Supporting Information

Cardiac Troponin I:
Ultrasensitive Detection Using Faradaic Electrochemical Impedance

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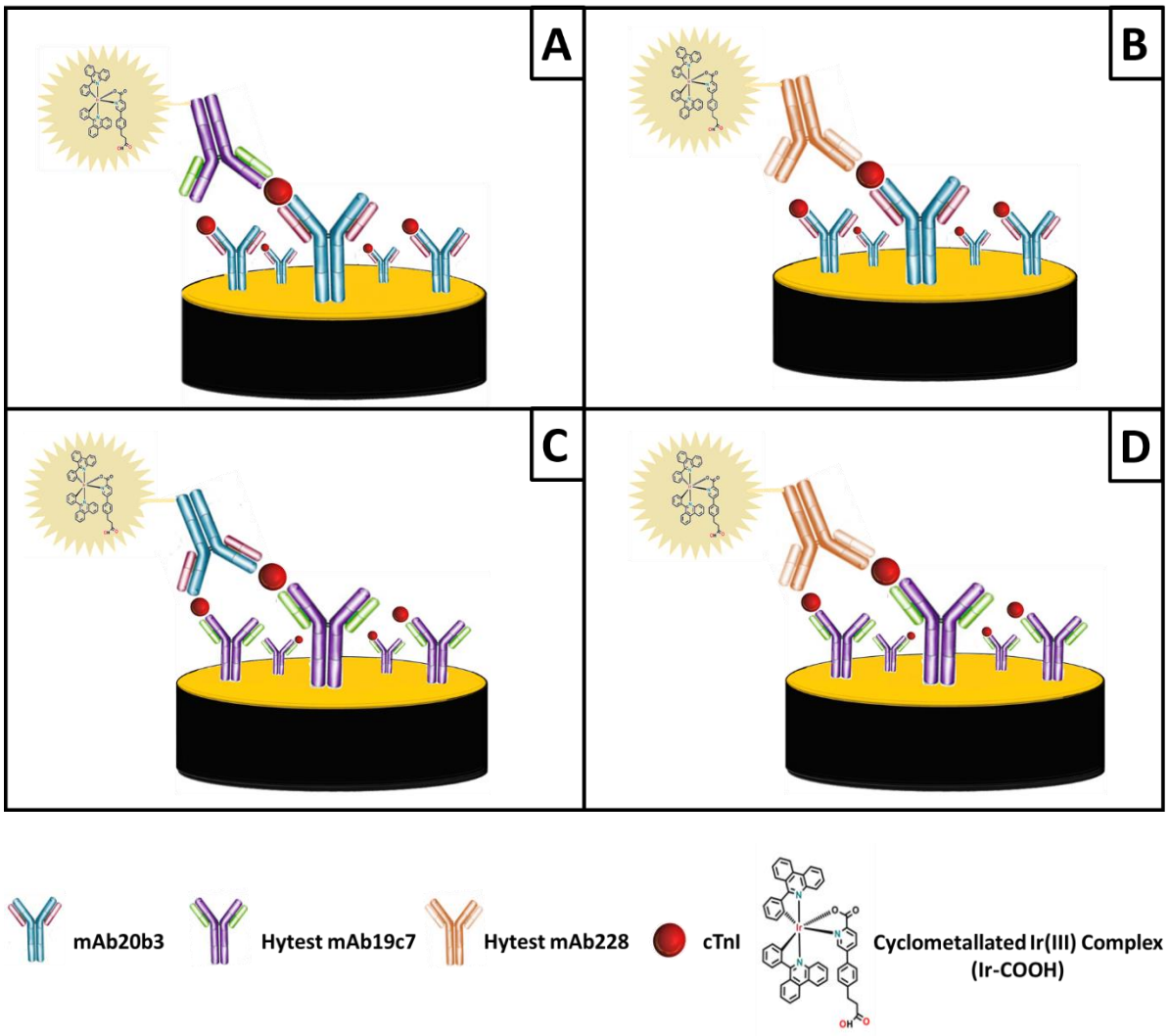
ABSTRACT: This supporting information includes SDS-PAGE and western blot analysis of the purification of monoclonal antibody, Nyquist plots of various immunoassay optimization, confocal images of control tests as well as 1 ag/mL – 1 ng/mL cTnI immunoassays with alternative primary and secondary mAbs and Lifetime and anisotropy rotational time of Ir-COOH and the conjugates.



