vate every PKA molecule in the cell. The morphological basis for diffusional restrictions is a fascinating subject for future study. With many of the necessary tools at hand, the time is right to expand our focus beyond the bounds of two-dimensional complexes to actual, four-dimensional signals.

JEFFREY W. KARPEN,* THOMAS C. RICH
Department of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, CO 80262, USA
*To whom correspondence should be addressed. E-mail: jeffrey.karpen@uchsc.edu

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Response

A macromolecular signaling complex is formed between the L-type Ca^{2+} channel $Ca_v1.2$ and the β_2 adrenergic receptor, heterotrimeric G proteins, an adenylyl cyclase (AC), and PKA, which regulate the activity of this channel by means of cAMP in a highly localized manner (*1*). Karpen and Rich perceptively ask: How can signaling by diffusible cAMP be localized so precisely?

As they showed earlier by mathematical analysis (*2*), simple diffusion cannot account for activation of PKA, even if it is close to the AC. Assuming a maximal catalytic rate of 59 per second for cAMP production by AC and a diffusion rate of 3 x 10^{-6} cm^2 per second, cAMP concentration will not exceed 5 nM in a distance of 10 nm from the catalytic site of the fully active AC if diffusion is unrestricted (*2*). Half-maximal activation of the various PKA isoforms is usually observed with a cAMP concentration in the range of 100 nM. ACs and PKA holoenzymes have molecular masses above 100 kilodaltons. The size of a protein with such a molecular mass is typically in the range of 5 nm. It is, therefore, likely that the distance between the catalytic site of the AC and the cAMP binding sites of PKA in our channel complex is 10 nm, if not larger.

The authors hypothesized in their work on cAMP-gated ion channels that elements of the endoplasmic reticulum might be localized underneath the plasma membrane, thereby limiting the diffusion of cAMP away from the AC and their channels in their system (*2*). This is a valid hypothesis that may also be true for the $Ca_v1.2$ channel complex in the heart. Cardiac $Ca_v1.2$ is precisely juxtaposed to elements of the sarcoplasmic reticulum (*3*) that could substantially limit cAMP diffusion by restricting it to two dimensions. However, the situation might be different in the system we studied, the neuronal cell body, and we propose here an alternative model for diffusional restriction of cAMP. We hypothesize that the AC and PKA might be arranged in such a way that cAMP is "channeled" from the AC to PKA by a molecular mechanism, thereby dramatically increasing the likelihood that newly synthesized cAMP will bind to PKA. Models of molecular product channeling are well established for metabolic enzymes, and mechanisms range from the formation of actual tunnels by proteins to electrostatic channeling, due to surface charges on the proteins that attract the substrate (*4*). A molecular mechanism of cAMP channeling would make activation of PKA by a neighboring AC more effective and spatially more restricted.

DUANE D. HALL, JOHANNES W. HELL
Department of Pharmacology, University of Iowa, Iowa City, IA 52242, USA. E-mail: johannes-hell@uiowa.edu

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CORRECTIONS AND CLARIFICATIONS

REPORTS: "A multispecies overkill simulation of the end-Pleistocene megafaunal mass extinction" by J. Alroy (8 Jun., p. 1893). In figure 1, some of the black or gray lines representing extant or extinct species, respectively, were the wrong color. The number and shape of the lines are correct, and the statistics are unaffected by the color error. The correct figure appears here.