

Supplementary Information

Amino acid-based biohybrids for nano-shellization of individual desulfurizing bacteria

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Supplementary Experimental section S1.

Medium: The sulfur-free medium was composed of glucose 10g, NH_4Cl 2g, KH_2PO_4 2.44g, Na_2HPO_4 5.57g, MgCl_2 0.2g, CaCl_2 0.04g, $\text{FeCl}_3 \cdot 7\text{H}_2\text{O}$ 0.04g, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.008g, ZnCl_2 0.001g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.004g, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 0.001g, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 0.001g, H_3BO_3 0.001g, $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 0.001g, sterilized water 1000 ml, pH 7.0.

Cell culture: *Gordonia sp.* WQ-01A, were grown in medium, incubated at 30°C and all the cells for experiments were harvested in the log phase with the same concentration (Fig. S9).

Preparation for biohybrid solution: The synthesis of 2-3 nm Au nanoparticles was performed via previously established method¹ that will be briefly described here. To summarize, $2.5 \times 10^{-4}\text{M}$ chloroauric acid and trisodium citrate was mixed in a flask, followed by the addition of 0.1M sodium borohydride. The Au colloid was preserved at room temperature. 0.05g L-lysine (Mw=146.19, powder, Sigma-Aldrich) was added to phosphate buffer saline (PBS) (pH=5.9) solution, followed by the addition of Au colloid into the solution under continuously stirring condition. Biohybrid nanoparticles were collected by centrifugation (8000r/min, 20 min) and immediately transferred into 3 ml PBS (pH=7.0)

Cell nano-shellization: Cells were harvested by centrifugation at 4000 rpm for 10 min and washed with aqueous PBS solution to get rid of excessive medium. Then the cells were immersed in the biohybrid solution and gently mixed at room temperature for 30 min. After mixing step, the cells were collected by centrifugation (4000 rpm, 15 min) and re-dispersed in medium and incubated under ambient conditions.

Characterization: SEM, EDX and TEM studies were conducted with S-4800 (HITACHI, Japan) and JEM2100F (JEOL, Japan), respectively. The samples were first collected by centrifugation and washed with distilled water for three times. The biological samples were dropped on to specimen stage and dried for 4h. The ultrathin section samples were fixed with glutaraldehyde, OsO_4 , and dehydrated in acetone. They were embedded in Epon 812/Araldite M resin. Thin sections (ca. 100 nm) were cut by using ULTRACUT UCT ultramicrotome (Leica, Germany) and characterized by TEM (Tecnai, Netherlands). Zeta potentials were measured by the electrophoretic mobility of particles/cells in an electric field (Ankersmid, Netherland).

Cell desulfurizing activity test, safety test and recycling test: Experiment was carried out using encapsulated cells or native cells as biocatalysts in 50 ml distilled water containing DBT (2mM,

using N, N-dimethylformamide as dispersant) and 5% glucose in a sterile flask with shaking (200 rpm) at 30°C. The amount of residual DBT was extracted with ethyl acetate after acidifying the samples to pH 2.0. The ethyl acetate phase was separated from aqueous-phase containing cells by centrifugation at 4000 rpm for 10 min. The concentration of DBT was analyzed by High Performance Liquid Chromatography (HPLC). HPLC was performed on a UFLC 20A (Shimadzu, Japan) liquid chromatography equipped, a reversed-phase C18 column (4.6 mm×250 mm; 5 µm) and an ultraviolet detector. The mobile phase was 90% of methanol in water (v/v, %) with a flow rate of 1.0 ml min⁻¹. For the quantification of DBT, the external standard method was used at 280 nm. For safety test, both of encapsulated cells and native cells were cultivated in fresh media to maintain cell ability to proliferate. Data of optical density at 600nm (OD₆₀₀) were measured by Microplate Reader. In order to mitigate the effect of biological variability, three technical replicates were performed for each experiment. Each biological replicate was treated independently with the same procedure. For recycling test, each cycle was carried out by re-collection of encapsulated cells and re-dispersion into fresh solution containing 5% glucose and 2 mM DBT.

