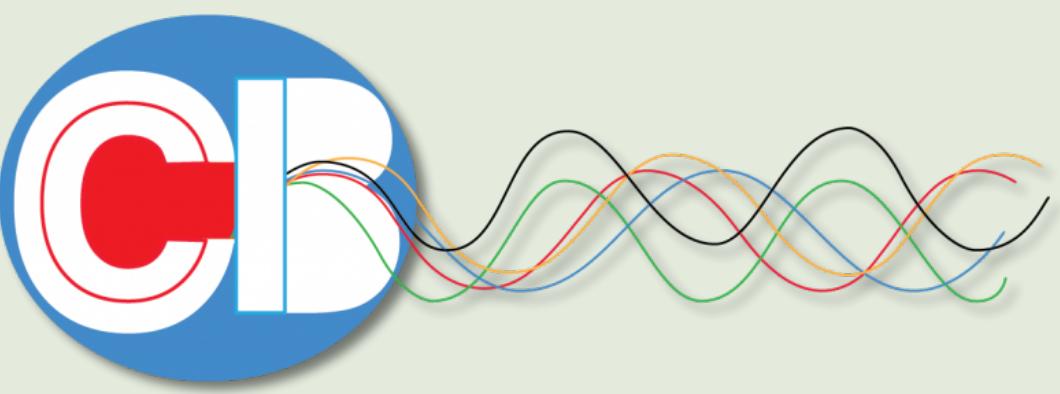


Hydrophobic specificity plays a role in determining the conformational landscape of a long intrinsically disordered protein

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Abstract

Recently, our lab demonstrated the Val66Met mutation in the disease-associated, long disordered prodomain of brain derived neurotropic factor (BDNF) increases protein compactness and tertiary interactions within the protein [1]. A notable discovery was the side-chain interaction between M66 and M95 which allows the backbone near M95 to form b-bridges with the residues near the N-terminus. These results demonstrate structural sensitivity to a charge-neutral mutation in an intrinsically disordered protein (IDP). It was unknown whether other charge-neutral mutations affect the structure of this IDP, or if our previous results were specific to M66 due to the special nature of Met-Met interactions. Here, we present the analysis of 2 μ s all-atomistic, temperature replica-exchange molecular dynamic simulations of the wild-type BDNF prodomain (V66) and mutated BDNF prodomains that contain a charge-neutral, hydrophobic mutation at position 66 of the amino acid sequence (A66, I66, L66, F66, Y66, M66). We discovered that in addition to M66, I66 and Y66 alter the conformation of the protein. Next, we applied our novel Blobulation method to the BDNF prodomain to identify regions of hydrophobicity. This method allowed us to easily determine unique self-interactions within the mutated proteins compared to the wild-type. These findings demonstrate that the conformational changes in the BDNF prodomain due to the mutations are not specific to M66 and extends to other charge-neutral amino acid mutations. This suggests that hydrophobic specificity plays a role in determining the protein compactness of the BDNF prodomain despite it being a long IDP.

Background

- The BDNF prodomain containing a Val66Met mutation has been shown to be associated with neuropsychiatric disorders [2].
- We applied the Blobulation method to the protein (Figure 1) which revealed unique self-interactions, specifically a Met-Met interaction that caused increased protein compactness [1].
- The BDNF prodomain does not have a defined structure therefore a small, charge-neutral amino acid mutation was not expected to change its conformation.
- We are interested in how other charge-neutral amino acid mutations may affect the conformation of the BDNF prodomain.

