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Characterization of antigenic peptide p17-1 from HIV-1 in nanostructured films

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Abstract – Nanostructured films of antigenic peptides are promising for the development of immunosensors. In this work, we analyze the peptide p17-1 (LSGGELDRWEKIRLRPGG), derived from the HIV-1 p17 protein, when immobilized into Layer-by-Layer (LbL) films and incorporated into Langmuir monolayers of phospholipids. Film assembly conditions with a polyelectrolyte were identified for immobilization of the peptide p17-1, as well as peptide structure. This system will be used to build immunosensors that could be highly specific and selective based on immune principles.

Nanostructured films of antigenic peptides are now used in immunosensors [1], in which the specific molecular recognition property of the antigenic peptide toward antibodies leads to a high selectivity employing immune principles without the necessity of using complex molecules such as proteins. In this study, we analyze the peptide p17-1 (LSGGELDRWEKIRLRPGG), derived from the HIV-1 p17 protein, immobilized in Layer-by-Layer (LbL) films and incorporated into Langmuir monolayers of phospholipids.The LbL film was assembled using poly(allylamine) hydrochloride (PAH) and the growth of each bilayer (PAH/p17-1) was monitored with UV.vis. absorption at 280 nm. The PAH/p17-1 film showed an exponential growth, as indicated in Figure 1, which may mean that the peptide is reorganized in each bilayer adsorbed. The p17-1 in solution and LbL films were investigated by fluorescence spectroscopy, where the interaction with the film did not induce an alpha helix conformation in p17-1, similarly to what occurs in an aqueous solution and in contrast to the organized peptide in a methanol solution. The maximum emission for p17-1 fluorescence occurred at 340 nm in methanol, compatible with buried tryptophan residues, and at 355 nm when immobilized in LbL films (Figure 2). This red shift is consistent with the tryptophan being exposed to the environment [2]. These results can be the cause for a low sensitivity in the amperometric sensor made with the LbL film (PAH/p17-1n) when it was tested in the presence of anti-p17.

The phospholipid dipalmitoyl phosphatidyl glycerol (DPPG) in Langmuir monolayers responds to the presence of p17-1. For instance, 0.5 mol% of the peptide was already sufficient to affect the surface pressure and surface potential isotherms, as shown in Figure 3. This system can be promising to build films due to the tendency of phospholipids to induce conformation of the peptides [3], which may be a possible way to achieve a suitable architecture for the immunosensor based on an antigenic peptide.

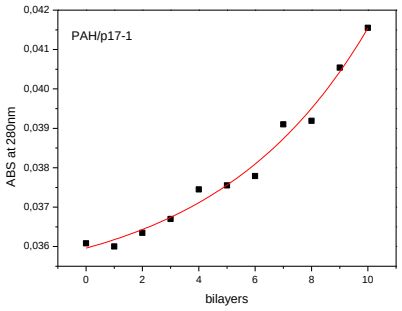

Figure 1: Exponential growth of 10-bilayer PAH/p17-1 film.

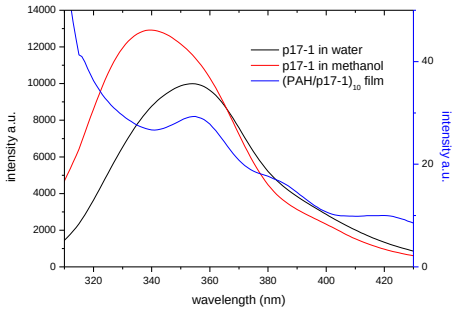

Figure 2: Fluorescence spectra for p17-1 in solution and LbL film.

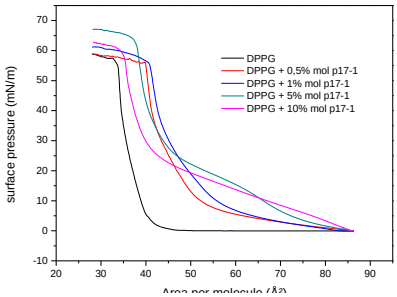

Figure 3: Surface pressure for DPPG and p17-1 Langmuir films.

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