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Supplemental Information

Sequence Context Influences the Structure and Aggregation Behavior of a PolyQ Tract

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Table S1 Experimental parameters used for the acquisition of the NMR experiments on 4Q for sequence-specific resonance assignment

	Spectral widths and maximal evolution times				No. of scans	Inter-scan delays (s)	No. of complex points (aq)	No. of hypercomplex points	Duration of the experiment	Relative data points density (%)
	Indirect dimensions			Direct dimension						
2D CON			2600 Hz (^{15}N) 197.0 ms	8800 Hz (^{13}C')	16	2.5	512	-	12 hours, 30 min	100
2D ^{1}H-^{15}N HSQC			1600 Hz (^{15}N) 160.0 ms	10500 Hz (^{1}H)	8	1.0	1024	-	1 hour, 20 min	100
4D HCBCACON	5000 Hz (^{1}H$^{\alpha/\beta}$) 20.0 ms	12500 Hz (^{13}C$^{\alpha/\beta}$) 7.5 ms	2600 Hz (^{15}N) 50.0 ms	8800 Hz (^{13}C')	8	0.9	512	850	1 day, 10 hours	0.07
4D HCBCANCO	5000 Hz (^{1}H$^{\alpha/\beta}$) 20.0 ms	12500 Hz (^{13}C$^{\alpha/\beta}$) 7.5 ms	2600 Hz (^{15}N) 32.0 ms	8800 Hz (^{13}C')	16	0.9	512	850	3 days, 2 hours	0.11
4D (HCA)CON(CA)CON	2200 Hz (^{13}C') 24.1 ms	2600 Hz (^{15}N) 24.2 ms	2600 Hz (^{15}N) 30.0 ms	8800 Hz (^{13}C')	16	0.9	512	930	3 days, 9 hours	0.34
4D (HN)CON(CA)CON	2200 Hz (^{13}C') 24.1 ms	2600 Hz (^{15}N) 24.2 ms	2600 Hz (^{15}N) 30.0 ms	8800 Hz (^{13}C')	32	0.5	512	910	4 days, 11 hours	0.33
3D TROSY HNCO		2700 Hz (^{13}C') 20.7 ms	2300 Hz (^{15}N) 21.7 ms	14200 Hz (^{1}H)	8	1.2	1024	560	7 hours	20.00
4D TROSY (H)NCO(CA)NNH	2300 Hz (^{15}N) 20.4 ms	2700 Hz (^{13}C') 23.9 ms	2300 Hz (^{15}N) 23.9 ms	13300 Hz (^{1}H)	8	1.2	1024	2660	2 days, 20 hours	1.60
4D TROSY HN(COCA)NNH	1500 Hz (^{1}H) 20.0 ms	2300 Hz (^{15}N) 23.9 ms	2300 Hz (^{15}N) 23.9 ms	13300 Hz (^{1}H)	8	1.2	1024	1450	1 day, 13 hours	1.60

Table S2 Experimental parameters used for the acquisition of the NMR experiments on 25Q for sequence-specific resonance assignment

	Spectral widths and maximal evolution times				No. of scans	Inter-scan delays (s)	No. of complex points (aq)	No. of hypercomplex points	Duration of the experiment	Relative data points density (%)
	Indirect dimensions			Direct dimension						
2D CON			2600 Hz (^{15}N) 197.0 ms	8800 Hz (^{13}C')	16	2.5	512	-	12 hours, 30 min	100
2D ^{1}H-^{15}N HSQC			1600 Hz (^{15}N) 160.0 ms	10500 Hz (^{1}H)	8	1.0	1024	-	1 hour, 20 min	100
4D HCBCACON	5000 Hz ($^{1}H^{\alpha/\beta}$) 20.0 ms	12500 Hz ($^{13}C^{\alpha/\beta}$) 7.5 ms	2600 Hz (^{15}N) 50.0 ms	8800 Hz (^{13}C')	8	0.9	512	850	1 day, 10 hours	0.07
4D (HN)CON(CA)CON	2200 Hz (^{13}C') 24.1 ms	2600 Hz (^{15}N) 24.2 ms	2600 Hz (^{15}N) 30.0 ms	8800 Hz (^{13}C')	32	0.5	512	910	4 days, 11 hours	0.33
3D HNCO		2000 Hz (^{13}C') 35.5 ms	2000 Hz (^{15}N) 24.0 ms	10500 Hz (^{1}H)	16	1.0	1024	1130	1 day	33.30
3D TROSY HN(CA)CO		1800 Hz (^{13}C') 25.0 ms	2400 Hz (^{15}N) 20.8 ms	13300 Hz (^{1}H)	16	1.2	1024	580	14 hours	26.00
4D TROSY HN(COCA)NNH	1500 Hz (^{1}H) 20.0 ms	2400 Hz (^{15}N) 22.9 ms	2400 Hz (^{15}N) 22.9 ms	13300 Hz (^{1}H)	8	1.2	1024	1720	1 day, 20 hours	1.90

