ALL-ATOM SIMULATIONS OF HUNTINGTIN'S N-TERMINAL: SOLVENT AND MEMBRANE EFFECTS

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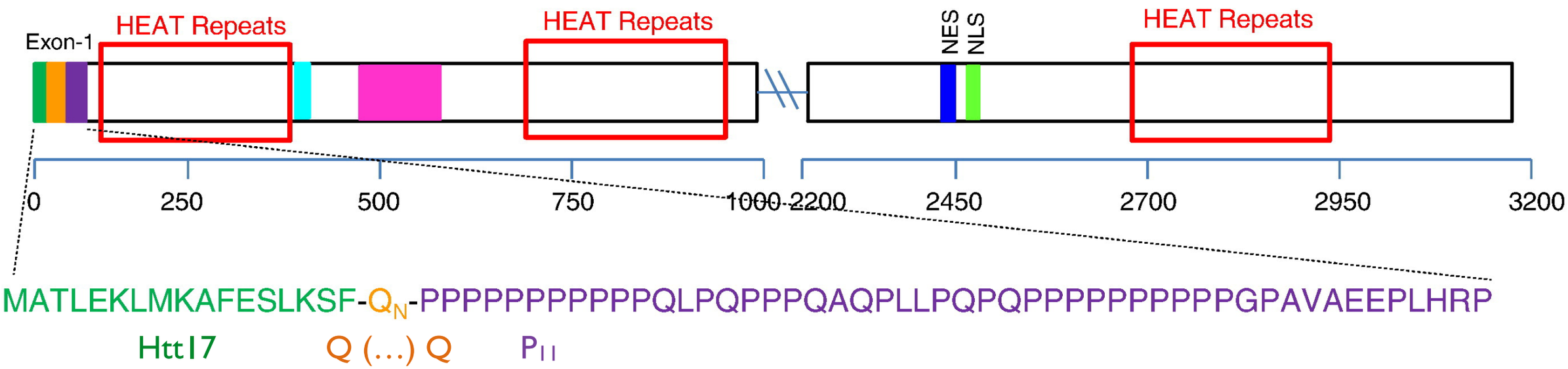
HUNTINGTON DISEASE & HUNTINGTIN PROTEIN

The first exon of Huntingtin is sufficient to reproduce the neurological phenotypes and aggregation.

The Exon1 is composed of an amphipathic sequence of 17 amino acids (Htt17) MATLEKLMKAFESLKSF, a polyglutamine region (Q_N) and a 38 amino acids proline rich domain (C38).

Htt17 is responsible for membrane anchoring and the acceleration of the fibrillation kinetics.

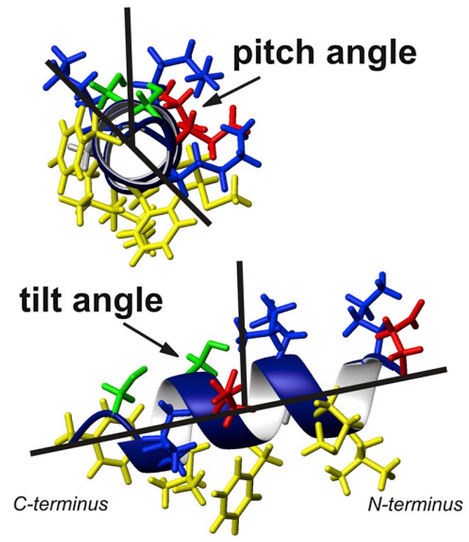


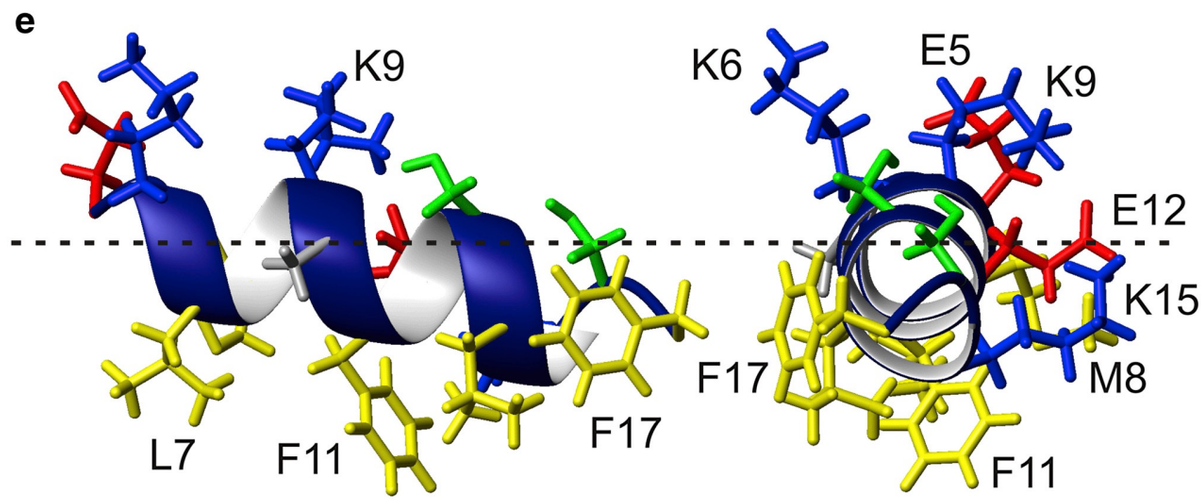
Wetzel, R. Journal of molecular biology, 421, 466-490. (2012)

HTT17'S STRUCTURE UNCOVERED BY NMR

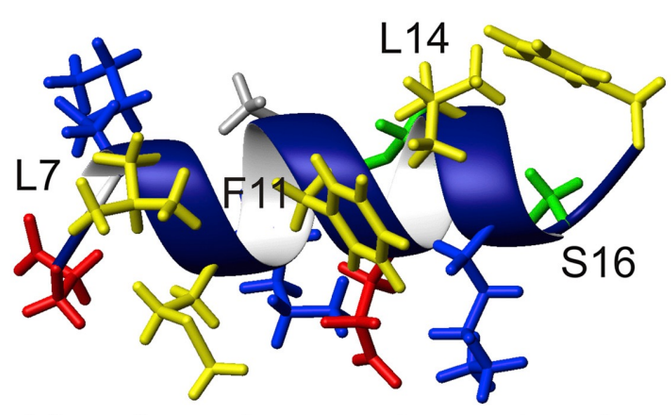
Recent solution NMR experiments in DPC micelles found that:

- Htt17 forms an amphipathic helix from residues 6 to 17.
- It lies almost parallel to the membrane surface.

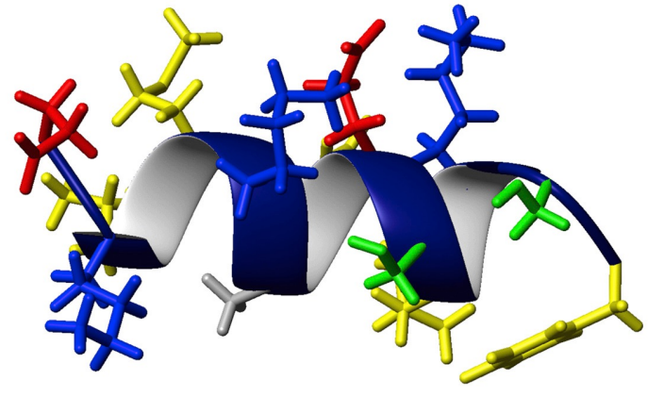




Such structure could form dimers which could lead to high local concentration of PolyQ at the membrane surface.



View from the membrane interior



View from the aqueous side

Michalek, M. et al. Biochemistry, 52, 847-858. (2013)
Michalek, M. et al. Biophysical journal, 105, 699-710. (2013)

THE PROJECT

All-atom, explicit solvent numerical simulation, combining:

- Molecular dynamics (MD)
- Hamiltonian replica exchange (HREX)
- Metadynamics (MetaD)

Probe the effect of the other Exon1's segments (Q_N and C38) on Htt17's structure.

Explore the stability and the properties of the proposed NMR model (PDB: 2LD2) in aqueous solution and in phospholipid bilayer and answer the following questions:

- Is the model stable in solution? In membrane?
- What is the secondary structure?
- What is the effect on the membrane?

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SIMULATION SETUP AND FORCEFIELD CHOICE

For the protein, we used the all-atom AMBER99sb*-ILDN forcefield[1], optimized by David Shaw's group.

The Berger forcefield[2] was used for the lipids.

The combination of HREX and Metadynamics was done with GROMACS 4.6.5[3] patched with PLUMED 2.0.2[4].

In aqueous solution:

Htt17: M A T L E K L M K A F E S L K S F

Htt17Q_{17}: M A T L E K L M K A F E S L K S F Q_1 (…) Q_{17}

Htt17$Q_{17}P_{11}$: M A T L E K L M K A F E S L K S F Q_1 (…) Q_{17} P_1 (…) P_{11}

In phospholipid bilayer:

We used two initial structure of Htt17: the **NMR model in micelles environment** and a fully formed α-helix.

In total: 10 to 24 simulations of 500 ns to 1000 ns for each system.

