Unlocking the (Reversal) Potential of SSM Electrophysiology:

Transporter Stoichiometry with the SURFE^{2}R N1

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Integrated Program in BIOCHEMISTRY

Stoichiometry is a crucial determinant of transporter function

LacY

1 proton per lactose

(West and Mitchell, 1972)

$PepT_{Sa}$

5 protons per dipeptide

(Parker, Mindell, and Newstead, 2014)

-160mV PMF can drive:

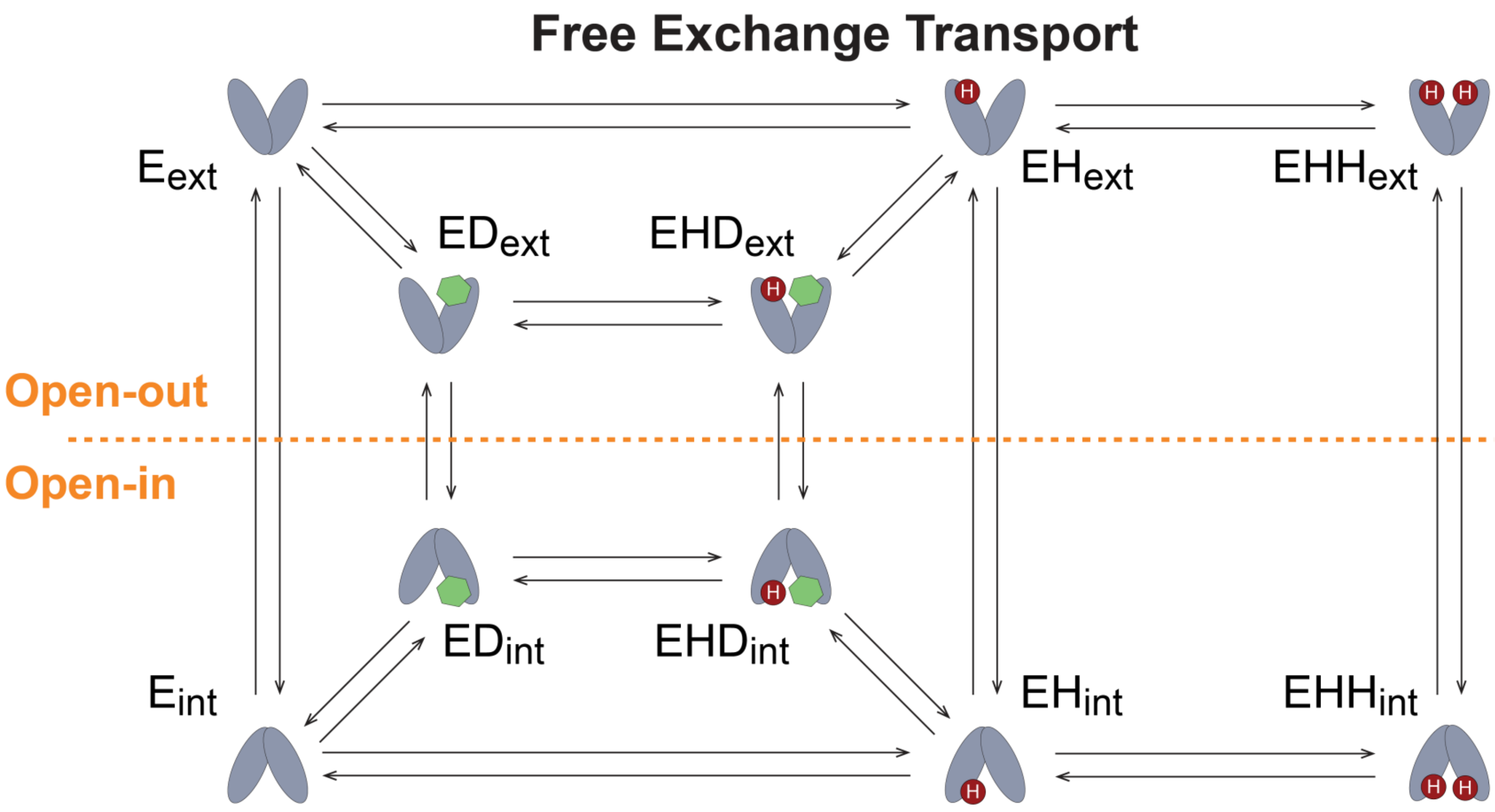
400-fold lactose gradient*

-160mV PMF can drive:

10 trillion-fold dipeptide gradient*

*Assumes tight coupling

MDR efflux pump EmrE is not tightly coupled



Multiple pathways – multiple stoichiometries?

How to measure transport stoichiometry

How to measure transport stoichiometry: Traditional Reversal Potential assay with GdX

$$\mathrm{n}H^{+}_{out} + \mathrm{m}Gdm^{+}_{in} \rightleftharpoons \mathrm{n}H^{+}_{in} + \mathrm{m}Gdm^{+}_{out}$$

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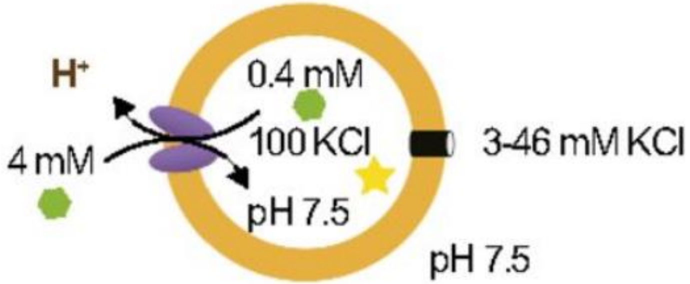

Kermani, et al. 2018

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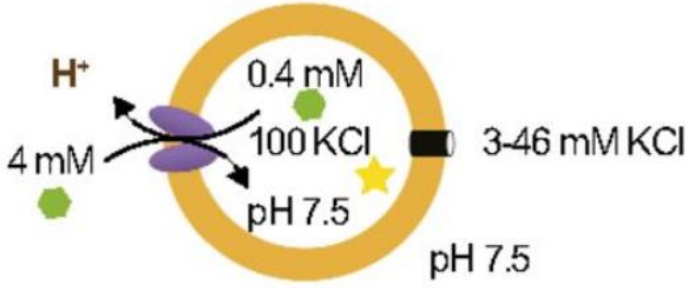
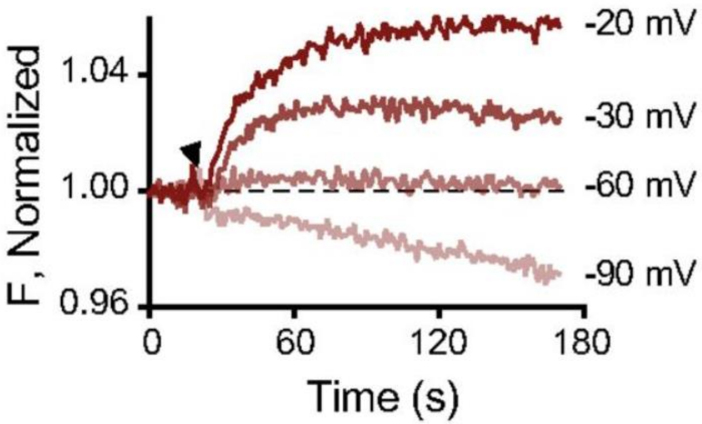
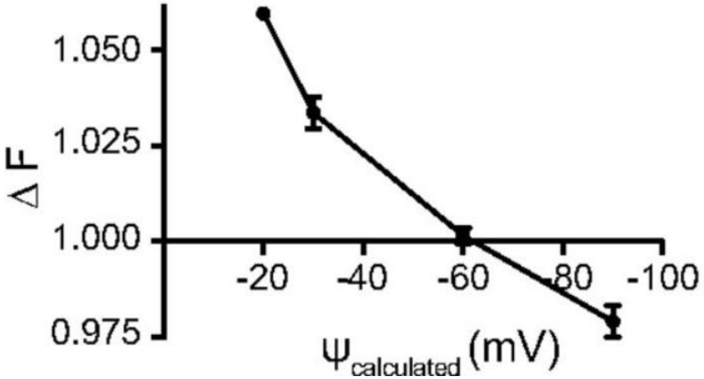




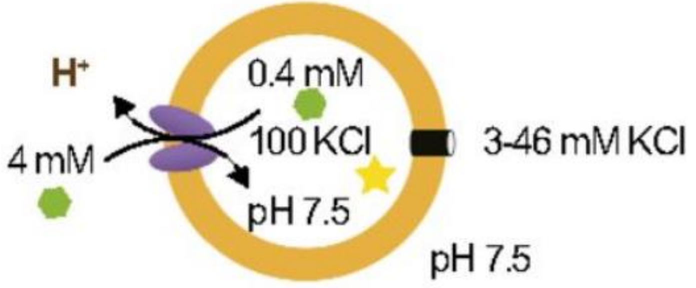


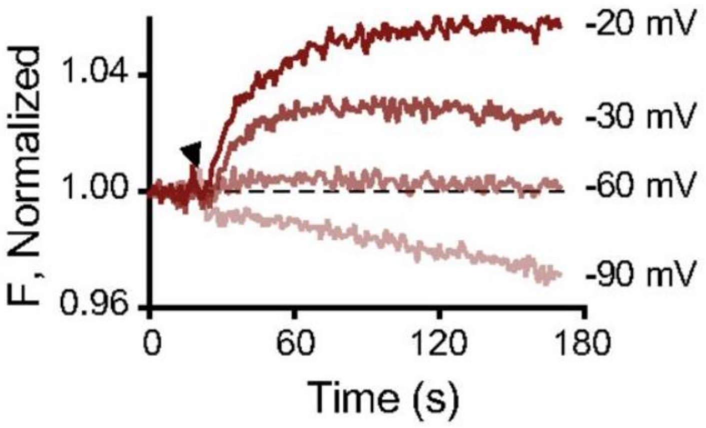
$$E_{rev} = \frac{n\mu_i + m\mu_s}{-F(nz_i + mz_s)}$$

