Designing membrane-like surfaces and virus detectors

Scientists at Biofilms – Research Centre for Biointerfaces at Malmö University – have developed so called rSRAMs, molecule-thin films that mimic the behaviour of living cell membranes.

Text **BOEL JÖNSSON**

Börje Sellergren and Sing Yee Yeung in the lab.

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professor of biomedical science

Self-Assembled Monolayers (SAMs) are thin layers, 2-5 nanometres in thickness, in which all molecules are oriented in a specific direction. Sing Yee Yeung, PhD student in professor Börje Sellergren's group, has managed to produce reversible versions of such layers. A simple adjustment of the pH level can make the layers detach from a functionalised surface of gold or glass.

The layers have been given the name rSAMs, reversible SAMs.

At an alkaline pH level, the surface is negatively charged and the rSAM molecules positively charged. In an acidic low pH solution, the surface will lose its charge, and the rSAM layer will detach from it. The surface can be reused many times following alkaline/acid treatment.

SAM is a well-established technology for biosensors (e.g. Biacore), a building block within nanotechnology, molecular electronics, and as a model system for building highly-controlled surfaces, and for copying biological membranes. It is one of the main areas within supramolecular chemistry.

DESCRIBING HIMSELF AS an "all-round" chemist, Börje Sellergren, professor of biomedical science, stumbled into the area around 20 years ago when he was developing a receptor for an antiviral drug (HIV), pentamidine.

"We studied gold surfaces coated with thiol molecules, the concept that has become most widely adopted."

Thiols are suitable as, in gentle conditions, they will spontaneously bind to the gold surface, forming semi-covalent bonds that are partly reversible.

"Our new system, rSAM, is reversible on a much more practical timescale. You can stabilise/destabilise the system very quickly by altering the pH."

In this context, quickly means in less than a minute. The limiting factors are the stirring method used in the system and the mass transport.

"We use so called bolaamphiphilic molecules, characterised by having hydrophilic groups at both ends of a hydrophilic chain of molecules. In our case, one of the groups is an amidine. The surface to be coated can be made of gold or glass, but must also be functionalised with an oxoacid, for instance, a carboxylic acid or a sulfonic acid. These two are what we have studied so far. The oxoacid develops a hydrogen-bonded ion-pair with the amidine, stabilising the rSAM layer."

"DEPENDING ON THE PKA VALUE of the oxoacid, we are able to regulate at what pH the surfaces will detach. However, that is not the central point of our new work. Instead, we show that these layers display similarities to how a flu virus meets its host cell – like a living cell membrane."

When the virus meets its host cell, it has to find the correct "handle" on the cell's surface, i.e. the right sugar molecules. In the case of a flu virus this would be sialic acids present on the membrane. These are bonded in various ways that distinguish human cells from animal cells, and that protect us from certain viruses. Lectins (hemagglutinin) on the surface of the virus grip hold of the sialic acids, attaching to them. Then, the virus can enter the cell.

"We have used the same sugar molecules in an rSAM. When the virus is binding, we get a dynamic system that mimics the membrane with lateral diffusion. This is what makes our system unique."

The rSAM layer acts as a bridge between the virus and the gold surface. The bridge is dynamic and its molecules can move parallel to the surface. This makes it easier for the virus to attach and develop many points of contact that together form a strong, multivalent bond.

"Currently, our system is not suitable for identifying different types of viruses. Others have done that using covalent chemistry. However, our system can detect far lower concentrations of viruses. Time will tell how far we will be able to combine that with a method for type classification."

GLYCOBIOLOGY IS A HOT TOPIC. The cell's surface is covered by sugar molecules that determine its identity; how it moves and interacts with viruses, antibodies or other cells. ▶

The results from the rSAM project are available in the article "Reversible Self-Assembled Monolayers (rSAM:s): Adaptable Surfaces for Enhanced Multivalent Interactions and Ultrasensitive Virus Detection", one of the 200 articles published annually in the journal ACS Central Science.

Pros and cons

▶ Börje Sellergren had enjoyed a long research career in Germany, from 1995 to 2012, when he was made an offer to join what is now Malmö University.

Any regrets about returning to Sweden?

"No, it has worked out really well, and here it is much easier to set up exchanges and contacts, including unexpected ones. In Germany, everything is very formal and a bit rigid. However, there is a lot of money in the German system, and it is easy to secure all-year-round funding from the research council. Also, there is no limit to the number of grants you can have, which is very good if you have many ideas."

"Unfortunately, the Swedish research council didn't want to cooperate with their German counterpart and transfer the grants I had there to support collaboration with German scientists. I found that very sad."

The proposed collaboration involved imprinting technology and plastic antibodies.

"Now we have started that within the EU's Marie Curie programme for PhD training. We are coordinating two such projects."

It is difficult to mimic the dynamics in the living membrane, which is one reason why it is hard to study glycobiology. It involves multivalent bonds, several sugar molecules simultaneously binding to, for example, a virus.

"That aspect is lost with covalent surfaces. One example is the tumour specific sugars that exist only on tumour cells. In that case, you want to find the correct way of blocking them, but in arrays it is easy to miss the multivalence," explains Börje Sellergren.

"We believe that our reversible, self-organising layers will be able to better mimic the cell membrane, and that they will be of interest within many areas where today SAMs are used. rSAM can better adapt to what is being measured and identified, offering a real opportunity for virus detection/recognition, which we have demonstrated in our recently published research.

ALTHOUGH THE RESEARCH HAS NOT included the technical part of the sensor system, Börje Sellergren believes it will be easy to use.

"You can work with the same surface all the time, covering it with different rSAMs."

"We are collaborating with a Canadian research group studying stem cells, and they are interested in using the technique to reversibly bind stem cells."

He believes that rSAM can also be used within tissue engineering. In addition, his group is initiating other work which indicates that the sensitivity of immunosensors in general can be improved.

HE DRAWS A COMPARISON with the molecular machines for which the Nobel Prize was awarded in 2016.

"However, we are considerably closer to realising applications, for instance, in glycoarrays where receptors are being studied – ligand interactions in order to mimic what happens in the cell membrane. This is an important technology for pharmaceutical development."

"Also, we have ideas that we wish to develop within nanotechnology, involving coating nanoparticles of gold with rSAMs and sugar ligands. Such particles are seen as potential antiviral drugs because they bind so effectively to viruses. They might be able to block the initial binding process between viruses and cells in the body."

"We have developed the chemistry around these layers, and now we know how it works. Especially Sing Yee Yeung, my latest PhD student, who has worked on this for four years. Her creativity and ambition have been decisive for the latest advances." KB