



BRINGING THE INVISIBLE TO LIGHT:

BIGELOW SCIENTISTS FIND UNEXPECTED VIRAL MEMBERS OF OCEAN COMMUNITY

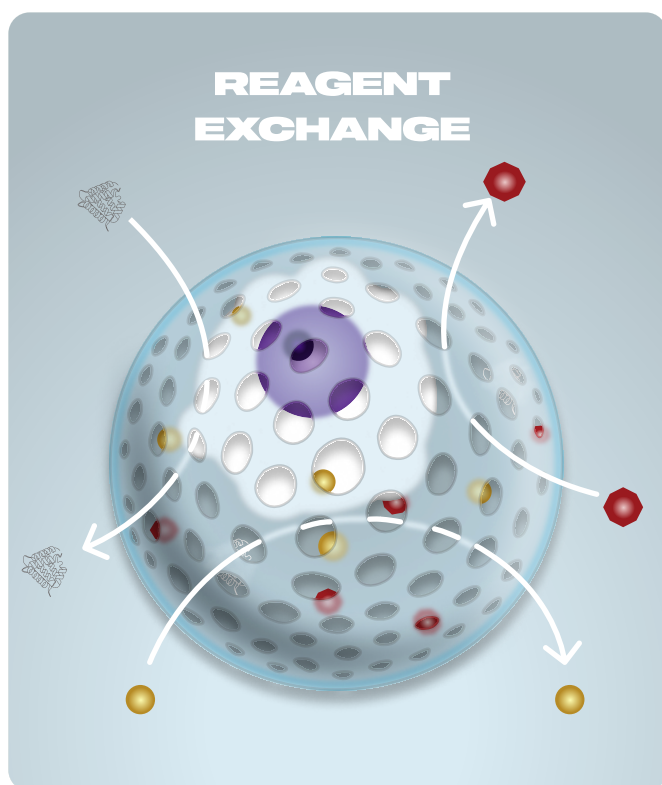
With a novel high-throughput genomic analysis workflow powered by Atrandi's Semi-Permeable Capsule (SPC) technology, scientists glean new insights from single cells in environmental samples.

At the Bigelow Laboratory for Ocean Sciences, scientists have a special mission: to study the health of our precious oceans and use those discoveries to build a better future for all life on Earth. They carry out this work through several pioneering labs at the institute's coastal campus in East Boothbay, Maine, in the USA.

A central hub for innovation at the institute is the Single Cell Genomic Center (SCGC). Launched in 2009 by **Ramunas Stepanauskas**, a senior research scientist, the center was the first in the world to focus entirely on single-cell genomics for characterizing microbes and microbial communities. Today, the SCGC has three key functions: 1) method development to spur technical innovation in this field;

2) experimental research focused on better understanding ocean environments and other ecosystems; and 3) providing services to process samples submitted from scientists around the world.

Recently, SCGC scientists published results in *Nature Microbiology* from an exciting study that deployed a new single-cell sequencing method and revealed novel insights about viral populations in seawater. The work was enabled by a collaboration with Atrandi Biosciences and the company's unique **Semi-Permeable Capsule (SPC) technology**.



CAPTURING CELLS

During the past decade, scientists pursuing a broad range of research avenues have shifted from bulk microbiome analyses to a single-cell approach. Typically, cells are separated into individual wells on a microplate for characterization and sequencing. This separation is often done via fluorescence-activated cell sorting, but this requires expensive instrumentation.

More recently, methods have emerged to use high-throughput droplet microfluidics; individual cells are enclosed in droplets generated in an oil-water emulsion. Droplet-based systems offer much higher throughput than microplates, but they are still limited. Reagents can be added but not removed, reducing efficiency and requiring compartmentalization to run reactions with incompatible processes.

At Atrandi, scientists have developed a more flexible approach for capturing and working with individual cells. **SPCs are aqueous compartments surrounded by semi-permeable shells.** Like droplets, they can hold individual cells; unlike droplets, reagents can be freely added or removed for more sophisticated experimental design

The permeable nature of the capsule shell means that larger elements, such as cells or large DNA fragments, are retained, while allowing small molecules, such as enzymes, buffers, and nutrients, to pass through.

