David T. Yue

A great teacher who spoke in parables.

As a professor in the Biomedical Engineering Department at Johns Hopkins, Dr. David T. Yue placed enormous value in teaching and put forth great effort in compiling coherent lectures that sought to describe biology in a truly quantitative manner. In this regard, he often used analogies or parables to provide an intuitive and often entertaining perspective of biophysical phenomena. Such analogies were an incredibly effective mode of instruction that we as students remember vividly. Professor Yue taught two courses that he absolutely cherished, (1) Systems Bioengineering I (formerly known as Physiological Foundations) and (2) Ion Channels of Excitable Membranes. The Systems Bioengineering I course is a core Biomedical Engineering course taught to mostly upper level undergraduate or early graduate students each fall. Dr. Yue's section covered topics of ion permeation, ion channel gating, and the generation of action potentials. The Ion Channels of Excitable Membranes course was in many ways an extension of the Systems Bioengineering I course and comprised of more advanced or current topics in ion channel biophysics. His exams and homeworks were often agonizingly difficult, forcing us to burn the midnight oil on many occasions. But those problem sets were always insightful and challenged his students to rise up to the occasion. He believed strongly in teaching as a way to elevate students to higher ground. To him, the pursuit of enlightening a next generation of thinkers was a worthy and noble cause – something he valued immensely throughout his life.

We describe, here, ten analogies that were particularly memorable and illuminating – things we pondered about in the *quietude of our own rooms*, as he asked us to at the end of every lecture.

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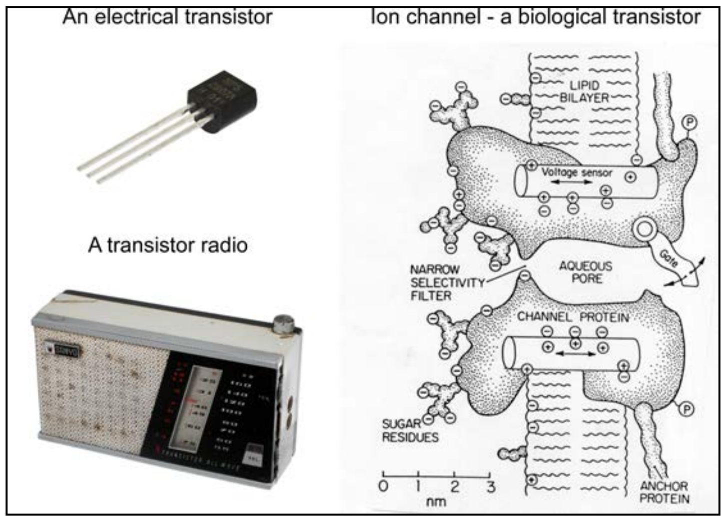
These memories are contributed by his students in the Calcium Signals Laboratory who were absolutely enthralled by his lectures and described them to be akin to watching thriller movies.

I. Ion channels as nonlinear electrical circuit elements – *A Christmas gift for the boys*

David admired both the beauty of biology and the rigor of engineering. In many ways, ion channel biophysics is a field that sits prominently at the confluence of both biology and engineering. David often recalled how he took apart old electronic equipment in his youth and this love for electronics was apparent in his later work on ion channels. In this manner, David often described ion channels as nature's very own transistors. In response to an appropriate voltage change, these biological macromolecules open to convey ion flux or a current, as a transistor might. Electrical impulses known in the biological realm as action potentials emerge as a result of the coupled nonlinear dynamics of many classes of ion channels. In his introduction to the Systems Bioengineering course, David reminisces over a particular event as a young father.



