Supplementary Information

Study of conformational transitions of i-Motif DNA by femtosecond fluorescence and MCR-ALS methods

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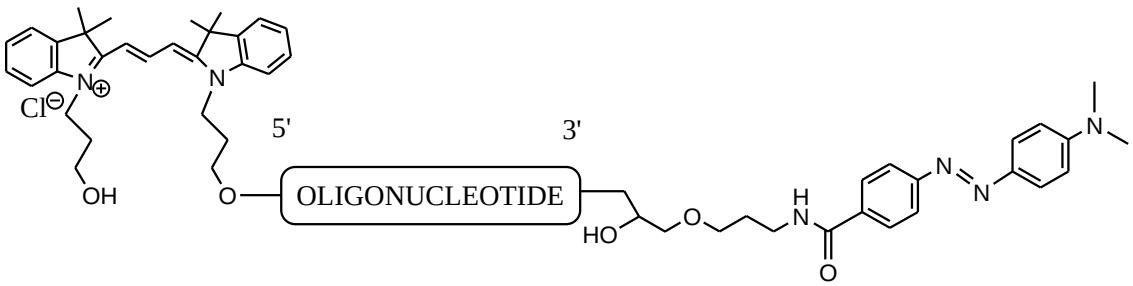
Contents

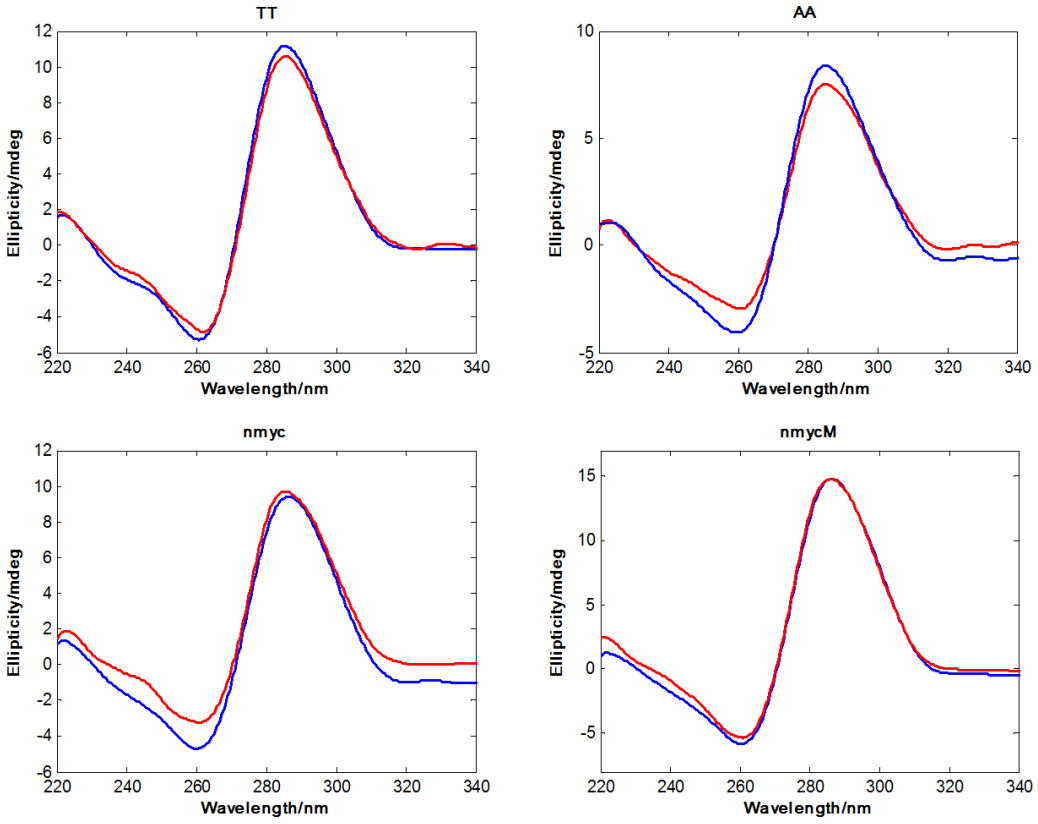
Supplementary Figure 1. Chemical structure of the 5'-CY3 and 3'-DABCYL modifications