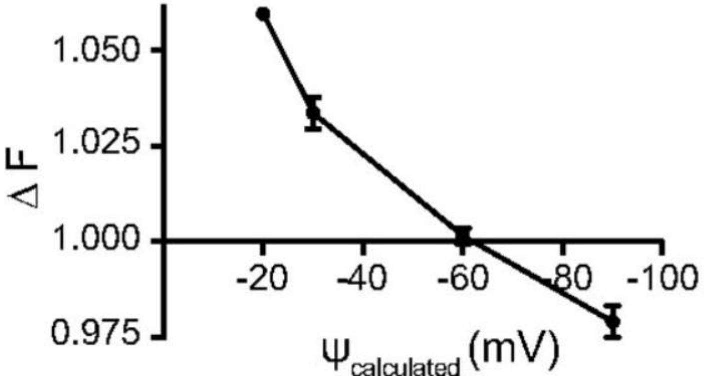
Kermani, et al. 2018

How to measure transport stoichiometry: Traditional Reversal Potential assay with GdX

$$2H^{+}_{out} + 1Gdm^{+}_{in} \rightleftharpoons 2H^{+}_{in} + 1Gdm^{+}_{out}$$







$$E_{rev} = \frac{n\mu_i + m\mu_s}{-F(nz_i + mz_s)}$$

Kermani, et al. 2018

Issues with reversal potential assays

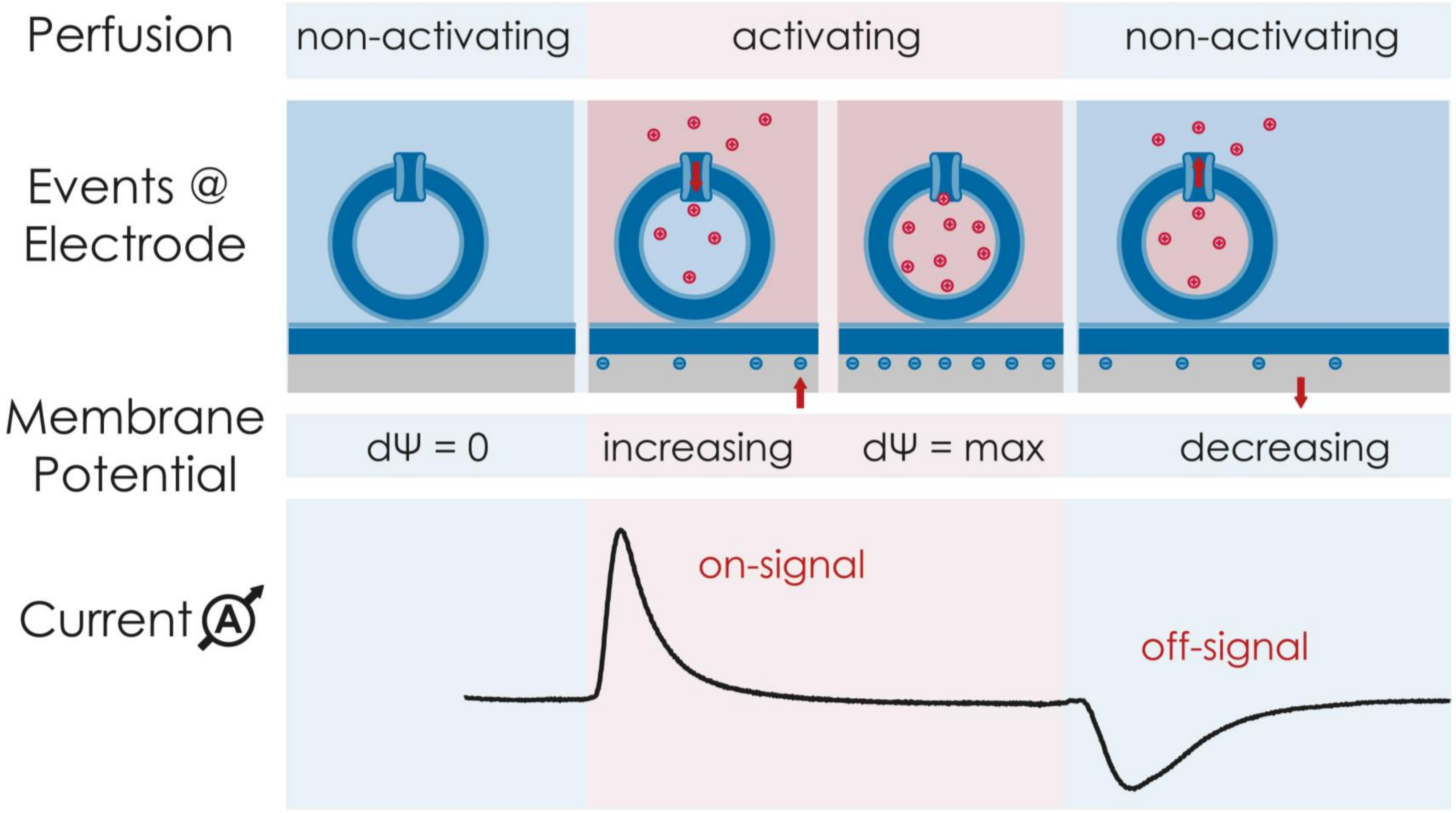
- Technically difficult and time consuming
  - Need to ensure internal solutions are accurate
  - Different internal conditions require separate reconstitutions

- Need fluorophores or radioactivity to monitor transport

- Relatively large amounts of sample required

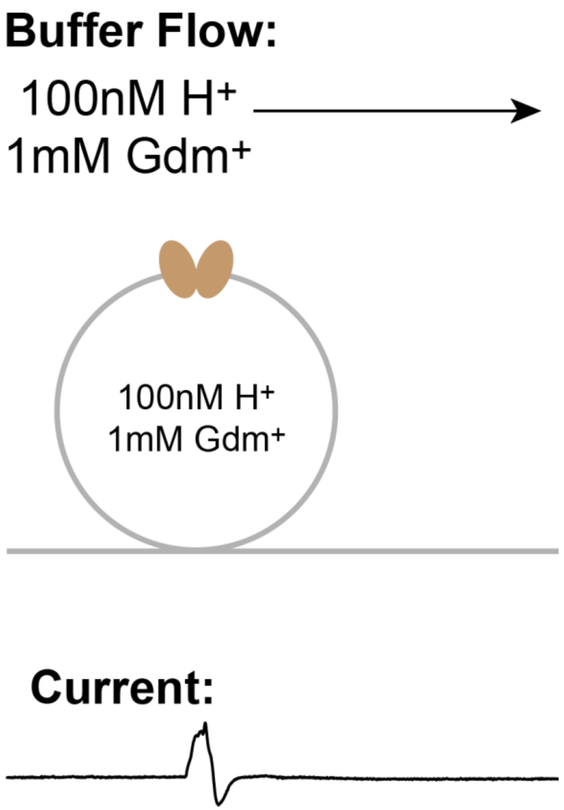
Can SSM-electrophysiology be used to measure stoichiometry?