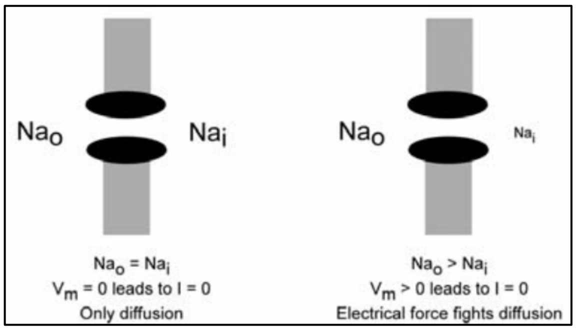
"So, when I was a younger professor, I had three young boys you know they were all below ten, were ten years and diapers, running around the house. A lot of times, they wasted their childhood in my sort of viewpoint – electronic video games and card games and things like that. And sort of as a Hopkins professor coming from a nerd world view, I wanted to interest them in the higher things in life. So, when it came to Christmas, I wanted to give them a gift that not only they can enjoy but that would edify them and elevate their purpose in life. What I wanted to do was to get them an electronics lab kit from RadioShack. I went to the RadioShack store and there was a plethora of kits you could buy. Some were very very expensive, but they had a lot of gadgets in them. Some were medium priced. Some of them still attractively wrapped – looking very good – but were more economical. Coming from sort of a Chinese American Asian background, I succumbed to my core cultural heritage and bought the more economical package. So I brought it home and the kids saw that package and were all excited and ripped it open and in five minutes they were bored to tears. Because inside of that electronics kit was wires and things like that – all the linear devices. And there was no magic. So I learned my trick. I went back to the RadioShack store and bought the Mondo kit – the exciting one – and I brought that back and they were very excited. They could build all sorts of things like a burglar alarm, and there were sirens going off – had the nonlinear devices. Those kits had the magic of electrical signals. So, for biological electrical signals, the magic are a class of molecules called ion channels. That is what we are going to be focusing on"

II. Nernst potential – *Population control through economics*

The concentration of ions in the extracellular and intracellular milieu of the cell often differs in magnitude. As ions are charged particles, this concentration gradient sets up an electrical potential across the cell. This potential is the very famous Nernst potential that David often expected his students to recall at the ready. So in this spirit, we recall this mathematical relation in a Yue-like nerdiness:

$$V_X = \frac{RT}{z_X F}\ln\left(\frac{[X]_o}{[X]_i}\right) = \frac{25}{z_X}\ln\left(\frac{[X]_o}{[X]_i}\right)$$

Here, V_X is the Nernst potential, R is the gas constant, T is the temperature, F is the Faraday's constant, and $[X]_O$ and $[X]_i$ are concentration of ions outside and inside the cell respectively. To the erudite, this relation is a consequence of thermodynamic balance between the chemical and the electrical potential of ions between the inside and the outside of the cells at equilibrium. As customary, David had an analogy of this principle as well.



Imagine you could teleport anywhere in the world. Where would you go? Perhaps somewhere with less people like Hawaii! You could work on your tan, make sand castles, surf, snorkel, hike, etc. If people could teleport, eventually at equilibrium, the population density in Hawaii would probably equal the population concentration here in Baltimore such the left subpanel of figure above. Thus, in the absence of an electric field the concentration of ions will equal on both sides of the membrane. Unfortunately, teleportation is not yet feasible so if you want to go to Hawaii you have to take a plane, which is extremely expensive. Thus, although the population density in Hawaii is less than that in Baltimore, travel to Hawaii is limited by economics such as it might be for ions in the presence of an electric field (right subpanel of figure above).

III. Ion permeation - *the Bathtub analogy*

An important feature of ion channels is the permeation properties of ions through the channel pore or in layman's terms, the propensity of ions to flow through the channel. A simple classic model for ion permeation is the battery-resistor model. Here, an electrical gradient is established as a result of differences in ion concentrations between extracellular and intracellular milieu of the cell (the Nernst potential). Accordingly, the channel behaves as a battery in series with a resistor. Though this view of ion flux is often useful, it is inaccurate. In fact, the conductivity of a solution of water depends on its ion concentration. A low amount of salt within the pore decreases electrical conductivity, while a high amount of salt enhances electrical conductivity. Thus, the concentration gradient between the extracellular and intracellular solutions of a cell would give rise to nonlinearities in ion permeation through the channel – a process known as rectification to the erudite. David had a particularly memorable analogy to describe this process – the infamous *bathtub analogy*.

