Insights into the Impact of Gold Nanoclusters $Au_{10}SG_{10}$ on Human Microglia

Assist. Prof. Željka Sanader Maršić
Department of Physics
Faculty of Science
Split

Europska unija - Zajedno do fondova EU.

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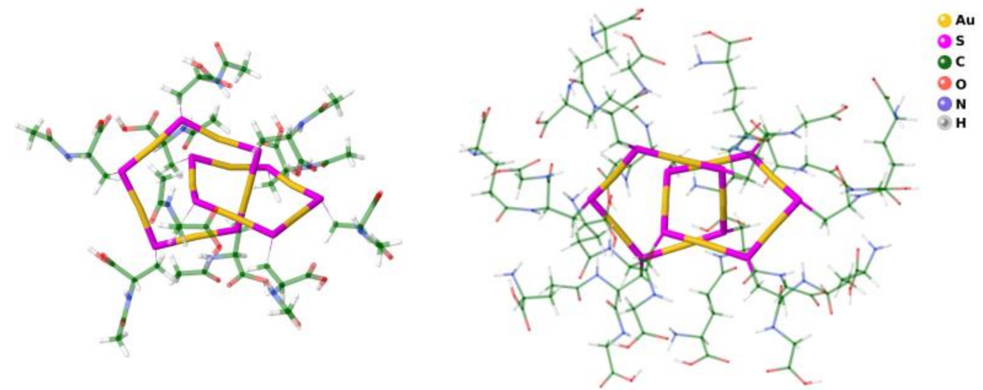
Introduction

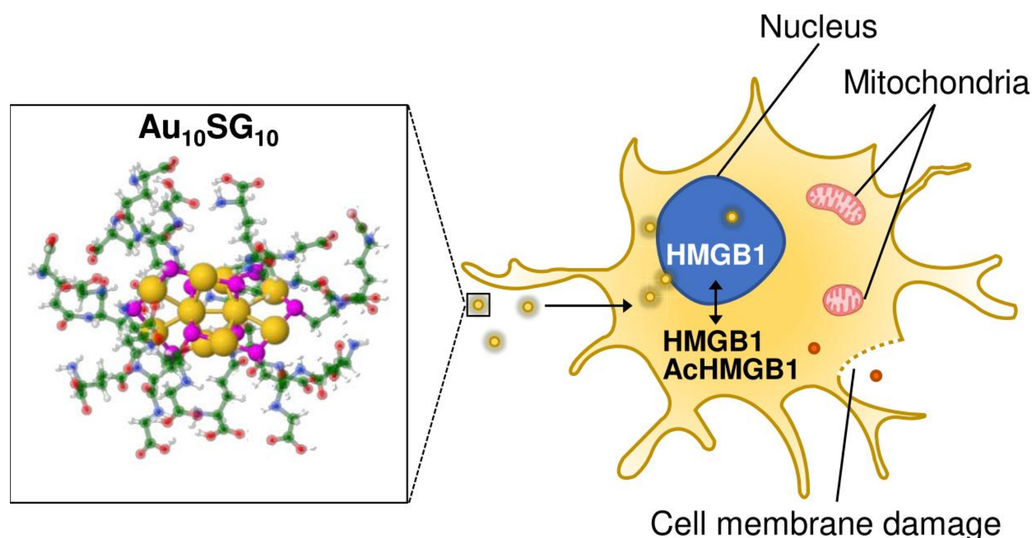


- **Ligated gold nanoclusters (AuNCs)**, in size regime in which **"each atom counts"** with **thiol-containing ligands**, **cysteine** and **glutathione (GSH)** , prevent toxicity and allow different biomedical applications: imaging, detection, and therapy[2-4]
- **Human microglia (part of central nervous system)** are the main surveyors of the **brain** and consumers of the nanostructures

- **HMGB1** is a highly redox-responsive alarmin molecule which changes its cellular location and functions depending on post-translational modifications (PTMs). Considering that AuNCs have catalytic and enzyme-like properties, it was anticipated that $Au_{10}SG_{10}$ could influence PTMs of HMGB1, which determine the intracellular location of the protein.