Synthesis of encapsulated desulfurizing bacteria post-functionalized with TiO₂ nanoparticles:

Encapsulated cells were collected by centrifugation and re-dispersed into PBS solution (7.0). 0.05g TiO₂ nanoparticles were dispersed into PBS solution and added into encapsulated cells solution. After gently shaking for 30 min, the product was collected by centrifugation and re-dispersed into PBS solution. For DBT degradation test, four groups of samples (native cells, cells@biohybrid shell, cells@biohybrid shell@TiO₂ layer and TiO₂ nanoparticles) were added into 80 ml PBS solution containing 5% glucose and DBT, respectively. All the samples were shaking under UV-visible light at room temperature. The residual DBT concentration of was analyzed by HPLC.

Synthesis of encapsulated desulfurizing bacteria post-functionalized with Fe₃O₄/SiO₂ nanocomposites: 11.8g FeCl₃·6H₂O and 4.3g FeCl₂·4H₂O in 300 ml distilled water under nitrogen with stirring at 85°C and then quickly added 30 ml of 7.1 M ammonium hydroxide. 16 ml of oleic acid was subsequently added. The magnetic precipitates were washed several times with 7.1 M ammonium hydroxide to form the hydrophilic magnetic nanoparticles.² Magnetic colloids and silicic acid were added and gently shaken for 30 min. The product was obtained by

centrifugation-washing steps. Encapsulated cells were collected by centrifugation and re-dispersed into PBS. The mixture of magnetic colloids and silicic acid was then added into cell solution and adjusted pH value to neutral by KOH solution.

Author Contributions

N.J. carried out all the experiments. G.L.Y., S.Y.L., L.S. and L.J.D. carried out precursor preparation. G.L.Y. measured zeta potentials. S.Y.L. prepared magnetic nanoparticles. J.H. synthesized titania nanoparticles. N. J. and T. G. analyzed the data. T. G. guided the precursor experiments. X.Y.Y. conceived the project, provided the idea, and designed and guided the experiments. B.L.S. conceived the project, and supported scientific and technological platform and guidance. N.J. and X.Y.Y. wrote and revised the paper.

Table S1 Zeta potential of native cells, cells@biohybrid shells and biohybrids.

Sample	Native cells	Cells@biohybrid shells	Biohybrids
Zeta potential	-16.5 mV	-20.4 mV	-23.8mV

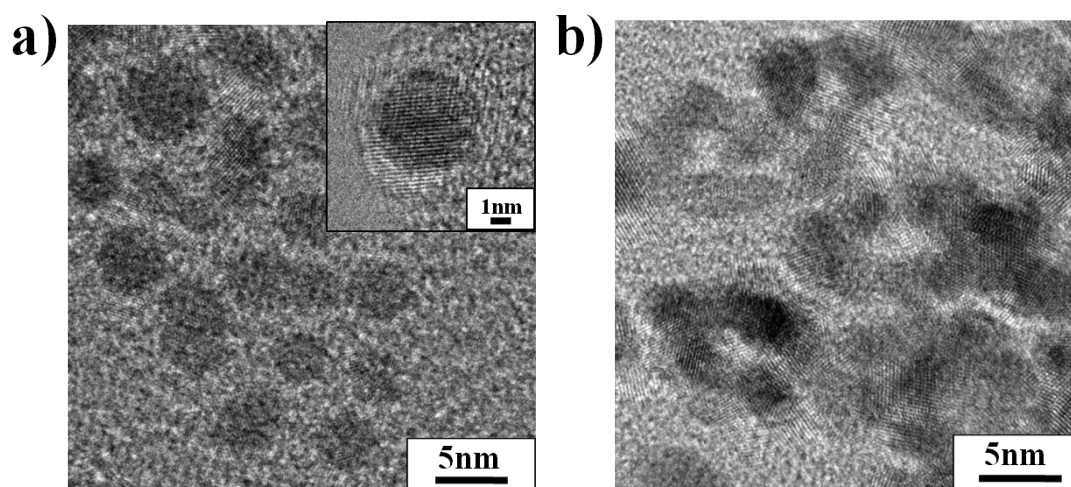


Fig. S1 Characterization of amino acid-based biohybrids: a) TEM and high-magnification images (inset) of mono-dispersed gold nanoparticles; b) TEM image of nanoporous-structured biohybrids.

