Theses of doctoral (Ph.D.) dissertation

Protein-stabilized gold/silver bimetallic nanoclusters

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2025

1. Introduction, background, objectives

Noble metal nanoclusters (NCs) are a kind of intermediate material between molecules and classical noble metal nanoparticles (NPs). They cannot be called NPs, because the localized surface plasmon resonance (LSPR), which gives rise to the plasmonic property, cannot be observed due to their extremely small size, meaning that the coherent motion of electrons under excitation at a given energy does not occur. In contrast, NCs exhibit distinct energy levels, more akin to molecular orbitals, which are associated with the emergence of new magnetic and optical properties, such as photoluminescence (fluorescence or phosphorescence). These properties can be controlled by the number of atoms that build up the NCs, the type of atoms and their oxidation state, as well as the ligands that stabilize the surface of the NCs (specifically, the type and number of electron-rich donor atoms). Further change can be achieved by building NCs from two different metals instead of one, which enables the formation of nanostructured materials whose structural, optical or catalytic properties differ from the unique characteristics of pure nanoclusters (NCs) created from either component alone. The application of mono- and bimetallic nanostructures in the biomedical field can be promising, making it essential to ensure their biocompatibility. This can mostly be achieved by choosing suitable stabilizing molecules, which can be amino acids or proteins. When selecting these materials, it is important that they should possess functional groups capable of ensuring the reduction of noble metal ions and the proper stabilization of NCs formed from the self-assembly of atoms. This is often true for proteins, as their sulfur-containing amino acids (cysteine and methionine) coordinate and stabilize metal ions by forming sulfur-gold bonds, while amino acids with aromatic side-chains (e.g., tyrosine) can effectively reduce them at high pH values.

Our research group has been working for a long time on the aqueous synthesis of noble metal nanostructures, including NCs, using both small molecules and macromolecules. At the beginning of my doctoral studies, our group was primarily focused on developing synthetic processes for monometallic NCs, as evidenced by the PhD theses of Ditta Ungor (2018) and Gyöngyi Gombár (2024). When I started my PhD work, only one article was published on the topic of bimetallic NCs, where the group developed a green chemistry protocol for producing adenosine-monophosphate-stabilized gold/silver NCs and demonstrated the promising role of these yellow-emitting, ~1 nm NCs in analytical testing. During my PhD research, continuing this new topic, we aimed to develop the production of new protein-stabilized bimetallic (Au/Ag) NCs. At the beginning of my work, there were no available data in the literature on the formation of bimetallic NCs using gamma-globulin (γG) and transferrin (Tf) proteins, so

determining their applicability on NCs' formation was the basis of the PhD dissertation. Additionally, for bovine serum albumin (BSA) and lysozyme (LYZ), we developed a new protocol for the synthesis of bimetallic NCs at room temperature. A key objective was to increase the fluorescence intensity of the nanostructured materials (as the only less favorable property of NCs is their low quantum yield), which we attempted to control by systematically varying the preparation parameters. During the optimization, we varied the molar ratio of the metals, the metal-to-protein mass ratio, the temperature, the metal concentration and the synthesis time. We attempted to utilize the purified and thoroughly characterized bimetallic systems (alongside the monometallic NCs) in various application areas. We investigated their potential role as fluorescent labeling agents for colloidal drug carrier particles, and we studied them as analytical markers for the selective identification of a biologically important family of compounds, the tryptophan metabolites. In the former case, we investigated the optical and colloidal stability of the adducts formed by the target colloids and the NCs, and we also attempted to determine and interpret the factors limiting their use. In the case of the design of sensing materials, we investigated the molecules of the kynurenine and dopamine degradation pathways, and for the compound that was selectively identified, we conducted detailed studies in order to determine the limit of detection (LOD) value and to investigate the parameters of the resulting interaction.

