Electrochemical DNA biosensors
– multitask tools for analytical and physicochemical purposes

Elaboration on scientific and other academic achievements on account of application for the degree of doctor of habilitation

Warsaw, 2013
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1. PERSONAL DATA

First name and last name:  Anna Maria Nowicka  
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Nationality:  Poland

2. INFORMATION ON RECEIVED DIPLOMAS AND SCIENTIFIC DEGREES

- PhD in Chemistry, defense with distinction, University of Warsaw, June 8, 2005.  
  Title of PhD thesis: „Metalocene derivatives as model compounds for examination of diffusional, migrational and convectional transport”.  
  Adviser: prof. dr hab. Zbigniew Stojek

- MSc in chemistry, defense with distinction, University of Warsaw, May 20, 2000.  
  Title of MSc thesis: „Diffusinal and migrational transport of monosubstituted ferrocene derivatives”.  
  Adviser: prof. dr hab. Zbigniew Stojek

3. INFORMATION OF EMPLOYMENT IN SCIENTIFIC INSTITUTIONS

Adjunkt, University of Warsaw, Faculty of Chemistry, Laboratory of Theory and Applications of Electrodes, from 2005

Anna M. Nowicka
4. INDICATION OF ACHIEVEMENTS ACCORDING TO ART. 16 PARAGRAPH 2 OF THE ACT OF LAWS FROM 14 MARCH 2003 ON ACADEMIC DEGREES (JOURNAL OF LAWS NO. 65, ITEM 595 AS AMENDED) THAT CAN BE TREATED AS BASIS FOR HABILITATION PROCEDURE

4.1. Title of achievement

Electrochemical DNA biosensors – multitask tools for analytical and physicochemical purposes

4.2. Subject-specific set of papers published in journals listed by Journal of Citation Reports

(* – candidate is corresponding author)


IFI = 2.283

My contribution: 55%; I have planned and overseen the experiments related to the DNA biosensors with gravimetric and electrochemical detection and to the influence of the presence of various forms of chromium on their work. I have synthesized streptavidin modified ferrocene and written the first draft of the paper. I have also edited the replies to comments of the reviewers.


IFI = 2.721

My contribution: 40%. I have planned and overseen the experiments related to the estimation of the usefulness of new anthraquinone derivatives for visualization of the hybridization process. I have also done the preliminary experiments, interpreted the results, written the publication and edited the answers to reviewers’ comments.


IFI = 2.721

My contribution: 50%. I have made the investigation plan and supervised the experiments. I have performed the experiments related to modification of electrode surface with colloidal gold, analysed the results, written the ms. corresponded with the editor and edited the replies to reviewers’ comments.

My contribution: 45%. I have planned and supervised the experiments. AFM measurements and impedance measurements with oligonucleotides with mismatching bases have been done by me. I have also analysed the results, written the ms. and edited the replies to reviewers’ comments.


My contribution: 75%. I have planned and overseen the investigations reported in this paper. I have done impedance measurements and analysed them. I have also written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


My contribution: 75%. I have planned and overseen the investigations reported in this paper. I have done EQCM measurements and taken AFM micrographs. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


My contribution: 40%. I have planned and overseen the investigations reported in this paper. I have done impedance measurements and taken AFM micrographs. I have also analysed the results, corrected the ms., and edited the replies to reviewers’ suggestions and comments.


My contribution: 60%. I have planned and overseen the investigations reported in this paper. I have done CD and EQCM measurements and analysed the results. I have also written the ms. and edited the replies to reviewers’ suggestions and comments.


My contribution: 55%. I have planned and overseen the investigations reported in this paper. I have done impedance measurements and prepared the samples for taking AFM micrographs. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.
4.3. Indication of scientific aim and discussion of most significant results

4.4. Summary

The set of selected papers presents the following issues:

- influence of the way of immobilization of single DNA strands (oligonucleotide probes) taken from bacteria *Listeria monocytogenes* and from the milk bacteria (strain: *Lactococcus lactis*) – on the quality of the sensing layer in the DNA biosensor;
- dependence of the efficiency of the hybridization process on the way of arrangement of the DNA strands in the sensing layer;
- monitoring of the DNA damages and the DNA transformation caused by reactive oxygen species and selected toxicants;
- comparison of sensitivity of DNA sensors coupled with various detection procedures;
tagging the DNA biosensor for the purpose of visualization of the hybridization process;

- influence of the presence and concentration of reactive oxygen species on the performance of DNA sensors.

