Physisorption of Cytochrome *c* to Nanostructured Gold Surfaces: Relevance for Bionano-Devices and Composites

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Gold materials in a planar or curved (*i.e*., gold nanoparticles-AuNPs) configuration can be conjugated with proteins either directly or *via* tailored ligands forming a capping monolayer[1]. These bionano-conjugates, due to their high surface areas and stability, are especially suited for utilization in bio-nano devices[2].

The functionalization of electroactive gold surfaces and AuNPs with the alkanethiol, mercaptoundecanoic acid (MUA) has been considered the method of choice for targeting protein surfaces through the establishment of complementary electrostatic interactions[3].

In the present work, the effects of surface curvature and pH of the medium on the physisorption of horse heart cytochrome *c* (Cyt *c*) to gold surfaces were evaluated and the importance of MUA functionalization of those surfaces was assessed. Two different types of gold surfaces were analyzed: planar gold surfaces of gold coated quartz crystals, and curved surfaces of 15 nm diameter AuNPs.

Quartz Crystal Microbalance with Dissipation (QCM-D) allowed the quantification of Cyt *c* adsorption to gold-coated quartz crystals with and without functionalization with a self-assembled monolayer (SAM) of MUA.

For curved surfaces, the effects of Cyt *c* electrostatic adsorption were analyzed by UV-visible spectroscopy and zeta-potential measurements of the bionanoconjugates formed between cytochrome *c* and citrate or MUA-capped AuNPs.

QCM-D assays conducted at pH 4.5 showed that when MUA-covered gold surfaces are positive or neutral and cytochrome *c* is globally positively charged, only 15% of the surface area was coated with protein (Figure 1). In contrast, at pH 7.4, when MUA-coated gold surfaces and cytochrome *c* have opposite charges, a protein coverage of 30% could be observed for the planar gold surfaces, implying an adsorption process mainly governed by electrostatic interactions (Figure 1).

In zeta potential measurements at pH 7.4, the cytochrome *c* binding to AuNPs revealed to be greatly favored by the presence of a MUA-capping layer on the AuNPs, presenting a binding constant three times larger than the binding constant obtained for citrate-capped AuNPs.

These results indicate that both planar and curved gold surfaces functionalized with MUA are suitable for cytochrome *c* electrostatic physiosorption, allowing simple pH control over the amount of adsorbed protein, with potential applications on biosensing devices and bionano-composites.

References

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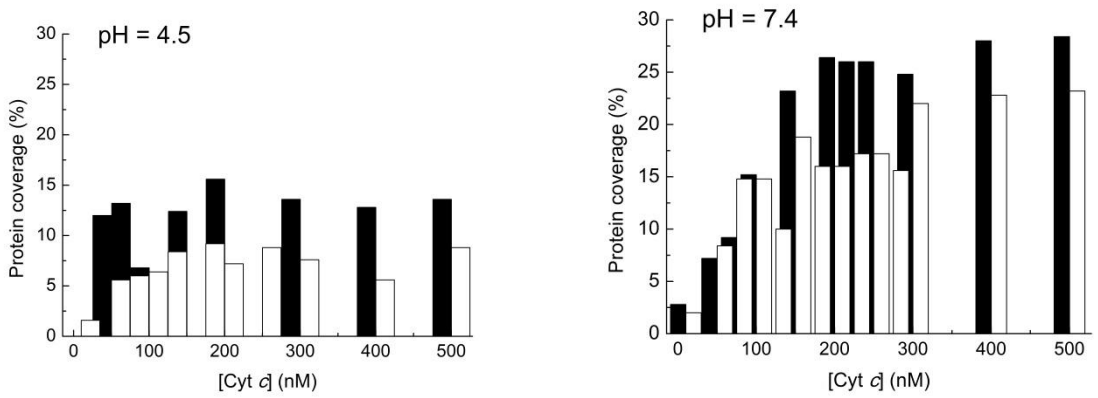

Figure 1. Cytochrome *c* coverage of gold-coated quartz crystals. Data are presented for experiments conducted at pH 4.5 and pH 7.4. Black bars are data from gold surfaces modified with MUA SAM and white bars are data from gold surfaces with no modification.