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A New Classic Arrives

By Jason Socrates Bardi

A few years ago, Scott Snyder was settling into the fall semester of his senior year at Williams College in Williamstown, Massachusetts, and his thoughts were some 3,000 miles away.

Surrounded by the gorgeous fall foliage on the Williams campus near the Vermont and New York borders, Snyder was nevertheless engrossed in a book that was written a few years prior on the temperate, California campus of The Scripps Research Institute (TSRI).

Titled *Classics in Total Synthesis* by TSRI's K.C. Nicolaou and Erik J. Sorensen (now at Princeton University), the book detailed several modern examples of the "total synthesis" or construction of an organic compound from precursor molecules in the laboratory—research that has often been compared to Herculean tasks.

"Reading this book was a strong driving force for me to come to TSRI," says Snyder. Presently a fifth year graduate student in TSRI's Kellogg School of Science and Technology, he immediately chose to study synthetic organic chemistry under Nicolaou, who is chair of TSRI's Department of Chemistry and holds the Aline W. and L.S. Skaggs Professorship of Chemical Biology and the Darlene Shiley Chair in Chemistry.

Now, four years later, Snyder is coauthor with Nicolaou of a new book—*Classics in Total Synthesis II* (2003, Wiley)—the sequel to the text that first steered his course.

Milestones in Science and Publishing

The total synthesis of organic compounds dates back to 1828, when Friedrich Wohler first combined cyanic acid with ammonium carbonate in a test tube to make urea. But it took much of the 20th century for the field to grow to its present, powerful state.

In the foreword to the new book, Nobel laureate E.J. Corey of Harvard University, who was Nicolaou's postdoctoral advisor, says that the first book "...stands as a milestone to mark the closing of the 20th century, which encompassed both the birth of the field and its growth to one of the most intellectually fulfilling and practically important areas of modern science."

Classics in Total Synthesis II takes up where the previous volume left off, and covers the reactions and synthetic methods that have come of age since the publication of *Classics in Total Synthesis*.

One of the new compounds featured is the anticancer compound diazonamide A, which Nicolaou, Snyder, and several members of the group synthesized last year. A picture of this natural product and the sea creature from which it was discovered, the colonial ascidian *diazona angulata*, adorns the cover.

Twenty other complex natural products are also described in *Classics in Total Synthesis II*, including several others from the Nicolaou group, two molecules tack-

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Professor K.C. Nicolaou (right) and graduate student Scott Snyder (center) sign copies of their new book at the American Chemical Society meeting.

Classics

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led by TSRI investigator Dale L. Boger, and one synthesized by former TSRI Professor Erik J. Sorensen. Mention is also made of several important synthetic discoveries that have emanated locally from the TSRI groups of M. Reza Ghadiri, Kim D. Janda, Subhash Sinha, and K. Barry Sharpless.

For each compound, the book carefully describes the synthetic route from chemical precursors to the final product, focusing special attention on the novel strategies and tactics that were employed. Each chapter ends with a lengthy bibliography, which is intended to enhance the pedagogic value of the book to all of its readers—professors, graduate students, advanced undergraduates, and researchers in chemical industry—by providing primary references for further enrichment.

Ultimately, say Nicolaou and Snyder, the goal is not only to present these classic examples as historical achievements of what can be accomplished in the laboratory, but, hopefully, to inspire the next generation of synthetic chemists on whose shoulders lie the responsibility of pushing the boundaries of the field even further.

A Sign of Things to Come

Last month, Nicolaou and Snyder held a book signing sponsored by their publishers at the American Chemical Society meeting in New York City. They were met with lines of students and teachers eagerly waiting to get their hands on an advanced copy of *Classics in Total Synthesis II*.

Nicolaou and Snyder joke that while it took a year and a half for them to put the book together, they sold out all their advanced copies in less than half an hour.

The success of this signing could not have been more thrilling for Nicolaou and Snyder, because most of the copies were purchased by students who were attending the meeting. It was in the spirit of educating these very students that Nicolaou and Snyder picked up their pens in the first place.

"It's important for us [as scientists] to motivate, inspire, educate and train," says Nicolaou. "We must think about the next generation of scientists."

Copies of *Classics in Total Synthesis II* will be available from the publisher, Wiley-VCH, and should be delivered by the end of October.



***Classics in Total Synthesis II* covers reactions and synthetic methods that have recently come of age.**

Young TSRI Scientists Receive Grants for Clinical Projects

By Mika Ono Benedyk

One of the hardest things about starting out as a scientist is finding money to fund those first few critical projects.

