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Biomimetic detection of aminoglycosidic antibiotics using polydiacetylene–phospholipids supramolecules†

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We rationally designed highly sensitive and selective polydiacetylene (PDA)–phospholipids liposomes for the facile detection of aminoglycosidic antibiotics. The detecting mechanism mimics the cellular membrane interactions between neomycin and phosphatidylinositol-4,5-bisphosphate (PIP_2) phospholipids. The developed PDA–PIP_2 sensory system showed a detection limit of 61 ppb for neomycin and was very specific to aminoglycosidic antibodies only.

Neomycin is a representative aminoglycosidic antibiotic prevalently used in hospitals and the livestock industry. The antibiotic mechanism of neomycin is based on the inhibition of protein synthesis by binding to a ribosomal RNA[1] and is very effective against most clinically harmful bacteria. However, neomycin is much more nephrotoxic compared to other aminoglycosides. Therefore, the abuse and misuse of neomycin can cause an allergic response,[2] organ damage (such as ear and kidney), and nerve system malfunction,[3] as well as the emerging super bacteria having a tolerance to antibiotics.[1] Consequently, many agriculture, food, and drug regulatory authorities such as World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) have set a tolerance limit of neomycin in meat and dairy products including milk and eggs. Accordingly, various detection strategies have been developed to detect neomycin including high performance liquid chromatography (HPLC),[4] enzyme-linked immunosorbent assay (ELISA),[5] aggregation based sensors,[6] and competitive impedimetric assay.[7] However, these techniques are complex, expensive, and often require highly sophiscated heavy equipment and skillful operation.

Polydiacetylene (PDA), a unique conjugated polymer, has been applied to various label-free and colorimetric sensory systems since PDA changes its color from blue to red upon exposure to various external stimuli such as heat,[8] mechanical stress,[9] ions,[10] chemicals,[11] bio-molecules,[12] and bacteria.[13] In addition to this convenient colorimetric self-signaling property, the transformed red phase PDA also emits red fluorescence, enabling the convenient and sensitive dual detection capability. The most commonly used and convenient form of PDA is liposome because amphiphilic PDA monomers can be easily designed and self-assembled into a liposome shape. After the self-assembly, PDA monomers in the liposomes are photopolymerized by 254 nm UV irradiation to become conjugated PDA having blue color.[14] Distortion of the conjugated yne–ene backbone of PDA by external stimuli is believed to cause the color change and the red fluorescence development.

PDA liposomes can accommodate various natural and/or synthetic lipids such as phospholipids. Molecular interactions *via* such a lipid inserted into a PDA liposome, for example, enzymes–lipids interactions,[12b] membrane permeabilization by antimicrobial peptides[12a,c] or bacterial toxin,[13c] and receptor–ligand interaction,[13b] have been investigated in various colorimetric biosensor development. We coined such a molecular interaction into a PDA sensory system design to selectively and sensitively detect possible residual neomycin in dairy products or meat. Neomycin is known to bind to phosphatidylinositol-4,5-bisphosphate (PIP_2) lipids in the cellular membrane.[15] Molecular and cellular biology research revealed that PIP_2 decomposes into diacylglycerol (DAG) and 1,4,5-triphosphate (IP_3) by phospholipase C (PLC) through stimulating various hormones and growth factors.[16] As shown in Scheme 1A, neomycin binds to PIP2, inhibiting the PIP_2 degradation by PLC and thus inhibiting the IP_3-related signal cascade, which is a known side effect of neomycin.[15] In our convenient colorimetric PDA sensor design, as schematically described in Scheme 1C PIP_2 was used as a selective receptor to detect neomycin and co-assembled into a PDA liposome (PDA–PIP_2). As designed and anticipated, the interaction between neomycin and PIP_2 exerted stress on the conjugated backbone of PDA–PIP_2 and consequently produced a sharp color change and fluorescence development as dual sensory signals.

