**Electronic Supplementary Information**

# Electrochemical impedimetric aptasensors based on hyper-cross-linked porous organic frameworks for determination of kanamycin

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## 1. Experimental section

### *1.1. Materials and methods*

All chemical solvents and reagents in this work were purchased from commercial sources and used without purification. The human serum with the purity of 99% is achieved from Solarbio. The sequence of kanamycin aptamer is 5'-TGG GGG TTG AGG CTA AGC CGA-3', which is synthesized and purchased by Shanghai Sangon Biotechnology Co., Ltd. The special aptamer has inherent binding affinity and specificity for kanamycin, which was selected by the SELEX approach. Thermogravimetric analysis (TGA) were measured on a Shimadzu DTG-60A thermal analysis machine in the temperature range from room temperature to 800 °C in air. Fourier-transform infrared (FT-IR) spectra with KBr pellets were measured on a Bruker ALPHA-T instrument. Powder X-ray diffraction (PXRD) data were taken on a Rigaku D/max-2500 diffractometer based on a Cu-$K_\alpha$ radiation with the $2\theta$ value from 5° to 50° at room temperature. $N_2$ sorption measurements were collected on a surface area ASAP 2020 analyzer at 77 K. X-ray photoelectron spectroscopy (XPS) were performed on an AXIS Ultra DLD X-ray photoelectron spectroscopy from Shimadzu. $^{13}C$ solid-state NMR spectra were measured on a Varian Infinity-plus 400 MHz spectrometer. The morphology was measured by using FEI Tecnai $G^2$ F20 transmission electron microscope (TEM) and Nova Nano SEM 230 scanning electron microscope (SEM).

### *1.2. Fabrication of electrochemical aptasensors*

These as-synthesized POFs were ground strongly in an agate mortar to decrease size and well dispersed in ethanol to obtain suspensions (1.0 mg·mL$^{-1}$) before fabricating POFs-based electrochemical aptasensors. Bare Au electrodes (AE) were polished by using the water

slurries of aluminum powder with the grain diameter of 0.3 and 0.05 μm, then immersing in a mixed solution of $H_2O_2$ (3 mL) and $H_2SO_4$ (7 mL) for 20 min. The AE was further ultrasonicated in a 6 mL mixed solution of water and ethanol (V/V = 1/1) for another 20 min. Phosphate buffer solution (PBS) was obtained by dissolving $KH_2PO_4$ (0.242 g), KCl (0.200 g), $Na_2HPO_4$ (1.445 g), and NaCl (8.003 g) in distilled water; meanwhile, the pH value of PBS was regulated to ~7.4 by adding NaOH (0.1 M) or HCl (0.1 M) aqueous solutions. $K_3Fe(CN)_6$ (1.650 g) and $K_4Fe(CN)_6$ (2.111 g) were both dissolved in 1 L PBS to prepare an electrolyte solution. The POFs suspension was slowly added and covered on the surface of clear AE, and then dried at room temperature for 8 hours to generate POF/AE. The POF/AE was soaked motionlessly in the aptamer aqueous solution to achieve apt/POF/AE. During this synthetic process, it can not only wipe off POFs with the betainteraction but also guarantee aptamers on the surface of POFs/AE until saturated. The apt/POF/AE was incubated in different kanamycin concentration solutions for electrochemical tests to fabricate kana/apt/POF/AE. The unused aptasensors were stored in a refrigerator at –20 °C.

*1.3. Kanamycin detection by electrochemical measurements*

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were performed on an electrochemical work station (Shanghai Chenhua CHI660D). All measurements were carried out by using a three-electrode system, including an Ag/AgCl reference electrode with saturated KCl solution, an Au work electrode with 3 mm diameter, and a platinum slide as counter electrode, respectively. The electrochemical measurements are similar with the reported work.[1–3] EIS results were collected in a mixed solution of $[Fe(CN)_6]^{3-/4-}$ (0.5 mM) and KCl (0.1 M) in the frequency range from 100 kHz to 0.1 Hz

