

Elucidating the Properties of Hydrophobic Clusters on Tertiary Interactions in Model Peptides

By: Ryan Lamb

Pathogenic Missense Mutations

- Pathogenic missense mutations are a frequent cause of pathogenic phenotypes
- Pathogenic missense mutation have been the cause for diseases such as cancer, neurodegeneration, and diabetes

Mutation in the alpha-synuclein gene identified in families with Parkinson's disease

M H Polymeropoulos ¹, C Lavedan, E Leroy, S E Ide, A Dehejia, A Dutra, B Pike, H Root, J Rubenstein, R Boyer, E S Stenroos, S Chandrasekharappa, A Athanasiadou, T Papapetropoulos, W G Johnson, A M Lazzarini, R C Duvoisin, G Di Iorio, L I Golbe, R L Nussbaum

Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17

M Hutton ¹, C L Lendon, P Rizzu, M Baker, S Froelich, H Houlden, S Pickering-Brown, S Chakraverty, A Isaacs, A Grover, J Hackett, J Adamson, S Lincoln, D Dickson, P Davies, R C Petersen, M Stevens, E de Graaff, E Wauters, J van Baren, M Hillebrand, M Joosse, J M Kwon, P Nowotny, L K Che, J Norton, J C Morris, L A Reed, J Trojanowski, H Basun, L Lannfelt, M Neystat, S Fahn, F Dark, T Tannenberg, P R Dodd, N Hayward, J B Kwok, P R Schofield, A Andreadis, J Snowden, D Craufurd, D Neary, F Owen, B A Oostra, J Hardy, A Goate, J van Swieten, D Mann, T Lynch, P Heutink

Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction

Agustin Anastasia ¹, Katrin Deinhardt, Moses V Chao, Nathan E Will, Krithi Irmady, Francis S Lee, Barbara L Hempstead, Clay Bracken

Insulin gene mutations as a cause of permanent neonatal diabetes

Julie Støy ¹, Emma L Edghill, Sarah E Flanagan, Honggang Ye, Veronica P Paz, Anna Pluzhnikov, Jennifer E Below, M Geoffrey Hayes, Nancy J Cox, Gregory M Lipkind, Rebecca B Lipton, Siri Atma W Greeley, Ann-Marie Patch, Sian Ellard, Donald F Steiner, Andrew T Hattersley, Louis H Philipson, Graeme I Bell; Neonatal Diabetes International Collaborative Group

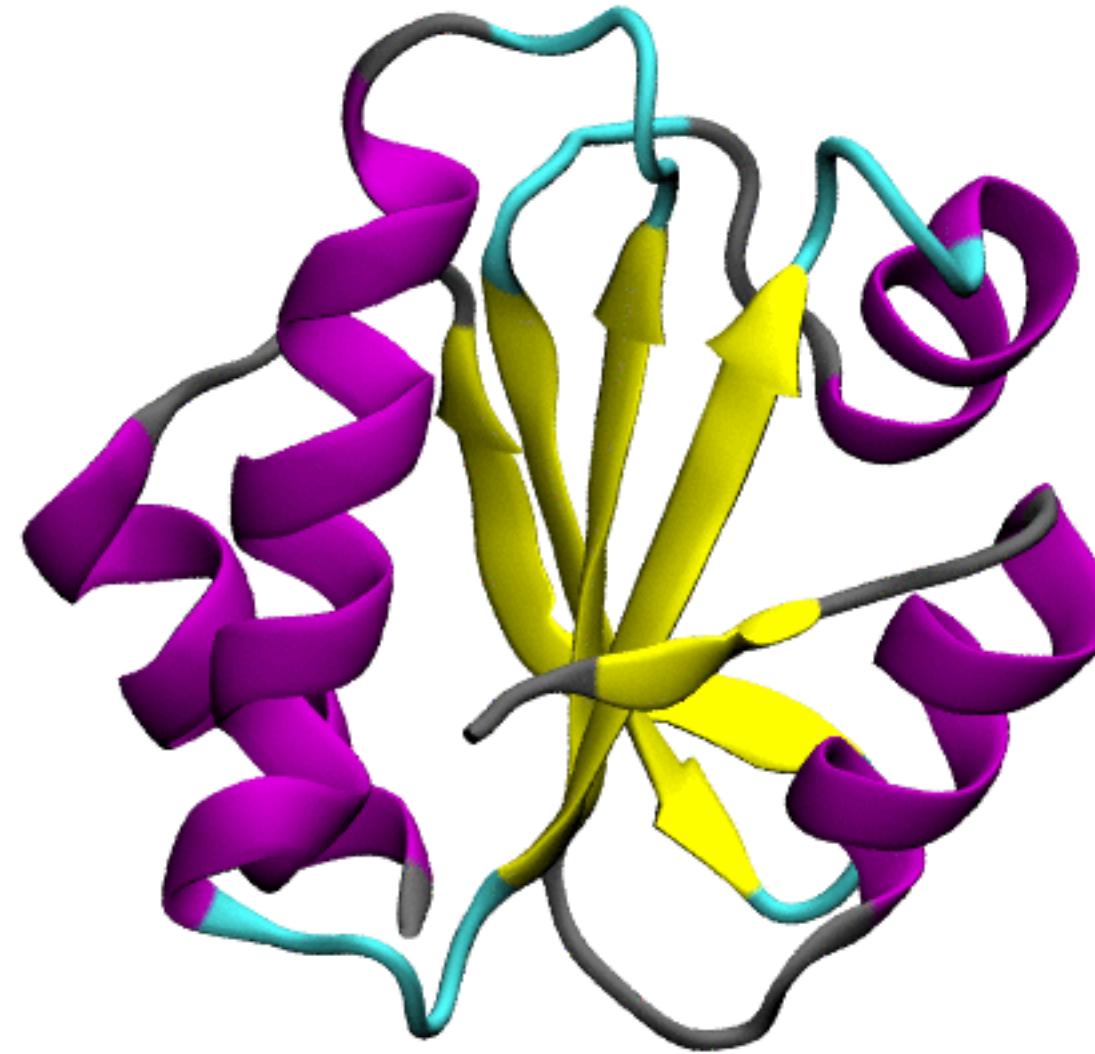
What initiates a pathogenic phenotype?

- Many missense mutations happen with no pathogenic phenotype
- However, mutations can become pathogenic under the right conditions
- Pathogenic mutations can alter the probability landscape of conformational ensembles

APADNAADA**Q**RFPWDWSWSI**N**K**I**Y**G**VNTL**E**
TYKVDGY**L**WA**Q**WTG**K**P**R**K**T**PGD**K**P**L**W**E**
PIERWW**M****M****G**LLWWWW**F****A**LE**F****A****M****M****W****G**S**P**D**T****G****N****K****R**
LMLFPDGR**L**IVYNARFLGSFSNDMDFREPPD
RQ**Q**EV**M****E****I****E****P****S****S****M****N****Q****Q****Q****R****R****S****D****D****Q****Q****V****T****E****N**
D**N****E****E****H****D****E****W****W****W****I****L****R****K****A****S****S****T****H****S****S****D****R****Y****D****H****L****S****S****X****Q****P****N**
Q**Q****E****I****E****S****S****R****I****N****T****R****I****Q****A****W****R****R****R****S****S****Y****M****W****S****S****E****F****P****P****G****G****I****A**
A**S****S****S****S****S****S****S****S****S****S****S****R****R****Q****Q****S****S****F****I****M****M****C****V****V****A****A****A****T**
F**Y****S****S****S****S****I****I****P****R****R****P****P****P****T****W****D****Q****Q****M****M****I****I****A****G****X****G****S****S****F****F****A****A****A****A****A**
FAHHRQAMGVEDDLLIQRCRLA**F**P**M****G****F****E****A****A**
GCVLVIRGIT

What is conformational probability?

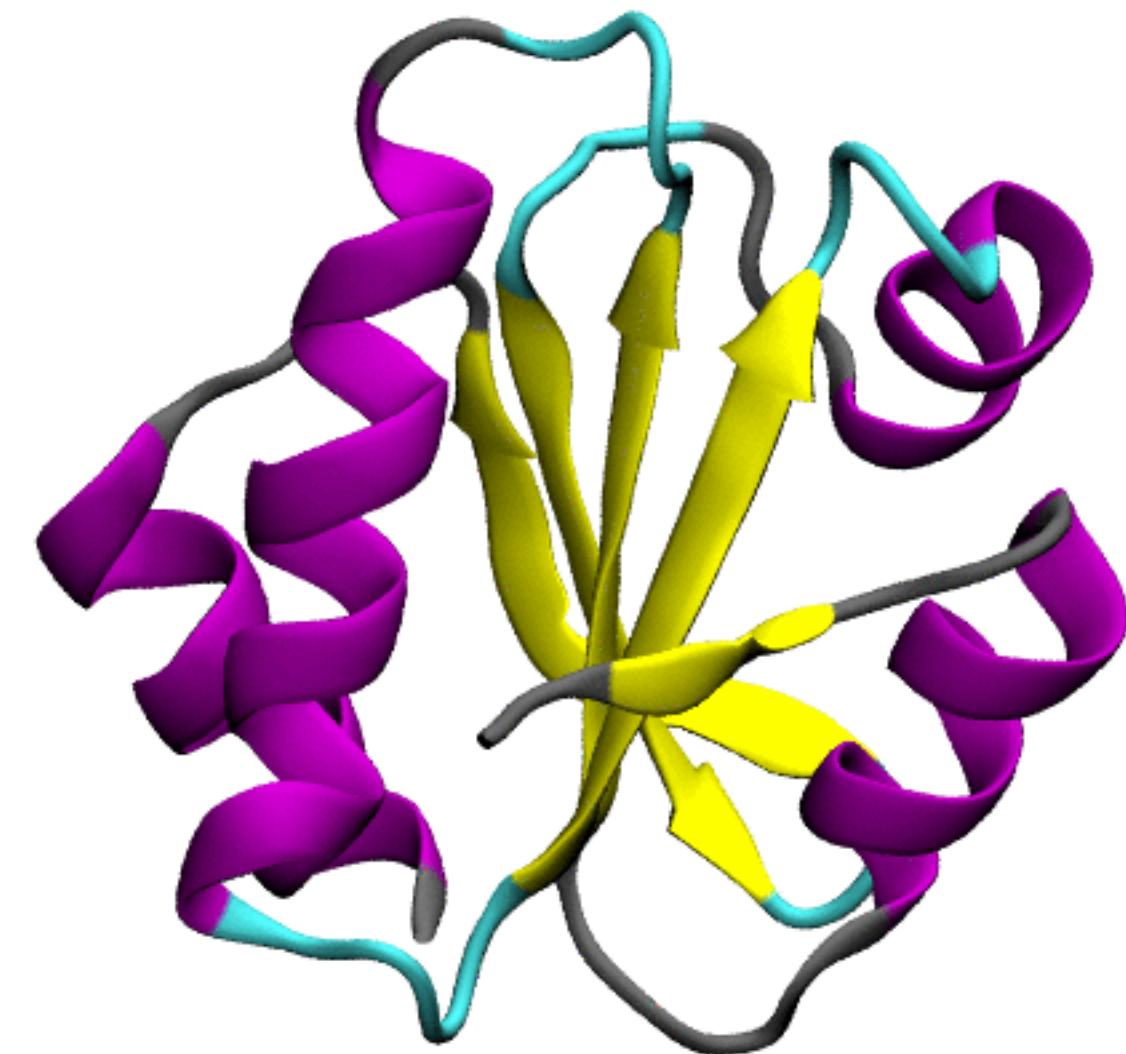
- Conformational ensembles is the macro states of all possible conformations that a protein can exhibit.
- A probability shift of of these conformational ensembles potentially increase the chance for a pathogenic phenotype to occur.



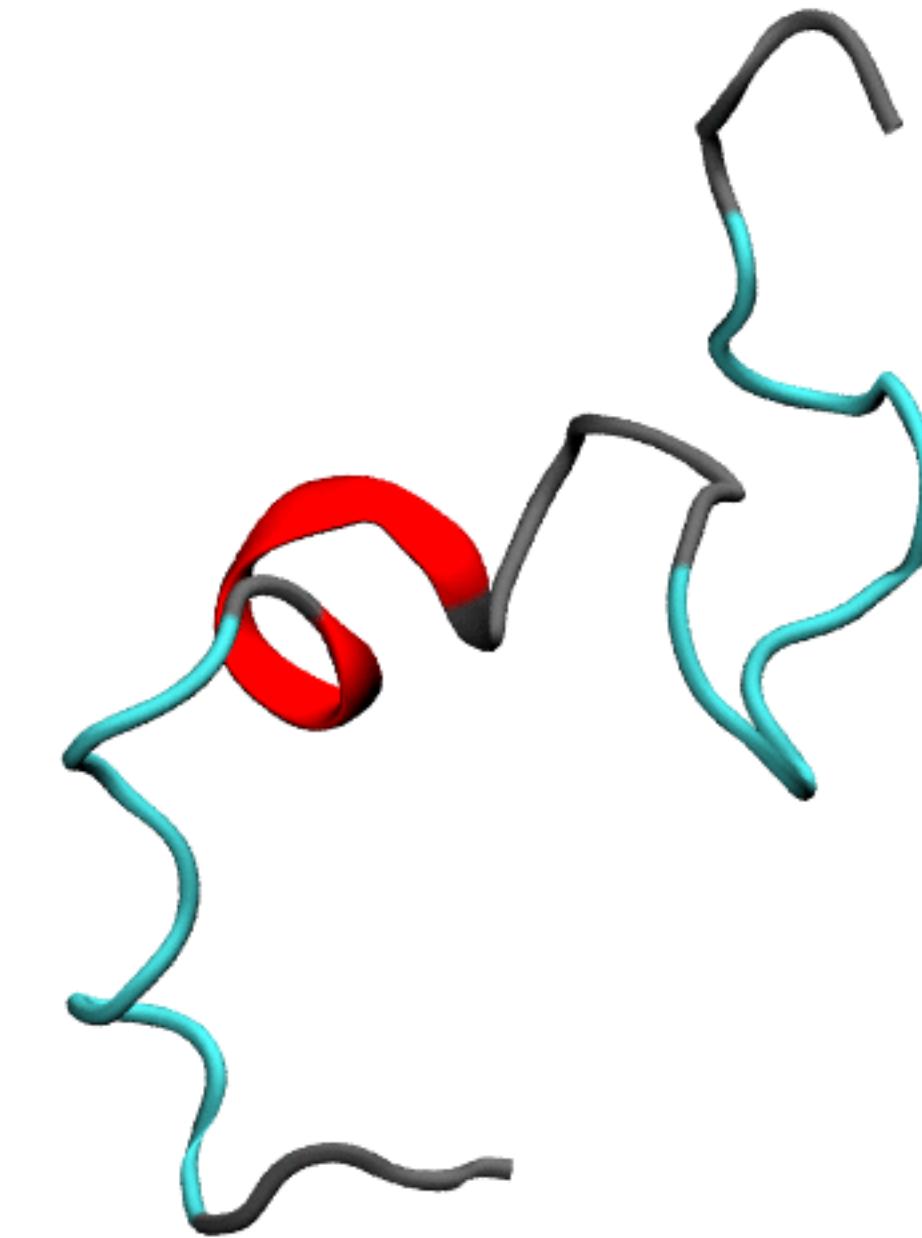
Structured Protein

Intrinsically Disordered Proteins

- Contains more charged residues and prolines which promote disorder
- Has many more possible conformations in its ensemble



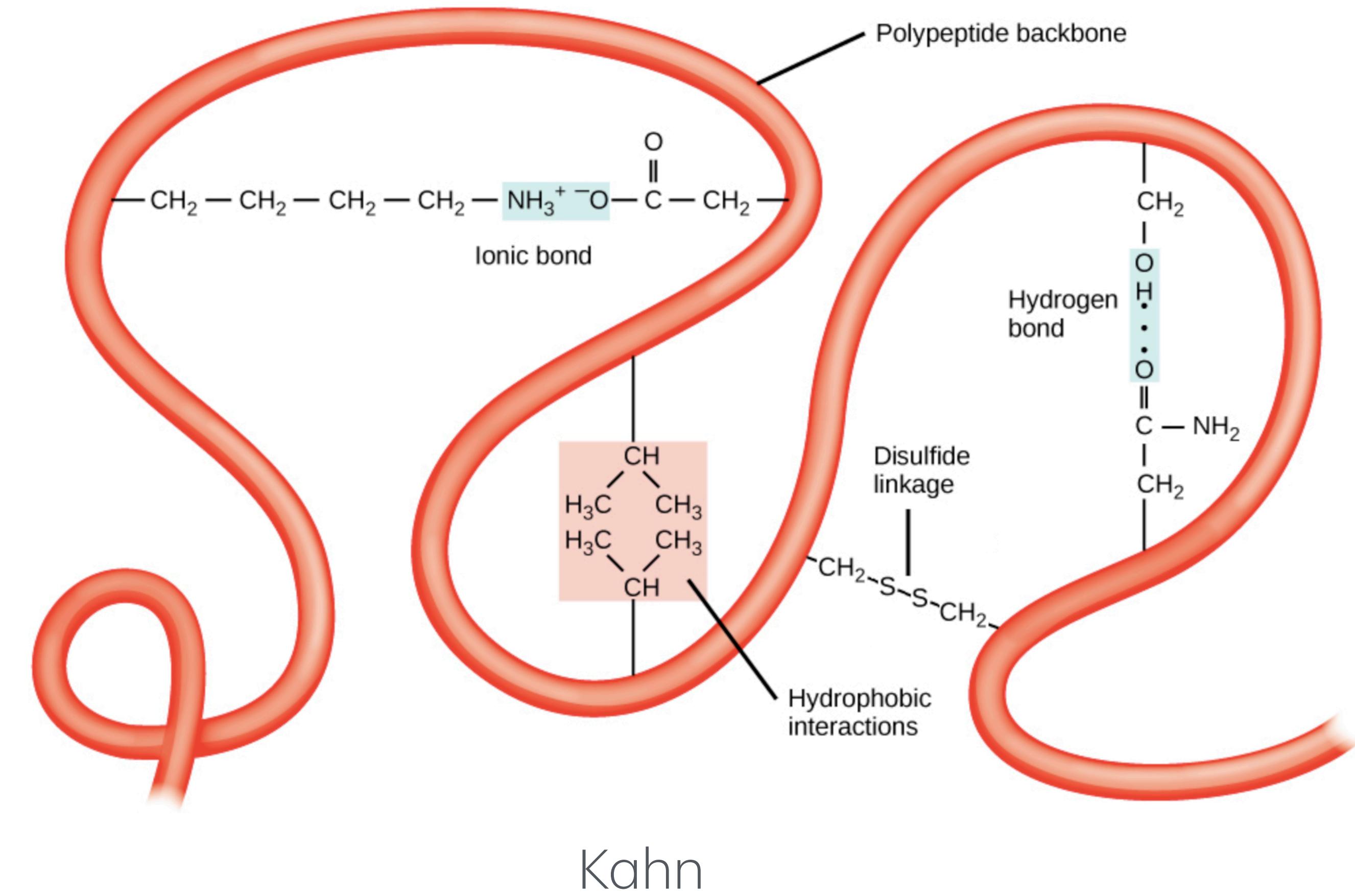
Structured Protein



Intrinsically Disordered Protein

Non-covalent interactions

- Non-covalent interactions are the predominate driving force for protein stability
- Non-covalent interactions such as: cation-pi, salt bridges, hydrogen bonding, sulfur-sulfur interactions, van der Waals, and hydrophobic interactions
- Main benefit is easy breaking of bonds, allowing the protein to explore more conformations
- Hydrophobic interactions are the majority of non-covalent interactions in any protein.



