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ARTICLE TYPE

On the chiroptical properties of Au(I)-thiolate glycoconjugate precursors and their influence on sugar-protected gold nanoparticles (glyconanoparticles)

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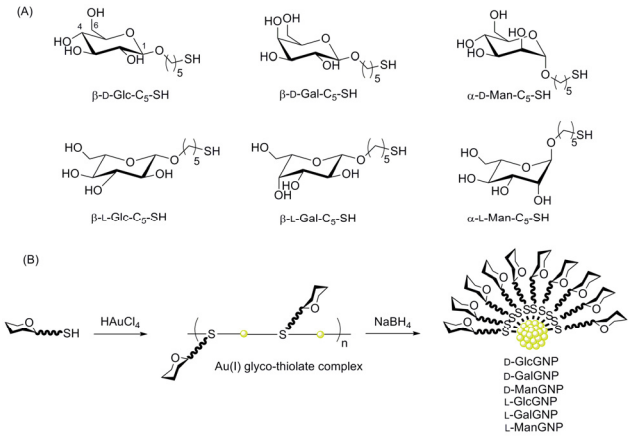
Chiral gold nanoparticles passivated with glycoconjugates were prepared. By extensive washing, they lost their chiroptical activity, whereas highly luminescent compounds isolated from the washing reproduced the original ellipticity. A systematic characterization of these compounds, Au(I) precursors and glyconanoparticles allowed us to unravel the origin of the observed chiroptical activity.

The physical phenomenon of chirality has fascinated chemists since the 19th century. In recent years, the optical activity of quantum sized gold nanoclusters has become an important topic of research, but its origin is not completely understood.[1] The crystal structure of thiolate-passivated nanoclusters have revealed that the arrangement of protecting $[Au(I)SR]_n$ staples on the gold surface seems to be the origin of the observed chirality.[2]

The preparation of thiolate-stabilized Au nanoclusters by reduction of a gold (III) salt in the presence of thiols is a two-step process. Firstly, gold (III) is reduced by the thiol species leading to polymeric gold (I)-thiolate complexes, which are then reduced by strong reductants as $NaBH_4$.[3] The understanding of the origin of chirality in quantum sized thiolate gold nanoclusters goes together with the understanding of the chirality evolution of their Au(I)-thiolate precursors. These intermediates seem to play an important role in the growth mechanism of thiolate-protected gold nanoclusters, but it still remains unclear how they intervene in the size and properties of the resulting nanoparticles. Although studies on the growth mechanism of glutathione-gold(I) polymers to yield nanoclusters have been reported,[4] the chiroptical properties of these thiolate-precursors and their relation with the chirality of the formed nanocluster have not been identified.

The great structural diversity that offers enantiomerically pure sugars makes them ideal molecules for a systematic study of the origin of the optical activity in Au(I)-thiolate precursors and the resulting nanoclusters. While the preparation of sugar–thiolate protected gold nanoclusters (glyconanoparticles, GNPs) as multivalent system has been explored in depth,[5] the chiroptical properties of the Au(I)-sugar precursors have not been addressed so far. A detailed characterization of the Au(I)-thiolate precursors may contribute to clarify the origin of ellipticity in thiolate-protected gold nanoclusters.

We now report on the origin of the chiroptical activity of Au(I)-thiolate glycoconjugate complexes of the enantiomerically



Scheme 1 (A) 5-mercaptopentyl conjugates of D/L glucose, galactose, and mannose used for the synthesis of gold glyconanoparticles. (B) Schematic representation of the synthesis of the D/L gold glyconanoparticles starting from the thiol-glycoconjugates, where the precursor Au(I)sugar-thiolate polymer is shown.

pure 5-mercaptopentyl glycosides of D and L glucose (Glc), galactose (Gal), and mannose (Man) and its relation with the corresponding gold nanoclusters (Scheme 1). Water-soluble GNPs (average size 1.6 nm) were prepared from the thiol-glycoconjugates by a previously described protocol.[6] ^{1}H-NMR spectra confirmed that the sugar conjugates form the organic shell protecting the GNPs (for experimental details, see ESI†). The UV-vis spectra of the nanoparticles showed an onset around 800 nm with absorption in the surface plasmon region (Fig. 1A). On the other hand, the CD spectra of the D-sugar GNPs showed two positive maxima at 238 and 327 nm and two negative at 187 and 292 nm, while the spectra of L-sugar GNPs are the mirror-like image of the D-sugars (Fig. 1B). The ellipticity bands are located in the UV range (λ<500 nm), which excludes a possible plasmonic contribution. It is worth noting that, although Glc, Gal and Man have different chiral carbons at positions 1, 2 and 4 of the pyranose ring (Scheme 1A), their corresponding GNPs show identical CD spectra shape, which indicates that the electronic transitions are not affected by the structure of the sugar epimer itself. The strong similitude among the ellipticity of different

