Supplementary Information

Optimising T_m of CProbes

Method: T* and FProbe were incubated in 10 mM Tris-HCl, 150 mM NaCl, 5 mM $MgCl_2$ (pH 7.4). Tubes were heated to 80 °C for 5 minutes and then subjected to a temperature gradient to 25 °C, falling by 1 °C every 60 seconds to achieve maximum hybridization. QDs were incubated with CProbe (either low, medium or high Tm), at a QD:CProbe ratio of 1:30 H_2O, for 60 minutes at RT in the dark. Final assay volume was 100 µL.

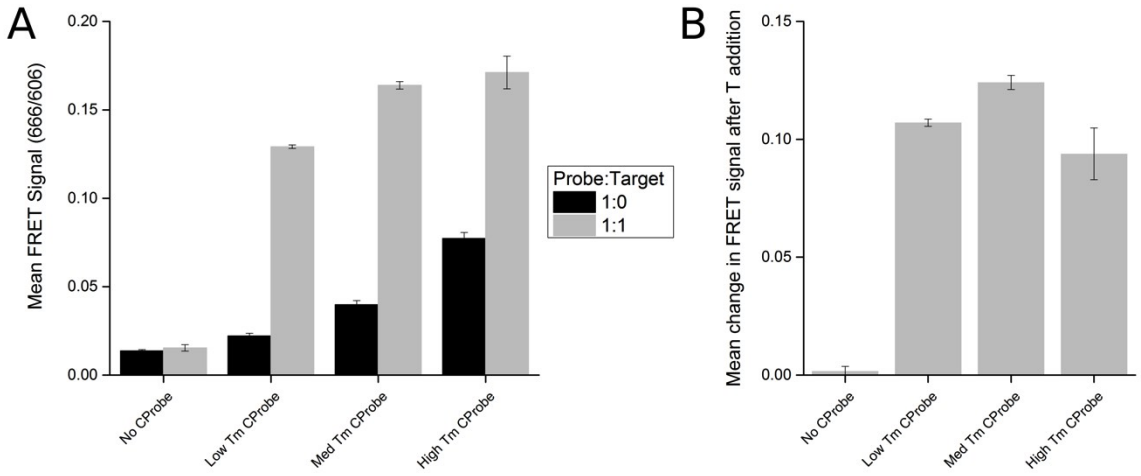

Figure S1. (a) FRET signal was measured for the detection system before and after target addition, using each CProbe. (b) The mean change in FRET signal following target addition was plotted for each CProbe (n=3, error bars represent standard deviation).

	Sequence (mismatches are underlined)	Modification	Length (bases)
GBP6 FProbe	ATA GAA CAG ATC CAT ACA CAC TTT GA	AF647 at 3'	26
High T_m GBP6 CProbe	AAT CAA AGT GTG TAT GGA TCT GTT CTA T	Biotin at 5'	28
Medium T_m GBP6 CProbe	AAT CAA AGT CTG TAT GGA TGT GTT CTA T	Biotin at 5'	28
Low T_m GBP6 CProbe	AAT CAG AGT CTG TACGGA TGT GTT CTA T	Biotin at 5'	28

Determining the Binding Capacity of the SA-QDs

Biotin functionalized capture probe (CProbe) and quencher probe (QProbe) was incubated at a 1:1 ratio in borate buffer (50 mM borate, 150 mM NaCl, 0.01% BSA, pH 8.3). This was heated to 80 °C for 5 minutes and then subjected to a falling temperature gradient to 25 °C, falling by 1 °C every 60 seconds to achieve maximum hybridization. The detection complex (hybridized CProbe:QProbe) was added to 10^{-13} moles QDs and made up to a final volume of 25 µL (QD concentration 4 nM) before reading fluorescence.

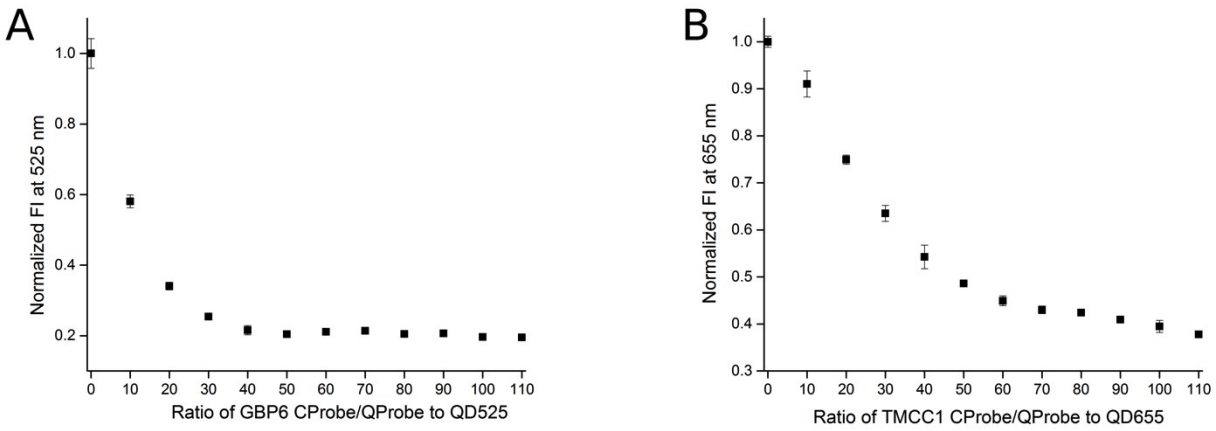

Figure S2. Binding capacity of the QD525 and QD655s. Increasing amounts of pre-hybridized CProbe/QProbe were added to a constant concentration of QDs. n=3, error bars represent standard deviation.

