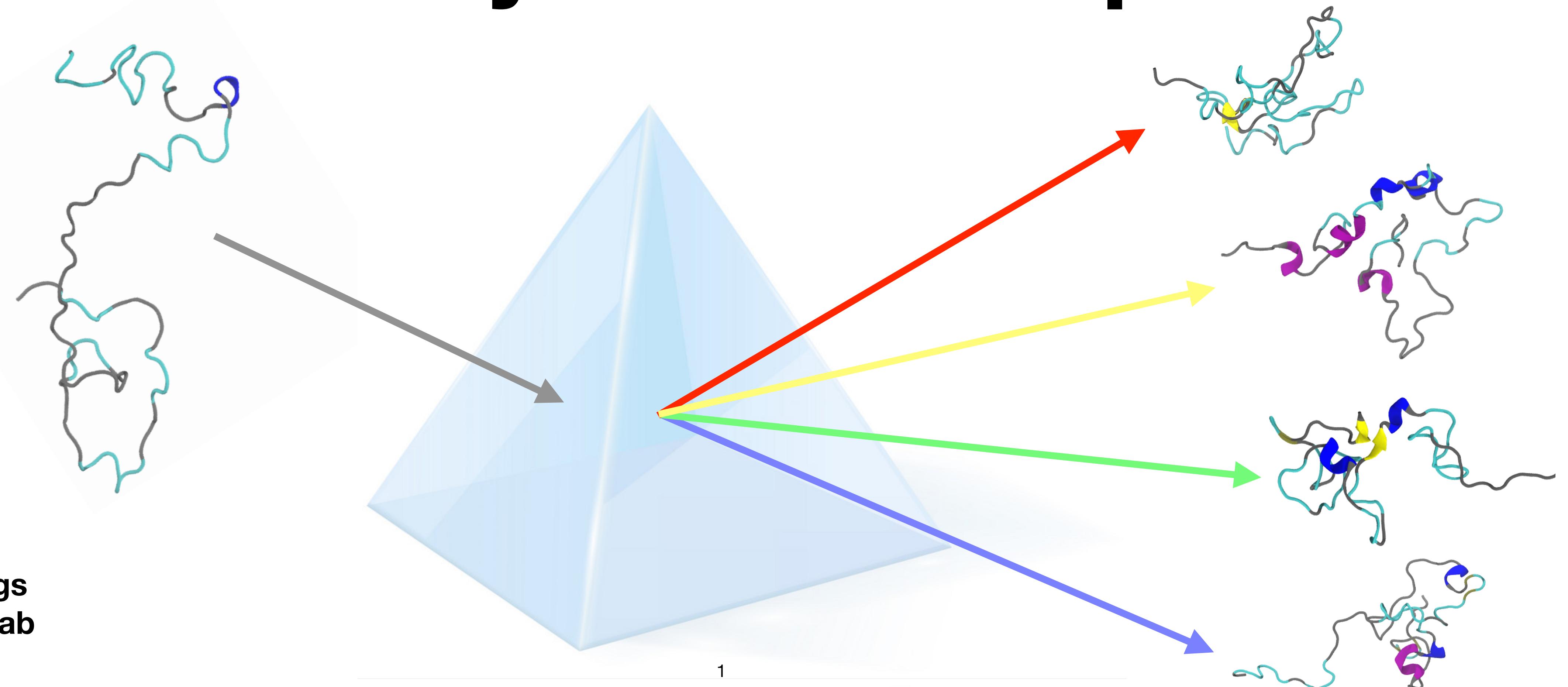


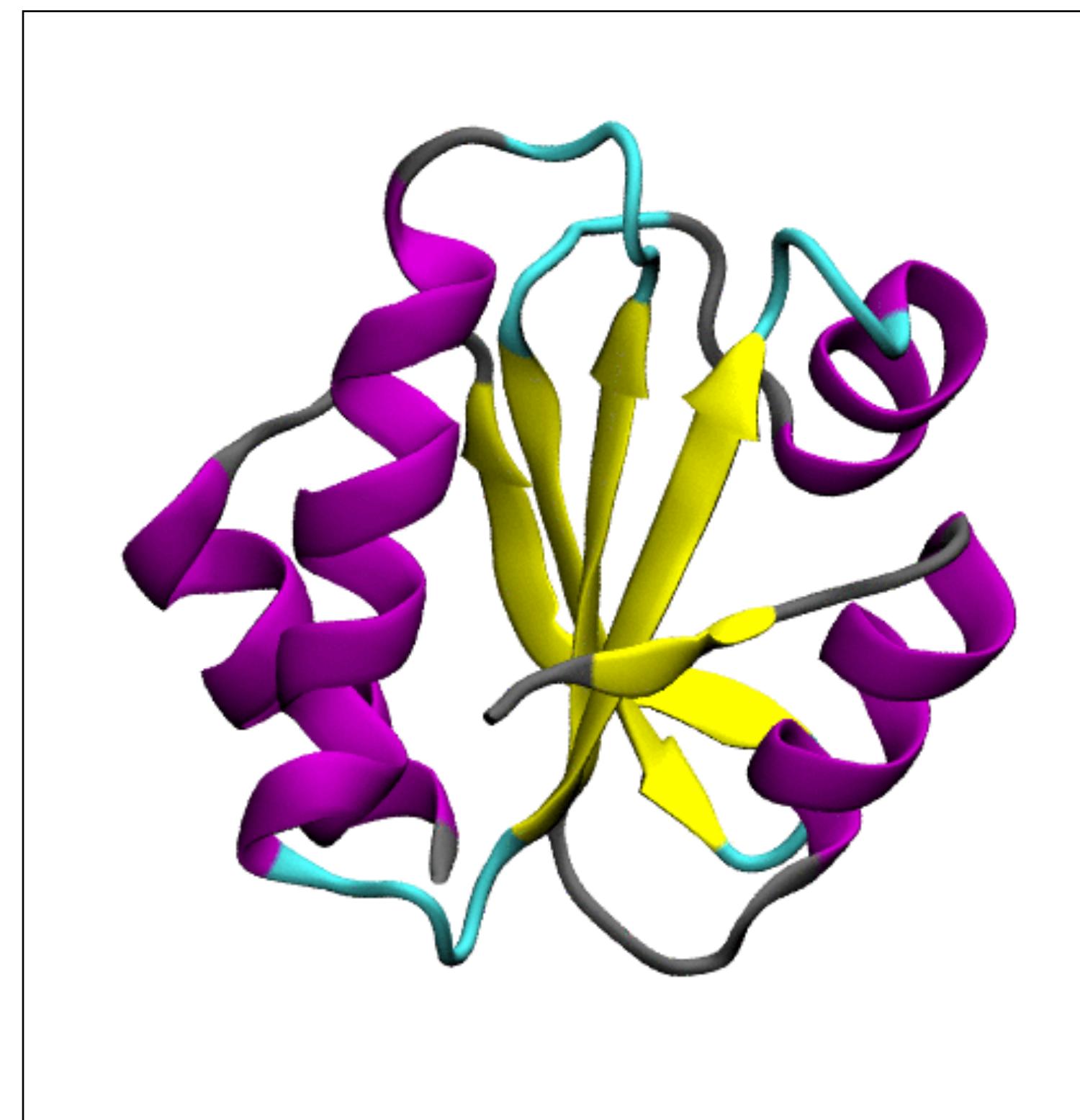
# Revealing hidden structures in the conformational ensemble of an intrinsically disordered protein



Lindsey Riggs  
Brannigan Lab  
4/15/25

# Intrinsically disordered proteins adopt many conformations

Structured



Yeast Thioredoxin

Unstructured

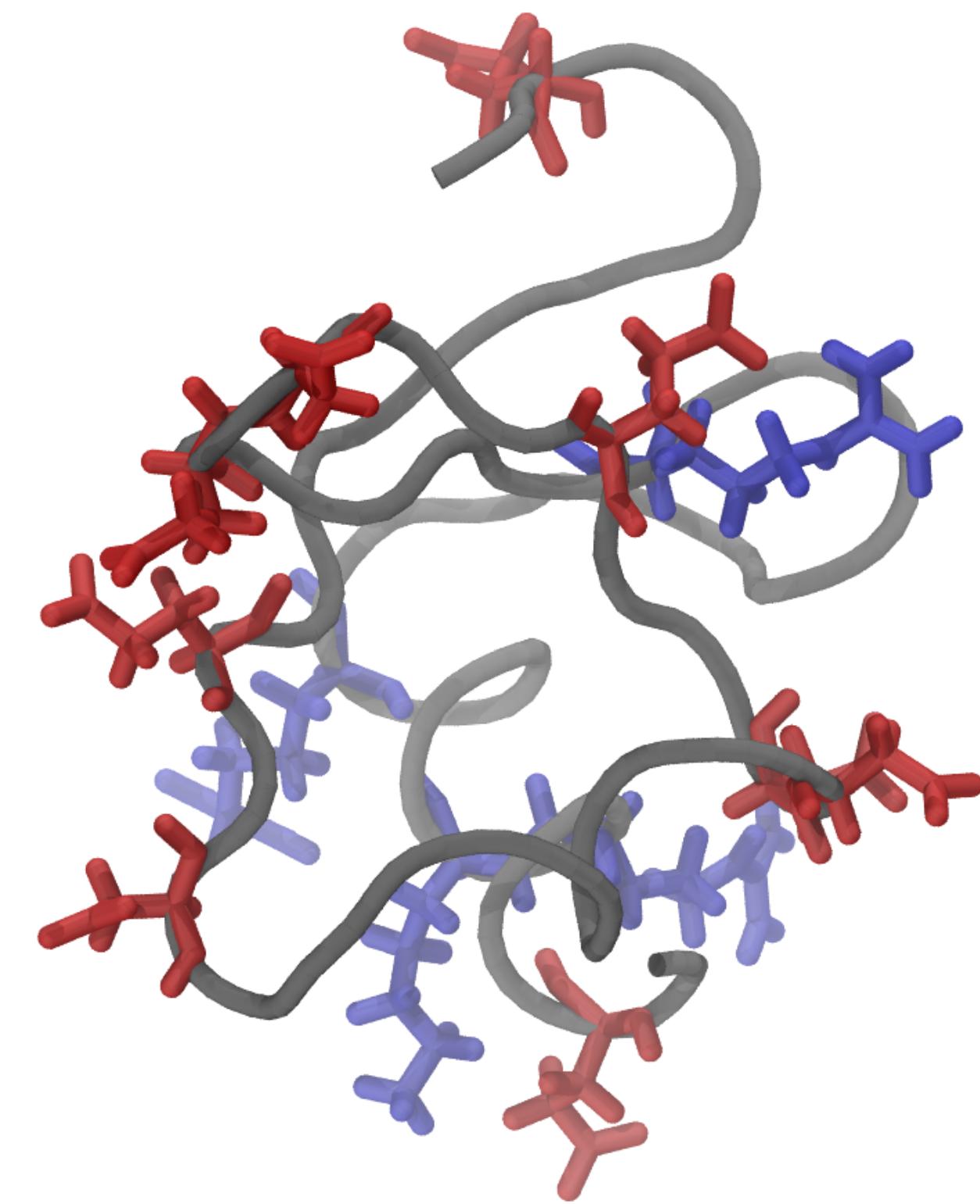


BDNF prodomain

Movie courtesy of Dr. Ruchi Lohia

# Intrinsically disordered proteins and regions (IDPs and IDR)s

- Play a role in many biological functions such as disease development and DNA regulation
- Contain many charged amino acids compared to structured proteins
- Highly dynamic and form a broad ensemble of conformations
- Many disease associated mutations are found in IDP/Rs ( $\alpha$ -syn, tau, p53)
- Demonstrates that even unstructured proteins are sensitive to subtle mutations



# Protein structure/function are sensitive to its sequence

- Missense single nucleotide polymorphisms (SNPs) can affect structure/function of proteins by altering intramolecular interactions.

