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Investigating the interactions of the first 17 amino acid residues of Huntingtin with lipid vesicles using mass spectrometry and molecular dynamics

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Abstract

The first 17 amino acid residues of Huntingtin protein (Nt17 of htt) are thought to play an important role in the protein's function; Nt17 is one of two membrane binding domains in htt. In this study the binding ability of Nt17 peptide with vesicles comprised of two subclasses of phospholipids is studied using electrospray ionization - mass spectrometry (ESI-MS) and molecular dynamics (MD) simulations. Overall, the peptide is shown to have a greater propensity to interact with vesicles of phosphatidylcholine (PC) rather than phosphatidylethanolamine (PE) lipids. Mass spectra show an increase in lipid-bound peptide adducts where the ordering of the number of such species is 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) > 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) > 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE). MD simulations suggest that the compactness of the bilayer plays a role in governing peptide interactions. The peptide shows greater disruption of the DOPC bilayer order at the surface and interacts with the hydrophobic tails of lipid molecules via hydrophobic residues. Conversely, the POPE vesicle remains ordered and lipids display transient interactions with the peptide through the formation of hydrogen bonds with hydrophilic residues. The POPC system displays intermediate behavior with regard to the degree of peptide-membrane interaction. Finally, the simulations suggest a helix stabilizing effect resulting from the interactions between hydrophobic residues and the lipid tails of the DOPC bilayer.

Graphical Abstract

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Introduction

The majority of cell membrane volume in biological systems is occupied by lipids and proteins. The interactions between such components within complex systems have been studied for years^{1–5}. Protein/peptide interaction with the membrane at its surface is an important topic in many fields of study including drug delivery, cell-cell recognition, signal transduction, as well as the study of disease processes^{6–16}.

Peptides that interact with the plasma membrane through processes of traversal, fusion, or residing directly at the membrane surface interface are termed membrane active¹⁷. It has been suggested that the manner in which the peptide locates within the membrane is highly dependent on the membrane lipid composition¹⁸. Broadly categorized, the interactions have been described as the “barrel-stave” and “carpet” mechanisms¹⁷. The former process involves the insertion of the peptide into the membrane core while the latter involves binding at the surface primarily through interactions with the polar head groups.

Recently a special type of peptide-membrane interaction has come under increased examination. This involves peptides that form amphipathic helices. In general amphipathic helices are polypeptide regions that form a helix upon interacting with hydrophilic/hydrophobic interfaces.¹⁹ A characteristic of such protein regions is the regular incorporation of non-polar amino acid residues at every third or fourth position. This allows the helix to form in which the non-polar face interacts with the lipid constituents of the membrane while the polar face is positioned in the aqueous environment. This anchors the protein to the membrane and orients the helical region parallel to the surface¹⁹.

Huntington's disease (HD) is one of several neurological conditions characterized by Huntingtin protein (htt) fibril formation in brain tissue. Fibril formation is caused by a polyglutamine (polyQ) expansion occurring in the N-terminal region of the htt²⁰. Studies have shown that the first 17 amino acid residues (Nt17) as well as a downstream proline-rich region of htt play important roles in protein stability as well as sub-cellular localization^{21–25}. The Nt17 peptide is suggested to play an important role in fibril formation²⁶ in which the self-association of the peptide favorably positions the expanded polyQ tracts for aggregation. The subcellular location of htt, necessitates its interaction with the lipid membrane bilayer and is mediated by posttranslational modifications of Nt17 residues^{27–29}. Additionally, the interaction of Nt17 with the lipid membrane bilayer has been shown to be an important factor in protein aggregation in HD^{14, 30–32} where Nt17 can participate in lipid-binding as well as protein-protein interactions.

The location of hydrophobic residues (L4, L7, A10/F11, L14, and F17) within the primary sequence of the Nt17 peptide suggests a propensity to form an amphipathic helix at the lipid membrane surface. This is supported by recent solid-state NMR studies which suggest that the peptide lies horizontally along the membrane surface³³. A recent study by Mousseau *et al.* examined effects of the Nt17 peptide on a phospholipid bilayer³⁴. The per-residue helix propensity in the presence of the POPC lipid bilayer was compared with the NMR structure. Overall general agreement was achieved for the two models; however, the MD study

suggested that residue numbers 1–4 are more ordered because they are less accessible to the solvent.

Here we present studies of the interaction of Nt17 with the different lipid bilayer systems [1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3 phosphoethanolamine (POPE)] using native mass spectrometry (MS)³⁵ and molecular dynamics (MD) simulations. MS reveals that Nt17 has a different propensity for interaction with vesicles of different lipid compositions. The data suggest that the level of peptide interaction is DOPC > POPC > POPE. Modeling these peptide-lipid systems using MD simulations also supports this interpretation of the MS data and helps to provide a molecular view of the tendency of the peptide to form non-specific interactions with lipids.

In general, the combined study presented here is representative of a growing body of work that utilizes native MS with a goal of elucidating protein interactions with lipid membranes^{36–40} as well as the effect of such interactions upon biomolecular structure⁴¹. Additionally, the work becomes part of a larger body of studies aimed at determining the mechanisms of aggregation for pathologically-relevant proteins.^{26, 42–52,53}

Experimental

Reagents.

The Nt17 peptide (>90% purity) was synthesized by GenScript (Piscataway, NJ, USA) and used without further purification. The peptide sequence is: MATLEKLMKAFESLKSF. The peptide was dissolved in ACS/USP absolute ethanol (Pharmco, Brookfield, CT) at a concentration of 1 mg/mL to make stock solution. The powder lipids (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3 phosphoethanolamine (POPE)) were obtained from Avanti Polar Lipids (Alabaster, AL) and dissolved in HPLC grade chloroform (Fisher Scientific, Fair Lawn, NJ) to yield a 10 mg/mL stock solution for each lipid.

Vesicle Capture-Freeze-Drying (VCFD).

Details about sample preparation using the vesicle production method are provided elsewhere⁴¹. Briefly, 100 μ L of the stock solution ($\sim$ 1200 μ M) for each lipid were dried using a speedvac (Thermo Scientific Speedvac) maintained under nitrogen gas flow. The dried samples were rehydrated in 1 ml of HPLC grade water with 10 mM ammonium acetate buffer and sonicated for 30 minutes. Then, the samples were subjected to 10 freeze-thaw cycles using liquid nitrogen and a $\sim$ 50 $^{\circ}$ C water bath. Subsequently, 100 μ L of the stock solution of the Nt17 peptide ($\sim$ 100 μ M) was added to each lipid solution. For all samples, lipids were maintained at a final concentration that was $\sim$ 10 times greater than the Nt17 peptide. The peptide/lipid complex solutions were stored over night at 37 $^{\circ}$ C for equilibration. Notably, a limitation of this study is that the physiological concentration of Nt17-containing protein is largely unknown although it has been estimated to be 0.15 μ M in the cytosol of neural cells⁵⁴. Experiments here were designed with higher peptide concentrations (10 μ M) to enhance the level of peptide-membrane interactions as well as to

abbreviate the timescale of such interactions as well as peptide-peptide interactions. Notably this approach is used to study htt aggregation using THT⁵⁵ and PDA⁵⁶ assays and to perform CD spectroscopy¹⁴ and AFM⁵⁷ and MS³⁸ measurements.

Electrospray-Mass Spectrometry (ESI-MS).

