

# Mechanism underlying conformational effects of a disease-associated hydrophobic-to-hydrophobic substitution on an intrinsically disordered region

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## Abstract

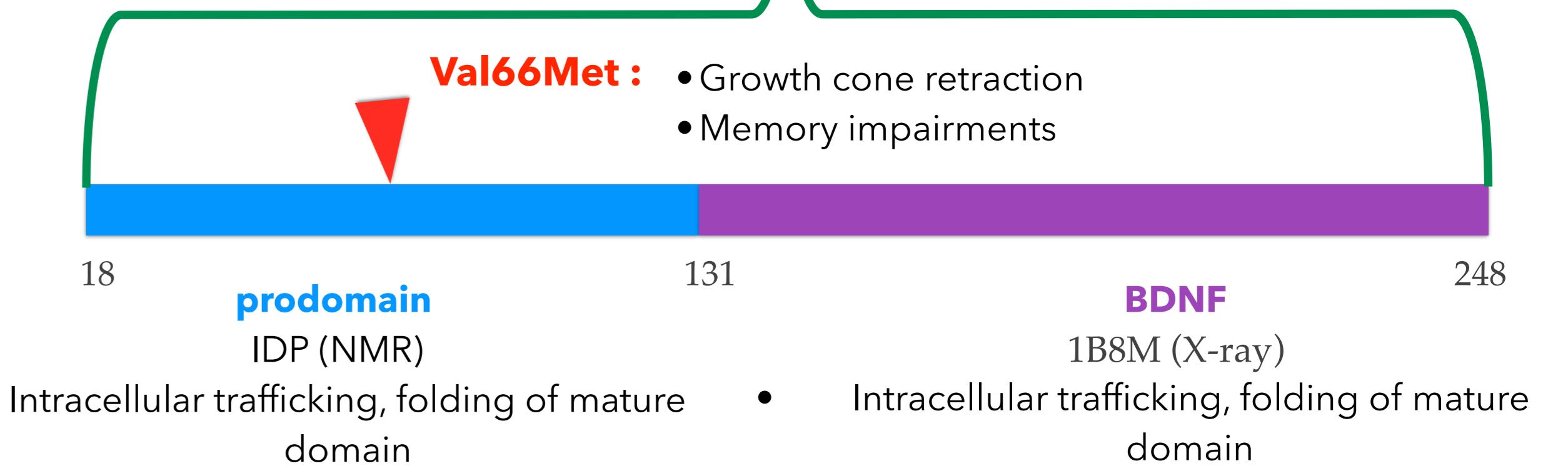
Disease-associated Single Nucleotide Polymorphisms (SNPs) are common in the disordered regions of proteins. Most mutational studies of IDP's consider loss or gain of a charged residue. In this study, we explore the local and global effect of a charge-neutral mutation between two hydrophobic residues, with known effects on function: the Val66Met SNP in the 100-residue disordered prodomain of Brain Derived Neurotropic Factor (BDNF). Val66Met is the most frequently found SNP in the BDNF disordered domain, and is associated with various neurological and psychiatric disorders such as bipolar disorder and Alzheimer's disease. Previously, NMR studies demonstrated that the prodomain is disordered with different secondary structure preferences for Val and Met at 275K (Anastasia et al 2013). We used large-scale, fully atomistic temperature replica exchange molecular dynamics simulations of both the Val and Met forms of the BDNF prodomain. MD simulations identify similar regions of residual secondary structure compared to NMR studies. Interestingly, we observe reversed temperature dependence of the secondary structure around the SNP for Val and Met. With increasing temperature, Val66 is less likely to assume helical secondary structure, while Met66 is more likely, consistent with established reduction of the valine side-chain entropy upon helix formation. At room temperature, we also observe an increase in the radius of gyration of the Met66 prodomain relative to the Val66 prodomain, which can be reliably attributed to differential hydrogen bonding preferences of SNP-adjacent residues, affecting their likelihood of hydrogen bonding with distant residues. These results indicate the neutral substitution may exert its effects by critically adjusting entropic cost of local secondary-structure elements, which, in turn, affects the conformational ensemble via differential long-range beta bridging patterns. Furthermore, although the SNP is neutral, it alters the exposure of charged residues around the SNP, which can potentially affect the binding characteristics of the prodomain.

## IDPs : structural middle zone

	Structured Proteins	Generic polymers	Intrinsically disordered proteins
Primary	High complexity	No/very low complexity	KAGSRG TSLADT FEHVI EELDEDQ KVRP
	Well-defined; short coil linkers	100% coil	Some transient secondary structure
	Well-defined; few accessible conformational states	Purely Statistical : (radius of gyration, end to end distance)	?
Effect of single mutation on tertiary structure	Often significant	Probably minimal	?
Example	Enzymes	Polyethene	> 21.7 % of missense disease mutations.
	Intracellular signaling		
			> 33 % of eukaryotic proteins have disordered regions

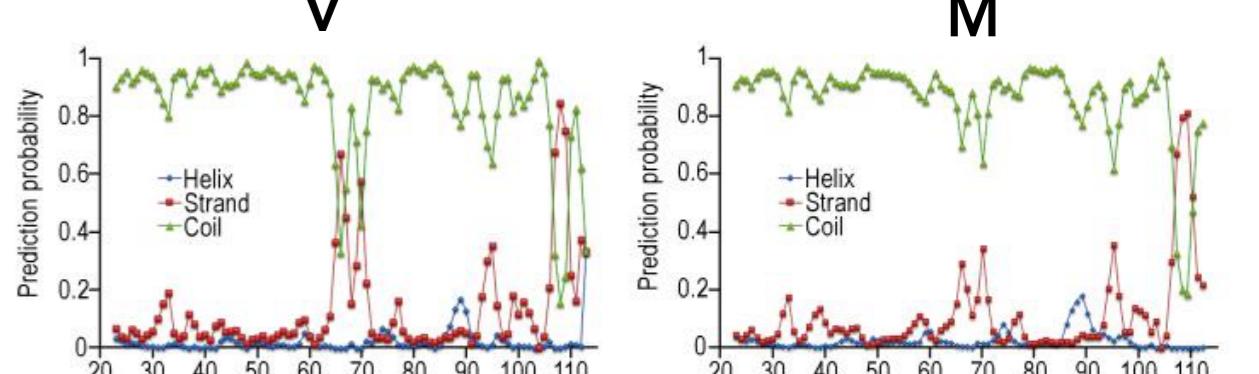
## BDNF and Val66Met SNP

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



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

- NMR (273K) and CD spectra (300K): Val66 and Met66 prodomain both intrinsically disordered.
- NMR prediction (273K): no helicity around residue 66 for Val or Met 66



## Methods

- Purpose: prodomain simulation for both V and M forms.
- Package: GROMACS 5.0.7.[C]
- Sampling method: replica exchange with explicit solvent [D]
- Total simulation time: 39μs
- Initial conditions: Different random coil for each replica
- Force field: Amber99sb-ildn [E]
- No. of replicas: 60 replicas
- Temperature range: 300K to 420 K
- Acceptance ratio: 14-20%.
- Exchange frequency: 1ps

## Summary

- Val66Met reverses effect of temperature on secondary structure around residue 66, consistent with different entropic cost of helix formation.
- The tertiary contacts changes are mediated by the residue 66 dihedral angle preference and are mostly dominant at lower temperatures.
- Colder M66 has reduced exposure of charged residues and increased exposure of hydrophobic residues, which might affect binding to SorCS2.

## References

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