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High Throughput Mathematical Modelling with the SyncroPatch

Nanion User Meeting 2019

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How do you describe ion current kinetics?

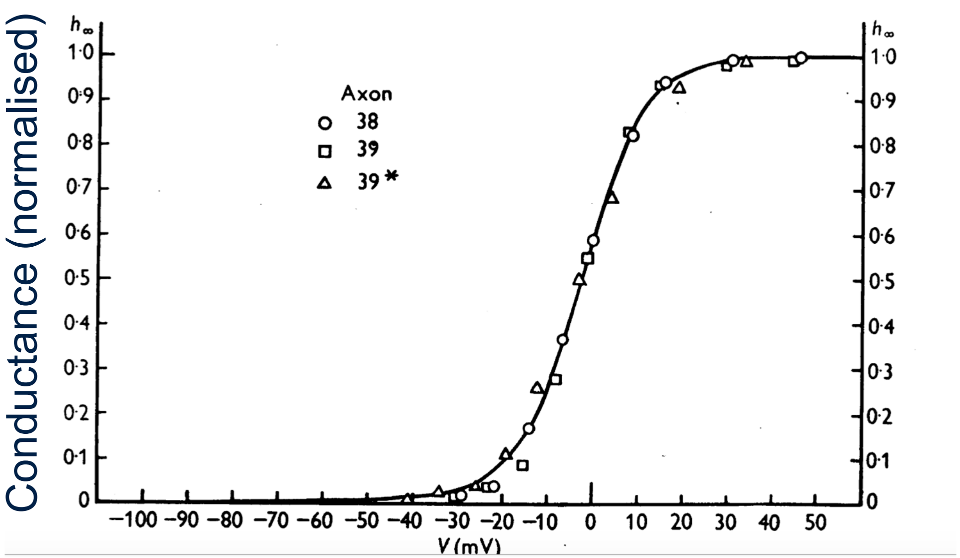
Describing Ion Current Kinetics

- Conventionally

- ~~Controversially?~~
- Conventionally!

518 *A. L. HODGKIN AND A. F. HUXLEY*

movement of a negatively charged particle which blocks the flow of sodium ions when it reaches the inside of the membrane. This is encouraging, but it must be mentioned that a physical theory of this kind does not lead to satisfactory functions for α_h and β_h without further *ad hoc* assumptions.



MEMBRANE CURRENT IN NERVE 519

$$\alpha_n = 0{\cdot}01\ (V+10)\Big/\left(\exp\frac{V+10}{10}-1\right),$$

$$\beta_n = 0{\cdot}125\ \exp\ (V/80),$$

$$\alpha_m = 0{\cdot}1\ (V+25)\Big/\left(\exp\frac{V+25}{10}-1\right),$$

$$\beta_m = 4\ \exp\ (V/18),$$

$$\alpha_h = 0{\cdot}07\ \exp\ (V/20),$$

$$\beta_h = 1\Big/\left(\exp\frac{V+30}{10}+1\right).$$

J. Physiol (1952) http://bit.ly/HH1952-updated

High throughput model building

High-information protocols

$$
\begin{tikzcd}[column sep=large, row sep=large]
(1 - r)(1 - a) \quad CI \arrow[r, shift left, "k_1"] \arrow[d, shift right, "k_3"'] & I \quad a\,(1 - r) \arrow[l, shift left, "k_2"] \arrow[d, shift right, "k_3"'] \\
r\,(1 - a) \quad C \arrow[r, shift left, "k_1"] \arrow[u, shift right, "k_4"'] & O \quad a\,r \arrow[l, shift left, "k_2"] \arrow[u, shift right, "k_4"']
\end{tikzcd}
$$

activation rate k_1

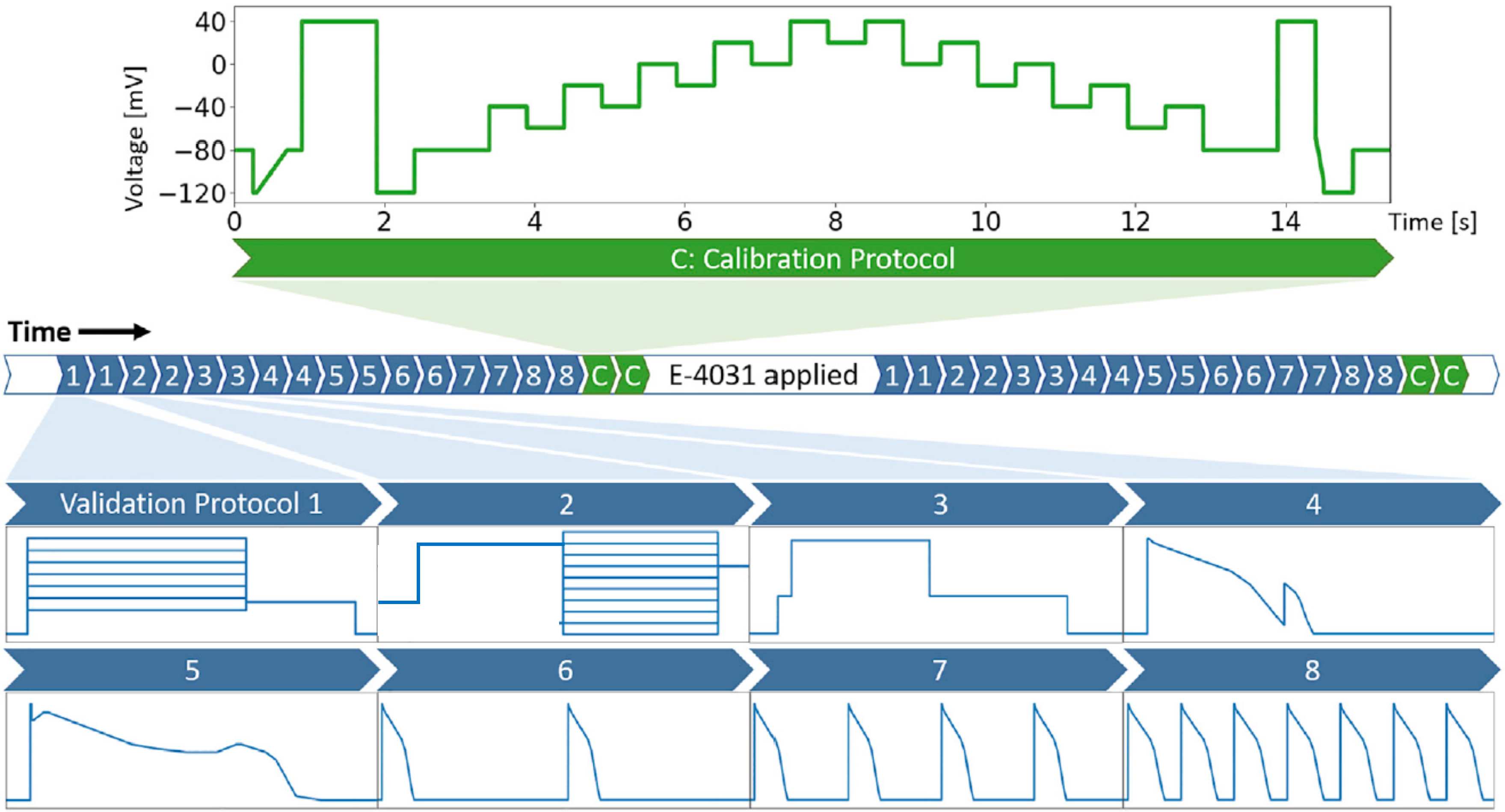
deactivation rate k_2

inactivation rate k_3

recovery rate k_4

$$
\begin{aligned}
k_1 &= p_1 \exp(p_2 V), & k_3 &= p_5 \exp(p_6 V), \\
k_2 &= p_3 \exp(-p_4 V), & k_4 &= p_7 \exp(-p_8 V).
\end{aligned}
$$