Case Study: Val66Met BDNF prodomain

- Previously, our lab was interested in the conformational differences between Val66 BDNF Prodomain and Met66 BDNF Prodomain

Val66Met Polymorphism of BDNF Alters Prodomain Structure to Induce Neuronal Growth Cone Retraction

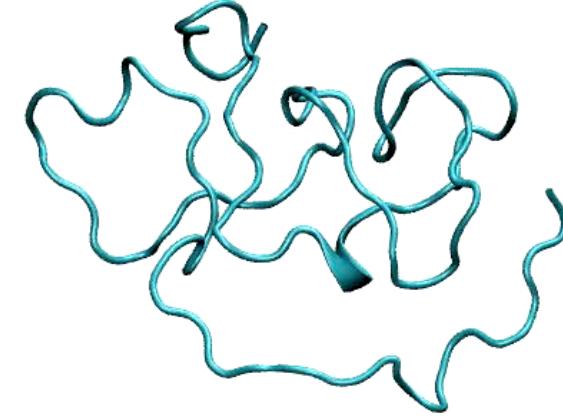
[Agustin Anastasia](#)¹, [Katrin Deinhardt](#)^{2,5}, [Moses V Chao](#)², [Nathan E Will](#)¹, [Krithi Irmady](#)¹, [Francis S Lee](#)³, [Barbara L Hempstead](#)^{1,*}, [Clay Bracken](#)^{4,*}

The BDNF Val66Met Prodomain Disassembles Dendritic Spines Altering Fear Extinction Circuitry and Behavior

[Joanna I. Giza](#)¹, [Jihye Kim](#)¹, [Heidi C. Meyer](#)¹, [Agustin Anastasia](#)², [Iva Dincheva](#)³,
[Crystal I. Zheng](#)¹, [Katherine Lopez](#)⁴, [Henrietta Bains](#)⁵, [Jianmin Yang](#)^{5 6},
[Clay Bracken](#)⁷, [Conor Liston](#)⁴, [Deqiang Jing](#)^{1 9}, [Barbara L. Hempstead](#)^{5 9}  
[Francis S. Lee](#)^{1 8 9 10}  

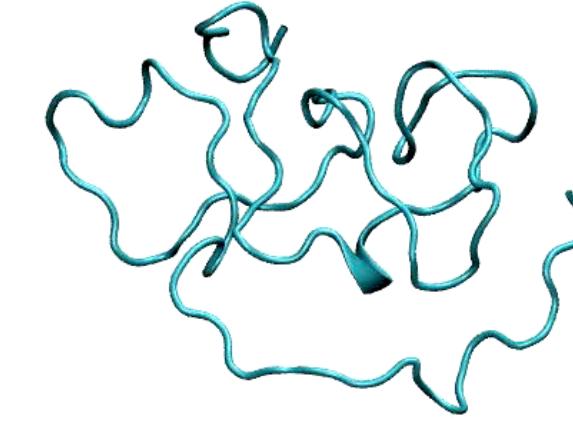
Molecular Dynamics simulations of BDNF prodomain

- Simulated Val66 and Met66 prodomain variants
- Simulated each for over 128 μ s
- Measured the inter residue contacts to see the change in h-blob contacts

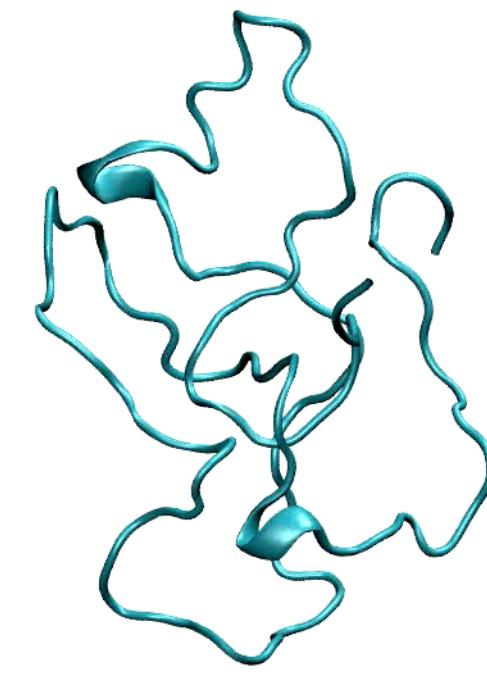


Simulation of BDNF prodomain simulation

Hard to Tell the Difference



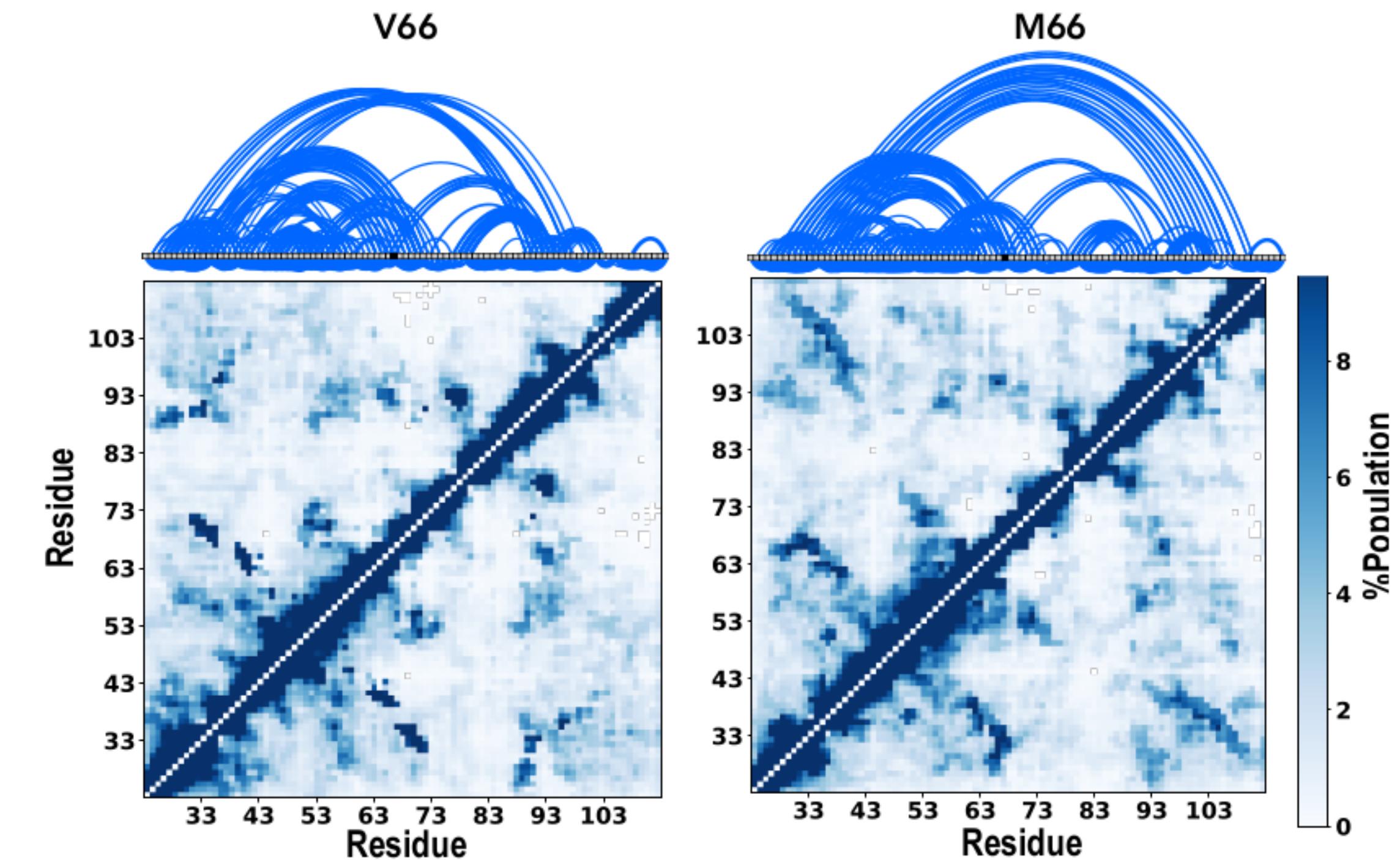
Video: Val66



Video: Met66

Interresidue Contact Map is hard to interpret

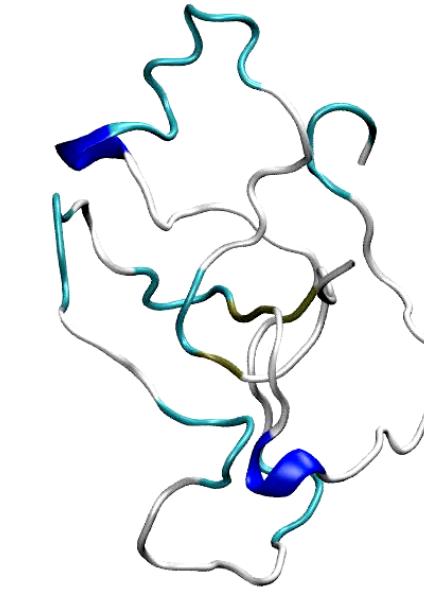
- Interresidue contact frequency map can not show subtle changes that occur from changing non-covalent interactions
- A segmentation method is needed to zoom out and see macro changes



Residue Contact Map of BDNF prodomain

IDPs can't be segmented using secondary structure

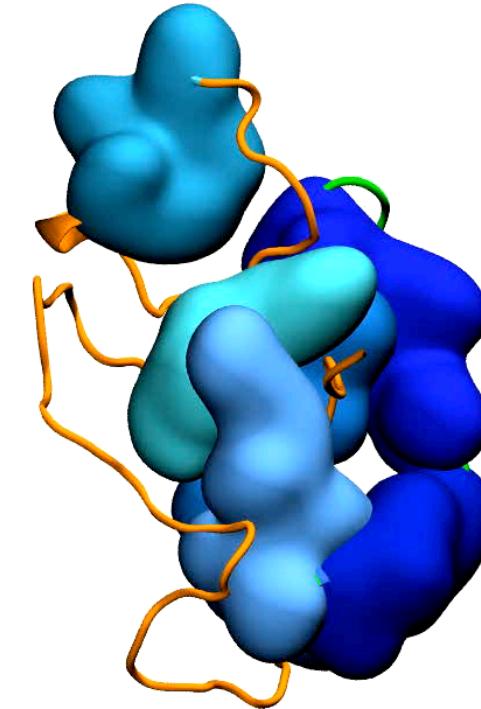
- IDPs conformational ensemble will contain transient secondary structural elements



BDNF prodomain simulation

Using Hydrophobicity to segment proteins

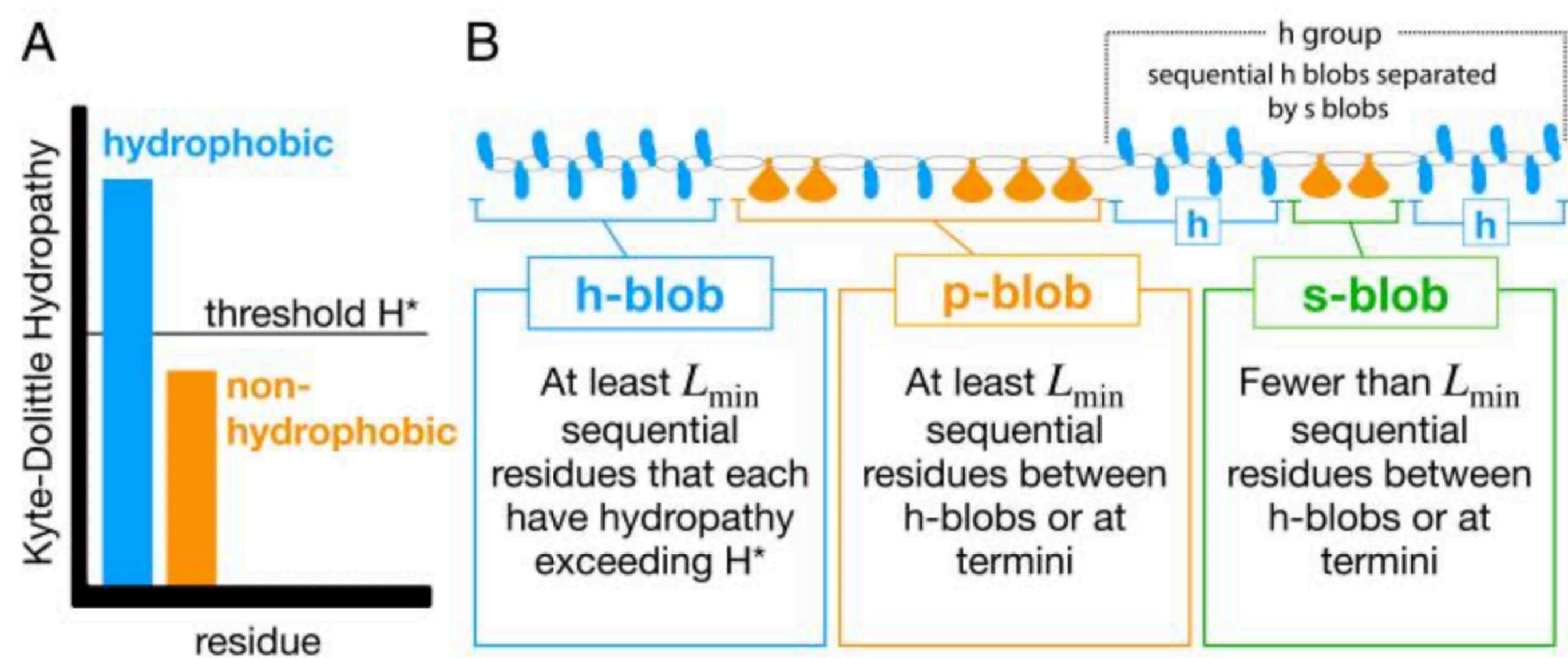
- Hydrophobic segments are found in most proteins
- These remain constant during the protein's lifetime



BDNF prodomain simulation
blobulated

Blobulation

- Applies scores to individual amino acids using the Kyte-Doolittle Hydropathy Scale
- Determines if smoothed average of residue meets or exceeds user defined hydrophobicity threshold
- Looks for continuous hydrophobic residues that meets user defined length threshold
- Meeting both criteria, segments of the protein are defined as hydrophobic and non-hydrophobic



