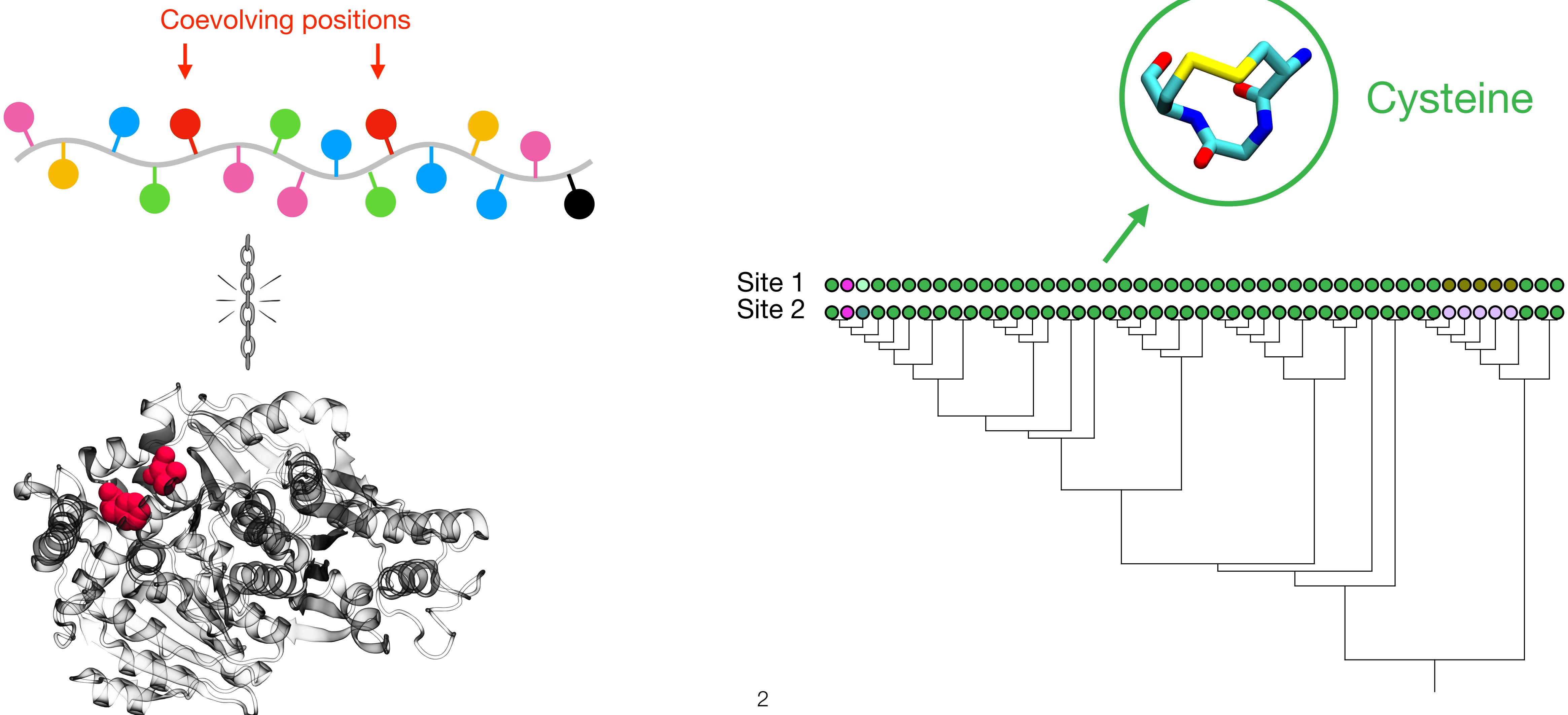


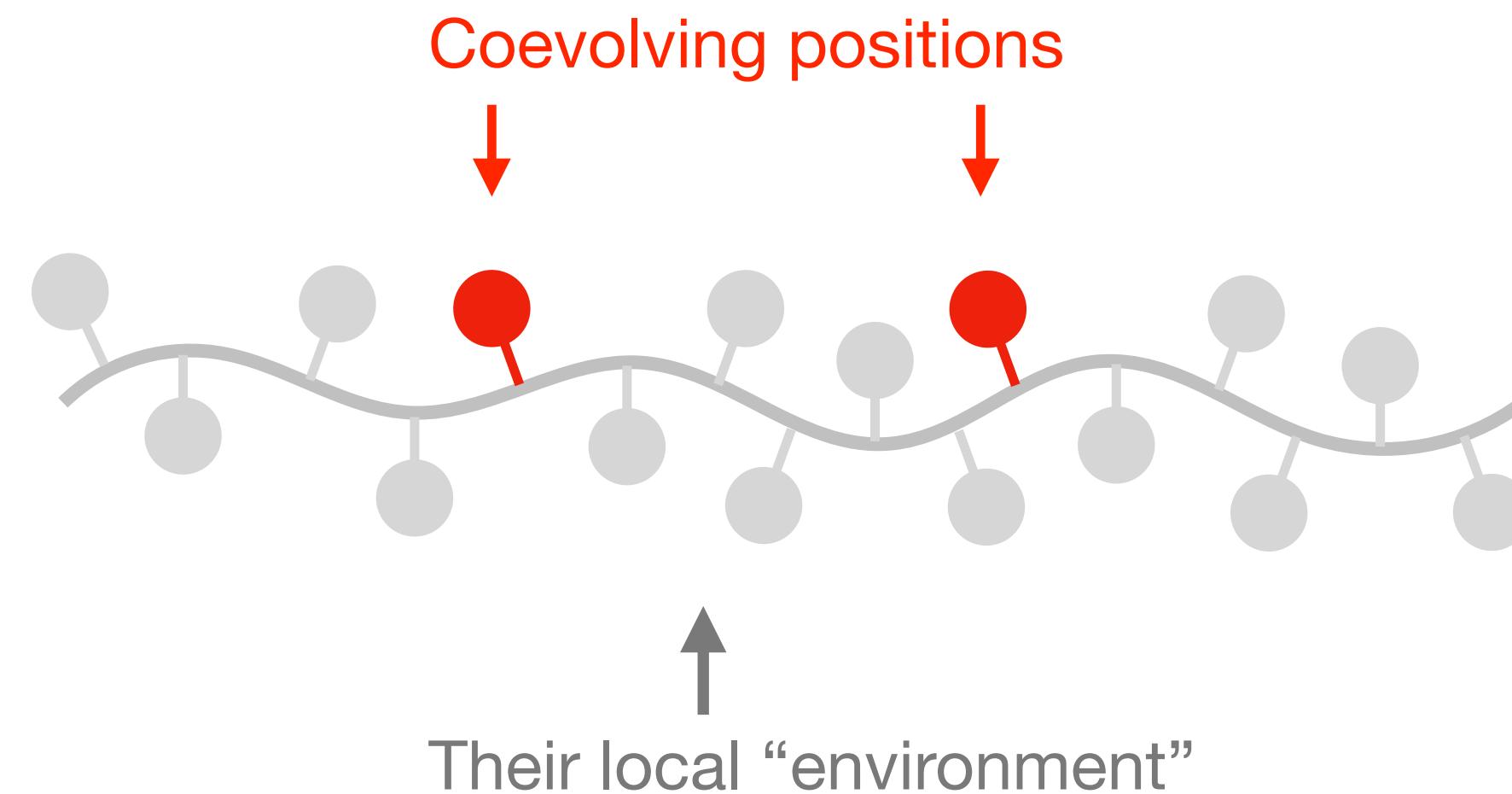
# **Elucidating Interaction Nodes Created by Hydrophobic Residues and Subsequences in Proteins**

