

Quantum Modeling: A Bridge between the Pumping and Signaling

Functions of the Na/K – ATPase

By

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Abstract

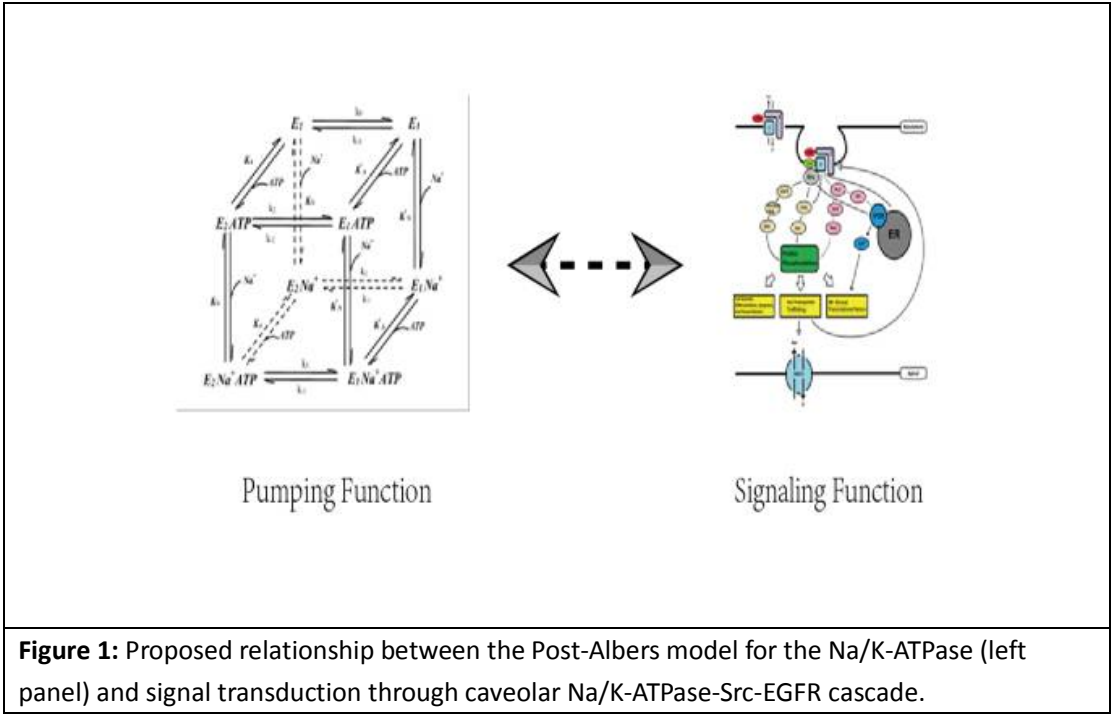
Although the signaling function of Na/K ATPase is been studied for decades, the chasm between the pumping function and the signaling function of Na/K – ATPase is still an open issue. This article explores the relationship between ion pumping and signaling with attention to the amplification of oxidants through this signaling function. Starting with some experimental observations published by our laboratories and others, we develop some predictions regarding cellular oxidant stress.

Keywords: Na/K – ATPase, Markov chain, master equation, Src, oxidant stress, reactive oxygen species, aging

Introduction

The Na/K – ATPase is a P-type ATPase discovered in 1950s by Skou¹. It is arguably one of the most important proteins in animal biology. Although it has been known since Skou's original discovery to play a key role in ion transport (hence its alternate name, "sodium pump"), studies from the Xie/Shapiro laboratories also showed that the Na/K – ATPase also plays an essential role in cell signal transduction and ROS regulation²⁻⁴. It has been postulated that this signaling function is related to a scaffolding function of the Na/K-ATPase controlling the activity of Src kinase⁵⁻⁷. Although this postulate has attracted some controversy⁸, we would argue that the effectiveness of therapeutics developed entirely on the basis of this scaffolding function demonstrate its validity⁹⁻¹². For the purpose of this exploration into modeling, we propose the following axioms based on this postulated scaffolding function.

1. The Na/K-ATPase alpha1 subunit inhibits associated Src kinase while in the E2 state.
2. Activation of Src kinase leads to transactivation of the epithelial growth factor receptor (EGFR) and downstream generation of reactive oxygen species.



