

Theses of doctoral (Ph.D.) dissertation



## **Functional gold nanoclusters with fluorescence property**

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## 1. Introduction, background, objectives

Understanding and describing changes related to small sizes has become crucial in scientific research, with key elements being nanoclusters (NCs, sub-nanometer particles), nanoparticles (NPs), and other nanostructured systems. Among metallic nanostructured materials, noble metals (especially gold (Au)) are extremely preferred due to their unique electrical, magnetic, and optical properties, and based on these features there are widely applied in medicine, healthcare, physics, electronics, catalysis, etc. By reducing the size of Au NPs, continuous conduction bands split into discrete energy levels, allowing for the emergence of a new optical property, fluorescence, instead of localized surface plasmon resonance (LSPR) bands, through the formation of these sub-nanometer-sized NCs. One of the main factors that determine the photoluminescence properties of noble metal NCs is the number of atoms that build up the cluster. The quality of the (bio)molecules bound on the surface of the metal clusters, the ligand-metal and the ligand-metal-metal charge transfer also play a crucial role in the tuning of the fluorescence. In addition, the oxidation state of the metal, as well as pH or temperature, also affect the appearance and characteristics of the optical property that can be detected. In case of nanomedicine, there is an increasing demand for the development of novel noble metal NCs with controllable size, composition, and function synthesized via "green chemistry" fabrication protocols. This was the main motivation of my research work.

For almost 10 years, our research group has been working on the fabrication and structural investigation of size-controlled mono- and bimetallic gold nanostructures, using the "template" method, in which macromolecules play the role of the "template" for the reduction of metal ions in aqueous medium. I started my PhD studies in autumn 2020, where I worked on the development of mainly one-step "green chemistry" synthesis processes, where the reduction of precursor tetrachloroaurate ions and the stabilization of the resulting nano-objects is mainly achieved by using small molecules. The research includes the study of key experimental conditions (metal ion/ligand molar ratio, pH, temperature, reaction time) and the comprehensive structural and optical characterization of nanodispersions.

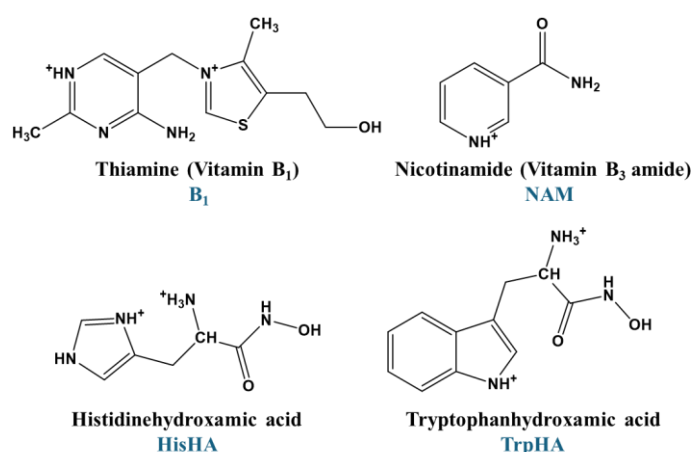
The aim of my PhD thesis is to investigate the effect of systematic variation of the above-mentioned experimental parameters on the production process and reproducibility of Au NCs. In order to achieve the reduction of metal ions, we utilized newly studied Vitamin B and the hydroxamic acid derivatives of amino acids having aromatic side chain. In addition to the development of syntheses and purification protocols, the potential usefulness of the products obtained was also a priority. On the one hand, the use of the synthesized and characterized blue-emitting Au NCs stabilized with the hydroxamic acid derivatives of the amino acids in sensing

areas was proposed. There is a considerable literature on the detection of metal ions with Au NCs, but few publications provide a more detailed understanding of the mechanisms. For the most promising metal ions, where possible, my task was to determine the minimum detectable limit and to interpret the fluorescence (PL) quenching and enhancement processes and to explore their mechanisms. In addition to the development of optical sensors, the preparation of Au NCs with antioxidant property, stabilized with vitamin B derivatives and their structural analysis was also part of my research. In addition, an attempt was made to study the interaction of these latter mentioned Au NCs with human serum albumin using specific measurement techniques (surface plasmon resonance (SPR) spectroscopy, isothermal titration calorimetry (ITC)), for which data are missing in the literature.

