Electronic supplementary information

Fingerprint signatures of lanthanide circularly polarized luminescence from proteins covalently labeled by a β-diketonate europium(III) chelate

Junpei Yuasa*, Tomoko Ohno, Hiroyuki Tsumatori, Rumi Shiba, Hironari Kamikubo, Mikio Kataoka, Yasuchika Hasegawa, Tsuyoshi Kawai*

Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayamacho, Ikoma, Nara 630-0192, Japan,

* To whom correspondence should be addressed.

E-mail: yuasaj@ms.naist.jp; tkawai@ms.naist.jp

Electronic Supplementary Material (ESI) for Chemical Communications

Experimental Section

2,5-dioxopyrrolidin-1-yl4'-(4,4,5,5,5-pentafluoro-3-oxopentanoyl)-[1,1'-biphenyl]-4-carboxylate (DK) was perched from KNC Laboratories Co., Ltd. ^{1}H NMR (300 MHz, $CDCl_3$) [deprotonated form]: δ = 8.26 (d, J = 8.7 Hz, 2H), 8.08 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.7 Hz, 4H), 6.70 (s, 1H), 2.94 (s, 4H). HRMS [ESI-MS (negative)]: m/z calcd. for $C_{22}H_{13}F_5N_1O_6$ ([M$^-$H]$^-$), 482.06630; found 482.06638. The labeling reaction of DK with proteins was performed as follows: DK (5 mg, 0.01 mmol) was introduced into 5 ml aqueous solution of proteins (5 mg). The cloudy mixture stirred for 24 h at 4 ºC, and was filtrated to remove unreacted DK molecules. In the emission lifetime measurements, the samples were excited by a N_2 laser (Usho KEC-160; wavelength, 337 nm; pulse width, 600 ps; 10 Hz). The emission profiles were recorded using a streak camera (Hamamatsu, picosecond fluorescence measurement system, C4780). Emission quantum yields of proteins and Lys labeled with DK-Eu^{3+} were measured using a calibrated integrating sphere system in aqueous solution.

The emission spectra were measured at room temperature using JASCO FP-6500. A schematic diagram of the CPL measurement system used in this study is given (see ESI†, S5).