All of the experiments were carried out using a Q Exactive Hybrid quadrupole Orbitrap mass spectrometer (Thermo Fisher, San Jose, CA). The experiments were conducted using the commercial HESI source available with the MS instrument. For both ionization sources MS spectra were recorded in positive ion mode over a mass-to-charge (m/z) range of 500 to 4000. The samples were infused at a rate of 10 μ L/min using a heated syringe and infusion line to ensure that the POPE vesicles did not undergo a phase transition. The needle was biased at 3,500 V relative to the instrument inlet. The instrument parameters used for ESI were: 450 °C for the capillary inlet temperature, 80 V for the S-lens assembly, 400 ms for the maximum injecting time, 1×10^6 for the AGC, and 70,000 for the MS resolution. Each spectrum was recorded in triplicate for 180 seconds each to ensure data collection reproducibility. To analyze the MS data, the Xcalibur 2.2 software suite (Thermo Scientific) was used.

A limited number of experiments were performed on a linear ion trap mass spectrometer (LTQ, Thermo Fisher, San Jose, CA) to examine product ions upon isolation of lipidated species (see below). For these experiments, the commercial HESI source was utilized and mass spectra were recorded in positive ion mode using a m/z range of 900 to 2000 m/z . The same flow rate and needle bias parameters were used for these experiments. Precursor ions were isolated using a 10 m/z window and no collisional activation was employed.

Molecular Dynamics (MD) Simulations.

For MD studies, two types of models were used. The first involved initiation with a linear structure to represent the initial interaction between the peptide and the membrane surface. Because full helix formation at the surface of the membrane appeared to be an unreachable event because of the long timescale, MD simulations were also performed in which the peptide was forced to be helical and placed directly above the membrane. Notably the NMR structure suggests that it is helical at the surface³³. For the latter simulations, the goal was to determine the degree of amphipathic helix interactions with the different lipid membranes.

For the initial random coil simulation of the Nt17 peptide, the fully linear structure was built in Avogadro⁵⁸ and subjected to 10 ns of simulation in pure water. The output was used as an input file for the Nt17-lipid bilayer simulation. For the helix model, the Nt17 peptide pdb structure (2LD2) was obtained from the protein data bank website³³ and used as the initial input file for the MD simulations. The CHARMM-GUI server (www.charmm-gui.org) was used to create the lipid bilayer system⁵⁹. The peptide was placed above the bilayer in the z axis and solvated (explicit model) with water. All the simulations were performed in triplicate. The M1 residue at the N-terminus as well as the lysine residues (K6, K9, K15) were protonated. The carboxylic acid of F17 at the C-terminus and the two glutamic acid residues (E5, E12) were deprotonated. This provided a net charge of +1 for the modeled peptides. The nano-scale molecular dynamics (NAMD 2.13) software⁶⁰ was utilized to

perform minimization and equilibration of the system based on the CHARMM-GUI protocol and CHARMM c36 force field for lipids and proteins⁶¹. The protocol consists of 1950 ps minimization and equilibration process in which restraints from the peptide backbone, lipids, water and ion molecules are gradually removed in six steps⁶². Parameters for all-atom MD simulations using the periodic boundary condition were: NPT ensemble, 2 fs for the timestep, 310 K for the temperature and 12 Å for the cutoff. The visual MD (VMD) suite was used to visualize and analyze the simulation data. The GridMAT-MD script was used to calculate the theoretical area per lipid⁶³.

Results and Discussion

Peptide-lipid interactions examined with native MS.

Studies have shown that the presence of the Nt17 flanking sequence in the htt protein is important for membrane interactions^{14, 21, 30}. This association has been suggested to serve to anchor huntingtin protein (htt) to the membrane⁶⁴. To investigate the peptide interactions with DOPC lipid, mass spectra were recorded for the sample in which the peptide was incubated with the DOPC vesicles. For these studies, experiments were conducted in duplicate.

Figure 1 shows a typical mass spectrum for the DOPC-peptide system. ESI produces ions of several different charge state for the Nt17-lipid system. In the mass spectrum, the doubly-charged monomer peptide ions ($[M+2H]^{2+}$ at $m/z \sim 988$) predominate. Dimer, trimer and tetramer ions for the Nt17 peptide are also observed at $m/z \sim 1317$, 1482, 1580, respectively. These species are of significantly lower abundance (Figure 1).

In addition to the ions described above, ions comprised of peptide-lipid complexes are also observed. These range in size from peptide monomer to tetramer ions. A complete list of the observed protein-lipid complex ions is presented in Table 1. Notably, monomer peptide ions with differing numbers of lipid adducts are observed. These range in size from 1 to 7 attached lipids. This relatively high number of adducts could, to some degree, indicate the propensity of the lipid to interact with this peptide (see discussion below). In addition to these ions, peptide-lipid complexes which include one lipid attached to dimer and trimer species are observed at $m/z \sim 1578$ and 1678, respectively, Figure 1). Table 1 shows the multimeric peptide-lipid ions that have been observed in these studies. In general, the number of separate ions as well as the number of attached lipids decreases with higher order peptide multimer. For example, >1 lipid adduct is observed for all the peptide multimers except for the tetramer where only 1 lipid is attached (Table 1).

Lipid preference of Nt17.

The affinity of amphipathic α -helices for lipid bilayers has been shown to be dependent on the physicochemical characteristics of the membrane^{65–67}. One characteristic showing importance is the packing defects of the membrane. Beyer *et al.* have suggested that a small radius of curvature is essential for increasing peptide-membrane interaction⁶⁸. In this study it was suggested that peptide binding to a membrane depends on the presence of defects in the membrane structure at the membrane-water interface. These short-lived defects create

temporary spacing between the lipid head groups which allows greater interaction between the lipid and the peptide. To investigate this phenomenon further by MS, experiments were also conducted using DOPC, POPC, and POPE lipid systems. All are zwitterionic lipids; however, the DOPC lipid contains a much bulkier head group which is expected to increase the overall area per lipid⁶⁹. Additionally, DOPC and POPE systems have different lipid tails, that can impact their fluidity at given temperatures. DOPC, POPC, and POPE have respective phase transition temperatures of -17, -2 and 25 °C. Thus the DOPC bilayer system is more fluid under experimental conditions (~37 °C) which can more readily create short-lived defects in the exposed membrane leaflet.

By comparing the mass spectral results from the Nt17-DOPC (Figure 1), and Nt-17-POPC and Nt17-POPE (Figure S1 in the Supporting Information section) systems, it may be possible to obtain some information about differences in types of interaction between the peptide and the respective lipid vesicles. The assigned peaks for the peptide-PC and peptide-PE complex ions are listed in Table 1. From Table 1, it is readily apparent that the most lipid adducts are observed in the DOPC system. For example, 1 Nt17 peptide is observed with up to 7 attached DOPC lipids. Conversely, in the POPE system, only up to 5 lipid adducts are observed for the monomer peptide. In comparison, this number is 6 for the POPC system.

The greater propensity of the peptide to bind with DOPC extends to the higher-order oligomers of Nt17. For the DOPC system up to 8 lipid adducts are observed with peptide dimers while this limit is 5 for POPC and 3 for POPE systems. There was no mass spectral feature corresponding to the peptide trimer and tetramer containing POPE lipid adduct but the peak for tetramer with one lipid adduct can be seen at $m/z \sim 2171.44$ for the DOPC system. Overall, 38 lipid adduct species are observed for the DOPC vesicle system while only 10 related peaks are observed for the POPE vesicle system suggesting the possibility of a different interaction type for the former lipid vesicles. Notably, the phenomenon of increased lipid adducts in the DOPC system is not a result of the relative number of peptide monomers or multimers believed to form in solution as these are observed in similar abundances for all peptide-lipid systems. Together these results suggest that differences in interaction between the Nt17 peptide individual lipid vesicles may be responsible for differences in the mass spectra.

