Mutating Proteins in the Computer

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What is a Protein?

* large biomolecules made of amino acids (AAs)

(image: www)

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* amino acid consists of backbone and an 'R' group (residue)

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What is a Protein?

* large biomolecules made of amino acids (AAs)
* amino acid consists of backbone and an 'R' group (residue)
* 20 different AAs
* proteins are ~25 to more than 2000 AAs long

(image: www)

Levels of Protein Structure

* The AA-sequence (primary structure)

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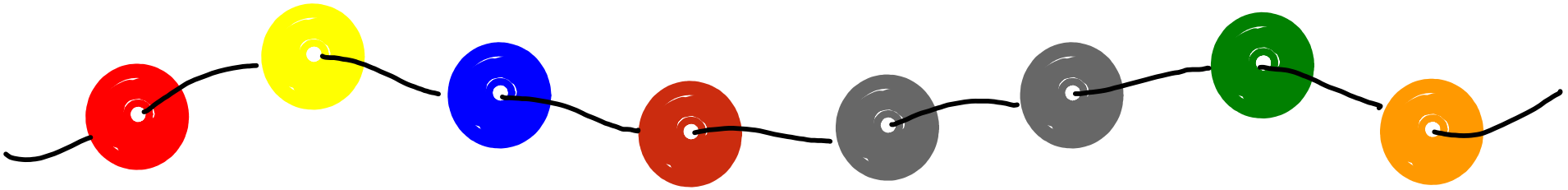
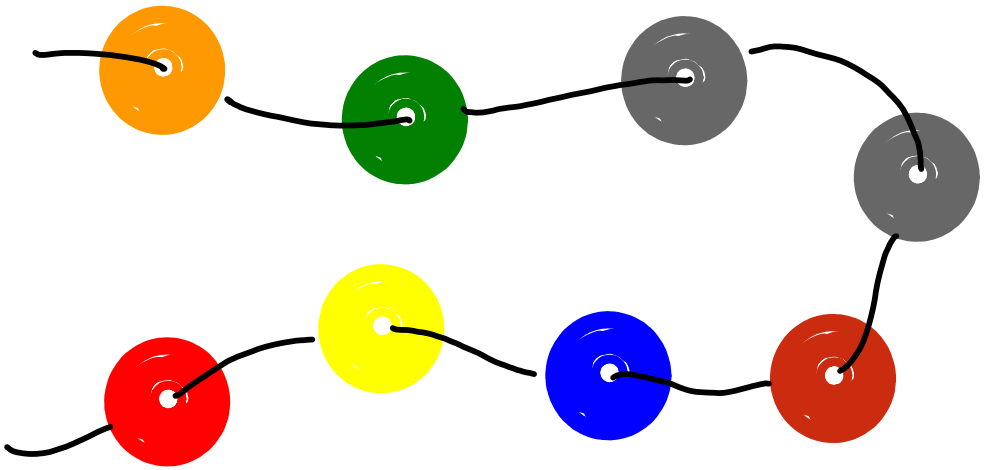
* Model - beads in a open necklace
  color of bead $\Leftrightarrow$ AA identity
  string $\Leftrightarrow$ backbone



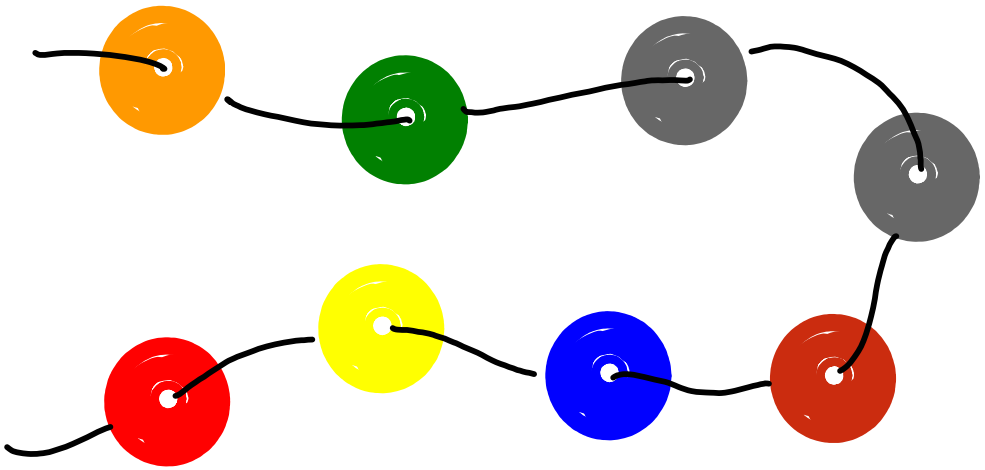
Secondary Structure

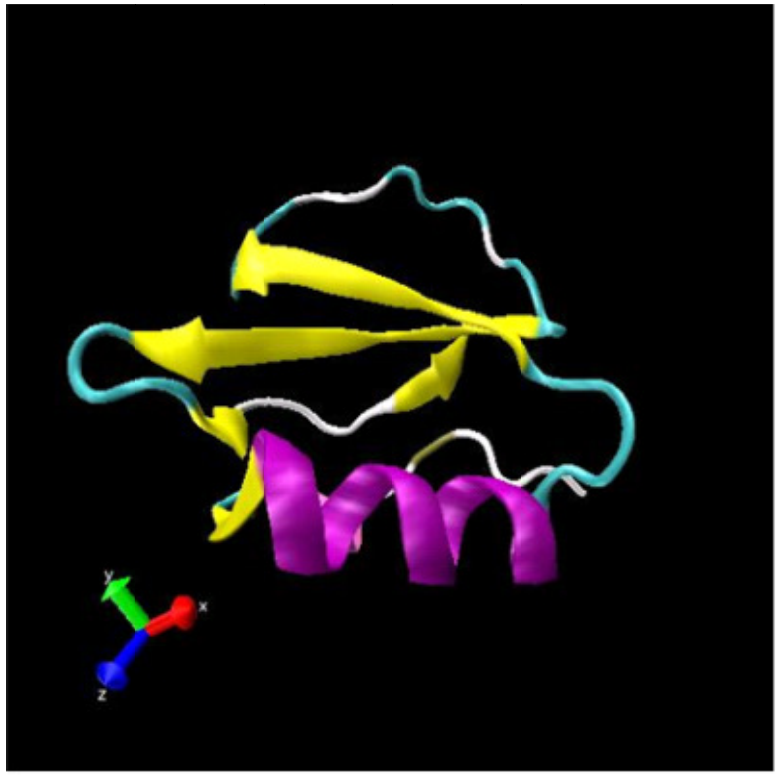
* folding of open necklace into 3D structural units



Secondary Structure

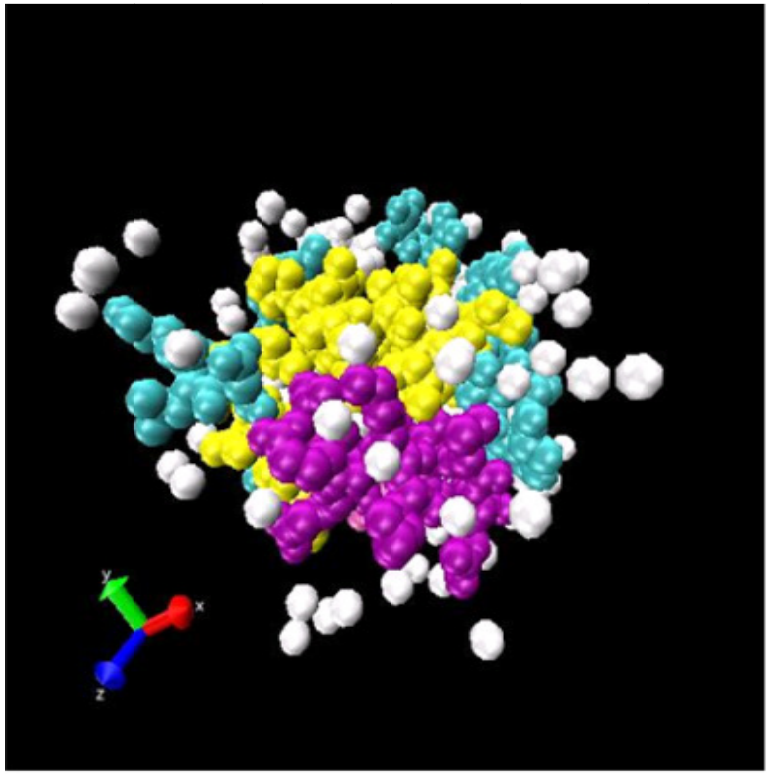
* folding of open necklace into 3D structural units
* alpha helices, beta sheets, and random coils