CYANINE 3 DABCYL

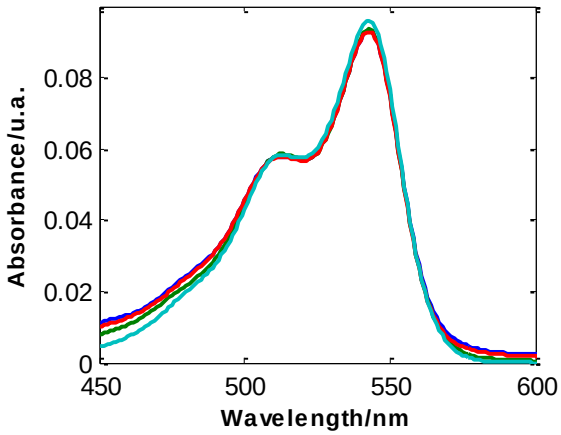
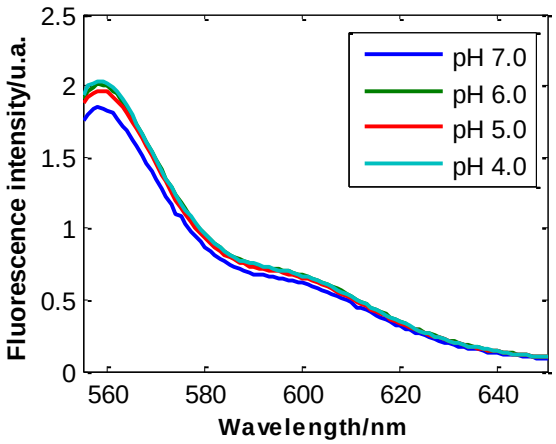
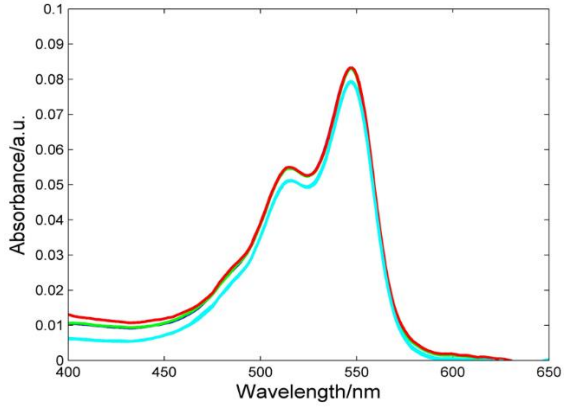
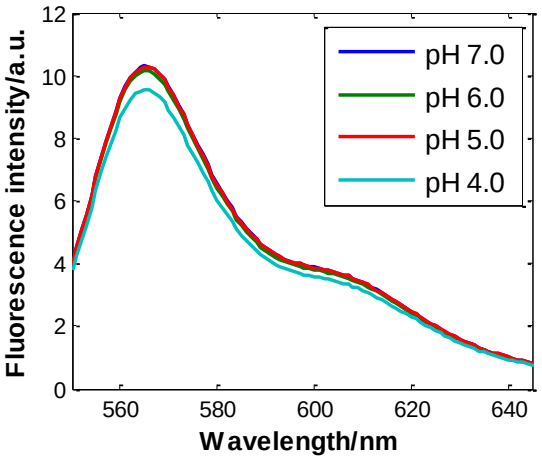
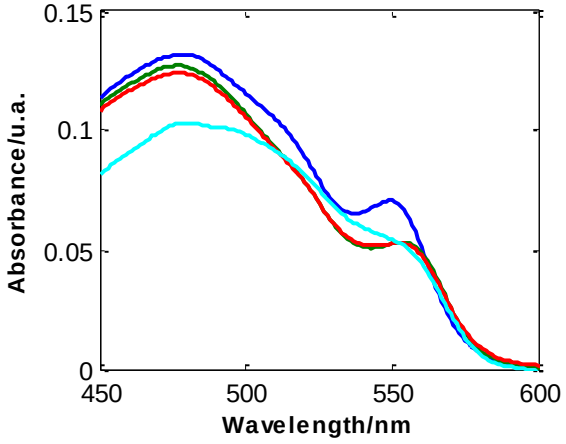
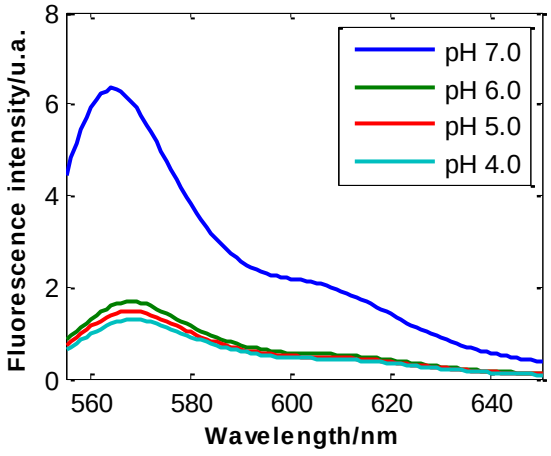
Supplementary Figure 2. CD spectra

