Review

N-Heterocyclic carbenes as “smart” gold nanoparticle stabilizers: State-of-the art and perspectives for biomedical applications

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\textbf{Abstract}

The unique properties and high synthetic flexibility of N-heterocyclic carbenes (NHCs) have made them highly attractive tools for the development of new nanomaterials and the fundamental study of their properties. In this review, we focus on the case of NHC-stabilized gold nanoparticles (NHC@AuNPs) with potential for biological applications. AuNPs are ubiquitous in biomedicine, where they serve as versatile scaffolds for drug/gene delivery, biosensing, imaging and therapy. In this context, our review aims at presenting an overview of the relatively few studies reporting on the synthesis and characterization of NHC@AuNPs, with emphasis on the strategies adopted to achieve water-soluble biocompatible nanoparticles. Overall, the possible combinatorial design of NHC ligand shell functionality opens to new perspectives for this relatively unexplored research area.

1. Introduction

Gold nanoparticles (AuNPs) have attracted much attention over the last few decades in the fields of materials, \cite{1,2} catalysis \cite{3} and medicine, \cite{4–6} also due to their optical properties, \cite{7,8} biocompatibility and well-established synthetic protocols to control their size and morphology. \cite{9} In such nano-assemblies, the size of the aggregates and interparticle distances within the structure govern the final collective properties of the resulting materials. For biomedical applications, these variations in morphology, size, and functionality are directly correlated to the AuNPs biodistribution, biological half-life, renal secretion, cellular internalization, and plasmon optical properties. \cite{10–13} Because of the latter, gold nanoparticles are inherently theranostic, providing imaging contrast through near infrared (NIR) fluorescence, X-ray, \cite{14,15} photoacoustic, \cite{16,17} and Raman enhancement, \cite{18} and act as a vehicle to enhance ablative therapies both for photothermal and X-ray radiation. \cite{19–23} In recent years, AuNPs have also been used as nanocarriers for transition metal catalysts for bio-orthogonal transformations in cells. \cite{24}

One major challenge in the field of nanomaterial design is to achieve sufficient metal nanoparticle stability for the desired application. In fact, 'stability' is a broad concept, describing the preservation of a particular nanostructure property, ranging from aggregation, composition, crystallinity, shape, size, and surface chemistry. \cite{25} In practice, the definition of nanoparticle stability depends on the targeted size-dependent property that is pursued and can only exist for a finite period of time given all nanostructures are inherently thermodynamically and energetically unfavorable relative to the respective bulk states. Over the years, in order to enhance the AuNPs' stability and to tune their surface properties, a number of capping ligands have been developed, through which colloidal stabilization originates from a combination of electrostatic repulsion and steric isolation. Several amongst such capping agents feature sulfur containing groups with high binding affinity to 'soft' Au surfaces. Thus, a variety of ligands have been employed to functionalize metallic gold, particularly sulfur-based compounds like thiols, sulfides or disulfides, with high packing density. \cite{26} In 1994, Brust et al. \cite{27} reported the first example of thiol stabilized AuNPs using the alkanethiol dodecanethiol, ranging between 1 and 3 nm in size, which remained a popular synthetic choice over the years. Later on, in a seminal paper, Rotello and coworkers developed water soluble amphiphilic AuNPs capped with octanethiol/11-thioundecanoic acid, whose assembly was controlled by pH. \cite{28}

Despite the numerous examples, the S–Au bond is not strong enough to confer long-term stability to the derived materials. Therefore, N-heterocyclic carbenes (NHCs) have emerged as surface ligands for metal NPs due to their ability to form strong covalent bonds to metallic surfaces. \cite{29} Another advantage of using NHCs...
as ligands is the possibility of modification of the N-heterocyclic scaffold with different functional groups affording a tunable surface, as it will be discussed in the next sections.

2. Properties of N-heterocyclic carbene ligands

Following the isolation of the stable carbene (1,3-bis(adamantyl)imidazol-2-ylidene) (IAd, Fig. 1A) in 1991, [30] N-heterocyclic carbenes have gained significant attention due to their great stability and ease of derivatization, allowing a broad library of NHCs to be produced within a relatively short period of time. [31–33] The \( \sigma \) -electron-withdrawing and \( \pi \) -electron-donating nitrogen atoms next to the carbene carbon stabilize the NHC by lowering the energy of the occupied \( \sigma \) -orbital and increasing the electron density in the empty p orbital. Furthermore, the cyclic NHC structure confers additional stabilization to the singlet sp\(^2\) hybridized carbene state (Fig. 1B).