Overview of an SSM-electrophysiology experiment

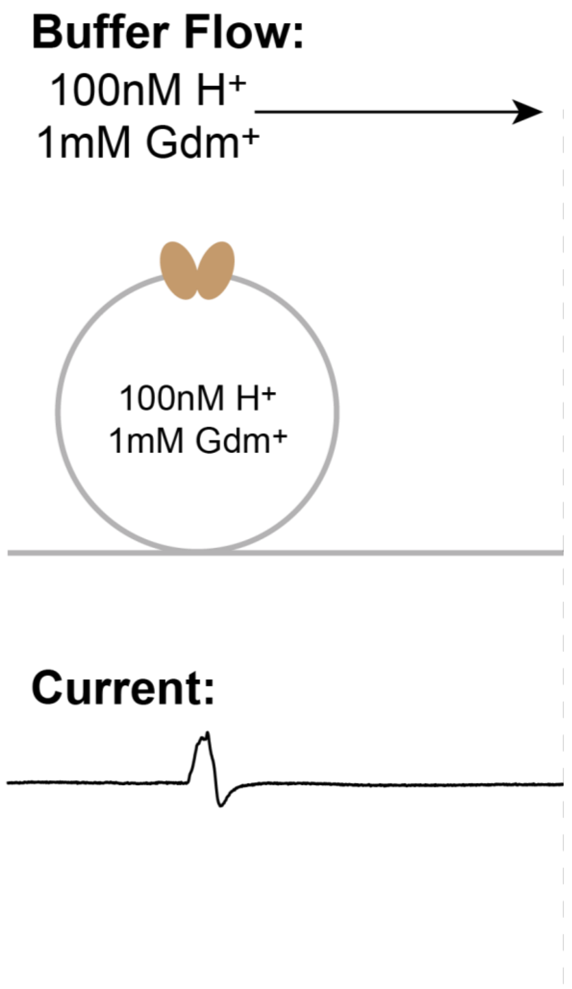


SURFE^{2}R N1
Reversal Potential Assay

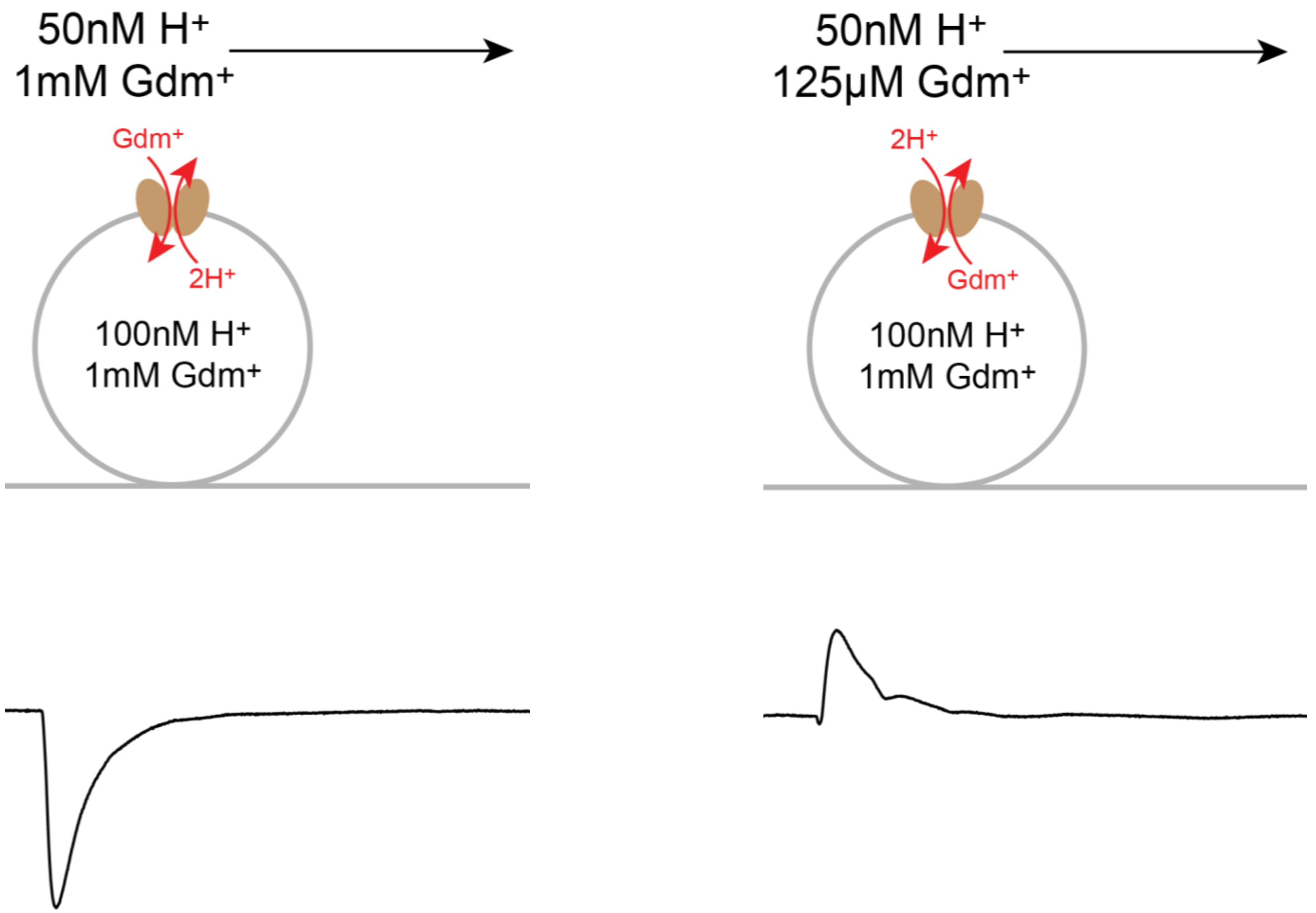
Assay Scheme – Proton gradient drives guanidinium transport



Assay Scheme – Guanidinium gradient drives proton transport



Transport current can be reversed



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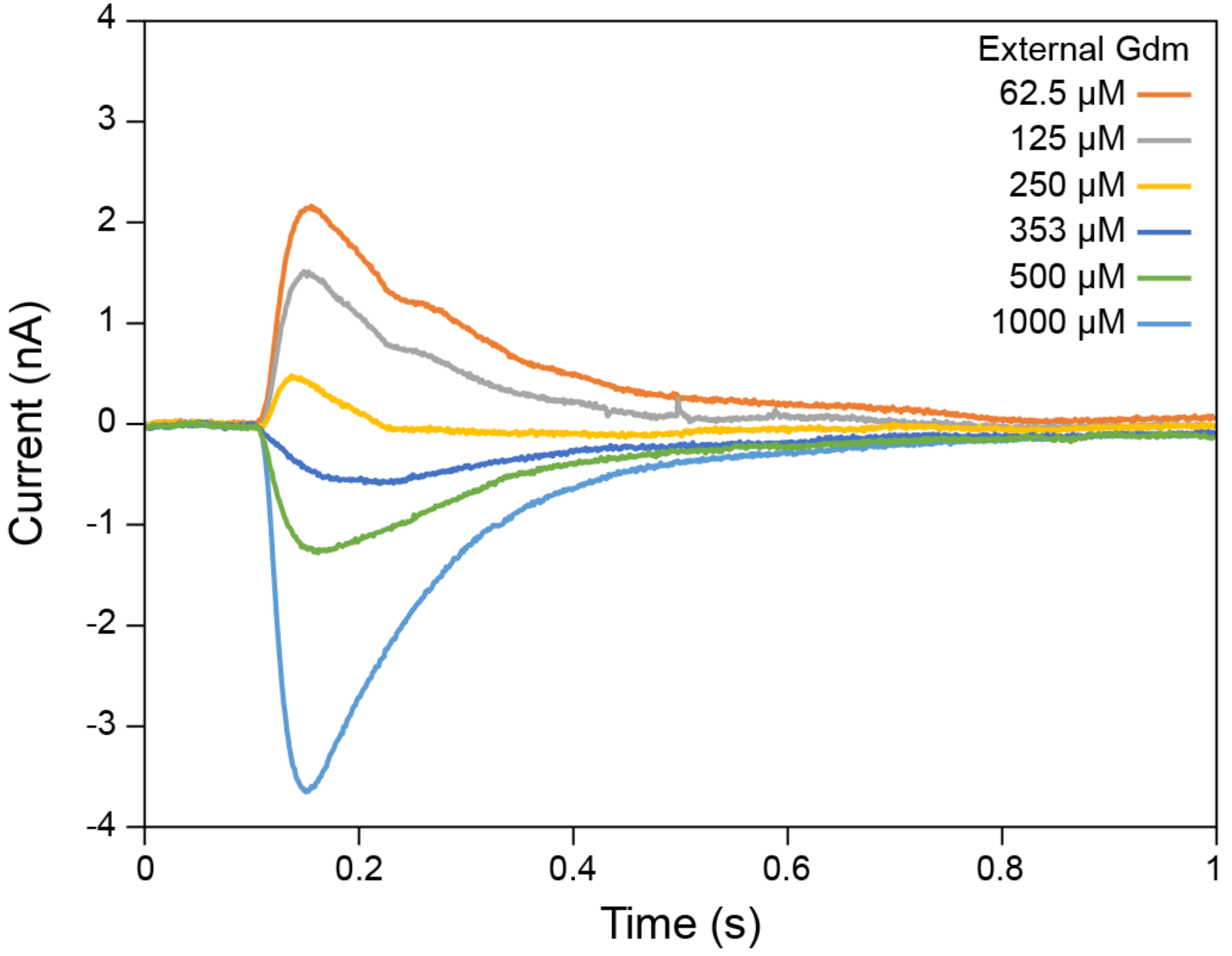
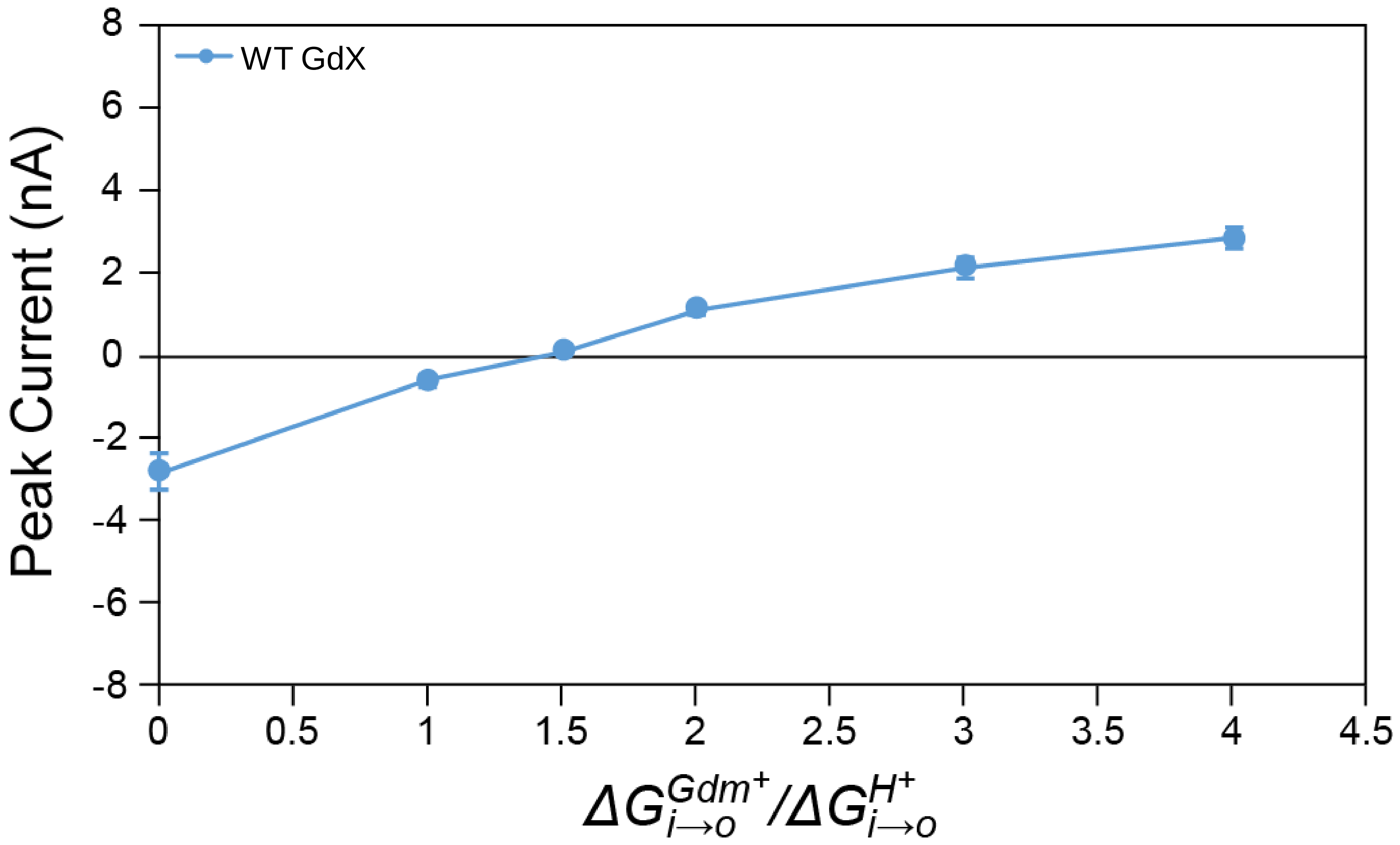