Blobulation Example

Hydrophobicity Threshold: 0.4 Length Threshold: 4

Take Sequence

ARKNPILFHIFRNGSNALIGILWNGNG

0 = Non-Hydrophobic 1 = Hydrophobic

Determine if residue is
Hydrophobic

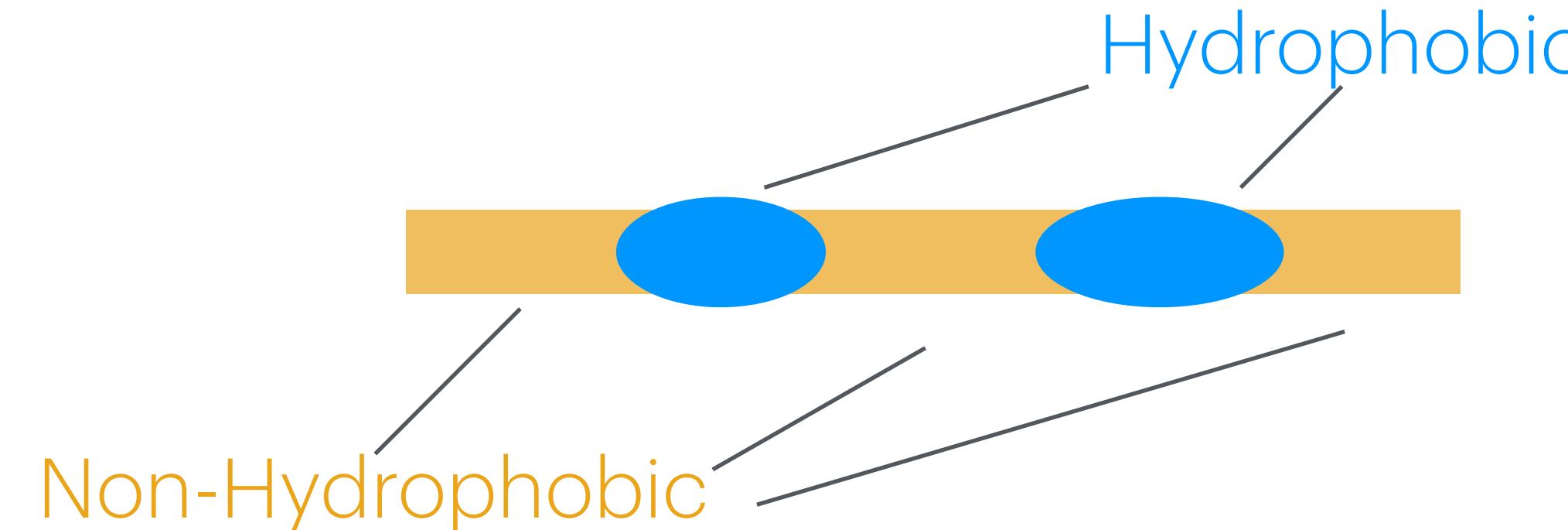
000011111000011111110000

Blob segments > length
threshold

000011111000011111110000



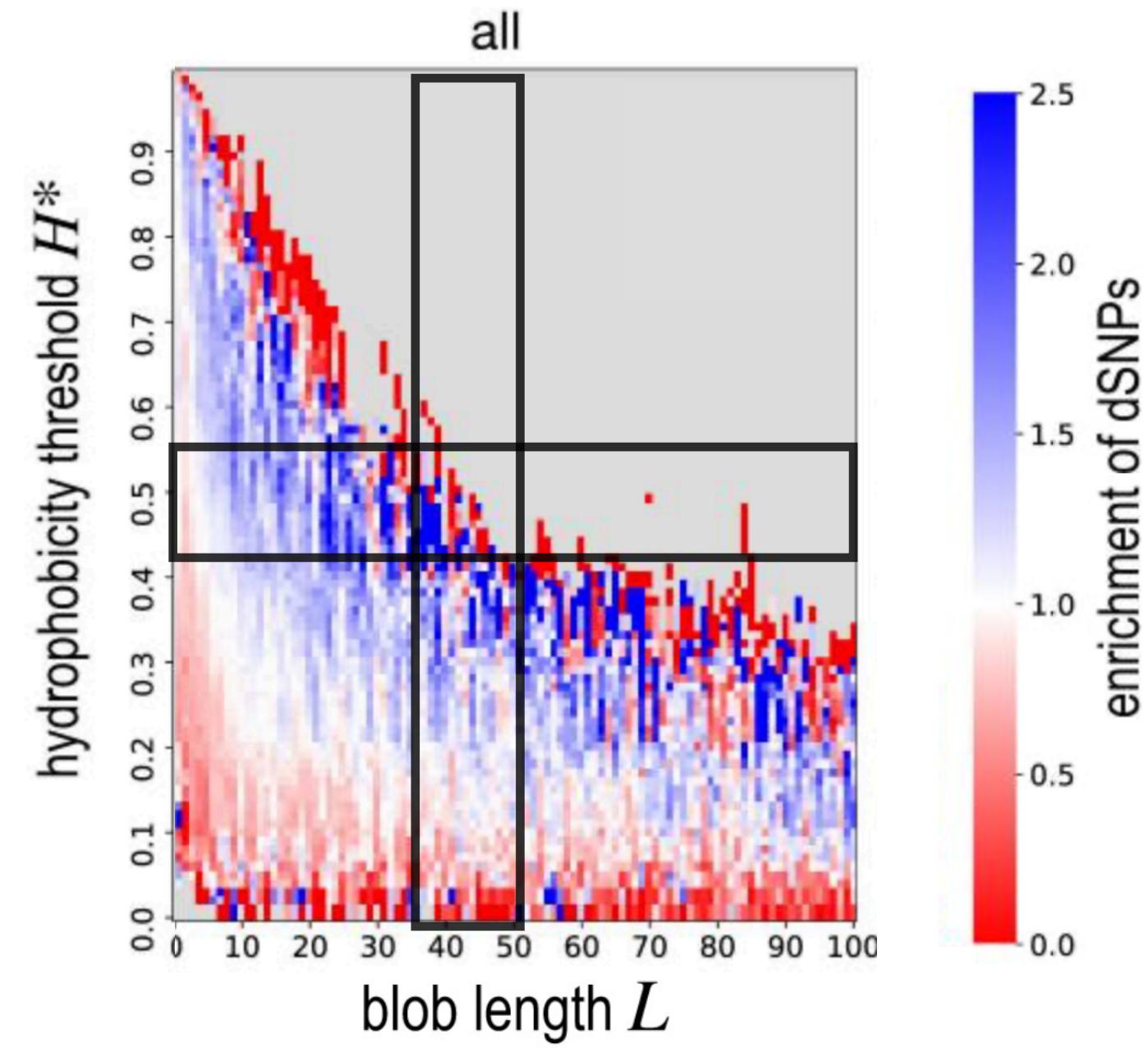
Define Blobs



aa	aa	Kyte-Doolittle
A	Alanine	1.80
C	Cysteine	2.50
D	Aspartic acid	-3.50
E	Glutamic acid	-3.50
F	Phenylalanine	2.80
G	Glycine	-0.40
H	Histidine	-3.20
I	Isoleucine	4.50
K	Lysine	-3.90
L	Leucine	3.80
M	Methionine	1.90
N	Asparagine	-3.50
P	Proline	-1.60
Q	Glutamine	-3.50
R	Arginine	-4.50
S	Serine	-0.80
T	Threonine	-0.70
V	Valine	4.20
W	Tryptophan	-0.90
Y	Tyrosine	-1.30

H-blobs are enriched for disease-associated single nucleotide polymorphisms

- Because hydrophobic regions of proteins frequently interact, a missense mutation in an h-blob could have more opportunity to interact with other residues.
- This could mean that h-blobs are enriched for pathogenic missense mutations
- Our lab performed a study on the human proteome looking for this correlation
- Disease associated single nucleotide polymorphism (dSNPs) are enriched in long and highly hydrophobic h-blobs
- dSNPs are depleted in short and h-blobs with low hydrophobicity



Lohia et al. 2022

We need to test the biophysical mechanism

Long and highly hydrophobic h-blobs increase h-blob contacts



Close residue proximity increases chance for a non-covalent interaction to occur



Long and highly hydrophobic h-blobs are enriched for dSNPs

Hypothesis

If h-blobs are long and more hydrophobic then they have more interactions with other h-blobs

Approach

Project Approach: Molecular Dynamics

- We need to see subtle conformation changes insilico peptides.
- Molecular Dynamics simulations allows us to simulate any non-natural structure that we want
- We can take these simulations and run analysis using Visual Molecular Dynamics (VMD)



Project Approach: Peptide Design

- We designed our peptides to test different blob topologies
 - We create peptides with modified lengths and h-blob residue compositions
 - We expect these peptide will behave differently depending on how the h-blobs are made

aa	aa	Kyte-Doolittle
A	Alanine	1.80
I	Isoleucine	4.50
V	Valine	4.20
L	Leucine	3.80

Project Approach: Analysis

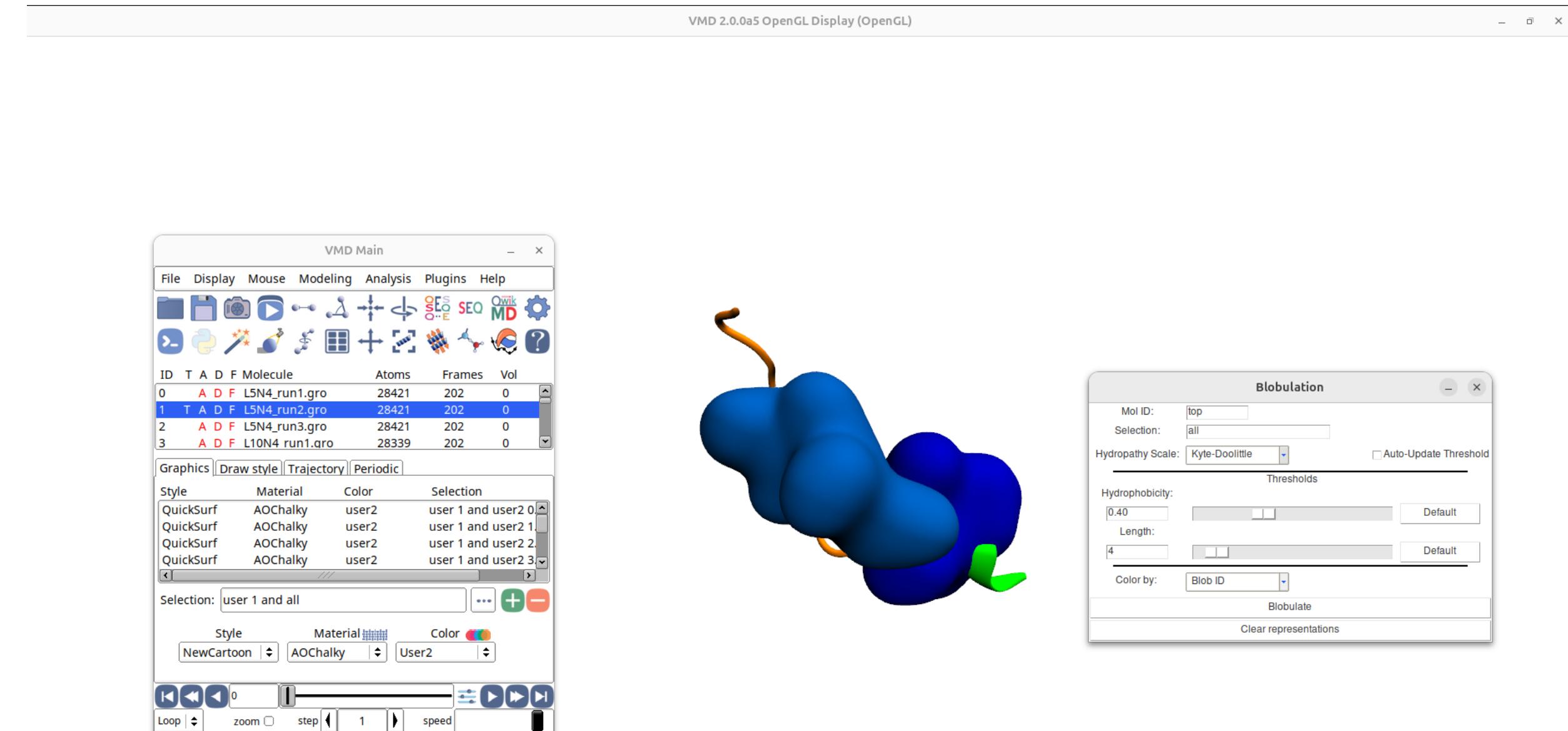
To examine properties of our peptides, we used these analysis

- Radius of Gyration
- Blob-Blob Contacts
- Clusters
- Secondary Structure Formation

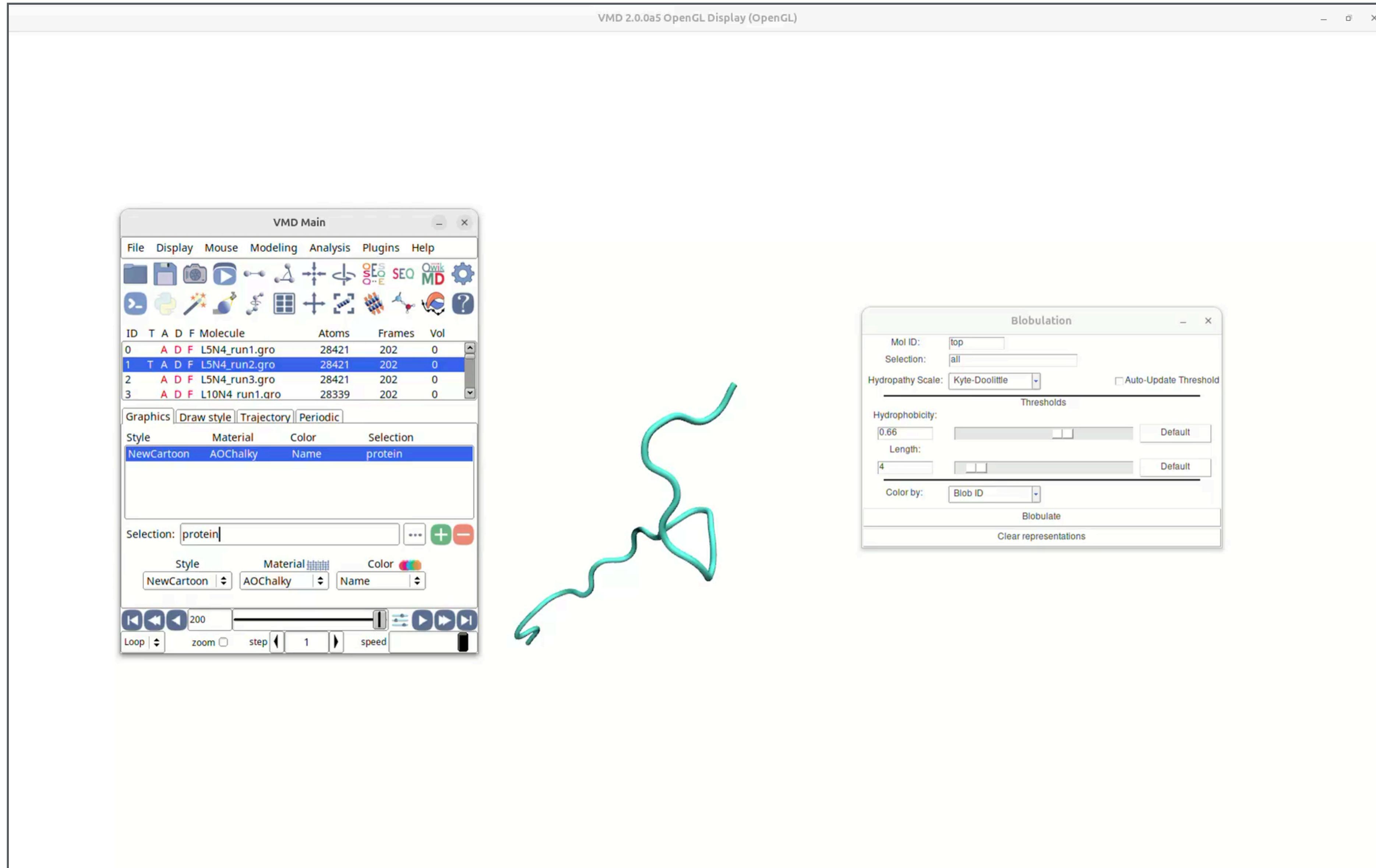


Blobulator VMD Plugin

- VMD offered analysis and visual tools needed to project
- A rewrite of the blobulator algorithm in TCL was needed.
- Creation of the GUI offered convenient use for analysis
- Sets blob residue ranges for blob-blob contacts

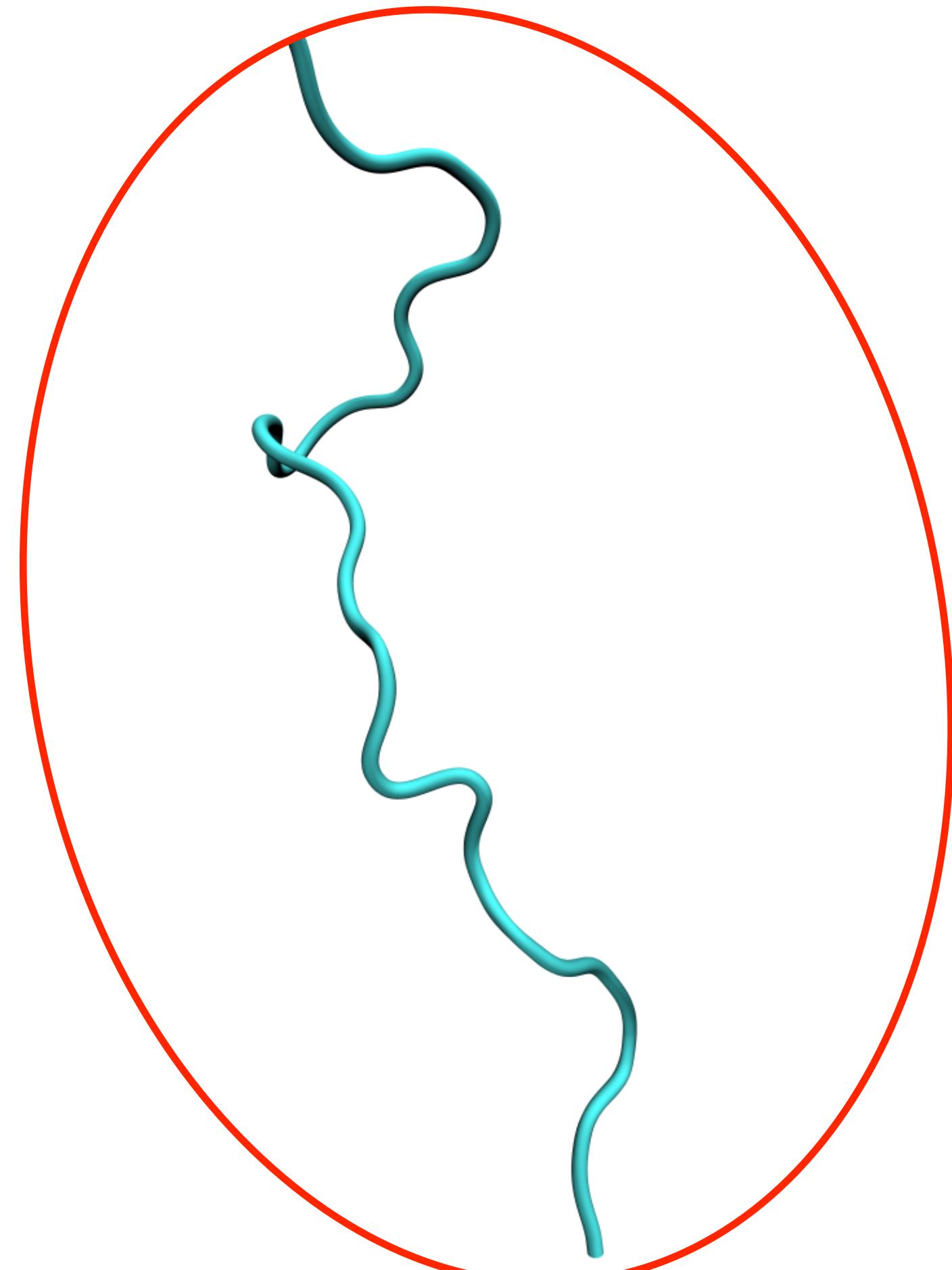


VMD Plugin: Demo



Methods

Radius of Gyration



Large Radius of Gyration

Radius of gyration is the area of space taken up by the peptide

$$r_{gyr}^2 = \frac{\left(\sum_{i=1}^n w(i)(r(i)) - \bar{r} \right)^2}{\sum_{i=1}^n w(i)}$$

