SUPPORTING INFORMATION

Interaction of Huntingtin Exon-1 peptides with lipid-based micellar nanoparticles probed by solution NMR and Q-band pulsed EPR

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Supplementary methods, 1 table and 12 figures
**Cloning, expression and purification of htt\textsuperscript{NTQ}_n peptides.** The plasmid encoding for the N-terminal domain of Htt-exon 1 (ATLEKLMKAFESLKSF) with 7 or 10 consecutive C-terminal poly-Q repeats (abbreviated as htt\textsuperscript{NTQ}_n where the superscript ‘NT’ denotes the N-terminal part only, and \( n \) is the number of C-terminal glutamines) was cloned into the GB1-fusion expression vector pET-21d(+) (Novagen) containing a Factor Xa cleavage site upstream of the peptide sequence. Note that the N-terminal methionine is removed during expression in *E. coli* and hence the sequence starts at Ala-1. A1C, S12C and S15C site specific mutations were introduced using the QuikChange kit (Agilent Technologies). The GB1-htt\textsuperscript{NTQ}_n fusion proteins were expressed in *E. coli* BL21 (DE3) cells grown in M9 minimal medium. Induction of protein over-expression was initiated by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 20°C. After induction, the cells were grown for 16 h, and harvested by centrifugation at 3500 x g at 4°C for 25 min. The cell pellet was re-suspended in 50 mM Tris-HCl buffer, pH 8.0 and 100 mM NaCl. Cell lysis was accomplished through temperature treatment (10 min at 80 °C). Following incubation on ice for 10 min, the suspension was centrifuged at 20,000 x g at 4°C for 30 min. The htt\textsuperscript{NTQ}_n peptides were cleaved off the GB1-htt\textsuperscript{NTQ}_n fusion protein by overnight incubation with Factor Xa (New England Biolabs) in 50 mM Tris-HCl buffer, pH 8.0, 100 mM NaCl and 2 mM CaCl\(_2\).

The GB1 tag and undigested fusion protein were removed by reverse phase high pressure liquid chromatography (HPLC) in an acetonitrile/trifluoroacetic acid mixture using a C4 column (250 x 22 mm, 10 µm particle size, Protein C4, Grace Vydac) on an AKTA Purifier system (GE Healthcare). Absorbance of the eluate was monitored at 210 nm. To prevent the formation of disulfide bonded oligomers, the samples carrying cysteine mutations were incubated at room temperature for 30 min with 2 mM dithiothreitol (DTT) prior to HPLC separation. A disaggregation procedure involving dissolution of peptides in a mixture of trifluoroacetic acid (TFA) and hexafluoroisopropanol (HFIP) was carried out as described previously,\(^2\) prior to lyophilization of the peptide solutions. Completion of the cleavage reaction was verified by liquid phase chromatography coupled with mass spectrometry (LC-MS).

**NMR samples.** Samples for NMR spectroscopy comprised uniformly \(^{15}\)N- or \(^{15}\)N/\(^{13}\)C-labeled samples of htt\textsuperscript{NTQ}_n peptides. Isotope labeling was obtained by using 1 g/L \(^{15}\)NH\(_4\)Cl and 3 g/L of \(^{13}\)C\(_6\)-D-glucose (Cambridge Isotope Laboratories, CIL) as the respective sole nitrogen and carbon sources in M9 minimal medium. The peptide was dissolved in 20 mM phosphate buffer, 10% D\(_2\)O/ 90% H\(_2\)O, pH 6.5, and 50 mM NaCl. Protein concentrations were determined by UV absorption measurements at 205 nm.\(^3\)
Nitroxide spin-labeling and EPR sample preparation. To prevent dimerization, cysteine-containing mutants of the htt\textsuperscript{NT}Q\textsubscript{n} peptides (A1C, S12C and S15C) were initially dissolved in 2 M Guanidine-HCl, pH 2.5. Immediately after dialysis the pH was restored to 8.0 by addition of 1 M Tris-HCl buffer, and a 10-fold molar excess of the nitroxide spin-label (R1), 1-oxyl-2,2,5,5-tetramethyl-δ-3-pyrroline-3-methylmethanethiosulphonate (MTSL; Toronto Research Chemicals), was added. The nitroxide labeling reaction was allowed to proceed for 2 h at 4°C. Unreacted MTSL was separated on a Superdex 10/300 GL column (GE Healthcare) pre-equilibrated with 15% (v/v) acetonitrile. HPLC fractions were lyophilized and stored in the dark at -20°C. Prior to EPR measurements, the nitroxide-labeled peptide was dissolved in 20 mM phosphate buffer, pH 6.5, and 50 mM NaCl, and diluted to a final EPR sample concentration (80 μM) with 30% (v/v) glycerol. (Note that glycerol is required to ensure that the samples freeze in a homogeneous manner). Labeling efficiency was verified by liquid chromatography-positive ion electron spray mass spectrometry (Waters Model LCT). In each instance only a single species was observed.

Methionine oxidation of htt\textsuperscript{NT}Q\textsubscript{n} peptides. The methionine-sulfoxide form (Met\textsuperscript{7}O) of the htt\textsuperscript{NT}Q\textsubscript{n} peptides was obtained by incubating the GB1-htt\textsuperscript{NT}Q\textsubscript{n} fusion proteins with 4% (v/v) H\textsubscript{2}O\textsubscript{2} (Sigma) for 2 h. Complete removal of H\textsubscript{2}O\textsubscript{2} was accomplished by exhaustive dialysis against a 50 mM Tris-HCl buffer, pH 8.0 and 100 mM NaCl, prior to addition of Factor Xa protease. Oxidation of MTSL-labeled cysteine htt\textsuperscript{NT}Q\textsubscript{n} mutants was achieved in a similar manner, except that the excess of H\textsubscript{2}O\textsubscript{2} was removed using Superdex 10/300 GL column (GE Healthcare). Oxidation of Met\textsuperscript{7} to a sulfoxide results in a +16 Da addition to the molecular mass of the respective peptides as verified by mass spectrometry.

