Published in final edited form as:

Langmuir. 2018 January 09; 34(1): 359–365. doi:10.1021/acs.langmuir.7b03718.

Switchable Photoacoustic Intensity of Methylene Blue via Sodium Dodecyl Sulfate Micellization

Junxin Wang[†], Ching-Yu Lin[†], Colman Moore[†], Anamik Jhunjunwala[‡], and Jesse V. Jokerst^{*,†}

[†]Department of NanoEngineering, University of California San Diego, La Jolla, California 92093, United States

[‡]Department of BioEngineering, University of California San Diego, La Jolla, California 92093, United States

Abstract

The interaction between methylene blue (MB) and sodium dodecyl sulfate (SDS) has been widely studied spectroscopically, but details about their interactions remain unclear. Here, we combined photoacoustic (PA) imaging with nanoparticle tracking analysis (NTA) and spectroscopy to further elucidate this interaction. PA imaging of 0.05 mM MB showed a 492-fold increase in intensity upon the addition of 3.47 mM SDS. Higher concentrations above SDS's critical micelle concentration (CMC) at 8.67 mM decreased the PA intensity by 54 times. Relative quantum yield measurements indicated that PA intensity increased as a result of fluorescence quenching. Meanwhile, NTA indicated an increased number of nonmicellar MB/SDS clusters at SDS concentrations below the CMC varying in size from 80 to 400 nm as well as a decreased number above the CMC. This trend suggested that MB/SDS clusters are responsible for the PA intensity enhancement. Comparison of PA intensities and spectral shifts with MB/hexadecyltrimethylammonium bromide, MB/sodium octyl sulfate, and MB/sodium chloride demonstrated that MB was bound to the sulfate moiety of SDS before and after micellization. Our observations suggest that MB forms aggregates with SDS at premicellar concentrations, and the MB aggregates disassociate as monomers that are bound to the sulfate moiety of SDS at micellar concentrations. These findings further clarify the process by which MB and SDS interact and demonstrate the potential for developing MB-/SDS-based contrast agents.

Graphical Abstract

*Corresponding Author: jjokerst@ucsd.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b03718.

Image of the experimental setup, pH-dependent PA change of MB as a function of the SDS concentration, measurement of size distribution and the concentration of the MB-SDS aggregate, and MB concentration-dependent changes to spectra and aggregation (PDF) Brownian motion of the aggregates (MPG)

The authors declare no competing financial interest.

1. INTRODUCTION

Photoacoustic (PA) imaging is a rapidly growing molecular imaging modality that combines the high temporal resolution of ultrasound with the strong contrast of optics.^{1–6} It can image both endogenous chromophores (e.g., deoxygenated/oxygenated hemoglobin and melanin) and exogenous contrast agents.^{7–14} Methylene blue (MB) is a useful cationic exogenous contrast agent for identifying sentinel lymph nodes.^{15–17} It has also been proposed for the measurement of biological functions based on the difference in PA intensity between its monomer and dimer forms. For example, Morgounova et al. measured the difference in PA lifetime contrast between MB monomers and dimers, suggesting potentials for an activatable probe using enzymatic cleavage.¹⁸ PA monitoring of MB can also measure changes in the local tissue environment. Ashkenazi used MB to measure dissolved oxygen in biological tissues because the PA lifetime of MB decreased due to energy transfer to oxygen.¹⁹ More recently, we showed that the PA signal of the cationic MB molecule increased in the presence of the polysulfated and hence negatively charged heparin.²⁰ This suggested that electrostatic interactions between MB and anionic materials could boost the PA signal.

Interactions between sodium dodecyl sulfate (SDS) and cationic dyes—especially MB—have drawn much attention because they present unique spectral profiles with peak shifts between SDS at premicellar and micellar concentrations.^{21–23} MB monomers exhibit a characteristic absorbance peak near 660 nm, but the addition of SDS at premicellar concentrations (<8 mM) blue-shifts the peak to 610 nm.²⁴ This hypsochromic shift is attributed to the formation of H-type dimers resulting from neutralization by SDS and π – π stacking between MB monomers.²⁵ The exciton theory suggests that the coupling between MB monomers splits the single excited state into two exciton states, and the energy absorption between the ground state and the upper exciton state causes a hypsochromic shift in the absorbance spectrum.^{26–28}

In addition to spectral data, Carroll et al. detected increased scattering signal²², and Morgounova et al. detected decreased triplet transient absorption at 660 nm¹⁸ of MB in SDS at premicellar concentrations. This suggested the presence of MB/SDS clusters. However, the exact nature of the MB/SDS interaction remains unclear including the binding site between SDS and MB. Numerous intermolecular forces besides the electrostatic force affect this interaction, and MB might bind to the hydrophobic tail of SDS because of hydrophobic attraction.¹⁸ Previous research has suggested that when the SDS concentration exceeds the critical micelle concentration (CMC), MB dimers disassociate into monomers as indicated by the increase of the primary monomer absorbance peak at 660 nm.^{18,22,25} However, the nature of this supposition has not been investigated or verified beyond absorption

spectroscopy, and the mechanism of the dimer to monomer transition at the CMC is unknown.