Axiom 1 has evidence in references ^{13, 14} whereas Axiom 2 is supported by data published in references ^{3, 4, 14-18}.

Although for many years, focus has been on the effect that a class of molecules referred to as cardiotonic steroids (CTS) signal through the Na/K-ATPase, more recent evaluation demonstrates that the Na/K-ATPase can function as a feed forward amplifier for oxidants ^{9-11, 13, 14}. Based on these data, we propose axiom

3. ROS both result from the activation of the Na/K-ATPase-Src-EGFR signal cascade and can initiate said activation by oxidation of the Na/K-ATPase which, in turn, decreases the tonic inhibition of associated Src Kinase. We further assume (candidly without much proof at present) that the oxidation of the Na/K-ATPase results in an increase in time that the Na/K-ATPase spends in the E2 state. The data for ouabain or other CTS increasing time in the E2 state are excellent.

To further incorporate understandings gleaned from extensive experiments probing the

functional relationship between the scaffolding function of the Na/K-ATPase and cell biology, we suggest that

4. The level of oxidant stress determines the rate of endocytosis of the Na/K-ATPase complex from the plasma membrane which results in a net disinhibition of membrane associated Src.

From these assumptions or axioms, we will attempt to model the relationship between signaling through the Na/K-ATPase and redox state within the cell. Recent studies show that the normal Na/K – ATPase has a rapid velocity of conformational change during its pumping function. For instance, some studies show that purified Na/K-ATPase hydrolyzed ATP at 1000-1500 $\mu\text{mol/mg protein/hr}^{19}$. As a result of this rapid changes in conformation, characteristics of the Na/K-ATPase, especially binding coefficients, will not hold constant. In fact, we have modelled this with respect to the scaffolding function – tonic inhibition of Src kinase based largely on molecular modeling presented in reference ¹³.

The Markov chain model: First, we assume that conformational change of a single Na/K – ATPase unit is simply (and sometimes incorrect) independent from adjacent conformations during its turnover process, therefore its turnover process could be depicted by Markov Chain model²⁰. Obviously, at the equilibrium state, this single Na/K – ATPase unit will essentially represent a superposition of a number of conformations which can be described as follows.

$$\{X_n, n \in T\} (T = 0, 1, 2, \dots)$$

Assume that after a very short time interval Δt , this single Na/K – ATPase unit will randomly switch its conformation from one to another, which only depends on its previous conformation.

Obviously, this probability could be described as:

$$P\{X_{n+1}=i_{n+1} | X_n=i_n\}$$

The term P means the probability of this single Na/K – ATPase unit changes its conformation from conformation i to conformation i+1, the time interval between n and n+1 is Δt . Similarly, between every possible conformation i and j ($i, j \in n$), a one-step transform probability could be defined as:

$$p_{ij}(n) = P\{X_{n+1}=j | X_n=i\}$$

Which means that for every given conformation i and j, there always have a corresponding probability p_{ij} . If we restrict ourselves to the 4 major states in the Post-Albers model:

	<i>E1</i>	<i>E1P</i>	<i>E2P</i>	<i>E2</i>
<i>E1</i>	p_{11}	p_{12}	p_{13}	p_{14}
<i>E1P</i>	p_{21}	p_{22}	p_{23}	p_{24}
<i>E2P</i>	p_{31}	p_{32}	p_{33}	p_{34}
<i>E2</i>	p_{41}	p_{42}	p_{43}	p_{44}

However, it is certainly possible (in fact likely) that there are any number of “hidden” states

(possibly representing those associated with binding different numbers of Na or K molecules). If

those “hidden states” need to be considered, the one-step transition matrix could be written as:

	<i>E1</i>	...	<i>E1P</i>	...	<i>E2P</i>	...	<i>E2</i>	...	E_{1j}
\vdots	\vdots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\vdots
<i>E1</i>	p_{11}	...	p_{12}	...	p_{13}	...	p_{14}	...	p_{2j}
\vdots	\vdots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\vdots
<i>E1P</i>	p_{21}	...	p_{22}	...	p_{23}	...	p_{24}	...	p_{3j}
\vdots	\vdots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\vdots
<i>E2P</i>	p_{31}	...	p_{32}	\ddots	p_{33}	...	p_{34}	...	p_{4j}
\vdots	\vdots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\vdots
<i>E2</i>	p_{41}	...	p_{42}	...	p_{43}	...	p_{44}	...	\vdots
\vdots	\vdots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	\ddots	p_{ij}