This week, three young investigators at The Scripps Research Institute (TSRI) got some help from a new source, the Clinical Research Feasibility Funds (CReFF), part of a grant from the National Institutes of Health to TSRI's General Clinical Research Center (GCRC). The CReFF program is intended to encourage young investigators to become engaged in clinically oriented research. Matching grants from the Skaggs Foundation and TSRI also made the awards possible.

The three TSRI researchers, each winning \$20,000 of support for pilot projects at TSRI's General Clinical Research Center (GCRC), are:

- Marc Arnush, a postdoctoral fellow working with Associate Professor K. Michael Pollard. He will conduct a project comparing Daf gene structure and expression of patients with and without a history of the lupus-related kidney disease glomerulonephritis, one of the most significant health problems associated with lupus.
- Emily Chen, a postdoctoral fellow working with Associate Professor Brunhilde Felding-Habermann. She will conduct a project to identify cell surface proteins that contribute to the propensity of metastatic breast cancer cells to colonize skeletal bone as a target organ.
- Alexander R. Shikhman, assistant professor. He will conduct a study analyzing the safety and efficacy of a potential therapy for osteoarthritis, N-acetylglycosamine, in patients with osteoarthritis of the knee.

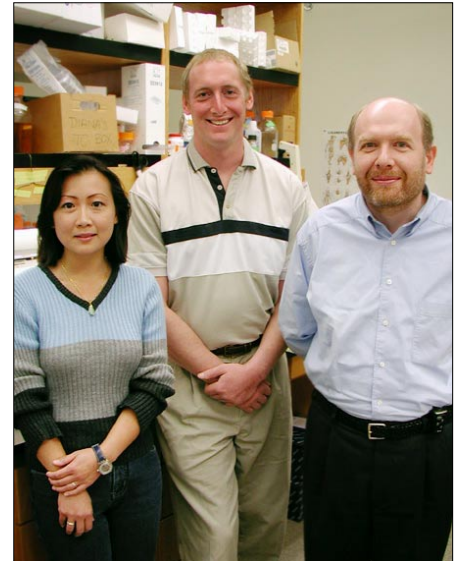
"Congratulations to the award winners," says TSRI Professor Dr. Frank Chisari, program director of the GCRC. "I'm delighted to see young investigators inspired to research such relevant clinical problems using the excellent resources available at TSRI's GCRC."

The winning projects were selected on the basis of scientific merit, clinical relevance of the project and/or need for the GCRC, and qualifications of the candidates. The GCRC Scientific Advisory Committee, which is comprised of over a dozen members representing four TSRI departments and three clinical divisions of Scripps Clinic, acted as the selection committee for the awards.

Arnush, Chen, and Shikhman will use the CReFF funds for such diverse items as lab supplies, lab procedures, payments to patients, rental of small equipment, experimental drug preparation, and partial salary support.

The GCRC, which has over 100 approved clinical protocols, provides investigators at TSRI and The Scripps Clinic Medical Group with the facilities to conduct clinical research, including:

- a seven bed inpatient unit, including a sleep lab;
- an adjacent outpatient suite;
- nursing staff specially trained to provide both excellent patient care and rigorous research data collection;
- a core laboratory staffed and equipped to perform specialized research assays and provide specimen preparation and storage; and



Investigators Emily Chen, Marc Arnush, and Alexander R. Shikhman (left to right) will conduct research in TSRI's General Clinical Research Center. Photo by Kevin Fung.

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GCRC

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- a computer center, staffed with a biostatistician and a systems manager, to help investigators design studies and perform sophisticated genetic and clinical data management and analysis.

In addition, the GCRC runs a blood donor program, which ensures that the blood used in laboratory experiments is properly drawn, screened, and categorized.

For more information about the GCRC, contact Beth Bieger, administrative manager, at tie line 554-2281 (x-3542281 if calling from the TSRI campus) or bieger@scripps.edu. More information is also available at the GCRC web site.

The Tail End of Integrin Activation

By Jason Socrates Bardi

There is no shortage of information on the Internet about integrins.

A recent Google search for the word "integrin," in fact, turned up 263,000 web pages devoted to the structure, chemistry, and biology of this important family of cell-surface proteins, which are involved in everything from early embryonic development to the development of heart diseases and cancer later in life. There was even one site that boasted an integrin chat room.

Such an ocean of preexisting information begs the question, is there anything more to say?

In this case, the answer is an emphatic yes.

Written by a team of scientists from The Scripps Research Institute (TSRI) and its neighboring La Jolla institution, The Burnham Institute, a paper appearing in this week's issue of the journal *Science* describes a crucial final step in the process of integrin activation—the binding of a protein called talin.

"Talin is required for the activation process," says TSRI Assistant Professor David Calderwood, who led the study. "This interaction is the last step."

The study is interesting because understanding the way in which integrins are activated is crucial to understanding their function in all the physiological processes in which integrins are involved.

