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# RudraGold™ – a Thermally Stable and Kinetically Inert Schmid's gold-55 with Smaller Hydrodynamic Radius.

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Our aim is to develop oligonucleotide-gold and oligonucleotide-semiconductor "quantum dot" (QD) labeled probes that will provide gold-labeled and QD-labeled messenger ribonucleic acid or mRNA targets following *in situ* hybridization (ISH). Such gold- and QD-labeled targets will enable their correlative detection using complimentary multi-modal microscopic methods at low- (optical imaging following further autometallographic (AMG) amplification), intermediate- (fluorescence imaging), and high-resolution (electron microscopy or EM) with improved sensitivities. We tested oligonucleotide-ultrasmall gold (commercial **Nanogold®** with 1.4 nm metal core diameter and ~2.7 nm overall diameter; Nanoprobes, NY 11980, USA) [1]. However, dark metal granules and precipitates were observed following hybridization [2]. One successful oligonucleotide-Nanogold® hybridization has been reported with under moderate conditions (46 °C for 3 h) [3]; the reported oligonucleotide-Nanogold® conjugate yield was up to 30% [3]. In general, higher temperatures and longer hybridization times are preferred for ISH, and Nanogold® and QDs do not survive the hybridization step(s)

Commercial Schmid's gold-55 clones (Au-55), as judged by their size and electronic absorption spectra, have major disadvantages: i) they are very pricy (Nanogold® ~\$4.5x$10^{+4}$/g; Arora® ~\$1.4x$10^{+6}$/g; ii) they are thermally unstable; both decompose above 50 °C [1,2,4]; and iii) the stabilizing ligand coatings increase their overall size and hydrodynamic radii, that in turn reduce spatial resolution at EM. Further, phosphine ligands on the Au-55 undergo rapid exchange even at room temperature [5]. Surface ligand dissociation expose gold to destabilizing species, e.g., metal ions and bio-ligands, leading to cluster breakdown; and above 50 °C both Au-55 clones, Nanogold® and Arora®, disintegrate

We have undertaken the task of producing thermally stable, affordable, and reduced-sized gold probes. For this purpose, we have designed supramolecular assembly of multidentate ligands that wrap around Au-55 and gold nanoparticles (AuNPs), and firmly grab them. The new ligands are designed using Hard-Soft Interaction Principle; they are derived from sulfur and nitrogen co-doped graphene like carbon (**N,S-GLC**), and strongly bind to Au-55 and AuNPs based on Pearson's Hard and Soft Acid and Base Theory. The ligand design utilizes Euler's formula [6], V + F – E = 2, that is related to five major components of polyhedrons, namely vertices (V), faces (F), edges (E), sides (S) and plane angles (P), and their interrelationship(s). Atoms are known to pack and form stable polyhedral arrangements (**Figure 1**). For example, truncated icosahedron or soccer ball has 12 regular pentagonal faces and 20 regular hexagonal faces (32 x F), 60 vertices (60 x V) and 90 edges (90 x E). The wraparound/grabbing N,S-GLC (**Figure 1** *h* and ***i***) can be synthesized by palladium-catalyzed cascade annulation of pentagonal/hexagonal aromatic rings, a strategy used to synthesize carbon "nanocones" [7]. The wraparound N,S-GLC derivatives produce thermally stable, reduced-sized Au-55 and AuNPs. **Figure 2** compares solution stability of our RudraGold™ stabilized with N,S-GLC with Nanogold® stabilized with triphenylphosphine derivatives. In phosphate buffered saline, pH 7.4, less than 10% of the original Nanogold® survives after 24 hours at 70 °C as compared to >90% of the RudraGold™. Further, because only one ligand wraps around Au-55 and AuNPs exclusively monofunctional probes are produced.

Currently, we are testing RudraGold™-thiol-phosphonamidite [8] and RudraGold-N-Fmoc-lysine-O-Bt [9] derivatives that enable the preparation of Au-55-labeled hybridization probes and peptide probes via automated solid-phase synthesis with high efficiency. Our thermally stable ~1.5 nm RudraGold™ will produce gold-labeled targets following ISH, that can be further amplified using AMG techniques for even higher detection sensitivities. Thermally stable, reduced-sized RudraGold™-labeled peptide and oligonucleotide probes prepared via solid-phase synthesis are expected to have significant impact on research and diagnostic studies.

