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Nucleation inhibition of Huntingtin protein (htt) by polyproline PPII helices: a potential interaction with the N-terminal α -helical region of htt

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Abstract

Huntington's disease (HD) is a genetic neurodegenerative disorder characterized by the formation of amyloid fibrils of the huntingtin protein (htt). The seventeen-residue N-terminal region of htt (Nt¹⁷) has been implicated in formation of early-phase oligomeric species, which may be neurotoxic. Because tertiary interactions with a downstream (C-terminal) polyproline (polyP) region of htt may disrupt oligomer formation which are precursors to fibrillar species, the effect of co-incubation of a region of htt with a 10-residue polyP peptide on oligomerization and fibrilization has been examined by atomic force microscopy (AFM). From multiple, time-course experiments, morphological changes in oligomeric species are observed for the protein/peptide mixture compared with the protein alone. Additionally, an overall decrease in fibril formation is observed for the heterogeneous mixture. To consider potential sites of interaction between the Nt¹⁷ region and polyP, mixtures containing Nt¹⁷ and polyP peptides have been examined by ion mobility spectrometry (IMS) and gas-phase hydrogen deuterium exchange (HDX) coupled with mass spectrometry (MS). These data combined with molecular dynamics simulations (MDS) suggest that the C-terminal region of Nt¹⁷ may be a primary point of contact. One interpretation of the results is that polyP may possibly regulate Nt¹⁷ by inducing a random coil region in the C-terminal portion of Nt¹⁷, thus, decreasing the propensity to form the reactive amphipathic α -helix. A separate interpretation is that residues important for helix-helix interactions are blocked by polyP association.

Graphical Abstract

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Introduction

Huntington's disease (HD), a fatal neurodegenerative disorder, is caused by an expanded polyglutamine (polyQ) region within the first exon of huntingtin (htt).¹ In HD, the expanded polyQ domain is directly associated with the formation and deposition of intranuclear and cytoplasmic inclusion bodies composed of fibrillar htt aggregates.^{2–4} Beyond the formation of inclusions, htt aggregates into a variety of oligomeric and fibrillar species,^{5–8} and these different aggregate types can be diffusely spread throughout the cell^{9, 10} and even have prion-like properties.¹¹

The seventeen-residue N-terminal (Nt¹⁷) region of huntingtin protein (htt) has been shown to modulate htt-exon1 oligomerization in bulk solution and in the presence of lipid bilayers.^{12–15} Several studies have sought to block Nt¹⁷ self-interaction as a means of preventing amyloid formation, or have shown that chemical modification results in aggregation pathways that may not be Nt¹⁷-mediated and that bypass the potentially toxic oligomeric state.^{16–20} One hypothesis suggests that the polyproline (polyP) region of htt-exon1 adopts a PPII helix and acts as an intrinsic regulator for Nt¹⁷.²¹ Disease-related polyQ expansion decreases the Nt¹⁷/polyP interaction. One potential mechanism is that expanded polyQ develops β -turn strand structure resulting in a rigid linker that separates the two domains.²¹ Alternatively, the polyQ domain could act via an entropic chain mechanisms where the additional length due to expansion increases the time interval for Nt¹⁷ and polyP to approach each other. Such a mechanism would be similar to the “ball and chain” mechanisms associated with voltage gated ion channels.²² Either mechanism would increase the intramolecular distance between Nt¹⁷ and polyP, thus freeing Nt¹⁷ for disease-related interaction, such as association with other Nt¹⁷ tracts or interaction with phospholipid

surfaces.²¹ Additionally, the free polyP region may act synergistically with Nt¹⁷ to decrease solubility of the polyQ tract and thus, drive aggregation.^{23, 24} Similarly, recent studies show that an Nt¹⁷ peptide co-incubated with a htt-exon1 peptide mimic was sufficient to force aggregation via a pathway not mediated by Nt¹⁷.¹⁸ By extrapolation, it may be hypothesized that a polyP peptide could interact with Nt¹⁷ and perhaps modify aggregation, or at least promote a pathway that is not mediated by Nt¹⁷.

Although Nt¹⁷ is a strong promoter of both oligomer formation and amyloid formation,^{13, 14, 24} the location and extent of helicity in Nt¹⁷ can vary in different experimental systems. In x-ray crystal structures of htt-exon1, Nt¹⁷ is entirely helical with the α -helix extending into the polyQ region.²⁵ In other solid state NMR and simulation experiments, the helix is not present in the monomeric form, but rather forms a helix when in proximity with a binding partner, such as a second Nt¹⁷ sequence or a lipid bilayer.^{26–29} In other cases, Nt¹⁷ is tightly associated with the polyQ region in a monomeric form.²⁴ Of particular importance are structural changes associated with early stages of Nt¹⁷ oligomer formation. With regard to this, several multimeric species (dimers, trimers, and tetramers) of Nt¹⁷ peptides (no glutamines attached) were detected using native electrospray ion mobility spectrometry-mass spectrometry (IMS-MS).³⁰ importantly, three distinct Nt¹⁷ dimers were detected. Using molecular dynamic simulations (MDS) to compare with experimentally obtained collisional cross-sections, the structure of Nt¹⁷ dimers were comprised of partially helical monomers arranged in an antiparallel geometry. More recently, atomic resolution structural studies of the oligomerization of the N-terminal region of htt have been reported using relaxation-based NMR.³¹ These studies used residues 2–17 of the Nt¹⁷ domain with the addition of six glutamines. At least two distinct dimer species were observed, one on pathway toward fibril formation and the other off-pathway to fibril formation. In addition tetramers were detected. For the on-pathway dimers and tetramers, the N-terminal region adopted an α -helical structure ranging from residues 3–17 arranged in an antiparallel geometry. In general, it appears that Nt¹⁷ is mostly helical in htt aggregates;^{13, 28, 32} thus, it represents an intriguing structural target for the amelioration of htt aggregation. With this regard, chemical modification of Nt¹⁷ via labeling of lysine with diethylpyrocarbonate³¹ or oxidation of methionine³¹ destabilized dimer species.

Several studies have modified Nt¹⁷ through S to D mutation, which mimics phosphorylation, at S13 and S16.^{16, 33} These mutations slowed the progression of Nt¹⁷-mediated htt aggregation, but did not prevent aggregation altogether, providing a framework for targeting Nt¹⁷. Similarly, Nt¹⁷ attached to a pathogenic polyQ tract has been targeted with a simple Nt¹⁷ peptide¹⁸ or by elimination by enzymatic digestion.¹³ These studies showed a net decrease in aggregation, most likely due to Nt¹⁷-Nt¹⁷ interactions where the fragment is included in the nucleation of pathogenic peptides, increasing the distance between polyQ fragments and thus, increasing the energy barrier required for amyloid formation. A similar idea, then, is to target the Nt¹⁷ region using a polyP peptide. It is possible that the polyP peptide would function the same as the Nt¹⁷ peptide alone in inhibiting nucleated amyloid formation. The present experiments test this hypothesis by monitoring oligomer and fibril formation associated with htt-exon1 in the presence or absence of a polyP peptide. In short, the presence of the polyP peptide significantly prevents fibrilization. Due to this, additional experiments using hybrid ion mobility spectrometry-mass spectrometry (IMS-MS)

techniques coupled to gas-phase hydrogen-deuterium exchange (HDX) were performed to probe the interactions between Nt¹⁷ and polyP peptides.

Materials and Methods

Purification of htt-exon1 construct.

Disease-length (51Q) htt-exon1 was purified from *Escherichia coli* according to previously published protocols.^{34, 35} Briefly, pelleted *E. coli* colonies containing glutathione S-transferase (GST) fused htt-exon1 were thawed and resuspended in a pH 8 phosphate buffer containing EDTA and a protease inhibitor cocktail. The suspension was lysed using lysozyme and mechanical sonication. The resulting slurry was centrifuged at 3500 RPM to pellet cellular debris. The solution containing the protein of interest was decanted and diluted 1:1 into the same phosphate buffer. Proteins were purified using a Biorad Profinity 5 mL GST affinity column by FPLC (Biorad Prologic, Hercules, Ca). Fractions containing protein of interest were dialyzed against a Tris-NaCl solution overnight. GST was cleaved from htt-exon1 using Factor Xa (ThermoFisher Scientific, Waltham, MA). Overall protein concentration was determined by a Bradford assay. The final working concentration of htt-exon1 was adjusted to 20 μ M in a Tris-NaCl buffer. It is important to note that after cleavage of GST by factor Xa that an additional three residues remain at the N-terminus of htt-exon1(51Q). These residues are glycine, isoleucine, and arginine. Additional N-terminal residues on htt can influence the self-association of Nt¹⁷.³⁴

Thioflavin T assay.

Purified htt-exon1(51Q) was diluted to a final concentration of 10 μ M in the presence of 125 μ M Thioflavin T (Sigma-Aldrich, St. Louis, MO). Conditions were run with and without the Nt¹⁷ peptide or the polyP peptide at various molar ratios to htt. Reactions were run in a black Costar 96-well plate with clear flat bottom and the ThT fluorescence was monitored using a SpectraMax M2 microplate reader. Experiments were run at 37 °C with 440 nm excitation and 484 nm emission, with readings every 5 minutes for 12 hours.

Atomic Force Microscopy (AFM).

The purified GST protein and co-incubated peptides were maintained at 37°C in Eppendorf tubes and 1400 rpm for the duration of the experiment in an orbital mixer. At 24 h after cleavage of GST with factor Xa protease, a 5.0 μ L aliquot of each protein incubation was spotted onto a freshly cleaved mica substrate (Ted Pella Inc., Redding, CA), for 30 seconds, washed with 200 μ L of ultra-pure water to remove salt and dried gently with a stream of clean air. The mica was placed on metal pucks and stored in a covered petri dish until imaging.