The main motivation for the PhD thesis was that the study of fluorescent noble metal NCs is a unique research area in Hungary, so we would like to contribute to the expansion of the field with new scientific results. In addition, the importance of sub-nanometer sized structures with tunable fluorescence properties was highlighted by the Nobel Prize in Chemistry 2023.

## 2. Syntheses and experimental methods

The chemicals used for synthesis and analysis were of analytical grade, so no further purification was carried out before their application. For the preparation of stock solutions and buffers, high purity Milli-Q water (Millipore, Milli-Q Integral3, Direct-Q3 UV, specific resistance 18.2 MΩ·cm, 25 °C) was always used. The structural formulas of the protonated forms of the small molecules used for the synthesis of NCs are summarized in **Figure 1**.



**Figure 1.:** Structural formulas of the protonated form of the small molecules used in the syntheses.

The following measurement techniques were used for the preparation and purification of fluorescent NCs and for the determination of their main optical and structural properties:

- Spectrofluorometry (ABL&E-JASCO, FP-8500)
- Absolute internal quantum yield (ABL&E-JASCO, FP-8500, which was equipped with the ABL&E JASCO ILF-835 integrating, for the calibration, WI light source ABL&E JASCO ESC-842 was applied)
- Fluorescence lifetime (Horiba DeltaFlex, a DeltaDiode pulsed laser served as an excitation light source)
- ORAC assay (Oxygen radical antioxidant capacity; to determine the antioxidant activity of NCs (TE = Trolox equivalent value)
- ABTS assay (to determine the antioxidant activity of NCs)
- UV-Vis spectrophotometry (JASCO V-770, two-ray)
- Dynamic light scattering and  $\zeta$ -potential measurement (Malvern Zetasizer NanoZS)
- Fourier-transformed infrared spectroscopy (FT-IR, ABL&E-JASCO, FT/IR-4700)
- High-resolution transmission electron microscopy (HRTEM, FEI Tecnai G2 20x-Twin)
- X-ray photoelectron spectroscopy (XPS, measurements were done by a SPECS instrument equipped with a PHOIBOS 150MCD9 hemispherical analyzer)
- Surface Plasmon Resonance Spectroscopy (SPR, Reichert2SPR two-channel, angle modulated)
- Isothermal Titration Calorimetry (ITC, MicroCal VP-ITC)
- Conductometry (Radelkis OK-114)
- Freeze drying (Christ Alpha 1-2 LD)

### 3. New scientific results

**(T1.) Few atomic gold nanoclusters with both fluorescent (blue emission) and antioxidant properties can be produced by the reaction of  $[\text{AuCl}_4]^-$ -ions with nicotinamide [1].**

T1.1. Simple and reproducible synthesis protocol has been developed in alkaline medium (pH = 8.0) for the preparation of nicotinamide (NAM)-stabilized gold nanoclusters (NAM-Au NCs) using NAM: $[\text{AuCl}_4]^-$ /10:1 molar ratio. We confirmed that these blue-emitting NCs ( $\lambda_{\text{ex}} = 335 \text{ nm}$ ,  $\lambda_{\text{em}} = 380 \text{ nm}$ ) possess average fluorescent lifetime in the nanosecond range ( $\tau_1 = 4.3 \text{ ns}$  (32%),  $\tau_2 = 6.7 \text{ ns}$  (68%) and  $\text{QY}(\%) = 1.9 \pm 0.3\%$  internal quantum yield. It was also highlighted for production of NCs containing a few atoms (ca. 3–4) that the reduction of

$[\text{AuCl}_4]^-$ -ions is further promoted using Na-citrate alongside the nicotinamide. The presence of  $\text{Au}^0$  atoms in the cluster core was identified by XPS studies.