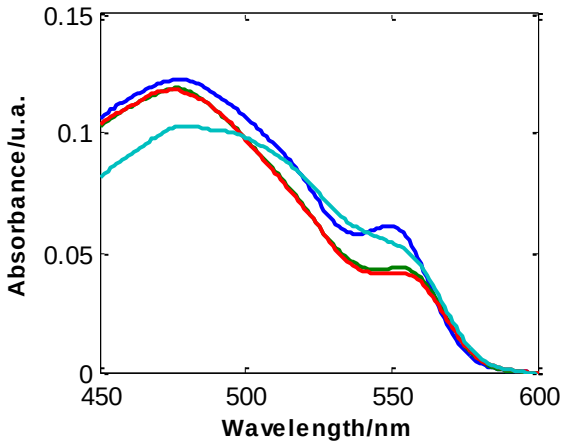
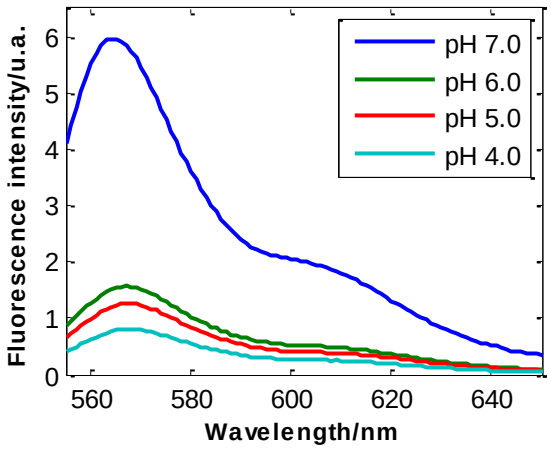
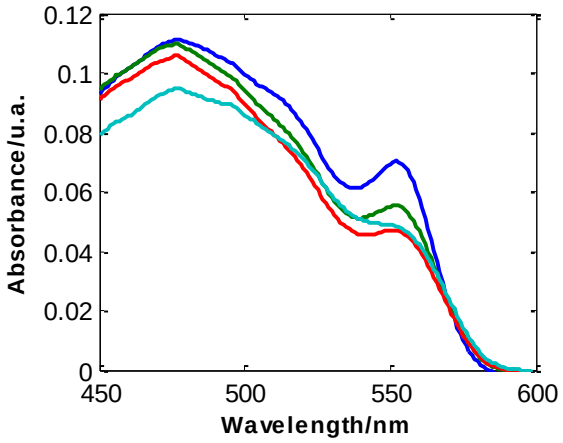
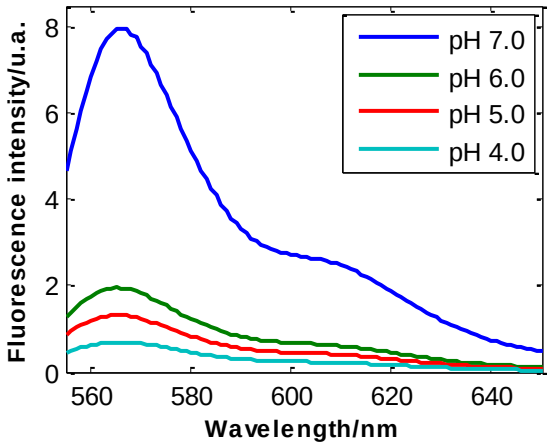
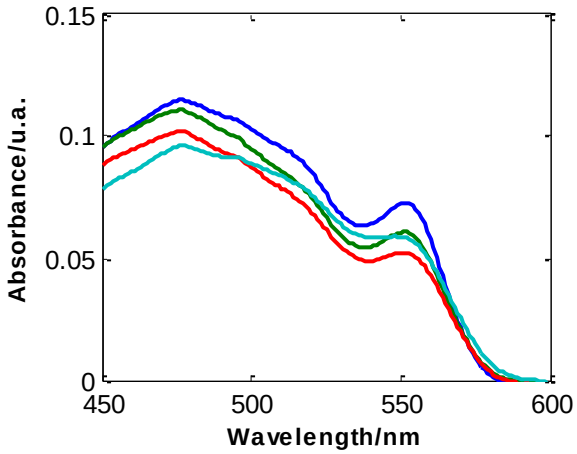
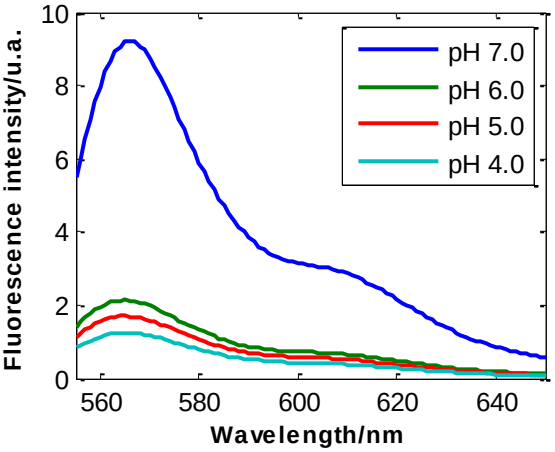
CD spectra of the unmodified (Blue) and 5'-Cy3, 3'-DABCYL-modified (red) TT, AA, nmyc and nmycM sequences at pH 4.



