Shortwave infrared imaging with J-aggregates stabilized in hollow mesoporous silica nanoparticles

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ABSTRACT: Tissue is translucent to shortwave infrared (SWIR) light, rendering optical imaging superior in this region. However, the widespread use of optical SWIR imaging has been limited, in part, by the lack of bright, biocompatible contrast agents that absorb and emit light above 1000 nm. J-aggregation offers a means to transform stable, near-infrared (NIR) fluorophores into red-shifted SWIR contrast agents. Here we demonstrate that hollow mesoporous silica nanoparticles (HMSNs) can template the J-aggregation of NIR fluorophore IR-140 to result in nanomaterials that absorb and emit SWIR light. The J-aggregates inside PEGylated HMSNs are stable for multiple weeks in buffer and enable high resolution imaging in vivo with 980 nm excitation.

Optical imaging with shortwave infrared (SWIR, 1000–2000 nm) light has emerged as a powerful method of fluorescence imaging in animals due to the superior resolution and contrast one can achieve with low energy light (Figure 1A). A primary challenge with SWIR imaging is the development of bright, biocompatible, SWIR contrast agents. Originally, the advantageous qualities of imaging in the SWIR region were showcased with carbon nanotubes, quantum dots, and rare earth nanomaterials. In efforts to set the stage for clinical translation, the past two years have seen a focus on the synthesis of non-toxic, SWIR-emissive organic fluorophores. This work has significantly expanded the suite of fluorophores that emit above 1000 nm; however, the low quantum yields of SWIR fluorophores present an inherent challenge in developing bright dye monomers for SWIR imaging. Consequently, we looked toward an alternative avenue to create SWIR organic materials: J-aggregation.

J-aggregation is the slip-stacked alignment of chromophores that leads to constructive coupling of the excited state transition dipoles (Figure 1B). The photophysical consequences of J-aggregation, namely bathochromically-shifted absorption and emission spectra, small Stokes shifts, shortened fluorescence lifetimes and cycling rates, enhanced absorbance coefficients (ε) and quantum yields (ΦF), are beneficial qualities for in vivo imaging. Red-shifted absorption and emission spectra will enable significant depth penetration during both the excitation and image acquisition, while the increased ε and ΦF combine for bright materials. Despite the significant photophysical advantages J-aggregates typically
have over the monomer, there are few reports of employing J-aggregates for in vivo imaging due to the difficulty in obtaining and stabilizing the necessary chromophore alignment in complex settings.9

Nanostructures can sequester and protect payloads, rendering nanomaterials a promising approach toward stabilizing J-aggregates in vivo. In 2016, Zheng and coworkers performed image-guided surgery with porphyrin lipids that formed J-aggregates upon self-assembly into nanovesicles.10 The following year, Xu and coworkers prepared pyrrolopyrrole cyanine J-aggregate-containing polymer micelles, which could be visualized after subcutaneous injection.11 In work very recently published, Fan and coworkers reported a squaraine J-aggregate, also stabilized in polymeric micelles, for SWIR image-guided photothermal therapy.12 Each of these reports utilizes self-assembled organic nanomaterials,13 which are prone to disassembly when diluted in the presence of hydrophobic biomolecules, leading to destabilization of the J-aggregate.14 Here, we employ robust, biocompatible, hollow mesoporous silica nanoparticles to template, stabilize and protect SWIR-emissive J-aggregates of IR-140 for in vivo imaging (Figure 1C/D).

Hollow mesoporous silica nanoparticles (HMSNs) have 2–4 nm pores that open into a large, tens of nm cavity, allowing these nanostructures to carry significant cargo.15 The surfaces of the HMSNs can be modified with alkoxy silane derivatives to alter the biodistribution and serum half-lives of the nanoparticles.16 Consequently, there are numerous reports of HMSNs as the core scaffold of multifunctional materials15a,16,17 Included in these studies are the loading or conjugation of visible18 and near-infrared15a,19 fluorophores and administering the resulting fluorescent nanomaterials for in cellulo and in vivo imaging. However, the controlled assembly of J-aggregates in HMSNs has yet to be demonstrated.