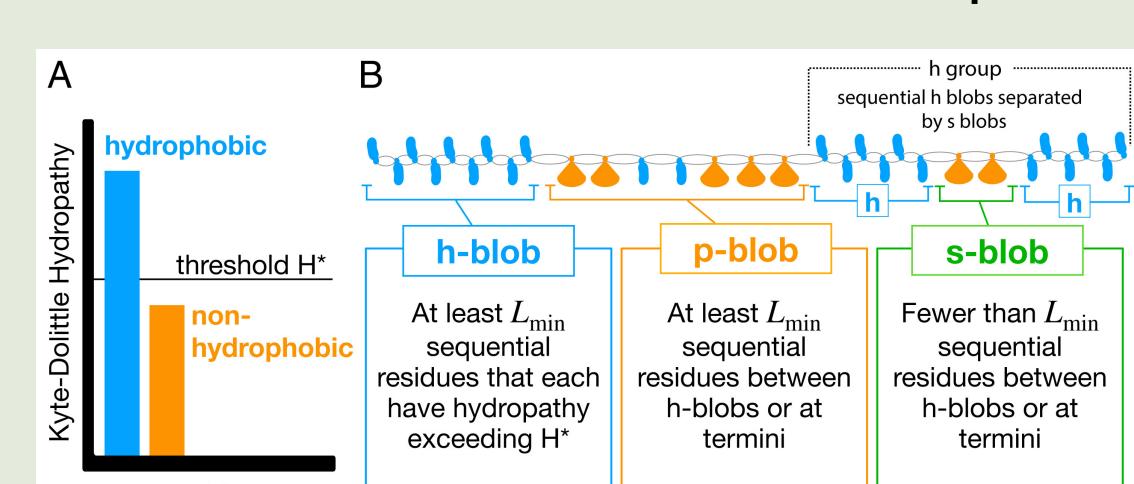


Figure 1: Graphical representation of the Blobulation method. (A) The amino acid sequence is digitized by the user defined hydrophobicity threshold (H^*). (B) Next, the sequence is clustered into 3 different blobs based on H^* and the minimum number of amino acids (L_{min}). Figure adapted [3].

Research Questions

- Are the conformational effects observed in our previous study specific to the Val66Met mutation or does it extend to other charge-neutral amino acids?
- How do charge-neutral mutations affect self-interactions in the BDNF prodomain?
- Is there a relationship between hydrophobic specificity and the conformation of an IDP?

Approach

- Blobulate each protein: A stretch (L_{min}) of 4 or more residues with $\langle H \rangle > 0.37$ (H^*) are termed hydrophobic blobs (h-blobs) and the remaining residues are classified as non-hydrophobic blobs (p-blobs).
- Analyze simulations: Calculate radius of gyration, blob contact enrichment, and residue contact enrichment.

