

Journal of Visualized Experiments

Bio-inspired Polydopamine Surface Modification of Nanodiamonds and its Reduction of Silver Nanoparticles --Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58458R2
Full Title:	Bio-inspired Polydopamine Surface Modification of Nanodiamonds and its Reduction of Silver Nanoparticles
Keywords:	Nanodiamonds, Polydopamine, Reduction, Silver nanoparticles, Surface modification, Self-polymerization.
Corresponding Author:	Risheng Wang UNITED STATES
Corresponding Author's Institution:	
Corresponding Author E-Mail:	wangri@mst.edu
Order of Authors:	Yun Zeng Wenyan Liu Risheng Wang
Additional Information:	
Question	Response
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US\$2,400)
Please indicate the city, state/province, and country where this article will be filmed . Please do not use abbreviations.	400 W 11th street, Rolla MO 65401

TITLE:

Bio-inspired Polydopamine Surface Modification of Nanodiamonds and its Reduction of Silver Nanoparticles

AUTHORS & AFFILIATIONS:

Yun Zeng¹, Wenyan Liu², Risheng Wang¹

¹Department of Chemistry, Missouri University of Science and Technology, Rolla, MO, USA

²Center for Research in Energy and Environment, Department of Chemistry, Missouri University of Science and Technology, Rolla, MO, USA

Corresponding Author:

Risheng Wang

Email Address: wangri@mst.edu

Tel: (573) 341-7729

Email Addresses of Co-authors:

Yun Zeng (yzeng@xidian.edu.cn)

Wenyan Liu (liuweny@mst.edu)

KEYWORDS:

Nanodiamonds, polydopamine, reduction, silver nanoparticles, surface modification, self-polymerization.

SHORT ABSTRACT:

A facile protocol is presented to functionalize the surfaces of nanodiamonds with polydopamine.

LONG ABSTRACT:

Surface functionalization of nanodiamonds (NDs) is still challenging due to the diversity of functional groups on the ND surfaces. Here, we demonstrate a simple protocol for the multifunctional surface modification of NDs by using mussel-inspired polydopamine (PDA) coating. In addition, the functional layer of PDA on NDs could serve as a reducing agent to synthesize and stabilize metal nanoparticles. Dopamine (DA) can self-polymerize and spontaneously form PDA layers on ND surfaces if the NDs and dopamine are simply mixed together. The thickness of a PDA layer is controlled by varying the concentration of DA. A typical result shows that a thickness of ~5 to ~15 nm of the PDA layer can be reached by adding 50 to 100 µg/mL of DA to 100 nm ND suspensions. Furthermore, the PDA-NDs are used as a substrate to reduce metal ions, such as Ag[(NH₃)₂]⁺, to silver nanoparticles (AgNPs). The sizes of the AgNPs rely on the initial concentrations of Ag[(NH₃)₂]⁺. Along with an increase in the concentration of Ag[(NH₃)₂]⁺, the number of NPs increases, as well as the diameters of the NPs. In summary, this study not only presents a facile method for modifying the surfaces of NDs with PDA, but also demonstrates the enhanced functionality of NDs by anchoring various species of interest (such as AgNPs) for advanced applications.

INTRODUCTION:

Nanodiamonds (NDs), a novel carbon-based material, have attracted considerable attention in recent years for use in various applications^{1,2}. For instance, the high surface areas of NDs provide excellent catalyst support for metal nanoparticles (NPs) because of their super-chemical stability and thermal conductivity³. Furthermore, NDs play significant roles in bio-imaging, bio-sensing, and drug delivery due to their outstanding biocompatibility and nontoxicity^{4,5}.

To efficiently extend their capabilities, it is valuable to conjugate functional species on the surfaces of NDs, such as proteins, nucleic acids, and nanoparticles⁶. Although a variety of functional groups (*e.g.*, hydroxyl, carboxyl, lactone, *etc.*) are created on the surfaces of NDs during their purification, the conjugation yields of the functional groups are still very low because of the low density of each active chemical group⁷. This results in unstable NDs, which tend to aggregate, limiting further application⁸.

Currently, the most common methods used to functionalize NDs, are covalent conjugation by using copper-free click chemistry⁹, covalent linkage of peptide nucleic acids (PNA)¹⁰, and self-assembled DNA¹¹. The non-covalent wrapping of NDs has also been proposed, including carbohydrate-modified BSA⁴, and HSA¹² coating. However, because these methods are time consuming and inefficient, it is desirable that a simple and generally applicable method can be developed to modify the surfaces of NDs.

Dopamine (DA)¹³, known as a natural neurotransmitter in the brain, was widely used for adhering and functionalizing nanoparticles, such as gold nanoparticles (AuNPs)¹⁴, Fe₂O₃¹⁵, and SiO₂¹⁶. Self-polymerized PDA layers enrich amino and phenolic groups, which can be further utilized to directly reduce metal nanoparticles or to easily immobilize thiol/amine-containing biomolecules on an aqueous solution. This simple approach was recently applied to functionalize NDs by Qin *et al.* and our laboratory^{17,18}, although DA derivatives were employed to modify NDs *via* Click Chemistry in earlier studies^{19,20}.

Here, we describe a simple PDA-based surface modification method that efficiently functionalizes NDs. By varying the concentration of DA, we can control the thickness of a PDA layer from a few nanometers to tens of nanometers. In addition, the metal nanoparticles are directly reduced and stabilized on the PDA surface without the need for additional toxic reduction agents. The sizes of the silver nanoparticles depend on the initial concentrations of Ag[(NH₃)₂]⁺. This method allows the well-controlled deposition of PDA on the surfaces of NDs and the synthesis of ND conjugated AgNPs, which dramatically extends the functionality of NDs as excellent nano-platforms of catalyst supports, bio-imaging, and bio-sensors.

PROTOCOL:

1. Preparation of Reagents

CAUTION: Please read and understand all relevant material safety data sheets (MSDS) before use. Some of the chemicals are toxic and volatile. Please follow special handling procedures and storage requirements. During the experimental procedure, use personal protective equipment,

such as gloves, safety glasses, and a lab coat to avoid potential hazards.

