Supporting information for

Structure-Guided Aggregation-Regulated and Coordination-Assisted Zn–Porphyrins for Decoding Aminoglycoside Recognition in Real-Life Samples

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Experimental Section

General Information: All chemicals, including starting materials, reagents, and substances, were purchased from local chemical suppliers and used as received without additional purification. Solvents were distilled and dried before their use.

FTIR Spectroscopy: FTIR spectra were recorded using a Perkin-Elmer FT-IR Spectrum BX system, and the results are reported in wave numbers. (cm^{-1})

NMR Spectroscopy: ^1H NMR spectra were acquired with a Bruker Advance DRX 400 spectrometer operating at 400 MHz for ^1H NMR. Chemical shifts are reported in ppm relative to the internal standard, tetramethylsilane (TMS).

Mass Spectrometry: Mass spectra were obtained using a Micromass Q-TOF Micro TM spectrometer.

Spectroscopic Studies: UV-Vis Spectroscopy: UV-Vis spectroscopic measurements were conducted on a Shimadzu model 2100 spectrometer. The slit-width used for the experiment was set to 5 nm.

Scanning Electron Microscopy. The samples were made under dust-free conditions and drop-cast over double-sided tapes attached to the brass stubs. Then the stubs were air-dried for 48 h. The coatings with gold vapor were done before analyzing the samples on a Quanta 200 SEM operated at 15 kV.

Dynamic Light Scattering Studies (DLS): DLS measurements were done using a Malvern Zetasizer NanoZS particle sizer (Malvern Instruments Inc., MA) instrument. Samples were prepared in water and examined under dust-free conditions. Reported mean hydrodynamic diameters were obtained from Gaussian analysis of the intensity-weighted particle size distributions.

Detection limit determination: The method used for the calculation of the detection limit is known as the blank variability method. In this method, the calibration curve was prepared by recording the fluorescence spectra of compound **1** in a water medium with different amounts of Neomycin. From the equation obtained from the calibration plot, the added Neomycin concentrations were calculated. Then another calibration curve was drawn between the C_{real} (added Neomycin, μM) vs. $C_{calc.}$ (Calculated amount of Neomycin, μM). This afforded a value of the slope (b). The fluorescence of compound **1** in water without added Neomycin was taken as a blank reading. A total of 10 replicates of the blank were measured. The standard deviation from the blank readings was calculated by fitting the fluorescence reading into the equation obtained from the first calibration curve (titration spectra). Using this standard deviation value, we calculated decision limit by this following equation.

$L_C = t_C \times s \times (1 + 1/N)1/2$……..……... (1)

where, N = the number of blank replicates taken; the value of tc for 10 blank readings is 1.833; and s = the standard deviation. The detection limit (L_D) was calculated as the double of the decision limit obtained,

$L_D = 2\ L_c$ ……. (2)

In concentration term, the detection limit appeared as,

$x_D = 2 \times C = 2\ L_C / b$ ……... (3)

where, b = slope of the calibration curve (C_{real} vs. $C_{calc.}$).

Synthesis of the probe molecules:

Zn(II)-porphyrin derivatives (Compounds 1–3) were synthesized via acid-catalyzed condensation of lpyrrole derivative with the corresponding substituted aromatic aldehydes, followed by DDQ oxidation and subsequent Zn (II) insertion using $Zn(OAc)_2$, following a modified literature-reported protocol. (**Chem. Eur. J. 2005, 11, 3427–3442)**