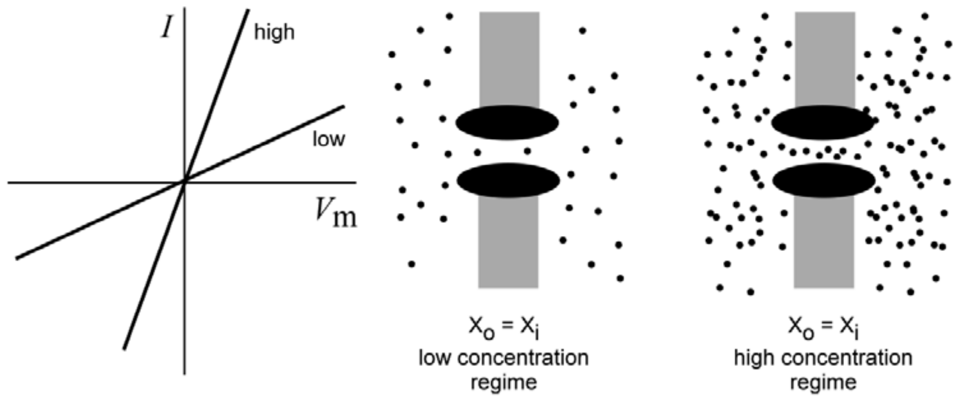


Figure 11. Consequence 1 of bathtub analogy

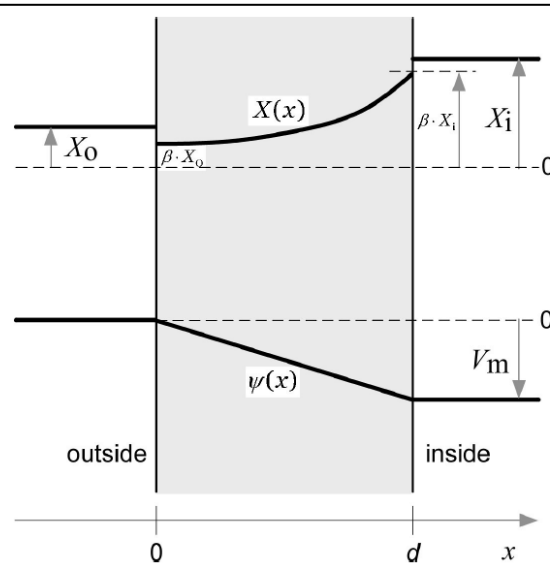
"Suppose you have your roommate from hell that you are forced to live with. And you are very good citizen at the beginning of the year. So you put up with them. But this roommate is really bad and by the time a few months in the semester, it is getting old. You have these very evil thought. So you want to get rid of this roommate but you don't know how. So one day, one of the things that bothers you about this evil roommate is that they are hogging the bathroom in the morning – nothing worse than that – right? Not only are they hogging it, they are taking a nice long bath. Who takes a bath in the morning? So you can't get ready for the day and you can't make it to SBE. And this bad roommate is not only bad to you but he is bad to others. He works in a lab and he is taking a bath using water stolen from his lab – the double distilled water – highest quality water. So you don't know that detail at first so you say, 'are you almost done with the bath tub?' He responds, 'Stop rushing me!' So you see this little radio by there with a power plug and you knock it into the bathtub and you are thinking you will electrocute this roommate and you will be done with it. So you knock it in there, and it splashes. Nothing happens! This evil roommate says, 'hey what are you doing?' And suddenly you remember the lecture from SBE I about permeation. With the double-distilled water there are hardly any ions in it. The conductivity is very low and you can't electrocute them. What I need is some salt in there. So you say, 'Just a moment.' You go back out to the kitchen and you get the salt shaker, you unscrew it and put a little a handful of white crystalline salt in there. And you come back in there, and you just flick it out there and as the salt is coming in you say, 'hasta la vista, babe!' He is electrocuted. So you will never forget it again."

