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Gold nanoclusters cause selective light-driven biochemical catalysis in living nano-biohybrid organisms

Electronic Supplementary Information

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Supporting Information:

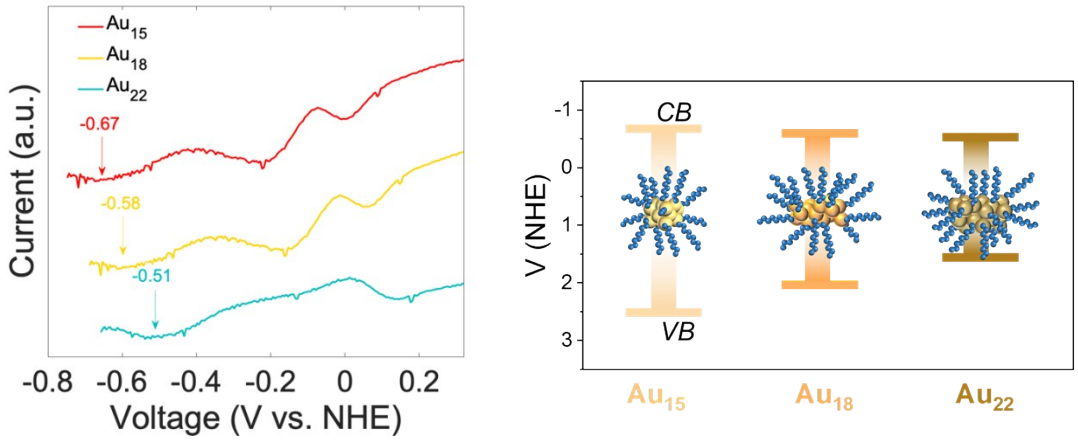

Figure S1. DPV curves and electrochemical potentials of Au_{15}, Au_{18}, and Au_{22} NCs. Differential pulse voltammetry curves for Au_{15}, Au_{18}, and Au_{22} determining their conduction band and valence band values relative to NHE.

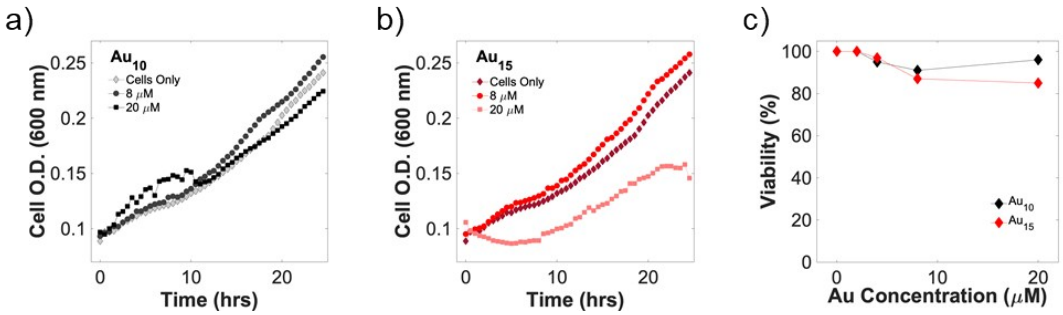

Fig. S2. Biocompatibility tests of small Au NCs. Growth curves for (a) $Au_{10\text{-}12}$ and (b) Au_{15}, compared to cell growth with no Au NCs, under light irradiation. (c) cell viability (resazurin dye assay, compared to no Au NCs as 100%) after treatment under light irradiation for 4 hours.

