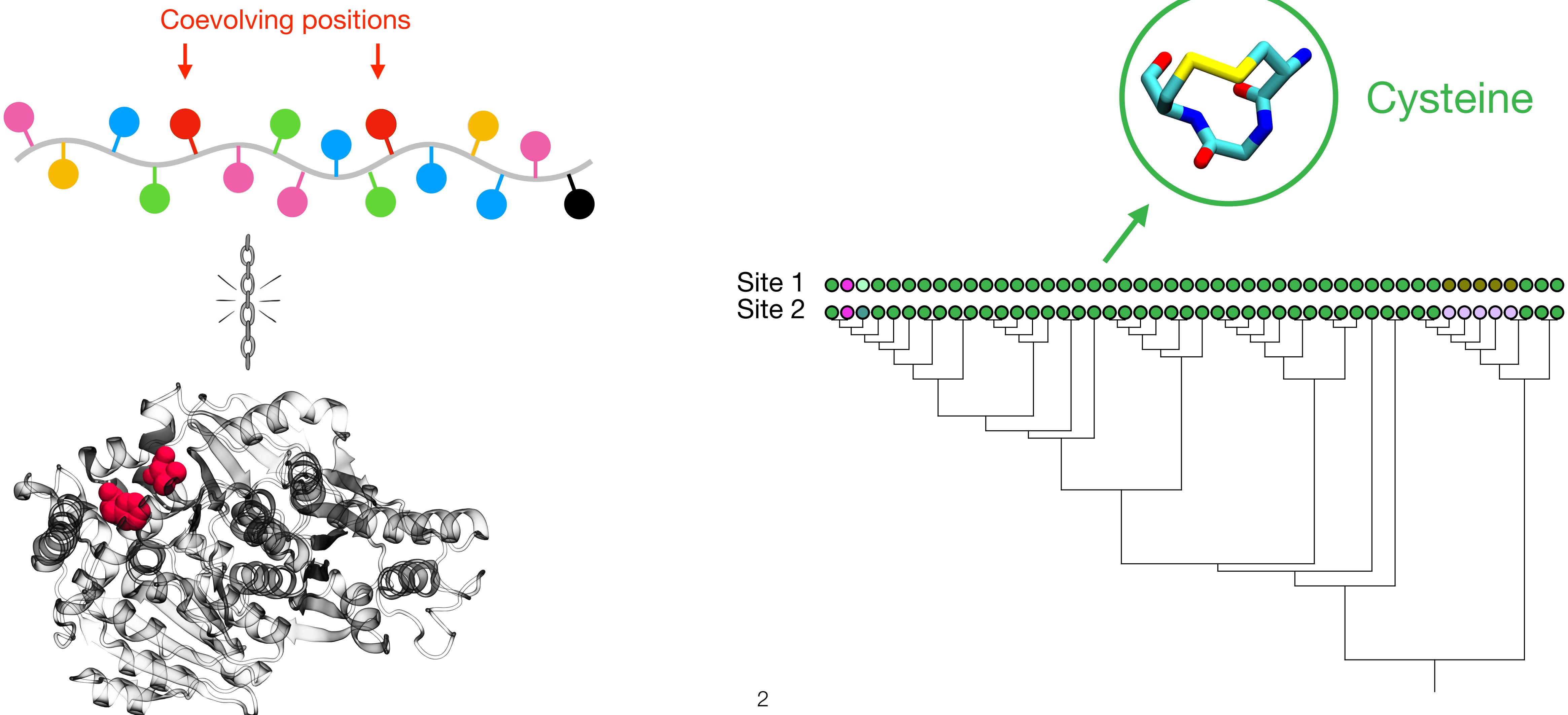


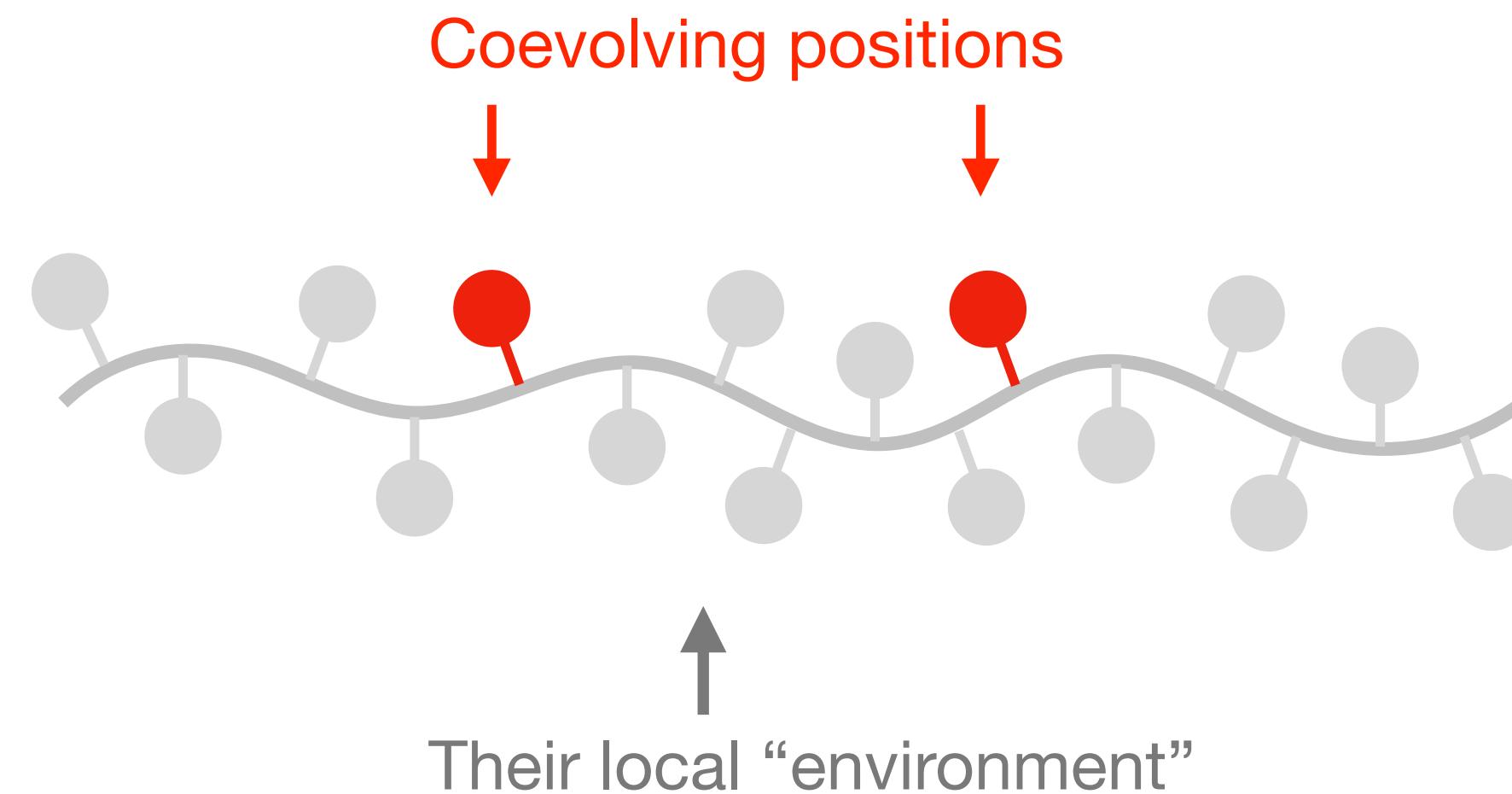
Elucidating Interaction Nodes Created by Hydrophobic Residues and Subsequences in Proteins

