

Peptide-based biosensors

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20.1 Introduction

Biosensors are analytical devices incorporating biological sensing elements with the processing power of microelectronics [1,2]. The main components of biosensors are biological recognition elements and physical/chemical transducers. Due to these biorecognition components, biosensors have significant advantages such as high sensitivity, high selectivity, and the ability for high-throughput processing [3,4]. Therefore, they have important applications in various fields such as clinical diagnosis and environmental monitoring. The construction of biosensors mainly relies on the incorporation of bioactive elements, which can be divided primarily into tissues, cells, and proteins [5,6]. These components can maintain high biosensitivity, however, biosensors fabricated with them still suffer from tough acquirement of biological components, short survival time, high cost, and other difficulties, which brought significant obstacles to the practical applications.

In recent years, with the development of biosynthetic technologies, including specific peptide screening and artificial peptide synthesis, the field of biosensor development has seen significant advances. Through standard Fmoc and *t*-Boc solid-phase synthesis, a wide variety of peptide chains with high specificity and versatility can be produced on a large scale and can be custom-made to meet specific requirements. Compared to other biological materials, peptides are stable and active for prolonged periods of time, and can be produced at low cost. Besides, peptides with high specificity can be facilely prepared, modified through mature and diverse methods, and used for rapid detections. Meanwhile, with the development of modern science and technology, peptides can be designed to have specific three-dimensional structures like proteins, thereby having new and improved biorecognition capabilities. As a result, increasing numbers of researchers have set out to develop peptide-based biosensors that can be commercialized.

Generally, the binding activities between peptides and target analytes (such as small molecules, nanoparticles, heavy metals, DNA, and proteins) will induce changes that cannot be easily detected. Thus, transducers or molecular beacons are usually conjugated with peptides to convert these changes into detectable signals, thus facilitating subsequent analysis and applications. Such conjugation will lead to the construction of peptide-based biosensors that combine the properties of peptides and sensors. Depending on the conjugation method, peptide-based biosensors can

be classified into electrochemical biosensors, optical biosensors, and other kinds of biosensors. In the following sections, a brief introduction to the development of each of these types of peptide-based biosensor is provided.

20.2 Peptide-based electrochemical biosensors

As one of the main sensing detection tools in modern analytical science, electrochemical detection technology has been proved to be of great value in the detection of important analytes such as proteins, metabolites, nucleic acids, and metal ions. It is an excellent quantitative detection method, with advantages that include low cost, high reliability, simple operation, and low limit of detection (LOD). Moreover, it provides direct information of the collected electrical signals, while its detection systems can be easily miniaturized and integrated. Hence, sensors based on electrochemical detection have been widely used in many fields including environmental monitoring, health care, and food regulation. Researchers have increasingly focused on the combination of peptides and electrochemical sensors in recent years. Depending on the sensing technology, peptide-based electrochemical biosensors can be divided into sensors operating on differential pulse voltammetry (DPV) mode, square wave voltammetry (SWV) mode, electrochemical impedance spectroscopy (EIS) mode, chronoamperometry mode, cyclic voltammetry (CV) mode, anodic stripping voltammetry (ASV), and so on. Some typical peptide-based electrochemical biosensing systems are described in [Table 20.1](#).

20.2.1 Peptide-based DPV biosensors

As can be seen in [Table 20.1](#), DPV is the most popular technique used in peptide-based electrochemical biosensors. In DPV, the base potential is incremented from the initial potential toward the final potential, with a potential pulse applied on it. Current is sampled before the potential pulse and at the end of the pulse. The difference between the two current samples is recorded as the response. This method can reduce the influence of background interferences such as the redox current induced by impurities. Therefore it gains a higher detection sensitivity and lower detection limit than other electrochemical approaches, which makes it a more sensitive tool in quantitative detection compared with chromatographic methods.

Based on these properties, DPV has advantages such as simultaneous detection of multiple substances, low demand for electrolytes, and less analytes needed. As a result, it has been widely used in the field of analysis, and is mainly used for quantitative determination of a variety of substances, the study of adsorption phenomena, the study of the mechanism of complex electrode reactions, and the detection of trace analytes. For example, Zhao et al. proposed a peptide-based DPV biosensor for the detection of prostate-specific antigen (PSA), which is correlated with prostate cancer [\[7\]](#). As [Fig. 20.1](#) shows, ferrocene-functionalized helix peptides with a specific molecular recognition site for PSA were assembled on the gold electrode

Table 20.1 Typical peptide-based optical biosensors

Sensing technique	Analytes	Peptides	References
DPV	Prostate-specific antigen (PSA)	CHSSLKQK	[7]
	Matrix metalloproteinase-2 (MMP-2)	Specific peptide (PLGVR)	[8,9]
	Protein kinase A	Kemptide or CGGALRRASLG	[10,11]
	Renin	Peptide aptamer (KLASSPLS)	[12]
	The breast cancer marker BRCA1	Zwitterionic peptide (EKEKEKE)	[13]
	Apoptotic cells	FNFRLKAGAKIRFGRGC and AFGNRGRAAKNFHARGC	[14]
SWV	Human papillomavirus DNA type 16	Peptide nucleic acid (AQ-CATACACCTCCAGC-LysNH ₂)	[15,16]
	BIR3 domain of X-linked inhibitor of apoptosis protein (XIAP-BIR3)	AVPFAQKG	[17]
	Caspase-3	Biotinylated DEVD-peptide (biotin-GDGDEV DGC)	[18]
EIS	<i>Escherichia coli</i> O157:H7	Antimicrobial peptide	[19]
	Human noroviral capsid proteins (rP2) and human norovirus	Noro-1 peptide (QHKMHKPHKNTKG GGGSC)	[20]
	Thrombin	Coiled-coil peptide (EKKLAQLEWENQALEKELAQGG-LVPRGS-GGAQLKKLQANKKELAQLWK)	[21]
Chronoamperometry	H ₂ O ₂	Peptide nanofiber (AEAKAEAKY WYAF AEAKAEAK)	[22]
	Phenolic compounds (catechol, phenol, and bisphenol A)	Silk peptide (a partly hydrolysis product of silk protein)	[23]
	Ochratoxin A	NFO4 synthetic peptide (VYMNRKYYK CCK)	[24]
CV	MMP-9	MB-peptide (methylene blue-GPLGMWSRC)	[25]
	Plasmin	KTFKG GGGGGC	[26]
ASV	Caspase 3	Biotinylated DEVD-peptide (biotin-GDGDEV DGC)	[27]

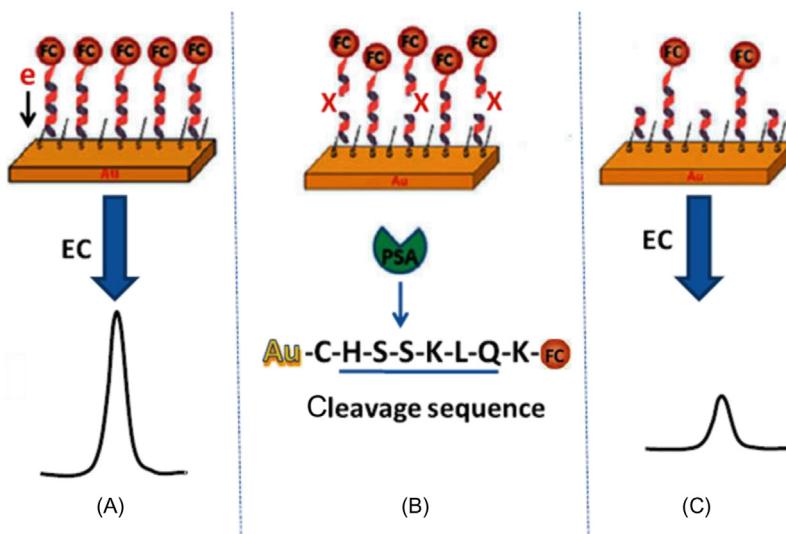


Figure 20.1 Sensing mechanism of the biosensor proposed by N. Zhao et al. (A) Ferrocene-functionalized helix peptides were assembled on a gold electrode surface and the DPV response showed a large current; (B) PSA can recognize the specific sequence on the peptides and cleave at the site; (C) after cleavage of PSA, the cleaved ferrocene and peptide fragments were removed from the electrode surface, resulting in a smaller current than before.

and could be cleaved when there was PSA in the environment. This led to a decrease in the DPV response of the biosensor. With the help of DPV and peptides, the biosensor could linearly respond to the analytes at a concentration range of 0.5–40 ng/mL with a competitive detection limit of 0.2 ng/mL.

Due to the small sizes of peptides, changes induced by interactions between peptides and analytes are usually small. DPV seems to be an ideal electrochemical approach to apply in peptide-based biosensors, as it can eliminate background noises, leading to a large signal-to-noise rate (SNR), and can be applied in the detection of substances with low electrical activity.

20.2.2 Peptide-based SWV biosensors

SWV is also a common detection method which is used for the construction of peptide-based electrochemical biosensors. It takes a rapid scanning stepped voltage as the baseline potential, on each step of which a small symmetric square wave is superimposed. At the end of the forward and reverse steps of the potential, currents are sampled and their differences are plotted against the voltage to obtain an SWV curve. SWV is more effective than DPV in suppressing background currents. Thus, with advantages such as high sensitivity and rapid detection, the method is widely

used in quantitative analyses of substances and kinetic studies, and it can detect organic and inorganic compounds with redox properties.

Wang et al. reported a reagentless SWV-based peptide sensor that detected the BIR3 domain of X-linked inhibitor of apoptosis protein (XIAP-BIR3) [17]. As Fig. 20.2 shows, the bioreceptor was based on a conducting copolymer film electro-synthesized from juglone (JUG) and a juglone–peptide conjugate (JP) that was designed for this experiment. The peptide–protein interactions generated an important increase in steric hindrance at the interface and a current decrease (signal off) of the redox reaction from quinone which was embedded in the polymer backbone. The biosensor allowed a specific and sensitive detection of XIAP-BIR3 with a detection limit of 1 nM. The peptide–protein complex could then be dissociated by adding the free precursor peptide (AVPFAQKG) into the solution, causing a shift-back on the signal, i.e., an increase in the current intensity (signal-on). This “off–on” detection sequence was used in this work as a double verification of the specificity and this approach can be employed as a general way to increase the reliability of results.

