Supporting Information

1. Cloning and expression of polypeptide scaffold

(a) Sequence optimization

	def	*gabcdef*	*gabcdef*	*gabcdef*	*gabcdef*	*gabcdef*	*gabcd*
ZE	LEI	EAAALEQ	ENTALET	EVAELEQ	EVQRLEN	IVSQYRT	RYGPL
ZR	LEI	RAAALRR	RNTALRT	RVAELRQ	RVQRLRN	EVSQYET	RYGPL

Figure 1: Sequence modifications of the heterodimeric leucine zipper

Compared to the leucine zipper sequences reported by Vinson et al., some modifications (shown in red) were made to enhance the application described here. Phenylalanine residues in the first heptads of both coils were replaced by alanine. In order to provide an additional salt bridge, glutamic acid in the fifth heptad of ZE was changed to arginine and isoleucine in ZR was changed to glutamic acid.

(b) Vector construction

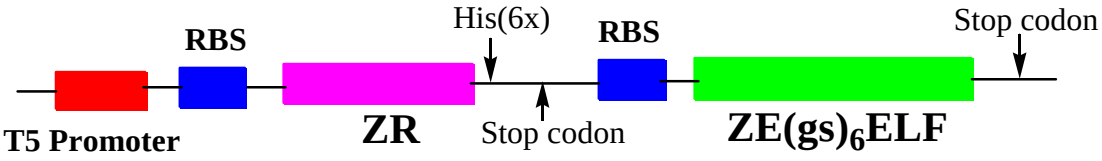

Figure 2: Dicistronic expression construct for the polypeptide scaffold

The genes for ZE and ZR were constructed by assembling synthetic oligonucleotides through PCR. A *BamH*I fragment encoding ZE with a C-terminal flexible linker $(Gly\text{-}Ser)_6$ was ligated in frame with a synthetic gene encoding ELF in a pQE60 (Qiagen) plasmid developed in this laboratory. To avoid *in vivo* degradation at 37°C and enhance the expression yield, a dicistronic construct was used to express $ZE(gs)_6ELF$. First, a *Nhe*I fragment encoding mutant *E. coli* PheRS (A294G) was inserted into expression plasmid pQE60 to yield pQE-FS*. Then the ZR gene was ligated into the *Bam*HI site of pQE-FS* to yield ZR-pQE-FS*. Finally, a DNA fragment encoding $ZE(gs)_6ELF$ and containing a ribosome binding site was amplified and ligated into the *Bpu*1102I site of ZR-pQE-FS*. The resulting plasmid (ZRhis-$ZE(gs)_6ELF$) was transformed into phenylalanine auxotroph *E. coli* strain AF-IQ.

(c) Protein expression and purification

Cultures were grown at 37°C in M9 minimal medium supplemented with glucose (0.4 wt %), thiamine (5 mg L^{-1}), $MgSO_4$ (1 mM), $CaCl_2$ (0.1 mM), 20 amino acids (15 mg L^{-1} Phe, 40 mg L^{-1} other amino acids), and antibiotics (200 µg/ml ampicillin and 35 µg/ml chloramphenicol). At an optical density of 1.0 at 600 nm (OD_{600}), the culture was supplemented with *para*-azidophenylalanine (0.3 g L^{-1}). After incubation for 15 minutes, IPTG (2 mM) was added to induce protein expression at 37°C for 5 hours. The cell pellet was lysed in 8 M urea solution, followed by freeze-thawing and sonication. The cell lysate was passed through a Ni-NTA column and washed with 8 M urea solution (pH=6.3). $ZE(gs)_6ELF$ was eluted with 6 M GuHCl solution (pH=7.0) and collected. The collected protein sample was subjected to dialysis against ddH_2O and freeze drying.