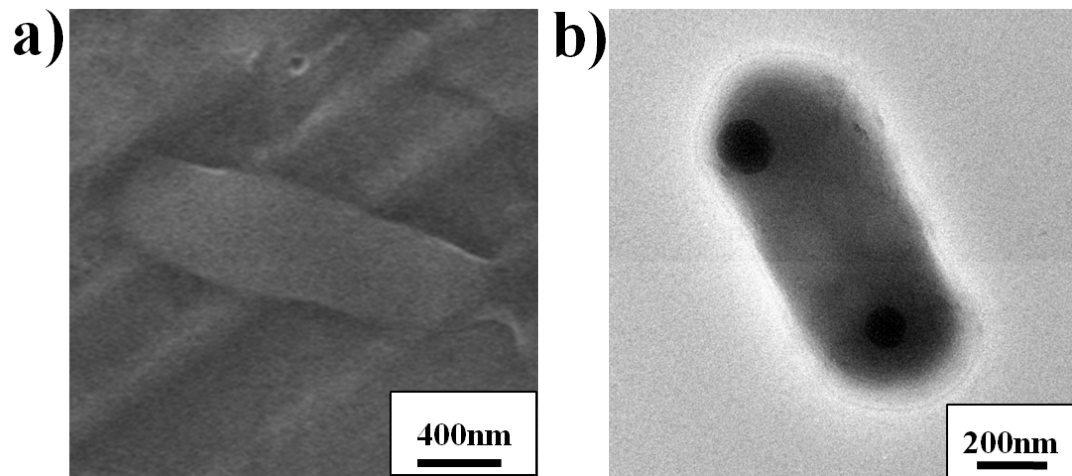


Fig. S2 SEM and TEM images of native *Gordonia* sp. WQ-01A. The native cell surface is smooth.

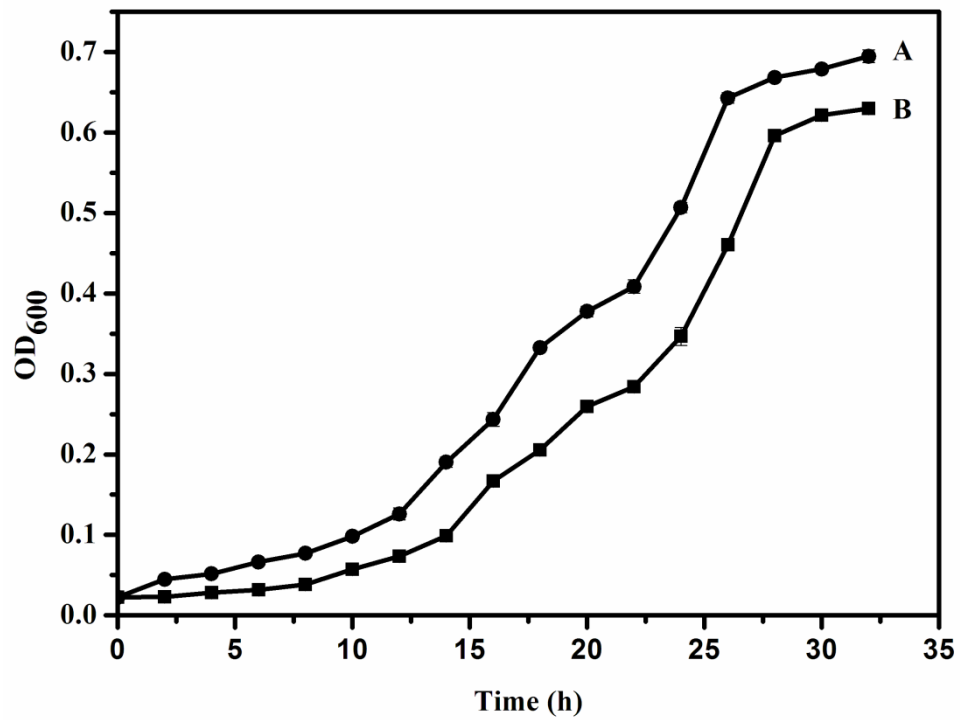
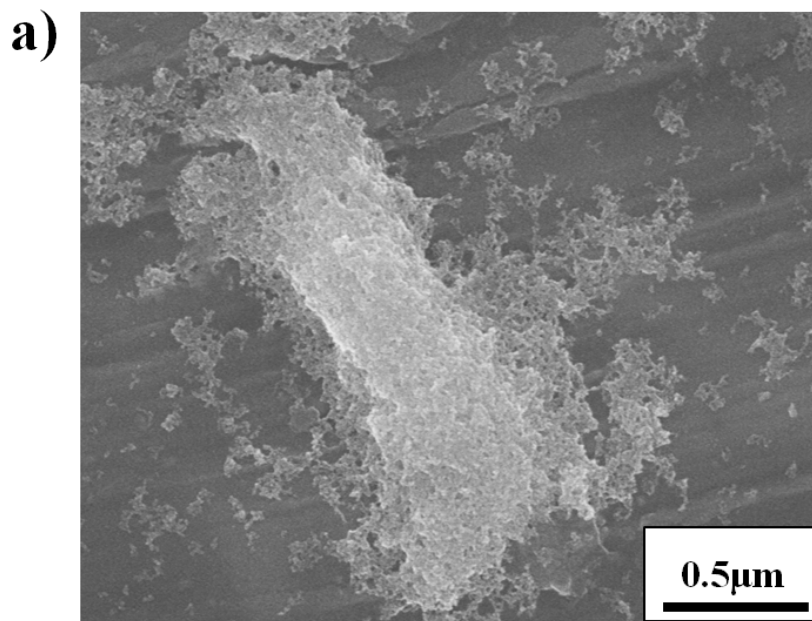


