New Trends in Electrochemical Protein Sensors

Elektrokimyasal Protein Sensörlerinde Yeni Yaklaşımlar

Invited Review / Davetli Derleme

Arzum Erdem*, Pembe Ece Canavar, Hakan Karadeniz, Gülşah Congur
Ege University, Faculty of Pharmacy, Analytical Chemistry Department, Izmir, Turkey

ABSTRACT

Proteins are essential parts of organisms and participate in every process within cells. Protein biosensors are becoming more essential for the through and systematic investigation of complex biological processes. Several methods have been used for development of protein biosensors e.g., surface plasmon resonance, quartz crystal microbalance, chemiluminescence, electrophoresis, fluorescence techniques and electrochemical methods. There has been growing interest for electrochemical metods in protein research (like protein-protein interactions, or some diseases related to proteins investigations). Herein, this paper will specifically focus on new trends in electrochemical protein sensors.

Key Words
Protein sensors, electrochemistry, aptamers, nanomaterials.

ÖZET

Proteinler organizmaların yapıtaşlarından birisi olup, hücre içerisindeki tüm mekanizmalarda görev alır. Karmaşık biyolojik sistemlerin araştırılmasında protein biyosensörleri önem taşımaktadır. Yüzey plazmon rezonansı, kuvartz kristal mikroterazisi, kemilüminesans, elektroforez, floresans teknikleri ve elektrokimyasal yöntemler protein biyosensörlerinde kullanılan tekniklerden bazılarıdır. Son yıllarda protein araştırmalarında (protein-protein etkileşimleri ve protein kaynaklı hastalıkların incelenmesi gibi çalışmalar) elektrokimyasal teknikler büyük ilgi görmektedir. Bu derlemede, özellikle elektrokimyasal protein sensörlerindeki yeni yaklaşımlara değinilmiştir.

Anahtar kelimeler
Protein sensörleri, elektrokimya, aptamerler, nanomalzemeler.

Article History: Received March 7, 2011; Revised April 12, 2011; Accepted May 16, 2011; Avaliable Online: June 5, 2011.

Correspondence to: A.Erdem, Analytical Chemistry Department, Faculty of Pharmacy, Ege University, 35100 Bornova, Izmir, Turkey
Tel: +90232 311 1010 / 5131 Fax: +90232 388 5258 E-Mail: arzum.erdem@ege.edu.tr

Abbreviations:

EIS : Electrochemical Impedance Spectroscopy,
SPR : Surface Plasmon Resonance,
DPV : Differential Pulse Voltammetry,
FT-IR : Fourier Transform Infrared Spectra,
SEM : Scanning Electron Microscopy,
CV : Cyclic Voltammetry,
2D IR : Two-dimensional Infrared Spectroscopy,
AFM : Atomic Force Microscope,
SWV : Square Wave Voltammetry,
LYS : Lysozyme,
THR : Human Thrombin,
AuNPs : Gold Nanoparticles,
MWCNTs : Multiwalled Carbon Nanotubes,
SWCNT : Single-Wall Carbon Nanotubes,
DNA : Deoxyribonucleic Acid,
RNA : Ribonucleic Acid.
QCM : Quartz Cyrstal Microbalance,
SAM : Self-Assembled Monolayer,
ECL : Chemiluminescence,
NFIS : Non-Faradaic Impedance Spectroscopy

INTRODUCTION

The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids. They are at the very core of biological function and essential parts of organisms and participate in every process within cells. Proteins are neccesary in every metabolic reaction. Catabolic and anabolic reactions necessary for the viability of the organism were catalyzed by enzymes and enzymes are in protein structure [1-3]. For example, protein kinase A, an important enzyme in regulation of glycogen, sugar, and lipid metabolism in the human body [4]. Becuse of all its important function, detection of protein is important and it gives researchers many information about organism. Protein detection plays an important role in detection and treatment of specialy diseases. There is a high demand for convenient methodologies for detecting and measuring the levels of specific proteins in biological and environmental samples because their detection, identification and quantification can be very complex, expensive and time consuming.

