

NewsBytes

Simulating Membrane Transport

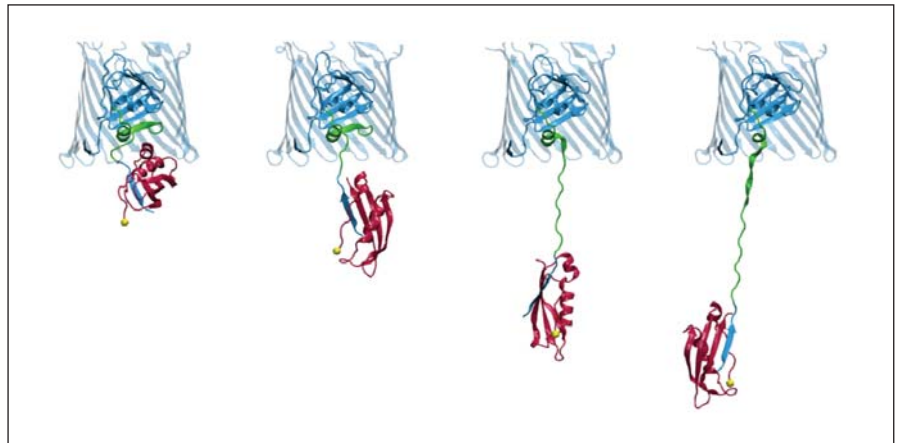
For a bacterium to admit certain large nutrients, a steady tug from inside might do the trick, according to computer simulations recently published in *Biophysical Journal*.

Bacterial membranes are loaded with a vast number of specialized transporters. For some of these to function, an energized inner membrane protein must transmit its energy to an outer membrane protein so that nutrients can enter the cell. The question is: How does that work? Does the inner protein shuttle across the membrane, unplug a pore, or simply yank open a gap?

"I don't know of any other model in which you energize a protein and then send it out to take energy to the outer membrane," says **Emad Tajkhorshid, PhD**, assistant professor of biochemistry, pharmacology and biophysics at the University of Illinois, Champaign, a co-author on the paper along with his student **James Gumbart**. "We are trying to improve the picture of this mechanism by doing this simulation," he says.

Bacteria can seem obsessed with finding and absorbing nutrients from the environment. Indeed, they dedicate more than 50 percent of their genetic material and 50 percent of their energy to membrane transport. For large nutrient molecules, such as vitamin B12, bacteria rely on TonB dependent transporters such as BtuB. These barrel-shaped molecules reside in the outer membrane with their tails (known as the plug domain) tucked in and plugging the barrel. TonB itself is anchored to the inner membrane with its tail end mating up with BtuB's plug domain, according to X-ray crystallographic results published last year in *Science*.

Based on experimental evidence, researchers know that when TonB is energized, nutrients can pass through the TonB dependent transporter. Scientists have proposed several possible mechanisms: TonB acting as a shuttle; or TonB forcibly pulling open the plugged barrel either by unfolding the luminal domain or by unplugging it entirely. Last year, with the new structural information about the



In these four snapshots, as the end (yellow bead) of Ton B (red) is pulled inside the cell, the plug domain of BtuB (green) unfolds allowing nutrients to enter. Courtesy of James C. Gumbart and Emad Tajkhorshid.

TonB/BtuB complex, Tajkhorshid and his colleagues decided to simulate the "pulling" theory in order to determine where the force would be felt first.

"It was possible that if you pull on TonB it might just come off," says Tajkhorshid. But that didn't happen. The connection between TonB and BtuB

transporter. But it's obvious to me that there is no way you can have 100 angstroms of pulling from something on the inner membrane. There must be other things going on."

Susan Buchanan, PhD, an investigator in the Laboratory of Molecular Biology at National Institute of Diabetes

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held, while the luminal domain of BtuB unfolded. After pulling for 100 angstroms, this produced an opening wide enough for vitamin B12 to pass through. The team also tested the "unplugging" theory, but found that it took ten times as much force to remove the entire luminal domain. "Unfolding is much easier to induce than coming off as one piece," says Tajkhorshid.