Co-mixing Nt17 peptide and lipids without vesicle formation.

To investigate the role of the ESI process on the numbers of Nt17 peptide ions with lipid adducts, experiments were performed in which the peptide and lipid were co-mixed and, after a similar incubation time, electrosprayed into the mass spectrometer. More specifically, the Nt17 peptide and lipids were mixed without any preparation (without sonication and freeze drying) for 5 hours in 10 mM ammonium acetate buffer. The assigned mass spectral peaks for these datasets are shown in bold in Table 1. Compared to the experiments in which lipid vesicles were generated before adding the peptide, in all cases, co-mixing peptide and lipid showed a significantly decreased number of assigned species. Only 4 ions ([Nt17+1DOPC+H]⁺, [Nt17+1DOPC+2H]²⁺, [Nt17+2DOPC+2H]²⁺, and [Nt17+3DOPC+2H]²⁺ at m/z 2760.64, 1380.82, 1773.62, and 2166.42 relatively) were assigned for the Nt17-DOPC system Table 1. For the Nt17-POPC and for the Nt17-POPE systems, only 6

and 3 species were observed, respectively. This indicates that vesicle formation is an important step to detect elevated peptide-lipid interactions. It also supports the premise that the mass spectral peak diversity is an indication of solution interaction as opposed to only processes occurring in the ESI droplets.

As a final consideration for co-mixing experiments, it is noted that only 2 of 13 assigned peaks are for Nt17 dimers with lipid adducts. This could suggest that Nt17 requires a membrane interface to form increased numbers of oligomeric species and this is revealed in the MS data. This would be consistent with the concept of mode of action for amphipathic helices as well as proposed mechanisms of oligomer formation for *htt*³³. That said, caution must be used in interpreting the mass spectral data as the dataset features could arise in another manner. For example, an increased degree of interaction with the lipid membrane could translate into a large population of adsorbed monomer. During the ESI process, this increased likelihood of being located at the membrane surface could result in an increase in peptide numbers within ESI progeny droplets resulting in multimeric ions. Therefore, no conclusions can be drawn with regard to the degree of membrane-induced oligomerization. To disentangle the routes of formation for such a heterogeneous mixture of oligomeric species is the subject of future, more extensive experiments employing a greater number of lipid systems as well as gas-phase separations and reactivity studies^{26, 70–74}. This process will also be examined by MD simulations where multiple peptides are examined with the membrane systems. This preliminary study does, however, show that vesicle formation is required to produce a greater number of oligomer ions with lipids attached in the mass spectrum.

Consideration of peptide-lipid ion intensities.

It is instructive to consider the ion intensities with regard to the degree of interaction between peptides and lipid vesicles. Figure S2 in the Supporting Information section shows the relative intensities of the various ions observed for the three separate lipid systems. Notably, the ions with PE adducts are of higher intensity especially for those species containing 1 lipid adduct. The question arises as to the origin of this higher intensity in light of the observation that a greater number of diverse lipidated species is observed for DOPC and POPC systems. In other words, is this value reflective of greater interaction overall (independent of type) at the membrane surface? Here, a note of caution is raised based on a few observations. First, separate experiments have been performed on a LTQ mass spectrometer in which the $[Nt17+L+2H]^{2+}$ (where L is DOPC or POPE) ions were isolated in the linear ion trap. Immediately upon isolation, the DOPC-containing ions are observed to dissociate to some degree to yield $[Nt17+H]^+$ ions. This did not occur for the POPE-containing ions as shown in Figure S3 in the Supporting Information section. Based on this observation, it is possible that many ions with differently-oriented lipid adducts are very fragile and do not survive to the point of mass detection. Additionally, this could extend to the oligomeric species where a greater number of unique ions is observed for DOPC (Table 1). Due to such differences, it may not be possible to fully determine whether or not ion intensity affords a clear indication of the degree of surface interaction. The results are presented here as being of interest and worthy of further MS and molecular modeling studies.

Molecular dynamics (MD) simulations: random coil interaction.

To consider initial interactions between the Nt17 peptide and the lipid membrane, MD simulations were performed in which the initial peptide structure was random coil (see Experimental Section). To ensure that the proper parameters had been utilized for the simulations, the area per lipid was determined as a function of MD trajectory time. These results are shown for the DOPC, POPC, and POPE lipid systems in Figure 2. The average area per lipid values for the respective lipid systems are 68.6, 65.1, 57.3 Å² (Figure 2A and Supplementary Table 1) This is consistent with the ordering and magnitudes of the reported areas per lipid and transition temperatures for DOPC⁷⁵, POPC⁷⁶, and POPE⁶⁹.

One measurement that provides some insight into interaction differences with the random coil peptide is the degree of helicity within the peptide as a function of MD trajectory time. Figure 2B shows the results for the three different lipid systems. Of note is the increased degree of helicity achieved for the peptide in the DOPC system simulation. There are two major times in which extensive helix formation is observed occurring at ~100 and ~470 ns. It is instructive to observe how helix formation proceeds near these time points. Figure 3 shows this for the first time point region. In both instances, the Nt17 peptide is observed to interact with the membrane via the first 2 to 3 amino acid residues. Over the next few ns in the simulation, a single turn to two helical turns is observed. This rapidly propagates to extensive helical formation (~50% in both cases) with concomitant detachment from the membrane surface. Notably, the degree of helix formation for the POPC and POPE lipid systems does not occur to the degree observed in the two instances for DOPC.

Molecular dynamics (MD) simulations: helix-membrane compatibility.

Although the simulations described above present an intriguing possibility regarding differences in helix initiation roles by different membrane types, it is possible that they do not fully represent the interactions expected for a system in which the peptide has been incubated extensively in the presence of the lipid vesicles. Because experimental evidence suggests that, at the membrane surface, the peptide is helical, MD simulations have also been performed in which the fully helical peptide is placed at the membrane surface. This was performed as the computational time required to observe full helix formation while at the membrane surface may be prohibitively long according to the random coil simulations.

For the helix-membrane studies, the peptide was placed equidistant (29 Å – center of the bilayer to peptide center of mass) above the three different lipid bilayer head groups. Figure 4A shows a heat map representing the distance between each residue in the peptide and the center of the lipid bilayer across the simulation time. For the peptide-DOPC system, the MD simulations suggest that the N-terminus section of the peptide helps to maintain the entire peptide at the surface of the bilayer. This occurs during the first 50 nanoseconds of the simulation. The process initiates with the N-terminal residues over this relatively short timescale. Specifically, the first three amino acid residues (M1, A2, and T3), are the first to locate closer to the lipid membrane (Figure 4A). This is followed by positioning successive residues (moving toward the C-terminus) within increased proximity of the lipid membrane. Notably, the first three residues are not associated with the helical character of the peptide obtained from the protein databank. Figure 4A also provides information regarding the

relative orientation of the peptide with respect to the DOPC lipid membrane. After 50 nanoseconds, distinct bands are observed in the heat map. This reveals increased proximity for specific regions of the peptide. For example, residues 1 through 4, 7 and 8, 10 and 11, and 14 and 15 are in much closer proximity to the lipid membrane. This result is consistent with the suggested structure and topology of the Nt17 peptide interacting with a lipid membrane from solid-state NMR (ssNMR) experiments³³.

