Supporting Information

Detection of Pathogenic Biofilms with Bacterial Amyloid Targeting Fluorescent Probe, CDy11

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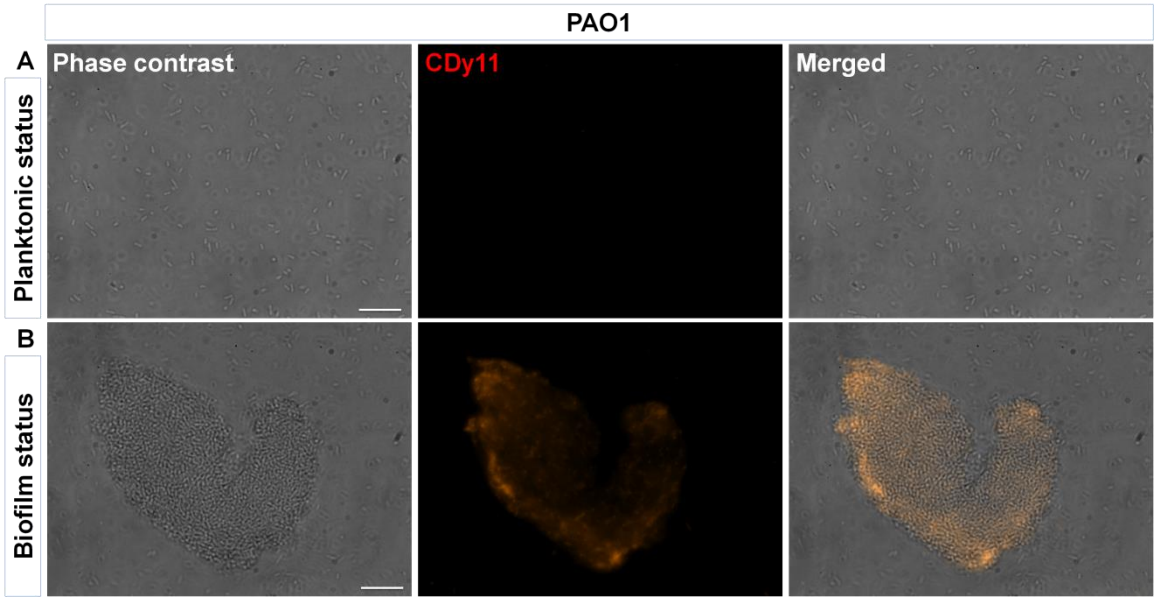


Figure S1. Test of CDy11 in two different growth states of *P. aeruginosa.* Same concentration (1 μM) of CDy11 was applied to the two different status of *P. aeruginosa* in order to investigate the specificity of CDy11. Planktonic cells were prepared from liquid cultures and incubated with CDy11. Biofilms were grown on cover glass and incubated for 1 hour with CDy11 before acquiring images. (A) No fluorescent signals were detected for the planktonic cells, whereas (B) biofilms were labeled by CDy11. Scale bars, 10 μm.

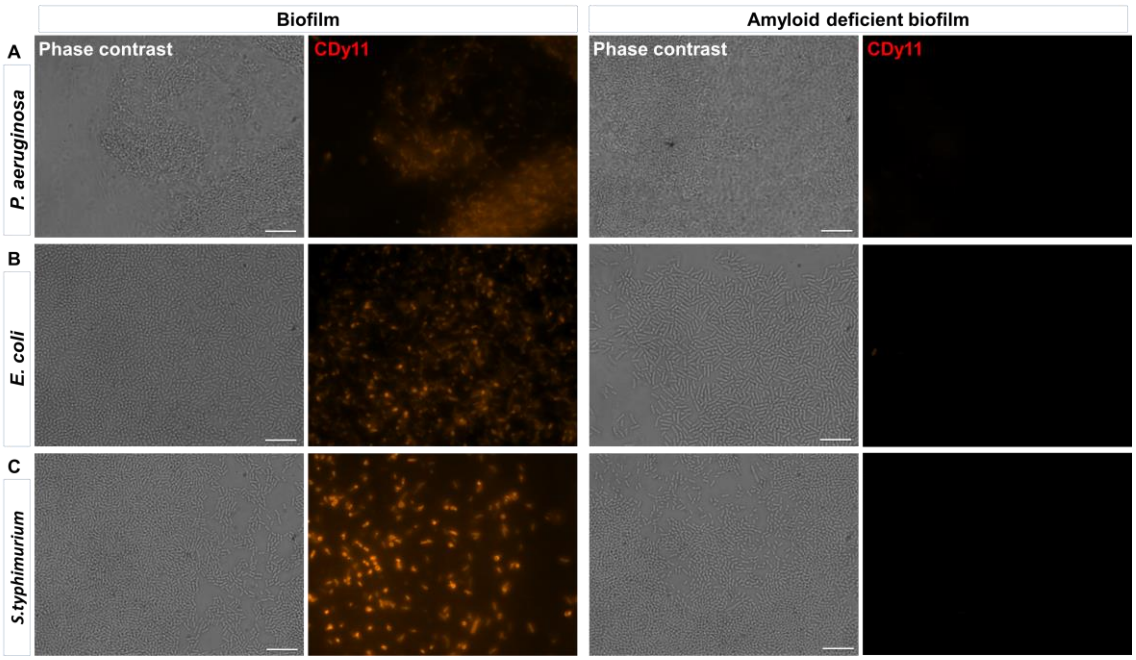


Figure S2. Test of CDy11 as different bacterial amyloid targeting probe. CDy11 signals were only observed in *P. aeruginosa*, *E. coli* and *S. typhimurium* when amyloids were synthesized in biofilm matrix. (A) Biofilms of *P. aeruginosa* (PAO1 and PAO1Δfap). (B) Biofilms of *E. coli* (UTI89 and UTI98Δcurli). (C) Biofilms of *S. typhimurium* (UMRI and MAE32). Scale bars, 10 μm.