When the single Na/K – ATPase reaches the equilibrium state ($n \rightarrow \infty$), this Markov process could be defined as a stationary distribution as a result of $p_{ij}(n)$ is independent from n :

$$\pi = (\pi_j, j \in I), \pi_j \geq 0$$

$$\lim_{n \rightarrow \infty} X^{(n)} = \lim_{n \rightarrow \infty} P X^{(n-1)} = \pi,$$

$$\pi_j = \sum_{i \in I} \pi_i p_{ij}, \sum_{j \in I} \pi_j = 1$$

As a result of motor protein, assume every p_{ij} has a corresponding k_{ij} between conformation i and j . Thus, the Consider the probability density $p_{ij}(t)$ denoted by the master equation:

$$\frac{d}{dt} p_{ij}(t) = \sum_{j \neq i} [k_{ij}^+(t) p_j(t) - k_{ij}^-(t) p_i(t)] \quad 1 - 1$$

The transition rate k_{ij}^+ and k_{ij}^- means the conformation change rate of Na/K – ATPase from conformation i to j and j to i , respectively. According to laws of thermodynamics, the number of reactions per unit time (J^{NESS}) and the amount of heat dissipated into the environment per unit time (e_p) could be written as²¹:

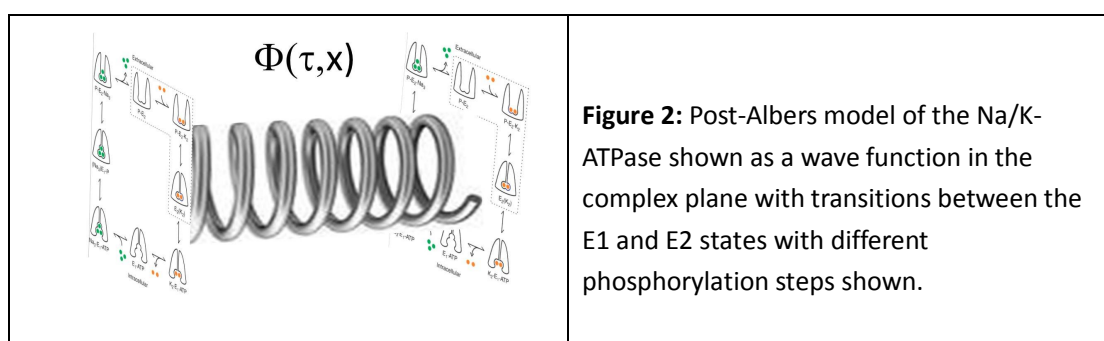
$$J^{NESS} = \frac{k_1^+ k_2^+ k_3^+ \dots k_n^+ - k_1^- k_2^- k_3^- \dots k_{ij}^-}{\{k_1^+ k_2^+ + k_1^- k_2^- \dots + k_{n-1}^+ k_{ij}^-\}} \quad 1 - 2$$

$$e_p = J^{NESS} \ln \left(\frac{k_1^+ k_2^+ \dots k_{ij}^+}{k_1^- k_2^- \dots k_{ij}^-} \right) \quad 1 - 3$$

Although nontrivial, this calculation suggests that a measurement of e_p would allow for some

