Supplementary Information

Methods

Expression and purification of SLAC-3ZF

A 1030 bp DNA fragment containing the gene encoding SLAC was generated by PCR from the template pSLAC (kind gift from G. Canters (Leiden University, The Netherlands) using the following primers: (forward) 5'-AGATATACATA*T*GGACAGGCGAGGCTTTAACCGACGC-3' and (reverse) 5'-ATGACTAAGCTTGTGCTCGTGTTCGTGTGCGGCCGC-3' (Integrated DNA Technologies). The forward primer introduced an NdeI site (underlined) upstream of the start codon (italics) and the reverse primer introduced a HindIII (underlined) site. The NdeI/HindIII-digested PCR fragment was ligated into a NdeI/HindIII-digested pSLAC vector, generating pSLAC-His that has a HindIII and an XhoI site between SLAC and a C-terminal His_6-tag (all restriction enzymes were purchased from New England Biolabs, MA). The gene encoding a 17 amino acid linker segment, followed by 3 Zinc-finger domains (Zif268, Uniprot ID P08046) was generated by PCR assembly using primers that introduced a HindIII site at the 5' end and XhoI site at the 3' end. The used primers are listed in Table S1. The HindIII/XhoI-digested gene was inserted into HindIII/XhoI-digested pSLAC-His, generating pSLAC-3ZF. DNA sequencing (Genewiz) confirmed the sequence of the cloned genes. The plasmids pSLAC-His and pSLAC-3ZF were transformed into *E.coli* BL21(DE3) (Invitrogen, CA) for SLAC-His and SLAC-3ZF-His protein expression, respectively.

For protein expression cells were grown in Terrific Broth media at 30°C until OD_{600} ~ 1.5. The temperature was then reduced to 25°C and expression was induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (Sigma). After 20 h of expression, the cells were collected by centrifugation. Cell pellets corresponding to 0.5 L cultures were resuspended in 50 mL Binding Buffer (50 mM Tris, 20 mM Imidazole, 500 mM NaCl at pH 8.0) supplemented with 0.1 mM phenylmethylsulphonyl fluoride and EDTA-free protease inhibitor cocktail tablet (Roche, IN), and subsequently sonicated using a microtip probe to lyse the cells. The insoluble material was removed by centrifugation at 24 000 x g for 30 min at +4°C. The resulting soluble fraction was supplemented with 1 mM $CuSO_4$ and 1 mM $ZnSO_4$ and incubated in a rotator at +4°C.

SLAC-3ZF-His and SLAC-His were purified from the soluble fraction by immobilized metal ion affinity chromatography using a 5 mL HisTrap FF crude column (GE Healthcare) that had been equilibrated with Binding Buffer. Bound proteins were eluted with Binding Buffer containing 260 mM imidazole. Fractions containing SLAC-3ZF or SLAC-His, blue in color, were pooled, imidazole concentration was lowered to ~0.01 mM and the protein was concentrated by ultrafiltration (Amicon, Millipore, MA). The protein preparations were about 70% pure based on SDS-PAGE (Figure S1). SLAC protein concentrations were determined by absorbance at 590 nm using the extinction coefficient 4400 M^{-1} cm^{-1}.[1]

Characterization of catalytic activities

Enzyme kinetics against 0 – 35 mM 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Sigma, MO) were studied at 24.5 nM protein concentration in air-saturated 50 mM sodium acetate buffer pH 5.0. Oxidation of ABTS was followed at 420 nm, ε = 36 000 M^{-1} cm^{-1}. 2, 6-Dimethoxyphenol (DMP) activities were assayed identically but in 10 mM sodium phosphate buffer at pH 7.3 and DMP oxidation was monitored at 468 nm, ε = 14 800 M^{-1} cm^{-1}.

