Supporting Information:

Functionalized Carbon Nanotube Adsorption Interfaces for Electron Transfer Studies of Galactose Oxidase

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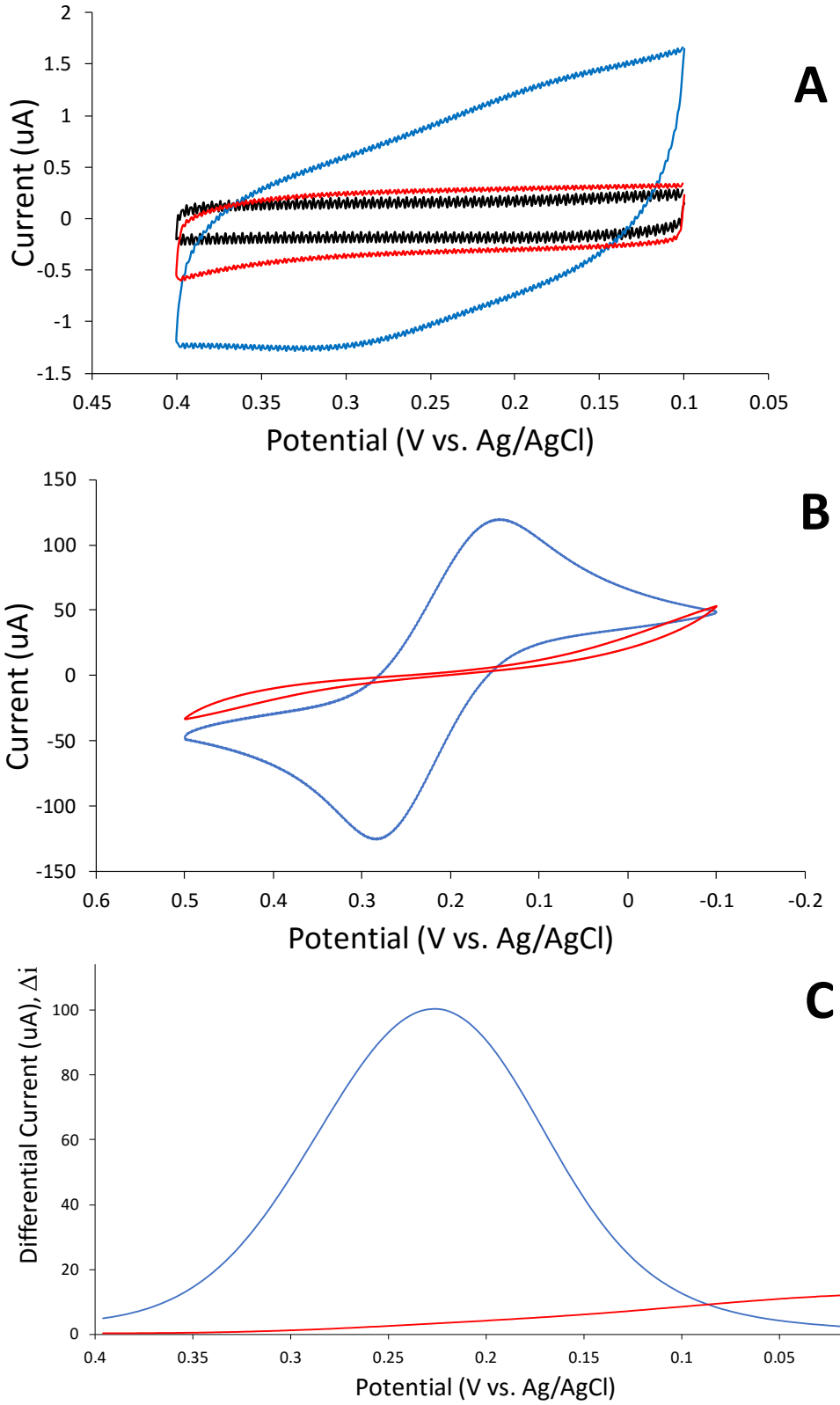

Figure SI-1. Electrochemical techniques to confirm modification of the gold electrode including **(A)** capacitance measurements of as received gold (black trace), clean bare gold (blue trace), and TA-SAM modified gold (red trace); **(B)** cyclic volammetry and **(C)** differential pulse voltammetry of 5 mM $Fe(CN)_6^{3-/4-}$ (0.5 M KCl) redox probe at clean bare (blue trace) and TA-SAM modified gold (red trace). Note: scan rate is 0.020 V/sec.

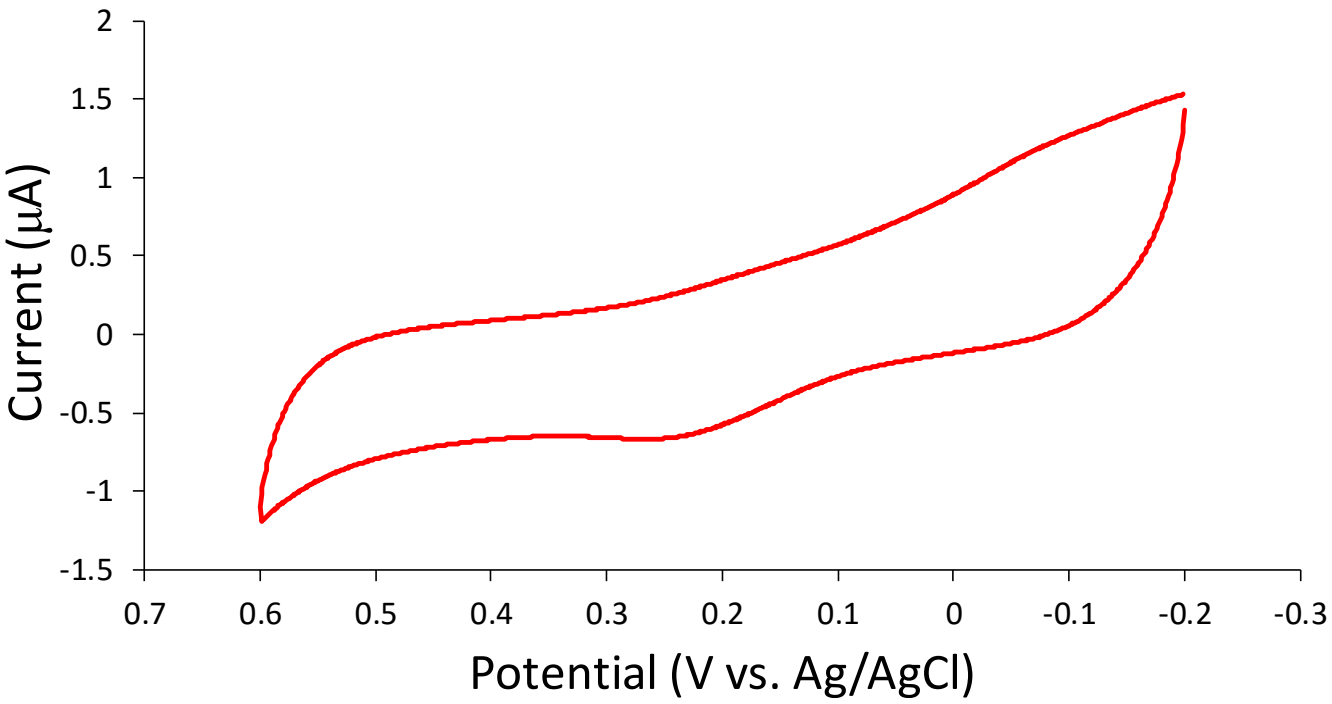

Figure SI-2. Typical cyclic voltammetry of GaOx adsorbed at a 4-mercaptoaniline (4-aminothiophenol, 4-ATP) modified electrode in 20 mM MES buffer (pH 7.5) at a scan rate of 0.020 V/sec. .

Table 1. Estimated Electrochemical Parameters for GaOx Adsorbed at Various Modified Electrode Platforms

Platform	Adsorption Interface	# Films	Est. ET Distance[a] (min-max) (nm)	$E_{p,c}$ (V)	$E_{p,a}$ (V)	$E^{\circ'}$ (V)	ΔE_p (V)	Γ_c[b] (pmol/cm^2)	k_{ET}^0[c] (s^{-1})	FWHM[d] (V)
Au	Au	6	~0.8-9	0.125 (0.01)	0.274 (0.02)	0.199 (0.01)	0.149 (0.03)	30.9 (8.3)	1.2 (0.08)	0.170 (0.030)
4-ATPh SAM	NH_2	3	~1.3-9.5	0.108 (0.02)	0.253 (0.01)	0.149 (0.02)	0.208 (0.06)	2.8 (0.52)	0.66 (0.12)	0.059 (0.009)
CYST-SAM	NH_2	3	~1.1-9.3	0.105 (0.02)	0.278 (0.00)	0.192 (0.01)	0.173 (0.02)	43.7 (9.4)	0.82 (0.03)	0.162 (0.003)
TA-SAM	COOH	2	0.78-9.7	0.086 (0.00_1)	0.232 (0.00_3)	0.159 (0.00_1)	0.146 (0.00_3)	64.8 (8.4)	0.42 (0.04)	0.139 (0.007)
TA-SAM + NH_2 MWCNT	NH_2	2	~150-250	0.061 (0.00_1)	0.288 (0.04)	0.175 (0.02)	0.227 (0.04)	35.5 (6.5)	0.676 (0.10)	0.160 (0.010)
TA-SAM + NH_2-SWCNT	NH_2	4	~20-30	0.073 (0.01)	0.277 (0.03)	0.162 (0.01)	0.231 (0.05)	40.2 (6.7)	0.55 (0.05)	0.123 (0.007)
CYST-SAM + COOH-MWCNT	COOH	4	~150-250	0.131 (0.01)	0.257 (0.01)	0.194 (0.00_3)	0.126 (0.01)	27.9 (7.3)	0.34 (0.04)	0.163 (0.008)
CYST-SAM + COOH-SWCNT	COOH	5	~20-30	0.107 (0.01)	0.260 (0.01)	0.183 (0.01)	0.153 (0.01)	68.1 (9.4)	0.74 (0.09)	0.174 (0.010)