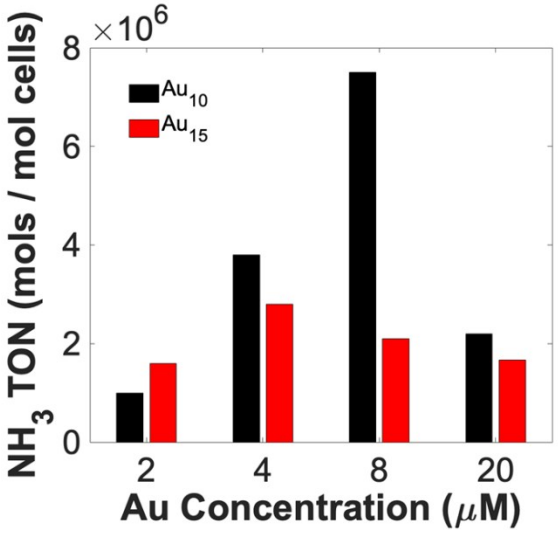

Fig. S3. Light-driven ammonia production using nanorgs with small Au NCs. Turnover number for the biocatalytic reaction of ammonia production from air using nanorgs formed from *A. vinelandii* DJ995 strain with $Au_{10\text{-}12}$, and Au_{15} NCs. The variation of ammonia production with increasing Au NC concentration tracks well with an initial increase in light absorption, subsequent saturation of nano-biohybrids, and subsequent loss of cell viability (Fig. S2) at higher concentrations.

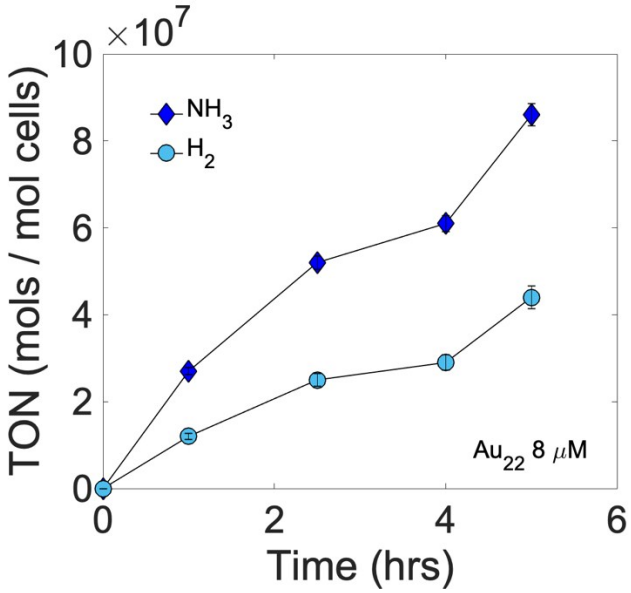

Fig. S4. Time-resolved light-driven ammonia and hydrogen production. Kinetic data for the production of H_2 and NH_3 over 5 hours for *A. vinelandii* DJ995 in the presence of 8μM Au_{22} NCs. The production of hydrogen in congruence with ammonia is facilitated by the nitrogenase enzyme which produces a 2:1 ratio of ammonia to hydrogen for each reaction.

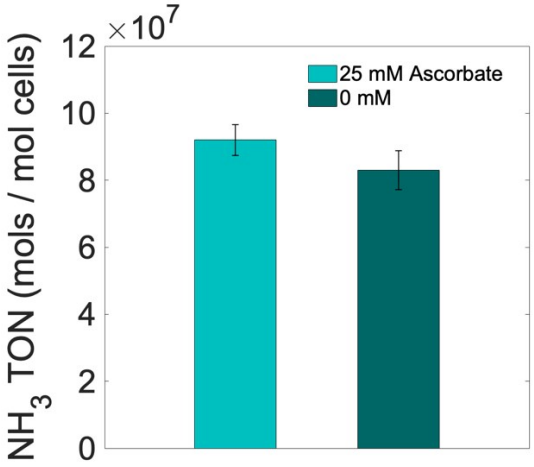

Figure S5. NH_3 production dependence on ascorbic acid. Au_{22}-*A. Vinelandii* nanorg tests were performed with and without ascorbic acid to demonstrate that ascorbic acid itself minimally affects the cellular metabolism. This indicates that the electrons powering N_2 reduction through nitrogenase come from Au NCs.