$r(i)$ = the position of the i-th residue

\bar{r} = the weighted center of the selection

$w(i)$ = the mass of the I-th residue

Normalizing Sequence Length

$$rgyR_g^2 \Rightarrow \sqrt{\frac{NR_b^2}{N}}$$

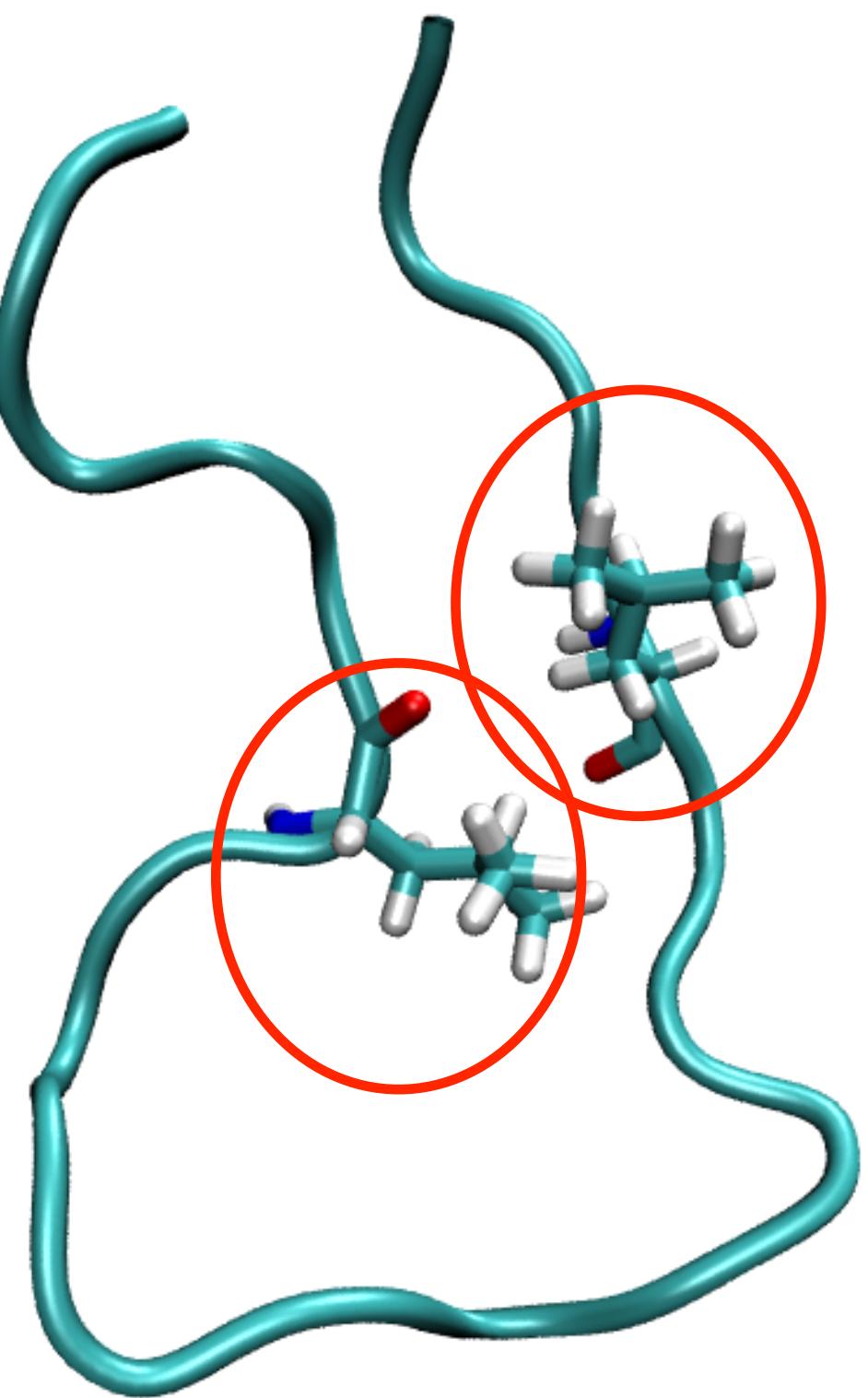
Need to compare peptides
of different lengths

N = Number of residues

b = residue length

Measure Contacts

- A contact is defined as two atoms of a residue, within a blob, that are within 5.5 \AA
- Used to measure contact frequency and clusters



Secondary Structure

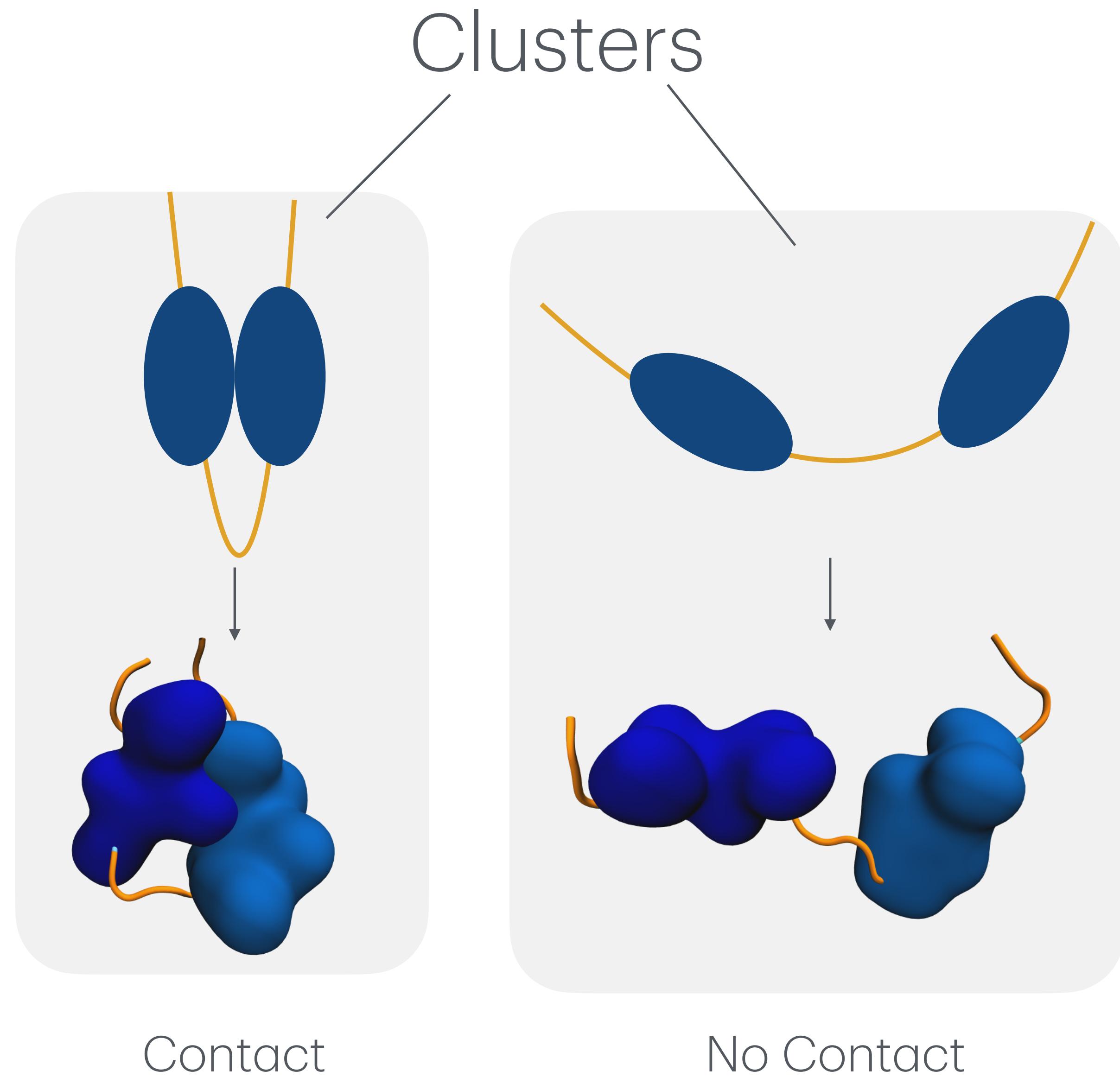
- Measure each residues secondary structure formation using sscache
- Create a graph of secondary structure formation over simulations
- Secondary Structure formations of interest are alpha-helices and beta-sheets



Video of Peptide with sscache

Clustering

We want to measure the frequency the peptides adopt conformations with h-blobs touching



Results

If h-blobs are **long** and **more hydrophobic** then they have more interactions with other h-blobs



Examine Residues
Effects on H-blob
Contacts

Expectation

We expect that h-blobs contacts frequency will follow this order

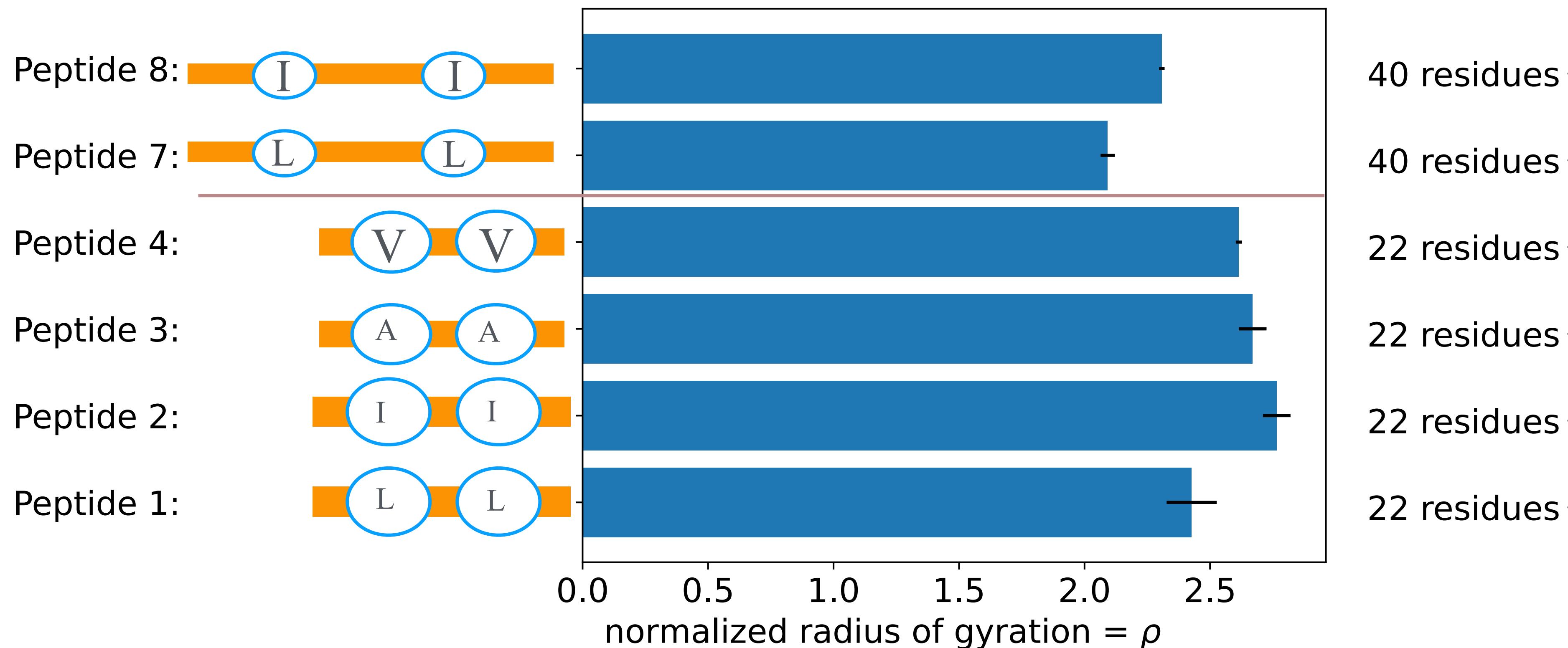
Using the Kyte-Doolittle Scale

In terms of h-blob contacts and peptide compaction

I	Isoleucine	4.50	>	V	Valine	4.20	>	L	Leucine	3.80	>	A	Alanine	1.80
---	------------	------	---	---	--------	------	---	---	---------	------	---	---	---------	------

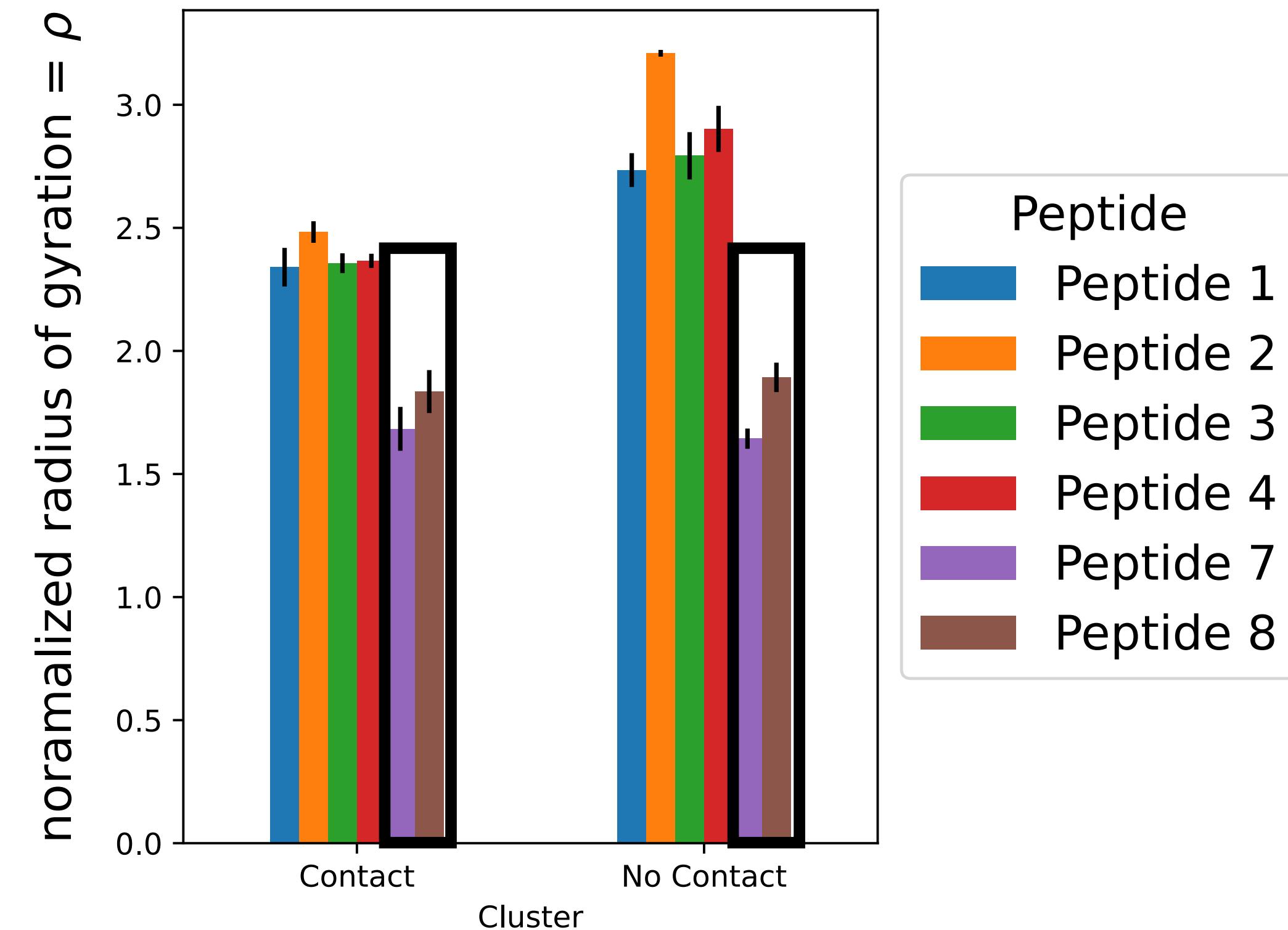
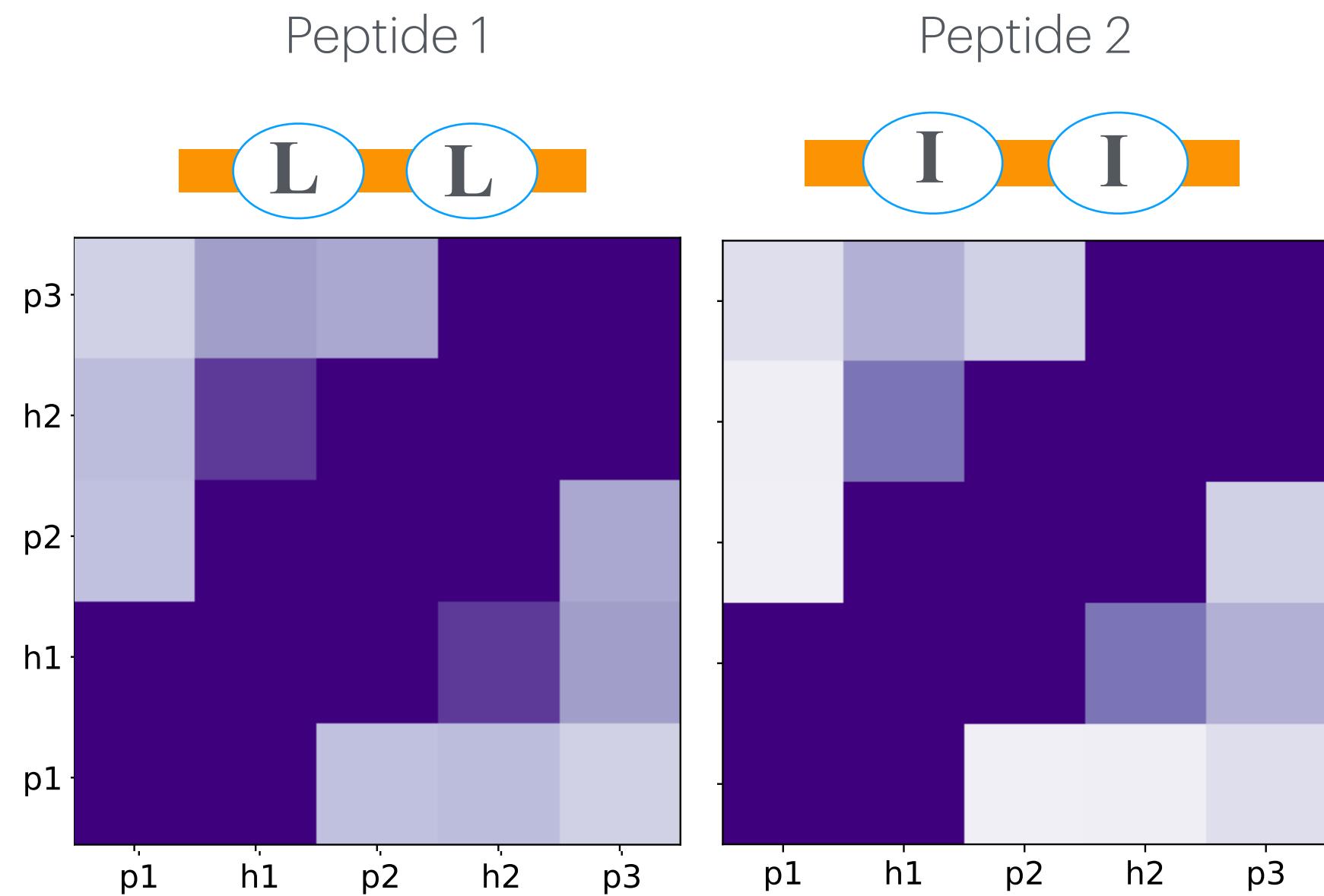
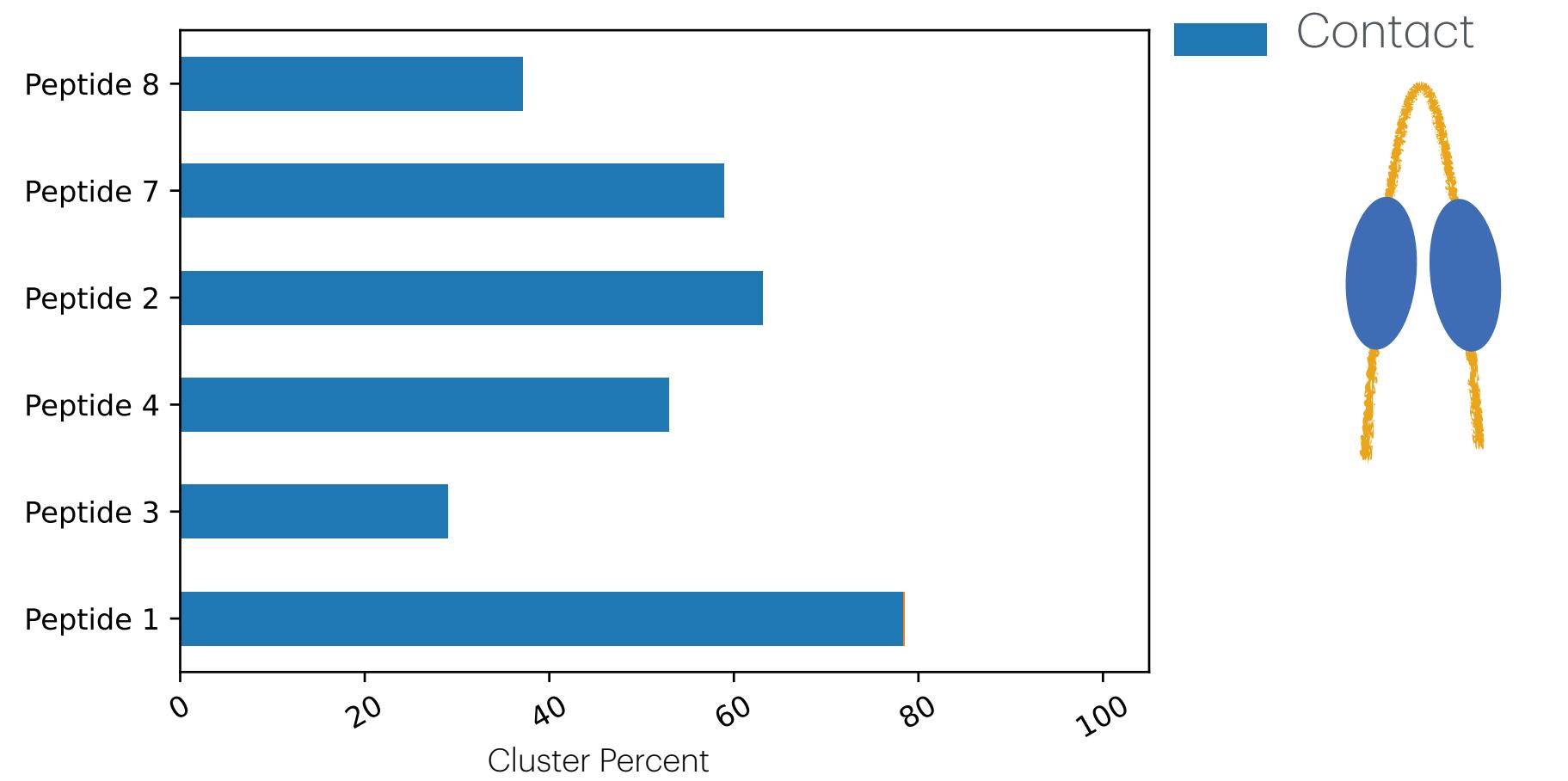
We expect isoleucine to have the most frequency h-blob contacts and the most compact peptides