AFM imaging Experiments were performed with a Nanoscope V Multi-Mode scanning probe microscope (Veeco, Santa Barbara, CA) equipped with a closed loop vertical engage J-scanner. All images were acquired with diving board shaped silicon-oxide cantilevers with a nominal spring constant of 40 N/m and resonance frequency of about ~300 kHz. Scan rates were set at 1–2 Hz with cantilever drive frequencies 10% below resonance. All images were analyzed using Matlab with the image processing toolbox (MathWorks, Natick, MA) as previously described.¹⁸

PDA lipid vesicle protein binding assay.

Total brain lipid extract (TBLE)/polydiacetylene (PDA) lipid binding assays were performed based on published protocols.^{36, 37} In short, diacetylene monomers 10,12-tricosadiynoic acid (GFS Chemicals, Columbus, OH) and TBLE were dissolved in a solution of 1:1 chloroform/ethanol. TBLE and PDA was always mixed in a 2:3 molar ratio. The solution was evaporated under a constant flow of nitrogen, leaving a thin, dry film. 1× Tris-buffered saline (TBS) heated to 70 °C was added to the film and sonicated for 5 min at 100 W using a sonic dismembrator (FisherSci). To enable self-assembly into vesicles, the resulting suspension was stored at 4 °C overnight. Polymerization of the diacetylene monomers was achieved by irradiation at 254 nm with 7 lumens for 10 minutes (room temperature with stirring), resulting in a brilliant blue color. As controls, each TBLE/PDA vesicle sample was exposed to 1× Buffer A (negative control) or NaOH (pH 12) (positive control). Polymerized TBLE/PDA vesicles were exposed to htt-exon1(51Q) at a final concentration of 20 μM. Experiments were performed in triplicate in a 96-well format, and the colorimetric response of each well was recorded over 12 h using a SpectraMax M2 microplate reader. Polymerized vesicles were exposed to htt-eson1(51Q) at a final concentration of 10 μM. The PB, defined by $A_{\text{blue}}/(A_{\text{blue}} + A_{\text{red}})$ will be calculated for the control (PB_0) and each sample condition (PB). The percent colorimetric response (% CR) indicates the extent of insertion or disruption of the lipid membrane by htt and was calculated using the following equation:

$$\% \text{ CR} = \left(\frac{PB_0 - PB}{PB_0} \right) \times 100 \quad (1)$$

IMS-MS Sample Preparation.

Nt¹⁷ (MATLEKLMKAFESLKSF) and polyP (Ac-P₁₀-NH₂) peptides were prepared according to established protocols.³⁴ All samples were reconstituted in 500 μL of 0.200 M ammonium acetate (Fisher Scientific) in 18 MΩ water that was purified using a Milli-Q purification system (Millipore). For binary samples, the first peptide was reconstituted, incubated, and vortexed. The resulting solution was then removed from the tube and used to reconstitute the second peptide film at a final concentration of ~5×10⁻⁴ M each.

Ion Mobility Spectrometry (IMS) – Mass Spectrometry (MS).

Over the last 2 decades, IMS-MS techniques have found extensive use in the study of peptide and protein aggregation phenomena.^{38–41} Here, IMS-MS was performed on a home-built hybrid instrument that consists of a stacked-ring drift tube coupled to a linear ion trap mass spectrometer (LTQ Velos Pro, Thermo Scientific, San Jose, CA). Design, operation, and figures of merit for the instrument have been described in detail elsewhere.^{42, 43} All samples were introduced at 0.5 μL/min. Ions were pulsed into the drift tube every 20 ms using a 150-μs-wide pulse. The time-delayed gate at the rear of the drift tube, was periodically lowered for 200 μs to allow the recording of mass spectra for mobility-selected ions. The delay between the front and rear gates is scanned to generate the multiple mass spectra necessary to produce the IMS-MS spectra. The pressure in the drift tube was maintained at approximately 2.5 Torr. The ion trap MS was set to scan every 400 μs, and

average every four microscans. Each mass spectrum in the drift tube experiment was collected for thirty seconds.

Gas-phase Hydrogen-Deuterium Exchange (HDX).

The history of biomolecular ion structure characterization by gas-phase HDX is similarly extensive.^{44–52} The development of non-ergodic ion dissociation techniques such as electron capture dissociation (ECD)⁵³ and electron transfer dissociation (ETD)⁵⁴ enabled HDX studies as demonstrated in seminal work showing deuterium location determination at the single amino acid level.⁵⁵ Although the first experiments coupling gas-phase HDX and IMS-MS measurements were performed nearly 20 years ago,^{56, 57} recently novel studies have combined these techniques with deuterium site localization using ETD.^{58, 59} For the current experiments, gaseous D₂O was introduced into the He buffer gas in order to determine the relative numbers of accessible sites in the various ion conformers. Liquid D₂O was measured into a sealed stainless steel tube connected with a Swagelok fitting, and flash frozen using liquid nitrogen. Using several freeze-thaw cycles, the headspace above the D₂O was pumped away, leaving only D₂O in the bulb. D₂O was allowed into the drift tube using a variable leak valve (MKS instruments, Andover, MA). The partial pressure of D₂O for all experiments was 0.01 Torr. The helium pressure was adjusted to compensate for the addition of D₂O, in order to maintain a total pressure of 2.50 Torr. This is a sufficient amount of the reagent to induce labeling of rapidly exchanging sites as determined previously.^{43, 60, 61}

Molecular Dynamics Simulations (MDS).

Simulated annealing with MDS was performed as described previously using the same molecular constraints.³⁰ Additionally, PolyP was constrained to an all trans conformation in CHARMM36 force field by limiting the ω angle to between 150° and 210°. An all-trans proline conformation was chosen because polyP tracts favor all-trans PPII helices in aqueous solvents,⁶² and the polyP tract in htt-exon1 forms a PPII-helix.^{63, 64} Simulated annealing was performed as described previously for two different starting structures with different charge configurations.³⁰ For the simulated annealing, the Nanoscale Molecular Dynamics (NAMD) software suite was employed and the two different ions were protonated at the K6, K9, and K15 amino acid residues and the K6 and K9 residues as well as the polyP N-terminus. Low-energy conformations within $\pm 1\%$ of the measured CCS were catalogued as candidate structures for exchange site accessibility determinations (see below).⁶⁰

HDX estimations using kinetics modeling.

Hydrogen accessibility scoring (HAS) was performed for each structure according to methods developed in house.^{60, 61} To calculate the HAS, each carbonyl oxygen within 7 Å of a charge site was scored (S_O) by scaling as the inverse distance to the charge site (d_{O-N}). This is termed a HDX-active carbonyl that represents the initial site of deuterium incorporation.⁶⁰ In the event that multiple charge sites were within the designated proximity of a carbonyl, the oxygen was scored as the sum of inverse distances. Next, each heteroatomic hydrogen within 7 Å of an (or multiple) active carbonyl was scored as:

$$S_H = \sum \frac{S_O}{d_{H-O}} \quad (2)$$

where S_H is the hydrogen score and d_{H-O} is the interatomic distance between the hydrogen and the HDX active carbonyl. D₂O accessibility was also assessed *in silico* by allowing a water molecule to sample the entire peptide's surface to obtain an excluded volume in order to provide a surface accessibility score (S_{SES}) for each site.

With S_H and S_{SES} , it was possible to produce theoretical, exchange site contributions to the experimentally observed HDX rate (k) for the ion conformers. Here, the rate contributions for each site were obtained from the S_H values as performed previously.⁶⁰ That is, each site's S_H value as a percentage of the total represented the respective percentage contribution to k . The new site-specific k values were then scaled by the relative (fractional) S_{SES} values. Finally, the HDX kinetics were simulated by stepping each ion through its respective drift time and computing the fraction of exchanges occurring at each timestep as calculated using Equation 3:

$$1 - \frac{A}{A_0} \quad (3)$$

where

$$\frac{A}{A_0} = e^{-kt_i} \quad (4)$$

In Equations 3 and 4, A/A_0 represents the fraction of sites that do not exchange during a given time increment (t_i). For each computation (each site at each t_i), the fraction altered was compared to a ratio of a random number to total possible random numbers generated. If the ratio was less than the fraction altered, then an exchange event was recorded for the exchange site. The process was repeated as the ion stepped through the time increments (t_i) up to a designated drift time allowing for the correct exchange levels to be achieved. In total, 10⁴ ions were used to represent each conformer type. The deuterium uptake levels from this algorithm were further used to filter the candidate ion structures obtained from MDS thereby requiring a collision cross section and HDX match.

Results and Discussion

Incubation with polyP reduces htt-exon1 fibril formation.

Inspired by recent work suggesting interaction between Nt¹⁷ and a flanking polyP sequence within htt²¹ as well as experiments that monitored htt aggregation in the presence of truncated Nt¹⁷ peptide,¹⁸ htt-exon1 aggregation was monitored in the presence and absence of a Nt¹⁷ peptide or a 10-residue polyP peptide with a ThT assay, which is commonly used to detect β -sheet formation associated with fibrils (Figure 1). Htt-exon1(51Q) at 10 μ M was incubated with either the Nt¹⁷ peptide or the polyP peptide at molar ratios of 1:1, 5:1, or 10:1 peptide to htt. Both the polyP peptide and the Nt¹⁷ peptide inhibited htt fibril formation in a dose dependent manner; although, the Nt¹⁷ peptide appeared to be slightly more

effective. While the ThT data suggests a reduction in fibril formation, there are several weaknesses associated with this assay. ThT fluorescence is typically associated with a common amyloid structure based on β -sheet formation.⁶⁵ However, ThT signal intensity varies based on the exact morphology of fibrils,⁶⁶ and a variety of non-amyloid structures and cavities can also invoke ThT fluorescence.⁶⁷ As a result, further experiments were performed to verify inhibition of fibrillization by Nt¹⁷ and polyP peptides and characterize any resulting aggregate morphologies that may have been promoted by the presence of these peptides.

