

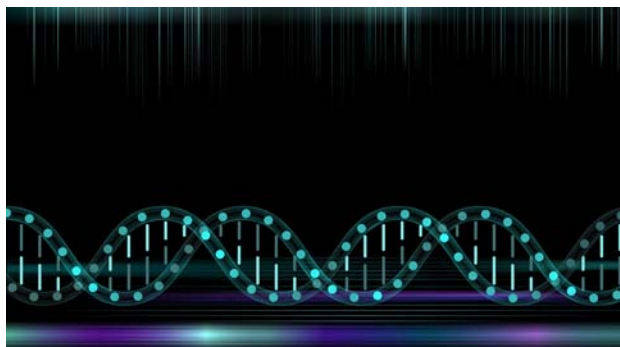
# Highlights in Chemical Biology

## Optically mapping DNA with nanometre resolution Visualising DNA sequences

A new, fast way to analyse stretches of DNA has been developed by European scientists. The technique could be used to sequence the genome of viruses and in the future help tackle genetic disorders such as schizophrenia and congenital heart defects.

Current DNA sequencing methods are able to sequence short regions of the genome (30 to 1500 bases in length). Regions that are either duplicated or deleted relative to a reference genome are an important cause of structural variation in the human genome with links to a variety of genetic disorders. Using current sequencing methods, studying these repeats is time consuming and labour intensive.

Now, Robert Neely and colleagues at the Catholic University of Leuven in Belgium and the Institute of Biotechnology in Vilnius in Lithuania have used a DNA methyltransferase enzyme to label the 5'-GCGC-3' DNA sequences with a fluorescent marker. Immobilising and stretching



the DNA on a surface then produces a unique and reproducible pattern when combined with the fluorescent markers. The result is a 'fluorocode' - a simple description of the DNA sequence, which can be read and analysed like a barcode.

DNA barcodes using fluorescent tagging can be read quickly as labelled samples pass a detector, but Neely's fluorocode gives significantly enhanced resolution and uses a much smaller number of DNA molecules. 'The method from unlabelled DNA

**Sequences of DNA are tagged with a fluorescent marker**

**Reference**  
R Neely *et al*, *Chem. Sci.*, 2010,  
DOI: 10.1039/c0sc00277a

to fluorocode can be achieved in less than 8 hours for a DNA molecule that is around 50 000 bases in length,' says Neely. Current single molecule mapping methods have a timeframe of around one week for analysing individual genomes.

Kalim Mir, an expert in DNA sequencing and genomics at the Wellcome Trust Centre for Human Genetics, University of Oxford, comments, 'the advantage the system has over conventional optical mapping is that it can provide ultra-high density mapping of genomic DNA and could easily be extended to much longer fragments from larger genomes, from bacteria to humans. The most significant challenge the authors face is to scale the technique up to the human genome.'

The group now plan to scale the fluorocode up from viral genomes to bacterial and on to eukaryotic genomes with the immediate aim of producing multi-coloured fluorocodes with even more detail.  
*Carl Saxton*

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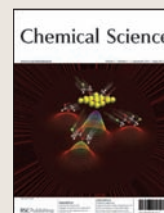
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# Research highlights

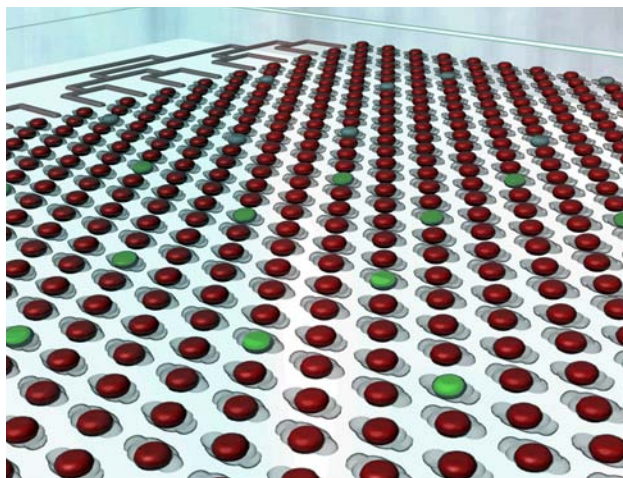
Deformation of blood cells investigated using a microfluidic device

## SlipChip performs PCR

Scientists in the US are using a simple microfluidic device to perform the polymerase chain reaction (PCR).