The formally divalent carbon species features several advantages, such as straightforward synthesis and robustness towards functionalization enabling structural diversity and catalytic activity. [31,32,34] Currently, NHCs are privileged ligands for a wide range of main group, transition metal, and f-block species, [35,36] being highly modular in nature leading to their large structural and stereoelectronic diversity. NHCs are prepared most commonly from (benz)imidazolylidenes, as well as from pyrazolylidene, triazolylidene, tetrazolylidene, thiazolylidene, oxadiazolylidenes and oxazolylidenes scaffolds or from their so-called ‘abnormal’ zwitterionic counterparts. The majority of NHCs feature a five-membered ring scaffold, although a number of ‘ring-expanded’ analogues based on tetrahydropyrimidine, triazine, diazepane and diazocane rings have also been reported. [31] Furthermore, cyclic (alkyl)(amino)carbenes (CAACs) were discovered in 2005 by Bertrand and coworkers, [37] differing electronic properties due to the substitution of one of the \( \pi \) -electron donating amino groups with a \( \sigma \) -donating alkyl group. This results in CAACs being more nucleophilic (\( \sigma \) -electron donating) and more electrophilic (\( \pi \) -electron accepting) than ‘classical’ NHCs. [38]

The neutral, electron-rich NHCs form a strong covalent bond with metallic surfaces, which is the key to the stabilization of metal NPs, enabling them to maintain their size-dependent properties. [39] Although NHC-stabilized mono/oligo-atomic elemental species have been synthesized and characterized as early as 1994, [40] the first evidence for NHC–metal NP interactions appeared in 2005 and 2007. [41,42] Since then a range of different NHC-functionalized metal NPs (NHC@MNPs) have been synthesized.

To fine-tune the strength of the metal coordination environment and therefore, the stability of the NHC-metal fragment, derivatization of the backbone scaffold has important influence, as well as modifications of the wingtip position (Fig. 1C). The latter can stericly affect the carbene-metal bond favoring stability of the resulting metal complexes, as well as preventing aggregation in the case of NHC@MNPs. [39] Moreover, wingtip-derivatization can increase water solubility [43] or be exploited to generate surface-bound initiators for catalytic applications. [44] The broad structural variety of NHCs contrasts with thiol ligands, whose interfa-

![Fig. 1](image1.png)

**Fig. 1.** (A) Structure of IAd (1,3-bis(adamantyl)imidazol-2-ylidene); (B) Representation of singlet ground state electronic structure of imidazol-2-ylidene-type NHC; (C) General structure of Au(I) NHC complexes and possible modifications.

3. NHC stabilized gold nanoparticles

Two main approaches have been applied for the synthesis of gold NPs stabilized by NHC ligands (NHC@AuNPs): i) the ‘top-down’ and ii) the ‘bottom-up’ approach, respectively (Fig. 2).

![Fig. 2](image2.png)

**Fig. 2.** General schemes of the (A) top-down synthesis and (B) bottom-up synthesis of NHC@AuNPs.

Special properties are mainly determined by the head group of the alkyl chain as a consequence of the close packing at the NPs surface. [26] Despite these advantages, to date, the number of ex-amples of NHC-stabilized metal (Au) NPs is still limited and numerous applications remain unexplored, particularly in the areas of biomedicine, photoacoustic (PA) imaging, photothermal therapy (PTT) and biosensing. The reader is referred to more comprehensive reviews for an overview of the field. [29,39,45]
NHCs via thermolysis of preformed or in situ generated heteroleptic Au(I) NHC complexes bearing pentafluorophenyl as ancillary lig-and, under solvent-free conditions. The AuNPs were found to be smaller than 2 nm and were applied as catalysts, loaded onto aminopropyl-functionalized silica support, in the reduction of 4-nitrophenol. [48] Such ultra-small AuNPs could be suitable for in-tracellular drug delivery due to their increased stability in the presence of serum components, [49] or as a near-infrared imaging agent for in vivo fluorescence detection of tumor cells. [50] An-other useful method to achieve NHC@AuNPs applied deprotonation of imidazolium halourate salts followed by their in situ re-daction; [51,52] however, spectroscopic characterization of the re-sulting particles suggested the presence of some unidentified NHC-derived impurities.

The effect of different NHC ligands for NP formation was investigated using 1,3-diethylbenzimidazol-2-ylidine, 1,3-bis(mesityl)imidazol-2-ylidine, and 1,3-bis(2,6-diPr2C6H3)imidazol-2-ylidine carbene scaffolds. [53] The latter scaffold, characterized by increased steric bulk, produced AuNPs of smaller average size (2.7 nm) and larger size distribution (12.5%) with respect to the other two NHCs (producing nanoparticles of 6.5 ~ 6.6 nm and near-row size distributions of 6.7% and 4.6%, respectively). Overall, the efficiency of the process and the average size and size distribution of the nanoparticles markedly depended on the nature of the NHC ligand, on the sequence of reactant addition (i.e., presence or absence of thiol during the reduction step), and on the presence of oxygen. [53]

