# Self-Assembled Peptide Nanostructures for the Development of Electrochemical Biosensors

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## Contents



**Abstract**

Biological building blocks such as peptides or proteins are able to self-organize into nanostructures with particular properties. There are several possibilities for their use in varying applications such as drug delivery, biosensing, clean-room fabrication methods, and tissue engineering. These biological nanostructures have recently been utilized for bionanotechnological applications thanks to their easy and low-cost fabrication, their stability, and their facile functionalization. These features suggest the usage of self-assembled peptide nanostructures in the development of biosensing platforms, and the present chapter explores their

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use for such purposes. Several immobilization strategies, mechanisms, and detected substrates are described. Moreover, different possibilities to functionalize and modify their structure toward utilization in sensing applications are also discussed.

**Keywords**
Self-assembly • Peptides • Biosensors • Amperometry • Biomedical analysis • Dielectrophoresis • Environmental analysis • Impedance • Cyclic voltammetry • Conductive polymers

## Introduction

Traditionally, nanomaterials such as carbon nanotubes (CNTs), silicon nanowires, as well as gold, platinum, and silver nanowires or nanoparticles have been used in the development of electrochemical and optical biosensors due to their large surface area, mechanical stability, electrochemical properties, and advantages in terms of signal amplification (Table 1). However, issues involving fabrication costs, biocompatibility, and functionalization have directed much attention to finding alternatives to overcome these challenges.

Self-assembled peptides are natural molecular building blocks able to self-organize into structures such as nanofibers, nanotubes, or nanoparticles. Peptides, e.g., the short aromatic dipeptides diphenylalanine or diphenylglycine, octapeptides such as NSGAITIG, or more complex linear peptides, have been reported to form nanotubes, nanofibers, or nanoparticles. These structures can, in most cases, be fabricated under very mild conditions: room temperature, aqueous media, and outside a clean room. Fabrication costs can thus be lowered as compared with the

**Table 1** Nanostructures commonly used in the development of electrochemical biosensors

| Nanostructure | Fabrication method | References |
|---|---|---|
| Metallic nanowires | Template assisted electrodeposition<br>Electrochemical deposition<br>Electroless deposition<br>Template filling | [1] |
| Si nanowires | Reactive ion etching<br>Photolithography | [2] |
| Carbon nanotubes | Chemical vapor deposition<br>Arc discharge<br>Laser ablation<br>Gas-phase catalytic growth | [3–5] |
| Polymer nanowires | Electrochemical deposition<br>Template filling<br>Reactive ion etching | [1, 6] |
| Graphene nanostructures | Exfoliation<br>Chemical vapor deposition<br>Epitaxial growth | [7] |

fabrication of CNTs or silicon nanowires. Due to these advantages and properties, self-assembled peptide nanostructures (SAPNs) have been used in several applications ranging from tissue engineering to microfabrication processes [8].

In addition to their easy fabrication, self-assembled peptide nanostructures have proven to be resistant to high temperatures and chemical attacks [9]. Moreover, SAPNs are easily functionalized through chemical modification with structures such as quantum dots, magnetic and metallic nanoparticles, or enzymes. This leads to new possibilities for their utilization in the development of ultrasensitive biosensing devices [10, 11].

Even though SAPNs are not yet mentioned in the literature review articles reporting the latest advances in the use of nanomaterials for electrochemical sensing [5, 12, 13], more and more reports are appearing, presenting the possibilities, advantages, and challenges to overcome when using SAPNs for the development of electrochemical sensing platforms [8, 14–22].

The use of SAPNs involves several challenges. Due to their biological origin, their conductivity is very low, and depending of the fabrication method, the control of the size of the final structure may prove to be difficult; the fabricated structure needs to be manipulated and immobilized in specific locations and some of the SAPNs are not stable in liquid environments [15]. These challenges need to be overcome in order to integrate them with transducers and accelerate their use in the fabrication of sensing devices.

The present chapter discusses these challenges and present various solutions for the use of SAPNs in the development of electrochemical biosensors, as well as methods to deposit these nanostructures on transducer surfaces and their decoration with functional molecules (such as enzymes, antibodies, conductive polymers) are listed and discussed.

## Fabrication and Deposition of Self-Assembled Peptide Nanostructures on Transducers

As previously mentioned, one of the features that make SAPNs an attractive option for bionanotechnological applications is the easy fabrication under very mild conditions. Numerous techniques have been reported for the synthesis of nanotubes, nanofibers, and nanoparticles using SAPs as building blocks. These synthesis techniques include very simple steps ranging from the dilution and mixing of two liquids containing the precursor compounds in a small container or the controlled mixing in a microfluidic chip to the use of more complex instruments like in the case of physical vapor deposition of SAPNs [23–25]. Cyclic, linear, chemically modified linear peptides and short aromatic dipeptides have been used for the self-assembling of nanostructures using the fabrication techniques mentioned above.

