Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Tracking the Delivery of Silver to Bacteria through Turn-on Fluorescence

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Experimental

All chemicals, solvents and reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise specified. The BODIPY derivative 8-(4-amino)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-a-indacene **3** was synthesized by following a previously reported procedure.[1] UV-Vis spectra were obtained using a Varian Cary 50 UV-Vis spectrophotometer. IR spectra were acquired using a Perkin-Elmer Spectrum One FT-IR spectrometer. Fluorescence spectra were recorded using an Agilent Cary-Eclipse florescence spectrophotometer. Bacterial growth curves were obtained using a Versa max microplate reader. Confocal microscopy images were acquired on a Solamere Spinning disk confocal microscope equipped with a Nikon TE2000 inverted stand, a CSU-X1 spinning disk, and a Hamamatsu ImageEMX2 camera. A 488 nm laser with a 500-550 nm band-pass emission filter was utilized as the excitation source, and a 100x (1.4 NA) Nikon Plan Apo utilized as the objective lens. *E. coli* suspensions were imaged at 1 frame per second for a total of 9.5 minutes with a 100 ms exposure time.

Synthesis of complexes

qBODIPY 2. 8-(4-amino)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-a-indacene (**3,** 170 mg, 0.50mmol) and 2-quinolinecarboxaldehyde (79 mg, 0.50 mmol) were dissolved in 25 mL of MeOH. The orange mixture was stirred magnetically and refluxed for 48 h forming a red/orange precipitate. The volume of solvent was decreased under reduced pressure and the precipitate was collected through vacuum filtration using a fritted funnel. The crude compound was recrystallized from MeOH to afford compound **2** (82%, 196 mg, 0.41 mmol) as a bright orange powder.

[Ag(qBODIPY)(CF_3SO_3)] 1. Compound **2** (100 mg, 0.21 mmol) was dissolved in 20 mL of DCM and a solution of silver trifluoromethanesulfonate (107.5 mg, 0.42 mmol) in 20 mL of MeOH was added dropwise into this solution while stirring. The mixture was magnetically stirred at room temperature for 24 hours yielding a dark red precipitate. The volume of the solvent was decreased under reduced pressure and the precipitate was collected through vacuum filtration using a fritted funnel. The obtained powder was recrystallized twice from MeOH to afford complex **1** (91%, 189 mg, 0.19 mmol) as a dark red microcrystalline powder.

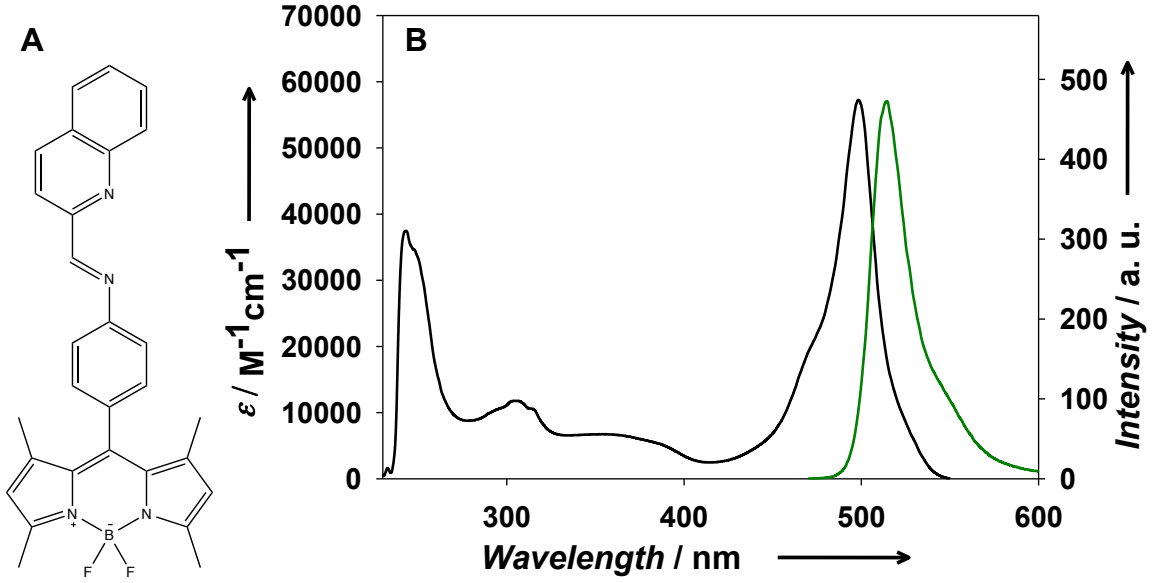

Fig. S1. (A) Chemdraw™ representation of qBODIPY **2**. (B) Absorbance and emission profile of qBODIPY in 1:10 DMSO:H_2O.