Scheme S1: Schematic representation of all 4 immunoassays carried out with alternating primary and secondary mAb combinations to compare the performance of the custom and commercial mAbs for ***capture*** efficiency of cTnI and increased ***detection*** enhancement of cTnI. The four possible configurations of the sandwich immunoassays with in house generated and commercially available antibodies, following exposure to the cTnI target are as follows: The in house generated mAb20B3 following exposure to increasing cTnI and the Ir(III) labelled commercial secondary antibody mAb19C7 **(A)** and mAb228 **(B)**. In a comparison study, the commercially available primary antibody mAb19C7 was used and this was exposed to cTnI target and the Ir(III) labelled in house generated secondary antibody mAb20B3 **(C)** and commercially secondary available mAb228 **(D)**.

Supplementary Figures and Tables

Figure S1

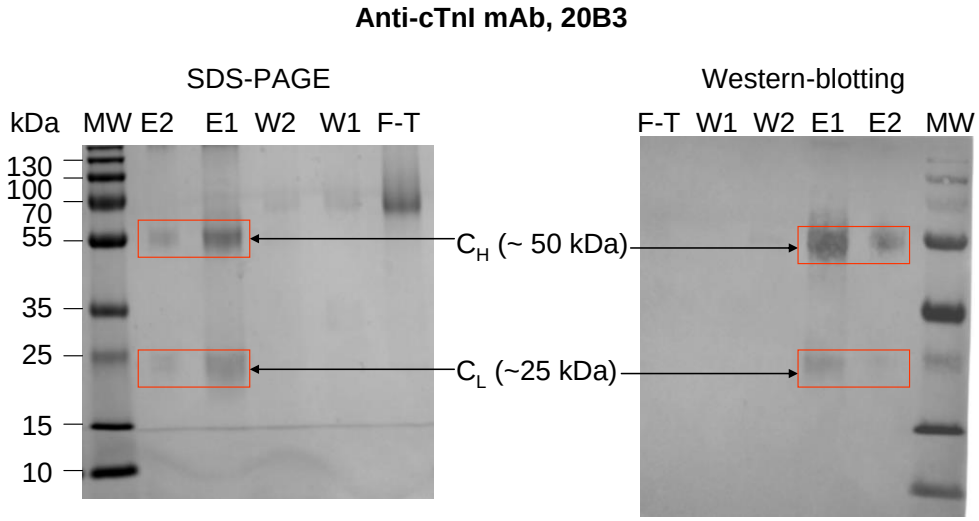


Figure S1: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot (WB) analysis of the purification of monoclonal antibody, 20B3 using protein G.

Figure S2

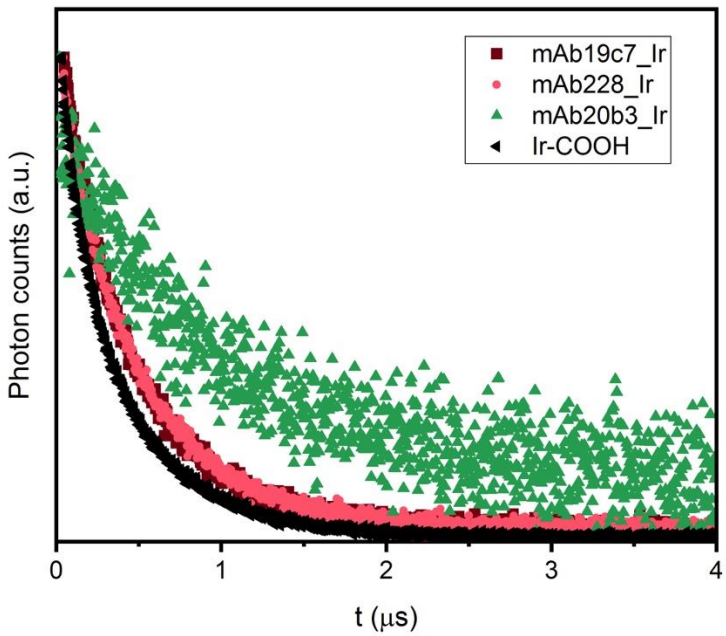


Figure S2: Luminescence lifetime of Ir-COOH and the conjugates in aerated PBS solution. λ_{exc} 405 nm, λ_{em} 650 nm.