The PDA–PIP_2 liposome containing PIP_2 showed a sharp color change upon exposure to a neomycin solution as shown

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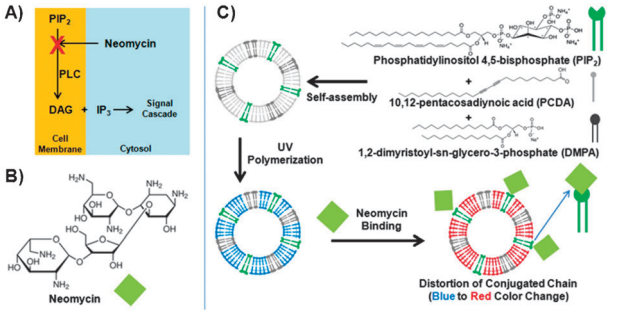

Scheme 1 (A) Schematic illustration of the inhibition of the PIP_2–PLC signal pathway by neomycin binding to PIP_2 lipid. (B) Chemical structure of neomycin. (C) Schematic illustration of a neomycin detection mechanism by the designed PDA–phospholipids liposome including PIP_2 phospholipids as a specific receptor toward neomycin.

in Fig. 1A. The optimized molar ratio of three components of the PDA–PIP_2 liposome was 7 : 2 : 1 (PCDA : DMPA : PIP_2) as Fig. 1B shows that the sensitivity at that composition is highest. The role of DMPA in our liposome design is to enhance the sensitivity by making the PDA backbone in the liposome more mobile.[13d] PDA liposomes having no PIP_2 did not show any noticeable color change as presented in Fig. 1B, indicating that the recognition of neomycin originates from PIP_2 in the PDA–PIP_2 liposome. The affinity between neomycin and PIP_2 stems from the charge–charge interaction between negative charges on the PIP_2 head group and positive charges of neomycin (Scheme 1).[15,17] The neomycin–PIP_2 complex formation exerts stress on the conjugated backbone of PDA either due to the steric hindrance between surface-bound adjacent neomycin or the reconstruction of the liposome surface induced by charge alteration.

We further developed a more convenient solid-state sensory system based on the solution results.[14] A PDA–PIP_2 microarray was fabricated by spotting the PDA–PIP_2 liposome on an amine-coated glass slide. Fig. 2A shows fluorescence microscope images of the PDA–PIP_2 liposome microarrays before and after a 20 minute exposure to a 50 μM neomycin solution. The fluorescence emission intensity of the liposome spot became stronger as the concentration increased (Fig. 2B). We could develop a good correlation between the fluorescence signal intensity and the neomycin concentration as plotted in Fig. 2C. The detection limit of the present system is 0.1 μM (61 ppb), which is good enough to detect the regulation limits

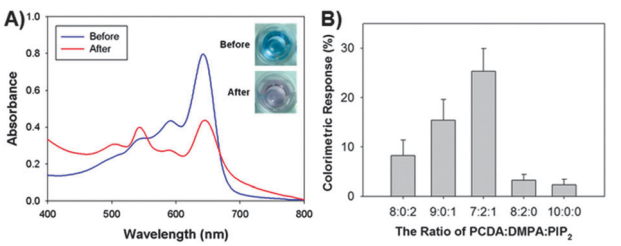

Fig. 1 (A) UV-vis spectra and corresponding optical images of a PDA–PIP_2 liposome solution after 20 minute of incubation with 50 μM neomycin at 37 °C. (B) Optimization of the mole ratio of PCDA : DMPA : PIP_2 in the PDA–PIP_2 liposome. Colorimetric response was measured after 20 minute incubation with 50 μM neomycin at 37 °C.

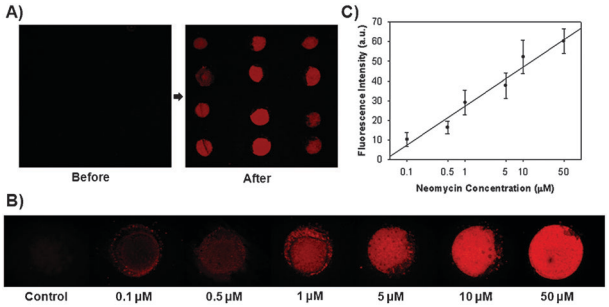

Fig. 2 (A) Fluorescence microscope images of PDA–PIP_2 liposome arrays before and after 20 minute reaction with 50 μM neomycin at 37 °C. (B) Enlarged fluorescence microscope images of PDA–phospholipid liposome spots after 20 minute incubation with various concentrations of neomycin from 0.1 to 50 μM at 37 °C. (C) Correlation curve between the fluorescence intensity and the concentrations of neomycin.

of neomycin defined by WHO and FAO (500 ppb in meat and egg and 1500 ppb in milk).