The experiments involved several independent techniques and this allowed me to draw several conclusions:

- stability and good quality of the sensor layers guarantees a satisfactory work of the DNA biosensors;
- the most uniform sensing layer with optimal density of the DNA strands can be obtained when the intermediate phenyl layers are formed by the way of appropriate electroreduction of a diazonium salt;
- the better quality of the phenyl intermediate layers compared to the thiol layers is a result of the way of binding of these layers with the substrate and and their stable orientation vs. the substrate surface;
- modification of the gold surface with a layer of colloidal gold can widen the working concentration range of any DNA biosensor and improve its detection limit by at least one order of magnitude;
- electrochemical quartz microbalance and electrochemical faradaic impedance allow to detect the hybridization process without necessity of labeling the DNA biosensor with a redox probe; the condition is that the formation of the sensing layers is done with very good reproducibility;
- electrochemical DNA biosensors pass very well the competency tests, which opens real chances for their application;
- DNA biosensors appeared to be a useful tool in the examination of properties and activity of DNA under various conditions.

5. DESCRIPTION OF OTHER SCIENTIFIC ACHIEVEMENTS

5.1. Summary of scientific output

Total number of publications: 42
List of scientific papers published after the doctor: 36
Total IF: 168.614
Report of citations according to the Web of Science (WoS 13.11.2013; publications H9, H10, D22, D23 and D24 accepted for publication are not indexed)

Total number of citations = 197 (5.32 citations / publication)
Total number of citation without self-citations = 129 (3.5 citations / publication)

Hirscha index H = 8

5.2. List of Journal-Citations-Reports papers (except for those listed in section 4) published before getting doctor degree


IF: 5.695

My contribution: 10 %. I have performed electrochemical experiments.


IF: 0.965

My contribution: 50 %. I have performed all experiments and synthesised differrocene derivatives. I have also analysed the results and written the first version of ms..


IF: 0.393

My contribution: 10 %. I have performed electrochemical experiments.


IF: 2.672

My contribution: 10 %. I have performed electrochemical experiments.

IF: 5.695
My contribution: 40 %. I have performed all experiments and synthesised ferrocene derivatives.


IF: 2.721
My contribution: 35 %. I have performed all experiments and synthesised ferrocene derivatives.

5.3. **List of Journal-Citations-Reports papers (except for those listed in section 4) published after getting doctor degree**
(* – candidate is corresponding author*)


IF: 3.947
My contribution: 40 %. I have performed all electrochemical experiments. I have also analysed the results and written the draft of ms..


IF: 2.721
My contribution: 35 %. I have performed all electrochemical experiments. I have also analysed the results and written the draft of ms..


My contribution: 45 %. I have performed all electrochemical experiments. I have also analysed the results, determined the binding parameters between potential anticancer drug C-1311and DNA. I have also analysed the results, written the draft of ms., corresponded with the editor and edited the replies to reviewers’ comments.


My contribution: 15 %. I have performed all electrochemical experiments.


My contribution: 40 %. I have planned and overseen the investigations reported in this paper. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


My contribution: 10 %. I have have performed the experiments on the stability of the DNA biosensor.


My contribution: 40 %. I have performed the experiments on the stability of the DNA biosensor.
My contribution: 30 %. I have planned and overseen the investigations reported in this paper. I have performed the part of the electrochemical measurements. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


IF: 3.829

My contribution: 10 %. I have performed the part of the electrochemical measurements.


IF: 5.695

My contribution: 30 %. I have planned and overseen the investigations reported in this paper. I have performed the part of the electrochemical measurements. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


My contribution: 10 %. I have performed the part of the electrochemical measurements.


IF: 13.455

My contribution: 70 %. I have putted the research problem, planned and overseen the investigations reported in this paper. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.

