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QBiC

QBiTs

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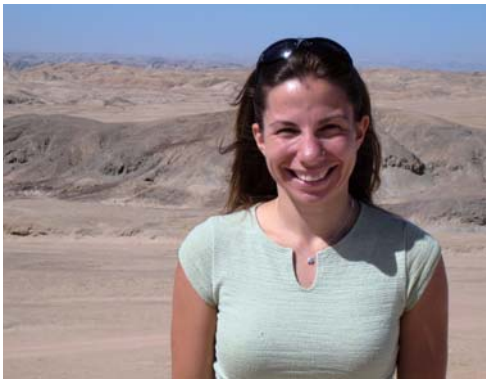
Talking With. . .

Margarita Marinova

SpaceX engineer and former NASA scientist



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New glass-based pumps for lab-on-chip technology

One key component of the research at QBiC is new technologies for systems biology. Yo Tanaka, a Unit Leader, has devoted himself to lab-on-chip devices, especially those made of glass. Glass is relatively inert compared with more common materials used in these chips, such as polymers. Much of the research done in the Tanaka lab has investigated the use of a flexible, ultra-thin glass sheet that is durable enough to be used as a pump. In his most recent work, published in *Micromachines*, Yo describes a glass-based peristaltic pump built entirely from commercially available products and assembled onto a 100% glass microchip.

To increase the pumping pressure, Yo designed the pump to have four serial valves that generate a circular flow. The flow rate was linearly proportional to the pumping frequency and was comparable with that seen in contemporary polymer-based pumps. A more universal measure of pump performance is the self-pumping frequency, which describes the ratio of the maximum flow rate at zero pressure delivery with the fluid volume of the pumping chambers or channels. His pump scored 0.6. Other peristaltic pumps have achieved this performance, but only when using an elastomer as the diaphragm material.

As a next stage, Yo is collaborating with other QBiC members to show the applicability of his all-glass microchip for biological study. One exciting promise is its feasibility with organic solvents, as he demonstrates in the paper they do not compromise the glass or leak from the chambers. These properties will allow for the separation and study of cells that require treatment with such solvents, along with expanding organic chemistry studies in general.

Talking with . . .

Margarita Marinova has always been fascinated by space, and career stops at NASA and SpaceX show it. She explains her unique career and how her space research contributes to our understanding of habitability and life in extreme conditions.


image from NASA

Very quickly, tell us a little about your education background and scientific interests.

I studied aerospace engineering as an undergraduate and did my Ph.D. in planetary science, specifically Mars surface processes and Mars geophysics. My research goal was to understand Mars better, as well as extreme environments on Earth. Extreme environments are especially interesting because we are making them go extinct. Our group was mostly focused on going [to these environments], putting the instruments out to understand how that environment behaved, modeling the processes and then applying the models to Mars.

This research seems very far removed from studying life. What is the connection?

On Earth, you have interactions of water and wind with the surface and that shapes everything we see. On Mars, that wind-surface interaction is even more important. It is pretty much the only interaction that has happened for billions of years. And that has an impact on life.

We use the Earth as an example of where life can and cannot live. So we [NASA] went to all sorts of extreme environments on Earth, looked at the physical environment, which was the part I was most involved with, and then we had biologists look at what kind of organisms live there, how they survive, how much they metabolize. From that we got a picture of what organisms could survive where. There are actually very few examples on Earth where life cannot survive. The Atacama Desert. Middle of Sahara. In both cases the limitation seems to be water. Liquid water. The Atacama Desert gets a lot of morning dew, but that is not enough to keep life going.

And what do the models suggest?

There is no way to confirm life did or did not exist on Mars without exploring the planet's surface. But at least these Earth-based studies give us a target. If life did not like extreme conditions on Earth, then it's very unlikely life was on Mars except maybe very early on when it was wetter. But given life can survive really dry, really cold, really hot, then it's easier to say here are some locations on Mars that had similar conditions for a long time – millions, maybe billions of years.

