DESIGNER DEBACLE

A high-profile scientist, a graduate student and two major retractions. **Erika Check Hayden** reports on a case that has rocked the chemistry community.

When Mary Dwyer was looking for a doctoral adviser, Homme Hellinga was her first choice. A biochemist at Duke University Medical Center in Durham, North Carolina, Hellinga had ground-breaking ideas and an exciting research programme. He also shared Dwyer's interest in the relationship between protein structure and function. But there was a problem: students in Hellinga's lab were warning Dwyer away. "It's pretty tough," they told her; "there are other good labs." One student even pulled her aside and told her flat out that working with Hellinga was so difficult that she should not join the lab. By that time, that student remembers, many more students had left Hellinga's lab than had earned doctoral degrees under his tutelage.

Yet Dwyer had done a short rotation with Hellinga's group, and had seen nothing alarming. "I felt like I would probably be able to handle it," she recalls — and so, about nine years ago, she decided to join the lab.

Dwyer's work under Hellinga led to major publications in journals including *Nature* and *Science*, adding sparkle to Hellinga's already shining career. But last year, another scientist found problems that forced the eventual retraction of two papers — and Hellinga turned on Dwyer, accusing her of fabricating data. The episode has sparked controversy and condemnation, while highlighting the pressures on scientists working in cutting-edge research.

Hellinga is a bold scientist with a sterling pedigree. From his first *Nature* paper[1] onwards, Hellinga has been fascinated by one question: how does a series of amino acids encode a protein's function? Cracking that code is one of the major goals of science, because it would enable researchers to design custom proteins. In 1991, Hellinga, together with his postdoctoral mentor Frederic Richards of Yale University in New Haven, Connecticut, published a computer program[2] intended to do just that. Called DEZYMER, the program predicts protein sequences that might adopt target structures and functions — some of which are new to nature.

It was fitting that Hellinga should take on such a problem. Those who know him describe Hellinga as highly confident in his intellect and interested only in grand challenges. One scientist recalls, for example, that Hellinga once asked a companion, "Do you think I'll be more famous than Darwin one day?" Asked whether he agrees with claims that he is arrogant, Hellinga replies, "I would say no. Can I appear to be personally arrogant? I would imagine yes. When you are trying to do a difficult experiment, you have to have a certain amount of self-confidence to say, 'All right, this is the moment and we think we have the techniques and ideas together to try and give this a go.'"

Shapely targets

Around 2002, Hellinga decided to embark on his most difficult challenge yet: radically reshaping a humble protein into a highly active enzyme — a biological catalyst — called triose

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Homme Hellinga is well known for his work in designing enzymes.

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phosphate isomerase (TIM). The enzyme is part of a biological chain of reactions called the glycolysis pathway that is found in most organisms. Hellinga's goal was audacious; other scientists had designed weak enzymes[3], but nothing as active as TIM — considered a 'perfect enzyme' because of its extremely high efficiency (see graphic).

Hellinga chose Dwyer and another student, Loren Looger, to work on the project in *Escherichia coli* bacteria. The pair were to transform *E. coli's* ribose-binding protein, which has no enzymatic activity, into a TIM. Looger and Hellinga wrote computer programs to model how the structure of the ribose-binding protein could be changed to make it work like a TIM. Dwyer used the program to design mutated ribose-binding proteins, dubbed "NovoTIMs", and tested whether they worked in the lab.

Dwyer, who describes herself as a "pretty conservative person", was sceptical that the project would pan out. "I had my doubts all the time," she says. After about 6 months testing 25 designs, Dwyer found that a couple of the designed proteins were active, but she also noticed some problems. The *E. coli* bacteria made much smaller amounts of the NovoTIM proteins than of their own natural, or native, proteins. And the NovoTIMs were very unstable.

Perhaps because of these issues, Dwyer's experiments yielded confusing data about NovoTIM activity. When she measured the enzymes' kinetic parameters — characteristics that describe how enzymes work — the tests didn't always give the same results. "I felt like we couldn't nail down the kinetic parameters because of the variability that we were seeing," Dwyer recalls. Even after she started working with another member of the lab, "we were also getting a lot of variability. We just didn't understand it," Dwyer says. Hellinga says that the variability was "no more than you would expect in [such] an experiment".