Connor Pitman
Center for Computational and Integrative Biology
Rutgers University – Camden

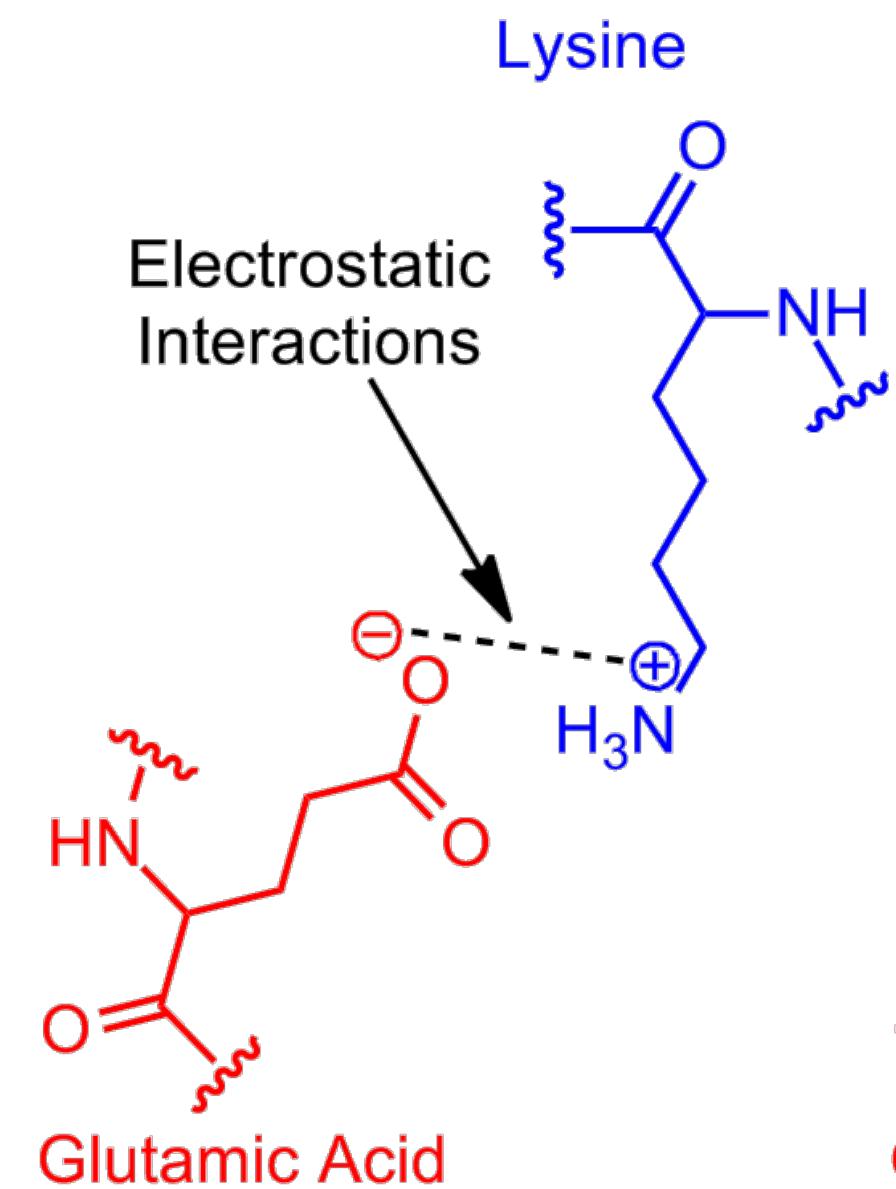
Protein evolution is constrained by intra-protein interactions