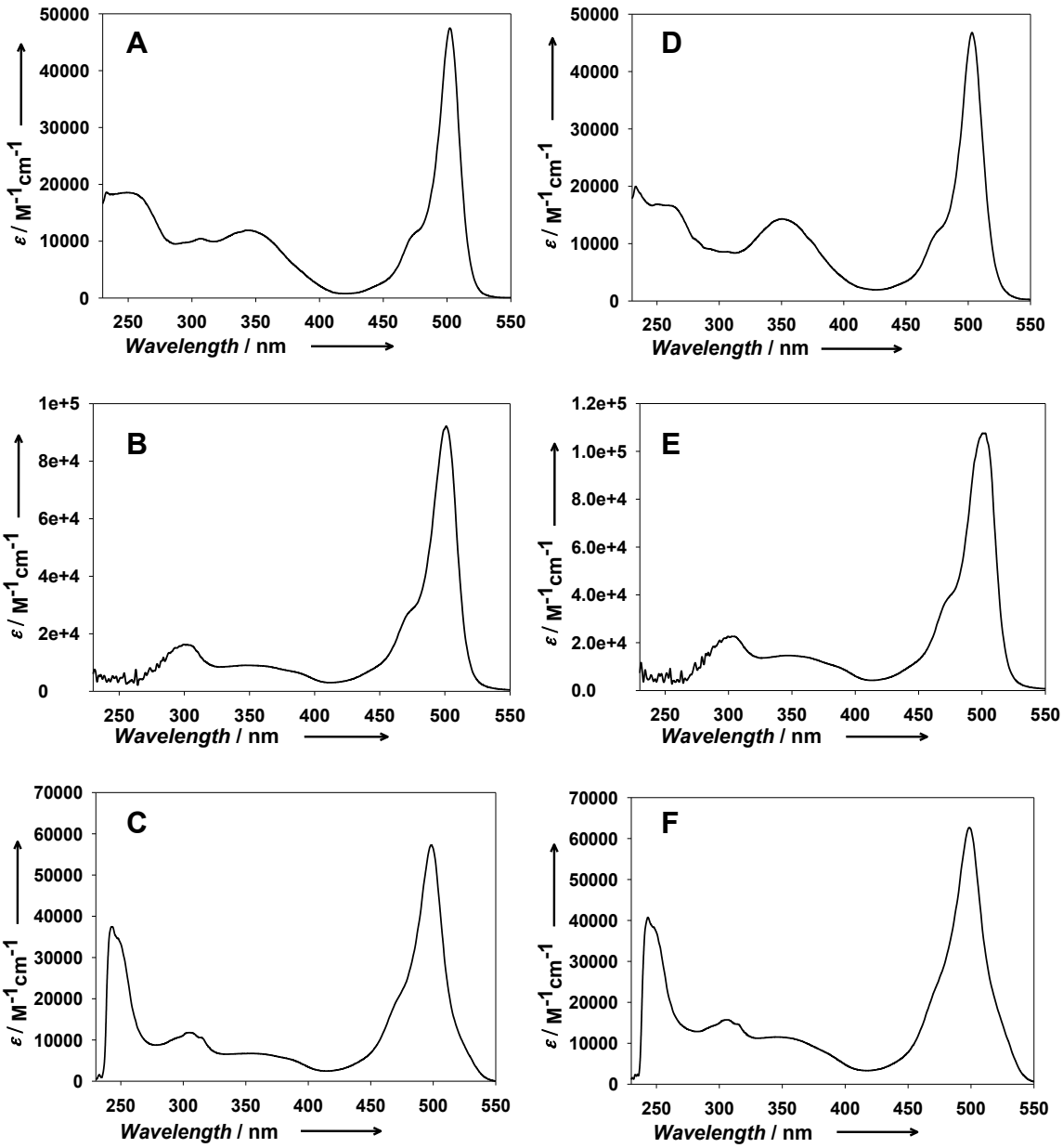

Fig. S2. Electronic absorption spectra of qBODIPY and [Ag(qBODIPY)(CF_3SO_3)] (**1**) in different solvents: (A) qBODIPY in DCM, (B) qBODIPY in DMSO, (C) qBODIPY in 1:10 DMSO:H_2O, (D) complex **1** in DCM, (E) complex **1** in DMSO, (F) complex **1** in 1:10 DMSO:H_2O.