To further evaluate the ability of the Nt¹⁷ peptide or the polyP peptide to inhibit htt aggregation, solutions of 10 μ M htt-exon1(51Q) alone and with either the Nt¹⁷ peptide or the polyP peptide at a 10:1 peptide to htt molar ratio were incubated at 37 °C and sampled at 1, 3, 5, 8, and 24 hours for AFM analysis (Figure 2A). To quantify the effect of the different peptides on htt aggregation, AFM images from all incubations were analyzed by counting the number of fibrils or oligomers per μ m² at each time point (Figure 2B–C). For this analysis, aggregates were defined as objects in the image taller than 2 nm. Fibrils were distinguished from oligomers by a length-to-width (aspect) ratio filter. Fibrils had an aspect ratio >3; oligomers had an aspect ratio < 3.³⁴

Htt-exon1(51Q) incubated without any small peptides formed a variety of oligomers and fibrils (Figure 2A). The number of oligomers per unit area fluctuated from 15–30 over 24 h. Fibrils first appeared in limited numbers after 1 h of incubation, but the population of fibrils steadily increased over 24 h. The length of fibrils also increased with time. When htt-exon1(51Q) was co-incubated with Nt¹⁷, the number of fibrils was significantly reduced compared with htt alone for the entire 24 h timeframe (approximately 5 fold fewer fibrils at 3 h and over 20 fold less at later time points). While fibrils were observed at the 3 h time point for htt incubated with Nt¹⁷, there was not an increase in their population at later time points. Previous experiments that reported co-incubation of htt-exon1 protein and Nt¹⁷ peptide showed similar morphologies to those reported in Figure 2 at the 24 hour time point. In their study, Mishra et al. report enhanced population of oligomers when htt-exon1 is co-incubated with Nt¹⁷ at shorter time points.¹⁸ Here, the number of oligomers increased between the 1 and 3 h time-points as the additional Nt¹⁷ peptides drive oligomerization. This initial increase in oligomers observed in the presence of Nt¹⁷ was followed by a sharp decline in the number of oligomers observed. The sample containing htt-exon1 co-incubated with polyP peptide did not form observable fibrils until the 8-hour time point, and the number of fibrils was significantly smaller than htt incubated alone (over 20 fold less). Therefore, for the protein samples co-incubated with polyP and Nt¹⁷ peptides, fibril formation was approximately 20 fold less than the htt-exon1 protein alone. In the presence of polyP, the number of htt oligomers was initially much smaller than the htt control, but the number of oligomers steadily increased with time. This increase resulted in a larger population of oligomers formed in the presence of polyP peptides compared to control after 24 h of incubation. This suggests that the addition of polyP initially blocks oligomer formation and has an amyloidogenic inhibitory effect in a model htt system.

Next, we compared the morphology of oligomers formed from htt alone or incubated with either Nt¹⁷ or polyP (Figure 3). For htt-exon1(51Q) incubated alone, the height distribution

of oligomers varied with time. At 1 and 3 h, the mode height of oligomers of htt alone was 2–3 nm, which increased to 7–8 nm at 5 and 8 h. At 24 h, the mode height was 5–7 nm. The distribution of oligomer height also spread with time for htt alone, which is consistent with the formation of a heterogeneous mixture of aggregate species. When co-incubated with Nt¹⁷, the initial distribution of oligomers was broad with a mode height of 3–6 nm. However, this distribution narrowed with time, and the mode height of oligomers decreased to 2–3 nm. When co-incubated with polyP, the initial mode height of oligomers was 3–5 nm. Unlike htt alone, oligomers formed in the presence of polyP did not appear to become larger with time, as the mode height remained below 5 nm for 24 h.

There appears to be a discrepancy between the extent of aggregation inhibition between the ThT assay and the AFM analysis. That is, the AFM data suggests a larger inhibition of fibril formation in comparison to the ThT assay. This discrepancy is likely due to several factors. ThT can bind not fibril-related β -sheet structure and cavities.⁶⁷ ThT also transiently interacts with some oligomers,^{68, 69} and this interactions enables the use of ThT for high resolution imaging of A β oligomers.⁷⁰ While ThT assays are commonly used to track fibril formation, the presence of non-fibrillar, ThT active species may enhance the signal. As such, it is plausible that the small oligomers observed by AFM are ThT active or even nascent fibrils that are trapped in a truncated state. It is also possible that fibrils forming in the presence of Nt¹⁷ or polyP peptides were not detected by AFM; however, this is unlikely for several reasons. The binding of proteins and aggregates to mica is based on electrostatic interactions. That is, polar and positively charged residues on proteins have strong attractive interactions with the negatively charged mica surface. As it is thermodynamically unfavorable to sequester polar and charged residues within an aggregate structure, there is likely sufficient charge on the periphery of any aggregate formed to stick to the mica surface. Such a notion is partially supported by the observation that distinct fibril polymorphs of A β all readily stick to mica despite their underlying difference in ultrastructure.⁷¹ Nevertheless, both assays agree that Nt¹⁷ and polyP peptides inhibit htt fibrillization.

While Nt¹⁷ and polyP can both inhibit fibril formation, they alter the oligomeric state of htt aggregation in different ways based on the number and size of observed oligomers. Nt¹⁷ peptide initially promotes oligomerization; however, these oligomers are incompetent with regard to nucleating fibrillization. As a result, a tighter population of oligomer species forms with time. Oligomer formation is inhibited by the presence of free polyP peptide. However, the number and heterogeneity of oligomers increases with time. While the distribution of oligomer sizes formed in the presence of polyP peptide was different than control, the heterogeneity of this distribution suggests that polyP peptides may interact with a variety of htt oligomers.

Another role of the Nt¹⁷ domain in htt is to bind lipid membranes,^{12, 26, 27} and lipid membranes can alter the way aggregation inhibitors interact with htt.⁷² To determine if the Nt¹⁷ peptide or the polyP peptide could interfere with the ability of htt to bind lipids, a polydiacetylene (PDA) lipid binding assay was performed to measure the total interaction between htt-exon1(51Q) and lipid membranes (Figure 4). Polydiacetylene is a lipid moiety that can be incorporated into vesicles and photo-crosslinked to create vesicles that are

sensitive to mechanical perturbations induced by interactions with peptides and proteins, resulting in a quantifiable colorimetric response (color shift from blue to red). We incorporated PDA into total brain lipid extract (TBLE) vesicles. First, the interaction of the Nt¹⁷ peptide and the polyP peptide with the TBLE/PDA vesicles was determined (Figure 4A). The Nt¹⁷ peptide, which is known to interact with lipid membranes, elicited a mild colorimetric response. The polyP peptide did not appreciably interact with the vesicles. Using these experiments as the backgrounds for the %CR calculation (based on Eq.1), the ability of the peptides to inhibit htt/lipid binding was assessed (Figure 4B). Compared to exposure to htt-exon1(51Q) alone, the addition of Nt¹⁷ peptide reduced the overall interaction of htt with the lipid vesicles. The polyP peptide had no effect.

Several interpretations of the observed polyP inhibitory effect on aggregation are possible (Figure 5). PolyP can interact directly with polyQ domains,^{63, 73} and this interaction could occur at a variety of aggregation stages. If polyP interacts with a htt monomer via the polyQ domain, this stabilize the polyQ domain by promoting it to adopt PPII character,^{63, 73} prohibiting nucleation (Figure 5A). Additionally, interactions between polyP and polyQ within higher order aggregates would sterically block the ability of polyQ to aggregate. In the oligomer, this could prevent nucleation (Figure 5B). In the fibril, this could prevent the attachment of additional htt monomers onto the fibril (Figure 5C). Previous studies have shown inhibition of aggregation by short polyQ fragments in a PPII conformation by N-methylated short peptide sequences.⁷⁴

Other scenarios for the ability of the polyP domain to inhibit aggregation could be associated with interaction of the peptide with the Nt¹⁷ domain of the htt-exon1 protein (Figure 5D–F). Nt¹⁷/polyP interactions within htt monomers would sterically block the formation of Nt¹⁷-mediated oligomer formation (Figure 5D). In addition, the interstitial insertion of polyP into the N-terminal htt-exon1 helical bundles associated with oligomers could act in the same manner as Nt¹⁷ peptides that inhibit aggregation.¹⁸ That is, by increasing distance between polyQ chains and thus, increasing the energy required to form β -sheet rich aggregates that lead to fibrillization (Figure 5E). However, different sized oligomers were stabilized by the addition of free Nt¹⁷ peptide compared to the addition of free polyP peptide, suggesting that the stabilized intermediates are different. In addition, this likely would have resulted in a decreased interaction of htt with lipid vesicles upon the addition of polyP peptide, which was not observed. Alternatively, the polyP region could partially shut down Nt¹⁷ helix formation in disease-length htt-exon1 constructs, thus decreasing kinetics of fibril formation, though still proceeding through an oligomeric intermediate. For the htt-exon1 protein here, although the polyQ-associated polyP region would no longer be in proximity of Nt¹⁷ for regulation, free polyP peptide could insert and act to regulate Nt¹⁷ in the same manner. This alternative strategy, depending on the structural mechanism and affinity of such an interaction, would not necessarily block the interaction of htt with lipid membranes. PolyP could also interact with Nt¹⁷ within putative fibril structures, blocking htt addition for fibril elongation (Figure 5F). This appears unlikely as the morphologies of the oligomers observed by AFM did not have similar thickness to htt fibrils. In any case, incubation of htt-exon1 with a polyP peptide reduced fibril formation. Because of the evidence for interaction between the Nt¹⁷ region and the polyP sequence in htt-exon1,²¹ IMS-MS experiments have been devised to consider potential interaction sites.

