*Int. J. Electrochem. Sci.*, 9 **(2014)** 5948 - 5960

International Journal of
ELECTROCHEMICAL
SCIENCE
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# Deposition of Ni(II)Porphyrin Monolayer on the Gold Electrode via Azide-Alkyne *Click* – Coupling and its Electrochemical Characterization

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*Received:* 28 May 2014 / *Accepted:* 9 July 2014 / *Published:* 25 August 2014

In this work, a Ni(II)porphyrin containing two azide groups ($(N_3)_2$-Ni(II)porphyrin) was applied for gold electrode modification via azide-alkyne *click* reaction. The $[CuBr(PPh_3)_3]$ catalyst was used for the cycloaddition of diazido Ni(II)porphyrin to alkyne functionalized thiols previously assembled onto the gold surface. The self-assembled monolayer (SAM) incorporating Ni(II)porphyrin was characterized in aqueous solutions by cyclic voltammetry (CV), Osteryoung square-wave voltammetry (OSWV) and differential pulse voltammetry (DPV). The attachment of a ssDNA probe via a strained bicyclononyne group to the developed redox active monolayer via copper-free alkyne-azide *click* ligation is presented.

**Keywords:** $(N_3)_2$-Ni(II)porphyrin, azide-alkyne *click* chemistry, self-assembled monolayers, redox active SAMs, gold electrode.

## 1. INTRODUCTION

The spontaneous formation of self – assembled monolayers (SAMs) by chemisorption of thiol-modified molecules on metallic surfaces (particularly on gold) has achieved tremendous interest during the last few decades [1-3]. SAMs with different functionalities, because of high stability, compactness and flexibility have been applied in biological [4], medical [5] and material chemistry research [6]. Special attention has been paid to monolayers containing metallomacrocyclic compounds [7].

Particularly metalloporphyrins [8-11] have gained interest, because of their potential application in chemical sensors and as models for studies of electron transfer reactions in redox SAMs. Unfortunately, direct chemisorption of thiol modified metalloporphyrins onto gold surface could be difficult, due to the fact that the thiol can coordinate the central metal of the porphyrin [12]. As a result, the formation of multilayers as well as blocking of the catalytic center could be observed.

Till now, a number of strategies have been applied, in order to avoid the above problems. The assembly of disulphide derivatives of metalloporphyrins on gold [13,14], the attachment of metalloporphyrins to the surface of gold modified with imidazole –terminated adsorbates [15] or insertion of different metals into thiol-derivatised free-base porphyrin monolayers previously assembled on gold [16] are examples of the strategies reported up to date. Recent studies have shown, that *click* chemistry [17-19] is very frequently used for the coupling of molecules or macromolecules to surfaces [20-22], with high efficiency and regioselectivity. The main advantages of *click* chemistry are the availability of a wide range of starting materials and that the process is carried out under mild reaction conditions, insensitive to oxygen and water [23]. In particular, the copper(I) catalyzed 1,3-dipolar cycloaddition of azides and alkynes has gained interest and it is called the "best click reaction" [24]. In the case of this reaction, predominantly, the copper(I) salts are generated from copper(II) salts and sodium ascorbate used as reducing agent. The reactions have been carried out with water tolerant substrates; addition of ligands, which protect the copper(I) centers from oxidation are used. The use of the commercially available catalyst, $[CuBr(PPh_3)_3]$, under very mild reaction conditions and low catalytic loadings as reported by Diez-Gonzales et al. is another possibility [25].

In the majority of the recently published papers, the use of free copper(I), generated and maintained in aqueous solutions by an excess of reducing agents is used. Unfortunately, DNA is susceptible to damage by copper(I) *via* the generation of reactive oxygen species in the presence of dioxygen. Therefore, either the use of triazolyl-amine ligands to stabilize the copper(I) and prevent damage to DNA or RNA, or the use of strained alkynes such as bicyclononyne, are very successful strategies [26, 27].

The electrochemistry of redox-active SAMs and their application in the construction of sensors has been an ongoing research topic in our group [28-30]. In the present study, a $(N_3)_2$-Ni(II)porphyrin was self-assembled to a gold surface via a *click* chemistry strategy, related to the $[CuBr(PPh_3)_3]$-catalyzed 1,3 dipolar cycloaddition between an azide and terminal alkyne groups with formation of the five-membered heterocycle [25]. The different steps of the gold electrode modification using a Ni(II)porphyrin containing two azide groups and C-C triple bond terminated thiols are shown in Scheme 1. The characterization of the SAMs was done by cyclic voltammetry (CV), Osteryoung square-wave voltammetry (OSWV) and differential pulse voltammetry in aqueous solutions. The suitability of azide-Ni(II)porphyrin SAM for attachment of oligonucleotide containing a bicyclononyne(BCNII) linker via alkyne-azide cycloaddition *click* reaction without copper(I) will be presented.