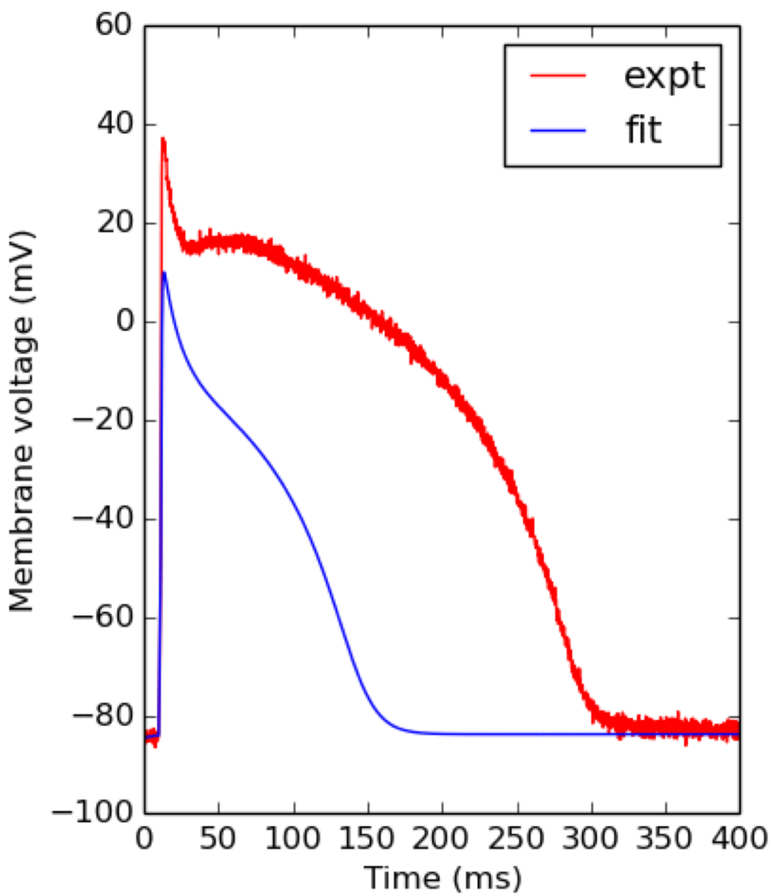
Voltage Clamp Protocols

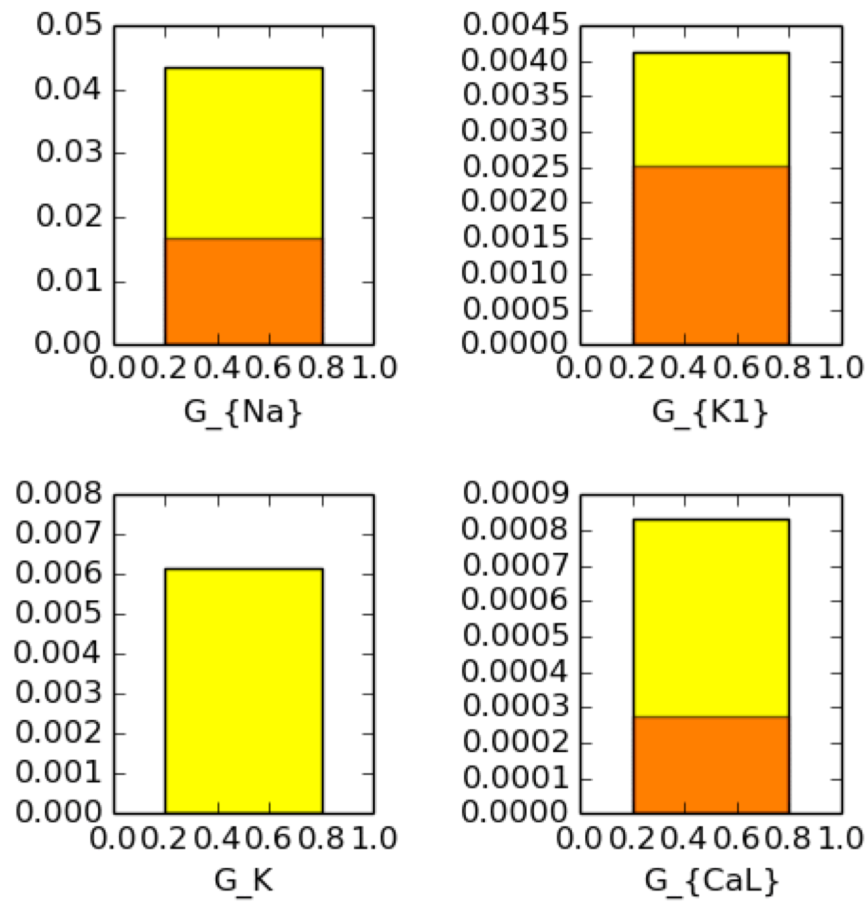


Applied in SyncroPatch 384PE to CHO cells stably expressing hERG1a at 25C

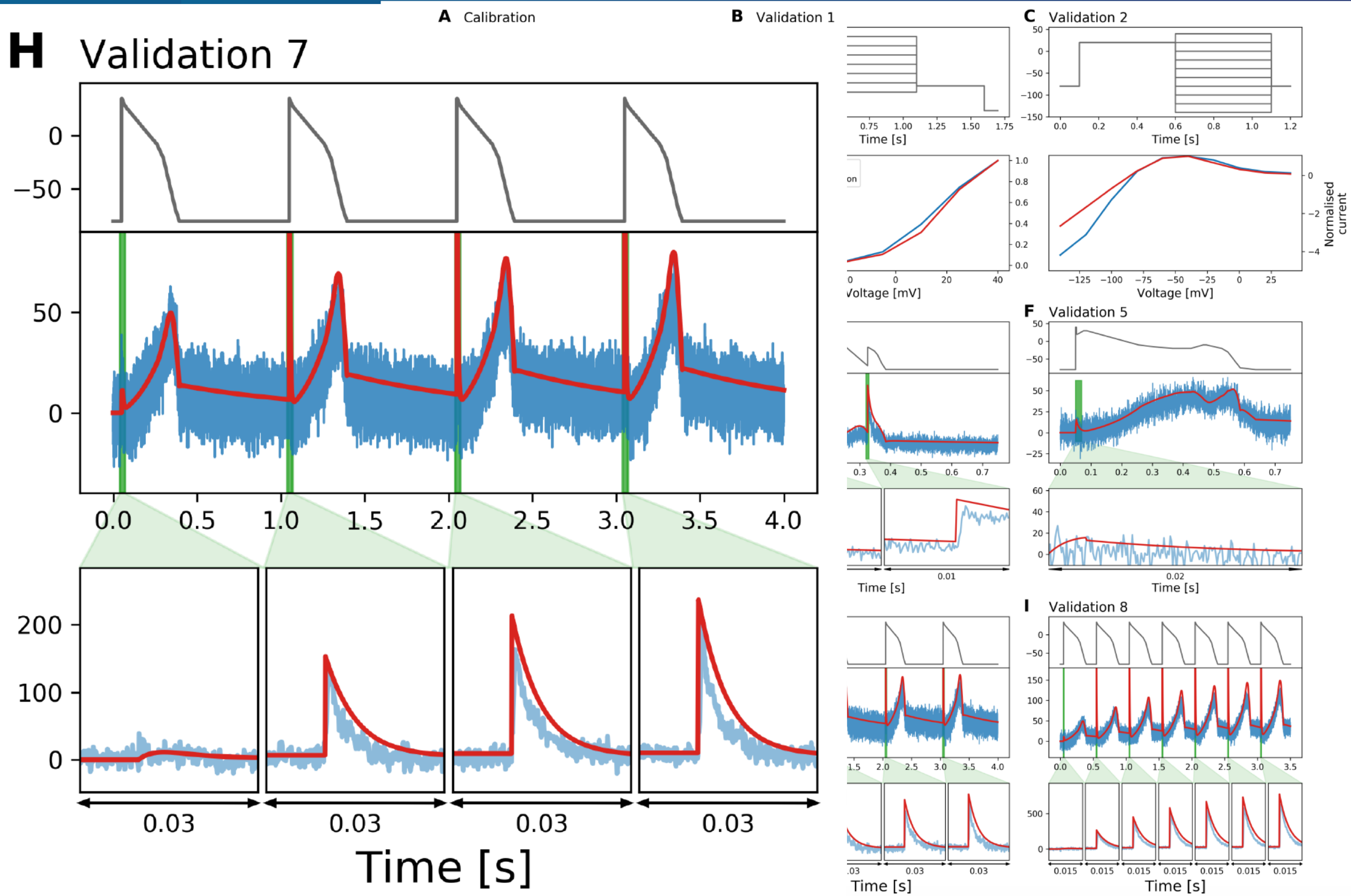
A (simpler) example of model fitting

Fitting four conductances to an action potential recording





An example of protocols recorded in a single well



Lei et al., Biophysical Journal, 117 (online ahead of print)

Enhanced Quality Control

Because we fit directly to the trace, it needs to be really accurate…

So we introduce more strict QC

Still left N=124 good wells out of 384 at room temp.

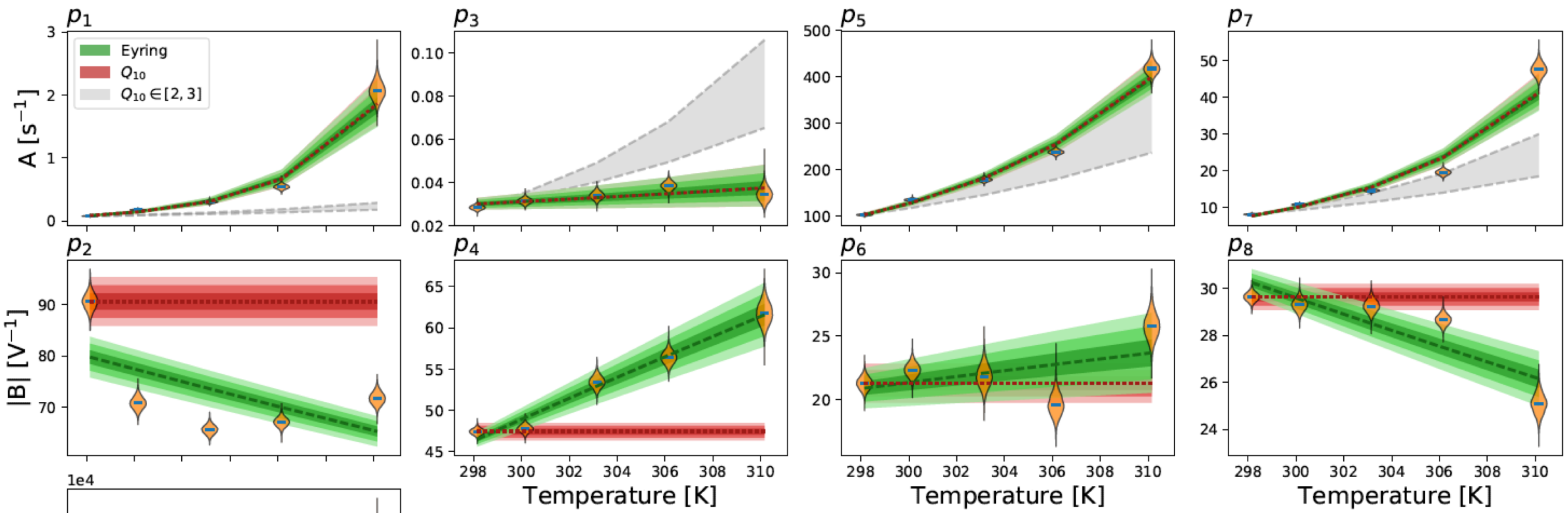
(slightly lower numbers at higher temps, but still tens)

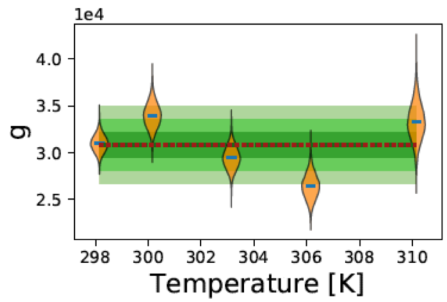
TABLE 1 A Summary of the Fully Automated Quality Control Criteria for the Staircase Protocol, QC1–QC6

QC Name	Criterion Description
QC1.Rseal	Check R_{seal} within [0.1, 1000] $G\Omega$.
QC1.Cm	Check C_m within [1, 100] pF.
QC1.Rseries	Check R_{series} within [1, 25] $M\Omega$.
QC2.raw	Check raw trace recording SNR is over 25 (SNR defined as var(trace)/var(noise)).
QC2.subtracted	Check subtracted trace SNR > 25.
QC3.raw	Check 2 sweeps of raw trace recording are similar by comparing the RMSD of the two sweeps $<$ mean(RMSD to zero of the two sweeps) $\times$ 0.2.
QC3.E4031	Check 2 sweeps of E-4031 trace recording are similar (same comparison as QC3.raw).
QC3.subtracted	Check 2 sweeps of subtracted trace recording are similar (same comparison as QC3.raw).
QC4…	Check R_{seal}, C_m, R_{series}, respectively, before and after E-4031 change (defined as std/mean) < 0.5.
QC5.staircase	Check the maximum current during the second half of the staircase changes by at least 75% of the raw trace after E-4031 addition.
QC5.1.staircase	Check RMSD to zero of staircase protocol changes by at least 50% of the raw trace after E-4031 addition.
QC6.subtracted	Check the first step up to $+40$ mV, before the staircase, in the subtracted trace is bigger than $-2\times$ estimated noise level.
QC6.1.subtracted	Check the first $+40$ mV during the staircase, with the same criterion as QC6.subtracted.
QC6.2.subtracted	Check the second $+40$ mV during the staircase, with the same criterion as QC6.subtracted.

RMSD, root mean-square difference; SNR, signal-to-noise ratio; std, standard deviation; var, variance.