Tertiary Structure

* α-helices, β-sheets, and coils coming together



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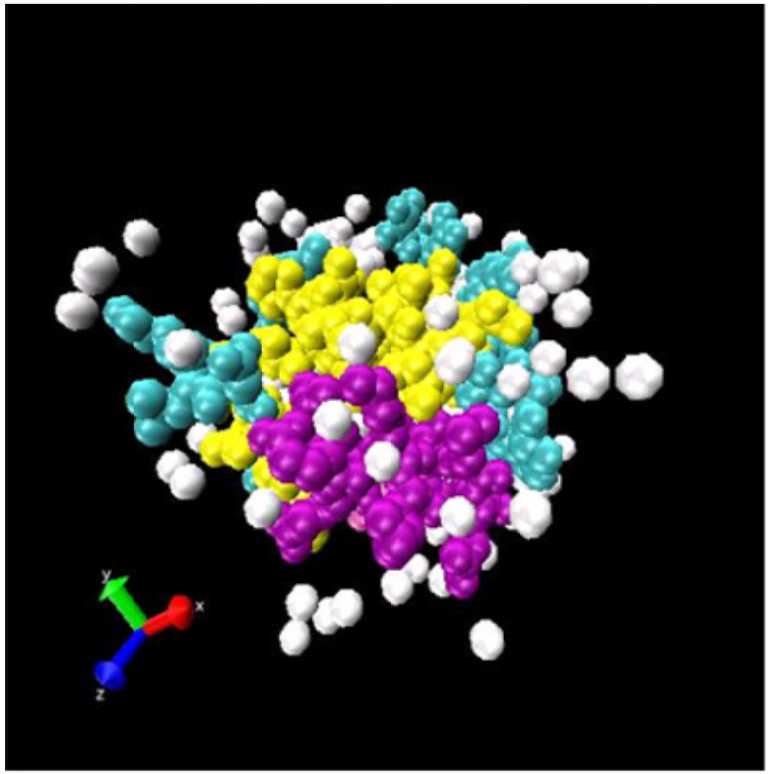

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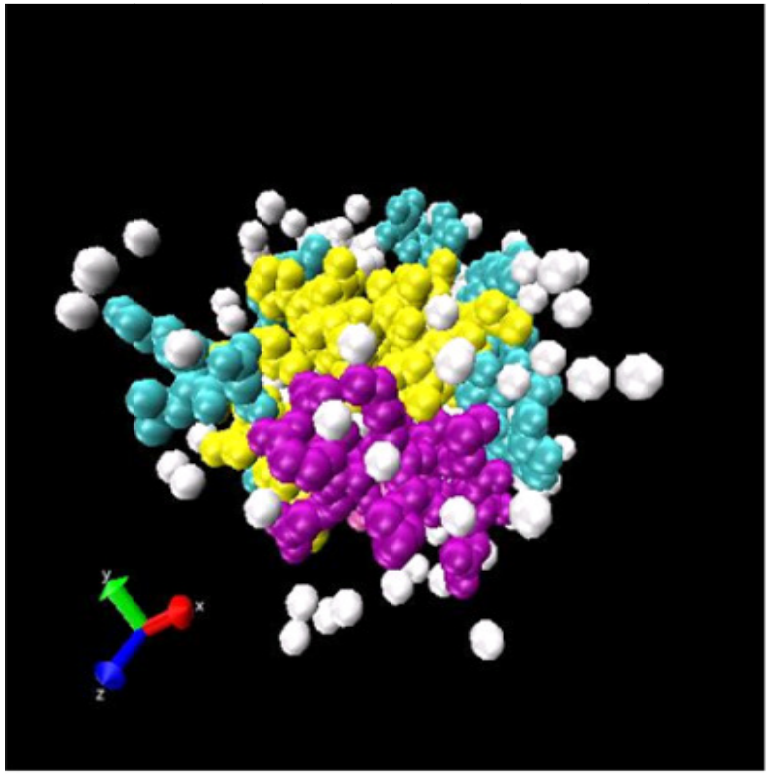

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* minimum energy conformation
* sequence $\Rightarrow$ structure $\Rightarrow$ function

Protein folding problem

Mutagenesis

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* want to make the protein more or less stable, reactive, soluble, etc.

* standard technique used in drug design, protein engineering, ...

Mutagenesis in the Lab

Lab Rule #1: If an experiment works, something's wrong ;)

(image: www)

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* long, hands-on process
* DNA is "spliced out" and replaced

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? Can we **predict** the effects of mutations computationally ?

(image: www)

Results

✓ stability mutagenesis
- four-body scoring function

✓ solubility mutagenesis
- three-body scoring function optimized using linear programming

✓ temperature-sensitive (Ts) mutants
- 133 features tested:
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  - mutual information of Ts mutants

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* Bioinformatics, 2007; with C. Deutsch
* predict whether stability increases or decreases after mutation(s)

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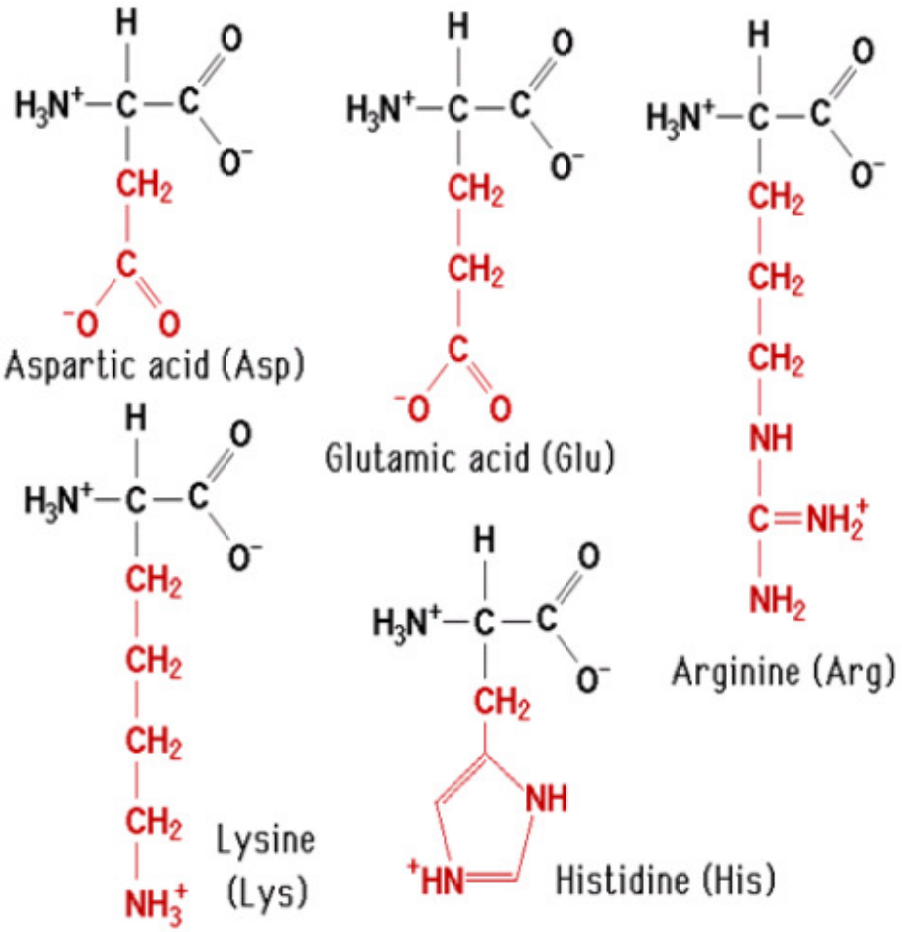
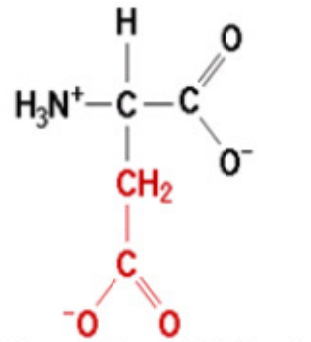
* scoring function – knowledge-based method
* probabilities of how often do groups of AAs appear close-by in proteins
* need (simpler) representation of protein structure

