Journal of Integrative Neuroscience, Vol. 9, No. 3 (2010) 1–15

DOI: 10.1142/S0219635210002482

ICP Imperial College Press
www.icpress.co.uk

"MEMORY BYTES" — MOLECULAR MATCH FOR CaMKII PHOSPHORYLATION ENCODING OF MICROTUBULE LATTICES

STUART R. HAMEROFF*, TRAVIS J. A. CRADDOCK† and J. A. TUSZYNSKI†,‡

*Departments of Anesthesiology and Psychology
Center for Consciousness Studies
The University of Arizona Health Sciences Center
Tucson, Arizona USA 85724

†Department of Physics
University of Alberta, Edmonton, Alberta
T6G 2G6, Canada

‡Division of Experimental Oncology
Cross Cancer Institute 11560
University Avenue, Edmonton
AB T6G 1Z2, Canada

Received 3 August 2010
Accepted 2 September 2010

Learning, memory and long-term potentiation (LTP) are supported by factors including post-synaptic calcium ion flux activating and transforming the hexagonal calcium-calmodulin kinase II (CaMKII) holoenzyme. Upon calcium-induced activation, up to six kinase domains extend upward, and up to six kinase domains extend downward from the CaMKII association domain, the fully activated holoenzyme resembling a robotic insect 20 nanometers in length. Each extended kinase domain can be phosphorylated, and able to phosphorylate other proteins, thus potentially further encoding synaptic information at intraneuronal molecular sites for memory storage, processing and distribution. Candidate sites for phosphorylation-encoded molecular memory include microtubules, cylindrical lattice polymers of the protein tubulin. Using molecular modeling, we find spatial dimensions and geometry of the six extended CaMKII kinase domains can precisely match those of microtubule hexagonal lattice neighborhoods (both A- and B-lattices), and show two feasible phosphorylation mechanisms. In one, phosphorylation sites (e.g. valine 208) on a CaMKII extended kinase domain interact with serine 444 on a C-terminal 'tail' of tubulin. In the second, the CaMKII kinase domain unfurls, enabling phosphorylation sites to contact threonine and serine sites on the tubulin surface. We suggest sets of six CaMKII kinase domains phosphorylate hexagonal microtubule lattice neighborhoods collectively, e.g. conveying synaptic information as ordered arrays of six "bits", and thus a "byte", with (minimally) 2^6 (64) possible bit states per CaMKII-microtubule interaction. We model two levels of interaction between CaMKII and microtubules, suggesting a testable framework for molecular memory encoding.

Keywords: Microtubule; calmodulin kinase; phosphorylation; dendrite; memory; neuron.

1. Introduction

Learning and memory are understood as synaptic plasticity among brain neurons, shaping activity through neuronal networks [36, 17], and supported by "long-term potentiation" (LTP) [28, 2, 4] an experimental paradigm in which brief repetitive pre-synaptic stimulation causes prolonged post-synaptic sensitivity, e.g. to glutamate. Glutamate receptor binding opens membrane calcium channels, causing calcium ion flux (Ca^{2+}) into dendritic spines, shafts and cell bodies which in turn results in various effects including (via Ca^{2+}/calmodulin) activation and phosphorylation of the hexagonal calcium-calmodulin kinase II (CaMKII) holoenzyme.

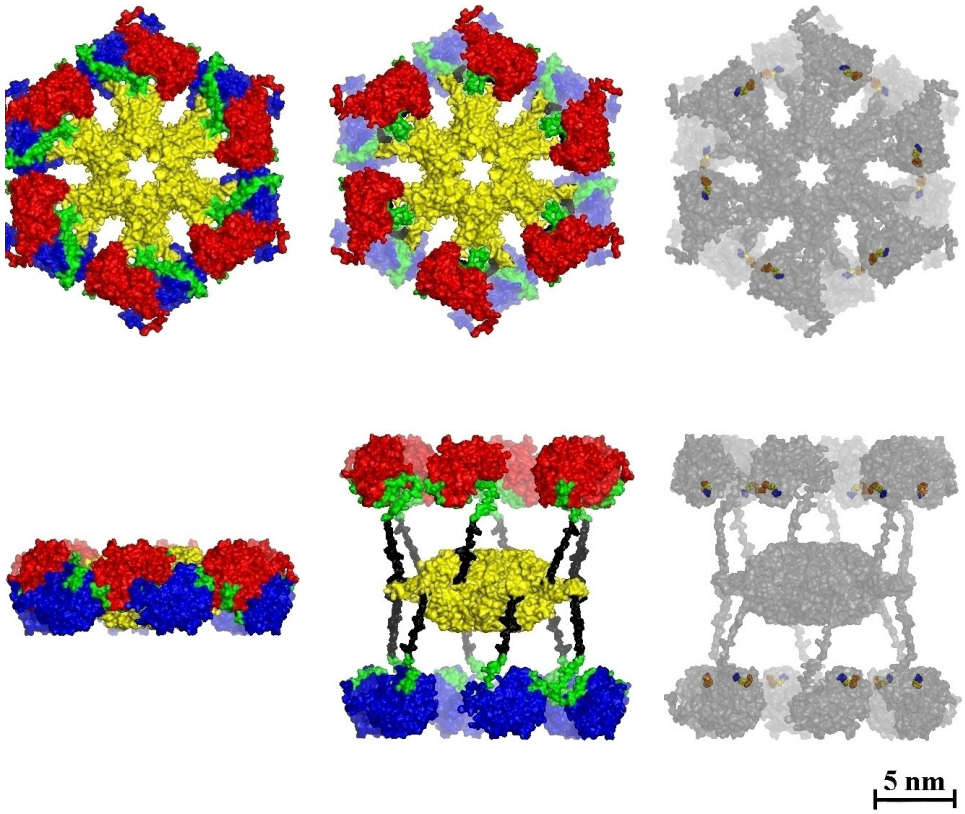
Activation/phosphorylation prolongs CaMKII activity, suggesting that memory of Ca^{2+} synaptic events is encoded in CaMKII structure [24–27, 30]. Ca^{2+} activation transforms CaMKII, with up to six kinase domains extending above, and up to six kinase domains extending below the association domain, the fully activated CaMKII resembling a robotic insect 20 nanometers in length (Figs. 1(a) and 1(b)) [39]. Each extended kinase domain can phosphorylate a substrate.