One of the simplest methods to fabricate nanostructures through the self-assembly of peptides is by mixing a peptide stock solution with a solution that will promote the self-assembly of the peptide nanostructure. A very well-documented method is the self-assembly of the short aromatic dipeptide diphenylalanine. The dissolution of this peptide in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and further dilution with

water cause multiwall nanotubes to form in seconds at room temperature [25]. Another example of the fabrication of peptide nanotubes involves dissolving bis(N-α-amido-glycylglycine)-1-7-heptane dicarboxylate in water. In this case, the nanotubes were formed after 1 week at room temperature [26].

In both cases, the size of the obtained nanotubes differed both in diameter and length. In order to obtain nanostructures with more defined dimensions, various methods exist, such as on-chip fabrication, where a more controlled mixing of the precursor solutions is possible due to a laminar flow, or the use of templates that define the final diameter of the fabricated nanostructures [24, 27, 28].

A novel solid-phase method to grow vertically aligned crystalline peptide nanofibers in the absence of water and driven by aniline vapor was reported by Ryu and coworkers [29]. By using a μ-channeled polydimethylsiloxane (PDMS) mold, a micropattern of peptide nanofibers was fabricated. Figure 1 shows vertically aligned self-assembled peptide nanofibers prepared with the aniline vapor aging method.

Thanks to this method, it was possible to integrate these biological nanofibers in metallic electrodes for the development of a cell culture-biosensing platform for the

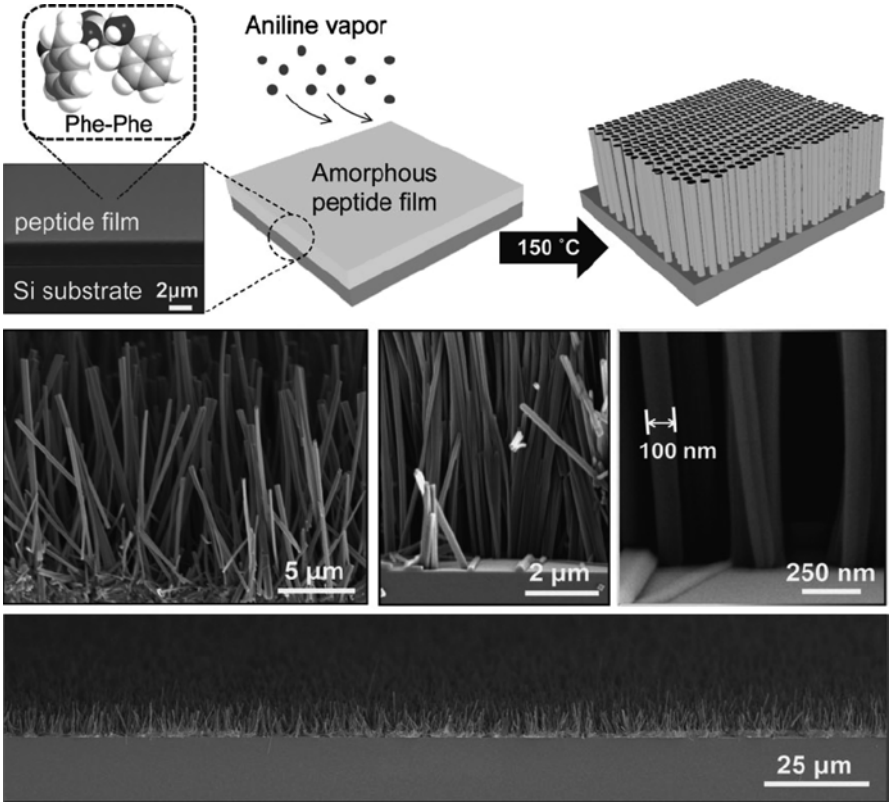


**Fig. 1** Growth of vertically aligned nanofibers from an amorphous diphenylalanine thin film by high-temperature aniline vapor aging (Figure from Ryu and Park [29] with permission from John Wiley & Sons)

detection of neurotransmitters from cells [30]. This method requires temperatures around 140 °C but assures the synthesis of vertically well-aligned peptide nanofibers.

Physical vapor deposition was used for the controlled fabrication of dense and homogeneous peptide nanostructures to be used in microelectronics. The employed technique requires temperatures above 200 °C and the use of more specialized equipment, vacuum chambers, heating control systems, and thickness control systems among others, but enabled a controlled deposition of either nanotubes or nanofibers [23].

Another advantage of the last two preparation techniques is that the peptide nanostructures can – as they are being fabricated – be deposited on specific locations such as metallic electrodes or $SiO_2$ wafers for the development of biosensing devices.

Apart from these two methods that require temperatures over 120 °C, other deposition techniques can be used at room temperature for the controlled deposition of peptide nanostructures on top of transducers. The simplest method to immobilize peptide nanostructures on top of electrochemical transducers is the deposition of droplets of a solution containing the biological modified or unmodified nanostructures; once the solvent is evaporated, the nanostructures are physically immobilized on the transducer surface. Although this approach is both simple and rapid, it does not ensure a stable layer of nanostructures on the transducer surface: when the modified transducer is dipped in the sample to be measured, some of the nanostructures may become detached. In order to prevent this, an additional layer of polymer, e.g., poly(allylamine hydrochloride) (PAH), glutaraldehyde, or polyethyleneimine (PEI), is added to trap and keep the peptide nanostructures in the desired position [31, 32]. Figure 2 displays the use of glutaraldehyde as a cross-linker to immobilize glucose oxidase on peptide nanotubes and PEI to keep the functionalized peptide nanotubes on top of gold electrodes.

