Atomically Precise Gold Nanoclusters: Towards an Optimal Biocompatible System from a Theoretical–Experimental Strategy

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Potential biomedical applications of gold nanoparticles have increasingly been reported with great promise for diagnosis and therapy of several diseases. However, for such a versatile nanomaterial, the advantages and potential health risks need to be addressed carefully, as the available information about their toxicity is limited and inconsistent. Atomically precise gold nanoclusters (AuNCs) have emerged to overcome this challenge due to their unique features,

such as superior stability, excellent biocompatibility, and efficient renal clearance. Remarkably, the elucidation of their structural and physicochemical properties provided by theory–experiment investigations offers exciting opportunities for site-specific bio-functionalization of the nanoparticle surface, which remains a significant concern for most of the materials in the biomedical field. This Concept highlights the advantages conferred by atomically precise AuNCs for biomedical applications and the powerful strategy combining computational and experimental studies towards finding an optimal biocompatible AuNC-based nanosystem.

1. Introduction

Gold nanoparticles (AuNPs) are one of the most exploited nanomaterials in different fields of science,[1–3] attracting special interest in biomedicine.[4,5] Over the past decade, diverse applications of AuNPs have been mainly focused on drug delivery, imaging techniques, and photothermal therapy.[6] Their high versatility has enabled them to show promise in diagnosis and therapy for several diseases, such as cancers, hepatitis, tuberculosis, and Alzheimer's disease.[7] However, despite the extensive *in vitro* and *in vivo* studies demonstrating the efficacy of a large variety of AuNPs, the *in vivo* toxicity remains a significant problem, directly related to their size and aggregation.[8,9] Either their large size (typically above 50 nm) or the eventual formation of large aggregates (around 20–100 nm) means that the AuNPs cannot be metabolized[10] and are unable to escape the reticuloendothelial system (RES), with the consequent accumulation in some organs, like liver and spleen.[7] Therefore, with the increasing progress of bio-applications, it becomes critical to evaluate the toxicity and biocompatibility, which particularly in the case of AuNPs, is still contradictory.[3]

In the past 10+ years, a singular type of ultra-small AuNPs, so-called gold nanoclusters (AuNCs), has emerged.[11,12] Unlike colloidal and polydisperse gold nanoparticles, AuNCs are composed of a few to some hundreds of gold atoms, which corresponds to NPs smaller than 2 nm metal core diameter with a uniform crystal structure. AuNCs are unique since their structures can be determined to atomic precision by using single crystal X-ray diffraction methods.[11,13,14] This fact has enabled accurate studies of their physicochemical properties, highlighting their atomically precise structure that can bring much more control for binding selectivity, offering a higher precision in the exploration of their potential applications, than what can be achieved with the colloidal noble metal NPs. In biomedicine, they have gained growing interest due to their extraordinary properties such as facile synthesis, superior stability, excellent biocompatibility and efficient renal clearance.[14,15]

Functionalization of their surface takes the AuNCs to the next level allowing them to exert their action at a specific site. However, it also creates one of the main challenges because the chemical and physical properties of AuNCs can be significantly affected by their surface modification.[16] Functionalization also increases the system's complexity due to the high number of atomic and molecular interactions involved, which, together with its small size, hinder its development at the experimental level. This is where the use of computational modeling begins to become particularly important. Computational tools provide useful approaches to addressing several challenges in the design of nanosystems, including the need for a better understanding of nanoparticle behavior and the prediction of different physicochemical properties at the atomic level. The advances in the field of thiolate-protected

AuNCs have been powered by this close interaction of experimental and theoretical studies. To date, several precise structures have been revealed either by X-ray diffraction or theoretically predicted by employing density functional theory (DFT) computation.[17] Combination of theory and experiment has permitted the elucidation of several properties, including electronic structures, luminescence, optical absorption, as well as the structural patterns of their high symmetric cores and their protecting ligand motifs.[17,18] This well-defined structural information offers a particularly attractive chance for a better understanding of the structure–property relationships of these intriguing systems and the unique opportunity for highly-controlled adjustments of specific features in order to increase the desired properties in different applications.

This Concept focuses specifically on the advantages conferred by atomically precise AuNCs for biomedical applications and the powerful strategic approach of using theory and experiment in a complementary manner towards finding an optimal biocompatible AuNC-based nanosystem.

2. The Unique Properties of Ultra-small AuNCs for Biomedical Applications

Potential biomedical applications of any nanosystem are mainly linked to some critical factors, such as size, physicochemical properties and surface chemistry.[19] AuNCs possess a unique combination of these features, which makes them particularly promising in the field of biomedicine. Their ultra-small size favors the renal clearance[20,21] and helps them evade the uptake by the RES.[22] Likewise, their small size and the benefit from the enhanced

permeability and retention (EPR)[23] and nanomaterials-induced endothelial cell leakiness (NanoEL)[24] effects allow exceptional tumor accumulation properties.

Highly stable optical properties are another advantage. AuNCs display molecular-like properties such as discrete electronic transitions, leading to photoluminescence (PL) properties tunable from the ultra-violet (UV) to near-infrared (NIR) region,[25,26] which offers great potential for their detection *in vivo* by multimodal imaging techniques with excellent performance, overcoming difficulties related to PL quenching of larger plasmonic AuNPs.[15]

Proper surface functionalization is also critical for the biocompatibility of the nanosystem. The surface ligands significantly influence the solubility, stability, and determine the interactions of the nanomaterial with the environment.[27] The biocompatibility of the AuNCs can be further improved by taking advantage of their facile surface modification. Several efficient strategies have been developed to synthesize AuNCs protected by thiolate ligands with atomic precision,[14] resulting in water-soluble AuNCs, which are especially desired in applications for biological systems. Atomically precise AuNCs functionalized with other biocompatible molecules offer unique opportunities in theranostics, allowing more control over such sensitive and challenging issues as protein corona formation, *in vivo* biodistribution, and cellular uptake efficiency, to name a few.[28] Considering this huge potential within the field of nanomedicine, investigations using atomically precise, water-soluble, and monodisperse AuNCs, need to be emphasized.

