October 2012 Issue No.1

QBiC QBiTs

NEWSLETTER OF RIKEN Quantitative Biology Center

To strengthen the QBiC community, the Science Communication Office is launching a quarterly newsletter, QBiC QBiTs. The newsletter is intended to a) announce and describe QBiC research, b) inform about activities and awards, and c) share interesting stories that you perhaps did not know about the people you see everyday. The format is still in its experimental stages, and we are always welcoming suggestions. We also very much welcome contributions (in English or Japanese), so if you have any interesting stories you want to share, please contact us.

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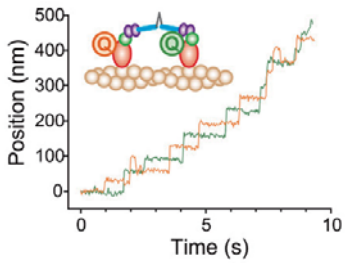
Berkeley University Assistant Professor and molecular motor researcher

Ahmet Yildiz



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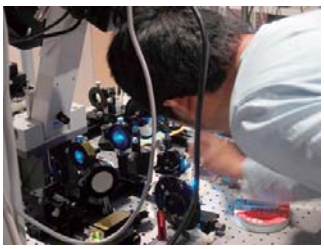

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Talking With...

Ahmet Yildiz, Assistant Professor at Berkeley University and creator of FIONA, a now ubiquitous algorithm in single molecule imaging, came to QBiC to talk about his research and move to the United States

Why the University of Illinois for his doctorate?

Our university was originally established by Americans. Robert College in Istanbul. It had American culture. Most of the graduates were going to America for Ph.D. A friend of mine [Burak Okmus] asked me to visit Urbana-Champaign. I met with a lot of faculty like Taekjip Ha, Paul Selvin and others. Was fascinated that you could use physical methods to study biological systems. I had no idea. The beauty of the single molecule data. Then Paul Selvin offered me a job and I took it.

> *"My mom didn't want me to be a scientist. There were no scientists in my family or in my town."*

The influence of his parents on his education

My mom didn't want me to be a scientist. There were no scientists in my family or in my town. I was raised in the countryside [Sakarya]. I came to Istanbul for high school. It was a special high school for science and engineering and I decided to become a physicist." *And why not make your mom happy?* "Self-esteem. I was pretty sure this [physics] was what I want.

His reasons for leaving Turkey

Yes I had to leave Turkey to do this. Preeminent Turkish schools don't even hire their own graduates for academic jobs. Most of our professors had American or English Ph.D. And in my time Turkey was a second-class research country.

I was actually excited at the time, because I really wanted to see different countries. I wanted to see where the science is really done. Of course, there are many options for that. I also had a lot of friends at the United States. I have more Turkish friends in the United States than in Turkey.

The development of FIONA

FIONA is a particle tracking problem. If you have anything that is moving inside a cell, FIONA will allow you to get better resolution. If you want to get detailed information and understand the mechanics, FIONA is very useful for those applications.

The idea was Paul's. I was a first year graduate student. Until that moment there was a lot of indirect evidence [on how molecular motors walk], but the field was craving for direct evidence. I accepted it because it was a risky project. I was looking for high risk/high gain projects. I have that attitude. The theory of FIONA is very simple. The biggest challenge in FIONA is having highly bright and stable fluorophores. The images Taekjip was getting were really good. So then I rotated in Taekjip's lab, learned the techniques and duplicated their microscope. We basically collaborated with Takejip who was very important in the FIONA development. Paul's lab had never done single molecules before. We didn't know how to do high-resolution microscopy. I rotated in multiple labs and got expertise from multiple people. Once everything was setup, it took us two weeks to get the experiment done. Theory and building the equipment and testing the equipment took about six months. In my Ph.D. including taking courses, exams…everything was done in two years and ten months.