Enzyme-linked immunosorbent assay (ELISA) is a fluorescent optical detection method widely used in protein detection is the "gold standard" for clinical diagnostics with high sensitivity [5-8]. By the advantages of recent developments, biosensors have become promising platforms for detecting proteins.

Biosensors are devices that combine with a biological recognition component and a physicochemical detector component as transducer. The transduction unit can be electrochemical, optical, piezoelectric, magnetic, or colorimetric. The recognition layer can be constructed using enzymes, antibodies, cells, tissues, nucleic acids, peptide nucleic acids, and aptamers. For monitoring of proteins by sensor technology, different techniques were used such as surface plasmon resonance (SPR) [9], quartz crystal microbalance (QCM) [8], electrophoresis [10,11], fluorescence [12], IR spectroscopy [13], amperometric [6,14], chemiluminescence (ECL), electrochemical impedance spectroscopy (EIS) [15] and voltammetric techniques [16].

Electrochemical biosensors are molecular sensing devices that couple a biological recognition element to an electrode transducer. Due to their excellent sensitivity, ease of miniaturization and low-cost, electrochemical biosensors are well suited for point-of-care applications [17-19].

The aim of this paper is to overview the new trends in electrochemical protein sensors that could be classified into three groups: (i) traditional, (ii) nanomaterial based and, (iii) aptamer based electrochemical protein sensors. Some representative studies were also summarized in Table 1.

Traditional Electrochemical Sensors in Protein Detection:

Electrochemical biosensors are well suited for urinary diagnostics due to their excellent sensitivity, low-cost, and ability to detect a wide variety of target molecules including nucleic acids and protein biomarkers [17]. The protein detection by traditional electrochemical sensors in combination with Differential Pulse Voltammetry (DPV), Cyclic Voltammetry (CV), Electrochemical Impedance Spectroscopy (EIS) techniques has been given in this part.

Le et al. described an electrochemical label-free immonusensor by using biotinylated single-chain

Table 1. Some representative studies for new trends in electrochemical protein sensors.

	Analyte	Material	Method	Detection Limit	Ref.
Traditional Electrochemical Sensors	C-reactive Protein and Myeloperoxidase		EIS	~1 pg/mL	[5]
	Biotinylated Single-Chain Variable Fragment / Antibody		SPR DPV FT-IR	1 pg/mL	[9]
	Glucose Oxidase		SEM CV 2D IR	-	[13]
	Bovine Serum Albumin		EIS RAMAN	-	[16]
	Lactoferrin		EIS	145 pg/mL	[17]
	Catechol		CV SEM AFM	5 μM	[20]
	A Uric Acid		Chronoamperometry	$1.8x10^{-7}$ mol/L	[21]
	Human İmmunoglobulin G and Protein A		EIS	5 ng/mL	[22]
Nanomaterial Based Electrochemical Sensors	Human İmmunoglobulin G	AuNPs	Amperometric CV DPV EIS	0.001 ng/mL	[23]
	H_2O_2	MWCNTs	CV	$1.0x10^{-6}$ M	[24]
	Lysozyme	AuNPs	SWV	0.1 pM	[25]
	Cytochrome c	SWCNT	CV	$1.0x10^{-5}$ M.	[26]
Aptamer Based Electrochemical Sensors	Lysozyme	Aptasensor	SWV	0.1 pM	[25]
	Protein	Nucleic Acid Aptamers	SPR	-	[27]
	Lysozyme and Human Thrombin	DNA aptamer	DPV	10.77 mg/mL for LYS and 2.00 mg/mL for THR	[28]
	C-reactive Protein	RNA Aptamers	NFIS	Working range within 100–500 pg/mL	[29]

variable fragment (Sc-Fv) antibody immobilized on copolypyrrole films [9]. A composit copolymer was formed from a mixture of pyrrole (py) as spacer and a pyrrole bearing a N-hydroxyphthalimidyl ester group on its 3-position (pyNHP) to immobilize antibody. Different techniques were used for characterization of the sensor and detection process, such as; SPR to monitor in real time the electropolymerization process, FT-IR spectroscopy to demonstrate the chemical copolymer composition and the efficiency of the covalent attachment of biomolecules, SEM to analyze the film morphology and DPV for direct detection of specific antigen.