1.1. Preparation of Tris-HCl buffer

1.1.1. Dissolve 121.14 g of Tris powder in 500 mL of deionized H₂O, ensure that the powder dissolves completely, and then transfer the solution to a 1,000 mL-volumetric flask.

1.1.2. Add deionized H₂O to the scale of 1,000 mL in the volumetric flask to give 1.0 M of Tris buffer.

1.1.3. Dilute the 1.0 M Tris buffer 100 times to give 0.01 M Tris buffer and adjust the pH to 8.5 by using 1.0 M HCl standard solution.

1.1.4. Use a pH-meter to calibrate the pH value of the 0.01 M Tris-HCl buffer.

1.2. Preparation of ND suspensions

1.2.1. Dilute 100 nm of monocrystalline ND suspensions (1.0 mg/mL) 50 times with the 0.01 M Tris-HCl buffer to give 0.02 mg/mL of ND suspensions.

1.3. Preparation of dopamine solution

1.3.1. Dissolve 20 mg of dopamine hydrochloride in 2.0 mL of 0.01 M Tris-HCl buffer to give 10 mg/mL DA solution.

Note: The DA solution must be freshly prepared and used within 15 min.

1.4. Preparation of Ag[(NH₃)₂]OH solution

1.4.1. Dissolve 100 mg of AgNO₃ solid in 10 mL of deionized H₂O to give 10 mg/mL AgNO₃ solution.

1.4.2. Add 1.0 M ammonium hydroxide (NH₃·H₂O) dropwise to the AgNO₃ solution until yellow precipitate forms, then continue to add the NH₃·H₂O solution until the precipitation disappears.

Note: Make the minimum volume required; prepare immediately before use and dispose immediately after use.

CAUTION: Add NH₃·H₂O in fume hood with face shields, gloves, and goggles.

2. Synthesis PDA Layer on the Surface of NDs (PDA-NDs)

2.1. Add the freshly prepared DA solution (10 mg/mL) to the ND suspensions to give varied final concentrations of 50, 75, 100 µg/mL of DA. Adjust the total reaction volume to 1.0 mL, transfer it to a 10 mL-test tube, and vigorously stir at 25 °C, in the dark for 12 h.

2.2. Centrifuge the PDA-NDs solution for 2 h at 16,000 x g, remove the supernatant and wash three times with deionized water for 1 h at 16, 000 x g each time.

2.3. Re-disperse the PDA-NDs in 200 μ L of deionized water with sonication for 30 s. The PDA-coated NDs will be ready for further use.

3. Reduction of AgNPs on the Surface of PDA-NDs (AgNPs-PDA-NDs)

3.1. Dilute 40 μ L of the pre-synthesized PDA-NDs in Step 2.3 two times with deionized water. Add Ag[(NH₃)₂]OH solution to give various final concentrations of Ag[(NH₃)₂]⁺ (0.08, 0.16, 0.24, 0.40, and 0.60 mg/mL).

3.2. Adjust the final volume to 100 μ L in a 1.5 mL-centrifuge tube by adding deionized water, followed by sonication for 10 min.

3.3. Centrifuge the AgNPs-PDA-NDs for 15 min at 16,000 x g to remove the free silver ions, discard the supernatant after centrifugation, add 100 μ L of deionized water, and wash three times with deionized water at 16,000 x g for 5 min each time.

3.4. Re-disperse the AgNPs-PDA-NDs in 100 μ L of deionized water with sonication for 30 s to prepare for further use.

4. Analysis of PDA-NDs and AgNPs-PDA-NDs Clusters

4.1. Ultraviolet-visible (UV) spectra

4.1.1. Use the UV spectra to monitor the average size distribution of AgNPs on PDA-ND surfaces. Transfer the AgNPs-PDA-NDs samples prepared in Step 3.4 with varied concentrations of Ag[(NH₃)₂]OH in 1 cm-quartz cuvette and monitor the absorption at a scan wavelength of 250 to 550 nm.

4.2. Transmission electron microscopy (TEM)

4.2.1. Place the carbon coated copper grids on a glass slide wrapped with parafilm to keep the grids in place. Insert the glass slide with attached TEM grids into the plasma cleaner. Turn on the plasma cleaner and the vacuum pump. After 5 min, turn on the plasma and discharge the grids with a medium power level for 3 min.

4.2.2. Deposit 5 μ L of the samples on the carbon film coated Cu-grids for 3 min. Use filter paper to wick off the extra sample from the edge of grid. Then, deposit a drop of deionized water on the grid for 15 s to remove salts, then wick off the water with filter paper. Repeat the washing procedure twice and allow the grid to air dry for further use.

4.2.3. Visualize the samples by TEM, typically at 38,000X magnification. Operate at 200 KV.

REPRESENTATIVE RESULTS:

The formation of PDA layers on ND surfaces were analyzed by TEM (**Figure 1**). Different thicknesses of PDA layers were observed as higher concentrations of DA led to thicker PDA layers. In addition, after an encapsulating reaction, the color of the NDs solution changed from colorless to dark, while the higher the initial concentration of DA was, the darker the solution became.

Figure 2 describes the reduction of $\text{Ag}[(\text{NH}_3)_2]^+$ to AgNPs on the surface of 100 nm PDA-ND surfaces. The size distribution of AgNPs, calculated by TEM, were used to determine the dependence of the original concentration of $\text{Ag}[(\text{NH}_3)_2]^+$ on the sizes of the AgNPs.

The flow chart in **Figure 3** presents the two-step procedure for functionalizing the surface of NDs by PDA and for reducing metal ions into NPs on the PDA-ND layers.

The formation of AgNPs on the ND surface was monitored by UV-vis spectra (**Figure 4**). The intensity of the peaks at ~ 400 nm increased, along with an increase in the concentration of silver solution while the peaks showed a red-shift, indicating the formation of AgNPs with increased sizes.

FIGURE AND TABLE LEGENDS:

Figure 1. Characterization of the thicknesses of PDA layers on the surfaces of 100 nm NDs with varied concentrations of DA (0, 50, 75, and 100 $\mu\text{g}/\text{mL}$) and their corresponding TEM images. The average thicknesses of each PDA layer are ~ 5 nm (B), ~ 10 nm (C), and ~ 15 nm (D), respectively. The inset photograph shows the colorimetric change in the corresponding samples.

