CSUN Department of Biology

BIOSPHERE

The Weekly Bulletin of Biology

No Biology Colloquium — Black Friday

No Classes Thursday–Saturday

Thanksgiving and recovery.

RSVP for Great Symposium

Biology Careers Outside of Academia Symposium

December 2nd
Panel: 12:00-2:00
Meet & Greet: 2:00-3:00
Location:Tujunga room, USU, East Conference Center

Love ecology, evolution, or marine biology? Want to learn more about interesting science careers that you can pursue with a B.S., M.S., or Ph.D. in biology? Speakers from various sectors (governmental, non-profit, consulting, science writing and education) will talk about the pros and cons of their careers, how they acquired their position, what qualities they look for in potential applicants, etc.

RSVP by Facebook or email for **FREE LUNCH**!

email: csun.wis@gmail.com

Facebook event page: goo.gl/Iylauw

Hosted by CSUN Women in Science Club

New Publication

Marine and Freshwater Research has published a paper by Peter Petraitis and Dr.**Steve Dudgeon**: "Cusps and butterflies: multiple stable states in marine systems as catastrophes."

Welcome our New Microbiologist

Dr. **Cristian Ruiz Rueda** joined our permanent faculty this fall. *Biosphere* sat down to find out more about him and his research.

Biosphere: Welcome to CSUN. Tell us where you're from.

Ruiz Rueda: I'm from Barcelona. That's where I grew up, went to college, and got my Ph.D.

B: When did you learn Spanish?

R R: My parents are Spanish, so I learned it as a baby. I learned Catalan back then too. I learned English at 11. Now they start at three, so kids grow up without an accent. I learned some English from songs.

B: In college, were there things you had to read in English?

R R: Yes, toward the end. You read papers in English. The conferences are in English. I chose to write my dissertation in English. Part of my research was done at the University of Rome, so I could apply for a European Union Ph.D. The best thing was moving here; now it's English 24/7.

B: What were the memorable things from your dissertation?

R R: I worked with microbial lipases. We were cloning and characterizing neo-enzymes. As an undergrad, I made a gene library from a strain isolated from

a rice field. Then I also had a big project in which I isolated about 800 strains and tested for activity at degrading lipids.

B: Like the break down of lipids in oil spills?

R R: Lipases are used in organic chemistry. They are really good for detergents and lots of other applications, like recycling paper or making ethanol from plant wastes. Lipases are also used in bread making.

B: Oh, I see. What does characterizing a lipase involve?

R R: You purify the enzyme. Then optimize pH and temperature. You study stability and how it behaves with different inhibitors. You do a biochemical characterization and then see if you have the features you want.

B: You use the pure lipase rather than just using the organism?

R R: No, you take the gene and put it in *E. coli* or something and engineer the bacteria to make a lot of it. That was the first part of my thesis: cloning genes and characterizing the enzymes biochemically. Then from the collaboration with Rome, we started working on inhibitors.

B: What kind of inhibitors? Like things that are made by cells?

R R: I was in a pharmacology department, so they were using inhibitors for treatment. There's one inhibitor called orlistat that inhibits lipases and is used by people who, for instance, are trying to lose weight. We studied how the inhibitors work. We were looking at natural compounds found in plants. Certain plant secondary metabolites inhibit this or that enzyme.

B: And were there particular lipases that you worked on?

R R: For a while we were mainly studying a lipase from *Candida*. Many of the inhibitors that work in *Candida* lipase also work in bacterial lipase. They are not homologous, but they are functionally similar biochemically. Later we worked on lipases from pathogens, like the one that causes acne, *Propionibacterium*. That bacterium breaks down the oils in your skin and causes inflammation. And I was also working on *Helicobacter*, the bacterium involved in ulcers. So then we had these two lipases from pathogens, and we could try out the inhibitors that had worked with other lipases. One flavonoid in particular we found worked. So that was pretty fun. I had the opportunity to live in Rome. I went three times, a total of nine months. I really love history and art, so that was like living in a candy store.

B: And then what happened?

R R: I moved to Boston, to Tufts Medical School, to the lab of Stuart Levy. He's one of the pioneers of studying antibiotic resistance. They were the first ones to find a gene that caused multiple resistances, a regulator of an efflux pump. When the regulator was mutated, the pump was over-expressed, and the bacteria were resistant to many things. They discovered the tetracycline efflux pump.

B: What's efflux?

R R: These pumps are in the membrane of the cell and they kick out toxins that get into the cell before they kill the cell. The efflux pump removes the tetracycline.

B: Antibiotic resistance, that's not just an ivory-tower interest.