Bovine serum albumin (BSA) has been used in fundamental researches for the development of better therapeutics to convert globular proteins, which is non-cytotoxic into fibrillar form that causes cell death [30]. Ignat and his coworkers reported a nanostructured gold substrates to adsorp BSA on the 11-mercaptoundecanoic acid (MUA) layer for further biomedical applications. The binding of protein to a single monolayer of MUA can be easily detected by CV and Raman spectroscopy analysis [16].

Biogenic nanoporous silica was used for developing a biosensor platform electrochemical detection of cardiovascular biomarkers by Lin et al [5]. This label-free electrochemical biosensor based on specific antibody-substrate interaction could be applied to pure and commercial human serum samples with high sensitivity and selectivity for detection of C-reactive protein (CRP) and myeloperoxidase (MPO).

A bi-protein/layered double hydroxide (LDH) ultrathin film was tested for catechol detection [20]. LDH was developed by deposition hemoglobin (HB) and horseradish peroxidase (HRP) molecules layer-by-layer. Structural and morphological characterization of this new surface was done by UV–vis spectra and XRD. Electrocatalytic behavior of the modified electrode and detection of catechol was investigated by CV. This catechol biosensor showed a wide linear response range (6–170 μM, r = 0.999), low detection limit (5 μM), high sensitivity and good reproducibility [20].

Langmuir–Blodgett (LB) technique was used to immobilize the uricase on chitosan/Prussian blue (CS/PB) prefunctionalized indium-tin oxide (ITO) electrode as an uric acid biosensor. The effects of ionic strengths, acidity of subphase, and uricase amount on the film were studied by chronoamperometric techniuqes The linear range of uric acid was found from 5 x 10^{-6} mol/L to 1.15 x 10^{-3} mol/L with a detection limit of 1.8 x 10^{-7} mol/L [21].

In the study of Qi et al. [22], a simple and sensitive biosensor was developed for the determination of human immunoglobulin G (IgG). Electrochemical impedance spectroscopy was used to detect Protein A. The surface morphology of the self-assembled layer before and after interaction with IgG was studied by AFM. Protein A can bind specifically to the Fc portion of IgG, and this could cause a change in the resistance of the interfacial electron transfer when using a ferrocyanide redox couple as a probe. The increase of the resistance of the electron transfer was found linearly related to the concentration of IgG in the range from 10 ng/mL to 1.0 μg/mL, with a detection limit of 5 ng/mL [22].

In the study of Wang and co-workers [13], the mechanism of the effects of thermal unfolding of proteins on their catalytic activities and conformational structures were studied. Two-dimensional infrared correlation spectroscopy (2D IR) and CV techniquies were used to elucidate the conformational structures.

Electrochemical immunosensor was fabricated for the direct detection of the urinary tract infection (UTI) biomarker lactoferrin from infected clinical samples by Pan et al. [17]. EIS was used to characterize the mixed SAM (Self Assambled Monolayer), consisted of 11-mercaptoundecanoic acid and 6-mercapto-1-hexanol. A sandwich amperometric immunoassay was developed for detection of lactoferrin from urine with a detection limit of 145 pg/mL [17].

Nanomaterial based Electrochemical Sensors

Electrical detection of protein biomarkers is a relatively new approach that can monitor a specific

electrical parameter during the protein detection event. The adventage of nanotechnology in the field of clinical diagnostics has resulted in the incorporation of nanoscale materials in designing diagnostics assays, especially for electrical detection. The major classes of nanomaterials that have been used for protein biomarker detection are: nanotubes, nanowires, nanoparticles and other nanotemplates. These materials have been frequently utilized for their improved surface area towards developing detectors with enhanced sensitivity and reduced use of reagents [5,20,31,32].

Carbon nanotubes (CNTs) have attracted much attention as a conducting material, suitable for use in biosensor fabrication [18,24,33-39].