Four small (<1.6 nm diameter) calix[4]arene NHC-bound gold clusters were synthesized following the bottom-up method. [54] The smallest calix[4]arene NHC-bound Au cluster consisted of a 1.2 nm gold core, and its number density of accessible and open surface sites was measured using a SAMSA (5-(2-(anil-3)-S-(acetylcyclopt- succinylimino)amin) fluorescein dye molecule. The number density of open Au sites on the new calix[4]arene NHC-bound AuNPs measured by the SAMSA fluorescein probe strongly supports the generality of a mechanical model of accessibility, which does not depend on the functional group involved in binding to the gold surface and rather depends on the relative radius of curvature of bound ligands and the gold cluster core. [54]

Conductive polymer-AuNP hybrids were also synthesized by disproportionation of Au(I) to Au(III) and Au(0), with concomitant reduction of Au(III) by oxidatively coupling bi thiophenes linked to the NHC ligands in the starting Au(III) complexes. [55] TEM images of the synthesized NHC CP/AuNP hybrids indicated that spherical AuNPs of average size ca. 3.6 nm were well-dispersed with near-row size distributions. Since the isolated NHC-CP/AuNPs were not soluble in organic solvents, their analysis was performed by 13C solid-state NMR spectroscopy, showing a signal in the region up-propionate for a carbene C–Au bond (185.5 ppm), supporting the idea that NHC groups help the dispersion of AuNPs in the polymer matrix. The NHC@AuNPs were also active towards the reduction of 4-nitrophenol. [55]

In 2014, Glorius, Ravoo, and coworkers [56] demonstrated that the introduction of long alkyl chains at C4 and C5 of the imi-dazolylidene ligand, and use of small or flexible N-substituents, enabled the preparation of “air”- stable and aggregation-stable NHC@AuNPs. These NPs were formed following the top-down strategy, via ligand exchange on thioether-stabilized AuNPs. The ef-fects of the type of NHC ligand on the AuNPs formation were fur-ther studied by Richter and coworkers, [57] who reported on the reaction of thioether-stabilized gold nanoparticles generated in situ with benzimidazol-2-ylidine ligands. In this case, the NHC@AuNPs were two times smaller than the thioether-stabilized NPs precursors, with a mean size of 2.8 ±0.6 nm, and more aggregated, as shown by TEM and by the broad SPR observed at λmax ~ 552 nm. [57] Such an evolution of the AuNP morphology suggests that lig-and exchange is not the only process occurring at the AuNPs sur-face, and that the reaction with NHCs also leads to etching. In-terestingly, using several characterization techniques in the solid state, including 13C solid state NMR and powder X-ray diffraction (XRD), it was possible to demonstrate that (i) the formation of bis-carbene Au(I) complexes occurs in concomitance to NHC@AuNPs formation, and that (ii) residual NHC ligands are present at the AuNPs surface. [57] One possible interpretation of the quan-tita-tive formation of the bis-carbene Au(I) complex would be that the initially generated NHC-Au containing species are weakly bound to the NP surface, and may be released to further react with the remaining NHC generated in situ. This process occurs also in the case of in situ generated imidazol-2-ylidines. Interestingly, the bis-NHC Au(I) complexes may be interacting with the AuNP surface by aurophilic interactions, as discussed in another study. [58] In the latter, starting from citrate capped AuNPs, the synthesis of NHC@AuNPs was achieved using dinuclear Au(I)-bridged flexible macrocycles whereby the gold centers were bound by the NHC scaffold. [58] Unfortunately, the binding of the NHCs to the NP sur-face could not be undoubtedly characterized.

A few years later, the binding modes of NHCs on the gold sur-face were studied in depth by a combination of scanning tunneling microscopy, XPS, and density functional theory (DFT) calculations. [59] In detail, the impact of alkyl side groups on the formation of NHC species at the Au(111) surface was elucidated, whereby two significantly different binding modes depending on the alkyl chain length were identified. Whilst longer alkyl groups resulted exclu-sively in NHC-Au-NHC complexes lying flat on the surface, stabi-lized by van der Waals interactions, the short alkyl substituents favored an up-standing NHC configuration with one Au adatom ex-tracted from the surface. [59]

In 2018, Cruden and coworkers [60] reported on AuNPs sta-hilized by bidentate N-heterocyclic carbene (NHC) ligands, syn-thesised by either top-down or bottom-up approaches. The se-lected NHCs featured a benzimidazolazole scaffold with the backbone appended by alkyl groups and a connecting alkyl chain bridging the NHC moieties via the wingtip positions (Fig. 3A). As a general trend, smaller nanoparticles resulted from the bottom-up reductive approach compared with the top-down ligand exchange method (Fig. 3B). The presence of the NHC on the AuNPs surface was con-firmed by XPS and thermogravimetric analysis (TGA). [60] Whilst the appearance of the N (1 s) signal at 401 eV typical of the pre-ence of NHCs on surfaces was detected, higher ligand desorption temperatures were also observed for NHC-stabilized AuNPs (ob-tained by the top-down approach) compared to thiol function-alized ones, as well as for alkylated or bidentate NHCs vs non-alkylated or monodentate NHCs, respectively. AuNPs prepared by the bottom-up method showed greater propensity to ripen upon heating, likely to be due to the smaller initial size of the NPs, or lower ligand density. [60] Finally, the stability of the NHC@AuNPs was tested towards thiols, such as DTT and thiophenol, by UV-Visible spectroscopy. Of note, while the top-down synthesized NPs with bidentate NHCs featured remarkable stability, the correspond-ing bottom-up NPs showed initial changes upon thiol treatment and more significant decomposition after 48 h. [60]

