## Testing fractal and Markov models of ion channel kinetics

Dear Sir:

Recent papers by Korn and Horn (1) and McManus et al. (2) compare models with a few exponential time processes to those with fractal kinetics. These few state exponential models (3) used for 35 years to model channel kinetics assume that: (*a*) there is only a small number of discrete states and (*b*) the kinetic rate constants connecting these states are independent. However, supported by the patch clamp data, and consistent with protein biophysical measurements and molecular dynamics simulations, we proposed (4–8) that channels have (*a*) a large number of states and (*b*) that the kinetic rates connecting these states were linked, and were not independent. Since the data suggested that this linkage has fractal properties, we called our model a fractal model. Korn and Horn (1) and McManus et al. (2) conclude that the few state exponential models fit the data better than the simplest fractal model and therefore imply that ion channels do indeed have a few states connected by independent, exponential kinetic rates. Such a conclusion is not supported by the evidence they present. First, they have ignored the extensive experimental and theoretical work over the last decade (9–20) that shows that proteins have a large number of conformational states and can have non-exponential dynamics; this contradicts their assumption of a small number of exponential time processes. Second, the few state exponential models fit the data not necessarily because they have any physical meaning, but simply because they have a large enough number of adjustable parameters, up to 15 in the paper by McManus and Magleby (21). Korn, Horn, McManus, Weiss, Spivak, Blatz, and Magleby have provided new evidence for the old adage that any model with enough adjustable parameters can fit anything.

It is not as simple as "Markov vs. Fractal." There are really two separate issues: (*a*) are there a small number or a large number (a continuum) of channel states, and (*b*) are the kinetic rates between these states independent or connected by a simple relationship, fractal or otherwise. All four combinations are possible. An exponential model with a few discrete states may have independent rates, or it may have a set of kinetic rates with a fractal scaling. A continuum model may not have a simple relationship connecting the states, or it may have a fractal scaling connecting the states.

The crux of our fractal model was that overall the channel behaves as if it has memory. That is, the longer the channel resides in a state, the less the probability per unit time that it exits that state. Different mechanisms can produce such a memory. For example, this occurs in the model proposed by Millhauser et al. (22) where one open state is connected to a long Markov chain of hundreds of closed states. When the channel has diffused a long way away from the open state, it will take a correspondingly longer time to reopen. Läuger has proposed a model (23) where a small piece of the channel moves to block the channel shut. The rest of the channel will then rearrange itself so that the longer the wait, the less probable per unit time is the return of the blocking piece to its original location to open the channel. Both these models have many states with overall fractal properties constructed from pieces whose fundamental processes are Markovian. Thus, these models suggest mechanisms to produce the features we proposed in the fractal model. The usefulness of the fractal way of thinking is to emphasize the overall memory, and thus to force one to search for the underlying physical processes that produce it.

We now give detailed responses to the issues raised by Korn and Horn and McManus et al.

### 1. Discrete or continuous?

Korn & Horn (1) and McManus et al. (2) fit Markov models with increasing numbers of states until the fit failed to improve. That is, they assumed a priori, that if two models equally well fit the data, then they prefer the model with fewer states. They did not test whether models with a much larger number of states could indeed fit the same data equally well. Thus, they did not test whether the channel data is best represented by models with few or many or a continuum of states.

### 2. Comparing models with different numbers of parameters

The few state exponential models add two adjustable parameters for every state added. The simplest fractal model has just two parameters. Hence, a few state exponential model with several states is likely to be a better fit to any data because it will have many more adjustable parameters than the simplest fractal model. Thus, as expected, McManus et al. (2) found that exponential models with 11 adjustable parameters better fit the data than the simplest fractal model with 2 adjustable parameters. Such a comparison, of course, tells very little about the relative merits of the two models.

To control for the improvement of fit added by additional parameters both Korn and Horn (1) and McManus et al. (2) use the Akaike Information Criterion (AIC) (24) to discriminate between models having different numbers of parameters. Does this criterion always work? No. We can see why by considering a simple example. Suppose we measure the perimeter of an object. We fit the data with polygons of $n$ sides, increasing $n$ and using the AIC to determine how many sides our polygon should have to appropriately represent the object. Let's say Nature has played a naughty trick on us and the object is not a polygon at all, but a circle! The AIC requires that to be accepted, each additional parameter must significantly improve the goodness of fit. However, when $n$ is large, increasing $n$ adds little improvement in the fit between our polygons and Nature's circle. Thus, the AIC will lead us to conclude that our object was a polygon with only a small number of sides. That is, the AIC will not choose the true answer, which is an infinite number of sides. This happens because the parameterization of the models tested is poorly matched to Nature's object tested. In the language of Akaike's paper (24), if the norm of $\theta - \theta_0$ is not small, then the assumptions used in deriving the AIC are no longer valid. Using the AIC to discriminate between different channel models is

