Insights into the Structure and Dynamics of the N-terminal Fragment of the Huntingtin Protein

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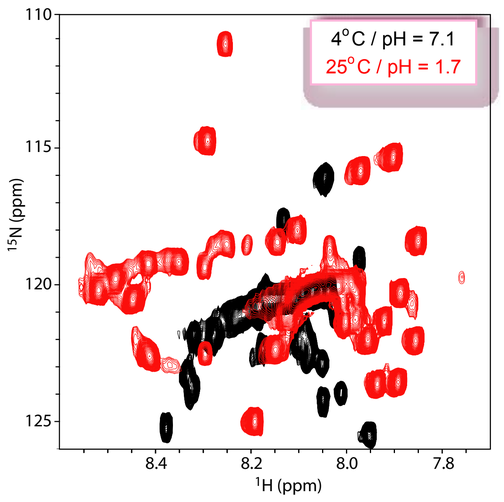
The problem with Huntingtin

Studying proteins at atomic resolution both in vitro and in their native environments, is fundamental to understanding protein folding and aggregation. This work studies a CAG expansion within the huntingtin (Htt) gene, that encodes a polymorphic glutamine tract near the protein N-terminus and that is associated with Huntington's disease[1]. The ensuing polyQ peptide is preceded by a 17 residue region that modulates the glutamine tract's behavior. To help elucidate the molecular basis of Htt aggregation, we investigated wild type Htt's 17 amino acid N-terminal segment with a 17 residue polyQ stretch (**HttN17Q17**). Studying Htt peptides presents a number of unique challenges: they display a high degree of conformational flexibility leading to averaging of NMR chemical shifts, and a large portion of their backbones are solvent-exposed leading to fast hydrogen exchange and causing extensive line broadening. To proceed with the NMR study, hydrogen exchange was suppressed by dissolving HttN17Q17 in a low pH solution. Resonances in the neutral (pH = 7.4) in vitro samples were then mapped to their low pH counterparts by performing NMR titration experiments. Molecular dynamics simulations starting from CS-ROSETTA derived structures based on the experimental chemical shifts were used to extract order parameters for the Htt peptide at low and neutral pH. All these data confirmed the high flexibility that the N17 residues display in solution at neutral pH.

N17 polyQ polyP His-Tag

MATLEKLMKAFESLKSFQQQQQQQQQQQQQQQPPPPPPLECHHHHHH

1 5 10 15 20 25 30 35 40 45



neutral pH:

- the fast conformational exchange reduces the chemical shift dispersion resulting in extensive spectral overlap
- the peptide's backbone is fully solvent exposed, thus labile amide hydrogens efficiently exchange with the solvent, broadening spectral lines