In PCR thousands of copies of a particular DNA sequence are generated via cycles of repeated heating and cooling – a process known as amplification. The DNA copies can then be used for cancer research, prenatal diagnostics and DNA profiling in forensic science. Previous attempts to miniaturise PCR have always required mechanical systems to open and close the valves controlling the reaction, but now Rustem Ismagliov and his team at the University of Chicago have used a simple device, called a SlipChip, to amplify *Staphylococcus aureus* DNA.

The SlipChip is a device that relies on the movement, or ‘slipping’ of two plates imprinted with wells and ducts. As the plates slip past each other the wells and ducts are brought in and out of contact to combine reagents and perform reactions.



Ismagliov designed the device in 2009 demonstrating its use in protein crystallisation. Now they are using it to amplify and copy small amounts of DNA by performing the PCR without needing mechanical valves or pump action.

**The SlipChip contains many wells to perform digital PCR**

Feng Sheng, a member of the team, says that their method ‘avoids both a complex fabrication process and a complex manipulation system’ and meets the need for ‘a simple and inexpensive platform to apply digital PCR in laboratories and resource-limited settings’.

Claus Poulsen, an expert in PCR from Dublin City University, Ireland, says ‘the SlipChip is a nice example of achieving the goal of microfluidics as the system makes an elaborate method (digital PCR) easy to perform due to automatic handling of minute samples by the device.’

Ismagliov and his colleagues are now working on using SlipChip-based digital PCR for the analysis of rare cells and the detection of mutations.

*Hilary Burch*

**Reference**

F Shen *et al*, *Lab Chip*, 2010, DOI:10.1039/c004521g

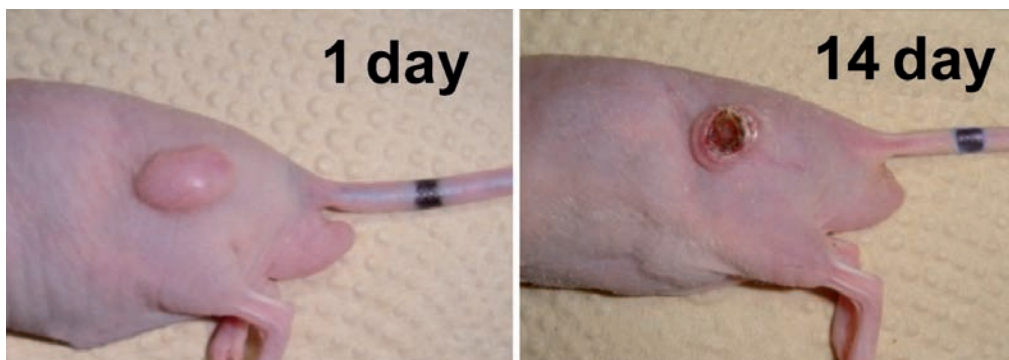
Polymeric micelle releases contents in response to pH of tumour tissue

## Directed tumour diagnosis and treatment

Researchers from Korea have developed a drug delivery system that simultaneously diagnoses and treats tumours within the body.

Photodynamic therapy (PDT) is an effective and non-invasive method used to treat prostate, skin and lung cancers. It works by injecting a photosensitiser into the body and then irradiating it at an appropriate wavelength. The photosensitiser produces singlet oxygen that damages the tissue around it. Encasing the photosensitiser in a nanosphere or polymer micelle makes delivery more successful and protects the photosensitiser from being removed from the body through the renal system.

Heebeom Koo, from the Korea Institute of Science and Technology and colleagues have developed a pH responsive polymeric micelle that releases its contents at the acidic extracellular pH of



**Improved delivery of photosensitiser improves PDT of tumours**

tumour tissue. When used in PDT, the photosensitiser is released producing fluorescence and singlet oxygen, simultaneously diagnosing and treating the tumour.

This research was undertaken to provide ‘solutions to overcome the urgent limitations in photosensitiser delivery and it will contribute to the clinical utility of PDT in cancer therapy,’ says Koo, and they hope their research ‘can

assist development of personalised medicine in the future.’

