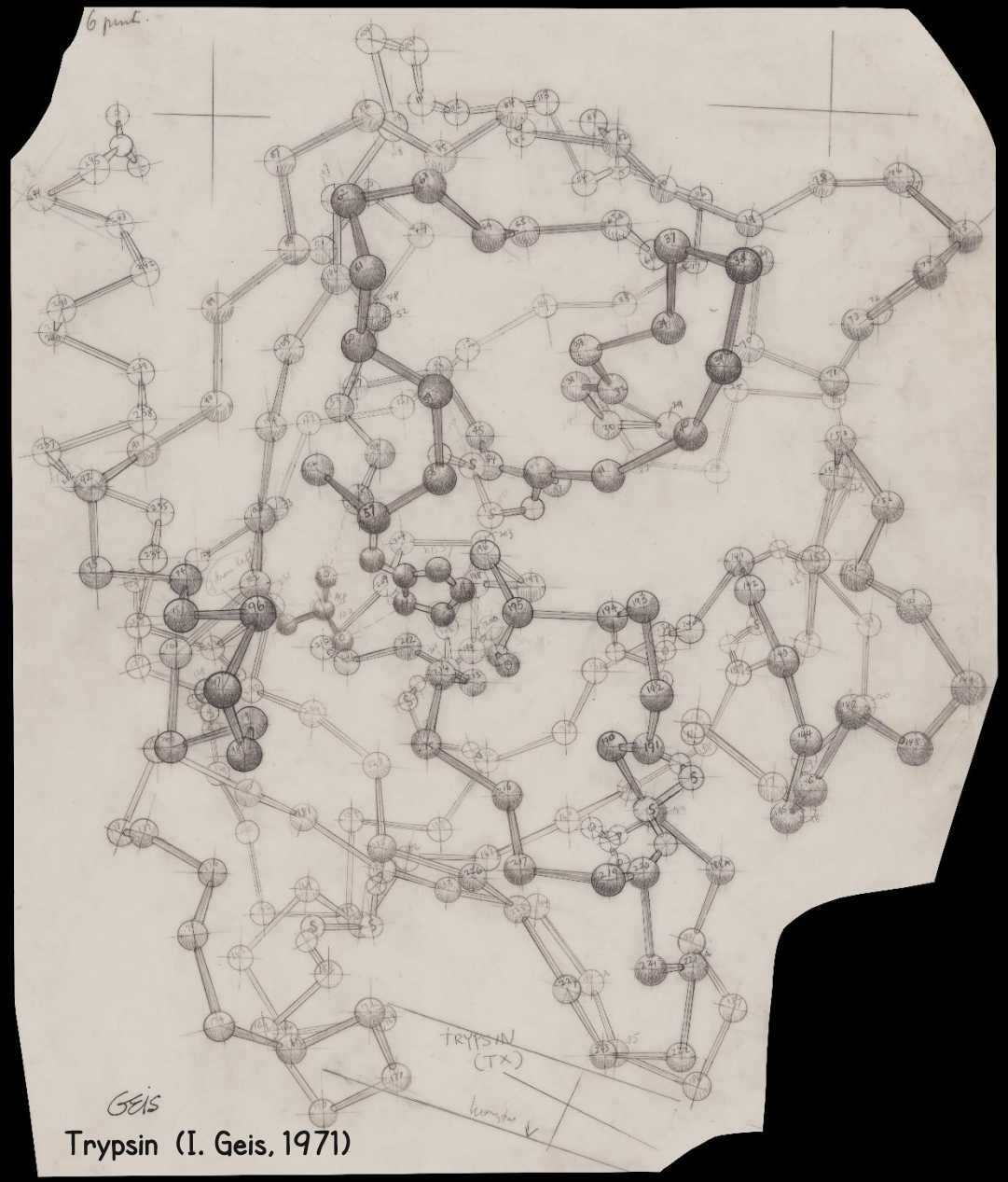




CATALYTIC site = ACTIVE + BINDING

"REST" = Scaffold to support and position active site

Muro, Ballesteros, Luque, & Bascompte (PNAS 2025)



Trypsin (I. Geis, 1971)

The function of protein is the outcome of collective interactions encoded in the gene

What are the principles of encoding?

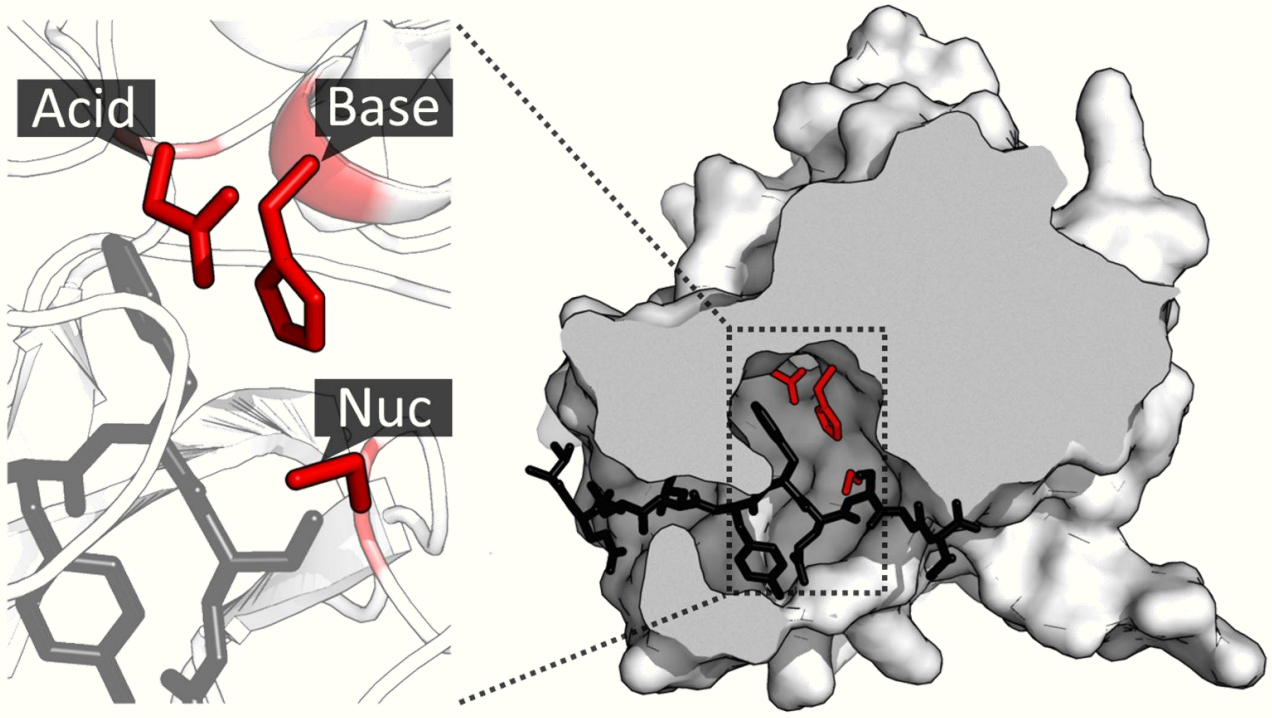
Collective interactions in heterogeneous matter ,

determined by long evolutionary processes.

☞ Interactions occur on multiple scales

In enzymes, the catalytic site is finetuned at the Angstrom scale

to match the shape and charge distribution of the transition state.



Matching accelerates reactions by many orders of magnitude (up to 10^{17})

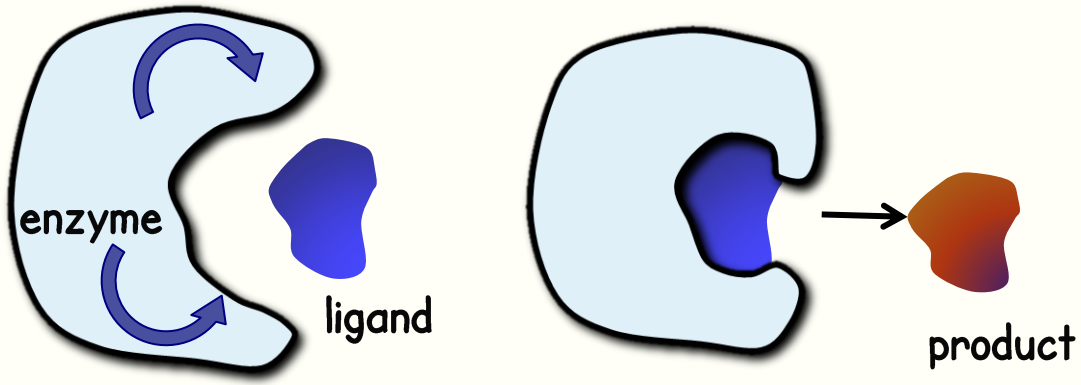
TEV protease (PDB)

The "REST" is more than a scaffold:

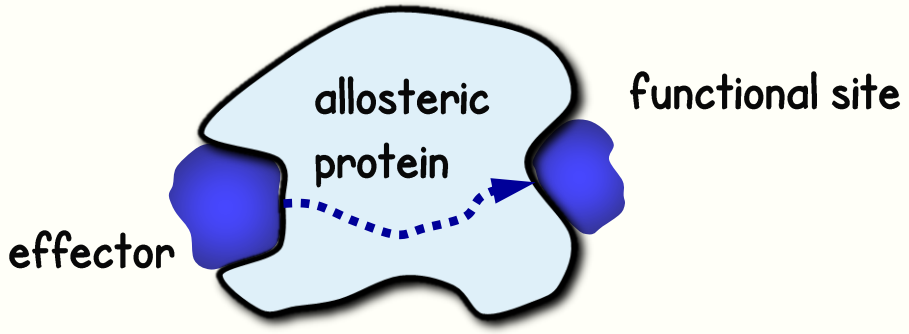
Large-scale motions allow enzymes to bind specifically,

reach the transition state, and release product

INDUCED FIT



ALLOSTERY



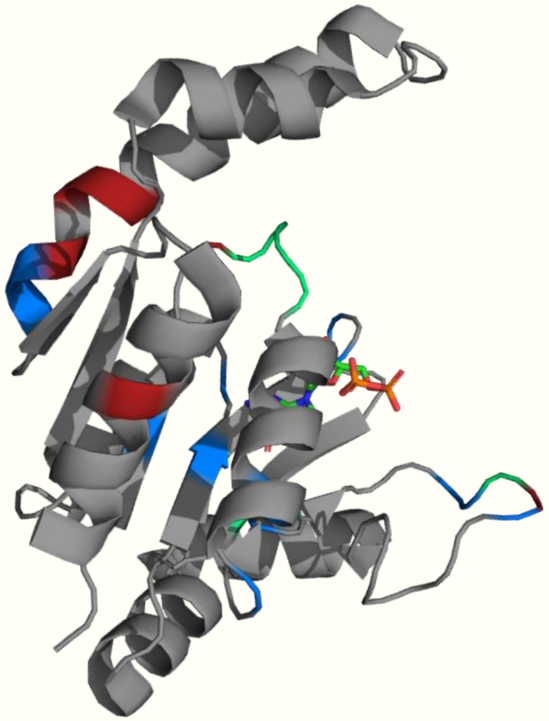
** catalytic step also affected by motion*

How can we understand, predict, and quantify the link

between large-scale motion and localized catalytic function of enzymes?

THE BASIC IDEA

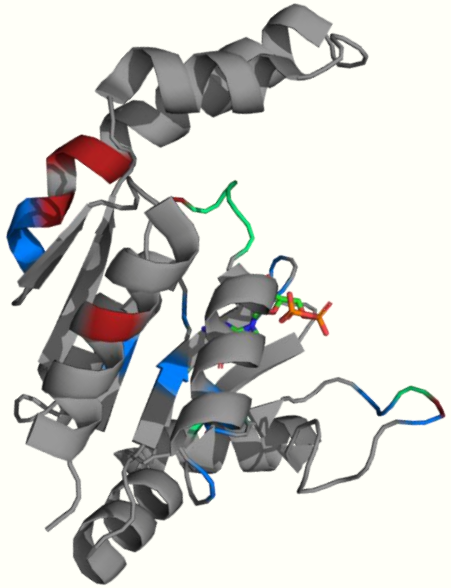
Proteins as sequence-encoded viscoelastic machines



- Proteins evolve a network of flexible viscoelastic elements that facilitate high-strain deformation essential for their function. This network is encoded in the gene.

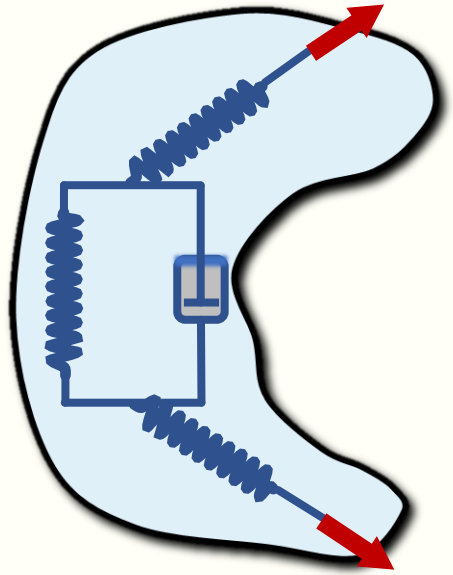
- Existence of these high-strain regions and their effect on function can be predicted and tested against experiments.

Outline



1. **Theory** of protein as viscoelastic matter predicts the evolution of functionally-relevant collective motion.

2. **Strain** – measure of deformation estimates effect of distant mutations on catalytic site.



