Supplementary Information for

The Folding Equilibrium of Huntingtin Exon-1 Monomer Depends on Polyglutamine Length

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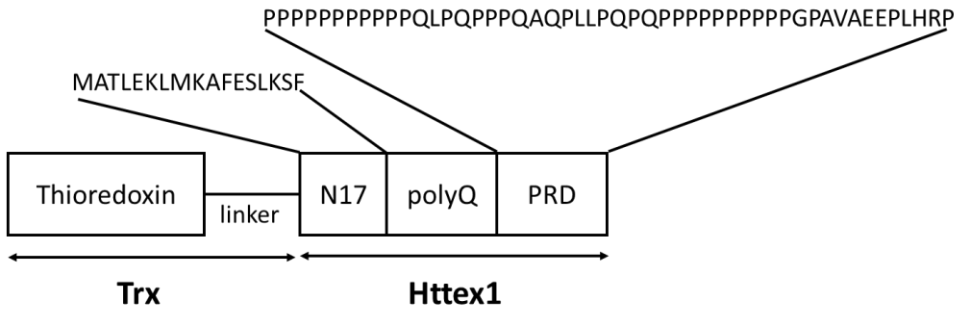
a)



b)

Construct name:	Domains:
N17 peptide	N17
Trx-Httex1(Q7)	Trx – N17 – Q_7 – PRD
Trx-Httex1(Q7)	Trx – N17 – Q_{16} – PRD
Trx-Httex1(Q7)	Trx – N17 – Q_{25} – PRD
Trx-Httex1(Q7)	Trx – N17 – Q_{46} – PRD
Trx-Httex1(Q7)	Trx – N17 – Q_{55} – PRD
Httex1(Q46)	N17 – Q_{46} – PRD

Fig. S1. Summary of constructs used in this study. (a) The domain organization of the thioredoxin fusion protein used in the present study. The sequences for N17 and PRD are displayed above the schematic. The sequence was the same as in Bennett *et al.* (1), except for the original two cysteine residues in the thioredoxin fusion partner, which were changed to serine by site-directed mutagenesis to prevent unwanted spin labeling. (b) List of constructs used and a breakdown of the domains contained. With the exception of the N1, which was obtained from peptide synthesis, all proteins were expressed recombinantly. Httex1(Q46) was generated from enzymatic cleavage of thioredoxin fusion protein.