with EIS parameters of open circuit potential and amplitude 5 mV. The Zview2 software was applied to analysis the detected signals. In addition, CV data were measured from –0.2 V to 0.8 V *vs* Ag/AgCl at a 100 $mV \cdot s^{-1}$ scan rate. The apt/POF/AE aptasensor was carefully incubated in the kanamycin aqueous solution for 2 h in a refrigerator at 4 °C before the electrochemical tests to estimate the detection capability. All data were confirmed by the average value of three parallel experiments. The selectivity of apt/POF/AE aptasensor toward kanamycin was investigated by soaking apt/POF/AE in chloramphenicol (CPL), thiamphenicol (THI), oxytetracycline dihydrate (OTC), sulfamethazine (SMZ), and ornidazole (ODZ) for 2 h in a refrigerator at 4 °C. These interferences are at the concentration of 1 $ng \cdot mL^{-1}$, which is as much as $1.0 \times 10^8$ times than the kanamycin concentration of $1.0 \times 10^{-5}$ $pg \cdot mL^{-1}$. The repeatability of the apt/POF-3/AE can be appraised by five fabricated aptasensors. The stability of this fabricated aptasensor was continuously kept at a refrigerator at –20 °C for 29 days by collecting the corresponding ESI data every day. Meanwhile, raw milk and human serum were both directly employed to investigate the real detectability of kanamycin.

*1.4. Amount of POF-3 on AE*

Different amounts of POF-3 suspension (1 $mg \cdot mL^{-1}$) were coated on the surface of bare AE and then dried at room temperature for 8 hours. The amount of POF-3 suspension is 1, 4, 7, 10, and 13 μL, respectively. The most appropriate dosage is 10 μL for preparing the POF-3/AE.

*1.5. Incubation time of the POF-3/AE in the aptamer solution*

The POF-3/AE was incubated in the aptamer (10 $ng \cdot mL^{-1}$) solution to fabricate the

apt/POF-3/AE. The immersing time is 30, 60, 90, 120, and 150 min, respectively. The POF-3/AE can reach up to the saturated adsorption capacity of aptamer after 120 min.

*1.6. Incubation time of the POF-3/AE in the kanamycin solution*

The apt/POF-3/AE was immersed in the kanamycin solution at the concentration of $1.0 \times 10^{-5}$ pg·mL$^{-1}$ for electrochemical measurements. The immersing time of apt/POF-3/AE is 30, 60, 90, 120, and 150 min, respectively. The best immersing time of apt/POF-3/AE is 120 min in the kanamycin solution.