2. Syntheses and experimental methods

2.1 Syntheses

Syntheses of protein stabilized monometallic nanoclusters: For newly fabricated NCs, the „template-assisted" preparation protocol was applied in every case using slight modifications. The syntheses were carried out in an exclusively aqueous medium. During these protocols, the given amount of protein (15 mg BSA, 20 mg LYZ, 15 mg γG, and 1.5 mg Tf) was dissolved in 5 ml of Milli-Q water (for Tf only in 920 µl). After dissolving time, fixed volumes of 10 mM $HAuCl_4$ were pipetted into these protein solutions (80 µl for Tf and 510 µl for other proteins), and then the pH was adjusted to 12.0–12.5 by using 1 M NaOH to promote the formation of NCs. The maximum fluorescence intensity was achieved at the end of the 24 h synthesis time at 40°C. To ensure the undisturbed formation of the NCs the samples were left without stirring, protected from light. The final products were purified by dialysis (applying 2−2 hours, using 1−1 liter of Milli-Q water). The purified dispersions were stored in the fridge (+10°C).

Syntheses of protein stabilized bimetallic nanoclusters: The synthesis of bimetallic Au/Ag NCs required numerous modifications to achieve the highest fluorescence intensity of the products.

In contrast to monometallic derivatives, higher amount of proteins (45 mg BSA, 45 and 60 mg LYZ, 90 mg γG and 2.7 mg Tf) were dissolved in 5 ml (for Tf only in 960 µl) Milli-Q water, and then less amount (ca. half) of metal precursors ($HAuCl_4$ and $AgNO_3$) were added into these protein solutions. The nominal molar ratios of metals were varied in the range of 6:1 and 10.5:1. After 5 min of homogenization, the pH of the samples were adjusted to 12,0–12,5 using 1 M NaOH solution. The samples were protected from light and were incubated at room temperatures (25°C) without stirring for 24 h. The formed fluorescent products were purified using same methods presented for monometallic derivatives. For LYZ the dialysis was carried out just once. The purified final products were stored in fridge (+10 °C) under dark conditions.

Used buffers:

- Phosphate-buffered saline (PBS): 0.2 M total concentration using NaH_2PO_4 and Na_2HPO_4, 0.9 m/m% NaCl, pH = 7.4
- Artificial cerebrospinal fluid (aCSF): 1.2 mM KH_2PO_4, 1.0 mM KCl, 127 mM NaCl, 2.4 mM $CaCl_2$, 1.3 mM $MgCl_2$, 26 mM $NaHCO_3$, 10 mM *D*-glucose-containing solution

2.2 Experimental techniques

Nanocluster synthesis, optimization: Jasco FP8500 spectrofluorometer with normal sample holder and Horiba Jobin Yvon Fluoromax-4 spectrofluorometer with normal sample holder

Fluorescence parameters (internal quantum yield, lifetime): ABL&E Jasco FP-8500 spectrofluorometer, ABL&E JASCO ILF-835 integrating sphere (with 100 mm diameter) + ESC-842 calibrated light source (tungsten) (for creating reference spectra)
Horiba DeltaFlex time-correlated single photon counting device (TCSPC) equipped with DeltaDiode pulsed laser as a light source (λ = 371 nm)

Creation of solid samples: Christ Alpha 1-2 LD plus and Flexi DryTM (FTS systems®, Inc.) lyophilization equipments

Determination of the metal content and oxidation states of the metals: Agilent 7700X inductively coupled plasma mass spectrometry (ICP-MS), Agilent I-AS type autosampler, a MicroMist microflow concentric pneumatic nebulizer and a Scott-type spray chamber with Peltier-cooling.
SPECS X-ray photoelectron spectroscopy (XPS) PHOIBOS 150 MCD 9 hemispherical analyzer, with an Al-K_α X-ray source operated at 200 W power
Hitachi S-4700 field emission scanning electron microscope using a Röntec XFlash X-ray detector (SEM-EDX)

Determination of the secondary structure of the proteins: BIO-RAD Digilab Division FTS-65 A/896 spectrometer, ATR Single-reflection accessory (Harrick's Meridian®SplitPea)
ABL&E Jasco FT/IR-4700 Fourier-transformed infrared spectroscopy (FT-IR), ATR PRO ONE Single-reflection accessory
ABL&E Jasco J-1100 spectrometer (CD)