The versatility of NHC@AuNPs was next showcased by Cao et al. in 2016, [61] where the efficient electrocatalytic reduction of CO2 to CO in water was reported. The nanoparticles were formed by the top-down approach, whereby oleylamine stabilized AuNPs were added to an excess of the ligand in anhydrous tolune, resulting in ligand exchange.

In an attempt to obtain water-soluble AuNPs via the bottom-up method, NHCs tethered to N-BOC-histidine-methyl ester group (BOC = tert-butylxycarbonyl) via the heterocyclic backbone were synthesized. [62] Thus, chiral histidine-derived Au(I) NHC complexes, chlorido-(1,3-dimethyl-N-BOC-O-methyl-L/D-histidin-2-
ylidene)Au(I), were reduced by $^t$BuNH₂•BH₃ (tert-Butylamine borane) in THF to afford AuNPs showing a bimodal size distribution. Nanoparticle size selection was carried out by centrifugation of the AuNP suspensions, enabling isolation of monodisperse nanoparticles. Circular dichroism (CD) spectroscopy showed optical activity of the AuNPs arising from the chiral NHC ligands, but not for the Au(I) NHC complexes, indicating that packing of the NHCs on the nanoparticle surface is crucial to enable chiral activity. Unfortunately, $N$-BOC deprotection did not afford water soluble AuNPs, but produced aggregates which were not soluble in a variety of protic and aprotic solvents.

An alternative application of chiral NHC ligands for AuNP synthesis was later published by Toste and coworkers, based on the concept of supported Dendrimer-Encapsulated Metal Clusters (DEMCs). The AuNPs were formed by reduction of the chiral Au(I) NHC complex with $^t$BuNH₂•BH₃, in the presence of the dendrimer. The resulting dendrimer-encapsulated AuNPs were then added to the silica support, followed by separation from the solution to prevent NP aggregation. XPS studies reflected the prevalence of Au(I) species at the surface of NHC@AuNPs, also confirmed by high-resolution mass spectrometry. To further understand how far beyond the NP surface the oxidation state differences persisted, a bulk analysis technique, namely X-ray absorption near edge structure (XANES), was applied. The obtained results indicated that over 95% of Au atoms were in the Au(0) phase. The NHC-ligated AuNP catalysts enabled a model lactonization reaction to proceed at 20 °C, while NHC-free AuNPs are inactive below 80 °C. Of note, the NHC-capping of the AuNPs led to asymmetric induction (up to 16% enantiomeric excess) in the lactonization transformations. Varying the chiral NHC ligands enabled initial structure-activity relationships to be inferred, including: i) steric bulk of the NHC wingtip groups did not correlate with catalytic activity.
3.1. Water-soluble NHC-stabilized AuNPs: synthesis and biological applications

The water-stability (solubility) of gold NPs is an important pre-requisite for their biomedical applications, and is a great challenge in nanotechnology. It should be noted that the first example of water-soluble metal NPs stabilized by NHC ligands was published by the groups of Chaudret and de Jesús in 2014, [64] reporting on highly stable NHC@PtNPs that could be formed using hydrophilic sulfonated NHC ligands via a bottom-up approach. Subsequently, following the same method, the first example of water-soluble NHC@AuNPs was reported by MacLeod and Johnson in 2015. [65] PEGylated AuNPs were formed by reduction of the corresponding Au(I)/Au(III) NHC complexes with t-BuNH₂-BH₃ in THF at room temperature (Fig. 4, Table 1), followed by dialysis against water. A mixture of Au(I) and Au(III) NHC complexes were used as precursors due to separation issues. TEM and dynamic light scattering measurements confirmed the presence of a polymeric shell around the gold core (Fig. 4B). The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the related imidazolium salt and NHC@AuNPs were very similar, confirming the presence of the PEGylated ligand onto the NP surface. However, the lack of the imidazolium key vibrational band (at ~1576 cm⁻¹) in the nanoparticle specimen (Fig. 4C) strongly suggested that the NHC is coordinated to the Au surface. UV-Vis spectroscopy of these PEGylated NHC@AuNPs revealed that they were stable for at least 3 months in aqueous solution, as well as in NaCl solutions below 250 mM for 6 h. Aqueous NP solutions above pH 3 were also very stable, i.e. after 8 weeks the SPR band decreased only slightly, with a small red shift. The NPs also showed durability against extreme temperatures (95 and ~78 °C) for 5 h (Fig. 4E), and remained dispersed and stable in cell culture media containing fetal bovine serum (FBS) for 26 h at 37 °C, as evidenced by the unaffected surface plasmon band (SPB). [65] The effect of thiols on the PEGylated NHC@AuNPs was monitored by UV-Vis spectroscopy and ¹H NMR, and different rates of reactivity and induced aggregation were observed. [65]

