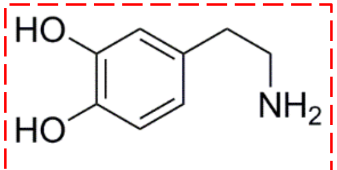
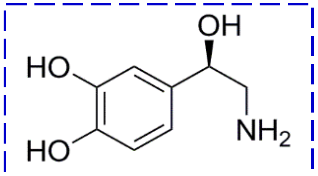


Dopamine (DA)



Norepinephrine (NE)

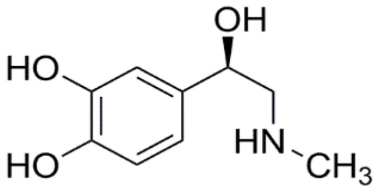
Development of Microelectrochemical Methods for Differentiation of the Catecholamine Neurotransmitters

Miguel Abrego, Mengjia Hu, Mahsa Lotfi-Marchoubeh, and Ingrid Fritsch
Department of Chemistry and Biochemistry
University of Arkansas

October 4, 2018

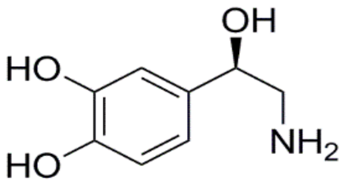
OUTLINE

- Catecholamines as neurotransmitters
- CA Mechanism Reaction
- Modeling framework
- Application of electrochemical redox cycling towards the differentiation of neurotransmitters.
- Physics description
- Redox cycling of the catecholamines
- Results
- Conclusions
- References

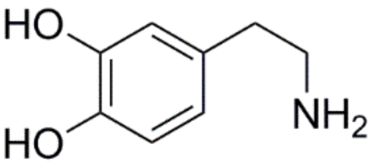


Epinephrine (EP)

Catecholamines as Neurotransmitters

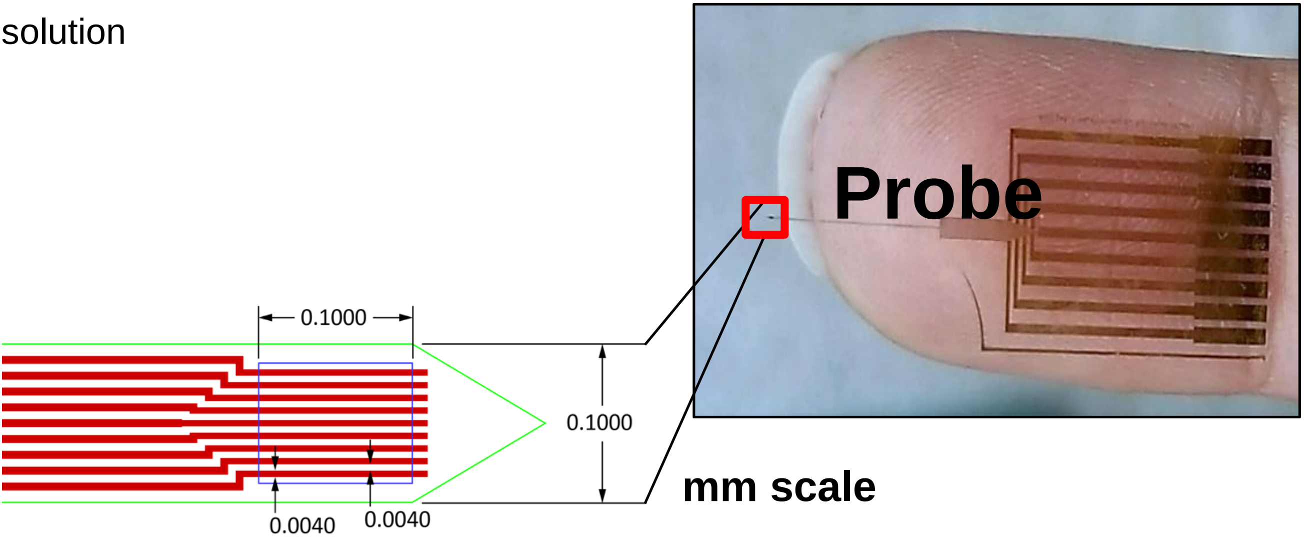


Norepinephrine (NE)