IV. The Goldman-Hodgkin-Katz equation – *Mom's home!*

A more sophisticated model of ion permeation through a channel is the electrodiffusion model also known as the Goldman-Hodgkin-Katz (GHK) equation. In principle, this model considers the flow of an ion through the channel pore as the result of diffusion or random walks and an added component that reflects the drift velocity of the ion through the pore. This drift velocity is attained as a result of the balance between an electrical force and a frictional force that the ion experiences as it travels through the pore. Though mathematically intimidating, Prof. Yue expressed this process using an analogy:

"… in the absence of electric field you are just bouncing – random walks that is the Fick's law part. This is like my boys and I before mom comes home. Often times we are just chilling out in the family room and it is a little bit messy and we got chips out, soda out. Dad doesn't care too much. We are just diffusing – random walk. But when the electric field is turned on right, that is like when mom is getting home. All of a sudden, we feel something. 'Oh, it is not cleaned up, and there is a certain thing.' … So if the electric field is on you will feel a force … So you move, but when you start moving, it is like Mom says "Dad, clean up the room – clean up this, that. But of course you can't clean things up instantaneously – you would run into things in the room and slow down. So there is a friction coefficient. Right? So eventually the electrical field is countered by the frictional force and you reach sort of a terminal velocity. There is only so fast that you can clean up the house, much to Mom's concern."

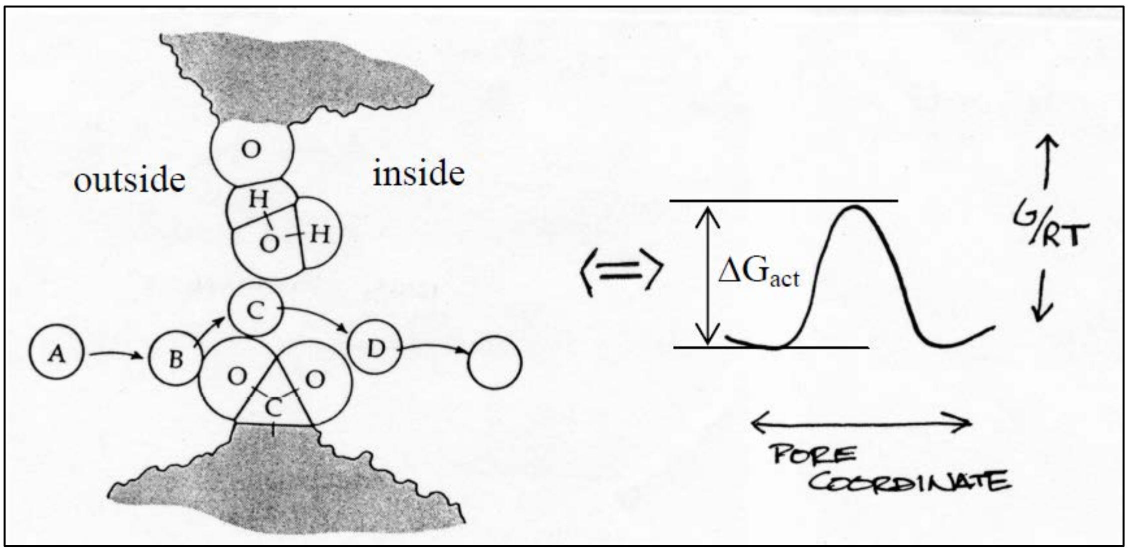
Figure 13. Layout and assumptions of GHK current equation model.



(CONSTANT FIELD
1 ASSUMPTION)
2 INDEPENDENCE

V. Eyring Rate theory – *The bliss of the basement!*

Eyring rate theory describes the kinetics of chemical reactions using underlying energetic parameters. In the case of an ion channel, one may model permeation as an ion crossing a narrow barrier inside the channel pore – the so-called selectivity filter. This process could be thought of as a chemical reaction with the transition state being the ion at the selectivity filter. As such, the formalism of Eyring rate theory relates the ionic flux to the energetics of the transition state through an exponential function. If the barrier were high (i.e. ΔG is large), then the rate of ion passage through the pore is slow. By contrast, if the barrier is shallow, then the ion readily passes through the pore. David described this chemical process using a personal recollection.