Protein sequence → Structure → Function

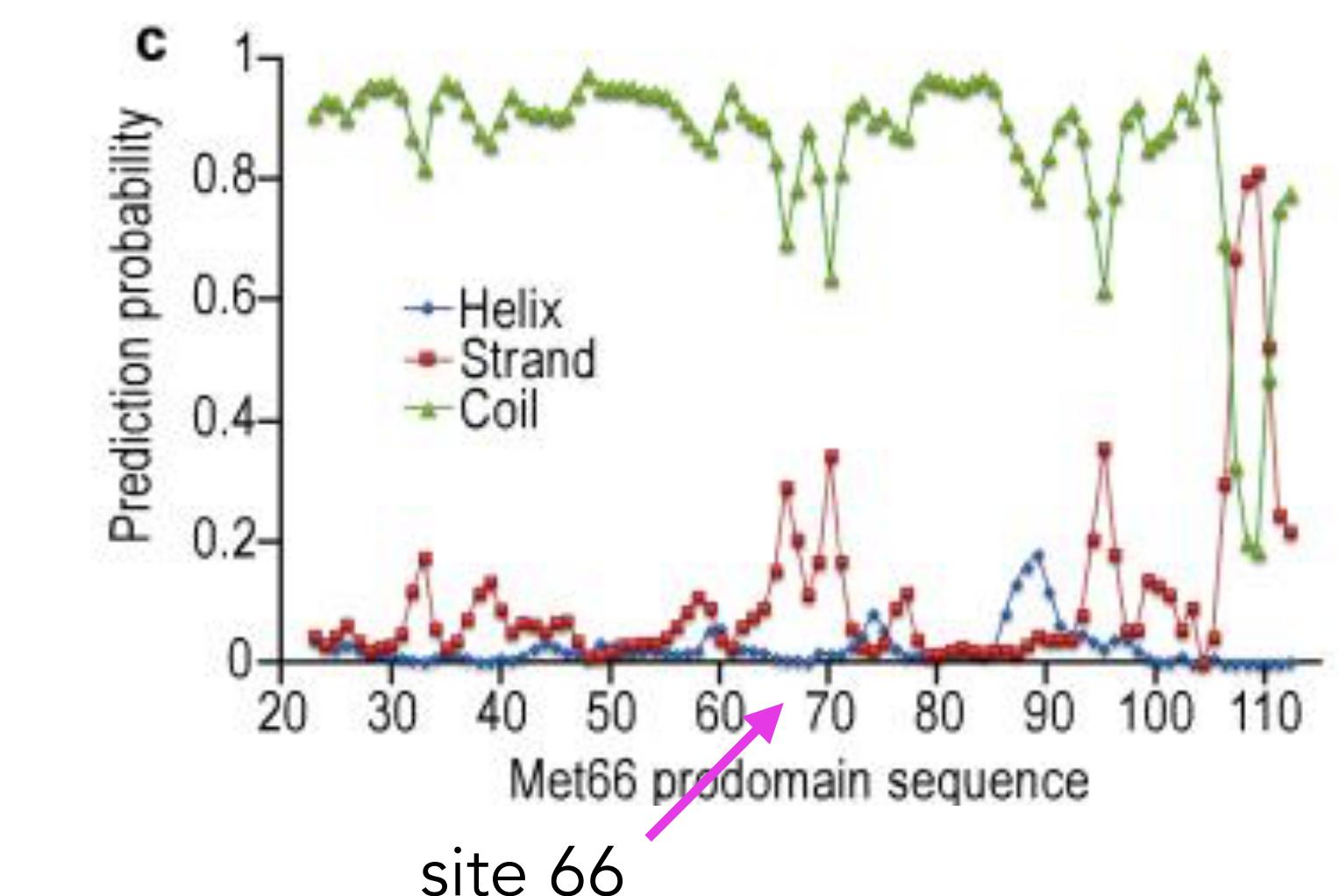
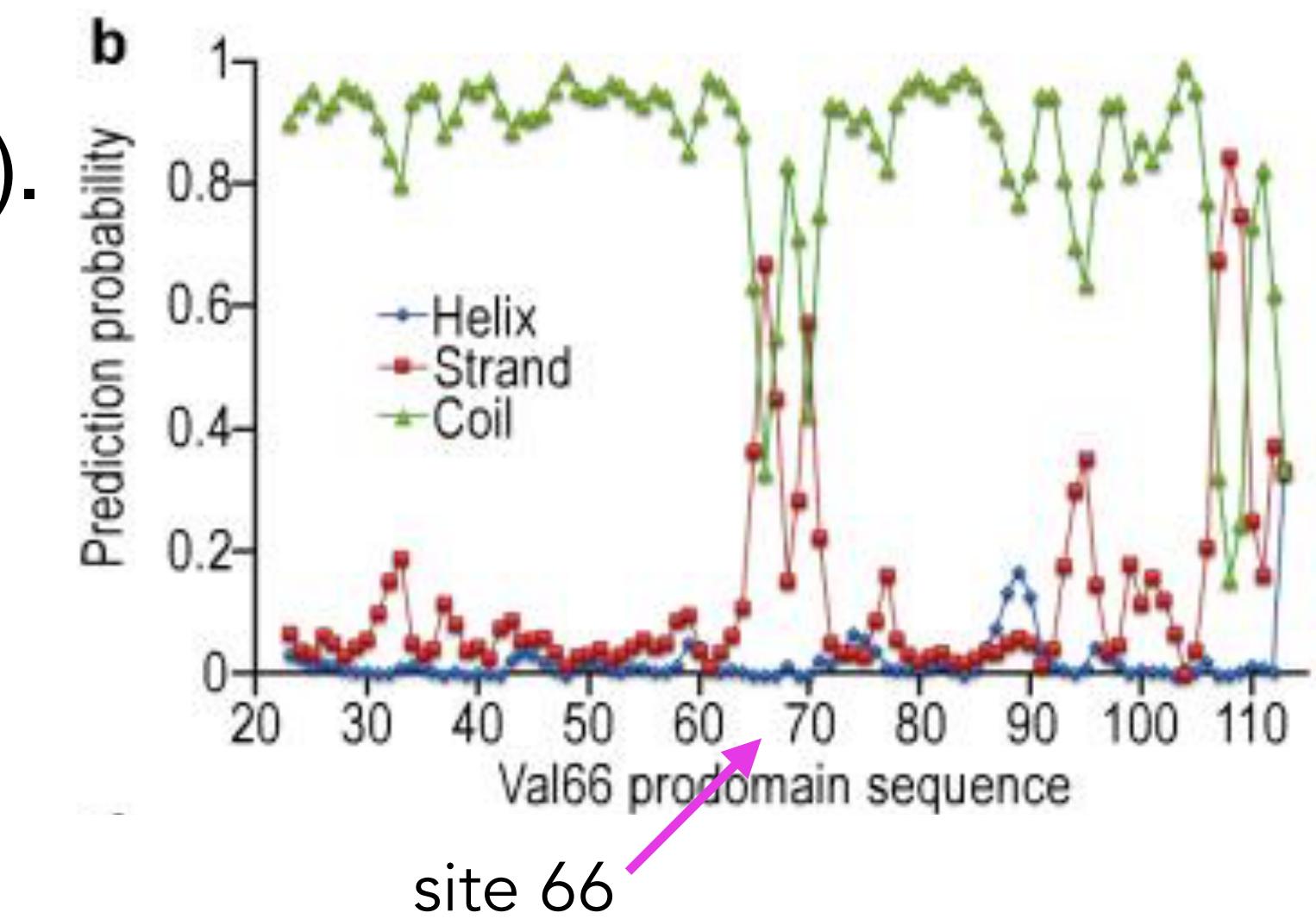
- For IDPs, which do not have a defined structure, it is unclear how SNPs alter its ensemble and function.

IDP sequence → ? Ensemble → ? Function

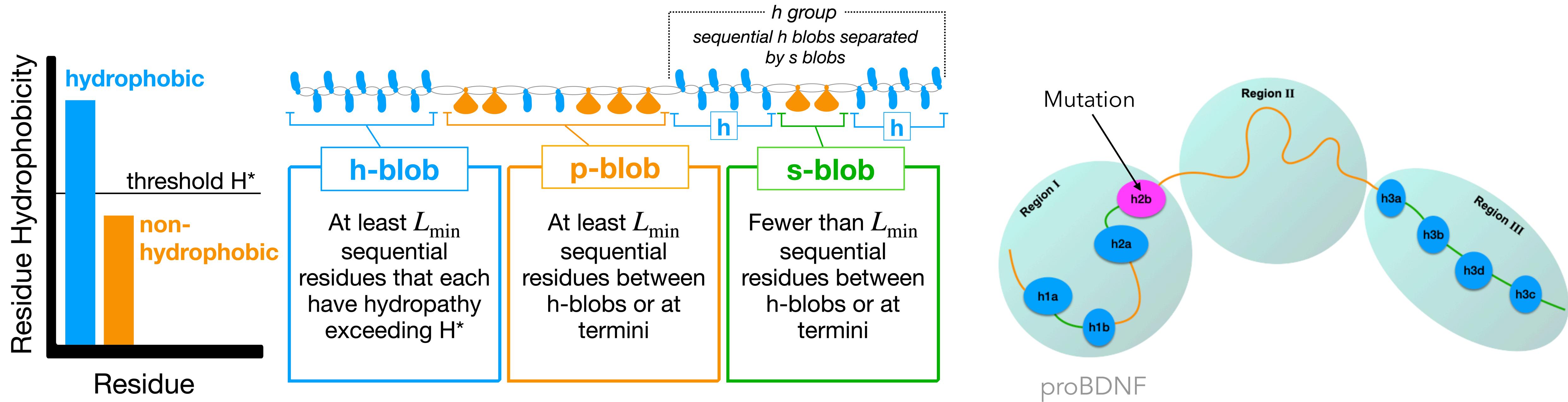
# Val66Met mutation shifts the conformational ensemble of the IDP proBDNF

- M66 affects the presence of secondary structural elements of the prodomain of Brain Derived Neurotropic Factor (proBDNF).
- Mutation causes neuronal growth retraction.
- Unexpected because Val and Met are both uncharged and hydrophobic amino acids.

