Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Assessment of presumed small-molecule ligands of telomeric i-DNA by BioLayer Interferometry (BLI)

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General details

Bio-layer interferometry experiments (BLI)

Bio-layer interferometry experiments were performed using sensors coated with streptavidin (SA sensors) purchased from Forte Bio (PALL). Prior to use, they were immersed for 10 minutes in a buffer before functionalization to dissolve the sucrose layer. Then, the sensors were dipped for 15 minutes in DNA containing solutions (**1**-**3** and RAFT-T23) at 100 nM and rinsed in the buffer solution (50 mM Tris AcOH, 35 mM NaCl, 50 mm KCl (pH 5.5 or 6.5), 0.05% v/v surfactant P20) for 10 minutes. The functionalized sensors were next dipped in the ligands containing solution at different concentrations for 10 minutes interspersed by a rinsing step in the buffer solution for 10 minutes. Reference sensors without DNA immobilization were used to subtract the non-specific adsorption on the SA layer. The dissociation equilibrium constant, K_D, was determined by the fitting of the Langmuir isotherm. The reported values are the means of representative independent experiments, and the errors provided are standard deviations from the mean. Each experiment was repeated at least two times.

Circular dichroism studies (CD)

Studies at pH 5.5 and pH 6.5: Circular dichroism studies were performed on a Jasco J-810 spectropolarimeter using 1 cm length quartz cuvette. Spectra were recorded at 25°C with wavelengths range from 220 to 340 nm and were an average of three scans with a 0.5 s response time, a 1 nm data pitch, a 4 nm bandwidth and a 200 $nm.min^{-1}$ scanning speed.
Samples of 2.5 µM of DNA were annealed at 90°C alone and cooled slowly overnight. Ligands (5 equivalents) were added and the samples were incubated at 25°C for 1 min. and the spectra were then recorded. All experiments were performed in the same buffer as for the BLI experiments without P20.

Studies at pH 6.2 (equilibrium point): CD spectra were recorded on a Jasco J-1500 spectropolarimeter in a quartz cuvette with a path length of 2 cm. Spectra were recorded at 20 °C in a wavelength range from 220 to 340 nm and were an average of five scans with a 1 s response time, 0.5 nm data pitch, 2 nm bandwidth, and a 50 nm min^{-1} scanning speed.
Samples of 2.5 µM h-telo DNA **1** in 10 mM $LiAsO_2Me_2$, 100 mM KCl, pH 6.2 buffer were annealed at 90 °C in the absence of ligands and cooled slowly overnight. Ligands (2 molar equiv.) or DMSO control (2.5 µL per 1 mL of sample) were added and the samples were incubated at 20°C for 30 min prior to first measurement. Then, additionally 3 molar equiv. of ligands (or DMSO control) were added, samples were incubated for 30 more minutes and measured again.

Structure of DNA sequences

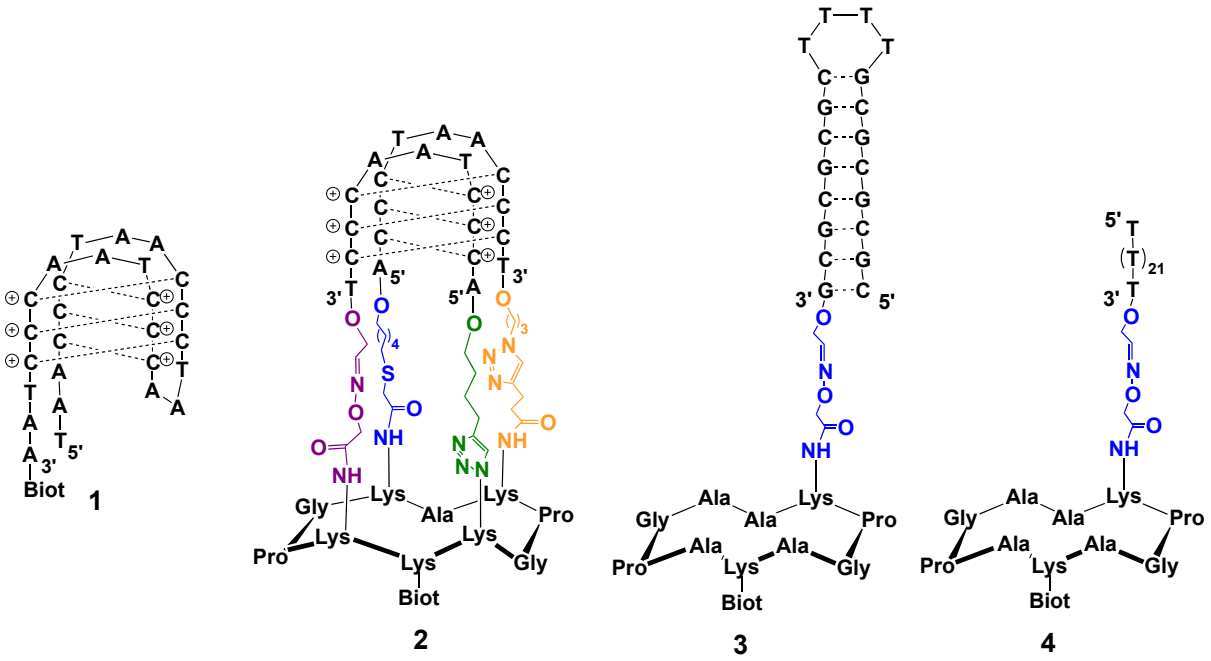

Figure S1. Structure of the native telomeric i-motif **1**, i-motif forming DNA-peptide conjugate **2** , hairpin forming DNA-peptide conjugate **3** and single strand forming DNA-peptide conjugate **4** (RAFT-T23) used in the study.

Bio-layer interferometry experiments

Sensorgrams obtained for RHPS4

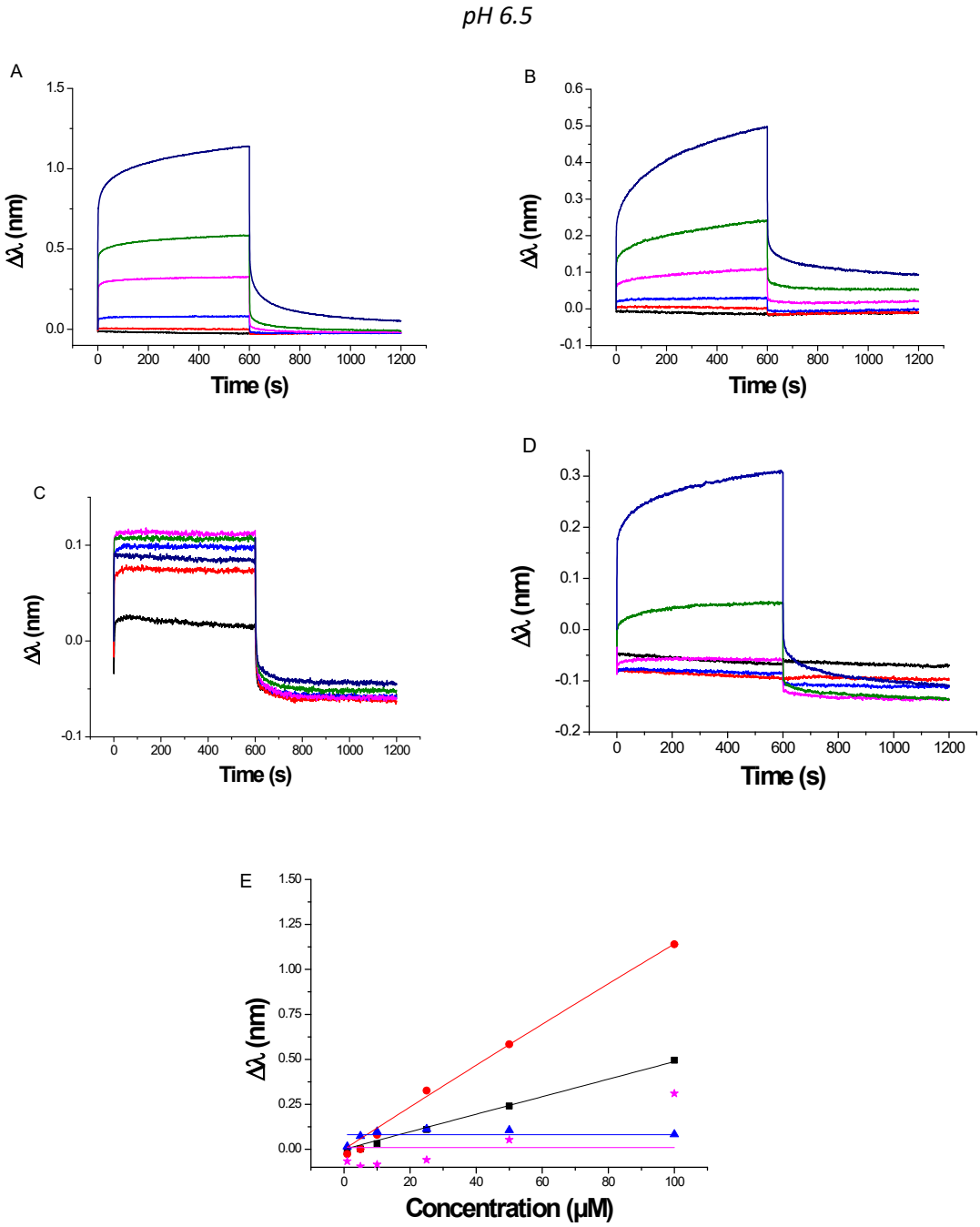

Figure S2. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 25, 50 and 100 µM.

