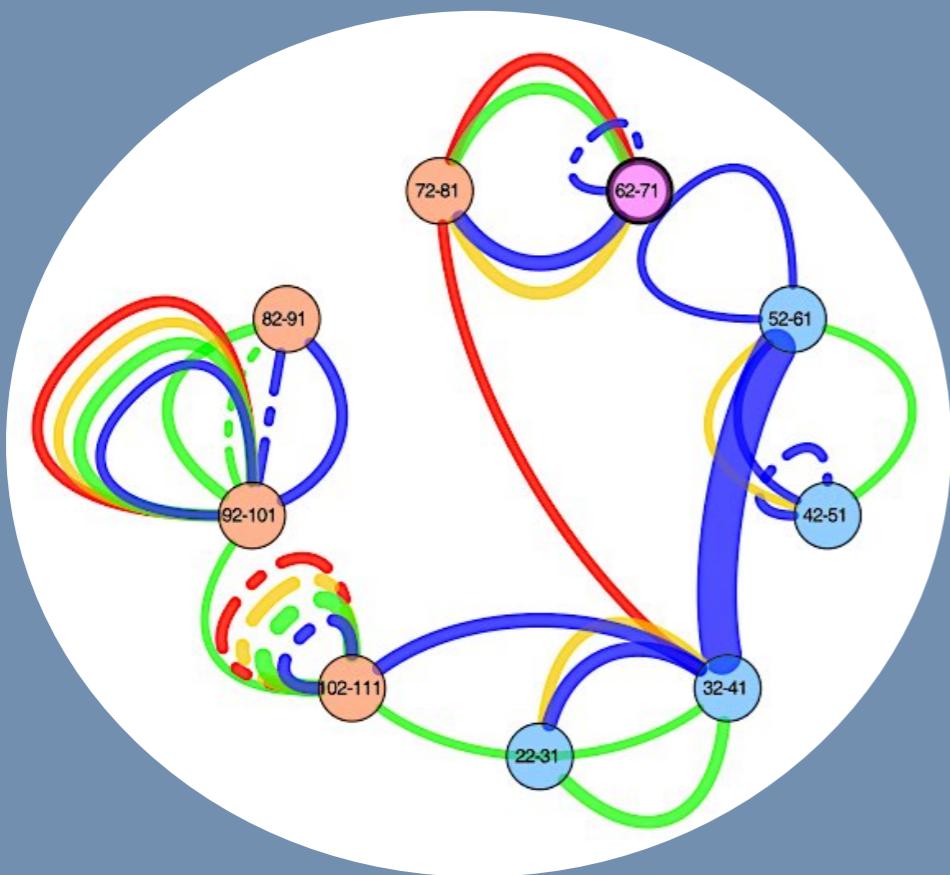


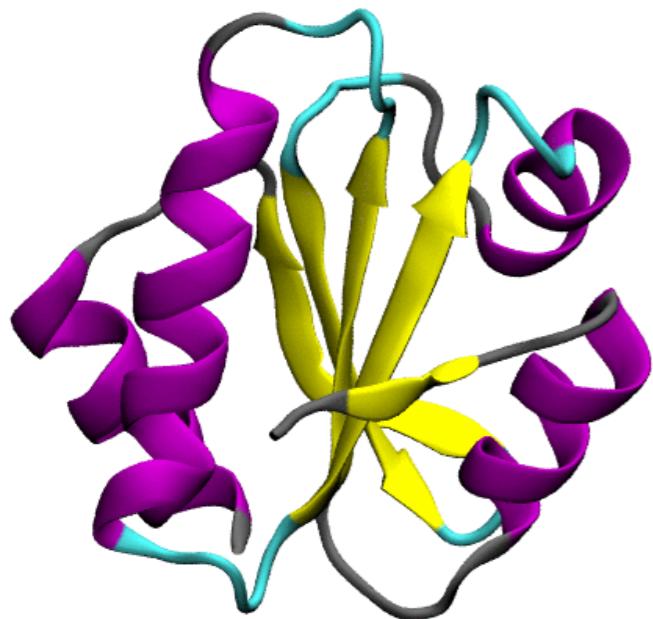
# Mechanism underlying conformational effects of a disease-associated hydrophobic-to-hydrophobic substitution on an intrinsically disordered region

Ruchi Lohia  
Brannigan group  
Center for Computational &  
Integrative Biology  
Rutgers University



# Proteins : disordered/structural continuum

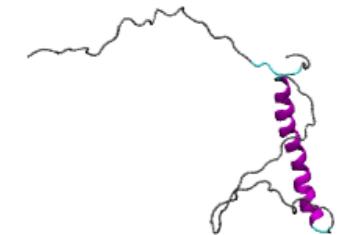
2n5a (nmr)  
yeast Thioredoxin



2l9q(nmr)  
HSP12 in SDS micelle



2ljl(nmr)  
HSP12 in DPC



## Structured

X-ray crystallography,  
NMR (secondary &  
tertiary)

Enzymes

## Intrinsically Disordered (IDP)

NMR (secondary)

NMR (secondary)

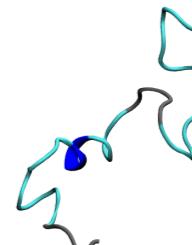
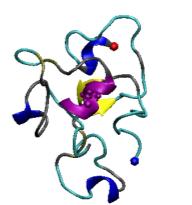
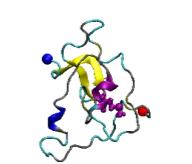
Signaling proteins

# IDPs : Abundance, Significance and Advantages

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- 33 % of eukaryotic proteins have long (>30 residues) disordered regions
- > 21.7 % of **missense disease mutations.**
- **roles in :**
  - transcriptional activation
  - intracellular signaling
  - frequent hubs in protein interaction networks
- implicated in: **cancer, cardiovascular disease, amyloidosis, neurodegenerative disease, diabetes, among others**
- **disordered advantages :**
  - Can bind multiple partners
  - Possibility of high specificity/low affinity binding
  - Reversible nature of their intermolecular interactions makes them extremely attractive targets for small molecule drugs

# IDPs : structural middle-zone

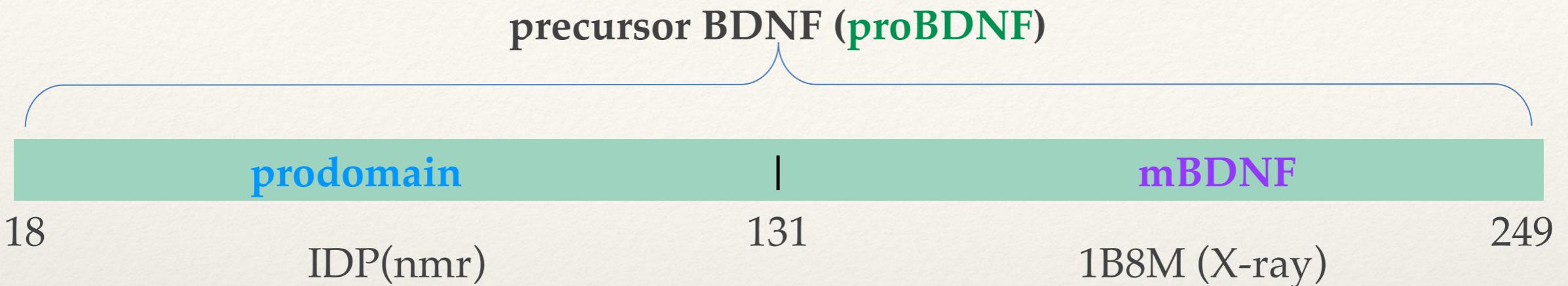
	Structured Proteins	Generic polymers	Intrinsically disordered proteins
Primary	High complexity	No/very low complexity	<b>KAGSRGLTSLA</b> <b>DTFEHVIEELL</b> <b>DEDQKVRPNEE</b> <b>NNKDADLYTSR</b>
Secondary (local)	Well-defined; short coil linkers	100% coil	Some transient secondary structure 
Tertiary (long-range/ global)	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	 

# Known effects of mutations on IDP's

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- What we known by now
  - **Experimental**
    - reduced/increased binding with receptor
    - increased/decreased aggregation
    - disorder to order transition or vise versa.
  - **MD**
    - local secondary structure changes
    - increased/decreased aggregation
    - change in dynamics due to loss/gain of electrostatic charges,
- Current Study
  - exploring the effects of a disease-associated hydrophobic-to-hydrophobic mutation (V to M) on the conformational ensemble and dynamics of pro domain.
  - Other than local secondary structure changes, the effects of point mutation on the overall conformation of an IDP.