Size and surface potential determination: Fei-Tecnai G2 20 X-Twin high-resolution transmission electron microscopy (HR-TEM)
Horiba SZ-100 NanoParticle Analyzer (DLS and ζ-potential), 532 nm Nd:YAG laser source
Malvern Zetasizer NanoZS Zen 4003 (DLS), 633 nm He-Ne laser source
Mütek PCD 04 particle charge detector (measuring streaming potential)

Fluorescent imaging: Leica DM IL LED inverted confocal fluorescent microscope

Analytical sensing: Metrohm DropSense μStat 400 Bipotentiostat/Galvanostat, Metrohm-manufactured screen-printed carbon electrode (SPCE), with a carbon film working and counter electrode, and a AgCl-covered Ag electrode served as the reference electrode.

VP-ITC microcalorimeter (MicroCal Inc., Northampton, MA, USA)

Biological studies: Antibacterial activity tests conducted on Gram-positive (*Staphylococcus aureus*, methicillin-sensitive and resistant strains) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria

Citotoxicity assays with MTT-probe on doxorubicin-sensitive Colo205 cell line and CCD-19Lu human normal fibroblast cells

Softwares: Microsoft Office
OriginPro 8.5 program
ImageJ software
Jasco SpectraManager 2.0 software + Secondary Structure Estimation program
Horiba EZTime program
CasaXPS software
Agilent Mass Hunter program

3. New scientific results

(T1) Green chemical synthesis of Au/Ag bimetallic nanoclusters can be achieved using bovine serum albumin and lysozyme proteins, where the internal quantum yield value can be increased from 1.5% to ~2.5−7.5% by adjusting the silver content to 3–10 wt%, depending on the applied protein [1].

T1.1 As new scientific result, we demonstrated that by using a "template-assisted" method in an aqueous medium (***Fig.1.***) – and systematically varying its reaction conditions – Au/Ag bimetallic NCs with increased fluorescence intensity (5–7 times higher with respect to metal content) can be produced using BSA and LYZ proteins. By replacing the 3–10 w/w% of gold to silver the fluorescence maximum of bovine serum albumin stabilized gold NCs (BSA-Au NCs) can be tuned from λ_{em} = 660 nm to 620 nm (BSA-Au/Ag NCs), while for antibacterial LYZ, fluorescent NCs (LYZ-Au/Ag NCs) having emission maxima at λ_{em} = 570 and 600 nm can be synthesized. We were the first in the literature to demonstrate the suitability of LYZ for the „template-assisted" synthesis of two (yellow and orange) types of Au/Ag bimetallic NCs, and we highlighted the possibility of syntheses at room temperature for both BSA and LYZ.

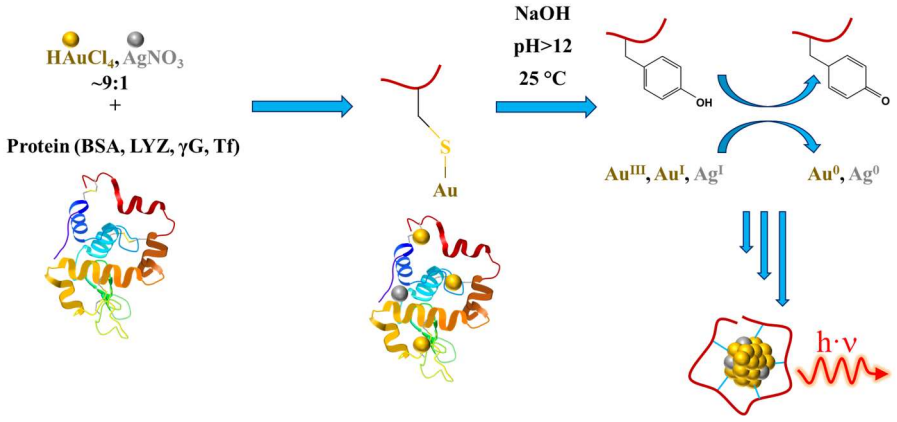

Figure 1. *Schematic representation of the formation process of protein-stabilized bimetallic Au/Ag NCs*

T1.2 By interpreting the optical and structural properties of purified bimetallic NCs, we confirmed that all products have an average lifetime in the μs range (based on fitting three components), and due to the synergistic effect resulting from the incorporation of silver, the internal quantum yield can be increased from 1.5% (characteristic of pure Au NCs) to as high as 7.5%. XPS measurements clearly indicated the metallic character of gold, while CD and FT-IR measurements demonstrated the effect of NC formation on the secondary structure of

the stabilizing proteins. Regarding BSA-Au/Ag NCs, we confirmed through antibacterial activity and cytotoxicity assays that they do not exhibit biological activity (IC_{50} > 50 μM).