You mention very dry extreme environments. What about very wet ones, like the absolute depths of the oceans? Can these places teach us something interesting about life on Mars?

I have never done anything in the deep ocean. It's just something that hasn't come up in my research. The deep ocean is very interesting. We often think of them as isolated ecosystems, but most of them are not. Most of them feed off biological material coming from the surface of the ocean.

You have said before that you are excited especially about the prospect of finding life on Mars that is unrelated to life on Earth. How would you determine whether the lifeforms are similar?

The idea there may have been life on another planet and we can go find it is really exciting. If we find life that still has DNA and RNA, the alien life is probably related to us. If the life has genetic material but is nothing like DNA, things that look like cells but don't have the inner parts that Earth life has, then we are pretty sure they are of separate and unique origin.

There is a lot of work done now on how to find life. How do you know if that's life or not? One thing that comes up a lot is that Earth life is picky. If you search somewhere and see a spike in say a certain type of amino acid, you might be able to tell this molecule is not in equilibrium with its environment. Earth life for example is only interested in left-handed amino acids. Life is really good at keeping chemistry out of equilibrium.

NASA took you to some fascinating places. What was it like and why did you go?

Somebody had to put the instruments in, and I was usually the one. A lot of modelers never go to the places they are modeling. I think that is a huge detriment. In modeling you have to make certain assumptions. Unless you really see the place, it is difficult to know what assumptions are reasonable.

Our expedition length was highly variable. Antarctica is very difficult to go to for less than a month. Some of our trips to Namibia were 10 days. 10 days were the shortest. The lodgings were anything from tents to research stations. The food – sometimes we'd grill steaks and chicken every night, and sometimes it was cheese sandwiches for a week. One of the times in Antarctica, we were there for Thanksgiving, so we decided to cook a full Thanksgiving meal!

Yet you left NASA for SpaceX. Why?

The planetary science funding at NASA became very difficult, partly due to the economic downturn. At the same time funding for other faculty and researchers also became scarce so more people were applying for NASA funding, in addition to [NASA] cutting a lot of the planetary science funding, so it became very difficult. Funding rates on proposals were 15%, and are even lower now. You had to write many proposals to get funding for yourself. Certainly some people were lucky; I wasn't. It was especially difficult as a new researcher. It just seemed like the right time to try something else.

Going to SpaceX…I always wondered whether I wanted to go back to engineering. In science you study something and you think you understand how it works, but you're not really sure. In engineering, you think you understand it and you test it. Hey, if the rocket flies then the rocket flies. There is something nice about making things work. At NASA I was a scientist; at SpaceX I do engineering.

image from NASA

What can you say about SpaceX?

SpaceX is very exciting to me because they want to go to Mars. That was the biggest draw. I am a propulsion engineer at SpaceX, responsible for the operation of the stage and the commands required to set the movement of fluids in the reusable rocket F9R.

Doing planetary science at NASA Ames was – pick an interesting question and study it in great detail, then publish all your work. At SpaceX, it's very different, but I think that difference is driven by the difference between building things and doing research. To accomplish SpaceX's goals, we have to actually build and fly rockets. That certainly requires us to understand how the systems work, but the main goal is building a working system rather than the pure goal of understanding how something works, which is at the center of research. And with that, the work at SpaceX is really focused on the company's main goals of building rockets and from there colonizing Mars. In research, generally there is unlimited freedom to study any interesting question you want.

When Curiosity landed, I sometimes found the video of the NASA researchers cheering more captivating than the Mars surface. When large projects like these are a success, how do teams celebrate?

It is definitely very exciting when we have a successful test or launch, and we certainly celebrate. What I found interesting is that there is so much work after a test, to make sure all the systems are safe and the rocket is happy, that you really have to just focus on what's next for a while. People always think of just the rocket, and forget all the other ground systems required to make it all work. So hours after the actual launch the success really sinks in and you can go celebrate.