This communication clarifies the unique dynamic behaviors between MB and SDS by studying the PA intensity, aggregate concentration, and spectral profiles of MB upon the addition of increasing SDS, hexadecyltrimethylammonium bromide (CTAB), sodium octyl sulfate (SOS), sodium octadecyl sulfate (SOD), and sodium chloride (NaCl).

2. MATERIALS AND METHODS

2.1. Reagents.

MB (98%) was purchased from Fisher Scientific. SDS was purchased from Calbiochem. CTAB was purchased from Fluka. Rhodamine 6G (R₆G) was purchased from EM Science. NaCl (99%), SOS, and SOD (>95%) were purchased from Sigma Aldrich. Laboratory polyethylene tubing (OD: 1.27 mm, ID: 0.85 mm) was purchased from Harvard Apparatus.

2.2. Preparation of Stock Solutions and High-Order MB Aggregates.

The MB stock solution was initially prepared at 2 M in Millipore water followed by filtration using a 0.22 μm filter. This solution was diluted as needed for each experiment. SDS, CTAB, NaCl, and SOS were prepared in Millipore water at 17.34 mM, 3 mM, 1 M, and 270 mM, respectively. MB dimer and high-order aggregates were prepared by adding 25 μL of 2 mM MB in 975 μL of 1 and 3 M sodium sulfate at 40 °C, respectively.

2.3. Preparation of the MB/SDS Complex.

The SDS stock solution was diluted to 17.34, 8.67, 3.47, 1.73, and 0.87 mM. Next, 25 μL of 2 mM MB was added into 975 μL of each diluted SDS solution. For studies on the effect of SDS at high MB concentrations, 900 μL of 2 mM MB was added to 100 μL of 86.7, 34.7, 17.3, and 8.7 mM SDS. For studies on the effect of MB concentration, 20 μL of 0.125, 0.25, 0.5, 1.0, and 2.0 mM MB was added into 180 μL of 3.47 mM SDS. MB/SDS solutions were maintained in water at final volumes of 1 mL.

To validate the PA phenomena of MB/SDS in basic and acidic conditions, pH 2 and pH 10 buffers were first prepared by mixing hydrochloric acid and sodium hydroxide in Millipore water, and the pH value of the solutions was determined with a pH meter (Milwaukee MW102). Next, 5 mg/mL SDS was dissolved in the buffer, and the pH value was confirmed again. The SDS stock solution was diluted at concentrations of 0.5, 1.0, and 2.5 mg/mL. MB (12.5 μL) was added to 487.5 μL of SDS solutions.

2.4. Preparation of MB/CTAB and MB/NaCl Solutions.

CTAB was diluted to 3, 1.5, 1, 0.5, and 0.25 mM. The MB/CTAB solution was prepared by adding 25 μL of 2 mM MB into 975 μL of each diluted CTAB solution. To study the effect of NaCl, 2.5 mg of SDS was added to 25 μL of 2 mM MB followed by 975 μL of 0.8 M NaCl. In the control sample, 2.5 mg of SDS was added to 25 μL of 2 mM MB followed by 975 μL of Millipore water.

2.5. Preparation of MB/SOS and MB/SOD.

The SOS stock solution was diluted to 270, 135, 67.5, 33.75, and 16.8 mM. Next, 25 μL of 2 mM MB was added to 975 μL of each diluted SOS solution. For the preparation of MB/SOD, 25 μL of 2 mM MB was added to 975 μL of 30, 60, 130, 250, and 500 μM SOD solution.

2.6. Absorbance and PA Imaging Characterization.

Absorbance spectra (400–850 nm) of these samples were characterized by a SpectraMax M5 spectrophotometer. The absorbance values $\left(A = -\log \frac{I_{\text{out}}}{I_{\text{in}}}\right)$ were exported from the software and plotted. The PA images of samples were obtained from a Vevo LAZR imaging system (Visualsonics). This PA imaging system was equipped with a 45 ± 5 mJ tunable laser outlet for excitation and a 21 MHz-centered transducer for ultrasound signal reception. The tunable laser had a pulse of 4–6 ns at 20 Hz via a flashlamp-pumped Q-switched Nd:YAG laser with an optical parametric oscillator and a second harmonic generator. The laser wavelength could be adjusted between 680 and 970 nm with a 1 nm step size. The ultrasound transducer had a 26 mm wide full field-of-view and acquired signals at 5 frames per second. The samples were held in polyethylene tubing fixed in a 3D-printed phantom holder²⁹ and aligned under the transducer at a depth of 11 mm for maximized laser exposure (Figure S1). The laser energy was optimized using the built-in energy power meter as well as an external power meter prior to measurements.