T1.2. Based on ORAC measurements it was confirmed that the synthesized and purified NAM-Au NCs show nearly the same ( $\text{TE} = 0.48 \pm 0.03 \mu\text{M}$ ) antioxidant properties than the pure nicotinamide ( $\text{TE} = 0.568 \pm 0.02 \mu\text{M}$ ), but in contrast to the NAM ligand the NCs possess more dominant fluorescent feature as well, so based on this double function they are promising agents in biocatalytic reactions.

T1.3. Based on the results of surface plasmon resonance studies, it was confirmed that these NAM-Au NCs form a substantially weaker interactions with bovine serum albumin compared to other blue-emitting Au NCs that are also stabilized with small molecules (e.g. thiamine, adenin monophosphate). This is established by the binding constant ( $K_A = 143 \pm 30 \text{ M}^{-1}$ ) calculated as a percentage of the rate constants ( $k_a, k_d$ ) (**Figure 2.**)

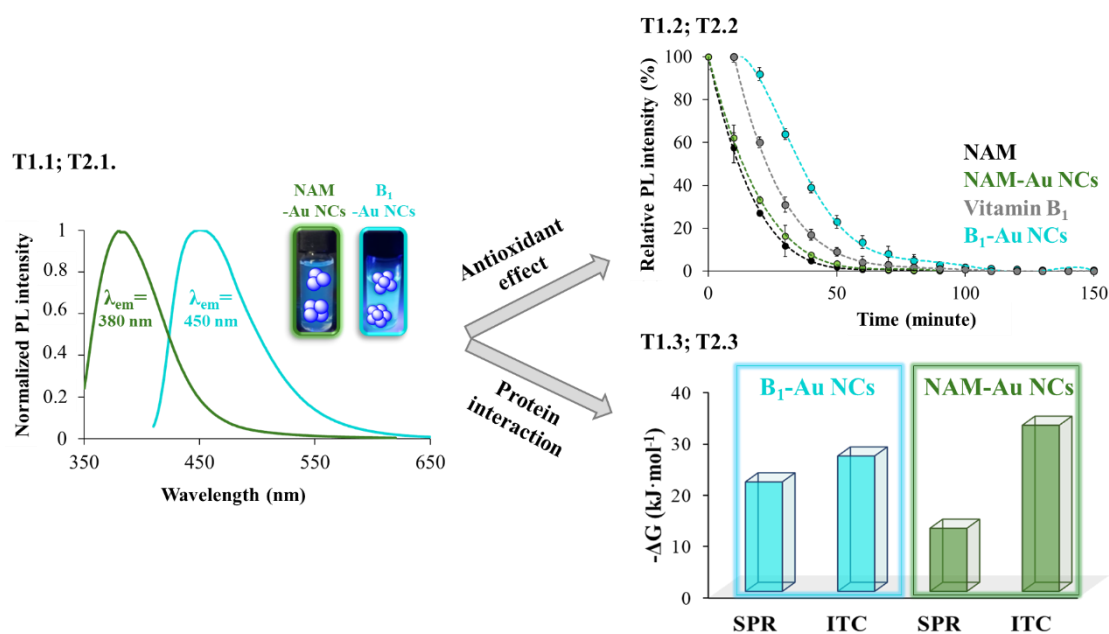
**(T2.) Vitamin B<sub>1</sub> (thiamine) is suitable to reduce the  $[\text{AuCl}_4]^-$ -ions in aqueous medium. Using this green synthesis protocol, sub-nanometer sized, fluorescent Au NCs can be prepared in room temperature. The antioxidant feature of the Vitamin B<sub>1</sub> can be increased by a factor of almost one and a half by the formation of Vitamin B<sub>1</sub>-stabilized NCs (B<sub>1</sub>-Au NCs) [2].**

