

Switches in the brain?

A potential mechanism for long-term memory storage

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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.

1. Introduction

Our brain is made up of roughly 100 billion neurons, joined together with over 100 trillion connections called **synapses**. 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, \dots, s_n . A subset of these synapses participate in a specific memory formation. For example, my memory of being chased by a ferocious street dog named *Lalu* (lets call it M_{Lalu}) is represented by the set of the synapses $M_{Lalu} = (s_{10}, s_{21}, s_{12}, \dots, s_{331})$ i.e. these connections were changed during my troubling encounter with *Lalu*. I sometimes recall this memory whenever I see a similar looking dog.

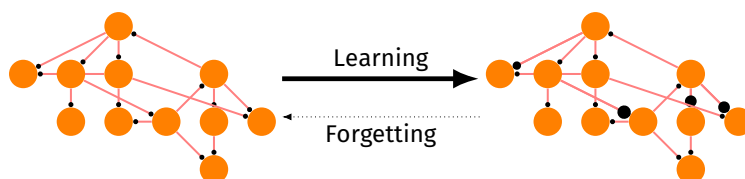
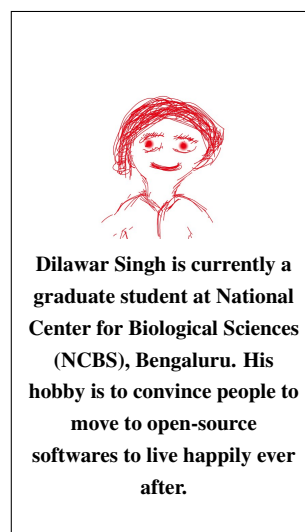
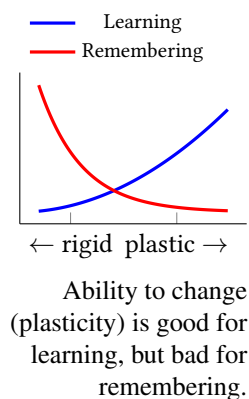


Figure 1. Memory formation and forgetting. During formation of a memory, some synapses become stronger. The longer you can maintain these connections, the longer you can hold on to this memory.

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 is 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



For M_{Lalu} (or any other memory) to remain intact, each of its component ($s_{10}, s_{21}, s_{12} \dots$) should also remain intact. The longer a synapse can keep itself unchanged, the better it will be at keeping the memory. Lets assume that somehow I create a synapse which maintain its state for a very long time (i.e. a rigid synapse). This synapse will not *forget* easily, but it causes another problem. Rigid synapse will not participate in any memory formation anymore since learning requires change and it can't change. It behaves like a read-only compact disk. On the other hand, if I create a synapse which is easily changeable (i.e. a plastic synapse), it will be good at learning new experiences but won't be able to retain it for long. Plastic synapse forgets easily. We know that not only we remember for long time, we are capable of learning quickly too. For example, honey bees can learn the location of food after one encounter with flowers. Indeed a good memory system is the one which learns quickly from new experiences and forgets old information as slowly as possible. Forgetting and remembering are the two sides of the same coin. They are conflicting demands – 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 ad-



clothing scheme. This process is very similar to looking up the index page in a reference book to find a chapter. 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 – while the rest of the person may be hidden behind others – you could easily identify the person. And 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*.