Temperature dependence





	ΔS ($JK^{-1}mol^{-1}$)	ΔH ($Jmol^{-1}$)	z_e	D (V^{-1})
k_1	389.543	195330.0	9.59193	-293.601
k_2	-235.457	11514.1	9.98987	-435.358
k_3	75.3932	84017.4	-1.85611	93.1319
k_4	125.715	105487.0	-2.71481	75.4196

	Q_{10}	α (s^{-1})	β (V^{-1})
k_1	13.1283	0.0843731	90.5512
k_2	1.19849	0.030066	-47.4353
k_3	3.0847	103.436	21.2742
k_4	4.08594	7.62406	-29.6366

Figure S9. Fitting of Generalised Eyring equation and Q_{10} equation to the mean distribution μ inferred using the simplified psuedo-MwG (orange violin plot). The obtained Generalised Eyring fits are shown as green fan charts with the first three standard deviations in green; the obtained Q_{10} fits are shown in red. The fitted parameters for the Generalised Eyring and Q_{10} equations are shown in the bottom right tables, one set for each k_i, $i = 1, 2, 3, 4$. For comparison to typical Q_{10} values in literature, where Q_{10} values are usually assumed to be around 2 to 3, we show the parameters prediction using $Q_{10} \in [2, 3]$ as the grey shaded region.

SEE CHON'S POSTER IN COFFEE ROOM!

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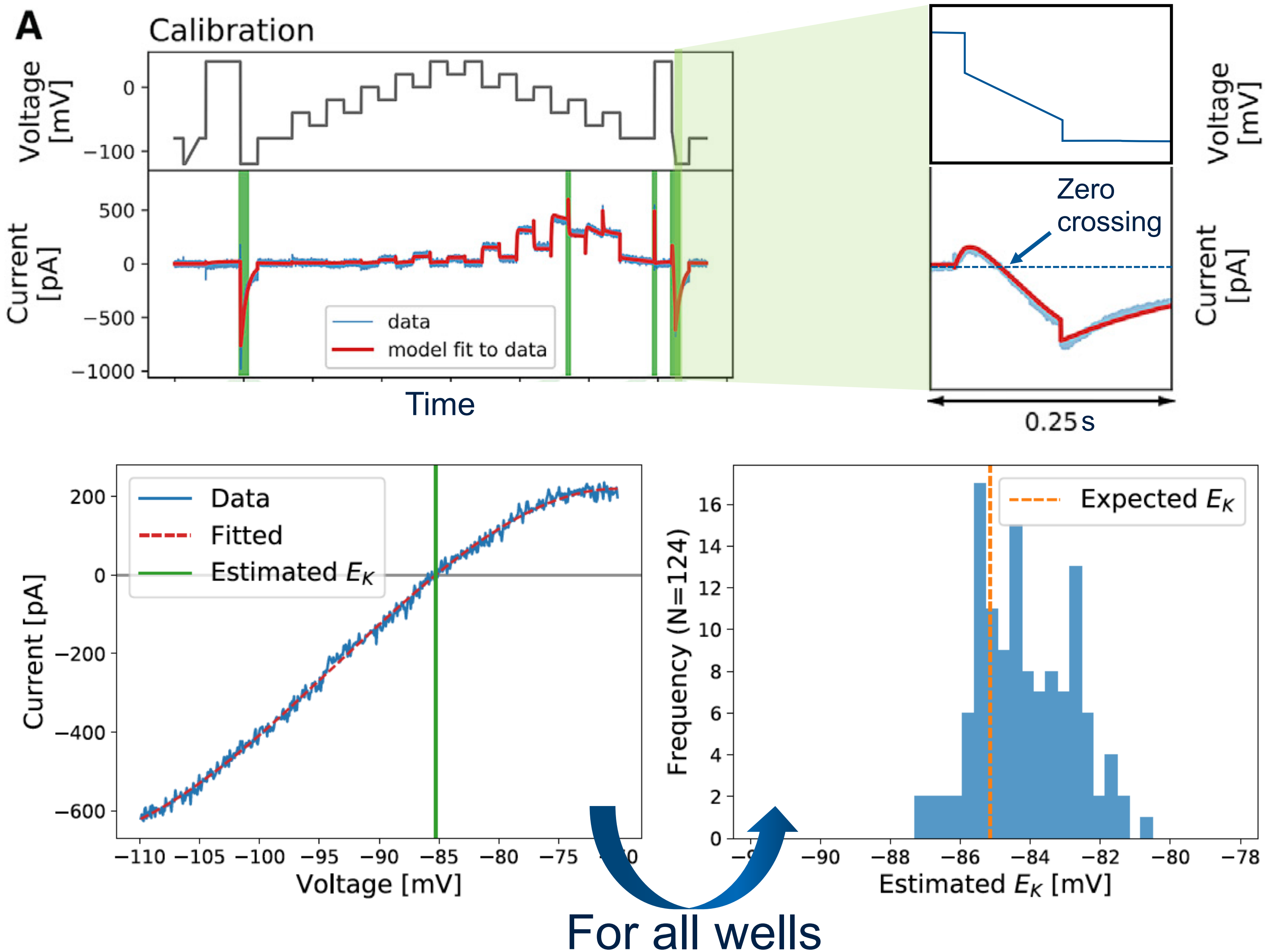
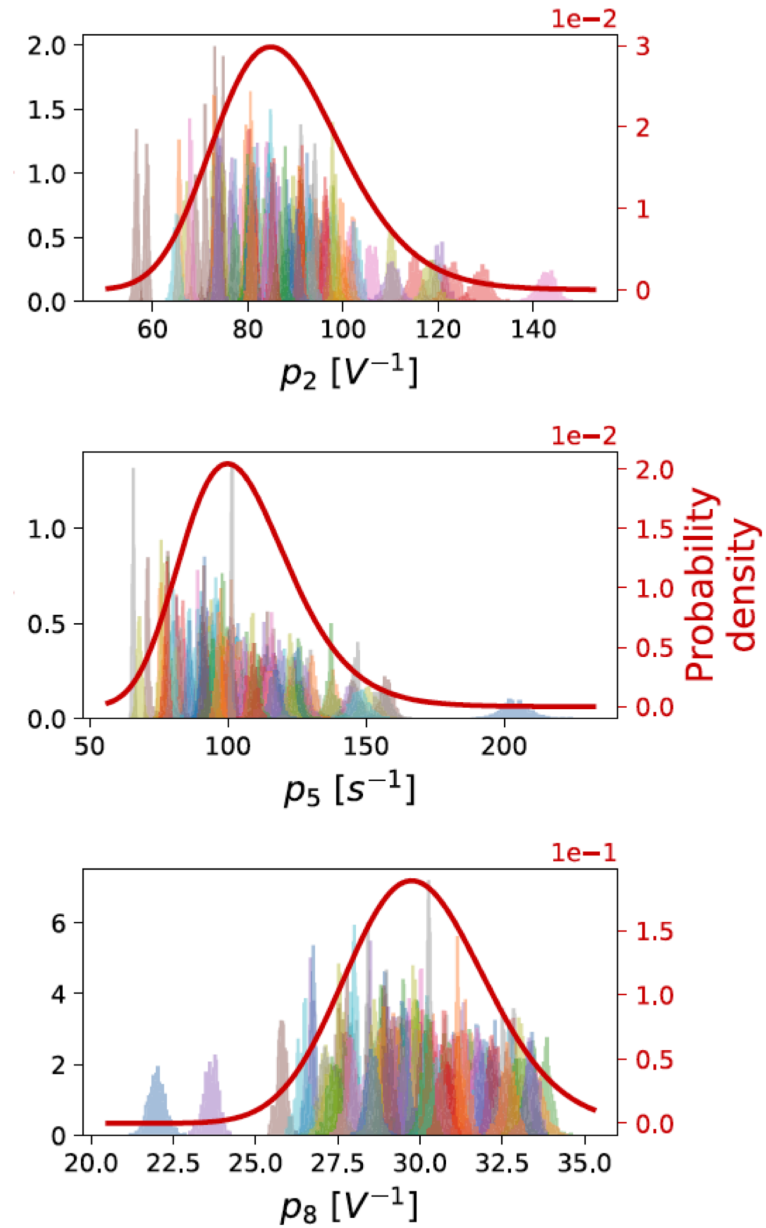
The Reversal Ramp and Variability