Fig. S3 Viability of (A) amino acid-based biohybrid shell encapsulated and (B) native *Gordonia* sp. WQ-01A. Both of the native cells and encapsulated cells are cultivated in fresh media for their living and proliferation. There is no obvious difference between native cells and encapsulated cells during the whole log phase in which cells are proliferating.



b)

Reaction time (h)	0	20	40	60
Degradation rate (%)	0	77.2	86.0	89.5

Fig.S4 a) SEM image of Au@L-cysteine biohybrid-encapsulated cell; and b) degradation rate of DBT by Au@L-cysteine biohybrid-encapsulated cells. The cell is encapsulated by nanoporous structured Au@L-cysteine shell. The rod-like morphology is maintained after encapsulation. Moreover, the encapsulated cells show good desulfurizing activity, indicating that Au@L-cysteine shells do not affect the activity of cells.

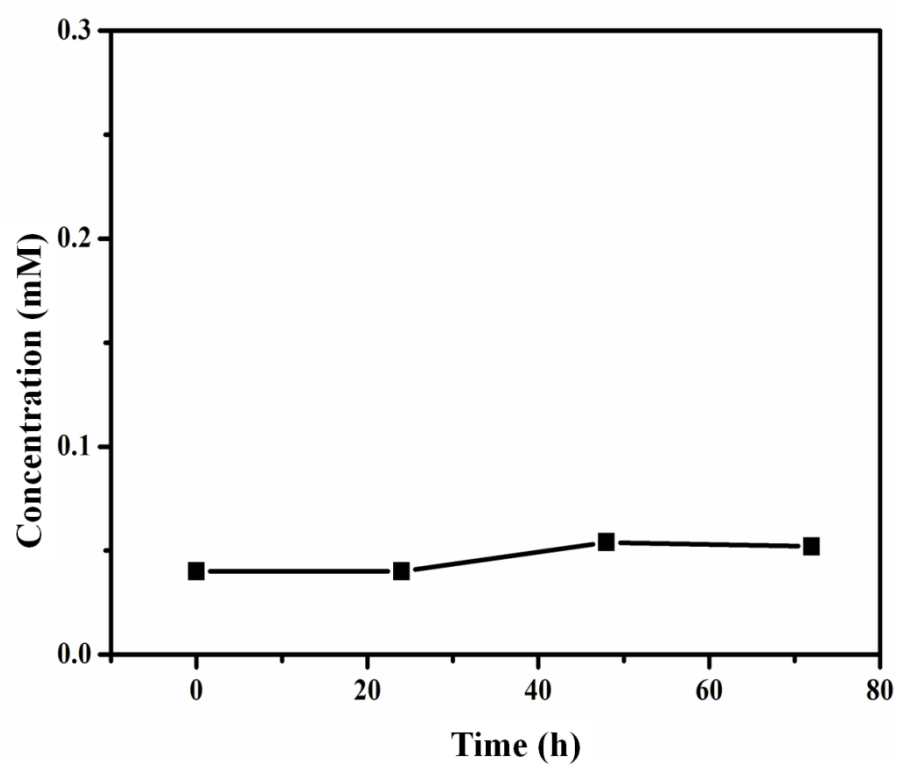


Fig. S5 Adsorption of DBT to biohybrids.

