Egypt. J. Chem. **Vol. 64,** No. 2 pp. 1057 - 1063 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biosynthesis and Biosensing Applications of Silver and Gold Metal Nanoparticles

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Abstract

Biogenic nanoparticles have gained major interest because of their eco-friendly approach, as non-toxic chemicals are involved in the biosynthesis, and the preparation process carried out at ambient pressure and temperature. In the present study, two fungal strains (MM1and MM2) showed the ability to biosynthesize silver and gold nanoparticles, respectively, were isolated from local Egyptian soil samples. Molecular 18srRNA techniques identified the two fungal strains as *Aspergillus terreus* MM1-EGY and *Aspergillus flavus* MM2-EGY isolate, respectively. The biosynthesized silver nanoparticles by *Aspergillus terreus* isolate MM1-EGY exhibited UV absorbance around 420 nm with particle size ranging from 15 to 35 nm. Gold nanoparticles biosynthesized using *Aspergillus flavus* isolate MM2-EGY showed an absorption maximum at 550 nm with particle size ranging from 20 to 50 nm. The biosynthesized AuNPs were preliminary tested for simultaneous voltammetric determination of neurotransmitter metabolite, vanillylmandelic acid (VMA) and homovanillic acids (HVA), while silver nanoparticles were applied for the fabrication of disposable screen-printed Ag/AgCl planner reference electrode.

Keywords: Metal nanoparticles biosynthesis; Silver nanoparticles; Gold nanoparticles, *Aspergillus terreus*; *Aspergillus flavus*; Electrochemical sensor

1. Introduction

Nanotechnology as a term is defined as the synthesis, characterizations and applications of nanoparticles, and/or nanostructures which have a small scale less than 100 nm. Recently, nanomaterials have been introduced for a broad range of applications in different technology areas [1]. In the last decades, metal nanoparticles such as platinum, silver, copper, gold, and others have attracted the attention due to their promising electronic, catalytic and optical properties [2].

Generally, the common approach to synthesis and prepare metal nanoparticles is carried out chemically, physically or biologically. Chemical methods involve using chemical reagents such as sodium borohydride and sodium citrate, alcohols. Such techniques have been found to be highly toxic to the environment in addition to the cost consideration. Contrary, biological methods [3, 4] using living bacterial cells [5], fungal strains [6], *Actinomyces sp*. [7], microalgae [8] or even plant extracts are eco-friendly protocols avoiding the application of highly toxic chemicals. Among the diversity of microorganisms, bacteria were found to be more efficient for metal nanoparticles biosynthesis [3, 5]. These microorganisms have the ability to produce a large amount of proteins that reduce metal to form metal-ion solution [2, 7, 9]. However, production of metal nanoparticles from fungi could be done either extracellularly (i.e. secretion of the nanoparticles to the supernatant) or intracellular through the bioaccumulation of the metal nanoparticles into the cells. The extracellular method was simply performed by the treatment of mycelium filtrate with the targeted metal salt solution and incubation for appropriate period while the colour change of the mixture

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Receive Date: 18 September 2020, Revise Date: 06 October 2020, Accept Date: 01 November 2020
DOI: 10.21608/EJCHEM.2020.43379.2878

considered as indication for the nanoparticle formation.

Biological methods show the advantages as a renewable resource for nanoparticles biosynthesis. The bio-reduction rate of metal to form metal ion solution is much faster compared with chemical methods even at ambient temperature and pressure. Furthermore, the size and phase of the biosynthesized metal nanoparticles could be controlled by modulating the temperature and pH of the reaction mixture. Generally, the biological synthesizing process is a low-cost technique, nontoxic and eco-friendly approach [10, 11].

Owing to its importance of gold nanoparticles (AuNPs) in nanoelectronics and nanooptics, their synthesis attracted considerable attention. Although several approaches have been used to synthesize GNPs [1-3]; new cost effective and environment friendly procedures are still needed.

