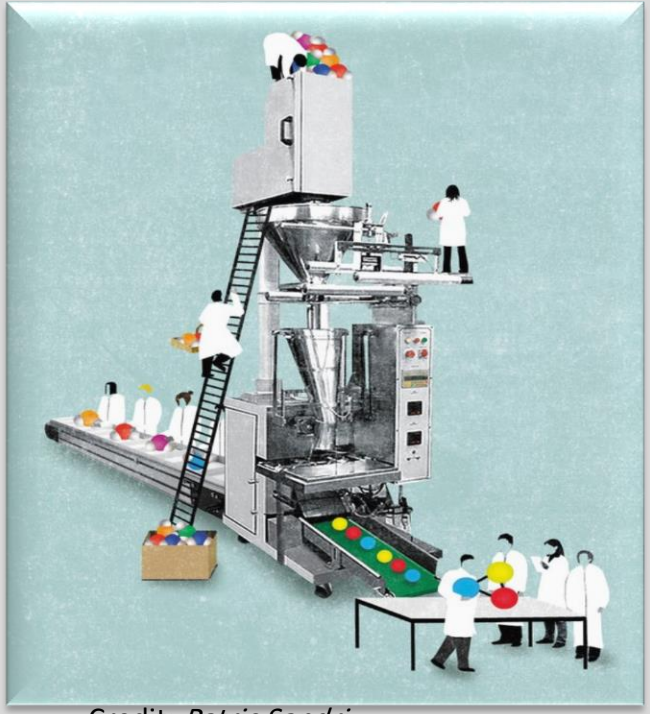


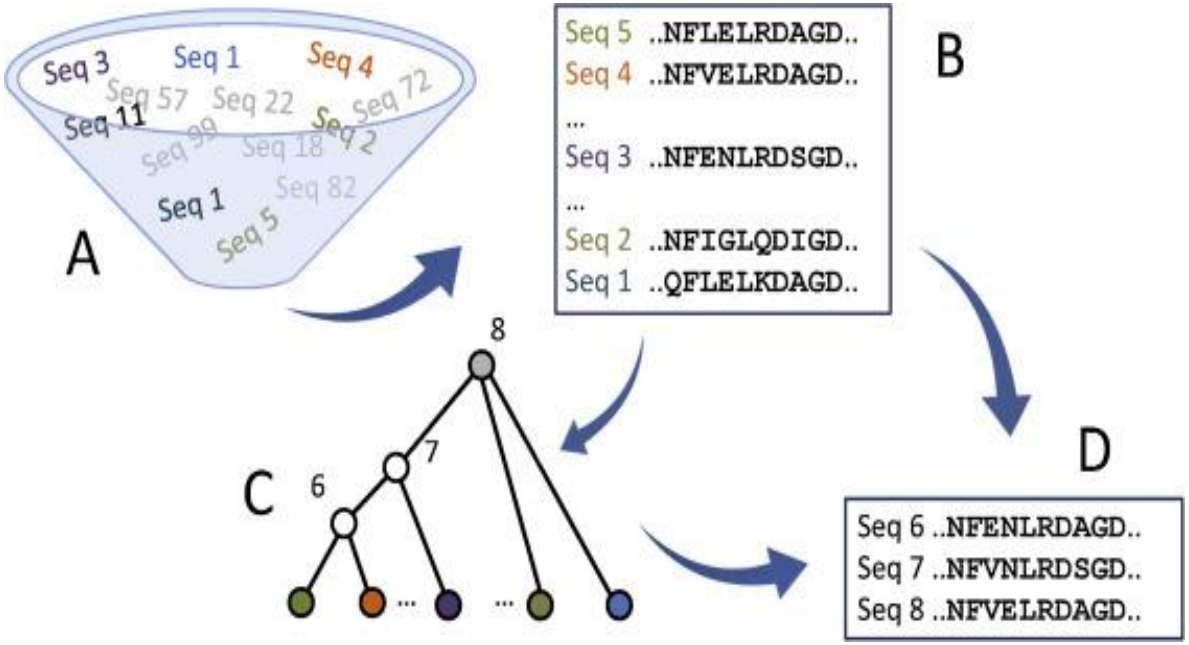
Credit: *Patric Sandri*

Biotechnological & protein-engineering implications of ancestral protein resurrection

Contents

- Introduction
- Utilizing ancestral reconstruction in protein biotechnology.
- Enhanced stability.
- Conformational flexibility/diversity.
- Promiscuity.
- Concluding remarks.