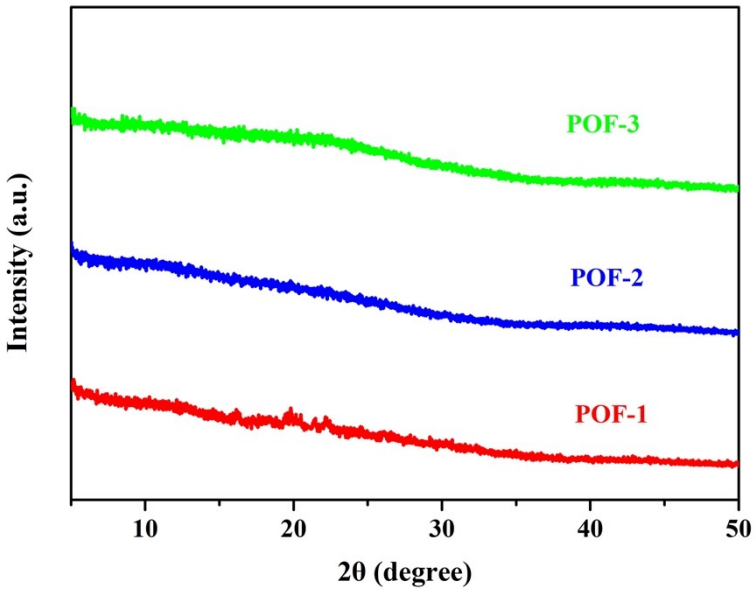


**Figure S1**. The PXRD patterns of as-synthesized POFs.

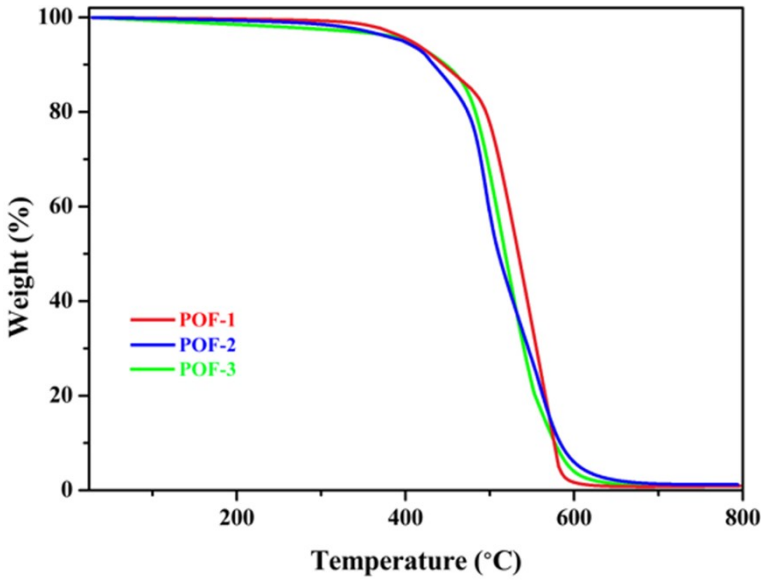


**Figure S2**. The TGA curves of as-synthesized POFs.

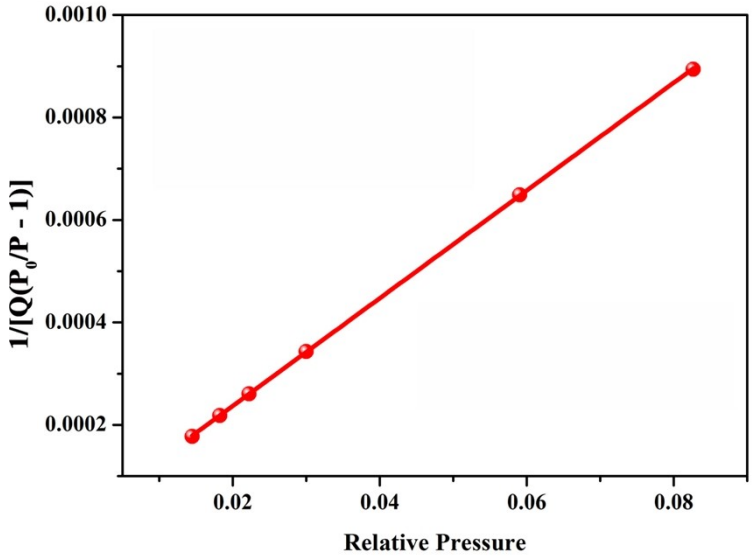


**Figure S3**. The BET surface area plots of POF-1.

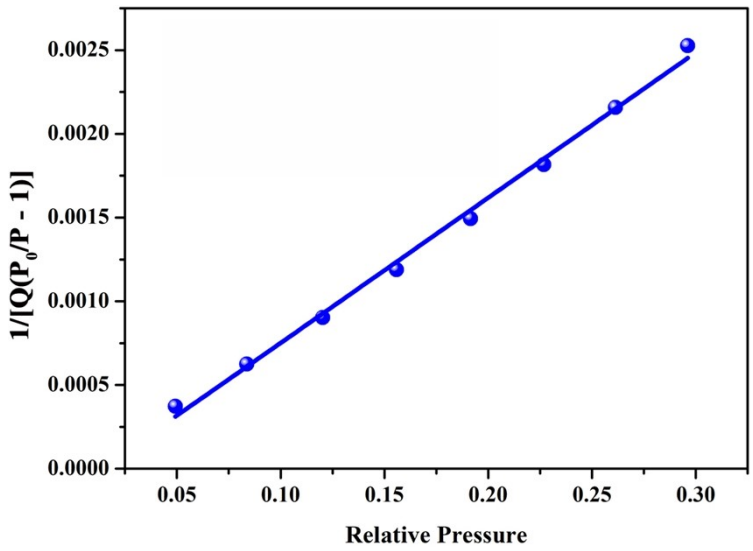


**Figure S4**. The BET surface area plots of POF-2.

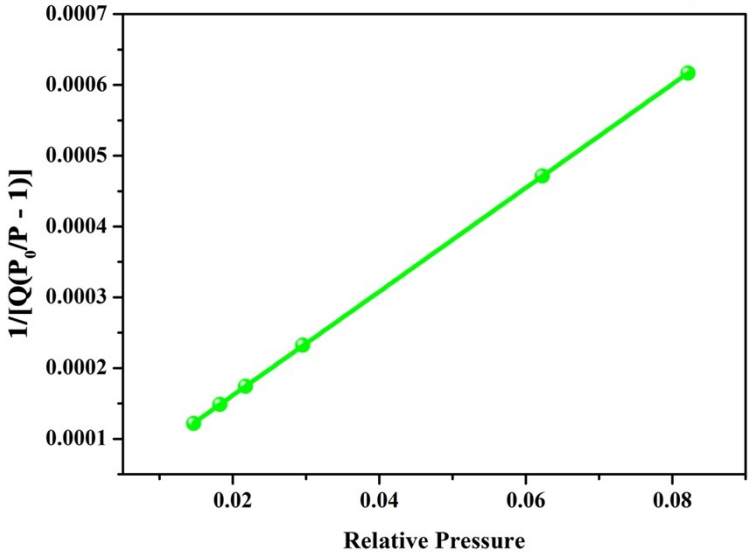


**Figure S5**. The BET surface area plots of POF-3.

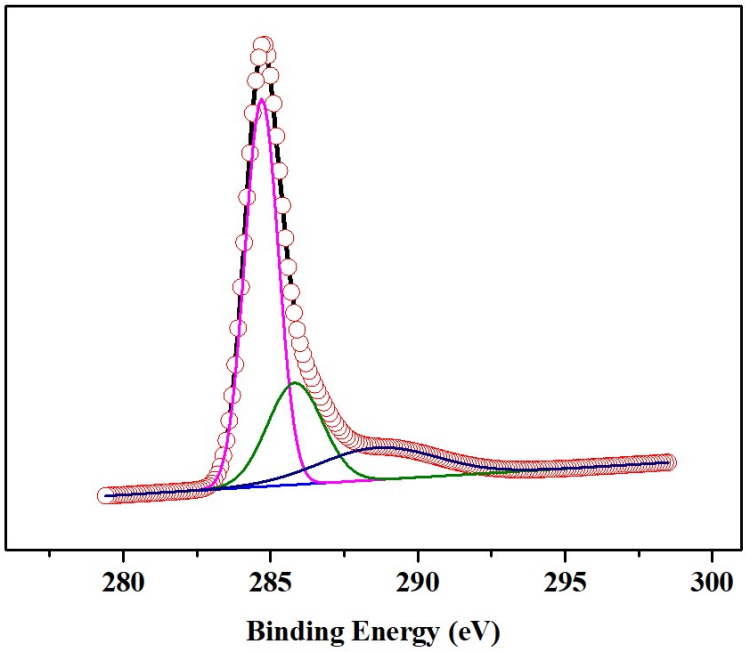