Nt¹⁷ and polyP form two stable heterodimeric complex conformers.

Nt¹⁷ and polyP were incubated together in an ammonium acetate buffer at a pH ~7.0. Direct electrospray of the mixture produced a mass spectrum that was dominated by the $[M+2H]^{2+}$ Nt¹⁷ monomer ion, as well as the polyP monomer ion of the same charge; however, several dimeric species were also present, including but not limited to, the Nt¹⁷ homodimer ($[2M+3H]^{3+}$) and a Nt¹⁷-polyP heterodimer ($[MN+3H]^{3+}$). Other multimers, such as the Nt¹⁷ homotrimer ($[3M+4H]^{4+}$) and the homotetramer ($[4M+5H]^{5+}$) were observed, but did not decrease in abundance in the presence of the polyP peptide. There was no evidence of higher charge heteromultimers (e.g., Nt¹⁷-polyP $[MN+4H]^{4+}$) forming. Additionally, polyP homomultimers were not detected, which does not support the previously reported mechanisms of inhibition of polyQ aggregation by cis-polyP-mediated oligomerization.⁷³ All collision cross sections for the various ion conformers relevant to this study can be found in Table 1.

Expanded regions of a representative IMS-MS distribution of a co-incubated Nt¹⁷-polyP sample demonstrate the presence of heterodimers, Nt¹⁷ monomers, and polyP monomers (Figure 6). The Nt¹⁷-polyP heterodimer possesses structural heterogeneity (Figure 6A). Two distinct multimer structures resolved in the gas phase [mass-to-charge (m/z) 1002.4] are evident in the distribution. It is possible that these complexes could arise from association of non-helical (more compact) and more helical (more elongated) Nt¹⁷ with polyP,³⁰ or possibly polyP association with the Nt¹⁷ helix via two separate faces. The more compact conformer is roughly 20% more abundant than its more elongated counterpart. It is important to note that gentle ion source and ion extraction conditions have been employed to ensure that conformers are not interconverting or that scrambling of the isotopic label in the HDX studies is occurring (see below).^{43, 60}

The first conformer has a peak centered at 9.0 ms on the drift time axis, with a peak width of 0.2 ms (FWHM; Figure 7A). This arrival time corresponds to a collision cross section of $539 \pm 1 \text{ \AA}^2$. Though distinct, the peak corresponding to the compact conformer is not fully resolved from the more elongated conformer as shown in the drift time distribution (Figure 7A). It is difficult to determine if multiple structures are present in the t_D range of the two conformers due to significant overlap; however, later deuterium exchange experiments suggest that these are the dominant conformer types of this species (see below). The second, more elongated conformation has an arrival time centered at 9.4 ms, with a calculated cross section of $565 \pm 1 \text{ \AA}^2$. Each conformation was mobility selected in the drift tube and then mass selected in the mass analyzer before being subjected to increasing normalized collision energy. In this manner, it was determined that the two conformations do not differ significantly in complex strength (Figure 7B); each conformer required a normalized collision energy (NCE) of ~15 for 50% signal decay.

Several conformations of monomeric Nt¹⁷ and polyP are apparent from the IMS-MS distributions in which the heterodimeric conformers are observed (Figure 6B–C). MDS suggests that the two most abundant Nt¹⁷ conformations can be represented by a semi-helical random structure (10.0 ms) and an elongated, more helical structure (11.2 ms).³⁰ These conformations are in good agreement with previous reports that suggest a significant degree of structural heterogeneity in solution. Higher mobility features (8.0 ms and 9.4 ms) may result from dissociation of separate or higher-order multimers after exiting the drift tube

as described previously.³⁰ For example, the conformer arriving at 9.4 ms may be due to limited dissociation of the Nt¹⁷-PolyP heterodimer complex at the same drift time. However, the similarity in ion complex stabilities (Figure 7) suggests this is not the case although it cannot be ruled out entirely. Analysis of the isotopic envelope indicated that no multimers with number of monomer units/charge (n/z) = 1/2 (e.g., $[2M+4H]^{4+}$, which would have the same m/z as $[M+2H]^{2+}$) were present in the sample. The more elongated conformer is roughly one-tenth the abundance of the more compact structure, suggesting that the solution structure giving rise to the more compact structure is the dominant motif. The same argument can be applied to the polyP peptide. Figure 6C shows an expanded drift time and m/z region for the polyP $[M+2H]^{2+}$ peptide ions. Both observed conformers are on the same cross section order as other gas-phase polyP peptides of similar length, which were identified as mostly PPII helices with one or two residues exhibiting residual *cis* PPI structure.⁶² The first, most compact, conformation has a drift time of 6.2 ms. The second, more elongated conformer, has a drift time of 7.0 ms, which corresponds to a gas phase cross section of $276 \pm 3 \text{ \AA}^2$. As with the Nt¹⁷ monomer, no evidence of any isobaric homomultimeric species was observed.

Nt¹⁷ homodimer dissociation (Figure 7C) occurs at approximately 20 NCE (50% signal decay). Interestingly, the more elongated Nt¹⁷ homodimer conformer (filled diamonds, Figure 7C) forms a slightly stronger complex than the more compact conformer (filled squares, Figure 7C). In general, however, both Nt¹⁷-polyP heterodimers require less dissociation energy (Figure 7B) compared with the Nt¹⁷ homodimers. Although CID experiments do not reveal the location of interacting peptide regions, the relative association strengths could have implications for the normal function of htt. If the polyP region does regulate the function of Nt¹⁷ as suggested by Truant and co-workers,²¹ then it may be expected to form a relatively weak interaction such that Nt¹⁷ can undergo its normal function. Additionally, the relative strength of the homodimeric Nt¹⁷ complex as well as the presence of the homodimer may suggest a greater self-affinity which is consistent with its potential role in aggregation.^{13, 14, 23, 75}

Heterodimer conformations vary in deuterium uptake.

Following the evidence of two heterodimeric species, gas-phase HDX was performed to determine differences in deuterium uptake by each conformer. Deuterium uptake may help determine sites of binding and provide insight into the location of exposed exchange sites in the proximity of charge sites. Figure 8A–C shows expanded drift time and m/z dataset regions encompassing the Nt¹⁷-polyP heterodimer, the Nt¹⁷ monomer, and the polyP monomer ions. Each of these species are the same as in Figure 6A–C. Overall, very little deviation in drift time was observed for all species; a 0.2 ms shift to higher drift times is observed, which is expected when using a heterogeneous drift gas mixture containing one species of larger mass and different polarizability.^{43, 56}

An interesting result is the differential deuterium uptake exhibited by the two Nt¹⁷-polyP heterodimer conformations (Figure 8A). Notably this difference in m/z for the two ions is further evidence that the ion conformers are not interconverting. That is, the different m/z ranges upon deuterium uptake provide clear evidence of unique reactivities due to different

physical properties. This has been thoroughly demonstrated over the last 30 years by numerous MS and IMS-MS experiments for biomolecular ions.^{44, 57, 58, 60, 61, 76–78} The more compact conformation (conformer 'a' from Figure 7A) shifted upward in m/z by 1.6. From multiple measurements, an average uptake of $\sim 4.0 \pm 2.0$ deuteriums (see Figure 9 for total deuterium uptake for the various ions) is observed for this conformer. Conversely, conformer 'b' incorporated an average of $\sim 10.8 \pm 0.5$ deuteriums, over twice the uptake of conformer 'a'. Both Nt¹⁷ monomer conformations exhibited the same uptake of ~ 13 deuteriums, which is higher than that of the complex, suggesting that several hydrophilic residues may be involved in intermolecular interactions. Finally, the polyP peptide ion only exhibited an average uptake of ~ 2 deuteriums. Because polyP does not contain exchangeable backbone amide hydrogens, these two deuteriums found in the polyP tract must result from charge site deuteration;^{45, 57} once the deuterium is transferred to the charge site, it cannot be redistributed as it is in a peptide ion containing no other labile heteroatom sites.

The disparate uptake data for the two [MN+3H]³⁺ ion conformers may indicate that polyP can associate with multiple faces of Nt¹⁷, perhaps via similar interactions; however, one site yields a gas-phase conformer with increased access to labile heteroatoms. Conversely, polyP could be associated in roughly the same location in both structures, but Nt¹⁷ could sample several conformations. Notably S13 and S16 have been implicated in Nt¹⁷-polyP interactions.²¹ Both S13E/S16E mutations and Nt¹⁷ hyperphosphorylation (M8P) by IKK kinase inhibition resulted in reduced Nt¹⁷-polyP interaction for pathogenic and non-pathogenic htt-exon1.²¹ When considered with the HDX data reported here, it may be that the more compact conformation, which has less deuterium incorporation than its more elongated counterpart, could include S13/16-polyP type interactions.

The relatively low level of deuterium incorporation into the higher-mobility [MN+3H]³⁺ heterodimer ions provides further insights. Because HDX is expected to occur near charge sites,^{45, 79} and considering the peptide sequence (MATLEKLMKAFESLKSF), it can be argued that for these ions, significant protection is observed for the lysine residues. That is, K6, K9, and K15 contribute 6 exchangeable side-chain hydrogens and 3 exchangeable backbone hydrogens. Several more might be expected due to the likelihood of these residues to serve as charge sites. With an exchange of only ~ 4 deuteriums (expected to occur near the charge sites), more than half of the exchangeable sites at lysine residues appear to be protected. This raises the question as to whether or not such residues interact with the polyP peptide. HDX suppression of these residues by interaction with polyP would be consistent with acetylation studies showing their importance in protein aggregation.^{20, 30} Indeed, together with studies implicating S13 and S16 in polyP interactions,^{21, 33} these HDX results may indicate an important regional interaction (i.e., the mid to C-terminal portion of Nt¹⁷). That said, larger deuterium uptake by the lower-mobility conformer indicates greater access to these residues. To consider heterodimer structures that could account for the observed HDX levels, MDS experiments were performed.

