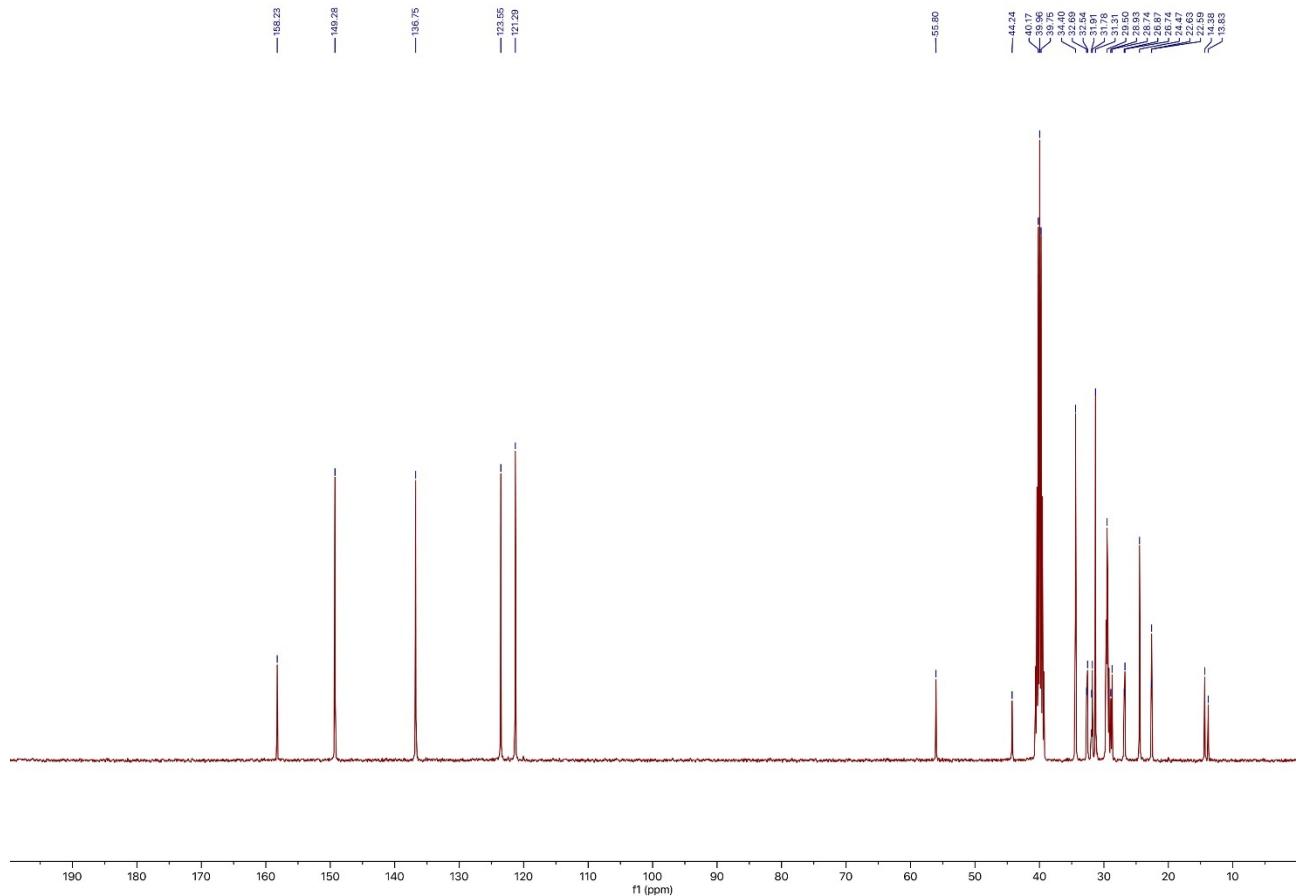


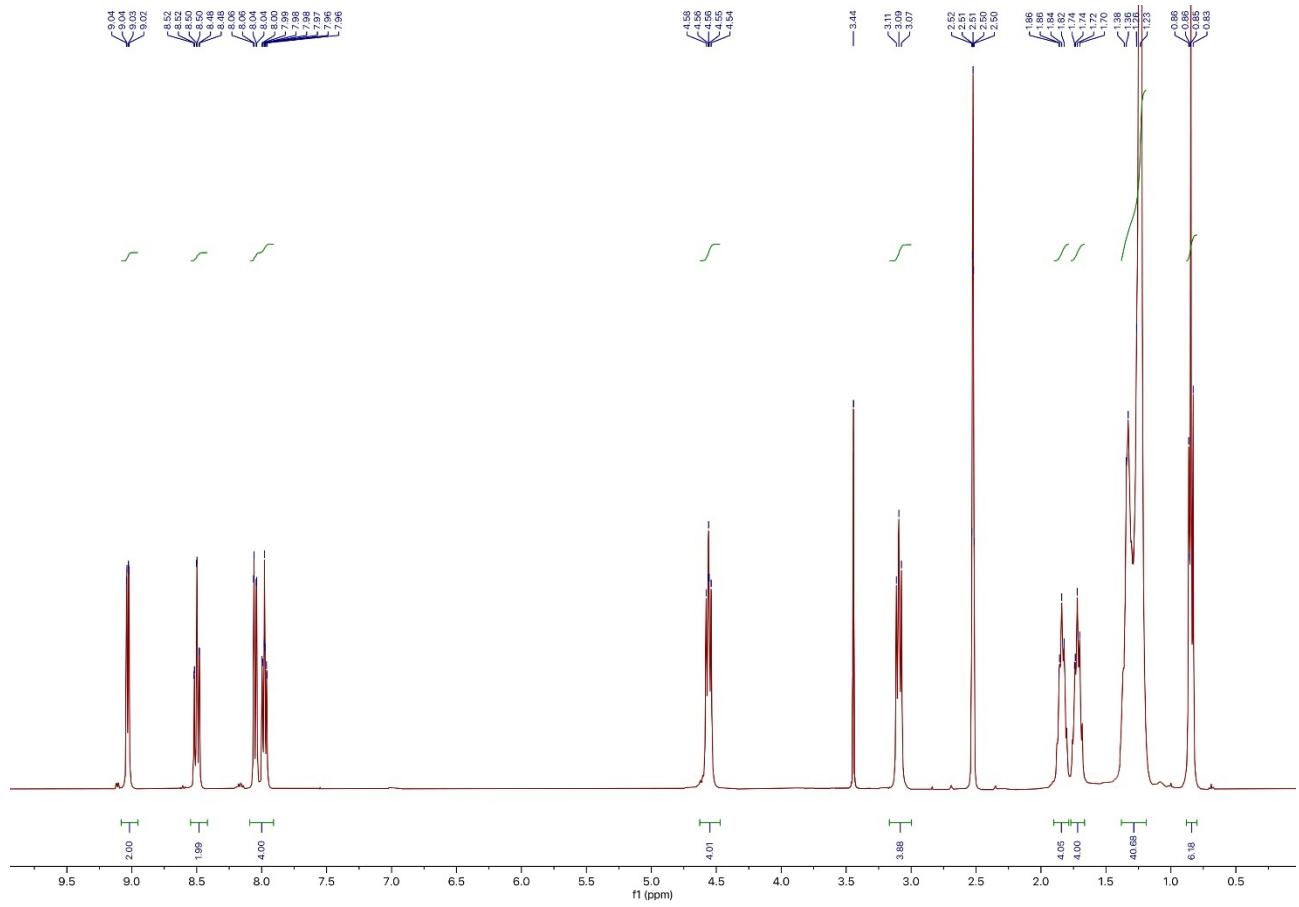
## SUPPORTING INFORMATION

# Chemico-Physical Properties of Some 1,1'-Bis-alkyl-2,2'-hexane-1,6-diyl-bispyridinium Chlorides Hydrogenated and Partially Fluorinated for Gene Delivery

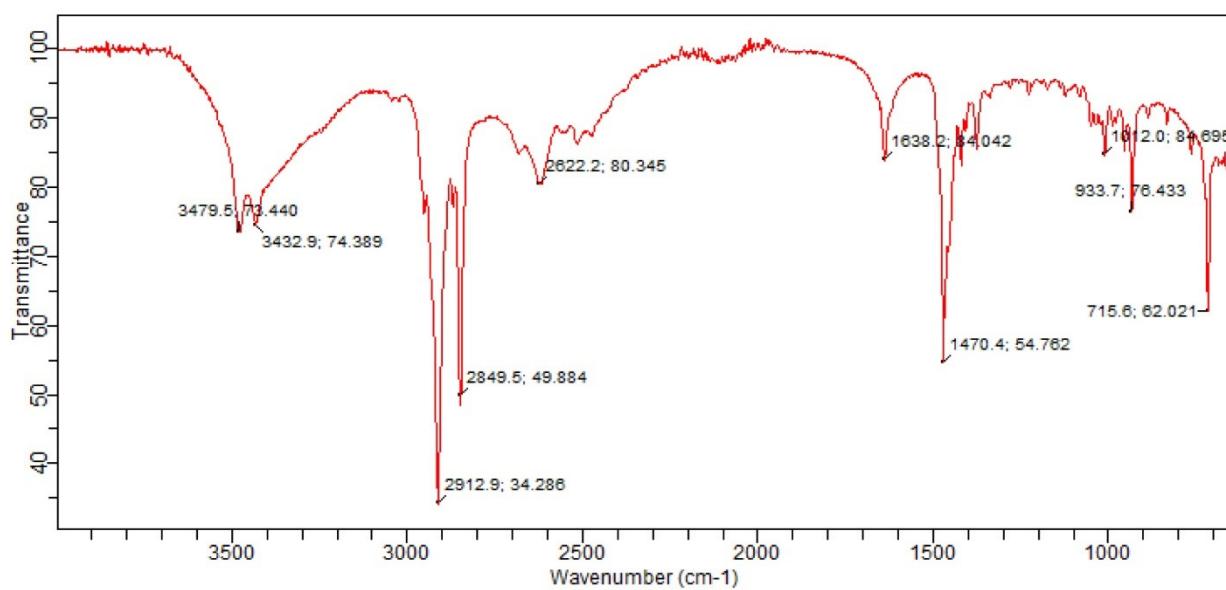
Michele Massa <sup>1</sup>, Mirko Rivara <sup>2</sup>, Thelma A. Pertinhez <sup>3</sup>, Carlotta Compari <sup>2</sup>, Gaetano Donofrio <sup>4</sup>, Luigi Cristofolini <sup>5</sup>, Davide Orsi <sup>5</sup>, Valentina Franceschi <sup>4</sup> and Emilia Fisicaro <sup>2,\*</sup>