R R: Right. When antibiotics were first used, they were miracle drugs, but bacteria continue to evolve more and more resistance, so now they increasingly fail. The Levy lab also discovered the mechanism of action of triclosan, which is an antibiotic that people put in soaps. That's is a terrible idea. The more antibiotics are used, the more selection for bacteria that are resistant to antibiotics. Then those bacteria transfer their resistances to pathogens. There's more and more bacteria that are basically resistant to everything we have. Also, 80% of antibiotics are used on livestock. That's a huge selective pressure for antibiotic resistance. Diseases that we used to treat in a week are now killing thousands of people every year.

B: So anyway, what were you doing in this lab?

R R: I was working on regulation of the antibiotic-resistance genes involved in multi-drug resistance. Regulation is much more complex than was once thought. You have a key regulator that regulates the gene for the pump, and you have multiple other genes that regulate the regulator, and those respond to stimulants and inhibitors in the environment. The bacteria can respond to nutrient availability. They can respond to changes in pH. They can respond to the presence of the antibiotic or other toxic compounds. The efflux pump itself turns out to be an autoregulator. So, for example, when I deleted one of the genes for the efflux pump, I saw that the promoter for the operan was overexpressed. It senses that there's not enough pump, and it tries to make more. I started to study the metabolism behind that, and I found that there are at least three different genetic pathways that the promoter uses to sense when the pump is not working. When you block one of these pathways, the metabolites accumulate and give you expression of the pump. I could pinpoint the metabolites. It's a very interesting system, how everything is integrated. Everything that the bacterium does is coordinated.

B: Is that work continuing?

R R: Yes, that's one of the main areas I'm going to try to pursue is to have a comprehensive understanding of all the regulatory pathways involved in this phenomenon. If we can find metabolites that inhibit the pump, then we can use that in therapy.

B: What bacteria are these in?

R R: I'm studying *E. coli*, which has more than 20 pumps, and there's a main pump that we're trying to understand. The part that I'm most interested in is how this pump is regulated by the cell's own metabolites. It's kind of like an excretory system. That's something very interesting to know.

B: That's the work you're coming back to now, that you got involved in at Tuft's.

R R: Right. Since then, for the last two years, I've been at Cornell Medical School in New York. There, I was using my knowledge of antibiotic mechanisms and inhibitors to develop new compounds. The idea was to develop new fluoroquinolones, which are effective antimicrobials, but more and more resistance has evolved. They target gyrase, the enzyme that

uncoils DNA. The fluoroquinolones have binding sites that are close enough so that they can interact and make a new compound inside the cell. So the idea is you give the fluoroquinolone as a monomer and it crosses the membrane, and then you have it dimerize itself. This gives it a larger binding area and much more affinity, so it is rare for bacterial mutations to arise that confer resistance to the dimer. In addition, the dimerized fluoroquinolones cannot be pumped out of the cell, so that mechanism of resistance is made ineffective. It won't be able to be effluxed out.

B: And what was it that you were developing?

R R: All we had to do was add a little tail to the fluoroquinolones so that they would dimerize inside the bacterium.

B: How is that working out?

R R: Well, we got some results, but not as good as we wanted. So, I started working on a new method for detecting cancer mutations. Here, the idea was to develop a method for detecting bits of DNA that are in the blood from the cancer cells. Most cancers can be cured if you catch them very early on. It's a screening method.

B: It sounds like finding a needle in a haystack.

R R: Yes, exactly. But we developed a three-step method that allows us to detect one mutant copy in 5000 or even 10,000 wildtype copies. And we are filing a patent. It's going to be a 20-marker approach, and if you detect all of those, then you go in for a biopsy or colonoscopy or whatever to confirm. The markers are like an early detection method. That project involved a lot of genetics and molecular biology. That method could be applied to many other things—detecting pathogens or even strokes. So that was what I was doing at Cornell Medical, until I came here.

B: What is the first thing you want to do at CSUN?

R R: The first big thing is to study the metabolites that regulate the pump. I have the preliminary data that I published in my last paper from my time in Boston. I'd like to thoroughly understand that whole phenomenon.

B: That ought to keep you busy, but you said "first" so what else?

R R: Another project that several professors at CSUN are joining together on is to look at the evolution of antibiotic resistance in bacteria in food. We are submitting a joint grant proposal to the USDA.

B: Well, that's about it. Is there anything you want to add?

R R: Well, I'm always eager to hear from students who are motivated and who are interested in what we do, anyone interested in work on antibiotic resistance.

Biosphere: The Weekly Bulletin of Biology

Department of Biology
California State University, Northridge
Editors: Paul Wilson and Robert Espinoza
BACK ISSUES | **JOBS, ETC.** | **GIVE TO BIOLOGY**