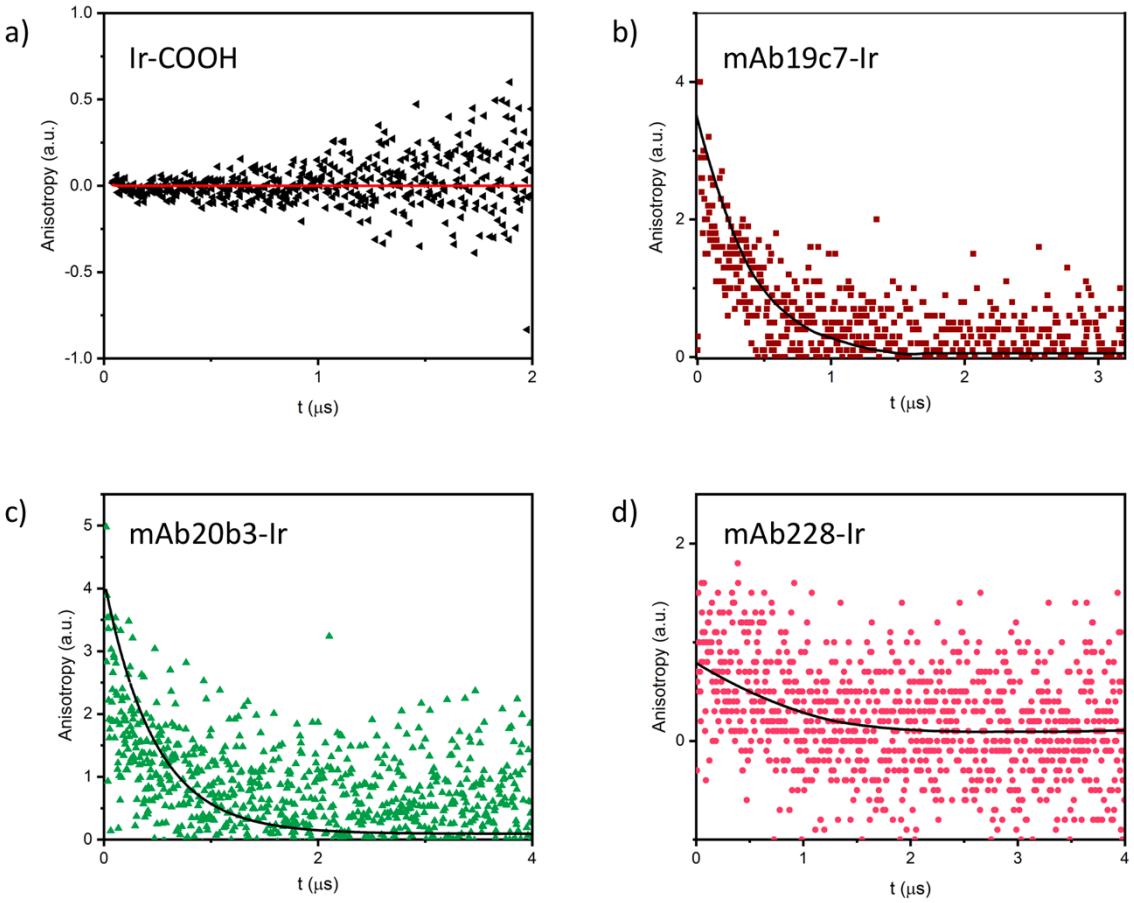


Figure S3: Anisotropy decays for Ir-COOH (**A**) and the conjugates (mAb19C7 (**B**), mAb20b3 (**C**), mAb228 (**D**)) in aerated PBS solution. λ_{exc} 405 nm, λ_{em} 650 nm. Sheet polarizers were used to select V polarized excitation from the diode source and the G-factor was estimated by tail fitting the VV and VH emission decays. The data were fit using Fluofit software with a single decay function tail fitting.