In radiotherapy, which is one of the leading types of cancer treatment, AuNCs with atomically precise structure provide a remarkable opportunity for radiosensitizer investigations (**Figure 1**). Beyond just showing enhancement of the radiotherapeutic activity, they allow to accurately associate radiosensitizer properties with the inner core structure and ligands.[29] For instance, Xie group (2014 and 2015) has developed and studied *in vivo* a series of sub-2 nm glutathione (GSH)-protected AuNCs with these features, such as $Au_{10-12}(GSH)_{10-12}$,[30] $Au_{25}(GSH)_{18}$,[31] and $Au_{29-43}(GSH)_{27-37}$[32] NCs, which showed a ultrahigh tumor uptake and low toxicity. They found that the AuNCs could readily escape from the RES and accumulate in tumors *via* the improved EPR effect, leading to enhanced cancer radiotherapy. In 2019, Jia and coworkers reported a structurally defined alkynyl(levonorgestrel)-protected Au_8 NC as an effective and biocompatible radiosensitizer that causes irreversible apoptosis of tumor cells due to their localized production of reactive oxygen species (ROS).[29] *In vivo* models showed a significant inhibition rate of 74.2 % when tumors are treated with Au_8(levonorgestrel)$_8$ NCs and an X-ray dose of 4 Gray (Gy) compared to tumors irradiated with X-ray alone. Recently, targeting capacity of radiosensitizers for prostate cancer cells has also been reported with promising results. Luo *et al*. (2019) designed Au_{25} NCs protected with a peptide-tagged prostate specific membrane antigen targeting ligand (CY-PSMA-1) with the ability to target PSMA receptor positive cancer cells (PC3pip).[33] The results demonstrated a significantly enhanced tumor-suppressing efficacy of the $Au_{25}S_{(18-m)}$(CY-PSMA-1)$_m$ (m=0–18) NCs *in vivo* when combined with X-ray irradiation (6 Gy) and showed fast renal clearance from the mice body.

Targeting strategies in AuNCs with atomic precise structures has expanded well beyond the cancer nanomedicine field. For example, by replacing GSH ligands with (4-

mercaptobutyl)triphenyl-phosphonium bromide (MTPB) on fluorescent $Au_{18}(GSH)_{14}$ NCs through a ligand exchange method, mitochondrial targeting was achieved.[34] While the unmodified $Au_{18}(GSH)_{14}$ preferably accumulated in lysosomes, the resulting water-soluble $Au_{18}(GSH)_{12}(MTPB)_2$ NCs showed higher accumulation at the mitochondrial site with their initial optical properties largely unaffected. Due to the central role of mitochondria in critical cellular processes such as metabolism and apoptosis, this approach is highly attractive to stimulate extensive studies and potential applications for diagnosis and treatment of several diseases, such as diabetes, obesity, neurodegenerative diseases, and cancers[35] at the cell and organelle levels.

Another growing global threat is antibiotic resistance.[36] Thus, alternatives to conventional antibiotics that address bacterial infections are highly desirable. For several years, the NP activity against multidrug-resistant pathogens has been demonstrated.[37,38] However, potential antimicrobial activity of AuNPs reduced to the NC range remained practically unexplored until 2017 when Zheng and colleagues studied the antibacterial potential of 6-mercaptohexanoic acid (MHA)-protected Au_{25} NCs on both Gram-positive and Gram-negative strains.[39] $Au_{25}(MHA)_{18}$ NCs showed a high wide-spectrum antimicrobial activity, killing more than 90 % of Gram-positive (*Staphylococcus aureus, Staphylococcus epidermis,* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) with a dose of 0.1 mM. They found that $Au_{25}(MHA)_{18}$ NCs could induce a metabolic imbalance in the cells, leading to a significant increase of intracellular ROS production, which confers the bacteria-killing effect. In sharp contrast, the observed antimicrobial effect is absent in the AuNPs counterparts protected with the same ligand. This

study provides a proof of principle for the utilization of AuNCs as an alternative wide-spectrum antimicrobial agent and, at the same time, with potential high biocompatibility in the host human cells due to the noble qualities of gold[40,41] (Figure 1). Even more interesting, AuNCs possess higher structural stability than silver (Ag) NPs and AgNCs,[42] which have led the investigations in this field due to the intrinsic antimicrobial capacity of Ag.[43] Hence, AuNCs could be one of the best candidates for the development of a new generation of antimicrobial agents.

