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Supporting Information

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Supporting Information

for

Prospecting the Proteome: Identification of Naturally Occurring Binding Motifs for Biarsenical Probes

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Figure S1. Binding affinity of peptides to FIAsH. A) Fluorescence increases upon peptide binding to FIAsH-EDT₂ (1 μM) normalized as fluorescence change with $F_{\min}=0$ and $F_{\max}=1$. B) Fluorescence increases upon peptide binding to FIAsH-EDT₂ (1 μM) normalized to FIAsH-EDT₂ fluorescence. Binding affinities of PG1_peptide (○, - - -), S_peptide (●, —), PG2_peptide (□, - - -), and E_peptide (■, —) in PBS buffer with 200 μM EDT. The solid lines are fits to Eqs. (4)–(7) (see below), and fitting results are listed in Table S1.

Figure S2. FIAsh is less solvent-exposed in the S_ in comparison to other peptide complexes. Stern-Volmer plots for acrylamide quenching of FIAsh-PG1_peptide (?), FIAsh-S_peptide (!) and FIAsh-E_peptide (?). The solid lines are fits to *eqn.* (8) (see below), and the resulting fitting parameters are listed in Table S1. The tighter-binding PG_1 peptide was chosen to represent both PG_peptides, as the difference between S_peptide and E_peptide was considered.

Figure S3. Labeling of S_{peptide}-tagged protein in *E. coli* lysate. *E. coli* TOP10 Cells expressing RNA polymerase with the PG1_{peptide} and S_{peptide} tags on the C-terminus of *S. oneidensis* RNA polymerase were grown in LB media at 37 °C to O.D. 0.6. Arabinose was then added to a 1 mM final concentration and cells were allowed to grow for another 16 h. Cells (1 mL) were collected by centrifugation and resuspended in 40 µL PBS buffer (20 mM, PH 7.6) with 1 mM TCEP, 500 µM EDT and 20 µL 4x LDS loading buffer (Invitrogen) with 5% beta-mercaptoethanol, followed by boiling at 100°C for 10 minutes to break the cells. Then FIAsH was added to the cell lysate to 1 µM final concentration and incubated at room temperature for 2 h before gel electrophoresis.

Table S1: Properties of the FIAsh-peptide complexes.

Peptide	Extinction Coefficient (ϵ [M ⁻¹ cm ⁻¹]) ^a	Fluorescence Maximum (λ_{max} [nm]) ^a	Fluorescence enhancement over FIAsh-EDT ₂ ^a	Quantum Yield ^e	K_{app} [μ M] ^b	$\langle t \rangle$ [ns] ^c	Solvent accessibility ^d	
							K_{SV} [M ⁻¹]	$k_q \times 10^{-8}$ [M ⁻¹ s ⁻¹]
PG1_peptide	40400	527	170	0.68	2.1 $\pm$ 0.5	5.0 $\pm$ 0.1	0.41 $\pm$ 0.02	0.82 $\pm$ 0.04
PG2_peptide	36000	529	140	0.63	3.9 $\pm$ 0.8	5.4 $\pm$ 0.1	ND	ND
E_peptide	44900	527	190	0.69	3.3 $\pm$ 0.5	5.1 $\pm$ 0.1	1.1 $\pm$ 0.1	2.1 $\pm$ 0.2
S_peptide	57900	523	280	0.78	1.9 $\pm$ 0.3	5.0 $\pm$ 0.1	0.26 $\pm$ 0.01	0.52 $\pm$ 0.02

a) Measured after in vitro labeling as described in Materials and Methods, b) The apparent binding constant was derived through titration (Supplementary Fig. 2) and fitting to a modified Hill equation as described in Materials and Methods and Supplementary Materials and Methods, c) Fluorescence lifetime measurements were made as described in Supplementary Materials and Methods, d) The bimolecular quenching (k_q) and Stern-Volmer (K_{SV}) constants as derived from eqn. 8 in Supplementary Materials and Methods from the acrylamide quenching data shown in Suppl. Fig. 2. e) Calculated relative to the average quantum yield for PG2_peptide measured by Martin et al. ⁴

METHODS

Materials. 4',5'-Bis(1,3,2-dithioarsolan-2-yl)fluorescein (FIAsh-EDT₂) was synthesized as previously described.^[1] All peptides employed in our current study were obtained at >90% purity from CPC Scientific (San Jose, CA). All reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

Cell growth. An *E. coli* K-12 SlyD gene deletion variant was a gift from Hirotada Mori and Andrei Osterman.⁵ The cultures of *S. oneidensis* MR-1 and *E. coli* K-12 strains were grown in LB media at 30 and 37 °C, respectively. All media were supplemented with 50 µg/mL kanamycin except for the wild-type *E. coli* K-12.