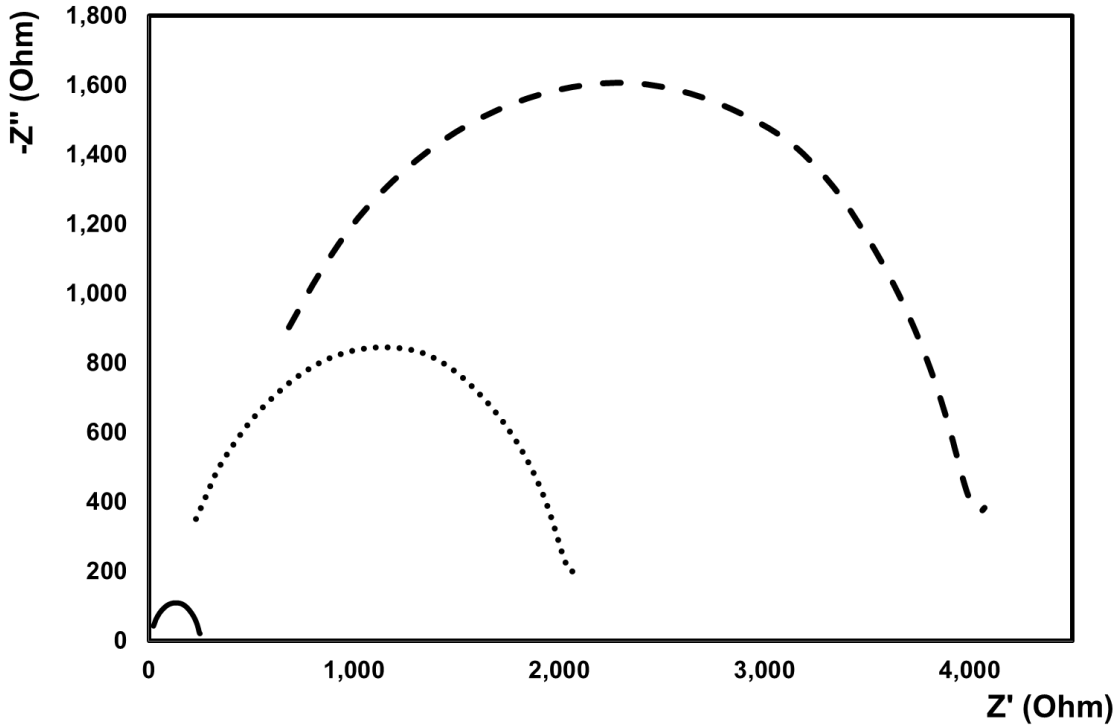


Figure S4: R_{ct} (Ω) of gold disc electrode (bold) modified with 16-MHDA SAM (dots) and in house generated 20b3 capture antibodies (dash). The EIS were recorded in the presence of 1 mM DPBS at a frequency range between 0.01 Hz and 100,000 Hz using an ac amplitude of 25 mV amplitude and the dc potential set to the open circuit potential.