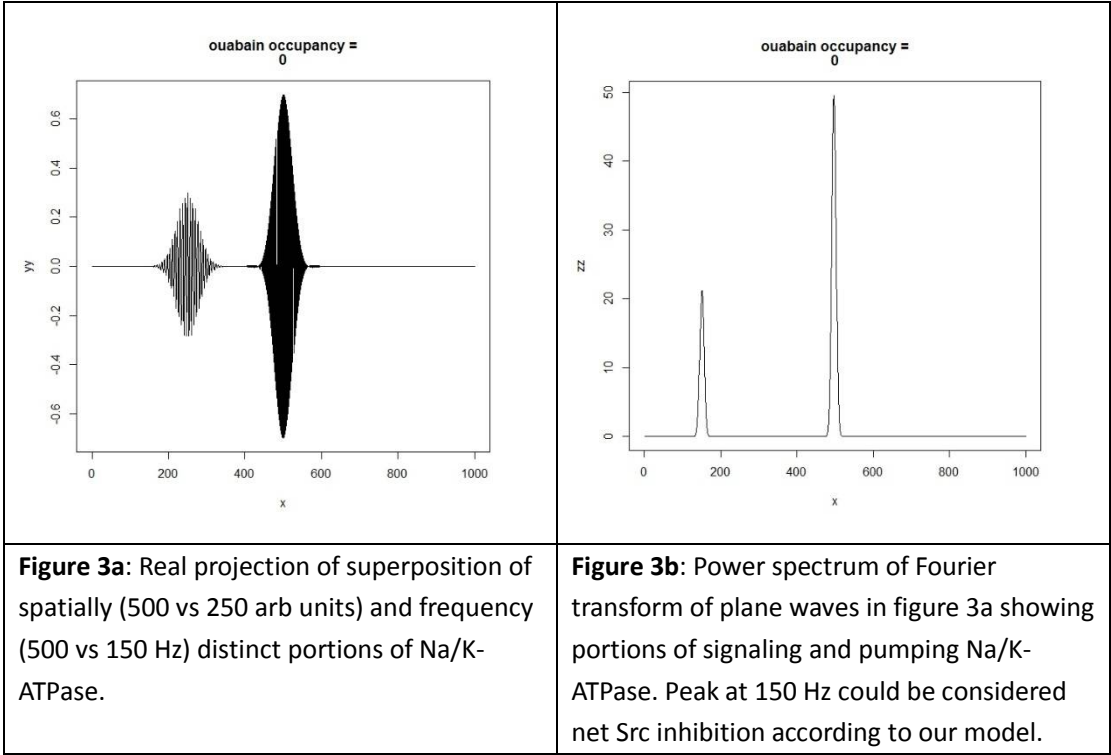
estimation regarding these transitions. Developing strategies to minimize and or maximize probability of different states could allow for calculation, at least on the level of an ensemble for these k values. Unfortunately, it is likely (as we proposed) that there are different pools of Na/K-ATPase which have different rates of transitions. Hence while the above calculations might be possible to describe one of these pools, we would need to separately consider those e_p and associated k values associated with the pumping and signaling portions of the Na/K-ATPase. Again, there are biochemical and molecular biological methods that are available to selectively deplete the signaling portion of the Na/K-ATPase allowing for at least the possibility of performing these measurements.

Superposition of states: A related but more simplistic approach would be as follows. If we imagine this in the complex plane, one could contemplate a wave function to describe the different states of the Na/K-ATPase as shown in Figure 2.



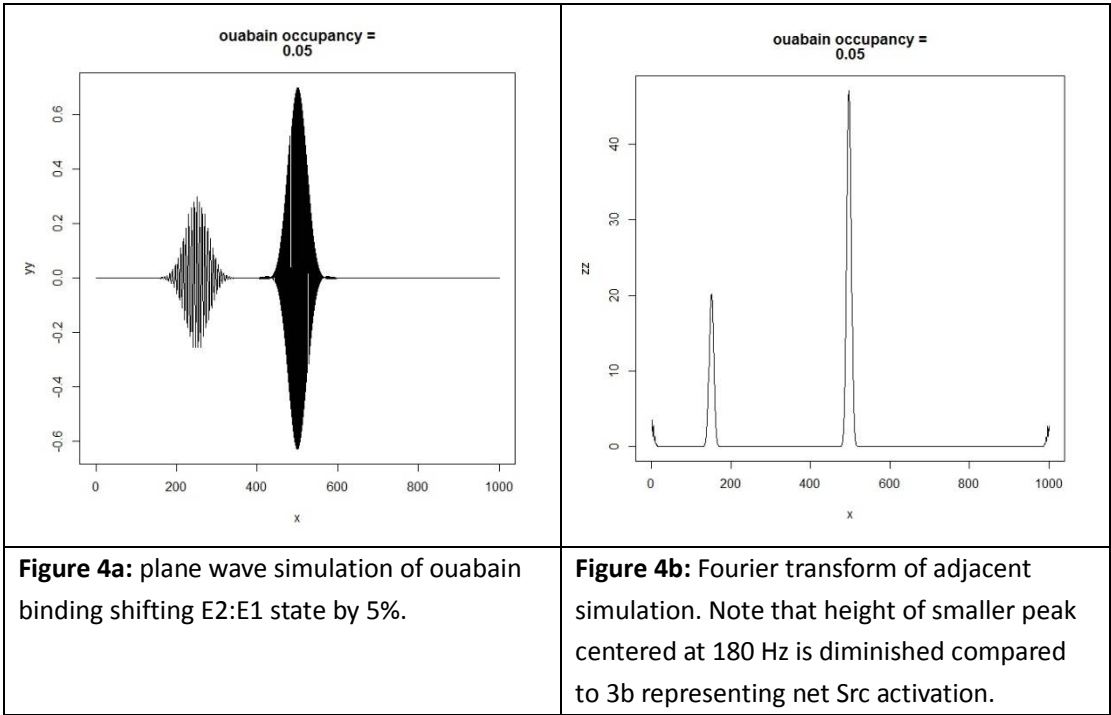
Arbitrarily, we could consider the “real” values to represent transitions between E1 and E2 whereas the “complex” values would represent the degree of phosphorylation. If we further assume that there are both spatial and frequency separation of the “signaling” Na/K-ATPase (i.e., that Na/K-ATPase present in caveolae and complexed with caveolin 1, Src and other signaling partners) and “pumping” pump (that Na/K-ATPase NOT associated with caveolin 1, Src and other

