Published in final edited form as:

Phys Biol. 2012 June ; 9(3): 036010. doi:10.1088/1478-3975/9/3/036010.

CaMKII activation and dynamics are independent of holoenzyme structure: an infinite subunit holoenzyme approximation

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Abstract

The combinatorial explosion produced by the multi-state, multi-subunit character of CaMKII has made analysis and modeling of this key signaling protein a significant challenge. Using rule-based and particle-based approaches, we construct exact models of CaMKII holoenzyme dynamics and study these models as a function of the number of subunits per holoenzyme, N . Without phosphatases the dynamics of activation are independent of holoenzyme structure unless phosphorylation significantly alters the kinase activity of a subunit. With phosphatases the model is independent of holoenzyme size for $N > 6$. We introduce an infinite subunit holoenzyme approximation (ISHA), which simplifies the modeling by eliminating the combinatorial complexities encountered in any finite holoenzyme model. The ISHA is an excellent approximation to the full system over a broad range of physiologically relevant parameters. Finally, we demonstrate that the ISHA reproduces the behavior of exact models during synaptic plasticity protocols, which justifies its use as a module in large models of synaptic plasticity.

1. Introduction

Calcium/calmodulin-dependent protein kinase II (CaMKII) is a non-specific serine/threonine kinase which plays an essential role in multiple forms of synaptic plasticity [1, 2]. CaMKII is one of the earliest upstream nodes activated during the processes of long-term potentiation (LTP) and long-term depression (LTD), which are changes in synaptic efficacy thought to underlie learning and memory [3]. LTP and LTD are controlled by a large and complex regulatory network whose mechanisms and functions are still being elucidated [4]. The properties of large regulatory networks are often emergent behaviors which are difficult to study without pairing experimental data with computational modeling and numerical simulations [5, 6]. Indeed, LTP and LTD have been studied using increasingly complex computational models over the past three decades [7]. (See Ref. [8] for an excellent recent review of synaptic plasticity models.)

All CaMKII isoforms are composed of four domains: an N-terminal kinase domain, a regulatory domain, a linker domain, and a C-terminal association domain [9]. CaMKII proteins cluster together through their association domains into 12-subunit holoenzymes [10, 11]. The subunits are arranged with kinase domains extending out from the central core of association domains, and this structure facilitates important regulatory interactions between the subunits [12]. Fig. 1(a) shows a schematic depiction of a 6-subunit holoenzyme.

Any useful model of CaMKII-dependent synaptic plasticity must include a realistic model of CaMKII activation and dynamics. However, CaMKII is notoriously difficult to model because the 12 interacting CaMKII subunits exist in multiple physiologically important states [2]. Exact deterministic models of CaMKII necessarily require many species to describe the hundreds of configurations of subunits within the holoenzyme structure. Such

large networks are undesirable because they are computationally taxing and, more importantly, because it is not practical to include such a large network as a component of the already large networks required to study synaptic plasticity.

To overcome combinatorial complexity and generate tractable networks, we propose an infinite-subunit holoenzyme approximation (ISHA). In this abstraction the holoenzyme is imagined to be infinitely large and interactions between subunits are described in terms of probabilities rather than concentrations. A related approach has been used (implicitly) in other models which describe intersubunit interactions through probabilities [13], but the accuracy of this approximation has never been determined. Here we compare the ISHA to several exact models of CaMKII dynamics in order to define a region of validity for this approximation. Such information will inform future modeling efforts by indicating when an ISHA is appropriate and when a more detailed model is required to accurately describe CaMKII activation.

There are several other modeling approaches which are useful for dealing with the combinatorial complexity of the CaMKII system. Particle-based stochastic simulations [14, 9] track the exact state of each subunit and the exact organization of each holoenzyme, without the need to pre-define each possible holoenzyme configuration. Hybrid approaches use a particle-based stochastic method to model CaMKII and a deterministic reaction-diffusion equation method to model other components of the system [15, 16]. However, such approaches become computationally taxing when modeling large systems for long time periods and frequently require custom-written software. A deterministic ODE-based approach is better suited for use with existing modeling platforms and allows for computationally efficient numerical simulations.

ODE based models of CaMKII usually rely on a combination of three approximations to simplify the system: minimizing the number of states per subunit, lumping several species together as a single species, and reducing the size of the holoenzyme. For example, a popular model by Zhabotinsky [17] employs the first two of these approaches: subunits exist in one of only two states, either inactive or active, and the spatial organization of the holoenzyme is ignored by lumping all holoenzymes according to their number of activated subunits. This model system exhibits bistable behavior as a function of calcium concentration, and Zhabotinsky [17] and others [18, 19] have speculated that this might be the mechanism behind long-lasting synaptic plasticity. This model also suggests that both the dynamic and steady state behavior of the system depend on the number of subunits in the holoenzyme. However, the validity of this model is difficult to ascertain because of the multiple uncontrolled approximations. In fact, subsequent experimental studies failed to detect the bistable behavior predicted by this model [20].

Here we use exact reaction networks for a model with three states per subunit to show that the activation and dynamics of CaMKII are independent of holoenzyme structure over a wide range of physiologically relevant parameters, which suggests that we are free to use any number of subunits to model the CaMKII system. The number of states per subunit in the real system is so large that even a dimer model introduces an undesirable amount of complexity. We therefore use the ISHA in order to overcome the combinatorial complexity associated with any finite holoenzyme structure. We examine the validity of this approximation by comparing it to the exact reaction networks. We show that the approximation is excellent over a broad range of physiologically relevant parameters, and we show how and why the approximation fails outside its region of validity. We show that these results apply to a more realistic model with six states per subunit. Lastly, we demonstrate the validity of the ISHA in models of synaptic plasticity.

