ACCOUNTS
of chemical research

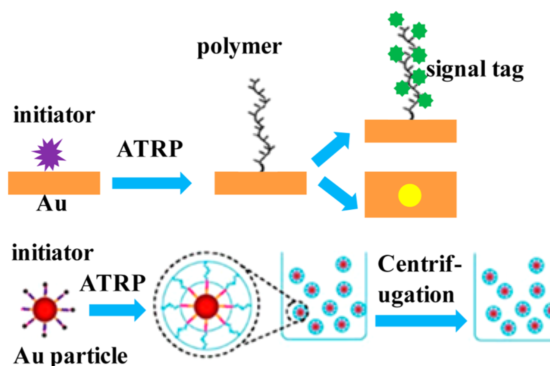
Target-Triggered Polymerization for Biosensing

YAFENG WU, WEI WEI, AND SONGQIN LIU*
State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 210096, People's Republic of China

RECEIVED ON NOVEMBER 29, 2011

CONSPECTUS

Because of the potential applications of biosensors in clinical diagnosis, biomedical research, environmental analysis, and food quality control, researchers are very interested in developing sensitive, selective, rapid, reliable, and low-cost versions of these devices. A classic biosensor directly transduces ligand–target binding events into a measurable physical readout. Because of the limited detection sensitivity and selectivity in earlier biosensors, researchers have developed a number of sensing/signal amplification strategies. Through the use of nanostructured or long chain polymeric materials to increase the upload of signal tags for amplification of the signal readout associated with the ligand–target binding events, researchers have achieved high sensitivity and exceptional selectivity.



Very recently, target-triggered polymerization-assisted signal amplification strategies have been exploited as a new biosensing mechanism with many attractive features. This strategy couples a small initiator molecule to the DNA/protein detection probe prior to DNA hybridization or DNA/protein and protein/protein binding events. After ligand–target binding, the in-situ polymerization reaction is triggered. As a result, tens to hundreds of small monomer signal reporter molecules assemble into long chain polymers at the location where the initiator molecule was attached. The resulting polymer materials changed the optical and electrochemical properties at this location, which make the signal easily distinguishable from the background. The assay time ranged from minutes to hours and was determined by the degree of amplification needed.

In this Account, we summarize a series of electrochemical and optical biosensors that employ target-triggered polymerization. We focus on the use of atom transfer radical polymerization (ATRP), as well as activator generated electron transfer for atom transfer radical polymerization (AGET ATRP) for in-situ formation of polymer materials for optically or electrochemically transducing DNA hybridization and protein-target binding. ATRP and AGET ATRP can tolerate a wide range of functional monomers. They also allow for the preparation of well-controlled polymers with narrow molecular weight distribution, which was predetermined by the concentration ratio of the consumed monomer to the introduced initiator.

Because the reaction initiator can be attached to a variety of detection probes through well-established cross-linking reactions, this technique could be expanded as a universal strategy for the sensitive detection of DNA and proteins. We see enormous potential for this new sensing technology in the development of portable DNA/protein sensors for point-of-need applications.

1. Introduction

The sensitive detection of trace amounts of target proteins in complex biological matrices has attracted considerable attention from many fields, such as clinical diagnosis,[1−3] biomedical research,[4−6] environmental analysis,[7−9] and food quality control.[10−12] To achieve high sensitivity for detection of biotargets, a number of sensing and signal amplification strategies have been developed through the use of various probes coupled with biorecognition steps, such as nucleic acid hybridization[13,14] or antibody/antigen sandwich-type procedure.[15,16] Among these, the target-responsive strategies are of particular interest due to their great utility in the design of simple and potentially portable biosensors with high sensitivity and exceptional selectivity.[17,18] For example, a typical immunosensor employs two-step immunoreactions: monoclonal antibody immobilized on a solid surface for capture of its antigen, followed by a reaction with the secondary antibody labeled with optical or electrochemical species. The binding process was transduced to either optical or electrochemical signals, which

Published on the Web 07/10/2012 www.pubs.acs.org/accounts
10.1021/ar200310f © 2012 American Chemical Society

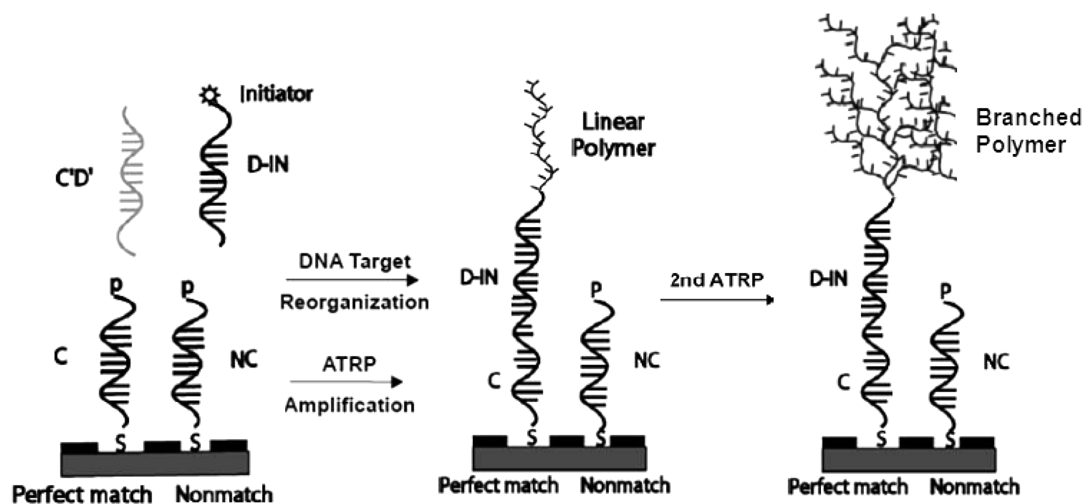


FIGURE 1. ATRP-based DNA sensing and two-stage 30-min ATRP reaction to form branched polymers. The figure shows the actual biosensor response after radical polymerization. In particular, a polymer brush grew at the point where cDNA (C) binding occurred, resulting in a visible change in the substrate color. The control spots (NC) remain unchanged. Adapted from ref 33.

relied on the immunoassay-induced physical enrichment of optical or electroactive tags. These tags were either covalently or noncovalently attached to the secondary antibodies on the solid surface. Also, a number of nanostructured materials and long chain polymeric materials have been adopted into these strategies to increase the upload of signal tags for further amplification of the signal readout associated with each ligand–target binding event.[19−21] Very recently, target-triggered polymerization-assisted signal amplification strategies have been exploited as a new biosensing mechanism with many attractive features. During this strategy, a small initiator molecule is first coupled to the DNA/protein detection probe prior to DNA hybridization or DNA/protein and protein/protein binding events, which did not disrupt the normal bioactivities of biomolecules. The in situ polymerization reaction is triggered after ligand–target binding is completed. Tens and hundreds of small monomer molecules, as signal reporters, were assembled into long chain polymers at the specific location where the initiator molecule was attached, that is, where DNA hybridization or sandwiched immunoassay occurred. The resulting polymer materials changed the optical and electrochemical properties of the sensing spot, which can be easily distinguished from the background. Assay time, which ranged from minutes to hours, was determined by the needed amplification degree.