From the analytical chemistry point of view, nanotechnology brought important and challenging opportunities for sensor construction and developing new electroanalytical approaches. Perhaps one of the most intuitive effects is due to the change in the surface to volume ratio [12, 13]. The use of such biosynthesized metal nanoparticles in diagnosis is becoming very important, as they can be used to construct effective bio-sensing platforms for rapid detection of analytes. Thus, they can be implemented as reliable and efficient field-based methods for biomedical monitoring, quality control and environmental pollution. Nowadays, electrochemical sensors and biosensors form an integrated issue of our modern life to provide us with accurate and continuous statistics about the quality of daily-used-products, product composition and all other utilities we consume or encounter in our daily life [14-17].

The present work aimed to synthesize silver and gold nanoparticles using two locally isolated fungi namely Aspergillus terreus isolate MM1-EGY and Aspergillus flavus isolate MM2-EGY. The biosynthesized nanoparticles will be characterized using UV, TEM and XRD techniques. Furthermore, the green synthesized gold and silver nanoparticles will be investigated for their possible applications in electrochemical analysis.

2. Experimental

2.1 Collection of soil sample and fungal isolation

Samples have been collected from the Egyptian National Research Centre (NRC, Cairo, Egypt) garden during November 2016. Samples were collected using disinfected spatula from 10 cm depths. The collected samples were coded, stored in a cooled sterilized container until they reached the lab. The samples were processed in an isolation process using the soil dilution method [18] and inoculated on Potato Dextrose Agar plates [19] for 10 days at 25 ^{o}C until the fungal colonies appeared. To inhibit any bacterial and fungal growth, 30 mg L^{-1} of streptomycin and rose Bengal were added separately to the medium culture. The separated fungal colonies were preserved on potato dextrose agar slants.

2.2. Genetic identification of fungal strains

The two fungal strains were isolated and cultured in potato dextrose broth media for 5 days at 25 ^{o}C. Pure distinct colonies were spread in Sterile Saline Solution (0.5 mL), then the suspension was centrifuged at 10,000 rpm for 10 min/RT. The DNA extraction was performed using (DNeasy Blood & Tissue Kits) the instruction is afforded by manufacturer instructions. Two primers were used: ITS2' 'GCTGCGTTCTTCATCGATGC' and ITS3 'GCATCGATGAAGAACGCAGC'. The amplification reaction mixture was performed as fellow: 1.0 µL of DNA solution was mixed with 20 µL of PCR reaction solution and PCR reaction profile was as fellow: denaturation step for 5 min at 94 °C, followed by 35 cycles of 30s at 94 °C, 30s at 55 °C, 90s at 72 °C, and a final extension step for 5 min at 72 °C. To remove unincorporated PCR primers and dNTPs from PCR products, Montage PCR Clean up kit (Millipore) were used. Sequencing of the purified PCR product was carried out via 2 primers TS1 'TCCGTAGGTGAACCTGCGG' and ITS4 'TCCTCCGCTTATTGATATGC' applying the Big Dye terminator cycle sequencing kit. The sequencing products were resolved on an Applied Bio-systems model 3730-XL automated DNA-sequencing system.

2.3. Fungal cultivation

Sabouraud dextrose broth (SD) is used for the cultivation of the isolated two fungal strains [20, 21], whereas each strain was incubated in a 100 mL broth medium for 10-days. Mycelia were removed by centrifugation at 5000 rpm for 30min, while the mycelial-free supernatants were used for the biosynthesis process.

2.4. Biosynthesis of silver nanoparticles

Aliquot of the mycelium-free the ***Aspergillus terreus*** culture filtrate was incubated with 45 mL of silver nitrate solution 10^{-3} mol L^{-1} at 25 °C and 150 rpm for 72h. The change of the colour of the mixture is an indication of silver ions reduction to form silver metal [22].

2.5. Biosynthesis of gold nanoparticles

The *Aspergillus flavus* cell-free filtrate (supernatant) was incubated with 45 mL of gold chloride solution at 25 °C and 150 rpm for 72h. In order to confirm the role of fungal extract on the nano-metal synthesis, the gold chloride and the solutions cell-free filtrate were incubated under the same conditions. The formation of purple colour was considered as an indication for the synthesis of gold nanoparticles [23].

2.6. Physico-Chemical Characterization of Bio-silver and Bio-gold nanoparticles

2.6.1. Size evaluation using Ultraviolet-Visible (UV-Vis) Spectroscopy

The conversion of Au^{3+} to Au^0 and Ag^+ to Ag^0 was monitored spectrophotometrically by sampling of aliquots of the incubated mixture at different time interval and measuring the UV-vis spectra of the solutions (Jasco-V-570-UV-Visible spectrophotometer with double-beam, 10 mm light-path cells for absorbance measurements). The recorded spectra were used as indicators for the formation of the metal nanoparticles.