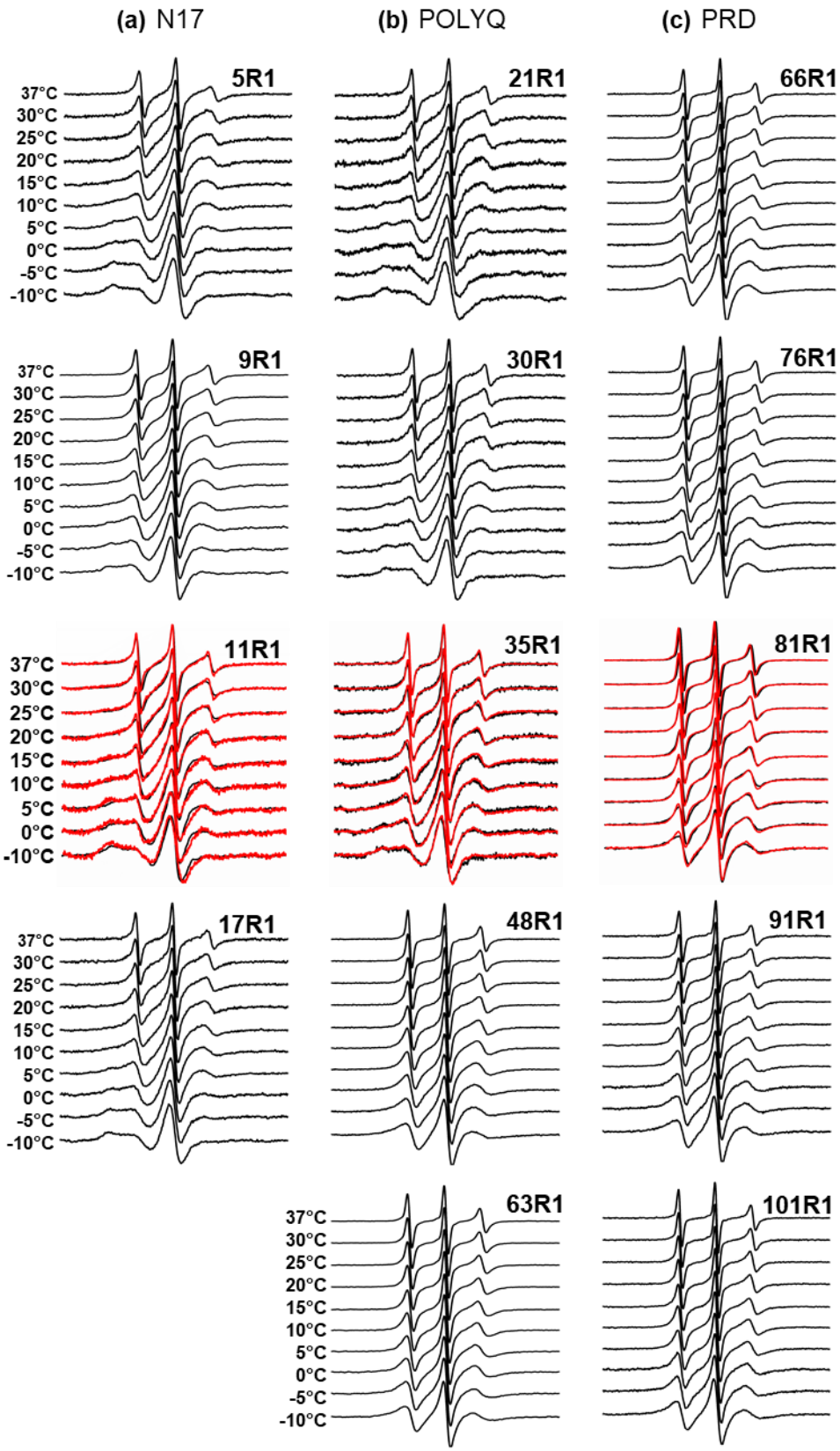

(a) N17
(b) POLYQ
(c) PRD
5R1
21R1
66R1
9R1
30R1
76R1
11R1
35R1
81R1
17R1
48R1
91R1
63R1
101R1
37°C
30°C
25°C
20°C
15°C
10°C
5°C
0°C
-5°C
-10°C

Fig. S2. EPR spectra of spin labeled Trx-Httex1(Q46) and Httex1(Q46) derivatives at different temperatures. The continuous wave EPR spectra of the remaining Trx-Httex1(Q46) labeling sites not shown in Fig. 2b are shown in black at the indicated temperatures. The left (a), middle (b) and right (c) columns are comprised of sites from the N17, polyQ and PRD, respectively. The red spectra are for Httex1(Q46) that was bound to beads. To better visualize the spectral features, the spectra are not normalized to the same number spins, rather they are all shown at comparable amplitudes.

