

Supporting Information

Dual-channel Nano-carbon-liquid/liquid Junction Electrodes for Multi-modal Analysis: Redox-active (dopamine) and Non-redox-active (acetylcholine)

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1. Electrode modification using FIB milling

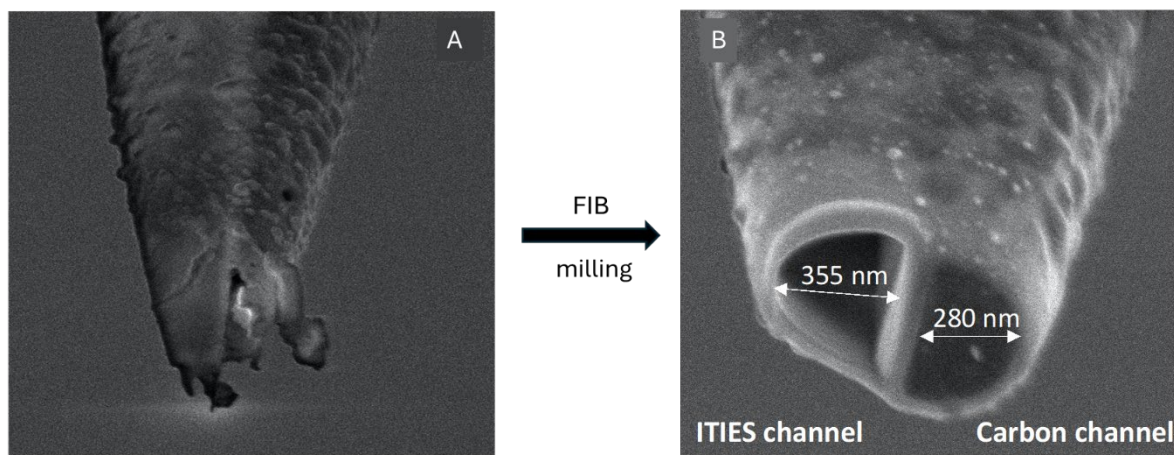


Figure S1. Scanning electron microscopic image of the carbon-deposited dual-functional nano-carbon-ITIES electrode A) before and B) after FIB milling.

After carbon deposition, when we observed tip end with roughness (Figure S1A), we performed FIB milling to generate a smooth surface (Figure S1B).

2. Radius determination of the carbon nanoelectrode channel using cyclic voltammogram (CV) of RuHex

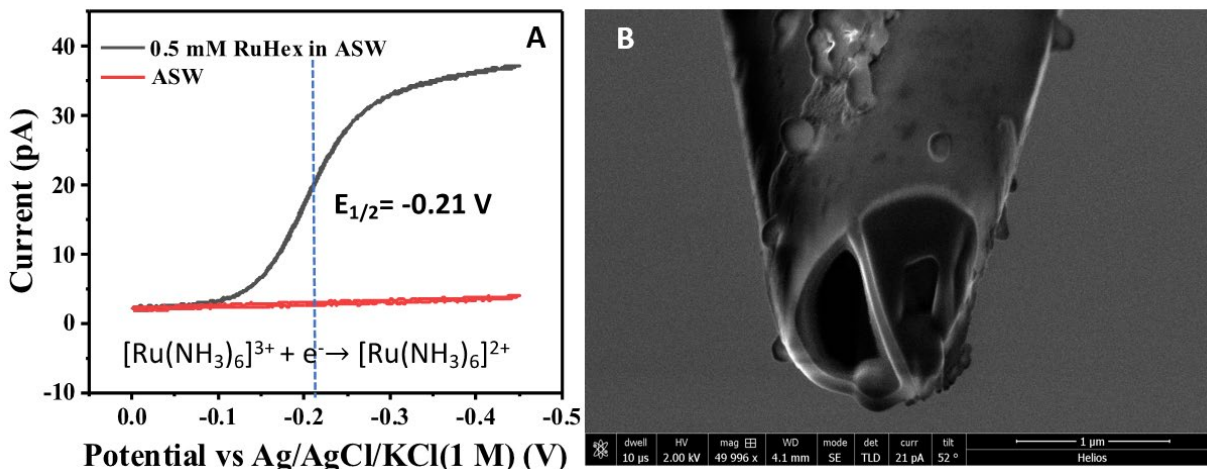


Figure S2. A) Cyclic voltammogram (CV) of 0.5 mM RuHex in ASW on the carbon nanoelectrode channel of the dual-channel nano-carbon-ITIES electrode (grey curve) overlapped with cellular background ASW CV (red curve). B) SEM image of the electrode after FIB milling with carbon channel on the right side.