## 2. MATERIALS AND METHODS

### *2.1. Chemicals*

2-Mercaptoethanol, $[CuBr(PPh_3)_3]$ chloroform, KCl, DMSO, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, were obtained from Fluka-Sigma-Aldrich (Poznań, Poland). Ethanol, methanol, THF, potassium hydroxide, sulphuric acid were obtained from POCh (Gliwice, Poland). 5-hexyne-1-thiol was obtained from Prochimia Surfaces, Sopot, Poland. All aqueous solutions were prepared with deionized and charcoal-treated water (resistivity of 18.2 MΩ cm), purified with a Milli-Q reagent grade water system (Millipore, Bedford, MA). All solutions were deoxygenated by purging with ultra-pure nitrogen.

### *2.2. Synthesis of $(N_3)_2$-Ni(II)porphyrin*

5,15-bis(2,4,6-trimethylphenyl)-10,20-bis(4-azidomethylphenyl) porphyrin [19] (40 mg, 0.05 mmol, 1 equiv) and $Ni(OAc)_2.4H_2O$ (122 mg, 0.5 mmol, 10 equiv) were added to a flask of 25 ml containing 10 ml of DMF. The resulting mixture was refluxed at 120$^o$C overnight under $N_2$ atmosphere. DMF was evaporated under reduced pressure to obtain crude product. Subsequently, $CH_2Cl_2$ (10 ml) was added to the flask and the solution was extracted three times with water. The organic layer was dried over $MgSO_4$ and the solvent was evaporated under vacuum to yield $(N_3)_2$-Ni(II)porphyrin in pure form in quantitative yield. $^1$H NMR (300 MHz, THF-$d_8$, 25$^o$C, TMS ): $\delta$ = 8.70 (d, $^3J_{H,H}$ = 4.89 Hz, 4 H, H-pyrrole), 8.58 (d, $^3J_{H,H}$ = 4.89 Hz, 4 H, H-pyrrole), 8.03 (d, $^3J_{H,H}$ = 8.1 Hz, 4 H, H-Ar), 7.64 (d, $^3J_{H,H}$ = 8.1 Hz, 4 H, H-Ar), 7.20 (s, 4 H, H-mesityl), 4.66 (s, 4 H, 2×$CH_2$), 2.51 (s, 6 H, 2×$CH_3$), 1.77 ppm (s, 12 H, 4×$CH_3$); $^{13}$C NMR (75 MHz, THF-$d_8$, 25$^o$C, TMS): $\delta$ = 134.76, 133.17, 131.77, 128.67, 127.49 (CH-Ar), 143.77, 143.52, 141.83, 139.57, 138.64, 138.13, 136.71, 119.14, 118.24 (C-Ar), 55.19 ($CH_2$), 21.45 ppm ($CH_3$). FTIR: 2083 cm$^{-1}$ ($N_3$).

### *2.3.Synthesis of BCN-oligonucleotide probe*

The DNA was synthesized using standard solid phase automated synthesis on an Expedite DNA synthesizer; reagents were obtained from Link Technologies (Bellshill, Scotland). The bicycle[6.1.0]nonyne was introduced at the 5'-end using 5'-Click-easy® BCN CEP II (Berry & Associates, Dexter, USA) and following the manufacturer's instructions. The sequence made was 5'-cctcaaggagagagagaagaag-3'.

### *2.4. Modification of the electrodes*

The gold disk electrodes (2 mm$^2$ area (Bioanalytical Systems (BAS), West Lafayette, IN) were polished mechanically with wet 0.3 and 0.05 μm alumina slurry (Alpha and Gamma Micropolish; Buehler, Lake Bluff, IL) on a flat pad for 5 minutes each and rinsed repeatedly with water. The polished electrodes were then dipped in 0.5 M KOH solution, deoxygenated by purging with argon for 15 min, and the potential was cycled between -400 and -1200 mV (versus an Ag/AgCl reference