Supplementary Figure 3. Absorption and emission spectra

Absorption and emission spectra at different pH for the fluorophore (Cy3) and all sequences studied. Conditions: 20mM phosphate buffer, 150mM KCl and 25°C

Sequence	Absorption spectra	Emission spectra
Cy3		
F-T10		
F-TT-Q		

F-AA-Q		
F-nmyc-Q		
F-nmycM-Q		

Supplementary Tables 1 and 2. Fluorescence decay fitting parameters

Supplementary Table 1. Fluorescence decay fitting parameters of F-T_{10}, F-AA-Q and F-TT-Q sequences, lifetime (τ) and the % of fractional amplitude. Experimental error : 20 ps. $^{\$}$: Number in parentheses is the first significant figure of error.

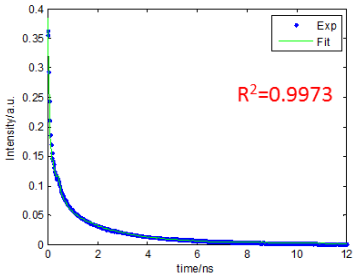
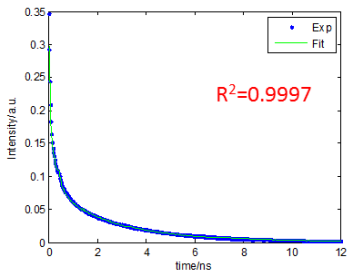
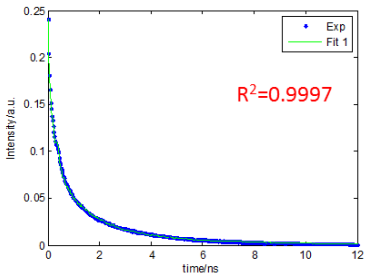
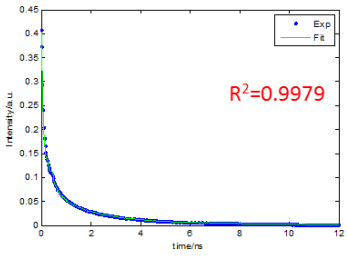
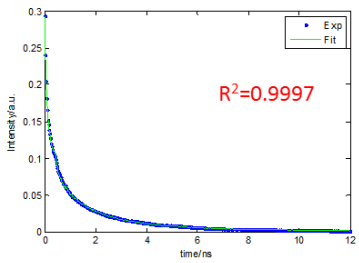
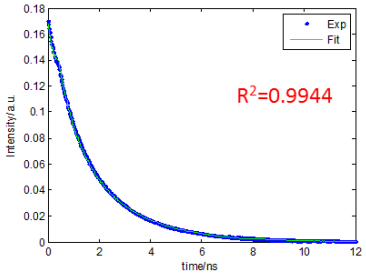
Sample	F-T_{10}				F-TT-Q				F-AA-Q			
pH	7	6	5	4	7	6	5	4	7	6	5	4
τ_1(ns)	1.88(8)$^{\$}$	1.98(1)	2.01(1)	1.94 (9)	2.35(7)	2.99(1)	3.08(1)	3.01(1)	2.38(7)	3.03(1)	3.06(1)	3.00(2)
% Amplitude	15.01	13.94	13.91	15.28	38.17	6.48	3.44	1.75	37.94	5.28	4.15	1.64
τ_2(ns)	0.71(3)	0.79(6)	0.81(7)	0.77(5)	1.11 (8)	0.89(2)	0.81(2)	0.88(1)	1.13(8)	1.02(2)	0.85(2)	0.88(2)
% Amplitude	44.04	42.48	42.82	42.28	44.09	5.4	2.94	2.59	43.72	4.73	3.39	2.58
τ_3(ns)	0.19(3)	0.24 (6)	0.25 (7)	0.23 (5)	0.35(2)	0.19(4)	0.17(3)	0.18(3)	0.34(2)	0.19(5)	0.16(3)	0.18(5)
% Amplitude	40.95	43.58	43.27	42.43	17.75	16.11	11.62	7.93	18.34	12.51	13.29	7.73
τ_4(ns)						0.04(7)	0.03(2)	0.03(2)		0.03(3)	0.03(3)	0.03(2)
% Amplitude						72.01	82.00	87.73		77.48	79.18	88.05