The MD simulations for the POPC lipid system (Figure 4B) show different behavior compared with the DOPC results. One similarity is that initial interaction at the N-terminus is observed for residues 1 and 2 after 50 ns. This, however, is shorter lived for the POPC system as it disappears after 50 ns. By 150 ns this initial interaction is replaced by primary interaction at residues 7 and 8 which persists for the remainder of the simulation. In a sense, this interaction anchors, to some degree, the peptide and allows other interactions to eventually occur. For example, the interaction for residue 1 and 2 is re-established at ~220 ns. Subsequent interactions for residues 3 and 4, 11, and 14 and 15 are established by ~330, 460, and 490 ns, respectively. The end result is the similar observance of bands associated with hydrophobic residues showing increased interaction; however, this association occurs at a much longer time point than that for the DOPC system.

The MD simulations for the POPE lipid membrane reveal a markedly different behavior regarding peptide-lipid interaction as shown in Figure 4C. As noted by the heat map, very little of the peptide is ever located within 20 Å of the lipid membrane. As with the DOPC model, the first three residues are located closest to the lipid membrane across the simulation time frame. Indeed, periodically, the M1 residue is the only residue that locates within 20 Å of the lipid membrane. Similar to the DOPC system, specific bands in the heat map (Figure 4C) reveal peptide residues exhibiting relatively increased proximity to the POPE lipid membrane; residues 1 and 2, 5 and 6, 9 and 10, 12 and 13, and 16 and 17 are clearly located in increased proximity to the membrane across the simulation timescale. The clear differences between the modeled membrane systems are: 1) the residue numbers that are in the closest proximity; and, 2) the relative degree of proximity (observed to be much greater for the DOPC modeling).

Molecular Dynamics (MD) Simulations: peptide orientation.

It is instructive to consider the origin of the bands presented in the heat maps in Figure 4. Notably, for all systems, the positioning of residues in closer proximity to the lipid membrane is ~3 to 4 residues apart. This distance in peptide primary sequence suggests a connection to helical structure. Additionally, the fact that different residues interact with the different lipid membranes suggests membrane-guided lipid orienting.

Because the Nt17 region is known to form an amphipathic helix^{77, 78}, it is instructive to examine the characteristics of the amino acid residues exhibiting greater lipid interaction. Figure 5A represents the nature of the peptide-lipid interaction for the DOPC system. Notably, because the same residues are involved for the POPC system (Figure 4), only the DOPC system is discussed here. As indicated by the heat map (Figure 4A), the peptide is noticeably close to the center of the bilayer. This can be observed pictorially in Figure 5A where the Nt17 peptide is located exactly at the membrane surface. At this moment in the

simulation, the A2, L4, L7, F11 and L14 residues exhibit the greatest interaction with DOPC lipid molecules which matches with results from the previously performed ssNMR study³³. Indeed, the general interaction is between these hydrophobic residues and the lipid tails (Figure 5C). These interactions necessarily cause the more hydrophilic residues of the amphipathic helix to extend into the aqueous environment. This result is in very good agreement with previous simulations using this lipid system³⁴.

Figure 5B shows that the Nt17 peptide interacts more distantly with the POPE membrane surface. Figure 5C presents the interaction type between the peptide and one POPE lipid. This shows a simulation frame in which the peptide is located near its closest overall proximity to the lipid bilayer. Notably, the protonated K9 and K15 residues are shown to interact with the zwitterionic head groups. Indeed, the residues that exhibit the closest proximity to the lipid membrane across the dynamics run (Figure 4C) are the hydrophilic K6, K9, S13, and S16 residues. It is noted that the S16 residue resides next to the protonated K15 residue. Overall, the peptide-lipid interactions are dominated by hydrophilic residues for the POPE system.

A question that arises is what is the net effect of the interactions described above on overall peptide orientation at the lipid membrane? Figure 5 helps to illustrate the net effect of these interactions. Overall, for the DOPC lipid system, the insertion of the hydrophobic residues to some degree anchors the peptide right at the lipid surface (i.e., among the head groups) as shown in Figure 5A. Therefore, there is greater disruption of the lipid bilayer at this location. Conversely, for the POPE system, the peptide is maintained at a distance from the lipid bilayer surface due to hydrogen bond interactions between charged residues and the head group constituents (Figure 5B). Therefore, the anchoring of the peptide occurs at several Å distance from the bilayer surface and the membrane integrity is largely preserved. It is important to note that the relative peptide orientation derived from the MD simulations (Figures 4 and 5) strongly supports the mass spectral data (Figure 1 and Table 1) discussed above. That is, the increased interaction with a greater number of lipid molecules for the DOPC system would suggest the observance of a greater number of lipid adducts³⁶ as is observed in the mass spectra. Furthermore, the greater difficulty to establish the strongly adsorbed peptide for the POPC system (Figure 4B) could indicate the intermediate number of lipid adducts associated with this peptide-lipid system.

MD simulations: potential origin of interaction differences.

Because the DOPC and the POPE lipids both contain zwitterionic head groups, it is instructive to consider differences that could account for the different predominant interactions observed with the MD simulations. One possibility is that the higher order of the POPE lipid bilayer disallows the interactions by the hydrophobic residues as are observed for the DOPC system. Here it is proposed that the POPE lipids can make strong intermolecular H-bonds between the phosphate group on one lipid and the carbonyl oxygen atom of an adjacent lipid resulting in a higher ordering of the POPE bilayer. In contrast, the terminal methyl groups of the DOPC system disrupt such hydrogen bonding networks⁷⁹ and, therefore, the head group spacing increases. As mentioned above, studies have hypothesized that the interaction between amphipathic α -helices and membranes are

influenced by short-lived defects created by membrane dynamics^{67, 68, 80}. Thus, when a membrane is more fluid there will be more defects, and the peptide will have more opportunity to explore those defects leading to increased interactions with the lipid tails. Additionally, greater spacing between the headgroups resulting from membrane curvature may also lead to a higher propensity of peptide binding.³⁰ Based on these hypotheses, it is instructive to compare the results with the average area per lipid calculations (Figure 2A). This analysis shows increasing numbers of lipidated species with increasing area per lipid. This supports the idea that the higher area per lipid value for the DOPC bilayer system could also provide more short-lived defects for the peptide interaction. The greater disorder (spacing) as well as the more fluid characteristics of the DOPC system would thus allow for increased interaction between the lipid tails and the hydrophobic residues as observed in Figure 5C.

MD simulations: peptide helical persistence.

As mentioned above, the formation of an amphipathic α -helix is hallmark of the Nt17 tract^{81–85} in htt. Notably, it has previously been proposed that lipid interactions with the Nt17 amphipathic helix could occur precisely as suggested by the DOPC MD simulations. It has also been proposed that helix-helix interactions are required to form early-stage oligomers that ultimately lead to protein aggregation associated with HD⁸². It has also been suggested that peptide-lipid interaction could ultimately induce helix formation⁸⁶. Therefore, it could be argued that suitable peptide-lipid interactions could favor the persistence of the amphipathic helix. The time-dependent helical character of the modeled Nt17 peptide has been assessed for the DOPC and POPE lipid systems investigated here. Figure S4 in the Supporting Information section shows the helical content of the Nt17 peptide across the two different simulations. For the DOPC simulations, the peptide begins as relatively helical (~65%). The helicity is almost immediately disrupted within the first few nanoseconds and is reestablished at the 68% level for the first 70 ns before stabilizing at ~75% for the remainder of the simulation. The initial collapse and the lower helicity correlate with the initial interaction of the N-terminal region with the lipid bilayer (Figure 3A). The relatively stable region (sustained helicity) corresponds with increased interaction with the lipid. In comparison, for the POPE system, no initial collapse is evident, however, a greater number of collapse events is observed across the entire simulation. Additionally, the overall average level of helicity trends downward across the simulation and is ultimately observed to be well below that for the DOPC simulation. These observations are the same for the replicate analysis.