His time with Ron Vale at UCSF for his post-doctorate

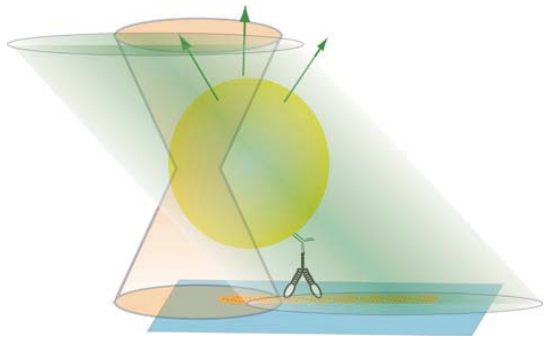
FIONA was done. I wanted to apply it to other systems. I basically wanted to learn two different things. First, I wanted to learn optical traps. But I also wanted to learn molecular biology because until that moment we always used someone else's motor protein. Ron was also starting dynein research at that time and I was actually quite excited about it. I was thinking about building my lab on dynein in the future.

Dynein was a really hot protein and a lot of work had not been done yet.

How his research expanded to include DNA Telomeres

That evolved pretty much out of nowhere. I also wanted to branch out of motors. Do more complicated and more directly health-related projects. I went to a lot of conferences out of my expertise like Cell Biology. There is also not much biophysics done with telomeres. When biochemistry and cell biology is more or less well established, that's when you want to start biophysics.

Our initial hypothesis failed, so we changed directions. It's actually very important to be flexible. Sometimes a project doesn't go anywhere or your hypothesis is wrong, then you really have to sit down and read a lot of literature, think really deep and try to understand what are the important questions and think how to make a direct contribution to that.



> *"I am much more interested in biology. I'm not trying to come up with new physical laws."*

His transition from physics to biophysics; scientist to professor

Biophysics is very loosely defined. Biology is the main part. Experimental biophysicists want to make big contributions to biological systems. Can you put anything new on the table? Now I am much more interested in biology. I'm not trying to come up with new physical laws. I am trying to use my physical expertise to make contributions to biology.

I am starting my fifth year. 10 people. Weird thing about academy, in your most productive time – the end of your post-doc – you have to sit down in your office and polish your grant. My most productive time I have to sit down do job applications, interview for one year, write grants, teach lectures, chase graduate students and recruit them. I had that transition. There is another level of transition where you are actually turning from a scientist to a manager. I haven't had that yet.

First two years was a painful transition. But now I do enjoy it. When I was a post-doc I could only follow one project. Now there are 10 things going on in parallel. Gets me much more excited. And I learn much more.

Single molecule imaging studies

The Yanagida lab recently published two reports that give insight on how biomolecular motor machines function inside a cell. Fujita et al. report in *Nature Communications* that the impact of noise on the work done by a motor is far more significant than originally thought. By labeling the heads of myosin V dimers with an elastic DNA-handle and high-resolution optical imaging, the authors show that myosin V actually switch between a deterministic and stochastic mechanism in accordance to the intracellular environment, a property that may explain how bio-machines are so readily adaptable to the capricious environment of a cell. At very high loads, the stochastic mechanism domniates, conributing nearly 80% of the work done. In another report now available in *Small*, Ikezaki et al. describe how myosin VI has the exceptional property of acting as both a transporter and an anchor. By labeling the myosin heads with quantum dots (QD) of different emission spectra, the authors show that the two heads can attach to an actin filament at one of two distances from each other, leading to adjacent- or distant-binding states. While the distant-binding state is common among transporters, the adjacent-binding state is unique to myosin VI and therefore may prove intrinsic to the VI anchor function. They find that the physical configuration of the two heads is the same during the adjacent-binding state, which makes any external load felt by the myosin molecule equally distributed on the two heads and therefore optimizes it for anchoring.

