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Modification of Near-infrared Cyanine Dyes by Serum Albumin Protein

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Methods

Cyanine dyes (Sigma Aldrich, MI), human serum albumin (Wako Pure Chemical, Osaka, Japan), bovine serum albumin (A4503, Sigma) and lysine-tryptophan (4:1) random copolymer (P9285, Sigma) were used without purification. These were dissolved in distilled water except IR786, which was dissolved in HPLC grade methanol. Absorption measurements were performed with a spectrometer (MCPD2000, Otsuka Electronics Co., Osaka, Japan). Emission measurements were performed with a fluorescence spectrometer (F-7000, Hitachi, Tokyo, Japan).

The fluorescence lifetime was measured using the time-correlated single-photon-counting (TCSPC) system (TRSF20S, Hamamatsu, Hamakita, Japan) with an excitation source mode-locked pico-second Ti:sapphire laser (Tsunami, Spectra Physics, CA). The emission signal was collected by a 3 mm ϕ bundle fiber and deliver to a multi-channel photomultiplier tube (R3809U-51, Hamamatsu). The emission signal was amplified, discriminated and then led to a time-to-amplitude converter (TAC) as the start pulse. For the reference, small amount of the pulse light was sampled and illuminated an avalanche photodiode. The signal of the avalanche photodiode was used for an external trigger of pulse generator (81110A/81112A, Agilent Technologies, Boeblingen, Germany) to give the stop pulse of TAC. For selecting the emission wavelength, proper filters were used; in water solution, IR820 film filter (Fuji film, Japan); in early stage of time, an interference filter D850/40 (Chroma, VT); in the later stage of time, an interference filter HQ817/25 (Chroma, VT). All the optical measurements were performed at room temperature.

In the ultrafiltration study, a centrifugal filter device (Amicon Ultra-4 30K NMWK, Billerica, MA) was utilized. Firstly, these filters were pre-rinsed with 2 ml MilliQ water under 7500 \times g for 10 minutes (Tomy MX305, Tokyo, Japan). The 2 ml of solution was filtered under 7500 \times g for 15 minutes at 20 °C. The volume of the retentate was about 50 μ l. The blue-shifted complex solution was prepared about 2 hours before the measurement to form the complex completely. Then, 8 M guanidine chloride was mixed into the solution to make 4 M guanidine chloride mixture solution. The red-shifted complex solution was prepared just before the measurement. Then, 8 M guanidine was mixed. The retentate and the ultrafiltrate were measured by the absorption and emission spectrometers to check the existence of the blue-shifted species.