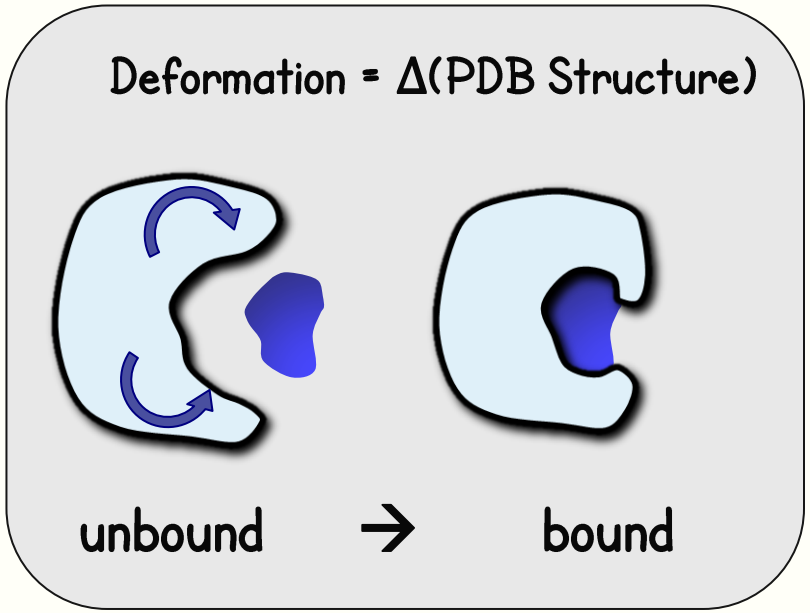
3. **Experimental test** – the enzyme guanylate kinase.

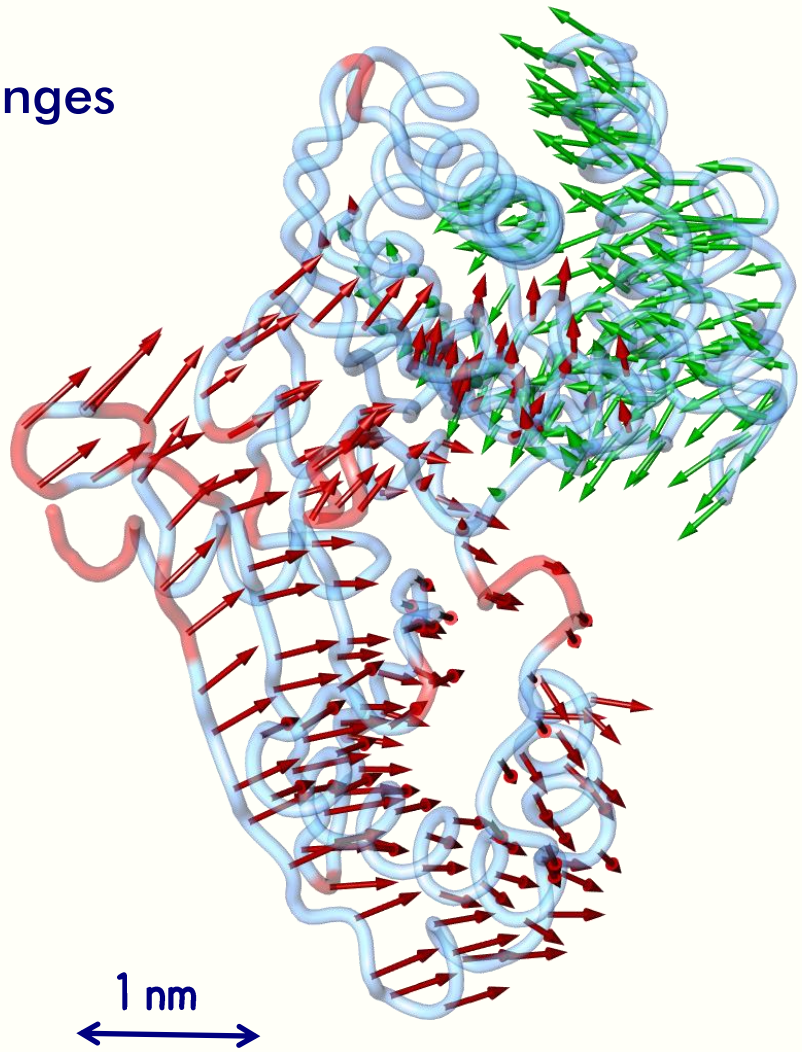
4. **Effective model** of protein as sequence encoded viscoelastic machine.

- Weinreb et al. "**Enzymes as viscoelastic machines**". *Nature Physics* 2025.
- McBride et al. "**AI-predicted protein deformation encodes energy landscape perturbation**". *Physical Review Letters* 2024.
- McBride et al. "**AlphaFold2 can predict single-mutation effects".** *Physical Review Letters* 2023
- Eckmann et al. "**Proteins: the physics of amorphous evolvable matter**", Review of Modern Physics, 2019

1. THEORY

Looking at motion and deformations identifies structural changes relevant to function and how they are encoded in the gene



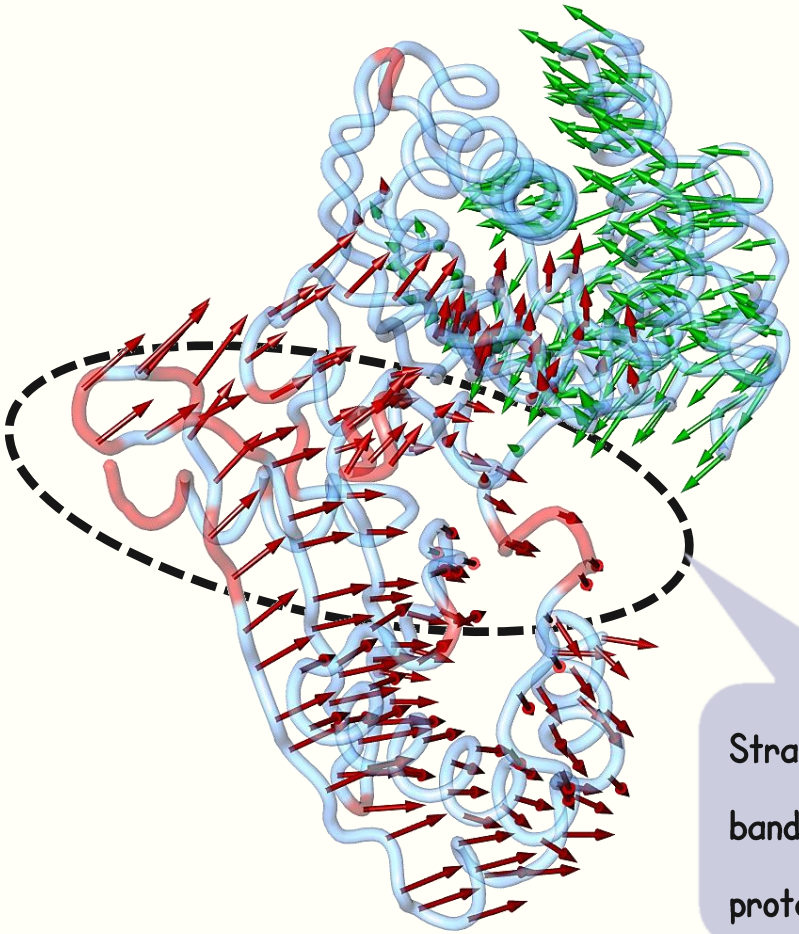


Glucokinase

(PDB: 1v4s → 1v4t)

Eckmann TT, *Bioessays* 2021
Rougemont Eckmann TT, *Rev Mod Phys* 2019
Dutta Eckmann Libchaber TT, *PNAS* 2018
Eckmann Libchaber TT, *PRX* 2017

Strain measures the internal motion of amino acids in the protein



Strain is localized in a band separating the protein into rigid domains

Glucokinase (PDB: 1v4s → 1v4t)

strain = how much amino acids move w.r.t. to neighbors

$$\text{affine strain} = \quad \varepsilon_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} - \sum_k \frac{\partial u_k}{\partial x_i}\frac{\partial u_k}{\partial x_j} \right)$$

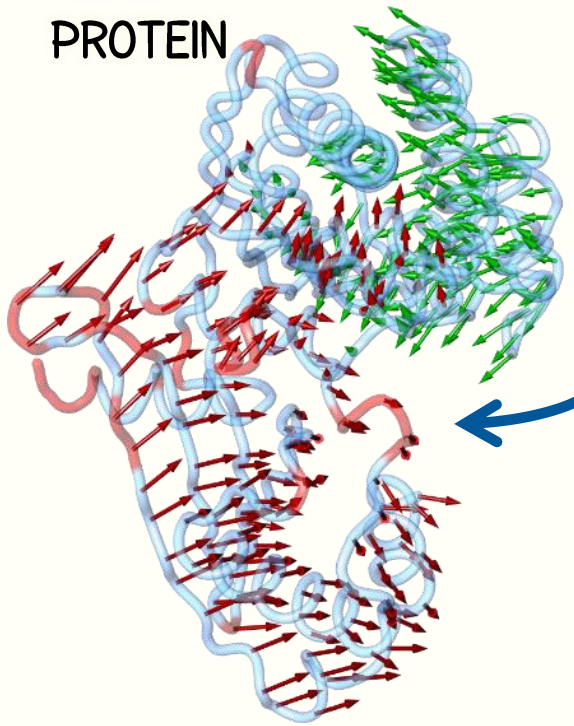
Geometric meaning: metric of deformation $\varepsilon = \frac{1}{2}(\mathbf{g} - \mathbf{1})$

Discrete version by tracing motion of neighboring atoms

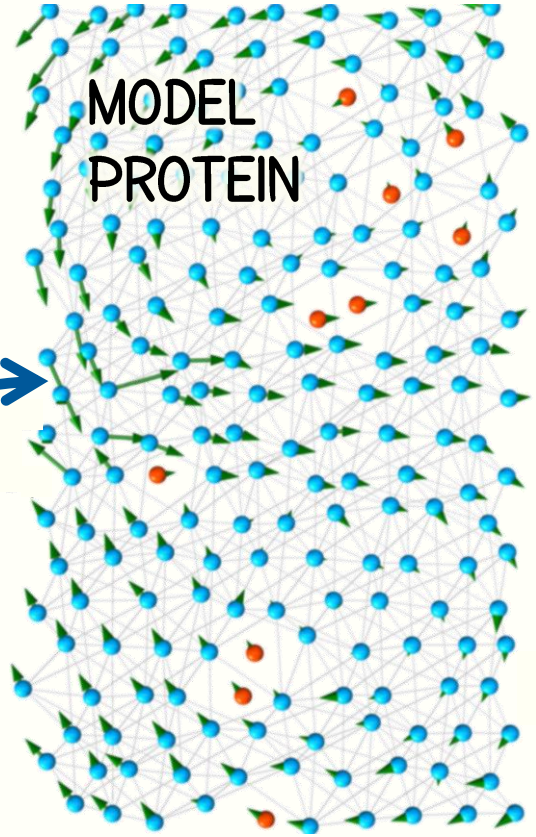
Physical theory of protein as evolving matter (Green functions)

links collective physical interactions among amino acids and genetic correlations

REAL PROTEIN



MODEL PROTEIN

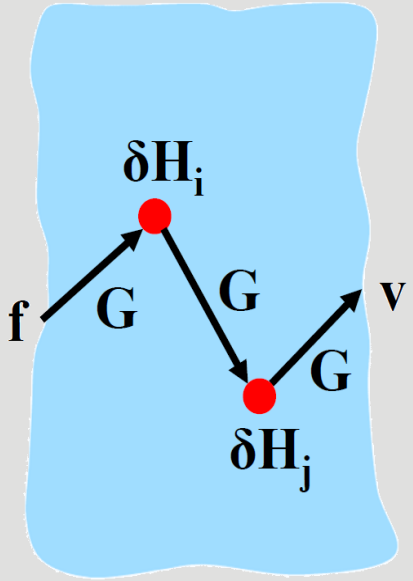


- "gene = 0111010100…

Two types of amino acids connected by "springs"

amino acids	H	P
H	strong	weak
P	weak	weak

GREEN FUNCTION FORMALISM



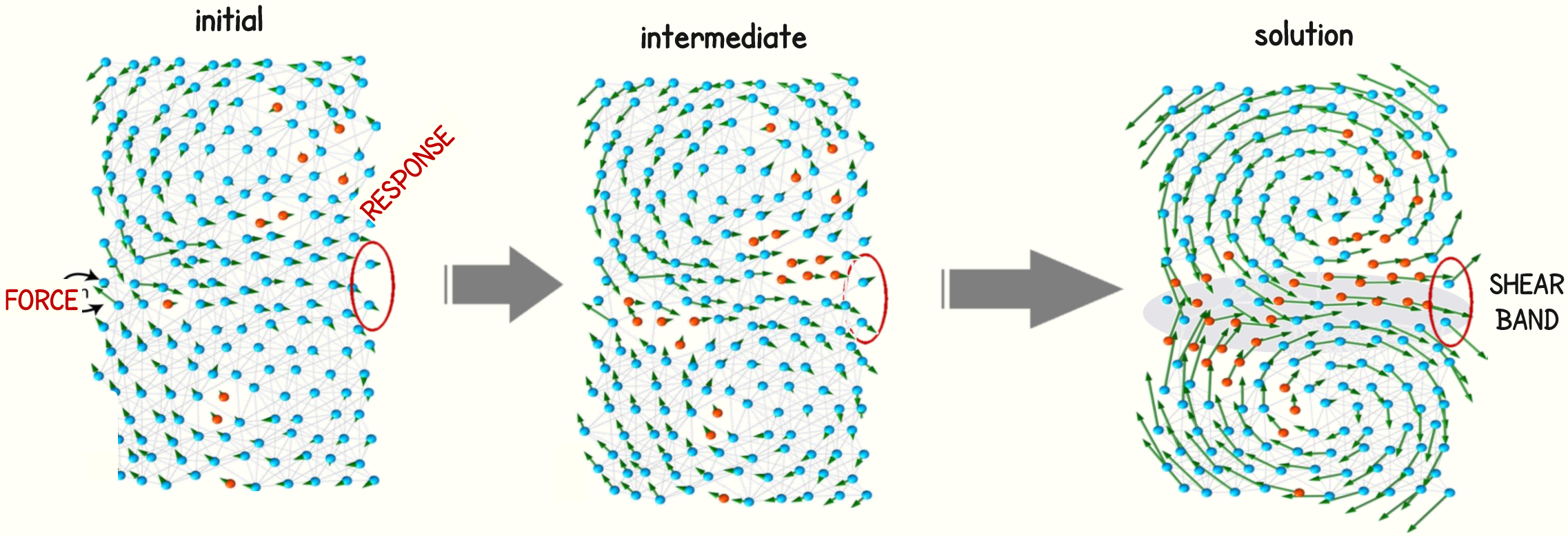
Mutations = local perturbations

Interactions → Dyson series, multiple scattering…

$$\delta \mathbf{G} = -\mathbf{G}\delta H\mathbf{G} + \mathbf{G}\delta H\mathbf{G}\delta H\mathbf{G} - \ldots$$

Dutta Eckmann Libchaber TT, PNAS 2018

Green function theory explains how functional proteins with high-strain regions emerge during evolution

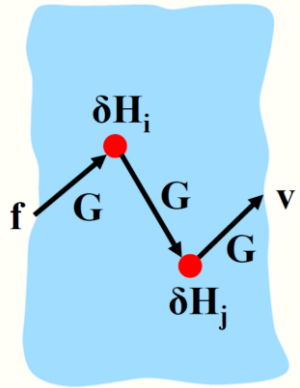


High-strain regions facilitate versatile, sophisticated functions: specific recognition, induced fit, allostery….

