Supplemental information on the Madonna models of ezrin distribution

This supplement is offered to readers of the journal to provide more detailed information about the Madonna computer models described in our ms, "A Possible Mechanism for Ezrin to Establish Epithelial Cell Polarity."

The Madonna files (EzMod_Stim.mmd, EzMod_TA.mmd, EzMod_wt.mmd, EzMod_TD1.mmd, EzMod_TD2.mmd) can be downloaded from this site, to be opened and studied by any who have the Madonna program or who wish to download a free trial version of the program from http://www.berkeleymadonna.com. The trial version cannot save newly created files, but can open and run pre-existing files.

An image of the Madonna flowchart for the most complicated of our models (TD2; described in Fig. 8D of ms), incorporating the shifts of membrane and F-actin from apical to basal domains, is included here as Fig. S1, which can be used to locate the various modules described in the following paragraphs.

The Stim model, shown in Fig. 8A of the ms, lacks the "Tot…" modules at the four corners, the two "…shift" modules, and the "…infect" module. The mutant expression models (TA, TD1, and TD2; Fig. 8B, C & D), along with the wt model, use the "…infect" module (described below) to feed new ezrin into E (for TA or wt infection) or into EP (for TD infection). Only the TD2 model uses the two "…shift" modules and the four "Tot…" modules (all described below). Although the basic structure of the model was maintained for TA and TD expression, the numerous "Phos" and "DePhos" flow modules are functional only in the Stim and wt models since the phosphorylation and dephosphorylation constants are set to zero for TA and TD expression.

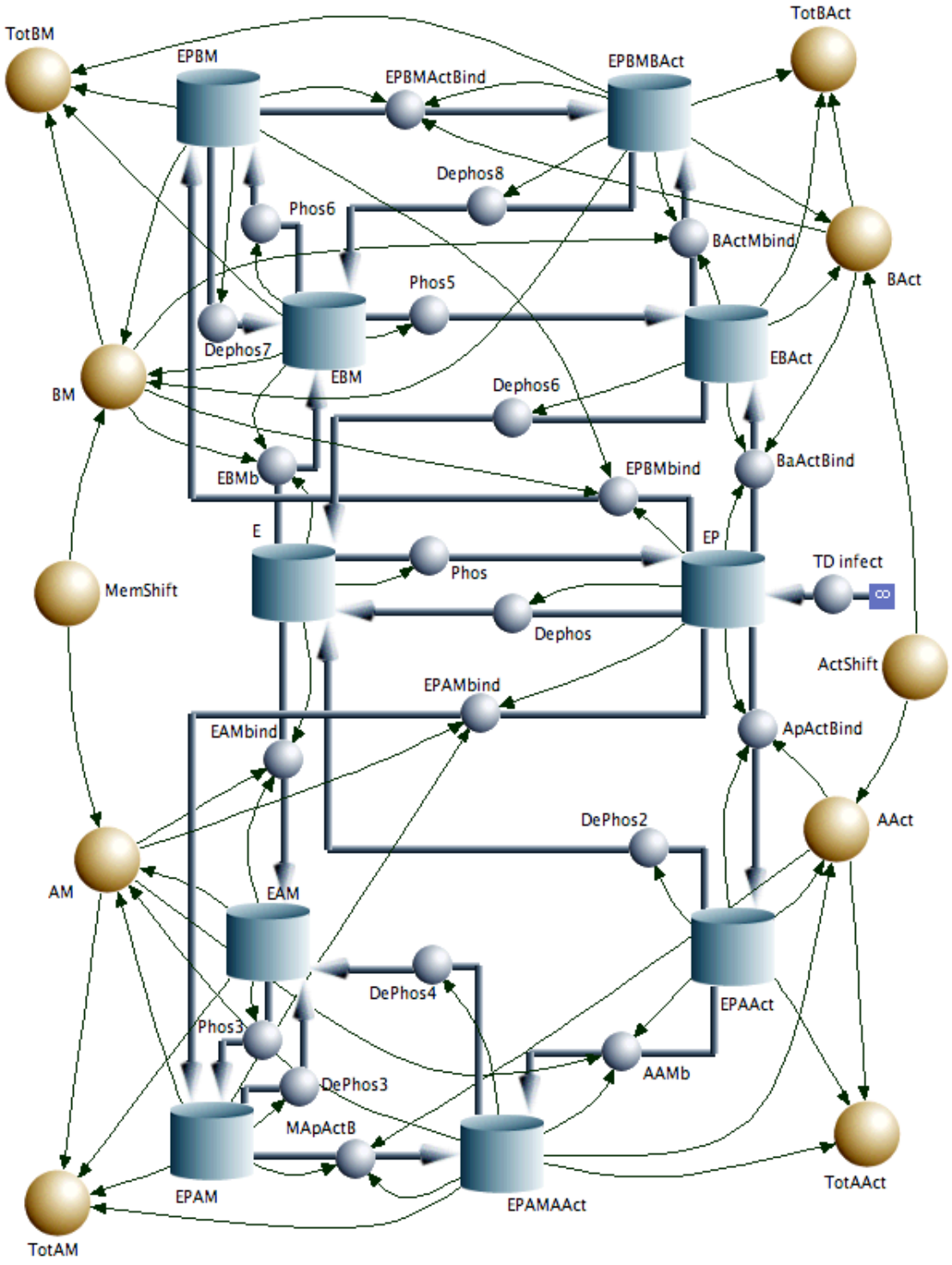
In all five versions of the model, the modules AM, BM, AAct, and BAct, used in the equilibrium equations listed in the manuscript (and embedded within various modules shown in the flowchart), return values representing the available (unoccupied) sites for ezrin binding. For the available binding sites, each module takes a total assigned value (4.0 for AM, 0.4 for BM, 3.0 for AAct, and 1.0 for BAct) and subtracts the amount of ezrin bound to that domain of membrane or F-actin to yield the unoccupied sites. The total assigned values (shifted toward basal in the TD2 model) represent numbers of ezrin-binding sites relative to a total endogenous ezrin amount of 1.0 (the four versions modeling overexpressed ezrin increase total ezrin to about 4 over 50 h).