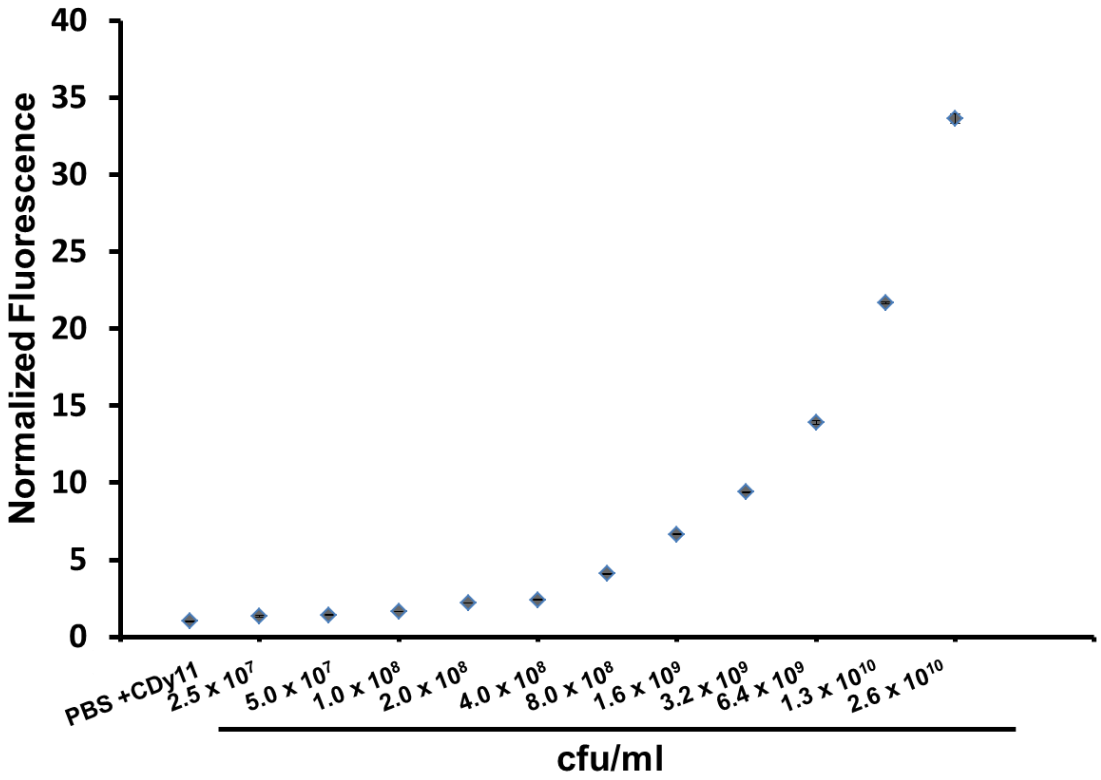


Figure S3. Response of CDy11 against serially diluted biofilms. Serially diluted biofilms were incubated with CDy11 (10 μM) and the intensity of CDy11 was normalized with CDy11 in PBS.

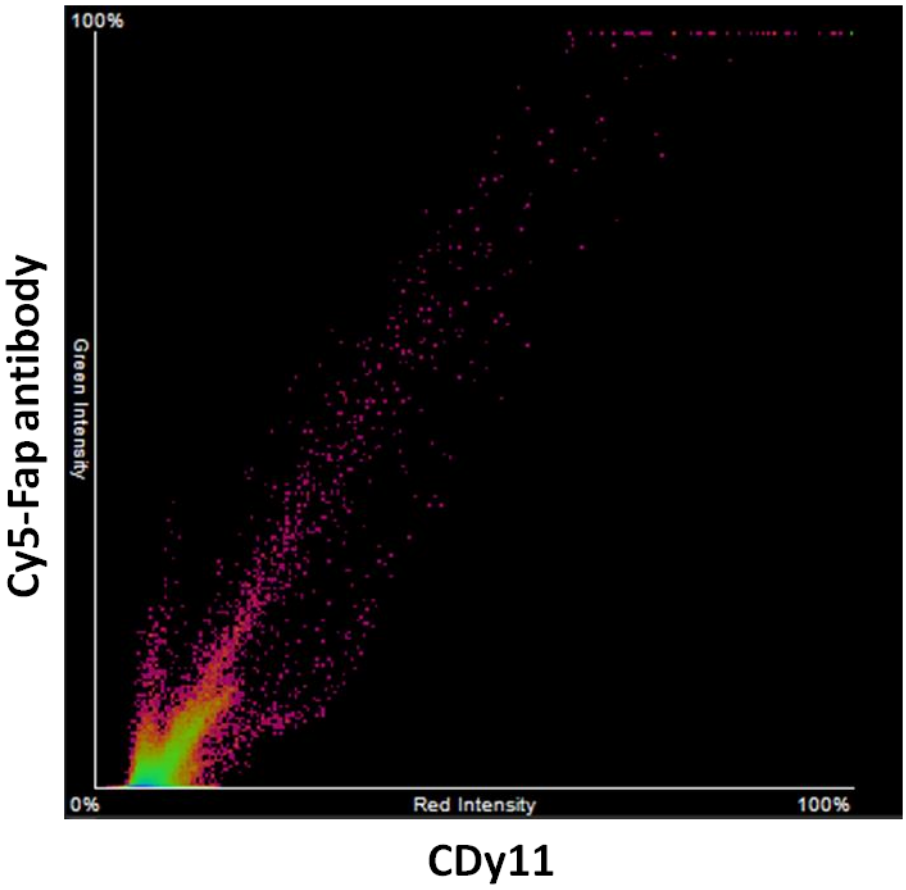


Figure S4. Analysis of Pearson′s correlations between CDy11 and Cy5-Fap signals in biofilms. PAO1 biofilm images by Cy-Fap antibody and CDy11 double labeled signals were analyzed by NIS-Elements software (Nikon, Japan). Pearson′s correlation value was calculated to 0.91±0.7 (x-axis,CDy11; y-axis, Cy5-Fap).