**CCIB Seminar 2025, Connor Pitman, Brannigan Lab**

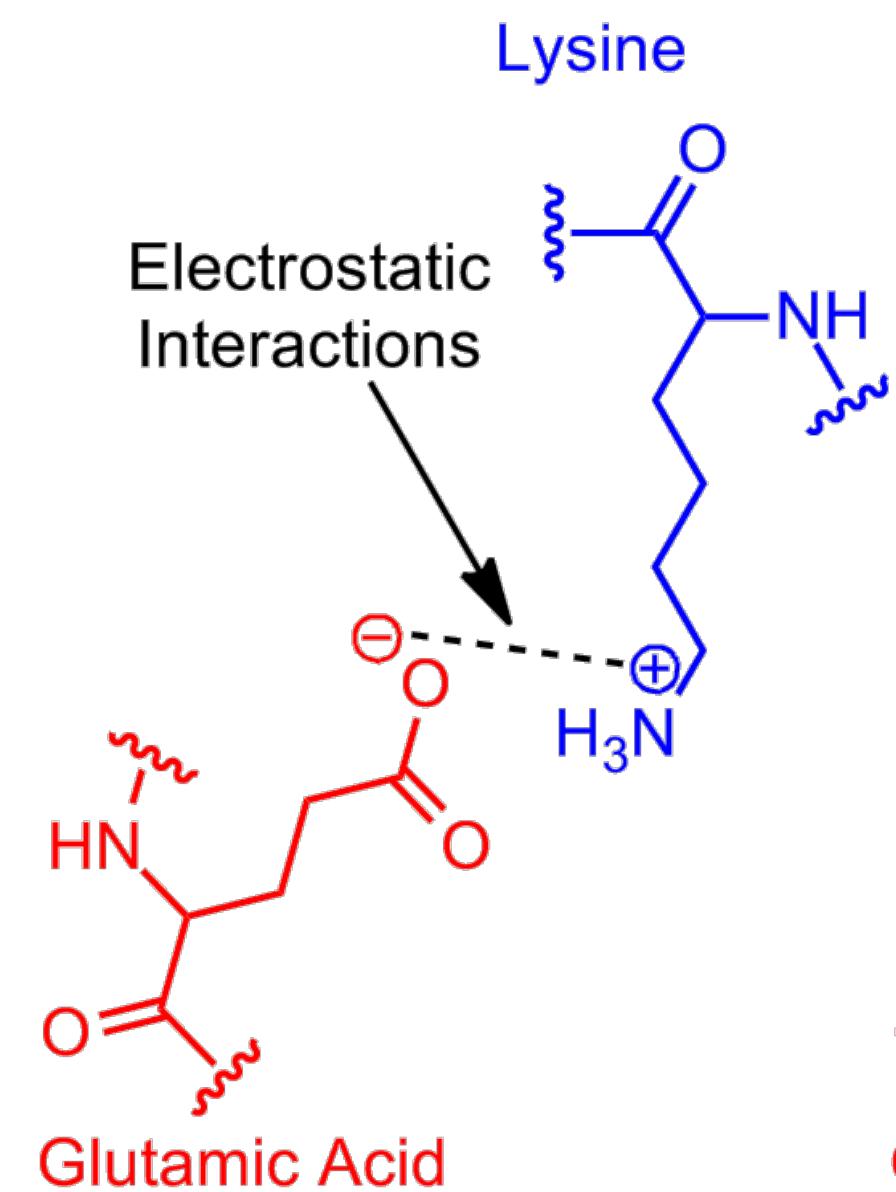
# Protein evolution is constrained by intra-protein interactions



# Coevolving pairs exist in the context of the residues around them

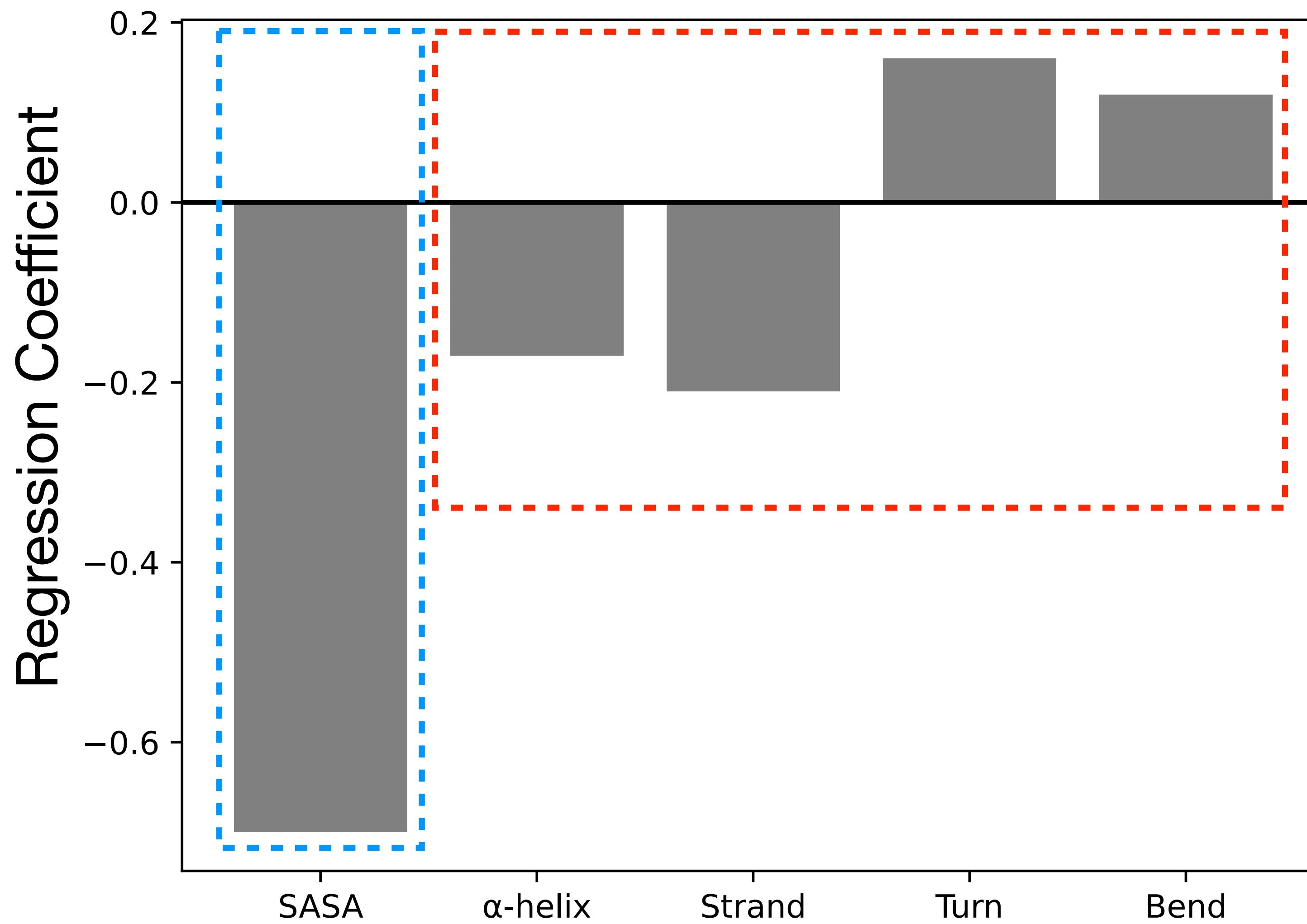


We have mechanistic hypotheses at the residue level

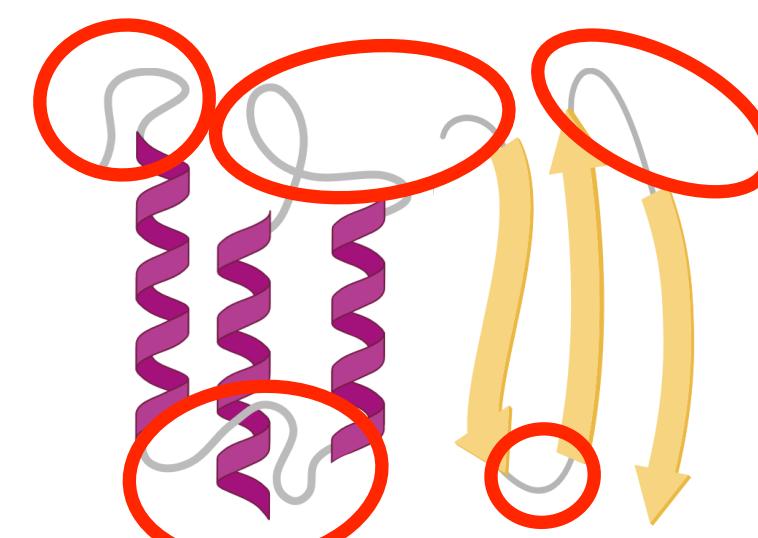


How do we incorporate the role of the protein “environment”?