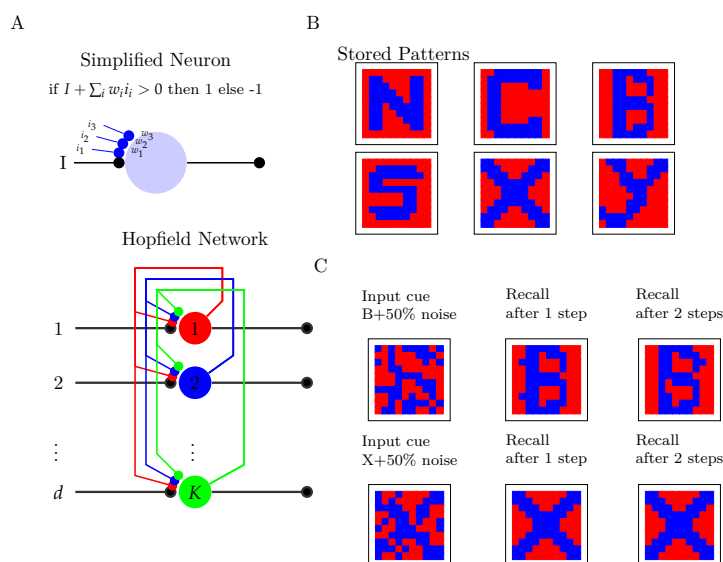


Figure 2. Hopfield network with 100 spiking neurons. These *recurrent* configurations give rise to interesting brain-like computation. **(B)** 6 patterns (memory) i.e. NCBSXY are stored in this network. **(C)** When a very distorted *cue* is applied to the network input, it *fetches* one of stored pattern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

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

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

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 strong 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 network provide us with a framework to concretely think about the problem of memory storage and its recall. We learn a great deal about these problem by pointing out the limitations and failure of these models. 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 fetch the right pattern. It often fetches a pattern which was not even stored; the retrieved pattern instead resemble some mixture of many stored patterns. When too many memories are stored, they corrupt each other by mixing up. One can ask more questions. What if connections are allowed to decay in these networks, and when memories starts disappearing, do the weakest memory disappearing first or the newest? When a new memory is added, what do happen to the 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 level – or 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.

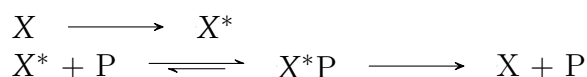


Figure 3. Phosphorylation and dephosphorylation of X . P is phosphatase.

Lets assume that X is converted to its ACTIVE form X^* by adding a phosphoryl group (PO_4^{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 if not all X has been turned into X^* during memory formation, how do we make sure that X^* does not turn back into X (lose memory)?

Let's mull over a solution to this problem of long term maintenance of X^* . Let's assume that somehow followings are true.

1. **(Amplification) X^* auto-phosphorylate** itself i.e. $X \xrightarrow{\quad} X^*$.
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 *pastic* synapse 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 $P' + X^* \rightarrow P'X \rightarrow$



The volume of a typical synapse is $1 \times 10^{-20} \text{ m}^3$.

At this volume, $1 \mu\text{M}$ concentration is roughly equal to 6 molecules.

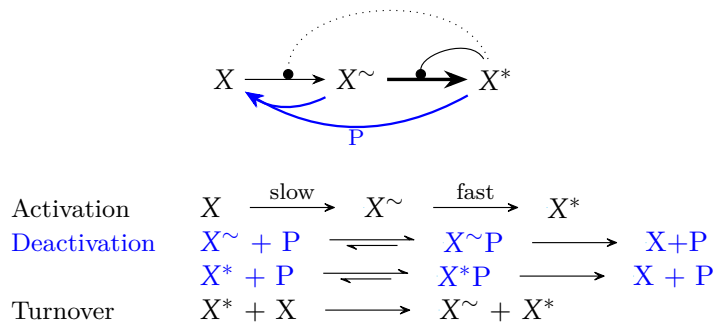
$P' + X$ which deactivates X^* when the *need* arise. Phosphatase P' is different than P . This adds another layer of control to an already complicated problem i.e. forgetting is now controlled by another process. This requires one more explanation: how does this new mechanism controlling *forgetting* works? And philosophically – if you care about it – it violates the principle of **parsimony** which recommends to pick 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 biochemical system operating in very small volumes, effect of chemical noise can be very strong. There are over 200 types of protein molecules in a typical synapse. Indeed, most of these protein molecules have few tens of molecules. The brain is always active and the chemical noise caused by the background activity in the brain will surely turn some molecules of X into X^* . Then due to auto-phosphorylation, sooner than later, all of the X will be turned into X^* . We have created a very stable memory of nothing but background noise. This is highly undesirable!