pH 5.5

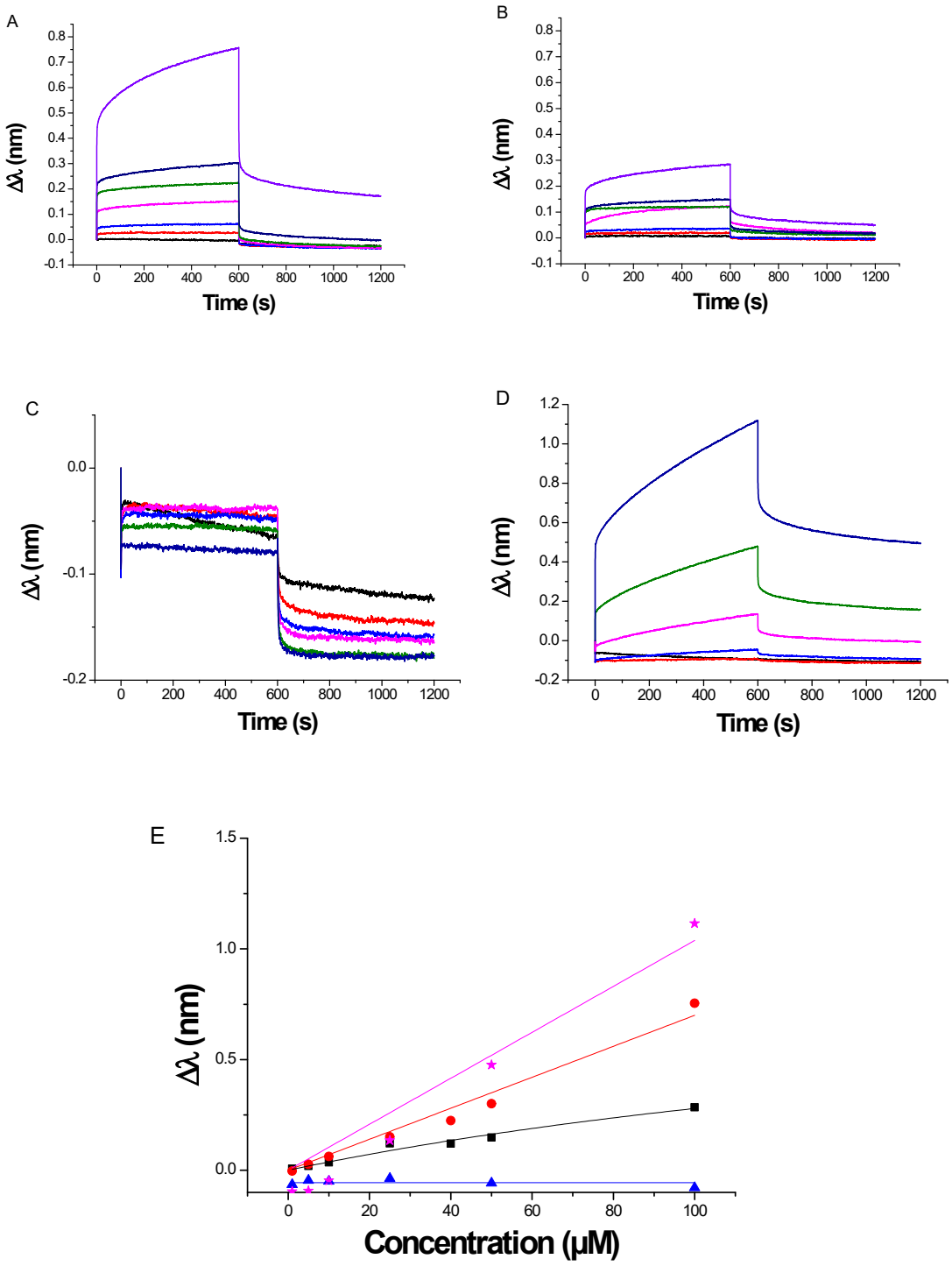

Figure S3. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 25, 40, 50 and 100 µM **1** and **2** and 1, 5, 10, 25, 50 and 100 µM for **3** and **4**.

Sensorgrams obtained for $Ru(Phen)_2dppz$

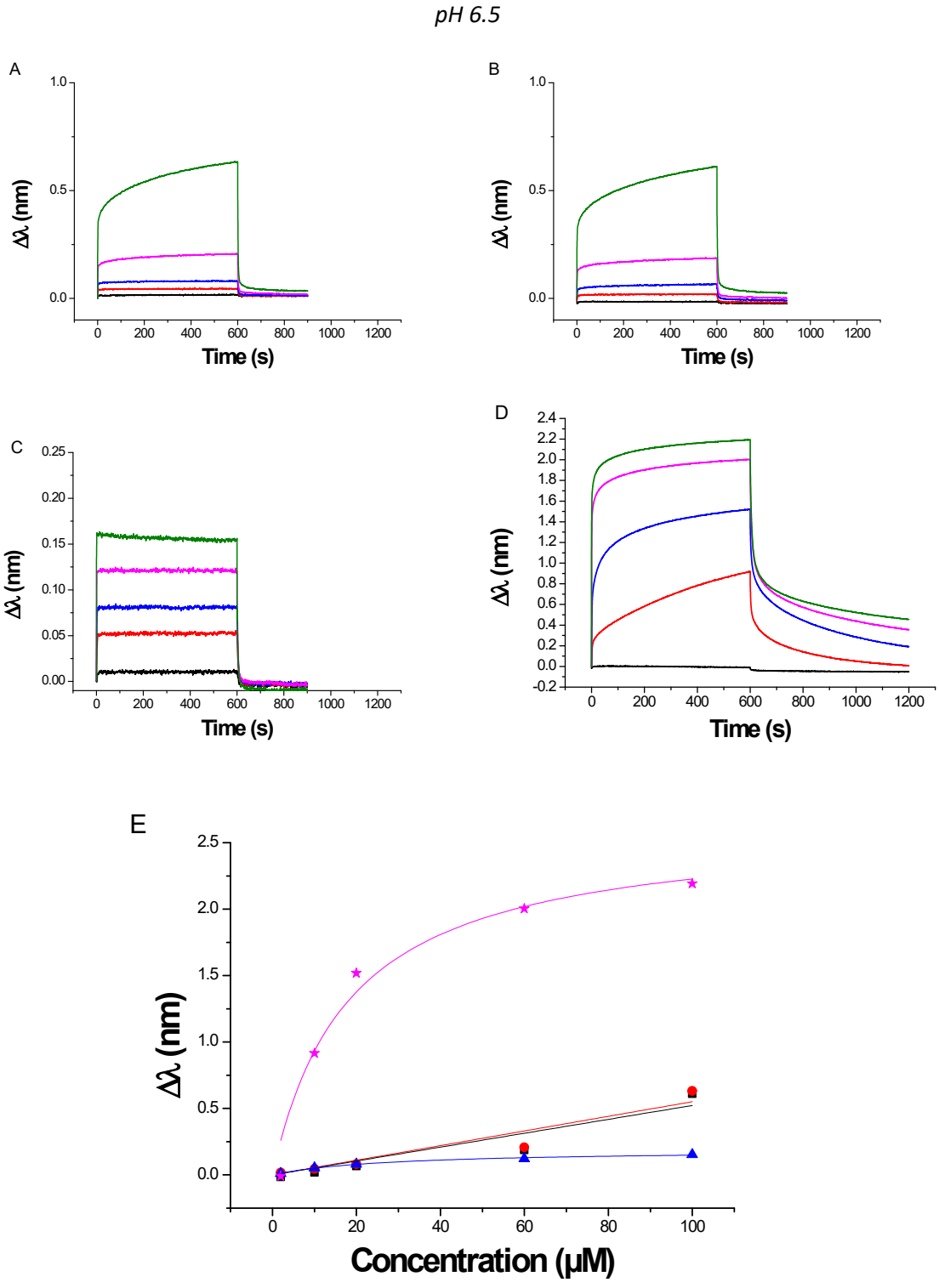

Figure S4. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 2, 10, 20, 60 and 100 µM.

pH 5.5

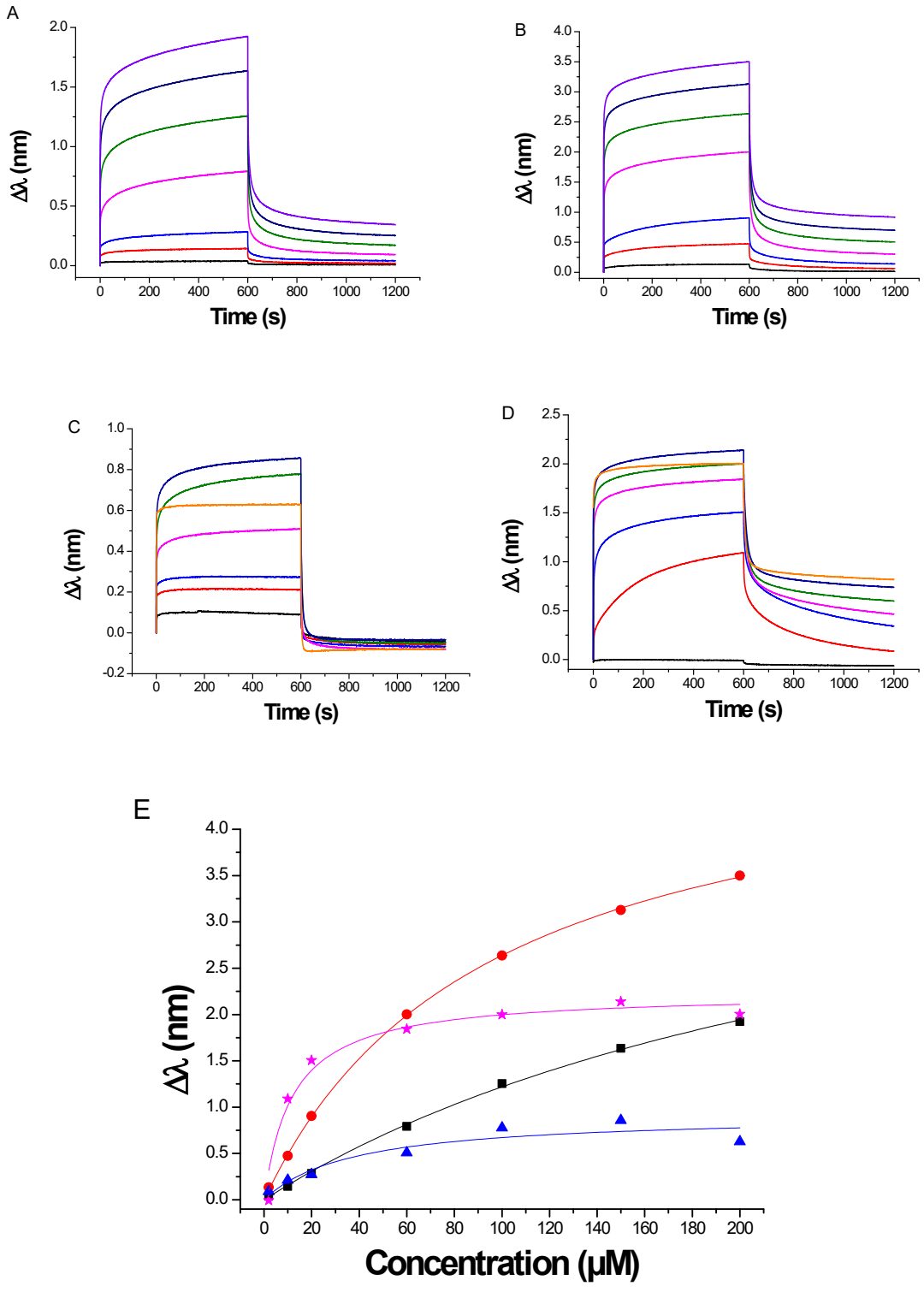

Figure S5. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 2, 10, 20, 60, 100, 150 and 200 µM.