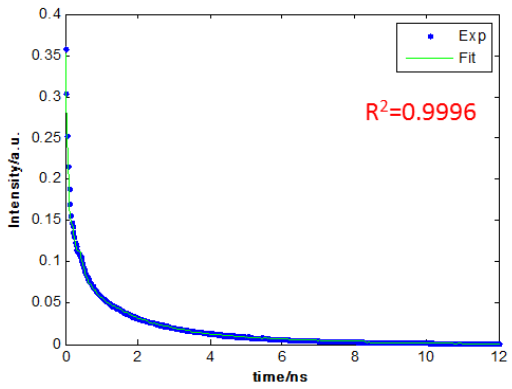
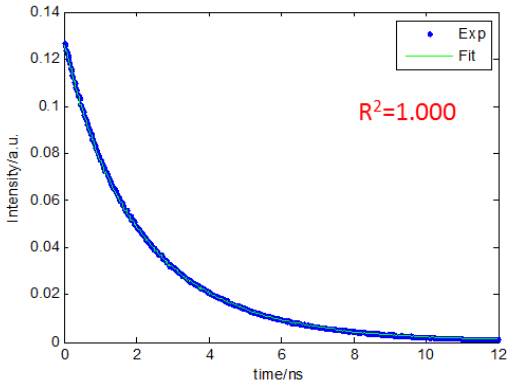
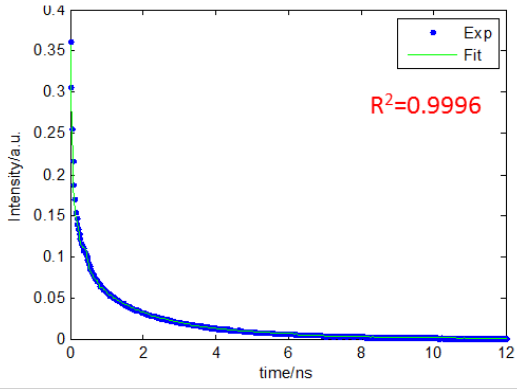
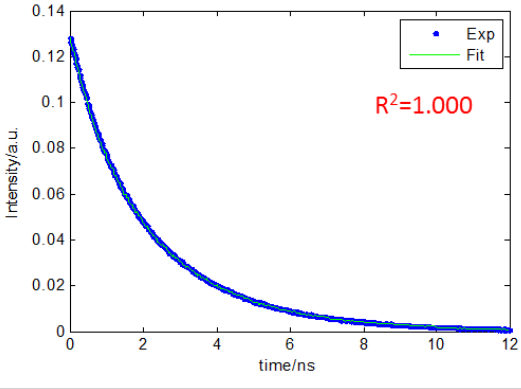
Supplementary Table 2. Fluorescence decay fitting parameters of F-nmyc-Q and F-nmycM-Q sequences, life time (τ) and the % of fractional amplitude. Experimental error : 20 ps. $^{\$}$: Number in parentheses is the first significant figure of error.