It is believed that the design and synthesis of a molecule that is capable of both molecular recognition and initiating a polymerization reaction is a prerequisite for implementation of this target-triggered polymerization-assisted signal amplification method. Polymers that are used for molecular recognition[22−24] or macroinitiation[25−27] have been reported in the literature. However, polymeric materials that possess both highly efficient initiation and biological recognition are still rare. In this Account, we focus on signal amplification strategies with target-triggered polymerization, which are largely based on the recent progress of both He's group at North Carolina State University and our laboratory. As will be discussed in the following sections, atom transfer radical polymerization (ATRP) and activators generated electron transfer for atomic transfer radical polymerization (AGET ATRP) were employed, on in situ formed polymer materials to optically or electrochemically transduce DNA hybridization and protein-target binding. Compared with other living polymerization techniques, ATRP is uniquely based on the repetitive addition of monomers to radicals that are generated from dormant alkyl halides in a reversible redox process.[28] ATRP and AGET ATRP were preferred in our studies because they not only were able to tolerate a wide range of functional monomers but also allowed the preparation of well-controlled polymers of narrow molecular weight distribution. This distribution could be predetermined by the concentration ratio of the consumed monomer to the introduced initiator.[29]

2. Target-Triggered Polymerization for DNA Detection

Design and synthesis of polymer materials has emerged as a sensitive, low cost, and easy-to-operate method for DNA analysis. Polymers with unique electrical or optical properties have been successfully involved in DNA detection by

FIGURE 2. (A) Surface chemistry used in the study of DNA-accelerated polymer growth. (B) Photograph showing two substrates in which PHEMA was formed atop ssDNA molecules using (top) CuBr/bpy = 1:2.2 and [CuBr] = 69 mM and (bottom) $CuCl/CuBr_2$/bpy = 1:0.3:2.9 and [CuCl] = 23 mM. Adapted from ref 35.

electrostatically attracting cationic polymers to highly negatively charged DNA or by chemical coupling of DNA on the polymer side chains.[30−32] However, the use of a dynamic growth process of macromolecules as an amplification tool for biosensing has not been widely exploited. He's group pioneered the use of target-triggered polymerization as a signal amplification method in DNA detection.[33,34] In their work, DNA hybridization and ligation were used to differentiate target DNA sequences from mismatches, which led to the attachment of ATRP initiators on a solid surface where specific DNA sequences were located. These initiators subsequently triggered the growth of poly(hydroxyethyl methacrylate) (PHEMA) at the end of immobilized DNA molecules and formed polymer brushes. The formed thick polymer film altered surface reflectivity, changed surface opacity, and rendered the spots directly visible to the naked eye (Figure 1). After that, a second ATRP reaction to form branched polymers on the as-synthesized thick polymer film drastically improved the visibility of occurring DNA binding events. Using this approach, they were able to visually detect target DNA molecules of interest with a detection limit of 1 fmol. Compared with other DNA detection strategies, this method possessed the following advantages: (i) small initiators instead of bulky detection tags were prelabeled on DNA probes, introducing minimal interference to DNA sequence recognition; (ii) amplification of detection signal was demonstrated; (iii) because detection of signal was independently conducted after DNA recognition, various chemical procedures could be used to optimize the polymerization reaction without the concerns over the stability of detection tags or DNA duplexes; (iv) no sophisticated detectors were needed to visualize femtomole target DNA in the sample. The fact that no sophisticated equipment is needed, for qualitative recognition of specific DNA sequences advocated this as a promising solution for portable sensing applications. This pioneer work demonstrated that the polymerization-assisted signal amplification method, which localized accumulation of small molecules, that is, monomers as signal reporter, could be adopted to further improve the selectivity and sensitivity of DNA detection.

There were two independent steps involved in this ATRP-assisted DNA detection: (i) sequence-specific hybridization and ligation and (ii) signal amplification by polymerization. To investigate the ATRP reaction kinetics in the presence of DNA molecules, single-stranded DNA molecules (ssDNAs) were used as the model system to mimic the surface chemistry that formed during the actual DNA detection (Figure 2A).[35] The initiator-coupled single-strand DNA (ssDNA) (1) and initiator-coupled small molecule (4) were immobilized on a Au surface to examine the effectiveness of polymer grafting. Meanwhile, the same ssDNA (2) and small molecules (5) without initiators were used as control molecules for background subtraction. Small alkanethiol molecules, 6-mercapto-1-hexanol (MCH) (3) or 1-undecanethiol (6), were used as diluting reagents to control the surface density of ATRP initiators. The results showed that the presence of DNA molecules significantly accelerated the growth rates at the beginning of the ATRP reactions, which was ascribed to a combined result of the highly charged sugar−phosphate backbones of DNA molecules and the formation of Cu complexes with DNA bases. After the initial polymer growth, a smaller yet constant polymer