The outstanding role of atomistically well-defined monodisperse AuNCs as contrast agents in biological imaging applications has also been demonstrated through site-specific conjugation to biomolecules[44,45] (Figure 1). AuNCs would allow addressing some fundamental challenges in this field, such as the long-term stability of markers and their biocompatibility for clinical applications,[46] offering distinctive features to the development of more direct and robust tracking strategies *in vitro* and *in vivo*. In 2014, Marjomäki *et al*. reported a site-specific covalent conjugation of water-soluble *para*-mercaptobenzoic acid (*p*MBA)-protected Au_{102} NCs, functionalized by a thiol-reactive linker (*N*-(6-hydroxyhexyl)maleimide) to target cysteine sites of capsid proteins in echovirus 1 (EV1) and Coxsackievirus B3 (CVB3) without compromising their infectivity.[44] The well-defined structures of both maleimide functionalized-$Au_{102}(p\text{MBA})_{44}$ NCs and viruses capsid allowed analyzing the specific spatial ordering of enterovirus−cluster conjugates in both virus types through transmission electron microscopy (TEM). One year later and taking advantage of this strategy, $Au_{102}(p\text{MBA})_{44}$ NCs were utilized for noncovalent linking to the capsid of EV1 and CVA9 enteroviruses.[45] AuNCs were conjugated to a Pleconaril drug-like molecule, which was used to target site-specifically the hydrophobic pocket of the virus capsid,

enabling the visualization of the viruses *via* TEM while retaining their infectivity. These approaches may be extended not only for structural studies of viral uncoating, but also for revealing more fundamentals of virus and other biomolecules entry pathways into cells. In this context, a AuNC-labeled fibroblast growth factor 21 (FGF21) tracking technique was reported in human primary adipocytes.[47] By using *meta*-mercaptobenzoic acid (3MBA)-protected Au_{144} NCs conjugated with FGF21 and cryo-electron tomography (cryo-ET), Azubel and coworkers (2019) were able to capture different states of activation, internalization, and traffic of the FGF21/FGFR1c/β-Klotho (FGF-receptor-cofactor) complex demonstrating its clathrin-dependent pathway endocytosis.[47] The paired or unpaired FGF21 functionalized-$Au_{144}(3MBA)_{\sim 40}$ NCs distribution in different vesicular compartments helped to confirm the overall 2:2:2 stoichiometry of the ternary complex in the human fat cells and observe its disruption at specific levels in the pathway. This is the first study in which, through $Au_{144}(3MBA)_{\sim 40}$ NCs labeling, a three-dimensional picture of the entire endocytosis pathway is achieved.

The photoluminescence of AuNCs is another significant feature that makes them a promising agent for biological imaging and medical diagnosis.[48] In a recent study, Liu *et al.* (2019) demonstrated the potential of water-soluble $Au_{25}(GSH)_{18}$ NCs as an efficient near-infrared II (NIR-II) fluorophore in *in vivo* models.[49] $Au_{25}(GSH)_{18}$ NCs can emit fluorescence at 1100–1350 nm and showed unique features of particular relevance for biological and clinical applications, such as high quantum yield (QY) that can be further increased by metal-atom doping, and efficient renal clearance even at an ultrahigh dose of 100 mg kg^{-1}. It is noteworthy that most of the nanomaterials (inorganic and organic) that have shown promising features as agents for bioimaging meet only one of these requirements.[49] Thus, it is time to expand the investigations of AuNCs-based agents in this field, but now including comparisons with

more promising and emerging nanostructures, such as palladium (Pd)-based nanomaterials.[50] Interestingly, Pd nanosheets (16 nm) were reported to have high photothermal efficiency and showed superior stability, measured against AuNPs (nanorods).[51] However, these findings might substantively differ when the point of comparison is AuNCs instead, due to their exceptional features described above, which can only be obtained when the size of NPs is reduced to the NC range.

3. Manipulation of the System at Atomic Level: In Pursuit of the Most Realistic Theoretical Model

Clearly, the extraordinary properties that atomically precise AuNCs have exhibited in experimental research, place them as one of the most promising nanomaterials within the field of biomedicine. However, in order to extend their applications and achieve their clinical translation, some current fundamental challenges need to be considered and understood in more detail, such as improving the PL quantum yield, increasing the yield of the synthesis, and precisely controlling the surface modification.[16] To achieve this purpose, an interdisciplinary strategy becomes critical. The use of theoretical models either as a predictive tool or as complementary to experimental work could provide crucial information for the design of optimal AuNC-based biocompatible systems, including their geometric structure, electronic structure, reaction mechanisms, structure–activity relationship as well as the effects of the surrounding environment (**Figure 2**).

DFT calculations have been extensively used and synergistically combined with experiments for AuNCs characterization, showing an outstanding performance in their structure and properties elucidation.[52] By this method, some aspects like general structures, core-shell geometries, and isomerization mechanisms of diverse atomically precise AuNCs have been predicted with high accuracy.[17,53,54] Furthermore, DFT has supported the development of a molecular mechanics force field for thiolate-protected AuNCs compatible with the well-known biomolecular force field AMBER.[55] This allows investigating the dynamics of the AuNCs in a more realistic biological environment (i.e., including solvent, counterions) by employing classical molecular dynamics (MD) simulations. Combined with the existing biomolecular force fields in AMBER, interactions between the gold clusters and biomolecules, such as proteins, cell membranes, or nucleic acids can be explored in long time scales (in principle, from hundreds of nanoseconds to microsecond by using supercomputers) with an all-atom description. The precise atomic structure of AuNCs is crucial in the construction of these theoretical models and particularly relevant for investigations of the bio–nano interface and addressing the challenge of protein corona formation in an exhaustive way. Correlation of these theoretical predictions with experimental data from methods like X-ray diffraction and Ultraviolet-visible (UV-vis) spectroscopy offers the possibility to determine which features of the AuNC can be adapted to increase the desired properties.

