Effects of Mutations on Disordered Proteins

Presented by:
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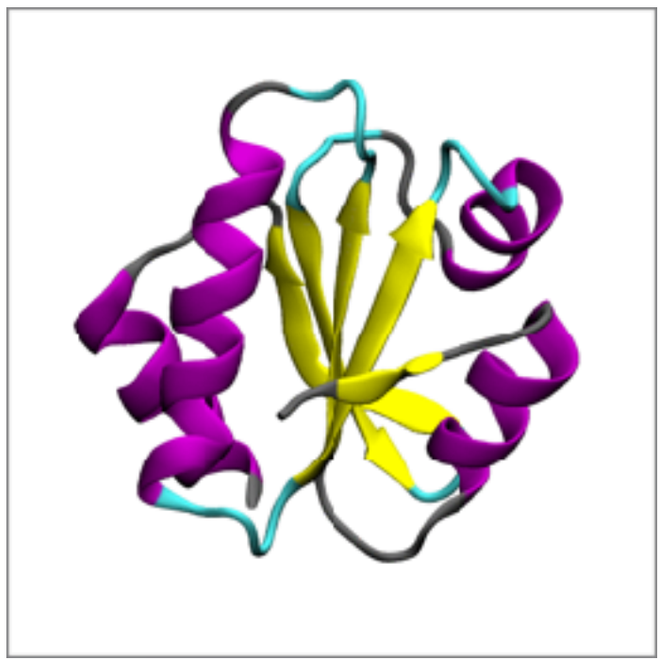
Intrinsically Disordered Proteins (IDP's)

2n5a (nmr)
yeast Thioredoxin



Structured

X-ray crystallography, NMR (secondary & tertiary)

Enzymes

2ljl(nmr)
HSP12 in DPC

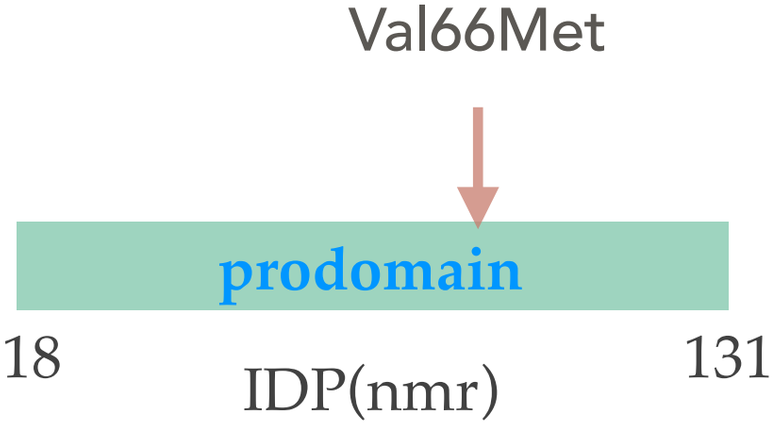
Intrinsically Disordered (IDP)

NMR (secondary)

Signaling proteins

- > 33 % of eukaryotic proteins have long (>30 residues) disordered regions
- Involved in critical biological functions including transcriptional activation and intracellular signaling