In 2017, Crudden and coworkers [66] reported on the synthesis of an amphiphilic NHC-Au(I) complex based on an asymmetry-ric triethylene glycol/dodecyl-functionalized benzimidazole, which was further used to prepare the corresponding stable amphiphilic NHC-decorated AuNPs, whose self-assembly behavior was studied in polar solvents (water and EtOH). [66] While relatively small islands of NHC@AuNPs ensembles were observed for the sample in water, higher order aggregation was evident for nanoparticles self-assembled in EtOH. It should be noted that it is the aggregation numbers of the AuNPs clusters as well as the interparticle dis-tances within the structures that change in this process, not the discrete AuNPs size. In fact, the average diameter of the AuNPs within the aggregates formed in water and EtOH, was found to be 4.1 ± 1.3 nm and 4.3 ± 1.3 nm after 24 h, respectively. [66] Unfortunately, due to the formation of NPs aggregates, these systems are not suitable for biological applications.

The same group presented water-soluble NHC@AuNPs formed by a ‘bottom-up’ approach. In detail, the [Au(NHC)Cl] complex and corresponding bis-NHC complex ([(NHC)₂Au]OTf (NHC = carboxy-lated benzimidazoyle, Fig. 5A, Table 1) were reduced using sodium borohydride (NaBH₄) in an aqueous solution of sodium hy-
Table 1
Examples of water-soluble AuNPs stabilized by NHC ligands, including method of synthesis, size, stability in biologically relevant conditions and explored application.

<table>
<thead>
<tr>
<th>NHC Ligand</th>
<th>Details</th>
<th>Size (nm)</th>
<th>Stability</th>
<th>Application</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Top-down approach</td>
<td></td>
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<tr>
<td>*Starting material DDS (didecylsulfide) capped AuNPs - NHC deprotonated \textit{in situ} with KO\textsuperscript{t}Bu in biphasic hexane/DMF system.</td>
<td>4.1 – 4.9</td>
<td>All NPs stable at pH &gt;4 for months in aqueous solution.</td>
<td>Not tested</td>
<td>Ravoo and Glorius, 2015 \cite{68}</td>
<td></td>
</tr>
<tr>
<td>*Starting material OAm (oleylamine) capped Au\textsubscript{x}Pd\textsubscript{y} NPs - NHC deprotonated \textit{in situ} with KO\textsuperscript{t}Bu in DMF.</td>
<td>4.0</td>
<td>N/A</td>
<td>Aerobic oxidation of d-glucose (aqueous solution), and semi hydrogenation of diphenylacetylene by transfer hy-drogenation and hydrogenation of nitroarenes (solid support)</td>
<td>Ravoo and Glorius, 2018 \cite{69}</td>
<td></td>
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<tr>
<td>*Starting material citrate capped AuNPs in DCM/H\textsubscript{2}O mixture.</td>
<td>ca. 18</td>
<td>N/A</td>
<td>Not tested</td>
<td>Jenkins and Camden, 2020 \cite{70}</td>
<td></td>
</tr>
<tr>
<td>Bottom-up approach</td>
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<tr>
<td>Au\textsubscript{(I)}/Au\textsubscript{(III)} NHC complex pre-synthesized before reduction with 2-t-BuNH\textsubscript{2}•BH\textsubscript{3} in THF, for 24 h.</td>
<td>4.2 ± 0.7</td>
<td>Stable in pH range of 3–14 over 2 months, and in various biologically relevant conditions. Moderate stability towards GSH over 26 h.</td>
<td>Not tested</td>
<td>Johnson, 2015 \cite{65}</td>
<td></td>
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<tr>
<td>Au\textsubscript{(I)} NHC complex pre-synthesized before reduction with NaBH\textsubscript{4} in water, for 5 h.</td>
<td>2.4 ± 0.3</td>
<td>Moderate stability in 150 mM NaCl solution after 7d, and after exposure to 2 mM GSH over 24 h.</td>
<td>Probe for photoacoustic imaging</td>
<td>Nambo and Crudden, 2017 \cite{67}</td>
<td></td>
</tr>
<tr>
<td>(i) Au\textsubscript{(I)} NHC complex pre-synthesized before reduction with 2-t-BuNH\textsubscript{2}•BH\textsubscript{3} in THF, 16 h. (ii) Hydrolysis of ethyl ester group with NaOH in H\textsubscript{2}O/EtOH for 1 h at 90 °C.</td>
<td>4.2 ± 1.2</td>
<td>Moderate stability in aqueous solutions (pH 3–13), stable up to 36 days in PBS (pH 7.4) and NaCl solution (150 mM), and stable for 48 h in GSH (2 mM, pH 8)</td>
<td>Not tested</td>
<td>Chin and Reithofer, 2019 \cite{71}</td>
<td></td>
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Table 1 (continued)