Summary: Theory predicts the evolution of high-strain regions in the protein

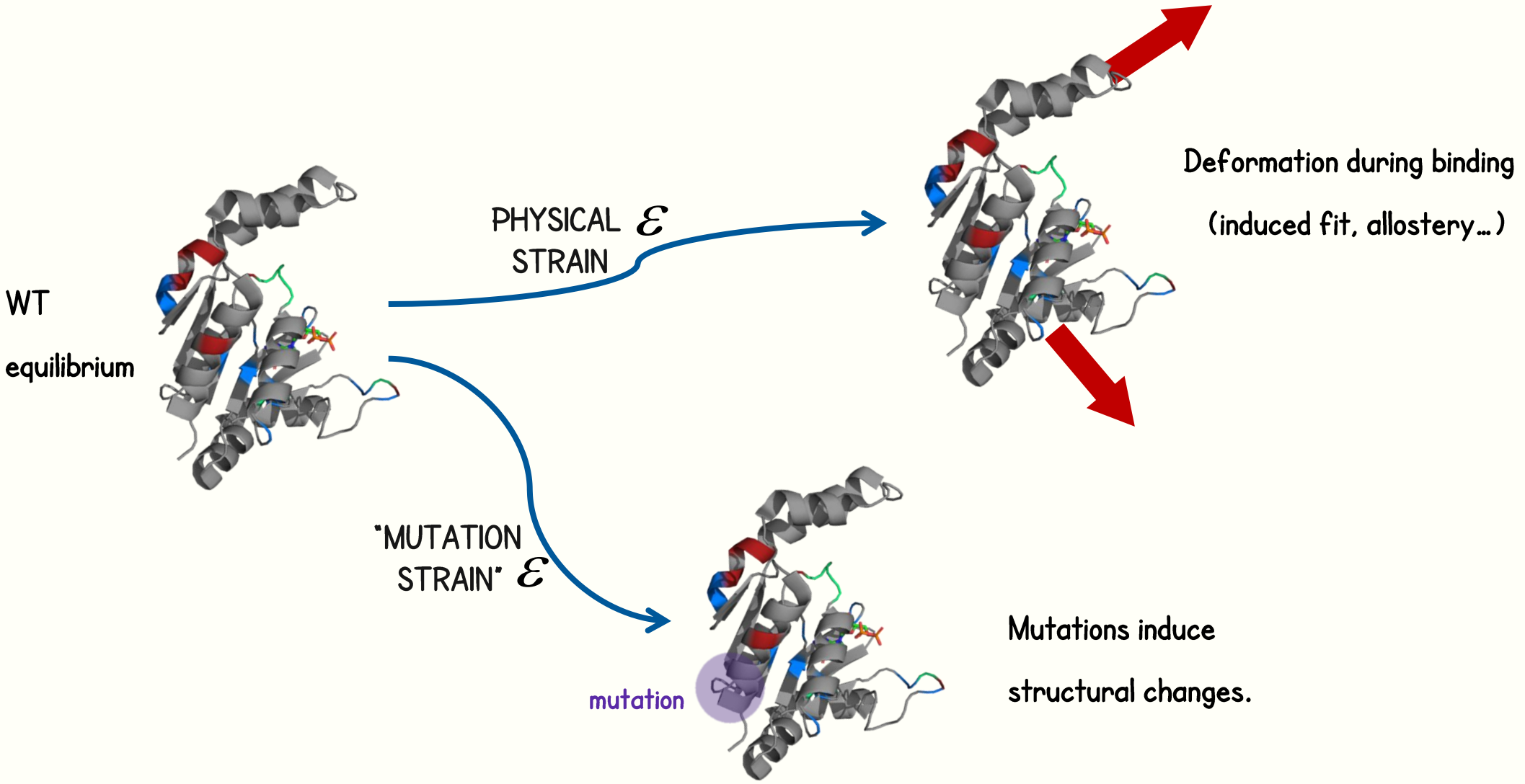
High-strain regions evolve that are:

- essential for biochemical function
- through large-scale motion and long-range mechanical interactions,
- and therefore, genetically conserved and correlated.



Eckmann TT, BioEssays 2022
Eckmann Rougemont TT Rev Mod Phys 2019
Dutta Eckmann Libchaber TT, PNAS 2018
Eckmann Libchaber TT, PRX 2017

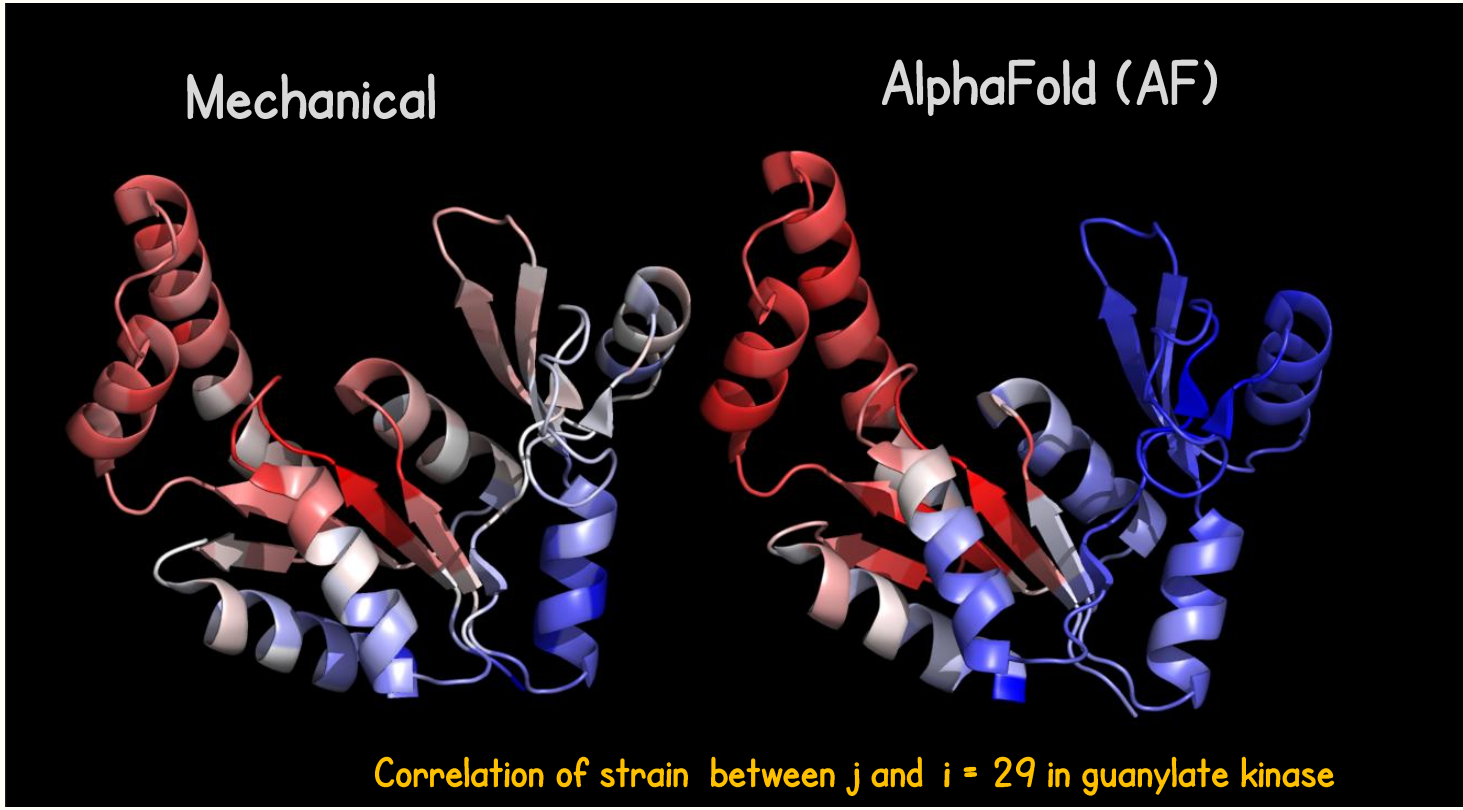
2. Strain (= localized rearrangement) can be induced by physical forces or mutation



Using AI-predicted strain as a sensitive probe single mutation effects

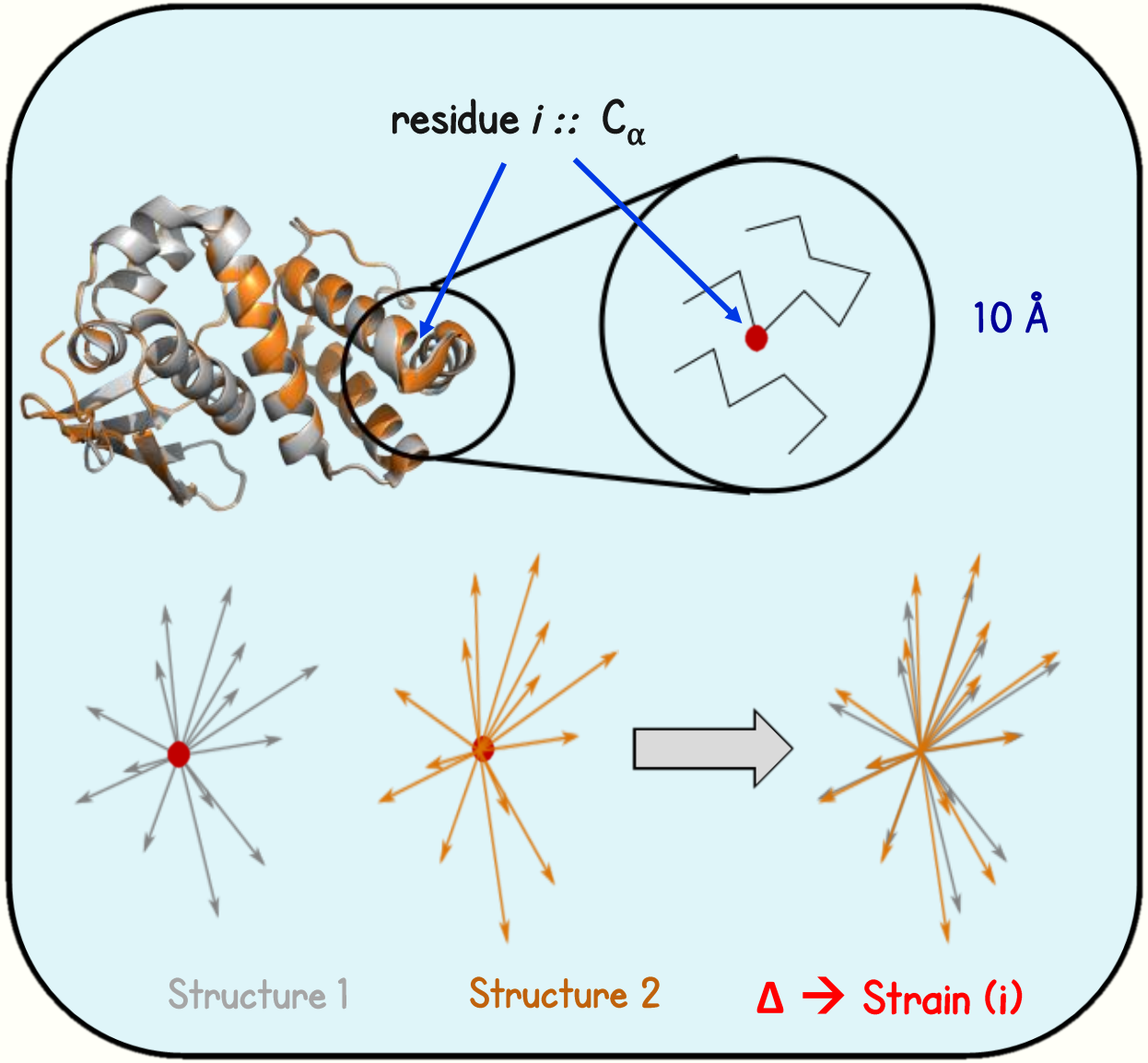
AlphaFold predicts protein structure at unprecedented accuracy, but it was not validated or expected to predict single mutation effects