Protein of interest: Brain-derived neurotrophic factor (BDNF) and Val66Met SNP



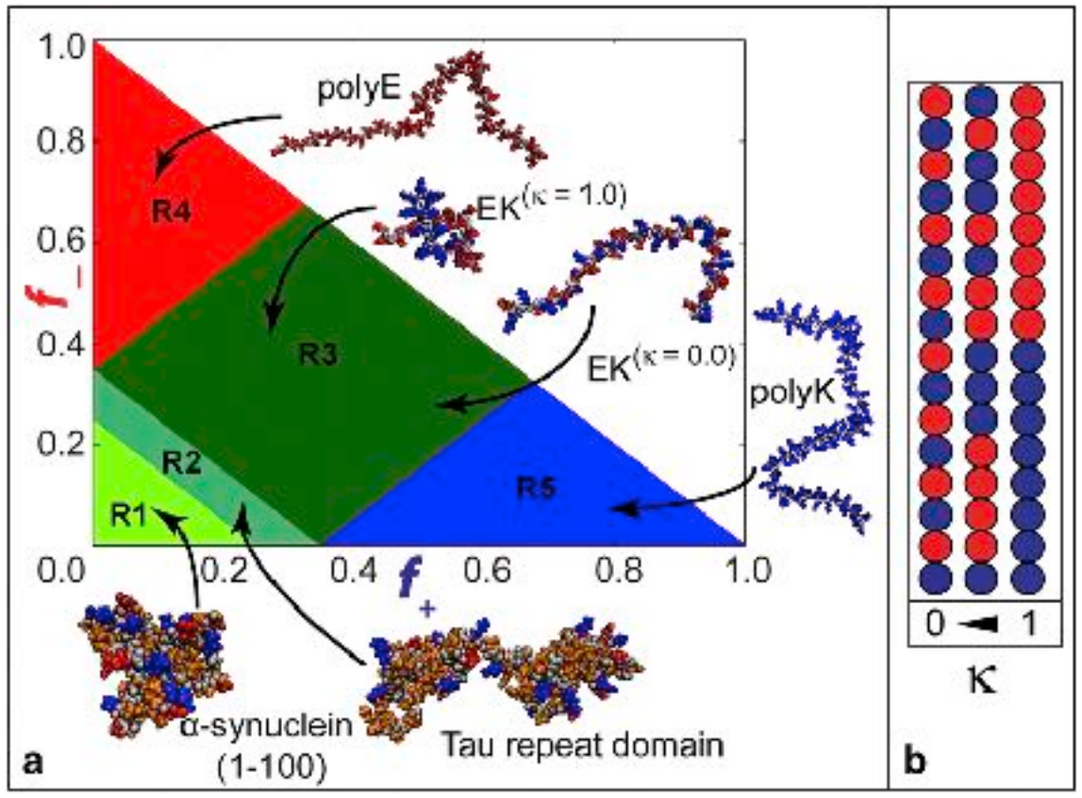
- Neurotrophin family of signaling proteins
- Val66Met SNP is most widely studied (>10,000 papers until 2017) and is associated with memory impairments among others

- NMR -> pro-domain is disordered with differential secondary structure preferences for V66 and M66
- Only Met66 proBDNF causes neuronal growth cone retraction by binding to SorCS2 (sortilin-related VPS10p-domain containing receptor 2)

(Anastasia et al.,Nat Commun., 2013)

Significance of mutations on IDP's conformational ensemble

- > 20 % of missense disease mutations are found in disordered regions
- >10% of these mutations are hydrophobic to hydrophobic mutations



(Das et al., PNAS, 2013)

f_+ fraction of positively charged residues
f_- fraction of negatively charged residues
κ sequence distribution of oppositely charged residues

negatively charged

positively charged

- Weak polyampholytes & polyelectrolytes: Globules & tadpoles
- Janus sequences: Collapsed or expanded - context dependent
- Strong polyampholytes: Coils, hairpins, & chimeras
- Negatively charged strong polyelectrolytes: Swollen coils
- Positively charged strong polyelectrolytes: Swollen coils

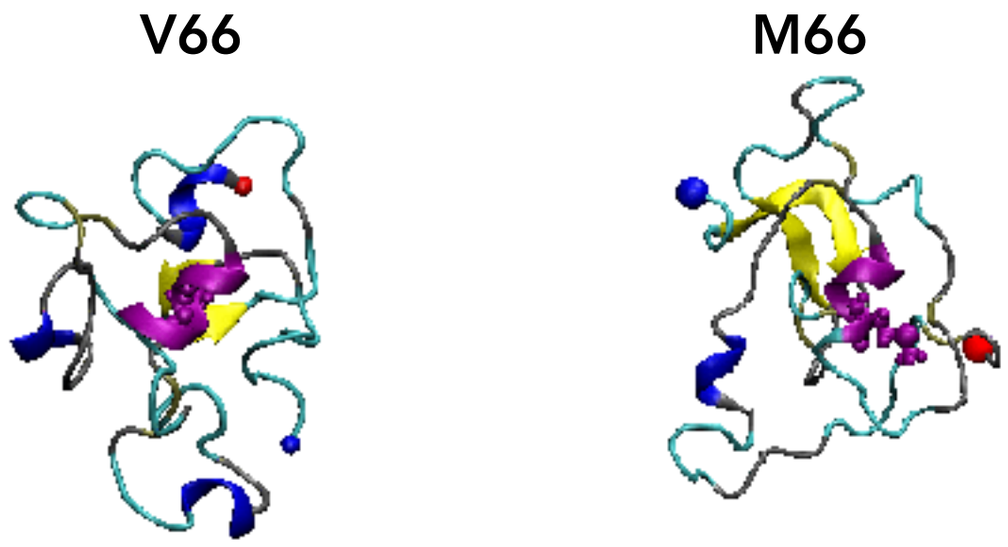
- Effect of hydrophobic mutations on IDPs conformational ensemble is not well understood

