SUPPORTING INFORMATION

$(NTA)_1$-Cy3

N-(5-amino-1-carboxypentyl)iminodiacetic acid,[1] (Dojindo; 26 mg, 80 μmol) was dissolved in 1.6 ml 0.1 M sodium carbonate and was added to Cy3 mono-succinimidyl-ester[2] ("Cy3 Mono-Reactive Dye"; Amersham-Pharmacia Biotech; 800 nmol). Following reaction for 1 h (with vortexing at 15-min intervals) at 25°C in the dark, the product was purified from excess N-(5-amino-1-carboxypentyl)iminodiacetic acid using a Sep-Pak C18 cartridge (Millipore; pre-washed with 10 ml acetonitrile and 10 ml water; washed with 20 ml water; eluted with 1 ml 60% methanol), dried, re-dissolved in 500 μl water, and further purified by flash chromatography [silica gel, NH_4OH:ethanol:water, 55:35:10, v/v/v; TLC $r_f = 0.6$]. The product was dried, re-dissolved in 2 ml water, and quantified spectrophotometrically ($\varepsilon_{550} = 150{,}000\ M^{-1}\ cm^{-1}$). Yield: 130 nmol, 16%.

$(Ni^{2+}:NTA)_1$-Cy3 (1a)

$NiCl_2$ (Aldrich; 350 nmol in 3 μl 0.01 N HCl) was added to $(NTA)_1$-Cy3 (70 nmol in 2 ml water), and the solution was brought to pH 7 by addition of 0.8 ml 50 mM sodium acetate (pH 7), 200 mM NaCl. Following reaction for 30 min at 25°C in the dark, the product was purified using a Sep-Pak C18 cartridge (Millipore; procedure as above) and dried. ES-MS: *m/e* 928.6 (calculated 928.2). Ni^{2+} content [determined by performing analogous reaction with $^{63}NiCl_2$ (New England Nuclear) and quantifying radioactivity in product by scintillation counting in Scintiverse II (Fisher)]: 0.92 mol Ni^{2+} per mol.

$(NTA)_1$-Cy5

N-(5-amino-1-carboxypentyl)iminodiacetic acid,[1] (Dojindo; 26 mg, 80 μmol) was dissolved in 800 μl 0.1 M sodium carbonate and was added to Cy5 mono-succinimidyl-ester[2] ("Cy5 Mono-Reactive Dye"; Amersham-Pharmacia Biotech; 800 nmol). Following reaction for 1 h (with vortexing at 15-min intervals) at 25°C in the dark, the product was purified from excess N-(5-amino-1-carboxypentyl)iminodiacetic acid using a Sep-Pak C18 cartridge (Millipore; procedure as above), dried, re-dissolved in 500 μl water, and further purified

by flash chromatography (silica gel, ethanol; TLC r_f = 0.2). The product was dried, re-dissolved in 100 µl water, and quantified spectrophotometrically (ε_{650} = 250,000 M^{-1} cm^{-1}). Yield: 77 nmol, 9.6%. ES-MS: *m/e* 896.7 (calculated 896.3).

$(Ni^{2+}$:NTA$)_1$-Cy5 (1b)

$NiCl_2$ (Aldrich; 50 nmol in 0.5 µl 0.01 N HCl) was added to $(NTA)_1$-Cy5 (30 nmol in 1 ml water), and the solution was brought to pH 7 by addition of 0.5 ml 50 mM sodium acetate (pH 7), 200 mM NaCl. Following reaction for 30 min at 25°C in the dark, the product was purified using a Sep-Pak C18 cartridge (Millipore; procedure as above) and dried. ES-MS: *m/e* 955.0 (calculated 954.2).