Paper Highlight

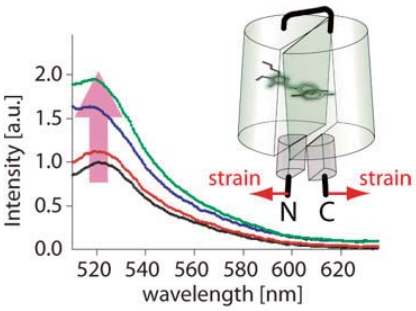
The Laboratory for Comprehensive BioImaging describes its new strain-sensitive nano-probe

May it be moving an object from one location to another inside a cell, or providing the rigidity that prevents a cell from imploding, intracellular molecules must remain robust to strain when conducting their function. Therefore, the strain-function relationship is very important when understanding the fundamental properties of a number of molecules. While techniques for measuring molecular responses to applied strain are well established, methods for when strain is unknown, however, are comparatively lacking.

Recently published work in *Chemical Communications* from the Tomonobu Watanabe lab that describes the design and properties of a strain-sensitive probe which may overcome this problem. The paper, first-authored by Taro Ichimura, presents a yellow fluorescent protein (YFP) probe that can be used to detect the strain felt by motor molecules in their intracellular environment. YFP is a G(reen)FP derivative that differs by a T203Y mutation. The result is a stacking effect of phenol rings that shifts the fluorescence spectrum. The group speculated that this stacking effect could offer a potential way to measure stress.

The team used circular permutation (cp) on the YFP to divide it and then added a spring between the two resulting ends. The theory was that cpYFP would changes its structure in response to strain, changes that could be seen in its fluorescence spectrum. A very important part of this strategy was the selection of the spring. The team "randomly tried 20 to 30 springs" before settling on a β-hairpin peptide.

After confirming the chemical properties of their cpYFPβ1 probe, the group tested it on kinesin molecules. By attaching the probe between the motor domain of a kinesin molecule and a second molecule (BDTC), the group showed that cpYFPβ1 had different fluorescence at different concentrations of microtubules and ATP, which they associated with the cross-bridge cycle for kinesin and therefore different strains sensed by the motor protein.



> *"I didn't think [the paper] would be accepted. But then it was"*

Typical of the Watanabe lab after a breakthrough, the group has moved on to other projects, leaving the application of their cpYFPβ1 probe to others. Now, the group is investigating probes that can be used to measure molecular responses to other physical properties of the intracellular environment like pressure and pH.

Tomonobu feels that someone interested in the biological results of the paper would be better to refer to the Supplementary Materials. Because Chemical Communications is a chemistry journal, its readers are more interested in the chemistry of a report, in this case the manufacturing and properties of his cpYFPβ1. So then, why did he choose that journal in the first place? "I didn't think [the paper] would be accepted, but I wanted to hear the opinions of chemists. But then it was accepted. I didn't expect that".

More work from the Watanabe group

In another report published in *PLOS ONE*, the Watanabe team describes a new high-resolution microscopy system that competes with other superresolution methods, but has the added bonus of being much simpler and cheaper to use. The Distinct Modulated Pupil Function System (DiMPS) does not depend on any complicated scanning hardware, making it no bigger than a shoebox. More importantly, the temporal resolution of the system is determined by the CCD camera rate, and therefore can reach as low as 2 ms. By incorporating subtraction imaging to DiMPS (siDiMPS), they could enhance their resolution even more, as demonstrated with crisp structural images of actin, E-cadherin, and autophagosomes.

Watanabe hopes DiMPS will appeal to researchers with limited budgets and knowledge for microscopy, but who nevertheless want to incorporate imaging into their work. "It is very good for surveying the specimen, and therefore may have application to the clinical setting"

Meet the QBiC Lab...

Cell-Free Protein Synthesis

Yoshihiro Shimizu

The lab's research focus is on the protein synthesis system in cells. Protein synthesis is fundamental to cell development and survival, and requires innumerous molecules that coordinate. We have previously constructed a reconstituted cell-free protein synthesis system known as the PURE system and are now working on the following topics:

1) The study of protein synthesis:
The PURE system offers a simple basis for complex systems, and can therefore be used as a foundation for frameworks and models. It can also be a tool for protein synthesis.

2) Development of protein biochemistry:
The PURE system makes it relatively easy to synthesize and purify proteins. Current work involves the incorporattion of gene synthesis and microfluidics technology into the PURE system.

