# SUPPLEMENTARY INFORMATION

## IMAB ANTIBODY BINDS SINGLE-STRANDED CYTOSINE-RICH SEQUENCES AND UNFOLDS DNA I-MOTIFS

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# Biolayer interferometry (BLI) studies

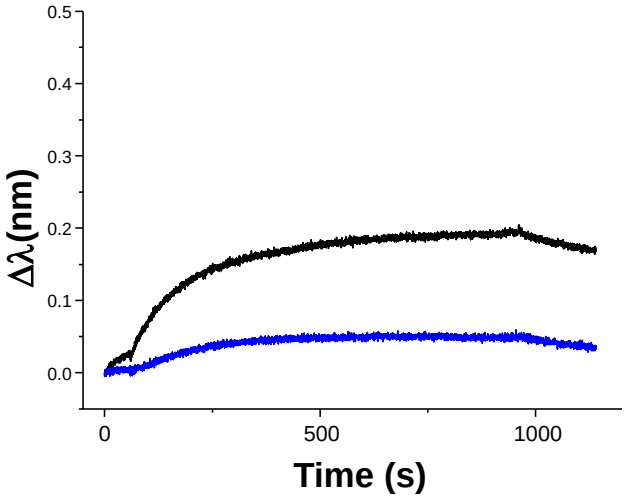



**Figure S1.** BLI signals observed during immobilization of hTeloC-27 at pH 6.0 (black) and 7.5 (blue).

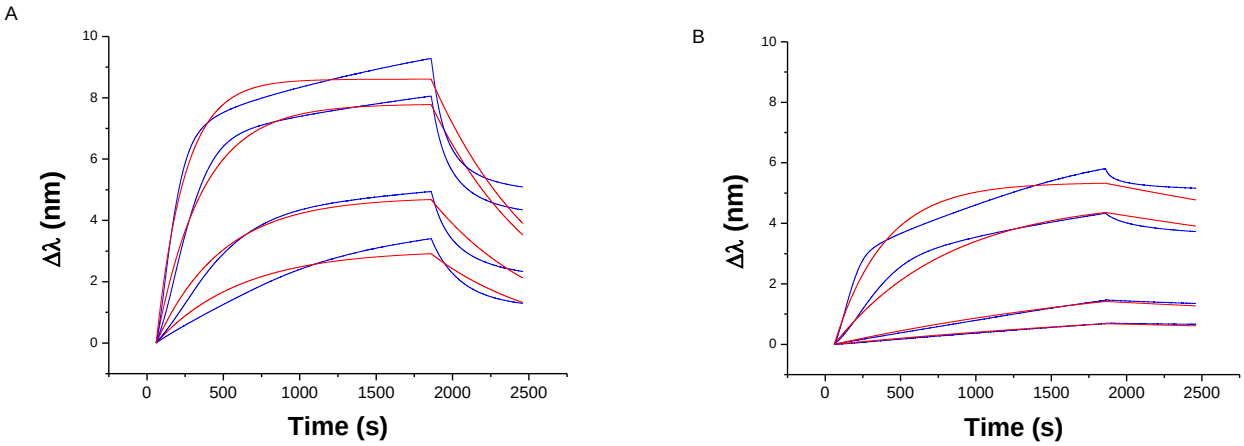



**Figure S2.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$-FLAG (62.5, 125, 250 and 500 nM) to hTeloC-27 at pH 6.0 (**A**) and pH 7.5 (**B**). Blue lines are experimental curves; red lines are the fits to the 1:1 model.

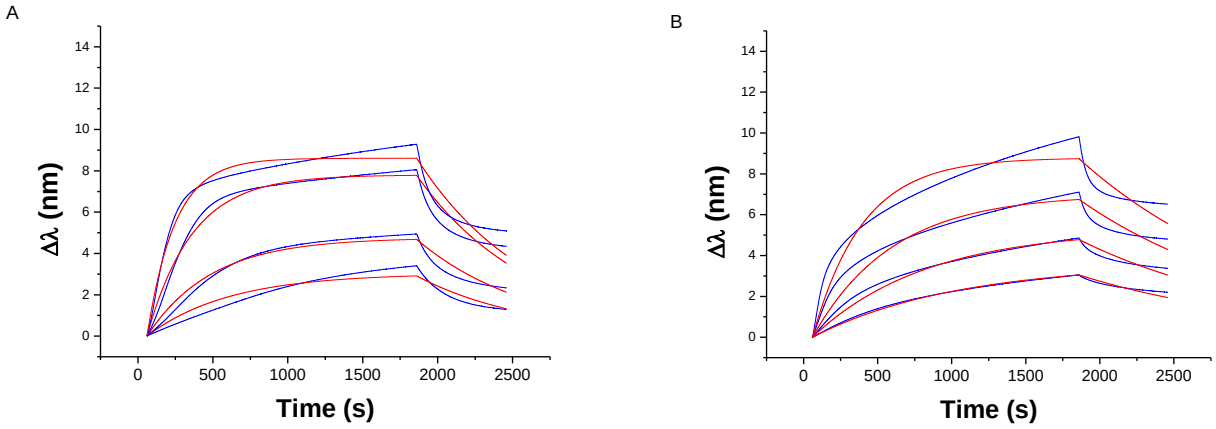
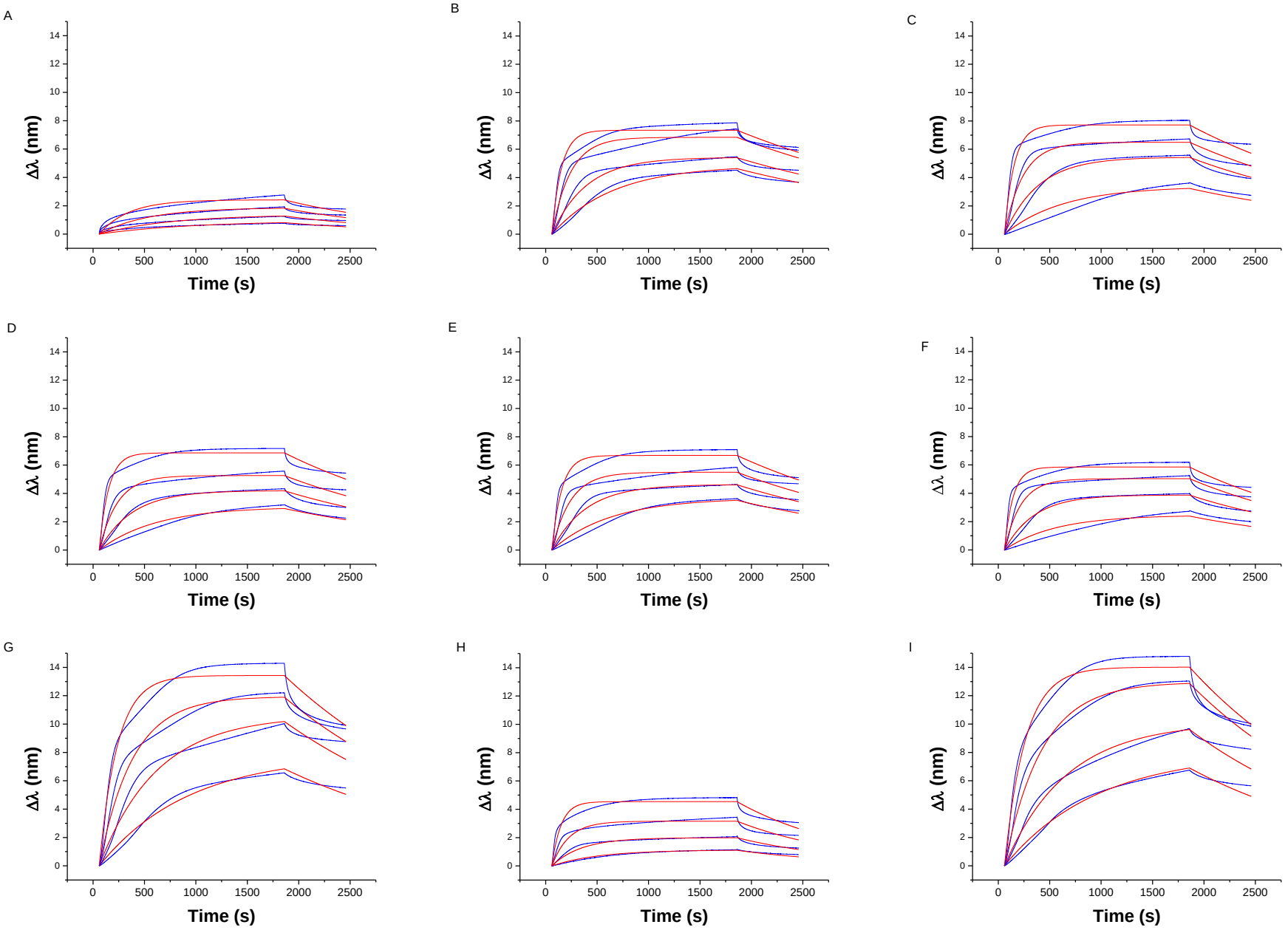



