*Sustainable autonomous system for nitrites/nitrates and heavy metals monitoring of natural water sources*

# Year 4 report (MFA, UP, Hungary)

**Project Acronym: WaterSafe**

**Project Coordinator: "Ilie Murgulescu" Institute of Physical Chemistry of the Romanian Academy, Romania**

Partners: NANOM MEMS SRL, National Institute for R&D in Microtechnology, Transilvania University of Brasov, Romania

Hungarian Partners: Institute for Technical Physics and Materials Science, Centre for Energy Research, Hungarian Academy of Sciences (MFA), University of Pannonia (UP)

**M-ERA.NET Transnational Call 2014**
**NKFI-ID: 117847 Type: NN**

## Project Summary

The project proposes to develop a new energy autonomous system based on micro (photo)electrochemical sensors and ultra-thin solar cells, for concentration measurement of different ionic species in natural water sources.

It focuses on three directions: 1) new materials with high efficiency in solar energy harvesting and fabrication of small ultra-thin solar cells together with the power stabilizing device able to supply the needed voltage to the sensors and electronics module; 2) new microsensors and materials for detection of nitrites/ nitrates and heavy metals in water; 3) low cost autonomous energy system integration and fabrication.

The materials proposed to be optimized as primary option are $SnO_2$, $TiO_2$ and ZnO for the sensors and $TiO_2$, ZnO and $Cu_xS$ for solar cell electrodes; additionally a CZTS solar cell type will be developed. Polymeric membranes (e.g PANI) deposited by electrochemical Cyclic Voltammetry on the working electrode will also be investigated as sensing material for nitrites/ nitrates and heavy metals. Bacterial flagellar filaments (special protein molecules) will be investigated and engineered as sensitive biolayer for heavy metal detection.

The goals of the project are to develop an integrated materials – technology – product – system concept, demonstrator and prototype for eliminating or minimising the use of chemical batteries, other energy sources, or complex wiring in microsensors/microsystems, increasing the autonomy of sensors, systems and networks, to provide affordable energy technologies with low $CO_2$ emissions and to deliver a portable, autonomous apparatus for water monitoring (detection of nitrates/ nitrides, heavy metals).

The project will provide a technology demonstrator and water monitoring system prototype.

Minimum one patent and several scientific papers will be submitted, two doctoral thesis and two workshops will be supported/ organised in the frame of the project.

**Short description of the WP's where MFA and UP are involved:**

### - WP2: Sensitive materials prepared by physical method and biolayer development (MFA, UP)

The aim of WP2 is to develop, characterise and compare new sensing materials with the traditional sensor materials. We plan to enhance the sensitivity of the nano-structures by bio-inspired (protein-based) materials. Activities include sample preparation, characterisation and modelling. Another aim of this package is the development of flagellin-based receptors capable of recognizing and binding heavy metal (Ni, Co, Cd, As) contaminants in fresh water samples.

**- WP3: Sensors development (NANOM, IMT, ICF, UTB, MFA, UP)**

A gold electrode and a silver electrode act as counter electrode and reference electrode respectively. A multiworking electrode sensor will be design and fabricated in order to reach the minimum area and a maximum sensitivity and reproducibility of signals.

Milestones of MFA and UP

**M1.** Optical models and characterization methods on nanostructured surfaces (MFA, *Month 12*)

**M2.** Metal binding flagellin variants (UP, *Month 12*)

**M3.** Investigation of immobilization and stability of proteins on nanostructured surfaces. (MFA, *Month 24*)

**M4.** Flagellar nanorods displaying thousands of metal binding sites. Immobilization protocols for various sensor (waveguide, nanowire) surfaces (UP, *Month 24*)

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Year 1 work:

We performed experiments to fabricate covalently immobilized protein filament layer on gold surface. Chemical cross-linking is perhaps the most commonly used method for immobilizing proteins on inert surfaces.