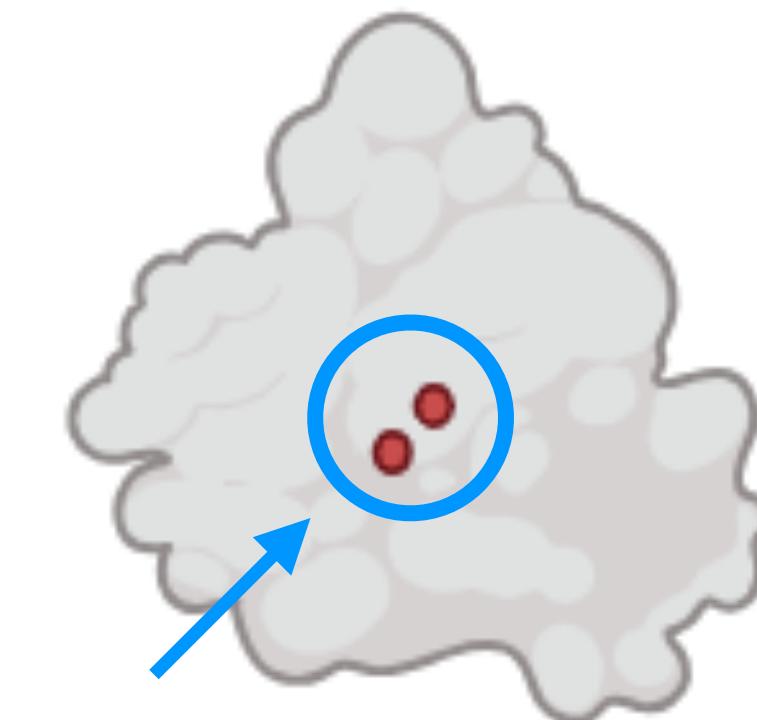
# Coevolution is unassociated with secondary structure



Coevolving pairs tend to be found...

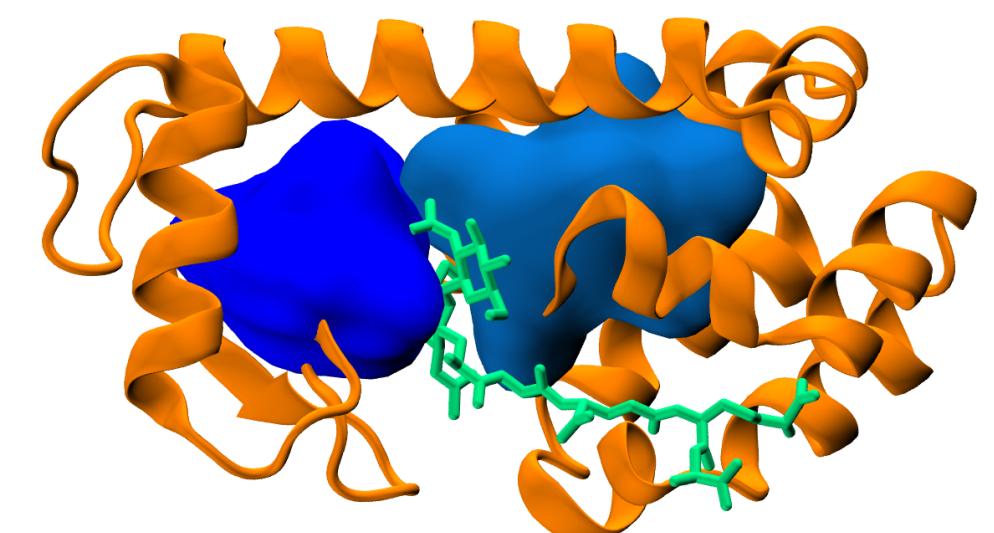
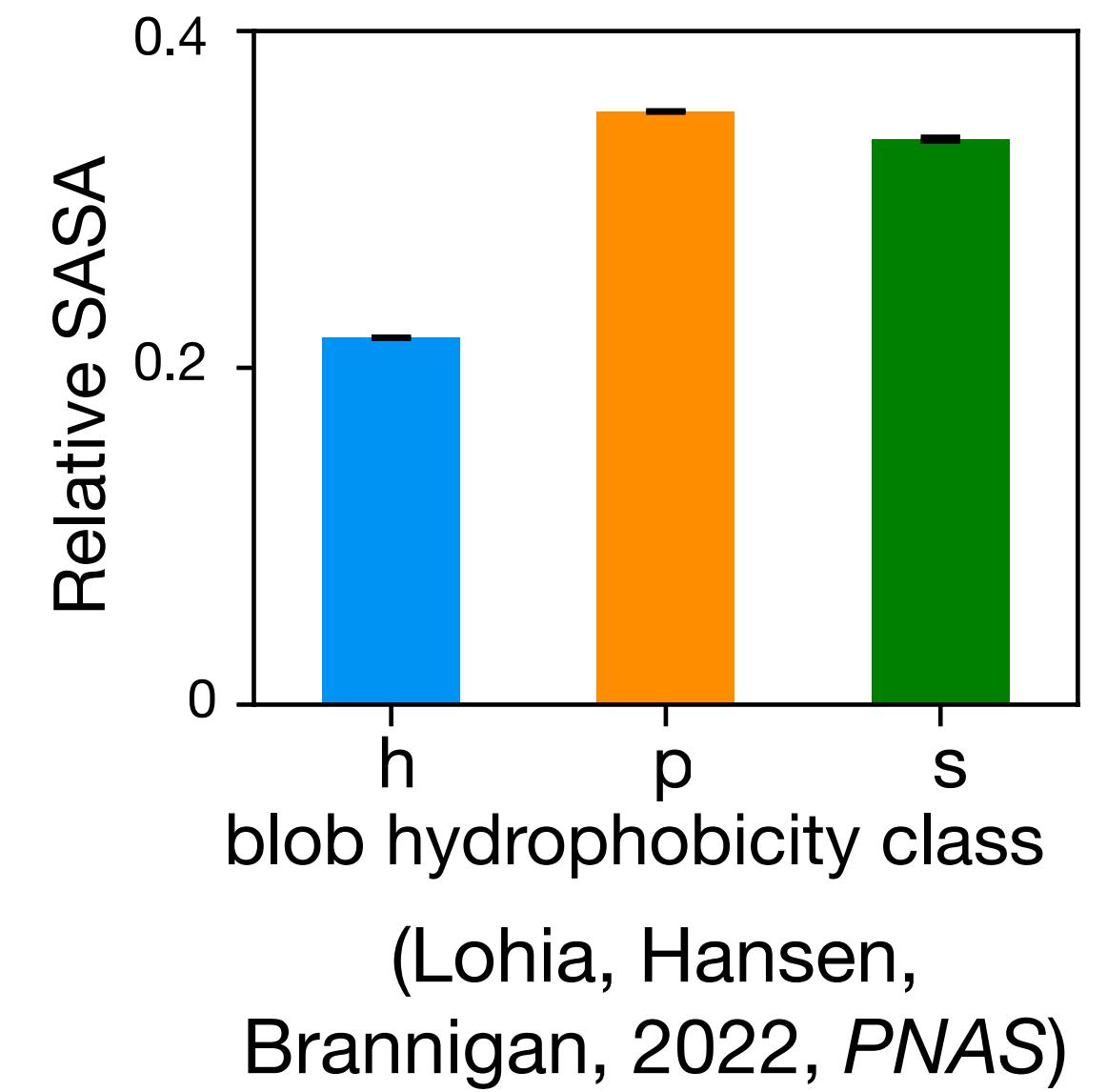
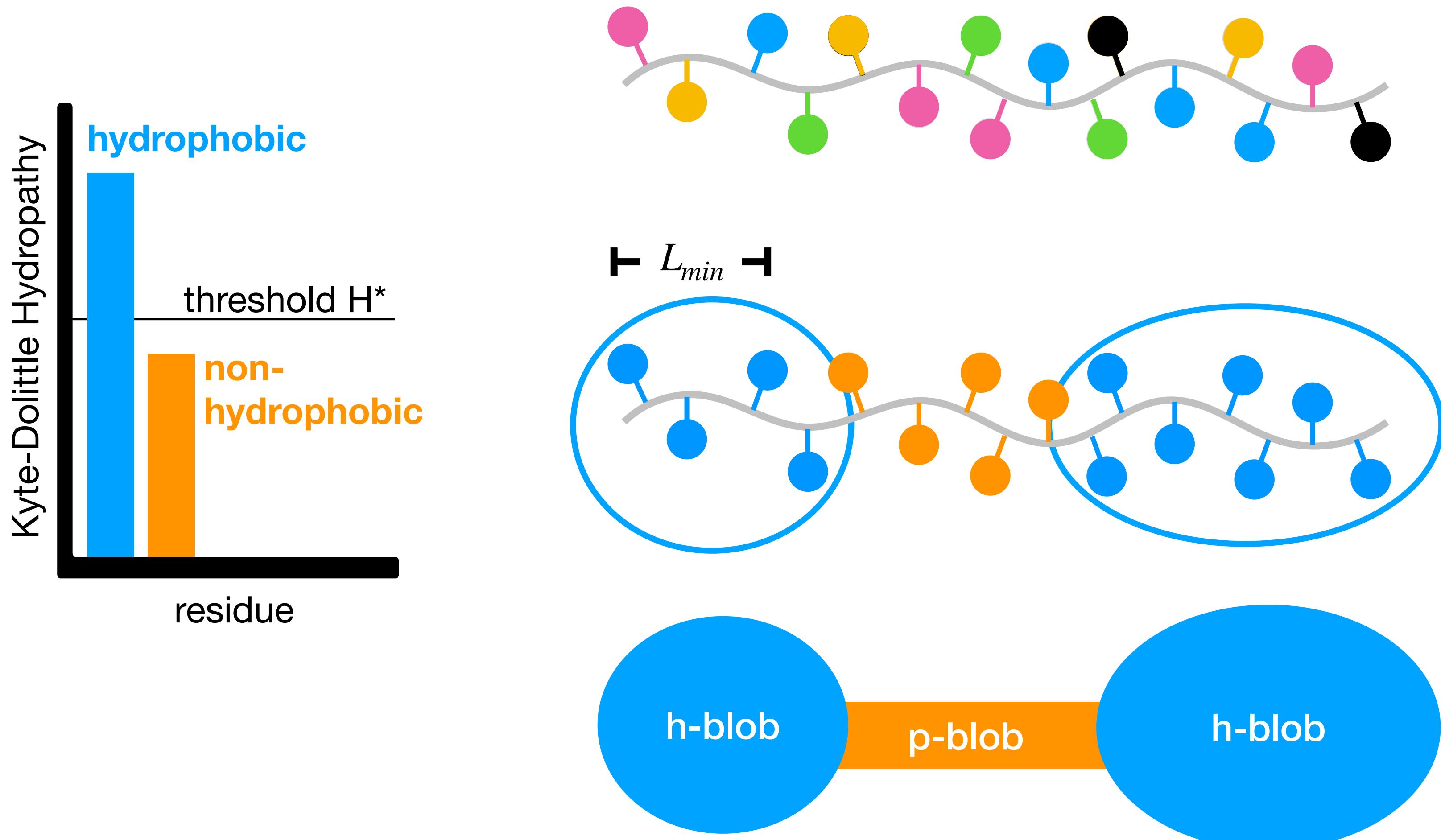