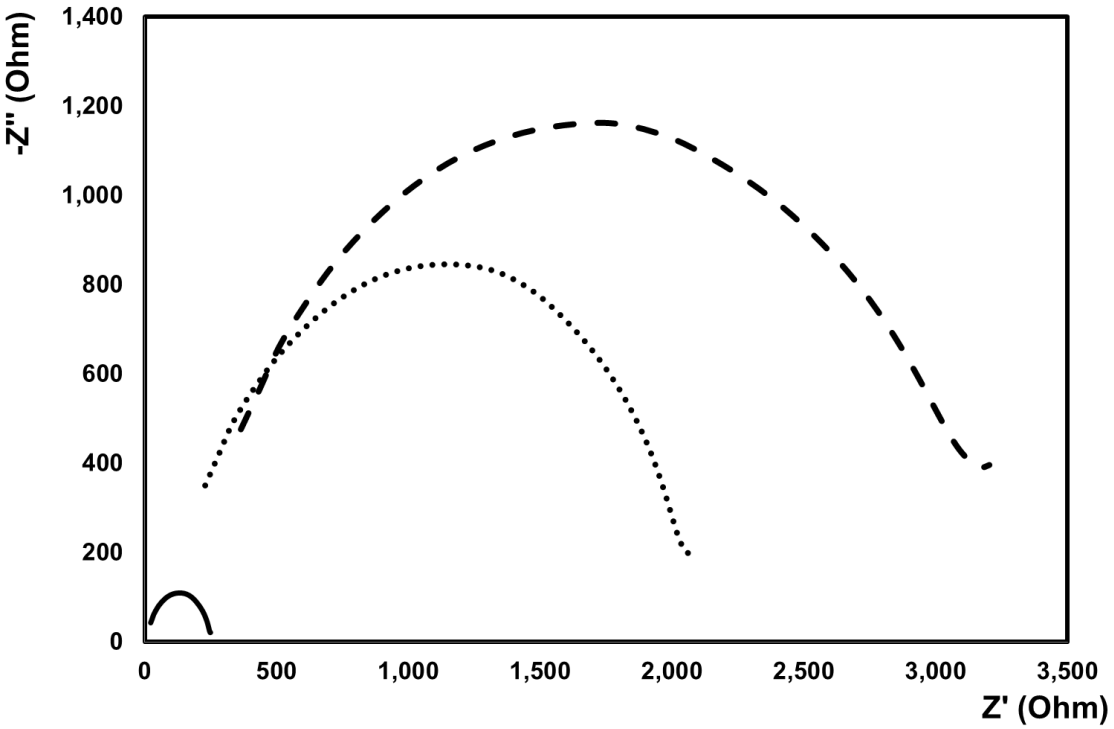


Figure S5: R_{ct} (Ω) of gold disc electrode (bold) modified with 16-MHDA SAM (dots) and commercially available monoclonal antibodies with a similar epitope to our custom synthesised mAb (Hytest 228, dash). The EIS were recorded in the presence of 1 mM DPBS at a frequency range between 0.01 Hz and 100,000 Hz using an ac amplitude of 25 mV amplitude and the dc potential set to the open circuit potential.