**Figure S3.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$-FLAG (62.5, 125, 250 and 500 nM) to hTeloC-27 (**A**) and constrained hTeloC **1** (**B**) at pH 6.5. Blue lines are experimental curves; red lines are the fits to the 1:1 model.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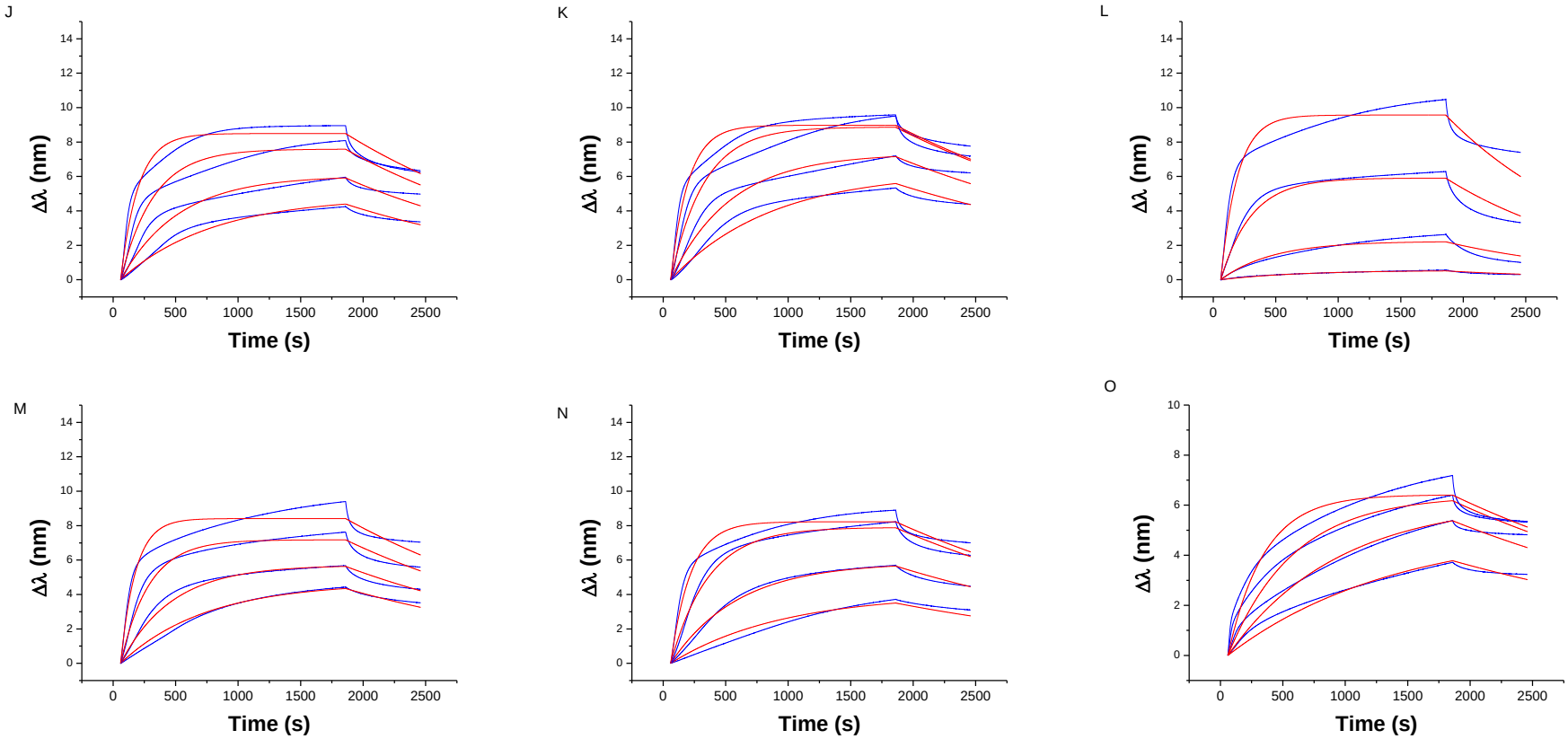
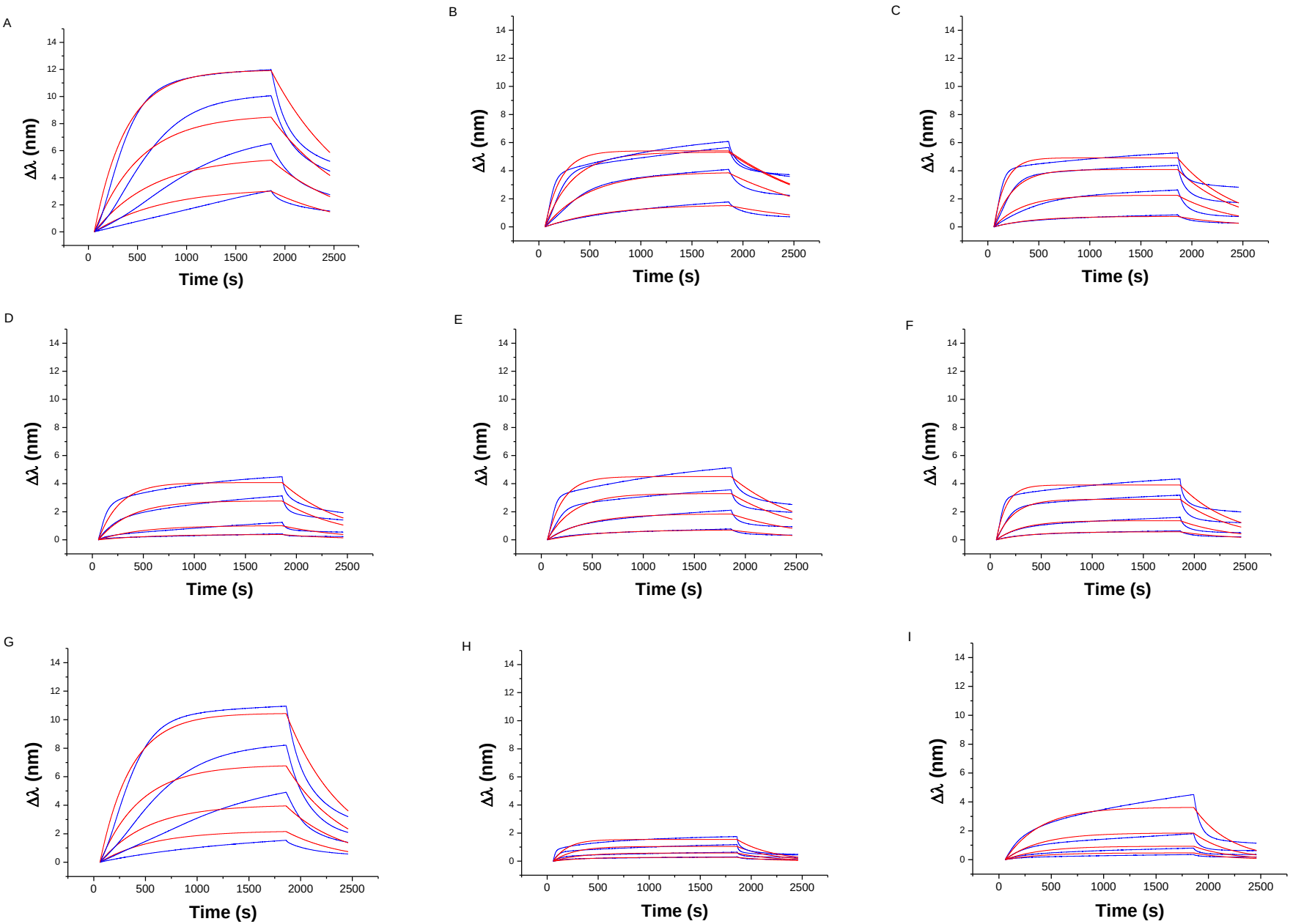