2.7. Relative Quantum Yield of MB/SDS.

The relative quantum yield of MB/SDS samples was measured following a standard protocol using the equation $\Phi_{f,x} = \Phi_{f,x} \times \frac{F_x}{F_{\text{st}}} \times \frac{f_x}{f_{\text{st}}} \times \frac{n_x^2}{n_{\text{st}}^2}$, where Φ , F , f , n , x , and st denote the quantum yield, integral photon flux, absorption factor, refractive index, sample, and standard, respectively.³⁰ R₆G in ethanol was selected as the fluorescence standard because of its fluorescence spectrum overlap with MB. The quantum yield and excitation wavelength of R₆G were 0.91 and 530 nm,³⁰ respectively. The excitation wavelength of MB/SDS was 660 nm (the plateau region of the absorbance spectrum). The refractive indices of ethanol (530 nm) and water (660 nm) are 1.3636 and 1.3311,³¹ respectively. The integral of the fluorescence spectrum of R₆G and MB/SDS was started 20 nm red-shifted from the excitation wavelength.

2.8. Nanoparticle Tracking Analysis.

The nanoparticle tracking analysis (NTA) uses Malvern Nanosight LM10. The system uses a 532 nm laser to excite the analyte in a liquid chamber. The camera records the scattered light from the analyte and measures the Brownian motion of the species to determine its size distribution and concentration using the Stokes–Einstein relation. The MB/SDS samples were injected into the system three times, and the system recorded 30 s videos of each injection for analysis.

2.9. Image Processing and Statistical Treatment.

Three images were generated at each scan: the ultrasound image (ultrasound signal only), the PA image (PA signal only), and the overlaid image of ultrasound and PA image. PA images were saved as TIFF files and quantified using ImageJ (1.48v).³² The RGB images were converted to 8-bit images. Eight equal-sized regions of interest (ROI) per tube were selected. The raw integrated density values of the pixels in each ROI were measured for statistical analysis. The mean and standard deviation (SD) were calculated using GraphPad Prism. Here, $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

The chemical structures of the molecules used here are shown in Figure 1A. We measured the switchable PA intensity of MB upon the addition of SDS, and the PA intensity was strongly correlated to the hypsochromic shift in its absorbance spectrum and the number of MB/SDS aggregates in solution. The binding site of the MB monomers at the premicellar concentration was then determined by control experiments using CTAB and SOS. The addition of NaCl at high concentration (i.e., 0.8 mM) in MB and SDS at micellar concentrations then confirmed the binding between the MB monomer and SDS. Finally, we present a scheme of the MB/SDS complex at premicellar and micellar concentrations.

3.1. SDS Concentration-Dependent PA Intensity.

For reference spectra, we locked MB into three different conformations with sodium sulfate: monomer, dimer, and aggregate³³ (Figure 1B). We then studied the MB PA intensity as a function of SDS concentration below the CMC ($\sim 8 \text{ mM}$ ³⁴). There was an increase in signal and then a rapid reverse at and above the SDS CMC. Here, the MB concentration was held at 0.05 mM to optimize its baseline PA intensity and maintain it in a majority of monomeric form (Figure 1B); MB starts to dimerize at 10^{-7} M .^{35,36}

The addition of 3.47 mM SDS increased the PA intensity of pure MB by 492 times (Figure 2A) at 680 nm excitation, and statistical analysis indicated a strong correlation between the PA intensity and the concentration of SDS (linear regression $R^2 > 0.99$) from 0.87 to 3.47 mM (Figure 2B). However, the PA intensity of MB/SDS solutions decreased significantly as the SDS concentration exceeded the CMC of SDS. The PA intensity at SDS concentrations of 8.7 mM was 54 times lower than that at 3.47 mM SDS (near the baseline).

The absorbance and fluorescence intensity of the sample at 680 nm followed the opposite trend—an initial decrease before the CMC of SDS and then an increase in the concentration above the CMC (Figure 2C,D). In many cases, the PA intensity is proportional to the absorption of the sample.³⁷ The decreased fluorescence intensity could not prove that PA enhancement was due to fluorescence quenching because the absorbance also decreased. To study the mechanism underlying the photoacoustic enhancement, we measured the relative quantum yield of the samples using R₆G as the fluorescence standard (right y axis in Figure 2B). The relative quantum yield of the MB/SDS sample decreased as the PA signal increased. We are aware of the limited precision of the absolute value because MB/SDS aggregates cannot fully dissolve in the solvent like fluorescent small molecules; however, we

are more interested in the trend of the relative quantum yield as a function of SDS concentration and its correlation with the PA intensity. This strong correlation suggests that the enhanced PA intensity is indeed a result of fluorescence quenching.

