

Class core values

1. Be **respectful** to yourself and others
2. Be **confident** and believe in yourself
3. Always do your **best**
4. Be **cooperative**
5. Be **creative**
6. Have **fun**
7. Be **patient** with yourself while you learn
8. Don't be shy to **ask "stupid" questions**



Week 2, Lecture 2

Rational design of proteins guided by structure

Learning Objectives

1. Describe the concept of rational design
2. Identify potential mutations to increase stability
3. Describe different methods for rational design
4. Apply tools for structure analysis, evolutionary information, and computational techniques to design
5. Identify the limitations of rational design approaches

Rational design



Rational design is often guided by prior knowledge, often structure

Structure-guided mutagenesis based on prior knowledge to improve features



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Structure-guided mutagenesis based on prior knowledge to improve features



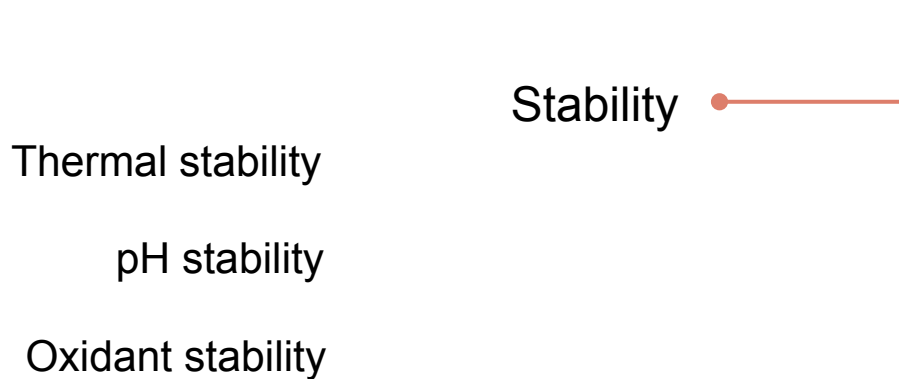
Advantage	Disadvantage
We're building off of what works → higher chance of success	Limited to what we know
Simpler if you have a good guess	Limited by what exists in nature
Requires fewer resources	You need to know the structure

The next step is to consider the features we want to improve (the what)

Structure-guided mutagenesis based on prior knowledge to improve **features**

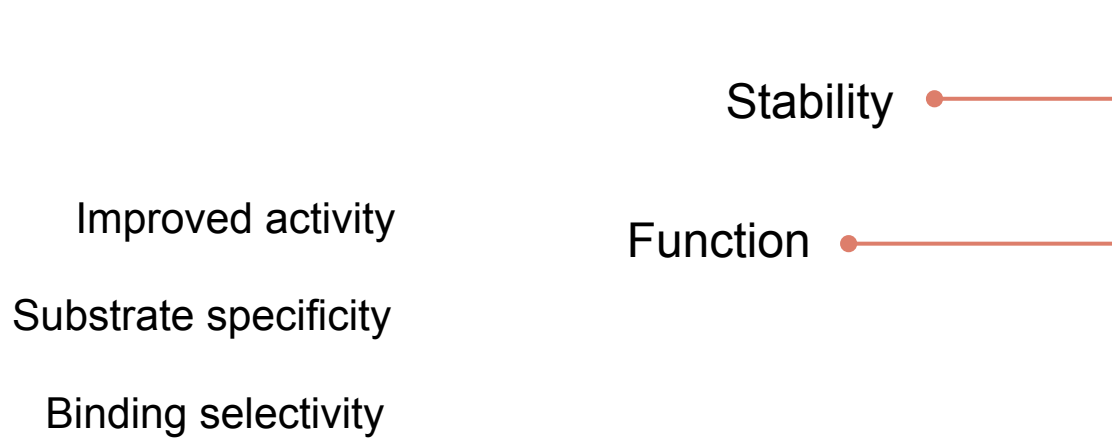
Enhancing proteins stability is one of the most commonly used engineering applications

Structure-guided mutagenesis based on prior knowledge to improve **features**



Another commonly used application of rational design is enhancement of function/activity

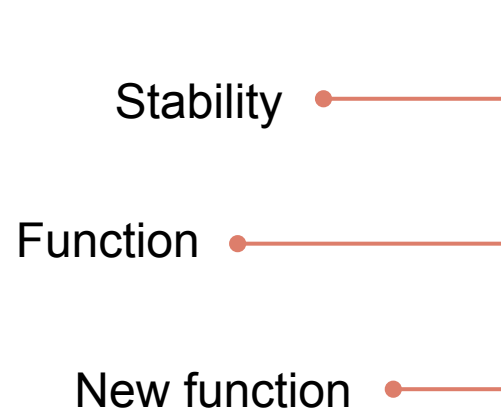
Structure-guided mutagenesis based on prior knowledge to improve **features**



Rational design can also be used to design biomimetics or create new function

Structure-guided mutagenesis based on prior knowledge to improve **features**

Mimicking other proteins
Changing substrates
Changing cofactors



If multiple features are desired, it's best to use a scaffold that satisfies one of the conditions

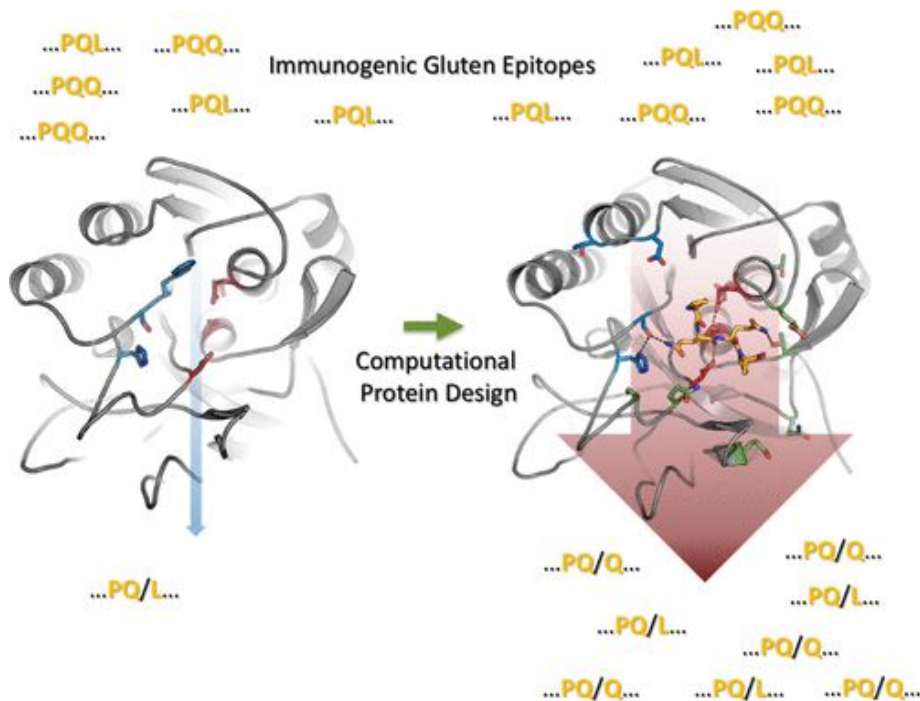
If multiple features are desired, it's best to use a scaffold that satisfies one of the conditions