**Figure S4.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$-FLAG (62.5, 125, 250 and 500 nM) to hTeloC-4x5 (**A**), hTeloC-3x4 (**B**), hTeloC 3x3 (**C**), hTeloC 3x2 (**D**), hTeloC 2x3 (**E**), hTeloC 2x3T (**F**), hTeloC-2x2 (**G**), hTeloC 1x3T (**H**), hTeloC-1x2T (**I**), hTeloC 1x6 T (**J**), hTeloC 2x6T (**K**), hTeloC-scr (**L**), hTeloC-Mut (**M**), hTeloC X3 (**N**) and hTeloC-22 (**O**) at pH 6.0. Blue lines are experimental curves; red lines are the fits to the 1:1 model.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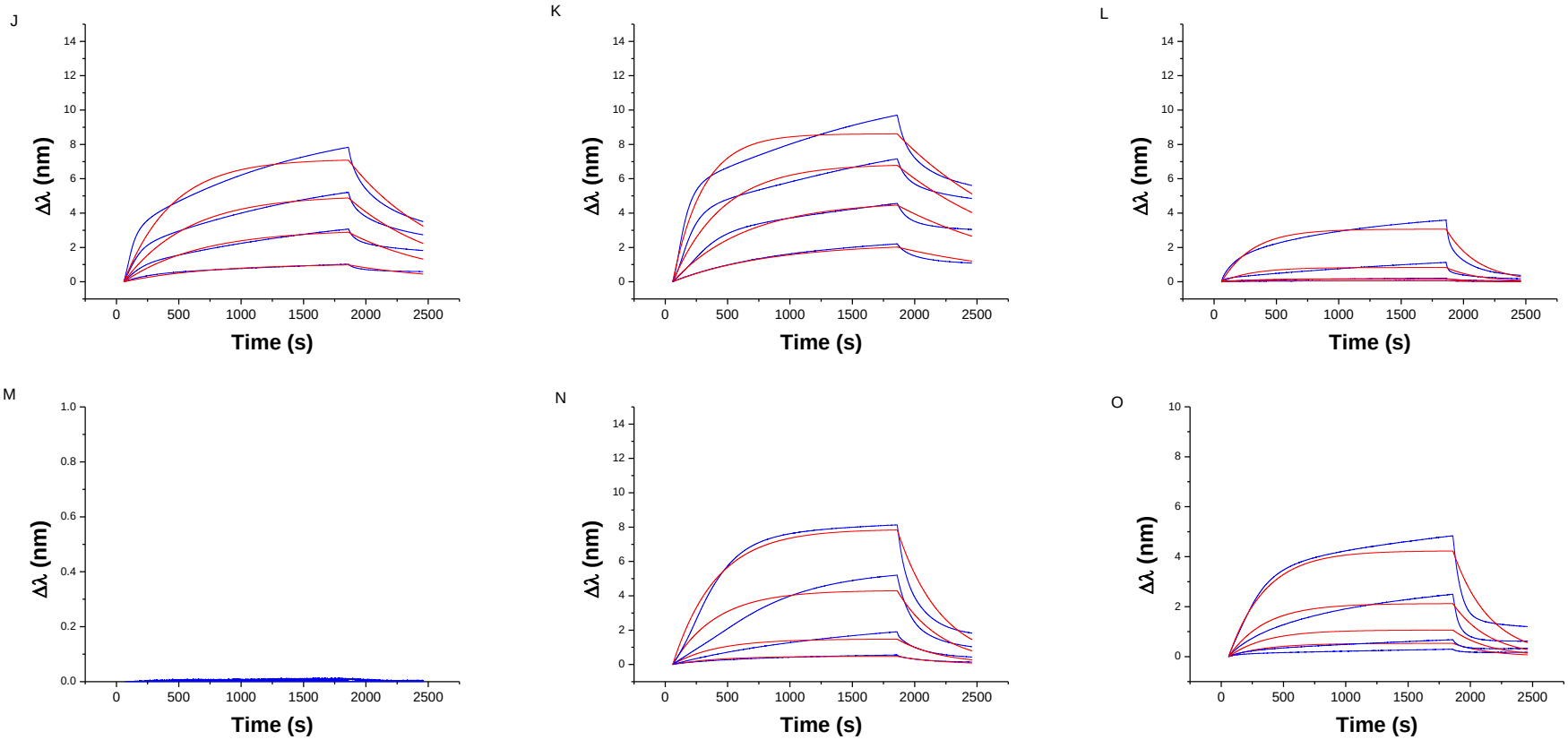
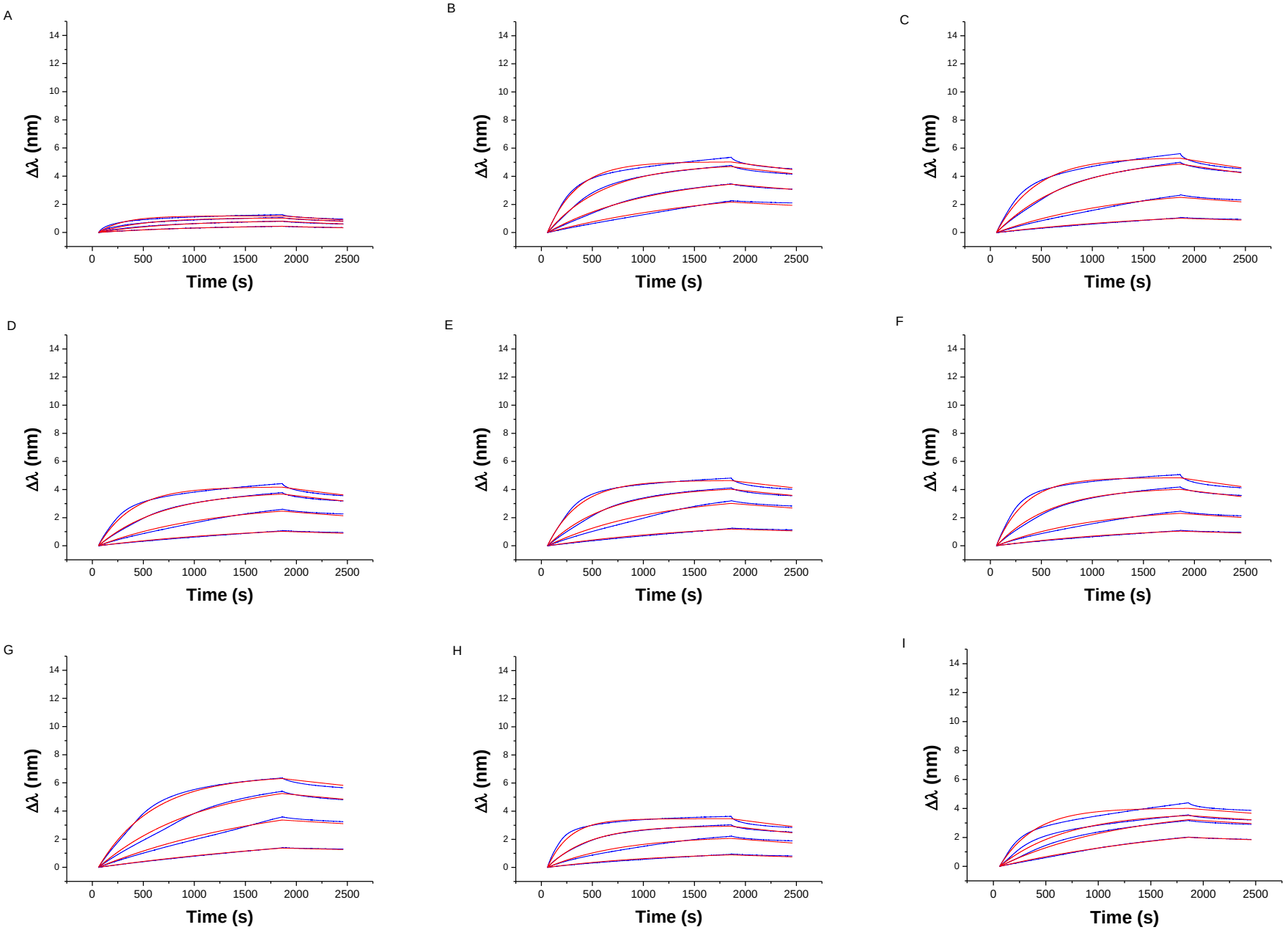



**Figure S5.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$-FLAG (62.5, 125, 250 and 500 nM) to hTeloC-4x5 (**A**), hTeloC-3x4 (**B**), hTeloC 3x3 (**C**), hTeloC 3x2 (**D**), hTeloC 2x3 (**E**), hTeloC 2x3T (**F**), hTeloC-2x2 (**G**), hTeloC 1x3T (**H**), hTeloC-1x2T (**I**), hTeloC 1x6 T (**J**), hTeloC 2x6T (**K**), hTeloC-scr (**L**), hTeloC-Mut (**M**), hTeloC-X3 (**N**) and hTeloC-22 (**O**) at pH 7.5. Blue lines are experimental curves; red lines are the fits to the 1:1 model.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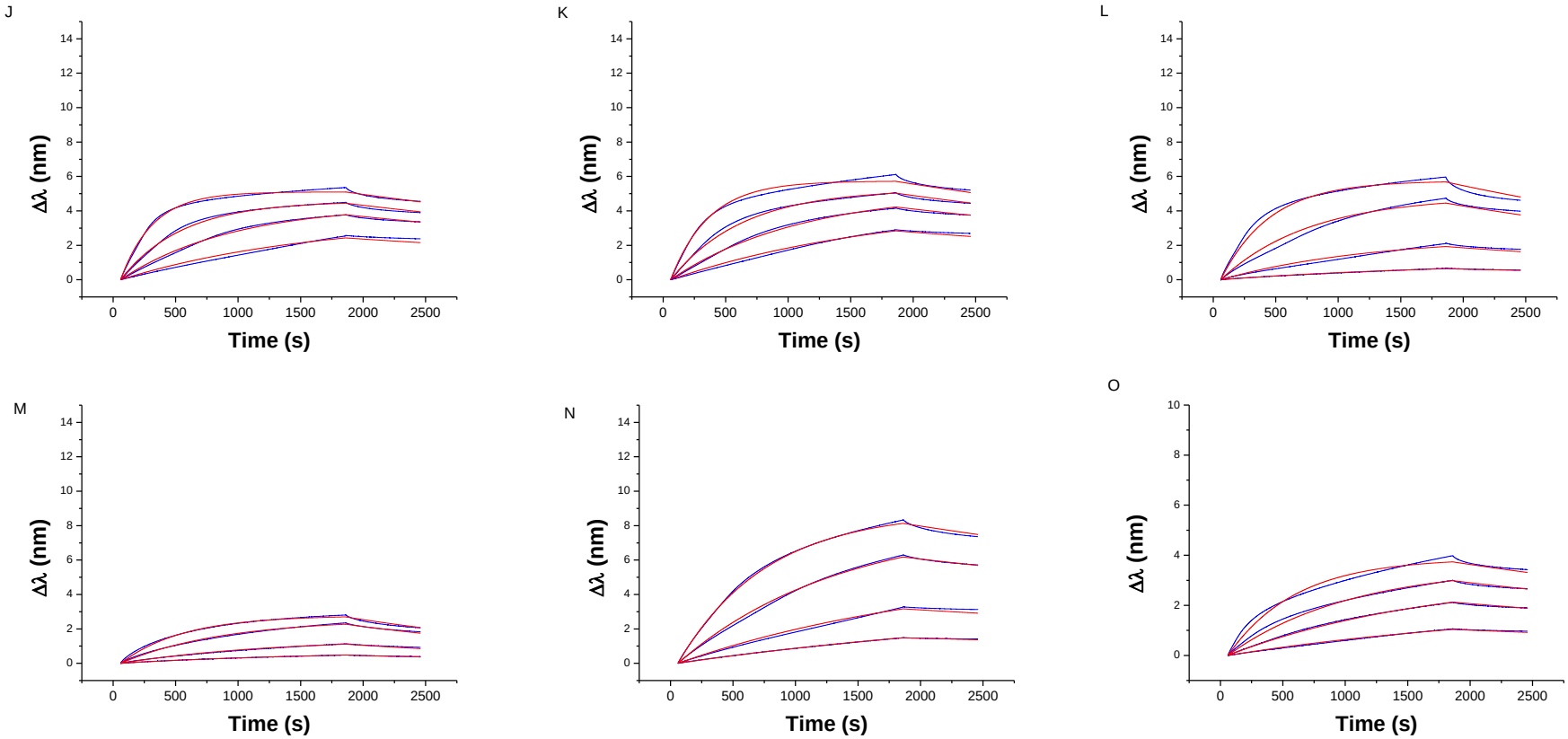
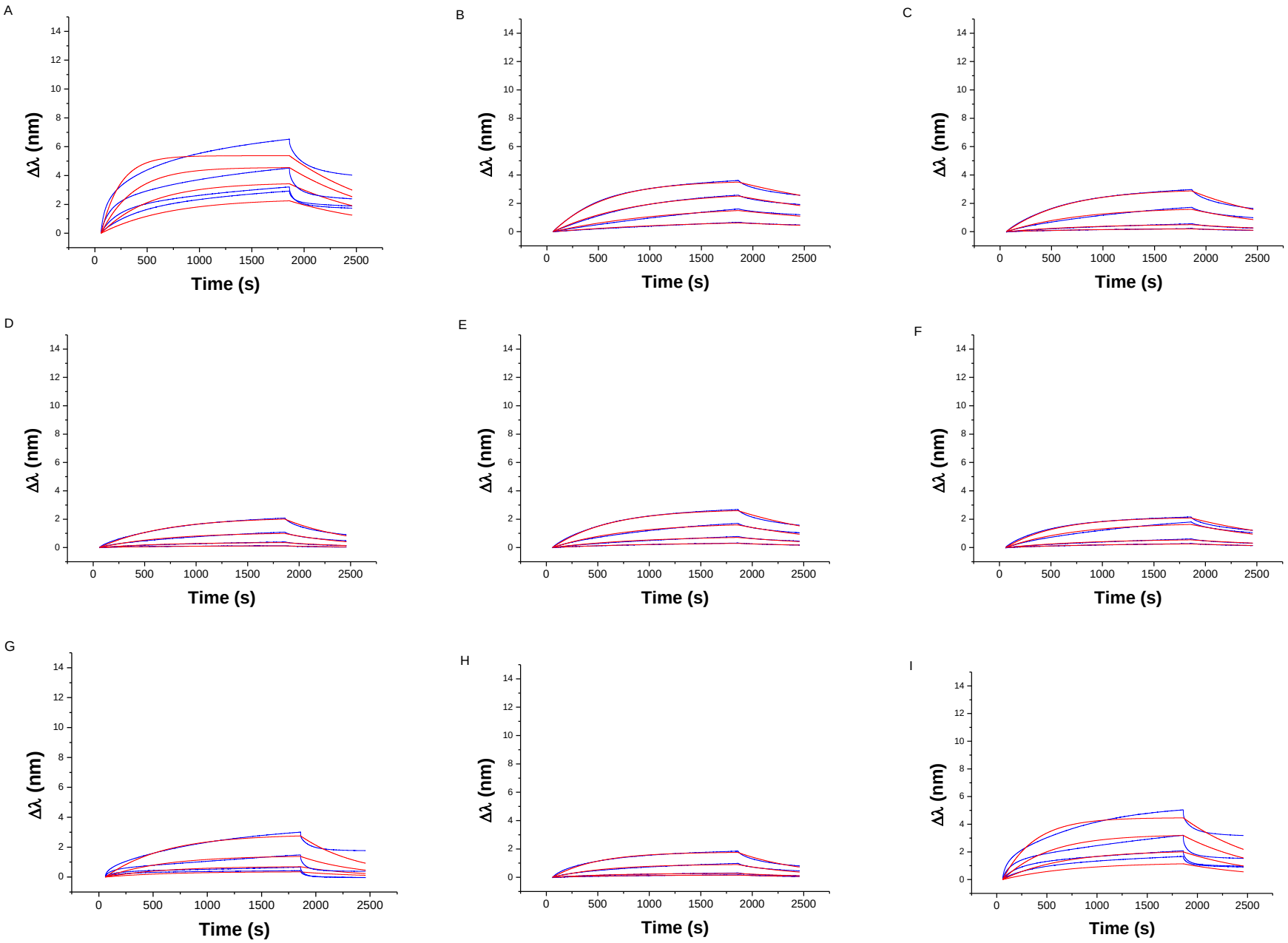