Dielectrophoresis is a technique where an inhomogeneous electric field is used to move a neutral but polarizable particle. It has been used for the controlled deposition of nanofibers and nanotubes on top of gold electrodes, as shown in Fig. 3. After deposition of the biological nanostructures on the electrodes, their electrical characterization and utilization as sensors were made possible, as previously reported. This deposition technique is a noncontact method ensuring that the peptide nanostructure does not become damaged during the manipulation step [33–35].

An inkjet printing technology was used for the deposition of peptide nanotubes and nanoparticles forming specific patterns on top of indium tin oxide electrodes, as shown in Fig. 4. This method was found to rapidly produce durable patterns at room temperature, making it very attractive for the deposition of peptide nanostructures at a high scale. However, challenges regarding clogging of the printing device need to be overcome [36].

Another way to immobilize peptide nanostructures on a transducer, both horizontally and vertically, involves the functionalization of peptide nanostructures with magnetic nanoparticles and then exposition of the modified tubes to an external magnetic field. With this technique, very highly organized peptide nanotube arrays were immobilized on siliconized glass [37].

A similar approach was used by Zhao and Matsui, in which case antibody-functionalized peptide nanotubes were accurately immobilized on protein-patterned arrays by optimizing their ligand-receptor interactions. In their work, peptide

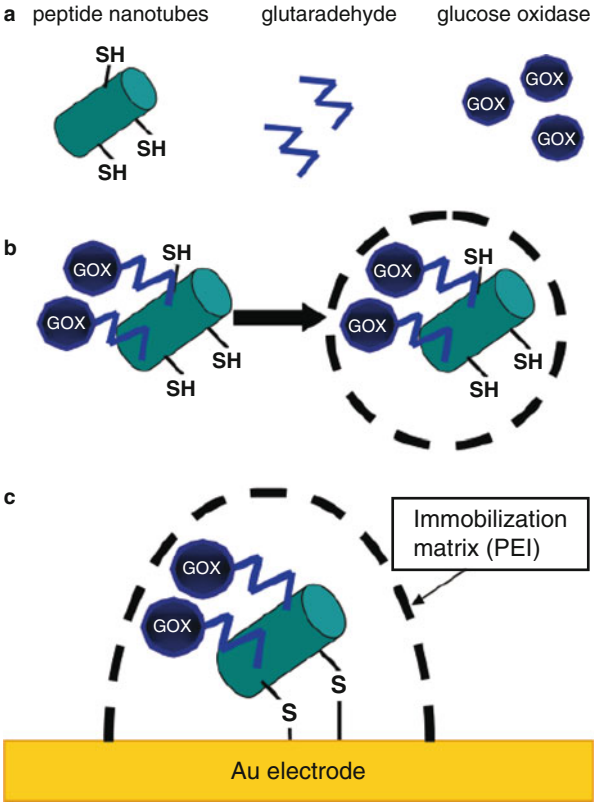


**Fig. 2** Immobilization of GOX-modified peptide nanotubes on Au electrodes using PEI as an immobilization matrix (Reprinted with permission from Yemini, M. et al. Peptide nanotube-modified electrodes for enzyme-biosensor applications. Anal. Chem. 77 (16): 5155–5159. Copyright (2005) American Chemical Society [32])

nanotubes self-assembled from bolaamphiphile peptide monomers were coated with antihuman-IgG antibody and immobilized on 150 × 600 nm trenches modified with human gamma immunoglobulin (IgG). Perfectly vertically aligned peptide nanotubes were deposited on the modified trenches with nearly 100 % efficiency [38, 39].

The direct transfer of octapeptide fiber arrays on gold surfaces was achieved using laser-induced forward transfer (LIFT). This immobilization technique involves a single pulse from a focused laser beam to transfer a peptide solution from a donor-coated surface to an acceptor surface [40]. The method provides the high-resolution, noncontact, direct, flexible, and parallel transfer of more than one type of material resulting in the deposition of peptide-based microarrays maintaining the biological functions of the nanostructures [41, 42]. As in the case of the physical vapor deposition method, this technique requires additional equipment such as laser systems, translation stage drivers, and microscopes.

In addition to the fabrication and manipulation-immobilization techniques mentioned in this section, there are a few others that could interest the reader. However, this chapter only presents techniques relevant for the development of electrochemical biosensors. The readers are thus invited to learn more about other methods to fabricate, manipulate, and immobilize SAPNs in some very good reviews and chapters focusing on these topics recently published [40, 43, 44].

