Supplementary Information

Biomimetic detection of aminoglycosidic antibiotic using polydiacetylene-phospholipids supramolecules

Do Hyun Kang,‡[a,b] Ho-Sup Jung,‡[a] Namyoung Ahn,[a] Jiseok Lee,[d] Sungbaek Seo,[d] Kahp-Yang Suh,[a,b,c] Jinsang Kim*[d,e] and Keesung Kim*[a,c]

[a]*School of Mechanical and Aerospace Engineering, Seoul National University, Seoul, 151-742, Korea.*

[b]*Division of WCU Multiscale Mechanical Design, School of Mechanical and Aerospace Engineering, Seoul National University, Seoul 151-742, Korea.*

[c]*Institute of Advanced Machinery and Design, Seoul National University, Seoul 151-742, Korea. Fax: 82-2-889-6205; Tel: 82-2-883-7301; E-mail: keesung@snu.ac.kr*

[d]*Macromolecular Science and Engineering, University of Michigan, 2300 Hayward St., Ann Arbor, MI 48109-2136, USA.*

[e]*Materials Science and Engineering, Chemical Engineering, Biomedical Engineering, University of Michigan, 2300 Hayward St., Ann Arbor, MI 48109-2136, USA. E-mail: Jinsang@umich.edu*

‡ Equally contributed to this work.

Materials

Phospholipids, L-α-phosphatidylinositol-4,5-bisphosphate (PIP_2) and 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA) were purchased from Avanti Polar Lipids. Other chemicals such as a PDA monomer, 10,12-pentacosadiynoic acid (PCDA), neomycin, other antibiotics, solvents and buffers were purchased from Sigma-Aldrich Chemicals. Amine-coated glass slides for the microarray experiment were fabricated similarly to a reference.[16] Glass slides were cleaned with chloroform, acetone, and IPA for a 5 minute bath sonication each and activated in sulfuric acid with ammonium persulfate for a 1 hr bath sonication. After thorough washing with water and drying, the slides were dipped in toluene containing 1 wt% 3-

aminopropyltriethoxysilane toluene for 1 hr. After washing with toluene and subsequent drying, the slides were baked at 110 ^{o}C for 15 minutes and the unreacted silane molecules were removed by bath sonication in toluene, toluene:methanol (1:1), and methanol for 3 minutes each.

Liposome Assembly

As schematically illustrated in Scheme 1B, PDA-PIP_2 liposomes were assembled by the following thin film method. PCDA, DMPA, and PIP_2 lipids were dissolved in chloroform (7:2:1 molar ratio) to have the final concentration of 0.5 mM. After removing chloroform by thorough N_2 blowing, the lipids were suspended in 10 ml 0.1X PBS and 120W probe-sonicated at 80 ^{o}C for 10 minutes. The liposome solution was filtrated through a 0.8 μm cellulose acetate syringe filter and stored at 5 ^{o}C for 24 hr before use.

Aminoglycosidic Antibiotic Detection with PDA-PIP2 Liposome in Solution

0.5 mM PDA-PIP_2 liposome solution was polymerized by 254nm UV irradiation for 1 minute. 50 μM aminoglycosidic antibiotic solution was introduced into the solution. UV/Vis adsorption spectra taken from PerkinElmer Lambda 45 UV/Vis spectrometer were used to quantify the colorimetric response by means of CR values.[8a] Fluorescence spectra were obtained on PTI QuantamasterTM spectrofluorometer.

Microarray Experiment with PDA-PIP2 Liposome

0.5mM PDA-PIP_2 liposome solution was spotted on amine-coated glass slides by using a manual microarrayer (V&P scientific, INC) and incubated at above 70% humidity for 24 hrs to prevent droplet drying out too fast. The liposomes were covalently immobilized on amine coated glass slides through the carbodiimide chemistry between the carboxylic groups on the liposome surface and the amine groups on the substrate. (Or the liposomes were physically immobilized on amine coated glass slides with interaction between negative-charged liposome and positive-charged amine.) After washing with 0.1X PBS for 10 minutes followed by drying with N_2 blowing, the immobilized PDA-PIP_2 liposomes on the glass slides were subsequently polymerized by 254 nm UV irradiation for 1 minute. For detection tests, the glass slide was dipped into an antibiotics solution at 37 ^{o}C for 20 minutes. Fluorescence microscopic images were obtained on Olympus BX 71 microscope with mercury lamp and 540 nm excitation, 600 nm cut-off emission filters.

Fluorescence Spectra of the PDA-PIP_2 Liposomes upon Exposure to Aminoglycosidic Antibiotics

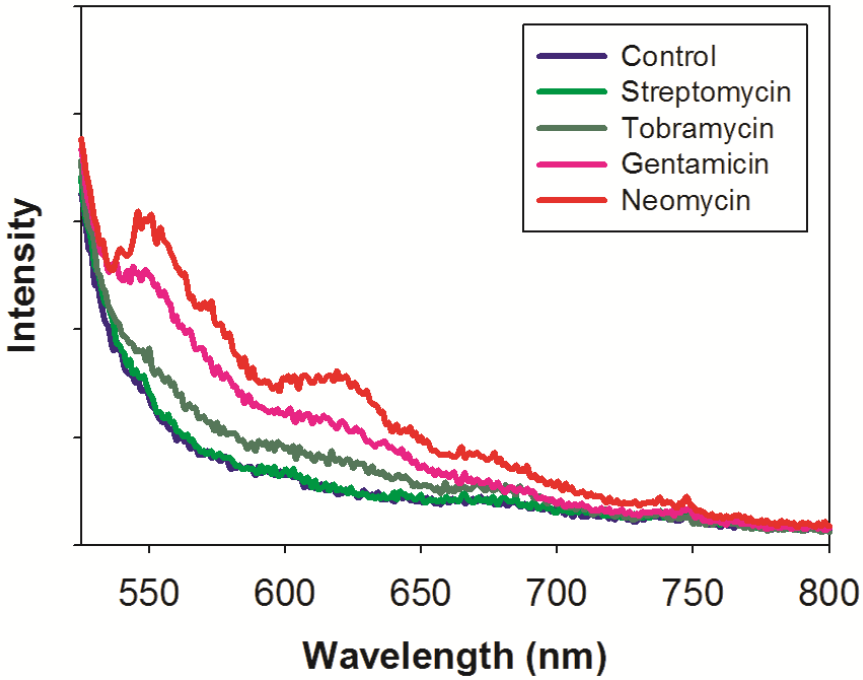

Figure S 1 Fluorescence Spectra of the PDA-PIP_2 Liposomes after 20-minute incubation with 50 μM concentration of various aminoglycosidic antibiotics at 37 ^{o}C and a physiological pH