Bacterial Growth Kinetics

Escherichia coli, *Pseudomonas aeuroginosa*, and *Staphylococcus aureus* cells were first grown by spreading frozen liquid cultures (20% glycerol, -72 °C) on Luria broth (LB) agar plates and incubating them at 37 °C overnight. From these plates, one individual colony was selected and used to inoculate a 3 mL aliquot of sterile liquid Mueller-Hinton broth (MHB) which was then incubated at 37 °C for 18 h with constant shaking at 250 rpm. Upon completion of this growth period, the bacterial cells were washed by centrifuging a 1 mL aliquot of each bacterial suspension at 5000 rpm for 5 min, discarding the supernatant, and re-suspending the pellet in 1 mL sterile MHB. This process was repeated once, with enough of the second re-suspension added to a 5 mL volume of sterile Mueller-Hinton Broth to bring the optical density at 600 nm to 0.100. This new suspension with 0.100 optical density was subsequently used for all inoculations. A 96-well plate (Costar) was utilized to obtain bacterial growth profiles, with each well having 20 μL of this bacterial inoculation suspension, 20 μL fresh MHB, varying volumes (2-100 μL) of complex **1** solutions, and enough 1:99 DMSO:H_2O to bring the final volume to 200 μL. Upon addition of all components, the wells were mixed by pipetting, then the 96-well plate was immediately placed into a Molecular Devices VERSAmax microplate reader maintained at a constant temperature of 37 °C with measurements of each well's optical density at 600 nm taken every 3 minutes over a 24 hr period with a 15 s mixing period between each acquisition. The concentrations at which no bacterial growth was observed in the plate after the incubation period was determined to be the minimum inhibitory concentration (MIC) for that compound.