Introduction



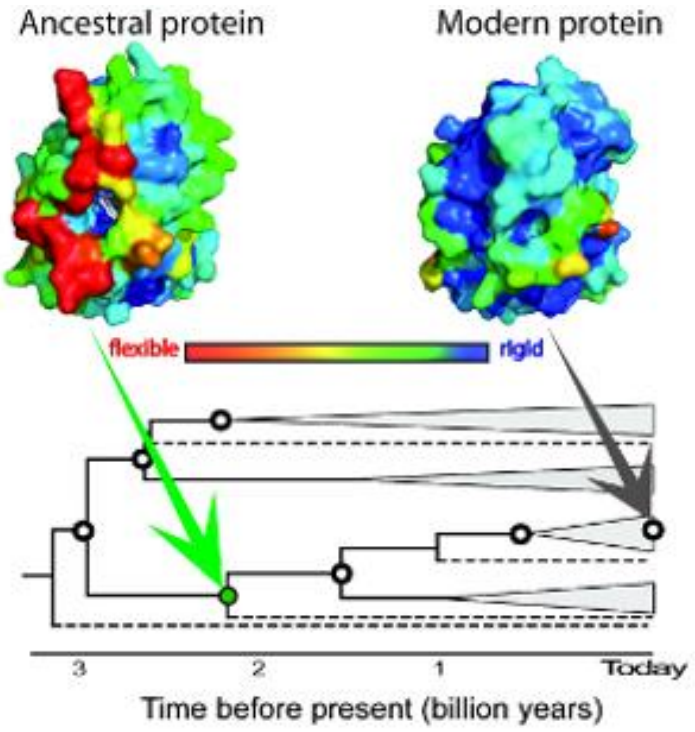
- Reasonable sequences of **ancestral proteins** can be derived from the sequences of their **modern descendants**.

Resurrected ancestral proteins

- Can be prepared in the laboratory.
- **Subjected to experimental works.**
- have **remarkable properties** reflecting ancestral adaptations.
- Differed from (modern/extant proteins).

‘resurrected ancestral proteins’

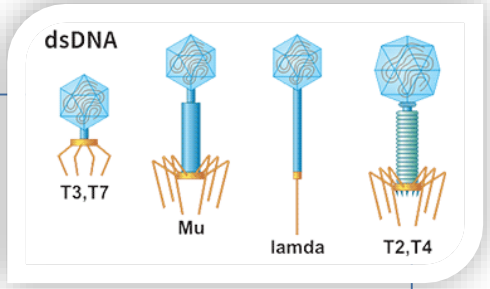
- **Precambrian proteins** show large numbers of amino acid differences with their modern descendants.



Risso…Sánchez-Ruiz {*Nat Commun* 8: 16113}

Utilizing ancestral reconstruction in protein biotechnology.

Example



- Phage T7 recruits E. coli **thioredoxin.**
- It can be replaced by **ancestral Precambrian thioredoxins that** could not be recruited by the phage and rendered E. coli **resistant to infection.**
- Applying this approach to **engineering of virus resistance in plants.**

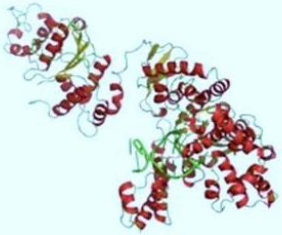
Primordial trait

- **High stability** is a very convenient property from a biotechnological point of view **because low stability compromises many practical applications of proteins**

Several approaches for protein stabilization

- Search for stable proteins in **thermophilic organisms**
- Ancestral proteins **higher thermal stability** compared to **extant proteins.**
- **Exception:** some extant proteins may exhibit higher stability.