Scientists can use SPCs to perform simple or multi-step reactions sequentially, each under optimal conditions. Reactions are initiated by simply replacing different buffers with a pipette. SPCs were designed to support high-throughput workflows. They can be used to analyze thousands to millions of cells simultaneously, enabling high-efficiency processing while maintaining single-cell resolution.

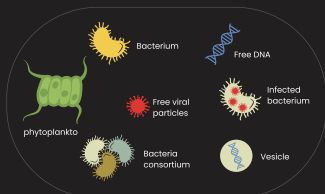
SINGLE CELLS IN SEAWATER

The first is that environmental samples are much more complex than controlled lab samples, and they require significant method optimization to be processed and analyzed reliably. The second challenge stems from the limitations of conventional analysis methods in providing a comprehensive view of what's in ocean water.

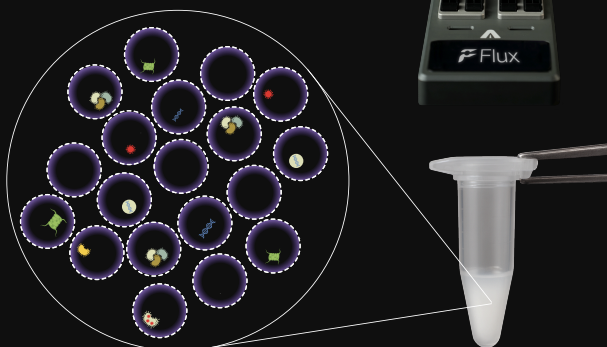
"We have an estimate of the abundance of different extracellular entities in the ocean based on microscopy and DNA staining, but it's hard to know what we're missing by using those methods," says SCGC postdoctoral researcher **Alaina Weinheimer**, an author on the Nature Microbiology paper. *"There are limitations in optical sensitivity for tiny things, and our methods for filtering and purifying samples are indirect since we often use size to estimate abundance of entities."*

So the Bigelow team has been left to wonder what they—and other marine researchers—might have missed over the years. That's why they turned to Atrandi's SPC technology, hoping it would offer a different and perhaps more effective means of elucidating microscopic communities in the seawater. In a collaborative effort between Atrandi's research team and SCGC, scientists developed an SPC-powered

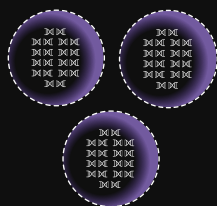
DNA-containing particles in seawater



Sample compartmentalization inside SPCs



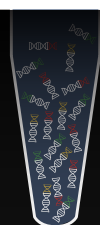
Lysis and gDNA amplification inside SPCs



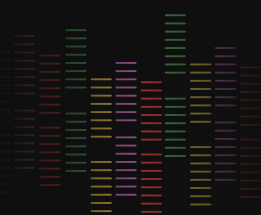
Combinatorial barcoding inside SPCs



Production and sequencing of barcoded libraries



De novo assembly and annotation of demultiplexed reads



workflow for environmental samples that captures and barcodes individual cells and other DNA-containing particles for downstream analysis. Each SPC operates as a microscopic compartment.

Bigelow's recent paper reports the results of those efforts. From a single 300 nL seawater sample, the environmental SPC-enabled workflow yielded genomic sequences for more than 2,000 particles. The team also ran a conventional metagenomic approach in order to compare results with those from the SPC protocol. The vast majority of sequences identified via the SPC-enabled workflow came from viruses, with some cellular DNA as well. Viral genome assemblies were mostly complete, and the overall community composition generally matched scientists' expectations based on previous analytical techniques. SPC-generated data and metagenomic data were largely concordant.

But the project also revealed important marine components that were completely unexpected. Nearly 2% of the viral genomes captured by the SPC-enabled workflow appear to belong to the family **Naomiviridae**, yielding an estimate of approximately 300,000 *Naomiviridae* per liter of seawater. Only two isolates from this family existed before the Bigelow study, and *Naomiviridae* was considered widespread but low in abundance. Members of the genus are distinguished by the non-canonical deoxyuridine (dU) nucleotide in their genomes. Standard metagenomic workflows, which rely on Illumina sequencing without preamplification, cannot detect these viruses because of their dU content.