IF: 13.455

My contribution: 50 %. I have planned and overseen the investigations reported in this paper. I have performed the part of the electrochemical measurements. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


IF: 4.425

My contribution: 15 %. I have prepared the samples for XPS and Ramman analysis. I have also analyzed the results.


IF: 3.829

My contribution: 40 %. I have planned and overseen the investigations reported in this paper. I have performed the measurements concerning the effects of hydroxyl radicals and UV irradiation on DNA electroactivity. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


IF: 2.279

My contribution: 35 %. I have planned and overseen the electrochemical investigations reported in this paper. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.

IF: 3.829

My contribution: 5 %. I have analysed the results.


IF: 2.672

My contribution: 5 %. I have discussed the results.


IF: 4.425

My contribution: 15 %. I have performed a part of electrochemical experiments and analysed them. I have also written the draft of ms.


IF: 3.659

My contribution: 5 %. I have performed the gravimetric experiments.


IF: 4.814

My contribution: 30 %. I have planned and overseen the electrochemical and gravimetric investigations reported in this paper. I have also analysed the results, written the draft of ms..

IF: 5.371

My contribution: 35 %. I have planned and overseen the electrochemical and gravimetric investigations reported in this paper. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


IF: 5.437

My contribution: 25 %. I have planned and overseen the electrochemical and gravimetric investigations reported in this paper. I have also analysed the results, written the ms., corresponded with the editor and edited the replies to reviewers’ comments.


IF: 3.777

My contribution: 20 %. I have performed the electrochemical investigations in the absence and presence of magnetic field. I have also analysed the results, written the draft of ms..


IF: 2.721

My contribution: 35 %. I have planned and overseen the investigations reported in this paper. I have done all gravimetric and voltammetric measurements and
analysed these results. I have written the ms., corresponded with the editor and edited the replies to reviewers’ comments.

Chapter in book


5.4. Summary of scientific activity related to papers listed in section 5.3.

During my PhD studies my research interests were focused on the migrational and convectional transport of analytes in solutions. Both theoretical and experimental complex approaches were undertaken to solve the selected problems. Finally, I got a good lesson of organic synthesis, spectroscopy, electrochemistry, modeling of compounds and computer calculations [M1-M6].

After PhD thesis defense I decided to concentrate on biochemical issues. I was particularly interested in such processes as denaturation and hybridization of DNA and in the effects of interactions between DNA and drugs. Electrochemistry gives a chance of deeper insight into these processes, since it allows monitoring chemical changes in particular molecules built into DNA strands. The first aim in my research was to perfect the spectroelectrochemical and electrochemical procedures to be able to characterize quantitatively the interactions between DNA and potential anticancer drugs [D2,D3,D5,D7,D10,D16].

The new possibilities of electrochemical examination of DNA were related to the electrochemical anodic activity of guanine and adenine. The previous lack of such data for DNA strands dissolved in a solution was caused by relatively fast decay of the electrooxidation signals and the corresponding poor reproducibility of the measurements. These obstacles were a consequence of contamination of DNA samples with proteins and inappropriate measurement methodology. I have found the conditions for good reproducibility of the electrooxidation of guanine and adenine: the solution and the electrochemical cell should be biologically and chemically pure [D1]. A possibility of independent analysis of electrochemical signals of DNA and a selected drug made possible the determination of the equilibrium constant of the drug-DNA interactions and the number of coordination sites needed to bind one drug molecule. Having this possibility I could examine how the binding capability of the drug changes when
a magnetic nanoparticle carrier is attached to the drug [D9,D19]. Cytotoxicity of anticancer drugs in healthy cells is very undesirable. It can be substantially limited by e.g. directing the drugs selectively to the cancer cells. One of good ideas is the attachment of a magnetic nanoparticle to the drug molecule. By applying the magnetic field it is possible to direct such a conjugate selectively to the ill cells. Some questions arise here: will the drug modified with a nanoparticle maintain its cytotoxicity? Will the interaction with DNA preserve its original character and strength? Will the nanoparticle be released in the cancer cell? I have tried to find answers to these questions. The corresponding work has been described in publication [D21]. The measurements of cytotoxicity of DOX-Fe₃O₄ Np. conjugate were done by dr. Ewa Augustin and dr. hab. Zofia Mazerska from Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology.