Increasing Hydrophobicity in h-blobs Does Not Correlate To More Compaction

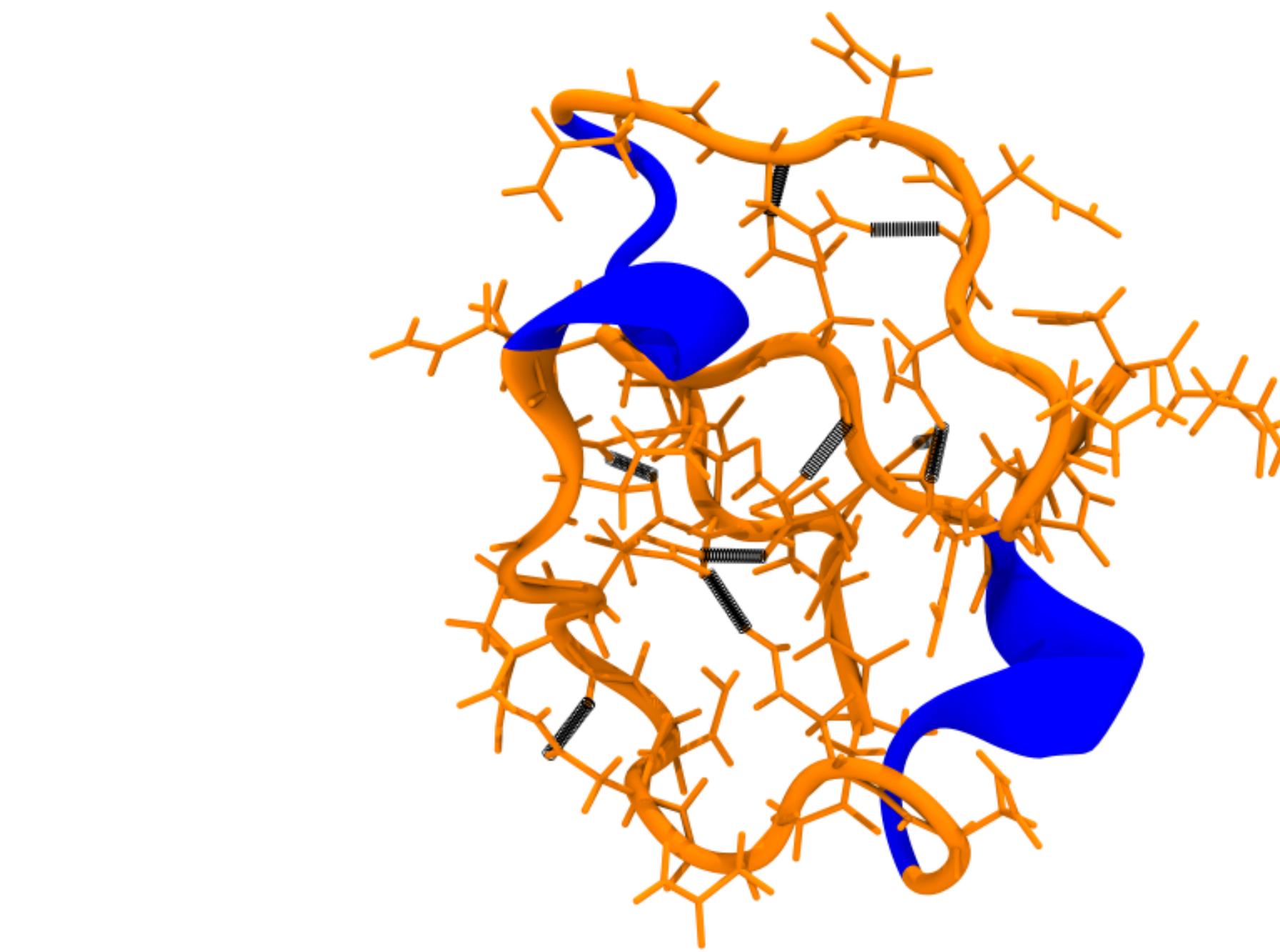
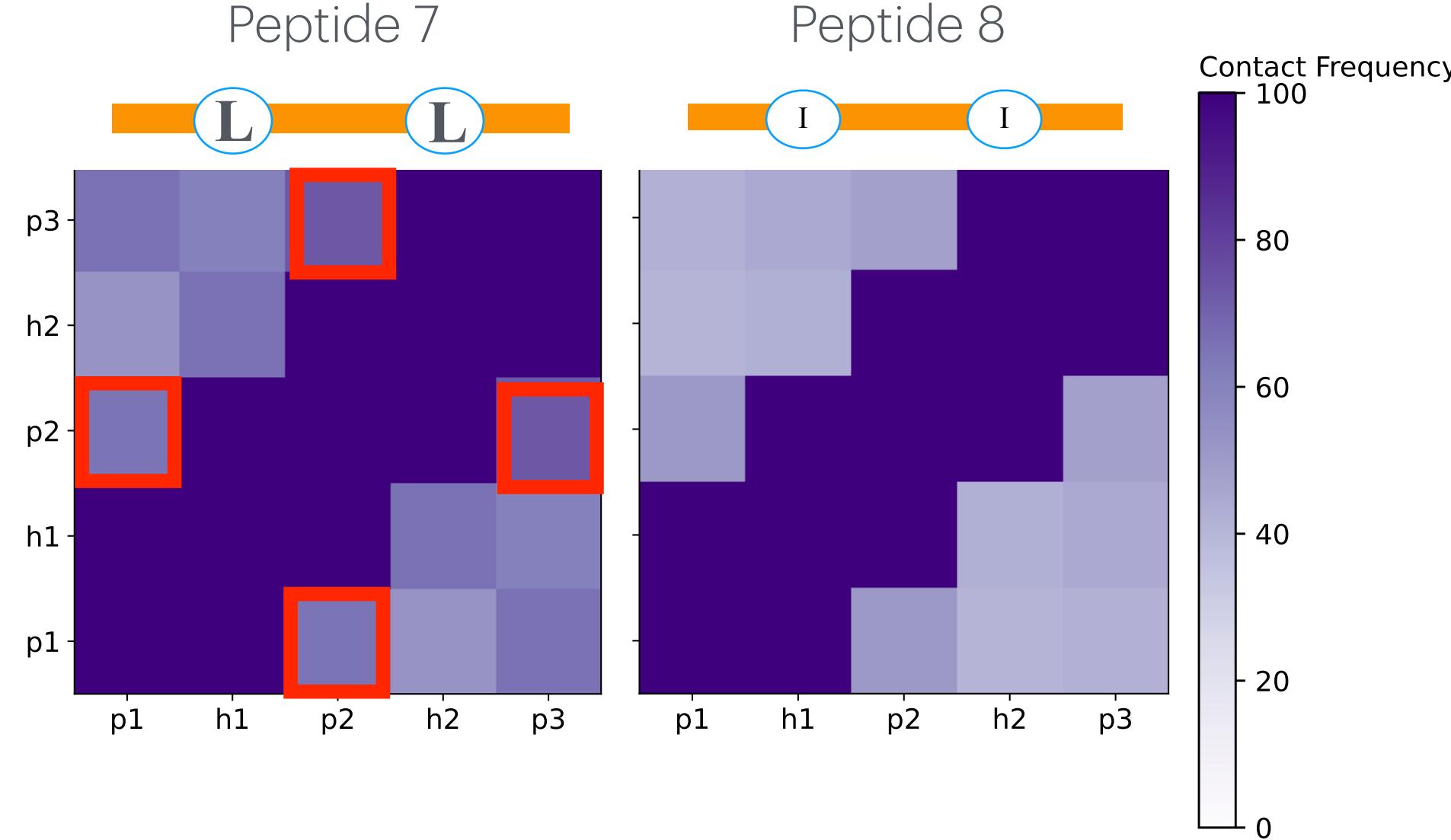


$$rgyr = \sqrt{\frac{\langle R_g^2 \rangle}{N}}$$

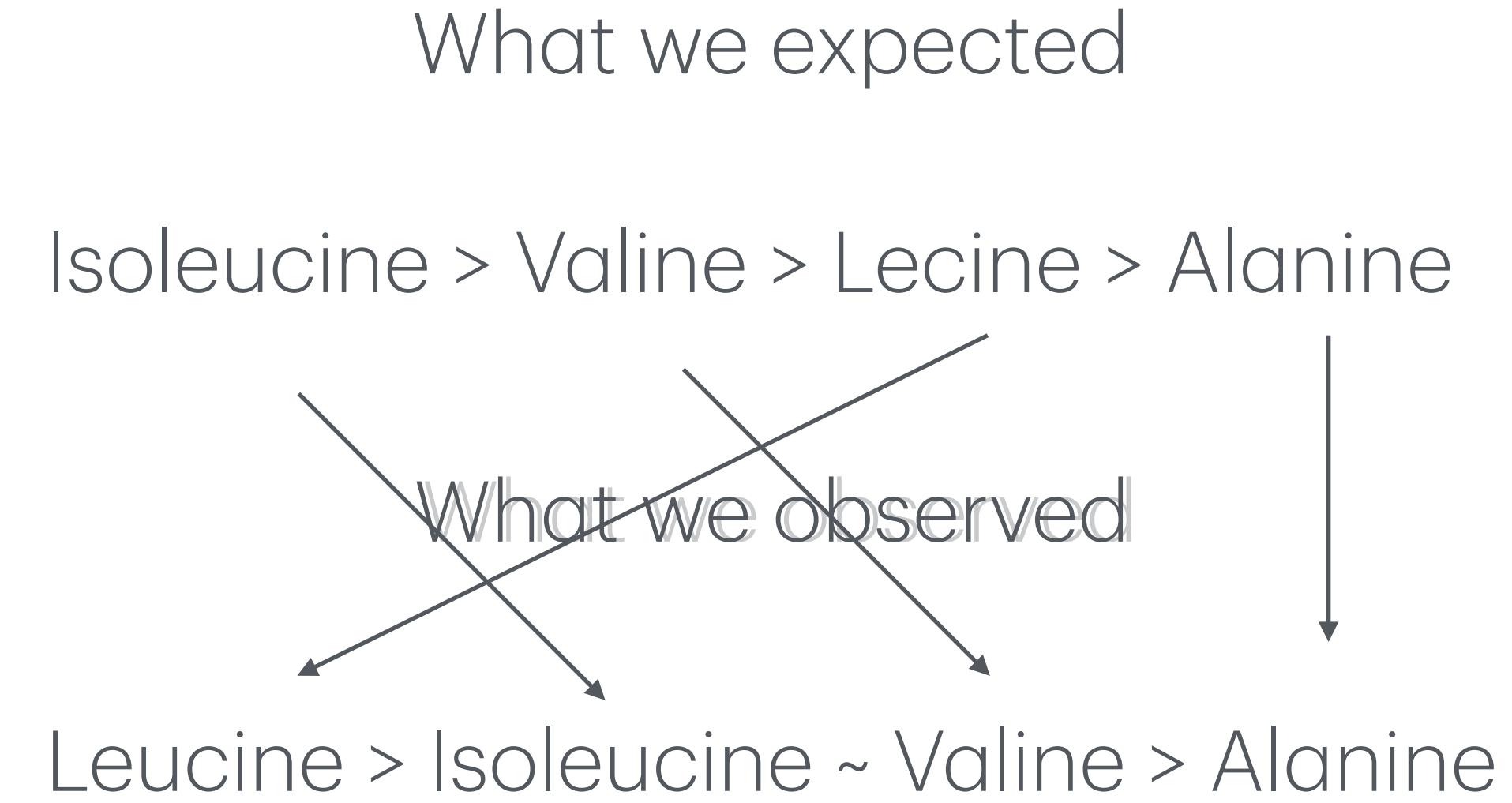
Increasing Hydrophobicity in H-blobs Doesn't Increase H-blob Contacts



Long p-blobs Exhibit Hydrogen Bonding



Increasing hydrophobicity in h-blobs doesn't increase h-blob contacts



Contradicts our Hypothesis

If h-blobs are long ~~and more hydrophobic~~ then they have more interactions with other h-blobs

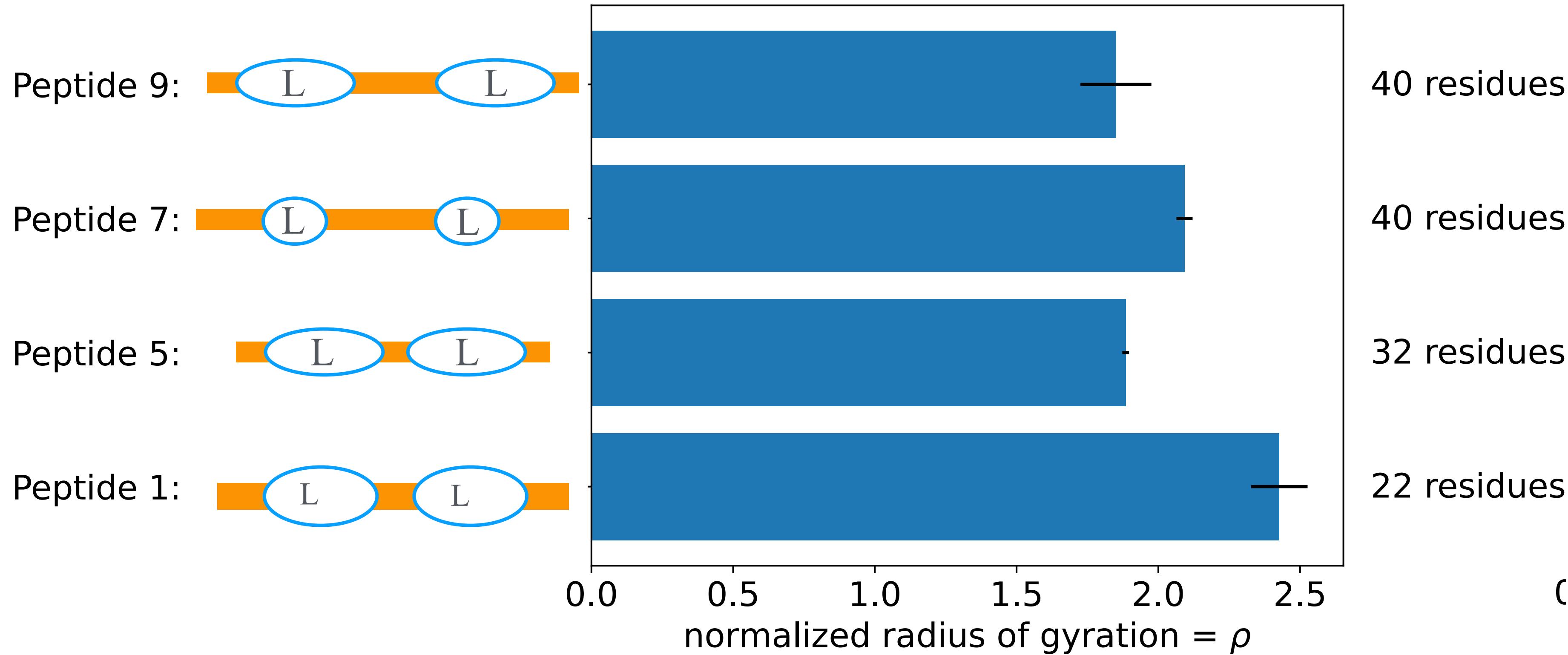
Results

If h-blobs are long and more hydrophobic then they have more interactions with other h-blobs