Outside of secondary structure elements



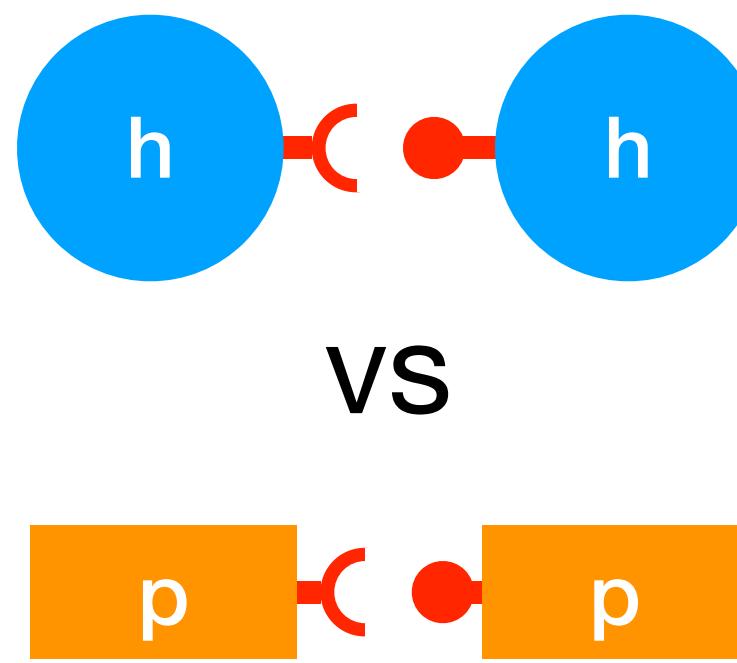
Near the hydrophobic core

# Blobulation: Subsequences from contiguous hydrophobicity



(Pitman et al. 2024, *bioRxiv*)

# Research Question



coevolving pairs in the same blob type

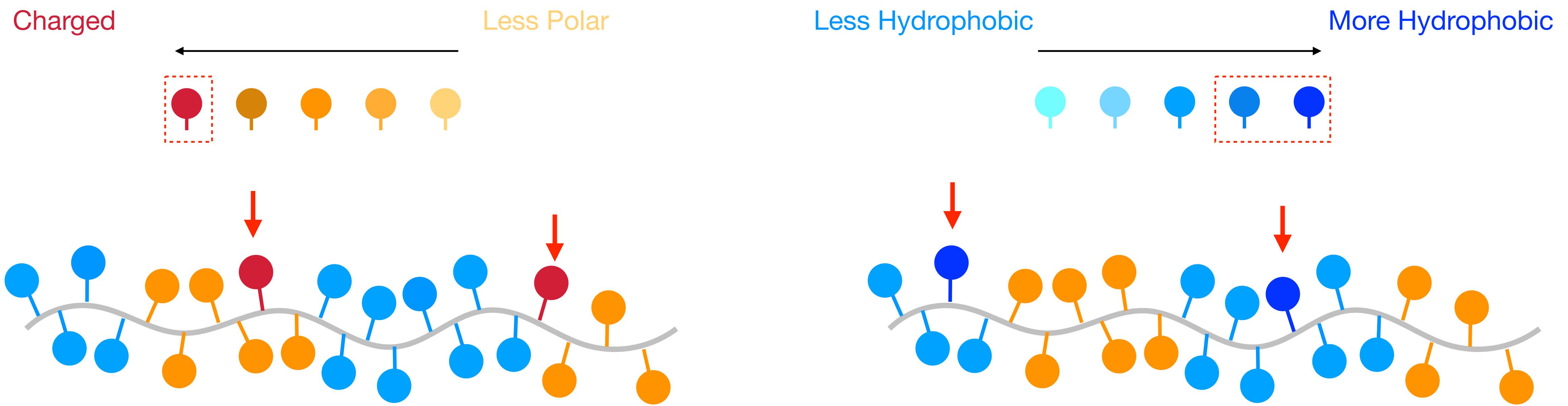


coevolving pairs in opposite blob types

**Do the types of amino acids that coevolve depend on the blob type they're found in?**

**Do blobs matter, from an evolutionary perspective?**

# 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

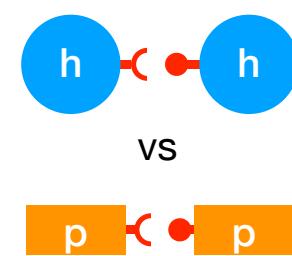
$$\text{Enrichment} = \frac{N_{ab, hh}^{\text{obs}}}{N_{ab, hh}^{\text{perm}}}$$