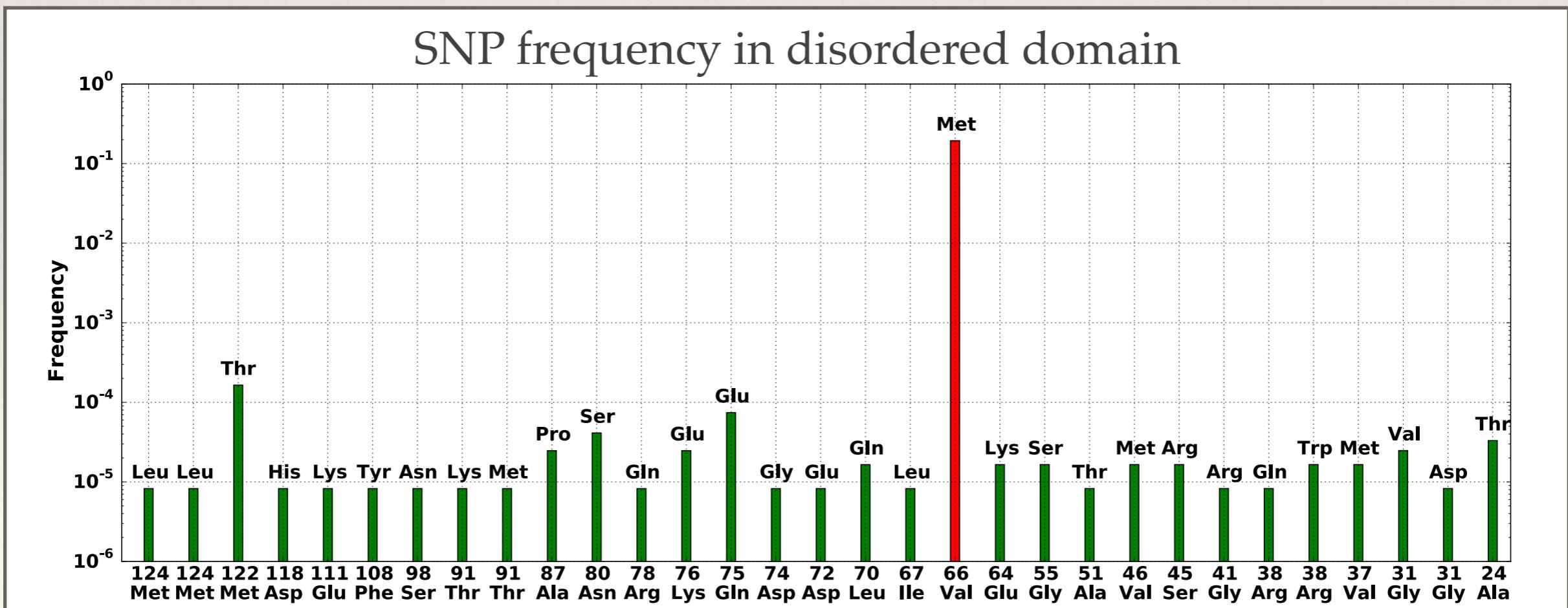
# brain-derived neurotrophic factor (BDNF)



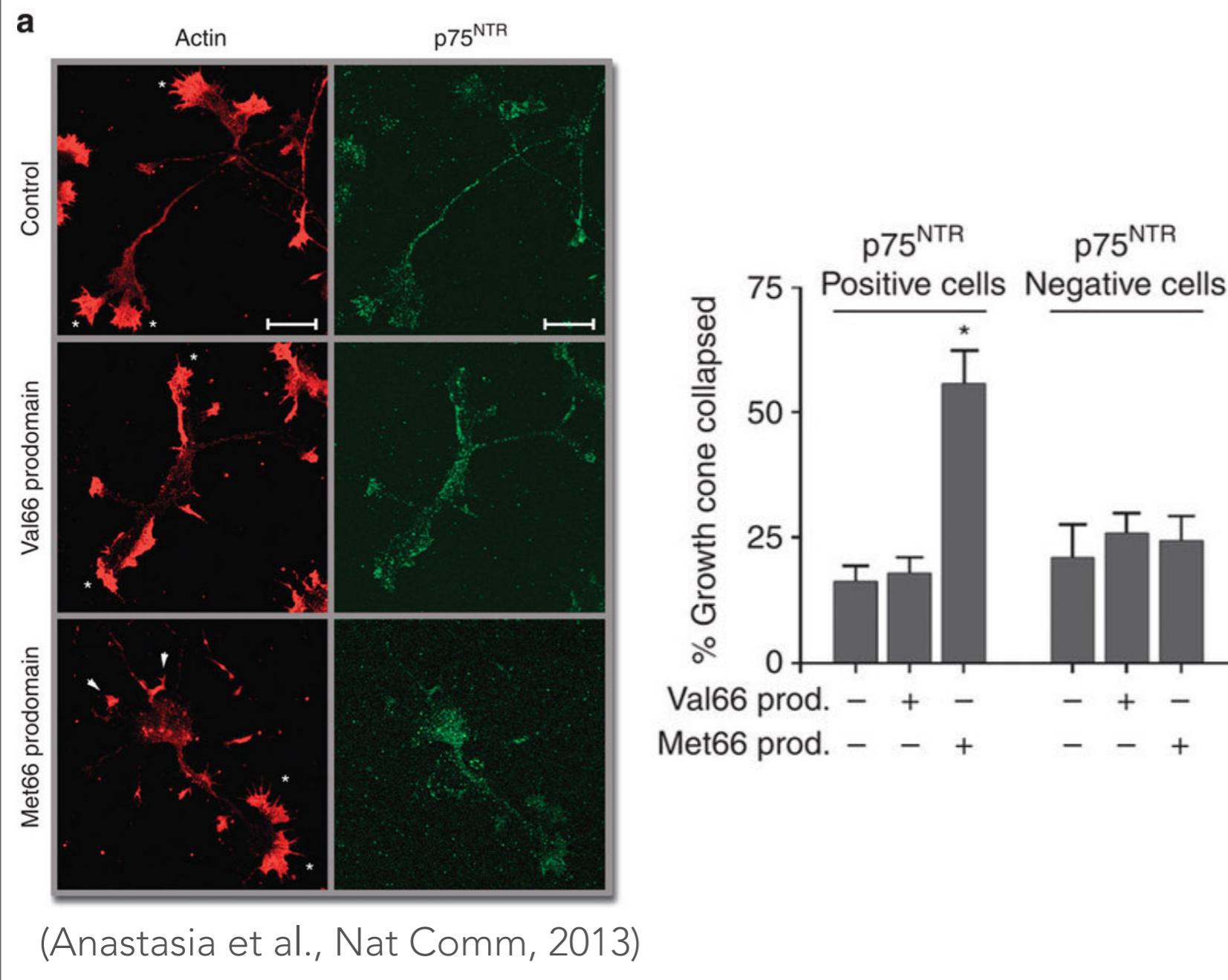
- Neurotrophin family of signaling proteins
- **proBDNF** - : apoptosis, refinement of correct target innervation during development.
- **mBDNF** -: growth ,differentiation , maturation and survival of neurons.
- **prodomain** : Intracellular trafficking, folding of mature domain.
- **prodomain** : SorCS2 : Growth cone retraction

# Val66Met significance

- The BDNF prodomain is highly conserved with a valine at or near position 66 in more than 70 species examined.
- Val66Met SNP is associated with
  - memory impairments; deficits in short term episodic memory
  - Lowers levels of BDNF has been associated with Alzheimer's disease, bipolar disorder, Parkinson's disease and Alcohol dependence among various other psychiatric disorders



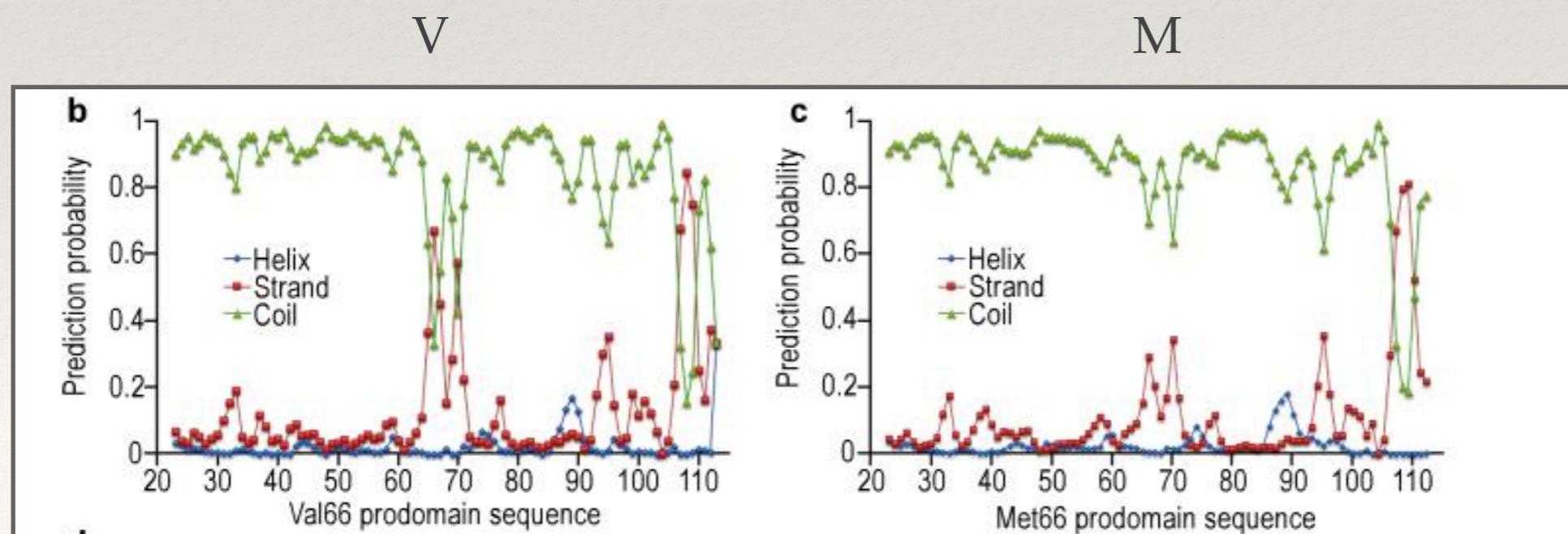
# Possible mechanism: Val66Met affects binding of pro-domain to SorCS2



- Met66 binds to SorCS2, but Val66 does not.
- 50% more growth cone retraction in the presence of Met66 ,  $p75^{\text{NTR}}$  and SorCS2
- binding does not affect SorCS2 structure.