1. Piana, S. et al. Biophysical journal, 100(9), L47-L49. (2011)
2. Cordomí, A. et al. Journal of chemical theory and computation, 8(3), 948-958. (2012)
3. Pronk, S. et al. Bioinformatics, 29(7), 845-854. (2013)
4. Tribello, G.A. et al. Computer Physics Communications, 185(2), 604-613. (2014)

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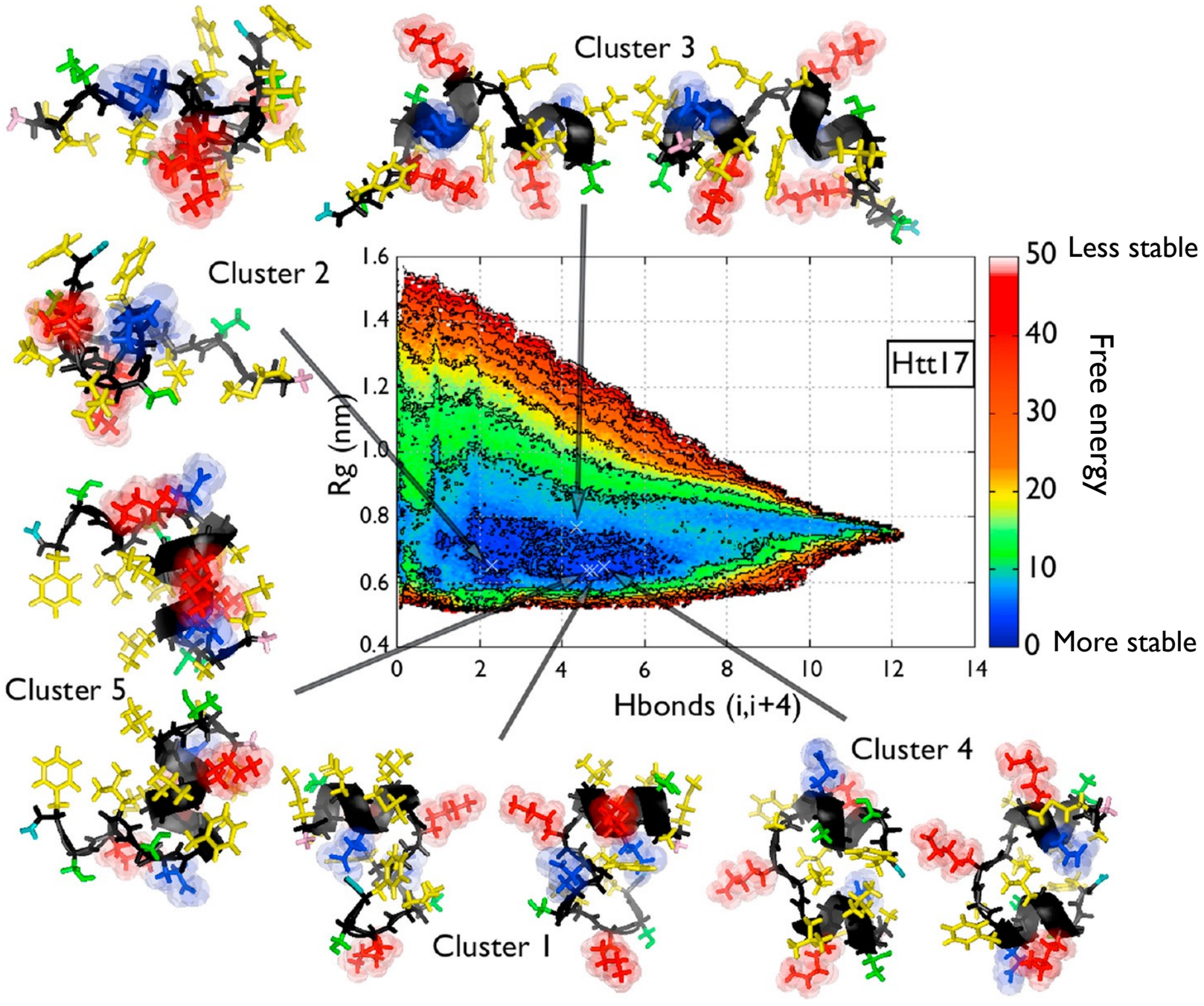
HTT17

Simulation details:
- HREX + MetaD
- NMR model
- 14.4µs (0.9µs x 16 replicas)

The FES is characterized by a single broad minimum bound by 2 to 6 (i,i+4) H-bonds and a gyration radius between 0.6 to 0.8 nm.

The minimum is populated by various two-helix bundle and fully random structures and the first half of Htt17 is more structured than the second one.



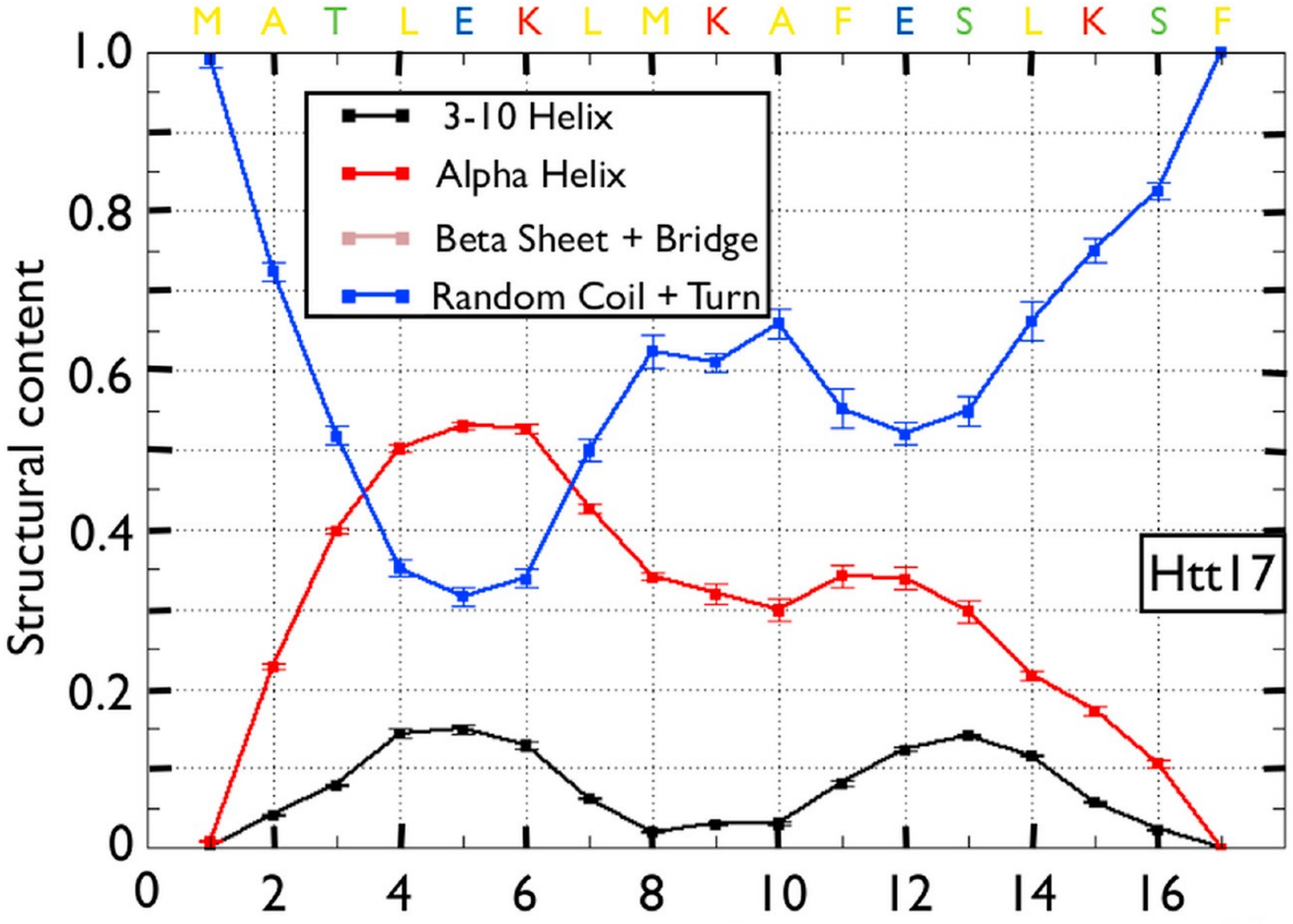
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

Htt17

Our simulations showed that Htt17 is mostly unstructured in solution with 29.3 ± 0.7% of α-helical content.

The helical propensity is notably high for residues 3-6 and could nucleate the helix formation.

Residues 10 to 13 form a turn after which the peptide is less structured.