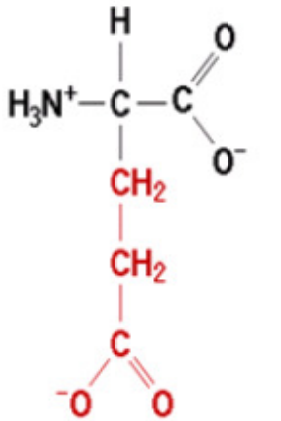
Representing the Amino Acids

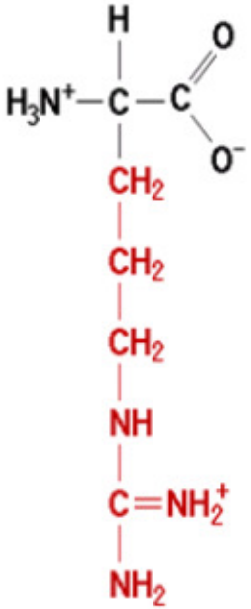


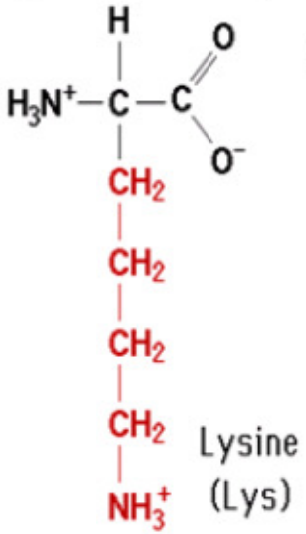
* 'R' group varies
* alpha C (C_α) is the backbone carbon atom
* beta C (C_β) is the first carbon atom in "R"

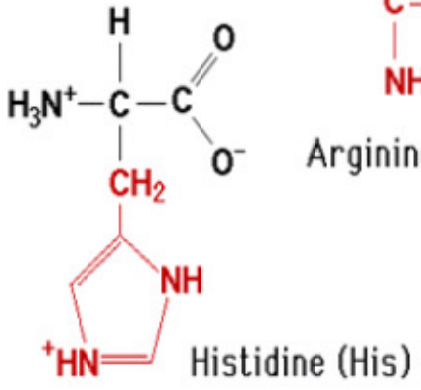
Representing the Amino Acids











* 'R' group varies
* alpha C (C_α) is the backbone carbon atom
* beta C (C_β) is the first carbon atom in "R"
* side chain center — centroid of all atoms in 'R' (including C_α)

Two-Body Potentials

* previous scoring functions counted how many times pairs of AAs are "close to each other"
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$\Rightarrow$ 7.99 A°: neighbors; 8.01 A°: not neighbors!

Voronoi/Delaunay Tessellation

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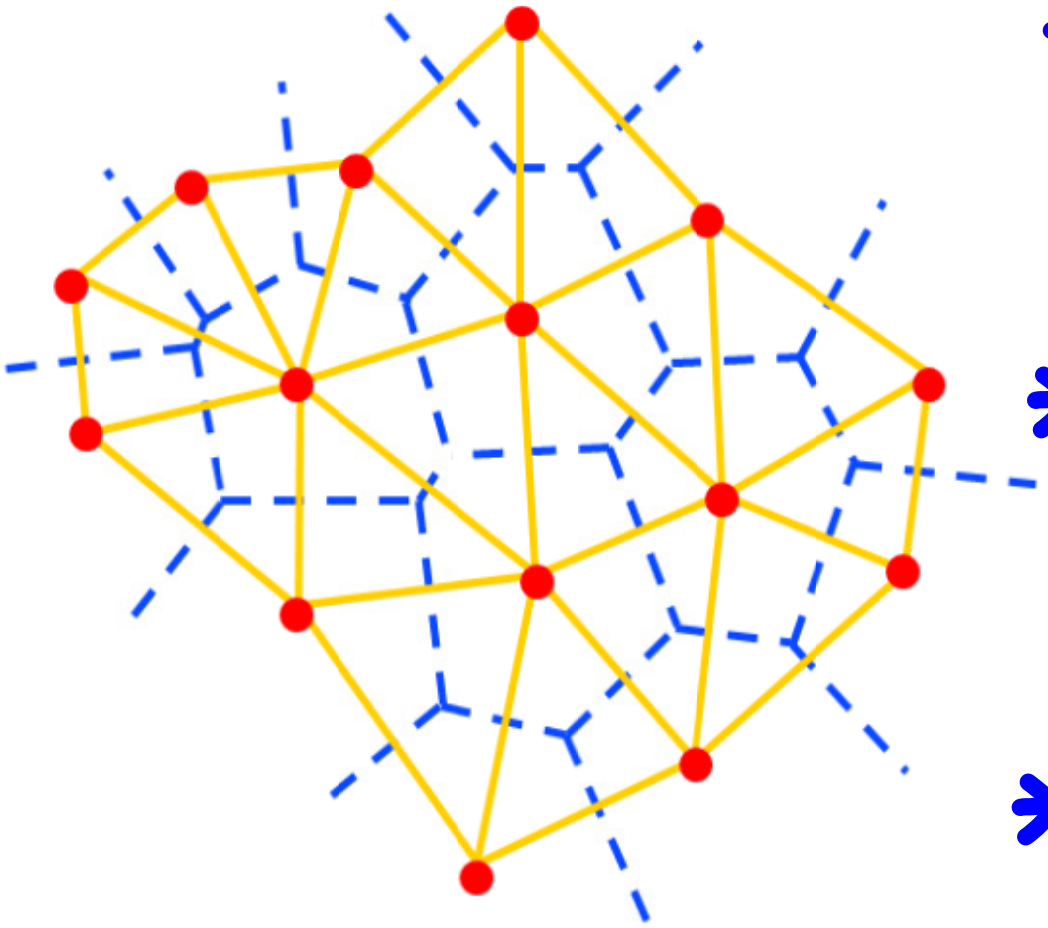
Voronoi/Delaunay Tessellation



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* Get tetrahedra in 3D

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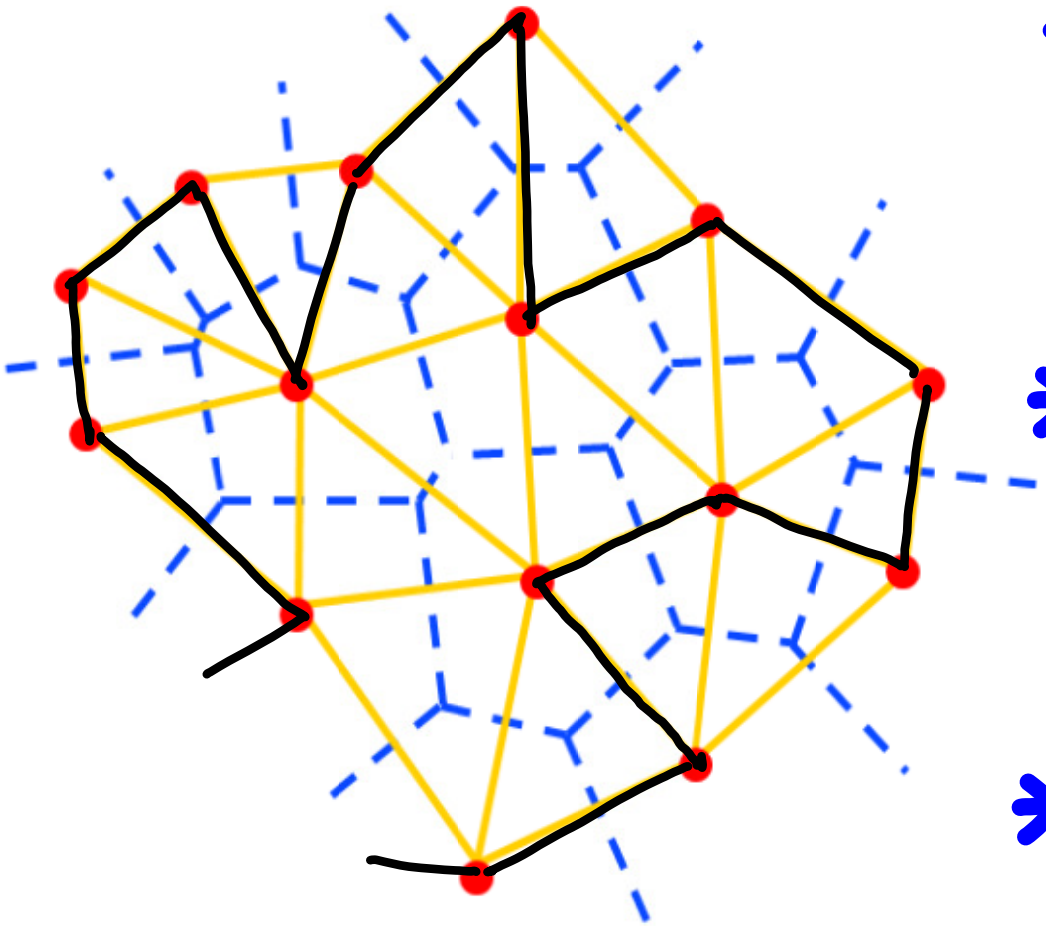