The results of investigation of the strength of the dsDNA – potential-drug interactions may be helpful in planning chemical modifications of the anticancer drugs. I want to stress here that the work in the anodic range gave not only a possibility of successful electrochemical and spectroelectrochemical investigation of interactions between dsDNA and chemical compounds but also enabled fuller analysis of the denaturation process and the influence of the potential drugs on that process. So far the electrochemical investigation of the DNA denaturation process was carried out with DNA accumulated on the electrode surface. For the accumulated DNA the changes in the voltammetric signal caused by the denaturation process appeared exactly at the same temperature range as they did in the spectrograms. The formation of the DNA solid state apparently hides some properties of individual strands. It became clear after voltammetric examination of dissolved DNA. Additional effects appeared in the voltammograms and that allowed drawing more significant conclusions. The differences appeared in a temperature range preceding the denaturation process [D8,D14]. They prompted me to do an examination with circular dichroism spectroscopy. Significant changes in the ellipticity of the dsDNA molecules were seen in the temperature range where new voltammetric effects appeared. The observed changes could be related to a conformation transformation of the helical dsDNA structure and, in consequence, to better exposition of guanine and adenine for the electrooxidation. I expected that in the presence of compounds that can interact with dsDNA, both: electrochemical and spectroscopic signals should change. My expectations were fulfilled.

A subject parallel to the above problems is using the DNA biosensors as multitask tools for analytical and physicochemical purposes [H1-H12]. The quality
of the sensing layer becomes the major issue here. It is strongly related to the way the biomolecules are immobilized on the substrate surface [D6,D20,D22].

I did not limit myself to the research with biomolecules. During my stay in Greifswald, Germany I spent some time investigating the action of hydroxyl radicals against the gold electrode surface. Classical Fenton solution was used as a source of the radicals. The results were astonishing [D11,D12]. They changed our view on the problem of the electrochemical activation and polishing of gold. These investigations were continued with other electrode materials [D13,D15,D17].

Recently, I have started the research on the influence of outer magnetic field on the intensity of voltammetric signals of paramagnetic substrates. [D18,D21,D23].

6. INFORMATION ON OTHER ACTIVITIES

6.1. Teaching activities

I took the following classes:

Faculty of Chemistry, integrated 5-year studies, undergraduate studies and graduate studies:

- repetitions and General Chemistry laboratory
- Analytical Chemistry laboratory
- Inorganic Chemistry I laboratory
- Inorganic Chemistry II laboratory

Faculty of Biology, integrated 5-year studies and undergraduate studies:

- repetitions and General and Analytical Chemistry laboratory

Interfaculty program, Engineering of Nanostructures, undergraduate studies:

- Analytical Chemistry laboratory

Interfaculty program, Environment Management, graduate studies:

- Environment Analytics laboratory, one exercise in Polish and English

Other forms of didactic activity:

- work for The National Fund for Children

Activity in the events aimed at popularization of science:

- participation in Science Festival in Warsaw, from 2005.
6.2. Scientific care over students

I have been scientific adviser for the following students:

Bachelor thesis:
- Edyta Matysiak (2010) – „Influence of length of oligonucleotide chain on electrooxidation of guanine”
- Marta Kaczyńska (2011) – „Ways of investigations of drug-DNA interactions”
- Magdalena Jastrzębska (2011) – „Influence of reactive oxygen species on physicochemical properties of oxygen”