2. Models

2.1. CaMKII structure

CaMKII subunits exist in at least six physiologically relevant states depending on the binding of calmodulin (CaM) and the phosphorylation state of T286 and T305/T306, as schematically illustrated in Fig. 1(b). CaMKII is autoinhibited in the absence of calcium and CaM because the regulatory domain blocks the ATP and substrate binding sites on the kinase domain [21]. CaMKII is activated when calcium-bound CaM binds to the regulatory domain and displaces it from the kinase domain [21]. This exposes T286, which can be phosphorylated by a neighboring active subunit [22, 23, 24]. Once phosphorylated on T286, a CaMKII subunit remains partially active even in the absence of calcium/CaM [25]. CaM dissociation exposes T305/T306 in the CaM binding domain, which are phosphorylated via an intrasubunit reaction [23]. This phosphorylation event prevents CaM from rebinding and thus provides an additional level of regulation [26]. A very slow basal autophosphorylation reaction phosphorylates T305/T306 in the absence of calcium/CaM [27] and produces a completely inert, inactive state by inhibiting CaM-binding. Phosphatases can also produce such a state from the triply phosphorylated state by dephosphorylating T286.

2.2. Six state model

CaM is fully activated by binding four calcium ions, two each at the C-lobe and the N-lobe [28]. The binding at each lobe is cooperative [29, 30], and the C-lobe has a higher calcium affinity but slower dynamics than the N-lobe [31]. Here we ignore the lobe structure and assume CaM binds four calcium ions sequentially. The five states of CaM are denoted CaM_i , where $i = 0, \dots, 4$ labels the number of bound calcium ions. Calcium binding is a standard bimolecular reaction, as shown in Fig. 1(c), where the flux between CaM_i and CaM_{i+1} is given by

$$J_i = -k_{\text{on},i}[\text{Ca}^{2+}]\text{CaM}_i + k_{\text{off},i}\text{CaM}_{i+1}, \quad (1)$$

and the CaM concentrations evolve according to

$$\begin{aligned} \frac{\partial \text{CaM}_0}{\partial t} &= J_0 \\ \frac{\partial \text{CaM}_1}{\partial t} &= -J_0 + J_1 \\ \frac{\partial \text{CaM}_2}{\partial t} &= -J_1 + J_2 + J_{\text{CaMKII}}^{(2)} \\ \frac{\partial \text{CaM}_3}{\partial t} &= -J_2 + J_3 \\ \frac{\partial \text{CaM}_4}{\partial t} &= -J_3 + J_{\text{CaMKII}}, \end{aligned} \quad (2)$$

where $k_{\text{on},i}$ is the on-rate constant for calcium binding to CaM_i , $k_{\text{off},i}$ is the off-rate constant for calcium dissociating from CaM_{i+1} , J_{CaMKII} is the flux describing CaM_4 binding to various forms of CaMKII, and $J_{\text{CaMKII}}^{(2)}$ is the flux describing the Ca^{2+} regulated dissociation of CaM from CaMKII (described below). Such a model is sufficient to describe equilibrium calcium binding and has been used by others to model CaMKII activation [13].

We allow a CaMKII subunit to exist in one of the six states depicted in Fig. 1(b). These six states are denoted as follows:

$$\begin{aligned}
 D_{uu} & \text{--Autoinhibited.} \\
 C_u & \text{--CaM-bound, not phosphorylated.} \\
 C_p & \text{--CaM-bound, phospho-T286.} \\
 D_{pu} & \text{--Phospho-T286, no CaM.} \\
 D_{pp} & \text{--Phospho-T286 and phospho-T305.} \\
 D_{up} & \text{--Phospho-T305.}
 \end{aligned} \tag{3}$$

For simplicity we treat the double phosphorylation event on T305/T306 as a single event on T305. Figure 1(d) shows the reaction diagram which describes conversion of a CaMKII subunit between these six states. We assume that only fully saturated CaM (CaM₄) can bind CaMKII. CaM binds CaMKII with different on rates depending on the phosphorylation state of T286 [32, 33], $k_{\text{on},u}$ and $k_{\text{on},p}$, where the subscripts u and p refer to unphosphorylated and phosphorylated T286, respectively. CaM dissociation can proceed through two pathways: either CaM₄ directly dissociates from CaMKII, or CaM₄ loses two calcium ions and then rapidly dissociates. We do not include the intermediate CaM₂ state, but account for this second pathway with a calcium dependent dissociation rate. The complete flux describing CaM dissociation from CaMKII, $J_{\text{diss},i}$ is given by

$$J_{\text{diss},i} = \left(k_{\text{off},i} + \frac{k_{\text{off},i}^{(2)}}{1 + ([\text{Ca}^{2+}]/K_{\text{Ca},i})^2} \right) C_i, \tag{4}$$

where i equals u or p depending on the phosphorylation state of T286. The first term in parentheses, $k_{\text{off},i}$ is the dissociation rate of CaM₄. The second term in parentheses is a Hill function describing the two step calcium dissociation-dependent pathway, where $k_{\text{off},i}^{(2)}$ is an effective first-order dissociation rate and $K_{\text{Ca},i}$ defines the calcium concentration where the rate is half-maximal.

Phosphorylation of T286 occurs via an intersubunit reaction [23]. The number of neighboring subunits accessible to a kinase domain is unknown. Here we make the common assumption [17, 18, 34] that phosphorylation is uni-directional and that each kinase domain phosphorylates a single neighbor. For example, for the six-subunit holoenzyme shown in Fig. 1(a), subunit (1) phosphorylates subunit (2), subunit (2) phosphorylates subunit (3), and so on. We allow the rate of intersubunit autophosphorylation to depend on the state of the kinase subunit, and we thus define three autophosphorylation rates: C_u phosphorylates C_u with a rate r_1 , C_p phosphorylates C_u with a rate r_2 , and D_{pu} and D_{pp} phosphorylate C_u with a rate r_3 .

CaMKII is dephosphorylated by a variety of protein phosphatases, including PP1 and PP2A [35]. We model dephosphorylation with the standard irreversible Michaelis-Menten reaction, and we assume equal phosphatase activity toward T286 and T305 with no preference for the order of dephosphorylation. The Michaelis-Menten constants have been determined for PP1 acting on CaMKII, and we use these numbers here [20]. These values, along with the values of all other rate constants in the six state model, can be found in Table S1.