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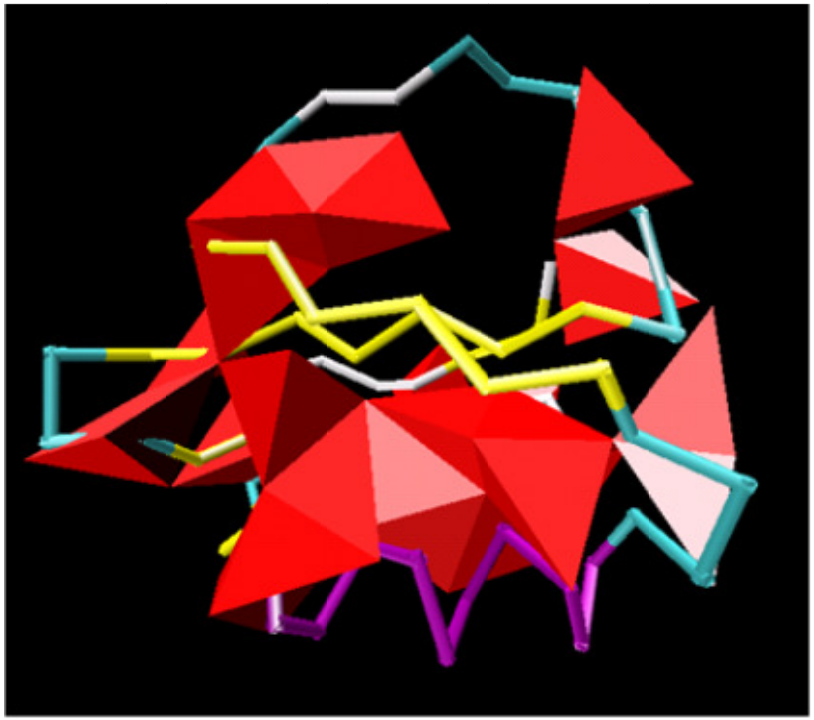
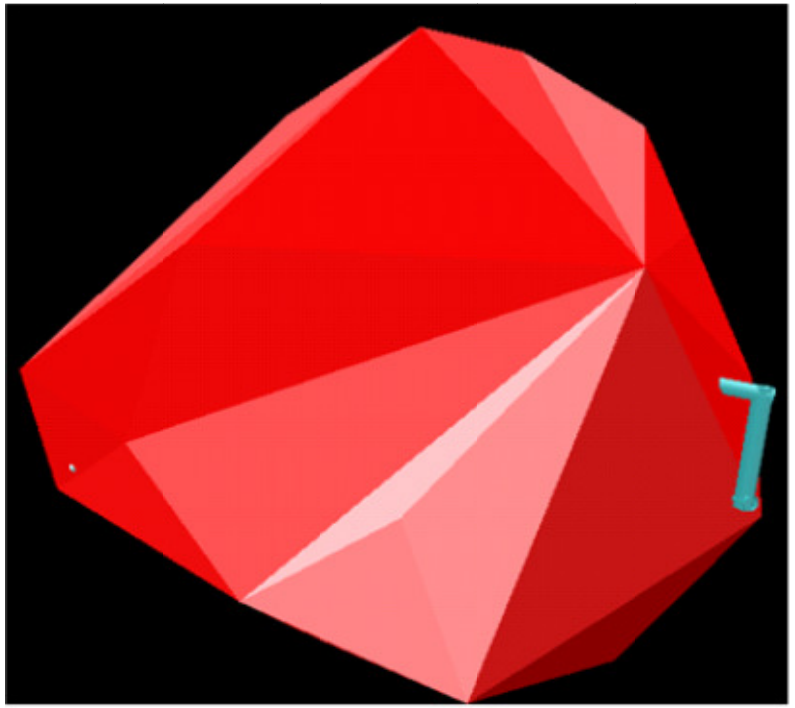
Delaunay Tessellation of Proteins

* represent each AA by a point at the side chain center
* discard biologically irrelevant tetrahedra (>12 Å°)





Types of Tetrahedra

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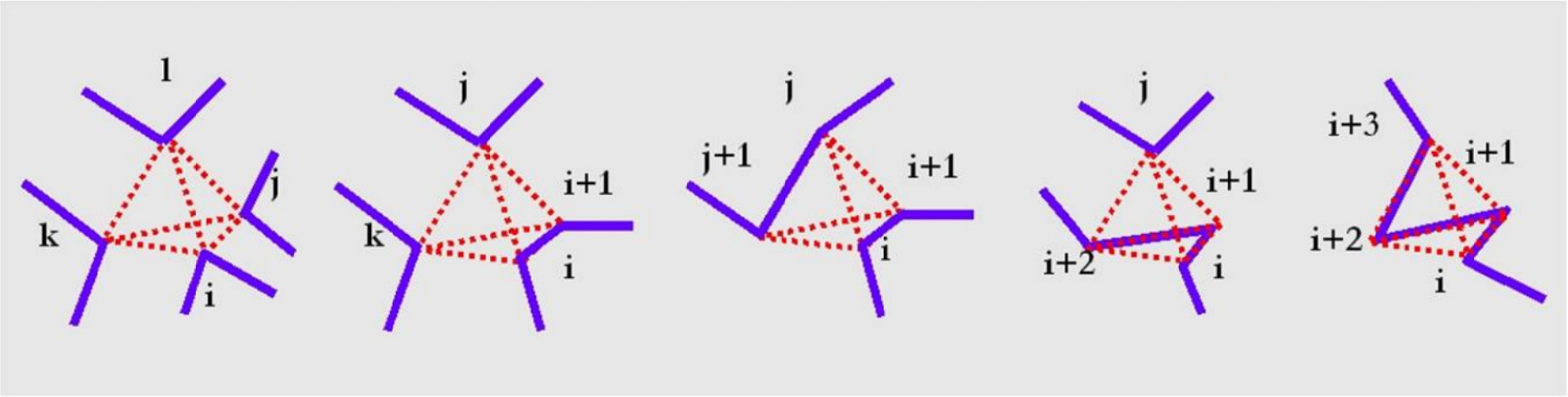
* 5 types of tets – based on how many AAs in the tet are connected



Score of a Tetrahedron

$$q^t_{ijkl} = \log\left(\frac{f^t_{ijkl}}{p^t_{ijkl}}\right)$$

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$$p^t_{ijkl} = C\, a_i a_j a_k a_l P_t$$

a_i = frequency of AA i

P_t = frequency of tet type t

C = combinatorial factor

Mutagenesis Score

* total score of protein $S = \sum_{\text{all tets}} q^{t}_{ijkl}$
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* $\Delta > 0 \iff$ increase in stability

A Dataset of Mutants

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* databases (e.g., ProTherm) and previous studies (e.g., Chang et al., 2006) have collections of single-point mutations handled in the same way by varying function
* comprehensive literature search to assemble database of single- and multi-point mutations from 24 papers

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* FOLD-X (Guerois et al., 2002) has an accuracy of 68% on our dataset

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Noteworthy:

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✓ more accurate for multi-point mutants

Solubility Mutagenesis

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* predominantly surface property
* solvent accessible surface area
  — measures exposure to solvent
* correlate propensities of groups of AAs to be on/near surface with solubility
* use the framework of Delaunay tessellation (DT)

