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COLLOIDAL SILICON QUANTUM DOTS: FROM PREPARATION TO THE MODIFICATION OF SELF-ASSEMBLED MONOLAYERS FOR BIOIMAGING AND SENSING APPLICATIONS

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ABSTRACT

In this paper we present recent advances in Förster resonance energy transfer (FRET) sensing and bioimaging using nontoxic silicon quantum dots. (SiQDs) In our work, we prepare SiQDs-dye conjugates, with SiQDs serving as the donor which are covalently attached to organic dye acceptors via self-assembled monolayer linkers. Enzymatic cleavage of the peptide leads to changes in FRET response which was monitored using fluorescence lifetime imaging microscopy (FLIM-FRET). The combination of interfacial design and optical imaging presented in this work opens new opportunities for bio-applications using nontoxic silicon quantum dots.

Keywords: Quantum dots, silicon, FRET, FLIM, sensor

1. INTRODUCTION

Colloidal quantum dots are ideal materials for biosensing due to their small size, tunable surface properties and unique optical signatures.[1-4] However, many optically active colloidal nanoparticles contain toxic heavy metal elements has compromised their ability for not perturbing the environment they are designed to monitor.[5-7] This has led to growing interest in developing quantum dots made with materials of low toxicity, represented by group IV elements such as carbon and silicon.[8-12] Currently, ultrasmall fluorescent colloidal silicon quantum dots (SiQDs) of a few nanometers in size have demonstrated the promise of low toxicity quantum dots in nanomedicine, with particularly intereting applications seen in imaging, sensing and drug delivery.[13-26]

The most well-established biosensing approach with quantum dots is via intermolecular dipole-dipole coupling effects, typically as Förster resonance energy transfer (FRET).[27-30] Quantum dot FRET sensing is usually achieved by appropriate selection of the donor/acceptor pair, as well as rational design of material interface.[29, 31, 32] Measurements could be performed intracellularly due to the small size of nanoparticles,[33] and toward various molecular targets by proper control of interfacial properties.[34-37] The first case of quantum dots FRET protease sensor was reported more than a decade ago, [28, 29, 38] while to date this concept has not been shown with nontoxic silicon quantum dots, regardless of enormous research efforts made with nanostructured silicon materials for their applications in nanomedicine, optoelectronics and energy harvesting devices in general.[11, 19, 39-42] This is partially because working with SiQDs usually requires the use of different surface modification strategies compared with metal containing nanoparticles, typically with hydrosilylation reactions or strong nucleophiles.[19, 43-50] The fact that silicon is an indirect bandgap semiconductor, where a lattice matched passivation layer is absent in all passivation methods imposes additional challenges because surface properties are particularly influential over the electron transfer behavior

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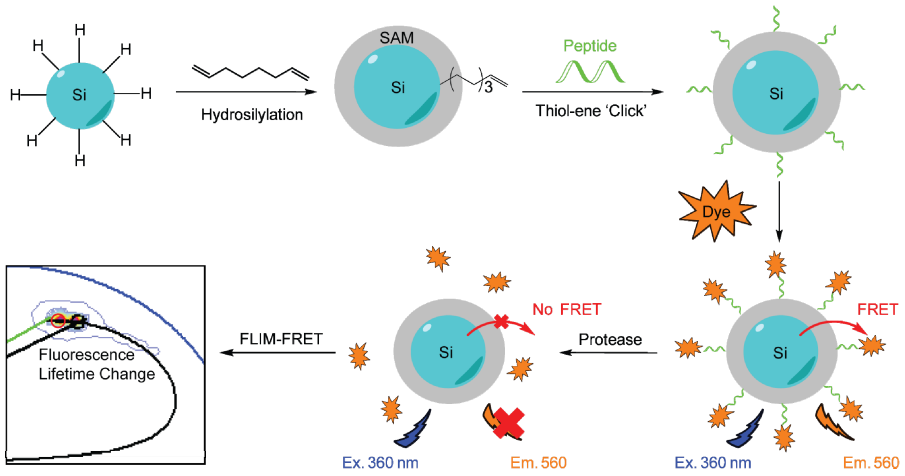
across the surface, where trap states and electron transition via capping molecules play key roles in controlling the photophysical properties of nanoparticles.[46, 51-57]



Scheme 1.Schematics of the FRET protease sensor based on silicon quantum dots. Alkene functionalized silicon quantum dots were first prepared in the solution phase via hydrosilylation, followed by surface modification with thiol-ene 'click' reaction utilizing the cysteine residue in the peptide. Dye molecules were then immobilized on the particle surface at the N terminus of the peptide. The resulting change of fluorescence signatures was characterized by both photoluminescent (PL) and lifetime based measurements, with the latter performed with fluorescence lifetime imaging microscopy (FLIM-FRET).