Moreover, the changes in PA intensity are reproducible in acidic (pH 2) or basic (pH 10) conditions (Figure S2) and are likely caused by the aggregation of MB because the increase and decrease of PA intensity were also accompanied by a distinct color change (Figure 2C, inset) indicating a transition between MB monomers and dimers.³⁸ The peak absorbance of MB blue-shifted from 660 to 610 nm at 3.47 mM SDS and returned to 660 nm when the SDS concentration was above the CMC (Figure 2C). We hypothesize that the hypsochromic shift and its reversal correspond to the formation and dissociation of electrostatically formed aggregates of MB and SDS rather than H-type dimers formed by ionic MB monomers.

3.2. MB Forms Aggregates with SDS at Premicellar Concentrations.

To verify the presence of aggregates, we used NTA to measure the size distribution and concentration of potential aggregates in MB/SDS solutions (Figure 3A). We found that SDS at 3.47 mM with 0.05 mM MB formed 2320 times more aggregates (inset of Figure 3A) than that of pure MB with an average size of 285.7 ± 5.1 nm (Figure S3). The Brownian motion of the aggregates is shown in the Video SV1. This number decreased 82 times when SDS was increased to 8.67 mM (above the CMC). The decreased concentration could be attributed to the disassociation of MB/SDS clusters to micelles (~ 1.8 nm³⁹) that were smaller than the detection limit of NTA (10 nm). Additionally, the concentration of aggregates was dependent on the concentration of MB. The concentration of aggregates decreased 84 times when the MB concentration was reduced from 0.05 to 0.00625 mM (Figure S4A). This decrease further demonstrated that MB formed aggregates with SDS.

More interestingly, the increase of PA intensity was specific to the addition of SDS rather than the dimerization of MB. We increased the concentration of MB from 0.05 to 2 mM so that it formed mostly dimers (0 mM SDS in Figure 3B). Here, 1.73 mM SDS decreased the magnitude of absorbance at 610 nm by 1.6 times (Figure 3B), suggesting fewer MB dimers for absorption at 610 nm (Figure S4B). The additional SDS increased the PA intensity by 12 times (Figure 3C), even though the relative increase of absorbance at 680 nm (limited by the wavelength range of the laser) was only 8.3%. Therefore, because of the decreased absorbance at 610 nm, we attributed the large increase of PA intensity at 680 nm to the formation of MB/SDS ionic pairs in the aggregates with a lower extinction coefficient than self-aggregated MB H-type dimers. The PA intensity decreased to a value slightly higher (1.58 times) than that of pure MB when the SDS concentration increased above the CMC at 8.67 mM. This indicated disassociation of the MB/SDS aggregates into MB dimers. The aggregation of MB dimers with SDS that had lower absorbance than ionic MB dimers verified that MB formed ionic pairs with SDS in aggregates rather than H-type dimers formed by ionic MB monomers.

3.3. Control Experiment with Alternative Surfactants.

Next, we used alternative surfactants to determine where MB interacts with SDS: the sulfate moiety or the hydrophobic tail. We substituted SDS with CTAB—a molecule similar in

structure to SDS but with a cationic ammonium group rather than the anionic sulfate of SDS. The addition of 0.25 mM CTAB decreased the PA intensity of 0.05 mM MB by 3 times, and the PA intensity remained low when the CTAB concentration surpassed its CMC (0.9 mM⁴⁰) to 3 mM (Figure 3D). The reduced absorbance (Figure 3E) and PA intensity indicated that CTAB further repulsed the MB monomers, suggesting that electrostatic forces govern the interaction between MB and charged surfactants.

Next, we studied SOS which has the same structure as that of SDS but four fewer carbons. SOS increased the PA intensity of 0.05 mM MB by 3.2 times at 33.8 mM, after which the intensity decreased. At a concentration slightly higher than the SOS CMC (135 mM), the PA intensity decreased to a value slightly lower (0.84 times) than the baseline of pure MB (Figure 3F). The PA intensity would be higher at 135 mM if MB interacted with SDS at a location between the SDS's head and tail because the MB monomer would be closer to enhance the PA intensity. These controls suggest that MB interacts with SDS at the sulfate moiety instead of the hydrophobic tail.