Table S3 Experimental parameters used for the acquisition of the ^{15}N relaxation NMR experiments on 4Q

	Spectral widths and maximal evolution times		No. of scans	Inter-scan delays (s)
^{15}N R_1	1600 Hz (^{15}N) 156.8 ms	10500 Hz (^{1}H) 97.6 ms	8	3.0
^{15}N R_2	1600 Hz (^{15}N) 156.8 ms	10500 Hz (^{1}H) 97.6 ms	8	3.0
Steady-state heteronuclear $^{15}N\{^{1}H\}$ NOEs	1600 Hz (^{15}N) 156.8 ms	10500 Hz (^{1}H) 97.6 ms	64	6.0
For the determination of R_1, 10 experiments were acquired changing the variable delay from 15 to 995 ms. For the determination of R_2, 10 experiments were acquired changing the variable delay from 30 to 565 ms.				

Table S4 Experimental parameters used for the acquisition of the ^{15}N relaxation NMR experiments on 25Q

	Spectral widths and maximal evolution times		No. of scans	Inter-scan delays (s)
^{15}N R_1	1600 Hz (^{15}N) 156.8 ms	10500 Hz (^{1}H) 97.6 ms	8	3.0
^{15}N R_2	1600 Hz (^{15}N) 156.8 ms	10500 Hz (^{1}H) 97.6 ms	8	3.0
Steady-state heteronuclear $^{15}N\{^{1}H\}$ NOEs	1600 Hz (^{15}N) 177.7 ms	10500 Hz (^{1}H) 97.6 ms	64	6.0
For the determination of R_1, 10 experiments were acquired changing the variable delay from 15 to 995 ms. For the determination of R_2, 10 experiments were acquired changing the variable delay from 30 to 315 ms.				

Supporting Figures

4Q:

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGPRHPEAASAAPPGASLLLLQQQQETSP
RQQQQQQGEDGSPQAHRRGPTGYLVLDEEQQPSQPQSALECHPERGCVPEPGAAVAASKGLPQQLP
APP

25Q:

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGPRHPEAASAAPPGASLLLLQQQQQQQQ
QQQQQQQQQQQQQQQQQQETSPRQQQQQQGEDGSPQAHRRGPTGYLVLDEEQQPSQPQSALECHPER
GCVPEPGAAVAASKGLPQQLPAPP

25QΔL4:

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGPRHPEAASAAPPGASQQQQQQQQQQQQ
QQQQQQQQQQQQQQETSPRQQQQQQGEDGSPQAHRRGPTGYLVLDEEQQPSQPQSALECHPERGCVP
EPGAAVAASKGLPQQLPAPP

Figure S1 Sequences of the constructs used in this work. The polyQ tracts are shown in purple, as in Figure 1 of the main text, and the Leu_4 motif is shown in green.