Gas-phase Nt¹⁷-polyP heterodimer structure.

Figure 10 shows low-energy conformations from MDS that are within $\pm 1\%$ of the measured CCS (Table 1). For structural comparisons, two different charge configurations were

examined. The first (Figure 10A) consisted of protonation of all K residues while the second simulation (Figure 10B) utilized protonation sites of K6 and K9 on Nt¹⁷ as well as the amino terminus on polyP. For the two separate annealing experiments, the polyP constituents in the compact conformers associate with Nt¹⁷ mostly by the C-terminal half (>K6) of the peptide. Although there is some small degree of helicity from residues M1 to K6, the extended helix that was prevalent in the modeled homodimeric complex, as well as monomeric Nt¹⁷ was not evident.³⁰ For the ion exhibiting the K6/K9/K15 protonation configuration, essentially all hydrophilic residues are buried in the interaction with the polyP tract. The inaccessibility of these residues could explain the lack of deuterium uptake in conformer 'a' (Figures 8 and 9). Because the heteroatom hydrogens are less accessible to collisions with D₂O (Figure 10A), HDX cannot proceed efficiently. For the other compact ion, these hydrogens appear to also be less accessible to the complex surface although this appears to occur primarily by being buried within Nt¹⁷ itself (Figure 10B).

Figure 10 also shows the more elongated conformer types for the different charge configurations. The larger ion conformers effectively spread apart the heteroatom hydrogens in the C-terminal portion of Nt¹⁷. This results in increased surface accessibility of S13 and S16 for the ion conformer protonated at K6, K9, and K15; for the ion conformer protonated at K6 and K9, greater accessibility to K6 hydrogens is observed. Increased access to surface collision events could explain the increased deuterium uptake for these more elongated ion conformers.

To further evaluate the MDS structures shown in Figure 10, HAS and surface accessibility have been incorporated into HDX kinetics modeling (see Materials and Methods section above) and deuterium uptake has been calculated for each candidate structure. The most compact candidate structures are calculated to exchange on average ~4.6 and ~4.4 deuteriums for the ions having all K residues protonated and those with protonated K6 and K9 residues as well as the polyP amino terminus, respectively. For the respective, more elongated candidate structures, values of ~8.9 and ~9.2 deuteriums are obtained. These calculated values are in good agreement with the experimental deuterium uptake data (Figure 9) making these plausible gas-phase conformer types. It must be stressed here that these are gas-phase structures and their resemblance to solution structures is unknown. However, for both compact ion conformations, the significant, wholesale migration of the polyP peptide to the C-terminal portion of the Nt¹⁷ peptide upon electrospray can be argued to be a less likely event than the preservation to some degree of interactions in this region by native MS.⁴⁰ Extreme conformation transformation, however, cannot be ruled out entirely. Therefore, the structures presented in Figure 10 present a framework from which to begin considering the effects of polyP binding which are discussed below; further work is required to refine peptide interaction characterization. In summary, the MD results suggest that: 1) the most plausible structures that would protect a significant number of hydrogens from exchange are species involving C-terminal interaction between Nt¹⁷ and polyP; 2) the formation of the more elongated ions results in a more diffuse configuration of heteroatom sites some of which become more accessible to surface collisions with D₂O; and, 3) this C-terminal interaction could be responsible for a lack of helicity in the solution phase that extends to a more globular gas-phase structure. Previous IMS-MS experiments have shown

that helical peptides in solution can give rise to gas-phase ions of considerable helicity while intrinsically disordered peptides produce globular gas-phase structures.⁸⁰

For the structures presented in Figure 10, the lack of the extended helical conformation in Nt¹⁷ stands in contrast with the matching low-energy MDS structures for several homodimer Nt¹⁷ ions that exhibit higher degrees of helicity,³⁰ as well as oligomeric structures studied by NMR that were virtually entirely α -helical.³¹ In solution, the Nt¹⁷ tract, both in the context of polyQ and without, adopts a helical conformation when in proximity of a binding partner: either another Nt¹⁷ tract^{13, 28} or a lipid bilayer.^{26, 81, 82} Additionally, the helical association is the driving force for oligomerization in htt-exon1.^{14, 18} Therefore, the association of polyP with the C-terminal half of Nt¹⁷ and the lack of extended heterodimer structures observed here could have implications in intrinsic polyP regulation of Nt¹⁷ in solution.²¹ For example, the polyP region may regulate Nt¹⁷ by associating with the C-terminal portion of the tract, preventing α -helix formation associated with oligomerization. This would explain the inherent lack of helicity in Nt¹⁷-Q_n-P_m and htt-exon1 monomeric structures.¹⁴ This could also explain the acceleration of aggregation observed when Nt¹⁷ is attached to extended polyQ regions without the proline-rich domain,^{23, 24} as the proline domain is absent and unable to regulate Nt¹⁷.

Interpretation of structural studies.

This study reports, for the first time, multiple conformations of a Nt¹⁷-polyP complex that could have implications in Huntington's disease-related amyloid formation. Previous work suggests that the C-terminal polyP region acts as a stabilizer of Nt¹⁷ for short polyQ lengths; however, as polyQ length increases the distance between Nt¹⁷ and polyP is increased, breaking down this regulation.²¹ The increased distance allows Nt¹⁷ to participate in intermolecular associations with other Nt¹⁷ tracts, or perhaps the associated polyQ, to initiate fibril formation. This is supported by the data in Figure 7, where increased interaction strength for the Nt¹⁷ homodimer relative to the Nt¹⁷-polyP heterodimer is observed; the increased complex strength can support the idea that Nt¹⁷ has a high self-affinity, and thus can act to nucleate aggregation. Additionally, no decrease in Nt¹⁷ homomultimer is observed upon addition of the polyP peptide, which, again may indicate the greater propensity of the Nt¹⁷ helix-helix interaction compared with the Nt¹⁷-polyP association.

The gas-phase structures reported in Figure 10 provide a potential structural context for Nt¹⁷-polyP complexes. A variety of studies suggest that Nt¹⁷ monomers are intrinsically disordered²⁶ while Nt¹⁷ multimers (dimers to tetramers) are comprised of stacked helical arrangements.^{13, 30, 31} In the Nt¹⁷/polyP heterodimers (Figure 10), however, Nt¹⁷ adopts a mostly random coil, compact motif. The differential deuterium uptake by conformation is then explained primarily by the burying of hydrophilic residues involved in stabilizing the complex. Based on these data, it could also be hypothesized that C-terminal Nt¹⁷ complexation with the polyP helix stabilizes (or induces) the random coil nature of Nt¹⁷, preventing a transition to the extended α -helix, as observed in early oligomers¹³ and amyloid fibrils.^{28, 32} A caveat to this work is the lack of a polyQ sequence. The polyQ linker would restrict the freedom of the polyP and Nt¹⁷ domains and may impart structure into

these regions; however, solid-state NMR results indicate that in a mature fibril, the polyP PPII helix abruptly stops immediately before the polyQ domain and the β -sheet character of the polyQ region only extends to the last two residues of Nt¹⁷.³² It is well-established that the addition of a polyP domain on the C-terminal side of aggregating polyQ peptides suppress the nucleation of fibril formation.^{63, 64, 83, 84} This effect has been attributed to PPII structure of the polyP region induces PPII-like structure into the polyQ domain⁶³ and that the rigidity of the PPII structured polyP domain can limit the conformational freedom of the adjacent polyQ domain. The ability of polyP to directly interact with Nt¹⁷ and influence polyQ structure may work in concert to reduce htt aggregation.

As described above, incubation of disease-length htt-exon1 with the polyP tract led to a reduction in fibril formation relative to control htt-exon1, which suggests that polyP has an inhibitory effect on htt-exon1. Together, the data described in this work suggest that the polyP region may inhibit formation of the amphipathic α -helix by Nt¹⁷, thus reducing the ability to participate in energetically favorable helix-helix interactions. A separate interpretation is the sequestering of critical residues required for helix-helix interactions. Although not conclusive, this would support the idea of suppression of aggregation by the mechanism shown in Figure 5D and Figure 5E. Overall, these experiments lay the groundwork for future studies seeking not just blockage of a critical residue, but perhaps blockage of a critical secondary structure as a therapeutic strategy.

