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Converging on RAS

Building on bench studies, supercomputer simulations aim to illuminate RAS in action on the plasma membrane

In 35 years of research, scientists have learned much about the signaling pathways controlled by *RAS* genes, but frustratingly little about how the proteins—responsible for approximately one third of all cancers when defective—can be targeted therapeutically. As such, the NCI launched its RAS Initiative in 2013, fostering open collaborations among government, academic, and industry researchers to address the long-standing problem of RAS being "undruggable."

The reasons for RAS's intractability are broadly known: It binds GDP or GTP with picomolar affinity during its "off" or "on" states, respectively, and because these nucleotides are present at millimolar concentrations in cells, "the idea of an inhibitor that's actually competitive is difficult to imagine," says Dwight Nissley, PhD, director of cancer research technology at the Frederick National Laboratory for Cancer Research (FNLCR) in Maryland, where the RAS Initiative is headquartered. Then, too, RAS is "essentially a small, smooth ball without obvious pockets on its surface that traditional inhibitors could exploit."

Notably, most studies have been done on the purified protein in solution, thereby missing an essential part of its biology: RAS is active only upon interacting with a plasma membrane. This was first shown by the laboratory of Frank McCormick, PhD, the RAS national program advisor, Nissley notes.

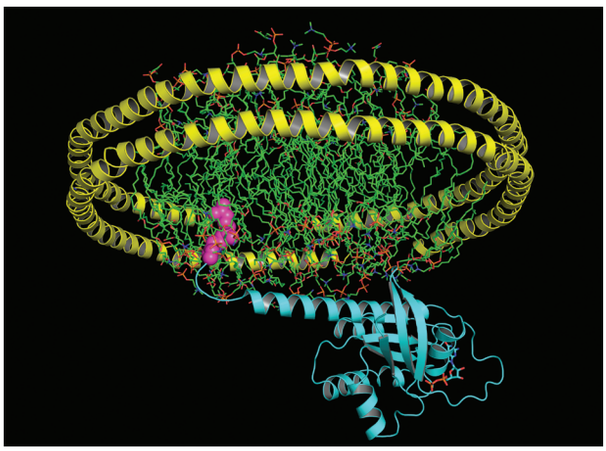
Two years ago, the Initiative's leaders were invited to participate in the Joint Design of Advanced Computing Solutions for Cancer, a collaboration between the NCI and the U.S. Department of Energy (DOE). Recognizing that current laboratory techniques for functional RAS characterization are inadequate to probe its membrane dynamics, they decided to enlist the DOE's powerful supercomputing abilities for this purpose.

"Computer simulations generate details at a really fine scale that's not possible with physical instruments," explains Frederick Streitz, PhD, chief computational scientist at the DOE's Lawrence Livermore National Laboratory (LLNL) in California. "Even so, unearthing what RAS actually does when it's floating around on a membrane will be devilishly tricky."

Nissley and Streitz are jointly overseeing this effort at convergence research—the integration of physical and life sciences to advance biomedical discoveries—which involves three DOE labs in addition to LLNL: Oak Ridge in Tennessee; Argonne in Illinois; and Los Alamos in New Mexico. Using data from FNLCR's structural, biochemical, and *in vivo* studies of RAS to inform their computer modeling, the DOE physicists and engineers aim to shed light not only on RAS's membrane interactions, but also on how contact prompts activation of RAF and other downstream kinases.

GEARING UP

Every protein consists of tens of thousands of atoms, Streitz says, which makes for "a grand challenge" when attempting to simulate RAS's membrane behavior with an atomistic level of precision. However, at least "we now have sufficient computing power to begin addressing this question," which wasn't the case a decade ago.



Dhirendra Simanshu, NCI RAS Initiative, FNLCR

A model depicting probable interaction interface between fully processed KRAS and a nanodisc—a small lipid bilayer used as a membrane surrogate in *in vitro* biophysical studies. KRAS and its tail are shown in ribbon (cyan) and sphere (magenta) representations, respectively; the nanodisc's lipids and membrane scaffolding proteins are shown in line (green) and ribbon (yellow) representations, respectively.

"We do need to design additional technology and write software before we can really get down to modeling RAS," he notes, "but this will also inform how the next generation of supercomputers are built and used." This collaboration, for instance, is part of the DOE's strategic plan to develop exascale computing—systems capable of at least a quintillion calculations per second—to solve problems across multiple scientific disciplines.

Nissley is excited to use DOE resources such as Oak Ridge's Spallation Neutron Source, a one-of-a-kind research facility that produces the most intense pulsed neutron beams in the world. It allows complex measurements to be made at greater sensitivity and higher resolution than would be possible elsewhere. "With this invaluable tool, we can start looking at just how RAS positions itself on a membrane," he says.

Both scientists acknowledge that communicating across their vastly different worlds of physics and biology can be challenging. "To me, a 'model' is built and run on a computer," Streitz says. "When biologists use the term, however, they think of animals like mice. These days, I usually say 'algorithm' instead."

Meanwhile, because he viewed biological responses that occur within 5 to 10 minutes as "really quick, and therefore easy to model," Nissley was surprised when "their [DOE collaborators'] faces turned white at the idea. In terms of a timescale that's possible to simulate computationally, I was completely off."

