Supporting Information

Reference Electrodes for Electrochemical Sensors Based on Redox Couples Immobilized within Nafion Films

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Section 1: Additional Figures

S1 Electrochemical fabrication of the Fc/Fc^+ Nafion film reference electrode

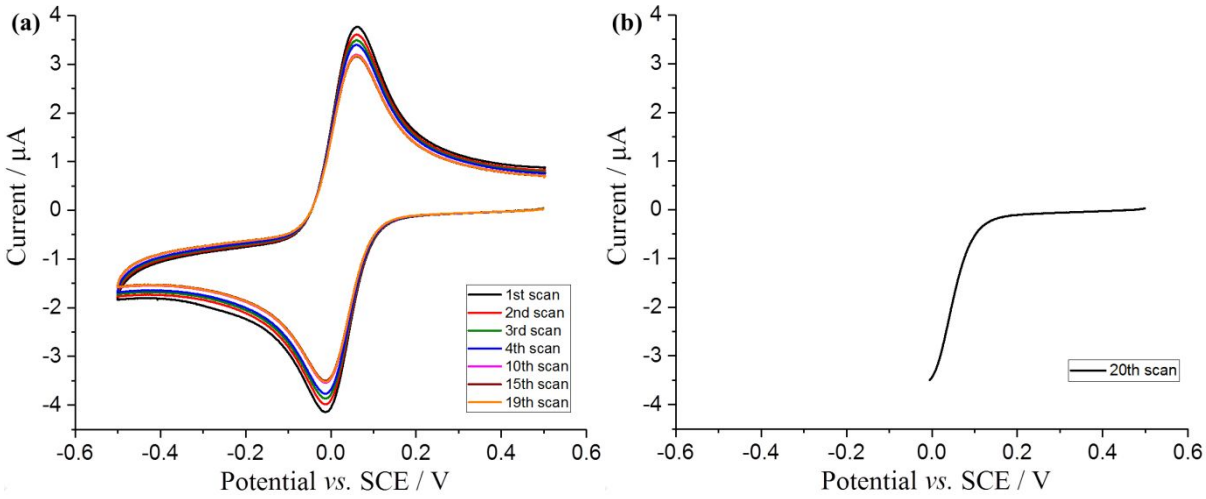

Figure S1. Voltammograms of a Fc^+-Nafion film (with the doping ratio of $Fc^+ : SO_3^- = 1 : 30$ and thickness of 35 µm) dropcasted glassy carbon electrode in pH 7.4 PBS buffer at a scan rate of 0.1 V s^{-1} for 20 successive scans. (a) 1st scan to 19th scan (b) the 20th scan

The Fc/Fc^+ Nafion film reference electrode was obtained electrochemically by sweeping the potential first cathodically from 0.5 V to -0.5 V *vs.* SCE then reversing back to 0.5V at 0.1 V/s for 20 repetitive scans in degassed pH 7.4 PBS buffer and in the final scan, the potential stopping at -0.005 V, as shown in Figure S1. At this point, the Fc^+ was partially reduced and a Nafion film of $Fc/Fc^+PF_6^-$ was produced. Note that the decrease in peak current after the 1st scan in Figure S1a is likely due to the slow mass transport of the electro-generated product within in the thick Nafion film, which can be easily misunderstood as the chemical depleting from the Nafion film, as detailed investigated in the previous work[1]. The current stops decreasing after a few scans (magenta, brown and orange lines in Figure S1a) confirming that current decrease is not caused by the redox depletion during the reaction. Figure S1b shows the 20th scan which is the final scan of the fabrication process, showing Fc^+ was partially reduced and a Nafion film of $Fc/Fc^+PF_6^-$ was produced.

S2 Voltammetric characterization of fabricated MV^{2+}/MV^{+} Nafion film reference electrodes

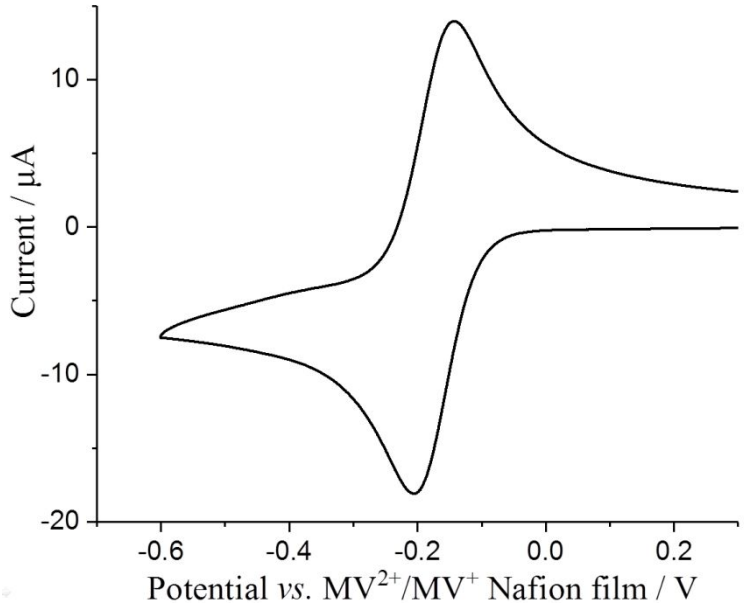

Figure S2. Voltammograms of a glassy carbon working electrode with a freshly prepared MV^{2+}/MV^{+} Nafion film reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1}.

Figure S2 shows a typical cyclic voltammogram (CV) of hexaammineruthenium(III) chloride recorded on a GC electrode with a freshly fabricated MV^{2+}/MV^{+} Nafion film reference electrode (with the doping ratio of MV^{2+} : SO_3^- = 1 : 30) and graphite counter electrode in a solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ supported with 0.1M KCl.

S3 Comparison of MV^{2+}/MV^{+} Nafion particles and film reference electrodes

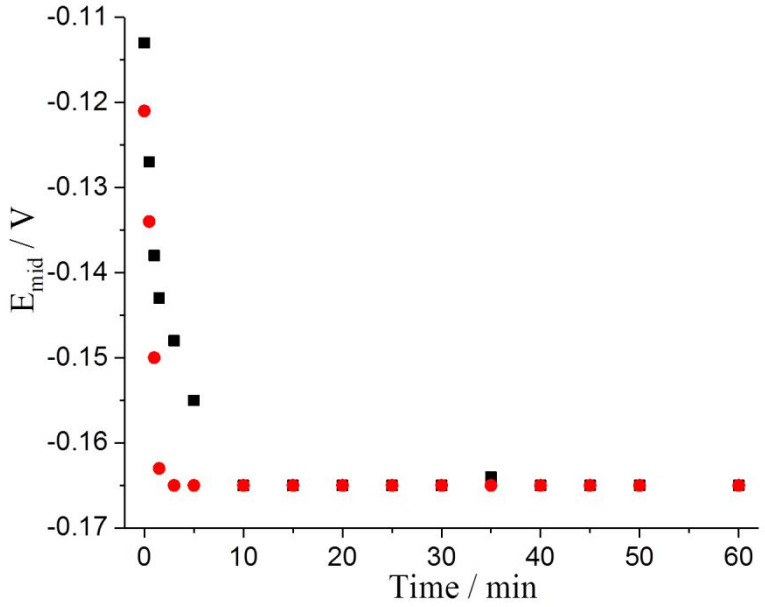

Figure S3. The midpoint potential, E_{mid}, of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* MV^{2+}/MV^{+} Nafion (with a doping ratio of 1 : 30) reference electrodes. Black dots: Nafion film with film thickness of 35.1 μm; red dots: Nafion particles with 38.7 μm particle layers thickness.