Integrins and Platelets

Integrins are large binary protein complexes made up of two different types of polypeptide chains (called the alpha and beta subunits) that come together to form a "heterodimer" that is expressed on the surface of a cell.

They are somewhat top-heavy. A huge portion of the protein is extracellular and sticks out on the outside of the cell, and just a tiny tail of a few dozen amino acids protrudes through the membrane on the inside of the cell.

The large extracellular portions are the domains that bind to molecules on the outside of the cells and mediate the interactions of the cell with other cells.



Collaborating with colleagues at TSRI and The Burnham Institute, TSRI Assistant Professor David Calderwood has published a paper in *Science* describing a vital step in integrin activation. Photo by Kevin Fung.

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Talin

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If tissues were trains and cells were the boxcars, then integrins would be the hooks that hold the boxcars together. They hold cells together and keep them bound to one another and to the extracellular matrix maintaining the integrity of tissues in mammals and other multicellular organisms. They are also important in early development for the formation of distinct tissues.

But integrins do more than just hold cells together. They are also crucial mediators of a host of other normal and abnormal biological processes. They are important for inflammation; they are essential for platelet aggregation after vascular injury; and they are involved in cell motility. As such, they are involved in diseases where the normal mechanisms of platelet aggregation go awry—as in heart attacks and strokes—and are implicated in cancer metastasis.

Not surprisingly, scientists have for years been interested in what integrins do, how they are involved in conditions like cancer, heart attacks, and stroke, and whether the mechanisms of integrin activation could be modulated to improve the prognosis of patients.

For instance, one of the molecules to which integrins bind is fibrinogen, a circulating dimeric protein that is present in large amounts in the blood and can bind integrins at both ends. This interaction is essential for mediating the aggregation of platelets—those flat, molecule-filled cytoplasmic disks in the blood.

Platelets are covered with integrins (typically 80,000 are on the surface of any given platelet). But the integrins need to be activated to bind fibrinogen. When they are not active, the platelets flow in the blood without sticking to each other or to blood vessel walls.

An injury will cause the integrins on the surface of platelets to become activated. The activated integrins then bind to fibrinogen, which then bind to other activated integrins on other platelets, cross-linking many platelets into a massive thrombus.

The body tightly controls this cascading reaction. Not enough thrombus formation could lead to massive blood loss, and too much could lead to a lethal, occlusive thrombus, causing a heart attack or stroke.

Understanding how integrins are activated, then, is a crucial question for scientists. Calderwood and his Department of Cell Biology colleagues, TSRI Professors Mark Ginsberg and Sanford Shattil investigated this topic thanks to support from the Program in Hemostasis and Thrombosis at the National Heart, Lung, and Blood Institute, one of the National Institutes of Health, and from the American Heart Association.

The Vital Step in Integrin Activation

How exactly the activation of integrins is controlled by the body has been an open question for several years, but in the last decade more and more evidence has pointed to the importance of the tiny tails of the integrins inside the cells.

How these small cytoplasmic domains activate integrins has been studied for some time. In fact, says Ginsberg, many—perhaps thousands—of scientific papers published on various steps in the pathway of integrin activation and molecules that perturb these steps.

What has not been known, until now, is the final step in this activation process.

The talin protein turns out to be key. Though the mechanism is not completely clear, Calderwood, Ginsberg, and their colleagues have evidence that shows when talin binds to the beta subunit of integrin, it causes a conformational change in the integrin, which is propagated across the membrane, changing the structures of the integrin domains on the outside.

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Talin

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"This is the vital step," says Calderwood. "Talin binds to the cytoplasmic tail, and that passes a signal that changes the large extracellular domain."

This work started a few years ago after Ginsberg's group discovered a salt bridge between the alpha and beta tails of the integrin. Salt bridges are favorable interactions formed by two oppositely-charged ionized groups within a protein, and Ginsberg knew that this salt bridge probably had a stabilizing effect on the interface between the two subunits—locking them in place, so to speak.

Ginsberg demonstrated that if he mutated the amino acid residues that formed this salt bridge disrupting these contacts, the integrins became activated.

This led him to speculate that under normal conditions, some sort of association between the inner tails of the alpha and beta subunits of the integrin held the protein in an inactive position.

"Whatever was activating [the integrins]," says Ginsberg, "was doing it by pulling the tails apart."

Around the same time, Calderwood arrived at TSRI as a postdoctoral fellow in the Ginsberg lab. Calderwood and Ginsberg began asking what cellular proteins might be disrupting the two tails of the integrin subunits, and they soon focused on talin.

Talin is a large intracellular protein more than 2,000 amino acids long and a major cytoskeleton protein on the inside of cells. Most of the talin protein binds to actin—the filamentous cellular protein that makes up the cytoskeleton and gives a cell its shape.