(T2) Fluorescent labeling of complex colloidal particles composed of polysaccharides functioning as drug carriers can be achieved using with orange/red-emitting BSA- and LYZ-stabilized mono- and bimetallic NCs. It can be proved that the stability of fluorescent NC-colloid particle adducts in an aqueous medium and the efficiency of fluorescent labeling with NCs are jointly determined by the composition of the cluster core, the quality and quantity of the protein stabilizing the NCs, and the composition, structure, and surface charge of the carrier particles [1, 2].

T2.1 By interpreting the size of the fluorescent NC-polysaccharide „complex" particles, we determined that the material quality of the protein ensuring sufficient stability is not decisive, as aggregation is observed at a very low m_{NC}:mc_{olloid} ratio (< 0.15) during the interaction of all monometallic NCs with chitosan/hyaluronic acid particles. The presence of significantly more stable „complex" particles with bimetallic NCs can be detected up to an m_{NC}:$m_{colloid}$ ratio of ~1.5, which can be attributed to the presence of a larger amount of stabilizing protein in the bimetallic system. We also pointed out that using NCs stabilized with BSA, we can identify a greater degree of aggregation at the same mass ratio. Under the labeling conditions (pH = 4.0), this could be due to the fact that BSA has a near-neutral net charge, as its isoelectric point (i.e.p) is ~4.0–4.5, while LYZ has a net positive charge (i.e.p ~10.5–11.3).

T2.2 Regarding the change in fluorescence intensity, we found that the values of BSA-stabilized NCs – whether monometallic or bimetallic – do not change significantly in the presence of carrier particles, while a substantial enhancement (2.8-fold) is observed for LYZ-Au NCs, which is attributed to the phenomenon of aggregation-induced emission (AIE). Comparing the type of carrier particles, we can state that the higher amount of negatively charged hyaluronic acid present in the surface layer of the core-shell structured particles was more accessible to the LYZ-stabilized NCs. The polysaccharide molecules were able to somehow inhibit the free movement of the LYZ molecules that stabilize the NCs, thereby reducing the likelihood of non-radiative decay and thus enhancing their fluorescence.

(T3) Fluorescent Au/Ag nanoclusters with outstanding kinetic stability in physiological medium can be produced at room temperature in an aqueous medium using the "template-assisted" synthesis method with gamma-globulin immunoprotein [3].

T3.1 As new scientific result, we demonstrated that the optical characteristics of gamma-globulin-stabilized Au NCs (γG-Au NCs) can be systematically tuned on one hand by the replacement of the 10% of gold atoms to silver atoms, and on the other hand by the change of other parameters of the synthesis (protein concentration, temperature, metal content, synthesis time). The fluorescence maximum of the new bimetallic NCs with a 1 μs average lifetime (γG-Au/Ag NCs) is almost identical to that of the starting monometallic derivative (634 nm instead of 637 nm), but the fluorescence intensity can be increased by nearly 2.3 times, which could improve their role in imaging and analytical applications.

T3.2 During the structural characterization of the purified γG-Au/Ag NCs, we demonstrated that the reduction of metal ions is almost fully achieved during the developed synthesis, which is confirmed by the nominal and ICP-MS-measured real composition of the cluster core. Using HR-TEM images, we confirmed that the cluster cores have an average diameter of $d = 1.61 \pm 0.54$ nm, while DLS measurements determined a hydrodynamic diameter of $d_H = 6.82 \pm 0.97$ nm for the protein-stabilized NCs solvated in an aqueous medium. The formation of the alloy cluster core can also be inferred from the results of electrochemical measurements. Through biological activity assays, we determined that the NCs do not exhibit antibacterial or cytotoxic effects even at concentrations above 50 μM.

