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Gold nanoparticles capped with sulfate-ended ligands as anti-HIV agents

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ABSTRACT

Gold nanoparticles coated with multiple copies of an amphiphilic sulfate-ended ligand are able to bind the HIV envelope glycoprotein gp120 as measured by surface plasmon resonance (SPR) and inhibit in vitro the HIV infection of T-cells at nanomolar concentrations. A 50% density of sulfated ligands on ~2 nm nanoparticles (the other ligands being inert glucose derivatives) is enough to achieve high anti-HIV activities. This result opens up the possibility of tailoring both sulfated ligands and other anti-HIV molecules on the same gold cluster, thus contributing to the development of non-cocktail based multifunctional anti-HIV systems.

Nanotechnology is nowadays opening new opportunities to chemists in the design and synthesis of assembled systems which can be used for biosensing, imaging, diagnosis and therapy.[1] The control of size, shape, dispersity, and surface chemistry of nanoparticles (NPs) are some of the characteristics which make this research field attractive. The similarity in size of NPs to biomolecules is another advantage when biological interactions want to be addressed. In recent years, functionalized gold nanoparticles have attracted much attention although their application in biomedicine is still in embryonic stage.[1c] In the development of therapeutics, the multimerization of synthetic molecules on NPs may afford new tools which are more effective at intervening in biomolecular processes than the monovalent parent compounds. Nanoparticle-based presentation of multiple ligands also creates a high local concentration of binding molecules which can assist the targeted biological interaction/process, as it is the case of multimerization of single strained oligonucleotide sequences into gold NPs which highly enhanced DNA-hybridization.[2] Metal NPs have been proposed as anti-viral systems taking advantage of the core material and/or the ligands shell. Silver nanoparticles have been shown to exhibit promising antimicrobial activities towards different types of viruses.[3] Multivalent presentation of a biologically inactive compound (a TAK-799 homologue) on gold nanoparticles transformed the monovalent small molecule into an active HIV fusion inhibitor.[4] We have recently reported that multivalent gold NPs coated with structural motifs of the high-mannose undecasaccharide $Man_9(GlcNAc)_2$ (*manno*-GNPs) which is present in the HIV-1 envelope glycoprotein gp120 are able to inhibit the viral transfer to T-cells. *Manno*-GNPs intervene at early steps of viral infection mainly acting as competitors of the gp120 towards the C-type lectin DC-SIGN expressed on dendritic cells, which can efficiently transfer the virus to T-lymphocytes where viral replication occurs.[5]

In an effort to develop alternative/complementary multivalent anti-HIV systems, we present in this Letter gold nanoparticles coated with multiple copies of sulfated ligands and their ability to inhibit the HIV infection of T-cells. These sulfated NPs have been designed to target the virus and thus potentially have a complementary anti-HIV activity with respect to the *manno*-GNPs which act as virus mimics. It is well known that polysulfates are potent inhibitors of various enveloped viruses, including HIV.[6] A variety of polyanionic compounds have been or still are in clinical trials as potential candidate microbicides.[7] The anti-HIV activity of polyanions is mainly due to their ability of binding the positively charged amino acids in the V3 loop and/or in a second binding site of the viral envelope glycoprotein gp120.[6a,8] Here we demonstrate that multivalent gold NPs capped with an amphiphilic sulfate-ended ligand (**1**-**Au**, Scheme 1) are able to bind gp120 with high affinity as measured by surface plasmon resonance (SPR) experiments and to inhibit in vitro HIV-1 infection in cellular models, depending on the sulfate density on the gold cluster. The half maximal inhibitory concentrations (IC_{50}) of the active NPs are in the same range with respect to PRO 2000 (a 5000-MW naphthalene

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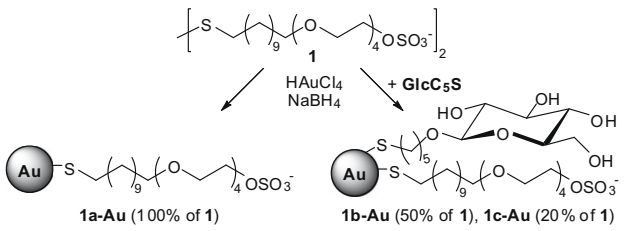

Scheme 1. Sulfated gold nanoparticles **1**-**Au** having different densities of the amphiphilic ligand **1** and the glucose conjugate **GlcC$_5$S**.