In the Stim model, the assigned kp goes from 0.006/min in the resting state to 0.2/min upon stimulation, while kdp goes from 0.03/min resting to 0.02/min stimulated. The various ezrin forms are set at starting values (shown below, all forms adding up to 1.0) that will maintain a steady state when kp and kdp are at their resting levels:

E = 0.04583	EAM = 0.717	EBM = 0.0613
EP = 0.002	EPAM = 0.03525	EPBM = 0.00419
	EPAAct = 0.00655	EPBAct = 0.0112
	EPAMAAct = 0.1054	EPBMBAct = 0.0113

These distributions reflect experimental observations that, in the resting parietal cell, ezrin is mostly unphosphorylated at T567 (thus mostly unbound to F-actin) and is mostly localized to the apical membrane.

Fig. S1


TotBM
EPBM
EPBMActBind
EPBMBAct
TotBAct
Dephos8
Phos6
BActMbind
BAct
Phos5
Dephos7
EBM
EBAct
BM
Dephos6
EBMb
EPBMbind
BaActBind
E
EP
Phos
TD infect
MemShift
Dephos
ActShift
EPAMbind
EAMbind
ApActBind
AAct
DePhos2
AM
EAM
DePhos4
EPAAct
Phos3
AAMb
DePhos3
MApActB
EPAM
EPAMAAct
TotAAct
TotAM