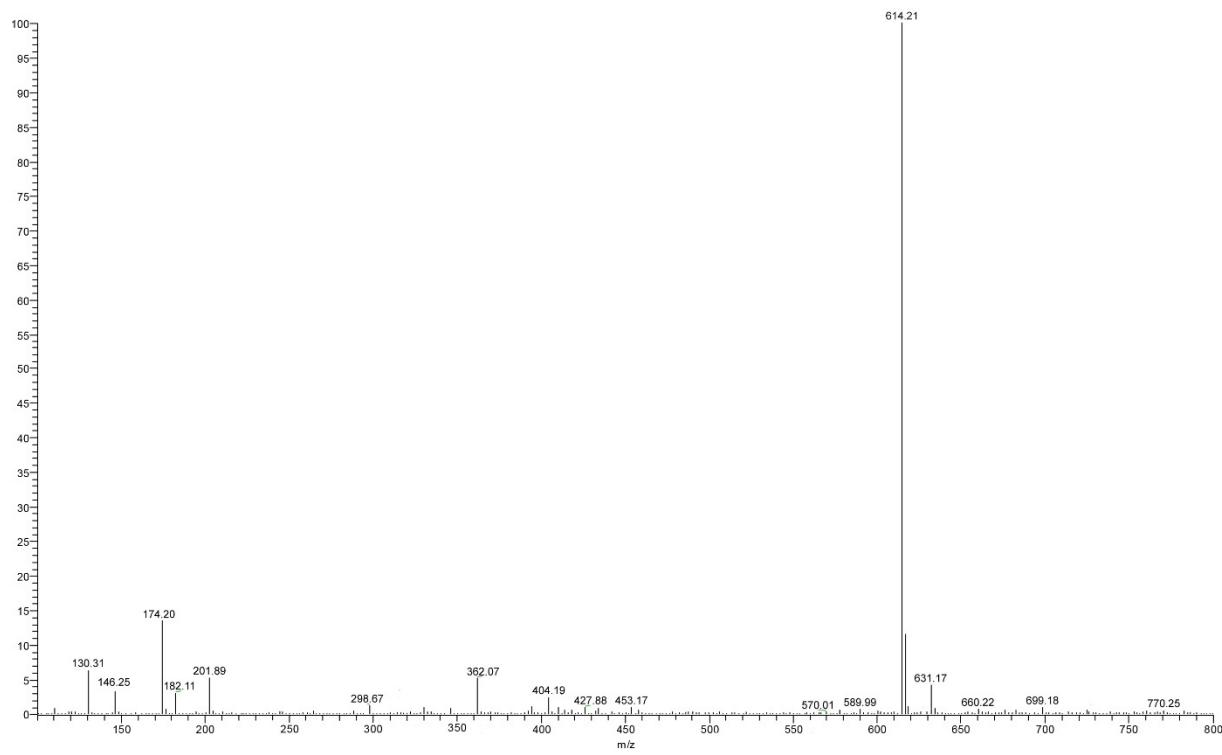
**Figure S1.** <sup>13</sup>C-NMR of 1,1'-bis-hexadecyl-2,2'-hexamethylenebispyridinium chloride (GP12\_6).



