The conjugation of fluorescent silica nanoparticles (SiO₂NPs) with biomolecules can be performed in different ways. We focus here on two strategies: adsorption of biomolecules through electrostatic interactions and covalent bonding through 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry. Using monodispersed and stable NPs with well-defined chemical-physical properties (e.g., size, shape, surface charge, and stability) facilitates the functionalization process and allows elevated control over the number and orientation of attached biomolecules. It is helpful to characterize the NPs at each modification step to have direct control over functionalization.

**Fluorescent Silica Nanoparticle Functionalization with DNA**

The functionalization of fluorescent silica nanoparticles (Prod. No. 797952) with oligonucleotides, plasmids, and siRNA is possible through molecular adsorption on the nanoparticle surface. The adsorption of biomolecules on a nanoparticle occurs via electrostatic interaction between differently charged moieties. Before the adsorption of DNA, NPs are functionalized with amine groups in order to create a positively charged surface that will interact with negatively charged DNA molecules (Figure 1). Generally, the silane 3-aminopropyltriethoxysilane (APTES) is most frequently used to introduce –NH₂ groups to the NPs surface. After the functionalization, it is advisable to characterize the NPs using Z-Potential measurements to confirm that the surface charge has changed from negative to positive. DNA-functionalized fluorescent NPs can be used in several applications, such as imaging, diagnostics, and gene and drug delivery.

**Protocol**

1. Prepare 3 microtubes with 100 µg of fluorescent SiO₂NPs each. One aliquot can be used as a control, whereas the other 2 can be used to test the adsorption of different amount of oligonucleotides, plasmid vectors, RNA, or DNA.
2. Disperse NPs in 1 mL of 1 mM acetic acid and sonicate for 5 minutes in a sonicator bath, or until the NPs are completely redispersed.
3. Add 50 µL of APTES to the NPs solution to introduce –NH₂ groups to the NPs surface.
4. Stir for 1 hour at room temperature.
5. Precipitate NPs by centrifuging for 30 minutes at 4,200 × g.
6. Remove the supernatant and add 1 mL of water.
7. Repeat steps 5−6 four times to ensure any excess of APTES is removed.
8. Verify the change in NP surface charge from negative to positive through the measurement of Z-Potential using a Zetasizer (Figure 2).
9. Precipitate the NPs in the three microtubes by centrifuging for 30 minutes at 4,200 × g.
10. Remove the supernatant and disperse 3 NP aliquots in TBE (45 mM Tris, 45 mM boric acid, and 1 mM EDTA).
11. To quantify the DNA binding capability of the amino-functionalized NPs, add increasing amounts of oligonucleotides (0.5 and 2 µg)¹ to the two aliquots and incubate for 2 hours. The third aliquot of NPs is used as a control.
12. After incubation, load the mixture of NPs and DNA (Figure 3, columns 2, 3) on a 1% agarose gel in TBE buffer containing SYBR® Green dye. Also, load two control samples in the gel: the amine modified NPs (Figure 3, column 4) and oligonucleotide (Figure 3, column 1).

**Materials**

- Fluorescent SiO₂NPs (See full product list at end of this section)
- Acetic acid (Prod. No. 45726)
- APTES (Prod. No. 440140)
- TBE (45 mM Tris, 45 mM boric acid, and 1 mM EDTA)
- 1% agarose gel
- SYBR® Green dye (Prod. No. L6544)