Figure 2. Characterization of AgNPs-PDA-NDs. TEM images of AgNP-PDA-NDs and the size distribution of AgNPs by adding 0.4 mg/mL (A), and 0.6 mg/mL (B) of $[\text{Ag}(\text{NH}_3)_2]^+$, respectively.

Figure 3. Wall chart diagram of the surface functionalization of NDs. Two-step functionalization of the surface of NDs: (1) the surface coating of NDs with DA polymerization; (2) the reduction of metal ions into NPs on the PDA layer.

Figure 4. Characterization of reduced AgNPs on the surfaces of NDs, via UV-vis spectroscopy. This figure has been modified and reprinted by permission from Zeng *et al.*¹⁷.

Table 1. The thickness of PDA layer and the size of reduced AgNPs. The validation of the mathematical models with experimental data. The thickness of PDA layer is determined by the initial concentration of DA, and the ratio of initial concentrations of $[\text{Ag}(\text{NH}_3)_2]^+$ is consistent with the average radius cubed of AuNPs.

DISCUSSION:

This article provides a detailed protocol for the surface functionalization of NDs with self-polymerized DA coating, and the reduction of $\text{Ag}[(\text{NH}_3)_2]^+$ to AgNPs on PDA layers (**Figure 3**). The

strategy is capable of producing various thicknesses of PDA layers by simply changing the concentration of DA. The size of the AgNPs can also be controlled by altering the original concentration of metal ion solution. The TEM image in **Figure 1A** displays the uncoated 100 nm NDs which tended to form microclusters and aggregates. When NDs were encapsulated with PDA, the PDA layers showed as a thin ring round the NDs. The thicknesses of the PDA layers, as measured in TEM images, were around 5 nm, 10 nm, and 15 nm, which corresponded to the final DA concentrations of 50 µg/mL, 75 µg/mL, and 100 µg/mL, respectively. The color of the NDs suspension was observed to change from colorless to dark following PDA coating, indicating the successful wrapping of PDA on ND surfaces and showing that the thickness of PDA was dependent on the concentration of DA. Please note: the critical factor that influences the DA polymerization is the pH condition (the most favorable value is 8.5²³). The accurate pH value of a solution is beneficial for controlling the thicknesses of PDA layers. In addition, fast agitation during polymerization is necessary for the disaggregation of NDs and the formation of a uniformed PDA layer. Therefore, this method is not effective for any particles that are unstable in alkaline solutions.

To describe the influential factors that contributed to the thickness of the PDA, we introduce **equation (1)** to describe the formation of a PDA layer on ND surfaces. This is based on the kinetic equation of PDA deposition on nanoparticles from previous reports^{21,22}. The initial concentrations of DA (C_1 , m/v), reaction time (t), and the thickness of the PDA layer (d), are as follows:

$$\frac{4}{3}\pi\rho_1N_1(d+R)^3 - \frac{4}{3}\pi\rho_1N_1R^3 = C_1V_1(1 - e^{-k_1t}) \quad (1)$$

R is the radius of NDs (assuming NDs are spheres), ρ_1 is the density of PDA, V_1 is the reaction volume, N_1 is the number of NDs, and k_1 is a constant related to pH values, partial pressure of O₂, ambient temperature and luminous intensity²³. Therefore, the thickness of a PDA layer can be written as **equation (2)**:

$$d = \sqrt[3]{\frac{3C_1V_1(1-e^{-k_1t})+4\pi\rho_1N_1R^3}{4\pi\rho_1N_1}} - R \quad (2)$$

Or if we rewrite **equation (1)** to **(3)**:

$$(d+R)^3 - R^3 = d^3 + 3d^2R + 3dR^2 = \frac{3C_1V_1(1-e^{-k_1t})}{4\pi\rho_1N_1} \quad (3)$$

Then, eliminate d^3 and $3d^2R$ because d is far less than R ($d \ll R$).

At last, the d can be expressed as **equation (4)**:

$$d = \frac{C_1V_1(1-e^{-k_1t})}{4\pi\rho_1N_1R^2} \quad (4)$$

The coating process required 12 h, with the DA being completely consumed and monitored by UV-vis spectra. Therefore, $\frac{V_1(1-e^{-k_1t})}{4\pi\rho_1N_1R^2}$ was a constant, and the value of d was directly proportional to the initial concentrations of DA (C_1), which were confirmed by our experimental results (**Table 1**). Please note, along with the increase in the thicknesses of the PDA layers, accumulation speeds of the layers were slower because of the increases in the surface areas of the NDs-PDA.

The presence of the catechol groups in PDA has been shown to directly induce the growth of the nanoparticles upon the reduction of metal precursors and their immobilization on a PDA-coated surface²⁴⁻²⁷. After coating 100 nm NDs with a PDA layer (~ 15 nm), the resulting PDA-NDs were used as a substrate to synthesize AgNPs from a metal ion solution, with the assistance of sonication. As seen in **Figure 2**, with the increase of $[\text{Ag}(\text{NH}_3)_2]^+$ concentration, the size of AgNPs increased from ~24 nm to ~28 nm, and the number of NPs raised from 97 to 117, corresponding to the $[\text{Ag}(\text{NH}_3)_2]^+$ concentration of 0.4 to 0.6 mg/mL, respectively. This phenomenon can also be characterized by UV-vis spectroscopy. The absorbance peak of nanoparticles gradually appeared as the concentration of $[\text{Ag}(\text{NH}_3)_2]^+$ increased (**Figure 4**). For example, the maximum absorbance of nanoparticles, formed by reducing 0.4 and 0.6 mg/mL of $[\text{Ag}(\text{NH}_3)_2]^+$, is 410 and 430 nm, which corresponds to AgNPs with the diameters of ~ 20 and ~ 30 nm, respectively. This is consistent with TEM observation¹⁷.