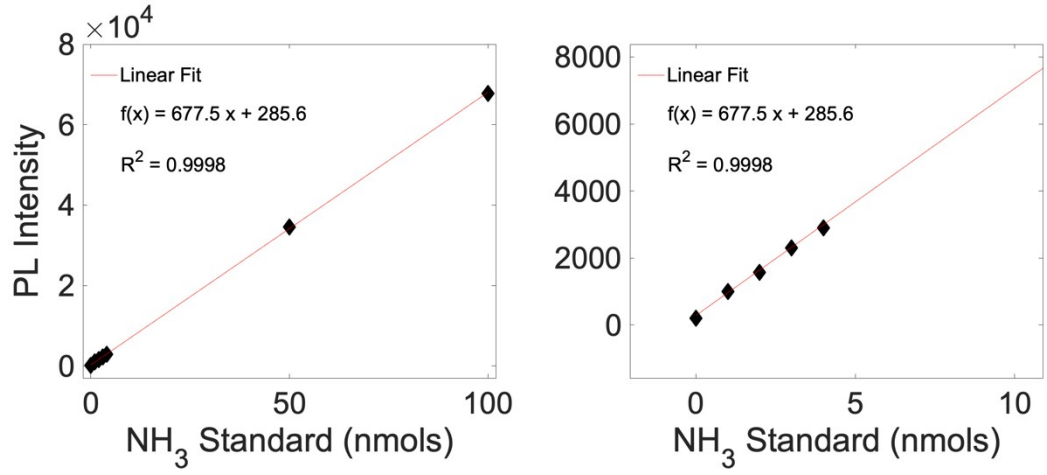

Fig. S6. Representative ammonia assay calibration curve. Calibration curve for the colorimetric ammonia assay to determine the amount of ammonia generated by the nano-biohybrids in solution.