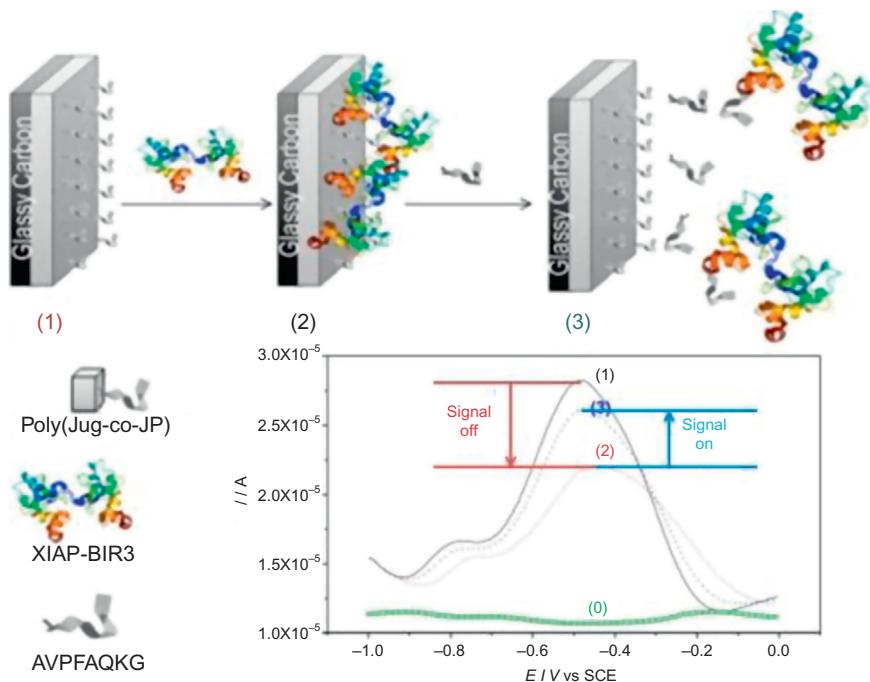


Figure 20.2 Strategy for label-free electrochemical detection of XIAP-BIR3 based on surface hindrance of the poly(JUG-co-JP)-modified electrode and their SWV response curves: (0) bare electrode (open circles); (1) poly(JUG-co-JP)-modified electrode; (2) after complexation with 10 nM XIAP-BIR3 (dash, signal-off); (3) after addition of 10 nM peptide into the assay solution (dash dot, signal-on).

20.2.3 Peptide-based EIS biosensors

EIS has been one of the most common electrochemical detection approaches in recent years. When the electrochemical electrode system is disturbed by an AC signal of a sine voltage (typically <10 mV) or current, a corresponding current or voltage response signal is generated from which the impedance or conductance of the electrode can be obtained. Then, the impedance spectrum can also be calculated from the disturbance of sine signals at a series of frequencies, known as EIS. Due to the small-amplitude disturbance, great impacts on the system are avoided, and an approximate linear relationship is shown between the disturbance and the system responses, which makes the data processing of the measurements straightforward. Responses of EIS can be used to deduce the equivalent circuit of the electrode, by which the kinetic process and excitation of the system can be analyzed. Kinetic parameters, such as capacitances of double electric layers, reaction resistances of the charge transfer process, and parameters of the diffusion mass-transfer process, can also be estimated by relevant element values in the equivalent circuit.

EIS can detect impedance spectra with a wide frequency range to study the electrode system and can provide more information of the kinetics and electrode interface structure than other conventional electrochemical methods. Therefore, EIS is suitable for the rapid detection of electrode processes (mass transfer process, diffusion process, etc.). For example, Kongsuphol et al. fabricated an ultra-sensitive, disposable, electrochemical thrombin biosensor with a comb-structured gold microelectrode array (CSGMA), which was functionalized with a self-assembled monolayer of thiol terminated coiled-coil peptide (CCP) [21]. Containing a thrombin-specific cleavage site (LVPRGS), the CCP provided sites for thrombin capture and detection, as shown in Fig. 20.3. The EIS technique was chosen to detect thrombin at different concentrations. Results revealed the sensor can respond to thrombin with a LOD of 28 fM and was able to detect the catalytic activity of thrombin within 30 min. CCP/CSGMA electrodes were found to be selective against other antibodies such as the p-53 antibody (clone DO1) and the hemagglutinin (HA) antibody. Thus, the sensor provided high specificity toward thrombin detection and mechanistic details of the binding and cleavage process.

Although EIS is a rapid detection tool in electrochemical sensors, it shows lower sensitivity and larger background noises than DPV and SWV. Thus, it needs more effort to be applied in peptide-based electrochemical sensors.

20.2.4 Peptide-based chronoamperometry biosensors

As the simplest method in electrochemical detection, chronoamperometry is a potential-controlled analytical method that measures the functional relationship between current responses and time by applying a single-potential step or a double-potential step to the working electrode. It is often used in research on electron transfer kinetics. With general usage of electrodes of fixed area, chronoamperometry is suitable to study the electrode process coupling chemical reactions and the reaction mechanism of organic electrochemistry.

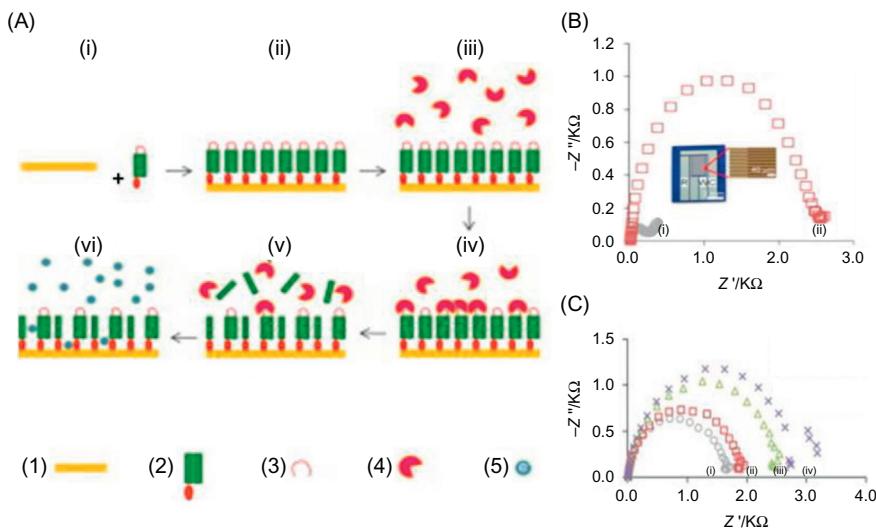


Figure 20.3 (A) Scheme of the sensor fabrication and thrombin detection mechanism. Symbols represent (1) gold electrode, (2) thiol-terminated CCP, (3) thrombin-specific cleavage site, (4) thrombin, and (5) redox molecule. (B) EIS verification of (i) CSGMA electrode, (ii) CCP/CSGMA electrode. Inset shows the optical image of a CSGMA electrode chip. (C) Nyquist plot of (i) CCP/CSGMA, (ii) 10 pg/mL p-53 antibody (clone DO1), (iii) 100 fg/mL thrombin, and (iv) 10 pg/mL DO1 + 100 fg/mL thrombin.

Li et al. designed a high-performance electrochemical hydrogen peroxide (H_2O_2) biosensor based on motif-designed peptides. These peptides form nanofibers (PNFs) and recognize graphene quantum dots (GQDs) and graphene oxide (GO) nanosheets specifically [22]. By designing the peptide sequence, ternary GQD-PNF-GO nano-hybrids were synthesized and applied to modify the working electrodes for the detection of H_2O_2 with the chronoamperometry method (Fig. 20.4). The biosensor exhibited high sensitivity and selectivity, a low detection limit, and wide linear range for sensing H_2O_2 , demonstrating the usefulness of peptides as biosensors.

The chronoamperometry approach is simple and easy-to-perform, however the method provides lower sensitivity and less information for biosensing detection compared with other electrochemical tools. As a result, it acts more like a quick test to investigate the performance of peptides in biosensors.

20.2.5 Other peptide-based electrochemical biosensors

Besides the methods introduced above, there have also been other electrochemical tools applied in peptide-based biosensors. For example, Lee et al. constructed a biosensor for detection of matrix metalloproteinase-9 (MMP-9) with CV [25]. As shown in Fig. 20.5, the biosensor was integrated with a methylene blue (MB)-labeled peptide, which was specific to MMP-9. Peptide cleavage by the protease caused the release of electroactive MB from the gold electrodes and

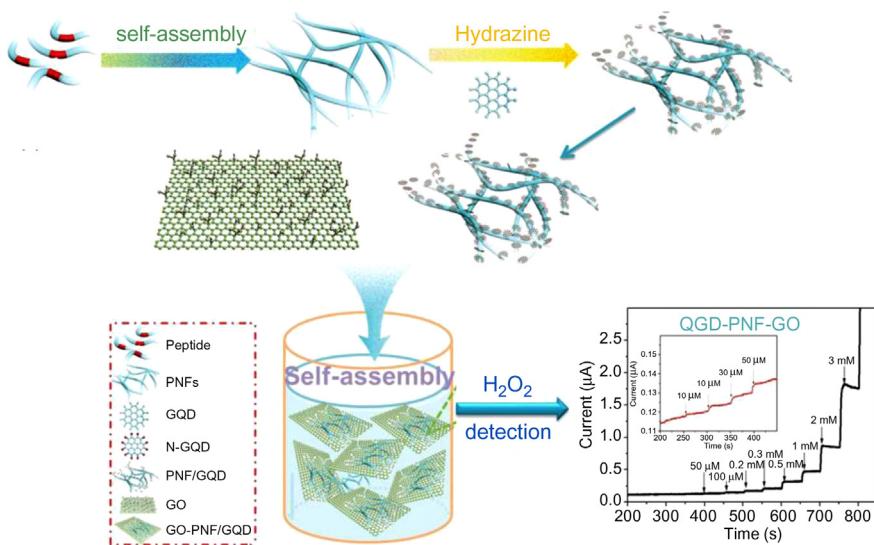


Figure 20.4 Schematic representation for the synthesis of PNFs, binary GQD-PNF nanohybrids, and ternary GQD-PNF-GO nanohybrids, and the chronoamperometry responses to different doses of H₂O₂ detected with GQD-PNF-GO.

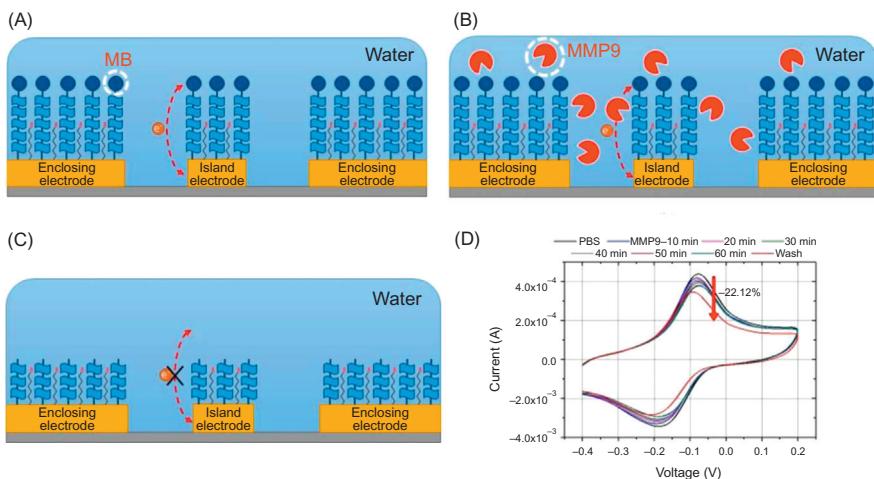


Figure 20.5 Sensing mechanism of the biosensor proposed by Lee et al. and its CV responses to MMP-9. (A) Tunneling of electrons in the presence of MB-peptide on an Au electrode. (B) Peptide cleavage reaction of the MB-peptide with MMP-9. (C) Decrease in the current of electron tunneling after the peptide cleavage reaction. (D) CV results after incubating an MB-peptide immobilized electrode challenged with 1 nM MMP-9.

resulted in a decrease in the electrical tunneling current. As CV can determine changes to voltage and current during the electrolysis process through repeated scanning with a triangular potential at different rates, this method was chosen to carry out quantitative and qualitative analysis of thrombin. With the help of a self-designed gold electrode with self-gating effect, the electrochemical biosensor exhibited a linear correlation between the concentration of MMP-9 and the current reduction in a range from 1 pM to 1 nM, and the LOD was determined to be at 7 pM.