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Three Body Contacts

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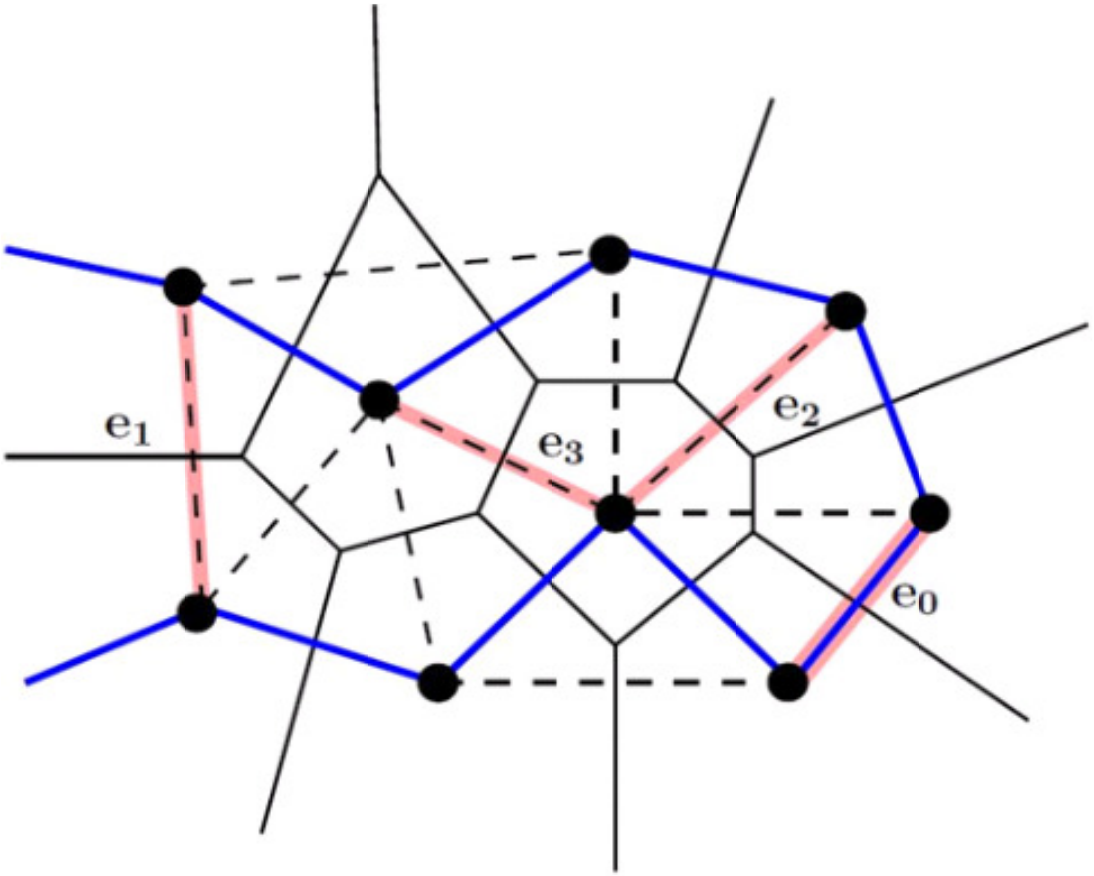
* 3-body log-likelihoods
* buriedness defined in the DT
  - combinatorial definition
  - combines sequence info (AA types)

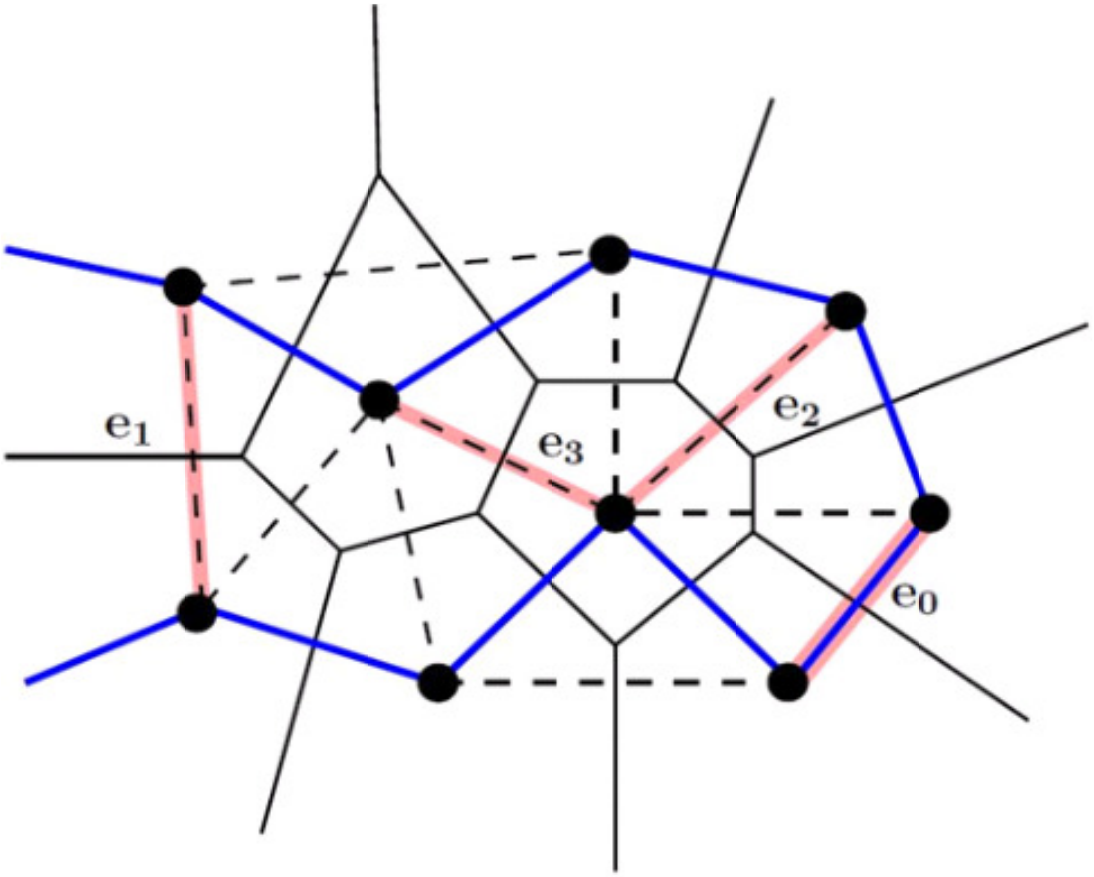
Delaunay Buriedness

* edge is buried if it is part of two triangles

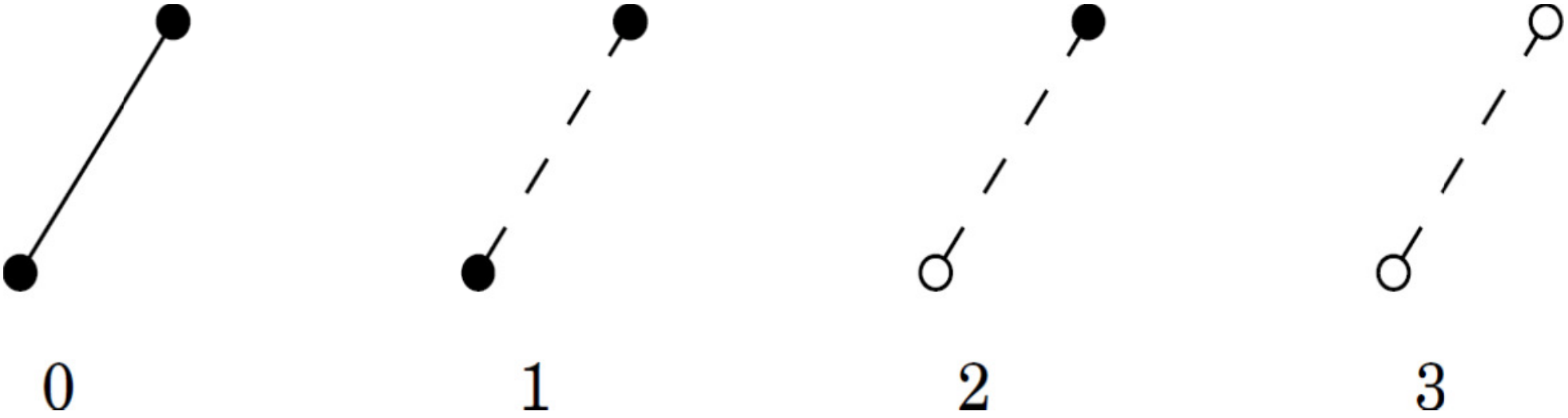


Delaunay Buriedness

* edge (triangle) is buried if it is part of two triangles (tetrahedra)