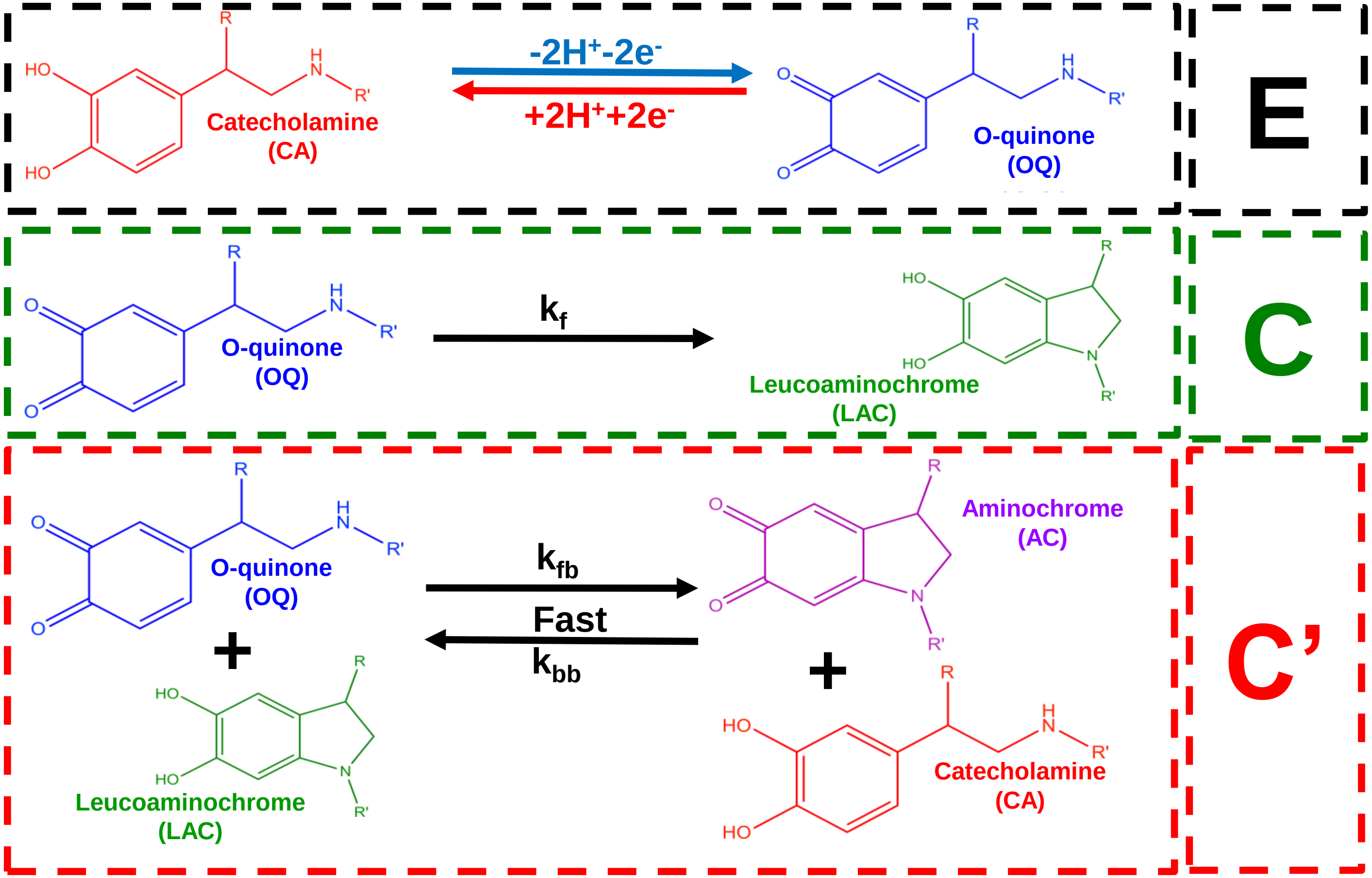


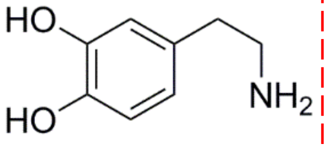
Dopamine (DA)

- **Importance:**
  - Related to neurological disorders including Parkinson’s disease, Huntington’s disease, Tourette’s syndrome and schizophrenia.
- **Detection**
  - *Desired features:*
    - High spatial and temporal resolution
    - Minimal tissue damage
  - *Electroactive*
    - Electrochemical detection
  - *Similar structure*
    - Difficult to differentiate

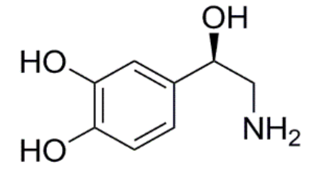


CA Mechanism Reaction





Dopamine (DA)



Norepinephrine (NE)

$$\frac{\partial C_1}{\partial t} = D\nabla^2 C_1 - k_{bb}C_1C_4 + k_{fb}C_2C_3 - k_{bb}C_1C_8 + k_{fb}C_2C_7$$

k_f (s^{-1}) at pH=7.4	
DA	0.13±0.05
NE	0.98±0.52

Modeling Framework

$$\frac{\partial C_1}{\partial t} = D\nabla^2 C_1 - k_{bb}C_1C_4 + k_{fb}C_2C_3 - k_{bb}C_1C_8 + k_{fb}C_2C_7$$

$$\frac{\partial C_2}{\partial t} = D\nabla^2 C_2 - k_f C_2 + k_b C_3 + k_{bb}C_1C_4 - k_{fb}C_2C_3 + k_{bb}C_1C_8 - k_{fb}C_2C_7$$

$$\frac{\partial C_3}{\partial t} = D\nabla^2 C_3 + k_f C_2 - k_b C_3 + k_{bb}C_1C_4 - k_{fb}C_2C_3 + k_{bb}C_5C_4 - k_{fb}C_6C_3$$

$$\frac{\partial C_4}{\partial t} = D\nabla^2 C_4 - k_{bb}C_1C_4 + k_{fb}C_2C_3 - k_{bb}C_5C_4 + k_{fb}C_6C_3$$

$$\frac{\partial C_5}{\partial t} = D\nabla^2 C_5 - k_{bb}C_5C_8 + k_{fb}C_6C_7 - k_{bb}C_5C_4 + k_{fb}C_6C_3$$

$$\frac{\partial C_6}{\partial t} = D\nabla^2 C_6 - k_f C_6 + k_b C_7 + k_{bb}C_5C_8 - k_{fb}C_6C_7 + k_{bb}C_5C_4 - k_{fb}C_6C_3$$

$$\frac{\partial C_7}{\partial t} = D\nabla^2 C_7 + k_f C_6 - k_b C_7 + k_{bb}C_5C_8 - k_{fb}C_6C_7 + k_{bb}C_1C_8 - k_{fb}C_2C_7$$

$$\frac{\partial C_8}{\partial t} = D\nabla^2 C_8 - k_{bb}C_5C_8 + k_{fb}C_6C_7 - k_{bb}C_1C_8 + k_{fb}C_2C_7$$

Initial Conditions:

$C_{CA}(x,y,0) = C^*_{CA} = 0.01\,m\mathrm{M}$

$C_{OQ}(x,y,0) = 0$

$C_{LAC}(x,y,0) = 0$

$C_{AC}(x,y,0) = 0$

Boundary Conditions:

$C_{CA}(\infty,\infty,0) = C^*_{CA} = 0.01\,m\mathrm{M}$

$C_{OQ}(\infty,\infty,0) = 0$

$C_{LAC}(\infty,\infty,0) = 0$

$C_{AC}(\infty,\infty,0) = 0$

Definitions:

$C_1 = [C_{CADA}(x,y,t)]$

$C_2 = [C_{OQDA}(x,y,t)]$

$C_3 = [C_{LACDA}(x,y,t)]$

$C_4 = [C_{ACDA}(x,y,t)]$

$C_5 = [C_{CANE}(x,t)]$

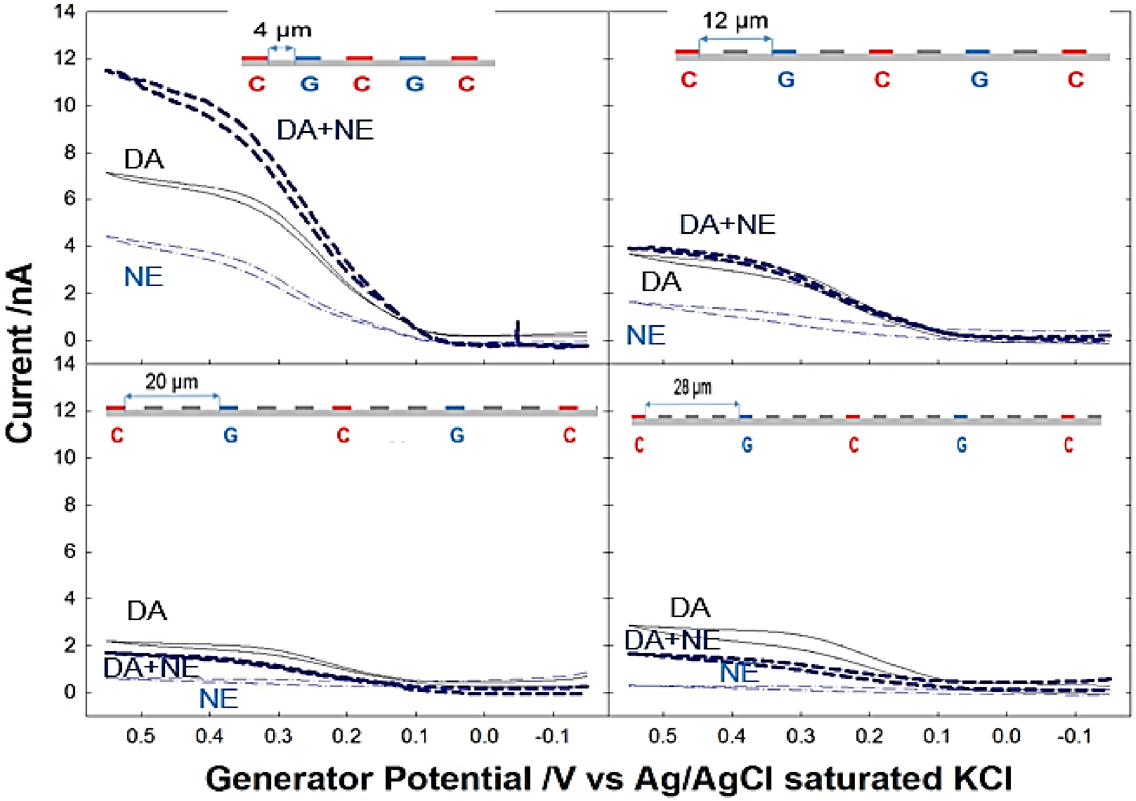
$C_6 = [C_{OQNE}(x,t)]$

$C_7 = [C_{LACNE}(x,t)]$

$C_8 = [C_{ACNE}(x,t)]$

Previous Work

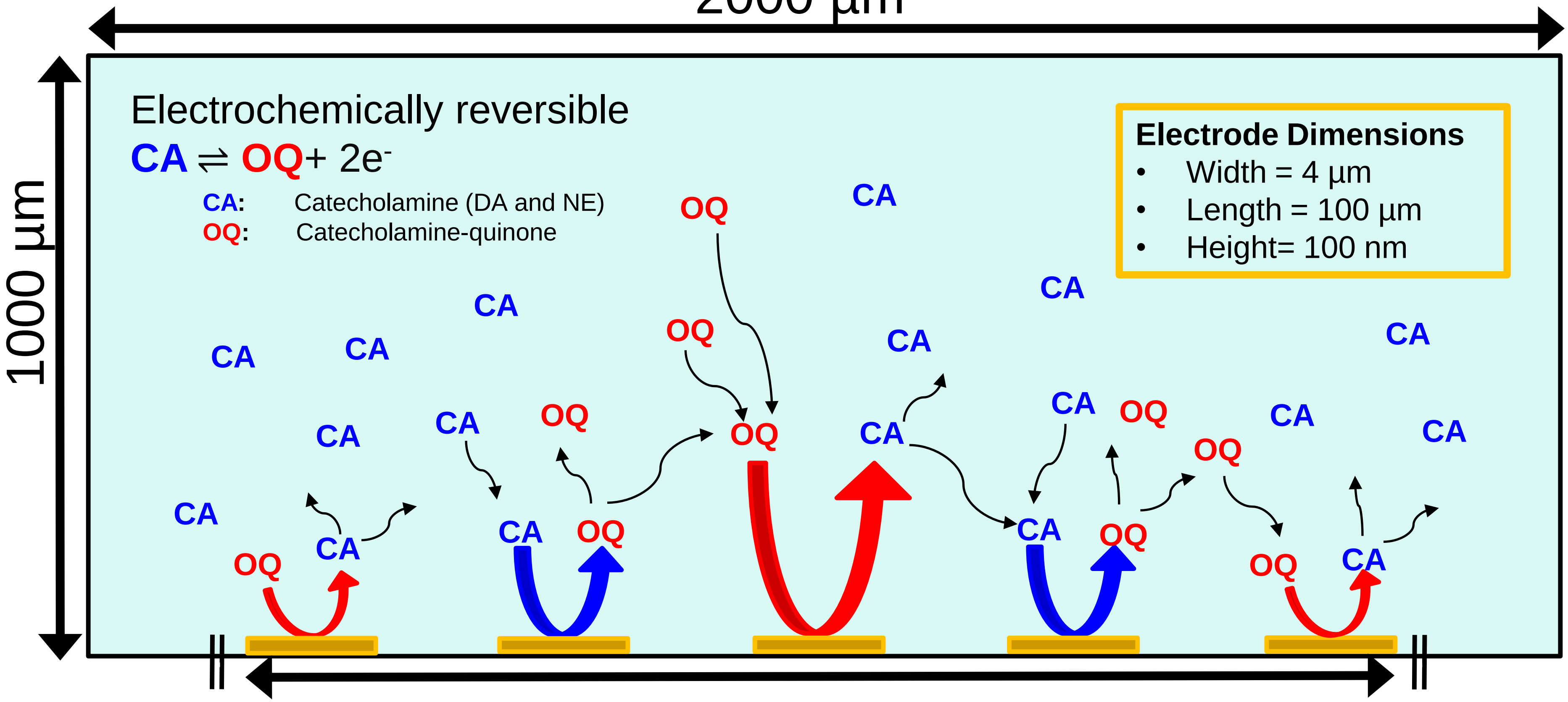
Application of Electrochemical Redox Cycling: Toward Differentiation of DA and NE



