Akihiro Kusumi

Cells make a living on the difference between inside and outside, with a bilayer lipid membrane standing between. In recent years, that membrane has been depicted as a fluid mosaic with the double layers of lipid molecules aligned heads-out and tails-in, behaving for all purposes like a two-dimensional liquid. Floating lipids and membrane proteins should spread out rapidly across a liquid-like membrane like ink drops in water, driven only by thermal inputs. In artificial bilayer membranes, they do. In living cells, they diffuse about 20 times more slowly on average, and sometimes they become completely immobilized.

To get at the critical difference between artificial membranes and those of cells, Akihiro "Aki" Kusumi has been studying membrane diffusion in living cells, one kinetic molecule at a time. Kusumi is a high-profile scientist in his native Japan, professor of biophysics at Kyoto University, and a prime mover behind the new Institute for Integrated Cell-Material Sciences (iCeMS). iCeMS, he hopes, will marry materials science and cell biology on the "mesoscale," that is, between the nano- and micron-scale worlds. Kusumi is equally at home in international science, where among other things he was just elected to a three-year term on the ASCB Council.

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Yet Kusumi is best known for his extraordinary experiments to capture single molecule movement. A biophysicist by background and a cell biologist by choice, Kusumi has assembled a large, multidisciplinary research team and adapted a high-speed digital camera originally designed to film explosions at the equivalent of 40,000 frames per second. He tethered single membrane protein and phospholipid molecules to colloidal gold tags and filmed them skittering across the surface of living cells in culture. Instead of diffusing randomly across a featureless liquid membrane, Kusumi's tagged single molecules bounced around inside tiny compartments 30–200 nm wide before "hopping" to a new compartment.

No one had seen this before Kusumi, says W. Karol Subcyznski, a longtime friend and collaborator (on other work) who is now at the Medical College of Wisconsin (MCW). "His work was the first to explain this problem." In living cells, diffusing molecules appeared to be moving much more slowly than in artificial systems, but that appearance was deceptive. Speed is time over distance, and until Kusumi filmed their movement in microseconds, the molecules didn't seem to be covering much ground. Yet Kusumi's camera showed them tracking round and round inside fenced compartments. Subczynski explains, "They diffuse very fast in small areas but only when they hop to another compartment can we see much movement, so they appear to be moving very slowly."

Hop Diffusion

Further experiments with optical traps, electron microscopy–computed tomography, and reagents that modify the cytoskeleton are helping Kusumi and his collaborators fill out the hop diffusion model. Kusumi now believes that membrane diffusion is directly influenced by the three-dimensional cytoskeleton beneath the plasma membrane, because the cytoskeleton and plasma membrane are closely and dynamically associated. These compartments confine moving phospholipids and membrane proteins, at least for a significant number of milliseconds, until they suddenly hop away into the next compartment. A fraction of transmembrane proteins are bound to the actin meshwork and thus catch against the actin network shapes and act like picket fence stakes on the cell surface. "Remember that the membrane is a fluid," says Kusumi, "and ruled by the laws of fluid dynamics." Hydrodynamic friction acts on the immobile pickets to form a diffusion barrier nearby.

Hop diffusion has been controversial in cell biology circles since Kusumi first reported

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his single molecule results for transmembrane proteins in 1993 and then for phospholipids in 2002. Some of the opposition stems from what Kusumi says is the misconception that hop diffusion contradicts or undercuts the leading model of plasma membrane organization, the so-called "lipid raft" model first proposed by Kai Simons, now at the Max Planck Institute in Dresden, and Gerrit van Meer of the University of Utrecht. "Some people think that rafts and compartments are conflicting ideas but I really don't think so, and I am sure that neither of the fathers of the raft does," says Kusumi. "I believe in rafts."

Kusumi's work extends to receptor action and cellular signaling. When glycosylphosphatidylinositol (GPI)-anchored receptors are stimulated, they form stabilized rafts, and interactions of raft-based lipids play key roles in this transgenesis, Kusumi reports. "We found that ligation of GPI-anchored receptors induces their clustering, which leads to the formation of stabilized rafts. With so many nanoscale transient rafts floating around, the plasma membrane is always ready to switch to larger events, which I think is at the heart of the raft thesis. Furthermore, we were able to use the new simultaneous two-color single-molecule tracking method that we developed to see the recruitment of intercellular signaling molecules, one by one, to these stabilized rafts." Surprisingly, intracellular signaling molecules stay in the raft of GPI-anchored receptor clusters for only a fraction of a second, which was totally unanticipated, he reports. "This might change our thinking about how the cellular signaling system works."

More recent results indicate that the plasma membrane is hierarchically organized, Kusumi says. "The entire plasma membrane is partitioned by picket fences and the raft domains are dotted within compartments, which are still undergoing hop diffusion across picket fences."

There is nothing in Kusumi's hop diffusion model that proves or disproves the existence of lipid raft domains, according to Genentech's Ira Mellman. Unfortunately, there are "raft loyalists" so determined to fight off any theoretical threat that they don't want to consider the cytoskeleton's possible role in surface dynamics, Mellman says. "A transmembrane segment that's buried within the lipid bilayer has an inside which faces the cytoplasmic domain, where it is constantly bumping into lots of stuff that could, in principle, impede its movement." Actin cable networks create cell shape and could well leave traces on the membrane, says Mellman.