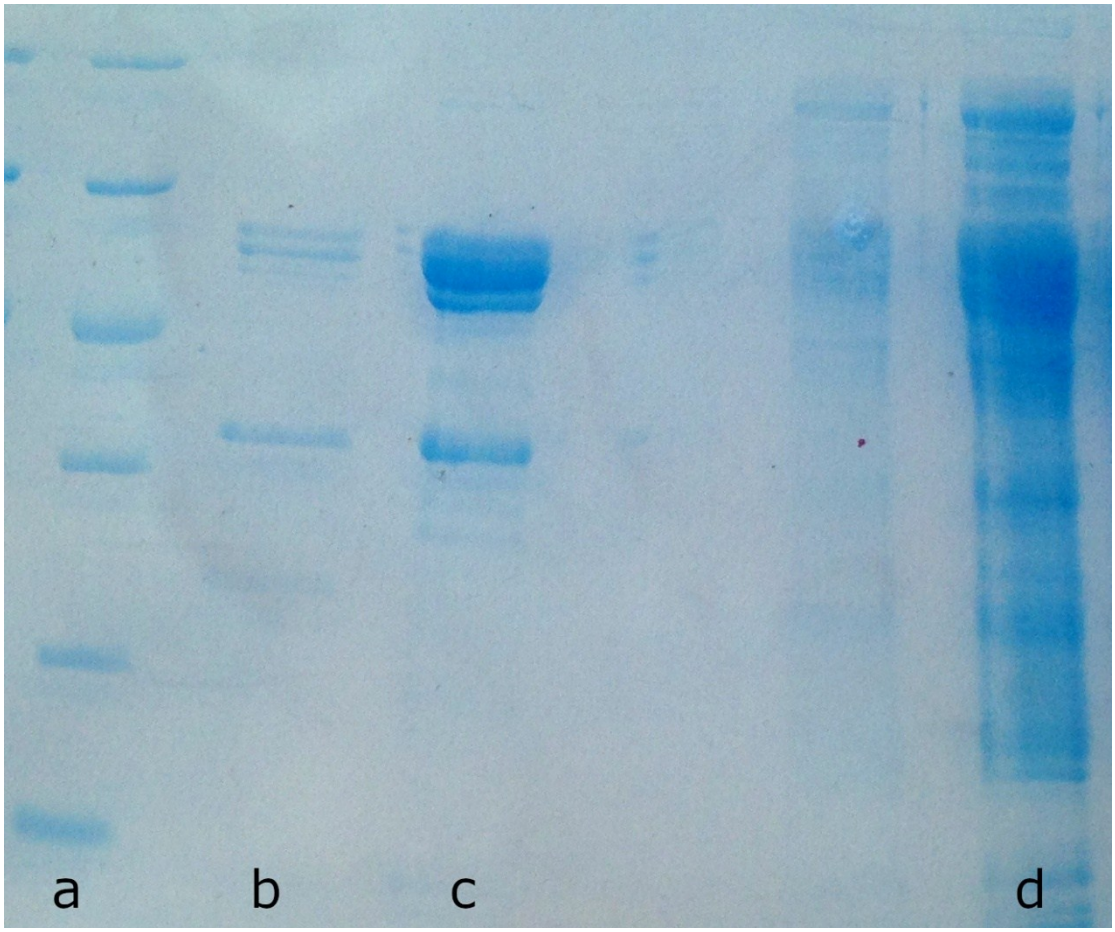

Fig. S7. Selective binding of nanoparticles to the targeted nitrogenase enzyme. Lane a: Protein molecular weight marker (From top to bottom: 116.0, 66.2, 45.0, 35.0, 25.0, 18.4 kDa). Lane b: Protein from *A. vinelandii* DJ995 cell lysate bound to nanoparticles (Three bands correspond to β, α-unit of MoFe protein and Fe protein, from top to bottom). Lane c: Purified His-tag MoFe-nitrogenase. Lane d: Cell lysate prepared from *A. vinelandii* DJ995.

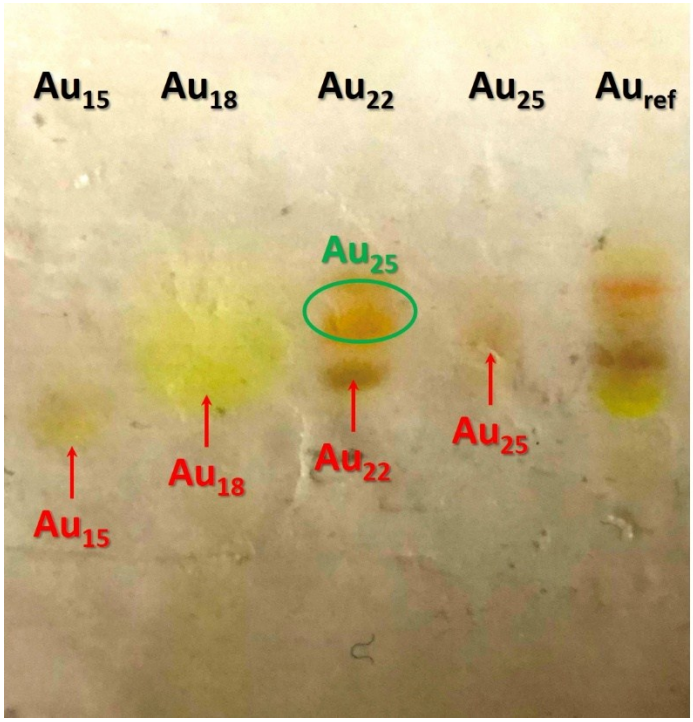

Fig. S8. Native polyacrylamide gel electrophoresis (PAGE) of Au NCs to characterize their purity.

Table S1. Table of representative data for the light-driven production of NH_3 by Au_{22} NC – *A. vinelandii* nano-biohybrid.

Sample	T_0 (0 hours, PL Intensity)	T_1 (4 hours, PL Intensity)	NH_3 in aliquot (nmols)	NH_3 in solution (nmols)
Au_{22} - 4 μM	307	1586	1.98	39.76
Au_{22} - 8 μM	362	2304	3.07	61.13
Au_{22} - 20 μM	406	2532	3.36	67.29
Au_{22} – 8 μM Dark	358	427	0.05	1.02
Au_{22} – 0 μM	276	309	0.03	0.63
Au_{22} – 8 μM No Cells	342	401	0.05	0.87

NH_3 Turnover Number and Turnover Frequency Calculations

NH_3 turnover numbers (TON) were calculated based on the known number of cells in the reaction medium and the measured amount of NH_3 generated by the light-driven process of the nanorgs. This value was taken to be $7.48 * 10^{-16}$ mols cells / mL at OD_{600} = 1.0.

$$TON = \frac{mols\ NH_3}{mols\ cells}$$

Similarly, turnover frequency (TOF) was calculated by using the slope of NH_3 TON as a function of time (Figure 4a in the main text), giving a TOF in units of h^{-1}.

