Electronic Supplementary Information (ESI)

Three Dimensional Multipod Superstructure based on $Cu(OH)_2$ as a Highly Efficient Nanozyme

Ren Cai,[†] Dan Yang,[#] Xigao Chen,[†] Yun Huang,[†] Yifan Lyu,[†,‡] Jinglin He,[†,‖] Muling Shi,[†,‡] I-Ting Teng,[†] Shuo Wan,[†] Weijia Hou,[†] and Weihong Tan[†,‡*]

[†]Center for Research at Bio/Nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611-7200, United States
[‡]Molecular Science and Biomedicine Laboratory, State Key Laboratory for Chemo/Bio-Sensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, and Collaborative Research Center of Molecular Engineering for Theranostics, Hunan University, Changsha 410082, China
[#]Department of Chemistry, National University of Singapore, 117574 Singapore
[‖] School of Chemistry and Biological Engineering, Changsha University of Science and Technology, 410004, China

Experimental Section

Chemicals

The following chemicals were obtained as indicated: copper (II) acetate ($Cu(CH_3COO)_2$, $Cu(Ac)_2$ 99.9%, Merck), copper chloride ($CuCl_2 \cdot 2H_2O$, 99.9%, Sigma-Aldrich), sodium borohydride ($NaBH_4$, 98%, Sigma-Aldrich), ammonia hydroxide ($NH_3 \cdot H_2O$, 29%, Fisher-Scientific), hydrogen peroxide (H_2O_2, 30%, Sigma-Aldrich), 3,3`,5,5`-Tetramethylbenzidine (TMB, 240.35 g/mol, Sigma-Aldrich), glucose oxidase (160 kDa, Sigma-Aldrich), polyvinylpyrrolidone (PVP, MW = 1,300,000, Sigma-Aldrich), polyvinylpyrrolidone (PVP, MW = 55,000, 99.9%, Sigma-Aldrich), methanol (absolute for analysis, 99%, Sigma-Aldrich), acetone and ethanol (absolute for analysis, ACS, 99.9%, Merck). All materials were used without further purification.

Synthesis of solid $Cu(OH)_2$ nanoparticles. The synthesis of solid $Cu(OH)_2$ nanoparticles follows the approach developed by Cai and coworkers, which involves reducing $Cu(Ac)_2$ with $NaBH_4$ in ethanol. In a typical experiment, $CuCl_2$ (12.75 mg), $Cu(Ac)_2$ (5 mg), NaCl (5.85 mg), (PVP (molecular weight 55,000) (65.5 mg), and ethanol (40 mL) were added to a 100 mL flat bottom flask one-by-one. After 10 min ultrasonication and 30 min stirring, 8.3 mg $NaBH_4$ were dissolved in 10 mL ethanol and quickly added to the solution under vigorous stirring. After 72h, the products were collected by centrifugation and washed with ethanol three times.

Synthesis of 3D $Cu(OH)_2$ hollow multipod superstructures (HMPS): In a typical experiment, $Cu(OH)_2$ nanoparticles (9 mg) and PVP (molecular weight 1,300,000) (21 mg) were dispersed in 20 mL DI-water by ultrasonication for 15 min in a 100 mL flat bottom flask. The solution was vigorously stirred for 15 min. In a separate flask, 5 mL $Cu(NO_3)_2{\cdot}3H_2O$ (24.16 mg) aqueous solution was added to 15 mL $NH_3{\cdot}H_2O$ (400 μL $NH_3{\cdot}H_2O$ (29%) mixed with 14.6 mL DI-water) to form $[Cu(NH_3)_4](NO_3)_2]$. Then, 20 mL $[Cu(NH_3)_4](NO_3)_2$ were added to the above $Cu(OH)_2$ nanoparticle mixture and stirred for 10 min. The products were collected by centrifugation and washed with acetone and methanol three times.