We tried to use several protocols for bonding our Flagellar Filament (FF) protein onto a gold surface using the chemical cross-linker Dithiobis(succinimidyl propionate) (DSP) or Dithiothreitol (DTT) to inhibit the aggregation of thiolized FF-s. The number of the surface pinning points used for the filament immobilization may be crucial, because few pinning points will not result in properly high level of filament coverage, while too many of them might cause conformation change in the filament structure, which would lead to the loss of its desired function (sensing).

We examined the dependence of the filament coverage with or without DSP or DTT by varying the concentration, using Spectroscopic Ellipsometry (SE) in liquid cell and Atomic Force Microscopy (AFM) in dry enviroment. MFA developed new high sensitive detection methods and UP developed metal-ion-bonding FF-s (see publications).

Year 1 publications:

Judit Nador, Benjamin Kalas, Andras Saftics, Emil Agocs, Peter Kozma, Laszlo Korosi, Inna Szekacs, Miklos Fried, Robert Horvath, Peter Petrik; *Plasmon-enhanced two-channel in situ Kretschmann ellipsometry of protein adsorption, cellular adhesion and polyelectrolyte deposition on titania nanostructures*, OPTICS EXPRESS 24:(5) pp. 4812-4823. (2016)

Agocs Emil, Kozma Peter, Nador Judit, Hamori Andras, Janosov Milan, Kalas Benjamin, Kurunczi Sandor, Fodor Balint, Ehrentreich-Förster Eva, Fried Miklos, Horvath Robert, Petrik Peter; *Grating coupled optical waveguide interferometry combined with in situ spectroscopic ellipsometry to monitor surface processes in aqueous solutions*, APPLIED SURFACE SCIENCE in press: p. in press. 6 p. (2016)

Fodor B, Kozma P, Burger S, Fried M, Petrik P; *Effective medium approximation of ellipsometric response from random surface roughness simulated by finite-element method*, THIN SOLID FILMS in press: p. in press. 5 p. (2016)

Farkas E, Patko D, Khanh NQ, Toth E, Vonderviszt F, Horvath R: *Self-assembly and structure of flagellin-polyelectrolyte composite layers: Polyelectrolyte induced flagellar filament formation during the alternating deposition process*, RSC ADVANCES 6: (95) pp. 92159-92167.

Éva Bereczk-Tompa, Mihály Pósfai, Balázs Tóth, Ferenc Vonderviszt; *Magnetite-binding flagellar filaments displaying the MamI loop motif*, CHEMBIOCHEM 17:2075-2082 (2016) (ISBN 1439-7633)

Hajnalka Jankovics, Éva Tóth, Ágnes Klein, Anett Sebestyén, Balázs Tóth, Ferenc Vonderviszt; *ENGINEERING HEAVY METAL BINDING FLAGELLIN-BASED NANOSTRUCTURES FOR SENSOR ELEMENTS MONITORING NATURAL WATERS*, In: Hungarian Chemical Society, 13th European Biological Inorganic Chemistry Conference (EuroBIC 13). Budapest, 2016.08.26-2016.09.01. Budapest: Hungarian Chemical Society (MKE), 2016. p. 110. (ISBN:9789639970670)

## Year 2 work:

In 2017 an As-binding flagellin variant was constructed (UP) by optimization of the length of the As-binding oligopeptide motif inserted into flagellin. As it has been proved, this fusion protein retains polymerization ability. As-binding capability is under study.

The variable D3 domain of flagellin was substituted (UP) by the C-terminal domain of E. coli NikR, a very strong Ni(II) binding protein (Kd ~ pM range) using gene technology tools. Due to the strong interaction between the Ni(II)-binding motifs in the monomeric form, this flagellin variant is not able to form filaments in vivo. For this reason, optimization of the protein expression and purification protocol is in progress.

Two other Ni(II) binding flagellin variants were engineered (UP) by substituting 2 or 3 surface-exposed amino acids by histidine or glutamic acid residues (HHE and HHH variant, respectively). TEM images and motility assays proved that the HHE-FliC variant was able to form filaments in vivo on the cell surface, unlike HHH-FliC. ITC measurements with Ni(II) ions gave Kd in the mM range.