Method - MD simulations

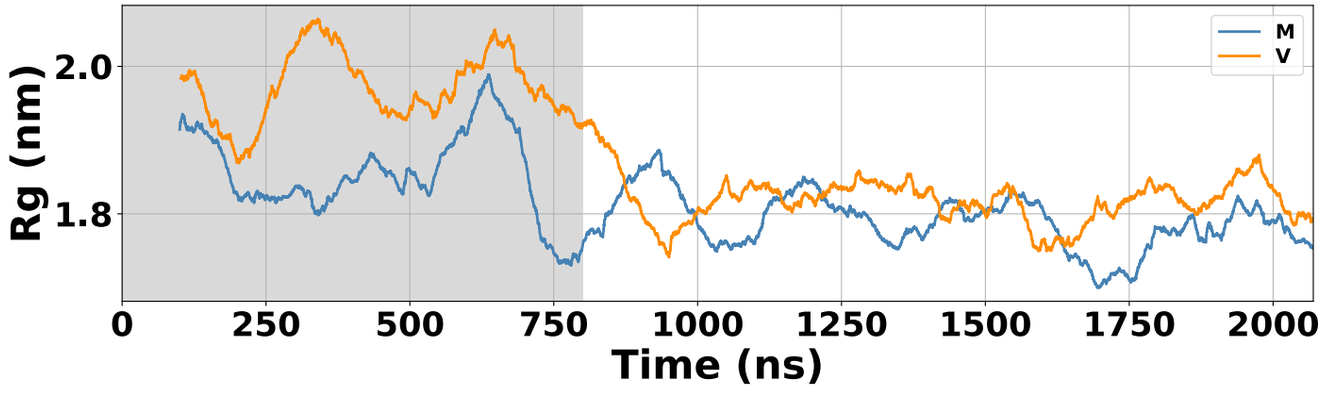
- The experimental method X-ray crystallography often fails for IDP's
- NMR gives us the average conformational ensemble properties
- Molecular dynamics (MD) simulations are an indispensable tool for studying IDP's. It gives us insight at microscopic levels
- Many recent studies involve IDPs of 40-70 residues using Temperature- replica exchange method (T-REMD) on micro-second timescale.
- We did the entire prodomain (**91 residues**) MD simulations for both Val and Met forms using T-REMD.
- No MD simulations of BDNF have been reported

Running on Caliburn and simulation convergence

- 256μs (2μsx64replicas) explicit solvent replica exchange simulation of the prodomain.