Notes: Uncertainty ( ) represents standard error. [a] Minimum and maximum ET distance estimated from modeling, prior studies of protein adsorption to SAMs [24], the GaOx structure (globular metalloprotein with dimensions of 9.8 x 8.9 x 8.7 nm and the copper redox center 0.8 nm from surface representing minimum ET distance within enzyme)[4], and analysis of the cross-sectional TEM of the CNT films (Figure 5) and Supporting Information; [b] Apparent surface coverage (Γ_c) is calculated from charge passed during cathodic cyclic voltammetry peak without de-convolution of two separate electron transfers (n=2) demonstrated in prior work and likely resulting in the observed elevated values [4]; [c] Calculated using Laviron's Theorem for diffusionless (adsorbed) electrochemical systems [41] assuming a charge transfer coefficient of 0.5; [d] As estimation calculated from convoluted peak and should not be interpreted as a single ET where ideal adsorbed, reversible behavior would result in a FWHM of .091 V.

Table SI-1. Electrochemical Parameters for GaOx Adsorbed at Various Modified Electrode Platforms

Platform	Adsorption Interface	n	$E_{p,c}$ (V)	$E_{p,a}$ (V)	$E^{\circ\prime}$ (V)	ΔE_p (V)	Γ_c (pmol/cm^2)	k_{ET}^0 (s^{-1})
TA-SAM	COOH	2	0.086 (0.00_1)	0.232 (0.00_3)	0.159 (0.00_1)	0.146 (0.00_3)	64.8 (8.4)	0.42 (0.04)
TA Exchanged With MUD	COOH/OH	1	0.009	NP	N/A	N/A	47.38	(N/A)
TA Exchanged With C8	COOH/CH_3	1	NP	NP	N/A	N/A	(N/P)	(N/A)
TA Exchanged With MHOL	COOH/OH	1	0.039	0.282	0.160	0.243	14.97	(N/A)
TA Exchanged With MUA	COOH	1	0.009	0.255	0.132	0.246	223.2	(N/A)
MHA SAM	COOH	5	0.064 (0.01)	0.248 (0.01)	0.156 (0.01)	0.184 (0.00_7)	169 (28.0)	0.082 (0.01)
MHA/MUA SAM	COOH	5	0.005 (0.01)	0.246 (0.01)	0.126 (0.01)	0.242 (0.00_8)	137 (19.2)	0.033 (0.00_6)
MHA/MUD SAM	COOH/OH	2	0.021 (0.00_9)	0.251 (0.02)	0.136 (0.02)	0.230 (0.01)	126 (9.3)	0.034 (0.00_9)
MHA/MHOL SAM	COOH/OH	2	0.038	0.268	0.153	0.230	50.2	0.031 (0.00_6)

Notes: TA = thioctic acid; MUD = 11-mercaptoundecanol; C8 = octane thiol; MHOL = mercaptohexanol; MUA = 11-mercaptoundecanoic acid; MHA = mercapto-hexanoic acid; COOH = carboxylic acid functionality; OH = hydroxyl group functionality; CH_3 = methyl group functionality. All parameters measured at 20 mV/sec except k_{ET}° (Laviron's Theorem); Uncertainty represents standard error.

Table SI-2. Electrochemical Parameters for GaOx Adsorbed at Various Modified Electrode Platforms

Platform	Adsorption Interface	n	$E_{p,c}$ (V)	$E_{p,a}$ (V)	$E^{\circ\prime}$ (V)	ΔE_p (V)	Γ_c (pmol/cm^2)	k_{ET}^0 (s^{-1})
MUA/ CSNPs	COOH	3	0.107 (0.00_8)	0.243 (0.00_6)	0.175 (0.00_6)	0.136 (0.00_8)	265.9 (71.8)	0.150 (0.03)
MHA/CSNPs	COOH	3	0.077 (0.01)	0.263 (0.01)	0.170 (0.00_3)	0.186 (0.02)	557.3 (43.5)	0.137 (0.07)
BPDT SAM/PLL /TASNP	COOH	3	0.023 (0.04)	0.295 (0.02)	0.114 (0.00_8)	0.363 (0.03)	9.137 (3.0)	0.483 (0.11)
C6-BPDT SAM/ TASNP	COOH	3	-0.058 (0.00_6)	0.135 (0.00_5)	0.039 (0.00_5)	0.192 (0.00_2)	2.606 (0.56)	0.122 (0.00)
C6-NDT SAM/ TASNP	COOH	3	-0.072 (0.00_8)	0.153 (0.00)	0.041 (0.00_3)	0.225 (0.00_8)	1.730 (0.19)	0.051 (0.00_5)

Notes: MUA = 11-mercaptoundecanoic acid; PLL = poly-L-lysine, cationic linker; CS-NP = citrate-stabilized gold nanoparticles; MHA = mercaptohexanoic acid; BPDT = biphenyldithiol; TAS-NPs = thioctic acid stabilized gold nanoparticles; C6 = hexanethiol; NDT = nonanedithiol. All parameters measured at 20 mV/sec except k_{ET}° (Laviron's Theorem); Uncertainty represents standard error.

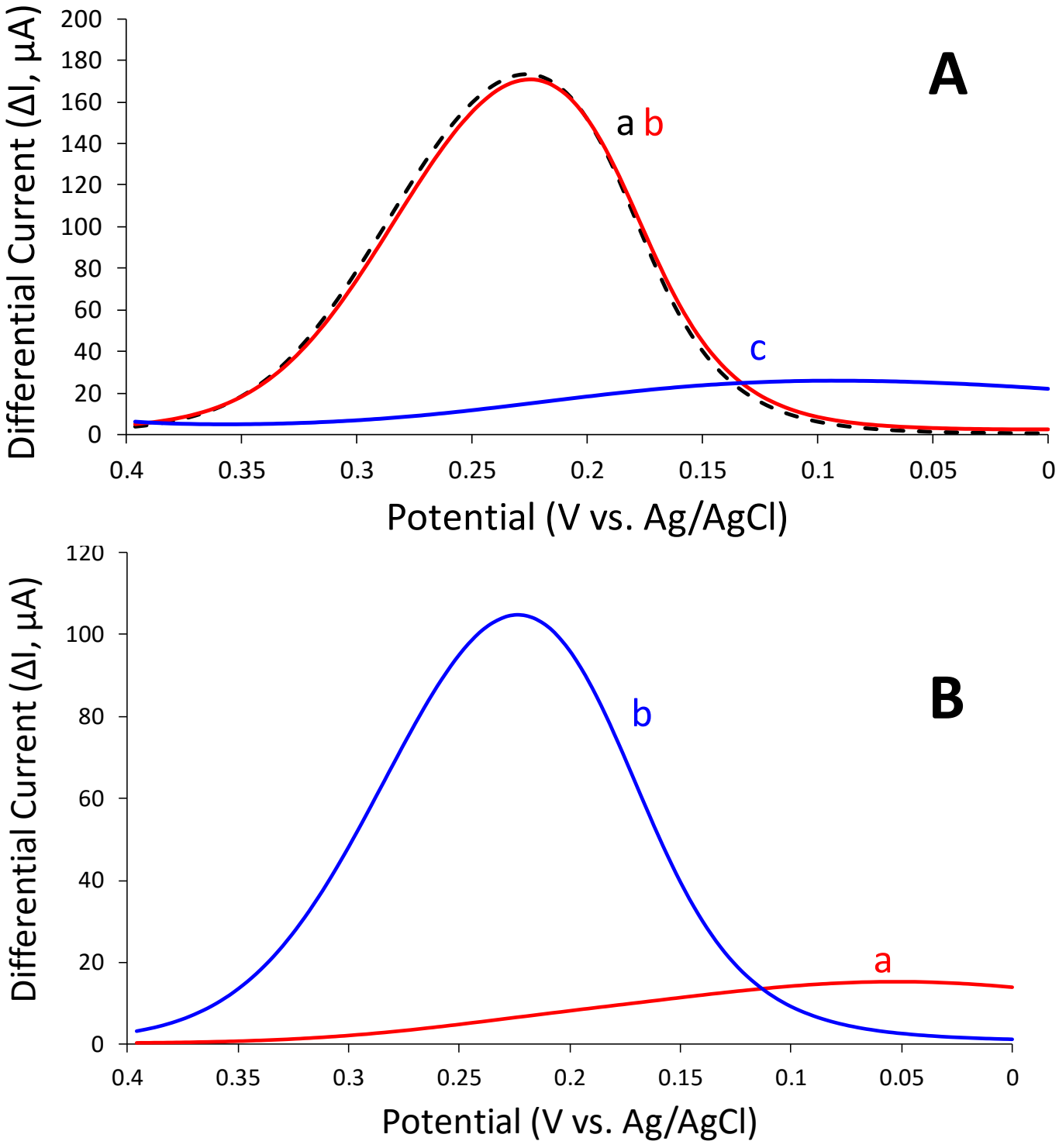

Figure SI-3. Typical differential pulse voltammetry (DPV) of 5 mM $Fe(CN)_6^{3-/4-}$ (0.5 M KCl) at the same interfaces described in **(A) Figure 2** and **(B) Figure 3** of the paper, respectively. Specific interfaces include (**top, A**) **(a)** CYST-SAM modified gold, **(b)** CYST-SAM with amide-coupled COOH-SWCNT, and **(c)** CYST-SAM with amide-coupled COOH-SWCNTs in 8 mM KPB (pH 10) and; (**bottom, B**) **(a)** TA-SAM modified gold, **(b)** TA-SAM with amide-coupled NH_2-SWCNTs.