<table>
<thead>
<tr>
<th>NHC Ligand</th>
<th>Details</th>
<th>Size (nm)</th>
<th>Stability</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction of H[AuCl₃]·3H₂O with NaBH₄ in water in the presence of the imidazolium salt.</td>
<td>5.6 ± 1.5</td>
<td>N/A</td>
<td>Catalyzed the A¹ coupling of aldehydes, terminal alkynes and amines in neat conditions to afford propargylamines, and the cycloisomerization of γ-alkynoic acids to enol lactones in toluene: H₂O (1:1)</td>
<td>Pleixats, 2020 [72]</td>
<td></td>
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</table>

Top-down and adatom approach

(i) Ligand exchange with CTAB coated Au nanorods.
(ii) Reduction of AuNHC with t-BuNH₂·BH₃ in THF, 24 h.

Length: 42.6 ± 6.7
Width: 9.9 ± 1.3
Nanorods stable over a range of different pHs and temperatures, as well as with excess GSH and in cell culture media.

Use in PTT. Preliminary tests successful in MCF7 cancer cells in vitro. | Johnson, 2019 [73] |

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Fig. 5. (A) Structures of mono- and bis-NHC Au(I) complexes used for the formation of water soluble AuNPs; (B) Acoustic wave signal recorded after irradiation of an aqueous solution of NHC@AuNPs (different concentrations) at 532 nm using a pulsed laser beam. Image adapted with permission from ref. [67] Copyright (2017) John Wiley and Sons.

NaOH is required to deprotonate the carboxylate group on the NHC ligand, forming water soluble AuNPs with a negatively charged surface. The size of the nanoparticles (between 2 and 4 nm) and the intensity of the SPR band could be controlled by varying the reaction time. [67] Interestingly, the evolution of the NHC-Au nanoparticles to larger sizes proceeded homogeneously, affording NHC@AuNPs with narrow size distributions after 7 and 24 h, as evaluated by TEM and gel electrophoresis. Next, the stability of the NPs was assessed in basic solutions at pH 8 and 10 for 2 months, showing very little change in UV–Vis spectra over time. [67] To better mimic biological media, the stability of the NHC@AuNPs was assessed in 150 mM NaCl solution, and showed high stability with only a slight sharpening of the SPR band after 7 days. The larger NPs were also moderately stable in excess GSH under slightly basic conditions (pH 8) over 24 h. For the first time, the NHC@AuNPs were tested for biomedical applications as possible probes for photoacoustic imaging. [67] Thus, an aqueous solution of AuNPs was prepared and irradiated with a pulsed laser beam (532 nm). Despite their weak SPR bands, the sample gave a reliable photoacoustic signal, which increased linearly with the NHC@AuNPs concentration (Fig. 5B). [67]

In 2019, the groups of Chin and Reithofer [71] achieved water-soluble NHC@AuNPs by the bottom up approach (Table 1) using NHC ligands derived from a N-acetyl-L-histidine ethyl ester scaffold and featuring either a methyl or isopropyl group on the wingtips of the imidazole ring. The Au(I) NHC complexes were subsequently reduced to AuNPs using t-BuNH₂·BH₃ in THF at room temperature for 16 h. [71] To obtain water soluble AuNPs, they were then saponified with NaOH in an EtOH/H₂O mixture for 1 h at 90 °C. TEM analysis confirmed the presence of AuNPs with a size range of ca. 4 nm. The NHC@AuNPs bearing methyl groups as wingtips showed much higher stability compared to the isopropyl derivatives across a range of different conditions, including phosphate buffer solution (PBS, pH 7.4), aqueous NaCl solution (150 mM) and in the presence of glutathione (GSH, 2 mM, pH 8). [71] The stability of the NPs in the presence of GSH was also investigated by ¹H NMR; in this case no change was observed over 24 h suggest-ing that the thiol group of GSH is unable to displace the NHC ligand on the AuNPs. The steric bulk of the isopropyl group was proposed to be the cause of reduced stability compared to the AuNPs with the methyl wingtips. [71] in line with previous studies on a family of sterically demanding NHC ligands. [56] UV–Vis spec-troscopy also showed the pH dependent reversible aggregation of the NHC@AuNPs, due to protonation of the carboxylic acid moiety. [71] Recently, the use of PEGylated imidazolium (bromide and tetrafluoroborate) and tris-imidazolium (bromide) salts containing triazole linkers, as stabilizers for the preparation of water-
soluble gold nanoparticles by reduction of tetrachloroauric acid (HAuCl₄) with NaBH₄ was reported (Table 1). TEM analysis confirmed the formation of spherical and well-dispersed nanoparticles of mean diameters from 4.5 to 5.6 nm. Unfortunately, whilst a number of spectroscopic data indicate that the heterocycle ligand is close to the surface of the metal, no conclusive experimental evidence with respect to its nature, either imidazolium or carbene, could be obtained. It should be noted that, in general, although NHCs have been demonstrated as suitable ligands for the stabilization of gold nanoparticles through a variety of methods, the mechanism by which such NHC@AuNPs form is yet to be fully elucidated. For example, XPS studies of chiral (L/D)-histidin-2-ylidenes stabilized gold nanoparticles, formed using well defined organometallic Au(I) complexes, showed the concomitant presence of Au(I) and Au(0) in the nanoparticles. Based on this observation, it was postulated that AuNPs synthesized from Au(I) NHC complexes exhibit a monolayer of Au(I) surrounding a Au(0) core, but further studies are necessary to elucidate this hypothesis.