Catalytic oxidation: Unless otherwise stated, steady-state kinetic assays were carried out at 25 ^{o}Cina1.5-ml tube with 30 μg HMPS or 300 ng HRP in 500 μL of reaction buffer (0.2 M NaAc, pH 4.5) in the presence of530 μM H_2O_2 for HMPS or HPR, using 800μM TMB as the substrate. In a typical experiment for different pH values at 25 ^{o}C, 30 μLH_2O_2were added to 400 μL reaction buffer and vortexed 4 min. Then, 40 μL TMB (10 mM) were added into the mixture and vortexed for another 4 min. Finally, 30 μL HMPS (1 mg/mL) were quickly added to the mixture. Immediately after the substrates were added, color reactions were observed. All reactions were monitored according to the maximum intensity of absorbance in time-scan mode at 652 nm using a Cary Bio-100 UV/Vis spectrometer (Varian).

In a typical experiment for different temperatures at pH=4.5, 400 μL of reaction buffer were kept at the desired temperature for 5 min. Then, 30 μL H_2O_2 were added to the reaction buffer and vortexed 1 min, and the mixture was held at that temperature for 4 min. Then, 40 μL TMB (10 mM) were added to the mixture and vortexed 1 min. The mixture was maintained at the desired temperature for another 4 min. Finally, 30 μL HMPS (1 mg/mL) were quickly added to the mixture, and absorbance was monitored at 652 nm.

Glucose detection was realized as follows: a) 20 μL of 1.3 mg/mL glucose oxidase and 100 mL of glucose of different concentrations in 0.5mM NaAc buffer (pH 5.1) were incubated at 37.8 ^oC for 2 h; b) 40 μL of 10 mM TMB, 30 μL of HMPS stock solution, and 310 μL of 0.2M NaAc buffer (pH 4.5) were added to the glucose solution, and the absorbance was monitored at 652 nm.

Urine stock solutions were obtained from two patients: Patient A and Patient B. Signed informed consent was obtained from each patient participating in this study before our test. For urine glucose determination, the urine stock solution was diluted 10-, 10^2-, 10^3-, 10^4-, 10^5-, or 10^6-fold, using 0.5 mM NaAc buffer (pH 5.1) for the measurement. Meanwhile, some of these urine stock solutions were sent to the University of Florida (UF) Student Health Care Center for analysis.

Characterization

Morphology of the samples was characterized with a transmission electron microscope (TEM) system (JEOL Model JEM-2010F) operating at 200 kV. The crystal phase of samples was investigated using a Bruker D8 Advance diffractometer X-ray diffraction (XRD) at the 2θ range of 10° to 80° with Cu Kα radiation. FTIR measurements were conducted in a Perkin Elmer Instruments Spectrum GX FTIR spectrometer at room temperature from 400 to 4000 cm^{-1}. A total of 32 scans were recorded at a resolution of 2 cm^{-1} to average each spectrum. The specific surface area was calculated by the Brunauer-Emmett-Teller (BET) method using nitrogen adsorption/desorption (Quantachrome Instruments, Autosorb AS-6B).

