

Conformational effects of various hydrophobic-to-hydrophobic substitution located at the midpoint of the intrinsically disordered region of proBDNF

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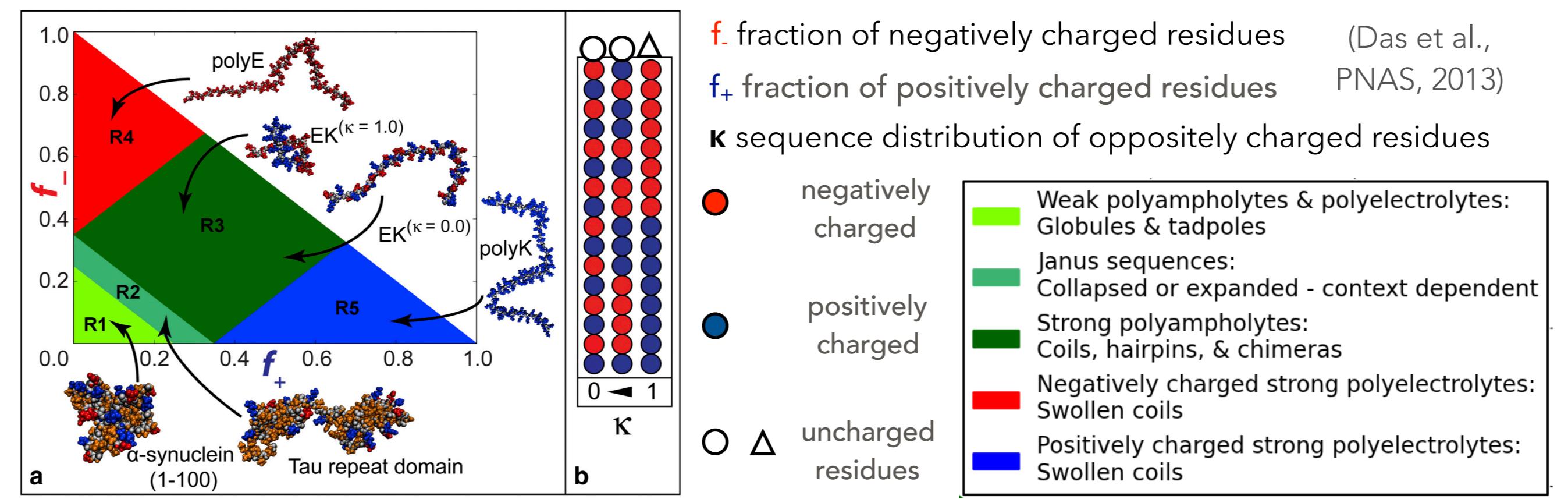
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Abstract

Although the role of electrostatic interactions and mutations that change charge states in intrinsically disordered proteins (IDPs) is well-established, many disease-associated mutations in IDPs are charge-neutral. Earlier, we studied the effects of the disease-associated Val66Met substitution at the midpoint of the prodomain of precursor brain-derived neurotrophic factor (proBDNF) using fully atomistic molecular dynamics simulations. Val66Met substitution is found in 25% of the American population, which has been widely studied for its association with aging-related and stress-related disorders, reduced volume of the hippocampus, and variations in episodic memory. We found that the local secondary structure, transient tertiary contacts, and compactness of the protein are correlated to backbone configuration around residue 66. The midpoint location and the substitution at the most highly charged region of the protein played a critical role in causing the conformational changes of Val66Met substitution. To gain further insight into the generalizability of the found mechanism with which a hydrophobic-to-hydrophobic substitution can effect the IDP's conformational ensemble and, to further establish the significance of substitution location, we studied 5 more hydrophobic substitutions at residue 66. We report on fully-atomistic temperature replica exchange molecular dynamics simulations of the 90 residue proBDNF for Ala66, Ile66, Leu66, Phe66 and Tyr66 sequence. Analyzing and comparing the residue level insight from all 5 simulations helped us in further establishing the significance of charge-neutral mutations in IDPs.