T2.1. We first demonstrated that Au NCs can be produced in aqueous medium at 25 °C using Vitamin B<sub>1</sub> as a reducing and stabilizing agent. It was confirmed that these blue-emitting NCs ( $\lambda_{\text{ex}} = 395 \text{ nm}$ ,  $\lambda_{\text{em}} = 450 \text{ nm}$ ) probably contain *ca.* 5–6 gold atoms in the metallic core and possess average lifetime in the nanosecond range ( $\tau_1 = 1.26 \pm 0.38 \text{ ns}$  (31%),  $\tau_2 = 2.46 \pm 0.13 \text{ ns}$  (49%),  $\tau_3 = 0.24 \pm 0.01 \text{ ns}$  (20%)), while the internal quantum yield is nearly 3.0%. Based on the experimental results of XPS and FT-IR measurements, it was identified that the gold atoms in the formed clusters are in a zero oxidation state, and the Vitamin B<sub>1</sub> stabilizes the metal core through pyrimidine-*N* and the oxidized thiochrome form of the ligand is also involved in the cluster core stabilization.

T2.2. Based on ORAC measurements performed to confirm the potential antioxidant activity of B<sub>1</sub>-Au NCs, we have demonstrated that the presence of metallic core containing a few gold atoms – due to the electron-rich surface – increases the antioxidant capacity by a factor of almost one and a half ( $\text{TE} = 0.53 \pm 0.01 \mu\text{M}$ ) compared to pure Vitamin B<sub>1</sub> ( $\text{TE} = 0.36 \pm 0.02 \mu\text{M}$ ). The  $\text{IC}_{50}$  values determined for ABTS test show a similar trend as the results of the ORAC assays; the increased antioxidant effect is also likely due to the presence

of oxidized thiochrome on the cluster surface (vitamin B<sub>1</sub>: IC<sub>50</sub> = 76.4 ± 3.7 μM, B<sub>1</sub>-Au NCs: IC<sub>50</sub> = 36.9 ± 2.1 μM).

T2.3. Based on the results of surface plasmon resonance studies, it was confirmed that these B<sub>1</sub>-Au NCs form a substantially stronger interactions with bovine serum albumin compared to NAM-Au NCs This is established by the binding constant (K<sub>A</sub> = 5468 ± 73 M<sup>-1</sup>) calculated as a percentage of the rate constants (k<sub>a</sub>, k<sub>d</sub>) (**Figure 2.**)



**Figure 2.:** Main optical and antioxidant features of NAM- and Vitamin B<sub>1</sub>-stabilized Au NCs and the presentation of their interaction with serum albumins.

**(T3.)** We have demonstrated for the first time that the synthesis of blue-emitting Au NCs in aqueous media is feasible through the interaction of hydroxamic acid derivative of histidine amino acid with [AuCl<sub>4</sub>]<sup>-</sup> ions. We have pointed out that these blue-emitting NCs may have a promising function for the design of turn-off (Cu<sup>2+</sup>) and turn-on (Zn<sup>2+</sup>) metal ion sensors [3].

T3.1. A new preparation protocol has been developed for the fabrication of histidine hydroxamic acid (HisHA)-reduced and -stabilized few-atomic Au NCs with QY% ~ 4% internal quantum yield and  $\tau = 4.2 \text{ ns}$  average fluorescence lifetime. It was verified that the formation of blue-emitting NCs ( $\lambda_{ex} = 365 \text{ nm}$ ,  $\lambda_{em} = 440 \text{ nm}$ ) is preferred at pH = 7.0 using HisHA:[AuCl<sub>4</sub>]<sup>-</sup>/5:1 ratio. We confirmed that the by using HisHA:Na-citrate:[AuCl<sub>4</sub>]<sup>-</sup>/5:10:1 molar ratio the yield of the fluorescent product can be doubled. Structural studies have demonstrated that the imidazole-*N* and amino-*N* donor atoms of HisHA can stabilize the formed metallic core and NCs do not show aggregation at relatively high inert salt concentrations ( $c_{\text{NaCl}} > 1 \text{ M}$ ).