Thiolate-for-thiolate ligand exchange reaction is a well-established technique and widely used for surface modification of monolayer-protected AuNCs.[56–58] Although this strategy had been utilized for many years since its origin, it was not until 2012 when Heinecke and colleagues reported the structural basis of this strategy by employing a computational-

experimental approach.[59] They used *p*-bromobenzene thiol (*p*BBT) as the incoming ligand and performed the exchange reaction to obtain the first single-crystal X-ray structure of a partially exchanged $Au_{102}(pMBA)_{40}(pBBT)_4$ NC. They studied the regioselectivity of the reaction based on the 22 symmetry-unique *p*MBA ligand sites of $Au_{102}(pMBA)_{44}$ and postulated the mechanistic pathway for the reaction by DFT calculations. Ligand place-exchange was exhibited in 2 of the 22 symmetrically unique ligand sites, each of them bonded to a different solvent-exposed gold atom. Together with a complete reaction path modeling both thiol and thiolate incoming ligands by DFT, the authors suggested an associative exchange mechanism. Following the same line, Rojas-Cervellera *et al*. (2017) studied the molecular mechanism of ligand exchange reaction involving AuNCs and proteins by combining classical and *ab initio* quantum mechanics/molecular mechanics (QM/MM) MD simulations.[60] Inspired by the experimental work of Ackerson group (2006), they modeled the conjugation of $Au_{25}(GSH)_{18}^-$ NC with a single chain Fv (scFv) fragment of the NC10 antibody in water in order to obtain an atomistic description of this reaction.[61] Two particular sites in the Au_{25} V-shaped staple were considered (side thiolate and apex thiolate) and thus, two different routes were explored to determine which one is more readily exchanged. Calculations showed that the reaction follows an associative S_N2-like reaction mechanism and the substitution of the side ligand is favored over the apex one, which interestingly, is in high accordance with the study described above using $Au_{102}(pMBA)_{40}(pBBT)_4$ NCs.[59] The results also suggested that the presence of positively charged residues surrounding the incoming ligand facilitates the adsorption of the protein on the AuNC surface. These approaches are attractive for site-selective modification of AuNCs displaying desired properties for stable and functional AuNC-biomolecule conjugates.

In 2016, Salorinne *et al*. reported a complete ^{1}H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts assignment for all ligands of the atomically precise $Au_{102}(pMBA)_{44}$ NC in water by employing a combination of multidimensional NMR methods, DFT calculations, and MD simulations.[62] By using these computational techniques, it was possible to obtain a precise structural and dynamical information of the ligand shell and correlate it with ligand symmetry environments observed in the single-crystal X-ray structure.[63] This study is particularly attractive for accurate functionalization strategies and gives the potential to control and understand in more detail the bio–nano interface and the transformations that AuNCs undergo in the biological environment.

Another interdisciplinary study was carried out in 2017, where the atomic structure and dynamics of the ligand layer of $Au_{68}(3MBA)_{\sim 32}$ and $Au_{144}(3MBA)_{\sim 40}$ NCs was revealed.[64] By a combination of MD simulations and DFT calculations, and supported by NMR, UV-vis absorption, and Infrared (IR) spectroscopy the authors suggested a distinct chemistry in the ligand−metal interface, which is absent in other known thiol-stabilized AuNCs. Interestingly, they found weak π–Au and O=C–OH···Au interactions protecting the metal core in addition to the covalent S–Au bond formation. This finding could support some particular features observed previously in 3MBA-protected AuNCs, such as their reactivity to thiol-modified DNA and proteins[65] and the unusually low ligand coverage in the $Au_{144}(3MBA)_{\sim 40}$ NC.[66] In addition to explaining or validating experimental evidence, this finding offers new possibilities for the development of novel hybrid nanosystems based on 3MBA-protected AuNCs (e.g., as drug delivery systems, biosensors) and understand their functionalization *via* a new and characteristic route.

Motivated by the ability of AuNCs as biocompatible contrast agents in virus imaging demonstrated experimentally and described in the previous section,[45] Pohjolainen and coworkers (2017) performed a series of all-atom MD simulations combined with non-equilibrium free energy calculations to study in detail the effect of $Au_{102}(pMBA)_{44}$ NCs on the binding affinity of the AuNC-linked pocket factor–virus complex.[67] Half- and fully deprotonated states of *p*MBA ligand layer were considered and included in a complete system containing the full EV1 virus and the hydrophobic pocket factors in water, making it able to obtain molecule-scale structural information and complement the experimental findings. They found that the binding affinity of AuNC-linked pocket factor–virus is pH-sensitive. Specifically, the affinity of this complex (before AuNC labeling) is unaffected when fully deprotonated $Au_{102}(pMBA)_{44}$ NCs are conjugated to the pocket factor, whereas the half deprotonated state induces a higher binding affinity, but more importantly, in none of two conditions a decrease in the affinity is observed. This theoretical approach exhibited results that agree qualitatively with the experiments[45] and represents a useful methodology that, combined with tools for drug design like virtual screening, could be used for designing new biomarkers and optimal strategies for virus-imaging applications.