The “Reversal Ramp”



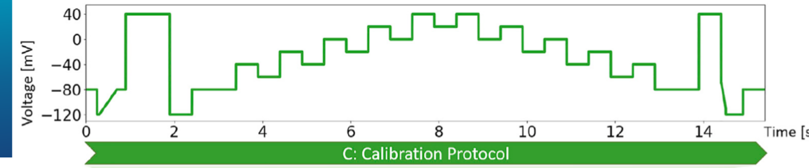
Sources of variability

- We saw a fair bit of variation in recordings and subsequent parameters in the models
- The ramp hinted this is due to slightly different patch artefacts / imperfect compensations and leak subtraction in each well.
- We can include artefacts in a larger mathematical model of what the amplifier does, to get consensus kinetics across all wells.
- Even better fits and predictions when we do this (not shown!).

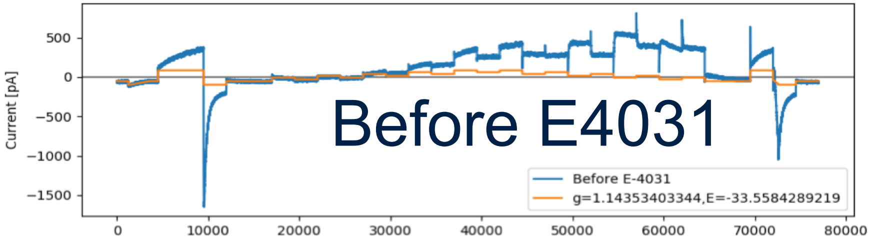


Nonlinear leak

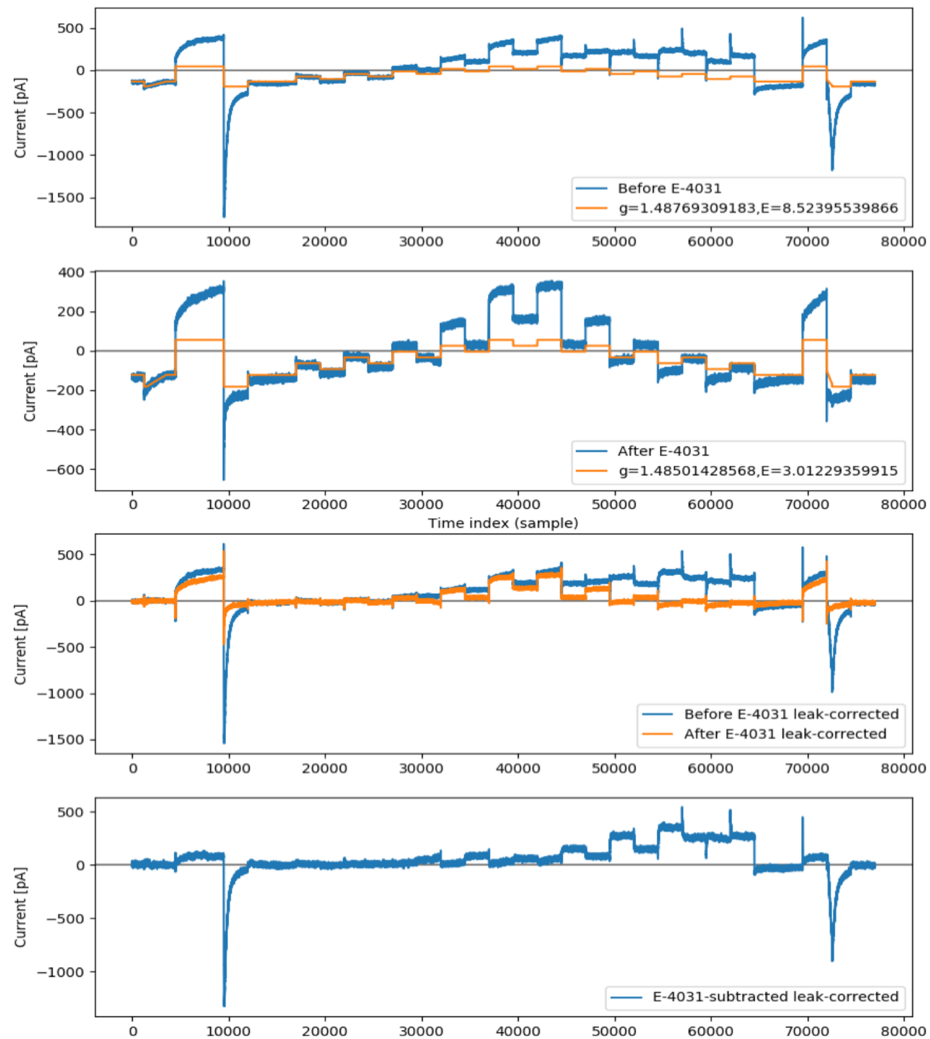
hERG measurements



Our measurements at Roche (Lei et al. 2019)



Monique, Adam & Jamie's measurements (Victor Chang, Sydney)