Interestingly, similar PA and spectral phenomena were not observed when we changed the surfactant to SOD, which has six more carbons than SDS (SOD in Figure 1A). A comparison of the absorbance spectrum between MB/SDS and MB/SOD complexes (Figure 3G,H) suggested that the SOD above the CMC did not split MB dimers because of the lack of increased absorbance at 660 nm (MB monomer peak). As a result, there is no decrease in the PA signal of the MB/SOD complex (Figure 3I). The discrepancy between SOD and SDS or SOS might result from the lower charge density of SOD micelles. SOD (CMC = 0.2 mM⁴²) requires fewer monomers to form micelles than SDS (CMC = 8 mM⁴³) or SOS (CMC = 134 mM⁴¹), and therefore, the electrostatic force may not be sufficient to overcome the π - π stacking of MB dimers.

3.4. MB Attaches to SDS after Micellization.

At SDS concentrations above the CMC, the reversal of the hypsochromic shift in the absorbance spectra and reduction in the PA intensity suggests the disassociation of MB/SDS aggregates, yet the precise form of the resultant MB molecules remained unknown. We investigated this question by adding NaCl to MB/SDS solutions to examine whether MB became ionic in solution or was bound specifically to the sulfate moiety. NaCl at a high concentration (e.g., 0.8 M) forms MB dimers.⁴⁴ In our experiments, it decreased the absorbance at 660 nm (MB + NaCl in Figure 4A). As a result, the remaining MB monomer dimerized and increased the PA intensity (Figure 4B). In contrast, additional NaCl in MB with 8.7 mM SDS did not induce a peak shift or decrease in the absorbance spectrum (MB + SDS + NaCl in Figure 4A), although the PA intensity was increased by 59%. This indicated that the resulting MB monomers disassociated from the MB/SDS aggregates were specifically bound to the sulfate moiety of SDS rather than that of free ions in solution.

On the basis of the overall observations, we propose a mechanism for the interaction between MB and SDS in Figure 5. MB forms aggregates or ionic pairs with SDS at premicellar concentrations. SDS brings MB monomers closer together, allowing the formation of MB dimers due to π - π stacking (Figure 5B). However, when the SDS concentration is increased above the CMC, SDS molecules form micelles and separate

individual MB monomers, overcoming the aromatic force between MB monomers. These MB monomers are bound to the sulfate moieties that form the perimeter of SDS micelles (Figure 5C).

4. CONCLUSIONS

A PA, spectroscopic, and nanoparticle tracking study was conducted to elucidate the interactions between MB and SDS. At premicellar concentrations, MB forms aggregates with SDS promoting dimerization. At the CMC and above, SDS disassociates the MB aggregates. The resulting MB monomers bind to the sulfate moieties of SDS micelles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We acknowledge funding from DP2 HL137187, R00 HL117048, and infrastructure from S10 OD021821. We also thank the GT3 core of Sanford consortium for providing the Nanosight LM10 instrument.

REFERENCES

- (1). Ermilov SA; Khamapirad T; Conjuteau A; Leonard MH; Lacewell R; Mehta K; Miller T; Oraevsky AA Laser optoacoustic imaging system for detection of breast cancer. *J. Biomed. Opt.* 2009, 14, 024007. [PubMed: 19405737]
- (2). Jokerst JV; Thangaraj M; Kempen PJ; Sinclair R; Gambhir SS Photoacoustic imaging of mesenchymal stem cells in living mice via silica-coated gold nanorods. *ACS Nano* 2012, 6, 5920–5930. [PubMed: 22681633]
- (3). Wang LV; Hu S Photoacoustic Tomography: In Vivo Imaging from Organelles to Organs. *Science* 2012, 335, 1458–1462. [PubMed: 22442475]
- (4). Bayer CL; Wlodarczyk BJ; Finnell RH; Emelianov SY Ultrasound-guided spectral photoacoustic imaging of hemoglobin oxygenation during development. *Biomed. Opt. Express* 2017, 8, 757–763. [PubMed: 28270982]
- (5). Zhang J; Zhen X; Upputuri PK; Pramanik M; Chen P; Pu K Activatable Photoacoustic Nanoprobes for In Vivo Ratiometric Imaging of Peroxynitrite. *Adv. Mater* 2017, 29, 1604764.
- (6). Hariri A; Fatima A; Mohammadian N; Mahmoodkalayeh S; Ansari MA; Bely N; Avanaki MRN Development of low-cost photoacoustic imaging systems using very low-energy pulsed laser diodes. *J. Biomed. Opt.* 2017, 22, 075001.
- (7). Razansky D; Distel M; Vinegoni C; Ma R; Perrimon N; Köster RW; Ntziachristos V Multispectral opto-acoustic tomography of deep-seated fluorescent proteins in vivo. *Nat. Photonics* 2009, 3, 412–417.
- (8). Wilson KE; Bachawal SV; Abou-Elkacem L; Jensen K; Machtaler S; Tian L; Willmann JK Spectroscopic Photoacoustic Molecular Imaging of Breast Cancer using a B7-H3-targeted ICG Contrast Agent. *Theranostics* 2017, 7, 1463–1476. [PubMed: 28529630]
- (9). Shakiba M; Ng KK; Huynh E; Chan H; Charron DM; Chen J; Muhanna N; Foster FS; Wilson BC; Zheng G Stable J-aggregation enabled dual photoacoustic and fluorescence nano-particles for intraoperative cancer imaging. *Nanoscale* 2016, 8, 12618–12625. [PubMed: 26731304]
- (10). Zhang Y; Wang D; Goel S; Sun B; Chitgupi U; Geng J; Sun H; Barnhart TE; Cai W; Xia J; Lovell JF Surfactant-Stripped Frozen Pheophytin Micelles for Multimodal Gut Imaging. *Adv. Mater* 2016, 28, 8524–8530. [PubMed: 27396479]
- (11). Yan R; Ye D Molecular imaging of enzyme activity in vivo using activatable probes. *Sci. Bull* 2016, 61, 1672–1679.