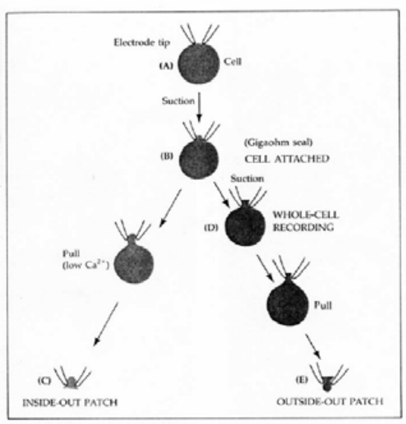


"You could almost intuit it. I like to think of it as back when I was a little bit younger – a young assistant professor at Hopkins. We worked very hard. We still work hard but back then we worked very hard. So when I got back home, we lived in a little duplex and had a basement, I would always go to the basement and I'd always be in the lowest coolest part of the basement. And my wife would come home, we didn't have as many kids back then and she would look around expecting someone to get dinner ready or something like that but she would find me down in the lowest part of the basement, cool, and supine on the floor there. 'Honey, Honey, what are you doing?' And I said, 'just following gravity.' I was at my low energy minimum. But that wouldn't do right. So, she tried to get me up to do stuff. Be responsible at that point. So, the rate of those inquiries to do something was k_bT/h related to the absolute temperature. This is basically the molecular vibration rate. It is the number of times that the ions would knock on the door, saying 'get up, get up.' How many times per second would I get those calls? But, not every one of those calls will be successful. There is a long stair up from the basement to the kitchen and all those tasks there. So I had to multiply it by the probability that a certain knock on the door would be enough to get me up from the basement. So that would be related to the height of the stair, which is this ΔG. So this is the success rate that a molecular knock on the door would actually result in the passage of an ion through that barrier."

VI. Patch clamping – *Bruce Lee and the broken beer bottles*

A revolutionary advance in the study of ion channel gating occurred with the discovery of "patch clamp," which resulted in a Nobel Prize for Erwin Neher and Bert Sakmann. This experimental technique allows for the interrogation of single molecules in its full glory – something David used repeatedly to advance our understanding of both Ca^{2+} and Na^{+} channel gating. He was particularly proud of his early recordings of Ca^{2+} current through a single voltage-gated Ca^{2+} channel in guinea pig ventricular myocytes. Briefly, the technique involves the formation of a 'Giga-ohm electrical seal' between a glass pipet and the cell membrane. This process, however, was quite challenging and required a key advance – the ability to create glass pipets with a smooth orifice that could interface perfectly with the cell. He described this process with great enthusiasm as transcribed below.

Erwin Neher (left) and Bert Sakmann in their laboratory (1985).



2 PATCH CLAMP RECORDING CONFIGURATIONS. (A) On contact with the cell membrane, the electrode forms a seal, which is converted to a gigaohm seal (B) by gentle suction. Records may then be made from the patch of membrane within the electrode tip (cell-attached patch), or a cell-free, inside-out patch may be made by pulling (C). Alternatively, the membrane within the electrode tip may be ruptured by further suction to obtain a whole-cell recording (D), or by subsequent pulling to obtain an outside-out patch (E). (After Hamill et al., 1981.)

"This story here encapsulates the magic that turns that jagged [pipe] into something smooth. So, back to 'Enter the Dragon' with Bruce Lee. And I think this is the number one bad guy or the chief evil doer. He is really bad. And bad guys although they fight really well they fight dirty. So, this guy is kind of fighting dirty. This guy earlier in the movie, actually killed Bruce Lee's sister in cold blood. He knows that so they are coming out for the match and so it is not just competition. It is personal. And the way these matches are they both hold their hands up there and no one tells them when the match starts –it is whoever moves first. That is when it starts. You have to have fast reflexes. And this bad guy is really confident because before the scene here, just to show off he had this like huge board and he goes in there and 'hoof!' and then just splinters it into bunch of little pieces just to intimidate Bruce Lee. And he just looks at that and says 'hmm, that is interesting.' And so they are up there like that. And Bruce lee who is super-fast, wow, he moves so fast. This guy couldn't react he was completely struck to the ground. They do that three times. So this bad guy is not going to lose fair and square. He is mad. So what he does - he goes to the side and he gets some bottles. They were like beer bottles. He breaks them and then Bruce Lee is walking backwards because he thinks he won and this guy is going to come and attack him from the back. He is going to fight him unfairly. I won't tell you what