**Figure S6.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$ (62.5, 125, 250 and 500 nM) to hTeloC-4x5 (**A**), hTeloC-3x4 (**B**), hTeloC 3x3 (**C**), hTeloC 3x2 (**D**), hTeloC 2x3 (**E**), hTeloC 2x3T (**F**), hTeloC-2x2 (**G**), hTeloC 1x3T (**H**), hTeloC-1x2T (**I**), hTeloC 1x6 T (**J**), hTeloC 2x6T (**K**), hTeloC-scr (**L**), hTeloC-Mut (**M**), hTeloC-X3 (**N**) and hTeloC-22 (**O**) at pH 6.0. Blue lines are experimental curves; red lines are the fits to the 1:1 model.. Blue lines are experimental curves; red lines are the fits to the 1:1 model.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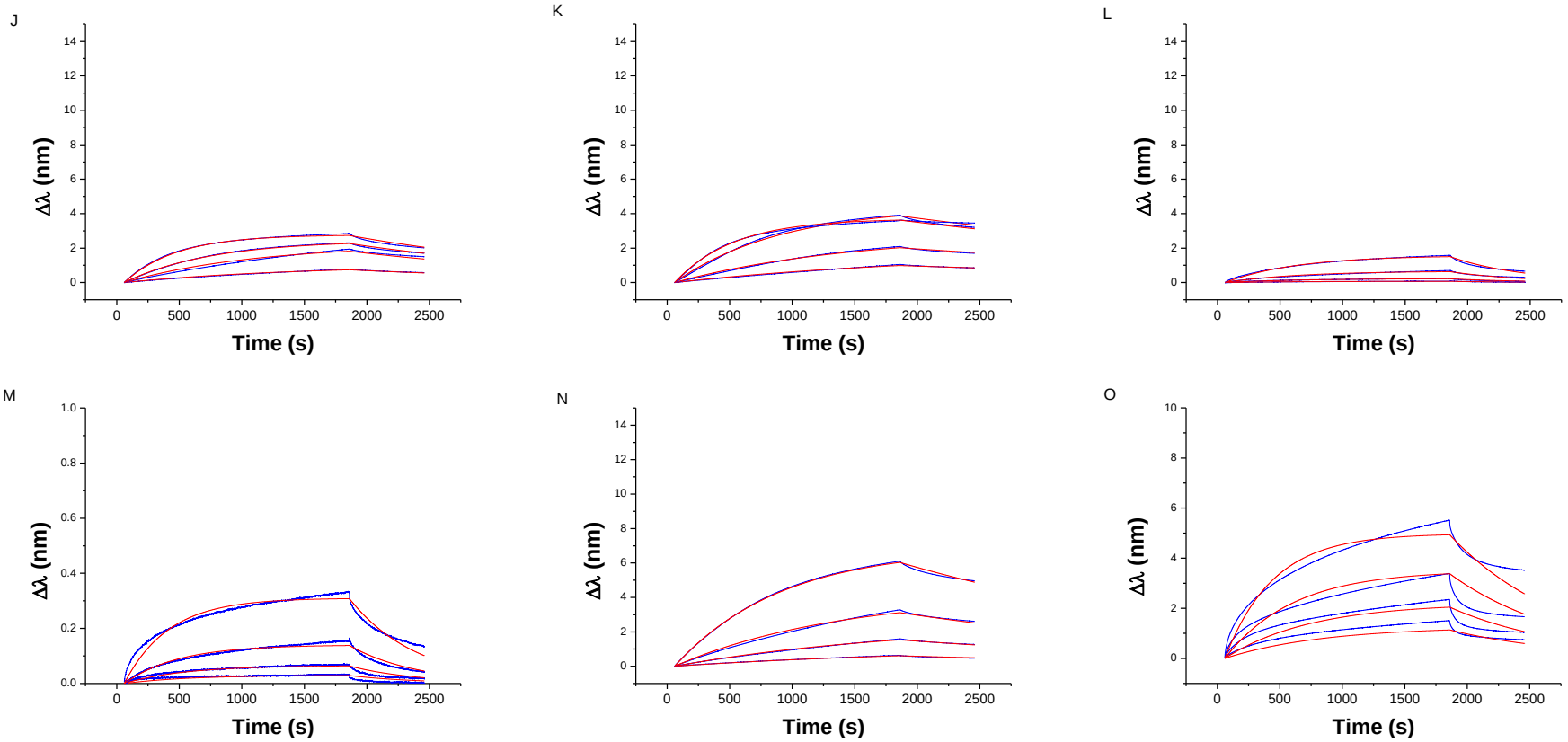


**Figure S7.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$ (62.5, 125, 250 and 500 nM) to hTeloC-4x5 (**A**), hTeloC-3x4 (**B**), hTeloC 3x3 (**C**), hTeloC 3x2 (**D**), hTeloC 2x3 (**E**), hTeloC 2x3T (**F**), hTeloC-2x2 (**G**), hTeloC 1x3T (**H**), hTeloC-1x2T (**I**), hTeloC 1x6 T (**J**), hTeloC 2x6T (**K**), hTeloC-scr (**L**), hTeloC-Mut (**M**), hTeloC-X3 (**N**) and hTeloC-22 (**O**) at pH 7.5. Blue lines are experimental curves; red lines are the fits to the 1:1 model.

