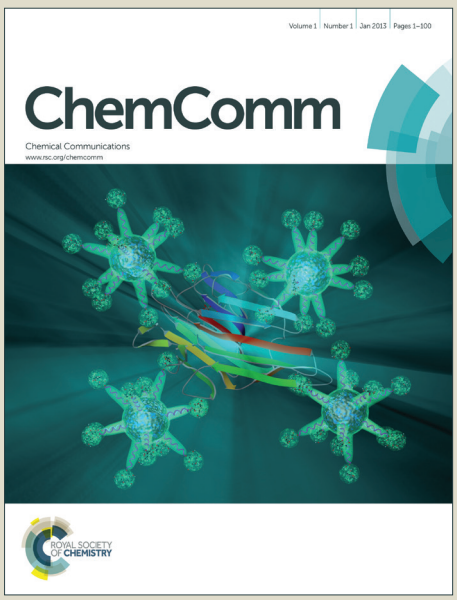
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

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

Nan Jiang,[a] Guo-Liang Ying,[c] Shao-Yin Liu,[a] Ling Shen,[a] Jie Hu,[a] Ling-Jun Dai,[a] Xiao-Yu Yang*[a] Ge Tian,*[a] and Bao-Lian Su*[a,b]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Amino acid-based biohybrids have been developed to self-assemble on desulfurizing bacteria surface to form nanothin and nanoporous shells. The shells not only endow the encapsulated cells with reusability, but also offer platforms to incorporate titania and magnetic nanoparticles to improve the desulfurizing activity and the separation efficiency.

Cell surface functionalization without complicated genetic manipulations, offering the cells with new structural and functional properties, has been received particular interest in research area of bio-nanotechnology.[1-3] Cell surface shellization as one of the most efficient approaches have been developed to enhance the cell stability and engineer non-biogenic cell surface.[4] The benefit of having durable nanostructured shells on cell surfaces lays in maintenance of cells integrity without perturbance by the encapsulation methods. This is because the nanostructured shell materials with tunable physico-chemical properties, such as hydrophilic silica, electrically conductive carbon materials, smart stimulus-responsive polymers, and other targeted functional materials, offer surface functionalities to cells both in *vitro* and in *vivo*.[5-14] However, the biocompatibility of interface between living cell surface and abiotic shell engineering materials is still a big challenge. Good understanding in the interaction of cell surface and nanostructured materials is essential for the shellization with desired structures and properties. For example, very careful control of the synthetic condition has been used to cover the problems of poor interactions, such as lack of active groups in inorganic materials and excessive electric charges in polymer-based material, which occurs in the interface of the living cells and nanomaterials. Biological molecules and/or aggregations, as perfect interfacing materials, have been therefore utilized to cell surface shellization due to their natural biocompatibility and biodegradability,[15-16] although the nanostructures are not well-defined compared to the traditional abiotic nanomaterials.

Amino acid molecules as one of typical small biological molecules not only present similar physico-chemical properties of small organic molecules to fabricate well-defined nanostructures via self-assembly with nanomaterials, due to their abundant functional groups, but also have very good biocompatibility for cells surface functionalization. However, instability of amino acid layer is the main drawback that restricts further development in cell-surface-functionalization. Gold nanoparticles are therefore used to bind with the groups of amino acids to form nanoporous and stable biohybrids nano-aggregations. L-lysine (one of α-amino acid molecules) possesses amino groups/carboxyl groups, which avails to bind with gold nanoparticles to form nano-aggregations and also interact with cell surface for shellization. Herein, nano-structured amino acid-based biohybrids, composed of gold nanoparticles and L-lysine molecules have been developed for cell surface shellization. The self-assembled biohybrid shell exhibits nanoporous and nanothin structure which facilitates mass communications and molecules diffusion. Most importantly, the biohybrid shell can provide a platform to incorporate with various inorganic materials to expand applicability of cells. For example, titania nanoparticles can be introduced to enhance the desulfurizing activity, and the magnetic iron oxide nanoparticles on the cells surface can endow the encapsulated cells with the magnetic separation behaviour.

Biodesulfurization (BDS), a biocatalytic process performed by microorganisms to specially remove sulfur from fossil fuels, has always been considered as a "green" way and extensively used.[17,18] *Gordonia sp.* WQ-01A, as an example of desulfurizing

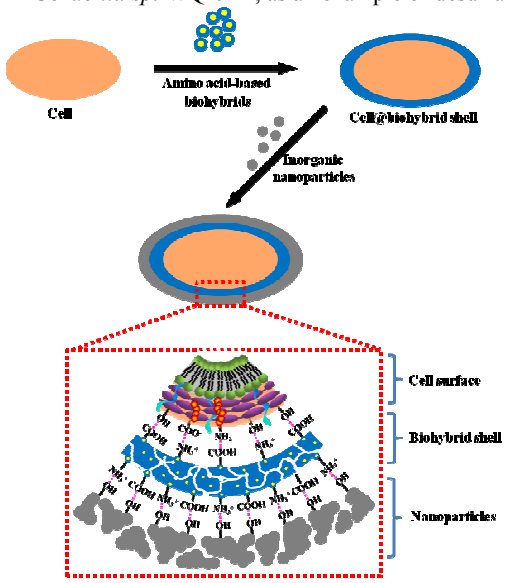


Fig. 1 Schematic representation of amino acid-based cell surface shellization and post-functionalization of the nanoshells. The formation of biohybrid shell and nanoparticles shell is achieved by hydrogen-bonding or electrostatic interaction.

