Supporting Information for

Microdialysis Coupled with Droplet Microfluidics and Mass Spectrometry for Determination of Neurotransmitters *in vivo* with High Temporal Resolution

Shane S. Wells, Ian J. Bain, Alec C. Valenta, Ashley Lenhart, Daniel J. Steyer, Robert T. Kennedy*

Department of Chemistry, University of Michigan, 930 N. University Ave, Ann Arbor, MI 48109-1055

Corresponding Author:

Robert Kennedy

Email: rtkenn@umich.edu

Tel: 734-615-4363

Table SI-1: List of all transitions, dwell times, and collision voltages used for MS/MS analysis.

Compound	Parent (m/z)	Daughter (m/z)	Dwell (s)	Collision (V)	Calibration High Point (nM)	Calibration Low Point (nM)
GABA	104	87	0.15	10	200	10
d6GABA	110	93	0.03	12		
AMPH	136.1	119.1	0.03	15		
ACh	146	87	0.1	17	100	5
Glu	148	130	0.1	8	600	30
d4ACh	150	91.1	0.03	17		
13C5Glu	153	135	0.04	10		
DA	154	137	0.15	13	100	5
d4DA	158	141	0.05	13		
5HT	177	160.1	0.15	12	100	5
d45HT	181	164	0.03	15		
Ado	268	136.1	0.05	18	200	10
d1Ado	269	137	0.04	18		

Table SI-2: Summary of conditions used for ESI-MS/MS analysis of dialysate samples collected in vivo and for calibration.

nESI	Flow Rate	Capillary, Cone Voltage	Emitter i.d.	Drying Gas
	50 nL/min	1.4 kV, 35 V	15 µm	150 L/hr
Sample	Matrix (cal//tune)	Matrix (Perfusion)	Additive	Dilution
	aCSF (33% PO4)	aCSF (no PO4)	0.1% Acetic Acid	1:1, H2O
Analyzer	LM Res 1, 2	HM Res 1, 2	Ion Energy 1, 2	Exit, Entrance
	14.7, 14.5	14.5, 14.5	0.5, 0.4	5, -1 V
MS/MS	Span	Interscan Delay	Transitions/CE	Dwell Times
	0.3 m/z	2.5 ms	Table SI-2	Table SI-2

