Supporting information for: Transcription Factor Beacons for the Quantitative Detection of DNA Binding Activity

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I-SUPPORTING METHODS

Fluorescent TF beacons: HPLC purified DNAs modifiedwith 5'-FAM (5-carboxyfluorescein) and internal BHQ-1 (black hole quencher 1) linked to the C5-position of a thymine were purchased from IBA (Goettingen, Germany) and Biosearch Technologies (Novato, CA). DNA binding sequences with optimal affinity and specificity for the TF of interest are selected for the design of each new TF beacon[3]. The predicted and observed equilibrium constants of the switches were, respectively, estimated using *mfold*[4] and determined experimentally using the fluorescence of the beacon in the absence or presence of saturating amount of TFs (see TF switch sequences in Supporting notes). DNA binding proteins: Recombinant TBP and Myc-Max were expressed, purified, and characterized as previously described[5,6]. Recombinant NF-κB (p50 homo-dimer) was purchased from ActiveMotif (Carlsbad, CA) and used as delivered. HeLa cell nuclear extract was purchased from Santa Cruz Biotechnologies (Santa Cruz, CA) and used as delivered.

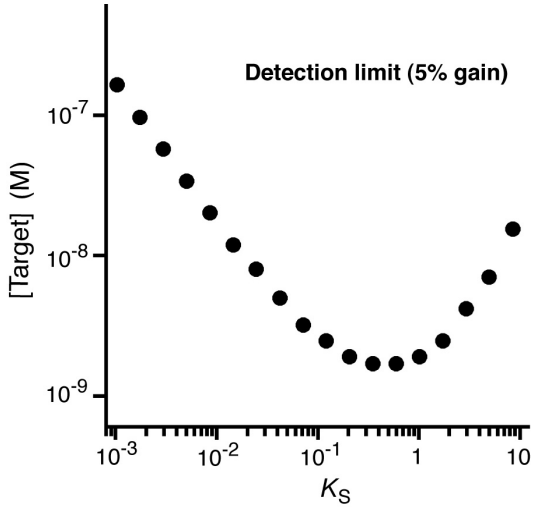
All fluorescence experiments were conducted at 25°C in 50 mM sodium phosphate, 150 mM NaCl at pH 7. This was supplemented with 5 mM $MgCl_2$ for all experiments with TBP, as this is essential for TBP binding. Equilibrium fluorescence measurements were obtained on a Cary Eclipse Fluorimeter with excitation at 480 (± 5) nm and acquisition at 517 (± 5) nm unless otherwise specified. Binding curves were obtained using 4 nM switch and fit to Langmuir isotherms[1]. Kinetic fluorescence data were obtained on an SM-18 Applied-Photophysics stopped-flow instrument, exciting at 480 (± 10) nm and monitoring the total fluorescence above 495 nm using a cut-off filter (except for the NFkB beacon kinetic, which was obtained on the Cary).

Measurement of TBP in nuclear extract. The fluorescence of the TF beacon in equilibrium with the endogenous TF in the sample, F_{smp}, was obtained by diluting commercial HeLa nuclear extract four-fold to 250 μg/ml in the above-described buffer (to control pH and ionic strength), mixing with 2 nM TBP switch and incubating 20 min before measuring fluorescence. The addition of excess (600 nM) unlabeled, non-switching, double-stranded competitor oligonucleotide *to the same tube* (this non-switching competitor binds the TF with a higher affinity than the switching beacon –see Supporting Fig. 2), followed by a 20 minutes equilibration, allows measurement of the fluorescence of the TF beacon in its unbound state, F_{bkg}. Finally, addition of an excess (600 nM) of a single-stranded DNA that stabilizes the emissive conformation of the TF beacon, *again to this same tube* and with a further 20 minutes equilibration, allows determination of the fluorescence of the fully bound beacon, F_{sat}. Alternatively, F_{sat} can be determined via the addition and equilibration of excess TF (1 uM TBP in Fig. 3), but this requires a supply of purified target protein, which can be difficult or expensive to obtain. With F_{bkg}, F_{sat} and F_{smp} measurements, the concentration of target protein, C, can be found via equation 1:

