Facile Production of Hexagonal Boron Nitride Nanoparticles by Cryogenic Exfoliation

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Supporting Information



ABSTRACT: Fluorescent nanoparticles with optically robust luminescence are imperative to applications in imaging and labeling. Here we demonstrate that hexagonal boron nitride (hBN) nanoparticles can be reliably produced using a scalable cryogenic exfoliation technique with sizes below 10 nm. The particles exhibit bright fluorescence generated by color centers that act as atomic-size quantum emitters. We analyze their optical properties, including emission wavelength, photon-statistics, and photodynamics, and show that they are suitable for far-field super-resolution fluorescence nanoscopy. Our results provide a foundation for exploration of hBN nanoparticles as candidates for bioimaging, labeling, as well as biomarkers that are suitable for quantum sensing.

KEYWORDS: Hexagonal boron nitride, nanoparticles, quantum dots, photostability, blinking

F luorescent nanoparticles are crucially important for a variety of applications, including imaging, sensing, drug delivery, energy, and quantum nanophotonics.¹⁻⁶ These applications impose a broad set of requirements on the nanoparticles of which the physical and optical properties, particularly size, brightness, and photostability, are among the most important. Sizes of or below 6 nm have been proven to enable live cell imaging and have been shown to minimally perturb biological systems. Moreover, nanoparticles smaller than 6 nm are usually eliminated from the body through the kidneys, whereas nanoparticles with sizes above 30 nm accumulate rapidly in the reticuloendothelial system.⁷

Fluorescent colloidal quantum dots, which can have diameters below 5 nm, are an appealing candidate that satisfy the size prerequisite. These quantum dots, however, suffer from two major drawbacks, low photostability and biological toxicity, thus limiting their use in imaging and biomarking applications.⁸ Research of alternative platforms such as fluorescent nanodiamonds have therefore garnered significant attention in the past decade.^{9,10} While nanodiamonds can

indeed host optically stable emitters in the form of color centers,¹¹ mass production of particles with diameters below 40 nm is still challenging.¹² Furthermore, achieving high fluorescence brightness requires cumbersome protocols of high energy irradiation and annealing that significantly increases the price and limits broad widespread use of nanodiamonds.

An emerging material that can host ultrabright fluorescent, optically stable defects is hexagonal boron nitride (hBN), a layered van der Waals crystal with a wide bandgap of ~ 6 eV. Similar to diamond, point defects in hBN can be isolated and exhibit single photon emission statistics which are necessary for on-demand single photon generation and associated quantum photonics applications.^{13,14} However, unlike diamond, hBN is a layered material, and a facile production of nanoparticles from larger hBN flakes is therefore easier. In

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Figure 1. Cryogenic exfoliation of hBN powder into nanoparticles and their characterization. (a) Schematic of the cryogenic exfoliation process. Commercial hBN powder (i) was soaked in liquid nitrogen (ii) for an hour before being dispersed into a room-temperature solvent of isopropanol/water (1:1) (iii) to induce peeling-off and breakdown of the hBN flakes. The resulting hBN nanoparticles were then separated from the larger clusters by centrifugation and filtration through a 200 nm pore membrane (iv). The solution containing small hBN nanoparticles was then spin-casted on marked silicon substrates and subsequently annealed at 850 °C in an argon atmosphere (v). (b) Photograph of samples obtained by centrifugation at 2000 rpm (S1), 6000 rpm +200 nm power filtration (S2), and 12 000 rpm + 200 nm power filtration (S3). (c-e) TEM images taken from hBN nanoparticles in samples S1, S2, and S3. (f) Representative electron diffractogram taken from sample S2. For consistency throughout the main text, the green, blue, and red dots are used to represent sample S1, S2, and S3, respectively, unless otherwise stated.

addition, hBN nanoparticles are biocompatible and display no cytotoxicity at concentrations of up to 0.4 mg/mL.^{15–17} This makes the material appealing for super-resolution imaging^{18–20} in biological environments and motivates fabrication of fluorescent hBN nanoparticles with diameters below 30 nm, particularly as the solutions discussed above are limited by low brightness or poor photostability. Far-field super-resolution fluorescence imaging techniques such as single molecule

localization microscopy (SMLM) require self-blinking fluorescent probes in the form of photoswitchable nanoparticles or recently introduced self-blinking organic dyes.²¹ Quantum emitters hosted by hBN have previously been shown to exhibit blinking,^{19,22} and hBN nanoparticles may therefore also be suitable probes for SMLM applications.