The second problem is *turnover* i.e. old molecules are constantly degraded and being replaced by newly minted molecules. Assume that at the time of memory formation, we had 100 molecules of X^* in synapse. And also assume that on average, every day one new molecule (i.e. X) replaces an old one (X^*). After 50 days, half of the synaptic strength is gone! We must have a *refresh* mechanism by which we make sure that the new molecule quickly changes its state according to the state of synapse i.e. newborn X becomes X^* most molecules at synapse are X^* .

Effectively, we want a stable ACTIVE state (where all X are X^*) and don't want chemical noise to turn X into X^* . We want a switch like behaviour – if it is *OFF* or ON it tends to stay *OFF* or ON respectively. A significant force is required to flip a switch; it does not get flipped by noise. If few X are turned into X^* by background noise, we expect them to be quickly turned back into X by phosphatase. And if during memory formation, a significant





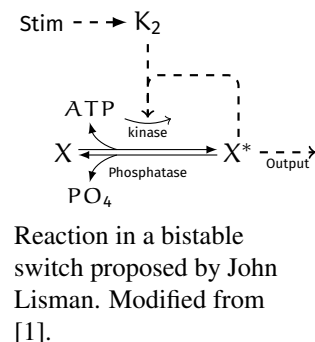
portion of X has been turned into X^* then we expect that any X^* turned back into X is quickly activated again into X^* . This system should operate like a switch which does not flip unless significant force is applied. These are called **bistable switches**.

Is there any proof that bistable systems are even possible? Do they occur at all in living cells? Bistability (and its close relative oscillations) are very common in biology; from cellular level to population levels. So if it won't be surprising if we find bistable switches in synapses as well. *Is there a set of chemical reactions which forms a bistable switch at synapse?* Various studies have shown that calcium/calmodulin dependant protein Kinase II (CaMKII) may form a bistable switch in synapse.

Figure 4. A hypothetical network which can solve the problem of chemical noise and turnover with suitable parameters (how?). The activation step is divided into slow and fast components such that fluctuations caused by background noise do not cause system to activate itself. X^* also partially activate X to X^{\sim} to overcome turnover.

4. Molecular bistable switch at synapse

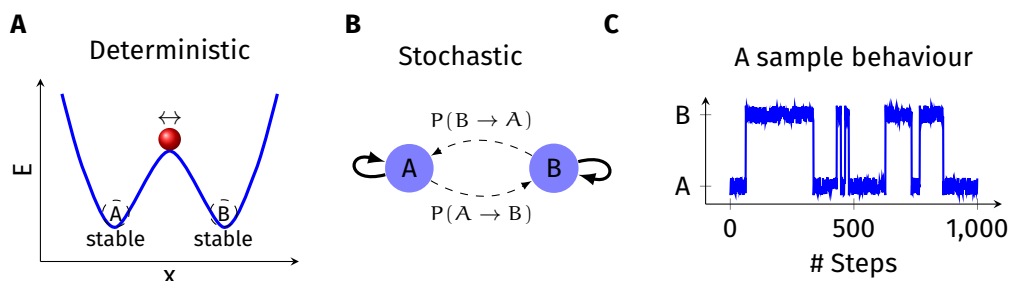
John Lisman hypothesised that a kinase and a phosphatase together (*Figure 4.*) can form a bistable switch which is stable against turnover. CaMKII and its phosphatase protein phosphatase 1 (PP1) were identified as the hypothesised kinase and phosphatase. This chemical system had been extensively studied using computational models for over two decades [3]. There is evidence that CaMKII is bistable *in vitro* conditions.