By early 2004, Hellinga was ready to publish. On 29 March, he submitted a paper describing the NovoTIMs to *Science*, which accepted it on 6 May. The paper did not mention the variability Dwyer had noticed. It included only her best data and claimed victory[4]. "We have successfully converted a protein devoid of catalytic activity into a triose phosphate isomerase, using computational design techniques," it stated.

Dwyer was the first author on the *Science* paper, which was co-authored by Hellinga and Looger, who left Duke that year and now works at the Howard Hughes Medical Institute's Janelia Farm in Virginia. But Dwyer did not celebrate the accomplishment. "It was kind of strange," she recalls. "I wanted to work more on the variability issue," along with other odd results she had seen. "I felt like we weren't quite there yet."

Dwyer says that she raised her concerns with Hellinga at the time. But Hellinga says he does not feel he pushed Dwyer or anyone else to publish prematurely. "These things were talked through very carefully with all the people involved," he says.

That September, the National Institutes of Health gave Hellinga one of its nine inaugural Director's Pioneer Awards, worth US$2.5 million over five years. In October, he received the $10,000 Feynman Prize for experimental work from the Foresight Nanotech Institute in Palo Alto, California. Around the same time, he says, he and his wife, Duke structural biochemist Lorena Beese, were considering multiple job offers, including one from Yale. But in April 2005, Duke named Hellinga a James B. Duke Professor of Biochemistry, and Beese received the same honour the following year. Duke also created a new institute co-headed by the couple, the Institute for Biological Structure and Design.

"It is a bush-league error not to purify your proteins well."
— Wallace Cleland

To the letter

As Hellinga's career was skyrocketing, it was perhaps easy for him to overlook a letter that crossed his desk in December 2004 amidst the flurry of accolades. "Dear Professor Hellinga," it began. "I was wondering if you would be interested in collaborating."

The letter was written by John Richard, a chemical biologist at the State University of New York in Buffalo. Richard had studied with giants of the enzymology field: Perry Frey at the University of Wisconsin-Madison, Bill Jencks at Brandeis University in Waltham, Massachusetts, and Irwin Rose, now at the University of California, Irvine, who shared the 2004 Nobel Prize in Chemistry for discovering how a protein called ubiquitin marks other proteins for destruction in cells.

Richard had developed a method to analyse reactions catalysed by TIM[5,6]. He had seen Hellinga's *Science* paper and wanted to compare the characteristics of the NovoTIMs with those of normal TIMs. Richard proposed such experiments to Hellinga, but received no response. "It wasn't a high priority," Hellinga says.

The two men come from very different scientific cultures. Richard was trained in mechanistic enzymology and is known for his work in physical organic chemistry — fields that are no longer in vogue, perhaps because "all the easy experiments have been done", Richard says. Richard has gained respect in these fields, which require carefulness and meticulousness. "John is clearly one of the best physical organic chemists in the world today working on enzymes," says Joseph Kappock, a biological chemist at Purdue University in West Lafayette, Indiana. By contrast, protein design — a hot field — requires daring, as it seeks not just to understand nature, but also to improve on it.

In July 2006, Richard was discussing the *Science* paper with another chemist, Jack Kirsch, an emeritus professor at the University of California, Berkeley, where Hellinga had given a seminar on his work. On 9 August, Kirsch sent Hellinga an e-mail. "[Richard] informed me recently that he had sent you an e-mail requesting materials," Kirsch wrote. "Is there any reason why you cannot comply with his request?"

That e-mail seemed to grease the wheels. On 20 October, Hellinga wrote to Richard, agreeing to send DNA templates for the NovoTIMs he had made for the *Science* paper. He also sent templates for a second batch of NovoTIMs made by Dwyer and another researcher the year before. A paper describing these new proteins was about to be published in the *Journal of Molecular Biology*[7]. Hellinga sent Richard instructions for expressing and purifying all the NovoTIMs, as well as a note: "I hope that your experiments will be successful, and look forward to seeing the profiles for these designs."