electrode) with a scan rate of 100 $mVs^{-1}$ until the cyclic voltammograms showed no further changes. Next, the electrodes were cleaned in 0.5 M sulphuric acid and the potential was cycled between -300 and 1500 mV (versus an Ag/AgCl reference electrode) with a scan rate of 100 $mVs^{-1}$ until the voltammograms were not changed. After cleaning, the gold surface of the electrodes was immediately immersed into a mixture of $10^{-3}$ M solution of 2-mercaptoethanol and $10^{-5}$ M in 5-hexyne-1-thiol in ethanol for 3 h. Then, the electrodes were modified with a solution of $10^{-4}$ M $(N_3)_2$-Ni(II)porphyrin and $10^{-5}$ M $[CuBr(PPh_3)_3]$ in THF:DMSO (volume ratio 1:1) for 18 h. The solutions used for modification were put into 8 mm diameter tubes (no flat bottom). After dipping the electrodes, the tubes were sealed with Teflon tape to prevent solvent evaporation. After modification, the electrodes were rinsed with ethanol or THF:DMSO from the modification solution. Then, 10 μl of 10μM solution of BCN-oligonucleotide dissolved in 1 M NaCl were dropped on the surface of gold electrode and left for 18 h at a temperature 4°C. After deposition of BCN-oligonucleotide, the modified electrodes were carefully washed and stored at 4 ºC in a 0.1 M KCl solution until use.

### *2.5. Electrochemical measurements*

All electrochemical measurements were performed with a potentiostat–galvanostat AutoLab (Eco Chemie, Utrecht, The Netherlands) with a three electrode configuration. Potentials were measured versus the Ag/AgCl electrode, and a platinum wire was used as the auxiliary electrode. Osteryoung square-wave voltammetry was performed in 0.1 M KCl using the following parameters: step potential 5 mV, square-wave frequency 25 Hz, and square-wave amplitude 50 mV. Differential pulse voltammetry was performed in 0.1 M KCl solution using the following parameters: step potential 1 mV, and modulation amplitude 25 mV. Cyclic voltammetry (CV) was performed in 0.1 M KCl solution in the presence of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (0.5 mM each) and the potential was cycled from 700 mV to -200 mV with the scan rate of 100 mV/s.

## 3. RESULTS AND DISCUSSIONS

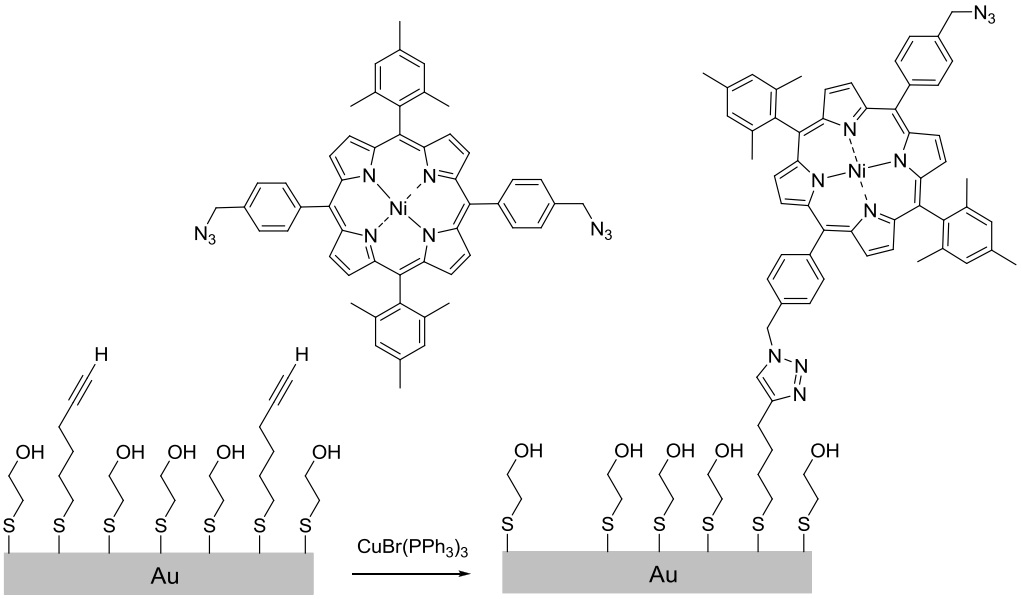
The electrochemical properties of many metalloporphyrins studied in nonaqueous solutions have been carefully described over last few decades. The redox reactions of metalloporphyrins depend on the selection of appropriate solvent and supporting electrolyte [31]. The air stable form of nickel porphyrins exists in a Ni(II) oxidation state and could be reduced or oxidized giving a variety of derivatives with Ni(I) or Ni(III) central metal ions together with Ni(II) π-cation radicals and dications or Ni(II) π-anion radicals and dianions in nonaqueous solutions [31].