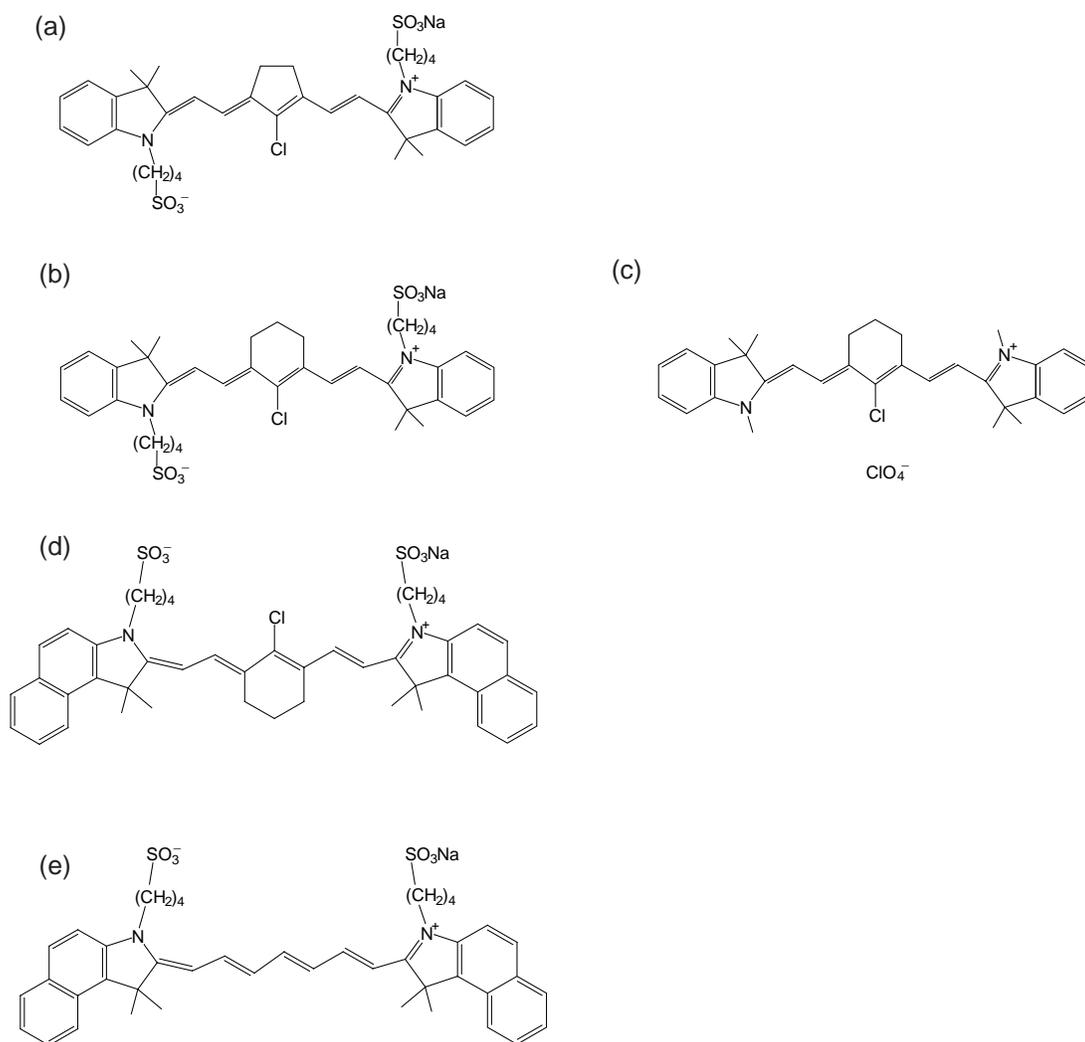


Fig.1S. Chemical structure of (a) IR806, (b) IR783, (c) IR786, (d) IR820 and (e) ICG.

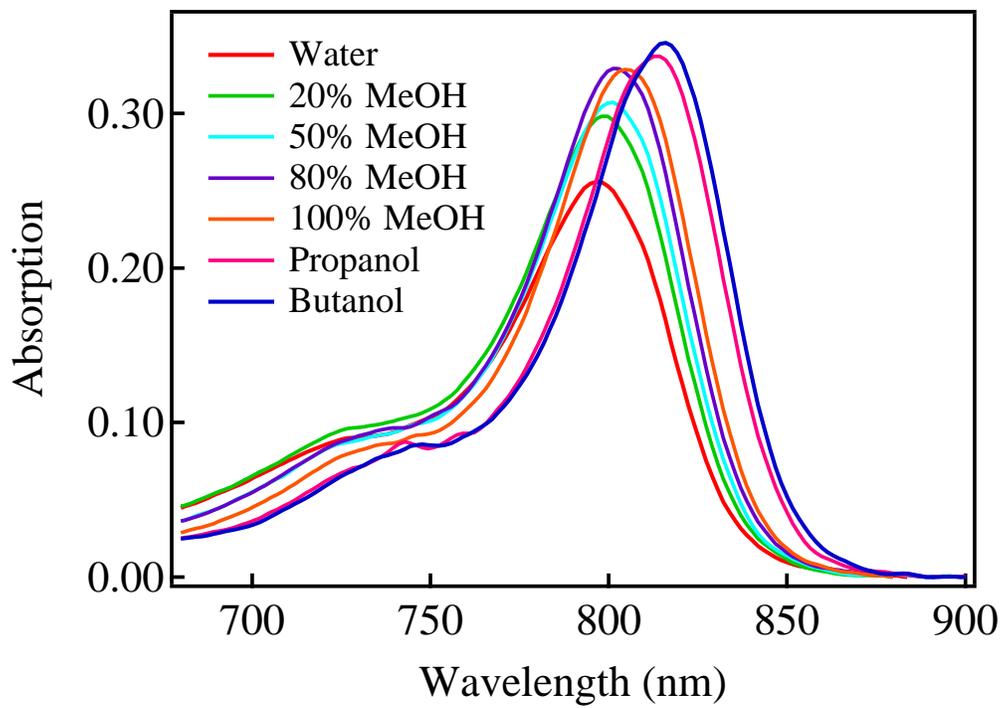


Fig.2S. Absorption spectrum of 1 μ M IR806 with respect to the solvent. The solvent corresponds to each spectrum is shown in the figure.