In another study by Zhang et al., ASW was introduced to a biosensing platform for the determination of caspase 3 activity and inhibition using a DEVD-peptide and quantum dots (QDs) [27]. As shown in Fig. 20.6, a gold electrode was immobilized by a biotinylated peptide which contained the specific cleavage site (DEVD) of caspase 3 and then blocked by 6-mercaptop-1-hexanol (MCH). Subsequently, the electrode was immersed into apoptotic cell lysates containing active caspase 3, which could specifically recognize and cleave the peptide. This procedure led to the loss of the biotin label on the electrode surface. The ratio of the uncleaved biotin-DEVD on the electrode surface was related to the activity of caspase 3 and evaluated by a signaling probe composed of CdTe QDs, carbon nanotubes (CNTs), and

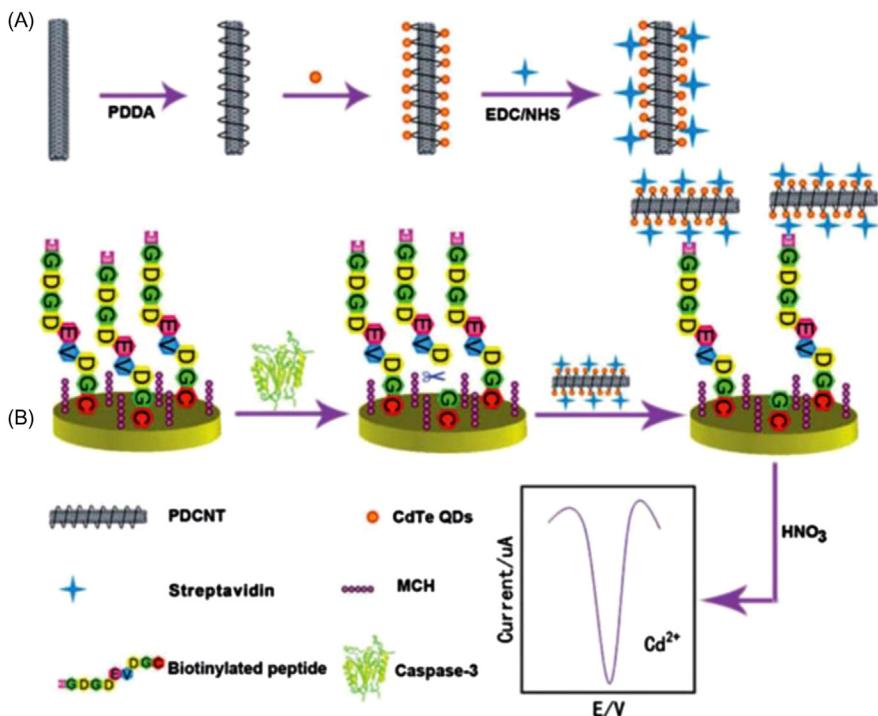


Figure 20.6 (A) Preparation process of CNT-QD-SA bioconjugates. (B) Schematic illustration of the ASV strategy for sensing caspase 3 activity.

streptavidin (SA) that can be captured by the uncleaved biotin-DEVD. Finally, the cadmic component obtained by subsequent dissolution of the electrode in HNO_3 can be quantified by ASV. The ASV method is suitable for detection of metal elements in dilute solutions. This electrochemical strategy allowed for the study of drug action in activating caspase 3 within a short time and at a low concentration, presenting a significant tool for efficient screening of potential caspase 3 inhibitors and anticancer drugs.

Although the sensors introduced above can also be used effectively in detection, electrochemical techniques in general are not widely applied due to difficulties in controlling the experimental conditions. However, taking all the electrochemical tools together, the peptide-based electrochemical sensors have broad potential in various fields because of their advantages such as high sensitivity, accurate quantitation, and simple operation. The significant progress in the field of portable electrochemical detection systems suggests that peptide-based electrochemical biosensors will likely see rapid development.

20.3 Peptide-based optical biosensors

Compared to electrochemical techniques, optical tools seem to be more popular in the development of peptide-based biosensors. With the help of molecules with significant optical characteristics, peptide-based optical biosensors are easier to construct than electrochemical ones. According to their different sensing technology, peptide-based optical biosensors can be mainly divided into fluorescence biosensors, colorimetric biosensors, surface plasmon resonance (SPR) biosensors, and surface-enhanced Raman scattering (SERS) biosensors. Some typical peptide-based optical biosensing systems are described in [Table 20.2](#).

20.3.1 Peptide-based fluorescence biosensors

Biosensors utilizing fluorescence signals represent the most common approach for the development of peptide based biosensors. This is due to the fact that the technology for the detection of fluorescence emitted by peptides tagged with a fluorescent probe is well established and optimized. There are several modes of this technology including fluorescence “turn-on” techniques, fluorescence resonance energy transfer (FRET), fluorescence quenching, fluorescence enhancement, and fluorescence lifetime imaging microscopy (FLIM).

Fluorescence “turn-on” indicates a process like turning on a light. The general preparation of such a biosensor is to modify each end of a peptide with a fluorescent probe and a fluorescent quencher. When target analytes are present in the solution, they will interact with the peptides, which results in separation of the probe and the quencher, leading to the “turning-on” of fluorescence. For example, Wang et al. reported a novel intracellular protease fluorescence “turn-on” sensor based on the nanoconjugate of GO and peptide substrates [30]. Apoptosis-related caspase-3 activation was chosen as the case study. GO is a carrier for delivering peptide

Table 20.2 Typical peptide-based optical biosensors

Sensing technique	Signal strategy	Analytes	Peptides	References
Fluorescence	Fluorescence turn-on	Cyclin A	H ₂ N-AKRRRLI-Dap(4-DMAP)-E	[28]
		Broad-spectrum cancer-related protein LAPTM4B	IHGHHIISVG (referred to as AP2H)	[29]
		Caspase-3	DEVD	[30]
		Human carbonic anhydrase II (HCAII)	KE2b-D(15)C(25)-5, a specific designed polypeptide	[31–33]
		Protein tyrosine kinases (PTKs)		[34]
		Protein kinase activity		[35]
	Fluorescence quenching	Matrix metalloproteinase-2 (MMP-2) and MMP-9	A triple-helical peptide with the type V collagen sequence GPPG ~ VVGEKGEQ	[36]
		Alkaline phosphatase (ALP)	Fmoc-K(FITC)FFYp	[37]
		Protease activity	CCAAAA	[38]
		Human immunodeficiency virus type 1 (HIV-1) matrix protein p17	CEKIRLRC	[39]
	Fluorescence enhancing	Protein kinase activity	Phosphorylated peptide	[40,41]
		Src and ab1 PTKs		[42]

(Continued)

Table 20.2 (Continued)

Sensing technique	Signal strategy	Analytes	Peptides	References
	FRET	Lipopolysaccharide (LPS, also known as bacterial endotoxin)	Tamra-QVPAQLLVGALRVLAYSRLK-εK(αFluo)-NH ₂ or Tamra-QVAKLLVKALRKLAYKRLK-εK(αFluo)-NH ₂	[43]
		Hg	GGTLAVPGMTCAACPITVKKGGW-CONH ₂	[44,45]
		Protein kinase C α (PKC α)	FKKQGSFAKKK-NH ₂	[46]
	FLIM	Abl kinase	GGEAIYAAPC _{Cys5} GGRKKRRQRRRPQ	[47]
Colorimetric	Color changes/density	Matrix metalloproteinase-7 (MMP-7)	HHHHHHRPLALWRSC	[48]
		HCAII		[49]
		<i>Escherichia coli</i> O157:H7 (<i>E. coli</i> O157:H7)	NH ₂ -Ahx-KVSRRRRGGDKVDRRRRGD-Ahx-Cys	[50]
		Zn ²⁺ and chymotrypsin	CCPGCAR-NH ₂ , CAYRA-NH ₂ , etc.	[51]
		Thrombin	CLVPRGSC	[52]
SPR	Refractive angle	His-tagged proteins	3-Mercaptopropionyl-LHDLHD	[53]
		Staphylococcal enterotoxin B (SEB)	LLADTTTHRPWTLLADTTTHRPWT	[54]
		Leukocyte function-associated antigen-1 (LFA-1)-derived peptides	CD11a _{237–261} (ITDGEATDSGNIDAQDIIRYIIGI) and CD11a _{456–465} (APLFYGEQRG)	[55]
SERS	Raman intensity and wavelength shift	Phosphorylation catalyzed by Abl kinase	EAIFYAAPVAKKKGGGGC	[56]
		Heparin, trypsin, and thrombin	RKGSGRRLVKC, RCFRGDD, and RCFLVPRGSDD	[57–59]

cargoes inside living cells. Therefore, the peptide is cleaved from the GO–peptide conjugate after being transported into cells by intracellular proteases. This results in a significant increase of the fluorescence emission due to the release of the fluorophore from GO.

Based on intracellular delivery of GO–peptide conjugate and caspase-3-mediated cleavage of substrate peptide, a sensitive, simple, and robust intracellular protease sensor for high-contrast imaging of apoptotic signaling in live cells was developed (Fig. 20.7A). Assays were performed using mixtures of GO–peptide conjugate and caspase-3 of varying concentrations to demonstrate the quantitative nature of the protease sensor (Fig. 20.7B). The fluorescence peaks dynamically increased with increasing caspase-3 concentration in the range from 7.25 to 362 ng/mL. A plot of fluorescence at the maximum emission wavelength of 520 nm versus caspase-3 concentration revealed a linear response characteristic of the protease sensor, with a readily achieved detection limit of 7.25 ng/mL.