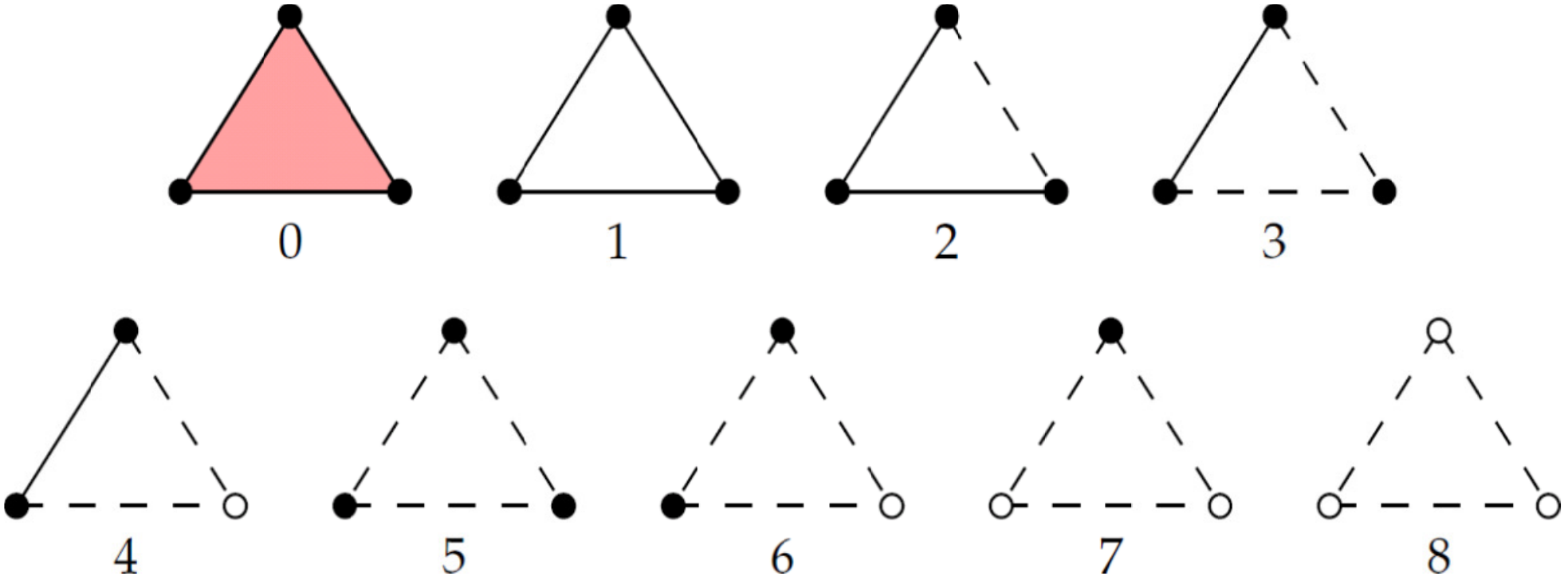


Edge Buriedness – 4 Levels



* varies from most non-buried (0), i.e., on the surface, to most buried (3)

Triplet Buriedness – 9 Levels



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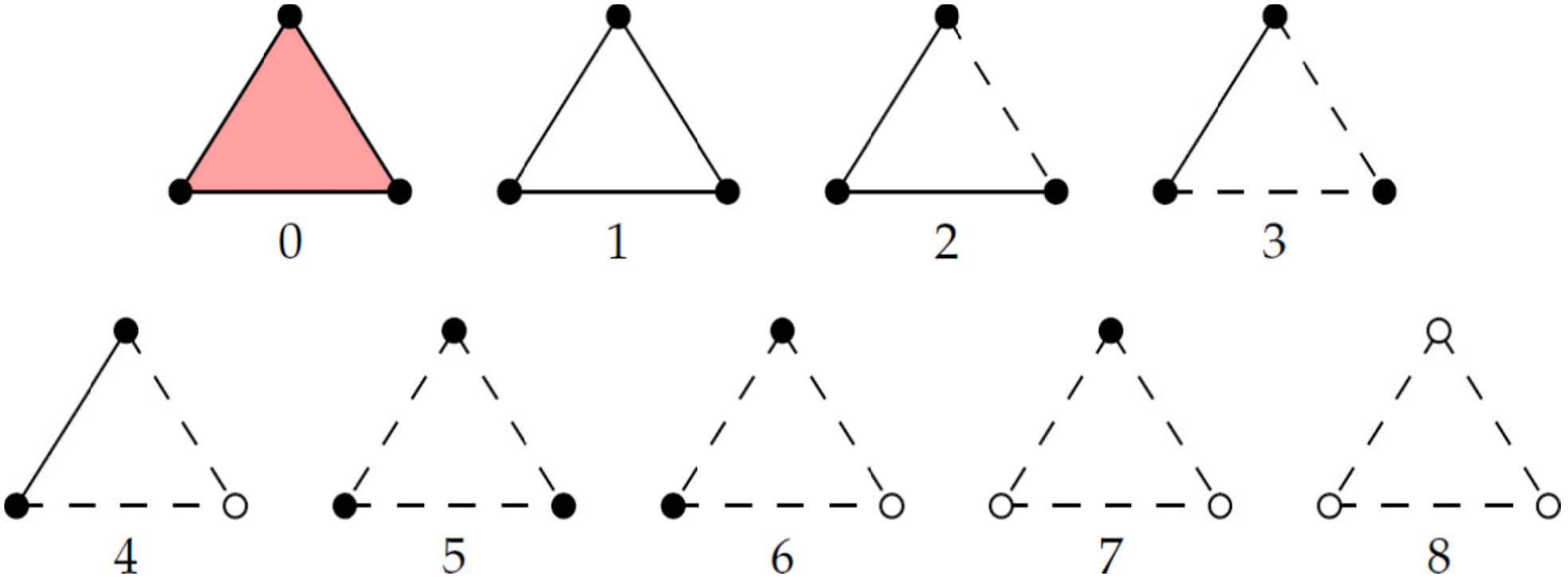
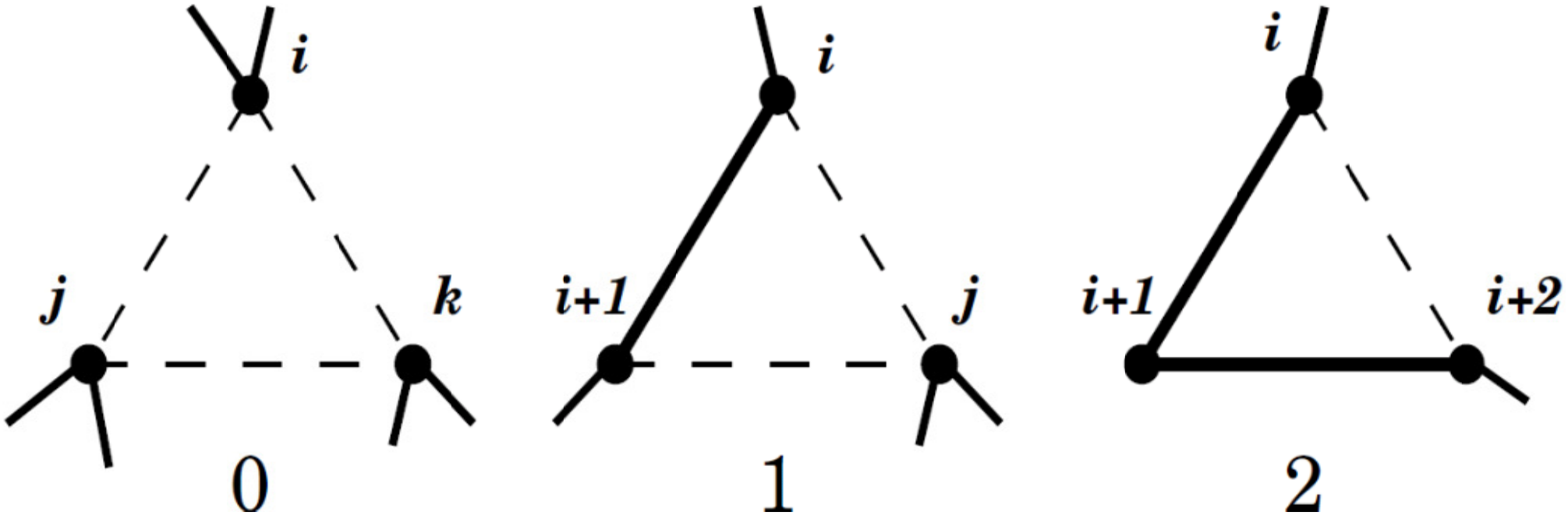


* from most non-buried (0) to most buried (8)
* use levels 0-4 (non-buried) for solubility mutagenesis

