The non-point of **discovery**

Tracing sources of water pollution is a difficult task for hydrological science, but **Dr Todd Walter**'s project working with nanobiotechnology points towards smarter, more effective watershed management

To begin, could you provide a brief background to your project?

One of the fundamental questions in hydrological science is: 'where does the rain water go after the storm is over?'. Someone standing in a stream, however, might ask the inverse question, 'where did this water come from?' One reason why the answer to this question is important is that water moving through the landscape picks-up pollutants and carries them to streams. This kind of pollution is called 'non-point source' pollution because we are not able to reliably locate the pollutant sources unless the pollutant is unique, ie. there is only one place in the landscape where it exists. This is almost never the case.

What inspired this unlikely partnership between nanobiotechnology and hydrological engineering?

The basic idea of our project is to place unique, and safe, 'pollutants' in locations where we suspect pollutant sources and then look for our pollutants in the stream. We call our pollutants 'tracers' because, if we find them in the stream, we can trace their origin back to where we initially placed them in the landscape. The idea of using nanobiotechnology evolved from a casual discussion with Dr Dan Luo, who at that time had spent several years developing ways to deliver drugs, DNA, and other materials to specific cells for medical applications. We quickly recognised that both of our areas of research shared the concept of 'targeting'; Dan was interested in targeting specific cells and tissues in humans to treat diseases and I was interested in targeting specific locations in a watershed to treat water quality problems.

How have you managed collaboration across your different disciplinary backgrounds?

One challenge was to find young researchers with the capabilities and interest in both nanobiotechnology and watershed management. We were extremely fortunate to find just such a person in Asha Sharma, who has a degree in Industrial Biotechnology from Anna University, Chennai, India, but with an interest in environmental engineering. Although Dan, Jay, and I understood one another enough to conceive this project, it has been invaluable to have a graduate researcher who is truly comfortable in each of our labs and fluent in our individual fields.

What characteristics are you looking for to determine flow path? What is the degree of accuracy?

Right now all we are looking at is whether a flow path is confirmation that a tracer, placed at known location in the landscape, is found at a downstream sampling point. From this we know the two points are connected by flowing water. The degree of accuracy of our tracer-system, based on our experiments to date, is much higher than that of most tracers used in environmental research. For addressing non-point source pollution, our accuracy has to be at least as precise as the scale at which land managers make decisions. In some cases it may be sufficient to link a specific agricultural field to a potential pollutant flow path. In others, we may need to link a specific toilet to a pollutant flow path.

What are your major achievements to date?

We are still in the nascent stages of this project but so far our results are very promising. The system we initially proposed has worked as we envisioned. One of our major accomplishments was to reduce the cost of fabricating our tracers by several orders of magnitude. Because when we were working with Dr Luo, we initially used medical-grade materials, but Asha quickly found more cost-effective alternatives. The polymer we were using, for instance, is essentially the same material used in biodegradable plastic cups, and costs a fraction of what the medical grade version costs.

What are your overall ambitions for this project? Is more research needed?

My ambitions for this project keep growing. I think this idea of applying nanoscale technologies to landscape systems is still very new and there is huge untapped potential. It would be great if we could start using our tracers to identify specific pollutant sources sometime in the near future. We should know by the end of next year how well our tracer idea can be scaled-up to whole watersheds, or at least how large we can scale-up. But regardless, we are continually coming up with new ideas with every experiment.

Cracking the codes of pollution

Science is seeing evermore innovative collaborations across disciplines. A new project at Cornell University, concerned with water pollution, is harnessing such an approach through applications of DNA barcode tracers

POLLUTION IS A major water quality issue not only in the US but worldwide, and can be caused by a multitude of factors. Identifying these factors and the source points of pollution has long been a challenge for hydrologists as well as land and water quality managers. In the past, 'non-point source' pollution (that is to say, pollution that could come from more than one unique source) has been identified by its composition, without consideration for its transportation through landscape. More recently, researchers have come to see the significance of how pollutants travel, and the movements of waterflow, as a way of pinpointing pollution sources. Dr Todd Walter's new project brings together his own background in hydrology with expertise in nanobiotechnology, to help develop new ways of identifying pollutant flow through the use of DNA nanobarcode tracers.

NON-POINT SOURCE POLLUTION

The causes of water pollution are diverse, and come from sources as varied as agricultural, military, industrial and domestic sites. Approaches to non-point source pollution have traditionally involved identification of composition, as Walter illustrates: "If phosphorus is identified as a pollution problem, we try to reduce all potential source of phosphorus including soaps, livestock manures, chemical fertilisers, etc". More recently some pollutants have been more precisely identified by their composition, most notably, nitrates and pathogens: "The molecular weight of nitrate often differs depending on its origin; for example, nitrates from human waste are slightly different than from cattle and from chemical fertilisers," Walter explains. As well as by molecular composition, pathogens can be 'fingerprinted' by DNA, which can help to indicate the type of source, if not information about its location.

DNA BARCODING

Walter's research is aimed at overcoming this nonpoint problem, by adding DNA barcoded pollutants to water sources. Employing the same technology as forensic scientists use to 'read' DNA at crime scenes, Walter's project utilises DNA to distinguish unique tracers from each other: "Genetic code is written with four letters, A, T, C, and G, which represent four different compounds that are the rungs of the DNA's molecular structure. We use the letters to label our tracers and use a process called Polymerase Chain Reaction (PCR) to read this label," he explains.