T3.2. In case of study of the interaction of HisHA-Au NCs with paramagnetic metal ions, we have shown that although several metal ions cause PL quenching ( $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ), the highest PL quenching detectable for  $\text{Cu}^{2+}$ -ions ( $I_0/I \sim 24$ ) can be increased almost twofold ( $I_0/I \sim 44$ ) by controlling (removing) the amount of Na-citrate in the system (**Figure 3.**). This is not possible for the other three metal ions. In addition to the determination of the limit of detection ( $\text{LOD} = 2.49 \mu\text{M}$ ), the identification of the quenching mechanism showed that after addition of  $\text{Cu}^{2+}$ -ions to the NCs dispersion, they form complexes with a predominant amount of "soft" HisHA molecules from the cluster surface, whose presence in the aqueous dispersion can be spectrally identified. At the same time, the stabilization of the metallic core is ensured by the Na-citrate molecules present in the dispersion, which is confirmed by the lifetime measurements. In the absence of Na-citrate, the stabilization of the cluster core is not occurred, resulting in nearly absolute quenching (**Figure 3.**).

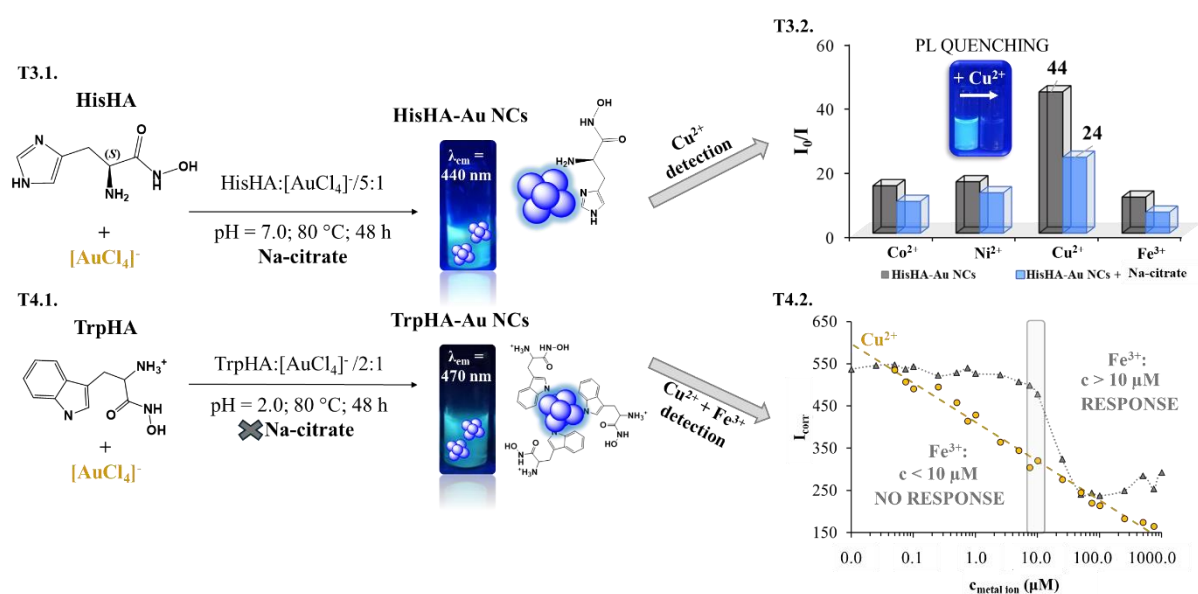
T3.3. In case of study of the interaction of HisHA-Au NCs with diamagnetic metal ions (e.g.  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ), we have shown that only the  $\text{Zn}^{2+}$ -ions cause dominant PL enhancement ( $\text{LOD} = 7.5 \mu\text{M}$ ). By contributing to the development of fluorescent labelling agents, we have demonstrated as a promising result, that the internal quantum yield is greatly controlled (increase from  $\sim 4\%$  to  $\sim 12\%$ ) via the binding of  $\text{Zn}^{2+}$ -ions to the hydroxamate-[O,O] donors on the cluster surface. For other metal ions this coordination is not preferred under the applied experimental condition.