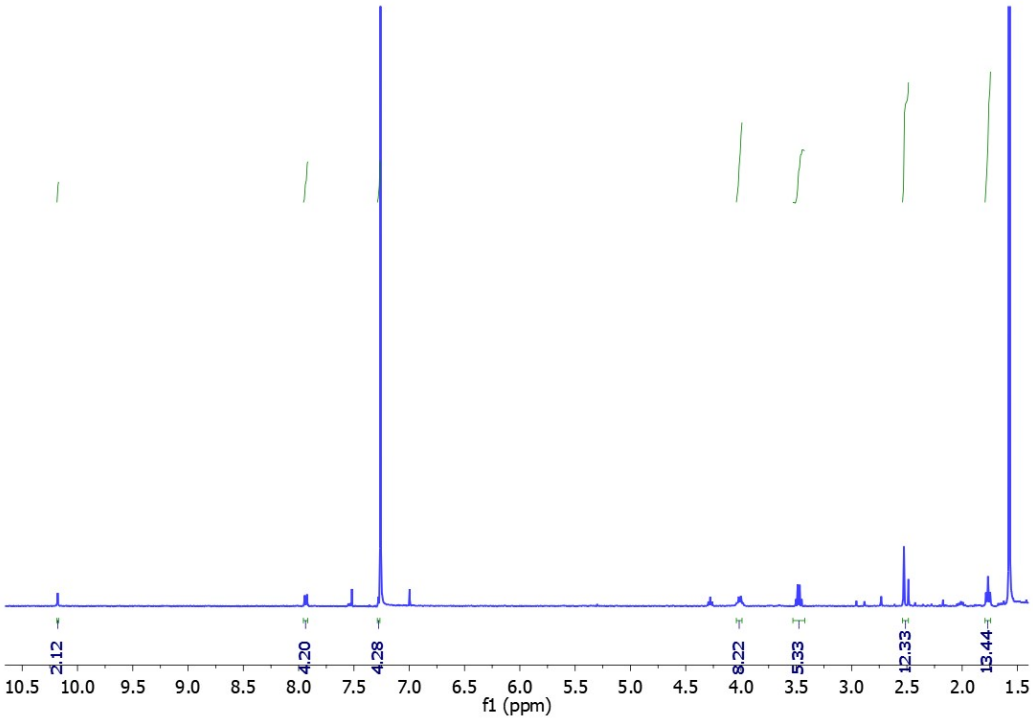
Characterisation

Compound-1: Chemical Formula: $C_{46}H_{48}N_4Zn$ Exact Mass: 720.3, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 2H), 7.96 – 7.92 (m, 4H), 7.27 (d, *J* = 5.9 Hz, 4H), 4.01 (d, *J* = 7.8 Hz, 8H), 3.48 (dt, *J* = 14.0, 5.7 Hz, 6H), 2.51 (d, *J* = 16.1 Hz, 12H), 1.77 (t, *J* = 7.6 Hz, 12H).

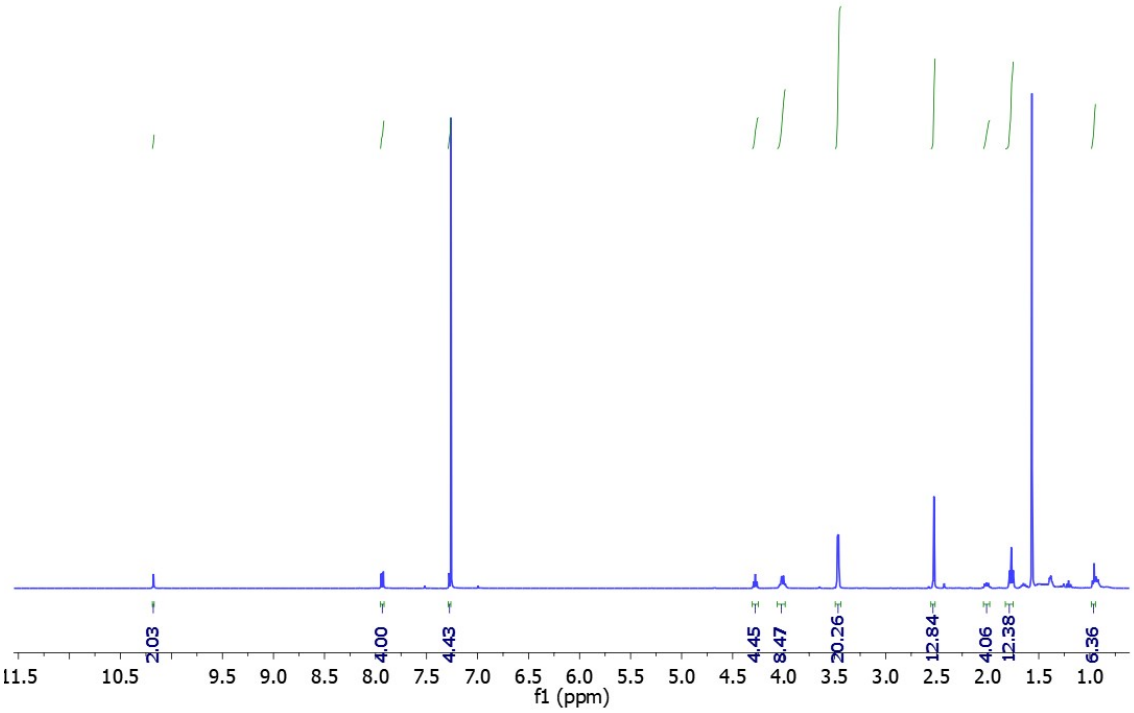
Compound-2: Chemical Formula: $C_{60}H_{76}N_4O_2Zn$, Exact Mass: 948.54, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 2H), 7.95 – 7.92 (m, 4H), 7.29 – 7.26 (m, 4H), 4.28 (t, *J* = 6.6 Hz, 4H), 4.06 – 3.99 (m, 8H), 3.47 (t, *J* = 5.2 Hz, 20H), 2.53 (d, *J* = 3.9 Hz, 12H), 2.01 (dd, *J* = 9.3, 5.8 Hz, 4H), 1.77 (t, *J* = 7.5 Hz, 12H), 0.96 (dd, *J* = 7.3, 4.6 Hz, 6H).