In this work, we leverage a cryogenic-mediated exfoliation approach²³ to convert hBN powder into hBN nanoparticles with diameters below 10 nm and as small as \sim 3 nm. We characterize the photophysical and photodynamic properties of the particles and show that they can host isolated single photon emitters that are photostable and suitable for super-resolution imaging.

The original hBN powder was purchased commercially from Graphene Supermarket Inc. with a mean particle size of ~100 nm. To fabricate smaller nanoparticles, we adopted a recently developed cryogenic exfoliation technique.²³ The process is shown schematically in Figure 1a. Briefly, hBN powder was soaked in liquid nitrogen for an hour and thermally shocked by dispersing it into a room-temperature solvent of isopropanol:water (1:1). After the cryogenic treatment, peeling-off of the hBN flakes and small cracks in the intralayer were formed, facilitating the breakdown of hBN powder into small nanoparticles. The solution of hBN nanoparticles was then exposed to an ultrasonication bath for an extended period of time to induce full liquid-phase exfoliation (LPE). The obtained solution was subsequently centrifuged and filtered to remove large hBN clusters from smaller nanoparticles, thus resulting in a narrow size distributions of hBN nanoparticles. Specifically, we prepared three different sample batches with increasing centrifugation speeds: S1-2000, S2-6000, and S3-12000 rpm. To obtain smaller hBN nanoparticles, we filtered S2 and S3 through 200 nm pore polymer membranes. The 200 nm pore filter was chosen to effectively filter out the very thin yet large hBN flakes that still remained at the supernatant of the solution after the centrifugation process without adversely



Figure 2. Size distributions of hBN nanoparticles. (a–c) AFM images taken from samples S1, S2, and S3, respectively. (d–f) Thickness histograms of hBN particles in samples S1, S2, and S3, respectively. The scale bars are 1 μ m. The sample sizes (i.e., number of *N*) for sample S1, S2, and S3 are 2745, 2145, and 1660, respectively. The size analysis was done using particle analysis in the NanoScope Analysis software (Bruker).



Figure 3. Optical properties of quantum emitters in hBN nanoparticles. (a-c) Three sets of five representative spectra, each set taken from samples S1, S2, and S3, respectively. (d-f) Three sets of five representative second-order autocorrelation functions, each set recorded from samples S1, S2, and S3, respectively. (g-i) Histograms of antibunching dip values extracted from 18 spots in sample S1, 24 spots in sample S2, and 10 spots in sample S3. A value below 0.5 indicates a quantum emitter. All the measurements were taken with a 568 nm long-pass filter in the collection path at room temperature. Insets represent the median values and standard deviations of antibunching dips measured from samples S1, S2, and S3, respectively.

affecting the yield of the nanoparticles. In order to perform optical characterization, a portion of each batch was dispersed onto a silicon substrate and annealed at 850 °C in an argon atmosphere to activate quantum emitters embedded within hBN nanoparticles. For convenience, throughout the paper we present the data for S1, S2, and S3 in green, blue, and red color, respectively. Because of smaller particle sizes, samples S2 and S3 are more transparent whereas S1 is more opaque because of light scattering from larger particles (Figure 1b).

To characterize the hBN nanoparticles structurally, we dropcasted them onto an ultrathin carbon film supported by a lacey carbon film on a copper grid and imaged them with a transmission electron microscope (TEM) operated using an accelerating voltage of 80 kV. Figure 1c-e shows HRTEM images of samples S1, S2, and S3, respectively. With higher centrifugation speeds and the use of a 200 nm pore filter, sample S2 (5.3 ± 0.8 nm) and sample S3 (3.0 ± 0.7 nm) exhibit significantly smaller hBN nanoparticle sizes than sample S1 (23.5 ± 4.6 nm). To confirm the nanoparticle symmetry and composition, diffraction patterns of the same samples were studied in different spots across the grid (Figure 1f). In addition, by analyzing the fast Fourier transform (FFT) of the most representative particles (Figure S2) and based on the distance in the reciprocal space, we found their lattice parameter to be equal to 2.54 Å, which matches that of hBN.²⁴