Preparation and characterization of lipid micelles. A micellar stock solution was prepared by dissolving 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phospho-(1'-rac-glycerol) (LPG) lipids in a 3:1 molar ratio in 20 mM phosphate buffer, pH 6.5 and 50 mM NaCl, to a final concentration of 48 mM (in total lipids). The micelle dispersions were stored in the dark at 4°C. The size distribution of micelles was determined by dynamic light scattering (DLS) measurements using a Malvern Zetasizer Nano ZS instrument, (Malvern Instruments, UK) operating at a wavelength of 633 nm (10 °C). The autocorrelation functions of the scattered intensity were interpreted by cumulant analysis to yield an effective diameter of 8 nm with a polydispersity index of ~0.15. The average of five DLS measurements is shown in Figure S2A.
The molecular mass of the monodisperse micelle solution was determined by size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) optimized for differential refractive index detection (Wyatt-925-H2, Wyatt Technology Inc., Santa Barbara, CA) (Figure S2B). A 100 µl aliquot of a 48 mM lipid solution was injected onto a pre-equilibrated Superdex-200 10/300 column (GE Healthcare). Elution was carried out with 20 mM phosphate buffer, pH 6.5 and 50 mM NaCl, under the conditions of supra-critical micelle concentration (~0.6 mM). A value of 0.136 mL/g was chosen as the lipid refractive index increment.4

**Thioflavin T (Tht) assay.** The time-course of httNTQn peptide aggregation (and cross β-sheet formation seen in amyloid-like fibers) in the presence of micelles was monitored using a Tht assay as follows. 25 µM httNTQ10 (or Met’O-httNTQ10) was incubated with 25 µM Tht and 50 µM LPC/LPG (3:1 molar ratio) in a 96-well microtiter plate. The plates were sealed with parafilm to prevent evaporation and incubated at 4°C between measurements. ThT fluorescence measurements were carried out using an Infinite M200 Pro (Tecan, Switzerland) plate reader with an excitation wavelength of 440 nm and emission wavelengths from 460 to 550 nm. The recorded values were averaged and background fluorescence (25 µM of ThT only) subtracted. The data are reported in Fig. S1B.

**Circular Dichroism (CD) measurements.** CD spectra were recorded on a JASCO J-810 spectropolarimeter and data were analysed using the Spectra Manager Software. Far-UV spectra (200 - 250 nm) were recorded in 20 mM phosphate buffer, pH 6.5 and 50 mM NaCl at 10 °C using a 0.1 cm path-length flat cell. The measured values of ellipticity (θ) were converted to mean residue ellipticity [θ]. Spectral changes at 222 nm (Δ[θ]222) (after subtraction of the contribution to ellipticity from the random coil peptide) were used to characterize the binding of httNTQn peptides to micelles.

The change in the mean residue molar ellipticity (Δ[θ]222) upon addition of micelles is given by Δ[θ]222 = pB×[θ]222,B, where [θ]222,B is the mean residue molar ellipticity at 222 nm for the bound peptide, and pB the fraction of the membrane-bound peptide. Since 15 and 17 residues of httNTQ7 and (Met’O)-httnTQ7, respectively, out of a total of 23 residues contribute to helix formation, as judged from the backbone NMR chemical shifts (see Figure 2 of the main text), it follows that [θ]222,B < [θ]222,max, where [θ]222,max corresponds to the mean residue ellipticity for a 100% helical peptide estimated from the relationship,5

\[
[θ]_{222,\text{max}} = (-44000 + 250T)(1 - k/N)
\]
where \( T \) is the temperature (in degrees Celsius, 10°C), \( N \) the number of residues (23 and 26 for htt\textsuperscript{NTQ_7} and htt\textsuperscript{NTQ_10}, respectively), and \( k \) a correction factor (3 for carboxyamidated peptides).\(^6\) The estimated values of \([\theta]_{222,\text{max}}\) for the htt\textsuperscript{NTQ_7} and htt\textsuperscript{NTQ_10} are -3.6x10\(^4\) and -3.7x10\(^4\) deg.cm\(^2\).dmol\(^{-1}\), respectively. The optimized values of \([\theta]_{222,\text{B}}\) are -2.3(±0.3)x10\(^4\) and -2.2(±0.1)x10\(^4\) deg.cm\(^2\).dmol\(^{-1}\) for htt\textsuperscript{NTQ_7} and htt\textsuperscript{NTQ_10}, corresponding to ~15 helical residues. The corresponding values for the Met\(^7\)-sulfoxide forms are -2.6(±0.3)x10\(^4\) and -2.8(±0.9)x10\(^4\) deg.cm\(^2\).dmol\(^{-1}\), respectively, corresponding to ~17 and ~20 helical residues.

**Pulsed Electron Spin Resonance (EPR) spectroscopy.** Pulsed EPR data were collected at Q-band (33.8 GHz) at a temperature of 50 K on a Bruker E-580 spectrometer equipped with a 150 W traveling-wave tube amplifier, a model ER5107D2 resonator, and a cryo-free cooling unit. Samples were placed in 1 mm internal diameter quartz tubes (Wilmad WG-221T-RB) and flash frozen in liquid nitrogen.

The observe and pump pulses were separated by ~85 MHz. The lengths of the observe \( \pi \) and \( \pi/2 \) pulses were 24 and 12 ns, respectively; the length of the ELDOR \( \pi \) pump pulse was 10 ns. The pump frequency was centered at the maximum of the Q-band nitroxide spectrum located at +40 MHz from the center of the resonator frequency. The \( \tau_1 \) value (350 ns) of the first echo period (2\( \tau_1 \)) was incremented ten times in 2 ns increments to average out \(^1\)H modulation; the position of the pump pulse was incremented in steps (\( \Delta \tau \)) of 2 ns . (Note that the data points in the DEER echo curves in Figures S5 to S8 are plotted at \( \Delta \tau \) intervals of 8 ns). The bandwidth of the overcoupled resonator was ~120 MHz. Data collection during the second echo period (2\( \tau_2 \)) was not carried out over the full \( \tau_2 \) range because of a persistent “2+1” echo perturbation of the DEER echo curve at a time of about \( \tau_1 \) from the final observe \( \pi \) pulse. The pulse gate time used for echo integration was 32–38 ns. Because of the short phase memory time \( T_m \) (~1.2 μs) of the samples arising from the use of protonated lipids, the maximum dipolar evolution time \( t_{\text{max}} \) was set to 0.6-3 μs.

