

# **Sequence Context Influences the Structure and Aggregation Propensity of a PolyQ Tract**

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## **Supplemental material**

**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 ( <sup>15</sup> N) 197.0 ms	8800 Hz ( <sup>13</sup> C')	16	2.5	512	-	12 hours, 30 min	100
2D <sup>1</sup> H- <sup>15</sup> N HSQC			1600 Hz ( <sup>15</sup> N) 160.0 ms	10500 Hz ( <sup>1</sup> H)	8	1.0	1024	-	1 hour, 20 min	100
4D HCBCACON	5000 Hz ( <sup>1</sup> H <sup>α/β</sup> ) 20.0 ms	12500 Hz ( <sup>13</sup> C <sup>α/β</sup> ) 7.5 ms	2600 Hz ( <sup>15</sup> N) 50.0 ms	8800 Hz ( <sup>13</sup> C')	8	0.9	512	850	1 day, 10 hours	0.07
4D HCBCANCO	5000 Hz ( <sup>1</sup> H <sup>α/β</sup> ) 20.0 ms	12500 Hz ( <sup>13</sup> C <sup>α/β</sup> ) 7.5 ms	2600 Hz ( <sup>15</sup> N) 32.0 ms	8800 Hz ( <sup>13</sup> C')	16	0.9	512	850	3 days, 2 hours	0.11
4D (HCA)CON(CA)CON	2200 Hz ( <sup>13</sup> C')	2600 Hz ( <sup>15</sup> N) 24.2 ms	2600 Hz ( <sup>15</sup> N) 30.0 ms	8800 Hz ( <sup>13</sup> C')	16	0.9	512	930	3 days, 9 hours	0.34
4D (HN)CON(CA)CON	2200 Hz ( <sup>13</sup> C')	2600 Hz ( <sup>15</sup> N) 24.2 ms	2600 Hz ( <sup>15</sup> N) 30.0 ms	8800 Hz ( <sup>13</sup> C')	32	0.5	512	910	4 days, 11 hours	0.33
3D TROSY HNCO		2700 Hz ( <sup>13</sup> C')	2300 Hz ( <sup>15</sup> N) 21.7 ms	14200 Hz ( <sup>1</sup> H)	8	1.2	1024	560	7 hours	20.00
4D TROSY (H)NCO(CA)NNH	2300 Hz ( <sup>15</sup> N) 20.4 ms	2700 Hz ( <sup>13</sup> C')	2300 Hz ( <sup>15</sup> N) 23.9 ms	13300 Hz ( <sup>1</sup> H)	8	1.2	1024	2660	2 days, 20 hours	1.60
4D TROSY HN(COCA)NNH	1500 Hz ( <sup>1</sup> H) 20.0 ms	2300 Hz ( <sup>15</sup> N) 23.9 ms	2300 Hz ( <sup>15</sup> N) 23.9 ms	13300 Hz ( <sup>1</sup> 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 ( <sup>15</sup> N) 197.0 ms	8800 Hz ( <sup>13</sup> C')	16	2.5	512	-	12 hours, 30 min	100
2D <sup>1</sup> H- <sup>15</sup> N HSQC			1600 Hz ( <sup>15</sup> N) 160.0 ms	10500 Hz ( <sup>1</sup> H)	8	1.0	1024	-	1 hour, 20 min	100
4D HCBCACON	5000 Hz ( <sup>1</sup> H <sup>α/β</sup> ) 20.0 ms	12500 Hz ( <sup>13</sup> C <sup>α/β</sup> ) 7.5 ms	2600 Hz ( <sup>15</sup> N) 50.0 ms	8800 Hz ( <sup>13</sup> C')	8	0.9	512	850	1 day, 10 hours	0.07
4D (HN)CON(CA)CON	2200 Hz ( <sup>13</sup> C')	2600 Hz ( <sup>15</sup> N) 24.2 ms	2600 Hz ( <sup>15</sup> N) 30.0 ms	8800 Hz ( <sup>13</sup> C')	32	0.5	512	910	4 days, 11 hours	0.33
3D HNCO		2000 Hz ( <sup>13</sup> C')	2000 Hz ( <sup>15</sup> N) 24.0 ms	10500 Hz ( <sup>1</sup> H)	16	1.0	1024	1130	1 day	33.30
3D TROSY HN(CA)CO		1800 Hz ( <sup>13</sup> C')	2400 Hz ( <sup>15</sup> N) 20.8 ms	13300 Hz ( <sup>1</sup> H)	16	1.2	1024	580	14 hours	26.00
4D TROSY HN(COCA)NNH	1500 Hz ( <sup>1</sup> H) 20.0 ms	2400 Hz ( <sup>15</sup> N) 22.9 ms	2400 Hz ( <sup>15</sup> N) 22.9 ms	13300 Hz ( <sup>1</sup> H)	8	1.2	1024	1720	1 day, 20 hours	1.90