$(NTA)_2$-Cy3

N-(5-amino-1-carboxypentyl)iminodiacetic acid,[1] (Dojindo; 26 mg, 80 µmol) was dissolved in 1.6 ml 0.1 M sodium carbonate and was added to Cy3 bis-succinimidyl-ester[2] ("Cy3 Reactive Dye"; Amersham-Pharmacia Biotech; 200 nmol). Following reaction for 1 h (with vortexing at 15-min intervals) at 25°C in the dark, products were purified from excess N-(5-amino-1-carboxypentyl)iminodiacetic acid using a Sep-Pak C18 cartridge (Millipore; procedure as above), dried, re-dissolved in 200 µl methanol, and purified by preparative TLC [silica gel, 1000 Å (Analtech); NH_4OH:ethanol:water, 55:35:10, v/v/v]. Three bands were resolved, corresponding to $(NTA)_2$-Cy3 (r_f = 0.2), $(NTA)_1$-Cy3 mono acid (r_f = 0.5), and $(NTA)_2$-Cy3 bis acid (r_f = 0.8). $(NTA)_2$-Cy3 was eluted with 60% methanol, dried, re-dissolved in 2 ml water, and quantified spectrophotometrically (ε_{550} = 150,000 M^{-1} cm^{-1}). Yield: 64 nmol, 8%. ES-MS: *m/e* 1197.0 (calculated 1197.4).

$(Ni^{2+}$:NTA$)_2$-Cy3 (2a)

$NiCl_2$ (Aldrich; 350 nmol in 3 µl 0.01 N HCl) was added to $(NTA)_2$-Cy3 (70 nmol in 2 ml water), and the solution was brought to pH 7 by addition of 0.8 ml 50 mM sodium acetate (pH 7), 200 mM NaCl. Following reaction for 30 min at 25°C in the dark, the product was purified using a Sep-Pak C18 cartridge (Millipore; procedure as above) and dried. ES-MS: *m/e* 1316.8 (calculated 1315.7). Ni^{2+} content [determined

by performing analogous reaction with $^{63}NiCl_2$ (New England Nuclear) and quantifying radioactivity in product by scintillation counting in Scintiverse II (Fisher)]: 1.4 mol Ni^{2+} per mol.

$(NTA)_2$-Cy5

N-(5-amino-1-carboxypentyl)iminodiacetic acid,[1] (Dojindo; 40 mg, 125 μmol) was dissolved in 0.8 ml 0.1 M sodium carbonate and was added to Cy5 bis-succinimidyl-ester[2] ("Cy5 Reactive Dye"; Amersham-Pharmacia Biotech; 800 nmol). Following reaction for 1 h (with vortexing at 15-min intervals) at 25°C in the dark, products were purified from excess N-(5-amino-1-carboxypentyl)iminodiacetic acid using a Sep-Pak C18 cartridge (Millipore; procedure as above), dried, re-dissolved in 200 μl methanol, and purified in 100 μl portions by preparative TLC [silica gel, 1000 Å (Analtech); NH_4OH:ethanol:water, 55:35:10, v/v/v]. Three bands were resolved, corresponding to $(NTA)_2$-Cy5 (r_f = 0.2), $(NTA)_1$-Cy5 mono acid (r_f = 0.6), and $(NTA)_2$-Cy5 bis acid (r_f = 0.8). $(NTA)_2$-Cy5 was eluted with 60% methanol, dried, re-dissolved in 2 ml water, and quantified spectrophotometrically (ε_{650} = 250,000 M^{-1} cm^{-1}). Yield: 60 nmol, 7.5%.

$(Ni^{2+}NTA)_2$-Cy5 (2b)

$NiCl_2$ (Aldrich; 90 nmol in 1 μl 0.01 N HCl) was added to $(NTA)_2$-Cy5 (30 nmol in 1 ml water), and the solution was brought to pH 7 by addition of 0.5 ml 50 mM sodium acetate (pH 7), 70 mM NaCl. Following reaction for 30 min at 25°C in the dark, the product was purified using a Sep-Pak C18 cartridge (Millipore; procedure as above) and dried. ES-MS: *m/e* 1341.0 (calculated 1341.7).