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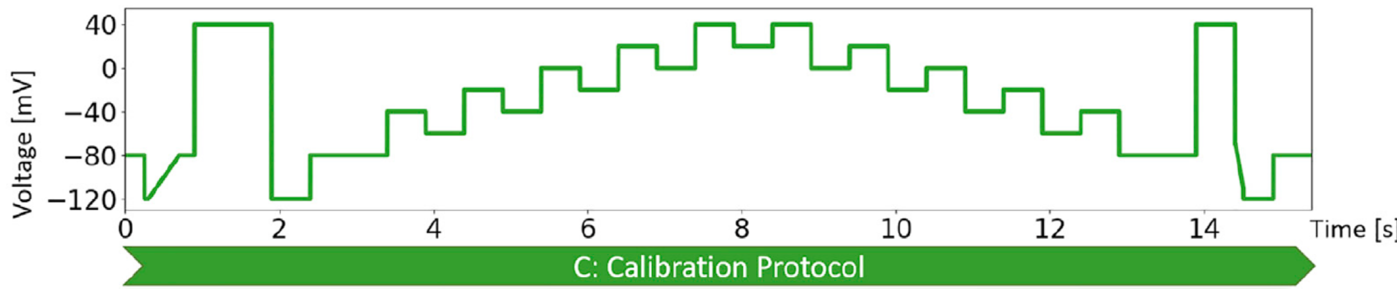
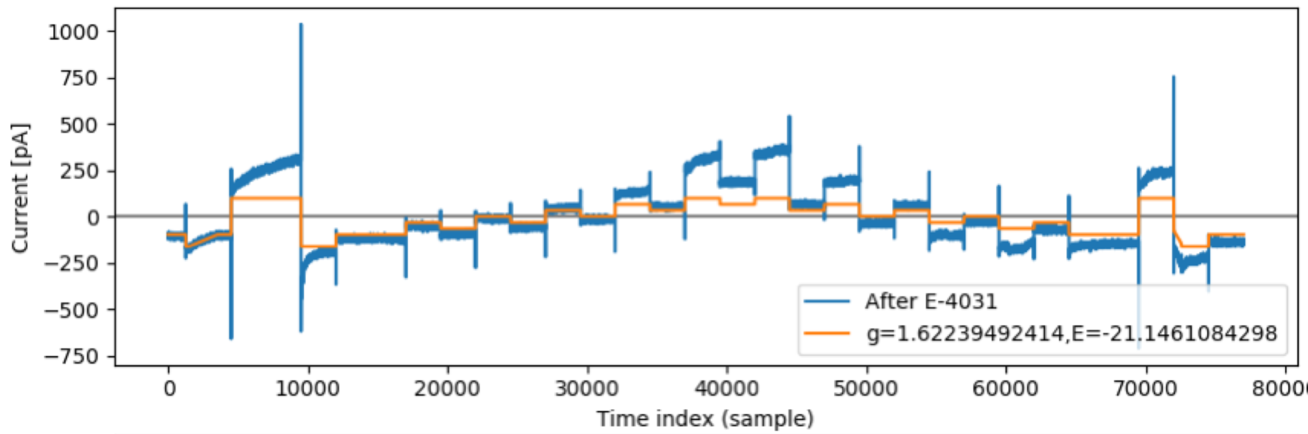
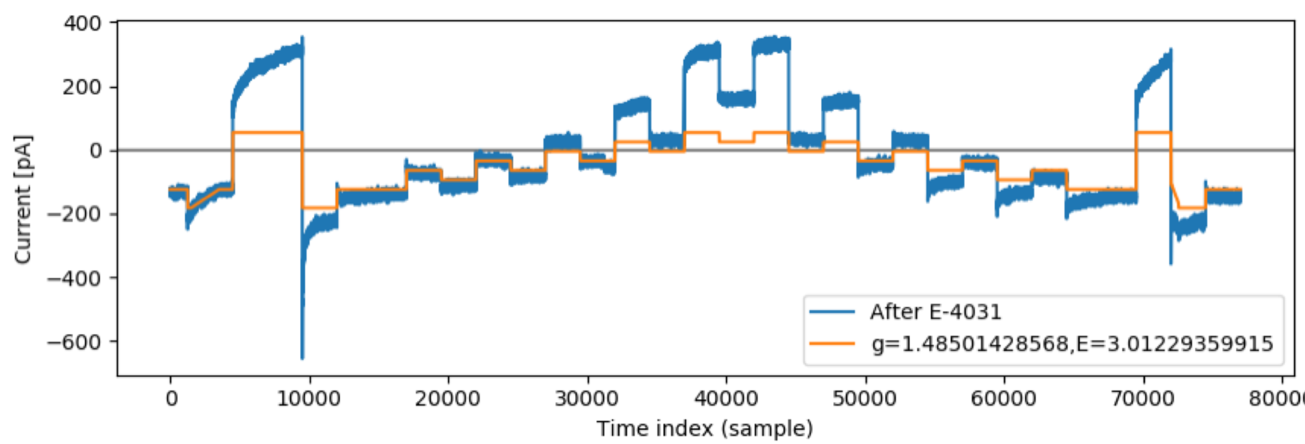
Post E-4031 (hERG blocker)



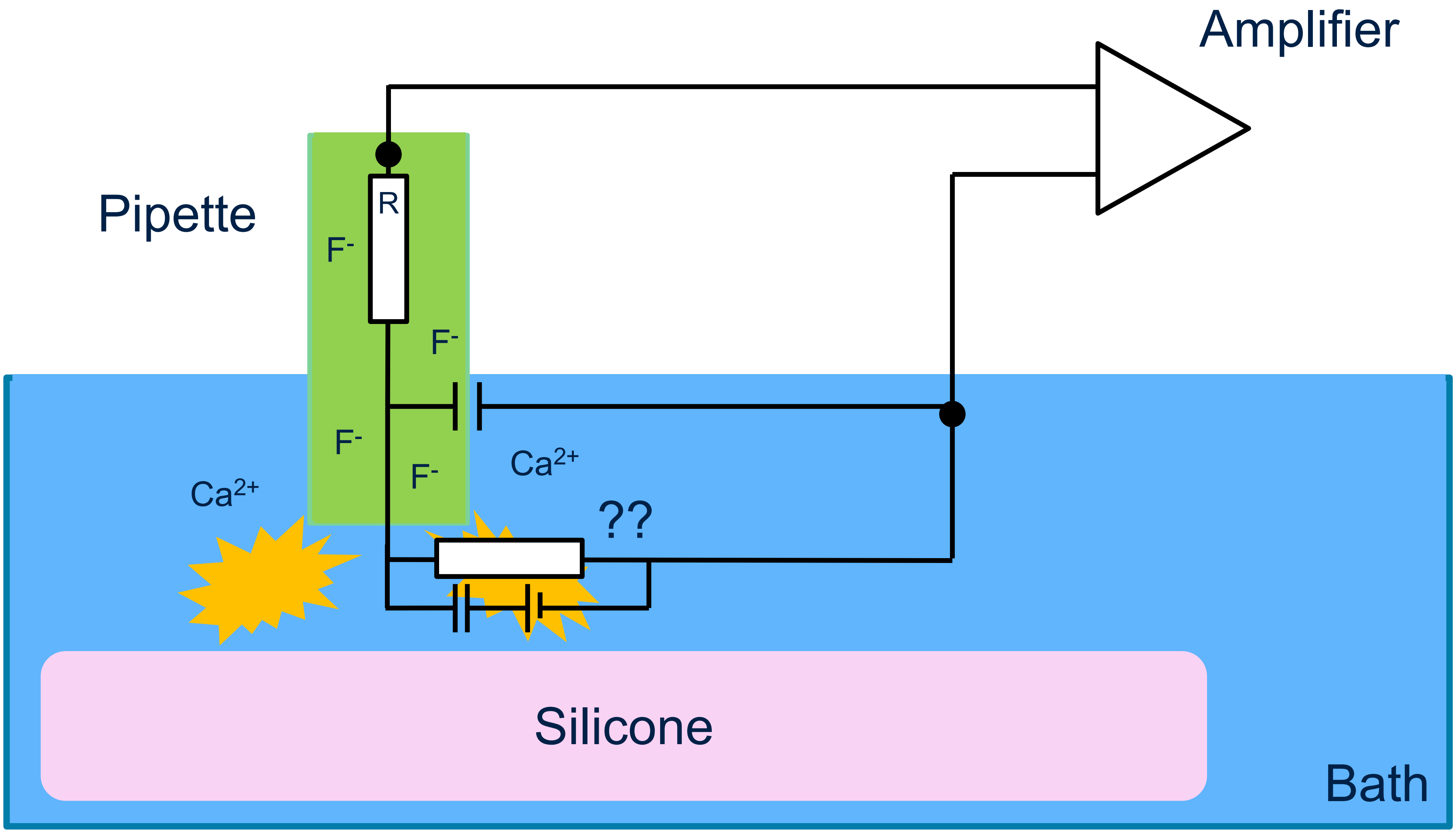
Ours at Roche
(Lei et al. 2019)



