

Supplementary Information

**Transformation and species identification of CuO
nanoparticles in plant cells (*Nicotiana tabacum*)**

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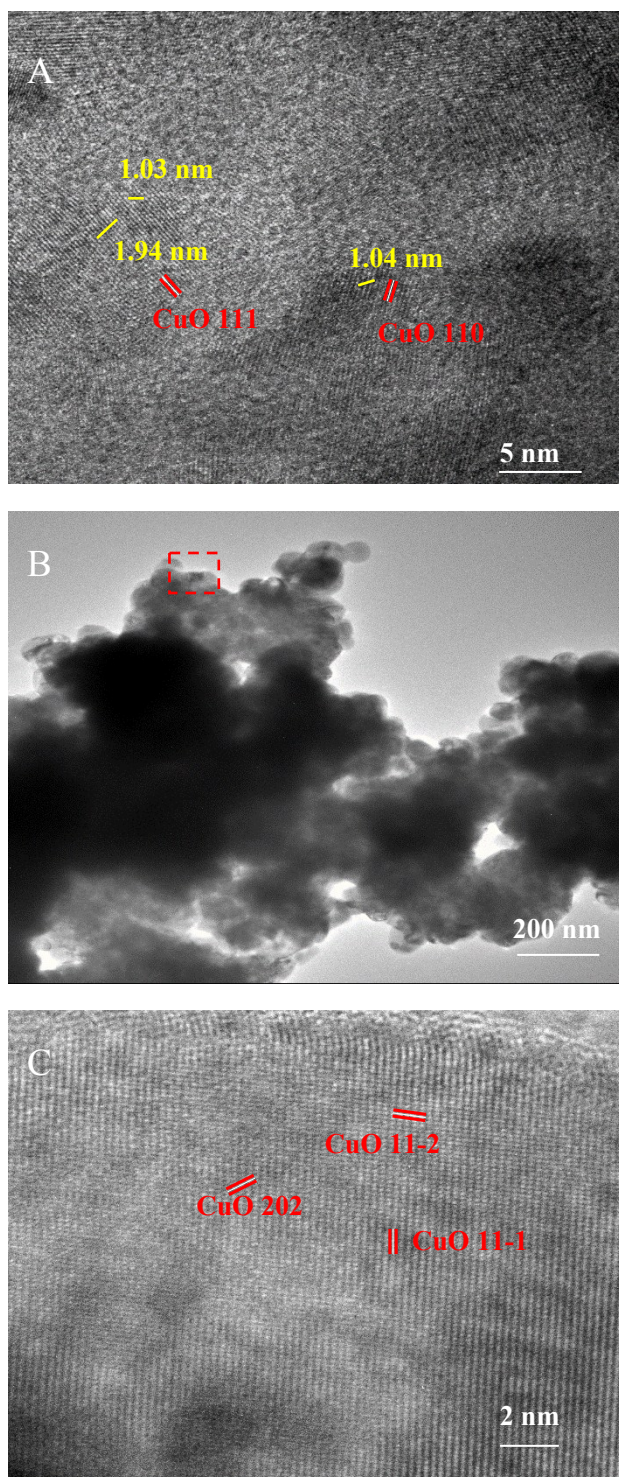


Figure S1. The TEM and HRTEM images of CuO NPs. A: HRTEM image of CuO NPs as suspended in ethanol. B: TEM image of CuO NPs as suspended in 1/2 MS medium. C: HRTEM image of CuO NPs enlarged from red dashed box in panel B. The yellow bars in panel A indicate the individual sizes of polycrystal CuO particles. The red bars indicate the crystal planes of CuO NPs. The crystal spacings of CuO (111) and (110) in Figure S1A are 0.234 and 0.274 nm, respectively. The crystal spacings of CuO (11-2), (202) and (11-1) in Figure S1C are 0.196, 0.156, and 0.254 nm, respectively.