Conclusions

Electrospray ionization - mass spectrometry (ESI-MS) and molecular dynamics (MD) simulations have been used to study the lipid interaction properties of the first 17 amino acid residues of Huntingtin protein (htt). In this study, phosphatidylcholine (PC), the most common lipid in the eukaryotic membrane, as well as phosphatidylethanolamine (PE) lipids were used.

From mass spectra data, a noticeable difference was observed in the number and type of peptide-lipid complex ions. The MS spectra indicate that the Nt17 peptide yields more adducts with the DOPC lipid in both monomeric and multimeric ion forms in comparison to the POPC and POPE lipids. MD simulations provide atomic insight into these interactions. The simulation showed that the residues of the Nt17 peptide were generally closer to the center of the DOPC bilayer throughout the simulation. The amphipathic peptide interacts with the DOPC bilayer with hydrophobic residues. However, when the POPE lipid bilayer was examined, the hydrophilic residues interact with the lipid headgroups. Further analysis utilizing the simulation data reveals that the area per lipid may play an important role in the Nt17-lipid interactions. The increased surface area for the DOPC bilayer results in a longer lasting interaction as the Nt17 peptide is imbedded within the lipid headgroups resulting in more effective interaction with the hydrophobic tails. The simulation also suggests that the increased interaction between the Nt17 peptide and the DOPC lipid bilayer leads to greater stabilization of helicity for the peptide. Conversely, as the peptide increases aqueous interactions as opposed to the lipid bilayer, it loses some helical character over time. As the Nt17 peptide is a lipid-binding domain, its interactions with the membrane can lead to the formation of helix bundles at the bilayer surface. Understanding the nature of these interactions is vital because they can trigger the aggregation process and ultimately result in disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Persaud R, Boggs JM, Wood DD, Moscarello MA. Interaction of glycosylated human myelin basic protein with lipid bilayers. *Biochemistry* 1989, 28:4209–4216. [PubMed: 2475162]
2. Poklar N, Fritz J, Macek P, Vesnaver G, Chalikian TV. Interaction of the pore-forming protein equinatoxin II with model lipid membranes: A calorimetric and spectroscopic study. *Biochemistry* 1999, 38:14999–15008. [PubMed: 10555982]
3. Shimizu M, Saito M. Lipid—Protein Interaction at an Emulsified Oil Surface: Protein Structures and Their Roles in Lipid Binding. 1996, 650:156–165.
4. Sanders MR, Clifton LA, Frazier RA, Green RJ. Role of Lipid Composition on the Interaction between a Tryptophan-Rich Protein and Model Bacterial Membranes. *Langmuir* 2016, 32:2050–2057. [PubMed: 26813886]
5. Nagy L, Milano F, Dorogi M, Agostiano A, Laczko G, Szebenyi K, Varo G, Trotta M, Maroti P. Protein/lipid interaction in the bacterial photosynthetic reaction center: phosphatidylcholine and phosphatidylglycerol modify the free energy levels of the quinones. *Biochemistry* 2004, 43:12913–12923. [PubMed: 15461464]
6. Guigas G, Weiss M. Effects of protein crowding on membrane systems. *Biochim Biophys Acta* 2016, 1858:2441–2450. [PubMed: 26724385]
7. Han SK, Kim KP, Koduri R, Bittova L, Munoz NM, Leff AR, Wilton DC, Gelb MH, Cho WH. Roles of Trp(31) in high membrane binding and proinflammatory activity of human group V

phospholipase A(2). *Journal of Biological Chemistry* 1999, 274:11881–11888. [PubMed: 10207008]

8. Yang J, Bahreman A, Daudey G, Bussmann J, Olsthoorn RC, Kros A. Drug Delivery via Cell Membrane Fusion Using Lipopeptide Modified Liposomes. *ACS Cent Sci* 2016, 2:621–630. [PubMed: 27725960]
9. Yin H, Flynn AD. Drugging Membrane Protein Interactions. *Annu Rev Biomed Eng* 2016, 18:51–76. [PubMed: 26863923]
10. Williams AF, Barclay AN. The immunoglobulin superfamily--domains for cell surface recognition. *Annu Rev Immunol* 1988, 6:381–405. [PubMed: 3289571]
11. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000, 1:31–39. [PubMed: 11413487]
12. Resh MD. Membrane targeting of lipid modified signal transduction proteins. *Subcell Biochem* 2004, 37:217–232. [PubMed: 15376622]
13. Gorbenko GP, Kinnunen PK. The role of lipid-protein interactions in amyloid-type protein fibril formation. *Chem Phys Lipids* 2006, 141:72–82. [PubMed: 16569401]
14. Pandey NK, Isas JM, Rawat A, Lee RV, Langen J, Pandey P, Langen R. The 17-residue-long N terminus in huntingtin controls stepwise aggregation in solution and on membranes via different mechanisms. *J Biol Chem* 2018, 293:2597–2605. [PubMed: 29282287]
15. Sparr E, Engel MF, Sakharov DV, Sprong M, Jacobs J, de Kruijff B, Hoppener JW, Killian JA. Islet amyloid polypeptide-induced membrane leakage involves uptake of lipids by forming amyloid fibers. *FEBS Lett* 2004, 577:117–120. [PubMed: 15527771]
16. Zhao H, Tuominen EK, Kinnunen PK. Formation of amyloid fibers triggered by phosphatidylserine-containing membranes. *Biochemistry* 2004, 43:10302–10307. [PubMed: 15301528]
17. Avci FG, Akbulut BS, Ozkirimli E. Membrane Active Peptides and Their Biophysical Characterization. *Biomolecules* 2018, 8.
18. Sani MA, Separovic F. How Membrane-Active Peptides Get into Lipid Membranes. *Acc Chem Res* 2016, 49:1130–1138. [PubMed: 27187572]
19. Gimenez-Andres M, Copic A, Antonny B. The Many Faces of Amphipathic Helices. *Biomolecules* 2018, 8.
20. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat Genet* 1997, 15:62–69. [PubMed: 8988170]
21. Rockabrand E, Slepko N, Pantalone A, Nukala VN, Kazantsev A, Marsh JL, Sullivan PG, Steffan JS, Sensi SL, Thompson LM. The first 17 amino acids of Huntingtin modulate its sub-cellular localization, aggregation and effects on calcium homeostasis. *Hum Mol Genet* 2007, 16:61–77. [PubMed: 17135277]
22. Nozaki K, Onodera O, Takano H, Tsuji S. Amino acid sequences flanking polyglutamine stretches influence their potential for aggregate formation. *Neuroreport* 2001, 12:3357–3364. [PubMed: 11711886]
23. Wang L, Tsai CC. Atrophin proteins: an overview of a new class of nuclear receptor corepressors. *Nucl Recept Signal* 2008, 6:e009.
24. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci* 2002, 5:731–736. [PubMed: 12089530]
25. Suopanki J, Gotz C, Lutsch G, Schiller J, Harjes P, Herrmann A, Wanker EE. Interaction of huntingtin fragments with brain membranes--clues to early dysfunction in Huntington's disease. *J Neurochem* 2006, 96:870–884. [PubMed: 16405500]
26. Arndt JR, Kondalaji SG, Maurer MM, Parker A, Legleiter J, Valentine SJ. Huntingtin N-Terminal Monomeric and Multimeric Structures Destabilized by Covalent Modification of Heteroatomic Residues. *Biochemistry* 2015, 54:4285–4296. [PubMed: 26098795]
27. Cariulo C, Azzollini L, Verani M, Martufi P, Boggio R, Chiki A, Deguire SM, Cherubini M, Gines S, Marsh JL, et al. Phosphorylation of huntingtin at residue T3 is decreased in Huntington's