If you are invested in your work, you have that moment of being really happy when something works. The reason for choosing these jobs – actually all the jobs I have had - is because I am really excited about space exploration and going to other planets. That has been my motivation. I remember the first time I put a weather station in Antarctica. You put it in and come back a year later hoping there is data there. That was very exciting for me, because I was so invested. Definitely F9R is very exciting, but when things work the personal happiness is very similar based on your personal investment in the project.

Whole Brain Imaging with Single Cell Resolution

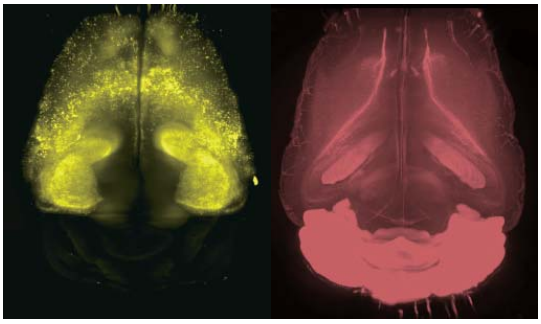
A simple method for clearing and transparency that gives exceptional resolution of the brain

A major challenge of systems biology is understanding how phenomena at the cellular level correlate with activity at the organism level. A concerted effort on this topic has been made especially in the brain, as scientists aim to clarify how neural activity is translated into consciousness and other complex brain activities. Accordingly, new technologies are needed, including whole-brain imaging with single-cell resolution. Normally, a highly transparent sample that minimizes light scattering is prepared and neurons tagged with fluorescent probes at different slices are imaged to produce a 3D representation. However, limitations in current methods prevent comprehensive study of the relationship between different biological levels. The Hiroki Ueda lab recently described a high-throughput method, CUBIC (Clear, Unobstructed Brain Imaging Cocktails and Computational Analysis), which offers unprecedented rapid whole-brain imaging with single cell resolution and a simple protocol to clear and transparentize the brain sample based on the use of aminoalcohols. The report is first authored by Etsuo Susaki and can be read in *Cell*.

Etsuo explains that the “aminoacids are the critical chemicals in the CUBIC reagents”. These were identified by a screening that considered 40 chemicals and resulted in the design of a simple tissue-clearing protocol that enhanced the transparency of the brain. The resulting protocol involves serially immersing fixed tissues into just two reagents for a relatively short time, which makes it simpler and therefore advantageous over other methods. Moreover, CUBIC is compatible with a large variety of fluorescent proteins, which makes it suitable for multi-color imaging, because the effective clearance minimizes fluorescent quenching. Such proteins include red fluorescent proteins, such as mCherry and mKate2, which offer better penetration depths for imaging. Indeed, two-photon microscopy could reach depths of 4 mm in mouse brain when using CUBIC.

The authors also showed that CUBIC, when combined with single-photon excitation microscopy, can achieve rapid whole-brain imaging of a number of mammalian systems, such as mouse and primate, which further demonstrates its scalability for brains of different size. Entire horizontal sections of mouse brain were acquired in a single plane with sub-cellular resolution. Making Z-stack images from these sections could then provide a 3D description of the spatial and temporal gene expression patterns in the hypothalamic circadian rhythm center, a region that is of high interest to the lab. Specifically, the paper compares wild type and Cry1-/-, Cry2-/- double knockout mice and shows that staining of the suprachiasmatic nucleus differed over circadian times between the two mouse types.