But, he says, there are still plenty of unknowns. They didn't try to simulate the shuttle theory because it would require too much computing power to do so. And, "in our simulation, we had to pull for 100 angstroms to observe enough opening to let the substrate permeate the

and Digestive and Kidney Disease, agrees that a linear movement of 100 angstroms is unlikely. But, she says, "The simplest thing to do in simulations is to apply a linear force as he did. *In vivo*, it could be a combination of some sort of rigid body movement, conformational changes, and rotation. But those things are hard to simulate." What's important, she says, is that this work provides a model that people can look at further. "With the recently solved crystal structure," she says, "it's important to do simulations at this point because no one's been able to do this *in vivo* yet."

—By Katharine Miller

Flexible Molecular Computer Functions Inside a Cell

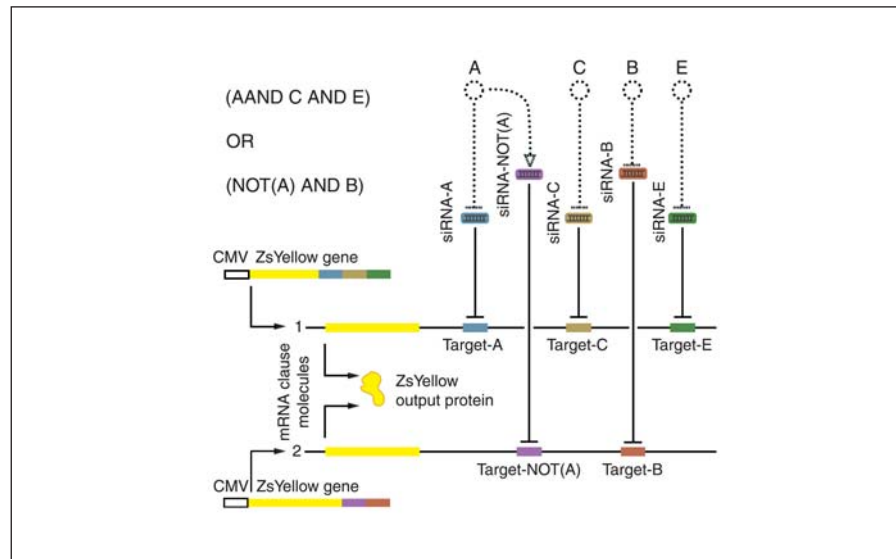
A newly created molecular computer works in human cells and offers the flexibility of a general-purpose circuit. The advance, described in *Nature Biotechnology* in May, brings closer the eventual possibility of placing bio-based computers inside cells to diagnose and treat disease on a cellular level.

“In theory, there is no limit to the decision-making complexity” that this system can handle, says bioengineer **Yaakov Benenson, PhD**, a Bauer Fellow at Harvard University’s Center for Systems Biology. Until now, molecular computers have mostly been test-tube prototypes that tackled just one specific task, such as tic-tac-toe. Benenson, along with **Ron Weiss, PhD**, assistant professor of electrical engineering at Princeton University, devised a way to engineer a general purpose circuit by taking advantage of the cell’s cell-regulation pathways.

The scientists first got their machinery to work inside a human kidney cell by mimicking a virus. They transfected the cell with genes that code for the circuit. The cell then took up the genes and created the computer network for them.

The network itself is made up of engineered mRNA strands that encode a chosen protein and smaller RNA strands that interfere with the translation of the mRNAs. Scientists can engineer these small, interfering RNAs (siRNAs) to bind any number of possible disease markers in a cell. (Weiss and Benenson did not experimentally validate sensing disease markers in this work.) In the simplest scenario, once an siRNA binds a disease marker, that siRNA can’t interfere with translation of the mRNA, and the protein is made. The protein can be whatever the designer likes: a fluorescent tag or therapy for the diseased cell, for example.

By adding interacting pairs of mRNAs and siRNAs, the researchers can individualize the network to handle any problem that can be expressed as a



Benenson's molecular networks utilize engineered messenger RNAs (mRNAs) and small interfering RNAs (siRNAs) to perform logic equations such as the one in this example diagram. If cellular marker A is present, it binds siRNA-A, keeping the siRNA from binding Target-A on mRNA-1. If siRNA-C and E are similarly occupied, mRNA-1 is freed for translation, and the output (ZsYellow output protein) is positive. The output is also positive if marker B is present, which occupies siRNA-B, and marker A is absent, because A activates siRNA-NOT(A) to bind Target-NOT(A). (This example uses human cytomegalovirus (CMV) immediate-early promoter.) Adapted and reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology, 25(7):795-801, 2007.

