

Chapter 13

Spectroscopy and Electrochemistry of Peptide-Based Self-Assembled Monolayers

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Mono and bi-component peptide-based self-assembled monolayers (SAMs) immobilized on a gold surface were studied by electrochemical and spectroscopic techniques. The peptides investigated were exclusively formed by C^α-tetrasubstituted amino acids. These residues, due to their peculiar conformational properties, constrain the peptide in a helical conformation, as confirmed by X-ray diffraction structure determinations, and Circular Dichroism and NMR experiments in solution. Both mono- and bi-component peptide SAMs were functionalized with electroactive, fluorescent chromophores strongly absorbing in the UV region. While electrochemical experiments indicated the formation of densely-packed films on the gold surface, fluorescence spectroscopy revealed the occurrence of aromatic-aromatic interactions between the pyrene units functionalizing the peptide chains, obtaining important information on the structural and dynamical properties of the peptide SAMs investigated.

1 Introduction

Hybrid materials obtained by functionalizing metals or semiconductors with biomolecules or bioinspired molecular systems have been recently synthesized, paving the way for the fast-growing field of nanobiotechnology [1]. Among these nanometer scale systems, peptide-based Self-Assembled Monolayers (SAM) have

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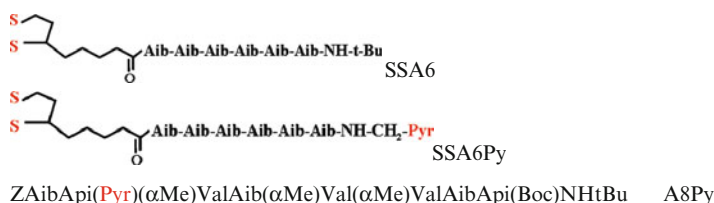


Fig. 13.1 Acronyms and molecular structures of the peptide building-blocks forming the mono- and bi-component SAMs investigated

been investigated as possible substrates for molecular recognition, biocompatible coating for cellular growth, conductive media for Electron Transfer studies over large distances [2].

In this contribution, the properties of peptide-based SAMs linked to gold substrates through Au-S bond were studied by optical spectroscopy (steady-state fluorescence) and electrochemical (Cyclic voltammetry, CV) methods. The peptides investigated were exclusively formed by C $^{\alpha}$ -tetrasubstituted amino acids (Fig. 13.1). These residues, due to their peculiar conformational properties, constrain the peptide in a helical conformation, as confirmed by X-ray diffraction structure determinations, and Circular Dichroism and NMR experiments in solution [3]. An Aib (α -aminoisobutyric acid) homo-hexapeptide was functionalized at the N-terminus with a lipoic group for immobilization to a gold substrate exploiting the strong Au-S affinity (≈ 40 kcal \cdot mol $^{-1}$). The peptide was further functionalized with a pyrene chromophore (**SSA6Py**) strongly absorbing in the UV-vis region to enhance the molecular photon capture cross-section of the SAM (*antenna effect*). A peptide with the same backbone, but lacking the pyrene chromophore (**SSA6**), was also synthesized as a reference compound. Furthermore, a photoactive octapeptide (**A8Py**), also formed by C $^{\alpha}$ -tetrasubstituted residues and comprising a pyrene chromophore but lacking the lipoic group, was prepared for obtaining a bi-component peptide SAM formed by inclusion of **A8Py** into the palisade of the **SSA6** SAM linked to the gold surface by Au-S interaction.

2 Results and Discussion

2.1 Cyclic Voltammetry (CV) Experiments

The formation and stability of the **SSA6Py** and **SSA6** SAMs on the gold electrode was checked by CV measurements in the presence of an electrochemical standard redox pair [$\text{K}_3\text{Fe}(\text{CN})_6$, $E^\circ(\text{Fe}^{3+}/\text{Fe}^{2+}) = 0.36$ V] (Fig. 13.2). The deposition of the peptide film partially passivated the gold surface, inhibiting the discharge of the redox pair to the electrode. The decreased activity of the redox pair can be directly related to the package density of the peptide film on the gold surface. Both the **SSA6**

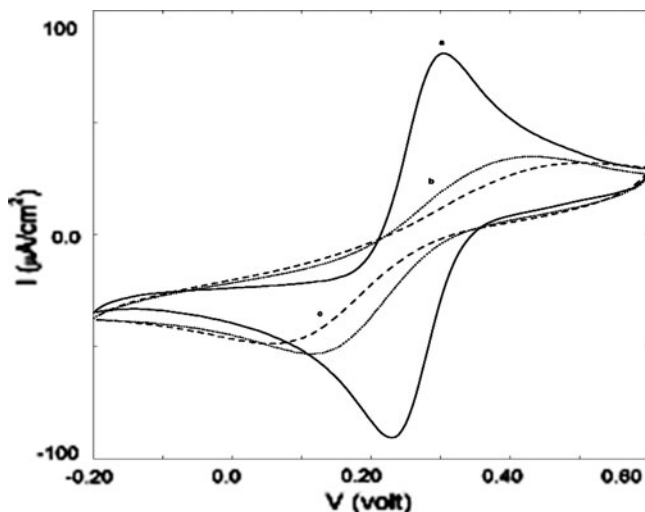


Fig. 13.2 Cyclic voltammety experiments in a 0.50 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$. (a) bare gold electrode; (b) gold electrode modified by SSA6 and (c) SSA6Py peptide SAMs

and **SSA6Py** SAMs inhibited the $\text{Fe}_3(\text{CN})_6^{3-}$ discharge on the gold electrode, although a residual capacitive current, most likely ascribable to diffusion of the buffer electrolyte (KCl), was still measured for the modified electrode. Interestingly, for the **SSA6/A8Py** bi-component SAM the discharge of the redox pair was found to be almost completely depleted indicating the formation of a densely-packed SAM.