Peak current does not reverse at published reversal potential



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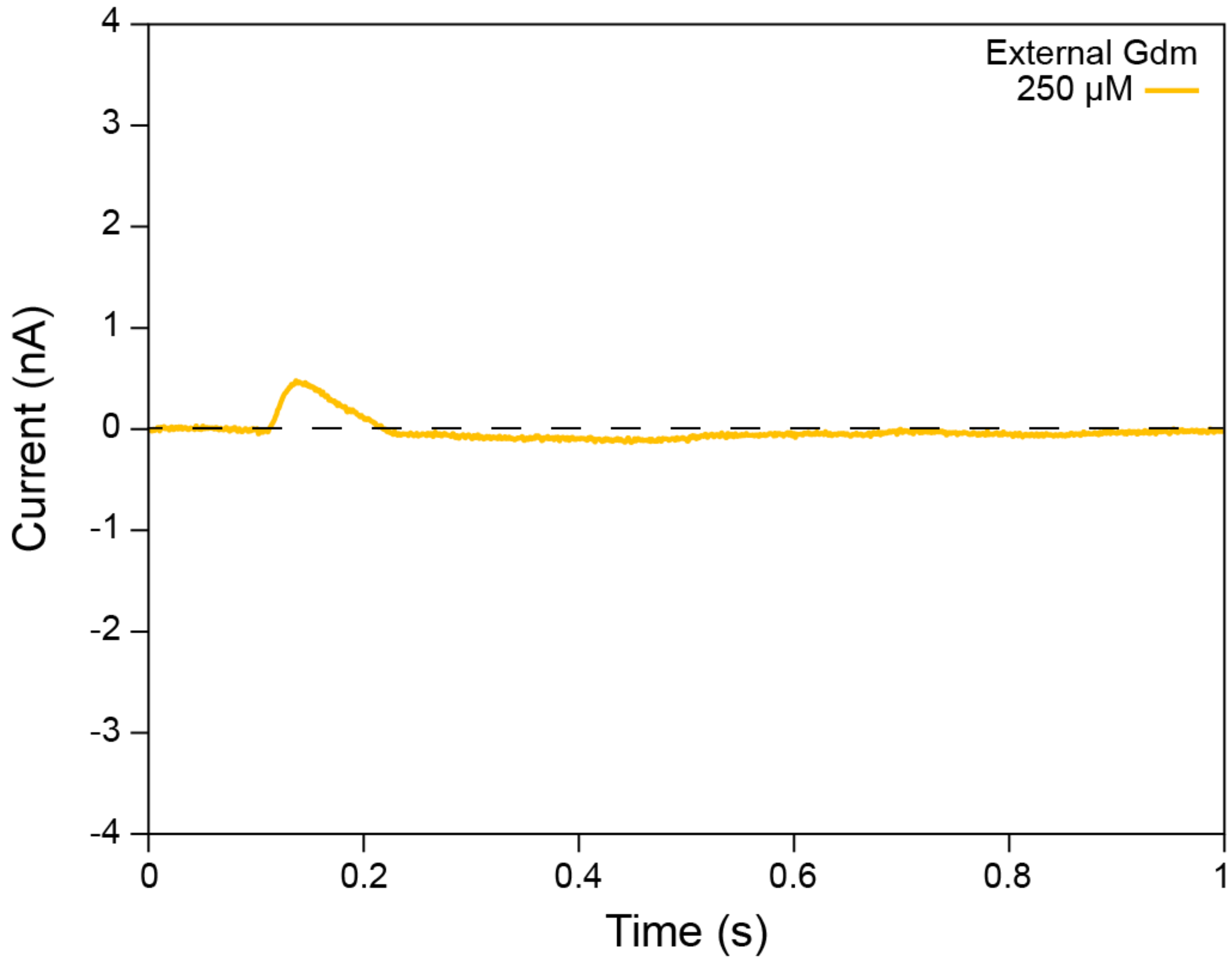
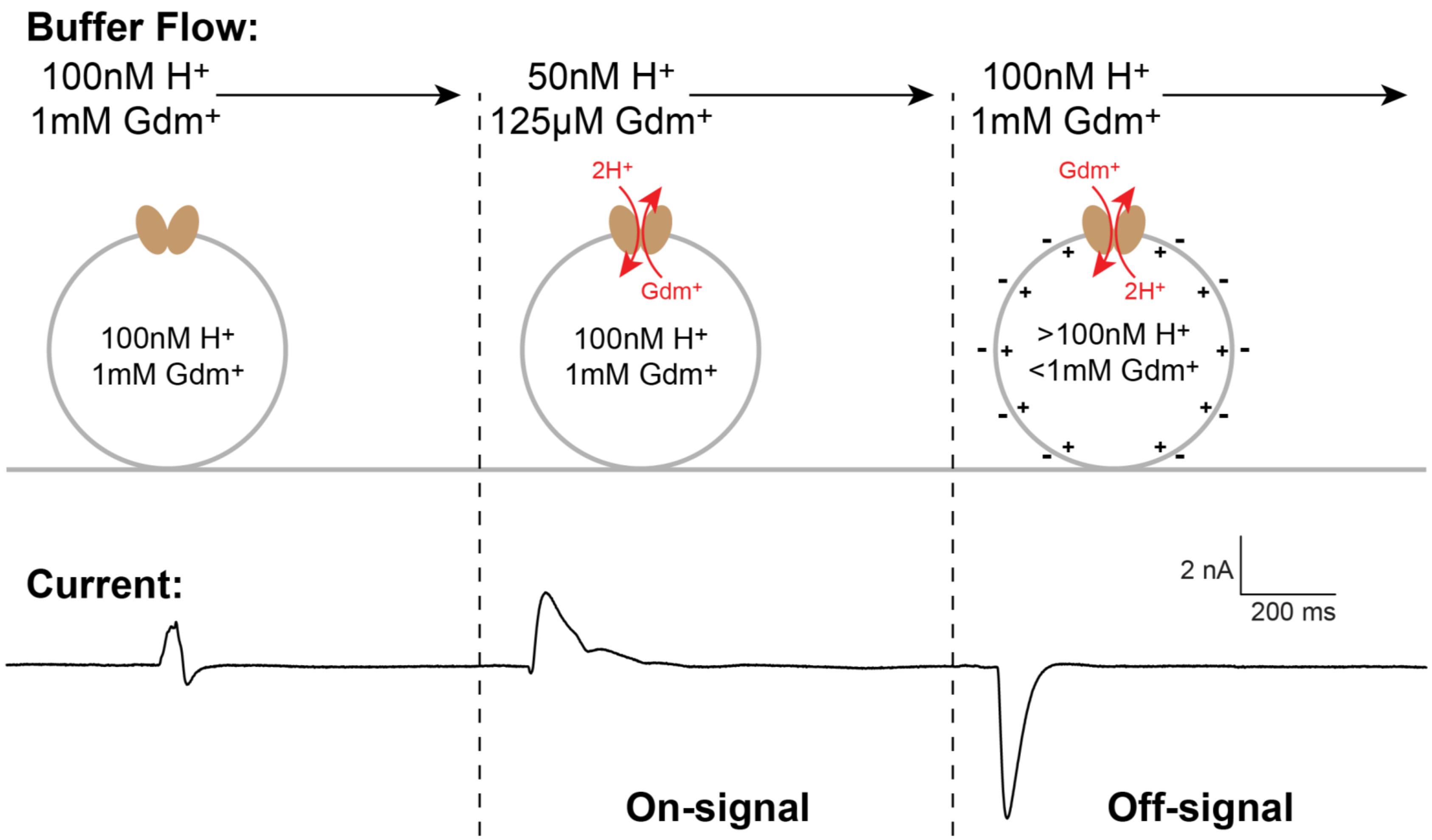
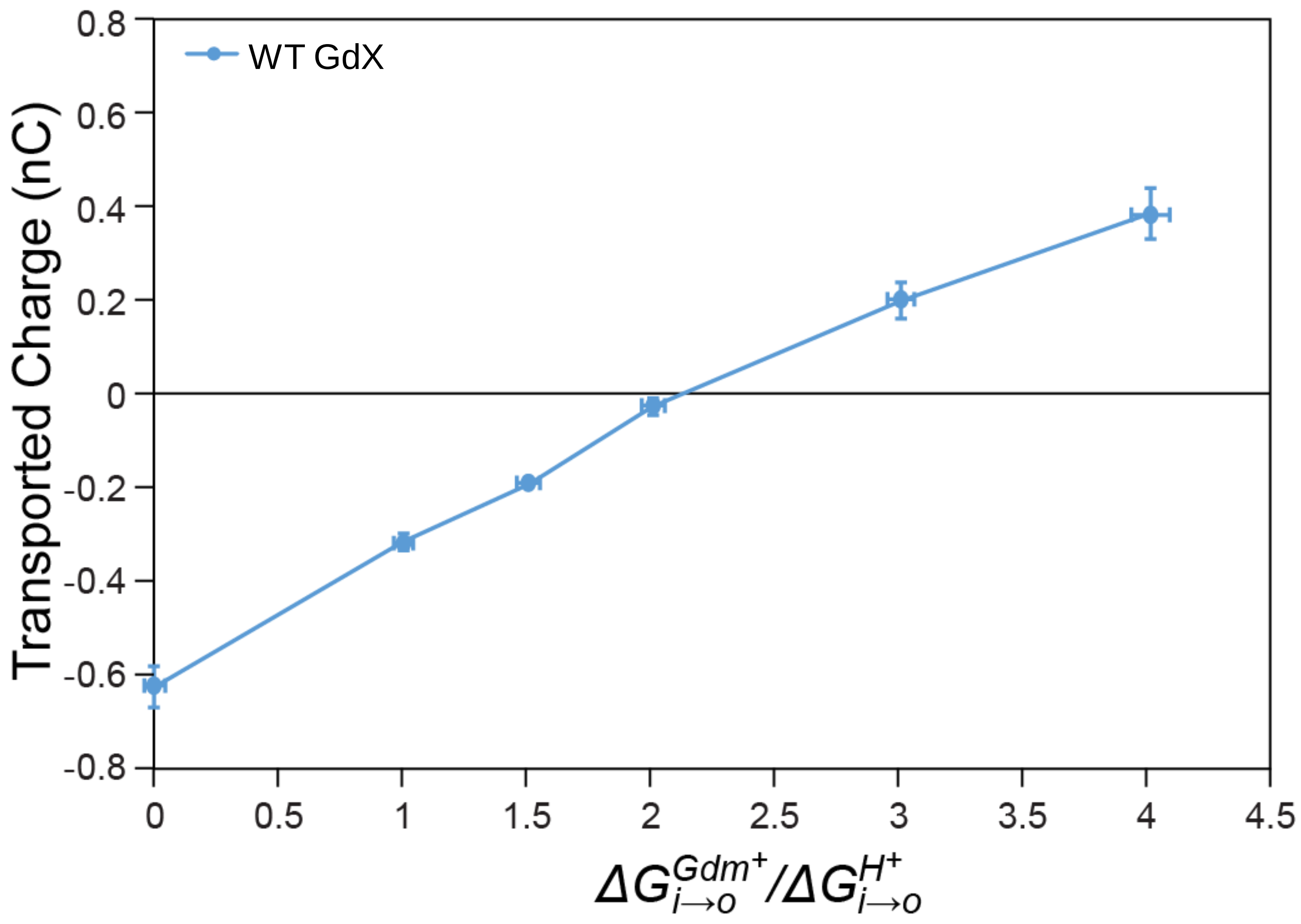


