

Synthesis of Poly-L-proline -Dye Conjugates (7a-d). Poly-L-proline peptides of lengths n proline residues, where $n = 6,9,12,15,18$, were individually synthesized by standard *t*-Boc solid phase methods, retaining the *n*-terminal *t*-Boc protecting group. All peptides were cleaved from resin by treatment with 20 % (v/v) anhydrous hydrazine in absolute ethanol for 18 hrs at room temperature. Peptides were purified by reverse-phase HPLC, and lyophilized to provide white powders. Purified peptides (2.5 μ moles) were treated with the 5-*N*-hydrosuccinimidyl ester of Oregon Green 488 (OG, Molecular Probes) (5.0 μ moles), DMF (100 μ L), and *N,N*-diisopropylethylamine (20 μ L), and purified by reverse-phase HPLC to yield *c*-terminal dye intermediates. The *N*-terminal *t*-Boc group was removed by treatment with 20% TFA in CH_2Cl_2 and the resulting molecules were treated with the 5-*N*-hydrosuccinimidyl ester of tetramethylrhodamine (TAMRA, Molecular Probes) (5.0 μ moles), DMF (100 μ L), and *N,N*-diisopropylethylamine (20 μ L). Purification by reverse-phase HPLC afforded dye conjugates **7a-e** (typical isolated yields: 20-40%). The identity of all conjugates was verified by MALDI-TOF mass spectrometry. Characterization: **7a** (TAMRA-Pro₆-OG): MALDI-TOF [M+H] (monoisotopic mass) calcd 1422.5, obsd 1422.6; **7b** (TAMRA-Pro₉-OG) MALDI-TOF [M+H] (monoisotopic mass) calcd 1712.7, obsd 1712.5; **7c** (TAMRA-Pro₁₂-OG): MALDI-TOF: [M+H] (monoisotopic mass) calcd 2003.8, obsd 2003.9; **7d** (TAMRA-Pro₁₅-OG): MALDI-TOF: [M+H] (monoisotopic mass) calcd 2295.0, obsd 2295.2.

Fluorescence Resonance Energy Transfer Experiments. Fluorescence emission spectra were collected on an ISS-K2 fluorometer. Solutions (25 nM) of conjugates **7a-e**, Oregon Green, and TAMRA were prepared in 1:1 methanol:bicarbonate buffer (150 mM, pH 8.3). The emission spectra of these solutions were collected from 500-640 nm (excitation wavelength: 495 nm).