3) Construction of a system for cell-free protein synthesis that can self-replicate:
This project aims to bulid artificial cells by beginning with a minimal set of biological macromolecules and perturbing their organizaton.

https://sites.google.com/site/labcfps/home/

Briefs

Probes...

In a collaborative effort with Osaka University's Yoshichika Yoshioka and Qilin University's (China) Qiang Ma, the Jin lab reports in *Biomaterials* a new probe that can be used for MRI imaging, but offers the sensitivity of quantum dots (QD). While doing a 10-month stay at QBiC as a JSPS fellow, Qiang synthesized an MQQ probe – a probe that consists of a magnetic nano-probe (M) and two QD (QQ). Each of the three probes serves a specific purpose. The most outer layer consists of a near infrared (NIR) QD, which minimizes absorbance and interference by the specimen and therefore provides a strong signal. The other QD emits at the red wavelength, and while serves no purpose for diagnostic imaging is necessary to evaluate whether the MQQ probe is potentially toxic to the cell. Finally, the third probe, found in the most inner layer, is the magnetic nano-probe (MNP) and gives the MQQ probe its magnetic properties for MRI. The authors show that their MQQ probe could identify the location of cancer cells inside a mouse and at the same time offer detailed MR imaging of blood vessels feeding the tumor.

...and more probes

Another report from the Jin lab that made the cover of *Analytical Methods* describes a new quantum dot (QD) that can be used to measure the viscosity inside a cell. By coating spherically shaped QD with bovine serum albumin (BSA), the group could measure the free diffusion of their BSA-QD by fluorescence correlation spectroscopy and thus evaluate the viscosity of the intracellular environment. First author Yuko Nakane explains that the spherical shape is key, because properties like viscosity depend not only on the size of the molecule, but also its shape. "Manufacturing and testing different sized spherical QD means we can develop a table that relates viscosity and size". Such a table could help with simulations and other predictive methods examining intracellular behavior. While others have reported viscosity probes, their irregular shapes means a similar comparison cannot be made.

Awards

Chikara Furusawa was nationally recognized twice for his research in the span of weeks. The Japanese Biophysical Society declared the submisson of work done by him and his colleagues as the Outsanding Biophysics Paper this past September, while different research got him accolades from the Japan Bioindustry Association at BioJapan 2012 in October.

Equal Work for Less Pay

Beginning this October all Riken employees including those at QBiC will see their salaries drop in accordance with government policy that had been in the works since the start of the year. The savings are ostensibly to be used for the reconstruction of the Tohoku area following last year's disaster. After many months of negotiations, Riken agreed on final numbers that vary with the title of employment. The policy is currently planned to be in effect for the next 24 months.

Interesting People

Johannes Frohnmayer, the Olympics, and QBiC

Despite his soft smile and timid demeanor, Johannes Frohnmayer has a physical presence that cannot be ignored. It is not surprising then to learn that he has his 2nd dan in judo and has been practicing the martial art for 20 years. Nor is it surprising when the Olympics roll around, as they did this year, more than swimming or gymnastics, the Olympic sport that most captures his interest is judo. This time, though, the London games had an even more special appeal. While living in Munich for his studies, Johannes befriended two fellow judoka, Christopher Völk and Tobias Eugelmeier. The three, along with many others, grappled regularly at TSV Großhaden Münich, a dojo that has graduated many of Germany's Olympic judo team members.

"I think both came to the World Championships in Japan in 2010", said Johannes. It was shortly after Johannes arrived at QBiC when it was announced that Christopher and Tobias would be joining Germany's Judo Team in London.

Because judo is not one of the Olympic's most popular events, matches are normally around noon local time, which made them a prime time event in Japan this year. Alas, no matches included Tobias or Christopher. Tobias lost his first match, and Christopher was out by his second.

