Electronic Supplementary Information of

"Highly efficient gold nanoparticle disaggregation dimer formation via controlled DNA hybridization"

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DNA oligonucleotide sequences used to functionalize gold nanoparticles

Three different DNA oligonucleotides lengths were used: 11/16/21 nts. The sequences were designed to have a ~50% of CG content and to have the thiol-capping modification on 3' end of the strands according to constructor information. The oligonucleotides were purchased from Sigma-Aldrich with HPLC purification.

SEQUENCE NAME	SEQUENCE	LENGTH (NTS)	GC CONTENT	MELT. TEMP. °C
D11	5'- ATT CGC ACT GA[ThiC3] -3'	11	45,50%	21.6
D11R	5'- TCA GTG CGA AT[ThiC3] -3'	11	45,50%	21.6
D16	5'- ATA AGG CTG CAT GGC A[ThiC3] -3'	16	50%	51.3
D16R	5'- TGC CAT GCA GCC TTA T[ThiC3] -3'	16	50%	51.3
D21	5'- GAT TTA CCG TAG CGA TAC GTA[ThiC3] -3'	21	42,90%	51.6
D21R	5'- TAC GTA TCG CTA CGG TAA ATC[ThiC3] -3'	21	42,90%	51.6

Table S1. The three sequences of the DNA oligonucleotides used to functionalize the gold colloid surface and their complementary strand.

Dimer formation with 16 and 21 nts sequences

The dimer formation was carried on with all the three sequence lengths. The agarose gel electrophoresis analysis was performed several times, here we report the representative results for 16 and 21 nts DNA oligonucleotides functionalized NP.

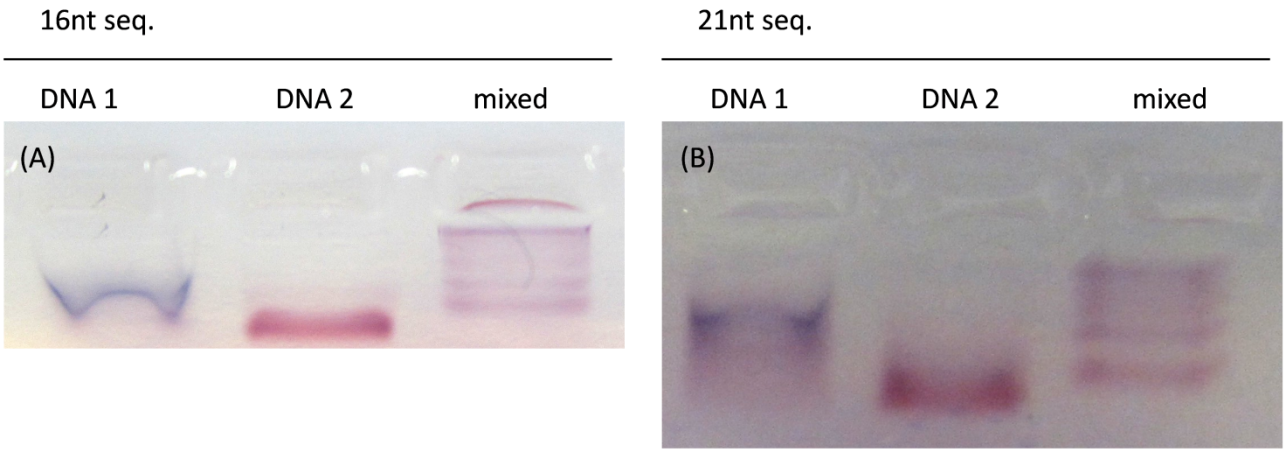

Figure S2. Agarose gel of 16 nt (A) and 21 nt (B) DNA 1 and 2 probes. In the mixed solution it's evident the separation of different discrete bands that demonstrate the formation of mono-di-trimer NP aggregates. The partial aggregation and not homogeneous run of the probe DNA 1 could be attributed to an affective secondary structure formation of DNA sequence.

Morphological characterization and statistical analysis of population of NP aggregates

To characterize morphologically and to demonstrate the correlation between 1st, 2nd, 3rd band and 1st, 2nd, 3rd order of NP aggregates, the gel band were electroeluted. The resulting solutions were analyzed with SEM. So performing a software analysis (ImageJ), a statistical investigation was carried on, in order to distinguish the different aggregates species. These data are represented in the graph in Figure S3(A), that demonstrates the presence of predominance of single, two and three particles in the first, second and third band of the S2(B) agarose gel respectively. The not negligible presence of crossover of the aggregates in all the bands it's ascribable to the inter-band contamination in the electro-elution process.