The majority of studies on metalloporphyrin monolayers self-assembled on solid surfaces is related to the catalytic action of the central metal for dioxygen reduction [32,33] or oxidation of biochemically important molecules in aqueous solutions [34-36]. However, it was reported, that the redox reactions of metalloporphyrins, when self-assembled in monolayers, show unclear peaks in aqueous deareated solutions [32,33], because of the formation of uncontrolled

structures. Therefore, appropriate attachment of metalloporphyrins to the surface in order to get a well-controlled structure is crucial for the possible observation of a redox reaction of these compounds on the surface. This is the most important step, because a functionalized surface with organic molecules is crucial for numerous applications of both fundamental and practical importance. Till now, several coupling strategies have been reported and adopted [13-16]. In this work, we propose a method of Ni(II)porphyrin monolayer creation on the gold surface using azide-alkyne cycloaddition strategy.

### *3.1. Immobilization of $(N_3)_2$Ni(II)porphyrin on gold electrode surface via azide-alkyne cycloaddition click reaction*

Scheme 1 illustrates the modification steps required for the gold electrode modification in order to obtain Ni(II)porphyrin SAM. The $(N_3)_2$-Ni(II)porphyrin was covalently attached to the surface of the gold electrode by copper catalyzed azide-alkyne cycloaddition with the formation of the five membered heterocycle. In the first step, a mixed monolayer of 2-mercaptoethanol and 5-hexyne-1-thiol in a ratio of 1:100 was formed on the surface of the gold electrode. Such modification is created in order to avoid the intermolecular interactions between the adjacent porphyrin molecules. In this step, a C-C-triple bond containing monolayer was assembled on the surface of gold electrode. In the second step, the commercially available $[CuBr(PPh_3)_3]$ catalyst was used for cycloaddition of the immobilized alkyne thiol with $(N_3)_2$-Ni(II)porphyrin. As reported by Diez-Gonzales [25], the formation of 1,2,3-triazoles in the presence of $[CuBr(PPh_3)_3]$ occurs under very mild reaction conditions. Moreover, the use of 5-hexyne-1-thiol as linker connecting the redox center and electrode ensures the exact distance between the redox active center and electrode. This is one of the most important parameters influencing the electron transfer kinetics of redox active SAMs [37].




**Scheme 1**. Chemical structure of $(N_3)_2$-Ni(II)porphyrin and schematic illustration of its attachment to mixed SAM of 2-mercaptoethanol and 5-hexyne-1-thiol via azide-alkyne *click* chemistry reaction.

*3.2. Electrochemical characterization of Ni(II)porphyrin self-assembled on the gold electrode*

In the first step, in order to prove the presence of Ni(II)porphyrin on the surface of gold electrode, cyclic voltammetry was performed. The redox peaks of the Ni(II)porphyrin SAM were clearly visible in 0.1 M KCl solution. The cyclic voltammetry was performed at different scan rates, changing from 10 to 500 mV/s in 0.1 M KCl. The representative voltammograms are presented on Figure 1.

The redox reactions of metalloporphyrins immobilized on the surface of electrodes investigated in aqueous solutions are not widely reported. According to the literature, unclear peaks are observed for these compounds immobilized on the solid surface [32,33], mainly because of formation of transition structures. In this work, we have developed a method of controlled Ni(II)porphyrin attachment to the gold electrode and its electrochemical characterization. We have observed the quasi-reversible noticeable peaks relating to $Ni^{II}/Ni^{III}$ redox processes at the negative potential window, as shown on Figure 1. Both peaks: oxidation and reduction increase linearly with the scan rate over the range studied, as seen on inset in Figure 1 indicating that the transfer of electrons is an adsorption - controlled process. The research on improving of electrochemical parameters of Ni(II)porphyrin SAM will be carried out in our laboratory.

The cyclic voltammetry performed at the scan rate of 100 mV/s showed quasi-reversible redox processes of Ni(II) with the peaks at the potential of -150 ± 23 mV and -215 ± 3 mV. According the literature, these peaks probably correspond to the oxidation of Ni(II) and reduction of Ni(III) [31]. As reported, the reduction of the metalloporphyrin metal centre (Ni(II)/Ni(I)) is only possible for non-planar systems, such as isobacteriochlorins or hydroporphyrins, in which a flexible macrocycle skeleton is present [9]. Ni(II) porphyrins are known to have a saddle type distortion geometry which agrees well with the observation of the redox potentials [38].