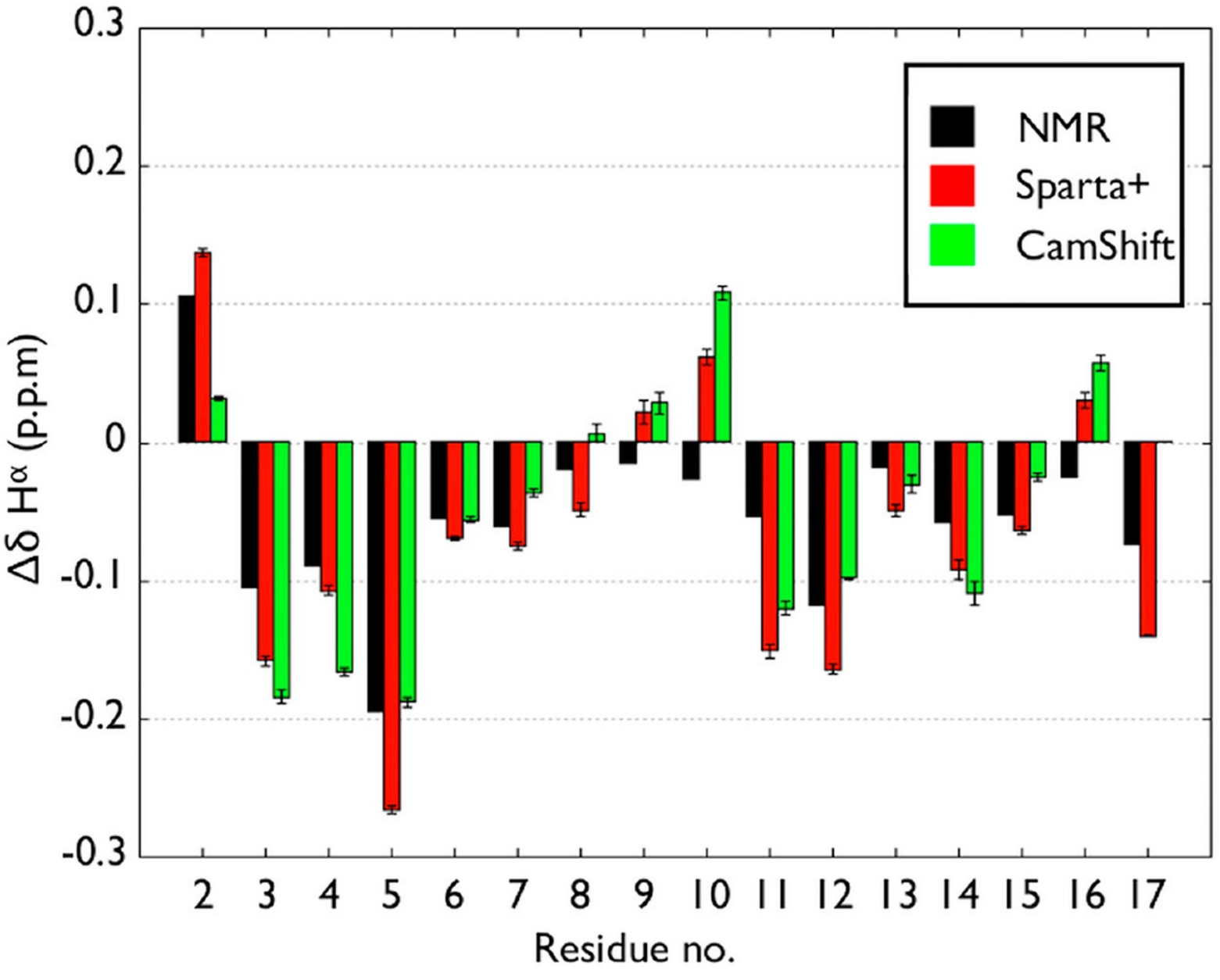
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

HTT17

NMR expriment[1] strongly suggests the absence of stable folded structure with transient α-helix between Thr3 and Glu5.

The negative H^{α} chemical shifts indicates helical features. Our simulations are in good agreement with the NMR results.

Multiple CD experiments indicate between 10% and 50% of helical content in solution.

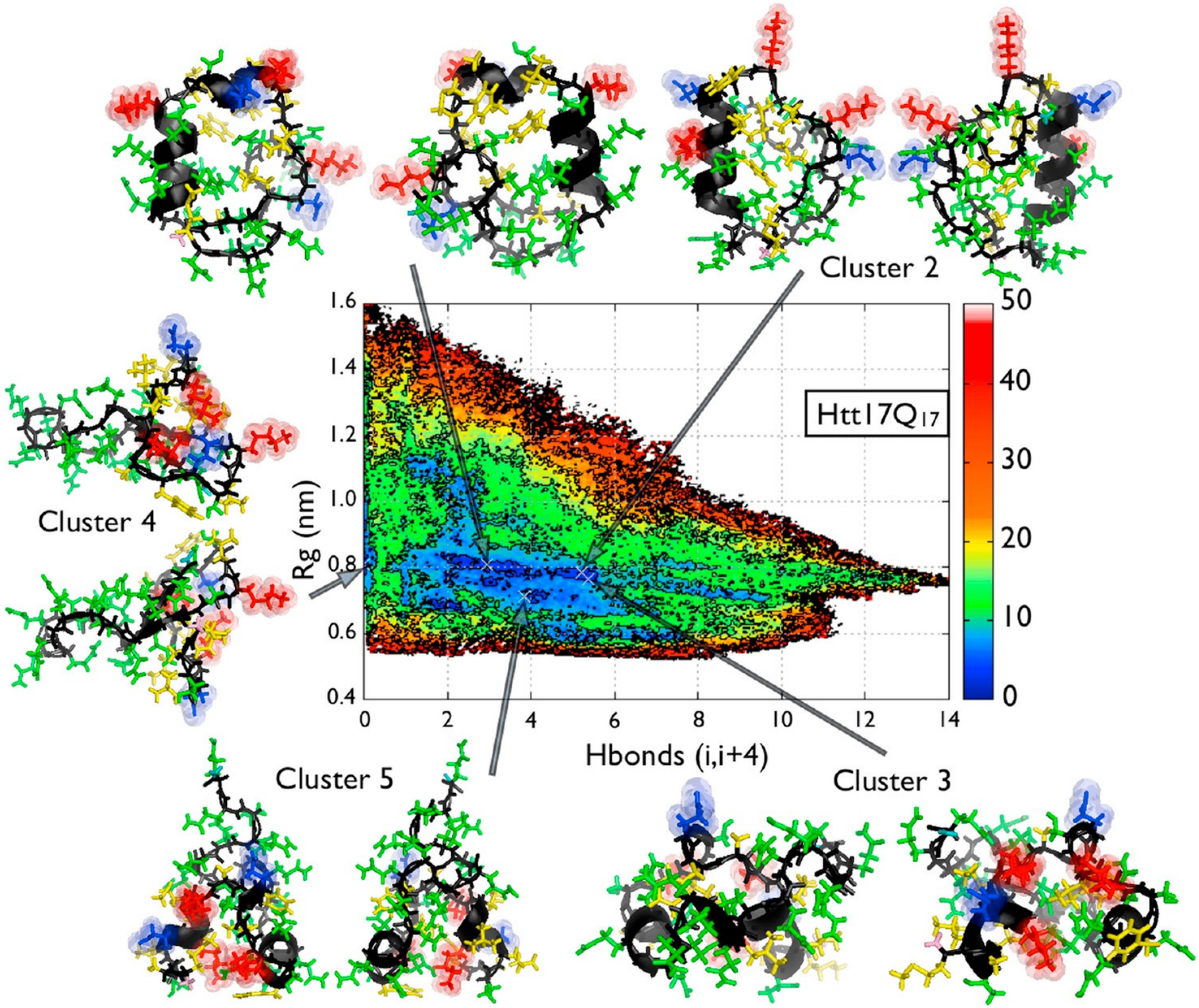


1. Thakur, A. K. et al. Nature structural & molecular biology, 16(4), 380-389. (2009)
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

HTT17 + Q_{17}

Simulation details:
- HREX + MetaD
- NMR model
- 21.6µs (0.9µs x 24 replicas)

The addition of Q_{17} leads to:
- Destabilization of highly helical structure and conformations with small gyration radius.
- Minimum populated by U-shaped Htt17 surrounding an hydrophobic core partially covered by the Q_{17} domain.