The direct outputs of this model are the concentrations of CaMKII subunits in each of the states listed in Eq. 3. However, we are usually interested in quantities such as the total concentration of phosphorylated subunits or the total number of active subunits. Thus, we define Σ_C to be the total concentration of CaM-bound subunits, and Σ_P to be the total

concentration of subunits phosphorylated at a particular site. (It will always be clear in context if Σ_P refers to phospho-T286 or phospho-T305.) In order to compare the results of simulations with different concentration of CaMKII, it is useful to plot our results in terms of the fraction of subunits in a certain state. We therefore define

$$F_C = \frac{\Sigma_C}{\text{CaMKII}_{\text{tot}}}, \quad (5)$$

$$F_P = \frac{\Sigma_P}{\text{CaMKII}_{\text{tot}}},$$

where F_C is the fraction of CaM-bound CaMKII subunits, and F_P is the fraction of phosphorylated CaMKII subunits.

2.3. Three state model

Intersubunit phosphorylation of T286 is the only reaction which depends on the holoenzyme structure. Therefore, we study the effect of varying the number of subunits per holoenzyme, N , using a minimal model which includes this reaction. This model consists of three CaMKII states, which we relabel as follows: an autoinhibited state (U), a CaM-bound state (C), and a CaM-bound, phospho-T286 state (P). The model is minimal in the sense that it is composed of the smallest subset of CaMKII states shown in Fig. 1(b) required to model CaMKII activation by CaM and to distinguish between the phosphorylated and unphosphorylated active states. For simplicity we ignore calcium binding and assume that all CaM is fully calcium saturated, CaM_4 . The reactions in this model are a subset of those described above. Notably, this model does not allow CaM_4 to dissociate from the phospho-T286 state. Below we study the dynamics of this system as we vary the number of subunits per holoenzyme. The dimer holoenzyme is described by 6 species, which we call M_{ij} , where i and j describe the state of each subunit: M_{UU} , M_{CU} , M_{CC} , M_{PU} , M_{PC} , and M_{PP} . The dynamics of this system are given by the following first-order ODEs:

$$\begin{aligned} \frac{\partial M_{UU}}{\partial t} &= -2k_{\text{on},u} \text{CaM}_4 M_{UU} + k_{\text{off},u} M_{CU} \\ \frac{\partial M_{CU}}{\partial t} &= 2k_{\text{on},u} \text{CaM}_4 M_{UU} - (k_{\text{off},u} + k_{\text{on},u} \text{CaM}_4) M_{CU} + 2k_{\text{off},u} M_{CC} + \frac{k_{\text{cat}}[\text{PP1}]M_{PU}}{K_m + \Sigma_P} \\ \frac{\partial M_{CC}}{\partial t} &= k_{\text{on},u} \text{CaM}_4 M_{CU} - 2k_{\text{off},u} M_{CC} - 2r_1 M_{CC} + \frac{k_{\text{cat}}[\text{PP1}]M_{PC}}{K_m + \Sigma_P} \\ \frac{\partial M_{PU}}{\partial t} &= -k_{\text{on},u} \text{CaM}_4 M_{PU} + k_{\text{off},u} M_{PC} - \frac{k_{\text{cat}}[\text{PP1}]M_{PU}}{K_m + \Sigma_P} \\ \frac{\partial M_{PC}}{\partial t} &= k_{\text{on},u} \text{CaM}_4 M_{PU} - k_{\text{off},u} M_{PC} + 2r_1 M_{CC} - r_2 M_{PC} - \frac{k_{\text{cat}}[\text{PP1}]M_{PC}}{K_m + \Sigma_P} + \frac{2k_{\text{cat}}[\text{PP1}]M_{PP}}{K_m + \Sigma_P} \\ \frac{\partial M_{PP}}{\partial t} &= r_2 M_{PC} - \frac{2k_{\text{cat}}[\text{PP1}]M_{PP}}{K_m + \Sigma_P}, \end{aligned} \quad (6)$$

where $\Sigma_P = M_{PU} + M_{PC} + 2M_{PP}$ is the total concentration of phosphorylated subunits. The kinetic equations for larger holoenzymes are similar but require more species and more reactions, as listed in Table 1.

In the ISHA, the phosphorylation of a subunit in state C depends on the probability of its neighbor being a C or a P . These probabilities are simply given by $(C/\text{CaMKII}_{\text{tot}})$ and $(P/\text{CaMKII}_{\text{tot}})$, respectively, where $\text{CaMKII}_{\text{tot}} = U + C + P$ is the total concentration of CaMKII subunits. This system is described by

$$\begin{aligned} \frac{\partial U}{\partial t} &= -k_{\text{on},u} \text{CaM}_4 U + k_{\text{off},u} C \\ \frac{\partial C}{\partial t} &= k_{\text{on},u} \text{CaM}_4 U - k_{\text{off},u} C - r_1 \left(\frac{C}{\text{CaMKII}_{\text{tot}}} \right) C - r_2 \left(\frac{P}{\text{CaMKII}_{\text{tot}}} \right) C + \frac{k_{\text{cat}}[\text{PP1}]P}{K_m + P} \\ \frac{\partial P}{\partial t} &= r_1 \left(\frac{C}{\text{CaMKII}_{\text{tot}}} \right) C + r_2 \left(\frac{P}{\text{CaMKII}_{\text{tot}}} \right) C - \frac{k_{\text{cat}}[\text{PP1}]P}{K_m + P}. \end{aligned} \quad (7)$$

This description is mathematically similar to a monomeric CaMKII model, which has been used by others to overcome combinatorial complexity [5, 36]. In a monomeric model the autophosphorylation reaction is a bimolecular interaction with a second-order rate constant k_i , and the ISHA is mathematically equivalent if we define $k_i \equiv r_i/\text{CaMKII}_{\text{tot}}$. The ISHA affords a better connection to the actual system because the autophosphorylation rates are written in terms of the measured first-order rate constants, r_i . Moreover, the ISHA can be directly compared to exact models because they depend on the same rate constants, whereas the physical interpretation of a monomeric model does not allow a direct comparison to exact holoenzyme models.

2.4. Modeling platforms

Virtual Cell [37] is a freely distributed, web-based modeling and simulation software package available at vcell.org. VCell allows the user to define a reaction network which can then be simulated in arbitrary geometries using a finite element method. All simulations used here were performed in a compartmental geometry using the Combined Stiff Solver (IDA/CVODE) with a variable time step.