Sample	F-nmyc-Q				F-nmycM-Q			
pH	7	6	5	4	7	6	5	4
τ_1(ns)	2.62(5)$^{\$}$	2.68(1)	2.64(1)	2.75(1)	2.52(5)	2.67(7)	2.74(1)	2.63(1)
% Amplitude	64.06	3.79	2.98	3.39	56.00	3.4	3.09	2.61
τ_2(ns)	1.12(1)	0.89(1)	0.80(1)	0.95(1)	1.06(1)	0.89(1)	0.97(2)	0.81(1)
% Amplitude	29.39	3.13	2.79	3.86	32.63	2.73	2.93	2.92
τ_3(ns)	0.24(4)	0.17(4)	0.16(3)	0.19(4)	0.27(3)	0.17(3)	0.18(4)	0.16(3)
% Amplitude	6.56	8.81	9.69	12.1	11.38	9.85	11.23	10.96
τ_4(ns)		0.03(3)	0.03(2)	0.03(4)		0.03(2)	0.03(4)	0.03(2)
% Amplitude		84.27	84.54	80.66		84.03	82.75	83.52

Supplementary Figure 4. Curve fitting analysis

Fitting of the resolved pure decay traces obtained by MCR-ALS for the dominant conformations at the different pH values. Fitting tasks are done by using the curve fitting tool (cftool) of Matlab.

Sequence	pH 4	pH 5 and 6	pH 7
F-TT-Q			
F-AA-Q			

Sequence	pH 4, 5 and 6	pH 7
F-nmyc-Q		
F-nmycM-Q		

Supplementary Table 3. Comparison of fitting on pure resolved traces by MCR-ALS and on raw experimental decay curves (cftool)

Fluorescence decay fitting parameters of F-TT-Q, F-AA-Q, F-nmyc-Q and F-nmycM-Q sequences, lifetime (τ) and the % of fractional amplitude. a) Fitting of experimental decay curves and b) pure resolved traces by MCR-ALS analysis by cftool. *Pure traces that could be associated with this pH range. $^{\$}$: Number in parentheses is the first significant figure of error.

a) Fitting of raw experimental decay curves (cftool)

Sample	F-T10	F-TT-Q			F-AA-Q			F-nmyc-Q		F-nmycM-Q	
pH	7&6&5&4	7	6&5	4	7	6&5	4	7	6&5&4	7	6&5&4
τ_1(ns)	1.79(4)$^{\$}$	2.6(2)	2.90(1)	2.7(5)	2.7(1)	2.88(3)	2.7(6)	2.67(2)	2.56(1)	2.6(4)	2.5(1)
% Amplitude	33	33	23	18	32	26	18	70	25	70	22
τ_2(ns)	0.610(1)	1.2(3)	0.5(4)	0.53(1)	1.25(3)	0.59(4)	0.5(1)	1.16(3)	0.55(4)	1.06(1)	0.5(2)
% Amplitude	67	67	29	33	68	27	33	30	28	30	27
τ_3(ns)			0.07(2)	0.064(3)		0.07(5)	0.06(2)		0.066(4)		0.067(1)
% Amplitude			63	59		47	49		47		51

b) Fitting of pure resolved traces by MCR-ALS analysis (cftool)

Sample	F-TT-Q			F-AA-Q			F-nmyc-Q		F-nmycM-Q	
pH	7*	6&5*	4*	7*	6&5*	4*	7*	6&5&4*	7*	6&5&4*
τ_1(ns)	2.8 (2)$^{\$}$	3.08(3)	2.9(2)	2.63(3)	2.66(3)	2.8(1)	2.69(2)	2.54(4)	2.61(2)	2.52(4)
% Amplitude	25	20	14	48	18	12	68	18	69	18
τ_2(ns)	1.33(5)	0.45(1)	0.60(5)	0.46(1)	0.469(9)	0.56(4)	1.23(3)	0.49(2)	1.11(2)	0.48(2)
% Amplitude	75	28	29	52	42	28	32	30	31	28
τ_3(ns)		0.065(1)	0.089(4)		0.048(1)	0.073(3)		0.065(1)		0.066(1)
% Amplitude		66	67		40	60		52		53