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Supporting Information

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for

Novel Conformationally Constrained Parallel G Quadruplex

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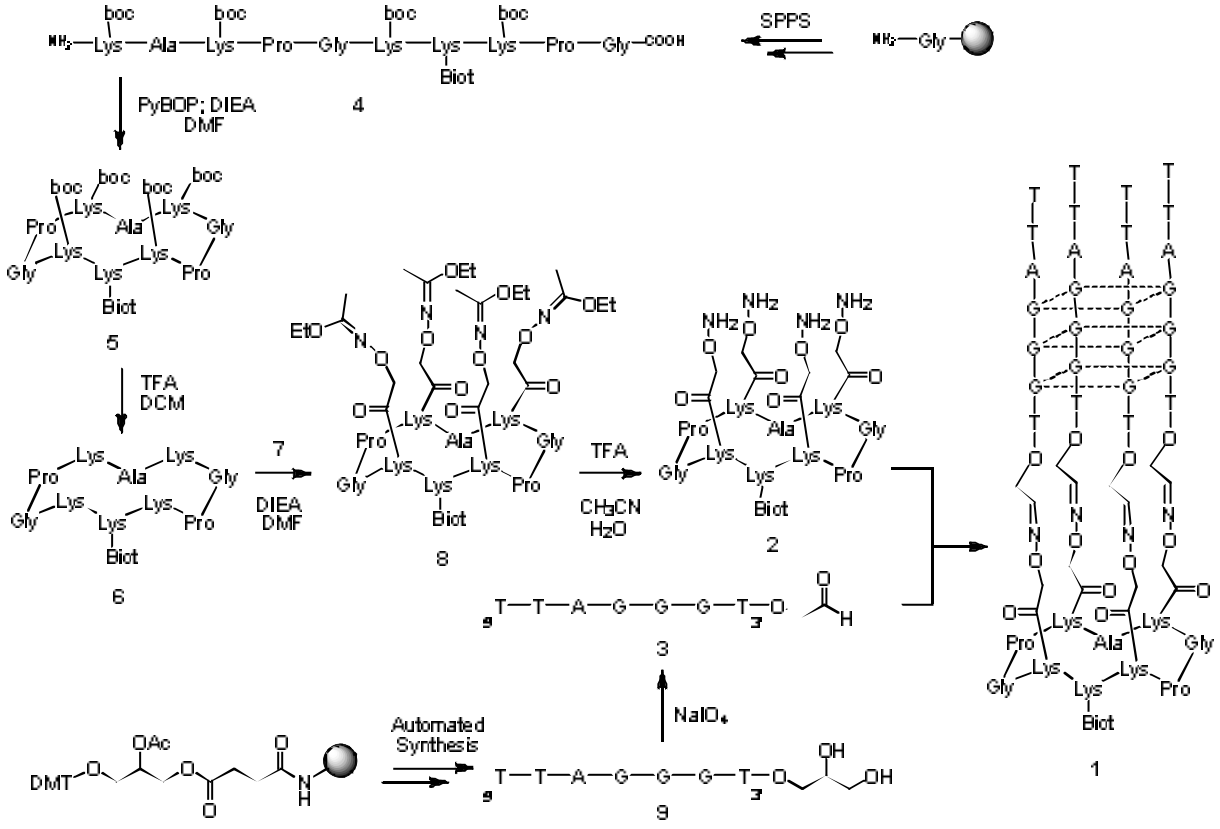
Abbreviations.

Biot: biotine; boc: *tert*-butyloxycarbonyl; CPG: controlled pore glass; DCM: dichloromethane; DIEA: diisopropylethylamine; ESI MS: electrospray ionisation mass spectrometry; Fmoc: fluorenylmethyloxycarbonyl; MALDI-Tof MS: matrix-assisted laser desorption ionization time of flight mass spectrometry; ODN: oligodeoxyribonucleotide; PyBOP: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; TEA: triethylamine; TFA: trifluoroacetic acid; Tris HCl: Tris (hydroxymethyl) aminomethane hydrochloride; RP-HPLC: reversed-phase high pressure liquid chromatography; SAX-HPLC: strong anion exchange high pressure liquid chromatography.