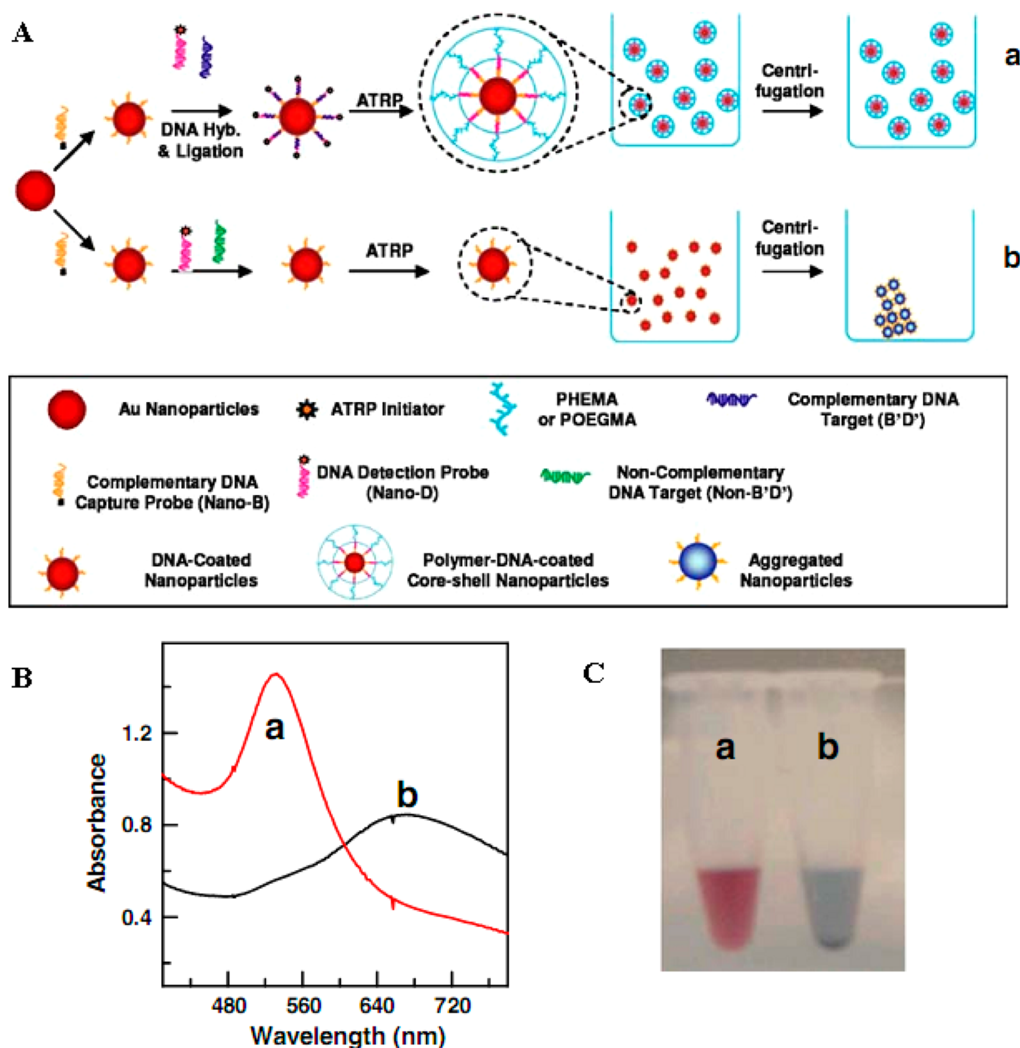


FIGURE 3. (A) Polymer-assisted reverse colorimetric particle-based DNA assays. (B) UV spectrum of a and b results from panel A. (C) The photograph of a and b results from panel A. Adapted from ref 36.

growth rate was observed, suggesting the reduced influence of DNA molecules as the ATRP reaction centers moved farther away from the surface. A sufficient amount of PHEMA was formed under the optimized ATRP reaction conditions that enabled the direct visualization of the spots where the ssDNA initiators were immobilized (Figure 2B, bottom). However, no discernible features were observed on the surface exposed to less-than-optimal ATRP reaction conditions (Figure 2B, top). All these results demonstrated that DNA molecules could be used as biocatalysts to expedite polymer grafting efficiency.

Following these spot visualization detections further, they developed a polymer-assisted reverse colorimetric method to monitor the occurrence of the DNA binding events.[36] The target-triggered polymerization was employed to form a thick polymer shell outside of particles, which acted as the physical barrier to keep Au particles apart. Particles without DNA hybridization aggregated, accompanied by a pronounced solution color change from red to blue (Figure 3). Under the optimized conditions, faster polymer chain growth on the surface overcame particle aggregation and preserved particle stability via steric stabilization. Unlike the conventional colorimetric method, polymer-assisted steric stabilization improved resistance of Au nanoparticles to environmental fluctuation and physically prevented close contact between particles, which consequently eliminated false-negative readouts.

Development of electrochemical sensors hold significant advantages due to their high chemical selectivity, excellent sensitivity, and more importantly, great portability.[37−39] We have recently adopted target-triggered polymerization signal amplification in combination with electrochemical detection for DNA biosensing. This was an attempt to further

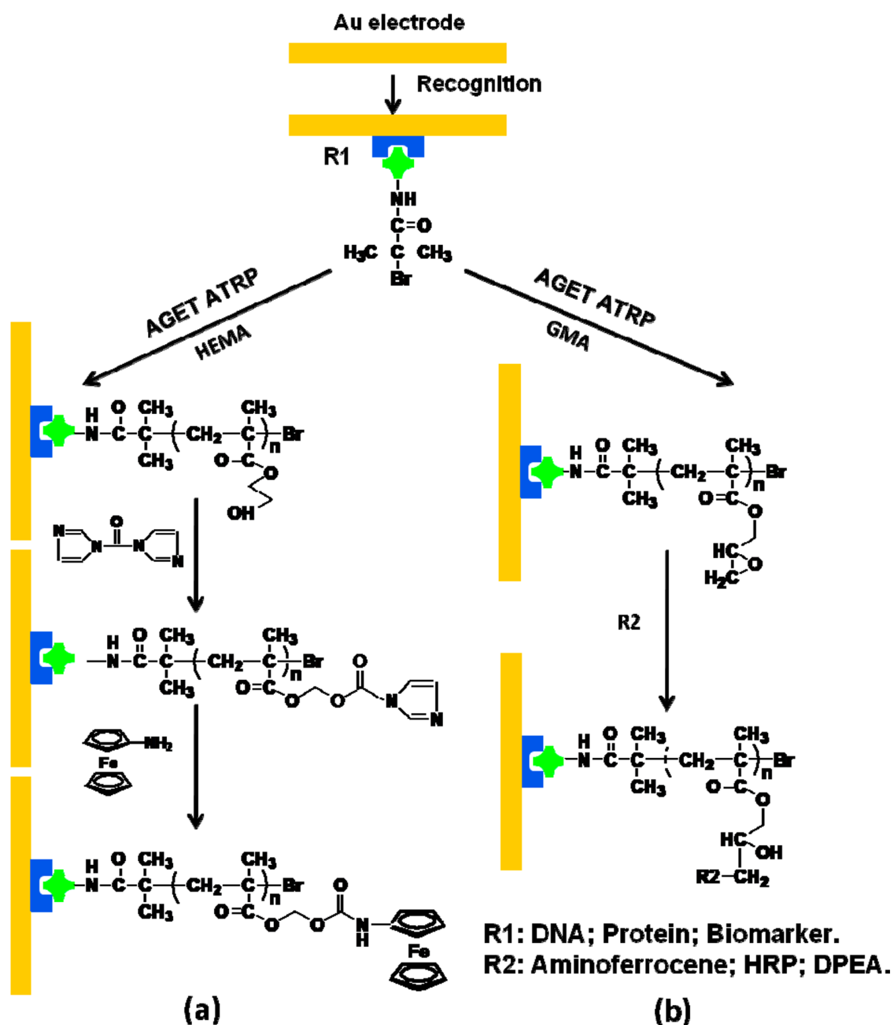


FIGURE 4. Scheme of polymerization-assisted signal amplification strategies for DNA and protein detection.