4. Conclusion and Future Perspectives

It is clear that atomically precise AuNCs have helped to revolutionize the field of nanomedicine due to their good biocompatibility and extraordinary physicochemical properties. Although the development of AuNCs-based strategies is still in its infancy, their optical features together with low toxicity demonstrated in varied applications, make them

promising to overcome challenges like biostability and cytotoxicity that remain pending for most of the nanomaterials. Notably, the increasingly detailed knowledge of the structural and physicochemical properties provided by the close connection between theoretical and experimental studies, offers exciting opportunities for site-specific bio-functionalization of the nanoparticle surface. Understanding the details of the internal structure and stability of the ligand shell at atomic level, which is particularly well-defined in these systems, allows accurate control of the potential surface functional groups. Thus, their precise atomic structure provides remarkable advantages for potential applications in drug delivery, biomolecule labelling and targeting. Recent advances in force fields will allow exploration of the interaction of the engineered AuNCs with the desired environment *via* atomistic simulations as well as gaining more insight on the nano–bio interface and protein corona complex, which play a pivotal role in the cellular uptake efficiency and biodistribution of the nanomaterials throughout the body.

Another aspect that needs to be addressed is the high-yield synthesis in order to contribute with new affordable strategies compared to conventional methods, as well as the improvement of their fluorescence QY with the aim to enhance their use as fluorescence imaging agents.

Undoubtedly, further exploration of biocompatible AuNC-based nanosystems must be accompanied by theory–experiment investigations for driving this field forward and ideally, to achieve their clinical translation in the near future.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] S. E. Skrabalak, J. Chen, Y. Sun, X. Lu, L. Au, C. M. Cobley, Y. Xia, *Acc. Chem. Res.* **2008**, *41*, 1587.

[2] M. Homberger, U. Simon, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2010**, *368*, 1405.

[3] P. Singh, S. Pandit, V. Mokkapati, A. Garg, V. Ravikumar, I. Mijakovic, *Int. J. Mol. Sci.* **2018**, *19*, 1979.

[4] Y.-C. Yeh, B. Creran, V. M. Rotello, *Nanoscale* **2012**, *4*, 1871.

[5] P. Chhour, P. C. Naha, R. Cheheltani, B. Benardo, S. Mian, D. P. Cormode, in *Nanomater. Pharmacol.*, Springer, **2016**, pp. 87–111.

[6] H. Daraee, A. Eatemadi, E. Abbasi, S. Fekri Aval, M. Kouhi, A. Akbarzadeh, *Artif. cells, nanomedicine, Biotechnol.* **2016**, *44*, 410.

[7] O. B. Adewale, H. Davids, L. Cairncross, S. Roux, *Int. J. Toxicol.* **2019**, *38*, 357.

[8] C. M. Goodman, C. D. McCusker, T. Yilmaz, V. M. Rotello, *Bioconjug. Chem.* **2004**, *15*, 897.

[9] Y. Pan, S. Neuss, A. Leifert, M. Fischler, F. Wen, U. Simon, G. Schmid, W. Brandau, W. Jahnen- Dechent, *Small* **2007**, *3*, 1941.

[10] J. F. Hainfeld, D. N. Slatkin, H. M. Smilowitz, *Phys. Med. Biol.* **2004**, *49*, N309.

[11] R. Jin, C. Zeng, M. Zhou, Y. Chen, *Chem. Rev.* **2016**, *116*, 10346.

[12] I. Chakraborty, T. Pradeep, *Chem. Rev.* **2017**, *117*, 8208.

[13] H. Häkkinen, *From gold nanoparticles physics, Chem. Biol.* **2012**, *9*, 233.

[14] T. Tsukuda, H. Häkkinen, *Protected Metal Clusters: From Fundamentals to Applications*, Elsevier, **2015**.

[15] E. Porret, X. Le Guével, J.-L. Coll, *J. Mater. Chem. B* **2020**, *8*, 2216.

[16] N. Kaur, R. N. Aditya, A. Singh, T.-R. Kuo, *Nanoscale Res. Lett.* **2018**, *13*, 302.

[17] Z. Ma, P. Wang, L. Xiong, Y. Pei, *Wiley Interdiscip. Rev. Comput. Mol. Sci.* **2017**, *7*, e1315.

[18] H. Häkkinen, *Nat. Chem.* **2012**, *4*, 443.

[19] S. Naahidi, M. Jafari, F. Edalat, K. Raymond, A. Khademhosseini, P. Chen, *J. Control. release* **2013**, *166*, 182.

[20] X.-D. Zhang, D. Wu, X. Shen, P.-X. Liu, F.-Y. Fan, S.-J. Fan, *Biomaterials* **2012**, *33*, 4628.

[21] C. N. Loynachan, A. P. Soleimany, J. S. Dudani, Y. Lin, A. Najer, A. Bekdemir, Q.

Chen, S. N. Bhatia, M. M. Stevens, *Nat. Nanotechnol.* **2019**, *14*, 883.

[22] A. K. Iyer, G. Khaled, J. Fang, H. Maeda, *Drug Discov. Today* **2006**, *11*, 812.

[23] R. A. Petros, J. M. DeSimone, *Nat. Rev. Drug Discov.* **2010**, *9*, 615.

[24] M. I. Setyawati, C. Y. Tay, B. H. Bay, D. T. Leong, *ACS Nano* **2017**, *11*, 5020.

[25] K. L. D. M. Weerawardene, E. B. Guidez, C. M. Aikens, *J. Phys. Chem. C* **2017**, *121*, 15416.

[26] H. Yu, B. Rao, W. Jiang, S. Yang, M. Zhu, *Coord. Chem. Rev.* **2019**, *378*, 595.

[27] R. Mout, D. F. Moyano, S. Rana, V. M. Rotello, *Chem. Soc. Rev.* **2012**, *41*, 2539.

[28] C. Auría-Soro, T. Nesma, P. Juanes-Velasco, A. Landeira-Viñuela, H. Fidalgo-Gomez, V. Acebes-Fernandez, R. Gongora, M. J. Almendral Parra, R. Manzano-Roman, M. Fuentes, *Nanomaterials* **2019**, *9*, 1365.