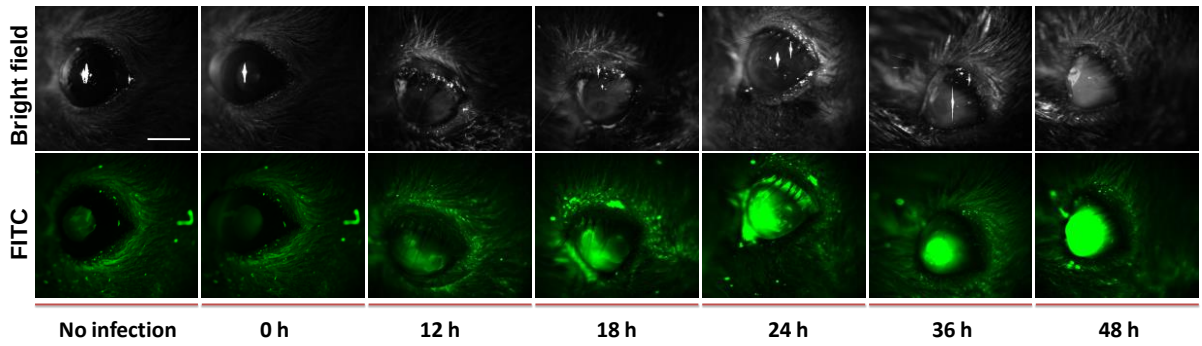


Figure S5. Propagation of *P. aeruginosa* post infection in the corneal infection model. Bright field images and corresponding GFP signals determined by stereomicroscopy in cornea at various time points post infection with *P. aeruginosa* (PAO1-GFP). GFP signal indicative of *P. aeruginosa* (PAO1-GFP) was detected in the mouse cornea 12 hours post infection. The GFP signal increased in a time dependent manner and was fully saturated 36 hours post infection. Scale bar, 2 mm.