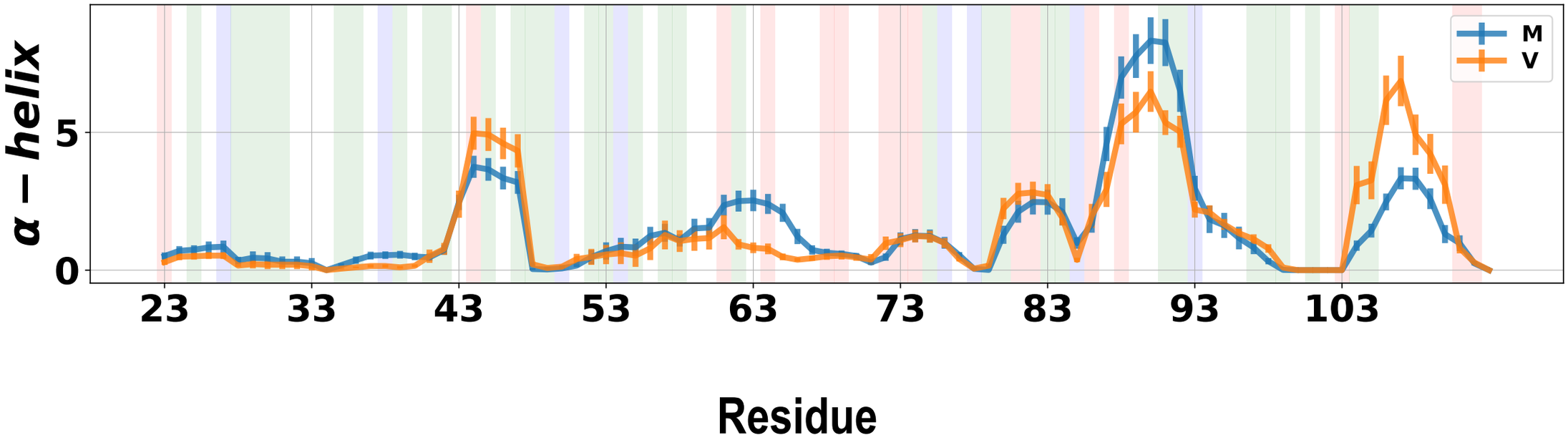


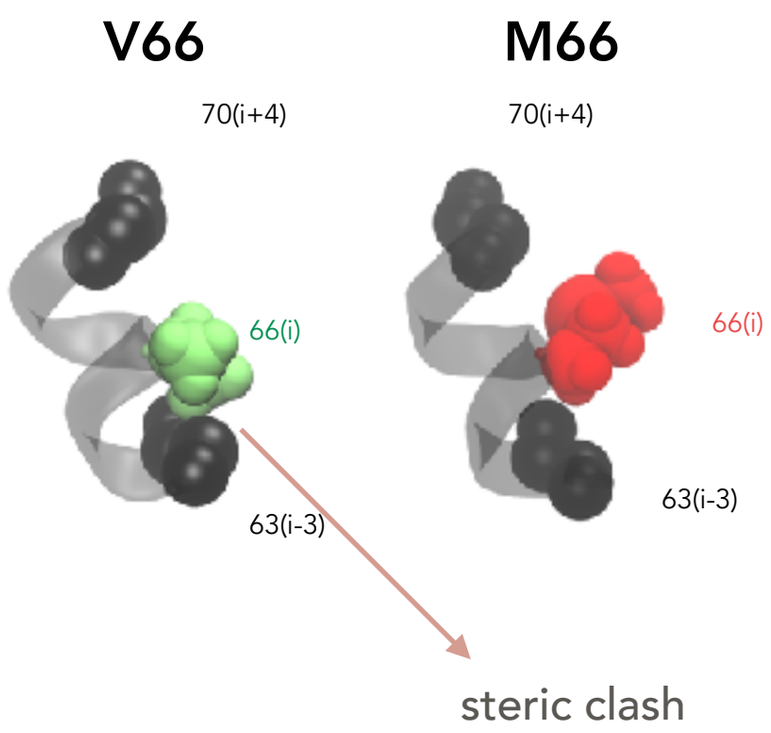
- Each replica was run on a single node parallelly on Caliburn



- The simulated hydrodynamic radii of V66 (2.27 nm) and M66 (2.21 nm) are in excellent agreement with the experimental values (2.24 nm and 2.20 nm respectively).