The fluorescence quenching mode is a process which involves two submodes: self-quenching and probe-pair quenching. These two submodes can be simply distinguished based on the quenching mechanism. For self-quenching, Oh et al. proposed a peptide beacon sensor for detection of human immunodeficiency virus type 1 (HIV-1) matrix protein p17 with a self-quenching fluorescence at 475 nm (Fig. 20.8A) [39]. The excimer-based PB architecture is composed of two *N*-(1-pyrenyl)maleimides conjugated to a recognition peptide. Intramolecular collisions and, perhaps, hydrophobic affinity occurring in the absence of target (left), allow for excimer formation and thus longer wavelength excimer emission. Upon target binding (right), the pyrene moieties are segregated, thereby eliminating the longer wavelength excimer emission. Complex formation between the PB (3 nM) and its target (here an antibody directed against the HIV protein p17) results in a twofold decrease in excimer emission (Fig. 20.8B). In the case of probe-quencher quenching mode, Gu et al. reported a novel fluorescent “turn-off” biosensing platform for protease activity assay using peptide-templated gold nanoclusters (AuNCs) [38]. As shown in Fig. 20.8C, the AuNCs synthesized using the peptides as templates formed a core–shell structure through interactions between the amino acids

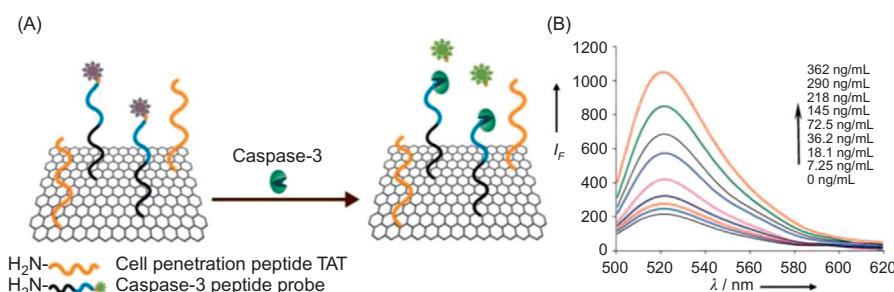


Figure 20.7 (A) Caspase-3 detection using GO–peptide conjugate. (B) Fluorescence spectral responses of the conjugate of GO with caspase-3-specific substrate peptide to caspase-3 of varying concentrations.

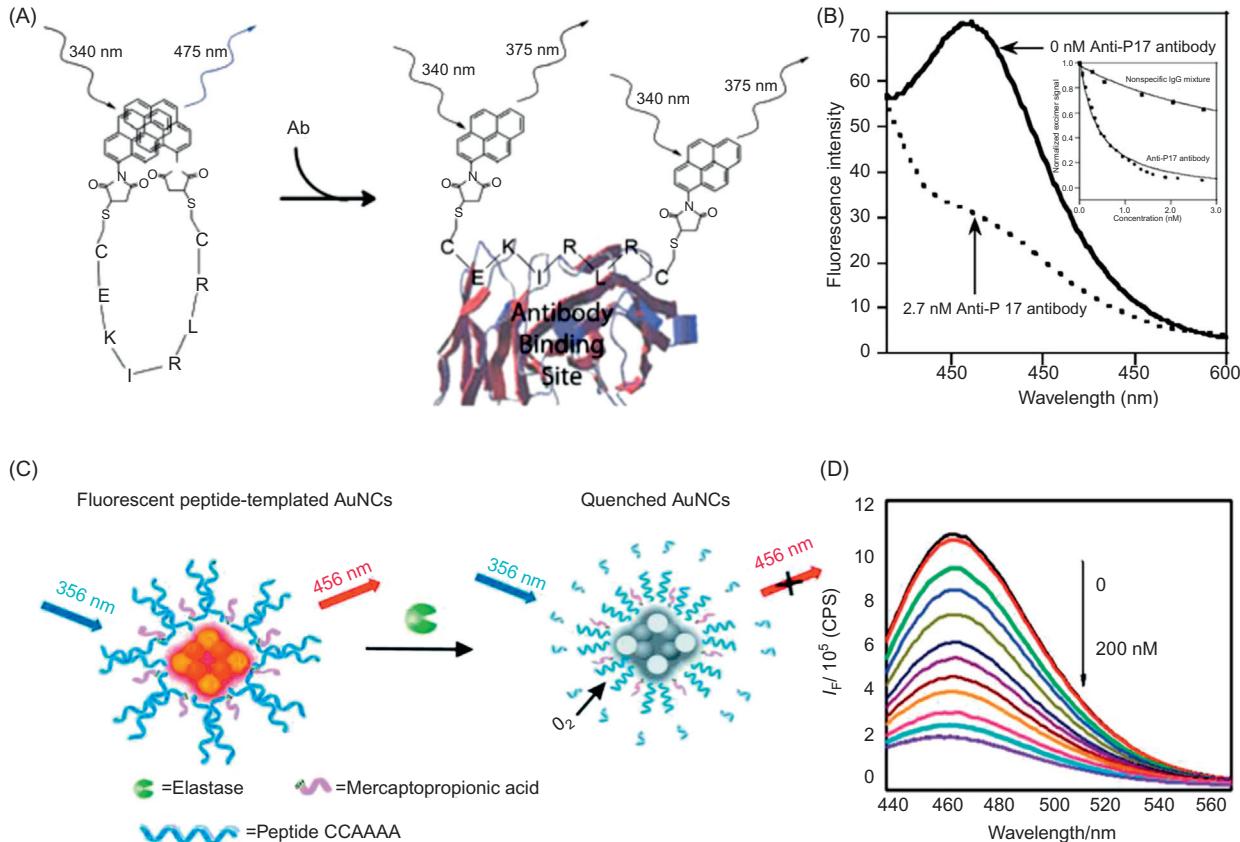


Figure 20.8 (A) The excimer-based peptide beacon sensor composition and recognition mechanism of antibody. (B) The fluorescent response of the biosensor to 2.7 nM Anti-P7 antibody. (C) Schematic illustration of the sensing mechanism for proteases using peptide-protected AuNCs. (D) Fluorescence responses of peptide-AuNCs to different concentrations of elastase (range from 0 to 200 nM).

and the AuNC core. The peptides acted as a compact coating layer, protecting the AuNCs from O₂-mediated fluorescence quenching. Cleavage of the substrate peptides by elastase removed the protective peptide coating, which allows O₂ to diffuse and interact with the AuNC cores and quench their fluorescence in a concentration-dependent mode (Fig. 20.8D).

Fluorescence enhancement, or sensitized fluorescence, is different from fluorescence “turn-on” as its initial fluorescence intensity is not zero. Shults and Imperiali introduced a versatile fluorescence-enhanced peptide probe of protein kinase activity (Fig. 20.9) [40]. The probe can be modified to target a desired kinase by changing the kinase recognition motif in the peptide sequence. The reporter motif contains the Sox amino acid, which generates a fluorescence signal when bound to Mg²⁺ when it is present in the reaction mixture. The phosphorylated peptide exhibits a much greater affinity for Mg²⁺ than its unphosphorylated analogue, resulting in greater fluorescence intensity. Product formation during phosphorylation by the kinase was easily followed by the increase in fluorescence intensity over time. These probes exhibited a three- to fivefold increase in fluorescence intensity upon phosphorylation, the magnitude of which depended on the substrate. Peptides containing the reporter functionality were phosphorylated on serine by protein kinase C and cAMP-dependent protein kinase and were shown to be good substrates for these enzymes.

Generally speaking, FRET is an energy transfer phenomenon taking place between two fluorescent molecules. Nonradioactive energy transfer will appear when the emission spectrum of the fluorescent donor molecule overlaps with the absorption spectrum of the fluorescent receptor molecule and the distance between the two moieties is within 10 nm. The FRET phenomenon results in significantly decreased fluorescence intensity of the donor while the emission intensity of the receptor is greatly enhanced. In the field of life sciences, FRET is a powerful tool for detection of the nanoscale distance and distance changes between large biomolecules *in vivo* and can be used to detect the direct interaction of two protein molecules inside the cell.

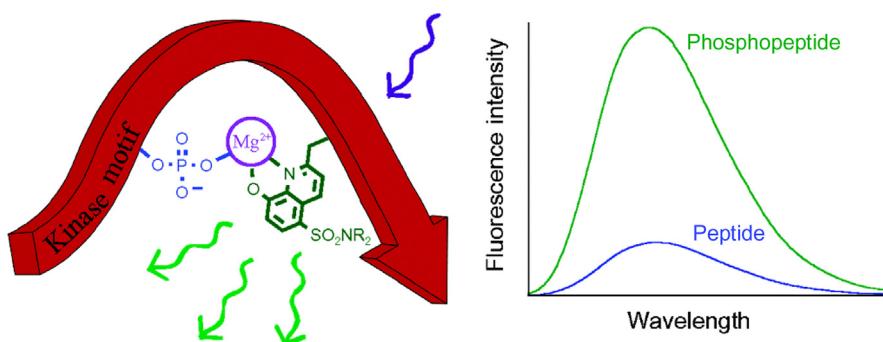


Figure 20.9 Schematic of the sensing mechanism of the fluorescence-enhanced peptide probe and its fluorescence response curve.

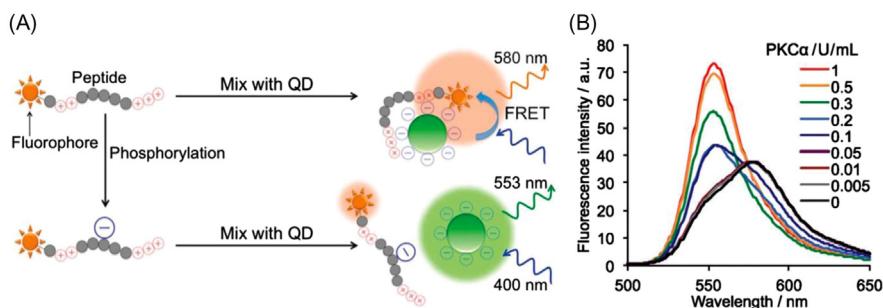


Figure 20.10 (A) Schematic illustration of the FRET-based detection principle. QDs and fluorophore-modified peptides electrostatically interact to induce FRET. (B) PKC α concentration-dependent emission spectra of peptide S–QD mixtures after the phosphorylation reaction with ATP.

Based on FRET, Shiosaki et al. developed a novel antibody-free assay using QDs without any surface modification [46]. As shown in Fig. 20.10A, a cationic substrate peptide modified with a fluorophore interacts electrostatically with anionic QDs causing FRET from the QDs to the fluorophore. In contrast, a phosphorylated peptide with reduced cationic charge due to the anionic phosphate group cannot induce efficient FRET. This simple assay was applied for the evaluation of protein kinase C α (PKC α) inhibitors. The phosphorylation reaction of peptide was carried out at various PKC α concentrations for 60 min at 30°C. The resulting solution was mixed with a solution containing QDs and it was allowed to interact for 15 min before measuring the emission spectra. Fig. 20.10B shows the emission spectra of the solutions. The QD fluorescence intensity increased with increasing PKC α concentration.