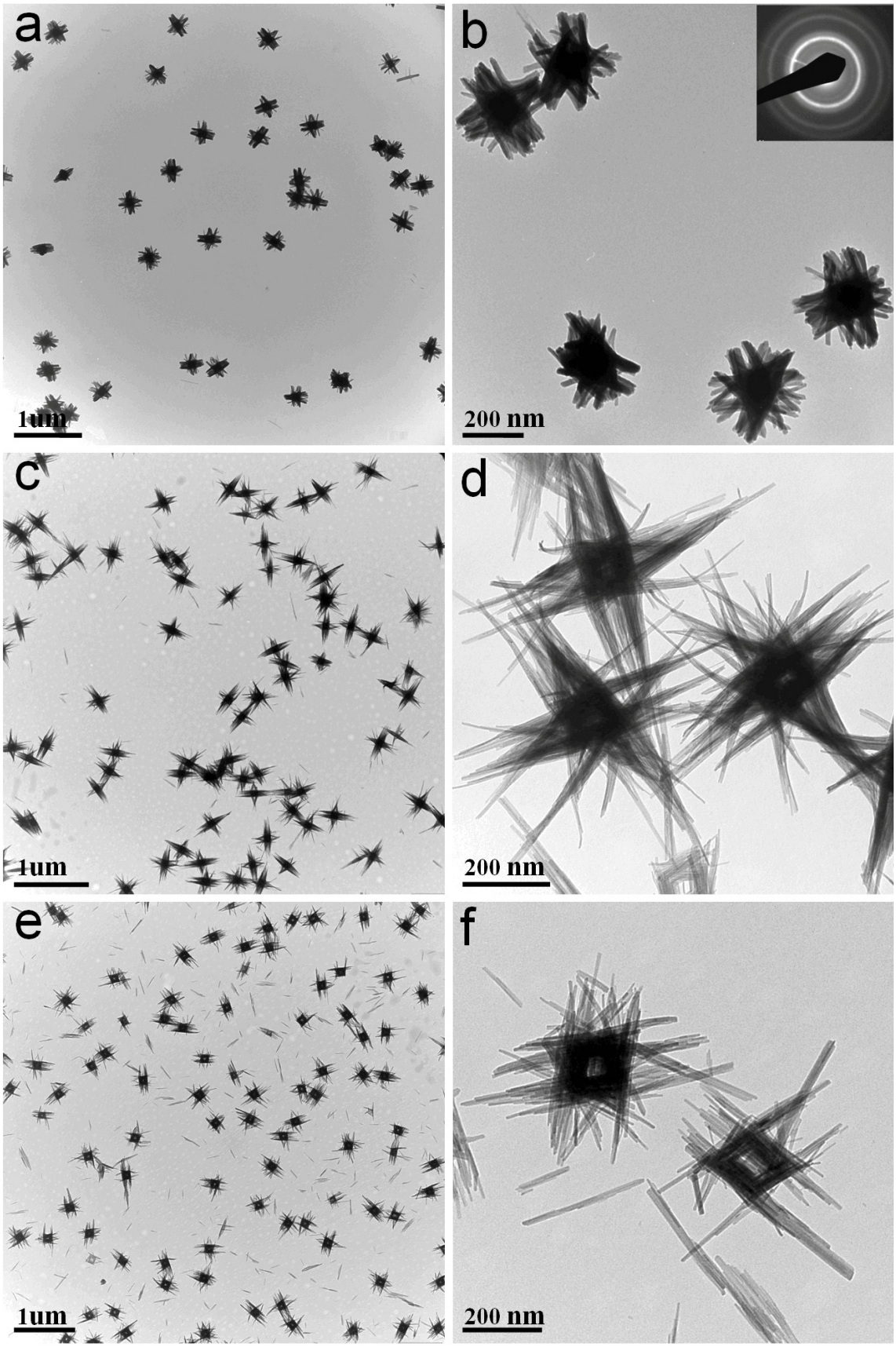

Figure S1 Characterization of irregular $Cu(OH)_2$ nanoparticles: (a) low-magnification and (b) high-magnification TEM images (inset is SAED). The samples prepared from the synthesis for TEM with reacted different concentration of NH_3: c) and d) 400 μL; e) and f) 800 μL.