After spin-casting of the hBN nanoparticles onto marked silicon substrates, we used atomic force microscope (AFM) to check their size distributions. The AFM images of the three samples are shown in Figure 2a-c. Because the lateral size measured by AFM is largely overestimated by tip convolution artifacts, we chose to measure the nanoparticle thickness instead of the lateral size. Figure 2d-f shows the nanoparticle thickness histograms. The average nanoparticle size is 119.5 \pm 46.0, 20.5 \pm 10.6, and 5.14 \pm 1.40 nm for samples S1, S2, and S3, respectively. The decrease in the nanoparticle size obtained from AFM measurements is in good agreement with that from the TEM measurements, confirming the effectiveness of the centrifugation and filtration processes.

Next, we turn to optical properties of the nanoparticles. We start with their spectral properties and the presence of quantum emitters, and then characterize their temporal photodynamics. Optical measurements were performed using a 532 nm excitation laser. The fluorescence signal was then analyzed by a spectrometer and a Hanbury Brown and Twiss (HBT) setup to confirm the presence of quantum emitters.

Figure 3a-c shows five representative spectra taken from each of the three samples. All spectra exhibit sharp lines, attributed to the zero-phonon lines (ZPL) and some broader peaks red-shifted from the ZPLs, regarded as phonon sideband (PSB) transitions. As expected, spectra taken from sample S1 show in some cases multiple ZPLs and PSBs due to the larger particle sizes and larger quantities of material probed by the excitation laser. On the other hand, spectra from samples S2 and S3, obtained with higher centrifugation speeds and a filter typically contain a single ZPL with a correlated PSB.

Figure 3d-f shows the corresponding second order autocorrelation measurements, $g^2(\tau)$, that can confirm whether the probed luminescence originates from single emitters. An antibunching dip at zero delay time, $g^2(0)$, of less than 0.5 is indicative of single photon emission characteristics. For sample S1, that is larger in size and hence likely to include multiple emitters in any single measurement, only a few true single emitters were observed with an average $g^2(0)$ value of ~0.5 ± 0.21. However, most of the emitters probed in samples S2 and S3 were in fact single point defects. Sample S3 exhibited only single emitters with an average value of $g^2(0) \sim 0.26 \pm 0.10$ with no background correction. The histograms of the dip values (Figure 3g-i) further confirm this observation with sample S3 showing highest single photon purity.

To analyze the photodynamic properties of the hBN nanoparticles prepared by cryogenic exfoliation, we use a wide-field SMLM approach that was recently applied to characterization of dense optically active defects in hBN monolayers.^{19,20,22} Such wide-field SMLM analysis enables rapid analysis of much larger numbers of particles than scanning confocal imaging. A successful demonstration of an SMLM-based technique on hBN nanoparticles also validates their potential use as fluorescent markers for biological applications.

The three solutions of hBN nanoparticles were first dropcasted/spin-coated onto glass coverslips and dried on a hot plate. The samples were then imaged using a 561 nm excitation laser in a widefield illumination mode (with an illumination



Figure 4. Photodynamics of quantum emitters obtained using SMLM on samples S1–S3. (a) Widefield (max intensity projection) and reconstructed image of an area in sample S2, based on 6000 frames, processed using ImageJ plugin ThunderSTORM.²⁵ Scale bar: 5 μ m. The inset shows a zoom of two emitters in the super-resolved image (scale bar: 100 nm) (b) Time trace of a localized emitter, derived from the localization table from (a), and an example of the detected ON and OFF times. Exposure time: 20 ms. (c) Normalized distribution of ON/OFF times for the localized emitters, calculated from the analysis of their time traces, as shown in (b). (d) Histogram of the number of blinks (transitions from OFF to ON state and back) per emitter, detected throughout the entire acquisition (8000 frames, 160 s).

power of ~150 W/cm2). Emission from optically active defects in hBN nanoparticles is collected by a 100× oilimmersion microscope (Numerical Aperture ~1.4) and projected onto an EMCCD camera with a projected pixel size of 105 nm. We observed localized blinking photoluminescece emission from individual diffraction-limited spots on the coverslip, which we attribute to the emission from individual hBN nanoparticles (sample S2) as shown in Figure 4a (widefield). Tuning the particle concentrations during dropcasting, we optimize the density of emitters on coverslips to achieve large coverage while allowing straightforward localization of single hBN nanoparticles within diffraction-limited spots. Fitting the emission intensity from individual emitters on successive frames by 2D Gaussian profiles allows the reconstruction of a super-resolved image (see Figure 4a, SMLM).