# Val66Met effect on secondary structure : NMR

- 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
- likelihood of beta strands : V66 > M66
- chemical shifts show overall weak signal



(Anastasia et al., Nat Comm, 2013)

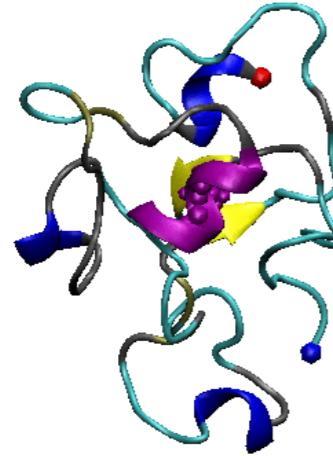
# MD Simulation

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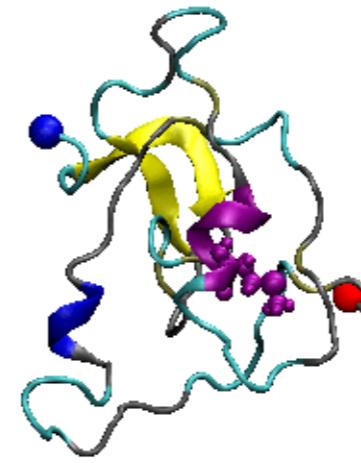
- 39 $\mu$ s explicit solvent replica exchange simulation of the **prodomain** region 23-113 (91 residues) for both Val and Met forms.
- GROMACS 5.0.7 simulation package with Amber99sb-ildn force field.
- 60 replicas in the temperature range of **300K to 420 K** were each run for 650 ns, exchanged every 1ps. Acceptance ratio was 14-20%.
- Initial conditions : Different random coil for each replica ( However, same starting structure for both V and M form )

# MD: conformations of 300K Replica

VAL



MET



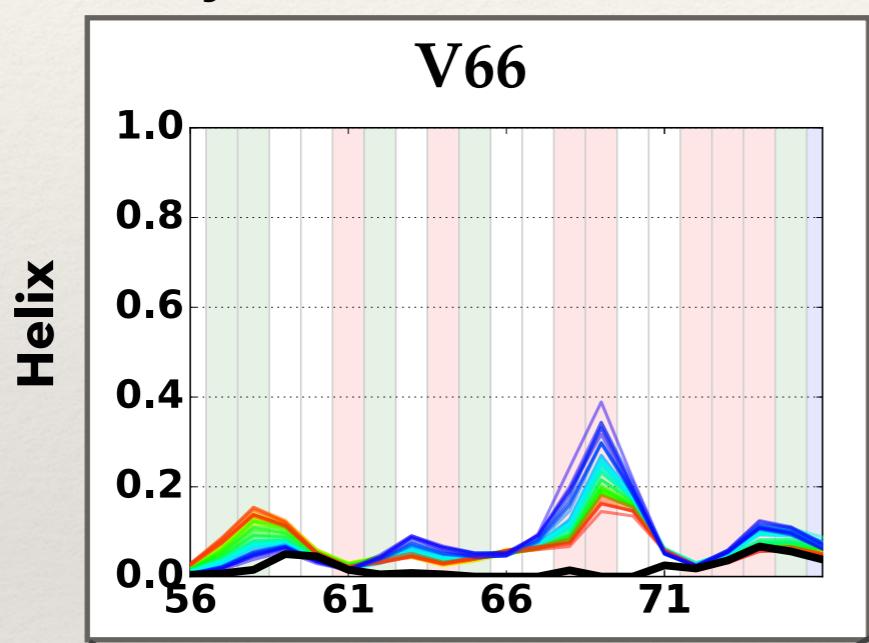
Both : Very disordered

Short regions of transient secondary structure

# Effects of temperature on secondary structure

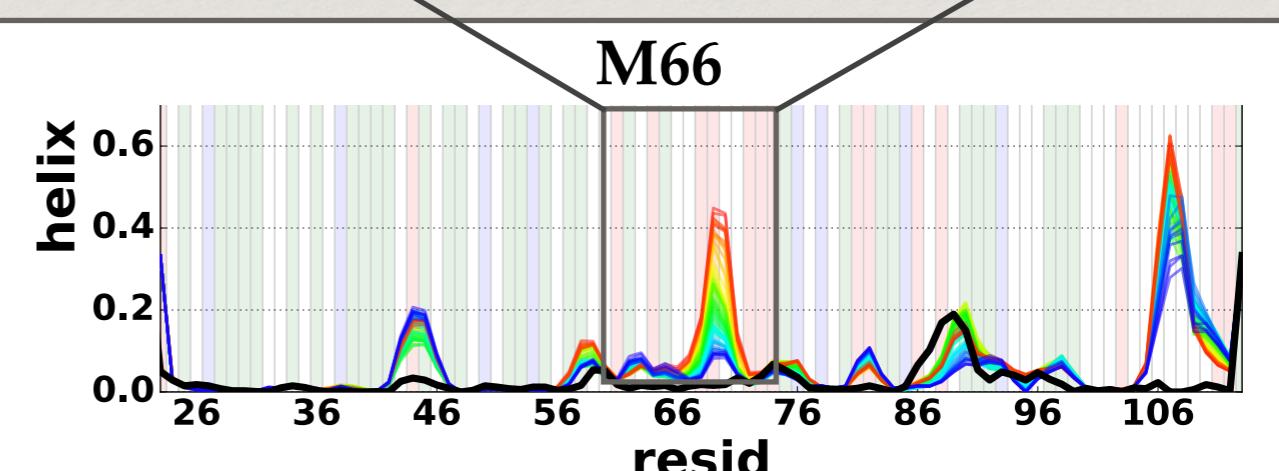
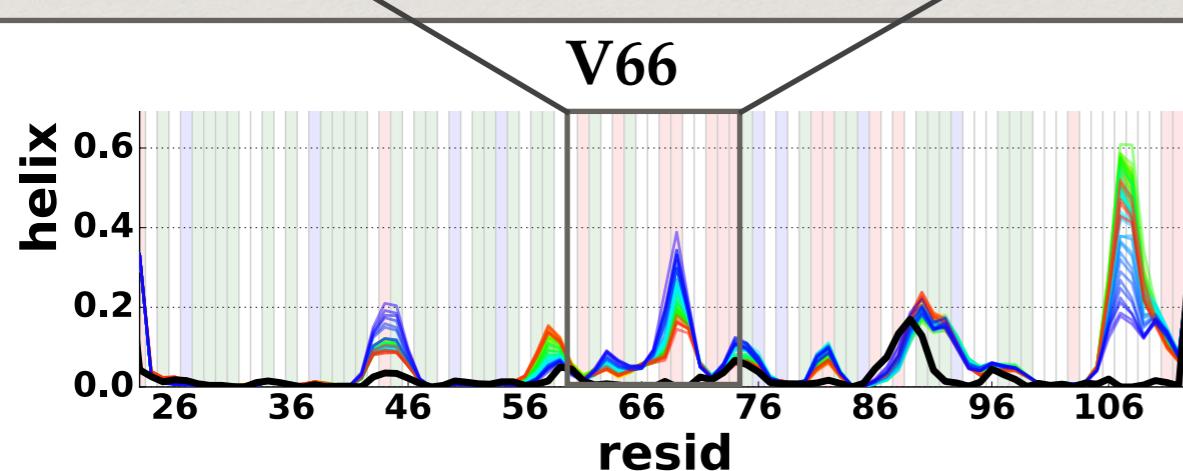
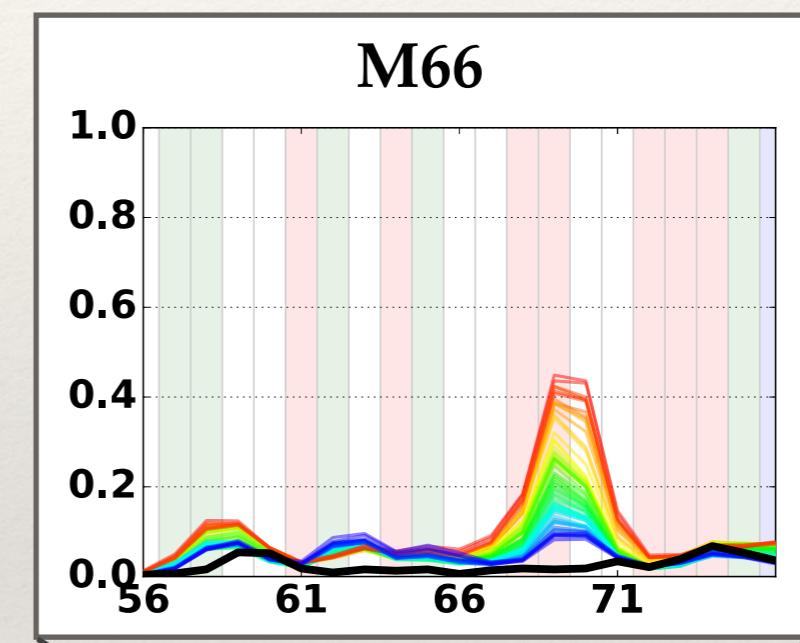
MD predicts more helicity near residue 66 - can this be explained by colder NMR temperature?