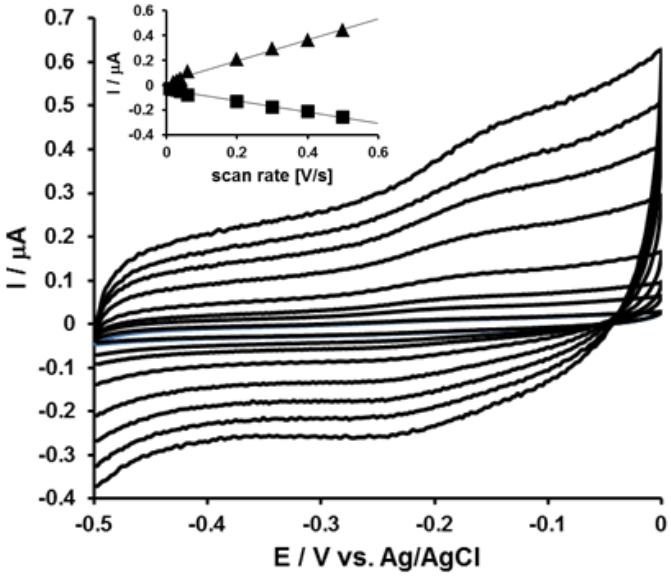
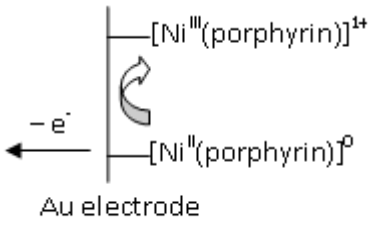



**Figure 1**. Cyclic voltammograms (CV) of Au-$N_3$-Ni(II)porphyrin electrode measured at different scan rates. Measuring conditions: scan rate: 10 - 500 mV/s, reference electrode: Ag/AgCl, counter electrode: Pt, solution composition: 0.1 M KCl. Inset: plots of anodic and cathodic peak current vs. scan rate.

The redox processes of metalloporphyrins can involve either the central metal or/and the porphyrin ligand [31]. In aqueous solutions, mainly the redox activity of central metal or its electrocatalytic activity are investigated for the metalloporphyrins [32,33]. In the case of our complex [$Ni^{II}$(porphyrin)], the $Ni^{II}$ was oxidized to $Ni^{III}$ and again reduced to $Ni^{II}$ at the applied potential according the scheme of reaction presented below:




**Scheme 2**. Redox processes of Ni(II)porphyrin

Active $Ni^{II}$ center is oxidized to $Ni^{III}$ and neutral form of complex changes to the plus charged, which has to be neutralized by anions present in the supporting electrolyte. The mechanism of this reaction should be studied more deeply and will be the subject of our future work.

The other electrochemical technique used for characterization of Ni(II)porphyrin SAM is Osteryoung square wave voltammetry (OSWV), which allows for the capacitive current decrease. Reproducible Osteryoung square wave voltammograms were obtained, with the peak position at the -234 ± 16 mV (Figure 2A).

The redox current of Ni(II) centers was in range 4.4 ± 1.3 x $10^{-8}$ A, determined in 0.1 M KCl (average value calculated from five different modifications). The electrochemical properties of Ni(II)/Ni(III)porphyrin SAM were also examined using differential pulse voltammetry (DPV), and representative voltammograms are presented in Figure 2B,C. When the potential was changed in the reduction direction, a well-defined peak was visible at the potential -254 mV, with a redox current of 1.3 x$10^{-8}$ A. Moreover, when the potential was changed in opposite direction, a similarly clear peak was observed, at a potential -269 mV, with the same redox current value as above.

There are only few reports concerning metalloporphyrins SAMs, and usually redox active peaks were reported which are not very well resolved [32,33].

The results presented in this work clearly demonstrate that the azide-alkyne *click* reaction is perfectly suitable for Ni(II)porphyrin immobilization on gold electrode surfaces, assuring the retention of the redox properties of nickel centers. To the best of our knowledge, the electrochemistry of Ni(II)porphyrin SAM in aqueous solutions has not been reported up to date.