Inversion-modulation DEER (IM-DEER) experiments to obtain the dependence of the normalized modulation depth (\( \Delta/\Delta_{\text{max}} \)) as a function of ELDOR pulse flip angle (\( \theta \) ~ 35° to 180°) were carried out exactly as described previously.\(^7\) The length of the 180° ELDOR pulse as a function of attenuation and the maximum inversion efficiency (\( \lambda_{\text{max}} \)) were determined using a spin-echo nutation experiment exactly as described previously.\(^7\) The attenuation settings for the ELDOR pulse were restricted to experimental \( \lambda_{\text{max}} \) values ≥ 0.7.\(^7\) The dependence of \( \Delta(\theta)/\Delta_{\text{max}} \) (where \( \Delta_{\text{max}} \) is the modulation depth at \( \theta = 180° \)) on pulse flip angle \( \theta \) and number of nitroxide spins \( N \) is given by: \(^7,8\)
Analysis of DEER and IM-DEER data. Q-band DEER four-pulse echo curves were analyzed using the program DeerAnalysis 2016. Background correction of the raw DEER echo curves to remove contributions to the signal decay arising from intermolecular interactions was carried out with a polynomial of order 3 (Fig. S5) rather than the exponential function conventionally used to model a homogeneous three-dimensional distribution of spin pairs. This was necessary because the intermolecular interactions include non-homogenous distributions of spin pairs arising from the presence of multiple peptide particles (either monomer and/or dimer) bound to a single micelle and from possible aggregation/clustering of micelles during the flash freezing process. The same phenomenon is observed when size exclusion effects become significant. The impact of polynomial baseline subtraction on the resulting distribution of residuals between the experimental DEER echo curves and those calculated following validated Tikhonov regularization, and on the corresponding \( P(r) \) distance distributions are shown in Fig. S5. The results of baseline correction with polynomials of order 3 and 4 are essentially identical and yield a random distribution of residuals together with a single major \( P(r) \) distance distribution corresponding to the inter-spin distance within the micelle-bound dimer. Baseline correction with polynomials of lower order (1 and 2) result in a non-random distribution of residuals and the presence of artifacts at longer distances in the corresponding \( P(r) \) distributions.

Modulation depths are directly obtained from the intercept of the polynomial baseline subtraction function at zero dipolar evolution time. Validated Tikhonov regularization was carried out by varying the modulation depth (11 steps), background density (11 steps) and background start (11 steps) for a total of 1331 permutations. The Tikhonov regularization parameter \( \alpha \) was automatically determined by DeerAnalysis for each iteration and ranged in value from 10 to 100.

Analysis of CD and EPR binding data. The binding of htt\(^{NTQ_n} \) peptides to micelles can be described by a simple equilibrium, \( P_F + L_F \rightleftharpoons P_B \), where \( P_F \) and \( L_F \) are free peptide and lipids, respectively, and \( P_B \) is the micelle-bound peptide. The fraction of the bound species \( p_B \) can be described by the Hill equation,

\[
p_B = \frac{[L_F]^n}{([L_F]^n + K_D^n)} \quad \text{(S3)}
\]

where \( K_D \) is the dissociation constant (in units of lipid concentration), and \( n \) the Hill coefficient. The latter accounts for deviations from a hyperbolic two state binding
isotherm at low lipid concentrations observed for htt<sup>NTQ<sub>7</sub></sup> and htt<sup>NTQ<sub>10</sub></sup> (Figure 3; main text). The value of [L<sub>F</sub>] in Eq. S3 was approximated by the total lipid concentration [L<sub>tot</sub>] which is reasonable for a binding stoichiometry of 1 peptide per lipid head group (see below). A value of the exponent \( n \) in Eq. S3 different from unity is phenomenologically similar to binding cooperativity exhibited by proteins with multiple binding sites and can possibly be attributed to exclusion of micelle surface area available for binding and/or repulsive interactions between surface-bound peptide molecules - both effects are expected to be more pronounced with increasing saturation of the micelle surface (at low lipid concentrations) and unfavorable for binding. The values of \( n \) obtained from the fits of the CD binding curves decrease steeply with decreasing affinity for micelles: from 1.74 for htt<sup>NTQ<sub>7</sub></sup> (\( K_D = 0.54 \) mM) to 1.12 in htt<sup>NTQ<sub>10</sub></sup> (\( K_D = 1.02 \) mM), while a value of \( n = 1 \) to within experimental error is obtained for the Met<sup>7</sup>O forms of htt<sup>NTQ<sub>7</sub></sup> and htt<sup>NTQ<sub>10</sub></sup> (\( K_D = 2.34 \) mM and 2.61 mM, respectively).

Although the stoichiometry of binding (defined as the number of lipid head groups \( F \) occupied by each molecule of htt<sup>NTQ<sub>n</sub></sup> on the micelle surface, that enters into the calculation in Eq S3 as \([L_F] = [L_{tot}] - F \cdot [P_{tot}] \) (where \([P_{tot}] \) is total protein concentration and \( p_B \) can be estimated approximately from the experimental CD data) is not known with certainty, very similar values of \( K_D \) are derived from the CD data fits irrespective of whether a molecule of the peptide binds to 1, 2 or 3 lipid head groups. Based on the length of the helix (~23 Å) for bound htt<sup>NTQ<sub>n</sub></sup> in relation to the diameter of a single head group (~10 Å), the factor \( F \) is unlikely to exceed ~3.

Analysis of observed normalized modulation depth (\( \Delta \theta/\Delta \theta_{180} \)) as a function of ELDOR pulse flip angle \( \theta \) obtained from the IM-DEER experiment indicates the presence of a dimers of htt<sup>NTQ<sub>7</sub></sup> and htt<sup>NTQ<sub>10</sub></sup> on the surface of the micelles (Figure 4A; main text). The three-state equilibrium used to analyze the dimerization of htt<sup>NTQ<sub>n</sub></sup> on the micellar surfaces is shown in Figure 4C (main text). Briefly, the free monomorphic state of htt<sup>NTQ<sub>n</sub></sup>, \( P_F^m \), binds to the micelle surface with a dissociation constant \( K_D \) (as determined from the CD data). The bound monomorphic species can reversibly dimerize on the micelle surface, with an equilibrium constant \( K_{eq} \) describing the equilibrium between the bound monomorphic species (\( P_B^m \)) and the bound dimeric species (\( P_B^d \)). The fraction of the total bound species (\( p_B \); defined for the two-state binding characterized by CD above) is partitioned between the monomorphic and dimeric forms according to the relationship,

\[
\{P_B^d\} = K_{eq} \{P_B^m\}^2
\]  
(S4)
where the curly brackets ‘{ }’ denote the concentration (number) of peptide particles of each type (bound monomeric or bound dimeric) per micelle. Assuming that the distribution of bound peptide particles is uniform among all available micelles, the average number of peptide particles of each type is given by: 

\[ \{P_{Bi}\} = N \times p_{Bi}' \times [P_{tot}] / [L_{tot}] \]

where \( p_{Bi}' \) is the fraction (population) of bound peptides of each type, \( i \in (‘m’, ‘d’) \), and \( N \) is the number of lipids forming a micelle (\( N = 183 \) as calculated from SEC-MALS measurements reported in Fig. S2B). Using Eq S4 and bearing in mind that \( \{P_{Bi}\} + 2\{P_{Bi}'\} = \{P_{B}\} \), where \( \{P_{B}\} \) is the total number of bound peptide particles per micelle (calculated from \( p_{B} \) as above), we obtain the following expressions for the average number of bound peptides in each oligomeric state,

\[ \{P_{B}^m\} = \frac{\sqrt{1 + 8K_{eq} \{P_{B}\}} - 1}{4K_{eq}} \quad (S5.1) \]

and

\[ \{P_{B}^d\} = \frac{\{P_{B}\} + 1 - \sqrt{1 + 8K_{eq} \{P_{B}\}}}{2} \quad (S5.2) \]

