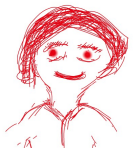
Switches in the brain?

A potential mechanism for long-term memory storage

Dilawar Singh

We forget often. But we remember some memories as long as we live. This means that our brain is capable of protecting memories for years. This is a remarkable feat given that the *biochemical hardware* involved in creating new memories is a hostile place for its storage. What are the challenges involved? And what type of biochemical mechanisms may overcome them? This article explores a major hypothesis that molecular switches may be behind our remarkable ability to remember for a lifetime.



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1. Introduction

Our brain is made up of roughly 100 billion neurons, joined together with over 100 trillion connections called **synapse**s. Each neuron on average makes 1000 connections. It is now widely accepted that memories are created by changing these connections.

Let's label these synapses as $s_1, s_2, \ldots s_n$. During memory formation, a subset of these synapses will undergo changes. For example, my memory of being chased by a ferocious street dog named *Lalu* (lets call it M_{Lalu}) is stored in the set of synapses $M_{\text{Lalu}} = (s_{10}, s_{21}, s_{12}, \ldots, s_{331})$ i.e., these connections were changed during my troubling encounter with Lalu. I sometimes recall this memory whenever I see a similar looking dog.

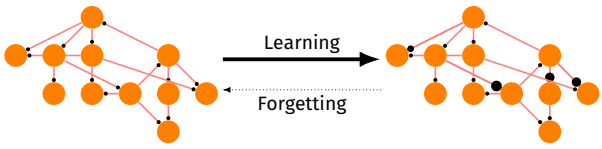
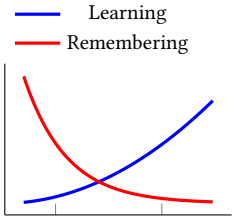


Figure 1. Memory formation and forgetting. During formation of a memory, some synapses become stronger (larger black dots). The longer you can maintain these connections, the longer you can hold on to this memory.

I can recall an experience as long as the set of synapses in which

the particular experience was stored remains intact. Therefore, our ability to remember is contingent on our brain's ability to keep its connections intact. But on the other hand, our ability to learn depends on our brain's ability to change its connections. And here is the first challenge!

1.1 *Learning quickly v/s forgetting slowly, a zero-sum game*



Ability to change (plasticity) is good for learning, but bad for remembering.

For M_{Lalu} (or any other memory) to remain intact, each synapse which participated in its formation (i.e., $s_{10}, s_{21}, s_{12} \ldots$) should remain intact. The longer a synapse can keep itself unchanged, the better it will be at keeping the memory safe. Let's assume that somehow I create a synapse which maintains its state for a very long time (i.e., a rigid synapse). This synapse will not *forget* easily, but it will not participate in any new memory formation either. Learning requires synapse to change and a rigid syanpse can't change. Rigid synapses behave like a read-only Compact Disk (CD). On the other hand, if I create a synapse which is easily changeable (i.e., a plastic synaspe), it will be good at learning new experiences but won't be able to retain them for long. Plastic synapse forgets easily. We know that we not only remember for long time, we are also capable of learning quickly. And not just us, many other animals are quick to learn. Honey bees can learn the location of food only after encounter with food source such as flowers. Indeed, a good memory system is the one which learns as quickly as possible from rewarding or painful experiences and forgets as slowly as possible. Forgetting and remembering are two sides of the same coin. They are conflicting demands i.e., improving one will deteriorate the other – a zero-sum game. The challenge is to strike a balance.

2. Hopfield network – associative memory network

Memory storage and retrieval is trivially done by a computer. It will be helpful to compare memory storage in the computer and the brain. In the computer, we always know the address of every stored memory and we access it by providing this address. The

file icon on your desktop is a graphical way of encoding this addressing scheme. This process is similar to looking up the index page in a reference book to find a topic. Our brain, on the other hand, is very unlikely to have such an indexing mechanism.