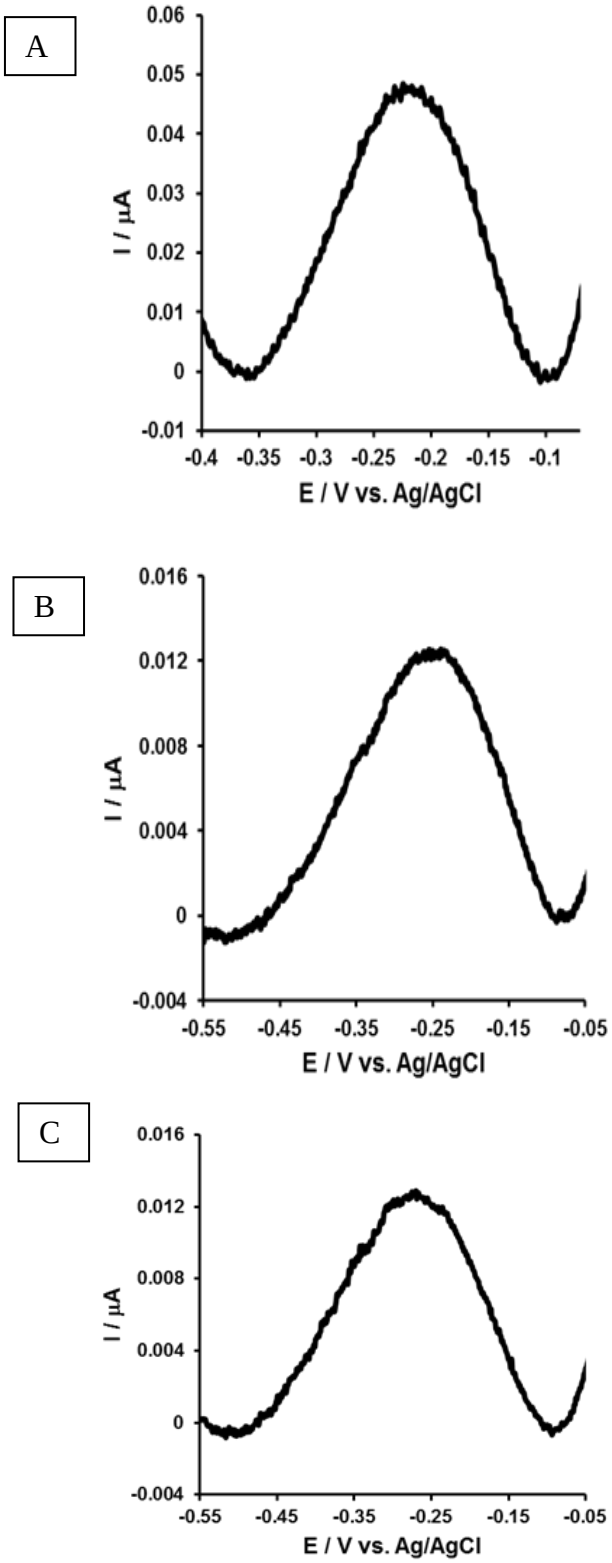
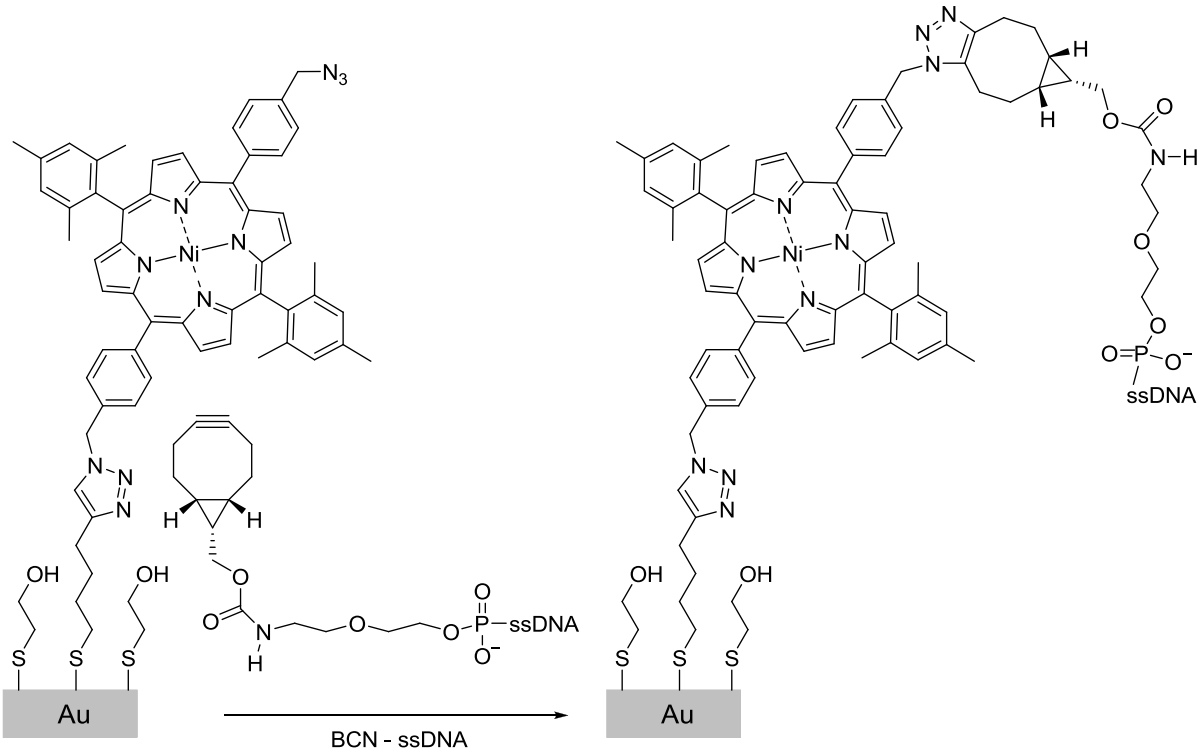