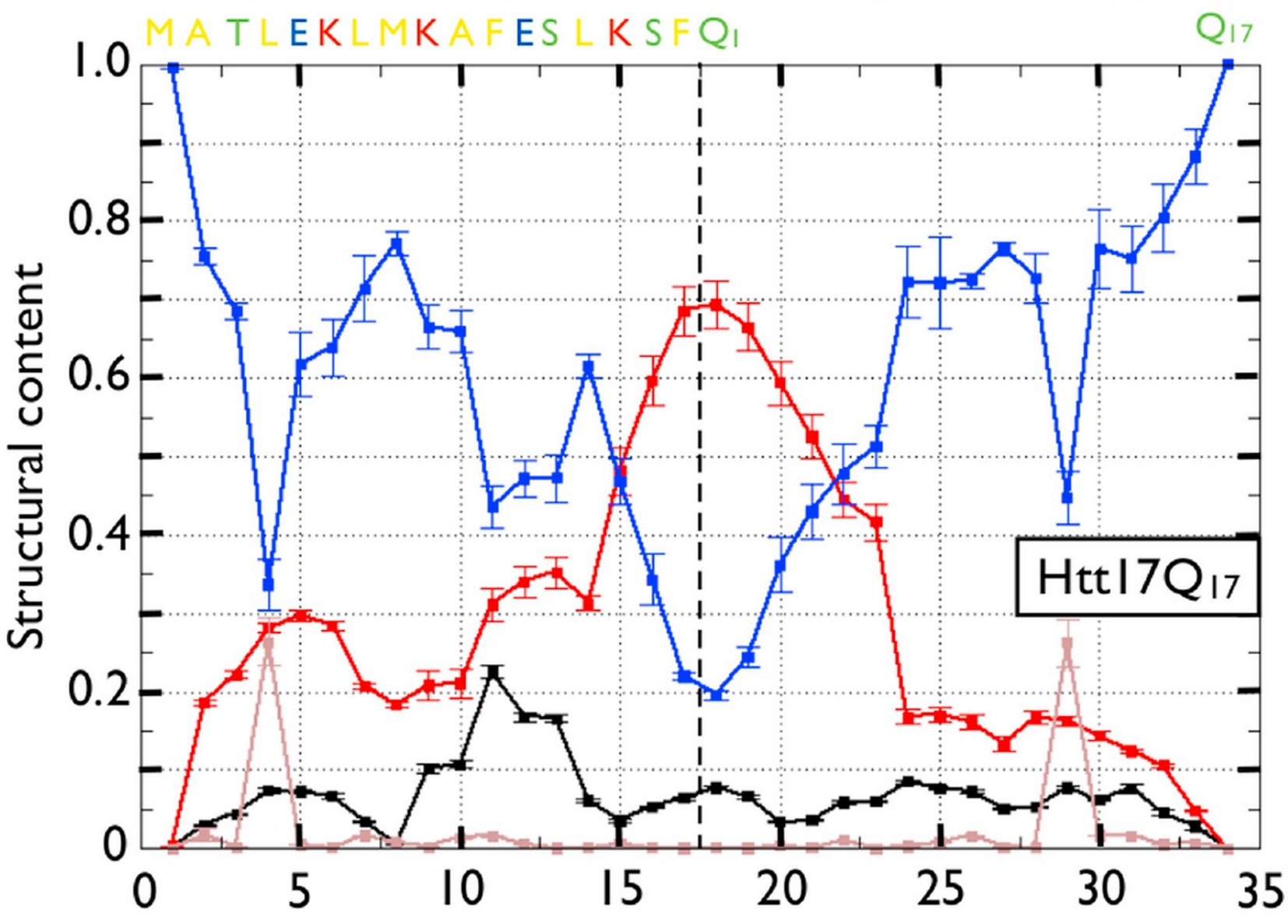
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

HTT17 + Q_{17}

Htt17 has 30.4 ± 1.4% of α-helical content.

FRET experiments[1] shows that Htt17 is in a collapsed state and becomes more extended with the addition of Q_{17}.

CD[2] suggests an increase in the α-helical content with the Q_N length.



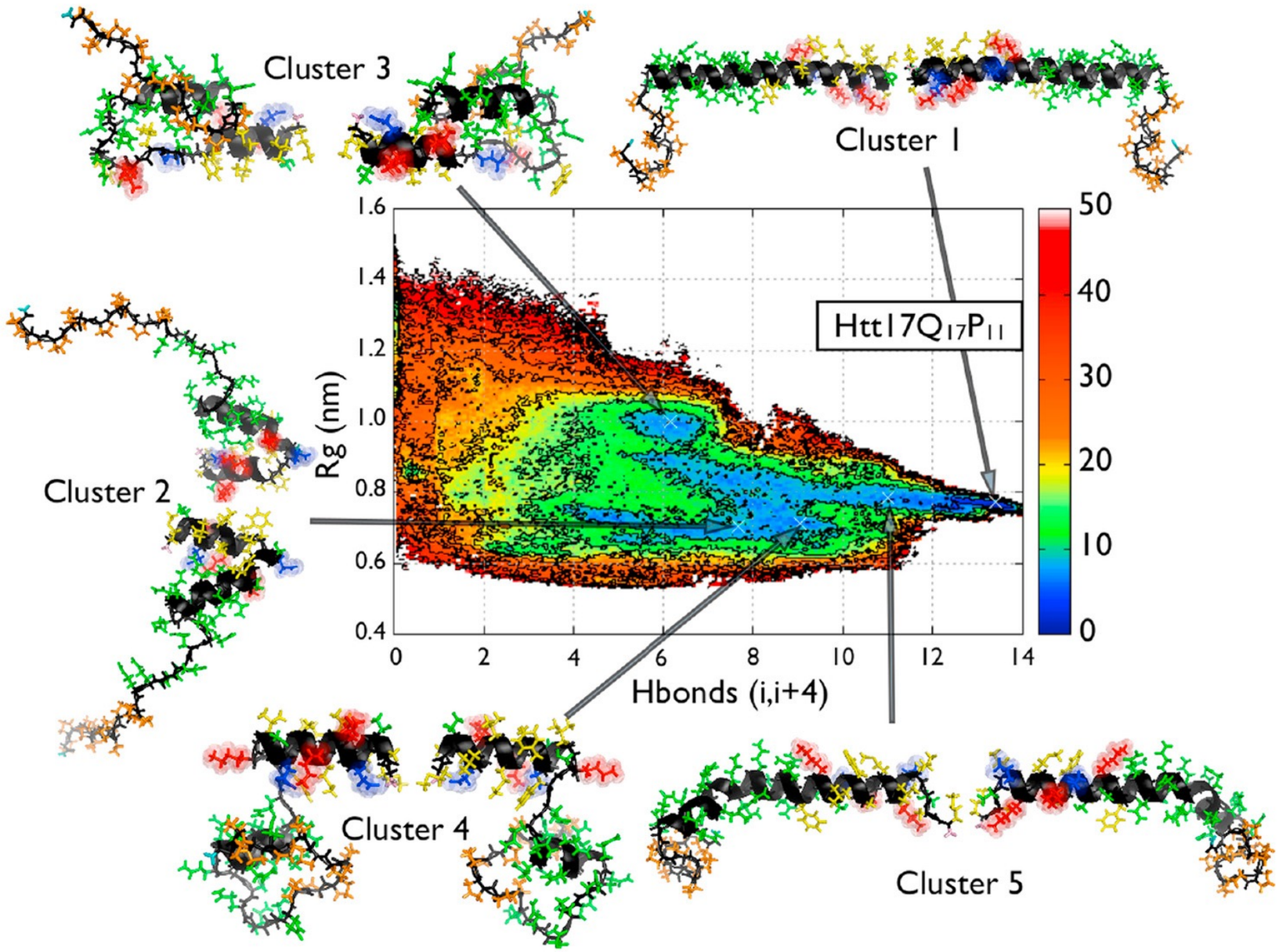
1. Thakur, A. K. et al. Nature structural & molecular biology, 16(4), 380-389. (2009)
2. Jayaraman, M. et al. Journal of molecular biology, 415(5), 881-899. (2012)
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

HTT17 + Q_{17} + P_{11}

Simulation details:
- HREX + MetaD
- NMR model
- 21.6μs (0.9μs x 24 replicas)

The FES minimum is populated by Htt17 in a fully formed α-helix with a clear separation between the polar and non polar residues.

The P_{11} domain extends away from Htt17 and Q17.



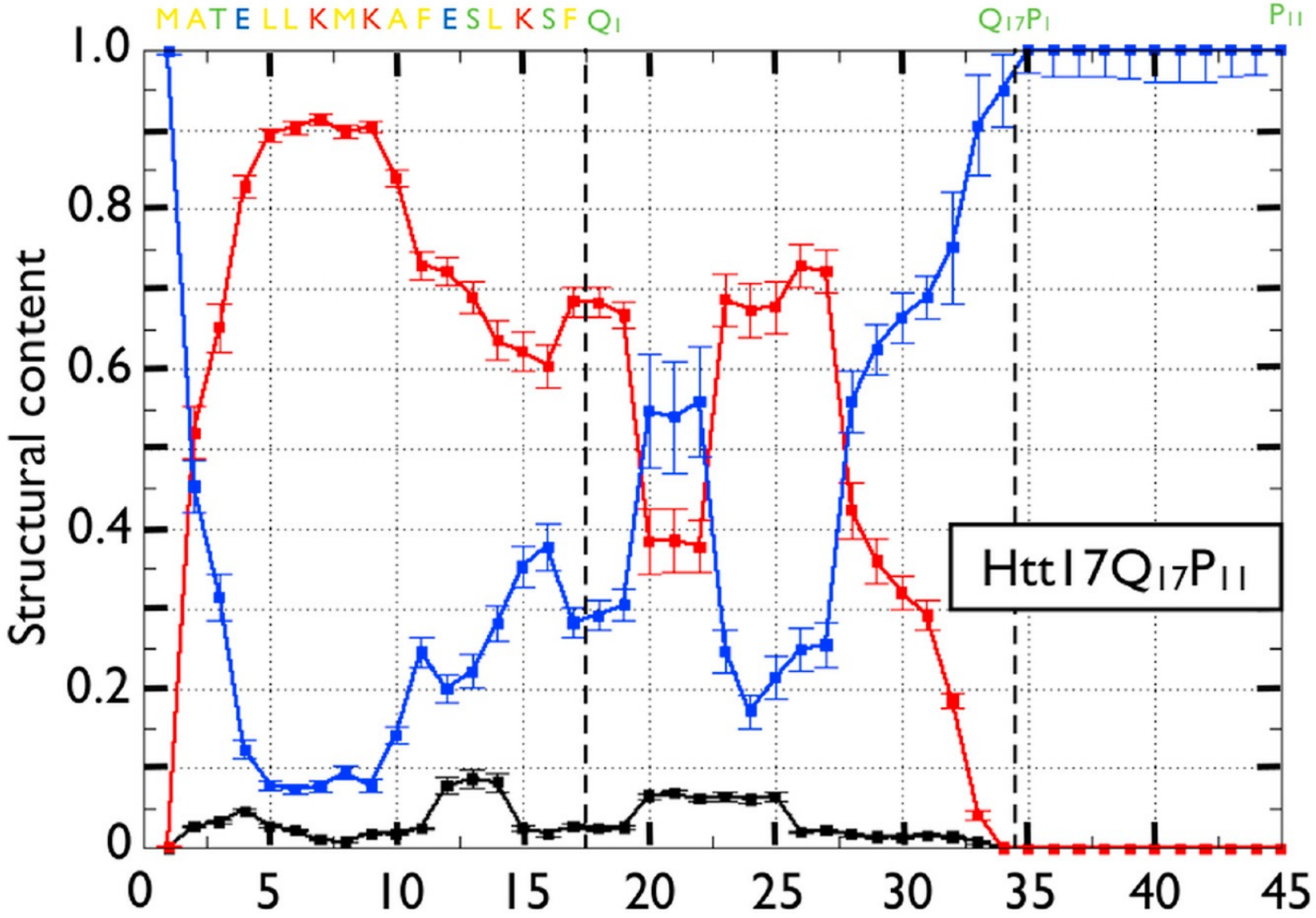
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