We extended our detection study to other aminoglycosidic antibiotics because PIP_2 lipids are known to bind to other aminoglycosidic antibiotics that have a similar chemical structure to neomycin.[15b,c] As anticipated, the PDA–PIP_2 liposome also developed a certain level of a fluorescence sensory signal to other aminoglycosidic antibiotics such as gentamicin, tobramycin, and streptomycin as shown in Fig. 3 and Fig. S1 (PL spectra, ESI†). The signal intensity for other aminoglycosidic antibiotics, however, was much weaker and showed an interesting trend. As one can see in Fig. 3A, the signal intensity displays an exponential drop rather than a linear decline as the net charge[18] of the aminoglycosidic antibiotics decreases. The signal intensity is likely a product of the molecular size as well as the charge density of the aminoglycosidic antibiotics. Neomycin having the largest charge density among the aminoglycosidic antibiotics also has the largest molecular weight of 614.64 g mol^{-1} compared to gentamicin (477.60), tobramycin (467.52), and streptomycin (581.57). Therefore, the larger charge density will cause stronger interaction with the PDA–PIP_2 liposome and the larger size will induce greater stress and a brighter sensory signal.

We also tested non-specific binding of non-aminoglycosidic antibiotics such as penicillin G, oxytetracycline, and sulfamethazine to the PDA–PIP_2 microarray. As shown in Fig. 4,

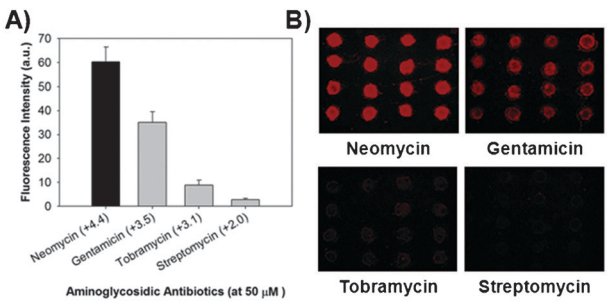

Fig. 3 (A) Fluorescence emission intensity and (B) fluorescence microscope images of the PDA–PIP_2 liposome microarrays after 20 minute incubation with 50 μM concentration of various aminoglycosidic antibiotics at 37 °C and a physiological pH. The net charge of each aminoglycosidic antibiotic is given in the parentheses.

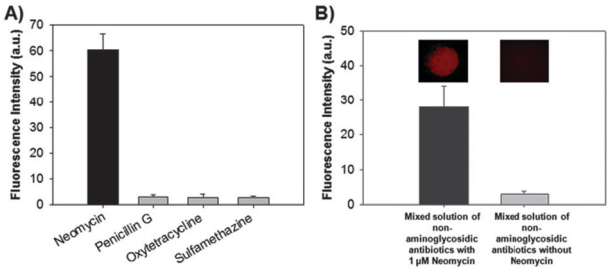

Fig. 4 (A) Fluorescence intensities of the PDA–PIP_2 liposome microarray after 20 minute reaction with 50 μM concentration of neomycin and non-aminoglycosidic antibiotics at 37 °C. (B) Fluorescence intensities and corresponding microscope images of the PDA–PIP_2 liposome microarray after 20 minute incubation with a mixed solution of 50 μM of non-aminoglycosidic antibiotics having or without having 1 μM of neomycin at 37 °C.

these antibiotics, having a different chemical structure from the aminoglycosidic antibiotics, did not produce any sensory signals, which confirmed a good selectivity of the developed PDA–PIP_2 sensory system toward aminoglycosidic antibiotics. Furthermore, we found that even a cocktail solution of 50 μM penicillin G, oxytetracycline, and sulfamethazine do not hinder the specific interaction between neomycin and the PDA–PIP_2 liposome. While the cocktail without having neomycin did not produce any noticeable signal generation, the same cocktail having 1 μM neomycin generated a fluorescence signal as demonstrated in Fig. 4B.

We developed a PDA-based biomimetic colorimetric sensory system for the detection of aminoglycosidic antibiotics by adapting the interaction between PIP_2 lipids and aminoglycosidic antibiotics into our PDA sensor design. Binding of aminoglycosidic antibiotics to the PDA–PIP_2 liposome imposes stress to the conjugated yne–ene PDA backbone and produces an ensuing color change and develops fluorescence emission as a dual sensory signal. The most commonly used neomycin has the high cation density among the aminoglycosidic antibiotics. Hence, the designed PDA–PIP_2 liposome microarray showed the best detection limit of 61 ppb for neomycin. The developed PDA–PIP_2 liposome microarray also displayed good sensitivity toward aminoglycosidic antibiotics. Non-aminoglycosidic antibiotics such as penicillin G, oxytetracycline, and sulfamethazine did not cause any false signaling nor hindered the detection capability of the PDA–PIP_2 liposome microarray for neomycin. We believe that the presented biomimetic sensory design principle can be readily applicable to the detection of various other biomolecules and the investigation of bio-interfacial phenomena.

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