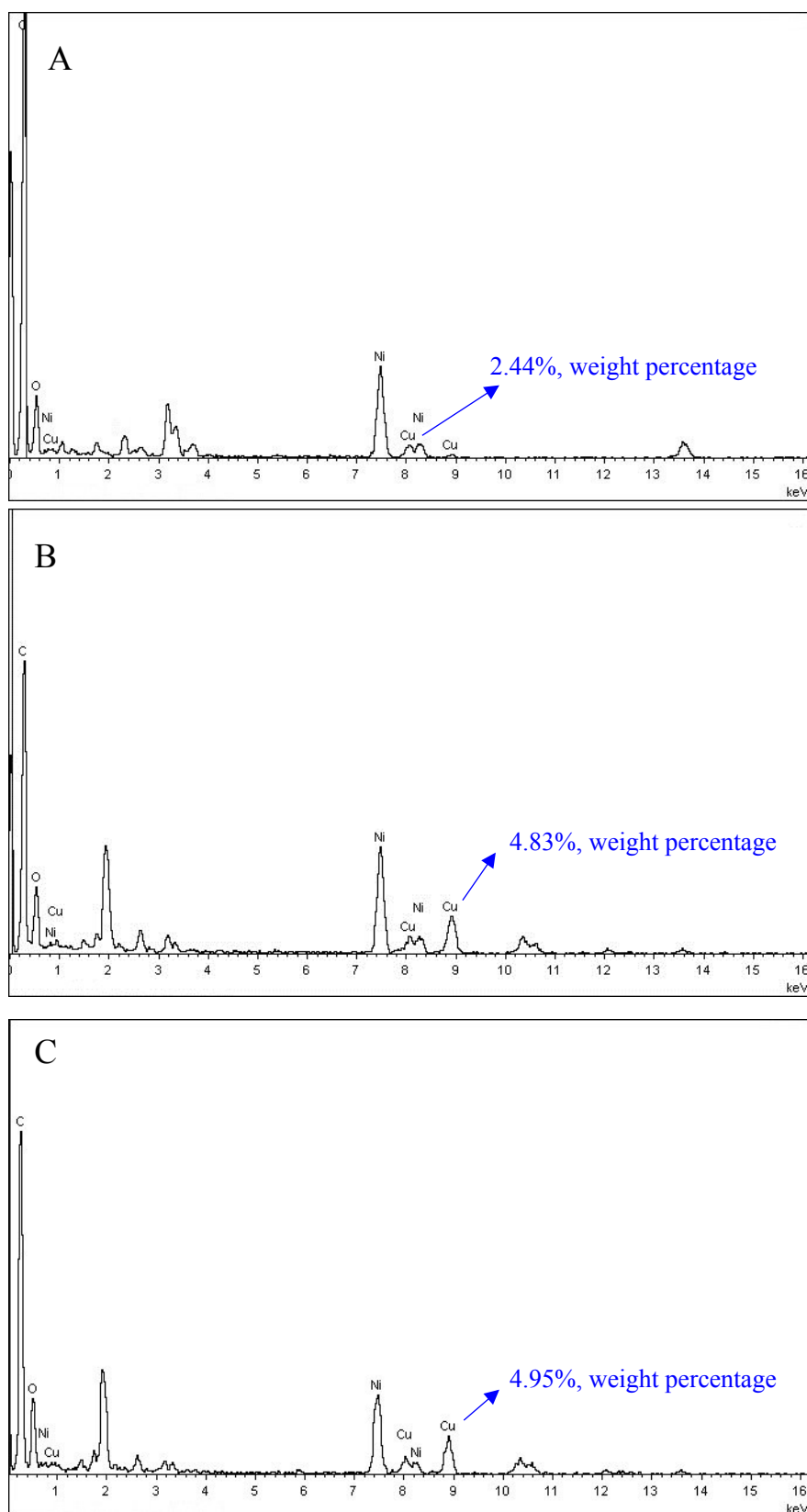


Figure S2. The EDS analysis of the dark particles in Figure 2. A-C: EDS spectra of dark particles marked by blue arrows in Figure 2A, 2B and 2D, respectively.

