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Chemistry as a Vector for Understanding Biology

When Jack Taunton was an undergraduate chemistry major at Trinity University in San Antonio, TX, natural products were a favorite focus for synthetic chemists. However, once chemists had developed an elegant synthesis, the fruits of their labor often remained locked in the freezer, their worth in biological studies sometimes ignored. Through a combination of good mentors and creative thinking, Taunton, a professor in the Department of Cellular and Molecular Pharmacology at the University of California, San Francisco (UCSF), developed an appreciation for using synthetic, biologically active small molecules to investigate biological mysteries. Now, using the tools of both biology and chemistry, Taunton and his colleagues are focusing on a few broad objectives: probing the signals that control actin assembly and force generation on cell membranes and synthesizing small molecules to dissect cellular functions.

Undergraduate Guidance. Taunton was born in Madison, WI, in 1968. "There was certainly some science in the family," he recalls. His father was a chemical engineer, and his mother taught science and math to junior high and elementary school students. Yet, Taunton says, his parents did not push him to study science. Instead, he pursued a variety of interests, including music and creative writing.

Toward the end of high school, Taunton decided to leave early. "I was extremely academically driven, but not engaged. I knew I had to do something else," he says. A high school counselor who supported his decision to leave encouraged him to try his luck with applying to college. Although he lacked a high school diploma, he was accepted to Trinity University. He started his freshman year there in the fall of 1985.

Though Trinity had initially been his fallback school, the school ended up having many unexpected benefits, such as engaging professors in religion and chemistry. After developing intense interests in both subjects, Taunton decided to take on a double major in those subjects. "I wanted to have the choice of going to grad school for religion or chemistry," he says.

Taunton did well in classes for both majors, though an important factor steered his interest toward science: Michael Doyle, who now chairs the Department of Chemistry and Biochemistry at the University of Maryland in College Park, taught organic chemistry at Trinity during Taunton's time there and remains a strong proponent for undergraduate research. Having demonstrated a knack for science in Doyle's class, Taunton received an invitation to do independent research in his laboratory. While juggling his regular course load, Taunton began characterizing insertion reactions of diazirines and diazo carbonyl compounds, which were a major focus of Doyle's laboratory.

Taunton's time in Doyle's laboratory was extremely productive, especially for an undergraduate, and netted him four publications (*1*–*4*). Taunton was inspired by Doyle's mentorship to pursue graduate education in chemistry. While searching for the right graduate school, Taunton met Tom Wandless, one of Doyle's former students who is now a professor of chemical and systems biology at Stanford University. At the time, Wandless was pursuing his own graduate degree in the laboratory of Stuart

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Schreiber at Harvard University. Wandless's project, which focused on using natural products to explore biological systems, sparked Taunton's interest in combining chemistry and biology.

"I was interested in synthetic chemistry, but this piqued my interest in a world beyond just making molecules," says Taunton. "I saw that there were amazing things you can do with molecules for learning biology. I became interested in chemical biology, though it didn't have that name then."

Biological Puzzles. Taunton was intrigued by Schreiber's broad focus on synthesizing biological molecules in the laboratory and using them to solve biological mysteries. Yet, with his undergraduate concentration on chemistry, he knew little about biology when he applied for a position at Schreiber's laboratory. "I was very naive and completely unsophisticated about biology," he recalls. "I wasn't sure that I wanted to study biology, but I knew I wanted to go to someplace where there was some possibility to explore biology using chemistry."

Taunton applied to Harvard with a plan to join Schreiber's laboratory. He remembers being nervous that perhaps he would not get in. "I had no real backup plan," Taunton recalls. In the end, a multiyear National Science Foundation fellowship he'd received as an undergraduate made him an attractive candidate. Schreiber soon approached Taunton with an idea for a project: wrapping up a senior graduate student's work on the total synthesis of dynemicin, a natural product that cleaves DNA. Taunton notes that he was hesitant to join the project. "It definitely wasn't something I was that psyched about," he remembers. "John Porco [now at Boston University] and John Wood [now at Colorado State University] had done all the really sexy chemistry and had made a lot of progress." Plus, because the molecule's function was already known, the lack of mystery dampened the project's excitement.

However, Taunton found that much work remained to round out the molecule's complicated, 30-step synthesis. After two years of effort, he still hadn't finished the synthesis of dynemicin, but he was able to publish the synthesis of a partially methylated derivative (*5*).

"By that time, I was tired of doing just complex natural product synthesis," he remembers. Yet, the experience of being in the Schreiber laboratory boosted his interest in biology. Taunton began teaching himself cell biology, reading extensively to educate himself not only on the basics but on the latest developments in the field. During this time, the makeup of Schreiber's laboratory also changed: it slowly morphed from a majority of chemists to roughly half chemists, half biologists.