TEST: using strain to predict mutation effect on structure and phenotype in a large data set of proteins



- McBride et al. "**AI-predicted protein deformation encodes energy landscape perturbation**". *Physical Review Letters* 2024.
- McBride et al. "**AlphaFold2 can predict single-mutation effects".** *Physical Review Letters* 2023

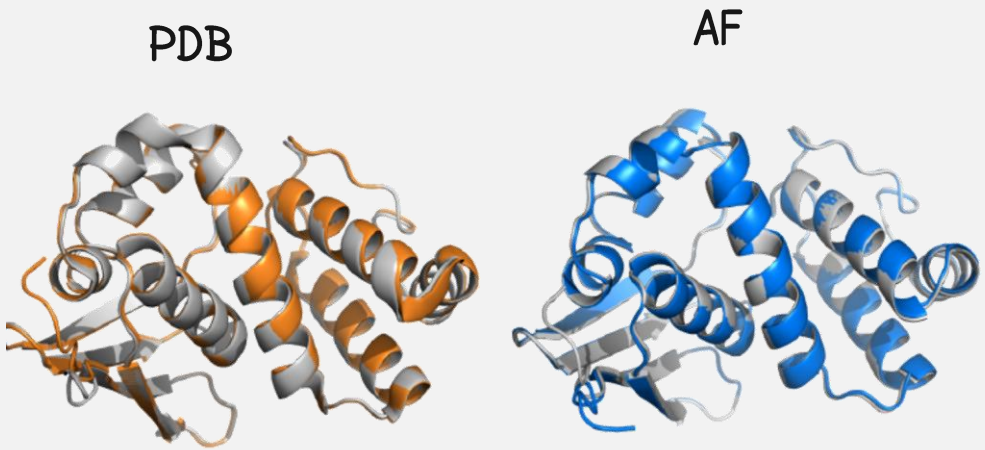
EFFECTIVE STRAIN is the mean relative change in distances to neighbors



$$\text{strain}_i = \left\langle \frac{\left|\mathbf{r}'_i - \mathbf{r}'_j\right| - \left|\mathbf{r}_i - \mathbf{r}_j\right|}{\left|\mathbf{r}_i - \mathbf{r}_j\right|} \right\rangle_{\text{neighbors } j}$$

We compared mutation strain from PDB and AF in ~5,000 pairs differing by 1-3 positions

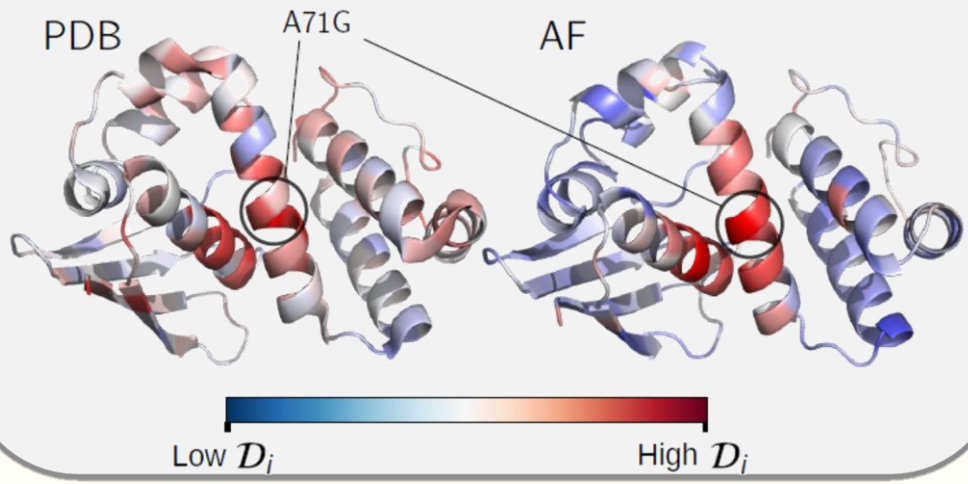
Structural change



Example: H-NOX (Heme-Nitric oxide/Oxygen binding)

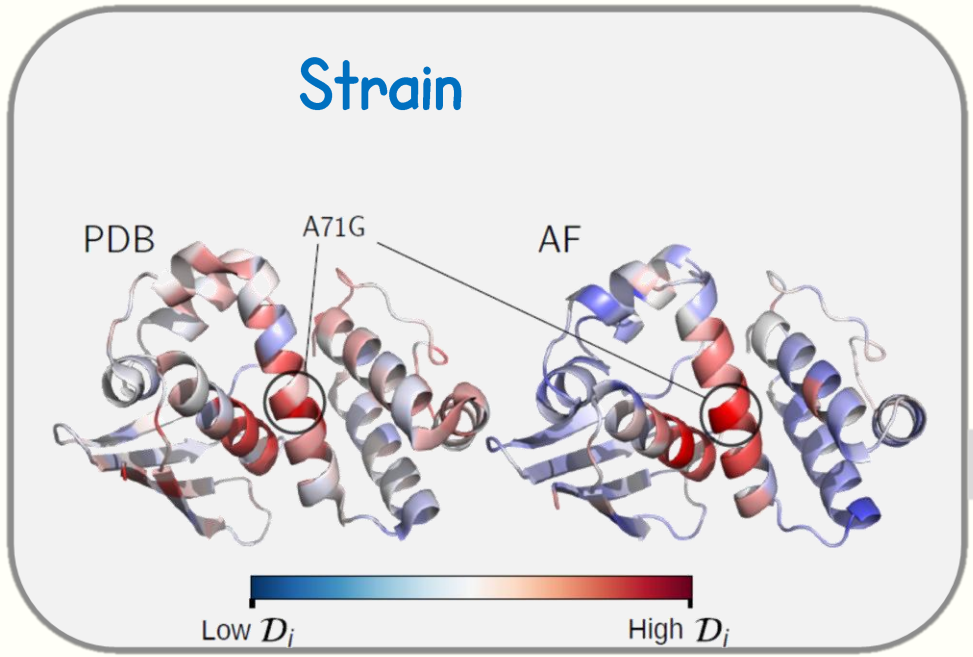
Mutation A71G

Strain

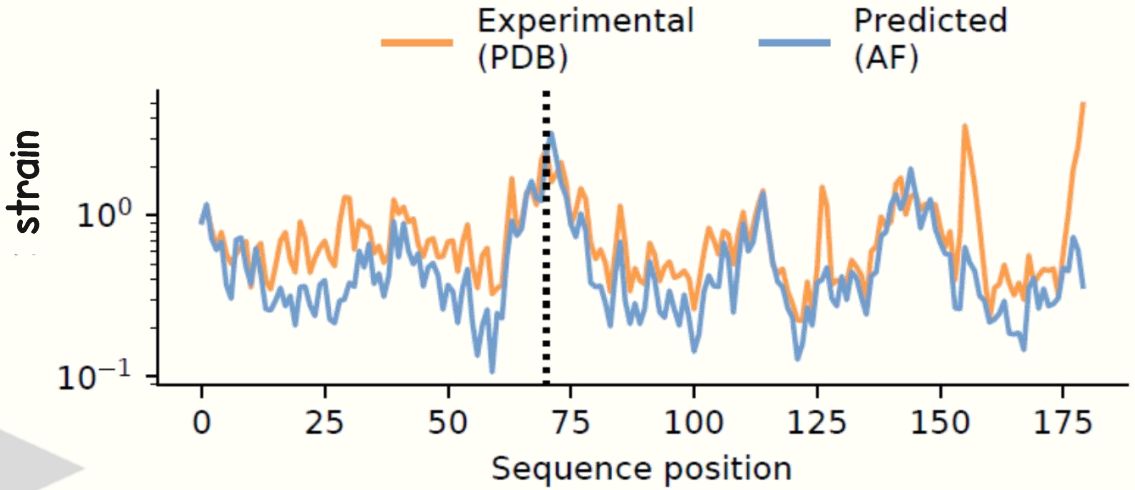


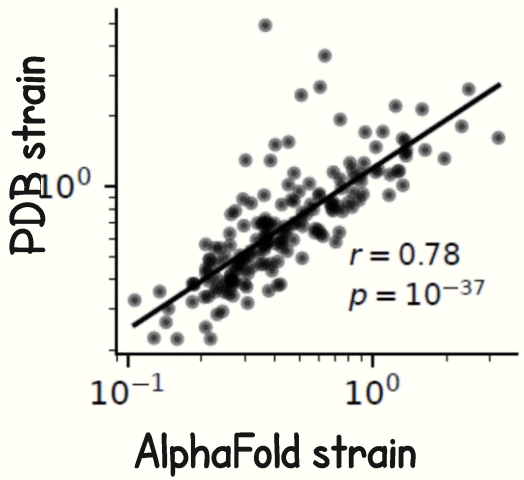
* Same crystallographic groups in PDB

Strain predicted from AF correlates with strain computed from PDB



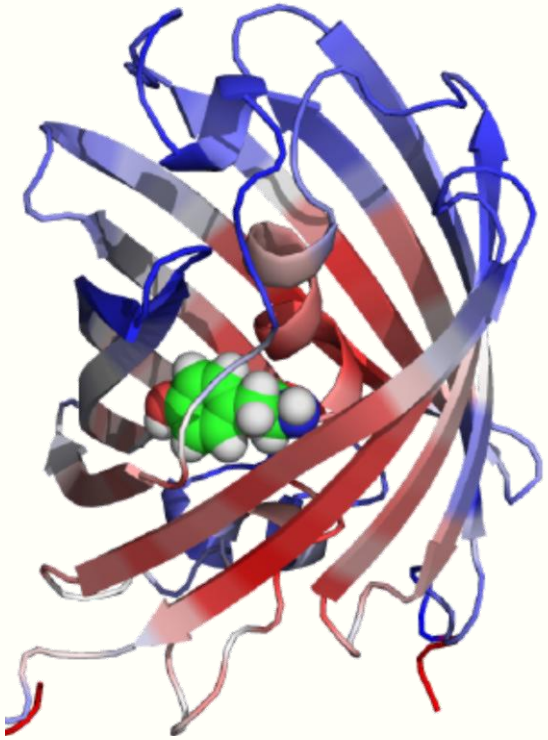
H-NOX = HEME-NITRIC OXIDE/OXYGEN BINDING





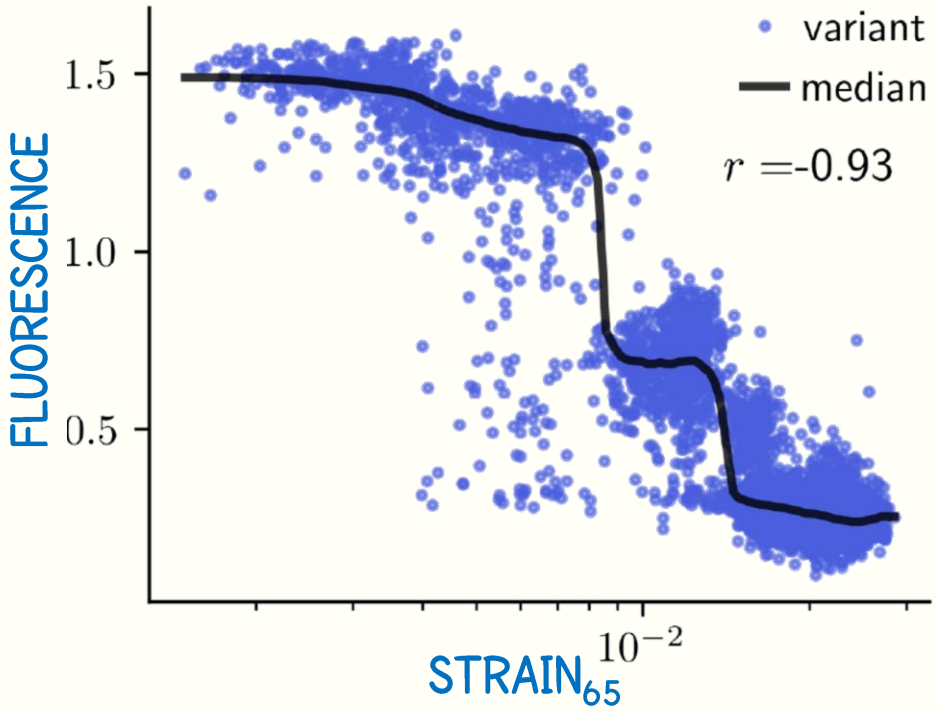
Strain correlates with phenotypic changes in fluorescence in BFP

Example: Blue fluorescent protein (BFP)



Fluorescence measured for 2^{13} = 8,192 mutations

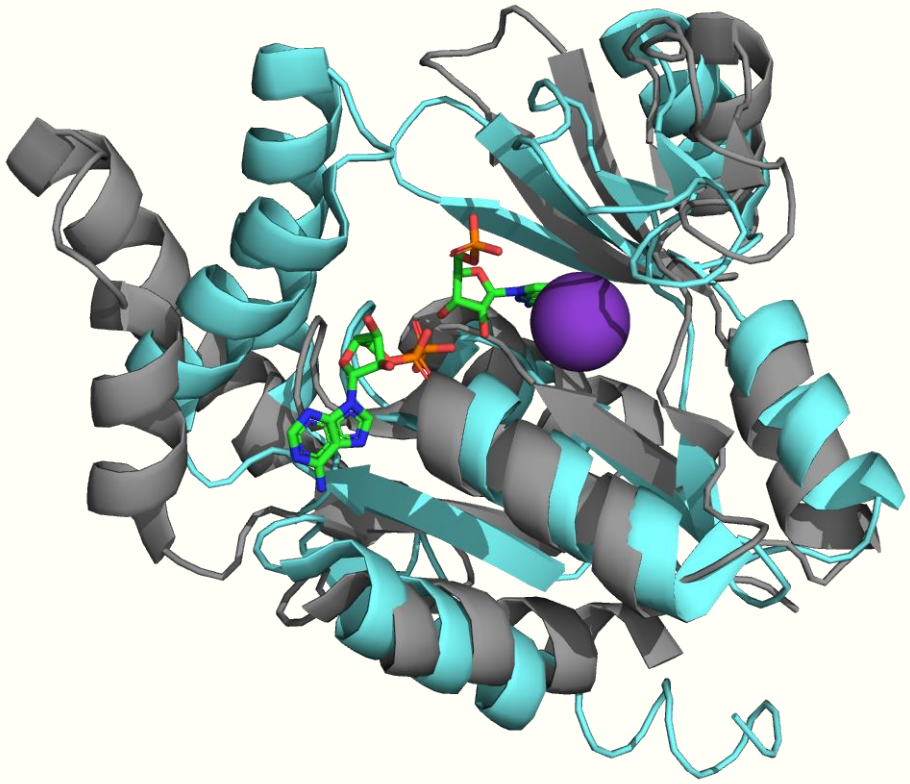
(Poelwijk et al. Nat. Comm.2019).