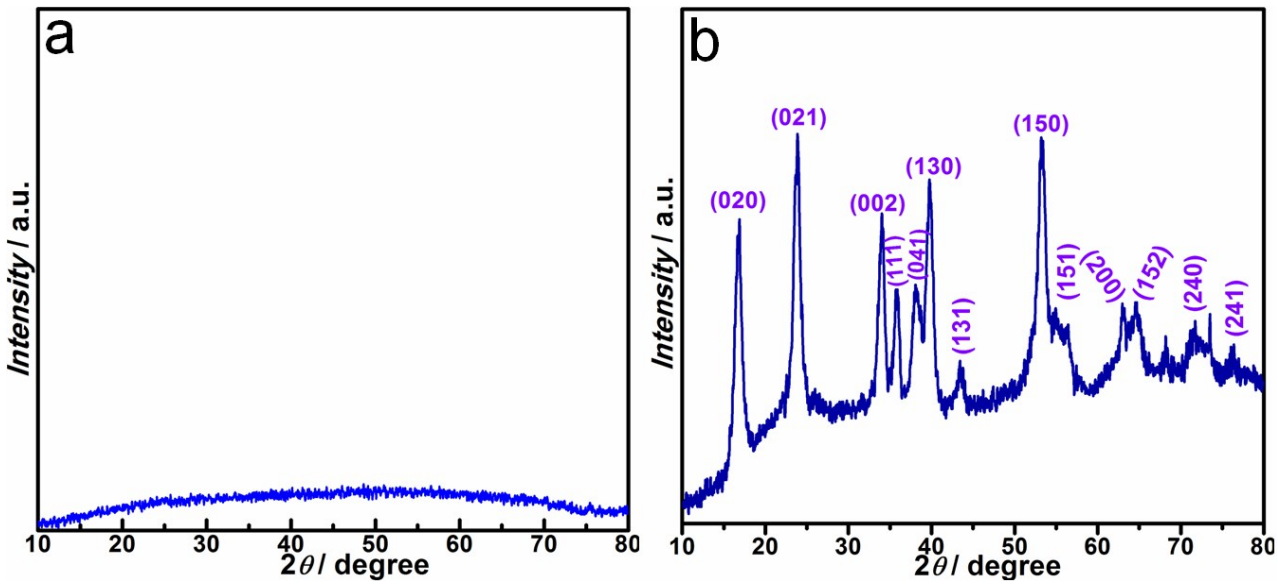

Figure S2. XRD patterns of a) $Cu(OH)_2$ nanoparticles and b) the samples prepared from the synthesis for TEM with reacted 800 μL NH_3 (Figure S1 c-d);.

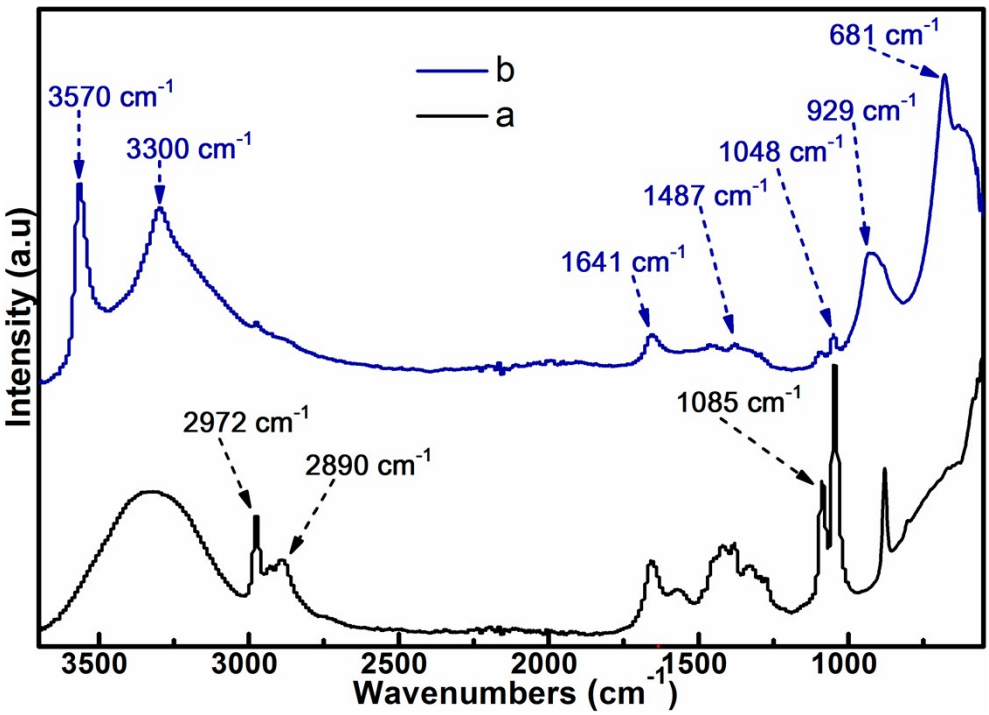

Figure S3 FTIR spectra of (a) initial solid nanoparticles (black line) and (b) hollow multipod $Cu(OH)_2$ superstructure (blue line).