(T4) Transferrin, known as a transport protein, is suitable for the formation of gold-silver bimetallic nanoclusters (Tf-Au_{85}/Ag_{15} NCs) with an average lifetime in the μs range and with an average diameter of d ~ 2.0 nm, exhibiting a fluorescence intensity nearly four times higher than that of pure gold derivatives, through the simultaneous reduction of $[AuCl_4]^-$ and Ag^+ ions in an aqueous medium [4].

T4.1 As a new scientific achievement, we highlighted that the properties of Tf-Au NCs can be dominantly improved via incorporating silver atoms into the gold cluster core, creating bimetallic Tf-Au/Ag NCs. Through the synthesis of the bimetallic derivative, the fluorescence intensity can be increased by almost four times, while the emission maximum can be shifted from λ_{em} = 626 nm to 595−610 nm. Experimental results of CD and FT-IR measurements confirmed that the formation of NCs strongly modifies the protein structure, significantly reducing the proportion of ordered secondary structural elements (especially α-helices). TEM measurements proved the formation of NCs with a metal core size of 2.0 ± 0.3 nm, while using the DLS technique, 7.2 ± 0.6 nm was determined for the hydrodynamic diameter of the protein-stabilized nanostructure. Through fluorometry, lifetime measurements, and the study of quenching reactions with iodide ions, we identified the specific processes contributing to the

fluorescence of the bimetallic product. We confirmed that the emission of NCs is primarily provided by two processes: one is a transition originating from the metal core, characterized by an emission wavelength of 583 nm and a lifetime of 250 ns, while the largest contribution comes from a ligand-to-metal transition with λ_{em} of 670 nm and a lifetime of 1.56 μs.

(T5) For Au/Ag NCs of nearly identical size (1.5–2.0 nm) and metal composition (10–15% Ag content) exhibiting orange/red emission (610–630 nm), their sensitivity to detecting molecules of the kynurenine pathway can be altered solely by replacing the stabilizing γG protein with Tf [4].

T5.1 During the detection of molecules belonging to the tryptophan degradation pathway, the kynurenine pathway, the group's colleagues previously demonstrated that *L*-kynurenine and kynurenic acid (in the appropriate order) can be selectively identified using γG-stabilized Au and Au/Ag NCs via the quenching of the NCs' fluorescence. We pointed out that by replacing γG with Tf, a third compound, 3-hydroxyanthranilic acid, can be detected in a fluorescence enhancement reaction (***Fig.2.***). Through concentration-dependent experiments, we determined the LOD value, which was 0.55 μM in phosphate-buffered saline (PBS) and 0.32 μM in artificial cerebrospinal fluid (aCSF), significantly lower compared to similar analytical applications (γG-Au NCs/*L*-kynurenine: 15–22 μM, γG-Au/Ag NCs/kynurenic acid: 60–80 μM in PBS and aCSF media). We also performed our investigations in diluted human blood serum as a medium, confirming the feasibility of using NCs in a biological environment.

T5.2 During the investigation of the interaction between 3-hydroxyanthranilic acid, Tf, and Tf-Au/Ag NCs using ITC measurements, we demonstrated that there is no measurable interaction with Tf (***Fig.2.***), while it is clearly detectable with the NCs, which supports their applicability in analytical tests. By interpreting the thermodynamic parameters of the reaction, we confirmed that the reaction is thermodynamically favored (ΔG^0 = −36.0 kJ/mol), endothermic (ΔH^0 = +13.1 kJ/mol), and entropy-driven (ΔS^0 = 165 J/mol K). Based on the applied model, we can assume one binding site (N = 1.03) on the surface-stabilizing protein, with a binding constant of $K_a = 2.17 \times 10^6$ M^{-1}.

