EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

	Experiment title: Reale time monitoring of structure variation of protein-containing layers and capsules	**Experiment number**: SC-3783
Beamline: ID10 (C09)	**Date of experiment**: from: 20/11/2013 to: 26/11/2013	**Date of report**:
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Report:

The functionalization of polyelectrolyte multilayers and microcapsules with specific proteins is of fundamental importance in order to modulate their properties and functionalities for biomedical applications, such as drug delivery.

The main aims of the project were the real time investigation of the structure variation of polyelectrolyte multilayers functionalized with two membrane proteins, bacteriorhodopsin (BR) and cytochrome ba3 oxidase (ba3), and the real time investigation of release of objects, with defined size and shape, from capsules with a shell composed from such layers.

In the first experiments, two different polyelectrolyte multilayers were studied. Specifically multilayers obtained by the deposition onto silicon slides of four bilayers of cationic poly (allylamine hydrochloride) (PAH) and anionic poly (styrene sulfonate) (PSS), and multilayers obtained by the deposition of cationic chitosan and anionic pectin were functionalized by the deposition of a final layer of BR and ba3. Before functionalization the multilayers were decorated by $CdCl_2$ treatment to increase electron density. The samples

were then placed in a teflon chamber, with kapton windows, and X-ray reflectivity measurements at the solid-liquid interface were started. In the case of BR functionalized multilayers, the experiments were carried out initially in dark, then the light was switched on and the reflectivity variation due to the activation of BR molecules was estimated. In the case of ba3 functionalized multilayers, the measurement was started then it was stopped and cytochrome c, which is the ba3 substrate, was added and the reflectivity variation was estimated. In this case, reflectivity curves from the multilayer covered by one last ba3 layer before and after treatment by cytochrome c are shown in Fig. 1 left and right, respectively. The experimental data revealed the increase of the multilayer roughness and whereas no thickness was detected indicating that the layer-by-layer structure was completely lost do to the reaction catalysed by ba3 in the presence of cytochrome c.

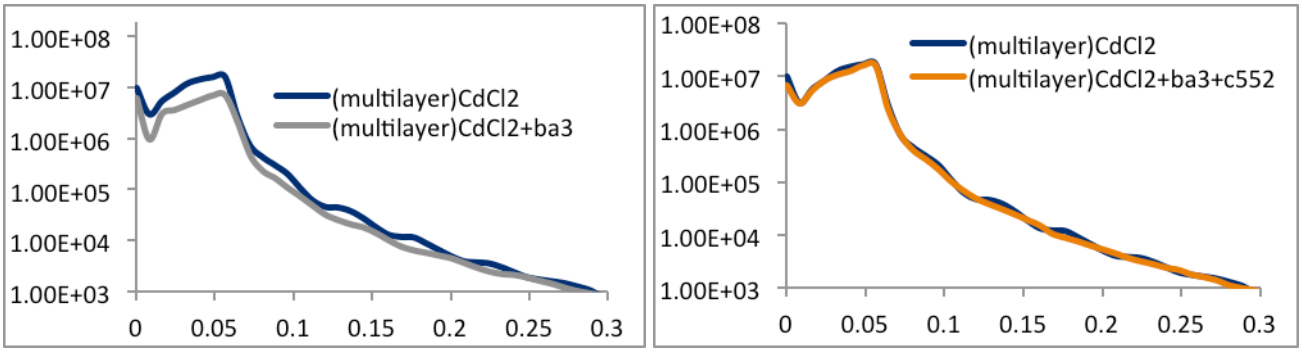

Fig. 1. X-ray reflectivity data Si/(PEI/PSS)/(PAH/PSS)/$(PAH/PSS)_{4CdCl2}$/ ba3 deposited onto silicon before (left) and after (right) interactions with cytochrome c.

In BR functionalized multilayers it was not possible to estimate the layer parameters with satisfectory accurance. Moreover, measurements, performed in the light and durk conditions revealed no difference, what can be due to two reasons: not complete dark conditions in the working chamber (even very weak light results in BR functioning, quantum efficiency is near 100%) or to the fact that proton transfer do not provide the layer reorganization.

The planar mulilayers, characterized by X-Ray reflectivity, were then deposited onto the surface of $CaCO_3$ microparticles followed by their removal in acidic environment to obtain hollow microcapsules. The microcapsules were then loaded with gold nanoparticles (d= 5 micron) and their real-time release under the action of ba3 was monitored by X-ray fluorescence measurements performed in a real time. The release of the particles was detected as an increase of Au fluorescence after the addition of cytochrome c.