Shawn Chen, from the National Institute of Biomedical Imaging and Bioengineering, USA, was impressed with the research. This study is highly significant that the combination of pH-responsive micelle and photosensitiser showed synergetic effects for both cancer imaging and therapy”

*Rebecca Brodie*

**Reference**

H Koo *et al*, *Chem. Commun.*, DOI: 10.1039/c0cc01413c

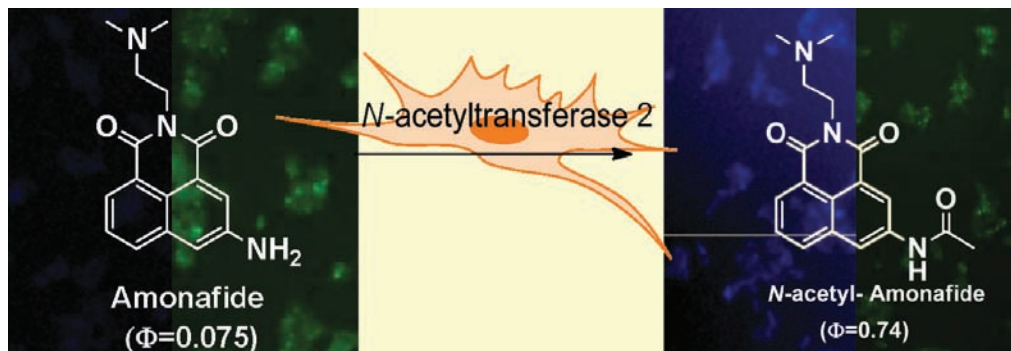
## A simple fluorescence assay could determine which drugs are best for a patient

# Predicting drug response

Scientists in China have developed a probe that could be used to test how well a patient will respond to certain drug treatments.

The new probe measures the activity of *N*-acetyltransferase 2 (NAT2), an enzyme that metabolises drugs and other toxins containing aryl amines and hydrazines. The activity of NAT2 differs between individuals, which affects how well a drug will work, and dysfunction of the enzyme has been linked to breast cancer, Parkinson's and other diseases. A simple measure of NAT2 activity could help ensure patients are given drugs that they can metabolise effectively with minimal side effects.

Xuhong Qian and colleagues at the East China University of Science and Technology found that the fluorescent molecule amonafide is metabolised specifically by NAT2. The enzyme acetylates the probe molecule, shifting its fluorescence



wavelength. Hence, this fluorescence change correlates to NAT2 activity. Current methods for predicting patient response to certain drugs require complex genetic analysis, but this probe could provide a simple and sensitive test.

AP de Silva, an expert in fluorescent sensors at Queen's University Belfast, UK, admires the team's use of fluorescence in two

**Acetylation by NAT2 changes the fluorescence wavelength of amonafide**

**Reference**  
L Cui *et al.*, *Chem. Commun.*, 2010, DOI:10.1039/c0cc01000f

colours to monitor an intracellular enzyme. He adds 'this work is likely to attract favourable attention.'

'The probe has significant potential applications in personal medicine,' Qian says. 'We also hope that it can be used to study the mechanism of different kinds of diseases related to NAT2.' The team now intends to design probes for other important enzymes. *Harriet Brewerton*

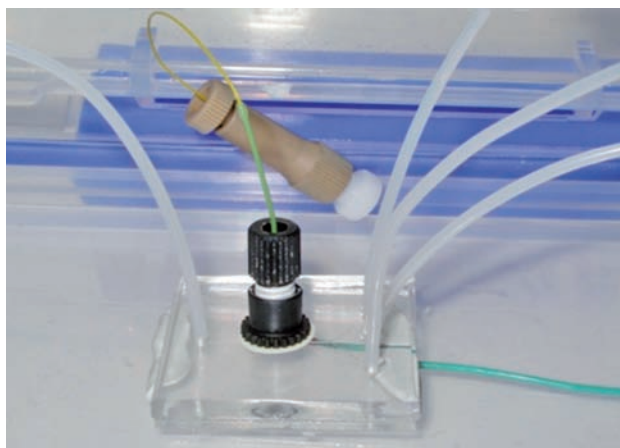
## Microfluidic device keeps tumour tissue alive for three days

# On chip tissue testing

A microfluidic device that keeps tumour tissue alive long enough to perform drug testing could reduce the need for animal testing and improve personalised medicine, claim researchers in the UK.