Note: In (b), the presence of peaks at 3570 $cm^{-1}$and 3300 cm^{-1} reflects stretching modes of the hydroxyl group in the $Cu(OH)_2$.[1] The peaks at 3300 cm^{-1} and 1641 cm^{-1} are from the hydrogen-bonded hydroxyl groups and bending mode of the hydroxyl group of water.[2] The peak at 1487 cm^{-1} indicates the Cu-OH bond. The peaks at 1085cm^{-1} and 1048 cm^{-1} correspond to the C−O stretching vibration of coordinated metal cations (Cu^{2+}) in $Cu(OH)_2$.[3,4] The peaks appearing at 929 cm^{-1} and 681 cm^{-1} are from the Cu-O-H bond.[5,6] Characteristic modes of CH_2 are observed at 2972 cm^{-1} and 2890 cm^{-1}, respectively, indicating the presence of the acetate ion on the surface of solid $Cu(OH)_2$ nanoparticles.

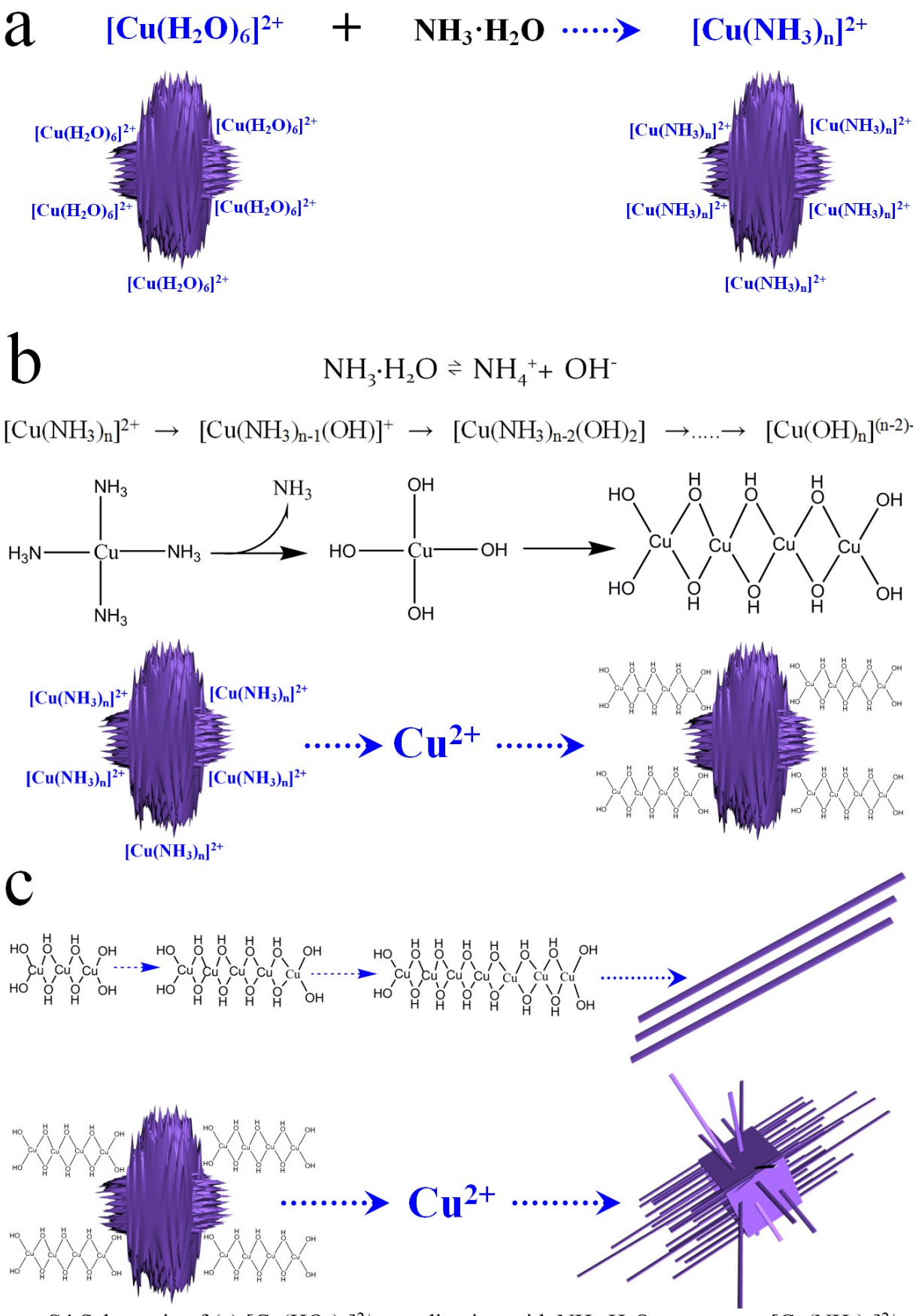

Figure S4 Schematic of (a) $[Cu(HO_2)_6]^{2+}$ coordinating with $NH_3 \cdot H_2O$ to generate $[Cu(NH_3)_n]^{2+}$; (b) coordinated growth of the lamellar structure; (c) formation of tiny branch and hollow multipod $Cu(OH)_2$ superstructure.

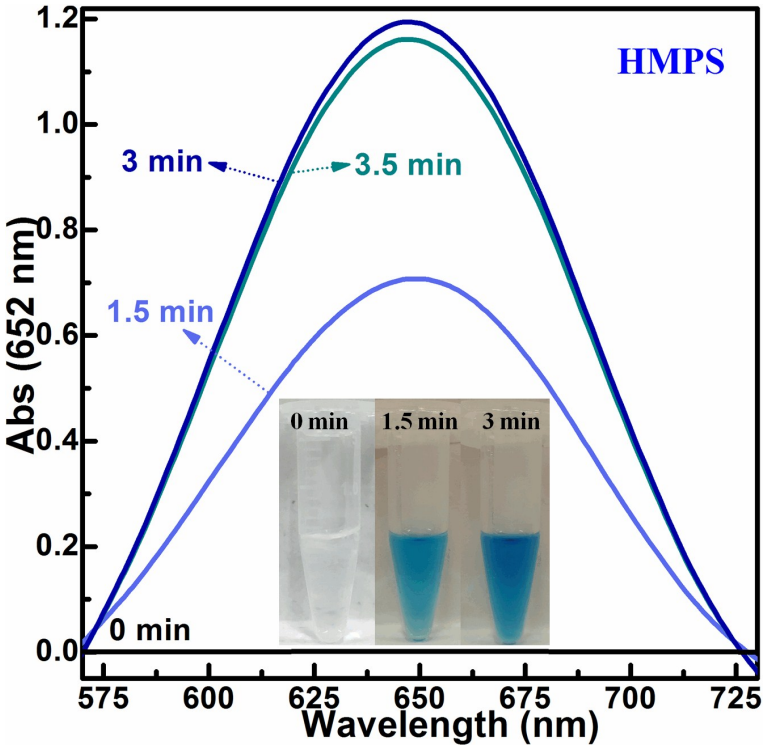

Figure S5 The absorption spectra for TMB oxidation by HMPS in the presence of H_2O_2 to produce blue ox-TMB (pH was 4.5 and temperature was 25 ^oC); the above inset corresponds to typical photographs of the reaction solution.