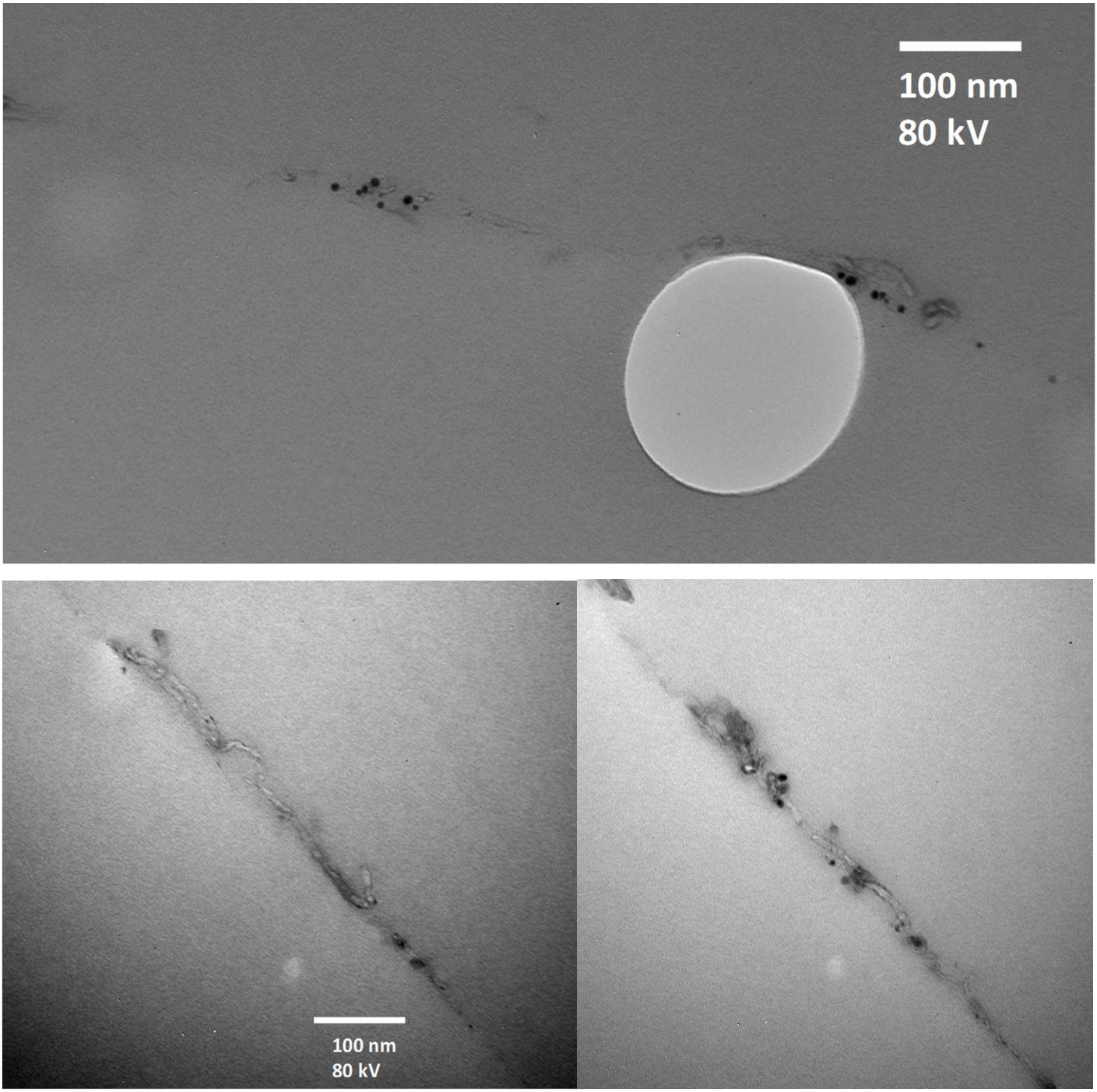

Figure SI-4. Cross-sectional TEM imaging of the COOH-SWCNT films that comprise the GaOx adsorption interface. Notes: small black circles are believed to be CNT overlap sites whereas the large white structure is believed to be a bubble in the epoxy resin used to lift the film (see Experimental Details).

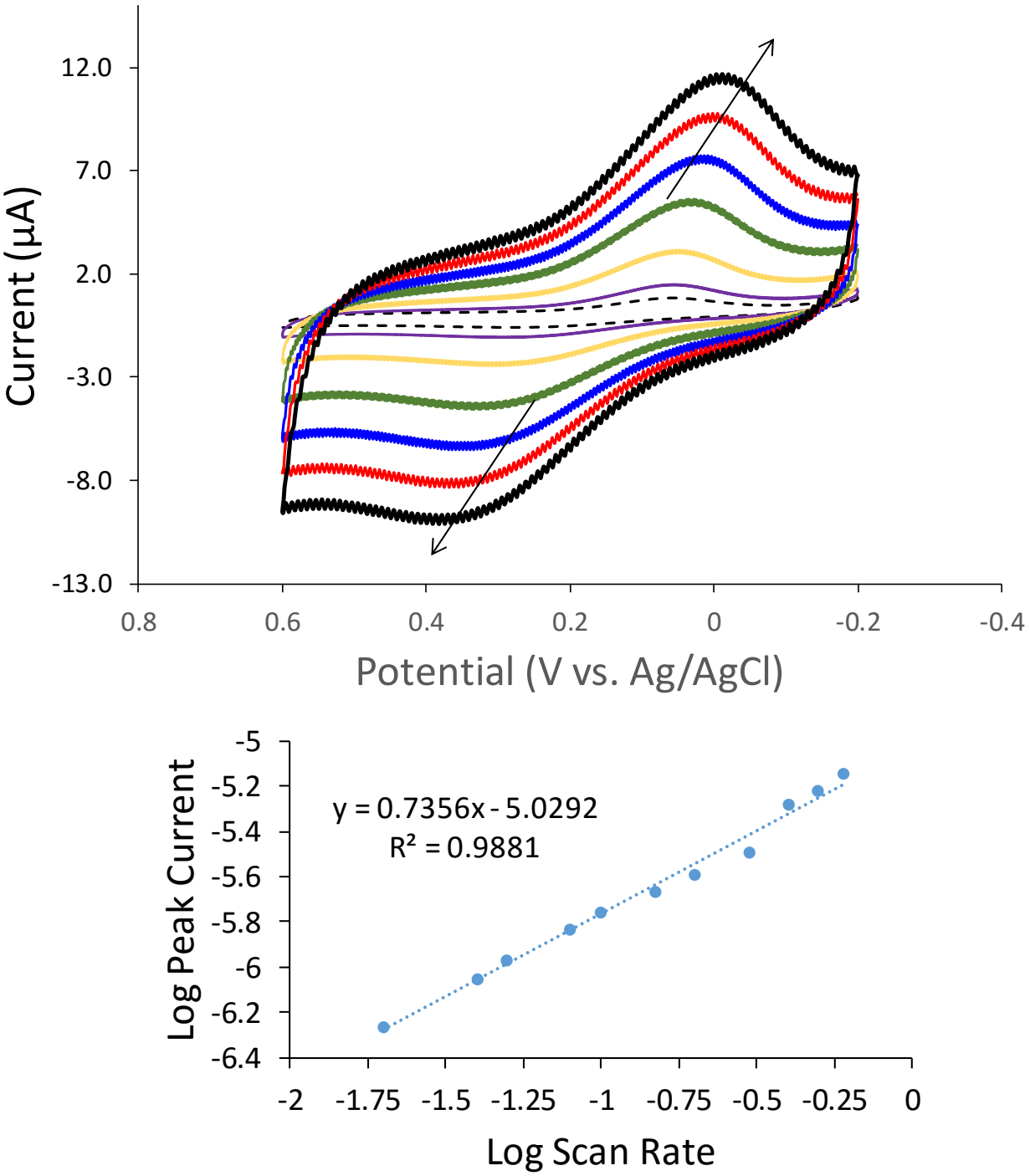

Figure SI-5. Cyclic voltammetry of GaOx adsorbed at TA-SAM modified electrodes amide-coupled with NH_2-**MW**CNTs at increasing scan rates (υ) with linear plot (**below**) of log peak current ($I_{p,c}$) versus log of scan rate υ, linear for non-diffusional (adsorbed) behavior.