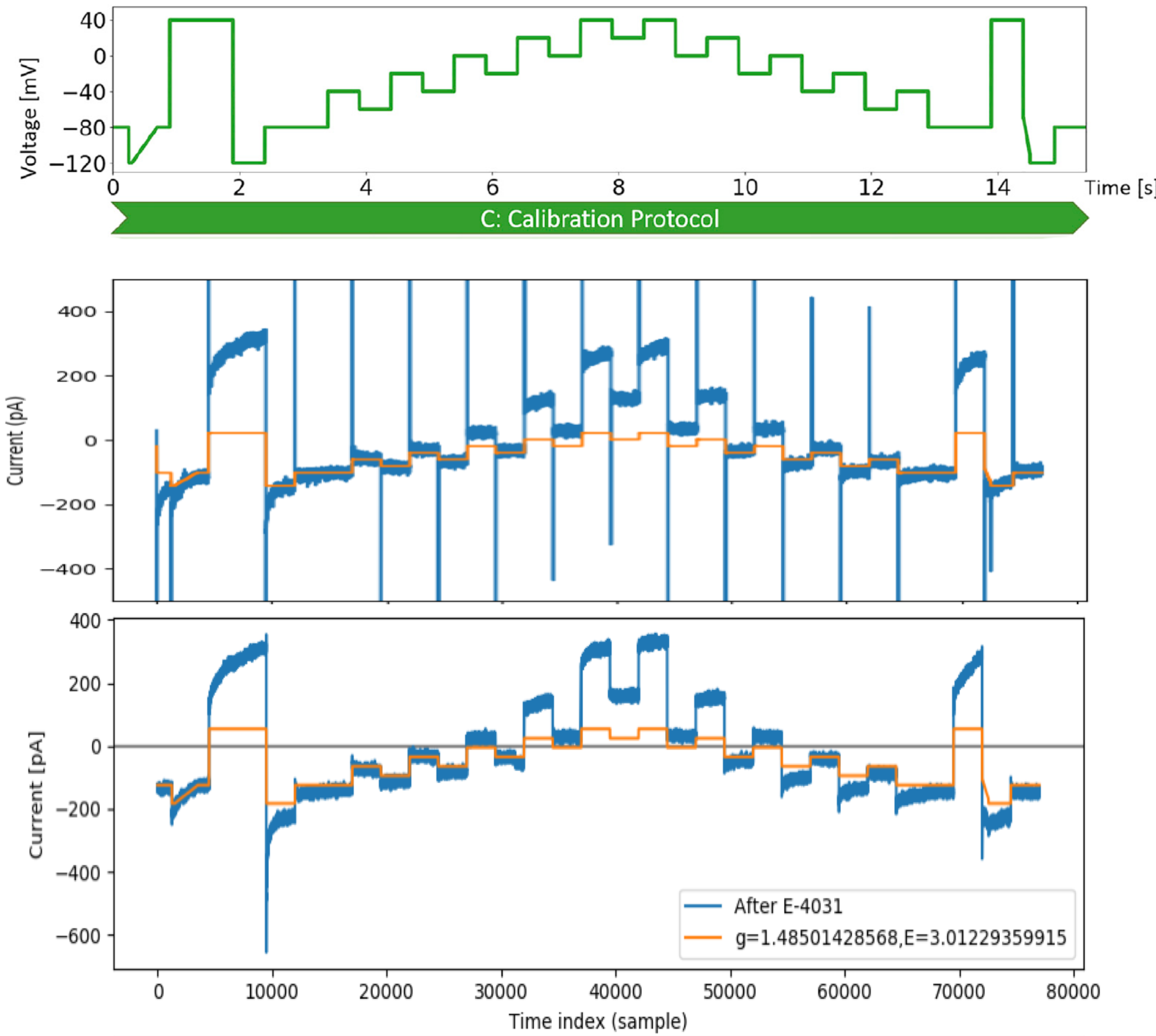
Victor Chang
measurements



Manual patch experiment



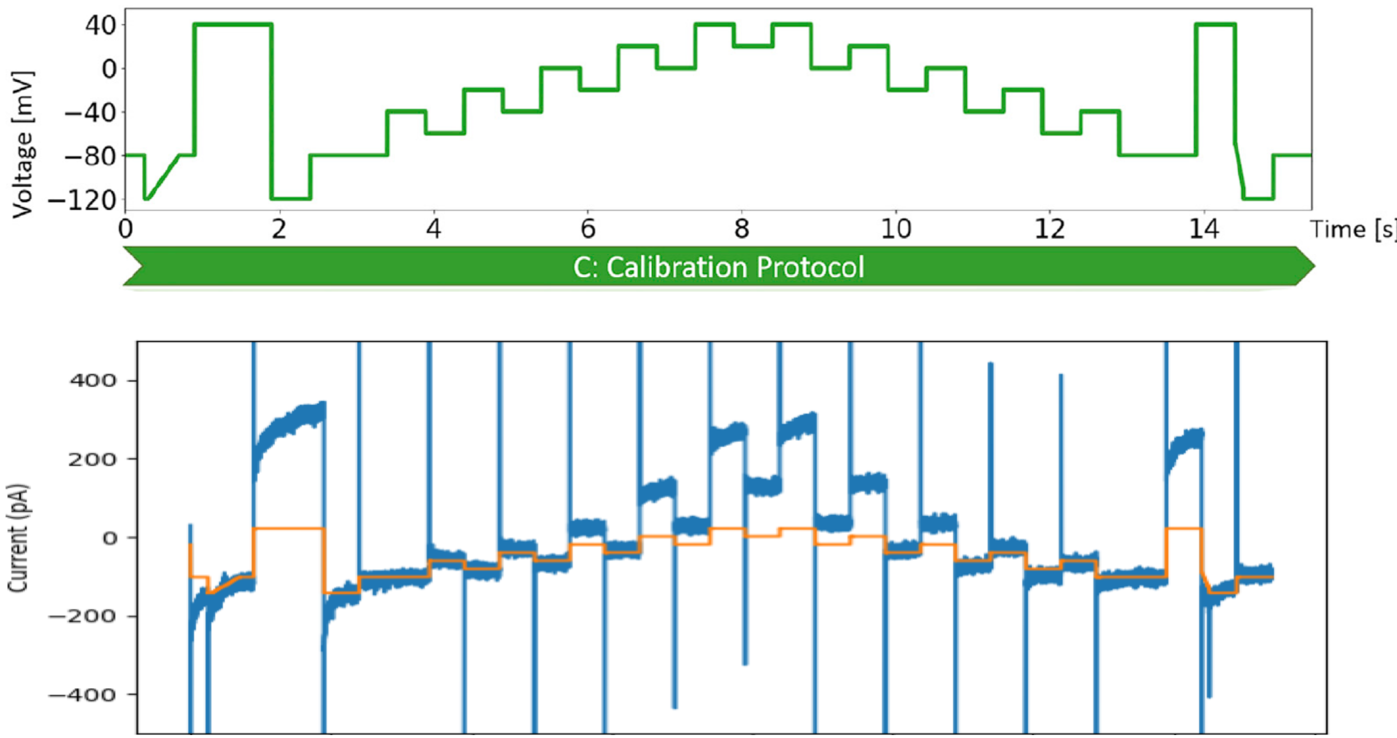
Definitely not endogenous currents!