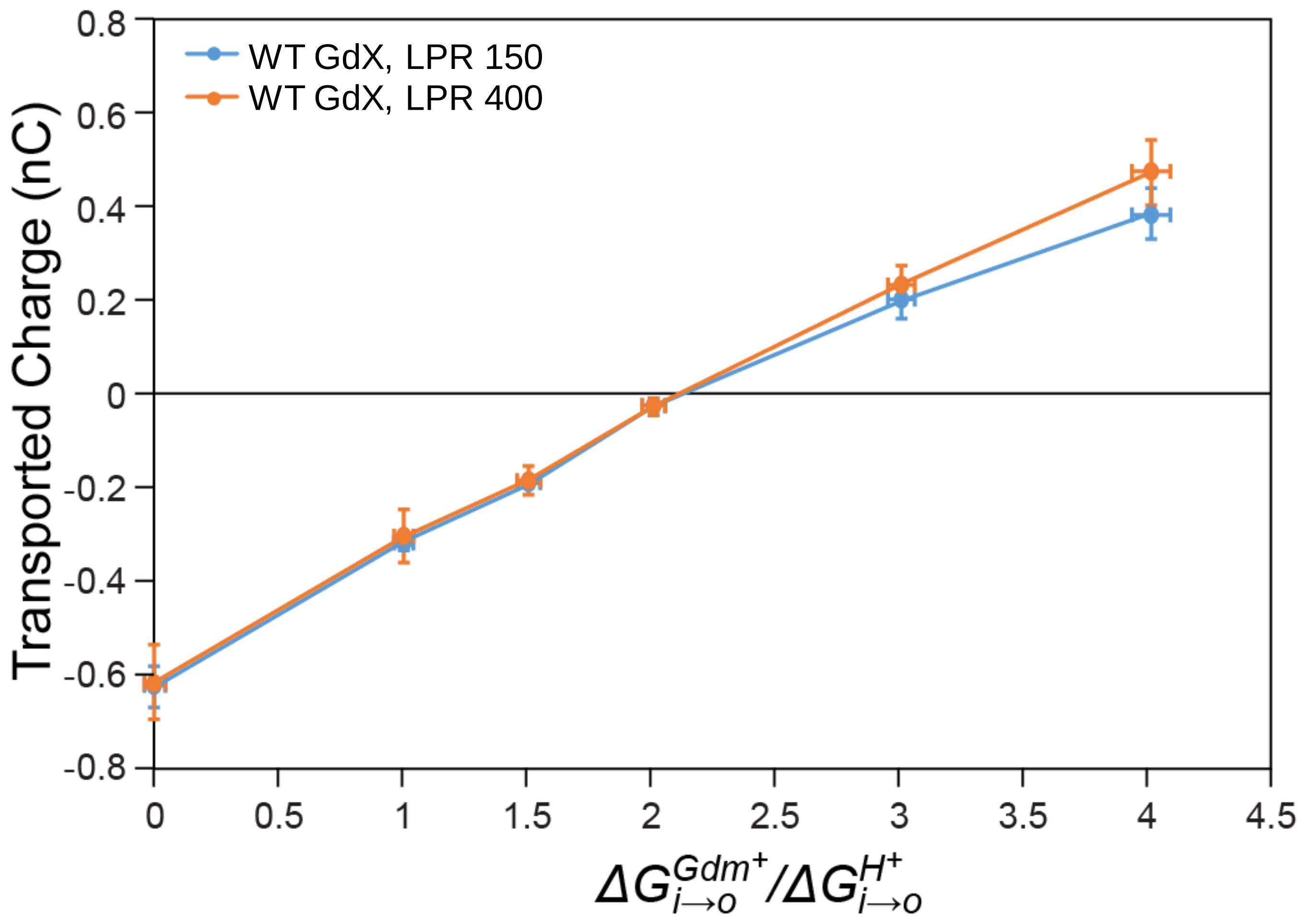
Integrate on-signal



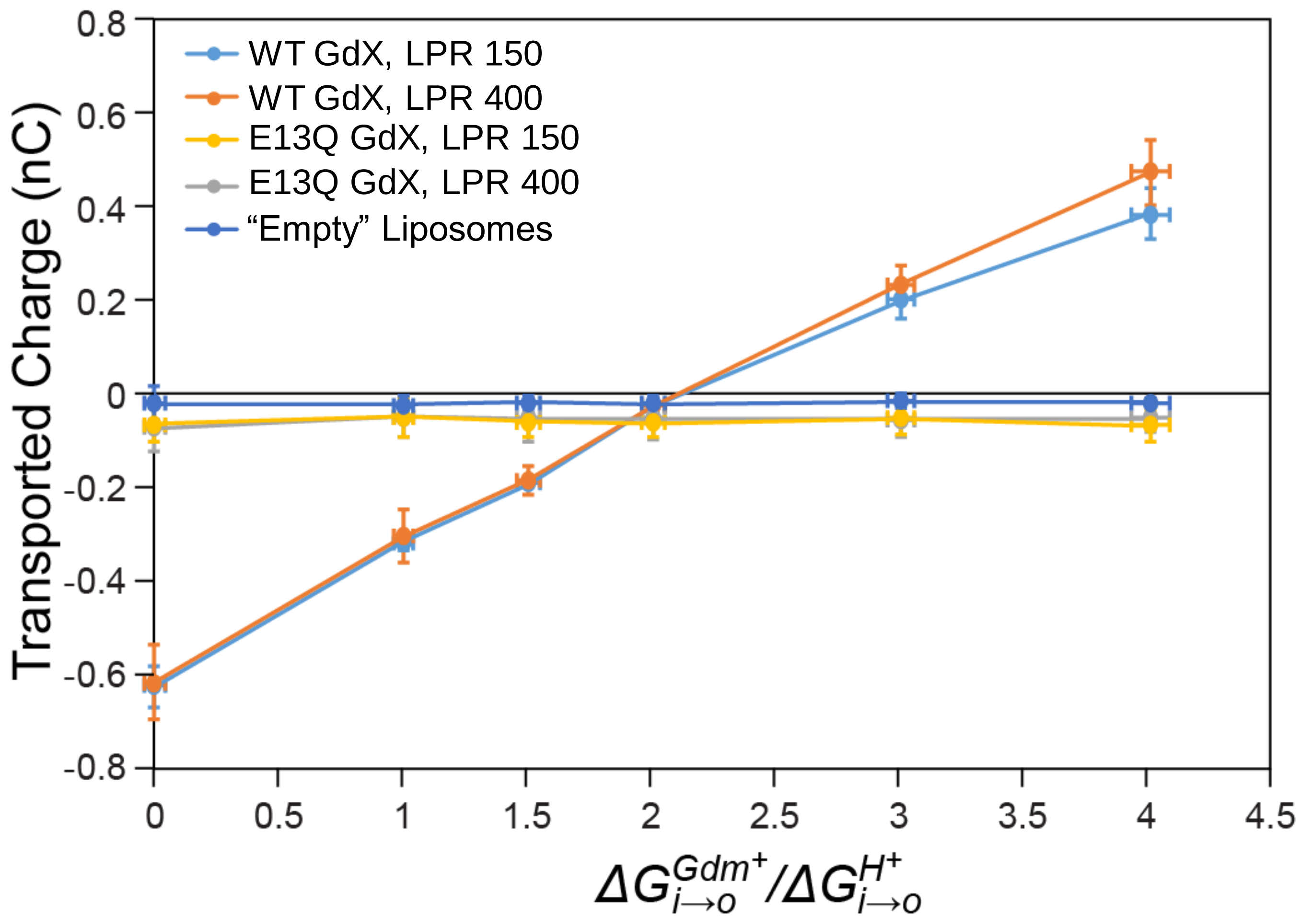
Integrated signal agrees with published stoichiometry



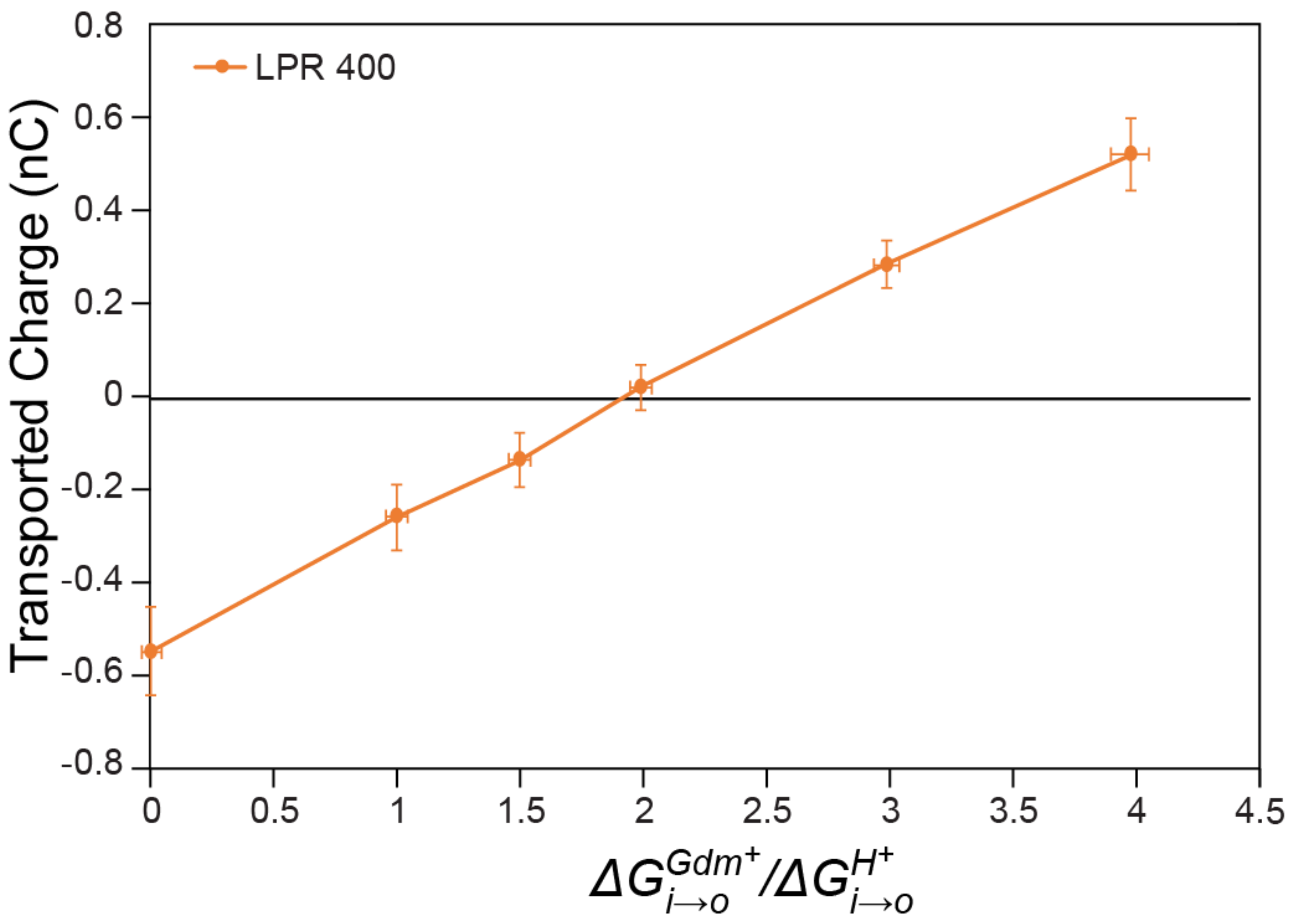
Signal is independent of protein concentration