Overall, CUBIC provides information on previously unattainable 3D gene expression profiles and neural networks at the systems level. Because of its rapid and high-throughput imaging, CUBIC offers extraordinary opportunity to analyze the localized effects of genomic editing and is also expected to identify neural connections at the whole brain level. Hiroki Ueda is optimistic about further application to even larger mammalian systems. "In the near future, we would like to apply CUBIC technology to whole-body imaging at single cell resolution." ■



Noise in information

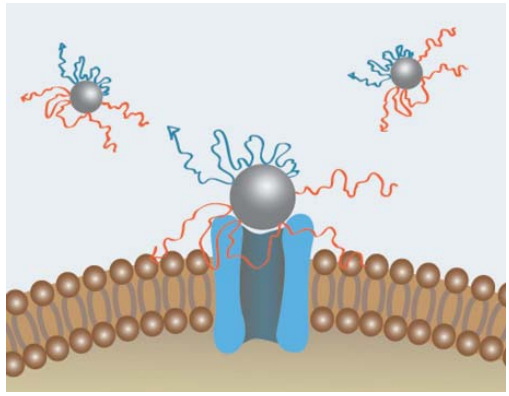
QBiC researchers show the limit at which a cell must operate with noise

For cells to survive, they must constantly adapt to an ever-changing environment. These changes are often recognized by signals that contact the cell membrane and trigger one or multiple signaling cascades that generate responses like growth, development and death. Cells can respond with remarkable reliability to infinitesimally small concentrations or concentration gradients of surrounding signals. It is assumed that the detector of these external signals is the membrane receptor. Occupancy of the receptor acts like the eyes of a cell and provides an estimate of the environment. As effective as this measure is, there must exist some limit in the detection capability. Understanding this limit provides insight on how much the cell response is based on actual information and how much depends on probabilities and stochastic behaviour, an important question when considering the design of sensors for synthetic biology systems.

Nearly 40 years ago, Berg and Purcell defined this limit by considering the occupation state of a receptor that interacts with a specific ligand at a fixed concentration. They showed the relationship between the concentration measured by the receptor and the flux of the ligand, which includes a factor for the binding kinetics. Their work assumed the movement of the signaling molecule is limited by diffusion. They argued, however, that the same conclusion can be extrapolated to conditions where that is not the case, such as when receptor-ligand associations are predominantly reaction-limited. Bialek and Setayeshgar, in a much more recent paper, make a different conclusion. Using the fluctuation-dissipation theorem, they find that the noise limit is independent of the binding kinetics.

This disagreement motivated Kazunari Kaizu and Koichi Takahashi to collaborate with theoretical biologists at AMOLF, the Netherlands, to investigate the matter further. They examined the sensitivity of a single receptor when assuming diffusion-influenced reactions. They decoupled the sensitivity into two components: one that describes the effect of diffusive transport on the ligand to and from the receptor and one that describes the effect of the intrinsic binding and unbinding kinetics of the receptor. Interestingly, while the paper, which can be seen in *Biophysical Journal*, shows the second component to be identical to that reported by Bialek and Setayshgar, it shows the first component to be the same as that published by Berg and Purcell. Because it is the first that represents the fundamental limit to accurately detecting external chemical concentrations, the paper concludes the original work by Berg and Purcell is the more appropriate description.

This conclusion depends on one essential assumption: each time a ligand dissociates from the receptor, it and the receptor are surrounded by a uniform distribution of other signaling molecules. This condition reduces the complexity of the problem significantly, as otherwise the study would have to solve a multi-body problem of more than two. This condition is reasonable, however, if the dissociation rate constant is low and allows the first of a series of equations to be solved that leads to the final result.



A new technique for genomic editing

Researchers show the promise of methylamine for DNA concatenation

Genetic recombination has become a fundamental tool for molecular biology. Although often restriction enzymes are used, non-enzymatic approaches are becoming increasingly popular because they are cheaper and more robust to pH and ionic concentrations. However, at the same time, they also have a propensity for mutations and DNA degradation.

The Hiroki Ueda lab has reported a novel non-enzymatic cleavage reaction, Quantitative Base-Induced DNA Cleavage (QBIC), which allows for DNA concatenation and minimizes the aforementioned drawbacks. In a paper seen in *PLOS One* and first authored by Shuji Ikeda, who has since joined Adaptmer Solutions in Singapore, the group explains how DNA oligonucleotides containing 5-ethynluracil (5EU) can have DNA cleavage induced when in methylamine aqueous solution. The authors for the most part come from a chemistry background, which they used to test and confirm that the cleavage reaction is triggered by methylamine making a nucleophilic attack. Because the cleavage only requires the addition and removal of methylamine, the procedure is considered far simpler than other contemporary ones.