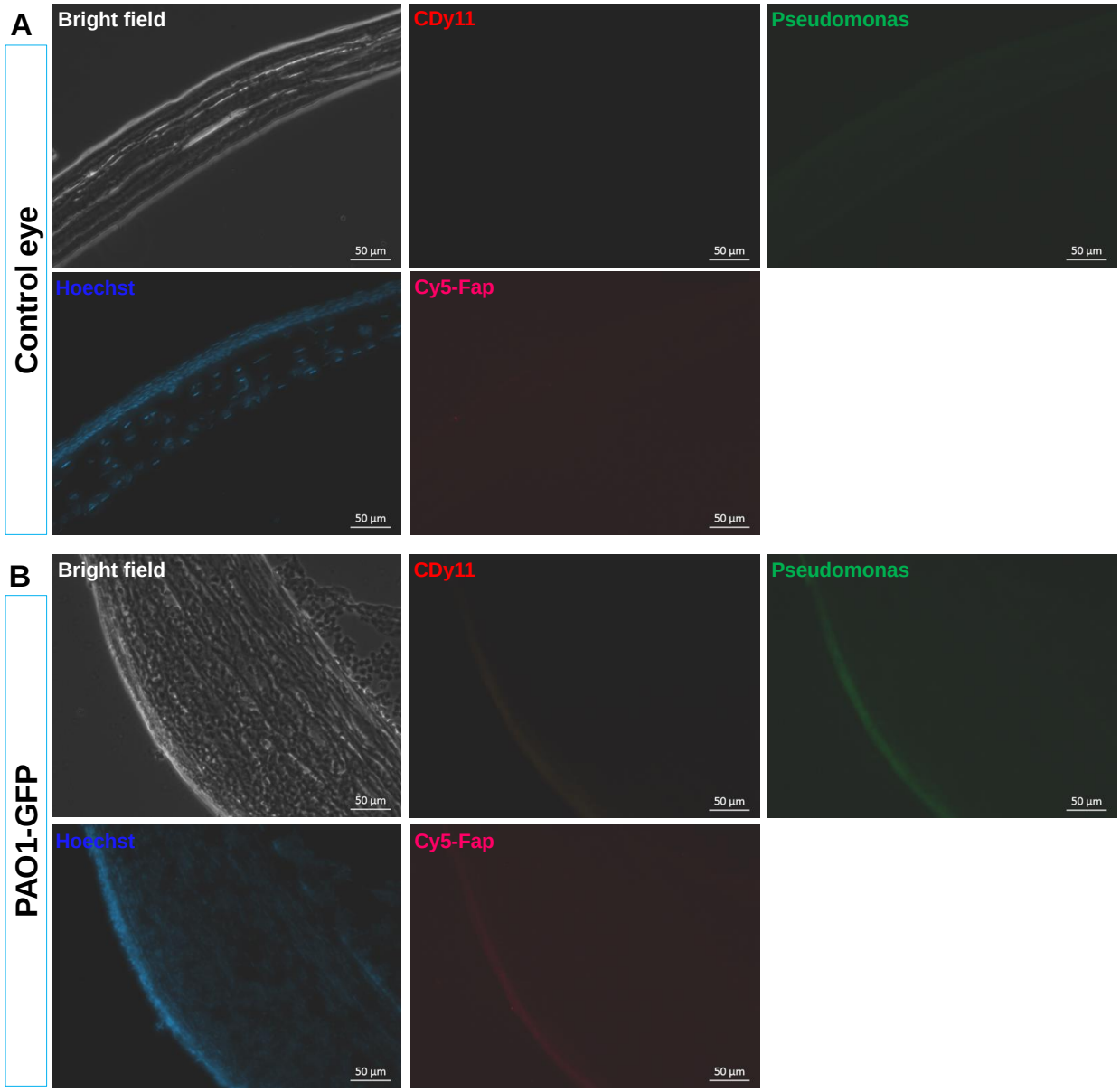


Figure S6. Quadruple-fluorescent images from eye section with CDy11/PAO1-*GFP*/Hoechst/Cy5-Fap antibody. (A) Eye sectioning samples from control eye without infection of *P. aeruginosa*. (B) PAO1-GFP infected eye sectioning sample. Clockwise from upper left; Bright field image, CDy11, PAO1-GFP, Hoechst and Cy5-Fap antibody. Eyes were frozen in OCT media after 1 hour incubation with 10 µM CDy11. Sectioning samples with 14 µm thickness was incubated with Fap antibody. Subsequently, Cy5-secondary antibody was incubated and Hoechst dye was treated before imaging under fluorescent microscopy.

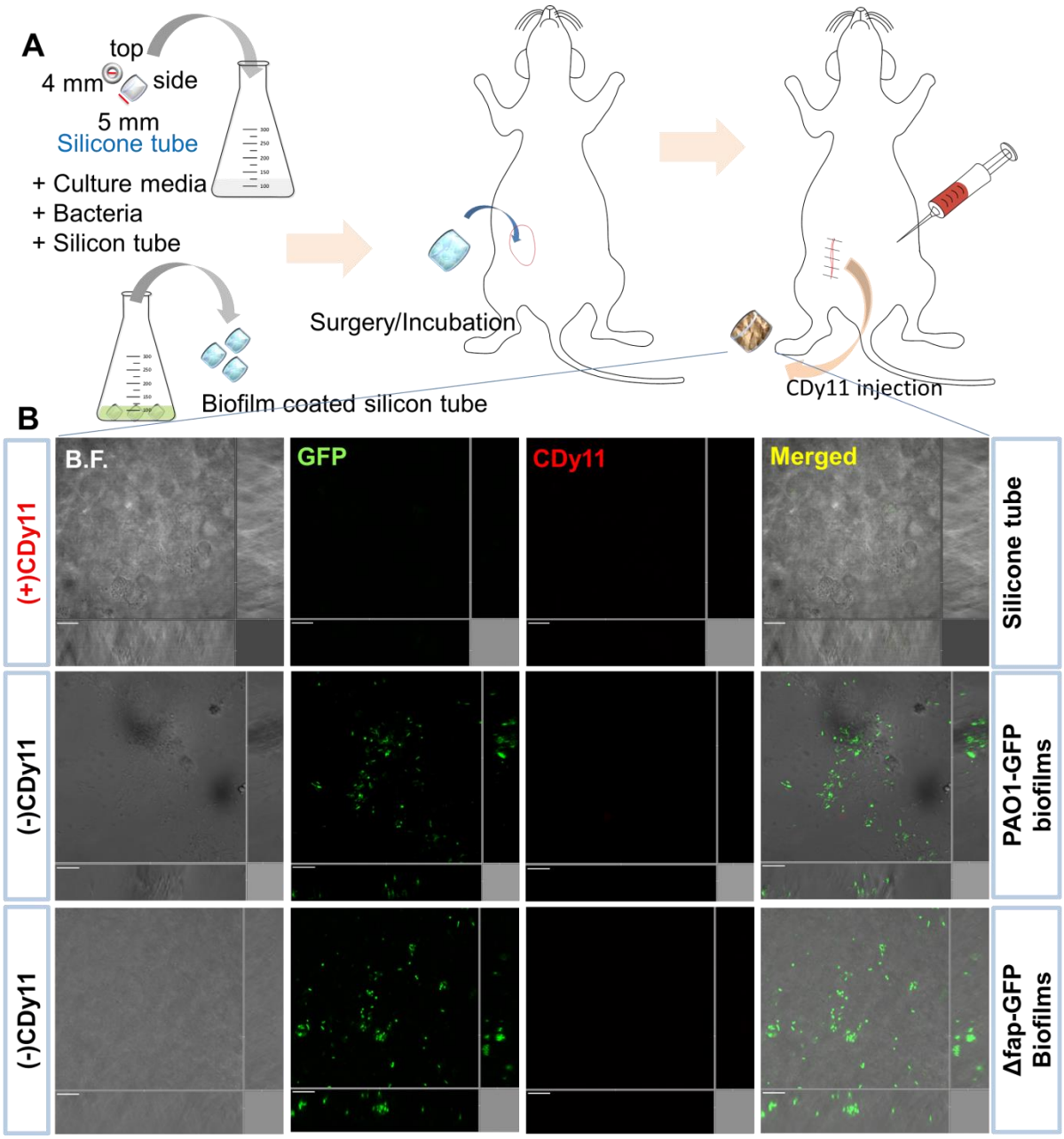
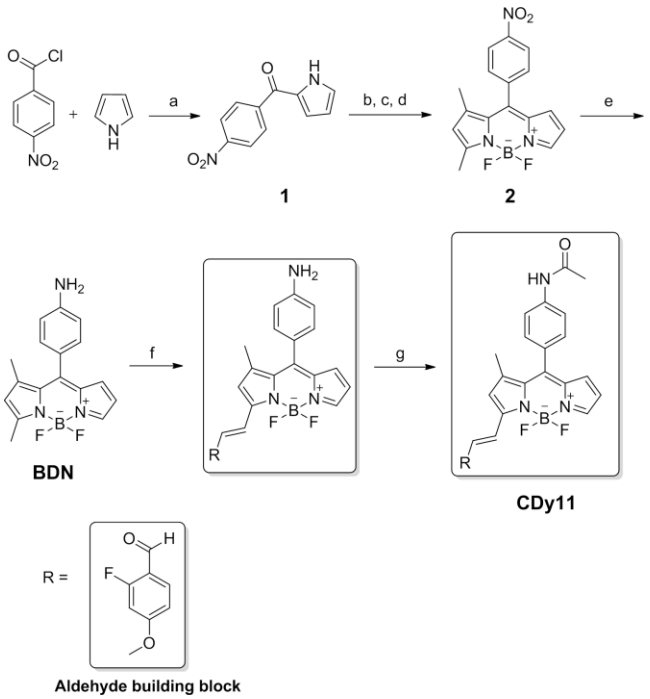