The fluorescence lifetime of the fluorescent probes can also be used for monitoring biological processes. This phenomenon is called FLIM. Fluorescence lifetime refers to the average time that a molecule can stay in an excited state upon excitation with light pulses before it returns to the ground state. Fluorescence lifetime depends on the microenvironment of the fluorescent molecules. Some researchers believe that FLIM can be more reliable than monitoring fluorescence intensity. Damayanti et al. reported a cell-penetrating peptide biosensor for dynamic monitoring of phosphorylation by Abl kinase [47]. FLIM, which is not confounded by photobleaching or cellular autofluorescence, was employed to detect phosphorylation-dependent fluorophore lifetime shifts (1–2 ns) in living cells (Fig. 20.11). The authors of this work studied the dependence of the fluorophore lifetime shift on phosphorylation by Abl kinase, mapped the fluorophore intensity and lifetime components to quantify subcellular phosphorylation, and monitored kinase inhibition in real time. This approach, which can be used for the study of other kinases, provides a new method for interrogating real-time, subcellular signalling activities in cell populations that are not amenable to expression of genetically engineered biosensor proteins.

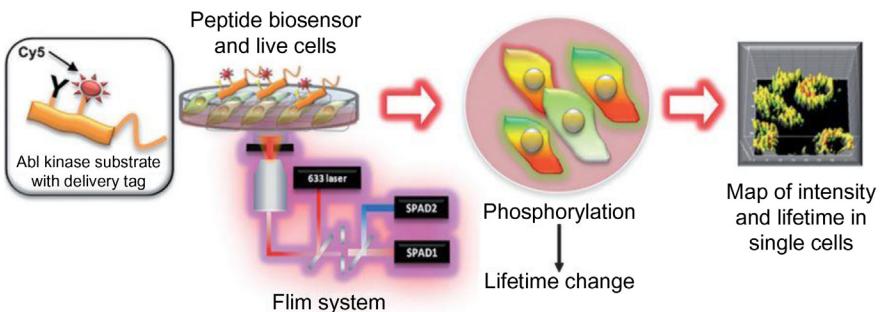


Figure 20.11 FLIM to detect phosphorylation-dependent fluorophore lifetime shifts for biosensors in living cells. A fluorophore-labeled peptide substrate is delivered to living cells by a cell-penetrating peptide. Phosphorylation of the substrate results in increased fluorophore lifetime by FLIM.

It can be seen from the above that there are various kinds of peptide-based fluorescence biosensors, which can be used for different occasions and requirements. However, the quantitative detection of fluorescence is not so convenient, thus demanding the cooperation of portable detection equipment with these sensors to expand its follow-up applications.

20.3.2 Peptide-based colorimetric biosensors

Colorimetry is an approach that determines the content of the target analytes by measuring or comparing colored substance solutions. It is based on Lambert's law and depends on the generation or cooperation of colored compounds to complete the colorimetric detection. Aili et al. described a novel strategy for the colorimetric sensing of proteins, based on peptide-functionalized gold nanoparticles [49]. Recognition is accomplished using a polypeptide sensor scaffold designed to specifically bind to the model analyte, human carbonic anhydrase II (HCAII). The extent of particle aggregation, induced by the Zn²⁺-triggered dimerization and folding of a second polypeptide which is also present on the surface of the gold nanoparticles, gives a readily detectable colorimetric shift that is dependent on the concentration of the target protein. In the absence of HCAII, particle aggregation results in a significant red-shift of the plasmon peak, whereas analyte binding prevented the formation of dense aggregates, significantly reducing the magnitude of the red-shift. The versatility of the technique is demonstrated using a second model system based on the recognition of a peptide sequence from the tobacco mosaic virus coat protein (TMVP) by a recombinant antibody fragment (Fab57P). Concentrations down to 10 and 25 nM are detected for HCAII and Fab57P, respectively. This strategy is proposed as a generic platform for robust and specific protein analysis that can be further developed to monitor a wide range of target proteins (Fig. 20.12).

Peptide-based colorimetric biosensors are mainly based on the combination of peptides and chromogenic nanomaterials, for example, gold or silver nanoparticles.

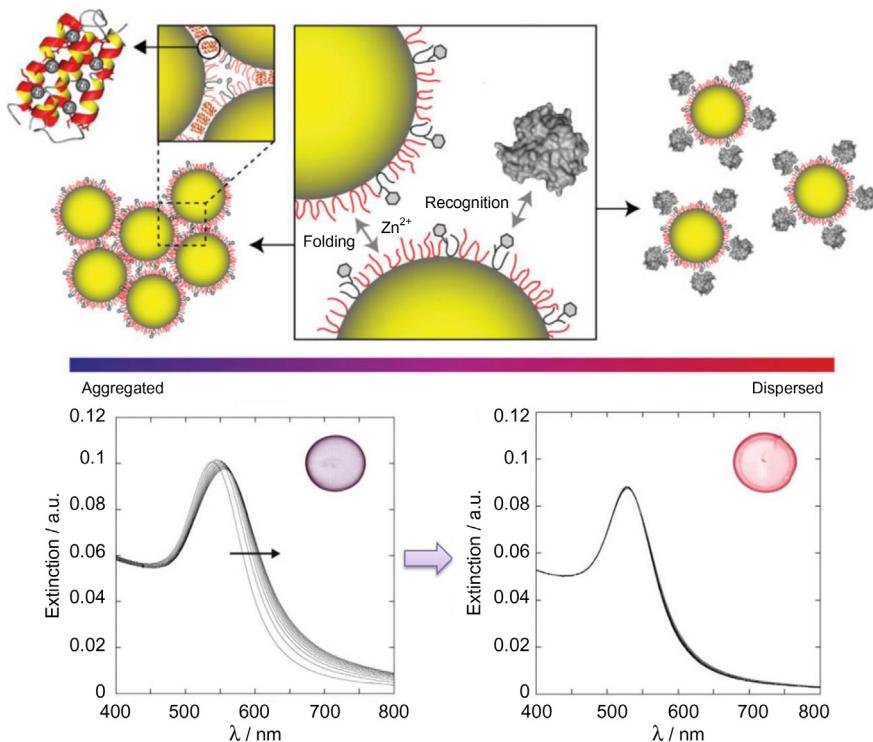


Figure 20.12 Gold nanoparticles were modified with a peptide designed to allow folding-induced particle aggregation triggered by Zn²⁺ and a polypeptide-based synthetic receptor for binding of protein analytes. In the absence of the analyte, addition of Zn²⁺ triggers dimerization and folding, resulting in particle aggregation and a rapid color shift of the suspension from red to purple. Analyte binding precludes aggregation and the dispersion remains red. Analyte binding can thus easily be determined even by the naked eye. The two spectra were recorded with 2-min intervals for 20 min after exposing the particles to 10 mM Zn²⁺ (pH 7), in the absence (left) and in the presence (right) of HCAII. The concentration of HCAII was 70 nM after dilution in the Zn²⁺-containing buffer. The insets show the corresponding suspensions after being dried on nitrocellulose membranes.

Compared with the fluorescence method, it has many advantages such as ease of the method, simple detection procedure, low cost, and ease of miniaturization. However, sensitivity is still its demerit, and more ingenious sensor designs are needed to improve its detection accuracy.

20.3.3 Peptide-based SPR biosensors

SPR is an opto-physical phenomenon with high sensitivity and high specificity. When a beam of P-polarized light is incident on the prism side within a certain angular range, a surface plasma wave can be generated at the interface between the prism and

the metal film (Au or Ag). When the propagation constant of the incident light matches that of the surface plasma wave, free electrons in the metal film will resonate, namely SPR. In biosensing detection, biological recognition molecules will be immobilized on the surface of the sensor chip, and can interact with target molecules. This can cause refractive index changes to the metal film surface, which ultimately leads to changes in the SPR angle. Through detection of the SPR angle changes, information on the analytes, such as dose, affinity, kinetic constants, and specificity can be obtained.

Dudak et al. developed a label-free biosensor based on SPR to evaluate the potential of peptide ligands as biorecognition molecules for the detection of bacterial toxins [54]. A 24-mer peptide, previously identified from a phage display library and modified for better binding was used for the detection of staphylococcal enterotoxin B (SEB). The peptide was immobilized covalently onto a gold-coated surface modified with self-assembly monolayer. The LOD for SEB was found to be 20 µg/mL. The selectivity of the peptide-based sensor for SEB was verified by injecting bovine serum albumin (BSA) and the sensor response for the BSA was negligible. Further, to probe the effectiveness of the sensor in complex food environments, the binding phenomenon was tested using milk samples spiked with SEB and the results showed that the system can be used in complex food matrices. The results of this study demonstrate the potential of peptides as recognition agents for detecting bacterial toxins using biosensors (Fig. 20.13).

Due to the sensitivity of the method, SPR can be used for the construction of peptide-based biosensors. Refraction angle changes appear to be more rapid and sensitive than wavelength shifts of optical spectra, which is an important factor for precise quantification. However, the SPR methodology is more complex.

20.3.4 Peptide-based SERS biosensors

SERS refers to the approach in which the sample is adsorbed on the surface of colloidal metal particles (e.g., gold, silver, or copper) or on the rough surface of sheet metal and then the sample is measured by conventional Raman spectroscopy. Due to the electromagnetic field enhancement of the surface or near surface of the

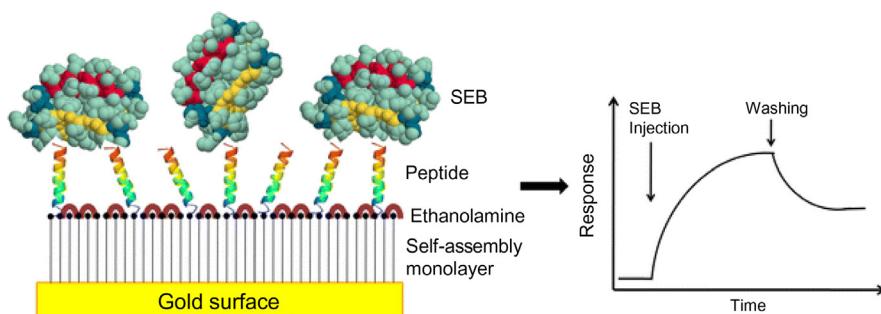


Figure 20.13 Schematic illustration of the peptide-based SPR sensor system for detection of SEB.

sample in the excitation region, SERS can increase the detection of Raman intensity by 10^3 – 10^6 times and can provide structural information that is not easily accessible through conventional biophysical methods of analysis. SERS has been widely used in conformational and structural studies of biomolecules.

Hu's group has developed a series of peptide-based SERS biosensors with 4-mercaptopbenzoic acid (4-MBA)-labeled gold nanoparticles (AuNPs). The method employed aggregation of 4-MBA-modified AuNPs by peptides after treatment with target protease. SERS signals of 4-MBA are sharply decreased or increased because of the increased or decreased electrostatic stability of AuNPs. Through this strategy, facile “turn-off” or “turn-on” SERS biosensors for proteases can be established with sensitivity, selectivity, and simplicity. Compared with other methods, this newly proposed method has improved sensitivity and can be easily adapted for the detection of many proteases. The method demonstrated the capability for application in complex matrix samples. The results also illustrated the potential and superiority of SERS biosensors as a general approach for proteases based on enzyme activity [57–59] (Fig. 20.14).

