The Role of Blob Composition in Determining Peptide Conformation

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Abstract

Three dimensional clusters of hydrophobic residues help stabilize protein structure via the hydrophobic effect. Hydrophobic residues with multiple contact residues are expected to be particularly sensitive to mutation, but traditionally, these residues have been identifiable only by consulting the 3D protein structure. Identifying these residues directly from a 1D sequence could inform sequence-based genomic and bioinformatics analyses. Our Blobulation method segments a 1D protein sequence into contiguous regions of hydrophobicity called "h-blobs", as well as non-hydrophobic "p-blobs" and "s-blobs". A previous study from our lab found that h-blobs are more likely to contain disease-associated mutations across the human exome. Additionally, we've discovered that a disease associated mutation found in an h-blob alters an intrinsically disordered protein's interaction network via molecular dynamics simulations. However, the effect of altering h-blob characteristics such as length and composition on intra-protein interactions still remains an open question. We created multiple peptide sequences containing h-blobs of varying lengths and compositions to examine the effects of these characteristics on intra-protein interactions. We performed an all atomistic molecular dynamics simulations for 1 μs after annealing and equilibration in ionized water under constant pressure on each peptide. We expect that peptides containing the longest h-blobs made of the most hydrophobic residues will be more compact and undergo a higher rate of intra-protein interactions compared to other peptides. By analyzing these simulations, we are able to quantify this effect by measuring the radius of gyration and intra-protein interactions of each peptide.

Blobulation

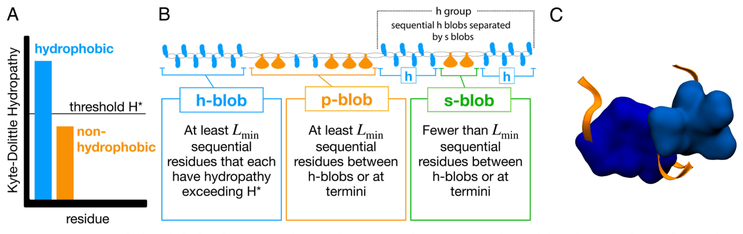

Figure 1. Diagram of the blobulation algorithm. A. The normalized hydropathy of each residue is compared to the threshold (H^*) to determine if its hydrophobic. B. Each stretch of hydrophobic residues greater than the length minimum (L_{min}) is considered an h-blob. C. Graphical representation of blobulation using a model peptide.

Background

- In a previous study, we found that disease-associated single nucleotide polymorphisms (dSNPs) are more likely to occur in longer and more hydrophobic blobs (h-blobs) than shorter and less hydrophobic blobs [1].
- Additionally, we found that h-blobs have a low solvent accessible surface area compared to p-blobs [1].
- In an aqueous environment, we expect h-blobs to be interacting with each other, as in the hydrophobic core of a globular protein.
- We aim to examine how h-blob characteristics (residue composition, length, and number) influence the tertiary interactions of peptides.

Research Questions

1. What is the effect of the residues that compose the h-blob on tertiary interactions?
2. Does blob length play a role in tertiary interactions?
3. Does the blob amount affect tertiary interactions?
4. Are peptides with clusters of contiguous hydrophobic residues more globular than peptides with distributed hydrophobic residues?

Approach

1. Select sequences with varying residue composition, length, and number of h-blobs (Table 1). Blobulation was done using default parameters (H^* = 0.4, L_{min}= 4).
2. Generate starting structures for peptides using I-Tasser [2].
3. Run atomistic simulations:
   - Software: Gromacs 5.1.2 [3], Force Field: Amber99sb-ildn-q [4]
   - Temperature : 300k
   - Peptide Simulations: 8 peptide (3 replicas), 2 μs
4. Analyze Simulations:
   - Average Radius of Gyration $\langle R_g \rangle$.
   - Blob - Blob Contact Frequency: The frequency of contacts between two blobs (0.55 nm) across each simulation [2].

#	Sequence	Topology	⟨Hydropathy⟩
1	NNNNLLLLLNNNNLLLLLNNNN	L L	-0.18
2	NNNNIIIINNNNIIIINNNN	I I	0.14
3	NNNNVVVVNNNNVVVVNNNN	V V	0
4	NNNNAAAANNNNAAAANNNN	A A	-1.1
5	NNNNLLLLLLLLLNNNNLLLLLLLLLNNNN	L L	1.1
6	NNNNLLLLNNNNLLLLNNNNLLLLNNNNLLLLNNNN	L L L L	0.15
7	NNNNNNNNNNLLLLNNNNNNNNNLLLLNNNNNNNNNN	L L	-1.7
8	NNNLNNNLNNNLNNNLNNNLNNNLNNNLNNNLNNNL		-1.7

Table 1. Numbered peptide sequences, topology cartoon, and average hydropathy. Topology cartoons are used to represent peptides throughout the poster. Peptides with 22 residues will be called short peptides.