In this work, we report the first experimental utilization of FRET sensing using SiQDs to detect protease activity. (Scheme 1) Abnormal expression of protease is also found in bacterial infection, cholesterol regulations, apoptosis and necrosis.[58, 59] Trypsin was chosen as the model protease. It is a serine protease produced mainly in the pancreas as a proenzyme trypsinogen. Upon activation, trypsin is one of the most important digestive enzymes in the body, and its expression level is closely associated with pancreatic diseases, such as cystic fibrosis and chronic pancreatitis where high concentration is associated with organ damage.[60-62] In this work, fabrication of the SiQDs FRET protease sensor was achieved with a step-wise manner by first preparing alkene passivated construct with hydrosilylation. Nanoparticles were then modified with thiol-ene 'click' chemistry to covalently attach substrate peptides onto the surface, and finally dye acceptors, allowing FRET to occur. Cleavage of the peptides with the target enzyme facilitated the removal of the dyes from the surface of SiQDs, with the concomitant decrease in the fluorescent signal due to FRET. This response was monitored with both intensity and fluorescence lifetime based methods, with the latter performed with far-field optical methods by fluorescence lifetime imaging microscopy (FLIM). The combination of interfacial design and imaging method shown in this paper allow new opportunities of sensing and imaging with nontoxic silicon nano probes.[63]

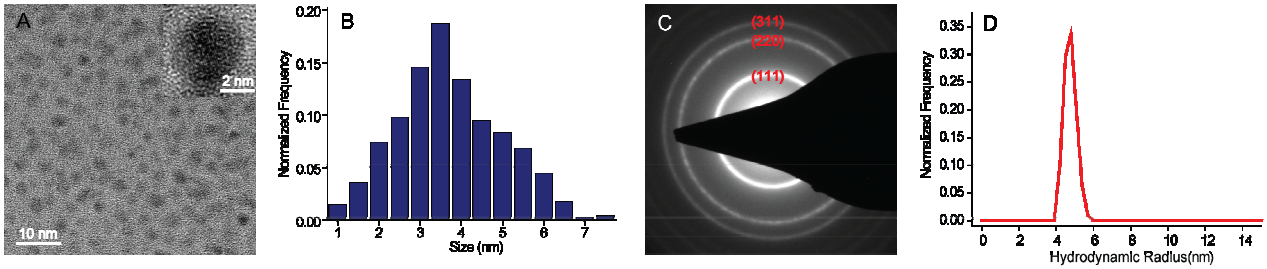


Figure 1 Characterization of morphologies of SiQDs used as the FRET donors. (A) Representative transmission electron microscopy (TEM) images and high resolution (HR-TEM) images showing the spherical morphology and lattice fringes of nanoparticles synthesized (B) Size distribution analysis based on TEM results (C) Selected area diffraction patterns

showing crystalline diamond structures at the core. (D) Dynamic light scattering measurements confirming the narrow size distribution of the nanoparticles.

Morphology and size distribution of SiQDs donors were characterized by a combination of methods including transmission electron microscopy (TEM), selected area diffraction and dynamic light scattering techniques. (Figure 1) TEM results indicated that the obtained nanoparticles were spherical in shape and relatively monodisperse with an average size of 3.4±0.7 nm (Figure 1A). High resolution TEM images revealed lattice fringes matching the (220) lattice spacing of silicon. The observed crystallinity was further confirmed by electron diffraction (Figure 1C) which shows ring patterns corresponding to the (111), (220), and (311) reflections respectively. Dynamic light scattering measurements showed that size of nanoparticles were 5.4 ± 0.6 nm, a value slightly higher than TEM measurements (Figure 1D). This was expected as this method measured the hydrodynamic radius of nanoparticles, which took into account of surface groups and surrounding water molecules, where in TEM only the inorganic core part was imaged.