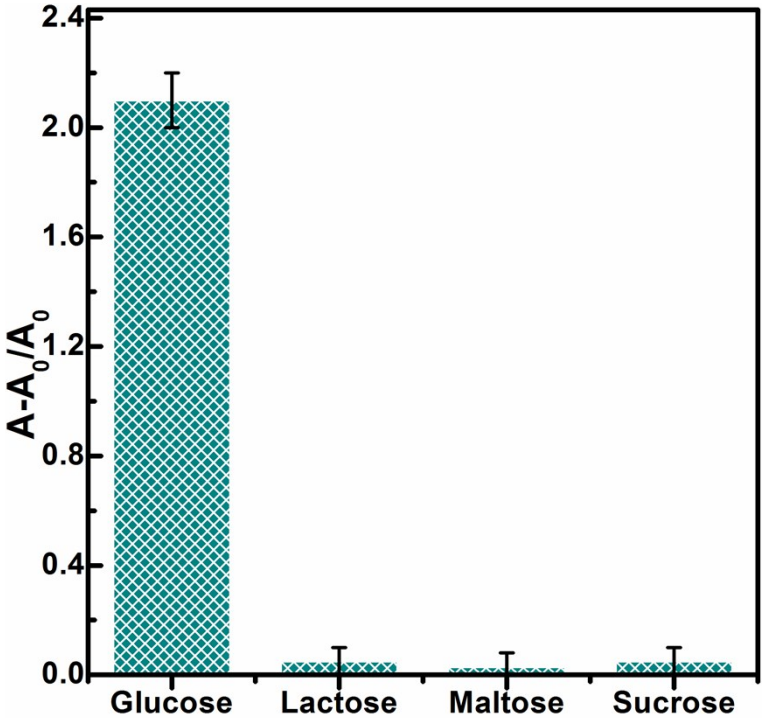

Figure S6 Selectivity analysis for glucose detection by monitoring relative absorbance in buffer (pH was 4.5 and temperature was 25 ^{o}C). Glucose concentration = 0.1 mM, and the other sugars are 5 mM.

Patient A

UF Student Health Care Center

UF Student Health Care Center
280 Fletcher Dr.
Gainesville, FL 32611
(352) 392-1161

01/14/2016 @ 2:03 PM

Laboratory Report

Result Status:F

Patient Information

Name: [redacted]
Address: [redacted] GAINESVILLE, FL 32603
ID#: [redacted]
Gender: Male
MRN:
DOB: [redacted]
Phone: (352) 665-[redacted]

Order Information

Code: 81001
Test Name: Urinalysis, automated with microscopy, complete

	Ordered	Collected	Received	Reported
Date:	01/11/2016	01/11/2016	01/11/2016	
Time:	01:56 PM	02:01 PM	02:01 PM	

Pyramed Order #: 1244486
Lab Reference #:
Accession #: L-1287-16
Speciman Source: Urine
Associated Diagnoses: Other abnormal findings in urine (R82.99)
Requestor Notes:

Provider: [redacted]
NPI: [redacted]

Lab Results

Test Name	Results	Units	Range	Flags	Status	Lab
URINALYSIS					F	ADA
Notes:						
COLOR	YELLOW		STRAW AMBER		F	ADA
Notes:						
CLARITY	CLEAR		CLEAR		F	ADA
Notes:						
GLUCOSE	NEGATIVE	Qual	NEG		F	ADA
Notes:						
BILIRUBIN	NEGATIVE	Qual	NEG		F	ADA
Notes:						
KETONES	NEGATIVE	QUAL	NEG		F	ADA
Notes:						
SPEC GRAV	1.020		1.005-1.030		F	ADA
Notes:						
PH	6.0	log 1/H+	6.0-8.0		F	ADA
Notes:						
PROTEIN	NEGATIVE	QUAL	NEG		F	ADA
Notes:						

Patient Information

Name: [redacted] **Address:** [redacted] GAINESVILLE, FL 32603 **ID#:** [redacted]
Gender: Male **MRN:**
DOB: [redacted] **Phone:** (352) 665-[redacted]

UROBILINOGEN	0.2 E.U./DL	EU/dL	0.2-1.0	F	ADA
Notes:					
NITRITE	NEGATIVE	Qual	NEG	F	ADA
Notes:					
BLOOD	NEGATIVE	Qual	NEG	F	ADA
Notes:					
WBC ESTERASE	NEGATIVE	Qual	NEG	F	ADA
Notes:					
WBC'S	0-1	#/hpf	0-5	F	ADA
Notes:					
RBC'S	0	#/hpf	0-2	F	ADA
Notes:					
BACTERIA-SEDIMENT	NONE SEEN	Qual		F	ADA
Notes:					
MUCOUS	NONE SEEN	Qual		F	ADA
Notes:					
SQUAM EPITH CELLS	RARE	Qual		F	ADA
Notes:					
CRYSTALS	NONE SEEN	Qual		F	ADA
Notes:					
CASTS	0	#/lpf		F	ADA
Notes:					
OTHER FINDINGS	SEE COMMENT			F	ADA
Notes: NONE					

Ascession Notes:
Performing Lab(s) Address(es):

Results	Negative (Normal)	+	++	+++	++++
Glucose (mg/dL)	0 ~ 100	100 ~ 250	250 ~ 500	500 ~ 1000	1000 ~ 2000

End of Report

Figure S7 Laboratory report providing diagnostic details of Patient A's urine glucose from UF Student Health Care Center.