No significant currents for empty liposomes or transport-dead mutants



Transported Charge is a linear function of chemical potential



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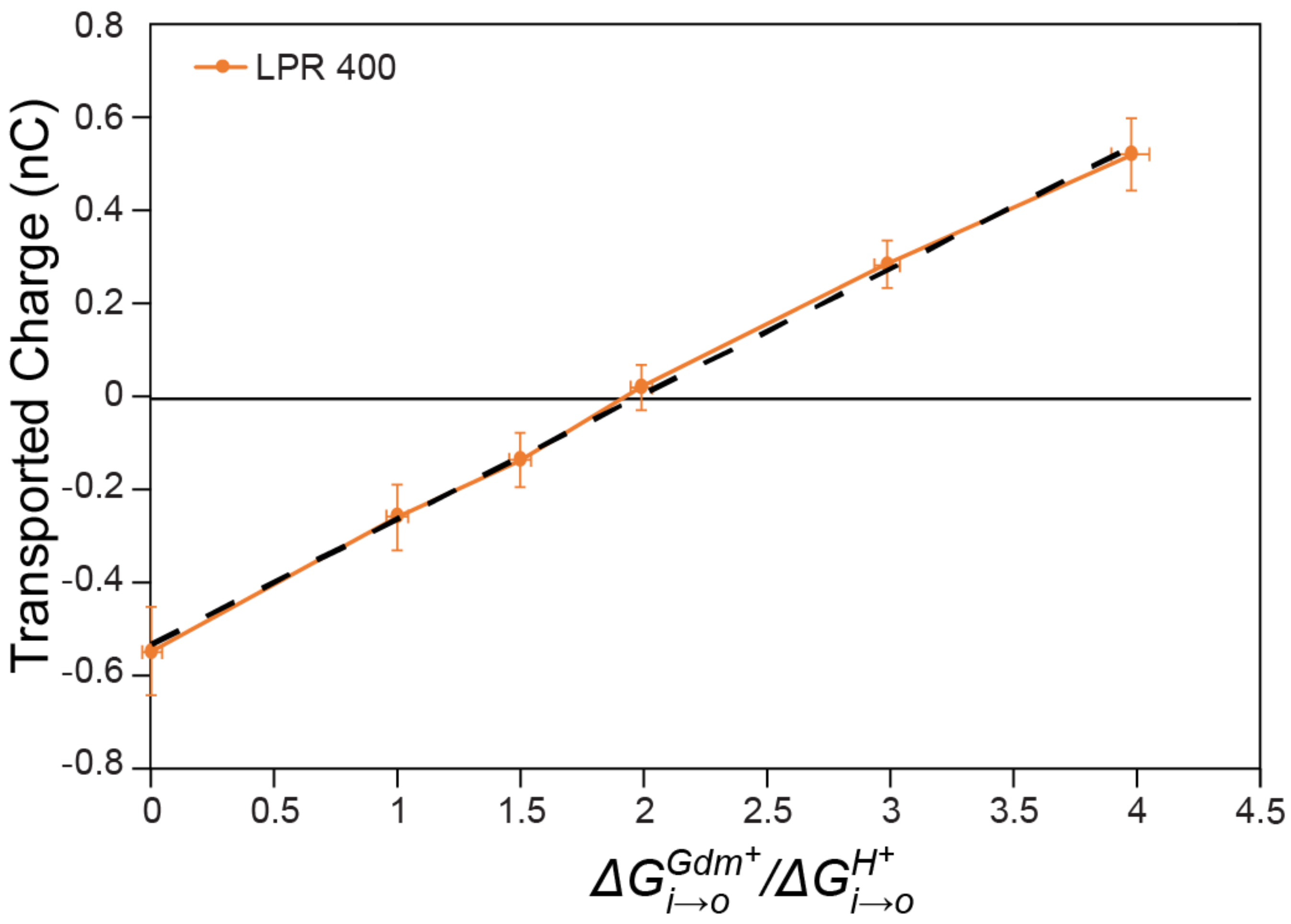


$R^2 = 0.998$

x-intercept = 1.98 ± 0.06

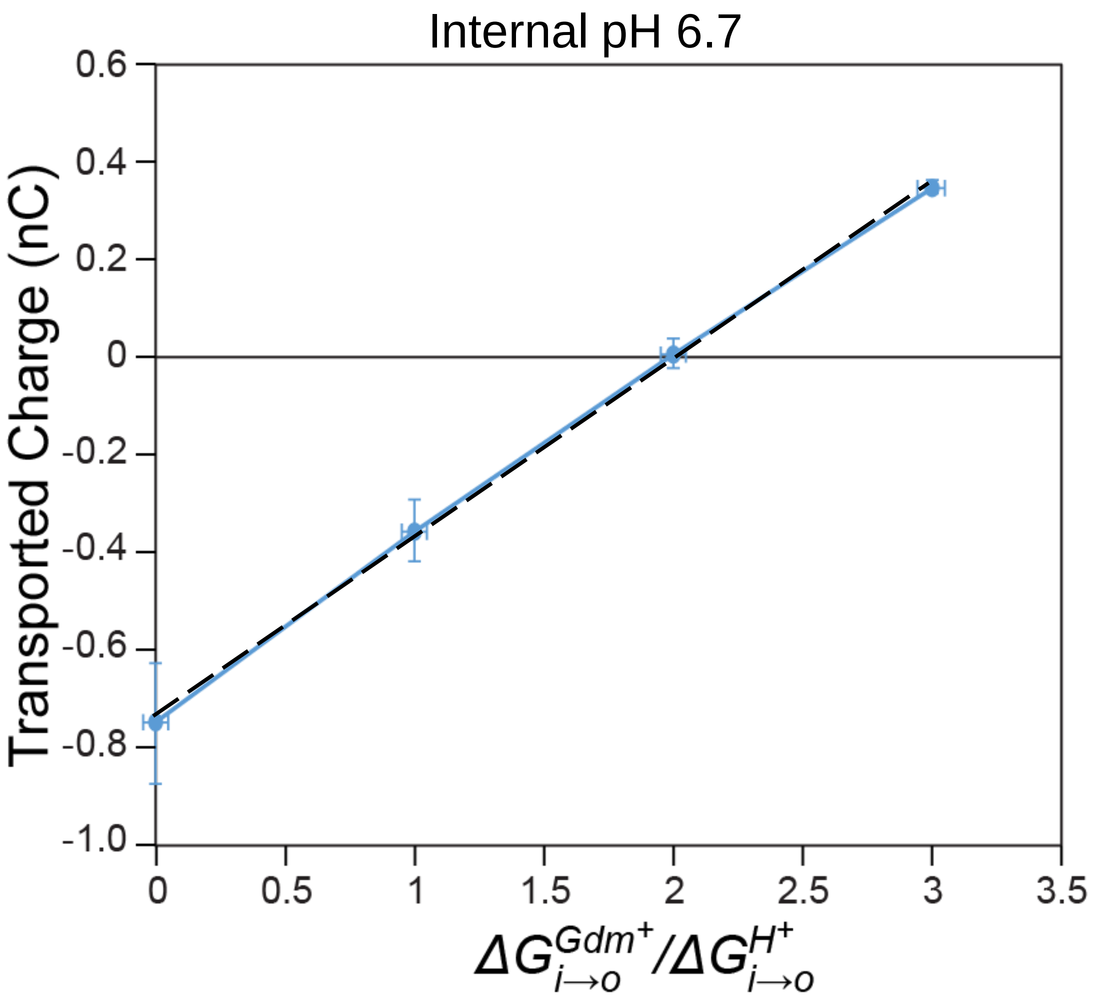
Sensor internal solution can be changed

- Reconstitute and prepare sensors at pH 7, 1 mM Gdm^+
- Flow pH 6.7, 1 mM Gdm^+ buffer over sensor
  - Monitor solution exchange until no current is observed – about 3 mL
- Repeat reversal potential assay
  - Internal buffer: 200 nM H^+, 1 mM Gdm^+
  - External buffer: 100 nM H^+, varied Gdm^+