DNA binding using surface plasmon resonance

Protein binding to DNA was analyzed using surface plasmon resonance using a DNA coated gold chip. Prior to surface modification, gold chips (50 nm thick gold with a 2 nm thick chromium underlayer purchased from Biosensing Instrument, Tempe, AZ) were cleaned with a 3:1 piranha solution, sulfuric acid: hydrogen peroxide (from chemical sources with analytical purity that were used as received), for 5 minutes and then washed thoroughly with water treated with a Millipore Milli-Q water purification system. After cleaning, physical adsorption was used to coat the gold chips with DNA. The 47 base DNA oligos (sequence shown below) purchased from Integrated DNA Technologies (IA) contain two Zif-268 zinc finger binding sites (underlined).

Sense
5'- TAC CAT GGA GCG TGG GCG TAT GAA TTC TAG CGT GGG CGT AAG CTT AT-3'

Antisense
5'- ATA AGC TTA CGC CCA CGC TAG AAT TCA TAC GCC CAC GCT CCA TGG TA -3

Sense and antisense strands were annealed at 95° C for 10 minutes and then allowed to cool to room temperature. The gold chips were soaked in 20 µM annealed DNA overnight. The gold chip was then washed with DI water followed by ethanol, DI water, and then dried with nitrogen gas.

The DNA coated gold chips were confirmed to be fully coated with DNA using SPR. Cysteine, a small thiol containing amino acid known to bind gold through gold-thiolate bonds, was used to detect uncoated gold surfaces.[2] Injection of 1 mg/ml cysteine (Sigma-Aldrich, St. Louis) resulted in an immediate and significant increase in SPR signal for clean gold chips which remained high for uncoated gold chips and returned to initial levels for DNA coated gold chips indicating that physical DNA adsorption successfully coated the gold chip in DNA.

The SPR measurements were conducted on a BI-SPR 2000 Instrument (Biosensing Instrument Inc. Tempe, Arizona) equipped with a dual-channel flow cell and two through-the-handle six-port injection valves. SPR conditions were the following: flow rate was 20 µl/min, room temperature, and 80 µl injection volume. Flow buffer was 0.05 M Tris-hydrochloride (EMD Chemicals, Taiwan), 0.5 M Sodium chloride (Mallinckrock, Inc., St. Louis, MO), 0.05% IGEPAL CA-630 (Sigma, St. Louis, MO) at pH6. SLAC-his and SLAC-3ZF-His at 20 µM, diluted in

flow buffer, were injected into the SPR. Figure S2 illustrates the SLAC-3ZF-His conjugate binding to a DNA coated gold surface.

DNA binding using magnetic bead capture assay

Protein binding to DNA was analyzed by a bead capture assay using streptavidin coated magnetic beads (NEB). Washed beads were resuspended in Assay Buffer (10 mM Tris-HCl, 500 mM NaCl, 0.05% (w/v) IGEPAL (Sigma), 1 mM dithiothreitol, 0.1mM $ZnSO_4$ at pH 8) and reacted with biotinylated double-stranded DNA (dsDNA) having the nucleotide sequence Biotin-5'-TAT GGA TCC TAC CAT GGA GCG TGG GCG TAA GCT TAT-3' (zinc finger binding sequence underlined) for at least 1 h at +4°C followed by washing with Assay Buffer. Control beads were reacted with biotinylated decoy DNA with the sequence Biotin-5'-TAT GGA TCC TAC CAT GGA CCT ATG TGC TAA GCT TAT-3' (no zinc finger binding site).[3]

SLAC-3ZF-His or SLAC-His protein was added in Assay Buffer and incubated with the dsDNA decorated magnetic beads for 1 h after which the beads were excessively washed. For determining the bound enzyme activity the beads were resuspended in 20 mM ABTS solution in 50 mM Na-acetate buffer at pH 5.0 and the absorbance read at 420 nm using a SpectraMax M2 (Molecular Devices, Sunnyvale, CA) spectrophotometer. The amount of bound protein was determined from the enzymatic activity of samples and from protein standards. The supplier of the magnetic beads reports that the DNA binding capacity of the beads is 500 pmol per mg of beads and this value was used to calculate the fraction of bound protein per available DNA binding site.