It would be almost too obvious to conclude that judo had sparked Johannes' interest in Japan and his one-year exchange at QBiC from Munich University. It would also be wrong. "I was always interested in Japan, but that interest did not come through judo". Unexpectedly, it grew at a museum for anthropology in his hometown of Stuttgart, Germany, that had an extraordinarily rich exhibit of Japanese art due to the donations of Erwin von Baelz, a physician who worked in Japan during the end of the 19th century. It was this exhibit that spawned Johannes' interest in Asian culture and a joy for kung-fu movies. "13 Assassins. That one is really good". In turn, these kung-fu movies stoked his interest in judo. "I decided I wanted to do the martial arts and had a long discussion with my mother because she thought it was too violent". Although "football is the big sport" in Germany, judo is the most popular martial art there. Johannes began at age 7 and continued at Stuttgart until he went to Edinburgh, Scotland for his military service. Germany still had military conscription at the time, but conscientious objectors could alternatively do civil service. Johannes decided to fulfill his military requirement by working at a home for the handicapped and, of course, continue his judo.

He then matriculated at Munich University to study physics. His senior-year project dealt with protein folding, but was mostly in silico. Johannes began his masters with the hope of doing experimental biophysics and ideally intertwining it with his interests in Japan. A professor at Munich suggested QBiC Director Prof. Yanagida, which led him to join Masahiro Ueda's lab to study the spontaneous waves of PTEN and PI3K and how this spontaneous behavior can lead to proper cellular function like cell motility. Johannes' work involves searching for a yet unproven binding partner that complexes with PTEN

> *"I was always interested in Japan, but that interest did not come through judo"*

Johannes returns to Munich this autumn pondering what to do now that his masters is nearing completion - continue to the Ph.D. or leave for industry? One thing that is not in doubt, however, will be his judo, which his time here has taught him is different between the two countries. "The Japanese are much more flexible; the Germans more strong. From an aesthetic point of view, Japanese judo is probably more beautiful."

Journal Editing

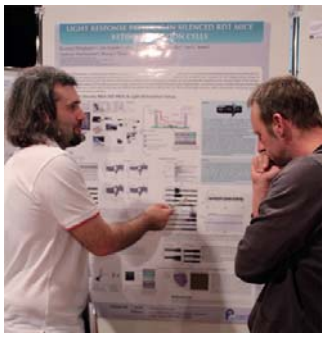
Renato Zenobi, Professor of Chemistry at ETH Zurich, recently visited QBiC to discuss his experience as an Associate Editor at Analytical Chemistry.

"Nobody does it for the money" explains Prof. Zenobi. Instead, the joy of being an editor comes from seeing the newest and best in the field. "It's an interesting job. You see the forefront…it's the best journal in the field, so you see a lot of interesting stuff". At Analytical Chemistry, Zenobi estimates that no less than 80% of submissions reach the associate editors after being considered by the Editor-in-Chief. In theory, Zenobi is supposed to be an expert of the submissions that reach his desk, but he admits, "occasionally I get an oddball and know nothing about it, but that happens to everybody". Also, because he is receiving 300-400 papers a year – an average of one a day – he concedes that seeing the name of an established researcher as the author makes it more likely the paper will be reviewed. However, he adds that does not mean these papers are more likely to be favorably reviewed. "That does not mean there is always good stuff from them, but they have a higher chance of being reviewed". While this may sound unfair, Zenobi notes that new authors will also be given generous consideration. "We give them usually a running chance more often than not". Regarding how to impress the reviewers, because of the large number of papers, Zenobi stresses that he will have an opinion of a paper by looking only at the abstract and figures. As for the cover letter, he deems these as "unimportant". "My own cover letters are two lines" he jokes. Of course, he acknowledges this is not true for other journals like Science and Nature. In the end, for those seeking tips on how to increase their chances of being published, Zenobi offers a simple hint, "send us the best stuff".

Science trekking in Europe

This past summer, Kosmas Deligkaris of the Frey Team parlayed his research results into a three-week sojourn to Germany and Switzerland.