Coevolving pairs exist in the context of the residues around them

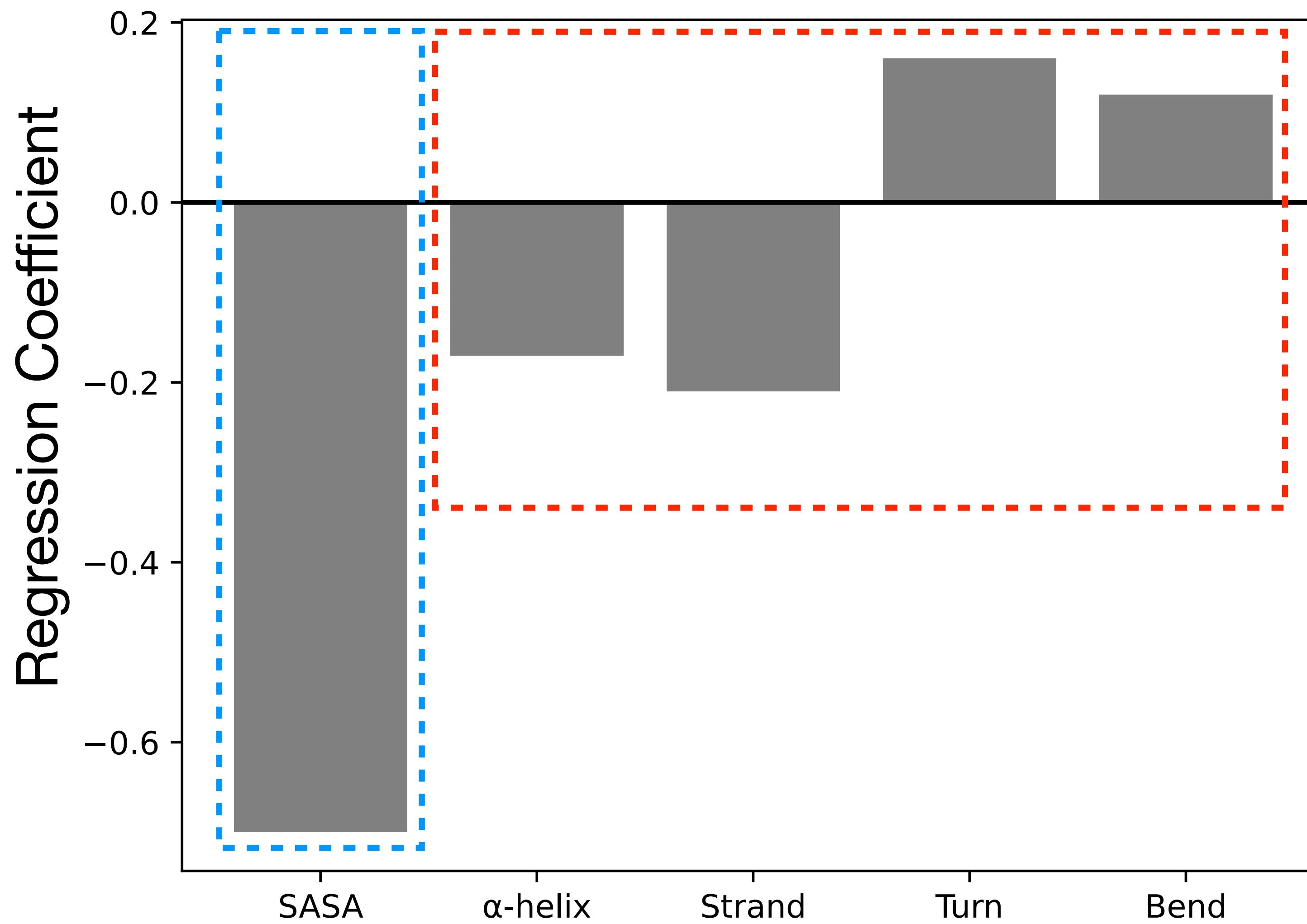


We have mechanistic hypotheses at the residue level

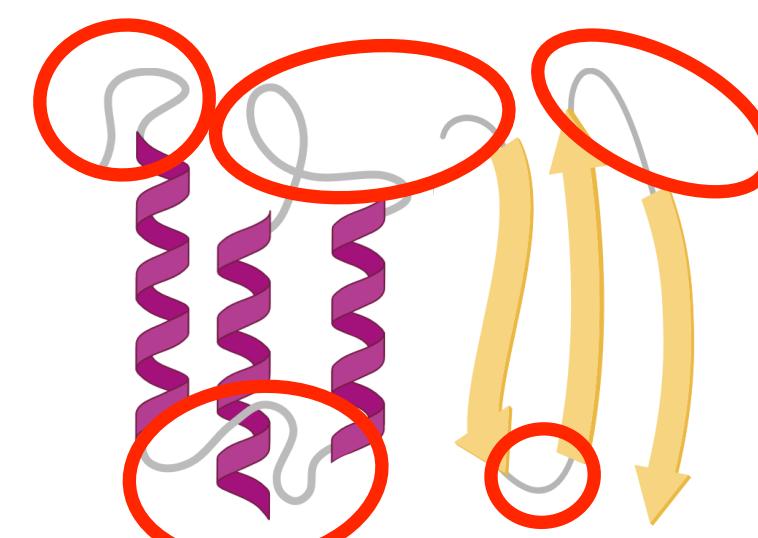


How do we incorporate the role of the protein “environment”?

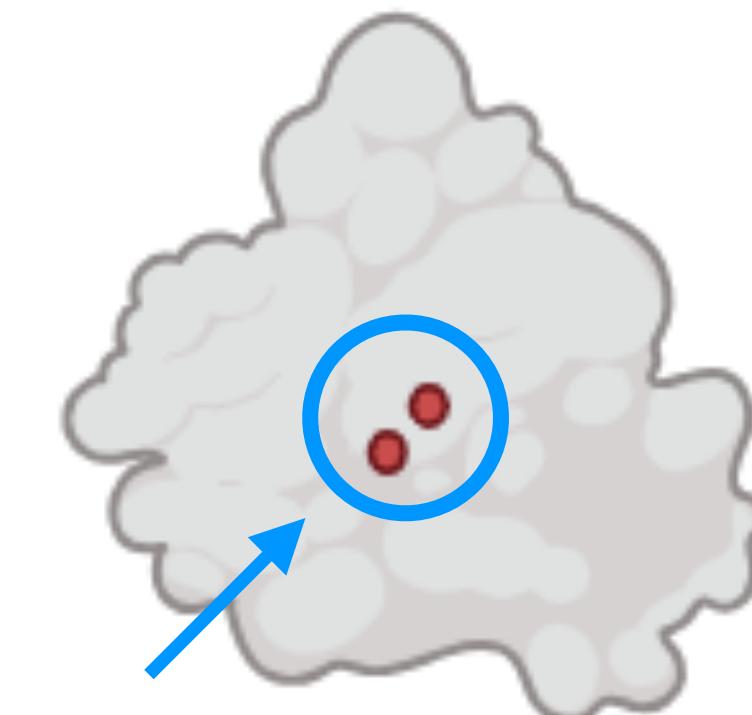
Coevolution is unassociated with secondary structure



Coevolving pairs tend to be found...