Oral Enzyme Therapy

to Treat Celiac Disease

1

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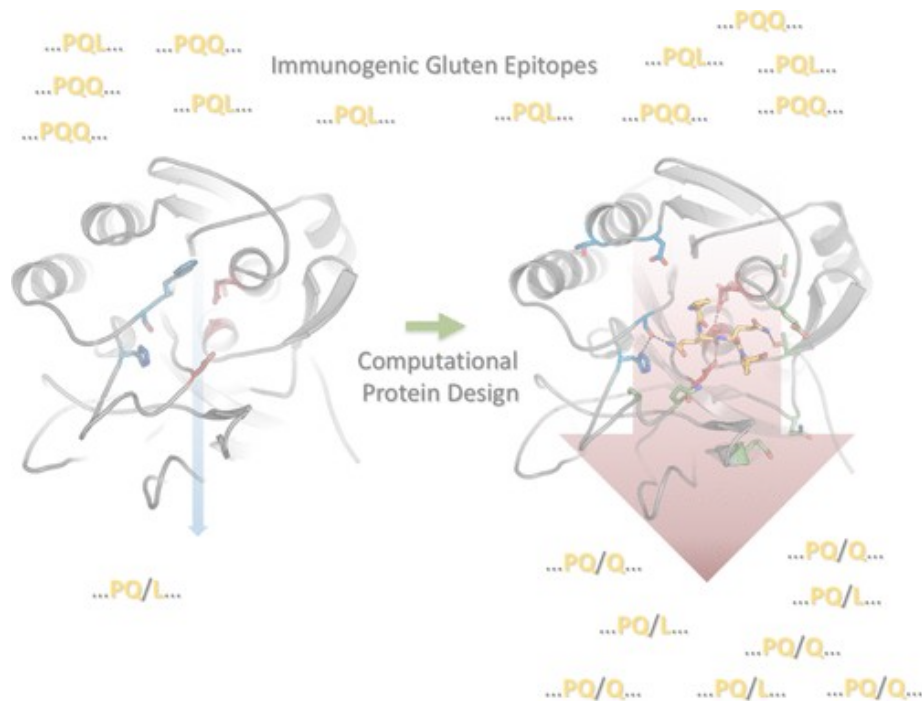


Oral Enzyme Therapy

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If multiple features are desired, it's best to use a scaffold that satisfies one of the conditions



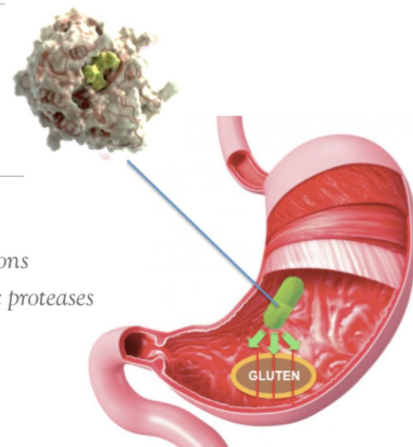
Oral Enzyme Therapy

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KumaMax

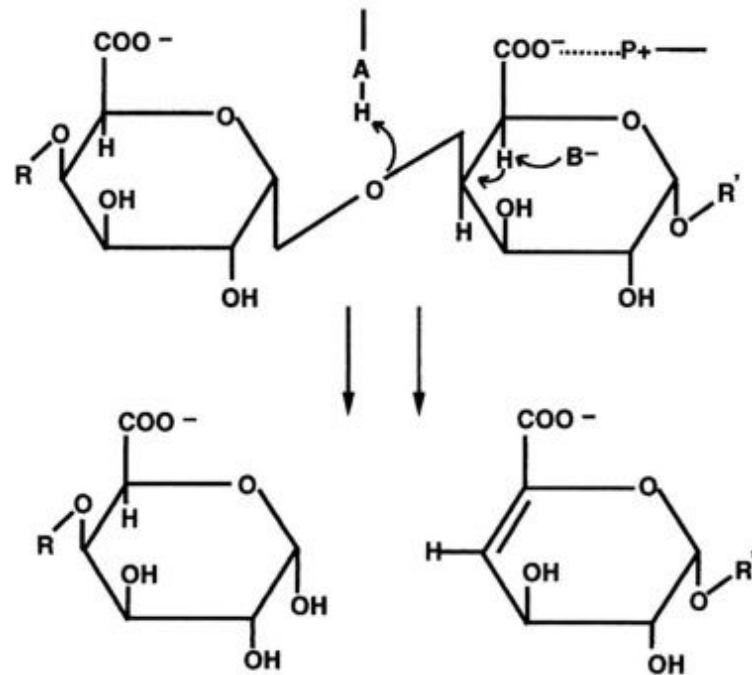
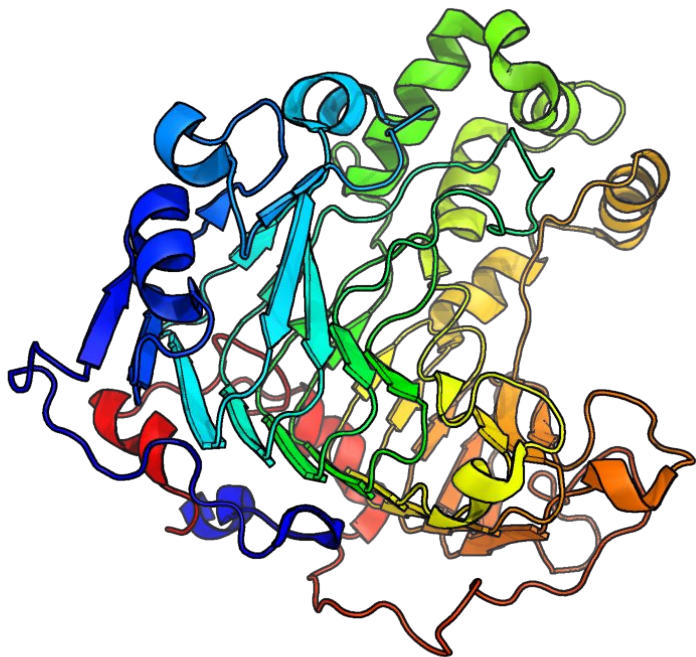
a novel gluten-degrading enzyme

- Degrades gliadin in gastric conditions
- Resistant to degradation by gastric proteases
- Naturally thermostable
- Readily produced and purified



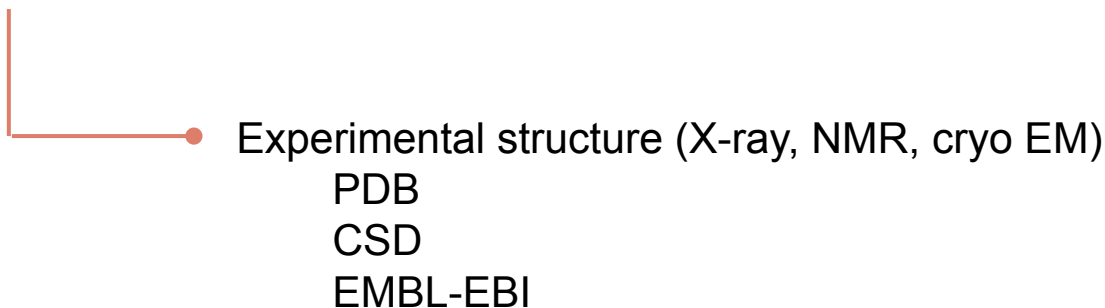
In-class activity:

Enhancing stability of PelN using Rational Design



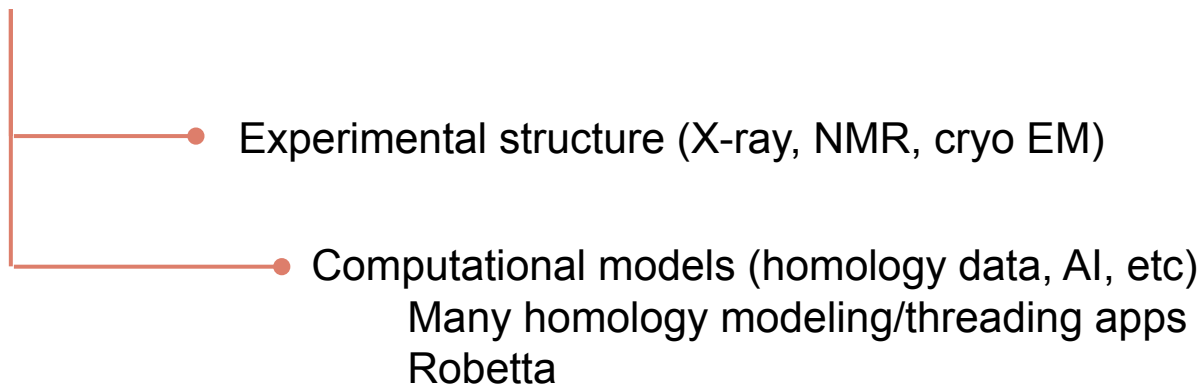
Many databases are available that include protein structures with statistics about quality

Structure-guided mutagenesis based on prior knowledge to improve features



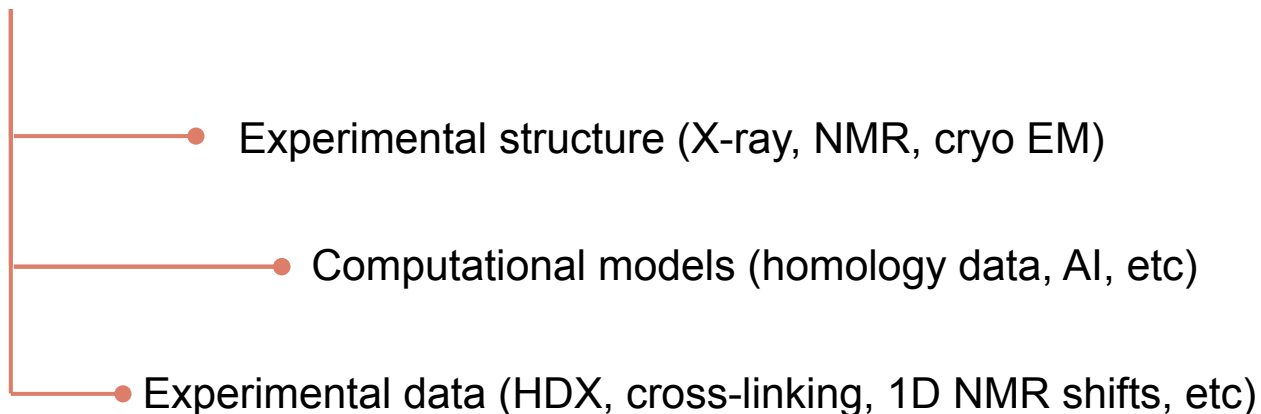
Computational modeling methods offer great insight about proteins without validated structure

Structure-guided mutagenesis based on prior knowledge to improve features



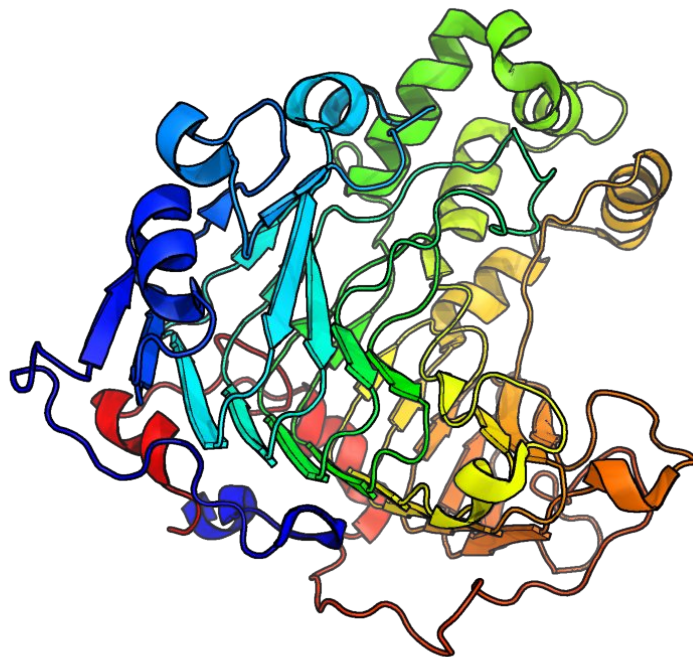
For more challenging cases, experimental data can provide a powerful intuition

Structure-guided mutagenesis based on prior knowledge to improve features



In-class activity:

Enhancing stability of *PeiN* using *Rational Design*



PeiN
PDB: 5GT5


The engineering process is often guided by some prior knowledge

Structure-guided mutagenesis based on **prior knowledge** to improve features

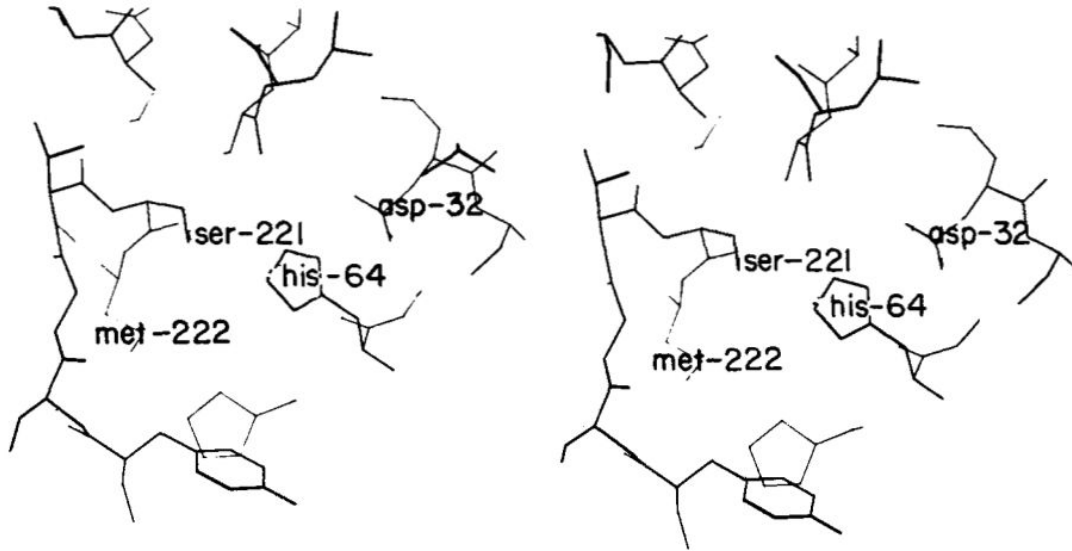
Our biophysical/biochemical knowledge can guide us in finding beneficial mutations