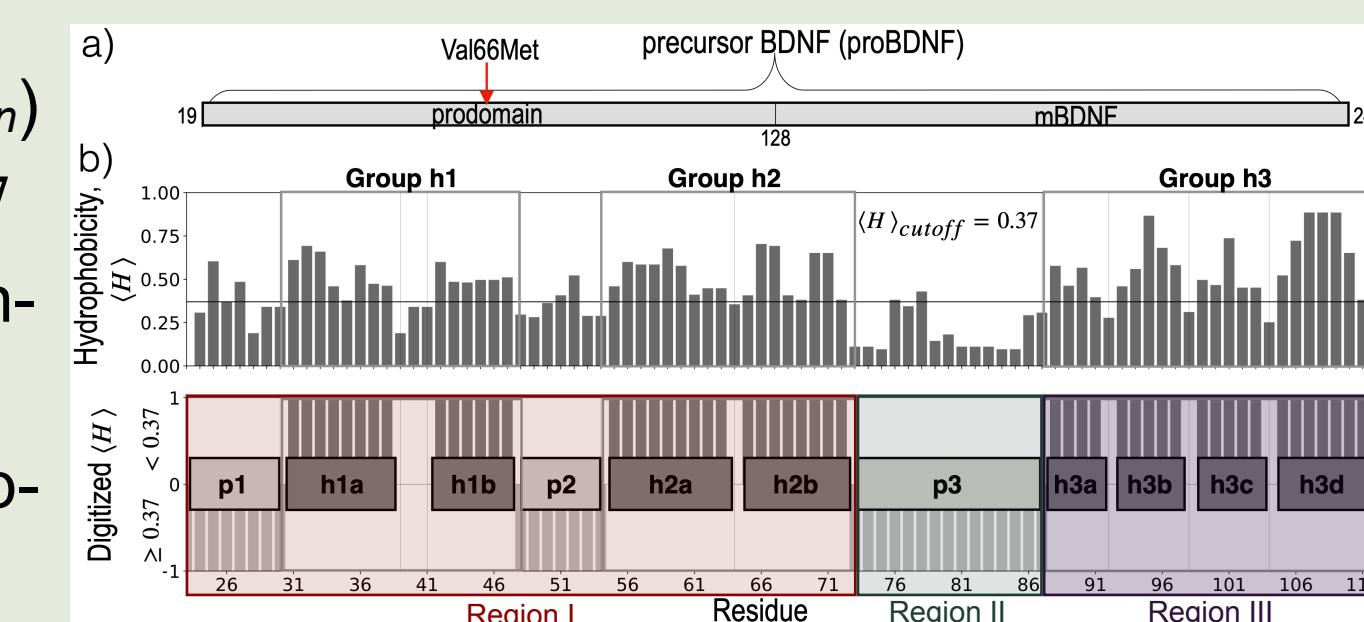


Figure 2: (a) Graphical representation of the BDNF prodomain location of the entire protein and (b) the Blobulation method applied to the protein. Figure adapted [1].

Effect of mutation on conformation

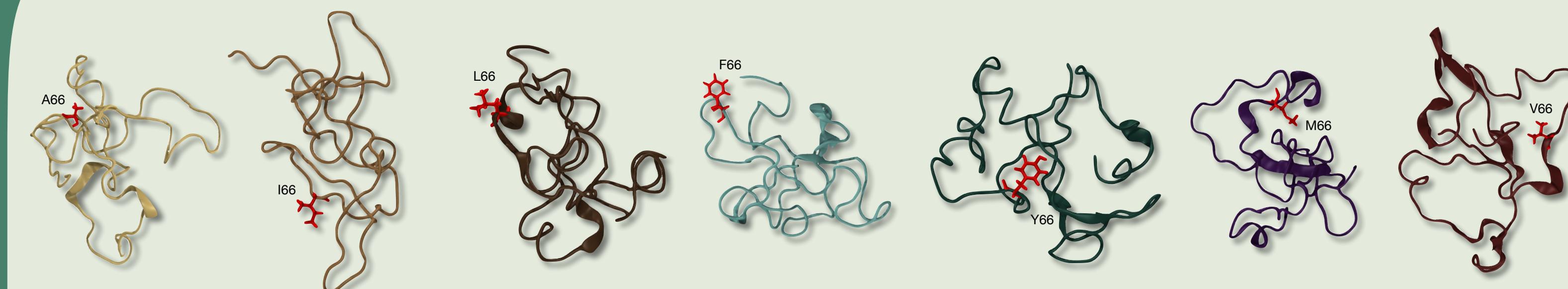


Figure 3: BDNF prodomain (New Cartoon) and its amino acid on position 66 (Licorice) made in Visual Molecular Dynamics.

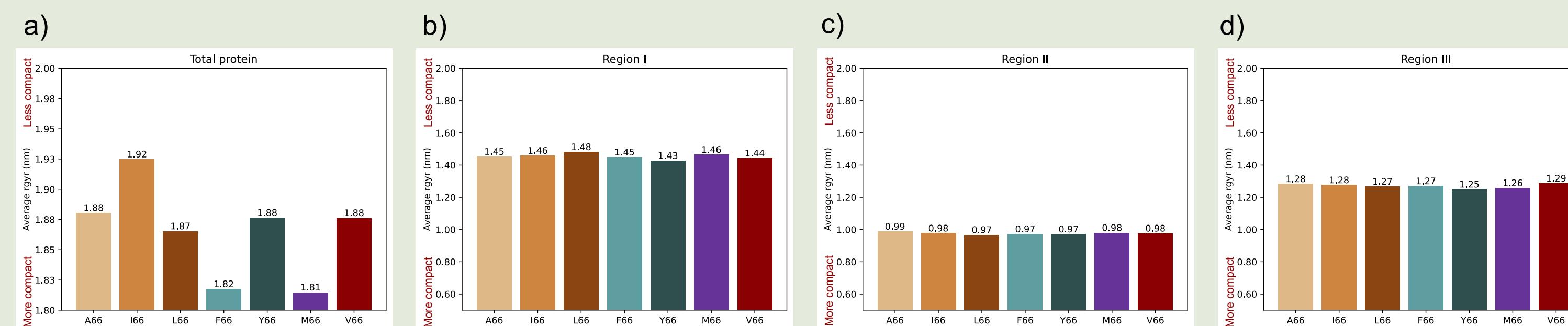


Figure 4: (a) Total radius of gyration ($rgyr$) and (b-d) radius of gyration of domain I, III, and III of each protein. Total radius of gyration is altered in BDNF prodomain containing mutations I66, F66, and M66 compared to V66. Radius of gyration of each region does not drastically fluctuate between mutations.