The diameter of reduced AgNPs follows the first order linear differential **equation (5)**, which is similar to the seeded growth synthesis of AuNPs²⁸, where the S is the surface area of PDA-NDs, C_2 is the initial concentrations of $\text{Ag}[(\text{NH}_3)_2]^+$, t is the reaction time, r is the radius of AgNPs, k_2 is a constant, ρ_2 is the density of Ag, V_2 is the reaction volume, N_2 is the number of AgNPs, and equals to $S \cdot n$, in which n is the average number of active catechol groups that can reduce $\text{Ag}[(\text{NH}_3)_2]^+$. The AgNPs are treated as spheres:

$$S \cdot n \cdot \frac{4}{3}\pi\rho_2r^3 = \frac{4}{3}\pi\rho_2N_2r^3 = C_2V_2(1 - e^{-k_2t}) \quad (5)$$

In the equation, the number of AgNPs was assumed to be directly proportional to the surface area of PDA, which depended on the thicknesses of the PDA layers. On the surface of the PDA layers, the AgNPs grew with the continuous reduction of $\text{Ag}[(\text{NH}_3)_2]^+$, while the metal (0) bonds at the O-site of the PDA served as the seed precursor of AgNPs. The number of AgNPs is proportional to the O-site on PDA, which is directly proportional to the surface area^{23,29-31}. On the other hand, the reduced AgNPs are distributed evenly on the PDA surface because the $\text{Ag}[(\text{NH}_3)_2]^+$ was reduced by the uniformed catechol groups on the PDA layers. Experimental results showed that the higher the initial concentrations of $\text{Ag}[(\text{NH}_3)_2]^+$ were, the larger the AgNPs were, but with a similar number of NPs on each ND. The ratio of initial concentrations of $[\text{Ag}(\text{NH}_3)_2]^+$ (C_2) ratio (0.6 mg/mL : 0.4 mg/mL=1.5) were consistent with the average radius cubed $[(14/12)^3=1.588]$. Therefore, if a higher density of particles is desired on the PDA-NDs, a thicker layer of PDA-NDs should be selected but, if larger sizes of NPs are needed, a longer reduction duration would meet the requirement.

To remove the unreacted $[\text{Ag}(\text{NH}_3)_2]^+$ during the purification process, a high centrifugation speed is recommended because of the low density of NDs. The higher the centrifugation speed is, the shorter the purification duration will be, which will provide better control of the sizes of AgNPs. In addition, sonication is an indispensable approach for obtaining uniform AgNPs. Samples should be sonicated for several minutes initially before $\text{Ag}[(\text{NH}_3)_2]^+$ solutions are added.

We have demonstrated a facile method for the surface modification of NDs with self-polymerized PDA. Compared with the Click Chemistry method, this strategy, not only enhances ND dispersity and stability, but also provides a reactive platform (PDA layer) for potential post-modification by reducing the metal nanoparticles or linking with amino/thiol attached species. The thickness of a PDA layer and the size of the nanoparticles on ND surfaces can be changed by varying PDA and $\text{Ag}[(\text{NH}_3)_2]^+$ concentrations. They can also be used to reduce AuNPs or other noble metal NPs. By combining the diversity of PDA chemistry and the unique properties of NDs, this method will open the door for extending ND's applications in the catalyst, energy, and biomedical areas.

ACKNOWLEDGMENTS:

This work is supported by the University of Missouri Research Board, Material Research Center, and the College of Arts and Science at Missouri University of Science and Technology.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Mochalin, V.N., Shenderova, O., Ho, D., Gogotsi, Y. The properties and applications of nanodiamonds. *Nature Nanotechnology*. **7**(1), 11-23 (2011).
2. Kucsko, G. *et al.* Nanometre-scale thermometry in a living cell. *Nature*. **500**(7460), 54-58 (2013).
3. Liu, J. *et al.* Origin of the Robust Catalytic Performance of Nanodiamond-Graphene-Supported Pt Nanoparticles Used in the Propane Dehydrogenation Reaction. *ACS Catalysis*. **7**(5), 3349-3355 (2017).
4. Chang, B.-M. *et al.* Highly Fluorescent Nanodiamonds Protein-Functionalized for Cell Labeling and Targeting. *Advanced Functional Materials*. **23**(46), 5737-5745 (2013).
5. Ho, D., Wang, C.H., Chow, E.K. Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine. *Science Advances*. **1**(7), e1500439 (2015).
6. Hsu, M.H. *et al.* Directly thiolated modification onto the surface of detonation nanodiamonds. *ACS Applied Materials and Interfaces*. **6**(10), 7198-7203 (2014).
7. Krueger, A. Diamond Nanoparticles: Jewels for Chemistry and Physics. *Advanced Materials*. **20**(12), 2445-2449 (2008).
8. Turcheniuk K., Trecuzzi C., Deeleepojananan C., Mochalin V.N. Salt-assisted ultrasonic deaggregation of nanodiamond. *ACS Applied Materials and Interfaces*. **8**(38), 25461-25468 (2016).
9. Akiel, R.D., Zhang, X., Abeywardana, C., Stepanov, V., Qin, P.Z., Takahashi, S. Investigating Functional DNA Grafted on Nanodiamond Surface Using Site-Directed Spin Labeling and Electron Paramagnetic Resonance Spectroscopy. *Journal of Physical Chemistry B*. **120**(17), 4003-4008

(2016).

10. Gaillard, C. *et al.* Peptide nucleic acid-nanodiamonds: covalent and stable conjugates for DNA targeting. *RSC Advances*. **4**(7), 3566-3572 (2014).

11. Zhang, T. *et al.* DNA-based self-assembly of fluorescent nanodiamonds. *Journal of the American Chemical Society*. **137**(31), 9776-9779 (2015).

12. Liu, W. *et al.* Fluorescent Nanodiamond-Gold Hybrid Particles for Multimodal Optical and Electron Microscopy Cellular Imaging. *Nano Letters*. **16**(10), 6236-6244 (2016).

13. Lee, H., Dellatore, S.M., Miller, W.M., Messersmith, P.B. Mussel-inspired surface chemistry for multifunctional coatings. *Science*. **318**(5849), 426-430 (2007).

14. Wang, C., Zhou, J., Wang, P., He, W., Duan, H. Robust Nanoparticle-DNA Conjugates Based on Mussel-Inspired Polydopamine Coating for Cell Imaging and Tailored Self-Assembly. *Bioconjugate Chemistry*. **27**(3), 815-823 (2016).