- (12). Lyu Y; Zhen X; Miao Y; Pu K Reaction-Based Semiconducting Polymer Nanoprobes for Photoacoustic Imaging of Protein Sulfenic Acids. *ACS Nano* 2017, 11, 358–367. [PubMed: 27997794]
- (13). Jiang Y; Pu K Advanced Photoacoustic Imaging Applications of Near-Infrared Absorbing Organic Nanoparticles. *Small* 2017, 13, 1700710.
- (14). Miao Q; Pu K Emerging Designs of activatable photoacoustic probes for molecular imaging. *Bioconjugate Chem.* 2016, 27, 2808–2823.
- (15). Grootendorst DJ; Jose J; Wouters MW; van Boven H; Van der Hage J; Van Leeuwen TG; Steenbergen W; Manohar S; Ruers TJM First experiences of photoacoustic imaging for detection of melanoma metastases in resected human lymph nodes. *Lasers Surg. Med* 2012, 44, 541–549. [PubMed: 22886491]
- (16). Song KH; Stein EW; Margenthaler JA; Wang LV Noninvasive photoacoustic identification of sentinel lymph nodes containing methylene blue in vivo in a rat model. *J. Biomed. Opt* 2008, 13, 054033. [PubMed: 19021413]
- (17). Erpelding TN; Kim C; Pramanik M; Jankovic L; Maslov K; Guo Z; Margenthaler JA; Pashley MD; Wang LV Sentinel Lymph Nodes in the Rat: Noninvasive Photoacoustic and US Imaging with a Clinical US System. *Radiology* 2010, 256, 102–110. [PubMed: 20574088]
- (18). Morgounova E; Shao Q; Hackel BJ; Thomas DD; Ashkenazi S Photoacoustic lifetime contrast between methylene blue monomers and self-quenched dimers as a model for dual-labeled activatable probes. *J. Biomed. Opt* 2013, 18, 056004.
- (19). Ashkenazi S Photoacoustic lifetime imaging of dissolved oxygen using methylene blue. *J. Biomed. Opt* 2010, 15, 040501. [PubMed: 20799768]
- (20). Wang J; Chen F; Arconada-Alvarez SJ; Hartanto J; Yap L-P; Park R; Wang F; Vorobyova I; Dagliyan G; Conti PS; Jokerst JV A Nanoscale Tool for Photoacoustic-Based Measurements of Clotting Time and Therapeutic Drug Monitoring of Heparin. *Nano Lett.* 2016, 16, 6265–6271.
- (21). Garcia MED; Sanz-Medel A Dye-surfactant interactions: a review. *Talanta* 1986, 33, 255–264. [PubMed: 18964075]
- (22). Carroll MK; Unger MA; Leach AM; Morris MJ; Ingersoll CM; Bright FV Interactions between Methylene Blue and Sodium Dodecyl Sulfate in Aqueous Solution Studied by Molecular Spectroscopy. *Appl. Spectrosc* 1999, 53, 780–784.
- (23). Sarkar M; Poddar S Studies on the Interaction of Surfactants with Cationic Dye by Absorption Spectroscopy. *J. Colloid Interface Sci* 2000, 221, 181–185. [PubMed: 10631018]
- (24). Ruprecht J; Baumgärtel H The influence of the electronic structure on the dimerization of phenthiazonium cations in aqueous solution. *Ber. Bunsenges. Phys. Chem* 1984, 88, 145–150.
- (25). Neumann MG; Gehlen MH The interaction of cationic dyes with anionic surfactants in the premicellar region. *J. Colloid Interface Sci* 1990, 135, 209–217.
- (26). Dean JC; Oblinsky DG; Rafiq S; Scholes GD Methylene Blue Exciton States Steer Nonradiative Relaxation: Ultrafast Spectroscopy of Methylene Blue Dimer. *J. Phys. Chem. B* 2016, 120, 440–454. [PubMed: 26781668]
- (27). Kasha M; Rawls HR; El-Bayoumi MA The exciton model in molecular spectroscopy. *Pure Appl. Chem* 1965, 11, 371–392.
- (28). Kasha M Energy Transfer Mechanisms and the Molecular Exciton Model for Molecular Aggregates. *Radiat. Res* 1963, 20, 55–70. [PubMed: 14061481]
- (29). Arconada-Alvarez SJ; Lemaster JE; Wang J; Jokerst JV The development and characterization of a novel yet simple 3D printed tool to facilitate phantom imaging of photoacoustic contrast agents. *J. Photoacoust* 2017, 5, 17–24.
- (30). Würth C; Grabolle M; Pauli J; Spieles M; Resch-Genger U Relative and absolute determination of fluorescence quantum yields of transparent samples. *Nat. Protoc* 2013, 8, 1535–1550. [PubMed: 23868072]
- (31). Kedenburg S; Vieweg M; Gissibl T; Giessen H Linear refractive index and absorption measurements of nonlinear optical liquids in the visible and near-infrared spectral region. *Opt. Mater. Express* 2012, 2, 1588–1611.
- (32). Schneider CA; Rasband WS; Eliceiri KW *Nat. Methods* 2012, 9, 671–675. [PubMed: 22930834]