Compound-3: Chemical Formula: $C_{60}H_{76}N_4Zn$, Exact Mass: 916.5, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (d, *J* = 4.4 Hz, 2H), 7.95 (d, *J* = 1.8 Hz, 4H), 7.81 (t, *J* = 1.8 Hz, 2H), 4.02 (q, *J* = 7.3 Hz, 8H), 2.45 (s, 12H), 1.78 (t, *J* = 7.6 Hz, 12H), 1.51 (s, 36H).

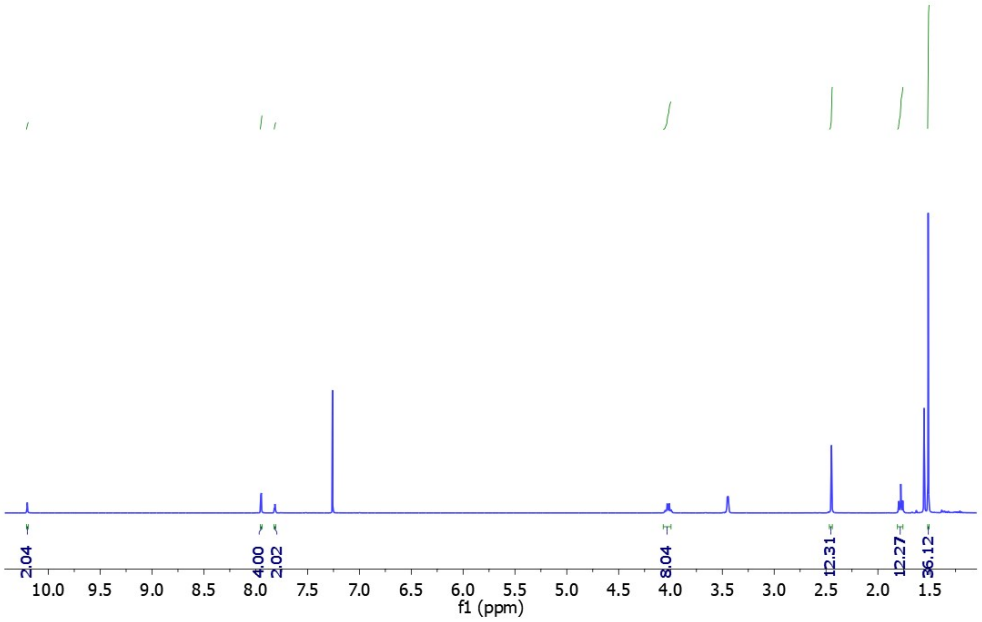
NMR Data



Partial NMR spectra of compound 1 in the $CdCl_3$ medium.



Partial NMR spectra of compound 2 in the $CdCl_3$ medium.



Partial NMR spectra of compound 3 in the $CdCl_3$ medium.

Additional Data

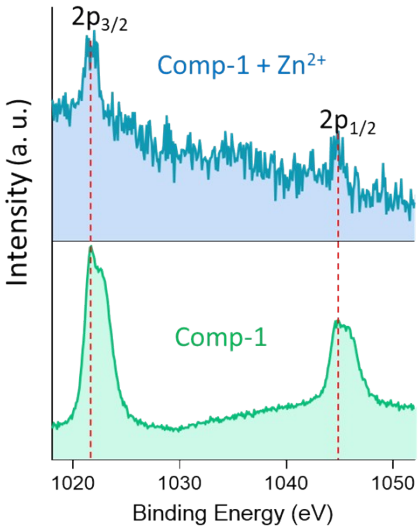


Figure S1: High-resolution Zn 2p XPS spectra of compound 1 before and after interaction with neomycin. The characteristic Zn $2p_{3/2}$ and Zn $2p_{1/2}$ signals show noticeable changes in their spectral profiles and local electronic environments upon neomycin addition, supporting coordination-assisted interactions between amino functionalities and the Zn^{2+} center of the porphyrin framework.