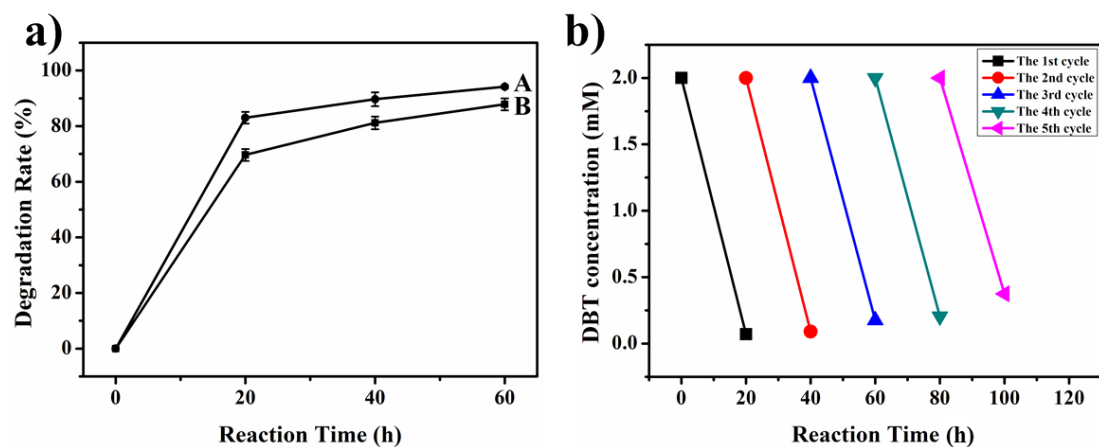


Fig.S6 a) Degradation rate of DBT by encapsulated (A) and native (B) *Gordonia sp.* WQ-01A; b) Stability of encapsulated *Gordonia sp.* WQ-01A. desulfurizing activity with the number of recycles.

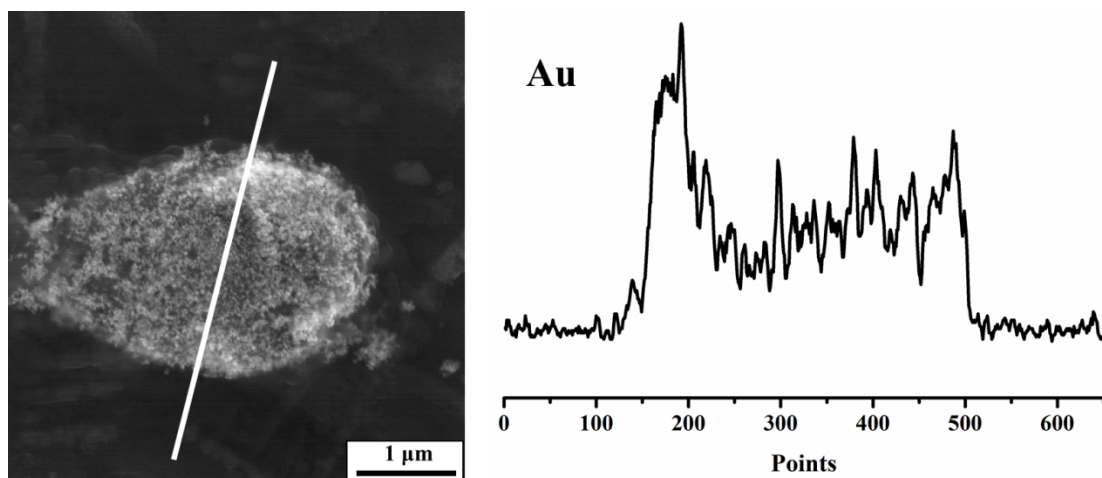


Fig. S7 SEM image and EDX line profiles of encapsulated cell for Au element after desulfurizing process.