Box 1. Bistability in reaction network

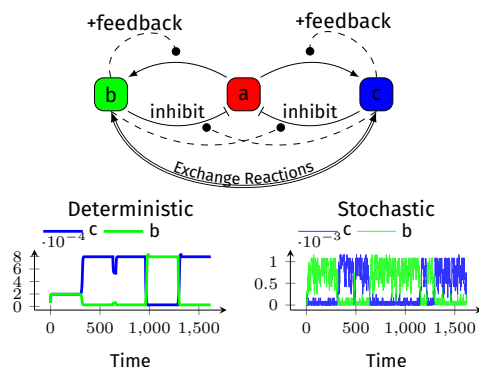
In a world full of fluctuations, stability is indeed very useful property. Life is remarkably stable even though it is made up of inherently noisy components. Bistable chemical networks are ubiquitous in biology. In bistable chemical networks, noisy components acts together to give rise to highly (bi)stable behaviour.

A bistable system – as its name implies – has two stable states. From the point of view of an experimentalist, if you observe 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 rest of the time in transition between ON and OFF).



In the figure above, **A** show a conceptual model of bistability. The energy landscape of a bistable system has two minima (\bar{A} and \bar{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, and from B to A set to be 0.01. Note that each time, this trajectory will be different but the average of many trajectories will be equivalent to a deterministic bistable system.



Such stochastic bistable motif are commonplace in biology [6], especially found in situations where cell has to make a decision. One such motif is show below in both deterministic and stochastic settings. 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 explosions[5].

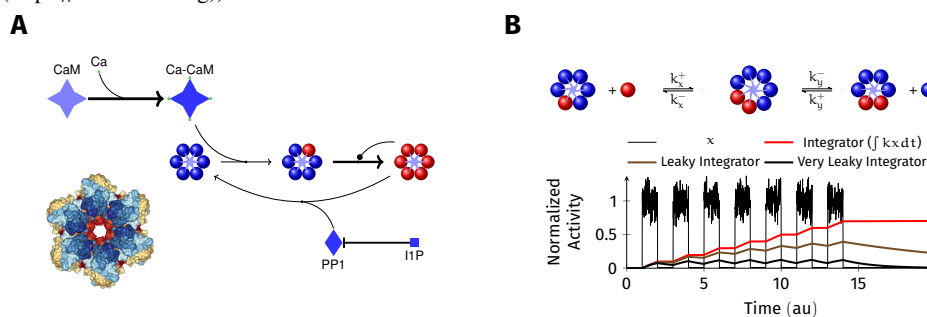


A bistable chemical system adapted from [6]. Species b and c catalyze their production (positive feedback) and inhibits production of a. **(below)** Two trajectories are shown in different settings. (Left) System is deterministic; (right) System is stochastic.

Box 2. CaMKII at synapse: A brief overview of its properties and function

Among more than 2000 proteins found in brain, CaMKII constitutes roughly 2% of them all. Furthermore, it is enriched in Hippocampus – a brain structure essential for memory formation. Indeed, CaMKII is known to play essential role in memory formation. 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. 12 or 14 subunits of CaMKII make up one holoenzyme, usually arranged in dodecameric (top view ) or tetradecameric form (top view ). CaMKII structure is shown in (A) inset below (from Protein Data Bank (<https://www.rcbs.org>)).



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

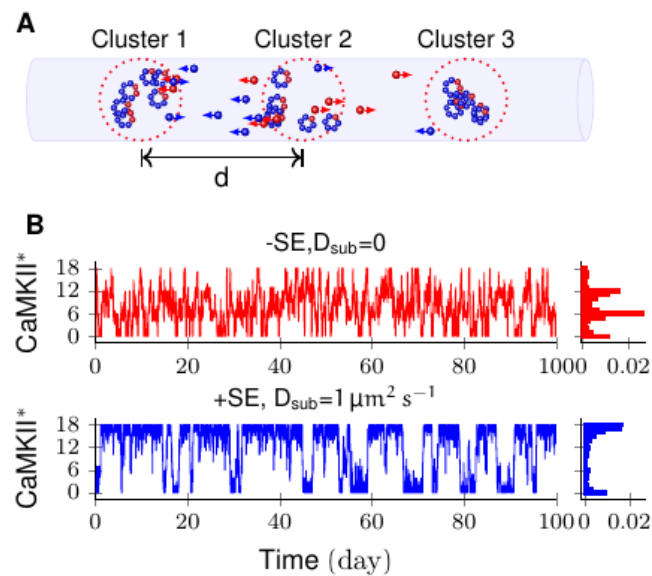
Note that first step is very slow for it requires binding of two $\text{Ca}^{++}/\text{CaM}$ simultaneously. Once a subunit has been activated, phosphorylation of its clockwise neighbour requires binding of only one $\text{Ca}^{++}/\text{CaM}$ and therefore further phosphorylation proceeds at much faster rate. Note that the first very slow step of CaMKII phosphorylation can be overcome by subunit exchange when an inactive holoenzyme picks up an active subunit released by other holoenzyme. Now this holoenzyme requires binding of only one $\text{Ca}^{++}/\text{CaM}$ for further phosphorylation. Therefore 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 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 if we model Ca^{++} activity as applied current and CaMKII activity as voltage across capacitor. Integrators are useful when you want accumulate *enough* information about 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.