signaling proteins), one can imagine the pump as a plane wave with two different spatial domains and 2 different frequencies as shown below (R program used to generate these figures shown in appendix 1).



Although we have previously described a pool of “non-pumping” pump to describe the signaling portion of the Na/K-ATPase ²², it is far more likely (and consistent with our other observations) that the signaling portion of the Na/K-ATPase cycles through different conformations more slowly and is thus much less relevant to ion transport than the non-signaling pool. While it is a gross oversimplification to consider residence within caveolae to define signaling vs pumping pump, it does appear that all of the signaling pump does associate with Src and caveolin ⁶. However, to illustrate the difference (figures 3 and 4), we will continue use both frequency and space to separate these two pools. If we look at ouabain as a pharmacological tool to shift more Na/K-ATPase into the E2 state (ultimately dis-inhibiting Src kinase), the wave

function simulations might look something like figure 4.



Extension of the Markov chain to define the oxidant amplification loop: If we accept the axioms stated above, it is clear that Src kinase activity is a master-controller of subsequent signal transduction and ROS generation. As ROS can also initiate this sequence through altering Na/K-ATPase conformation and dis-inhibiting Src kinase, feed forward amplification results ¹³.

Although a number of additional assumptions must be made to construct the model, we chose to attempt to look at ROS regulation from this system. The assumptions necessary for this simple system of differential equations include.....(detailed R program shown in appendix 1)

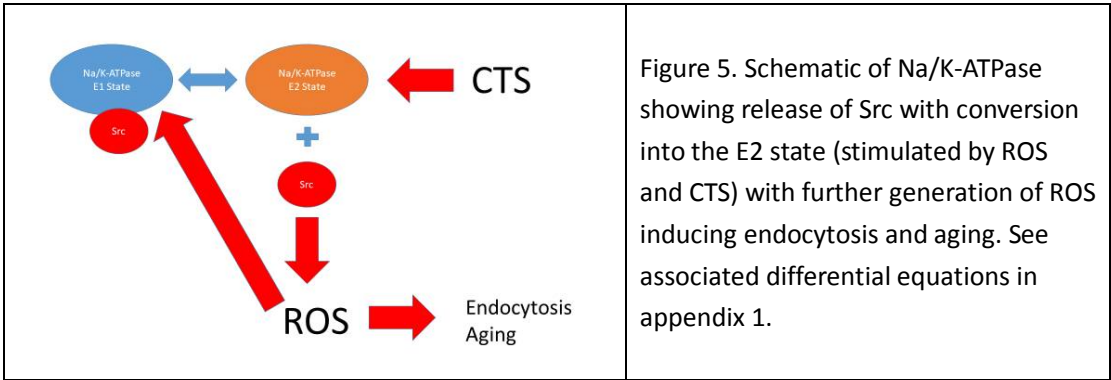
- a. The Na/K-ATPase has a basal synthesis rate
- b. ROS shift the Na/K-ATPase into the E2 state (as do CTS).
- c. There is a basal rate of ROS production from other sources.
- d. There is a link between Src phosphorylation and ROS production (oversimplifying the