Figure S3 compares the initial potential drift of MV^{2+}/MV^{+} Nafion film reference electrode with MV^{2+}/MV^{+} Nafion particles reference electrode of similar thickness. The initial potential stabilization of MV^{2+}/MV^{+} Nafion particles reference electrode completed much faster than that of Nafion films (ca. 45 mV drift within 1.5 min for 38.7 μm particle layers vs. 52mV drift within 5 min for 35.1 μm film), consistent with the faster diffusional transport of both cations inside the particles, as reported in our previous studies,[1] consequently a short stabilization time for a homogeneous distribution in Nafion.

S4 Morphology of Uniform Nafion Films and Films with an obvious surface roughness

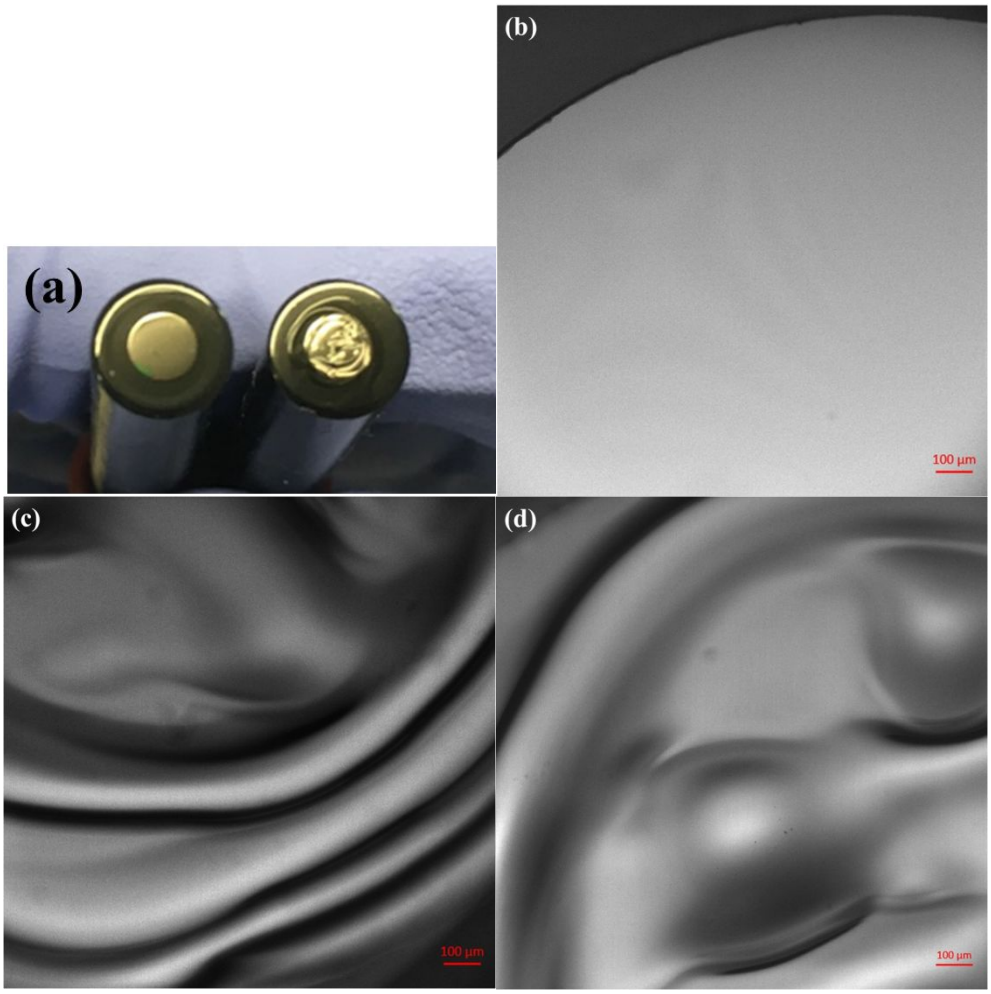

Figure S4. (a) Photo of a uniform Nafion film prepared by dropcasting (left) and a film with an obvious surface roughness as judged visually (right); (b) Optical microscope images of a uniform Nafion film; (c) and (d) Optical microscope images of a film with an obvious surface roughness.

Figure S4 shows the morphology of uniform Nafion films and films with an obvious surface roughness. The uniform film was prepared via dropcasting and a very homogeneous and uniform film obtained both visually and microscopically in Figure S4b. Literature also confirms via AFM that a film prepared in this way is highly uniform.[2] The Nafion films with an obvious surface roughness were prepared by dropcasting same amount of Nafion stock mixture not once but five times (2 µL each time). A highly rough film surface at visual level was obtained. Microscope images show the big difference in morphology between the uniform and rough films.

S5 Stability test of Nafion film reference electrode with a uniform or rough film surface

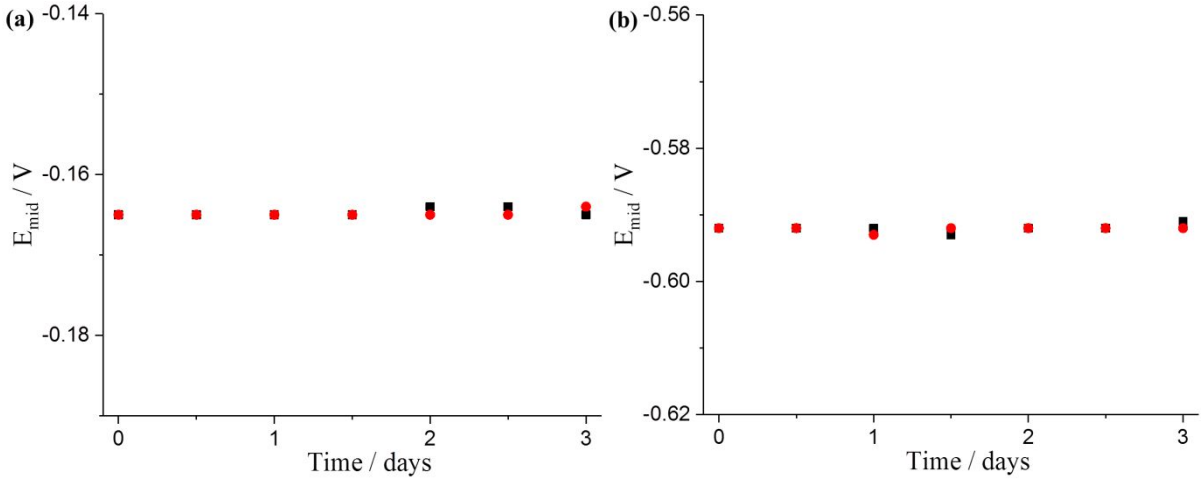

Figure S5. (a) The midpoint potential, E_{mid}, of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* MV^{2+}/MV^{+} Nafion film (with a doping ratio of 1 : 30 and film thickness of 35 μm) reference recorded after the electrodes being fabricated and left in in air without any protection (uniform film surface, black dot; rough film surface, red dot). (b) E_{mid} of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film (with a doping ratio of 1 : 30 and film thickness of 35 μm) reference electrodes recorded after the electrodes being fabricated and left in in air without any protection (uniform film surface, black dot; rough film surface, red dot).