- disease and modulates mutant huntingtin protein conformation. *Proc Natl Acad Sci U S A* 2017, 114:E10809–E10818. [PubMed: 29162692]
28. Ehrnhoefer DE, Sutton L, Hayden MR. Small changes, big impact: posttranslational modifications and function of huntingtin in Huntington disease. *Neuroscientist* 2011, 17:475–492. [PubMed: 21311053]
29. Perutz MF, Pope BJ, Owen D, Wanker EE, Scherzinger E. Aggregation of proteins with expanded glutamine and alanine repeats of the glutamine-rich and asparagine-rich domains of Sup35 and of the amyloid beta-peptide of amyloid plaques. *Proc Natl Acad Sci U S A* 2002, 99:5596–5600. [PubMed: 11960015]
30. Chaibva M, Burke KA, Legleiter J. Curvature enhances binding and aggregation of huntingtin at lipid membranes. *Biochemistry* 2014, 53:2355–2365. [PubMed: 24670006]
31. Chaibva M, Gao X, Jain P, Campbell WAt, Frey SL, Legleiter J. Sphingomyelin and GM1 Influence Huntingtin Binding to, Disruption of, and Aggregation on Lipid Membranes. *ACS Omega* 2018, 3:273–285. [PubMed: 29399649]
32. Gao X, Campbell WAt, Chaibva M, Jain P, Leslie AE, Frey SL, Legleiter J. Cholesterol Modifies Huntingtin Binding to, Disruption of, and Aggregation on Lipid Membranes. *Biochemistry* 2016, 55:92–102. [PubMed: 26652744]
33. Michalek M, Salnikov ES, Bechinger B. Structure and topology of the huntingtin 1–17 membrane anchor by a combined solution and solid-state NMR approach. *Biophys J* 2013, 105:699–710. [PubMed: 23931318]
34. Cote S, Binette V, Salnikov ES, Bechinger B, Mousseau N. Probing the Huntingtin 1–17 membrane anchor on a phospholipid bilayer by using all-atom simulations. *Biophys J* 2015, 108:1187–1198. [PubMed: 25762330]
35. van den Heuvel RH, Heck AJ. Native protein mass spectrometry: from intact oligomers to functional machineries. *Curr Opin Chem Biol* 2004, 8:519–526. [PubMed: 15450495]
36. Landreh M, Costeira-Paulo J, Gault J, Marklund EG, Robinson CV. Effects of Detergent Micelles on Lipid Binding to Proteins in Electrospray Ionization Mass Spectrometry. *Anal Chem* 2017, 89:7425–7430. [PubMed: 28627869]
37. Walker LR, Marzluff EM, Townsend JA, Resager WC, Marty MT. Native Mass Spectrometry of Antimicrobial Peptides in Lipid Nanodiscs Elucidates Complex Assembly. *Anal Chem* 2019, 91:9284–9291. [PubMed: 31251560]
38. Liu Y, Cong X, Liu W, Laganowsky A. Characterization of Membrane Protein-Lipid Interactions by Mass Spectrometry Ion Mobility Mass Spectrometry. *J Am Soc Mass Spectrom* 2017, 28:579–586. [PubMed: 27924494]
39. Marcoux J, Wang SC, Politis A, Reading E, Ma J, Biggin PC, Zhou M, Tao H, Zhang Q, Chang G, et al. Mass spectrometry reveals synergistic effects of nucleotides, lipids, and drugs binding to a multidrug resistance efflux pump. *Proc Natl Acad Sci U S A* 2013, 110:9704–9709. [PubMed: 23690617]
40. demmers JA, Haverkamp J, Heck AJ, Koeppe RE 2nd, Killian JA. Electrospray ionization mass spectrometry as a tool to analyze hydrogen deuterium exchange kinetics of transmembrane peptides in lipid bilayers. *PNAS* 2000, 97:3189–3194. [PubMed: 10725361]
41. Patrick JW, Gamez RC, Russell DH. Elucidation of conformer preferences for a hydrophobic antimicrobial peptide by vesicle capture-freeze-drying: a preparatory method coupled to ion mobility-mass spectrometry. *Anal Chem* 2015, 87:578–583. [PubMed: 25522119]
42. Scarff CA, Ashcroft AE, Radford SE. Characterization of Amyloid Oligomers by Electrospray Ionization-Ion Mobility Spectrometry-Mass Spectrometry (ESI-IMS-MS) In: Eliezer D, ed. *Protein Amyloid Aggregation: Methods and Protocols*. New York, NY: Springer New York; 2016, 115–132.
43. Ionut Iurascu M, Cozma C, Tomczyk N, Rontree J, Desor M, Drescher M, Przybylski M. Structural characterization of beta-amyloid oligomer-aggregates by ion mobility mass spectrometry and electron spin resonance spectroscopy. *Anal Bioanal Chem* 2009, 395:2509–2519. [PubMed: 19838688]

44. Young LM, Cao P, Raleigh DP, Ashcroft AE, Radford SE. Ion mobility spectrometry-mass spectrometry defines the oligomeric intermediates in amylin amyloid formation and the mode of action of inhibitors. *J Am Chem Soc* 2014, 136:660–670. [PubMed: 24372466]
45. Wang JS, Whitehead SN, Yeung KK. Detection of Amyloid Beta (Abeta) Oligomeric Composition Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS). *J Am Soc Mass Spectrom* 2018, 29:786–795. [PubMed: 29464543]
46. Bernstein SL, Wytenbach T, Baumketner A, Shea J-E, Bitan G, Teplow DB, Bowers MT. Amyloid β -Protein: Monomer Structure and Early Aggregation States of A β 42 and Its Pro19 Alloform. *Journal of the American Chemical Society* 2005, 127:2075–2084. [PubMed: 15713083]
47. Bernstein SL, Dupuis NF, Lazo ND, Wytenbach T, Condrón MM, Bitan G, Teplow DB, Shea JE, Ruotolo BT, Robinson CV, et al. Amyloid-beta protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nat Chem* 2009, 1:326–331. [PubMed: 20703363]
48. Do TD, Economou NJ, Chamas A, Buratto SK, Shea JE, Bowers MT. Interactions between amyloid-beta and Tau fragments promote aberrant aggregates: implications for amyloid toxicity. *J Phys Chem B* 2014, 118:11220–11230. [PubMed: 25153942]
49. Pryor NE, Moss MA, Hestekin CN. Unraveling the early events of amyloid-beta protein (Abeta) aggregation: techniques for the determination of Abeta aggregate size. *Int J Mol Sci* 2012, 13:3038–3072. [PubMed: 22489141]
50. Doherty CPA, Young LM, Karamanos TK, Smith HI, Jackson MP, Radford SE, Brockwell DJ. A peptide-display protein scaffold to facilitate single molecule force studies of aggregation-prone peptides. *Protein Sci* 2018, 27:1205–1217. [PubMed: 29417650]
51. Osterlund N, Kulkarni YS, Misiaszek AD, Wallin C, Kruger DM, Liao Q, Mashayekhy Rad F, Jarvet J, Strodel B, Warmlander S, et al. Amyloid-beta Peptide Interactions with Amphiphilic Surfactants: Electrostatic and Hydrophobic Effects. *ACS Chem Neurosci* 2018, 9:1680–1692. [PubMed: 29683649]
52. Osterlund N, Luo J, Warmlander S, Graslund A. Membrane-mimetic systems for biophysical studies of the amyloid-beta peptide. *Biochim Biophys Acta Proteins Proteom* 2019, 1867:492–501. [PubMed: 30468884]
53. Knight PD, Karamanos TK, Radford SE, Ashcroft AE. Identification of a novel site of interaction between ataxin-3 and the amyloid aggregation inhibitor polyglutamine binding peptide 1. *Eur J Mass Spectrom (Chichester)* 2018, 24:129–140. [PubMed: 29334808]
54. Chen M, Wolynes PG. Aggregation landscapes of Huntingtin exon 1 protein fragments and the critical repeat length for the onset of Huntington's disease. *Proc Natl Acad Sci U S A* 2017, 114:4406–4411. [PubMed: 28400517]
55. Jayaraman M, Thakur AK, Kar K, Kodali R, Wetzel R. Assays for studying nucleated aggregation of polyglutamine proteins. *Methods* 2011, 53:246–254. [PubMed: 21232603]
56. Sokolovski M, Sheynis T, Kolusheva S, Jelinek R. Membrane interactions and lipid binding of casein oligomers and early aggregates. *Biochim Biophys Acta* 2008, 1778:2341–2349. [PubMed: 18675247]
57. Burke KA, Kauffman KJ, Umbaugh CS, Frey SL, Legleiter J. The interaction of polyglutamine peptides with lipid membranes is regulated by flanking sequences associated with huntingtin. *J Biol Chem* 2013, 288:14993–15005. [PubMed: 23572526]
58. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J Cheminform* 2012, 4:17. [PubMed: 22889332]
59. Jo S, Kim T, Iyer VG, Im W. CHARMM-GUI: a web-based graphical user interface for CHARMM. *J Comput Chem* 2008, 29:1859–1865. [PubMed: 18351591]
60. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. *J Comput Chem* 2005, 26:1781–1802. [PubMed: 16222654]
61. Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I, et al. CHARMM general force field: A force field for drug-like molecules