To realize SWIR-emissive J-aggregates inside HMSNs, we utilized the heptamethine dye IR-140 (1). IR-140 is a commercially available NIR fluorophore (λmax,abs = 826 nm, λmax,em = 875 nm) that has been applied as a photopolymerization initiator,20 fluorescent payload,21 component of plasmonic arrays,22 as well as Raman23 and two-photon24 imaging agents. In 2016, Wang and Weiss reported that introduction of IR-140 to glutathione-coated quantum dots results in J-aggregate formation with two aggregates observed: J1, (λmax,abs = 965 nm, non-emissive) and J2 (λmax,abs = 1040 nm, λmax,em = 1047 nm).21 We envisioned that similar IR-140 J-aggregates could be formed on the negatively charged interior of HMSNs. Further, once the aggregates were assembled inside the particles, the hydrophobic nature of IR-140 would make them unlikely to disassemble in aqueous environments, rendering J-aggregates stable in vivo.

We prepared HMSNs via synthesizing a mesoporous silica coating on a Stöber sphere core that was subsequently removed via etching with sodium carbonate. The resulting HMSNs were ~85 nm in diameter and contained 3.25 nm pores. Importantly, the pore size was large enough to allow IR-140 to traverse into the cavity of the nanoparticles. The HMSNs were then treated with varying amounts of IR-140 in different solvents (Figure 2A). J-aggregate formation was assayed by UV/Vis/NIR spectroscopy evaluating loss of monomeric IR-140 at 826 nm and formation of the J-aggregates at 965 nm (J1) and 1040 nm (J2). Upon optimization, we found that SWIR J-aggregates could be obtained when IR-140 dissolved in dimethyl sulfoxide (DMSO) was combined with HMSNs and washed. The washing procedure proved essential for templating the desired J2 aggregate formation (Figure 2B). Washing with water yielded a mixture of monomer and both J-aggregates, while washing with phosphate buffered saline (PBS) resulted in predominantly the desired SWIR-emissive J2 aggregate. If sonication in PBS was performed, the undesired J1 aggregate was the dominant species. After arriving at optimal loading conditions, the presence of IR-140 associated with the mesoporous silica shell of the HMSNs was confirmed by transmission electron microscopy.

![Figure 2](image_url)

Figure 2. A) Schematic of loading IR-140 into HMSNs. B) Washing conditions help template J-aggregation. 10 mg/mL HMSNs were combined with 10 mM IR-140 in DMSO and washed with water (gray), PBS (dark blue) and PBS with sonication (teal). Pre-wash spectrum, diluted 1:350 is shown in yellow. C/D) Transmission electron microscopy images of HMSNs with (D) and without (C) IR-140 treatment. Scale bars represent 100 nm in the main image and 50 nm in the inset.
The photostability of monomeric IR140 laser (power density = 97 mW/cm²) aggregate in PBS were continually irradiated with a 23 kDa PEG-carboxylate to the surface using carbodiimide chemistry. Successful PEG conjugation was verified by changes in hydrodynamic diameter and zeta potential. Gratifyingly, the loading of IR-140 into the functionalized HMSNs proceeded similarly to that of the original HMSNs assayed, yielding analogous loading of IR-140 and a higher ratio of J2:J1. Control loading experiments performed with Stöber spheres treated or untreated with APTS support that IR-140 is protected on the interior of the HMSNs as minimal IR-140 J-aggregate was templated on the surface of APTS-modified Stöber spheres.

We evaluated the photophysical properties of the PEGylated HMSNs (HMSNs-PEG) containing IR-140 in comparison to IR-140 in solution as the monomer and J-aggregate (Figure 3A). Monomeric IR-140 has been well-characterized; however, the non-templated J-aggregate of IR-140 had previously not been reported. We found that 35% DMSO/0.9% NaCl in water afforded formation of the desired SWIR J-aggregate with a λ_{max,abs} = 1042 nm, λ_{max,em} = 1043 nm, ε = 3.9 x 10⁸ M⁻¹cm⁻¹, and ϕₑ = 0.01%. The IR-140-containing HMSNs-PEG had similar spectral properties with a λ_{max,abs} = 1038 nm, λ_{max,em} = 1047 nm. When solutions of IR-140 in DMSO, IR-140 in 35% DMSO/0.9% NaCl in water, and IR-140 loaded HMSNs-PEG in PBS were excited with a 980 nm laser, the wavelength to be used for in vivo imaging experiments, the IR-140 J-aggregate in solution and in the particles were similarly emissive, while the monomer was not excited by 980 nm light (Figure 3B). Thus, J-aggregation is essential for SWIR imaging with low energy excitation.

