Supplementary Information

Sugar-Powered Nanoantimicrobials for Combating Bacterial Biofilms

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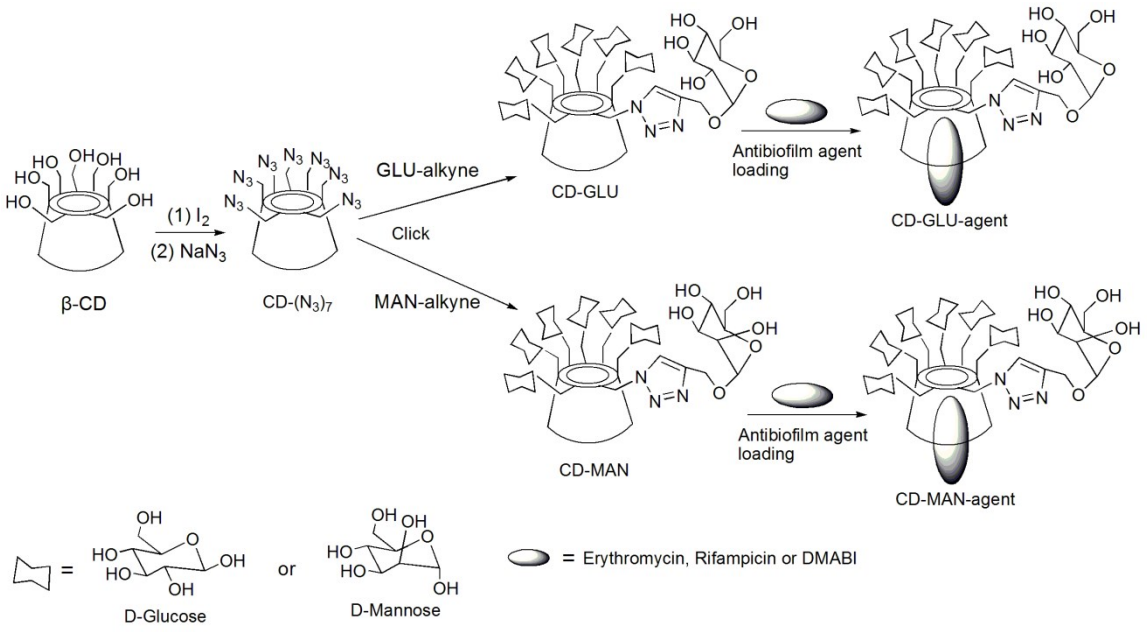
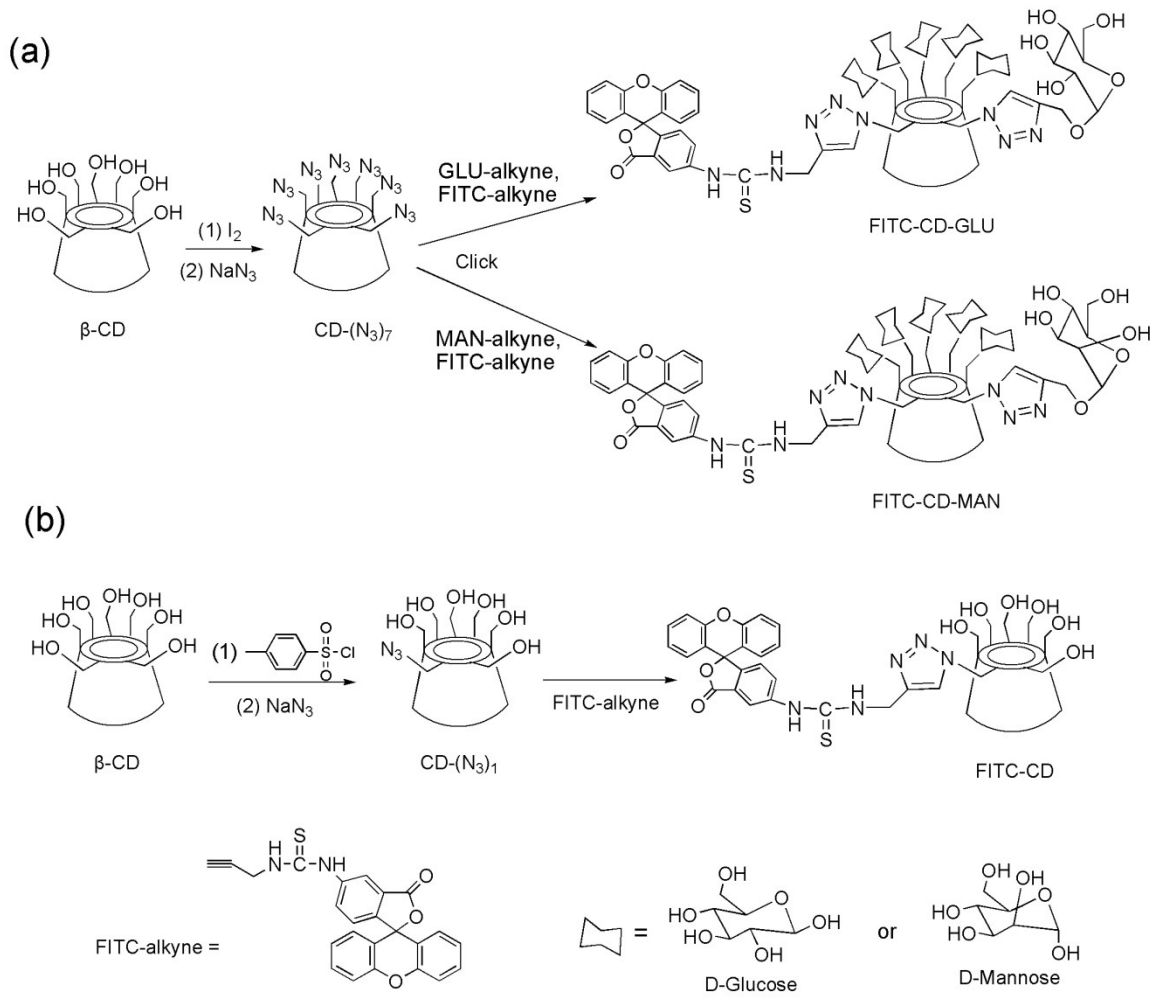
Preparation of FITC-CD-MAN, FITC-CD-GLU and FITC-CD carriers