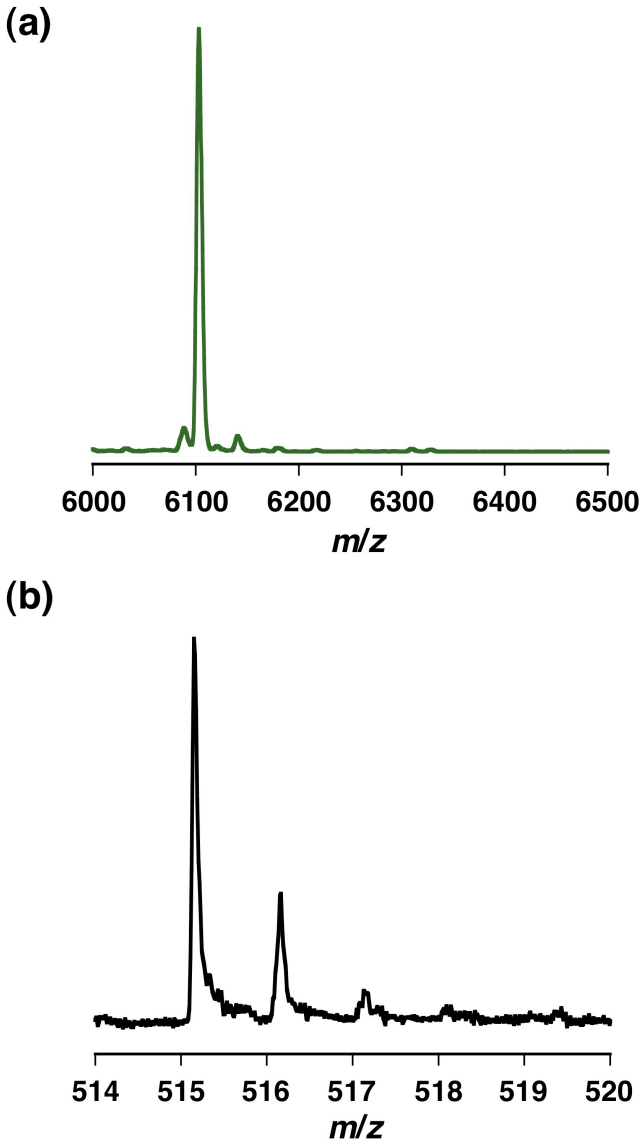


Fig. S1 MALDI-TOF positive ion [M + H] mass spectra of (a) Ins-DK and (b) Lys-DK.

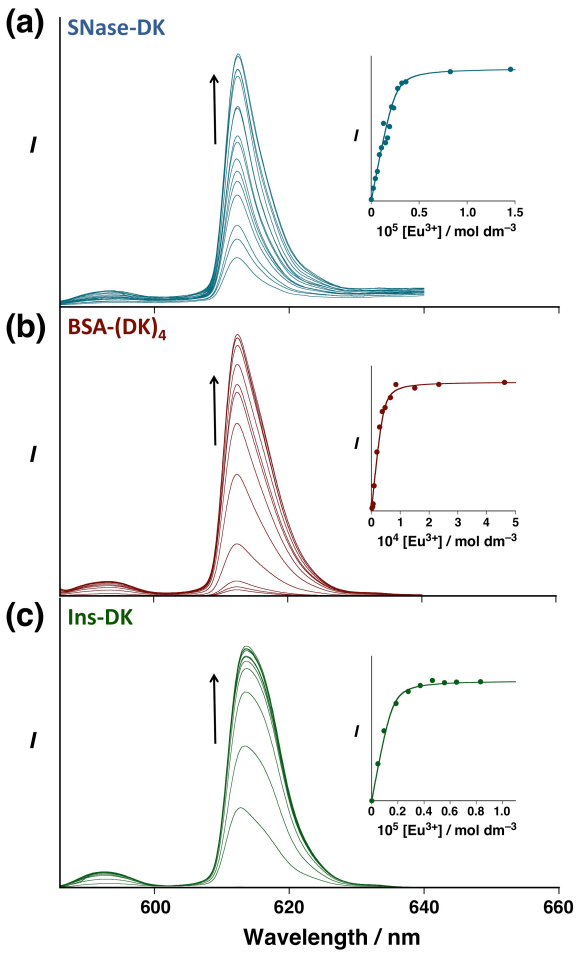


Fig. S2 Emission spectral changes observed upon addition of $EuCl_3$ to an aqueous solution of (a) SNase-DK (1.6×10^{-6} mol dm^{-3}), (b) BSA-$(DK)_4$ (1.1×10^{-5} mol dm^{-3}), and (b) Ins-DK (2.5×10^{-6} mol dm^{-3}) at 298 K. Excitation wavelength $\lambda = 290$ nm. Inset: plot of emission intensity at $\lambda = 613$ nm *vs*. [Eu^{3+}]. The titration curves can be well fitted using eqn [$K([Eu^{3+}] - \alpha[L]_0) = \alpha(1 - \alpha)^{-1}$] representing a 1:1 interaction model, where $\alpha = (I - I_0)/(I_\infty - I_0)$ at $\lambda = 613$ nm, I_0 and I_∞ are the emission intensity due to DK and a 1:1 complex DK–Eu^{3+} bound to proteins at $\lambda = 613$ nm, respectively. K denotes the binding constant between Eu^{3+} and DK bound to proteins.