MSc thesis:
- Agnieszka Laba (2005) – „Voltammetric investigation of interactions between DNA and anticancer drug C-1311”;
- Agata Kowalczyk (2007) – „Interactions of dsDNA and drugs in polymeric matrix”;  
- Barbara Klim (2008) – „Investigation of interactions between dsDNA and C-1305 at various pH”;
- Michał Fau (2010) – „Application of 4-aminoethylbenzenediazonium tetrafluoroborate for construction of DNA hybridization biosensor with electrochemical impedance detection”;
- Stefania Iwanowska (2010) - „Electrochemical and spectroscopic investigation of AG99p – a cis-Pt derivative”;
- Edyta Domel (2010) – „Influence of number of guanine molecules in strand on denaturation of dsDNA. Electrochemical and spectroscopic investigations”;
- Aleksandra Sikora (2010) – „Electrochemical and spectroscopic characterization of dsDNA interactions with free and modified with a magnetic nanoparticle doxorubicin”;
- Marcin Maćkiewicz (2011) – „Influence of way of binding of oligonucleotide molecular probe to substrate on DNA biosensor work”;
- Edyta Matysiak (2012) – „Influence of UV irradiation and Tl(I) ions on physicochemical properties of physiologically important DNA sequences”;
- Magdalena Jastrzębska (2013) – „Influence of Tl(III) complexes on physicochemical properties of physiologically important DNA sequences”;
- Anita Jarzębińska (2013) – „Biological activity of doxorubicin-magnetic nanoparticle conjugate against cancer cells”;
- Marta Kaczyńska (2013) – „Modification of gold substrate with phenyl groups of diazonium salt formed in situ”.

PhD thesis:
- mgr Ewelina Zabost (2010) – „Changes in properties of double stranded DNA caused by chemical interactions and external physicochemical parameters”; auxiliary advisor
- mgr Agata Kowalczyk (2012) – „Improvements of layer modifying electrode and detection of hybridization process in DNA biosensors”; auxiliary advisor
- mgr Michał Fau (graduate student) – Mono- and policomponent layers for control of adsorption of biomolecules on different materials; auxiliary advisor

6.3. Engagement in scientific projects
- Grant KBN for graduate students 4 T09A 052 24 (2004/05): „Metalocene derivatives as model compounds for investigation of diffusional and migrational transport”; main investigator (project completed)
- Grant KBN 3 T09A 087 27 (2004/07): „Physicochemical investigation of gel- and ionic liquid media and investigation of transport of ions and molecules in those media”; investigator (project completed)
- Grant KBN N-N204 244534 (2008/11): „Chemical reactions associated with electron and ion transfer at boundary of three phases and their application for synthesis of new materials”; investigator (project completed)
- BW-175609 (2007): „Sensors based on enzymatic reaction and red-ox polymers – mediators for amperometric detection of DNA hybridization”; coordinator (project completed)
- BW-179213 (2008): „Application of intercalators as factors that modify dsDNA structure and their use in biosensors based on hybridization process of DNA”; coordinator (project completed)
- BW-175609 (2009): „Change in DNA structure caused by attack of toxicants, free radicals and ionizing irradiation. Influence of base sequence in strands”; coordinator (project completed)
- Grant Iuventus Plus IP2010 028570 (2011): „Mono-component and complex nanostructural materials of desired properties”; coordinator (project completed)
Grant Iuventus Plus IP2011 025971 (2012/13): „New compounds for immobilization of biomolecules on surfaces of various materials”; investigator (project completed)

Grant Iuventus Plus IP2011 038871 (2012/14): „Influence of sequence of nitrogen bases in DNA strand on its conformation, activity against drugs and its resistance to toxicants”; coordinator (project in progress)

6.4. Scientific visits in other scientific and academic centers

- 2003 – USA, City University of NY, New York, 3 months;
- 2006 – Ireland, National University of Ireland, Galway, 1 month;
- 2009 – USA, City University of NY, Potsdam, 3 months;
- 2009 – Germany, Institut für Biochemie, Universität Greifswald, 2 months.

6.5. Scientific awards/prizes

- 2005 r. – W. Kołos prize for PhD thesis
- 2008 r.– Scientific stipend from UW Rector
- 2010 – W. Kemula prize for works on preparation of gold and graphite surfaces for molecular sensor layers
- 2010/11 – Scientific stipend for best PhD students and young doctors
- 2011/14 – Stipend of Minister of Science and Higher Education for outstanding young scientist
- 2013 r.- Scientific prze, Faculty of Chemistry UW

6.6. Participation in local and international scientific conferences

Participation in scientific conferences during PhD studies:

A: Local meetings

2. A.M. Nowicka, Z. Stojek; Mogilany (2001), Elektroanaliza w teorii i praktyce IV, poster presentation: „Chronoamperometria monopodstawionych pochodnych ferrocenowych.”