HTT17 + Q_{17} + P_{11}

The addition of $Q_{17}P_{11}$ favors:

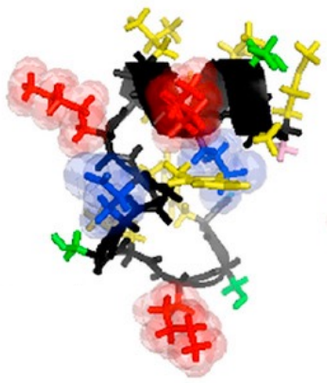
- Highly helical Htt17 structure with 70.9 ± 1.6% of α-helix content.
- Q_{17} adopts an α-helix for its first half and unstructured for the rest.
- P_{11} adopts a PP type-II helix.

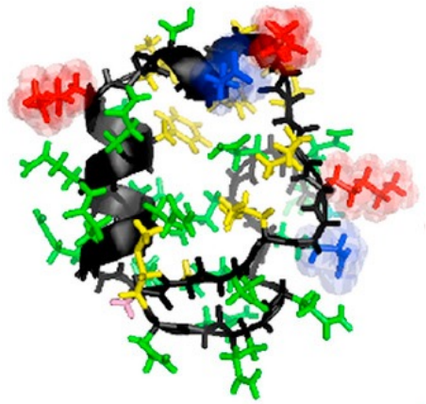
X-ray crystallography[1] suggests that Htt17 adopts an α-helix structure that extend to the first few residues of Q_N while the rest are mostly unstructured and the PolyP domain adopts a PP-helix.

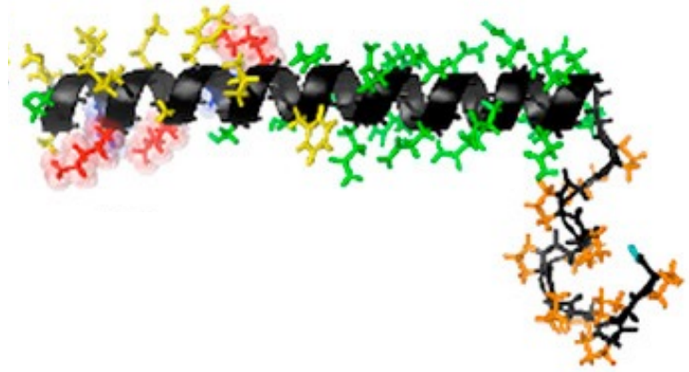


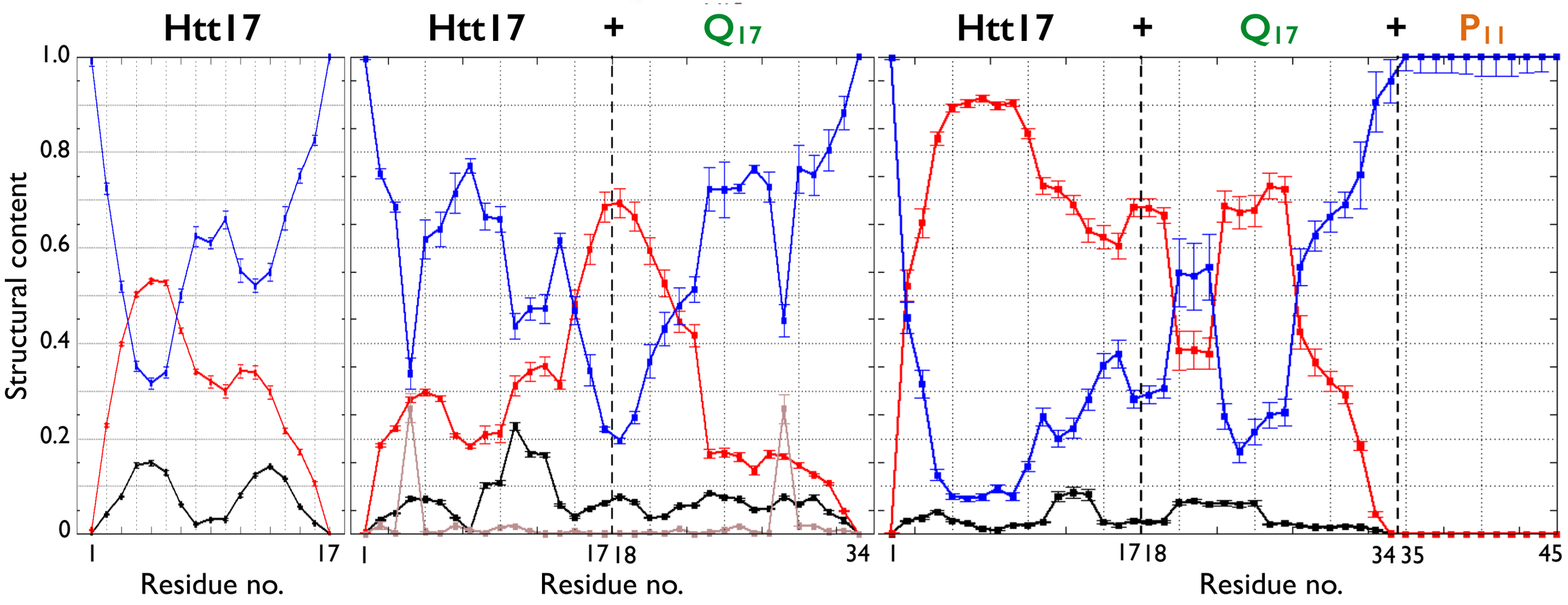
1. Kim, M.W. et al. Structure, 17(9), 1205-1212. (2009)
Binette, V. et al. Biophysical journal, 110(5), 1075-1088. (2016)

