A self-assembling protein-DNA complex with an inbuilt DNA release system for quantitative immuno-PCR applications

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ESI

Materials and Methods

Oligonucleotides

Two oligonucleotides were designed to include the *E. coli bioO* target sequence:

5'-TAATCGACTTGTAAACCAAATTGAAAAGATTTAGGTTTACAAGTCTACAC-3'

5'-GTGTAGACTTGTAAACCTAAATCTTTTCAATTTGGTTTACAAGTCGATTA-3'

The qPCR *bioO* template was annealed at a final concentration of 50 μM (20 mM Tris pH 8.0, 150 mM NaCl) following denaturation for 2 min at 80°C and slow cooling. The qPCR *bioO* template was stored at -20°C.

Sequences for qPCR primers:

Forward 5'-TAATCGACTTGTAAACCAAATTG-3'

Reverse 5'-GTGTAGACTTGTAAACCTAAATC-3'

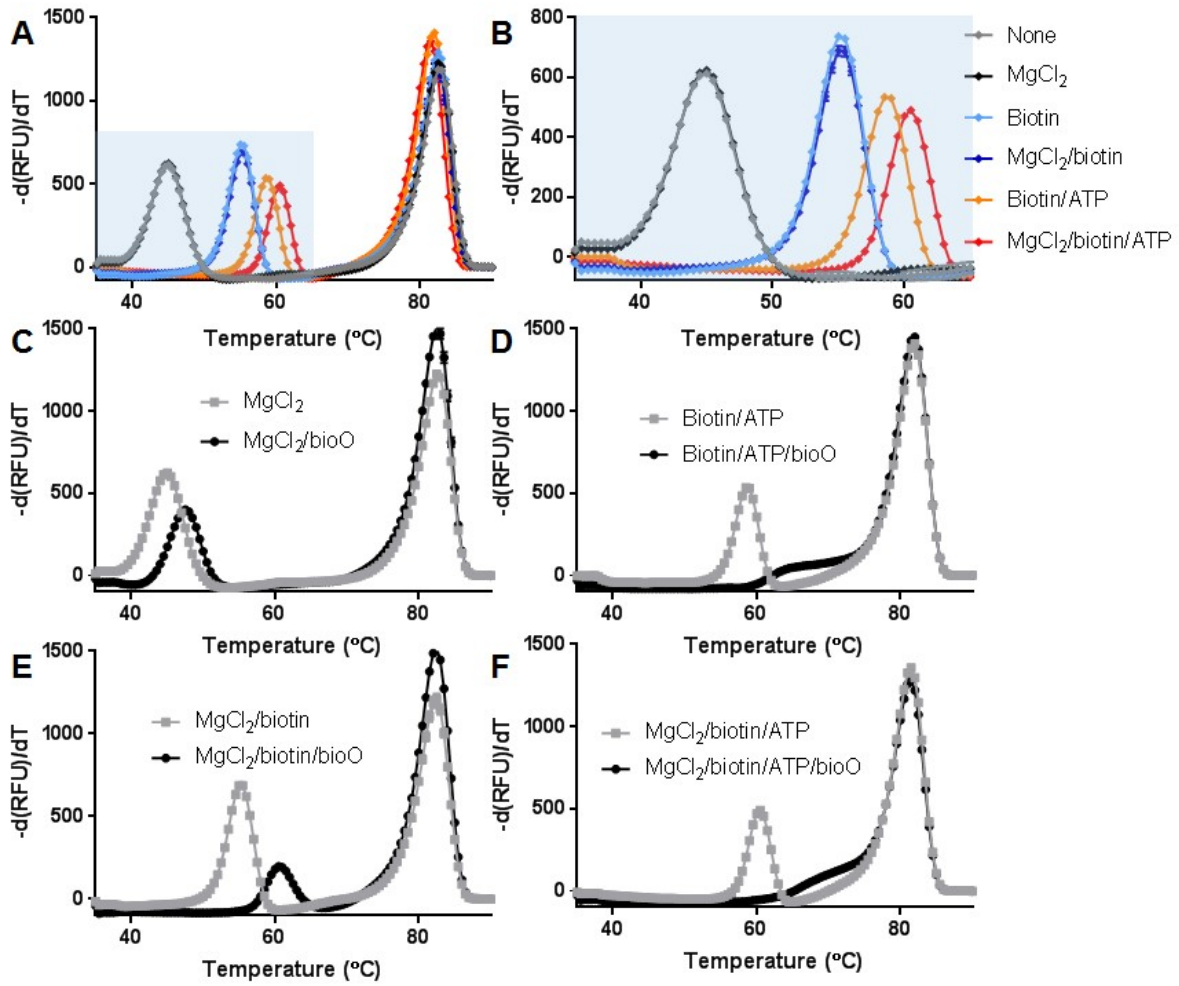

Fig. S1 Ligand effects on Ec BirA-GFP melt-curves in PBS-T. A) Ec BirA-GFP (1 µM) was incubated for 10 min at RT with combinations of biotin (1 mM), ATP (1 mM) and $MgCl_2$ (5 mM) in PBS-T