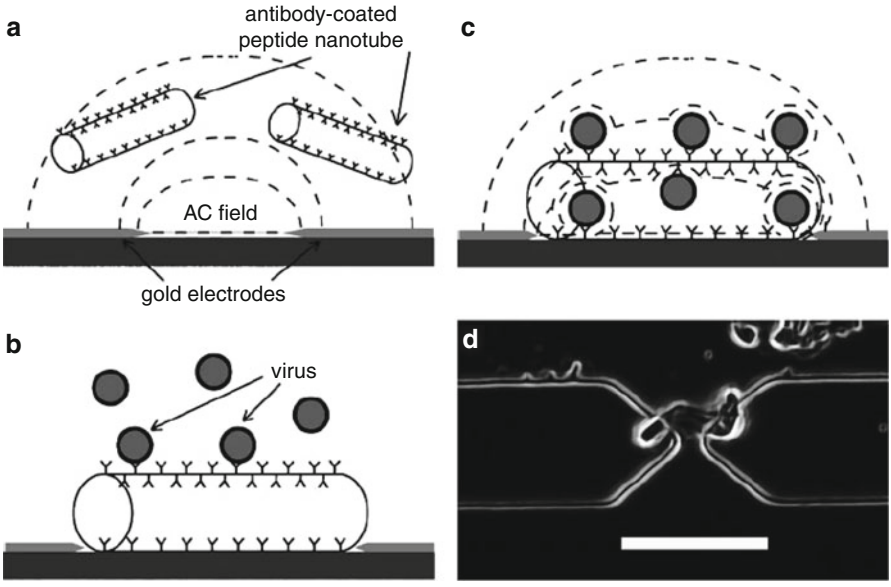


**Fig. 3** Immobilization of antibody-coated peptide nanotubes using dielectrophoresis (Figure from de la Rica et al. [34] with permission from John Wiley & Sons)

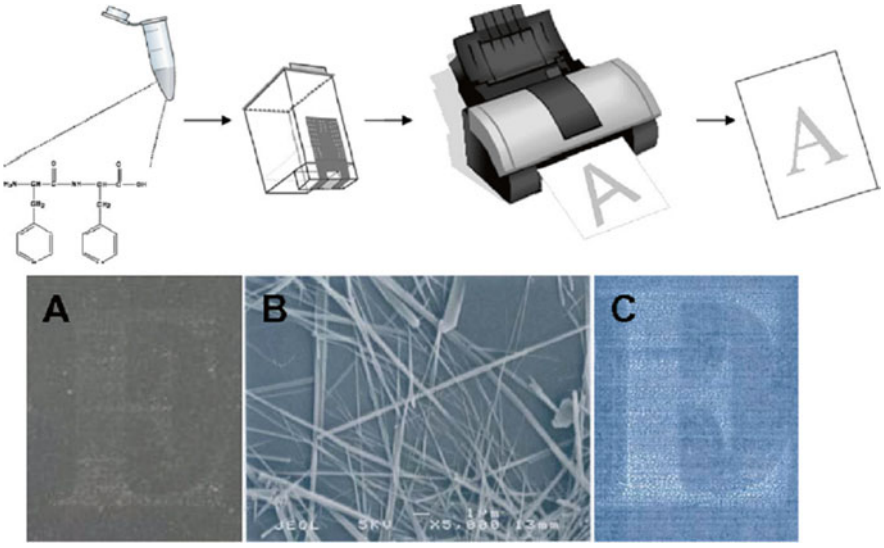


**Fig. 4** Scheme of the inkjet printing deposition of self-assembled peptide nanotubes. (**a**) Image of a single printing cycle. (**b**) Scanning electron microscopy image of the printed area in **a**. (**c**) Image of a 10-cycle print on transparent foil (Figure adapted from Adler-Abramovich and Gazit [36] with permission from John Wiley & Sons)

## Functionalization of Self-Assembled Peptide Nanostructures

An important advantage of self-assembled peptide nanostructures, when compared with carbon nanotubes or silicon nanowires, is how easily these biological substrates can be decorated with functional compounds that increase the sensitivity and selectivity to the biosensing device. Thanks to the amino acids present on the structure of the self-assembled peptide, a variety of possible chemical interactions between the peptide nanostructure and the functional compound are available and have been utilized to decorate the surface of SAPNs.

If we focus only on the functionalization of SAPNs with the purpose of using them in electrochemical biosensing, we can find that these bionanostructures have been decorated with enzymes, antibodies, conductive polymers, metallic nanoparticles, and organic acids or integrated with inorganic nanomaterials, just to mention a few.

The manner in which SAPNs are functionalized varies depending of the type of peptide used to fabricate the nanostructure and the functional groups available on its surface. For instance, by taking advantage of the amino groups exposed on the external wall of diphenylalanine nanotubes, a biotinylation procedure was employed to decorate these nanotubes with gold nanoparticles, InGaP quantum dots, and a fluorescent labeling (Ato-610). The functionalization was performed through a rapid chemical reaction without any special requirements regarding equipment or temperature [45].