Figure S5 depicts the stability of both MV^{2+}/MV^{+} Nafion film and $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrodes with a uniform or rough film surface over three days of the same film electrode after being fabricated and left in in air without any protection. Both types of Nafion reference electrodes show good stability and suggest the morphology of Nafion films has a significant effect on initial performance (an initial potential drift, as detailed discussed in the main text) of the reference electrode but plays a negligible role in terms of stability.

S6 Examination of fabricated Fc/Fc^+ Nafion film reference electrodes

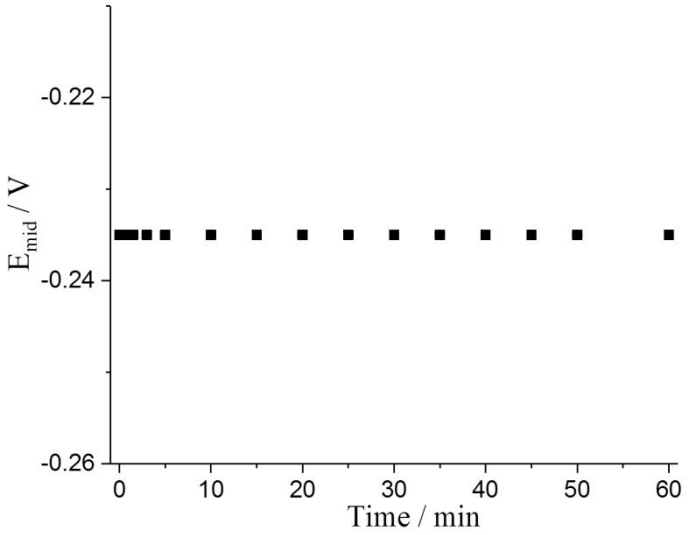

Figure S6. The midpoint potential, E_{mid}, of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* Fc/Fc^+ Nafion film (with a doping ratio of 1 : 30 and film thickness of 35 μm) reference electrodes recorded over first one hour after being fabricated.

Figure S6 shows the midpoint potential of $[Ru(NH_3)_6]^{3+}$ reduction against a freshly prepared Fc/Fc^+ Nafion film reference electrode. The initial potential drift has been successfully and fully eliminated suggesting the inhomogeneous distribution of generated product within the film plays a predominant role to cause an initial potential drift and this drift could be fully eliminated if the product is insoluble, as detailed discussed in the main text. The electrode demonstrates a good stability with the E_{mid} potential stabilized at 235 ± 0.5 mV over one hour.

S7 Examination of Fc/Fc^+ Nafion film reference electrodes with a rough film surface

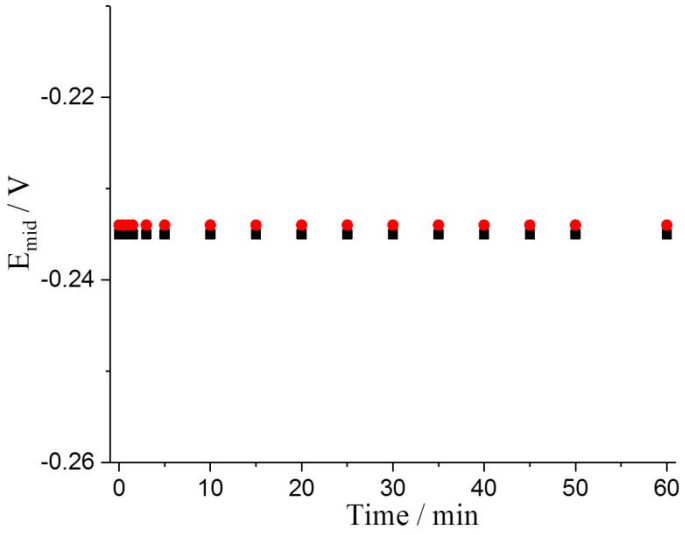

Figure S7. The midpoint potential, E_{mid}, of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* Fc/Fc^+ Nafion film (with a doping ratio of 1 : 30 and film thickness of 35 μm) reference electrodes recorded over one hour (uniform film surface, black dot; rough film surface, red dot).

Figure S7 shows that there is no initial potential drift for a Fc/Fc^+ Nafion film reference electrode with a high surface roughness, contrasting with the behaviour of MV^{2+}/MV^+ Nafion film and $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrodes as shown in Figure 3 in main text. This further confirms the hypothesis that the inhomogeneous distribution of generated product within the film plays a predominant role to cause an initial potential drift. The electrode with a high film surface roughness also demonstrates a good stability with the E_{mid} potential stabilized at 234 ± 0.5 mV over one hour.

S8 Examination of fabricated PVFc/PVFc$^+$ Nafion film reference electrodes

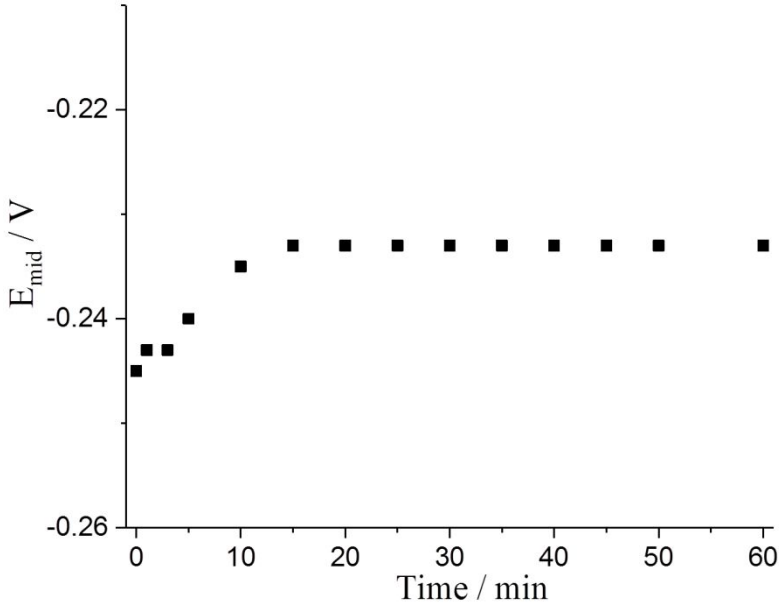

Figure S8. The midpoint potential, E_{mid}, of $[Ru(NH_3)_6]^{3+}$ reduction *vs.* PVFc/PVFc$^+$ Nafion film (with a doping ratio of 1 : 30, film thickness of 35.1 μm and uniform surface) reference electrodes recorded over one hour.

