Supporting Information

Antibacterial and Potentiation Properties of Charge-Optimized Polyrotaxanes for Combating Opportunistic Bacteria

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Supplementary Information Outline

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1. Materials and Instruments

α-Cyclodextrin (α-CD) and 1,1′-Carbonyldiimidazole (CDI) were purchased from VWR International; 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO), potassium bromide (KBr), Poly (ethylene glycol) 2000 (PEG 2000), N-Boc-ethylenediamine, ethanolamine, Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA) and Fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich. The free base form 1-adamantanamine was purchased from Alfa Aesar. Other chemicals were purchased from Fisher Scientific. All reagents were of experimental grade and used without further purification. Mouse macrophage/monocyte cell line J774A.1 was purchased from American Type Culture Collection (ATCC).

$^1$H NMR spectra were recorded using a Bruker Advance 400 MHz spectrometer with deuterated chloroform (CDCl₃) and deuterium dimethyl sulfoxide (d₆-DMSO) as the solvent. UV−Vis spectra were recorded with a Spectramax® Plus 384 Microplate Reader (Molecular Devices). Confocal laser scanning microscopy (CLSM) images of samples were captured with an LSM710 confocal microscope (Carl Zeiss). 3D-SIM super-resolution images were captured with Zeiss ELYRA S1(SR-SIM) Super Resolution Microscope. Transmission electron microscopy (TEM) was performed on a Philips/FEI Tecnai20 instrument with an acceleration voltage of 120 kV and particle size and Zeta potential were measured with a Zeta-sizer Nano ZS instrument (Malvern).

2. Methods

Cationic polyrotaxanes were synthesized following a previously reported procedure.$^{1-2}$

2.1 Synthesis of PEG-COOH

PEG-COOH was synthesized according to previous literature.$^{2-3}$ Briefly, PEG (5g, 2.5 mmol, Mw 2000), TEMPO (1.56g, 10mmol) and KBr (1.2 g, 10 mmol) were dissolved in 100 mL water. Next, 30 mL NaClO was added to the flask while stirring at room temperature for 10–15 min at pH 10–11. The oxidation was quenched by addition of 10 mL of ethanol, followed by acidification with HCl to pH < 2 and three extractions with 100 mL aliquots of chloroform. The combination of organic layers was dried over
anhydrous Na$_2$SO$_4$. The solvent was removed by rotary evaporator under reduced pressure. Final PEG-COOH was obtained after recrystallization from hot isopropyl alcohol and vacuum-drying gave PEG-COOH in 90% yield. $^1$H-NMR (CDCl$_3$ p.p.m) $\delta=3.4$–$3.8$ (m, CH$_2$CH$_2$O of PEG), 4.15 (s, CH$_3$CO).

2.2 Preparation of Polyrotaxane with PEG-COOH.
PEG-COOH (0.1 g, 0.05 mmol) was added to a saturated solution of $\alpha$-CDs (7.25 g per 50 ml dH$_2$O). The solution was stirred for 24 h at room temperature to obtain a white paste-like inclusion complex. The precipitate was collected by centrifugation and lyophilized. Next 1-Adamantanamine (0.39 g, 2.6 mmol), BOP reagent (1.15 g, 2.6 mmol), HOBt (0.35 g, 2.6 mmol) and DIEA (0.45 ml, 2.6 $\times$ 10$^{-3}$ mol) were dissolved in 4.5 ml DMF and the inclusion complex (2.0 g) was added to the solution. The suspension was stirred for 24 h at room temperature. Afterwards, the suspension was poured into 100 ml diethyl ether to precipitate the crude product and the precipitate was collected by centrifugation (3,000 rpm 20 min) and washed by stirring three times successively in abundant acetone, methanol, and water. Finally, the white precipitate was dried in vacuo to obtain the capped polyrotaxane in 70% yield (1.4 g). $^1$H-NMR (DMSO-d$_6$ p.p.m) $\delta=5.4$–$5.6$ (m, $\alpha$-CD of O(2)H and O(3)H), 4.75 (m, $\alpha$-CD of C(1)H), 4.49 (m, $\alpha$-CD of O(6)H), $\delta=3.8$–$3.2$ (m, C(3)H, C(5)H, C(6)H, C(2)H and C(4)H), 3.45 (s, CH$_2$CH$_2$O of PEG).