sulfonate polymer in human phase III efficacy trials as HIV microbicide) in similar cellular assays.[9]

The preparation of **1a**-**Au** was realized using an aliphatic-tetraethyleneglycol which has a terminus ending in a disulfide group and the other one in a sulfate group (compound **1**, Scheme 1). The synthesis of **1** was realized in two steps starting from [1-(thioacetyl)undec-11-yl]tetra(ethylene glycol) (see Supplementary data). The long aliphatic chain guarantees the formation of ordered and stable self assembled monolayers on gold, while the tetraethyleneglycol moiety ensures flexibility, assists water solubility and defends against non-specific adsorption of other biomolecules and cells.[10] Hybrid NPs **1b,c**-**Au** incorporating ligand **1** in different ratios with the biocompatible and inert 5-(thio)pentyl D-glucopyranoside (**GlcC$_5$S**)[11] were also prepared (Scheme 1). The one-pot synthesis and characterization of NPs, as well as the control of the different ligands proportions, were achieved following previously described procedures (see Supplementary data).[11] The average molecular weights and number of sulfated ligand **1** per NP (see below, Table 1 and Table 1S in Supplementary data) were calculated from the gold cluster size (as determined by transmission electron microscopy, TEM) and elemental analysis. Compound **1a**-**Au** displayed around 140 sulfated ligands in a gold cluster of 1.7 nm average diameter. Compound **1b**-**Au** was bigger in size (2.6 nm diameter) and approximately accommodated the same number of sulfated ligands, in addition to **GlcC$_5$S** as second ligand. Similarly to **1b**-**Au**, **1c**-**Au** had an average gold cluster diameter of 2.3 nm, but displayed less sulfated ligands (~55), the rest being the inert component **GlcC$_5$S**. TEM images of as-synthesized NPs also showed that **1**-**Au** are well dispersed. No gold surface plasmon signal around 520 nm was observed in UV–vis spectra due to the small size of the NPs.

The binding activities of sulfated NPs **1**-**Au** and ligand **1** to gp120 were qualitatively evaluated by SPR using a ProteOn™ XPR36 system. Gp120 was covalently immobilized on the sensorchip by standard methodology at a level of 6000 RU and the substrates were injected at six different concentrations (2, 1, 0.5, 0.25, 0.125, and 0.0625 μg mL^{-1}) in TRIS buffered saline at 25 °C (see Supplementary data). NPs **1a**-**Au** and **1b**-**Au** showed dose-dependent binding affinity to gp120 in this concentrations range (Fig. 1S), while NPs **1c**-**Au** (Fig. 1) did not give apparent binding at these concentrations. Taking as a reference point the binding response unit (RU) at 30 s in the association phase of the sensorgrams, **1a**-**Au** and **1b**-**Au** gave 97 RU and 25 RU (respectively) at 2 μg mL^{-1} (Fig. 1) which corresponds to 2.6 μM and 1.3 μM average concentration in terms of sulfate ligands, respectively (Table 2S). To ensure that the binding effect was due to the sulfate moieties, gold NPs in which the sulfate group of **1a**-**Au** is formally substituted by a phosphate group were also tested. These NPs (**Au**-**OPO$_3$$^{2-}$**) were prepared using the same methodology and resulted practically identical in size to **1a**-**Au** (Supplementary data). The phosphate group was chosen because, in spite of its anionic character, it is known to have poor anti-HIV activity with respect to the sulfate.[12] No significant binding of **Au**-**OPO$_3$$^{2-}$** to gp120 was detected at 2 μg mL^{-1} (<5 RU, Fig. 1). These data discard an unspecific effect of the gold core and demonstrate that the sulfate group is essential to achieve efficient binding activity. No detectable binding to gp120 was observed with compound **1**, which presents two sulfate units, at 2 μg mL^{-1} (4.2 μM in terms of sulfate ligands) (Fig. 1 and Table 2S).