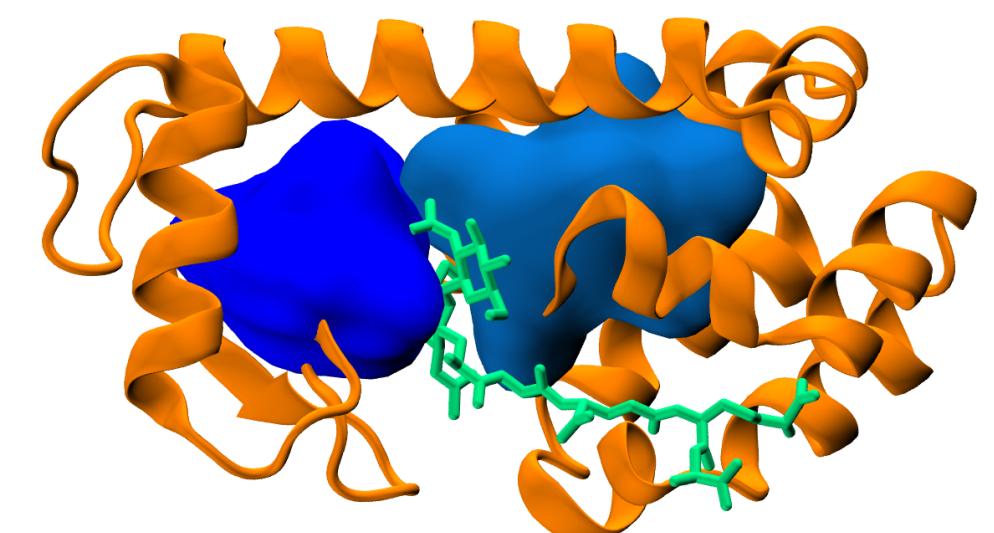
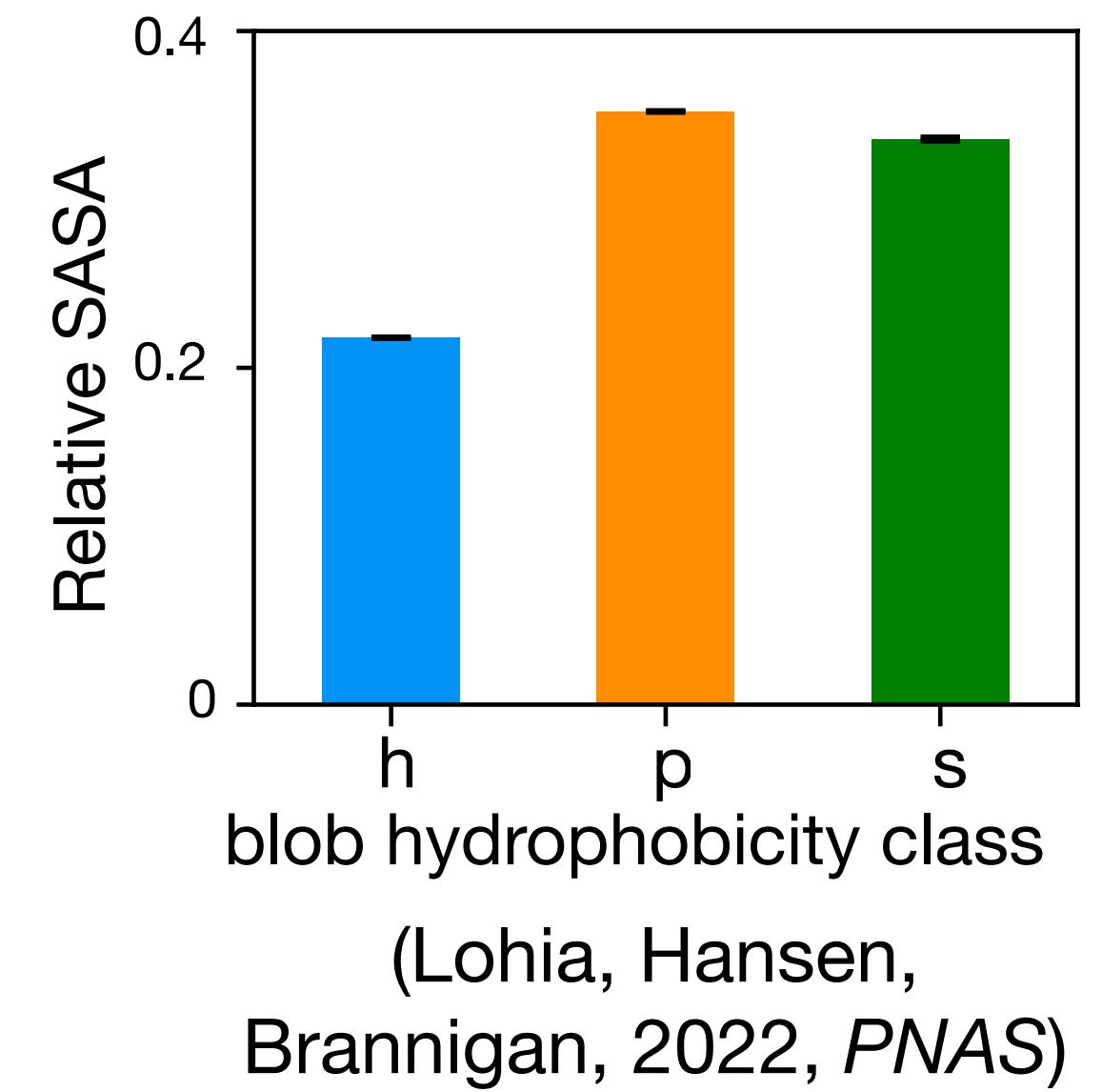
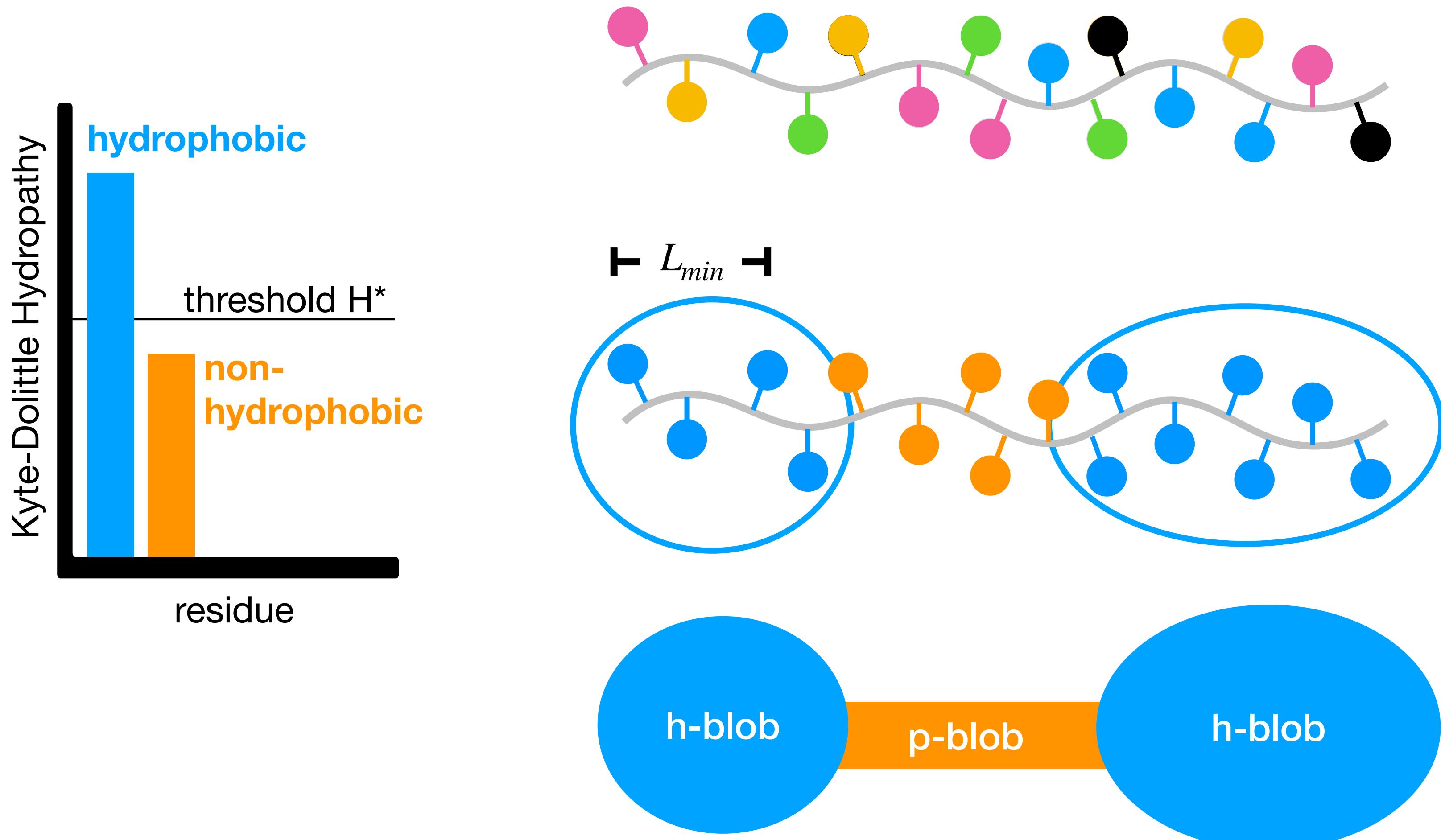


Outside of secondary structure elements



Near the hydrophobic core

Blobulation: Subsequences from contiguous hydrophobicity



(Pitman et al. 2024, *bioRxiv*)



blobulator™

hydrophobic modularity in protein sequences
Brannigan Lab • Rutgers University – Camden

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Protein Details: Alpha-synuclein

Uniprot Entry: SYUA_HUMAN

Genomic Location (GRCh Build 38, longest transcript): 4: 89835667-89835547 (+)

ID: [P37840](#)

Hydrophobicity Scale: Kyte and Doolittle

D	E	T
E	Q	S
R	K NH	P Y W G
A	M	C F
		L V

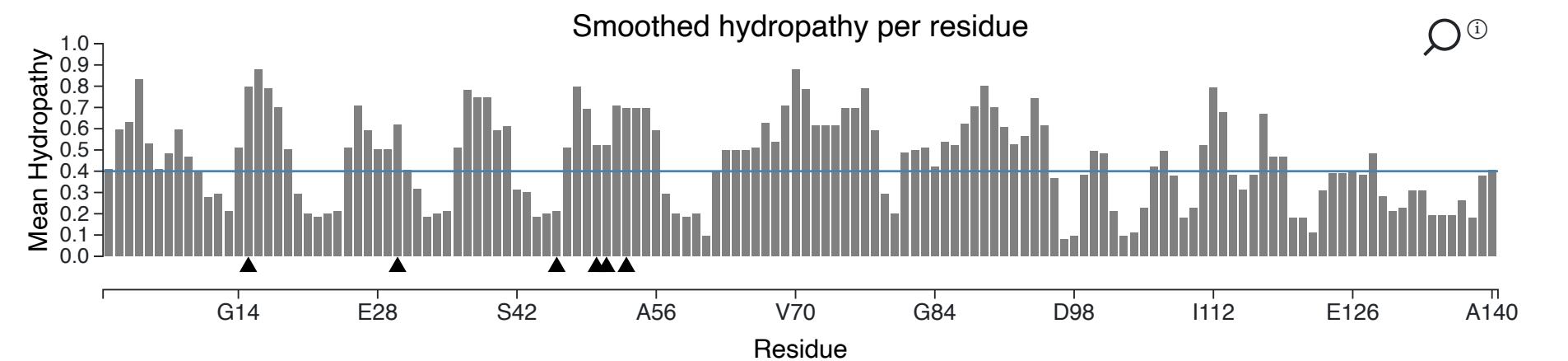
Hydropathy cutoff: 0.4

Minimum blob size: 4



Mutate residue: 1 (M) to R - Arg

[Print Screen](#) [Download data!](#) [Reset Zoom](#) [Clear Mutation](#) [Lock Control Panel](#)