The 7-arm azide-functionalized β-cyclodextrin (CD-$(N_3)_7$), 1-arm azide-functionalized β-cyclodextrin (CD-$(N_3)_1$), 1-(2'-propargyl)-D-mannose (MAN-alkyne), 1-(2'-propargyl)-D-glucose (GLU-alkyne) and alkyne-functionalized FITC (FITC-alkyne) were synthesized using the procedures reported in earlier studies.[1-3] FITC-CD-MAN was prepared via azide-alkyne click reaction between the CD-$(N_3)_7$, MAN-alkyne and FITC-alkyne. Briefly, CD-$(N_3)_7$ (0.133 g, 0.1 mmol), MAN-alkyne (0.14 g, 0.64 mmol), FITC-alkyne (0.048 g, 0.11 mmol) and PMDETA (13 mg, 0.075 mmol) were dissolved in DMF (5 mL). The solution was degassed by purging with argon for 30 min and CuBr (10.76 mg, 0.075 mmol) were added. The reaction mixture was further purged with argon for 10 min. The reaction was then allowed to proceed under continuous stirring at 60 ^{o}C for 24 h. After reaction, the solution was poured into excess methanol. The precipitate was collected after filtration and re-dissolved in doubly distilled water. The aqueous solution was then subjected to dialysis against doubly distilled water for 3 days followed by freeze-drying to obtain the FITC-CD-MAN product. FITC-CD-GLU was prepared using similar procedures with GLU-alkyne instead of MAN-alkyne. FITC-conjugated β-cyclodextrin (FITC-CD) was prepared as follows: CD-$(N_3)_1$ (0.116 g, 0.1 mmol), FITC-alkyne (0.048 g, 0.11 mmol) and PMDETA (1.91 mg, 0.011 mmol) were dissolved in DMF (5 mL). The solution was degassed by purging with argon for 30 min and CuBr (1.57 mg, 0.011 mmol) were added. The FITC-CD product was then obtained using the same reaction conditions and purification procedures as described above for FITC-CD-MAN. The alkyne-azide click reaction is a well-known method for the preparation of molecular architectures because it is a facile, high-yield and selective reaction under mild

conditions with little or no by-products.[4] Based on the MAN-alkyne (or GLU-alkyne)/FITC-alkyne reactant ratio used in the preparation of the FITC-CD-MAN (or FITC-CD-GLU), the product is expected to be grafted with 6 sugar and 1 FITC moieties. On the other hand, the FITC-CD is expected to be grafted with 1 FITC moiety only. The fluorescence spectra of 10 μM of fluorescein, FITC-CD, FITC-CDGLU and FITC-CD-MAN in PBS were measured to confirm that a similar level of fluorescence intensity was obtained for a similar molar concentration of these compounds.



Scheme S1. Schematic illustration of the preparation procedures for CD-GLU (or CD-MAN) and CD-GLU-agent (or CD-MAN-agent) complex.



Scheme S2. Schematic illustration of the preparation procedures for FITC-CD-GLU (or FITC-CD-MAN) (a) and FITC-CD (b) based on the selected reagent ratios.

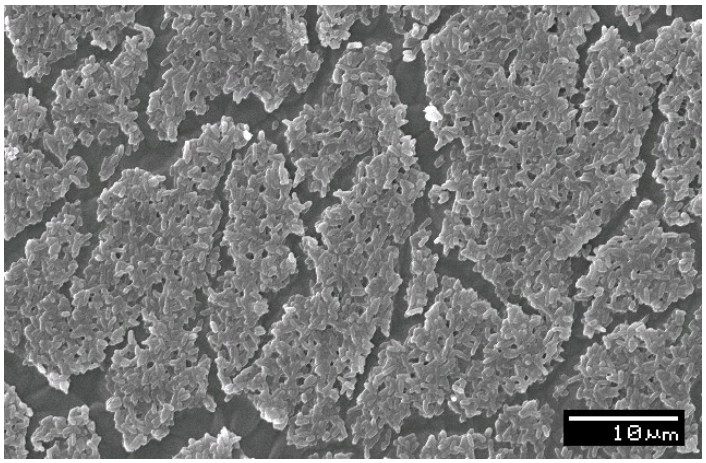


Figure S1 SEM image of *P. aeruginosa* biofilm on silicone film after incubation in growth medium containing 1×10^8 CFU/mL for 24 h. Scale bar is 10 µm.

