

## **Chemistry 681: Student Seminar Series**

Speaker: Yong-Gu Kim

Date: February 25, 2002

Time: 4:00 p.m.

Room: 2102

Advisor: Dr. Richard M. Crooks

## **Electrochemical Detection of DNA**

### **I. Introduction**

Since the elucidation of DNA structure by Watson and Crick, many scientists have endeavored to study the sequence of DNA and its' role in the origin of life. After completion of the human genome project, attention has been refocused and devoted to new ways of making artificial organs and seeking cures for cancer. Therefore detection of DNA becomes an important issue and many attempts to design fast and reliable methods are ongoing in this field. There are various analytical approaches applied to the detection of DNA including fluorescence,<sup>1-2</sup> electrochemiluminescence,<sup>3-4</sup> and direct electrochemical methods.<sup>5-19</sup> Among these analytical techniques, electrochemical methods are of special interest because of their speed and low-cost.

Electrochemical detection methods take advantage on the fact that DNA typically exists in a conjoined double-helix form. Therefore, a single-strand DNA (ssDNA) is allowed to react with a target-DNA-strand to spontaneously form a double strand (dsDNA) and as a consequence of this hybridization process, an observable electrochemical response will be changed. Electrochemical detection methods for DNA usually fall into three categories. First, a probe-DNA strand can be immobilized on an electrode surface and form a dsDNA with the target-DNA. This process is monitored by electrochemical signal changes as intercalation is observed. Second, probe-DNA can be similarly immobilized on the surface of nanoparticles and form dsDNA with the target-

DNA. In this case, electrochemical detection is based on the oxidation of gold or silver clusters which are attached to the target-DNA. The third method is based on the electrochemical oxidation of nucleotides in the DNA. In this approach, electrochemical signals from oxidation of individual base or sugar residues of label free ssDNA and dsDNA are tested.

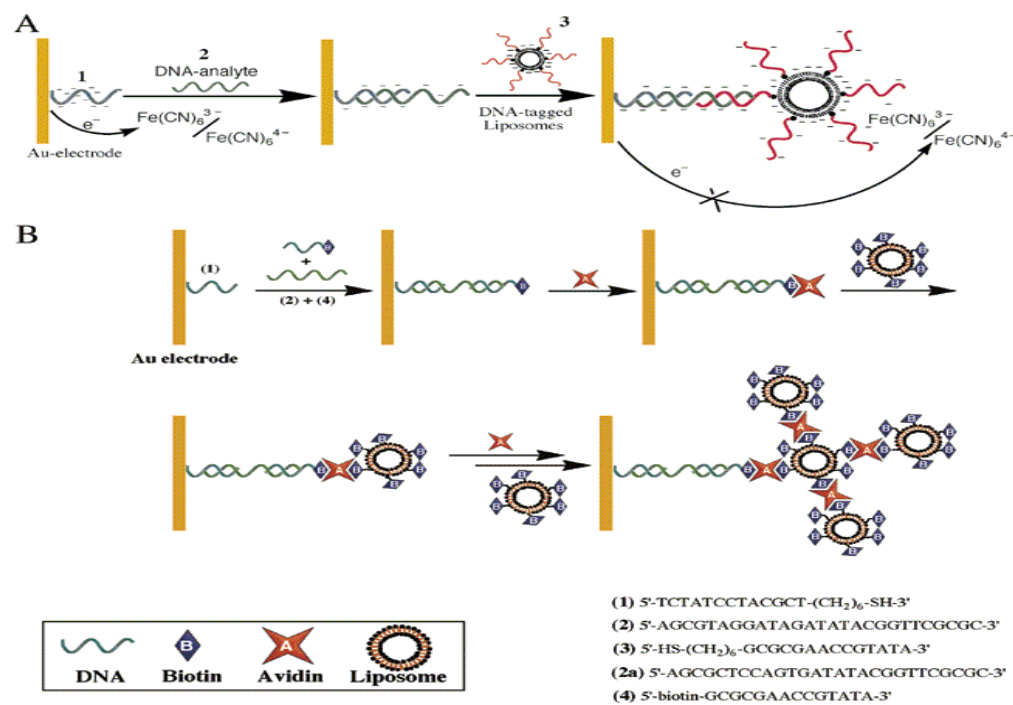
## **II. Target DNA detection via probe DNA immobilized on the electrode**