prior to analysis by DSF-GTP melt-curve protocol (35-90°C at 0.5°C/30 s). B) Detail of the BirA region of the Ec BirA-GFP curves from (A). Comparison between Ec BirA-GFP curves in the presence and absence of *bioO* (1 µM) with (C) $MgCl_2$, (D) biotin/ATP, (E) $MgCl_2$/biotin and (F) $MgCl_2$/biotin/ATP. Data represent averages and SD of three melt-curves.

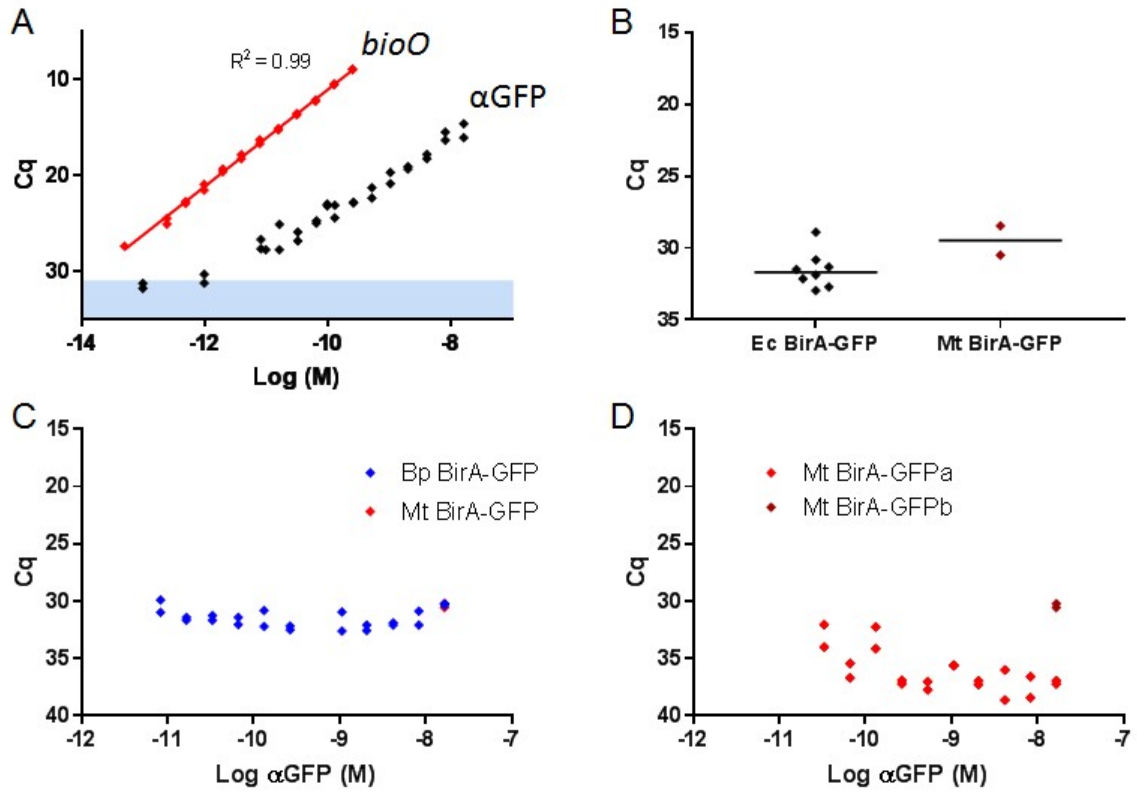

Fig. S2 A) qPCR standard curve of *bioO* and comparison with qIPCR detection of preadsorbed polyclonal anti-GFP IgG (αGFP) with preassembled Ec BirA-GFP:*bioO* in PBS-TBA (cf Fig. 3 in main article). See ESI methods for details of the *bioO* template and primer sequences. B) No anti-GFP IgG qIPCR with preassembled Ec BirA-GFP:*bioO* or Mt holoBirA-GFP in the presence of *bioO*. C) qIPCR with Bp holoBirA-GFP in the presence of *bioO*. D) qIPCR with Mt BirA-GFP in the presence of *bioO*. Concentrations of anti-GFP IgG are as indicated. All replicates are shown.