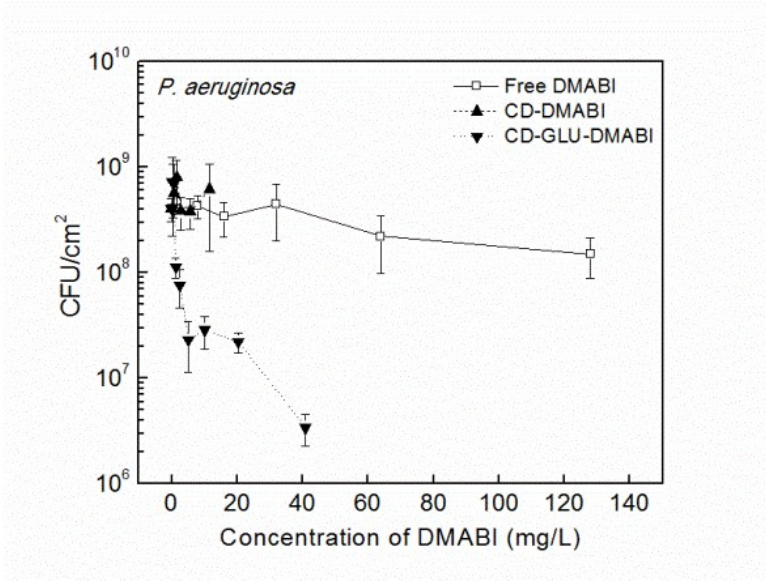


Figure S2 Number of adherent *P. aeruginosa* cells per cm^2 of silicone film surfaces after incubation in growth medium containing 1×10^8 CFU/mL for 24 h in the presence of different concentrations of free DMABI, CD-DMABI and CD-GLU-DMABI as determined by the spread plate method.

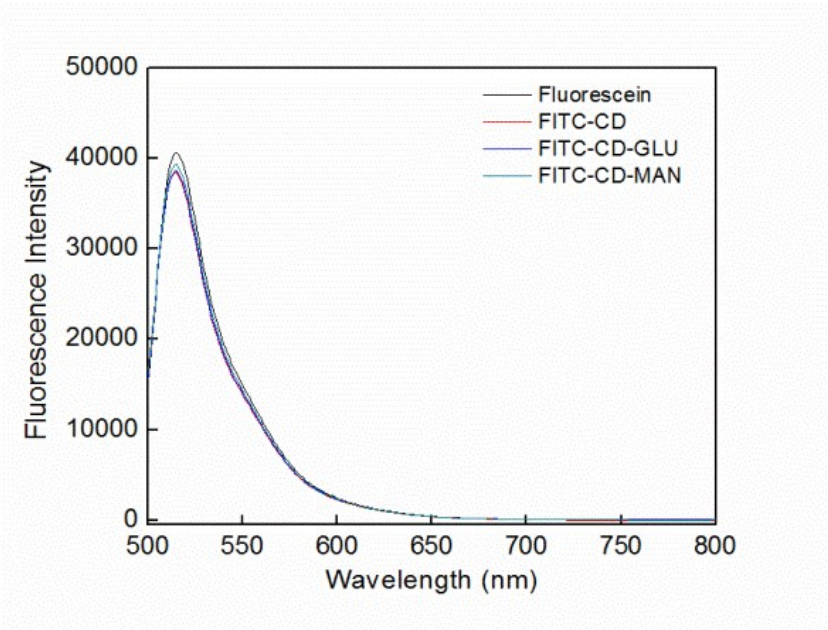


Figure S3 Fluorescence spectra of 10 μM of fluorescein, FITC-CD, FITC-CD-GLU and FITC-CD-MAN in PBS. Excitation wavelength was 488 nm.

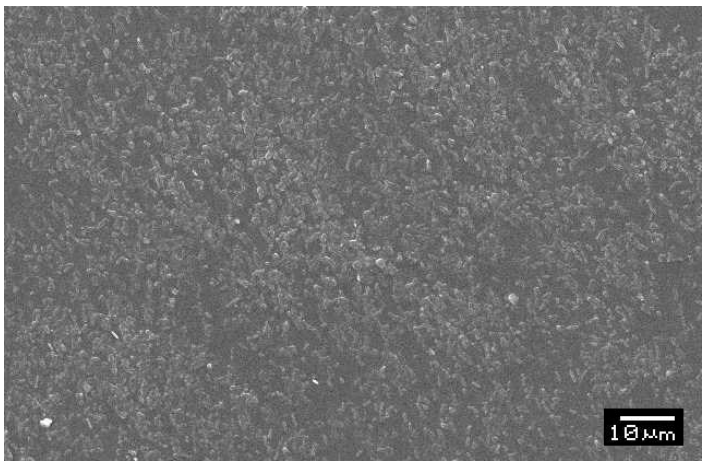


Figure S4 SEM image of *P. aeruginosa* biofilm on silicone film after incubation in growth medium containing 1×10^5 CFU/mL for 24 h. Scale bar is 10 μm.

