DNA Nanotechnology

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Thermostable and Monoconjugable Gold Clusters with a Dodecadentate Thioether Ligand Gripper**

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The Schmid cluster,[1] $[Au_{55}(PPh_3)_{12}Cl_6]$, has stimulated many different areas of technology ranging from catalysis research[2] to the concept of quantum electronics.[3,4] Monofunctionalized water-soluble derivatives of this cluster[5] have become commercially available and found numerous applications.[6–14] Recently, gold clusters have been employed as universal fluorescence quenchers in molecular beacons[15] and as nanoscale antennas for the reception of radio radiation in the GHz frequency region, which causes local and selective inductive heating of cluster-labeled biomolecules.[16] These applications as well as current experiments in DNA nanotechnology[17–23] point to the need for the increased thermostability of these gold clusters. We have shown that replacement of the monopodal triphenylphosphane ligands in such clusters with water-soluble tripodal thioethers based on 1,3,5-tris(thiomethyl)benzene leads to gold clusters with improved stability.[24]

Herein, we report a new generation of biocompatible gold clusters that are surrounded by a single dodecadentate, nanoscale thioether-based "gripper". This method allows nanoparticles to be singly functionalized without the otherwise necessary step of purification of mixtures by HPLC.[25] We demonstrate that "gripped" clusters in oligonucleotide conjugates can survive the temperature conditions of polymerase chain reaction (PCR) and hybridization experiments.

The concept behind our ligand is sketched in Figure 1. The Au_{55} cluster was proposed to possess a cuboctahedral core, the surface of which is composed of eight corner-sharing triangles (111) and six squares (110).[1] Although there is an ongoing debate[26,27] about the cluster geometry, we decided to adopt this proposal as a working hypothesis. Au_{55} is expected to bind four tripodal ligands provided that the binding mode of the ligands is comparable to that of the Schmid cluster, in which the 12 triphenylphosphanes most likely occupy the corners of the cuboctahedron. If the four tripodal ligands are connected by suitable linkers, a dodecadentate ligand will result. The synthesis was also designed so that the final material carries a single functional group to allow conjugation to (bio)molecules.

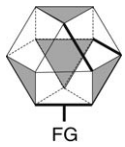

Figure 1. General concept of a gold cluster gripper. Four tripodal units (1,3,5-tris(thiomethyl)benzene groups) occupy four (111) faces of the cuboctahedral cluster. The tripodal units are linked (bold lines) to give a dodecadentate ligand, which also bears a functional group (FG) suitable for conjugation to a biomolecule.

Scheme 1 shows the synthesis of the target ligand **8**. We selected a monomaleimido group as the monoconjugable unit to ensure functional comparability with the commercially available variant of the Schmid cluster. Thioether **2** was generated from trisbromide **1** as a key precursor that allowed the synthesis of **8** by a divergent–convergent route in only six steps with an overall yield of 31%. The gold cluster was synthesized from ligand **8** by phase-transfer synthesis, as previously described for the tristhioether derived from L-cysteine.[24] High-resolution transmission electron microscopy revealed a narrow size distribution of the clusters with a mean diameter of 1.4 nm, which is as expected for Au_{55} particles. The cluster was conjugated with a 5′-thiol-modified 27-mer oligonucleotide, the complement of which was labeled with fluorescein in the 3′ position.

To study the thermostability of the label, a thermal-stress experiment was performed in which the fluorescence signal of a mixture of both oligonucleotides was monitored during 100 cycles of temperature variation. Each cycle consisted of a period of heating from 20 to 95 °C at 10 K min^{-1}, a thermal-stress period for 6 min at 95 °C, and a period of cooling at a rate of 20 K min^{-1}, which corresponds to an average temperature of 70.5 °C. The process of the temperature cycling experiment is depicted in Figure 2. At low temperatures, the duplex holds the gold cluster and the fluorescent dye within spatial proximity. Quenching of fluorescence takes place, which results in low fluorescence intensity. At high temperatures, the fluorescent oligonucleotide exists in its single-strand form, and an increase in fluorescence signal is observed