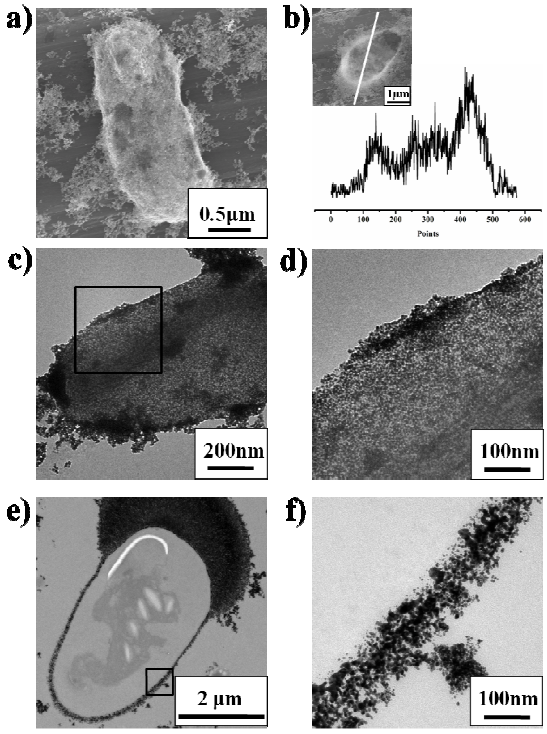


Fig. 2 Characterization of the cell@biohyrbid shell: a) SEM image of a native cell; b) EDX line profiles of encapsulated cell for Au element; c) and d) TEM images of cell encapsulated by biohybrid shell and the magnified nanoporous structure of the shell; e) and f) TEM and magnified images of microtome-sliced encapsulated cell.

bacterium, is here harnessed as our model cell for the investigation of amino acid-based surface nano-shellization. In a typical synthesis (Fig. 1), the self-assembled Au@L-lysine biohybrids are chosen as the precursors for shellization on surfaces of desulfurizing bacteria. Subsequently, the cell surface is gradually covered with the biohybrids formed by the self-assembled Au@L-lysine biohybrids. Biohybrids possess functional groups (amino groups and carboxyl groups, supported by L-lysine molecules), which enables the hydrogen bonding or electrostatic interaction with abundant hydroxyl groups, amino and carboxyl moieties of cell surface (magnified image in Fig.1). More importantly, these functional groups of biohybrid shell can also be used to interact with hydroxyl groups of nanoparticles during the hydrolysis and condensation. Post-functionalization could therefore be achieved by the formation of secondary shell constructed by nanoparticles.

Transmission electron microscopy (TEM) image in Fig. 2a shows that size of the mono-dispersed gold nanoparticles is 3-5 nm (inset in Fig. S1a). After the addition of the L-lysine solution, the Au@lysine biohybrids form. TEM image (Fig. S1b) further shows that the biohybrids are uniformly dispersed in solution and formed a wormlike nanoporous structure (3-6 nm of pore size). Scanning Electron Microscopy (SEM) in Fig. 2a clearly presents that biohybrids have been successfully self-assembled onto cell surface without affecting cell original integrity (Fig. S2). The presence of Au in biohybrid shell is characterized by the line-scan analysis of energy-dispersive X-ray (EDX) spectroscopy (Fig. 2b). According to TEM images in Fig. 2c and 2d, the cell is encapsulated by biohybrid shell with worm-like nanoporous structure. These mesopores around whole cell surface are very