subject to this limitation. Since we do not know if either the few state exponential or the simplest fractal model is indeed a close parameterization of Nature's channel, we are not guaranteed that the AIC analysis is valid. In fact, there is no foolproof method to determine how many parameters are needed to model a given data set. This is at present an unsolved statistical problem. Although many techniques have been proposed, none has gained universal acceptance.

However, we can compare the few state exponential model to a null hypothesis model, with the same number of parameters. Since open and closed time histograms $f(t)$ are often nearly linear when $\log f(t)$ is plotted against $\log t$, a good null hypothesis would be a logarithmic polynomial. That is, we can compare the fit of the physically meaningless model $\log f(t) = a_1 + a_2 \log(t) + a_3[\log(t)]^2 + a_4[\log(t)]^3 + a_5[\log(t)]^4 + a_6[\log(t)]^5$ to the fit of the model $f(t) = a_1 \exp(-a_2 t) + a_3 \exp(-a_4 t) + a_5 \exp(-a_6 t)$, both having 6 parameters $a_i$. The fit of both these models to the data recorded by Korn and Horn (1) is shown in Fig. 1. The physically meaningless log polynomial is a closer fit to the data than the few state exponential model. The sum of the squares of the residuals between the data and the fit by Korn and Horn is 10.4, while that of the physically meaningless log polynomial is 2.1 This is a statistically significant difference (F-test, $F = 4.95$, $\nu_1 = \nu_2 = 98$, $P < 2.6 \times 10^{-14}$). Hence, a completely physically meaningless model is better fit to the data than the three-state exponential model if both have the same number of parameters. Thus, we conclude that the few state exponential models fit the data not because they necessarily have any physical meaning, but simply because they have enough adjustable parameters.

## 3. Can sums of exponentials be reliably fit to open and closed time histograms?

The author of a textbook on numerical methods (25, p. 253) writes: "For it is well known that an exponential equation of this type [$f(t) = a_1 \exp(-a_2 t) + a_3 \exp(-a_4 t)$] in which all four parameters are to be fitted is extremely ill-conditioned." He adds, that those who try to determine the parameters of such equations from experimental data "must be spanked or counselled. At the very least, keep them from obstructing Progress and the computer!" A recent review of protein dynamics (16, p. 34) states that: "The fitting of experimental data to sums of exponentials is a notoriously ill-conditioned problem, and the determination of individual exchange rates with [$f(t) = \Sigma_{i=1,n} \exp(-a_i t)$] is not possible." This is because exponential functions form neither a complete nor an orthogonal basis set and because the determinant of the information matrix is often close to zero. Ill-conditioned means that many different values of the parameters $a_i$ will equally well fit the data to within the experimental or numerical accuracy so that one cannot determine the "correct" values of the parameters. This is "well known" in applied mathematics, physics, and chemistry, but apparently not in the patch clamp field. For example, when we were cautious about using such methods, a patchologist reviewer wrote that these methods are "relatively simple ... standard procedures which are readily implemented." In one of the best