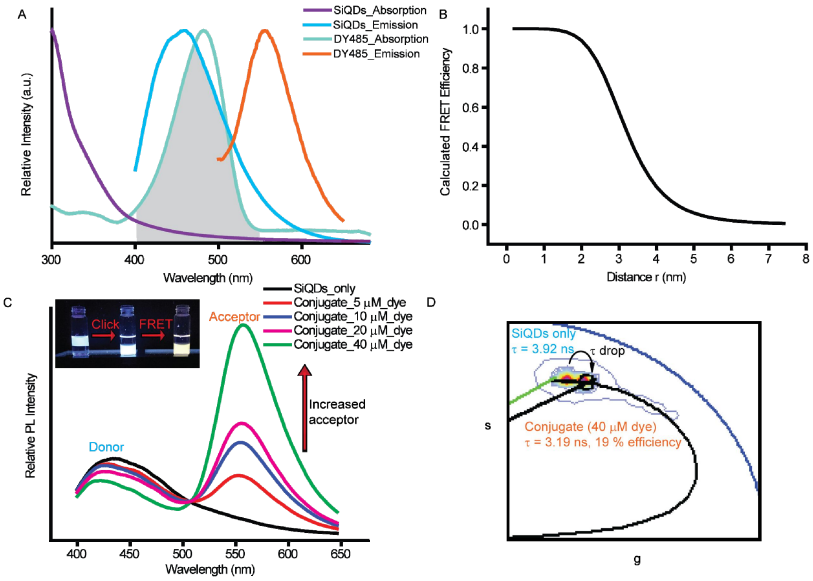


Figure 2 Optical properties of the SiQDs FRET protease sensor. (A) Spectral overlap between SiQDs (donor) and DY485 (acceptor). (B) Calculated FRET efficiency in relation to distance. (C) Photoluminescence spectra of SiQDs FRET conjugate. (Inset) Picture showing the fluorescence of the nanoparticles in different phases obtained from each surface conjugation step. The nanoparticles were dispersed in solvents containing hexane (top layer) or Milli-Q water (bottom layer) under direct excitation using a 365 nm UV lamp. (D) FLIM phasor plots showing decrease of SiQDs fluorescence lifetime (τ) after modification with DY485. The phasor cluster of the conjugate is located on a FRET trajectory defined by the donor/acceptor alone, indicating the occurrence of FRET.

With each surface modification step, SiQDs exhibited varied dispersity in different solvents (Figure 2C, inset). When modified with 1,7-octadiene, nanoparticles were dispersed in nonpolar solvents such as hexane, while after attachment of the hyrdrophilic peptide molecules onto the surface, SiQDs became dispersible in water. Further modification with dye molecules yielded orange fluorescence from SiQDs, which were initially blue upon UV excitation of 365 nm. Photophysical properties of the fabricated FRET sensor were then characterized. The organic dye acceptor used in this study, DY485, was chosen for two reasons. The first was that this particular dye acceptor has a large Stokes shift of ~85 nm. The large separation between the absorption and emission peaks allowed minimal influence of acceptor signals when observing from the donor channel, especially when considering the full width at half maximum of SiQDs emission peak was quite broad (FWHM ~90 nm). The second reason for choosing DY485 was that its absorption profile overlaps well with the emission peak of SiQDs. (Figure 2A) This is important as FRET efficiency depends strongly on the spectral overlay between donor/acceptor, and a high overlapping factor is preferred for high transfer rate.[29] With the donor-acceptor pair presented herein, the Förster radius was calculated to be ~3.2 nm, (Figure 2B) where the spacing

between the dye and the nanoparticle was ~2.5. Figure 2A shows the change of photoluminescence intensity when an increasing amount of dye was modified onto the surface of SiQDs. Increasing the amount of dye used resulted in a gradual decrease in intensity of the SiQDs photoluminescence and corresponding growth in intensity of the acceptor peak. A closer inspection of this trend revealed that surface coupling occurred quickly within 30 min after the introduction of the dye, and was nearly completed within ~90 min after the start of the reaction. This provided direct evidence that the enhanced emission at the acceptor channel was indeed from FRET, instead of direct excitation of the dye. Considering the quantum yield of SiQDs donor used in our case was quite low relative to many organic dyes with comparable spectral features (< 8 %), the more than doubling of the photoluminescence intensity of the acceptor peak was somewhat unexpected. This large enhancement of photoluminescence intensity was comparable to quantum dots FRET sensors made with direct bandgap semiconductors, [29, 30, 38] and this led to a conclusion that SiQDs can indeed be good FRET donors if the interface is properly designed.

