Discussion

Shoji Takeuchi

Q: How do you insert the protein into the artificial membrane?
A: The biologist who we collaborate with purifies or prepares the lipid vesicle with membrane proteins. These vesicles can be easily fused to the lipid bilayer in micro-chambers. (Of course, preparation of vesicle with membrane protein is difficult).

Q: How small can you go down the size of micro-chamber?
A: We can create micro-chamber of 1um in diameter. Other researchers can create micro-chamber of 1nm in diameter by using electron beam lithography.

Q: Can you create the compartment (ex. ER or nucleus) within the cell?
A: Not yet, but we have some idea to use two kind of bilayer to create small vesicle within the large vesicle.

Q: What is the remaining challenge in micro-confinement?
A: In static micro-chamber, it will be to maintain micro-chamber for long days like 3-4 days because PDMS that we usually use absorbs the water during a long time. In dynamic micro-confinement, it will be to divide the droplet into the two.

Cristina Smolke.

Q: In your logic example, you used temperature and molecular input. Is it possible to use the aptamers to do a kind of combinatory logic?
A: Yes, it should be. Instead of using the thermo sensor switch, we are trying to use two switches in combinations. In addition, there was a recent paper published in Science from Breaker's laboratory, which characterized a natural ribo-switch system which took two different inputs in bacteria.

Q: How do you control the expression of aptamers in cells?
A: It is possible to control the strength and timing of aptamer expression by changing its promoter. But you have to deeply understand the nature of the promoters.

Q: When you create the binding domain, the aptamer obviously perturbed the structure pretty strongly to attach it to the computational element. Is it possible to design the strong aptamer to couple it to the rest of the structure?
A: It is possible because there are some modularity in three elements (computation, sensor, and actuator). You can design it using the information of binding affinity of aptamer.

Q: How can you design RNA structure precisely?
A: We only use folding program based on secondary structure. But we checked the functionality of them by comprehensive experiments and elucidate the important parameters for design.

Q: How about scalability of the logic?
A: It depends on how you implement the logic.
In cells, you have to control the relative levels of small non-coding RNA and target RNA, which is very difficult in cells. We cannot do this yet because we are limited by the promoter system. In vitro, you can control the relative amount of these molecules, and you can realize high-order logic.

Q: How about time constant?
A: Our system responds immediately and instantaneously.