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

	Spectral widths and maximal evolution times		No. of scans	Inter-scan delays (s)
$^{15}\text{N}$ $R_1$	1600 Hz ( $^{15}\text{N}$ ) 156.8 ms	10500 Hz ( $^1\text{H}$ ) 97.6 ms	8	3.0
$^{15}\text{N}$ $R_2$	1600 Hz ( $^{15}\text{N}$ ) 156.8 ms	10500 Hz ( $^1\text{H}$ ) 97.6 ms	8	3.0
Steady-state heteronuclear $^{15}\text{N}\{^1\text{H}\}$ NOEs	1600 Hz ( $^{15}\text{N}$ ) 156.8 ms	10500 Hz ( $^1\text{H}$ ) 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  $^{15}\text{N}$  relaxation NMR experiments on 25Q

	Spectral widths and maximal evolution times		No. of scans	Inter-scan delays (s)
$^{15}\text{N}$ $R_1$	1600 Hz ( $^{15}\text{N}$ ) 156.8 ms	10500 Hz ( $^1\text{H}$ ) 97.6 ms	8	3.0
$^{15}\text{N}$ $R_2$	1600 Hz ( $^{15}\text{N}$ ) 156.8 ms	10500 Hz ( $^1\text{H}$ ) 97.6 ms	8	3.0
Steady-state heteronuclear $^{15}\text{N}\{^1\text{H}\}$ NOEs	1600 Hz ( $^{15}\text{N}$ ) 177.7 ms	10500 Hz ( $^1\text{H}$ ) 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:**

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGRHPEAASAAPP GAS **L L L L Q Q Q Q** E T S P  
R Q Q Q Q Q Q G E D G S P Q A H R R G P T G Y L V L D E E Q Q P S Q P Q S A L E C H P E R G C V P E P G A A V A A S K G L P Q Q L P  
A P P

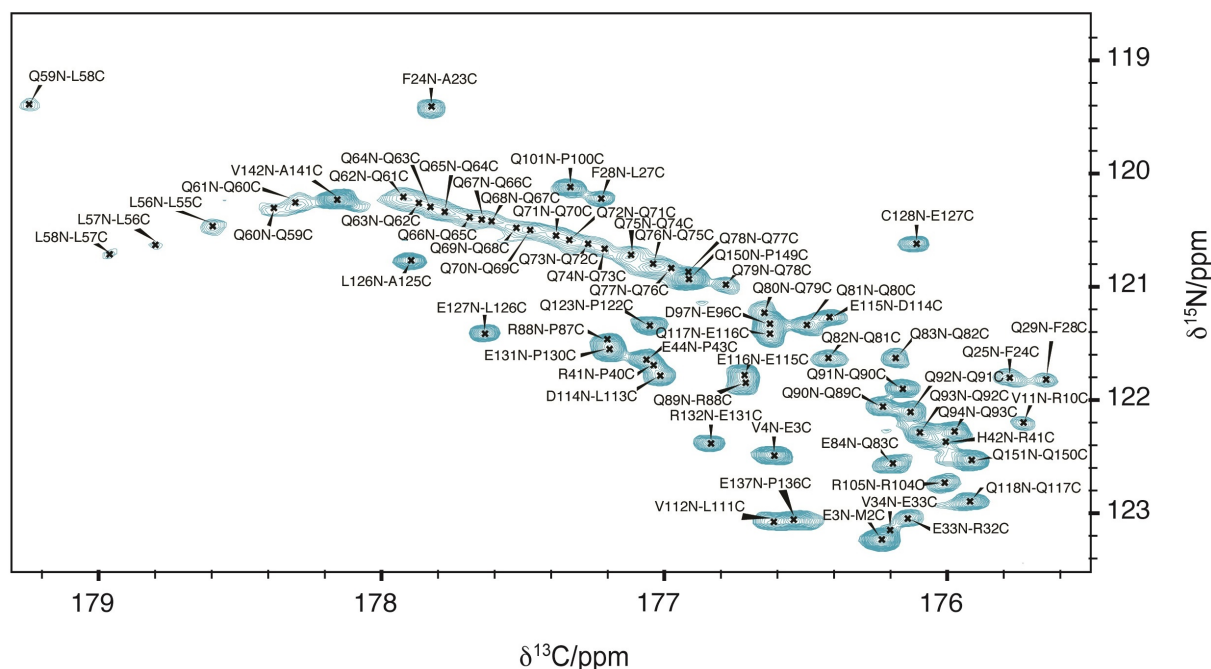
**25Q:**

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGRHPEAASAAPP GAS **L L L L Q Q Q Q Q Q Q Q**  
**Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q** E T S P R Q Q Q Q Q Q G E D G S P Q A H R R G P T G Y L V L D E E Q Q P S Q P Q S A L E C H P E R  
G C V P E P G A A V A A S K G L P Q Q L P A P P

