

Supporting Information

Fast and Selective Photoreduction of CO₂ to CO catalyzed by a Complex of Carbon Monoxide Dehydrogenase, TiO₂ and Ag Nanoclusters

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Experimental section

General Considerations

All chemicals were of the highest available quality and used as received without any further purification. All protein film electrochemistry experiments (PFE, see below for detailed experimental procedures) were carried out in 0.2 M MES buffer (pH 6 at 25 °C), which was prepared by dissolving 2-(N-morpholino)ethanesulfonic acid (MES, Melford) in purified H₂O (Milli-Q, 18 MΩ cm). The pH was adjusted to the correct value using NaOH (Sigma-Aldrich). For the photocatalysis experiments a pH 6.0 buffer consisting of 0.1 M triethanolamine (TEOA), 0.1 M NaCl and 25 mM EDTA (ethylenediaminetetraacetic acid tetrasodium salt) was used. The nanoparticles (TiO₂ P25 particles from Evonik Industries) were an anatase/rutile (8:2) mixture with an average size of 21 nm. Poly(methacrylic acid) (PMAA) was supplied from Sigma-Aldrich (MW 4000 - 6000). Tetrahydrofuran (THF) was obtained from Sigma-Aldrich (99.9%). Carbon monoxide dehydrogenase I (CODH) from *Carboxydotherrmus hydrogenoformans* was prepared as described previously.¹ All manipulations involving CODH were performed in an anaerobic glove box (VAC glovebox, O₂ < 3 ppm) under a nitrogen gas atmosphere.

Fabrication of electrodes from TiO₂ particles

Indium Tin Oxide (ITO) slides (SPI Supplies, USA) of approximate dimensions 1 x 2 cm were cleaned by sonication for 15 min in ethanol, followed by acetone, and finally in deionized H₂O. The slides were dried in the oven. TiO₂ powder (20 mg) and iodine (5 mg) were suspended in 20 mL acetone (Sigma-Aldrich). The suspension was sonicated for 30 min and then electrophoretic deposition was performed (10 V, 3 min) to create a film of TiO₂ on the surface of the ITO slides. The slides were converted into electrodes by making an ohmic contact with Cu wire on the uncoated area of the conducting glass and sealing with nonconducting epoxy.

Modification of TiO₂ electrodes and TiO₂ nanoparticles by AgNCs

The TiO₂ electrodes were modified by soaking in a 10 mL as-prepared solution of AgNCs-PMAA in the dark for 24 hours at room temperature. The electrodes were thoroughly

washed using H₂O before testing.

The modification of TiO₂ nanoparticles were prepared by the following procedure: 100 mg TiO₂ nanoparticles were suspended into 20 mL purified AgNCs (5mg/mL) in ethanol under vigorous stirring. The resulting mixture was stirred at room temperature overnight under protection from light by aluminum foil. Then, AgNCs-PMAA/TiO₂ nanoparticles were centrifuged, washed in water, then acetone and dried. The ratio of Ag/Ti was determined by ICP-MS.

Attachment of CODH to electrode surfaces

To study the electrochemistry of CODH on TiO₂ and AgNCs-PMAA/TiO₂ electrodes, aliquots of enzyme were drop-casted onto the electrode and left for a few minutes until partially dry. In a typical experiment, 15 μL of 10 μM enzyme was applied to a TiO₂ electrode with area 0.25 cm² (0.5 cm × 0.5 cm).

Spectral measurements

Optical absorption spectra of the AgNC solutions were acquired in the wavelength region from 200 nm to 800 nm using a Perkin Elmer Lambda 19 UV/Vis/NIR spectrophotometer. Emission spectra and photoluminescence titrations were recorded on a Fluorolog fluorescence spectrometer (HORIBA Scientific). All spectra were recorded with quartz cells of 10 mm path length. The lifetimes were measured by the time-correlated single photon counting (TCSPC) technique on Microtime-200 (Picoquant). Diode lasers (405 nm, ~100 ps, 1 MHz repetition rate) were used for excitation. From the measured decay traces, each lifetime was evaluated following a reconvolution fitting procedure.

Supporting tables and figures

Table S1. The lifetimes of AgNCs and complexes.