- cascade which we believe involve transactivation of the EGFR and other steps)
- e. There is a detoxification rate of ROS which is proportional to ROS concentration (an assumption that is at least partially true, based on kinetics of superoxide dismutase and catalase)
 - f. There is a relationship coupling ROS concentration to rates of endocytosis of the Na/K-ATPase ²³.
 - g. There is a decay rate of phosphorylated Src which is proportional to the amount of phosphorylated Src.

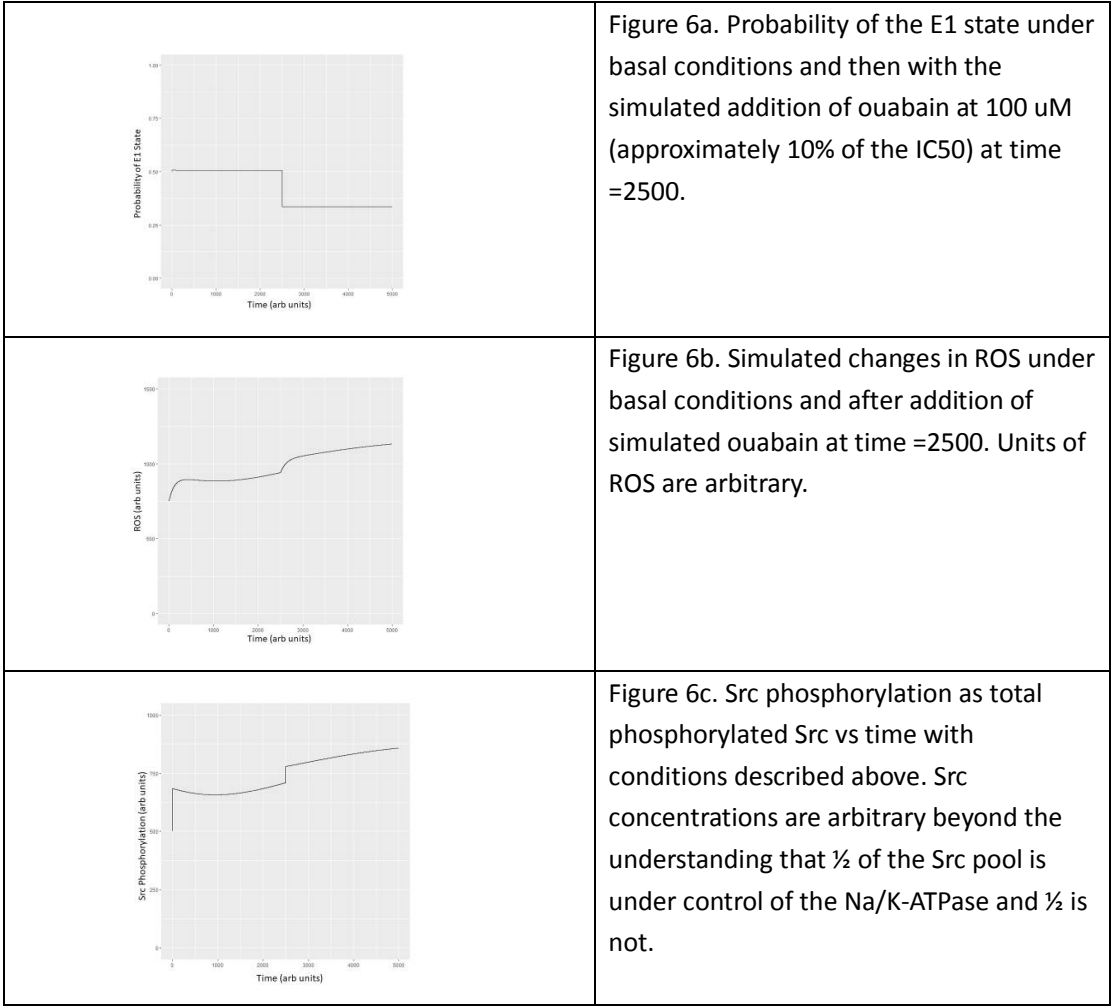
For the purpose of the model, we will further assume that the cells in question have approximately 2M Na/K-ATPase units on the surface with about ½ of Src regulated by this pool of the “signaling” Na/K-ATPase. This is similar to what we’ve seen with LLC-PK1 cells. We further assume (and model) that the usual proportion of the Na/K-ATPase in the E1 state is about 50% with units of ROS being entirely arbitrary (*vida infra*). This is consistent with the observed co-dependence of the velocity of Na/K-ATPase on both intracellular Na and extracellular K concentrations ²⁴.