Average Radius of Gyration

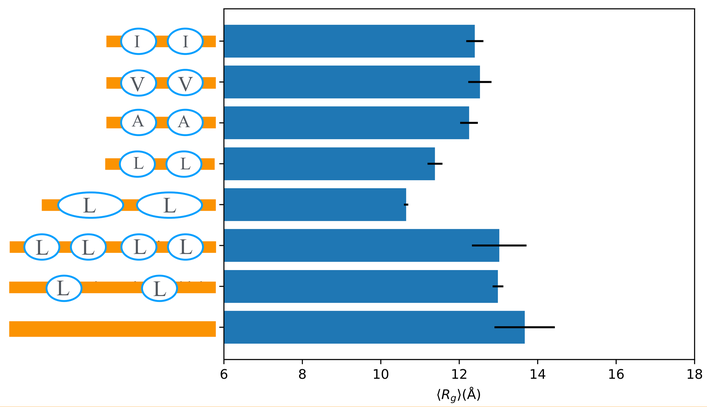

Figure 2. Average radius of gyration ($\langle R_g \rangle$) for each model peptide simulation across 3 replicates.

Blob - Blob Contacts

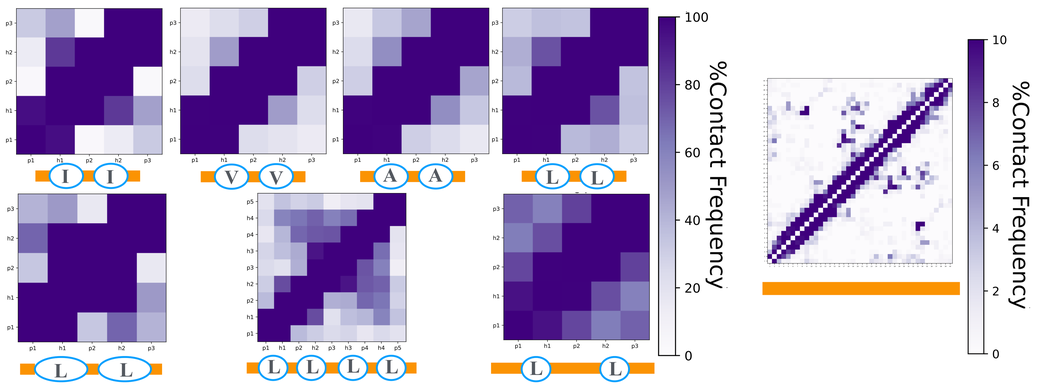

Figure 3. Blob to blob contact frequency heat maps. Top row shows "short" peptides, bottom row shows varying number and length of h-blobs. Peptide with alternating Leucines (#8) shown to the right.

Summary

1. Of the aliphatic residues, peptides with leucine blobs (#1) are the most compact.
2. Additionally, adding more leucines (#5) results in a peptide that is even more compact, despite being longer overall.
3. Peptides with 4 leucine blobs (#6) and peptides with 2 leucine blobs (#7) are similarly compact.
4. Peptides with non-adjacent leucines (#8) had similar $\langle R_g \rangle$ to those that had the same number of leucines in blobs (#7).

VMD Plugin

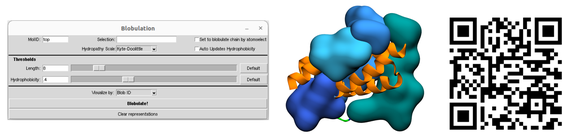

Figure 4. The blobulation plugin for Visual Molecular Dynamics (VMD). The VMD plugin used for the blob - blob contact maps, and to create the graphical representations shown in this poster. To access the VMD plugin, visit our GitHub page by using the QR code above.

References

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3. Berendsen H.J.C., van der Spoel D., van Duren R. *CPC* (1995)
4. H. Bekker, H.J.C. Berendsen, E.J. Dijkstra, S. Achterop, R. van Drunen, D. van der Spoel, A. Sijbers, and H. Keegstra et al. Physics computing (1993).

Acknowledgments

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