An important parameter for characterizing FRET is the change of fluorescence lifetime. (τ)[28, 64] It is a parameter associated with the inherent property of the fluorophore, which is not susceptible to intensity fluctuations and this is particularly relevant for measurements in complex environments.[65, 66] As cuvette based technique cannot be applied to cellular samples where the SiQDs sensor presented herein are expected to be used, we tested if change in □ of our FRET conjugates can be monitored by far-field optical imaging methods with fluorescence lifetime imaging microscopy. (FLIM) Since the number of photons collected in FILM experiments are usually limited, where fitting decays are difficult,[67, 68] we processed the results by Fourier transformation then plotted the sin and cos of the lifetime components onto their respective phasor diagrams. (Figure 2B) It was observed that as an increased amount of dye was added, the phasor of the FRET conjugate moved along a curved trajectory, defined by the lifetimes of both the donor and acceptor, which was distinct to any linear combination of the two which would be indication of no energy transfer occuring.[69-71] By measuring the extend of change of donor lifetime, FRET efficiency was determined to be between ~3-20 % using dye concentration of ~5-40 μM. It should be noted that by this method, knowing the exact value of fluorescence lifetime is not necessary, as FRET efficiency can still be obtained by measuring the degree of movement of phasor cluster along the FRET trajectory.

For protease sensing, the FRET conjugate was treated with trypsin, which cleaves at the C terminus of glycine of the peptide sequence and FRET response was monitored over time. (Figure 3A) With an increasing amount of enzyme added, the respective photoluminescence measurements indicated a more significant change of FRET response until a maximum change was reached at ~460 μg/mL. This was in comparison to the control group treated with phosphate buffer saline (PBS), which did not show appreciable photoluminescence change throughout the experiment, providing evidence that the observed change in FRET response was due to the cleavage of the peptide. Interestingly, it was noted that the Id/Ia peak did not fully recover to its initial value after enzyme treatment, which could be due to incomplete cleavage of surface bound peptides.

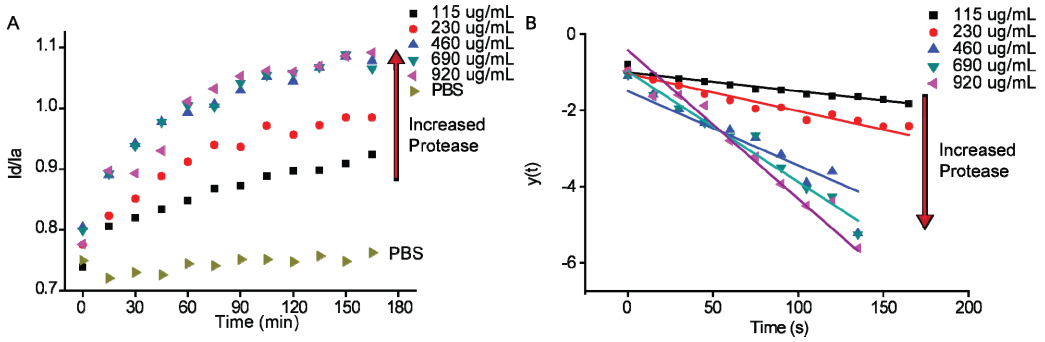


Figure 3 Enzymatic response of the SiQDs protease sensor. (A) Intensity ratio of the donor/acceptor peak plotted as a function of time. (B) Initial stage surface reaction fitted with Michaelis-Menten kinetics model. y(t) is defined as $\ln(\varepsilon[I_d/I_a][S]_0)-k_{cat}[E]_0t/K_m$, where $\varepsilon[I_D/I_A]$ stands for molar concentration FRET response of (I_d/I_a), k_{cat}: catalytic constant; K_m: Michaelis constant; $[S]_0$: initial substrate concentration; $[E]_0$:initial enzyme concentration. The relationship is linear in relation to time, confirming the enzyme concentration, [E], is in excess to enzyme-substrate complex concentration [ES].