Modified figure, reprinted with permission from Hu, M.; Fritsch, I. "Application of Electrochemical Redox Cycling: Toward Differentiation of Dopamine and Norepinephrine", Anal. Chem., 2016, 88 (11), pp 5574–5578. Copyright 2016 American Chemical Society. Figure has been modified from original

- **DA and NE exhibits different concentration profiles based on gap width and can be differentiated using this technique.**
- **NE has a greater dependence on gap width than DA.**

Physical Description



Electrochemically reversible

$\mathbf{CA} \rightleftharpoons \mathbf{OQ} + 2e^-$

CA: Catecholamine (DA and NE)
OQ: Catecholamine-quinone

Electrode Dimensions

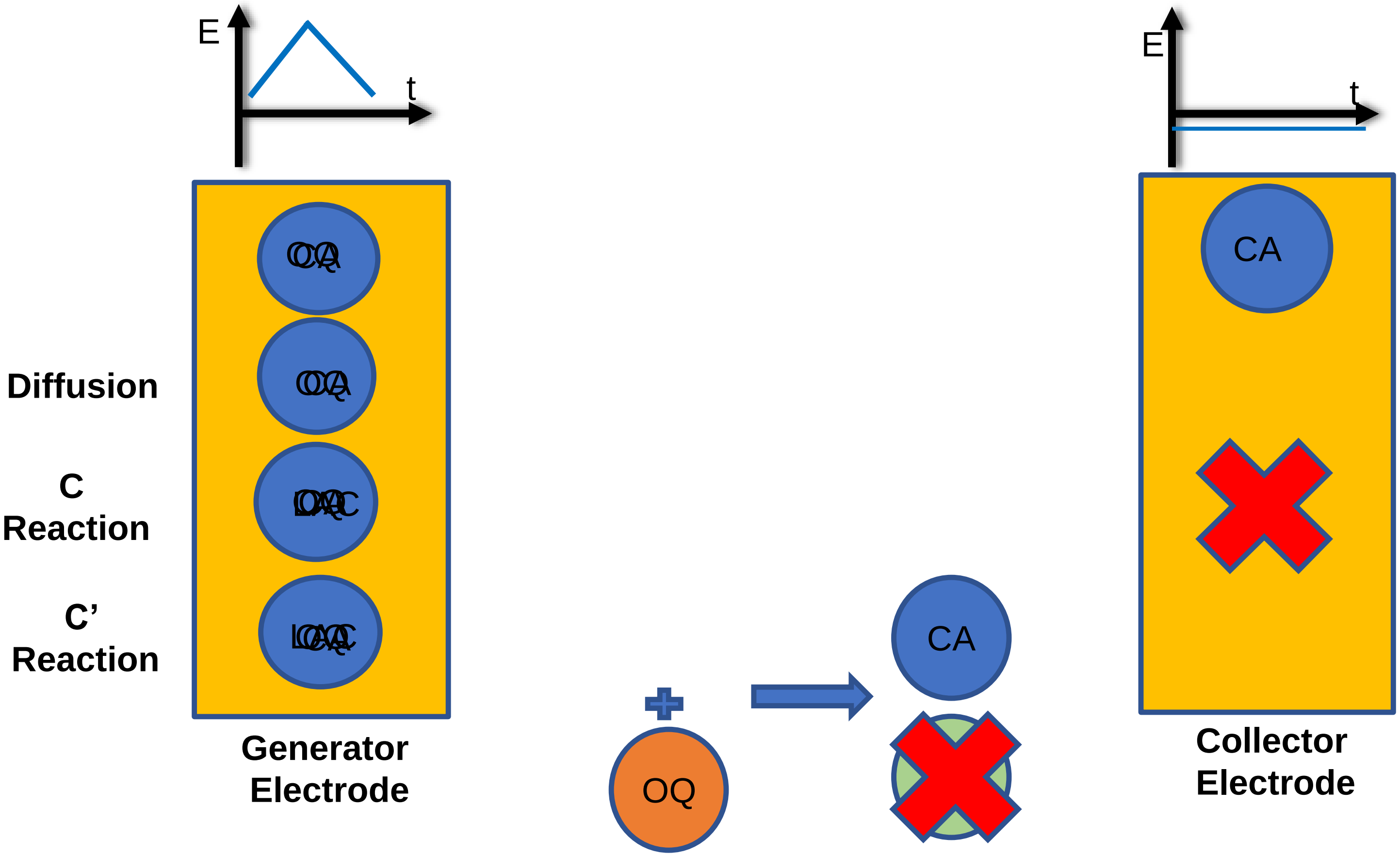
- Width = 4 µm
- Length = 100 µm
- Height= 100 nm