Sensorgrams obtained for mitoxantrone

pH 6.5

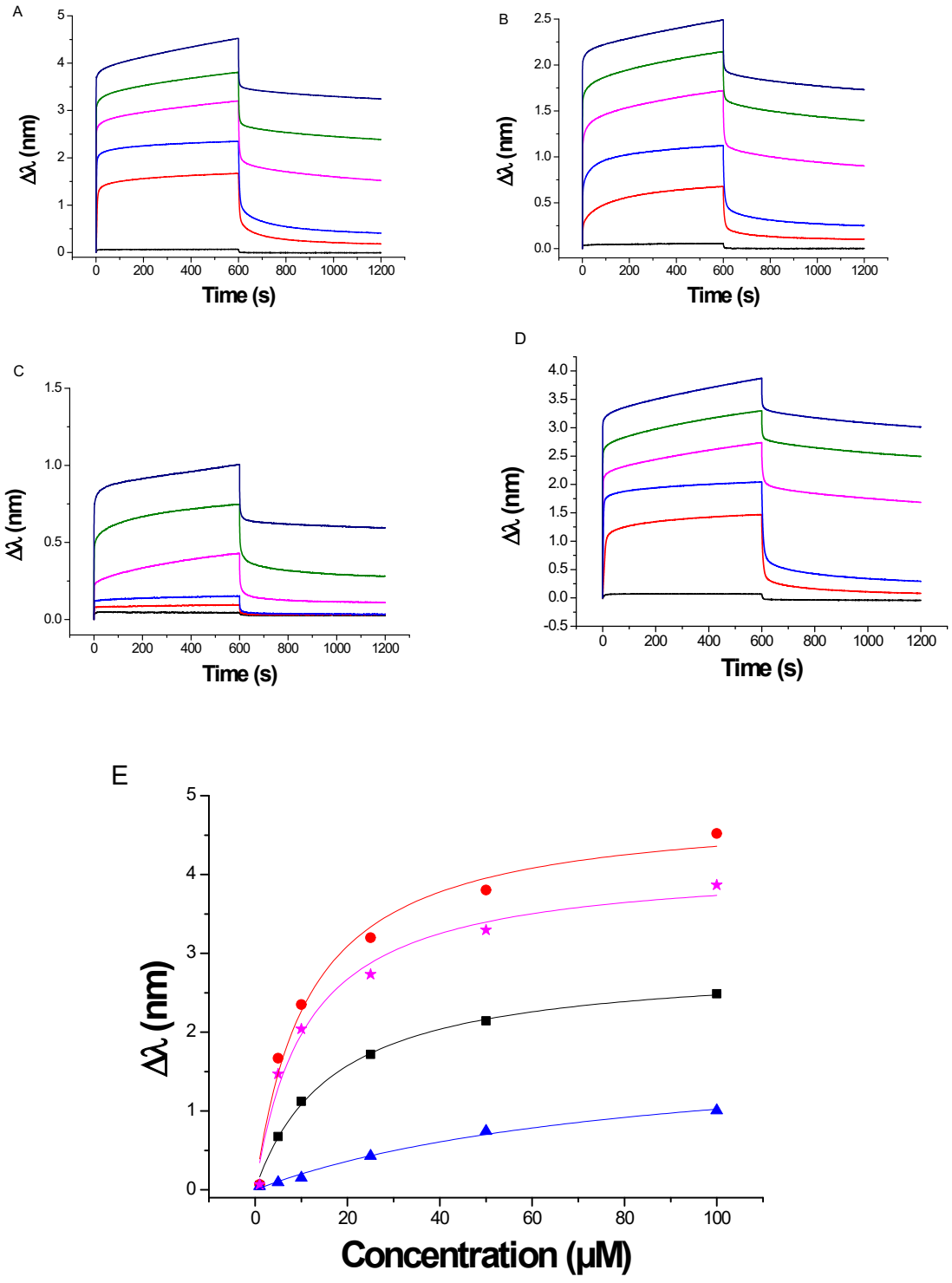

Figure S6. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 25, 50 and 100 µM.

pH 5.5

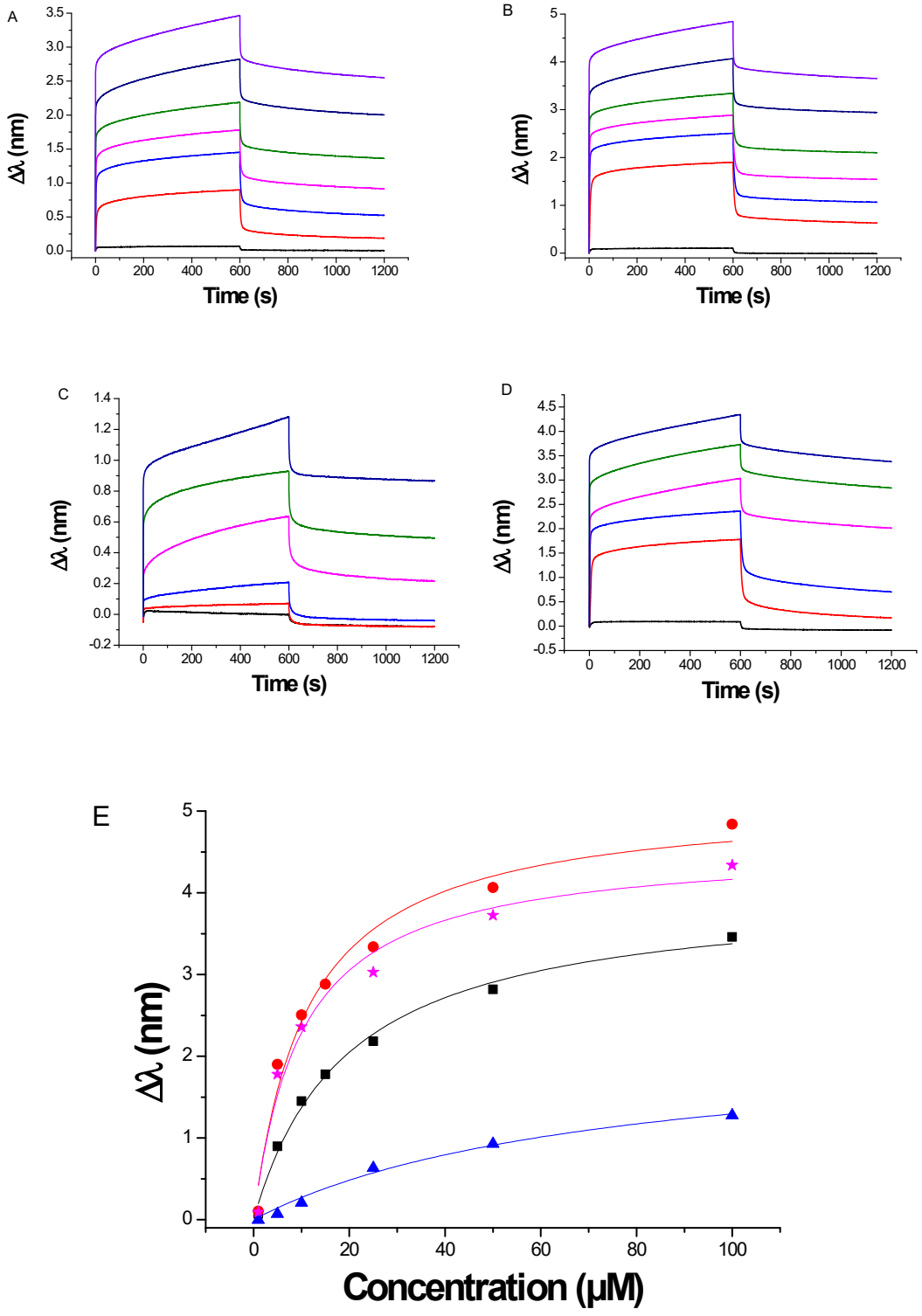

Figure S6. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 15, 20, 50 and 100 µM for **1** and **2** and 1, 5, 10, 20, 50 and 100 µM for **3** and **4**.

Sensorgrams obtained for Braco-19

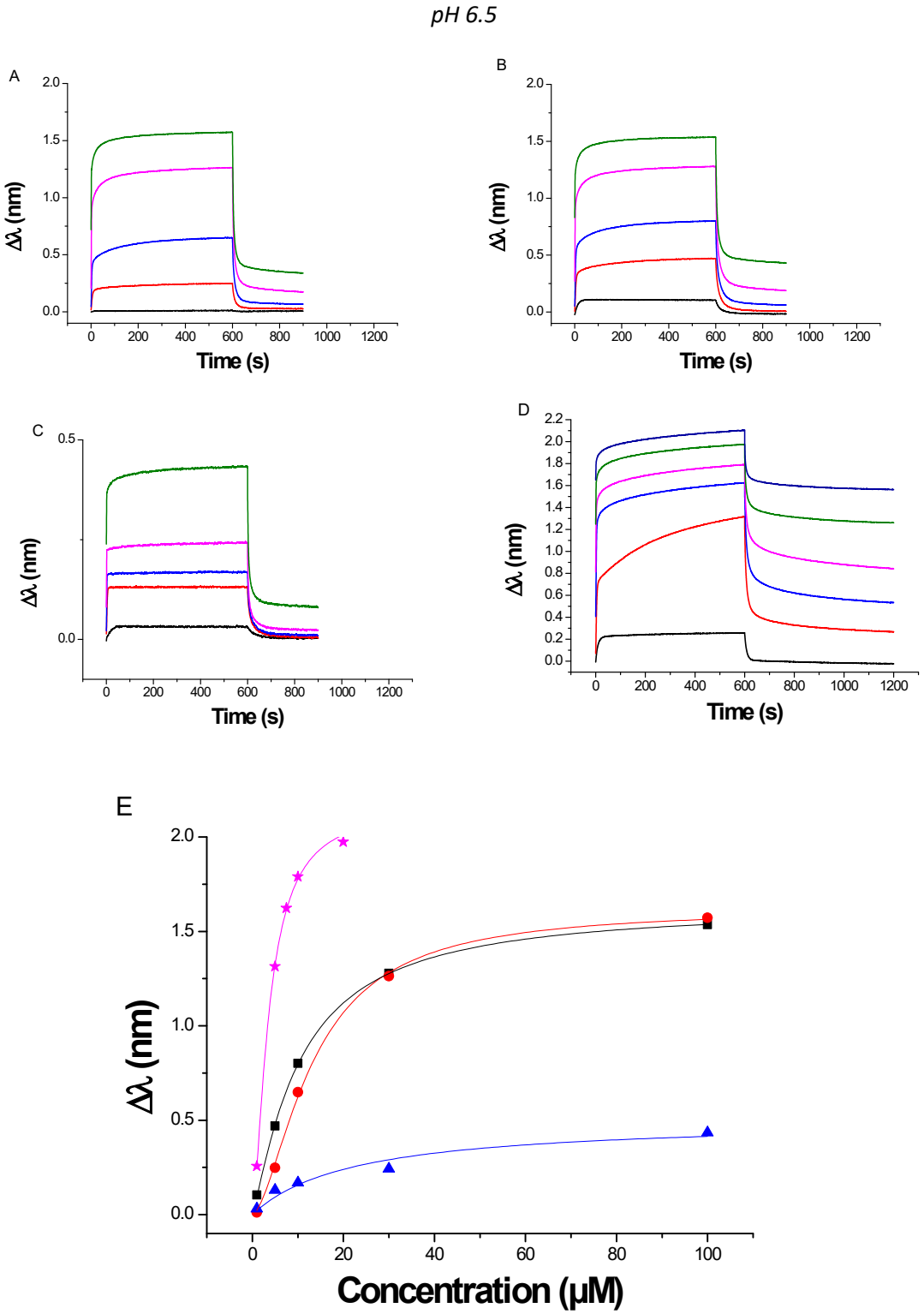

Figure S8. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 30 and, 100 µM for **1-3** and 1, 5, 7.5, 10, 20 and 30 µM for **4**.