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Abbreviations:

HD	Huntington's disease
htt-exon1	exon 1 region of Huntingtin protein
AFM	atomic force microscopy
IMS-MS	Ion mobility-mass spectrometry
Nt¹⁷	N-terminal 17-residue amphipathic α -helix of the huntingtin protein
polyP	10-residue polyProline peptide
polyQ	polyGlutamine tract
MDS	Molecular dynamics simulations
HDX	hydrogen deuterium exchange
HAS	hydrogen accessibility scoring

S_H	site-specific hydrogen accessibility score
S_{SES}	site specific solvent excluded surface area score

References

1. The Huntington's Disease Collaborative Research Group. (1993) A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes Cell 72, 971–983. [PubMed: 8458085]
2. Davies S, Turmaine M, Cozens B, DiFiglia M, Sharp A, Ross C, Scherzinger E, Wanker E, Mangiarini L, and Bates G (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation, Cell 90, 537–548. [PubMed: 9267033]
3. Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, and Li XJ (1999) Nuclear and neuropil aggregates in Huntington's disease: Relationship to neuropathology, J. Neurosci 19, 2522–2534. [PubMed: 10087066]
4. Wagner AS, Politi AZ, Ast A, Bravo-Rodriguez K, Baum K, Buntru A, Stempel NU, Brusendorf L, Hänig C, Boeddrich A, Plassmann S, Klockmeier K, Ramirez-Angueta JM, Sanchez-Garcia E, Wolf J, and Wanker EE (2018) Self-assembly of Mutant Huntingtin Exon-1 Fragments into Large Complex Fibrillar Structures Involves Nucleated Branching, J. Mol. Biol 430, 1725–1744. [PubMed: 29601786]
5. Sahoo B, Arduini I, Drombosky KW, Kodali R, Sanders LH, Greenamyre JT, and Wetzel R (2016) Folding Landscape of Mutant Huntingtin Exon1: Diffusible Multimers, Oligomers and Fibrils, and No Detectable Monomer, PLoS One 11, e0155747. [PubMed: 27271685]
6. Legleiter J, Mitchell E, Lotz GP, Sapp E, Ng C, DiFiglia M, Thompson LM, and Muchowski PJ (2010) Mutant Huntingtin Fragments Form Oligomers in a Polyglutamine Length-dependent Manner in Vitro and in Vivo, J. Biol. Chem 285, 14777–14790. [PubMed: 20220138]
7. Adegboyiro A, Sedighi F, Pilkington AW, Groover S, and Legleiter J (2017) Proteins Containing Expanded Polyglutamine Tracts and Neurodegenerative Disease, Biochemistry 56, 1199–1217. [PubMed: 28170216]
8. Landrum E, and Wetzel R (2014) Biophysical Underpinnings of the Repeat Length Dependence of Polyglutamine Amyloid Formation, J. Biol. Chem 289, 10254–10260. [PubMed: 24596088]
9. Sahl SJ, Weiss LE, Duim WC, Frydman J, and Moerner WE (2012) Cellular inclusion bodies of mutant huntingtin exon 1 obscure small fibrillar aggregate species, Sci. Rep 2, 895. [PubMed: 23193437]
10. Sahl SJ, Lau L, Vonk WI, Weiss LE, Frydman J, and Moerner WE (2016) Delayed emergence of subdiffraction-sized mutant huntingtin fibrils following inclusion body formation, Q. Rev. Biophys 49.
11. Masnata M, Sciacca G, Maxan A, Bousset L, Denis HL, Lauruol F, David L, Saint-Pierre M, Kordower JH, Melki R, Alpaugh M, and Cicchetti F (2019) Demonstration of prion-like properties of mutant huntingtin fibrils in both in vitro and in vivo paradigms, Acta Neuropathologica 137, 981–1001. [PubMed: 30788585]
12. Burke KA, Kauffman KJ, Umbaugh CS, Frey SL, and Legleiter J (2013) The Interaction of Polyglutamine Peptides With Lipid Membranes is Regulated by Flanking Sequences Associated with Huntingtin, J. Biol. Chem 288:14993–15005. [PubMed: 23572526]
13. Jayaraman M, Kodali R, Sahoo B, Thakur AK, Mayasundari A, Mishra R, Peterson CB, and Wetzel R (2012) Slow Amyloid Nucleation via alpha-Helix-Rich Oligomeric Intermediates in Short Polyglutamine-Containing Huntingtin Fragments, J. Mol. Biol 415, 881–899. [PubMed: 22178474]
14. Jayaraman M, Mishra R, Kodali R, Thakur AK, Koharudin LMI, Gronenborn AM, and Wetzel R (2012) Kinetically Competing Huntingtin Aggregation Pathways Control Amyloid Polymorphism and Properties, Biochemistry 51, 2706–2716. [PubMed: 22432740]
15. DeGuire SM, Ruggeri FS, Fares M-B, Chiki A, Cendrowska U, Dietler G, and Lashuel HA (2018) N-terminal Huntingtin (Htt) phosphorylation is a molecular switch regulating Htt aggregation,

- helical conformation, internalization, and nuclear targeting, *J. Biol. Chem* 293, 18540–18558. [PubMed: 30185623]
16. Mishra R, Hoop CL, Kodali R, Sahoo B, van der Wel PCA, and Wetzel R (2012) Serine Phosphorylation Suppresses Huntingtin Amyloid Accumulation by Altering Protein Aggregation Properties, *J. Mol. Biol* 424, 1–14. [PubMed: 22999956]
 17. O'Rourke JG, Gareau JR, Ochaba J, Song W, Rasko T, Reverter D, Lee J, Monteys AM, Pallos J, Mee L, Vashishtha M, Apostol BL, Nicholson TP, Illes K, Zhu YZ, Dasso M, Bates GP, Difiglia M, Davidson B, Wanker EE, Marsh JL, Lima CD, Steffan JS, and Thompson LM (2013) SUMO-2 and PIAS1 Modulate Insoluble Mutant Huntingtin Protein Accumulation, *Cell Rep* 4, 362–375. [PubMed: 23871671]
 18. Mishra R, Jayaraman M, Roland BP, Landrum E, Fullam T, Kodali R, Thakur AK, Arduini I, and Wetzel R (2012) Inhibiting the Nucleation of Amyloid Structure in a Huntingtin Fragment by Targeting alpha-Helix-Rich Oligomeric Intermediates, *J. Mol. Biol* 415, 900–917. [PubMed: 22178478]
 19. Aiken CT, Steffan JS, Guerrero CM, Khashwji H, Lukacsovich T, Simmons D, Purcell JM, Menhaji K, Zhu YZ, Green K, LaFerla F, Huang L, Thompson LM, and Marsh JL (2009) Phosphorylation of Threonine 3 IMPLICATIONS FOR HUNTINGTIN AGGREGATION AND NEUROTOXICITY, *J. Biol. Chem* 284, 29427–29436. [PubMed: 19710014]
 20. Chaibva M, Jawahery S, Pilkington A. W. t., Arndt JR, Sarver O, Valentine S, Matysiak S, and Legleiter J (2016) Acetylation within the First 17 Residues of Huntingtin Exon 1 Alters Aggregation and Lipid Binding, *Biophys. J* 111, 349–362. [PubMed: 27463137]
 21. Caron NS, Desmond CR, Xia J, and Truant R (2013) Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin, *P. Natl. Acad. Sci. USA* 110, 14610–14615.
 22. Zandany N, Lewin L, Nirenberg V, Orr I, and Yifrach O (2015) Entropic clocks in the service of electrical signaling: 'Ball and chain' mechanisms for ion channel inactivation and clustering, *FEBS Lett.* 589, 2441–2447. [PubMed: 26112606]
 23. Crick SL, Ruff KM, Garai K, Frieden C, and Pappu RV (2013) Unmasking the roles of N- and C-terminal flanking sequences from exon 1 of huntingtin as modulators of polyglutamine aggregation, *P. Natl. Acad. Sci. USA* 110, 20075–20080.
 24. Williamson TE, Vitalis A, Crick SL, and Pappu RV (2010) Modulation of Polyglutamine Conformations and Dimer Formation by the N-Terminus of Huntingtin, *J. Mol. Biol* 396, 1295–1309. [PubMed: 20026071]
 25. Kim MW, Chelliah Y, Kim SW, Otwinowski Z, and Bezprozvanny I (2009) Secondary structure of Huntingtin amino-terminal region, *Structure (London, England : 1993)* 17, 1205–1212.
 26. Michalek M, Salnikov ES, and Bechinger B (2013) Structure and Topology of the Huntingtin 1 17 Membrane Anchor by a Combined Solution and Solid-State NMR Approach, *Biophys. J* 105, 699–710. [PubMed: 23931318]
 27. Michalek M, Salnikov ES, Werten S, and Bechinger B (2013) Membrane Interactions of the Amphipathic Amino Terminus of Huntingtin, *Biochemistry* 52, 847–858. [PubMed: 23305455]
 28. Sivanandam VN, Jayaraman M, Hoop CL, Kodali R, Wetzel R, and van der Wel PCA (2011) The Aggregation-Enhancing Huntingtin N-Terminus Is Helical in Amyloid Fibrils, *J. Am. Chem. Soc* 133, 4558–4566. [PubMed: 21381744]
 29. Ceccon A, Schmidt T, Tugarinov V, Kotler SA, Schwieters CD, and Clore GM (2018) Interaction of Huntingtin Exon-1 Peptides with Lipid-Based Micellar Nanoparticles Probed by Solution NMR and Q-Band Pulsed EPR, *J. Am. Chem. Soc* 140, 6199–6202. [PubMed: 29727175]
 30. Arndt JR, Kondalaji SG, Maurer MM, Parker A, Legleiter J, and Valentine SJ (2015) Huntingtin N-Terminal Monomeric and Multimeric Structures Destabilized by Covalent Modification of Heteroatomic Residues, *Biochemistry* 54, 4285–4296. [PubMed: 26098795]
 31. Kotler SA, Tugarinov V, Schmidt T, Ceccon A, Libich DS, Ghirlando R, Schwieters CD, and Clore GM (2019) Probing initial transient oligomerization events facilitating Huntingtin fibril nucleation at atomic resolution by relaxation-based NMR, *P. Natl. Acad. Sci. USA* 116, 3562.