Synthesis of mimic 1

General. All solvents and reagents used were of highest purity commercially available. *N*-a-Fmoc-*N*-?-biotinyl-*L*-lysine was obtained from VWR International (Fontenay sous Bois, France). *N*-Hydroxysuccinimidyl 2-(1-ethoxyethylideneaminooxy) acetate **7** was synthesized as previously described (see below). 3'-aldehyde containing oligonucleotide **3** was prepared as previously described from 3'-diol containing oligonucleotide **9** (see below). RP-HPLC and SAX-HPLC analysis and purification were performed on a Waters HPLC system with dual wavelength detector. RP-HPLC analysis of oligonucleotides **3** and **9** was performed on a Nucleosil C18 column (Macherey Nagel, 250 x 4.6 mm, 5 µm) using the following solvent system: solvent A, 50 mM triethylammonium acetate buffer containing 5% acetonitrile; solvent B, acetonitrile containing 5% water; flow rate of 1 mL.min^{-1}; a linear gradient of 0-30 % B was applied in 20 minutes. UV absorbance was monitored at 260 nm and 280 nm simultaneously. Purification was performed on a µ-Bondapak C-18 column (Macherey-Nagel Nucleosil, 10 x 250 mm, 7 µm) with similar gradient at a flow rate of 4 mL.min^{-1}. RP-HPLC analysis of peptides **2, 4, 5, 6** and **8** was performed on a Nucleosil C18 column (Macherey Nagel, 120 Å, 30 x 4.6 mm, 3 µm) using the following solvent system: solvent A, water containing 1% TFA; solvent B, acetonitrile containing 1% TFA and 10% H_2O; flow rate of 1.3 mL.min^{-1} was employed with a linear gradient (5 to 100% B in 15 min.). UV absorbance was monitored at 214 nm and 250 nm simultaneously. Semipreparative column (Delta-Pak™ 100 Å, 200 x 2.5 mm, 15 µm) were used to purify peptides by using an identical solvent system at a flow rate of 22 mL.min^{-1}. Conjugate **1** was analyzed and purified on a Nucleogel anion exchange column (Macherey Nagel, SAX 1000-8 VA 50/4.6) using the following solvent system: solvent A,

Tris HCl buffer 20 mM; solvent B, Tris HCl buffer 20 mM, 1 M NaCl; flow rate of 3 mL min^{-1}; a linear gradient of 0-100% B was applied in 30 min. ESI mass spectra of **4, 5, 8** and **9** were measured on an Esquire 3000 spectrometer from Bruker. The analyses were performed in negative mode for **9** and in positive mode for **4, 5** and **8**. Aqueous acetonitrile (50%) was used as eluent for **4, 5** and **8** and a 2% TEA aqueous acetonitrile solution (49%) was used as eluent for **9**. MALDI-ToF mass spectrum of **1** was performed on an Autoflex Bruker using hydropiccolinic acid (HPA, 45 mg; ammonium citrate 4 mg in 500 µL H_2O/CH_3CN) as matrix. Prior to MALDI-ToF analysis, samples were exchanged on DOWEX 50 W X8 resin (ammonium form).



Scheme 1. Synthesis of the G-quadruplex mimic **1**.

*Linear peptide **4***. The synthesis was performed on an Advance ChemTech 348O synthesizer using Fmoc/tBu strategy on a SASRIN® resin**. 4** was released from the resin using a solution of TFA/DCM (1/99). After evaporation under vacuum, peptide **4** was precipitated in ether. ESI-MS (+): *m/z* calcd: 1665.0, *m/z* found: 1664.9 $[M+H]^+$.

*Cyclic peptide **5***. Linear peptide **4** (1 equiv) was dissolved in DCM (0.5 mmol.L^{-1}) then PyBOP (1.5 equiv) and DIEA (to obtain pH 8-9) were added. The solution was stirred at RT for 2h and the solvent was removed under vacuum and the peptide **5** precipitated with ether. ESI-MS (+): *m/z* calcd: 1647.0, *m/z* found: 1647.4 $[M+H]^+$.

*N ? -free cyclic peptide **6***. Peptide **5** was dissolved in DCM / TFA (1:1) and stirred at RT for 1 h. The solvent was removed under vacuum and the peptide **6** precipitated with ether. ESI-MS (+): *m/z* calcd: 1246.7, *m/z* found: 1247.0 $[M+H]^+$.