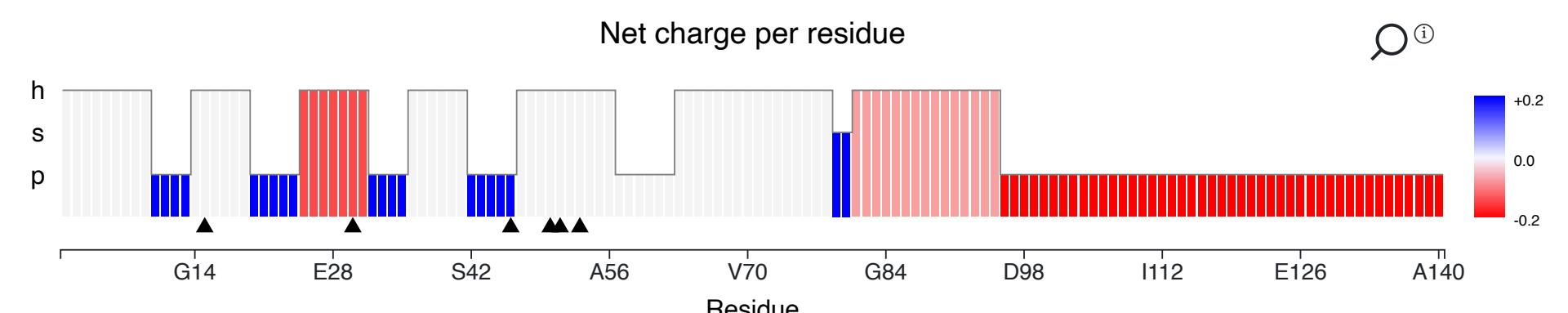
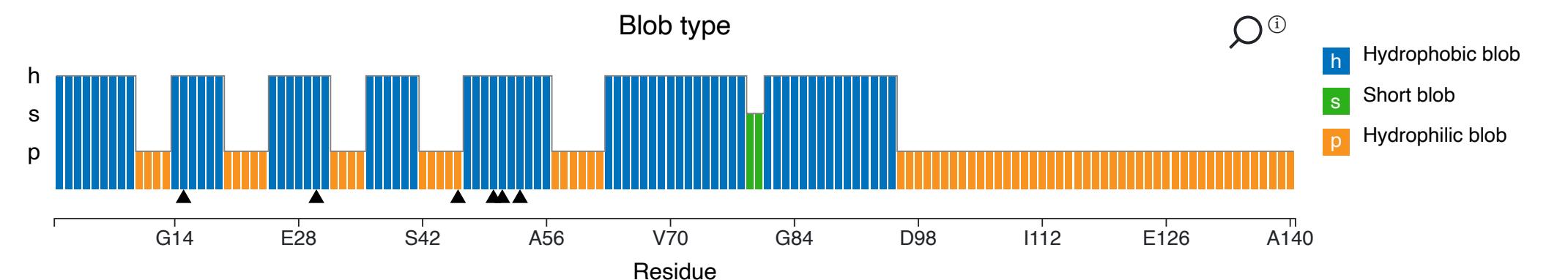


Webtool:

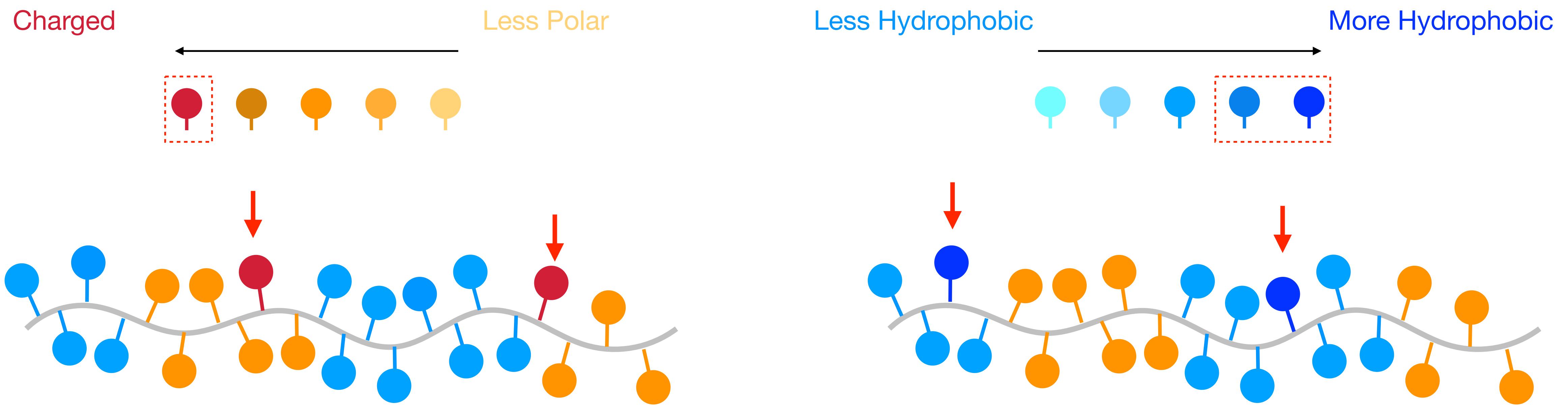


blobulator.branniganlab.org

Blobs colored according to ...



Hypothesis



Coevolving residues in polar blobs
tend to be charged

Coevolving residues in hydrophobic
blobs tend to be highly hydrophobic

Approach

1. Detect Coevolving Pairs

...VFQD**A**LLYP...IATP**L**VTRI...
...VFQD**F**LLYP...IATP**V**VTRI...
...VFQD**I**LLYP...IATP**A**VTRI...
...VFQD**I**LLYP...IATP**Q**VTRI...
...VFQD**A**LLYP...IATP**W**VTRI...

2. Blobulate

...VF**Q**D**A**LLY**P**...IATP**L**VTRI...
...VF**Q**D**F**LLY**P**...IATP**V**VTRI...
...VF**Q**D**I**LLY**P**...IATP**A**VTRI...
...VF**Q**D**I**LLY**P**...IATP**Q**VTRI...
...VF**Q**D**A**LLY**P**...IATP**W**VTRI...