enhance sensing sensitivity, as well as to provide an interface compatible with existing commercial sensing techniques based on electrochemical readouts.[40] In this case, ATRP was modified by using a reducing agent to bring the Cu(II) complex back to its corresponding ATRP-active, lower oxidative state of catalytic complexes.[41,42] During this redox cycling process, oxygen was consumed prior to the occurrence of polymer growth, thus eliminating the need for air-purging to remove oxygen. This elimination step allowed AGET ATRP to obtain high efficiency in polymer grafting and better tolerance toward oxygen in air. 2-Hydroxyethyl methacrylate (HEMA) and glycidyl methacrylate (GMA) were used as the monomers to provide excess hydroxyl or epoxy groups for immobilization of electrochemical tags, that is, aminoferrocene ($FcNH_2$). Growth of long chain polymeric materials atop the ATRP-initiator coupled DNA molecules that were anchored on the electrode surface provided numerous sites for aminoferrocene coupling. In turn, the increase coupling amounts of $FcNH_2$ significantly enhanced electrochemical signal output. The target-triggered polymerization signal amplification in combination with several detection technologies for DNA/protein biosensing has been summarized in Figure 4 and Table 1. A couple of well-defined peaks corresponding to the oxidation and reduction of the anchored aminoferrocene on long chain polymeric materials were clearly observed in its cyclic voltammogram. Quantitative analysis of the target DNA concentration showed that the measured peak current was proportional to the logarithm of DNA concentration over 5 orders of magnitude. About 30 amol of target DNA could be effectively detected by using this polymerization amplified electrochemical sensing strategy. This confirmed successful electrochemical sensing using target-triggered polymerization signal amplification for sensitive detection of a target DNA of interest.

Aside from AGET ATRP, the photoinitiated free-radical polymerization was also examined to suit the target-triggered

TABLE 1. Target-Triggered Polymerization Signal Amplification by Coupling Signal Tags on Polymer Side Chains in Combination with Different Detection Technologies

detection biomolecules	binding mode	signal tags	detection method	linear range (ng/mL)	LOD (pg/mL)	ref
DNA	DNA hybridization	aminoferrocene	electrochemical	0.1−1000 nM	15 pM	40
ovalbumin	carbohydrate recognition	aminoferrocene	electrochemical	0.1−500	0.07	40
CEA	sandwiched immunoassay	aminoferrocene	electrochemical	0.0005−40	0.1	47
PSA	sandwiched immunoassay	aminoferrocene	electrochemical	0.001−40	0.14	47
PSA	sandwiched immunoassay	HRP	flow injection CL/electrochemical	0.005−20	1.3	48
CEA	sandwiched immunoassay	DPEA	ECL	0.001−1000	0.5	49

polymerization-assisted signal amplification sensing strategy.[43] Dual-functional macrophotoinitiators with highly efficient initiation and biological recognition were synthesized. This was accomplished through coupling of water-soluble photoinitiators and NeutrAvidin, to a fraction of the carboxylate residues from a high-molecular-weight copolymer of acrylic acid and acrylamide, using aqueous carbodiimide coupling chemistry.[44] The as-prepared macrophotoinitiator displayed the ability to recognize biotin-labeled oligonucleotides and to initiate polymerization of water-soluble monomers. After a 10 min dose of light, a macroscopically observable polymer grew from the spots at which biotinylated oligonucleotides were located, which resulted in an easily observable color change from gold to blue.[45] With this approach, as few as $\sim$1000 recognition events (10 zmol) were easily visible to the unaided eye.

3. Target-Triggered Polymerization for Protein Detection

Target-triggered polymerization has been successfully used for quantitative detection of specific DNA sequences with better reproducibility, simpler assay procedure, and faster assay turn-around than those using a conventional PCR-based DNA detection method.[46] The reaction initiator could be attached to potentially any detection probes through well-established cross-linking reactions; inspired by this property, the application scope of this sensing concept could be extended beyond DNA detection. We have recently adopted target-triggered polymerization signal amplification strategy for protein detection by coupling of different detection techniques as shown in Figure 4 and Table 1.

First, concanavalin A (Con A) was covalently immobilized on a Au electrode to allow permanent attachment of the initiator-coupled ovalbumin.[40] AGET ATRP was conducted in a reaction mixture containing glycidyl methacrylate (GMA) as the monomer. The pendant epoxide groups on PGMA were used for direct coupling of aminoferrocene. More than 7-fold signal enhancement in ovalbumin detection has been achieved, compared with the unamplified method. We further adapted this polymerization-assisted signal amplification strategy to electrochemically detect biomarkers in serum.[47] A sandwich immunoassay process was employed to immobilize a polymerization reaction center, the initiator-conjugated polyclonal prostate specific antigen (PSA), or polyclonal carcinoembryonic antigen (CEA) antibodies on the surface of the electrode. AGET ATRP subsequently triggered the local accumulation of GMA monomers, which led to multiple detection probes consequently being introduced to each binding event, thusly enhancing detection sensitivity of immunosensing. The clinical validation was illustrated by examining of a medium number of 100 clinical sera. Results showed that the proposed immunosensor was highly sensitive and selective and matched well with the clinical electrochemiluminescent method.

Second, horseradish peroxidase (HRP) was introduced as a signal tag for flow injection chemiluminescent (CL) and electrochemical detection.[48] The initiator-conjugated polyclonal prostate specific antigen (PSA) antibodies were immobilized on the substrate surface through sandwiched immunoreactions to trigger AGET ATRP. Growth of long chain polymeric materials provided excess epoxy groups for HRP coupling, which in turn significantly increased the loading of signal molecules and enhanced the chemiluminescent and electrochemical readouts. With this method, more than 13- and 14-fold enhancement in the chemiluminescent intensity and electrocatalytic current was achieved, compared with the traditional sandwiched immunoassays using HRP-conjugated antibody directly.

Third, to further improve the sensitivity, small molecule 2-(diisopropylamino)ethylamine (DPEA) was introduced as a signal tag for electrochemiluminescence (ECL) detection of biomarkers.[49] ECL has attracted considerable attention due to its low cost, wide range of analytes, low background signal, and high sensitivity.[50] A number of ECL analytical methods based on ruthenium complex ($Ru(bpy)_3{}^{2+}$) with tertiary amine (TA) or diketone containing compounds, as coreactants,[51] have been developed for environmental assays, such as food and water testing, biological warfare agent detection, and clinical diagnostics.[52] We adapted the target-triggered polymerization-assisted signal amplification

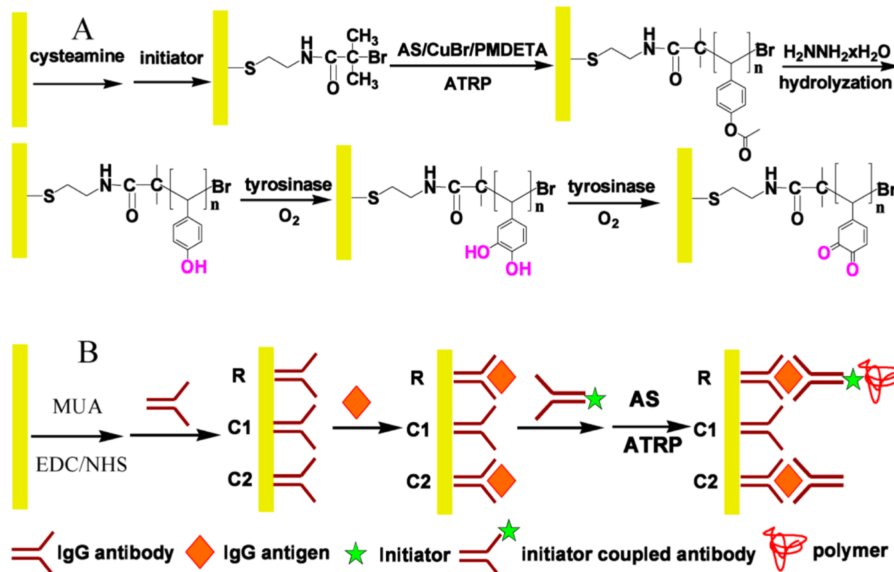


FIGURE 5. (A) Schematic illustration of the strategies used in the coupling of polymerization-assisted signal amplification with electrochemical detection for biosensing. (B) Schematic illustration of SI-ATRP of AS for immunosensing. Adapted from ref 53.