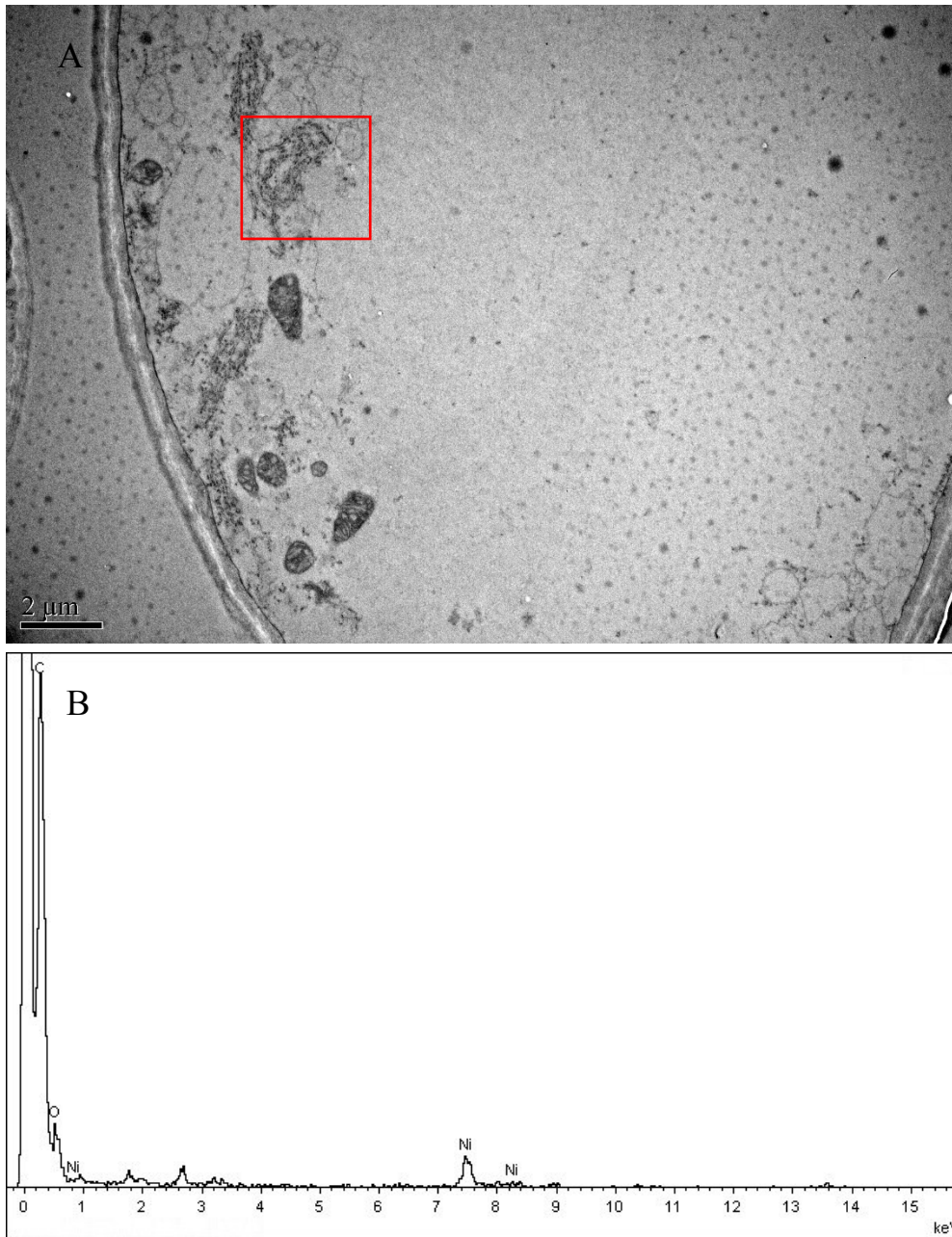


Figure S3. TEM image (A) and EDS analysis (B) of control cells without CuO NP exposure. The elemental composition of the red square in panel A was analyzed by EDS, and shown in Figure S2B.