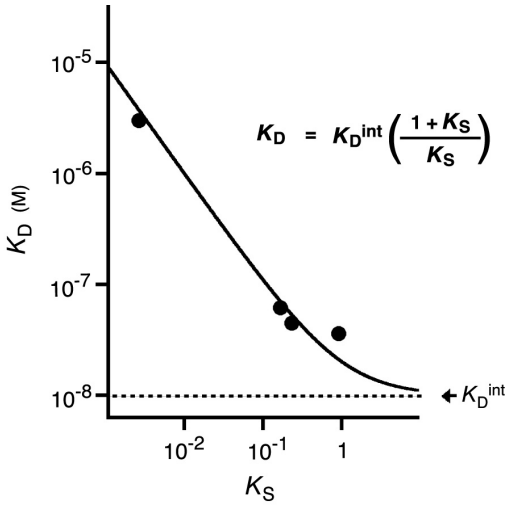
$$C = \frac{K_D\left(F_{smp} - F_{bkg}\right)}{F_{sat} - F_{smp}} \qquad (1)$$

where K_D is the dissociation constant of the TF switch (which we determined in Fig. 2 to be 45 ± 3 nM). The errors reported represent the standard error of 4 independent measurements. The concentration of TBP determined in 250 μg/ml nuclear extract, 5.8 ± 1.6 nM, is in reasonable agreement with the ~3 nM estimated from the number of cell equivalents in a given sample and the number of TBP molecules per cell[7,8]. The stabilizer and competitor oligonucleotides (sequences in Supporting notes) were purchased from Sigma-Aldrich (St. Louis, MO).

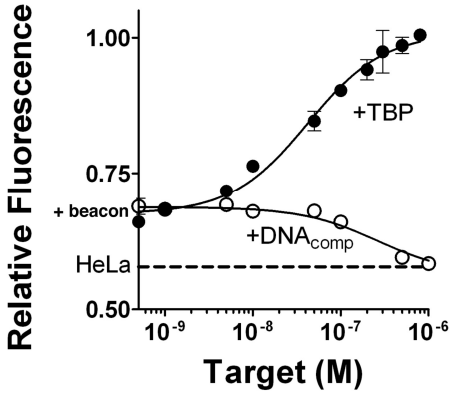
II-SUPPORTING FIGURES



Supporting Figure 1 | Simulated detection limit of TF beacon (here arbitrarily defined as the minimum target concentration that produces at least a 5% change in the fluorescence of the beacon) as function of the beacon's switching thermodynamics. Optimal detection limits are obtained at a switching equilibrium constant, K_S, of between 0.2 and 1, which balances the trade-off between high gain (optimal at lower K_S) and enhanced binding affinity (optimal at higher K_S) (see **Fig. 1b-c**)[1]. In this work we employed K_S = 0.2 as this leads to optimal detection limits and enhanced signal gain (300% at saturated concentration of TF). The simulations were performed with eq. 4 in Supporting Reference 1 using the experimental parameters taken from the TBP beacon (**Fig. 1c**): $F_{BACKGROUND}$= 0.02; $F_{NON\text{-}BINDING}$ = 0.23; $F_{BINDING\ COMPETENT}$= 1. K_A^{int} (or $1/K_D^{int}$) was set at 100,000,000 M^{-1} (see **Supporting Fig. 2)**.



