FINAL SCIENTIFIC REPORT

Noble metal nanostructures with tunable catalytic and optical properties (FK 131446)

Within the framework of the research project (FK 131446) in the period of 2019-2023 (46 months) the design, synthesis, as well as optical and structural characterization of different type of mono- and bimetallic nanoparticles (NPs) and mono- and bimetallic nanoclusters (NCs) containing noble metals have been successfully carried out; the synthesized systems have been efficiently used for the development of optical biosensors and electrocatalyst particles. The potential utilization of the fluorescent NCs as biolabeling agents has also been studied. The main results of the research during the last 4 years have been published in 31 reputed journals (15 directly related, 3 partly related in the noble metal' topic, 13 others not directly related, IF ~ 190) and in 1 book chapter. For 27 articles/book chapter Edit Csapó is the last/corresponding author. The planned research work was summarized in 5 different work packages. The experimental results confirmed by the publications are presented in accordance with the work packages.

1. Design and reproducible syntheses of novel, gold-containing bimetallic catalyst NPs for controlled decomposition of CO_2.

In 2019, we synthesized bimetallic alloyed Au–Ag NPs with uniform morphology. The electronic properties of Au–Ag alloy electrocatalysts were tuned with the composition, while keeping other structural parameters fixed. The average diameters of the particles were varied in the range of 25-32 nm. We have shown that the Fermi level shifts downwards linearly with increasing Au content, which likely resulted in the decrease of the binding strength of *COOH and *CO intermediates. Careful comparative experiments carried out on Au–Ag alloy and core–shell NPs (with similar bulk composition), as well as with pure Ag and Ag@Au core–shell NPs (with similar electronic structure), indicated differences in both the selectivity and activity. We confirmed that both the electronic and geometric effects need to be considered during the design of the bimetallic catalyst for selective and efficient CO_2 reduction (***Electrochimica Acta,*** *313 (**2019**) 171-178, D1/Q1, IF = 6.215*) In 2020, we have synthesized a series of Au−Pb bimetallic catalysts with different Au/Pb interfaces and studied their CO_2-reduction performance. The structural and composition characterizations by XRD, TEM, and XPS proved that the Au−Pb catalyst consists of Au NPs deposited on the top of Pb NPs with a native Pb oxide (PbO_x). These structural moieties work synergistically to transform CO_2 to >2e− reduction product (namely CH_4) on a Cu-free catalyst. Control experiments on Au, Pb, or their physical mixture yielded only trace amounts of CH_4, further proving our notion on the role of nanoscale interfaces. In situ Raman spectroelectrochemistry confirmed the existence and stability of PbO_x under the reduction conditions on the bimetallic catalyst (unlike for bare Pb), which seems to be necessary for CH_4 formation. (***ACS Catalysis,*** *10 (**2020**) 5681-5690, D1/Q1, IF = 12.350*).

2. Syntheses, optical and structural characterization of noble metal (gold, silver, and their alloys) nanoclusters having fluorescent feature.

In the last 4 years the several synthesis protocols have been designed for the fabrication of highly fluorescent Au- and Ag-based NCs as well as their bimetallic derivatives. As a result,