Huang et al. [23] published a novel experimental method based on a Prussian blue (PB) and gold nanoparticles (AuNPs) modified carbon ionic liquid electrode (CILE) for the sensitive detection of human immunoglobulin G as a model protein. The CILE was fabricated by using the ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate as binder. Controllable electrodeposition of PB on the surface of the CILE and coating with 3-aminopropyl triethylene silane (APS) formed a film with high electronic catalytic activity and large surface area for the assembly of AuNPs in order to further immobilization of IgG antibody. The electrochemistry of the formed nanocomposite biofilm was investigated by electrochemical techniques including CV, DPV, and EIS. The detection limit was found of 0.001 ng/mL (S/N=3) [23].

Multiwalled carbon nanotubes and ciprofloxacin (CF, fluoroquinolone antibiotic) were used for modifying the glassy carbon and indium tin oxide-coated glass electrode for Cytochrome C (Cyt-C) as a model protein. The Cyt-C adsorbed MWCNTs/CF film was characterized by SEM, UV–visible spectrophotometry (UV-vis) and CV. Cyt-C/MWCNTs/CF film modified electrode can be used as a biosensing material for sensor applications [24]. Another Cyt-C biosensor was investigated by Wang et al [26]. A pair of well-defined redox waves of Cyt-C were obtained at a GCE modified with single-wall carbon nanotubes (SWCNTs).

Smaller sample volumes and higher toxicity in very low sample consentration can promote the researchers to find high sensitive, selective and rapid detection. Due to their dimensions, nanostructured materials display unique properties not traditionally observed in bulk materials. Characteristics such as increased surface area along with enhanced electrical, optical properties make them suitable for numerous applications such as nanoelectronics, photovoltaics and chemical, biological sensing. Their small size is also responsible for superior electronic and optical properties which, due to quantum confinement effects, are very sensitive to minor perturbations. Thus, nanomaterials can be used to facilitate label-free detection, and to develop biosensors with enhanced sensitivities and improved response times [40].

Aptasensors based Electrochemical Sensors

Analogous to protein-based antibodies, aptamers are nucleic acid-based molecules that can be selected to bind essentially to any molecule of choice [41,42]. Chemical synthesis, selection through the SELEX (systematic evolution of ligands by exponential enrichment) process, easy modification, high stability, target versatility, easy-to-stock, and resistant to denaturation and degradation makes aptamer more advantageous beside to antibodies. These properties make aptamers ideal candidates as protein recognition elements in a wide range of bioassays and for the development of disease diagnostics [25,43]. A variety of techniques has been used in aptamer-based protein recognition and detection such as colorimetric [44], fluorescence [45], microgravimetric [46], QCM [47,48], electrochemical [49-53]. Among these, electrochemical techniques received a great attention conspicuousness with its sensitivity, selectivity, low-cost and high efficiency.

Nucleic acid aptamers a devoloping class of synthetic ligands can play an important role in deveolopment of biosensor technology. In principle, nucleic acid aptamers can be discovered to recognize any molecule of interest with high affinity and specificity. In addition, unlike most ligands in nature, synthetic nucleic acid aptamers are usually tolerant of harsh chemical,

physical, and biological conditions. These eminent characteristics make aptamers attractive molecular recognition ligands for biosensing applications. Several label-free electrochemical biosensors were developed based on changes in conductivity, or electrochemical signal because of highly specific aptamer–target interactions [1,11,47,54].

An electrochemical deoxyribonucleic acid (DNA) aptamer biosensor was investigeted by Erdem et al. [28]. Two cognate protein lysozyme (LYS) and human thrombin (THR) was selected as a target protein. The interaction of DNA aptamer with its target was existed on magnetic particle surface, and electrochemical detection was then performed by using disposable graphite electrode (PGE) in combination with DPV. The detection limits were estimated of 10.77 mg/mL LYS (769 nM) and 2.00 mg/mL THR (54.5 nM).

Various in situ injectable hydrogels have been developed for protein delivery sistems in treating human diseases and most of this hydrogels are highly permeable. According to Soontornworajit et al., nucleic acid aptamers aptamers can be used to functionalize an in situ injectable hydrogel model to control the release of proteins [27]. The aptamers were studied using secondary structural predictions and binding analyses, and the affinities of the aptamers were measured with SPR. The results showed that the structural predictions were different from the experimental measurements in numerous cases and aptamer-functionalized hydrogel could prolong protein release [27].