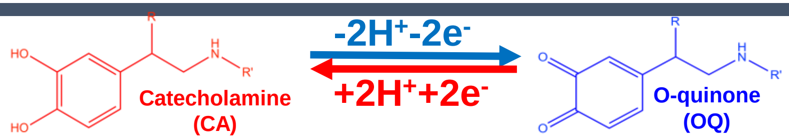
$$d = (5 \times 4\mu m \ \textbf{wide elctrodes}) + (4 \times Gap) = 36\mu m$$

$$d = (5 \times 4\mu m \ \textbf{wide elctrodes}) + (4 \times Gap) = 100\mu m$$

E $\mathrm{CA} \rightleftharpoons \mathrm{OQ} + 2e^{-} + 2\mathrm{H}^{+}$
C $\mathrm{OQ} \rightarrow \mathrm{LAC}$
C’ $\mathrm{LAC} + \mathrm{OQ} \rightarrow \mathrm{CA} + \mathrm{AC}$

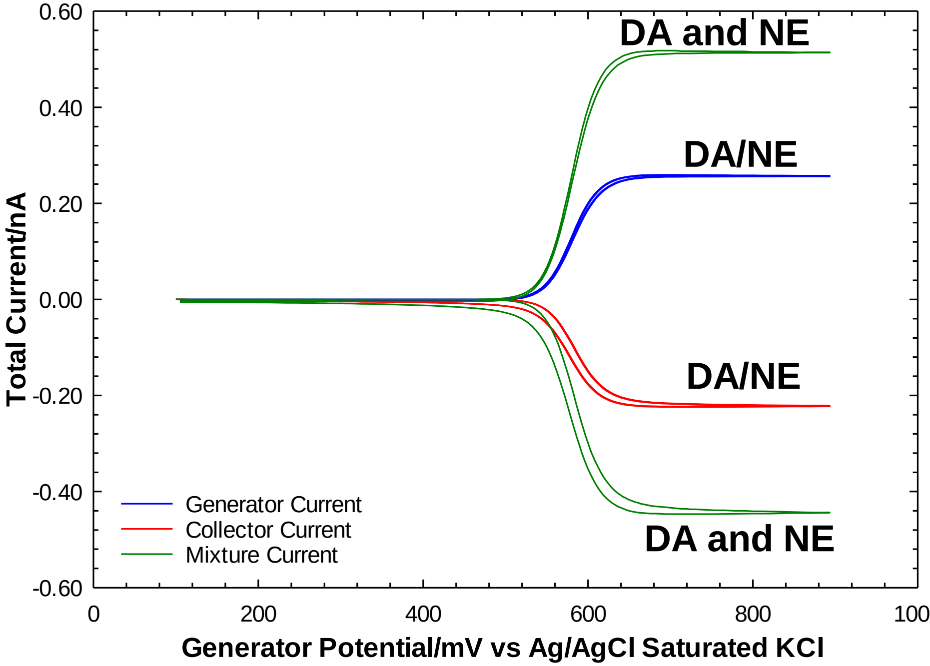
Redox Cycling of Catecholamines

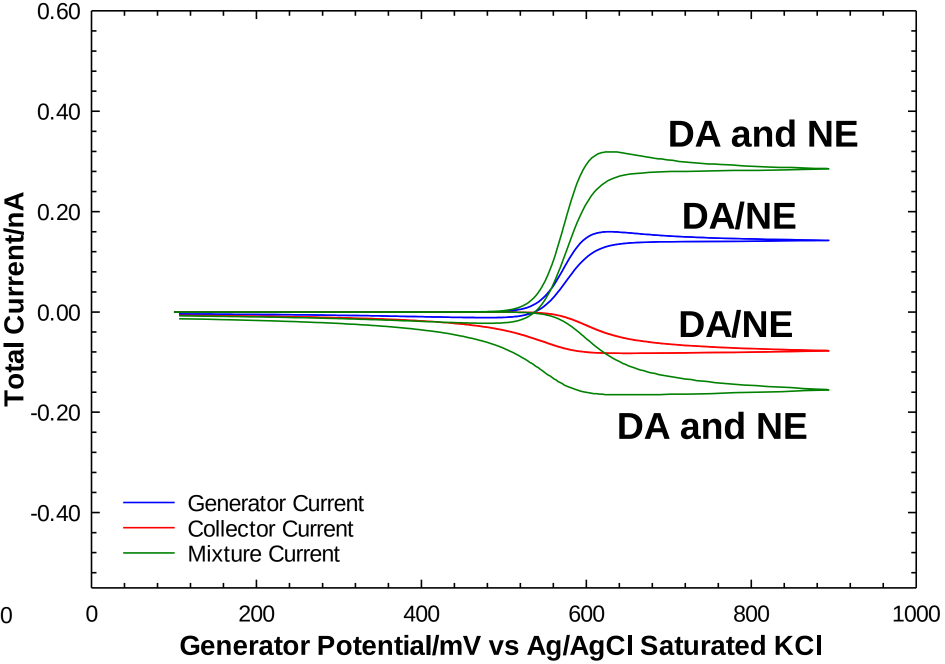




Results

E mechanism


E Mechanism at 4mm

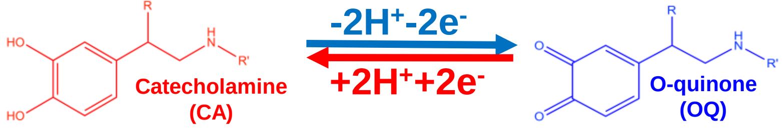

E Mechanism at 20mm

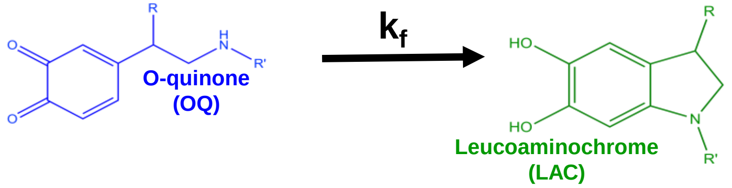
- The collector efficiency of NE has a greater dependence on the gap width than DA. The different shape in the collector electrode response is due to the longer distances allowing the solution to diffuse away from the electrodes.