In a different study, antibodies were anchored via hydrogen bonding on the amide groups of self-assembled nanotubes of the bolaamphiphile peptide bis(N-α-amido-glycylglycine)-1,7-heptane dicarboxylate [38]. Using the same type of bolaamphiphile peptide nanotubes, *Candida rugosa* lipase – an enzyme previously used for the potentiometric detection of pesticides [46] – was encapsulated inside the nanotubes with a simple incubation process. The immobilization of the enzyme was possible via hydrogen bonding between amide groups present in the nanotube structure and the complementary functional groups of the enzyme [26]. This functionalization process required the incubation of the enzyme with the nanotubes during 1 week at 4 °C. A scheme of the functionalization process is shown in Fig. 5. This encapsulation process resulted in a catalytic activity of the enzyme which was 33 % higher than for a free-standing enzyme at room temperature.

Horseradish peroxidase and glucose oxidase, enzymes used for the electrochemical detection of hydrogen peroxide and glucose, were encapsulated within the internal cavity of diphenylalanine peptide nanotubes by capillary effect [47, 48]. The self-assembled nanotubes were incubated in the respective enzyme solutions at 5 °C during 1 week with constant shaking. The encapsulation of the enzymes inside the peptide nanostructures was confirmed by scanning transmission electron microscopy (STEM).

Kasotakis and coworkers presented a means to incorporate metallic nanoparticles at specific locations of nanofibers formed by self-assembly of the octapeptides: NSGAITIG, NCGAITIG, CNGAITIG, and CSGAITIG from the fiber protein of adenovirus [49]. The functionalization involved the mixing and incubation during 18 min of the peptide fibril solution with an aqueous solution of the metal salt

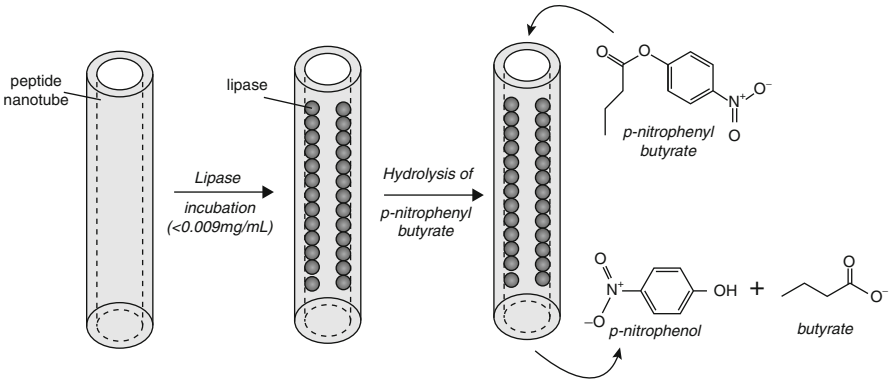


**Fig. 5** Scheme of the immobilization of *Candida rugosa* lipase inside bolaamphiphile nanotubes (Reprinted with permission from Yu, L.T. et al. Fabrication and application enzyme-incorporated peptide nanotubes. Bioconjugate Chem. 16 (6): 1484–1487. Copyright (2005) American Chemical Society [26])

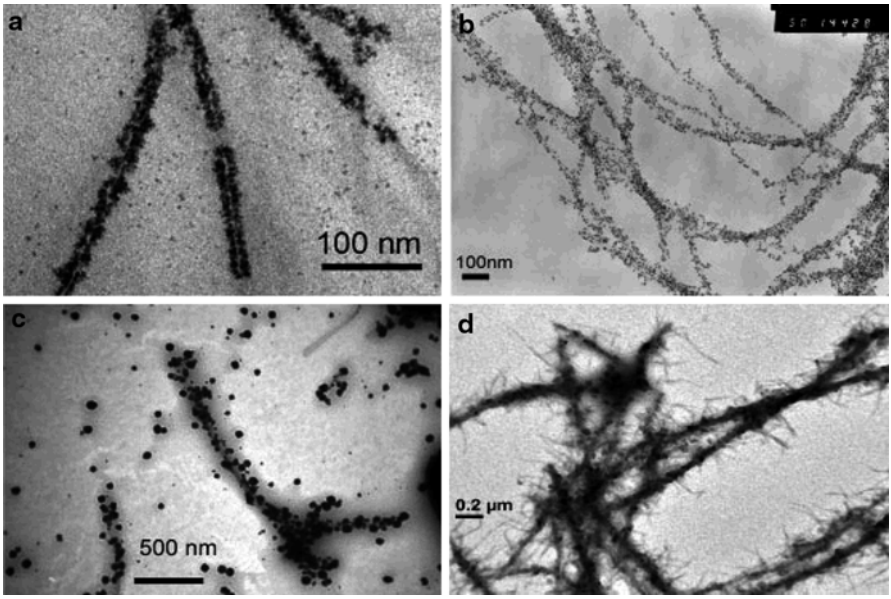


**Fig. 6** Transmission electron microscopy images of the deposition of platinum on octapeptide nanofibrils. (**a**) Fibrils formed from the NSGAITIG peptide; (**b**) NCGAITIG peptide; (**c**) CNGAITIG peptide; (**d**) CSGAITIG peptide (Figure from Kasotakis et al. [49] with permission from John Wiley & Sons)

($AgNO_3$, $HPtCl_6$ $H_2O$, or $HAuCl_4$ $3H_2O$). After the incubation, a reducing agent was added (1 % citric or ascorbic acid depending of the salt used). The mixed solution was then incubated during 1 h at room temperature. Figure 6 illustrates the NSGAITIG nanofibers after incubation with a platinum solution.

