

## Sticky DNA Problem-solving chemists begin to build three-dimensional tissue.

DEVELOPING ORGANISMS KNOW JUST WHERE TO POSITION various cells so they can communicate and cooperate with their neighbors to establish three-dimensional structures. For biologists, emulating the body's architectural dexterity, even for simple tissues, has been a frustrating pursuit.

"The spatially encoded information in tissues is very difficult to replicate outside the body, but also really important for function," says HHMI investigator Carolyn Bertozzi at the University of California, Berkeley.

"In the lab, we typically culture cells on flat substrates like tissue culture plastic. But we don't live in a flat world," adds Zev Gartner, who began to study cell-to-cell interactions as a postdoctoral fellow in Bertozzi's lab. "Cells in our bodies are surrounded on all sides by their neighbors and by an extracellular matrix, and the behavior of cells in 3-D is very different from when they are grown on these rigid, inert surfaces."

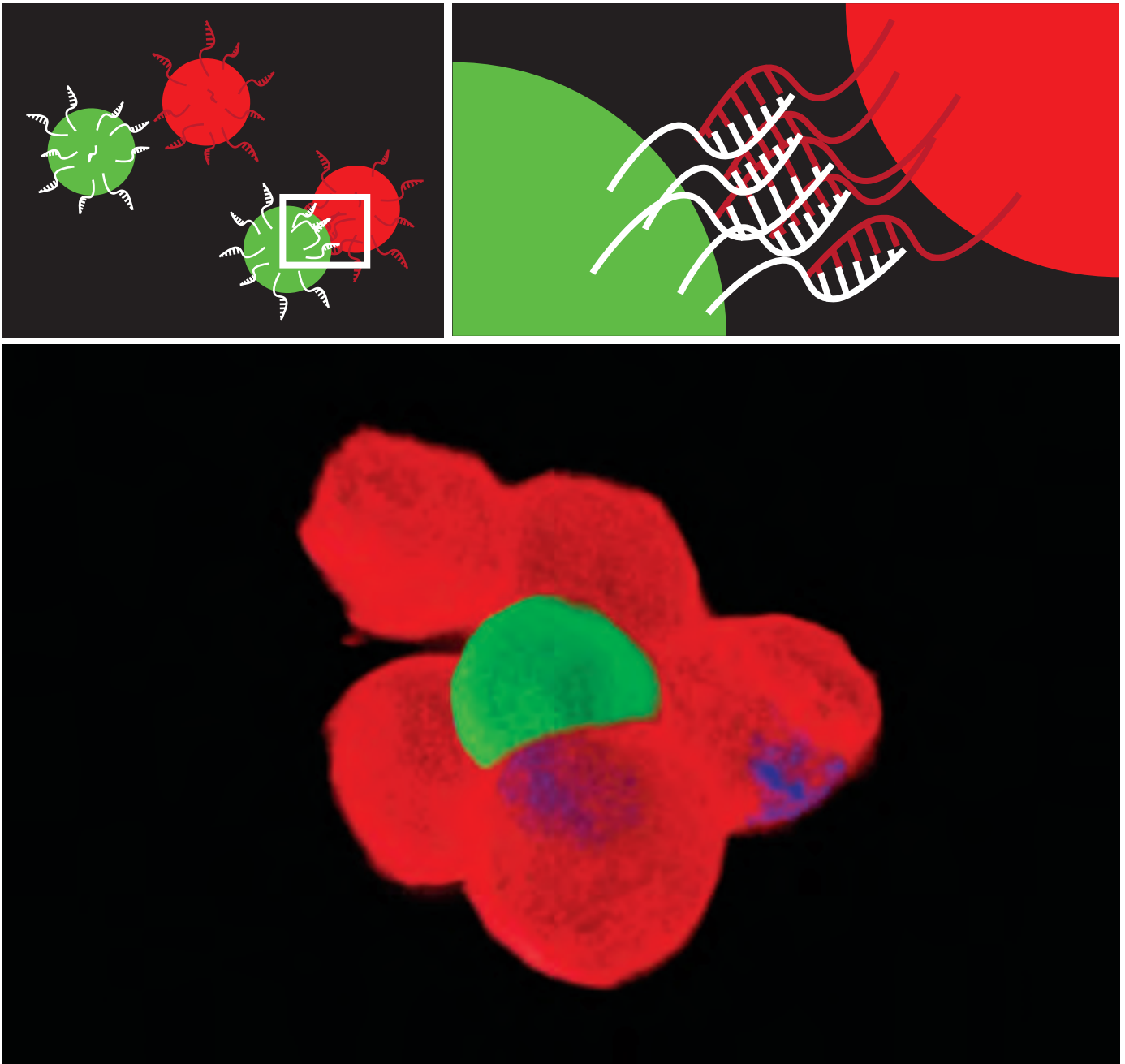
If they could control tissue assembly, researchers could design biomaterials to replace or repair injured tissue and would have more true-to-life models of tissues, both healthy and diseased, for study in the lab. To Bertozzi and Gartner, the challenge of organizing cells into more complicated structures looked like the kind of problem they solve best: a chemistry problem. Chemists synthesize complex molecules by performing a series of reactions with simpler building blocks. The team's building blocks were short single-stranded sequences of DNA linked to the outer surface of cells to make the cells selectively "sticky."