20.4 Other peptide-based biosensors

The two techniques described above are the most important techniques used in peptide-based biosensors. However, other technologies have also been developed such as electrochemical luminescence (ECL), microcantilever-based biosensors, quartz crystal microbalance (QCM), and field-effect transistors (FET). Some typical such peptide-based biosensing systems are described in Table 20.3.

ECL is a specific luminescence reaction initiated by electrochemical phenomena on the electrode surface involving both electrochemistry and chemiluminescence. Advantages of ECL include the ease of operation and imaging, fast analysis, high throughput, and ease of automating the process. ECL has been widely used in the fields of biomedicine, food safety, environment monitoring, and clinical diagnostics. Wu et al. proposed a simple and novel “signal-on” ECL biosensor for the detection of PSA. The system is composed of multiwalled carbon nanotubes (MWCNTs), polyamidoamine (PAMAM) dendrimers, and Au nanoparticle (NP) film on a glassy carbon electrode (GCE) to improve the electron transfer, provide abundant amine group for the immobilization of biomolecules, and amplify the ECL signal. Au nanorod (Au NR)-labeled peptide is modified on the electrode surface to serve as an ECL-RET acceptor due to the excellent overlap between the ECL emission spectrum of Ru(bpy)₃²⁺ and the absorption spectrum of Au NRs, leading to a significant reduction of the ECL signal. Upon cleavage of the peptide by PSA, both Au NRs and peptides are released from the electrode surface, resulting in the high recovery efficiency of the ECL signal. The proposed approach exhibits a wide linear range from 0.1 pg/mL to 10 ng/mL with a detection limit of 0.03 pg/mL. The results revealed that the recoveries were between 95% and 108%, indicating good accuracy of the method

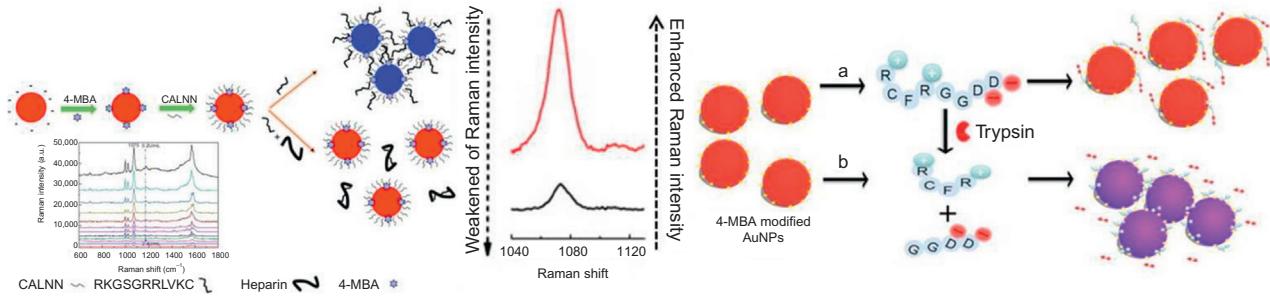


Figure 20.14 Schematic diagram of SERS-based enzymatic assay for heparin and trypsin.

Table 20.3 Other typical peptide-based biosensors

Sensing technique	Analytes	Peptides	References
Electrochemical luminescence	Cyclin A ₂ (CA2)	RRLIFGGGGG & TGQSWPESLIRKTGYTLESLKPCLMGGGGG	[60]
	PSA	CHSSKLQK	[61,62]
	MMP-2	CGPLGVRGK	[63]
	Casein kinase II (CK2) and cAMP-dependent protein kinase (PKA)	CRRRADDSDDDDD and CLRRASLG	[64]
QCM	Rheumatoid arthritis (RA)	A 14-mer cyclic filaggrin peptide derived from the amino acid sequence deduced from cDNA of human filaggrin	[65]
	Hepatitis B virus	5'-Bio-OO-TCCTTTTT-OOO-TTTTTTCCT-Lys	[66]
	Recombinant human interferon-β	NH ₂ -IAASLQESKQVVAH-OH and NH ₂ -IAASLEESKEVVAH-OH	[67]
Microcantilever	Vancomycin	CGGGG-L-Lys-d-Ala-d-Ala	[68]
	Breast cancer cells	Peptide 18-4 (WxEAYQrFL)	[69]
FET	Streptavidin	IMVTESSDYSSY	[70]
	Trinitrotoluene (TNT)	HSSYWYAFNNKTGGGGWFVI	[71]

for PSA detection. In addition, the biosensor exhibited specificity for the detection of PSA [62] (Fig. 20.15).

In QCM the resonance frequency of the quartz crystal will change depending on the size and mass of the molecules deposited on the surface. QCM can detect very small mass changes on the surface of the sensor in real time and is commonly used for detection of unlabeled molecules and the study of biomolecular interactions. Drouvalakis et al. demonstrated a label-free peptide-coated carbon nanotube-based immunosensor for the direct assay of human serum [66]. A rheumatoid arthritis (RA)-specific (cyclic citrulline-containing) peptide was immobilized onto functionalized single-walled carbon nanotubes deposited on a QCM sensing crystal. Serum from RA patients was used and antibody binding was detected by QCM sensing. Specific antibody binding was determined by comparing the assay of two groups (normal and diseased sera) and the native unmodified peptide. The sensitivity of the nanotube-based sensor (detection in femtomolar range) was higher than that of the established enzyme-linked immunosorbent assay (ELISA) and recently described microarray assay systems, detecting 34.4% and 37.5% more RA patients with anticitrullinated peptide antibodies than those found by ELISA and microarray, respectively. The performance of their label-free biosensor enables its application in the direct assay of sera in research and diagnostics (Fig. 20.16).

In the case of the microcantilever technology, the resonance frequency of the microcantilever shifts in response to mass loading from molecular interaction as in the case of any other biosensors. Through vibration changes, the microcantilever platform provides information about receptor–ligand, antibody–antigen, or enzyme–substrate interactions. Its dynamic range is large, with a resonant frequency ranging from tens of hertz to several megahertz and it can perform analyses with high resolution. In the study of Bai et al. a reversible detection method for vancomycin was developed utilizing the cantilever array sensor functionalized by a designed peptide consisting of a cysteine (Cys-), a space linker (-GGGG-), and a molecular recognition ligand (-L-Lys-D-Ala-D-Ala) [68]. It was found that the peptide space linker was necessary for the response of the cantilever array sensor. The sensing cantilevers in the array were functionalized with the peptide, while the

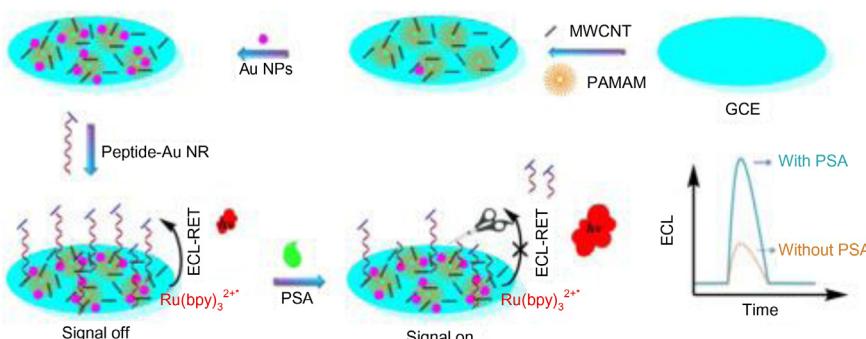


Figure 20.15 A simple “signal-on” ECL biosensor for detection of PSA.

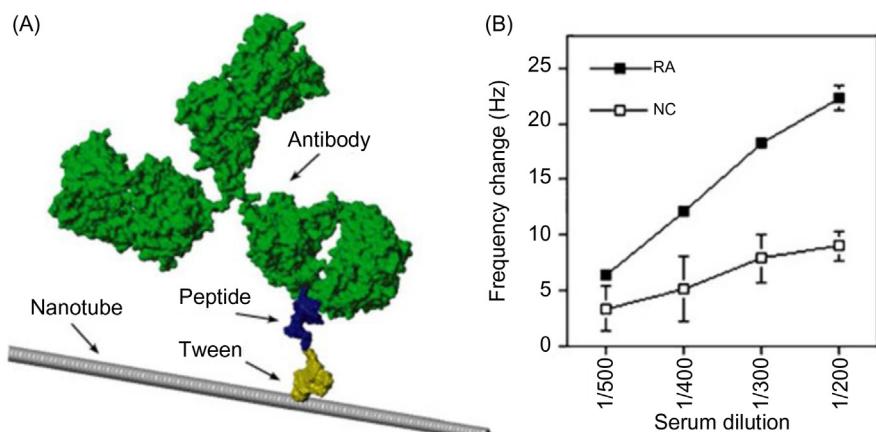


Figure 20.16 (A) A visual representation of an antibody bound to a peptide–Tween–nanotube complex that comprises the nanotube biosensor. (B) A sample representation of a dilution curve of an RA and normal control (NC) serum in the nanotube assay measuring antibody binding to citrullinated peptide-coated nanotubes as a change in frequency (Hz).

reference cantilevers were modified by 6-mercapto-1-hexanol (MCH) to eliminate the influence of environmental disturbances. The binding between vancomycin and the peptide induced a change of surface stresses in the sensing cantilevers, resulting in a differential deflection between the sensing and reference cantilevers. The reciprocal of the differential deflection is linear with the reciprocal of vancomycin concentration within the range of 2–100 µM at a detection limit of 0.2 µM. The reversible detection was performed by regenerating the sensing cantilevers with running buffer solution. Other antibiotics such as doxycycline, streptomycin, and kanamycin have a negligible effect on the response of the sensor. The sensor can also be utilized for the reversible detection of vancomycin in serum, which clearly shows the potential of the sensor for vancomycin detection in real biological samples (Fig. 20.17).