Localization of activated CaMKII correlates in some way with memory. In LTP, activated CaMKII rapidly distributes in dendrites via diffusion, molecular motors and/or cytoskeletal actin and microtubules [43, 11]. CaMKII binds to microtubules [22], whose depolymerization prevents rapid CaMKII distribution [1]. Disruption of microtubules into neurofibrillary tangles occurs in Alzheimer disease. First suggested to store memory by Cronly-Dillon *et al.* [5], microtubules are logical sites for CaMKII phosphorylation and memory encoding.

Microtubules are polymers of tubulin, a peanut-shaped protein heterodimer composed of alpha and beta monomers (Fig. 2(a)). Negatively charged C-terminal tails of amino acids protrude from both monomers. Tubulins self-assemble into microtubules (Fig. 2(b)), 25 nanometer diameter hollow cylinders of 13 tubulin chains (protofilaments) aligned alpha-to-beta. Lateral tubulin interactions between protofilaments result in helical winding pathways and two types of lattices (A-lattice and B-lattice, Figs. 3(b) and 3(c)). Unlike microtubules in non-neuronal cells which continuously assemble and disassemble, microtubules in brain neurons are stabilized by specialized proteins [12]. Lattice structure and organizational functions have prompted theoretical models of microtubules as computational automata [13, 14, 46].

Free, unpolymerized tubulin is phosporylated by activated CaMKII on or near the tubulin C-terminal tail [47]. CaMKII generally phosphorylates proteins at the amino acid sequence arginine-X-X-serine/threonine, where X can be any amino acid [33, 20, 44]. Several sites on alpha and beta tubulin follow this sequence [48, 49], another potential site being serine 444 on the C-terminal tail of beta tubulin [41, 7].

Phosphorylation interactions between CaMKII and tubulin assembled in microtubule lattices are unknown. In this study we used molecular modelling to examine possible binding, phosphorylation and information encoding between activated CaMKII and microtubule lattices.



(a) (b) (c)

Fig. 1. The calcium/calmodulin-dependent protein kinase II holoenzyme. Upper – face view, Lower – side view. Kinase domains are in red and blue, association domain in yellow, autoregulatory domains in green and extenders/legs in black. (a) Non-activated, (b) activated, (c) activated with phosphorylation sites in "S-T hydrophobic site" highlighted: yellow – valine 208, orange – tryptophan 237, blue – threonine 286. Scale bar: 5 nanometers.

2. Materials and Methods

We used molecular modeling to construct the activated CaMKII holoenzyme, tubulin dimers, microtubules and microtubule A- and B-lattice neighborhood patches.

To construct the activated CaMKII holoenzyme, sequences of the human α CaMKII kinase, autoregulatory, and linker domains as defined by Dosemici *et al.* [9] and association domain were taken from Tombes *et al.* [45] and used to build homology models. The crystal structures 1HKX [18] and 2VZ6 [35] were used as templates to build the association domain, and the kinase and autoregulatory domains respectively using MODELLER 9V6 [10]. The CaMKII holoenzyme structure was built with PYMOL 0.99rc6 [6] using the geometry described in Rosenberg *et al.*, [39] with the linker region constructed as a linear chain of residues joining the autoregulatory region and the association domain. Specific kinase domain amino acids associated

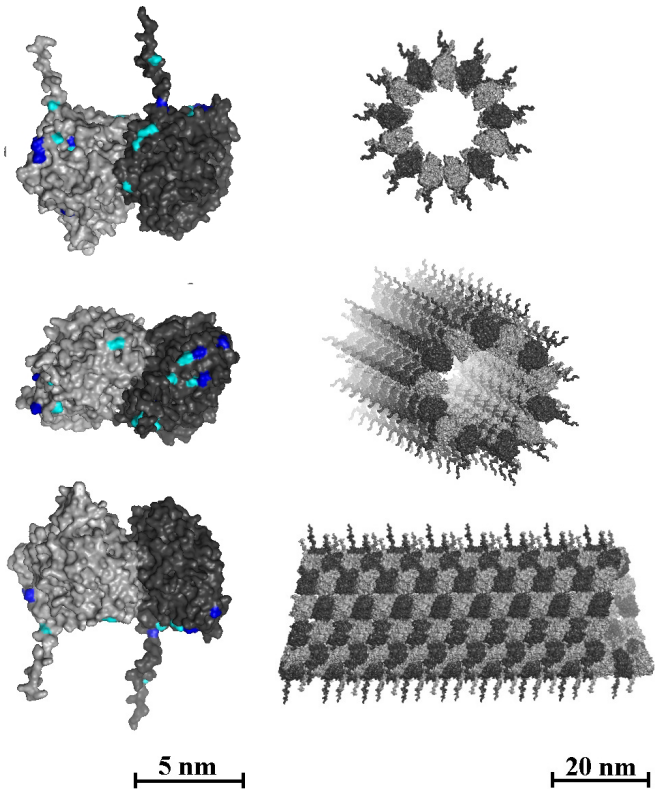


Fig. 2. Microtubule constituent protein tubulin and assembled microtubule composed of tubulins. Light gray – alpha tubulin, Dark gray – beta tubulin. Negatively charged C-termini tails extend from each alpha and beta tubulin. (a) Top, middle and bottom – tubulin in 90 degree rotation. In the middle picture, the C termini are oriented toward the viewer, and not seen. Potential phosphorylation sites are highlighted: Blue – threonine residues, Cyan – serine residues. Serine 444 (cyan) is on the beta tubulin C terminus (top). A cluster of serines (Ser 335, 338, 339) is on the beta tubulin surface along with threonines (top, middle). Scale bar: 5 nanometers. (b) A-lattice microtubule: Upper – Along the longitudinal axis through the microtubule lumen, Middle – angled view, Lower – side view. Scale bar: 20 nanometers.

with phosphorylation (valine 208, tryptophan 237, threonine 286) were identified and color-coded.

The tubulin protein structure 1JFF [29] was repaired by adding missing residues from 1TUB [32]. The repaired 1JFF dimer was then solvated, neutralized and energy-minimized using NAMD [34]. The minimized and repaired 1JFF structure was used as a template to build basic homology models of TUBA1A and TUBB3 using MODELLER 9V6 [10]. Specific amino acids appropriate for phosphorylation (serines and threonines) were identified and color-coded. Using this dimer, microtubules and the microtubule A- and B-lattice structures were built with PYMOL 0.99rc6 [6] using the microtubule geometry described by Li *et al.* [23] and Sept *et al.* [40]. Changes in positional geometry to illustrate interactions between CaMKII and

tubulin/microtubule lattices were modeled using PYMOL 0.99rc6 [6]. All images were generated in PYMOL 0.99rc6 [6].