The approach relies on DNA's discriminating nature: because each building block or nucleotide that makes up a DNA sequence has a preferred partner, a strand of DNA will bind only to another strand whose sequence is complementary to its own. For years,

Bertozzi's lab and colleagues in the chemistry department have been exploiting this property of DNA to create simpler, two-dimensional patterns of cells. By linking a strand of DNA to a cell, and a complementary DNA strand to a spot on a flat surface, the researchers can direct the cell to bind there—a useful strategy for tasks such as designing cell-based biosensors or drug-screening technologies. When Gartner joined the lab in 2006, he realized the approach could be taken, literally, to another dimension.

To jump from two to three dimensions, Gartner linked complementary strands of DNA to the outer membranes of different types of cells. Then he allowed those cells to interact so they could bind to one another. By varying the length of the DNA strand, the complexity of its sequence, and its abundance on a cell's surface, he could control how quickly the cells came together and how stable the resulting complexes would be. He incorporated fluorescent markers so that, after the cells had mingled, the sought-after multicellular assemblies could be sorted from cells that remained solo.

Gartner used the approach to create a tiny sphere in which two onion-like layers of cells encase one another. The innermost cells secrete a protein growth factor that the outer layer of cells needs to survive. This intercellular signaling depends on the close proximity of the cells to one another due to their DNA "glue." The team calls the structure a "microtissue"—simpler than most of the tissues the body builds but a useful model for studying specific cell-to-cell interactions in the lab. The contact-dependent exchange of survival factors in their system mimics signaling that spurs the growth of tumors and immune cells, according to their paper, published March 24, 2009, in the *Proceedings of the National Academy of Sciences*.



**TOP ROW:** Researchers coax cells of different types to find and form contacts with one another, using complementary strands of DNA as their “glue.” **BOTTOM ROW:** That “glue” on the surfaces of red- and green-labeled cells promotes their assembly into a defined structure. The shape and stability of this “microtissue” can be manipulated by altering the features of the DNA glue or the way in which cells are combined.

Courtesy of PNAS and Zev Gartner

This first microtissue demonstrates that Bertozzi and Gartner’s technique works. The method should be readily accessible to researchers in other labs who want to design their own microtissues, and because the DNA “glue” on the surface of each cellular building block “really has nothing to do with the individual cell type,” the possibilities for directing different assemblies are virtually limitless, Gartner says.

Gartner, now a faculty member at the University of California, San Francisco, is experimenting with building larger microtissues that include more cell types, and encoding more structural information into the cells so researchers can direct their orientation and construct asymmetric assemblies, such as the layers of cells in the skin. “We’re trying to see how much further we can push the basic idea,” he says. ■ —JENNIFER MICHALOWSKI