happens. Imagine that broken beer bottle there. So how will you make it smooth and make this a peaceful outcome. This is what Erwin Neher did. So what Erwin Neher did, he subjected this to high heat wire. It is like you have the broken beer bottle and stick it in a Bunsen burner. And it melts and gets gooey. Then when you take it away, it is smooth. So by fire polishing his pipets he made it very very smooth. And then on a nerdy weekend when he was working away, instead of leading a well-rounded life. He kissed up to a cell with these polished electrodes and he formed the first gigaseal. For that they got the Nobel Prize."

VII. Ion gradients in living cells – *Bruce Lee and the salty wound*

A key aspect of cells that enables electric signaling is the concentration gradients of various ions between the intracellular and extracellular milieu of the membrane. Dr. Yue expected his students to remember these concentration gradients as they determined the Nernst potential of each ion. As typical of him, he did not leave the students on a ledge. He helped out his students with a mnemonic.

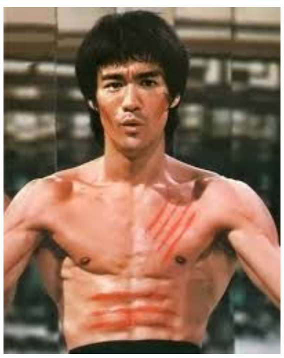


TABLE 3. FREE IONIC CONCENTRATIONS AND EQUILIBRIUM POTENTIALS FOR MAMMALIAN SKELETAL MUSCLE

Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	$\frac{[\text{Ion}]_o}{[\text{Ion}]_i}$	Equilibrium potential[a] (mV)
Na^+	145	12	12	+67
K^+	4	155	0.026	−98
Ca^{2+}	1.5	$<10^{-7}$ M	>15,000	>+128
Cl^-	123	4.2[b]	30[b]	−90[b]

[a] Calculated from Equation 1-11 at 37°C.
[b] Calculated assuming a −90-mV resting potential for the muscle membrane and that Cl^- ions are at equilibrium at rest.

"Remember what happens to Bruce Lee when he gets into a fight. Bruce lee is like probably the greatest Kung Fu artist that has ever lived. It is incredible. He fought with a lot of wicked villains. One that I remember is Mr. Han man. If you remember Bruce Lee, the fights didn't always go smoothly. At the end victory would prevail but initially it was kind of rough. Usually he would come in there and there would be some wicked villain and Bruce Lee wouldn't be quite ready and the wicked villain of course would strike first. He was struck and it was kind of unfair, because the wicked villain didn't fight with human hands. He had an attachment with sharp blades on it. So, the villain would scratch Bruce Lee. Bruce Lee wasn't fast enough in the initial phase to get out of the way. So there is blood, and what do you do in this sort of thing here – tasted that blood and that got him going. When he tasted that blood what did he taste? What was it like? Salty! Haven't you ever been punched in the face? It is salty. The reason it is salty is because your blood is like the extracellular solution of cells and there is a lot of NaCl. So there is high sodium concentration. And on the inside it is lower. What about potassium, potassium is kind of the opposite."

VIII. Laplace transforms – *Beam me up, Scotty*

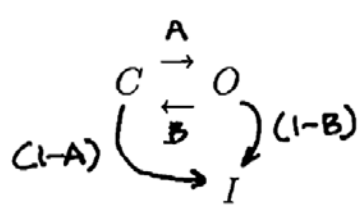
In the mathematics of linear time-invariant systems, Laplace transforms are an invaluable tool that allows one to transform systems from the time domain into the Laplace domain or the frequency domain. Such transformation often simplifies the problem at hand by converting systems of hairy differential equations into simpler systems of algebraic equations that are easy to manipulate. Once the simplification is performed in the Laplace domain, it may be transformed back into the time domain to obtain a final solution. David often admired the simplicity and power of Laplace transforms. He likened this magical mathematical transformation to a teleportation device from his favorite television series, Star Trek.