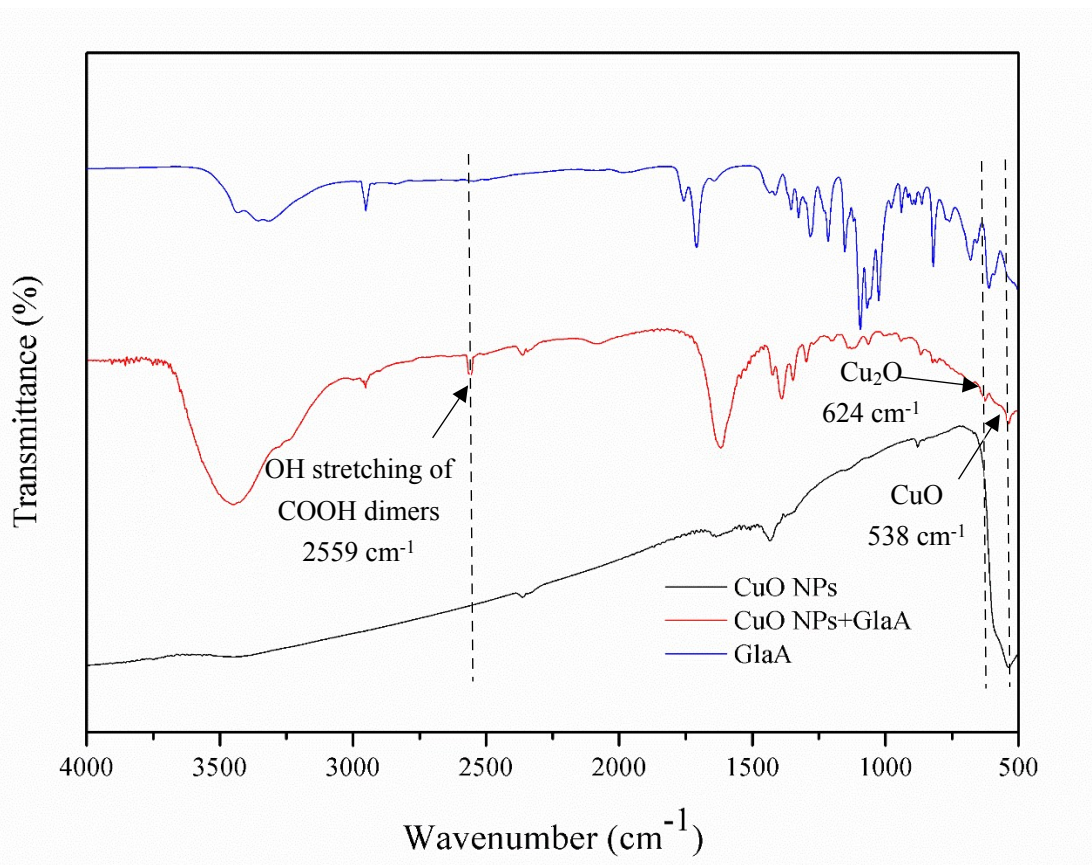


Figure S4. The FTIR spectra of the 4000-400 cm^{-1} region of CuO NPs, “CuO NPs+GlaA”, and GlaA.