*Protected aminooxy peptide **8***. Peptide **6** was dissolved in anhydrous DMF (0.01 mol L^{-1}) in presence of DIEA (to obtain pH 8-9) and *N*-Hydroxysuccinimidyl 2-(1-ethoxy-ethylideneaminooxy) acetate **7** (4.5 equiv) was then added. The solution was stirred at RT for 2 h and the solvent was removed under vacuum. The peptide was purified using RP-HPLC and freeze-dried. ESI-MS (+): *m/z* calcd: 1819.0, *m/z* found: 1819.6 $[M+H]^+$.

For a complete description of the synthesis of **7**, see: S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturyn, P. Dumy, *J. Org. Chem.* **2008**, *73*, 983- 991.

*Aminooxy peptide **2***. Peptide **8** was dissolved in a solution of TFA/CH_3CN/H_2O (25: 25:50) and stirred at RT for 10 min. The solution was concentrated under vacuum and used for the coupling without further purification.

*3' Diol containing oligonucleotide **9***. Automated synthesis of oligodeoxyribonucleotides was performed on a 3400 DNA synthesizer from Applied Biosystems by using standard ß-cyanoethyl phosphoramidite chemistry on a 3'-glyceryl CPG resin (from Eurogentec). The resin was cleaved with an aqueous ammoniac solution (28%) for 16h at 55°C and the ODNs were purified on a C_{18} column. Removal of the dimethoxytrityl ether was carried out by dissolving the crude in an aqueous acetic acid solution (80%) for 1h at RT. After freeze-drying the residue was dissolved in water and washed with ether. ESI-MS (-): *m/z* calcd: 2304.4, *m/z* observed: 2303.9 $[M-H]^-$.

*3' Aldehyde containing oligonucleotide **3***. The oligonucleotide was dissolved in an aqueous solution of sodium metaperiodate (20 equiv) to reach a concentration of 10^{-3} M. The reaction mixture was stirred at room temperature for 1.5 h then the aldehyde was purified on a C_{18} column.

For some examples of synthesis and use of $^{3'}$aldehyde containing oligonucleotides, see: O. P. Edupuganti, Y. Singh, E. Defrancq, P. Dumy, *Chem. Eur. J.* **2004**, 10, 5988 – 5995.

*Conjugate **1***. 3'-Aldehyde containing oligonucleotide **3** (0.268 µmol, 5 equiv) was dissolved in an ammonium acetate buffer (0.4 M) at a concentration at 10^{-3}M, then the peptide **2** (0.053 µmmol, 1 equiv) was added. The solution was stirred at 50°C for 20 h. The crude mixture was purified by anion exchange-HPLC and desalted on a C_{18} column. The mimic **1** was obtained in a 51% yield (0.027 µmol). MALDI-ToF: *m/z* calcd: 10554.3, *m/z* observed: 10550.2 $[M\text{-H}]^-$.