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

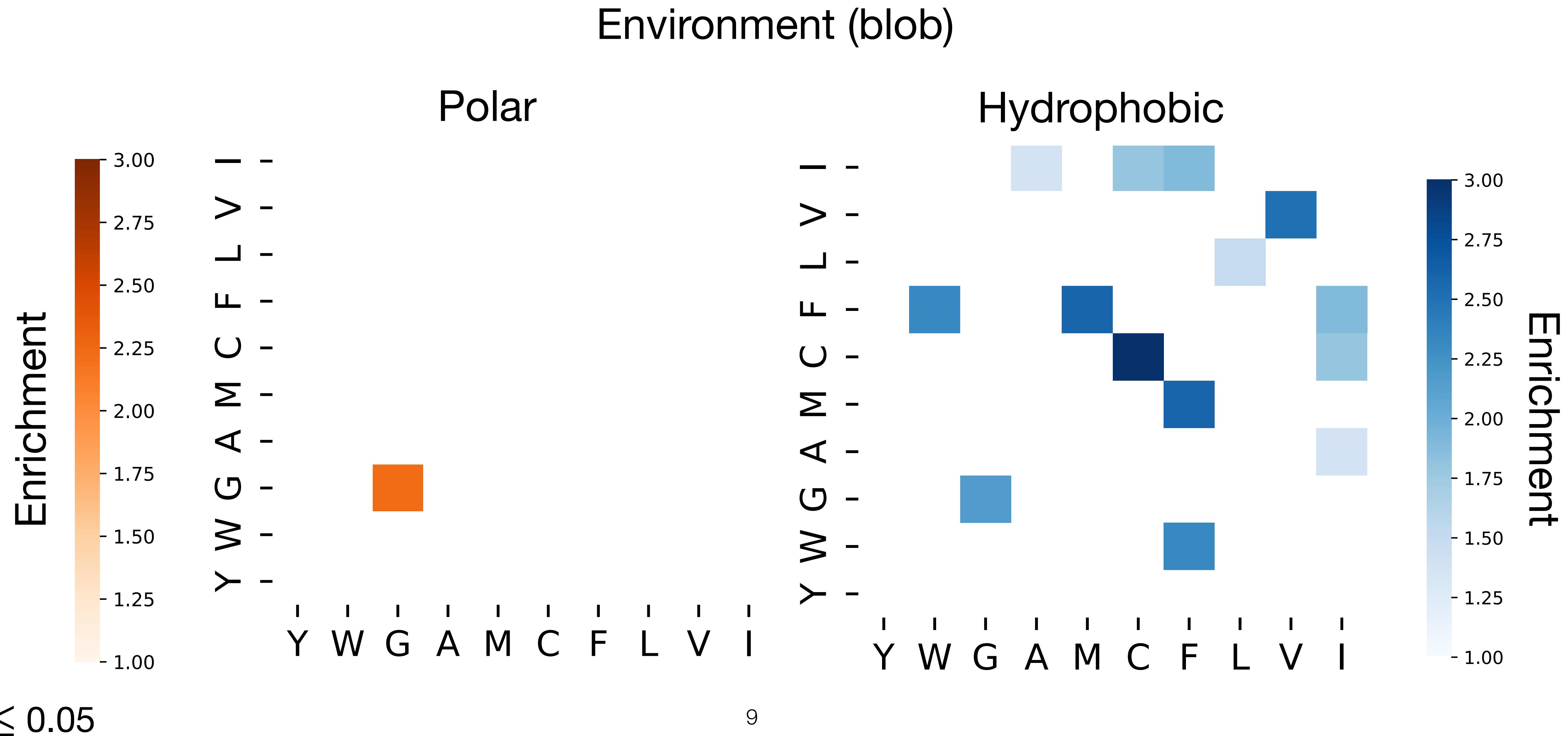
$$\text{Enrichment} = \frac{N_{ab, hp}^{\text{obs}}}{N_{ab, hp}^{\text{perm}}}$$

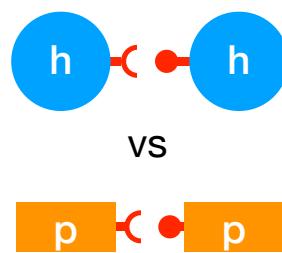
Software: CoMap

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



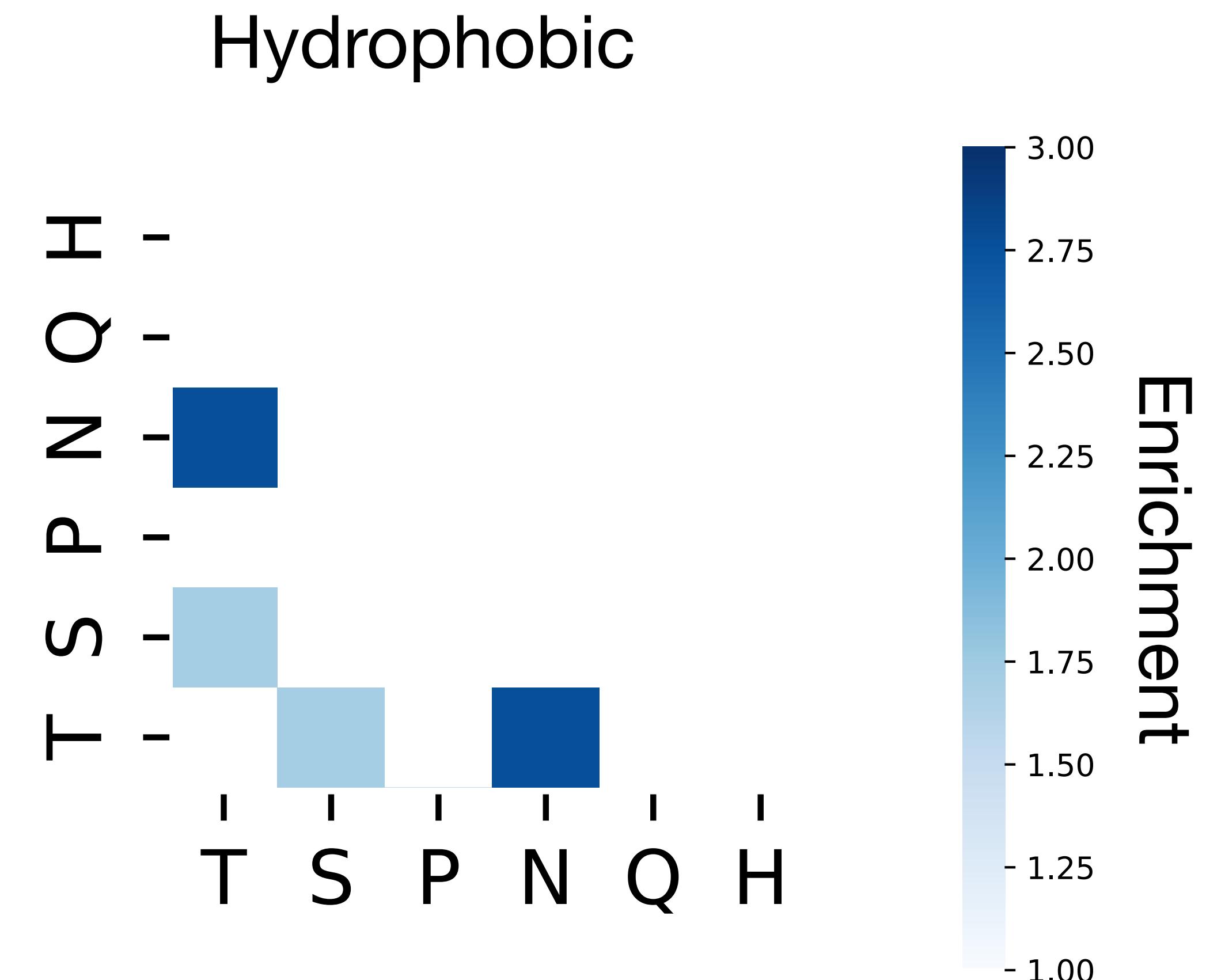
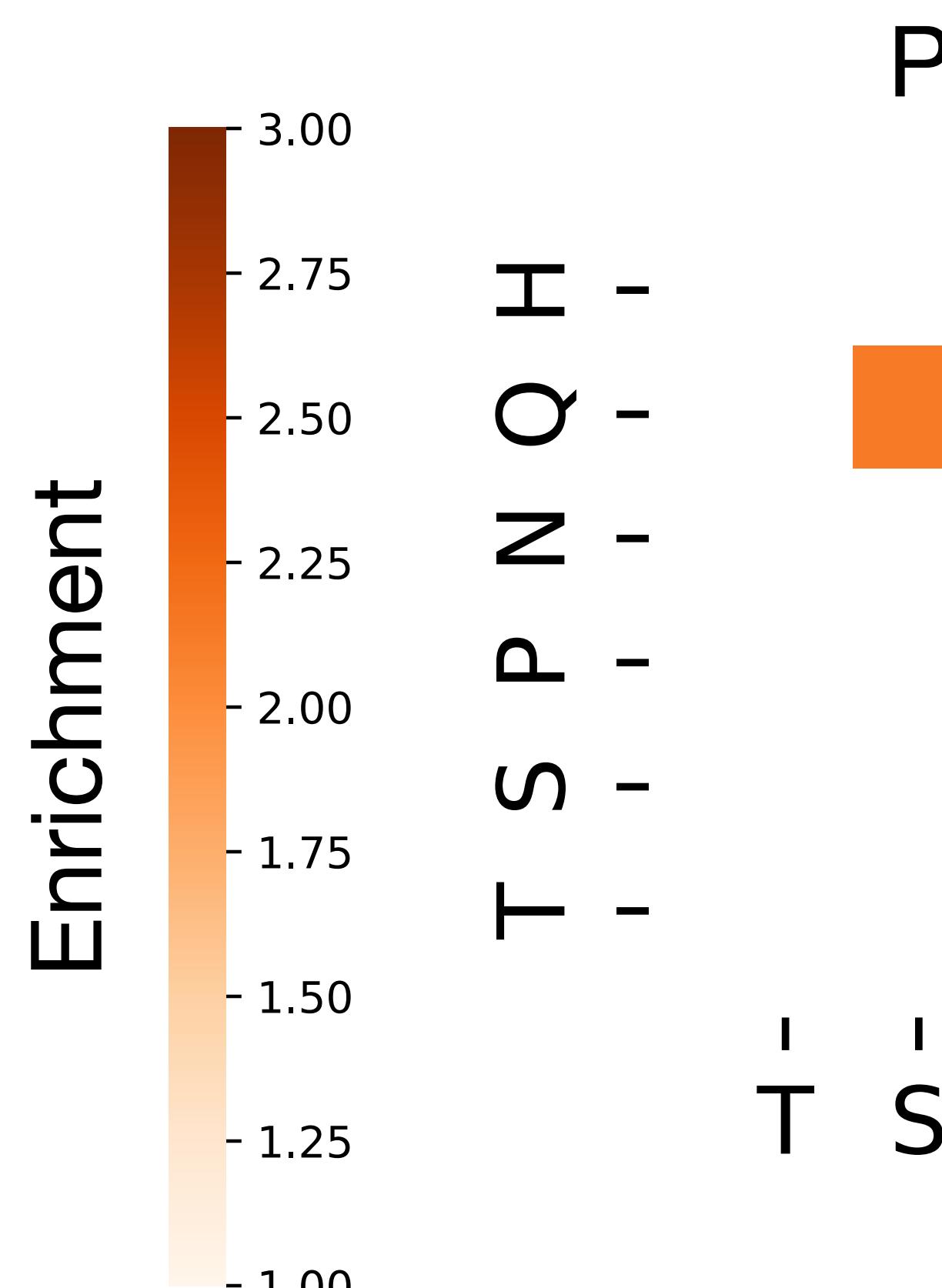
# Hydrophobic coevolving residues tend to be found in **hydrophobic** blobs