The size (a) of the carbon channel was calculated from the steady state current of the RuHex CV using equation (1)^{1,2} and found to be 518 nm.

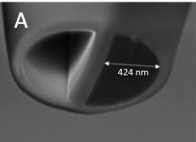
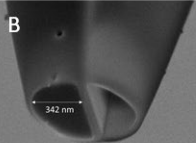
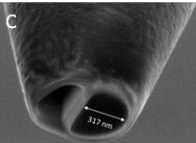

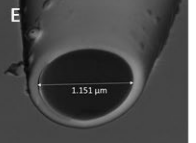
$$i_{ss} = 2\pi nFDCa \quad (1)$$

where x is a function of R_g , which is the ratio of the outer radius to the inner radius of the electrode. We estimated R_g to be 1.33 based on the capillary dimensions before pulling. n is number of electrons transferred during the reaction (1), F is Faraday's constant (96485.3 C/mol), D is diffusion coefficient of RuHex ($5.48 \times 10^{-6} \text{ cm}^2/\text{s}$),¹ C is concentration of RuHex (0.5 mM), a is distance from the septum to the circumference, designated as ' a ' in Figure 2B, and i_{ss} is the steady state current of RuHex detection. The average background-subtracted forward current from -0.35 V to -0.45 V vs Ag/AgCl/KCl (1 M) was used as i_{ss} for the size determination and is 32.8 pA. Background-subtracted current is acquired by subtracting the average of three consecutive background ASW CVs from the average of three consecutive 0.5 mM RuHex CVs. While we have fabricated numerous dual-functional nano-carbon-ITIES electrodes with smooth surfaces (Figures S1 and S3), this electrode seems to have surface irregularities after FIB milling, preventing accurate comparison of electrochemical size and geometric size as determined using SEM.

We carried out more studies to characterize the size of the carbon nanoelectrode channel using RuHex CVs and further compared that to the size obtained from the SEM images. The ratio between geometrical size and electrochemical size is closer to 1 when the RuHex CVs were measured directly on the freshly FIB-milled electrodes following a water wash (Table S1, C, D and E). In contrast, when electrodes (Table S1, A and B) underwent dopamine cyclic voltammetry

experiments before RuHex experiments, the electrochemical sizes appeared to be smaller than the SEM measured size. This decrease in the electrochemical size after dopamine measurements may be attributed to the potential carbon electrode fouling by dopamine oxidation.

Table S1. Comparison between the geometric (obtained from SEM) and electrochemical sizes (calculated from cyclic voltammograms of RuHex) of the carbon nanoelectrodes.

SEM image of the electrode	a /radius measured from SEM image. (geometrical size)	a /radius from RuHex current (electrochemical size)	Ratio between geometrical size and electrochemical size	History of the electrode (RuHex experiment before or after the dopamine detection)
	424 nm (a)	159 nm	2.667	RuHex current measured after dopamine CV experiments
	342 nm (a)	165 nm	2.073	
	317 nm (a)	293 nm	1.082	RuHex current measured immediately after FIB milling
	661 nm (r)	590 nm	1.120	
	575.5 nm (r)	609 nm	0.945	

3. Additional cyclic voltammograms exhibiting sigmoidal shapes for dopamine detection on the carbon nanoelectrode channel of the FIB-milled dual-channel nanoelectrode