**Figure S2.** <sup>1</sup>H-NMR of 1,1'-bis-dodecyl-2,2'-hexamethylenebispyridinium chloride (GP12\_6).

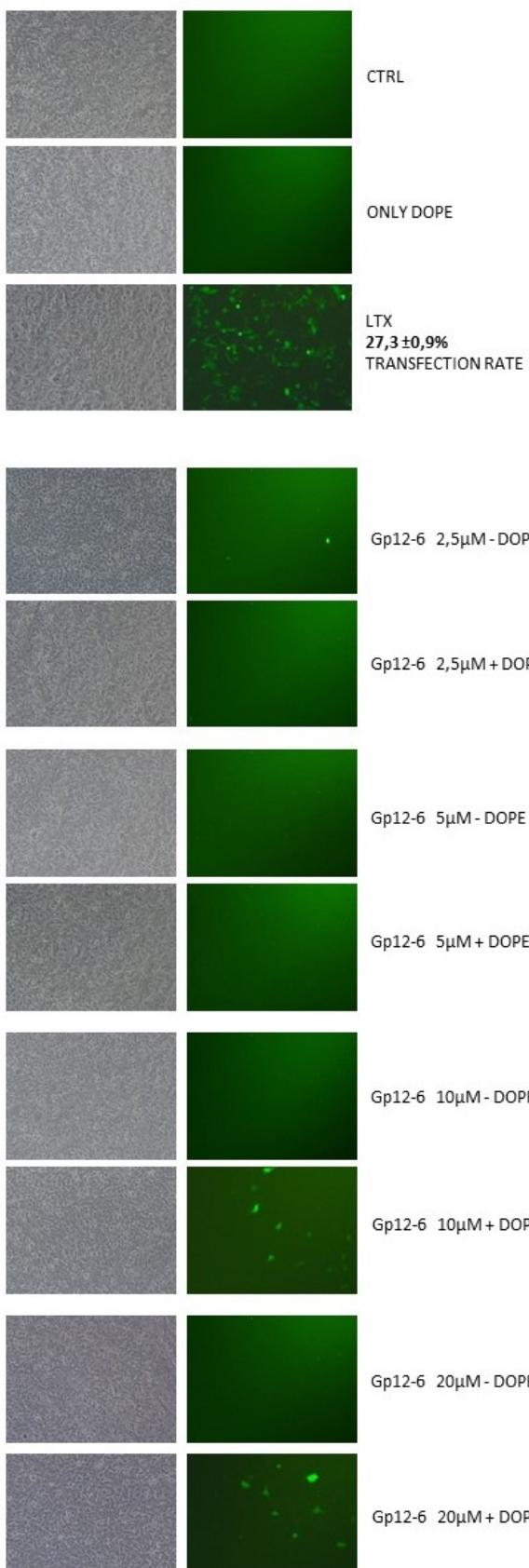


**Figure S3.** IR spectrum of 1,1'-bis-dodecyl-2,2'-hexamethylenebispyridinium chloride (GP12\_6).



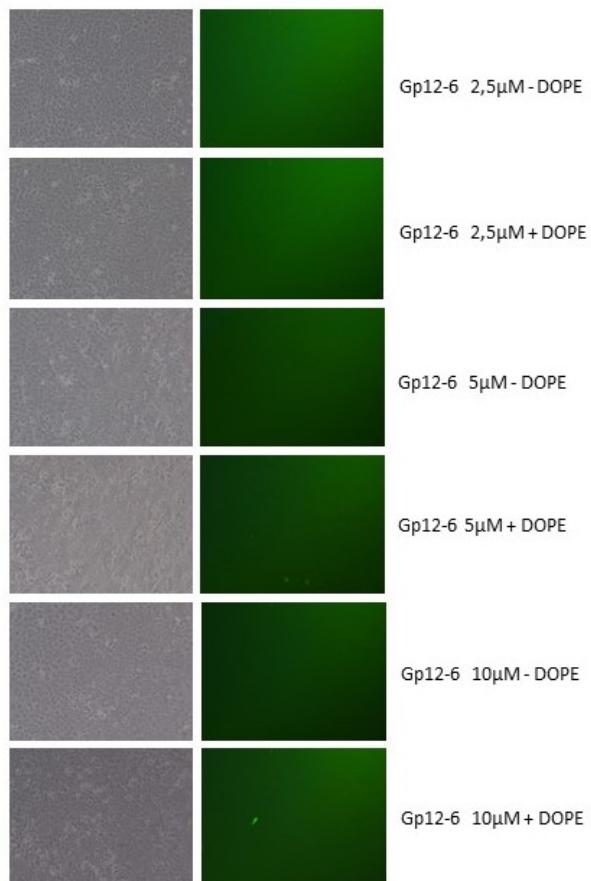
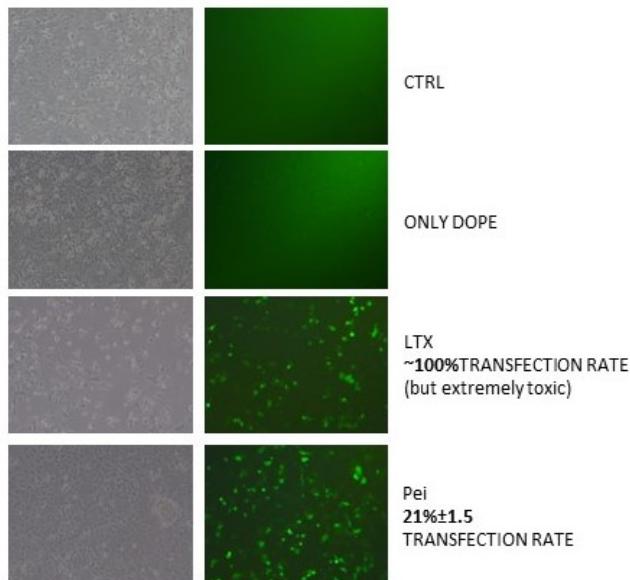
**Figure S4.** Mass spectrum of 1,1'-bis-dodecyl-2,2'-hexamethylenebispyridinium chloride (GP12\_6).