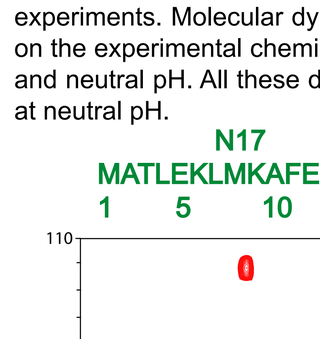
solution: **move to lower pH**

Backbone resonance assignment at low pH

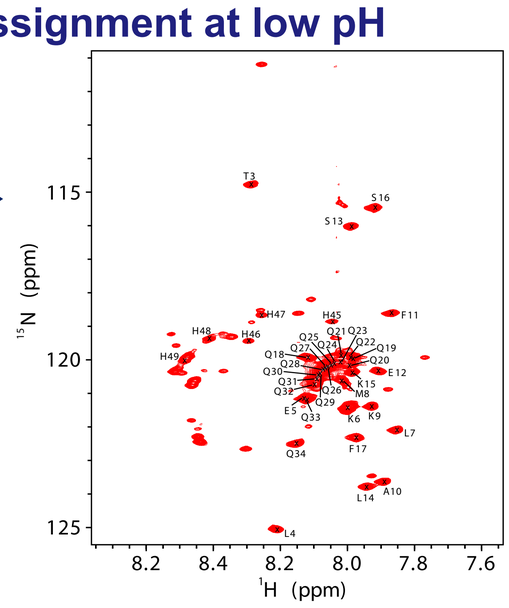
^{15}N-^{1}H HSQC

+

3D & 5D BEST^{2} NMR

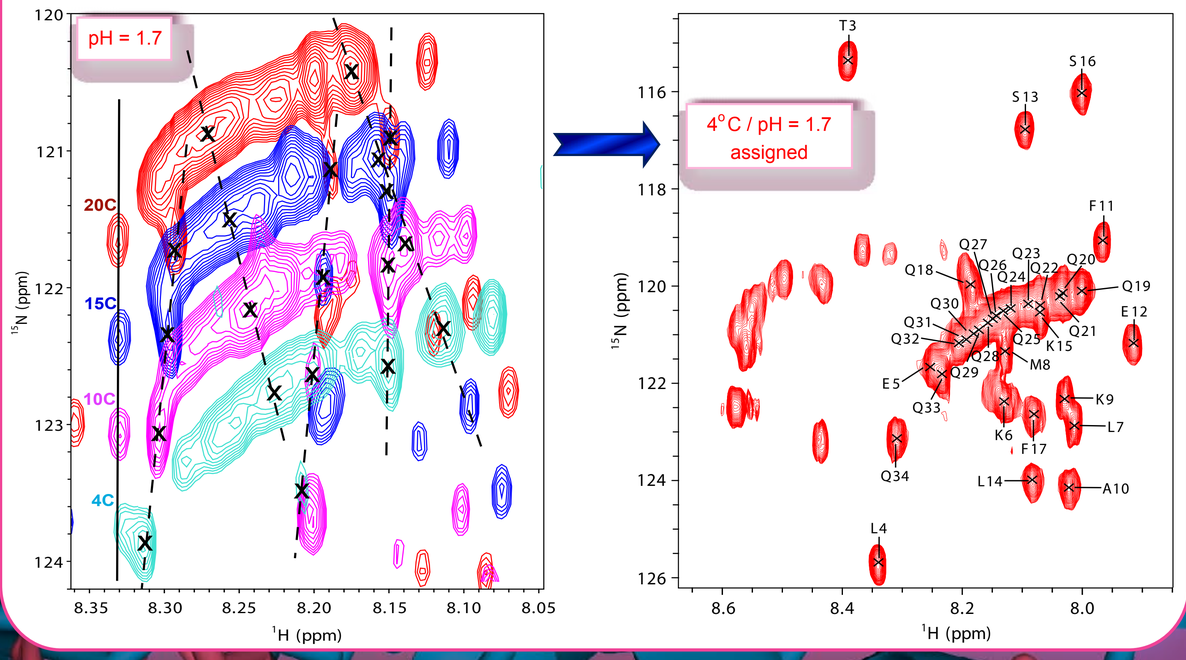


assignment of the amino acids from the **N17** terminal fragment of HttN17Q17 at low pH