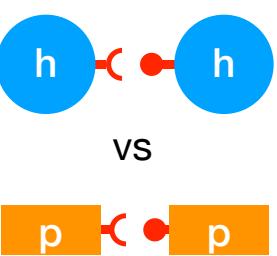
# Polar coevolving residues are found in both **polar** and **hydrophobic** blobs

Environment (blob)

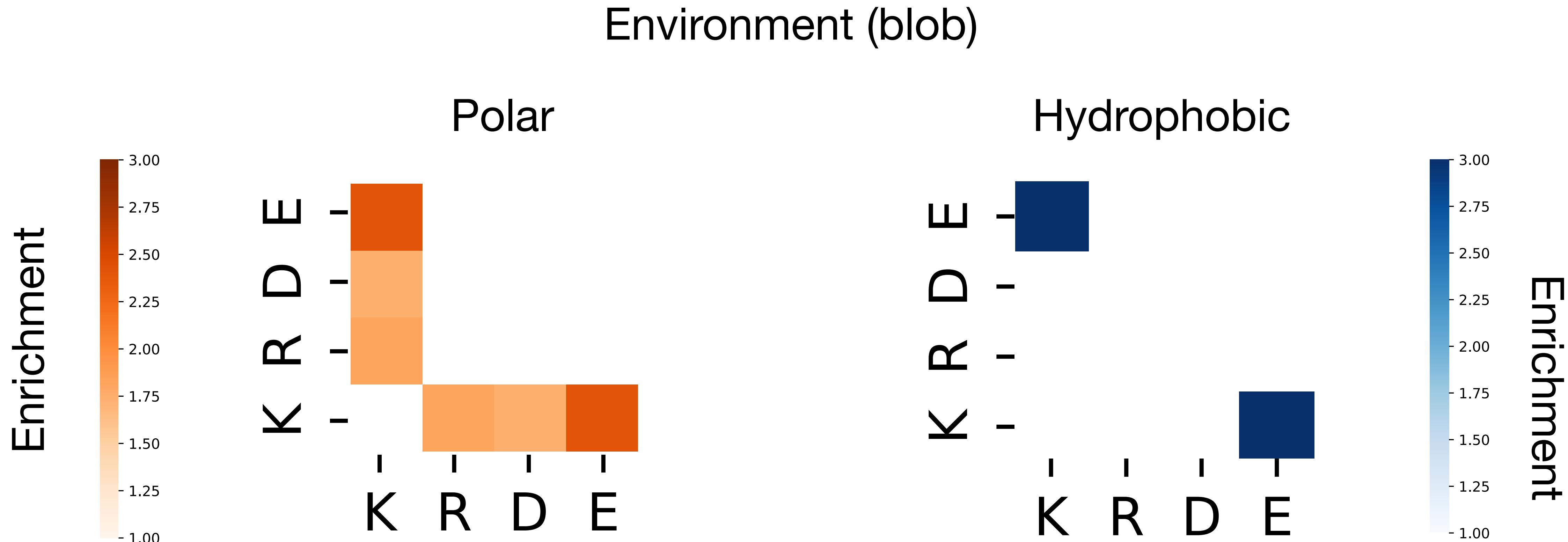


FDR  $\leq 0.05$

10

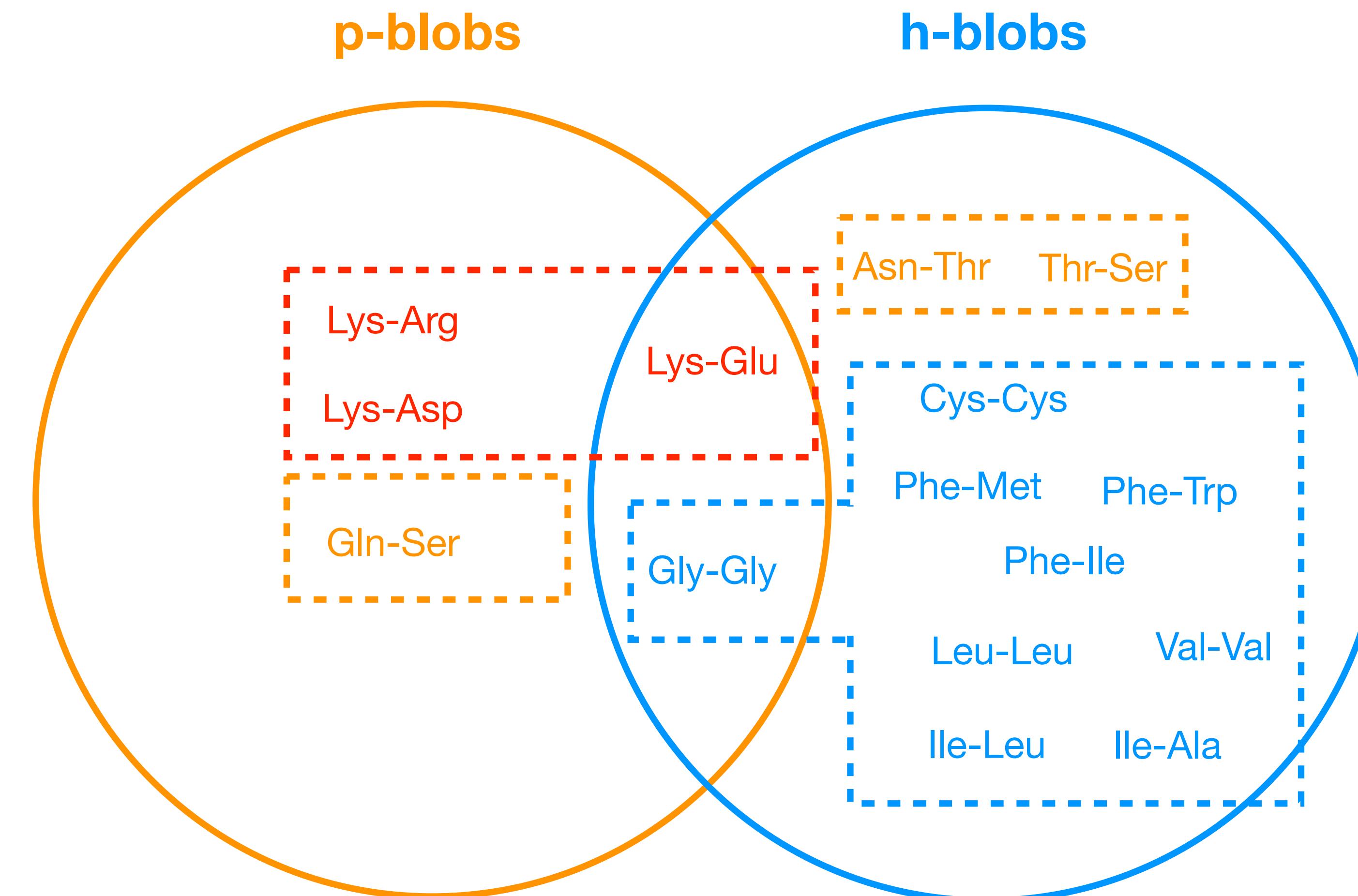


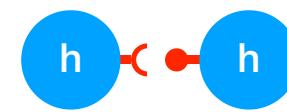
# Charged coevolving residues are found in both polar and hydrophobic blobs