We recall when we are provided with *cues*. For example, when you see some part of a familiar person in a wedding album you could easily identify the person even though most of the person is hidden behind others. Many other memories of that person will also be recalled. A famous class of recurrent neural network popularly known as Hopfield network can do just the same as shown in *Figure 2*.

Put simply, recurrent networks have *loops*, usually from output to input.

How does this recurrent network work is beyond the scope of article. Readers are encouraged to explore more by themselves. *"How well can we explain biological memory by these networks?"* is an active research area. Though these networks are extremely successful in accomplishing various *brain-like* computations (*a la* machine learning), I would like to advise the reader to be skeptical by noting the following:

- Neurons used in these networks are highly simplified. *Real* neurons are not this simple. Even though these simplified neurons

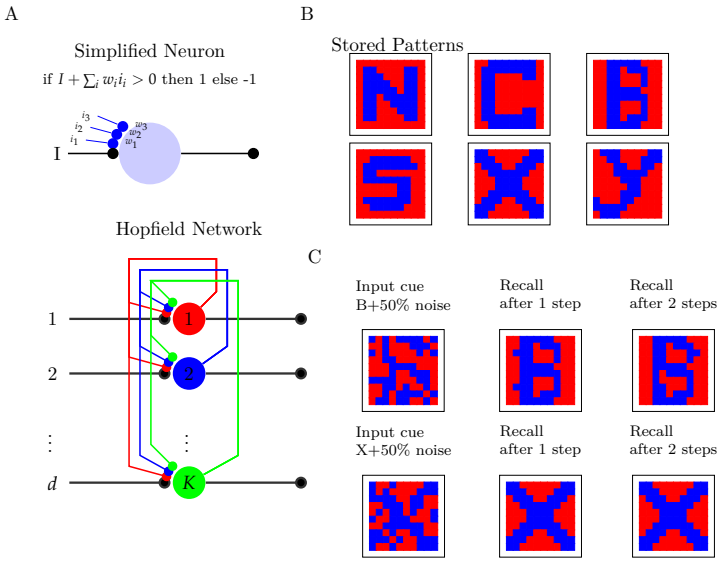


Figure 2. Hopfield network with 100 spiking neurons. These *recurrent* configurations give rise to interesting brain-like computation. **(B)** 6 patterns (memory) i.e. NCBSXY are stored in this network. **(C)** When a very distorted *cue* is applied to the network input, it *fetches* one of stored pattern which is the *closest* to the applied cue.

capture the essential *all-or-none* (electrical spike) way of communication and learning by changing synaptic connections, they do ignore rich local computations which can be accomplished by branches of these neurons called *dendrites*.

- There is no evidence that neurons make such dense recurrent connections. However, some studies have shown that Hopfield network can work with very sparse recurrent connections as well.

- Activity in these networks does not match usually observed activity in the primate brain during memory-recall experiments.

Solutions contributed by other disciplines are helpful for comparison and contrast and often provide very useful insight. But in the end, these solutions must be tested under the constraints imposed by biology.

Nonetheless, these networks provide us with a framework to concretely think about the problem of memory storage and recall. We learn a great deal about a problem by pointing out the limitations and failures of models which describe it.

Hopfield network has properties which will sound very natural to us. Can you store as many memories as you like in these networks? No. There is an upper limit. Adding more patterns over maximum limit causes distortion in memories. When a cue is given, the network no longer fetches the right pattern. It often fetches a pattern which was not even stored; the retrieved pattern instead resemble some mixture of stored patterns. When too many memories are stored, they corrupt each other by mixing up. One can ask more questions. When connections decay in these networks and memories start disappearing, which memory disappear first: the weakest or the newest? And, when a new memory closely associated with an old memory is added, what happens to that old memories?

After this necessary detour, lets go back to the main theme: how do synapses maintain their state?