- compatible with the CHARMM all-atom additive biological force fields. *J Comput Chem* 2010, 31:671–690. [PubMed: 19575467]
62. Jo S, Kim T, Im W. Automated builder and database of protein/membrane complexes for molecular dynamics simulations. *PLoS One* 2007, 2:e880.
63. Allen WJ, Lemkul JA, Bevan DR. GridMAT-MD: a grid-based membrane analysis tool for use with molecular dynamics. *J Comput Chem* 2009, 30:1952–1958. [PubMed: 19090582]
64. Kegel KB, Sapp E, Yoder J, Cuiffo B, Sobin L, Kim YJ, Qin ZH, Hayden MR, Aronin N, Scott DL, et al. Huntingtin associates with acidic phospholipids at the plasma membrane. *J Biol Chem* 2005, 280:36464–36473. [PubMed: 16085648]
65. Drin G, Antonny B. Amphipathic helices and membrane curvature. *FEBS Lett* 2010, 584:1840–1847. [PubMed: 19837069]
66. Hatzakis NS, Bhatia VK, Larsen J, Madsen KL, Bolinger PY, Kunding AH, Castillo J, Gether U, Hedegard P, Stamou D. How curved membranes recruit amphipathic helices and protein anchoring motifs. *Nat Chem Biol* 2009, 5:835–841. [PubMed: 19749743]
67. Cornell RB, Taneva SG. Amphipathic helices as mediators of the membrane interaction of amphitropic proteins, and as modulators of bilayer physical properties. *Current Protein & Peptide Science* 2006, 7:539–552. [PubMed: 17168787]
68. Nuscher B, Kamp F, Mehnert T, Odoy S, Haass C, Kahle PJ, Beyer K. Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study. *J Biol Chem* 2004, 279:21966–21975. [PubMed: 15028717]
69. Jambeck JP, Lyubartsev AP. An Extension and Further Validation of an All-Atomistic Force Field for Biological Membranes. *J Chem Theory Comput* 2012, 8:2938–2948. [PubMed: 26592132]
70. Khakinejad M, Ghassabi Kondalaji S, Tafreshian A, Valentine SJ. 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* 2017, 28:960–970. [PubMed: 28315238]
71. Ranganathan N, Li C, Suder T, Karanji AK, Li X, He Z, Valentine SJ, Li P. Capillary Vibrating Sharp-Edge Spray Ionization (cVSSI) for Voltage-Free Liquid Chromatography-Mass Spectrometry. *J Am Soc Mass Spectrom* 2019, 30:824–831. [PubMed: 30793264]
72. Karanji AK, Khakinejad M, Kondalaji SG, Majuta SN, Attanayake K, Valentine SJ. 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* 2018, 29:2402–2412. [PubMed: 30324261]
73. Ghassabi Kondalaji S, Khakinejad M, Tafreshian A, S JV. Comprehensive Peptide Ion Structure Studies Using Ion Mobility Techniques: Part 1. An Advanced Protocol for Molecular Dynamics Simulations and Collision Cross-Section Calculation. *J Am Soc Mass Spectrom* 2017, 28:947–959. [PubMed: 28211014]
74. Majuta SN, Maleki H, Kiani Karanji A, Attanayake K, Loch E, Valentine SJ. Magnifying ion mobility spectrometry-mass spectrometry measurements for biomolecular structure studies. *Curr Opin Chem Biol* 2018, 42:101–110. [PubMed: 29241076]
75. Kucerka N, Nagle JF, Sachs JN, Feller SE, Pencier J, Jackson A, Katsaras J. Lipid bilayer structure determined by the simultaneous analysis of neutron and X-ray scattering data. *Biophys J* 2008, 95:2356–2367. [PubMed: 18502796]
76. Kucerka N, Nieh MP, Katsaras J. Fluid phase lipid areas and bilayer thicknesses of commonly used phosphatidylcholines as a function of temperature. *Biochim Biophys Acta* 2011, 1808:2761–2771. [PubMed: 21819968]
77. Adegbuyiro A, Sedighi F, Pilkington AWt, Groover S, Legleiter J. Proteins Containing Expanded Polyglutamine Tracts and Neurodegenerative Disease. *Biochemistry* 2017, 56:1199–1217. [PubMed: 28170216]
78. Burke KA, Godbey J, Legleiter J. Assessing mutant huntingtin fragment and polyglutamine aggregation by atomic force microscopy. *Methods* 2011, 53:275–284. [PubMed: 21187152]
79. Murzyn K, Rog T, Pasenkiewicz-Gierula M. Phosphatidylethanolamine-phosphatidylglycerol bilayer as a model of the inner bacterial membrane. *Biophys J* 2005, 88:1091–1103. [PubMed: 15556990]

80. Drin G, Casella JF, Gautier R, Boehmer T, Schwartz TU, Antonny B. A general amphipathic alpha-helical motif for sensing membrane curvature. *Nat Struct Mol Biol* 2007, 14:138–146. [PubMed: 17220896]
81. Bhattacharyya A, Thakur AK, Chellgren VM, Thiagarajan G, Williams AD, Chellgren BW, Creamer TP, Wetzel R. Oligoproline effects on polyglutamine conformation and aggregation. *J Mol Biol* 2006, 355:524–535. [PubMed: 16321399]
82. Jayaraman M, Kodali R, Sahoo B, Thakur AK, Mayasundari A, Mishra R, Peterson CB, Wetzel R. Slow amyloid nucleation via alpha-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. *J Mol Biol* 2012, 415:881–899. [PubMed: 22178474]
83. Jayaraman M, Mishra R, Kodali R, Thakur AK, Koharudin LM, Gronenborn AM, Wetzel R. Kinetically competing huntingtin aggregation pathways control amyloid polymorphism and properties. *Biochemistry* 2012, 51:2706–2716. [PubMed: 22432740]
84. Thakur AK, Jayaraman M, Mishra R, Thakur M, Chellgren VM, Byeon IJ, Anjum DH, Kodali R, Creamer TP, Conway JF, et al. Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nat Struct Mol Biol* 2009, 16:380–389. [PubMed: 19270701]
85. Tam S, Spiess C, Auyeung W, Joachimiak L, Chen B, Poirier MA, Frydman J. The chaperonin TRiC blocks a huntingtin sequence element that promotes the conformational switch to aggregation. *Nat Struct Mol Biol* 2009, 16:1279–1285. [PubMed: 19915590]
86. Wieprecht T, Apostolov O, Beyermann M, Seelig J. Interaction of a mitochondrial presequence with lipid membranes: role of helix formation for membrane binding and perturbation. *Biochemistry* 2000, 39:15297–15305. [PubMed: 11112515]

Figure 1.