SAX-HPLC chromatogram of Crude conjugate 1

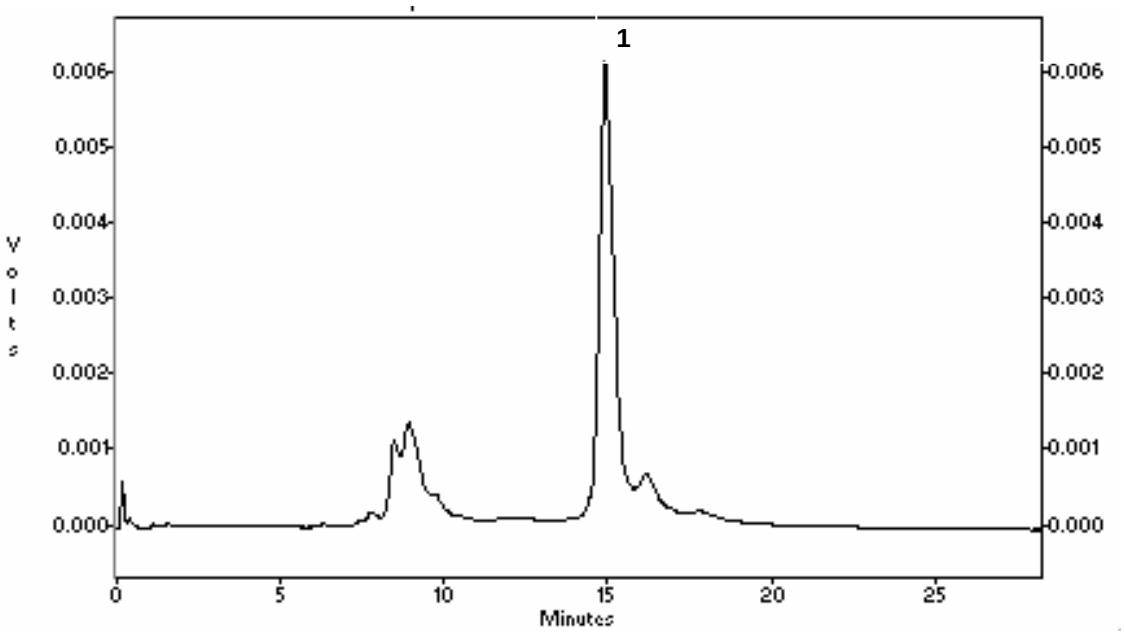


Figure S1. SAX-HPLC chromatogram of crude conjugate **1**.

MALDI-ToF MS spectrum of purified conjugate 1

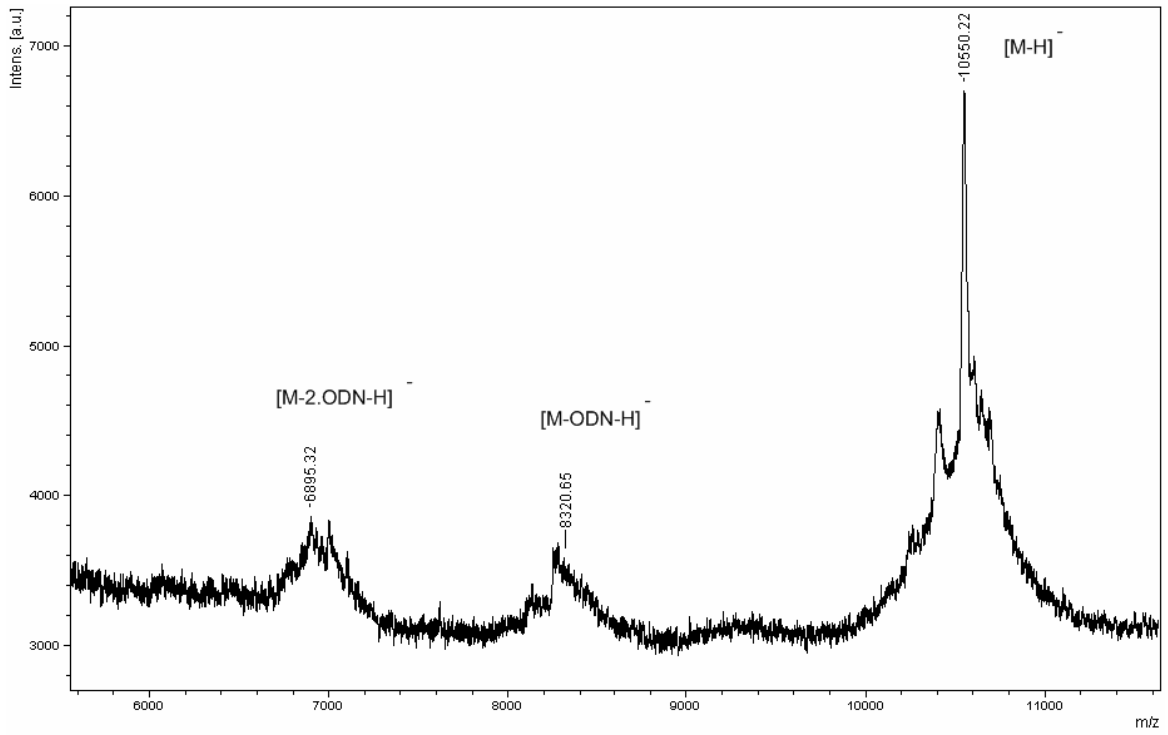


Figure S2. MALDI-ToF Ms spectrum (HPA) of purified conjugate **1**. *m/z* (calcd) 10552.

