Virtual NEURON: a Strategy for Merged Biochemical and Electrophysiological Modeling

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S1 Supplemental Text

S1.1 Reduction Methods

Our first attempt at parameterizing our own reduced model to account for the decrease in surface area took into account the generally parallel nature of branching in neurons. Though we did not require that dendrites are mapped to equivalent cylinders based on electrotonic distance from the soma, we initially used the method developed by Bush and Sejnowski (Bush and Sejnowski, 1993) to determine the length and radius of each equivalent cylinder. The length of an equivalent cylinder was calculated as the average length of all the dendrites to be represented by the cylinder. The average radius of each equivalent cylinder was calculated as the square root of the sum of the square of the radii of all the dendrites to be reduced to that cylinder. Using these formulae for equivalent cylinder radius and length, Bush and Sejnowski manipulated the surface area of the neuron, and did not attempt to conserve it as Rall and others had done (Rall and Agmon-Snir, 1998). They instead conserved axial resistivity (R_a) and the RC time constant (τ), as well as input resistance (R_{in}). They iteratively scaled down membrane resistance, R_m, in their reduced model of a pyramidal neuron until R_{in} in their reduced model matches R_{in} in their full model. They calculated the resulting scaling factor and used it to scale up the capacitance to conserve τ between the two models. Conceptually, scaling the membrane resistance down and scaling the membrane capacitance up corrects for the decreased surface area of the reduced model, relative to the full model. Indeed, Bush and Sejnowski found that taking the ratio of the surface area of the full model to the surface area of its reduced counterpart yielded a value very close to their scaling factor.

Reduced model scaled by 5.7. Following their system, we determined the ratio of the surface area of our full complex Purkinje geometry to the surface area of the reduced geometry to be approximately 5.7. The membrane resistance in all of the compartments in the reduced model was scaled down by this factor. The membrane capacitance was scaled up by 5.7, to preserve the membrane time constant between the full and reduced models, to be consistent with the method developed by Bush and Sejnowski (Bush and Sejnowski, 1993). Current injection in this version of the reduced model depolarized the soma to a plateau potential of approximately -44.6mV (Supplemental Fig. S2*b*). The R_{in} in this model is smaller than that of the full model, since the soma in the full model depolarized to -37.5mV (in Fig. 2*a*) from a resting potential of -67.8mV. This suggests that the appropriate scaling factor for matching R_{in} in the reduced model to the R_{in} of the full model may not solely or strictly depend on the ratio of the entire surface areas of the full and reduced geometries. In this case, it seems that scaling R_m by this surface area ratio underestimates the input resistance of the reduced model.

Reduced model scaled by 4.5 (Bush-inspired model). In our second attempt to correct for the reduction in surface area, we iteratively increased R_m until R_{in} in the reduced model matched the R_{in} of the full model, as originally attempted by Bush and Sejnowski. The resulting calculated scaling factor was approximately 4.5. We used this scaling factor to adjust C_m in all of the compartments of the reduced model. Current injection in this model (Supplemental Fig. S2*c*) gave the same input resistance as in the PPR model and in the full model (Fig. 2*a*). Next, we added channels to the soma and

applied the same current injection. The channels retained the same conductances in the reduced model as in the full version of the model. An action potential with an amplitude of 41mV was obtained, followed by a plateau potential of -54mV (Supplemental Fig. S3*a*), similar to the PPR model and the full model (in Fig. 2*b*). However, the single action potential in the Bush-inspired model slightly lagged the action potential in the other two models. Results in the Bush-inspired model diverge from those in the full model, when active conductances are added to the dendrites (Supplemental Fig. S3*b*). A single action potential is obtained, followed by a plateau at approximately -54mV for the duration of the current pulse. We then scaled the ion channel conductances in the equivalent cylinders by 4.5. Supplemental Figure S3*c* shows that action potentials are still not obtained with current injection. For the rest of this section, we will refer to this model with ion channel conductances scaled in the equivalent cylinders as the *Bush-inspired model*. Analyzing the resting potential for each model indicates that the Bush-inspired model (Supplemental Fig. S3*c*) is hyperpolarized, relative to the full model (Fig. 2*c*) and the PPR model (Fig. 2*d*). This could explain why the Bush-inspired model gives a suppressed action potential pattern relative to both the PPR model and the full model, given that it is more difficult to fire action potentials in a hyperpolarized cell. When calcium influx is appropriately scaled down in this model, the Bush-inspired model fires a single action potential, followed by a gradual depolarization, which results in action potential firing with transient plateaus during the last 100ms of the current pulse (Supplemental Fig. S3c).