Figure S8 shows the midpoint potential of $[Ru(NH_3)_6]^{3+}$ reduction measured against a freshly prepared PVFc/PVFc$^+$ Nafion film reference electrode as a function of time. PVFc/PVFc$^+$ Nafion film reference electrodes were fabricated in a contrast condition with Fc/Fc$^+$ Nafion film where the waster-insoluble PVFc was initially doped into Nafion and soluble PVFc$^+$ was electrochemically generated. The initial potential drift of PVFc/PVFc$^+$ Nafion film reference electrode may reflect re-arrangement of the polymer film and/or some solubility of the PFVc$^+$. This observation is consistent with that of MV^{2+}/MV^+ and $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ and in contrast with that of Fc/Fc$^+$ Nafion film where the initial potential drift being fully eliminated, confirming again the hypothesis as discussed in details in main text.

S9 Determination of midpoint potential, E_{mid}, of reduction of $[Ru(NH_3)_6]^{3+}$ *vs.* SCE

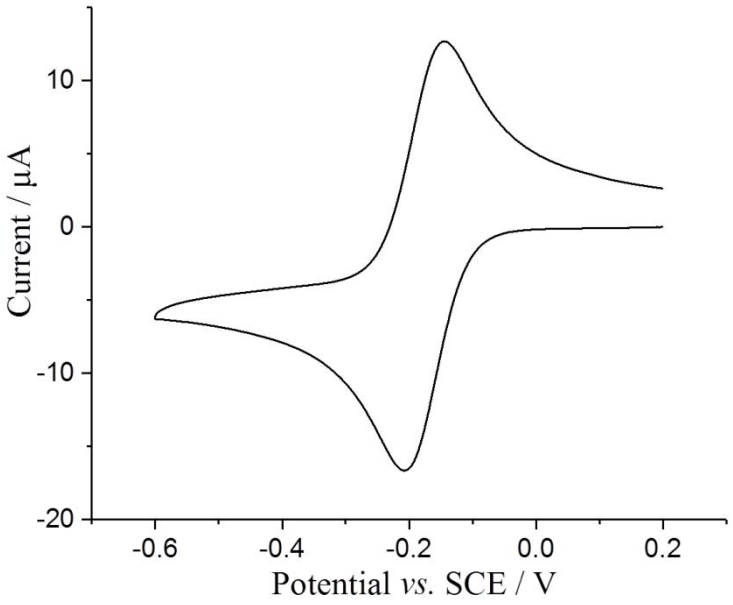

Figure S9. Voltammograms of a glassy carbon working electrode with a SCE reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1}.

The midpoint potential, E_{mid}, of reduction of $[Ru(NH_3)_6]^{3+}$ *vs.* SCE is -0.176 ± 0.001 V.

S10 Stability study of the Fc/Fc$^+$ Nafion film reference electrode

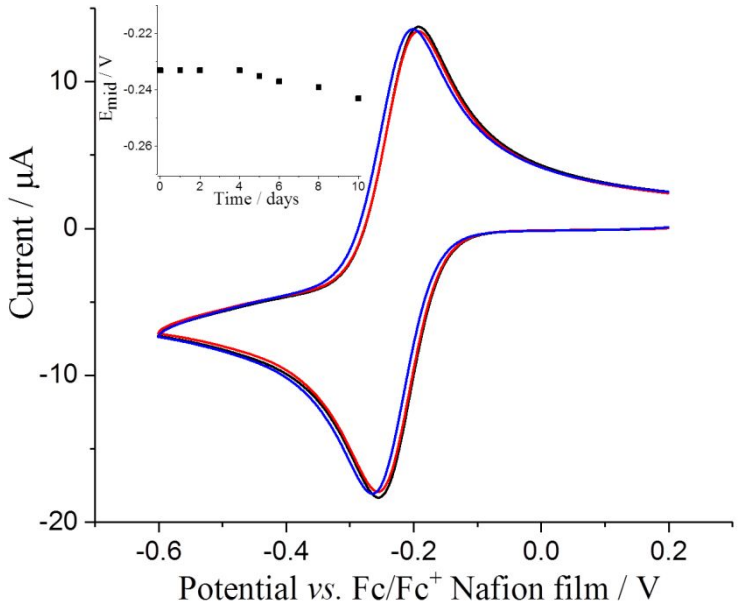

Figure S10. Voltammograms of a glassy carbon working electrode with the same Fc/Fc$^+$ Nafion film reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1} recorded after the electrodes being fabricated and left in in air without any protection (fresh, black line; 4 days kept in air, red line; 10 days kept in air, blue line). Inset shows the midpoint potential, E_{mid}, over the time.

Figure 10 shows Voltammograms every one or two days over a period of 10 days , with the same Fc/Fc$^+$ Nafion film electrode after washing with water and kept in air without any protection. A superior stability was observed with no significant drift of the E_{mid} within 10 mV over 10 days and no deviation in the first 4 days, clearly demonstrating the high stability and strong robustness of Fc/Fc$^+$ Nafion film.

Table S1. Raw data for the inset of Figure 4a - the midpoint potential, E_{mid}, of a freshly fabricated Fc/Fc^+ Nafion films over 24h immersing in solution.

Time / h	E_{mid} potential / V
0	-0.233
1	-0.233
2	-0.233
4	-0.233
6	-0.233
8	-0.234
10	-0.234
12	-0.235
16	-0.236
20	-0.237
24	-0.238

Table S2. Raw data for the inset of Figure 4b - the midpoint potential, E_{mid}, of a freshly fabricated Fc/Fc^+ Nafion films over 1000 successive scans.

No. of cycles	E_{mid} potential / V
1	-0.233
100	-0.233
200	-0.233
300	-0.233
400	-0.233
500	-0.233
600	-0.233
700	-0.234
800	-0.234
900	-0.235
1000	-0.236

Table S1 and S2 show a superior stability of the fabricated reference electrodes in aqueous solution and over a series of measurements.

S11 Reproducibility of the Fc/Fc^+ Nafion film reference electrodes

Table S3. Summary of the midpoint potential, E_{mid}, of 3 freshly fabricated Fc/Fc^+ Nafion films

	Fc^+/Nafion 1	Fc^+/Nafion 2	Fc^+/Nafion 3
GC substrate 1	-0.235 V	-0.233 V	-0.236 V
GC substrate 2	-0.236 V	-0.234 V	-0.235 V
GC substrate 3	-0.235 V	-0.235 V	-0.237 V

Table S4. E_{mid} potential drift between 1st scan and the 1000th scan of the Fc/Fc^+ Nafion films listed in Table S1 after 1000 successive scans

	Fc^+/Nafion 1	Fc^+/Nafion 2	Fc^+/Nafion 3
GC substrate 1	2 mV	4 mV	-1 mV
GC substrate 2	3 mV	-2 mV	1 mV
GC substrate 3	-1 mV	2 mV	3 mV

Table S3 and S4 show an excellent reproducibility of the fabricated reference electrodes and all the Nafion film electrodes exhibit superior stability over a series of measurements (1000 successive scans).