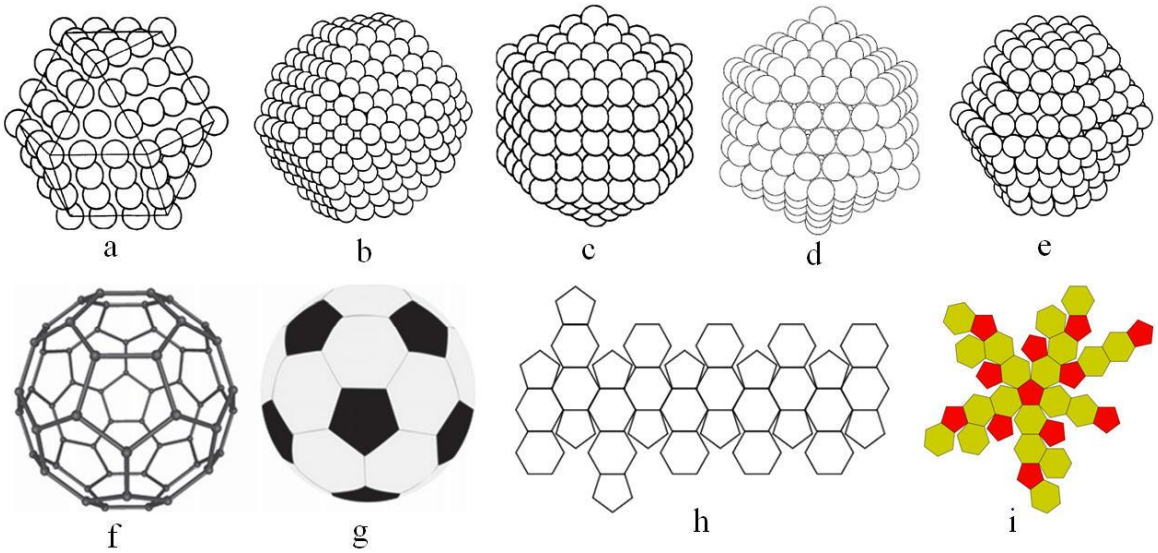


**Figure 1.** a) cuboctahedron; b) truncated octahedron; c) truncated decahedron; d) truncated icosahedron; e) truncated hexagonal bipyramid with triangular, square, pentagonal and hexagonal faces; f) truncated icosahedron; g) soccer ball, h) and i) arrangements of twenty hexagons and twelve pentagons that wrap around or grab truncated icosahedron as in soccer ball.

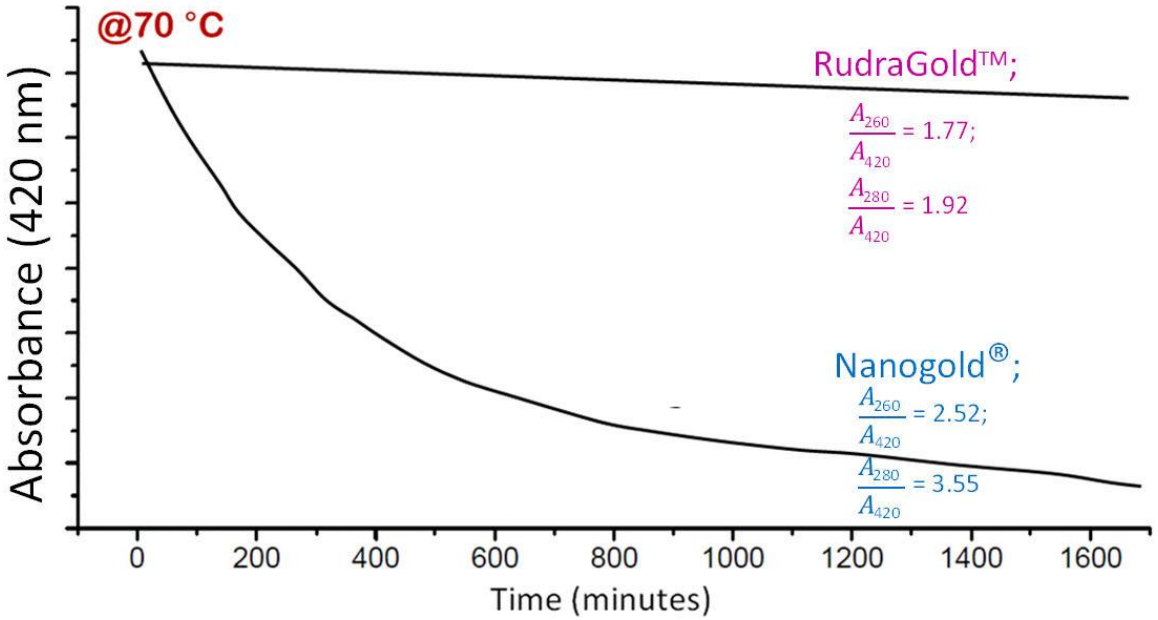


**Figure 2.** Thermal stability of equimolar RudraGold™ and Nanogold® in PBS, pH 7.4.

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