**Figure 2**. (**A**) Representative Osteryoung square wave voltammogram (OSWV) obtained for electrode modified with $N_3$-Ni(II)porphyrin in 0.1 M KCl solution. The OSWV was performed with step potential: 5 mV, square wave frequency: 25 Hz and amplitude: 50 mV; (**B**) Representative differential pulse voltammograms (DPVs) obtained for electrode modified with $N_3$-Ni(II)porphyrin in 0.1 M KCl solution when scanned in the reduction direction, and (**C**) in oxidation direction. The DPV was performed with step potential: 1 mV, and modulation amplitude: 25 mV.

*3.3 Application of bicyclononyne substituted ssDNA probe for attachment to azide modified Ni(II)porphyrin SAM.*

Scheme 3 illustrates the structure of the BCN linker attached to an oligonucleotide sequence and its attachment to the $N_3$-modified Ni(II)porphyrin SAM. The application of bicyclononynes has become popular for chemoselective ligation of biomolecules during the past few years [27]. This reaction does not require cytotoxic copper(I) catalyst, therefore the reaction is not damaging to biomolecules and is perfectly bioorthogonal.




**Scheme 3**. Chemical structure of BCN linker and its attachment to $N_3$-Ni(II)porphyrin redox active SAM.

The immobilization of BCN-oligonucleotide on the $N_3$-Ni(II)porphyrin SAM was monitored by Osteryoung square wave voltammetry in 0.1 M KCl solution. Before immobilization of oligonucleotide probe, as described above, the Ni(II) redox peak current was observed at ~ -224 mV, with the maximum value of current of ~$5x10^{-8}$ A (Figure 3, curve a). The attachment of the BCN-oligonucleotide to $N_3$-Ni(II)porphyrin caused a decrease of the Ni(II) redox peak and the peak potential was recorded at ~-255 mV with a maximum current value of ~2.7 x $10^{-8}$ A. The decrease of Ni(II) redox current was accompanied with a shift of the peak potential to negative direction (Figure 3, curve b).

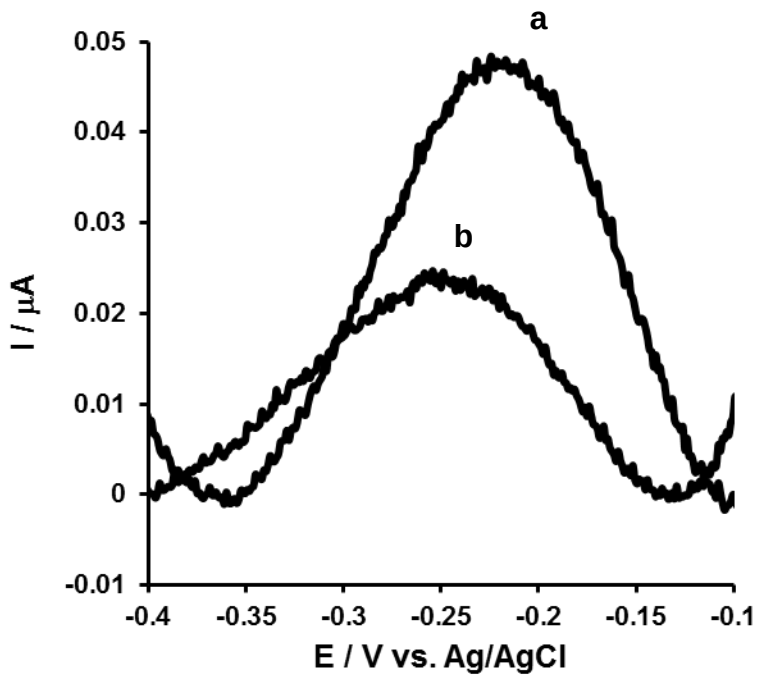



**Figure 3**. OSWVs of a gold electrode modified with $N_3$-Ni(II)porphyrin (line a) and after attachment of the BCN-oligonucleotide probe to $N_3$-Ni(II)porphyrin (line b). Measuring conditions: 0.1 M KCl, step potential: 5 mV, square wave frequency: 25 Hz and amplitude: 50 mV.