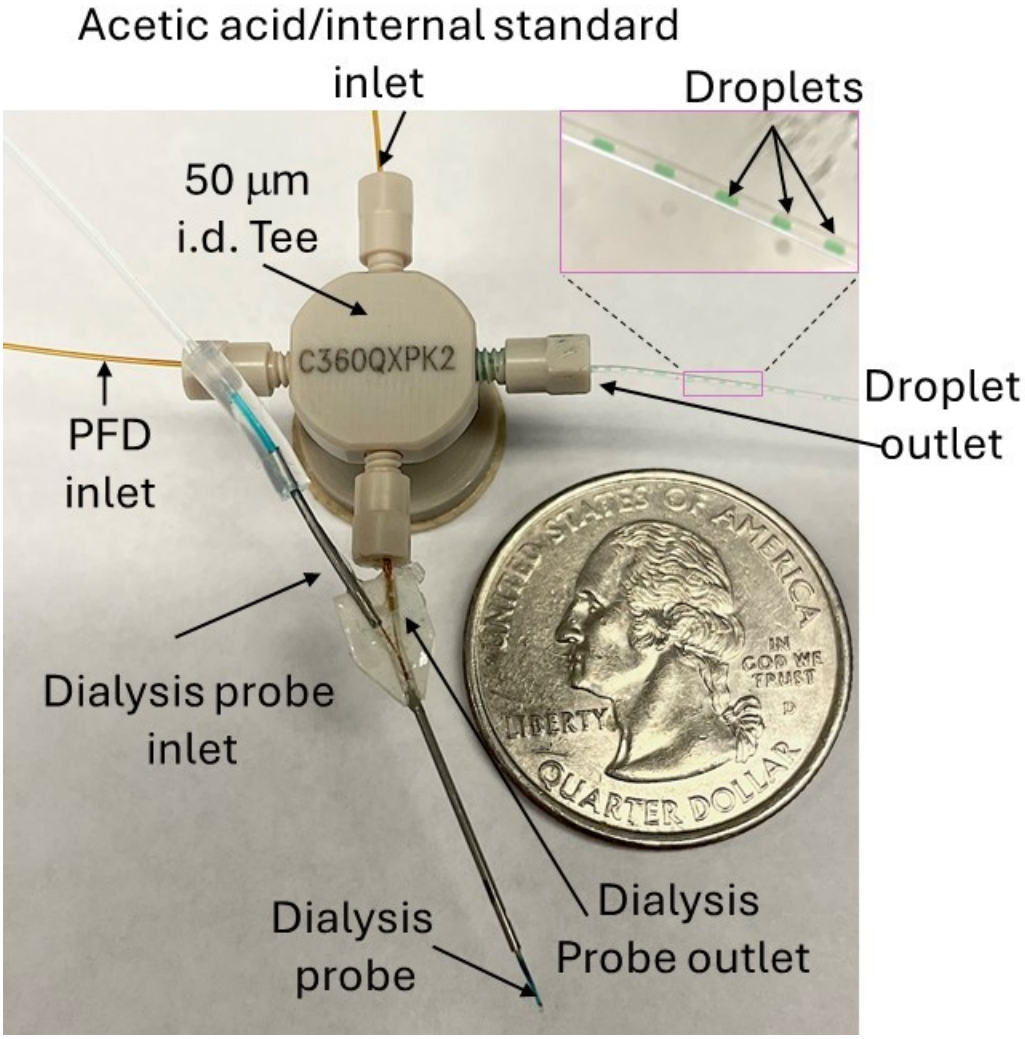

Figure SI-1. Photograph of probe and droplet formation system. Figure corresponds to drawing in Figure 1 in text, except this probe does not have an injection shank on it.