OVERLOOK









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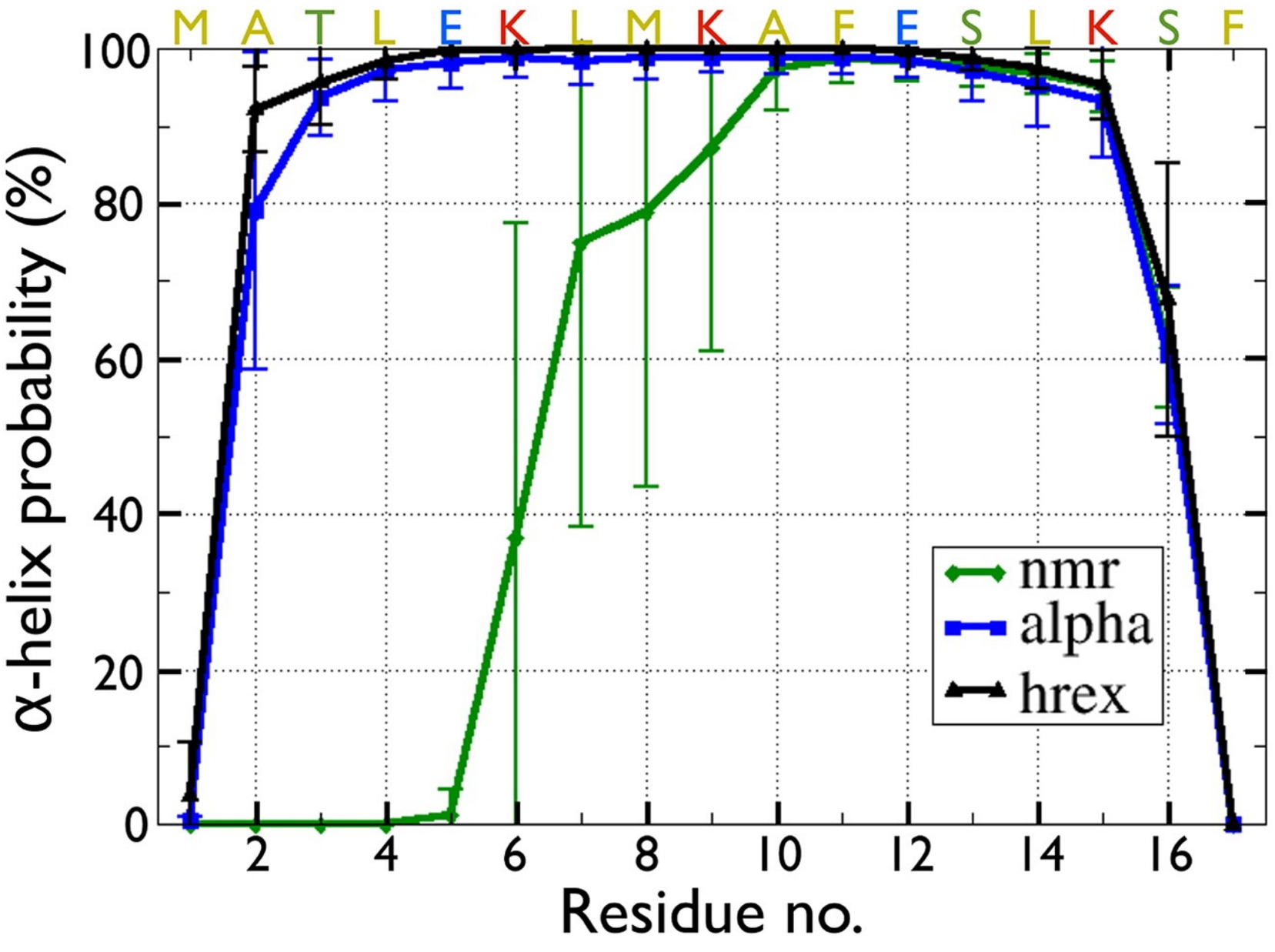
HTT17 + MEMBRANE

Simulation details:

- MD, NMR, (1000ns x 11 simulations)
- MD, α-helix, (1000ns x 11 simulations)
- HREX, α-helix, (250ns x 16 replicas)

Simulations started with the NMR model leads to large structural fluctuations around residues 6 to 9 due to the unfolding of the helix.

Simulations started with the fully helical model stay stable with small fluctuations and more than 80% of α-helix.



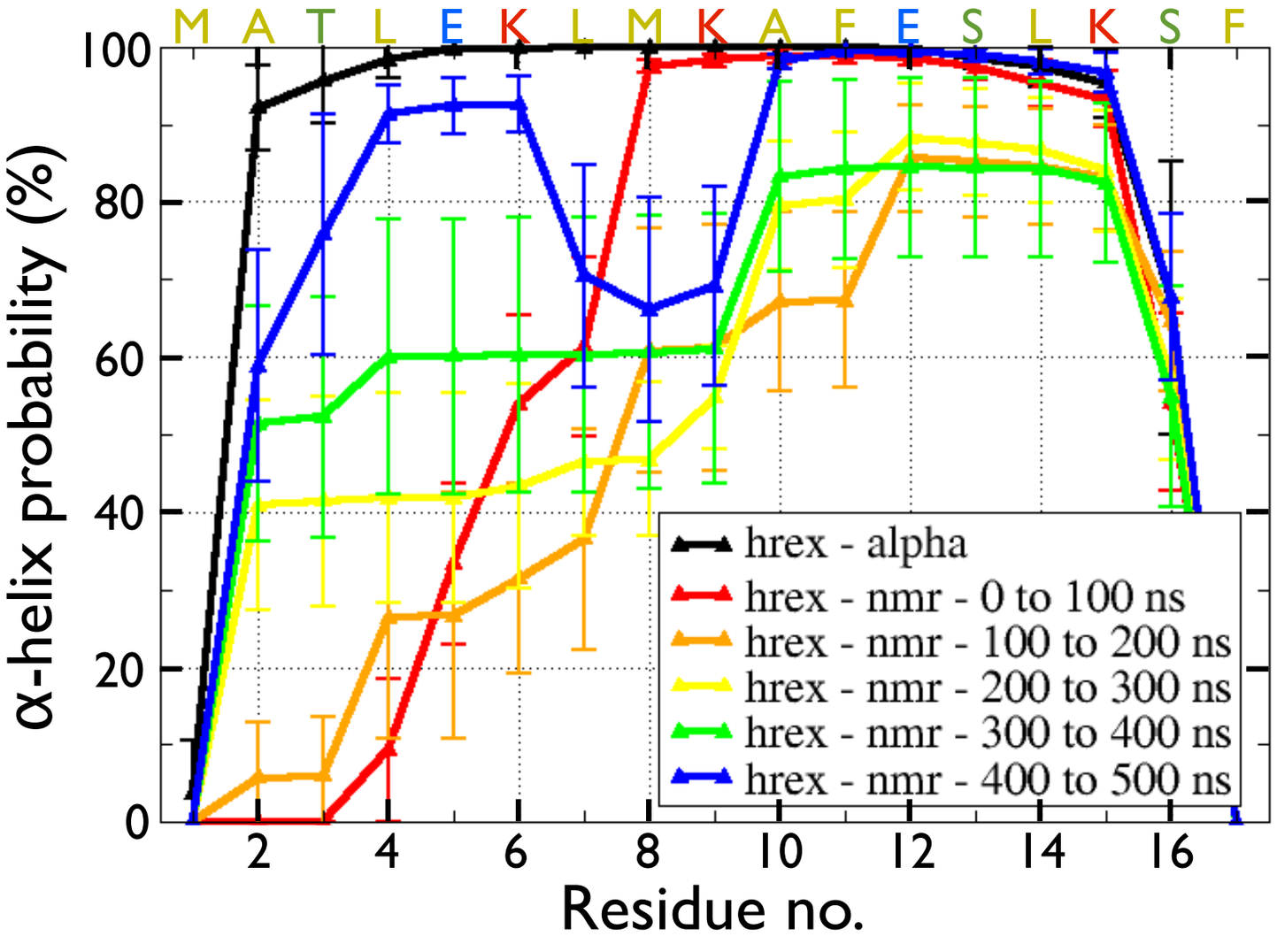
It indicates that Htt17 could be more structured in the membrane than the proposed NMR model.

Côté, S. et al. Biophysical journal, 108(5), 1187-1198. (2015)

HTT17 + MEMBRANE

Simulation details: HREX, NMR, (500ns x 16 replicas)

Starting from the NMR model, HREX is able refold Htt17 toward the α-helix.



Côté, S. et al. Biophysical journal, 108(5), 1187-1198. (2015)

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CONCLUSION

Using state-of-the-art all-atom numerical simulation combining molecular dynamics, Hamiltonian replica exchange and Metadynamics we found:

In Solution:

- Htt17 has 29.3 ± 0.7% of α-helical content in agreement with the various CD measurements.
- Htt17's residues 3 to 6 have the highest helical propensity in agreement with NMR experiments. They could nucleate the helix formation.
- The addition of Q_{17} destabilizes highly helical structures.
- The addition of $Q_{17}P_{11}$ greatly stabilizes the α-helix.

In Membrane:

- Htt17 could be more structured in the membrane than the proposed NMR model.

The $Q_{17}P_{11}$ domain could play an important role in Htt17's functions as it drastically changes its structure in a way that could favor the solution/membrane transition.