Reduced model scaled by 10. We then determined a new scaling factor based on the ratio of the surface areas not of the entire geometry in the full and reduced models, but only of the dendrites that will actually be reduced and their resulting equivalent cylinders. That new scaling factor was approximately 10. This version of the reduced model (Supplemental Fig. S2*d*) does not reproduce the result in the full model (Fig. 2*a*). In fact, it appears that the input resistance in this reduced model is smaller than the input resistance in the full model. Since the core resistivity, R_a, is conserved, the resistance in the dendritic tree depends on the distribution of lengths and radii of the dendrites. We surmised that we were not properly accounting for the fact that more than one thousand dendritic compartments were a part of the dendritic tree that contained only 17 branch points from the explicit path from the spine to the soma. In other words, we were not adequately treating the series versus parallel nature of the connectivity of the dendrites branching off of the preserved path. Our final scaling step was to adjust our voltage-gated ion channels destined for the dendrites by the same scaling factor used for R_m and C_m. We placed these adjusted conductances in the equivalent cylinders. We kept the conductances at their original values in the unreduced explicit path from the soma to the spine of interest. We then applied the same current injection at the soma. We obtained a single action potential, followed by a plateau (Supplemental Fig. S3*d*). We did not obtain action potential oscillations as in the full model (in Fig. 2*c*).

Similar results are obtained at the spine for all of the considered cases (Supplemental Fig.s S3, S6 and S7).

S1.2 Electrotonic Distance/Length

In Rall's traditional reduction method, all terminal branches to be reduced to an equivalent cylinder are assumed to end at the same electrotonic distance from the soma (Rall, 1969, Rall and Agmon-Snir, 1998). The electrotonic distance from the soma, *X*, is defined as

$$X = \sum_i L_i \quad , \qquad \textit{Eq. S1}$$

and L_i, the electrotonic length of each individual cylinder, is determined by

$$L_i = \frac{l_i}{\lambda_i} \qquad ; \qquad \lambda_i = \sqrt{\frac{R_m}{R_a} * \frac{d_i}{4}} \quad , \qquad \textit{Eq. S2}$$

where l_i is the anatomical length of the cylinder, λ_i is the characteristic length constant calculated for each cylinder, R_m is the membrane resistance, R_a is the axial resistivity, and d_i is the diameter for each cylinder. This assumption allows the voltage decrement in a dendritic tree to be mapped onto an equivalent cylinder by means of the electrotonic distance, *X*, measured from the soma. Our method, in contrast, allows the voltage response to be mapped based on where in the explicit path the branch points are made. Many of the associated dendrites are at similar electrotonic distances from the soma, though that is not a requirement for this method. In this study, all the smooth dendrites giving input to a particular explicit compartment are mapped to a single smooth equivalent cylinder, regardless of electrotonic length or distance. Similarly, all the related spiny dendrites are mapped to a single spiny equivalent cylinder connected to the smooth equivalent cylinder. Maindendrite is the only explicit compartment that receives input from a smooth equivalent cylinder (*viii* in Fig. 1*b*) with multiple subsequent connections (*ix*, *x*, and indirectly *xi*). This is because this branch point is associated with more than half the reduced dendrites in the model.