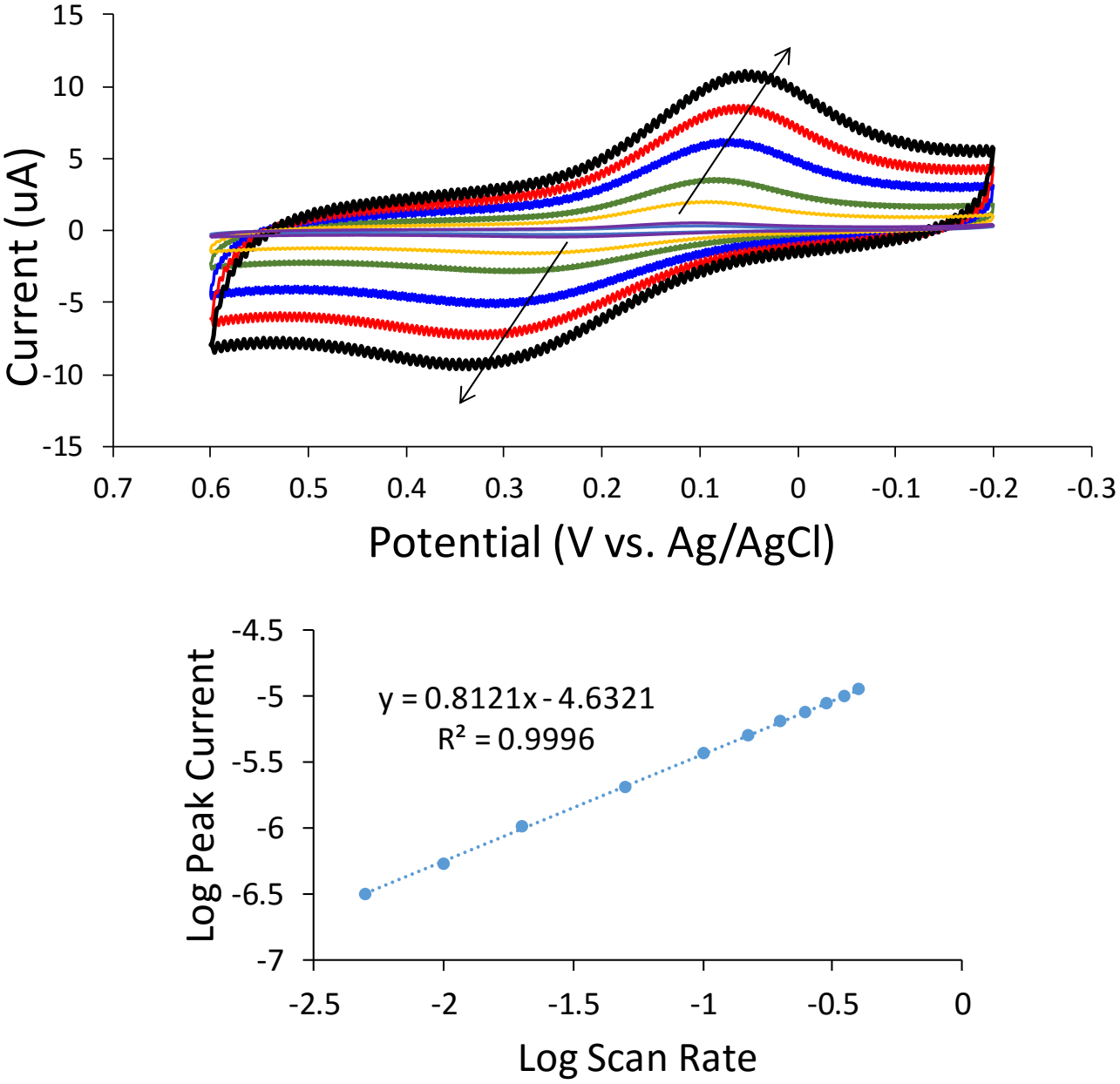

Figure SI-6. Cyclic voltammetry of GaOx adsorbed at TA-SAM modified electrodes amide-coupled with NH_2-**SW**CNTs at increasing scan rates (υ) with linear plots (**below**) of log peak current ($I_{p,c}$) versus log of scan rate υ, linear for non-diffusional (adsorbed) behavior.

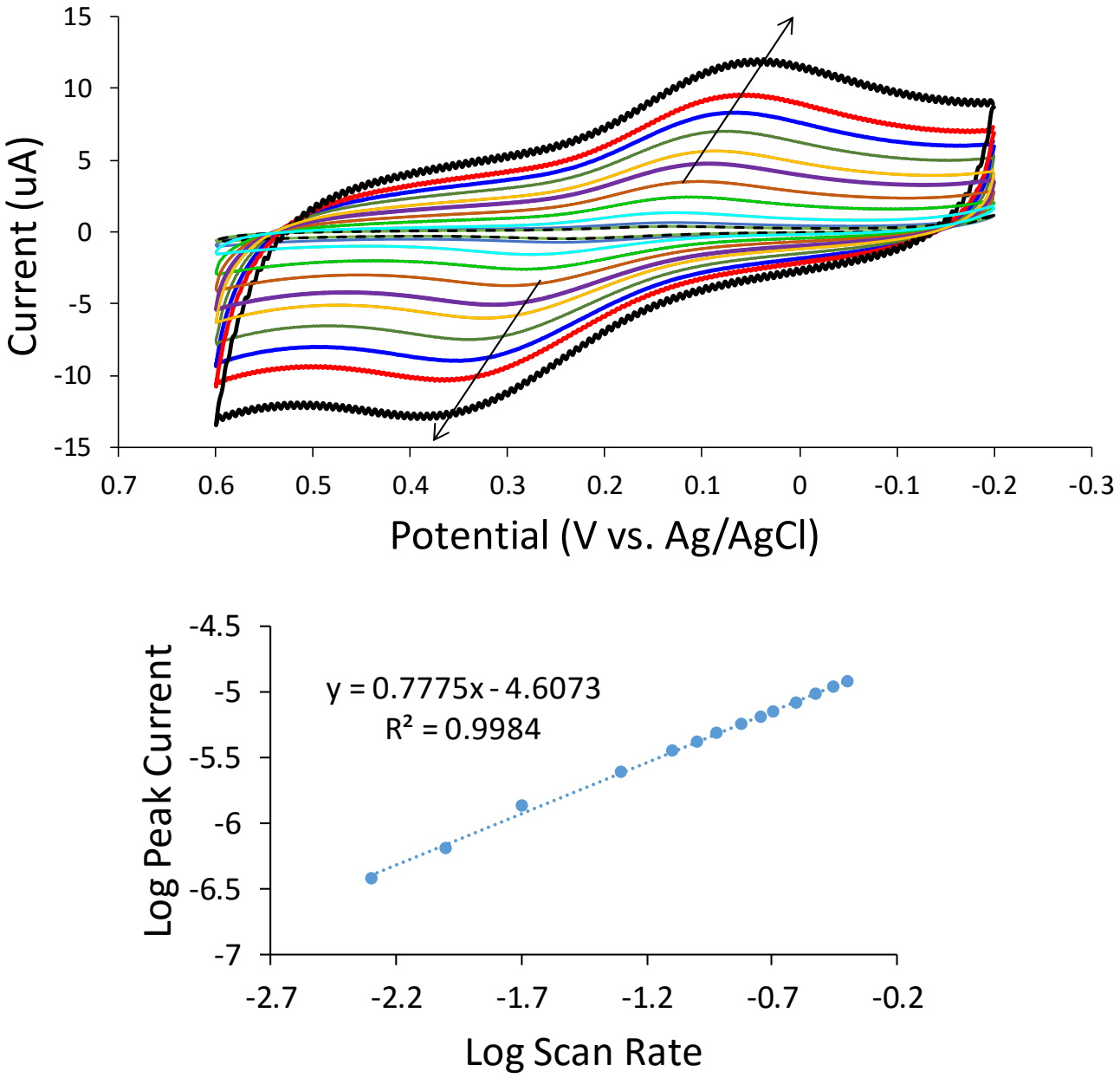

Figure SI-7. Cyclic voltammetry of GaOx adsorbed at CYST-SAM modified electrodes amide-coupled with COOH-**MW**CNTs at increasing scan rates (υ) with linear plots (**below**) of log peak current ($I_{p,c}$) versus log of scan rate υ, linear for non-diffusional (adsorbed) behavior.