- DA and NE present a peak shape in the generator electrode due to mass transfer restrictions.

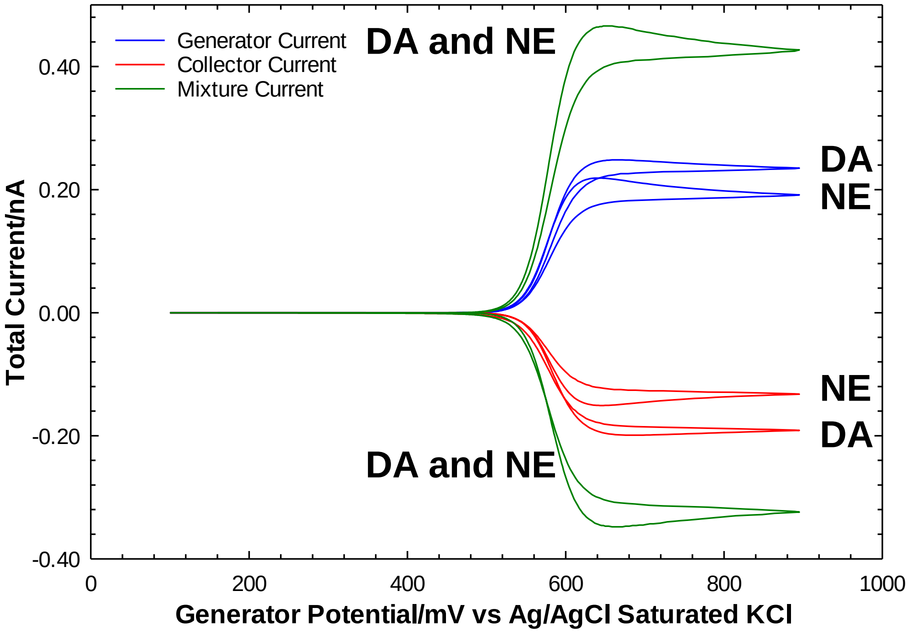
Results

EC mechanism

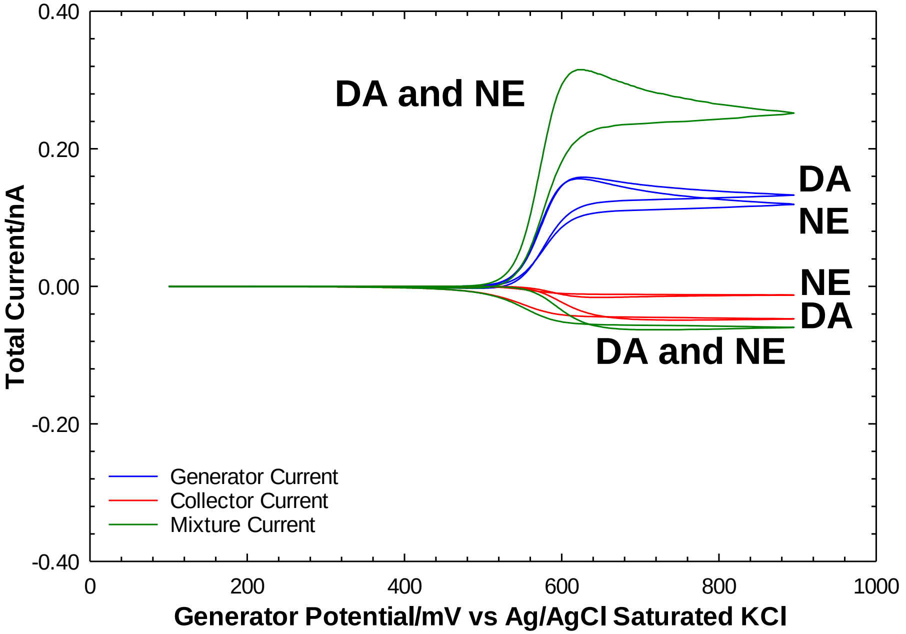




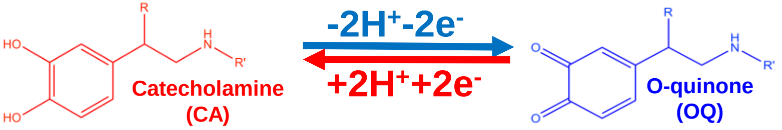
EC Mechanism at 4mm



EC Mechanism at 20mm

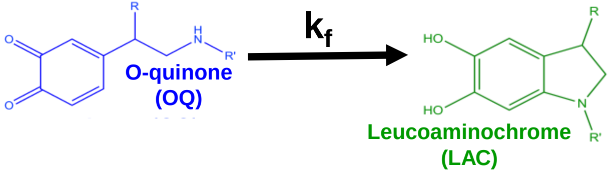


- DA and NE rate constant differ by a factor of 7.5. This provides the opportunity to differentiate them because NE is almost **silent**.
- **Hysteresis** appears. Phenomenon that occurs when OQ diffuses away from the electrode to the solution resulting in no OQ at the surface to be reduced allowing OQ to be converted into LAC