**(T4.) Tryptophanhydroxamic acid (TrpHA) can be used for the synthesis of blue-emitting Au NCs in aqueous medium through their interaction with  $[\text{AuCl}_4]^-$ -ions without the addition of other reducing and stabilizing agents. For sensor applications, it has been demonstrated that by replacing the tryptophan carboxyl group with a hydroxamic group, detection of  $\text{Cu}^{2+}$ -ions can be achieved even in the presence of  $\text{Fe}^{3+}$ -ions ( $c_{\text{Fe(III)}} < 10 \mu\text{M}$ ) with a  $\text{LOD} = 3.16 \mu\text{M}$  detection limit [4].**

T4.1. Tryptophanhydroxamic acid (TrpHA) can be used to synthesize blue-emitting Au NCs as a result of its interaction with  $[\text{AuCl}_4]^-$ -ions in aqueous media without the addition of other reducing and stabilizing agents. For use in the sensing field, it has been demonstrated that by replacing the carboxyl group of tryptophan with a hydroxamate group, the detection of  $\text{Cu}^{2+}$ -ions in the presence of  $\text{Fe}^{3+}$ -ions ( $c_{\text{Fe(III)}} < 10 \mu\text{M}$ ) can be achieved with a  $\text{LOD} = 3.16 \mu\text{M}$  detection limit.

T4.2. We have shown that the presence of a hydroxamate group, which does not undergo structural changes during the clustering process, results in tunable selectivity and sensitivity of

TrpHA-Au NCs and even allows simultaneous identification of metal ions. Based on temperature-dependent fluorescence quenching and quantitative calorimetric studies, it is confirmed that the metal ions can interact with clusters in different ways. On the one hand, we have demonstrated that for  $\text{Cu}^{2+}$ -ions, the stabilizer TrpHA ligands of the cluster surface are probably able to coordinate  $\text{Cu}^{2+}$ -ions, which in acidic media strongly generates the coupling (aggregation) of NCs resulting in combined PL quenching. It was also pointed out that for  $\text{Fe}^{3+}$ -ions, the extent of PL quenching is strongly dependent on the concentration of the metal ion and only occurs through aggregation of NCs when NCs cannot bind more  $\text{Fe}^{3+}$ -ions on the surface ( $c_{\text{Fe(III)}} < 10 \mu\text{M}$ ). As a result, the identification of  $\text{Cu}^{2+}$ -ions in aqueous media is feasible in the presence of  $\text{Fe}^{3+}$ -ions at concentrations of  $10 \mu\text{M}$  (Figure 3).



**Figure 3.:** Production process and the results of sensor studies of HisHA-Au NCs and TrpHA-Au NCs based on PL quenching.



#### **4. Expected use of the results**

With the emergence of nanobiotechnology, the development of nanostructures with diverse functionalities is becoming increasingly important. The noble metal nanoclusters with fluorescent properties produced by "green chemistry" have good biocompatibility, low cytotoxicity, high photostability and can be easily functionalized with different biomolecules. By exploiting these properties, NCs can be efficiently applied in many fields, including biomedical applications for selective and sensitive detection of various analytes, fluorescent labelling, (bio)imaging, drug delivery, and play an important role in diagnostics and therapeutics.