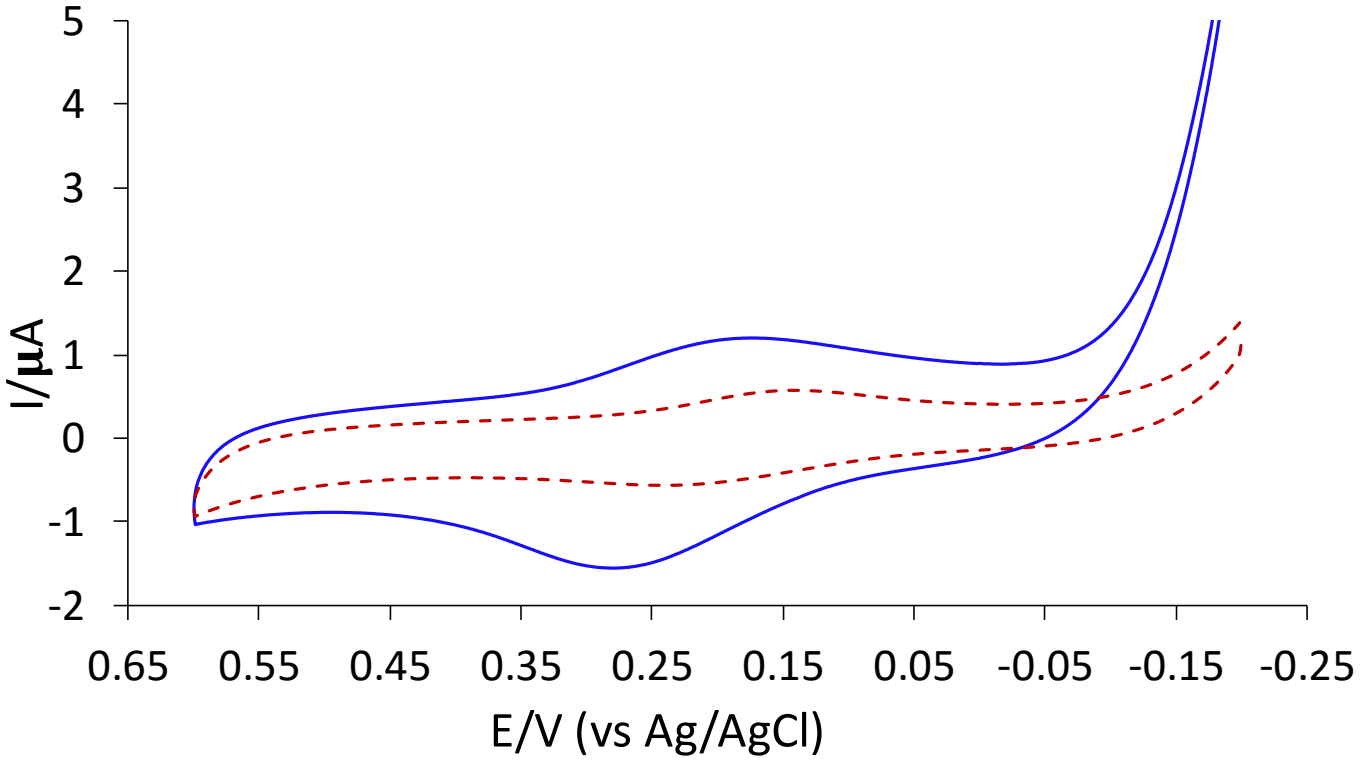

Figure SI-8. Comparison of representative cyclic voltammetry of GaOx adsorbed at day 0 (*solid, blue trace*) and day 8 (*red, dashed traces*) for GaOx adsorbed to bare gold. Notes: Scan rate is 0.020 V/sec;

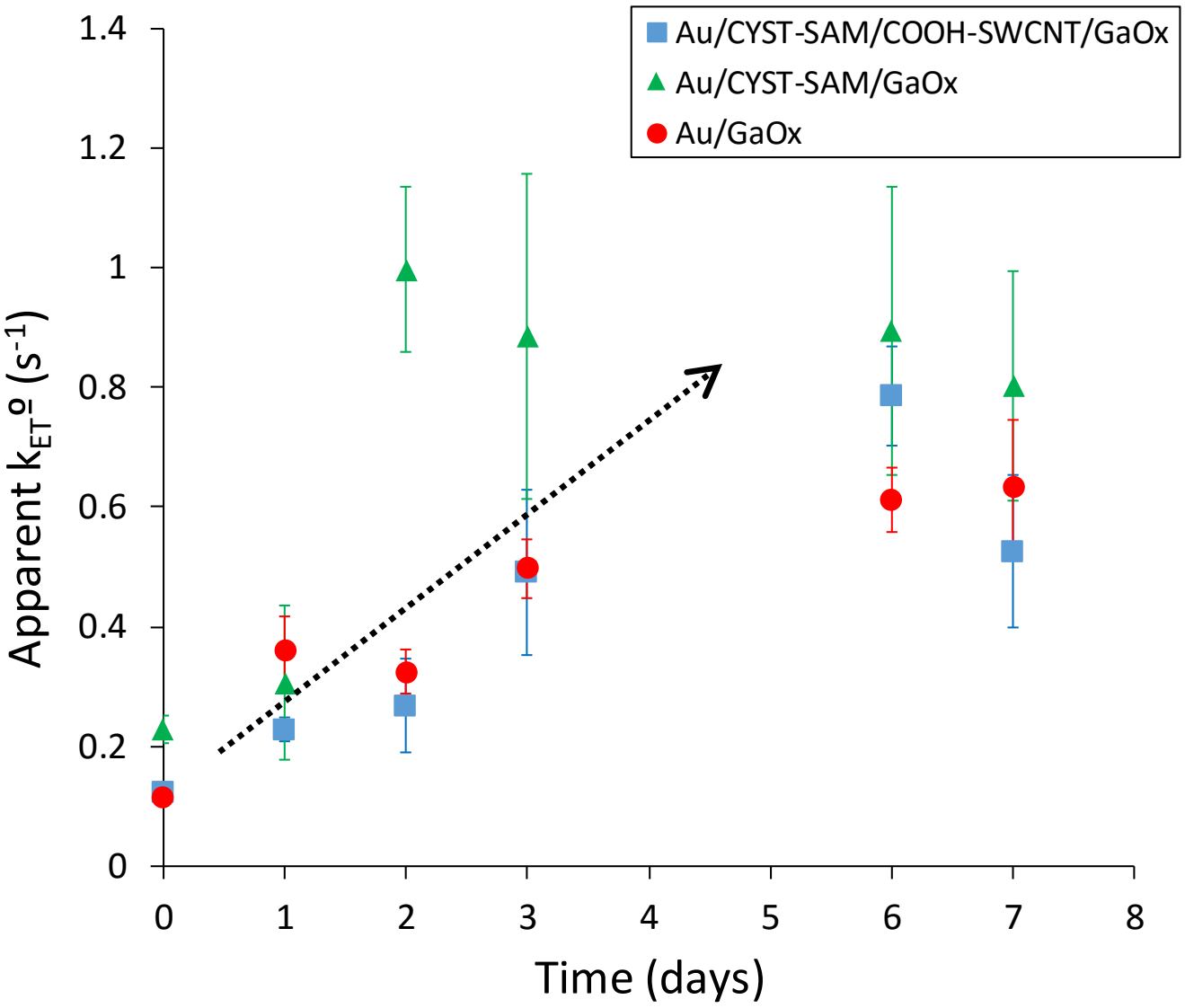

Figure SI-9. Tracking of apparent ET rate constant (k_{ET}^{o}) over time for GaOx at bare gold, CYST-SAM and CYST-SAM/COOH-SWCNT platforms.

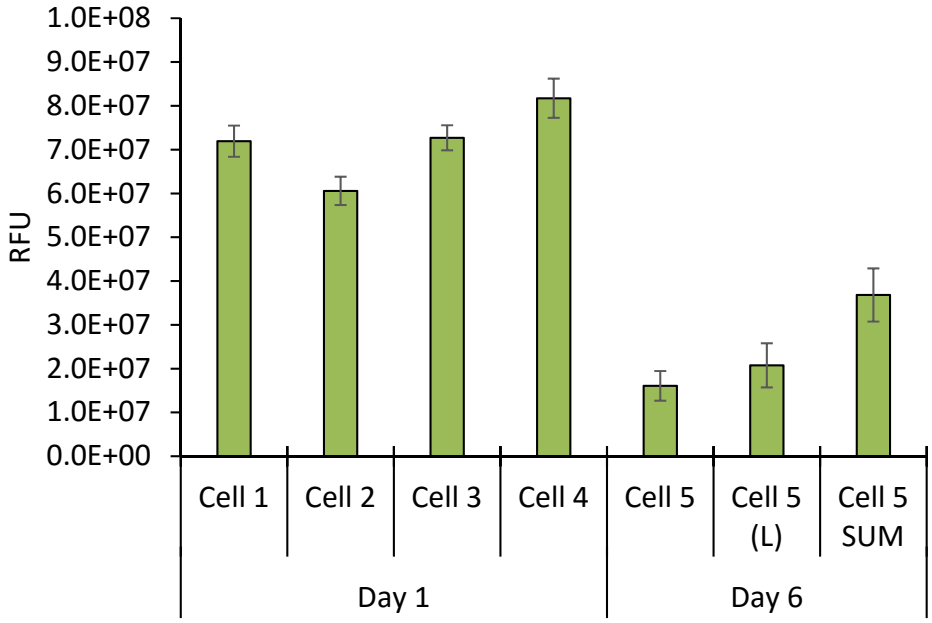

Figure SI-10. H_2O_2 fluorescence assay results showing relative fluorescence intensity representing GaOx activity (H_2O_2 production) in the presence of galactose substrate from aliquots sampled from electrochemical cells with Au/CYST-SAM/COOH-SWCNT/GaOx films on Day 1 (Cells 1-4) and Day 6 (Cell 5), the latter including measurements of the soaking buffer (L) and new buffer after 5 minutes of exposure to 50 μL of 10 mM galactose.