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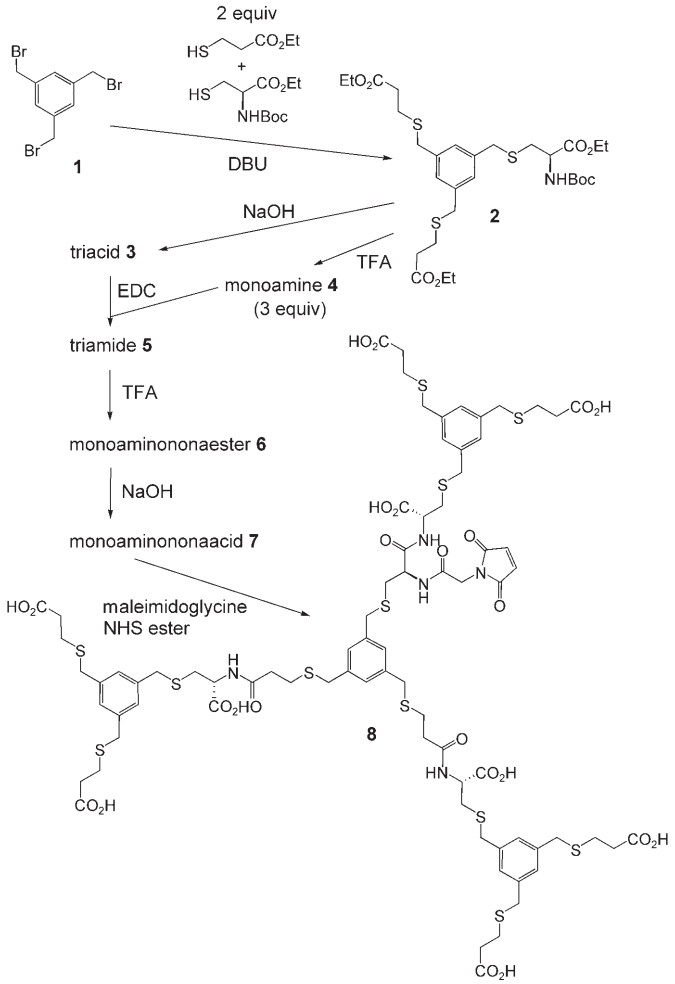
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Structure and synthesis of the dodecadentate ligand **8** bearing a monomaleimido functional group. Boc = *tert*-butyloxycarbonyl; DBU = 1,8-diazabicyclo[5.4.0]-7-undecene; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TFA = trifluoroacetic acid; NHS = *N*-hydroxysuccinimide.

because the gold cluster is no longer in proximity to act as a quencher. If the gold cluster escapes the grip of the ligand during a cycle, or if the conjugation with the complementary strand breaks, no fluorescence quenching will be observed in the following cycle. Thus, kinetic information about the stability of clusters and their oligonucleotide conjugation can be derived from the increase in low-temperature fluorescence as a function of the cycle number (and thus duration of thermal stress). Figure 3 depicts the development of the

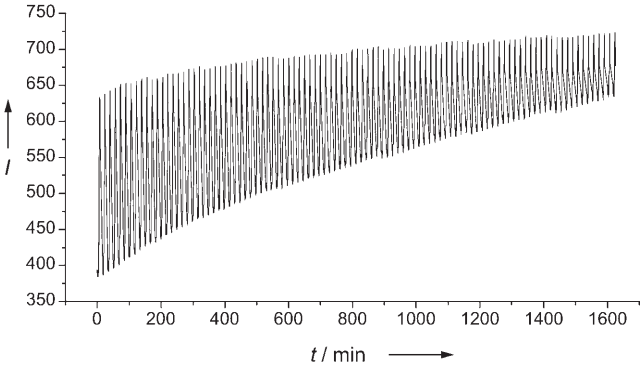


Figure 3. Fluorescence intensity (*I*) during 100 temperature cycles of a solution containing 1 μM 5′-ATGCACCCATTGGACATAACCGGGAAT-3′-FAM, 3 μM 5′-SH-modified complementary oligonucleotide conjugated with a mixture of gold-filled and unfilled grippers **8**, 300 mM NaCl, and 100 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) at pH 7.5.

temperature-driven fluorescence oscillations as a function of time. Fitting of the fluorescence minima to a model involving first-order decomposition of the cluster yielded a half-life of $\tau_{1/2} = 55 \pm 5$ cycles, which corresponds to 900 min for an average temperature of $T = 70.5\,^{\circ}\mathrm{C}$. The increase of the maxima of fluorescence oscillations in Figure 3 is due to a slight evaporation of water from the cuvette, which could not be avoided completely at the high temperatures employed.