Residue-level contact enrichment

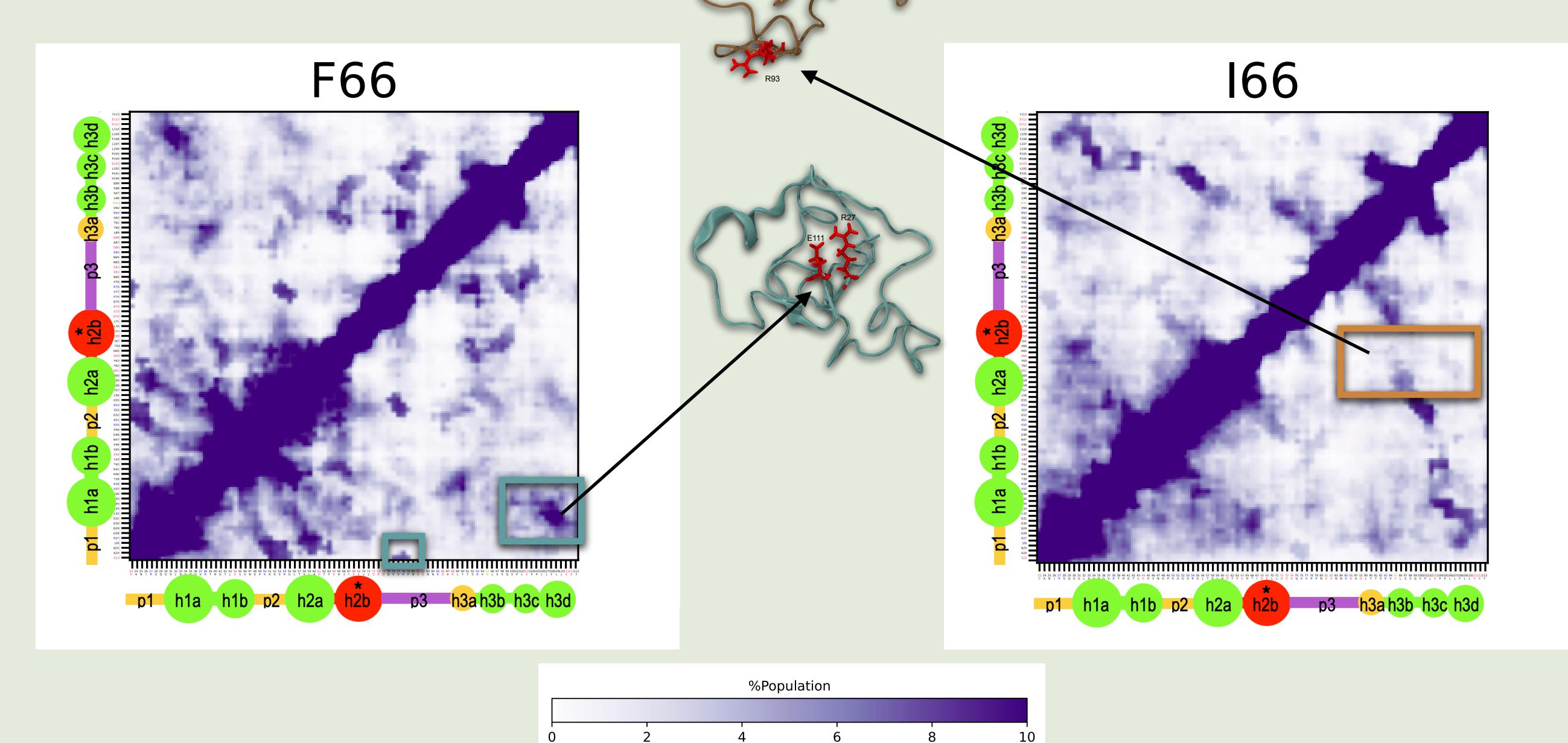