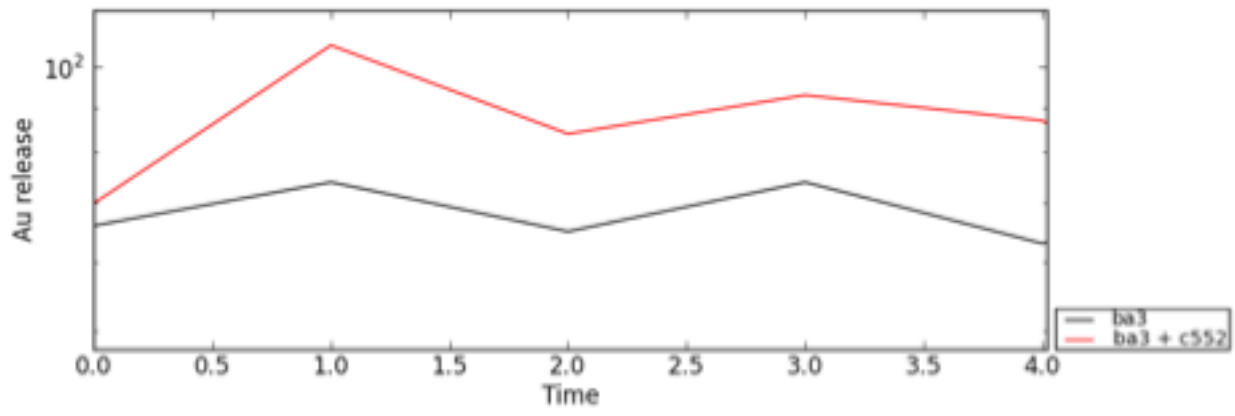

Fig. 2. Temporal [h] dependences of the intensities of Au fluorescence lines for capsules, loaded with 5 nm gold nanoparticles.

The results indicated that the reaction, catalysed by ba3, resulted in the rupture of the capsules and thus in the release of gold nanoparticles. This results confirmed our observation with planar samples that the action of ba3 strongly affected the multilayered structure resulting in its destruction.
In the case of BR we have obtained very interesting and, initially, unclear results that are shown in Fig. 3.

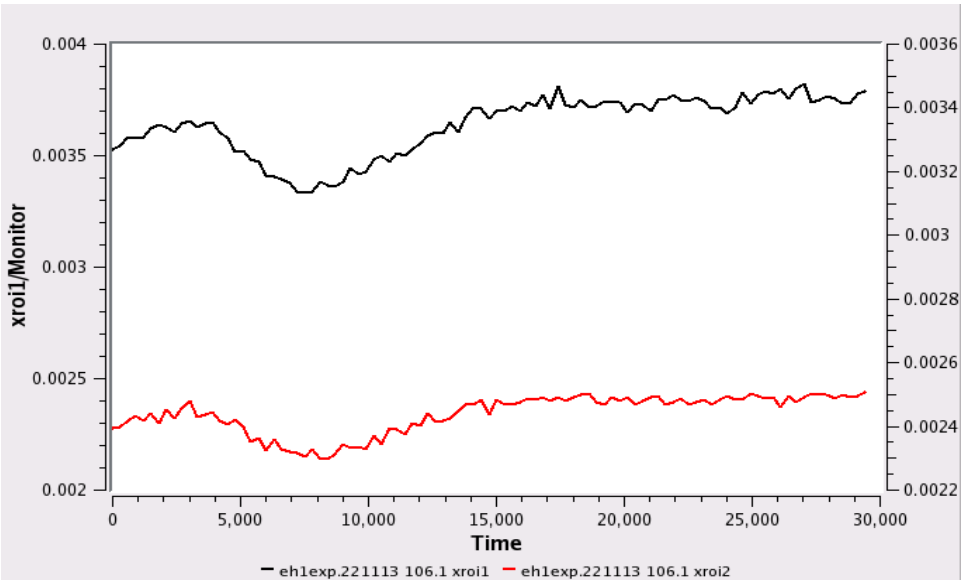

Fig. 3. Time dependence of the intensity of two Au fluorescence lines: light was switched on at the point, corresponding to 3000.