Using the constructed models for CaMKII, tubulin and microtubule neighborhood lattice patches, we then (1) compared size and hexagonal geometry of CaMKII extended kinase domains with those of hexagonal microtubule A- and B-lattices, (2) evaluated proximity of phosporylation sites on CaMII kinase domains with those on tubulin, and (3) calculated information capacity for collective phosphorylation encoding between sets of six CaMKII extended kinase domains and hexagonal microtubule lattices.

3. Results

Figure 1(c) shows key phosphorylation sites on inner surfaces of the CaMKII extended kinase domains (top of each "foot"), facing the association domain and consisting of an "S" site and "T" site, apparently related to shorter and longer term phosphorylation respectively [3]. The S and T sites are adjacent arrays of largely hydrophobic amino acid residues including valine 208 (yellow) and tryptophan 237 (orange), occupied by threonine 286 (blue) in the inactive state. (Point mutations at this threonine 286 site in mouse models result in impaired Ca^{2+}-dependent synaptic plasticity, learning and memory [19].) We consider these sites together as the "S-T hydrophobic site", represented by valine 208. The scale bar is 5 nanometers.

Figure 2(a) shows a tubulin dimer, with the alpha monomer in light gray, and the beta monomer in dark gray. Negatively charged C-termini tails extend from each monomer. Tubulin phosphorylation sites are highlighted in color, with threonine residues in dark blue, and serine residues in cyan (blue/green). Serine 444 (cyan) is on the beta tubulin C terminus (top). A cluster of serines in cyan (ser 335, 338, 339) is on the beta tubulin surface along with several blue threonines (top, middle). The scale bar is 5 nanometers.

Figure 3 compares size and geometry of the activated hexagonal CaMKII holoenzyme to microtubule A- and B-lattice neighborhood patches. The scale bar (10 nanometers) refers to all parts of the figure. The CaMKII holoenzyme is shown in face view with snowflake-like radial symmetry. The upper kinases are removed, leaving the association domain (yellow) and six extended kinase domains (red), with regulatory domains in green. Figures 3(b) and 3(c) show the microtubule A- and B-lattice neighborhood patches, respectively, in a two-dimensional plane (microtubule curvature here is neglected).

Figure 3(d) shows the CaMKII holoenzyme overlying the microtubule A-lattice. With minimal realignment (e.g. in the extenders between association and kinase domains) to account for microtubule curvature and lattice asymmetry, CaMKII kinase domains precisely match the microtubule A-lattice geometry, i.e. six extended kinases can interface collectively and simultaneously with six tubulins in an ordered microtubule hexagonal A-lattice. Figures 3(e) and 3(f) show CaMKII overlying a nine-tubulin neighborhood of the microtubule B-lattice, revealing two different