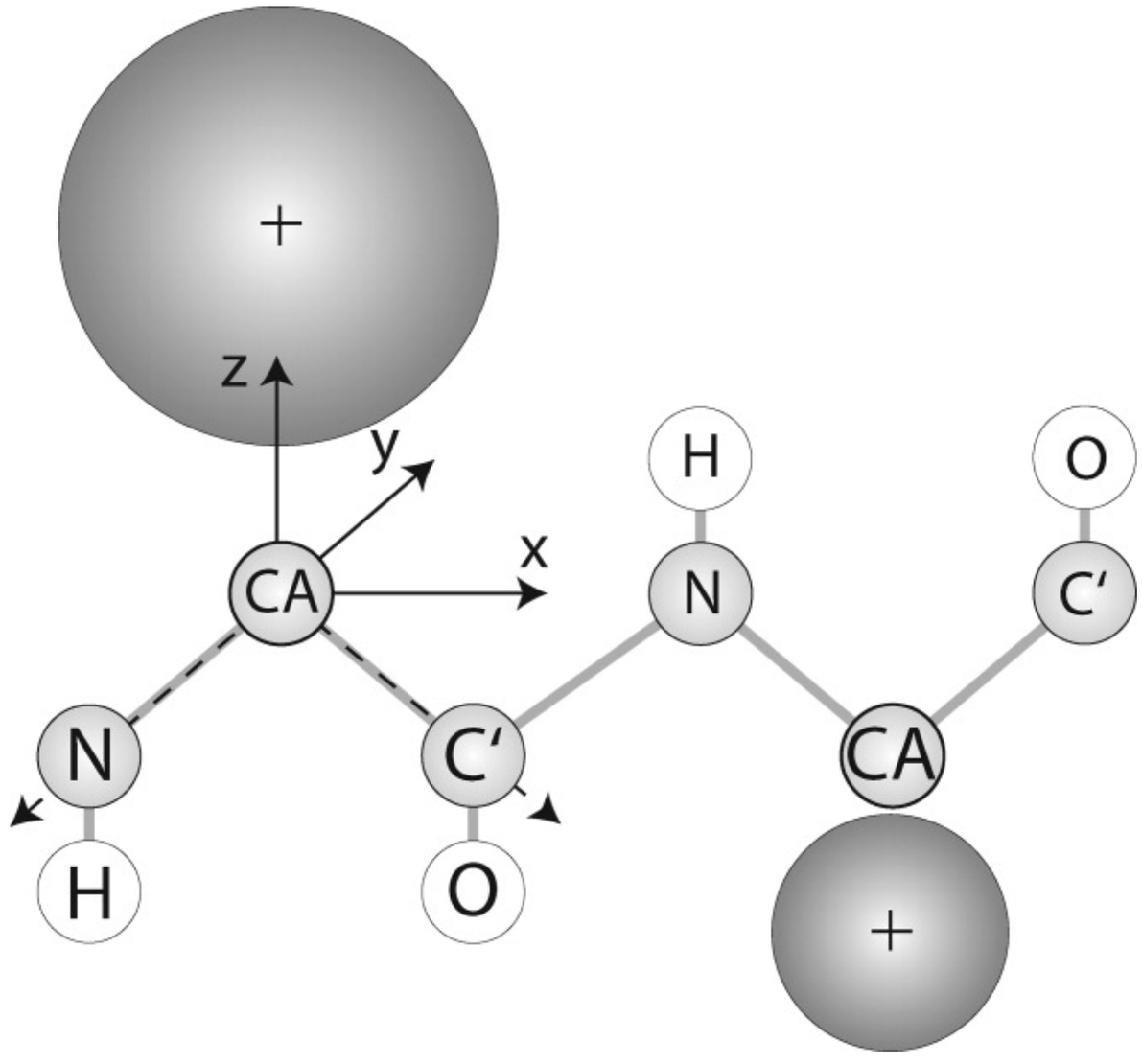
Our results are consistent with Ht17 functions of membrane anchoring and favoring the dimerization.

FUTURE WORK

Currently in development:

- Optimization of the coarse-grained sOPEP[1] potential.
- Development of the **aaOPEP** potential, a new all-atom version of the successful OPEP potential!
- Refine the PEP-FOLD[2] prediction and investigate protein folding and function.

Stay tuned!



1. Maupetit, J. et al. Journal of computational chemistry, 31(4), 726-738. (2010)
2. Shen, Y. et al. Journal of chemical theory and computation, 10(10), 4745-4758. (2014).

ACKNOWLEDGMENTS

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- Burkhard Bechinger (NMR experiments on Htt17)

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- GEPROM
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- CRSNG

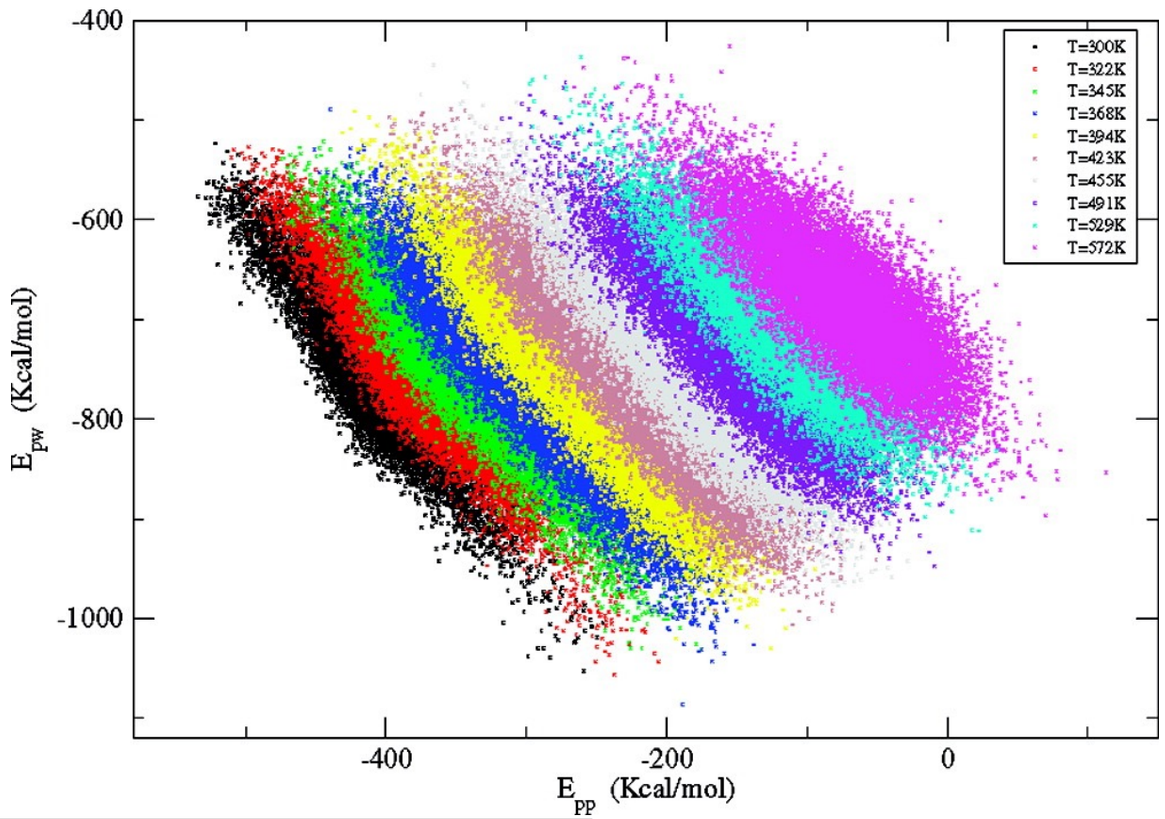
ADDITIONAL SLIDES

HAMILTONIAN REPLICA EXCHANGE

The REST2 protocol of Hamiltonian replica exchange (HREX) allows different replicas to evolve according to different Hamiltonians.

It scales down the forcefield terms contributing to energy barriers: Electrostatic, Lennard-Jones and proper dihedrals.

The reduction of the energy barrier allows us to spend less time inside local minima and therefore enhances the sampling power.



Wang, L. et al. The Journal of Physical Chemistry B, 115, 9431-9438. (2011)
Bussi, G., Molecular Physics, 112(3-4), 379-384. (2014)

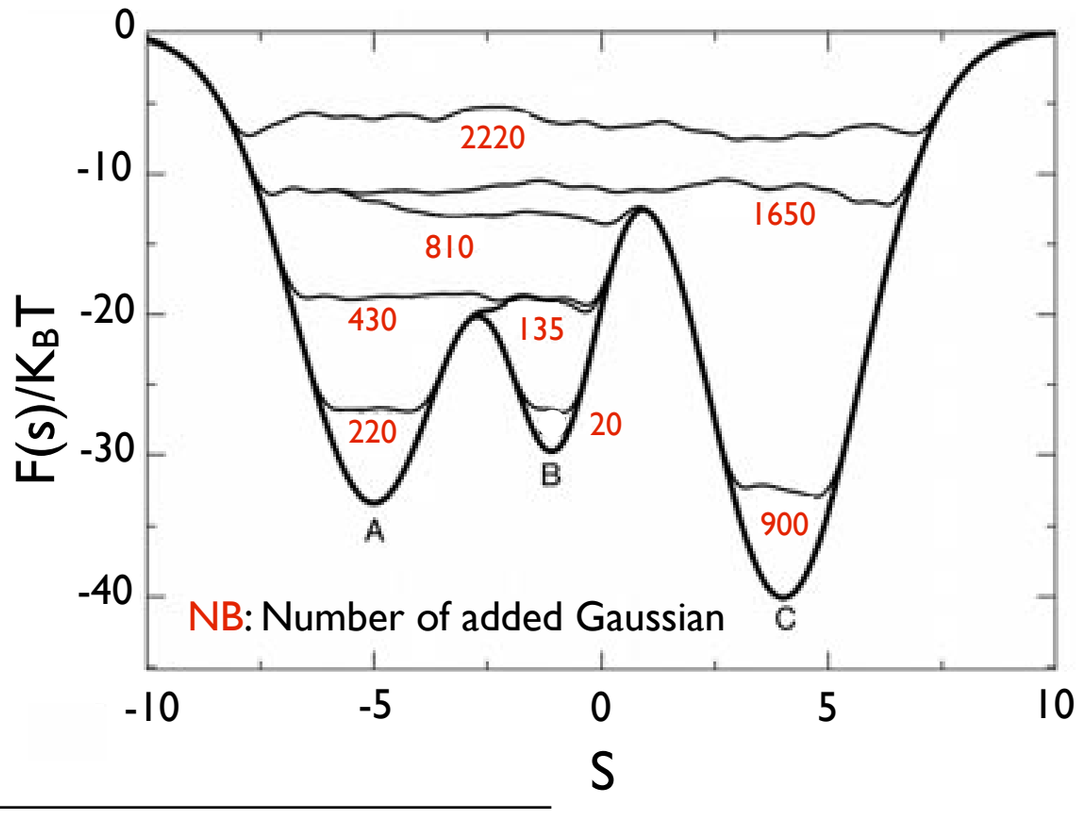
WELL-TEMPERED METADYNAMICS

WT-MetaD adds an external history-dependent bias potential of a few degrees of freedom called collective variables (CV).