maybe(reverse trend)



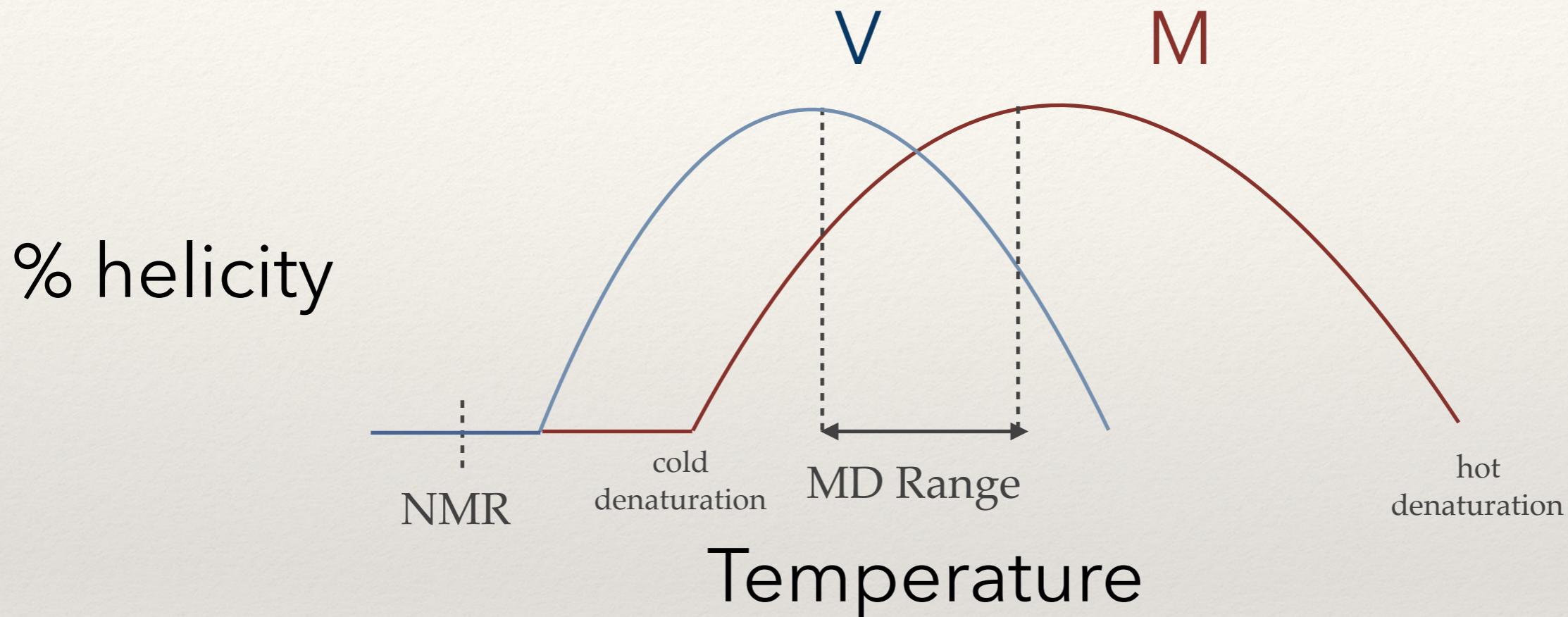
MD: 420K  
MD: 300K  
NMR: 270K

yes (straightforward trend)



# Non-monotonic temperature dependence: V vs M

consistent explanation for discrepancy:



Structured proteins: NMR vs MD/body T not that different compared to stable protein T range.

But - disordered proteins very sensitive to temperature!

# non-local interactions & tertiary “structure”

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not obtainable by NMR

normal MD methods for structured proteins not practical

characterize here 3 ways :