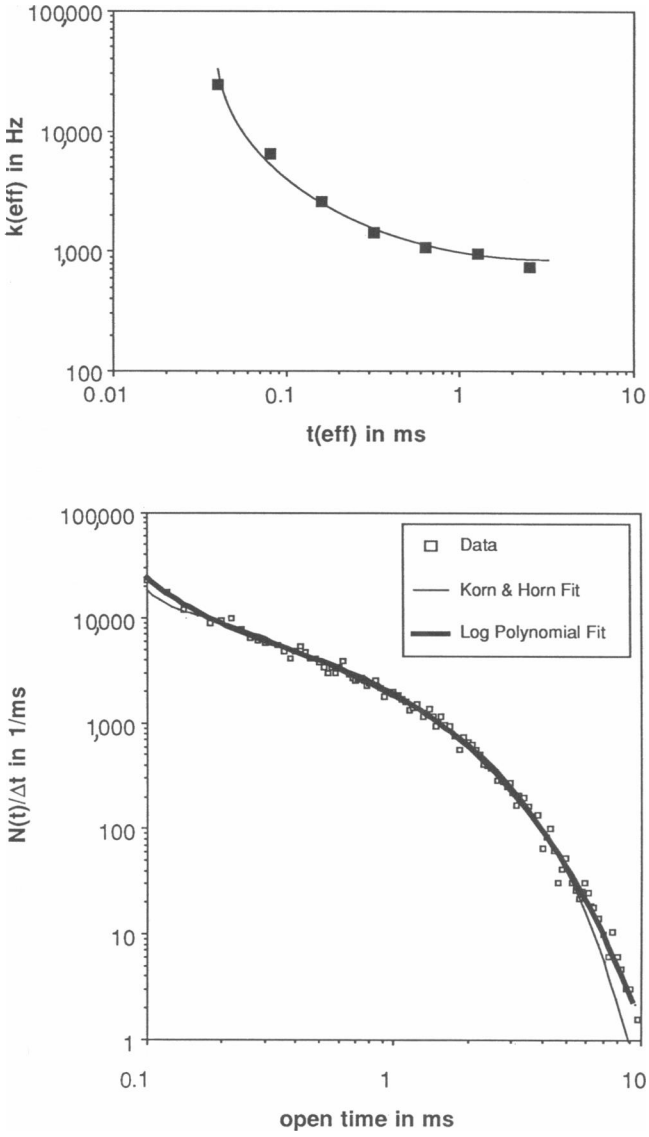


FIGURE 1 Re-analysis of the open times of Korn and Horn (1). (*Top*) Logarithm of the effective kinetic rate constant vs. logarithm of the effective time scale. There is no evidence for multiple plateaus that would corroborate the existence of three distinct states connected by exponential processes. (*Bottom*) Using the same number of adjustable parameters, the open time histogram of this data (□), is better fit by a log polynomial model with no physical meaning (*thick line*), than by the three-state exponential model fit by Korn and Horn (*thin line*). Kinetic models based on the sum of a small number of exponential time processes fit the single channel data not necessarily because they have any physical meaning, but simply because they have enough adjustable parameters.

review articles on the analysis of single channel data, two leaders in the field, Colquhoun and Sigworth, describe these methods as working quite well. However, in their tables and figures, the error bounds on the kinetic rate constants are large and after providing a very clear example fit by three exponentials they

add (26, p. 235): "However, if the same data were fitted by the sum of two exponentials, the fit still looked quite good, at least on the short and long time scales, although the parameter estimates were appreciably different." Moreover, since they provide all the terms of the information matrix (26, p. 252), one can evaluate its determinant which is found to be $1.4 \times 10^{-12}$, which is not a very big number, indicating that the fitting matrix is ill-conditioned. The question is: do these methods work? There is no discussion in Korn and Horn (1) or McManus et al. (2) on the reliability of the fitting procedures used to determine the parameters of their sum of exponential models. Since many other scientific fields have found fitting the sums of exponentials to be unreliable, the burden falls on those in the patch clamp field who use such methods to provide clear evidence that these methods yield reliable, accurate, unique solutions for the parameters given noisy experimental data.

## 4. Are the kinetic processes independent?

As noted by Millhauser, et al. (22), open or closed time distributions are often power laws or approximately power laws. Sums of exponentials will only form such power laws when the time constants of different kinetic processes are related, so that an overall power law scaling is produced. Although such data is clearly shown by Blatz and Magleby (27, Fig. 6) and McManus and Magleby (21, Fig. 12), they do not discuss if this lack of independence affects their statistical fitting procedures or suggest a mechanism responsible for it. On the other hand, the self-similar scaling of the fractal model leads to just such power law distributions (5, 6). Millhauser et al. (personal communication) have analyzed this lack of independence by studying the correlation between the time constants of the states and their relative frequency.

## 5. Plotting log $k_{eff}$ vs. log $t_{eff}$

A very useful method to study single channel data is to plot the effective kinetic rate constant, $k_{eff}$, against the effective time scale, $t_{eff}$ (4, 5). These plots provide a model-independent tool so that we can see forms in the data that we might not have anticipated. This is a much more powerful method than that used by Korn and Horn (1) or McManus et al. (2) who decide a priori what models to fit to the data and then compare the goodness of fit between these models. It is disappointing that they have not used these $k_{eff}$ vs. $t_{eff}$ plots. The usefulness of such plots in discovering new information was shown recently in the analysis by French and Stockbridge (28, 29) of the calcium dependence of potassium channels in avian and human fibroblasts.