The reaction kinetics of the enzyme cleave was then investigated. (Figure 3B) In this work, the step-wise, covalent interaction based surface modification strategy was distinct to classic quantum dots or other metal nanoparticles, where

ligand exchange chemistries were predominantly used where both the substrate concentration and nanoparticle/ligand ratios were known.[72, 73] For this reason, a slightly modified approach for studying the surface reaction kinetics of our system was needed. Here, plots of I_d/I_a as a function of enzyme reaction time was fitted with a global non-linear regression model using an integrated form of the Michaelis-Menten equation using Lambert function and known enzyme concentration.[74] By this method, we were able to obtain an estimate on both the k_{cat}/K_m = 1.06 $mM^{-1}s^{-1}$, and the total substrate concentration $[S]_0$ = 0.48 μM As the initial amount of peptide introduced into the system was known, this suggested only less than 1% of peptides were coupled onto the surface of the nanoparticles while at the same time has a dye molecule attached. It should be noted that with the method devised was not possible to obtain independent values of K_m and k_{cat}, but only their ratio as the the results suggested [E]>>[ES], which was confirmed by the linear relationship by introducing a new parameter, $y(t) = \ln(\varepsilon[I_d/I_a][S]_0) - k_{cat}[E]_0 t/K_m$, and time at the initial stage of the reaction. The fitting also suggested the the value of k_{cat}/K_m was about 3-5 times lower than reported value for trypsin catalyzed proteolytic cleavage of a longer peptide conjugate on conventional quantum dots,[73] suggesting that not all substrate peptide were coupled with a dye, and also possibly trypsin interacts more efficiently with longer substrate.

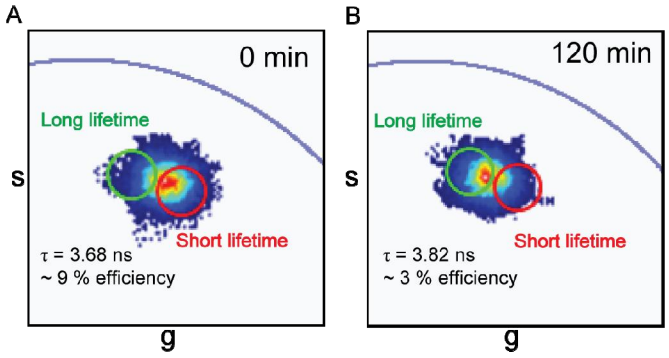


Figure 4 Fluorescence lifetime imaging microscopy (FLIM) studies of SiQDs conjugates upon treatment of trypsin. (A) – (B) FLIM phasor plots of SiQDs FRET sensor treated with trypsin at 0 min and 120 min, showing clear drop of fluoresce lifetime of the mixture when observing from the donor channel. Green circle: original SiQDs phasor cluster; red circle: acceptor phasor cluster. A shift of the phasor was observed after 120 min which is correlated with increase of donor lifetime.

We performed fluorescence lifetime imaging microscopy (FLIM) of SiQDs FRET sensor in PBS buffer to test if the enzymatic cleavage process could be monitored by FLIM. Figure 4 shows the impacts of trypsin treatment after 120 min on the fluorescence lifetime of the FRET sensor when observing from the donor channel. A clear shift of the conjugate clusters was seen which corresponded to decrease of FRET efficiency from ~9% to ~3%. Our previous studies demonstrated such FLIM signals of SiQDs FRET conjugates could be easily de-convoluted from biological scattering signals and autofluorescence with both one and two photon excitations,[75] and concentration of particles used with those cellular studies showed no sign of cytotoxicity. [63, 75] However, it should be noted that the actual change of fluorescence lifetime of the donor was very small (i.e. < 1 ns), which was expected as the emission was likely to be dominated by the surface states and this provided evidence that the temporal resolution of FLIM is sufficiently high for studying quantum dots FRET system in general.

In summary, we report the first proof-of-concept study of FRET protease sensing with quantum dots made from nontoxic material crystalline silicon. This was achieved by a combination of proper materials synthesis, interfacial design and advanced far-field imaging techniques. Mechanistic study revealed that the surface reaction follows Michaelis-Menten kinetics model. The general applicability of wet chemistry and thiol based surface modification strategy presented allow a simple way of preparing colloidal silicon quantum dots sensors for immobilizing target molecules onto the surface, and the use of microscopic method suggested measurements can be performed in cellular contexts. The concepts brought by this paper aim to bring new opportunities of bio-applications using nontoxic quantum dots in general.

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