Next, we analyzed the role of the HMSNs in stabilizing IR-140 J-aggregates. Over two weeks in PBS at room temperature, we observed only a ~10% decrease in absorbance from the IR-140 loaded HMSNs-PEG and no evidence that the packing of the IR-140 within the nanoparticles was changing (Figure 3C, blue). Comparatively, only ~8% of the J-aggregate in solution remained after 1 day (Figure 3C, red). Not only do the HMSNs stabilize the assembly of the J-aggregate, but they also enhance the photostability. The fluorescence of solutions containing IR-140 J-aggregate in 35% DMSO/0.9% NaCl in water and HMSNs-PEG containing IR-140 J-aggregate in PBS were continually irradiated with a 980 nm laser (power density = 97 mW/cm²) and the fluorescence intensity was measured with an InGaAs camera. The photostability of monomeric IR-140 in DMSO was also evaluated via excitation at 785 nm (power density = 97 mW/cm²). As seen in Figure 3D, the J-aggregates within the HMSNs-PEG are 4-fold more stable than the J-aggregates in solution and ~60-fold more stable than the monomer. This result is consistent with the use of silica shells to overcome the poor photostability that is characteristic of J-aggregates by limiting the amount of reactive oxygen species that can access the aggregate. Taken together, our data show that the HMSNs are critical for not only templating the J-aggregates but also enhancing their stability to light and solution.
Finally, with bright SWIR-emissive nanoparticles prepared and characterized, we evaluated their biocompatibility and in vivo imaging performance. In vitro studies showed no cytotoxicity of the IR-140 loaded HMSNs-PEG over 6 hours at concentrations up to 200 µg/mL. These data are consistent with other studies regarding mesoporous silica, which is generally considered nontoxic to animals. We performed in vivo imaging experiments using the IR-140 loaded HMSNs-PEG as a SWIR contrast agent with excitation at 980 nm and collection from 1000–1700 nm. The SWIR-emissive HMSNs-PEG were intravenously injected into nude mice and the mice were immediately imaged (Figure 4). The HMSNs-PEG rapidly clear from the blood stream and intense signal can be seen in the lungs, liver, and spleen. Fifty minutes after injection, the signal intensity within these organs remained constant.

In summary, we have presented J-aggregation as an approach to prepare biocompatible, SWIR contrast agents and demonstrated this concept by templating J-aggregates of the NIR fluorophore IR-140 inside HMSNs. The bathochromically-shifted absorption and emission and small Stokes shifts of the IR-140 J-aggregate allow imaging with 980 nm excitation and 1000–1700 nm acquisition, providing high resolution in vivo images. J-aggregate theory also indicates enhanced absorption coefficients and quantum yields, facilitating bright contrast agents. While we did not observe an enhanced quantum yield with the IR-140 J-aggregate, work is ongoing to access a SWIR J-aggregate that exhibits the superradiance phenomena predicted by Kasha. The modularity of the HMSNs will enable facile exchange of the imaging agent as well as the addition of targeting agents and/or therapeutics, poising these materials to become SWIR theranostics. Collectively, the use of J-aggregates stabilized in HMSNs as SWIR imaging agents has the potential to overcome the stability, toxicity, and brightness challenges of contrast agents for this compelling region of the electromagnetic spectrum.

Figure 4. Whole-mouse imaging at 16 fps (980 nm, 91 mW/cm² excitation; 1000–1700 nm collection) upon i.v. delivery of IR-140-loaded HMSNs-PEG. Background subtracted stills were averaged over 5 frames at 3 s (A), 8 s (B), 25 s (C), and 120 s (D) post injection. Scale bar represents 1 cm. Data are representative of two replicate experiments.

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