Section 2 Stability of other Nafion film electrodes under optimized conditions

S12 Stability of MV^{2+}/MV^{+} Nafion film electrodes

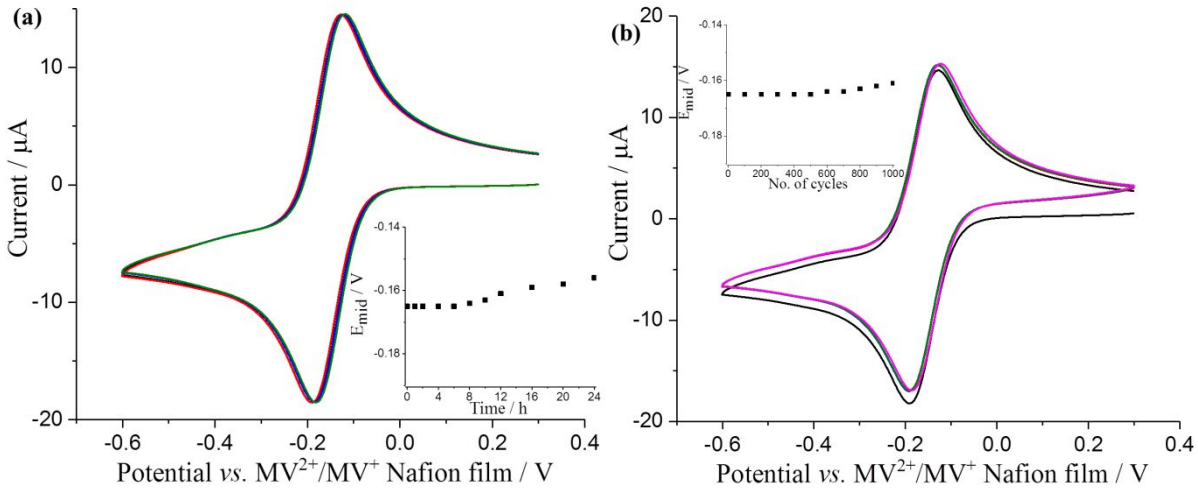

Figure S11. (a) Voltammograms of a glassy carbon working electrode with a MV^{2+}/MV^{+} Nafion film reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1} recorded after the electrodes immersing in solution (fresh, black line; 2 h, red line; 12 h, blue; 24 h, green line). Inset shows the midpoint potential, E_{mid}, over the time. (b) Voltammograms recorded over 1000 successive scans (1st scan, black line; 100th scan, red line; 200th scan, blue; 600th scan, green line; 1000th scan, magenta line). Inset shows the midpoint potential, E_{mid}, over the number of scans.

The potential of the optimized MV^{2+}/MV^{+} Nafion film reference electrode and its stability were also assessed electrochemically. The midpoint potential, E_{mid}, of the mediator was -0.165 V *vs.* MV^{2+}/MV^{+} Nafion film. The E_{mid} of reduction of $[Ru(NH_3)_6]^{3+}$ *vs.* SCE is -0.176 V, as shown in Figure S9. Therefore, the potential of fabricated MV^{2+}/MV^{+} Nafion film reference electrode in aqueous solution was determined as +0.011 V *vs.* SCE or +0.252 V *vs.* HNE. Figure S12 depicts a good stability of MV^{2+}/MV^{+} Nafion film reference electrode at optimized conditions (doping ratio of 1 : 30, film thickness of 35 µm and uniform film surface). The varication of E_{mid} with experimental time was within an average deviation of 4 mV over 12 hours and less than 9 mv after 24 hours in solution. The MV^{2+}/MV^{+} Nafion films also shows high stability over a series of measurements with only 1 mV drift after 600 successive cycles and 4 mV drift after 1000 successive cycles.

S13 Stability of $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film electrodes

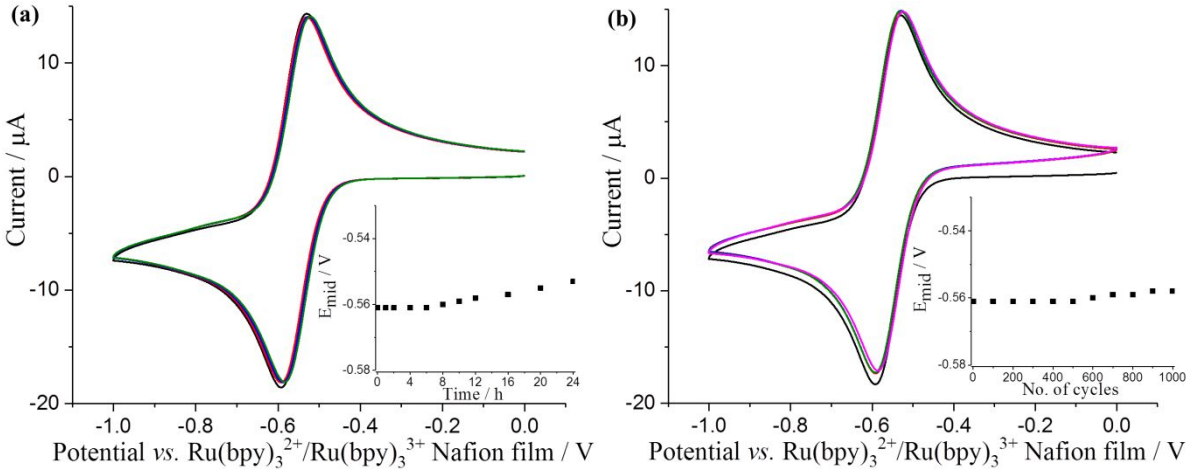

Figure S12. (a) Voltammograms of a glassy carbon working electrode with a $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1} recorded after the electrodes immersing in solution (fresh, black line; 2 h, red line; 12 h, blue; 24 h, green line). Inset shows the midpoint potential, E_{mid}, over the time. (b) Voltammograms recorded over 1000 successive scans (1st scan, black line; 100th scan, red line; 200th scan, blue; 600th scan, green line; 1000th scan, magenta line). Inset shows the midpoint potential, E_{mid}, over the number of scans.

The potential of the optimized $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrode and its stability were also assessed electrochemically. The midpoint potential, E_{mid}, of the mediator was -0.561 V *vs.* $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film. The E_{mid} of reduction of $[Ru(NH_3)_6]^{3+}$ *vs.* SCE is -0.176 V, as shown in Figure S9. Therefore, the potential of fabricated $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrode in aqueous solution was determined as -0.385 V *vs.* SCE or -0.144 V *vs.* HNE. Figure S12 depicts a good stability of the $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrode at optimized conditions (doping ratio of 1 : 30, film thickness of 35 µm and uniform film surface). The varication of E_{mid} with experimental time was within an average deviation of 3 mV over 12 hours and less than 8 mv after 24 hours in solution. The $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion films also shows high stability over a series of measurements with only 1 mV drift after 600 successive cycles and 4 mV drift after 1000 successive cycles.