3. How does a synapse maintain its state?

Very complex biochemistry plays out during learning that changes the synapse. Surprisingly, the net effect of this complex biochemistry can be summarised by a simple mathematical expression.

Ah, *the unreasonable effectiveness of mathematics*[4]! Let's assume that synaptic strength w is tightly correlated with a chemical species X found at synapse i.e. w changes with X. The problem of maintenance of w can be rephrased as the problem of maintenance of the activity of X. Therefore, the problem of "*synapse maintaining its state*" becomes the problem of "*molecule X maintaining its state*" – a more concretely defined problem.

Let's assume that X is converted to its active form X^* by adding a phosphoryl group (PO_3^{2-}). The phosphoryl group is removed by a phosphatase and X^* is turned back into inactive X. The phosphorylation and its counterpart dephosphorylation are a very common motif for controlling various chemical reactions by activating and inactivating protein molecules. Once most X has been turned into X^* during memory formation, how do we make sure that X^* does not turn back into X (lose memory)?

$$X \longrightarrow X^*$$
$$X^* + P \rightleftharpoons X^*P \longrightarrow X + P$$

Let's mull over a solution to this problem of long term maintenance of X^*. Here is one potential solution.

1. **(Amplification)** X^* **auto-phosphorylates** itself i.e. $X \xrightarrow{\curvearrowright} X^*$. If we manage to get sufficient X^* somehow, it will act as a catalyst to its own production. X^* will always remain high.

2. Dephosphorylation of X^* is minimized by controlling the number of P or reducing the reaction rate.

Can you think of other set of hypotheses? It must conform to laws of chemistry!

Both (1) and (2) help in making X^* highly stable. Problem solved? No. Now we have constructed a rigid synapse. Recall the *rigid* v/s *plastic* synpase dilemma discussed previously (section **1.1**). This synapse will definitely remember for longer but it will not participate in any new learning anymore.

As long as we are in the realm of theory, let's propose a solution to this problem. We add another reaction say $P' + X^* \rightarrow P'X \rightarrow P' + X$ which deactivates X^* when the *need* arises. Phosphatase P' is different from P. This adds another layer of control to an already complicated problem i.e. forgetting is now controlled by another process. This requires one more explanation: how does this new

mechanism controlling *forgetting* work? And philosophically – if you care about it – it violates the principle of **parsimony** which recommends picking the simplest explanation.

We still have two big problems hiding underneath. We have not considered the underlying biological hardware i.e. synapse in any detail where this biochemical network suppose to function. The first problem is chemical noise. For a biochemical system operating in very small volume, effect of chemical noise can be very strong. There are over 200 types of protein molecules in a typical synapse. Indeed, most proteins found in synapse has numbers as low as few tens. The brain is always active and the chemical noise caused by the brain's background activity will surely turn some molecules of X into X^*. Then due to auto-phosphorylation, sooner than later, all X will be turned into X^*. We have created a very stable memory of nothing but background noise. This is highly undesirable!

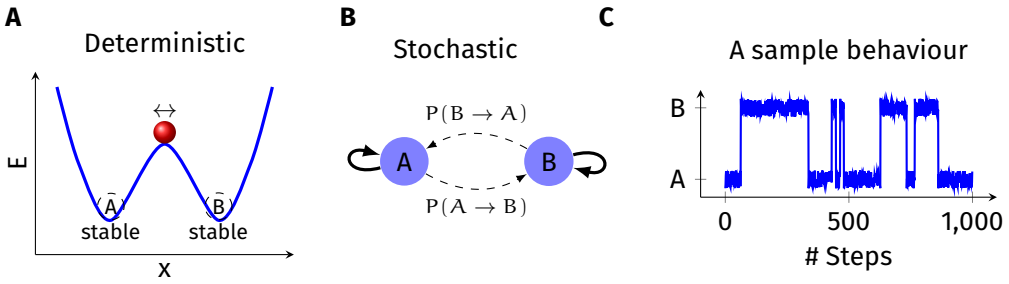
The volume of a typical synapse is 10^{-20} m^3. At this volume, 1 μM concentration is roughly equal to 6 molecules.