3. Calculate Enrichment

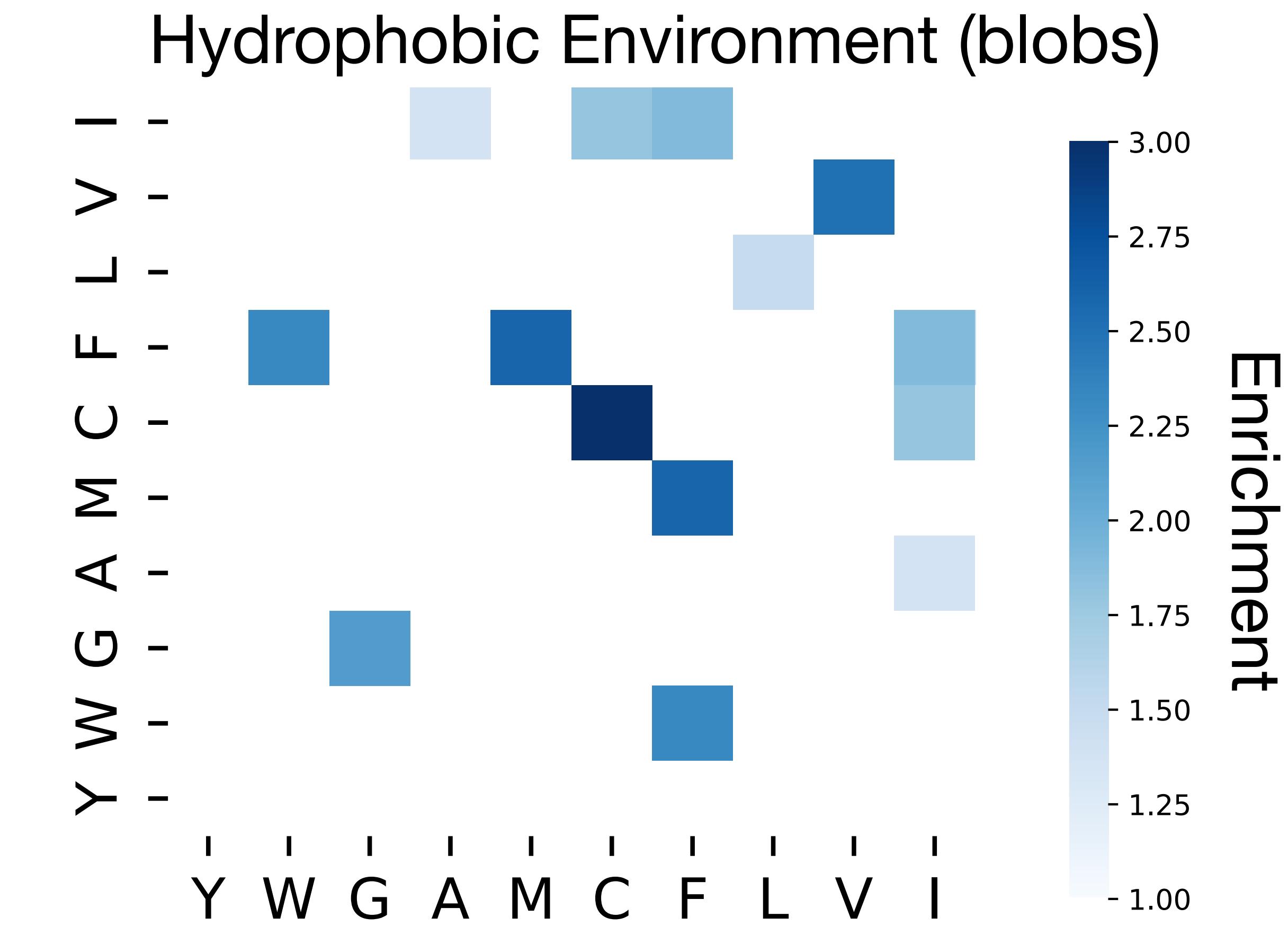
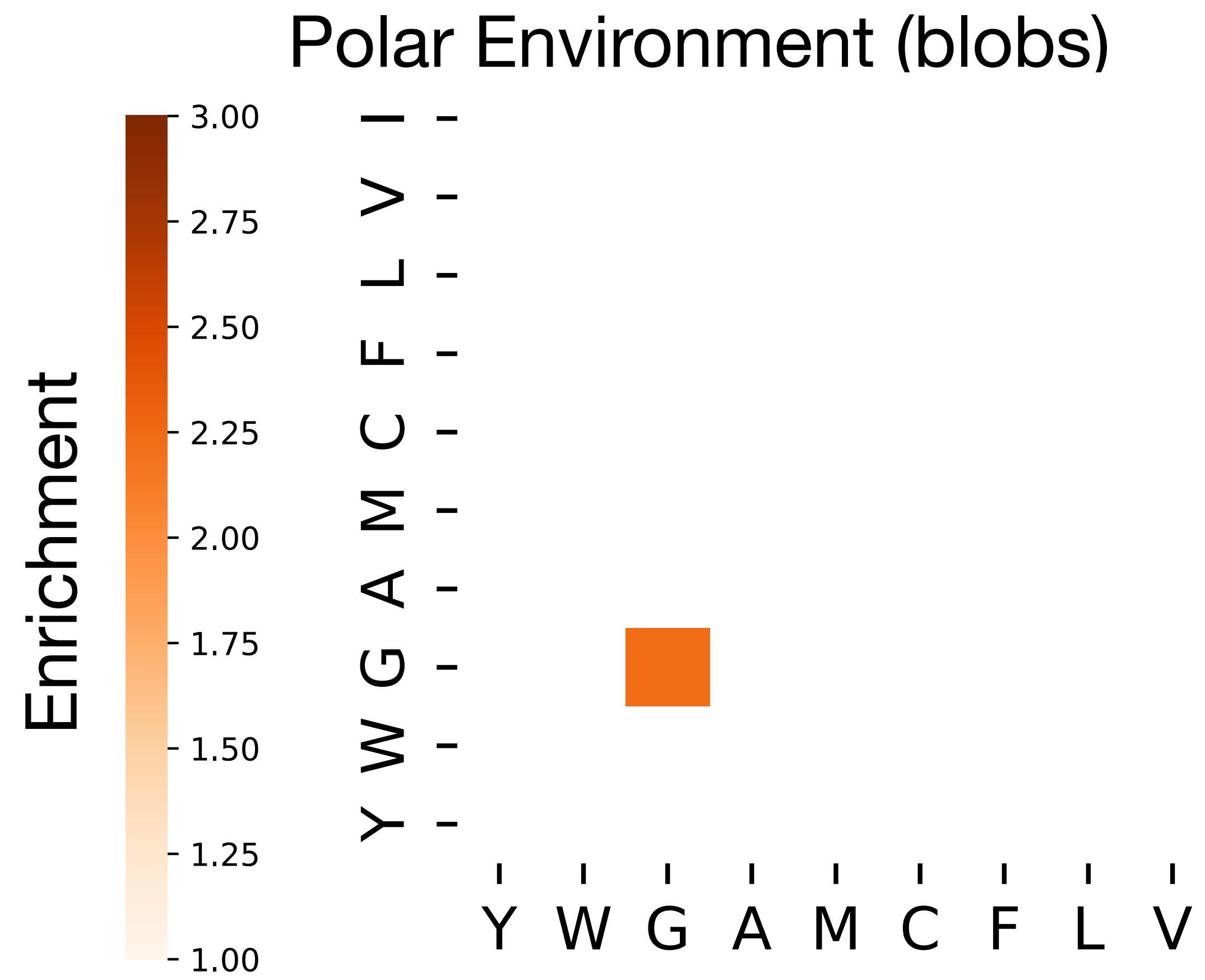
$$\text{Enrichment} = \frac{N_{ab, hh}^{\text{obs}}}{N_{ab, hh}^{\text{perm}}}$$