To obtain the emission dynamics of individual emitters, we analyze localization events throughout successive frames. The intensity of individual emitters is traced through the whole image stack, assuming that emitter is OFF (nonemitting) when it is not detected by the localization algorithm and ON when it is detected within the radius of its localization precision. A typical intensity trace for one of the localized emitters is shown in Figure 4b, showing successive ON-OFF events.

Analyzing such events for every localized emitter, we obtain the distribution of ON and OFF times for the emitters in the three samples S1–S3 (plotted on a log–log scale in Figure 4c). The distribution follows a well-defined power-law scaling with $P(t_{ON/OFF}) \sim t^{\alpha_{-}ON/OFF})$, consistent with recent characterization of blinking kinetics of emitters in hBN monolayers.²⁰ We do not observe any significant differences between the three samples, which suggests that blinking is actually dependent on the environment in the immediate vicinity of the defects, as it appears to be independent of particle size within the studied size range. The power-laws are found to be, respectively, $\alpha_{\rm ON} \approx -2.0$ and $\alpha_{\rm OFF} \approx -1.5$ for ON and OFF times.

Finally, we plot in Figure 4d the histogram of the number of blinking events per particle detected during the whole acquisition period (2.5 min) for the three samples. We observe a broad distribution in the number of blinks. For samples S2 and S3, corresponding to the smallest sizes, a significant proportion of emitters blink only a few times possibly due to irreversible photobleaching whereas other emitters demonstrate repeatable and consistent blinking throughout the acquired image stack. For sample S1, the distribution is shifted toward larger number of blinks per particle. This enhanced stability could be attributed either to a smaller number of defects per particle or to intrinsic stabilization of the emitters due to reduced interactions with the substrate for the largest particles. All in all, this implies that the produced hBN nanoparticles are indeed suitable as labels for SMLM.

In conclusion, we demonstrated the fabrication of hBN nanoparticles as small as 3.0 ± 0.7 nm. The particles contain fluorescent point defects that act as photostable single photon emitters. The emitter photodynamics were analyzed by SMLM, which demonstrates their potential use as fluorescent markers for bioimaging applications. The facile nanoparticle production method, together with their favorable optical properties and the established biocompatibility of hBN, makes this system a promising platform for super-resolution imaging and quantum sensing applications.

Experimental Section. Materials and Methods. Cryo-Mediated Exfoliation and Fracturing Process to Produce hBN Nanoparticles. Commercially available hBN nanopowder was purchased from Graphene Supermarket. All solvents were purchased from commercially available sources as a laboratory grade and used as supplied without purification. First, 60 mg of hBN powder was soaked in liquid nitrogen (77 K) for 1 h, then the powder were immediately dispersed into 20 mL of IPA/ H_2O (the volume ratio of IPA/ H_2O is 1:1) to induce thermal shock. The hBN flakes solution was subsequently exfoliated by a commercially available bath-ultrasonication for 4 h. The concentration of the dispersions were $\sim 2 \text{ mg/mL}$. After the cryomediated exfoliation and fracturing processing, the resultant solution was divided into three plastic vials with equal volume with each vial containing ~6.5 mL. These solutions were then centrifuged at 2000, 6000, and 12000 rpm (rounds per minute) for 30 min to remove big clusters. The supernatants of each sample were then centrifuged for another 30 min. To further separate the nanoparticles from the dispersions, the resultant supernatants were filtered through ultrafine membranes with a pore size of 200 nm.

AFM Characterization. For the AFM characterization, the as-obtained nanoparticle solution was diluted and then directly dropped onto a new silicon with thermal oxide layer substrate. The samples were annealed at 850 $^{\circ}$ C in 1 Torr argon atmosphere for 30 min to activate the single photon emitters in h-BN.