pH 5.5

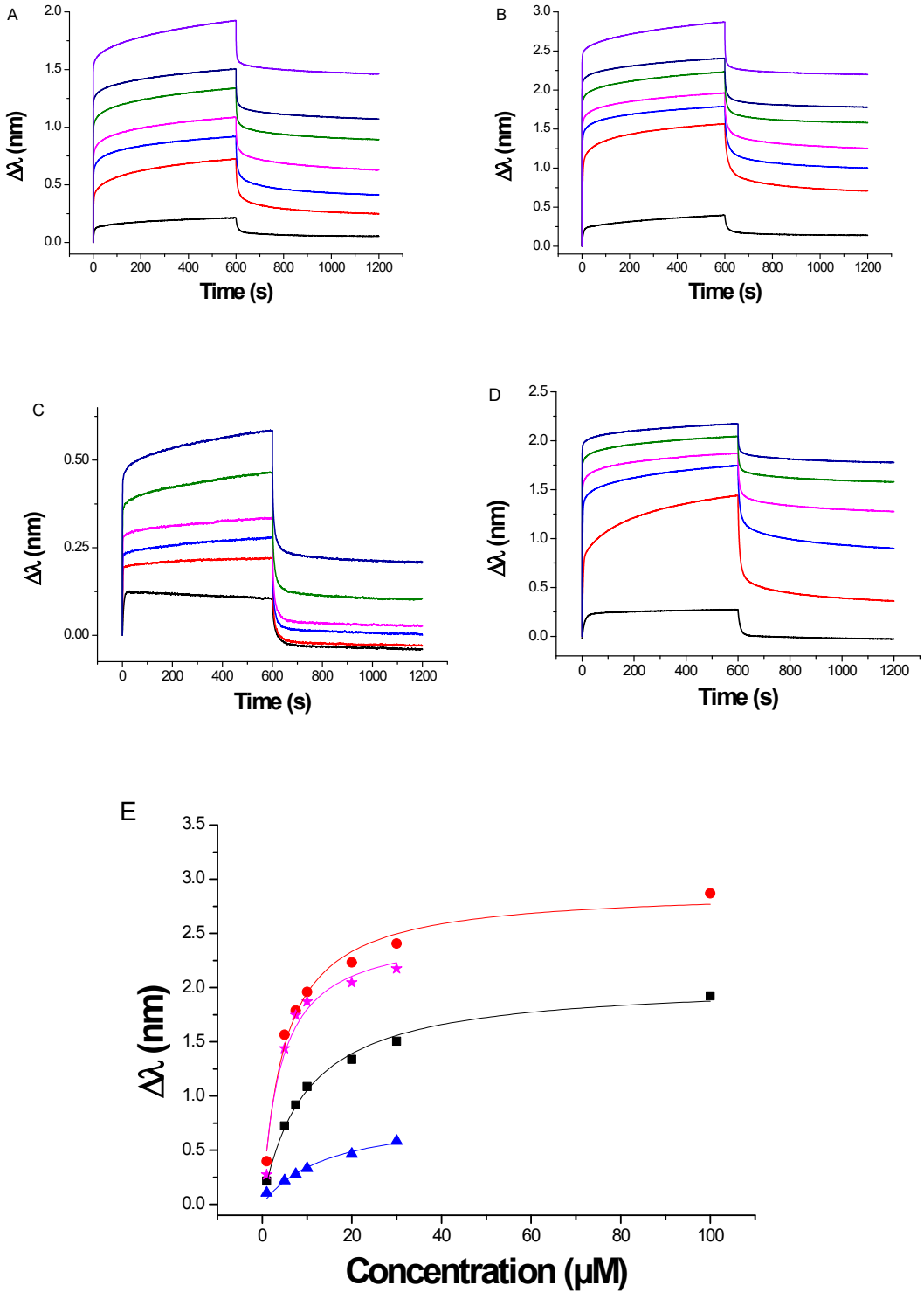

Figure S-9. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 7.5, 10, 20, 30 and 100 µM for **1** and **2** and 1, 5, 7.5, 10, 20 and 30 µM for 3 and 4.

Sensorgrams obtained for PDS

pH 6.5

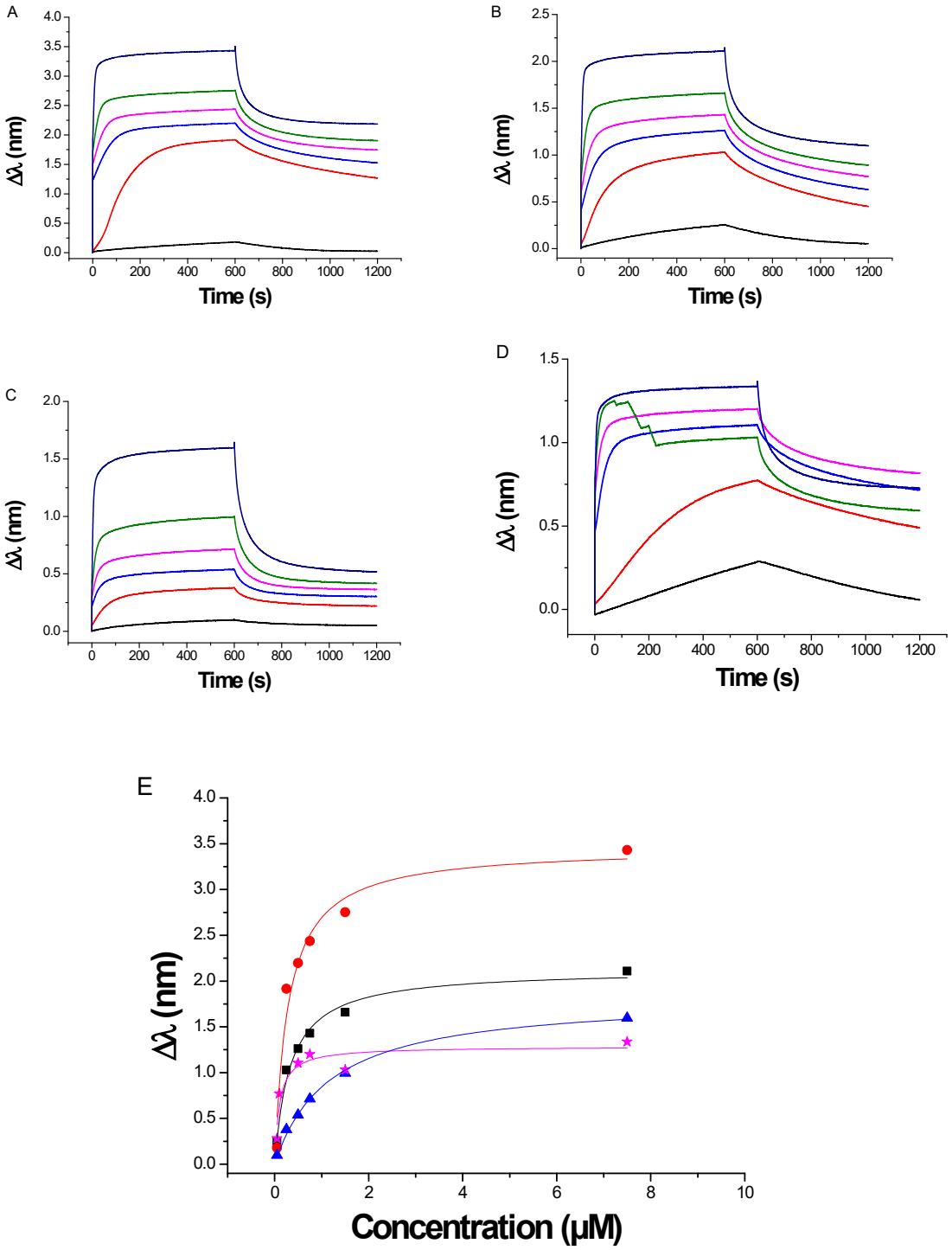

Figure S10. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.05, 0.25, 0.5, 0.75, 1.5 and 7.5 µM for **1-3** and 0.05, 0.1, 0.5, 0.75, 1.5 and 7.5 µM for **4**.

pH 5.5

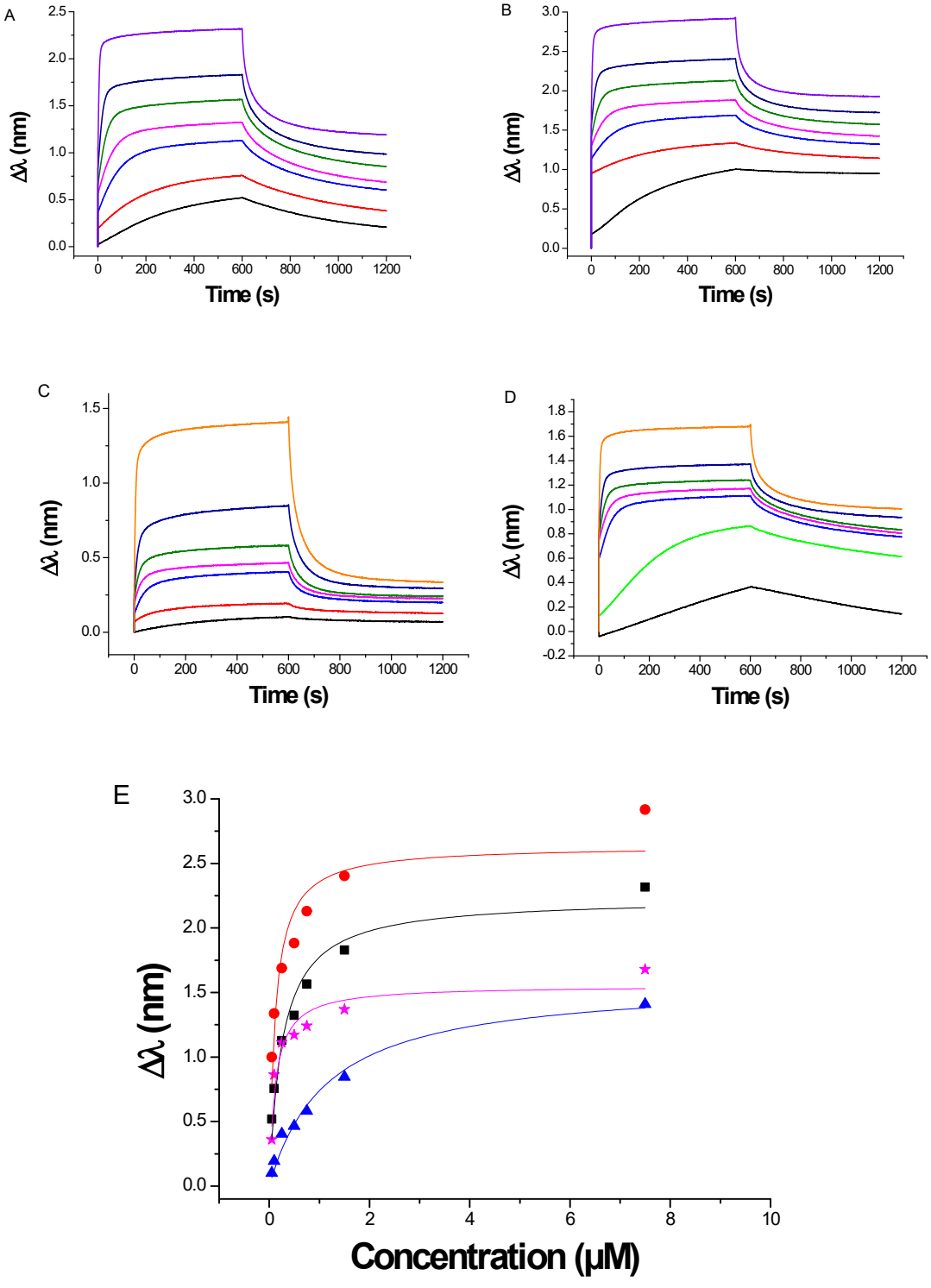

Figure S11. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.05, 0.1, 0.25, 0.5, 0.75, 1.5 and 7.5 µM.