The second problem is *turnover* i.e. old molecules are continuously degraded and being replaced by newly minted molecules. Let's assume that at the time of memory formation, we had 100 molecules of X^* in the synapse. And let's also assume that on average, every day one new formed molecule replaces an old one ($X^* \rightarrow X$). After 50 days, half of the synaptic strength is gone! To counter this, we must have a *refresh* mechanism by which the newly added molecule quickly changes its state according to the state of synapse i.e. newborn X becomes X^* if most molecules at synapses are X^*.

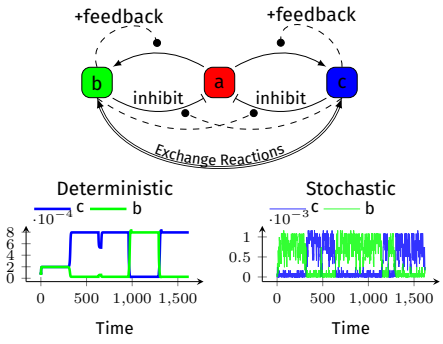
Box 1. Bistability in reaction network

In a world full of fluctuations, stability is indeed very useful property. Life is remarkably stable even though it is made up of inherently noisy components. Bistable chemical networks are ubiquitous in biology. In bistable chemical networks, noisy components acts together to give rise to highly (bi)stable behaviour. *Also note that even though the underlying reactions are almost always reversible, the bistable switch is usually not reversible*. Isn't it a neat way to make a very decisive cell?

A bistable system – as its name implies – has two stable states. From the point of view of an experimentalist, if you obverse a bistable system for very long time, you would almost always find it in either of its two stable states. Just like an electrical switch which you would almost always find in either `ON` or `OFF` state (and rarely in transition between states).



In the figure above, **A** show a conceptual model of bistability. The energy landscape of a bistable system has two minima ($\widetilde{A}$ and $\widetilde{B}$), therefore, the system always ends up in either of them. It is a deterministic bistable system. **B** shows a conceptual representation of stochastic bistable system as state transition diagram. The arrows depict the probability of transition from one state to the other. In **C**, we simulate the system described in **B** with probability of transition from A to B ($P(A \rightarrow B)$), and from B to A ($P(B \rightarrow A)$) set equal to 0.01. Such bistable motif are commonplace in biology [6], especially found in situations where cell has to make a decision or store information (memory). One such motif is shown below in both deterministic and stochastic settings.



A bistable chemical system adapted from [6]. Species b and c catalyze their production (positive feedback) and inhibits production of a. **(below)** Two trajectories are shown in different settings. (Left) System is deterministic; (right) System is stochastic. There are three necessary conditions a network must have to be able to show bistability: positive feedback, mechanism to filter out small stimuli, and a mechanism to prevent explosion [5].

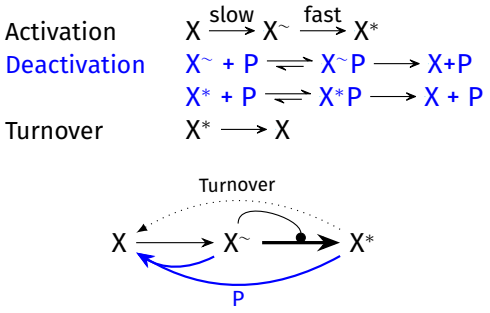


Figure 3. A hypothetical network which can solve the problem of chemical noise and turnover with suitable parameters. The activation step is divided into slow and fast components such that fluctuations caused by background noise do not cause the system to activate itself. X^* also partially activate X to $X^{\sim}$ to overcome *turnover.*

Effectively, we want a stable `ON` state (all X are X^*) which is immune to turnover. We also want a stable `OFF` state so that noise does not turn X into X^*. We want a switch like behaviour i.e. if it is `OFF` or `ON` it tends to stay `OFF` or `ON`. If a few X are turned into X^* by background noise, we want them to be quickly turned back into X by the phosphatase P. And if during memory formation, a significant portion of X has been turned into X^* then we want that any X^* deactivated into X is quickly activated again into X^*. This system should operate like a switch which does not flip unless significant force is applied. These are called **bistable switch**.