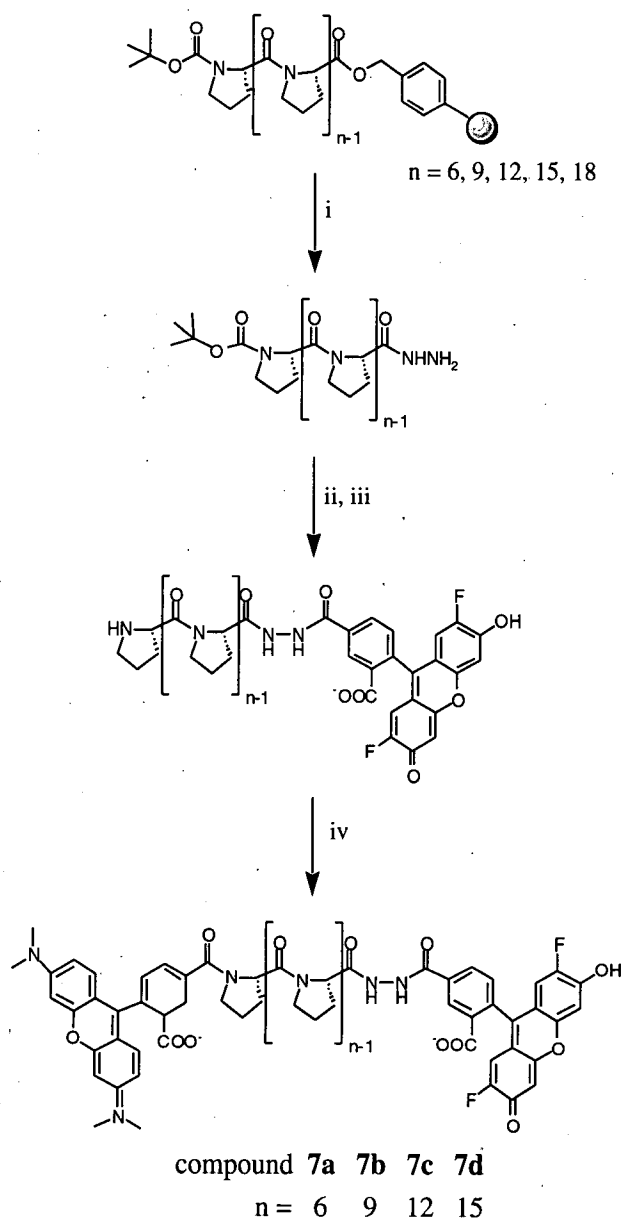


Figure S1. Synthesis of polyproline-dye conjugates: (i) 20% hydrazine in ethanol; (ii) Oregon Green-NHS ester, DMF, and DIEA; (iii) 20% TFA in CH_2Cl_2 ; (iv) TAMRA-NHS ester, DMF, and DIEA.

Figure S1

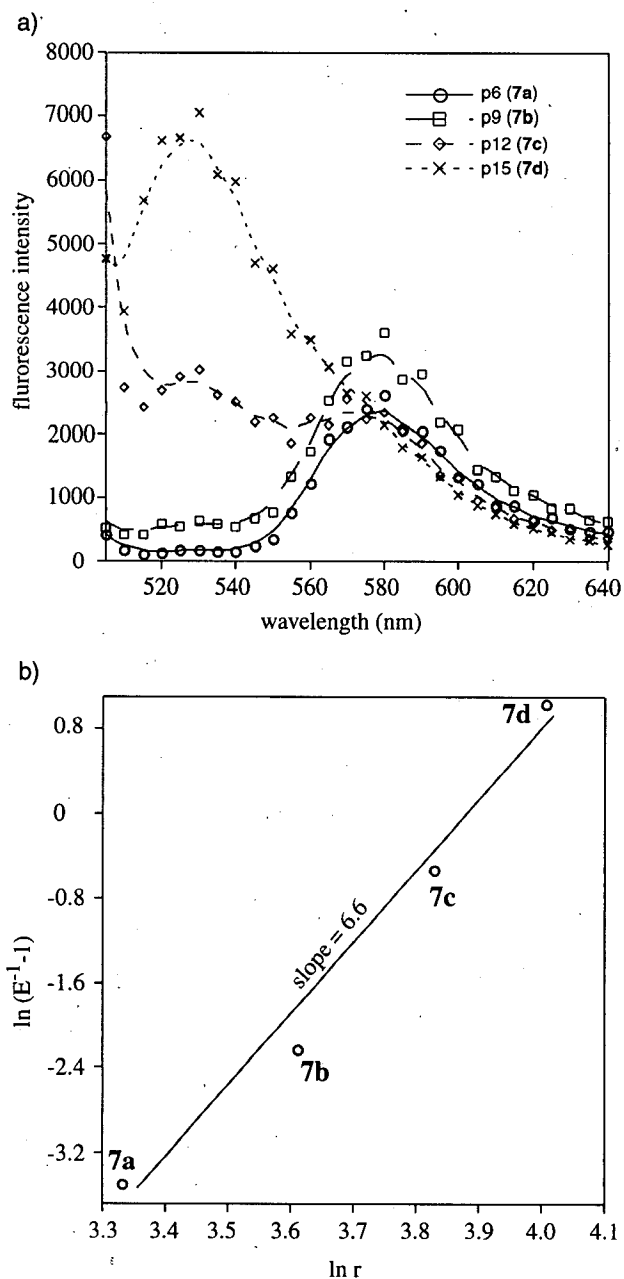


Figure S2. (a) The emission spectra of OG-Pro_n-TAMRA conjugates. The conjugates were excited at 495 nm. (b) The dependence of the efficiency of energy transfer on distance is given in this plot of $\ln(E^{-1} - 1)$ versus $\ln r$. The slope is 6.6, in good agreement with the predicted r^{-6} dependence.