Sensorgrams obtained for TMPyP4

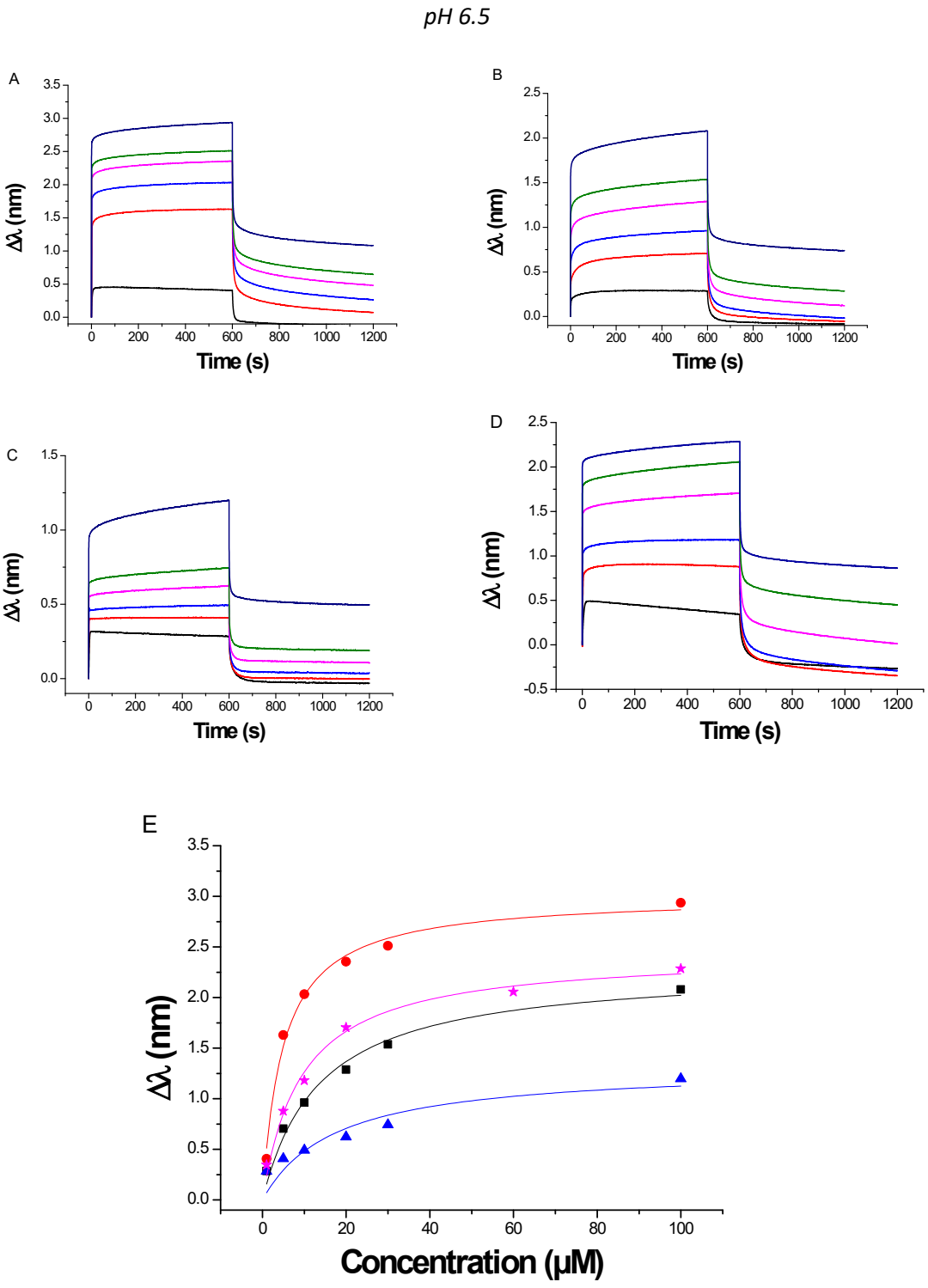

Figure S12. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 10, 20, 30 and 100µM for **1-3** and 1, 5, 10, 20, 60 and 100 µM for **4**.

pH 5.5

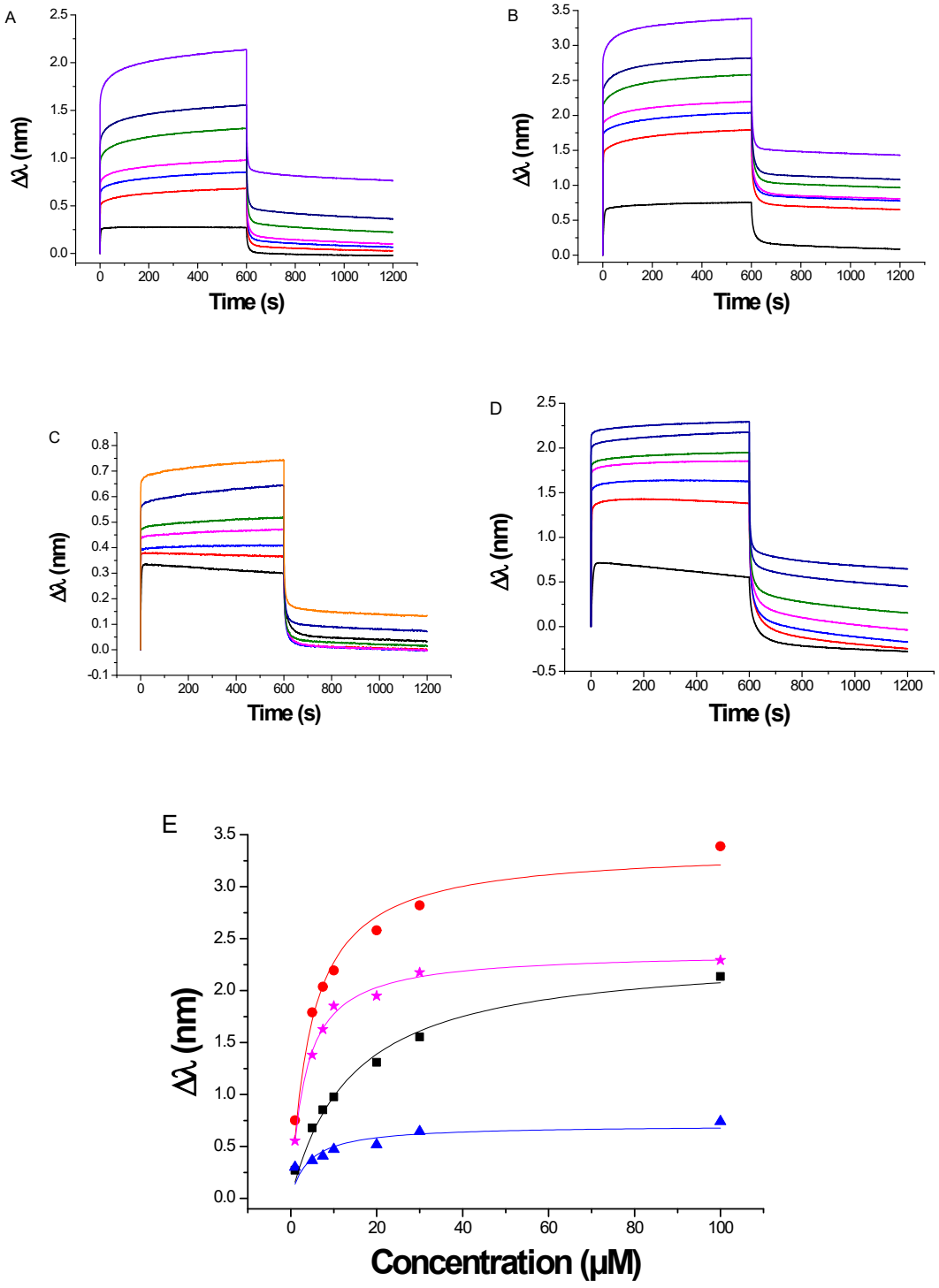

Figure S13. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 1, 5, 7.5, 10, 20, 30 and 100 µM.

Sensorgrams obtained BisA

pH 6.5

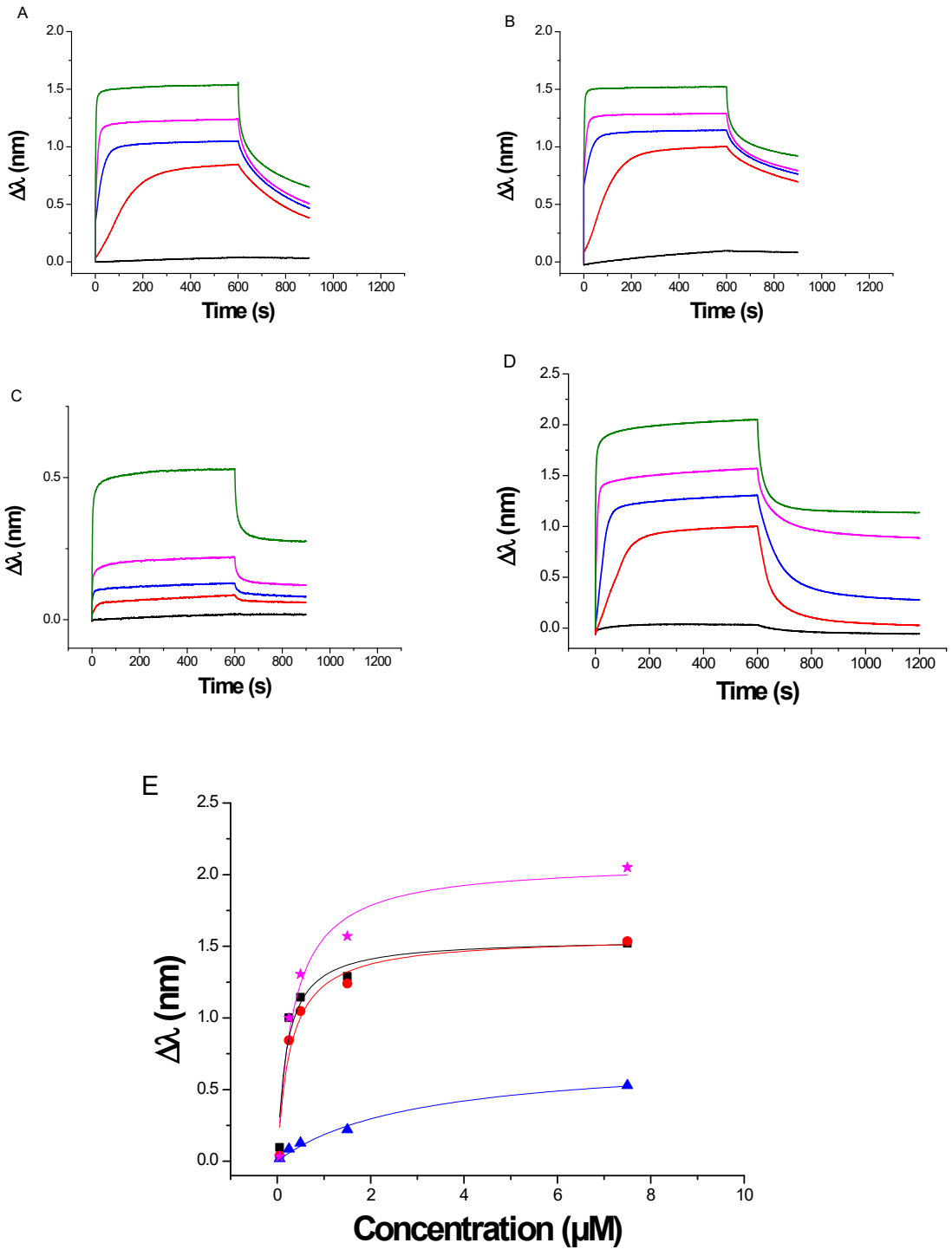

Figure S14. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.05, 0.25, 0.5, 1.5 and 7.5 µM.

