Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Quantitative, solution-phase profiling of multiple transcription factors in parallel

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Fig. S1. Binding buffer pH Analysis. Both specific and nonspecific binding were quantified at different pH values. pH 6.5 was selected, because it was closest to physiological pH while maintaining reasonable total specific signal and acceptable specific/non-specific ratio. Y-axis is cpm by scintillation counting of radiolabeled DNA probes recovered from the beads following binding to their specific (blue) or nonspecific (red) protein target, separation, and elution.

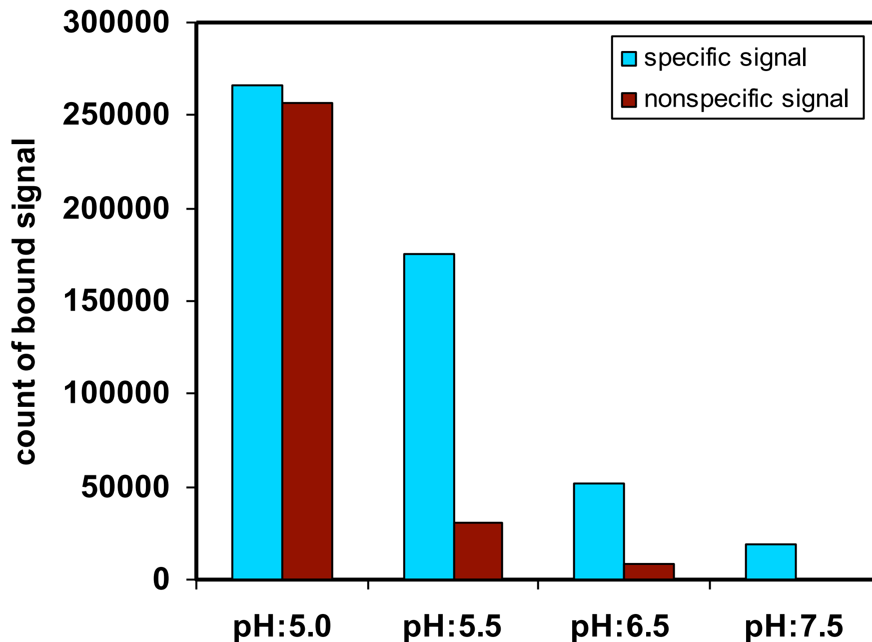


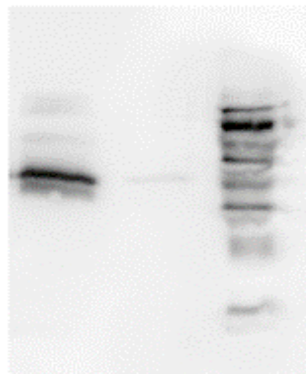
Fig. S2. The amount of protein immobilized on the beads was analyzed using a Bradford assay (to detect all proteins) and Western blot (with biotin antibody to detect biotinylated proteins). The Bradford results confirm that more than 83% of proteins from the nuclear extracts were retained by the beads during immobilization. This suggests that all or nearly all of the proteins in the original extract were biotinylated. This was further supported by the Western blots showing that essentially all of the biotinylated protein in the sample was retained on the beads during the immobilization process.

Bradford results

	Before immobilization	After immobilization	percent immobilized
TNFa	0.494	0.013	97.3
Ctrl	0.467	0.053	88.5
IKK inh.	0.514	0.083	83.8

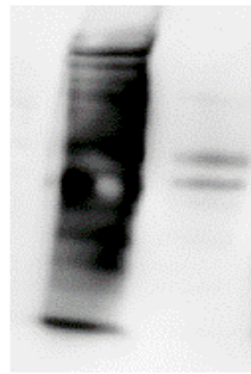
Western results

Pure Protein Immobilization



Before ⟶ Immobilization
After ⟶ Immobilization
Biotin Marker ⟶

Nuclear Extract Immobilization



Before ⟶ Immobilization
After ⟶ Immobilization

Fig. S3. Binding analysis of biotinylated protein. The binding of the DNA probe to biotin labeled NF-kB and un-labeled NF-kB was analyzed by EMSA. As shown in the gel, the binding properties were not altered by biotinylation.