Li et al. investigated an electrochemical lysozyme (LYS) aptasensor developed by using gold nanoparticles. The gold nanoparticles were applied to increase the immobilization of DNA probes due to the surface effect of nanomaterials, thus it could offer a significant amplification for the detection of lysozyme. The square wave voltammetry (SWV) peak current changed with increasing concentration of lysozyme with a lower detection limit of 0.1 pM LYS [25].

Rodriquez et al. [55] developed the EIS-based label-free biosensor for monitoring aptamer interactions. A biotinylated aptamer for lysozyme was linked to streptavidin-functionalised electrode. In another study Kawde et al., a highly specific and sensitive aptasensor was developed for detecting protein interactions using aptamer-coated magnetic beads by using chronopotentiometric stripping [56].

An aptamer-based impidimetric sensor was developed for the detection of IgE by Xu et al. [57]. Human IgE was used as a model target protein and incubated with the aptamer array consisting of single-stranded DNA with a hairpin loop. AFM analysis demonstrated that human IgE could be specifically captured by the aptamer and stand well above the SAM surface. The results of this method presented a good correlation for human IgE in the range of 2.5 - 100 nM. A detection limit of 0.1 nM (5 fmol in a 50 μL sample) was obtained, and an average of the relative standard deviation was found less than 10%.

Liu et al. investigated an electrochemical aptasensor for the detection of thrombin based on thrombin-binding aptamer (TBA) as a molecular recognition element by using MWCNTs modifed GCE (Glassy Carbon Electrode) and DPV. The decreased peak current was in proportion to the concentration of thrombin in a range from 1.0 x 10^{-12} to 5.0 x 10^{-10} M with a detection limit of 5 x 10^{-13} M [58].

A novel electrochemical assay based on the aptamer and the signal of amplification of nanoparticles for the determination of thrombin was fabricated by Ding et al. Aptamers immobilized on the electrode and AuNPs could be assembled with the target protein to form a sandwich structure in the presence of the latter. DPV was employed to detect the CdS nanoparticles loaded on the surface of the AuNPs through the linker DNA, which was related to the concentration of the target protein. Thrombin was detected in this assay in the linear range of 1.0×10^{-15} to 1.0×10^{-11} M with the detection limit of 5.5×10^{-16} M of target protein [59].

CONCLUSION

Identification, characterization and detection of proteins is significant for human health, environmental monitoring, treat diseases and food analysis. Although several metods have been used for protein detection, in this review we focused on electrochemical sensors for protein detection, especially to the most sensitive, more rapid and low-cost ones.

We overviewed herein the new trends in electrochemical protein sensors classified into three groups: (i) traditional, (ii) nanomaterial based and, (iii) aptamer based electrochemical protein sensors. It shows us how sensor technology can be progressed in recent years from traditional way to aptamer technology. According to this progress, the interaction of analyte with sensing element becomes more complicated, but it can yield more selective detection for protein monitoring.

Aptmers can offer us a different benefit from the nanomaterials. By the way of SELEX technique we can desing novel and synthetic, but spesific like antibody, unlimited macromolecules. Most of recent studies in literature discuss the events on the surface of the electrode, immobilization the material, characterization of electrode surface related to aptamer based biosensor development. But there have been rarely studies on direct measurement of protein, mostly based on similar ones. In near future, we believe that the challenging electrochemical aptasensor protocols will be developed for highly spesific and more sensitive detection of proteins.

ACKNOWLEDGEMENTS

A.E acknowledges to the Turkish Academy of Sciences (TUBA) as an Associate member for their support.