In the series Star Trek, Captain Kirk leads his heroic team on various adventures throughout the galaxy. Montgomery "Scotty" Scott, a brilliant engineer in Kirk's team, developed an ingenious algorithm that allows him to transport people great distances. Thus, Captain Kirk would shout "beam me up, Scotty!" and Scotty would beam him out of many challenging situations. Similarly, the Laplace domain acts as a method by which mathematically inclined individuals may rescue themselves from intractable problems and achieve higher ground by more elegant means.

IX. Transition state theory – *the parable of the Roach Motel*

David loved to think of ion channel gating using *transition state theory*. The ion channels in their gating process transition through a variety of states. Some of these states are conductive while others are not. This sort of thinking is very powerful to understanding biological molecules like ion channels. Accordingly, there are two types of states, a *transient* (*T*) state or an *absorbing* (*A*) state. The transient states, as the name implies, are ones where you can both enter and leave the state. By contrast, the absorbing states are ones where if you enter them, then you cannot leave.

Example 1 *An example of this classification of states comes from the ACS analysis.*



Here, $\mathcal{T} = \{C, 0\}$ *and* $\mathcal{A} = \{I\}$.

David had a very funny analogy for these two kinds of states: *There are these little traps for roaches called the Roach Motel. Roaches can go into these traps and there is some kind of a sticky paper that prevents the roaches from getting out once they get in. So the way they advertised it was – 'they check in but they don't check out.' That is how you can think of these absorbing states – you can check in but you can't check out. The transient states are outside of the roach motel.*

X. Lifetime of sojourn in a state – *the Chinese banquet hypothesis*

David loved to impose the formalism of *Markov processes* to describe the gating of ion channels. One can imagine that during the gating process, a channel transitions between multiple states corresponding to physical processes such as movement of a voltage-paddle. Indeed, the transitions between states are governed by characteristic rate constants that determine the lifetime of sojourn in a given state. In the simplest case, there are only two states. Thus, the lifetime in a given state is determined by the singular rate constant for leaving this state. But channel gating being more complex involves multiple states requiring multiple rate constants. It is here that a paradox emerges (see figure below). One might imagine that by simply observing the lifetime of sojourn in the central *O* state, one could determine the propensity of leaving state *O* towards either state *A* or state *B*. This turns out to not be the case. The lifetime in state *O* is governed by both transitions and cannot be disentangled by lifetime measurements of state *O*. David clarified this challenging concept through an analogy – the Chinese banquet hypothesis.

$$A \xleftarrow[a]{} O \xrightarrow{b} B$$

" … the best Chinese restaurant in Baltimore, Szechuan House, not saying it is a good Chinese restaurant but the best Chinese restaurant in Baltimore. And one of their best dishes is Kung Pao chicken. So imagine we are seated at a table with a plate of Kung Pao chicken sizzling – I love it, can't wait to bite into that with the hot peppers, sometimes in the mood, I eat a hot pepper. And, that is wonderful. Now, imagine there is a barrier between two people. This barrier doesn't prevent you from eating from the plate, but it inhibits you from seeing anyone else eating from it. So in front of this plate, one of them, we have this very slender well-mannered soft spoken young woman, she is at the meal picking at the plate with a lone pair of chopsticks. On the other side, we have this gentleman, massive, big, and hungry. He has got a pair of chopsticks too. And can use it very well. They both eat from this plate, but they can't see each other. She doesn't know there is this big guy, and this big guy doesn't know there is this diminutive girl. So what we do is we calculate the average lifetime of a piece of Kung Pao chicken disappears going one way versus another. Our intuition is, well, the lifetime going of the piece of chicken going [towards the big guy] is a lot shorter than towards [the slender young woman]. It is obvious. Look at the relative size of these individuals. But in actuality, the average lifetime of a piece of Kung Pao chicken that goes towards the slender woman is exactly the same as the average lifetime of the piece that goes towards the big guy. The answer to this paradox – is what we will cover in next lecture."