S14 Stability of PVFc/PVFc$^+$ Nafion film electrodes

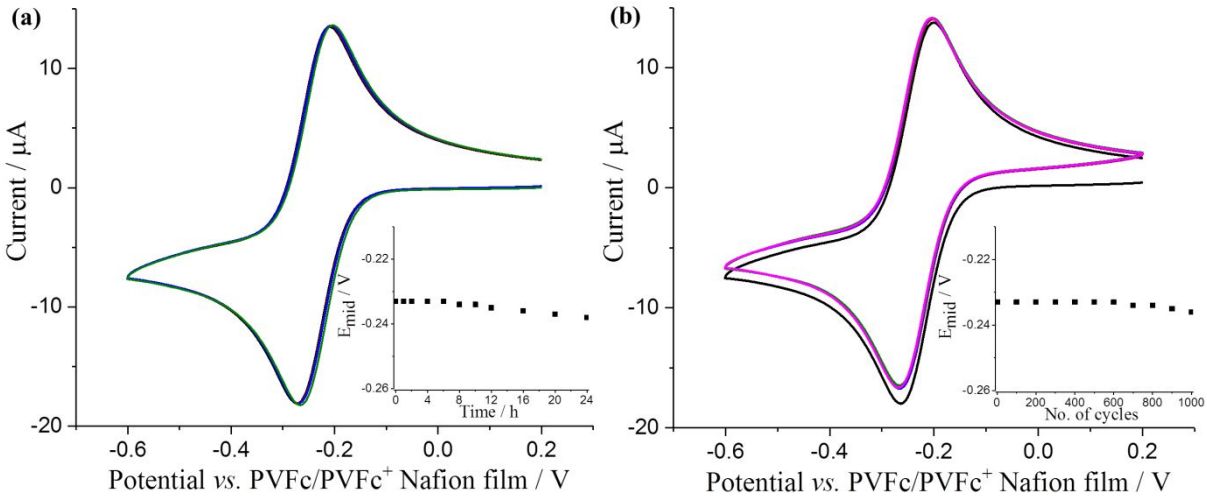

Figure S13. (a) Voltammograms of a glassy carbon working electrode with a PVFc/PVFc$^+$ Nafion film reference electrode in 0.1 M KCl aqueous solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ at a scan rate of 0.1 V s^{-1} recorded after the electrodes immersing in solution (fresh, black line; 2 h, red line; 12 h, blue; 24 h, green line). Inset shows the midpoint potential, E_{mid}, over the time. (b) Voltammograms recorded over 1000 successive scans (1st scan, black line; 100th scan, red line; 200th scan, blue; 600th scan, green line; 1000th scan, magenta line). Inset shows the midpoint potential, E_{mid}, over the number of scans.

The potential of optimized PVFc/PVFc$^+$ Nafion film reference electrode and its stability were also assessed electrochemically. The midpoint potential, E_{mid}, of the mediator was -0.233 V *vs.* PVFc/PVFc$^+$ Nafion film. The E_{mid} of reduction of $[Ru(NH_3)_6]^{3+}$ *vs.* SCE is -0.176 V, as shown in Figure S9. Therefore, the potential of fabricated PVFc/PVFc$^+$ Nafion film reference electrode in aqueous solution was determined as -0.057 V *vs.* SCE or +0.184 V *vs.* HNE. Figure S12 depicts a good stability of PVFc/PVFc$^+$ Nafion film reference electrode at optimized conditions (doping ratio of 1 : 30, film thickness of 35 µm and uniform film surface). The varication of E_{mid} with experimental time was within an average deviation of 2 mV over 12 hours and less than 5 mv after 24 hours in solution. The PVFc/PVFc$^+$ Nafion films also shows high stability over a series of measurements with no drift after 600 successive cycles and only 3 mV drift after 1000 successive cycles.

Section 3 Estimation of the Nafion film thickness

10 μL of doped Nafion stock mixture was drop-cast on the GC electrode and the film dry thickness can be estimated as followed:

Mass of 10 μL Nafion is

$$m = V \cdot \rho \cdot wt\% = 10\mu L \times 0.921 g / cm^3 \times 5\% = 4.61 \times 10^{-4} g$$

where ρ is the density of Nafion perfluorinated resin solution, whose value obtained from the supplier Sigma-Aldrich.

The density of dry Nafion is relevant to its storage and drying conditions and in this case the dry density of Nafion, $\rho_{DryNafion}$, is 1.965 g cm^{-3}.[3]

Therefore the dry volume of Nafion is

$$V = \frac{m}{\rho_{DryNafion}} = 2.48 \times 10^{-10} m^3$$

The diameter of GC electrode is 3 mm, hence the area is $A = D^2 / 4 = 7.07 \times 10^{-6} m^2$

The dry thickness for 10 μL of MV-Nafion thin film on the GC electrode is

$$h = \frac{V}{A} = \frac{2.48 \times 10^{-10} m^3}{7.07 \times 10^{-6} m^2} = 35.1 \mu m$$

Similarly, the thickness for 0.1 μL, 0.5 μL, 2 μL, 5 μL and 15 μL of Nafion film on the GC can be determined as 0.35 μm, 1.75 μm, 7.01 μm, 17.5 μm and 52.6 μm respectively.

Section 4: Experimental details for the fabrication of other Nafion film electrodes

S15 Fabrication of the MV^{2+}/MV^{+} Nafion film reference electrodes

The MV^{2+}/Nafion stock samples (with the doping ratio of MV^{2+} : SO_3^- = 1 : 30) were prepared by directly dissolving 0.5 mg of $MVCl_2$ into 1.40 mL Nafion perfluorinated resin solution. The mixture was shaken on a vortex (Whirlmixer, Loughborough, UK) for 5 min. MV^{2+}/Nafion films with film thickness of 35 μm were obtained by drop-casting 10 μL of MV^{2+}-Nafion solution on a bare glassy carbon (GC) macroelectrode and dried in a nitrogen environment at room temperature. Prior to use, the GC surface was cleaned by successively polishing with three grades of alumina (1.0, 0.3 and 0.05 μm, Buehler, UK) in a decreasing particle size order followed by sonication in water and drying with nitrogen. The MV^{2+}/MV^{+} Nafion film reference electrode was then obtained electrochemically by sweeping the potential first cathodically from -0.20 V to -0.95 V *vs.* SCE then reversing back to -0.20 V at 0.1 V/s for 20 repetitive scans in degassed pH 7.4 PBS buffer and in the final scan, the potential stopping at -0.73 V, as shown in Figure S15. At this point, the MV^{2+} was partially reduced and a Nafion film of $MV^{2+}/MV^{+}Cl_2$ was produced. The relative amount of MV^{+} and MV^{2+} in Nafion films after fabrication is estimated to be 1 : 133, as shown in Section S5.

Note that for the doping ratio of MV^{2+} : SO_3^- = 1 : 4, 1 : 50, 1 : 100 and 1 : 200 , 0.5 mg of $MVCl_2$ were directly dissolved into 0.19 mL, 2.32mL, 4.64 mL, 9.29 mL of Nafion perfluorinated resin solution respectively.