15. Liu, R., Guo, Y., Odusote, G., Qu, F., Priestley, R.D. Core-shell Fe₃O₄ polydopamine nanoparticles serve multipurpose as drug carrier, catalyst support and carbon adsorbent. *ACS Applied Materials and Interfaces*. **5**(18), 9167-9171 (2013).

16. Liu, R. *et al.* Dopamine as a Carbon Source: The Controlled Synthesis of Hollow Carbon Spheres and Yolk-Structured Carbon Nanocomposites. *Angewandte Chemie International Edition*. **50**(30), 6799-6802 (2011).

17. Zeng, Y., Liu, W., Wang, Z., Singamaneni, S., Wang, R. Multifunctional surface modification of nanodiamonds based on dopamine polymerization. *Langmuir*. **34**(13), 4036-4042 (2018).

18. Qin, S. *et al.* Dopamine@Nanodiamond as novel reinforcing nanofillers for polyimide with enhanced thermal, mechanical and wear resistance performance. *RSC Advances*. **8**(7), 3694-3704 (2018).

19. Barras, A., Lyskawa, J., Szunerits, S., Woisel, P., Boukherroub, R. Direct functionalization of nanodiamond particles using dopamine derivatives. *Langmuir*. **27**(20), 12451-12557 (2011).

20. Khanal, M. *et al.* Toward Multifunctional "Clickable" Diamond Nanoparticles. *Langmuir*. **31**(13), 3926-3933 (2015).

21. Rad, M.H., Zamanian, A., Hadavi, S.M.M., Khanlarkhani A. A Two-Stage Kinetics Model for Polydopamine Layer Growth. A Two-Stage Kinetics Model for Polydopamine Layer Growth. *Macromolecular Chemistry and Physics*. 1700505 (2018).

22. Ball, V., Frari, D.D., Toniazzo, V., Ruch, D. Kinetics of polydopamine film deposition as a function of pH and dopamine concentration: Insights in the polydopamine deposition mechanism. *Journal of Colloid and Interface Science*. **386**(1), 366-372 (2012).

23. Liu, Y., Ai, K., Lu, L. Polydopamine and its derivative materials: synthesis and promising applications in energy, environmental, and biomedical fields. *Chemical Reviews*. **114**(9), 5057-5115 (2014).

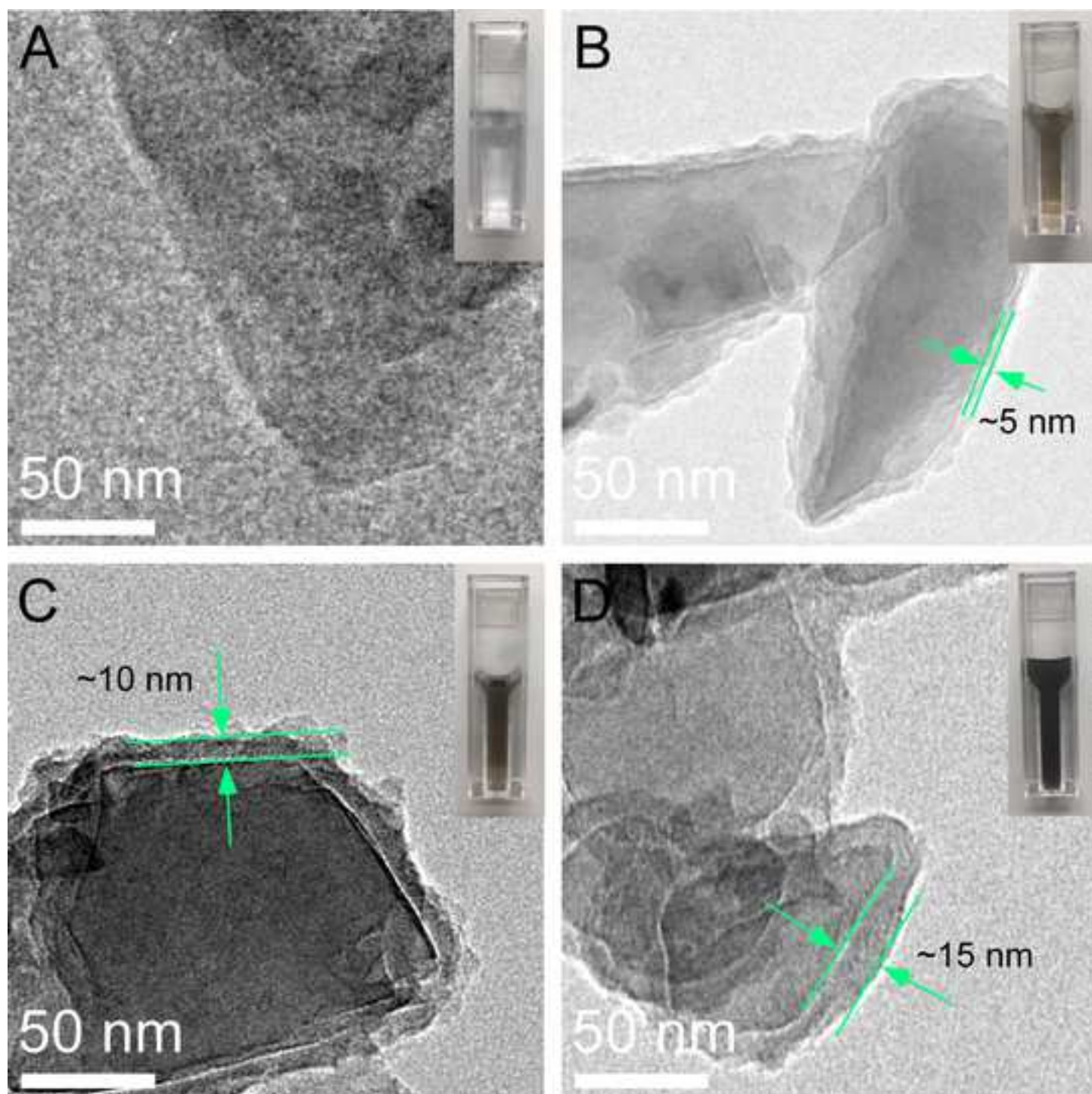
24. Hu, J., Wu, S., Cao, Q., Zhang, W. Synthesis of core-shell structured alumina/Cu microspheres using activation by silver nanoparticles deposited on polydopamine-coated surfaces. *RSC Advances*. **6**(85), 81767-81773 (2016).