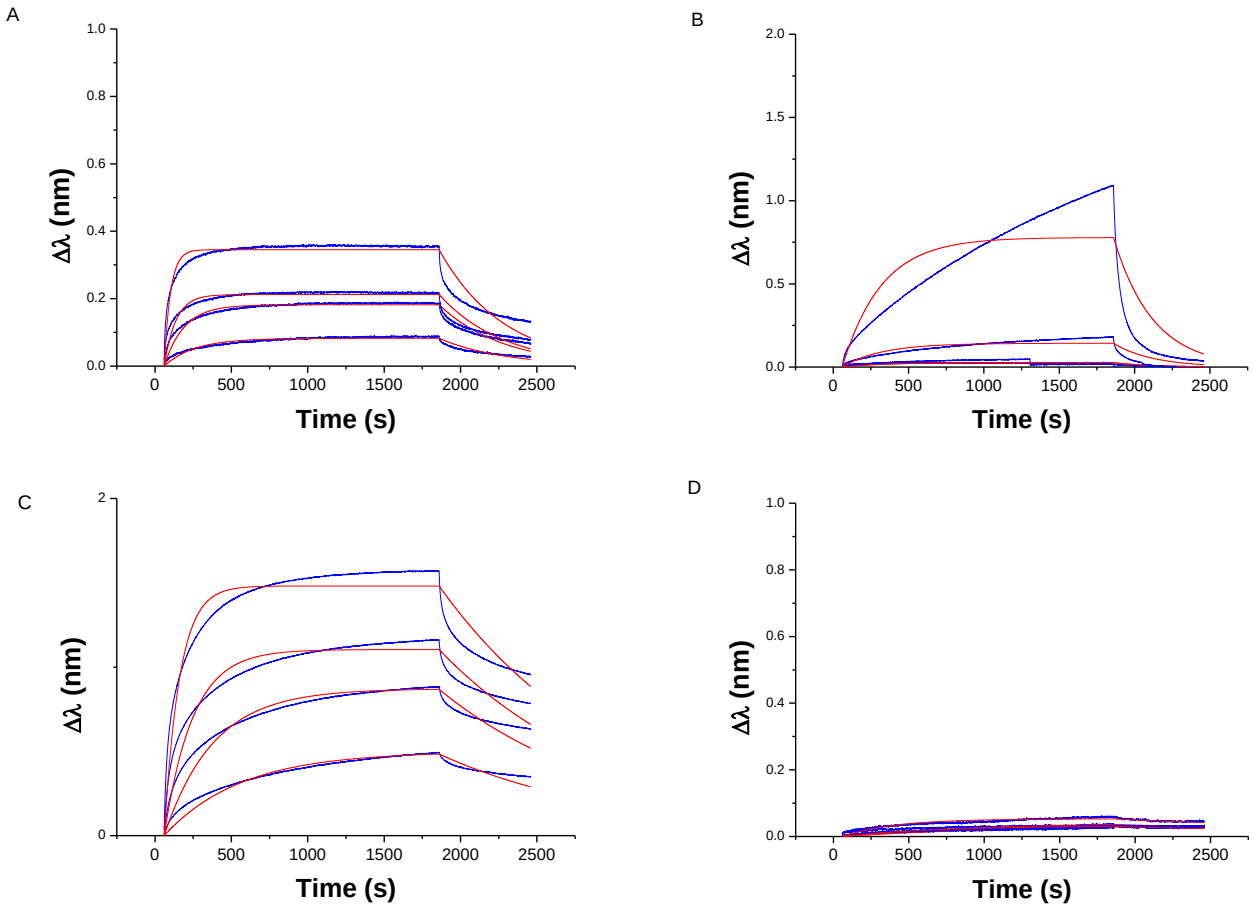


**Figure S8.** Bio-layer interferometry (BLI) analysis of binding of different concentrations of iMab scFv-$His_6$-FLAG at pH 6 (**A** and **C**) and 7.5 (**B** and **D**) for hp-ATT (**A**−**B**) and ss-DNA (**C**−**D**). The red lines are the fitting of the experiment. Concentration range: 62.5, 125, 250 and 500 nM.

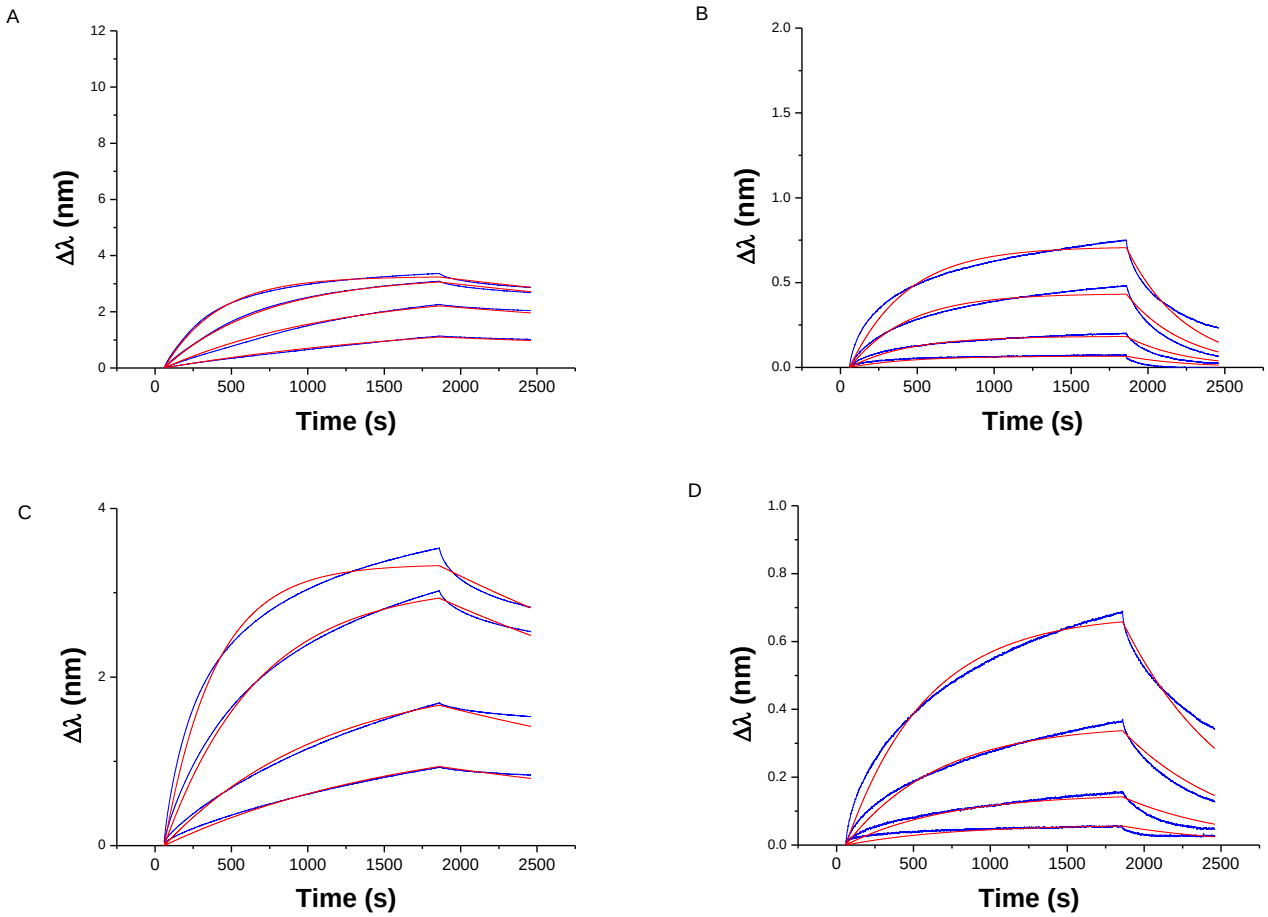


**Figure S9.** Bio-layer interferometry (BLI) analysis of binding of different concentrations of iMab scFv-$His_6$ at pH 6 (**A** and **C**) and 7.5 (**B** and **D**) for hp-ATT (**A**−**B**) and ss-DNA (**C**−**D**). The red lines are the fitting of the experiment. Concentration range: 62.5, 125, 250 and 500 nM.

**Table S1.** Oligonucleotide sequences used in this study and the corresponding $K_D$ values for the two variants of iMab, obtained from BLI experiments at pH 6.0 or 7.5 and 20 °C.[a]