V66 vs M66 helicity at residue 66 : M66 forms more helix at residue 66

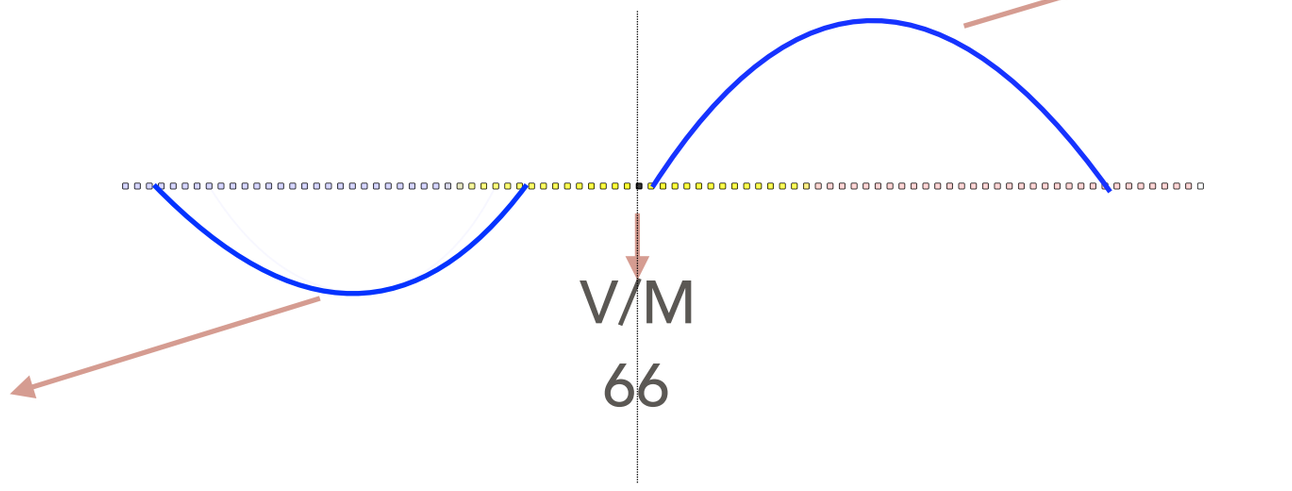




- M66 forms longer helix at residue 66

Pseudo-tertiary structure: long-range contacts at 300K

contacts present (top): |i-j| > 20

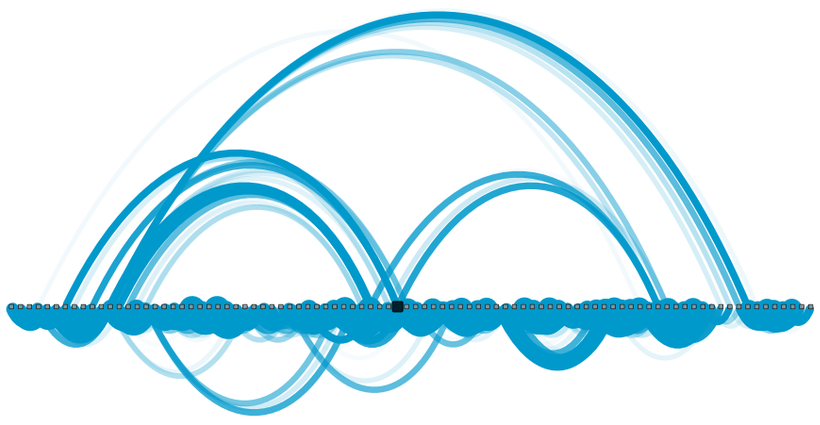


contacts present (bottom): |i-j| < 20

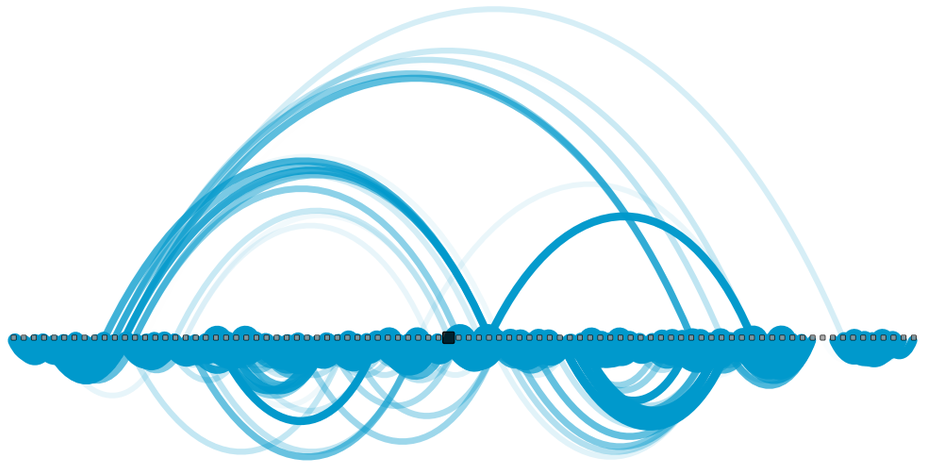
- The line thickness increases with increase in the contact strength

- V66 and M66 forms similar long range contacts network.