Once the average number of peptide particles on micellar surfaces is calculated using Eqs. S5.1-S5.2, \( P_{Bi}^m \) and \( P_{Bi}^d \) can be re-cast as the fractions (populations) of the total peptide present in solution according to: \( p_{Bi}' = ([L_{tot}] / N[P_{tot}])\{P_{Bi}\} \). We note that alternative units for concentration of peptide particles on the micelle surface can be chosen. For example, instead of the number of peptide molecules per micelle, the number of peptides per surface area of a micelle (~200 nm²) could be used which would result in a corresponding 200-fold up-scaling of the derived values of \( K_{eq} \).

The modulation depth, \( \Delta \), measured in DEER experiments for a series of spin-labeled htt\(^{N\text{T}}\)Q\(_{n}\) samples with different total lipid concentrations, is a good quantitative reporter of dipolar interactions between (proximal) electron spins present in the dimeric peptide species under conditions when exchange between the different species is effectively quenched (by freezing to 50 K). The value of \( \Delta \) at each lipid concentration is therefore directly proportional to the fraction of dimeric bound peptides present in each sample, \( p_{B}^d \). Small but non-zero modulation depths (\( \Delta \)) measured in the absence of lipids ([\( L_{tot} = 0 \)]) for both htt\(^{N\text{T}}\)Q\(_{7}\) and htt\(^{N\text{T}}\)Q\(_{10}\) (Figure 4D; main text) might arise from the presence of an additional low populated oligomeric state in equilibrium with the main monomeric free state \( P_{F}^m \). To account for this (small) offset of the modulation depth, we introduced a semi-empirical correction \( \kappa \) to the calculated modulation depths,

\[ \kappa = \Delta_0 (1 - p_{B}^m - 2p_{B}^d)^2 \quad (S6) \]
where $\Delta_0$ is the modulation depth measured in the absence of lipids ($\Delta_0 = 0.01$ and 0.07 for htt$^\text{NT}Q_7$ and htt$^\text{NT}Q_{10}$, respectively). The relationship in Eq. S6 is approximate and was derived under the assumption that the additional state is an ‘off-pathway’ dimer that exchanges with the free monomeric species $P_t^m$. It is straightforward to show that under these assumptions the population of the free dimeric species is proportional to $(p_n^m)^2 \sim (1-p_n^m - 2p_n^m)^2$, where the sum in the brackets is a good approximation to $p_n^m$ provided that the concentration of the additional (dimeric) free species is small and can be neglected. Note that per Eq. S6, the correction $\kappa$ decays rapidly with increasing $[L_{\text{tot}}]$ (Figure S11), but is relatively small even at low lipid concentrations. For example, for $[L_{\text{tot}}] = 1.2$ mM, the values of $\kappa$ are ~0.4 and ~5% of the measured $\Delta$ for htt$^\text{NT}Q_7$ and htt$^\text{NT}Q_{10}$, respectively.

Bearing in mind the proportionality of the measured $\Delta$ to $p_n^d$, as well as all the other considerations listed above, the calculated value of $\Delta$ is given by,

$$
\Delta = A \left( \frac{p_n^d}{2} - \frac{1}{8K_{eq}N[P_{\text{tot}}]} + \frac{1 + 8K_{eq}(N[P_{\text{tot}}]/[L_{\text{tot}}])p_n^d}{8K_{eq}N[P_{\text{tot}}]}[L_{\text{tot}}] \right) + \kappa + B 
$$

(S7)

where the correction factor $\kappa$ is calculated using Eq. S6, and $K_{eq}$, $A$ (proportionality constant) and $B$ (offset) are adjustable parameters. As the DEER experiments were conducted at total lipid concentrations $[L_{\text{tot}}]$ different from those used for CD measurements, the values of $p_n^d$ have to be re-calculated using Eq. S3, with $[L_{\text{tot}}]$ assumed to be equal to $[L_{\text{tot}}]$ and the values of $n$ and $K_D$ established for each htt$^\text{NT}Qn$ peptide from CD measurements, leading to the following expression for $\Delta$,

$$
\Delta = A \left[ K'[L_{\text{tot}}] \left( 1 - \frac{[L_{\text{tot}}]^{-1}}{K'([L_{\text{tot}}]^n + K_D^n)} \right) + \frac{[L_{\text{tot}}]^n}{2([L_{\text{tot}}]^n + K_D^n)} \right] + \kappa + B 
$$

(S8)

where $K' = (8K_{eq}N[P_{\text{tot}}])^{-1}$. As can be appreciated from Eq. S8, the relationship between $\Delta$ and $[L_{\text{tot}}]$ is quite complex. Numerical simulations using Eq. S8 for the range of $[L_{\text{tot}}]$ values used for the DEER experiments, show that for certain combinations of $K_{eq}$ (fitted parameter in our analysis) and $K_D$ (derived from the CD data), a characteristic, well-defined maximum can be obtained in the plots of $\Delta$ vs $[L_{\text{tot}}]$, consistent with experimental observations in Figure 4D (main text). More detailed analysis of Eq. S8 using an analytical expression for the derivative $d\Delta/d[L_{\text{tot}}]$ shows that the value of $K_{eq}$ is very sensitive to the position of the maximum (the value of $[L_{\text{tot}}]$ for which $d\Delta/d[L_{\text{tot}}] = 0$; i.e. for a given $K_D$, higher values of $K_{eq}$ correspond to the
maximum shifted to higher \([L_{\text{tot}}]\) values. In a similar manner, higher values of the exponent \(n\) for a given set of \((K_D; K_{eq})\) values correspond to higher \([L_{\text{tot}}]\) at the maximum \(\Delta\).