The attachment of the oligonucleotide to the porphyrin SAM using *click* chemistry was also confirmed using cyclic voltammetry performed with the $[Fe(CN)_6]^{-3/-4}$ redox marker present in the sample solution. For an electrode modified with $N_3$-Ni(II)porphyrin SAMs, a peak to peak separation of $\Delta E_p = 69 \pm 5$ mV was observed (Figure 4, curve a). This confirmed the almost reversible behavior of the $[Fe(CN)_6]^{-3/-4}$ marker. After attachment of the oligonucleotide to the $N_3$-Ni(II)porphyrin SAM, the reversibility of the system decreased, and a peak to peak separation of $190 \pm 12$ mV was observed (Figure 4, curve b). The direct influence of DNA immobilization onto the SAM is that the electrode surface gained negative charge, which decreases the $[Fe(CN)_6]^{-3/-4}$ marker accessibility. This clearly demonstrates that the oligonucleotide probe was successfully attached to the $N_3$-Ni(II)porphyrin SAM.

A decrease of the redox peak current upon immobilization of different proteins to the redox active SAMs was observed in our previous reports of different analytical systems [39,40]. We claimed that this phenomenon occurs because of the hindering of the counter ions accessibility towards the redox centers upon binding events. Here a similar phenomenon was observed upon DNA probe attaching to the $N_3$-Ni(II)porphyrin SAM. This indicates that the presented redox active SAM might be useful for the development of a new type of genosensor in which Ni(II)porphyrin centers could follow the hybridization processes, analogous to our recently reported Co(III)porphyrin DNA sensor [41].

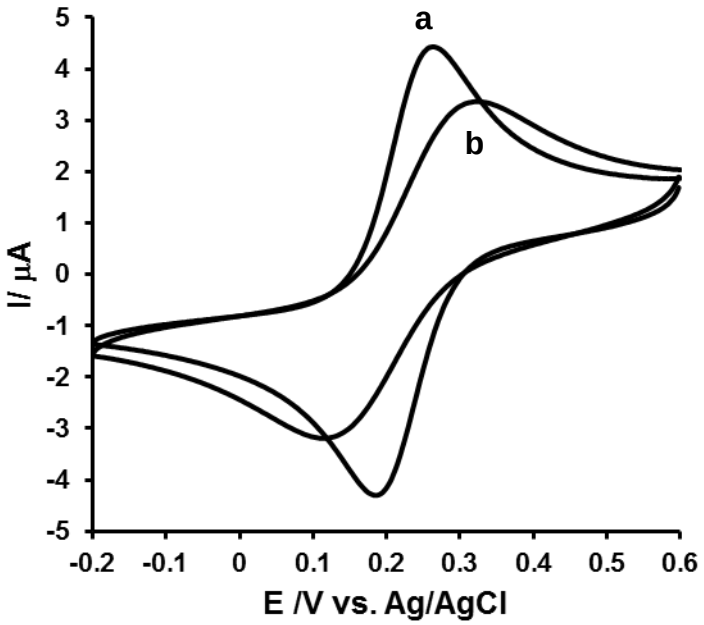



**Figure 4**. CV curves measured for electrodes modified with $N_3$-Ni(II)porphyrin (line a) and after attachment of the BCN-oligonucleotide probe to $N_3$-Ni(II)porphyrin (line b). Measuring conditions: scan rate: 100 mV/s, reference electrode: Ag/AgCl, counter electrode: Pt, solution composition: 0.1 M KCl + 1 mM $[Fe(CN)_6]^{-3/-4}$.

## 4. CONCLUSIONS

The azide-alkyne *click* reaction was suitable for proper immobilization of $(N_3)_2$-Ni(II)porphyrin to alkyne – alcohol terminated mixed SAM deposited on gold electrode surface. The redox active $N_3$-Ni(II)porphyrin SAM displayed good electrochemical parameters recorded in aqueous solutions. A well-defined oxidation/reduction peak measured with Osteryoung square wave voltammetry was observed at -234 ± 16 mV with redox current of 4.4 ± 1.3 x $10^{-8}$ A.

The $N_3$-Ni(II)porphyrin SAM was suitable for BCN oligonucleotide attachment via copper(I) free *click* reaction. The presented modification procedure might the base of the development of new type of genosensors in which metallo-porphyrin centers could track the hybridization processes.

ACKNOWLEDGEMENTS
This work was supported by the EU project COST CM1005 “Supramolecular Chemistry in Water”, project 679/N-BELGIA/2010/0 and the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland.

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