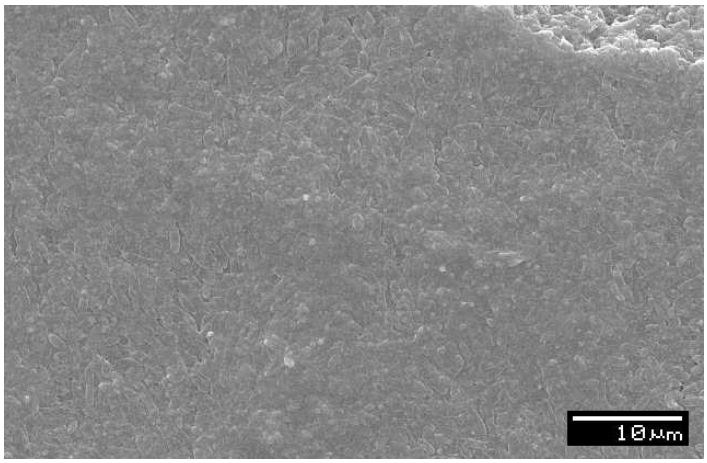


Figure S5 SEM image of *P. aeruginosa* biofilm on silicone film after incubation in growth medium containing 1×10^7 CFU/mL for 72 h. Scale bar is 10 µm.

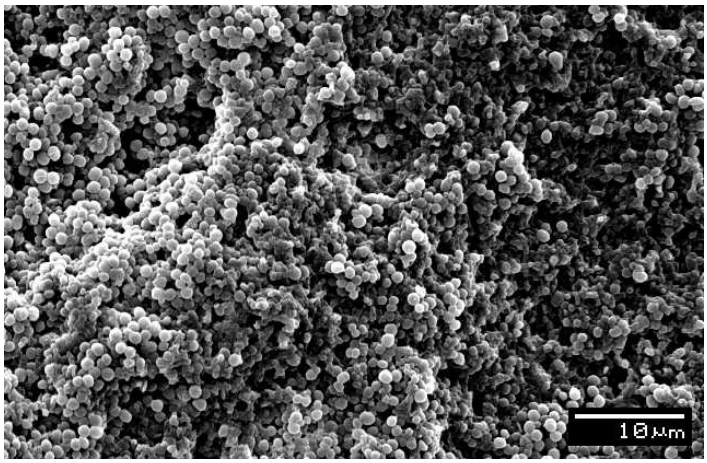


Figure S6 SEM image of *S. aureus* biofilm on silicone film after incubation in growth medium containing 1×10^7 CFU/mL for 72 h. Scale bar is 10 µm.

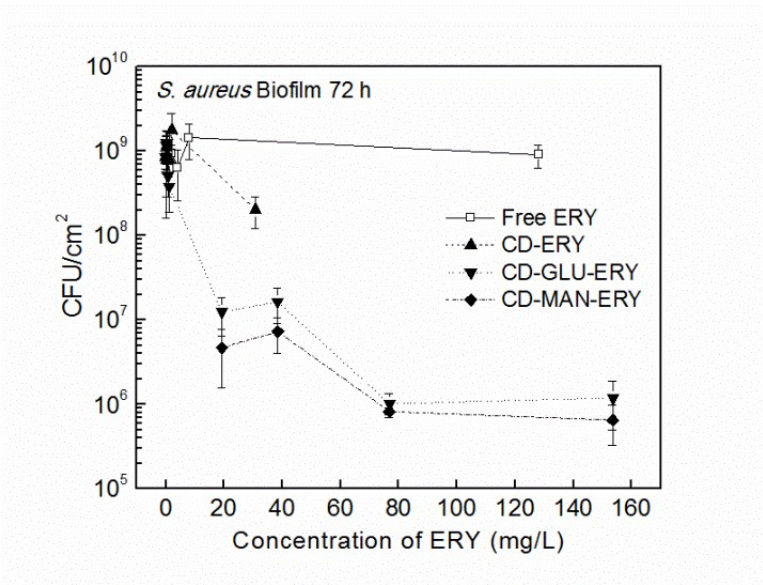


Figure S7 Number of adherent *S. aureus* cells in pre-formed 72 h mature biofilm on silicone film after treatment with different concentrations of free ERY, CD-ERY, CD-MAN-ERY and CD-GLU-ERY. Mature biofilm was pre-formed by incubating a silicone film in growth medium containing 1×10^7 CFU/mL for 72 h.