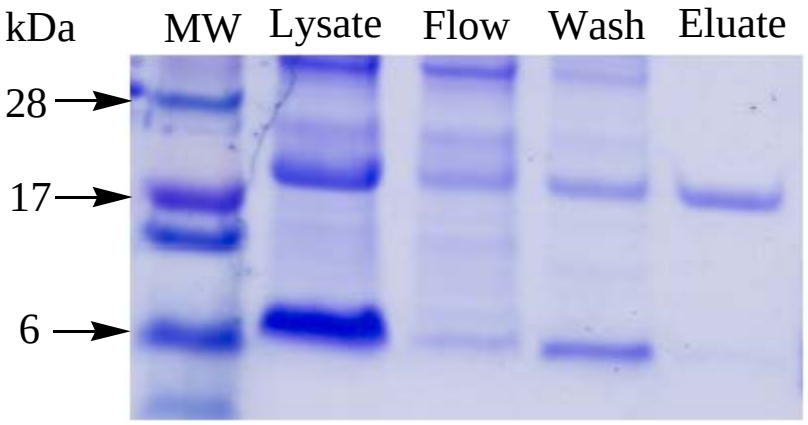

Figure 3: Purification of $ZE(gs)_6ELF$ by Ni-NTA affinity chromatography

2. Cloning and expression of fusion proteins

A GFP gene fragment was PCR amplified from pGFPuv (Clontech) and cloned into the *Pst*I site of pQE9 (Qiagen) to yield pQE9-GFP. The ZR gene assembled from synthetic oligonucleotides was digested by *Hind*III and ligated into pQE9-GFP to yield the expression vector pQE9-GFPZR. To construct the GST-ZR fusion protein, the ZR fragment was inserted into the *Bam*HI site of expression vector pGEX-2TK (Amersham Pharmacia Biotech). The resulting expression plasmids were transformed into *E. coli* strain BL-21 and protein expression was performed in 2XYT rich medium induced by 1 mM IPTG. GFP and GFP-ZR, both of which carry N-terminal hexahistidine tags, were purified on Ni-NTA spin columns, while GST and GST-ZR were purified on glutathione columns.

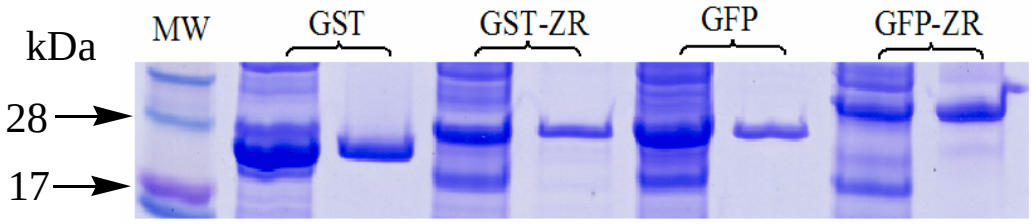

Figure 4: Expression and purification of model proteins

3. Protein array procedure

a) Substrate preparation

Standard glass slides (Corning) were immersed into concentrated H_2SO_4 for 1 hour. After washing thoroughly with water, they were immersed into a boiling solution of 1/1/5 (v/v) H_2O_2 (30%) /NH_4OH (30%)/H_2O for 30 minutes. Then the slides were gently shaken in 1% octyltrichlorosilane in toluene for 30 minutes. Finally they were cleaned twice in methanol and twice in DI water. The functionalized slides were cured at 110^oC for 30 minutes.

b) Polypeptide film preparation

A solution of $ZE(gs)_6ELF$ (80 µl, 0.8 mg/ml) in 50% trifluoroethanol was applied to a glass slide and spun at 1,500 RPM for 45 seconds. The resulting film was irradiated in a photochemical reactor equipped with 254 nm wavelength UV lamps for 5 minutes. After thorough washing with 80% DMSO and ddH_2O, the slides were dried for microarray experiments. They were blocked with 1% casein for 1 hour and then stored at 4^oC for later use.

c) Protein spotting and detection

GFP and GST fusion or control proteins diluted in printing buffer (0.5% casein, 0.5% polyvinylpyrrolidone, 15% sorbitol, 0.05% sarkosyl, 1X PBS) to a final concentration of 5 µM were spotted on slides with a custom-built microarrayer equipped with a MicroQuill® 2000 Array Pin (Majer Precision). After incubation for 1 hour in a humid chamber (saturated K_2SO_4), the slides were washed twice, each time for 15 minutes with PBS-Tween buffer (PBS plus 0.5% Tween-20). Finally, the immobilized proteins were probed with a mixture of cy3-antiGST and alexa647-antiGFP (4 µg/ml each) for 2 hours. After thorough washing with PBS-Tween buffer, fluorescence scans were acquired on a Genepix 4200A microarray scanner .

For cell lysate experiments, cell pellets from 100 ml 2XYT expression cultures were lysed with 5 ml 8 M urea solution and centrifuged to obtain clear supernatants. Printing solutions were prepared by diluting the supernatants 20-fold into printing buffer. The protocol for microarray preparation was the same as that described above.