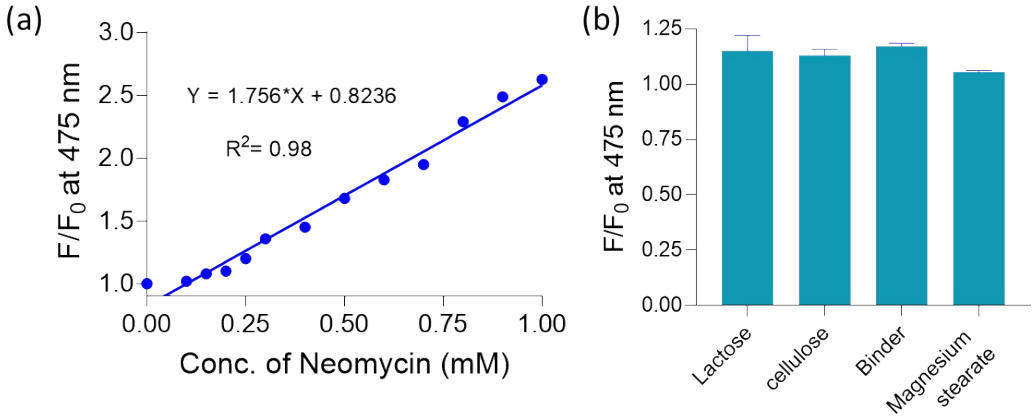


Figure S2: (a) Linear correlation between the normalized fluorescence response (F/F_0) of compound 1 at 475 nm and neomycin concentration in aqueous medium (pH 7.4) (b) Normalized fluorescence response (F/F_0) of compound 1 at 475 nm in the presence of common pharmaceutical excipients, including lactose, cellulose, binder, and magnesium stearate.

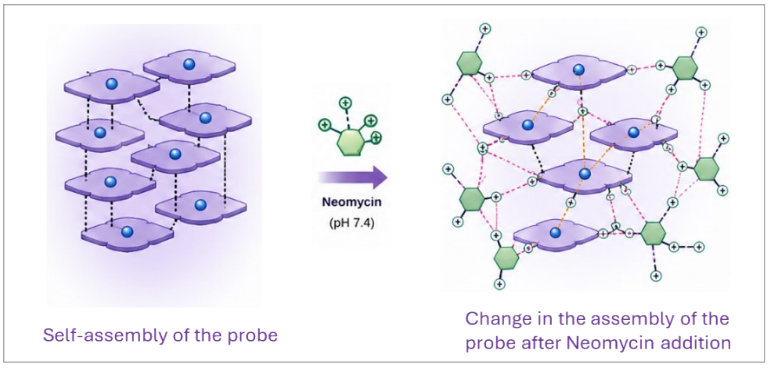


Figure S3: Change in the aggregation of the probe upon interaction with Neomycin.

Method	LOD (μM)	Recovery (%)	Detection Time	DOI
STAR (microbial inhibition)	2439.024	N/A	6-8 hours	10.17221/91/2011-CJFS
One-step ELISA	0.130	85-110	1.5 hours	10.1080/09540105.2011.569882
LC-MS/MS	5.691	N/A	3-4 hours	No DOI (kjvr-56-4-233)
ELISA/Immunochromatographic	4.439	N/A	20 min	10.1016/j.bios.2005.06.004
HPLC (RP-IP)	243.902	94 (CV 6.5%)	2 hours	10.1080/01483918908049520
Copan Milk Test/Delvotest MCS	<24.390	N/A	4-6 hours	10.3168/jds.2009-2412
RNA aptasensor	36.260	91.7-109	45 min	10.1016/j.foodcont.2015.03.026
Lateral flow immunoassay	5.854	N/A	10 min	10.3390/bios10050086
ELAA kit	1.805	100% specificity	1 hour	No DOI (VJBT)
Porphyrin-based fluorescence sensor	0.75	NA	5 Mins	This Work

Table 1: Comparison of different competitive detection techniques for the detection of neomycin residues in milk samples.