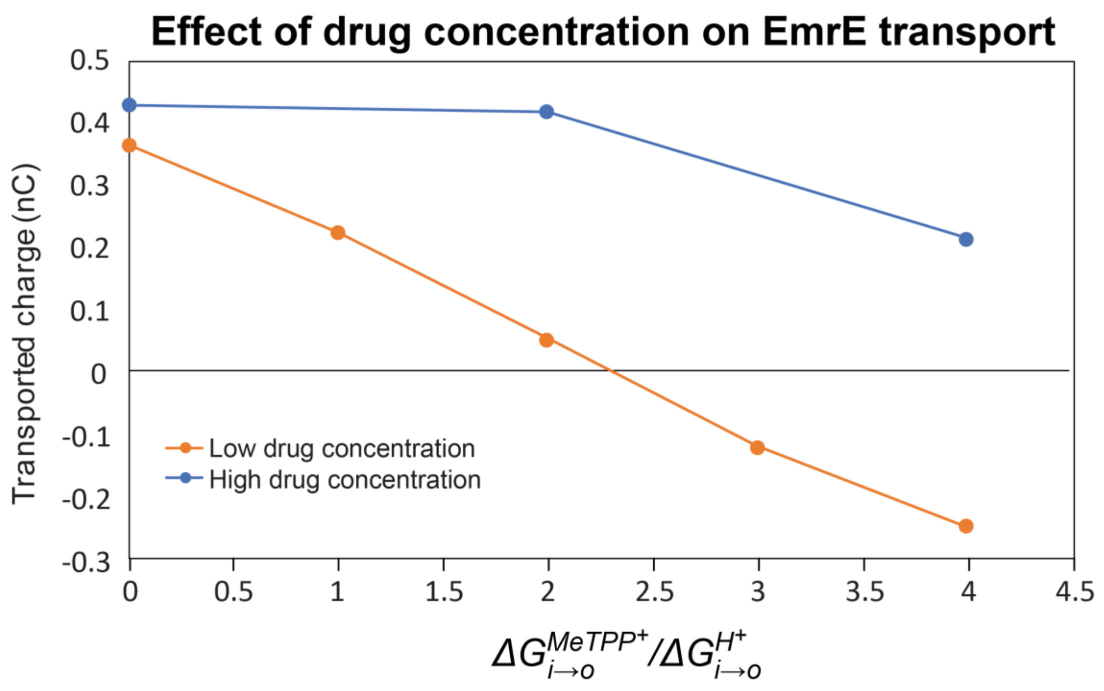
Sensor internal solution can be changed

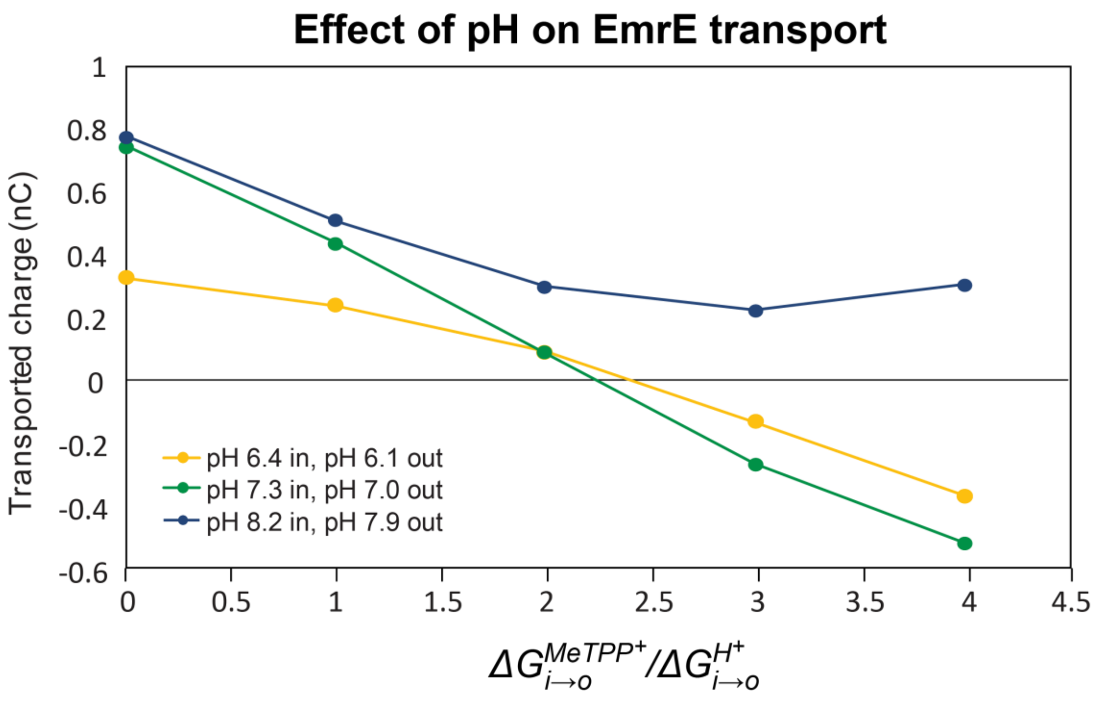
$R^2 = 0.999$

x-intercept = 2.02 ± 0.06



EmrE has variable stoichiometry





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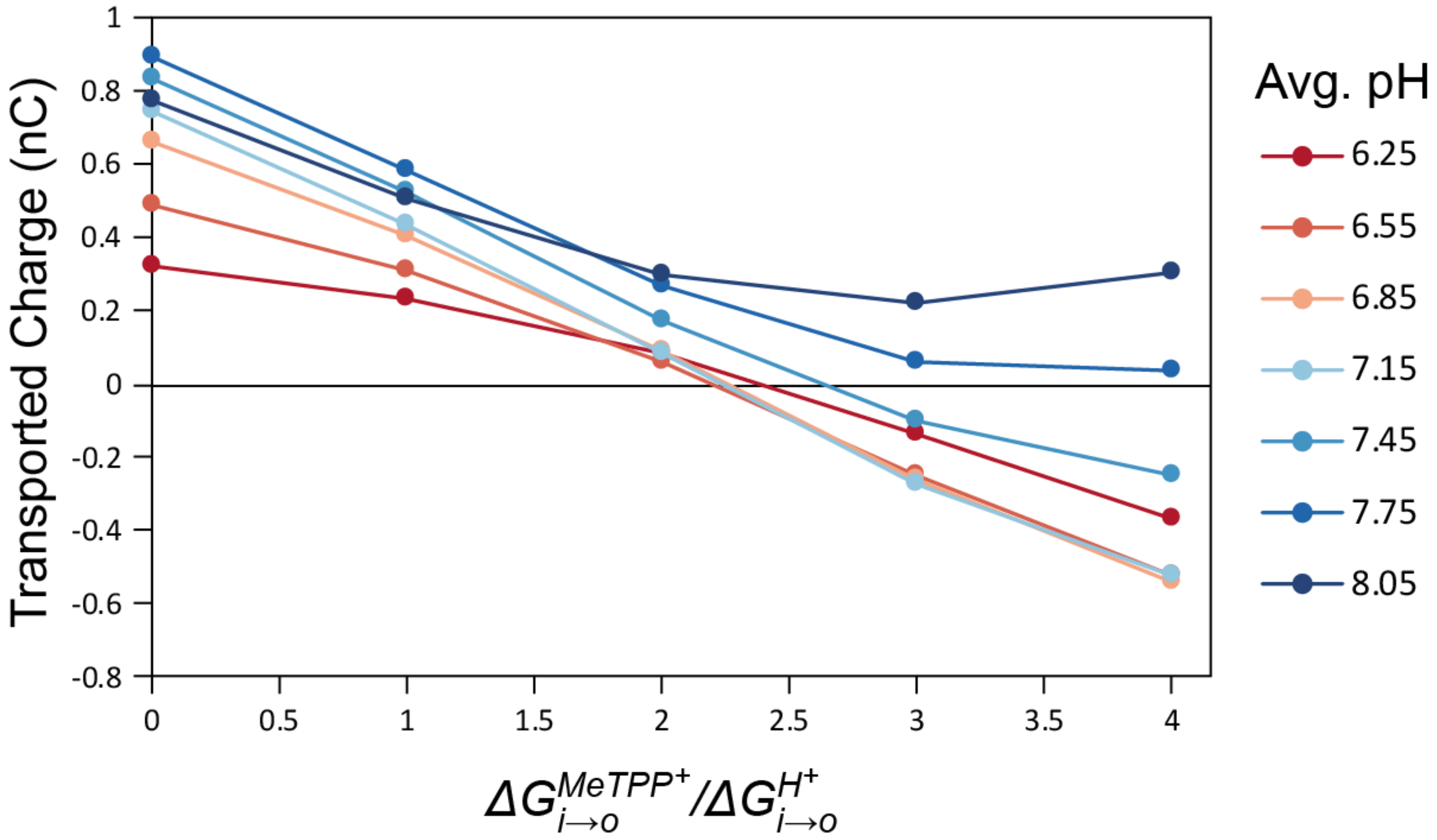

35 experimental conditions tested <3 hours, using <50 picomol protein

Advantages of SURFE^{2}R Reversal Potential

- No need for fluorescent probes or radioactive isotopes
- Much higher throughput – hundreds of measurements in a day
- Requires very little sample
  - 10^{-10} mol protein per sensor
  - Up to 100 measurements per sensor
  - Can measure multiple conditions on each sensor