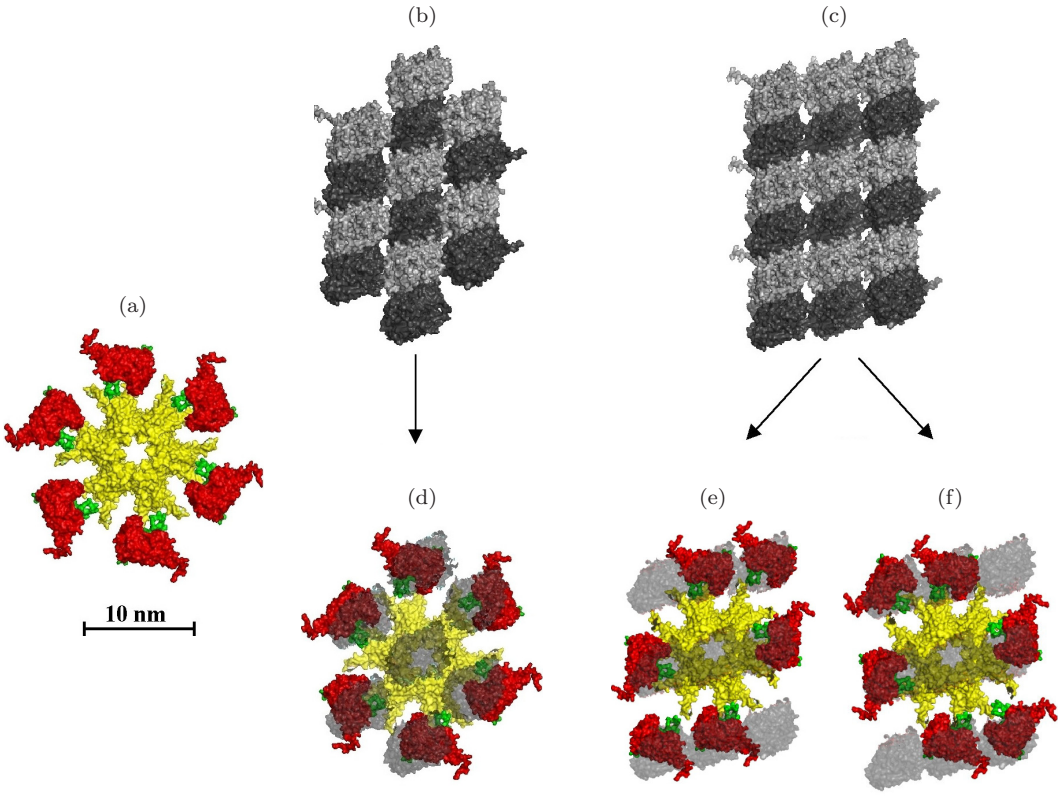


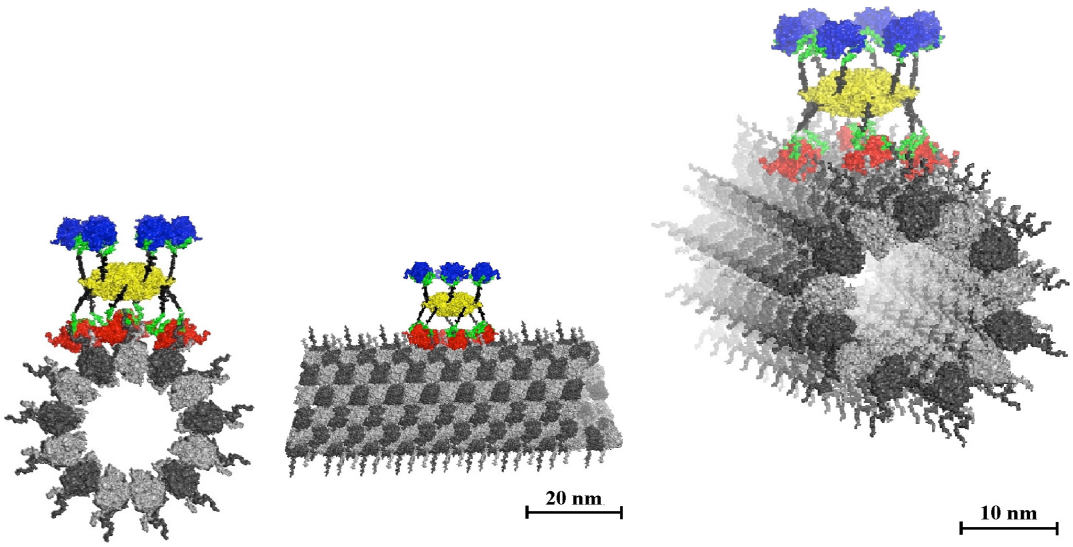
Fig. 3. Alignment of CaMKII holoenzyme with microtubule lattices. Upper kinases not shown. (a) Unaligned CaMKII holoenzyme without microtubule. (b) A seven tubulin dimer neighborhood patch of the microtubule A-lattice, and (c) A nine dimer neighborhood patch of the microtubule B-lattice, both from the viewpoint of the microtubule lumen looking out. In B and C (as in Fig. 2(a)) alpha tubulins are in light gray, beta tubulins in dark gray. In (d) through (f), red CaMKII kinase domains overlay light gray tubulin monomers. (b) shows alignment of activated CaMKII kinase domains with the geometry of the A-lattice. (c), and (d) show two alignments of CaMKII kinase domains with B-lattice geometry.

Scale bar: 10 nanometers.

CaMKII alignments precisely matching the B-lattice geometry. Such interactions can occur with either alpha or beta monomers.

Figure 4 shows three-dimensional views of the activated CaMKII holoenzyme with six extended kinase domains interacting collectively with six tubulins in the microtubule lattice. Scale bars for Figs. 4(a) and 4(b) are 20 nanometers, and 10 nanometers for Fig. 4(c).

Figure 5 shows two possible modes of docking and phosphorylation between CaMKII extended kinase domains and tubulin in a microtubule. We show interactions with beta tubulin in an A-lattice, but similar interactions can occur with alpha tubulin and/or B-lattice (not shown). Figure 5(a) is a close-up view of Fig. 4(b) showing red kinase domains interacting with beta tubulins (autoregulatory domains



(a) (b) (c)

Fig. 4. Potential docking of the calcium/calmodulin-dependent protein kinase II (CaMKII) activated holoenzyme to a segment of A-lattice microtubule. (a) Face view – looking through the microtubule lumen. (b) Side View. (c) Angled view.

in green). Figure 5(b) shows one possible phosphorylation mechanism. With the S-T hydrophobic site on the upper side of the kinase domain, a C-terminal "tail" extends upward from a beta tubulin on the microtubule surface. Serine 444 on the tubulin C-terminal tail is able to contact, and be phosphorylated by valine 208 in the CaMKII kinase S-T hydrophobic site.

A second potential phosphorylation mechanism is shown in Fig. 5(c), in which the extended kinase domain unfurls at the autoregulatory domain, exposing the S-T hydrophobic site directly to the beta or alpha tubulin surface, allowing e.g. valine 208 (yellow) to phosphorylate serines 335, 338 and/or 339, among others, on either alpha or beta tubulin surfaces.

We then analyzed and calculated the information capacity of collective phosphorylation of microtubule A- and B-lattice neighborhood patches by a set of six CaMKII kinase domains. We assumed each CaMKII extended kinase domain can either be phosphorylated at the S-T hydrophobic site, or not. Accordingly each extended kinase domain can either phosphorylate a tubulin substrate, or not, and thus encode one bit of information to a given tubulin (e.g. phosphorylation = 1, no phosphorylation = 0). Each set of six extended kinases on each side of a CaMKII holoenzyme can thus act collectively as 6 bits of information. Ordered arrays of bits are termed "bytes".

Figure 6 shows three possible scenarios for CaMKII encoding of microtubule lattices. On the far left column, a microtubule lattice neighborhood patch is identified, with individual tubulin dimers numbered. In all instances the central dimer