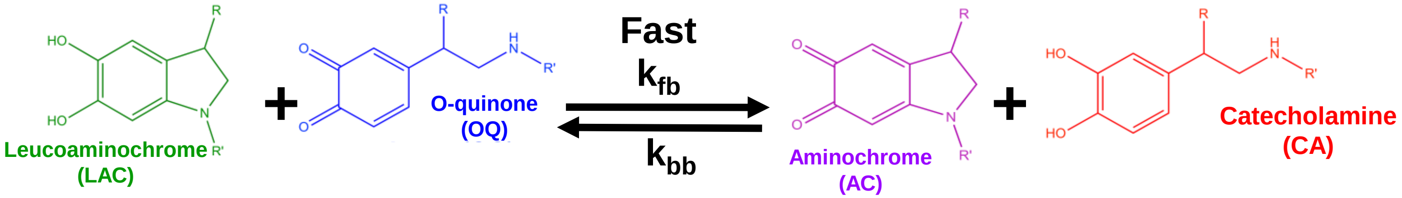
Results

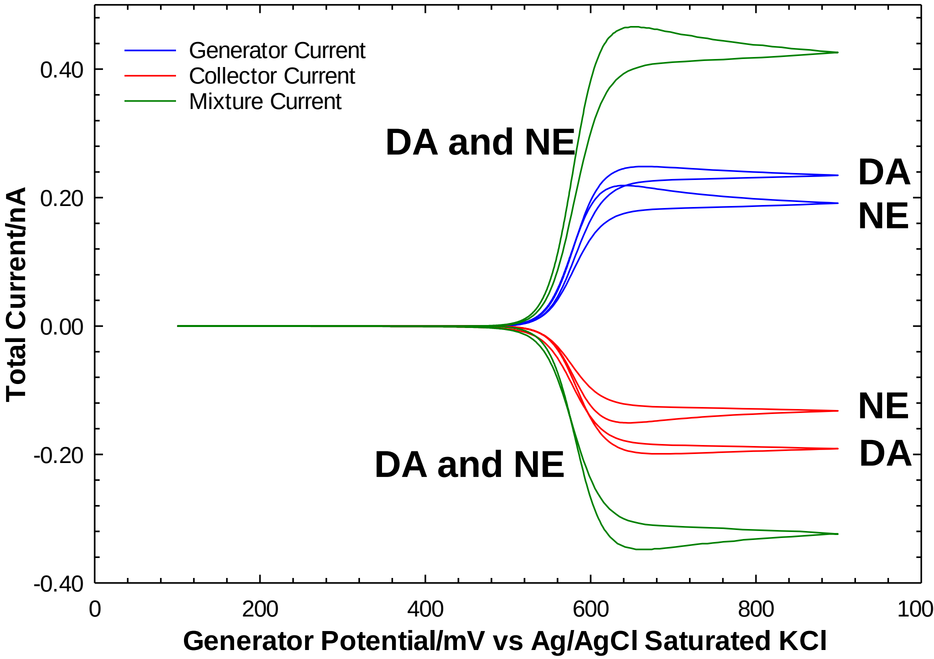
ECC' mechanism



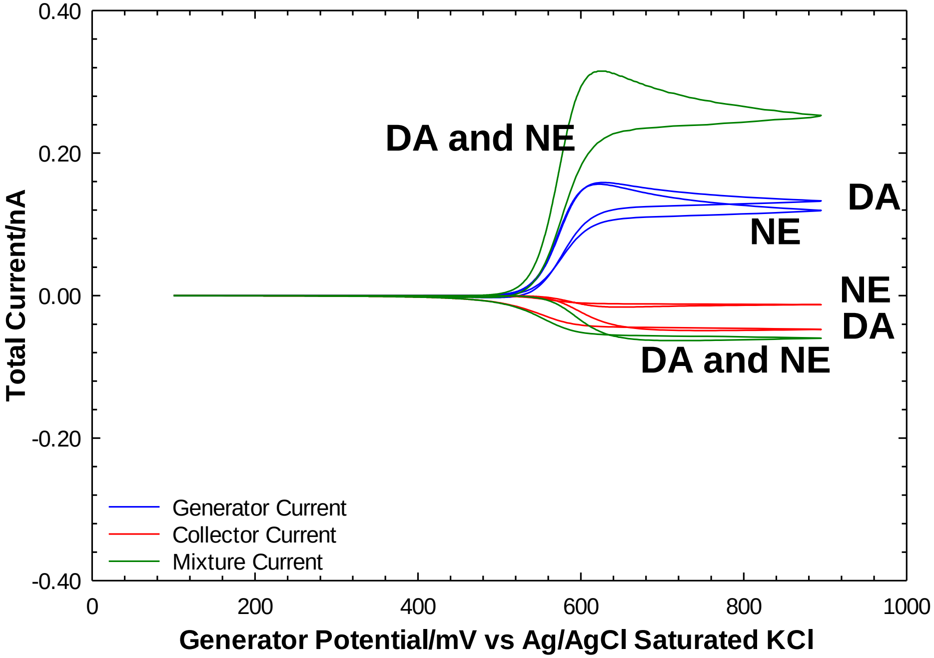
$$K = \frac{k_{fb}}{k_{bb}} = 10^{26}$$

$$kfb = 2\times10^{4}\ \frac{cm^3}{s-mol}$$





Complete Mechanism at 4mm

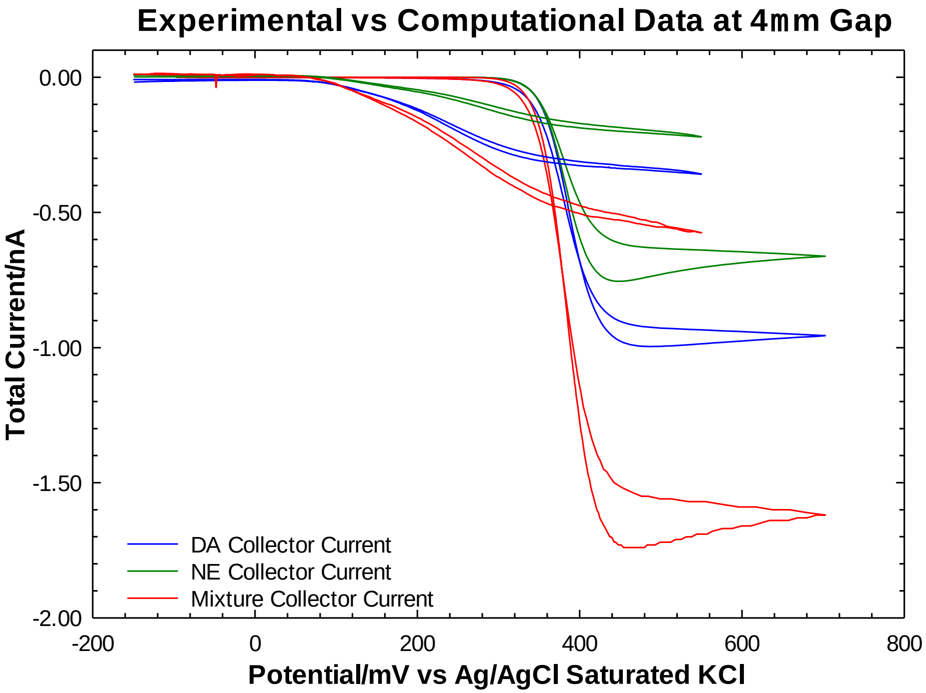


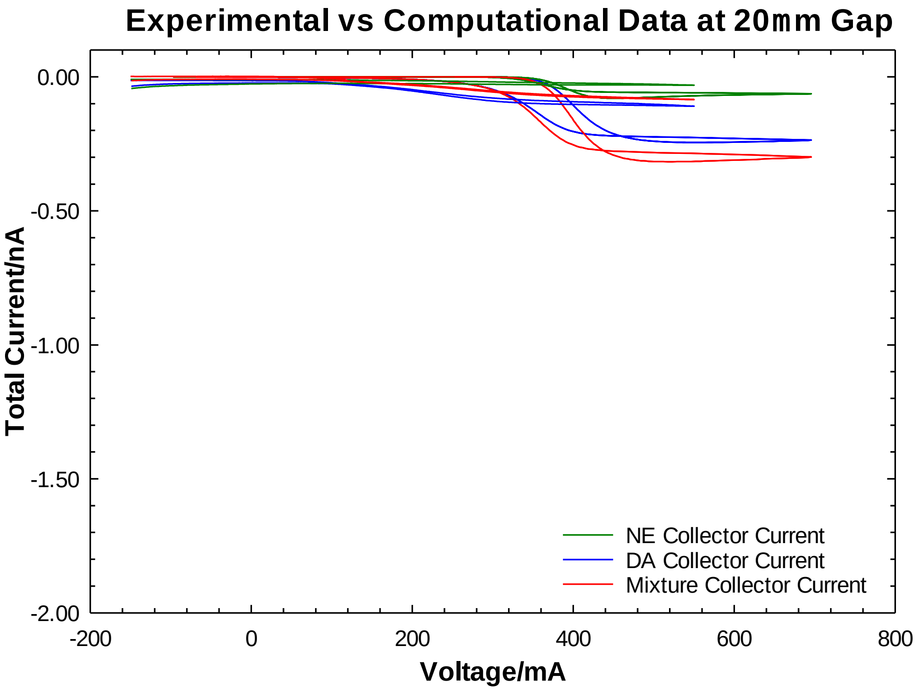
Complete Mechanism at 20mm

- k_{fb} and k_{bb} were estimated using an equilibrium constant.
- Based on the estimated K_{eq}, C mechanism shows no effect to the overall reaction.

Results

Experimental vs Computational Data


Experimental vs Computational Data at 4mm Gap


Experimental vs Computational Data at 20mm Gap

NE becomes “near **silent**” at 20 µm gap

Conclusions

- ☐ The catecholamines can be ***distinguished*** based on their different cyclization rates by redox cycling methods.
- ☐ **C mechanism** can be considered the most predominant mechanism, allowing the differentiation between the catecholamines.
- ☐ Additional studies are being developed to further **minimize the probe dimensions** to achieve lower detection limits.
- ☐ **Adaptable for analysis in small spaces**–probes for in vivo applications
- ☐ **Electrochemical generation-collection at arrays:** Investigation of chemistry through the relationship of space and time resulting from mass transfer and reaction kinetics
- ☐ **Experimental verification of equilibrium constant** is needed to refine simulations further

Acknowledgments

- Fritsch Research Group