In our computational study of this pathway, we explored the ef-

fect of subunit exchange on CaMKII pathway [7]. We found that the subunit exchange improves information retention capacity of CaMKII. Individual CaMKII acts as an leaky integrator of Ca^{++} activity (see Box 2.). With subunit exchange, the leaky integrator becomes less leaky and hold information for longer time. If many CaMKII holoenzymes come together and form a cluster, then they give rise to a bistable switch. A synapse is likely to have many such clusters i.e. many bistable switches. We found subunit exchange synchronizes these distributed switch. That is multiple bistable switches act as a single more stable bistable switch. To summarise, we found the subunit-exchange makes CaMKII molecule better at retaining information and it is likely that CaMKII is bistable in special micro-environments. To prove it, one needs to observe single molecule activity in synapse near surface of synapse which is a very challenging experiment.

Figure 5. (A) Three clusters of CaMKII in a small volume. (B) (red) 3 individual bistable switches without subunit exchange flipping independently. The distribution is binomial similar to the distribution of 3 coins flipped together. (blue) When subunit exchange is enabled, these 3 independent switches synchronizes and flip together. Notice that the 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. Most sup-



port for this idea has come from computational studies. To really prove it, we need experimental data supporting this hypothesis. There is growing experimental evidence that synapse change in *all-or-none* manner, a finding which is consistent with this idea. Some studies claim that changes are graded i.e. synapse changes in step-wise manner much like a **multi-stable** synapse. A multi-stable synapse is an ensemble of many bistable components. Whether CaMKII is bistable in synapse (or in some part of it) is still an open question. So far there is no concrete evidence that it is. There could be other still unknown mechanisms which can give rise to bistability. Given that bistable chemical motifs are widespread, it is reasonable to believe that there are indeed switches in our brain – much like flip-flops in your pen-drives and memory cards – which are evolved to keep our memories safe from the onslaught of time and noise.

Acknowledgements

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Suggested Reading

- [1] Lisman J. E., *A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase*. Proc. Natl. Acad. Sci. USA, May 1985
- [2] Christof Koch *Biophysics of computations*. Oxford University Press, 1999
- [3] Malin Sandstorm, *Models of CaMKII activation*, Master Thesis, Royal Institute Of Technology Sweden
- [4] Eugene Wigner, *The Unreasonable Effectiveness of Mathematics in the Natural Sciences*, Communications in Pure and Applied Mathematics, vol. 13, No. 1 (February 1960)
- [5] Thoman Wilhelm, *The smallest chemical reaction system with bistability*, BMC Systems Biology, vol. 3, No 1 (Sep 2009)
- [6] Naren Ramakrishnan, Upinder S. Bhalla *Memory Switches in Chemical Reaction Space*, PLOS Computational Biology, No 7 vol. 4 (July 2008)
- [7] Dilawar Singh, Upinder S Bhalla, *Subunit exchange enhances information retention by CaMKII in dendritic spines*, biorxiv, doi:10.1101/372748 (2018)

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