Electrochemical detection of urinary microRNAs via sulfonamide-bound antisense hybridisation

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Table S1 Sequences of miRNA and complementary DNA strands, with mismatches in bold. The DNA was purchased from Sigma Aldrich® and the RNA from IDT® (Integrated DNA Technologies).

| Species name | Sequence |
|--------------|----------|
| miR-21       | UAG CUU AUC AGA CUG AUG UUG A |
| miR-16       | UAG CAG CAC GUA AAU AUU GGC G |
| miR-21 1 mm  | UAG CUU AUC GGA CUG AUG UUG A |
| miR-21 2 mm  | UAG CUU AUC GGA CUG AUG UUG C |
| miR-21 3 mm  | AAG CUU AUC GGA CUG AUG UUG C |
| Anti-miR-21 (DNA strand) | 5’NH₂-C₆-TCA ACA TCA GTC TGA TAA GCT A |
| Anti-miR-16 (DNA strand) | 5’NH₂-C₆-CGC CAA TAT TTA CGT GCT GCT A |

Figure S2 Change in coulometric response when the electrode is regenerated through a thermal denaturation procedure to remove the hybridised miR-21 followed by another hybridisation event with an identical concentration of 10⁻¹⁰ M miR-21.

Procedure

The electrode was submerged in a 1 mL Eppendorf containing the TMD (50 mM Tris-HCl, 20 mM MgCl₂ and 1 mM dithiothreitol pH 8.0) buffer at 95 °C for 20 minutes to remove the RNA from the duplex. Following this, the electrode was submerged into ice cold TMD buffer for 10 minutes. Finally, the electrode was sonicated in the buffer for 2 minutes to remove any residual adsorbed RNA.
Figure S3 Change in charge transfer resistance between the complementary strand alone and following RNA incubation (ΔR2) for a glassy carbon electrode modified with anti-miR-21 with variation in concentration of miR-21. Performed in a solution of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 0.1 M KCl. Performed in triplicate.

The equivalent circuit model used to interpret electrical impedance data using EIS spectrum analyser.
Figure S4 An overlay of representative Nyquist impedance plots obtained for a glassy carbon electrode modified with anti-miR-21 with variation in concentration of miR-21. Performed in a solution of 5 mM K₃[Fe(CN)]₆/K₄[Fe(CN)]₆ in 0.1 M KCl.
Figure S5 An overlay of representative coulometry plots obtained for a glassy carbon electrode modified with anti-miR-21 with variation in concentration of miR-21. Performed in a solution of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 0.1 M KCl.
Figure S6 Change in charge transfer resistance ($\Delta R2$) between the anti-miR-21 DNA probe and DNA/RNA hybrid upon exposure to $10^{-8}$ M solution of mismatched RNA. The $10^{-8}$ M miR-21 (light blue), one mismatch (orange), 2 mismatches (gray), 3 mismatches (yellow) and miR-16 (dark blue). Performed in a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. Performed in triplicate.
Figure S7 Change in charge transfer resistance ($\Delta R_2$) with concentration of miR-21 (blue) and miR-16 (orange) with anti-miR-21 and anti-miR-16 DNA probes respectively. Performed in a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. Performed in triplicate.
Figure S8 A bar chart to indicate the change in signal intensity observed for a $10^{-10}$ M solution of miR-21 after storing the DNA modified probe at varying temperatures over 24 hours. Performed in a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. Performed in triplicate.

**Note**

This data shows that over 50% of the response is maintained after storage of the probe for 24 hours at elevated temperatures of 40 °C and 50 °C.
Figure S9 Coulometric analysis ($\Delta Q$) of miR-21 solutions ($10^{-10}$ M) containing sodium chloride using an anti-miR-21 modified electrode. Performed in a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. Performed in triplicate.
Figure S10 Charge transfer resistance (ΔR2) of an anti-miR-21 modified electrode exposed to miR-21 solutions (10⁻¹⁰ M) containing sodium chloride. Performed in a solution of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 0.1 M KCl.
Performed in triplicate.
Figure S11 Coulometric response change (ΔQ) of an anti-miR-21 modified electrode incubated with $10^{-11}$ M miR-21 in the presence and absence of urea (155 mM, 9.3 g/L). Performed in a solution of 5 mM $K_3[Fe(CN)]_6/K_4[Fe(CN)]_3$ in 0.1 M KCl.
Figure S12 An overlay of the Nyquist impedance response obtained using an electrode modified with anti-miR-21 (blue), followed by a hybridisation with a miR-21 solution containing 1 mg of BSA (orange) and then following a second incubation in proteinase K for 30 minutes (gray). Performed in a solution of 5 mM K$_3$[Fe(CN)$_6$]/K$_4$[Fe(CN)$_6$] in 0.1 M KCl.