Introduction

- The following questions will be addressed:
  - **What are the effect of $Au_{10}SG_{10}$ in human microglia in the oxidative stress and does the cluster enhance the oxidative stress?**
  - **Does cluster tretmant translacates HMGB1 ?**
  - **Do $Au_{10}SG_{10}$ NCs interact with HMGB1 in its different PTMs states: (A) reduced, (B) oxidized and (C) acetylated, and if so, what are the predicted sites of these interactions?**



Methods

- **Model of HMGB1 Protein:** The NMR structure of reduced HMGB1 (pdb: 2YRQ) and the oxidized crystal structure of the HMGB1 (pdb: 2RTU) were taken from the Protein Data Base.

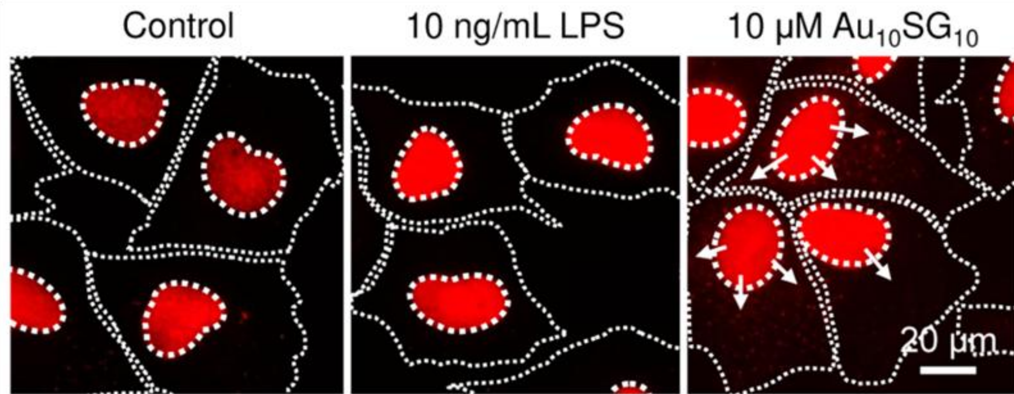
- **$Au_{10}SG_{10}$ Model**. The initial model of $Au_{10}SG_{10}$ was adopted from the crystal structure of $Au_{10}L_{10}$ and $Au_{25}L_{18}$ and reoptimized using PBE and SVP levels of theory.

- **Modeling of the Post-Translational Modifications**.
- Post-translational modifications investigated include oxidation (at Tyr78, Met82, and Tyr85) and acetylation (Lys10, Lys15, and Lys19) ⟶ introduced to the structure of HMGB1 using Chimera[6] and its Structure Editing tool.

- **QM/MM (PM7; UFF)** studies included three different systems: HMGB1 (reduced, oxidized, and acetylated) in proximity to $Au_{10}SG_{10}$. Side chains of PTM residues and $Au_{10}SG_{10}$ form the QM part of the system, and the rest of the protein is in the MM part.

Results:

(A) redHMGB1



Experimental evidence of location of reduced HMGB1 (without cluster, left) in the nuclei, and its increased concentration in nuclei due to higher concentration of $Au_{10}SG_{10}$. (right side figure)

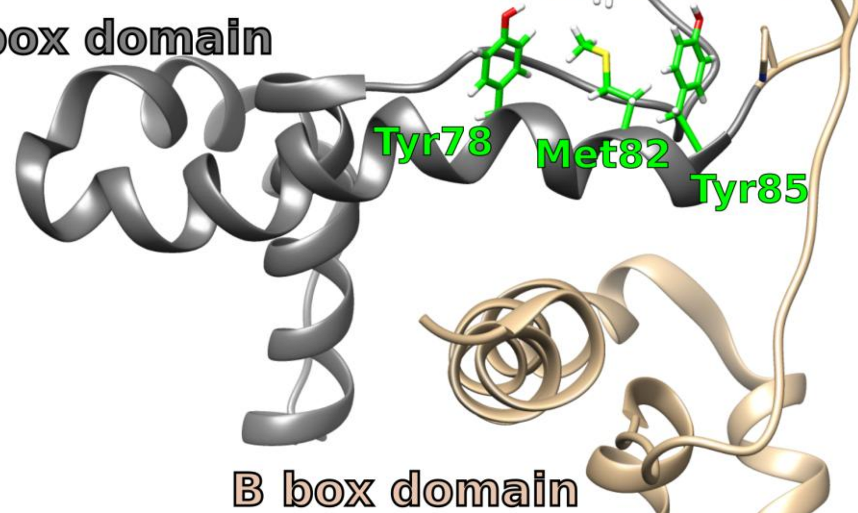
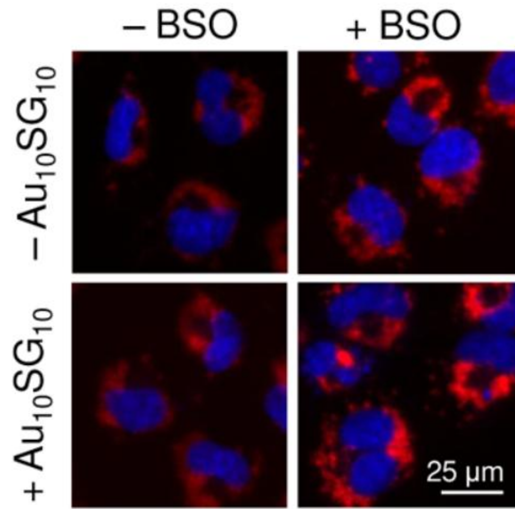