REFERENCES

1. M.Vestergaard, K.Kerman and E.Tamiya, An overview of label−free electrochemical protein sensors, Sensors 7 (2007) 3442.
2. B. Strehlitz, N. Nikolaus, and R. Stoltenburg, Protein detection with aptamer biosensors, Sensors 8 (2008) 4296.
3. H.R. Schnerr, Lead identification and optimization in crude samples using label free resonant acoustic profiling, J. Mol. Recognit. 23 (2010) 597.
4. S. Xu, Y. Liu, T. Wang, and J. Li, Highly sensitive electrogenerated chemiluminescence biosensor in profiling protein kinase activity and ınhibition using gold nanoparticle as signal transduction probes, Anal. Chem 82 (2010) 9566.
5. K.C. Lın, V. Kunduru, M. Bothara, K. Rege, S. Prasad, and B.L. Ramakrishna, Biogenic nanoporous silica−based sensor for enhanced electrochemical detection of cardiovascular biomarkers proteins, Biosens. Bioelectron. 25 (2010) 2336.
6. M.S. Belluzo, M.É. Ribone, C. Camussone, I.S. Marcipar, and C.M. Lagier, Favorably orienting recombinant proteins to develop amperometric biosensors to diagnose Chagas' disease, Anal. Biochem., 408 (2011) 86.
7. A. Kummer, and E.C. Li−Chan, Application of an ELISA−elution assay as a screening tool for dissociation of yolk antibody-antigen complexes, J. Immunol. Methods, 211 (1998) 125 .
8. J.S. Yu, H.X. Liao, A.E. Gerdon, B. Huffman, R.M. Scearce, M.McAdams, S.M.Alam, P.M.Popernack, N.J. Sullivan, D. Wright, D.E. Cliffel, G.J. Nabel, and B.F.Haynes, Detection of Ebola virus envelope using monoclonal and polyclonal antibodies in ELISA, surface plasmon resonance, and a quartz crystal microbalance immunosensor, J. Virol. Methods, 137 (2006) 219.
9. H.Q.A. Lê, H. Sauriat−Dorizon, and H. Korri−Youssoufi, Investigation of SPR and electrochemical detection of antigen with polypyrrole functionalized by biotinylated single−chain antibody: A review, Anal. Chim. Acta, 674 (2010) 1.
10. S. Xie, and S.P. Walton, Development of a dual−aptamer−based multiplex protein biosensor, Biosens. Bioelectron. 25 (2010) 2663.
11. K. Maehashi, T. Katsura, K. Kerman, Y. Takamura, K. Matsumoto, and E. Tamiya, Label−free protein biosensor based on aptamer−modified carbon nanotube field−effect transistors, Anal. Chem. 79 (2007) 782.
12. L.S. Barak, S.S. Ferguson, J. Zhang and M.G. Caron, Alpha beta−arrestin/green fluorescent protein biosensor for detecting G protein−coupled receptor activation, J. Biol. Chem. 272 (1997) 27497.