In Buffalo, Richard hired a technician, Astrid Koudelka, to work on the NovoTIM project. Koudelka followed

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Local Encoding of Computationally Designed Enzyme Activity
Malin Allert[1], Mary A. Dwyer[2] and Homme W. Hellinga[1,3*]

Computational Design of a Biologically Active Enzyme
Mary A. Dwyer,[1] Loren L. Looger,[1*] Homme W. Hellinga[1]

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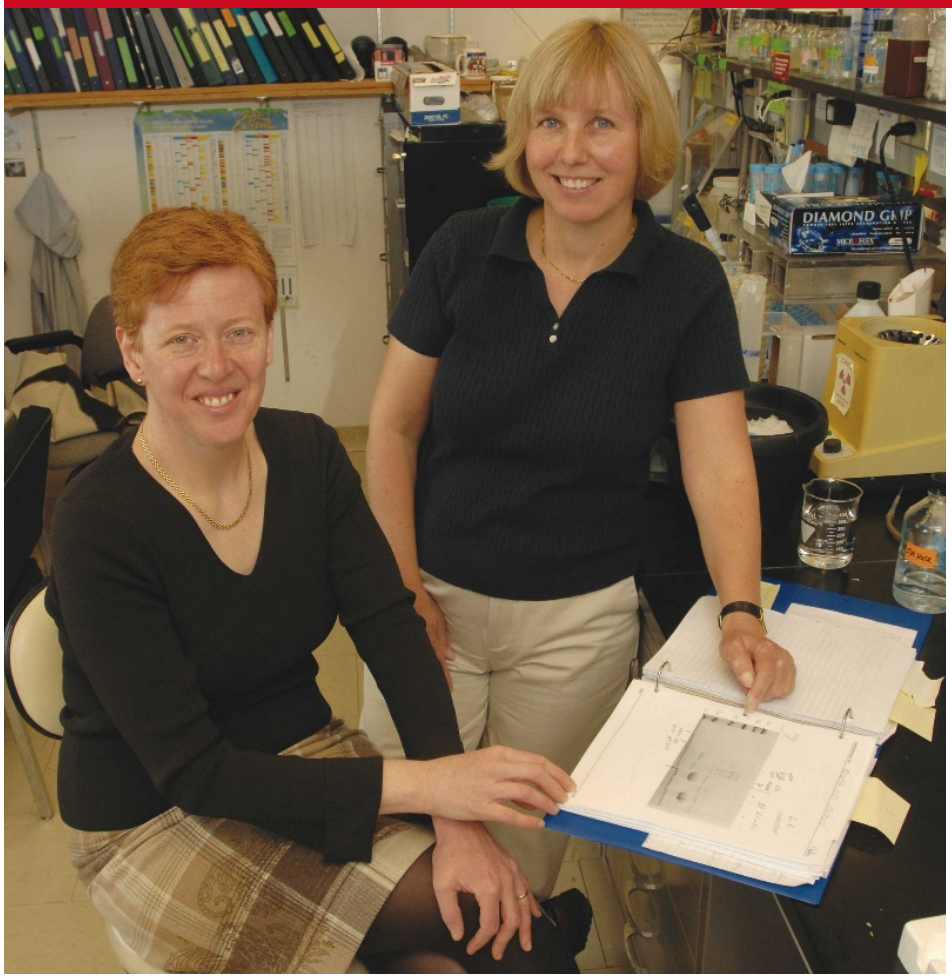

Tina Amyes (left) and Astrid Koudelka were unable to replicate Hellinga's work.

N.J. PARISI

Hellinga's notes, which instructed her to purify the NovoTIMs using a method called step gradient elution. But there was a problem: the step gradient could not separate the NovoTIMs from other contaminating proteins.

Then Richard's wife, chemist Tina Amyes, measured a kinetic parameter of the NovoTIMs — a value called the Michaelis constant. She found that it was different from the one reported in Hellinga's *Science* paper, but similar to that of natural, or wild-type, *E. coli* TIM. As Amyes studied the NovoTIMs throughout the first half of 2007, nothing about them was as Hellinga had reported, and everything suggested that they were wild-type TIMs.

Koudelka then modified Hellinga's procedures by using a continuous gradient elution, a more powerful purification method than the step elution. The new method cleanly separated the NovoTIMs from the contaminants. But when Amyes analysed the pure NovoTIMs, they had no enzymatic activity. Instead, the contaminating proteins were active — and looked just like wild-type *E. coli* TIMs.

By last July, the Buffalo group was convinced that something had gone wrong with Hellinga's experiments. By using step purification, they felt, Hellinga's lab had failed to separate the NovoTIMs from the TIMs found naturally in *E. coli*. The NovoTIMs were inactive; instead, all the activity that Hellinga had reported in his papers was probably due to contaminating wild-type TIM. "I was sort of distressed," says Richard. "We spent quite a bit of time, money and resources to basically do nothing, to show something was wrong." Yet the team felt an obligation to try to correct the scientific record. "Just saying, 'This is not right, let's discard it and move on' — that's not fair to the scientific community," Koudelka says.