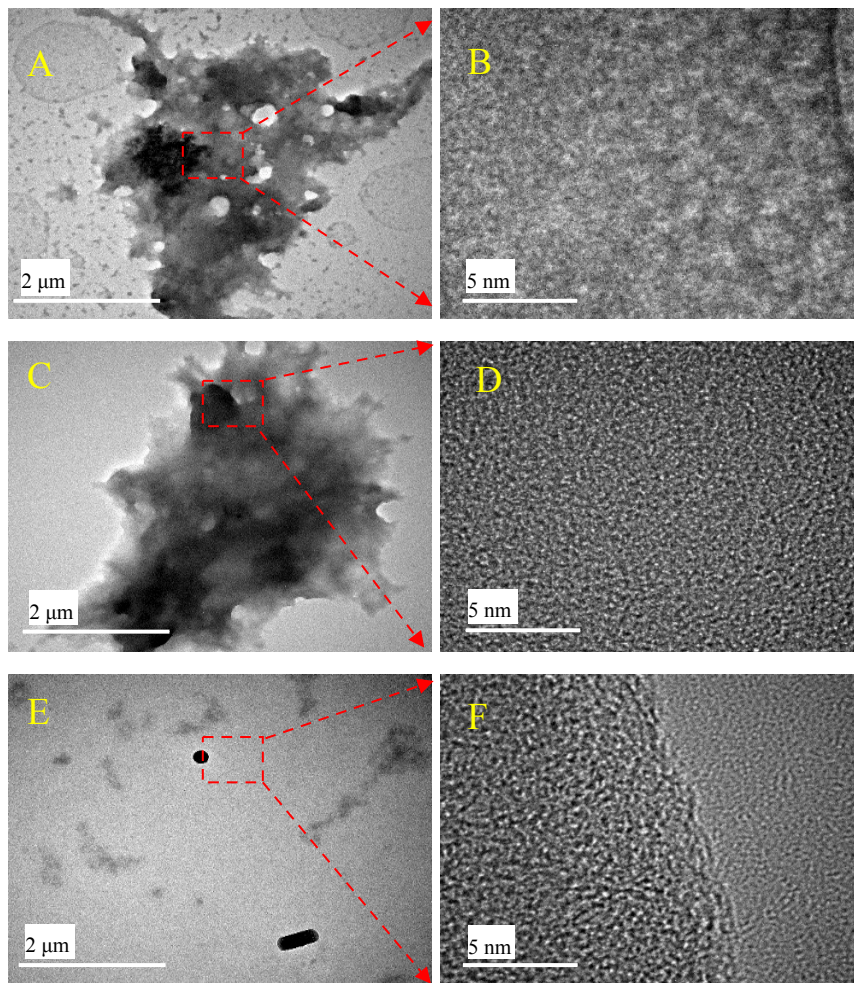


Figure S5. TEM images of cell walls and EPS treated with Cu^{2+} . (A): Cell walls extracted from un-exposed cells, and then exposed to Cu^{2+} (0.8 mg/L) for 12 h. (C): Extracted cell walls from cells incubated with Cu^{2+} (0.8 mg/L, 12 h). (E): EPS under the exposure of Cu^{2+} (0.8 mg/L) for 12 h. Cells cultured 3 days and then filtered with 18- μm stainless steel sieve. The filtrate was considered as EPS. Images (B), (D), (F) are enlarged from (A), (C), (E) marked with red dashed box.

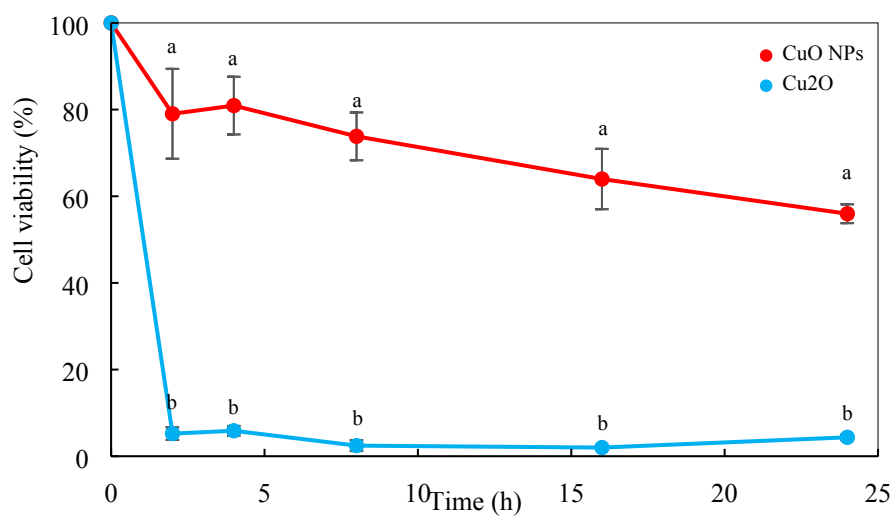


Figure S6. Cell viabilities after exposure to CuO (12 mg/L) or Cu₂O NPs (10.8 mg/L) for 2, 4, 8, 16 and 24 h. Different letters (a–b) denote significant difference between different treatments at the same exposure time ($p < 0.05$, LSD, $n=3$).