method for the development of a novel $Ru(bpy)_3^{2+}$/TA ECL system for immunosensing.[49] In our case, the accumulation of GMA monomer provided numerous epoxy groups for the immobilization of DPEA and thus enhanced the ECL signals. Detection of 17 serum specimens by using this approach produced similar results compared with the clinical electrochemiluminescent method. That is, the developed immunoassay provided a promising alternative tool for determining CEA in human serum in the clinical laboratory.

In the aforementioned cases, signal tags ($FcNH_2$, HRP, DPEA) were introduced to polymer side chains after polymers formed. To simplify the assay process and improve assay throughput, optimization of the preliminary process and searching for other suitable monomers with direct electrochemical activity were done.[53] The novel strategy included utilizing a sandwiched immunoassay to immobilize a polymerization reaction center on a gold surface, local accumulation of monomers by surface-initiated ATRP, and electrochemical detection of phenolic hydroxyl groups in the presence of tyrosinase. 4-Acetoxystyrene was chosen as the monomer to provide acetoxyl groups, which could be converted into phenolic hydroxyl groups through a simple hydrazine hydration process. Concurrently, tyrosinase catalyzed the oxidation of phenol derivatives to the corresponding catechol and *o*-quinone derivatives in the presence of O_2. Catechol/*o*-quinone systems were well-known redox couples that display good electrochemical behavior and thus can be electrochemically monitored to follow the ATRP of 4-acetoxystyrene. The growth of long-chain polymeric materials provided numerous acetoxyl groups, which in turn significantly enhanced the electrochemical signal output in the presence of tyrosinase (Figure 5).

As an alternative to electrochemical, flow injection chemiluminescent, and ECL detection, target-triggered polymerization-assisted signal amplification could also be integrated with other detection methods. For example, Liu and coworkers[54] proposed a novel surface plasmon resonance (SPR) sensing strategy by integrating target-triggered polymerization and SPR readout for highly sensitive detection of proteins. In their work, bacterial cholera toxin (CT) was chosen as the model protein covalently immobilized on gold with 11-mercaptoundecanoic acid linkage. The immobilized CT recognized biotinylated anti-CT and allowed initiators with a biotin tag to be fixed at the protein binding site through a neutravidin bridge. The localized growth of polymers of poly(hydroxyl-ethyl methacrylate) (PHEMA), via an ATRP mechanism, increased the SPR readout. In addition, by coupling 2-bromoisobutyryl bromide to the hydroxyl groups on the PHEMA side chains, the signal was further enhanced (Figure 6). These two consecutive ATRP steps significantly enhanced the sensitivity of SPR detection, which allowed low amounts of CT to be quantified with large signals. Using this signal amplification strategy, they were able to detect the surface coverage of CT in a range from 8.23×10^{-15} to 3.61×10^{-12} mol/cm^2, with a detection limit of 6.27×10^{-15} mol/cm^2.

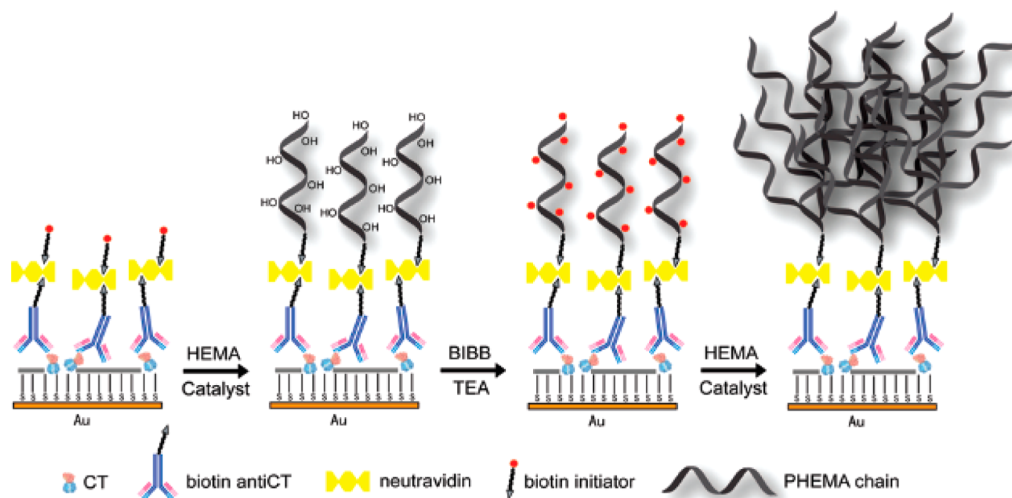


FIGURE 6. Cartoon representation of the biotinylated initiator coupled surface and two consecutive steps of in situ surface ATRP reactions for SPR signal amplification. Adapted from ref 54.

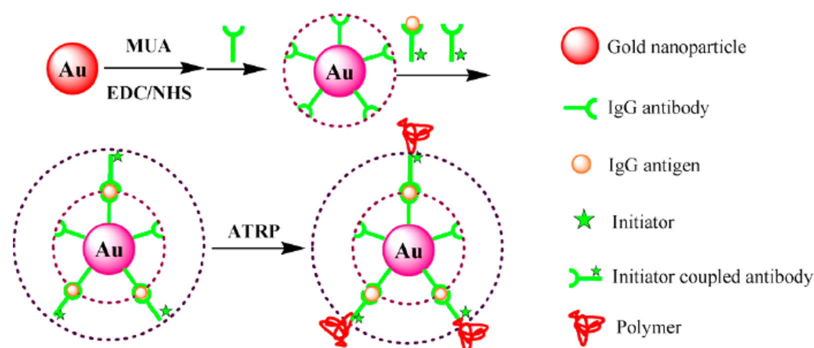


FIGURE 7. Schematic illustration for the colorimetric immunosensing approach by using protein-modified GNP probes that were functionalized with ATRP. Adapted from ref 55.