**Our lab sought out to investigate the cause of the shift in proBDNF's ensemble.**



# Key interactions can be revealed by Blobulation

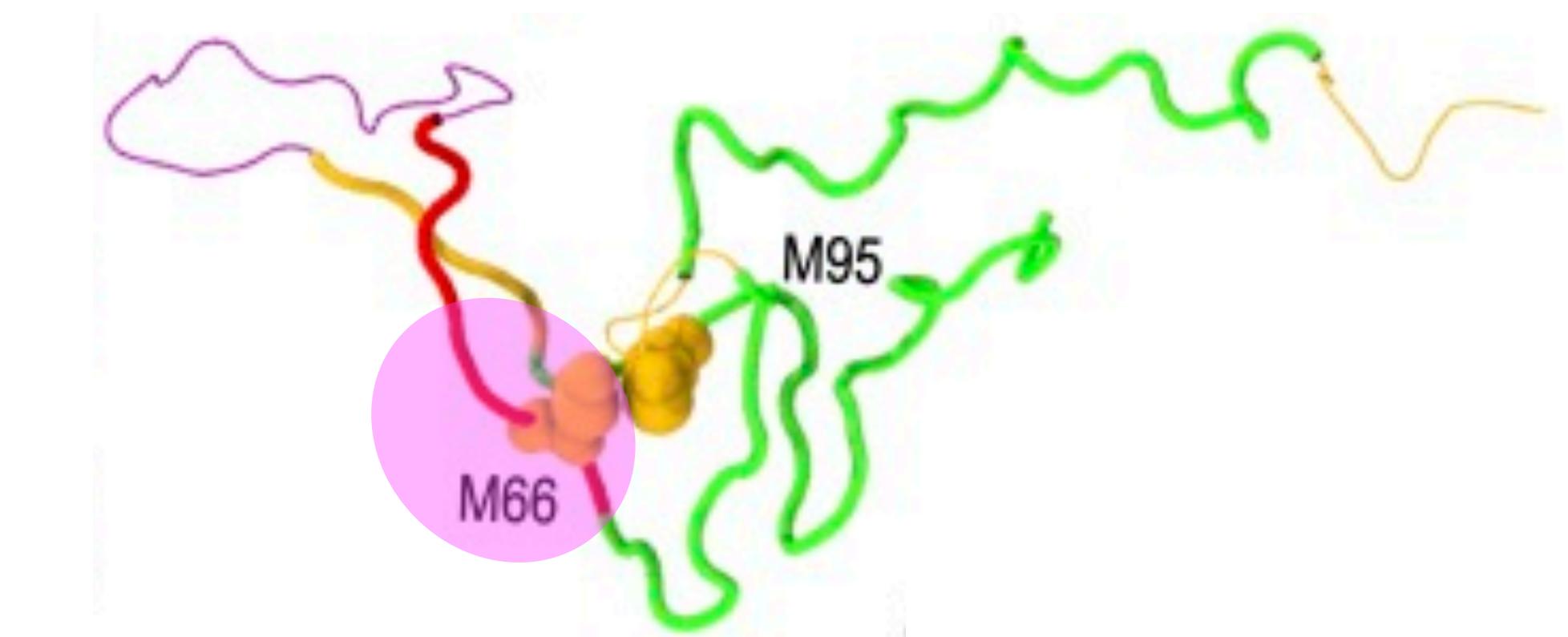
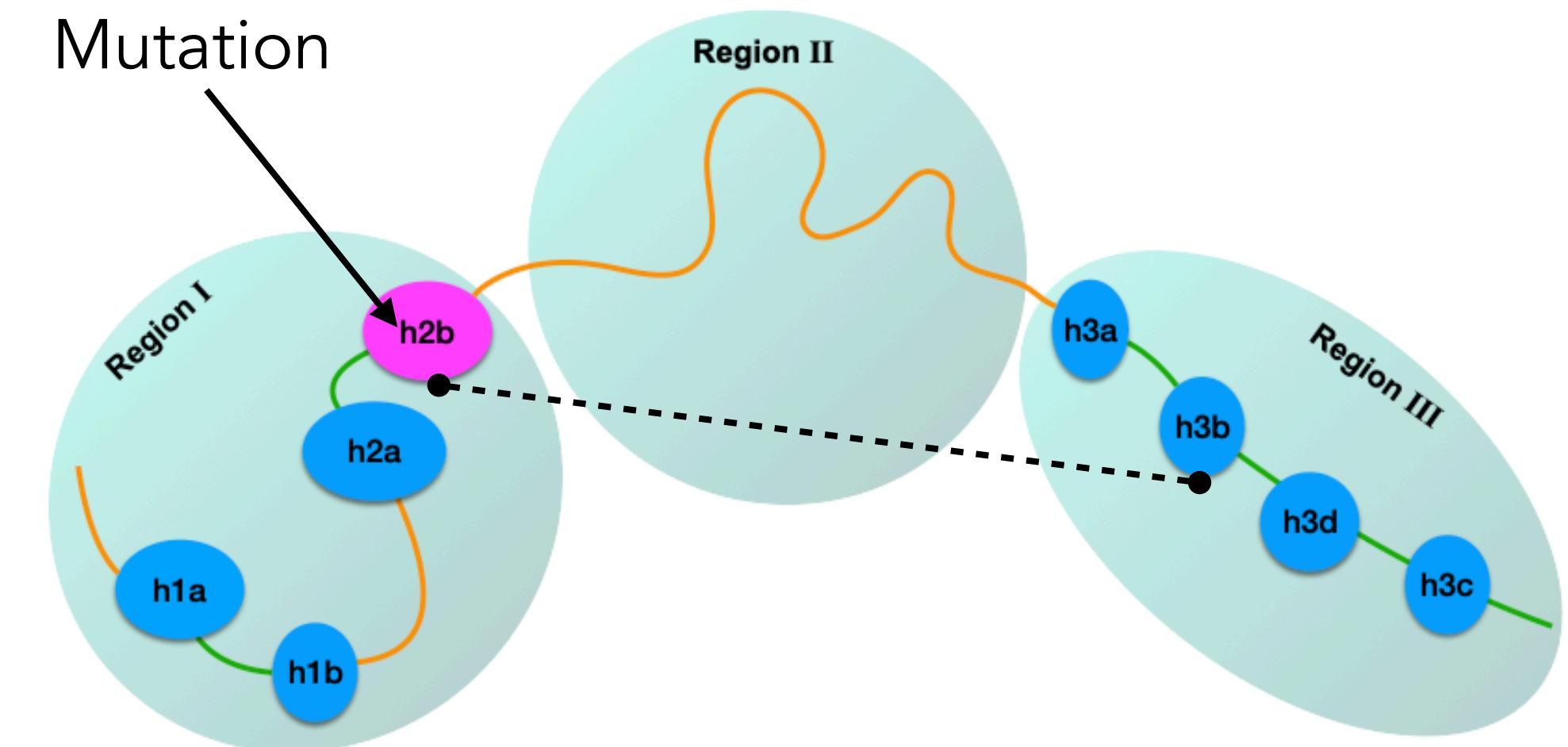


R. Lohia, M.E.B. Hansen, and G. Brannigan. *PNAS*, 2022.

Allows us to focus on the bigger picture

# M66 has a specific Met-Met interaction that is not possible in V66

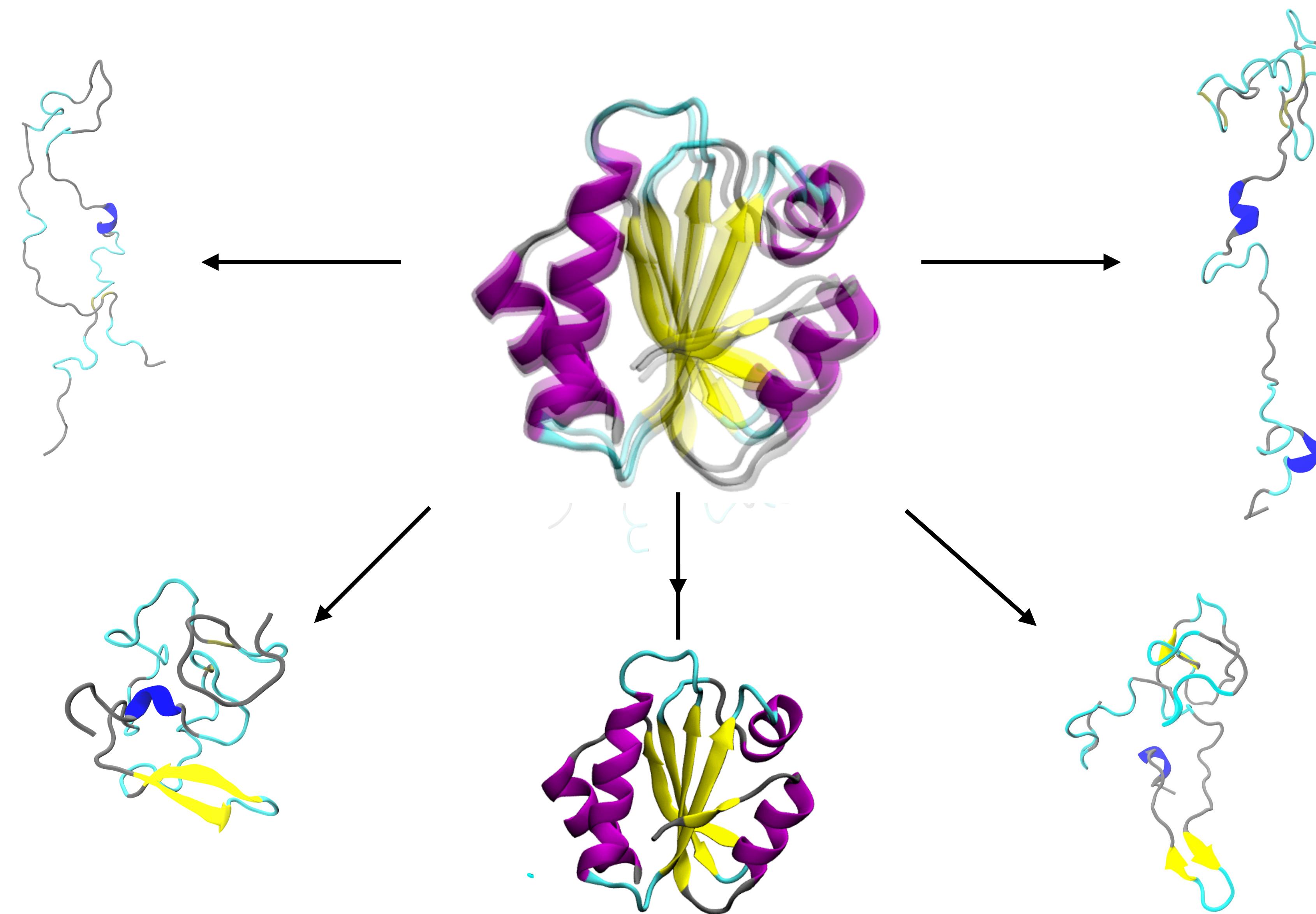
- Blob-based clustering revealed a key interaction between blobs h2b and h3b.
- Despite the mutation being uncharged, it alters tertiary interactions and compactness of the protein.
- Questioned whether conformational effects extend to other hydrophobic amino acids at site 66 of the sequence.



Lohia, Ruchi et al. PLOS Comp Bio, 2019

**Main Question:** Can the function of a “structureless” protein be generally sensitive to subtle changes in sequence? And if so, how?

# Clustering approach has the potential to filter conformations of a protein



# What makes a good clustering approach for IDPs?

1. Preserves key intramolecular interactions that make up the conformations.
2. Minimizes the number of clusters while representing the diversity of the ensemble.
3. Results in conformations that have the same compactness within a cluster.

Simulate 7 sequences with a different hydrophobic amino acid at site 66.

Site 66

RGLTSLADTFEHFIEELLDEDQKVR  
RGLTSLADTFEHMIEELLDEDQKVR  
RGLTSLADTFEHVIEELLDEDQKVR  
RGLTSLADTFEHLIEELLDEDQKVR  
RGLTSLADTFEAHIAEELLDEDQKVR  
RGLTSLADTFEHYIEELLDEDQKVR  
RGLTSLADTFEHIIIEELLDEDQKVR

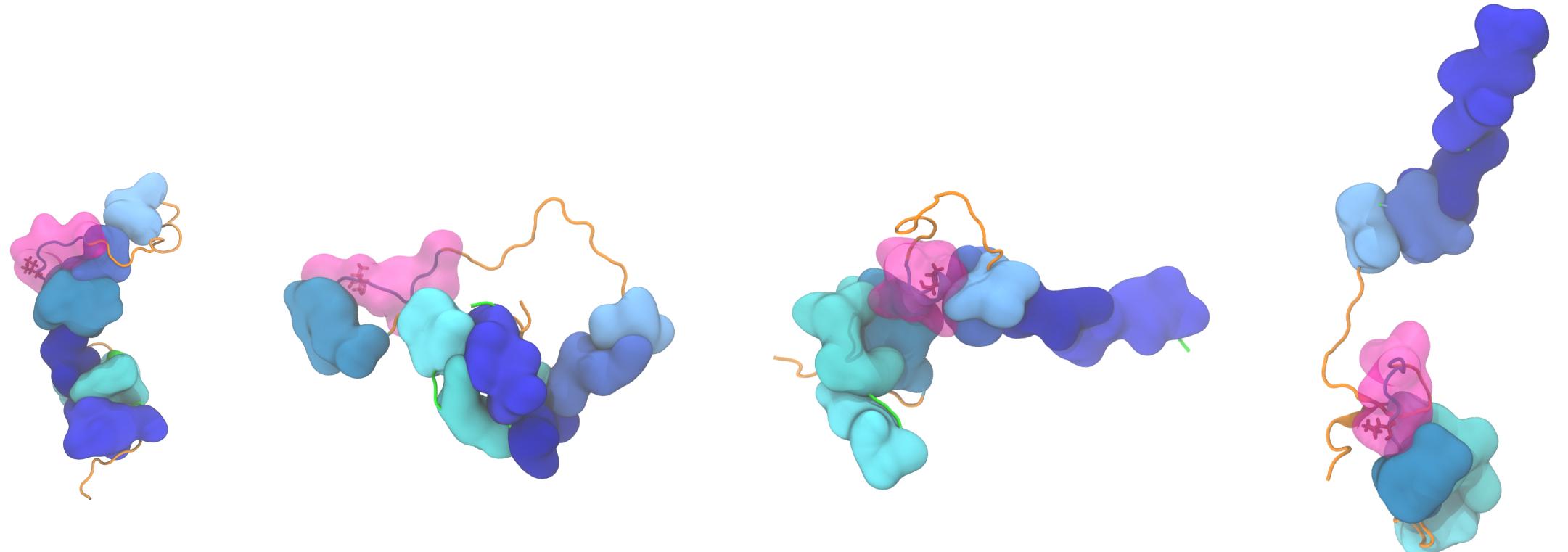
# Approach

Develop an approach for clustering conformations based on blob interactions



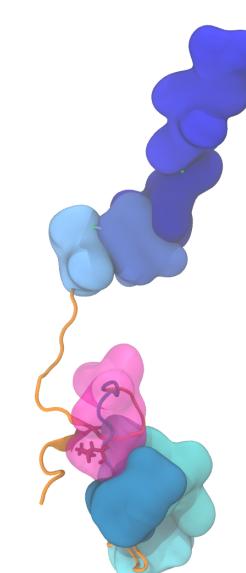
- Preserves key intramolecular interactions
- Minimizes the number of clusters
- Same compactness within a cluster

Determine the distribution of clusters in each sequence.