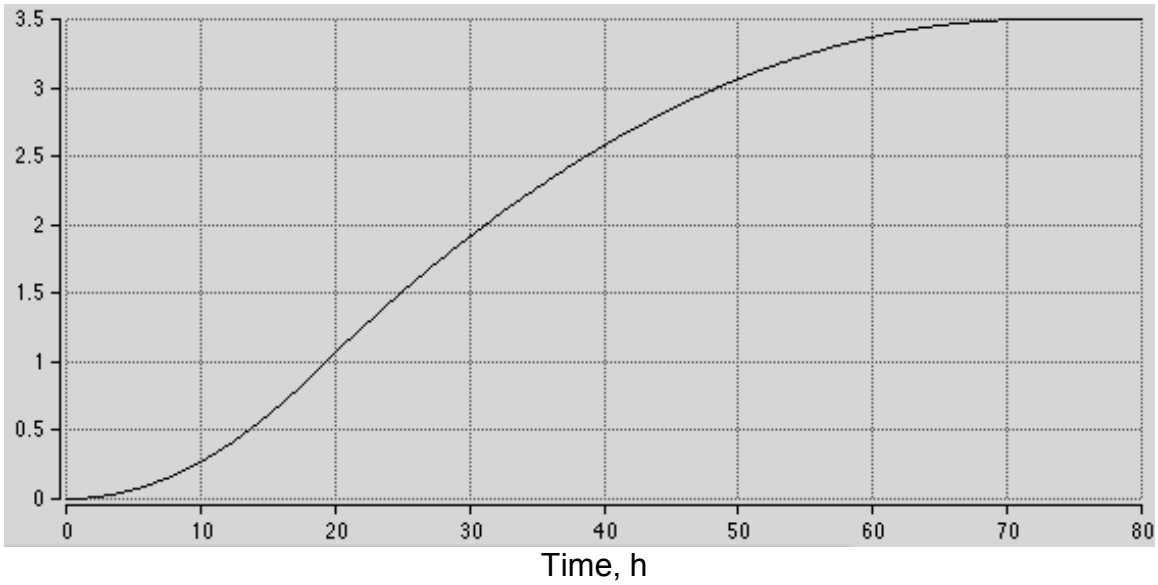
The membrane-binding constant, kMbnd, is set at 0.04/min (for the Stim model, which has time in minutes) and 2.4/hr (for the expression models). The unbinding constants are 5-fold lower for E (kEMunb) and 8-fold lower for EP (kEPMunb), reflecting an experimentally observed tendency for EP to bind membrane a little more tightly than E. While the assigned kActbnd = 0.04/min and 2.4/hr, values set for kActunb = 0.008/min and 0.48/hr.

In the expression models (TA, wt, TD1, and TD2), overexpression of the mutant ezrin, or wt, is accomplished with a "flow module" ("TA infect" or "wt infect" into the pool of E, or "TD infect" into the pool of EP) that has the formula,

IF TIME>72 THEN 0 ELSE (IF TIME<18 THEN time*0.0054 ELSE (72-time)*0.0018),

yielding unphosphorylated ezrin (for TA and unstimulated wt) or phosphorylated ezrin (for TD), added to the pre-existing endogenous ezrin (= 1) with the following time course (in hours):

Fig. S2 Accumulation of overexpressed ezrin (T567A, T567D, or wt)



In the TD2 model, ezrin-binding sites for F-actin and membrane are moved from apical to basal domains with the "ActShift" and "MemShift" modules, having the following sigmoid-curve formulas, the amounts being subtracted from the apical domain and added to the basal domain. The membrane shift lags behind the actin shift by 1 hour.