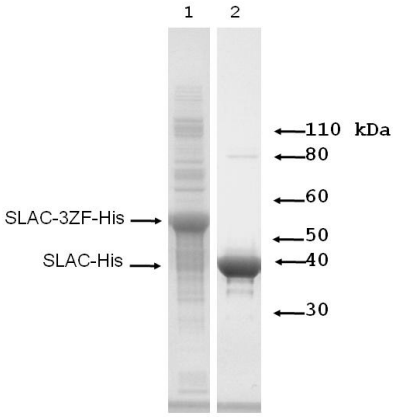

Figure S1. SDS-PAGE of purified SLAC-3ZF-His (1) and SLAC-His (2) protein. Molecular weight standards are indicated.

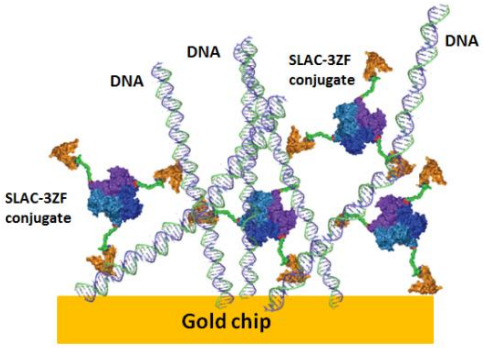

Figure S2. Illustration of nanostructure DNA-SLAC-3ZF complex formation on gold sensor surface in SPR.

Table S1. Oligonucleotide primers used for PCR assembly of linker segment and Zif269 zinc fingers.

Oligonucleotide name	DNA sequence
L-3ZF Fwd amplification	TTCGTTAAGCTTGGCGGTTCAGG
L-3ZF Rev amplification	AACGAACTCGAGGTCCTTCTGCCGC
L-3ZF 1	GGCGGTTCAGGCGGTGGGGGTTCTGGCGGGGGTGGGTAC
L-3ZF 2	CCCGGGGAACGCCCTTACGCTTGCCCAGTGGAGTCCTGT
L-3ZF 3	GATCGCCGCTTCTCCCGCTCCGACGAGCTCACCCGCCAC
L-3ZF 4	ATCCGCATCCACACAGGCCAGAAGCCCTTCCAGTGCCGC
L-3ZF 5	ATCTGCATGCGCAACTTCAGCCGCAGCGACCACCTCACC
L-3ZF 6	ACCCACATCCGCACCCACACAGGCGAAAAGCCCTTTGCC
L-3ZF 7	TGCGACATCTGTGGAAGAAAGTTTGCCAGGAGCGATGAA
L-3ZF 8	CGCAAGAGGCATACCAAGATCCACTTGCGGCAGAAGGAC
L-3ZF rev 1	GTCCTTCTGCCGCAAGTGGA
L-3ZF rev 2	TCTTGGTATGCCTCTTGCGTTCATCGCTCCTGGCAAACT
L-3ZF rev 3	TTCTTCCACAGATGTCGCAGGCAAAGGGCTTTTCGCCTG
L-3ZF rev 4	TGTGGGTGCGGATGTGGGTGGTGAGGTGGTCGCTGCGGC
L-3ZF rev 5	TGAAGTTGCGCATGCAGATGCGGCACTGGAAGGGCTTCT
L-3ZF rev 6	GGCCTGTGTGGATGCGGATGTGGCGGGTGAGCTCGTCGG
L-3ZF rev 7	AGCGGGAGAAGCGGCGATCACAGGACTCCACTGGGCAAG
L-3ZF rev 8	CGTAAGGGCGTTCCCCGGGGTACCCACCCCCGCCAGAAC
L-3ZF rev 9	CCCCACCGCCTGAACCGCC

References

(1) M. C. Machczynski, E. Vijgenboom, B. Samyn, G. W. Canters, *Protein Science*, 2004, **13**, 2388-2397.
(2) T. Baas, L. Gamble, K. D. Hauch, D. G. Castner, T. Sasaki, *Langmuir*, 2002, **18**, 4898-4902.
(3) C. I. Stains, J. R. Porter, A. T. Ooi, D. J. Segal, I. Ghosh, *JACS*, 2005, **127**, 10782-10783.