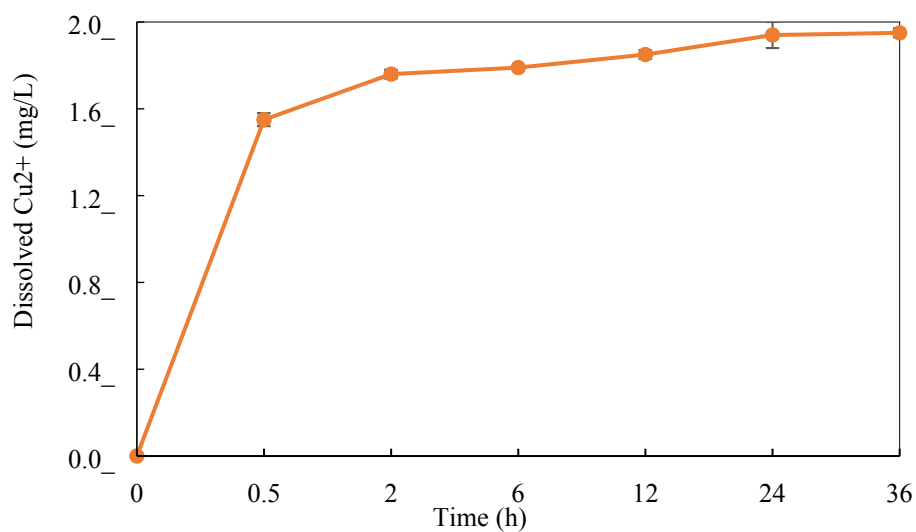


Figure S7. Dissolution of Cu₂O NPs (10.78 mg/L) in the medium as a function of incubation time (0.5, 2, 6, 12, 24, and 36 h).

Table S1. The components of 1/2 MS medium for BY-2 cell culturing

	Component	1/2 MS medium (mg/L)	
Inorganics	KNO ₃	975	
	NH ₄ NO ₃	875	
	CaCl ₂ ·2H ₂ O	220	
	MgSO ₄ ·7H ₂ O	185	
	KH ₂ PO ₄	112.5	
	EDTANa ₂ ·2H ₂ O	18.65	
	FeSO ₄ ·7H ₂ O	13.9	
	MnSO ₄ ·4H ₂ O	11.15	
	ZnSO ₄ ·7H ₂ O	4.3	
	H ₃ BO ₃	3.1	
	KI	0.415	
	Na ₂ MoO ₄ ·2H ₂ O	0.125	
	CuSO ₄ ·5H ₂ O	0.0125	
	CoCl ₂ ·6H ₂ O	0.0125	
	Organics	Sucrose	15000
		Myo-inositol	50
Glycine		1	
Thiamine-HCl		0.5	
Pyridoxine-HCl		0.25	
Nicotinic acid		0.25	

Table S2. The zeta potentials and hydrodynamic diameters of CuO NPs and Cu₂O NPs in ultrapure water and 1/2 MS medium, respectively

NPs	Zeta potential (mV)		Hydrodynamic diameter (nm)	
	ultrapure water	1/2 MS medium	ultrapure water	1/2 MS medium
CuO NPs	-5.14±0.98	-8.16±0.51	391.73±4.83	557.5±28.60
Cu ₂ O NPs	-4.74±0.53	-8.67±0.06	574.33±25.87	570±31.45

Table S3. The crystal planes and crystal spacings of the detected particles in Figure 2E,

2F, 2G

Image	CuO		Cu ₂ O		Cu ₂ S	
	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)
E	111	0.238	200	0.212	— ^a	— ^a
	110	0.279				
	11-2	0.204				
F	110	0.272	110	0.296	002	0.33
	11-2	0.199				
G	11-2	0.193	110	0.300	002	0.340
	20-2	0.187				
	110	0.271				
	002	0.258				

^a The corresponding crystal plane of Cu₂S was not detected.

Table S4. The crystal planes and crystal spacings of images (D), (E), (F) in Figure 4

Image	CuO		Cu ₂ O		Cu ₂ S	
	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)
D	022	0.142	111	0.244	102	0.27
	110	0.27				
	11-1	0.254				
E	11-1	0.252	221	0.143	103	0.187
F	113	0.139				
	11-1	0.25				

Table S5. The crystal planes and crystal spacings of Cu species in Figure 6

Image	CuO		Cu ₂ O		Cu ₂ S	
	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)	Crystal plane	Crystal spacing (nm)
B	11-1	0.256	200	0.213	— ^a	— ^a
	110	0.269	110	0.290		
	20-2	0.183				
	202	0.158				
C	— ^a	— ^a	— ^a	— ^a	002	0.343
E	110	0.280	110	0.301	102	0.241
	11-1	0.253				
F	110	0.270	111	0.249	— ^a	— ^a
	021	0.162	200	0.211		
	20-2	0.190				
	11-2	0.199				

^a The corresponding Cu species were not detected.