Cell cultures are widely used to test responses to drugs and other external stimuli but a more accurate result can be gained from using tissue samples. This is because cell-cell interactions are maintained, providing a closer representation of physiological environments. Microfluidic devices can be designed to maintain tissue samples, but so far this has only been done for ultra-thin brain tissue slices for a maximum of three hours.

Now, Charlotte Dyer and colleagues at the University of Hull have designed a device that can maintain tissue cultures for at least three days. 'The tissue is maintained in the microfluidic device by continuous flow systems



delivering essential nutrients and removing waste products in a highly controllable manner and with highly sensitive monitoring,' explains Dyer.

The team tested the system using both normal and cancerous colon tissue, showing for the first time that a tumour biopsy sample can be

**The device delivers a continuous flow of nutrients to the tissue and removes waste**

maintained and made to respond to external stimuli, such as drugs.

Yuan Wen, an expert in tissue engineering at biotechnology tool provider Life Technologies in Grand Island, US, says 'long-term culturing and interrogation of tissue constructs or biopsies with well controlled environmental parameters is one of the most sought applications of microfluidic technology in drug testing and clinical research.' According to Wen, this prototype microdevice allows in-depth investigation of tumour treatment in a more physiologically-relevant environment than previously available.

The team now intend to test other tissue types, and use the device to test the response of individual patient biopsy tissue samples to chemotherapeutic drugs.

*Harriet Brewerton*

**Reference**  
A Webster *et al.*, *Anal. Methods*, 2010, **2**, 1005 (DOI: 10.1039/c0ay00293c)

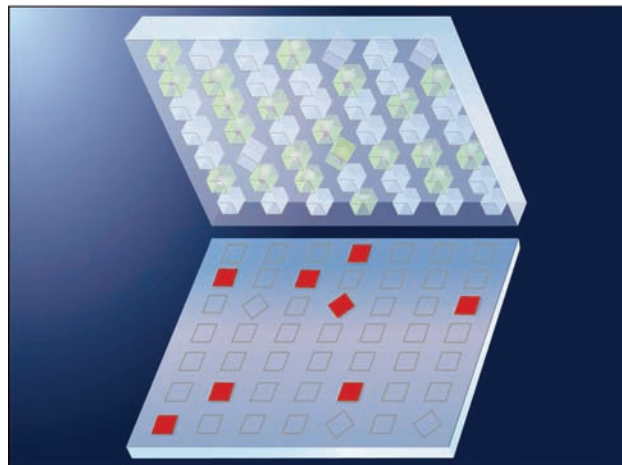
# Multiwell device measures cellular activity in thousands of cells at once

## Parallel detection of gene expression

US chemical engineers have developed a tool to measure gene expression and how those genes affect cellular activity in thousands of cells at once. This could aid research into treatments for viruses such as HIV or tuberculosis.

The relationship between the expression of certain genes and the subsequent functional activities of a cell is a central question in cell biology. Traditional tests that study genetic and proteomic responses to applied external stimuli typically require more than 1000 cells for each analysis, but the resulting averages obscure variations that may exist among individual cells, which can lead to misinterpretations of the biology. Now Christopher Love at Massachusetts Institute of Technology in Cambridge has developed a simple, one-step process for detecting the expression of specific genes in thousands of single cells in parallel.

Love's method involves using a multiwell device to detect copies of mRNA transcripts from individual cells in a one-step, single-cell, reverse transcription polymerase chain reaction (PCR). A mRNA transcript is an exact copy of a corresponding DNA coding region and by looking at the mRNA transcripts present in



a cell, scientists can find out which genes are expressed in that cell at different stages of development and under different conditions.

Simultaneous measurements to detect the presence of particular gene transcripts can be carried out for thousands of cells in parallel. A major advantage of the new approach is that it can be integrated with other techniques that capture additional information about the same cells, such as the secretion of particular proteins, says Love.

'This method should allow the detection of cells harbouring replicating intracellular pathogens

**Arrays of microwells allows thousands of cells to be monitored at once**

#### Reference

Y Gong, A O Ogunniyi and J C Love, *Lab Chip*, 2010, DOI: 10.1039/c004847j

such as HIV, endogenous retroviruses, or tuberculosis,' says Love. The ability to integrate this detection with other measures of phenotypes or functions should make it possible to correlate it with the functional profiles of those cells as well, he adds.