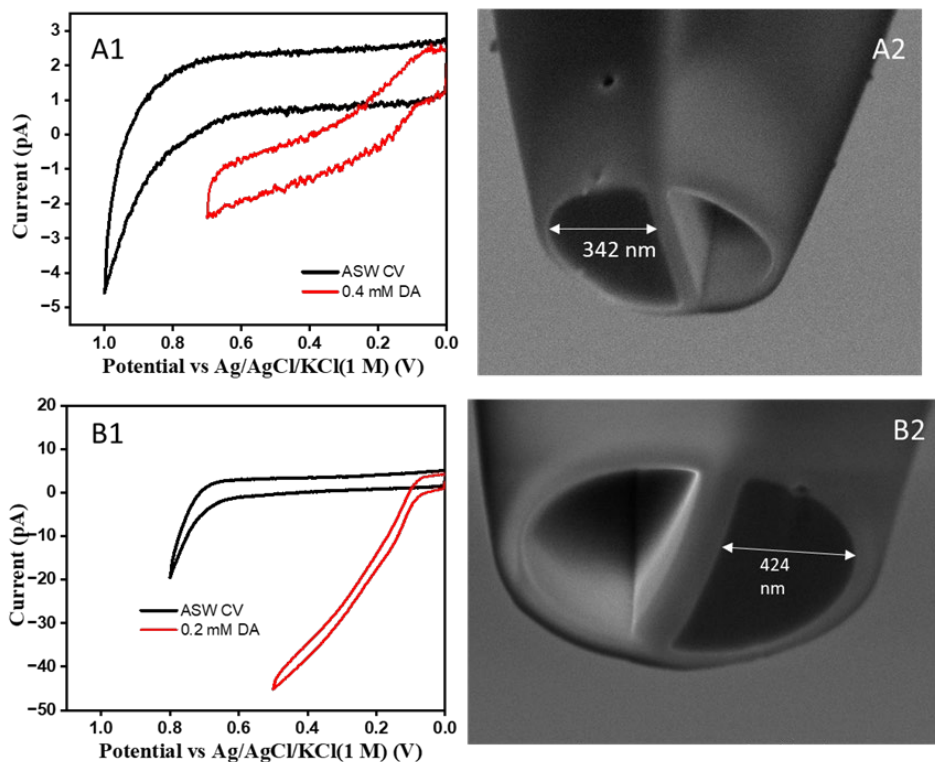


Figure S3. Additional cyclic voltammograms (A1, B1) that exhibited sigmoidal shapes for dopamine detection on the carbon nanoelectrode channel of the FIB-milled dual-channel nanoelectrodes and corresponding SEM images (A2, B2). It is unknown why the current of DA detection did not correlate to the sizes observed using SEM.

4. Radius determination of the nanoITIES electrode channel of the nano-carbon-ITIES electrode based on the cyclic voltammogram (CV) of TBA

The size of the nanoITIES channel was calculated from the diffusion-limiting current of the TBA CV (Figure 5A) using equation (1) and found to be 419 nm. Background-subtracted current was used to calculate the size of the electrode. The average forward current from -0.172 V to -0.237 V vs Ag/AgCl/KCl (1 M) was used as i_{SS} for the size determination and is 49.4 pA. Background-subtracted current is the average of three consecutive background ASW CVs subtracted from the average of three consecutive 1 mM TBA CVs.

5. Halfwave potential of dopamine detection measured using cyclic voltammogram

The halfwave potential ($E_{1/2}$) of dopamine detection was calculated from the cyclic voltammogram of dopamine detection. Forward current of the dopamine cyclic voltammogram was used to calculate the $i_{1/2}$ (Figure S5), which corresponds to $E_{1/2}$.

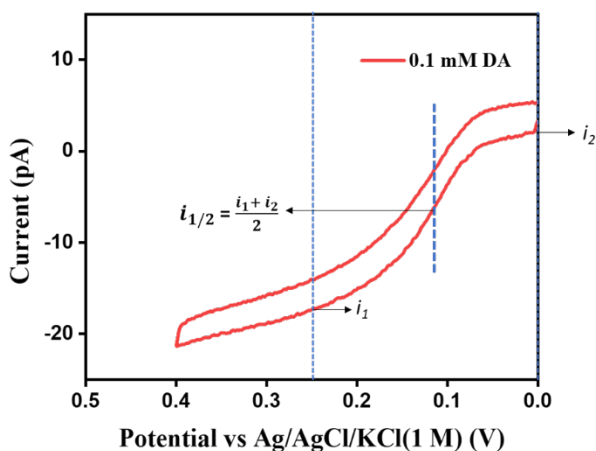


Figure S4. Cyclic voltammogram of 0.1 mM DA at carbon nanoelectrode channel of the dual-functional nano-carbon-ITIES electrode.

$E_{1/2}$ for dopamine was calculated based on the average from five different FIB-milled carbon nanoelectrodes. CVs of dopamine at 0.1 mM or 0.2 mM DA were used. The average $E_{1/2}$ was measured to be 0.108 ± 0.013 V vs Ag/AgCl/KCl (1M).

Table S2. $E_{1/2}$ of dopamine detection using five different FIB-milled carbon nanoelectrodes.

Serial Number of Electrode	Serial No.	Conc. of DA used for the calculation	$E_{1/2}$ (V) vs Ag/AgCl/KCl(1 M)
Dual-channel electrode	1	0.1 mM	0.1244
	2	0.2 mM	0.1039
	3	0.1 mM	0.0877
Single-channel electrode	4	0.2 mM	0.1046
	5	0.2 mM	0.1208
	Average		0.108
	Standard deviation		± 0.013

6. Cyclic voltammograms of acetylcholine and dopamine used to confirm the detection potentials in chronoamperometry experiments in Figure 6B.