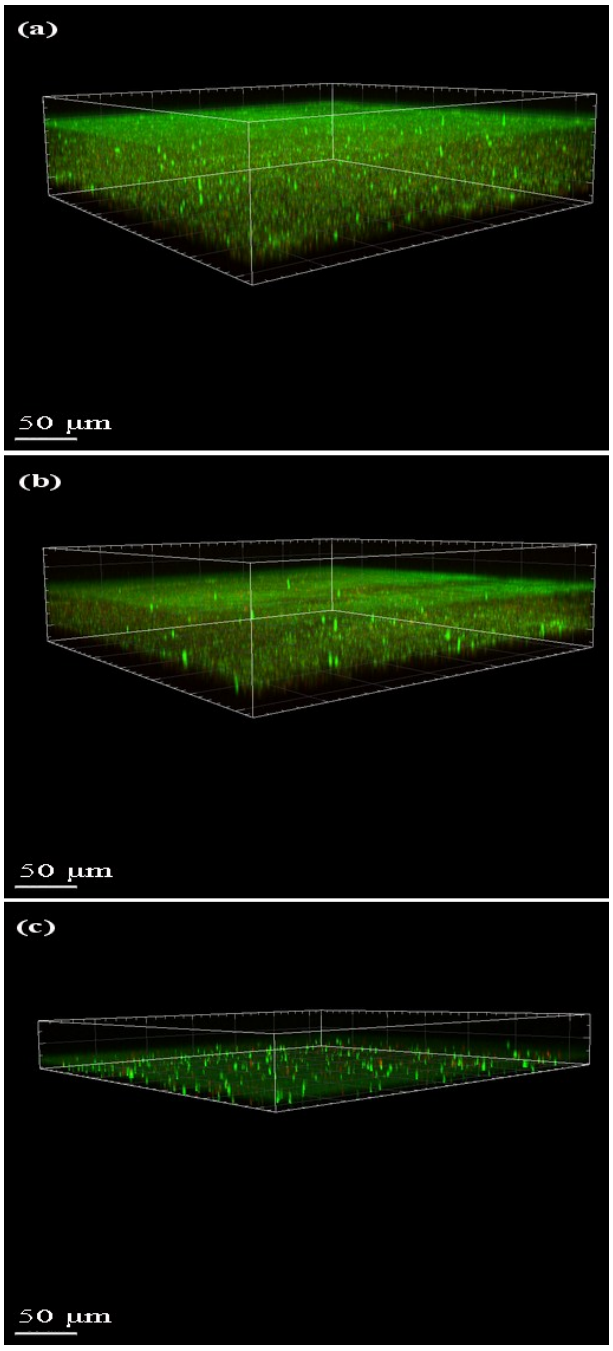


Figure S8 CLSM images of Live/Dead stained *P. aeruginosa* mature biofilm on silicone films after treatment with medium only (a), 8×MIC of CD-GLU-ERY (b) and 32×MIC of CD-GLU-ERY (e). Mature biofilm was pre-formed by incubating a silicone film in growth medium containing 1×10^7 CFU/mL for 72 h. Scale bar is 50 µm.