BioNetGen [38] is a rule-based modeling platform which may be launched from Virtual Cell [39]. The software generates reaction networks based on a minimum set of reaction rules. It is particularly convenient here because the CaMKII system involves relatively few reaction types which are repeated many times for each of the different holoenzyme structures. The models generated within BioNetGen were exported via SBML to Virtual Cell for simulations and data collection. Two example BioNetGen files are included in the supplementary material, one for the three state trimer and another for the six state trimer.

We used BioNetGen to generate Virtual Cell models of the three state system for the dimer holoenzyme through the hexamer holoenzyme. The number of species and reactions in these systems are listed in Table 1. For example, the eight species of the dimer model (with phosphatase) are CaM_4 , $PP1$, and the six CaMKII dimers described in Eq. 6. The eight reactions of this model are three CaM-binding reactions (to M_{UU} , M_{CU} , and M_{PU}), two autophosphorylation reactions (for M_{CC} and M_{PC}), and three dephosphorylation reactions (for M_{PU} , M_{PC} , and M_{PP}). Models with larger holoenzymes require more species and reactions because of the combinatorial complexity discussed above, but all reactions fall into the same three classes of either CaM-binding, autophosphorylation, or dephosphorylation. These Virtual Cell models and all others used in this paper are publicly available under username pjmachal. The Virtual Cell model names are listed in Table S2.

Conventional rule-based modeling generates a complete reaction network whose dynamics are given by a set of coupled first order ODEs. For relatively small networks, it is straightforward to solve these equations using standard ODE solvers. For the CaMKII system, the size of the reaction network increases non-linearly (approximately exponentially) with holoenzyme size, as shown in Table 1, and the reaction networks quickly grow to a size which overwhelms available computational resources. For example, the time required to generate the network by iterating the rules, which depends on processor speed, grows exponentially with holoenzyme size. A 2.53 GHz Intel Xeon processor took 6 hours to generate the network for a six-state pentamer model, and an exponential fit suggests it would take over 290 years to generate the network for a six-state, 10-subunit-holoenzyme model. Likewise, the memory required to store the resulting network is proportional to the total number of species and reactions, and thus also grows exponentially with network size. For these reasons, conventional rule-based modeling is not suitable for systems, such as large holoenzymes, which require large reaction networks[40].

To overcome such difficulties we used a custom Java program to run particle-based stochastic simulations. (This approach is frequently described as “network free” modeling [41].) In this approach the simulation keeps track of the current state of each individual CaMKII subunit. The simulation proceeds according to Gillespie’s exact stochastic simulation algorithm (SSA)[42], but transition probabilities are calculated for individual subunits instead of holoenzyme species. There is no need to enumerate the total number of possible holoenzyme configurations because it is the individual subunits which undergo transitions, and different holoenzyme configurations arise naturally as the subunits evolve. The results of such a simulation will differ from those obtained by directly solving the ODE system, but it has been proved that the resulting average of many stochastic simulations agrees with the results of the deterministic system[42].

In our Java program CaMKII holoenzymes were stored as $N \times 1$ arrays, where each element in the array stored a single CaMKII subunit. Each subunit could undergo one of three types of reactions depending on its current state and the state of the system: a CaM binding/unbinding reaction, a phosphorylation reaction (including both intersubunit T286 phosphorylation and intrasubunit T305/T306 phosphorylation), and a phosphatase-mediated dephosphorylation reaction. The i th element could phosphorylate the $(i + 1)$ th element, and the M th element could phosphorylate the 1st element. Simulations were run using the SSA. The Java code is available upon request.

3. Results

3.1. Three state model

We begin by studying a model with three states per subunit - an autoinhibited state (U), a CaM-bound state (C), and CaM-bound, phospho-T286 state (P) - as described in the methods. Intersubunit phosphorylation of T286 is the only reaction that depends on the holoenzyme structure, and thus this is the minimal system required to study the function of holoenzyme size. The three state model is also sufficient to describe the classic experiments of De Koninck and Schulman [25], which were constructed to keep calcium-saturated CaM buffered, and occurred on short enough time scales that CaM dissociation from the “trapped” state can be ignored. Our purpose here is simply to study the dependence of the model on the number of subunits per holoenzyme. Thus, we simplify the analysis by setting $k_{on,u} = 1 \mu\text{M}^{-1} \text{s}^{-1}$, $k_{off,u} = 0.0667 \text{s}^{-1}$, and $r_1 = 1 \text{s}^{-1}$, where $k_{off,u}$ was chosen to give a dissociation constant $K_D = 66.7 \text{nM}$ [25]. Initially we assume $r_2 = r_1$, but we vary this below, assuming $r_2 > r_1$.

3.1.1. Dimer through Hexamer—We used BioNetGen to generate exact reaction networks of the three state model as N varied from 2 (dimer) through 6 (hexamer) and ran these models using VCell. Figure 2 shows the fraction of phosphorylated subunits, $F_P = \sum P / \text{CaMKII}_{\text{tot}}$, after a 1 second and a 6 second autophosphorylation reaction in the absence of phosphatases, as a function of calcium-saturated CaM. Note that the system cannot achieve equilibrium without phosphatases, and thus these curves show that the time evolution is nearly independent of holoenzyme size. The dimer result is always within 2% of the hexamer result, and all other holoenzymes are always within 1% of the hexamer result.

CaMKII autonomy depends on the frequency of stimulation [25], and this property is a direct result of the autophosphorylation reaction which depends on the holoenzyme structure. We thus investigated the frequency dependence of activation as a function of holoenzyme size. We subjected the system to CaM pulses of varying duration (500 ms, 200 ms, or 80 ms), and varied the pulse number to keep the total CaM exposure constant. The frequency was varied by changing the time between pulses. The results of such simulations are shown in Fig. 3, where we plot the fraction of phosphorylated CaM as a function of

frequency. Again, there are no significant differences between holoenzymes of different sizes.