S1.3 Branch Points

The first branch point (*viii*) from the explicit path gives input to the main dendrite. More than 50% of the dendrites in the entire geometry stem from this branch point. This set of dendrites is reduced to four compartments based on their anatomical distance from the soma and their original classification as smooth or spiny dendrites (*viii, ix, x, xi*). Smooth dendrites are in green; spiny dendrites are in blue. There are two branch points giving input to compartment *iii*. Dendrites stemming from this branch points represent less than 10% percent of all the dendrites in the geometry. They are all reduced to 1 smooth (*xii*) and 1 spiny (*xiii*) compartment. There are a total of nine branch points from compartment *iv*, but all of the associated dendrites make up less than 30% of the dendrites in the geometry. These dendrites are thus also reduced to 1 smooth (*xiv*) and 1 spiny (*xv*) compartment. There are one and four branch points from compartments *v* and *vi*, respectively, consisting exclusively of spiny dendrites, which represent less than 2% of the dendrites in the geometry combined. Their dendrites are reduced to *xvi* and *xvii*, respectively.

S1.4 Further Analysis of the PPR Model

Adding active channels to the soma only completes the steps applied by Bush and Sejnowski for their reduction method of the pyramidal cell. However, unlike results from Bush and Sejnowski for the pyramidal neuron, Figure 2*b* shows that placing active conductances only in the soma in either our full or our reduced (PPR) model does not produce a train of action potentials as in the original study (full model in Fig. 2*c*) with active conductances throughout the dendritic tree (Miyasho et al., 2001). This could be due to the large dendritic arbor of the Purkinje neuron serving as a passive sink for the current injected at the soma.

The PPR model diverges from the full model when active conductances are added to the dendrites, without correcting for calcium flux into a smaller volume. Supplemental Figure 3*a* shows that current injection in the PPR model with active conductances in the equivalent cylinders adjusted according to our scaling factor described in *Model Features* gives a single action potential followed by a gradually depolarizing plateau at about -50mV. Action potential oscillations are observed during the last 50ms of the current pulse. This action potential pattern lacks the transient plateaus observed in the last 50ms of the current pulse in the full model. These transient plateaus are observed intermittently in the full model, in both the soma (Fig. 2*c*) and the dendrites (Supplemental Fig. 4) (Miyasho et al., 2001). They are due to calcium spiking (membrane potential transients) in the dendrites, as the dendrites become more and more depolarized during backpropagating action potentials. The calcium spikes propagate to the soma and give the transient plateaus seen in the pattern of action potentials in the full model (Fig. 2*c*). Similar results are obtained at the spine for all of these cases considered (Supplemental Fig. S3*b* and S5).

The resting potential of the PPR model appear to be hyperpolarized relative to the full model. This suggests that either potassium channels may be too active or calcium or sodium channels may not be sufficiently active. The most likely candidates are overactive potassium channels, since we already know that (i) calcium-activated voltage-gated potassium channels are sensitive to cytosolic calcium concentration, and (ii) scaling up the density of voltage-gated calcium channels may result in inadvertently increased and uncompensated calcium influx into the equivalent cylinders. In our reduction method, R_m and C_m were scaled, as were the active conductances, including the voltage-gated calcium channels. However, we have not yet taken into account the decreased volume of the cylinder cytosol into which calcium flows when the voltage-gated calcium channels are open. As a result, the same calcium load as in the full model encounters smaller volumes in the PPR model, leading to increased calcium concentrations in the PPR model. This overactivates the calcium-sensitive voltage-gated potassium channels present in the model, BK and IK. BK is the large (or big)-conductance channel (~200 pS), and IK is the intermediate-conductance channel. SK, the small-conductance channel, is not included in the model (see *Discussion*).