Boolean logic formula—equivalent to Boolean operations run on traditional silicon-based computers. The formula could be simple: “If marker A or marker B is present, then make the protein.” Or it could be much more complex: “If marker A is present and marker B is absent, or if marker A is present and marker C is present, or if marker D and E are both absent, then make the protein.”

Weiss and Benenson tested their system using a network of five siRNAs and two mRNAs. Complex functions, Benenson says, are limited by the scalability of the molecular components.

According to **Darko Stefanovic, PhD**, associate professor of computer science at the University of New Mexico, many functions “will require unacceptably complex forms.” Yet, Stefanovic comments, “the paper presents an innovative way of accomplishing logic computation using transcriptional networks.” It’s a promising direction, he says, for synthetic biology.

—By **Louisa Dalton**

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The Spontaneous Brain

When people sit peacefully at rest, doing and thinking nothing in particular, their brains still buzz merrily along. In scans called functional MRIs, they light up in characteristic patterns. No one knows the purpose of this spontaneous chatter, but it accounts for up to 98 percent of the brain's activity and burns about three-quarters of the brain's energy. To help unravel its origins and significance, researchers at Indiana University built a new computational model of a macaque monkey brain, which they describe in the June 12 issue of the *Proceedings of the National Academy of Sciences*.

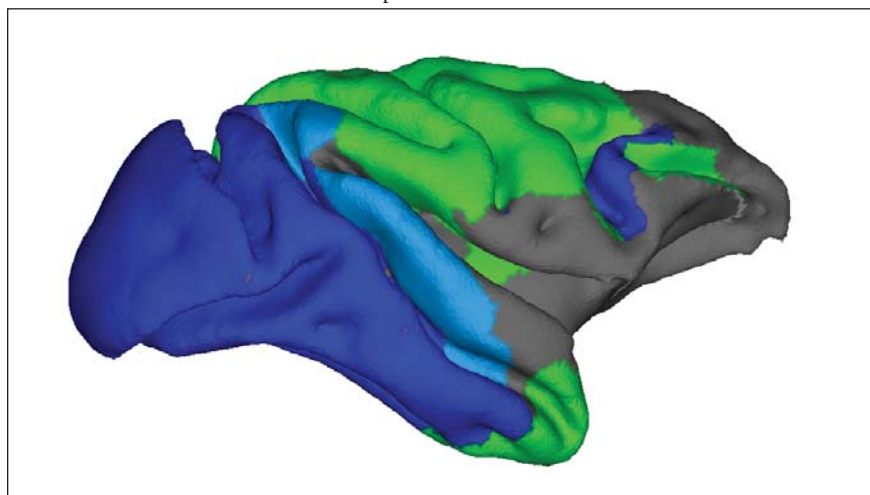
"With this work, we can shed some light on what is actually driving the pattern of activation and deactivation that is seen in the resting brain," says **Olaf Sporns, PhD**, associate professor of psychology, who worked on this project with his graduate student, **Christopher J. Honey**. The brain's activity at rest may ultimately influence how individuals think and behave and how the brain responds to injury and disease.

Sporns and Honey chose the macaque brain because its wiring diagram is well understood. Researchers have done hundreds of tracer experi-

ments—where they inject dye into one area of the brain and trace its spread to other areas—to establish the connectivity patterns of the macaque brain. From these data, Sporns and Honey built a "connection matrix" that specifies which of 47 brain areas are connected and which are not. On top of this roadmap, they superimposed differential equations that describe the electrical activity of each brain area. Then they ran a simulation to see how their virtual brain lights up when it is just talking to itself, with no external inputs.

The resulting patterns of brain activity closely resembled those seen in imaging studies of the human brain at rest. Interestingly, though the model operates at a very fast time scale (sub-millisecond resolution) it generates the slower fluctuations seen on fMRI (seconds to tens-of-seconds resolution). "Despite the fact that we have fast dynamics, we get these very slow processes to unfold," Sporns says.

When they randomly scrambled the connection matrix in their model, they no longer saw the characteristic activity patterns of the resting brain. "So we have a good argument that what we see is actually because of the specific pattern of the connectivity," he says.