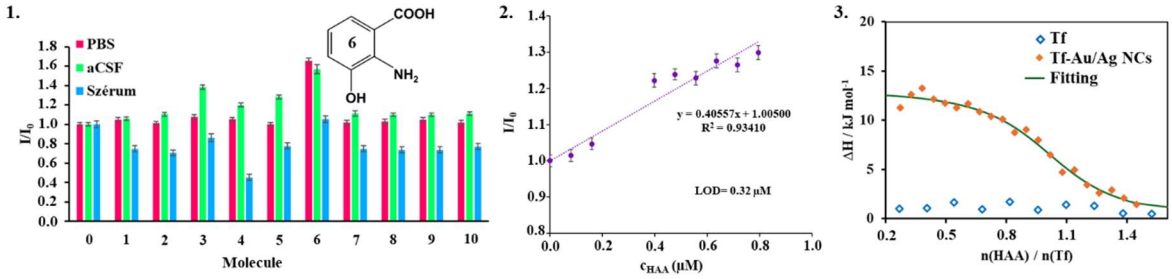

Figure 2. *(1) Changes in the fluorescence intensity values of NCs upon interaction with kynurenine pathway molecules (1-10) in different media; (2) determination of the limit of detection in aCSF medium for ligand (6); (3) enthalpograms recorded during the study of the interaction of Tf and Tf-Au/Ag NCs with ligand (6)*

4. Expected use of the results

I started my doctoral studies in the research group of Dr. Edit Juhászné Csapó. The group is unique in Hungary in the research topic on fluorescent metal nanoclusters, and the research and knowledge gained over several years can be well utilized in the production and application of further materials with tunable photoluminescent properties. These materials possess numerous attributes that promise potential applications in various biomedical fields (e.g., greater photostability, suitable biocompatibility). The newly created materials opened up a new field of application in fluorescent labeling and imaging, as, unlike previous utilization methods, they allowed for the labeling not only of cells and tissues but also of drug delivery systems. Their application for this purpose allows the tracking of the carrier particle's path within the organism, its accumulation sites, and the release of the active substance, and also aids in the development of drug carriers. In the field of analytical sensing, new nanosized analytical tests have been successfully produced that enable the selective detection of molecules in the kynurenine pathway, thus laying the foundation for the creation of a sensing kit targeting the molecules of the tryptophan degradation pathway. Developing such a kit allows the rapid, simple, and reliable detection of substances that play a significant role in the human body, highlighting neurodegenerative diseases where changes in the concentration of certain compounds can indicate the onset of a specific condition. In such cases, systems that allow for monitoring changes (e.g., simple paper-based tests) can become important tools for the rapid detection of diseases and the initiation of therapy as early as possible.

5. Publication list

Repository of Hungarian Scientific Works (MTMT) identifier: **10067812**

Scientific publications directly related to the doctoral thesis:

[1] **Á. Turcsányi**, D. Ungor, E. Csapó; Fluorescent Labeling of Hyaluronic Acid-Chitosan Nanocarriers by Protein-Stabilized Gold Nanoclusters; Crystals; 10(12) (2020) 1113.

https://doi.org/10.3390/cryst10121113

Q2, IF = 2.589

[2] **Á. Turcsányi**, D. Ungor, M. Wojnicki, E. Csapó; Protein-stabilized bimetallic Au/Ag nanoclusters as fluorescent reporters: Synthesis, characterization and their interactions with biocolloids; Journal of Molecular Liquids, 370 (2023) 121002.

https://doi.org/10.1016/j.molliq.2022.121002

Q1, IF = 5.3

[3] D. Ungor, **Á. Turcsányi**, B. Torma, E. Csapó; Gold/silver bimetallic nanoclusters stabilized by immunoprotein: Tuning the selectivity for identification of kynurenine pathway metabolites; Journal of Molecular Liquids, 402 (2024) 124756.

https://doi.org/10.1016/j.molliq.2024.124756

Q1, IF = 5.2

[4] **Á. Turcsányi**, Á. Juhász, E. Csapó; Transferrin-stabilized bimetallic nanoclusters: Design of a new fluorescent biosensor to identify tryptophan metabolites; Materials Today Chemistry, 47 (2025) 102835.