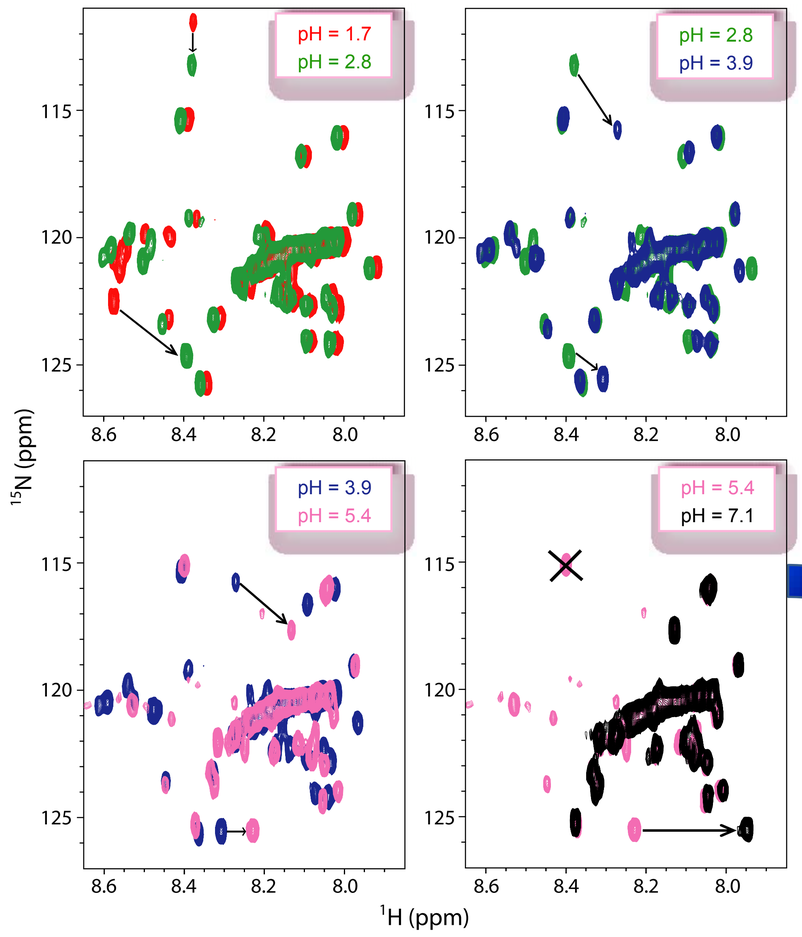
Variable temperature experiments

Monitoring the change in ^{1}H and ^{15}N chemical shifts when gradually decreasing the temperature allows for the assignment of HttN17Q17 at low temperature and low pH. This assignment will then be used as a starting point into assigning the low temperature spectrum at neutral pH, when gradually increasing the pH of the sample during pH titration experiments.

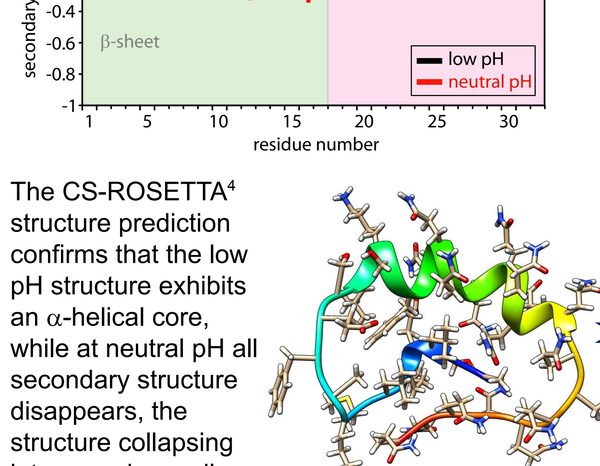


pH titration experiments

The assignment of the low pH spectrum at 4° C was followed by a series of titration experiments at this temperature, where the pH of the sample was gradually increased from 1.7 to 7.1. The chemical shift of each resonance was monitored with the help of ^{15}N-^{1}H HSQC spectra, as exemplified below.



Backbone resonance assignment at neutral pH



chemical shifts at neutral pH

assignment of the amino acids from the N-terminal fragment of the Huntingtin protein: **HttN17Q17 at neutral pH**

Structure and dynamics of HttN17Q17

Secondary structure propensities[3] (left) indicate that the low pH structure has higher propensities of forming secondary structure compared to the neutral pH structure, which has a smaller SSP score. This is confirmed by the order parameters (right) extracted from molecular dynamics simulations, with smaller values for the neutral pH structure compared to the low pH structure.

The CS-ROSETTA[4] structure prediction confirms that the low pH structure exhibits an α-helical core, while at neutral pH all secondary structure disappears, the structure collapsing into a random coil conformation.

Conclusions and Outlook

Using multidimensional correlation NMR experiments on a uniformly doubly [^{13}C, ^{15}N] labeled peptide, we successfully assigned the HttN17Q17 N-terminal region of the huntingtin protein at acidic pH. These assignments were then mapped to physiological pH conditions with the aid of variable temperature and pH titration NMR experiments. At low pH HttN17Q17 possesses an α-helical secondary structure in the central region of the peptide, characterized by high dipolar order parameters indicating less backbone mobility in this region. However, this structural feature disappears in neutral pH environments, and as a result the protein becomes more mobile, as indicated by the low order parameters. The results presented here are expected to facilitate understanding of the behavior of soluble Htt exon 1 protein fragments. Our forthcoming investigations will leverage the structural and dynamical knowledge gained from these experiments as well as the assignments presented here to study Htt exon 1 fragments in complex in-cell and brain extract environments.

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