2.3 Conjugation of ligands to $\alpha$-CDs in the polyrotaxane.
PR (600 mg, 4.56 $\times$ 10$^{-5}$ mol) was dissolved in 20 ml dry DMSO and CDI (3.06 g, 1.89 $\times$ 10$^{-2}$ mol) was added to the solution. The mixture was stirred for 3 h under a nitrogen atmosphere and then precipitated in ether. The CDI modified PR was dissolved in 20 ml dry DMSO and N-Boc-ethylenediamine (6 ml) was slowly added. The mixture was stirred for 24 h at room temperature and then precipitated in ether. Boc groups were removed with concentrated HCl, dialyzed, and finally lyophilized. For the mixed ligands of N-Boc-ethylenediamine as the positive ligand (P) to ethanolamine as the neutral ligand, a ratio of 1:2 and 1:4 was used to prepare cPR$_2$ and cPR$_3$. Note that cPR$_0$
contained only neutral ligands and cPR$_1$ contained only positive ligands.

2.4 FITC labelled cPR$_2$.

cPR$_2$ was labeled with FITC through mixing 1 mg/mL cPR$_2$ solution with 0.01 mg FITC in DMSO. Excess dye was removed from labeled cPR$_2$ by dialysis against deionized water and final FITC-cPR$_2$ was collected after lyophilization.

Fig. S1 $^1$H NMR spectra of PR in DMSO–d$_6$ reveal that, on average, 16 $\alpha$-CDs were threaded per PR. For PEG2000, this corresponds to a theoretical threading efficiency of 72%.
Fig. S2 $^1$H NMR spectra of PR modified with N-Boc-ethylenediamine (Boc-EDA) in DMSO–d$_6$. Peaks of O(2)H, O(3)H and O(6)H groups seen previously in Figure S1 disappeared and implies that hydroxyl groups of CD were efficiently conjugated to the ligand.
3. Characterization of Materials

Fig. S3 TEM images of cPRs: cPR\(_0\) (A), cPR\(_1\) (B), cPR\(_3\) (C); scale bar: 100 nm.

Fig. S4 Dose response curves of the antimicrobial activity of cPRs against *P. aeruginosa* PAO1 (A), *E. coli* ATCC 25922 (B) and *S. aureus* ATCC 25923 (C) after 24 h incubation in MHB medium. Error bars are SD (n=3).
**Fig. S5** Dose response curves for evaluating the cytocompatibility of cPRs. (A) Cytotoxicity of cPRs in the J774A.1 mouse macrophage cells; all assays were performed in DMEM media supplemented with 10% fetal bovine serum and incubated at 37°C, 5% CO₂. Error bars are SD (n=3). (B) Hemolysis of cPRs; 1% Triton-X served as the positive control (100% hemolysis) and DPBS served as the negative control (0% hemolysis); error bars are SD (n=4).
Fig. S6 Additional 3D-SIM images of *E. coli* ATCC 25922 before (A) and after treatment with 200 µg/mL FITC-cPR$_2$ in MHB for 90 min (B–D). Scale bar: 2 µm.
Fig. S7 Dose-response curves investigating the antibiotic potentiation properties of cPR$_2$ with vancomycin, erythromycin, and rifampicin against *P. aeruginosa* PAO1. Error bars are SD (n=3).

Fig. S8 Dose-response curves investigating the antibiotic potentiation properties of cPR$_2$ with vancomycin, erythromycin, and rifampicin against *E. coli* ATCC 25922. Error bars are SD (n=3).
References