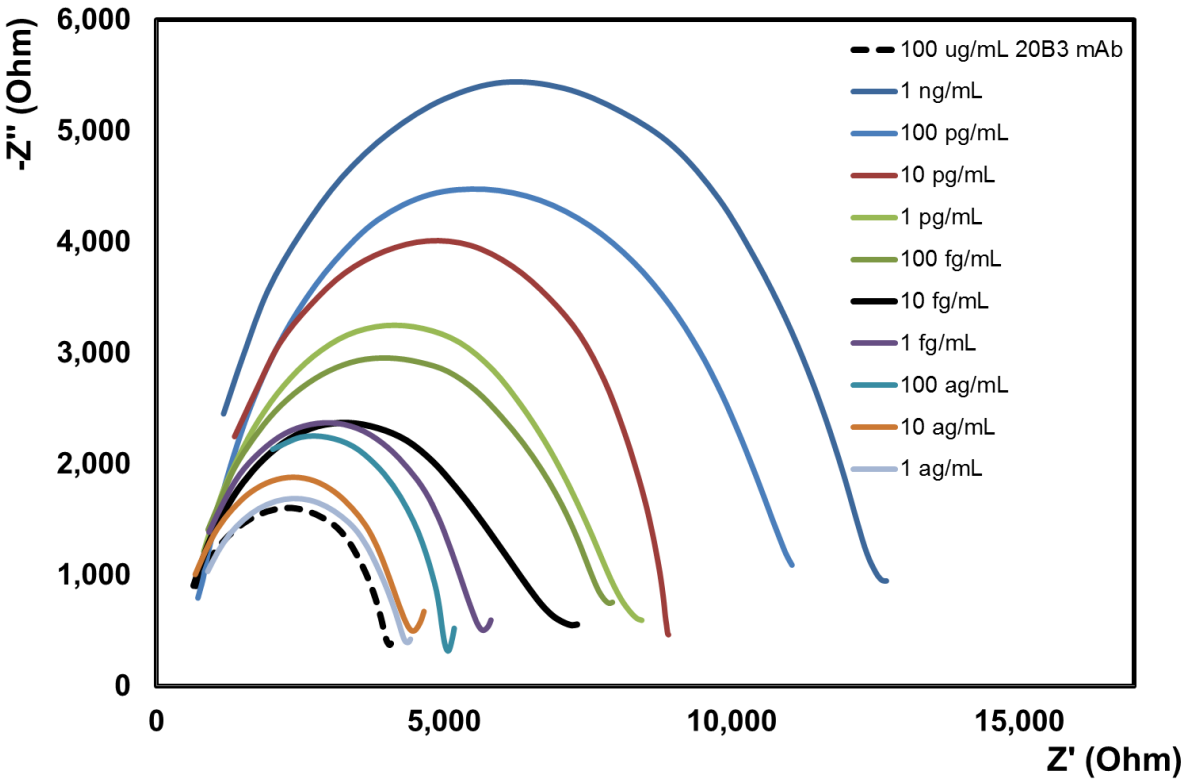


Figure S6: Nyquist plots of mAb20b3_cTnI bound to modified gold electrodes with the concentration of cTnI systematically varied from 1 ag/mL to 1 ng/mL in 1 mM DPBS (N=3). The EIS were recorded in the presence of 1mM DPBS using an amplitude of 25mV amplitude and the dc potential set to the open circuit potential. The frequency range was between 0.01Hz and 100,000Hz.

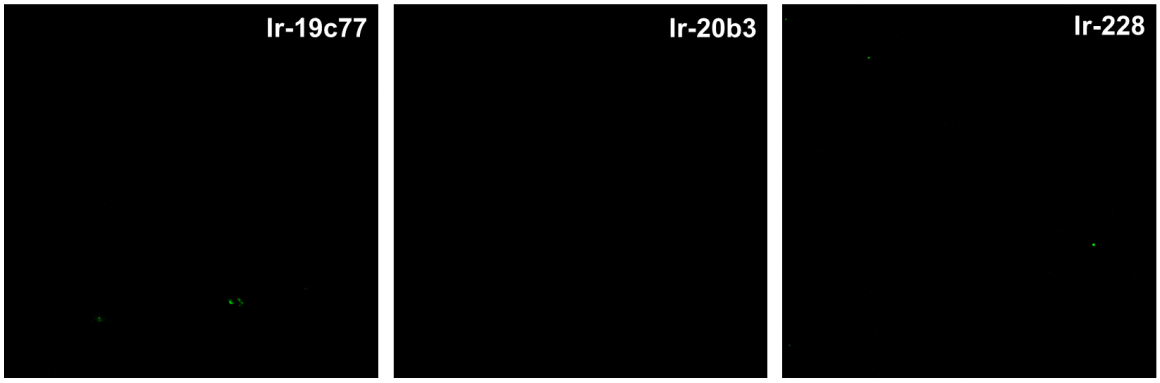


Figure S7: *Confocal Images of 16-MHDA monolayer modified gold electrodes after incubation with Ir-COOH-mAb (mAb = 19C7, 20b3, 228) in the absence of primary antibody and cTnI complex. In all cases, scale bar 20 µm and luminescence images were recorded live on a Zeiss LSM510 Meta confocal microscope using a 40× oil immersion objective lens (NA 1.4) and a 488 nm argon ion laser applied for Iridium labelled antibody imaging.*