Another example was a colorimetric immunosensing approach for biomarker assay.[55] Colloidal gold nanoparticles (GNPs) have a beautiful wine-red color, which is ascribed to the collective oscillation of the conduction band surface electrons on interaction with light of suitable wavelengths, the localized surface plasma resonance.[56] When GNPs were functionalized with polymers, both the position and bandwidth of the surface plasmon band of GNPs were changed. In our case, ATRP-initiator-conjugated IgG could be accumulated onto the surface of GNPs through a competive immunoreaction.[55] The immobilized ATRP initiators prompted polymer chain growth to form polymers outside of GNPs, which altered the optical property of GNPs and produced a distinct color change (Figure 7). Furthermore, the surface grafting of polymer chains on GNPs was controlled by the amount of initiator center being coated on the GNP surface. In turn, the amount of initiator center being coated was decided by the amount of free initiator-coupled antibodies in the incubation solution. This allowed the detection of IgG by the absorption spectra and imagework color-analysis software, with a linear range of 0.5−25 ng mL^{-1} and a detection limit of 0.03 ng mL^{-1}.

All above cases showed that the amplification-by-target-triggered polymerization concept was suitable for ultrasensitive detection of proteins in buffer and serum, but special instruments were needed. However, Qian and He described a source of promising potential in future development of point-of-need devices using target-triggered polymerization for grafting visualization, particular, visualization of distinguishable spots on the sensor surface in order to amplify the occurrence of protein binding events.[57] Con A and streptavidin were immobilized to a mercaptoundecanoic acid-coated Au surface in an array format for specific detection of ovalbumin, biotinylated insulin, or biotinylated BSA, respectively (Figure 8). The change of substrate reflectance at the spots demonstrated the occurrence of the protein binding. This also indicated the survival of protein complexes in AGET ATRP and the successful formation of organic films

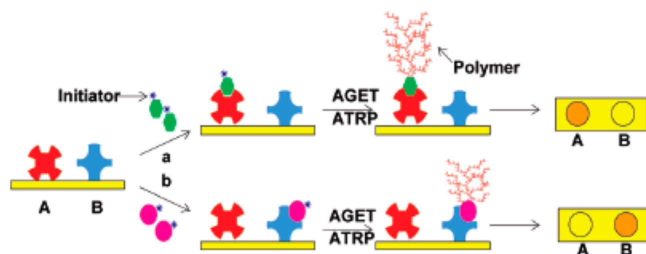


FIGURE 8. Major steps for polymerization-amplified protein detection. Adapted from ref 57.

upon protein binding. With this approach, the binding of femtomole ovalbumin was clearly differentiated from the background using ellipsometry, while binding of subpicomole protein led to visually distinguishable spots on the sensor surface.

4. Conclusions and Perspective

In this Account, we summarize recent research activities in target-triggered polymerization-assisted signal amplification for biosensor applications. We have demonstrated that target-triggered polymerization is an effective method to provide multiple binding sites to tag active probes for DNA/protein biosensing. Both DNA hybridization and sandwiched immunoassay led to immobilization of polymerization reaction centers on the surface; subsequently triggered polymer growth results in local accumulation of monomers that altered the optical properties of the substrate and provided multiple binding sites to tag active probes, which can be detected by many techniques.

This Account is not a comprehensive review and only summarizes a fraction of research activities that exploit target-triggered polymerization. Given that the reaction initiator can be attached to potentially any detection probes through well-established cross-linking reactions, in addition to the DNA/protein described above, it could be applied as a universal strategy for highly sensitive detection of nearly all DNA and proteins. It can be also foreseen that the new sensing technology possesses enormous potential for development of portable DNA/protein sensors for point-of-need applications.

We also expect to apply the target-triggered polymerization-assisted signal amplification strategy to microarray analysis for high-throughput screening of complex biological systems. On the other hand, given the availability of a large variety of signal amplification methods (e.g., PCR,[58] signal amplification based on conjugated polymers,[59] signal amplification based on enzymes,[60] and signal amplification based on nanoparticles/nanomaterials[61]), it is possible to further improve the sensitivity and selectivity of DNA/protein sensors by combining the target-triggered polymerization-assisted signal amplification strategy with other signal amplification methods.

This work is supported by the Key Program (21035002) from the National Natural Science Foundation of China, National Natural Science Foundation of China (Grant Nos. 20875013, 21175021), National Basic Research Program of China (No.2010CB732400), and the Key Program (BK2010059) from the Natural Science Foundation of Jiangsu province, Open Foundation from State Key Laboratory of Bioelectronics, and Significant Scientific Research Guidance Foundation from Southeast University.

BIOGRAPHICAL INFORMATION

Yafeng Wu was born in Jingjiang, China, in 1985. She received her B.S. degree (2006) in School of Chemistry from Huaihai Institute of Technology. After that, she studied in School of Chemistry and Chemical Engineering of Southeast University as a Ph.D. student.

Wei Wei was born in Henan, China, in 1975. She received the Ph.D. degree from the University of Nanjing in 2004. She joined Southeast University in 2004 and has been an Associate Professor since 2009. Her interests are in the study of new analytical methods based on spectroscopy, electrochemical methods, and chromatography. In recent years, she has studied many new methods for food, environment, pharmacy and clinic analysis. Her studies were funded by several national and provincial Nature Science Foundations of China.

Songqin Liu was born in Jingjiang, China, in 1965. He received the Ph.D. degree from the University of Nanjing in 2003. He has been a Professor in the Department of Chemistry since 2005. His major research areas include the fabrication and development of biosensors for the sensitive recognition of glycoprotein molecules in blood. To establish the new methods and novel techniques for the detecting of glycoproteins, reveal the relationship between these glycoproteins or their expressed level and illness or curative effects, and develop novel clinical diagnostic approaches with high selectively and sensitivity are his main interests.

FOOTNOTES

*To whom correspondence should be addressed. Tel: 86-25-52090613; Fax: 86-25-52090618. E-mail address: liusq@seu.edu.cn.
The authors declare no competing financial interest.

REFERENCES

1 Knox, K.; Carrigan, D.; Simmons, G.; Teque, F.; Zhou, Y. C.; Hackett, J.; Qiu, X. X.; Luk, K. C.; Schochetman, G.; Knox, A.; Kogelnik, A. M.; Levy, J. A. No evidence of murine-like gammaretroviruses in CFS patients previously identified as XMRV-infected. *Science* **2011**, *333*, 94–97.

2 Dacres, H.; Wang, J.; Leitch, V.; Horne, I.; Anderson, A. R.; Trowell, S. C. Greatly enhanced detection of a volatile ligand at femtomolar levels using bioluminescence resonance energy transfer (BRET). *Biosens. Bioelectron.* **2011**, *29*, 119–124.

3 Chen, Y. H.; Snyder, M. R.; Zhu, Y.; Tostrud, L. J.; Benson, L. M.; Katzmann, J. A.; Bergen, H. R. Simultaneous phenotyping and quantification of alpha-1-antitrypsin by liquid chromatography-tandem mass spectrometry. *Clin. Chem.* **2011**, *57*, 1161–1168.