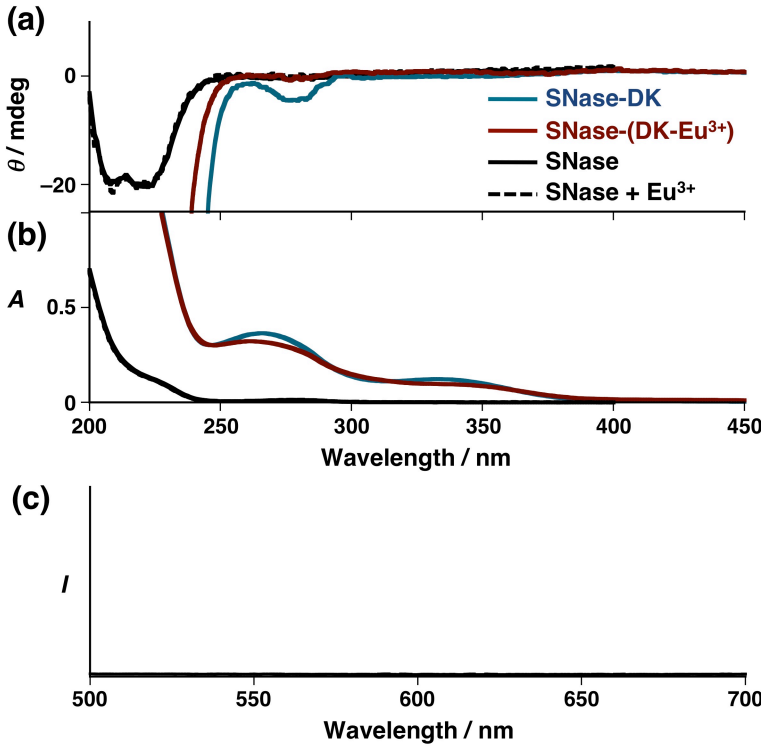


Fig. S3 (a) CD spectra of aqueous solutions of SNase-DK (2.4×10^{-5} M: blue line), SNase-(DK-Eu^{3+}) (4.8×10^{-6} M, [Eu^{3+}] = 5.3×10^{-6} M: red line), SNase (3.9×10^{-7} M: black line), and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). (b) UV-vis absorption spectra of aqueous solutions of SNase-DK (8.5×10^{-6} M: blue line), SNase-(DK-Eu^{3+}) (8.5×10^{-6} M, [Eu^{3+}] = 9.4×10^{-6} M: red line), SNase (3.9×10^{-7} M: black line), and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). (c) Emission spectra of aqueous solutions of SNase (3.9×10^{-7} M: black line) and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). Excitation wavelength λ = 360 nm.

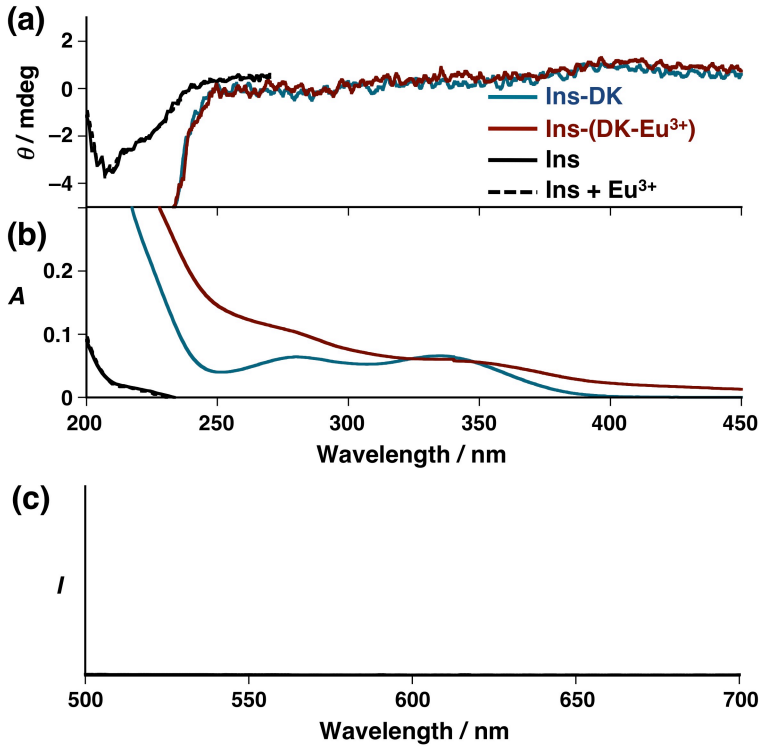
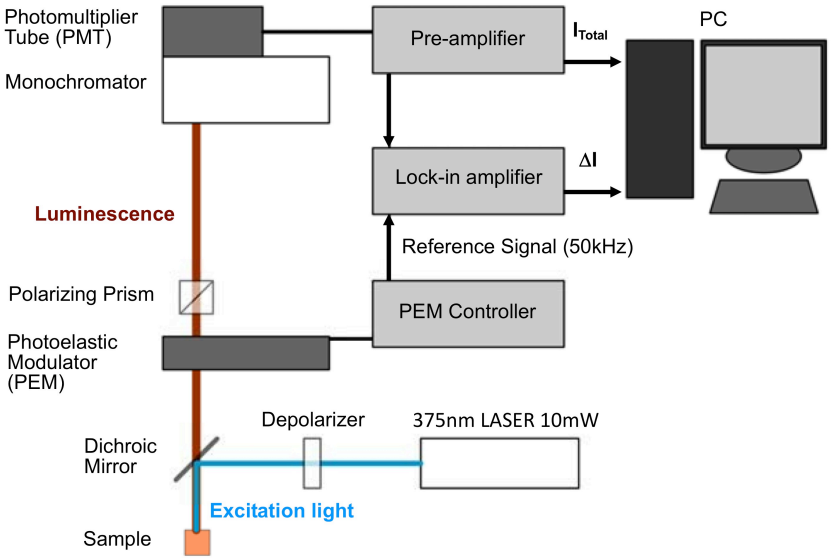