2.6.2. X-ray diffraction (XRD)

The synthesized silver and gold nanoparticles solutions were drop-casted onto the glass substrate where the X-Ray diffraction patterns were measured using a PANalytical X'Pert Pro X-ray diffractometer, The Netherlands. The XRD patterns were recorded at 2θ from 10° to 80° with the scanning speed of 0.02°/min.

2.6.3. Transmission electron microscopy analysis (TEM)

Both the size and morphology of the synthesized nanoparticles were measured using the Philips 10 Technai-TEM via coating of the carbon-coated copper grids with 2-4 µl of the sample suspension.

2.7. Voltammetric measurements

The working carbon paste electrodes were prepared by intimate mixing of 0.5 g graphite powder (synthetic 1– 2 µm, *Aldrich*) with 0.2 g of paraffin oil (PO; Merck, Germany). The resulting pastes were packed into Teflon piston holders [24]. The electrode surface was polished using a wet filter paper and coated with three-uniformed layers of each nanostructured gold suspension (2 mg/ml in DMF) where 10 µL were applied for each layer. As a supporting electrolyte for the electrochemical measurements, Britton– Robinson (BR) buffer was applied while the desired pH value was adjusted using 2×10^{-1} mol $L^{-1}$of NaOH solution. The stock solution of homovanillic acid ($C=10^{-3}$ mol L^{-1}) was prepared by dissolving 4.67mg of the HVA (Sigma-Aldrich) in 25 ml of distilled water into a volumetric flask. Similarly, the stock solution of VMA ($C=10^{-3}$ mol L^{-1}) was prepared by dissolving 4.95g of the pure substance in 25 ml of distilled water into a volumetric flask.

For testing the electrochemical performances of modified electrodes, the voltammetric signals were recorded using PSTrace 3.6-PalmSens potentiostat having the conventional three electrode systems consisting of the fabricated carbon paste electrodes (working), Ag/AgCl (either fabricated or commercial reference electrodes) and platinum wire (counter electrode).

For exploiting the silver nanoparticles to fabricate printed bio-reference electrodes (Ag/AgCl), the bio-silver based ink was prepared by mixing the biosynthesized silver nanoparticles with Polyvinyl chloride (PVC) solution (8% in cyclohexanone-acetone mixture as the proper solvent) and printed (5×35 mm) on plastic substrate as a single printed reference electrode [25]. The silver layer was transformed to AgCl by immersing the printed silver track in 0.1M $FeCl_3$ for 5 min at 25ºC. The protective polymer layer doped with KCl was then deposited on the electrode surface.

3.Results and Discussion

3.1. Fungal identification

The two isolated fungal strains, labelled MM1 and MM2, were identified genetically by DNA isolation, amplification and sequencing of their ITS region using 4 primers. The sequence of 18S rRNA gene for the two fungal strains MM1 and MM2 was obtained and aligned with other existing sequences available in the GenBank database to identify the similarity score and to calculate the statistical significance of the matches using BLAST tool (http://www.blast.ncbi.nlm.nih.gov/Blast). The results confirmed a very close similarity of the obtained gene sequences for MM1 and MM2 with 99.83% homology for isolate MM1 with *Aspergillus terreus* ATCC 1012, and 99.32% homology for MM2 with *Aspergillus flavus* ATCC 16883. The phylogenetic tree for the two strains were constructed using the neighbor-joining

method [26, 27] **(Fig. 1, 2)** by MEGA 7 program according to Kumar et al. [26].

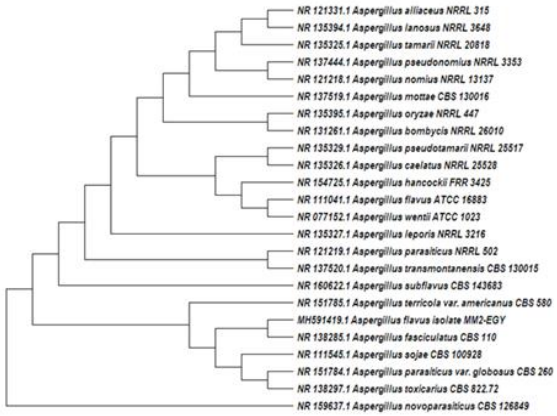

Fig. 1: Constructed phylogenetic tree for *Aspergillus flavus* isolate MM1-EGY.

