ESRF	**Experiment title:** Stuctural study of biocatalytic Langmuir-Blodgett assemblies	**Experiment number**: LS-1975
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Report:

Langmuir-Blodgett (LB) and related methods were used in numerous works to incorporate biologically active molecules into organized molecular films. Considerable efforts were applied to make sensing media for the developments of biosensors and laboratory bioanalytic techniques. However, up to now no approach was proposed for the development of biocatalysts based on LB films suitable for practical applications. The difficulty is obvious. One of the requirements of industrial biocatalysis is the immobilization of enzymes onto the carriers of very large effective surface. On the other hand, if such an approach is proposed, the application of the LB technique and combinations of the latter with the other methods of deposition may offer new possibilities for the production of biocatalysts with the improved efficiency and stability. In this experiment we characterized the X-ray reflectivity and GID from LB and LS layers of two particular model-proteins, penicillin G- acylase (PGA), an industrial enzyme, immobilized and protected by layers of polycyanoacrylate (PCA) and glutaraldehyde (GA), and Gluthathione-S-transferase (GST) as monocomponent multilayer and in superlattices comprising also a bilayer of fatty acid salt.

We first measured multilayers of PGA immobilized and protected by PCA and GA. In this case one would expect that the protein would retain its structure. However both the reflectivity curves and the GID patterns measured were poorly structured and prevented any accurate structural determination, so that no further data were taken.

We then measured the reflectivity of pure GST multilayers, which resulted in a poorly structured curve (figure 1), whose only feature are the Kiessig fringes from which the average thickness per layer could be extracted d=27.3±0.1 Å /layer**.** This is in contrast with XRD results on GST crystals which yield an average size of about 60 Å [1]. The discrepancy can be explained by assuming that in the LB film the GST dimeric-molecule is flattened out in some fashion. Such hypothesis is confirmed by other data [2] obtained by our group by interference microscopy and ellipsometry, and warrants further investigation. We should remark also, from the damping of Kiessig fringes, the small value of the surface roughness (σ_{RMS}=15-20Å), indicating a very smooth film surface and well defined thickness, whereas the other data indicate a strong dependence of the thickness on the details of the preparation method.

We obtained somehow better results when inserting also a fatty acid salt (Ba-stearate) in the structure. This is not surprising, since Ba-St itself induces ordering. The reflectivity curve measured from a superlattice of structure [2 LS GST, PCA, 2 LB Ba-St]$_{10}$ is shown in the left panel of figure 2, it is much less structured

than that measured on similar heterostructures in which the "active" part is made by a photosensitive poly-azo-acrylate, in place of the GST protein, shown in the right panel of the same figure. In the latter case Bragg peaks can be clearly seen, and their position is observed to shift [3] as a result of photoperturbation of the system, as shown in the right panel of the same figure. However, in the GST related superlattice (fig. 2 left panel) it is possible to observe a residual structure of the Ba-St matrix, whose main features are consistent with the expected dimensions of the superlattice periodicity.

In conclusion, the measurements show that pure GST yields very homogeneous but highly disordered films. When incorporated into a highly ordered matrix, GST seems to interact with such matrix, as evidenced by the relative lack of features probably due to a disordering effect. The overall structure of the superlattice is maintained, but strong disordering seems to have taken place over shorter distances. This is probably the most interesting result of this set of measurements, which however must be supplemented by further work, both with XRR and other techniques in order to find out what specifically is the role of GST in partially destroying the Ba-St order.

References

[1] X. Ji, P. Zhang, R.N. Armstrong, G.L. Gilliland, Biochemistry, 31, 10169 (1992)

[2] T. Berzina et al, unpublished work.

[3] L. Cristofolini, M.P. Fontana, T. Berzina, O. Konovalov - Phys. Rev. E 66, 041801 (2002).

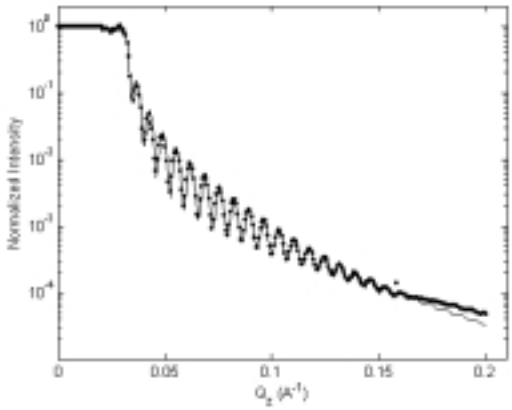

Figure 1: Kiessig fringes from an homogeneous multilayer of 32LS layers of GST, (points: experimental data, line: model fit) from which the average thickness d= d=27.3±0.1 Å /layer is obtained

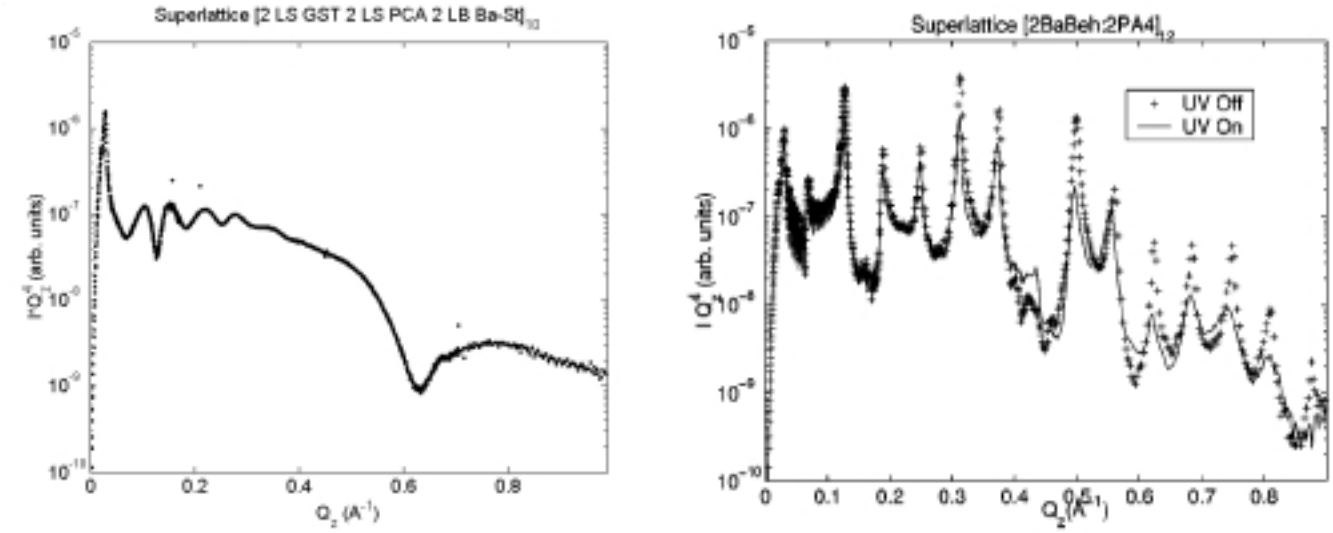

Figure 2: Left panel: Reflected intensity, scaled by Q_z^4 from a superlattice structure [2 LS GST, 2 LS PCA, 2 LB Ba-St]$_{10}$. **Right panel**: Reflected intensity, scaled by Q_z^4 from a photosensitive superlattice [2 LB BaBeh: 2 LS PA4]$_{12}$. Crosses, reflectivity measured on the pristine sample; line, reflectivity under UV illumination.