Structure-guided mutagenesis based on **prior knowledge** to improve features

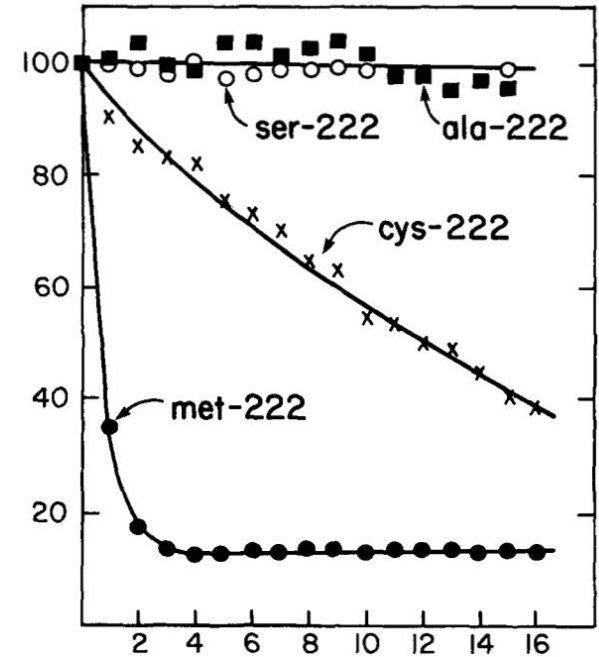
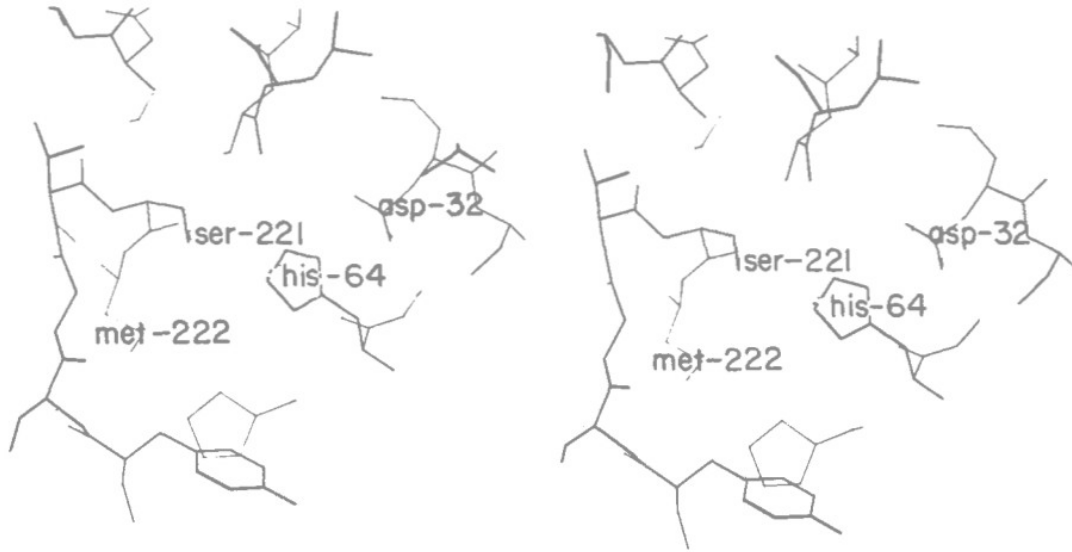
Biophysical and Biochemical knowledge
(aka physics-based)



Observations about amino acid properties can lead to significant outcome: the case of Subtilisin




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Structure-guided mutagenesis based on **prior knowledge** to improve features

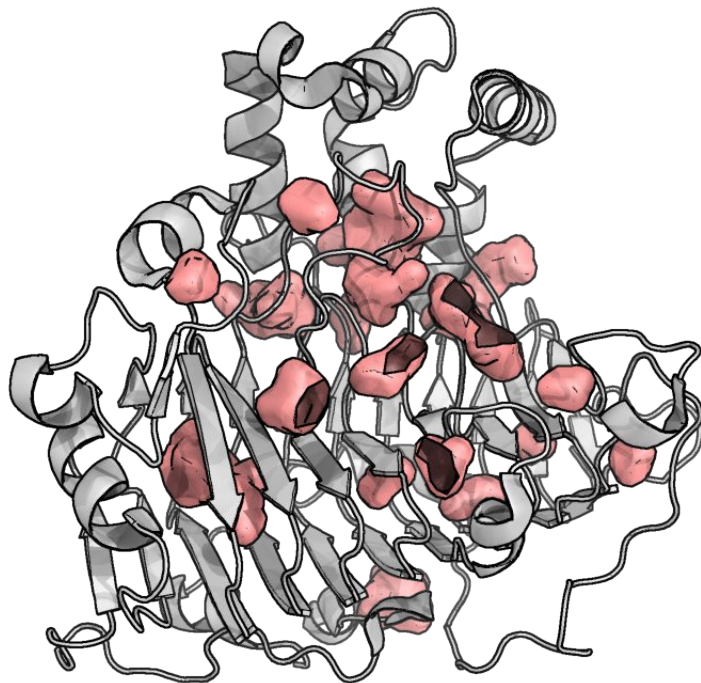
Biophysical and Biochemical knowledge
(aka physics-based)



- Burying polar residues in the core of a protein has a huge energy cost
- New hydrogen bonds are energetically favorable
- The size of an enzyme pocket affects its substrate scope
- ...

In-class activity:

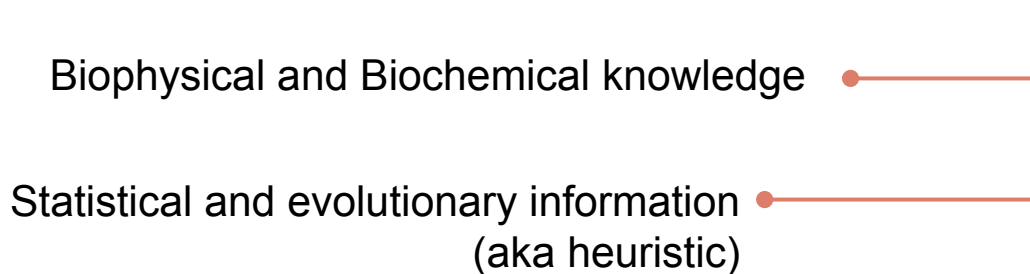
Enhancing stability of PelN using Rational Design



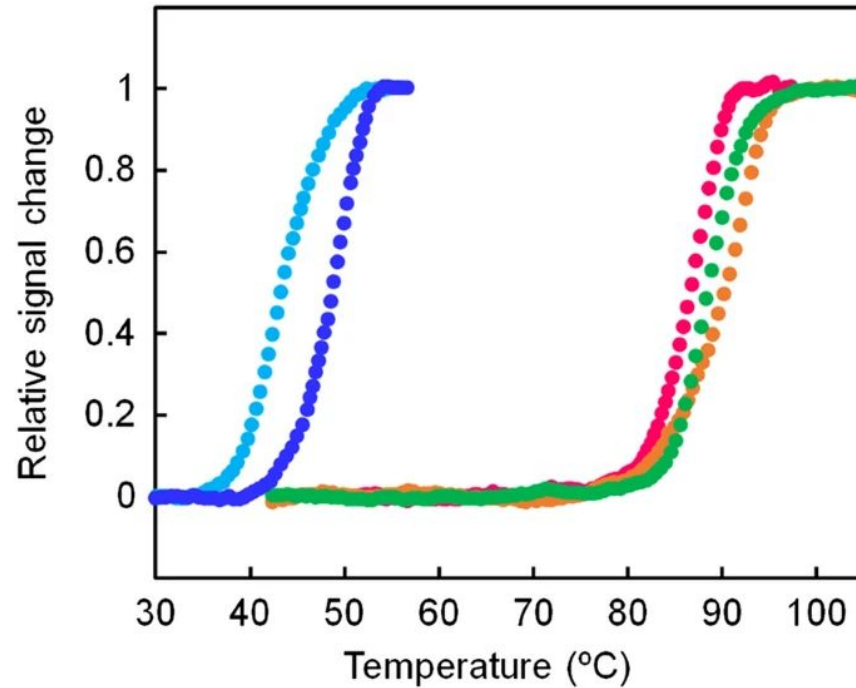
Cavities in PelN
PDB: 5GT5

Evolutionary data and close relatives contain rich information about protein fold/function