Based on the DNA sequence analysis and the morphological characteristics, the two strains MM1 and MM2 were identified as *Aspergillus terreus* isolate MM1-EGY and *Aspergillus flavus* isolate MM2-EGY and the sequences were deposited in GenBank under the accession no. MH591418 and MH591419, respectively.

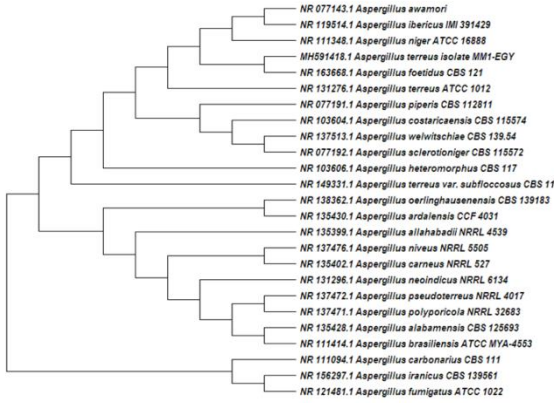

Fig. 2: Constructed phylogenetic tree for *Aspergillus terreus* isolate MM2-EGY

3.2.Biosynthesis of gold and silver nanoparticles

Synthesis of silver and gold nanoparticles was accompanied by color change of their salts within the reducing agents. Results in **figure 3** demonstrated the formation of reddish brown and purple colors due indicating the formation of silver and gold nanoparticles, respectively. The culture filtrate from the two fungal isolates, *Aspergillus terreus* MM1-EGY, and *Aspergillus flavus* MM2-EGY has the ability to reduce the metal ions to their corresponding metal particles in the nanoscale range (**Fig. 4**). These findings were also supported by the UV/Vis spectrophotometric measurements. It has been found that the biosynthesized silver nanoparticles exhibited maximum absorbance at 420 nm (**Fig. 4a**), while the formed gold nanoparticles exhibited maximum absorbance at 550 nm (**Fig. 4b**).

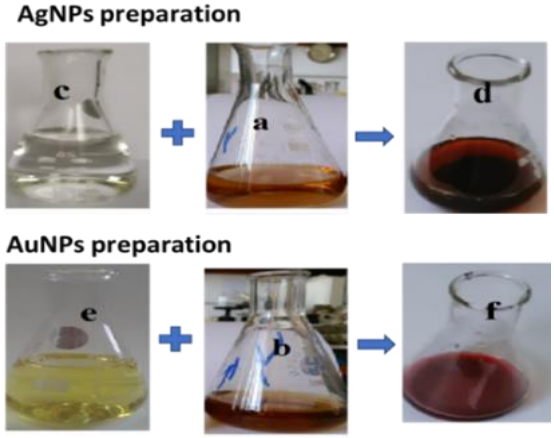

Fig. 3: Colour change of 1mM silver nitrate colourless (c) and 1mM gold nitrate yellow (e) into reddish brown (d) and purple colour (f) using cell-free culture filtrate of fungus *Aspergillus terreus* MM1-EGY (a) and *Aspergillus flavus* MM2-EGY (b).

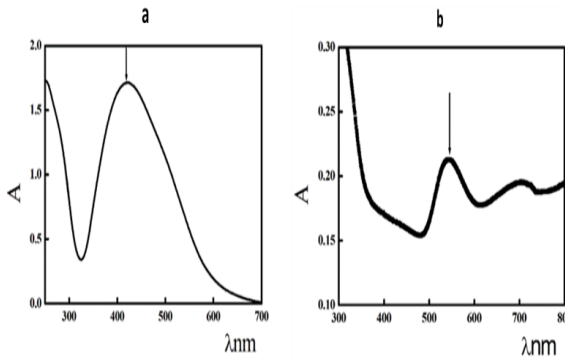

Fig. 4: The UV/Vis spectra of the a) biosynthesized silver nanoparticles (AgNPs), and b) biosynthesized gold nanoparticles (AuNPs) by the supernatants of the fungal isolate *Aspergillus terreus* MM1-EGY and *Aspergillus flavus* MM2-EGY, respectively.