Circular Dichroism (CD) studies

CD spectra were recorded on a Jasco J-810 circular dichroism spectropolarimeter using 10 mm path length quartz cuvette. Scans were performed at controlled temperature (20°C) over a wavelength range of 200-340 nm (only 220-320 nm is shown), with response time of 0.5 s, 1 nm pitch, 4 nm bandwidth and a scanning speed of 200 nm min^{-1}. Blank spectra of sample containing buffer were subtracted from collected data. The CD spectra represent an average of five scans and are zero-corrected at 340 nm.

Quadruplexes were annealed by heating the oligonucleotides and the mimic **1** at 90°C for 10 min in a Tris-HCl buffer (10 mM, pH 7.4) with 100mM of KCl or NaCl or without any cations added, and cooled slowly to room temperature to favor the thermodynamic form. The samples were prepared at 2.5 µM according to UV measurements.

Thermal denaturation studies. The samples prepared as described above were incubated at each temperature for a suitable time to achieve the equilibrium before re-

cording the CD spectra. The whole spectrum was recorded to follow the behavior of the quadruplex. The denaturation curves were constructed by reporting the intensity of the maximum peak in function of the temperature. The mimic **1** displayed a maximum peak at 263 nm and a minimum peak at 239 nm in the different salt conditions. The intramolecular quadruplex based on the sequence $d(^{5'}TTAGGG^{3'})_4$ displayed a maximum peak at 295 nm and a minimum peak at 265 nm in presence of Na^+, and a maximum peak at 295 nm, a shoulder at 260 nm and a minimum peak at 240 nm in presence of K^+.

Influence of the concentration on the melting temperature. In order to check if the mimic **1** forms an intramolecular or an intermolecular quadruplex, we compared the melting temperatures in function of the concentration of the mimic. As expected the melting temperature is concentration-independent and thus confirmed that the mimic is an intramolecular quadruplex (see Figure S3).

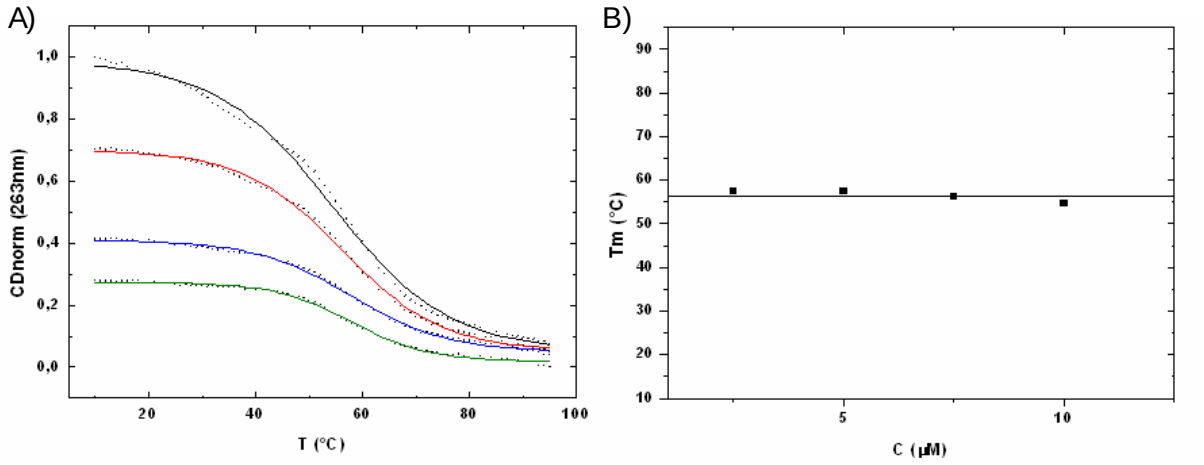


Figure 3. A) Melting curves for the mimic **1** in Tris.HCl pH 7.4 10mM + 100mM NaCl at different concentrations 2.5 (green), 5 (blue), 7.5 (red) and 10µM (black); B) $T_m = f(C)$

Thermodynamic studies

Principles: In the case of intramolecular equilibrium, the ?*H*° could not be derived from the classical concentration dependency of the T_m (the melting temperature is concentration-independent), therefore some thermodynamic analysis of the denaturation profile was performed in this sense. From the classical Van't Hoff relation $\Delta G° = -RT \ln K = \Delta H° - T\Delta S°$ one can deduce that $\ln K = -(\Delta H°/R)(1/T) + (\Delta S°/R)$. Thus, $\ln K$ can be expressed as a linear function of $1/T$. The equilibrium constant K can be

written as $K = ? / (1 - ?)$ for an intramolecular equilibrium where $?$ is the fraction of folded oligonucleotide. The use of this model leads to straight lines ($r > 0.99$), the slope of which is $-\Delta H°/R$ and y intercept $\Delta S°/R$.