Taunton became inspired to find his own natural product to study, searching for a molecule with a clear-cut biological mystery: an unknown target, function, or both. While reading the *Journal of Antibiotics*, he found an intriguing candidate, a molecule called trapoxin (*6*). Derived from a fungus, *Helicoma ambiens*, trapoxin had been shown by researchers in Japan to exhibit a particularly mysterious activity when added to mouse fibroblasts that have been transformed with an oncogene. Transformed fibroblasts usually change their morphology, going from cells that grow in a monolayer with prominent actin stress fibers to rounded cells that grow in multiple layers on top of each other. However, in the presence of trapoxin, the cells lose their transformed qualities, reverting to a normal-looking form. "I thought, wow, this is new, this is mysterious, this is cool; I have to find out how it works," Taunton says.

He set out to determine the direct biological target of trapoxin, a cyclic tetrapeptide. His efforts received an enormous boost when one of the Schreiber laboratory's postdoctoral fellows completed the synthesis of trapoxin along with a radiolabeled derivative of the molecule.

Taunton notes that trapoxin has an unusual epoxyketone side chain, a functional group rarely seen in peptides outside of trapoxin's molecular family. He immediately noticed that the electrophilic side chain might allow trapoxin to form a covalent bond with its protein target.

To study trapoxin's action, he replaced one of its side chains with a linker connected to a solid support. He then used this affinity reagent to pull trapoxin's protein target out from cell lysates. After two years of effort, he identified the target, which turned out to be similar to the protein encoded by a yeast gene whose function was then unknown. This gene, *RPD3*, had previously been recovered in several genetic screens for transcriptional regulators.

Taunton found an important clue about the protein's function in a paper that came out shortly after he started the project: the paper suggested that trapoxin not only reverted cell transformation but also inhibited a histone deacetylase activity (*7*). He recalls, "My initial reaction was that there could be nothing more boring than an enzyme that removes acetates from histones. How could this obscure enzyme activity be related to oncogene transformation?" As he read more on the potential link between histone acetylation and transcriptional regulation, he realized that the effect of trapoxin on actin filaments and cell morphology was probably indirect, essentially a side effect of changes to the cells' genetic wiring. Working with fellow graduate student Christian Hassig, he cloned the human ortholog of the yeast *RPD3* gene and showed that the recombinant protein had histone deacetylase activity. They published the first paper describing a histone deacetylase gene in 1996 (*8*).

Given the enormous focus on epigenetics research today, Taunton points out how important it is to nurture fundamental research on natural products such as trapoxin. "Even though this molecule doesn't have what it takes to make it as a drug, it's

a great example of how basic research can lead to discoveries that are relevant to medicine," he says. Just last year, a histone deacetylase inhibitor (vorinostat, Merck) was approved by the U.S. Food and Drug Administration for the treatment of cutaneous T cell leukemia.

Broad Interests. After reading extensively on actin for his trapoxin study, Taunton developed an intense interest in this cytoskeletal protein. Upon receiving his doctoral degree, he decided to scout out postdoctoral fellowships that would allow him to indulge his interest. He eventually settled on Tim Mitchison's laboratory, then at UCSF. Mitchison, whose group studies cellular locomotion and cell division, "was developing new chemical approaches for imaging cells, but that wasn't the main focus of his lab," says Taunton. "His main focus was to get to the bottom of an unsolved problem in cell biology, and he'd invent new techniques to do that." For example, Taunton explains, Mitchison had developed photoactivatable fluorescent dyes to view the action of cytoskeletal proteins by using sophisticated imaging experiments.

In talking with his new mentor, Taunton learned that actin nucleation seemed to occur preferentially on cell membranes. However, the details behind the signaling mechanism were not known. For the next few years, Taunton set up an *in vitro* system using *Xenopus* egg extracts to study a signaling pathway that triggers actin nucleation on membranes. He developed a fractionation procedure to identify the signaling molecules and spent most of his postdoctoral fellowship building and characterizing this cell-free system.

Four years after his arrival at Mitchison's laboratory, Taunton decided to act on a long-standing job offer at UCSF. After accepting the offer and moving across the country, he picked up where he left off in his investigation of actin signaling. His studies had suggested that N-Wiskott-Aldridge syndrome protein (N-WASP), a member of the WASP family of proteins, nucleates actin assembly to move endosomes (*9*). However, it was not clear how N-WASP, a soluble cytoplasmic protein, becomes enriched on membrane surfaces, where it promotes assembly of dynamic actin networks. "I wanted to get a thorough mechanistic understanding of how this happened when I arrived here," says Taunton. "The same is true today."

Taunton's laboratory at UCSF currently has three main foci, one of which is discovering the mechanism behind actin signaling. His team recently published findings suggesting that the WH2 domains of N-WASP play a previously unanticipated role in vesicle movement by transiently attaching actin filament barbed ends to the membrane. The researchers dissected the attachment mechanism by using lipid bilayer-coated glass beads and a minimal set of actin assembly proteins. Their results suggest that N-WASP's WH2 domains not only may create an attachment force but also may locally amplify the actin signaling pathway (*10*).