Equations can also be derived for cases when the bound species is partitioned between monomers and higher order oligomers on the micelle surface. By way of an example, when trimers \((P_B^t)\) are formed, Eqs. S4-S5 are transformed to,

\[
\{P_B^m\} = \left(\frac{\{P_B^t\}}{6 K_{eq}} + \sqrt{(9 K_{eq})^{-3} + \left(\frac{\{P_B^t\}}{6 K_{eq}}\right)^2}\right)^{\frac{1}{3}} - \\
(9 K_{eq})^{-1}\left(\frac{\{P_B^t\}}{6 K_{eq}} + \sqrt{(9 K_{eq})^{-3} + \left(\frac{\{P_B^t\}}{6 K_{eq}}\right)^2}\right)^{\frac{1}{3}}
\]  

(S9.1)

and,

\[
\{P_B^t\} = K_{eq} \left(\frac{\{P_B^m\}}{K_{eq}}\right)^3
\]  

(S9.2)

The rest of the operations to obtain expressions for \(P_B^t\) and \(\Delta\) remain the same as in the case of a dimer.

The set of optimized parameters used in the minimization of the target function constructed of the squared differences between the experimental value of \(\Delta\) and those calculated using Eq S8, comprised: \([K_{eq}; A; B]\). The minimization was performed using an in-house MatLab program (MathWorks Inc. MA) and the uncertainties in the values of the optimized parameters, corresponding to confidence intervals of \(\pm 1\) standard deviation were determined from the Jacobian matrix of the non-linear fit.

The modulation depths \((\Delta)\) measured in DEER experiments on the Met\(^7\)O forms of htt\(^{NTQ_n}\) are smaller in magnitude than those observed for native htt\(^{NTQ_n}\). As a result, the order of the oligomer for the Met\(^7\)O peptides on the micelle surface could not be determined unequivocally by IM-DEER (see Fig. S12A). Although the same general trends in the dependence of \(\Delta\) versus \([L_{\text{tot}}]\) were observed for the Met\(^7\)O peptides (Fig. S12B), we chose not to interpret these data quantitatively. Merely by way of an example, the modulation depth of DEER echo curves for (Met\(^7\)O)-htt\(^{NTQ_7}\) as a function of lipid concentration is fitted to the model with the bound species partitioned between monomers and trimers (solid curve in Fig. S12B; see Eqs. S9.1-9.2).

**NMR spectroscopy.** All NMR experiments were recorded on a Bruker Avance III 700 MHz spectrometer equipped with a z-gradient triple-resonance cryogenic probe operating at 10 °C.
\(^{15}\text{N}-\text{CEST}\) experiments\(^{12}\) were recorded as described earlier\(^{13}\) on 150 \(\mu\text{M}\) \(^{15}\text{N}\)-labeled Hi\(^{NT}\)Q\(_7\) and (Met\(^7\text{O})\)-htt\(^{NT}\)Q\(_7\) samples using 3:1 LPC:LPG (mol/mol) micelles with a protein/l lipid ratio of 1:2 (mol/mol). The final concentration of (reduced or oxidized) htt\(^{NT}\)Q\(_7\) upon addition of the micelle solution was 0.15 mM. The exchange time \(T_{EX}\) was set to 200 ms, and \(B_1\) continuous wave (CW) saturation fields of 6, 17 and 28 Hz were applied. \(^{15}\text{N}\) \(B_1\) fields were calibrated using a 2D nutation experiment\(^{14}\) focusing on residue Glu\(^{23}\) that did not show broadening due to chemical exchange. \(^{13}\text{Ca},\) \(^{13}\text{C}\beta\) and \(^{13}\text{C}'\)-CEST data were acquired using a set of triple-resonance CEST experiments described previously\(^{15}\) on 960 \(\mu\text{M}\) U-[\(^{15}\text{N}/^{13}\text{C}\)] peptide samples in the presence of micelles with a protein/lipid ratio of 1:0.5 and 1:2 (mol/mol) for htt\(^{NT}\)Q\(_7\) and (Met\(^7\text{O})\)-htt\(^{NT}\)Q\(_7\), respectively. The exchange time \(T_{EX}\) was set to 100 ms for \(^{13}\text{Ca}\) and \(^{13}\text{C}\beta\)-CEST, and to 130 ms for \(^{13}\text{C}'\)-CEST. A single \(B_1\) CW field strength of 25 Hz was applied for \(^{13}\text{Ca},\) \(^{13}\text{C}\beta\) and \(^{13}\text{C}'\)-CEST experiments performed on htt\(^{NT}\)Q\(_7\), while \(B_1\) saturation field strengths of 25 and 35 Hz were used for \(^{13}\text{Ca},\) \(^{13}\text{C}\beta\) and \(^{13}\text{C}'\)-CEST experiments acquired on (Met\(^7\text{O})\)-htt\(^{NT}\)Q\(_7\). Reference spectra for \(^{15}\text{N}\)- and \(^{13}\text{C}\)-CEST were acquired with the saturation (\(T_{EX}\)) period included and the \(B_1\) field strength set to 0.

\(^{15}\text{N}\)-\(R_{1\rho}\) and \(R_1\) rates were measured in the absence of micelles using experiments described previously.\(^{16}\) A \(^{15}\text{N}\) spin-lock field strength of 1.0 kHz was used in \(^{15}\text{N}\) \(R_{1\rho}\) experiments to suppress contributions from chemical exchange-induced line broadening. \(R_{1\rho}\) experiments were recorded with variable spin-lock periods of 5, 25, 75, 100, 125 and 200 ms, while the following relaxation delays were used in \(R_1\) experiments: 40, 160, 320 and 400 ms. Relaxation decays were fit to a single exponential function \(Ae^{-RT}\), where \(A\) is a scaling factor, and \(R\) and \(T\) are the \(R_{1\rho}\) relaxation rate and the relaxation delay, respectively. \(^{15}\text{N}\) \(R_2\) values were extracted from the relationship, \(R_2 = (R_{1\rho} - R_1\cos^2\theta)/\sin^2\theta\), where \(\theta\) is the angle between the effective spin-lock field and the z-axis of the laboratory frame. NMR spectra were processed using the nmrPipe/nmrDraw suite of programs.\(^{17}\) Errors in the extracted rates were estimated from Monte Carlo simulations.