$$\text{Enrichment} = \frac{N_{ab, pp}^{\text{obs}}}{N_{ab, pp}^{\text{perm}}}$$

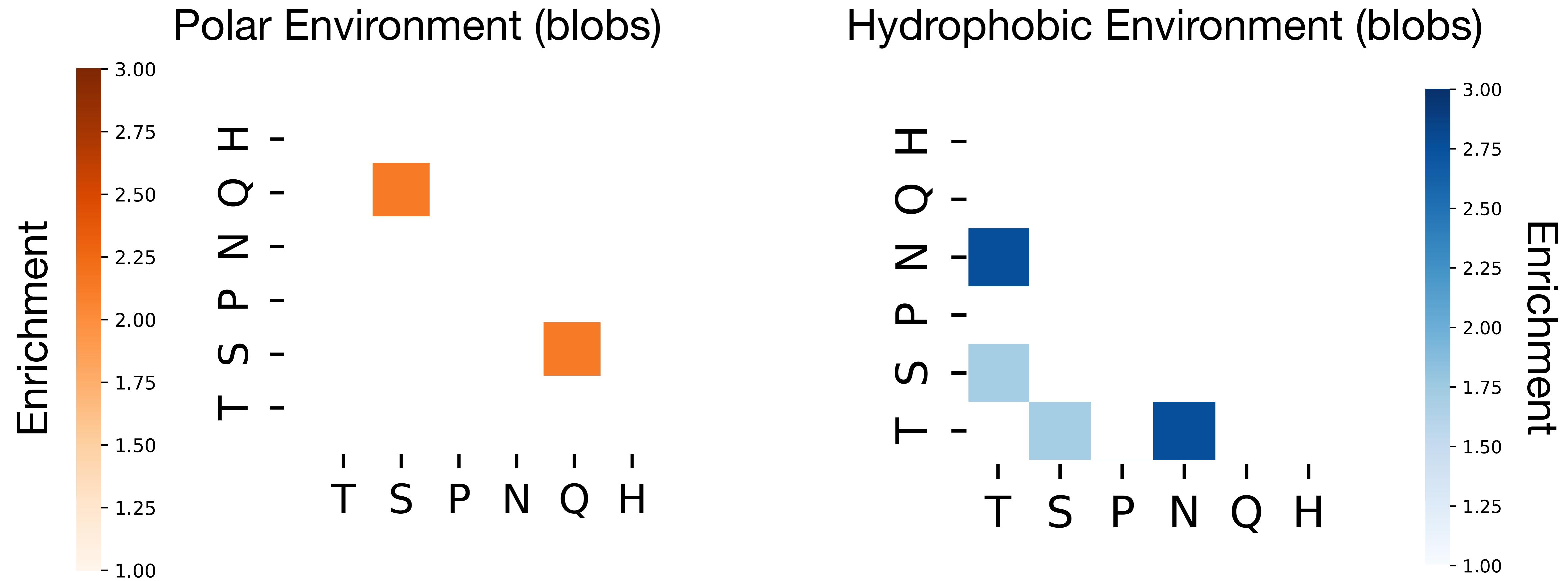
Software: CoMap

Inputs: ~250,000 aligned bacterial sequences across ~1,600 protein families

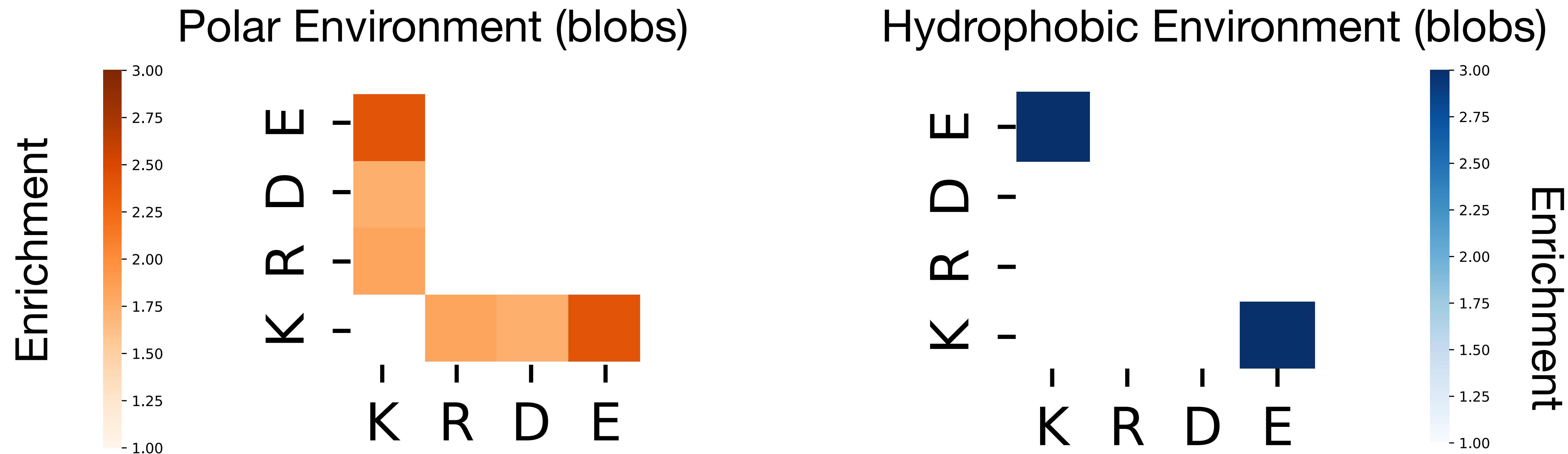
Hydrophobic coevolving residues tend to be found in **hydrophobic** blobs



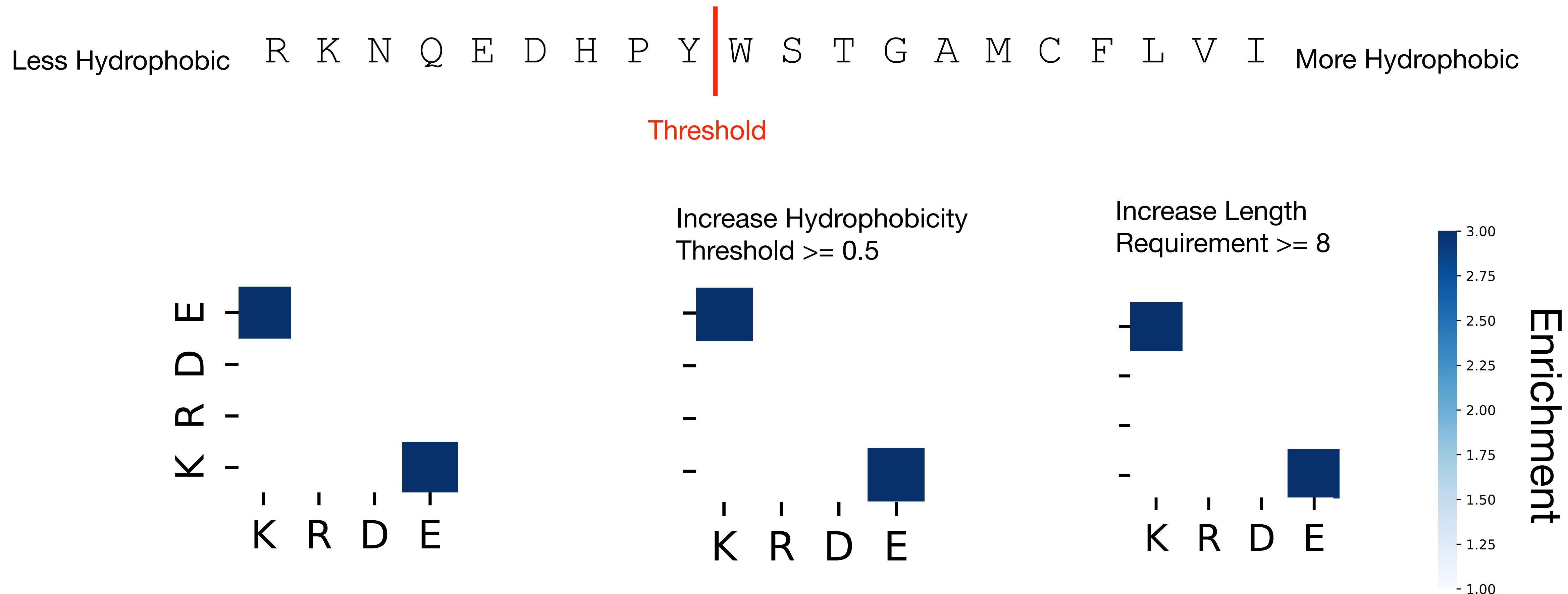
Polar coevolving residues are found in both **polar** and **hydrophobic** blobs



