Application note

Observation of gold nanoshell plasmon resonance shifts after bioconjugation

Using the NanoDrop One Microvolume UV-Vis Spectrophotometer

Authors

Kejian Li[1], Megan N. Dang[1], Alexis B. Duffy[1] and Emily S. Day[1,2,3]

[1] University of Delaware, Dept. of Biomedical Engineering, Newark, DE, USA

[2] University of Delaware, Department of Materials Science and Engineering, Newark, DE, USA

[3] Helen F. Graham Cancer Center and Research Institute, Newark, DE, USA

RNA interference (RNAi)-based therapy has shown great potential in improving the study and treatment of diseases whose genetic underpinnings are known. However, challenges such as susceptibility to nuclease degradation, low cellular uptake, or rapid clearance from circulation impede the successful preclinical and clinical application of RNAi therapeutics.[1] To overcome these limitations, small interfering RNAs (siRNAs) or microRNAs (miRNAs) can be conjugated to nanoparticles (NPs), such as nanoshells (NS), to improve their stability, cellular uptake, and blood circulation time, thus resulting in increased effectiveness.[2, 3, 4]

Prior to using RNA-NP conjugates in therapeutic applications, it is critical to confirm successful RNA conjugation to the NP. One common method to confirm molecule loading onto gold-based NPs involves evaluating the surface plasmon resonance (SPR) spectra of the NPs before and after functionalization; successful RNA attachment will typically cause a slight red-shift in the peak SPR wavelength. Traditionally, UV-Vis spectrophotometers are used to analyze the optical properties of gold-based NPs. For example, the peak absorbance can be utilized to determine NP concentration via Beer's Law and to evaluate changes due to any surface modification. However, conventional cuvette-based UV-Vis spectrophotometers have limited linear range due to the use of a standard fixed pathlength (10 mm) cuvette, and they often require relatively large sample volumes (ranging from 0.5 mL to 3 mL). This is not ideal for conserving precious samples such as NPs coated with expensive RNA molecules. Furthermore, the need to dilute samples to fit the operating range of the instrument is time-consuming and increases the likelihood for inaccurate measurements. Alternative measurement techniques that require less volume and allow analysis of concentrated samples without dilution would be ideal.

Recent work has shown that the Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer can be used to accurately measure highly concentrated NP samples without dilution, owing to its surface tension system and auto-ranging pathlength technique.[5, 6] For example, 150 nm diameter NS can be measured at concentrations up to 100 pM with high reproducibility.[5] In this application note, the use of the Nanodrop One instrument to observe shifts in the SPR of NS after conjugation to thiol-modified siRNA duplexes and

methoxy-poly(ethylene glycol)-thiol (mPEG-SH; a passivating agent) was investigated. The results indicate that the Nanodrop One instrument can serve as a microvolume alternative to traditional cuvette-based spectrophotometers for qualitatively confirming RNA and PEG loading on gold-based NPs via plasmon resonance shifts.

Experimental procedures

NS were synthesized by published protocols via the Oldenburg method.[7] First, 3-5 nm diameter gold colloid was made by the Duff method[8] from hydrogen tetrachloroaurate (III) hydrate (HAuCl4) (VWR), tetrakis(hydroxymethyl)phosphonium chloride (VWR), and 1 N sodium hydroxide (Fisher Scientific). The gold colloid was then combined with 120 nm diameter silica spheres functionalized with 3-aminopropyltriethoxysilane (Nanocomposix) and 1 M sodium chloride (NaCl) and rocked for 3-4 days at room temperature to create "seed" nanoparticles. The seed was purified twice via centrifugation at 3000 rpm for 30 minutes each and resuspended in Milli-Q® water (Sigma) to an optical density at 530 nm (OD530nm) of 0.1, as determined using a cuvette-based UV-Vis spectrophotometer. The diluted seed was mixed with additional HAuCl4 diluted in potassium chloride followed by addition of a small volume of 37% formaldehyde (VWR). The mixed solution was rapidly agitated to form complete gold shells and purified twice via centrifugation at 500 g for 15 minutes each. Additionally, NS were treated with 0.1% diethyl pyrocarbonate (DEPC) (Sigma) for 3 days rocking at 37°C to render the NS RNase-free. All materials described were purchased or treated with DEPC to be RNase-free prior to use.