The potential is built as a sum of Gaussian deposited along the trajectory in the CVs space.

This technique forces the system to explore high free-energy region.

In WT-MetaD, the Gaussian's height decreases with the simulation time.



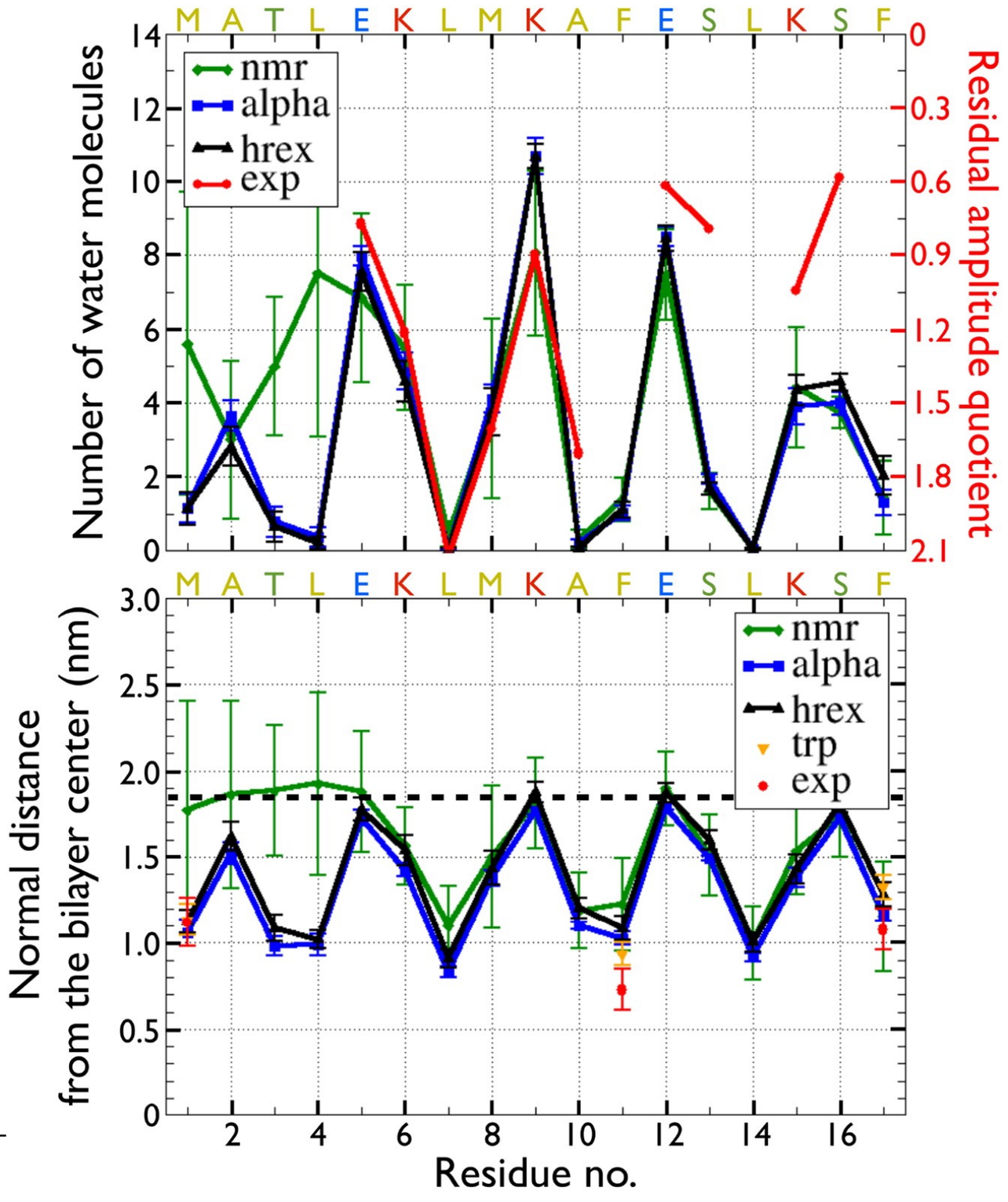
Barducci, A. et al. Physical review letters, 100, 020603. (2008)

HTT17 + MEMBRANE

In simulations starting from the α-helix (MD and HREX), the insertion depth of residues Met1, Phe11 and Phe17 are in good agreement with the fluorescence experiments.

In simulations starting from the NMR model, the first four residues are much more exposed to the solvent since they stay unstructured.

These results also indicates that Htt17 could be more structured inside the phospholipid membrane than the NMR model found in micelles.



Côté, S. et al. Biophysical journal, 108(5), 1187-1198. (2015)

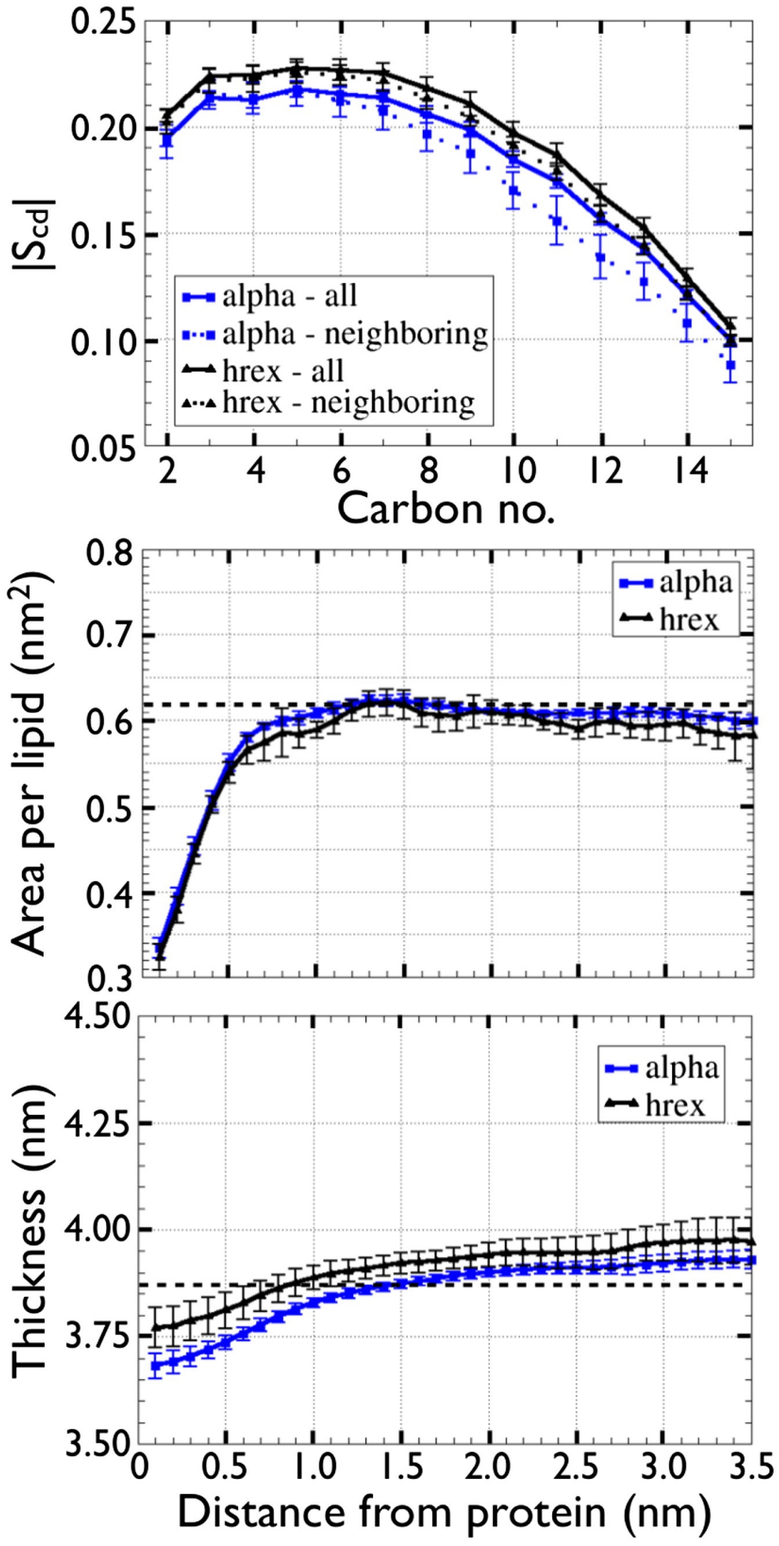
MEMBRANE PERTURBATION

The presence of Htt17 monomer leads to local perturbation of the membrane properties.

Htt17 allows its neighboring phospholipids to extend their acyl chain to cover its nonpolar surface.

These local membrane deformations around a peptide promote dimerization of the inserted amphipathic peptides.

Htt17 could play a fundamental role by initiating the binding of monomer, perturbing the membrane properties and favor the oligomerization.



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