Red region: strain strongly correlated with phenotype

…on average, strain is a sensitive probe of mutation effects predicted by AF

McBride et al., Phys Rev Lett 2023, Phys Rev Lett 2024



ATP + GMP ⇌ ADP + GDP

3. Measurements of Guanylate Kinase mutants link dynamics – function – structure

Predicting Physical strain- bound\unbound

Evolutionary strain- deformation AlphaFold

Measuring: Biochemical function - enzymatic kinetics

Viscoelastic dynamics - nano-rheology

Enzymes as viscoelastic catalytic machines.

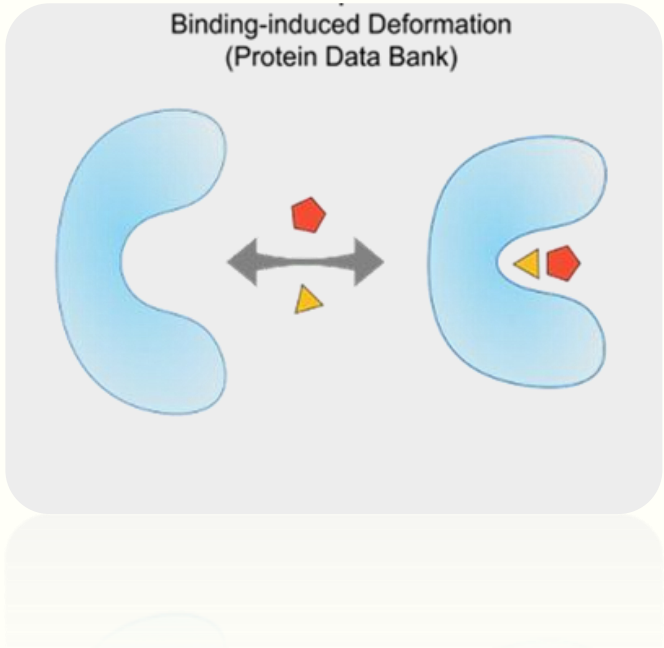
Eyal Weinreb, John M. McBride, Marta Siek, Jacques Rougemont, Renaud Renault, Yoav Peleg, Tamar Unger, Shira Albeck, Yael Friedman-Sirkis , Sofya Lushchekina, Joel L. Sussman, Giovanni Zocchi, Jean-Pierre Eckmann, Elisha Moses, TT *Nature Physics*. 2025

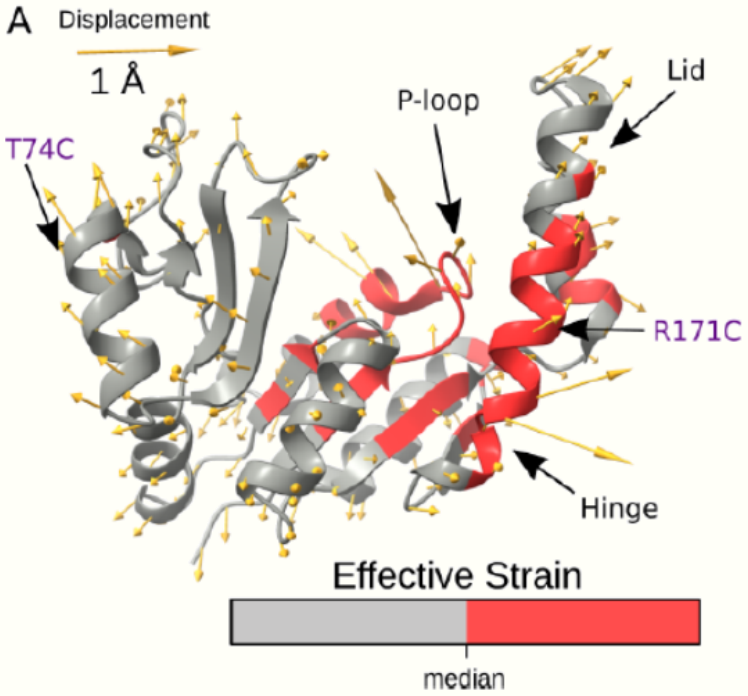
We calculate the strain and introduce 34 mutations in three regions:

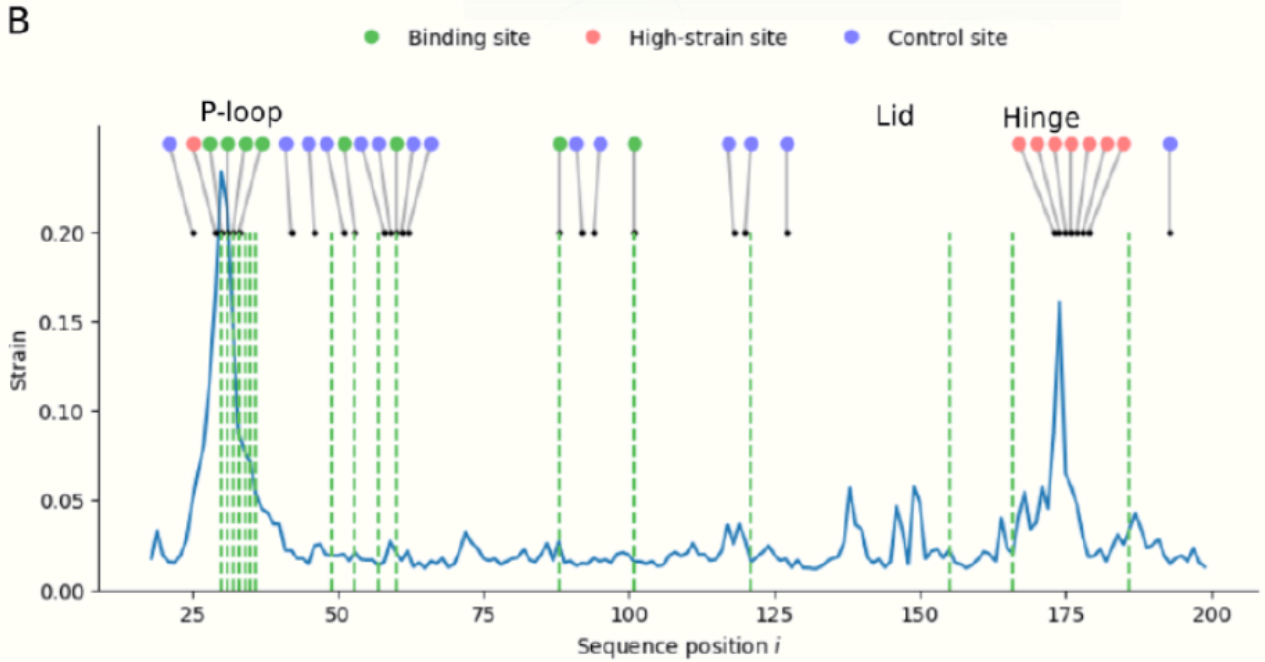
(i) high-strain

(ii) binding site

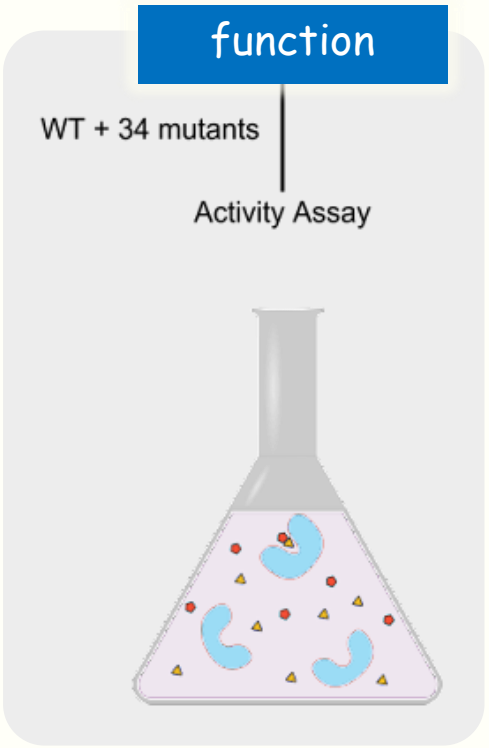
(iii) control



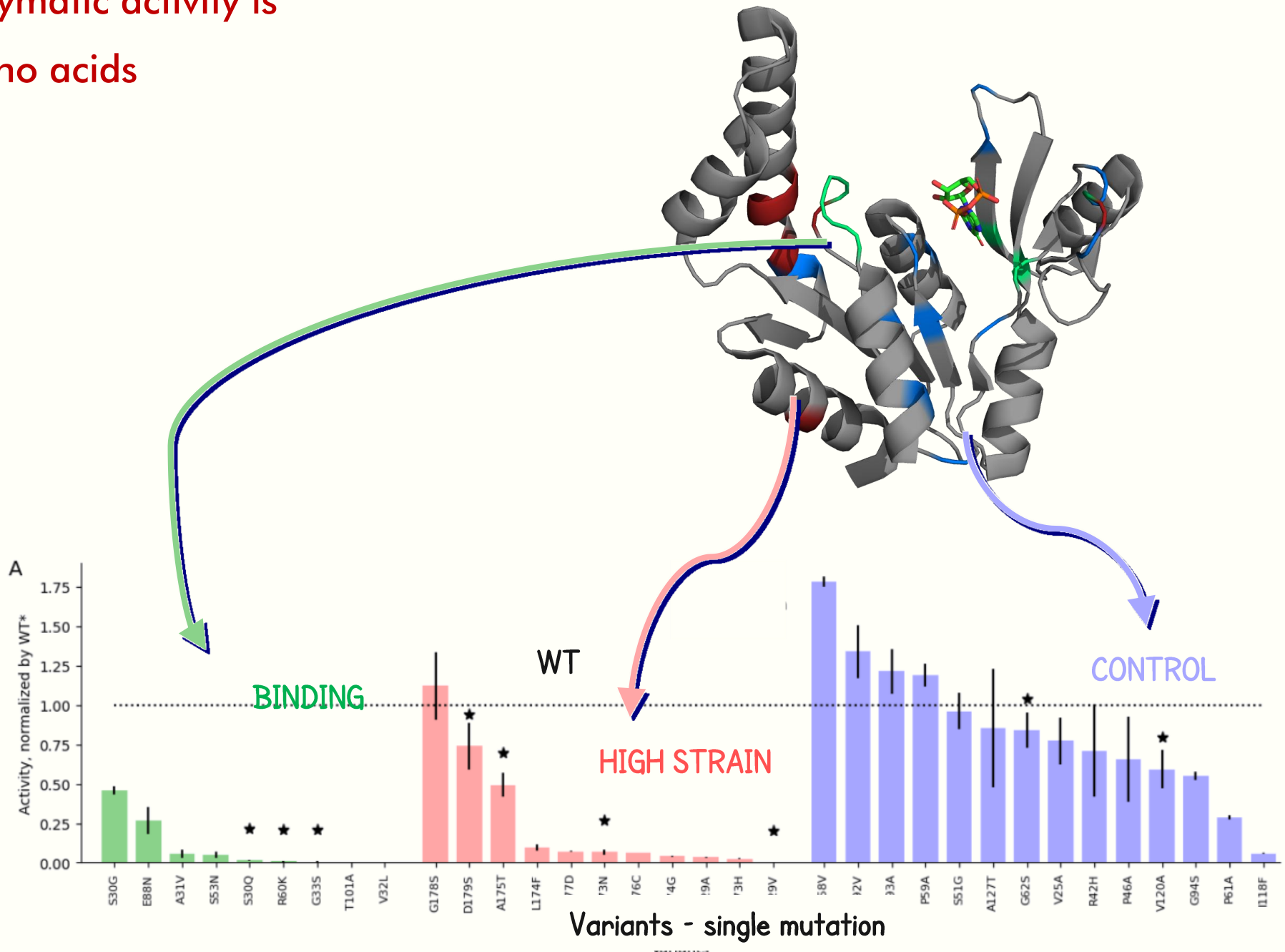




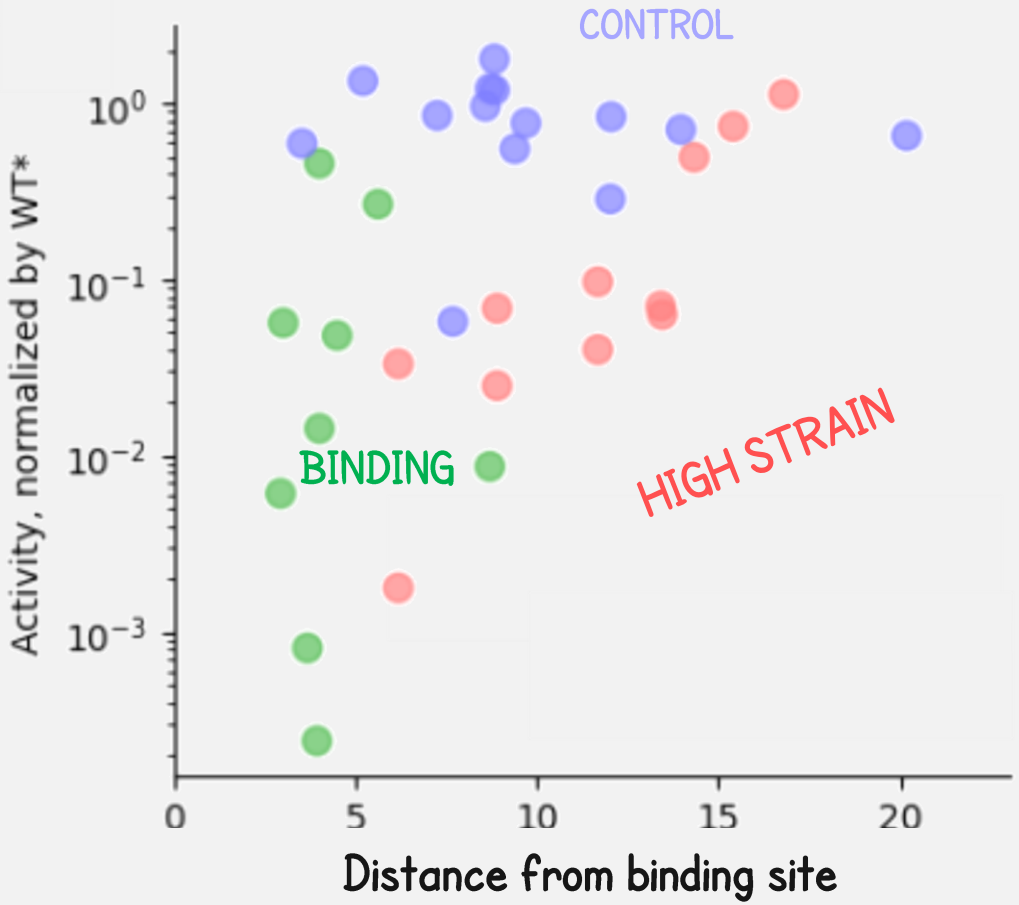
Effect of mutation on enzymatic activity is strong at high-strain amino acids

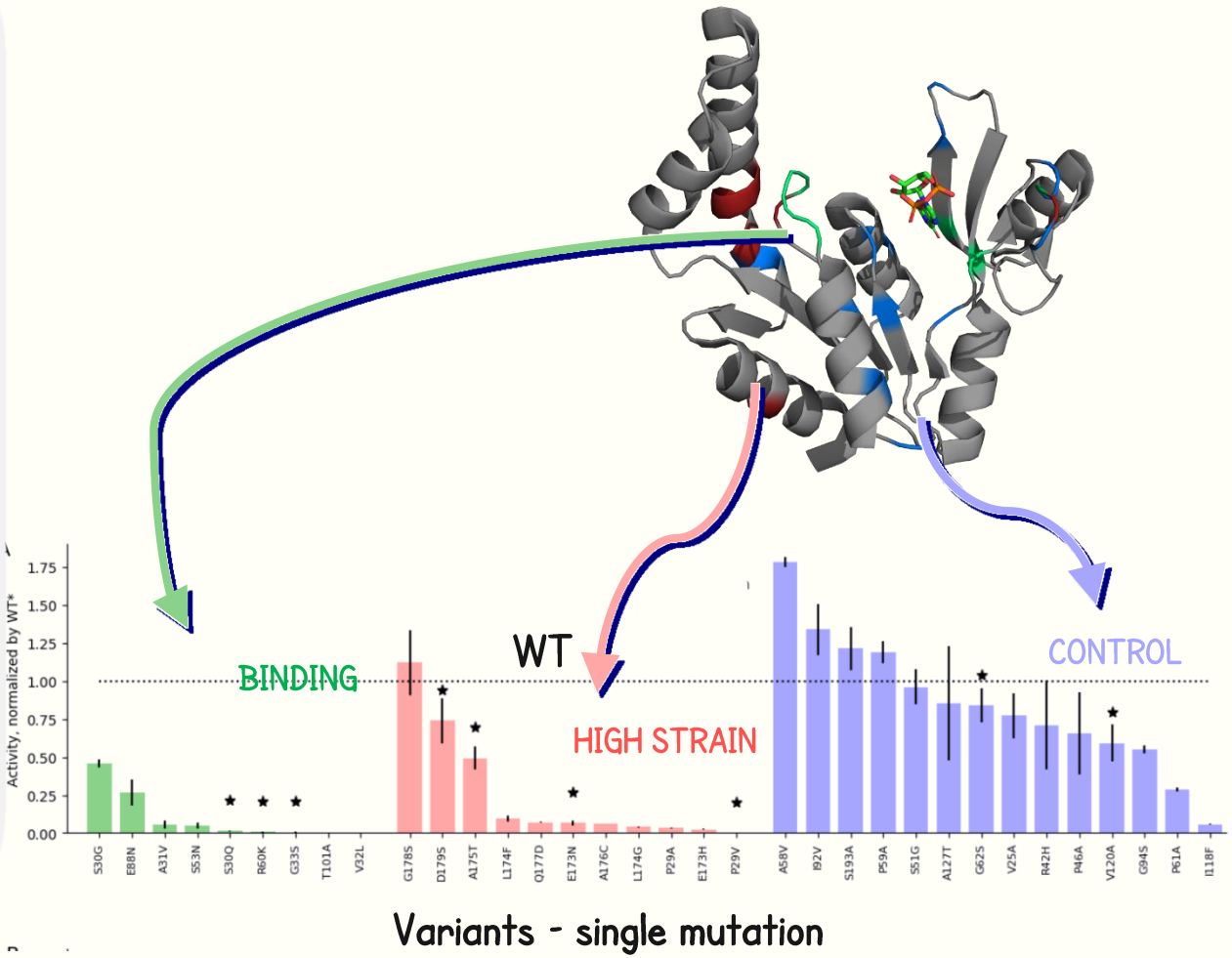


$$ATP + GMP \rightleftharpoons ADP + GDP$$



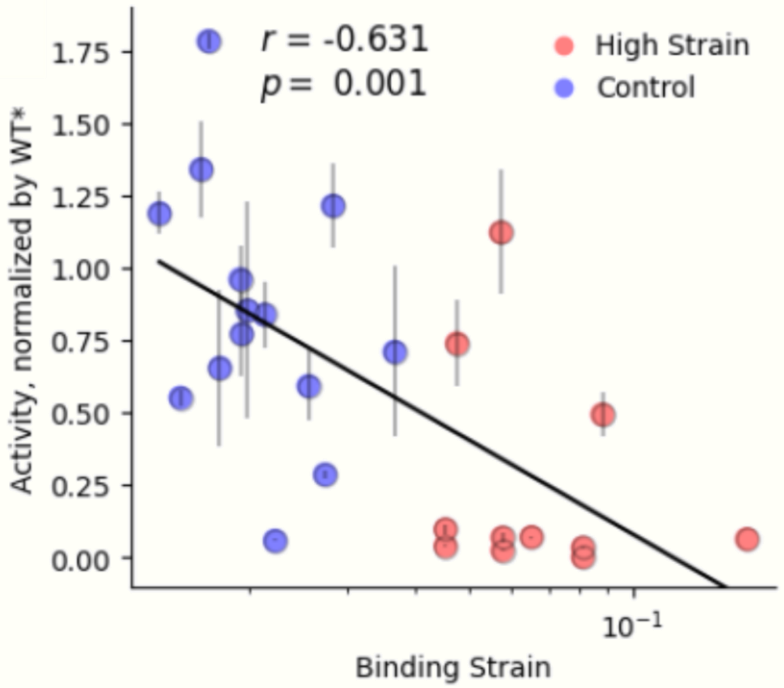
High-strain mutation have significant effect even far from binding site

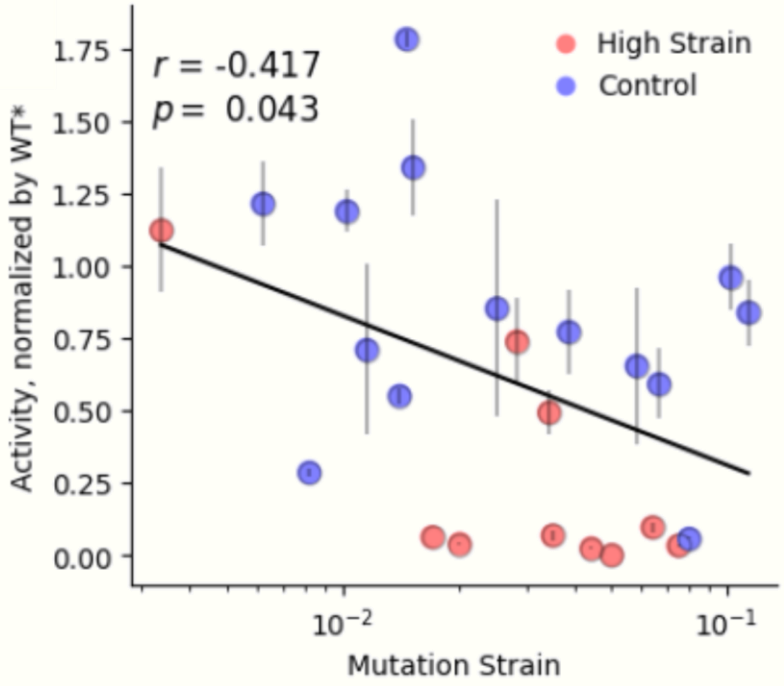




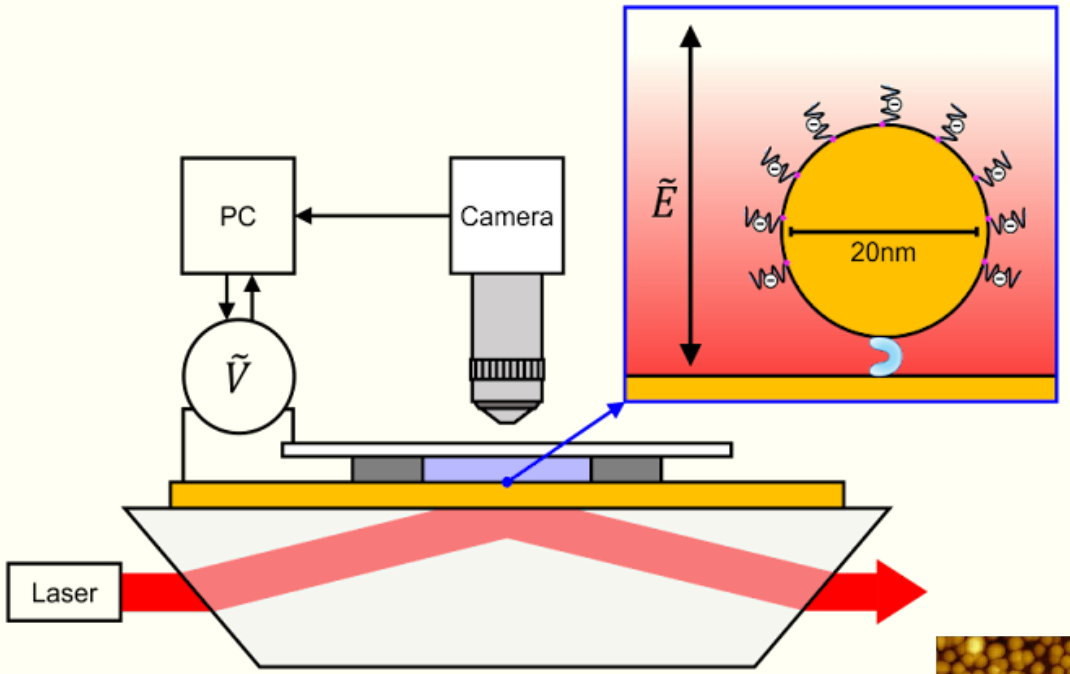
Mutations at high-strain regions have a strong effect on enzymatic function

– of both PDB (binding strain) and AlphaFold (mutation strain)

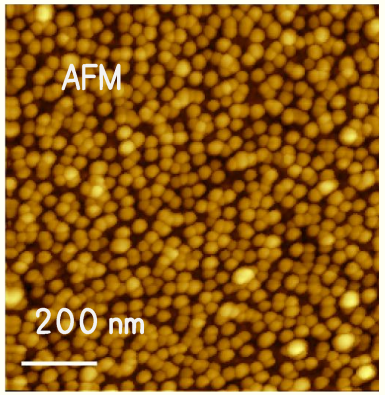




Nano-rheology of guanylate kinase exhibits viscoelastic dynamics in dependence of mechanical response on frequency