Interaction of other His-rich variants (4His-FliC and HG12-FliC) and different divalent metal ions (Ni(II), Co(II), Cd(II)) was studied by ITC. In vitro reconstructed filaments of 4His-FliC with controlled length were prepared for immobilization experiments on sensor surface.

Fe(II/III) binding M6A polypeptide motif of Mms6 protein (playing an important role in magnetite nanoparticle synthesis in bacteria) was also incorporated into the D3 deficient flagellin and proved to form filaments in vivo.

We (MFA) performed more experiments to fabricate covalently immobilized protein filament layer on gold surface. We optimized protocols for bonding our Flagellar Filament (FF) protein onto a gold surface using the chemical cross-linker Dithiobis(succinimidyl propionate) (DSP) to inhibit the aggregation of thiolized FF-s.

We (MFA) examined the dependence of the filament coverage with or without DSP by varying the concentration and process time, using Spectroscopic Ellipsometry (SE) in liquid cell and Atomic Force Microscopy (AFM) in dry environment. MFA developed new high sensitive detection methods and UP developed metal-ion-bonding (Ni, As) FF-s (see publications). We successfully bonded these metal-ion-bonding FF-s and performed promising electrochemical measurements with these new types of sensor-chips. The latest results were checked by comparative control measurements performed by roman partners from NANOM, proving that the sensor-chip (protein) stability is good enough.

## Year 2 publications:

Nador J., Saftics A., Kalas B., Illes L., Kovacs B., Moldovan C., Romanenko A., Gartner M., Fried M., Vonderviszt F., Petrik P.: "*Fabrication of genetically modified bacterial filament coatings to develop sensor surfaces for detecting water pollution*", 5th Conference on Sustainable Energy Brasov, 19-21. October 2017, oral presentation.
Andras Saftics, Judit Nádor, Benjámin Kalas, Barbara Türk, Ferenc Vonderviszt, Sándor Kurunczi, Robert Horvath, Péter Petrik, Miklós Fired: "*Ellipsometric characterization of carbohydrate and protein layers for biosensor applications*" 5th Conference on Sustainable Energy Brasov, 19-21. October 2017, oral presentation.
Hajnalka Jankovics; É. Tóth; Á. Klein; A. Sebestyén; B. Tóth; Ferenc Vonderviszt "*Engineering Heavy Metal Binding Flagellin-Based Nanotubes for Sensing Layers*" 5th Conference on Sustainable Energy Brasov, 19-21. October 2017, oral presentation.
Judit Nador, Andras Saftics, Benjamin Kalas, Levente Illes, Carmen Moldovan, Mariuca Gartner, Boglarka Kovacs, Sandor Kurunczi, Miklos Fried, Éva Tóth, Ferenc Vonderviszt, Peter Petrik: "*Sensor surface preparation using genetically modified bacterial filaments*", E-MRS 2017 Fall Meeting in Warsaw (Poland), September 18-21, 2017, Symposium S: Materials- nanoelectronics & -nanophotonics. poster presentation
Benjamin Kalas , Judit Nádor, Miklós Fried, Péter Petrik: "*Protein adsorption on different nanostructures monitored by Kretschmann ellipsometry*", E-MRS 2017 Fall Meeting in Warsaw (Poland), September 18-21, 2017, Symposium S: Materials- nanoelectronics & -nanophotonics. poster presentation
Judit Nador, Andras Saftics, Benjamin Kalas, Levente Illes, Carmen Moldovan, Mariuca Gartner, Miklos Fried, Ferenc Vonderviszt, Peter Petrik: "*Bevonatok létrehozása genetikailag módosított bakteriális filamentumokból vízszennyező anyagok detektálására alkalmas érzékelőfelületekhez*", MAGYAR BIOFIZIKAI TÁRSASÁG XXVI. KONGRESSZUSA SZEGED, 2017. AUGUSZTUS 22-25., poster presentation
Kovács B, Székács I, Kurunczi S, Kakasi B, Vonderviszt F, Horváth R: "*Biomimetic coatings from genetically enegineered flagellin variants to control cell adhesion*" Biological Surfaces and Interfaces: Interface Dynamics, Spanyolország, Sant Feliu de Guixols, 2017. július 2-7. poster presentation
Vonderviszt F: "*Functionalized self-assembling flagellar nanotubes*" 7th Annual World Congress of Nano Science and Technology-2017, Fukuoka, Japan; October 24-26, 2017. Oral presentation
Bereczk-Tompa É, Vonderviszt F, Horváth B, Szalai I, Pósfai M (2017) "*Biotemplated synthesis of magnetic filaments*" Nanoscale 9, 15062-15069. IF: 7.367
Kovács B, Patkó D, Klein Á, Kakasi B, Saftics A, Kurunczi S, Vonderviszt F, Horváth R (2018) "*Bacteria repellent layer made of flagellin*" Sensors Actuators B Chem. 257, 839–845. IF: 5.401