V66

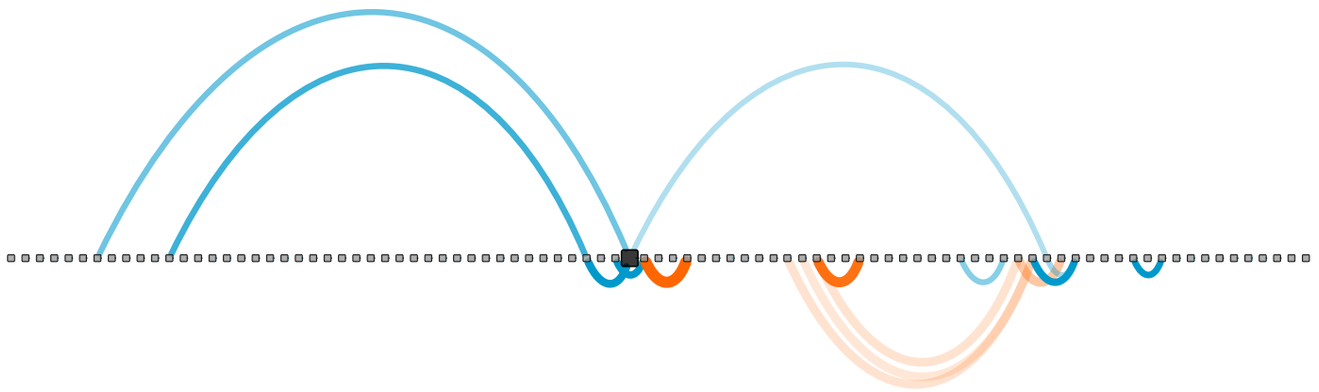


M66



Cutoff: 10%

V66 vs M66 ‘tertiary structure’ : M66 forms stronger contacts at residue 66



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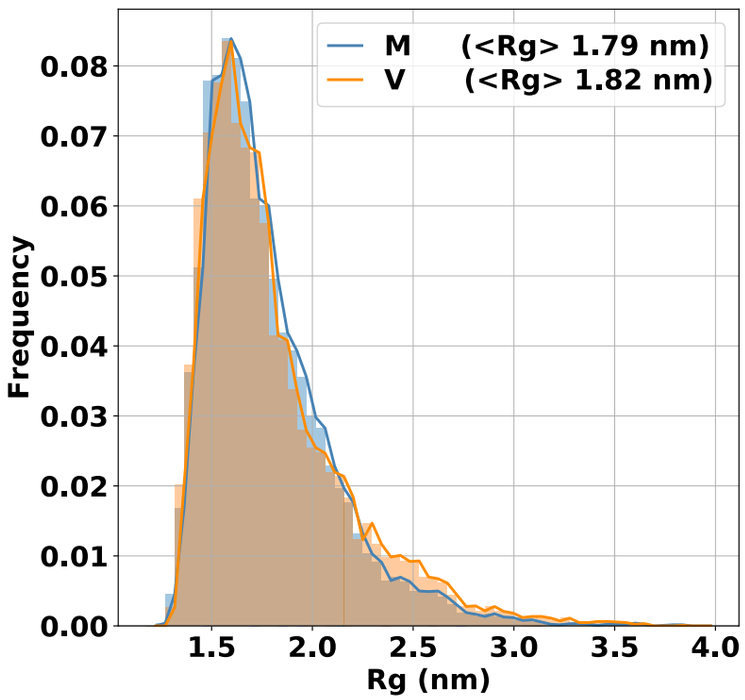

- Residue 66 forms weaker hydrophobic contacts in V66 leading to it’s slightly expanded Rg.

Main Questions Underlying Research so far and their found answers

- Why does the V66M mutation affect residual local secondary structure? Differential entropic cost of helix formation
- Is there a meaningful way to characterize tertiary structure of IDPs? Yes
- Does this mutation also affect the protein packing (Rg), even though it is charge-neutral? Yes
- Is this effect mediated by direct interactions of V vs M with rest of sequence? Yes

Acknowledgements

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Thank you for listening!