Quick response

On 26 July, Richard sent a long e-mail to Hellinga that laid out his team's evidence, and pointed out what he saw as additional problems in some of Hellinga's other papers. Richard copied in the editors of the *Science* and *Journal of Molecular Biology* papers and two other chemists. "I think that these issues need to be dealt with in an expedient manner," Richard wrote, adding, "Please understand how difficult it has been for me to write this letter."

This time, Hellinga responded quickly. In a 30 July e-mail, Hellinga wrote that the key experiments "have been repeated several times by different individuals in my research group". The experiments included the tests that detected NovoTIM activity, and a set of negative control experiments. These negative controls — not shown in either paper — found no activity in purified ribose-binding proteins, Hellinga said. But he agreed to look again at the NovoTIMs: "We will carry out a purification similar to the one that you describe," he wrote.

All this time, Dwyer had heard nothing about Richard's communication with Hellinga. After earning her doctorate in 2004, she had left Hellinga's lab in 2005 to pursue postdoctoral research in a different department. So she was not seriously concerned when Hellinga e-mailed her on the Labor Day holiday on 3 September last year, asking her to meet with him later in the week to discuss issues about NovoTIM. But Dwyer's new adviser, Donald McDonnell, a professor of pharmacology and cancer biology, advised her not to meet Hellinga alone; he felt she should go with someone who could advocate on her behalf. McDonnell arranged a meeting later that week at which he, Dwyer and Hellinga were joined by two other faculty members from the biochemistry department. And that's when Hellinga dropped the bombshell. "He said, 'I find it really hard to believe that you didn't make this up', and he kept saying that kind of statement over and over again," Dwyer says. "It was horrible."

Dwyer's adviser defended her, and she proclaimed her innocence. "I said, 'That's ridiculous, no, I didn't do that'," she says. "What he was saying wasn't true."

A few weeks later, McDonnell, Hellinga, Dwyer and the head of the biochemistry department met again. Dwyer's husband, who is also a scientist, was there. Dwyer showed Hellinga the data from her lab notebooks that, she thought, exonerated her. But, she recalls, "he didn't want to look at any of that. It was just flat out my fault, and that was it." Hellinga remembers it differently. "That's not true," he says. "Of course I looked at the data. I also had people in my lab repeat the experiments," he says.

On 8 October, Hellinga wrote to Richard. "We have completed our repeat experiments on NovoTIM," he wrote. "I concur with your finding that the NovoTIM designs do not exhibit enzymatic activity, and that the reported activity is due to a contaminating activity which is very likely to be the endogenous, wild-type triose phosphate isomerase." The repeat negative control experiments, Hellinga wrote, had found "TIM activity in the wild-type [ribose-binding protein] preparations prepared by the step gradient elution method."

He added that the repeat experiments were done by three people, "but NOT Mary Dwyer, the author responsible for executing the experiments described in the *Science* paper, and responsible in large part for the negative control experiment in the *Journal of Molecular*

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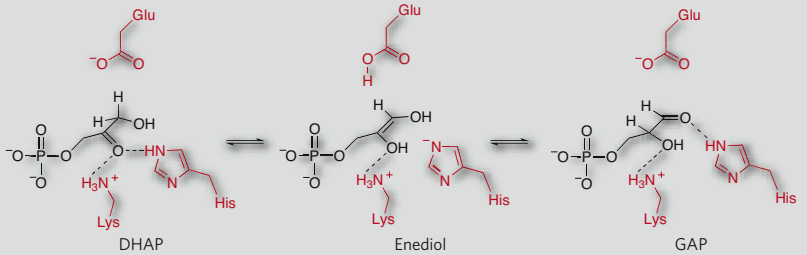
SOURCE: SCIENCE

HOW TIM WORKS

The enzyme called triose phosphate isomerase (TIM) catalyses one important step in sugar metabolism, and is found in most living organisms. The enzyme (active sites shown in red) allows cells to efficiently convert two sugars, called DHAP and GAP, into one another through the intermediate molecule enediol. Computer programs could enable researchers to design such efficient enzymes from scratch.



Biology paper." By naming Dwyer as the scientist primarily responsible for the experiments, Hellinga seemed to contradict his 30 July e-mail to Richard, in which he said "different individuals" had been involved. However, Hellinga clarified to *Nature* that his July e-mail was "slightly inaccurate"; at that time, Dwyer was the only person who had performed the negative controls, he says.