"If I could simulate 5 whole minutes, I could probably simulate the start of the universe," Streitz observes wryly. Instead, mere nanoseconds of time, on a nanometer scale, are about as much as current systems typically model. He and his colleagues will need to work on ratcheting up this capacity by several orders of magnitude to account for the timescale of RAS biology: milliseconds and micrometers.

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Communication challenges aside, both agree that converging on RAS from such disparate scientific viewpoints is turning out to be one of the most invigorating, satisfying aspects of this project. "We're creating a new culture, if you will, and it's been a blast so far," Nissley says.

TAKING OFF

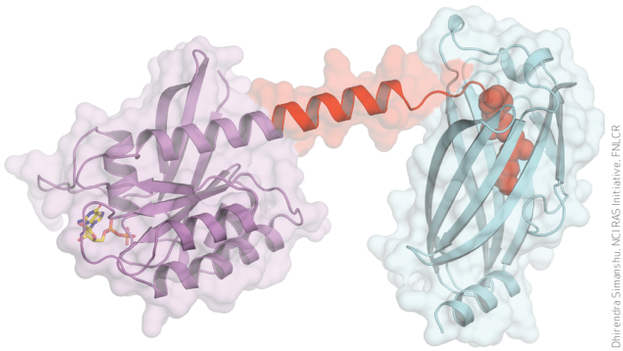
Nissley and Streitz are now forging ahead with their collaboration, after taking the better part of a year to determine its feasibility and then get the FNLCR and DOE teams in place.

"There's an iterative cycle established, where experimental results inform [computer] modeling and generate hypotheses," Nissley says. "We meet quarterly and, over 2 days, discuss this project and where to go with it."

Last November, his group successfully solved the structure of full-length, biologically processed KRAS in complex with PDEδ, a chaperone protein important for KRAS-membrane shuttling (Proc Natl Acad Sci U S A 2016;113:E6766–75). "We showed, for the first time, what KRAS's hypervariable region [HVR] looks like—or at least one form in which it could exist," Nissley says.

"If you think of RAS as a balloon, the HVR is the string that will eventually tether it to the membrane," Streitz explains. Nissley's team "figured out how this string curls itself up, so we can now do a series of simulations and ask, 'Does it stay in this conformation, or curl around in other ways?'"

Nissley points out that simulations could also reveal other unknown details of RAS's membrane interactions, possibly yielding new treatment strategies.



Dhirendra Simanshu, NCI RAS Initiative, FNLCR

Structure of full-length farnesylated and methylated KRAS4b in complex with PDEδ. KRAS4b and PDEδ are colored violet and cyan, respectively; atomic details of KRAS4b's hypervariable region (red) are shown for the first time.

"We'd love to get RAS-targeted therapies into the clinic within 5 to 10 years," he says. "I wouldn't be bold enough to predict this will happen, but it's certainly what we aspire to."

"Both groups are pushing the forefront of what's humanly possible here," Streitz observes: Laboratory researchers are striving to increase foundational knowledge of a wily oncoprotein while computer scientists do their best to accurately simulate every biological detail uncovered.

"I'm confident we'll have a far better understanding of RAS by the time this collaboration is done," he adds. "It'd be fabulous if some obvious way of introducing an inhibitor immediately emerges, but we could just as easily learn this is the wrong approach. Scientific data is agnostic like that." *–Alissa Poh* ■

SPEED-READING RAS LITERATURE

Through its Big Mechanism program, the Defense Advanced Research Projects Agency (DARPA), part of the U.S. Department of Defense, is also contributing to a greater understanding of RAS (http://www.darpa.mil/program/big-mechanism).

According to Program Manager Paul Cohen, PhD, Big Mechanism aims to build technologies that facilitate human comprehension of highly complex systems. "I was introduced to the RAS Initiative, and Frank McCormick said, 'How about you try modeling RAS-driven cancers?'" Cohen says. "Knowing absolutely nothing about this protein, I responded, 'Yes, let's do that.'" (Frank McCormick, PhD, is the RAS national program adviser.)

DARPA-developed software in an area of artificial intelligence called Natural Language Processing (NLP) plays a key role in this effort to advance RAS knowledge. "NLP gives ordinary computers the ability to process text and figure out what it means," Cohen explains. "Our machines can speedily read hundreds to thousands of journal articles—well beyond human capacity—and, in each case, pick out potentially useful nuggets related to RAS."

The hope is that these fragments of information can eventually be assembled into a mechanistic model of RAS-driven cancers, he adds. Additional algorithms are being developed so man and machine can essentially debate emerging models and possible iterations, as well as formulate new hypotheses.

For now, Big Mechanism's RAS project is chiefly driven by DARPA-funded computer scientists and biologists, although they do meet with the RAS Initiative crew at the Frederick National Laboratory for Cancer Research (FNLCR) in Maryland approximately every 6 months.

"We're not yet at the point where I'm inclined to suggest to [FNLCR] that they should do particular wet-lab experiments based on our models," Cohen says. "Having said that, our machines can already read and understand journal articles pretty well. It's not perfect by any means—mistakes are still made—but we're seeing steady improvement." *–AP*

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AACR American Association for Cancer Research

CANCER DISCOVERY

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