Samples	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)
AgNCs-PMAA	0.47	4.55	
AgNCs-PMAA/GSH	0.55	1.83	6.79
AgNCs-PMAA/CODH	0.45	2.69	8.29

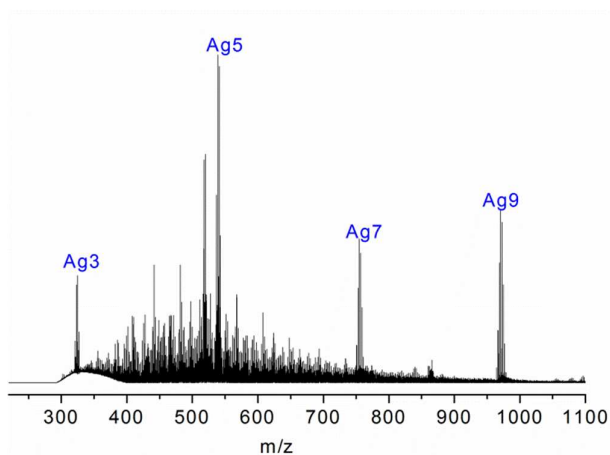


Figure S1 MALDI-TOF mass spectrum of as-prepared Ag nanoclusters.

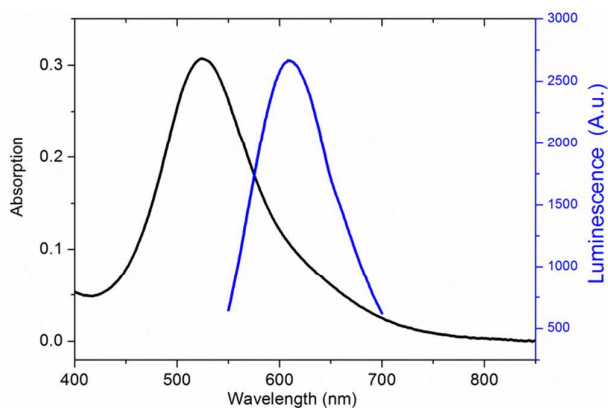


Figure S2 Absorption spectrum (black, $\lambda_{\text{abs,max}}=525$ nm) and luminescence spectrum of as-prepared Ag nanoclusters, $\lambda_{\text{ex}}=520$ nm and $\lambda_{\text{em,max}}=610$ nm.

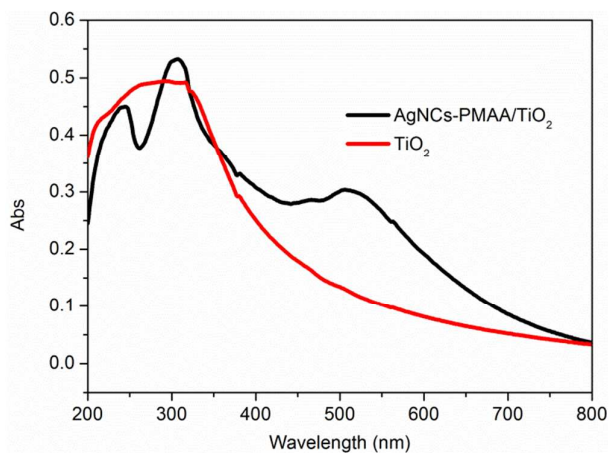


Figure S3 Absorption spectra of AgNCs-PMAA/TiO₂ and TiO₂ (15µg/mL) suspension.

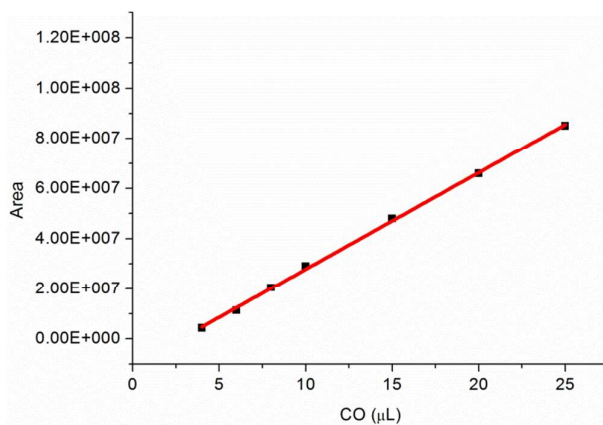


Figure S4 GC calibration for CO using pure CO at 1 atm, 25 °C. The slope is 3.846E6 and the intercept is 1.061E7. R² is 0.999.