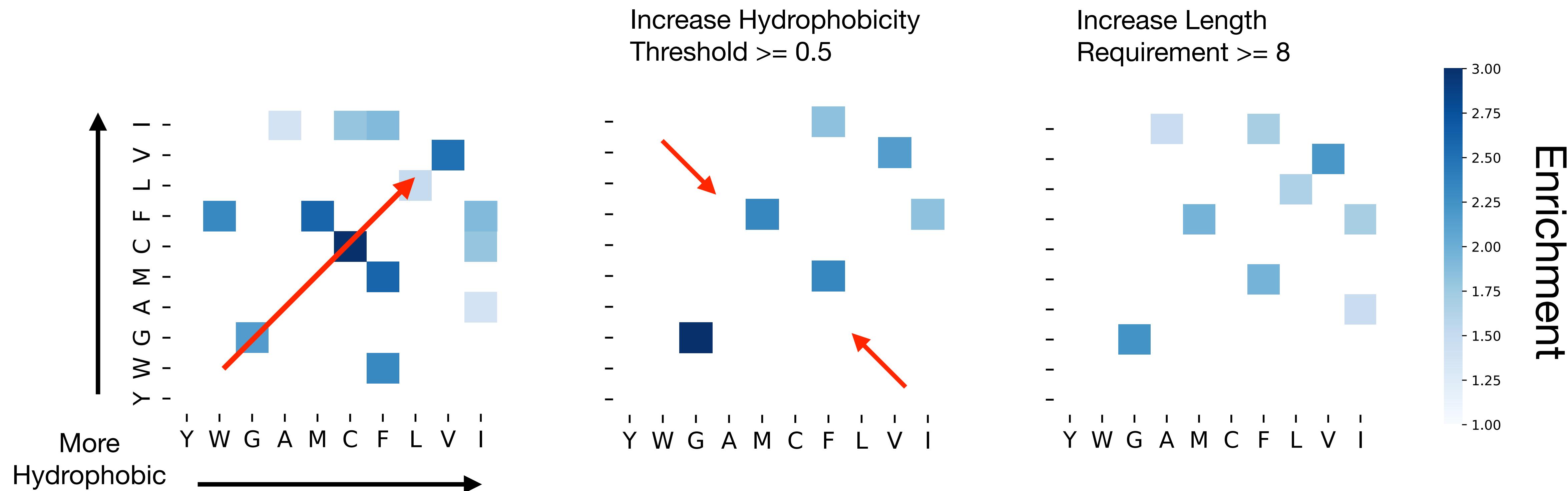
Charged coevolving residues are found in both polar and hydrophobic blobs



Charged residues in **hydrophobic blobs** remain enriched under more stringent parameters



Restrictive parameters do not simply rule out pairs of less hydrophobic amino acids in **hydrophobic blobs**



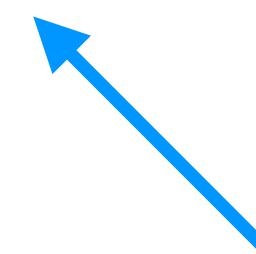
Summary

The identity of coevolving residues differ between blob types

Some shared pairs (Lysine-X, Glycine-Glycine) but many distinct pairs between blob types.

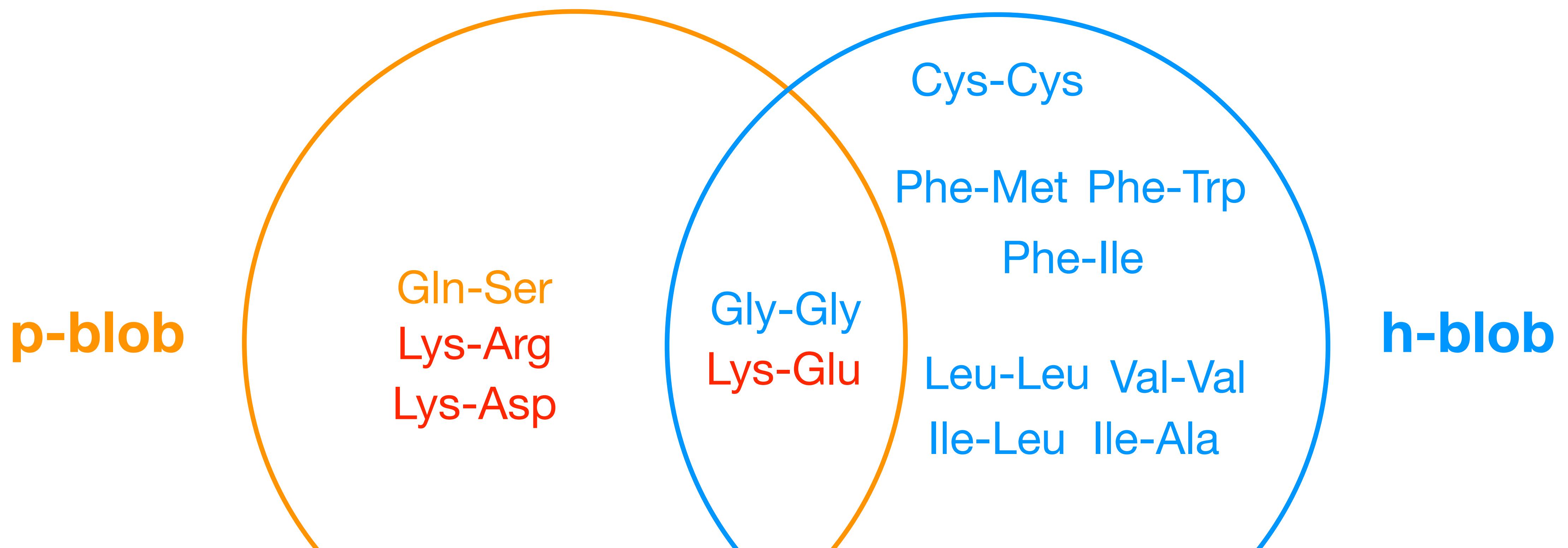
In h-blobs, hydrophobic pairs tended to be more similar to each other (pairs of aliphatic residues, CC pairs, aromatic pairs - but not many “cross-type” pairs).

The charged pair KE was found in h-blobs.



Insensitive to parameter changes

Summary: Enriched coevolving residues



Blobs matter!

Acknowledgements



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Computing

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Lindsey Riggs
Ryan Lamb
Regina Salzer

Collaborators:



Matt Hansen



Anthony Geneva



Questions?

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