Structure-guided mutagenesis based on **prior knowledge** to improve features

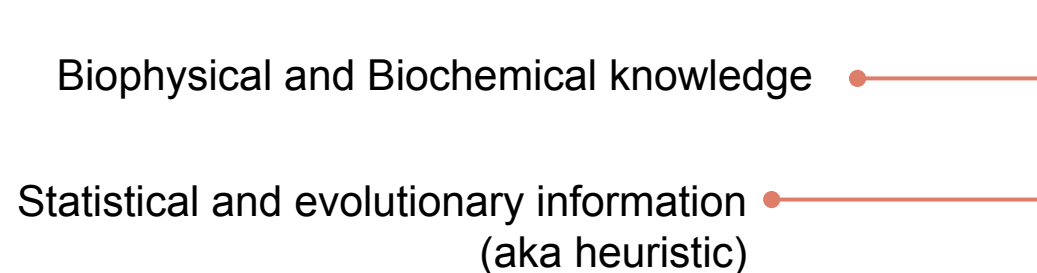


Ancestral sequence reconstitution can be used for enhancing stability of a protein



Evolutionary data and close relatives contain rich information about protein fold/function

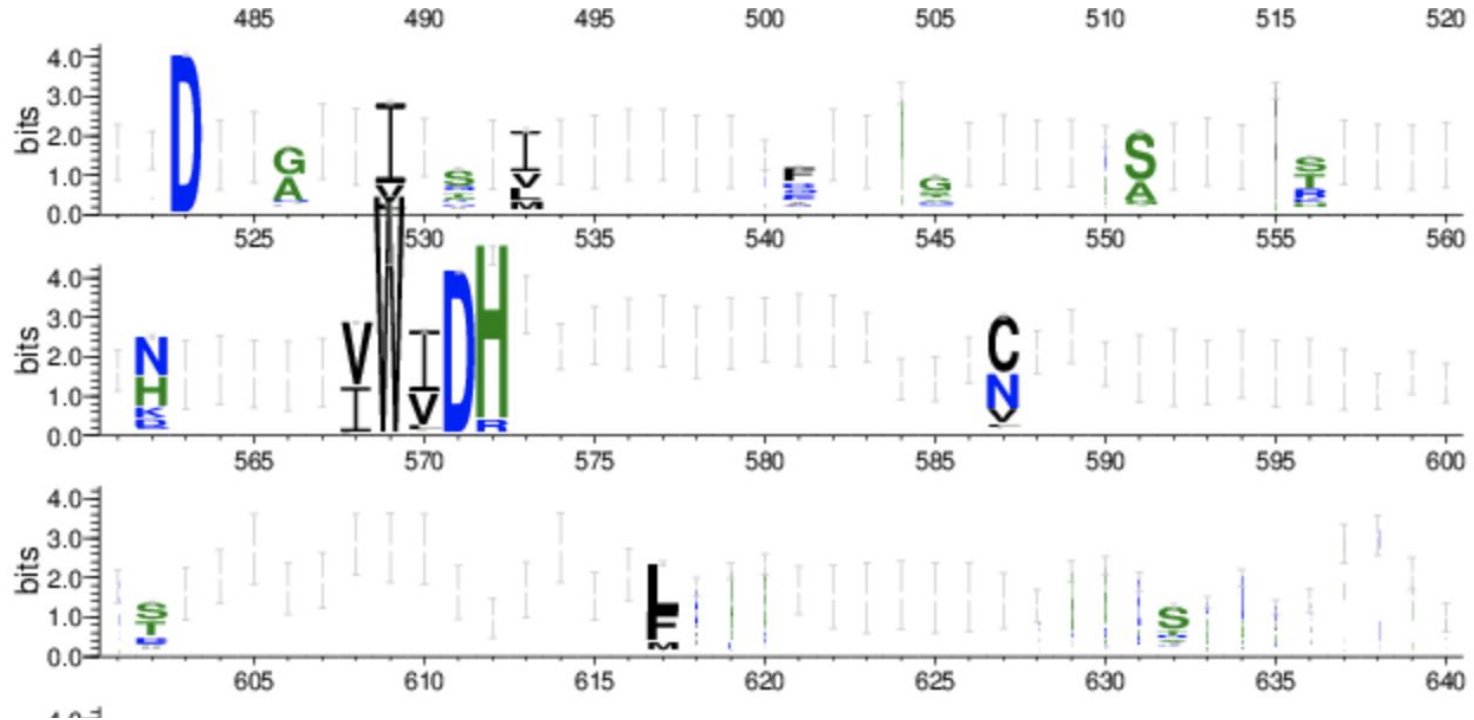
Structure-guided mutagenesis based on **prior knowledge** to improve features



- Multiple Sequence Alignment
 - NCBI, JackHmmer, Pfam

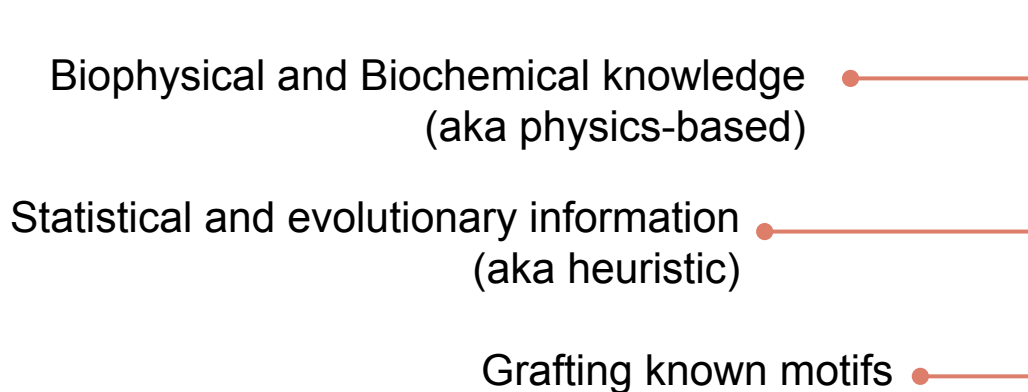
In-class activity:

Enhancing stability of PelN using Rational Design



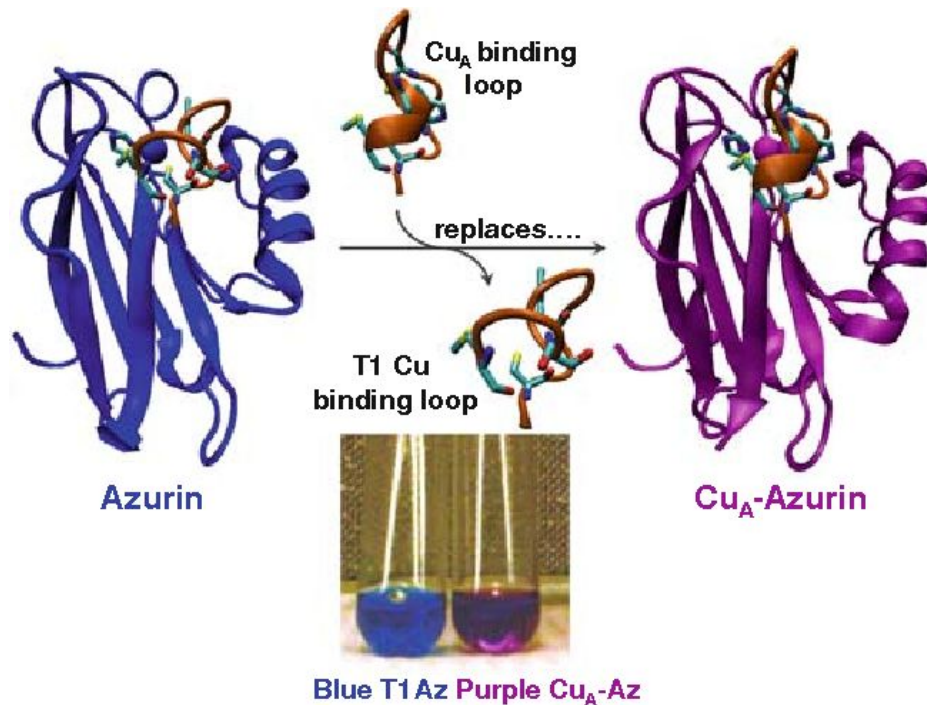
Structural features from one protein can be grafted onto other proteins to exert new function

Structure-guided mutagenesis based on **prior knowledge** to improve features



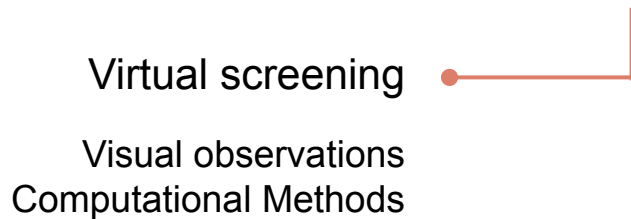
- Grafting loops onto structures
- Adding binding ligands inside other cavities
- Placing binding motifs onto other scaffolds
- ...

Loop grafting was used to change metal binding features of Azurin



Visual or computational screening can help narrow down the number of experiments

Structure-guided mutagenesis based on prior knowledge to **improve** features



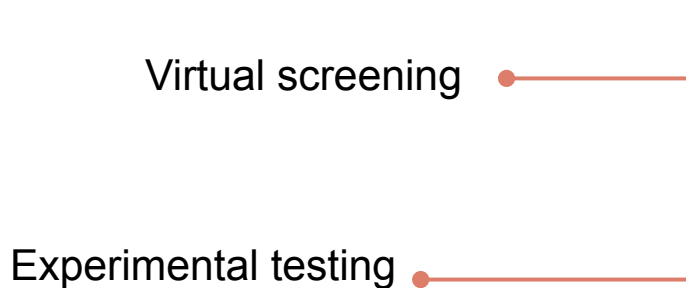
In-class activity:

Enhancing stability of PeIN using Rational Design



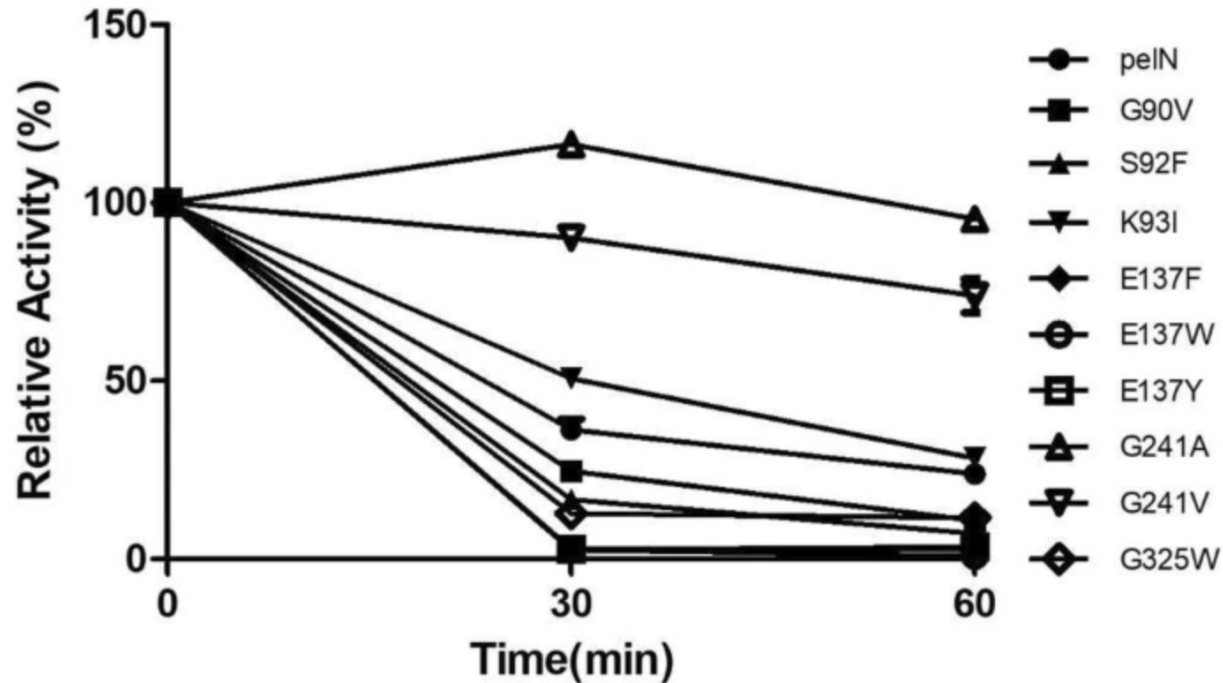
Experimental testing is the ultimate method for assessing the effectiveness of our designs

Structure-guided mutagenesis based on prior knowledge to **improve** features



In-class activity:

Enhancing stability of PelN using Rational Design

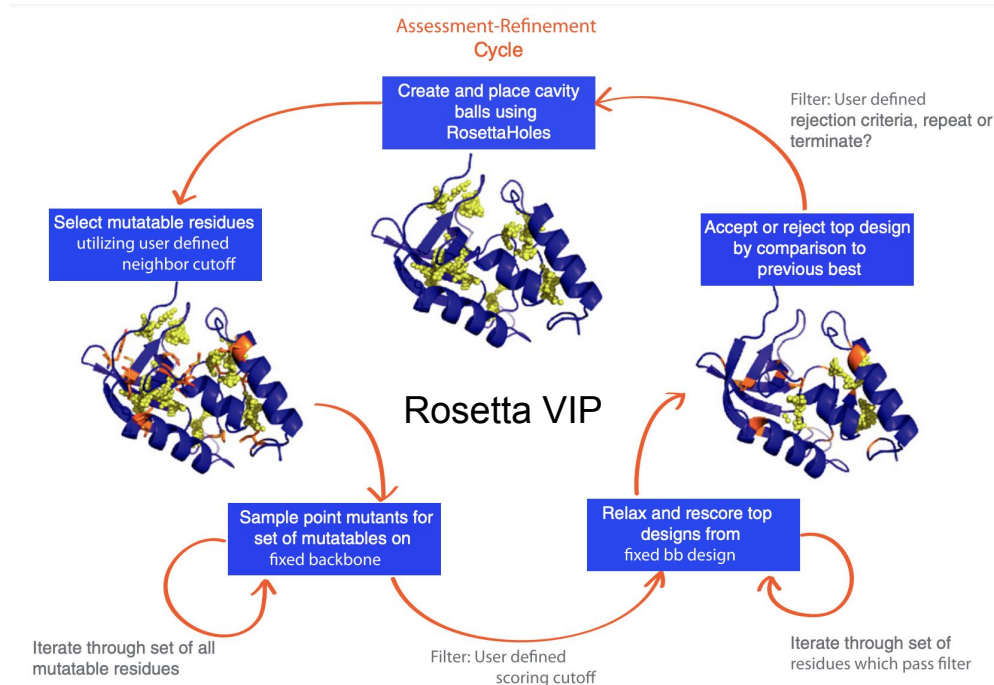


Possible search space is extremely large

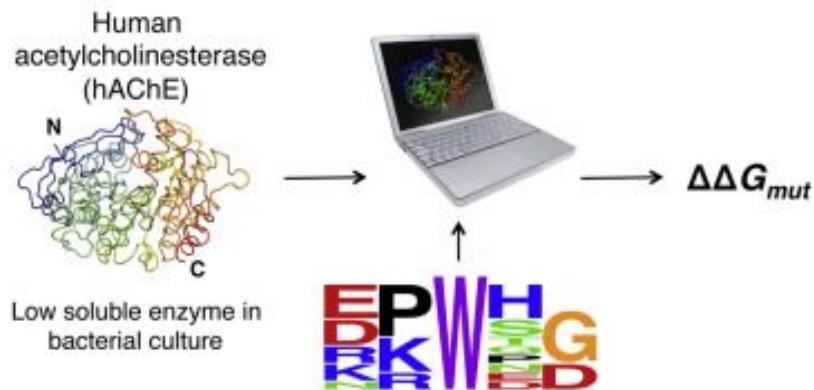
Possible search space is extremely large =>

Computational methods can be used to
speed up the rational design process

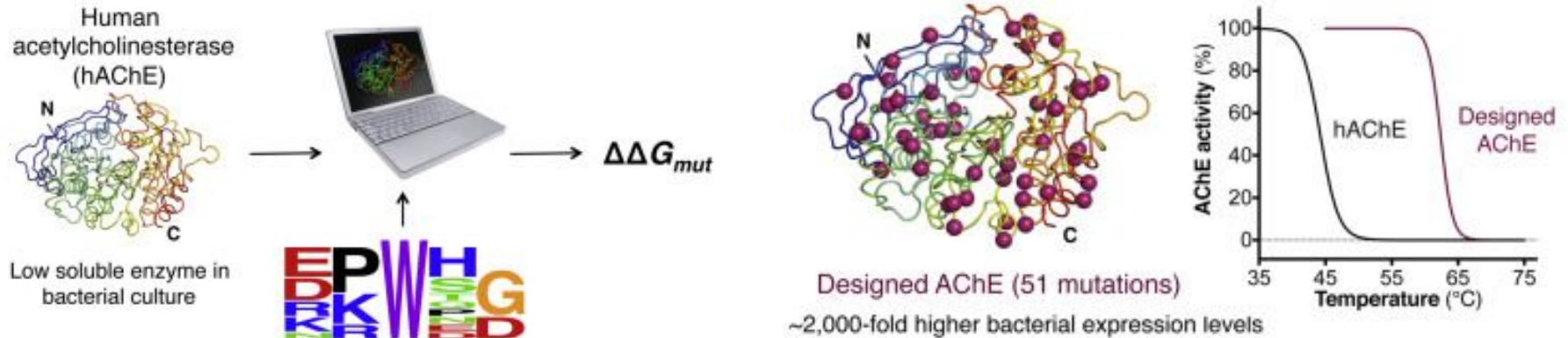
Automated search can be used to fill in core cavities and enhance stability



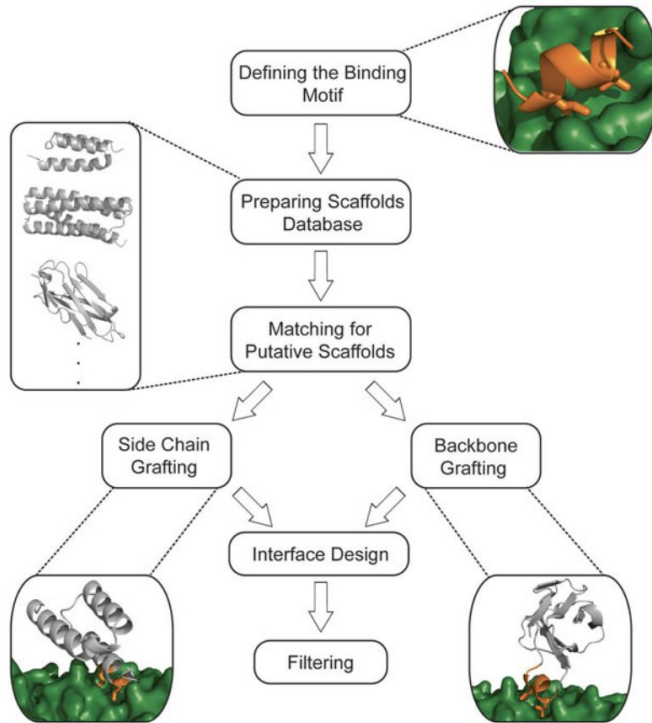
MSA information can be automatically used to find stabilizing mutations