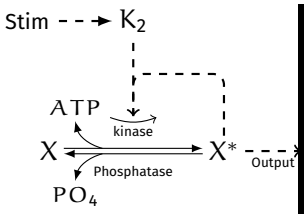
Is there any evidence that bistable chemical reaction networks exist? Do they occur at all in living cells? Bistability (and its close relative oscillations) are very common in biology from cellular level (genetic switch) to population levels (predator-prey dynamics)? What is the reason for their *selection* by evolution? And what do they accomplish? Bistability, from one point of view, is an irreversible way of taking YES or NO decisions. A phage λ virus can use them to decide if to remain dormant or become active; a cell can use them to decide whether to move to M phase from G1 phase or whether to enter apoptosis or not. From other point of view, bistability is a way to store 1 bit of memory (1 and 0, `ON` or `OFF`, `ACTIVE` or `INACTIVE`). Memory is older than brain! There are plenty of living organism without brain as we define them but I can't imagine a living organism without memory. So it won't be surprising if we discover bistable switches operating at syanpse as well. Indeed, various studies have shown that calcium/calmodulin dependent protein Kinase II (CaMKII)

may form a bistable switch in synapse.

4. Molecular bistable switch at synapse

Late John Lisman hypothesised that a kinase and a phosphatase together can form a bistable switch immune to *turnover*. CaMKII and its phosphatase Protein phosphatase 1 (PP1) were identified as the hypothesised kinase and phosphatase. This chemical system has been extensively studied using computational models for over two decades [3]. There is some evidence that CaMKII is bistable *in vitro* conditions. Whether CaMKII is bistable inside a living neuron is still an open question (see Box 2 for overview of CaMKII properties at synapse.).

In our computational study of this pathway, we explored the effect of subunit exchange on CaMKII pathway [7] (See Box 2.). A CaMKII molecule is made up to 12 to 14 subunits which it can exchange with other molecules. This makes CaMKII a rare molecule, perhaps unique. Subunit exchange enables CaMKII to act at position away from its current location. Due to its unique properties, CaMKII is often called *the memory molecule*.



Reaction in a bistable switch proposed by John Lisman. Modified from [1].

We found that the subunit exchange improves information retention capacity of CaMKII. Individual CaMKII acts as leaky integrator of calcium (Ca^{++}) activity (see Box 2.). With subunit exchange, the leaky integrator becomes less leaky, therefore maintains its active state for longer time. Many CaMKII holoenzymes often come together and form a cluster, then they give rise to a bistable switch (*Figure 4*). Such clustering by various molecules have been observed in experiments and is now thought to play a very important role. We found that subunit exchange synchronizes bistable activity of distributed clusters. That is, multiple bistable switches act as a single but much more stable bistable switch. In nutshell, we found the subunit exchange makes CaMKII molecule better at retaining information.