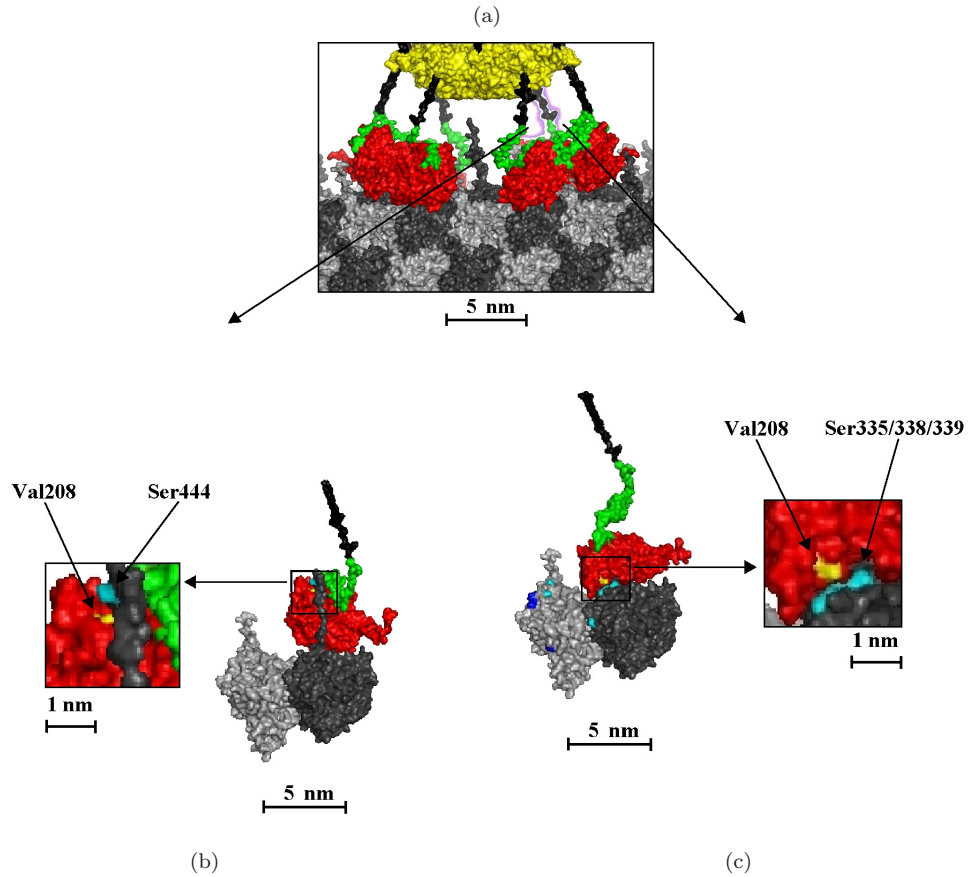


Fig. 5. Two possible modes of CaMKII phosphorylation of tubulin in a microtubule. In both modes, valine 208 in the "S-T hydrophobic site" on the inner surface of the extended kinase domains phosphorylates tubulin. (a) CaMKII docking on microtubule from Fig. 4, scale bar 5 nm. (b) The alpha tubulin C-terminal tail extends up to the top side of the kinase domain, adjacent to or beneath the (green) autoregulatory domain (scale bar 5 nm). Left: close-up showing proximity of CaMKII valine 208 (S-T hydrophobic site) and serine 444 on tubulin C-terminus, scale bar 1 nm. (c) A shift of the autoregulatory region allows the foot-like kinase domain to unfurl, exposing valine 208 (yellow) and the S-T hydrophobic site to serines 335/338/339 (cyan) on the beta tubulin surface.

is omitted for information encoding, and assumed to act as an address identifier for the surrounding six tubulins and lattice neighborhood patch. The column second from left shows possible phosphorylation/information states of each dimer, with red signifying phosphorylation (1), and no red (hence underlying light or dark gray) signifying no phosphorylation (0) for each dimer.

Row (A) in Fig. 6 shows a neighborhood patch of seven tubulins in an A-lattice microtubule. As the central dimer is not considered for phosphorylation, 6 dimer bits (one byte) are available. Beta tubulins are illustrated but the same scenario would hold for alpha tubulin phosphorylation (not shown). Six possible

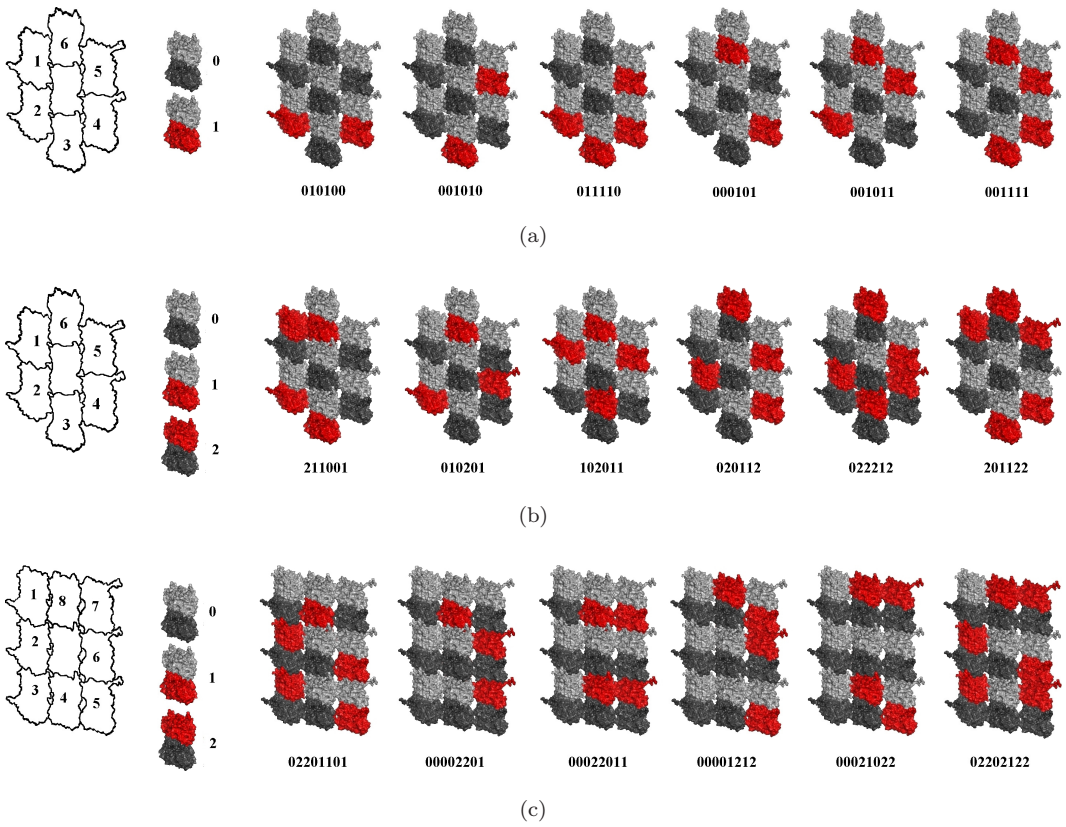


Fig. 6. Information capacity in microtubule lattice neighborhood patches due to phosphorylation by a single CaMKII holoenzyme. On far left are schematic microtubule lattice neighborhood patches with individual tubulin dimer locations identified by numbers (central dimer neglected). Second from left are phosphorylation/information states (red) of individual tubulin dimers with binary or trinary representations (0, 1, 2). On the right are six sample lattice neighborhoods with possible phosphorylation states in red. (a) A-lattice seven tubulin dimer neighborhood patch with beta tubulin phosphorylation states. A similar situation can occur with alpha tubulin phosphorylation (not shown). Six out of 2^6 (64) possible states are shown. (b) A-lattice seven tubulin dimer neighborhood in which either alpha or beta tubulins on each dimer may be phosphorylated, i.e. three possible states per tubulin. Six out of 3^6 (729) possible states are shown. (c) B-lattice nine tubulin neighborhood patches in which either alpha or beta tubulins can be phosphorylated. Six out of 5281 possible states are shown.

binary configurations, or bits, have 2^6 (64) possible states (one byte). Below each neighborhood patch is a binary string of bit states, with the key for dimer numbering shown in far left column.