The autophosphorylation reaction will depend on the holoenzyme size when $r_2 \neq r_1$. To see this, consider the limiting system with $r_2 = 0$. In this limit there can be only one phosphorylation reaction (per holoenzyme) in the dimer, because the phosphorylated subunit has no activity towards its neighbor. Thus, at long times only half the subunits in the dimer system will be phosphorylated. For the trimer, similar reasoning shows that the initial phosphorylation event creates an inactive kinase which will prevent one of the three subunits from being phosphorylated. Thus, in the long time limit two-thirds of the subunits will be phosphorylated in the trimer system. An exact analysis shows that

$$F_p = \sum_{n=1}^N \frac{(-1)^{n+1}}{n!} \quad (8)$$

for a holoenzyme of size N . A derivation of this result is beyond the scope of this text, but it is worth noting that as N increases this fraction converges very rapidly to its asymptotic limit, $F_p \rightarrow 1 - e^{-1} = 0.63$. Equation 8 shows that F_p is largest for the trimer. The physical reason for this is that all larger holoenzymes can evolve to states which have fewer than $2/3$ of their subunits phosphorylated. For example, the final configuration of the tetramer has a 50% chance of having three phosphorylated subunits and a 50% chance of having 2 phosphorylated subunits, giving $F_p = 5/8 = 0.625$. In Fig. 4 we plot the fraction of phosphorylated subunits after a 6 second autophosphorylation reaction with $r_2 = 0$. As expected from Eq. 8, in the large CaM limit this fraction goes to $1/2$ for the dimer, $2/3$ for the trimer, and rapidly approaches 0.63 for larger holoenzymes. The convergence is such that the differences between the tetramer, pentamer, and hexamer are negligible (less than 1%). As $r_2 = 0$ is a limiting case, the agreement between the models only improves as r_2 increases, until we recover the near perfect agreement at $r_2 = r_1$ seen in Fig. 2.

Up to now we have studied the system in the absence of phosphatases. In the presence of PP1 the autophosphorylation reaction can depend on the holoenzyme structure. It is easiest to see this by comparing the dimer to larger holoenzymes. Consider the case where $r_2 = r_1$ and assume that the concentrations of CaM and PP1 are very low, such that $\text{CaM}_4/K_D \ll 1$ and $k_{cat}[\text{PP1}]/K_m \ll r_1$, in which case autophosphorylation is much faster than dephosphorylation. The probability of two adjacent CaMKII subunits binding CaM₄ is very low, proportional to $[\text{CaM}_4]^2$, independent of N . In the dimer both subunits can act as a substrate for the other activated kinase, and thus both get phosphorylated. In larger holoenzymes only one of these subunits can act as the substrate (because phosphorylation is directional), and thus only one of the two gets phosphorylated. Furthermore, as autophosphorylation is much faster than dephosphorylation, in the dimer most dephosphorylation events are immediately counteracted by an autophosphorylation event on the same subunit. In larger holoenzymes, the rare autophosphorylated subunit is usually not paired with another active subunit, and dephosphorylation is not countered as strongly in these cases. Thus, at equilibrium we expect the dimer to have a larger fraction of phosphorylated subunits than larger holoenzymes. The argument can be extrapolated to show that, in this limiting case of low CaM and low PP1, F_p is a decreasing function of N , but such an argument does not indicate the size of the effect.

To quantify the effect of phosphatases, in Fig. 5(a) we plot the equilibrium fraction of phosphorylated subunits as a function of CaM₄ for three different concentrations of PP1. We see that there are significant differences between the dimer and hexamer at low and intermediate CaM concentrations for the two lower PP1 concentrations, as expected from

the argument above. The system appears to converge to a limiting value as N increases, but convergence is slow at these low values of N . The discrepancies between the models at low PP1 and low CaM₄ are shown in Fig. 5(b), where we replot the curves for 0.1 μM PP1 on a log-log scale. At the lowest CaM₄ concentrations there are about 10 times as many phosphorylated subunits in the dimer as in the hexamer, and even the pentamer differs from the hexamer by about 10% at certain CaM₄ concentrations.

The results of this section show that CaMKII activation depends only very weakly on holoenzyme structure. If $r_2 = 0.5r_1$, then the differences between the dimer and the hexamer are negligible in the absence of phosphatases. Even if $r_2 \ll r_1$, then the system converges to a limiting value at the tetramer. The dependence on holoenzyme structure is more pronounced with low concentrations of phosphatases, but even here the system appears to reach a limiting value at the hexamer.

3.1.2. Larger Finite Holoenzymes—In the previous section we restricted our study to holoenzymes with less than 6 subunits because significantly larger holoenzymes produce networks that are too large to efficiently simulate with VCell. To study larger holoenzymes we used a custom particle-based stochastic model, as described in the methods. We used this code to simulate the three-state model both with and without phosphatases. In Fig. 6(a) we plot both F_P and F_C , the fraction of CaM-bound, unphosphorylated subunits, as a function of holoenzyme size after a 6 second autophosphorylation reaction with 0.1 μM CaM₄. The stochastic results agree with the VCell results for $N = 6$, as expected. Furthermore, there are no significant differences for $N = 100$. In Fig. 6(b) we plot the equilibrium values of F_P and F_C as a function of holoenzyme size at 0.01 μM CaM₄ and 1.0 μM PP1. We saw above that in this limit of low CaM and low PP1, the system converged very slowly to a limiting value. For $N = 6$, the stochastic results show the same slow convergence to a limiting value, in agreement with the VCell results. Importantly, the stochastic simulations show that the results are independent of holoenzyme size once $N \gtrsim 10$.

3.1.3. Infinite Holoenzyme—The results of the previous sections show that CaMKII activation and phosphorylation are independent of holoenzyme structure over a wide range of parameters. This observation motivates the study of an infinite subunit holoenzyme, which should be an excellent approximation to a finite holoenzyme when the system properties are independent of holoenzyme size. In this approximation the intersubunit phosphorylation reaction depends on the probability of having an active neighboring subunit, as described in the methods. In this section we investigate the validity of this approximation.

In Fig. 7(a) we compare the ISHA to the hexamer by plotting F_P after a 1 second and a 6 second autophosphorylation reaction without PP1 as a function of CaM₄. We see that the two systems agree at intermediate and high CaM concentrations, but that the ISHA significantly overestimates F_P at low CaM. This behavior is easy to understand. At very low CaM most holoenzymes will bind less than two CaMs. Even if every holoenzyme in the system bound a single CaM, the autophosphorylation rate would be zero. (Recall that both the kinase and substrate subunits must bind CaM.) However, in the ISHA even a single binding event produces a “population” of activated subunits which drives autophosphorylation, and this necessarily overestimates the extent of phosphorylation in the finite system. In Fig. 7(b) we compare the ISHA to the hexamer by plotting F_P as a function of CaM₄ in the presence of phosphatases. The ISHA dramatically overestimates F_P for low CaM and low PP1, for the same reasons discussed above.