Fig. S4 (a) CD spectra of aqueous solutions of Ins-DK (4.6×10^{-6} M: blue line), Ins-(DK-Eu^{3+}) (4.6×10^{-6} M, [Eu^{3+}] = 5.1×10^{-6} M: red solid line), Ins (2.2×10^{-7} M: black line), and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). (b) CD spectra of aqueous solutions of Ins-DK (4.6×10^{-6} M: blue line), Ins-(DK-Eu^{3+}) (4.6×10^{-6} M, [Eu^{3+}] = 5.1×10^{-6} M: red solid line), Ins (2.2×10^{-7} M: black line), and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). (c) Emission spectra of aqueous solutions of Ins (2.2×10^{-7} M: black line) and that in the presence of $EuCl_3$ (4.5×10^{-4} M: black dashed line). Excitation wavelength λ = 360 nm.



Scheme S5 illustrates schematic diagram of our CPL measurement system in which an excitation laser of 375 nm in wavelength is irradiated to the sample from the same-side to the detection in the epi illumination con-figuration. The retardation of the emitted light was controlled by a photo-elastic modulator (Hinds, PEM-90) that is modulated with the frequency of 50kHz. The circularly polarized component was converted to the linearly polarized light and detected by a photomultiplier tube (Hamamatsu, H7732-10) after passing through the linearly polarized cubic prism (200,000:1) for analyzing circularly polarized luminescence (CPL) signal. In order to maintain a circularly polarized component in the emission, the dichroic filter was precisely settled to be 45° against the optical path. The AC component of the PMT output with frequency of 50kHz was analyzed by a lock-in amplifier (EG&G, Model 7265), which can be modulated by the reference frequency signal from the PEM. A PC system controls a monochromator and the PEM for the appropriate detection wavelength. The emission intensity and the CPL dissymmetry, $g_{lum} = 2(I_L - I_R)/(I_L + I_R)$, were evaluated by the DC component and ratio of AC and DC components, respectively, as a function of wavelength. The calibration of the EI-CPL was performed by reference to obtained data from a CPL spectrometer (JASCO, CPL-200). A description of other components in the measurement system as follows; depolarizer (SIGUMA KOKI CO., LTD., DEQ-S2), dichroic mirror (Chroma Technology Corp., Z375RDC), polarizing prism (Edmund Optics Japan Ltd., 47045-K), monochromator (SHIMADZU CORPORATION, SPG-120S), pre-amplifier (Stanford Research Systems, SR570).