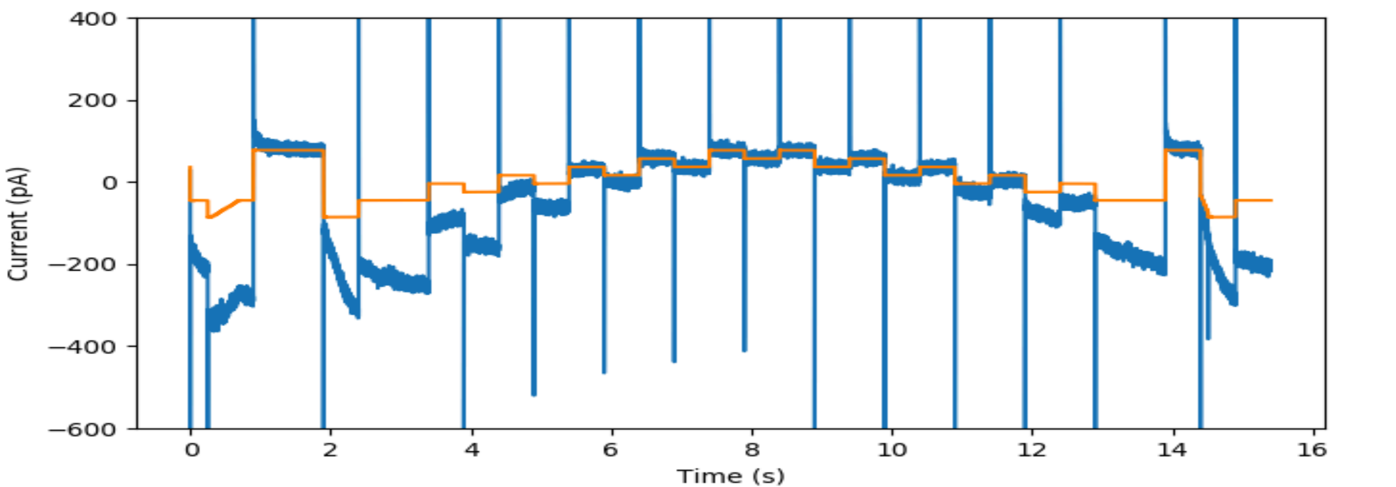
Silicone experiments (in manual patch)

Victor Chang Measurements (with cell, after hERG block)

Definitely not endogenous currents!

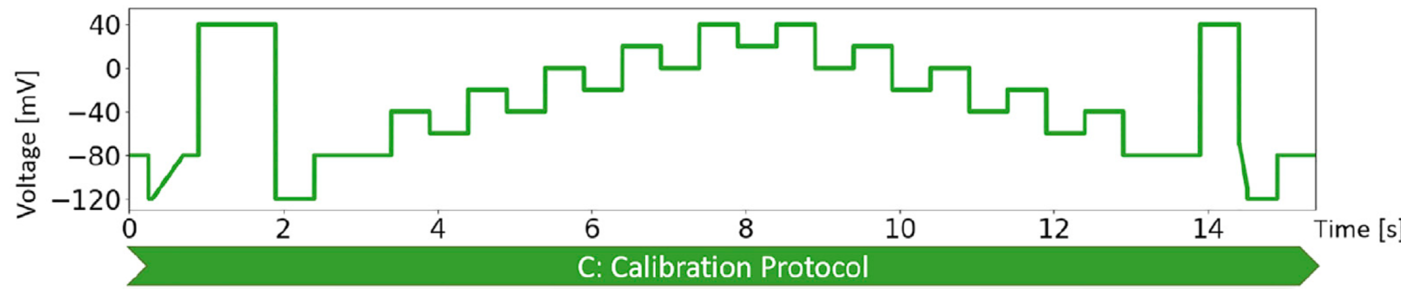


Silicone manual leak experiments

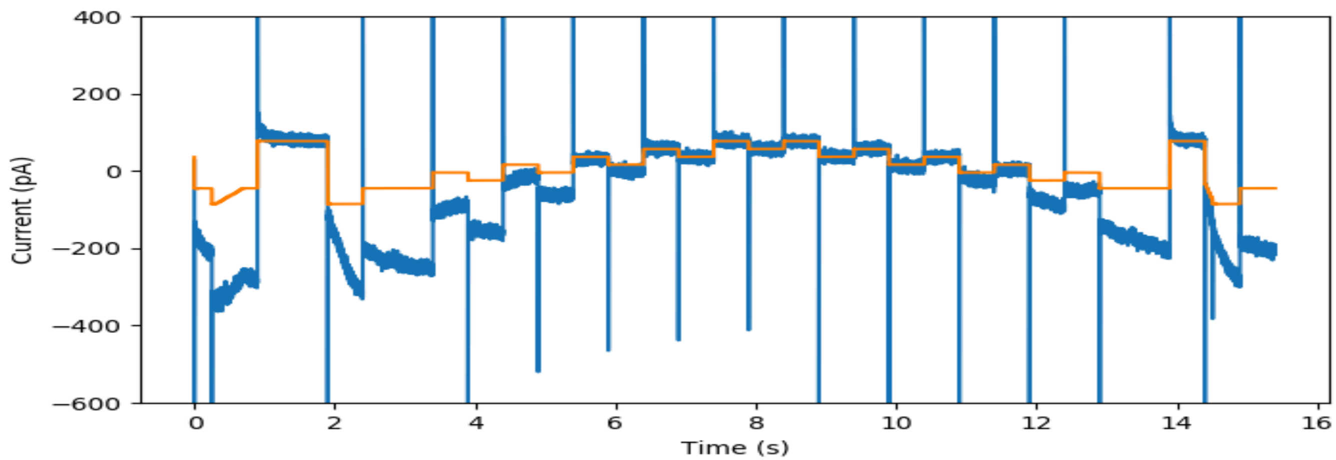


And if you swap pipette and bath solutions!

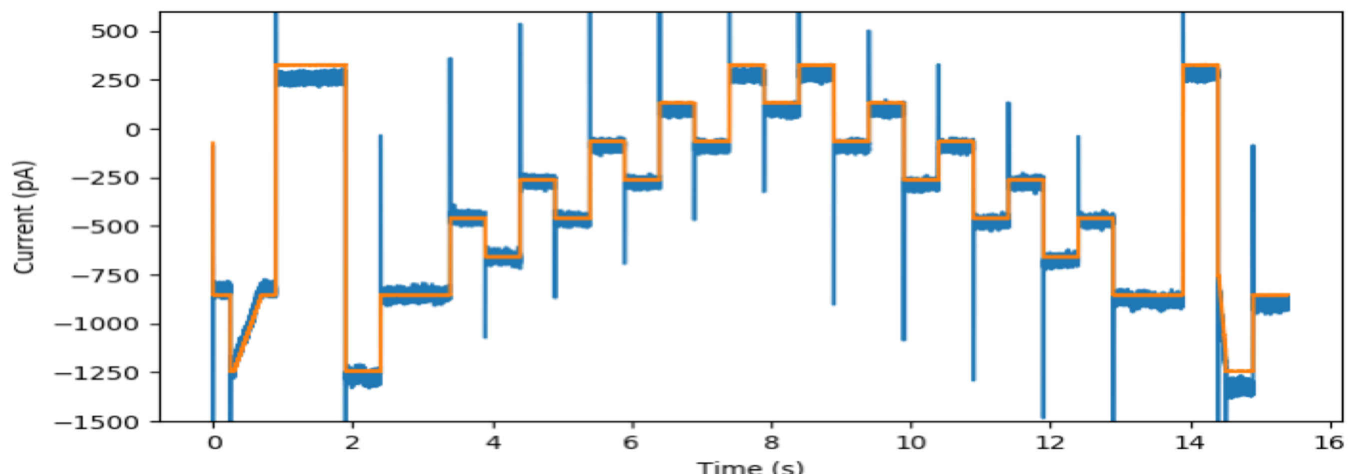
So it must be the seal enhancer solution…



Swapped pipette and bath solutions



Wash out bath and replace with manual solution (No F^-)



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3yr Research Fellow Job Available!

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Solutions

	Ca^{2+} External (mM)	F^- Internal (mM)
Our Study (Roche)	2.05	100
Ng et al. hERG variants (Victor Chang)	2	110
Kang et al. K_v2.1 variants (NorthWestern)	2	60