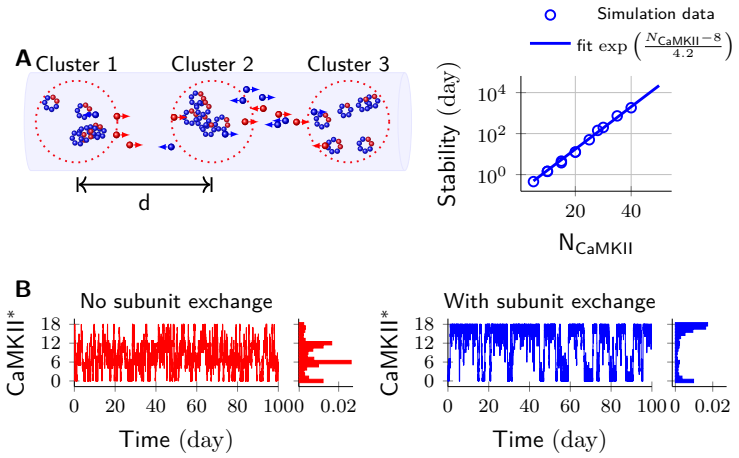


Figure 4. **(A)** (left) Three clusters of CaMKII in a synapse . (Right) This system ability to hold memories increases exponentially with system size. 52 holoenzymes can keep the memory intact roughly for 100 years! **(B)** Subunit exchange improves CaMKII ability to store information. **(Red)** 3 individual bistable switches independently without subunit exchange. **(Blue)** When subunit exchange is enabled, these independent switches synchronize. Notice that the now system spends more time in its stable states.

5. Conclusion

In this article, we have discussed why bistable motif is an attractive candidate for storing biological memories. They are ubiquitous in biology and are natural solution to the problem of memory maintenance under chemical noise and turnover. We have also discussed a potential pathway (CaMKII) which may be bistable at synapse. Now let's put all of this to a reality check. Let's assume that bistables mechanism exists at synapse. If the underlying mechanism is bistable, then I *must* observe a bimodal distribution. We know that size of a synapse is tightly correlated with its strength. That means we can observe synapse size as the proxy of its strength. If we observe synapse for long time and record its size continuously, what would we observe for our hypothesis to be correct? If the distribution of size is bimodal (two peaks) than it is a strong suggestion (not a proof!) that underlying mechanism is bistable.

There is now growing experimental evidence that synaptic size change in *all-or-none* manner, a finding which is consistent with this idea. Some other studies have claimed that changes are graded i.e. synapse changes in step-wise manner much like a **multi-stable** synapse. A multi-stable synapse is an additive ensemble of many bistable components (*Figure 5*).

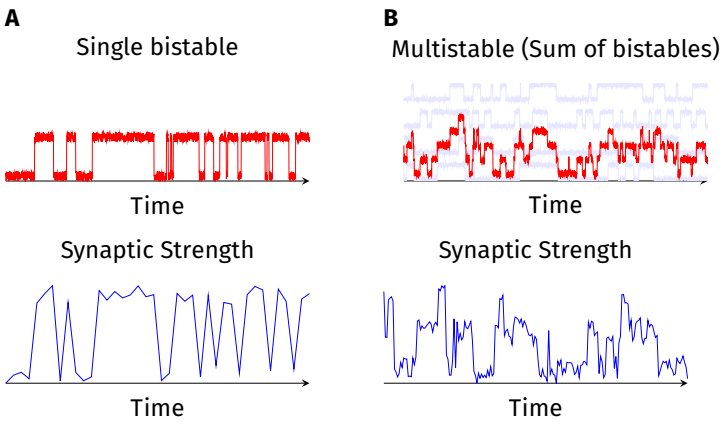


Figure 5. *All-or-none* v/s graded synapse and mechanism which may give rise to them. **(A)** A bistable mechanism (red) controlling the synaptic strength (blue). Synaptic strength changes in *all-or-none* fashion. **(B)** A multi-stable mechanism (red) gives rise to a graded synapse (blue). Synaptic strength changes in step-wise manner. Note that a multi-stable mechanism can be constructed by adding multiple bistable switches. In this case, 4 bistable switches were used (shown in light blue in background. Red curve is algebraic sum.).