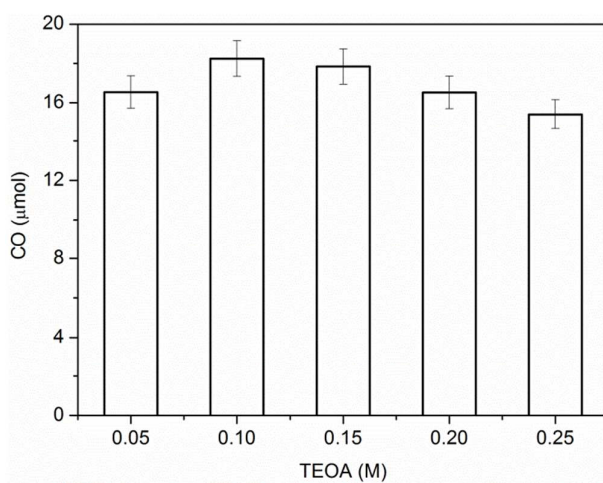


Figure S5 GC assays of CO production after 30 minutes of visible light irradiation using different concentrations of TEOA. All solutions were buffered at pH 6 containing 0.1M NaCl and 25 mM EDTA at 25°C.

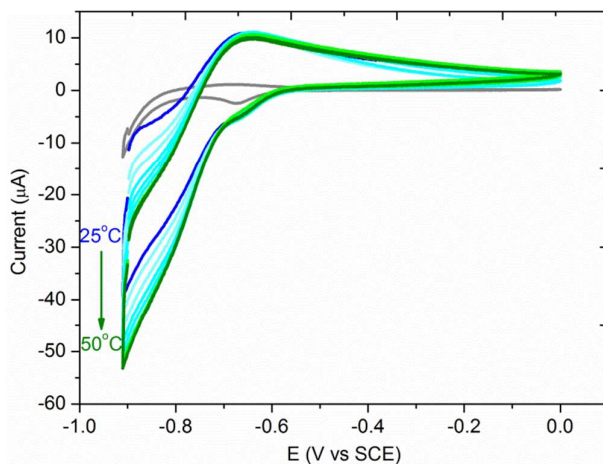


Figure S6 Cyclic voltammograms (10 mV s^{-1}) of CODH I on a AgNCs-PMAA/TiO₂ electrode. Voltammograms recorded in 0.2 M MES buffer (pH 6, with 0.1 M NaCl), from 25 to 50 °C. Current-potential curves of the bare electrodes are depicted in gray; experiments with bound CODH are shown in blue (25 °C) and green (50 °C) under 100% CO₂.

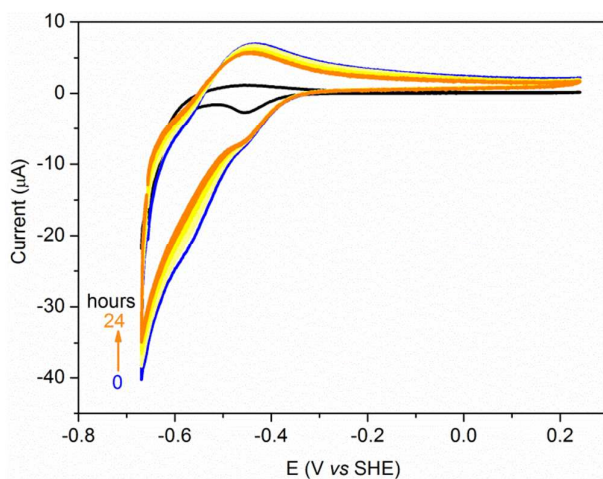


Figure S7 Cyclic voltammograms CV scans of CODH/AgNCs-PMAA/TiO₂ electrode recorded every two hours under a flow of 100% CO₂ in 0.2 M MES buffer (pH 6, 0.1 M NaCl) at 25 °C. Scan rate is 10 mV s^{-1} .