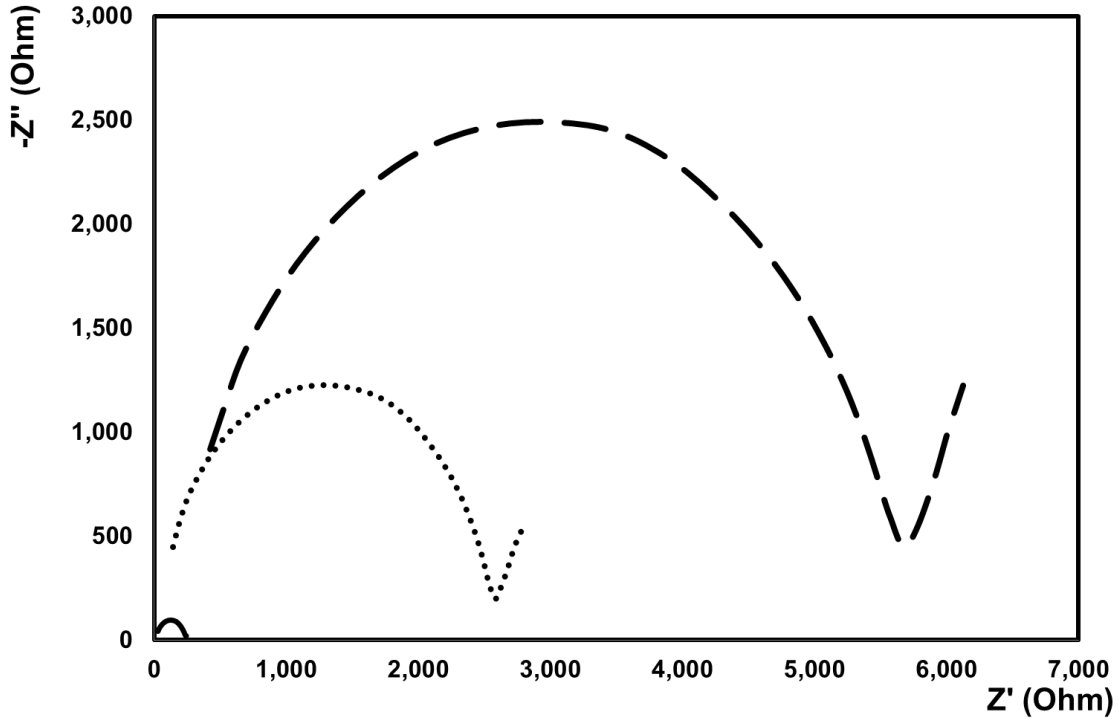


Figure S8: R_{ct} (Ω) of gold disc electrode (bold) modified with 16-MHDA SAM (dots) after incubation with 19C7 capture antibodies (dash). The EIS were recorded in the presence of 1 mM DPBS at a frequency range between 0.01 Hz and 100,000 Hz using an ac amplitude of 25 mV amplitude and the dc potential set to the open circuit potential.