The team examined a number of conditions for optimizing the reaction, including different temperatures and times, and different primary amine solvents. Importantly, although higher temperatures resulted in faster reactions, QBIC was successful at room temperature. Additionally, all the tested solvents showed successful cleavage, although none as effectively as methylamine.

As demonstration of their technique, concatenation was done using PCR-amplified fragments. The reaction led to sticky ends, which enabled control of the order of the concatenation. Following the cleavage, the methylamine was removed and the fragments were heated and cooled, resulting in stable concatenated forms. Moreover, despite observing this same pattern in over a dozen plasmids, the use of 5EU did not cause any mutations.

Overall, the authors are optimistic that the simplicity of QBIC will make available a number of DNA recombinant sequences not accessible before. ■

Meet the QBiC Lab . . .

The Takahashi Lab

We use in silico modeling and simulations of cellular dynamics to investigate cell heterogeneity. A large amount of our attention is on molecular crowding and its effects on cellular function. Most standard models do not sufficiently consider molecular crowding, despite the effects proteins, macromolecules, and other intracellular components can have on a given molecule or network of molecules. The foundation of our work is E-Cell, a software platform for integrative cell modeling and simulations, which has led to new and faster reaction-diffusion methods, such as enhanced Green's Function Reaction Dynamics and Spatiocyte. We also have easy access to the largest supercomputer in Japan on which we do a number of our studies. Furthermore, we often collaborate with other QBiC groups, which provides opportunities for our group members to do wet experiments along with their computational work.

Overall, we aim to comprehensively describe all the reactions in large-scale cellular networks. We believe that these details will explain cell heterogeneity, which is a primary goal at QBiC and also a key requirement for targeted therapies and medicines. Our most ambitious project is a complete model of the *E. coli* cell, which will include the entire genome (about 4700 genes) and a whole cell-scale metabolic network to simulate the entire cell cycle. ■

Interesting People

Every wine is like a snowflake

Like many students, university matriculation was the first time Yosuke Kawai lived on his own. His hope was to both stay close to home in Ehime and enroll in a strong pharmacy program. The result was him joining Hiroshima University, which was still 4 hours away by car. A more interesting and shorter trip he occassionally took was by ferry, but even that required a 30 minute bike ride to the port, then two hours on the boat, and finally another 30 minutes on the bike again.

It was at the university he met then faculty member Tsutomu Masujima. Yosuke chose the Masujima lab for his 4th year project even though Tsutomu explained that in order to continue the research, Yosuke would have to move to Osaka, since Tsutomu would be taking his lab to QBiC. Yosuke had no reservations and became a graduate student at Osaka University.

The appeal of Tsutomu's lab was live single cell mass spectroscopy (LSCMS). "I wanted to do something based on theory and apply it to biological systems". His original project involved investigating the resting B lymphocyte (RBL). Yosuke was responsible for identifying quantitative differences between dormant and stimulated RBL by using the spectrums produced from LSCMS. Classes interrupted his research during his first year of graduate studies. Now in his second year, he had planned to resume his experiments. However, Tsutomu decided to change the project. Rather than looking at biological systems, Tsutomu wanted to demonstrate the sensitivity of his technique by having Yosuke use LSCMS to measure wine with the hopes of identifying the key molecules that give each type its distinctive flavour. In other words, Tsutomu hopes to reveal the fingerprint of a wine by LSCMS. Yosuke was not sure why Tsutomu proposed the wine study. "I don't really drink". Tsutomu countered, "I thought it would be helpful to get a job, especially in food companies".

Currently, Yosuke is looking at whether samples of only pL volume are sufficient for producing informative spectra. The small sample size makes the Masujima lab one of the most popular at QBiC, because after opening the bottles there is little else they can do but share the remaining wine with other institute members. Moreover, the sample size is much less than what is normally needed for biological samples, like the plant cells and organelles commonly studied by the lab, which minimizes contaminants and simplifies handling of the sample.