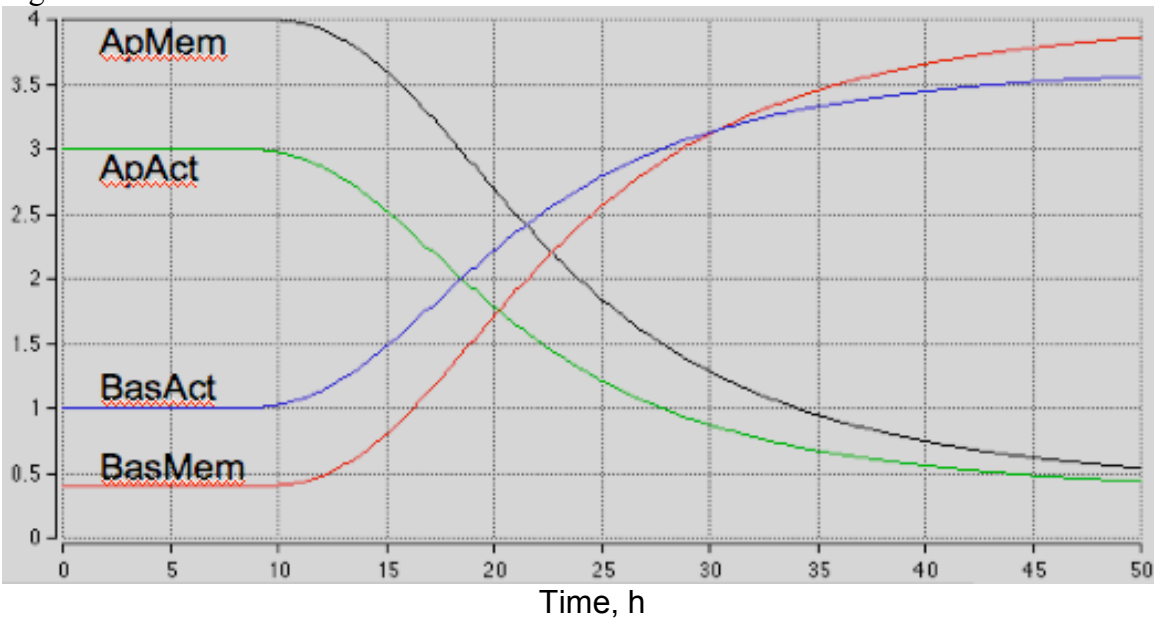
F-actin:

IF TIME<8 THEN 0 ELSE (TIME-8)^2.5*2.7/((TIME-8)^2.5+13^2.5)

Membrane:

IF TIME<9 THEN 0 ELSE (TIME-9)^2.5*3.7/((TIME-9)^2.5+14^2.5)