siRNA oligonucleotides were purchased as single strands from IDT DNA, with sequences listed in **Table 1.** Thiolated sense strands were mixed with complementary non-thiolated antisense strands in equimolar amounts, boiled at 95° C for 5 min in a thermomixer, and then slowly cooled to 37° C over 1 hour to facilitate siRNA duplexing. RNase-free NS were diluted to OD800nm = 1.5 in Milli-Q water (as measured on a cuvette-based spectrophotometer). Next, 10% Tween-20 and 5 M NaCl were added to final concentrations of 0.2% and 12 mM, respectively, and the NS incubated for 5 min at room temperature. Then, siRNA duplexes were added to a final concentration of 200 nM, and the solution was bath sonicated and rocked at 4° C for 3 hours. NaCl was then added incrementally to a final concentration of 400 mM prior to rocking overnight at 4° C. The following day, 5 kDa mPEG-SH was diluted in Milli-Q water to 1 mM and added to NS to a final concentration of 10 µM. After rocking for 4 hours at 4° C, the NS solution was purified via centrifugation at 500 g for 5 minutes 3 times, resuspended in RNase-Free 1X phosphate buffered saline (PBS) with 100 X less volume of the starting NS, and stored at 4° C until use.

For conventional spectrophotometry, bare NS and siRNA-NS (diluted 100-fold in water) were placed in 1-cm pathlength disposable cuvettes and analyzed on a reference UV-Vis spectrophotometer from 1,100 nm to 400 nm. The NS concentrations were calculated from Beer's Law using the peak extinction (OD at ~800 nm) as determined by the spectrophotometer and the theoretical extinction coefficient of NS with 120 nm diameter silica cores and 15 nm thick gold shells. This revealed the initial bare NS and siRNA-NS had a concentration of 6.9 pM and 150 pM, respectively. To prepare samples for measurement with the NanoDrop One Spectrophotometer, the bare NS were concentrated by centrifugation at 500 g for 15 minutes, followed by removal of the supernatant and dilution in water to 100 pM. The siRNA-NS were directly diluted in water to 100 pM. The 100 pM bare NS and siRNA-NS solutions were measured on a NanoDrop One Spectrophotometer from 850 nm to 190 nm by pipetting 2 µL aliquots directly onto the sample pedestal. Between measurements, the NanoDrop One instrument sample pedestal was cleaned using a lint-free lab wipe. The auto pathlength option was turned on in the NanoDrop One Spectrophotometer software for each measurement.

Name	Sequence
siRNA sense	GCU GAU AUU GAC GGG CAG UAU / iSpPC//iSpPC//3ThioMC3-D/
siRNA antisense	AUA CUG CCC GUC AAU AUC AGC

Table 1: siRNA sense and antisense RNA sequences used in this work, denoted 5' to 3'. iSpPC is a photo-cleavable 10-atom spacer molecule, while 3ThioMC3-D is a thiol modification that facilitates attachment to gold NS.

Results

The absorption spectra of 150 nm NS, before (bare NS) and after (siRNA-NS) conjugation to thiolated siRNA and mPEG-SH at concentrations of 100 pM are shown in **Figure 1**. These spectra reveal the bare NS and siRNA-NS have a peak plasmon resonance at ~795 nm and ~804 nm, respectively, which is consistent with the spectra obtained using a reference spectrophotometer. The slightly red-shifted peak post functionalization, which maintains the overall shape and intensity of the spectra, provides evidence of successful siRNA and mPEG-SH conjugation. This was corroborated by dynamic light scattering and zeta potential measurements, as well as by siRNA loading quantification via OliGreen assay.[2, 4, 9] Notably, the spectra produced by the NanoDrop One Spectrophotometer were highly accurate and reproducible. Very little sample volume (2 µL) was used in the measurement, and no dilution was required for the analysis of highly concentrated samples (100 pM).

