SUPPLEMENTARY MATERIAL

Table S1. Rate constants of the **D** triplex, obtained at differents flow rates

Flow rate (µl/min)	k_{ass} (M^{-1} s^{-1})	K_{diss} (10^{-6} s^{-1})
40	1430±27	60±6
20	1520±52	58±6
10	1420±23	61±4
5	1560±27	60±3

The measures were conducted in a 10mM sodium cacodylate buffer (pH6.2), 100 mM NaCl and 10 mM $MgCl_2$, at 30°C. 10 concentrations of TFO were used to determine the rate constants, as described in the manuscript.

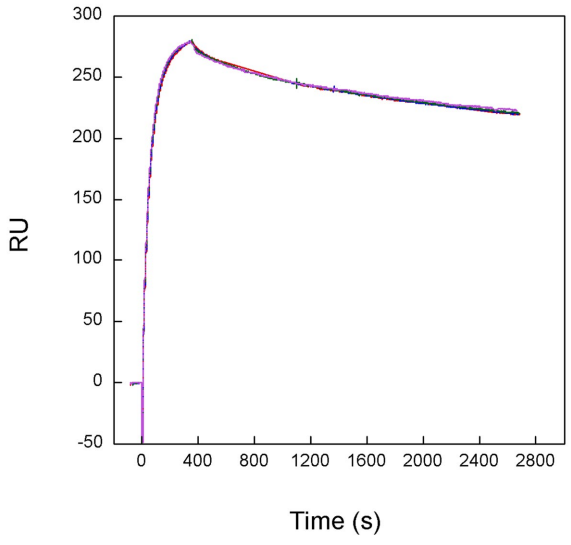

Figure S1. Sensorgrams obtained for the **D** system, on injection of 12 µM TFO, at different flow rates (5, 10, 20, 40 µl/min): the sensorgrams are superimposable

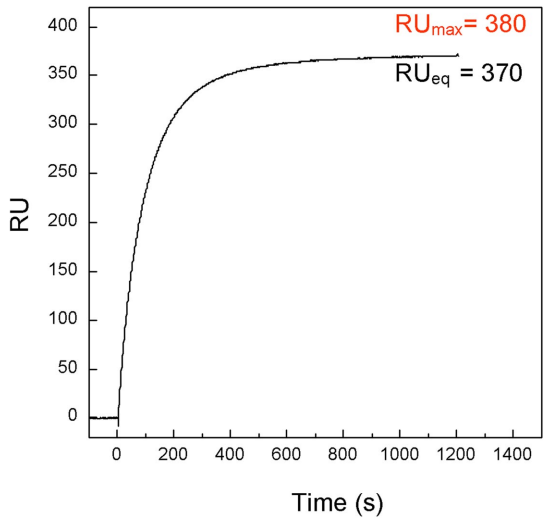

Figure S2. Predicted maximum SPR response (RU_{max}) and measured SPR response at equilibrium (RU_{eq}) for the triplex system **D**, in a 10 mM sodium cacodylate buffer (pH6.2), 100 mM NaCl and 10mM $MgCl_2$, at a temperature of 20°C. RU_{max} is the expected response if all of the binding sites at the sensor chip surface were saturated; it was calculated as RU_{max}= [(TFO molecular weight) / (DNA hairpin molecular weight)] x (response of DNA hairpin immobilized at the surface). RU_{eq} is proportional to the amount of bound TFO at equilibrium. The ratio r = RU_{eq}/RU_{max} provides the number of TFO bound to each DNA hairpin attached to the sensor chip surface. A value r = 1 is indicative of a stoechiometry 1:1 for the system TFO/DNA hairpin duplex.