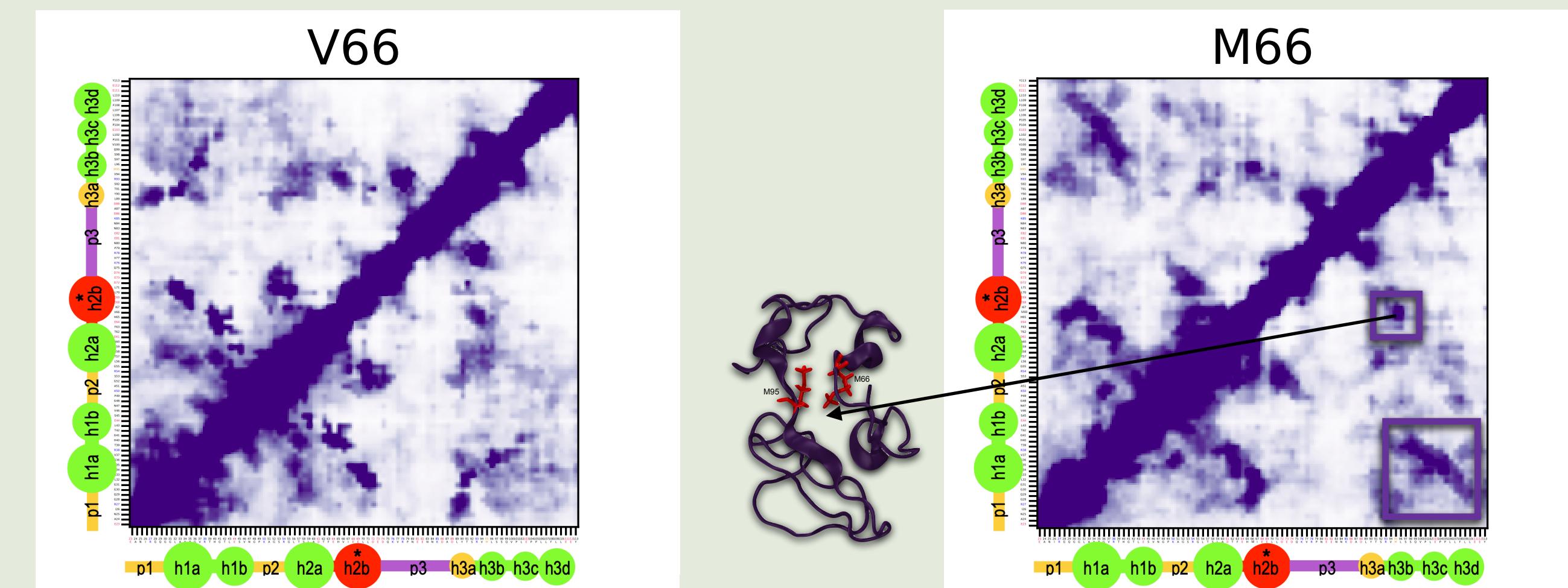