B: International meetings

1. A.M. Nowicka, W. Hyk, Z. Stojek; San Francisco (2001), 52nd ISE Meeting, poster presentation: „Extension of classical relations used in electroanalysis to conditions of mixed diffusion – migration transport.”


Participation in scientific conferences after PhD studies:

A: Local meetings


5. A.M. Nowicka, A. Kowalczyk, M. Donten, Z. Stojek; Kraków(2010), VIII Polska Konferencja Chemii Analitycznej, oral presentation: „Doskonalenie warstwy receptorowej i metody detekcji w bioczujnikach hybrydyzacji DNA”


B: International meetings


deoxyribonucleic acid (DNA) – hybridization, denaturation and its interactions with drugs.”


A.M. Nowicka, M. Fau, A. Kowalczyk, P. Olejnik; Portoroz (2012), ESEAC Meeting, oral presentation: „Phenyl groups perpendicular to gold surface as a way for successful attachment of DNA.”


24. A.M. Nowicka, A. Kowalczyk, M. Bystrzejewski, M. Donten, Z. Stojek; Prague (2012), 63rd ISE Meeting, oral presentation: „Carbon-encapsulated iron nanoparticles used to generate magnetic field and to enhance substrate transport at electrode surface.”


27. M. Mackiewicz, B. Krasnodebska-Ostrega, A.M. Nowicka; Prague (2012), 63rd ISE Meeting, poster presentation: „Monitoring of interactions between thallium(I) ions and physiologically important DNA sequences by electrochemical, spectroscopic and gravimetric techniques.”


presentation: „Targeting tumor cells by using drug-magnetic nanoparticle conjugate.”


33. A.M. Nowicka, A. Kowalczyk, M. Fau, M. Karbarz, M. Donten, Z. Stojek; Santiago de Queretaro (2013), 64th ISE Meeting, oral presentation: „Hydrogel with polymer chains grafted and functionalized with carboxyl groups as universal 3D platform for specific immobilization of DNA strands.”

6.7. Reviewer for scientific agencies and scientific journals

Reviewer for scientific journals:
- Analytical and Bioanalytical Chemistry: 1
- Chemia Analityczna: 2
- Electroanalysis: 5
- Electrochimica Acta: 3
- Langmuir: 2
- Journal of Physical Chemistry: 2
- Journal of Solid State Electrochemistry: 2
- Talanta: 2

Reviewer for scientific agencies:
- Polish Scientific Agency NCN discipline ST4
Certified translation:

[Rectangular stamp: Certified to be a true
the original
Date 12.03.2008
Signature] [illegible signature]

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02-093 Warszawa

Polish national emblem
THE REPUBLIC OF POLAND
UNIVERSITY OF WARSAW
Faculty of Chemistry
(name of the organizational unit of university or other research organization)

DIPLOMA

ANNA MARIA NOWICKA
(full name)
born on 8th December 1976 in Maków Mazowiecki

on the basis of the presented PhD thesis with the title
"The metalocene derivatives as the model compounds to the study the
migrational, diffusional and convective transport"

and after passing required examinations received a university

DOCTOR’S DEGREE

OF CHEMICAL SCIENCES
in the scope of CHEMISTRY
(details of the degree)
granted by the resolution of the Council
of the Faculty of Chemistry of the University of Warsaw
(name of the council and name of the university or other research organization)
of 8th of June 2005

Thesis supervisor in the registration and conferment procedure of a doctoral degree:
Prof. Zbigniew Stojek, PhD.

Reviewers in the registration and conferment procedure of a doctoral degree:
Prof. Zbigniew Gahuc, PhD.
Prof. Władysław Kubiak, PhD.
Warsaw, 28th December 2005
(location, date)
I, the undersigned Maria – Magdalena Charylo – Samul – a sworn English translator entered into the list of sworn translators under the number TP/1180/05 by the Minister of Justice, the Republic of Poland, do hereby certify that the above translation is consistent with the original of the document prepared in Polish language.

Warsaw 21st November 2008
Rep. number 3471/2008