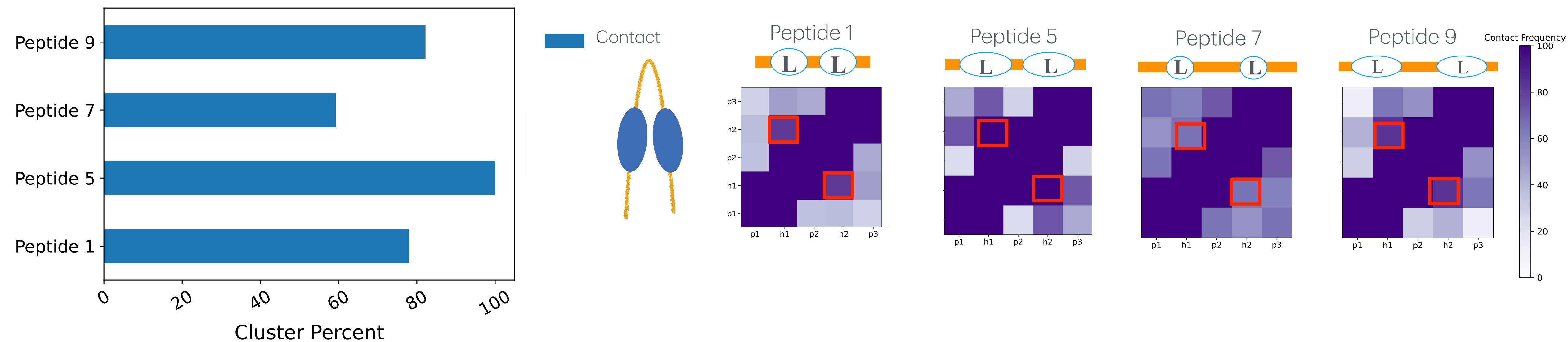
Examine How Long H-blobs Effect H-blob Contacts

Increasing the Length of h-blobs Produces More Compaction

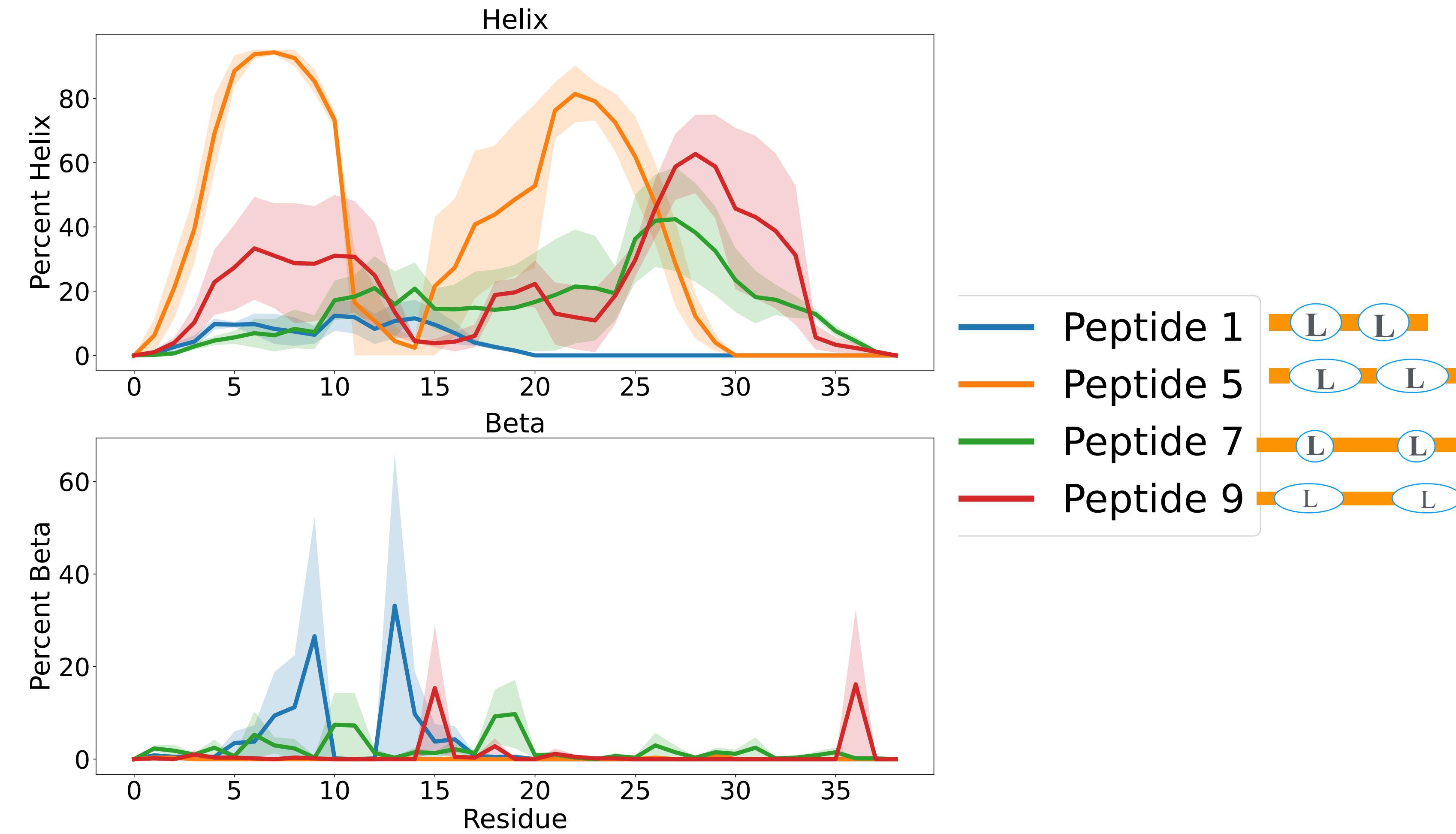


$$rgyr = \sqrt{\frac{\langle R_g^2 \rangle}{N}}$$

Increasing Length of H-blobs Increases H-blob Contacts

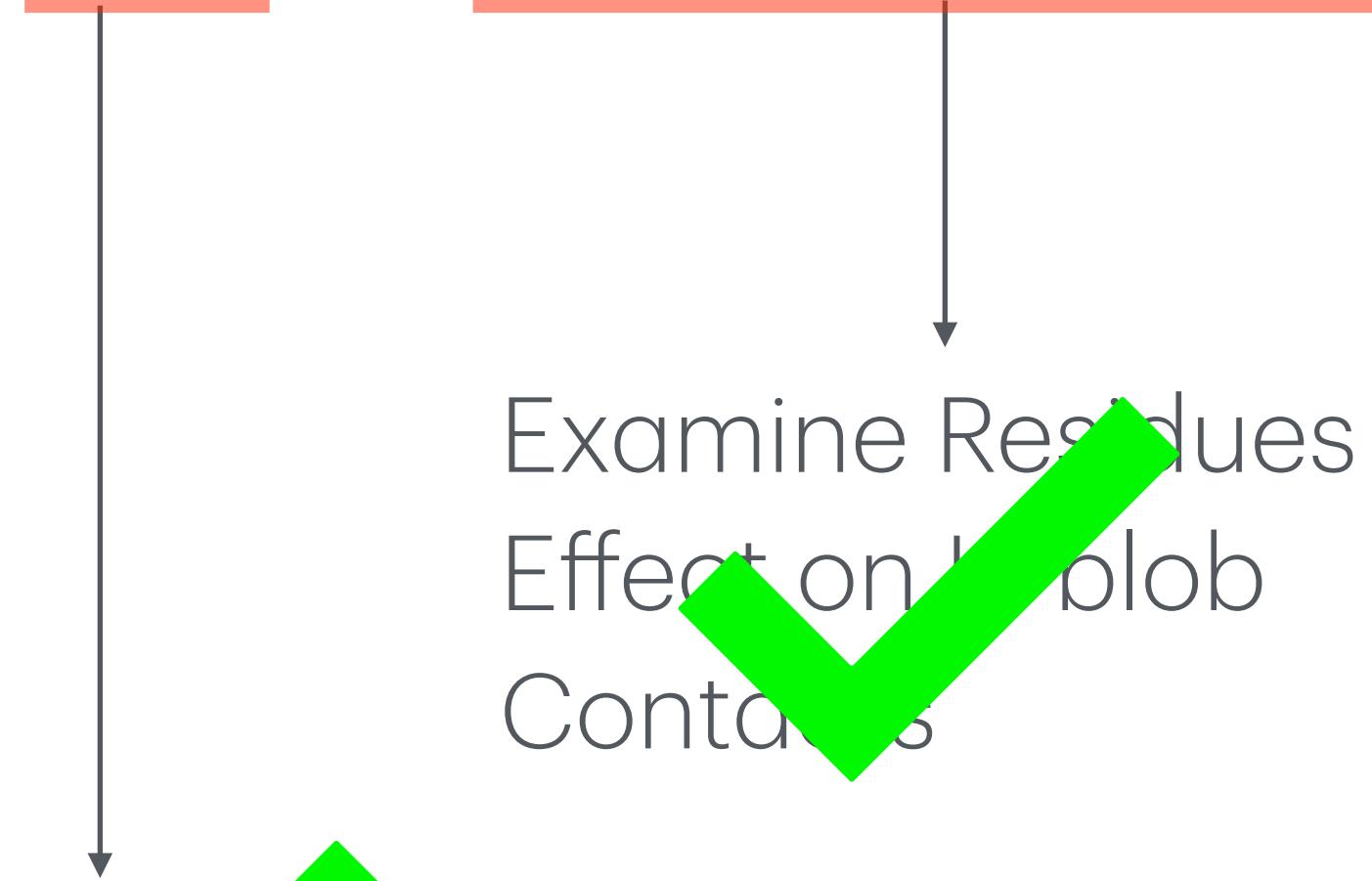


Longer h-blobs Tend to Form Alpha Helices



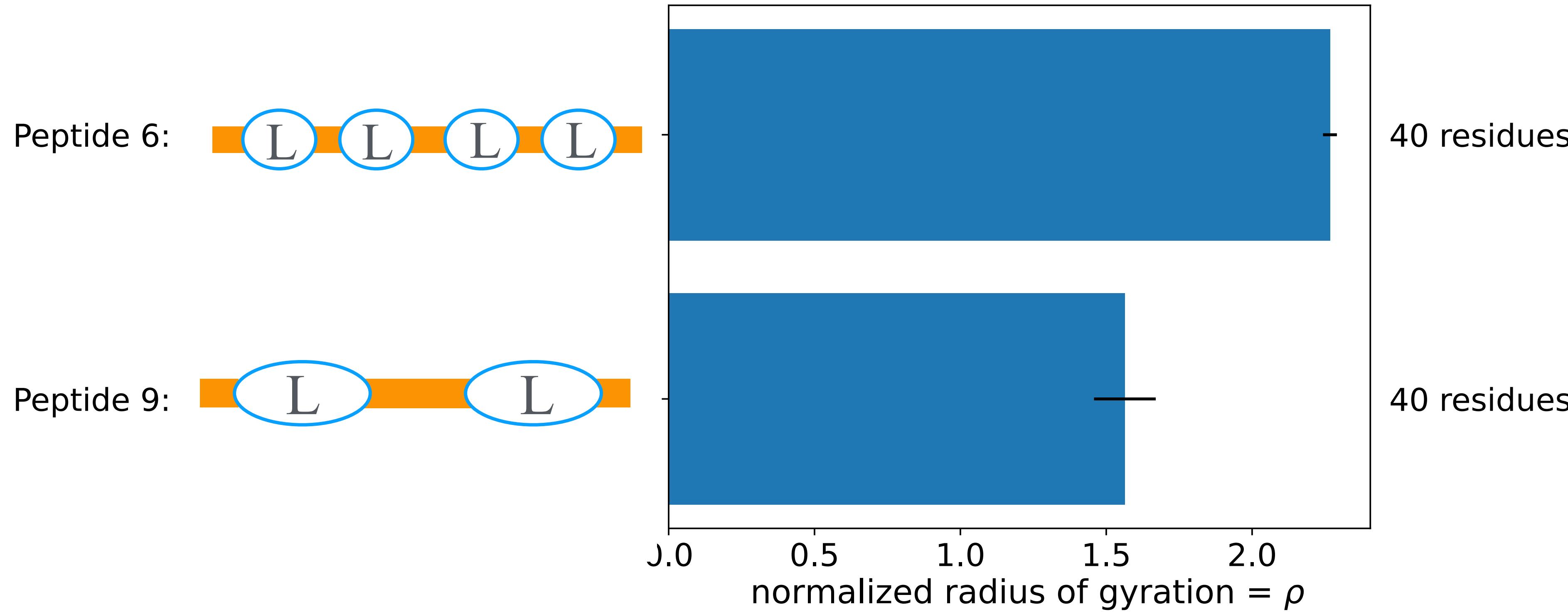
Results

If h-blobs are **long** and **more hydrophobic** then they have more interactions with other h-blobs



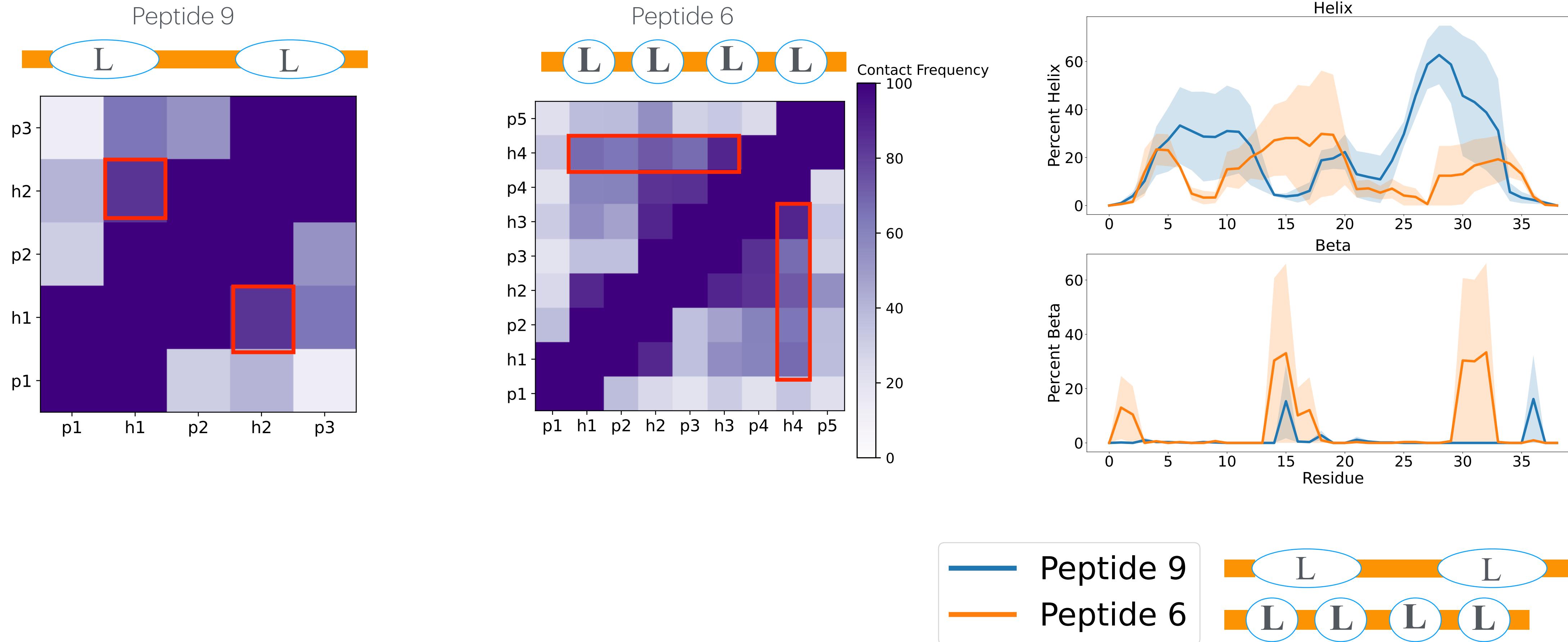
Examine How Long H-blobs Effect H-blob Contacts → Examine Long H-blobs to Four Short H-blobs

Longer h-blobs Produces More Compaction than Multiple h-blobs



$$rgyr = \sqrt{\frac{\langle R_g^2 \rangle}{N}}$$

Long h-blobs and multiple short h-blobs exhibit different h-blob contacts



Results

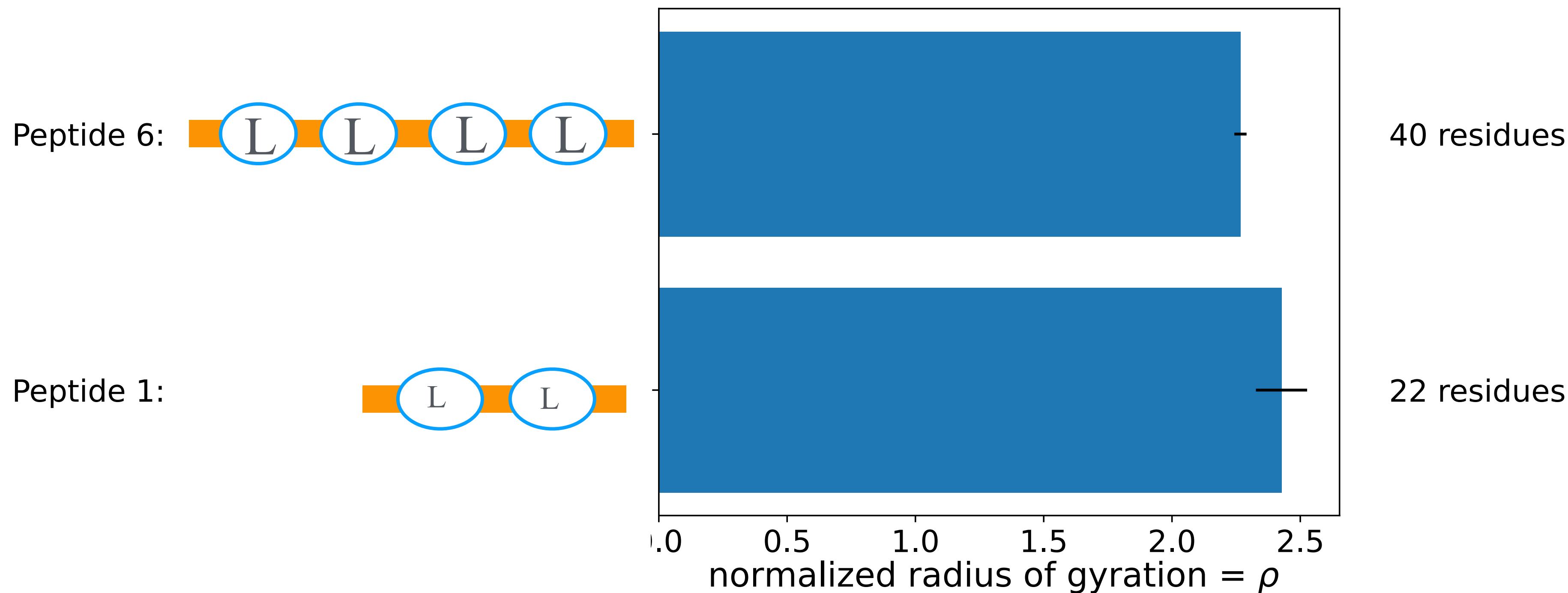
If h-blobs are long and more hydrophobic then they have more interactions with other h-blobs