Row B in Fig. 6 shows a microtubule A-lattice in which either alpha or beta tubulins within each dimer may be phosphorylated. Omitting the central dimer (which may act as an address identifier) gives six available tubulin dimers, each with three possible states — no phosphorylation (0), beta tubulin phosphorylation (1), or alpha tubulin phosphorylation (2). These are trinary states, or "trits" (rather

than bits). An ordered array of trits is a "tryte". Six possible tryte states are shown out of 3^6 (729) possible states, with a corresponding trinary string below each lattice neighborhood patch.

Row C in Fig. 6 shows a microtubule B-lattice neighborhood patch with nine tubulin dimers. As in B, either alpha or beta tubulin monomers may be phosphorylated, giving three possible states (0, 1, 2) per tubulin as in B. The central dimer is again not available for phosphorylation, and able to act as address identifier for the neighborhood lattice patch. But in the B-lattice (Row C), six tubulin dimers out of eight possible dimers may be phosphorylated, each in three possible ways. This gives $3^6 - 2^8 - 8(2^7) = 5281$ unique possible states encoded (per CaMKII "tryte") in a B-lattice neighborhood patch.

4. Discussion

Learning and memory are understood as synaptic plasticity, experimentally represented by long-term potentiation (LTP). But while synaptic membrane components are transient, memory can last a lifetime. Memory-related synaptic activity must be somehow converted to a more enduring form, e.g. at a molecular level within post-synaptic dendritic spines, shafts and cell bodies. In communication technology, a code converts (encodes) information from one form of representation to another. Is there a biomolecular code for memory in brain neurons?

In LTP, synaptic-level information is encoded as calcium ion flux (Ca^{2+}), which in turn appears to then be encoded as phosphorylation states of activated calcium calmodulin kinase II (CaMKII) holoenzymes [24–27, 30]. Activation by Ca^{2+} (via calmodulin) causes CaMKII to undergo a remarkable transformation: up to six catalytic kinase domains extend above, and up to six kinase domains extend below the association domain, the fully activated holoenzyme resembling a robotic insect with two sets of six spindly legs and large feet [39], (Fig. 1(b)).

Activated CaMKII may then phosphorylate other proteins which are candidates for meta-stable memory encoding, storage and processing. Following the trail of activated CaMKII phosphorylation in post-synaptic neurons may reveal molecular sites for memory.

In LTP, activated CaMKII rapidly distributes after Ca^{2+} influx, accumulating within seconds at the post-synaptic density [43], too fast for protein synthesis [11]. In dendritic spine LTP, activated CaMKII is redistributed from dendritic shaft to the targeted spine, which becomes enlarged [21, 50], and to nearby spines [16]. When multiple dendritic synapses are stimulated simultaneously, activated CaMKII moves into enlarged spines, followed by a secondary redistribution of activated CaMKII throughout the dendrite, and often throughout the entire dendritic arbor, cell body and axon [38]. Early steps in learning, memory and LTP appear to involve rapid intraneuronal distribution of CaMKII, presumably via diffusion and transport along cytoskeletal actin and microtubules.

CaMKII binds to both actin [42] and microtubules [22], and depolymerization of actin and microtubules prevents rapid CaMKII accumulation [1]. Actin and microtubules grow, reshape and modify neurons and synapses (though here we focus exclusively on microtubules). Various microtubule-associated proteins ("MAPs") interconnect microtubules in scaffolding networks which define neuronal and synaptic architecture. Motor MAPs dynein and kinesin convey synaptic cargo along microtubules, guided by yet another MAP, "tau" [8], whose separation from microtubules is associated with Alzheimer disease. Could microtubules encode, store and process memory-related information?

Microtubules are cylindrical polymers of tubulin, a peanut-shaped protein heterodimer composed of alpha and beta monomers arranged in two types of lattices (A- and B-lattices – Figs. 3(b) and 3(c)). Microtubules' ability to organize complex spatiotemporal intracellular activities (synaptogenesis, mitosis, etc.) and their geometric lattice structure of interactive subunits have prompted theoretical models of microtubule computation and automata function (e.g. [13–15, 37, 46]). In such proposals, variable states of individual tubulins are generally held to represent simple binary bits which interact with neighboring tubulin bits in microtubule lattices to process information. However, the actual biology of tubulin is likely to be far more complex than binary states, and can include variables such as tubulin phosphorylation, conformation, dipole, genetic isotype, post-translational modification, C-terminal configuration, ligand, ion or MAP binding. Here we consider only CaMKII phosphorylation of tubulins in microtubule lattices as potential memory encoding.

Using molecular modeling, we consider microtubule lattice neighborhood patches of either seven tubulins (A-lattice) or nine tubulins (B-lattice, Figs. 3 and 6) in which the central tubulin is excluded from phosphorylation (and whose lattice location can serve as the identifying address for particular neighborhood patches, thus enabling associative memory).

We show that size and geometry of the six extended feet-like kinase domains of activated CaMKII holoenzymes can precisely match hexagonal arrays of tubulin in both A-lattice and B-lattice microtubules (Figs. 3(d)– 3(f)). This demonstrates that six kinase domains on a single CaMKII holoenzyme can align with, and potentially phosphorylate and encode, six tubulins in a microtubule lattice collectively.

At a smaller scale on both CaMKII and tubulin, we show two plausible mechanisms for direct phosphorylation of tubulin by the "S-T hydrophobic site" on CaMKII kinase domains (Fig. 5). In one, the CaMKII S-T hydrophobic site (e.g. valine 208) interacts with serine 444 on a C-terminal "tail" of tubulin. In the second mechanism, the CaMKII kinase domain unfurls, enabling the S-T hydrophobic site (e.g. valine 208) to contact and phosphorylate threonine and serine sites on the tubulin surface. These interactions can happen collectively, simultaneously, between six kinase domains on one CaMKII and six tubulins in a microtubule lattice.

Phosphorylation of individual tubulins by an extended kinase domain is potentially equivalent to a binary bit (phosphorylation = 1, no phosphorylation = 0), and six CaMKII kinase domains acting collectively on A-lattice neighborhood of six tubulins may constitute an ordered array of 6 bits, or a "byte" with 2^6 (64) possible states, six of which are shown in Fig. 6(a).