13. Q. Wang, W. Xu, P. Wu, H. Zhang, C. Cai and B. Zhao, New Insights into the effects of thermal treatment on the catalytic activity and conformational structure of glucose oxidase studied by electrochemistry, IR spectroscopy, and theoretical calculation, J. Phys. Chem. B 114 (2010) 12754.
14. Z. Zhao, W. Lei, X. Zhang, B. Wang, and H. Jiang, ZnO–Based amperometric enzyme biosensors, Sensors 10 (2010) 1216.
15. A.A.P. Ferreira, M.J.M. Alves, S. Barrozo, H. Yamanaka, and A.V. Benedetti, Optimization of incubation time of protein Tc85 in the construction of biosensor: Is the EIS a good tool? J. Electroanal. Chem., 643 (2010) 1.
16. T. Ignat, M. Miu, I.Kleps, A. Bragaru, M. Simion, and M. Danila, Electrochemical characterization of BSA/11–mercaptoundecanoic acid on Au electrode, Mater. Sci. Eng. B, 169 (2010) 55.
17. Y. Pan, G.A. Sonn, M.L.Y. Sin, K.E. Mach, M.C. Shih, V. Gau, P.K. Wong, and J.C. Liao, Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis, Biosens. Bioelectron. 26 (2010) 649.
18. J. Wang, Nanomaterial–based electrochemical biosensors, Analyst 130 (2005) 421.
19. J. Wang, Electrochemical biosensors: Towards point–of–care cancer diagnostics, Biosens. Bioelectron., 21 (2006) 1887.
20. X. Kong, X. Rao, J. Han, M. Wei, and X. Duan, Layer–by–layer assembly of bi–protein/layered double hydroxide ultrathin film and its electrocatalytic behavior for catechol, Biosens. Bioelectron. 26 (2010) 549.
21. X. Wang, F. Yin, and Y. Tu, An uric acid biosensor based on langmuir–blodgett film as an enzyme–immobilizing matrix, Anal. Lett.43 (2010) 1507.
22. H. Qi, C. Wang, and N. Cheng, Label–free electro–chemical impedance spectroscopy biosensor for the determination of human immunoglobulin G, Microchim. Acta 170 (2010) 33.
23. K.J. Huang, D.J. Niu, J.Y. Sun, X.L. Zhu and J.J. Zhu, Label–free amperometric immunobiosensor based on a gold colloid and Prussian blue nanocomposite film modified carbon ionic liquid electrode, Anal. Bioanal. Chem. 397 (2010) 3553.
24. A.S. Kumar, S.F. Wang, C.T. Yeh, H.C. Lu, J.C. Yang and Y.T. Chang, Direct electron transfer of cytochrome C and its electrocatalytic properties on multiwalled carbon nanotubes/ ciprofloxacin films, J Solid State Electrochem. 14 (2010) 2129.
25. L.D. Li, Z.B. Chen, H.T. Zhao, L. Guo and X. Mu, An aptamer–based biosensor for the detection of lysozyme with gold nanoparticles amplification, Sens. Actuator., B 149 (2010) 110.
26. J. Wang, M. Li, Z. Shi, N. Li, and Z. Gu, Direct electrochemistry of Cytochrome c at a glassy carbon electrode modified with single–wall carbon nanotubes, Anal. Chem.74 (2002) 1993.
27. B. Soontornworajit, J. Zhou, Z. Zhang and Y. Wang, Aptamer–functionalized in situ Injectable hydrogel for controlled protein release, Biomacromolecules 11 (2010) 2724.
28. A. Erdem, H. Karadeniz, G. Mayer, M. Famulok and A. Caliskan, Electrochemical sensing of aptamer–protein interactions using a magnetic particle assay and single–use sensor technolog, Electroanalysis 21 (2009) 1278.
29. A. Qureshi, Y. Gurbuz, S. Kallempudi and J.H. Niazi, Label–free RNA aptamer–based capacitive biosensor for the detection of C–reactive protein, Phys. Chem. Chem. Phys. 12 (2010) 9176.
30. C.Y. Huang, C.M. Liang, C.L. Chu and S.M. Liang, Albumin fibrillization induces apoptosis via integrin/ FAK/Akt pathway, BMC Biotechnol. 9 (2009) 1.
31. J.F. Rusling, G. Sotzing and F. Papadimitrakopoulosa, Designing nanomaterial–enhanced electrochemical immunosensors for cancer biomarker proteins, Bioelectrochemistry 76 (2009) 189.
32. Z. Cao, X. Jiang, Q. Xie and S. Yao, A third – generation hydrogen peroxide biosensor based on horseradish peroxidase immobilized in a tetrathiafulvalene – tetracyano quinodimethane/multiwalled carbon nanotubes film, Biosens. Bioelectron.24 (2008) 222.
33. K. Balasubramanian and M. Burghard, Chemically Functionalized Carbon Nanotubes Small 1 (2005) 180.
34. H. Dai, Carbon Nanotubes: Synthesis, Integration, and Properties, Acc. Chem. Res.35 (2002) 1035.
35. M. Trojanowicz and M. Szewczynska, Biosensing in high–performance chemical separations, Trend. Anal. Chem. 24 (2005) 92.
36. J. Wang, Carbon–Nanotube Based Electrochemical Biosensors: A Review, Electroanalysis 1(2005) 7.
37. C.E. Banks, and R.G. Compton, New electrodes for old: from carbon nanotubes to edge plane pyrolytic graphite, Analyst 131 (2006) 15.
38. L. Agüí, P. Yanez–Sedeno and J.M. Pingarron, Role of carbon nanotubes in electroanalytical chemistry: A review, Anal. Chim. Acta 622 (2008) 11.
39. S. A. Kumar, and S.M.Chen, Electroanalysis of NADH using conducting and redox active polymer/carbon nanotubes modified electrodes–a review, Sensors 8 (2008) 739.
40. E. Lahiff, C. Lynam, N. Gilmartin, R. O'Kennedy and D. Diamond, The increasing importance of carbon nanotubes and nanostructured conducting polymers in biosensors, Anal. Bioanal. Chem. 398 (2010) 1575.