| Acronym | Sequence (5′→3′) | iM folding,[a] at pH: | | number of C / length (nt) | $K_D$ at pH 6.0 (nM) | | $K_D$ at pH 7.5 (nM) | |
|---|---|---|---|---|---|---|---|---|
| | | 6.0 | 7.5 | | scFv-$His_6$ | scFv-$His_6$-FLAG | scFv-$His_6$ | scFv-$His_6$-FLAG |
| hTeloC-4x5 | T**CCCCC**T**CCCCC**TA**CCCCC**T**CCCCC**TA | + | – | 20 / 27 | 63 *(50; 75)* | 137 *(112; 162)* | 102 *(80; 128)* | 22 *(15; 28)* |
| hTeloC-27 | TAA**CCC**TAA**CCC**TAA**CCC**TAA**CCC**TAA | + | – | 12 / 27 | 34 *(24; 44)* | 28 *(23; 31)* | 108 *(63; 153)* | 91 *(18; 164)* |
| hTeloC-3x4 | TAATAA**CCCC**TAA**CCCC**TAA**CCCC**TAA | + | – | 12 / 27 | 29 *(17; 41)* | 22 *(18; 26)* | 178 *(135; 221)* | 138 *(67; 209)* |
| hTeloC-2x6T | TTTTAA**CCCCCC**TAA**CCCCCC**TAATTT | + | – | 12 / 27 | 65 *(54; 76)* | 31 *(31; 31)* | 67 *(48; 86)* | 170 *(118; 222)* |
| hTeloC-scr | TA**C**A**C**T**C**A**C**A**C**T**C**A**C**A**C**T**C**AA | – | – | 12 / 27 | 62 *(50; 74)* | 57 *(53; 61)* | n.b.[c] | n.b |
| hTeloC-3x3 | TAATAATAA**CCC**TAA**CCC**TAA**CCC**TAA | –[d] | – | 9 / 27 | 49 *(31; 67)* | 27 *(24; 30)* | 716 *(466; 966)* | 325 *(205; 445)* |
| hTeloC-3x2 | TAATAA**CC**TAA**CC**TAA**CC**TAATAATAA | – | – | 6 / 27 | 43 *(16; 70)* | 30 *(25; 35)* | n.b. | n.b. |
| hTeloC-2x3 | TAATAATAA**CCC**TAA**CCC**TAATAATAA | – | – | 6 / 27 | 32 *(22; 42)* | 30 *(24; 36)* | 475 *(438; 512)* | 195 *(109; 281)* |
| hTeloC-2x3T | TTTTTTTAA**CCC**TAA**CCC**TAATTTTTT | – | – | 6 / 27 | 35 *(27; 43)* | 30 *(26; 34)* | 396 *(206; 586)* | 241 *(148; 334)* |
| hTeloC-1x6T | TTTTTTTTTAA**CCCCCC**TAATTTTTTT | – | – | 6 / 27 | 27 *(23; 31)* | 47 *(38; 56)* | 125 *(113; 137)* | 493 *(468; 518)* |
| hTeloC-2x2 | TAATAA**CC**TAAT**C**TAA**CC**TAATAATAA | – | – | 5 / 27 | 40 *(30; 50)* | 50 *(35; 65)* | n.b.[c] | 58 *(40; 76)* |
| hTeloC-1x3T | TTTTTTTTTTAA**CCC**TAATTTTTTTTT | – | – | 3 / 27 | 33 *(25; 41)* | 60 *(38; 82)* | 891 *(408; 1374)* | 346 *(338; 354)* |
| hTeloC-1x2T | TTTTTTTTTTAA**CC**TAATTTTTTTTTT | – | – | 2 / 27 | 26 *(12; 40)* | 65 *(43; 87)* | 303 *(252; 354)* | 108 *(62; 154)* |
| hTeloC-22 | **CCC**TAA**CCC**TAA**CCC**TAA**CCC**T | + | – | 12 / 22 | 62 *(58; 66)* | 61 *(55; 66)* | 250 *(100; 400)* | 45 *(10; 80)* |
| hTeloC-mut | **C**GGTAA**C**GGTAA**C**GGTAA**C**GGT | – | – | 4 / 22 | 141 *(117; 165)* | 169 *(102; 236)* | n.b.[c] | n.b. |
| hTeloC-X3 | **CCC**TAA**CCC**TAA**CCC**TAA | –[d] | – | 9 / 18 | 54 *(35; 73)* | 20 *(8; 32)* | 215 *(170; 260)* | 396 *(272; 520)* |
| hp-ATT | G**C**G**C**G**C**GCATTG**C**G**C**G**C**G**C** | – | – | 8 / 19 | 38 *(23; 53)* | 26 *(8; 44)* | n.b. | n.b. |
| ss-DNA | GG**C**ATAGTG**C**GTGGG**C**G | – | – | 3 / 17 | 50 *(27; 73)* | 43 *(24; 62)* | n.b. | n.b. |

[a] Data are means ± s.d. from $N$ = 2 independent measurements. [b] according to CD spectra (cf. **Supplementary Figure S12**). [c] n.b. = Not determined, due to weak BLI signals (no binding) or uncoherent value obtained during the fitting ($R_{max}$ too high). [d] partial iM formation was observed at pH 5.5.

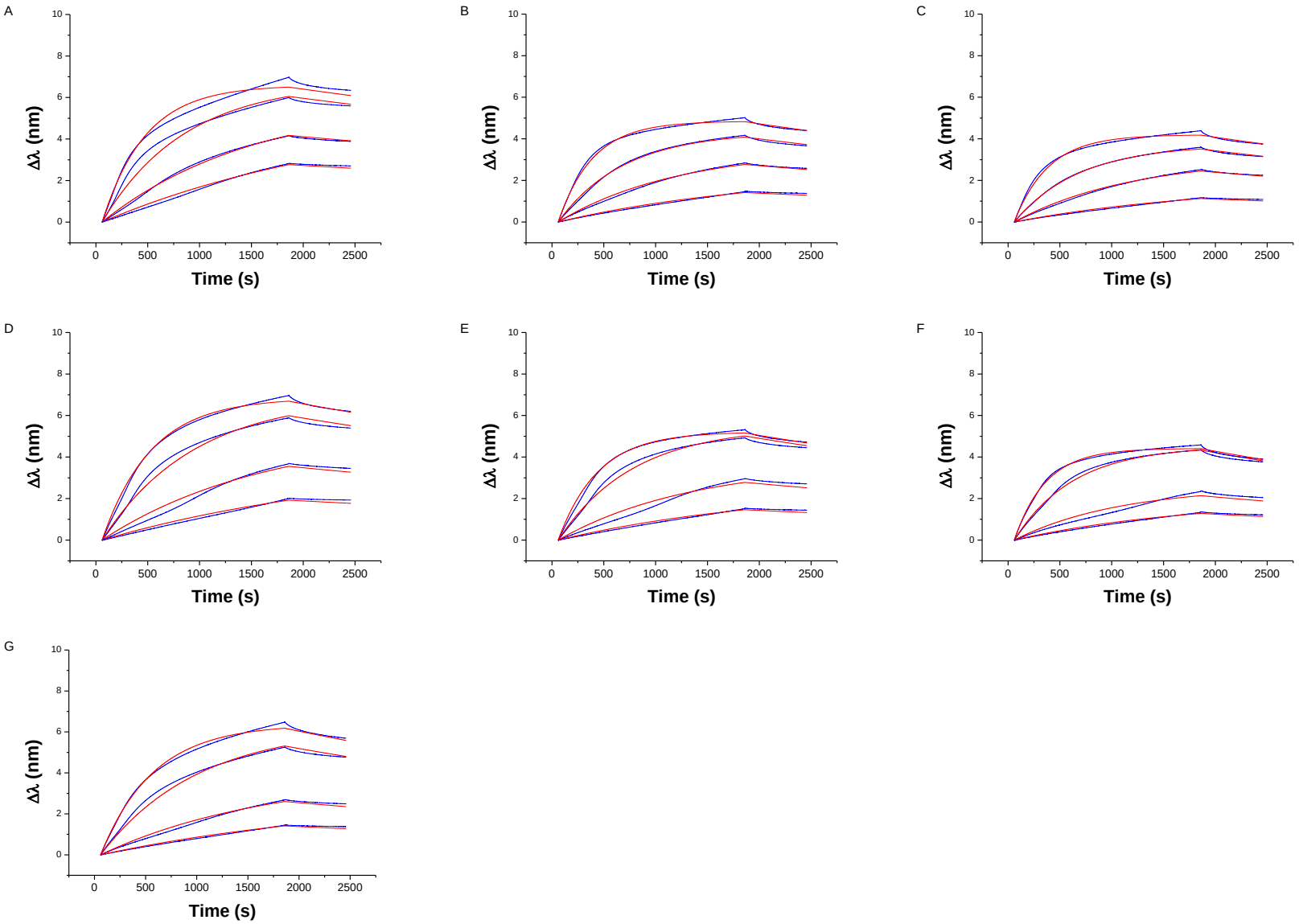


**Figure S10.** BLI analysis of binding of different concentrations of iMab scFv-$His_6$ (62.5, 125, 250 and 500 nM) to hTeloC-27 (**A**), hTeloC-3x4 (**B**), hTeloC 2x6T (**C**), hTeloC 3x4 (**D**), hTeloC 2x3 (**E**), hTeloC 1x3T (**F**), hTeloC X3 (**G**) at pH 6 after dipping of the immobilized structured on ammoniac solution at pH 10, 50°C during 10 minutes. Blue lines are experimental curves; red lines are the fits to the 1:1 model.

**Table S2.** Kinetic constants ($k_{on}$ and $k_{off}$) and calculated dissociation equilibrium constants ($K_D$) for interaction of iMab (scFv-$His_6$) with several HTeloC substrates at pH 6 and 20 °C, prior to and after dipping in $NH_3$ solution (pH 10, 50 °C during 10 min).[a]

| Substrate | pH 6.0 (prior to dipping in $NH_3$ solution) | | | pH 6.0 (after dipping in $NH_3$ solution) | | |
|---|---|---|---|---|---|---|
| | $k_{on}$ ($10^3$ $M^{-1}$ $s^{-1}$) | $k_{off}$ ($10^{-4}$ $s^{-1}$) | $K_D$ (nM) | $k_{on}$ ($10^3$ $M^{-1}$ $s^{-1}$) | $k_{off}$ ($10^{-4}$ $s^{-1}$) | $K_D$ (nM) |
| hTeloC-27 | 2.7; 4.5 | 1.1; 1.2 | 24; 44 | 4.1; 5.1 | 0.5; 1.6 | 12; 36 |
| hTeloC-3x4 | 5.9; 7.0 | 1.2; 2.4 | 17; 41 | 5.0; 6.4 | 0.9; 2.1 | 18; 32 |
| hTeloC-2x6T | 2.8; 3.6 | 1.5; 2.7 | 54; 76 | 5.1; 6.5 | 1.1; 2.4 | 21; 37 |
| hTeloC-X3 | 2.0; 2.9 | 0.7; 2.2 | 35; 73 | 2.9; 4.1 | 1.1; 2.2 | 38; 54 |
| hTeloC-3x3 | 4.0; 4.9 | 1.2; 3.3 | 31; 67 | 3.6; 4.1 | 0.9; 2.0 | 25; 54 |
| hTeloC-2x3 | 5.2; 6.7 | 1.5; 2.2 | 22; 42 | 4.2; 5.3 | 1.1; 2.1 | 26; 40 |
| hTeloC-1x3T | 8.6; 9.1 | 2.2; 3.7 | 25; 41 | 5.8; 6.5 | 1.7; 2.5 | 26; 43 |

[a] Data are individual values obtained from two independent measurements.