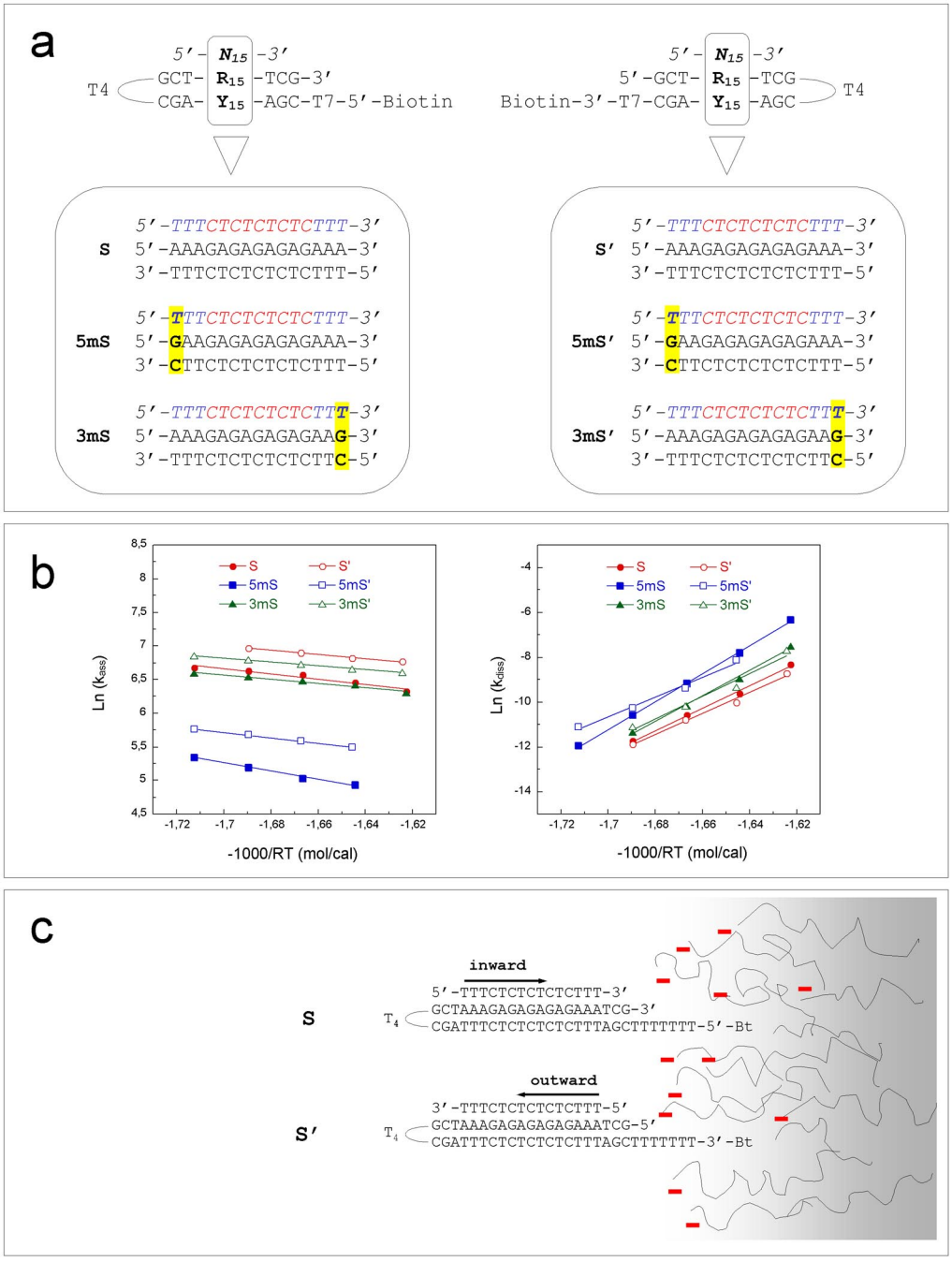

Figure S3. (a) SPR experiments were performed on two symmetrical systems differing in the position of the hairpin loop, located at at the 5'-side (left) and 3'-side (right) of the oligopurine strand, respectively. **(b)** Arrhenius plots of the rate constants of the two symmetrical systems. The

measures were performed in a 10 mM sodium cacodylate buffer (pH6.2), 100 mM NaCl and 10 mM $MgCl_2$. **(c)** Schematic illustration of the triplexes **S** and **S'** at the sensor chip surface. The directional nucleation-zipping model could explain the influence of the location of hairpin loop on the association rate constants: a directional triple helix formation should take place inward (when the loop was at the 5'-side of the oligopurine strand) or outward (when the loop was at the 3'-side of the oligopurine strand) from the sensor chip surface. Negatively charged carboxymethylated dextran layer might concentrate a higher mount of TFOs, therefore it should enhance the nucleation at the 5'-side of triplex closer to the surface.