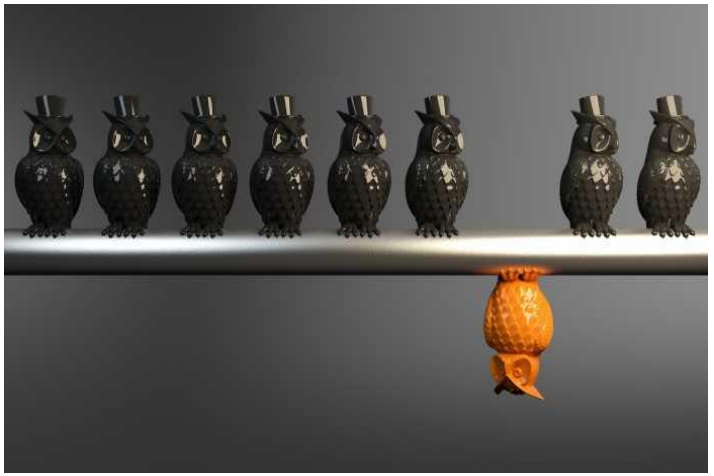
Thermus aquaticus



Structure of Taq Polymerase

Sequence differences

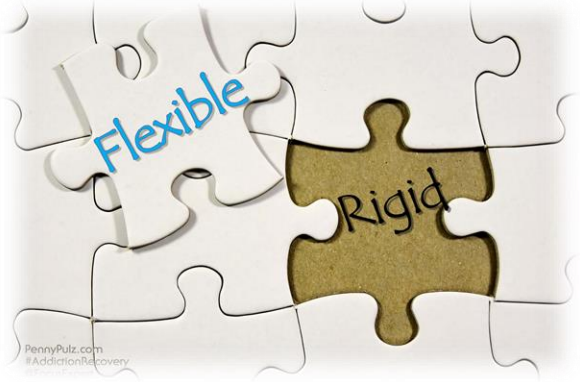
- Exploring the **differences** between molecular mechanism of stability of the ancestral and that of thermophilic extant sequences would provide **additional tools for biotechnology.**



Conformational flexibility/diversity

Proteins dynamically interconvert between conformations in the native state to achieve their function, including:

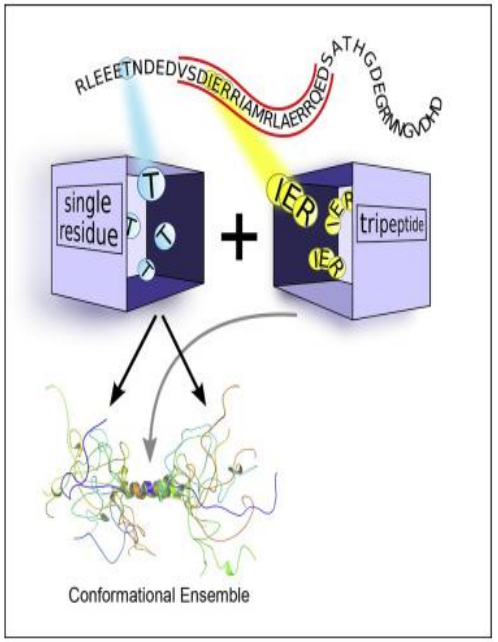
- Allosteric signaling
- Protein–ligand recognition
- Electron transfer
- Catalysis



Ensemble model

Protein causes a variety of conformations through:

- Side-chain rotations
- Global changes through domain rearrangement.
- Allostery



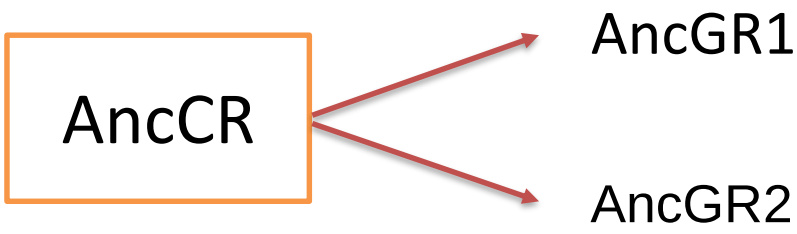
Cont.