The calcium load considers ion flow into a submembrane shell, a depth of cytoplasm (~100nm), right beneath the plasma membrane (Miyasho et al., 2001). The rate at which the calcium concentration increases due to influx through the voltage-gated calcium channel is given by (Destexhe et al., 1993, Miyasho et al., 2001)

$$\frac{dCa_i}{dt} = \frac{I_{Ca}}{2 * F * depth} \quad , \qquad \textit{Eq. S3}$$

where Ca_i is calcium concentration, I_{Ca} is the calcium current through all the calcium channels combined, F is Faraday's constant, and the depth is 100nm.

The PPR model closely fits the full model and is used for the rest of this study, for analysis in NEURON and reproduction in Virtual Cell. In every part of this study, the current input is injected at the soma, and the voltage is measured at the soma and at the spine. The input resistance, R_{in}, is calculated for the soma, and is equal to the ratio of the voltage response at the soma to the current input. The transfer resistance, R_t, is calculated at the spine, and is equal to the ratio of the voltage at the spine to the current input injected at the soma. The attenuation factor, A_f, between the soma and the spine is calculated as the ratio of the voltage at the soma to the voltage at the spine. A depolarizing current (2nA) was used to investigate these electrical properties in the passive models (Supplementary Table S1, Fig. 2*a*). A hyperpolarizing current was used to investigate these properties in full and PPR models with active conductances (Supplemental Fig. S8).

Table S1 shows various electrical properties of the passive full and PPR models when compartments *viii-xi* are grouped by anatomical distance from the soma versus electrotonic distance from the soma. The table shows that the PPR model with the compartmetns grouped by anatomical distance from the soma (as described in *Model Features*) more closely matches the full model than the PPR model that has the compartments grouped based on electrotonic distance. This suggests that the system we developed works more effectively with anatomical distance than electrotonic distance. This may be due to the scaling factor expression described in Model Features. The scaling factor depends on the radii of the individual dendrites, as well as the anatomical distance of terminal branches from the branch point with maindendrite, rather than the electrotonic length or the electrotonic distance from the soma. Mapping based on electrotonic distance from the soma works well for current accepted reduction methods. However, this approach appears to be incompatible with our novel reduction method, which is highly dependent on individual branch points from a preserved path.

Table S2 shows representative computer run times for the PPR and full models simulated in NEURON. The table shows that the passive PPR model runs three times faster than its original counterpart. The PPR model with active conductances runs at least 10 times faster than the full model. This indicates that reducing the geometry to fewer compartments significantly increases the computational efficiency of the model. It also indicates that there is a greater benefit to reducing the complex geometry when it will be modeled with active conductances versus with passive dendrites.

S1.5 Synaptic Stimulation

Transient stimulation of the glutamate receptor AMPAR in nerve cells can be represented by a synaptic current with alpha function conductance as described below (Rall and Agmon-Snir, 1998):

$$I_{AMPAR} = g * (V - E) \qquad \textit{Eq. S4}$$

where I_{AMPAR} represents the synaptic current through AMPAR, V is the spine membrane potential, E is the reversal potential for the postsynaptic conductance change, and g is defined by

$$g = 0 \text{ for } t < tstart, \qquad \textit{Eq. S5}$$

$$g = g\max * \frac{(t - tstart)}{tpeak} * e^{-\frac{t - tstart - tpeak}{tpeak}} \text{ for } t > tstart, \qquad \textit{Eq. S6}$$

where *gmax* is the maximum conductance of the postsynaptic conductance change, *tstart* is the time of stimulus onset, and *tpeak* is the time it takes for the transient conductance change to peak at *gmax*. The alpha function conductance change is applied at the spine and leads to a voltage response, the E.P.S.P.