**Analysis of \(^{15}\text{N}\)- and \(^{13}\text{C}\)-CEST profiles.** Quantitative analysis of \(^{15}\text{N}\)-CEST profiles followed the original work of Kay and co-workers\(^{12}\) and our earlier publications.\(^{13,18}\) \(^{15}\text{N}\)-CEST profiles obtained for htt\(^{NT}\)Q\(_7\) and Met\(^7\text{O})\)-htt\(^{NT}\)Q\(_7\) in the presence of micelles were best-fit globally to a two-state exchange between free (A) and membrane-bound (B) states by propagating a set of homogeneous Bloch-McConnell equations\(^{19}\) as described previously.\(^{13,18}\) As the contributions of chemical exchange and life-time line-broadening to the linewidths of htt\(^{NT}\)Q\(_7\) exceed the intrinsic linewidths by 3-4 fold (determined by the exchange-free transverse spin relaxation rate of the free state, \(R_2\text{,A}\)),
R2_A constitutes a relatively small portion (~20-25%) of the total relaxation rate. As a result, reliable extraction of R2_A from the fits to the 15N-CEST profiles is problematic. R2_A was therefore eliminated from the list of optimized parameters and the residue specific R2_A values were fixed to those measured separately from a combination of 15N-R1_p and R1 experiments as described above. The list of residue-specific (local) fitting parameters thus comprised: \{σ_B, R_{2,B}, I_{0}^{RF_1}, I_{0}^{RF_2}, I_{0}^{RF_3}\}, where σ_B is the chemical shift of the bound state B, R_{2,B} the transverse spin relaxation rate of the bound state, and I_{0}^{RF_i} denotes initial intensities for each RF irradiation field i. The list of global parameters consisted of: \{k_{on}^{app}, p_B\}, where k_{on}^{app} is the apparent pseudo-first order association rate constant of binding, and p_B is the population of state B. The rest of the exchange parameters were calculated from: k_{ex} = k_{on}^{app} / p_B and k_{off} = k_{ex} - k_{on}^{app}. Best-fitting was performed using an in-house MatLab program (MathWorks Inc. MA), and uncertainties in the values of the optimized parameters, corresponding to confidence intervals of ±1 standard deviation, were determined from the Jacobian matrix of the non-linear fit.

Because of the complications associated with 13C-13C J couplings in [U-13C]-labeled samples, quantitative analysis of 13C-CEST profiles was restricted to the extraction of accurate 13C (13Ca, 13Cβ and 13C′) chemical shifts of the bound state (13C-ωB), and otherwise closely followed the previously described procedures where the components of magnetization are evolved for each line of a 13C multiplet, and the values of relaxation rates and chemical shift differences (Δω) are assumed to be the same for all lines of the multiplet.

Structure calculation of micelle-bound htt^{NTQ_n} monomer from chemical shifts using CS-Rosetta. NMR structures of the membrane-bound htt^{NTQ_7} and (Me^7O)-htt^{NTQ_7} peptide were calculated from the bound 15N, 13Ca, 13Cβ and 13C′ chemical shifts (listed in Table S1) using the program CS-ROSETTA. The best 10 low energy structures clustered with Ca r.m.s.d values less than 0.1 Å from the lowest energy structure.

Modeling the structure of the micelle-bound htt^{NTQ_n} dimer from DEER data using Xplor-NIH. Dimer structures were calculated with the program Xplor-NIH on the basis of three DEER-derived distances between nitroxide spin labels (S15C-R1/S15C-R1, S12C-R1/S12C-R1 and A1C-R1/A1C-R1) using the ansatz that the subunit orientations are predominantly determined by interactions with the membrane (i.e. contacts between hydrophobic residues and the membrane). Toward that end, an initial monomer htt^{NTQ_7} subunit was calculated in an EEFx implicit membrane force field with rigid backbone atom coordinates taken from the CS-Rosetta calculations (see
above) so that a reasonable initial estimate of the peptide’s orientation and depth relative to the membrane could be ascertained.

The structure of the micelle-bound dimer was then calculated by randomizing the position and orientation of two subunits in space, and then performing a simulated annealing calculation after initial gradient-based docking. The simulated annealing schedule included high-temperature dynamics at 3000 K followed by cooling to 25 K in increments of 25K. This calculation was performed using the EEFx membrane force field\(^2\) with the torsion DB database potential of mean force\(^3\) used as the dihedral energy term.

The following energy terms were employed in the calculation:

1. Each subunit was restrained to have the same orientation relative to the surface normal of the membrane and the same relative depth to the membrane using a modified version of Xplor-NIH’s PosDiffPot non-crystallographic symmetry restraint. In this modification there is no penalty for rotation about the membrane surface normal or translation parallel to the membrane surface. With this term, the membrane’s effect on helix orientation was strongly enforced during initial docking.

2. Three distance restraints (applied as a square-well potential of width ±1 Å) derived from the DEER data were applied for the three spin-label locations (18.6, 17.1 and 19.7 Å for htt\(^{NTQ10}\) A1C-R1, S12C-R1 and S15C-R1, respectively). Each spin label was represented by three sets of coordinates, and the experimentally determined distances were restrained to the average distance of these three spin-label conformations.

3. A gyration volume term\(^4\) was used to pull the subunits of the dimer to a reasonable protein packing distance. This term is valuable since the DEER-derived distance restraints are sparse (three distances) and also quite loose (±1.5 Å).

4. The low-resolution residue contact term ResidueAffPot\(^5\) was used to encourage favorable interactions between subunits.

The initial docking calculation was followed by a calculation with the PosDiffPot term disabled so that the subunits could rotate and translate arbitrarily in the implicit membrane environment subject to the remaining restraints. In this refinement calculation the lowest 10 structures from the docking calculation were subject to simulated annealing from 1000 K to 25K.

**Predicted \(P(r)\) distance distributions between spin labels from the Xplor-NIH model dimer.** The program SCWRL4.0\(^6\) was used to optimize side chain positions of the model dimer before loading the coordinates into the program MMMv2013.2\(^7\) to generate rotamer probabilities for each spin-label pair from which predicted \(P(r)\)
distributions, depicted as a histogram of the resulting distances between spin labels, were obtained (see Fig. 4B and S9A).