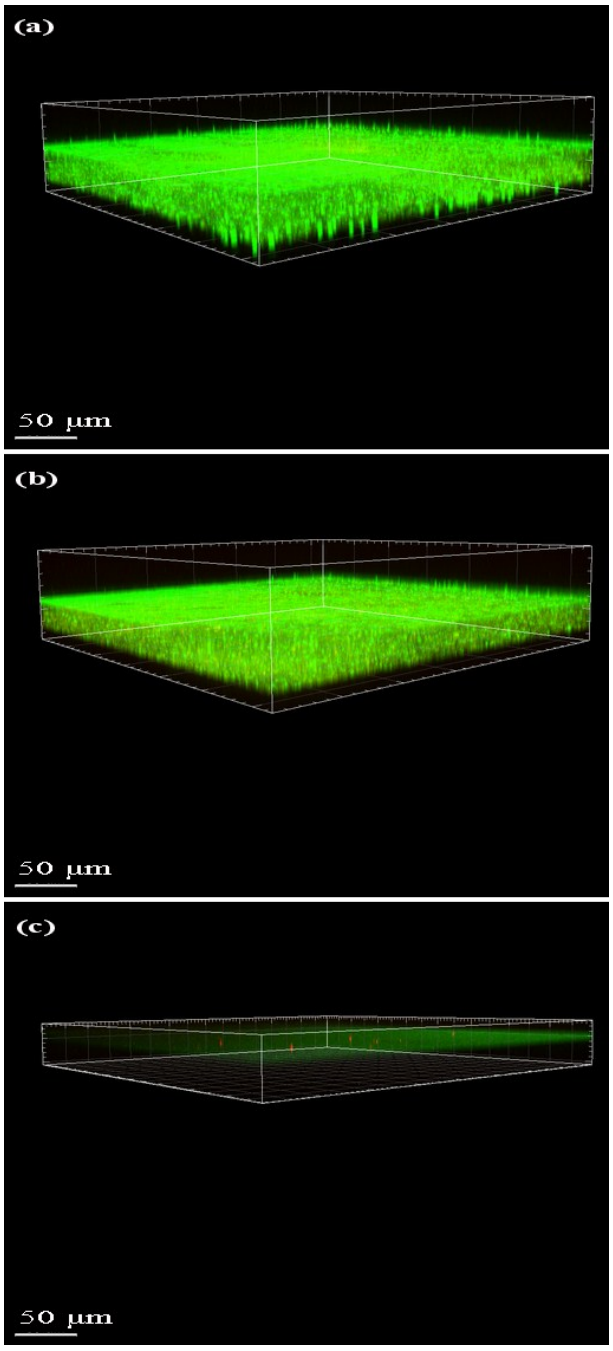


Figure S9 CLSM images of Live/Dead stained *S. aureus* mature biofilm on silicone films after treatment with medium only (a), 16×MIC of CD-GLU-ERY (b) and 256×MIC of CD-GLU-ERY (e). Mature biofilm was pre-formed by incubating a silicone film in growth medium containing 1×10^7 CFU/mL for 72 h. Scale bar is 50 µm.

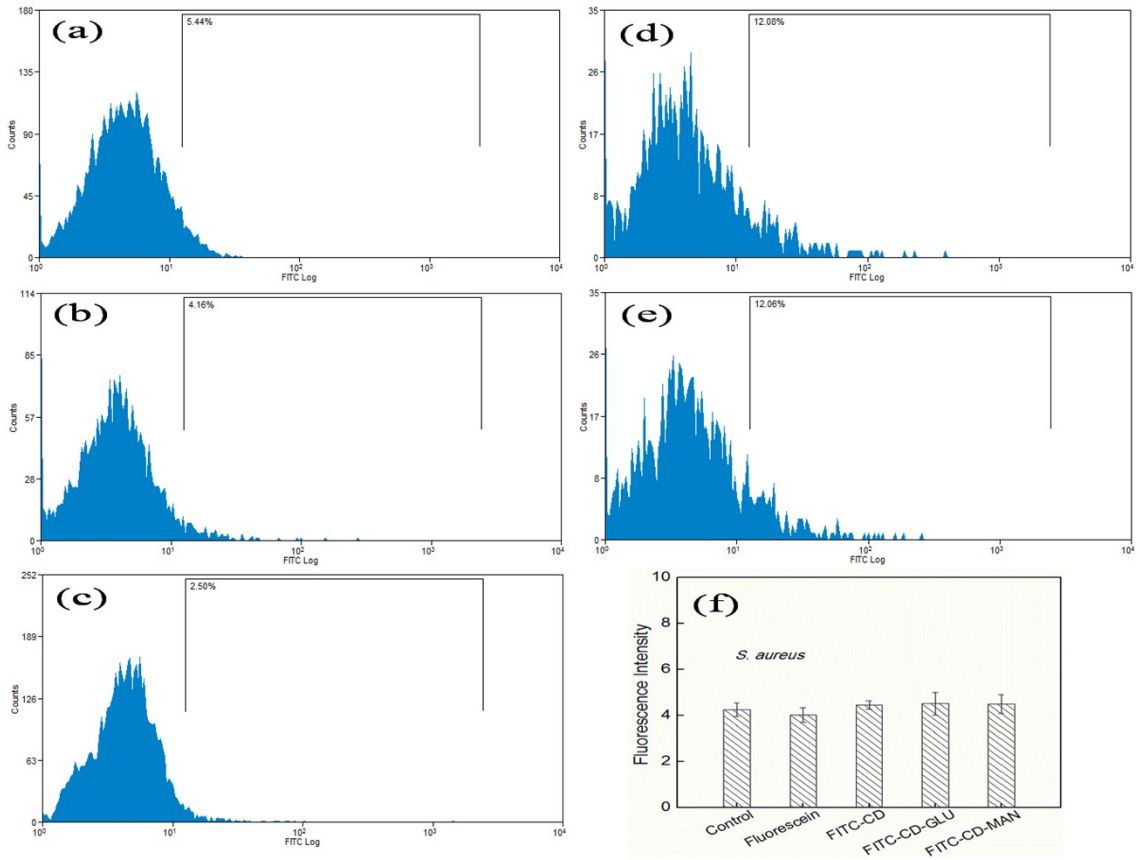


Figure S10 Flow cytometric assessment of *S. aureus* cells in mature biofilm: control (incubated with medium only) (a), labeled with free fluorescein (b), labeled with FITC-CD (c), labeled with FITC-CD-GLU (d) and labeled with FITC-CD-MAN (e). Plot of mean fluorescence intensity of the tested *S. aureus* cells (f).