[29] T.-T. Jia, G. Yang, S.-J. Mo, Z.-Y. Wang, B.-J. Li, W. Ma, Y.-X. Guo, X. Chen, X. Zhao, J.-Q. Liu, *ACS Nano* **2019**, *13*, 8320.

[30] X. Zhang, Z. Luo, J. Chen, X. Shen, S. Song, Y. Sun, S. Fan, F. Fan, D. T. Leong, J. Xie, *Adv. Mater.* **2014**, *26*, 4565.

[31] X. Zhang, J. Chen, Z. Luo, D. Wu, X. Shen, S. Song, Y. Sun, P. Liu, J. Zhao, S. Huo, *Adv. Healthc. Mater.* **2014**, *3*, 133.

[32] X.-D. Zhang, Z. Luo, J. Chen, S. Song, X. Yuan, X. Shen, H. Wang, Y. Sun, K. Gao, L. Zhang, *Sci. Rep.* **2015**, *5*, 8669.

[33] D. Luo, X. Wang, S. Zeng, G. Ramamurthy, C. Burda, J. P. Basilion, *Small* **2019**,

15, 1900968.

[34] Y. Yang, S. Wang, S. Chen, Y. Shen, M. Zhu, *Chem. Commun.* **2018**, *54*, 9222.

[35] L. Milane, M. Trivedi, A. Singh, M. Talekar, M. Amiji, *J. Control. release* **2015**, *207*, 40.

[36] E. Peterson, P. Kaur, *Front. Microbiol.* **2018**, *9*, 2928.

[37] P. V Baptista, M. P. McCusker, A. Carvalho, D. A. Ferreira, N. M. Mohan, M. Martins, A. R. Fernandes, *Front. Microbiol.* **2018**, *9*, 1441.

[38] N.-Y. Lee, P.-R. Hsueh, W.-C. Ko, *Front. Pharmacol.* **2019**, *10*, 1153.

[39] K. Zheng, M. I. Setyawati, D. T. Leong, J. Xie, *ACS Nano* **2017**, *11*, 6904.

[40] B. Hammer, J. K. Norskov, *Nature* **1995**, *376*, 238.

[41] N. Lewinski, V. Colvin, R. Drezek, *small* **2008**, *4*, 26.

[42] J. Tang, H. Shi, G. Ma, L. Luo, Z. Tang, *Front. Bioeng. Biotechnol.* **2020**, *8*, 1019.

[43] L. P. Silva, A. P. Silveira, C. C. Bonatto, I. G. Reis, P. V Milreu, in *Nanostructures Antimicrob. Ther.*, Elsevier, **2017**, pp. 577–596.

[44] V. Marjomäki, T. Lahtinen, M. Martikainen, J. Koivisto, S. Malola, K. Salorinne, M. Pettersson, H. Häkkinen, *Proc. Natl. Acad. Sci.* **2014**, *111*, 1277.

[45] M. Martikainen, K. Salorinne, T. Lahtinen, S. Malola, P. Permi, H. Häkkinen, V. Marjomäki, *Nanoscale* **2015**, *7*, 17457.

[46] Y. Yang, L. Wang, B. Wan, Y. Gu, *Front. Bioeng. Biotechnol.* **2019**, *7*, 320.

[47] M. Azubel, S. D. Carter, J. Weiszmann, J. Zhang, G. J. Jensen, Y. Li, R. D.

Kornberg, *Elife* **2019**, *8*, e43146.

[48] L. Shang, S. Dong, G. U. Nienhaus, *Nano Today* **2011**, *6*, 401.

[49] H. Liu, G. Hong, Z. Luo, J. Chen, J. Chang, M. Gong, H. He, J. Yang, X. Yuan, L. Li, *Adv. Mater.* **2019**, *31*, 1901015.

[50] Y. Liu, J. Li, M. Chen, X. Chen, N. Zheng, *Theranostics* **2020**, *10*, 10057.

[51] L. Nie, M. Chen, X. Sun, P. Rong, N. Zheng, X. Chen, *Nanoscale* **2014**, *6*, 1271.

[52] K. L. D. M. Weerawardene, H. Häkkinen, C. M. Aikens, *Annu. Rev. Phys. Chem.* **2018**, *69*.

[53] S. Malola, H. Häkkinen, *J. Am. Chem. Soc.* **2019**, *141*, 6006.

[54] M. F. Matus, S. Malola, E. K. Bonilla, B. Barngrover, C. M. Aikens, H. Häkkinen, *Chem. Commun.* **2020**, *56*, 8087.

[55] E. Pohjolainen, X. Chen, S. Malola, G. Groenhof, H. Häkkinen, *J. Chem. Theory Comput.* **2016**, *12*, 1342.

[56] M. J. Hostetler, A. C. Templeton, R. W. Murray, *Langmuir* **1999**, *15*, 3782.

[57] R. Guo, Y. Song, G. Wang, R. W. Murray, *J. Am. Chem. Soc.* **2005**, *127*, 2752.

[58] M. Montalti, L. Prodi, N. Zaccheroni, R. Baxter, G. Teobaldi, F. Zerbetto, *Langmuir* **2003**, *19*, 5172.

[59] C. L. Heinecke, T. W. Ni, S. Malola, V. Mäkinen, O. A. Wong, H. Häkkinen, C. J. Ackerson, *J. Am. Chem. Soc.* **2012**, *134*, 13316.