Encouraged by the SPR screening, ligand **1** and sulfated NPs **1**-**Au** were tested as anti-HIV systems in cell-based experiments. To evaluate their antiviral activity, MT-2 cells were infected with the X4 tropic NL4.3 Renilla HIV-1 recombinant virus in the presence or absence of the tested compounds. In this neutralization assay, inhibition of HIV-1 infection of MT-2 cells was assessed by use of a recombinant virus carrying the Renilla reporter genes.[13] Briefly, NL4.3 Renilla HIV-1 recombinant virus was pre-incubated with different concentrations of the tested compounds for 30 min. Afterwards, viral supernatants were used to infect target MT-2 cells. Viral replication was assessed 48 h post-infection by luciferase activity in cell lysates (see Supplementary data). The obtained results (Fig. 2) indicate that the degree of sulfate density on the gold surface has an important effect on the inhibition of HIV-1 infection of MT-2 cells. Gold nanoparticles **1a**-**Au** and **1b**-**Au** covered with 100% and 50% density of the sulfated ligand, respectively, were more active than **1c**-**Au** (20% density) at concentrations of 10 μg mL^{-1} and higher. In this range of concentrations, NPs **1c**-**Au** and ligand **1** displayed a similar behavior. In the same assay, the phosphated NPs **Au**-**OPO$_3$$^{2-}$** were unable to significantly inhibit viral replication even at 100 μg mL^{-1} (Fig. 2), demonstrating that the inhibitory effect of **1a**-**Au** and **1b**-**Au** was neither due to the gold core of the nanoparticles nor to the aliphatic-tetraethyleneglycol moiety of the ligands. The inactivity of NPs **1c**-**Au** (80% density of **GlcC$_5$S**) at the concentrations tested shows that glucose moieties do not contribute to antiviral activities of **1b**-**Au**.

The IC$_{50}$ values were calculated from concentration–response curves as reported in Figure 2S. As shown in Table 1, NPs **1a**-**Au** and **1b**-**Au** coated with 100% and 50% density of the sulfated ligand inhibited HIV-1 infection with an IC$_{50}$ of 1.29 and 2.32 μg mL^{-1} respectively. Ligand **1** and NPs **1c**-**Au** coated with 20% density of

Table 1
Inhibitory activity of compounds against HIV-1 infection of MT-2 cells in comparison with the candidate microbicide PRO 2000

Compd	MW, KDa[a]	N_{TL}[a]	N_{CL}[a]	IC$_{50}$ (μg mL^{-1})	IC$_{50}$ (μM)	IC$_{50}$[b] (μM)
1	0.963	2	2	>10	>10.4	>20.8
1a-**Au**	108	140	140	1.29	0.012	1.68
1b-**Au**	216	272	136	2.32	0.011	1.46
1c-**Au**	202	272	55	>10	>0.050	>2.7
Au-**OPO$_3$$^{2-}$**	108	140	140	>100	>0.93	>130
PRO 2000	5	—	21[c]	1.9[d]; 0.43[e]	0.38[d]; 0.086[e]	7.98; 1.81

[a] Average molecular weights (MW), average number of total ligands (N_{TL}) and average charged ligands (N_{CL}) per compound.
[b] IC$_{50}$ expressed in concentration of charged ligands.
[c] Taken from http://chemdb2.niaid.nih.gov/struct_search NIAID Database.
[d] Taken from Ref. 9a; PM-1 cells and HIV-1 RF (X4) were used.
[e] Taken from Ref. 9b; U87.CD4.CXCR4 cells and HIV-1 HxB2 (X4) were used.

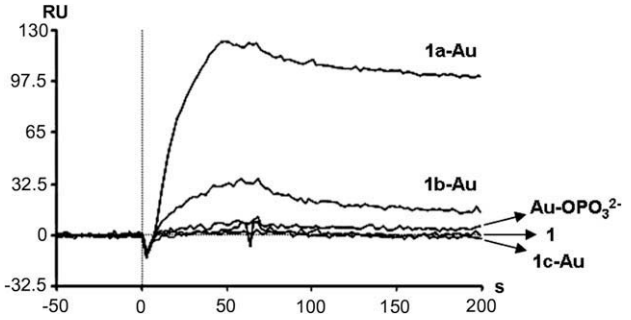

Figure 1. Analyses of substrates' binding to gp120 at 2 μg mL^{-1} (sensorgrams of **1** and **1c-Au** overlap at the baseline).