the applicability of various small biomolecules (like AMP (***J. Mol. Liquids,*** *338* ***(2021)*** *116695, Q1, IF = 6.165),* Vitamin B_3 (***J. Mol. Liquids,*** *359* ***(2022)*** *119372, Q1, IF = 6.633),* Vitamin B_1 (***Antioxidants,*** *12(4)* ***(2023)*** *874. Q1, IF = 7.00)*), amino acids (Tyr: ***Int. J. Mol. Sci***, 23 **(2022)** 977. *D1/Q1, IF = 6.208*; Glu, Phe, Arg: ***Chemistry-A Eur. J***, *29* ***(2023)*** *e202300720*) *D1/Q1, IF = 4.300*) and amino acid derivatives like HisHA (***J. Mol. Liquids***, *387* ***(2023)*** *122597, Q1, IF = 6.00*) has been confirmed for the synthesis of noble metal NCs. In all cases the preparation protocols were optimized and the dominantly blue-emitting NCs containing only a few atoms have been characterized in detail by several physico-chemical and colloid chemistry techniques. In case of Vitamin B derivatives-containing systems their potential antioxidant feature was also confirmed. Within the framework of the present FK research project, our new goal was to study the cytotoxicity of the previously and the newly prepared NCs. The biological measurements were carried out at Jerzy Haber Institute of Catalysis and Surface Chemistry PAS (Krakow, Poland). We clearly focused on the comparative cytotoxicity investigations of several protein-stabilized Au NCs (lysozyme (LYZ)-, bovine serum albumin-, human serum albumin-, and gamma globulin (γG)) towards lymphocytes B (COLO-720 L) and lymphocytes T (HUT-78) and promyelocytic leukaemia (HL-60) cells. We confirmed that the cytotoxicity can be clearly controlled by the quality of the proteins (***Coll. Surf. A,*** *620* ***(2021)*** *126569. IF = 4.539; Q2*; ***Coll. Surf. B,*** *200* ***(2021)*** *111593. IF = 5.268; Q1).* The results of this period were summarized in two reviews (***Nanomaterials,*** *9* ***(2019)*** *1229*; ***Advances in Coll. Int. Sci****., 301 (2022) 10261*; and a book chapter (*E. Csapó: Metal-based nanoclusters for biomedical applications in Targeted Metallo-Drugs Design, Development and Modes of Action* ***(2023)***).

3. Development of “turn-on” or “turn-off” optical sensors using noble metal based NCs for detection of metal ions/anions and small biomolecules/drug metabolites.

Three different newly produced Au, Ag and Au/Ag NCs have been tested as potential optical sensors for detection of metal ions and small ligands. On one hand, the potential usability of the AMP-stabilized Au/Ag alloy NCs as the optical biosensor of folic acid (FA) was described based on the fluorescence quenching, where the LOD was 0.109 μM with 0.15–2.50 μM dynamic range. To analyze the nature of the quenching process, it was established that a quasi-static quenching with steric shield and charge effect was identified. Moreover, an etching reaction was also discovered based on the time-dependent behavior of the sensor reaction and ICP-MS measurements. To compare the chemical properties of the studied analytes (Vitamin B molecular family and their derivatives) we can established that the only the FA has suitable reduction capacity to reduce the noble metal ions to zero oxidation sate confirmed by several publications. Based on this feature only the FA can cleave the larger yellow-emitting alloy NCs to few-atomic bimetallic NCs having blue emission. In addition to the standard liquid phase measurements, a paper-based quick test was also developed to recommend future usability (***J. Mol. Liq.,*** *338* ***(2021)*** *116695. IF = 6.165; Q1*). On the other hand, the prepared Tyr-AgNCs have been successfully used for design of biosensors. During these studies, the interactions with several metal ions from the tap water and wastewater were investigated. Among the studied cations, four different ions (Cu^{2+}, Ni^{2+}, Fe^{3+}, and Rh^{3+}) had a dominant effect on the fluorescence of NCs. Based on the detected quenching processes, the limit of detection (LOD) of the metal ions was determined. The best LOD value is determined for

Cu^{2+}, which is 2.07 ± 0.18 μM. Static quenching (formation of a non-luminescent complex) was observed in all cases by temperature-dependent measurements. The calculated thermodynamic parameters showed that the interactions are spontaneous ranked in the following order of strength: $Cu^{2+} > Fe^{3+} > Rh^{3+} > Ni^{2+}$. Based on the sign and relations of the standard enthalpy ($\Delta H°$) and entropy changes ($\Delta S°$), the dominant forces were also identified (***Int. J. Mol. Sci***, 23 **(2022)** 977. *D1/Q1, IF = 6.208)*. In third case, we firstly demonstrated a new biomolecule, Histidinehydroxamic acid (HisHA), to produce few-atomic fluorescent gold nanoclusters (Au NCs) in aqueous medium. Verifying its ability to detect metal ions, dual strategies were discovered. We confirmed that the copper ions cause fluorescence quenching (LOD = 2.49 μM) by pushing the higher amount of soft HisHA ligand from the metallic surface and forming complexes with dominantly hydroxamate-[O,O] coordination mode in the aqueous medium. For Zn^{2+}-ions, a "turn-on" sensing mechanism was observed; the smallest detectable amount of Zn^{2+} is 7.5 μM. Linear increase of the quantum yield (from ~ 4% to ~ 11.5%) was identified above 75 μM of Zn^{2+} due to the binding of the Zn^{2+}-ions on the cluster surface via hydroxamate-[O,O] donors (***J. Mol. Liquids***, *387* ***(2023)*** *122597, Q1, IF = 6.00)*.