Note that for Nafion films with film thicknesses of 0.35 μm, 1.75 μm, 7.0 μm and 17.5 μm were obtained by dropcasting 0.1 μL, 0.5 μL, 2 μL and 5 μL of MV^{2+}-Nafion solution on the GC electrode, respectively.

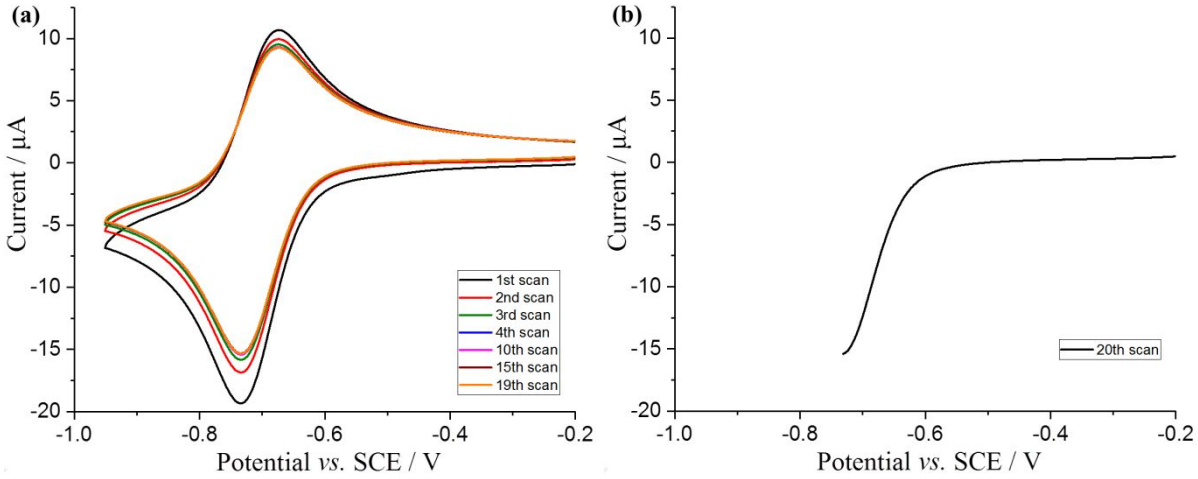

Figure S14. Voltammograms of a MV^{2+}-Nafion film (with the doping ratio of MV^{2+} : SO_3^- = 1 : 30 and thickness of 35 µm) dropcasted glassy carbon electrode in pH 7.4 PBS buffer at a scan rate of 0.1 V s^{-1} for 20 successive scans. (a) 1st scan to 19th scan (b) the 20th scan

S16 Fabrication of the $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrodes

The $Ru(bpy)_3^{2+}$/Nafion stock samples (with the doping ratio of $Ru(bpy)_3^{2+}$: SO_3^- = 1 : 30) were prepared by directly dissolving 1.4 mg of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ into 1.34 mL Nafion perfluorinated resin solution. The mixture was then shaken on a vortex for 5 min. $Ru(bpy)_3^{2+}$/Nafion films with film thickness of 35 μm were obtained by drop-casting 10 μL of $Ru(bpy)_3^{2+}$/Nafion solution on a bare GC macroelectrode and dried in a nitrogen environment at room temperature. The $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film reference electrode was then obtained electrochemically by sweeping the potential first anodically from 0.60 V to 1.25 V *vs.* SCE then reversing back to 0.60 V at 0.1 V/s for 20 repetitive scans in degassed pH 7.4 PBS buffer and in the final scan, the potential stopping at 1.0 V, as shown in Figure S16. At this point, the $Ru(bpy)_3^{2+}$ was partially oxidised and a Nafion film of $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}Cl_2$ was produced. The relative amount of $Ru(bpy)_3^{3+}$ and $Ru(bpy)_3^{2+}$ in Nafion films after fabrication is estimated to be 1 : 174, as shown in Section S5.

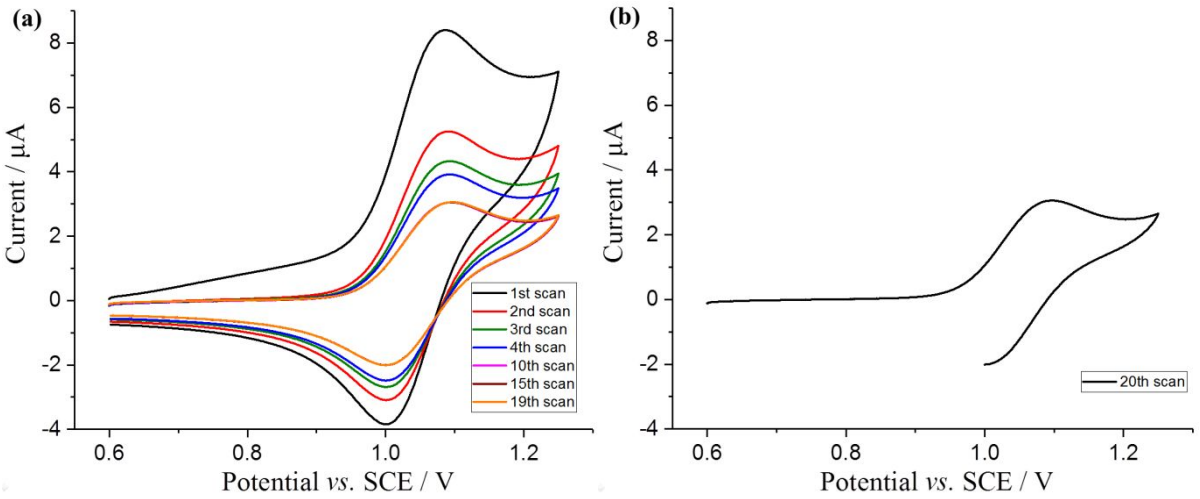

Figure S15. Voltammograms of a $Ru(bpy)_3^{2+}$-Nafion film (with the doping ratio of $Ru(bpy)_3^{2+}$: SO_3^- = 1 : 30 and thickness of 35 μm) dropcasted glassy carbon electrode in pH 7.4 PBS buffer at a scan rate of 0.1 V s^{-1} for 20 successive scans. (a) 1st scan to 19th scan (b) the 20th scan

S17 Fabrication of the PVFc/PVFc$^+$ Nafion film reference electrodes

The PVFc/Nafion stock samples (with the doping ratio of VFc : SO_3^- = 1 : 30) were prepared by directly dissolving 0.8 mg of PVFc into 2.70 mL Nafion perfluorinated resin solution. The mixture was then shaken on a vortex for 5 min. PVFc/Nafion films with film thickness of 35 µm were obtained by drop-casting 10 µL of PVFc/Nafion solution on a bare GC macroelectrode and dried in a nitrogen environment at room temperature. The PVFc/PVFc$^+$ Nafion film reference electrode was then obtained electrochemically by sweeping the potential first anodically from -0.20 V to 0.80 V *vs.* SCE then reversing back to -0.20 V at 0.1 V/s for 20 repetitive scans in degassed pH 7.4 PBS buffer and in the final scan, the potential stopping at 0.51 V, as shown in Figure S17. At this point, the PVFc was partially oxidised and a Nafion film of PVFc/PVFc$^+$ was produced. The relative amount of PVFc$^+$ and PVFc in Nafion films after fabrication is estimated to be 1 : 903, as shown in Section S5.