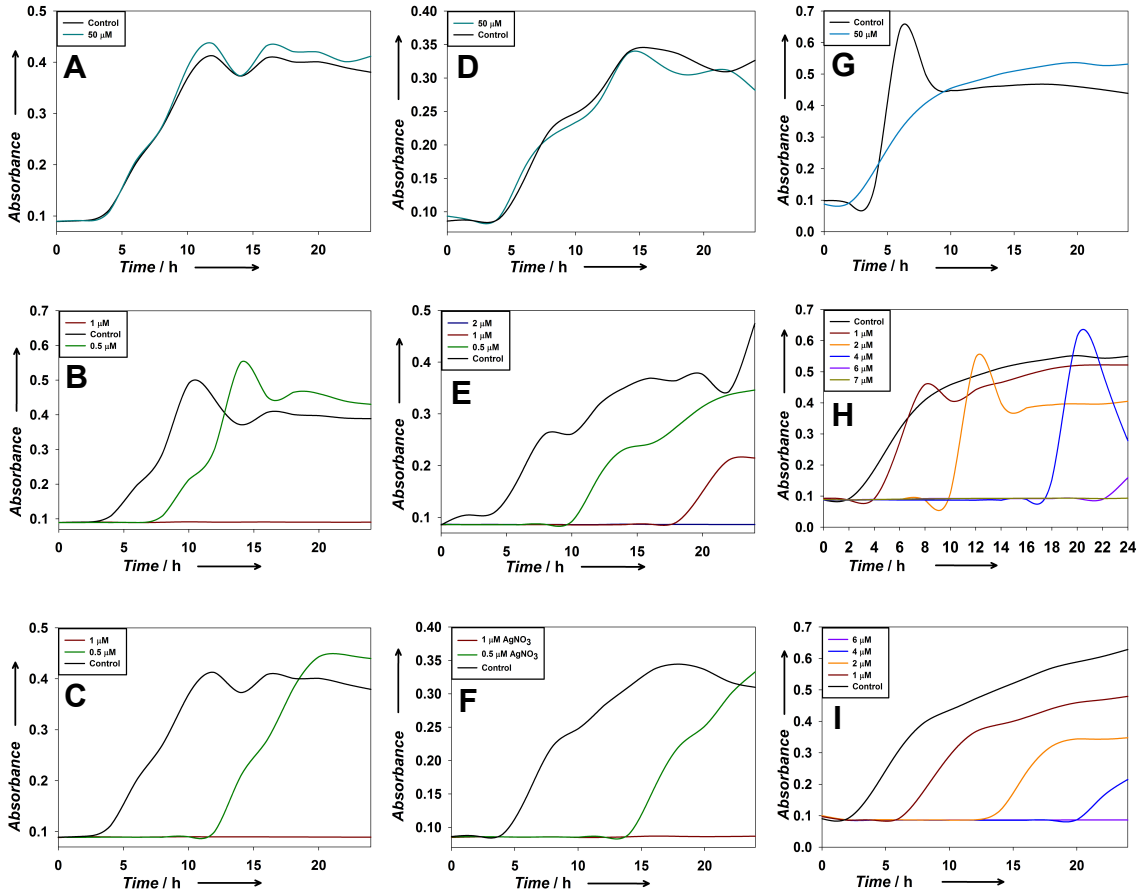

Fig. S3. Bacterial growth kinetics. *E. coli* in the presence of: (A) qBODIPY, (B) complex **1**, (C) $AgNO_3$. *P. aeuroginosa* in the presence of: (D) qBODIPY, (E) complex **1**, (F) $AgNO_3$. *S. aureus* in the presence of: (G) qBODIPY, (H) complex **1**, (I) $AgNO_3$.

As shown in Fig. S3, no growth inhibition was observed for *E. coli*, *P. aeuroginosa,* or *S. aureus* treated with the free ligand qBODIPY (Fig. S3A, S3D, and S3G) up to a concentration of 50 μM. Complete growth inhibition of *E. coli* was observed at 1 μM for both complex **1** and $AgNO_3$. For *P. aeuroginosa* complete growth inhibition was observed at 2 μM for complex **1** and 1 μM for $AgNO_3$. Finally for *S. aureus*, complete growth inhibition was observed at 7 μM for complex **1**, whereas $AgNO_3$ had an MIC of 6 μM.

Fluorescence Microscopy. *Escherichia coli* (ATCC 25922) cells were prepared by first spreading frozen liquid cultures (20% v/v glycerol, -72 °C) on Luria broth (LB) agar plates and incubating them at 37 °C overnight. From these plates, an individual colony was selected and used to inoculate 3 mL of sterile, liquid LB and subsequently incubated at 37 °C for 18 h with constant shaking at 250 rpm. Upon completion of this growth period, a 1 mL aliquot of this bacterial suspension was washed by centrifuging a 1 mL aliquot at 5000 rpm for 5 min, discarding the supernatant, and re-suspending the pellet in 1 mL sterile nanopure water. This process was repeated once, and a 2 μL drop of the washed suspension was placed onto a 1.5 microscope cover slip (0.17 mm thickness) and used for fluorescence imaging. *E. coli* suspensions were imaged at 1 frame per second for a total of 9.5 minutes with a 100 ms exposure time. After 30 seconds, a 2 μL aliquot of a 5 μM complex **1** or qBODIPY solution (1:99 DMSO:H_2O) was added to the 2 μL drop of the *E. coli* suspension being imaged, and the increase in qBODIPY fluorescence was noted over the remaining time.

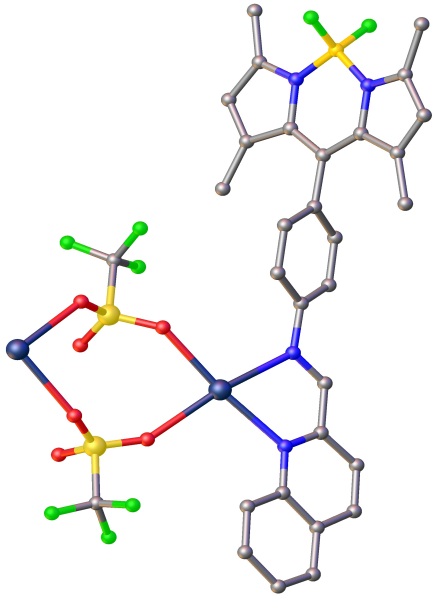

Fig. S4. Ball-stick representation of the molecular structure (asymmetric unit) of complex **1**. The hydrogen atoms are omitted for clarity.