- Research was supported partially through the National Science Foundation (Grant CHE-1808286), the University of Arkansas Women's Giving Circle, and the Arkansas Biosciences Institute, the major research component of the Arkansas Tobacco Settlement Proceeds Act of 2000.

References

E_{DA}=0.65 V at pH 7.4 vs. Ag/AgCl(saturated KCl):

Song, Yuanzhi, et al. "The electrochemical behavior of dopamine at glassy carbon electrode modified by Nafion multiwalled carbon nanotubes." *Russian journal of physical chemistry* 80.9 (**2006**): 1467-1474.

E_{NE}=0.66 V at pH 7.4 vs. Ag/AgCl(saturated KCl):

Song, Yuanzhi. "Theoretical study on the electrochemical behavior of norepinephrine at Nafion multi-walled carbon nanotubes modified pyrolytic graphite electrode." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 67.5 (**2007**): 1169-1177.

k_{DA}=0.13; k_{NE}=0.98; k_{EP}=87 (s^{-1})

Ciolkowski, E.; Cooper, B.; Jankowski, J.; Jorgenson, J.; Wightman, R., Direct Observation of Epinephrine and norepinephrine Cosecretion from Individual Adrenal-Medullary Chromaffin Cells. *Journal of the American Chemical Society* **1992,** *114* (8), 2815-2821.

Arnsten, A. F. T.; Pliszka, S. R. *Pharmacology, biochemistry, and behavior* **2011,** *99* (2), 211-216.

References

Arnsten, A. F. T.; Pliszka, S. R. *Pharmacology, biochemistry, and behavior* **2011,** *99* (2), 211-216.

$D=5.40\times10^{-6}$ cm^2/s, $k^0=0.0034 cm/s^{-1}$

Corona-Avendaño S., Alarcón-Angeles G., Ramírez-Silva M. T., Rosquete-Pina G, Romero-Romo M., Palomar-Pardavé M.; Journal of Electroanalytical Chemistry, **2007**,609(1), 17-26.