pH 5.5

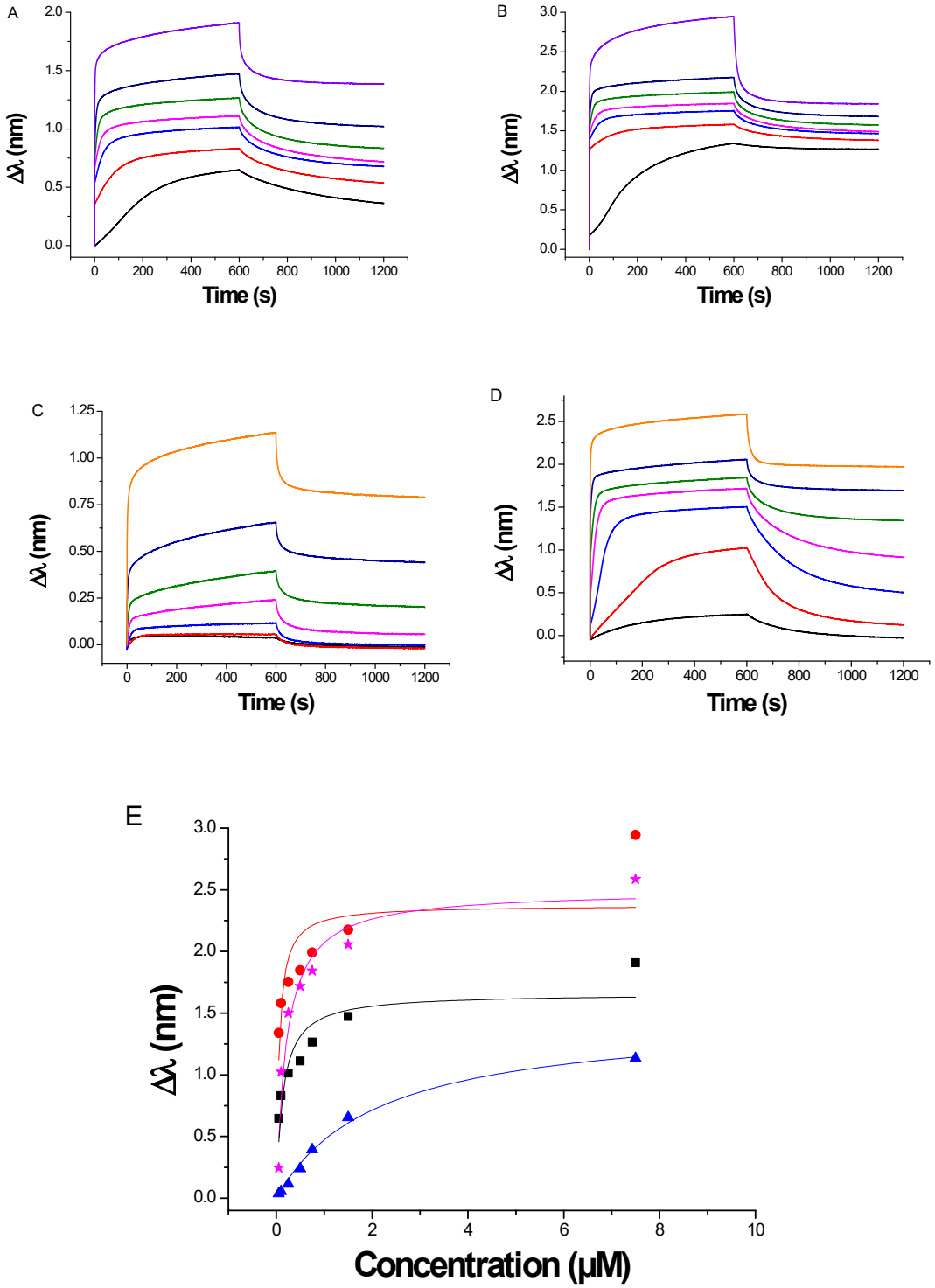

Figure S15. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.05, 0.1, 0.25, 0.5, 0.75, 1.5 and 7.5 µM.

Sensorgrams obtained for PhenDC3

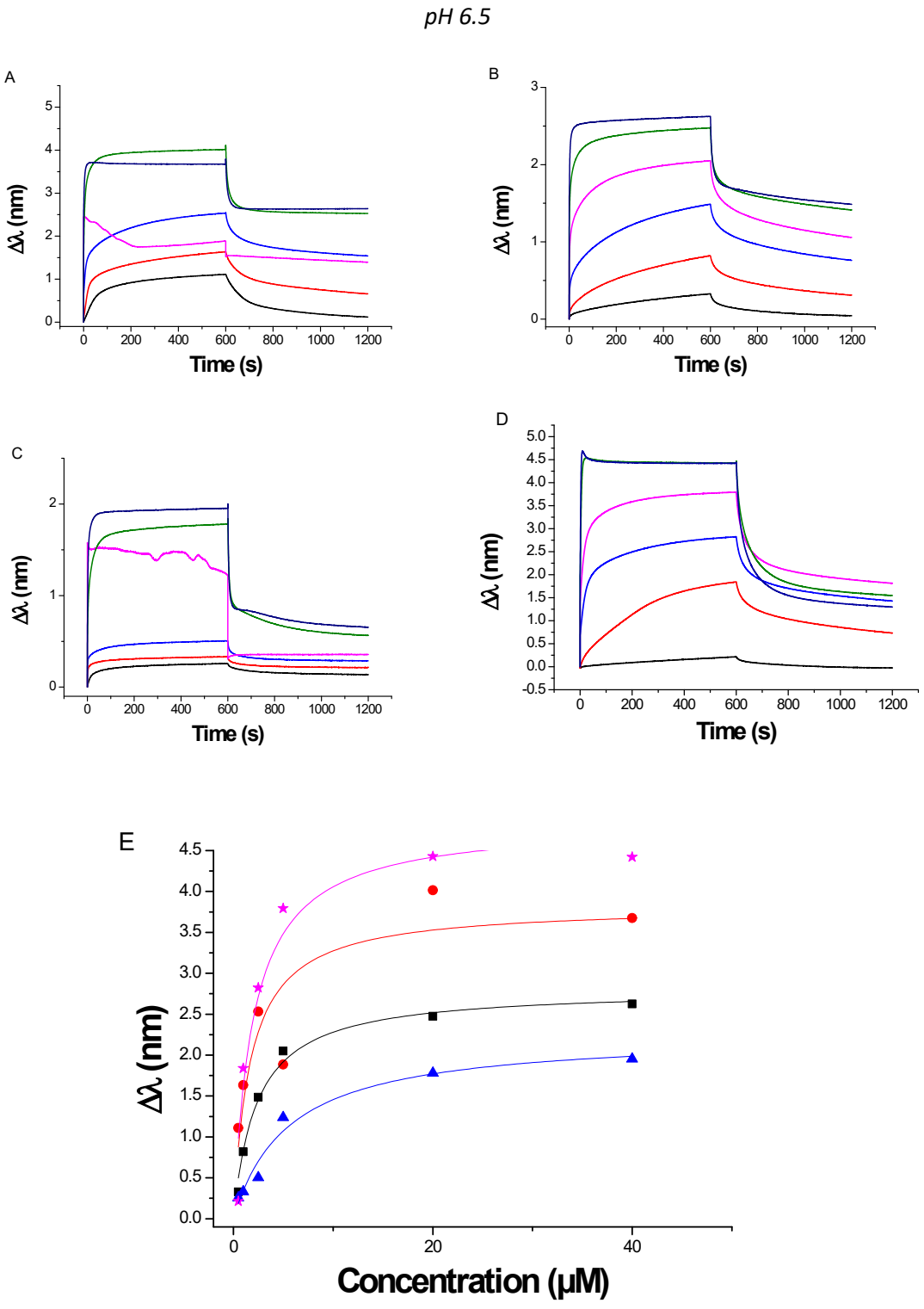

Figure S16. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.5, 1.5, 2.5, 5, 20 and 40 µM.

pH 5.5

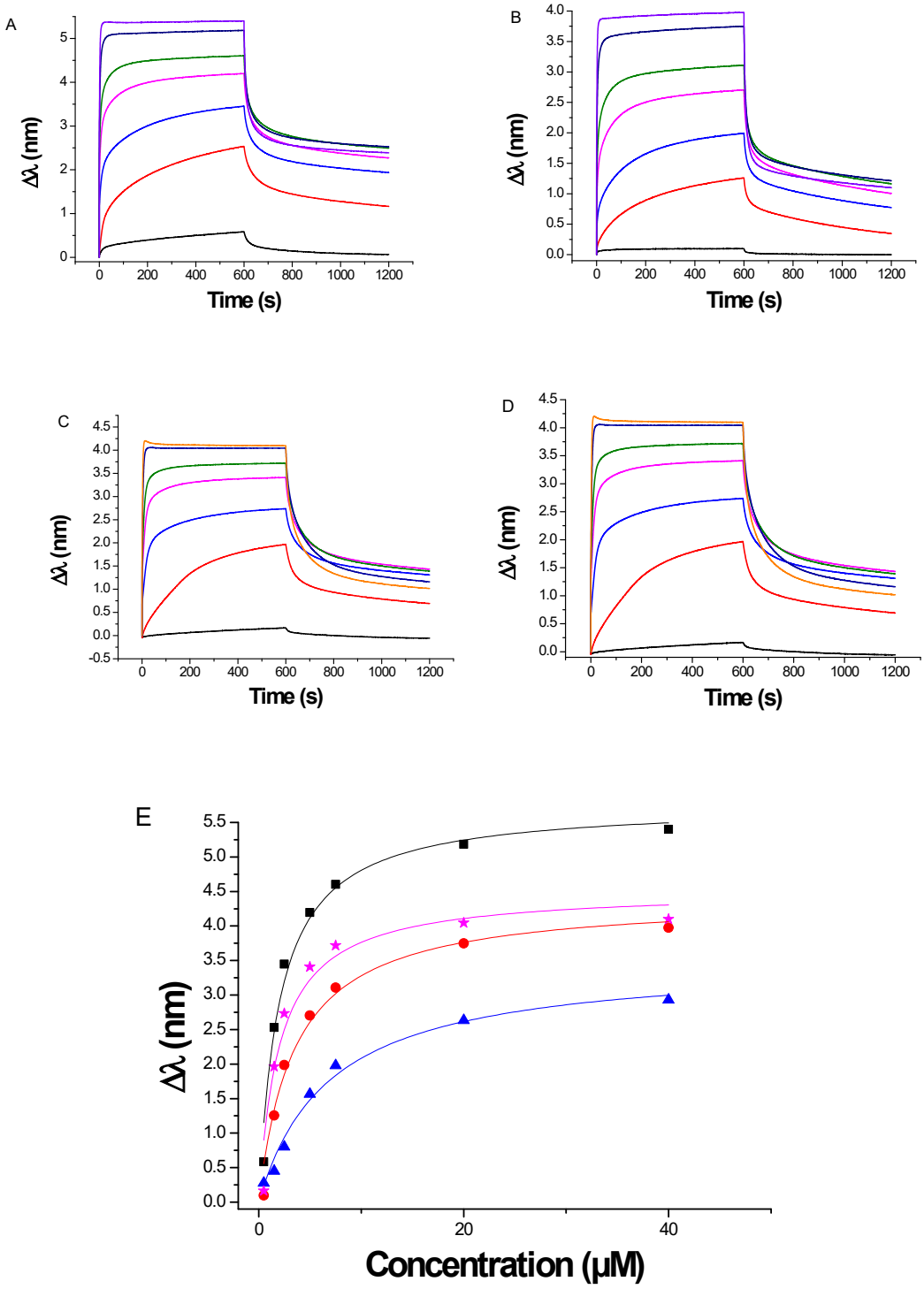

Figure S17. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 0.5, 1.5, 2.5, 5, 7.5, 20 and 40 µM.