Figure S7. Application of CDy11 in implant model. (A) Preparation of silicone tube for coating with *P. aeruginosa* and insertion of silicone tubes that were pre-coated with PAO1-GFP and PAO1Δfap-GFP biofilms into BALB/c mice. After 1 day incubation, CDy11 into peritoneal cavity was injected for testing the feasibility of CDy11. (B) CLSM images were acquired from silicone tubes removal from mice. Green fluorescent areas represent the distribution of *P. aeruginosa*. The fluorescence intensity of CDy11 was very low and the same for all sample. Scale bars, 10 μm.

General synthetic procedure and characterization of compound CDy11.

General information: All the chemicals and solvents were purchased from Sigma Aldrich, Alfa Aesar, Fluka, MERCK or Acros and used without further purification. Normal phase purifications were carried out using Merck Silica Gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh). Analytical characterization was performed on a HPLC-MS (Agilent-1200 series) with a DAD detector and a single quadrupole mass spectrometer (6130 series) with an ESI probe. Analytical method, unless indicated, gradient solvent system was water: acetonitrile (ACN) (95:5 to 5:95) with 0.1% HCOOH in run time of 10 min; C18 (2) Luna column (4.6 × 50 mm 2, 5 mm particle size). 1H-NMR and 13C-NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer, and chemical shifts were expressed in parts per million (ppm). All the photo-physical studies of CDy11 were performed in SpectraMax®M2 spectrophotometer (Molecular Devices) instrument and the obtained data were analyzed using the Microsoft Office Excel.

Syntheis of CDy11.

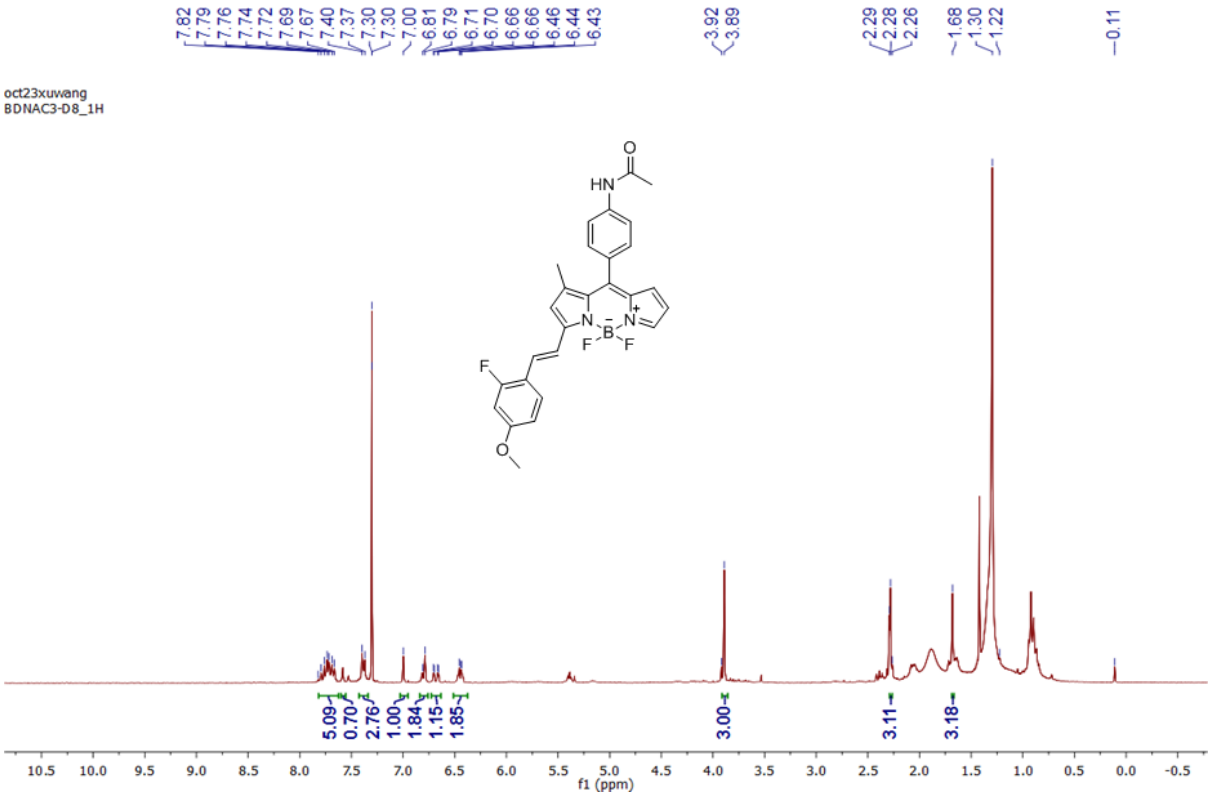


Scheme 1. (a) MeMgBr, -78 ˚C → 25 ˚C, overnight; (b) NaBH4, 0 ˚C → 25 ˚C, THF/H2O (10:1), 2h; (c) 2, 4-dimethyl pyrrole, InCl3, CH2Cl2; (d) i. DDQ in CH2Cl2, ii. BF3.Et2O, TEA; (e) Pd/C, hydrazine monohydrate, EtOH, reflux, 2h; (f) RCHO, pyrrolidine, 85 ˚C, acetonitrile, 5 min.; (g) acetyl chloride (AcCl), NaHCO3, CH2Cl2, 30 min.