Glucose oxidase was covalently immobilized on the surface of EAK16-II nanofibers using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) coupling. The functionalized EAK16-II nanofibers were deposited on highly ordered pyrolytic graphite for the amperometric detection of glucose. As in the case of the *Candida rugosa* lipase encapsulated on bolaamphiphile nanotubes, the activity and stability of the immobilized glucose oxidase were increased [50].

A recent study reported on the use of EDC as a linker agent between folic acid and diphenylalanine peptide nanotubes [51]. The functionalized peptide nanotubes were then deposited on a graphene electrode in order to capture cancer cells overexpressing folate receptors. The folic acid-functionalized self-assembled nanotubes were characterized using atomic force microscopy (AFM).

The templated polymerization of polyaniline (PANI), a conductive polymer, on the external wall of self-assembled peptide nanofibers was reported by Ryu [52]. For this, the formed nanofibers were immersed in a polymerizing solution of aniline for a desired time without stirring. The result was the formation of peptide nanofibers/PANI core/shell nanostructures, as shown in Fig. 7. Through doping/dedoping tests and electrochemical characterization, it was confirmed that the peptide/PANI nanofibers were electrochemically active.

Another example of the integration of SAPNs with conductive polymers was demonstrated by Hamedi and coworkers. Their work involved the decoration of amyloid fibrils synthesized from bovine insulin with poly(3,4-ethylenedioxythiophene)/poly(styrenesulfonate) (PEDOT/PSS). The PEDOT-functionalized amyloid fibrils were used to fabricate an electrochemical transistor device [53].

The same type of nanofibers was functionalized with $Co_3O_4$ nanostructures by treating the peptide nanofibers with 1 mM $CoCl_2$ solution in 10 mM Tris (pH 7.0)

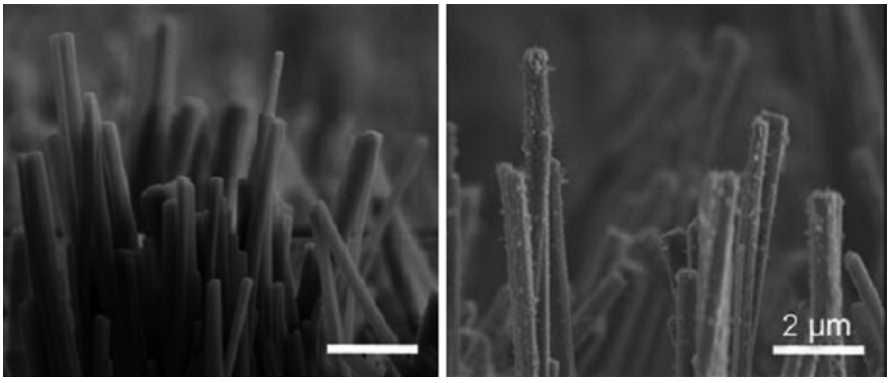


**Fig. 7** Cross-sectional scanning electron microscope images of bare (*left image*) and PANI covered (*right image*) self-assembled peptide nanofibers (Figure from Ryu and Park [52] with permission from John Wiley & Sons)

overnight and reducing the adsorbed $Co^{2+}$ ions with 5 mM $NaBH_4$. The resultant peptide/$Co_3O_4$ composite nanofibers were then subjected to structural and electrochemically characterizations [54].

Finally, an interesting example of the integration of SAPNs with carbon nanostructures was developed in order to fabricate peptide/graphene hybrid assemblies into core/shell nanowires by a single-step solution process. The prepared core/shell nanowires exhibited electroconductivity suggesting their use as a supercapacitor electrode [55].

As presented in the previous examples, there are many possibilities to functionalize SAPNs with a variety of functional molecules in order to improve the performance of the developed electrochemical sensing platform. These functionalization methods vary depending on the peptide used for the synthesis of the nanostructure and the target of the biosensing platform.

## Applications

A majority of the electrochemical biosensing devices developed using SAPNs are used for the detection of relevant compounds in two main fields: biomedical and environmental applications. For the detection of these compounds, electrochemical techniques such as amperometry, cyclic voltammetry, and square wave voltammetry and impedance have been applied.

As mentioned before, SAPNs can be employed to encapsulate or support the biorecognition element in its structure. Additionally, SAPNs have been integrated with carbon nanomaterials such as graphene or carbon nanotubes in order to add extra functionalities.

Table 2 summarizes some of the electrochemical biosensors fabricated using SAPNs.