Figure 8: Probability of two residues in contact in the simulation for each protein. Two residues are in contact if the distance between them is below 0.8 nm. Mutated proteins show values of enrichment (M66 & F66) and depletion (I66) for certain residue contacts.

Blob-blob contact enrichment

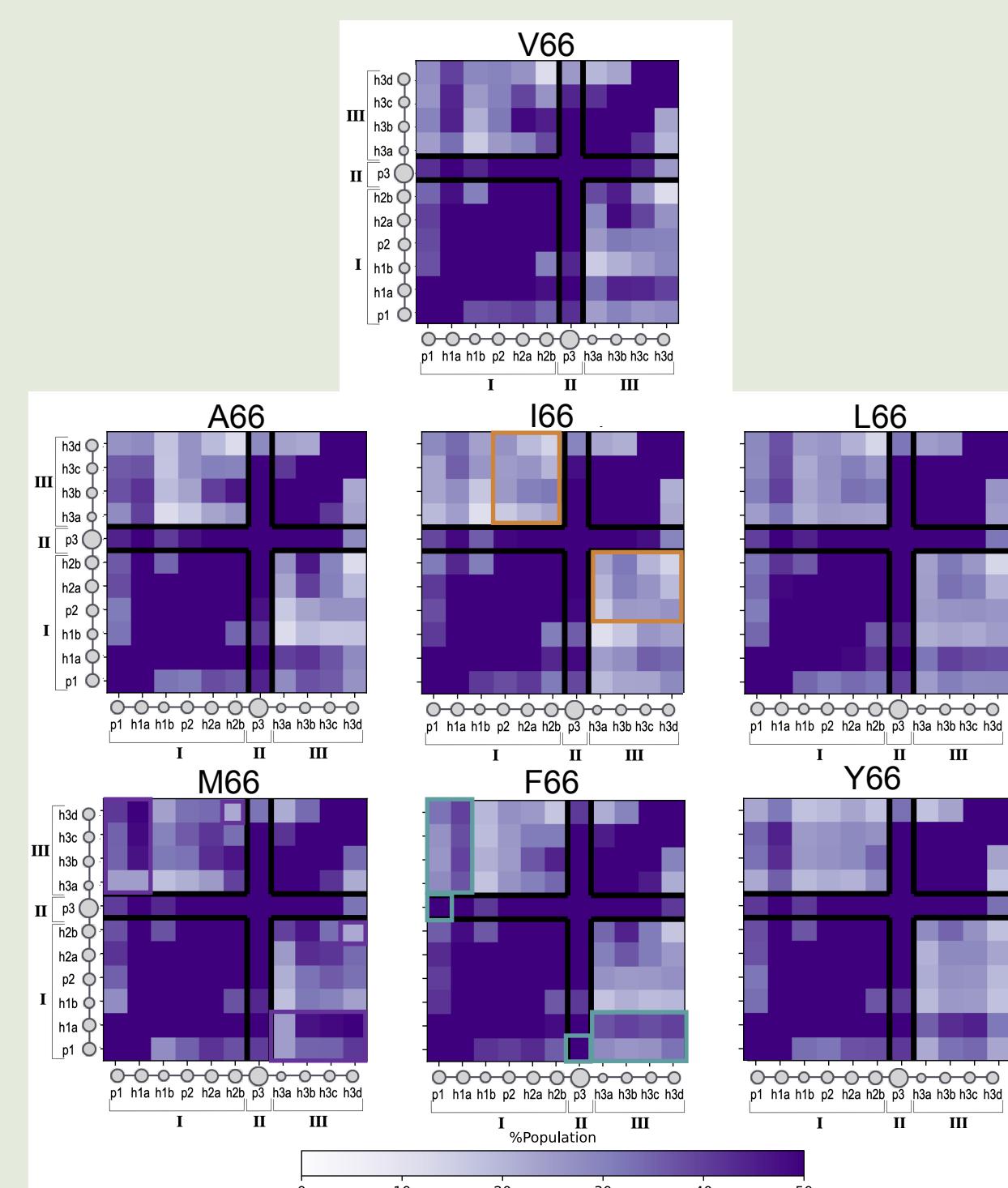


Figure 6: Probability of two blobs in contact in the simulation for each protein. Two blobs are in contact if the distance between them is below 0.55 nm. The probability is measured by determining the contact frequency or the number of instances where two blobs are in contact in the simulation.