GNPs indicates that the optical activity is dominated by the configuration of the sulphur atoms in the thiolate species.[1a,7]

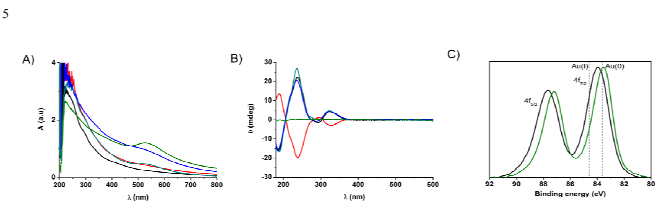


Fig. 1 UV-vis (A) and CD (B) spectra of original D-GlcGNP (black), L-GlcGNP (red), D-GalGNP (blue) and D-ManGNP (cyan) and extensively washed D-GlcGNP (green). XPS (C) of the original (black) and extensively washed D-GlcGNP (green). For simplicity only L-GlcGNP is shown.

In order to investigate more precisely the role of thiolate species on the overall chirality, we focused first on the D-GlcGNPs and their Au(I)-precursor. By extensive washing of the D-GlcGNPs with MeOH, a water-soluble white cotton-like compound that emitted photoluminescence (PL) under UV light was isolated. The washed GNPs exhibit a higher surface plasmon absorption in the visible and a shifting of the Au band to Au(0) energies in the XPS spectrum, indicating a more metallic character (Fig. 1A and 1C). Nevertheless, the original ellipticity disappeared (Fig. 1B and Fig.S1-S3, ESI†). By contrast, the "byproduct" isolated from the methanolic washing reproduced the optical activity observed in the original GNPs. Comparison of this compound and the Au(I)-thiolate precursor by size exclusion chromatography, XPS, UV-vis and CD indicates a strong similarity among them (Fig. 2). The UV-vis and CD spectra of both entities exhibit similar electronic and structural characteristics (Fig. 2B and C). The UV-vis spectra showed an onset around 400 nm and molecule-like discrete electronic transitions, while the onset of GNPs takes place at about 800 nm (Fig. 1A). Furthermore, both exhibit luminescence with emission around 585 nm (ex: 420 nm) characteristic of Au···Au interactions (Fig. S4, ESI†). Moreover, Au $4f_{7/2}$ binding energies of both entities exhibit a maximum binding energy at 84.4 eV, in accordance with the values of Au(I) state reported in literature (Fig 2D). Mass spectrometry identifies a common major fragment (m/e 1935.7) that corresponds to an eight member cyclic tetramer $(Au\text{-}SSugar)_4$ for "byproducts" and Au(I)-polymers (Fig. 2E and S4, ESI†). Finally, XRD experiments indicated that the GNPs did not show any well-resolved peak in the explored region, while both isolated "byproduct" and Au(I)-precursor exhibit characteristic peaks corresponding to 5.5 and 4.8 Å distance (arrows in Fig. 2F), which agree with the Au···Au across-ring distance (5.69-4.74 Å) observed for the 8-member structures (tetramer) of thiomalate[8] and thiopronine [9] polymers. All these results indicate that the "byproducts" isolated from the extensively washed GNPs are Au(I)-thiolate oligomers formed during the reduction of the Au(I)-thiolate precursors. Similar behaviour was observed for the rest of the GNPs (Fig. S2 and S3, ESI†).

To investigate how the structure of the glycoconjugate ligands influences the chiroptical properties of Au(I)-sugar-thiolate precursors and GNPs, a similar study was performed changing the 5-mercaptopentyl aliphatic linker of the $GlcC_5$-SH conjugate

