Discussion

Masahiro Takagi

Q: Are there examples showing that thermostability plays a role in the pathogenesis of some diseases?
A: Thermostability is very important in converting the aggregation-prone.

Q: Are there any reports showing the view that in some proteins that may change, thermostability is related to humans or something?
A: I don't know about the relationship between thermostability and point mutation. But point mutation will create more chances to form fibrils. There are so many reports, genetic disease, in those cases.

Q: You talked about how you got the ion pairs and C-value protein. Have you also tried introduction ion pairs?
A: That's the next part we are designing. To maintain structure is very important, but the enzymes are always flexible. It is a very difficult to deal with flexibility and stability.

Q: Do you think that oral administration of this polyamine can inhibit aggregation in the human body?
A: Those studies are now ongoing, but those chemicals are not so toxic. We inject them into mice.
Q: When you are looking at your proteins' aggregation, and you are desorbing them with polyamines, is that a charged effort? Are you disturbing the ion pairs between the protein's aggregates?
A: Yes.

Q: You look at protein aggregation versus protein-protein interaction. Do you disturb protein-protein interactions?
A: Yes. To minimize the chance of protein-protein interaction is one recent method to prevent aggregation formation.

Atsushi Miyawaki

Q: Do fluorescent proteins have very similar structures?
A: Those chromophores are the same, but the amino acid sequences are very different.

Q: I have a question about the color change across molecules. Is color generation reversible in the opposite direction for transfer?

A: It is actually irreversible, because the peptide chains change.

Q: You followed the inversion process that you observed. First of all, have you ever observed further conversion to the infrared?
A: No, we haven't.

Q: Can you comment at all about this process? This is a crazy question, but maybe this is a new form of optical sources, for either sensing or communication.
A: Yes. We are thinking about making white ones. And many times we see memory by using this convertible protein as a medium.

Q: I would like to know about some of the frontiers of in-place systems. For example, is there any trial applied for in vivo, for example, transgenic mice? Also, in addition to the brain and sensory events, accumulating evidence showing that the chromo-modification is involved in the expression? I wonder if histo-modification might be monitored by your system.
A: We have made transgenic animals producing this kind of protein. Ideally, if we avoid using cell-type-specific promoters, and then we wish to see some migration of cells, electrolytical cells, at specific times, and maybe angiogenesis. Now we are now struggling with how to obtain specific signals very efficiently from the animals.

Q: Have you ever tried to put your reporter protein in stem cells to look at the differentiation of process solve?
A: Actually, we have done it. The relationship between plurality and the differentiational stage is very interesting.

Q: What is the advantage of your fluorescent proteins compared to quantum dots?
A: We can make a transgenic animal and influence any organ. And we can target.

Q: Are there any colors that cannot be realized in such molecules right now?
A: It's unpredictable, because the protein is about 30 kD. And it's quite large compared with conventional small chemical contrast, chromophors. As I told you, it is surrounded by many side chains of the amino acid.

Forest M. White

Q: You mentioned that phosphorylated proteins are about 30 percent of proteins. In your method, what percentage of phosphorylated proteins were rejected?
A: We get rid of all of our non-phosphorylated peptides. So we no longer have information about the ratio of phosphorylated to non-phosphorylated peptides. All we have is the intensity of the phosphorylated peptides.

Q: What are the advantages and disadvantages of your method compared with other methods?
A: One of the methods that people have used is to treat the cell with a phosphor paste, for

example, and dephosphorylate all of the proteins, and then run it in a 2-D gel, and see which of them shows this change. The only issue with doing that is that you have to be able to go back to identify those proteins so that becomes an issue of mass spectroscopy and dynamic range in the gel. The dynamic range in the gel is a big limiting factor, and that's something that we are doing a little bit better on, compared with the gel technique.

Q: How many cells are required?
A: Right now we are down to about 100,000 cells. We've got it a little bit lower. I've tried it at 10,000 cells. The problem with going with a lower numbers of cells now is that our sensitivity is not good enough to go deeper.

Q: In the case of vascular carcinoma cells, do we need to calculate that?
A: In that case, we had plenty of samples. We were using something like 1/100 of the clinical tissue to do our analysis. So we were actually able to reproduce those data a couple of times and still had a lot of samples left over. A lot of it depends on the tumor. If we are looking at breast tumors, for example, it's a lot more heterogeneous. And then you have to do the same experiments for normal tissue, in which case the cell number becomes limited because people have to laser capture or use microdissection or something to purify it.

Q: How in this mixture of proteins do you keep things from dephosphorylating?
A: There is no kinase or phosphorylation activity because we kill the enzyme activities in the process of preparation of the peptide samples.

Q: What is the time resolution?
A: We are taking a snapshot. Maybe we could do 15 seconds.

Q: Are you finding real patterns or motif sequences?
A: Yes. We started collaboration. Basically, we are going to use my data to try to go in and try to figure out new motifs, as well as try to pull out which kinases might have done these phosphorylation events in the cell. It's a pretty hot topic right now because we have all these data, and what we are trying to do is something like that

Q: Have you ever compared protein expression data with the messenger RNA expression? Do you have any data on that?
A: Yes. That's one of the big questions that we are getting into. Other people have done similar analyses. The specific group is T. Fiji and Ruby Andersol. They published a paper in *Proceedings of the National Academy of Sciences* on that subject. And they saw a relatively poor correlation between the heaps of data on the mRNA expression level and the protein expression level.