3.3.XRD studying the structure properties of metals nanoparticles

X-ray diffraction (XRD) along with the electron microscopic analysis (TEM) are considered as the most important characterization techniques to study structural, and morphological properties of nanomaterials. Herein, the biosynthesized nanoparticles were examined via the XRD diffraction pattern as shown in **Figure 5 a and b**. Characteristic

peaks of metallic Ag found at 37.5°, 43.4° and 63.8° corresponding to the crystallographic planes (1 1 1), (0 0 2), and (0 2 2) of Ag, respectively, creates a characteristic of crystalline metallic Ag phase [28] **(Fig. 5a)**. The Au-NPs attained in existence of $AuCl_4^-$ analogous diffraction peaks are allocated to metallic Au phase with the most important characteristic peaks which appeared at 38.5°, 44.2° and 64.5° accredited to the crystallographic planes (1 1 1), (2 0 0) and (2 2 0), respectively [23].

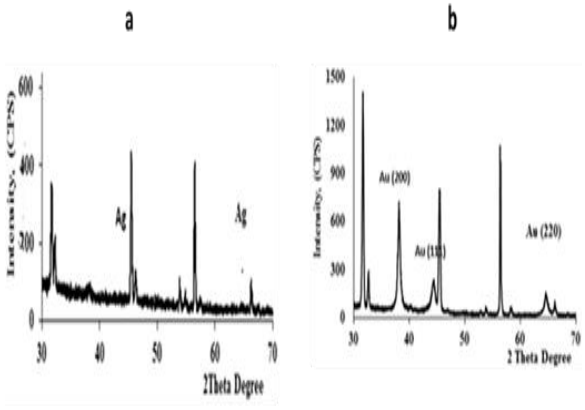

Fig. 5: XRD spectrum of a) silver nanoparticles (AgNPs), and b) gold nanoparticles (AuNPs) biosynthesized by culture filtrate of fungal isolate *Aspergillus terreus* MM1-EGY and *Aspergillus flavus* MM2-EGY.

3.4. Transmittance electron microscopy studies (TEM)

Results in Figure 6 showed the structures and sizes of both gold and silver nanoparticles biosynthesized by fungus strains under investigation.

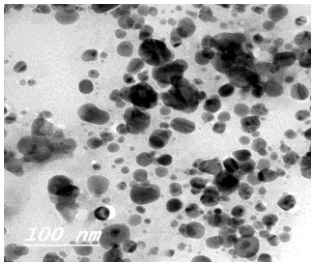

Fig. 6 a: TEM images of silver nanoparticles biosynthesized by culture filtrate of fungal isolate *Aspergillus terreus* MM1-EGY.

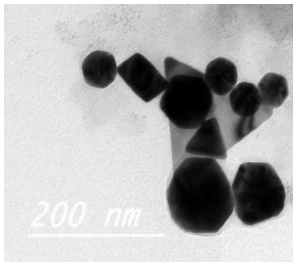

Fig. 6b: TEM images of gold nanoparticles biosynthesized by culture filtrate of fungal isolate *Aspergillus flavus* MM2-EGY.

Silver nanoparticles exhibited different sizes and shapes the sizes ranged from 15-35 nm while the gold nanoparticles formed exhibited different sizes (20-50 nm) with different shapes dominated by prism shapes and hexagonal.

3.5. Voltammetric measurements

After the morphological characterizations, cyclic voltammetric analysis was conducted to identify the electrochemical properties of the biosynthesized nanostructures, whereas the iron ferricyanide (FCN) was used as the redox probe. As a result, a higher catalytic current was achieved with CPE modified with both silver and gold nanoparticles due to their high conductivity compared with bare-CPE, as can be displayed in Figure **7**.