In 2015, following the top-down approach, water soluble PdNPs and AuNPs, stabilized by negatively charged NHC ligands bearing either sulfonate or carboxylate groups as wingtip substituents, were reported. The NPs were formed using the didecylsul-fide (DDS)-stabilised Pd or AuNPs as precursors in a biphasic mixture of hexane and DMF (dimethylformamide). In detail, the re-ative NHCs were formed in situ in DMF from their corresponding imidazolium salts by addition of KO²Bu, before addition of DDS-metal NPs (Fig. 6, Table 1). Of note, the NHC AuNPs showed a size-nificant decrease in size upon ligand exchange, with DDS@AuNPs having a mean size of 8.5 ± 1.7 nm, whereas NHC@AuNPs (with an organic co-solvent). The resulting surface-tethered Au(I) NHC complexes, the gold atom at-tached to the NHC complex is added to the surface as an adatom (Fig. 7, Table 1). Upon mild reduction (with 2 equiv. BuNH₂BH₃ in THF) of the re-sulting surface-tethered Au(I) NHC complexes, the gold atom-at-tached to the NHC complex is added to the surface as an adatom (Fig. 7). The concept of bidentate NHCs on nanoparticles was first described by Ravoo, Glorius and coworkers using similar bidentate hybrid NHC-thioether ligands to stabilize PdNPs, which remained spherical and small in size and were successful cata-lysts for the chemoselective hydrogenation of olefins. This strategy avoids the need for reorganization of the underlying surface-lattice upon NHC binding. Such an elegant approach was in-spired by the often observed etching of gold surfaces by free NHCs, and by previous data showing that when such NHCs are de-posted onto planar gold substrates under ultrahigh vacuum, they tend to abstract a gold atom from the surface lattice to generate translationally mobile NHC@Au adatom complexes. The re-sulting thiolate-NHC-stabilized gold nanorods were water soluble thanks to the functionalization of the NHC backbone with triazole-conjugated polyethylene glycol. The nanoparticles were also stable towards excess glutathione for up to six days, and under conditions with large variations in pH (from 2 to 14), low and high temp-eratures (between −78 °C to 95 °C), high salt concentrations (up to 1 M NaCl), or in biological and cell culture media. Of note, in this work the obtained NHC@AuNPs were applied for PTT in vitro, showing selective cancer cell-killing only upon laser irradiation.

To broaden the scope of NHC@AuNPs, the synthesis of non-spherical NPs was reported by Johnson and coworkers, who used a bidentate thiolate-NHC-Au(I) complex grafted onto com-mercial cetyl trimethylammonium bromide (CTAB)-stabilized gold nanorods through ligand exchange. First, exchange of CTAB ligands on commercial CTAB@Au nanorods with a photogenerated thio-late was achieved, followed by NHC installation (Fig. 7, Table 1). Upon mild reduction (with 2 equiv. BuNH₂BH₃ in THF) of the re-sulting surface-tethered Au(I) NHC complexes, the gold atom at-tached to the NHC complex is added to the surface as an adatom (Fig. 7). The concept of bidentate NHCs on nanoparticles was first described by Ravoo, Glorius and coworkers using similar bidentate hybrid NHC-thioether ligands to stabilize PdNPs, which remained spherical and small in size and were successful cata-lysts for the chemoselective hydrogenation of olefins. This strategy avoids the need for reorganization of the underlying surface-lattice upon NHC binding. Such an elegant approach was in-spired by the often observed etching of gold surfaces by free NHCs, and by previous data showing that when such NHCs are de-posted onto planar gold substrates under ultrahigh vacuum, they tend to abstract a gold atom from the surface lattice to generate translationally mobile NHC@Au adatom complexes. The re-sulting thiolate-NHC-stabilized gold nanorods were water soluble thanks to the functionalization of the NHC backbone with triazole-conjugated polyethylene glycol. The nanoparticles were also stable towards excess glutathione for up to six days, and under conditions with large variations in pH (from 2 to 14), low and high temp-eratures (between −78 °C to 95 °C), high salt concentrations (up to 1 M NaCl), or in biological and cell culture media. Of note, in this work the obtained NHC@AuNPs were applied for PTT in vitro, showing selective cancer cell-killing only upon laser irradiation.