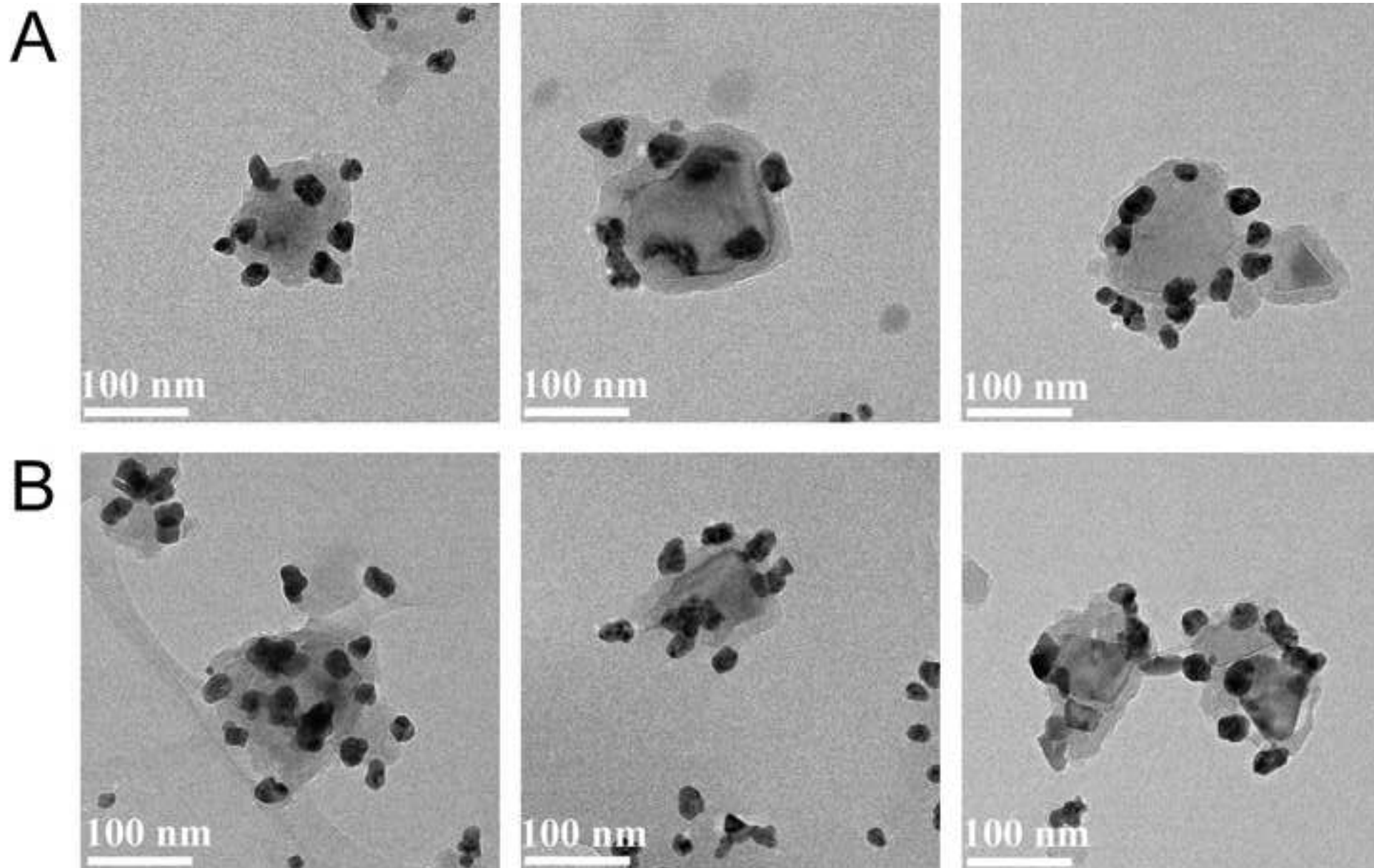
25. Orishchin, N. *et al.* Rapid Deposition of Uniform Polydopamine Coatings on Nanoparticle Surfaces with Controllable Thickness. *Langmuir*. 2017, 33, 6046-6053.