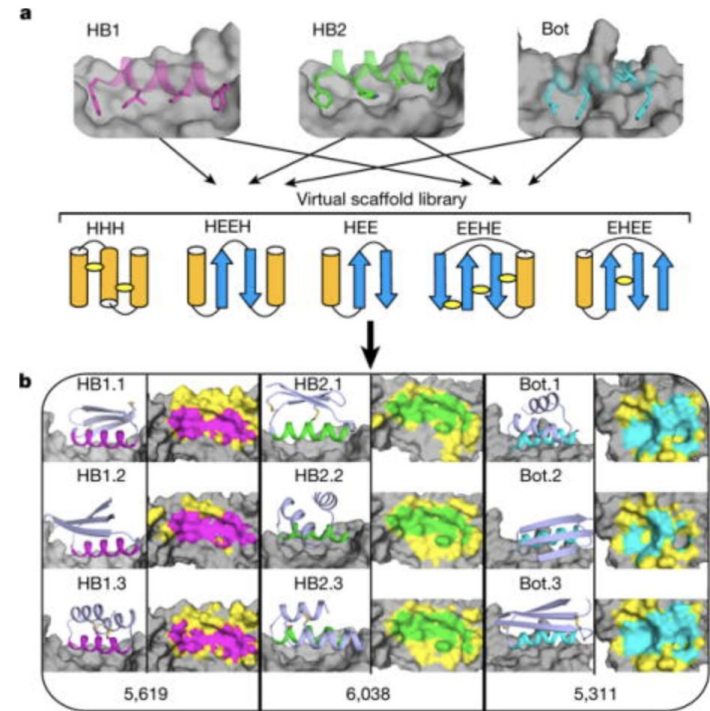
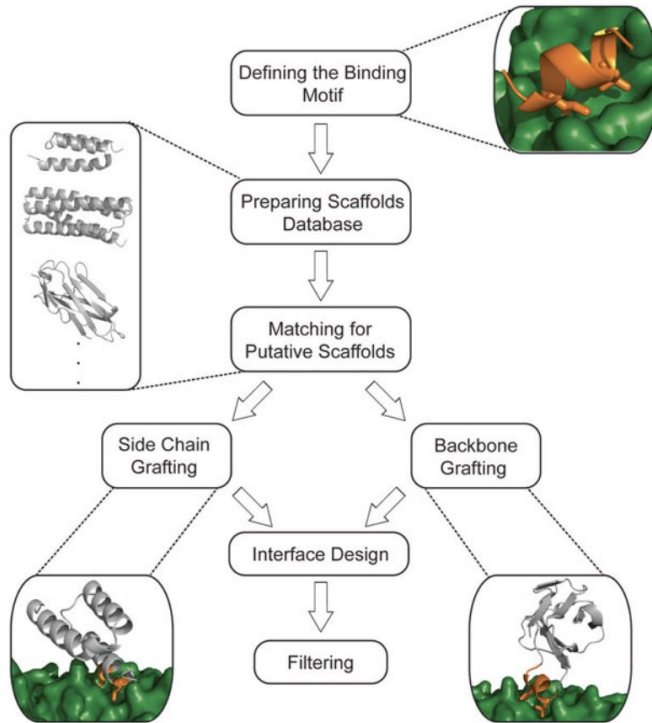
MSA information can be automatically used to find stabilizing mutations



Secondary structure motif grafting can be fully automated (Rosetta motif graft)

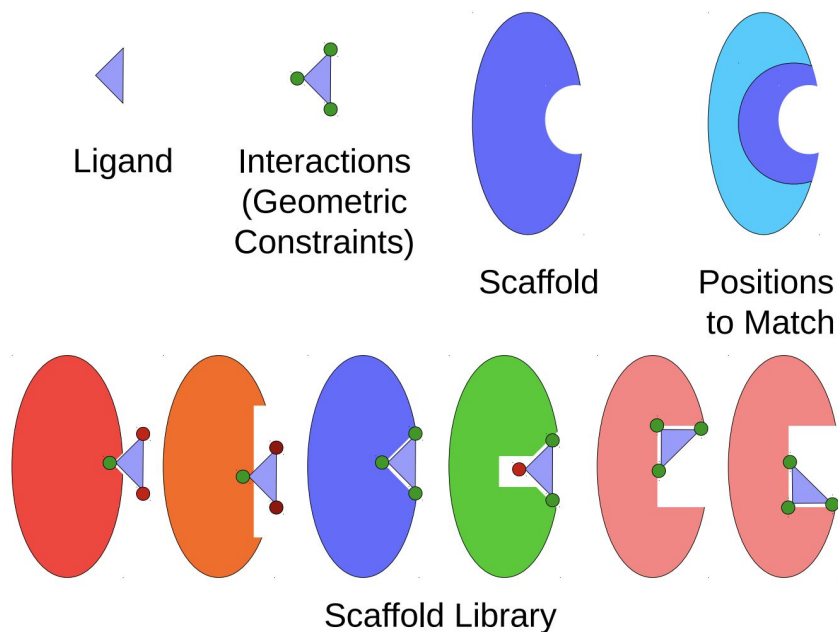


Secondary structure motif grafting can be fully automated (Rosetta motif graft)



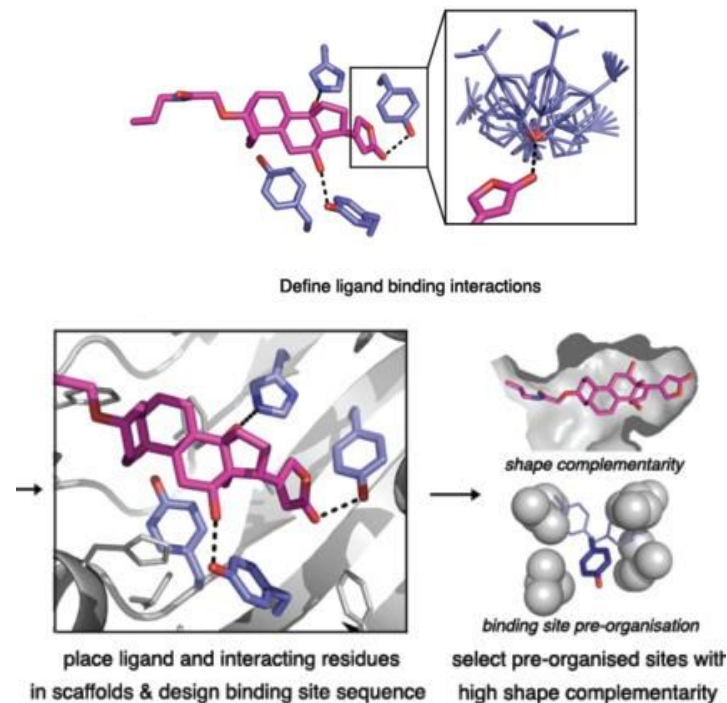
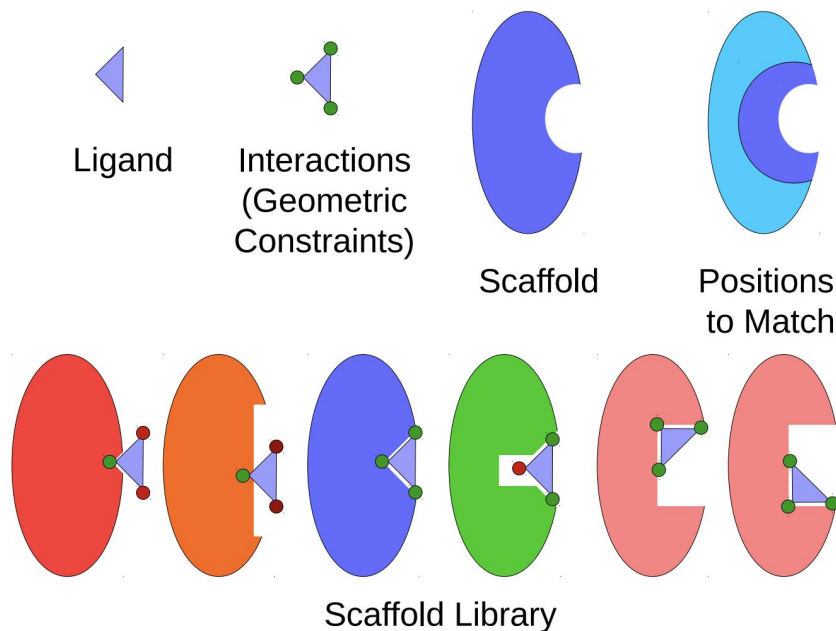
Interacting residues required for binding can also be placed in new scaffolds in an automated way

Rosetta matcher



Interacting residues required for binding can also be placed in new scaffolds in an automated way

Rosetta matcher



For the next lecture:

1. Proposal draft: Due next week (discussion in class)
2. First journal: Will be discussed next week
3. Post-class assignment for this lecture: Rosetta-guided SSM
 - a. Due next week

Next lecture:

A designed heme-[4Fe-4S] metalloenzyme