The ISHA will also overestimate total phosphorylation if $r_2 \ll r_1$. To see this, consider the limiting case $r_2 = 0$. As we saw above, at long times all finite holoenzymes evolve to a

steady state where only a fraction of subunits are phosphorylated. This fraction is 1/2 for the dimer, 2/3 for the trimer, and approaches 0.62 for large holoenzymes. This effect is a direct manifestation of spatial correlations within the holoenzyme. In the ISHA all spatial information is lost and the autophosphorylation reaction never stops. Thus, the infinite holoenzyme evolves towards a state with all subunits phosphorylated, even in the limit $r_2 = 0$. As the ISHA is a good approximation when $r_2 = r_1$, there must be some critical value of r_2/r_1 below which the ISHA fails. In Fig. S1 we show that the ISHA is an acceptable approximation to the hexamer for $r_2/r_1 \gtrsim 0.4$.

We conclude that the ISHA is an excellent approximation to a finite holoenzyme provided that (1) the concentration of CaM_4 is not too small and (2) r_2 is not significantly less than r_1 . The first condition can be made more explicit by noticing that, with our scaling, CaM_4 is short for $k_{\text{on},u}\text{CaM}_4/r_1$. Examining Fig. 7 then shows that the ISHA is an excellent approximation provided $k_{\text{on},u}\text{CaM}_4/r_1 \gtrsim 0.5$. In the real system, $k_{\text{on},u} \approx 30 \mu\text{M}^{-1} \text{s}^{-1}$ and $r_1 \approx 1\text{--}10 \text{s}^{-1}$. Therefore, we expect the ISHA to be accurate for $\text{CaM}_4 \gtrsim 50 \text{nM}$, depending on the value of r_1 .

3.2. Six State Model

Here we consider a more detailed six state model (as described in the methods) to demonstrate that the conclusions of the last section do not depend on the use of the simplified three state model. Exact models of finite holoenzymes showed only weak dependence on the size of the holoenzyme, in line with the differences in the three state model (data not shown). For convenience we compare the ISHA to the trimer, as larger holoenzymes generated extremely large and computationally taxing networks.

In Fig. 8 we compare the ISHA to the trimer by plotting the fraction of phospho-T286 subunits (a–b) and the fraction of phospho-T305 subunits (c–d) as a function of Ca^{2+} (assumed buffered) after a 10 second autophosphorylation reaction for a system with $0.005 \mu\text{M}$ CaMKII and $1.0 \mu\text{M}$ total CaM. Figures 8(a) and (c) show the ISHA is an excellent approximation to the trimer in the absence of PP1. Notably, both models predict that the amount of phospho-T305 is a non-monotonic function of Ca^{2+} . This behavior is easy to understand: at low calcium the curve increases because adding more calcium generates more CaM-bound CaMKII, which generates more phospho-T286 and phospho-T305. At high calcium the curve decreases with increasing calcium because CaM gets “trapped” on CaMKII, which protects T305 from phosphorylation. Figures 8(b) and (d) show that the ISHA overestimates equilibrium phosphorylation levels in the presence of PP1, as expected from the previous section, but that the deviations at $1 \mu\text{M}$ PP1 minimal. Again, both models capture the non-monotonic behavior of phospho-T305.

All of the systems considered so far have $\text{CaMKII}_{\text{tot}} = 0.005 \mu\text{M}$. The results of the three state model with CaM_4 buffered are independent of total CaMKII, but with a fixed amount of CaM the accuracy of the ISHA may depend on the total CaMKII. In Figs. S2 and S3 we compare the ISHA to the trimer model for varying amounts of CaMKII both with and without phosphatases. We find that the ISHA is best if $\text{CaMKII}_{\text{tot}} < \text{CaM}_{\text{tot}}$, but that the approximation is still acceptable (within 20%) even if $\text{CaMKII}_{\text{tot}} \gg \text{CaM}_{\text{tot}}$.

These results confirm that the ISHA remains valid in the same parameter region described above, even for a more complicated model. This is to be expected, as the three state model already includes phosphorylation of T286, which is the only holoenzyme-structure dependent reaction in the system. Subunits act independently for all other reactions, including phosphorylation of T305.

3.3. The ISHA in Synaptic Plasticity

Our primary reason for characterizing the ISHA was to determine its suitability for use in synaptic plasticity models, and we are now in a position to make this determination. Estimates of the CaMKII concentration in the post-synaptic density (PSD) range from 50–200 μM [36]. CaM interacts with many partners and thus the total concentration of CaM is difficult to quantify, but typical estimates are from 10–40 μM [36]. Basal Ca^{2+} concentrations are around 100 nM, but synaptic activity can drive this to 10 μM or higher [43]. The concentration of active PP1 in the PSD has not been directly measured. Previously published models typically require PP1 to be in the 0.1–1.0 μM range in order to get good agreement with experimental data [17, 18, 19].

Thus, for our spine model we use 50 μM CaMKII, 5 μM CaM (which assumes about half the total CaM is unavailable for CaMKII binding), and 0.5 μM PP1. We allow the system to equilibrate at basal (100 nM) calcium, and then subject it to one of two protocols. The first protocol is a high frequency, high amplitude stimulation of the sort used to induce LTP: 10 μM Ca^{2+} pulses at 100 Hz for 0.5 seconds. The second protocol is a low frequency, lower amplitude stimulation of the sort used to induce LTD: 3 μM Ca^{2+} pulses at 1 Hz for 5 minutes. The results of the first protocol are shown in Figs. 9(a) and (b), where we plot the fraction of activated CaMKII, the fraction of phospho-T286 CaMKII, and the fraction of phospho-T305 CaMKII, as a function of time. Here the ISHA is seen to be in excellent agreement with the trimer. The largest difference between the two models is the fraction of phospho-T286, but this difference is not considered significant for our purposes. The results of the second protocol are shown in Figs. 9(c) and (d), where the same fractions are plotted as in Figs. 9(a) and (b). Again, there is excellent agreement between the ISHA and trimer model. The differences between the models are well within the uncertainties of the protein concentrations and rate constants, and thus we conclude that the ISHA is appropriate for use in larger synaptic plasticity models.