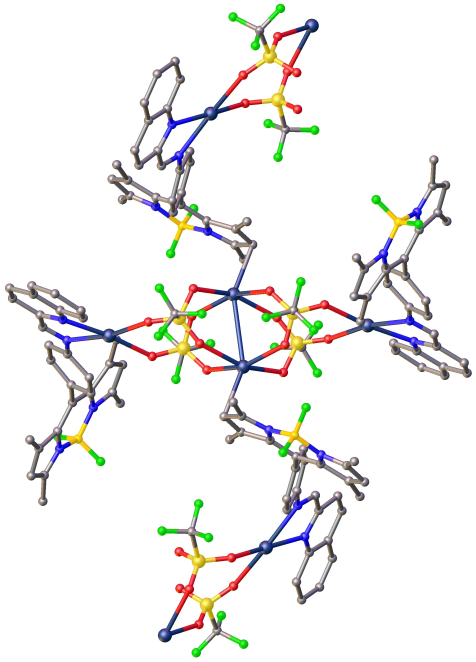

Fig. S5. Ball-stick representation of a partial extended pattern of complex **1** showing two different Ag coordination environments. The hydrogen atoms are omitted for clarity.

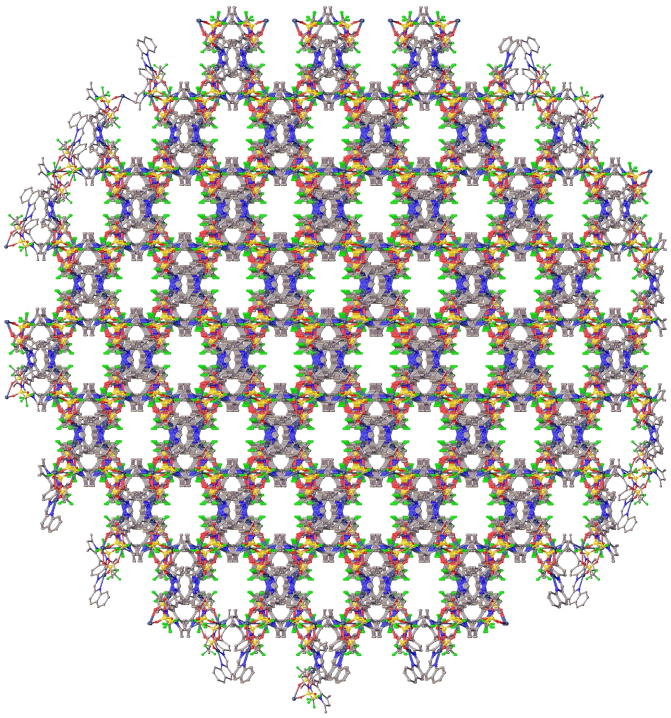

Fig. S6. Packing pattern of complex **1** along c axis (generated with the aid of the program Olex2).

X-ray data collection and structure refinement

Single crystals of the complex were obtained by layering hexanes over its dichloromethane solution. A suitable crystal was mounted on a MitiGen loop with Paratone-N oil (Hampton Research) and transferred to the diffractometer. Data were collected on a Bruker APEX II single crystal X-ray diffractometer equipped with PHOTON 100 detector with synchrotron radiation source by the f and w-scan technique in the range $3 \leq 2\theta \leq 51$ (Supplementary Table S1). The data integration and reduction were done with SAINT[2] module. The multi scan semi-empirical absorption correction was applied to the collected reflections using SADABS. The structure was generated using ShelXT (intrinsic phasing)[3] using Olex2[4] graphical user interface and subsequently refined by full-matrix least squares procedure on F^2 using ShelXL refinement package.[5] All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in calculated positions. Calculations were also performed using the SHELXTL 2014 program package.[6] The SQUEEZE[7] module within the PLATON[8] program was used to alleviate the Alert As emerged due to solvent accessible voids in the structure.

Table S1: Crystal data and structure refinement parameters for **1.**

	1
Empirical formula	$C_{31}H_{25}N_4O_6BF_8S_2Ag_2$
FW	992.22
Temp (K)	293
Crystal System	Monoclinic
Space group	C2/c (no. 15)
a (Å)	26.8250(13)
b (Å)	14.8903(7)
c (Å)	22.4731(11)
α (°)	90
β (°)	115.102(2)
γ (°)	90
V (Å^3)	8128.7(7)
Z	8
Density (calcd) (g cm^{-3})	1.622
Abs coeff (mm^{-1})	1.146
No. of unique reflns	7509 (R_{int} = 0.0471)
R_1[b] (I > 2s(I))	0.0742
wR_2[c]	0.2462
GOF[a] on F^2	1.137

[a]GOF = $[S[w(F_o^2-F_c^2)^2]/(N_o-N_v)]^{1/2}$ (N_o = number of observations, N_v = number of variables).

[b]$R_1 = S||F_o|-|F_c||/S|F_o|$. [c]w$R_2 = [(Sw(F_o^2-F_c^2)^2/S|F_o|^2)]^{1/2}$

References

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