Gold and carbon electrodes are widely used for the immobilization of probe-DNA because of their strong affinity. It is well known that sulfur atoms and gold surfaces form strong bonds. This allows easy target-DNA immobilization on the gold electrode surface by using bifunctional molecules that have a thiol group on one end and a DNA-reactive functional group on the other. Fang's group used aminoethanethiol for this purpose. They made monolayers of aminoethanethiol on the gold electrode surface via self-assembly and then immobilized probe-DNA on the electrode surface by the formation of phosphoramidate bonds between the phosphate group of probe-DNA and the amine groups on the surface. Daunomycin was used as the electrochemical active intercalator and the redox signal of daunomycin was increased when dsDNA was produced.<sup>5</sup> In another case, 5'-mercaptohexyloligonucleotide was synthesized to attach probe-DNA directly to the gold electrode surface without coupling reagents. Takenaka et al. synthesized thiol-terminated dT<sub>20</sub> (denatured-thymine) and directly immobilized this oligonucleotide on the gold electrode surface and studied the electrochemical response of ferrocenyl-naphthalene diimide during the hybridization events. A large enhancement in redox peak current for ferrocenyl-naphthalene diimide was observed upon the hybridization with dA<sub>20</sub> (denatured-adenine) but this effect was negligible when dT<sub>20</sub> was used as target-DNA.<sup>6</sup>

A sandwich-type DNA complex was studied by Ihara and co-workers. This sandwich-type complex was formed from surface immobilized oligodeoxynucleotide (ODN) reacting with

target-DNA, tagged with ODN-ferrocene.<sup>7</sup> Differential pulse voltammetry (DPV) experiments using a ferrocene moiety showed differing responses based on base-pair matching of the target-DNA. A strong current peak was observed when target-DNA is complementary to the surface confined ODN. On the contrary, the signal is repressed when target DNA is not complementary to the surface confined ODN.

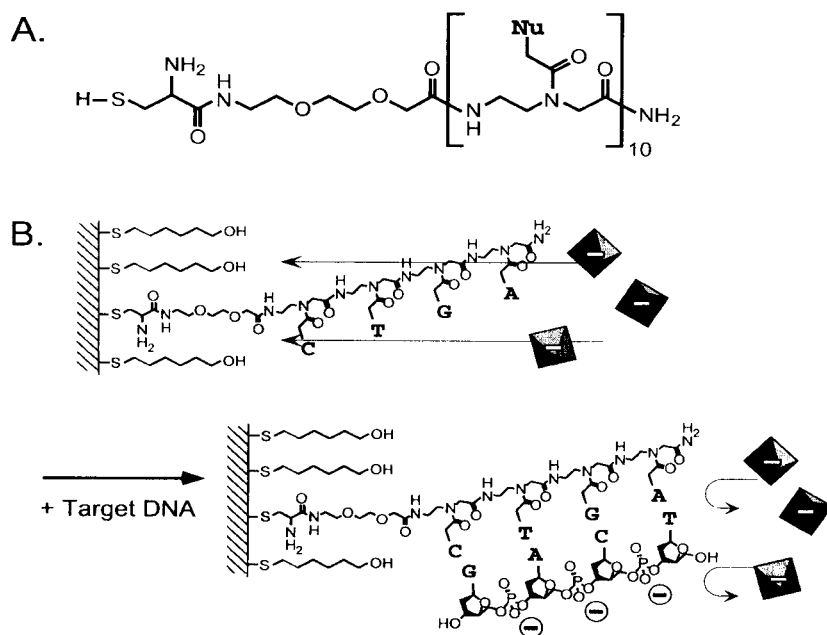
Lowering detection limits has always been a challenge for analytical chemists, and molecular signal amplifiers have been utilized by Willner to achieve low detection limits. Liposomes are closed and solvent filled vesicles that are sealed by only a single bilayer. Liposomes are widely employed as drug delivery vesicle or signal amplifiers because their large surface area and a large internal volume.<sup>8</sup> Therefore, it was thought possible that introduction of liposomes to the oligonucleotide or target-DNA film on the surface would alter the surface