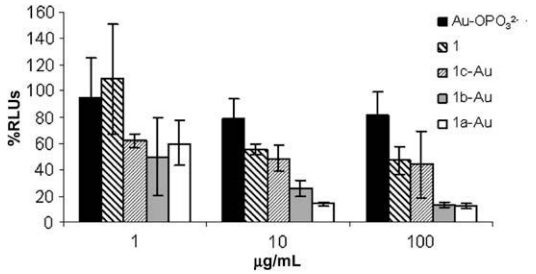

Figure 2. Neutralization assays. Inhibition of recombinant virus NL4.3 Renilla HIV-1 (X4) infection of MT-2 cells at different concentrations of tested compounds (sulfated ligand **1** and sulfated nanoparticles **1a-Au**, **1b-Au** and **1c-Au**). **Au-OPO$_3$$^{2-}$** was used as a negative control. Results are expressed as percentages of infection related to untreated control. Data represent the mean of three independent experiments ± SEM (Standard Error of the Mean).

the sulfated ligand were not able to reach an IC_{50} at the concentrations tested. This trend is in agreement with the results obtained by SPR. In order to better rationalize the results, the IC_{50} values were also expressed in terms of molar concentration of each compound and further transformed into molarity of charged ligands (Table 1S). NPs **1a-Au** and **1b-Au** afforded IC_{50} in the nanomolar range in NPs concentration and in the low micromolar range in terms of sulfated ligands (Table 1). The high degree of sulfation and molecular weight of the Au-NPs,[6a,7] in tandem with the higher local concentrations of the ligands compared to the disulfide dimer **1** and a supposable multivalent effect,[14] may origin the increased anti-HIV activity that results from attaching the sulfated ligand to the gold nanocluster. The anti-viral activity of sulfated NPs **1a-Au** and **1b-Au** is comparable to that reported for PRO 2000. In similar assays, Shattock[9a] and Sattentau[9b] reported that PRO 2000 has IC_{50} values of 1.9 μg mL^{-1} and 0.43 μg mL^{-1}, respectively. The differences in IC_{50} values are mainly related to the cell and virus types used. Expressed in molarity, these values are in the same order of magnitude of what we found for **1a-Au** and **1b-Au** in our experiments (Table 1). NPs **1a-Au** and **1b-Au** thus proved to be potent inhibitors of X4 HIV. This also indicates that a 50% density of the sulfated ligand on the gold platform is enough to obtain a significant anti-HIV effect.

To ensure that NPs antiviral activity was not due to toxicity, cell viability was measured in the same conditions of the neutralization assay, but no virus was added to the cell culture. Compounds were tested up to a concentration of 100 μg mL^{-1} and were compared to untreated controls. No toxicity was found and only mild toxic effect was observed with **1a-Au** at 100 μg mL^{-1} (70% of cell viability, see Fig. 3S).

In conclusion, multivalent gold nanoparticles coated with sulfated ligands can be considered a novel alternative to the known anti-HIV systems for targeting the adsorption/fusion process of the virus infection. Depending on the ligand density, these NPs can bind gp120 with high affinity as shown in SPR-based experiments and neutralize the in vitro HIV direct infection of T-lymphocytes in the nanomolar range. We demonstrate that a 50% percentage density of sulfated ligands on ~2 nm nanoparticles is enough to achieve anti-HIV activities which are comparable to those obtained with NPs completely coated with sulfated ligands, opening up the possibility of tailoring other anti-HIV molecules on the same gold cluster. The sulfated NPs are stable and well dispersible in water or physiological buffers, and not cytotoxic to MT-2 cells. Since many pathogens (especially enveloped viruses) suffer polysulfates-mediated inhibition in their early steps of infection, these Au-NPs may be potentially applied for the prevention of other infectious agents. We have recently demonstrated that *manno*-GNPs inhibit the DC-SIGN-mediated HIV-1 *trans*-infection of human T-cells even at medium density of high-mannose oligosaccharides on the nanometric gold clusters.[5] On the contrary, polyanionic compounds that strongly inhibit HIV entry by targeting selected basic surfaces of gp120[6a,8] are almost ineffective in impeding the transfer of HIV from DC-SIGN-expressing cells to T-lymphocytes.[15] In pursuit of microbicide candidates, more potent and/or less toxic agents may rise from combining different anti-HIV systems. Sattentau observed that cocktails of polyanions and other antiretroviral agents with microbicidal potential can act synergistically.[9b] The possibility of simultaneously tailoring on the same gold nanoplatform both sulfated ligands and other active molecules which target different stages of the HIV life cycle makes gold nanoparticles an appealing scaffold which can contribute to the development of new multifunctional anti-HIV systems.

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Supplementary data

Synthesis and characterization of ligands and nanoparticles used in this work. Selected SPR sensorgrams, cytotoxicity study and cellular based experimental details are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.079.

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