4. Discussion

We have investigated the validity of the ISHA by comparing it to exact models of finite holoenzymes using both a simplified three state model and a more complete six state model. The ISHA is an excellent approximation provided (1) that the enzymatic activity of the several active forms of CaMKII are similar ($r_1 \approx r_2 \approx r_3$), and (2) that autophosphorylation is not too much faster than calmodulin binding, $k_{\text{on},u}\text{CaM}_4/r_1 \gtrsim 0.5$.

It is well established that, at physiological ATP concentrations, the kinase activity of autonomous (not CaM-bound) CaMKII towards exogenous substrates is about 40% to 80% of the kinase activity of CaM-bound CaMKII [44]. If we assume that autonomous activity towards neighboring subunits is similar, then this gives $0.8 > r_3/r_1 > 0.4$. The ISHA is an excellent approximation to the real holoenzyme at the upper end of this range, and even at the lower end the approximation is still adequate.

CaM-bound CaMKII has the same activity toward exogenous substrates regardless of the phosphorylation state of T286 [25]. The simplest assumption is that the activity towards neighboring CaMKII subunits is also independent of CaMKII phosphorylation, in which case $r_2 = r_1$ and the ISHA is in excellent agreement with exact models. In fact, all of the available experimental data can be fit with this assumption. However, fits to different experiments give autophosphorylation rates that vary by more than an order of magnitude [45]. In Ref. [16] it was proposed the discrepancies in the measurements might be resolved if $r_2 < r_1$. By fitting autophosphorylation time courses with a hybrid deterministic-stochastic model, they found a best fit required $r_2/r_1 \lesssim 0.08$ [16]. However, the noisy data could also be

reasonably well fit with $r_2 = r_1$ [16], and thus it is difficult to draw any firm conclusions from this study.

De Koninck and Schulman [25] showed that autonomous activity after a 6 second autophosphorylation reaction is about 80% of maximal CaM-stimulated activity. There are two ways to interpret this result: either the phosphorylated CaMKII has the same activity as CaM-bound CaMKII, but only 80% of the subunits are phosphorylated in a 6 second reaction, or all of the subunits are phosphorylated but an autonomous subunit only has 80% of the activity of a CaM-bound subunit. More recent data would favor the latter explanation [44]. Either way, the data clearly show that $r_2 \neq 0$. In fact, the data require $r_2 > 0.1 \text{ s}^{-1}$, but do not put an upper bound on r_1 , and thus are not useful for determining r_2/r_1 .

Bradshaw, et al. [45] provides time courses of phosphate incorporation and autonomous activity, and the experimental conditions are such that a two state model of CaMKII is appropriate. In Fig. S4 we show that the autonomous activity data (from Fig. 2(a) in Ref. [45]) is best fit by $r_2 = r_1$, and generally requires $r_2/r_1 > 0.6$ for a good fit. In Fig. S4 we also show that the phosphate incorporation data (from Fig. 2(b) in Ref. [45]) is best fit with $r_2/r_1 = 0.66$, although this data is noisier and admits acceptable fits even with $r_2/r_1 \approx 0.03$. Nevertheless, the preponderance of data from Ref. [45] suggest that $r_2/r_1 > 0.5$.

Thus, it is reasonable to assume that $r_2/r_1 \gtrsim 0.5$ and $r_3/r_1 \gtrsim 0.5$, in which case we can expect the ISHA to be valid. It is worth noting that these autophosphorylation rates are dependent on both ATP concentration and temperature [45], and thus their ratios may not be constant. If it turns out that our assumption is wrong and $r_2 \ll r_1$, then not only does the ISHA fail but also a dimer model poorly describes CaMKII dynamics (see Fig. 4). In this case the best approximation is to consider a modified ISHA where the subunits are grouped into dimers on the infinite lattice and the dynamic variables are the joint probability distributions of these dimers. In this scheme half of the subunits have exact information about the state of their neighbor and half of the subunits have only probabilistic information about their neighbor, as in the original ISHA. This model requires as many species as the dimer model, but gives phosphorylation levels that are within 7% of the hexamer result even when $r_2 = 0$, as opposed to the finite dimer model which is only within 20% of the hexamer in this limit.

The most complicated model considered here allows for six states per subunit. In reality the number of physiologically relevant states is much greater than six. CaMKII contains several other phosphorylation sites with no known regulatory function [46]. In addition, CaMKII can bind unsaturated CaM [47], although it is unclear if unsaturated CaM can fully displace the regulatory domain and activate the enzyme [48]. CaMKII binding to unsaturated CaM is a low affinity interaction, but the affinity of CaM for calcium significantly increases upon CaMKII binding [49, 50], and thus this can be a significant CaMKII activation pathway [36]. Lastly, recent crystallographic and biochemical studies suggest that CaMKII subunits pair up within the holoenzyme [51, 52], which generates positive cooperativity in CaM-binding and activation [53]. Here we ignored other phosphorylation states, the binding of unsaturated CaM, and cooperative CaM binding. It is straightforward to include such processes in the ISHA, but they add additional complexity and are not required to study the validity of the ISHA.

We have shown that the activation and dynamics of CaMKII are well described with an infinite subunit holoenzyme approximation over a wide range of parameters, both with and without phosphatases. Importantly, the concentrations of various proteins in the PSD and the relevant in vivo rate constants make the ISHA appropriate for use in models of synaptic plasticity. The ISHA completely eliminates the combinatorial complexity that has plagued previous modeling efforts, and its simplicity makes it ideal either as a module in a larger

model or as a means to investigate more detailed mechanisms of CaMKII activation. With its validity verified, we expect future modeling efforts will make extensive use of this approximation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Igor Novak, Greg Huber, and Boris Slepchenko for helpful discussions regarding spatial correlations in the $r_2 = 0$ limit. We would also like to thank Michael Blinov for his suggestions and expert technical assistance with BioNetGen.