**Figure S6.** The C1*s* XPS of apt/POF-3.

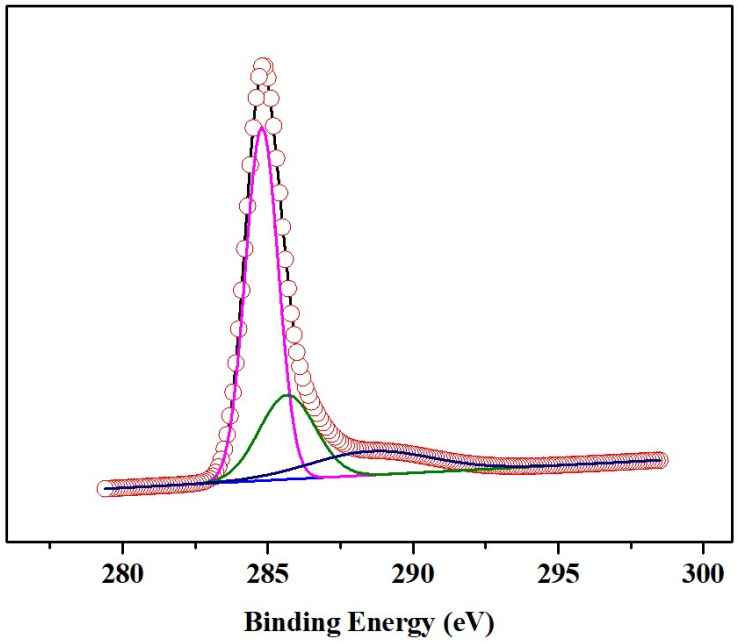


**Figure S7.** The C1*s* XPS of apt/POF-3 after binding to kanamycin.

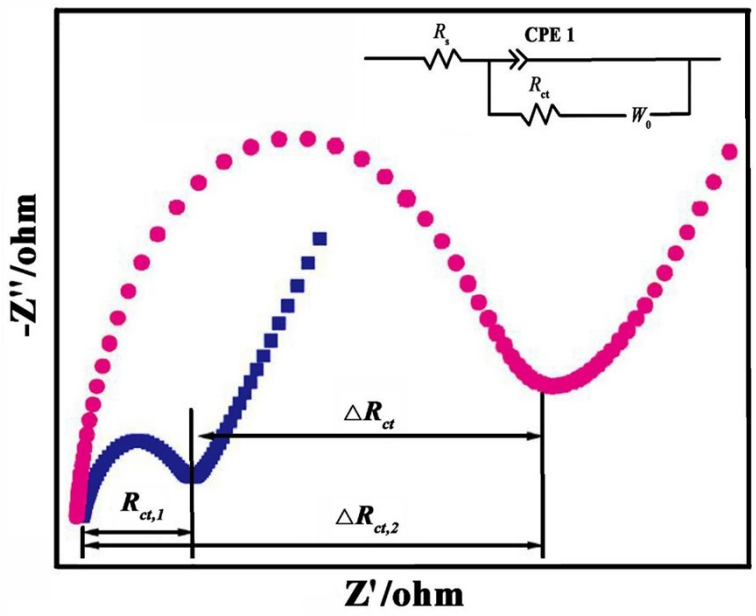


**Figure S8.** The EIS Nyquist plots and equivalent circuit.

**Table S1** The BET surface area report of POFs.

| Material | Sample weight (g) | BET ($m^2 g^{-1}$) | Slope ($g cm^{-3}$) | Y-Intercept ($g cm^{-3}$) | C | Qm ($cm^3 g^{-1}$) | Correlation Coefficient |
|---|---|---|---|---|---|---|---|
| POF-1 | 0.0605 | 413.0068±0.8517 | 0.010512±0.000022 | 0.000027±0.000001 | 392.020586 | 94.8879 | 0.9999915 |
| POF-2 | 0.0490 | 508.5516±0.0589 | 0.012346±0.000002 | 0.000021±0.000000 | 585.155503 | 80.8618 | 1.0000000 |
| POF-3 | 0.0597 | 592.9220±0.7215 | 0.007326±0.000009 | 0.000015±0.000000 | 486.062758 | 136.2232 | 0.9999970 |

## 2. Reference


[1] C.-S. Liu, Z.-H. Zhang, M. Chen, H. Zhao, F.-H. Duan, D.-M. Chen, M.-H. Wang, S. Zhang and M. Du, *Chem. Commun*., 2017, **53**, 3941.

[2] Y. Liu, S. Huang, J. Li, M. Wang, C. Wang, B. Hu, N. Zhou and Z. Zhang, *Microchim. Acta*, 2021, **188**, 69.

[3] S. Shahrokhian and S. Ranjbar, *Analyst*, 2018, **143**, 3191–3201.