We also considered phosphorylation in which either a beta monomer (1), alpha monomer (2), or neither (0) on each tubulin are phosphorylated by a CaMKII kinase domain, leading to trinary states, or "trits", an ordered array of which would be a "tryte". Figure 6(b) shows 6 out of 729 possible CaMKII trinary "trit" states (a "tryte") in a microtubule A-lattice neighborhood patch. In a microtubule B-lattice, CaMKII can phosphorylate/interact with 6 out of 8 possible tubulins (the central dimer being excluded). Figure 6(c) shows 6 out of 5281 possible CaMKII trinary "trit" states (a "tryte") in a microtubule B-lattice. Collective phosphorylation of microtubule lattices by activated CaMKII can enable large capacity encoding of memory-related information.

Memory-related information encoded as patterns of phosphorylated tubulins in microtubules could function to (1) determine binding sites for MAPs which interconnect microtubules to form scaffolding networks defining neuronal and synaptic architecture and extension, (2) regulate motor MAPs dynein and kinesin conveying precursors which maintain and regulate synapses, (3) transfer/encode information to particularly long-lasting and stable structures such as neurofilaments for memory storage, (4) regulate axonal firing threshold at the axon initiation segment following integration of synaptic inputs [31] (5) interact in microtubule computational (e.g. cellular/molecular automata) activity regulating synapses and intracellular activities, and relating in some way to memory and conscious experience.

5. Conclusions

LTP activation of CaMKII results in extension of up to six "leg-like" kinase domains, presumed to encode memory of synaptic activity via Ca^{2+} (e.g. [24]). Each CaMKII kinase domain can then phosphorylate additional protein substrates, and potentially further encode synaptic information. These additional substrates are candidates for molecular encoding of memory. We consider microtubules as such substrates.

Microtubules are cylindrical lattice polymers of peanut-shaped tubulin dimers, each composed of an alpha and beta monomer. Using molecular modeling, we find the six extended CaMKII kinase domains can precisely match size and geometry of hexagonal lattices of tubulin dimers in microtubules. At a smaller scale, we show juxtaposition of phosphorylation sites on CaMKII kinase domains (e.g. valine 208) and on tubulin in microtubules (e.g. serine 444).

Each CaMKII kinase domain can either phosphorylate (1), or not phosphorylate (0) a specific individual tubulin dimer, and thus encode binary "bits" of information. Six CaMKII kinase domains can collectively phosphorylate ordered arrays of six tubulin bits in a microtubule hexagonal lattice neighborhood, encoding an

addressable "memory byte". If either the beta monomer (1), alpha monomer (2), or neither (0) on each dimer can be phosphorylated by a kinase domain, trinary trits constituting memory "trytes" are obtained.

We propose the basis for a biomolecular code for memory in microtubules encoded by CaMKII and other factors. Understanding and interfacing with such a code would offer a wide range of new opportunities in diagnosis and therapy of a host of conditions.

References

[1] Allison DW, Chervin AS, Gelfand VI, Craig AM, Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: Maintenance of core components independent of actin filaments and microtubules, *J Neuroscience* **20**(12):4545–4554, (2000).

[2] Andersen P, A prelude to long-Term potentiation, *Philos Trans R Soc B* **358** (1432): 613–615, 2003.

[3] Bayer KU, De Konick P, Leonard AS, Hell JW, Schulman H, Interaction with the NMDA receptor locks CaMKII in an active conformation, *Nature* **411**:801–805, 2001.

[4] Bliss T, Collingridge G, Morris R, *Long-Term Potentiation: Enhancing Neuroscience for 30 Years* (Oxford, Oxford University Press, 2004).

[5] Cronly-Dillon J, Carden D, Birks C, The possible involvement of brain microtubules in memory fixation, *J Exp Biol* **61**:443–454, 1974.

[6] DeLano W, PyMOL Release 0.99. DeLano Scientific LLC, Palo Alto, CA, 2002.

[7] Díaz-Nido J, Serrano L, López-Otin C, Vandekerckhove J, Avila J, Phosphorylation of a neuronal-specific β-tubulin isotype, *J Biol Chem* **265**(23):13949–13954, 1990.

[8] Dixit R, Ross JL, Goldman YE, Holzbaur EL, Differential regulation of dynein and kinesin motor proteins by tau, *Science* **319**(5866):1086–1089, 2008.

[9] Dosemeci A, Reese TS, Petersen JD, Choi C, Beushausen S, Localization of the linker domain of Ca^{2+}/calmodulin-dependent protein kinase II, *Biochem Biophys Res Commun* **263**:657–662, 1999.

[10] Eswar N, Eramian D, Webb B, Shen MY, Sali A, Protein structure modeling with modeller, *Methods Mol Biol* **426**:145–159, 2008.

[11] Griffith LC, Lu CS, Sun XX, CaMKII, an enzyme on the move: Regulation of temporospatial localization, *Molecular Interventions* **3**(7):386–403, 2003.

[12] Guillard L, Bose C, Fourest-Lieuvin A, Denarier E, Pirollet F, Lafanechere L, Job D, STOP proteins are responsible for the high degree of microtubule stabilization observed in neuronal cells, *J Cell Biology* **142**(1):167–179, 1998.

[13] Hameroff SR, Watt RC, Information processing in microtubules, *J Theor Biol* **98**:549–561, 1982.

[14] Hameroff SR, Ultimate computing: biomolecular consciousness and nanotechnology, North-Holland, Amsterdam, 1987.

[15] Hameroff S, Penrose R, Orchestrated reduction of quantum coherence in brain microtubules: A model for consciousness, *Math Comput Simulat* **40**:453–480, 1996.

[16] Harvey CD, Svoboda K, Locally dynamic synaptic learning rules in pyramidal neuron dendrites, *Nature* **450**:1195–1200, 2007.

[17] Hebb DO, Organization of Behavior: A Neuropsychological Theory, New York, John Wiley and Sons, 1949.

[18] Hoelz A, Nairn AC, Kuriyan J, Crystal structure of a tetradecameric assembly of the association domain of Ca^{2+}/calmodulin-dependent kinase II, *Mol Cell* **11**:1241–1251, 2003.