CAP-His_6

Plasmid pAKCRP-His_6 encodes CAP-His_6 under the control of bacteriophage T7 gene 10 promoter. Plasmid pAKCRP-His_6 was constructed from plasmid pAKCRP[3] by use of site-directed mutagenesis[4] to insert six His codons (CAC-CAC-CAC-CAC-CAC-CAC) after codon 209 of the *crp* gene.

To prepare CAP-His_6, a culture of *Escherichia coli* strain BL21(DE3) (Novagen) transformed with pAKCRP-His_6 was shaken at 37°C in 1 L LB[5] containing 200 mg/ml ampicillin until OD600 = 0.5, induced by addition of isopropyl-thio-β-D-galactoside to 1 mM, and shaken an additional 3 h at 37°C. The culture was

harvested by centrifugation (4,500 x g; 15 min at 4°C), the cell pellet was re-suspended in 15 ml buffer A [20 mM Tris-HCl (pH 7.9), 500 mM NaCl, 5 mM imidazole], cells were lysed by sonication, and the lysate was cleared by centrifugation (30,000 x g; 30 min at 4°C). The sample was adjusted to 15 ml with buffer A, adsorbed onto 2 ml Ni^{2+}-NTA agarose (Qiagen) in buffer A, washed with 12 ml buffer A containing 20 mM imidazole, and eluted with 6x1 ml buffer A containing 200 mM imidazole. Fractions containing CAP-His_6 were pooled, desalted twice into buffer B [40 mM Tris-HCl (pH 8), 100 mM NaCl, 1 mM dithiothreitol, 5% glycerol] by gel-filtration chromatography on NAP-10 (Amersham-Pharmacia Biotech), quantified spectrophotometrically ($\varepsilon_{278,\ protomer}$ = 20,000 M^{-1} cm^{-1}), and stored in aliquots at -80°C. Yield: ~20 mg/L culture. Purity: >99%.

CAP

CAP was prepared as in ref. 3.

DNA^F

DNA^F was prepared as in ref. 3 (where it is referred to as "$ICAP52^{F,-9}$").

Fluorescence anisotropy experiments

Reaction mixtures [200 µl, in 100 µl quartz micro-cuvettes (Starna)] contained 50 nM **1a** or **1b**, or 10 nM **2a** or **2b,** in buffer C [40 mM Tris-HCl (pH 8), 100 mM NaCl, 1 mM dithiothreitol, 0.5 mM imidazole, 0.2 mM cAMP, 100 µg/ml bovine serum albumin, and 5% glycerol]. Reaction mixtures were titrated with 0-3 µM CAP-His_6 (or CAP) by successive addition of 0.5-4 µl aliquots of 2-4 µM CAP-His_6 (or CAP) in the same buffer. Fluorescence anisotropy was determined at the start of the titration and 5 min after each successive addition in the titration. All solutions were maintained at 25°C.

Fluorescence measurements were performed using a commercial steady-state fluorescence instrument (QM-1, PTI) equipped with T-format Glan-Thompson polarizers (PTI). Excitation wavelengths were 530 nm for **1a** and **2a**, and 630 nm for **1b** and **2b**; emission wavelengths were 570 nm for **1a** and **2a**, and 670 nm for **1b** and **2b**. Slit widths were 10 nm. Fluorescence emission intensities were corrected for background by subtraction of fluorescence emission intensities for control reaction mixtures containing identical concentrations of CAP-His_6

or CAP but not containing **1a**, **1b**, **2a**, or **2b**.

Fluorescence anisotropy, A, was calculated using:[6]

$$A = (I_{VV} - GI_{VH})/(I_{VV} + 2GI_{VH})$$

where I_{VV} and I_{VH} are the fluorescence intensities with the excitation polarizer at a vertical position and the emission polarizers at vertical and horizontal positions, respectively, and G is the grating correction factor. Data were plotted as:

$$(A-A_0)/A_0$$

where A is the fluorescence anisotropy in the presence of the indicated concentration of CAP-His_6 or CAP, and A_0 is the fluorescence anisotropy in the absence of CAP-His_6 or CAP. Equilibrium dissociation constants were calculated using non-linear regression.[7]

Similar results are obtained in experiments using buffers lacking imidazole or cAMP (data not shown).