Blob-blob contact enrichment

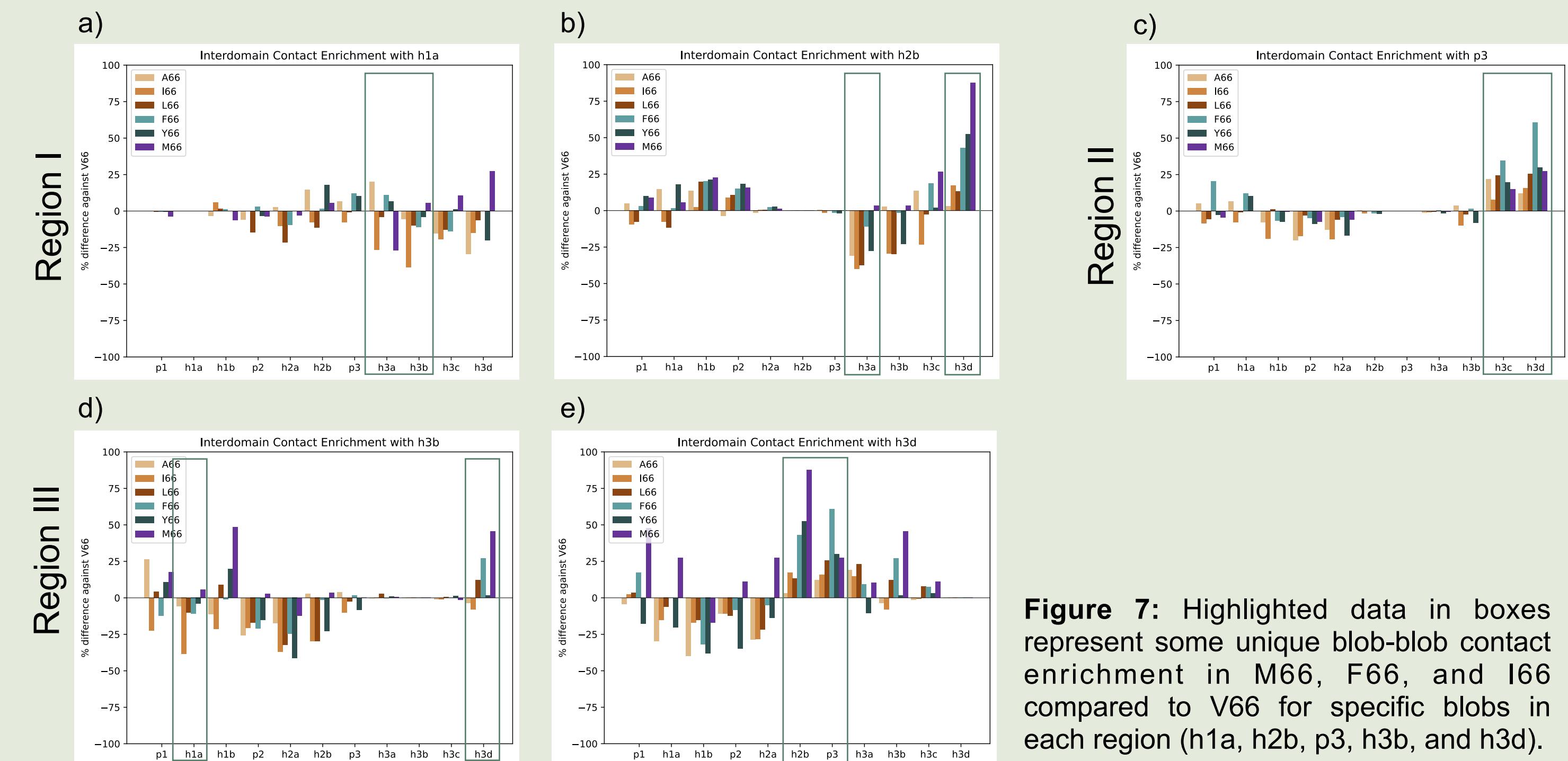


Figure 7: Highlighted data in boxes represent some unique blob-blob contact enrichment in M66, F66, and I66 compared to V66 for specific blobs in each region (h1a, h2b, p3, h3b, and h3d).

Summary

- Sensitivity of a small, neutral mutation at site 66 of the amino acid sequence is not specific to M66.
- Protein compactness decreases in F66 and increases in I66.
- F66 mutation shows increased self-interactions within regions I & III, II & III, and III & III.
- I66 mutation shows decreased self-interactions within regions I & III.
- Residue-residue contacts show specific interactions between residues that influence blob-blob contacts in each mutation.
- Hydrophobic sensitivity extends to mutations I66 and F66 suggesting that a single mutation can influence the structure of an IDP and thus their function

References

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