Sensorgrams obtained for berberine

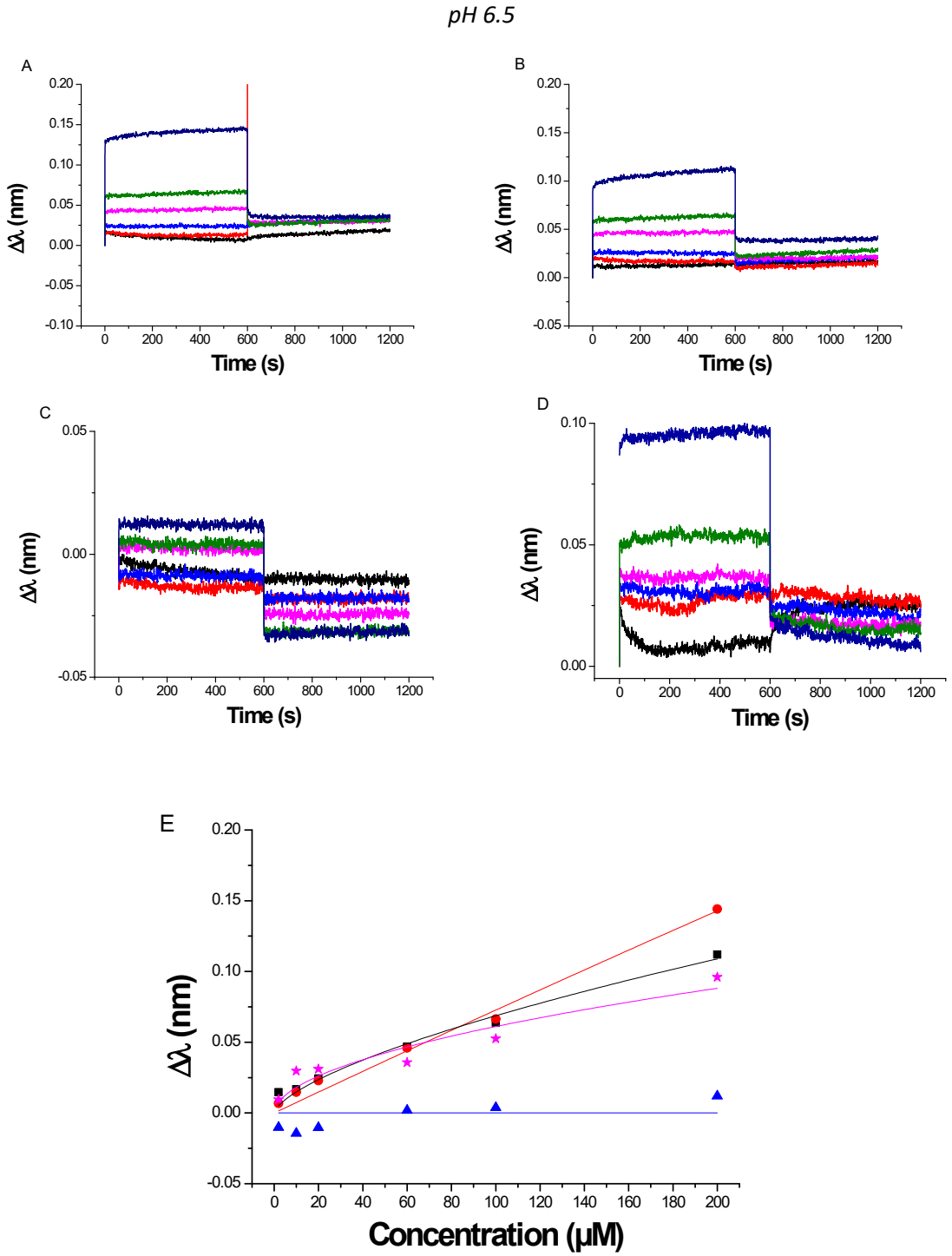

Figure S18. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 6.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 2, 10, 20, 60, 100 and 200 µM with 1 % DMSO.

pH 5.5

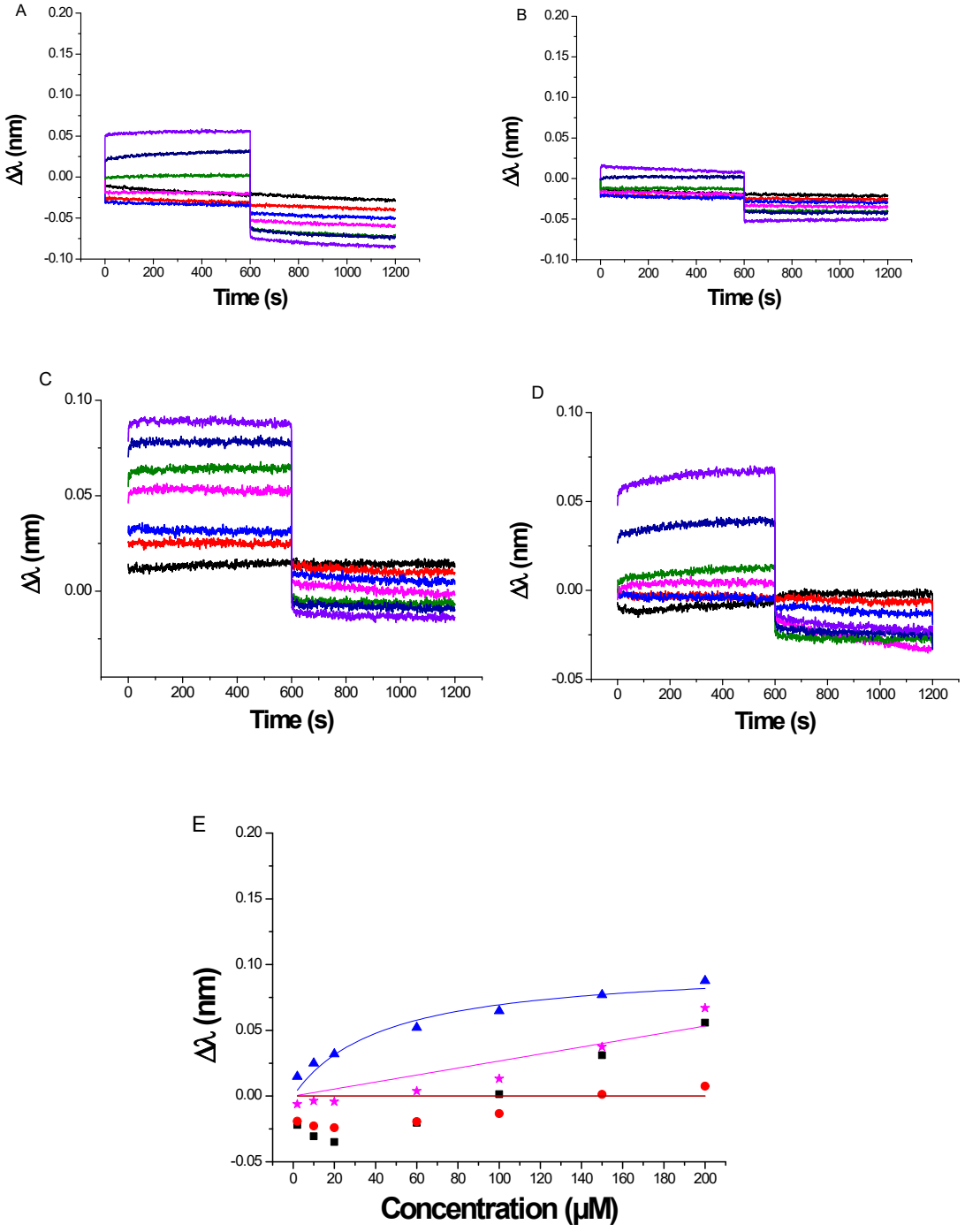

Figure S19. Sensorgrams obtained with A/ h-TeloC **1**, B/ constraint I-motif **2**, C/ control hairpin **3** and D/ RAFT T23 **4** at pH 5.5. E/ Langmuir isotherm with the plateau value at the equilibrium for **1** (red), **2** (black), **3** (blue) and **4** (pink), and the Langmuir fitting in full line. The concentrations injected were 2, 10, 20, 60, 100, 150 and 200 µM with 1 % DMSO.

Circular dichroism studies (CD)

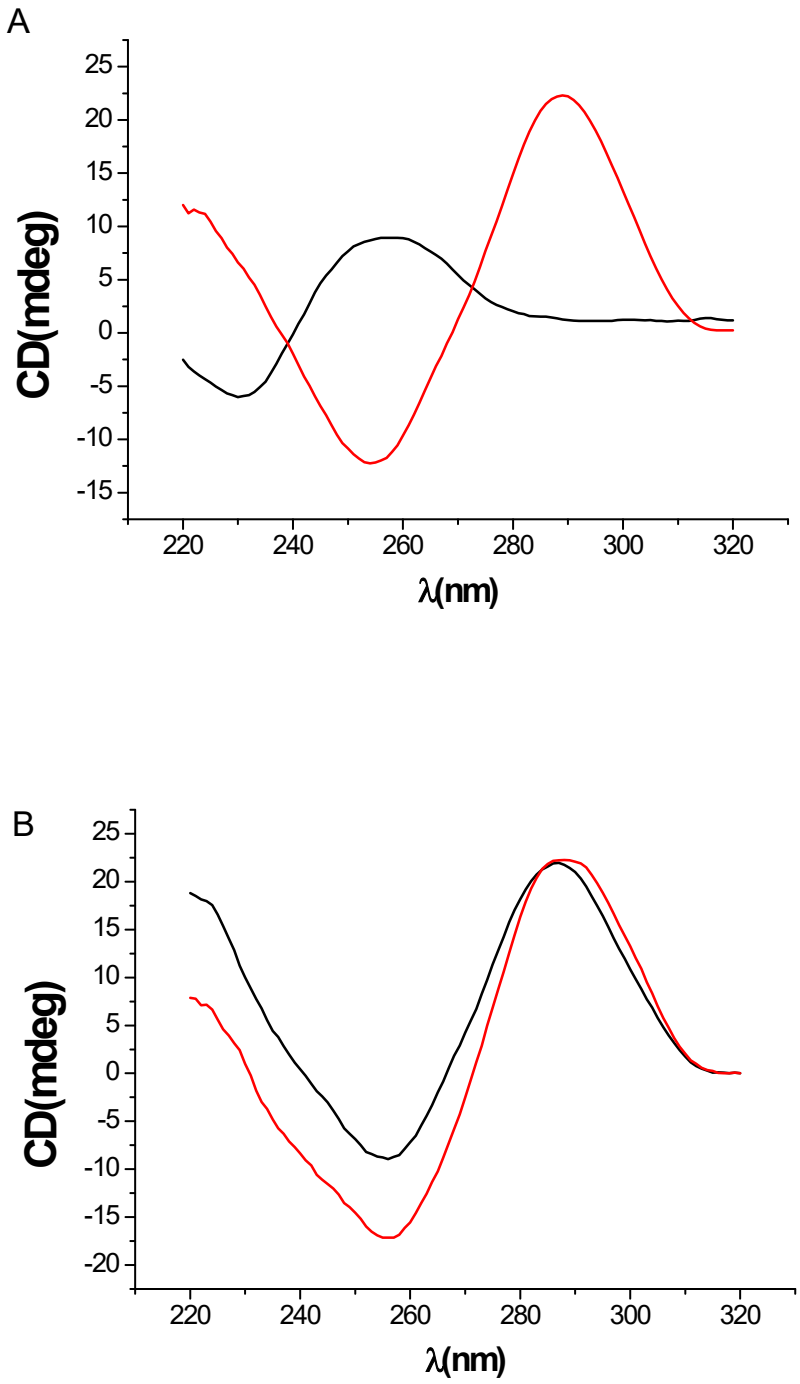

Figure S20. CD spectra of **A/** native h-telo **1** at pH 6.5 (black) and at pH 5.5 (red); **B/** constrained h-telo **2** at pH 6.5 (black) and at pH 5.5 (red). Buffer: 50 mM Tris AcOH, 35 mM NaCl, 50 mM KCl, adjusted at the desired pH.

CD spectra after addition of ligands

CD spectra at pH = 5.5

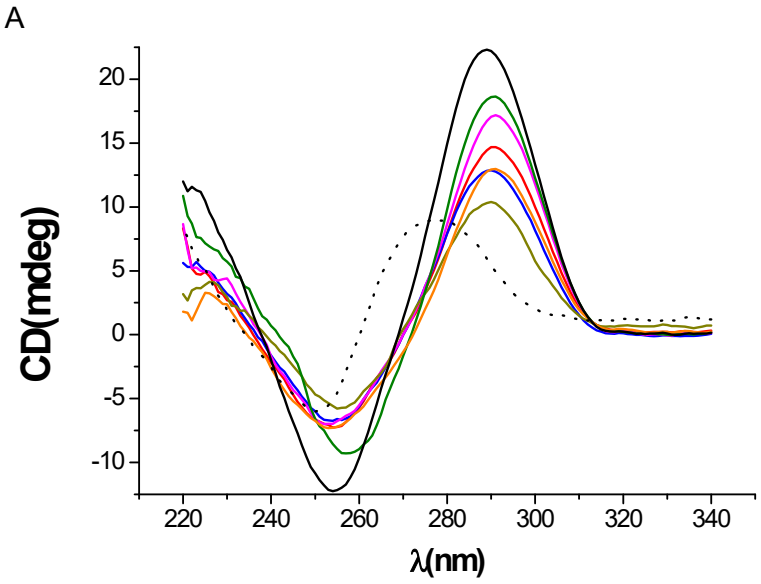

Figure S21. CD spectra obtained for native h-telo **1** alone (black) and after the addition of 5 equivalents of ligand (colored lines) at pH 5.5. PhenDC3 (red), PDS (blue), TMPyP4 (pink), Braco-19 (brown), Bis A (green) and mitoxantrone (orange). Dash line: native h-telo **1** alone at pH 6.5.