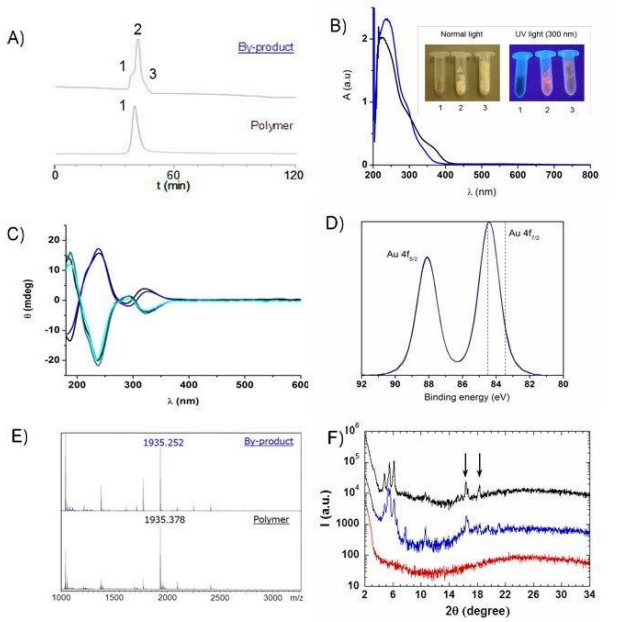


Fig. 2 SEC chromatograms (A); UV-vis (B); CD (C); XPS of Au4f binding energies (D), MALDI-TOF (E) and small-angle XRD (F) spectra of D-$GlcC_5$S-Au(I) byproduct (blue) and D-$GlcC_5$SAu(I) polymer (black) and D-GlcGNP (red, only for XRD). The black, green and cyan curves in (C) correspond to the L-$GlcC_5$SAu(I), L-$GalC_5$SAu(I) and L-$ManC_5$SAu(I) polymers. Inset in B: Luminescence of D-GlcGNP (1), D-$GlcC_5$S-Au(I) byproduct (2) and D-$GlcC_5$S-Au(I) polymer (3)

by a longer linker that consists of an eleven CH_2 aliphatic chain and a tetra-ethylene glycol chain (D-$GlcTEGC_{11}SH$). The TEM, NMR, XPS, UV and photoluminescence data showed similar results as those described for 5-mercaptopentyl monosaccharides (Fig. S5, ESI†). In contrast, neither ellipticity nor well resolved peaks in XRD were observed in the new Au(I)-thiolate precursor. Furthermore, the peak of the new Au(I)-thiolate precursor in the mass spectrum corresponds to the open tetramer. It is remarkable that despite the high similitude of the data for the Au(I) polymer precursors and "byproducts" among the sugars, only monosaccharides conjugated to the 5-mercaptopentyl short chain were able to organize polymers and oligomers in structures showing chiroptical activity.

On the basis of these results, we believe that the origin of the chiroptical activity observed in the Au(I)-polymers and oligomers is due to the formation of a chiral Au(I)sugar polymeric helix (or stacked planes) with a tetrameric repeating unit $(AuSR)_4$, similar to the double helix reported by Bau for the crystal structure of gold(I)-thiomalate polymer.[8] The helical organization via aurophilic and non-covalent interactions explains both the luminescence and the chiroptical activity observed in the 5-mercaptopentyl D/L monosaccharides gold conjugates. Sugars with D-configuration will organize in an opposite-handed supramolecular structure than the L-sugars. The presence of the same major peak at m/z 1935 in all the spectra of the oligomers and Au(I)-thiolate polymers and the absence of significant peaks

above this value confirm the cyclic 8-member tetramer [Au(I)-SC_5monosaccharide]$_4$ as the most stable structure resulting from the fragmentation of the helix. The lack of ellipticity in the D-Glc$TEGC_{11}$S-Au(I) polymers can be due to the high flexibility of the long conjugated chain, which avoids the formation of a stable helical chiral structure and indicates the strong influence of the glycoconjugate structure on the chiroptical organization of the Au(I)-thiolate complexes.

In the case of the 5-mercaptopentyl glycoconjugates, the original GNPs are the result of the formation of the chiral luminescent sugar-Au(I) polymers that by subsequent reduction with $NaBH_4$ evolve to the 1.6 nm "chiral" GNPs. Starting from the disulfide derivatives we never observed chiroptical activity during the formation of the GNPs. Therefore, the formation of the gold nanoclusters seems to follow different mechanisms depending on the oxidation state of the protecting ligands and experimental conditions. The thiol-ligands form highly luminescent Au(I) polymers that can evolve to ultra-small luminescent gold nanoparticles under different conditions as shown for Au(I)-glutathione polymers.[10] In the case of the disulfides, the Au(III) salt is directly reduced by the $NaBH_4$ and other mechanism is at work during the formation of the gold nanoclusters. This can explain why gold nanoclusters protected by glutathione with the same number of gold atoms exhibit different photophysical and chiroptical properties depending on the preparation method. For example, the $Au_{22}(SG)_{18}$ clusters obtained by Xie et al.[11] from Au(I)-glutathione polymers in the absence of $NaBH_4$ are highly luminescent, while $Au_{22}(SG)_{16}$ and $Au_{22}(SG)_{17}$ nanoclusters isolated by Negishi *et al.*[12] in the presence of a strong reducing agent exhibit weak luminescence and also different absorption spectra.