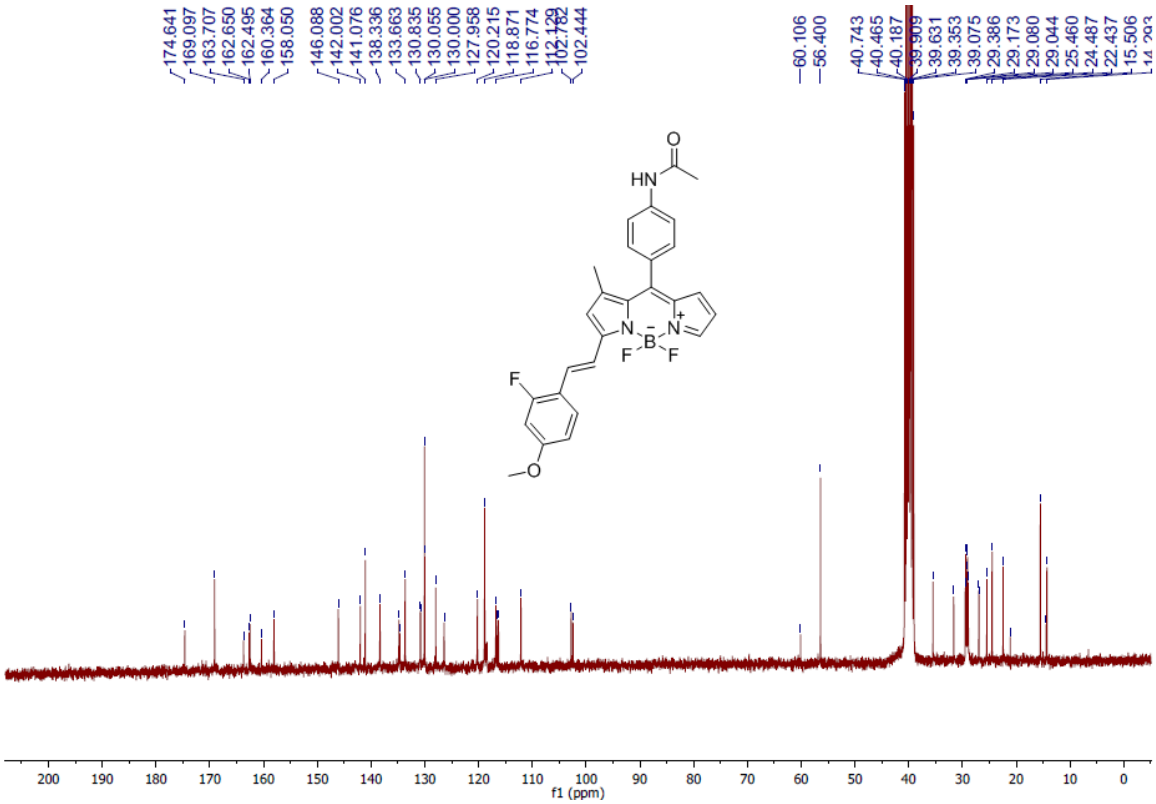
General Procedure for the Synthesis of compound CDy11. To a solution of BODIPY Aniline (BDN)1 (x eq.) in dry acetonitrile (ACN) was added with corresponding aldehyde (4x eq.), followed by pyridine (6x eq.) and refluxed at 85 ºC for 5 min. The crude condensed BODIPY compound was finally purified by normal silica gel chromatography in 7:3 hexane and ethyl acetate mixture. The purified compound (0.02 mili moles) from the above step was

dissolved in dichloromethane (DCM) and added with 100 µL of saturated solution of NaHCO3, followed by acetyl chloride (5 eq.) at 0 ºC. Then the reaction mixture was stirred at room temperature for 30 minutes. The acetylated compound was purified by silica gel chromatography in 7:3 hexane and ethyl acetate mixture. 1H NMR (CDCl3, 300 MHz): δ 1.69 (s, 3H), 2.28 (s, 3H), 3.89 (s, 3H), 6.45 (m, 2H), 6.68 (m, 1H), 6.8 (m, 2H), 7 (s, 1H), 7.38 (m, 3H), 7.74 (m, 5H). 13C NMR (DMSO-d6, 75 MHz): δ 15.5, 24.5, 56.4, 102.4, 112.1, 116.3, 116.4, 116.7, 118.8, 120.2, 126.4, 127.9, 130.0, 130.1, 130.7, 130.8, 133.6, 134.8, 138.3, 141.1, 142.0, 146.1, 158.0, 162.5, 162.6, 169.1, 174.6. HRMS: m/z (C27H22BF3N3O2) calculated: 488.1767, found: 488.1751 (M-H). Extinction coefficient (ε): 12456 M-1cm-1 (Solvent: Ethanol, Wavelength (λ): 558 nm).