Examine Residues
Effect on H-blob
Contacts

Examine How Long H-blobs Effect H-blob Contacts → Examine Long H-blobs to Four Short H-blobs

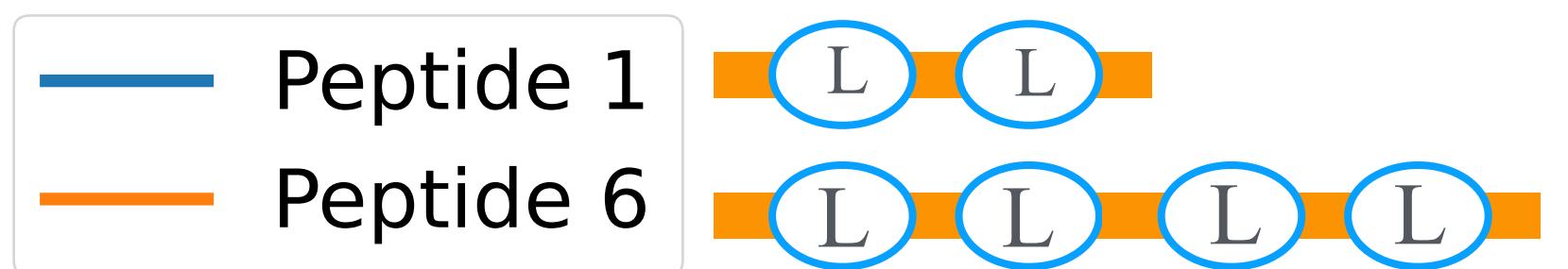
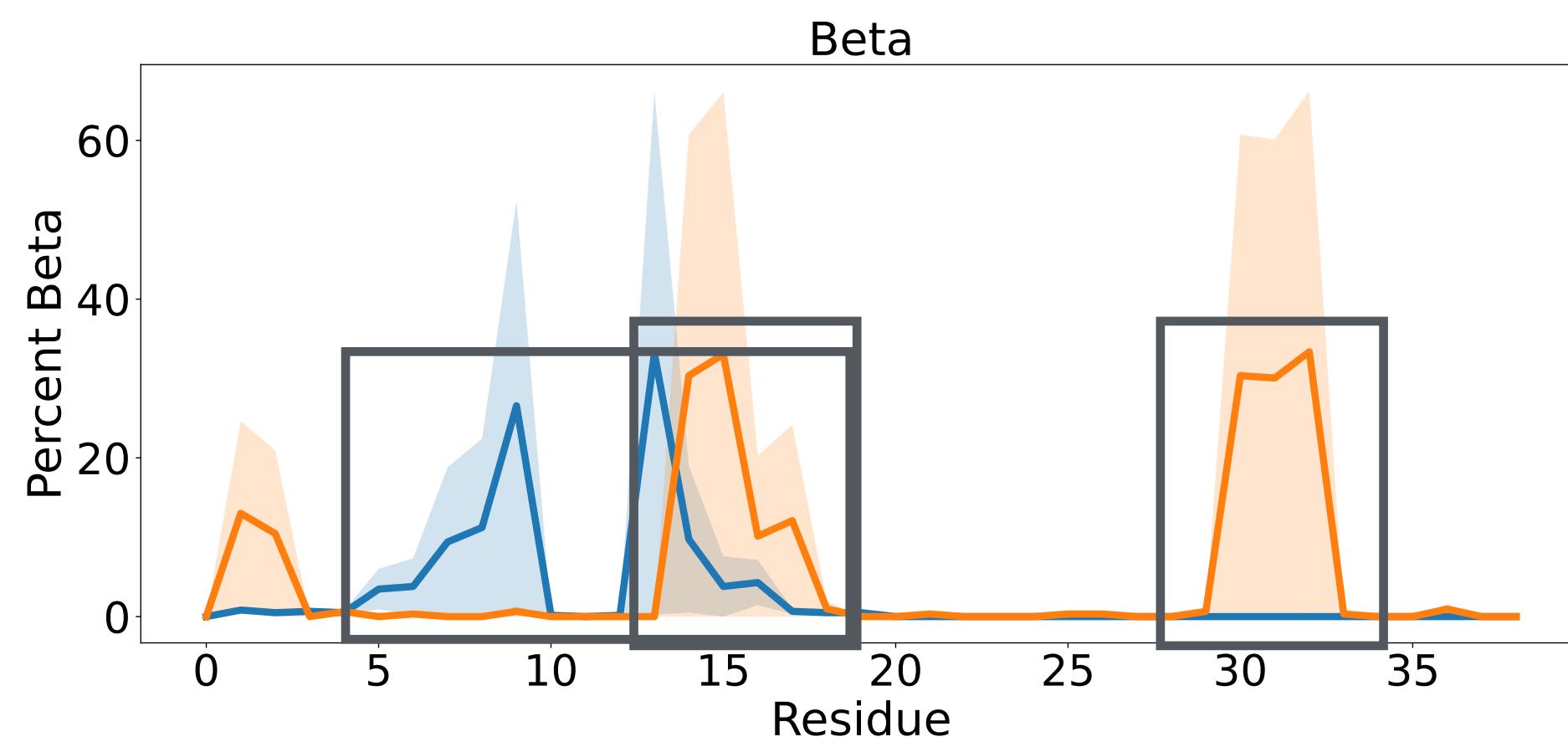
Examine Two H-blobs to Four H-blobs

Increasing Number of h-blobs Produces More Compaction

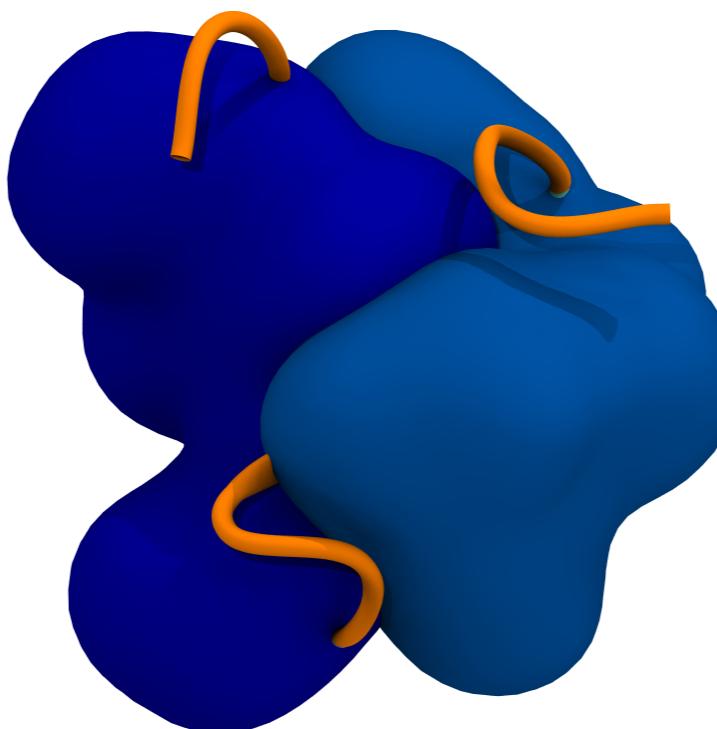


$$rgyr = \sqrt{\frac{\langle R_g^2 \rangle}{N}}$$

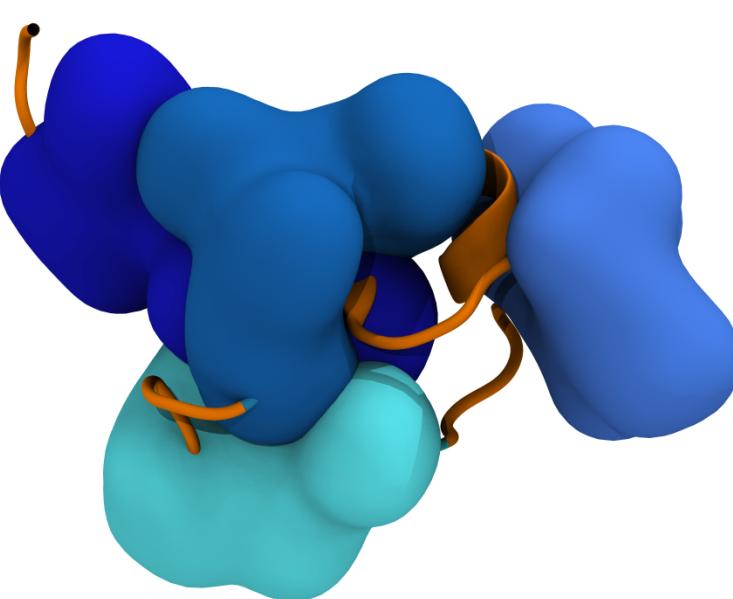
Increasing Number of h-blobs Influences Long Range Beta Pairings



Peptide 1



Peptide 6

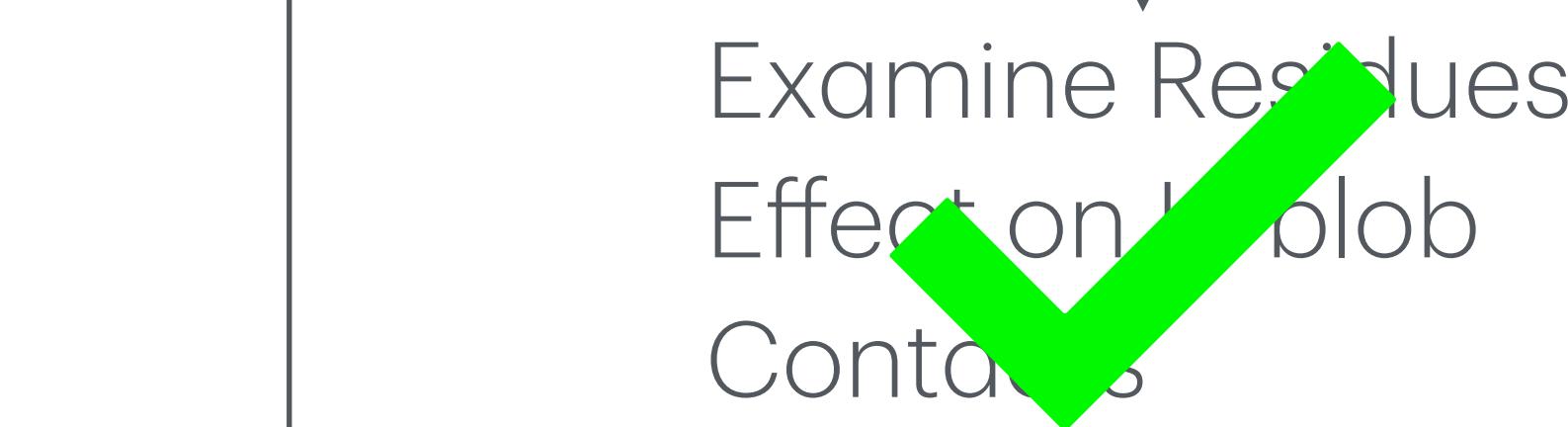


Results

If h-blobs are **long** and **more hydrophobic** then they have more interactions with other h-blobs



Examine Residues
Effect on H-blob
Contacts



Examine how long h-blobs effect H-blob contacts



→ Examine Long H-blobs to Four Short H-blobs



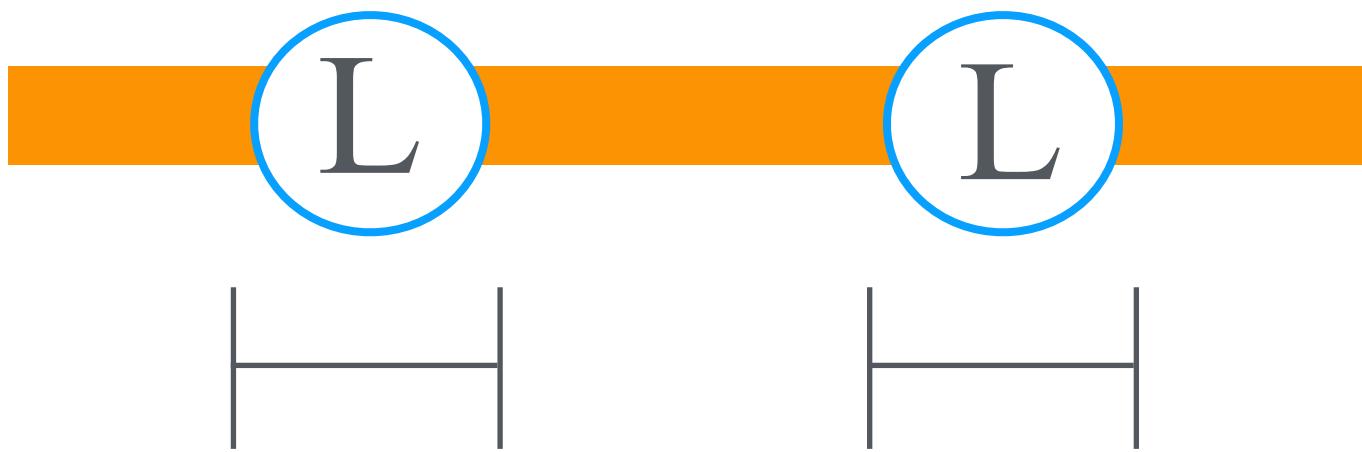
Contiguous Hydrophobicity
Increases H-blob Contacts