### Biomedical Applications

Glucose and hydrogen peroxide are compounds of biomedical relevance that are connected with the diagnosis of diseases such as diabetes. The detection of glucose constitutes one of the biggest markets in the electrochemical biosensing industry [56, 57]. SAPNs offer a new alternative for the development of electrochemical biosensors aimed to follow changes in the concentrations of glucose, hydrogen peroxide, neurotransmitters, and metals involved in different pathologies.

Glucose oxidase was attached to peptide nanotubes through Traut's reagent for the electrochemical detection of glucose [32]. The modified nanotubes were then attached to a gold electrode using glutaraldehyde as a cross-linker as depicted in Fig. 2.

The electrochemical detection of tumor necrosis factor α (TNF-α) was reported using a biosensor combining ferrocene carboxylic acid-functionalized peptide nanofibers [58]. The sensor response was linear from 5 pg/mL to 10 ng/mL with a calculated detection limit of 2 pg/mL.

**Table 2** Electrochemical biosensors fabricated using SAPNs

| | Peptide | Immobilization method | Functionalization | Application | References |
|---|---|---|---|---|---|
| **Biomedical** | Boc-Phe-OH<br>H-Phe-OMe | Physical adsorption | Gold nanoparticles<br>Anti-TNF-α antibody | Detection of protein biomarker for tumor necrosis factor α | [58] |
| | (Cyclo[(Gln-D-Leu)4]) | Physical adsorption | Antibody | Detection of *E. coli* cells | [63] |
| | Diphenylalanine | Physical adsorption | Horseradish peroxidase | Hydrogen peroxide | [64] |
| | | Thiol modification and cross-linking with PEI | Glucose oxidase | Glucose and NADH | [26] |
| | | Physical adsorption | | Dopamine detection from PC12 cells | [30, 59] |
| | | Physical adsorption | Folic acid | Cancer cells | [51] |
| | | Cross-linking with glutaraldehyde | | Ethanol and NADH | [65] |
| | | EDC/NHS cross-linking | Glucose oxidase | Glucose | [48] |
| | | Physical adsorption | Horseradish peroxidase | Hydrogen peroxide | [47] |
| | Octapeptides: NSGAITIG | Physical adsorption | | Cu ion detection | [66] |
| | EAK16-II | Physical adsorption | Glucose oxidase | Glucose | [50] |
| **Environmental** | Bolaamphiphile | Dielectrophoresis | TAR-2-Asp | Pb ion detection | [61] |
| | Diphenylalanine | Physical adsorption | Tyrosinase | Phenol detection | [62] |

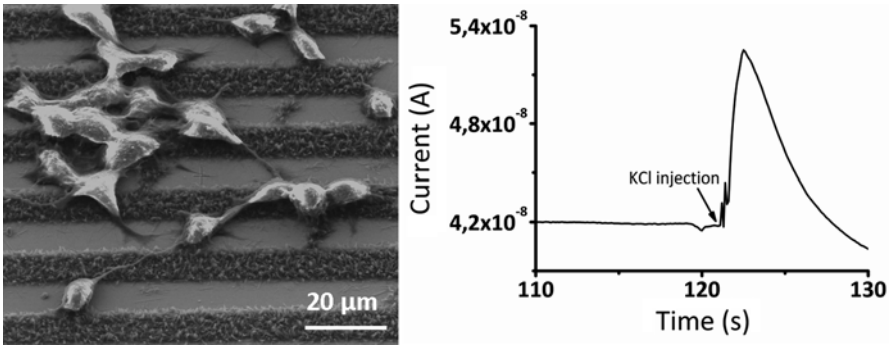


**Fig. 8** Scanning electron microscope image of PC-12 cells on top of vertically aligned peptide nanofibers for the electrochemical detection of dopamine (*left*). Amperometric current-time curve corresponding to the dopamine release from PC12 cells triggered with KCl (*right*) (Reprinted with permission from Taskin, M. et al. Combined cell culture-biosensing platform using vertically aligned patterned peptide nanofibers for cellular studies. ACS Appl. Mater. & Interf. 5 (8): 3323–3328. Copyright (2013) American Chemical Society [30])

Vertically aligned self-assembled peptide nanofibers patterned on a microchip containing gold electrodes were used to fabricate a combined cell culture-biosensing platform for the detection of dopamine released from PC12 cells [30, 59]. The advantage of this combined platform was that it offered a 3D environment mimicking the situation experienced by cells in vivo and at the same time enabled the in situ detection of the neurotransmitter release upon stimulation with KCl, decreasing the loss of the signal due to the diffusion of the sample in the electrolyte.

Figure 8 shows PC12 cells grown on top of vertically aligned peptide nanofibers and the amperometric signal corresponding to the release of dopamine.

Folic acid, a ligand used for targeting cell membranes was deposited on the external wall of self-assembled peptide nanotubes; these functionalized nanotubes were then immobilized on graphene electrodes for the electrochemical detection of cancer cells over-expressing folate receptors [51]. A limit of detection of 250 cells/mL was obtained with the developed biosensor. This sensing platform could be used also with cells infected with parasites causing tropical disease such as leishmaniasis or Chagas disease over-expressing folate receptors.