Monkey Brains. This virtual macaque monkey brain lights up in the characteristic patterns of a brain "at rest." Courtesy of Olaf Sporns; Reproduced from Figure 4c of Christopher J. Honey, Rolf Kötter, Michael Breakspear, and Olaf Sporns. *Network structure of cerebral cortex shapes functional connectivity on multiple time scales. PNAS 2007 104: 10240-10245. Copyright 2007 National Academy of Sciences, U.S.A.*

"With this work, we can shed some light on what is actually driving the pattern of activation and deactivation that is seen in the resting brain," says Olaf Sporns.

"Their work makes this very important step of linking the anatomy—the connections between the brain areas—to the patterns of spontaneous activity. I think this is really the first study that makes this link explicitly," comments **Giulio Tononi, MD, PhD**, a professor of psychiatry at the University of Wisconsin. "They are able to explain many of the features that are observed in studies using fMRI."

The next step is to apply this modeling approach to the human brain, Sporns says. Though people cannot undergo invasive tracer studies, a new non-invasive technique—diffusion tensor imaging—is providing the connectivity data for human brains.

Using human models, Sporns plans to study how brain lesions interrupt the brain's network—its connectivity, spontaneous activity, and ultimately performance. "There is great potential here for understanding brain injury and recovery processes," he says.

He also plans to study how the resting brain's activity influences people's thoughts and behaviors. Every person has a unique pattern of spontaneous activity. "The open question is whether this spontaneous activity actually colors or somehow interacts with our ability to do a task," Sporns says. "If that were the case, that would be really interesting."

—By Kristin Sainani, PhD

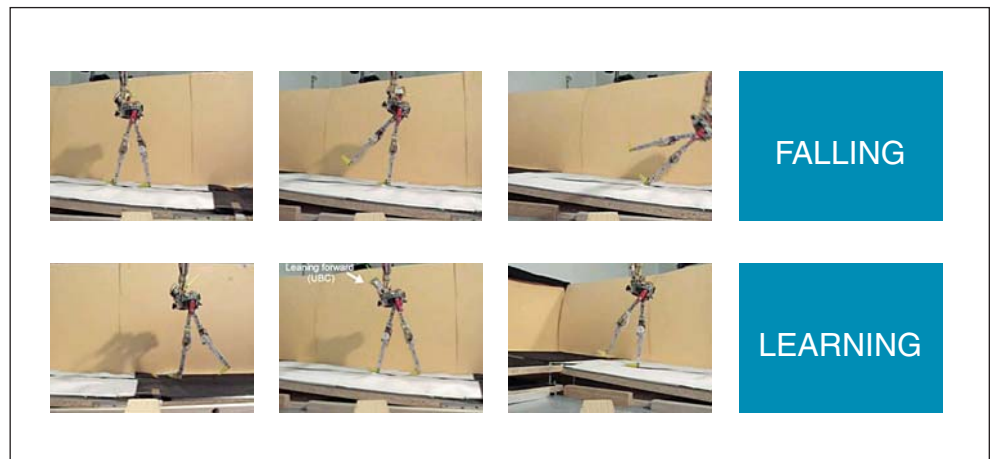
An Uphill Challenge

RunBot, already the world's fastest bipedal robot, has now also learned to keep its balance when walking up ramps. "We have achieved a synthesis of different functionalities, between biomechanics, neuronal reflexive control, and adaptive control, which allows the machine to learn," says **Florentin Wörgötter, PhD**, head of the Computational Neurosciences Group at the University of Göttingen in Germany, and leader of the team that built RunBot. The work was published in *PLoS Computational Biology*.

Creating a robot that walks as smoothly as a human is a long-standing challenge. Many walking robots are plodding and methodical, precisely calculating the trajectory of each step. The human gait is much more dynamic; our center of gravity is constantly shifting as we swing our legs forward. Last year, Wörgötter's group produced RunBot, a successful dynamic walker that could swing its legs almost as quickly as a human. However, RunBot was limited to walking on level surfaces, unable to adjust its balance to walk up an incline.

To address that shortcoming, Wörgötter's team added a learning mechanism that simulates synaptic plasticity, enabling RunBot to learn in a manner similar to humans. The learning mechanism allows RunBot to associate an infrared (IR) sensor, which detects changes in the angle of the floor, with an accelerometer, which detects the rapid acceleration of falling.