The structural evolution of Au(I)-thiolate polymers to nanocluster formation and growth is a very complex and fascinating process that seems to depend on many factors. Because the type of thiol derivatives and protocols for the preparation of many of the described chiral thiolate-protected nanoclusters are different, there are not enough data to correlate the evolution of the polymers with the resulting nanoclusters. Special attention has to be paid to the experimental conditions where the nanoclusters are prepared. We can mention two examples of this influence, one is the evolution of the colourless aqueous solution of the GlcC_5S-Au(I) polymer that in the presence of light at room temperature turns yellow, loses its luminescence and chiroptical activity and the UV-vis spectrum changes the onset from 420 nm to 800 nm, showing similar absorption pattern than the GlcGNPs (Fig. S6A, ESI†). The other example is the evolution observed in the luminescent non-chiral Glc$TEGC_{11}$S-Au(I) polymer by TEM. A drop of an aqueous solution was placed on a carbon grid, left 20 days at room temperature in a small box and then analyzed. The micrographs obtained in different regions of the grid (Fig. S6B, ESI†) showed a series of beautiful different structures (triangular, rhomboidal, octahedral, decahedral, rod-like) with sizes around 30 nm. What type of intermediates links the Au(I)-thiolate and these structures? Are the Au(I) oligomers forming part of a core/shell glyconanoparticle as described for thiomalate-protected nanoclusters[13] or are simple Au(I)-sugar thiolate oligomeric fragments resulting from the reductive fragmentation of the Au(I)-thiolate precursor during the gold nanoparticles' formation? More systematic studies are necessary to answer these questions.

To summarize, for the first time D and L Au(I)-thiolate glycoconjugate polymers and the corresponding nanoclusters have been prepared. A strong ellipticity firstly observed in the 1.6 nm sized GNPs was indeed due to compounds present at the surface of the dialyzed nanoparticles. The comparison of these compounds with the Au(I)-thiolate polymers confirms that the chiroptical properties observed for GNPs come from Au(I)-glycoconjugate complexes. The formation of the polymer and the chiroptical activity were strongly dependent on the structure and oxidation state (thiol or disulfide) of the glycoconjugates, but was not affected by the structure of the sugar epimer itself. Under the light of the mass spectrometry, CD and PL data, we believe that the chiroptical activity has its origin in a three-dimensional supramolecular architecture (helixes or stacked planes) of the tetrameric repeating units [$Au_4(SR)_4$]. These results suggest the importance of the original structure of the Au(I)-thiolate precursors to better understand the origin of the chirality observed in nanoclusters and nanoparticles, even though the thiolates themselves are achiral.

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Notes and references

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† Electronic Supplementary Information (ESI) available: The synthesis and characterization of glycoconjugate, polymers and glyconanoparticles are available. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

1 *(a)* H. Häkkinen, *Nat. Chem.* 2012, **4**, 443; *(b)* H. Qian, M. Zhu, Z. Wu and R. Jin, *Acc. Chem. Res.* 2012, **45**, 1470; *(c)* C. Gautier and T. Bürgi, *ChemPhysChem,* 2009, **10**, 483; *(d)* S. Knoppe and T. Bürgi, *Acc. Chem. Res.* 2014, **47**, 1318.

2 *(a)* P. D. Jadzinsky, G. Calero, C. J. Ackerson, D. A. Bushnell and R. D. Kornberg, *Science* 2007, **318**, 430; *(b)* M. W. Heaven, A. Dass, P. S. White, K. M. Holt and R. W. Murray, *J. Am. Chem. Soc.* 2008, **130**, 3754; *(c)* M. Zhu, C. M. Aikens, F. J. Hollander, G. C. Schatz and R. Jin, *J. Am. Chem. Soc.* 2008, **130**, 5883; *(d)* H. Qian, W. T. Eckenhoff, Y. Zhu, T. Pintauer and R. Jin. *J. Am. Chem. Soc.* 2010, **132**, 8280; *(e)* C. Zeng, H. Qian, T. Li, G. Li, N. L. Rosi, B. Yoon, R.N. Barnett, R. L. Whetten, U. Landman and R. Jin, *Angew. Chem. Int. Ed.* 2012, **51**, 13114; *(f)* C. Zeng, T. Li, A. Das, N. L. Rosi and R. Jin, *J. Am. Chem. Soc.* 2013, **135**, 10011; *(g)* C. Zeng, C. Liu, Y. Chen, N. L. Rosi and R. Jin, *J. Am. Chem. Soc.* 2014, **136**, 11922; *(h)* P. R. Nimmala, S. Knoppe, V. R. Jupally, J. H. Delcamp, C. M. Aikens, and A. Dass, *J. Phys. Chem.* B 2014, DOI: 10.1021/jp506508x.