CV experiments also showed that the Pyrene group in the **SSA6Py** SAM gave rise to irreversible oxidation at 0.95–1.0 V. After that, a new peak at 0.2–0.4 V can be observed, ascribable to the discharge of diol/diketone species, stable byproducts of Pyrene oxidation. This peak could be observed after repeated scans, signaling the integrity of the peptide SAM on the gold surface at these applied potentials. Disruption of the Au–S linkages was only observed at negative applied potentials (≤ -0.8 V).

2.2 Steady-State Fluorescence Experiments

Peptides functionalized with Pyrene chromophores allowed for easy determination of the onset of interchain interactions, due to the characteristic emission properties of pyrene groups. While the monomer emission is characterized by well-resolved vibrational transitions, pyrene-pyrene excited state interaction gives rise to a broad and intense red-shifted emission associated to the formation of dimeric excited-state complexes (*excimers*). As can be observed in Fig. 13.3, the emission spectrum of the **SSA6Py** SAM, linked to a 5 nm gold film supported on quartz, showed a

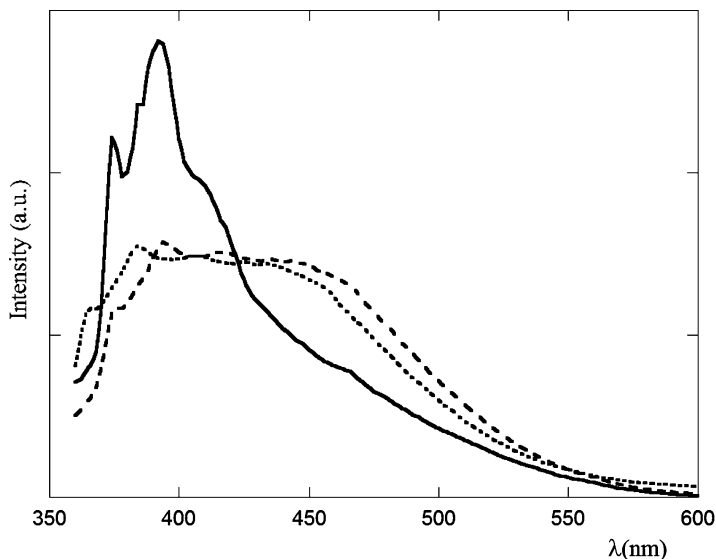


Fig. 13.3 Emission spectra of mono- and bi-component peptide SAMs: SSA6Py (*dashed line*); SSA6Py:SSA6 (1:10) (*continuous line*); A8Py:SSA6 (1:1) (*dotted line*)

broad red-shifted fluorescence, indicating the formation of excimer species. On the contrary, the fluorescence spectrum of the bi-component SAM obtained by a (1:10) **SSA6Py/SSA6** millimolar deposition solution, also reported in Fig. 13.3, was characterized by the typical emission of pyrene monomer species.

Interestingly, the fluorescence spectrum of the bi-component SAM formed by a (1:1) **A8Py** and **SSA6** millimolar deposition solution revealed an excimer-like emission, as also shown in Fig. 13.3. This finding confirmed the inclusion of **A8Py** in the **SSA6** palisade, linked to the gold surface by the strong Au-S electrostatic interaction. The densely-packed nature of this bi-component SAM, stabilized by favorable dipole-dipole interaction between the **A8Py** and **SSA6** peptide chains, was confirmed by the CV experiments.

The observation of excimer emission strongly suggests the formation of **A8Py** segregated domains (*rafts*) within the **SSA6** SAM. This effect is most likely ascribable to the dynamic nature of the processes leading to the formation of self-assembled monolayers. The relatively free diffusion of **A8Py**, lacking the lipoic group, allowed for the slow organization of **A8Py** domains during the SAM deposition (18 h). This conclusion was strengthened by the absence of excimer-like emission in bi-component SAMs formed by (1:1) **SSA6/SSA6Py** millimolar deposition solution, both strongly linked to the gold surface through Au-S interaction (data not shown).

3 Conclusions

The packing density and stability of mono- and bi-component peptide-based self-assembled monolayers were characterized by electrochemical and spectroscopic measurements. Cyclic Voltammetry experiments showed that all the peptide SAMs are densely-packed despite the shortness of the oligopeptides used as building blocks in the self-assembly process. This is probably due to the conformationally-constrained character of peptides rich in C $^{\alpha}$ -tetrasubstituted amino acids. Fluorescence experiments revealed that aromatic-aromatic interactions contribute to the stabilization of the peptide film on the electrode surface, forming separated domains whenever possible. The characteristic emission of excited state complexes (excimer) was exploited for monitoring the onset of interchain interactions between the peptide building blocks.

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