Korn and Horn (1) found that the simplest fractal model is not a very good fit to the open time distribution of their data. This is also clearly shown in Fig. 1 in the plot of $k_{eff}$ vs. $t_{eff}$ that we determined from their open times. The simplest fractal model, which is a straight line on such a plot, is clearly not an adequate fit to these data. However, much more additional information can also be obtained from this $k_{eff}$ vs. $t_{eff}$ plot. Since, there are no resolved multiple plateaus, there is no evidence to support the three discrete states postulated by the few state exponential model fit. Rather, the plot can be interpreted as either: (*a*) a power law indicating many states and a fractal scaling at short time scales, and a plateau indicating a stable isolated Markov state at long time scales, similar to that found by French and Stockbridge (28, 29); or (*b*) as a continuous scaling that is only slightly more complex than the simplest fractal model, such scalings are called multifractals (30).

## 6. There is no physicochemical justification for fitting exclusively exponential time processes to open or closed time durations

Extensive experimental and theoretical studies over the last decade have lead to a revolution in our understanding of protein dynamics (9–20). It is now well established that proteins exhibit both exponential and non-exponential time processes. It used to be thought that proteins were adequately modeled as having a few stable conformations separated by static activation energy barriers of a single value. These ideas correspond to the assumptions of the channel models having a few states separated by exponential time processes. However, it is now known that those assumptions do not adequately represent protein dynamics or kinetics. Proteins have many nearly equal energy minima. For example, Elber and Karplus (18) found that over a 300-ps interval myoglobin sampled ~2,000 energy minima. Multiple pathways between such large numbers of substates result in a distribution of activation energy barriers. For example, such continuous distributions of activation energy barriers have been measured for myoglobin (9, Fig. 13) and lysozyme (16, p. 35, Fig. 2). Since we now know that the dynamic properties of proteins often display non-exponential processes it is simply no longer justified to model ion channel kinetics exclusively as the sum of a small number of exponential rates.

## 7. Beyond the simplest fractal model

Korn and Horn (1) and McManus et al. (2) have indeed shown that the simplest fractal model (4–8) does not have all the wealth of detail seen in the single channel data. This can be seen in Fig. 1 where the plot of $k_{eff}$ vs. $t_{eff}$ is more complex than a straight line. Protein kinetics can be thought of as the diffusion of a system between different states in a state space of energy conformations. Thus, we can gain insight in how to expand the fractal model from such theoretical models done by Huberman et al. (31–34), Millhauser et al. (22), and Liebovitch (8). As shown in Fig. 2 we can represent channel kinetics as (*A*) many closed and many open states connected through many pathways (22, 31–34), (*B*) many closed and many open states connected by one pathway (4, 5), or (*C*) many closed states that do not communicate with each other but that are connected by parallel pathways to one open state (8) or to many open states that do not communicate with each other. Although models of type *C* have only recently been applied to ion channel kinetics (8), they are now widely used to interpret results from experiments in globular proteins. Such models are interesting because one can