Affinity purification. Cell lysates were incubated with FIAsh-derivatized beads at 4 °C overnight. Subsequently, beads were washed sequentially with 1) 50 mM phosphate (pH 7.5) and 150 mM sodium chloride buffer (PBS buffer) with 5 mM 2-mercaptoethanol (BME) and 5 mM TCEP, 2) 100 mM ammonium bicarbonate (pH 7.5), 5 mM 2-mercaptoethanol and 10 mM TCEP, and 3) 100 mM ammonium bicarbonate (pH 7.5) and 5 mM 2-mercaptoethanol. Proteins bound to FIAsh resin were then eluted with an elution buffer containing 100 mM ammonium bicarbonate (pH 7.5), 5 mM 2-mercaptoethanol, and 50 mM dithiothreitol after incubation with the same buffer for 1.5 h.

FIAsh labeling. In vivo FIAsh labeling involved addition of 1 µM FIAsh-EDT₂, 100 µM EDT, and 100 µg/mL protamine into a 1-mL cellular suspension at mid-exponential growth phase for 2 h. The labeled bacteria were then washed three times with 1 mM 2-mercaptoethanol in PBS buffer, prior to SDS-PAGE. For in vitro FIAsh labeling, all tetracysteine-containing peptides or proteins were pretreated with 5 mM TCEP in PBS buffer for 30 min at room temperature to allow complete reduction of disulfide bonds before addition of FIAsh-EDT₂. In all cases, the final labeling concentration of FIAsh-EDT₂ was 1 µM. All samples were incubated overnight at 4°C before fluorescence measurements or gel electrophoresis.

SDS-PAGE. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (PAGE) of FIAsh-labeled samples was performed using 4-12% Bis-Tris gels (Invitrogen, Carlsbad, CA). The FIAsh-protein complexes were visualized on the unstained gel with a Kodak Imaging Station 2000MM (Kodak, Rochester, NY), containing appropriate filters for FIAsh detection (Ex: 465 nm; Em: 535 nm) prior to visualization of all proteins using GelCode Blue (Pierce, Rockford, IL).

Steady-state fluorescence measurements. Emission spectra of FIAsh-protein/peptide complexes were collected with a Fluoro Max-2 fluorometer (SPEX, Edison, NJ). All samples were excited at 500 nm, using excitation and emission slits of 5 nm. Binding affinity of different peptides to FIAsh (1 μM) was characterized by measuring fluorescence of FIAsh-peptide complexes as a function of peptide concentration in the presence of 200 μM EDT, where the fluorescence intensity of FIAsh-peptide complex is assumed to be proportional to its concentration. K_{app} was then determined by fitting to a modified Hill equation, as described in Supplementary Information.

Derivation of the Hill equation for fluorescence binding measurements: When EDT is in excess, the overall binding scheme can be simply described as

where

$$[FIAsh]_{tot} = [FIAsh - EDT_2] + [FIAsh - Peptide] \quad (2)$$

and

$$[Peptide]_{tot} = [Peptide] + [FIAsh - Peptide] \quad (3)$$

where FIAsh-Peptide is peptide-bound FIAsh, and accordingly, the dissociation constant K_d , and apparent dissociation constant K_{app} are defined as follows:

$$K_d = \frac{[FIAsh - EDT_2][Peptide]}{[FIAsh - Peptide][EDT]^2} \quad (4)$$

and

$$K_{app} = K_d [EDT]^2 = \frac{[FIAsh - EDT_2][Peptide]}{[FIAsh - Peptide]} \quad (5)$$