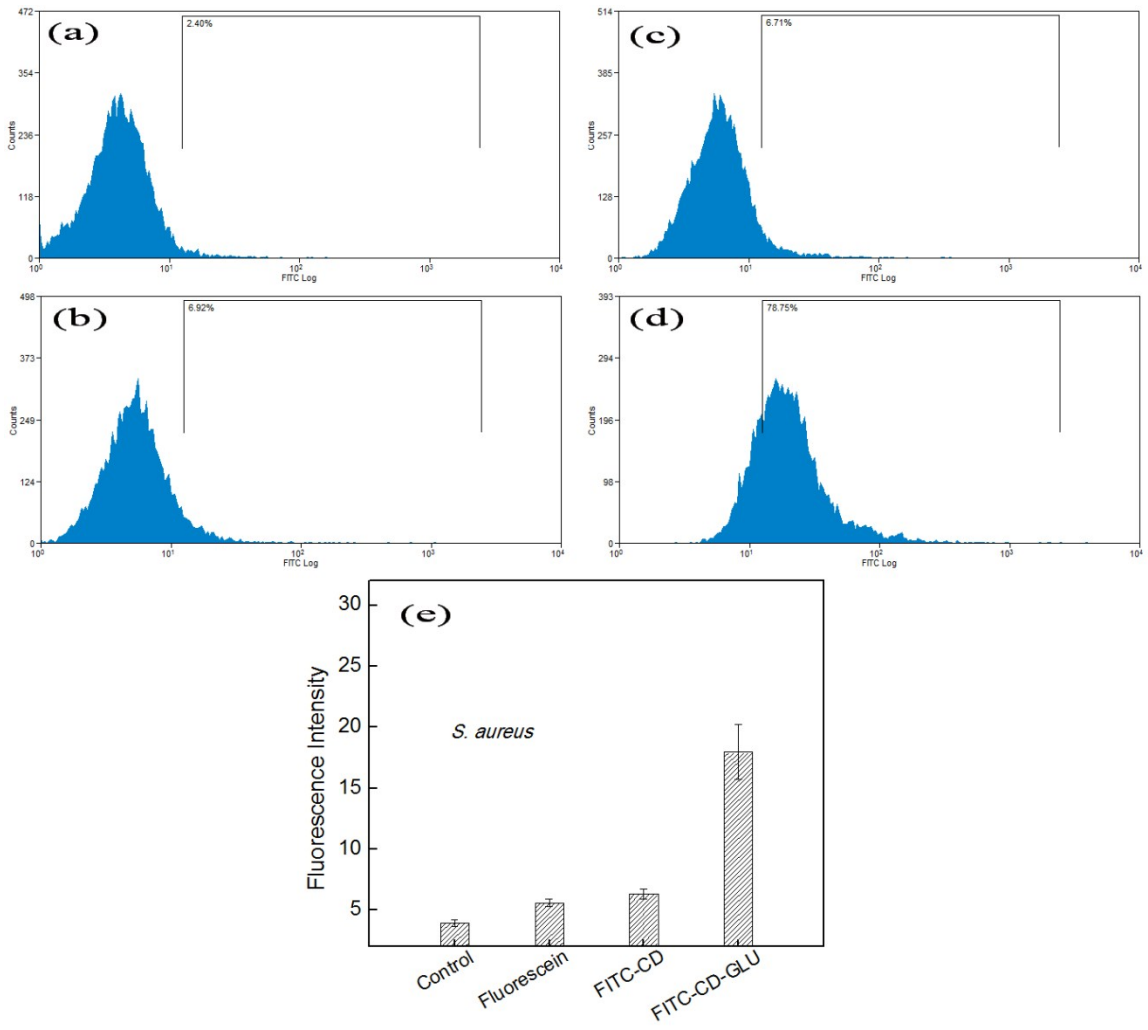


Figure S11 Flow cytometric assessment of planktonic *S. aureus* cells: control (incubated with medium only) (a), labeled with free fluorescein (b), labeled with FITC-CD (c) and labeled with FITC-CD-GLU (d). Plot of mean fluorescence intensity of the tested *S. aureus* cells (e).