**FIG.1.** The amplified sensing of a target DNA (A) with oligonucleotide-liposomes and (B) avidin and liposomes labeled with biotin (This figure is reprinted from Alfonda L., Singh A. K., Willner I. *Anal. Chem.* **2001**, 73, 91-102).

properties and amplify electrochemical response. Willner's group prepared DNA tagged-anionic liposomes and biotin labeled liposomes. These liposomes are introduced to double stranded structures formed on the electrode surface. The liposomes with tagged DNA form second double stranded assembly with the surface target-DNA. Biotin labeled liposomes bind to the surface via biotin-avidin interactions after introduction of biotinylated ssDNA and avidin on the surface (Fig.1). In both cases, the association of liposomes leads to formation of negatively charged membranes on the surface and alters the surface properties. The negatively charged membrane on the surface prevents access of negatively charged ions ( $[\text{Fe}(\text{CN})_6]^{4-/3-}$ ) by electrostatic repulsive force and in consequence, impedance increases.<sup>9</sup>

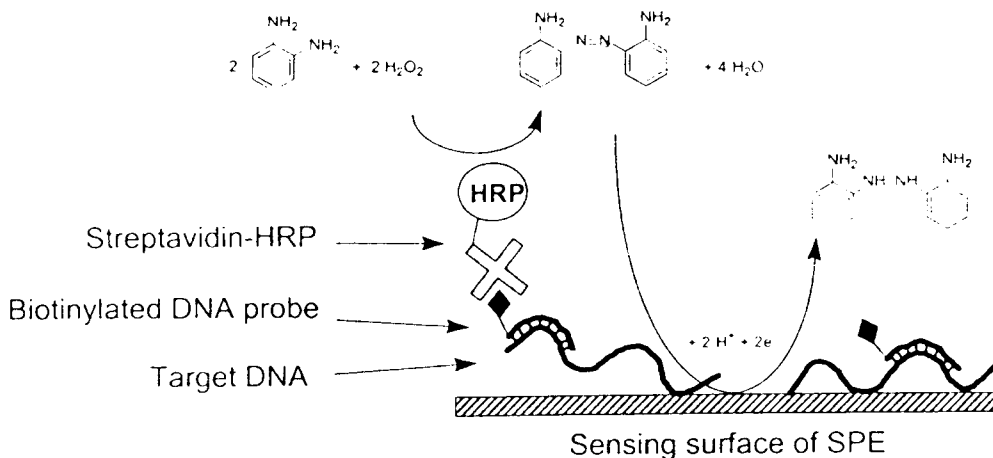
Peptide nucleic acid (PNA) has been used for detection of target-DNA. PNA is a DNA analogue but DNA bases are attached to an N-(2-aminoethyl)glycine backbone instead of a



**FIG 2.** (A) Chemical structure of the peptide nucleic acid (PNA) (B) working principles of the detection of target DNA. The PNA/DNA duplexes at the electrode surface hinder the redox reaction of the marker. This figure is reprinted from Aoki H., Buhlmann P., Umezawa Y. *Electroanalysis* **2000**, 12, 1272-1276).

charged phosphate backbone. PNA has a higher base affinity than its' DNA counterpart and higher discrimination ability towards mismatched DNA. Umezawa's group demonstrated the detection of target-DNA by using PNA-modified gold disk electrodes. They fabricated mixed monolayers of probe-PNA and 6-mercapto-1-hexanol on gold electrodes and observed the electrochemical response of  $[\text{Fe}(\text{CN})_6]^{4-/3-}$  (Fig. 2). 6-mercapto-1-hexanol was employed to fill the void areas between PNA strands and induced a more perpendicular orientation to the PNA strands. In consequence, the permeability of  $[\text{Fe}(\text{CN})_6]^{4-/3-}$  was increased and the intensities of redox peaks in cyclo voltamogram were also increased.<sup>10</sup>