# Circular dichroism (CD) and gel electrophoresis studies

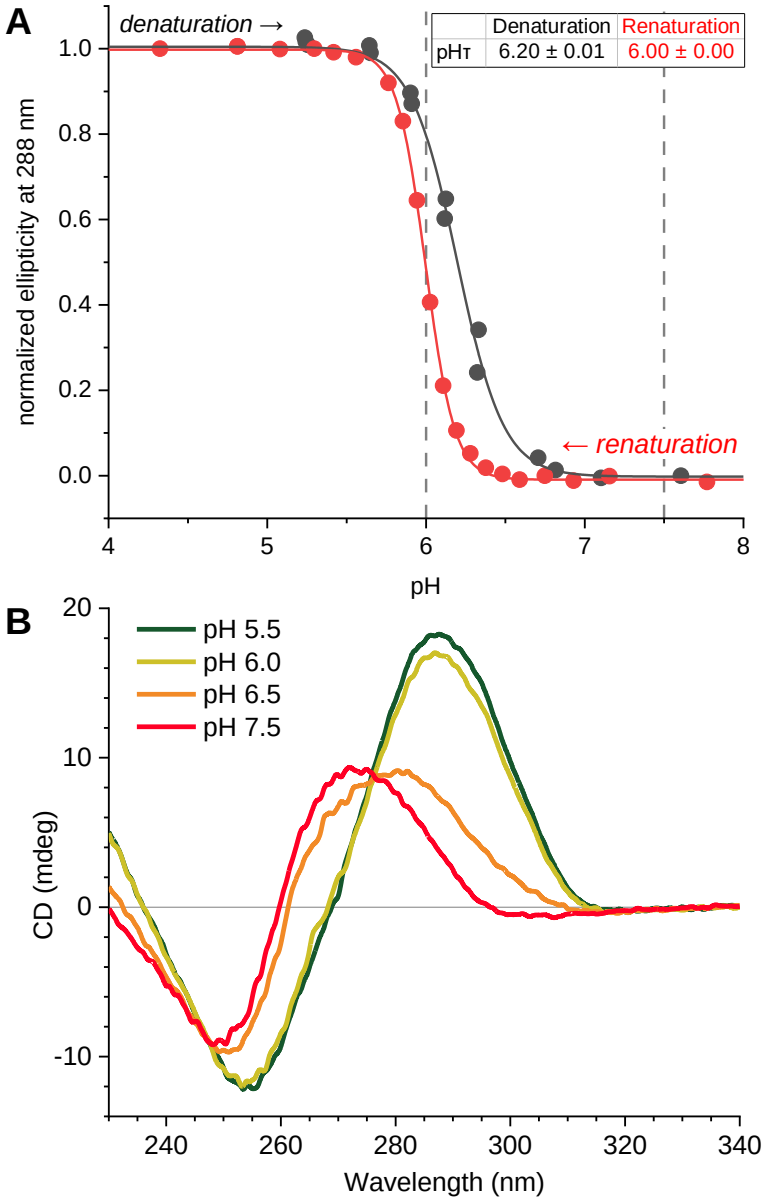


**Figure S11.** (**A**) pH-dependent denaturation/renaturation curves of hTeloC-27 (2.5 µM in 10 mM lithium cacodylate, 100 mM potassium chloride buffer). Titrations were performed as described elsewhere (Boissieras and Granzhan, *Methods Enzymol*. **2024**, *695*, 233–254). Dashed lines indicate the two conditions (pH 6.0 and 7.5) used in BLI experiments. (**B**) CD spectra of hTeloC-27 (2.5 µM) at pH 5.5, 6.0, 6.5 and 7.5.

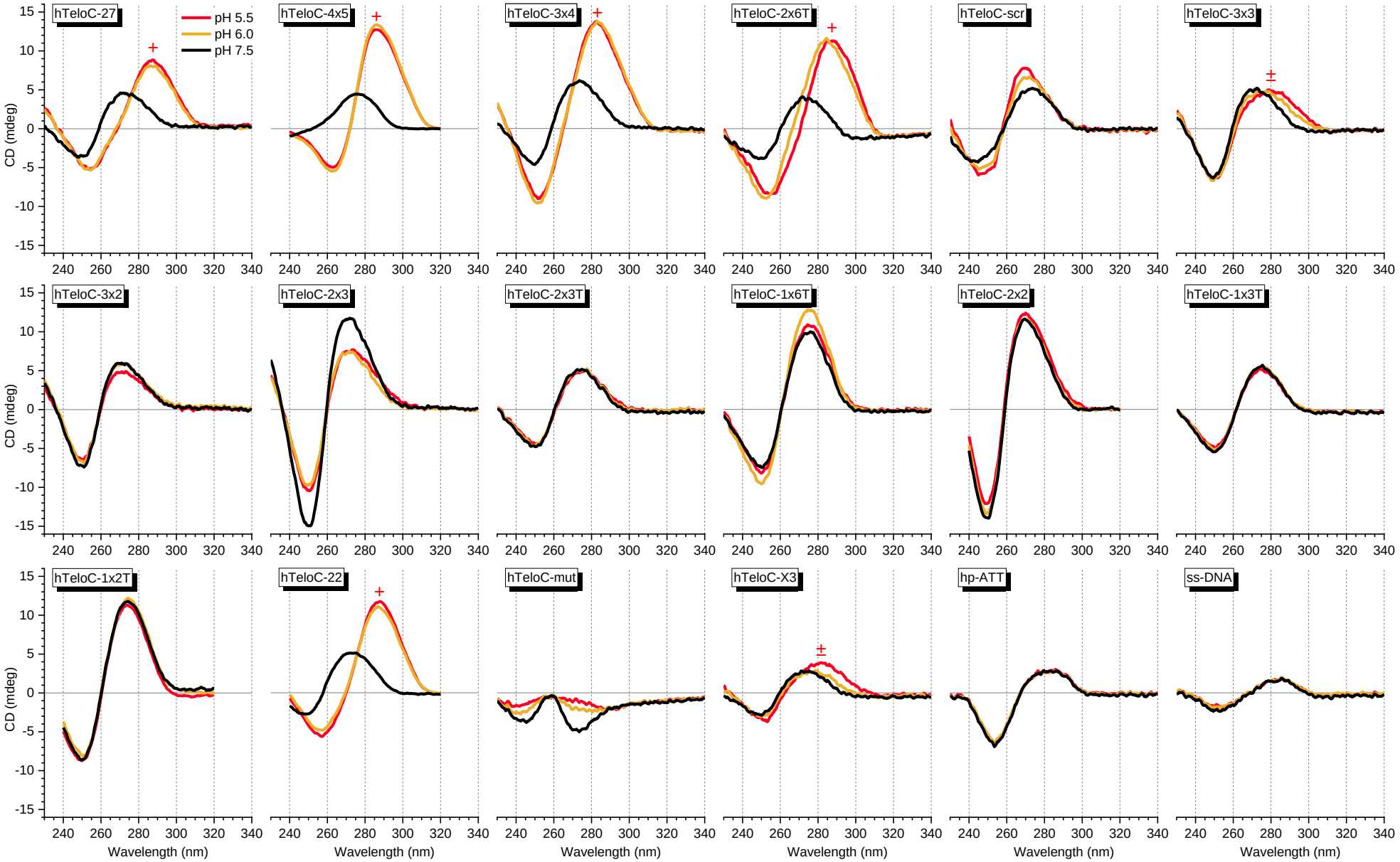


**Figure S12.** CD spectra of the sequences used in this study, obtained at pH 5.5 (red), 6.0 (dark yellow) and 7.5 (black). Oligonucleotide concentration *c* = 2.5 μM in all cases. iM-characteristic peaks are labelled with a plus (+) symbol; peaks suggesting partial formation of iMs are labelled with a plus-minus symbol (±). Note that the peculiar shape of hTeloC-mut spectrum is in agreement with literature data (M. Zeraati *et al.*, *Nature Chem.* **2018**, *10*, 631–637).

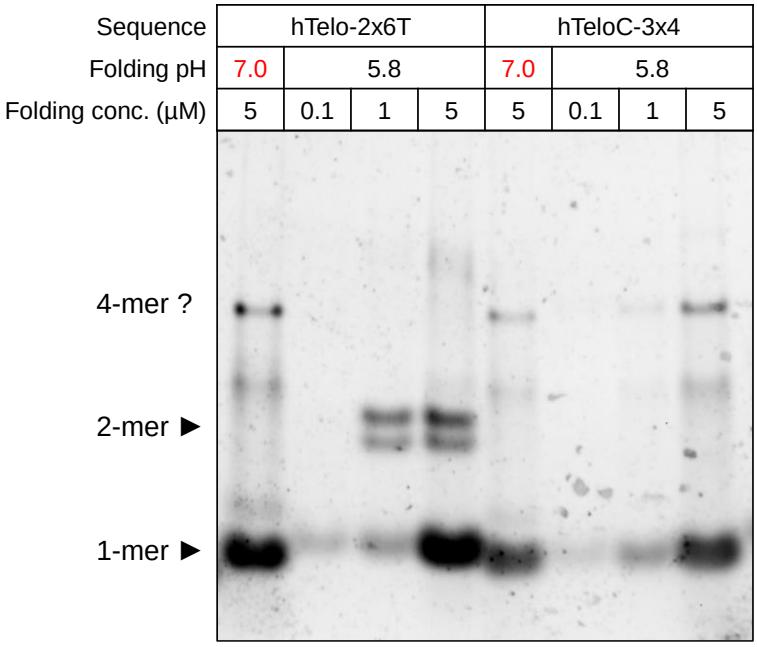


**Figure S13.** Native gel electrophoresis of hTeloC-2x6T and hTeloC-3x4. Oligonucleotides samples (10 µL each) were annealed at indicated concentrations for 10 min at 95 °C and then cooled overnight in Tris-AcOH, pH 5.8 or 7.0 (control), supplemented with 2 µL of a 6X Ficoll-based loading buffer and migrated on a 20% acrylamide gel with TAE buffer adjusted to pH 5.8, overnight at 4 °C. DNA bands were observed after a 15-min incubation in a 1:100 SYBR Green/TAE mixture in a BioRad GelDoc XR device.

