# Supplemental information: Enhancing Surface Immobilization of Bioactive Molecule via Silica Nanoparticle Base Coating

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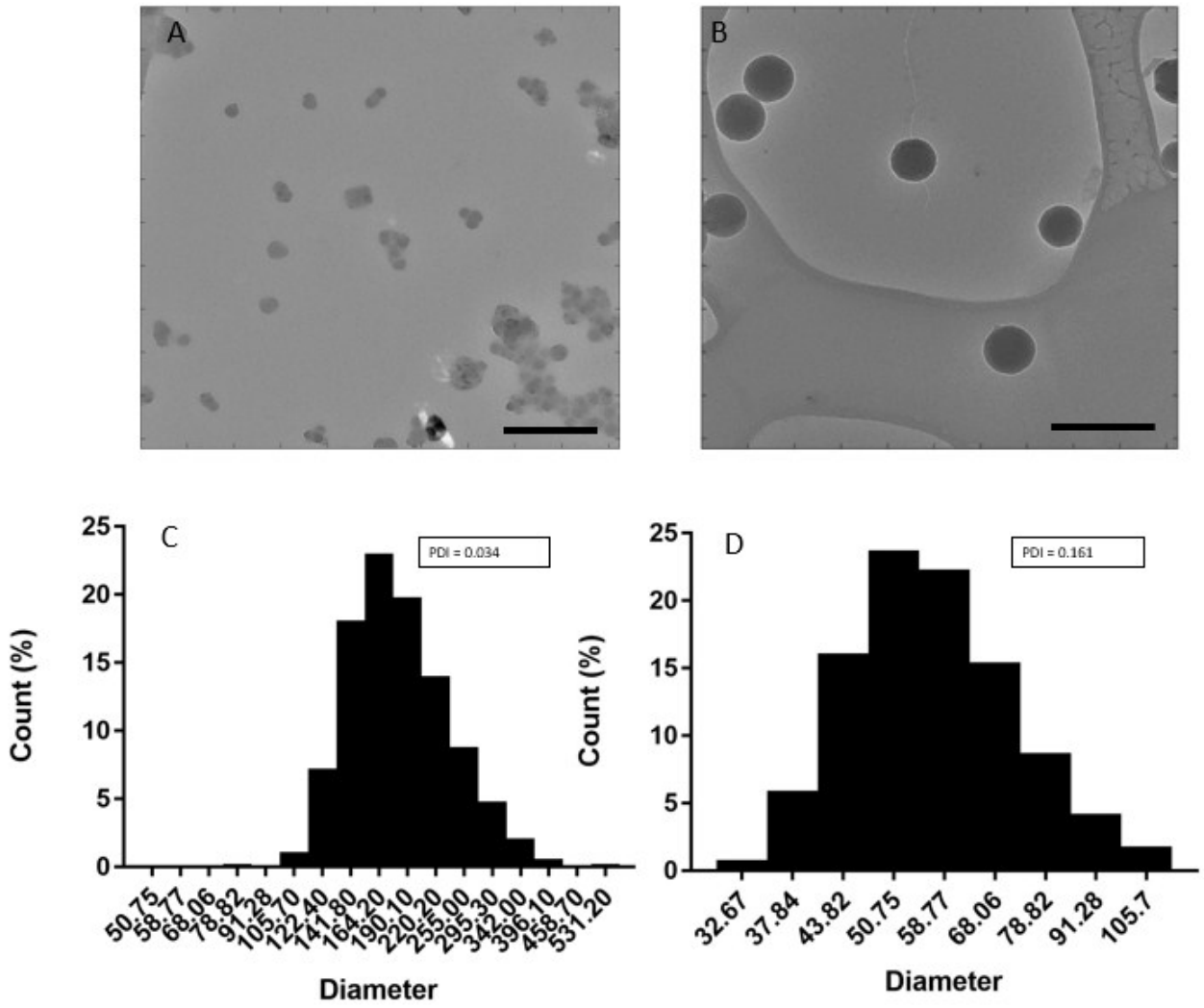
## Methods:

Fluorescently labelled TNP (fTNP) were synthesized in a similar manner to TNP. 50mL of 0.014M NaOH in $H_2O$ was heated to 70$^o$C. Prior to the addition of TEOS, 200mg of Fluorecein sodium salt was added to the solution. From this point, everything was kept isolated from light to avoid photobleaching. While vigorously stirring, 500µl of tetraethyl orthosilicate (TEOS) was quickly pipetted into the flask. After 5 minutes, 100µL of mercaptopropyl trimethoxysilane (MTS) was added and the solution reacted for 2h to form a slightly cloudy nanoparticle suspension. Nanoparticles were collected by centrifugation. fTNP were immobilized in the same fashion as TNP.

Scotch tape assay was performed on silicon with fTNP immobilized. fTNP on Silicon samples were split into 2 group, 1 was thoroughly washed to remove any unbound particles, while 1 was gently rinsed to keep loosly bound particles attached. For washed samples, substrates were rinsed under direct $H_2O$ stream with a water squirt bottle for 2-3 minutes then dried under vacuum. Gently rinsed samples were dipped and swirled in water, then air dried. Samples were place fTNP-side down on the scotch tape with firm pressure from behind. Once the silicon was removed, the scotch tape was placed over a glass microscope slide and examined by fluorescence microscopy.