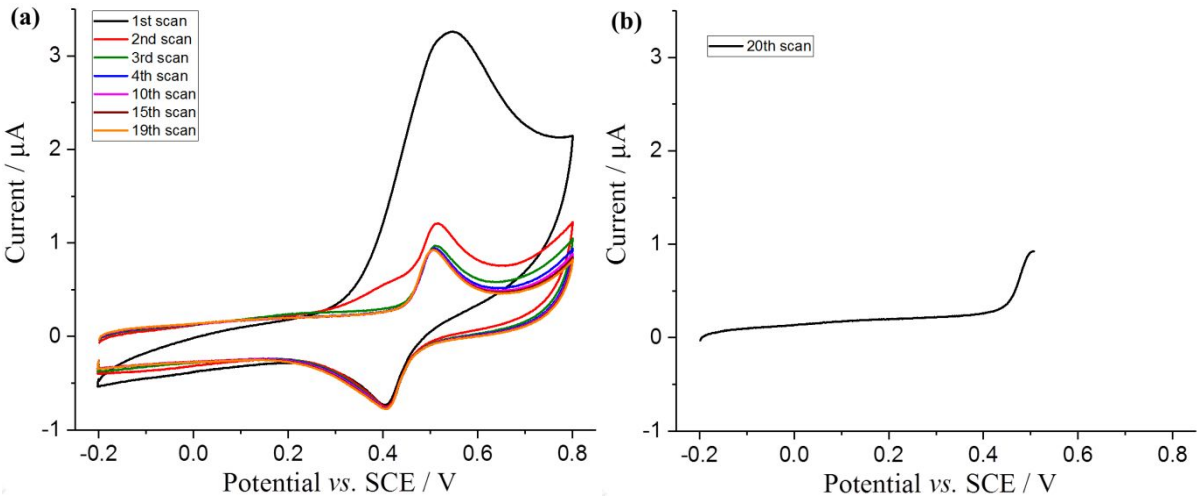

Figure S16. Voltammograms of a PVFc-Nafion film (with the doping ratio of PVFc : SO_3^- = 1 : 30 and thickness of 35 µm) dropcasted glassy carbon electrode in pH 7.4 PBS buffer at a scan rate of 0.1 V s^{-1} for 20 successive scans. (a) 1st scan to 19th scan (b) the 20th scan

Section 5 Estimation of the relative amount of dopant redox species in Nafion film reference electrodes

The relative amount of Fc and Fc^+ in Nafion films under optimized conditions (the doping ratio of Fc^+ : SO_3^- = 1 : 30 and thickness of 35 μm) can be estimated as followed:

Mass of 10 μL Nafion film (thickness of 35 μm) is

$$m = V \cdot \rho \cdot wt\% = 10\mu L \times 0.921 g/cm^3 \times 5\% = 4.61 \times 10^{-4} g$$

where ρ is the density of Nafion perfluorinated resin solution, whose value obtained from the supplier Sigma-Aldrich.

Equivalent weight (EW) of Nafion is defined as the number of grams of dry Nafion per mole of sulfonic acid groups when the material is in the acid form.[4] Nafion used in this work is 1100 EW.

The amount of SO_3^- can be then estimated as

$$n_{SO_3^-} = \frac{4.61 \times 10^{-4} g}{1100 g/mol} = 4.19 \times 10^{-7} mol$$

The doping ratio of Fc^+ : SO_3^- is 1 : 30 hence the total amount of Fc^+ in the film is

$$n_{Fc^+} = \frac{4.19 \times 10^{-7} mol}{30} = 1.40 \times 10^{-8} mol$$

The Fc was electro-generated via partial reduction of Fc^+ as shown in Figure S1 and the charge of this partial reduction can be estimated, with the aid of the software Origin, from Figure S1b to be 2.46×10^{-6} C.

Hence the number and mole of Fc generated within the film is

$$N_{Fc} = \frac{2.46\times10^{-6}C}{1.60\times10^{-19}C} = 1.53\times10^{13}; n_{Fc} = 2.54\times10^{-11}mol$$

The relative amount of Fc and Fc^+ in Nafion films is

$$\frac{n_{Fc}}{n_{Fc^+}} = \frac{2.54\times10^{-11}mol}{1.40\times10^{-8}mol - 2.54\times10^{-11}mol} = \frac{1}{548}$$

Similarly, the relative amount of dopant redox species in Nafion film of other three reference electrodes under optimized conditions are estimated to be:

For the MV^{2+}/MV^+ Nafion film reference electrodes, $MV^+ : MV^{2+} = 1 : 133$

For the $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ Nafion film electrodes, $Ru(bpy)_3^{3+} : Ru(bpy)_3^{2+} = 1 : 174$

For the $PVFc/PVFc^+$ Nafion film reference electrodes: $PVFc^+ : PVFc = 1 : 903$

Section 6 Fabrication of the Nafion particle reference electrodes

Nafion particles doped with MV^{2+} were synthesized by the re-precipitation method, as described in detail previously.[1, 5] Briefly, 5 μL of 300 mM MV^{2+} aqueous solution was added into 50 μL concentrated 12.5 wt% Nafion. Note that 12.5 wt% Nafion was obtained by bathing the commercial available 5 wt% Nafion® perfluorinated resin solution in a 52 ± 0.5°C water bath. The mixture of MV^{2+} and Nafion was stirred with a pipette tip for 200 circles then injected into 1 mL ultrapure water dropwise under stirring by magnetic vortexing at 600 rpm for 5 minutes. The resulting mixture was subjected to sonication (FB15050, Fisher Scientific, 50/60 Hz, Germany) for 30 min at room temperature. The suspension was then centrifuged (Eppendorf Centrifuge 5430 R) at 14000 rpm for 10 min and the precipitate was washed with water thoroughly. This washing-step was repeated three times to fully remove excess MV^{2+}. The obtained MV^{2+}/Nafion particles were characterised as previously described giving an average radius of 0.43 ± 0.26 μm with a doping amount (ratio of MV^{2+}:SO_3^-) of 1:20.[5, 6] MV^{2+}/Nafion particles were dispersed evenly in 0.5 mL water by sonication for 5 min. 30 μL of this suspension was drop-cast on the GC electrode and dried in a nitrogen environment at room temperature. 45 layers of MV^{2+}/Nafion particles were dropcasted on the surface of GC electrode suggesting an approximate 38.7 μm total thickness. The MV^{2+}/MV^+ Nafion particle based reference electrode was then obtained electrochemically, similarly to the MV^{2+}/ MV^+ Nafion film, by sweeping the potential first cathodically from -0.20 V to -0.95 V *vs.* SCE then reversing back to -0.20 V at 0.1 V/s for 20 repetitive scans in degassed pH 7.4 PBS buffer and in the final scan, the potential stopping at -0.73 V. At this point, the MV^{2+} was partially reduced and a MV^{2+}/MV^+Cl_2 Nafion particle based reference electrode was obtained.

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