Estimation of Quantum Efficiency (QE)

The quantum efficiency (QE), defined as the ratio of electron production to the total amount of photon absorbed, was estimated based on the following parameters:

1. NH_3 and H_2 turnover frequency: 6250 and 3075 s^{-1}, respectively.
2. Au_{22} QDs: 8 μM concentration (c), the extinction coefficient at 400 nm = 10000 $M^{-1}cm^{-1}$.
3. Cell OD_{600} = 1.0, corresponding to 4.5×10^8 cell / ml for *Azotobacter vinelandii*.
4. Light source: 400 nm LED, with 1.6 mW/cm^2 irradiation intensity (I).

5. Reactor: a glass vial with ~ 1 cm inner diameter (d), with total reaction volume (V) of 1 ml. Therefore, the irradiation cross section $S = \pi d^2/4 = 0.52$ cm^2 and the light path b = V/S = 1.91 cm.
6. The cellular uptake of the Au_{22} NCs is 92%.
7. The copy number of 7x His-tag MFN per cell is ~ 10^5, and assuming one Au_{22} NC bind to one histidine on the His-tag to form the $(Au_{22})_7$-MFN bioconjugates.

The total number of Au_{22} NC bound to the MFN in 1 ml mixture is:

$N = 4.5 \times 10^8 \times 10^5 \times 7 = 3.15 \times 10^{14}$

This corresponds to a concentration of $c' = \frac{N}{N_A V} = \frac{3.15 \times 10^{14}}{6.02 \times 10^{23} mol^{-1} \times 1\ ml} = 0.523\ \mu M$, which is lower than the total uptaken Au_{22} NCs.

Based on the Lambert-Beer's law, the light absorbed A = εbc = 0.01, and the transmittance can be calculated by A = 2 – log(%T), and T = 97.7% and the absorbed part is 1 – T = 2.3 %.

The incident photon number can be calculated from the irradiation intensity:

$$N' = \frac{total\ energy}{photon\ energy} = \frac{intensity * area * time}{hc/\lambda} = \frac{ISt}{hc/\lambda}$$

And the incident photon flux (F_{inc} = N'/t) is $F_{inc} = {IS\lambda}/{hc} = 1.676 \times 10^{15} s^{-1}$.

The absorbed photon by the nanorgs is $F_{abs} = F_{inc} \times (1 - T) = 3.82 \times 10^{13} s^{-1}$.

The total electrons produced from the nanorgs can be calculated from the TOF of NH_3 (3 electrons per NH_3 molecule) and H_2 (2 electrons per H_2 molecule), and for one cell, the electron flux is $F_{e/cell} = 3 \times T(NH_3) + 2 \times TOF(H_2) = 24900$ s^{-1}.

With OD_{600} = 1.0 cell in 1 ml total volume, the total electron flux is $F_e = 1.12 \times 10^{13} s^{-1}$.

Therefore, QY = F_e / F_{abs} × 100% = 29.3 %.

Photon-to-Fuel Conversion Efficiency Calculations

To calculate the photon-to-fuel conversion efficiency (PFCE), the following set of parameters were used:

Ammonia: 382.6 KJ/mol

Hydrogen: 286 KJ/mol

For 1 ml nanorgs, the total energy output (as for the formation of ammonia and hydrogen) per second is:

$$E_{out} = (E_{ammonia} \times TOF(ammonia) + E_{hydrogen} \times TOF(hydrogen)) N_A = (382.6 \times 6250 + 286 \times 3075) \times 4.5 \times 10^8/(6.02 \times 10^{23}) = 2 \times 10^{-9} kJ = 2.445\ \mu J$$

The energy input (as of 400 nm photon absorbed by the nanorgs) per second is:

$E_{in} = I \times S \times (1 - T) = 1.6 \times 10^{-3} \times 0.52 \times 0.023 = 18.94\ \mu J$

Photon-to-fuel conversion efficiency PFCE = E_{out}/E_{in} = 12.9 %.