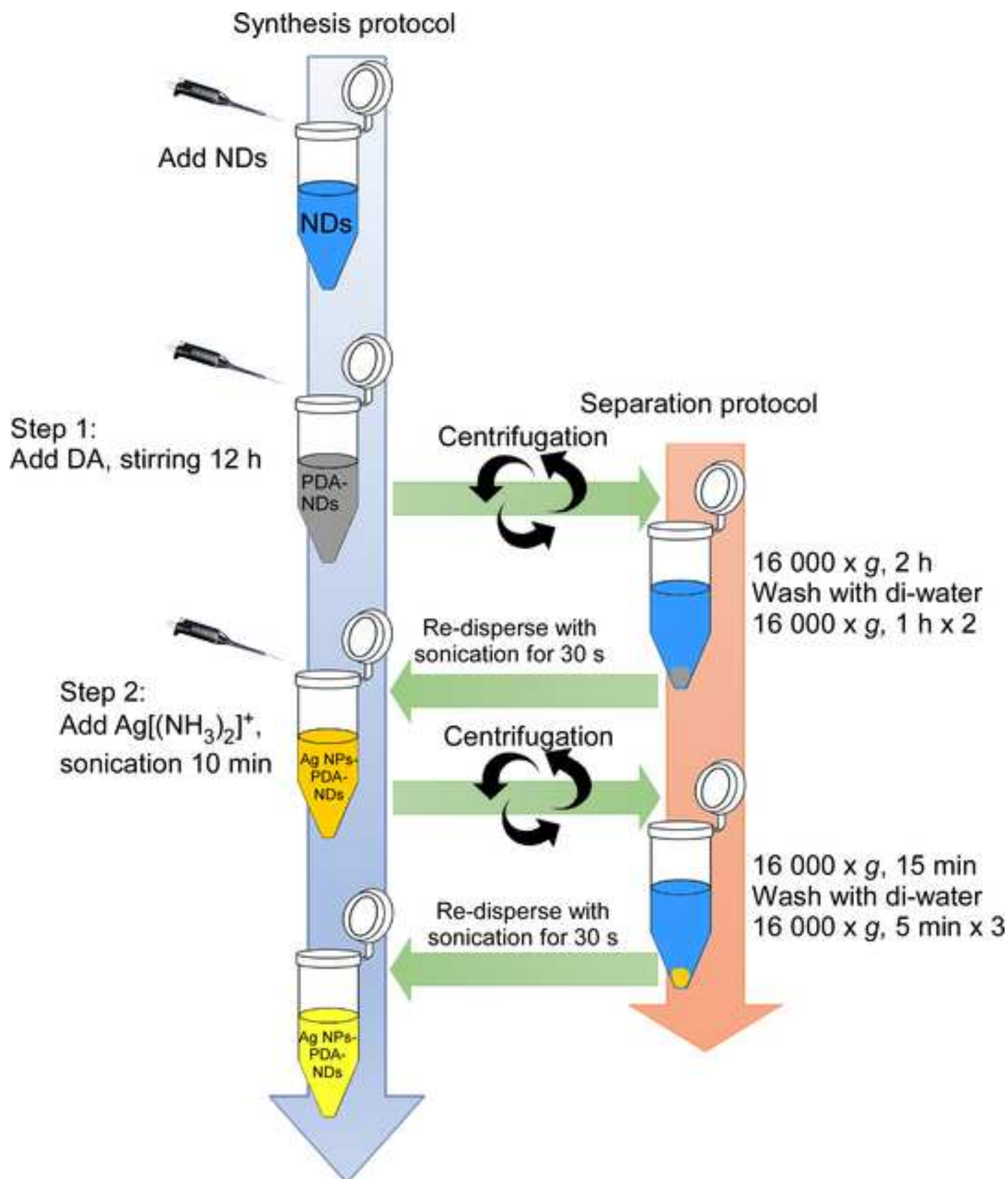
26. González, A.L., Noguez, C., Beránek, Barnard, A.S. Size, Shape, Stability, and Color of Plasmonic Silver Nanoparticles. *Journal of Physical Chemistry C*. **118**(17), 9128-9136 (2014).

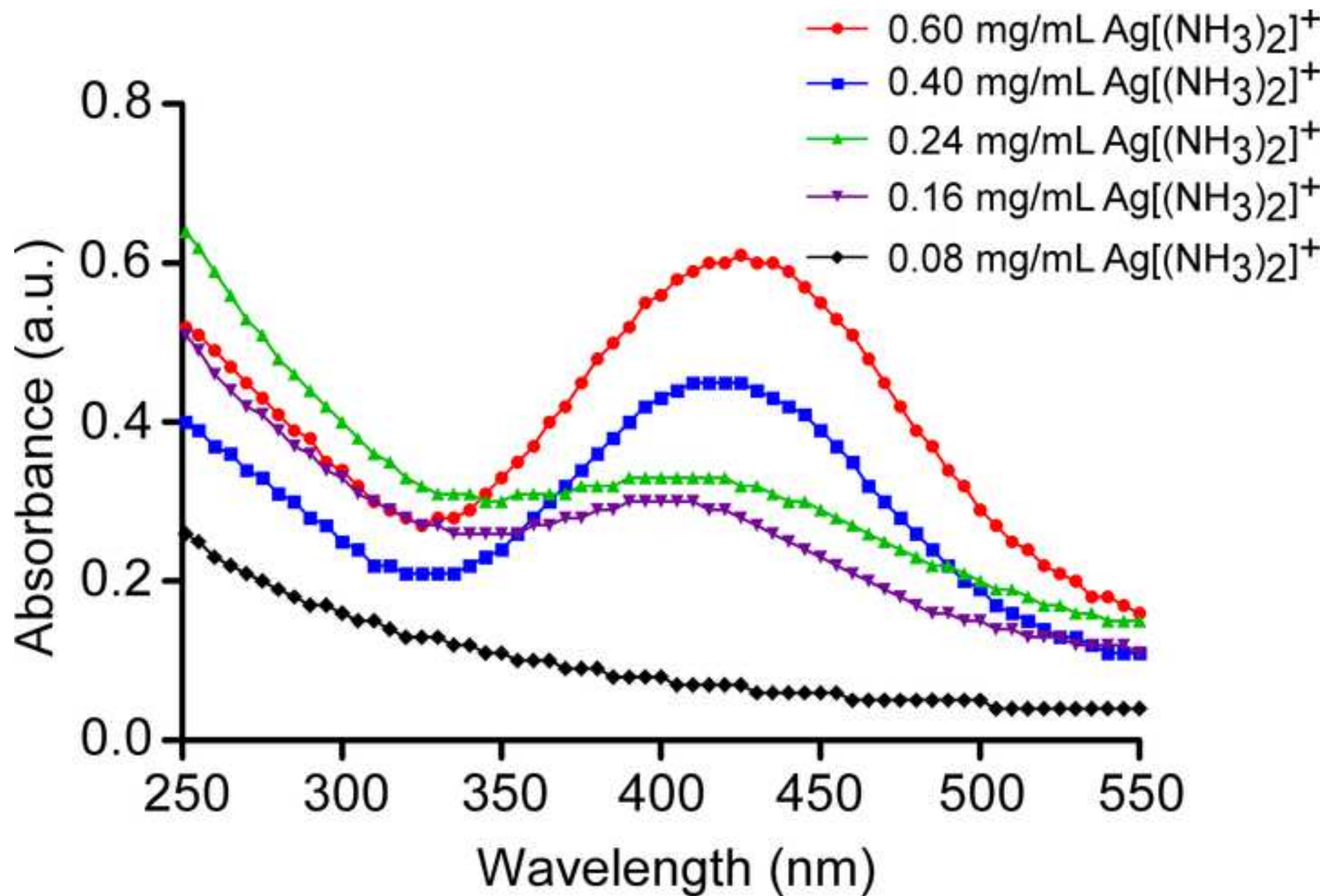
27. Muthuchamy, N., Gopalan, A., Lee, K.-P. A new facile strategy for higher loading of silver