Triplet Connectivity - 3 Classes



3-Body Scoring Function

* score of a triplet with AAs $\{i,j,k\}$, connectivity class c(0-2), buriedness class b(0-4)

$$q_{ijk}^{cb} = \log\left(\frac{f_{ijk}^{cb}}{p_{ijk}^{cb}}\right)$$

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  predict whether protein is soluble using only sequence info
* our dataset is for increase/decrease of solubility

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$$
\begin{array}{lrcl}
\max & \mu & & \\
\text{s.t.} & \sum_{t\in M_i} w_t Q_t - \sum_{t\in W_i} w_t Q_t & \geq & 1+\varepsilon_i, \forall i \in I; \\
& \sum_{t\in M_i} w_t Q_t - \sum_{t\in W_i} w_t Q_t & \leq & -1-\varepsilon_i, \forall i \in D; \\
& \mu & \leq & \varepsilon_i, \forall i \in I, D; \\
0 \leq & w_t & \leq & 2, \forall t.
\end{array}
$$

← mutants seeing increase & decrease in solubility

M_i, W_i – triplets seeing changes in mutant and WT

Solubility Mutagenesis – Results

* leave one out cross validation (LOOCV)

Measure	LP	SVM	Lasso
Accuracy	0.810	0.708	0.701
MCC	0.617	0.405	0.423
Precision (class I)	0.762	0.661	0.909
Precision (class D)	0.851	0.735	0.661

* 10-fold CV

Measure	LP	SVM	Lasso
Accuracy	0.766	0.752	0.708
MCC	0.545	0.496	0.448
Precision (class I)	0.719	0.705	0.952
Precision (class D)	0.822	0.790	0.664

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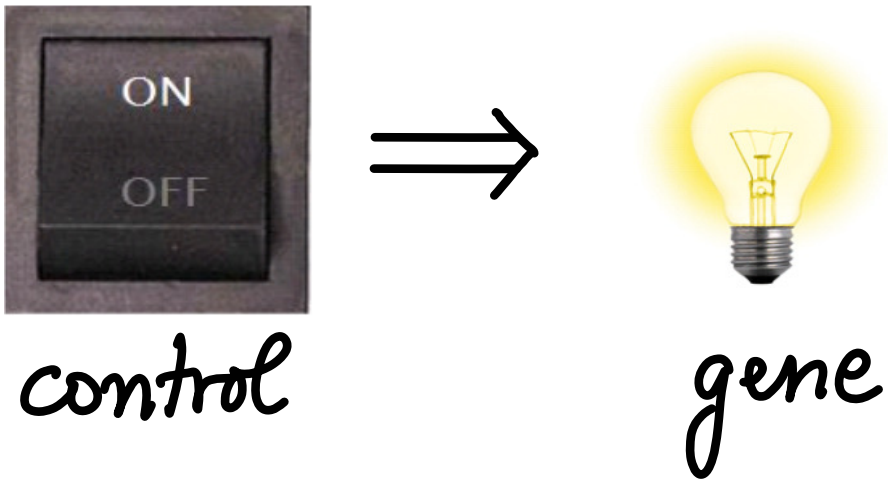
* scoring function most accurate for surface mutations
* DT-based scoring functions can predict stability and solubility mutagenesis simultaneously

Temperature-Sensitive (Ts) Mutants

* with P. Ye (Molecular Biosciences) and S. Lockwood (EECS)

Temperature-Sensitive (Ts) Mutants

* with P. Ye (Molecular Biosciences) and S. Lockwood (EECS)
* control of gene expression



Temperature-Sensitive (Ts) Mutants

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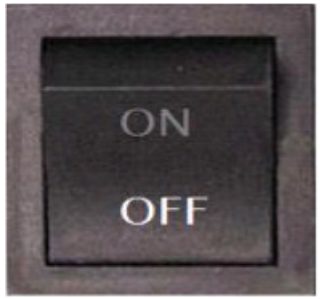

control ⟹ gene

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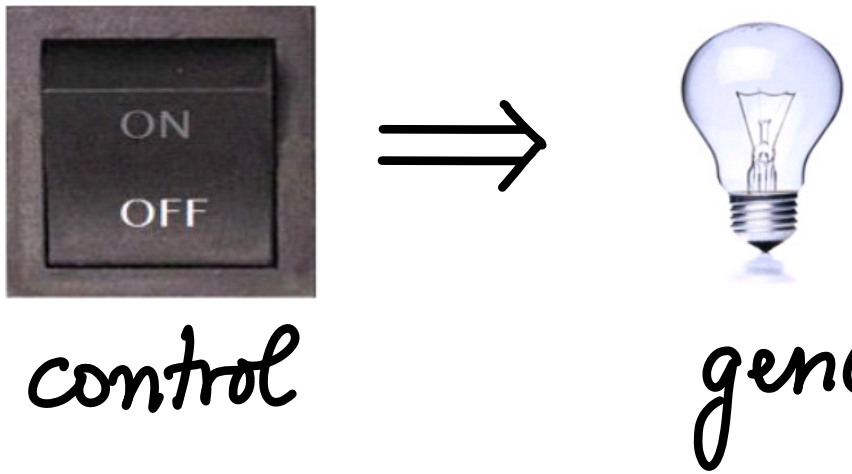
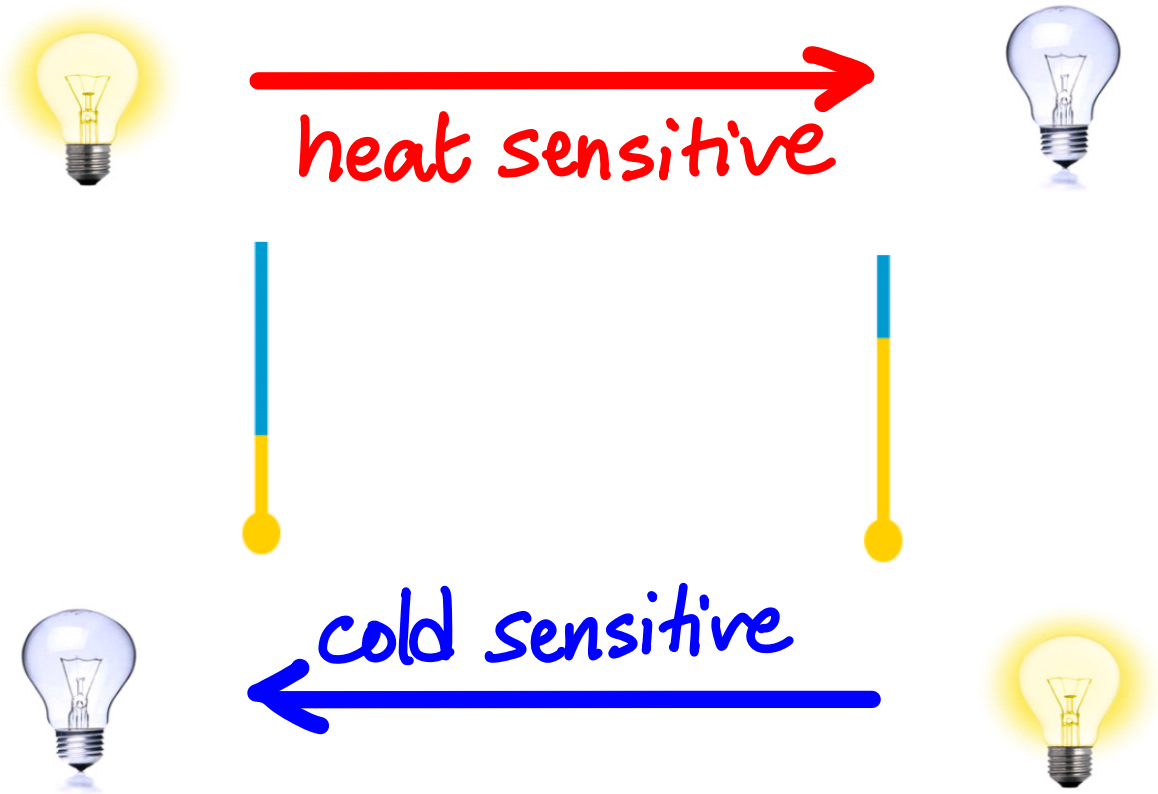


* useful for essential gene studies

Ts Mutants

Ts Mutants



Ts Mutants

Ts Mutants

* Explain mechanism of Ts mutants computationally ?