Figure 1. redHMGB1 interacts with **SGH** ligand of the $Au_{10}SG_{10}$.

- Intracts with $Au_{10}SG_{10}$ through functional groups COOH, NH2, C=O and N=H, which are present in the gluthatione ligand
- Formation of hydrogen bonds (with residues Lys19 and Tyr85), ionic (Lys19), hydrophobic (Tyr85) and polar (residues Met78, Tyr85) bonds

(B) oxiHMGB1



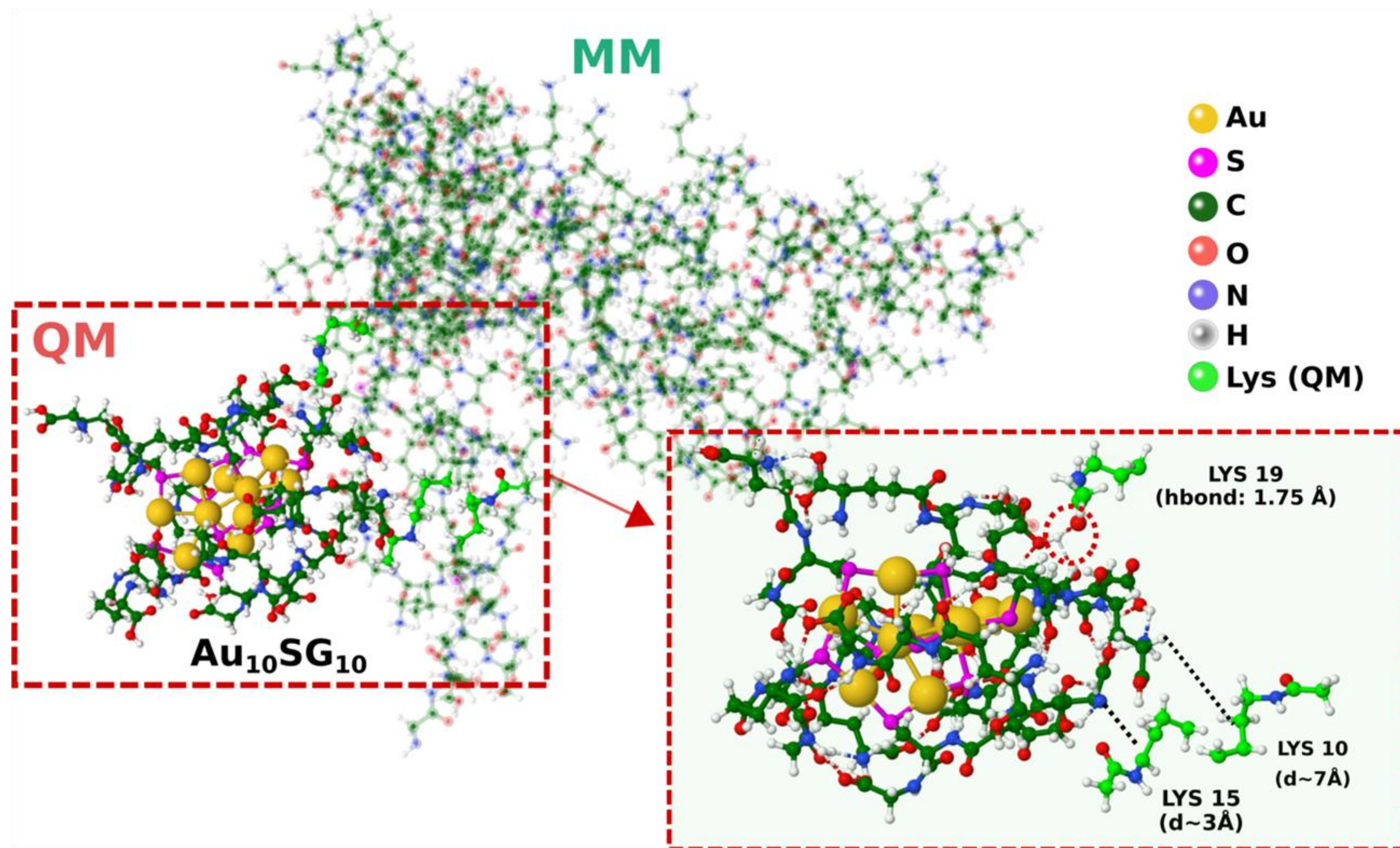
Increase of the oxidative stress in the presence of BSO and $Au_{10}SG_{10}$.