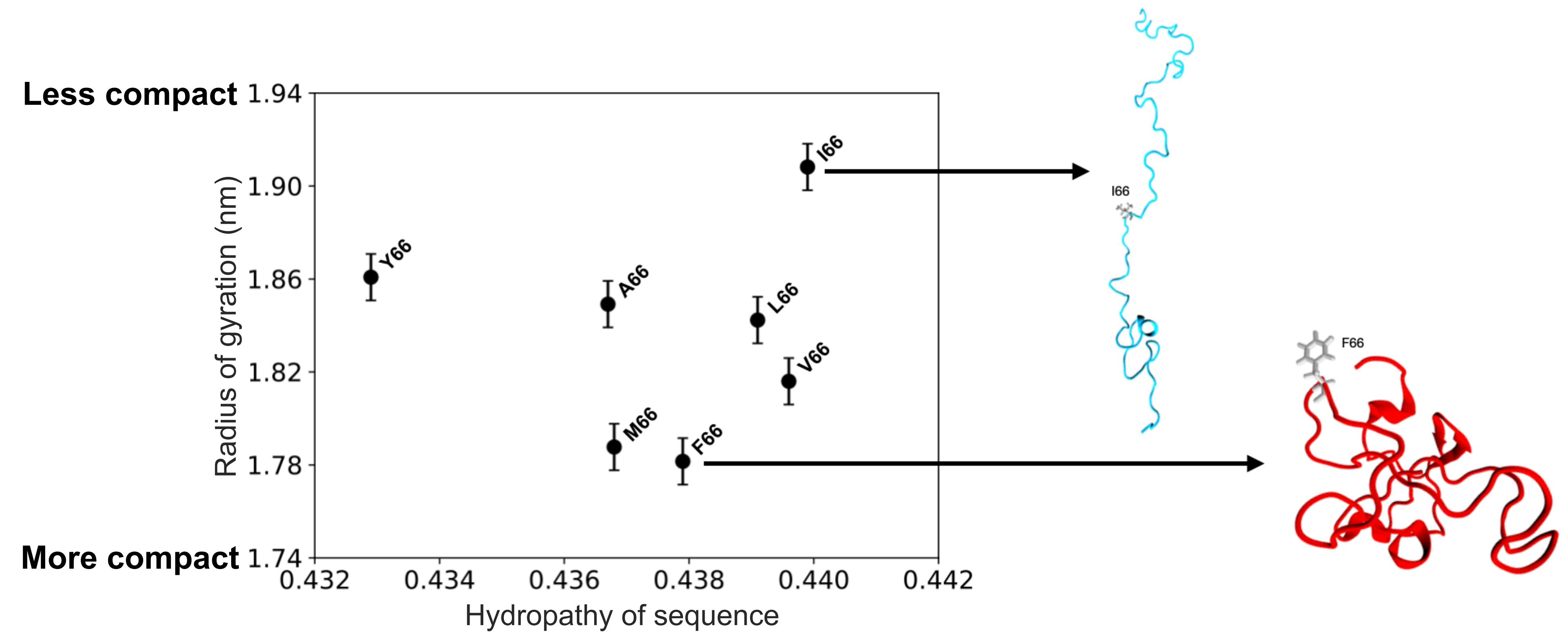


Determine if there is a correlation between cluster distribution and compactness of the protein.

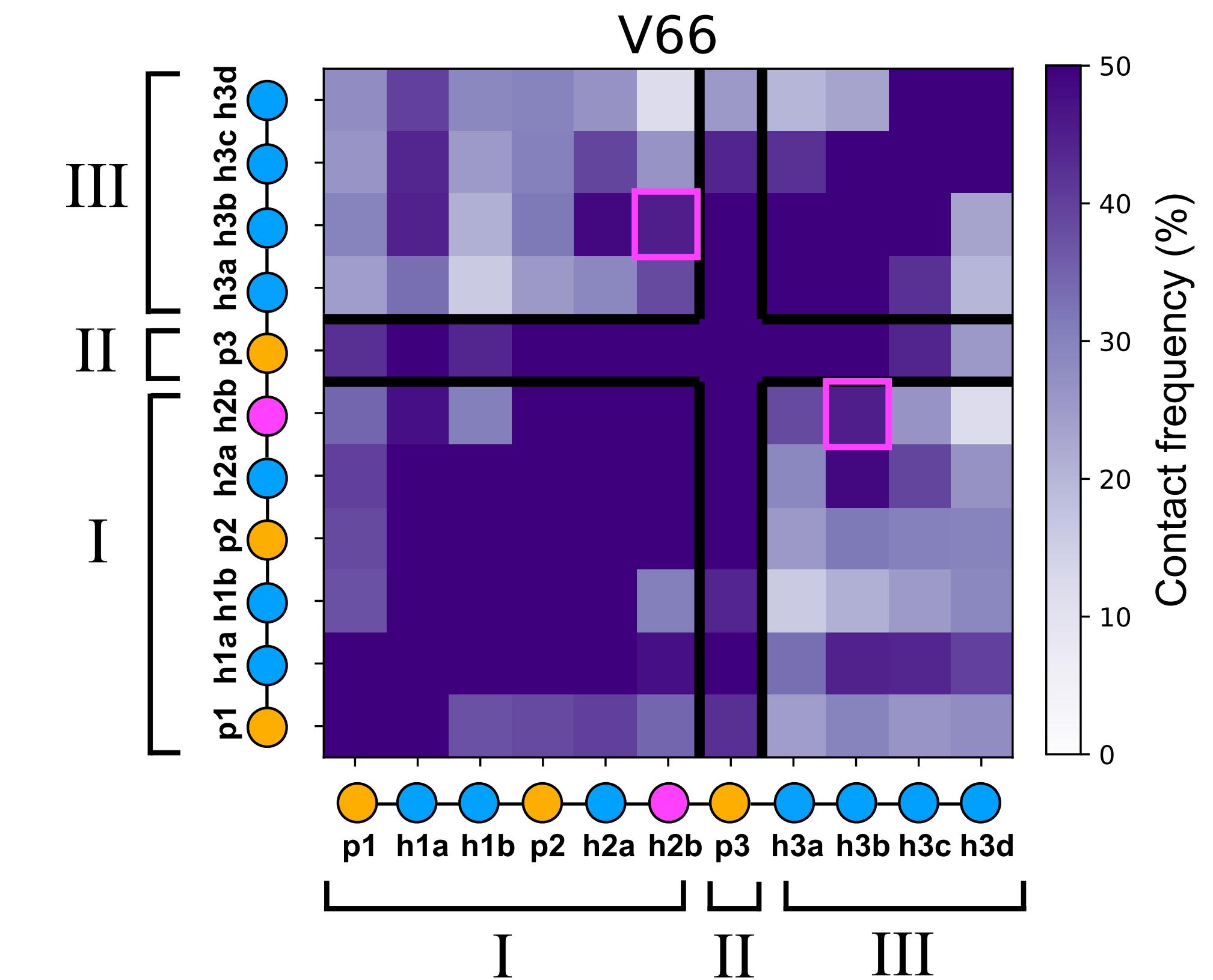
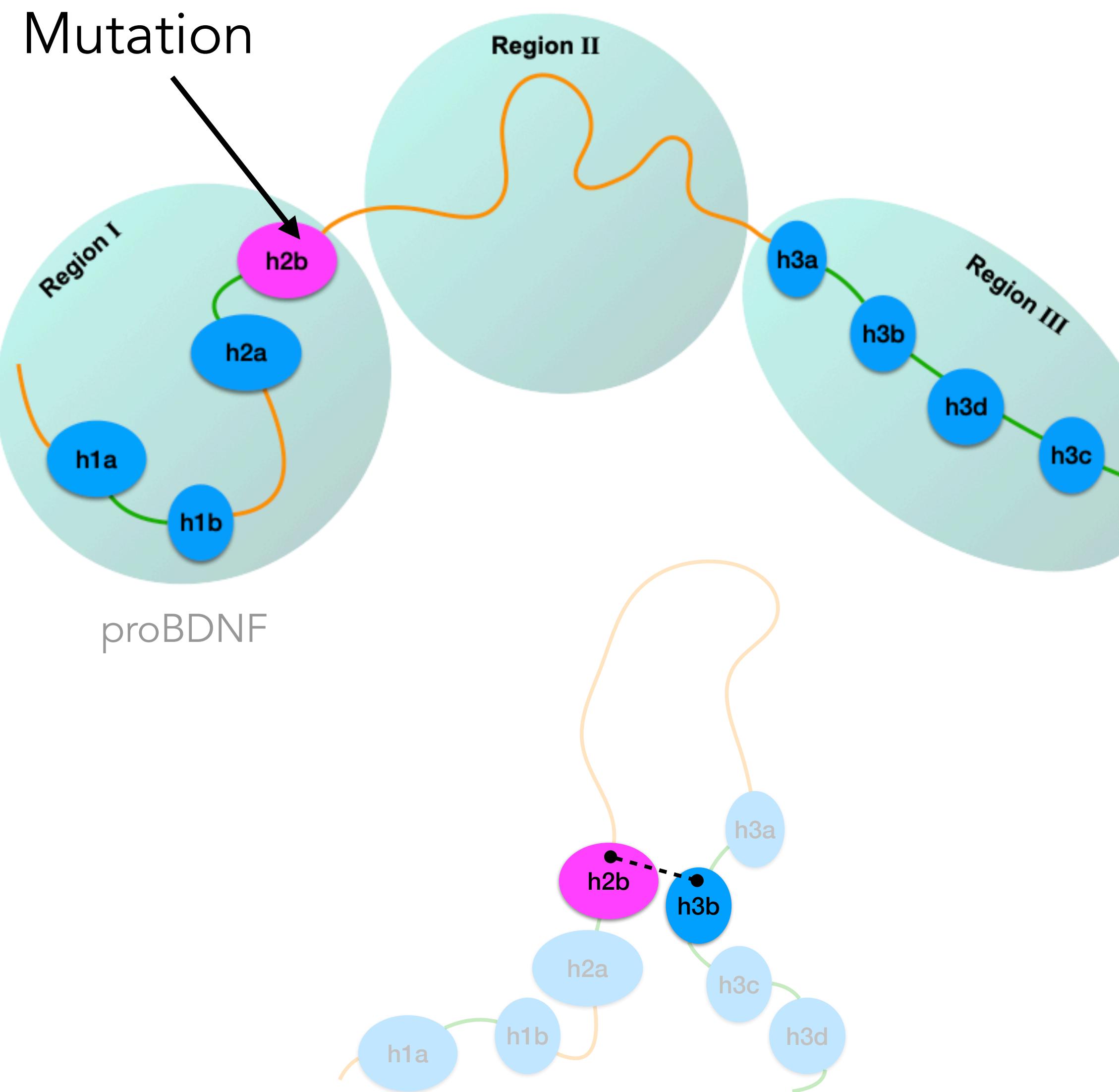
Less compact protein = more elongated conformations?