**Utility of transferred NOE measurements.** The transferred NOE (TRNOE) provides an alternative approach for obtaining structural information on bound species in a rapidly exchanging system by transferring \(^1\text{H}-\text{H}\) cross-relaxation information from the bound state to the observable free species.\(^{28}\) In this instance we made no attempt to carry out TRNOE measurements as the backbone chemical shifts provide a far more reliable and robust indicator of helix extent than sparse NOE data\(^{20,29}\) and further the TRNOEs provide no kinetic information on the binding process. Moreover, interpretation of TRNOE measurements in this instance would be complicated by (a) the presence of negative NOEs for the free peptide which would have to be deconvoluted from the TRNOEs arising from the bound state, (b) extensive cross-peak overlap due to very limited chemical shift dispersion, and (c) extensive spin-diffusion through the lipids of the 90 kDa micelle particle in the absence of perdeuteration of the micelles.
The backbone chemical shifts for the free peptides are close to random coil values, and analysis with TALOS-N indicates a probability of ≥85% coil for all residues, consistent with the CD spectra recorded in the absence of micelles (Fig. S4) that are characteristic of random coil. There are no measurable Δω values for residues 20-23 of httNTQγ. The absence of any negative 13Ca/13Cβ and positive 13Cβ Δω values from Gln18 onwards indicates that the current data provide no evidence for the pre-formation of any β-sheet structure between C-terminal polyglutamines in the dimeric micelle-bound population of httNTQγ. Moreover, the lower 15N- 2H values for residues 18-23 in the bound state obtained from the CEST fits suggest the C-terminal glutamines remain disordered.

Table S1. Backbone chemical shift differences, Δω, between micelle-bound and free forms of (A) httNTQγ and (B) (Met' O)-httNTQγ derived from CEST.

<table>
<thead>
<tr>
<th>Residue</th>
<th>15N (ppm)</th>
<th>13Ca (ppm)</th>
<th>13Cβ (ppm)</th>
<th>13C' (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. httNTQγ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.36 ± 0.01</td>
<td>3.58 ± 0.06</td>
<td>1.00 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-4.35 ± 0.25</td>
<td>2.65 ± 0.06</td>
<td>-0.68</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>-4.05 ± 0.04</td>
<td>3.06 ± 0.06</td>
<td>-0.85</td>
<td>2.40 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>-3.48 ± 0.05</td>
<td>2.71 ± 0.06</td>
<td>-0.85</td>
<td>2.72 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>-2.87 ± 0.08</td>
<td>2.65 ± 0.06</td>
<td>-0.34</td>
<td>2.38 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>-2.91 ± 0.06</td>
<td>2.26 ± 0.02</td>
<td>-0.51</td>
<td>2.69 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>-1.73 ± 0.06</td>
<td>2.12 ± 0.04</td>
<td>-0.68</td>
<td>1.53 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>-1.07 ± 0.01</td>
<td>2.34 ± 0.02</td>
<td>-0.51</td>
<td>2.62 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>-1.20 ± 0.01</td>
<td>2.76 ± 0.01</td>
<td>-0.34</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>10</td>
<td>-5.63 ± 0.06</td>
<td>1.47 ± 0.04</td>
<td>-0.57</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>11</td>
<td>-7.19 ± 0.05</td>
<td>2.00 ± 0.02</td>
<td>-0.34</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>-2.84 ± 0.04</td>
<td>1.61 ± 0.01</td>
<td>-0.34</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>13</td>
<td>-0.90 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-0.69 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>-0.46 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| B. (Met' O)-httNTQγ |
| 1       | 0.32 ± 0.01 | 4.67 ± 0.01 | -0.71      | 1.11 ± 0.01 |
| 2       | -4.58 ± 0.14 | 2.79 ± 0.02 | -1.22      | 1.06 ± 0.01 |
| 3       | -3.45 ± 0.03 | 3.13 ± 0.01 | -1.01      | 2.54 ± 0.01 |
| 4       | -3.64 ± 0.03 | 2.98 ± 0.01 | -0.07      | 2.92 ± 0.01 |
| 5       | -3.68 ± 0.04 | 2.54 ± 0.02 | -0.11      | 0.59 ± 0.01 |
| 6       | -3.32 ± 0.02 | 4.17 ± 0.02 | -0.88      | 2.80 ± 0.01 |
| 7       | -3.61 ± 0.04 | 2.45 ± 0.01 | -0.98      | 3.00 ± 0.01 |
| 8       | -2.27 ± 0.05 | 2.47 ± 0.03 | -0.90      | 1.38 ± 0.01 |
| 9       | -1.50 ± 0.01 | 2.72 ± 0.01 | -0.34      | 1.16 ± 0.01 |
| 10      | -4.21 ± 0.03 | 2.65 ± 0.01 | -1.00      | 3.19 ± 0.01 |
| 11      | -0.65 ± 0.01 | 3.40 ± 0.01 | -0.66      | 1.42 ± 0.21 |
| 12      | -1.07 ± 0.03 | 2.07 ± 0.02 | -0.36      | 0.08 ± 0.01 |
| 13      | -3.55 ± 0.02 | 2.51 ± 0.01 | -0.61      | 1.46 ± 0.01 |
| 14      | -1.97 ± 0.01 | 2.00 ± 0.01 | -0.51      | 1.16 ± 0.03 |
| 15      | -0.45 ± 0.10 | 1.49 ± 0.01 |           | 0.37 ± 0.01 |
| 16      | -2.68 ± 0.02 | 0.97 ± 0.01 |           | 0.62 ± 0.01 |
| 17      | -2.10 ± 0.02 | 0.10 ± 0.01 |           |           |
| 18      | -1.31 ± 0.02 |           |           |           |
| 19      | -1.09 ± 0.02 |           |           |           |
| 20      | -1.40 ± 0.02 |           |           |           |
| 21      | -0.71 ± 0.10 |           |           |           |
| 22      |           |           |           |           |
**Figure S1.** Aggregation of htt\(^{NTQ_7}\) peptides monitored by NMR and ThT assay. (A) Time courses at 10°C of the integrated intensity (from 7.9 to 8.4 ppm) of the amide proton envelope (obtained from the first increment of a \(^1\)H-\(^{15}\)N HSQC correlation spectrum) of htt\(^{NTQ_10}\) alone (filled-in red circles) and htt\(^{NTQ_7}\) (blue circles), htt\(^{NTQ_10}\) (red circles) and (Met\(^7\)O)-htt\(^{NTQ_10}\) (red squares) in the presence of LPC/LPG micelles at a peptide concentration of 150 µM and a protein to lipid molar ratio of 1:2. (B) Time course of ThT emission of 25 µM htt\(^{NTQ_10}\) (left) and (Met\(^7\)O)-htt\(^{NTQ_10}\) in the presence of micelles at a peptide to lipid molar ratio of 1:2 and 10°C.