FROM INNOVATION TO APPLICATION

Whilst the concept of nanobarcoding has been used in microbiology for some time, Walter's application of it in hydrology is most innovative. Once his team has generated unique tracer labels in the lab, they wrap them in a polymer commonly used for sutures and drug delivery, which biodegrades at a rate that can be controlled during fabrication. This is important to minimise any long term impact they will have in the environment, as Walter outlines: "Our tracers will eventually disappear and not constitute another source of pollution. Also, the DNA we use is completely synthetic and, as such, should have no genetic ramifications". In Walter's proposed application, batches of differently-labelled DNA barcoded tracers are produced and then placed at different locations in a watershed. Over time, stream water samples are collected and analysed using PCR to identify any of the tracers. If a tracer is found, and identified by its unique DNA barcode, it can be matched to the location, especially useful if that location is one suspected of being a pollution source.

Students from Walter's lab starting a tracer experiment in simulated runoff. From left, Meredith Kueny, Angela Rigden, Emily Stockwell and Asha Sharma.

ELIMINATING 'NONPOINT:' USING NANOTECHNOLOGY TO IDENTIFY POLLUTION SOURCES IN THE LANDSCAPE

OBJECTIVES

To identify the source of pollutants once they reach receiving water, as it is one of the major problems of nonpoint-source pollution management, and to develop a ìtracer-system for identifying and characterizing different flowpaths at field and watershed scales by labeling or coding ìtracers with unique DNA-based nanobarcodes so that many flowpaths can be simultaneously identified. The goal is to have the capacity of introducing ìtracers at different points in a watershed and collect them elsewhere in the watershed; using a portable nanobarcode reader, the hydrological linkages and transport times between the collection point(s) and the points of ìtracer-introduction can then be determined.

KEY COLLABORATORS

Dr Dan Luo, Department of Biological and Environmental Engineering, head of the Molecular BioEngineering Lab (MolBEL), Cornell University • **Dr Asha Sharma**, Candidate responsible for carrying-out most of the proposed, published, and presented research • **Dr John Regan**, Civil and Environmental Engineering, Pennsylvania State University, State College, PA

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TODD WALTER is an Associate Professor of Ecohydrology at Cornell University. His interests are in the intersections of biological and hydrological systems and, as such, involve collaborations with ecosystem scientists, microbiologists, ecologists, and molecular-biotechnology engineers.

SCALES OF MOVEMENT

As well as the DNA barcoding, another important factor in the fabrication of the tracers is their ability to move through water. It was important that the differently-labelled tracers have identical properties so that they move just as well in flowing water. An important collaborator of Walter's, Dr Dan Luo, is a specialist in molecular biotechnology and thus used to working on quite different scales to Walter. So they began their experiments small, initially adding tracers to tubes of sand only 30 cm long. When the DNA-labelled tracers moved through the material as anticipated, they scaled the experiment up to several meters in length, across a car park next to the laboratory. Using a hosepipe to rain a steady flow of water across the asphalt, they found that in this context too, the two tracers used not only flowed safely across the study plot but transported identically. The next upscale of the project was to a 100 m stream reach, again using two different tracers, this time released at five minute intervals. "The stream allowed us to consider a larger size experiment and provided more possibilities for losing the tracers, eg. in weeds, in the sediments, in eddies," Walter reflects. The experiment not only showed that the tracers would travel through such a stream, but highlighted the sensitivity of PCR detection, as Walter explains: "If anything, we found that our detection with PCR is very sensitive and we had to dilute our samples substantially to get the DNA concentrations low enough for our equipment. Theoretically, PCR can detect a single strand of DNA."

> Employing the same technology as forensic scientists use to 'read' DNA at crime scenes, Walter's project utilises DNA to distinguish unique tracers from each other

THE REAL TEST

The next step for Walter and his colleagues is to run a real watershed experiment that mimics the proposed application of the tracers. Whilst Walter is confident about the experiment, he is aware that there are factors of uncertainty too: "What if the tracers get caught in the soil when it dries out? What if the tracers biodegrade faster than we thought they would? We lose a lot of sleep thinking about all the things that could go wrong; but that is the nature of research," he reflects. If the experiment is successful, the project will then need to find a way to fabricate the tracers in larger quantities and with less effort, but considering the potential value of the technology, it seems likely that commitment and resources to do this will be found.

Students Lauren McPhillips and Vania Pereira collecting stream water samples during a tracer experiment.

AFFECTING WATER MANAGEMENT

Eradicating non-point source pollution would be a significant achievement, helping to reduce water pollution through the development of improved policies for watershed management. Being able to identify pollution sources more effectively would mean better management and protection of specific locations, as Walter illustrates: "This may mean, in the case of agricultural landscapes, taking land out of production to protect water quality. In my experience, land owners and managers want to avoid polluting water resources, especially those near their homes". Combining improved identification technologies like those developed by Walter with improved management policies could mean that rather than using it to simply attribute blame on certain polluters, it can allow a community to focus resources efficiently to protect their water: "We have had some watershed protection groups ask us to use our tracers to, for example, identify leaky septic systems by flushing tracers down everybody's toilets and seeing which tracers are ending up in the stream", suggests Walter.

MORE EFFECTIVE WATERSHED MANAGEMENT

If successful, it seems certain that Walter's project could have important impact in watershed management strategies and policies. Meanwhile, work in the laboratory continues, with Luo developing a new nanobarcode employing a branched DNA structure with different coloured fluorescent markers, which can be read using a laser technology called cytometry. "Cytometry can read markers much more quickly and easily than the currently used PCR-approach," enthuses Walter. The implications of this for Walter's project and its application remain to be seen, but certainly bode well for its success, and we watch with anticipation the impact it will have for the future of watershed management.