## RD4



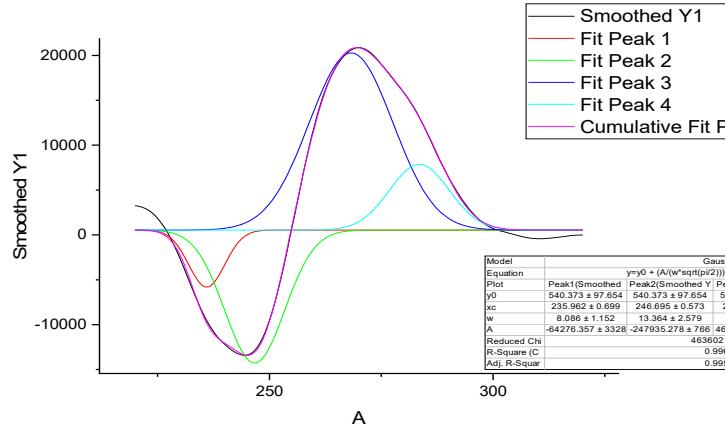
**Figure S5.** Transfection of RD4 by GP12\_6 as a function of concentration. On the left, phase contrast and, on the right, fluorescence microscope observation of the transfected cells (as shown by green cells expressing EGFP) are shown. The experiments were done with the surfactant alone and with surfactant:DOPE=1:2. Cells are not transfected with DOPE alone.

# A549

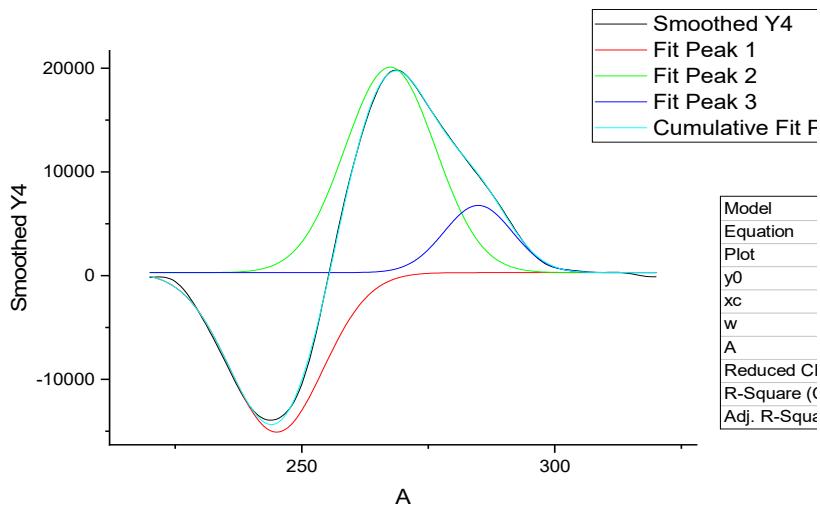


**Figure S6.** Transfection of A549 cells (right) by GP12\_6 as a function of concentration. On the left, phase contrast and, on the right, fluorescence microscope observation of the transfected cells (as shown by green cells expressing EGFP) are shown. The experiments were done with the surfactant alone and with surfactant:DOPE =1:2. Cells are not transfected with DOPE.