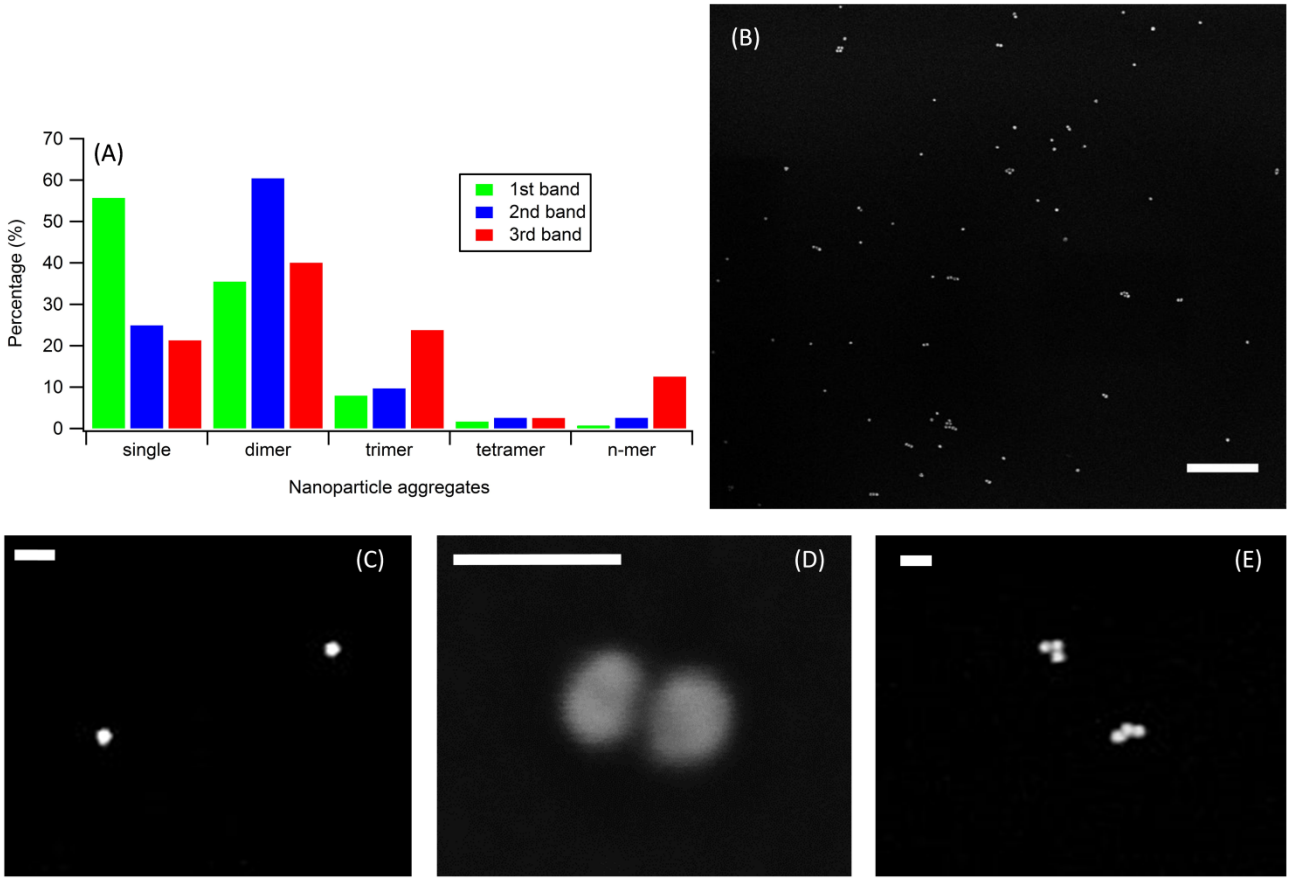

Figure S3. (A) Graph that shows the statistical distribution of single/dimer/trimer/tetramer and n-mer (>4) in the different bands. The calculated percentage of events regards the electro-eluted solution from the agarose gel (S2B) of the first, second and third band. It's evident a correlation between first, second and third band with one two and three NP aggregates respectively. Representative SEM micrographs of the solution with all the aggregates species (B), scale bar 300 nm. SEM micrographs of monomer (C), dimer (D), and trimer (E) NP aggregates, scale bars 40 nm.