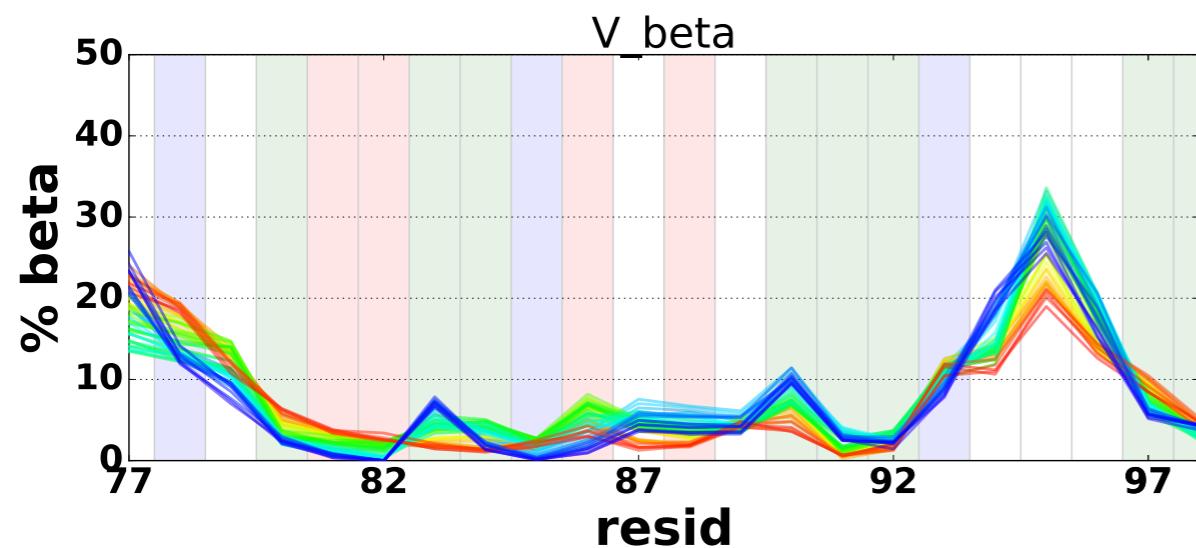
-- secondary structure far from 66

-- radius of gyration

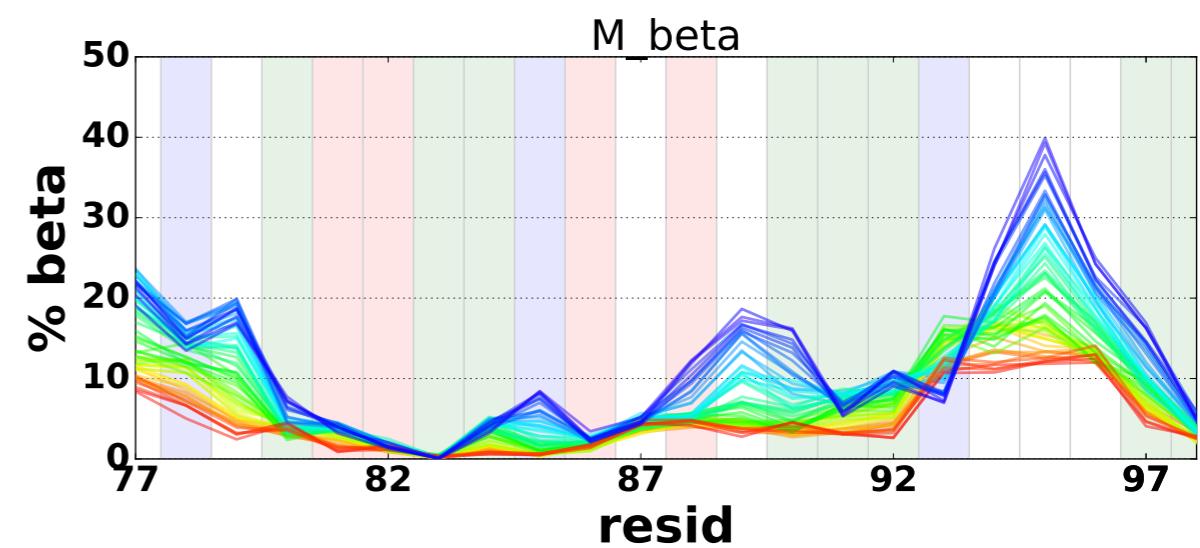
-- coarse-grained segment contact maps

# non - local secondary structure

V66



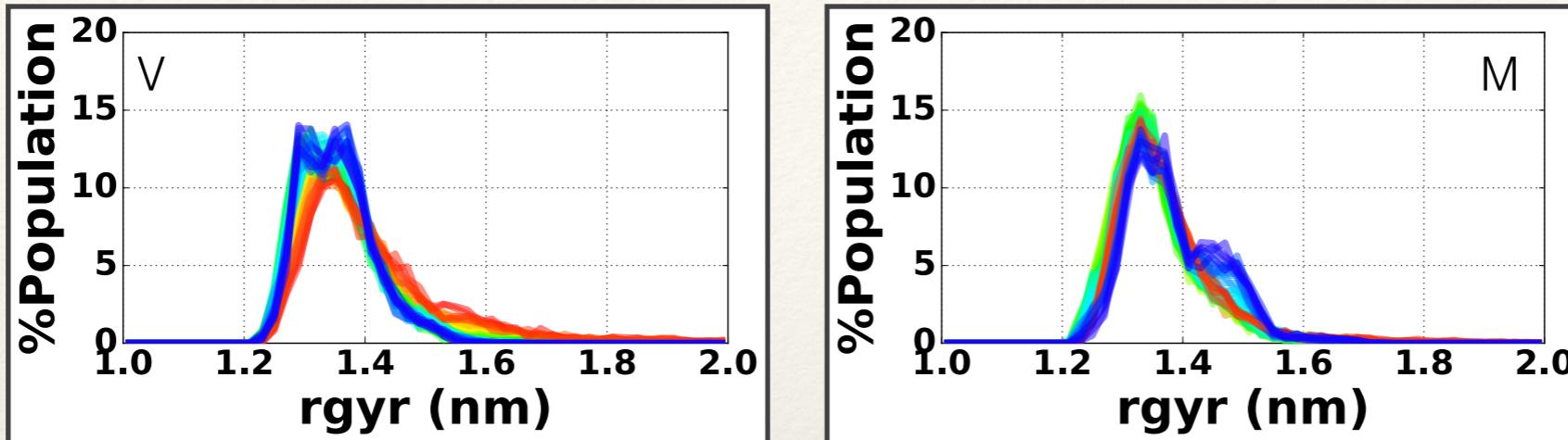
M66



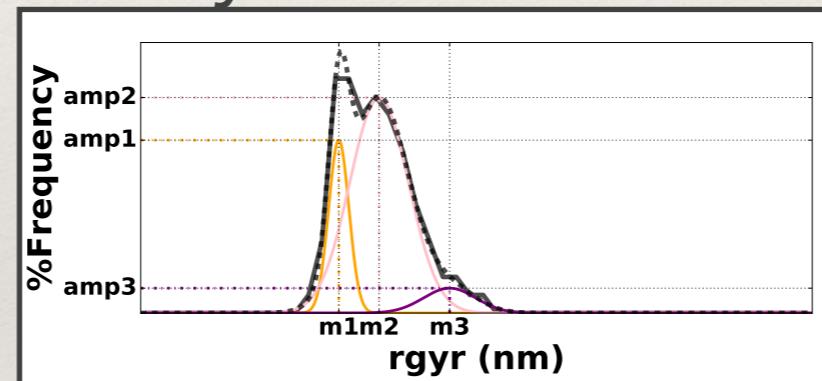
Any meaningful difference ? if so, why ?

# radius of gyration

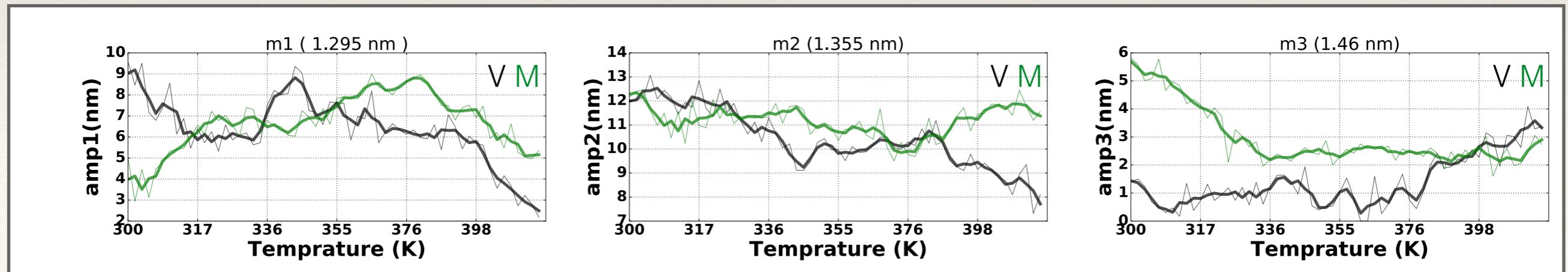
- describes how extended/compact the protein is



-distributions well fit by sum of 3 Gaussians with 3 means:

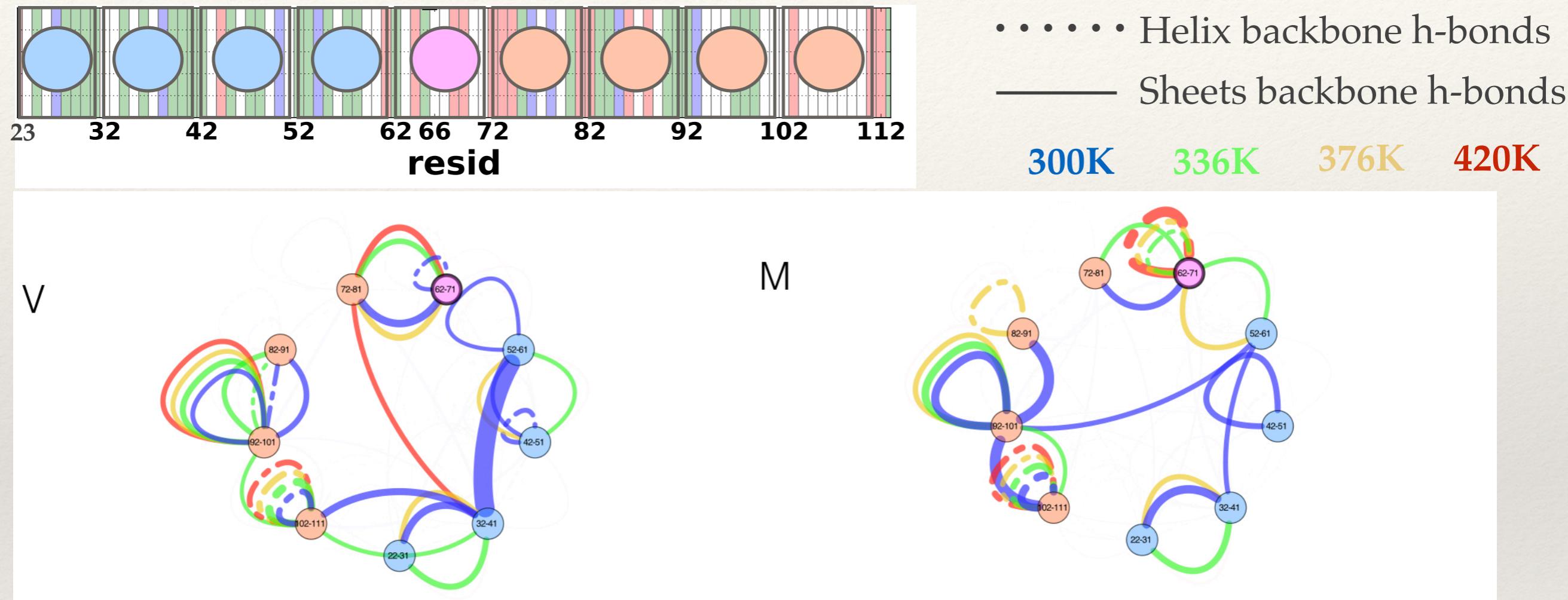


-possibly systematic differences at low temperature for mode 1 & 3



# Coarse-grained segment contact maps

Divided the protein into 10 segments of 10 residues each



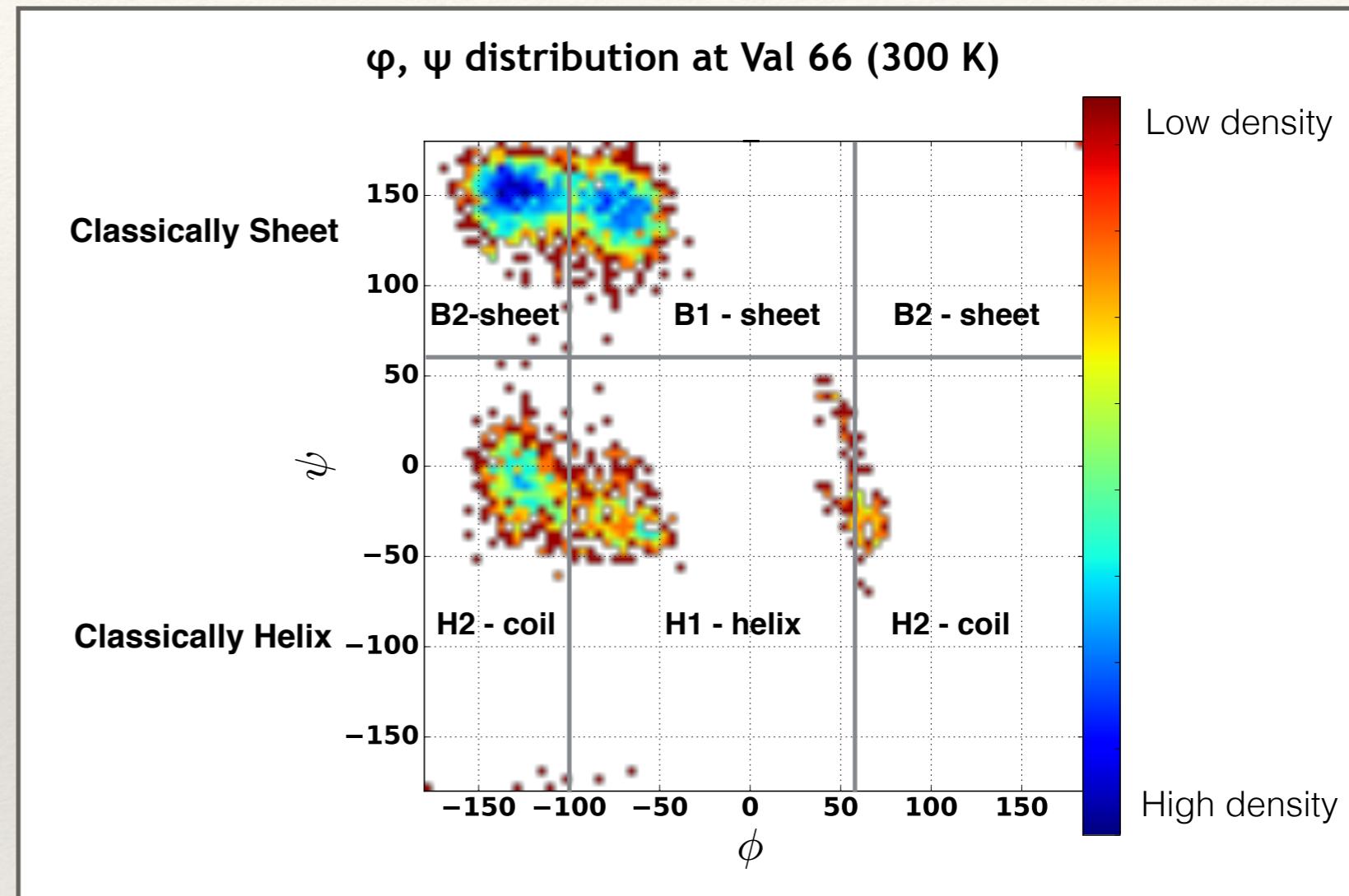
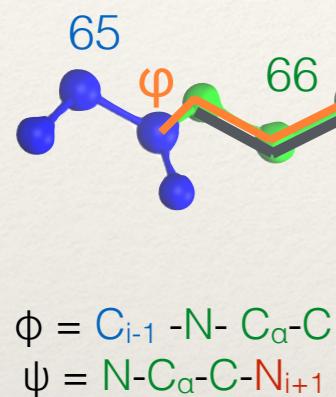
Long-range pairing:

at cold temperatures for M

at high temperatures for V - entropically surprising?

# More insight : clustering

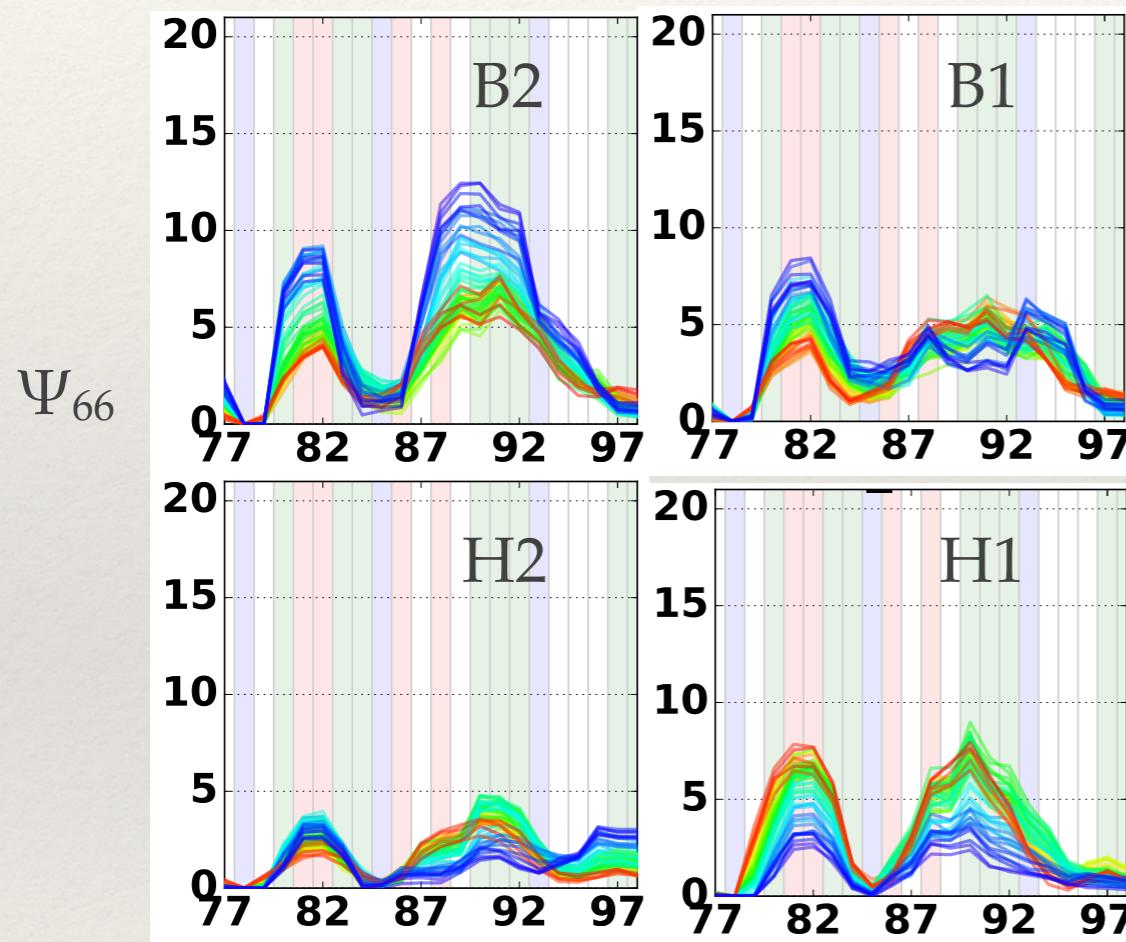
## Dihedral angle clustering



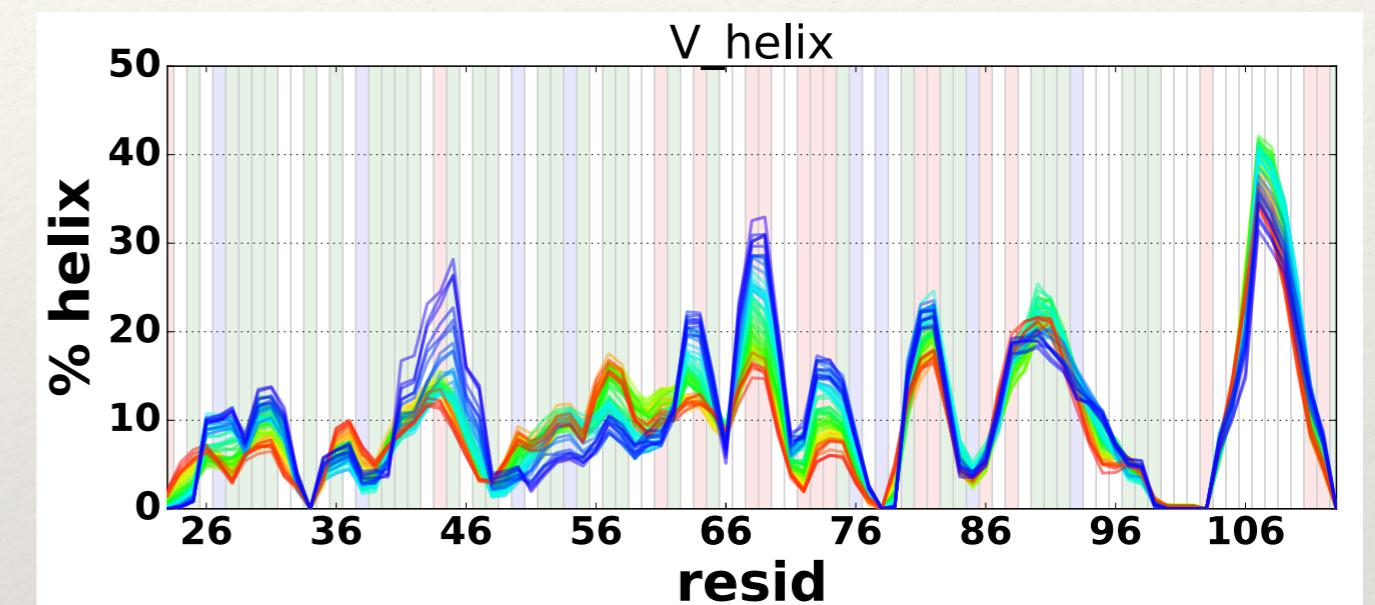
- $\varphi, \psi$  distribution depends on the side-chain (R group) of an amino acid.
- IDPs - clusters (related structures) - based on dihedral angles ( $\varphi, \psi$ ) for every residue.
- Our study - 4 clusters (H1,H2,B1,B2) - based on residue 66 dihedral angle.