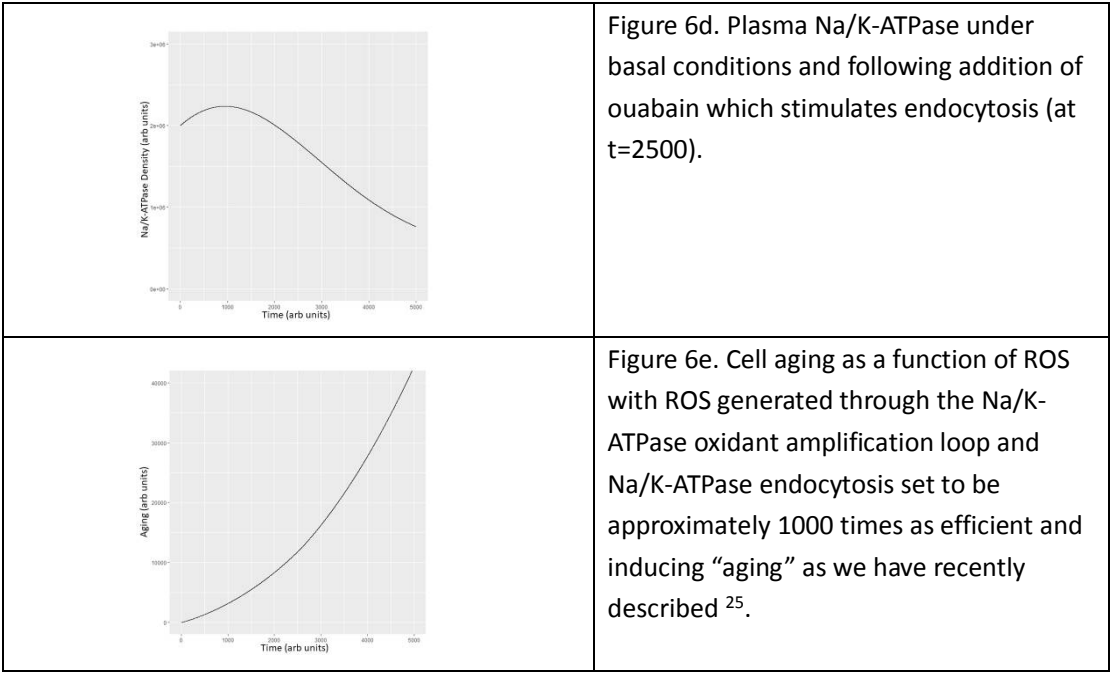
The ROS concentrations are more difficult. What we know from mammalian biology is that there are a number of interdependent processes leading to ROS generation including the NaK-ATPase oxidant amplification loop ²⁵. Simplistically, we know that oxidization of sulfhydryl groups occurs with oxidant stress whereas reduction occurs because of the generation of reducing equivalents, largely (although not entirely) through the hexose monophosphate shunt. Rate constants to make the model “work” were arrived at with an iterative approach, seeking stability

prior to the “shift” if E1/E2 states achieved a specific time point (see appendix).



What we see in experimental manipulations that shifting a portion of the Na/K-ATPase into the E2 state with ouabain results in a surge in ROS and endocytosis of the Na/K-ATPase ⁴.





The models discussed above are clearly simplistic and need validation of assumptions used in their construction. However, it is fair to say that the model (as it was constructed to simulated this) does give roughly similar changes in Src phosphorylation and ROS generation (as well as cellular aging) as that seen in experimental systems ^{3, 4, 13, 22, 25, 26}. In addition, there are additional several points which can be drawn.