← Examine Two H-blobs to Four H-blobs

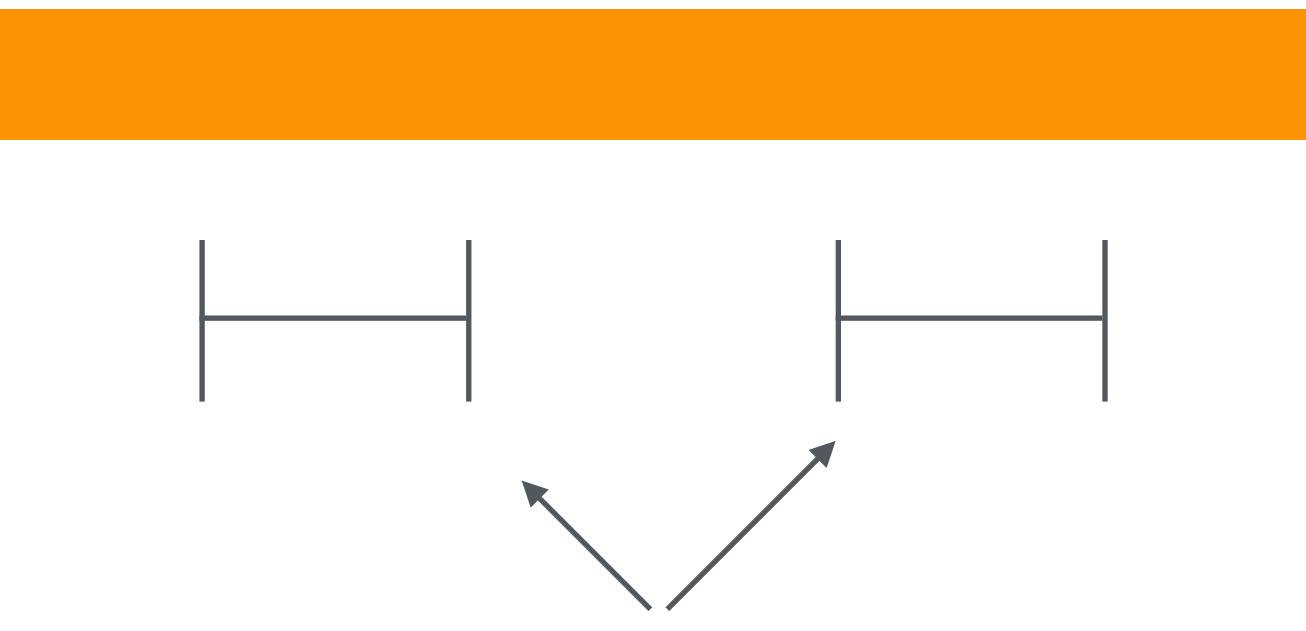


Measure Contacts by having shadow blobs for polar peptide

Peptide 7

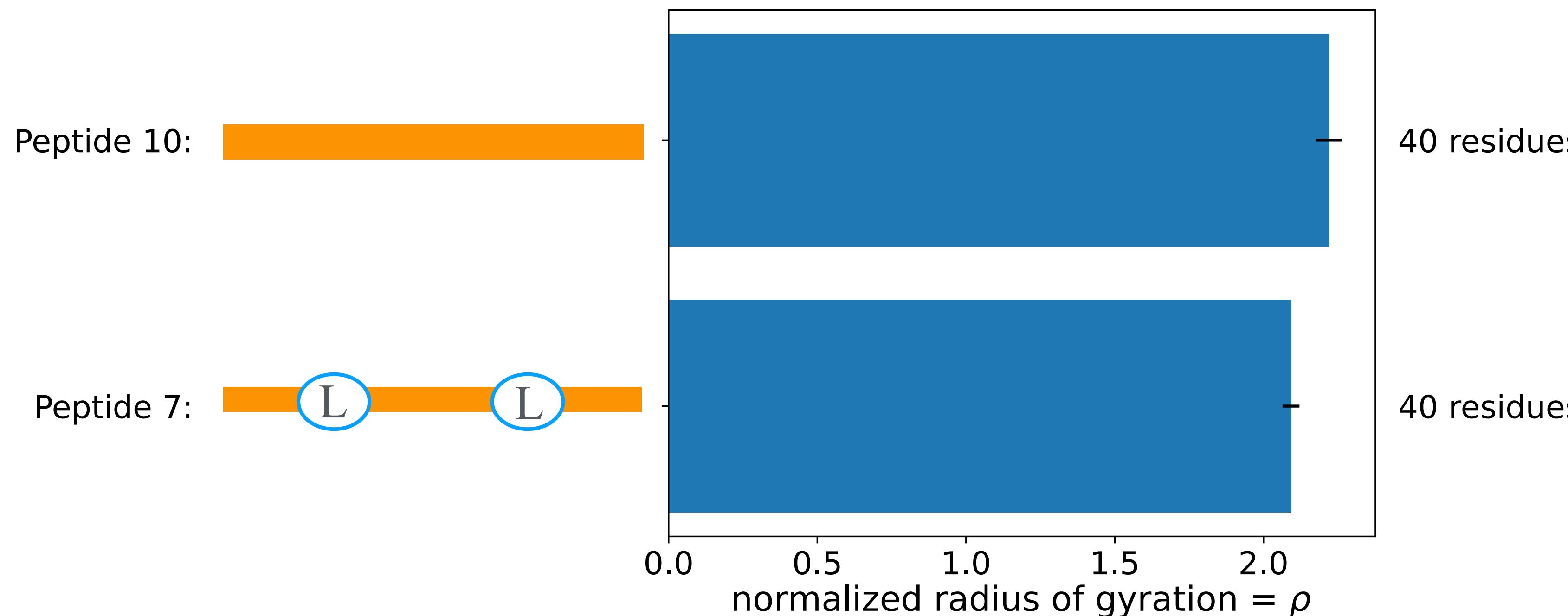


Peptide 10



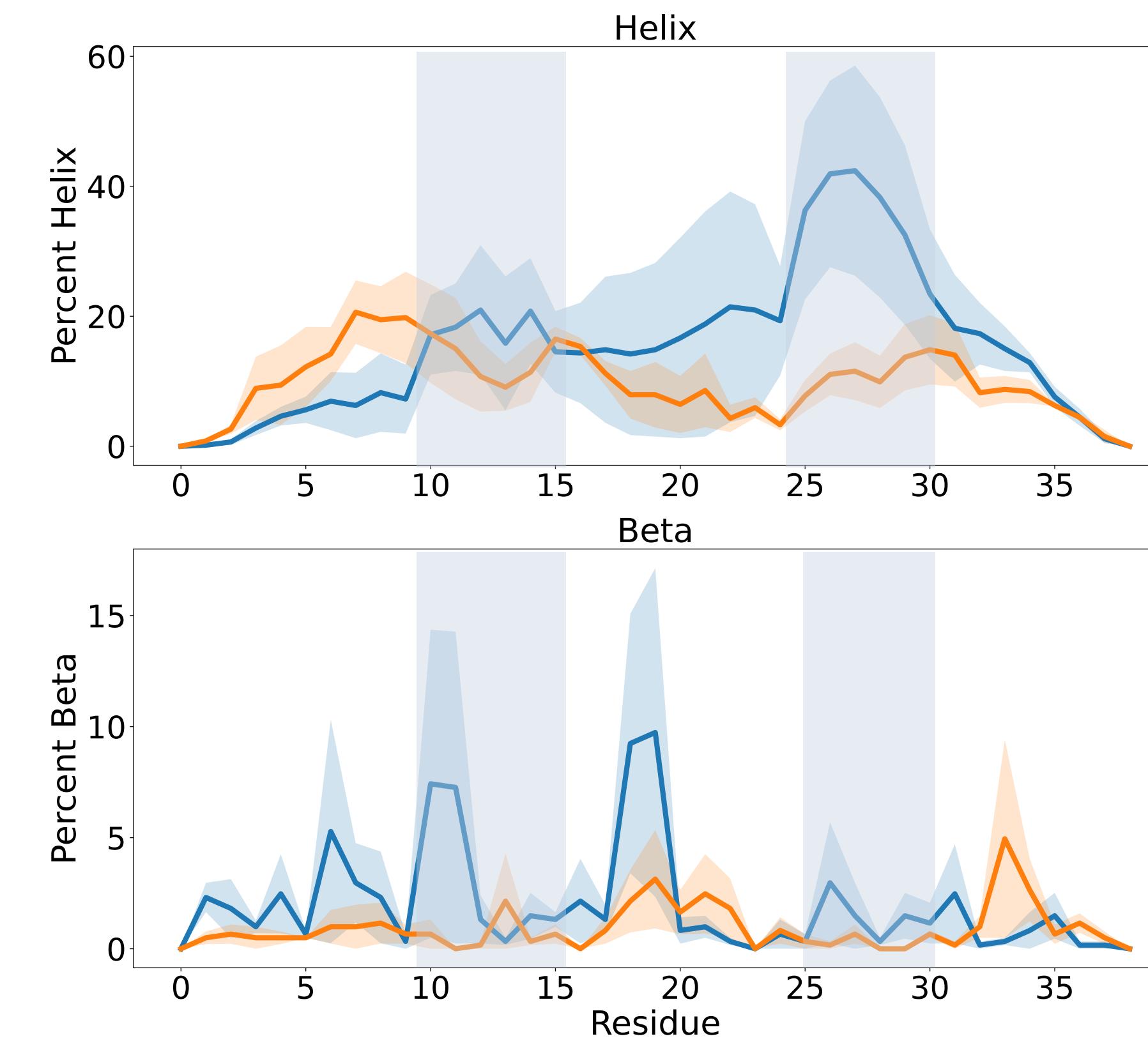
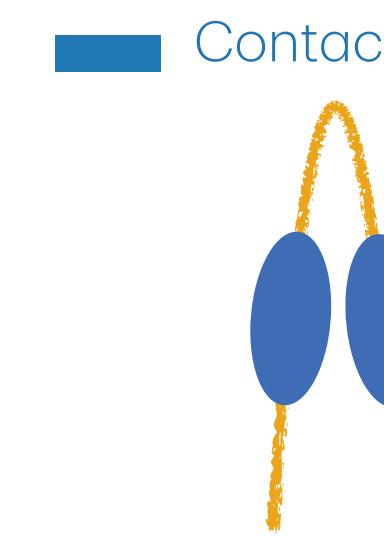
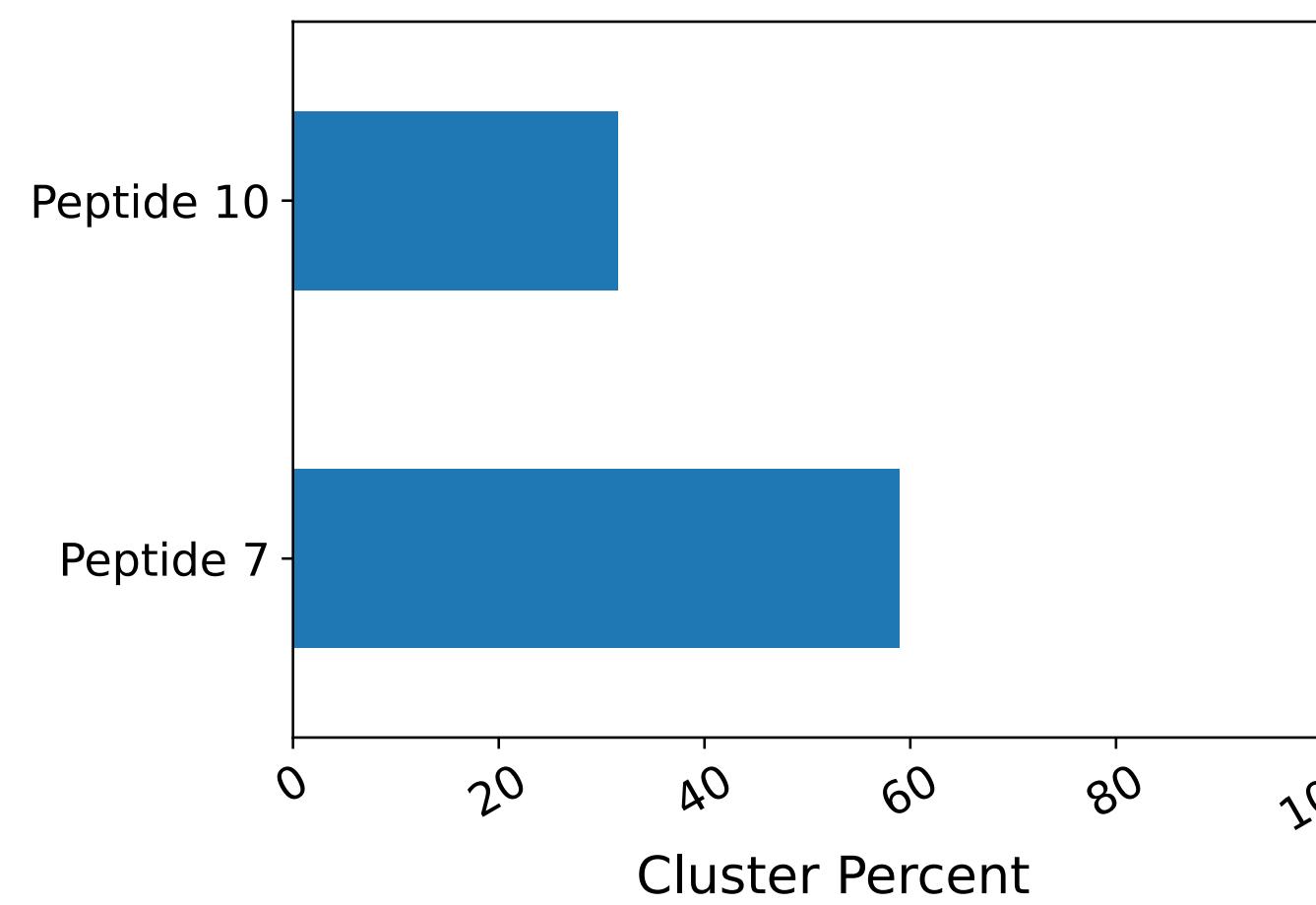
Fake Blobs

Contiguous Hydrophobicity Produces More Compaction

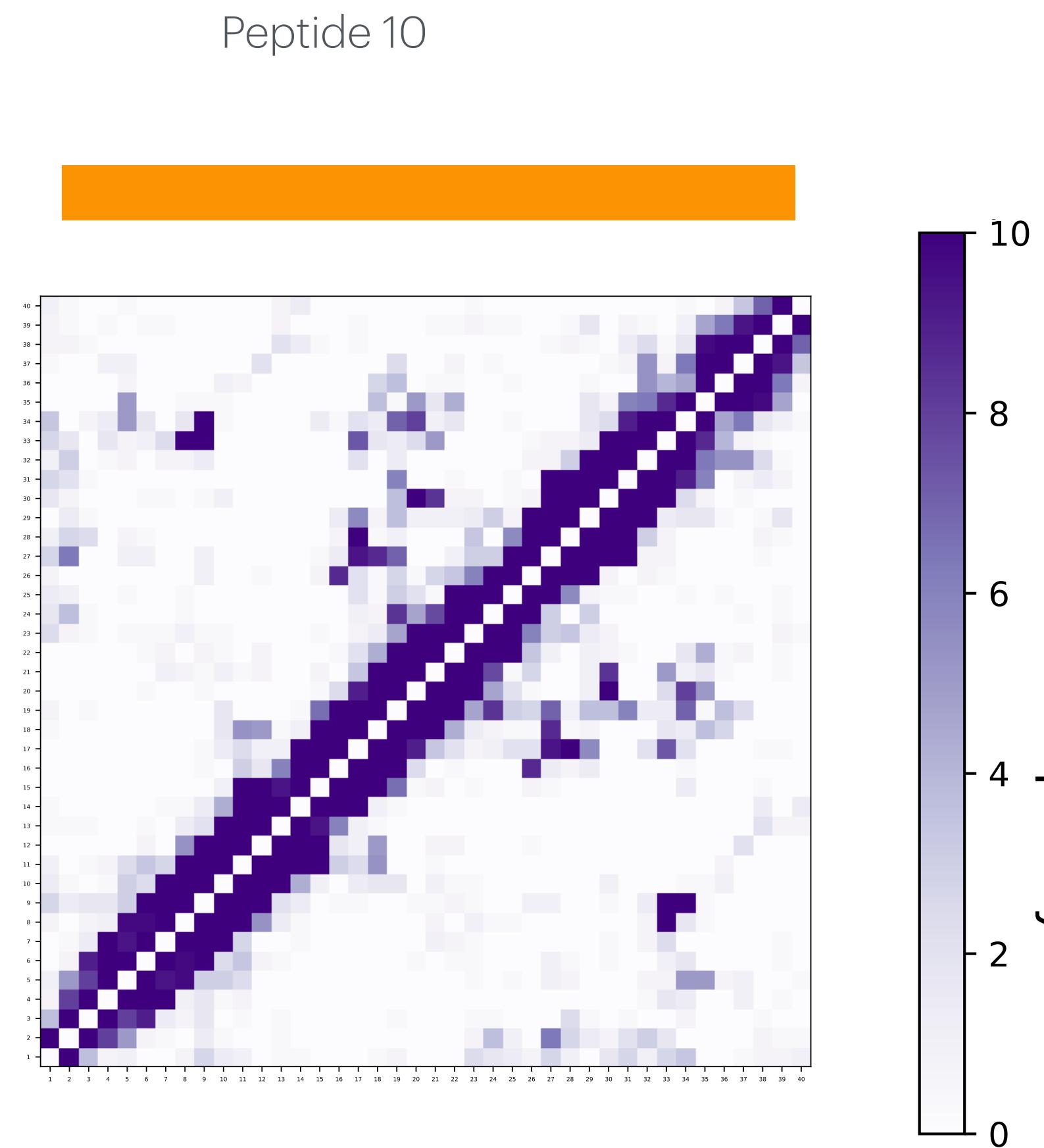
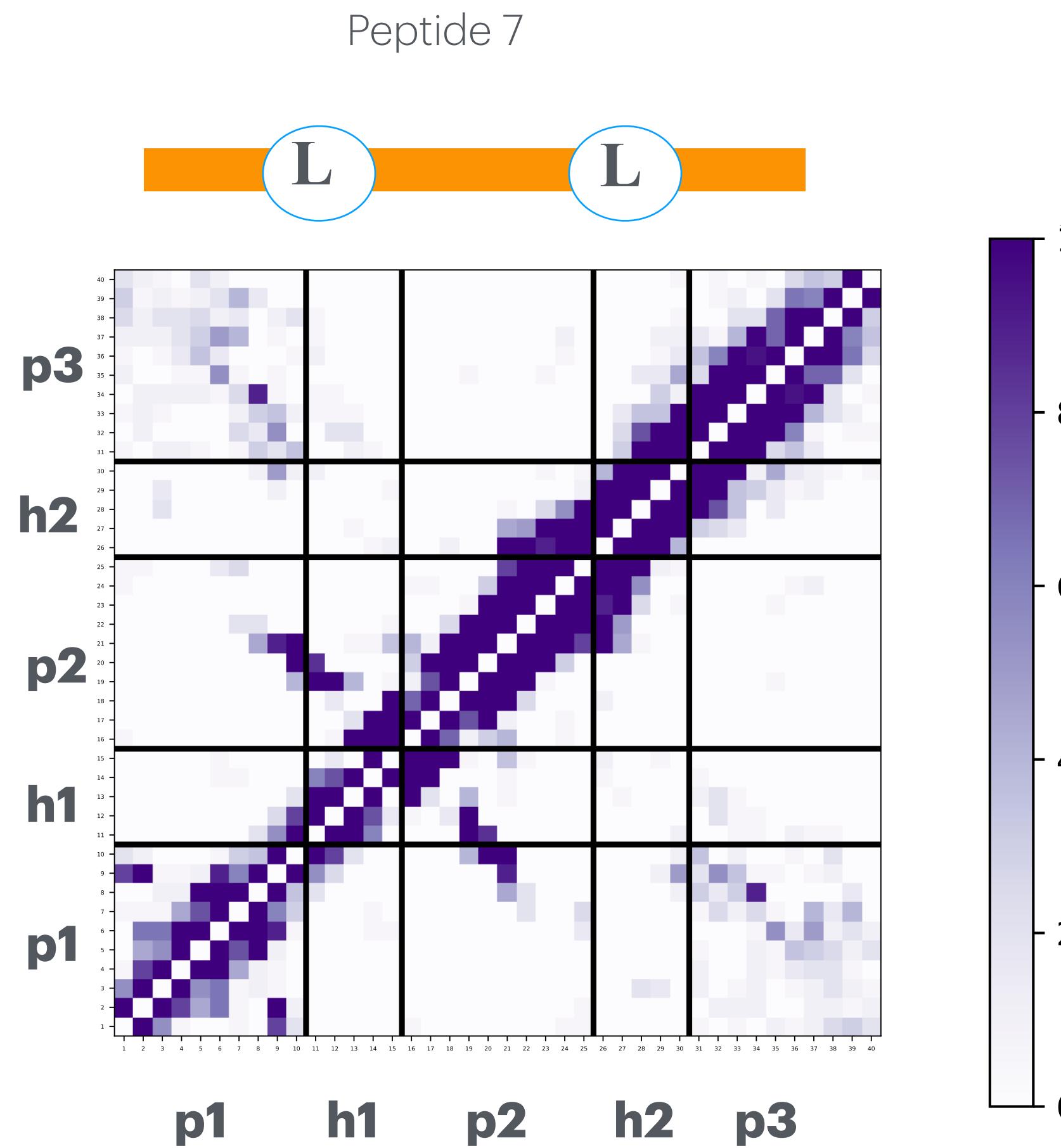


$$rgyr = \sqrt{\frac{\langle R_g^2 \rangle}{N}}$$

Contiguous Hydrophobicity Increases h-blob Contacts



Contiguous Hydrophobicity Increases h-blob Contacts



Results

If h-blobs are long and more hydrophobic then they have more interactions with other h-blobs

Examine Residues
Effect on H-blob
Contacts

Examine how long h-blobs effect H-blob contacts

→ Examine Long H-blobs to Four Short H-blobs

Contiguous Hydrophobicity
Increases H-blob Contacts

← Examine Two H-blobs to Four H-blobs

In Summary

- Increasing hydrophobicity, with Kyte-Doolittle scale, does not increase h-blob contacts
- Increasing h-blob length increases h-blob contacts
- Splitting long h-blobs into multiple short h-blobs does not promote greater compaction
- Increasing hydrophobic content increases compaction
- The presence of h-blobs increases h-blob contacts

Acknowledgements



Office of Advanced Research
Computing