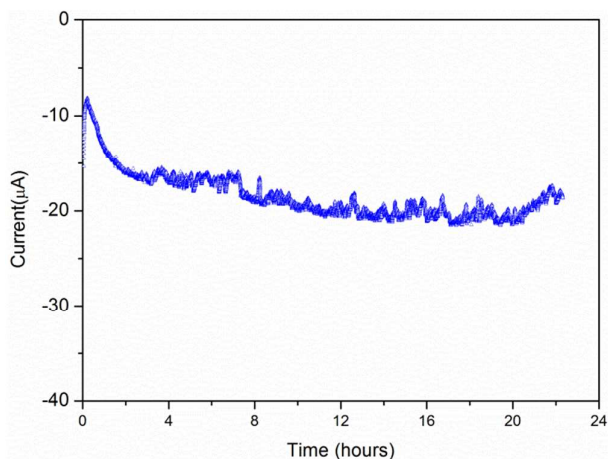


Figure S8 Chronoamperometry with a CODH/AgNCs-PMAA/TiO₂ electrode under a flow of 100% CO₂ with the potential held at -0.66 V vs SHE in 0.2 M MES buffer (pH 6, 0.1 M NaCl) at 25 °C.

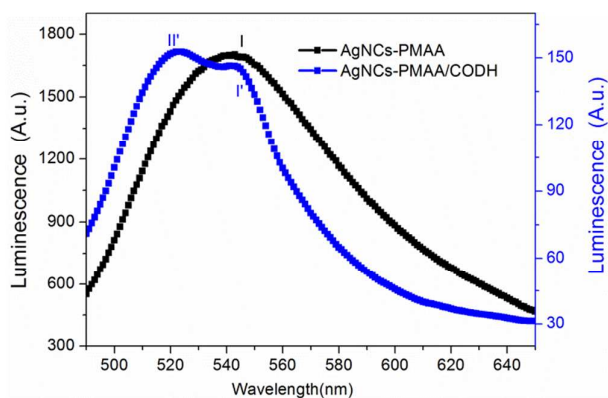


Figure S9 Luminescence spectra of AgNCs-PMAA (Ag atom concentration 20 μM determined by ICP-MS) and AgNCs-PMAA/CODH complex (CODH concentration 7.56 μM) excited at 460 nm.

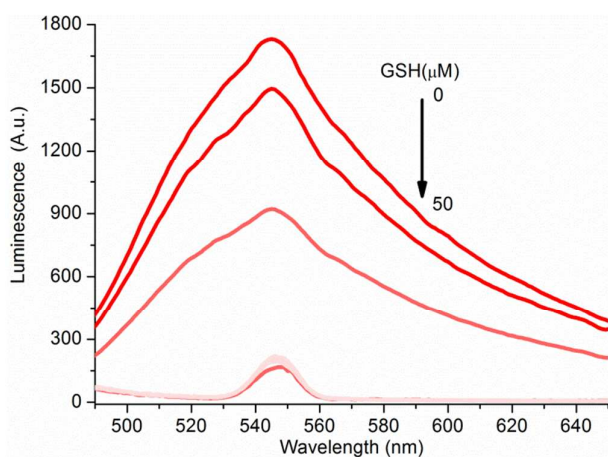


Figure S10 Photoluminescence titration of AgNCs-PMAA (Ag atom concentration 20 μM determined by ICP-MS) with GSH (0-50 μM) in 0.2 M MES buffer (500 μL, pH 6.0). λ_{ex}: 460 nm.