Electrophoretic gel bands purity analysis

To investigate better the content of the electrophoretic gel bands, purity analysis were performed. In comparison to the electro-elution extraction process, the bands were extracted using D-Tube™ Dialyzers that permits to reduce drastically the cross contamination between the different bands. After the collection, the nanoaggregates were concentrated and imaged by Scanning Electron Microscope to perform statistical analysis. The latter highlights an high purity of dimers in the second band: 80 ± 10%. Otherwise was found 45 ± 10% of trimers in third band; this datum could be affected by the third band higher dispersion in the gel electrophoretic process. In total around 100 events were counted for each band.

Absence of free DNA oligonucleotides mediated NP aggregation

The removal of free DNA strands in a complete manner was reached performing 3 centrifugal steps (10 min at 16000 x g) discarding the supernatant. The absence of DNA not attached to the NP was tested with agarose electrophoresis with Gel Red™ DNA staining (Biotium).

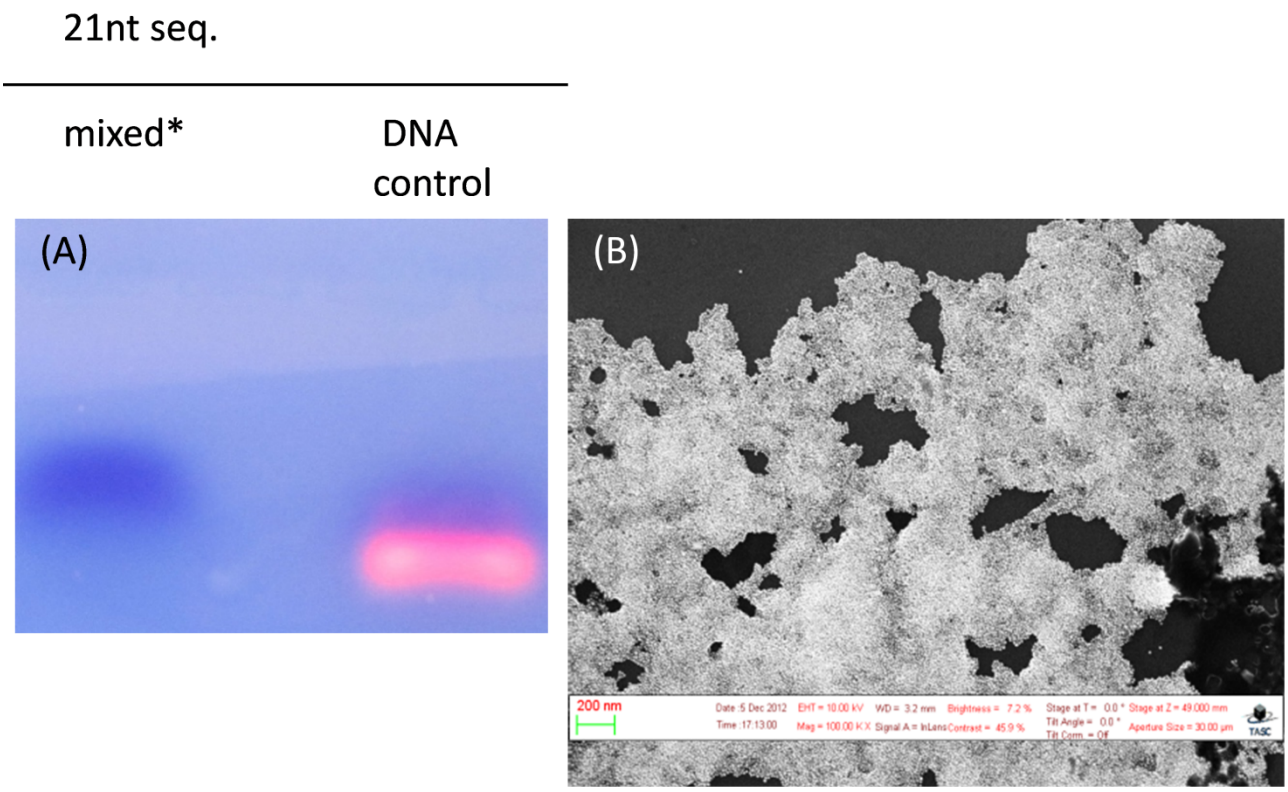

Figure S4. (A) Agarose gel panel that demonstrate the complete absence of DNA in mixed solution that was previously centrifuged 3 times discarding the supernatant (mixed*). In the DNA control was added only 21 nts sequence. The blue bands are due to the presence of Bromophenol blue

staining used to check the run front. (B) A representative SEM micrograph of the NP of mixed* sample: the evident complete aggregation do not allow the run into the gel.