4. Application of noble metal NCs (GNCs, SNCs) as bioimaging and bio-labelling agents.

In medical research the visualization of drug carrier accumulation and release of the loaded drugs *in vivo* is an important field. The interaction of BSA and LYZ-stabilized mono- and bimetallic NCs with polysaccharide (hyaluronic acid/chitosan) biocolloid particles was interpreted (***Int. J. Biol. Macromol.,*** *148 (**2020**) 218., IF = 6.953; Q1*; ***Crystals,*** *10(12) (**2020**) 1113. IF = 2.589, Q2**; J. Mol. Liquids,*** *370 (**2023**) 121002., IF = 6.000; Q1*). It was pointed out that the stability of fluorescent NCs-biocolloid particle complexes in aqueous media and the efficiency of fluorescent labelling by the presence of NCs should be interpreted together for a given system. The composition of the metallic core, the material quality and quantity of the proteins stabilizing the NCs, and the composition, structure (*e.g.* polyelectrolyte complex (PEC) or core-shell structure) and surface charge of the biocolloid particles are all factors that determine whether a noble metal-based NCs can function as a potential fluorescent marker.

5. Surface plasmon resonance (SPR) investigations.

During these application-oriented developments, in general, there is not enough time to systematically examine the interaction between nanoclusters and relevant biomolecules/proteins from a thermodynamic viewpoint. In this way, the primary motivation of this article was to carry out a series of tests to partially fill this scientific gap. Besides the well-known fluorescent probes, the mentioned interactions were investigated using such unique measurement methods as surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). These 2D (at the solid/liquid interface) and 3D (in the bulk phase) measuring techniques provide a unique opportunity for the thermodynamic characterization of the interaction between different gold nanoclusters containing various surface functionalizing ligands and bovine serum albumin (BSA) (***Int. J. Mol. Sci,*** *24(23) (**2023**) 16760., IF = 5.600; D1/Q1*).

Moreover, 13 other publications were also published, which are not directly related to the topic of the FK OTKA project.

In conclusion, we have successfully completed the planned research work which is confirmed by the publications. The scientific results were presented at several international and hungarian conferences. Namely, in 2022 1 keynote lecture (in English, Edit Csapó, *Nanomed2022* (2022. okt. 26-28, Athén, Greec.), 3 oral presentations (in English, Árpád Turcsányi (*European Student Colloid Conference*, 2022. jún. 26-30, Szeged, Hungary), Norbert Varga, *Nanomed2022* (2022. okt. 26-28, Athén, Greece) and Viktória Varga, ECIS 2022, 2022. szept. 4-9, Crete, Greece), 2 oral presentations (in Hungarian, Edit Csapó (MTÜ2022, Szeged, 2022. nov. 16, László Seres (KEN2022); 9 posters (*ICC2022*, 2022. jún. 12-15, Lisbon, Portugal). In 2023, 2 lectures (in English, Á. Juhász and N. Varga, *Mat2023* (2023. 09. 26-28, Valencia, Spain), 2 lectures (in Hungarian, E. Csapó (MTA Kolloid Munkabiz. (online) 2023. 05. 30.; 7 posters (*ICC2023*, 2023. 06. 10-13, Palma de Mallorca, Spain).