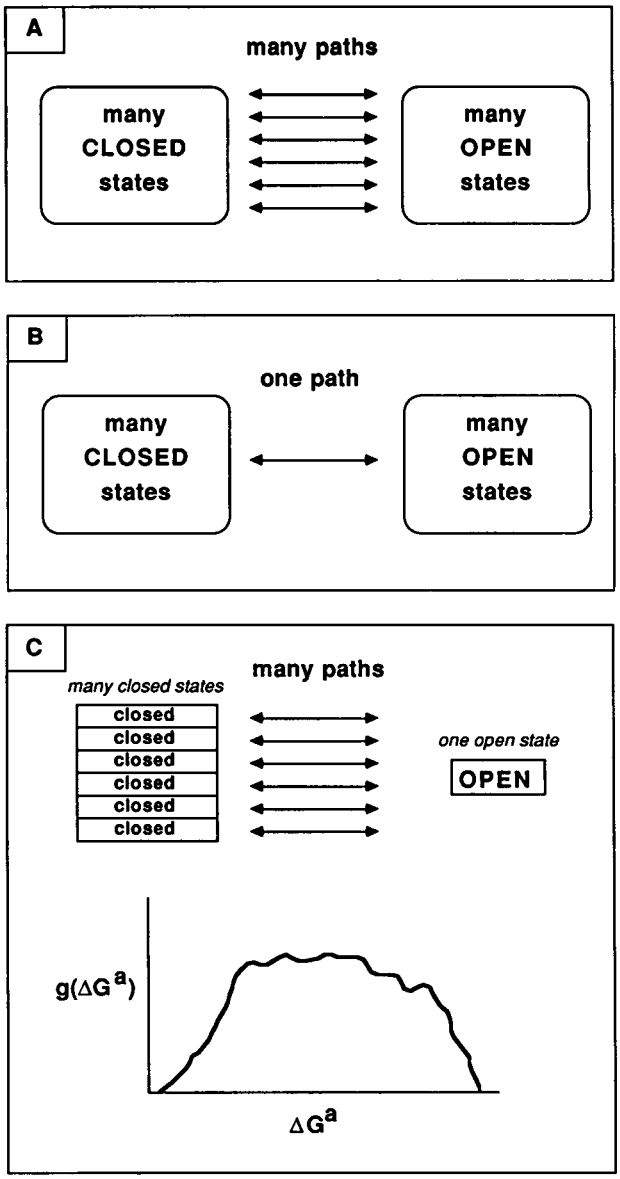


FIGURE 2 Three classes of models of ion channel kinetics. Models of type *C* were only recently applied to ion channels (8) although they have been widely used to model globular proteins. Such models have many closed states that do not communicate with each other but are connected by many parallel pathways to one open state (or many open states that do not communicate with each other). These models are interesting because all the details of the dwell times can be used to construct the distribution $g(\Delta G^a)$ of activation energy barriers $\Delta G^a$. The distribution of activation energy barriers is proportional to the inverse Laplace transform of the cumulative dwell time distribution.

calculate the distribution of kinetic rates and thus their associated activation energy barriers that separate the open and closed states. That is, the cumulative dwell time probabilities $P(t)$ are the sum of all the parallel processes and thus $P(t) = \int_{k=0,\infty} g(k) \exp(-kt)\, dk$ where $g(k)$ is the number of pathways with rate constant $k$ (35, p. 208; 16, p. 34). Hence, $P(t)$ is the Laplace transform of $g(k)$. The distribution of activation energy barriers $g(\Delta G^a)$ is proportional to that of their associated kinetic rates $g(k)$. Thus, the number of activation energy barriers $g(\Delta G^a)$ with energy $\Delta G^a$, is proportional to the inverse Laplace transform of the cumulative dwell time histogram. The detailed evaluation of $g(\Delta G^a)$ provides a model that can fit all the fine structure seen in the open and closed histograms. Perhaps the activation energy spectrum determined by this method may provide insight into the molecular mechanisms responsible for these activation energy barriers.

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## REFERENCES

1. Korn, S. J., and R. Horn. 1988. Statistical discrimination of fractal and Markov models of single channel gating. *Biophys. J.* 54:871–877.
2. McManus, O. B., D. S. Weiss, C. E. Spivak, A. L. Blatz, and K. L. Magleby. 1988. Fractal models are inadequate for the kinetics of four different ion channels. *Biophys. J.* 54:859–870.
3. Colquhoun, D., and A. G. Hawkes. 1983. The principles of the stochastic interpretation of ion-channel mechanisms. *In* Single-Channel Recording. B. Sakmann and E. Neher, eds. Plenum Publishing Corp., New York. 135–175.
4. Liebovitch, L. S., J. Fischbarg, J. P. Koniarek, I. Todorova, and M. Wang. 1987. Fractal model of ion-channel kinetics. *Biochim. Biophys. Acta.* 896:173–180.
5. Liebovitch, L. S., J. Fischbarg, and J. P. Koniarek. 1987. Ion channel kinetics: a model based on fractal scaling rather than Markov processes. *Math. Biosci.* 84:37–68.
6. Liebovitch, L. S., and J. M. Sullivan. 1987. Fractal analysis of a voltage-dependent potassium channel from cultured mouse hippocampal neurons. *Biophys. J.* 52:979–988.
7. Liebovitch, L. S. 1988. The fractal random telegraph signal: signal analysis and applications. *Ann. Biomed. Engr.* 16:483–494.
8. Liebovitch, L. S. 1989. Analysis of fractal ion channel gating kinetics: kinetic rates, energy levels, and activation energies. *Math. Biosci.* In press.
9. Austin, R. H., K. W. Beeson, L. Eisenstein, H. Frauenfelder, and I. C. Gunsalus. 1975. Dynamics of ligand binding to myoglobin. *Biochemistry.* 14:5355–5373.
10. Careri, G., P. Fasella, and E. Gratton. 1975. Statistical time events in enzymes: a physical assessment. *CRC Crit. Rev. Biochem.* 3:141–164.