*J. Nador and A. Saftics on 5th Conf. on Sustainable Energy Brasov, October 2017, oral presentation.*

***Dissertations:***

1. Kovács Enikő (2017) Ni(II)-ionok megkötésére képes flagelláris fehérjék előállítása bioszenzorok érzékelő elemeihez (biomérnök **BSc theses**, Pannon Egyetem; témavezető: Jankovics Hajnalka,)

2. Sipos Tímea (2017) Adhéziós motívumokkal ellátott flagellinvariánsok létrehozása (ITDK 2. helyezett dolgozat és biomérnök **BSc theses**, Pannon Egyetem; témavezetők: Vonderviszt Ferenc és Kakasi Balázs)

3. Szekér Patrik (2017) Arzén(III) megkötésére képes flagelláris fehérjék tervezése és előállítása természetes vizek monitorozására alkalmas bioszenzor érzékelő elemeihez (OTDK work és biomérnök **BSc theses**, Pannon Egyetem; témavezető: Jankovics Hajnalka)

4. Takács Vivien (2017) Vas- és nikkelkötő flagelláris filamentum templátok létrehozása mágneses nanocsövek szintéziséhez (**ITDK work** és **MSc theses** Pannon Egyetem; témavezető: Vonderviszt Ferenc)

5. Kovács Boglárka (2017) *CHARACTERIZING OF FLAGELLIN BASED BIOMIMETIC COATINGS AND MONITORING CELL ADHESION WITH LABEL-FREE OPTICAL BIOSENSORS - Flagellin alapú biomimetikus felületek jellemzése és élő sejtek adhéziójának nyomon követése jelölésmentes optikai bioszenzorokka*. (**PhD theses**, Pannon Egyetem Vegyészmérnöki és Anyagtudományok Doktori Iskola; témavezető: Vonderviszt Ferenc és Horváth Róbert)

## Year 3 work:

In 2018 UP stabilized and optimized the protein expression and purification protocol of Ni- and As-binding flagellin variants. NikR is a DNA-binding repressor protein present in bacteria which is activated by an extremely low Ni(II) concentration. Genetic engineering techniques are used to insert the NikR protein into the middle variable part of flagellin to develop a high-affinity Ni(II)-binding flagellin variant for the detection of a very low Ni(II) amount from i.e. natural waters. Further efforts are made to produce novel As-binding flagellin variants exhibiting high binding affinity. UP can produce optimized packages of the Ni- and As-binding flagellins for MFA within 2 weeks to prepare sensor chips. The sensors can deliver by normal mail into Romania and can store in frigider more than 6 months.

We (MFA) performed more experiments to fabricate covalently immobilized protein filament layer on gold surface. We optimized protocols for bonding Ni- and As-bonding Flagellar Filament (FF) proteins onto a gold surface using the chemical cross-linker Dithiobis(succinimidyl propionate) (DSP) to inhibit the aggregation of thiolized FF-s.