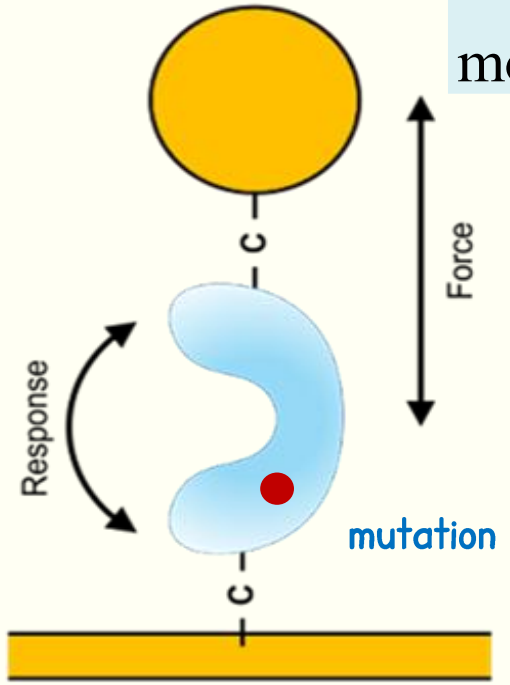


Averaging over 10^6 nanoparticles allows a resolution of 0.1 angstrom (Zocchi et al.)



$$\text{force} \sim e^{2\pi i \omega t}$$

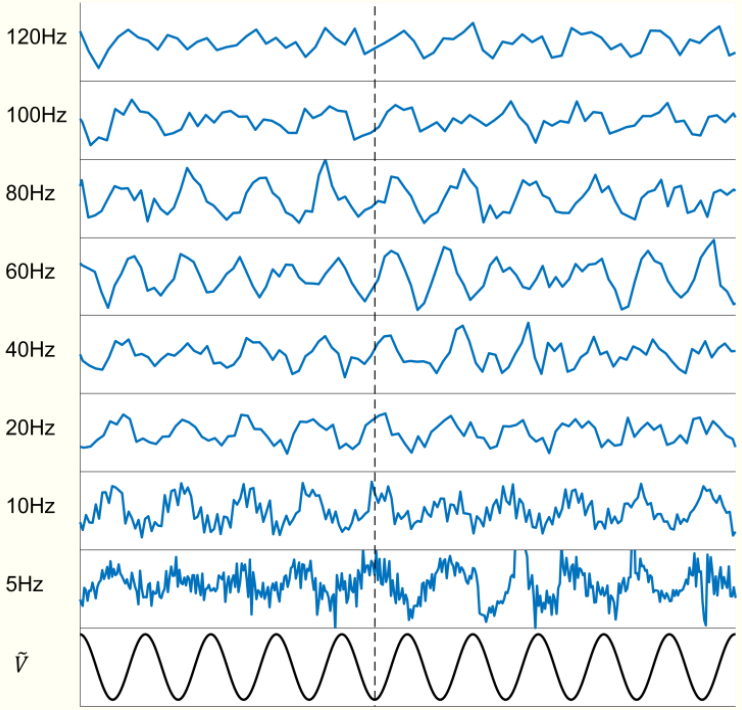
$$\text{motion} \sim e^{2\pi i(\omega t+\varphi)}$$



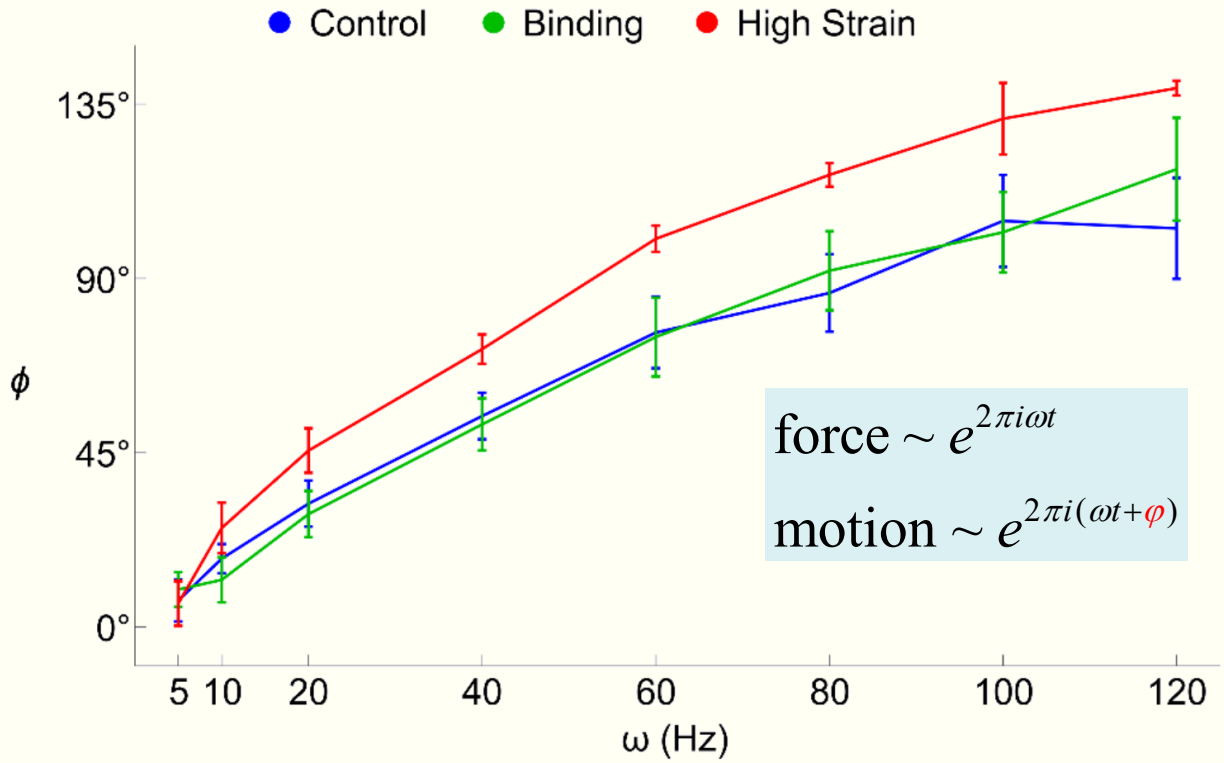
Weinreb, McBride et al. (in review Nat Phys)

Nano-rheology exhibits phase lag between applied electric force and internal motion and its frequency dependence is different for high strain

MOTION



FORCE



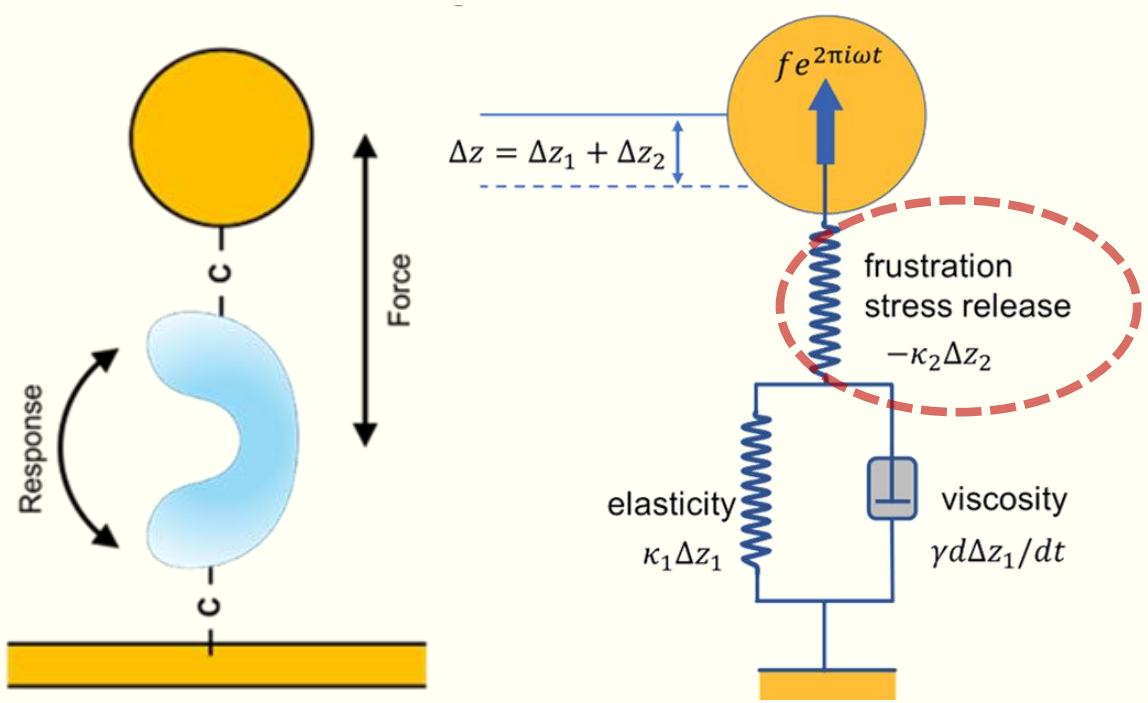
HIGH-STRAIN MUTATIONS AFFECT THE VISCOELASTIC RESPONSE OF THE PROTEIN, MAKING IT STIFFER AND MORE VISCOUS

How can we understand, predict, and quantify the link

between large-scale motion and localized catalytic function of enzymes?

4. Viscoelastic protein model

Protein viscoelasticity captures stress released from frustrated bonds



Pre-stressed bonds

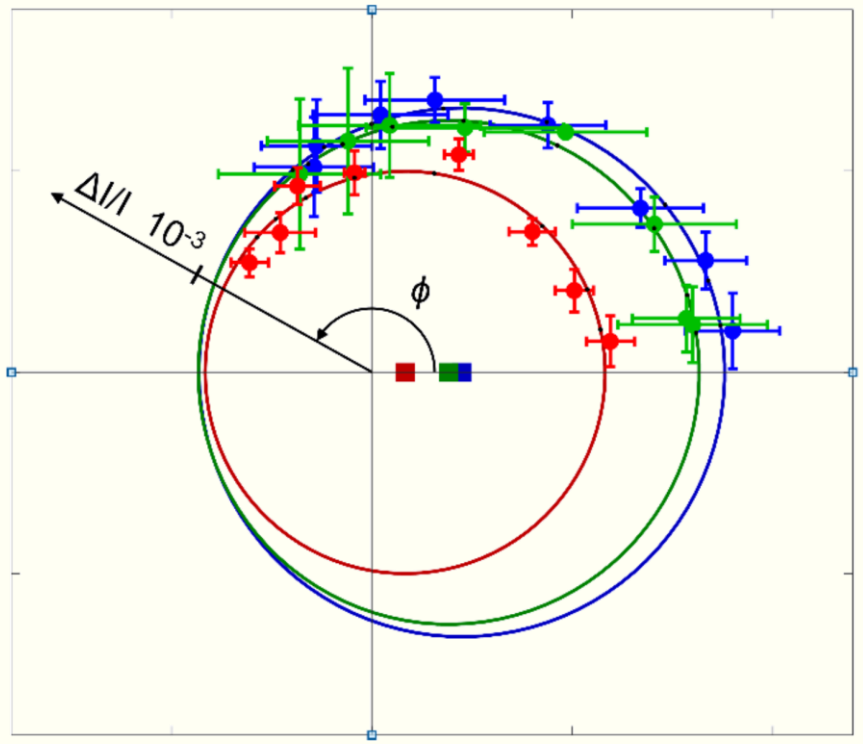
[Shlomo Alexander 1998: Physics of Amorphous solids]

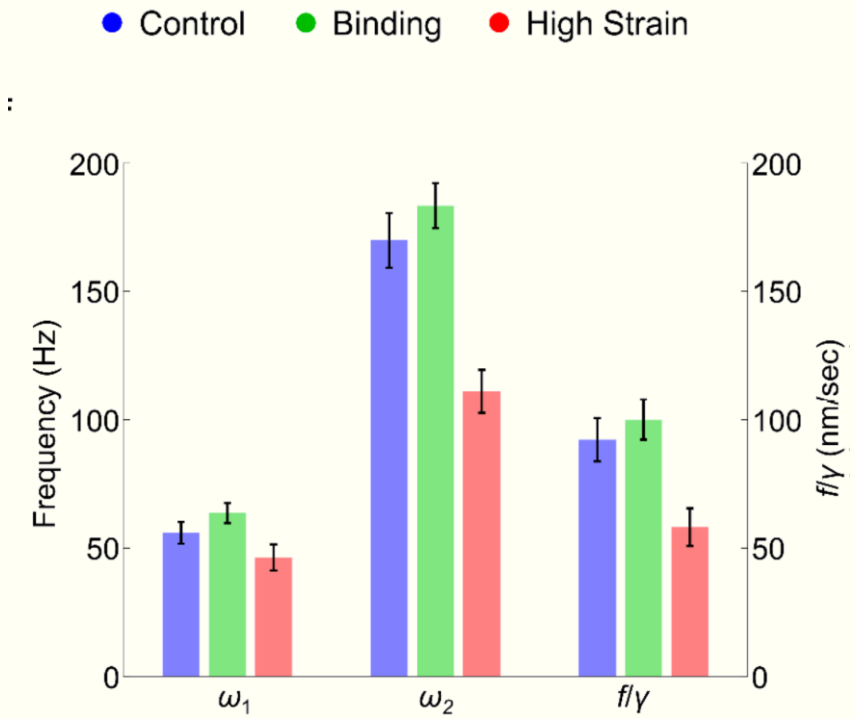
$$\Delta z(\omega) = \Delta z_1 + \Delta z_2 = \frac{f}{\gamma}\left(\frac{1}{\omega_1 + i\omega} - \frac{1}{\omega_2}\right)$$