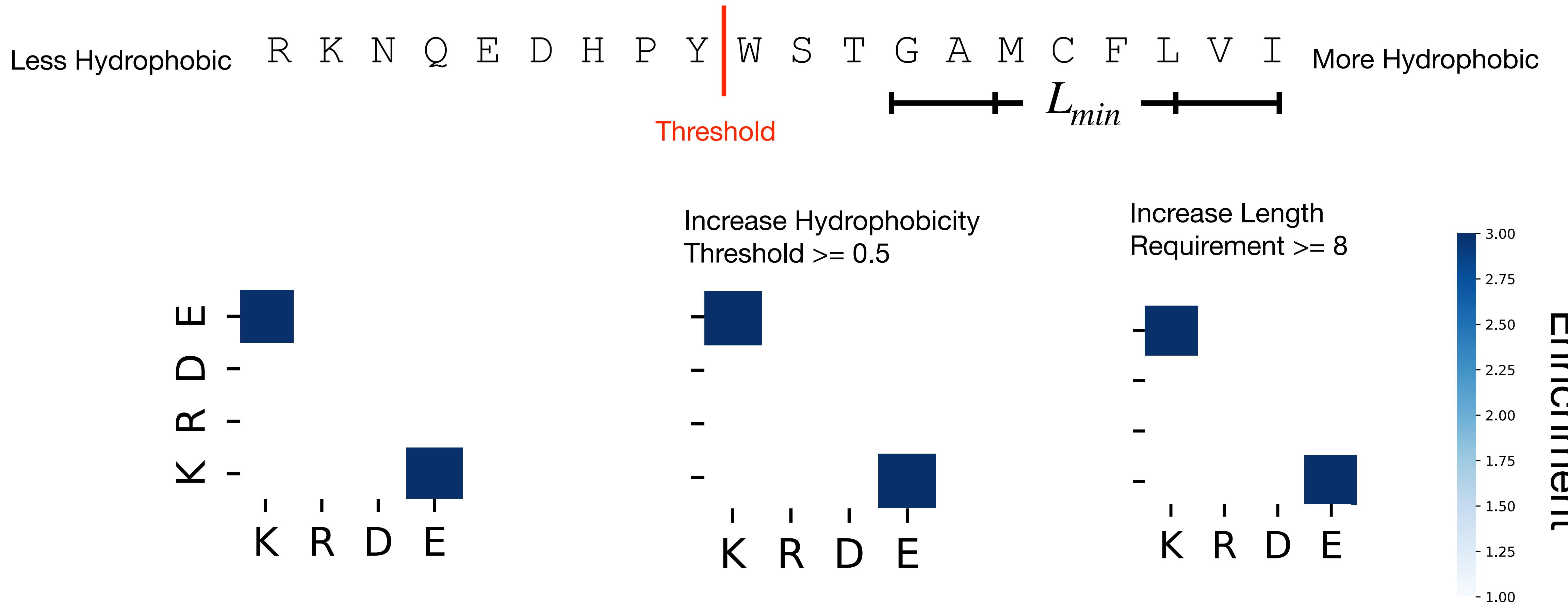
h h  
vs  
p p

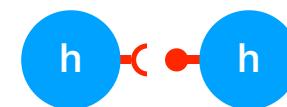
# Summary of pairs both in h-and p-blobs