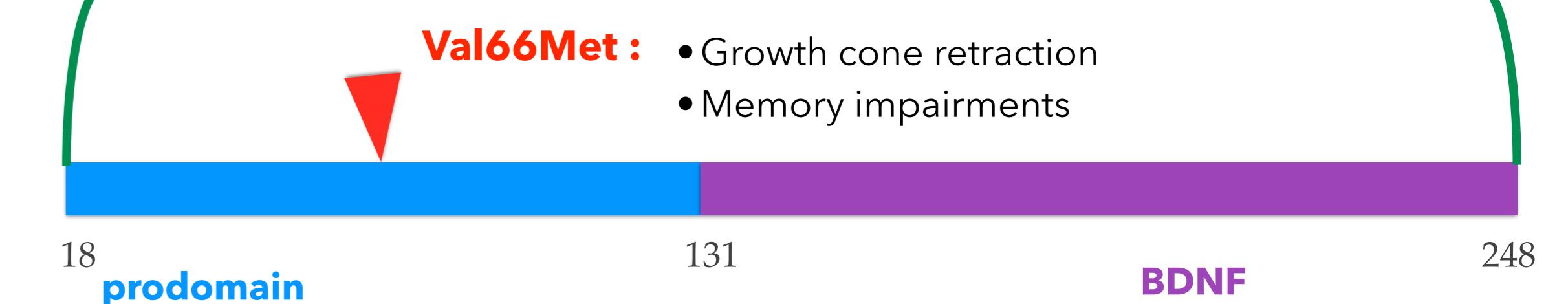
Challenges in predicting IDP's sequence ensemble relationship

- >75% the IDP's are polyampholytes and shows the sequence ensemble relationship as predicted by Pappu's lab
- ~70% of the IDP's fall in the R1,R2 region
- The current theory deals with sequence composition and sequence patterning and is completely based on charged residues
- It does not deal with specific residues; for e.g. different polar side-chains will have different effects on the conformational properties and solubility profiles of IDPs
- > 20 % of missense disease mutations are located in disordered regions
- >10% of these mutations are hydrophobic to hydrophobic mutations



BDNF and Val66Met SNP

- precursor BDNF (proBDNF):**
- Neurotrophin family of signaling proteins
 - Apoptosis, refinement of correct target innervation during development



- Val66Met:**
- Growth cone retraction
 - Memory impairments
- Intracellular trafficking, folding of mature domain
 - Growth, differentiation, maturation and survival of neurons.

Earlier Studies (NMR) Anastasia et al 2013[A]

- NMR (273K) and CD spectra (300K) : Val66 and Met66 prodomain both intrinsically disordered.
- NMR prediction (273K) : slightly increased helicity around residue 66 for M66 than V66.
- NMR diffusion measurements reported slightly higher hydrodynamic radius for V66 than M66. (V66 - 2.24nm, M66 2.20nm at 298K).

Methods

- Purpose: prodomain simulation for both V and M forms.
- Package: GROMACS 5.1.2.[C]
- Sampling method: replica exchange with explicit solvent Tip4P-D [D]
- Total simulation time: 300 ns per replica for each mutation
- Initial conditions : Different random coil for each replica
- Force field: Amber99sb-ildn-q [E]
- No. of replicas: 64 replicas
- Temperature range: 300 K to 385 K
- Acceptance ratio : 20-25%.
- Exchange frequency: 1ps

Summary

- Val66Met increases the formation of long helices around residue 66, consistent with different entropic cost of helix formation.
- Clustering at residue 67 segregates the long range contacts and Rg among clusters
- The difference in the Rg observed between V and M is seen only when residue 67 is in B clusters.
- B1 and B2 clusters have more long range contacts in M, consistent with the low Rg observed among these clusters when compared with V.
- For each hydrophobic mutation at residue 66 clustering at residue 67 segregates Rg among clusters.

References

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