$\omega_1 = \frac{\kappa_1}{\gamma}$ viscoelastic relaxation frequency

$\omega_2 = \frac{\kappa_2}{\gamma}$ stress release frequency (frustration)

High strain mutant are stiffer and more viscous



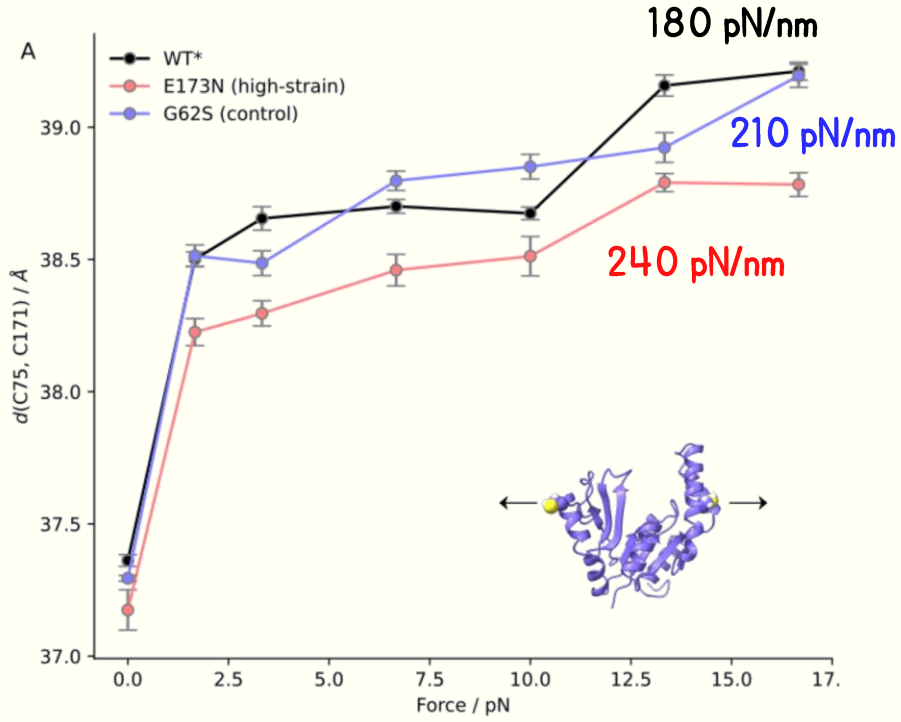


$$\Delta z(\omega) = \Delta z_1 + \Delta z_2 = \frac{f}{\gamma}\left(\frac{1}{\omega_1 + i\omega} - \frac{1}{\omega_2}\right)$$

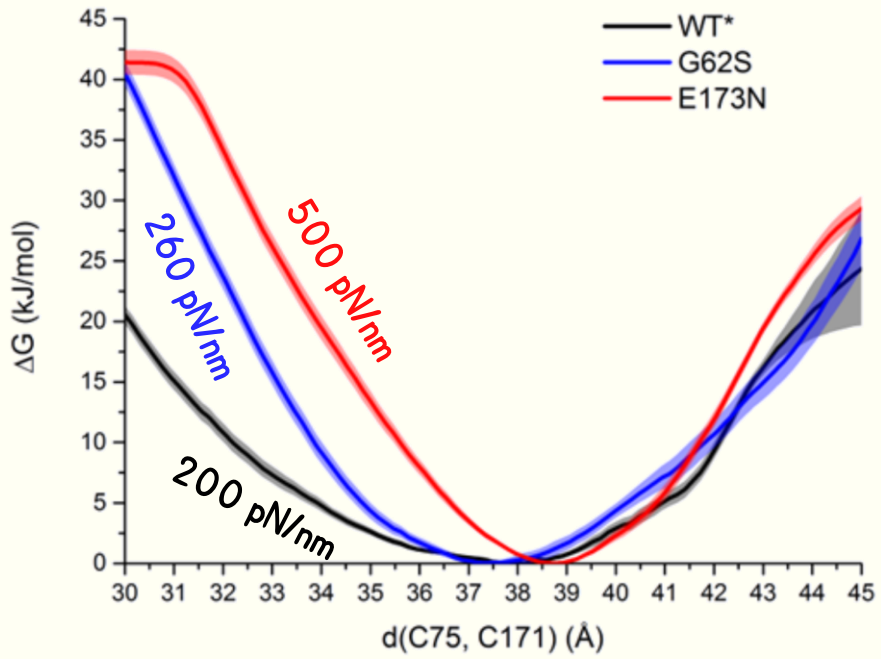
$\omega_1 = \frac{\kappa_1}{\gamma}$ viscoelastic relaxation frequency

$\omega_2 = \frac{\kappa_2}{\gamma}$ stress release frequency (frustration)

MD simulations link coarse-grained and microscopic descriptions



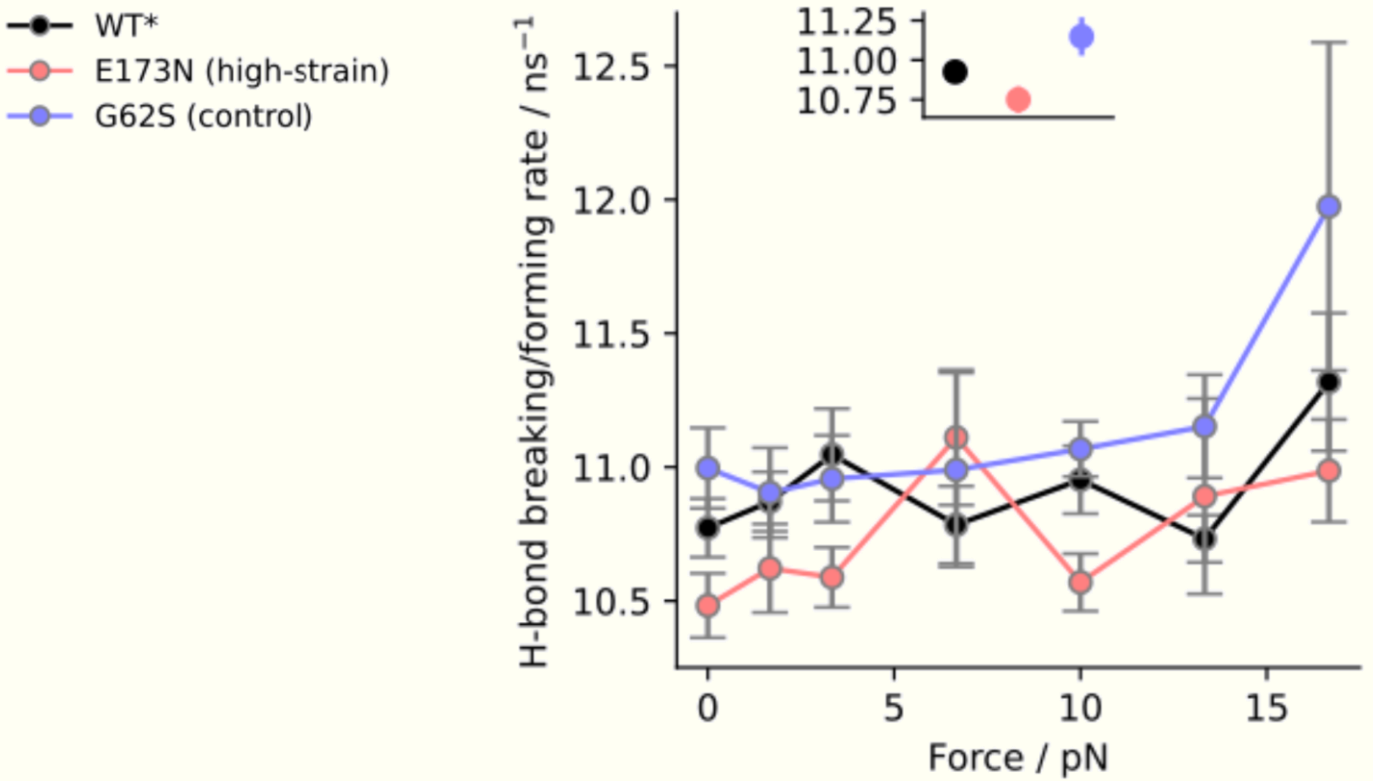
Force pulling simulations



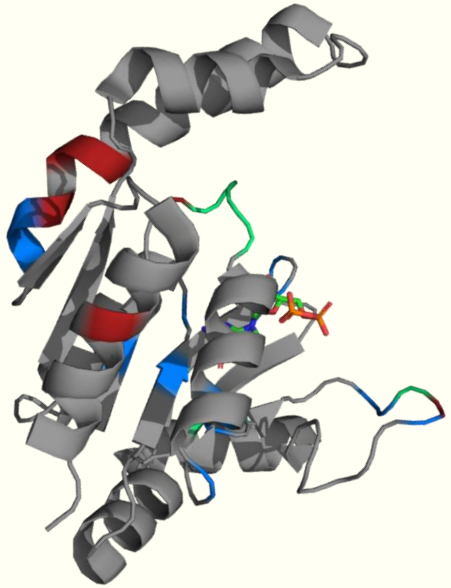
accelerated weight histogram (AWH)

Weinreb, McBride et al. (in review Nat Phys)

Viscosity correlates with h-bond forming\breaking rates

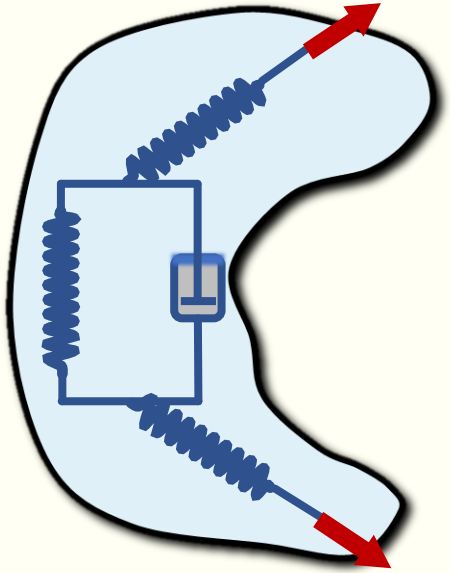


Summary



1. Theory of protein as viscoelastic matter predicts the evolution of functionally-relevant collective motion with high-strain regions.

2. Strain - measure of deformation estimates effect of distant mutations on catalytic site.



3. Experimental test - the enzyme guanylate kinase links enzymatic function to predicted high-strain region and their effect on viscoelasticity.

4. Effective model of protein as sequence encoded viscoelastic machine.

E Weinreb, E Moses,

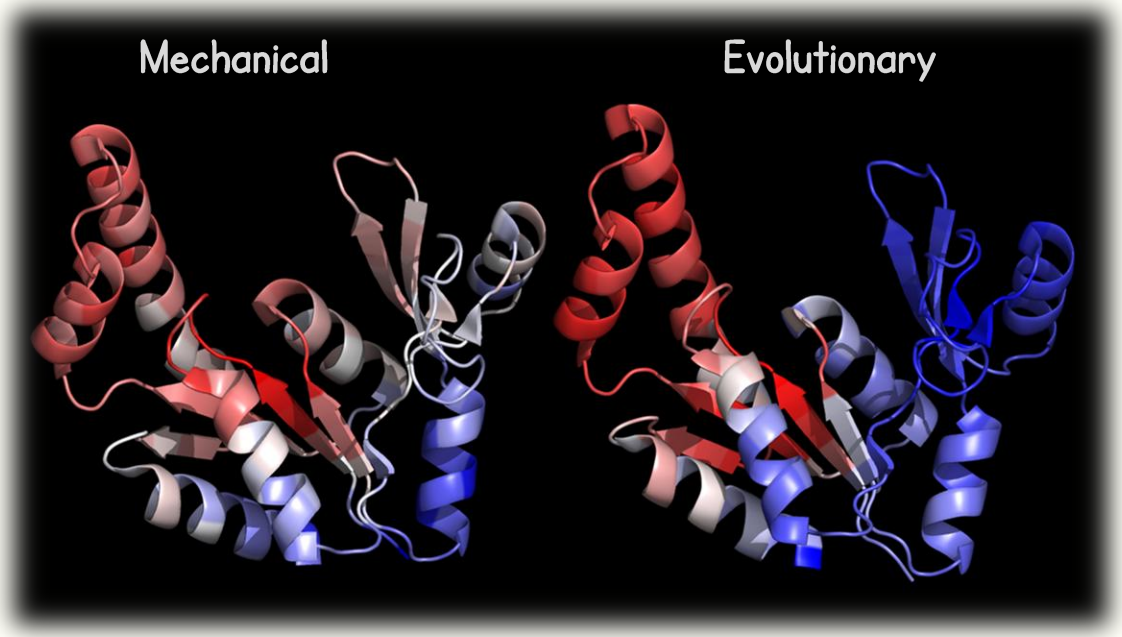
Y Peleg, T Unger, S Albeck, Y Fridmann-Sirkis,

S Lushchekina, JL Sussman (Weizmann)

J-P Eckmann, J Rougemont (Geneva)

R Renault (Qnergy), G Zocchi (UCLA)

JM McBride, MM Siek (IBS)



- Weinreb et al. "**Enzymes as viscoelastic machines**". *Nature Physics,* 2025.
- McBride et al. "**AI-predicted protein deformation encodes energy landscape perturbation**". *Physical Review Letters* 2024.
- McBride et al. "**AlphaFold2 can predict single-mutation effects".** *Physical Review Letters* 2023
- **Work on protein theory**: McBride et al. Mol. Biol. Evo 2022; Eckmann et al., Bioessays 2021; Eckmann et al., Rev Mod Phys 2019; Eckmann et al. PRX 2017; Dutta et al., PNAS 2018; Mitchell et al. PNAS 2016