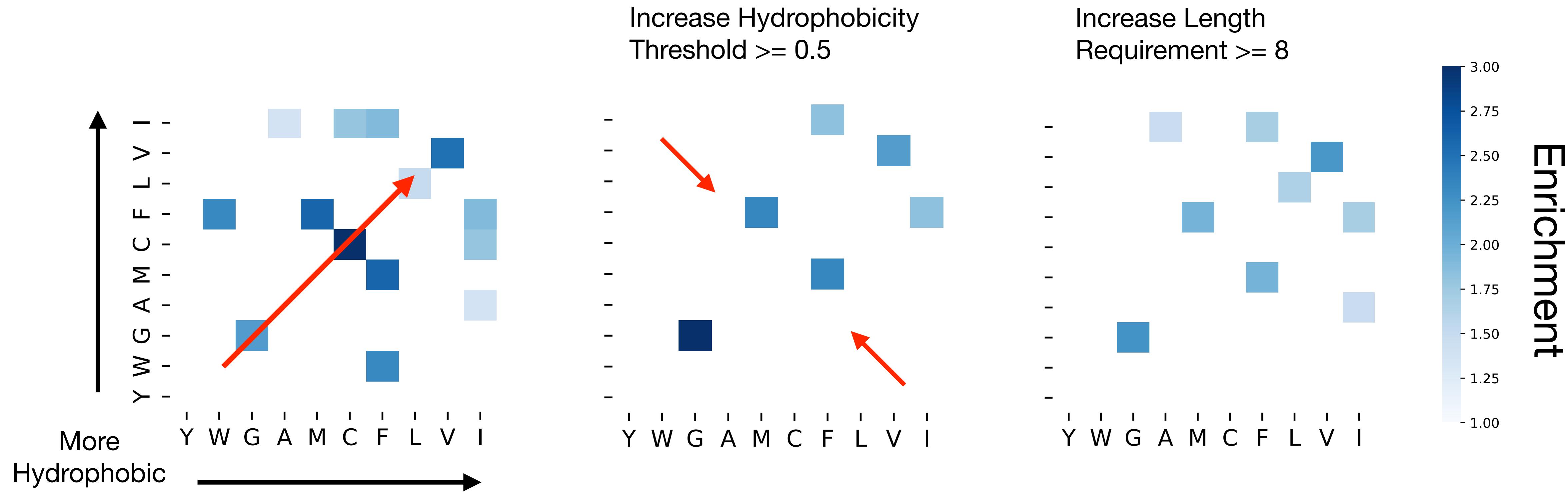


# Charged residue enrichment in hydrophobic blobs is not parameter-sensitive



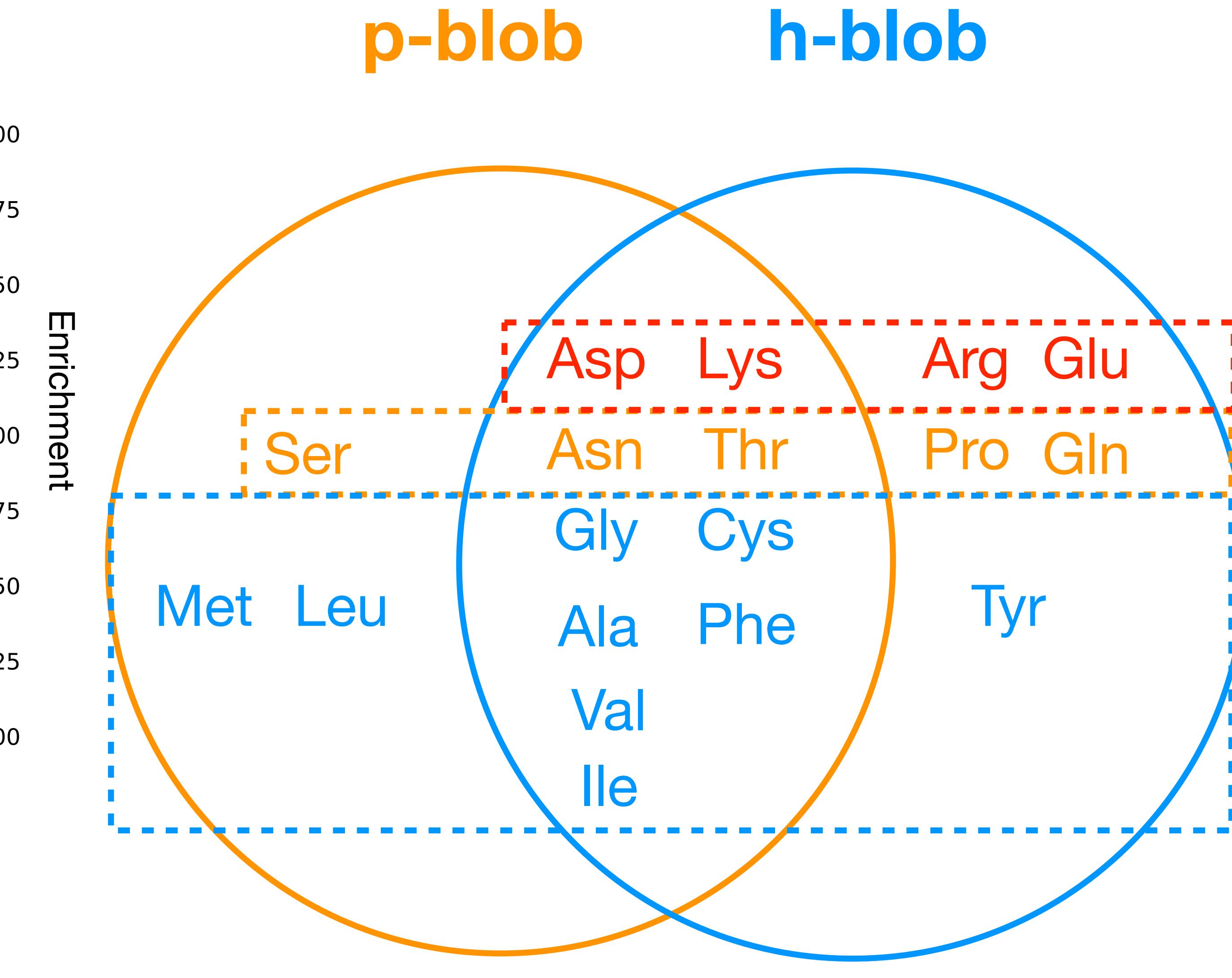
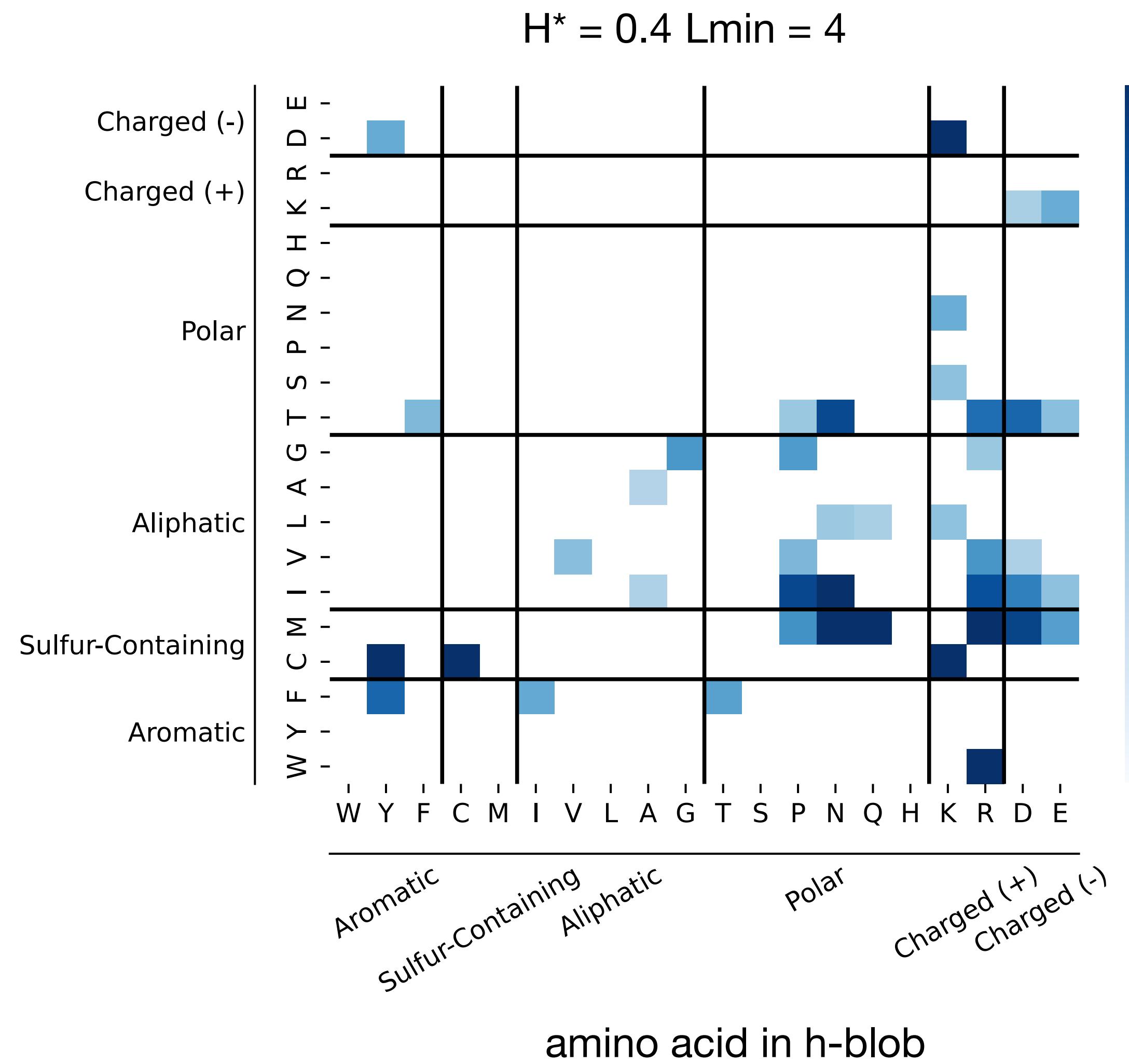


# Less hydrophobic residue enrichment in **hydrophobic blobs** is not parameter-sensitive

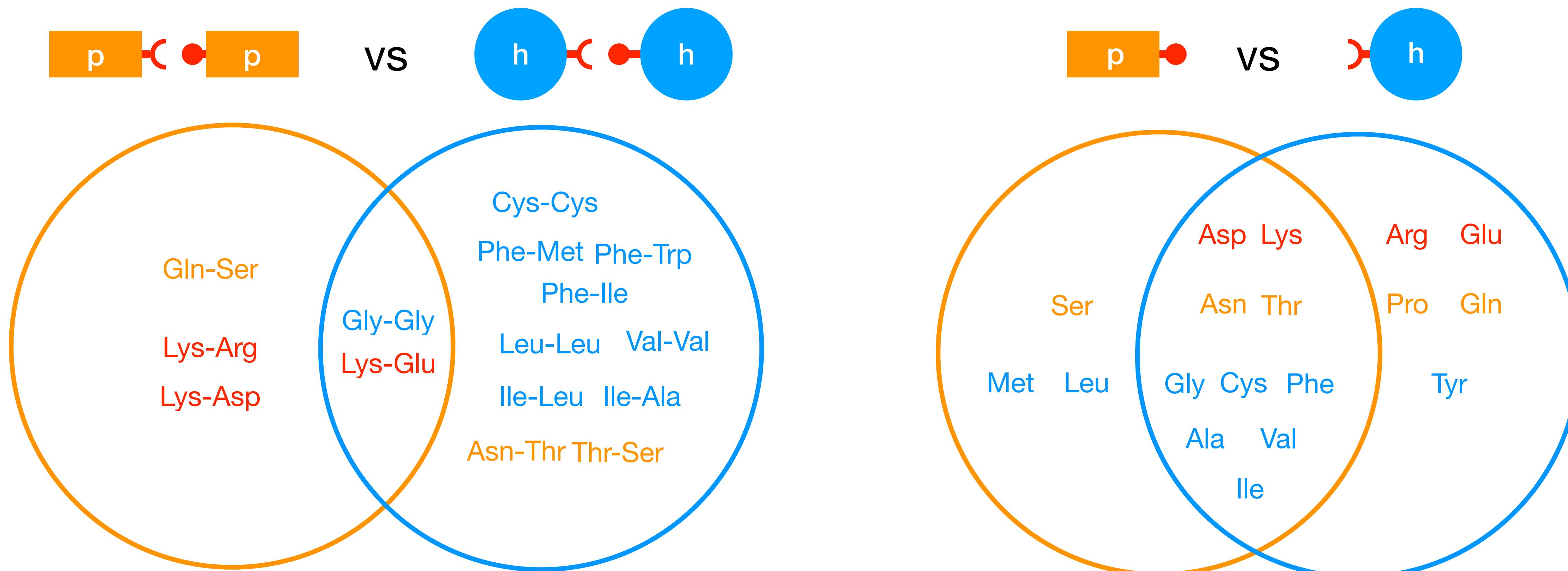


h vs p

# Charged residues are found in h-blobs, and residues tend to be unlike containing blob



# Summary



Do the types of amino acids that coevolve depend on the blob type  
Blobs matter!  
they're found in?

# Acknowledgements



Brannigan Lab



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## Lab Members:

Grace Brannigan (PI)  
Ezry Santiago McRae  
Jesse Sandberg  
Jahmal Ennis  
Lindsey Riggs  
Ryan Lamb  
Regina Salzer  
Asim Dave

## Collaborators:



Matt Hansen



Anthony Geneva



# Questions?