41. A.D. Ellington, and J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818.
42. C. Tuerk and L. Gold, Systematic evolution of ligands by exponential enrichment: RNAligands to bacteriophage, T4DNApolymerase, Science 249 (1990) 505.
43. S. Tombelli, M. Minunni and M. Mascini, Analytical applications of aptamers, Biosens. Bioelectron. 20 (2005) 2424.
44. M.N. Stojanovic and D.W. Landry, Aptamer–based colorimetric probe for cocaine, J. Am. Chem. Soc. 124 (2002) 9678.
45. H.X. Chang, L.H. Tang, Y. Wang, J.H. Jiang and J.H. Li, Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection, Anal. Chem. 82 (2010) 2341.
46. M. Minunni, S. Tombelli, A. Gullotto, E. Luzi and M. Mascini, Development of biosensors with aptamers as bio–recognition element: the case of HIV–1 Tat protein, Biosens. Bioelectron. 20 (2004) 1149.
47. M. Liss, B. Petersen, H. Wolf and E. Prohaska, An aptamer–based quartz crystal protein biosensor, Anal. Chem. 71 (2002) 4488.
48. M. Liss, B. Petersen, H. Wolf and E. Prohaska, An aptamer–based quartz crystal protein biosensor, Anal. Chem.74 (2002) 4488.
49. B.R. Baker, R.Y. Lai, M.S. Wood, E.H. Doctor, A.J. Heeger and K.W. Plaxco, An electronic, aptamer–based small–molecule sensor for the rapid, label–free detection of cocaine in adulterated samples and biological fluids, J. Am. Chem. Soc. 128 (2006) 3138.
50. X.L. Zuo, S.P. Song, J. Zhang, D.Pan, L.H. Wang, and C.H. Fan, Atarget–responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP, J. Am. Chem. Soc. 129 (2007) 1042.
51. Y. Jin, X. Yao, Q. Liu and J.H. Li, Hairpin DNA probe based electrochemical biosensor using methylene blue as hybridization indicator, Biosens. Bioelectron. 22 (2007) 1126.
52. M. Zayats, Y. Huang, R. Gill, C.A. Ma and I.J. Willner, Label–free reagentless aptamer based sensors for small molecules, J. Am. Chem. Soc.128 (2006) 13666.
53. I. Willner and M. Zayats, Electronic aptamer–based sensors, Angew. Chem. Int. Ed. 46 (2007) 6408.
54. Y. Xu, L. Yang, X. Ye, P. He and Y. Fang, An aptamer–based protein biosensor by detecting the amplified impedance signal, Electroanalysis 18 (2006) 1449.
55. M.C. Rodriquez, A.N. Kawde and J. Wang, Aptamer biosensor for label–free impedance spectroscopy detection of proteins based on recognition–induced switching of the surface charge, Chem. Commun. (2005) 4267.
56. A.N. Kawde, M.C. Rodriquez, T.M.H. Lee and J. Wang, Label–free bioelectronic detection of aptamer–protein interactions, Electrochem. Commun. 7 (2005) 537.
57. D. Xu, X.Yu, Z. Liu, W. He and Z. Ma, Label–free electrochemical detection of aptamerbased array electrodes, Anal. Chem. 77 (2005) 5107.
58. X.R. Liu, Y.Li, J.B. Zheng, J.C. Zhang, Q.L. Sheng, Carbon nanotube–enhanced electrochemical aptasensor for the detection of thrombin, Talanta 81 (2010) 1619.
59. C.F. Ding, Y. Ge and J.M. Lin, Aptamer based electrochemical assay for the determination of thrombin by using the amplification of the nanoparticle, Biosens. Bioelectron. 25 (2010) 1290.