Figure S9

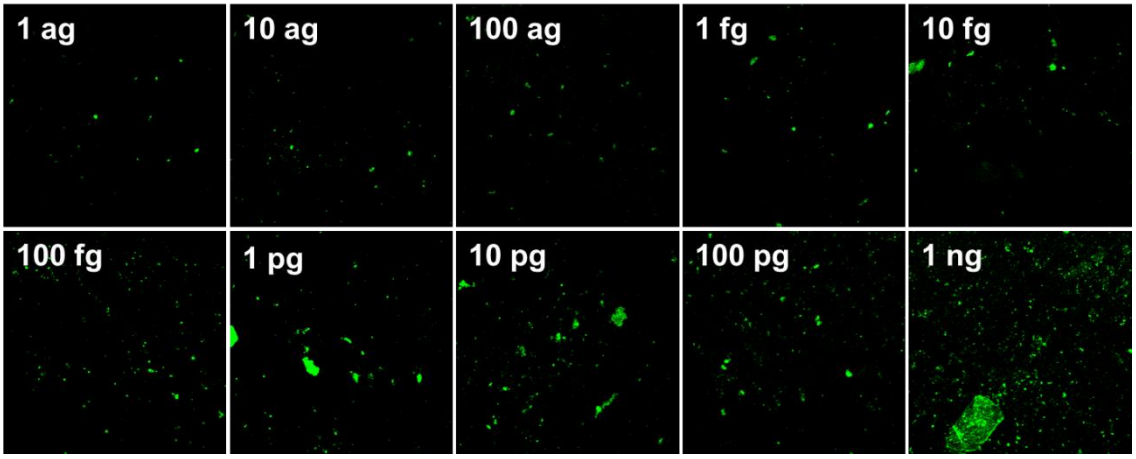


Figure S9: Confocal images of a 16-MHDA monolayer modified gold electrode following 100 µg/mL commercially available Hytest mAb19C7 antibody conjugation with cTnI target (cTnI concentration range 1 ag/mL-1 ng/mL) coupled to 100 µg/mL Ir(III) labelled antibody (***mAb20b3***). Scale bar 20 µm. Luminescence images were recorded live on a Zeiss LSM510 Meta confocal microscope using a 40× oil immersion objective lens (NA 1.4) and a 488-nm argon ion laser applied for Iridium labelled antibody imaging.

Figure S10

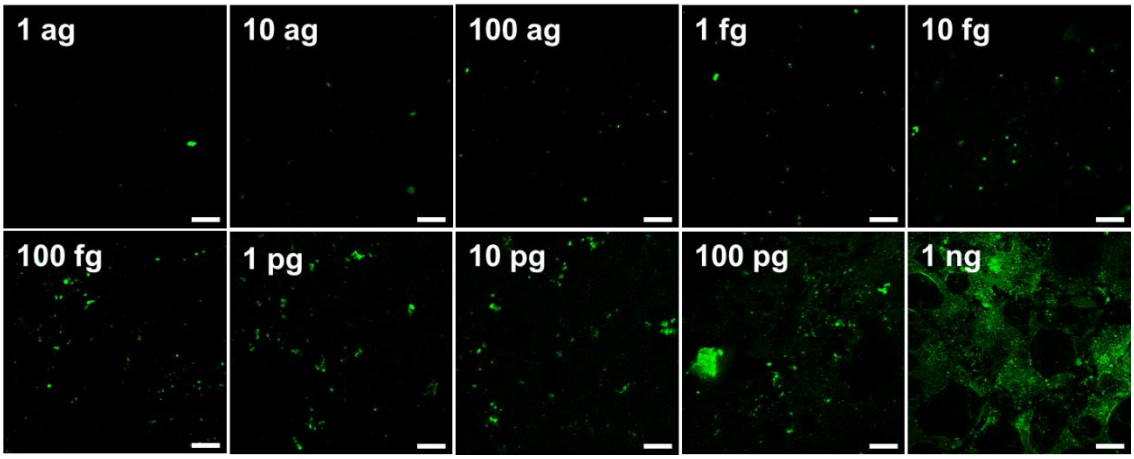


Figure S10: Confocal images of a 16-MHDA monolayer modified gold electrode following 100 µg/mL commercially available Hytest mAb19C7 antibody conjugation with cTnI target (cTnI concentration range 1 ag/mL-1 ng/mL) coupled to 100 µg/mL Ir(III) labelled commercially available antibody (***mAb228***). Scale bar 20 µm. Luminescence images were recorded live on a Zeiss LSM510 Meta confocal microscope using a 40× oil immersion objective lens (NA 1.4) and a 488-nm argon ion laser applied for Iridium labelled antibody imaging.

Table S1

	Lifetime (ns)	θ (ns)	β
Ir-COOH	430 (56%) 150 (44%)	0	0
Ir-mAb19c7	470	480 (r_0=-0.02)	37°
Ir-mAb228	466	760 (r_0=0.009)	40°
Ir-mAb20b3	924	490 (r_0=-0.014)	37°

Table S1: Lifetime, anisotropy rotational time θ in ns and angle between the absorption and emission transition moment β of Ir-COOH and the conjugates. Time resolved luminescence anisotropy measurements were analyzed to yield anisotropy (rotational correlation time, τ_{rot}) values using the mono-exponential model shown in Equation $r(t)=r_0 \exp(-\tau/(\tau_{rot}))$, where r(t) is the time dependent anisotropy, r_0 is the initial anisotropy, τ is the luminescent lifetime of the sample. Because of the long time-scale of the anisotropy decay and consequently the relatively low resolution of the data over short time-scales, a tail fit rather than re-convolution was used to fit the anisotropy data according to this model.