One of the problems of electrochemical detection of DNA is that the cost of DNA detection is typically higher than that of traditional spectroscopic measurements. For this purpose, disposable screen printed carbon electrodes (SPE) were investigated as a low cost DNA sensor. It was possible to immobilize target-DNA on the carbon electrode surface and attach biotinylated probe-DNA by forming dsDNA. P. Brossier's group tested this system using human



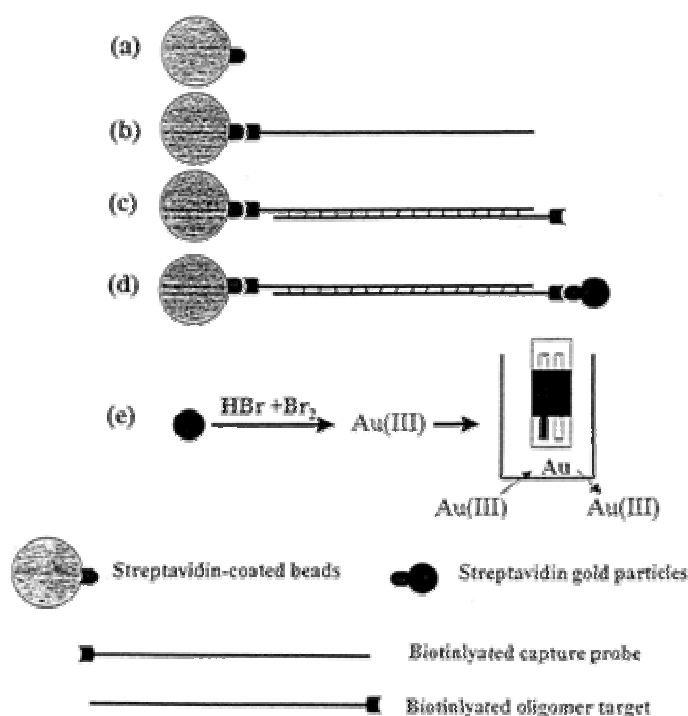
**FIG. 3.** Scheme of the electrochemical DNA hybridization assay on a screen-printed electrode (This figure is reprinted from Azek F., Grossiord C., Joannes M., Limoges B., Brossier P. *Anal. Biochem.* **2000**, 284, 107-113) .

cytomegalovirus (HCMV). HCMV was adsorbed on the carbon electrode surface by applying potential and its' dsDNA was formed with a biotinylated probe-DNA. Streptoavidin labeled horseradish peroxidase (HRP) can then be attached to the surface via a avidin-biotin interaction. This surface-confined HRP can convert o-phenylenediamine into 2,2'-diaminoazobenzene (DAA) (Fig. 3). Therefore DNA detection on the SPE electrode is based on the measurement of current generated by reducing DAA. The peak magnitude in the DPV reflects the amount of HRP anchored to the dsDNA hybridized on the surface. They observed an enhancement in peak magnitudes for DPV when HCMV is on the surface.<sup>11</sup> Probe-DNA labeled with aminoferrocene (AFC) was also used in the electrochemical detection of target-DNA. Fang's group could reduce the interference generated by intercalators by using AFC labeled probe DNA.<sup>12</sup> When surface immobilized target-DNA is complementary to probe DNA, AFC labeled probe DNA was strongly adsorbed on the surface and generated significant redox current.

Another method of probe-DNA immobilization on the surface is based on using conducting polymers. H. Korri-youssoufi et al. demonstrated that DNA could be immobilized on a 3-acetic acid pyrrole/3-N-hydroxyphthalimide copolymer surface prepared by electrochemical polymerization.<sup>13</sup> Polymer films have good leaving ester group on the surface and this allowed covalent attachment of amino-substituted probe-DNA via substitution reaction. They also reported that hybridization reactions with target DNA significantly altered voltammograms of polymer films.