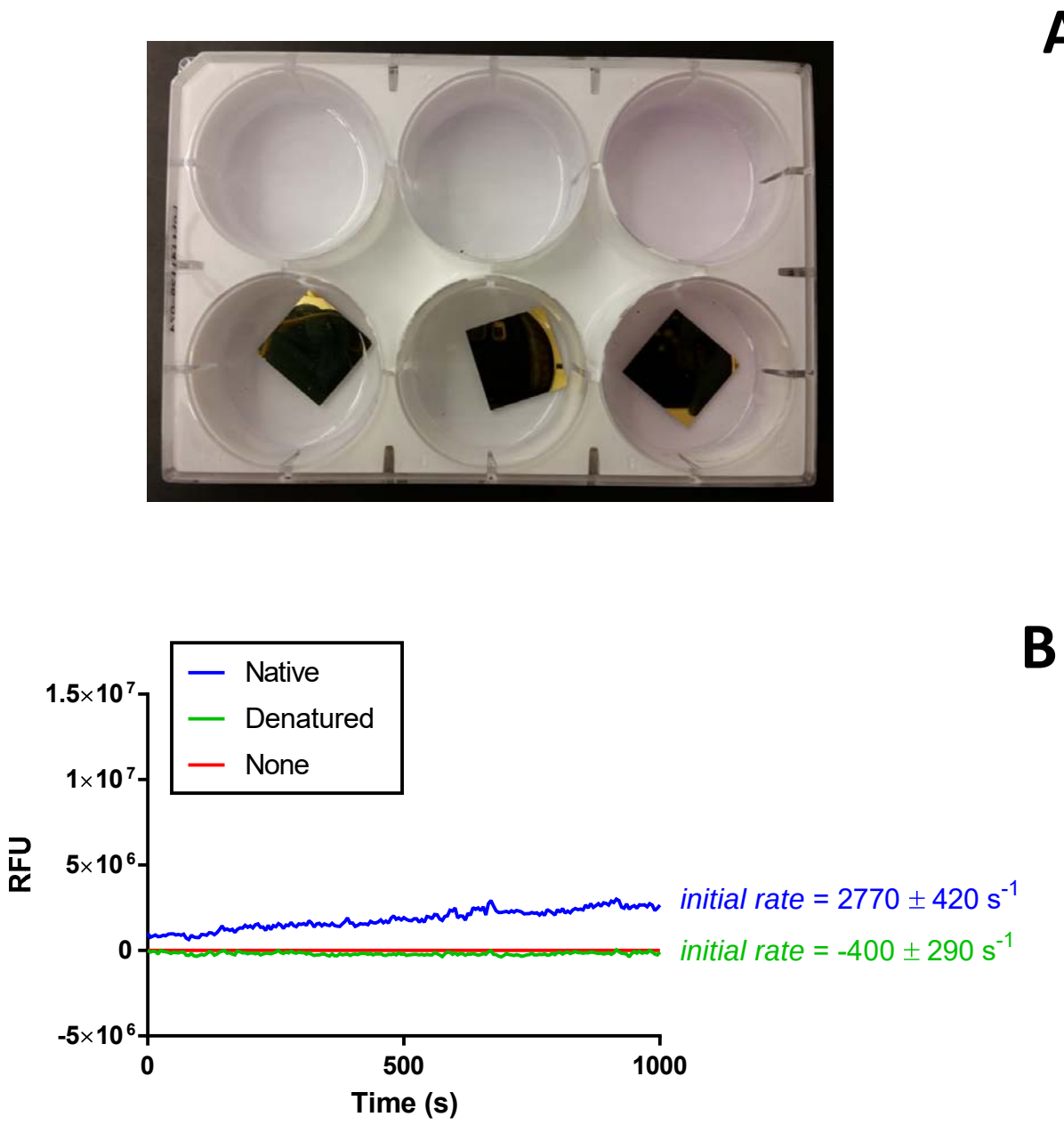

Figure SI-11. HRP-coupled fluorescence kinetic assay for hydrogen peroxide production experiment including (A) example set-up using whole films of Au/CYST-SAM/COOH-SWCNT/GaOx constructed within the electrochemical cell (film area ~ 0.32 cm^2) submerged in a 6-well plate and exposed to 10 mM galactose; (B) fluorescence generation (λ_{ex} = 540 nm, λ_{em} = 590 nm) over time comparing films with native GaOx, GaOx denatured using heat, urea, and glycine, and without GaOx (control). Initial rates were determined from linear fit of the first 180 seconds of data.

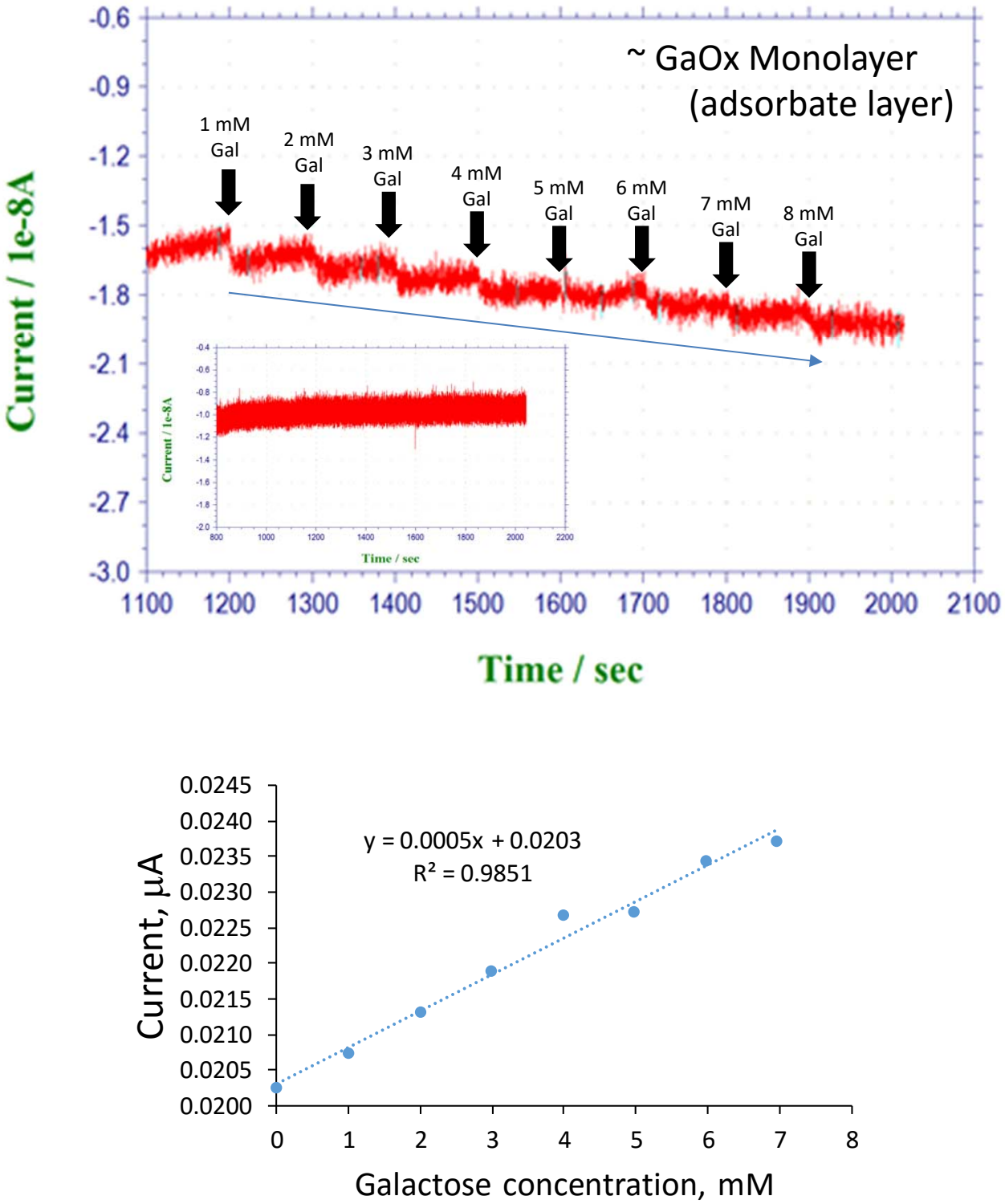

Figure SI-12. Representative amperometric I-t curve **(A)** and corresponding calibration curve **(B)** during injection of galactose (Gal) with 1 mM increases every 100s, **black arrows**) into a stirred buffer solution at a gold electrode (+0.65V) modified with CYST-SAM/COOH-SWCNT/GaOx (≤ monolayer) and capped with polyurethane help retain H_2O_2 that is generated by the enzymatic reaction. **Inset:** The same experiment without enzyme (control).