Control experiments (see the Supporting Information) allow us to draw the following conclusions: There is evidence

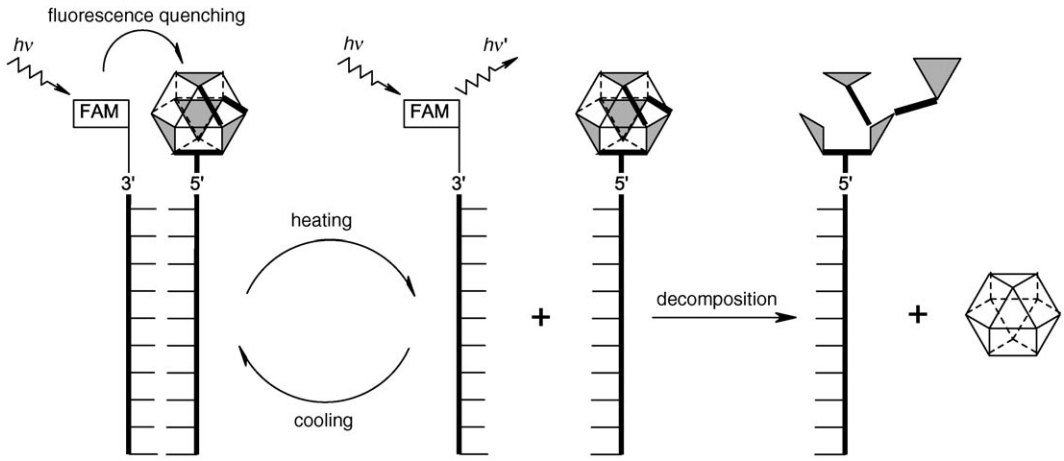


Figure 2. Chemical and physical processes underlying the determination of the cluster stability in the thermal-stress experiment. The fluorescence of the 3′-fluorescein labeled oligonucleotide in the presence of the complementary 5′-gold cluster is measured during the heating/cooling cycles. FAM = fluoroscein amino modifier.

from a comparison of experiments involving the empty and the filled gripper that it is the gold cluster rather than the gripper itself that is the responsible for the quenching, which is as expected in view of the UV-absorption properties of the ligand. Moreover, the fluorescence of both the duplex without the gripper and the labeled single strand decreases with the temperature; the latter, however, shows a higher level of fluorescence intensity, which is attributable to the poorer stacking of the 3′-fluorescein with the single strand. The different baseline courses for the single- and double-stranded species also explain why a slight increase in fluorescence is observed at the melting transition for the duplex with the empty gripper.

Any conceivable application of gold clusters in DNA bio- and nanotechnology in which their remarkable properties are employed will require compatibility of the label with at least the basic procedures of molecular biology. To the best of our knowledge, the gripped cluster presented here is the first example of an Au_{55} monolabel surviving the temperature conditions of PCR and hybridization protocols. A typical PCR experiment may be equivalent to around 100 min of the heating experiments described above, considering that the exposure to a temperature of 95 °C lasts for only around 1 min in PCR whereas each of these heating cycles lasted 6 min at 95 °C. The average temperature in our experiment is close to that of PCR, so that one cycle in our experiment may be equivalent to four to six typical PCR cycles. It is evident from Figure 3 that only a small fraction (less than 10 %) of the gold nanocrystals did not survive the first 100 min of treatment. It should be emphasized that the full potential of universal fluorescence quenching (e.g. in multiplexed quantitative PCR of gene sets by employing beacon sets with different dyes in the same tube) and radio-frequency-induced single-molecule heating (e.g. for nanoscale robotics) can only be realized with thermostable cluster materials.[28]

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