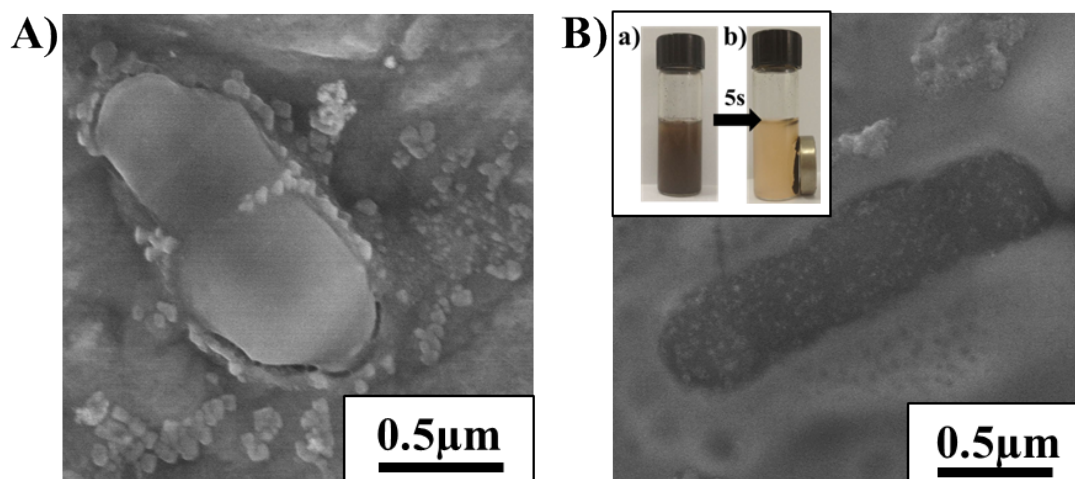


Fig. S8 A) SEM image of cells with TiO_2 nanoparticles; TiO_2 nanoparticles are deposited around cell, but a small amount of nanoparticles can be found on cell surface; B) SEM image of cells with magnetic nanoparticles without biohybrid layer. Inset shows magnetic behavior of cell with Fe_3O_4 nanoparticles a) before and b) after external magnet.

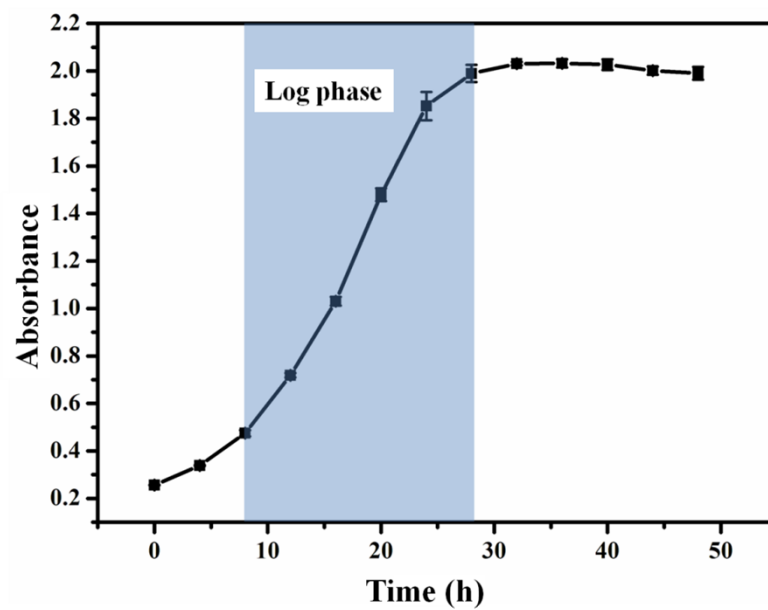


Fig. S9 Growth curve of native *Gordonia* sp. WQ-01A. Log phase is shown in shadow area.

References:

1. N. R. Jana, L. Gearheart and C. J. Murphy, *Langmuir*, 2001, **17**, 6782
2. G. B. Shan, J. M. Xing, H. Y. Zhang and H. Z. Liu, *Appl. Environ. Microbiol.*, 2005, **71**, 4497