CD spectra at pH = 6.5

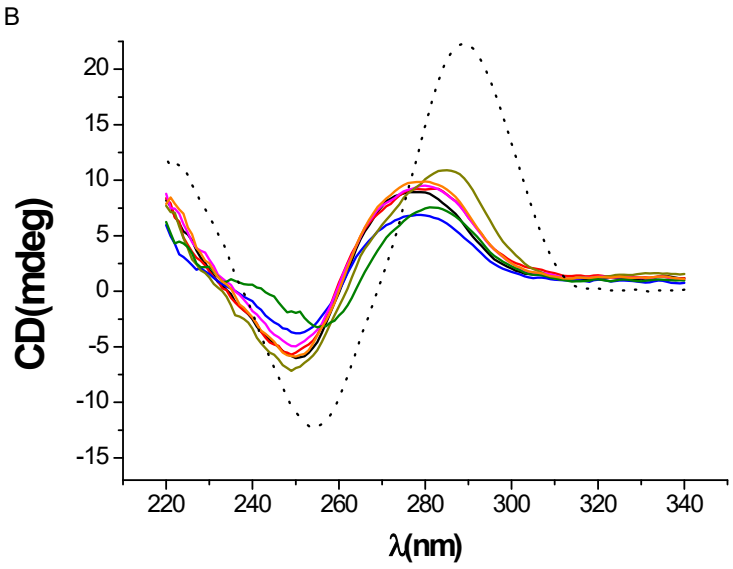

Figure S22. CD spectra obtained for native h-telo **1** alone (black) and after the addition of 5 equivalents of ligand (colored lines) at pH 6.5. PhenDC3 (red), PDS (blue), TMPyP4 (pink), Braco-19 (brown), Bis A (green) and mitoxantrone (orange). Dash line: native h-telo **1** alone at pH 5.5.

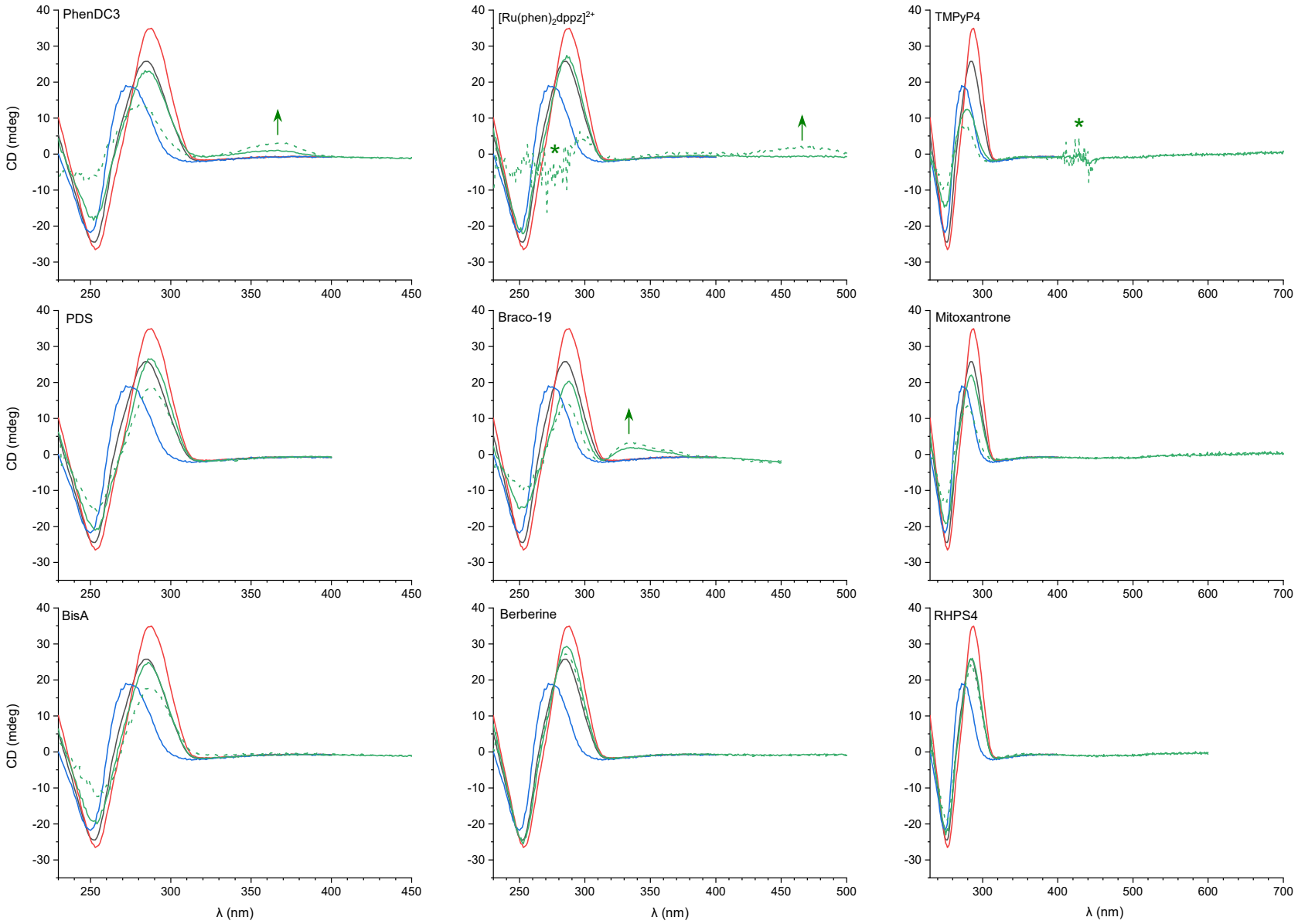
PhenDC3
[Ru(phen)2dppz]2+
TMPyP4
PDS
Braco-19
Mitoxantrone
BisA
Berberine
RHPS4
CD (mdeg)
λ (nm)

Figure S23. CD spectra of h-telo DNA (2.5 µM in 10 mM $LiAsO_2Me_2$, 100 mM KCl buffer, **pH 6.2**) in the absence (black curves) and in the presence of 2 (solid green) and 5 (dashed green curves) molar equivalents of ligands. The spectra at **pH 5.0** (i-DNA, red curves) and at **pH 7.3** (unstructured, blue curves) are plotted for reference. The induced CD signals are indicated with arrows. High noise areas (indicated with an asterisk) are due to strong absorbance due to added ligand.

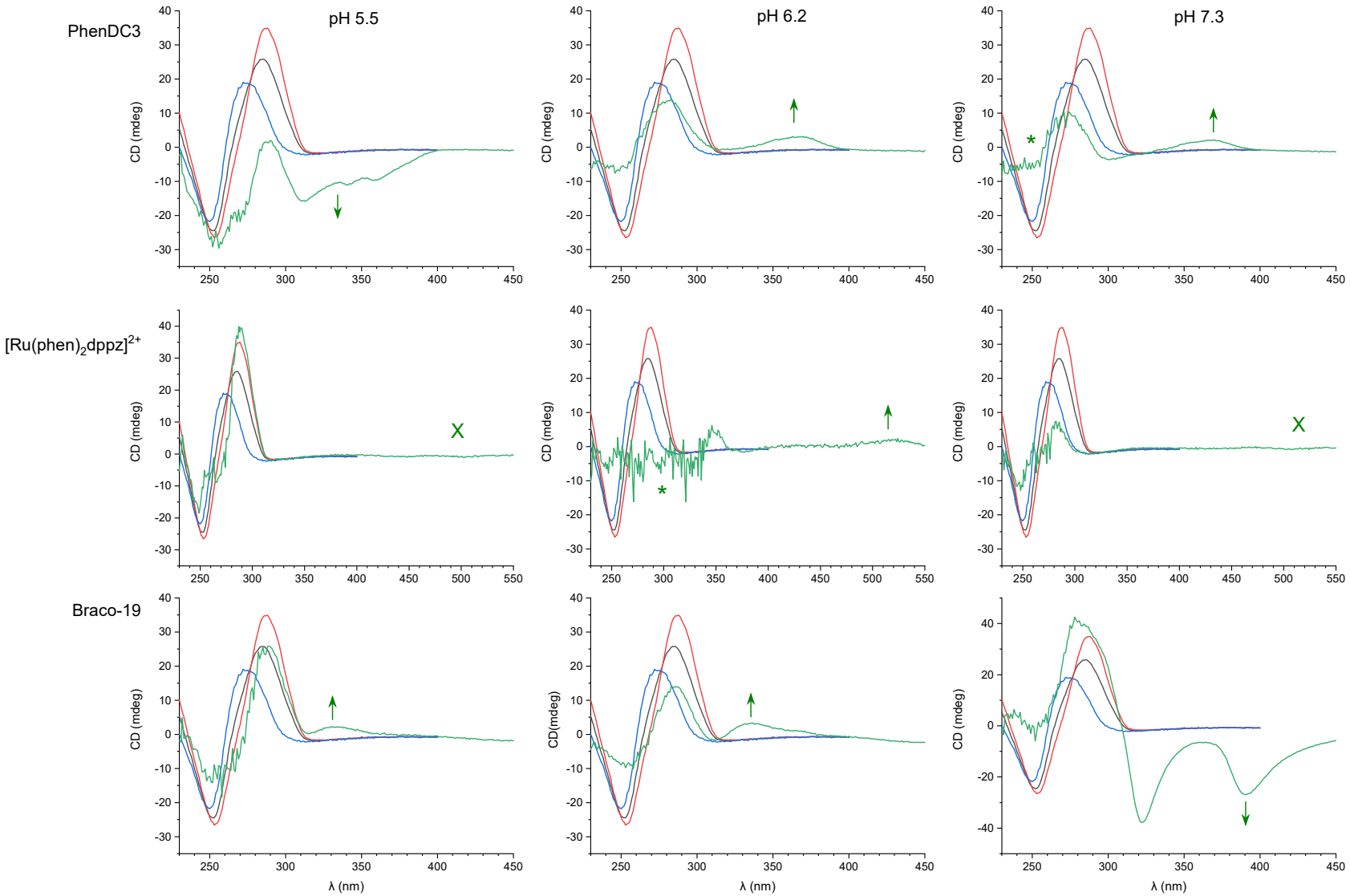

Figure S24. CD spectra of h-telo DNA (2.5 µM in 10 mM $LiAsO_2Me_2$, 100 mM KCl buffer) in the absence (black curves) and in the presence of 2 molar equivalents of selected ligands (PhenDC3, $[Ru(phen)_2dppz]^{2+}$ and Braco-19, green lines), at **pH 5.5**, **6.2** and **7.3**. The spectra of h-telo in the at **pH 5.0** (i-DNA, red curves) and at **pH 7.3** (unstructured, blue curves) are plotted for reference. The induced CD signals are indicated with green arrows. High noise areas (indicated with an asterisk) are due to strong absorbance due to added ligand.