S1.6 The PPR Model in Virtual Cell & NEURON

The PPR model was created in NEURON, and solved using the Euler method; the full model created by Miyasho et al. (Miyasho et al., 2001) was also solved with Euler. Euler is a fixed step method, and it is not computationally efficient for large models. CVODE, on the other hand, is a variable step method, and it is more computationally efficient than Euler; CVODE is also more stable. Consequently, the PPR model in NEURON was converted to a format that could be used with CVODE and various other variable step solvers, as well as implicit fixed methods such as Euler. This version of the model is readily available in ModeldB (http://senselab.med.yale.edu/ModelDb/). Further, The PPR model in Virtual Cell can be simulated with any of the 6 ODE solvers available in that software; the results presented in this paper were simulated with CVODE. As described in the main paper, the Virtual Cell model and all simulation results are accessible in the Virtual Cell database. The VCML code for the PPR model is also provided as Supplementary Material.

S1.7 The PPR Method Generalized Using Rallpacks

The PPR method is an algorithm that can be implemented in various software packages using their respective codes. It can be applied in general to neurons with complex morphologies. This work has reported results for the cerebellar Purkinje neuron, a specific and prototypical case. To test the generality of the algorithm, the PPR method was also applied to Rallpacks. Rallpacks are ideal models and geometries created by Bhalla et al (Bhalla et al., 1992). They are primarily used to validate new software packages, particularly those designed to solve neuronal electrophysiology problems. In our case, we are not validating a new software package. However, Rallpacks still proved useful in providing simple and complex morphologies for algorithm testing. We used

simple descriptions in a published report from Bhalla et al (Bhalla et al., 1992) to create codes for Rallpacks in NEURON. In particular, the report includes results for passive voltage propagation in Rallpack 2. Rallpack 2 is a highly branched passive geometry with 1023 compartments. We compared results from our code with those in the report. Results for passive voltage propagation were identical (data not shown). This validates our code for the passive Rallpack 2 geometry. Although the authors did not add active channels to the branched Rallpack 2 geometry, we added Hodgkin-Huxley Na+ and K+ channels to the geometry to test whether the PPR method could be generalized to branched *active* geometries. We used the PPR method to simplify the geometry from 1023 compartments to approximately 20. A current injection of 1nA at the root compartment in this modified full Rallpack2 geometry (Fig. S11a) gives virtually identical voltage changes at the root compartment and a terminal branch as in the corresponding simplified geometry (Fig. S11b). Note that the terminal branch is unreduced in the simplified geometry. This is because, as is characteristic of the PPR method, an unreduced explicit path is preserved from the root compartment, culminating in that terminal compartment (called compartment 1022). Thus, Figure S11 demonstrates that the PPR method can be generalized to another complex neuronal geometry with active ion channels.

Supplementary Figure Legend

Supplemental Fig. S1 Passive electrical properties of various reduced models. *a, unscaled reduced model 1. b, reduced model scaled by 5.7. c, Bush-inspired model. d, reduced model scaled by 10.* In each case, membrane potential is measured at the soma. IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S2 Electrical properties of various reduced models with a depolarizing current injection at the soma. a, the Bush-inspired model with active channels in the soma only, measured at the soma. *b*, the Bush-inspired model with unscaled active channels localized to the soma, dendrites, and spine, measured at the soma. *c*, the Bush-inspired model with channels scaled in the equivalent cylinders and unscaled channels in the soma, spine, and dendrites of the explicit path. *d*, the reduced model scaled by 10 with unscaled channels in the soma, spine, and dendrites. In each case, membrane potential is measured at the soma. IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S3 Electrical properties of the PPR model and the Bush-inspired model. *a*, In the PPR model, a depolarizing current injection at the soma gives an action potential followed by a plateau, then followed by membrane potential oscillations, when calcium influx in the equivalent cylinders is not adjusted by the scaling factor. *b*, Corresponding membrane response at the spine in the PPR model. *c*, In the Bush-inspired model with channels scaled only in the equivalent cylinders, a depolarizing current injection at the soma gives a single action potential, then a plateau, then a few action potential oscillations with calcium spike-induced plateaus at the soma. *d*, Corresponding calcium spikes at the spine in the Bush-inspired model with channels scaled only in the equivalent cylinders. IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S4 Membrane potential oscillations measured at various locations. Membrane response in the PPR model in NEURON, measured in the compartments maindendrite (*a*), smoothdistaldendriteshort (*b*), adjacentdendrite (*c*), and spine (*d*). IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S5 Membrane potential changes at the spine in the PPR and full models in NEURON. *a*, Passive electrical properties of the full and PPR models. *b*, Electrical properties of the full and PPR models with active channels only in the soma. IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S6 Passive electrical properties measured at the spine in various reduced models in NEURON. Membrane potential response due to depolarizing current injection at the soma in the unscaled reduced model (a), reduced model scaled by 5.7 (b),