In this work, we have reported for the first time the potential of Au NCs (NAM-Au NCs, B1-Au NCs) stabilized with vitamin B derivatives and having antioxidant property and proposed the application of newly synthesized and characterized intense blue emission NCs (HisHA-Au NCs, TrpHA-Au NCs) in the sensing field. In addition to this, it is worth mentioning that this field is developing not only in biomedical aspects besides materials science, but also in life sciences and plant biology, where nanoclusters can be produced in a reproducible, cost-effective and environmentally friendly way.

## 5. Publication list

Repository of Hungarian Scientific Works (MTMT) identifier: 10073950

### Scientific publications directly related to the doctoral thesis:

- [1] **G. Gombár**, D. Ungor, G. F. Samu, O. Dömötör, E. Csapó: *Synthesis and characterization of novel blue-emitting nicotinamide-gold nanoclusters with “chain-breaker” antioxidant property*, Journal of Molecular Liquids, 359 (2022) 119372. doi: 10.1016/j.molliq.2022.119372 Q1, IF<sub>2022</sub> = 6.0
- [2] D. Ungor, **G. Gombár**, Á. Juhász, G. F. Samu, E. Csapó: *Promising Bioactivity of Vitamin B1-Au Nanocluster: Structure, Enhanced Antioxidant Behavior, and Serum Protein Interaction*, Antioxidants 12 (2023) 874. doi: 10.3390/antiox12040874 D1, IF<sub>2022</sub> = 7.0
- [3] **G. Gombár**, P. Simon, D. Ungor, I. Szatmári, E. Csapó: *Histidinehydroxamic acid as new biomolecule to produce molecular-like fluorescent gold nanoclusters: Possible mechanisms for metal ion sensing*, Journal of Molecular Liquids, 387 (2023) 122597. doi: 10.1016/j.molliq.2023.122597 Q1, IF<sub>2022</sub> = 6.0
- [4] **G. Gombár**, D. Ungor, I. Szatmári, Á. Juhász, E. Csapó: *Tryptophanhydroxamic Acid-Stabilized Ultrasmall Gold Nanoclusters: Tuning the Selectivity for Metal Ion Sensing*, Nanomaterials, 14(5) (2024) 434. doi: 10.3390/nano14050434 Q1, IF<sub>2022</sub> = 5.3
- Σ IF = 24.3**

### Other scientific publications:

- [5] A. N. Kovács, N. Varga, **G. Gombár**, V. Hornok, E. Csapó: *Novel feasibilities for preparation of serum albumin-based core-shell nanoparticles in flow conditions*, Journal of Flow Chemistry, 10 (2020) 497-505. doi: 10.1007/s41981-020-00088-4 Q1, IF<sub>2022</sub> = 2.786
- [6] Á. Ágoston, L. Balassa, Á. Deák, G. F. Samu, P. Sz. Tallósy, **G. Gombár**, L. Janovák: *Photocatalytic and antimicrobial activity of sulfur functionalized TiO<sub>2</sub> containing composite films*, Chemical Engineering & Technology, 46 (2023) 927-933. doi: 10.1002/ceat.202200489 Q2, IF<sub>2022</sub> = 2.1

- [7] R. Béltéki, L. Kuklis, **G. Gombár**, D. Ungor, E. Csapó: *The Role of the Amino Acid Molecular Characteristics on the Formation of Fluorescent Gold- and Silver-Based Nanoclusters*, Chemistry-A European Journal, 29 (2023) e2023007  
doi: 10.1002/chem.202300720 D1, IF<sub>2022</sub> = 4.3
- [8] Á. Juhász, **G. Gombár**, E. F. Várkonyi, M. Wojnicki, D. Ungor, E. Csapó: *Thermodynamic Characterization of the Interaction of Biofunctionalized Gold Nanoclusters with Serum Albumin Using Two-and Three-Dimensional Methods*, International Journal of Molecular Sciences, 24(23) (2023) 16760.  
doi: 10.3390/ijms242316760 D1, IF<sub>2022</sub> = 5.6  
**Σ IF = 14.79**  
**Σ Σ IF = 39.10**