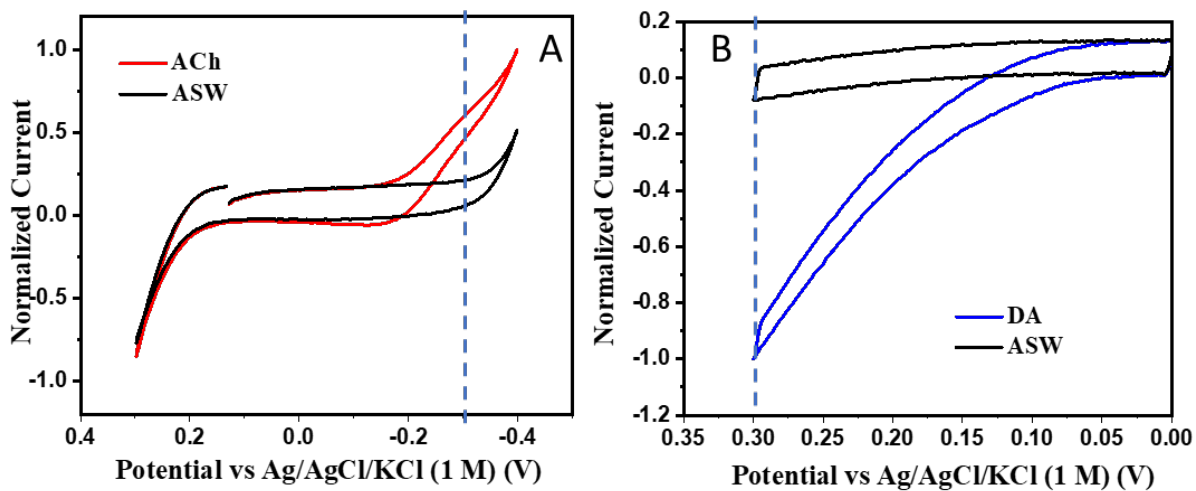


Figure S5. The CVs of A) acetylcholine (ACh) detection on the nanoITIES channel and B) dopamine (DA) detection on the nano-carbon channel collected after the injection experiments, overlapped with the background ASW CVs.

After the injection experiments, the detection potential was confirmed by adding ACh and DA into the background ASW solution and cyclic voltammograms were measured.

