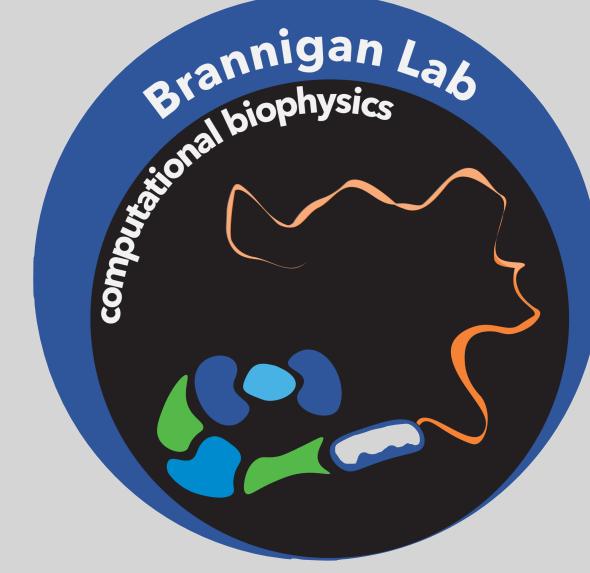


Hydrophobic specificity plays a role in determining the conformational landscape of a long intrinsically disordered protein



Lindsey Riggs¹, Ruchi Lohia¹, Grace Brannigan^{1,2}
 Center for Computational and Integrative Biology¹, Rutgers - Camden, NJ, Department of Physics, Rutgers - Camden, NJ²

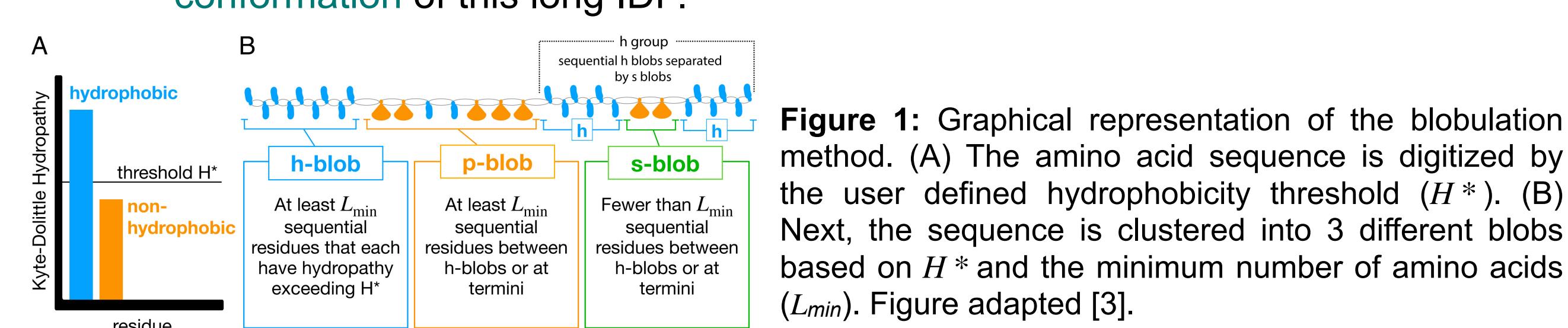


Abstract

A Val66Met mutation in the disease-associated prodomain of brain derived neurotrophic factor (BDNF) is known to alter the conformational ensemble of the protein. It was unclear how a small, charge-neutral mutation would affect the conformation or function of the BDNF prodomain, a long intrinsically disordered protein (IDP). Our lab aimed to determine the mechanism behind the conformational shift in the mutated protein (M66). We discovered that this effect is due to the special nature of Met-Met interactions within the protein. This raised the question whether other charge-neutral mutations at site 66 affect the conformation of the BDNF prodomain, or if our results were specific to M66. Here, we present the analysis of 2 μ s all-atomistic, explicit solvent, 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 sequence (A66, I66, L66, F66, Y66, and M66). We found that, in addition to M66, F66 also increases the radius of gyration, while A66, I66, L66, and Y66 decrease it compared to V66. We also applied our Blobulation method to the BDNF prodomain and found unique interactions between contiguous regions of hydrophobicity. This method was key to identifying the mechanism behind the conformational effects due to M66. 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. Our results suggest that even IDPs are sensitive to specific interactions between non-aliphatic, hydrophobic residues, which can detectably shift the ensemble toward a more globular conformation.

Background

- The prodomain of BDNF (proBDNF) with a Val66Met mutation is associated with neuropsychiatric disorders [1].
- We applied our “blobulation” algorithm on our simulations of proBDNF (Fig. 1) which identifies clusters of adjacent hydrophobic residues to help characterize tertiary interactions of the protein.
- Specifically, we discovered a Met-Met interaction that caused increased protein compactness and a shift in the blob contact network [2].
- We are interested in how other charge-neutral amino acid mutations affect the conformation of this long IDP.



Research Questions

- How does a single charge-neutral mutation affect the tertiary interactions in a long IDP?
- What is the mechanism behind the conformational shift due to the charge-neutral mutation on site 66 in proBDNF?

Approach

- Run simulations:** 900 μ s of fully atomistic, explicit solvent, replica exchange Molecular Dynamics [4] simulations of 7 proBDNF sequences with a different hydrophobic mutation at site 66: V, M, F, Y, A, L, I. Simulations used the Amber99sb*-ildn-q forcefield [5] and GROMACS 5.1.2 [6].
- Blobulate 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 the radius of gyration ($\langle R_g \rangle$), contacts between blobs and residues, and secondary structure formation.