'In the context of HIV, this approach could allow detailed phenotypic analysis of latently or lytically infected cells,' says Love. Lytic infection is when the virus enters the cell, makes copies and kills the cell. Latently infected cells have HIV-1 DNA as a provirus but are not producing the virus. Such information about the characteristics of cells harbouring the pathogen may facilitate the development of therapies specifically targeted to those populations, explains Love.

Eric Rubins, a professor of immunology and infectious diseases at the Harvard School of Public Health, says: 'this work provides an approach to extend the study of single-cells as opposed to populations. One can envision employing multiple modalities to monitor cells simultaneously, a capacity that extends the power of other single cell methods such as fluorescence activated cell sorting.' Sarah Corcoran

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## Interview

## Rising research

Charles Boone talks to Laura Howes about yeast, Canada and hockey



**Charles Boone**

**Charles Boone is a professor at the University of Toronto. His research focuses on developing an automated approach for genetic analysis in yeast. This work has enabled the mapping of genetic networks on a large-scale and the determination of the function of all genes in a yeast model system. The function of many yeast genes is conserved in humans and this work has the potential for discovery of therapeutic drug targets.**

**You started as a chemist but then moved to biology, how did that all happen?**

When I went to university I decided to study things I'm not very good at to learn more, so I studied maths and chemistry. I'm not a mathematician but by the end of my math course I was in classes with the geniuses. They'd be reading a book in class and I'd be trying to figure out what fuzzy logic was. I really liked chemistry but I'm like those guys that were looking for the chemical basis of life. Once I'd seen what you can do with microbiology, I thought it was really exciting so even though we didn't have much of that at our university at the time, once I read about it I had no problem switching over straight away.

**I know several people who've moved from chemistry to biology, is the biology to chemistry switch more difficult do you think?**

There's probably some problems making the shift either way. I don't have the best grounding in some bits of biology and in some of the breadth of biology I'd like. But I'd agree that generally biologists don't shift to being mathematicians or chemists. Chemistry is a major foundation of almost all sciences so that may make it easier to move.

**Can you tell us more about what you do in your lab?**

There is a fairly large community working in yeast molecular genetics and genomics – about a thousand labs around the world. And there's an incredible database called the *Saccharomyces* genome database that houses all the information. The idea is to understand how yeast works at the level of almost every nucleotide in the genome and one of the ways we do that is to delete each gene individually. This has defined 1000 of the 6000 genes to be essential in yeast – so if you delete an essential gene the yeast dies. This indicates that most genes in eukaryotic organisms are non essential and we think one of the reasons for this is that pathways in the cell are wired with many back up pathways for any individual essential process in the cell. So there are many ways to solve a particular problem that the cell may encounter and we try to decipher which pathways are working together to solve essential functions and come up with a wiring diagram for the cell.

To do this we have identified all possible double mutants of yeast – which comes to the order of 18 million. We've developed a system that we call synthetic genetic array analysis that allows you to take a gene and either delete it if it is non essential or make a partially functional version if it is essential. Then make all possible double mutants with that gene and score if the mutant dies or has a fitness phenotype than is worse than we'd expect

by combining the two single mutations, so it's quantitative.

We call that a genetic interaction – so we're scoring genetic interactions and have come up with our genetic interaction map. We hope to link each gene to those of related function and sort all the genes in a cell into clusters that work together and thereby provide a global view of how the cell is wired functionally.

**Million dollar question, why do we care about yeast genetics?**

Because it is a fundamental eukaryotic cell and if we can understand how it works we'll have a much better understanding of how human cells or higher order cells in a eukaryotic organism work and then the genetic network problem is important because it is a model for the genotype to phenotype problem where if you sequence an individual and you know their variations, which combinations of mutations or alleles of genes control pathways and lead to inherited phenotypes. And no-one knows how to solve that problem beyond just the single gene.