FRET experiments: standard titrations

Reaction mixtures [200 µl, in 50 µl quartz micro-cuvettes (Starna)] contained 5 nM DNA^F and 50 nM CAP-His_6 (or CAP) in buffer C. Reaction mixtures were titrated with 0-3.2 µM **2a** or **2b** by successive addition of 0.3-1.2 µl aliquots of 30-300 µM **2a** or **2b** in the same buffer. Fluorescence anisotropy was determined at the start of the titration and 5 min after each successive addition in the titration. All solutions were maintained at 25°C. Fluorescence emission intensities, F, were measured using a commercial steady-state fluorescence instrument (QM-1, PTI) equipped with L-format Glan-Thompson polarizers (PTI) set at 54.7° ("magic angle"). Excitation wavelength was 480 nm; emission-wavelength range was 500-600 nm (titrations with **2a**) or 500-700 nm (titrations with **2b**); excitation slit width was 10 nm; emission slit width was 15 nm. Fluorescence emission intensities were corrected for background (by subtraction of fluorescence emission intensities for control reaction mixtures containing identical concentrations of **2a** or **2b**, but not containing CAP-His_6 or CAP) and for dilution.

Efficiencies of FRET, E, were calculated as:

$$E = 1 - (F^{520,480}/F^{520,480}{}_o)$$

where $F^{520,480}$ is the fluorescence emission intensity of the fluorescein label at the indicated concentration of **2a** or **2b**, and $F^{520,480}{}_o$ is the fluorescence emission intensity of the fluorescein label at 0 μM **2a** or **2b**.

Data were plotted as E vs. titrant concentration, and binding curves and equilibrium dissociation constants were calculated using non-linear regression.[7]

Donor-acceptor distances, R, were calculated using:[8]

$$E = R_o^6/(R_o^6 + R^6)$$

where R_o is the Förster parameter:

$$R_o \text{ (in Å)} = 0.211 \times 10^{-5}(n^{-4} Q_D \kappa^2 J)^{1/6}$$

where n is the refractive index of the medium (1.4 for dilute protein solutions[8]), Q_D is the donor quantum yield in the absence of acceptor [0.4; measured using quinine sulfate in 0.1 N H_2SO_4 as standard ($Q_{QS} = 0.51$)[9]], κ^2 is the orientation factor relating the donor emission dipole and acceptor excitation dipole [approximated as 2/3 in this work--justified by the observed low fluorescent anisotropy of the donor[3]], and J is the spectral overlap integral of the donor emission spectrum and the acceptor excitation spectrum:

$$J = [\int F_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, d\lambda]/[\int F_D(\lambda)\, d\lambda]$$

where $F_D(\lambda)$ is the normalized corrected emission spectrum of donor, $\varepsilon_A(\lambda)$ is the molar extinction coefficient of acceptor, and λ is the wavelength.

Similar results are obtained in experiments using buffers lacking imidazole or cAMP (data not shown).

FRET experiments: stoichiometric titrations

Stoichiometric titrations were performed analogously to standard titrations (preceding section), using reaction

mixtures containing 0.6-2.6 μM DNA^F-CAP-His_6 [prepared by equilibration of DNA^F with excess CAP-His_6 for 20 min at 25°C, followed by removal of unbound CAP-His_6 by filtration through Bio-Rex 70 (Bio-Rad; methods as in ref. 3)], and titrating with 0-12 μM **2a** or **2b** by successive addition of 0.3-1.2 μl aliquots of 30-300 μM **2a** or **2b**.

Fluorescence emission intensities were corrected for dilution and background, and values of E were corrected for non-specific interactions (by subtraction of values of E for control reaction mixtures omitting CAP-His_6). Corrected values of E were plotted as E/E_{sat} vs. titrant concentration (where E_{sat} is the E at saturating titrant concentrations).

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