No Single Crisp Experiment

But neither "side" has proved its case so far, says Mellman, because it's extremely hard to devise experiments or visualization methods that do not perturb the membrane system in unknown ways. "There's never going to be a single crisp experiment," Mellman predicts. "It's going to be an accumulation of correlations. That's what makes this field so difficult."

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"Wherever the truth may ultimately lie," Mellman says, "Aki has been enormously influential, and creatively so, in his impact on how we think about membrane microdomain organization and how we study it. He's really had an impact far outside his field." "You have only to look at my own lab group," Mellman continues. "We're not professional biophysicists and yet we're very much influenced in our thinking by the type of stuff that Aki has come up with over the years." It's also one reason that Mellman says he persuaded Kusumi to run for ASCB Council. The Society needs more biophysicists.

Kusumi likes to joke that a U.S. colleague once told him that he had the résumé of an American. Kusumi has studied, worked as a postdoc, or held faculty appointments in Kyoto, Milwaukee, Princeton, Milwaukee again, Kyoto again, Tokyo, Nagoya, and Kyoto for the third time. Returning to Kyoto in 2005 brought Kusumi full circle to the city where he was born, where he grew up the son of junior high school teachers of science and Japanese literature, and where he started at Kyoto University in "mainstream" physics. In his junior year, an applied mathematics lecture was cancelled and before Kusumi could slip out to a café, a friend dragged him off to a lecture on developmental

biology. The lecturer was the legendary embryologist Tokindo Okada. Kusumi was enthralled and soon after switched to biophysics.

As was the custom, Kusumi stayed at Kyoto for his DSc. He was taken into the laboratory of Shuni-chi Ohnishi, another renowned figure in Japanese science and a pioneer in spin-labeling proteins and membranes for analysis by electron paramagnetic resonance (EPR). Ohnishi was also unusual among his generation of post-war Japanese scientists for having gone abroad for postdoctoral training, working at Stanford with Harden McConnell.

Needed Cell Biology

With Ohnishi's encouragement and U.S. connections, Kusumi left Japan in 1979 for a postdoc with Jim Hyde at MCW in Milwaukee. Hyde was a leader in adapting EPR and nuclear magnetic resonance for biological and medical uses. In the Hyde lab, Kusumi used EPR to study rhodopsin diffusion in artificial membranes but began to chafe at the limits of these benchtop model systems. To pursue a dream of working in living cells, Kusumi decided he needed cell biology. He revamped his job talk and somehow convinced cell and developmental biologist Malcolm Steinberg that a biophysicist would be a useful postdoc in his differential cell adhesion lab at Princeton.

In 1984, Kusumi went back to Milwaukee, lured by a U.S. National Institutes of Health–funded instrumentation grant to set up the Microphotonic Center, a fluorescence lifetime imaging facility at MCW. But his mentor in Kyoto hadn't forgotten Kusumi. Ohnishi offered him an unusual part-time faculty position at Kyoto University that allowed Kusumi to commute between Milwaukee and Kyoto. Over the next 10 years, Kusumi's schedule gradually shifted from mostly American to mostly Japanese, as his lab moved from Kyoto to Tokyo University to Nagoya University. In 2005, Kyoto invited Kusumi and his Membrane Organizer Project to join its expanding Institute for Frontier Medical Sciences.

Through all this trans-Pacific flying and marathon high-speed camera sessions, Kusumi has been married for 25 years to Taeko Kusumi, an ear, nose, and throat surgeon. They have two children, Natsuko, 22, who just graduated from university after majoring in international studies, and Mashahiro, 17, who is just finishing high school. When they were first married, Kusumi says he promised Taeko to split the housework 50–50. Commuting to and from Wisconsin, he discovered that Taeko meant a 50–50 split overall and not just when he happened to be home. "So when I'm home, I do a lot of cooking," he says. "But it is not a hobby."

Back in Milwaukee, Subczynski looks with pride on nearly 30 years of collaborating with Kusumi. "The best of my papers are co-authored with him," Subczynski declares. Their friendship dates to Subczynski's arrival from Poland for a postdoc only a few months after Kusumi's arrival from Japan. They shared a background in biophysics and the challenge of learning day-to-day English. Subczynski says that Kusumi had an immediate effect on his scientific thinking. "His background is like mine—physics—but Aki always said that being a physicist was good but we had to be biologists too. It was Aki who was always trying to understand the biological significance of our results."

Old Guys and Uncles

Their scientific work has moved in different directions in recent years, although Subczynski adds, "Even a small discussion with Aki can be very valuable to my work." It's their long friendship that he values most these days. "I am 'Uncle' to his kids and he is 'Uncle' to my kids and now my grandkids." His friend is not much for drinking but "Aki is a very good eater," says Subczynski. "We're getting to be the old guys now so maybe we'll have more time to visit."

Certainly ASCB business will be bringing Kusumi to this country more often. At the 50th Anniversary Annual Meeting, Kusumi was glad to see a renewed appreciation of the ASCB's early history. "This Society when it was set up was a forum for collaborations between physicists, chemists, and biologists," Kusumi explains. "I don't see any other society that tries to really enhance these collaborations, and this is a concept which I have been cherishing for a long time. Also cell biology is becoming more and more interested in quantification so I think biophysics is becoming more a part of the mix. Basically, I like the style of this Society."

Kusumi's style should suit ASCB, says Mellman. "I find him one of our most engaging and creative scientists. He is committed to biophysical approaches and yet he is accessible and tries to think very biologically about the work he does. That's something that many biophysicists are unable to do." ■

—John Fleischman