This work was supported by NIH postdoctoral fellowship 1F32 NS077751-01 and NIH grant P41 RR013186.

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Figure 1. Model states and reactions

(A) A schematic of the CaMKII holoenzyme structure. For simplicity, the holoenzyme is shown with 6 subunits instead of 12. The subunit numbering is arbitrary. A, association domain. K, kinase domain. (B) Six of the physiologically relevant states of CaMKII considered here. D_{UU} - Autoinhibited. C_U - CaM-bound, unphosphorylated. C_P - CaM-bound, phosphorylated on T286. D_{PU} - Phosphorylated on T286. D_{PP} - Phosphorylated on T286, T305, and T306. D_{UP} - Phosphorylated on T305 and T306. States D_{UU} and D_{UP} are inactive; the other states have varying activity binding to CaM. CaM_I binds Ca²⁺ in a standard bimolecular levels. (C) Calcium reaction to become CaM_{I+1}. (D) Reaction diagram describing conversion between the six states of CaMKII. Phosphatase-mediated dephosphorylations are not shown. See the text for details.

Figure 2. Time dependent autophosphorylation of CaMKII using the three state model

The fraction of phosphorylated subunits after a 1 second and 6 second autophosphorylation reaction is plotted as a function of CaM_4 for a system without phosphatase. The inset shows an expanded view of the values for the 1 second reaction at 1 μM CaM_4 . At this scale the minute difference between the dimer result and the other results is visible. The results from all other holoenzyme sizes remain indistinguishable.

Figure 3. Frequency dependent phosphorylation of CaMKII using the three state model
 The fraction of phosphorylated CaMKII subunits is plotted as a function of the frequency of $2 \mu\text{M}$ CaM_4 pulses, for pulse durations of 500 ms, 200 ms, and 80 ms. The total number of pulses was varied to keep the total time of CaM_4 exposure constant at 6 seconds: 12 pulses for 500 ms, 30 pulses for 200 ms, and 75 pulses for 80 ms. The frequency was varied by changing the time between the pulses. This sets maximum frequencies of 2 s^{-1} , 5 s^{-1} , and 12.5 s^{-1} for the 500 ms, 200 ms, and 80 ms runs, respectively.

Figure 4. Time dependent autophosphorylation of CaMKII using the three state model with $r_2 = 0$.

The fraction of phosphorylated CaMKII subunits after a 6 second autophosphorylation reaction is plotted as a function of CaM₄ for the limiting case $r_2 = 0$.

Figure 5. The effect of phosphatase on CaMKII autophosphorylation in the three state model
 (A) Equilibrium fraction of phosphorylated CaMKII subunits as a function of CaM_4 for three concentrations of phosphatase: 0.1 μM , 1.0 μM , and 10 μM . (B) The curve for 0.1 μM PP1 is replotted on a log-log scale to illustrate the dramatic differences in model results at low CaM_4 and low PP1.

Figure 6. The CaMKII system is nearly independent of holoenzyme size

F_P (blue) and F_C (red) obtained using a stochastic, particle-based simulation are plotted as a function of holoenzyme size after a 6 second autophosphorylation reaction without phosphatases (A) or in equilibrium with phosphatases (B). Systems in (A) contained $0.1 \mu\text{M}$ CaM_4 . Systems in (B) contained $0.01 \mu\text{M}$ CaM_4 and $1.0 \mu\text{M}$ PP1. Error bars represent the standard deviation of 5 simulations (A) or the standard deviation over the last 200 seconds (once the system reached equilibrium) of a 1000 second simulation (B).

Figure 7. Comparison of the ISHA to the hexamer in a three state model

(A) F_P as a function of CaM_4 for a 1 second (open symbols) and 6 second (closed symbols) autophosphorylation reaction. (B) Equilibrium value of F_P as a function of CaM_4 for varying amounts of PP1.

Figure 8. Comparison of the ISHA to the trimer in a six state model

(A–B) Fraction of phospho-T286 subunits as a function of Ca^{2+} after a 10 second phosphorylation reaction without PP1 (A) or in equilibrium with PP1 (B). (C–D) Fraction of phospho-T305 subunits as a function of Ca^{2+} after a 10 second phosphorylation reaction without PP1 (C) or in equilibrium with PP1 (D). All simulations contained $0.005 \mu\text{M}$ total CaMKII and $1.0 \mu\text{M}$ total CaM. Simulations in (B) and (D) contained $1.0 \mu\text{M}$ PP1.

Figure 9. Comparing the ISHA to the trimer under proto-typical synaptic plasticity protocols (A–B) LTP protocol: at 1 s the system is subjected to $10 \mu\text{M}$ Ca^{2+} pulses at 100 Hz for 0.5 seconds. The duration of each pulse was 50 ms. (A) The fraction of active CaMKII, defined as the sum of all active CaMKII species, is plotted as a function of time for the trimer (black) and the ISHA (red). (B) The fraction of phospho-T286 (solid lines) and the fraction of phospho-T305 (dashed lines) is plotted as a function of time for the trimer (black) and the ISHA (red). (C–D) LTD protocol: at 1 s the system is subjected to $3 \mu\text{M}$ Ca^{2+} pulses at 1 Hz for 300 seconds. The duration of each pulse was 300 ms. (C) The fraction of active CaMKII as a function of time for the trimer (black) and the ISHA (red). The inset shows an expanded view between 175 and 181 seconds, and shows the 1 Hz oscillation in activated CaMKII. (D) The fraction of phospho-T286 (solid lines) and phospho-T305 (dashed lines) as a function of time for the trimer (black) and the ISHA (red). Total protein concentrations were the same for both protocols: $50 \mu\text{M}$ total CaMKII, $5 \mu\text{M}$ total CaM, and $0.5 \mu\text{M}$ PP1.

Table 1

The number of species and reactions required in the three state model as the holoenzyme size increases from the dimer through the hexamer. Models with and without phosphatase are considered separately. See the text for more details.

	Phosphatase	Species	Reactions
Dimer	–	7	5
	+	8	8
Trimer	–	12	14
	+	13	22
Tetramer	–	25	43
	+	26	68
Pentamer	–	52	127
	+	53	202
Hexamer	–	131	396
	+	132	632