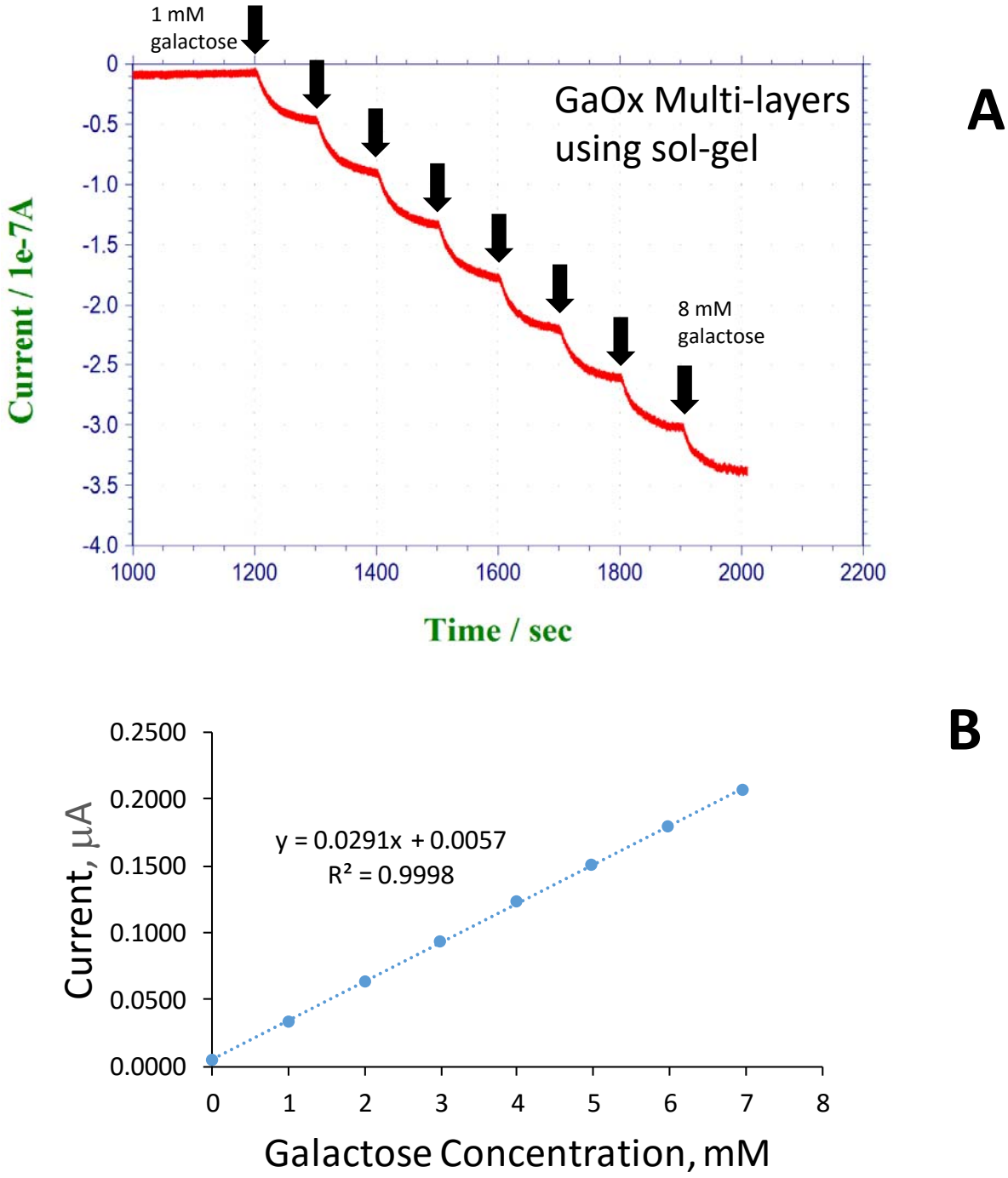

Figure SI-13. Representative amperometric I-t curve **(A)** and corresponding calibration curve **(B)** where GaOx was used in a traditional 1st generation biosensing scheme involving the complete encapsulation of GaOx **multi-layer** within a isobutyltrimethoxysilane (IBTMS) sol-gel layer at a platinum electrode (+0.65V), capped with another IBTMS layer and a polyurethane layer to help retain H_2O_2 generated by the enzymatic reaction produced with successive injections of galactose (1 mM every 100s, **black arrows**). Note that as more enzyme is immobilized on the surface (multi-layer vs. monolayer), the signal is increased by an order of magnitude as expected from more active enzyme being available.

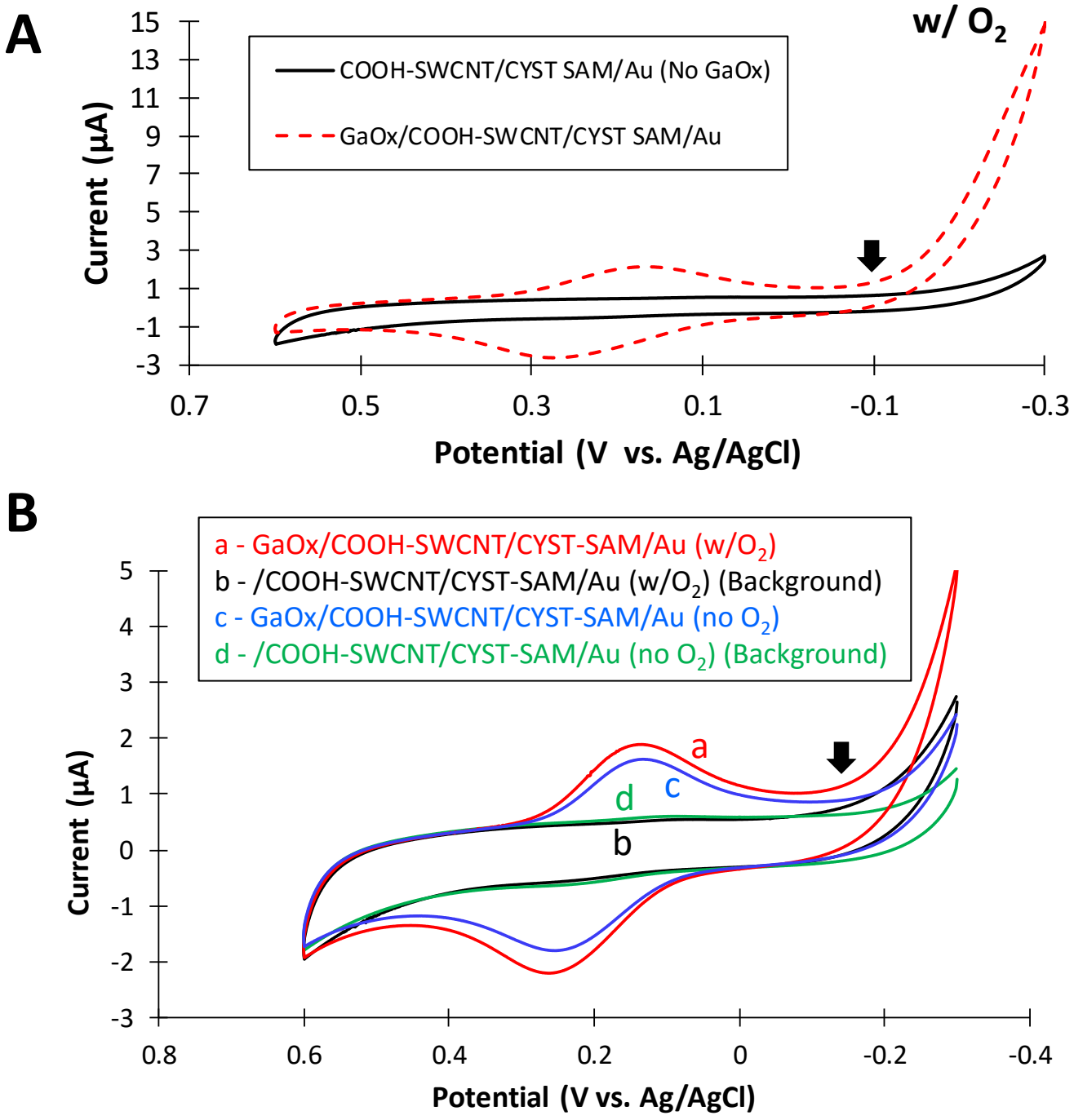

Figure SI-14. Representative examples of cyclic voltammetry of **(A)** GaOx adsorbed to a COOH-SWCNT/CYST SAM/Au platform and corresponding background (no GaOx) in the presence of oxygen and; **(B)** the same platforms in the presence and absence of oxygen. Notes: Solution is 20 mM MES buffer (pH 7.5) at a scan rate of 0.020 V/sec.); solutions were scrubbed with catalase agarose beads prior to conducting the experiments.

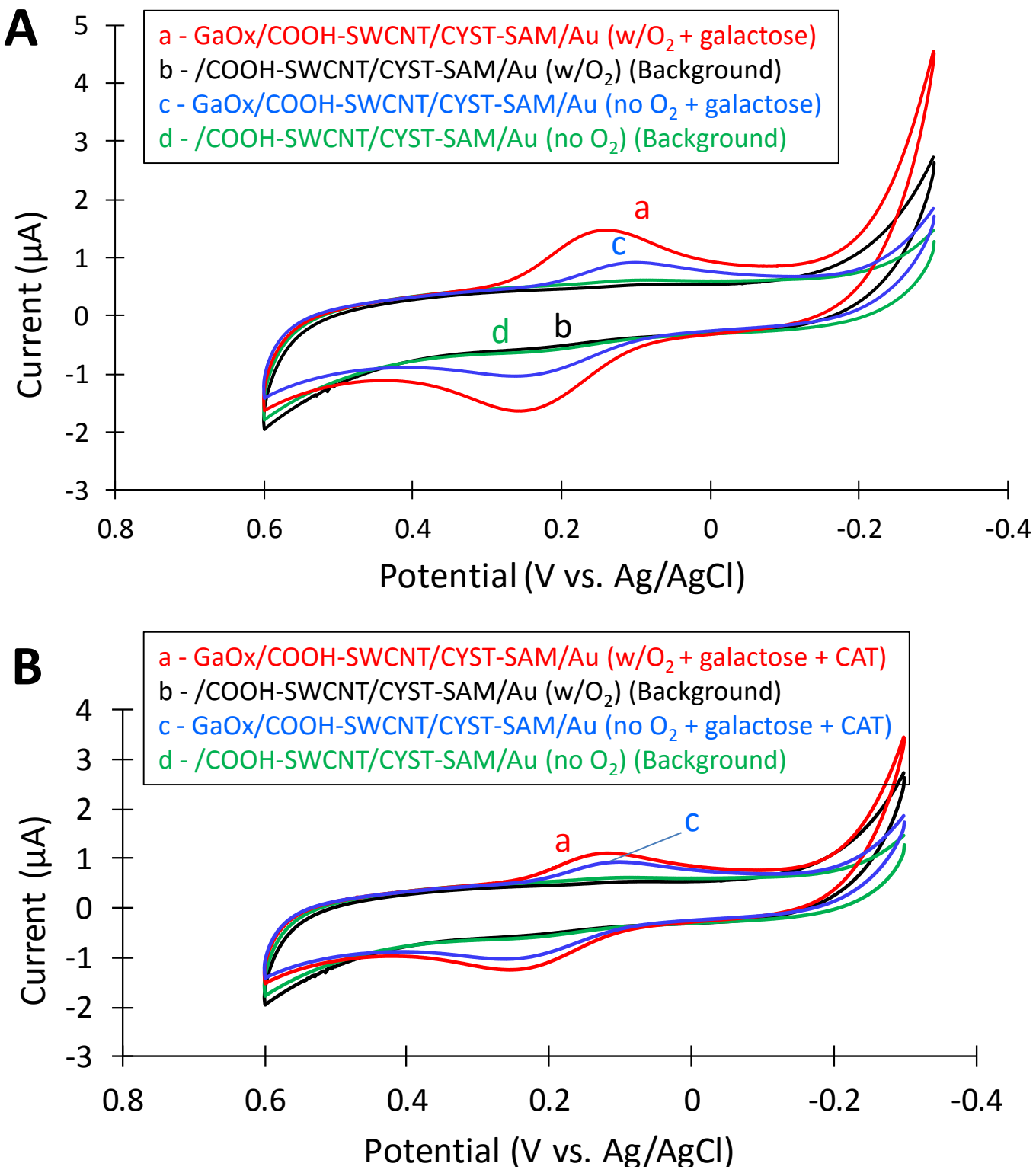

Figure SI-15. Representative examples of cyclic voltammetry of **(A)** GaOx adsorbed to a COOH-SWCNT/CYST SAM/Au platform and corresponding background (no enzyme) in the presence/absence of oxygen with galactose (10 mM) and; **(B)** the same platforms in the presence/absence of oxygen with galactose (10 mM) and catalase enzyme (CAT, 10 µM). Notes: Solution is 20 mM MES buffer (pH 7.5) at a scan rate of 0.020 V/sec; solutions were scrubbed with catalase agarose beads prior to conducting the experiments.