Characterization of Quantum Emitters in h-BN QDs. Optical properties of h-BN nanoparticles were characterized using the lab-built confocal microscope. A continuous wave (CW) 532 nm laser (Gem 532, Laser Quantum Ltd.) was used for excitation. The laser was directed through a half-wave plate and was focused onto the sample using a high-numericalaperture objective lens (NA = 0.9, TU Plan Fluor $100 \times$, Nikon). The fluorescence of the sample was collected by scanning laser across via an x-y piezo scanning mirror (FSM-300, Newport). The collected light was filtered using a 532 nm dichroic mirror (532 nm laser BrightLine, Semrock) and an additional 568 nm long pass filter (LP Filter 568 nm, Semrock). The signal was then coupled into a graded-index fiber, where the fiber aperture served as a pinhole. A fiber splitter was used to direct the light to a spectrometer (Acton SpectraPro, Princeton Instrument Inc.) and to an avalanche photodiode (SPCM-AQRH-14-FC, Excelitas Technologies) for time-resolved measurement. The laser excitation power was maintained at 300 μ W for all the sample characterizations. Correlation measurements were done using a time-correlated single photon counting module (PicoHarp300, PicoQuant). The autocorrelation measurements were not background corrected. Photodynamics of quantum emitters in h-BN nanoflakes was characterized using a widefield inverted microscope.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nano-lett.9b01913.

Additional transmission electron microscopy analysis, photoluminescence, and dynamic light scattering studies of the hBN nanoparticles (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wolfbeis, O. S. An overview of nanoparticles commonly used in fluorescent bioimaging. *Chem. Soc. Rev.* **2015**, *44*, 4743–4768.

(2) Alkahtani, M. H.; Alghannam, F.; Jiang, L.; Almethen, A.; Rampersaud, A. A.; Brick, R.; Gomes, C. L.; Scully, M. O.; Hemmer, P. R. *Nanophotonics* **2018**, *7*, 1423.

(3) Hanifi, D. A.; Bronstein, N. D.; Koscher, B. A.; Nett, Z.; Swabeck, J. K.; Takano, K.; Schwartzberg, A. M.; Maserati, L.; Vandewal, K.; van de Burgt, Y. Redefining near-unity luminescence in quantum dots with photothermal threshold quantum yield. *Science* **2019**, *363*, 1199–1202.

(4) Zhang, J.; Cheng, F.; Li, J.; Zhu, J.-J.; Lu, Y. Fluorescent nanoprobes for sensing and imaging of metal ions: Recent advances and future perspectives. *Nano Today* **2016**, *11*, 309–329.

(5) Panfil, Y. E.; Oded, M.; Banin, U. Colloidal Quantum Nanostructures: Emerging Materials for Display Applications. *Angew. Chem., Int. Ed.* **2018**, *57*, 4274–4295.

(6) Liu, J.-H.; Cao, L.; LeCroy, G. E.; Wang, P.; Meziani, M. J.; Dong, Y.; Liu, Y.; Luo, P. G.; Sun, Y.-P. Carbon "Quantum" Dots for Fluorescence Labeling of Cells. *ACS Appl. Mater. Interfaces* **2015**, *7*, 19439–19445.

(7) Schipper, M. L.; Iyer, G.; Koh, A. L.; Cheng, Z.; Ebenstein, Y.; Aharoni, A.; Keren, S.; Bentolila, L. A.; Li, J.; Rao, J. Particle Size, Surface Coating, and PEGylation Influence the Biodistribution of Quantum Dots in Living Mice. *Small* **2009**, *5*, 126–134.

(8) Valizadeh, A.; Mikaeili, H.; Samiei, M.; Farkhani, S. M.; Zarghami, N.; kouhi, M.; Akbarzadeh, A.; Davaran, S. Quantum dots: synthesis, bioapplications, and toxicity. *Nanoscale Res. Lett.* **2012**, *7*, 480.

(9) Vlasov, I. I.; Shiryaev, A. A.; Rendler, T.; Steinert, S.; Lee, S.-Y.; Antonov, D.; Voros, M.; Jelezko, F.; Fisenko, A. V.; Semjonova, L. F.; et al. Molecular-sized fluorescent nanodiamonds. *Nat. Nanotechnol.* **2014**, *9*, 54–58.

(10) Ho, D.; Wang, C.-H. K.; Chow, E. K.-H. Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine. *Sci. Adv.* 2015, *1*, e1500439.

(11) Aharonovich, I.; Neu, E. Diamond Nanophotonics. *Adv. Opt. Mater.* **2014**, *2*, 911–928.