Tyr78
Lys15
Met82
Arg17
disulfide bridge
A box domain

Pro13
Lys15
Arg17
Lys19
Lys72
Lys75
Tyr78
Glu79
Met82

- **Figure 2.** Oxidation induces **a disulfide bridge** between Cys30, at the first α-helix, and Cys52, at the second α-helix show by red stick representation
- The structure of the oxiHMGB1 is more rigid and spatially less exposed to the environment, as shown by the solvent-accessible surface area (SASA) calculation. for oxiHMGB1 is ∼7100 Å2 with respect to redHMGB1 ∼13 200 Å2
- oxiHMGB1 has additional interactions formed with Pro13, Lys72, and Glu79, while Pro83 and Glu87 are no longer included in the interactions.

(C) AcHMGB1



- **Figure 3. Lys10, Lys15, and Lys19 ⟶ low frequency acetylation site of HMGB1**. High frequency acetylation site is not convenient for interaction with $Au_{10}SG_{10}$.
- Both AcHMGB1 and redHMGB1 formed hydrogen bonds (the inset of Figure 3) and ionic and polar interactions with $Au_{10}SG_{10}$.

Conclusions

- The first step in investigating AuNC–HMGB1interactions predicts sites and types of binding between specific positions in $Au_{10}SG_{10}$ and HMGB1 in reduced, oxidized, and acetylated forms.
- (A) **redHMGB1 vs.** (B) **oxiHMGB1 :**
  - **More interactions** (hydrogen, polar, ionic, and hydrophobic bonds) **are present between redHMGB1 than between oxiHMGB1 and $Au_{10}SG_{10}$ according to the statistical analysis.**
  - **increase** in **nuclear** and in **cytosolic** HMGB1 **abundance** in **oxidized** microglia cells has been found
  - the structure of the oxiHMGB1 is more rigid and spatially less exposed to the environment due to formation of **a disulfide bridge**
- (C) **AcHMGB1 not affected by $Au_{10}SG_{10}$**
- The analysis of acetyl groups on Lys15 and Lys19 suggests that these sites are not favorable $Au_{10}SG_{10}$ interactions in the HMGB1 structure.

Conclusions

- HMGB1 isoforms are useful biomarkers for inflammation associated pathologies
- **The aim of the study** was to determine the state of human cells in the central nervous system (e.g. inflamatory processes) by difference in binding of AuNC to different isoforms of HMGB1.

  OxiHMGB1 is more aboundant in inflamated cells.
- highly fluorescent and stable AuNCs with a multishell will be used in follow-up studies for the determination of binding constants in order to study aboundance in inflamation cells.

Collaborations:

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- Université Claude Bernard Lyon 1, France
- McGill University, Montréal, Canada

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Dusica Maysinger, Željka Sanader Maršić, Evan Rizzel Gran, Adeola Shobo, Jun-Ray Macairan, Issan Zhang, Martina Perić Bakulić, Rodolphe Antoine, Gerhard Multhaup and Vlasta Bonačić Kouteckỳ

Thank you!