Q1, IF = 6.7 ΣIF = 19.789

Other scientific publications:

[5] L. Janovák, **Á. Turcsányi**, É. Bozó, Á. Deák, L. Mérai, D. Sebők, Á. Juhász, E. Csapó, M. M. Abdelghafour, E. Farkas, I. Dékány, F. Bari; Preparation of novel tissue acidosis-responsive chitosan drug nanoparticles: Characterization and in vitro release properties of Ca^{2+} channel blocker nimodipine drug molecules; European Journal of Pharmaceutical Sciences, 123 (2018) 79–88.

https://doi.org/10.1016/j.ejps.2018.07.031

Q1, IF = 3.532

[6] K. Majrik, **Á. Turcsányi**, Z. Pászti, T. Szabó, A. Domján, J. Mihály, A. Tompos, I. Dékány, E. Tálas; Graphite Oxide-TiO_2 Nanocomposite Type Photocatalyst for Methanol Photocatalytic Reforming Reaction; Topics in Catalysis, 61 (2018) 1323–1334.

https://doi.org/10.1007/s11244-018-0989-z

Q1, IF = 2.226

[7] N. Varga, **Á. Turcsányi**, V. Hornok, E. Csapó; Vitamin E-Loaded PLA- and PLGA-Based Core-Shell Nanoparticles: Synthesis, Structure Optimization and Controlled Drug Release; Pharmaceutics, 11(7) (2019) 357.

https://doi.org/10.3390/pharmaceutics11070357

Q1, IF = 4.421

[8] **Á. Turcsányi**, N. Varga, E. Csapó; Chitosan-modified hyaluronic acid-based nanosized drug carriers; International Journal of Biological Macromolecules, 148 (2020) 218–225.

https://doi.org/10.1016/j.ijbiomac.2020.01.118

Q1, IF = 6.953

[9] N. Varga, L. Seres, N. A. Kovács, **Á. Turcsányi**, Á. Juhász, E. Csapó; Serum albumin/hyaluronic acid nanoconjugate: Evaluation of concentration-dependent structural changes to form an efficient drug carrier particle; International Journal of Biological Macromolecules, 220 (2022) 1523–1531.

https://doi.org/10.1016/j.ijbiomac.2022.09.125

Q1, IF = 8.2

[10] K. W. K. Amin, Á. Deák, M. Csanády, Jr., N. Szemerédi, D. Szabó, **Á. Turcsányi**, D. Ungor, G. Spengler, L. Rovó, L. Janovák; pH-Triggered Hydrogel Nanoparticles for Efficient Anticancer Drug Delivery and Bioimaging Applications; Pharmaceutics, 16 (2024) 931.

https://doi.org/10.3390/pharmaceutics16070931

Q1, IF = 5.5 ΣIF = 50.621

Conference participation (poster, presentation) on the topic of the PhD thesis:

1. **Turcsányi Á.**; Kitozán/Hialuronsav típusú hatóanyag-hordozó rendszerek szintézise és jelölése fluoreszcens arany nanoklaszterekkel; XXIV. Tavaszi Szél Konferencia, 28–30 May, 2021, Miskolc, Hungary (presentation in Hungarian)

2. **Á. Turcsányi**, E. Csapó; Synthesis of chitosan/hyaluronan type drug carriers and their fluorescent labeling with protein/gold nanoclusters; 13th International Conference on nanomaterials – Research and Application, 20–22 October, 2021, Brno, Czech Republic (poster)

3. **Á. Turcsányi**, D. Ungor, E. Csapó; Fluorescent labeling of drug carriers with gold nanoclusters and bimetallic silver-gold nanoclusters; 11th International Colloids Conference, 12–15 June, 2022, Lisbon, Portugal (poster)

4. **Á. Turcsányi**, D. Ungor, E. Csapó; Utilization of gold nanoclusters and bimetallic silver-gold nanoclusters as fluorescent reporters; 18th European Student Colloid Conference, 26–30, June, 2022, Szeged, Hungary (presentation in English)

5. **Á. Turcsányi**; Utilization of protein-stabilized bimetallic nanoclusters for the detection of kynurenine pathway molecules; 16th International Conference on Nanomaterials – Research & Application, 17–19 October, 2024, Brno, Czech Republic (presentation in English)