## Environmental Applications

Lead is a highly toxic heavy metal and environmental pollutant that can be poisonous at very low concentrations [60]. By taking advantage of the high affinity and specificity of some peptides to bind target metals, a Pb ion biosensor could be fabricated by integrating a gold transducer with self-assembled peptide nanotubes able to bind Pb ions and template the growth of Pb crystals via molecular recognition [61]. The biosensor was highly selective, displaying a linear response between 0 and 1 nM $Pb^{II}$, and the signal was not affected by the presence of other heavy metals such as $Hg^{II}$, $Zn^{II}$, $Co^{II}$, or $Cu^{II}$ as shown in Fig. 9.

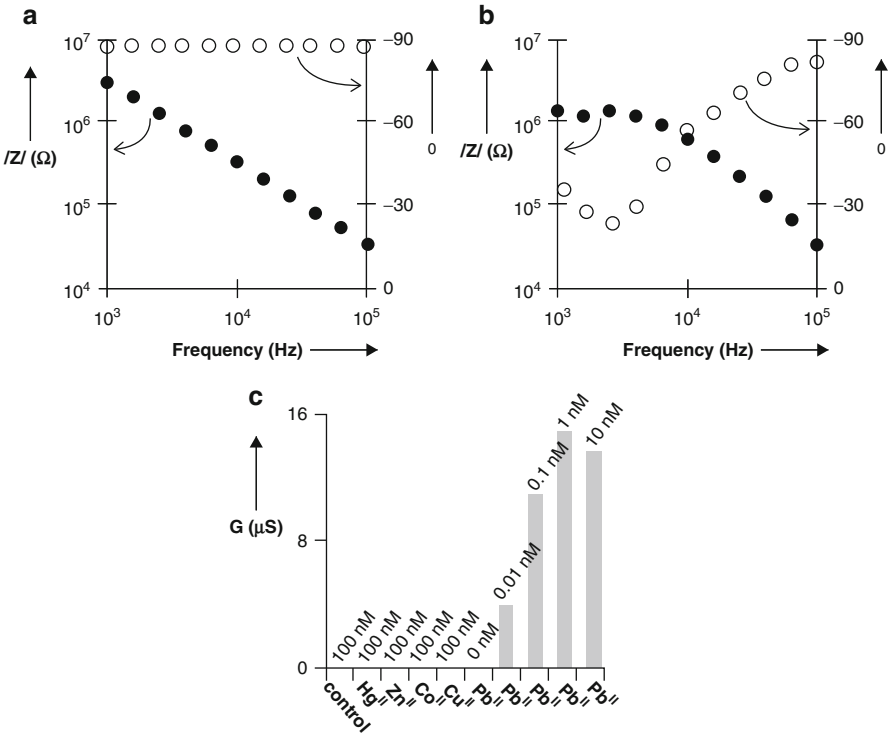


**Fig. 9** Ultrasensitive detection of Pb (II) using an electrochemical biosensor with peptide nanotubes (**a**) in the absence of Pb (II) and (**b**) in the presence of 0.01 nm Pb (II); (**c**) conductance of the peptide nanotubes after incubation with different heavy metal ions (Figure from de la Rica et al. [61] with permission from John Wiley & Sons)

In a study aimed to detect phenol, a graphite electrode was coated with tyrosinase-functionalized diphenylalanine peptide nanotubes [62]. Phenol concentrations as low as 50 nM were recorded using the developed electrochemical biosensor. The deposition of the tyrosinase-functionalized nanotubes resulted in an increased surface area between 0.06 and 0.07 $cm^2$ compared with an unmodified graphite electrode.

## Conclusions and Outlook

Due to its mild fabrication conditions, low-cost synthesis, and easy functionalization, self-assembled peptide nanostructures are being used in the development of electrochemical biosensors. SAPNs can be immobilized on electrochemical transducers using cross-linking agents, physical adsorption or deposited using dielectrophoresis. Different functional molecules are used to decorate the structure of SAPNs in order to improve the sensitivity and selectivity of the biosensing device.

Additionally, SAPNs were integrating with carbon materials (e.g., carbon nanotubes and graphene) and conductive polymers (e.g., PANI, PPY) in order to produce hybrid nanomaterials. Up to now electrochemical biosensors fabricated using SAPNs were applied for the detection of samples in two main areas: biomedical and environmental. Samples such as neurotransmitter metal, ions, and cancer cells among others are some of the compounds detected using this type of electrochemical sensors.

Studies evaluating the immunogenicity and toxicity of SAPNs could accelerate its use in implantable electrochemical biosensors. Additionally, new immobilization methods aiming to produce a more stable layer containing SAPNs together with the biorecognition element will improve the stability and reproducibility of the biosensing platforms and will expand its use in new application fields. Deposition methods such as inkjet print or airbrush will be convenient techniques for the industrial production of SAPNs paper-based electrochemical biosensors for point-of-care devices.

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