3 *(a)* M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, and R. Whyman, *Chem. Commun.* 1994, **7**, 801; *(b)* A. C. Templeton, W. P. Wuelfing and R. W. Murray, *Acc. Chem. Res.* 2000, **33**, 27.

4 *(a)* Y. Negishi, Y. Takasugi, S. Sato, H. Yao, K. Kimura and T.Tsukuda, *J. Am. Chem. Soc.* 2006, **110**, 1218. *(b)* R. P. Briñas, M. Hu, L. Qian, E. S. Lymar and J. F. Hainfeld, *J. Am. Chem. Soc.* 2008, **130**, 975; *(c)* C. Zhou, C. Sun, M. Yu, Y. Qin, J. Wang, M. Kim and J. Zheng, *J. Phys. Chem. C* 2010, **114**, 7727; *(d)* Z. Lou, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, *J. Am. Chem. Soc.*, 2012, **134**, 16662.

5 *(a)* J. M. de la Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Cañada, A. Fernández, and S. Penadés, *Angew. Chem. Int. Ed. Engl.* 2001, **40**, 2258; *(b)* M. Marradi, F. Chiodo, I. García and S.Penadés, *Chem. Soc. Rev.* 2013, **42**, 4728; *(c)* J. Frigell, I. García, V. Gómez-Vallejo, J. Llop, S. Penadés, *J. Am. Chem. Soc.* 2014, **136**, 449.

6 A. G. Barrientos, J. M. de la Fuente, T. C. Rojas, A. Fernández and S. Penadés, *Chem. Eur. J.* 2003, **9**, 1909.

7 S. Knoppe, N. Kothalawala, V.R. Jupally, A. Dass and T. Bürgi, *Chem. Commun.* 2012, **48**, 4630.

8 R. Bau, *J. Am. Chem. Soc.* 1998, **120**, 9380.

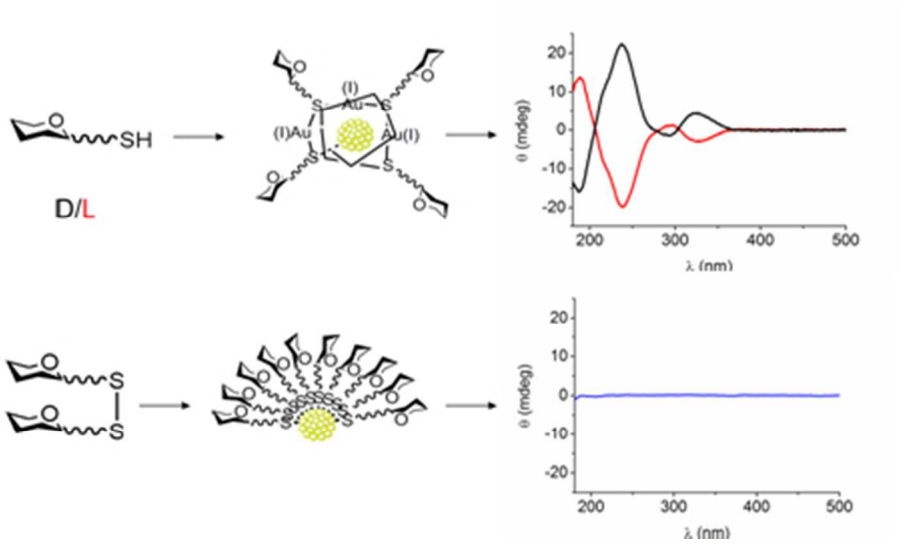
9 C. A. Simpson, C. L. Farrow, P. Tian, S. J. L. Billinge, B. J. Huffman, K. M. Harkness and D. E. Cliffel, *Inorg. Chem.*, 2010, **49**, 10858.

10 Z. Lou, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, *J. Am. Chem. Soc.*, 2012, **134**, 16662.

11 Y. Yu, Z. Luo, D. M. Chevrier, D. T. Leong, P. Zhang, D. Jiang and J. Xie, *J. Am. Chem. Soc.*, 2014, **136**, 1246.

12 Y. Negishi, K. Nobusada and T. Tsukuda, *J. Am. Chem. Soc.*, 2005, **127**, 5261

13 G. Corthey, L. J. Giovanetti, J. M. Ramallo-López, E. Zelaya, A. A. Rubert, G. A. Benitez, F. G. Requejo, M. H. Fonticelli and R. C. Salvarezza, *ACS Nano* 2010, **4,** 3413.



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