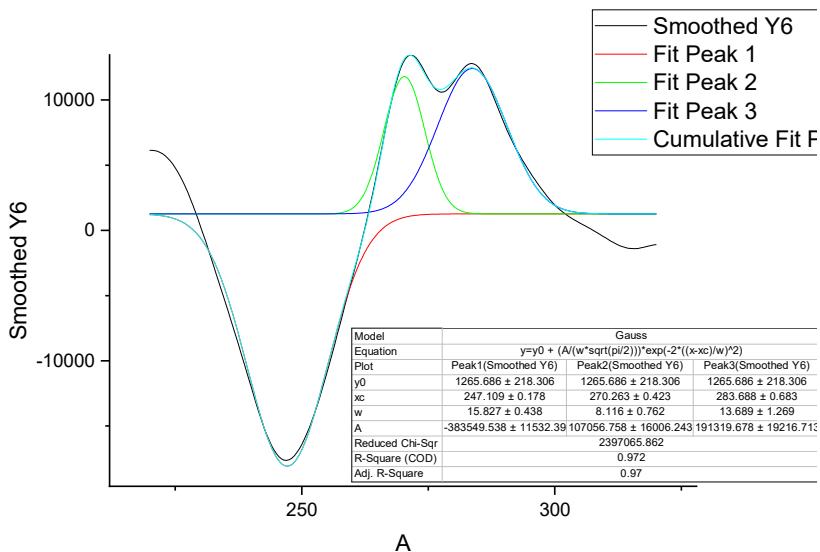
## DNA



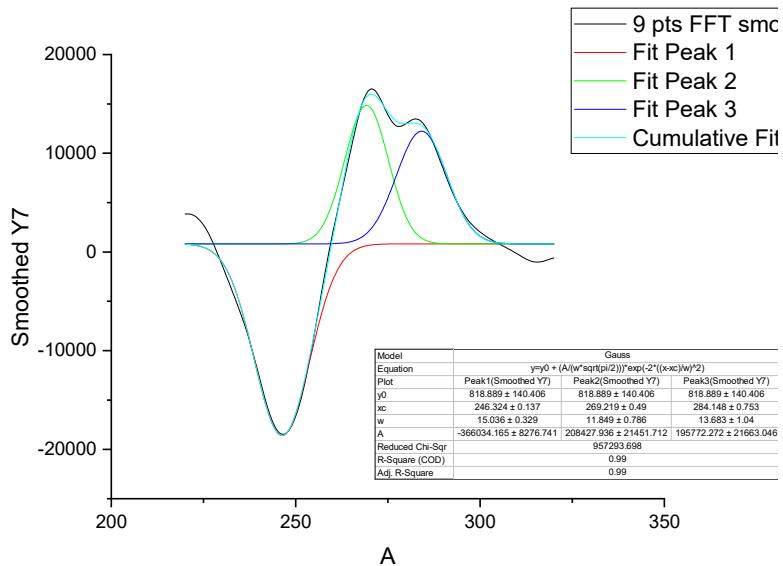
## GP12-6 1:4



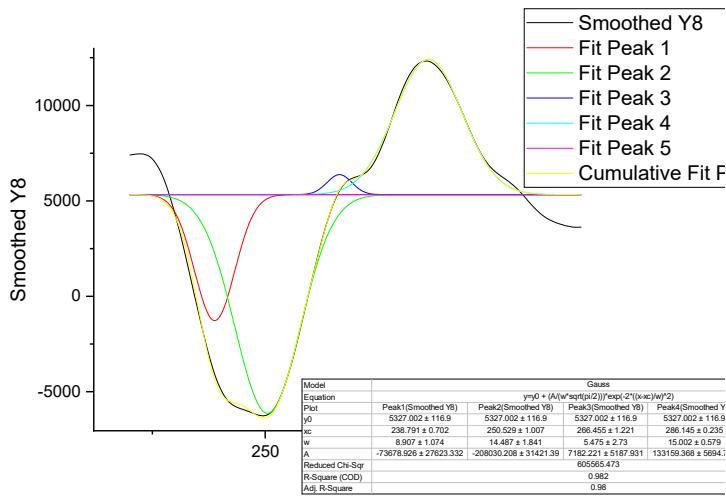
## GP16-6 1:2



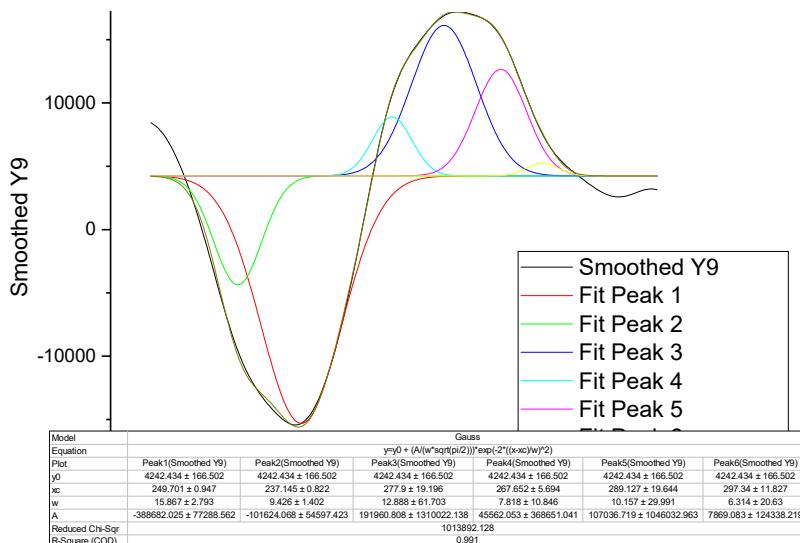
### GP16-6 1:4



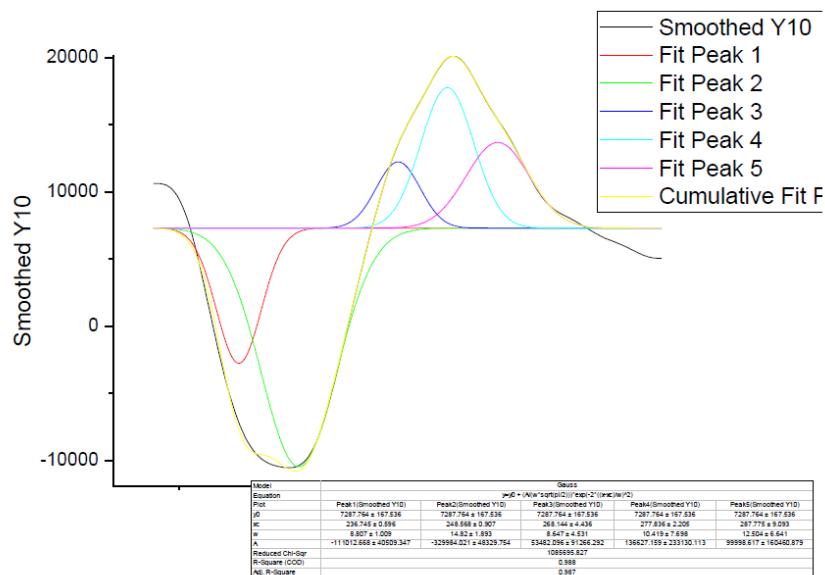
### FGP6 1:1



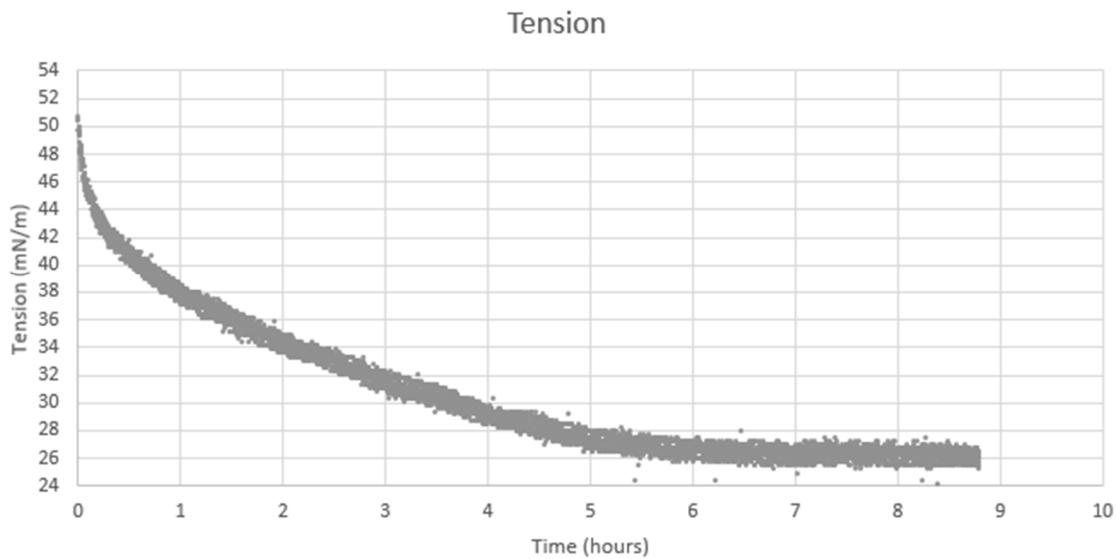
### FGP6 1:2



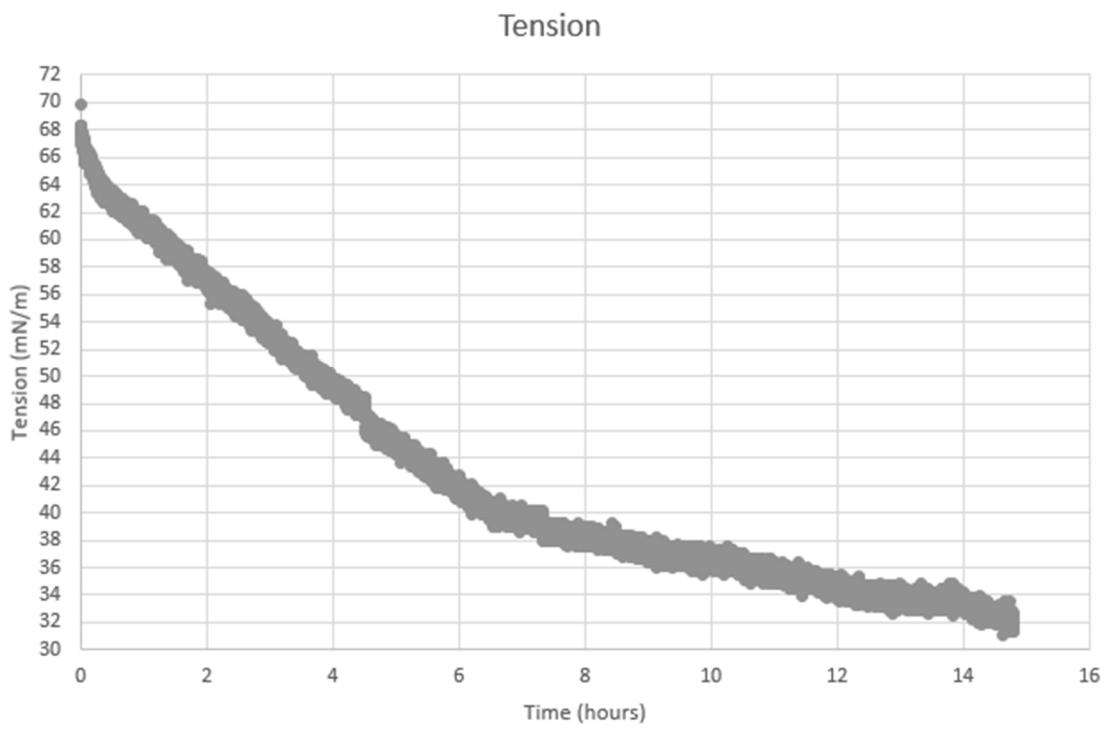
FGP6 1:4



**Figure S7.** Deconvolution of the spectra using Origin Pro, gaussian model.



**Figure S8.** Kinetics of surface layer formation for FGP4 100  $\mu\text{M}$ . It takes about 6 hours to reach the equilibrium. Surface tension is lowered to 26mN/m.



**Figure S9.** Kinetics of surface layer formation for FGP4 50  $\mu\text{M}$ . After 15 hours, the equilibrium is not reached.

**Table S1.** Deconvolution of the spectra. DNA: A-form yellow, B-form green.

Wavelength (nm)