4 Mitsakakis, K.; Gizeli, E. Multi-sample acoustic biosensing microsystem for protein interaction analysis. *Biosens. Bioelectron.* **2011**, *26*, 4579–4584.

5 Vangala, K.; Yanney, M.; Hsiao, C. T.; Wu, W. W.; Shen, R. F.; Zou, S. G.; Sygula, A.; Zhang, D. M. Sensitive carbohydrate detection using surface enhanced raman tagging. *Anal. Chem.* **2010**, *82*, 10164–10171.

6 Yang, K.; Zhang, C. Y. Improved sensitivity for the electrochemical biosensor with an adjunct probe. *Anal. Chem.* **2010**, *82*, 9500–9505.

7 Huang, J.; Chen, Y.; Yang, L.; Zhu, Z.; Zhu, G. Z.; Yang, X. H.; Wang, K. M.; Tan, W. H. Amplified detection of cocaine based on strand-displacement polymerization and fluorescence resonance energy transfer. *Biosens. Bioelectron.* **2011**, *28*, 450–453.

8 Boonjob, W.; Miro, M.; Segundo, M. A.; Cerda, V. Flow-through dispersed carbon nanofiber-based microsolid-phase extraction coupled to liquid chromatography for automatic determination of trace levels of priority environmental pollutants. *Anal. Chem.* **2011**, *83*, 5237–5244.

9 Zhu, X. X.; Kriegel, A. M.; Boustany, C. A.; Blake, D. A. Single-chain variable fragment (scFv) antibodies optimized for environmental analysis of uranium. *Anal. Chem.* **2011**, *83*, 3717–3724.

10 Whitcombe, M. J.; Chianella, I.; Larcombe, L.; Piletsky, S. A.; Noble, J.; Porter, R.; Horgan, A. The rational development of molecularly imprinted polymer-based sensors for protein detection. *Chem. Soc. Rev.* **2011**, *40*, 1547–1571.

11 Vashist, S. K.; Zheng, D; Al-Rubeaan, K.; Luong, J. H. T.; Sheu, F. S. Advances in carbon nanotube based electrochemical sensors for bioanalytical applications. *Biotechnol. Adv.* **2011**, *29*, 169–188.

12 Zeleny, R.; Schimmel, H. Towards comparability of ELISA results for peanut proteins in food: A feasibility study. *Food Chem.* **2010**, *123*, 1343–1351.

13 Browne, K. A.; Deheyn, D. D.; El-Hiti, G. A.; Smith, K.; Weeks, I. Simultaneous quantification of multiple nucleic acid targets using chemiluminescent probes. *J. Am. Chem. Soc.* **2011**, *133*, 14637–14648.

14 Choi, J.; Love, K. R.; Gong, Y.; Gierahn, T. M.; Love, J. C. Immuno-hybridization chain reaction for enhancing detection of individual cytokine-secreting human peripheral mononuclear cells. *Anal. Chem.* **2011**, *83*, 6890–6895.

15 Tian, D. Y.; Duan, C. F.; Wang, W.; Cui, H. Ultrasensitive electrochemiluminescence immunosensor based on luminol functionalized gold nanoparticle labeling. *Biosens. Bioelectron.* **2010**, *25*, 2290–2295.

16 Zheng, Y.; Chen, H.; Liu, X. P.; Jiang, J. H.; Luo, Y.; Shen, G. L.; Yu, R. Q. An ultrasensitive chemiluminescence immunosensor for PSA based on the enzyme encapsulated liposome. *Talanta* **2008**, *77*, 809–814.

17 Li, D.; Song, S. P.; Fan, C. H. Target-responsive structural switching for nucleic acid-based sensors. *Acc. Chem. Res.* **2010**, *43*, 631–641.

18 Li, W.; Nie, Z.; Xu, X. H.; Shen, Q. P.; Deng, C. Y.; Chen, J. H.; Yao, S. Z. A sensitive, label free electrochemical aptasensor for ATP detection. *Talanta* **2009**, *78*, 954–958.

19 Kong, L. M.; Huang, S. S.; Yue, Z. L.; Peng, B.; Li, M. Y.; Zhang, J. Sensitive mediator-free tyrosinase biosensor for the determination of 2,4-dichlorophenol. *Microchim. Acta* **2009**, *165*, 203–209.

20 Xu, F. J.; Cai, Q. J.; Li, Y. L.; Kang, E. T.; Neoh, K. G. Covalent immobilization of glucose oxidase on well-defined poly(glycidyl methacrylate)-Si(111) hybrids from surface-initiated atom-transfer radical polymerization. *Biomacromolecules* **2005**, *6*, 1012–1020.

21 Cui, H. F.; Ye, J. S.; Zhang, W. D.; Sheu, F. S. Modification of carbon nanotubes with redox hydrogel: Improvement of amperometric sensing sensitivity for redox enzymes. *Biosens. Bioelectron.* **2009**, *24*, 1723–1729.

22 Mosbach, K.; Ramstrom, O. The emerging technique of molecular imprinting and its future impact on biotechnology. *Nat. Biotechnol.* **1996**, *14*, 163–170.

23 Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Adv. Mater.* **2006**, *18*, 1345–1360.

24 O'Connor, N. A.; Paisner, D. A.; Huryn, D.; Shea, K. J. Screening of 5-HT1A receptor antagonists using molecularly imprinted polymers. *J. Am. Chem. Soc.* **2007**, *129*, 1680–1689.

25 Hawker, C. J.; Bosman, A. W.; Harth, E. New polymer synthesis by nitroxide mediated living radical polymerizations. *Chem. Rev.* **2001**, *101*, 3661–3688.

26 Dai, C. A.; Yen, W. C.; Lee, Y. H.; Ho, C. C.; Su, W. F. Facile synthesis of well-defined block copolymers containing regioregular poly(3-hexyl thiophene) via anionic macroinitiation method and their self-assembly behavior. *J. Am. Chem. Soc.* **2007**, *129*, 11036–11038.

27 Li, X. J.; Qian, Y. F.; Liu, T.; Hu, X. L.; Zhang, G. Y.; You, Y. Z.; Liu, S. Y. Amphiphilic multiarm star block copolymer-based multifunctional unimolecular micelles for cancer targeted drug delivery and MR imaging. *Biomaterials* **2011**, *32*, 6595–6605.

28 Matayjaszewski, K.; Xia, J. H. Atom transfer radical polymerization. *Chem. Rev.* **2001**, *101*, 2921–2990.

29 Coessens, V.; Pintauer, T.; Matyjaszewski, K. Functional polymers by atom transfer radical polymerization. *Prog. Polym. Sci.* **2001**, *26*, 337–377.

30 Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. DNA hybridization detection with water-soluble conjugated polymers and chromophore-labeled single-stranded DNA. *J. Am. Chem. Soc.* **2003**, *125*, 896–900.

31 Gibbs, J. M.; Park, S.-J.; Anderson, D. R.; Watson, K. J.; Mirkin, C. A.; Nguyen, S. T. Polymer-DNA hybrids as electrochemical probes for the detection of DNA. *J. Am. Chem. Soc.* **2005**, *127*, 1170–1178.