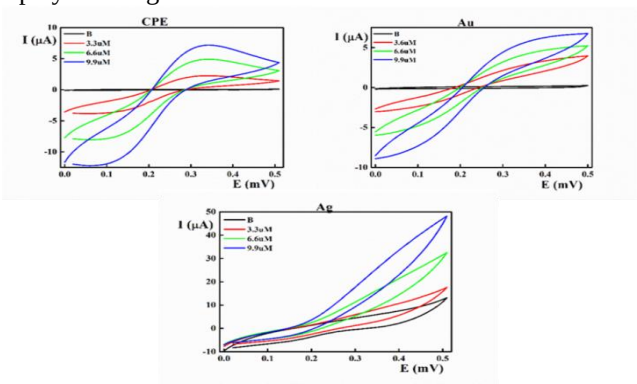

Fig. 7: Cyclic voltammograms of the redox reactions of the $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ on CPE modified with the biosynthesised Au and Ag nanomaterials in 10^{-1} mol L^{-1} KCl as the supporting electrolyte.

Next to the CV studies, the surface modified electrodes were applied for testing of two important cancer biomarkers namely homovanillic (HVA) and vanillylmandelic acid (VMA). At pH 2, VMA exhibited two oxidation peaks at 700 and 880 mV corresponding to the decarboxylation of VMA to form vanillin and further oxidation to o-quinone [29]. One oxidation peak at 720 mV was achieved for HVA, therefore; their simultaneous determination is not possible **(Fig. 8).**

The electrochemical behaviours of both VMA and HVA on carbon paste electrodes modified with gold nanoparticles were represented in **Figure 8.** Gold modified electrodes showed oxidation peak in the blank electrolyte (in absence of either VMA or HVA) at about 900 mV which increase by rising the pH value and interfere with the VMA main oxidation peak at lower pH value; while at pH higher than 5, complete shifting of the VMA peak was observed avoiding this peak overlapping. Using gold material as working electrodes, VMA showed a clear oxidation peak at 890 mV with improved peak current (about 4-fold compared with CPE) with a small hump at 650 mV. Homovanillic acid showed a well-defined oxidation peak at 690 mV. Oxidation peak was shifted to lower potential and the peak current was the maximum at pH 3 (more than two-fold of CPE). Comprehensive

studies for application of such sensors for biomedical analysis of VMA and HVA in biological samples are in progress.

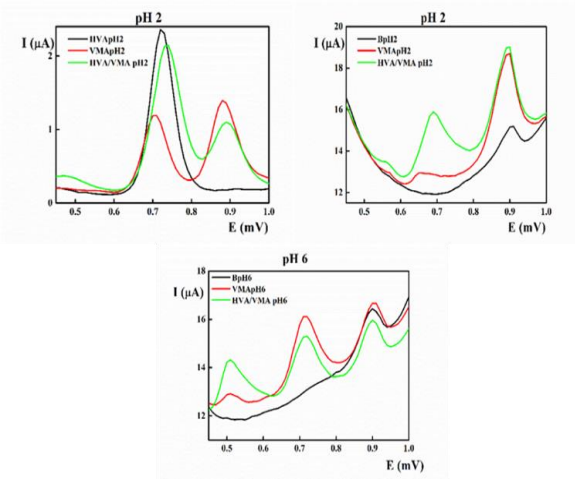

Fig. 8: Differential pulse voltammograms of 3×10^{-4} molL^{-1} vanillylmandelic acid and homovanillic acid at CPE and Au nanoparticles /CPE.

The performance of Ag/AgCl screen printed sensors fabricated with the synthesized silver nanoparticles was compared with those commercially available reference electrodes (Metrohm) using Potassium ferricyanide (FCN 1.0×10^{-3} mol L^{-1}) and carbon paste electrodes as working electrodes. There is no noticeable difference in peak current with a slight shift in the peak potential towards the positive direction suggesting application of such disposable reference electrodes in potentiometric and voltammetric measurements.

4.Conclusions

In this study, it was shown that sensors incorporated with green synthesized metal nanoparticles showed promising performance towards simultaneous voltammetric determination of neurotransmitter metabolite, vanillylmandelic acid (VMA) and homovanillic acids (HVA) with enhanced sensitivity. Comprehensive studies for application of such sensors for biomedical analysis of VMA and HVA in biological samples are in progress. The biosynthesized nanoparticles were characterized with spectrophotometric, XRD and TEM techniques.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

The project (ID: 11090306) is internally funded by the National Research Centre (NRC).

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