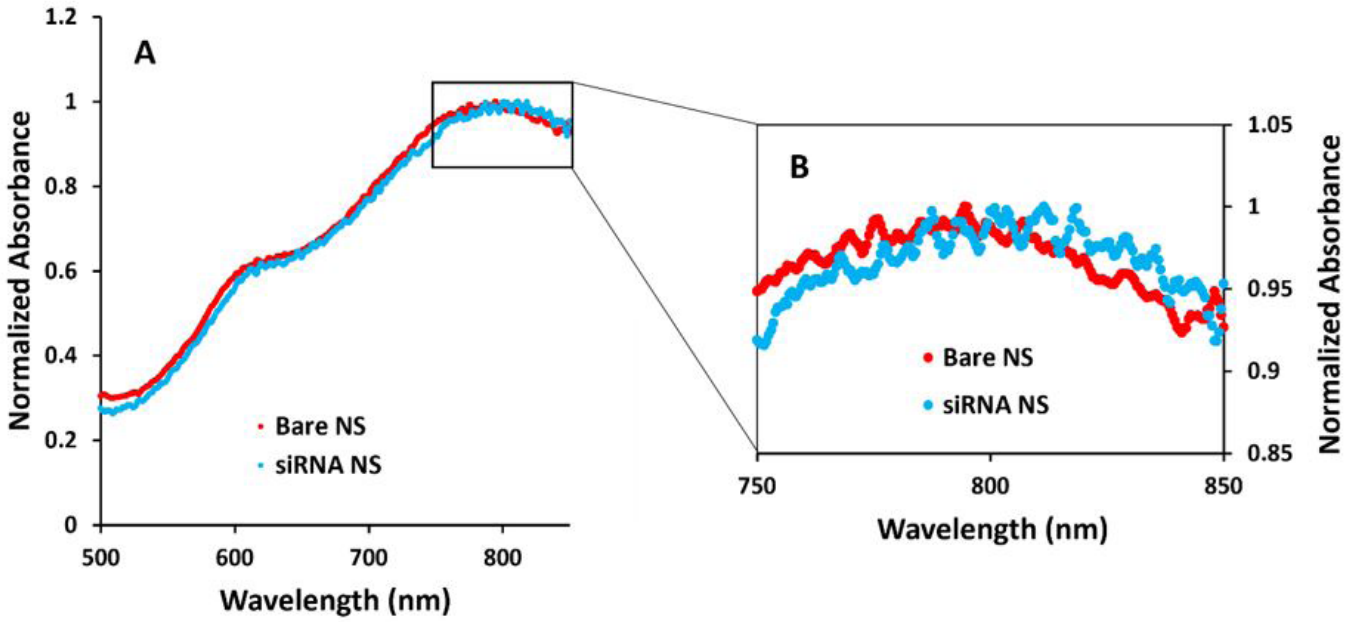

Figure 1: (A) UV-Vis spectra for Bare NS and siRNA-NS at concentrations of 100 pM, as measured on the NanoDrop One Spectrophotometer. n=3. (B) Zoom in of the UV-Vis spectra peak for Bare NS and siRNA-NS (750 nm to 850 nm).

Conclusions

This study demonstrates that the NanoDrop One Spectrophotometer can be used as a simple and reliable method to evaluate the surface modification of NS. The NanoDrop One Spectrophotometer can produce highly reliable results due to its built-in Thermo Scientific™ Acclaro™ Sample Intelligence Technology, which identifies common contaminants or other anomalies that may impact measurement accuracy. Additionally, the NanoDrop One Spectrophotometer allows the users to measure highly concentrated samples in 1–2 µL without dilution and produce full spectral data in seconds compared to a traditional cuvette-based spectrophotometer. These advantages save valuable time and money and help determine the quality and quantity of the sample before use in downstream applications. The ease of operation and small sample size requirement make the NanoDrop One Spectrophotometer an ideal and valuable instrument to characterize the properties of surface-modified NPs.

References

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