The first time RunBot's IR sensor detected a change in slope, the signal had no meaning, and RunBot continued to walk as normal until it fell, triggering the accelerometer. Over the next few trials, RunBot learned that the signal from the IR sensor requires a change in gait to avoid triggering the accelerometer. With guidance from the researchers, who predefined the direction in which RunBot could alter the parameters controlling its gait, RunBot experimented with different magnitudes of those parameters, resulting in different postures and stride lengths. After



On its first try at a slope, Runbot teeters backward and falls (top). But it learns from its mistakes: On subsequent efforts, Runbot makes it to the top of the hill (bottom). Courtesy of Florentin Wörgötter.

four or five trials, RunBot learned to lean forward and take shorter steps, similar to what humans do when walking up a slope.

RunBot is able to easily change its gait because of the hierarchical structure of its control systems. On the bottom level, each step is controlled by a reflexive neural network. Sensors in the feet, knee, and hip monitor the position of each joint relative to the other joints and the ground, and artificial motor neurons make minor adjustments to maintain stability. In this manner, the reflex control level autonomously generates a repetitive walking motion.

On top of the reflexive control lies an adaptive neural network, which controls RunBot's posture. By tweaking the activation parameters of the reflexive motor neurons, the adaptive control system causes RunBot to lean forward and take shorter steps when its IR sensor detects an upcoming slope.

In addition to creating robots with a more human looking stride, Wörgötter's work may be applicable to prosthetic legs. His lab recently started working with a major supplier of prosthetic devices, to apply similar neural networks in advanced prosthetics.

"RunBot is a successful demonstration of a small-scale 2-D biped that uses a controller that approximates a static

neural network and a novel learning algorithm," says **Steven Collins**, president of Intelligent Prosthetic Systems and a doctoral candidate at the University of Michigan.

—By **Matthew Busse, PhD**

"We have achieved a synthesis of different functionalities, between biomechanics, neuronal reflexive control, and adaptive control, which allows the machine to learn," says Florentin Wörgötter.

Modeling Early Evolution

The fittest organisms survive and produce offspring, according to the Darwinian theory of natural selection. And the changes that make an organism fit happen at the molecular level: when genes mutate they produce different proteins generating traits that may or may not benefit the organism. Yet the relationship between proteins and organism fitness is not well understood.

Now, for the first time, a computer model has attempted to connect the dots between organism evolution and the evolution of proteins.

“People understand that somehow the properties of proteins determine the evolution of populations, but this is only words,” says **Eugene L. Shakhnovich, PhD**, a professor of chemistry and chemical biology at Harvard University and lead author of the paper that appeared in *PLoS Computational Biology* in July 2007. “There’s no detailed microscopic picture of how these two biologies

ing a certain probability of occurring). The life expectancy of the organism is directly related to the stability of its proteins. The latter was determined using a “lattice” model that approximates a protein’s actual structure. It’s a useful approximation, however, because—for purposes of this model—a given amino acid sequence produces a specific measure of the stability of the native state of the protein, says Shakhnovich.

In about half of the 50 simulation runs, the organisms died off. But the successful organisms showed a characteristic pattern of protein evolution the researchers called “Big Bang” behavior. “At some point there is a discovery of a small number of advantageous protein structures and sequences that have evolvability properties,” says Shakhnovich. “These serve as a nucleus for expansion of the protein universe.”

Over time, the model reproduced other quantitative features of the existing protein universe, says Shakhnovich. “This makes us think that this model,

“[We] think that this model, while not the whole truth, captures essential aspects of early evolution,” says Eugene Shakhnovich.

talk to one another.”

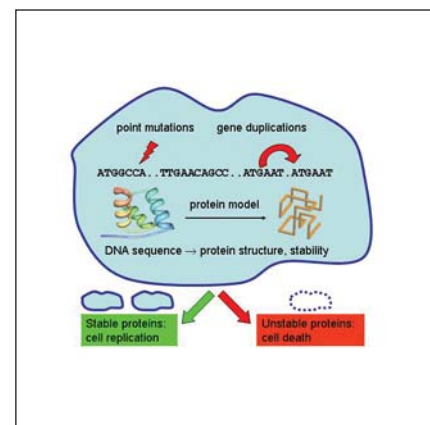
So Shakhnovich and his colleagues simulated an evolving set of proteins under selection pressure. “We developed a model, simpler than life, but still it’s microscopic with these two levels intimately connected,” he says. “So the properties of proteins in the model—like stability—are directly related to properties of model organisms that carry these proteins.”