For a complete article, see: J.-L. Mergny, A. T. Phan, L. Lacroix, *FEBS Lett.* **1998**, *435*, 74-78.

Protocol: The melting curves of the different systems are constructed as described above and fitted using Origin software and a Boltzmann sigmoid. All the fitted sigmoid were obtained with a correct approximation ($r > 0.99$). The melting temperatures T_m were obtained with the derivative of the melting curve and the different thermodynamic parameters with the plot of $\ln K = f(1/T)$ (see Figure S4).

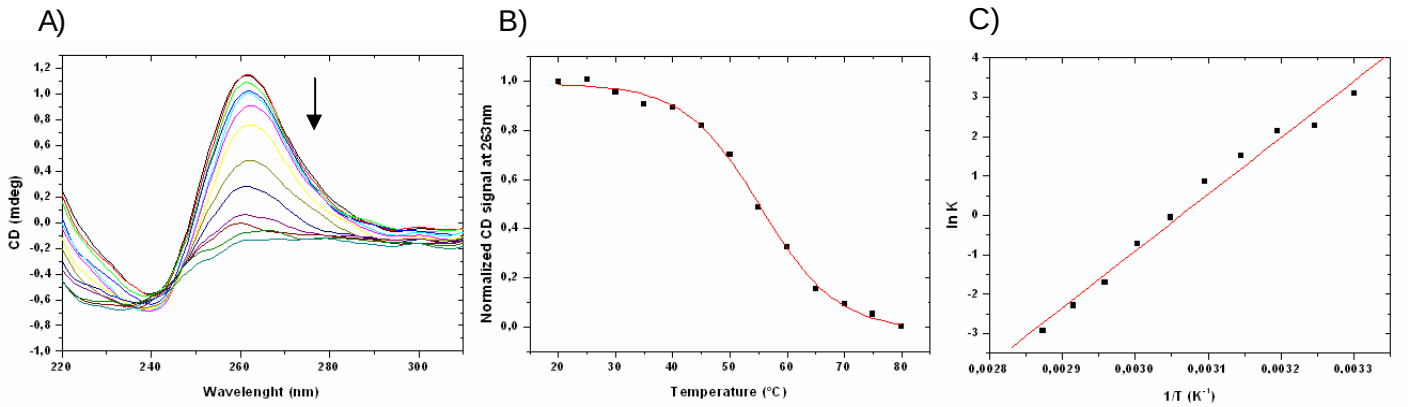


Figure S4. Thermodynamics studies of the mimic **1** in Tris.HCl pH 7.4 10mM + 100mM NaCl; A) CD spectra at different temperatures (20-80°C), B) Melting curve, C) $\ln K = f(1/T)$.

SPR measurement

The SPR measurements were performed with a BIAcore T100 (Biacore AB, Sweden) operated with BIAcore T100 evaluation Software 1.1. All measurements were performed at 25°C, using a working buffer (W.B.) composed of HEPES buffered saline 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% *v/v* surfactant P20 and 0.2 M KCl. G quadruplex mimic **1** was immobilized on a streptavidin-coated surface (SA sensor chip, BIAcore AB). After conditioning the surface with a 1 min injection of a 5 M NaCl/ 0.2 M NaOH solution of mimic **1** (50 nM in W.B.) was injected at 2 µL min^{-1} until a final response of 400 RU.

Binding experiments were conducted at 15 µL.min^{-1} by injection of ligand, the *N*,*N*'-Bis-(2-(dimethylamino)ethyl)-3,4,9,10-perylenetetracarboxylic acid diimide, dissolved in W.B at concentrations of 1 - 50 nM (injection time: 1400 s, dissociation time: 900 s). Regeneration of the surface was achieved by four successive injections (300 s, 30 µL.min^{-1}) of glycine 10 mM pH 2. A non-modified channel was used as reference. Curves obtained on the reference surface were deduced from the curves recorded on the recognition one, allowing elimination of refractive index changes due to buffer effects. A linear Scatchard binding plot as well as the non dependence of the dissociation curves to the association time (see Figure S5) support the use of a 1:1 interaction model to fit the SPR data. Equilibrium dissociation constant, K_D = 3.35 nM, was thus obtained by fitting the steady-state response versus the concentration by using BIAcore T100 evaluation Software 1.1(see Figure S6).