A productive collaboration with the Bio Engineering Lab (BEL) at ETH Zurich got Kosmas to Basel, Switzerland, the location of BEL, this past summer, where he talked to a number of people about new techniques for his experiments. Of special interest was the work done by Michele Fiscella, who had given a talk a week earlier at the 8th International Meeting on Substrate-Integrated Microelectrode Arrays in Reutlingen, Germany, which Kosmas had also attended. "Michele demonstrated step by step his retina protocol". Kosmas was also able to solve frustrating problems with his neural cultures. "Douglas Bakkum, having years of culturing experience, advised me to use the same batch of serum for the same batch of experiments which was really practical". BEL members have already come to Japan as part of an active collaboration, with more scheduled in the near future. With luck, Kosmas hopes to get back to Europe in the near future too.



After that, Kosmas returned to Germany to attend the summer school, Advanced Statistical Modeling of Neuronal Data, organized by the Institute of Cognitive Sciences at the University of Osnabrueck. The school focused on probabilistic models and stimulus encoding and decoding of neuronal populations. "I think advanced analysis methods is something missing from my research community. I want to bridge that gap". As a social event, scientists were split into teams and raced not by foot, but rather by raft. Kosmas showcased both his rowing and coxswain technique, the latter by counting up to ten in Japanese.

A Nobel Thought

Scientists have to constantly try to translate what they do into language that most people can understand.

Stanley B. Prusiner.
Winner of the 1997 Nobel Prize
in Physiology or Medicine
(see the Nobel website for the full interview)

Retreat 2012

The 2nd annual QBiC retreat was held this past July on Awaji Island. Because the institute is still awaiting its own building, researchers are currently divided in two locations more than an hour away, making the retreat a rare moment where all gather and discuss their work. The retreat included 120 participants and 6 presentation sessions including introductions by the Group Directors, 4 sessions divided by the strategies and techniques done at QBiC, and one by guest lecturer Tetsuya Yomo of Osaka University, whose talk addressed the question, What changes are needed so that an assembly of non-living organic matter gives life? Chikara Furusawa ended the retreat by providing a review of the sessions and moderating a discussion on the future direction of the organization.

The large screen, dimmed lights, and casually dressed audience at times gave more an impression of being at the cinema than a science meeting. Director Toshio Yanagida was given the stage for the first talk where he emphasized that while the researchers at QBiC may use very different techniques to study what may appear as only slightly related scientific problems, all share the common goal of creating ways to control self-organizing systems. This challenge was best described in a later talk by Mitsuhiro Iwaki, who quoted Johan van Neumann, "How do we constitute reliable systems from unreliable elements"?

The retreat was spread over three days and two nights, enough time to reflect on all the talks and posters. Indeed, the excellent weather, nearby sea, and a QBiC futsal match left very little complaints. Perhaps the biggest had to do with the excessive quantity of tasty food that caused many to overeat. Except for the desserts, which vanished far too quickly.

Newcomers at QBiC

Me: Osamu Akiyama
Lab: Team Furusawa
Hobbies: Movies, Art
Cheers:
Fukuoka Softbank Hawks

Me: Alexandra Dudina
Lab: Team Frey
Hobbies: Piano, Sports
Cheers: Searching for a favourite team

Me: Kazuya Nishimura
Lab: Team Taniguchi
Hobbies: Swimming
Cheers:
HANSHIN TIGERS

Me: Yasuko Takeuchi
Lab: Team Watanabe
Hobbies: Ballet, Farming
Cheers:
HANSHIN TIGERS

THE CHOW DOWN

A sweet and sour onion salad that complements many dishes

Ingredients

- 400 g of baby onions
  (about the length of your pinky finger)
- 1/4 cup wine vinegar or white wine
- 3 tbsp olive oil
- half a can of tomatos
- 2 tbsp white sugar
- 1/3 cup raisins (+1/3 cup dried apricot)
- parsley and pepper

Put all the ingredients into a pot. Add water until the onions are just submerged. Bring to a boil and then low heat for 45 minutes. The onions should be tender when done. Let cool and bon appetit.

Have a recipe you want to share, contact us at:
qbits@riken.jp

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