[60] V. Rojas-Cervellera, L. Raich, J. Akola, C. Rovira, *Nanoscale* **2017**, *9*, 3121.

[61] C. J. Ackerson, P. D. Jadzinsky, G. J. Jensen, R. D. Kornberg, *J. Am. Chem. Soc.* **2006**, *128*, 2635.

[62] K. Salorinne, S. Malola, O. A. Wong, C. D. Rithner, X. Chen, C. J. Ackerson, H. Häkkinen, *Nat. Commun.* **2016**, *7*, 1.

[63] P. D. Jadzinsky, G. Calero, C. J. Ackerson, D. A. Bushnell, R. D. Kornberg, *Science (80-.).* **2007**, *318*, 430.

[64] T.-R. Tero, S. Malola, B. Koncz, E. Pohjolainen, S. Lautala, S. Mustalahti, P. Permi, G. Groenhof, M. Pettersson, H. Häkkinen, *ACS Nano* **2017**, *11*, 11872.

[65] M. Azubel, R. D. Kornberg, *Nano Lett.* **2016**, *16*, 3348.

[66] M. Azubel, A. L. Koh, K. Koyasu, T. Tsukuda, R. D. Kornberg, *ACS Nano* **2017**, *11*, 11866.

[67] E. Pohjolainen, S. Malola, G. Groenhof, H. Häkkinen, *Bioconjug. Chem.* **2017**, *28*, 2327.

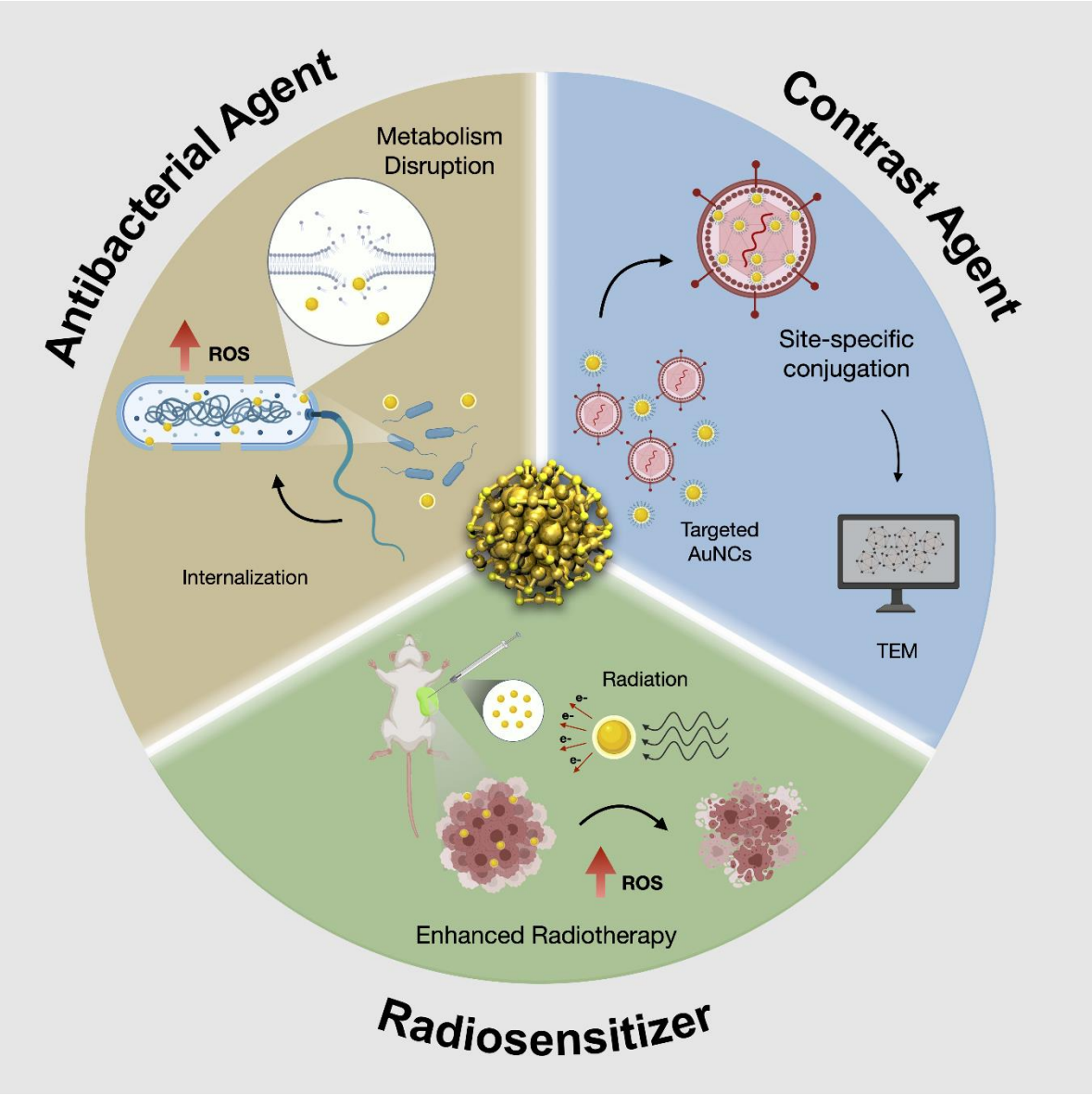


Figure 1. Representative examples of biomedical applications reported by atomically-precise AuNCs: Antimicrobial applications, biological imaging, and radiation therapy. ROS: Reactive Oxygen Species; TEM: Transmission Electron Microscopy.