32 Kim, W. J.; Sato, Y.; Akaike, T.; Maruyama, A. Cationic comb-type copolymers for DNA analysis. *Nat. Mater.* **2003**, *2*, 815–820.

33 Lou, X. H.; Lewis, M. S.; Gorman, C. B.; He, L. Detection of DNA point mutation by atom transfer radical polymerization. *Anal. Chem.* **2005**, *77*, 4698–4705.

34 Lou, X. H.; He, P.; Geoffrey, O. O.; He, L. Radical polymerization in biosensing. *Anal. Bioanal. Chem.* **2006**, *386*, 525–531.

35 Lou, X. H.; He, L. DNA-accelerated atom transfer radical polymerization on a gold surface. *Langmuir* **2006**, *22*, 2640–2646.

36 Zheng, W. M.; He, L. Particle stability in polymer-assisted reverse colorimetric DNA assays. *Anal. Bioanal. Chem.* **2009**, *393*, 1305–1313.

37 Cash, K. J.; Ricci, F.; Plaxco, K. W. An electrochemical sensor for the detection of protein-small molecule interactions directly in serum and other complex matrices. *J. Am. Chem. Soc.* **2009**, *131*, 6955–6957.

38 Ricci, F.; Bonham, A. J.; Mason, A. C.; Reich, N. O.; Plaxco, K. W. Reagentless, electrochemical approach for the specific detection of double- and single-stranded DNA binding proteins. *Anal. Chem.* **2009**, *81*, 1608–1614.

39 Freeman, R.; Li, Y.; Tel-Vered, R.; Sharon, E.; Elbaz, J.; Willner, I. Self-assembly of supramolecular aptamer structures for optical or electrochemical sensing. *Analyst* **2009**, *134*, 653–656.

40 Wu, Y. F.; Liu, S. Q.; He, L. Electrochemical biosensing using amplification-by-polymerization. *Anal. Chem.* **2009**, *81*, 7015–7021.

41 Jakubowski, W.; Matyjaszewski, K. Activator generated by electron transfer for atom transfer radical polymerization. *Macromolecules* **2005**, *38*, 4139–4146.

42 Esteves, A. C. C.; Bombalski, L.; Trindade, T.; Matyjaszewski, K.; Barros-Timmons, A. Polymer grafting from CdS quantum dots via AGET ATRP in miniemulsion. *Small* **2007**, *3*, 1230–1236.

43 Kloosterboer, J. G. Network formation by chain crosslinking photopolymerization and its applications in electronics. *Adv. Polym. Sci.* **1988**, *84*, 1–61.

44 Staros, J. V.; Wright, R. W.; Swingle, D. M. Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal. Biochem.* **1986**, *156*, 220–222.

45 Jenison, R.; La, H.; Haeberli, A.; Ostroff, R.; Polisky, B. Silicon-based biosensors for rapid detection of protein or nucleic acid targets. *Clin. Chem.* **2001**, *47*, 1894–1900.

46 Qian, H.; He, L. Surface-initiated activators generated by electron transfer for atom transfer radical polymerization in detection of DNA point mutation. *Anal. Chem.* **2009**, *81*, 4536–4542.

47 Wu, Y. F.; Liu, S. Q.; He, L. Polymerization-assisted signal amplification for electrochemical detection of biomarkers. *Analyst* **2011**, *136*, 2558–2563.

48 Wu, Y. F.; Liu, S. Q.; He, L. Activator generated electron transfer for atom transfer radical polymerization for immunosensing. *Biosen. Bioelectron.* **2010**, *26*, 970–975.

49 Wu, Y. F.; Shi, H. Y.; Yuan, L.; Liu, S. Q. A novel electrochemiluminescence immunosensor via polymerization-assisted amplification. *Chem. Commun.* **2010**, *46*, 7763–7765.

50 Dai, H.; Chi, Y. U.; Wu, X. P.; Wang, Y. M.; Wei, M. D.; Chen, G. N. Biocompatible electrochemiluminescent biosensor for choline based on enzyme/titanate nanotubes/chitosan composite modified electrode. *Biosens. Bioelectron.* **2010**, *25*, 1414–1419.

51 Yuan, J. P.; Li, T.; Yin, X. B.; Guo, L.; Jiang, X. Z.; Jin, W. R.; Yang, X. R.; Wang, E. K. Characterization of prolidase activity using capillary electrophoresis with tris(2,2′-bipyridyl)ruthenium(II) electrochemiluminescence detection and application to evaluate collagen degradation in diabetes mellitus. *Anal. Chem.* **2006**, *78*, 2934–2938.

52 Richter, M. M. Electrochemiluminescence (ECL). *Chem. Rev.* **2004**, *104*, 3003–3036.

53 Yuan, L.; Wu, Y. F.; Shi, H. Y.; Liu, S. Q. Surface-initiated atom-transfer radical polymerization of 4-acetoxystyrene for immunosensing. *Chem.—Eur. J.* **2011**, *17*, 976–983.

54 Liu, Y.; Dong, Y.; Jauw, J.; Linman, M. J.; Chen, Q. Highly sensitive detection of protein toxins by surface plasmon resonance with biotinylation-based inline atom transfer radical polymerization amplification. *Anal. Chem.* **2010**, *82*, 3679–3685.

55 Shi, H. Y.; Yuan, L.; Wu, Y. F.; Liu, S. Q. Colorimetric immunosensing via protein functionalized gold nanoparticle probe combined with atom transfer radical polymerization. *Biosens. Bioelectron.* **2011**, *26*, 3788–3793.

56 Teichroeb, J. H.; Forrest, J. A.; Ngai, V.; Jones, L. W. Anomalous thermal denaturing of proteins adsorbed to nanoparticles. *Eur. Phys. J. E* **2006**, *21*, 19–24.

57 Qian, H.; He, L. Detection of protein binding using activator generated by electron transfer for atom transfer radical polymerization. *Anal. Chem.* **2009**, *81*, 9824–9827.

58 Zhou, L.; Ou, L. J.; Chu, X.; Shen, G. L.; Yu, R. Q. Aptamer-based rolling circle amplification: A platform for electrochemical detection of protein. *Anal. Chem.* **2007**, *79*, 7492–7500.

59 Lee, K.; Povlich, L. K.; Kim, J. Recent advances in fluorescent and colorimetric conjugated polymer-based biosensors. *Analyst* **2010**, *135*, 2179–2189.

60 Alfonta, L.; Willner, I.; Throckmorton, D. J.; Singh, A. K. Electrochemical and quartz crystal microbalance detection of the cholera toxin employing horseradish peroxidase and GM1-functionalized liposomes. *Anal. Chem.* **2001**, *73*, 5287–5295.

61 Wang, J. Nanoparticle-based electrochemical DNA detection. *Anal. Chim. Acta* **2003**, *500*, 247–257.