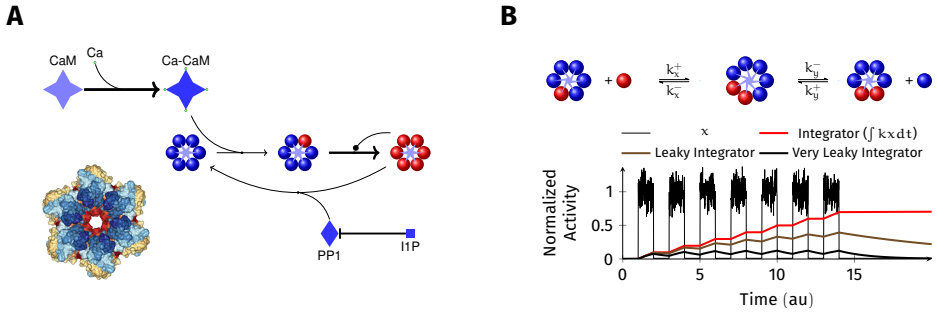
Whether CaMKII is bistable in the synapse is still an open question; neither there is any concrete evidence that it is nor it has been ruled out that it is not, especially near the membrane. Our aim in this article was to point out why bistable mechanism is a viable solution to the problem of storing memory for long time. Even if CaMKII turns out not to be bistable at synapse, there could still be other mechanisms which are bistables – a few of them have been proposed. Given that bistable chemical motifs are widespread, it is reasonable to suggest that there are indeed switches in our brain – much like flip-flops in the digital memory card of your phone – which are evolved to keep our memories safe from the onslaught of time and noise.

Simulations presented in the article are available at https://gitlab.com/dilawar/Resonance2018.

Box 2. CaMKII at synapse: A brief overview

Among more than 2000 proteins found in brain, CaMKII constitutes roughly 2% of them all. It is enriched in the hippocampus – a brain structure necessary for memory formation. Indeed, CaMKII is known to play essential role in learning and memory. In experiments involving mice, deactivating CaMKII in any way has always resulted in impairment of memory formation and learning.

CaMKII molecule has interesting properties which makes it an attractive candidate for storing information. It is unique among proteins for its dodecameric/tetradecameric structure (A, structure from Protein Data Bank), its ability to exchange part of it with others (B) and its complex response to calcium signal. Usually it acts as a integrator of calcium activity in synapse (C).



(A) Summary of CaMKII pathway of. Upon its influx into synapse, Ca^{++} binds to calmodulin (CaM) and create a complex calcium/calmodulin complex (Ca^{++}/CaM). Ca^{++}/CaM binds to CaMKII and phosphorylate it's subunit (red balls). CaMKII is dephosphorylated by PP1. **(B)** Subunit exchange between two holoenzymes i.e. a fully active CaMKII holoenzyme can lose an *ACTIVE* subunit which can be picked up by another holoenzyme which becomes partially active.

Activation of CaMKII requires two steps: the first step is very slow for it requires binding of two Ca^{++}/CaM simultaneously. Once a subunit has been activated (red ball), phosphorylation of its neighbours requires binding of only one Ca^{++}/CaM and therefore second step proceeds at much faster rate. Note that the first very slow step can be overcome by subunit exchange when an inactive holoenzyme picks up an active subunit released by other holoenzyme. Thus subunit exchange helps in spreading CaMKII activation.

CaMKII integrates Ca^{++} signal A single CaMKII holoenzyme acts as a leaky integrator of Ca^{++} activity i.e. it sums up Ca^{++} activity in time. And it also decays with a time-constant (leaky). See **(B)** to compare behaviour of three integrators of calcium activity (x): a non-leaky integrator and two leaky integrators with small and large amount of leakage. Mathematically, it is similar to a leaky capacitor charged by current source. Integrators are useful when you want to accumulate *enough* information about something (say x) before taking a decision e.g. a plant can *decide* to flower or shed leaves only if integration of moisture in air and/or temperature during the day crosses a threshold value.

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Suggested Reading

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[6] **Naren Ramakrishnan, Upinder S. Bhalla** ***Memory Switches in Chemical Reaction Space*****, PLOS Computational Biology, No 7 vol. 4 (July 2008)**

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