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

1. **G. Gombár**, D. Ungor, E. Csapó: *Small molecule-stabilized fluorescent gold nanostructures: preparation and structural analysis*, 18<sup>th</sup> European Student Colloid Conference, June 26-30, 2022, Szeged, Hungary (oral presentation in English)
2. **G. Gombár**, E. Csapó: *Fluoreszcens arany nanoszerkezetek előállítása és szerkezetvizsgálata*, Tavasz Szél Konferencia, 2021. május 28-30, Miskolc, Hungary (oral presentation)
3. **G. Gombár**, Á. Juhász, E. Csapó: *Felületi plazmon rezonancia spektroszkópia alkalmazása receptor-ligandum jellegű kölcsönhatások termodinamikai jellemzésére*, XLIII. Kémiai Előadói Napok, 2020. október 27-29, Szeged, Hungary (oral presentation)
4. **G. Gombár**, D. Ungor, E. Csapó: *Production and structural analysis of small molecule-stabilized fluorescent gold nanostructures*, 11<sup>th</sup> International Colloid Conference, 12-15 June, 2022, Lisbon, Portugal (poster) – *Best Poster Award*
5. **G. Gombár**, D. Ungor, E. Csapó: *Hydroxamic acid derivatives of amino acids: a new family of ligands for production of fluorescent gold nanoclusters*, 12<sup>th</sup> International Colloid Conference, 11-14 June, 2023, Palma, Mallorca, Spain (poster)

**Other conference appearances (poster, presentation):**

1. Á. Juhász, L. Seres, **G. Gombár**, E. Csapó: *Calorimetric Study of Binary Surfactant Mixtures for Design of Nanoscale Drug Carriers*, Nanocon, 20-22 October, 2021, Brno, Czech Rep. (poster)
2. N. Varga, L. Seres, Á. Turcsányi, **G. Gombár**, E. Csapó: *Design and characterization of the hyaluronic acid-based colloidal drug delivery systems* , 11<sup>th</sup> International Colloid Conference, 12-15 June, 2022, Lisbon, Portugal (poster)
3. E. Csapó, D. Ungor, **G. Gombár**, R. Béltéki: *Fluorescent noble metal nanoclusters with tunable optical features: synthesis, characterization, and biomedical applications* , 11<sup>th</sup> International Colloid Conference, 12-15 June, 2022, Lisbon, Portugal (poster)
4. Á. Turcsányi, D. Ungor, **G. Gombár**, E. Csapó: *Production of bimetallic noble metal nanoclusters by etching with biologically active vitamin B derivatives* , 12<sup>th</sup> International Colloid Conference, 11-14 June, 2023, Palma, Mallorca, Spain (poster)
5. E. Csapó, D. Ungor, **G. Gombár**, R. Béltéki, Á. Turcsányi, L. Kuklis: *Fluorescent noble metal nanoclusters with tunable optical features: synthesis, characterization, and biomedical applications*, SMS/EGF/Nanomed/Sensors, 26-28 October, 2022, Athens, Greece

## Statement

Signed Edit Juhászné Dr. Csapó, as the supervisor of the PhD thesis and the corresponding author of the T2. - D. Ungor, G. Gombár, Á. Juhász, G. F. Samu, E. Csapó\*: Promising Bioactivity of Vitamin B<sub>1</sub>-Au Nanocluster: Structure, Enhanced Antioxidant Behavior, and Serum Protein Interaction, *Antioxidants* 2076-3921, 12, 874 (2023) doi: 10.3390/antiox12040874 – article I hereby declare that the role of the candidate was crucial in achieving the results related to Thesis T2. No co-author has used the named publication to obtain a PhD degree and will not do so in the future.

Szeged, 2024. 05. 20.

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Edit Juhászné Dr. Csapó  
Ph.D. supervisor