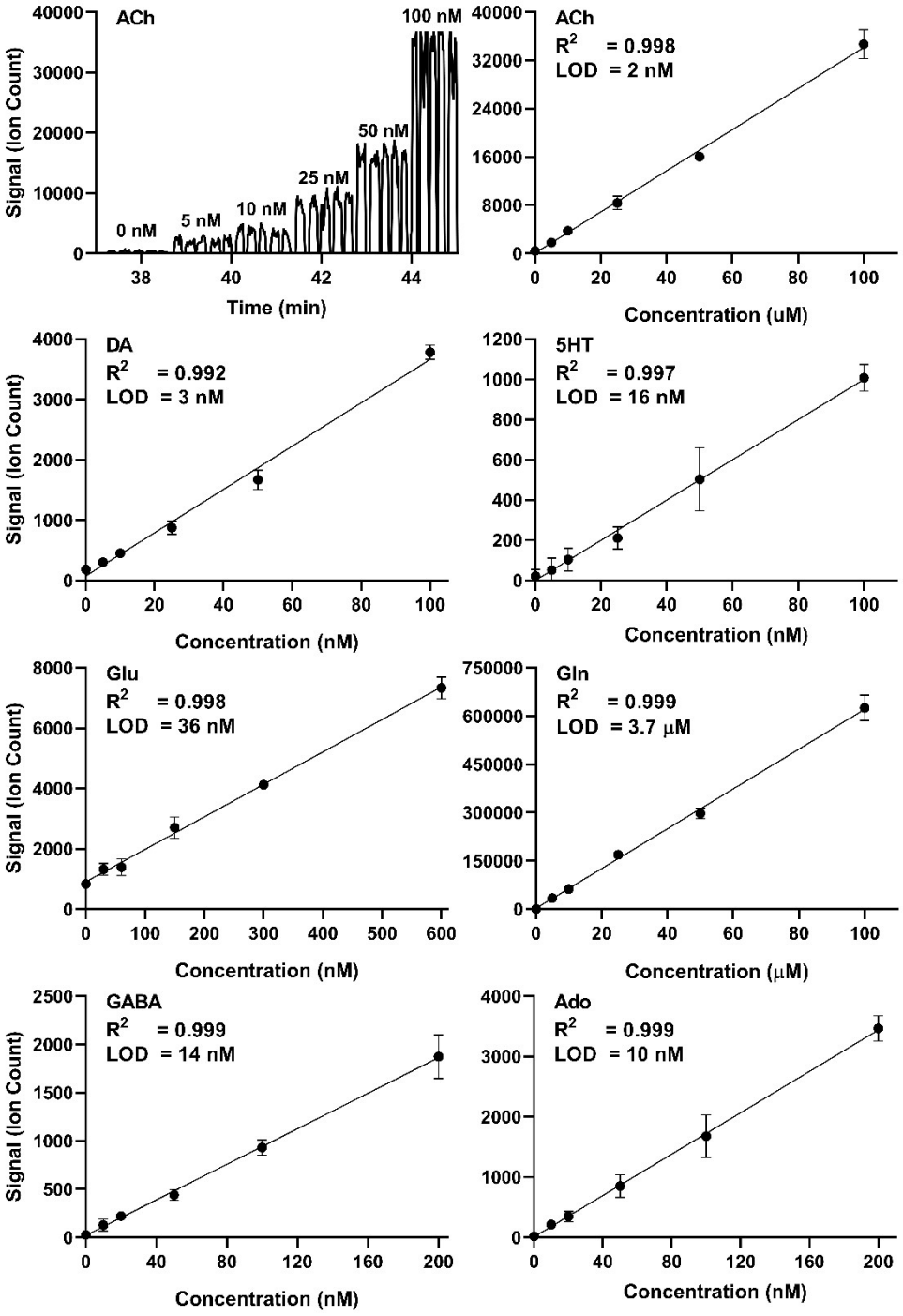

Figure SI-2. Calibration data for ESI-MS/MS of droplet samples of neurotransmitters. (Top Left) MS/MS trace of calibration data for ACh with 5 droplets for each of the 6 calibration levels. Samples were prepared from a well-plate and infused into the mass spectrometer as described in the text. (All other graphs) Average signal intensity versus standard concentration. Error bars are +/- 1 standard deviation for 3 replicates. These are the curves used for quantitation during one day of in vivo experiments. LOD is calculated using the limit of the blank method.

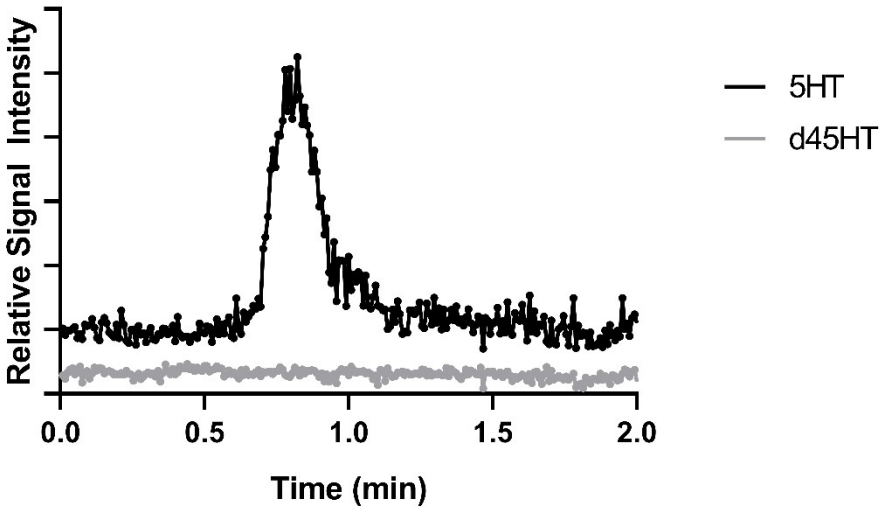

Figure SI-3. Example of single replicate that showed significant 5HT response to AMPH stimulation. The black dots and connecting lines represent MS/MS single for 5HT concentrations collected during AMPH stimulation and the gray dots and lines represent the internal standard d45HT used during droplet generation and nESI-MS/MS.