![Fig. 6. (A) Structures of imidazolium salts L1-L4 used as stabilizers for water soluble MNPs (M = Au and Pd); (B) Reaction scheme to form NHC@MNPs from L1-L4.](image-url)
Recent work by Ravoo, Glorius and coworkers [69] described, for the first time, bimetallic NPs stabilized by NHC ligands, combining the advantageous properties of both Au and Pd nanoparticles. The water soluble NPs (ca. 4 nm average diameter) were formed by the ‘top-down approach’ involving the ligand exchange of oleylamine (OAm)-stabilized Au$_3$Pd$_4$ with the in situ generated NHC ligand (Table 1). [69] The NHC@Au$_3$Pd$_4$NPs were explored as biomimetic catalysts for the aerobic oxidation of D-glucose, which is catalyzed naturally by glucose oxidase, and showed consider-able catalytic activity. [69] Considering that the NP performance increases with higher Au content, gold was supposed to be the ac-tual active metal, while the alloy effect confers superior colloidial stability with respect to the monometallic NHC@AuNPs analogues. The Au$_3$Pd$_4$NPs were also supported on TiO$_2$, and, as such, success-fully catalyzed the semihydrogenation of diphenylacetylene, show-ing switchable selectivity towards the (Z)- or (E)-stilbene product, and the hydrogenation of nitroarenes. [69]

4. Conclusions and perspectives

The outstanding properties of gold, including its high stabil-ity, ease of use, unique spectral properties and biocompatibility, make this noble metal a key material in nanotechnology. In recent years, the remarkable resilience of N-heterocyclic carbene chem-istry and NHC-gold bond s has quickly made them the ‘smart’ ligands of choice when functionalizing gold surfaces. Thus, a num-ber of NHC-coated AuNPs has been reported, mainly synthesized by reducing gold NHC complexes or by replacing labile ligands at the AuNP surface with NHCs. Results show that the size, size dis-tribution, shape and stability of NHC@AuNPs strongly depend on the structure of the NHC ligands and the reaction conditions used for their synthesis.

Although NHCs have demonstrated outstanding potential for use as surface anchors, this research area is still in its infancy, and synthetic challenges, limiting their application to either large planar metal substrates-surfaces or very small spherical particles, need to be addressed. For example, gold nanorods, which are inter-mEDIATE species between planar surfaces and nanoparticles, have received little attention with regard to the use of NHCs as sta-bilizing ligands. Nevertheless, these would be ideal nanomaterials for PTT. [85] For biomedical applications, further tuning of the wa-ter solubility/stability properties could be achieved using different types of hydrophilic NHCs, [43,86] including those based on amino acids, [87] whose Au(I) complexes have already the tendency to form AuNPs in aqueous solution. [88] The effect of different car-bene scaffolds would also be worth exploring. In 2020, Fuchs, Glo-rius and coworkers [89] were the first to report the binding of CAACs on a Au(111) surface, which could lead to their possible ap-plications as stabilizing ligands for AuNPs. To favor formation from non-symmetric car-bene scaffolds, nitrogen acyclic car-benes (NACs) can also be used, which were already shown to efficiently stabilize AuNPs. [90]

It is worth mentioning that understanding the precise ligand behavior at the surface of the AuNPs is another crucial step to-wards the control of NHC stabilizers. To some extent the problem relates to the complexity of the characterization techniques that can be applied to AuNPs and the different ways in which the nano-materials can be prepared. [91] Most of the reported examples in-volve the ex situ examination of the AuNPs. Eventually, more ef-forts should be devoted to the study of the nanomaterials under real operating conditions, including using (i) in situ/operando ex-tended X-ray absorption fine structure/X-ray absorption near edge structure (EXAFS/XANES), (ii) in situ Fourier transform infrared (FTIR) measurements, as well as (iii) by environmental TEM. [91]

In the near future, we envisage the combinatorial design of NHC ligand shell functionality, which would disclose the potential of metal NPs for adaptive chemistry, whereby, the use of the ro-bust NHC scaffold to tethering the AuNP surface to different moi-eties (e.g. peptides) [79] would enable the discovery of artificial receptors, sensors, as well as targeted biocatalysts and theranostic agents.

Declaration of Competing Interest

The authors declare that they have no known competing finan-cial interests or personal relationships that could have appeared to influence the work reported in this paper.

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