The binding affinity of different peptides to FIAsh under this condition is further determined by fitting eqns. (6)-(7):

$$\frac{F_{530}}{F_{max}} = \frac{F_{530min}}{F_{max}} + \frac{(1 - \frac{F_{530min}}{F_{max}})[FIAsh - Peptide]}{[FIAsh]_{tot}} \quad (6)$$

where:

$$[FlAsH - Peptid\ddot{a}] = \frac{\{([FlAsH]_{tot} + [Peptid\ddot{a}]_{tot} + K_{app}) - (([FlAsH]_{tot} + [Peptid\ddot{a}]_{tot} + K_{app})^2 - 4[FlAsH]_{tot}[Peptid\ddot{a}])^{\frac{1}{2}}\}}{2} \quad (7)$$

where F_{530} is measured fluorescence at the wavelength of 530 nm, F_{530min} and F_{530max} are the fluorescence minimum and maximum at 530 nm with $[FlAsH]_{tot} = 1 \mu M$, respectively.

Solvent accessibility measurements. Solvent accessibility of peptide-bound FlAsH was assessed through collisional quenching by adding variable concentration of acrylamide into $1 \mu M$ FlAsH-peptide complexes. The data were analyzed according to the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q <\tau> [Q] \quad (8)$$

F_0 and F are the fluorescence intensity in the absence and presence of a quencher, respectively, K_{SV} is the Stern-Volmer constant, k_q is the bimolecular quenching constant and corresponding to the slope of the plot of F_0/F versus the product of the average lifetime of the fluorophore in the absence of quencher ($<t>$) and the concentration of quencher ($[Q]$).

Frequency domain lifetime measurements. The fluorescence lifetime of FlAsH-peptide complexes was measured on an ISS (Urbana, IL) K2 fluorometer^{1, 2}, using Alexa488 as a reference. Excitation used the 488-nm output of a Coherent (Santa Clara, CA) Innova 400 argon ion laser; emitted light was collected after it had passed through a 515nm long pass filter. All measurements were taken at RT. The frequency lifetime data were fitted by a nonlinear least-squares analysis.³ The time-dependent fluorescence intensity decay $I(t)$ is generally described as:

$$I(t) = \sum_{i=1}^n \alpha_i e^{-t/\tau_i} \quad (9)$$

Where a_i is the pre-exponential factor, t_i is the decay lifetime, and n is the number of components required to fit the fluorescence decay. The intensity decay law is obtained from the frequency response of amplitude-modulated light and is character-

ized by the frequency-dependent values of the phase and the extent of demodulation. The values are compared with the calculated values from an assumed decay law until a minimum of squared deviation (χ^2) is reached. After the measurement of the intensity decay, the average lifetime was calculated:

$$\langle \tau \rangle = \frac{\sum_{i=1}^n \alpha_i \tau_i^2}{\sum_{i=1}^n \alpha_i \tau_i} = \sum_{i=1}^n f_i \tau_i \quad (10)$$

where f_i represents the fractional intensity associated with each pre-exponential term and $\langle \tau \rangle$ is directly related to the average time during which the fluorophore is in the excited state.

Protein cloning and expression: C-terminal amino acid sequences were appended onto the R-subunit of RNA polymerase from *Shewanella oneidensis* (SO0256) cloned into the pBAD202/D-TOPO vector (Invitrogen) containing in the following order the tetracycline tag adjacent to a V5 epitope (KGGRADPAFLYKVVINSKLEGKPIPNPL-LGL) and a His6 sequence at the C-terminus, as previously described. The forward primer

(5'-CACCCAGAGTACGGTTTACAGTTACGC) was used to amplify both coding and promoter region of *rpoA*. To add the PG_ tag on the C-terminus of RpoA, the reverse primer (5'-**CTTGCAACAACCAGGGCAACATGCAGCTAGGTCGTCTGCTAACTAGCTG**) contained the coding sequence for AACCPGCCCK (marked in bold). To add the S_peptide tag on the C-terminus of RpoA the reverse primer (5'-**ACCAC-CACAACAACCACCAGCATCATTACCAGAACCACCACAACCTAGGTCGTCTGCTAACTAGCTG**) contained the coding sequence for GCCGGSGNDAGGCCGG. The pBAD/D-TOPO expression system contains the promoter of the *araBAD* (arabinose) operon, which can be upregulated by arabinose and down-regulated by glucose to permit optimal expression. Plasmids were maintained by addition of 20 μ g/mL kanamycin. The proteins were induced in *E. coli* TOP10.

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