As it is clear from the figure, we see the increase of the Au fluorescence in the initial dark conditions. Then, when the light was switched on, we can see a decrease of the fluorescence, followed by its increase and saturation. Such behavior is absolutely different from the expected on: we expected the increase of the Au fluorescence due to the pore opening and release of the encapsulated gold nanoparticles to the solution, where the measurements were performed. However, according to our present understanding of the process, such behaviour has a rational explanation.
In order to understand it we must have in mind the following items. First, our stabilized gold nanoparticles are charged negatively. Second, BR was deposited in such a way, that the external part of the capsule had a net positive charge. Thus, the initial increase of the Au fluorescence intensity can be connected to the weak detachment of Au particles, mainly adsorbed to the capsule shell, in the presence of the electric field, facilitating their motion. In addition, we cannot exclude the presence of some holes in the shell due to the not compleate darckness of the experimental conditions.
Illumination of light resulted in two phenomena going in parallel: pumping of protons into the capsule core (increaseing, therefore, shells permeability) and charging negatively the external side of the shell. As these processes have very different time constants, the first effect must be due to the shell surface charging. Thus, we associate the decrease of the fluorescence to the blocking of the Au nanoparticle release possibility due to the electrostatic repulsion of the

negatively charged nanoparticles and negatively charged capsule shell. The fact that we see decrease of the fluorescence intensity instead of the its constant level is due to the fact, that the precipitation of nanoparticles and their adsorption to the walls of the working chamber always takes place, as it was direcly demonstrated in our previous experiments on the different types of capsules [1].
In parallel, pumping of protons into the core results in the significant decrease of the pH in this confined volume, accompanied by the pore formation. When the pores size are rather large, the release of Au nanoparticles, due to the concentration gradient, becomes a predominant process, resulting in the increase of the Au fluorescence intensity.
As this result is very interesting and absolutely not obvious, we are carrying out SEM and microanalysis experiments in order to confirm the finding.
Other part of X-ray fluorescence experiments was carried out on capsules, loaded with different substances. We have investigate capsules with Fe, Ti, and Au particles in the flow conditions. Typical experimental curve is shown in Fig. 4 for Au particles.

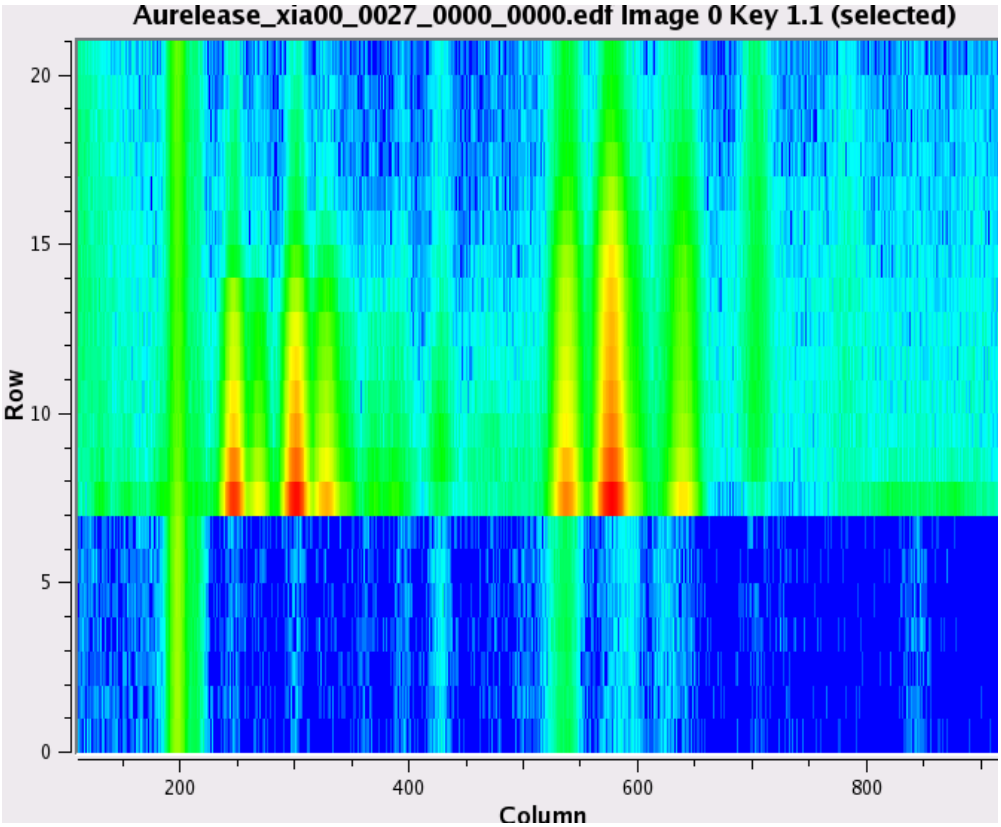

Fig. 4. Au particles release from polymeric capsules: horisontal line corresponds to the beginning of the peristaltic pump function.

Rational explanation of the obtained results demands additional independent experiments with other techniques (SEM, element mapping), that are in progress.