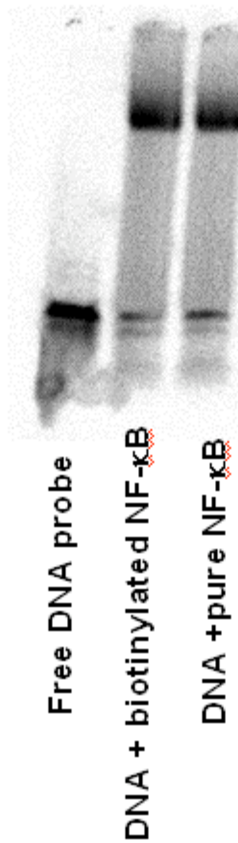


Table S1. An example of the cpm measured for each step of pure protein detection. In this case, 3 nM NF-kB was used.

Before	After	Wash1	Wash2	Wash3	Elution
$1.68x10^5$	$1.33x10^5$	$1.43x10^3$	554	250	$3.24x10^4$

Table S2. Sequences of DNA probes and PCR primers. TF binding consensus sequences are in bold. Complementary sequences of primers to DNA probes are underlined.

Transcription Factor	Probe name	Probe sequence
NF-kB	NF-kB oligo	5'-AGTTGAG**GGGACTTTCC**CAGGA-3'
	NF-kB complement	5'-TCCTG**GGAAAGTCCC**CTCAACT-3'
Ap1	Ap1 oligo	5'-CGCTTGA**TGAGTCA**GCCGGA-3'
	Ap1 complement	5'-TCCGGC**TGACTCA**TCAAGCG-3'
TBP	TBP oligo	5'-CGCCTACCTCATT**TTATA**TGCTCTGC-3'
	TBP complement	5'-GCAGAGCA**TATAA**AATGAGGTAGGCG-3'
Negative control	NC oligo	5'-TATTTAGGAGGAGTTCACCACATAG-3'
	NC complement	5'-CTATGTGGTGAACTCCTCCTAAATA-3'
NF-kB primer	NF-kB forward primer	5'-GTTTCTTCGACTTCGCGGCCTCCTGGGAAAG -3'
	NF-kB reverse primer	5'-GTTTCTTCCTGCGGCGACCGAGTTGAGGGGA -3'
Ap1 primer	Ap1 forward primer	5'- GCTGCCTGCCCGCTTGATGA-3'
	Ap1 reverse primer	5'- CTGCACGTCGTCCGGCTGAC-3'

Analysis of Transcription Factor:DNA binding

For single site DNA (D) binding to a transcription factor (T), the binding reaction can be written as;

D + T → DT

where:

$$K_D = \frac{[D]*[T]}{[DT]}$$

[D], [T] and [DT] represent the concentration of DNA, transcription factor and DNA:transcription factor complex at equilibrium, respectively.
Using $[D]_0$ and $[T]_0$ for the initial concentrations of DNA and transcription factor, respectively, the equation becomes:

$$K_D = \frac{([D]_0 - [DT]) * ([T]_0 - [DT])}{[DT]}$$

At $[D]_0 >> [T]_0$, the equation can be simplified to:

$$K_D = \frac{[D]_0 * ([T]_0 - [DT])}{[DT]}$$

Then:

$$\frac{[DT]}{[T]_0} = f = \frac{[D]_0}{K_D + [D]_0}$$

If $[D]_0 >> K_D$, then $[DT] = [T]_0$, meaning that all the transcription factor in the sample is bound to DNA. This condition gives maximal senstitivity. However, separation of complexes from the free DNA needs to be perfect, given the huge excess of free DNA relative to TF.

On the other hand, if protein is in excess ($[T]_0 >> [D]_0$), the equation can be simplified to:

$$K_D = \frac{([D]_0 - [DT]) * [T]_0}{[DT]}$$

Then:

$$\frac{[DT]}{[D]_0} = f = \frac{[T]_0}{K_D + [T]_0}$$

While limited in sensitivity by the relative magnitude of $[T]_0$ and K_D, this case allows more straightforward separation as the labeled molecule, the DNA, is limiting.