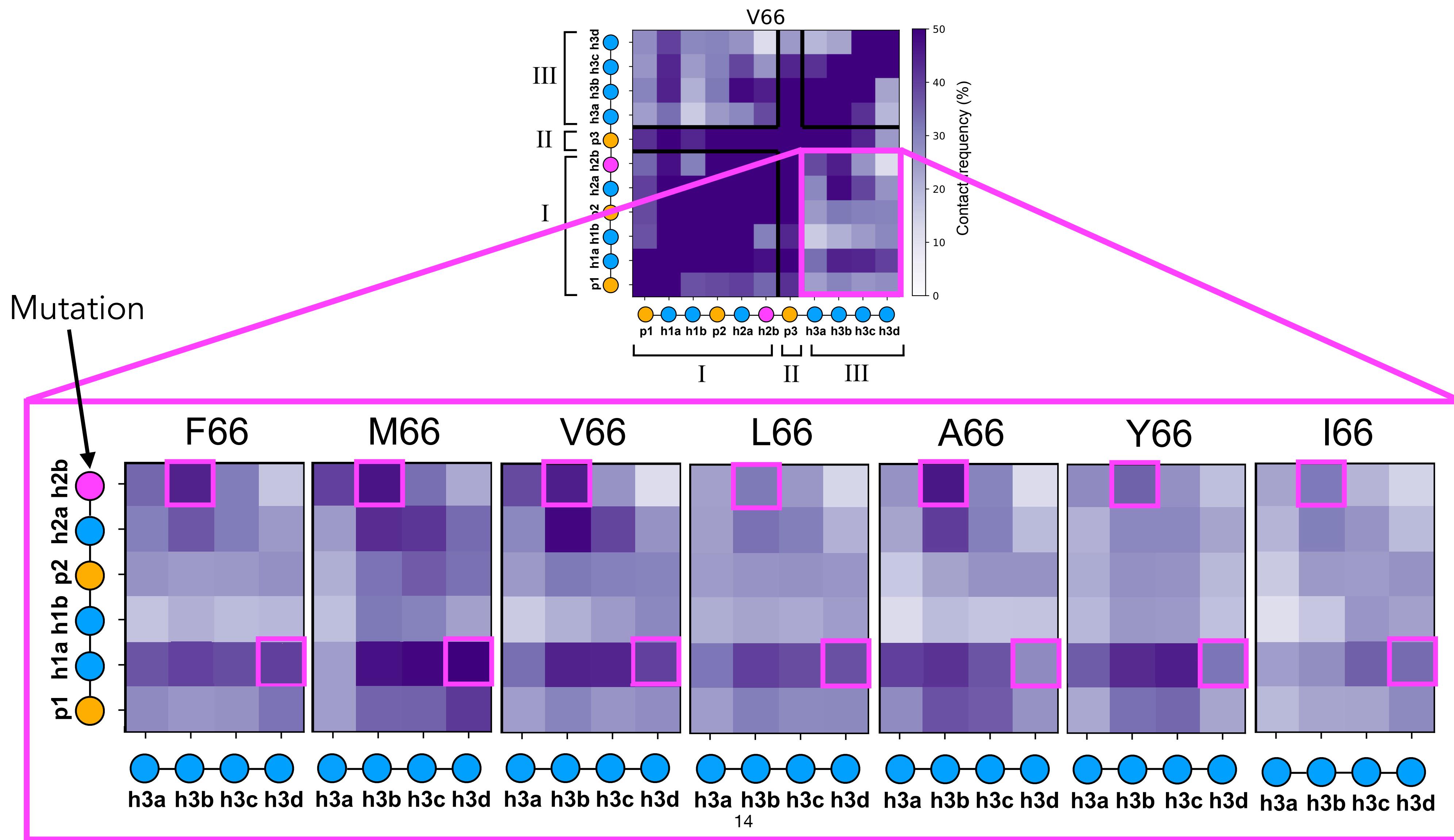
# Each mutation exerts a different effect on the ensemble of proBDNF



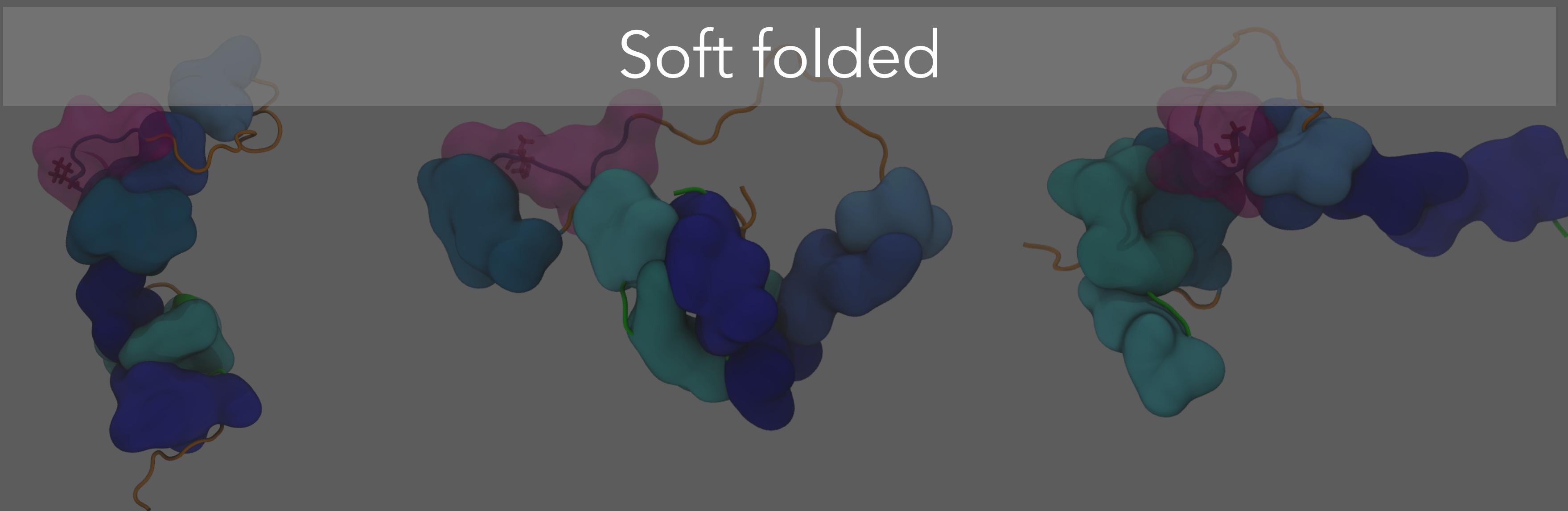
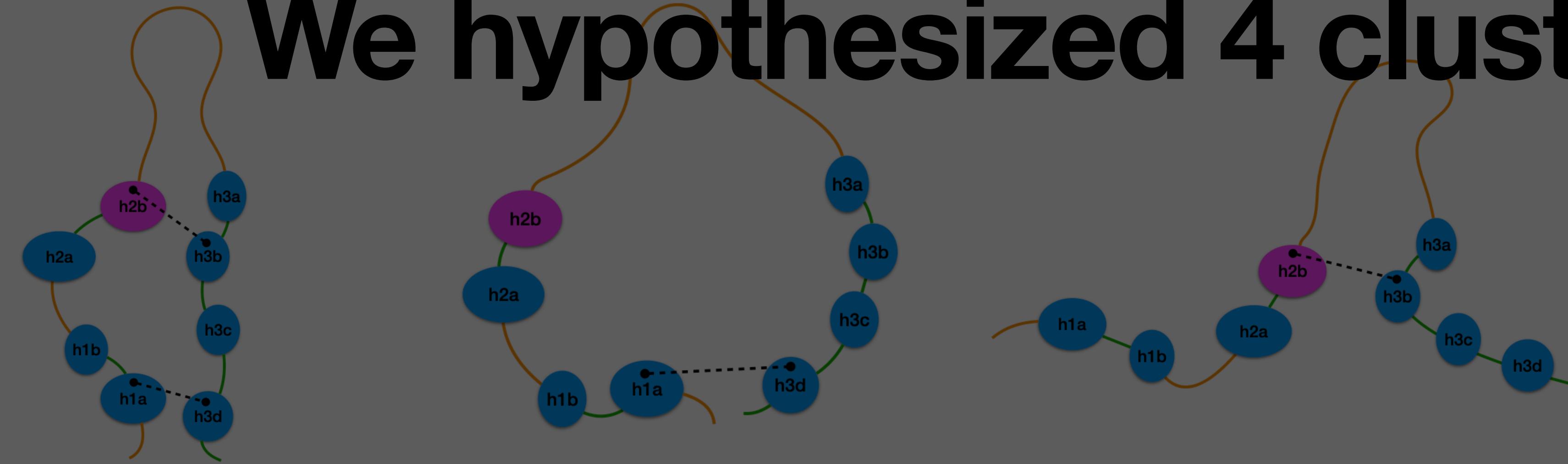
# Interactions between blobs influence tertiary interactions