We (MFA) investigated the developed (by UP) metal-ion-bonding (Ni, As) FF-s by optical (Spectroscopic Ellipsometry) and electrochemical (Cyclic Voltametry) methods (see publications). We performed promising electrochemical measurements with these new types of sensor-chips. The latest results were checked by comparative control measurements performed by roman partners from NANOM, proving that the sensor-chip (protein) stability is good enough and even the sensor chips can be re-used after 1 month and chemical cleaning. This later investigation series prove that the prepared chips can be used after several months and more than once.

## Year 3 publications:

A. Saftics, B. Kalas, J. Nador, A. Romanenko, É. Tóth, Z. Labadi, M. Gheorghe, L. Illes, B. Kovacs, C. Moldovan, M. Gartner, F. Vonderviszt, M. Fried, P. Petrik, "COATINGS FOR ELECTRONIC WATER

POLLUTION SENSORS", http://www.imt.ro/cas/2018/CAS2018%20Program_final.pdf , Workshop "Microsystems for Energy Harvesting and Environment Monitoring", organized in connection with the projects "PiezoMEMS", "WaterSafe", "PiezoHARV" and "SENSIS", INTERNATIONAL SEMICONDUCTOR CONFERENCE, CAS 2018, October 10-12, 2018, Sinaia, ROMANIA

*B. Kalas and Z. Labadi on CAS 2018, October 2018, Sinaia, ROMANIA, oral presentation.*

Vonderviszt, Ferenc ; Jankovics, Hajnalka ; Klein, Ágnes ; Muskotál, Adél ; Kovács, Mátyás ; Tóth, Éva ; Kakasi, Balázs ; Kovács, Noémi ; Szekér, Patrik ; Husztiné, Nagy Georgina et al., "Funkcionalizált flagelláris nanocsövek és flagellin alapú érzékelőrétegek fejlesztése": "Development of functionalized flagellar nanotubes and flagellinbased sensing layers" p. 109 In: Balogh, András; Klein, Mónika (szerk.) Műszaki Kémiai Napok, Veszprém, Magyarország : Pannon Egyetem, (2018)

M. Gartner, C. Lete, M. Chelu, H. Stroescu, M. Zaharescu, C. Moldovan, M. Gheorghe, S. Gheorghe, A. Duta, Z. Labadi, B. Kalas, A. Saftics, M. Fried, P. Petrik,E. Tóth, H. Jankovics, F. Vondervisztt, "Electrochemical sensors for detection of different ionic species (nitrites/nitrates and heavy metals) in natural water sources" submitted to IEEE CAS 2018 (Paper ID = 5032) (2018)

Kovács B, Patkó D, Klein Á, Kakasi B, Saftics A, Kurunczi S, Vondervisztt F, Horváth R (2018) Bacteria repellent layer made of flagellin. Sensors Actuators B Chem. 257, 839–845. IF: 5.401

Á. Klein, M. Kovács, A. Muskotál, H. Jankovics, B. Tóth, M. Pósfai, F. Vondervisztt (2018) Nanobody-displaying flagellar nanotubes. *Scientific Reports* 8:3584.

Kovács B, Saftics A, Bíró A, Kurunczi S, Szalontai B, Kakasi B, Vondervisztt F, Dér A, Horváth R (2018) Kinetics and Structure of Self-Assembled Flagellin Monolayers on Hydrophobic Surfaces in the Presence of Hofmeister Salts: Experimental Measurement of the Protein Interfacial Tension at the Nanometer Scale. *J. Phys Chem. C* **122**, 21375-21386.

Jankovics H, Kovács E, Tóth É, Kovács N, Vondervisztt F (2018) Development and characterisation of a high affinity nickel(II)-binding flagellin variant. 14th European Biological Inorganic Chemistry Conference (EuroBic 2018) Birmingham, UK; August 26-30, 2018.