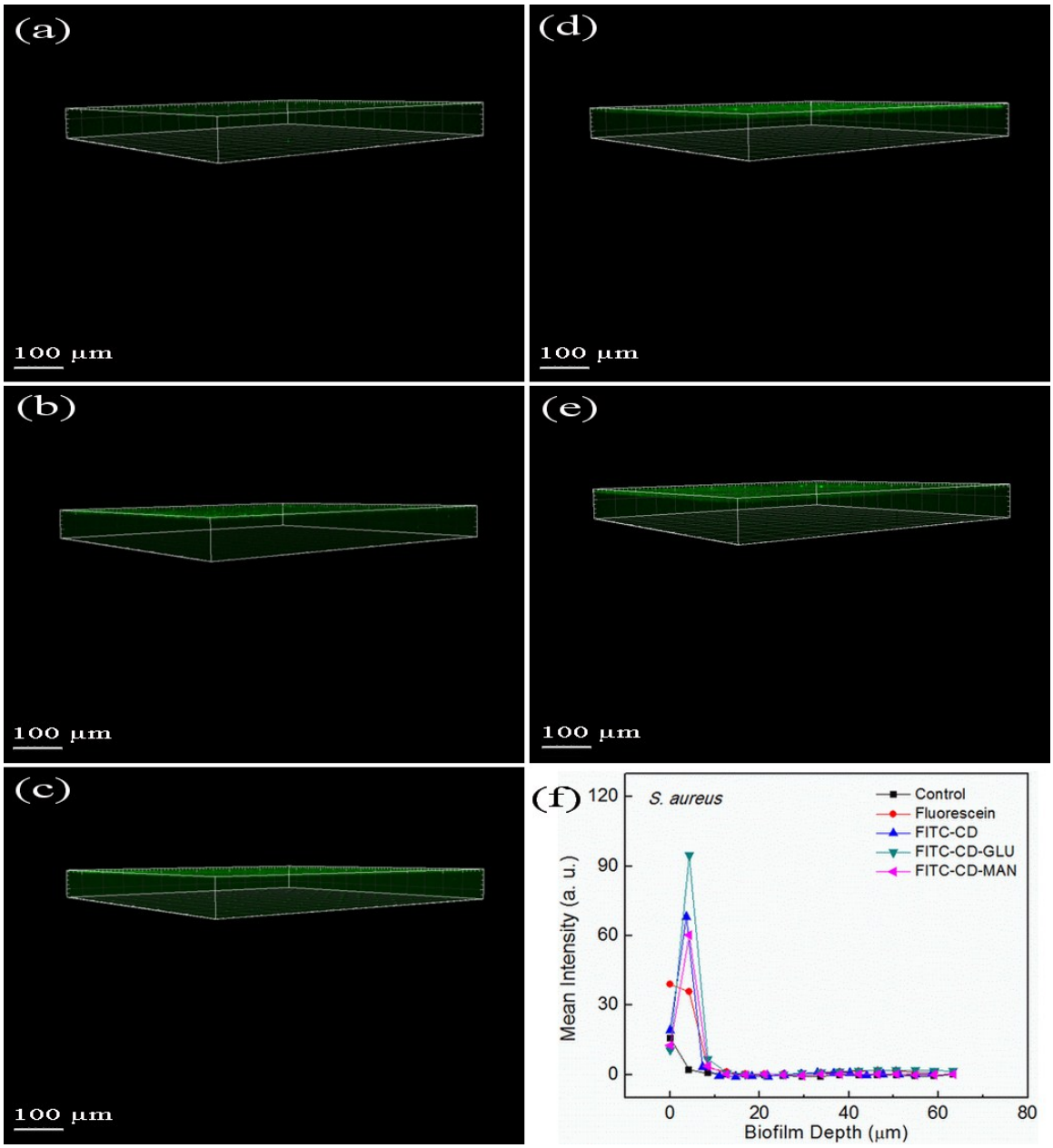


Figure S12 CLSM images of pre-formed 72 h *S. aureus* mature biofilm on silicone films after 24 h in medium only (a), and medium containing free fluorescein (b), FITC-CD (c), FITC-CD-GLU (d) and FITC-CD-MAN (e). Mature biofilm was pre-formed by incubating a silicone film in growth medium containing 1×10^7 CFU/mL for 72 h. Scale bar is 100 μm. Plot of mean intensity of the fluorescence per unit area against the depth into biofilm. The biofilm depth "0" refers to the top of biofilm (f).

Table S1. MIC of antibiotic in CD-GLU-antibiotic, CD-MAN-antibiotic, CD-antibiotic complex, and free antibiotic for *S. aureus* and *P. aeruginosa.*

Bacterium	**Gram**	**MIC (mg/L)**			
		ERY in CD-GLU-ERY	ERY in CD-MAN-ERY	ERY in CD-ERY	Free ERY
S. aureus ATCC 25923	Positive	0.075	0.075	0.12	0.5
P. aeruginosa PAO1	Negative	19.2	19.2	29.4	64
		RIF in CD-GLU-ERY	RIF in CD-MAN-ERY	RIF in CD-ERY	Free RIF
P. aeruginosa PAO1	Negative	4.92	4.92	12.99	32

Table S2. Hydrodynamic diameter (D_h) and zeta potential of CD-GLU and CD-MAN carriers and complexes with antibiofilm agent or antibiotic.

Sample	**D_h (nm)**	**Zeta potential (mV)**
CD-GLU	3.8±1.5	-3.51±0.19
CD-MAN	3.9±1.7	-3.85±0.45
CD-GLU-DMABI	15.0±13.0	-2.01±0.73
CD-MAN-DMABI	29.9±2.9	-3.18±0.96
CD-GLU-ERY	21.0±3.5	-3.4±0.77
CD-MAN-ERY	24.4±2.6	-3.77±3.35
CD-GLU-RIF	12.3±1.3	-11.02±4.62
CD-MAN-RIF	12.6±4.5	-8.05±5.22

References

1 P. R. Ashton, R. Koniger, J. F. Stoddart, D. Alker and V. D. Harding, *J. Org. Chem.*, 1996, **61**, 903-908.

2 Q. Zhang, S. Slavin, M. W. Jones, A. J. Haddleton and D. M. Haddleton, *Polym. Chem.*, 2012, **3**, 1016-1023.

3 A. Lancuski, S. Fort and F. Bossard, *ACS Appl. Mater. Interfaces*, 2012, **4**, 6499-6504.

4 K. Ladomenou, V. Nikolaou, G. Charalambidis and A. G. Coutsolelos, *Coord. Chem. Rev.*, 2016, **306**, 1-42.