32. Hoop CL, Lin H-K, Kar K, Hou Z, Poirier MA, Wetzel R, and van der Wel PCA (2014) Polyglutamine amyloid core boundaries and flanking domain dynamics in huntingtin fragment fibrils determined by solid-state NMR, *Biochemistry* 53:6653–66. [PubMed: 25280367]
33. Gu XF, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, Steffan JS, Thompson LM, Wetzel R, and Yang XW (2009) Serines 13 and 16 Are Critical Determinants of Full-Length Human Mutant Huntingtin Induced Disease Pathogenesis in HD Mice, *Neuron* 64, 828–840. [PubMed: 20064390]
34. Burke KA, Godbey J, and Legleiter J (2011) Assessing mutant huntingtin fragment and polyglutamine aggregation by atomic force microscopy, *Methods* 53, 275–284. [PubMed: 21187152]
35. Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, and Hartl FU (2000) Hsp70 and Hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils, *P. Natl. Acad. Sci. USA* 97, 7841–7846.
36. Sokolovski M, Sheynis T, Kolusheva S, and Jelinek R (2008) Membrane interactions and lipid binding of casein oligomers and early aggregates, *BBA - Biomembranes* 1778, 2341–2349. [PubMed: 18675247]
37. Zheng F, Wu Z, and Chen Y (2012) A quantitative method for the measurement of membrane affinity by polydiacetylene-based colorimetric assay, *Anal. Biochem* 420, 171–176. [PubMed: 22019766]
38. Counterman AE, Hilderbrand AE, Barnes CAS, and Clemmer DE (2001) Formation of peptide aggregates during ESI: Size, charge, composition, and contributions to noise, *J. Am. Soc. Mass Spectr* 12, 1020–1035.
39. Counterman AE, Valentine SJ, Srebalus CA, Henderson SC, Hoaglund CS, and Clemmer DE (1998) High-order structure and dissociation of gaseous peptide aggregates that are hidden in mass spectra, *J. Am. Soc. Mass Spectr* 9, 743–759.
40. Bernstein SL, Dupuis NF, Lazo ND, Wyttenbach T, Condron MM, Bitan G, Teplow DB, Shea JE, Ruotolo BT, Robinson CV, and Bowers MT (2009) Amyloid-beta protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease, *Nat. Chem* 1, 326–331. [PubMed: 20703363]
41. Bleiholder C, Do TD, Wu C, Economou NJ, Bernstein SS, Buratto SK, Shea JE, and Bowers MT (2013) Ion mobility spectrometry reveals the mechanism of amyloid formation of Aβ(25–35) and its modulation by inhibitors at the molecular level: epigallocatechin gallate and scyllo-inositol, *J. Am. Chem. Soc* 135, 16926–16937. [PubMed: 24131107]
42. Donohoe GC, Maleki H, Arndt JR, Khakinejad M, Yi J, McBride C, Nurkiewicz TR, and Valentine SJ (2014) A New Ion Mobility–Linear Ion Trap Instrument for Complex Mixture Analysis, *Anal. Chem* 86, 8121–8128. [PubMed: 25068446]
43. Khakinejad M, Kondalaji SG, Maleki H, Arndt JR, Donohoe GC, and Valentine SJ (2014) Combining ion mobility spectrometry with hydrogen-deuterium exchange and top-down MS for peptide ion structure analysis, *J. Am. Soc. Mass Spectr* 25, 2103–2115.
44. Suckau D, Shi Y, Beu SC, Senko MW, Quinn JP, Wampler FM, and McLafferty FW (1993) Coexisting stable conformations of gaseous protein ions, *P. Natl. Acad. Sci. USA* 90, 790–793.
45. Campbell S, Rodgers MT, Marzluff EM, and Beauchamp JL (1995) Deuterium exchange reactions as a probe of biomolecule structure. Fundamental studies of gas phase H/D exchange reactions of protonated glycine oligomers with D₂O, CD₃OD, CD₃CO₂D, and ND₃, *J. Am. Chem. Soc* 117, 12840–12854.
46. Evans SE, Lueck N, and Marzluff EM (2003) Gas phase hydrogen/deuterium exchange of proteins in an ion trap mass spectrometer, *Int. J. Mass Spectrom* 222, 175–187.
47. Freitas MA, and Marshall AG (2001) Gas phase RNA and DNA ions 2. Conformational dependence of the gas-phase H/D exchange of nucleotide-5'-monophosphates, *J. Am. Soc. Mass Spectr* 12, 780–785.
48. Chipuk JE, and Brodbelt JS (2007) Investigation of the gas-phase hydrogen/deuterium exchange behavior of aromatic dicarboxylic acids in a quadrupole ion trap, *Int. J. Mass Spectrom* 267, 98–108.

49. Tian Z, Lis L, and Kass SR (2008) Hydrogen-deuterium exchange and selective labeling of deprotonated amino acids and peptides in the gas phase, *J. Am. Chem. Soc* 130, 8–9. [PubMed: 18067298]
50. Pan JX, Heath BL, Jockusch RA, and Konermann L (2012) Structural Interrogation of Electrosprayed Peptide Ions by Gas-Phase H/D Exchange and Electron Capture Dissociation Mass Spectrometry, *Anal. Chem* 84, 373–378. [PubMed: 22129032]
51. Hemling ME, Conboy JJ, Bean MF, Mentzer M, and Carr SA (1994) Gas phase hydrogen/deuterium exchange in electrospray ionization mass spectrometry as a practical tool for structure elucidation, *J. Am. Soc. Mass Spectr* 5, 434–442.
52. Kaltashov IA, Doroshenko VM, and Cotter RJ (1997) Gas phase hydrogen/deuterium exchange reactions of peptide ions in a quadrupole ion trap mass spectrometer, *Proteins* 28, 53–58. [PubMed: 9144790]
53. Zubarev RA, Kelleher NL, and McLafferty FW (1998) Electron capture dissociation of multiply charged protein cations. A nonergodic process, *J. Am. Chem. Soc* 120, 3265–3266.
54. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, and Hunt DF (2004) Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry, *P. Natl. Acad. Sci. USA* 101, 9528–9533.
55. Zehl M, Rand KD, Jensen ON, and Jorgensen TJD (2008) Electron Transfer Dissociation Facilitates the Measurement of Deuterium Incorporation into Selectively Labeled Peptides with Single Residue Resolution, *J. Am. Chem. Soc* 130, 17453–17459. [PubMed: 19035774]
56. Valentine SJ, and Clemmer DE (1997) H/D exchange levels of shape-resolved cytochrome c conformers in the gas phase, *J. Am. Chem. Soc* 119, 3558–3566.
57. Wyttenbach T, and Bowers MT (1999) Gas phase conformations of biological molecules: the hydrogen/deuterium exchange mechanism, *J. Am. Soc. Mass Spectr* 10, 9–14.
58. Rand KD, Pringle SD, Murphy JP 3rd, Fadgen KE, Brown J, and Engen JR (2009) Gas-phase hydrogen/deuterium exchange in a traveling wave ion guide for the examination of protein conformations, *Anal. Chem* 81, 10019–10028. [PubMed: 19921790]
59. Mistarz UH, Brown JM, Haselmann KF, and Rand KD (2014) Simple Setup for Gas-Phase H/D Exchange Mass Spectrometry Coupled to Electron Transfer Dissociation and Ion Mobility for Analysis of Polypeptide Structure on a Liquid Chromatographic Time Scale, *Anal. Chem* 86, 11868–11876. [PubMed: 25375223]
60. Khakinejad M, Kondalaji SG, Tafreshian A, and Valentine SJ (2015) Gas-Phase Hydrogen-Deuterium Exchange Labeling of Select Peptide Ion Conformer Types: a Per-Residue Kinetics Analysis, *J. Am. Soc. Mass Spectr* 26, 1115–1127.
61. Khakinejad M, Ghassabi Kondalaji S, and Valentine SJ (2017) Comprehensive gas-phase peptide ion structure studies using ion mobility techniques: Part 2. Gas-phase hydrogen deuterium exchange for ion population estimation, *J. Am. Soc. Mass Spectrom* 28, 960–970. [PubMed: 28315238]
62. Shi L, Holliday AE, Shi H, Zhu F, Ewing MA, Russell DH, and Clemmer DE (2014) Characterizing Intermediates Along the Transition from Polyproline I to Polyproline II Using Ion Mobility Spectrometry-Mass Spectrometry, *J. Am. Chem. Soc*
63. Darnell G, Orgel J, Pahl R, and Meredith SC (2007) Flanking polyproline sequences inhibit beta-sheet structure in polyglutamine segments by inducing PPII-like helix structure, *J. Mol. Biol* 374, 688–704. [PubMed: 17945257]
64. Bhattacharyya A, Thakur AK, Chellgren VM, Thiagarajan G, Williams AD, Chellgren BW, Creamer TP, and Wetzel R (2006) Oligoproline effects on polyglutamine conformation and aggregation, *J. Mol. Biol* 355, 524–535. [PubMed: 16321399]
65. LeVine H (1995) Thioflavine T interaction with amyloid β -sheet structures, *Amyloid* 2, 1–6.
66. Pedersen JS, Dikov D, Flink JL, Hjuler HA, Christiansen G, and Otzen DE (2006) The changing face of glucagon fibrillation: structural polymorphism and conformational imprinting, *J. Mol. Biol* 355, 501–523. [PubMed: 16321400]
67. Groenning M, Olsen L, van de Weert M, Flink JM, Frokjaer S, and Jorgensen FS (2007) Study on the binding of Thioflavin T to beta-sheet-rich and non-beta-sheet cavities, *J Struct. Biol* 158, 358–369. [PubMed: 17289401]