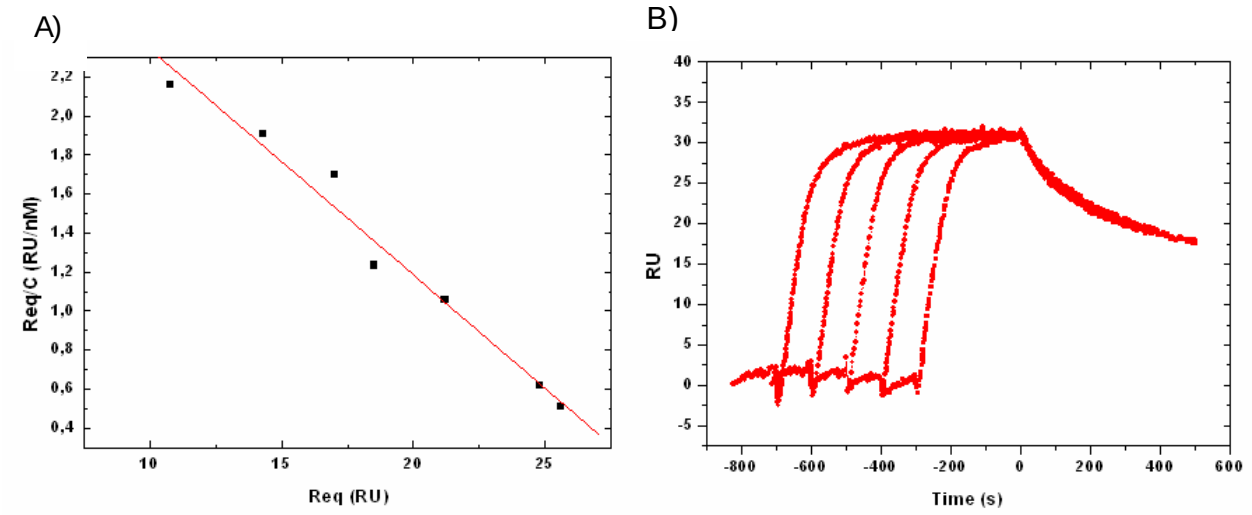


Figure S5. a) Scatchard binding plot of the interaction between ligand and mimic **1** immobilized on surface. Req is the response obtained from the sensorgramm (presented in the manuscript, Figure 3) for the steady state and C the concentration of the ligand solutions injected b) Association/dissociation curves obtained for various injection times (from left to right: 300, 400, 500, 600 and 700 s) of a 25 nM ligand solution at 30 µL/min.

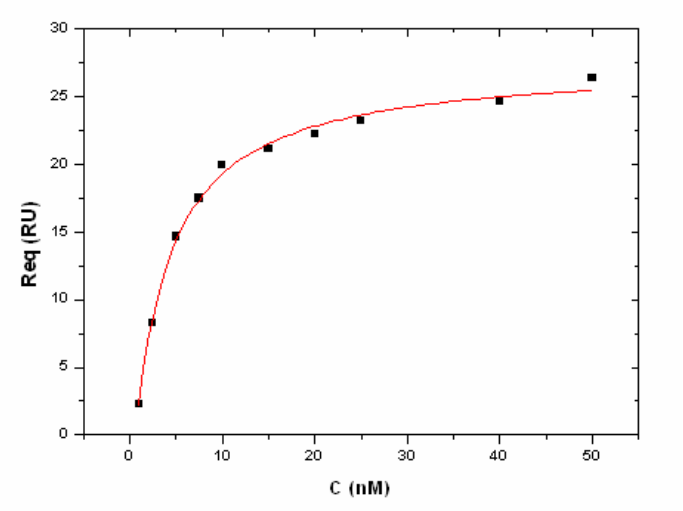


Figure S6. Determination of the dissociation constant (K_D) for ligand /mimic **1** interaction according to Langmuir model.