Supporting Figure 2 | The relationship between the switching equilibrium constant of the TBP beacon, K_S, and its target affinity, K_D, is well described by the three-state "population-shift" model (inset equation)[1]. As the switching equilibrium constant is reduced (*i.e.,* as the non-binding conformation is stabilized) the affinity of the switch is proportionally reduced, resulting in a higher K_D. K_D^{int} represents the affinity of the TF for the double-stranded recognition element that does not undergo switching (~ 6-fold higher affinity than for the beacon with a K_S of 0.2). The K_S values used in this figure were either determined experimentally (**Fig. 1b:** K_S^{exp}) or, for values which are difficult to measure precisely with experiments (below 0.1 or above 10), estimated by predicting the switching equilibrium constant using *mfold*[4] (K_S^{pred}) and correcting this to account for a systematic 2 kJ/mol discrepancy observed between predicted and experimental switching thermodynamics (inferred from the average difference between K_S^{exp} and K_S^{pred}). We believe this arises due to stacking of the fluorophore and quencher in the non-binding state[2].



Supporting Figure 3 | TF beacons are selective enough to deploy directly in crude nuclear extracts. A titration of our TBP beacon with active TATA binding protein in the presence of 250 μg/mL of HeLa nuclear extracts (filled circles) produces the expected hyperbolic binding curve albeit with a small offset from the same curve obtained in buffer (apparent K_D = 36 ± 6 nM *versus* 45 ± 3 nM in buffer), which is consistent with the presence of endogenous active TBP at low nanomolar concentrations in the extract as this would push the former curve to a lower apparent dissociation constant. Titration with an unlabelled, non-switching, double-stranded competitor DNA (open circles) produces a loss in fluorescence following a Langmuir isotherms curve thus further suggesting that the above offset is due to the presence of endogenous TBP (see **Fig. 3**).

Supporting Figure 4 | Detection of endogenous, active TATA binding protein (TBP) in HeLa nuclear cell extract using an electrophoretic mobility shift assay[9]. The observed shift is consistent with the presence of endogenous active TBP protein in the extract. The TBP lane (extreme left) contains 50 pmol of purified TBP, and the HeLa NE lanes contain from 2.5 μg to 0.25 μg nuclear extract (dilution range from 25% to 2.5%). The quantified intensity differences allow estimation of ~5 nM TBP activity in 250 μg/mL nuclear extracts (or 25% dilution), in alignment with the values determined using the TF beacon (5.7 ± 1.7 nM –see **Fig. 3**) and with the value estimated from the known number of TBP molecules per cell (see **Methods**). The mobility shift assay was performed using a fluorescein-labeled TBP competitor oligonucleotide in a 4-20% gradient Tris-glycine gel with 1x TBE (Tris borate EDTA) running buffer at a running voltage of 200 V. Reactions were pre-equilibrated for 20 minutes before loading, and after running the gel was imaged on a Typhoon Trio from GE Healthcare (Piscataway, NJ).

III-SUPPORTING NOTES

TF beacon sequences:
TBP red: 5'-FAM-TAC TTT TAT ATA AAT AAG TT(BHQ)G TGA ATT TTA TAT ATA TCA C-3'
TBP yellow: 5'-FAM TAC TTT TAT ATA AAT AAG TT(BHQ)G TGA TTT TTA TAT ATT TCA C -3'
TBP green: 5'-FAM- TCC CT TAT ATA AAT GGG T(BHQ) T GGA TTT TTA TAT ATT TCC A -3'
TBP blue: 5'-FAM- TAC CTT TAT ATA AAT AGG TT(BHQ) G TGA TTT TTA TAT ATT TCA C -3'
TBP black: 5'-FAM- TGC CTT TAT ATA AAT AGG CT(BHQ)G CGA TTT TTA TAT ATT TCG C -3'

Myc-Max:
5-FAM- TAA ATT AAC CAC GTG GTT TAT TTT(BHQ) ATG ATG ACC ACG TGT TCA TCA T -3'

NFkB:
5'-FAM- AGT ATG GGA CTT TCC ATA CT(BHQ)T ATT TGA GGA AAG TCC CTC AAA T 3'

TBP Competitor:
5'-CGT ATA TAA AGG TTT TTT TCC TTT ATA TAC G-3'

Beacon Stabilizer:
5'- GTG AAA TAA AAA GTA -3'

IV-SUPPORTING REFERENCES (Supporting Information)

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