Another focus of Taunton's laboratory is the rational design of small-molecule inhibitors that form covalent bonds with proteins, inspired in part by the epoxyketone of trapoxin. At the moment, his team is focusing on protein kinases, with a plan to expand to other classes of proteins in the future. Taunton and his colleagues recently invented small molecules that specifically bind to the protein kinase RSK, based on recognition of two amino acids, a threonine and a cysteine, on the protein (*11*). "We hope to generalize from this and target at least a small subset of nonconserved cysteines found in human proteins and in human pathogens," he says. Because many cysteines occupy or sit near a drug binding pocket on proteins, he notes, such a strategy could eventually identify candidates for new pharmaceuticals.

Taunton's third focus also mirrors his previous work on trapoxin: studying natural products that could give new insight to biological processes. His latest interest is a molecule called HUN-7293, which potently blocks expression of the cell adhesion molecule VCAM1. A series of experiments in Taunton's laboratory pointed toward the endoplasmic reticulum as the likely site of action for HUN-7293. Though this molecule was initially thought to affect transcription of the VCAM1 gene, recent experiments by Taunton's team have shown that the molecule prevents VCAM1 from getting to the cell surface by blocking a protein-conducting channel in the endoplasmic reticulum that is essential for protein secretion (*12*). "This channel is an ancient machine for getting proteins inside the cell to the outside of the cell," he says. Because VCAM1 cannot exit the cell, it is rapidly degraded in the cytoplasm.

The mechanism by which HUN-7293 and its analogues block this channel is unknown. It is just one of the biological mysteries that Taunton and his team are trying to solve by using chemical tools. Taunton notes that these many mysteries will likely fill his time for years to come. And, like all good scientists, he expects more mysteries to arise in his areas of interest and more interests to arise as he works.

—Christen Brownlee, Science Writer

REFERENCES

1. Doyle, M. P., Pieters, Roland, J., Taunton, J., Pho, H. Q., Padwa, A., Hertzog, D. L., and Precedo, L. (1991) Synthesis of nitrogen-containing polycycles via rhodium(II)-induced cyclization-cycloaddition and insertion reactions of *N*-(diazoacetoacetyl)-amides. Conformational control of reaction selectivity, *J. Org. Chem. 56*, 820–829.
2. Doyle, M. P., Taunton, J., and Pho, H. Q. (1989) Conformational and electronic preferences in rhodium(II) carboxylate and rhodium(II) carboxamide catalyzed carbon-hydrogen insertion reactions of N,N-disubstituted diazoacetoacetamides, *Tetrahedron Lett. 30*, 5397–5400.
3. Doyle, M. P., Taunton, J., Oon, S.-M., Liu, M. T. H., Soundararajan, N., Platz, M. S., and Jackson, J. E. (1988) Reactivity and selectivity in intermolecular insertion reactions of chlorophenylcarbene, *Tetrahedron Lett. 29*, 5863–5866.

4. Bagheri, V., Doyle, M. P., Taunton, J., and Claxton, E. E. (1988) A new and general synthesis of α-silyl carbonyl compounds by silicon-hydrogen insertion from transition metal-catalyzed reactions of diazo esters and diazo ketones, *J. Org. Chem. 53*, 6158–6160.
5. Taunton, J., Wood, J. L., and Schreiber, S. L. (1993) Total syntheses of di- and tri-*O*-methyl dynemicin A methyl esters, *J. Am. Chem. Soc. 115*, 10378–10379.
6. Itazaki, H., Nagashima, K., Sugita, K., Yoshida, H., Kawamura, Y., Yasuda, Y., Matsumoto, K., Ishii, K., Uotani, N., and Nakai, H. (1990) Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumor agents, *J. Antibiot. 43*, 1524–1532.
7. Kijima, M., Yoshida, M., Sugita, K., Horinouchi, S., and Beppu, T. (1993) Trapoxin, an antitumor cyclic tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase, *J. Biol. Chem. 268* 22429–22435.
8. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p, *Science 272*, 408–411.
9. Taunton, J., Rowning, B. A., Coughlin, M. L., Wu, M., Moon, R. T., Mitchison, T. J., and Larabell, C. A. (2000) Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP, *J. Cell Biol. 148*, 519–530.
10. Co, C., Wong, D. T., Gierke, S., Chang, V., and Taunton, J. (2007) Mechanism of actin network attachment to moving membranes: barbed end capture by N-WASP WH2 domains, *Cell 128*, 901–913.
11. Cohen, M. S., Zhang, C., Shokat, K. M., and Taunton, J. (2005) Structural bioinformatics-based design of selective, irreversible kinase inhibitors, *Science 308*, 1318–1321.
12. Garrison, J. L., Kunkel, E. J., Hegde, R. S., and Taunton, J. (2005) A substrate-specific inhibitor of protein translocation into the endoplasmic reticulum, *Nature 436*, 285–289.