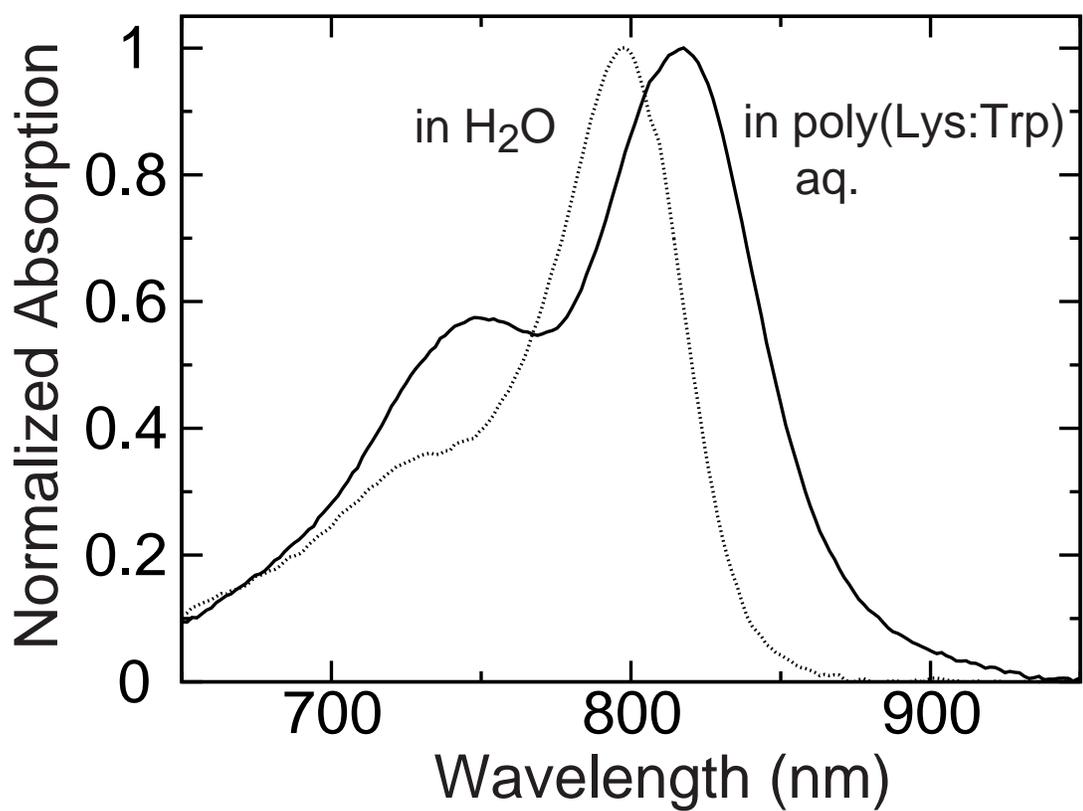


Fig.3S. Normalized absorption spectrum of 1 μM IR806 in water and in about 50 μM lysine-tryptophan random copolymer solution.

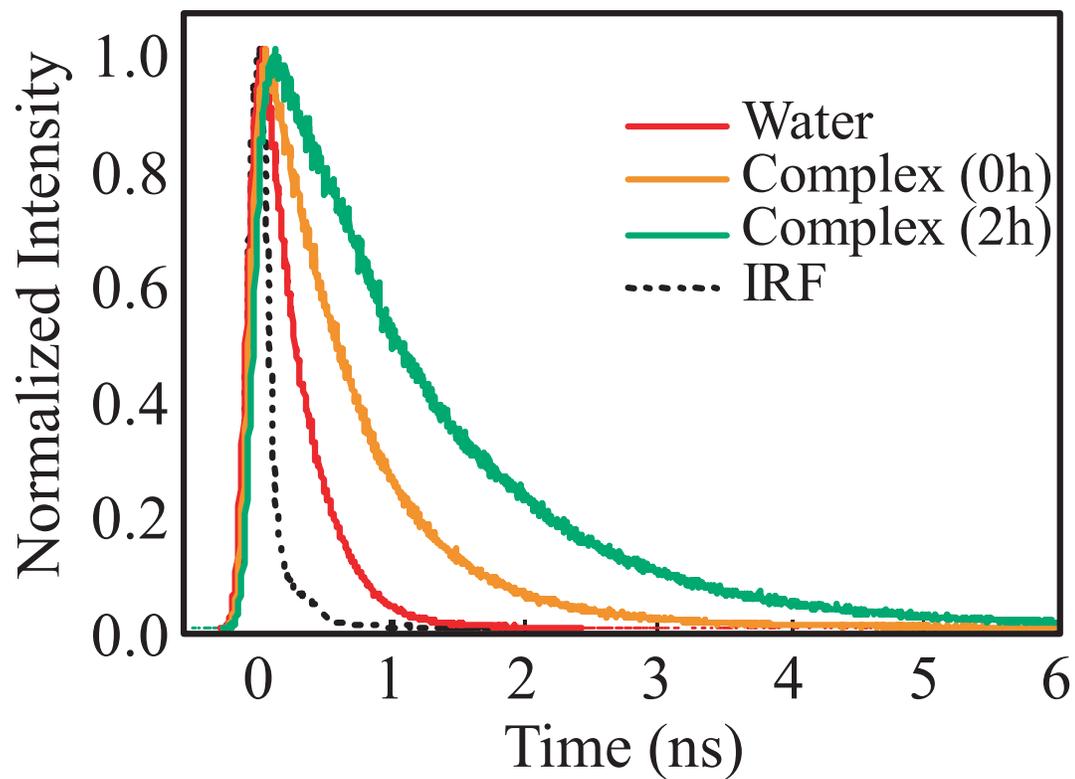


Fig.4S. Decay profiles of IR806 in water and HSA. Instrumental function is shown by dotted line.

	Water	Human serum albumin	
		early stage	later stage
Abs. (nm)	798	822	786
Em. (nm)	827	841	813
Lifetime (ns)	0.25(100%)	0.44(44%)	0.35(4%)
		0.80(56%)	1.2 (96%)

Table 1: Optical properties of IR806 in different environments