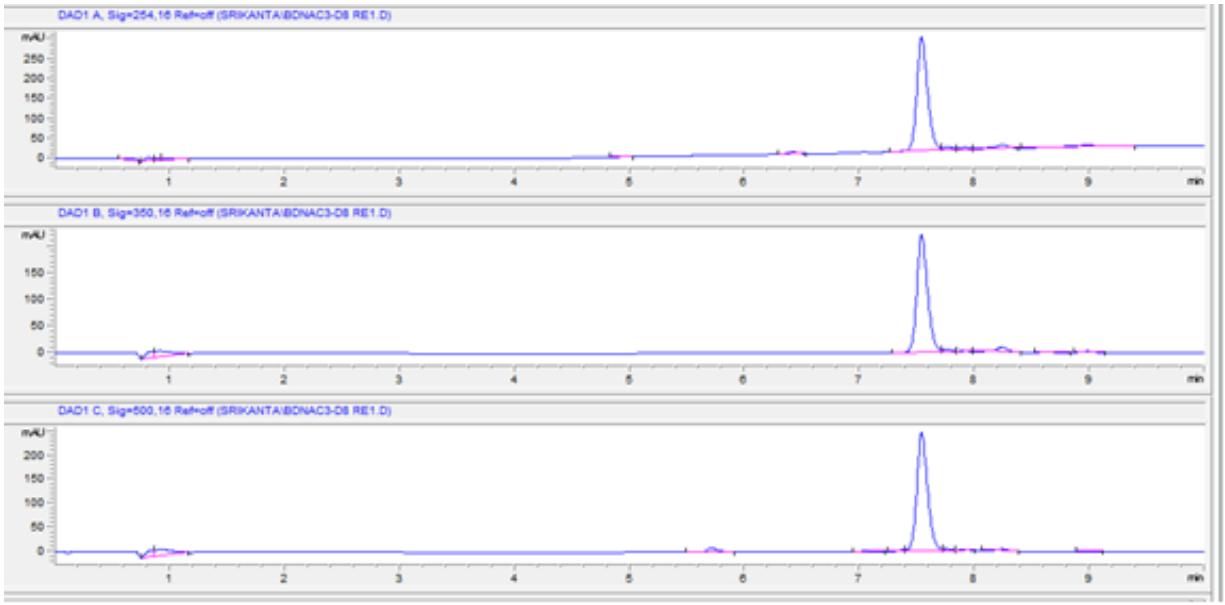
1H NMR (300 MHz, CDCl3) spectrum of CDy11



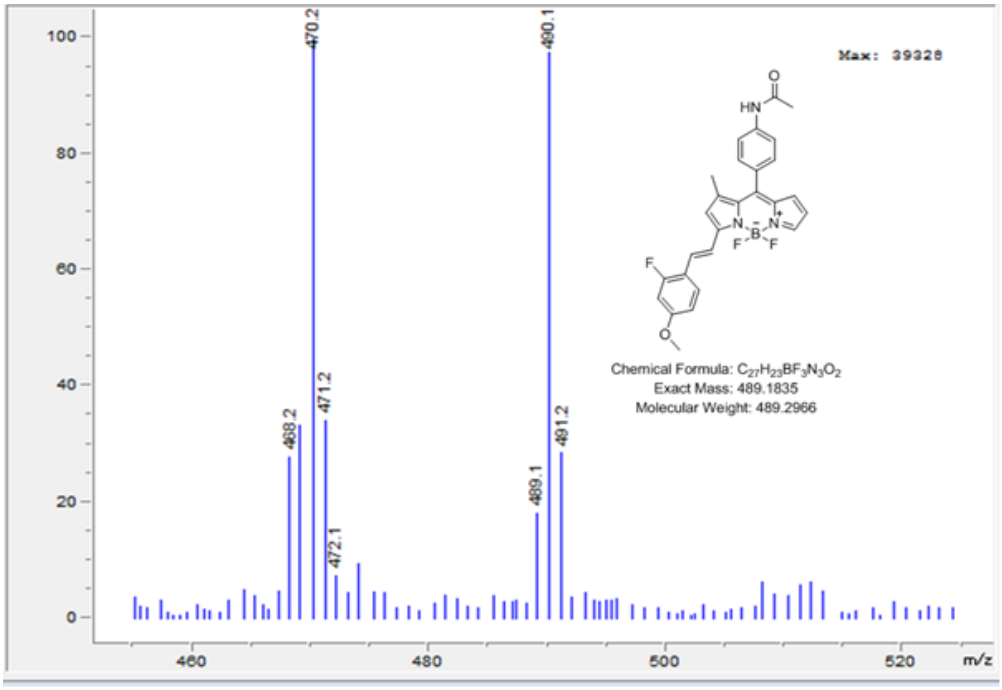
13C NMR (75 MHz, DMSO-d6) spectrum of CDy11