**Figure S2.** Characterization of 3:1 LPC/LPG (mol/mol ratio) micelles by DLS and SEC-MALS. (A) Particle size distribution from DLS (diameter = 8±1 nm, polydispersity index ~ 0.15). (B) SEC-MALS with the refractive index of the elution peak in black and the molecular mass in blue. The molecular mass is 91±3 kDa, corresponding to 183±6 lipid molecules per micelle. All measurements were performed in 20 mM sodium phosphate buffer, pH 6.5, 50 mM NaCl at 10°C.
Figure S3. Representative $^{13}$C' and $^{13}$C$\beta$ CEST profiles recorded on 960 $\mu$M (A) htt$^{NTQ_7}$ and (B) (Met$^O$)-htt$^{NTQ_7}$ in the presence of micelles at protein to lipid ratios of 1:0.5 and 1:2, respectively. The experimental data are shown as circles. The continuous lines for the $^{13}$C' CEST profiles represent the global best-fits using a two-state exchange model; the dotted lines for the $^{13}$C$\beta$ profiles are drawn to guide the eye. ($^{13}$C$\beta$ CEST profiles were not fit owing to poor signal-to-noise, and the $^{13}$C$\beta$ chemical shifts of the minor state were therefore extracted from the maxima of the minor dips.) The CW saturation pulse (RF field strength = 25 Hz) was applied for a duration $T_{EX} = 130$ and 100 ms for $^{13}$C' and $^{13}$C$\beta$, respectively.

Figure S4. Examples of CD spectra of (A) htt$^{NTQ_7}$ and (B) (Met$^O$)-htt$^{NTQ_7}$ recorded in the absence of micelles (dashed black line) and at different protein to lipid molar ratios ranging from 1:2 to 1:12. Measurements were carried out at 10°C in 20 mM phosphate buffer, pH 6.5, 50 mM NaCl.
Figure S5. Impact of polynomial baseline correction on the analysis of Q-band DEER echo curves recorded on 80 μM htn\textsuperscript{N10}Q\textsubscript{10} (S15C-R1) in the presence of micelles at a peptide to lipid molar ratio of 1:20. (A) Left panel: raw (green) and baseline-corrected (blue) DEER echo curves after application of polynomials (black line) of order \(n = 1\) to 4. Center and right panels: corresponding residuals between baseline-corrected and calculated DEER echo curves and \(P(r)\) distance distributions, respectively, after Tikhonov regularization. (B) Modulation depth obtained directly from the baseline-corrected DEER echo curves as a function of the polynomial order \(n = 1\) to 4 used for baseline correction. All calculations were carried out using DeerAnalysis 2016\textsuperscript{9}. 
Figure S6. Examples of raw (left) and baseline-corrected (right) DEER echo curves obtained in the IM-DEER experiments for 80 µM htt^{NTQ_7} (top) and htt^{NTQ_10} (bottom) in the presence of micelles at a protein to lipid molar ratio of 1:30. The baseline correction function is a polynomial of order 3 and all calculations were carried out using DeerAnalysis 2016.⁹
Figure S7. Q-band DEER echo curves used to obtain the $P(r)$ distributions reported in Fig. 4B (main text) were recorded on 80 µM htn^15NQ spin-labeled at A1C-R1, S12C-R1 and S15C-R1 in the presence of micelles at a peptide to lipid molar ratio of 1:80. The high lipid to peptide ratio was used to ensure that intermolecular interactions present in the samples could be approximated as closely as possible to a homogeneous three-dimensional distribution of spin pairs such that the results of background correction with either a polynomial of order 3 or an exponential were very similar. The raw and baseline-corrected DEER echo curves are shown in green and blue, respectively. The polynomial baseline correction functions of order 3 are shown in black, and the calculated DEER echo curve following Tikhonov regularization to obtain the $P(r)$ distributions (reported in Fig. 4B) are shown in red. All calculations were performed with DeerAnalysis 2016.
Figure S8. Examples of Q-band DEER echo curves recorded on 80 µM htt^{NT}_{7} (left) and htt^{NT}_{10} (right) S15C-R1 in the presence of micelles at varying lipid concentrations. The black lines represent the best-fit polynomial baseline of order 3 obtained with DeerAnalysis 2016. The modulation depth $D$ is the difference between the intensity of the DEER echo curve and the intercept of the baseline at zero dipolar evolution time.
Figure S9. Model of the micelle-bound htt\textsuperscript{NT}\textsubscript{Q}\textsubscript{6} dimer derived from DEER distance restraints (see Fig. 4B) using Xplor-NIH\textsuperscript{21b} (A) Distribution of nitroxide spin labels calculated with the program MMM\textsuperscript{v2013.2}\textsuperscript{27} and used to determine the predicted $P(r)$ distributions shown in Fig. 4B of the main text. (B) Distribution of hydrophobic residues (left panel) and interactions of hydrophobic residues with the membrane (right panel). (C) Intersubunit interactions between the two chains of the dimer. The backbones are shown as grey ribbons with a transparent molecular surface. In (A) the nitroxide spin labels are shown as bonds with the oxygen atoms as spheres. In (B) the hydrophobic residues are displayed in green and the membrane is shown as a grey mesh in the right panel. In (C) the side chains involved in interactions at the dimer interface are shown in red.
**Figure S10.** Average number of monomer and dimer particles bound per micelle calculated using the optimized values of $K_{eq}$, $K_D$ and $n$ (cf. Eq. S8) obtained from the fits of the modulation depth data as a function of lipid concentration shown in Fig. 4D.

**Figure S11.** Dependence of the semi-empirical correction term $\kappa$ (Eq. S6) on lipid concentration. The correction factor $\kappa$ represents the modulation depth attributable to a small population of free oligomer in equilibrium with the monomeric free state.
Figure S12. Oligomerization of Met\(^7\)O-htt\(^{NT}\)Q\(_7\) on the micelle surface characterized by Q-band pulsed EPR. (A) Normalized modulation depth (\(\Delta/\Delta_{\text{max}}\)) versus ELDOR pulse flip angle obtained for micelle-bound Met\(^7\)O-htt\(^{NT}\)Q\(_7\) (S15C). The experimental data are shown as circles, and the theoretical curves for a dimer (two spins), trimer (three spins) and tetramer (four spins) are shown in red, green and blue, respectively. An additional theoretical curve is shown for the case of an equal mixture of dimer and trimer (yellow). The peptide:lipid molar ratio was 1:30. (B) Modulation depth of DEER echo curves as a function of lipid concentration. The experimental data are shown as red circles; the dotted line represents the best fit to the data using the model reported in Fig. 4C whereas the solid line is the best-fit curve for the case when the bound species is partitioned between monomers and trimers (see Eqs. S9.1 and S9.2). All the data were recorded on a 80 µM Met\(^7\)O-htt\(^{NT}\)Q\(_7\) S15C-R1 sample.
Supplementary references

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(11) Hill, A. S. V. *J. Physiol. (Lond)* 1910, 40, 4-7.