FET sensors are based on the field effect principle and can be fabricated with semiconductor technology, which has advantages such as high density and dynamical detection. The technology is also used in combination with nanomaterials for higher sensitivity. Kuang et al. described the rational design of a peptide recognition element (PRE) that is capable of noncovalently attaching to SWCNTs on the FET sensor as well as binding to trinitrotoluene (TNT) [71]. The PRE contains two domains, a TNT binding domain derived from the binding pocket of the honeybee odor binding protein ASP1, and an SWCNT binding domain previously identified from the phage peptide display library. The PRE structure in the presence of SWCNT was investigated by performing classical all-atom molecular dynamics simulations, circular dichroism spectroscopy, and atomic force microscopy. Both computational and experimental analyses demonstrate that the peptide retains two

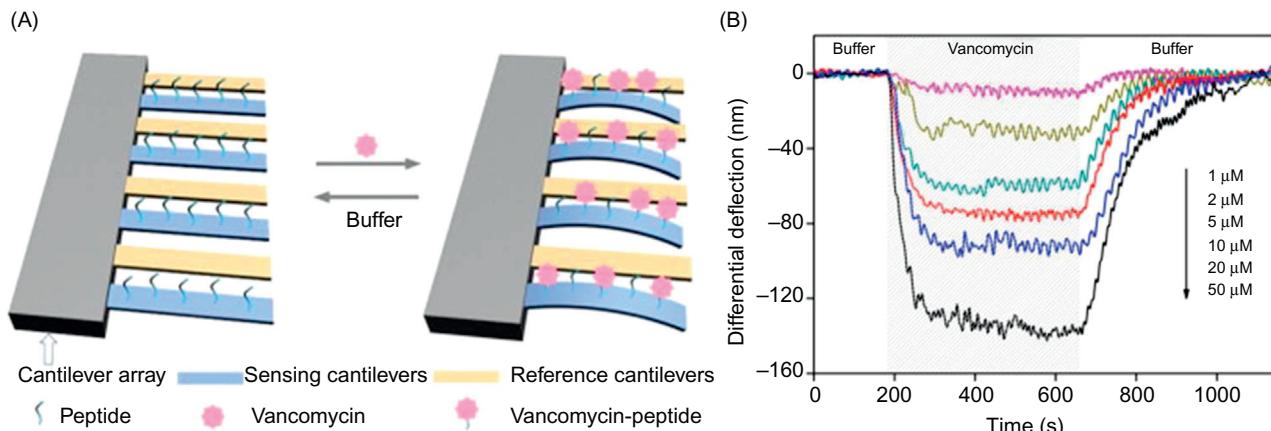


Figure 20.17 (A) Schematic illustration of reversible vancomycin detection. Sensing cantilevers were functionalized with peptides while reference cantilevers were modified with MCH. Binding of vancomycin to the peptide induced a change in cantilever surface stress and produced a differential cantilever deflection between the sensing and reference cantilevers. (B) Differential deflection as a function of time for different concentrations of vancomycin: 1, 2, 5, 10, 20, and 50 μ M (downward).

functional domains for SWCNT and TNT binding. The binding motif of the peptide to SWCNT and to TNT was revealed from interaction energy calculations by molecular dynamics simulations. The potential application of the peptide for the detection of TNT is theoretically predicted and experimentally validated using an SWCNT-FET sensor functionalized with a designer PRE. Results from this study demonstrate the creation of chemosensors using designed PRE as selective surface coatings for targeted analytes (Fig. 20.18).

All these biosensors were developed on the basis of traditional sensing technology with various forms and prominent features. Although most of these biosensors exhibited very high sensitivity, their construction and operation procedures are complicated. Other methodologies, such as the peptide-based ECL biosensors are in rapid development, and show broad potential in a variety of fields.

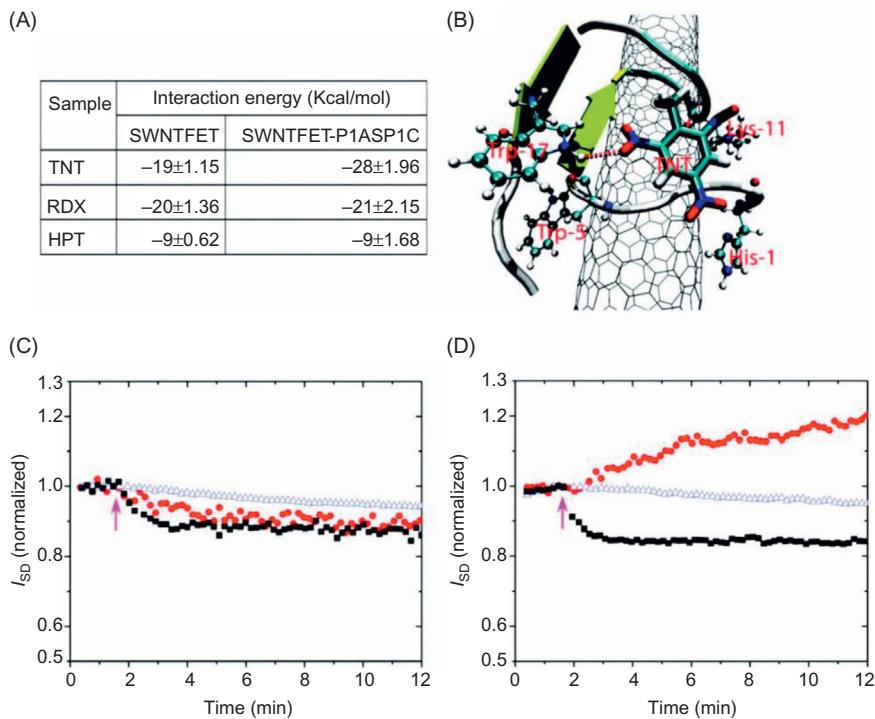


Figure 20.18 (A) Interaction energy of TNT, RDX, and HPT with bare SWCNT or P1ASP1C-decorated SWCNT. (B) Computational modeling predicts that TNT binds to P1ASP1C-SWCNT hybrid through a H bond with Trp17 and $\pi-\pi$ interaction with the SWCNT surface. Response of (C) bare SWCNT-FET and (D) P1ASP1C-coated SWCNT-FET to TNT (red circles), RDX (blue triangles), and HPT (black squares). Arrow indicates when the vapor was introduced into the device. Note that the chemical agents were exposed under ambient conditions.

20.5 Construction of peptide-based biosensors

Based on the diverse advantages of peptide-based sensors, they will continue to be the focus of development of biosensors in the next few years. Therefore, the construction approaches of peptide-based biosensors will be important and helpful to researchers. This section introduces some simple methods to fabricate peptide-based sensors with applications ranging from environment monitoring to disease diagnosis using examples from our work. The aim of this section is to offer technical support to those who want to work in this direction.

20.5.1 Environment monitoring

Environment monitoring has always been one of the main applications of biosensors because they can detect analytes in complex environments with high sensitivity, fast responses, and specificity. In the case of explosive detection, such as 2,4,6-trinitrotoluene (TNT), our group has constructed a novel nano-scale optical biosensing platform for TNT detection with TNT-sensitive peptides and derivatives of graphene oxide (GO) [72].

GO is a 2D nanomaterial with many oxygen-containing groups on single graphene sheets. These polar oxygen-functional groups give GO unique optoelectronic properties and make it easy to modify using nanoparticles, nucleic acids, and proteins [73]. Moreover, it is also an electronically hybrid material in which a large fraction of carbon atoms that are sp^3 hybridized bind to oxygen in the form of epoxy and hydroxyl groups, whereas the other carbon atoms that are sp^2 hybridized bind to neighboring carbon atoms or to oxygen in the form of carboxyl and carbonyl groups [74]. The strongly heterogeneous atomic and electronic characteristics of the material give GO unique optoelectronic and chemical properties. Researchers have confirmed that tuning the ratio of the sp^2 and sp^3 fractions can tune the band-gap of GO and therefore fine-tune the electrical, optical, and/or chemical properties of GO, such as enhancement of the conductivity and shift of the absorption peak [75]. In our design, GO was used as a transducer based on its tunable optical property.

According to the report of Jaworski et al., peptides containing the WHWQRPLMPVSI sequence can specifically interact with TNT molecules through partial charge–charge interactions or hydrogen bonding [76]. We synthesized TNT-specific peptides by solid-phase peptide synthesis (SPSS) and conjugated them with GO through a dehydration condensation reaction. Using *N*-hydroxysuccinimide (NHS) and *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide (EDC) the amino groups of the peptides can form stable covalent bonds with the carboxyl groups on the GO sheets, as shown in Fig. 20.19A. Through the ultraviolet (UV) absorbance spectra (Fig. 20.19B), it can be observed that the absorption peak of peptide-functionalized GO had a red shift in the wavelength compared to GO, which was due to changes in the molecular structure of GO and confirmed the tunable properties of the material. The peptide-modified GO combined the sensitivity of peptides

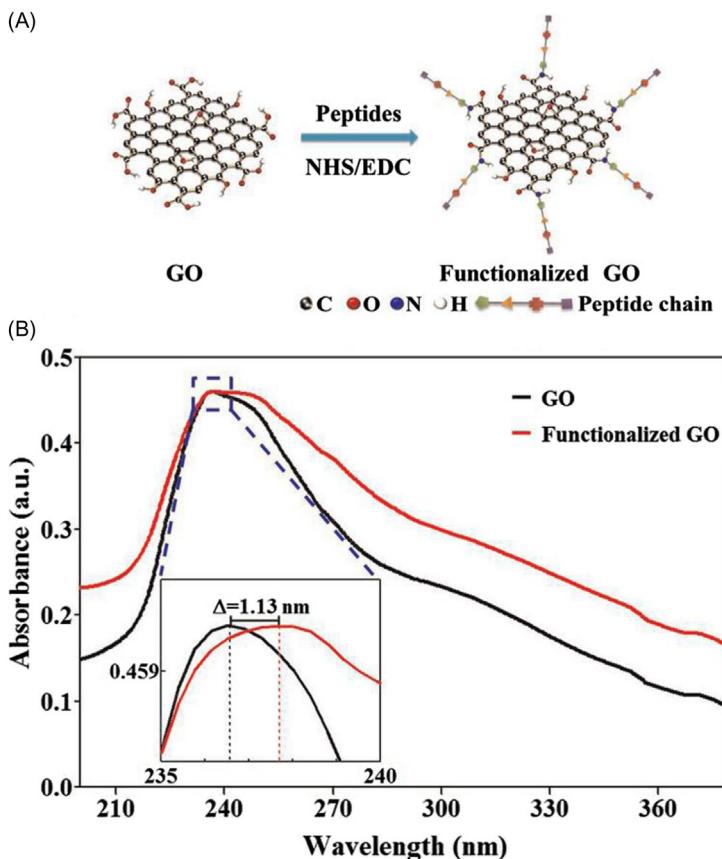


Figure 20.19 Schematic of synthesis (A) and UV absorption spectra (B) of biofunctionalized GO. The insert show that the absorption peak of biofunctionalized GO has a red shift in the wavelength.

and the optical properties of GO and it was used as an optical biosensor for TNT detection.

In the presence of TNT molecules, the peptide chains that are tethered to the GO sheets specifically interact and capture TNT molecules, as described in Fig. 20.20A. Donor–acceptor and $\pi-\pi$ interactions between peptide chains and TNT molecules result in changes in the optical properties of the peptide–GO complex which resulted in biosensor response upon binding TNT (Fig. 20.20B). The results showed that the addition of TNT can change the absorption magnitude of the biosensor, showing a linear relationship against log doses of TNT ranging from 4.40×10^{-9} to $4.40 \times 10^{-4} \text{ mM}$ with a detection limit of $4.40 \times 10^{-12} \text{ mM}$ in 38/ slope. Therefore, through modification of GO with designed peptides, an easy-to-construct peptide-based optical biosensor can be fabricated with a relatively high-sensitivity response to molecules such as TNT.