Fig. S3 Shifts of membrane and F-actin toward basal domain

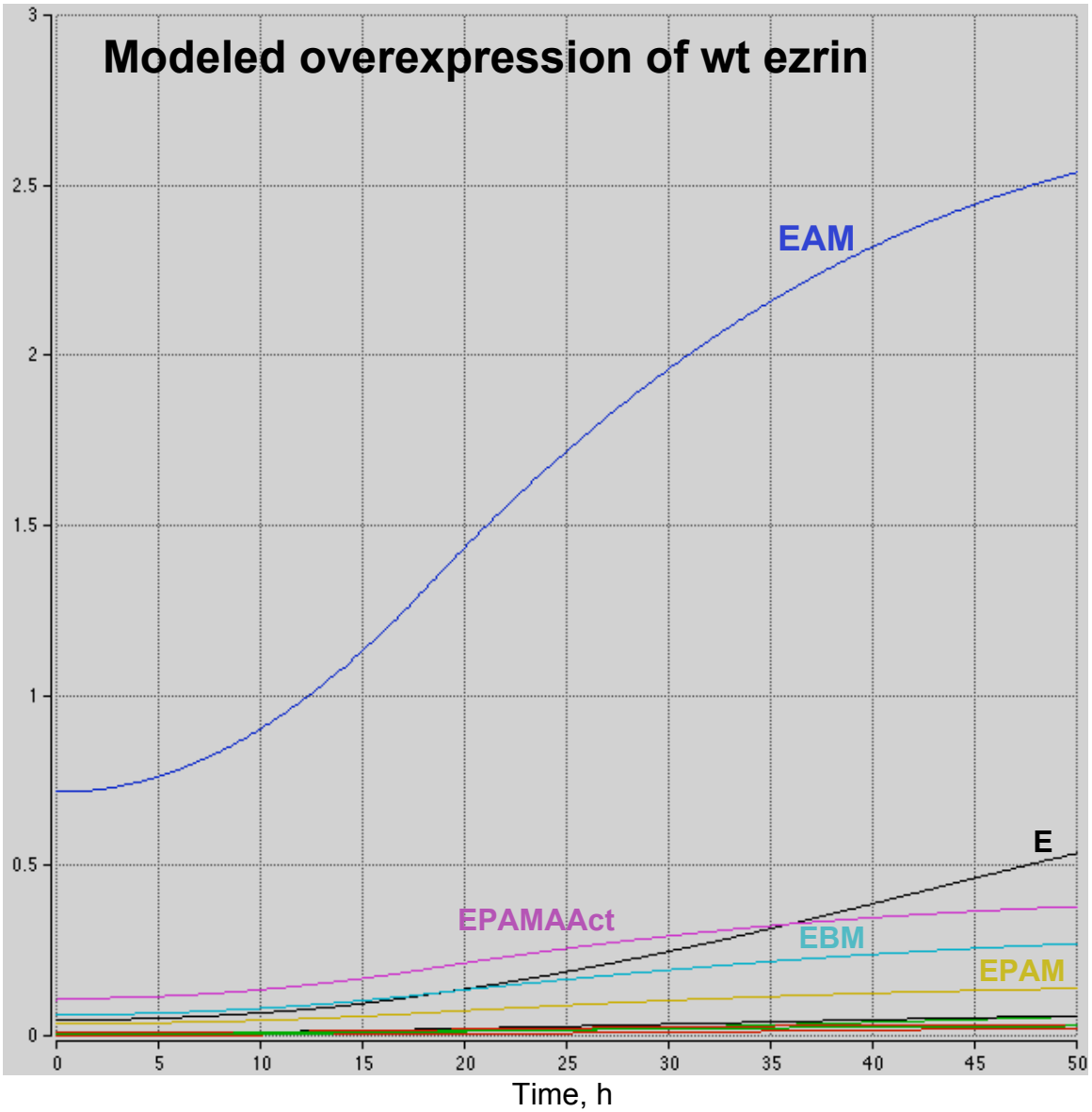


The above values are returned by the modules, TotAM, TotAAct, TotBM, and TotBAct, in the TD2 model and include occupied as well as unoccupied ezrin-binding sites.

Although the new basal F-actin would be formed from a cytoplasmic pool of G-actin, accompanied by a depolymerization of apical F-actin into the G-actin pool, our previous work has shown parietal cell actin to exist mainly as F-actin, requiring a very small pool of G-actin to be rapidly replenished by apical depolymerization to match basal polymerization. Thus, we feel that modeling a direct apical-to-basal shift of F-actin is an adequate simplification. While our experiments show tubulovesicle (H,K-ATPase-containing) cytoplasmic membranes to be among the membranes transferred to the basal membrane with T567D overexpression, tubulovesicles seem to lack direct ezrin-binding components, allowing us to model the shift of ezrin-binding membranes as a simple apical-to-basal shift.

Overexpressed wt ezrin, without stimulation by secretagogues, would be expected to be mostly unphosphorylated at T567. Here, we slightly altered the TA model, with overexpressed wt feeding into E, and with kp = 0.36/h and kdp = 1.8/h (as in the resting part of the Stim model, but /h instead of /min) in case a small kinase activity might make some difference compared to the TA model (in which kp = 0). Results were similar, in terms of the overwhelming accumulation of unphosphorylated ezrin bound to apical membrane (consistent with experimental results), so output of a wt model run was not shown in the published ms but is included here:

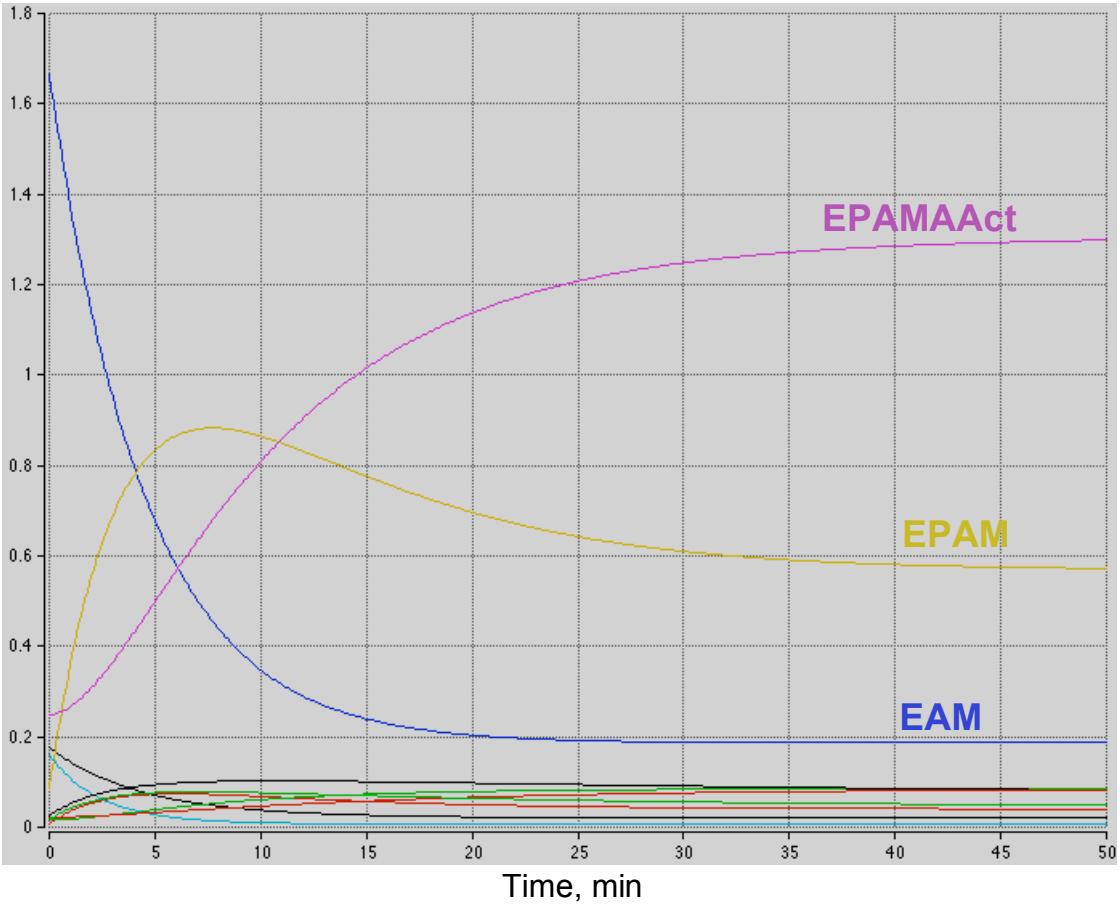
Fig. S4



Unlike Fig. 8 in the ms, this vertically expanded graph shows all ten ezrin forms (labeling the five that reach more than 3% of the total at 50 h) to illustrate the typical appearance of many of the lines that were omitted from Fig. 8.

Since the published ms included an experiment (Fig. 2) with stimulation of cells after 24 h of wt ezrin overexpression, we show here a modeling of that response. The stimulated portion (after 10 min) of the Stim model (kp = 0.2/min, kdp = 0.02/min) was used, with the starting amounts of all ezrin forms being set to the amounts found at the 24-h point in the unstimulated 50-h wt model run (Fig. S4, above). At this point, the wt model showed total ezrin being 2.4 times greater than endogenous ezrin and we modeled no further increase for the brief 50-min run shown in Fig. S5.

Fig. S5. Modeled stimulation of parietal cells after 24 h of wt ezrin overexpression:



The preponderance of ezrin forms at the apical membrane reflects the experimental results.