The simulation starts with 100 organisms, each with the same single primordial gene in their genomes. At each time step, the organism can replicate, die, undergo a gene mutation or duplication, or do nothing (with each event hav-

while not the whole truth, captures essential aspects of early evolution.”

For example, in nature, one finds large and small protein families co-existing. This is inconsistent with a random process. “It has been a mystery as to why this type of distribution pops up in protein science and genetics,” says Shakhnovich. “Our model suggests the source of it is in the evolutionary dynamics of proteins.”

Next steps include adding more complexity: e.g., protein-protein interactions and immune responses. The researchers also hope to gain a better understanding of protein stability, possibly even using that information to develop more stable



This schematic depicts a first-principles simulation of early evolution. One hundred organisms, each with the same single gene, begin to evolve. At each time step, the organism can replicate, die, undergo a gene mutation or duplication, or do nothing. The organism’s life expectancy depends on the stability of its proteins as determined by a protein lattice model. Courtesy of Eugene Shakhnovich.

proteins, useful in drug discovery.

“This is the first paper where people have used a simple but realistic model of protein folding to simulate genomes containing multiple genes,” says **Claus Wilke, PhD**, assistant professor of integrative biology at the University of Texas Center for Computational Biology and Bioinformatics. “I think that’s an interesting approach, and I think that over time those kinds of simulations will lead to all kinds of interesting insights.”

—By Katharine Miller

Teaching Resource: Computing Life

Explaining biocomputation to non-scientists can leave a person tongue-tied. Technical jargon gets in the way, and the breadth of the field resists encapsulation.

To help out, and to reach out to a new generation of future scientists, the National Institute of General Medical Sciences (NIGMS) has now published *Computing Life*. Due out in September 2007, the 24-page booklet presents snapshots of scientists' labs and brief overviews of what's happening across the field. The intended audience: high school and early college students.

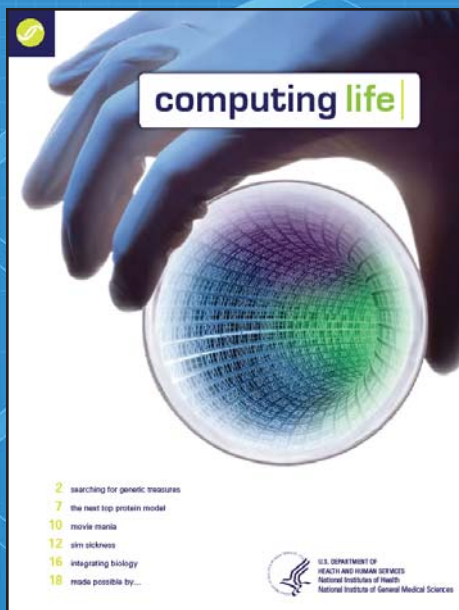
"With tremendous challenges ahead, we need to encourage young people to think about science with excitement and creativity," says **Karin Remington, PhD**, Director of the Center for Bioinformatics and Computational Biology at NIGMS. "Publications such as *Computing Life* help to light a spark, channeling what might've been untapped intellectual power toward the sciences, and building appreciation for the relevance and importance of scientific research in our lives."

To that end, *Computing Life* reads like a magazine. It's very visual, with plenty of colorful and intriguing graphics and

tight, explanatory captions. "The booklet brings pop culture to the science," says editor **Emily Carlson** of the NIGMS Office of Communications and Public Liaison.

Topics covered include genomics, protein folding, infectious disease modeling, molecular dynamics simulation, and systems biology, among others. The booklet also provides links to online material including simulations and movies. "We plan to maintain a complementary web site," says Carlson. "We'll post new material there as our way of keeping the publication up to date."

—By **Katharine Miller** □



"With tremendous challenges ahead, we need to encourage young people to think about science with excitement and creativity," says Karin Remington.

Contents Include:

Searching for Genetic Treasures
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