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Nanion Technologies

Dr. Maria Barthmes
Dr. Andre Bazzone

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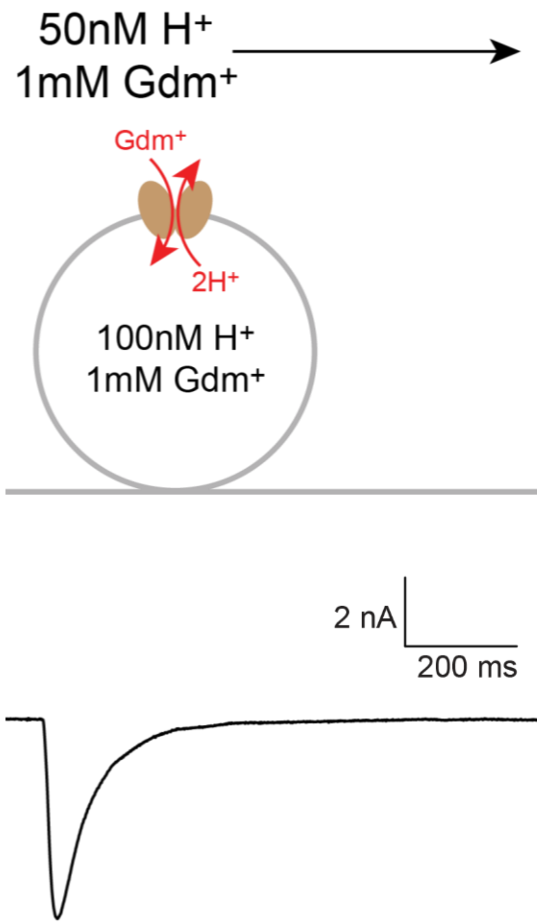
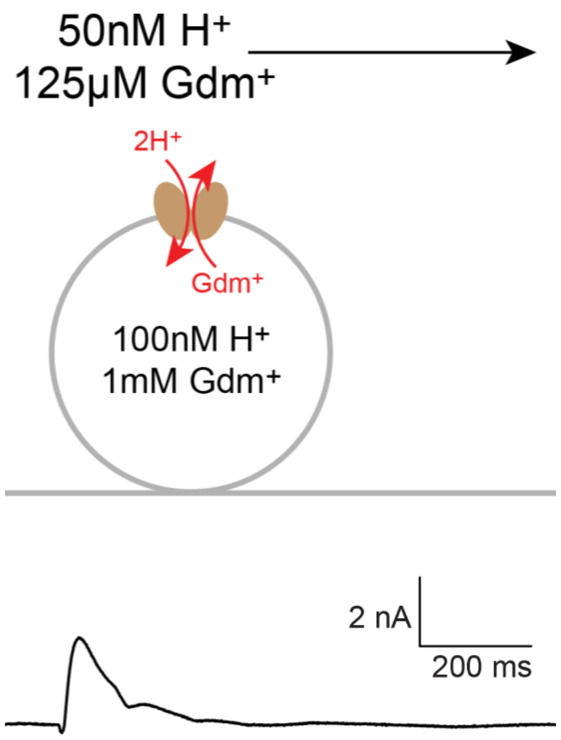
Nanion SURFE^{2}R N1 Grant 2018

SURFE^{2}R Reversal Potential





Derivation of reversal potential equation

For coupled antiport:

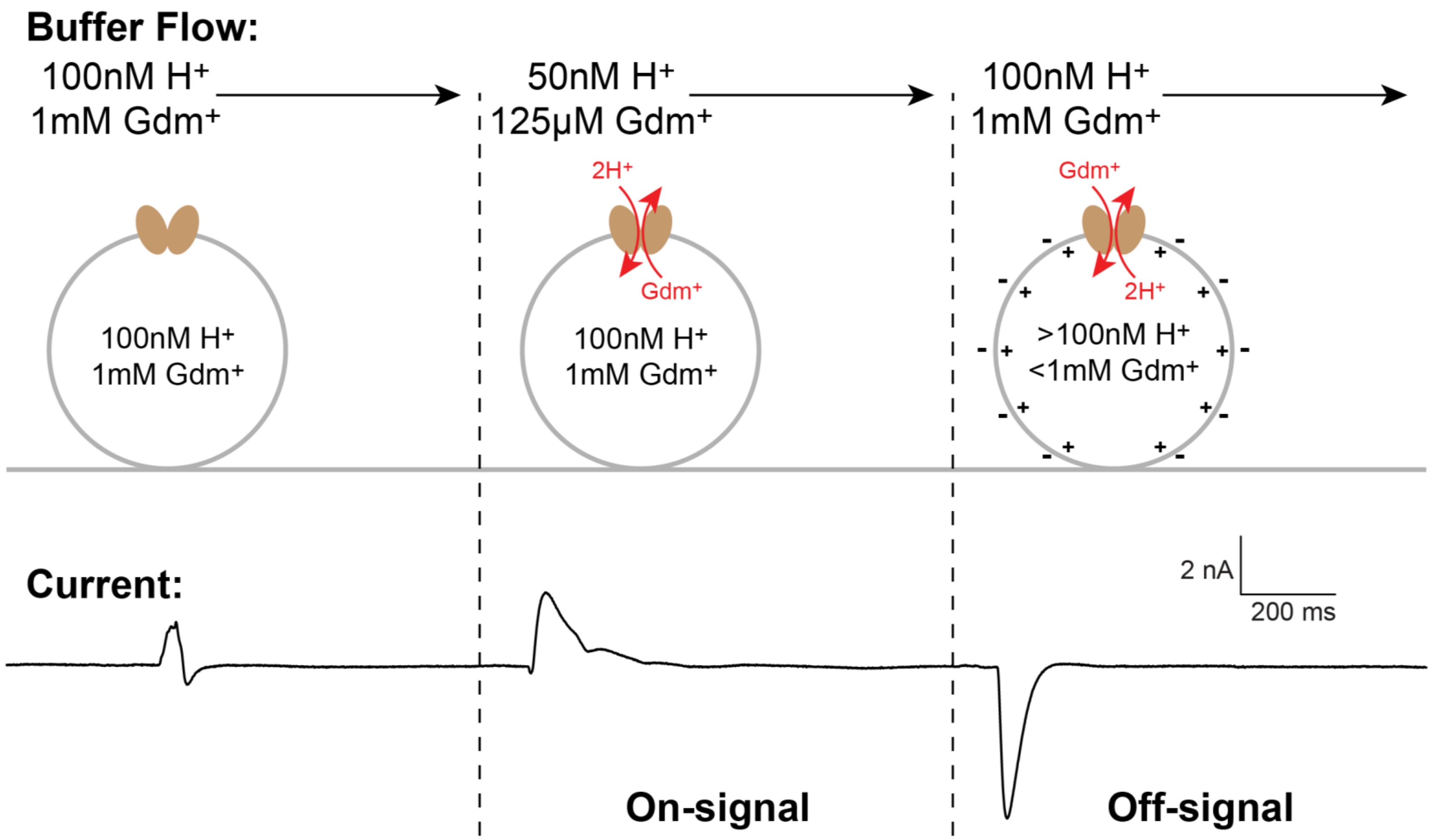
Eq. 1 $$nGdm^{+}_{i} + mH^{+}_{o} \rightleftharpoons nGdm^{+}_{o} + mH^{+}_{i}$$

Eq. 2 $$\Delta G_{antiport} = n\Delta G^{Gdm^{+}}_{i\to o} - m\Delta G^{H^{+}}_{i\to o}$$

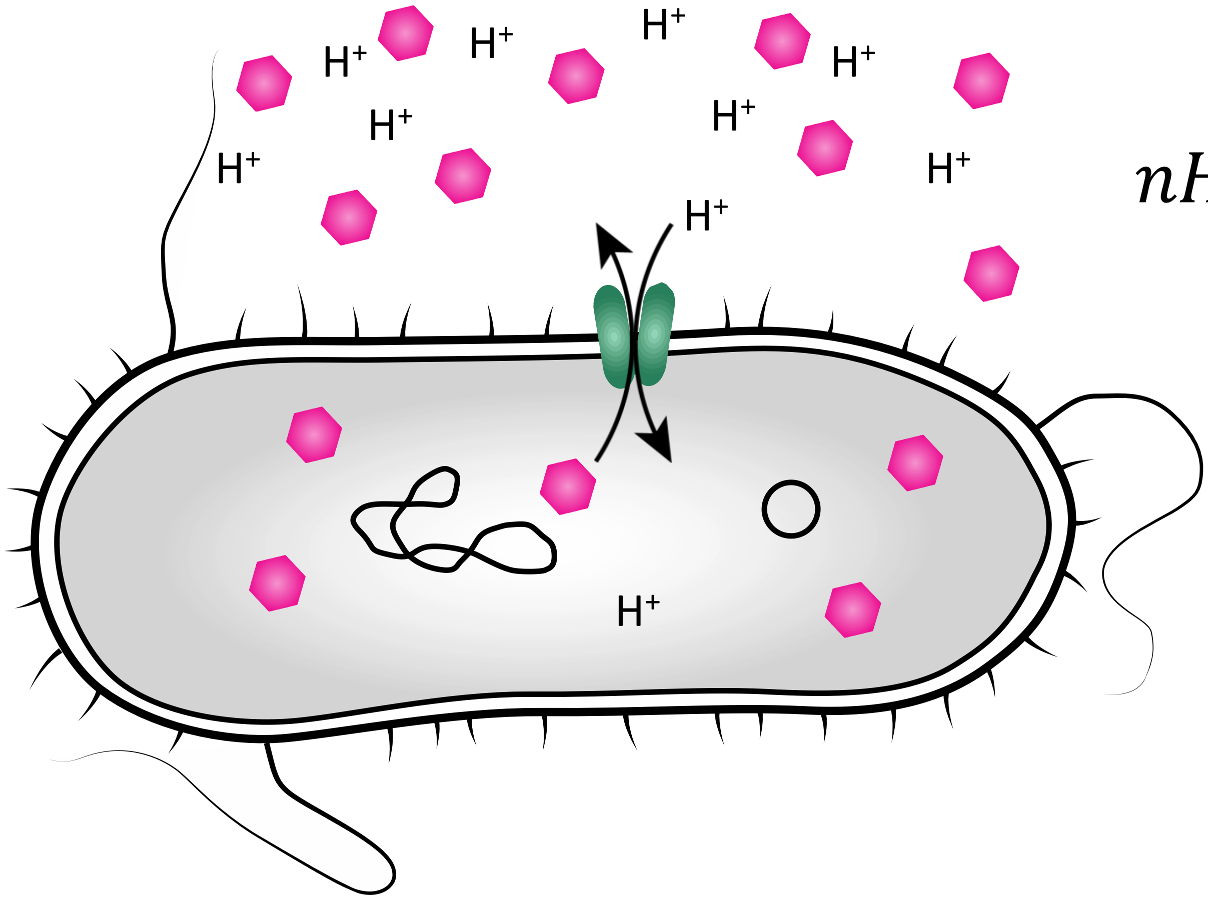
When $\Delta G_{antiport} = 0$,

Eq. 3 $$\frac{m}{n} = \frac{\Delta G^{Gdm^{+}}_{i\to o}}{\Delta G^{H^{+}}_{i\to o}}$$

Assay Scheme – Guanidinium gradient drives proton transport



Thermodynamics of proton coupled drug efflux



Antiport

$$nH^+_{out} + mDrug_{in} \rightleftharpoons nH^+_{in} + mDrug_{out}$$

$$\left(\frac{H^+_{out}}{H^+_{in}}\right)^n = \left(\frac{Drug_{out}}{Drug_{in}}\right)^m$$

E. coli **EmrE is a model drug/proton antiporter**

SSM Reversal Assay is quantitative