68. Rovnyagina NR, Sluchanko NN, Tikhonova TN, Fadeev VV, Litskevich AY, Maskevich AA, and Shirshin EA (2018) Binding of thioflavin T by albumins: An underestimated role of protein oligomeric heterogeneity, *Int. J. Biol. Macromol* 108, 284–290. [PubMed: 29208556]
69. Maezawa I, Hong HS, Liu R, Wu CY, Cheng RH, Kung MP, Kung HF, Lam KS, Oddo S, Laferla FM, and Jin LW (2008) Congo red and thioflavin-T analogs detect Abeta oligomers, *J. Neurochem* 104, 457–468. [PubMed: 17953662]
70. Spehar K, Ding T, Sun Y, Kedia N, Lu J, Nahass GR, Lew MD, and Bieschke J (2018) Super-resolution Imaging of Amyloid Structures over Extended Times by Using Transient Binding of Single Thioflavin T Molecules, *Chembiochem* 19, 1944–1948. [PubMed: 29953718]
71. Yates EA, and Legleiter J (2014) Preparation Protocols of A β (1–40) Promote the Formation of Polymorphic Aggregates and Altered Interactions with Lipid Bilayers, *Biochemistry* 53, 7038–7050. [PubMed: 25349919]
72. Beasley M, Stonebraker AR, Hasan I, Kapp KL, Liang BJ, Agarwal G, Groover S, Sedighi F, and Legleiter J (2019) Lipid Membranes Influence the Ability of Small Molecules To Inhibit Huntingtin Fibrillization, *Biochemistry*
73. Darnell GD, Derryberry J, Kurutz JW, and Meredith SC (2009) Mechanism of Cis-Inhibition of PolyQ Fibrillation by PolyP: PPII Oligomers and the Hydrophobic Effect, *Biophys. J* 97, 2295–2305. [PubMed: 19843462]
74. Lanning JD, Hawk AJ, Derryberry J, and Meredith SC (2010) Chaperone-like N-Methyl Peptide Inhibitors of Polyglutamine Aggregation, *Biochemistry* 49, 7108–7118. [PubMed: 20583779]
75. Zheng ZQ, Li AM, Holmes BB, Marasa JC, and Diamond MI (2013) An N-terminal Nuclear Export Signal Regulates Trafficking and Aggregation of Huntingtin (Htt) Protein Exon 1, *J. Biol. Chem* 288, 6063–6071. [PubMed: 23319588]
76. Wood TD, Chorush RA, Wampler FM, Little DP, O'Connor PB, and McLafferty FW (1995) Gas-phase folding and unfolding of cytochrome c cations, *P. Natl. Acad. Sci. USA* 92, 2451–2454.
77. Valentine SJ, Counterman AE, and Clemmer DE (1997) Conformer-dependent proton-transfer reactions of ubiquitin ions, *J. Am. Soc. Mass Spectr* 8, 954–961.
78. Freitas MA, Hendrickson CL, Emmett MR, and Marshall AG (1999) Gas-phase bovine ubiquitin cation conformations resolved by gas-phase hydrogen/deuterium exchange rate and extent. Dedicated to Professor Michael T. Bowers on the occasion of his 60th birthday, *Int. J. Mass Spectrom* 185–187, 565–575.
79. Valentine SJ, and Clemmer DE (2002) Temperature-dependent H/D exchange of compact and elongated cytochrome c ions in the gas phase, *J. Am. Soc. Mass Spectr* 13, 506–517.
80. Kiani Karanji A, Ghassabi Kondalaji S, Khakinejad M, Valentine SJ (2018) Comparison of Peptide Ion Conformers Arising from Non-Helical and Helical Peptides Using Ion Mobility Spectrometry and Gas-Phase Hydrogen/Deuterium Exchange, *J. Am. Soc. Mass Spectrom* 29(12), 2402–2412 [PubMed: 30324261]
81. Côté S, Wei G, and Mousseau N (2014) Atomistic mechanisms of huntingtin N-terminal fragment insertion on a phospholipid bilayer revealed by molecular dynamics simulations, *Proteins* 82, 1409–1427. [PubMed: 24415136]
82. Nagarajan A, Jawahery S, and Matysiak S (2013) The Effects of Flanking Sequences in the Interaction of Polyglutamine Peptides with a Membrane Bilayer, *J. Phys. Chem. B* 118, 6368–6379.
83. Chen M, and Wolynes PG (2017) Aggregation landscapes of Huntingtin exon 1 protein fragments and the critical repeat length for the onset of Huntington's disease, *P. Natl. Acad. Sci. USA* 114, 4406–4411.
84. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, L Byeon I-J, Anjum DH, Kodali R, Creamer TP, Conway JF, M Gronenborn A, and Wetzel R (2009) Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism, *Nat. Struct. Mol. Biol* 16, 380–389. [PubMed: 19270701]

Figure 1. Aggregation assay.

ThT assay demonstrating the aggregation of 10 μ M htt-exon1(51Q) aggregation in the presence of (A) a Nt¹⁷ peptide or (B) a polyP peptide at molar ratios of 1:1, 5:1, and 10:1 peptide to htt.

Figure 2. Representative AFM images.

A) Images of htt-exon1(51Q) aggregation as a function of time. Protein samples are deposited on a mica surface. Top row: htt-exon1. Middle row: htt-exon1 co-incubated with Nt¹⁷. Bottom row: htt-exon1 incubated with polyP. B) Fibril density as a function of time for the same samples shown in A. C) Oligomer density as a function of time for the same samples shown in A.

Figure 3. Oligomer distribution heat plots.

Heat plots showing the oligomer height distributions for each sample at the time points indicated in Figure 1. Left, middle, and right plots represent htt-exon1(51Q), htt-exon1 co-incubated with Nt¹⁷, and htt-exon1 co-incubated with polyP, respectively. Intensities have been normalized and the legend shows the intensity scale.

Figure 4. Lipid binding assay.

TBLE/PDA lipid binding assay monitoring the interaction of (A) Nt¹⁷ or polyP peptides with lipid vesicles directly and (B) the ability of Nt¹⁷ or polyP peptides to inhibit the interaction of htt-exon1(51Q) with lipid vesicles. The concentration of the peptides was 100 μ M, and the concentration of htt-exon1(51Q) was 10 μ M.

Figure 5. Schematic illustrating potential interactions of polyP peptides with htt-exon1 and their impact on aggregation.

Htt-exon1 aggregations proceeding via an oligomeric intermediate that is facilitated by associating Nt¹⁷ domains that lead to fibril nucleation and elongation is depicted in the middle of the schematic. Yellow arrows represent potential polyP interactions with the polyQ domain in (A) monomeric, (B) oligomeric, and (C) fibrillar species. Green arrows represent potential polyP interactions with Nt¹⁷ in (D) monomeric, (E) oligomeric, and (F) fibrillar species.

Figure 6. Expanded m/z Regions of Representative IMS-MS distributions.

A, distribution of the Nt^{17} -PolyP $[\text{MN}+3\text{H}]^{3+}$ heterodimers. B, distribution of the $[\text{M}+2\text{H}]^{2+}$ Nt^{17} monomer. C, distribution of the $[\text{M}+2\text{H}]^{2+}$ polyP monomer. For comparison purposes, each panel is shown over the entire drift time range. Nt^{17} -polyP heterodimer conformer drift times are indicated as conformer 'a' and conformer 'b'.

Figure 7. Nt¹⁷ and polyP heterodimer and Nt¹⁷ homodimer strength.

A, arrival time distribution of the heterodimer species with labeled conformers 'a' (9.0 ms) and 'b' (9.4 ms). B, normalized ion signal as a function of normalized collision energy for each heterodimer conformer. Circles: conformer 'a'. Triangles: conformer 'b'. C, normalized ion signal as a function of normalized collision energy for two elongated Nt¹⁷ homodimer conformers.

Figure 8. Expanded m/z regions of deuterated IMS-MS distributions.

A, deuterated $[MN+3H]^{3+}$ Nt¹⁷-polyP heterodimer complex. Conformers 'a' and 'b' are labeled the same as they are in Figure 6 – 7. B, deuterated $[M+2H]^{2+}$ Nt¹⁷ monomer distribution. C, deuterated $[M+2H]^{2+}$ polyP monomer. Each panel is shown over the entire drift time range. White boxes indicate the m/z shift for the two heterodimer complexes upon undergoing HDX in the drift tube.

Figure 9. Deuterium uptake by ion species.

Calculated deuterium uptake for species highlighted in Figure 6. polyP: $[M+H]^{2+}$ polyproline peptide ions; Nt^{17a} and Nt^{17b}: $[M+2H]^{2+}$ Nt¹⁷ peptide ions at 10.6 and 11.6 ms, respectively; N-P^a and N-P^b: $[MN+3H]^{3+}$ Nt¹⁷-polyP heterodimer complex at 9.2 and 9.8 ms, respectively. Two asterisks denote a highly significant statistical difference ($p < 0.01$)

Figure 10. Nt^{17} -polyP heterodimer gas-phase structures.

A. Low-energy, ion conformers having protonation sites of K6, K9, and K15 obtained from MDS having matching collision cross sections and modeled HDX levels as the experimental conformers. B. Low-energy, ion conformers having protonation sites of K6, K9, and the polyP N-terminus having matching collision cross sections and modeled HDX levels as the experimental conformers. Structures on the left and right in A. and B. best represent the experimental conformers labeled 'a' and 'b', respectively, in Figures 6 – 8. For all ion structures, the Nt^{17} polypeptide chain is distinguished by the shading of Glutamic Acid (yellow), Serine (red), and Lysine (blue) amino acid residues. The corresponding side-chain heteroatom hydrogens are shown as balls of the same color for all structures. The N-terminus for each polyP peptide is indicated with the letter 'N'.

Table 1.

Collision Cross Sections for All Ion Conformers.

Species ^a	Charge State ^b	Drift Time (ms) ^c	Cross Section (Å ²) ^d
Nt ¹⁷	[M+2H] ²⁺	10.0	401 ± 1
		11.2	443 ± 2
polyp	[M+2H] ²⁺	7.0	276 ± 3
Nt ¹⁷ -polyP	[M+3H] ³⁺	9.0	539 ± 1
		9.4	565 ± 1

^aSpecies name as it is referred to in the body of the manuscript and in Figure 6.^bCharge state determined by peak spacing in isotopic envelope for drift-selected species.^cDrift time determined by peak center in the IMS dimension. See Figure 6 for the IMS-MS heat map.^dGas-phase ion collision cross sections.