[19] Hudmon A, Schulman H, Structure–function of the multifunctional Ca^{2+}/calmodulin-dependent protein kinase II, *Biochem J* **364**:593–611, 2002.

[20] Kennelly PJ, Krebs EG, Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases, *J Biol Chem* **266**:15555–15558, 1991.

[21] Lee S-JR, Escobedo-Lozoya Y, Szatmari EM, Yasuda R, Activation of CaMKII in single dendritic spines during long-term potentiation, *Nature* **458**:299–304, 2009.

[22] Levine H, Sahyoun NE, Two types of brain calmodulin-dependent protein kinase II: Morphological, biochemical and immunochemical properties, *Brain Res* **439**(1–2):47–55, 1988.

[23] Li H, DeRosier DJ, Nicholson WV, Nogales E, Downing KH, Microtubule structure at 8Å resolution, *Structure* **10**:1317–1328, 2002.

[24] Lisman JE, A mechanism for memory storage insensitive to molecular turnover: A bistable autophosphorylating kinase, *Proc Natl Acad Sci USA* **82**:3055–3057, 1985.

[25] Lisman JE, Goldring MA, Feasibility of long-term storage of graded information by the Ca^{2+}/calmodulin-dependent protein kinase molecules of the postsynaptic density, *Proc Natl Acad Sci USA* **85**:5320–5324, 1988.

[26] Lisman J, Goldring M, Evaluation of a model of long-term memory based on the properties of the Ca^{2+}/calmodulin-dependent protein kinase, *J Physiol (Paris)* **83**:187–197, 1988.

[27] Lisman JE, Zhabotinsky AM, A model of synaptic memory, A CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly, *Neuron* **31**:191–201, 2001.

[28] Lømo T, The discovery of long-term potentiation, *Phil Trans R Soc (Lond)* **358**:617–620, 2003.

[29] Löwe J, Li H, Downing KH, Nogales E, Refined structure of $\alpha\beta$-tubulin at 3.5 Å resolution, *J Mol Biol* **313**:1045–1057, 2001.

[30] Miller SG, Kennedy MB, Regulation of brain type II Ca^{2+}/calmodulin-dependent protein kinase by autophosphorylation: A Ca^{2+}-triggered molecular switch, *Cell* **44**(6):861–870, 1986.

[31] Naundorf B, Wolf F, Volgushev M, Unique features of action potential initiation in cortical neurons, *Nature* **440**(7087):1060–1063, 2006.

[32] Nogales E, Wolf SG, Downing KH, Structure of the $\alpha\beta$ tubulin dimer by electron crystallography, *Nature* **39**:199–203, 1998.

[33] Pearson RB, Kemp BE, Protein kinase phosphorylation site sequences and consensus specificity motifs: Tabulations, *Methods Enzymol* **200**:62–81, 1991.

[34] Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kalé L, Schulten K, Scalable molecular dynamics with NAMD, *J Comput Chem* **26**(16):1781–1802, 2005.

[35] PDB ID: 2VZ6, Pike ACW, Rellos P, King O, Salah E, Parizotto E, Fedorov O, Shrestha L, Burgess-Brown N, Roos A, Murray JW, Von Delft F, Edwards A, Arrowsmith CH, Wikstroem M, Bountra C, Pike S, Knappa CW, Rellos P, King O, Shrestha

L, Structure of human calcium calmodulin dependent protein kinase type II alpha (Camk2A) in complex with indirubin E804.

[36] Ramon-y-Cajal SR, The croonian lecture: La fine structure des centres nerveux, *Proc R Soc Lond* **55**:444–468, 1894.

[37] Rasmussen S, Karampurwala H, Vaidyanath R, Jensen KS, Hameroff S, Computational connectionism within neurons: A model of cytoskeletal automata subserving neural networks, *Physica D* **42**:428–449, 1990.

[38] Rose J, Jin S, Craig A, Heterosynaptic molecular dynamics: Locally induced propagating synaptic accumulation of CaM Kinase II, *Neuron* **61**(3):351–358, 2009.

[39] Rosenberg OS, Deindl S, Sung RJ, Nairn AC, Kuriyan J, Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme, *Cell* **123**:849–860, 2005.

[40] Sept D, Baker NA, McCammon JA, The physical basis of microtubule structure and stability, *Protein Science* **12**(10):2257–2261, 2003.

[41] Serrano L, Díaz-Nido J, Wandosell E, Avila J, Tubulin phosphorylation by casein kinase II is similar to that found in vivo, *J Cell Biol* **105**:1731–1739, 1987.

[42] Shen K, Teruel MN, Subramanian K, Meyer T, CaMKII beta functions as an f-actin targeting module that localizes CaMKII alpha/beta heterooligomers to dendritic spines, *Neuron* **21**:593–606, 1998.

[43] Shen K, Meyer T, Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation, *Science* **284**:162–167, 1999.

[44] Swulius MT, Waxham MN, Ca^{2+}/Calmodulin-dependent protein kinases, *Cell Mol Life Sci* **65**:2637–2657, 2008.

[45] Tombes RM, Faison MO, Turbeville JM, Organization and evolution of multifunctional Ca^{2+}/CaM-dependent protein kinase genes, *Gene* **322**:17–31, 2003.

[46] Tuszynski JA, Brown JA, Hawrylak P, Dielectric polarization, electrical conduction, information processing and quantum computation in microtubules, are they plausible? *Trans R Soc* **356**:1897–1926, 1998.

[47] Wandosell F, Serrano L, Hernández MA, Avila J, Phosphorylation of tubulin by a calmodulin-dependent protein kinase, *J Biol Chem* **261**:10332–10339, 1986.

[48] Yoshimura Y, Aoi C, Yamauchi T, Investigation of protein substrates of Ca^{2+}/calmodulin-dependent protein kinase II translocated to the postsynaptic density, *Mol Brain Res* **81**:118–128, 2000.

[49] Yoshimura Y, Shinkawa T, Taoka M, Kobayashi K, Isobe T, Yamauchi T, Identication of protein substrates of Ca^{2+}/calmodulin-dependent protein kinase II in the postsynaptic density by protein sequencing and mass spectrometry, *Biochem Biophys Res Commun* **290**:948–954, 2002.

[50] Zhang Y-P, Holbro N, Oertner TG, Optical induction of plasticity at single synapses reveals input-specific accumulation of alphaCaMKII, *Proc Natl Acad Sci USA* **105**(33):12039–12044, 2008.