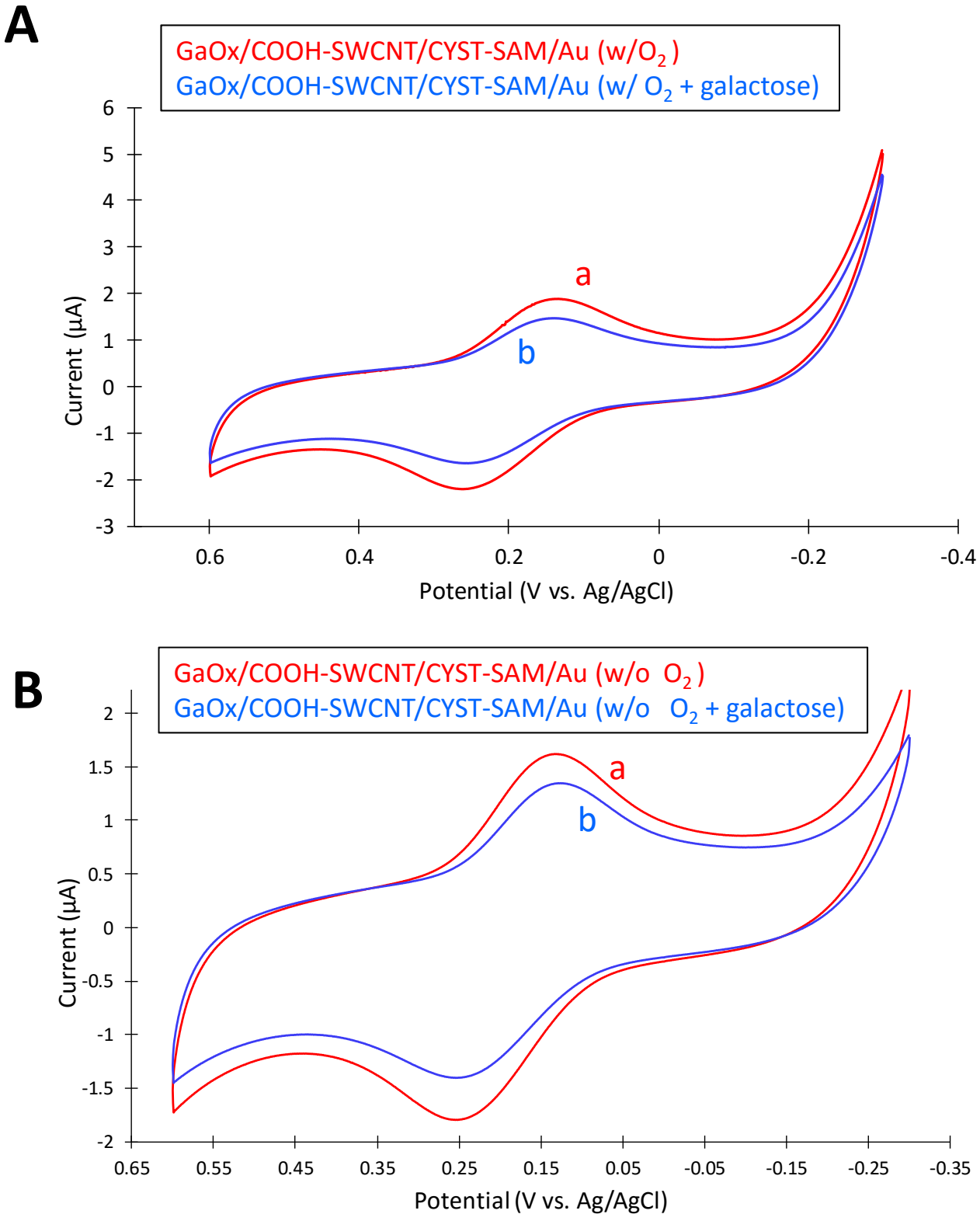

Figure SI-16. Representative examples of cyclic voltammetry of GaOx adsorbed to a COOH-SWCNT/CYST SAM/Au platform **(A)** in the presence and **(B)** absence of oxygen before **(a)** and after **(b)** exposure to galactose (10 mM). Notes: Solution is 20 mM MES buffer (pH 7.5) at a scan rate of 0.020 V/sec; solutions were scrubbed with catalase agarose beads prior to conducting the experiments.

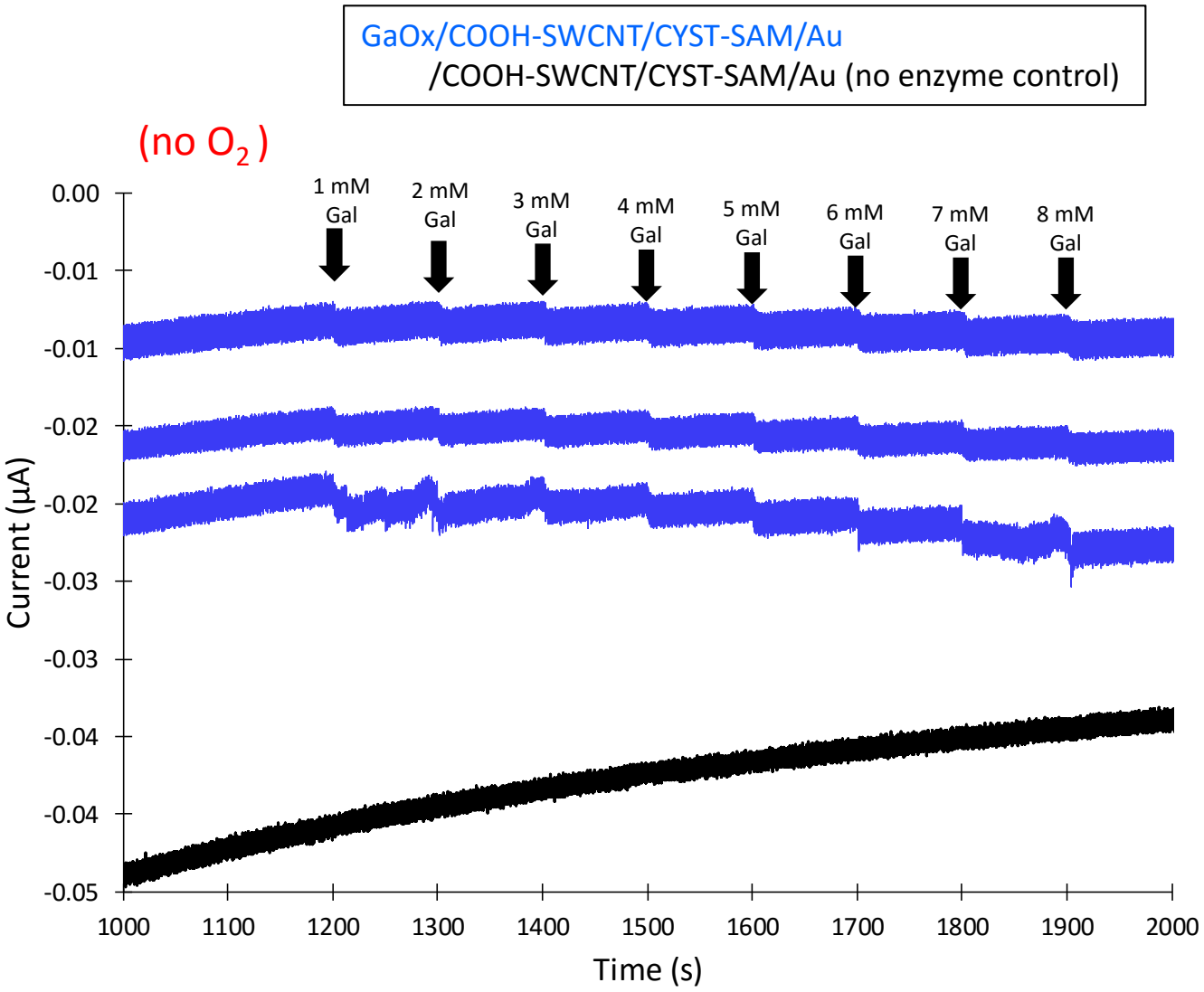

Figure SI-17. Representative amperometric I-t curves during injection of galactose (Gal) substrate injections (1 mM increases every 100s, **black arrows**) into a stirred PB solution that has been purged of O_2 at a gold electrode (+0.65V) with GaOx (*top three traces, blue)* and without GaOx (*bottom trace, no enzyme control)* adsorbed to a COOH-SWCNT/CYST-SAM modified gold electrode - each capped with polyurethane. Notes: Solutions were scrubbed with catalase agarose beads prior to conducting the experiments.