Supplementary Material (ESI) for Analyst

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Modified peptide monolayer binding His-tagged biomolecules for small ligand screening with SPR biosensors

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Experimental details

Synthesis and characterization of peptide-based self-assembled monolayers

The peptides were synthesized according to a previously described protocol [1]. LC-MS was used to verify the products at each step of the synthesis. The conformation of the peptides in solution was measured with circular dichroism on a Chirascan™ spectrometer (Applied Photophysics ltd) using a 1

mg/mL peptide solution in PBS. Microscope slides were coated with a 0.5 nm thick chromium adhesion layer and then with a 50 nm thick gold layer using a sputter coater (Cressington Model 308R). These SPR sensors were reacted for at least 16 h with a 5 mM peptide solution in DMF. The SAM formed on the SPR sensors was extensively rinsed with DMF and ethanol and dried. The mid-IR spectrum of the peptide monolayers immobilized on the SPR sensors was measured in attenuated total reflectance (ATR). Mid-IR spectra were recorded using a Bruker Tensor 27 equipped with a Ge-ATR module.

The synthesis of the modified peptide layer binding His-tagged biomolecules was performed directly on the SPR sensors (Figure 1), based on the optimal peptide 3-MPA-LHDLHD-OH. The following solutions are aqueous and the SPR sensors were rinsed in ultrapure water following each step. The peptide monolayer immobilized on the SPR sensor was reacted with a solution composed of 100 mM EDC and 20 mM NHS for 2 minutes, followed by a 1 hour reaction with 40 mM Nα,Nα-bis(carboxymethyl)-L-lysine hydrate. The final step was a 10-minute exposition to 100 mM $CuSO_4$, during which Cu^{2+} binds to the modified peptide layer. The SPR sensors were rinsed in ultrapure water and dried using a moderate flow of nitrogen. The product of each reaction was monitored using FTIR. In this configuration, the peptide monolayer chelates copper and copper chelates His-tagged biomolecules. Thus, the surfaces were analyzed using x-ray photoelectron spectroscopy (XPS) to ensure the presence of Cu^{2+} on the SPR sensors modified with the modified peptide layer binding His-tagged biomolecules. A VG ESCALAB 3 MKII equipped with a Mg Kα source running at 300W scanning from 50 to 100 Å deep provided the XPS spectral information.

Characterization of modified peptide layer binding His-tagged proteins

The peptides were produced in large amount (hundreds of mg) and stored in an opaque and sealed container at room temperature without any further care. They were used over a period of 30 days

without any change in the analytical signal. Peptides exhibit a good absorption signature in the mid-IR domain. Thus, the reactions performed on the SPR substrates were followed using FTIR as a convenient way to rapidly obtain information about the composition of the modified peptide layer at the surface of a gold-coated sensor. Every spectral acquisition was preceded by the acquisition of a blank measurement with a bare gold-coated slide. The amide I band is of primary importance in the analysis of a peptide-based self-assembled monolayer to determine the secondary structure of the peptide on the SPR sensor. The amine I band for 3-MPA-LHDLHD-OH is located at 1645 cm^{-1} typical for a α-helix. The C=O stretch of the carboxylic acid functional groups of the aspartic acid were observed at 1720 cm^{-1} and disappeared once coupled with Nα,Nα-bis(carboxymethyl)-L-lysine hydrate using EDC/NHS chemistry. This reaction was confirmed with FTIR, with the appearance of two bands at 1670 and 1740 cm^{-1} also observed on the spectra of pure Nα,Nα-bis(carboxymethyl)-L-lysine hydrate. The XPS spectrum of the modified peptide layer chelated with copper exhibited the Cu_{2p} band at 934.07 eV confirming the presence of copper at the surface of the sensors. The relative peak areas observed for sulphur, nitrogen, carbon and oxygen correspond to the values expected for this SAM. The XPS response corresponding to Au and Cu indicate a strong presence of these two metals as expected. Other metals such as Co or Ni can be used for His-tagged protein binding, the most common being Ni. Thus, Ni was also tried with the current modified peptide layer, which in this case did not appear on the XPS spectrum after exposure of the modified peptide layer to Ni. For this reason and because of its lower environmental impact, copper-functionalized surfaces were used thereafter.

References

1. O. R. Bolduc, J. N. Pelletier and J. F. Masson, *Analytical Chemistry*, 2010, **82**, 3699-3706.