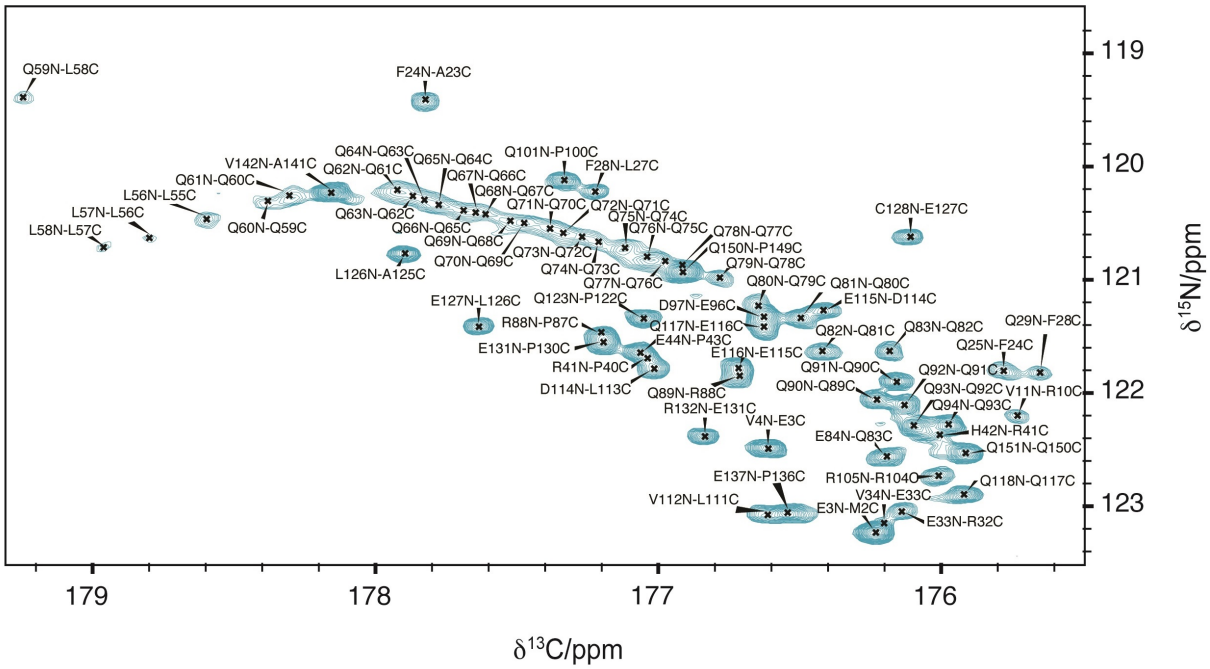

Figure S2 Close up view of the CON-IPAP spectrum of 25Q with the full assignment of the polyQ tract. ^{13}C dimension increases the chemical shift dispersion of the resonances and reduces cross-peak overlaps in the polyQ region.

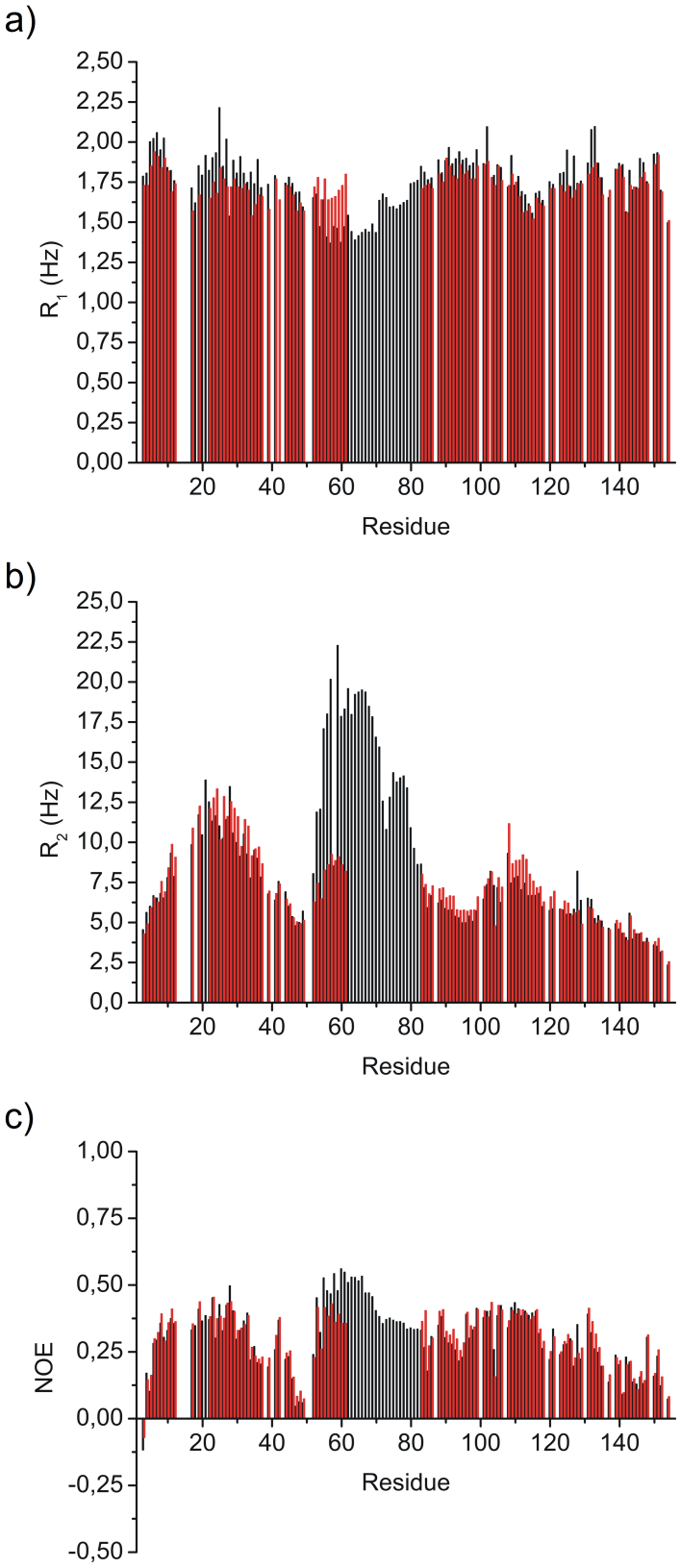

Figure S3 ^{15}N relaxation rates of 4Q (red) and 25Q (black). ^{15}N R_1, ^{15}N R_2 relaxation rates and {^{1}H}-^{15}N NOEs are reported as a function of residue number. To facilitate the comparison, error bars have been removed and values for residues of 4Q which are C-terminal to the polyQ tract have been shifted to the right by 21 units.