But Calderwood and Ginsberg discovered a small domain on the amino-terminus end of talin that binds to the beta-subunit tail of the integrin.

A Fruitful Collaboration

This week's report in *Science* shows that talin, indeed, is essential to integrin activation. The report is the result of a fruitful collaboration between Calderwood, Ginsberg and several other scientists at TSRI and The Burnham Institute.

TSRI Professor Sanford Shattil and his former TSRI postdoctoral fellow Seiji Tadakoro contributed their expertise with a technique called RNA interference.

RNA interference involves delivering small, 20- to 30-base pieces of double-stranded RNA into a cell. Once inside the cell, these short sequences anneal to complementary regions of cellular RNA and trigger an intracellular response that specifically destroys the target RNA. The technique allows scientists to selectively shut off normal cellular genes and permits them to study the impact of the absence of the corresponding gene products on cellular function.

The team used RNA interference to remove the talin from a type of cell called a megakaryocyte, a precursor of platelets, which TSRI postdoctoral fellow Koji Eto derived from embryonic stem cells. These megakaryocytes have the same machinery as platelets and respond to certain stimuli the same way that platelets do.

One of these stimuli is the chemical adenosine 5' diphosphate (ADP). When platelets are exposed to ADP, they become activated and the integrins on their surface switch from low to high affinity. The same is true of the megakaryocytes.

However, Calderwood and his colleagues showed that when the talin was removed from the megakaryocytes by RNA interference, the ADP no longer worked.

"It could not activate the integrins," says Calderwood, adding that they were able to rescue the activation by adding talin back into the cells from which it had been removed.

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Talin

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"This is a great example of a [scientific] collaboration," says Shattil. "It provided the critical evidence that talin was required for integrin activation."

The TSRI scientists also collaborated with Robert C. Liddington and Jose M. de Pereda of The Burnham Institute, with whom they had previously solved the crystal structure of talin bound to the cytoplasmic domain of integrin. This structure enabled Liddington and de Pereda to suggest places to mutate the talin and the beta subunit of the integrin to selectively disrupt the interaction between the two proteins.

"When [TSRI Research Assistant] Vera Tai introduced those mutations into full-length integrins, those integrins are inactive," says Calderwood. In the paper, the team also points out that overexpressing talin normally activates integrins. Overexpressing the mutant form of talin has no effect.

Further Questions

The importance of this discovery is enhanced by the fact that talin binds to almost all of the various tails of the beta subunits of integrins (eight of which are known).

The next step for Calderwood, Ginsberg, Shattil and their colleagues is to ask how the cell controls talin binding.

Figuring out these mechanisms is particularly interesting from a therapeutic point of view, since integrins are involved in such major killers as heart disease and cancer. Because talin binding is the final step in integrin activation, it might be a good target for keeping the integrins from becoming active.

"It's theoretically possible to perturb this interaction pharmaceutically," says Calderwood.

In Brief

Pilot Project: Point-of-Information Screen

If you have been in the Beckman Building lately, you may have noticed a new feature on the ground floor between the elevators. A point-of-information screen has been installed on a trial basis as an additional source of information about campus events, primarily scientific lectures and seminars. For more information, contact the Office of Communications, x4-8133.

Why Did Research Computing Cross the Road?

Research Computing is bringing its antivirus seminar "across the road" on Monday, October 6 at noon in CarrB/CIMBio Room 107. The seminar "Antivirus and You—The First Line of Defense" will help you become more familiar with the McAfee Antivirus software, and teach you how to check for the latest virus definition files, download new definition files, and maintain safe computing habits.

All TSRI employees can download McAfee VirusScan from the Research Computing web site. TSRI employees are authorized to install and use McAfee VirusScan on any campus computer AND on any home computer they own while employed at TSRI.

Good News about Health Care Flexible Spending Accounts

If you participate in the Health Care Spending Account or if you are thinking about enrolling during Open Enrollment in November, Benefits Administration has good news to share! The IRS has recently ruled that over-the-counter drugs can be paid for with pre-tax dollars through a health care flexible spending account. The ruling applies to medicines and drugs purchased by an employee without a physician's prescription when used for medical care. These include allergy and cold medications, pain relievers, and antacids. Vitamins and other dietary supplements, including weight loss medication, that are simply beneficial to general health are not reimbursable. Other non-reimbursable items include band-aids, gauze and toothpaste. The ruling is retroactive to January 1, 2003, so receipts for any over-the-counter medications purchased in 2003 can be submitted to Barney and Barney for reimbursement with a completed request for reimbursement form. For more information, contact Barney and Barney at (858) 587-7437 or Benefits Administration at x4-8487 or benefits@scripps.edu.

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