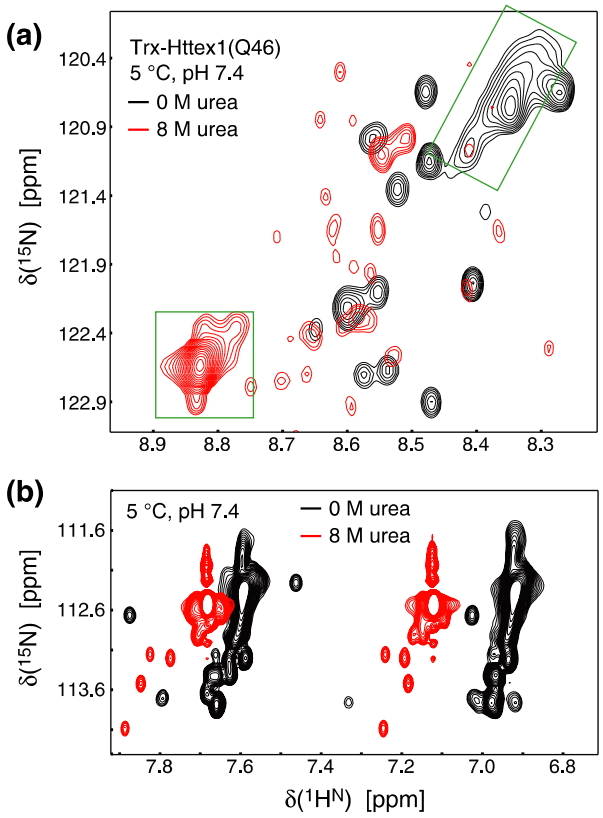


Fig. S3. NMR spectra of Trx-Httex1(Q46) at pH 7.4. Spectral regions containing the most intense **(a)** backbone and **(b)** sidechain H^N-N resonances are shown in the absence (black) and presence (red) of 8 M urea. The putative polyQ backbone resonances are boxed in *green*. The spectra were recorded in 25 mM NaH_2PO_4/Na_2HPO_4, pH 7.4 at 5 °C and 700 MHz using a protein concentration of 20 µM.

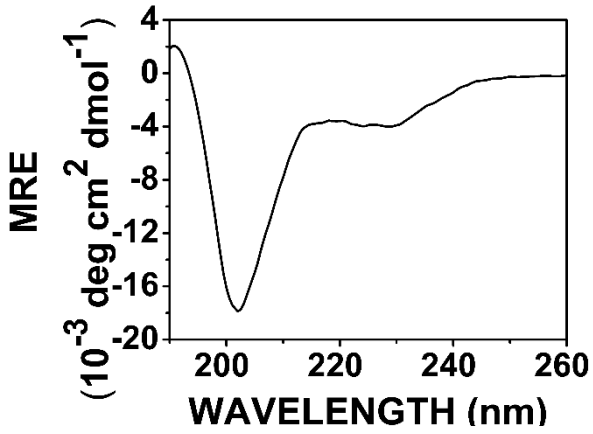


Fig. S4. CD spectrum of N17 peptide. The CD spectrum of a C-terminally amidated peptide containing the N17 sequence (obtained from Peptide 2.0) was recorded at 10 μM in 20 mM sodium phosphate buffer, pH 7.4. According to spectral analysis using the Dichroweb suite of programs (2), the CD spectrum indicates 80 to 85% random coil structure.

Table S1. PolyQ chemical shifts and predicted order parameter S^2.

Peak[a]	$\delta(^1H^N)$	$\delta(^{15}N)$	$\delta(^{13}C^\alpha)$	$\delta(^{13}C^\beta)$	$\Delta\delta(^{13}C^\alpha)$[b]	S^2
1	8.31	120.42	57.67	28.69	1.89	0.69
2	8.37	120.60	57.45	28.76	1.67	0.68
3	8.42	120.91	56.91	29.01	1.12	0.57
4	8.46	121.24	56.59	29.14	0.80	0.48
5	8.31	120.51	57.18	28.98	1.40	0.63
6	8.38	120.82	56.92	29.09	1.13	0.58
7	8.41	121.10	56.49	29.24	0.70	0.43

[a]See Fig. 3b-c for peak designations.
[b]$\Delta\delta(^{13}C^\alpha)$ denotes the secondary $^{13}C^\alpha$ shift.

References

1. Bennett MJ, *et al.* (2002) A linear lattice model for polyglutamine in CAG-expansion diseases. *Proc Natl Acad Sci U S A* 99(18):11634-11639.
2. Whitmore L & Wallace BA (2004) DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res* 32(Web Server issue):W668-673.