***Dissertations:***

Name of candidant: György Benjamin Kalas, Thesis Title: "Development of highly sensitive in situ optical methods for investigating interfaces", Date of expected defense: 2020/02, University: Doctoral School of Physics, Faculty of Science, University of Pécs, Hungary, Thesis supervisor(s): Péter Petrik

Éva Bereczk-Tompa (2018) *Synthesis of magnetic nanotubes using engineered flagellar filaments as templates- Mágneses nanocső-szintézis módosított flagelláris filamentumok segítségével.* (**PhD**

**theses**, Chemical Engineering and Material Sciences Doctoral School, University of Pannonia; Supervisors: Ferenc Vonderviszt , Mihály Pósfai)

Szekér Patrik (2018) Arzén(III) megkötésére kifejlesztett flagelláris fehérjék létrehozása és jellemzése bioszenzorikai alkalmazásra (TDK dolgozat, Pannon Egyetem; témavezető: Jankovics Hajnalka)

Pap Lejla (2018) Mágneses nanoszálak előállítása magnetitkötő flagelláris filamentum templátok alkalmazásával (TDK dolgozat, Pannon Egyetem; témavezető: Vonderviszt Ferenc, Husztiné Nagy Georgina)

## Year 4 (2019jan-2019aug) work:

This 8-month period is an extension of the project but only for the Hungarian groups (MFA and UP) The Romanian groups finished and reported their work, see http://www.icf.ro/pr_2016/WaterSafe/Obtained_results.html

In 2019 UP developed new flagellin variants with stronger Ni- and As-binding capability. The protein expression and purification protocols were improved, and functionalized flagellar filaments (FFs) of higher stability were prepared. UP can produce the Ni- and As-binding flagellin samples requested by MFA for sensor chip preparation within two weeks. The produced sensors could be delivered by normal mail to Romania and stored in fridge for more than 6 months.

MFA performed more experiments to fabricate covalently immobilized protein filament layer on gold surface and investigated the developed (by UP) metal-ion-bonding (Ni, As) FFs by optical (Spectroscopic Ellipsometry) and electrochemical (Cyclic Voltammetry) methods (see conference publications and ongoing publications). Promising electrochemical measurements were performed with these new types of sensor-chips. The measurements proved that the stability of proteinous sensing layer is good enough and the sensor chips can be re-used even after 2-3 month and chemical cleaning. This later investigation series prove that the prepared chips can be used after several months and more than once.

## Year 4 (2019jan-2019aug) publications:

A. Romanenko, B. Kalas, A. Nemeth, J. Nador, F. Vonderviszt, M. Fried, P. Petrik; *Real Time Characterization of Filamental Nano-objects at Solid-liquid Interfaces – Numerical Reconstruction from SE Measurements*, ICSE-8, 8. Int. Conf. on Spectroscopic Ellipsometry, 2019.05.26-31 Barcelona, Spain, poszter

B. Kalas, A. Romanenko, A. Saftics, K. Ferencz, M. Fried, P. Petrik; *Performance comparison of planar nanostructures for biosensing applications by Total Internal Reflection Ellipsometer*, ICSE-8, 8. Int. Conf. on Spectroscopic Ellipsometry, 2019.05.26-31 Barcelona, Spain, poszter

Jankovics H, Szekér P, Tóth É, Vonderviszt F: *Flagellin alapú arzén- és nikkelkötő érzékelő rétegek létrehozása és kötőtulajdonságuk vizsgálata*. Műszaki Kémiai Napok 2019, 2019. április 16-18, Veszprém.

Siddiqui J, Jankovics H, Vonderviszt F: *Protein library design for the directed evolution of specific binder flagellin variants*. Műszaki Kémiai Napok 2019, 2019. április 16-18, Veszprém.

Kakasi B, Gerecsei T, Kovács B, Horváth R, Jankovics H, Vonderviszt F: *Flagellin-based monolayers with tuneable characteristics for cell adhesion studies*. 6th Nano Today Conference, 16-20 June, 2019, Lisbon, Portugal.

***Dissertations:***

**PhD Theses**: Andras Saftics, **Theses title**: *Development of dextran-based hydrogel layers for biosensor applications* (the thesis is written in English), **Doctoral school**: George A. Olah Doctoral School, Budapest University of Technology and Economics, **Supervisor**: Dr. Sándor Kurunczi, **Date of defence**: February 8, 2019, **Venue**: Budapest University of Technology and Economics

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