Supporting Information

Noncovalent Interactions with Proteins Modify the Physicochemical Properties of a Molecular Switch

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1. Synthesis of 1

A solution of 2,3,3-trimethylindolenine (7.7 g; 48.4 mmol) and methyl iodide (7.5 mL; 120.5 mmol) in acetonitrile (90 mL) was refluxed for 18 h under a nitrogen atmosphere. The solvent was removed under reduced pressure and the residue was triturated with diethyl ether to yield a powder. The solid was collected by filtration, washed with diethyl ether, and dried under high vacuum. The resulting solid was added to a solution of KOH in water and stirred for 30 min. The product was extracted with diethyl ether and the combined organic extracts were washed with brine, dried over MgSO₄, and evaporated under reduced pressure to afford 1,3,3-trimethyl-2-methyleneindoline (80%) as a yellow oil (which gradually turned pink).

1,3,3-trimethyl-2-methyleneindoline (5.5 g; 31.7 mmol) was dissolved in ethanol (350 mL) and refluxed with 2-hydroxy-5-nitrobenzaldehyde (6.9 g; 41.3 mmol) under a nitrogen atmosphere for 15 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in CH₂Cl₂ and washed three times with an aqueous NaOH solution. The organic layer was dried over MgSO₄ and evaporated under reduced pressure. The residue was purified by crystallization from ethyl acetate to afford pure spiropyran 1 (75%).

¹H NMR (300 MHz, CDCl₃) δ 8.04–8.00 (m, 2H), 7.21 (dt, J = 7.5, 1.2 Hz, 1H), 7.09 (dd, J = 7.2, 1.2 Hz, 1H), 6.93 (d, J = 10.3 Hz, 1H), 6.89 (dt, J = 7.3, 0.9 Hz, 1H), 6.77 (d, J = 9.4 Hz, 1H), 6.56 (d, J = 7.5 Hz, 1H), 5.86 (d, J = 10.3 Hz, 1H), 2.74 (s, 3H), 1.30 (s, 3H), 1.19 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 159.8, 147.6, 140.9, 136.0, 128.2, 127.8, 125.8, 122.6, 121.6, 121.5, 119.7, 118.6, 115.4, 107.0, 106.3, 52.2, 28.8, 25.8, 19.9.
Figure S1 | $^1$H NMR spectrum of 1 (300 MHz, CDCl$_3$).

Figure S2 | $^{13}$C NMR spectrum of 1 (75 MHz, CDCl$_3$).
2. Quenching of HSA fluorescence by 1

The interaction between a fluorophore (in our case, the tryptophan residue of HSA) and a quencher (here, 1) is usually described by the Stern-Volmer equation, \( F_0/F = 1 + K_{SV} [Q] \), where \( F_0 \) and \( F \) are the fluorescence intensities in the absence (i.e., HSA only) and in the presence of the quencher, Q (i.e., 1), [Q] is the concentration of the quencher (i.e., \( c_1 \)), and \( K_{SV} \) is the Stern-Volmer quenching constant. Linear fitting of a plot of \( F_0/F \) as a function of \( c_1 \) (Fig. S3) gives \( K_{SV} \) of \( 8 \times 10^4 \) M\(^{-1}\).

Figure S3 | Changes in \( F_0/F \) as a function of the concentration of 1. The slope of the linear fitting corresponds to the Stern-Volmer quenching constant.

3. Solvatochromic properties of 1

We prepared solutions of 1 in five different solvents and exposed them to UV light in order to determine the position of the main absorption band of the open-ring isomer (merocyanine). In solvents of low dielectric constants (DCM, THF, and in particular, toluene), the absorption band featured a vibrational structure. The maxima of absorption were located at \( \lambda = 606 \) nm for toluene, 585 nm for THF, and 579 nm for DCM (Fig. S4 a, b, and c, respectively). In the more polar acetonitrile and methanol, only one well-defined band was observed. Absorption maxima were at \( \lambda = 558 \) nm for acetonitrile and 526 nm for methanol (Fig. S4 d and e, respectively).

Figure S4 | UV-Vis absorption spectra of 1 in various solvents: a) toluene (\( c = 56 \) µM), b) tetrahydrofuran (\( c = 62 \) µM), c) dichloromethane (\( c = 93 \) µM), d) acetonitrile (\( c = 87 \) µM), e) methanol (saturated solution), before (red traces) and after different times of UV irradiation. The path length was 10 mm.
4. Absorption and emission spectra of 1 complexed by other proteins

**Figure S5** | Ill-defined absorption spectra of insulin fibrils binding 1 (in blue) before and after UV irradiation. The high absorbance across the whole spectrum is due to aggregation. For comparison, the spectra of HSA·1 before and after UV are also shown (in green).

**Figure S6** | UV-Vis absorption spectra of 1 in the PBS buffer in the presence of a) insulin monomers, b) lysozyme, and c) glucose oxidase. All samples were prepared using a 1:1 molar ratio of 1 to the protein.

**Figure S7** | Fluorescence spectra of 1 in the PBS buffer solution in the presence of different proteins (equimolar with respect to 1). Colors correspond to those in Figs. S5 and S6; green: HSA; blue: insulin fibrils; olive: insulin monomers; maroon: lysozyme; navy: glucose oxidase. All spectra were recorded on samples containing the same concentration of 1 and each protein, except the insulin fibrils (the molar ratio of the protein to 1 was about 10:1). The weak emission in the glucose oxidase sample centered at ~530 nm is due to the native fluorescence of the protein and is not due to 1. In all cases, the excitation wavelength was 390 nm.