# Mutations affect interactions between blobs



# We hypothesized 4 clusters

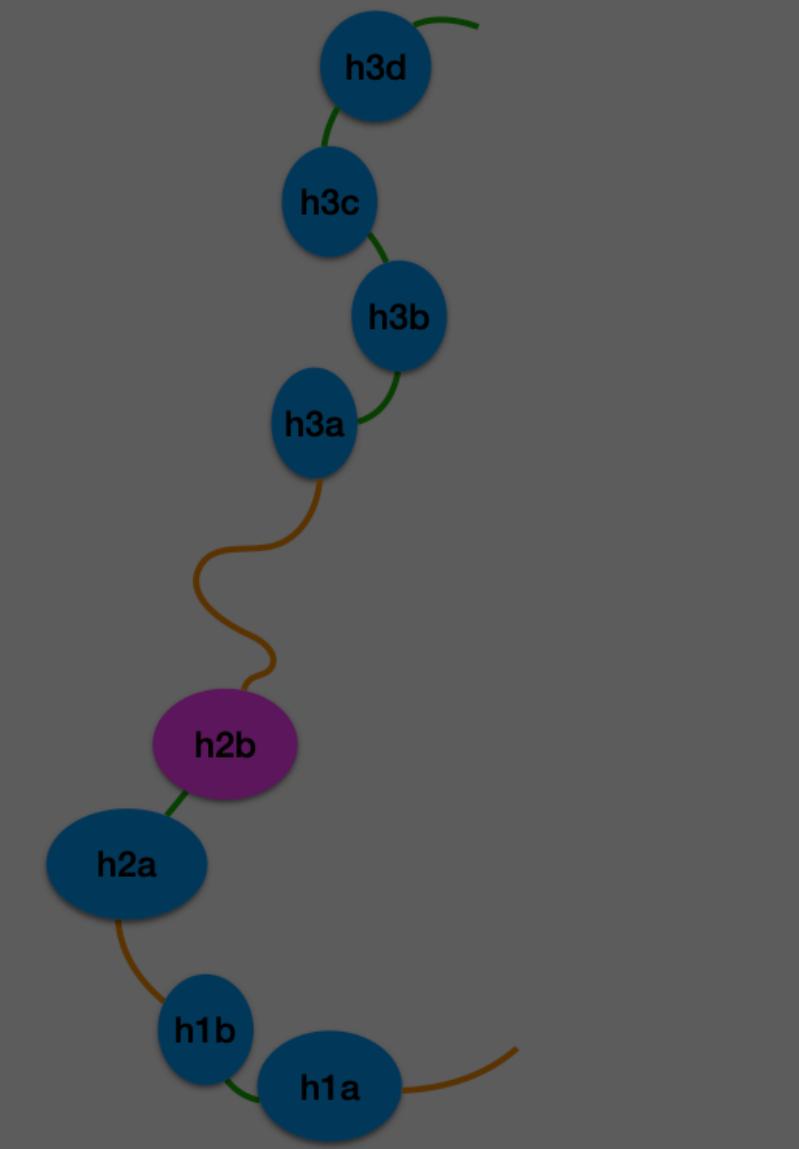


Pressed

Looped

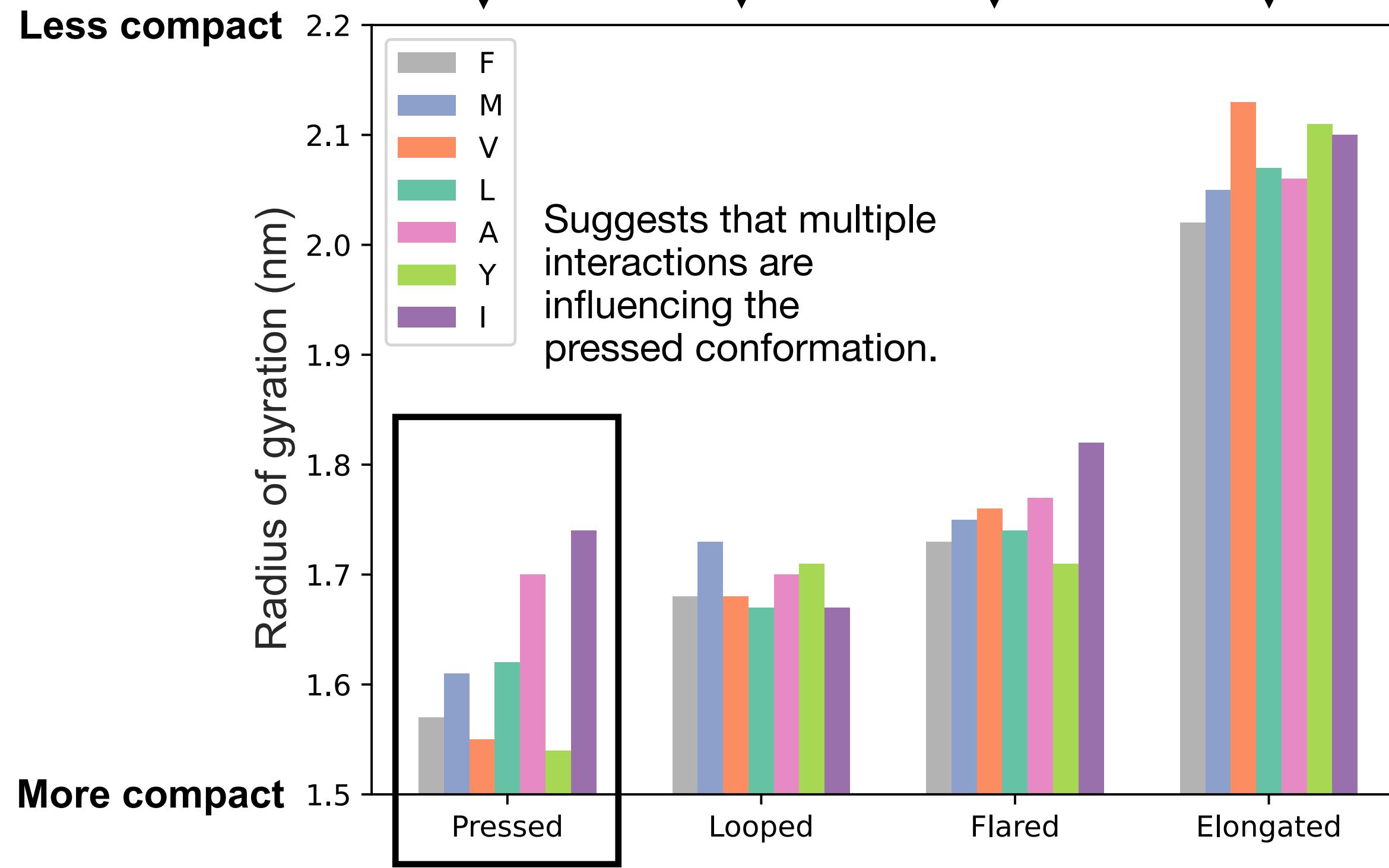
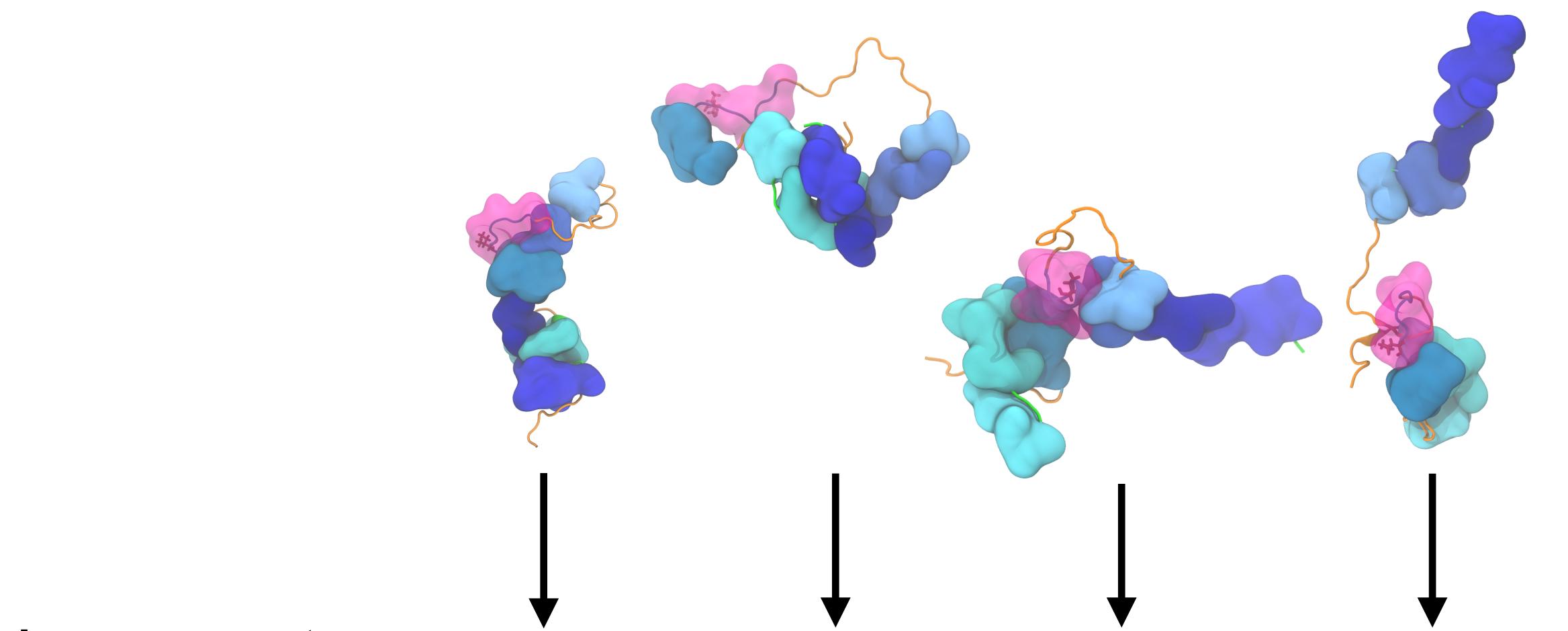
Flared