# V Secondary structure : by cluster

SS at residue 66 predicts  
SS trends elsewhere



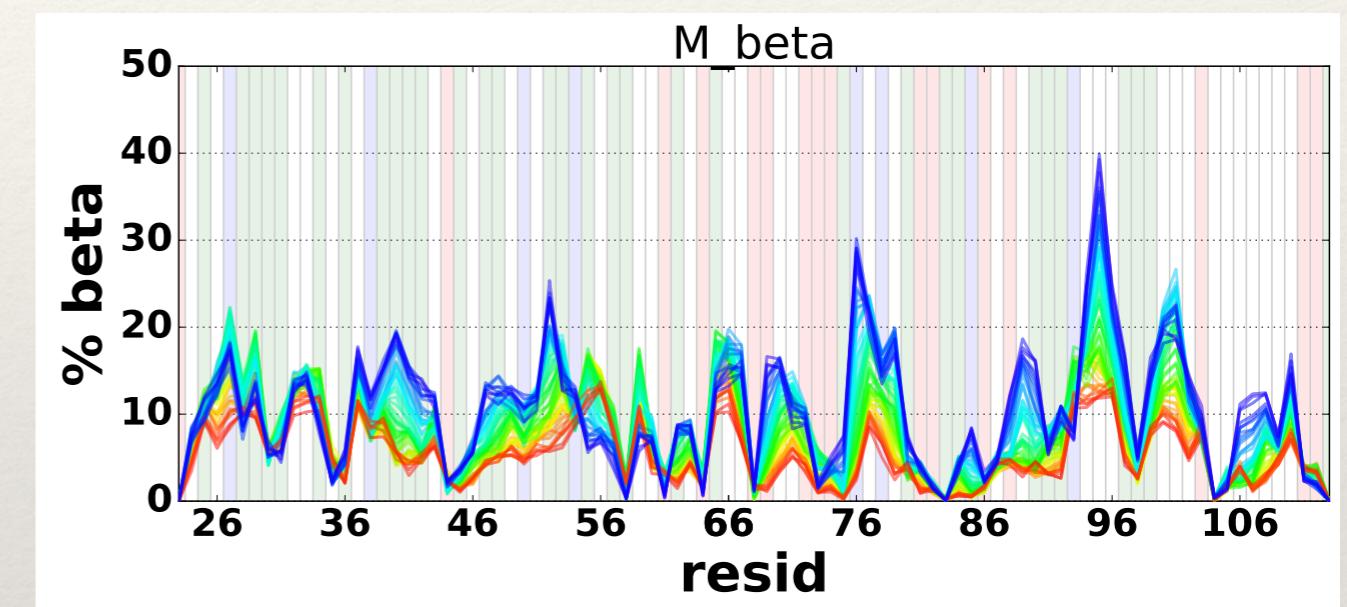
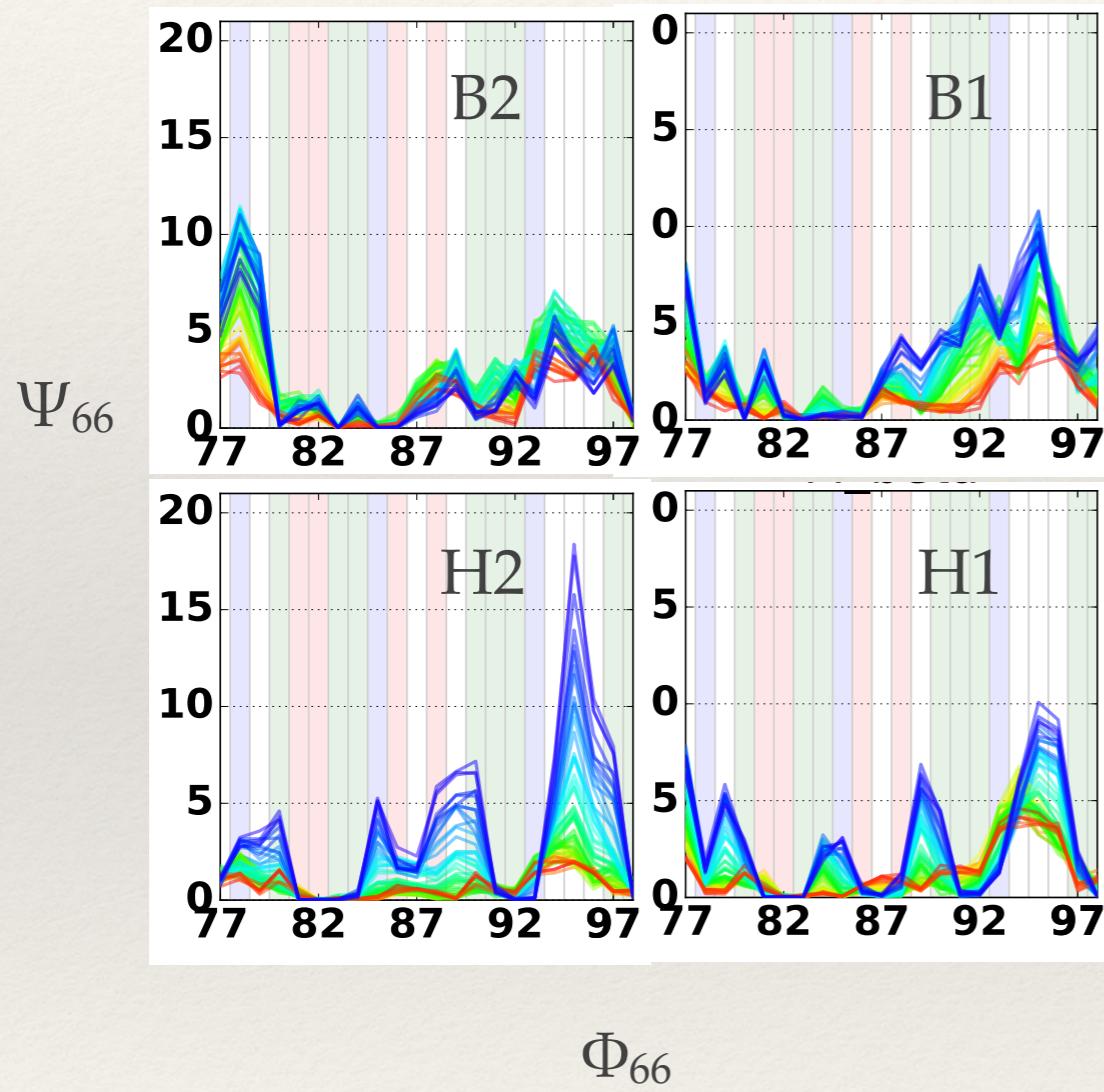
$\Phi_{66}$



- Clustering at 66 also separates non-local secondary structure.
- Residue 76 and residue 91 (no net trend) shows different trends in each cluster with temperature.