- (33). Jockusch S; Turro NJ; Tomalia DA Aggregation of Methylene Blue Adsorbed on Starburst Dendrimers. *Macromolecules* 1995, 28, 7416–7418.
- (34). Moroi Y; Motomura K; Matuura R The critical micelle concentration of sodium dodecyl sulfate-bivalent metal dodecyl sulfate mixtures in aqueous solutions. *J. Colloid Interface Sci* 1974, 46, 111–117.
- (35). Rabinowitch E; Epstein LF Polymerization of dyestuffs in solution. thionine and methylene blue. *J. Am. Chem. Soc* 1941, 63, 69–78.
- (36). Braswell E Evidence for trimerization in aqueous solutions of methylene blue. *J. Phys. Chem* 1968, 72, 2477–2483.
- (37). Cox BT; Laufer JG; Beard PC The challenges for quantitative photoacoustic imaging. *Proc. SPIE* 2009, 7177, 717713.
- (38). Zhang Y; Lovell JF Reversible Micro- and Nano- Phase Programming of Anthraquinone Thermochromism Using Blended Block Copolymers. *Langmuir* 2015, 31, 13488–13493. [PubMed: 26626998]
- (39). Duplâtre G; Marques MFF; da Graca Miguel M Size of Sodium Dodecyl Sulfate Micelles in Aqueous Solutions as Studied by Positron Annihilation Lifetime Spectroscopy. *J. Phys. Chem* 1996, 100, 16608–16612.
- (40). Li N; Liu S; Luo H A new method for the determination of the first and second CMC in CTAB solution by resonance Rayleigh scattering technology. *Anal. Lett* 2002, 35, 1229–1238.
- (41). Ranganathan R; Tran L; Bales BL Surfactant- and Salt-Induced Growth of Normal Sodium Alkyl Sulfate Micelles Well above Their Critical Micelle Concentrations. *J. Phys. Chem. B* 2000, 104, 2260–2264.
- (42). Porter MR Handbook of surfactants Blackie; Chapman and Hall: Glasgow, NY, 1991; ix, p 227.
- (43). Rahman A; Brown CW Effect of pH on the critical micelle concentration of sodium dodecyl sulphate. *J. Appl. Polym. Sci* 1983, 28, 1331–1334.
- (44). Severino D; Junqueira HC; Gugliotti M; Gabrielli DS; Baptista MS Influence of Negatively Charged Interfaces on the Ground and Excited State Properties of Methylene Blue. *Photochem. Photobiol* 2003, 77, 459–468. [PubMed: 12812286]

Figure 1.

Chemical structures of chemicals used in the experiment and absorbance spectra of MB at different orders of aggregates. Panel A shows the chemical structure of MB, SOS, SDS, SOD, and CTAB. Panel B shows the spectral profile of MB monomer (black), MB dimer (blue), and MB high-order aggregates (red) prepared by adding increasing concentrations of sodium sulfate. The absorbance peak of MB monomer, dimer, and higher-order aggregates occurs at 660, 610, and 580 nm, respectively.