- **Mutations** throughout protein evolution alter **conformational dynamics**, change the distribution of the ensemble and lead to new functions by **dynamic motions** and **adaption** to different environments.

Protein design

- Computational protein design methods have been used to introduce completely novel enzymatic functions in protein scaffolds initially lacking these abilities

Example regarding to change in **flexibility**



- **AncCR** & **AncGR1** have **a promiscuous binding affinity** to both aldosterone , cortisol.
- **AncGR2** specifically binds to cortisol.
- **AncGR1** and **AncGR2**, diverge functionally through **36 mutations**, have highly similar structures.

Cont.

- **AncCR** and **AncGR1** have a flexible binding pocket, suggesting **flexibility** plays a role.

- In contrast, the mutations of **AncGR2** lead to a **rigid binding pocket**, suggesting that binding pocket becomes cortisol specific.

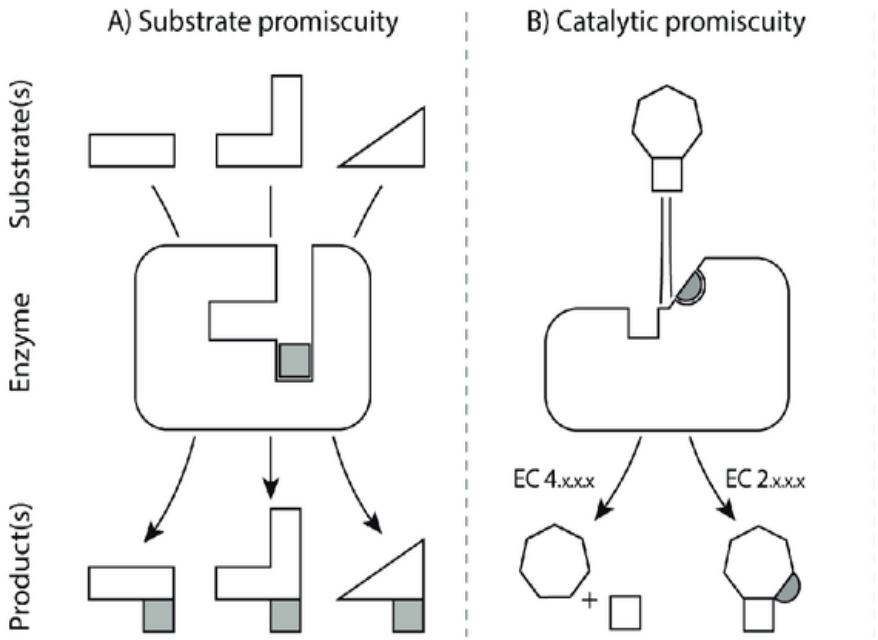
Promiscuity

- One physiologically relevant function.
- **de-toxification** , are highly promiscuous: can degrade a wide variety of toxics.
- This kind of promiscuity is often considered as generalist nature of **ancestral enzymes.**

classification of enzyme promiscuity

(A)Substrate promiscuity/multispecificity: A certain enzyme can perform the same catalytic reaction on a diverse set of substrates.

(B) Catalytic promiscuity: Different chemical transformations are allowed by the same enzyme, according to which this can be classified with various E.C. numbers



(Piedrafita, Keller, & Ralser, 2015)

Concluding remarks

- Ancestral proteins **show enhanced levels of activity** compared to their **modern descendants.**

- **Resurrected ancestral proteins** can display **high stability** and **enhanced promiscuity features** that are advantageous in biotechnological applications.

References

- Merkl, R. and R. Sterner, *Reconstruction of ancestral enzymes.* Perspectives in Science, 2016. **9**: p. 17-23.

- Piedrafita, G., M. Keller, and M. Ralser, *The Impact of Non-Enzymatic Reactions and Enzyme Promiscuity on Cellular Metabolism during (Oxidative) Stress Conditions.* Biomolecules, 2015. **2015**: p. 2101-2122.
- And main review in cover page.


THANK
YOU