Calculating a Theoretical LOD

QDs were incubated with CProbe at a molar ratio of 1:20 for QD525:GBP6 CProbe and 1:30 for QD655:TMCC1 CProbe, in borate buffer. The final QD concentration was 4 nM. QProbe was added in increasing concentrations, maintaining a constant reaction volume of 25 μL. The limit of detection (LOD) was calculated by subtracting three times the standard deviation for the signal at 0 nM target from the mean signal at 0 nM target and extrapolating to the next highest target concentration value to the resulting value.

	GBP6	TMCC1
Mean signal at 0 nM [T]	9379.33333	84160.33333
SD at 0 nM [T]	71.52855	1178.17924
Mean – 3 x SD	9164.74768	80625.79561
Theoretical LOD	< 1.6 nM	< 6.4 nM

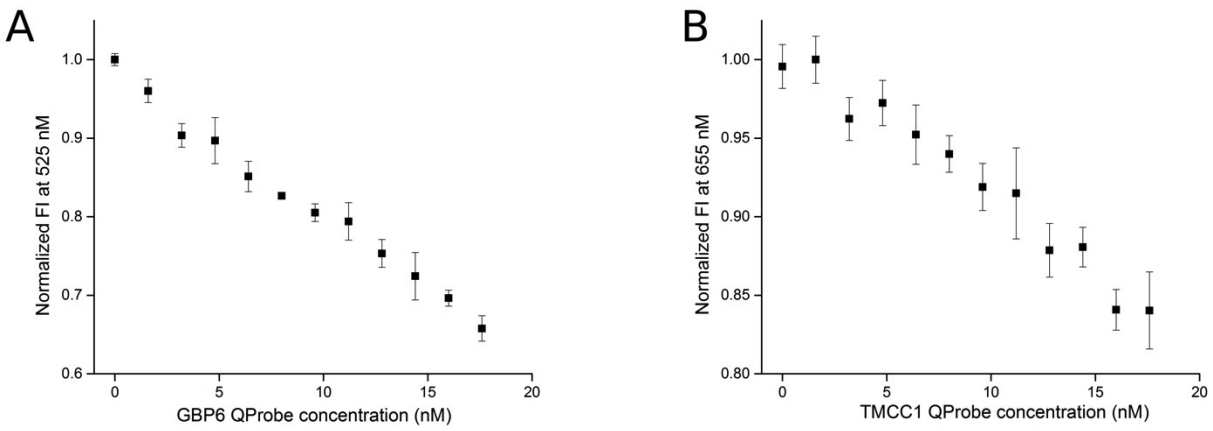

Figure S3. The theoretical LOD of each system was determined by adding Qprobes to preincubated QD/Cprobe. n=3, error bars represent standard deviation.

Calculating the Assay LOD

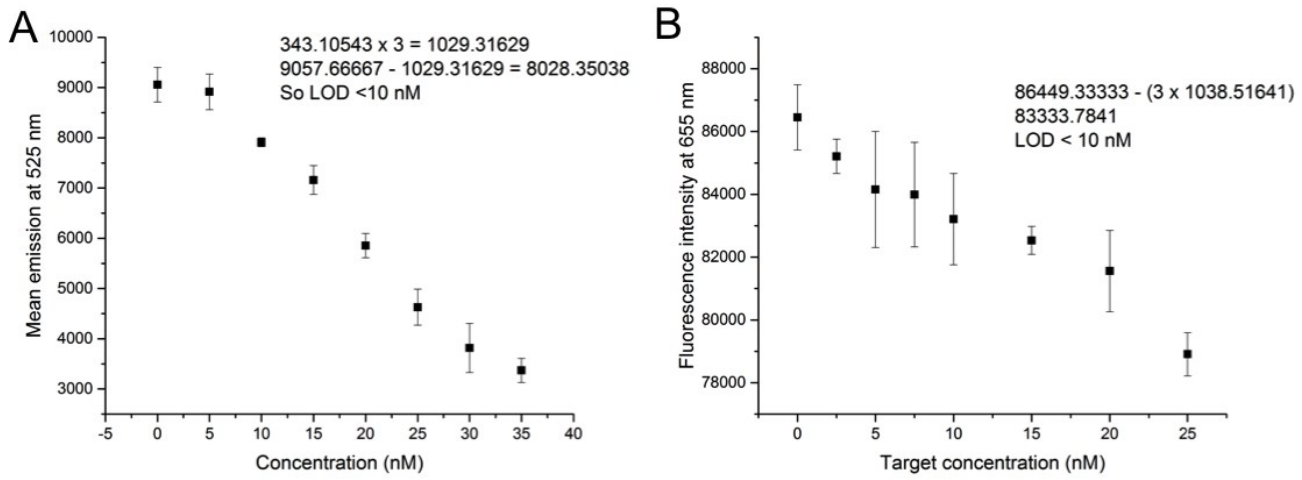

Figure S4. The assay LOD of each system was determined. n=3, error bars represent standard deviation.

LOD and Saturation Point calculations for monoplex assays:

	GBP6	TMCC1
Mean signal at 0 nM [T]	9057.66667	86449.33333
SD at 0 nM [T]	343.10543	1038.51641
Mean – 3 x SD	8028.35038	83333.7841
LOD	<10 nM	<10 nM

	GBP6	TMCC1
Mean signal at 0 nM [T]	2463.66667	63821
SD at 120 nM [T]	106.61301	1551.9739
Mean – 3 x SD	2783.5057	68476.9217
Saturation point	> 70 nM	> 70 nM