Elongated

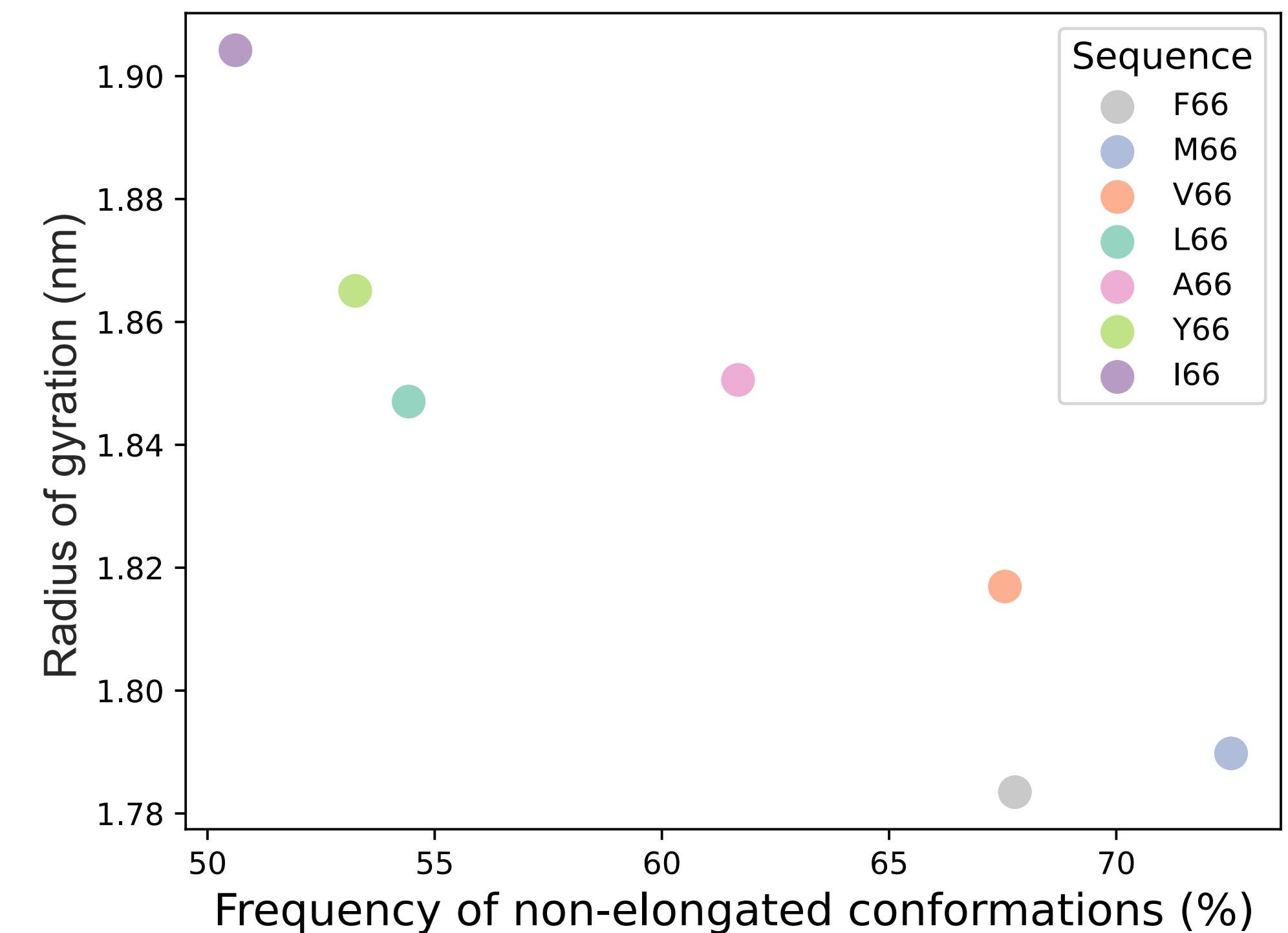


Unfolded

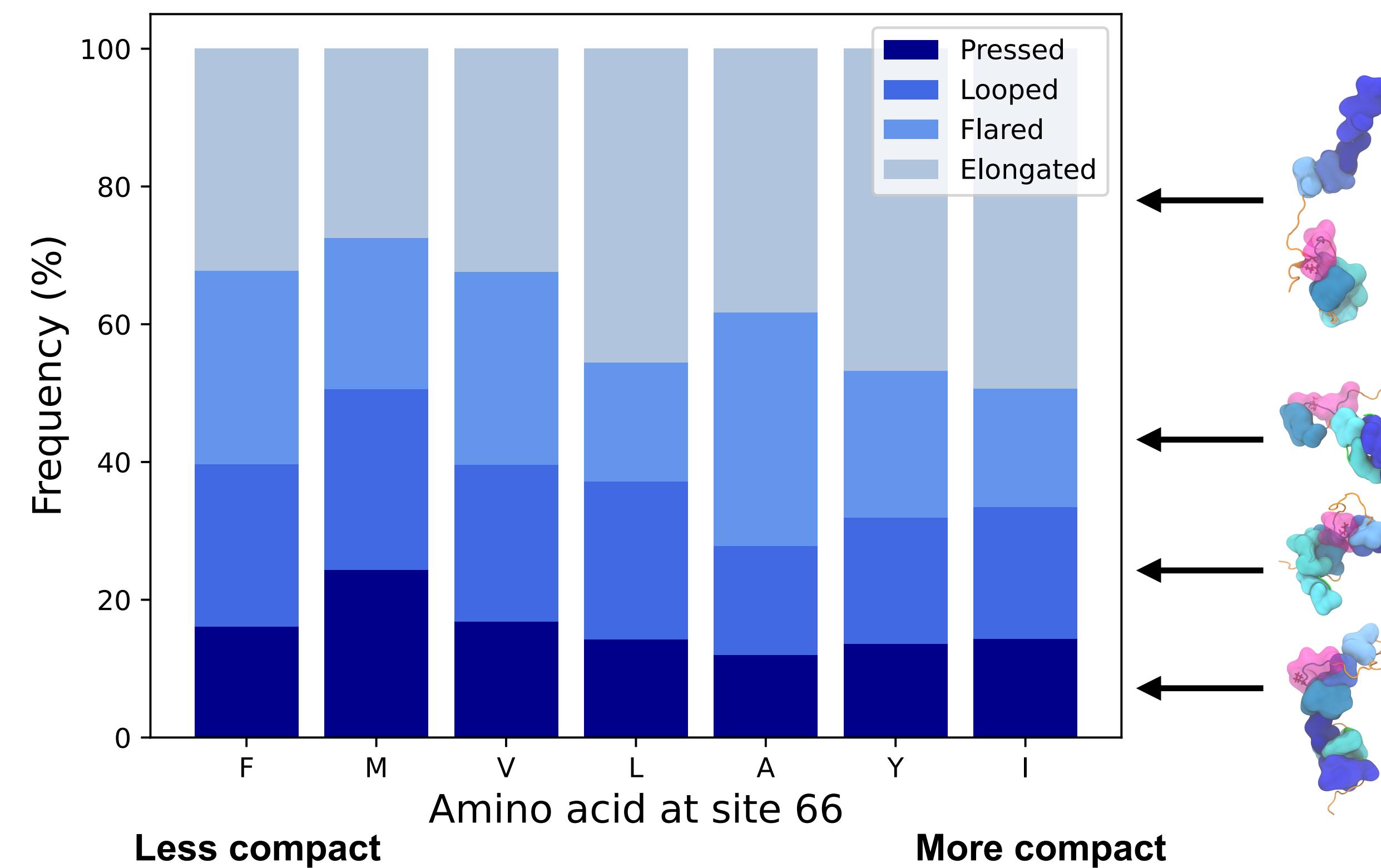
# Our clustering approach works



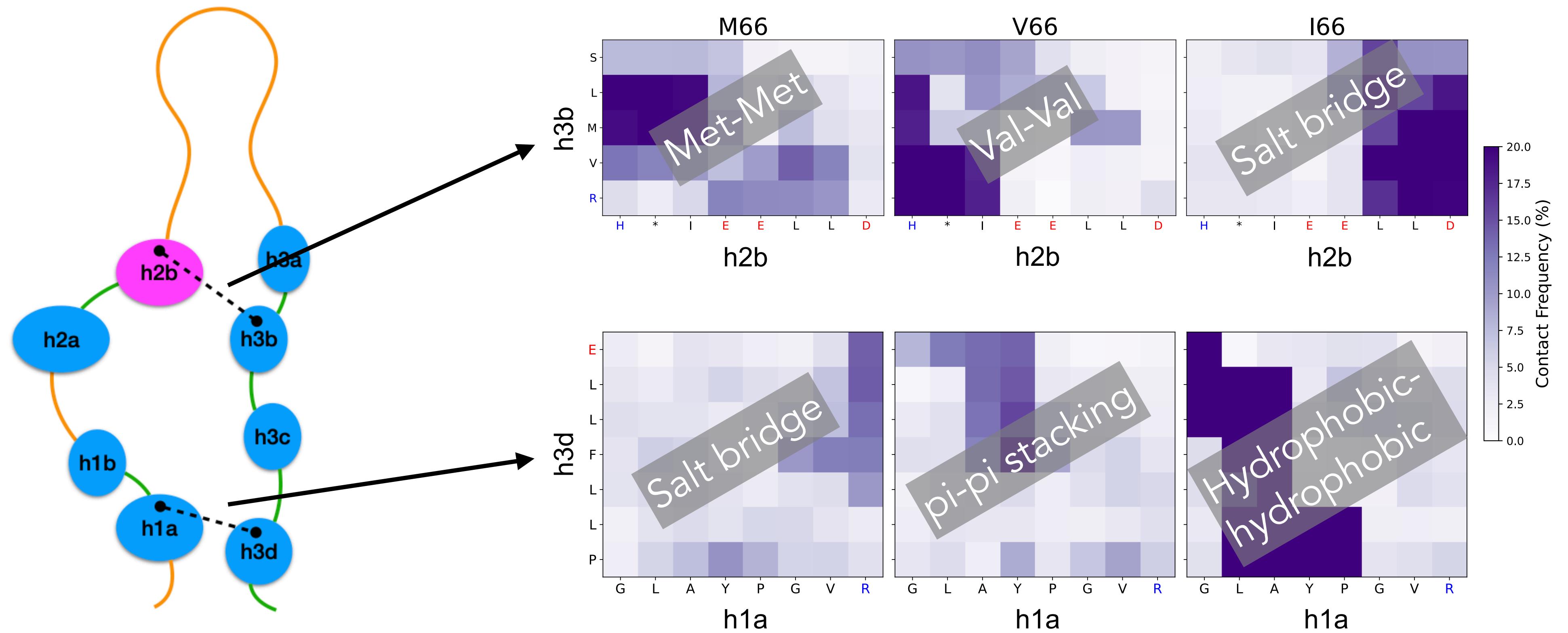
Correlation between compactness and % of non-elongated clusters



# Mutation affects frequency of clusters



# Residue interactions in the pressed conformation



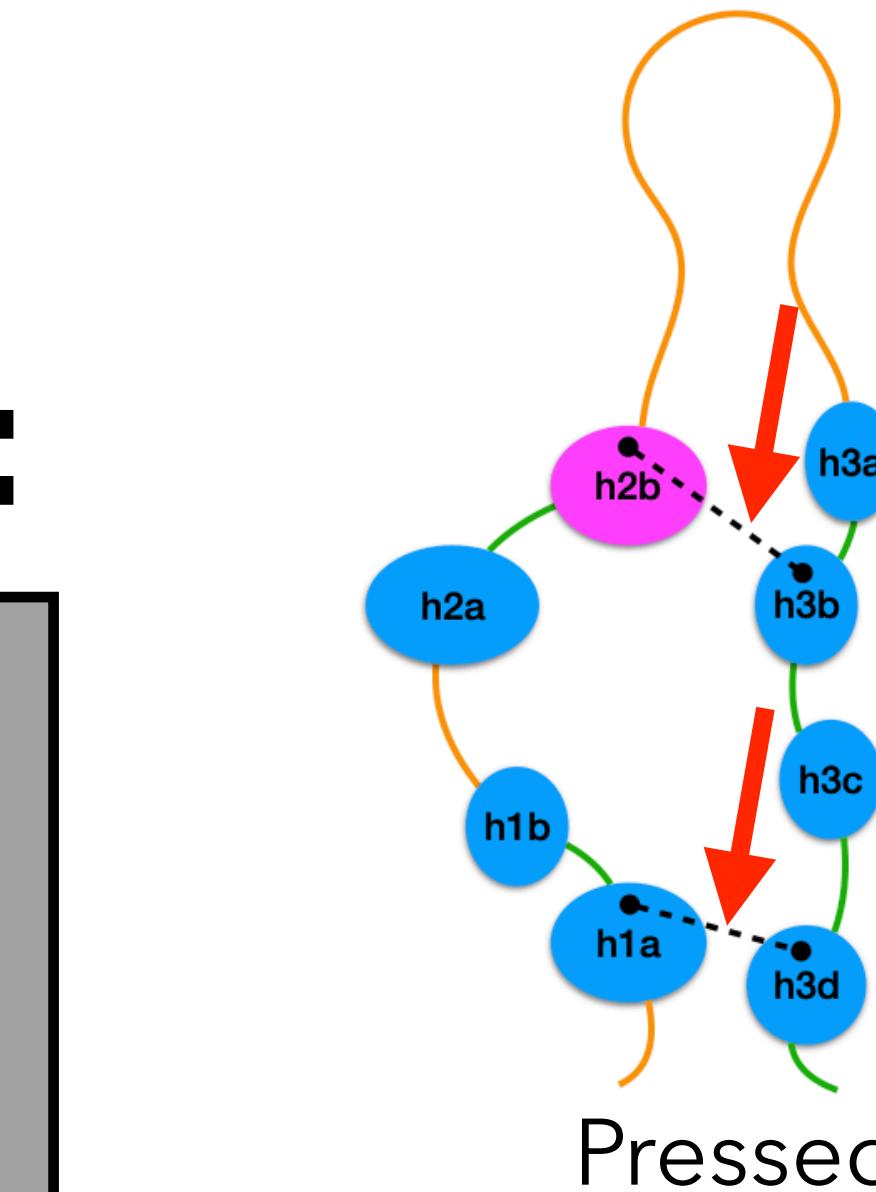
Different residue interactions  
influence pressed conformation

# Our clustering approach:

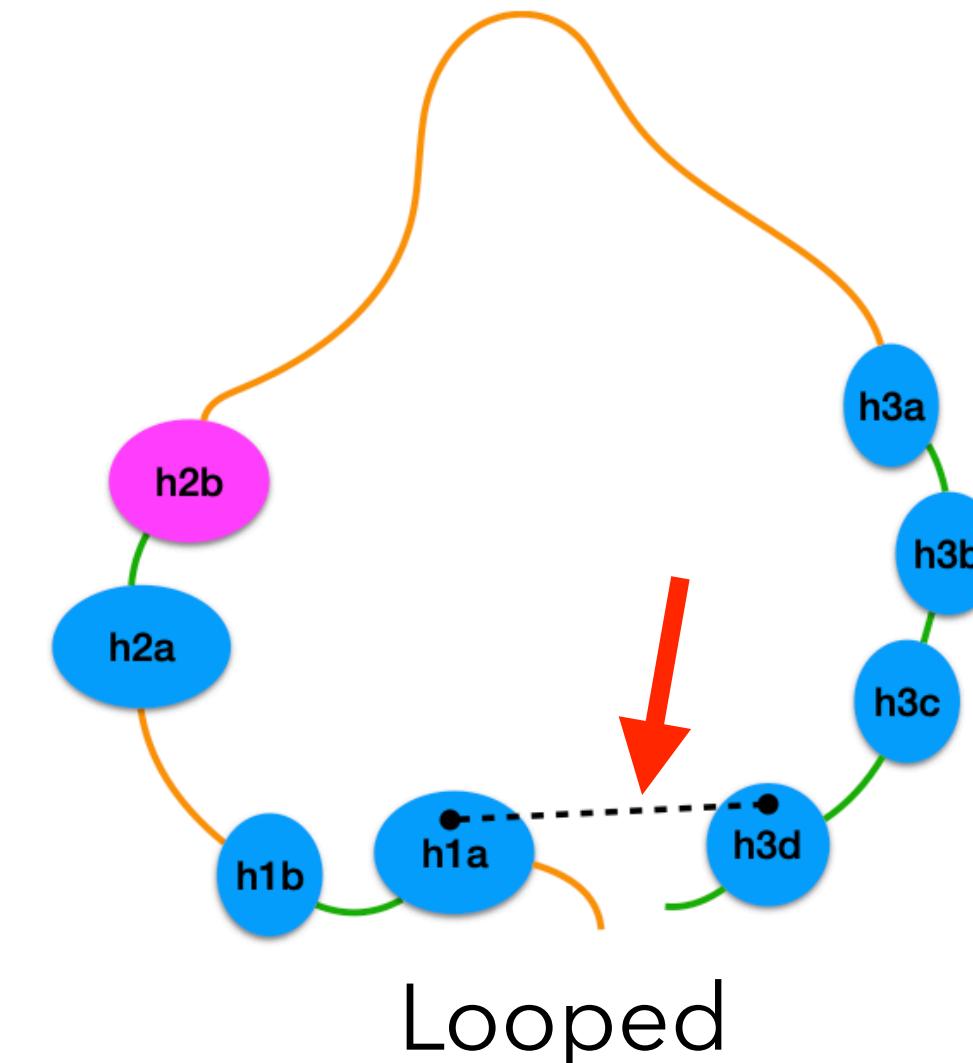
Preserves key intramolecular interactions