1. The Na/K-ATPase can be viewed as existing in a superposition of states which relate to each other as a Markov chain.
2. The Na/K-ATPase interacts with Src and its other signaling partners using principles also seen with Markov chains. This allows modeling to be developed using a system of ordinary differential equations.
3. As seen in biological systems, oxidant stress results from the shift in the Na/K-ATPase to the E2 state which then becomes ineffective in inhibiting Src ¹³. This leads to a cascade

generating oxidants which further stimulates the Na/K-ATPase
oxidant amplification loop (feed forward amplification) as well
as oxidant injury consistent with aging.

As we consider these points, we realize that none of these insights require the modeling efforts made in this paper. Moreover, there are large number of assumptions used in creation of the models that are very difficult to validate with experimental data. However, we do believe that we can use these models and further refinements of these models to make predictions in different cells and tissues and ultimately expand our understanding of this pathway which is the topic of this symposium.

Acknowledgments

This work was supported by National Institutes of Health Grants HL109015, HL071556, and HL105649, by the Brickstreet Foundation and by the Huntington Foundation, Inc.

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Appendix 1: R code for Oxidant Stress/Aging Simulation

```

#NKA signaling simulation
library(deSolve)

duration=5000
ko=function(t,duration){
  if(t<duration/2){
    return(0)
  }else{
    return(1e-3)
  }
}

#parameters
parms=c(GNKA=800 #rate of NKA synthesis
        ,K1=9.5e-4 #rate constant shift to E2 state
        ,K_1=1e-3 #rate constant shift to E1 state
        ,K2=3e-8 #effect of ROS on E1 E2 states
        ,KSR=1e-2 #link between Src and ROS
        ,GROS=5e-1 #basal rate of ROS synthesis
        ,K3=1.2e-3 #dephosphorylation rate of Src
        ,K21=8e-3 #decay of ROS
        ,K4=-2.5e-10 #conversion of ROS into endocytosis
        ,K5=0
        ,K6=10 #link between endocytosis and ROS and aging"
        ,K7=.001 #link between ROS and aging
        ,K8=1.1e-6 #phosphorylation of Src
        )

state=c(N=2E6,T=1E6, p=5e2, R=750, E=-0.00015, PE1=0.5,A=0)

SIMNKA=function(t,state,parms){
  with(as.list(c(state,parms)),{
    dN=GNKA+E*N
    dT=K5*T
    dp=K8*((1-PE1)*N/4 + T-N/4)*(T-p)-K3*p*T
    dR=GROS+KSR*p-K21*R
    dE=K4*R
    dPE1=-(K2*R+K1+ko(t,duration))*(PE1*N)+K_1*(1-PE1)*N
    dA=(K6*-1*E+K7)*R
  })
}

```

```

        list(c(dN,dT,dp,dR,dE,dPE1,dA))
    })
}

times=seq(from=0,to=duration, by=0.1)
out=lsoda(y=state,times=times, func=SIMNKA,parms=parms)

library(ggplot2)
out=as.data.frame(out)

windows()
p=ggplot(out)+geom_line(aes(x=out[,1],y=out[,2]))+coord_cartesian(xlim=c(0,5000),ylim=c(0,3e6))
)+ggtitle("plasmalemmal Na/K-ATPase")
plot(p)

windows()
p=ggplot(out)+geom_line(aes(x=out[,1],y=out[,4]))+coord_cartesian(xlim=c(0,5000),ylim=c(0,1e3))
)+ggtitle("Src phosphorylation")
plot(p)

windows()
p=ggplot(out)+geom_line(aes(x=out[,1],y=out[,5]))+coord_cartesian(xlim=c(0,5000),ylim=c(0,1.5e3))
)+ggtitle("ROS")
plot(p)

windows()
p=ggplot(out)+geom_line(aes(x=out[,1],y=out[,7]))+coord_cartesian(xlim=c(0,5000),ylim=c(0,1))
)+ggtitle("Prob of E1 state")
plot(p)

windows()
p=ggplot(out)+geom_line(aes(x=out[,1],y=out[,8]))+coord_cartesian(xlim=c(0,5000),ylim=c(0,4000))
)+ggtitle("Aging (arb units)")

plot(p)

```