**Note**

Protein fouls the electrode surface, however in the presence of proteinase K this effect is greatly reduced.
Figure S13 A CV overlay of the electrode with anti-miR-21 (blue), following incubation with urine (green) and after proteinase K incubation for 10 minutes at 50 °C (orange). Performed in a solution of 5 mM $K_3[Fe(CN)]_6/K_4[Fe(CN)]_3$ in 0.1 M KCl.
Figure S14 An overlaid CV of the electrode with anti-miR-21 (orange) and after incubation in filtered urine containing $10^{-9}$ M anti-miR-21 PNA (blue). Performed in a solution of 5 mM $K_3[Fe(CN)]_6/K_4[Fe(CN)]_6$ in 0.1 M KCl.

**Note**

Treatment of urine with a PNA complementary to the target miRNA resulted in negligible signal. This implies that responses obtained from proteinase K treated and filtered urine are from the miRNA target rather than protein fouling.
Figure S15 An overlaid CV of the electrode with anti-miR-16 (blue) and after incubation in filtered urine containing $10^{-9} \text{M}$ anti-miR-21 PNA (orange). Performed in a solution of $5 \text{mM} \text{K}_3[\text{Fe(CN)}_6]/\text{K}_4[\text{Fe(CN)}_6]$ in $0.1 \text{ M KCl}$.

Note

This experiment shows that the positive control, using a PNA sequence specific to a miRNA that is not the probe target, resulted in a significant signal change being obtained upon hybridisation. A further implication is that the responses obtained from the proteinase K treated and filtered urine are from the specific miRNA target.
Figure S16 An overlaid CV of the electrode with anti-miR-16 (orange), after incubation with RNase A treated urine for 20 minutes prior to proteinase K digestion and filtration (green) and following a ‘spike’ of the filtered urine solution with $10^{-11}$ M miR-16 (blue). Performed in a solution of 5 mM $K_3[Fe(CN)_{6}]/K_4[Fe(CN)_{6}]$ in 0.1 M KCl.

**Note**

Addition of RNase degrades RNA in the urine resulting in negligible current change. Finally, a miR-16 ‘spike’ is used to show that the signal can be restored, and also that the RNase used previously (and likely any originally present in the urine) is removed upon filtration through the 10 kDa spin filter.
The results of urine sample concentrations obtained by comparing the CT values of each urine sample to the calibration plot from the samples of known concentration. Performed using miR-21 specific primers.

Figure S17
Figure S18 Change in coulometric response (ΔQ) obtained using an electrode modified with anti-miR-21 for 5 urine samples. Performed in a solution of 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] in 0.1 M KCl. Performed in triplicate.
Figure S19 Coulometric responses of Figure S16 converted into concentration using the calibration plot shown in figure 1 of the manuscript.

**Note**

Higher bar is a lower concentration.
Figure S20 Change in charge transfer resistance ($\Delta R_2$), obtained using an electrode modified with anti-miR-21 for 5 urine samples. Performed in a solution of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 M KCl. Performed in triplicate.
Figure S21 Charge transfer resistance ($\Delta R2$) of Figure S18 converted into concentration using the calibration plot shown in figure S2.
Figure S22 Charge transfer resistance ($\Delta R_2$) plot converted into concentration (blue) using the calibration plot shown in figure S2 overlaid with the PCR concentration data (orange) for the same samples.
Figure S23 An overlay of the result of all electrochemical techniques used to analyse the urine samples.
Figure S24 The difference in CT values obtained upon PCR amplification of a range of synthetic miR-21 solutions, one set where RT was directly performed, and one where an extraction step was performed first.
The loss of concentration observed when an extraction step is performed prior to RT-qPCR amplification of a synthetic miR-21 solution compared to one where the RT is performed using the solution directly.

Note

The difference in RT-qPCR response, between extracted and directly analysed samples, decreased with decreasing initial miRNA concentration. With an initial concentration of $10^{-8}$ M, a decrease of approx. $7000 \times$ upon extraction is observed; this is lowered to approx. 700 at $10^{-10}$ M and further decreased at $10^{-12}$ and $10^{-14}$ M. The electrochemical analyses do not require extraction and so are not susceptible to these losses at higher miRNA concentrations.
Figure S26 Coulometric response change ($\Delta Q$) as a function of incubation time for $10^{-10}$ M miR-16. The optimised time is 30 minutes.