6. **Á. Turcsányi**, G. Spengler, E. Csapó; Bimetallic nanoclusters: Synthesis optimization and biological studies, Chemistry Physics and Biology of Colloids and Interfaces, 15−19 June, 2025, Eger, Hungary (presentation in English)

Other conference appearances (poster, presentation):

7. **Á. Turcsányi**, E. Tálas, G. Szijjártó, Á. Veres, I. Dékány, A. Tompos, T. Szabó; Synthesis and Characterization of Titanium Dioxide Based Ternary Nanocomposites for Photocatalytic Hydrogen Production, 22nd International Symposium on Analytical and Environmental Problems, 10 October, 2016, Szeged, Hungary (poster)

8. **Á. Turcsányi**, L. Janovák, T. Szabó; Chitosan Nanoparticle/Graphite Oxide Nanocomposites for Controlled Drug Release; 11th Conference on Colloid Chemistry, 28–30 May, 2018, Eger, Hungary (poster)

9. T. Szabó, **Á. Turcsányi**, L. Janovák; Controllable release of ketoprofen from chitosan nanoparticles deposited on graphene oxide platelets; 32nd Conference of the European Colloid and Interface Society, 2–7 September 2018, Ljubljana, Slovenia (poster co-author)

10. Majrik K., **Turcsányi Á.**, Vass Á., Szabó T., Pászti Z., G. Bonura, Tompos A., Tálas E.; AgO_x/TiO_2 katalizátorok viselkedése glicerin fotokatalitikus reformálási reakciójában: a ko-katalizátorok hatása; XLI. Kémiai Előadói Napok, 15–17 October, 2018, Szeged, Hungary (presentation co-author)

11. Á. Juhász, N. Varga, **Á. Turcsányi**, E. Csapó; Relation between rheological, structural and dissolution properties of covalently and ionically modified hyaluronic acid-based drug carriers; 10th Anniversary International Conference on Nanomaterials – Research & Application, 17–19 October, 2018, Brno, Czech Republic (presentation co-author)

12. **Á. Turcsányi**, N. Varga, V. Hornok, Á. Juhász, E. Csapó; Synthesis, structure and encapsulation efficiency of chitosan, hyaluronan and chitosan/hyaluronan composite nanoparticles; 9th International Colloids Conference, 16–19 June 2019, Sitges, Barcelona, Spain (poster)

13. N. Varga, A. N. Kovács, **Á. Turcsányi**, V. Hornok, E. Csapó; Design of poly(lactide-co-glycolide) particles: synthesis optimization and controlled drug release; SMS / EGF / Sensors / NanoMed 2022 Joint International Conferences, 26–28 October 2022, Athens, Greece (presentation co-author)

14. E. Csapó, D. Ungor, Gy. Gombár, R. Bélteki, **Árpád Turcsányi**, Loretta Kuklis: Fluorescent noble metal nanoclusters with tunable optical features: synthesis, characterization, and biomedical applications, SMS/EGF/Nanomed/Sensors, 26–28 October, 2022, Athens, Greece (poster co-author)

15. **Á. Turcsányi**, D. Ungor, Gy. Gombár, E. Csapó; Production of bimetallic noble metal nanoclusters by etching with biologically active vitamin B derivatives; 12th International Colloids Conference, 11–14 June, 2023, Palma, Mallorca, Spain (poster)

Statement

Signed Edit Juhászné Dr. Csapó, as the supervisor of the PhD thesis and the corresponding author of the *[3] Ditta Ungor, Árpád Turcsányi, Bianka Torma, Edit Csapó; Gold/silver bimetallic nanoclusters stabilized by immunoprotein: Tuning the selectivity for identification of kynurenine pathway metabolites; Journal of Molecular Liquids, Volume 402, 2024, 124756, https://doi.org/10.1016/j.molliq.2024.124756, Q1, IF 5.2* article, I hereby declare that in this article Árpád Turcsányi optimized the fabrication protocol of the nanoclusters and performed their optical and structural characterization as well, which results are the basis of the thesis point T3. The additional sensing studies were carried out by Bianka Torma, so the dissertation does not contain her experiments.

Szeged, 2025. 09. 15. ……

Edit Juhászné Dr. Csapó

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