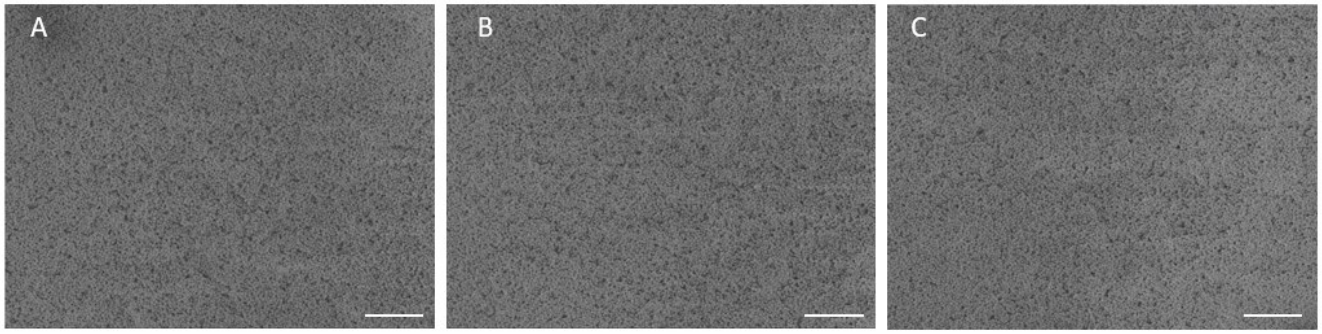
Results and discussion:




**Sup. Figure 1.** (A) TEM image of TNP particles without prior reduction treatment with TCEP, causing aggregation. Scale bar = 500nm. (B) TEM image of TNP particle with prior reduction in TCEP. Scale bar = 100nm. DLS measurements of TNP diameter without (C) or with (D) prior reduction treatment with TCEP. DLS measurements of the TNP diameter displayed as a histogram of percentage per bin

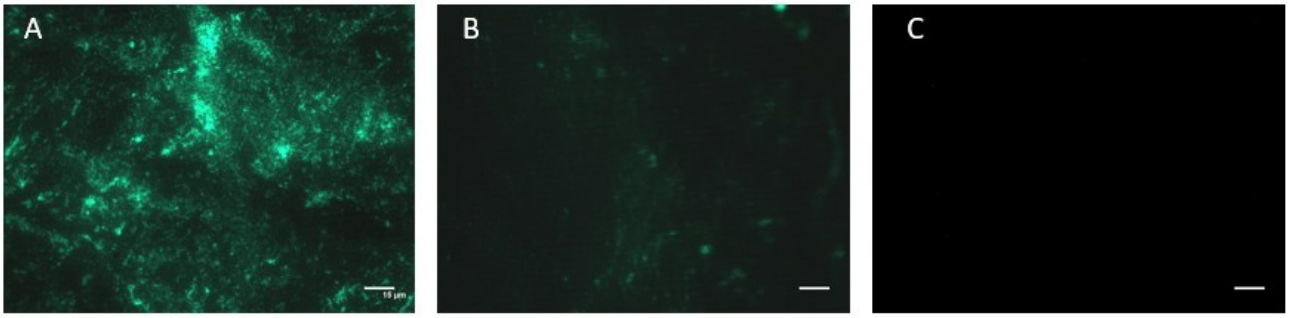
Nanoparticle size was observed by TEM and measured by dynamic light scattering (DLS) (Sup Fig. 1). Thiolated NPs have the tendency to react with each other via disulphide bonds, causing aggregations as seen in the TEM image (Sup Fig 1A). To dissociate the TNPs. TCEP was used to pre-treat the NPs and reduce the disulphide bonds. Upon TCEP treatment, TNPs were successfully dissociated with each other as seen in the TEM image (Sup Fig. 1B). The DLS measurement showed that nanoparticles have a median diameter around 50nm, with a poly dispersity index of 0.161 (Sup Fig. 1D). Without the reduction treatment by TCEP, DLS measurement showing a larger mean diameter, larger range, and much lower PDI of 0.034 compared to the reduced form. Since DLS analysis assumes spherical particle shape, the DLS measurement of the aggregated particles is no longer reliable, as can be seen from the unusually small poly dispersity index of 0.034. Further, small presence of large particles may greatly affect the accuracy of the measurement[1].

Particle coatings were tested for mechanical stability by sonication. Prior to sonication, select samples were removed to be examined by SEM (Sup Fig. 2A). Similarly, after 5 and 30 minutes of sonication, SEM was performed on the perturbed samples (Sup Fig. 2B,C). Qualitatively, there is no differences between to coating conditions. Coating roughness is further quantified by ellipsometry (figure 1).




**Sup. Figure 2.** SEM of nanoparticle coated silicon un-sonicated (A), after 5 minutes of sonication (B), and after 30 minutes of sonication (C), scale bar = 1µm

Stability was further tested qualitatively by a scotch tape assay (Sup Fig. 3). fTNP were substituted for TNP prior to nanoparticle immobilization. Fluorescent imaging of the washed fTNP surface shows clear binding of the nanoparticles to the silicon substrate. The tape was examined under fluorescence microscopy to determine if fTNP were removed from the substrate. Samples which were not thoroughly washed showed nanoparticle detachment, demonstrating the ability of these particle to be detected by our microscope. Thoroughly washed samples did not leave a fluorescent outline after removal, suggesting a strong interaction between the silicon and the fTNP.




**Sup. Figure 3.** (A) Fluorescent image of fTNP immobilized on GTS modified silicon after thorough washing. (B) without washing, a fluorescent residue is left on the surface of the tape by the poorly bound fTNP. (C) After washing, only well adhered particles remain, resulting in no residue left on the tape, scale bar = 15 µm

Supplemental references:

1. Filipe, V.; Hawe, A.; Jiskoot, W., Critical Evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the Measurement of Nanoparticles and Protein Aggregates. *Pharmaceutical Research* **2010,** *27* (5), 796-810.