Another reason wine makes an excellent model is because of its ionic properties. LSCMS depends on a nanospray ionization technique. The ethanol in wine, and any alcoholic beverage for that matter, is very compatible. In contrast, water is much more difficult to ionize and less applicable. Once the wine study is complete, Yosuke plans to expand his studies to other drinks that have age-minimum requirements. Although he spoke as though he has uncertain what would be the next model, when given a list that ranged from beer to brandies, he abruptly pushed it aside and tersely answered, "sake".

Getting In Shape

From their childhood, many Japanese are inculcated to Radio Taisou, which literally translates to "radio calisthenics". It is a custom that lasts a lifetime, as people of all ages and at all places can be seen doing Radio Taisou to begin their day. Typically, Radio Taisou is about 10 minutes of simple stretches and movements and is not intended for those who want a 6-pack. QBiC staff began Radio Taisou nearly two years ago, as each morning over half a dozen staff gather around the television. No time for the gym? We don't accept that excuse here!

Annual Retreat

The 4th QBiC retreat took place this past June 23-25 in Wakayama, the first time on the Kii peninsula. Each year the retreat offers QBiC members the best opportunity for collaboration, bringing together the labs from Osaka, Kobe and Yokohama for three days and two nights. The retreat has traditionally been divided into sessions designed to stimulate collaborations and new strategies to scientific problems. This year, however, to emphasize the scientific accomplishments at QBiC since its founding, researchers were invited to give an overview of their progress. Most of the speakers were young researchers late in their doctorate work or 1st post-doctorate. Additionally, two guest speakers were invited to provide their vision of quantitative biology and how it applies to modeling of the cell: Kaoru Amano, who investigates vision awareness at CiNet in Osaka using psychophysics and imaging techniques; and Jay Shin, who is developing single cell screening methods for the identification of the key factors that enable cell reprogramming at the Riken Center for Life Science Technologies.Finally, Hideki Ukai from the Hiroki Ueda lab and Akihito Komatsuzaki from the Jin lab were awarded best posters for their works on genome engineering and non-invasive in vivo imaging, respectively.

Artificial Intelligence Workshops

On June 2nd, QBiC co-sponsored the 4th Brain Architecture Study Workshops, bringing together over 100 neuroscientists, cognitive scientists, and engineers to discuss artificial general intelligence, a term that describes artificial intelligence that can surpass the human brain in any capacity including conscience, i.e. the moment of singularity. Singularity was once limited to science fiction stories, but is now being anticipated within a generation if not less. Although already reaching its 4th, the first workshop took place just last year. This one in Osaka was the first outside of Tokyo. It was also the longest, with six speakers who were each given the stage for 30 min. The final speaker was QBiC's Makoto Taiji, who presented brain computing, and QBiC's Koichi Takahashi was co-organizer of the event.

THE CHOW DOWN
Lasagna

Bolognese Sauce

1 onion
1 carrot
400 g mince meat
200 mL red wine
1/2 a stalk of celery
1 can of tomatoes
dash of nutmeg
bay leaf

Chop the onion and carrot and simmer on medium heat with half a table spoon of olive oil for 3-5 minutes. Add the meat and wine for 10 minutes, then add the remaining ingredients and continue to cook for 30 minutes.

Bechamel Sauce

60 g butter
40 g flour
500 mL milk
50 mL cream
dash of salt and pepper

Melt the butter on low heat and then mix in the flour. Once well mixed, add the milk in 4 parts, stirring thoroughly. Add the cream and the salt and pepper.

Lasagna

Butter a square glass dish. Place a layer of lasagna followed by the Bolognese sauce and Bechamel. Continue this pattern until the last layer of lasagna. Once the last layer is down, sprinkle parmesan cheese on top. Cook for 15 min at 200 °C.

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