Figure 2.

PA and spectroscopic changes of MB upon SDS addition. Panel A shows the combined PA/ultrasound image of tubes filled with 0.05 mM MB and increasing SDS concentration from 0.9 to 17.3 mM. The image is a maximum projection intensity of the overlay of ultrasound (black to white) and PA (red to white) signals (scale bar = 2 mm). The intensities were quantified in panel B. Statistical analysis showed a linear response from 0 to 3.47 mM SDS ($R^2 > 0.99$) with a 492-fold increase ($\lambda = 680$ nm) at 3.47 mM SDS. The PA intensity then decreased 54 times at 8.67 mM. The trend of PA intensity corresponds closely to the relative quantum yield shown in the right y axis. The relative quantum yield measurement using R₆G as a fluorescence standard revealed an initial decrease before the CMC and a subsequent increase beyond the CMC of SDS, which indicates that the PA enhancement is a result of fluorescence quenching. The PA intensity also corresponds to the absorbance spectral shift and the reversal of fluorescence intensity shown in panel C and panel D. The absorbance peak of pure MB blue-shifts from 660 to 610 nm at premicellar concentrations, suggesting the formation of MB/SDS aggregates. The shift reverses to 660 nm at the SDS concentration of 8.67 mM, a micellar concentration. The inset in panel C shows the color change and reversion of MB solution as the SDS concentration approaches and surpasses the CMC. Error bars in panel B denote SD.

Figure 3.

Investigation of MB/SDS aggregates and the binding between MB and SDS using 2 mM MB (dimer-promoting), CTAB, and SOS. Panel A shows that the concentration of MB/SDS aggregates increased from 1.28×10^6 to 2.97×10^9 particles/mL upon the addition of 3.47 mM SDS. The concentration decreased to 3.64×10^7 particles/mL when the SDS concentration was further increased to 8.68 mM because the MB/SDS aggregates disassociated to MB/SDS micelles that were too small to be detected. Panel B shows the absorbance spectra of 2 mM MB with an increasing SDS concentration from 0.9 to 8.7 mM. The peak absorbance of pure MB (black line) at 610 nm indicates the strong presence of MB dimers. The inset in panel C is the PA image of samples in panel B and panel C, which quantifies the PA intensities of the same solutions. Statistical analysis shows a 12-fold increase of MB upon 1.73 mM SDS addition. The PA signal begins to decrease at 3.47 mM SDS and eventually to a value similar to that of pure MB at 8.67 mM. Panel D shows the quantified PA intensity with a PA/ultrasound image (inset) of 0.05 mM MB with 0, 0.25, 0.50, 1.00, 1.50, and 3.00 mM CTAB. While additional 0.25 mM CTAB decreased the PA intensity of MB by 3 times, no hypsochromic shift such as the addition of SDS was observed in the absorbance spectra (panel E). Panel F quantifies the PA image (inset) of 0.05 mM MB with an increasing SOS concentration from 0 to 270 mM. The PA intensity of MB increased 3.2 times with the addition of 33.8 mM SOS and reversed to a value 0.84 times lower than that of the pure MB at 135 mM (the CMC of SOS is 134 mM^{41}). Panel G shows the absorbance spectrum of MB with an increasing concentration of SOS. Panels H and I show

the absorbance spectrum and PA intensity of MB with increasing SOD, respectively. Error bars and scale bars in panels A, C, D, F, and I represent SD and 2 mm, respectively.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

Mechanistic determination of the MB/SDS interaction at micellar concentrations using NaCl. Panel A shows the effect of 0.8 M NaCl in 2 mM MB with and without 8.67 mM SDS. The peak absorbance at 610 nm indicates that pure MB monomers form dimers at 2 mM. The addition of NaCl significantly reduces the monomer peak at 660 nm, implying that more dimers form. The peak absorbance of MB red-shifts to 660 nm in the presence of 8.67 mM SDS, and the addition of NaCl does not induce a hypsochromic shift. The corresponding PA image of the samples in panel B shows that the additional NaCl increases the PA intensity of MB by 1.7 times without SDS and 0.59 times with 8.67 mM SDS. This suggests that MB binds to SDS at the micellar concentration. Scale bars and error bars in panels B denote 2 mm and SD, respectively.

Figure 5. Interaction scheme between MB and SDS at pre-micellar and micellar concentrations. Panel A shows the chemical structure of MB and SDS. MB is blue, and SDS has a red hydrophilic head and a black hydrophobic tail. Panel B illustrates the potential forms of MB/SDS aggregates at pre-micellar concentrations. When SDS forms micelles (panel C), MB dimer disassociates, leaving MB monomers bound to the sulfate moieties of SDS micelles.