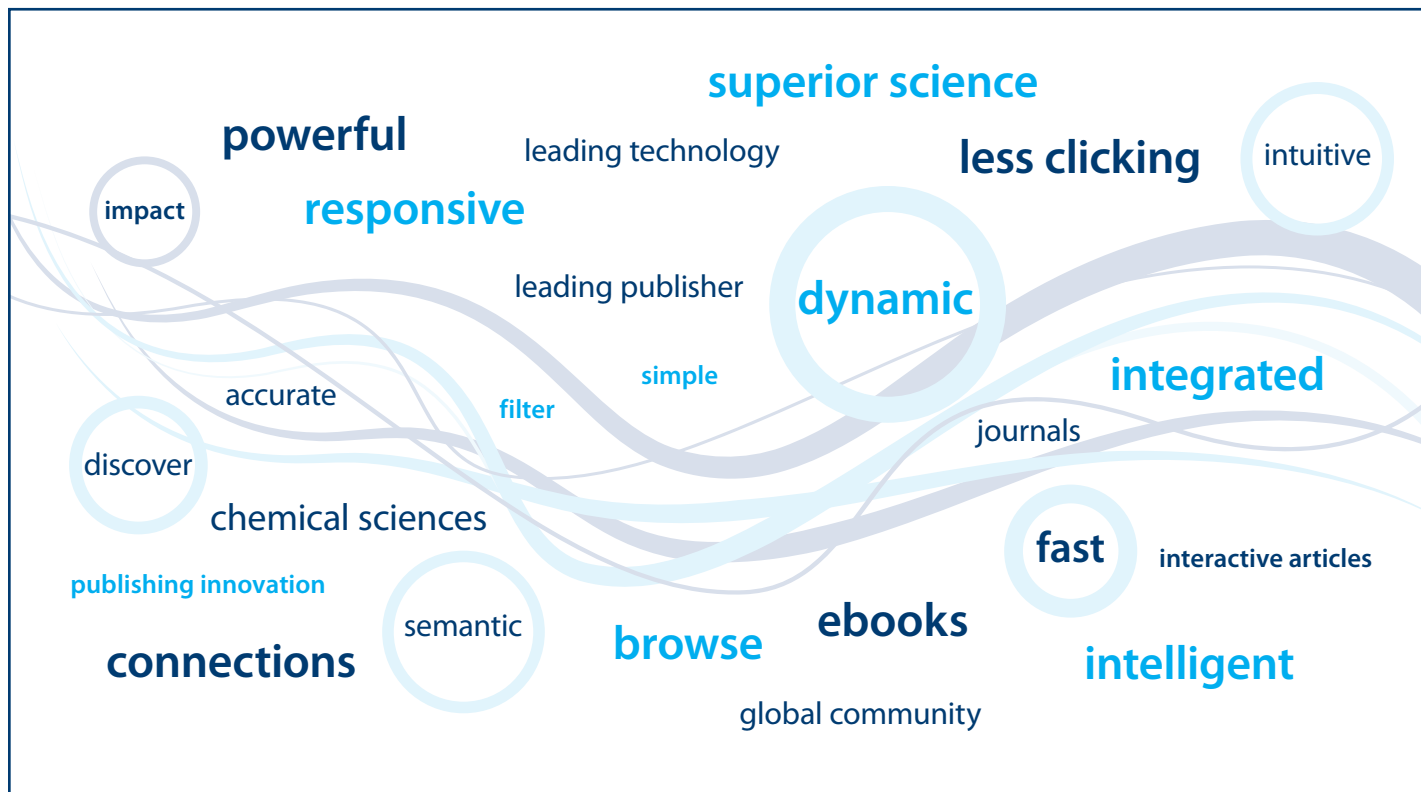
**How well funded is science in Canada, what are your thoughts on the implications for science?**

Our current government is not very interested in basic research. A model for them might be the US administration in terms of how they view basic research. In the budget they increased the funding to the three main funding bodies by this year, but what they failed to communicate was that the increase was less than what they'd cut from the budget the previous year. So that in the end there is still a net loss in funding. They don't seem interested in basic science as far as we can tell, and that's a massive problem that scientists have at the moment in Canada.

It's so short-sighted. There's a report by Harold Varners who used to be head of the NIH that outlines how if you just had faith in basic research, it would end up providing far greater impact than a directed approach that a government could apply because you just can't anticipate what will come out of the most basic research.

**What would you be if you weren't a scientist?**

I would like to have been an explorer, but it's such a drag that the world's been figured out by all those great British explorers – that would have been fun. And being a hockey player would have been way up on the list but maybe not as realistic as being a scientist. I think the science path I took is related to exploring because it's a great avenue for coming up with dreams and going out and trying to find them.



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