Bush-inspired model (c), reduced model scaled by 10 (d). IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S7 Active properties measured at the spine in various reduced models in NEURON. Membrane response in Bush-inspired model with active channels only in the soma (a). Membrane potential response with active channels in the soma, dendrites, and spine in the (b) Bush-inspired model with channels unscaled everywhere, (c) Bush-inspired model with channels scaled in equivalent cylinders, (d) reduced model scaled by 10. IClamp at the soma: onset 20ms, duration 400ms, amplitude 2nA, as in Miyasho et al, (Miyasho et al., 2001).

Supplemental Fig. S8 Membrane hyperpolarization in the full and PPR models in NEURON. *a*, Membrane response at the spine and at the soma due to a hyperpolaring current injection at the soma in the full model. *b*, Membrane potential response at the spine and at the soma in the PPR model. IClamp at the soma: onset 20ms, duration 400ms, amplitude -2nA.

Supplemental Fig. S9 PPR model results compared in Virtual Cell and NEURON. *a*, Membrane potential depression due to hyperpolarizing current injection at the soma in Virtual Cell and NEURON. *b*, Membrane potential response at the spine due to hyperpolarizing current injection in NEURON and Virtual Cell. IClamp at the soma: onset 20ms, duration 400ms, amplitude -2nA.

Supplemental Fig. S10 Membrane potential and submembrane calcium responses at the spine and soma in Virtual Cell. Membrane response at the soma (*a*) and the at the spine (*b*), due to sustained hyperpolarizing current injection at the soma. Submembrane calcium transients at the soma (*a*) and at the spine (*b*), due to a hyperpolarizing current injection at the soma. The hyperpolarizing current injection is the same as in Supplemental Fig. S8. In each case, the dashed line represents the membrane potential with no current injection. IClamp at the soma: onset 20ms, duration 400ms, amplitude -2nA.

Supplemental Fig. S11 The PPR method generalized using Rallpack. Active membrane potential changes at the root and terminal compartments in the full (*a*) and reduced (*b*) Rallpack 2 geometry coded in this study, with Hodgkin-Huxley sodium and potassium channels. Rallpack descriptions are available in the report by Bhalla et al (Bhalla et al., 1992).

Supplementary Table S1: Attenuation comparison of the PPR model with the full model

	Steady current input (nA)	Steady voltage response at the soma (mV)	Steady voltage response at the spine (mV)	Input resistance (Mohm)	Transfer resistance (Mohm)	Attenuation factor
Full model - passive dendrites	2	-37.5955	-53.951	18.79775	-26.9755	0.696845286
Reduced model - passive dendrites	2	-37.2381	-51.2741	18.61905	-25.63705	0.726255556
Reduced model - passive dendrites[a]	2	-35.4616	-49.8349	17.7308	-24.91745	0.711581643

[a] *Using electrotonic distance from the soma to allocate dendrites to reduced compartments viii-xi (see Fig. 1b).*

Supplementary Table S2: Representative computer run times for the PPR and full models

	Passive dendrites - depolarizing current (2nA)	Dendrites with active conductances - hyperpolarizing current (-2nA)	Dendrites with active conductances - depolarizing current (2nA)
Full model	30s	400s	400s
Reduced model	10s	30s	40s

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