# M Secondary structure : by cluster

SS at residue 66 predicts  
SS trends elsewhere



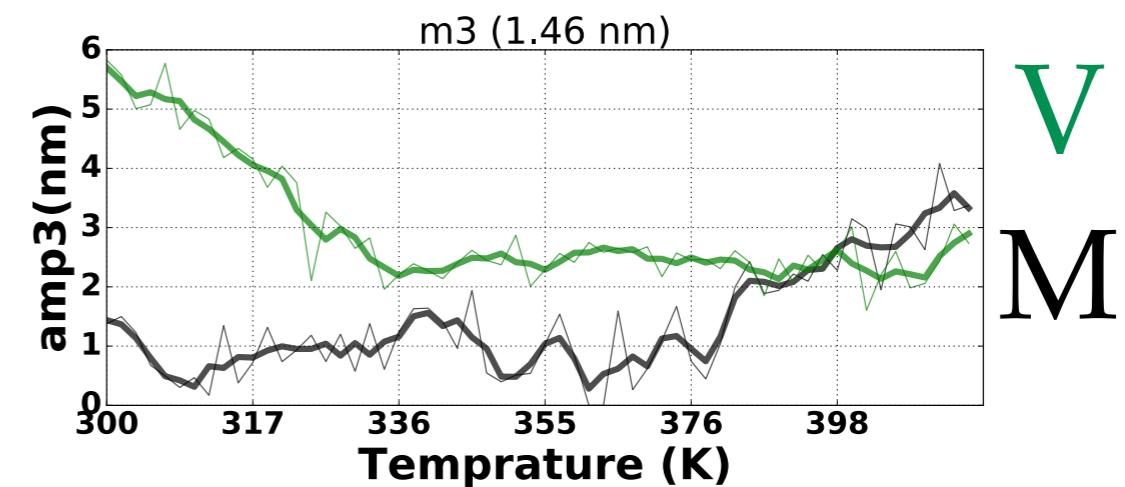
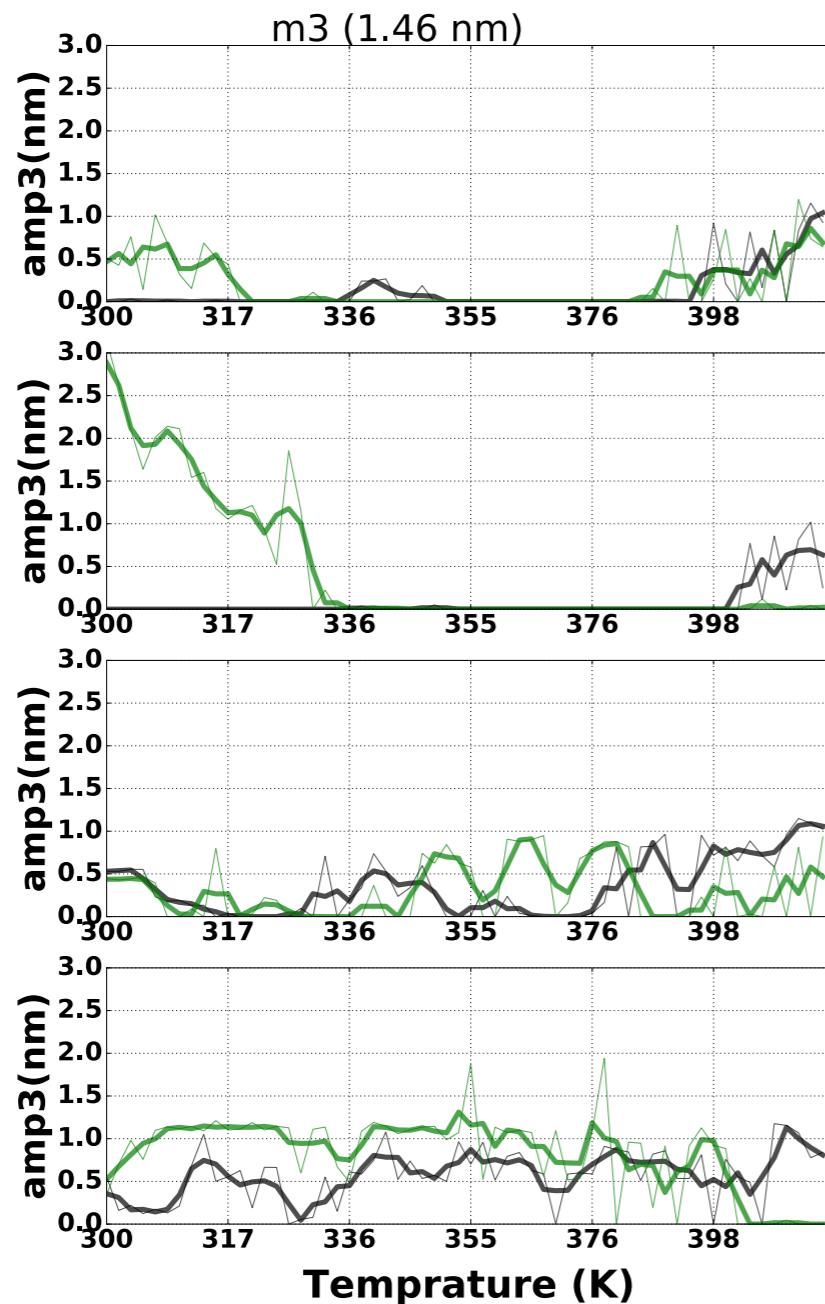
- Clustering at 66 also separates non-local secondary structure.
- Residue 77 and residue 95 shows different trends in each cluster with temperature.

# radius of gyration : by cluster

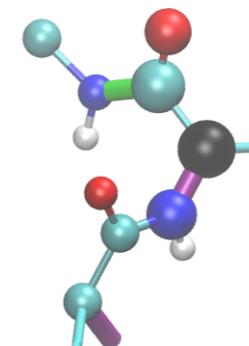
previous : possible systematic effect of SNP for most extended mode?

total:

fit by cluster:

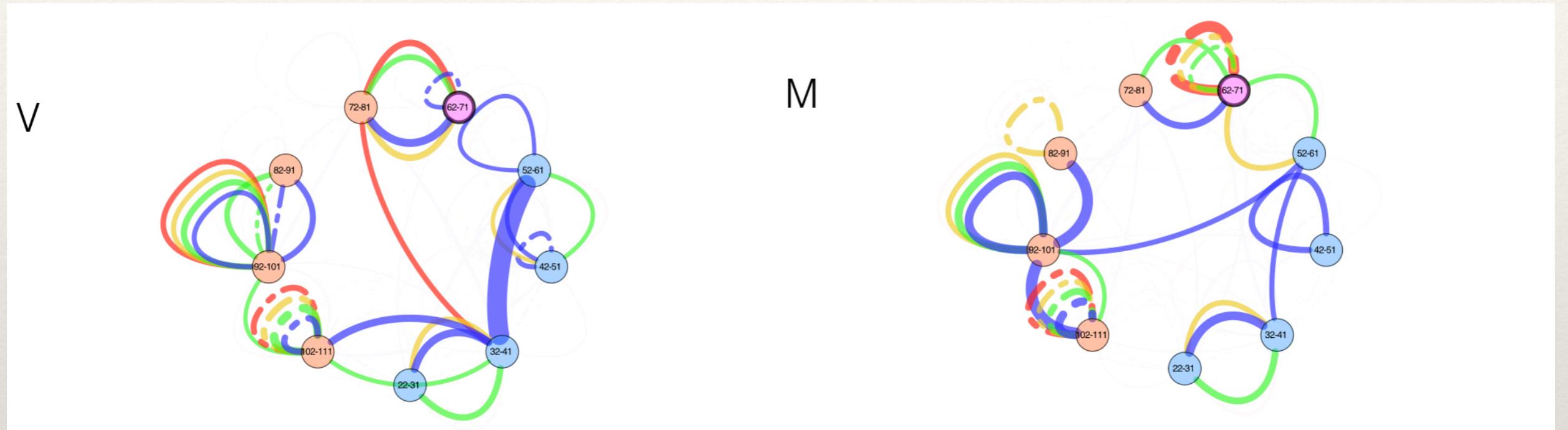


restricted to cluster H2: when residue 66 (MET) in conformation



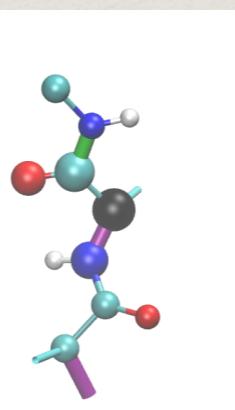
# Coarse-grained segment contact maps: cluster contribution

Previously : Divided the protein into 10 segments of 10 residues each

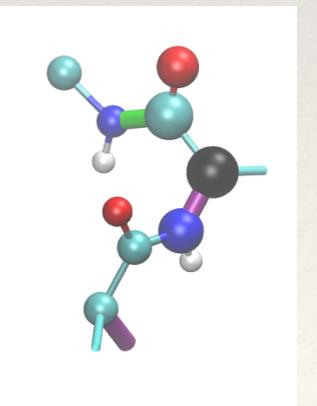


Dividing into clusters reveals:

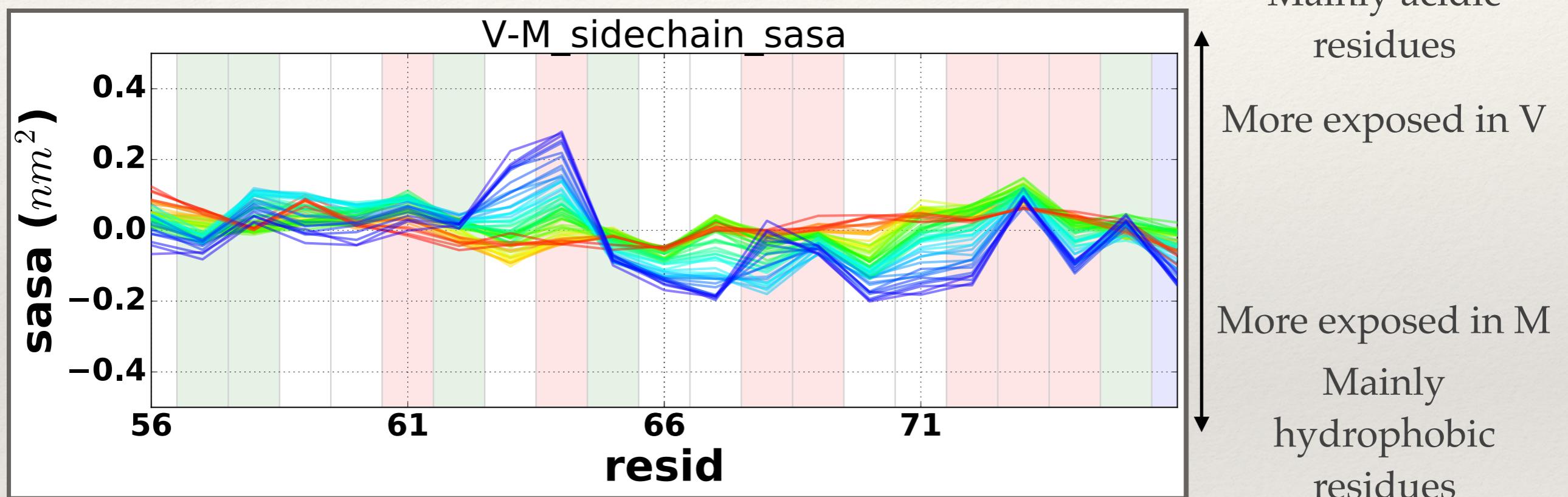
Warm V66 long-range pairs : mainly from B1 (larger Rg)



Cold M66 long-range pairs : mainly from H2 (larger Rg)



# Possibilities for Binding : Solvent accessible surface area



# Summary

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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.