11. Beece, D., L. Eisenstein, H. Frauenfelder, D. Good, M. C. Marden, L. Reinisch, A. H. Reynolds, L. B. Sorensen, and K. T. Yue. 1980. Solvent viscosity and protein dynamics. *Biochemistry.* 19:5147–5157.
12. Karplus, M., and J. A. McCammon. 1981. The internal dynamics of globular proteins. *CRC Crit. Rev. Biochem.* 9:293–349.
13. Karplus, M., and J. A. McCammon. 1983. Dynamics of proteins: elements and function. *Ann. Rev. Biochem.* 52:263–300.
14. Clementi, E., G. Corongiu, M. H. Sarma, and R. H. Sarma, editors. 1985. Structure and Motion: Membranes, Nucleic Acids and Proteins. Adenine Press, New York.
15. Sarma, R. H., editor. 1986. Biomolecular Stereodynamics. Adenine Press, New York.
16. Welch, G. R., editor. 1986. The Fluctuating Enzyme. John Wiley & Sons, Inc., New York.
17. Ehrenberg, A., R. Rigler, A Gräslund, and L. Nilsson, editors. 1987. Structure, Dynamics and Function of Biomolecules. Vol. 1. Springer-Verlag, New York.
18. Elber, R., and M. Karplus. 1987. Multiple conformational states of proteins: a molecular dynamics analysis of myoglobin. *Science (Wash. DC).* 235:318–321.
19. Alcala, J. R., E. Gratton, and F. G. Prendergast. 1987. Interpretation of fluorescence decays in proteins using continuous lifetime distributions. *Biophys. J.* 51:925–936.
20. McCammon, J. A., and S. C. Harvey. 1987. Dynamics of Proteins and Nucleic Acids. Cambridge University Press, New York.
21. McManus, O. B., and K. L. Magleby. 1989. Kinetic states and modes of single large-conductance calcium-activated potassium channels in cultured rat skeletal muscle. *J. Physiol. (Lond).* In press.
22. Millhauser, G., L. Salpeter, and R. E. Oswald. 1988. Diffusion models of ion-channel gating and the origin of the power-law distributions from single-channel recording. *Proc. Natl. Acad. Sci. USA.* 85:1503–1507.
23. Läuger, P. 1988. Internal motions in proteins and gating kinetics of ionic channels. *Biophys. J.* 53:877–884.
24. Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Auto. Cont.* AC-19:716–723.
25. Acton, F. S. 1970. Numerical Methods That (Usually) Work. Harper & Row, New York.
26. Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single-Channel Recording. B. Sakmann and E. Neher, eds. Plenum Publishing Corp., New York. 191–263.
27. Blatz, A. L., and K. L. Magleby. 1986 Quantitative description of three modes of activity of fast chloride channels from rat skeletal muscle. *J. Physiol. (Lond).* 378:141–174.
28. French, A. S., and L. L. Stockbridge. 1988. Kinetic analysis of cation channels in fibroblasts: Markov and fractal behavior. *Biophys. J.* 53:157. (Abstr.)
29. French, A. S., and L. L. Stockbridge. 1988. Fractal and Markov behavior in ion channel kinetics. *Can. J. Physiol. Pharm.* 66:967–970.
30. Cederbaum, L. S., E. Haller, and P. Pfeifer. 1985. Fractal dimension function for energy levels. *Phys. Rev. A.* 31:1869–1871.
31. Huberman, B. A., and M. Kerszberg. 1985. Ultradiffusion: the relaxation of heirarchical systems. *J. Phys. A.* L331–L336.
32. Bachas, C. P., and B. A. Huberman. 1986. Complexity and the relaxation of heirarchical structures. *Phys. Rev. Lett.* 57:1965–1969.
33. Keirstead, W. P., and B. A. Huberman. 1987. Dynamic singularities in ultradiffusion. *Phys. Rev. A.* 36:5392–5400.
34. Ceccatto, H. A., and B. A. Huberman. 1988. The complexity of heirarchical systems. *Phys. Scripta.* 37:145–150.
35. Frauenfelder, H. 1983. Ligand binding and protein dynamics. *In* Structure and Motion: Membranes, Nucleic Acids and Proteins. E. G. Clementi, E. G. Corongiu, M. H. Sarma, and R. H. Sarma, eds. Adenine Press, New York 205–217.

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