Patient B

UF Student Health Care Center

UF Student Health Care Center
280 Fletcher Dr.
Gainesville, FL 32611
(352) 392-1161

01/12/2016 @ 10:25 AM

Laboratory Report

Result Status:F

Patient Information

Name: [redacted]
Gender: Male
DOB: [redacted]
Address: [redacted]
4
GAINESVILLE, FL 32603
Phone: (352) 281-[redacted]
ID#: 8[redacted]

Order Information

Code: 81001
Test Name: Urinalysis, automated with microscopy, complete

	Ordered	Collected	Received	Reported
Date:	01/11/2016	01/11/2016	01/11/2016	
Time:	01:36 PM	02:03 PM	02:11 PM	

Pyramed Order #: 1244470
Lab Reference #:
Accession #: L-1288-16
Speciman Source: Urine
Associated Diagnoses: Headache (R51)
Unspecified abnormal findings in urine (R82.90)
Chest pain, unspecified (R07.9)
Requestor Notes:

Provider: [redacted]
NPI: [redacted]

Lab Results

Test Name	Results	Units	Range	Flags	Status	Lab
URINALYSIS					F	ADA
Notes:						
COLOR	YELLOW		STRAW AMBER		F	ADA
Notes:						
CLARITY	CLEAR		CLEAR		F	ADA
Notes:						
GLUCOSE	NEGATIVE	Qual	NEG		F	ADA
Notes:						
BILIRUBIN	NEGATIVE	Qual	NEG		F	ADA
Notes:						
KETONES	NEGATIVE	QUAL	NEG		F	ADA
Notes:						
SPEC GRAV	1.020		1.005-1.030		F	ADA
Notes:						
PH	7.0	log 1/H+	6.0-8.0		F	ADA
Notes:						

Patient Information

Name: [redacted] **Address:** [redacted] **ID#:** [redacted]
Gender: Male
DOB: [redacted]
4
GAINESVILLE, FL 32603
Phone: (352) 281 [redacted]

PROTEIN	NEGATIVE	QUAL	NEG	F	ADA
Notes:					
UROBILINOGEN	0.2 E.U./DL	EU/dL	0.2-1.0	F	ADA
Notes:					
NITRITE	NEGATIVE	Qual	NEG	F	ADA
Notes:					
BLOOD	NEGATIVE	Qual	NEG	F	ADA
Notes:					
WBC ESTERASE	NEGATIVE	Qual	NEG	F	ADA
Notes:					
WBC'S	0-1	#/hpf	0-5	F	ADA
Notes:					
RBC'S	0	#/hpf	0-2	F	ADA
Notes:					
BACTERIA-SEDIMENT	NONE SEEN	Qual		F	ADA
Notes:					
MUCOUS	NONE SEEN	Qual		F	ADA
Notes:					
SQUAM EPITH CELLS	NONE SEEN	Qual		F	ADA
Notes:					
CRYSTALS	NONE SEEN	Qual		F	ADA
Notes:					
CASTS	0	#/lpf		F	ADA
Notes:					
OTHER FINDINGS	SEE COMMENT			F	ADA
Notes: NONE					

Ascession Notes:
Performing Lab(s) Address(es):

Results	Negative (Normal)	+	++	+++	++++
Glucose (mg/dL)	0 ~ 100	100 ~ 250	250 ~ 500	500 ~ 1000	1000 ~ 2000

End of Report

Figure S8 Laboratory report providing diagnostic details of Patient B's urine glucose from UF Student Health Care Center.

Reference

1. Pramanik, A.; Maiti, S.; Mahanty, S., Metal hydroxides as a conversion electrode for lithium-ion batteries: a case study with a $Cu(OH)_2$ nanoflower array. *Journal of Materials Chemistry A* **2014,***2* (43), 18515-18522.
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3. Nakamoto, K., *Infrared and Raman spectra of inorganic and coordination compounds*. Wiley Online Library: 1986; p 251.
4. Aguirre, J. M.; Gutierrez, A.; Giraldo, O., Simple Route for the Synthesis of Copper Hydroxy Salts. *J Brazil Chem Soc* **2011,***22* (3), 546-551.
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