392 nanoparticles onto silica for efficient catalytic reduction of 4-nitrophenol. *RSC Advances*. **5**(93),
393 76170-76181 (2015).
394 28. Bastus, N.G., Comenge, J., Puntès, V. Kinetically Controlled Seeded Growth Synthesis of
395 Citrate-Stabilized Gold Nanoparticles of up to 200 nm: Size Focusing versus Ostwald Ripening.
396 *Langmuir*. **27**(17), 11098-11105 (2011).
397 29. Jana, J., Gauri, S.S., Ganguly, M., Dey, S., Pal, T. Silver nanoparticle anchored carbon dots for
398 improved sensing, catalytic and intriguing antimicrobial activity. *Dalton Transactions*. **44**(47),
399 20692-20707 (2015).
400 30. Zamudio, A. *et al.* Efficient anchoring of silver nanoparticles on N-doped carbon
401 nanotubes. *Small*. **2**(3), 346-350 (2006).
402 31. Chen, K., Li, T. Modification of membranes with polydopamine and silver nanoparticles
403 formed *in situ* to mitigate biofouling. *U.S. Patent Application* 14/689,085 (2016).
404









(1) PDA layer deposition			
Group No.	Initial concentrations of DA (C_1 , $\mu\text{g/mL}$)	Thickness of the PDA layer (d , nm)	Notes:
G ₁	50	5	The thickness of PDA layers (d) is directly proportional to the initial concentrations of DA (C_1), and it was discussed in DISCUSSION part.
G ₂	75	10	
G ₃	100	15	
(2) AgNPs growth synthesis			
Group No.	Initial concentrations of $[\text{Ag}(\text{NH}_3)_2]^+$ (C_2 , mg/mL)	AgNPs average radius (r , nm)	Notes:
G ₁	0.4	12	$(G_2/G_1)^3=(14/12)^3=1.588$ $G_2/G_1=0.6/0.4=1.5$
G ₂	0.6	14	The ratio of initial concentrations of $[\text{Ag}(\text{NH}_3)_2]^+$ is consistent with average radius cubed.

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Nanodiamond	FND Biotech, Inc.	brFND-100	dispersed in water, and used without further purification
Dopamine hydrochloride	Sigma	H8502-25G	prepare freshly
Silver Nitrate	Fisher	S181-25	
Ammonium Hydroxide	Fisher	A669S-500	highly toxic
Tris Hydrochloride	Fisher	BP153-500	
TEM grid carbon film	Ted Pella	01843-F	300 mesh copper



1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

Bio-inspired Polydopamine Surface Modification of Nanodiamonds
and its Reduction of Silver Nanoparticles

Author(s):

Yun Zeng, Wenyan Liu, Risheng Wang

Item 1 (check one box): The Author elects to have the Materials be made available (as described at

<http://www.jove.com/author>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):



The Author is NOT a United States government employee.



The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.



The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: **"Agreement"** means this Article and Video License Agreement; **"Article"** means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; **"Author"** means the author who is a signatory to this Agreement; **"Collective Work"** means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; **"CRC License"** means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; **"Derivative Work"** means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; **"Institution"** means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; **"JoVE"** means MyJoVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; **"Materials"** means the Article and / or the Video; **"Parties"** means the Author and JoVE; **"Video"** means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4 and 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

ARTICLE AND VIDEO LICENSE AGREEMENT

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such

statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's

expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:

RISHENG WANG

Department:

Chemistry


Institution:

Missouri University of Science and Technology

Article Title:

Bio-Inspired Polydopamine Surface Modification of Nanodiamonds and its Reduction of Silver Nanoparticles

Signature:



Date:

5/11/2018

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy of the document as a pdf on the JoVE submission site;
- 2) Fax the document to +1.866.381.2236;
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Dear Dr. Wu,

We are grateful to you and the editors for carefully reviewing our manuscript. We have made revisions to the paper in response to those comments. These changes are detailed in the attached pages. We hope that with these modifications, you will find our manuscript suitable for publication.

If you have any questions regarding our manuscript, please feel free to contact me directly by email at wangri@mst.edu or by phone at +573-341-7729.

Many thanks for your consideration.

Yours,

Risheng Wang on half of coauthors

Department of Chemistry
Missouri University of Science and Technology
Wangri@mst.edu
Phone: (573)-341-7729

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response:

We thank the editor for his/her comments. The manuscript has been proofreading with no spelling or grammar issues.

2. Please remove the embedded Table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

Response:

We thank the editor for his/her comments. The Table has been removed from the manuscript and uploaded separately. The title and description has been added in the manuscript.

3. JoVE cannot publish manuscripts containing commercial language. This includes company names of an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include Milli-Q, etc.

Response:

We thank the editor for his/her comments. The commercial language has been removed from the manuscript.

4. Figure 3: Please add a short description in addition to the figure title in Figure Legend. Please use “x g” instead of “g” for centrifugation rate. Please use “s” instead of “sec” for time unit.

Response:

The short description of Figure 3 has been added in the figure legend. The new Figure 3 has been uploaded with “x g” instead of “g” for centrifugation rate, and “s” instead of “sec” for time unit.

5. Please revise the table of materials to include all essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.

Response:

We revised the Table containing the reagents name, company, and catalog number.

**RightsLink®**[Home](#)[Create Account](#)[Help](#)

Title: Multifunctional Surface Modification of Nanodiamonds Based on Dopamine Polymerization

Author: Yun Zeng, Wenyan Liu, Zheyu Wang, et al

Publication: Langmuir

Publisher: American Chemical Society

Date: Apr 1, 2018

Copyright © 2018, American Chemical Society

[LOGIN](#)

If you're a **copyright.com user**, you can login to RightsLink using your copyright.com credentials.

Already a **RightsLink user** or want to [learn more?](#)

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.

[BACK](#)[CLOSE WINDOW](#)

5/11/2018

Rightslink® by Copyright Clearance Center

Copyright © 2018 [Copyright Clearance Center, Inc.](#) All Rights Reserved. [Privacy statement.](#) [Terms and Conditions.](#)
Comments? We would like to hear from you. E-mail us at customercare@copyright.com