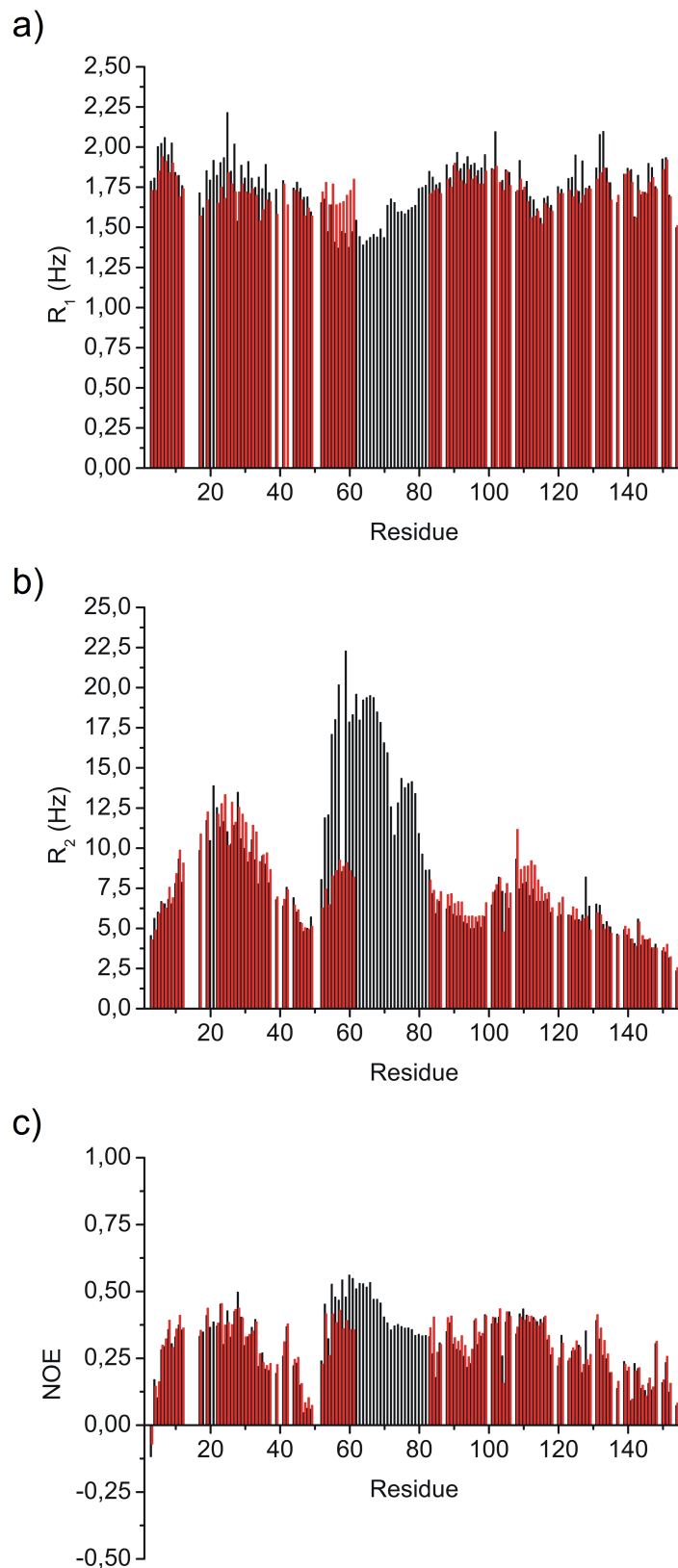
**25Q $\Delta$ L4:**

GMEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGRHPEAASAAPP GAS **Q Q Q Q Q Q Q Q Q Q Q Q**  
**Q Q Q Q Q Q Q Q Q Q Q Q Q Q** E T S P R Q Q Q Q Q Q G E D G S P Q A H R R G P T G Y L V L D E E Q Q P S Q P Q S A L E C H P E R G C V P  
E P G A A V A A S K G L P Q Q L P A P P

**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<sub>4</sub> 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. <sup>13</sup>C dimension increases the chemical shift dispersion of the resonances and reduces cross-peak overlaps in the polyQ region.



**Figure S3**  $^{15}\text{N}$  relaxation rates of 4Q (red) and 25Q (black).  $^{15}\text{N}$   $R_1$ ,  $^{15}\text{N}$   $R_2$  relaxation rates and  $\{^1\text{H}\}$ - $^{15}\text{N}$  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.