# Bulk-FRET experiments

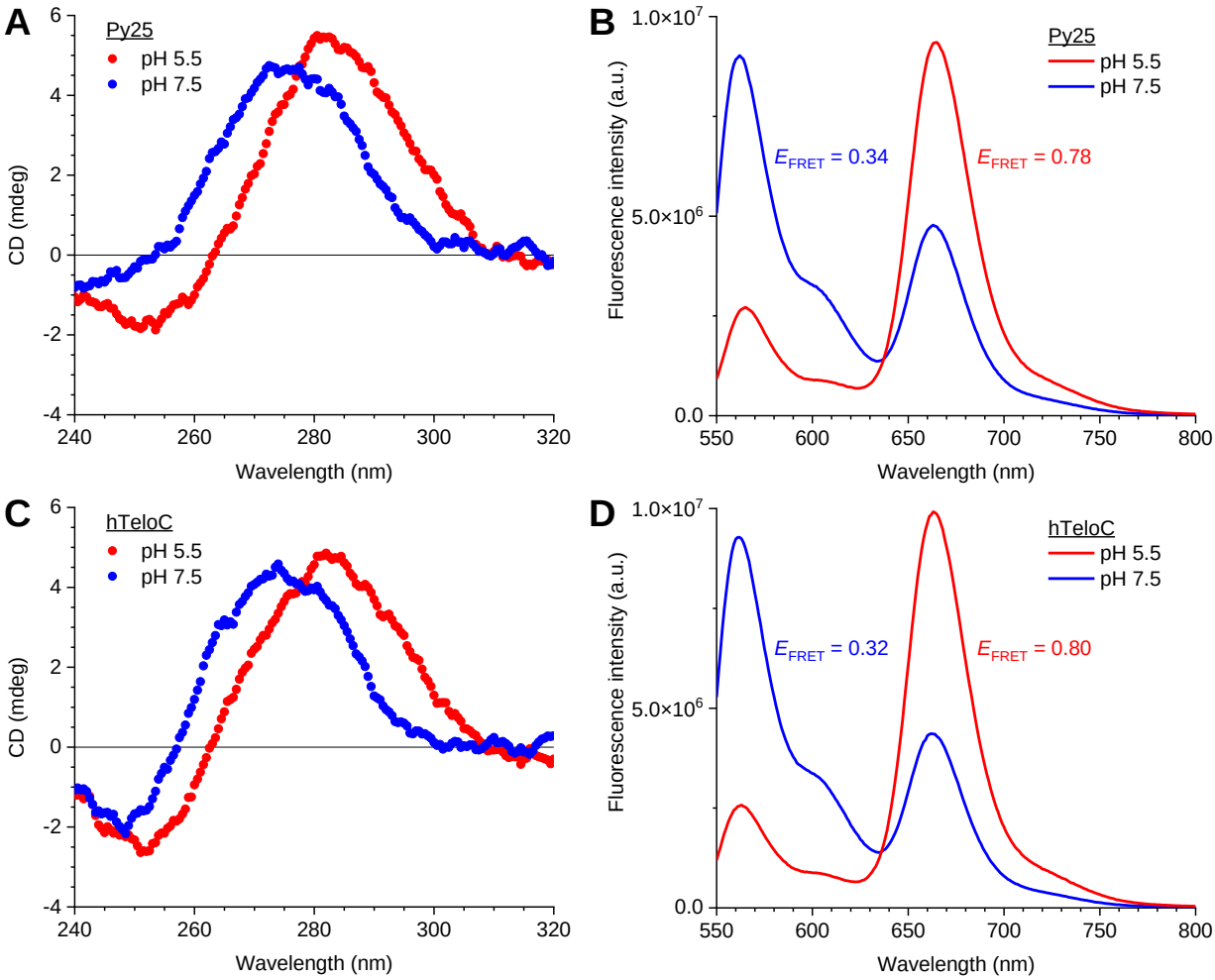


**Figure S14.** Circular dichroism (**A**, **C**) and fluorescence emission spectra (**B**, **D**) of Cy3-Py25/Cy5-CS (panels **A–B**) and Cy3-hTeloC/Cy5-CS (panels **C–D**) substrates (0.5 μM in bulk-FRET buffer), both recorded at pH 5.5 (red dots and lines) and pH 7.5 (blue dots and lines).

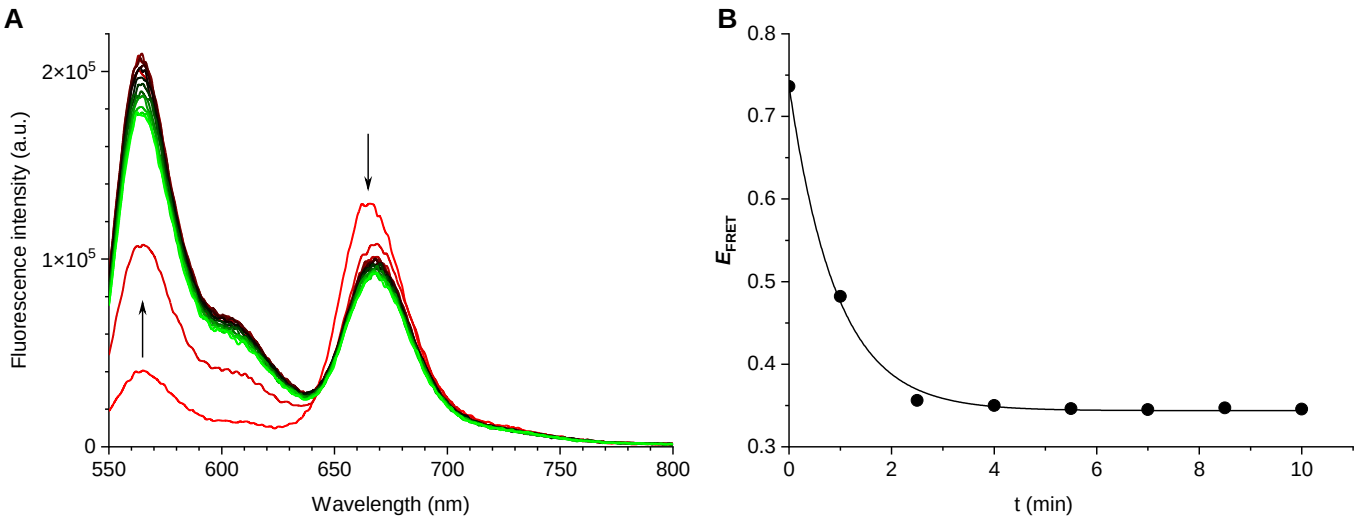


**Figure S15.** Time-dependent variation of fluorescence emission spectra (**A**) and $E_{FRET}$ value (**B**) of Cy3-Py25/Cy5-CS substrate (4 nM) after addition of recombinant hnRNP K (800 nM) at pH 5.8. Spectra were recorded in ~2-min intervals.

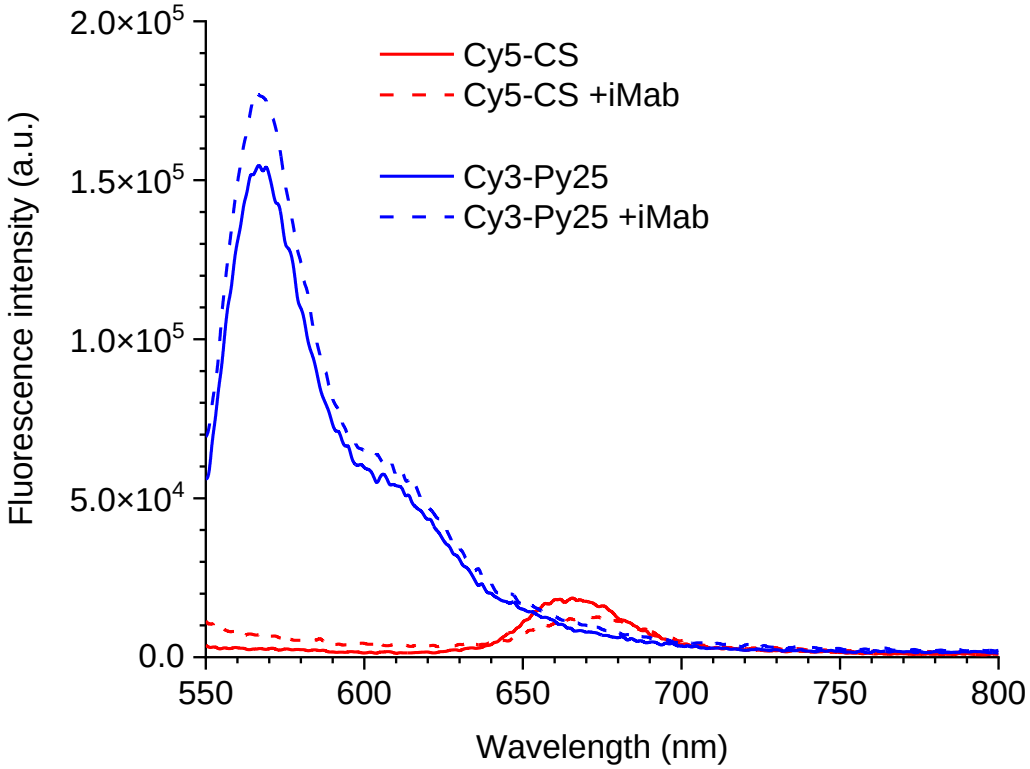


**Figure S16.** Fluorescence emission spectra of Cy3-Py25 (blue) and Cy5-CS (red) strands (4 nM in bulk-FRET buffer, pH 5.8), in the absence (solid lines) and in the presence of 800 nM of iMab (dashed lines). No additional changes were observed after ~1 hour of incubation time.