7. Control experiments for the simultaneous detection of dopamine and acetylcholine

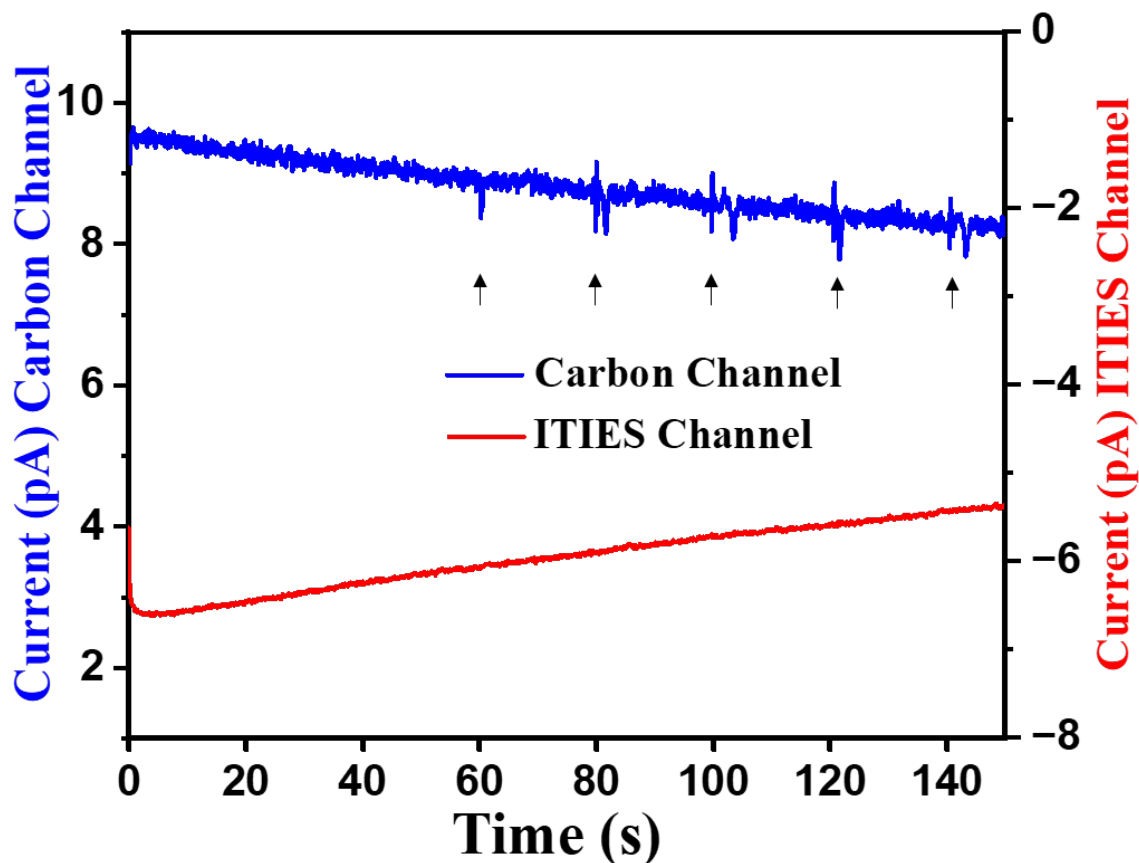


Figure S6. Control chronoamperometry experiments where the carbon nanoelectrode channel and the nanoITIES channel were biased at potentials where both DA and ACh were not measurable. The injection of the mixture of DA and ACh were indicated by arrows. No current peaks were observed. NanoITIES and carbon nanoelectrode channels were both biased at 0 V vs Ag/AgCl/KCl (1 M). Red and blue curves were current-time traces for the nanoITIES and carbon nanoelectrode channels, respectively.

8. Simultaneous detection of acetylcholine (ACh) and dopamine (DA) using chronoamperometry on the dual-functional nano-carbon-ITIES electrode over a longer period of time

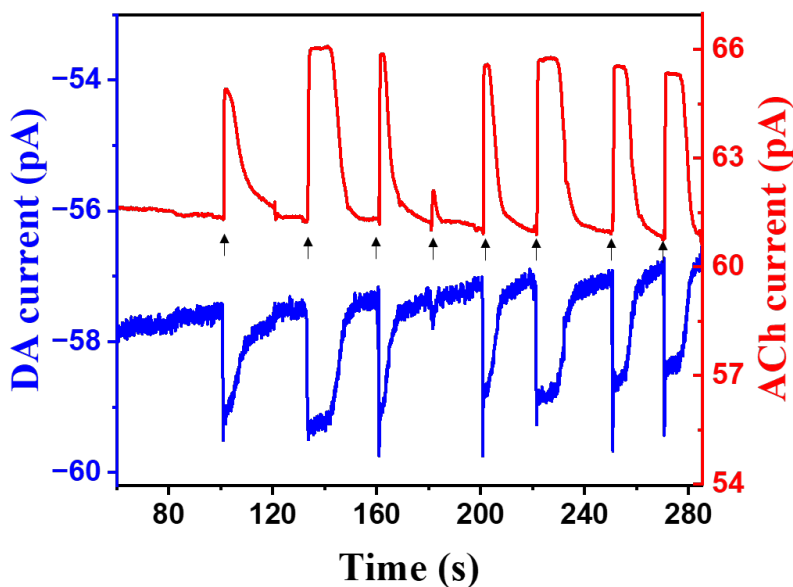


Figure S7. Current-time trace recorded following the injection of a mixture of dopamine (DA) and acetylcholine (ACh) with nanoITIES and nano-carbon channels biased at -0.3 V and 0.3 V vs Ag/AgCl/KCl (1 M), respectively. The red curve corresponds to ACh detection on the nanoITIES channel (y-axis, right). The blue curve corresponds to the DA detection on the nano-carbon electrode channel (y-axis, left). Black arrows indicate the injection of dopamine and acetylcholine mixture. The difference in the duration of current peaks might be related to the variation in the injection duration of ACh and DA cocktail solution.

The difference in peak shape of the detection corresponds to the difference in the force of the injection which released different amount of ACh and DA molecules. An injection with a feeble force during manual injection (fourth peak at 180s) resulted in a smaller current peak, which likely was related to a smaller amount of ACh and DA molecules at the electrode surface during the detection.

9. References

- (1) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods Fundamental and Applications*, 2nd Edition; 2001.
- (2) Lefrou, C. A unified new analytical approximation for positive feedback currents with a microdisk SECM tip. *Journal of Electroanalytical Chemistry* 2006, 592 (1), 103-112. DOI: 10.1016/j.jelechem.2006.05.003.
- (3) Harris, D. C. *Quantitative chemical analysis*; Macmillan, 2010.