HPLC Spectrum of CDy11



MS spectrum of CDy11



ESI HRMS of CDy11

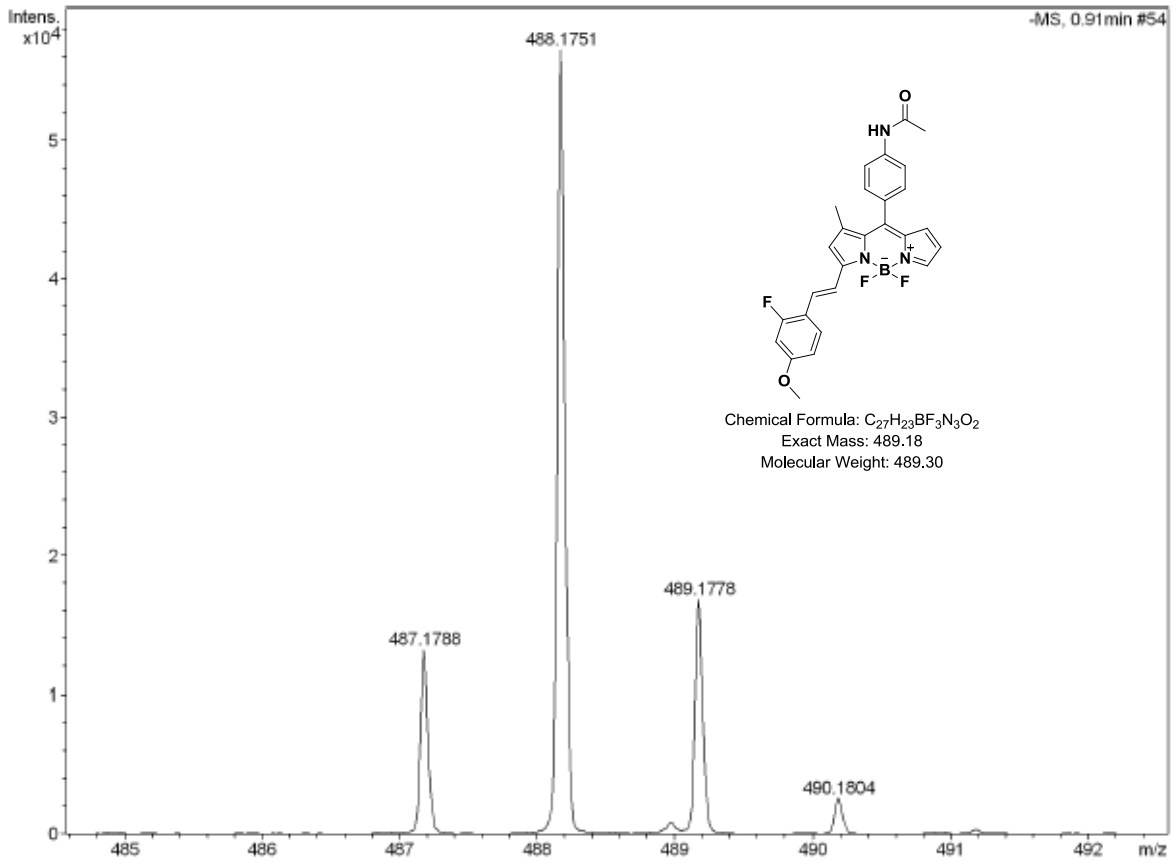
Analysis Info

Analysis Name	D:\Data\Chemistry\2014 Sample\Dec 2014\BDNAC3-D8-2n.d	Acquisition Date	12/15/2014 10:21:43 AM
Method	YCH_Pos-150-1800.m	Operator	default user
Sample Name	BDNAC3-D8	Instrument / Ser#	micrOTOF-Q II 10269
Comment	Prof. Chang Young-Tae		

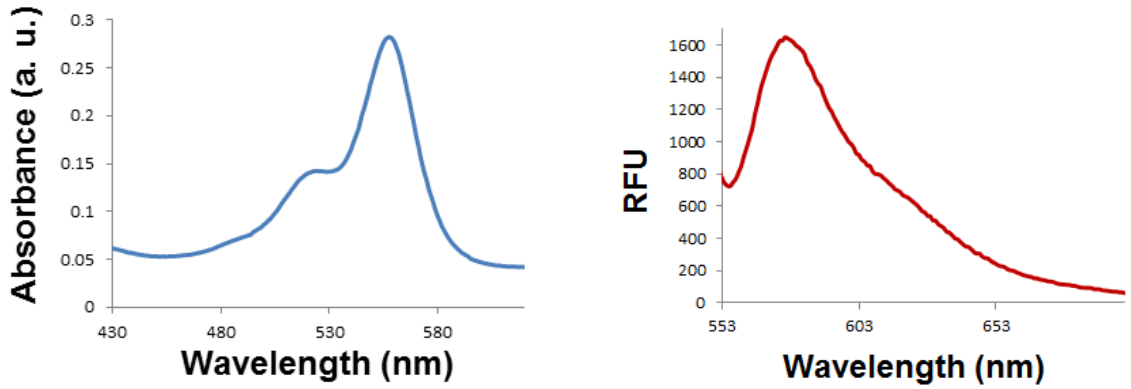
Acquisition Parameter

Source Type	ESI	Ion Polarity	Negative	Set Nebulizer	2.0 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	6.0 l/min
Scan End	1800 m/z	Set Collision Cell RF	200.0 Vpp	Set Divert Valve	Waste

Meas. m/z	#	Formula	m/z	err [ppm]	rdb	e⁻ Conf	N-Rule
488.1751	1	C 27 H 22 B F 3 N 3 O 2	488.1767	3.3	17.5	even	ok



Absorption and emission spectra of CDy11



Absorption and emission spectra of CDy11 were measured at the concentration of 20 μM in Ethanol, Excitation wavelength (λex) = 510nm.

Table S1. Spectroscopic properties and purity table for CDy11: absorbance maximum (λabs), fluorescent emission maximum (λem), and quantum yield (QY).

Compound	M^+ (cal)	M^+ 1(exp)	Abs (nm)	Em (nm)	QY	Purity (%)
CDy11	489.1	490.1	558	576	0.047	97

Reference:

Kang, N. Y.; Lee, S. C.; Park, S. J.; Ha, H. H.; Yun, S. W.; Kostromina, E.; Gustavsson, N.; Ali, Y.; Chandran,Y.; Chun, H. S.; Bae, M.; Ahn, J. H.; Han,W.; Radda, G. K.; Chang, Y. T. Angew. Chem. Int. Edit. 2013, 52, 8557.