Q: I'm struck by the outcome. The message system is, and I kind of like to think of nature as, finding the direct, simple solution.
A: That's a really good point, because a lot of research out there now (none of it published by my lab yet!), has started looking at these different growth factors coming into the cells, and a lot of them view similar signal transduction cascades. But the phenotype is different. In other words, if you treat with external growth factors or platelet-derived growth factors

or insulin, they all use the same protein inside the cells, but the signaling ends up in different places. And trying to figure out exactly that complicated signaling network is really difficult.

Q: Is the whole network complicated?
A: I think there is a good reason for that because there has to be a lot of fine tuning and control of what is happening in the cell. In other words, the cell has this apoptosis thing, this suicide signal. And it's actually fairly easy to put these cells in a suicide pattern. There has to be really tight regulation because the cell has to sense itself and figure out when it's doing something wrong and try to kill itself before it kills the entire organism. And that kind of fine tuning controls the signaling network means that it has to be somewhat complex.

Cynthia L. Stokes

Q: I'm interested in understanding in this interpretive process that you have at what levels of the modeling you do the checking, because it seems to me that by the time you get to the clinical stage it becomes rather qualitative.
A: That's a very good question. And the answer is, it depends. We do have to change for which things we need a qualitative batch gram versus those for which we need a quantitative one. And "Where's the impossible?"

The thing I would say is that there is less good qualitative data to which to compare things against in the inflammatory diseases than in metabolic diseases. So in our metabolism platform, for obesity and type-2 diabetes, they have hundreds of very carefully controlled quantitative experiments measuring all kinds things, all glucose tolerance tested, all kinds of manipulations with patients, different types, fasting, non-fasting, etc. And not only people's weight but their insulin, their glucose, the fatty-acid levels.

Q: How do you use published nonreproducible data in your model? In clinical research usually the data is from a few patients. The model is very limited, which I understand, because reproducible data from many different patients is needed. So we have very limited information about this. I respect and love the modeling. But do you think that we are supposed to make a standardized bioassay first and then get enough reproducible data that we can model, instead of making a model and then trying to fit into some published data? Any comment on this?
A: Often times it's amazing how behaviors of the models don't depend on the exact values of many various individual parameters, so you get a very similar response out of them. And I think that would have to be, because we have to put up with so many different inputs: "Did we eat today?" "Did we exercise today?" "Did we sleep enough?" "What is our pollution like?" So we have to be pretty robust against changes in our environment and even in our genomes and still be able to function.

But the other thing we can do, and lead to a problem like that PDE4 problem, is that we can say, "We know that there is uncertainty over here, because there are two inconsistent pieces of data, or however many. These four are answers to PDE4, but do those two things make a difference?" If they don't, then don't worry about them. If they did, then

go to parameters that you feel are going to be able to reconcile the differences and make you confident in what those values are worth. It tells you, "Look at this one, but don't worry about these other ones."

At the clinical level, again, as in my answer to a previous question, depending on the disease you are looking at, you need very good quantitative data or very good quality of data or, as you mentioned, in some diseases very little data at all.

Q: I think we need to look at what we mean by "model," because there seems to be a difference between, pre-fitting or matching existing data, and predicting a behavior based on knowledge of how certain chemicals actually interact with cells and express a result. Would you comment on that?
A: Sure. I'll try to give it a different direction. I think you are asking with respect to the model, as opposed to all possible modeling. We have a view toward what we need to be able to reproduce. And for some of those data we do use them to constrain places we don't know very well. If you have the parameters and are creating something, you can fit anything. It would be easy to model and reproduce one set of data. So if all I had to do was reproduce that asthma attack for untreated patients, it would be easy. We have to figure out multiple ways we can make it happen.

Q: Can you cure my dog with this method? I guess that the identification of the hypothetical function might be very important in your exact method. How accurately can you identify the hypothetical function?
A: So your first question was can I cure your dog?

Q: Can you use a dog as a pilot for this method?
A: Oh, you could have virtual bodies, and a virtual human. What you could do is adjust for the known differences between humans and the dog, which could be a lot, so that might be actually the problem.

Q: So that you can monitor the behavior with the dog.
A: Yes, that's exactly true. You can monitor the effects in the dogs. So theoretically, yes. I haven't done it.

Q: If there were two candidates of hypothesized function, which can you choose?
A: So you've got an area and we need to hypothesize its functions, and how do we know it's this one versus that one? You can put one in the model, put the other in the model, and run simulations and see if one of them gives a better representation of the whole system. If one of them makes it work, and the other completely just doesn't work at all, then clearly one of them is more reasonable than the other. It's possible they might both give similar system behaviors, in which case we actually would keep both of them in the model, turn one or the other off, depending what you wanted to look at, until you find the data that would help us choose one over the other.

Q: So sort of error minimization.
A: Essentially yes.

Q: To establish the position of the capability right now of this model, I think that on one end we have the view that it is possible to do systematic and comprehensive analysis of a problem, similar to the previous proteomic approaches. The other extreme will be to have a predictive capability.

A: Let me state something, not just for the asthma model but the different models. I think it's somewhere in between, and more towards one end than the other, depending on the model that day we have been able to use. We can do the first. That's not a problem. And then how far you can get to doing really rock-solid predictions is another matter.