To Richard, Hellinga continued: "Dwyer has been contacted in an attempt to seek an explanation … The matter has been referred to the Office of the Dean of the Medical School for further enquiries, which are now in progress."

A committee on research misconduct convened a formal inquiry hearing in December, at which Dwyer was asked to address the claims against her. On 4 February, she received a letter from Wesley Byerly, an associate dean in the medical school, clearing her of the allegation of falsifying and fabricating results.

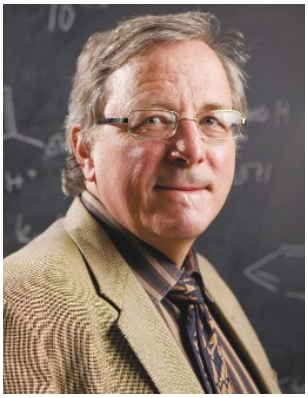
Culture of blame

But word about the inquiry had already spread, outraging chemists who felt it was wrong for a mentor to accuse a student of fraud. "It is reprehensible," says Frey. "It is up to the adviser to instruct the student, to guide the student to find out what problems exist with the data and their interpretation of it, and to show the student what the pitfalls are."

This February, both the *Science* and *Journal of Molecular Biology* papers were formally retracted. "The triose phosphate isomerase activity observed in our reported preparations can be attributed to a wild-type TIM impurity," stated the *Science* retraction; the other retraction was similar. Other chemists were surprised that Hellinga's lab had been fooled by a simple contamination problem. "It is a bush-league error not to purify your proteins well, especially in a paper like this," says Wallace Cleland of the University of Wisconsin-Madison.

Still, exactly what happened remains murky. On 10 March, *Science* published letters from Richard and Kirsch listing issues they said were not resolved by the retractions. For instance, they wrote, the kinetic values Hellinga reported for NovoTIM are not the same as those of wild-type TIM, which is difficult to understand, given that all the activity in Hellinga's papers was supposed to have come from the wild-type enzyme.

Kirsch also raised questions about other experiments in the *Science* paper that "would make sense only if the design were successful". For instance, the paper reported that NovoTIMs could substitute for wild-type TIMs in *E. coli* that lack TIM enzymes. And a different test supposedly showed that mutated NovoTIMs became less active, just as DEZYMER had predicted. Neither of these results makes sense if the designed enzymes never worked.



D. LEVERE, UNIV. BUFFALO

John Richard flagged issues with potential contamination.

Hellinga does not have explanations for the issues Kirsch and Richard have raised. Dwyer thinks that the issues with protein expression and assay variability are partly to blame, and says that in retrospect, the apparent decreased activity of NovoTIM mutants was actually insignificant, once experimental error is taken into account. But no one has offered a clear answer for what went wrong. That is frustrating to Richard, who has spent considerable time and resources trying to get to the truth.

But thanks to Richard's work, another research team has been able to earn credit for the breakthrough Hellinga once claimed. In March, a team led by biochemist David Baker from the University of Washington in Seattle published two papers showing that computer programs could indeed be used to design working enzymes[8,9].

Meanwhile, other scientists have questioned whether Hellinga himself should be investigated. Some point to a Duke policy that states that if an allegation of misconduct is found to be "baseless and malicious or reckless, the matter will be dealt with in accordance with existing university policies and mechanisms".

Hellinga says he has received no formal notification that he is under investigation. Duke would not comment specifically, saying only: "We are aware the retraction by Dr Hellinga has generated considerable debate in the scientific community. Duke continues to follow this debate and is evaluating various points that are being raised."

Asked whether he would have done anything differently in the NovoTIM experiments, Hellinga says, "I would like to not have the problem that we encountered." When asked whether the lab moved too quickly, he says: "Given how we understood things to be at the time, no. Obviously if we had known things had gone wrong, we wouldn't have moved forward with the speed we did."

As for Dwyer, she still feels rattled by the experience. "I feel incredibly guilty that I didn't catch it, but I didn't, and I just have to live with that. It's been really hard," she says. She is trying to move forwards with her life and career, she says, and is working in a new lab in a new field — endocrinology — with McDonnell. But sometimes, Dwyer says, she thinks back to the people who tried to steer her away from Hellinga's lab so many years ago. And she wonders how different things might have been if she had heeded their advice. "Everybody gets warned, but nobody listens," she says. "Maybe now they will." ■

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