(12) Chang, Y. R.; Lee, H. Y.; Chen, K.; Chang, C. C.; Tsai, D. S.; Fu, C. C.; Lim, T. S.; Tzeng, Y. K.; Fang, C. Y.; Han, C. C.; et al. Mass production and dynamic imaging of fluorescent nanodiamonds. *Nat. Nanotechnol.* **2008**, *3*, 284–288.

(13) Tran, T. T.; Bray, K.; Ford, M. J.; Toth, M.; Aharonovich, I. Quantum emission from hexagonal boron nitride monolayers. *Nat. Nanotechnol.* **2016**, *11*, 37–41.

(14) Toth, M.; Aharonovich, I. Single Photon Sources in Atomically Thin Materials. *Annu. Rev. Phys. Chem.* **2019**, *70*, 123. (15) Horváth, L.; Magrez, A.; Golberg, D.; Zhi, C.; Bando, Y.; Smajda, R.; Horváth, E.; Forró, L.; Schwaller, B. In Vitro Investigation of the Cellular Toxicity of Boron Nitride Nanotubes. *ACS Nano* **2011**, *5*, 3800–3810.

(16) Kıvanç, M.; Barutca, B.; Koparal, A. T.; Göncü, Y.; Bostancı, S. H.; Ay, N. Effects of hexagonal boron nitride nanoparticles on antimicrobial and antibiofilm activities, cell viability. *Mater. Sci. Eng., C* **2018**, *91*, 115–124.

(17) Merlo, A.; Mokkapati, V. R. S. S.; Pandit, S.; Mijakovic, I. Boron nitride nanomaterials: biocompatibility and bio-applications. *Biomater. Sci.* **2018**, *6*, 2298–2311.

(18) Kianinia, M.; Bradac, C.; Sontheimer, B.; Wang, F.; Tran, T. T.; Nguyen, M.; Kim, S.; Xu, Z.-Q.; Jin, D.; Schell, A. W.; et al. All-optical control and super-resolution imaging of quantum emitters in layered materials. *Nat. Commun.* **2018**, *9*, 874.

(19) Feng, J.; Deschout, H.; Caneva, S.; Hofmann, S.; Lončarić, I.; Lazić, P.; Radenovic, A. Imaging of optically active defects with nanometer resolution. *Nano Lett.* **2018**, *18*, 1739.

(20) Comtet, J.; Glushkov, E.; Navikas, V.; Feng, J.; Babenko, V.; Hofmann, S.; Watanabe, K.; Taniguchi, T.; Radenovic, A. Wide-Field Spectral Super-Resolution Mapping of Optically Active Defects in Hexagonal Boron Nitride. *Nano Lett.* **2019**, *19*, 2516–2523.

(21) Grimm, J. B; Muthusamy, A. K; Liang, Y.; Brown, T. A; Lemon, W. C; Patel, R.; Lu, R.; Macklin, J. J; Keller, P. J; Ji, N.; Lavis, L. D; et al. A general method to fine-tune fluorophores for live-cell and in vivo imaging. *Nat. Methods* **2017**, *14*, 987.

(22) Stern, H. L.; Wang, R.; Fan, Y.; Mizuta, R.; Stewart, J. C.; Needham, L.-M.; Roberts, T. D.; Wai, R.; Ginsberg, N. S.; Klenerman, D.; et al. Spectrally Resolved Photodynamics of Individual Emitters in Large-Area Monolayers of Hexagonal Boron Nitride. *ACS Nano* **2019**, *13*, 4538–4547.

(23) Wang, Y.; Liu, Y.; Zhang, J.; Wu, J.; Xu, H.; Wen, X.; Zhang, X.; Tiwary, C. S.; Yang, W.; Vajtai, R. Cryo-mediated exfoliation and fracturing of layered materials into 2D quantum dots. *Sci. Adv.* **2017**, *3*, e1701500.

(24) Paszkowicz, W.; Pelka, J. B.; Knapp, M.; Szyszko, T.; Podsiadlo, S.J.A.P.A. Lattice parameters and anisotropic thermal expansion of hexagonal boron nitride in the 10–297.5 K temperature range. *Appl. Phys. A: Mater. Sci. Process.* **2002**, *75*, 431–435.

(25) Ovesný, M.; Křížek, P.; Borkovec, J.; Svindrych, Z.; Hagen, G. M. ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics* **2014**, *30* (16), 2389–2390.