### **III. Target DNA detection via probe DNA immobilized on nanoparticles**

DNA hybridization detection based on nanoparticles has attracted many scientists recently. UV-vis spectroscopy and colorimetric methods were previously applied to detect DNA hybridization in nanoparticle-based systems. An electrochemical approach is relatively new



**FIG. 4.** Schematic representation of the analytical protocol

(This figure is reprinted from Authier L., Grossiord C.,

Brossier P. *Anal. Chem.* **2001**, 73, 4450-4456).

to ferrocenecarboxaldehyde labeled target DNA. Enhanced current response in DPV and CV was observed when target-DNA was in the hybridization solution.<sup>14</sup> In contrast, detection of target-DNA immobilized on the surface by oligonucleotide-modified gold colloids was also possible. In this scheme, the target-DNA hybridized with the probe DNA attached to the gold colloid and followed by the release of the colloidal gold particle. Indirect determination of gold ions by anodic stripping voltammetry (ASV) represented the degree of hybridization between the target and probe DNAs.<sup>15</sup> In the second approach, target and probe DNA is immobilized on the nanoparticles via streptavidin–biotin binding. In this method, biotinylated probe DNA is immobilized on a streptavidin-coated magnetic bead. Probe-DNA attached to the magnetic bead

compared to these techniques but offers significantly enhanced sensitivity.

There are two different kinds of experiments related to nanoparticles.

At first, Fang et al. assembled gold colloids on a cysteamine-modified gold electrode. This method enhanced the amount of the probe DNA immobilized on the gold electrode.

Oligonucleotide bearing mercaptohexyl group was easily attached to the gold

colloid in larger amounts compared

to the bare gold electrode. The

hybridization with target-DNA was

induced by exposure of probe-DNA

hybridized with biotinylated target-DNA and the biotin on the target-DNA captures streptoavidin-coated gold nanoparticle. Finally, gold tags are dissolved and detected by potentiometric stripping measurement (Fig. 4). Further enhancement of signal was possible by precipitation of gold or silver onto the gold nanoparticle. Wang's group achieved 1 pM detection limit by utilizing this method.<sup>16</sup>

#### **IV. Direct electrochemical detection of DNA**

Direct electrochemical detection of underivatized ssDNA and dsDNA is highly advantageous because contamination problems or losses while handling samples can be avoided. Kuhr and his group investigated the possibility of detecting individual base molecules by sinusoidal voltammetry.<sup>17</sup> His group tested both purine and pyrimidine based nucleic acids and showed that these bases could be measured at a submicromolar detection limit at a copper electrode. They could differentiate the adenine-containing and cytosine-containing nucleotide based on the difference in the frequency domain response. The detection limit of adenine (purine based nucleic acid) was much lower than that for cytosine (pyrimidine based nucleic acid). They reported 70 – 200 nM of detection limit and this value is 2 orders of magnitude lower than that of traditional UV absorbance detection. This high sensitivity makes this approach applicable to DNA sequencing coupled with separation methods. In another approach, they were able to detect ssDNA and dsDNA based on the electrocatalytic oxidation of sugars and amines since all nucleotides and DNA molecules have ribose sugars and amines.<sup>18</sup> The observed signal of dsDNA was almost two times higher than that of ssDNA and in both cases, the sensitivity was better than that of smaller oligonucleotides due to the large number of sugar backbones present.

Wang's group reported a new approach for label-free DNA detection by doping nucleic acid probes within electropolymerized polypyrrole films. They demonstrated that direct label-



free electrochemical detection of DNA hybridization could be achieved by observing changes in the conductivity of mixed polymer films. Current changes were observed when hybridization between surface-confined probe oligonucleotides and target oligonucleotides occurred.<sup>19</sup>

## V. Conclusion

Traditionally, electrochemical detection of DNA is based on the observation of changes in electrochemical signals of electrochemical active intercalator or other electrochemically active tags attached to the DNA strand when a double helix forms. Several methods were recently introduced to improve the detection limit of these earlier methods. These include signal amplification using magnetic beads or metal colloids. In addition, direct detection methods, which can detect DNA without labels, are being vigorously studied.

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