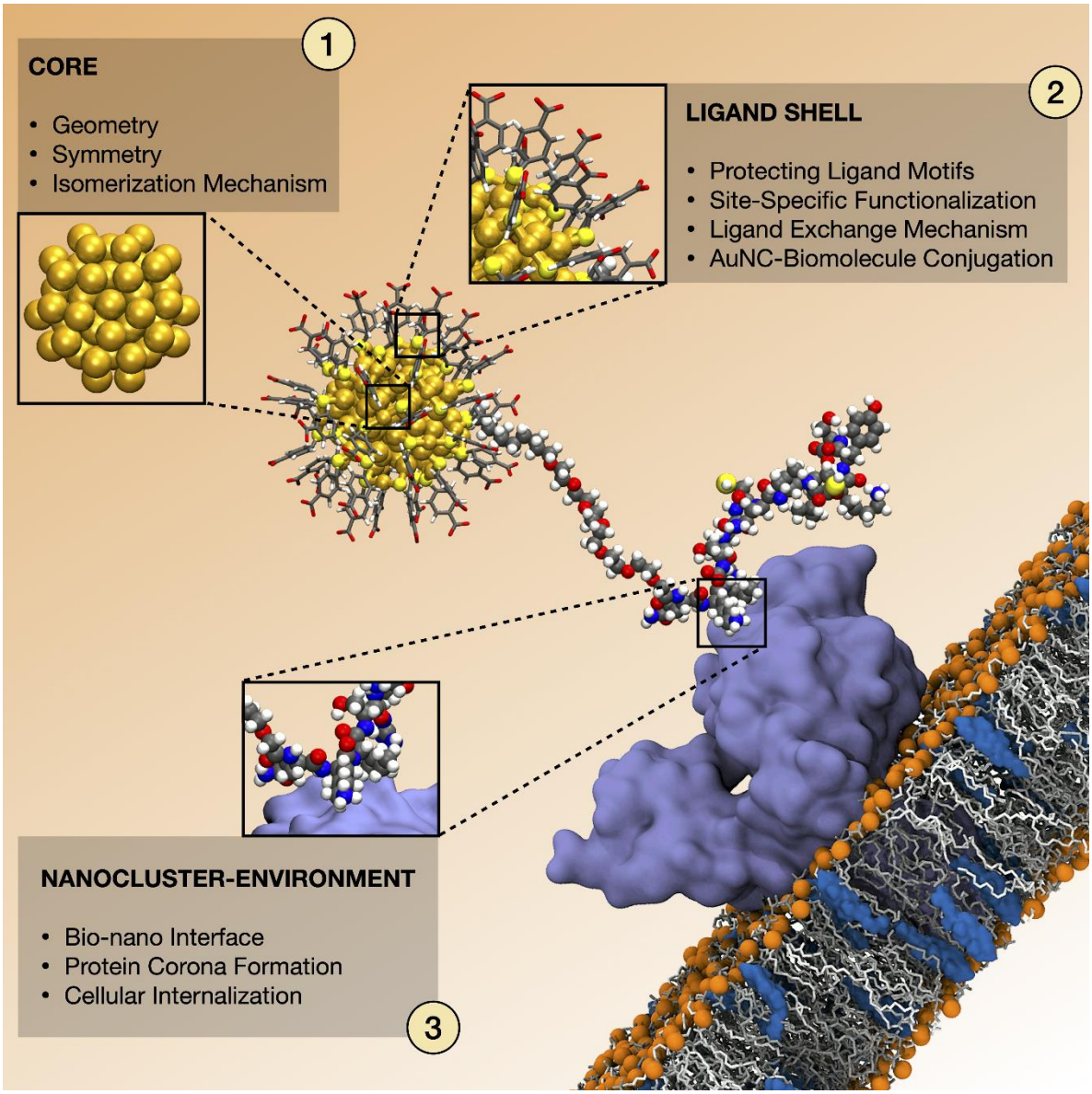
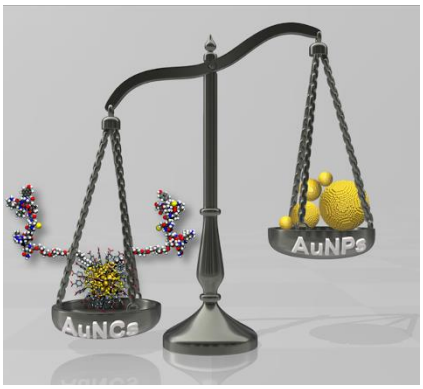


Figure 2. Schematic illustration showing the main aspects that can be precisely explored in the design of biocompatible gold nanoclusters (AuNCs)-based systems by employing a theoretical–experimental approach. A partially exchanged $Au_{102}(p\text{MBA})_{43}(\text{peptide})_1$ nanocluster is depicted near a typical bilayer of phospholipids. Different physicochemical properties of AuNCs can be either predicted or revealed by a combination of theory (quantum mechanics or molecular mechanics methods) and experimental studies at three levels: 1) core, 2) ligand shell, and 3) nanocluster-environment interface.

ToC Figure



Atomically precise gold nanoclusters (AuNCs) have helped revolutionize nanomedicine's field due to their excellent biocompatibility and extraordinary physicochemical properties. They have emerged as promising materials to overcome the current challenges of cytotoxicity and biostability present in colloidal gold nanoparticles. Although this revolution is still in its infancy, theoretical/experimental strategies show that the near future is promising!

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