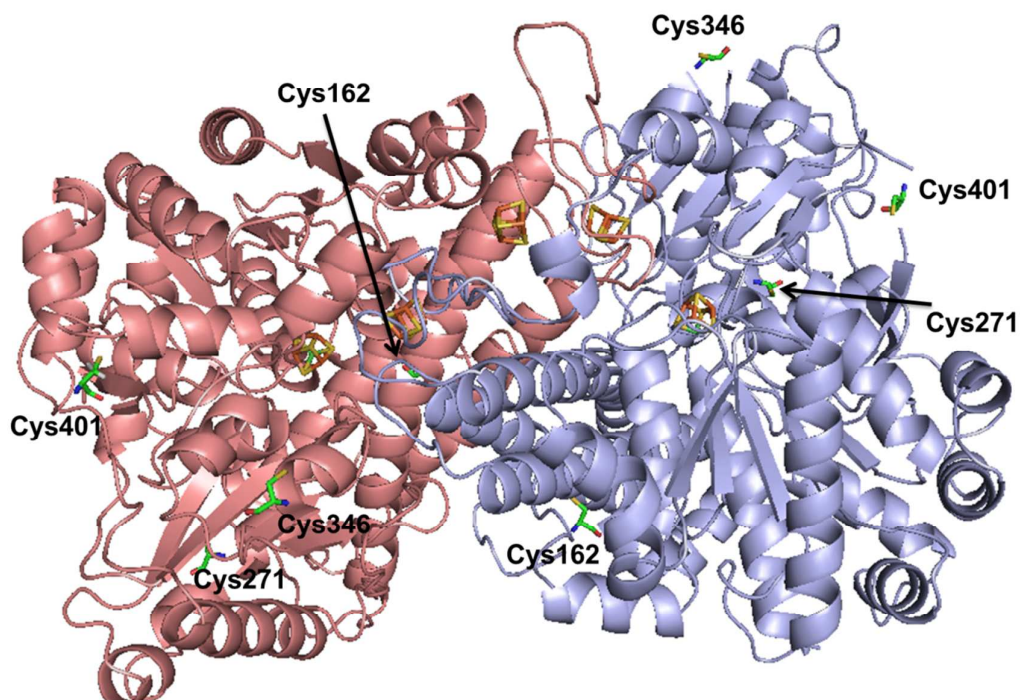


Figure S11 CODH simulative structure produced using SWISS-MODEL web-program. Surface exposed cysteines are colored in green. They are cys 162, cys271, cys346 and cys401.

Approximate calculation for CODH loading on a single AgNCs-PMAA/TiO₂ nanoparticle

TiO₂ nanoparticle diameter = 21 nm

Surface area (assuming spherical nanoparticle) = $1.39 \times 10^{-15} \text{ m}^2$

Volume = $4.85 \times 10^{-24} \text{ m}^3 = 4.85 \times 10^{-18} \text{ cm}^3$

Density = 3.9 g/cm^3

Mass = $1.89 \times 10^{-17} \text{ g}$

1 mg AgNCs-PMAA/TiO₂ containing 0.61 mg TiO₂ and 0.39 mg AgNCs-PMAA:

0.61 mg contains TiO₂ 3.22×10^{13} of nanoparticles ($6.1 \times 10^{-4} / 1.89 \times 10^{-17} = 3.22 \times 10^{13}$).

According to the ICP-MS results: 1 mg AgNCs-PMAA contains 0.53 mg of Ag atoms.

0.39 mg AgNCs-PMAA contains 1.91×10^{-6} mol of Ag atoms ($0.39 \text{ mg} \times 0.53 = 0.206 \text{ mg} = 1.91 \times 10^{-6} \text{ mol}$).

AgNCs-PMAA (assuming cube nanoparticles) size_{max} = 2 nm (According to TEM AgNCs-PMAA < 2 nm). AgNCs-PMAA 'footprint' (square of 2 nm) = $4 \times 10^{-18} \text{ m}^2$

Therefore, the approximate number of AgNCs-PMAA that can fit each TiO₂ nanoparticle is 348 ($1.39 \times 10^{-15} \text{ m}^2 / 4 \times 10^{-18} \text{ m}^2 = 348$).

Size of CODH enzyme molecule (using dimensions of the closely related enzyme CODH II) = 12 nm (approx.)

CODH enzyme 'footprint' = $1.44 \times 10^{-16} \text{ m}^2$

For bare TiO₂,

One TiO₂ nanoparticle = $1.39 \times 10^{-15} \text{ m}^2 / 1.44 \times 10^{-16} = 10$ enzyme molecules per nanoparticle.

For the standard conditions of photo-reduction of CO₂, in 5 mL colloidal solution contains 5 mg AgNCs-PMAA/TiO₂ and 0.5 nmol CODH, thus:

5 mg AgNCs-PMAA/TiO₂ contains 1.61×10^{14} TiO₂ nanoparticles ($5 \times 3.22 \times 10^{13} = 1.61 \times 10^{14}$).

5 mg AgNCs-PMAA/TiO₂ contains 9.55×10^{-6} mol Ag atoms ($5 \times 1.91 \times 10^{-6} = 9.55 \times 10^{-6} \text{ mol}$)

CODH = 0.5 nmol = 3.01×10^{14}

Therefore one AgNCs-PMAA/TiO₂ nanoparticle has 2 CODH molecules ($3.01 \times 10^{14} / 1.61 \times 10^{14} = 2$).

References

1. Seravalli, J.; Ragsdale, S. W., ^{13}C NMR Characterization of an Exchange Reaction between CO and CO_2 Catalyzed by Carbon Monoxide Dehydrogenase. *Biochemistry* **2008**, *47*, 6770-6781.