sample	A-form		B-form		
	neg band	pos band	neg band 2	pos band 2	pos band 3
DNA	235.96 ± 0.7	268.25 ± 1.46	246.69 ± 0.57	283.52 ± 2.41	
GP12-6 (1:4)		267.48 ± 0.12	245.03 ± 0.20	284.85 ± 0.39	
GP16-6 (1:2)		270.26 ± 0.42	247.11 ± 0.18	283.69 ± 0.68	
GP16-6 (1:4)		269.22 ± 0.49	246.32 ± 0.14	284.15 ± 0.75	
FGP8-6 (1:1)	238.80 ± 0.71	266.45 ± 1.22	250.53 ± 1.0	286.14 ± 0.23	
FGP8-6 (1:2)	237.15 ± 0.82	267.65 ± 5.69	249.70 ± 0.95	277.9 ± 19.2	289.13 ± 19.64
FGP8-6 (1:4)	236.74 ± 0.6	268.14 ± 4.43	248.57 ± 4.43	277.84 ± 2.2	287.77 ± 9.09
intensity					
sample	A-form		B-form		
	neg band	pos band	neg band 2	pos band 2	pos band 3
DNA	-5751.1	20219.7	-14035.3	8070.3	
GP12-6 (1:4)		20126.2	-15065.3	6695.6	
GP16-6 (1:2)		14855.7	-18505.9	12296.3	
GP16-6 (1:4)		10302.0	-17605.8	12448.0	
FGP8-6 (1:1)	-1219.4	6373.5	-6120.1	12340.5	
FGP8-6 (1:2)	-4256.0	9009.1	-15231.0	16058.1	12657.6
FGP8-6 (1:4)	-2810.6	12191.8	-10363.0	17760.7	13681.8

**Table S2.** Surface tension values of FGP4, FGP6 and FGP8 after 2 hours. . For each concentration, the mean and its relative positive and negative error are reported.

Concentrations	FGP4			FGP6			FGP8		
	Mean	Error +	Error -	Mean	Error +	Error -	Mean	Error +	Error -
<b>2.5</b>	68.32	69.1	67.54	64.1	64.67	63.53	63.52	64.27	62.77
<b>5</b>	69.1	69.77	68.43	61.135	61.86	60.41	62.895	63.31	62.48
<b>10</b>	62.7	63.2	62.2	56.44	57.07	55.81	55.04	55.78	54.3
<b>20</b>	61.515	62.06	60.97	52.62	53.46	51.78	46.27	47.02	45.52