Descriptive Framework

* logistic regression $y = \frac{1}{1+e^{-z}}$ where $z \to \sum_{j=1}^{n} \beta_j x_j$
  - predict 0/1 response
  - variables need not be independent
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* 10-fold CV
* dataset available : 6231 mutants
  - 747 (12%) are Ts mutants

133 Features Studied

Structure

86

sequence

47

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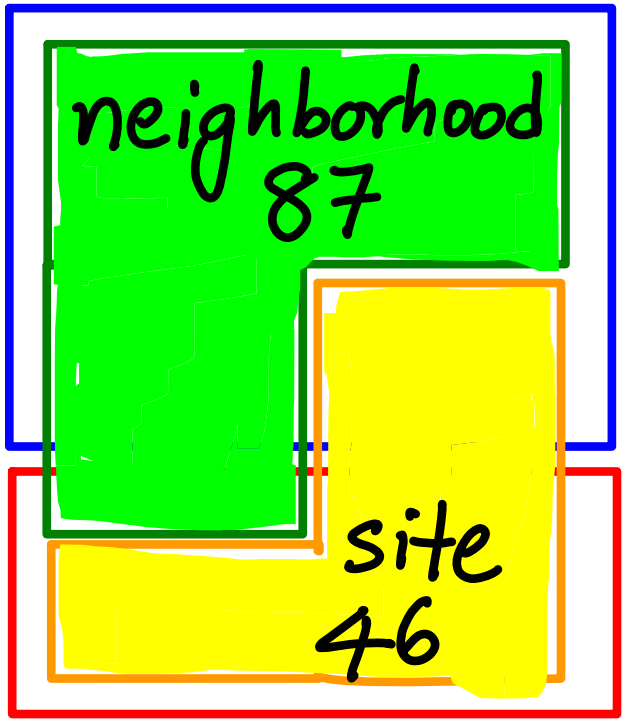
Structure



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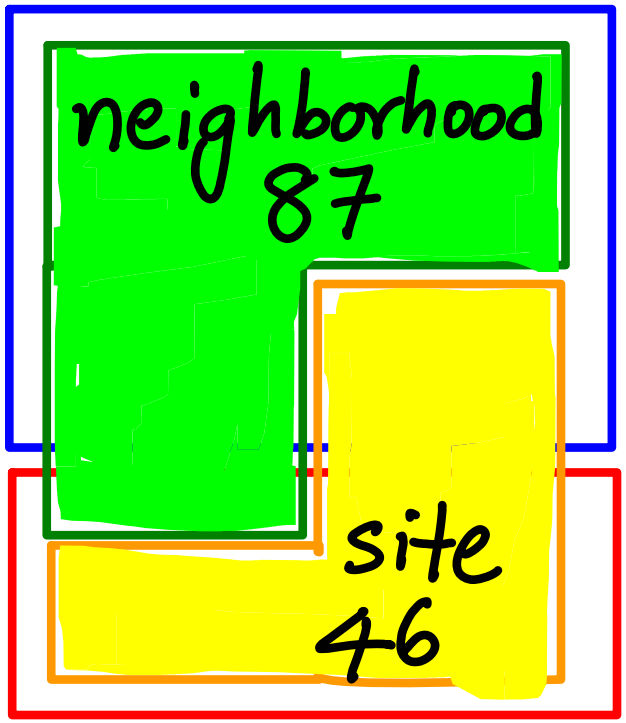

structure 86

sequence 47

* neighborhood further divided into sequence, Euclidean, and Delaunay

Mechanism of Ts Mutants

* evaluate predictive power of each feature individually

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* evaluate predictive power of each feature individually
* most predictive features explain Ts mutation mechanism
* Can build predictive models using subsets of features, e.g., sequence-only, site-only, neighborhood only, all, etc.

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traditional view – amino acid & position
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✓ 11 of top 20 most predictive features are neighborhood-based

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New view – neighborhood features are as (more) important as (than) site features in defining a mutation as Ts.

Prediction Models for Ts Mutants

Model name	ACC	MCC	AUC	KL	DD
Site features	0.78	0.39	0.87	0.17	0.48
Neighborhood features	0.82	0.46	0.91	0.10	0.68
Sequence neighborhood	0.79	0.37	0.84	0.11	0.48
Euclidean neighborhood	0.81	0.41	0.88	0.11	0.55
Delaunay neighborhood	0.78	0.39	0.86	0.15	0.52
All features	0.84	0.51	0.93	0.08	0.80
Sequence features	0.81	0.45	0.89	0.11	0.60
Structural features	0.83	0.49	0.92	0.09	0.75

Do Proteins Inspire New Mathematics?

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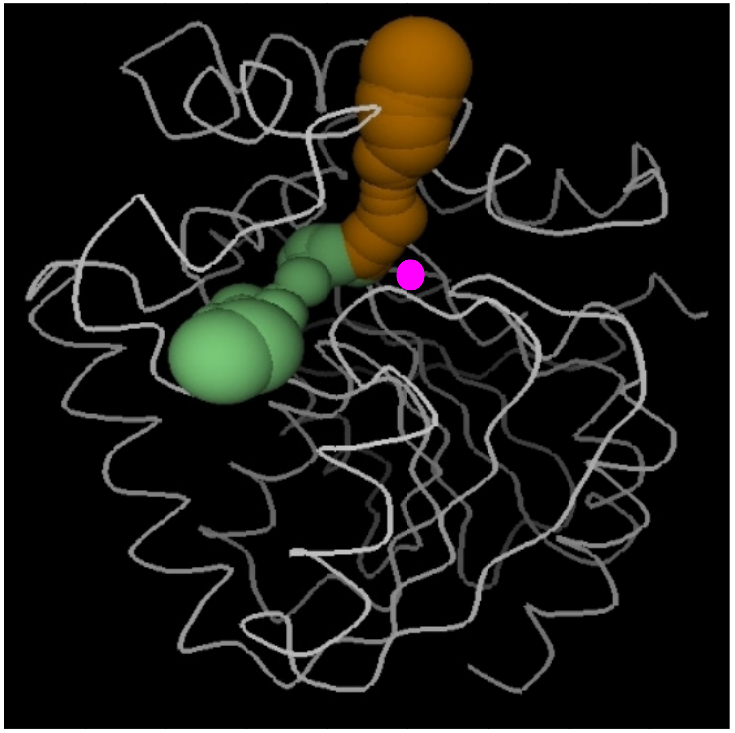
tunnels in proteins —
access to active site



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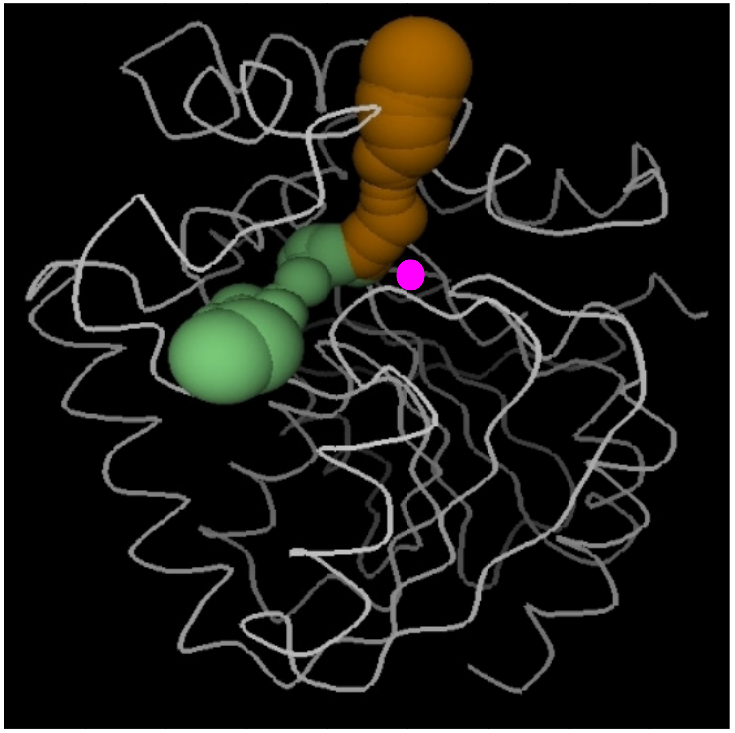

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Substrate can react with protein if the "narrowest neck" of tunnel is "big enough"

Proteins Do Inspire New Mathematics!

✓ finding bottlenecks in tunnels $\Longrightarrow$
optimal homologous chain problem (OHCP)

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? How to handle moving proteins? ⟹
inspires more fundamental questions!