[Supplementary Information]

Polythiophene derivative on quartz resonators for miRNA

capture and assay

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Figure S1: QCM response on miRNA adsorption on polythiophenes with varying pendant groups.

Polythiophenes with varying pendant group were synthesized in order to investigate their interactions with miRNA. As observed from Fig. S1, a higher frequency shift was observed for miRNA adsorption on PT with triethylamine pendant groups, suggesting that cationic triethylamine pendant groups facilitate miRNA adsorption. Polythiophenes with other pendant groups, PT (a) and PT (b), did not favor miRNA adsorption as compared to PT, suggesting that PT may be utilized for miRNA assay.



Figure S2: Normalized frequency response for varying concentrations of PT loading on QCM.

Real time QCM measurements were carried out using Q-Sense E4. Upon attaining stable resonant frequency, 250 μ L of varying concentrations of PT was injected (at a flow rate of 50 μ L/min) into the QCM chamber. As observed from Fig. S2, maximum normalized frequency was observed for 0.1 mg/ml indicating higher PT loading on QCM surface, compared to other test concentrations. Therefore, 0.1 mg/ml PT was used for development of miRNA assay.

S3: SPR measurement for the determination of the PT thickness

The surface plasmon resonance (SPR) measurement was carried out in an angular spectroscopy as described in our previous reports.¹ Briefly, a transverse magnetically (TM) polarized beam from a HeNe laser (2 mW, λ =632.8 nm) was coupled to a 90° LASFN9 glass prism and reflected from the prism base at the angle of incidence θ . To the prism base, a sensor chip was optically matched by using immersion oil. The sensor chip consisted of a BK7 glass substrate that was coated with 50 nm thick gold film. A flow-cell with the volume of approximately 10 µL was pressed against the sensor chip surface in order to flow liquid samples at a flow rate of 50 µL/min. The excitation of surface plasmon (SP) waves by the laser beam hitting the gold layer was observed from the angular reflectivity spectra R(θ) measured by using a photodiode detector and a rotation stage. The resonant coupling to SP is manifested as a dip in the reflectivity spectrum R(θ). In order to determine reflectivity spectra R(θ) were fitted by a transfer matrix-based model that was implemented in the software Winspall (developed at the Max Planck Institute for Polymer Research in Mainz, Germany). The angular spectra was measured before and after incubation the gold chip with PT for 5 min at a flow rate of 50 µL/min followed by rinsing with PBST for 3 min.

As shown in the angular spectra (Fig. S3A), the resonant angle shifts to higher angle after the modification of PT on the gold chip as measured in air, with the resonant angle changes of $\Delta\theta$ =0.12°. Fitting the angular spectra, we are able to estimate the thickness of PT on the gold chip about d_p =0.7 nm in dry state, providing the refractive index n_p =1.45 for the polymer. The surface mass coverage of PT molecules can be estimated based on the following equation,²

$$\Gamma = \frac{\left(n_p - n_b\right)d_p}{\partial n \,/\,\partial c}$$

where, n_p and n_b are the refractive index of polymer and buffer solution, respectively. d_p is the thickness of the polymer, and $\partial n/\partial c \sim 0.2 \ \mu L \ mg^{-1}$ is the refractive index of the polymer changes with the concentration of the polymer. Accordingly, we are able to estimate the surface mass coverage of the polymer Γ =158 ng/cm², which is corresponding to 1.2×10^{13} molecule/cm² for the PT molecular weight of MW_{PT}=8 kDa. Similarly, in PBST

buffer, the angular spectra shown in Fig. S3B indicate the resonant angle shifts about $\Delta\theta$ =0.2° after the modification of PT, which is corresponding to a thickness of d_p =2.8 nm in swollen state. The thickness is estimated based on the assumption of the polymer refractive index n_p =1.37 in swollen state, which is comparable to the refractive index of polymer brush and hydrogel as reported in previous reports.^{2,3}



Figure S3: The SPR angular spectra for the bare gold before and after modification with PT, measured in (A) air and (B) PBST buffer. Solid lines are the fitting curves.

S4: miRNA isolation protocol



Figure S4: miRNA isolation protocol



Figure S5: The UV-vis spectra of samples at each step of miRNA isolation protocol

In order to ascertain mir21 isolation, UV-vis spectra of the elute was recorded at each process step described in Fig. S4. Due to the complex composition of plasma samples as well as the proprietary chemicals provided with the isolation kit, the characteristic mir21 peak (at 260 nm) is not observed after 'plasma precipitation' and 'RNA spike in' steps. However, the shoulder at 260 nm for 'plasma after precipitation' curve hints at the presence of residual RNA/DNA in plasma. An increase in peak shoulder at 260 nm (Fig. S5) for 'RNA spike in' confirms the spiking of mir21. Subsequently, the shift in peak labelled 'after RNA loading' and the disappearance of peak shoulder at 260 nm indicates loading of mir21 to the separation column. Final curve labelled 'nucleospin eltue' is the isolated mir21 confirmed by a distinct peak at 260 nm. As a control measurement, same amount of mir21 was spiked in DI water followed by extraction process. Identical UV-vis spectra (at 260 nm) for the elute obtained upon extraction for mir21 spiked in plasma and DI water indicates successful mir21 isolation from plasma.

Supplementary Information References:

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