IMRA NanoBio Division

Technical Note T12

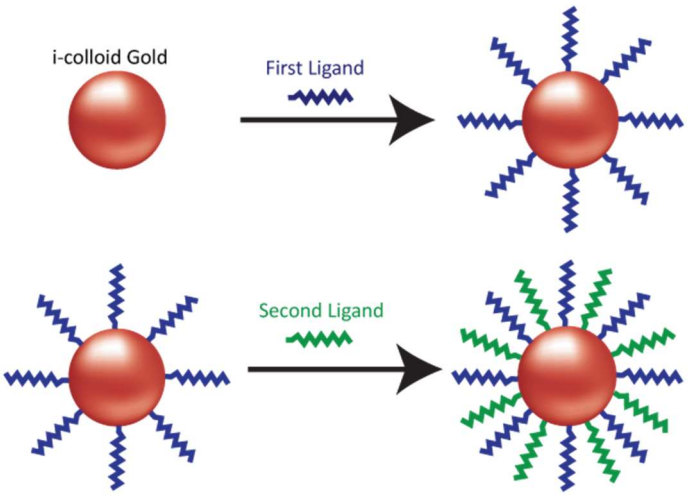
Sequential Conjugation of Multiple Ligands to i-colloid Gold

Multi-functional i-colloid gold conjugates

i-colloid gold can be multi-functionalized by sequential conjugation of ligands that bring various properties to the conjugates, such as targeting, detection, and stability.

A sub-saturating amount of first ligand is quantitatively bound to the gold surface (top), followed by subsequent binding of a different ligand that occupies the surface areas still available for binding (bottom). Both ligands can be bound by strong thiol-gold bonds.

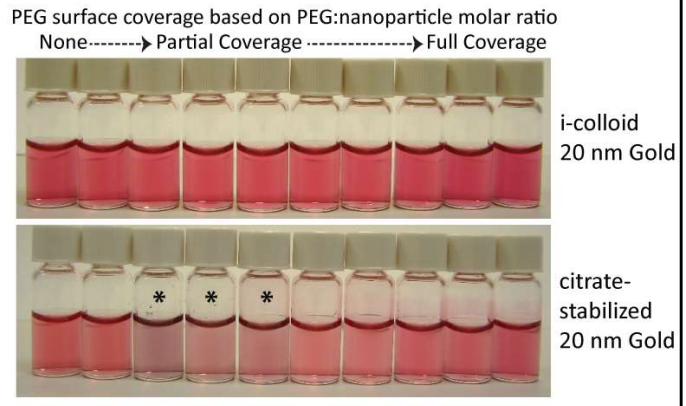
The density of multiple ligands on the gold surface can be quantitatively controlled to engineer multi-functional conjugates with tunable properties.



i-colloid gold stability during partial PEGylation

Sequential conjugation of multiple ligands onto i-colloid gold is enabled by the colloidal stability of nanoparticles with partial coverage of a first ligand, such as PEG-thiol molecules.

While i-colloid gold retains stability across a wide range of PEG surface coverage from partial to full saturation, citrate-stabilized gold nanoparticles can aggregate at partial PEG coverage making them unsuitable for sequential conjugation. A stability comparison of i-colloid and citrate-stabilized 20 nm gold at various PEG-5K surface coverage is shown.

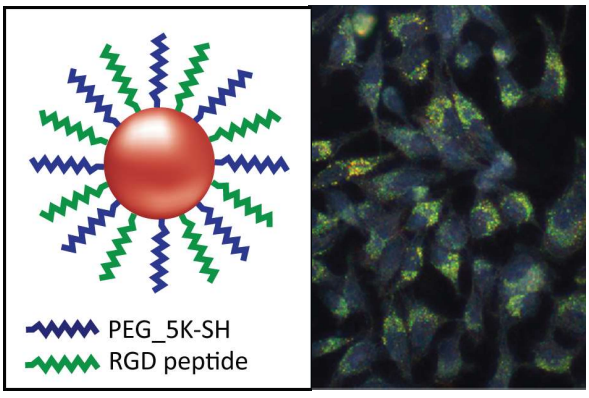


(Top) i-colloid 20 nm gold is stable at all PEG quantities
(Bottom) Citrate-stabilized 20 nm gold aggregates at sub-saturating PEG surface coverage, as indicated purple tint in vials marked *.

Cell imaging with gold conjugates

20 nm gold conjugates designed for cell imaging were generated by first binding PEG-5K-SH ligand at ~50% surface coverage followed by binding RGD peptide to occupy all remaining surface binding areas. The RGD peptide allows cell targeting, and the PEG provides conjugate stability in cell culture media.

The conjugates were incubated with HeLa cells for 1-2 hours, and dark-field microscopy detected green light scattered from the gold nanoparticles, demonstrating their utility for cell imaging.



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