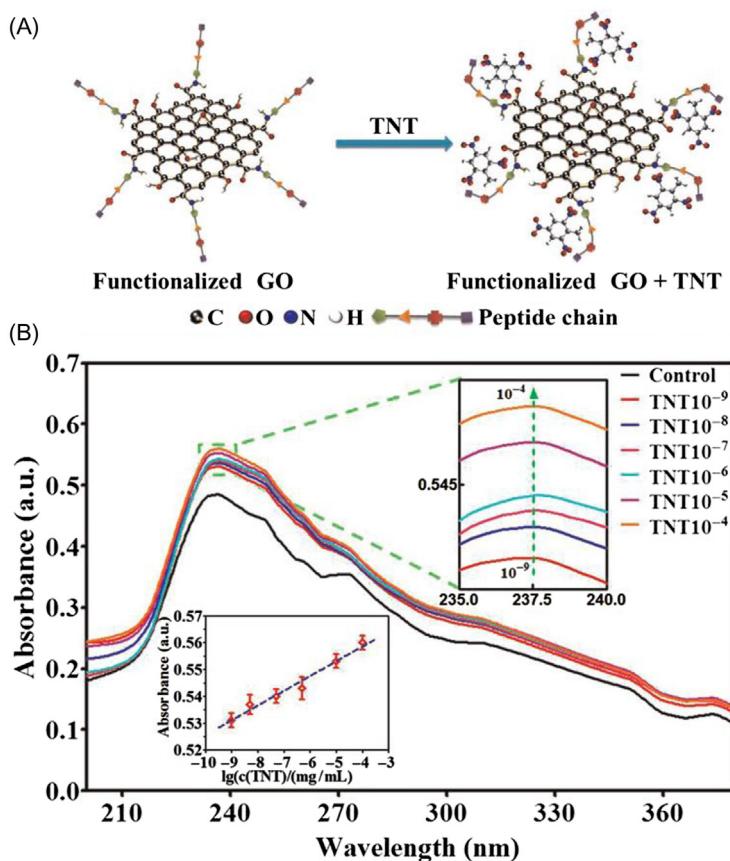


Figure 20.20 Schematic illustration (A) and UV absorption spectra (B) of TNT detection. The insert is the linear plot of statistical results of the absorption peak values ($n = 4$).

The peptide-based optical biosensor for TNT detection was based on the unique characteristics of GO and of the peptides. This biosensor was characterized by high sensitivity, versatility, and easy construction and modification. With the development of nanomaterials such as GO, such biosensors can exhibit great potential in high-throughput detection, not only in environmental monitoring but also in other fields such as food safety and health care.

20.5.2 Disease diagnosis

Disease diagnosis is one of the most important applications of biosensors. The detection of various biomarkers with different biosensing technologies represents one of the major challenges in the field of biosensor technology. Using the detection of thrombin detection as an example, we present here the development of a peptide-based optical biosensor for colorimetric detection.

Thrombin is a serine protease that plays a key role in the blood-clotting process. Modern medical research shows that thrombin is associated with diseases such as arteriosclerosis, liver fibrosis, and dementia [77]. Numerous efforts have been made towards thrombin detection and, among them, peptide-based biosensors stand out for their biosensitivity, high selectivity, and fast response. Thrombin can selectively cleave the arginine–glycine (RG) bond at Arg residues, which allows for the development of peptide-based biosensor construction.

As suggested previously, GO can be used in combination with peptides for the development of biosensor devices [78]. The GO surface can also be modified compounded with nanoparticles to obtain unique physical and chemical properties such as ultrasensitive features and excellent catalytic properties [79]. Herein, we proposed a peptide-based optical biosensor for thrombin detection [52]. Different from the biosensor constructed above, this sensor was fabricated by linking peptides containing specific cleavage sites of thrombin with composites of GO and gold nanoparticles (GNPs). The composition of GO and GNPs can not only change the responses of the sensor from the UV region to the visible region but also it can further improve the sensor sensitivity and allow for colorimetric detection.

Fig. 20.21A shows the synthesis and thrombin detection process of the biosensor. The nanocomposites of GO and GNPs (GO/GNPs) were prepared by the reduction of chloroauric acid with trisodium citrate on the surface of graphene sheets. Such a process resulted in nucleation and growth of gold particles attaching to the oxygen groups of the graphene material without internal bridges due to electrostatic interactions [80]. GO/GNPs can exhibit two absorption peaks at both the UV and visible regions. With a blue shift compared to the absorption peak of GO and a broader absorption band compared to that of GNPs, the nanocomposites were able to combine the optical properties of both materials (Fig. 20.21B,C). Then, specifically designed octapeptides (CLVPRGSC) were synthesized by SPSS with the recognition site of thrombin and cysteine groups at both ends of the peptides. Thus, the peptides can self-assemble on the nanocomposites covalently bonded to the GNPs through the sulphydryl groups. Self-assembly could also induce the aggregation of the composites when two gold nanoparticles on different GO/GNPs were connected by one peptide chain (Fig. 20.21A). This may be seen from the spectral comparison of GO/GNPs in Fig. 20.21C, which is based on the localized plasmon surface resonance (LSPR) characteristics of GNPs. Subsequently, when thrombin molecules were added, the peptides could be cleaved, resulting in separation of the aggregated GO/GNP complexes. Thus, composites clustered together by peptides would redisperse in the solution, and the spectrum of the solution could be expected to change and resemble the spectrum of the individual GO/GNPs (Fig. 20.21C). As a result, a peptide-based biosensor for thrombin detection was constructed successfully based on GO and GNPs.

When the biosensor was used for the spectroscopic detection of thrombin, the proteolytic activity of thrombin which cleaves the octapeptides at Arg-Gly sites results in redispersion of the nanocomposite complexes which aggregated due to the presence of the octapeptides (Fig. 20.21C). An increase of the thrombin

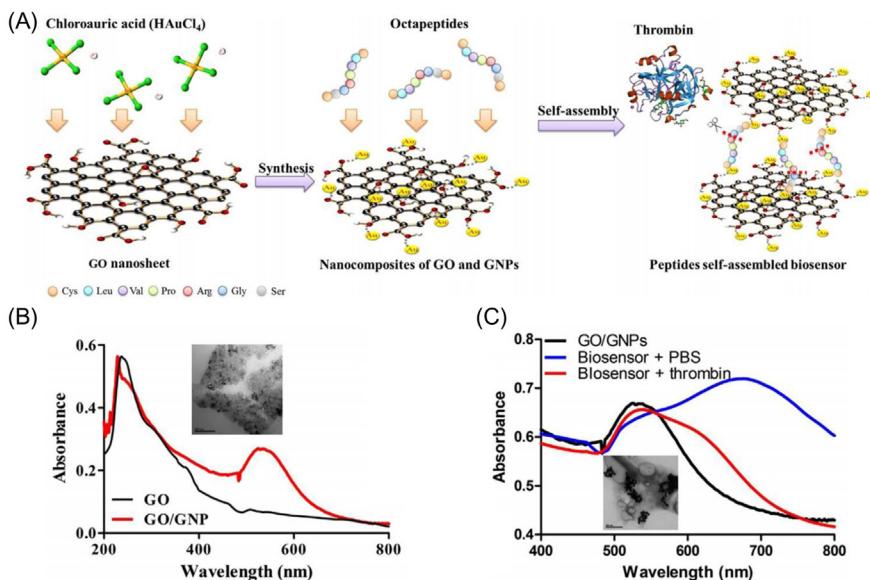


Figure 20.21 (A) Synthetic process of composites of graphene and oxide, self-assembly process of octapeptides on the nanocomposites, and detection mechanism of the biosensor for thrombin. (B) Optical spectroscopy for characterization of the nanocomposites and graphene oxide. The TEM image of the composites is also shown. (C) Optical spectroscopy for characterization of the octapeptide self-assembled biosensor before and after addition of thrombin at $27 \mu\text{M}$. The TEM image shows the peptide self-assembled nanocomposites before addition of thrombin.

concentration in the solution of the nanocomposite complexes resulted in an increase of the absorption peak at $\sim 528 \text{ nm}$, while the absorbance decreased at wavelengths larger than 600 nm (Fig. 20.22A). With a $528\text{-}680 \text{ nm}$ absorbance ratio as the sensitive detection parameter, the biosensor had good linearity, stability, and specificity against logarithms of thrombin concentrations (Fig. 20.22B), showing good performance in detection of such proteases.

As the biosensor fabricated here was composed of GO, GNPs, and thrombin-specific peptides, all three elements made a contribution to the performance of the biosensor. With various oxygen-containing groups on the surface of GO, the latter acted as the building block for GNPs and peptides and enabled the stability of the whole system. GNPs provided the nanocomposite system with an absorption peak in the visible region and aggregation-to-discoloration characteristics, which was the detection basis of the biosensor. Peptides played the role of biorecognition to thrombin. These three factors combined into one system gave rise to a sensor for thrombin detection with high sensitivity. Such a biosensor could also be designed for sensitive detection of other proteases and even other biomolecules such as important biomarkers of diseases.

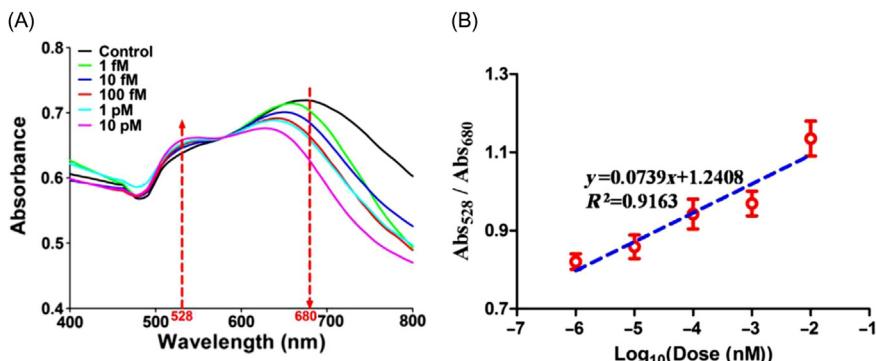


Figure 20.22 Dose-dependent measurements for thrombin detection with the biosensor system that is based on the aggregation of nanocomposite complexes consisting of GO, GNPs, and octapeptides. (A) Absorption spectra for detection of thrombin at different concentrations (1 fM–10 pM). (B) The statistical results of the 528–680 nm absorbance ratios ($\text{Abs}_{528} / \text{Abs}_{680}$) compared with those of the control ($n = 3$).

20.6 Conclusion

In this chapter, a number of peptide-based biosensors have been discussed including electrochemical, optical, etc. We aimed at providing the reader with an introduction to the peptide-based biosensor field. We believe that peptide-based biosensors will be further developed to advance sensitivity.

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