"We did not expect Naomiviridae to be abundant," Weinheimer says. "It was only discovered a few years ago. Metagenomics was missing these viruses entirely, so no one knew how abundant this group was until we could use a method like this."

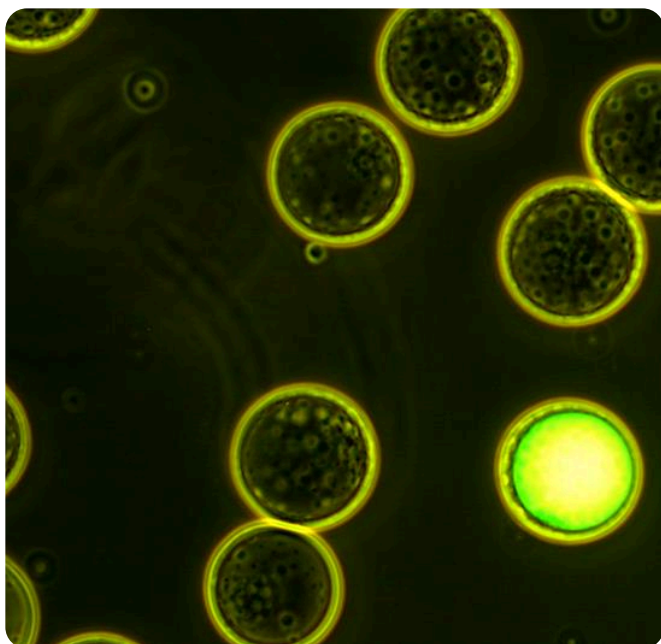


Photo: A micrograph shows several individual microcapsules including one, brightly lit one in the lower right corner, that contains amplified DNA from a single particle (Credit: Brian Thompson).

WHAT'S NEXT

The Bigelow team has already integrated the SPC workflow into its routine research. Now part of SCGC's service offering, scientists can send environmental samples to the lab for analysis using SPCs. The lab has already validated the process using different sample types, including sea floor sediment.

Ultimately, the value of implementing SPCs for microbial ecosystem research lies in the ability to study individual members of those communities holistically. Viruses, bacteria, and other biological entities in a sample are sequenced together quantitatively, enabling more accurate assessments of their functions and contributions to the community.

"There's a lot you can learn about viruses by studying them as single particles regarding their diversity and interactions with the microbial realm," Weinheimer says.

As the study authors report, the SPC method "provides opportunities for high-throughput, quantitative, and cost-effective genome analyses of individual cells and extracellular particles across complex microbiomes."

Semi-Permeable Capsules (SPCs) are microcapsules that have a liquid core surrounded by a thin, crosslinked hydrogel shell with size-selective permeability. Small molecules (e.g., salts, buffers, enzymes, primers, antibodies) diffuse freely; cells, genomic DNA and large complexes stay inside. Practically, that means you can wash, exchange, and stage any biochemical reaction all while preserving single-cell resolution. Thousands to tens of thousands of SPCs can be processed in the same tube at the same time.

SPCs are made by employing the principle of aqueous two-phase system (ATPS). Capsules are first formed as water-in-oil droplets prepared by co-flowing two immiscible aqueous polymers on a droplet microfluidic device (or chip). The polymers are chosen so, instead of mixing, they separate, with one polymer forming an outer shell, and the other – staying in the center (core). Visible-light crosslinking locks the shell, and droplets are broken to release capsules, yielding monodisperse SPCs whose diameters can range from 30 to 300 μm depending on flow and chip geometry.

The shell is the key element – a "sieve" that enables full reagent exchange without losing the cell or its genomic DNA. That lets you complete incompatible steps under their own optimal conditions: lysis, purification, amplification, labeling; or swap media for culture, fix and process. SPCs effectively combine the modularity of wells with the throughput of droplets and let you run bulk-like reactions at single-cell resolution. Published reports show SPCs are tolerant to temperature cycling, wide pH range, solvents, and enzymatic treatments, enabling both culture and molecular workflows within the same capsule.

Read more [here](#).