Full range mass spectrum of the peptide-DOPC system. For labeling purposes, a large red circle represents a single Nt17 peptide, a green triangle represents a single DOPC lipid molecule. Insets show expanded regions for a number of peptide-lipid complex ions. Ion identities are shown as symbol representations.

Figure 2.

A) Theoretical area per lipid calculations of the 100-member lipid bilayer for the DOPC, POPC and POPE. The linear relationship shows that the average area per lipid number for each DOPC, POPC, and POPE are 68.59, 65.12, 57.26 respectively. B) the relative degree of peptide helicity as a function of simulation time for all three lipid systems. Here the initial structure for the Nt17 peptide was completely random coil.

Figure 3. Schematic representations of random coil Nt17 peptide interacting with the DOPC bilayer. A) The Nt17 peptide in solution having no contact with the bilayer. B) The peptide interacting with the bilayer surface after 93 ns simulation time. C) DOPC bilayer inducing some peptide helical formation. D) The Nt17 peptide leaves the bilayer surface with ~65% helicity at a time of 120 ns. The peptides and DOPC lipid bilayer are shown with the “new cartoon” and “surf” drawing models, respectively.

Figure 4.

Heat maps showing the distance between α -carbons of each residue on the Nt17 peptide to the center of the bilayer as a function of MD simulation time. Panels A, B and B the results for the DOPC, POPC and the POPE bilayer systems, respectively. The legend in the figure shows the distance (Å) represented by each color.

Figure 5.

Molecular representations of the position/orientation of the Nt17 peptide on the surface of (A) the DOPC lipid bilayer, and (B) the POPE lipid bilayer. The peptides are shown with the ribbon rendering of the polypeptide backbone. Hydrophobic residues interacting with the DOPC lipids are rendered with the “licorice” drawing method and hydrophilic residues are represented by the “CPK” model. The lipid bilayer is depicted as an electron cloud rendering and the lipid head groups are emphasized. Panels A and B show results for the MD simulations for the DOPC and POPE lipid systems, respectively. Note that the DOPC system

requires visualization from a top-down perspective because a side-on visualization does not reveal the peptide which is essentially interspersed within the lipid head groups. Schematic representations of the Nt17 peptide interacting with lipid molecules of the DOPC and POPE lipid systems are provided in panels C and D, respectively. The peptides are shown with the ribbon rendering of the polypeptide backbone. Hydrophobic residues interacting with the DOPC lipids are rendered with the “licorice” drawing method and hydrophilic residues are represented by the “CPK” model. Interacting residues are labeled. A distance measurement is used to represent a hydrogen bond in the POPE system.

Table 1.

Assigned compounds and their corresponding mass to charge ratio

Ion ^a	m/z ^b	Charge state	Ion ^a	m/z ^b	Charge state	Ion ^a	m/z ^b	Charge state
Nt17 + 1DOPC	2760.64	1	Nt17 + 1POPC	2734.63	1	Nt17 + 1POPE	2692.59	1
Nt17 + 1DOPC	1380.82	2	Nt17 + 1POPC	1367.81	2	Nt17 + 1POPE	1346.79	2
1Nt17 + 2DOPC	3546.24	1	Nt17 + 2POPC	1747.61	2	Nt17 + 2POPE	1705.57	2
Nt17 + 2DOPC	1773.62	2	Nt17 + 3POPC	2127.40	2	Nt17 + 3POPE	2064.34	2
Nt17 + 3DOPC	2166.42	2	Nt17 + 4POPC	2507.2	2	Nt17 + 4POPE	2423.12	2
Nt17 + 4DOPC	2559.22	2	Nt17 + 5POPC	2886.99	2	Nt17 + 5POPE	2781.89	2
Nt17 + 5DOPC	2952.02	2	Nt17 + 6POPC	3266.79	2	2Nt17 + 1POPE	2333.81	2
Nt17 + 6DOPC	3344.82	2	2Nt17 + 1POPC	2354.83	2	2Nt17 + 1POPE	1556.21	3
Nt17 + 7DOPC	3737.62	2	2Nt17 + 1POPC	1570.22	3	2Nt17 + 2POPE	1795.39	3
2Nt17 + 1DOPC	2367.84	2	2Nt17 + 2POPC	1823.42	3	2Nt17 + 3POPE	2034.57	3
2Nt17 + 1DOPC	1578.89	3	2Nt17 + 3POPC	2076.61	3			
2Nt17 + 2DOPC	2760.84	2	2Nt17 + 4POPC	2329.81	3			
2Nt17 + 2DOPC	1840.76	3	2Nt17 + 5POPC	2583.01	3			
2Nt17 + 3DOPC	3153.44	2	3Nt17 + 1POPC	2228.23	3			
2Nt17 + 3DOPC	2102.62	3	3Nt17 + 2POPC	2481.43	3			
2Nt17 + 4DOPC	3546.24	2	3Nt17 + 1POPC	1671.42	4			
2Nt17 + 4DOPC	2364.49	3	3Nt17 + 2POPC	1861.32	4			
2Nt17 + 5DOPC	3939.04	2	4Nt17 + 1POPC	2164.93	4			
2Nt17 + 5DOPC	2626.36	3						
2Nt17 + 5DOPC	1970.02	4						
2Nt17 + 6DOPC	2888.22	3						
2Nt17 + 7DOPC	3150.09	3						
2Nt17 + 8DOPC	3411.96	3						
3Nt17 + 1DOPC	3354.86	2						
3Nt17 + 1DOPC	2236.90	3						
3Nt17 + 1DOPC	1677.93	4						
3Nt17 + 2DOPC	2498.77	3						
3Nt17 + 2DOPC	1874.33	4						
3Nt17 + 3DOPC	2070.73	4						
3Nt17 + 4DOPC	3022.50	3						
3Nt17 + 4DOPC	2267.13	4						
3Nt17 + 5DOPC	3284.37	3						
3Nt17 + 5DOPC	2463.53	4						
3Nt17 + 6DOPC	2659.93	4						
3Nt17 + 7DOPC	2856.33	4						
3Nt17 + 8DOPC	3052.73	4						
4Nt17 + 1DOPC	2894.92	3						
4Nt17 + 1DOPC	2171.44	4						

^aThe following average masses has been used to assign the peaks: 1974.04 for Nt17 peptide, 785.60 for DOPC lipid and 717.54 for POPE lipid.

Bold lines were assigned in the control experiment (when the peptide and lipids were co-mixed) as well as the experiment in which the lipid vesicles were formed.

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