Minimizes the number of clusters

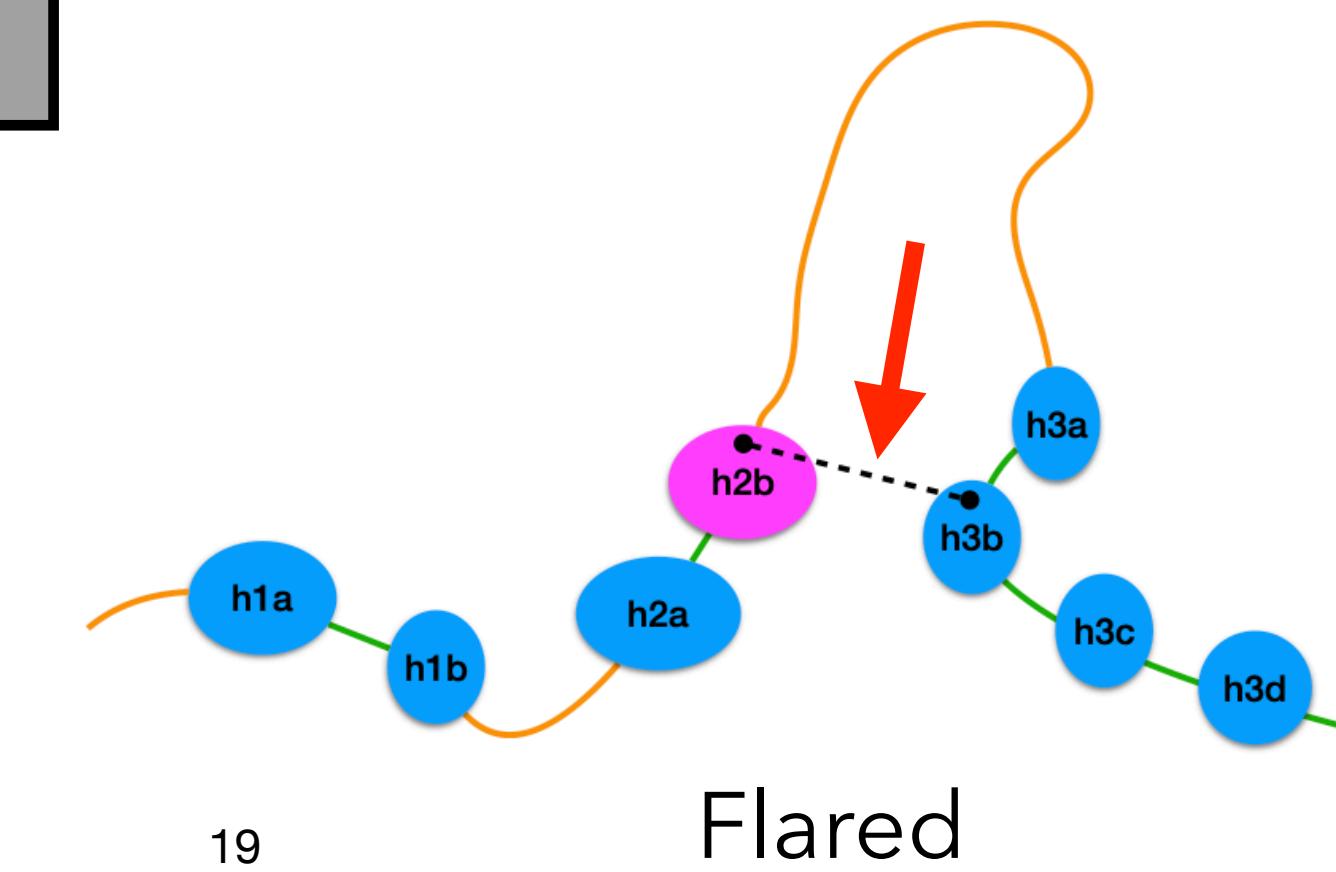
Same compactness within a cluster



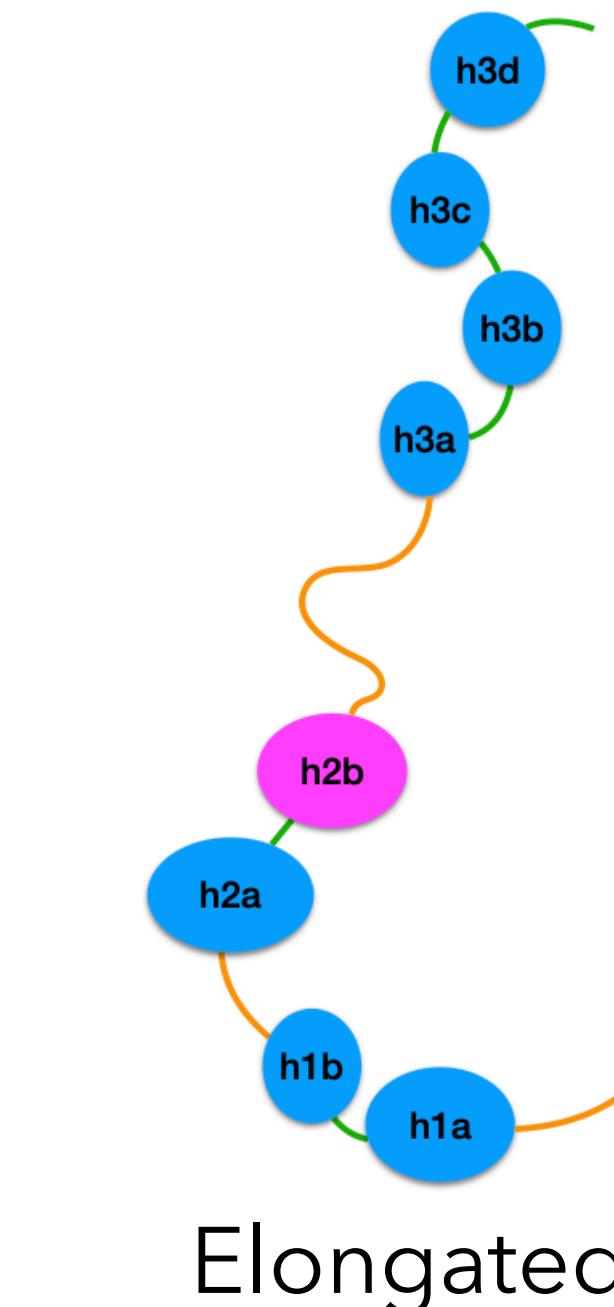
Pressed



Looped



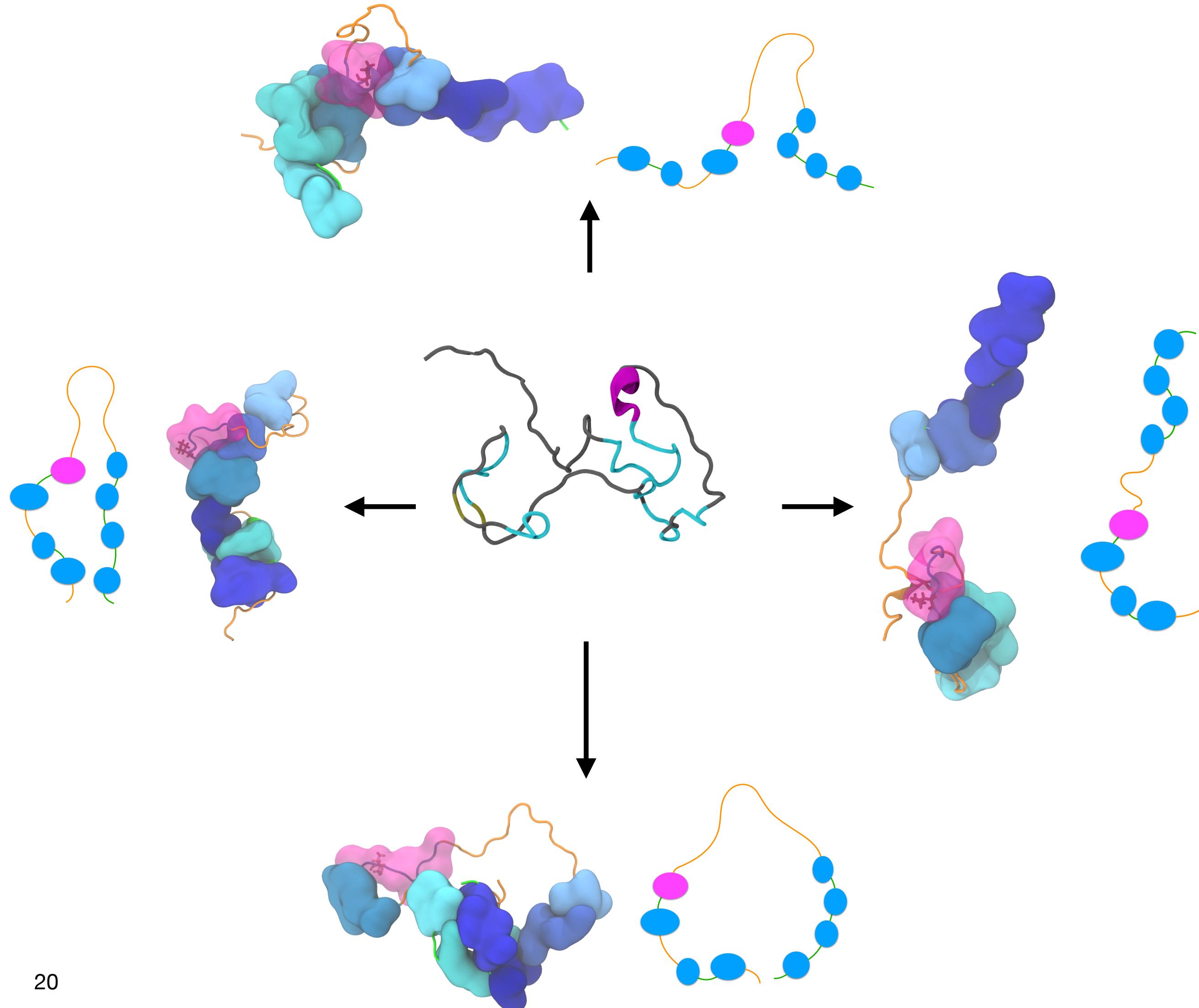
Flared



Elongated

# Summary: zooming out to see the big picture

- Conformational effects due to M66 sequence is not specific to Met-Met interaction.
- Ensemble of proBDNF can be clustered based on interactions between blobs.
- A subtle change in the sequence of proBDNF shifts frequency of clusters
- The frequency of unfolded and soft-folded clusters is anti-correlated to the compactness of the proBDNF.



# Thank you!

## Lab members:

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Ryan Lamb

Asim Dave

Regina Salzer

## Resources

Rutgers Office of Advanced  
Research and Computing (OARC)  
NRT, NSF DGE 2152059