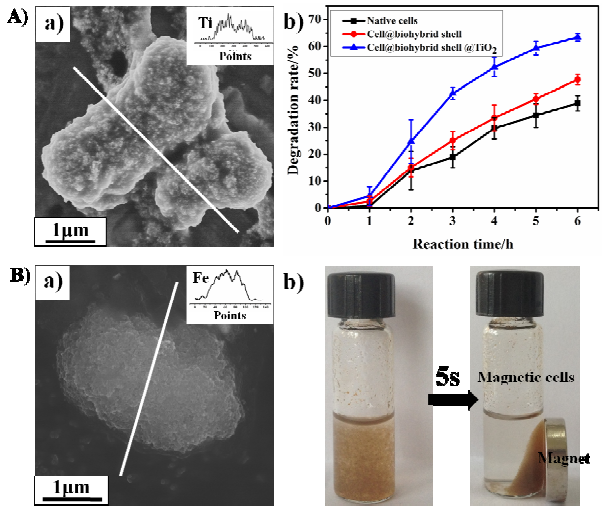


Fig 3 A) Post-functionalization with TiO_2 nanoparticles: a) SEM image and EDX line profile for element Ti (inset in a) in cell@biohybrid shell@TiO_2 shell; b) degradation rate of cell@biohybrid shell@TiO_2 shell, cell@biohybrid shell and native cells; B) Post-functionalization with Fe_3O_4/SiO_2 nanocomposites: a) SEM image and EDX line profile for elements Fe (inset in a) in cell@biohybrid shell@Fe_3O_4/SiO_2 shell, and b) magnetic behavior of cell@biohybrid shell@Fe_3O_4/SiO_2 shell.

uniform, suggesting that the shells have molecules selectivity which could enable the permeability to reaction molecules diffusion and retard toxic macromolecules into cells.[19] The ultrathin section TEM images of the encapsulated cell confirm that cytoplasm maintains its inherent integrity, and the artificial shell densely covers the whole cell (Fig. 2e and f). In addition, the higher magnified image shows that the average thickness of the biohybrid shell is 100 nm (Fig. 2f). These evidences imply that the biohybrids have been successfully self-assembled on cell surface to form artificial nanoshells. Furthermore, long-term experiment has also proven that the artificial shell does not affect cell viability, indicating the biohybrids is cyto-compatible to cells (Fig. S3).

The formation of the biohybrid shell is possibly attributed to weak interaction of self-assembly, such as surface charge and hydrogen bonding. Surface charges are positive proportional to zeta potentials (Table S1).[20] The cationic ions in solution might absorb or assemble to the negatively charged cell surfaces.[15] The negatively charged biohybrids would then assemble onto cell surface. After this process, the biohybrids are thought to actively interact with cell surface for shellization. Furthermore, cell surface often contains abundant hydroxyl groups and a little amino and carboxyl moieties. Such a surface hardly induces inorganic nanomaterials deposition due to its relatively low density of active groups which are essential to interact with functional materials for shellization. Interestingly, cells after encapsulation are stable in solution, due to its improvement of surface zeta potential (Table S1).[21] Furthermore, different amino acid molecules (such as L-cysteine) can also be easily introduced to biohybrids for cell surface shellization without loss of activity (Fig. S4). This not only suggests the extension of our method, but also further confirms that the hydrogen bonding plays an important role to the interaction between amino acid and the cell surface after surface charge effect.

The *Gordonia sp.* WQ-01A can effectively remove sulfur

from DBT (dibenzothiophene, a typical sulfur compound in fossil fuels) by the sulfur-specific pathway.[17] Desulfurizing activities of encapsulated cells and native cells are investigated by measuring residues concentration of DBT. It has been proven that biohybrids do not affect DBT concentration in solution (Fig. S5) and also maintain metabolically activities of cells (Fig. S6a). The result also illustrates that the DBT molecules are accessible to cell surface through the nanopores, which ensures the normal process of desulfurization after shellization. Moreover, the encapsulated cells show good recycling stability (Fig. S6b) and the biohybrid shell is stable on cell surface after desulfurizing process (Fig. S7), which are crucial factors in BDS.

More importantly, the amino acids-based biohybrid shell with amine and carboxyl functionalities enables an active platform to bind with nanoparticles. TiO_2 nanoparticles have been reported to absorb UV light for efficient DBT desulfurization.[22] In this regard, it can be thought that the enhanced desulfurizing efficiency could be achieved by incorporating encapsulated cells with TiO_2 nanoparticles. The SEM image clearly shows that the cell is encapsulated by TiO_2 nanoparticles and the presence of Ti element is confirmed by the line-scan analysis of EDX spectroscopy (Fig. 3A (a)). The desulfurizing activity of post-functionalized cells is carried out under ultraviolet-visible light, cell@biohybrid shell and native cells are also used as comparisons. The post-functionalized desulfurizing bacteria show a higher desulfurizing activity in DBT degradation in the comparison with cells@biohybrid shells and native cells. After 6 hours degradation, the degradation rate of cells@biohybrid@TiO_2 (63%) is higher than cells@biohybrid (48%) and native cells (39%). Obviously, the extra degradation rate of post-functionalized cells is mainly ascribed to the protective and photocatalytic effect of TiO_2 layer. These results suggest that the cell shellization process can more simply and directly improve the cell desulfurizing capacity, comparing with complex genetic process. Furthermore, magnetic nanoparticles possess magnetic properties which can be easily handled by external magnetic field.[23] Incorporating cells with magnetic nanoparticles are unquestionably useful for re-collection and orientation. Herein, we post-functionalize the encapsulated cell with a magnetic layer by the introduction of biocompatible Fe_3O_4/SiO_2 nanocomposites in which elements Fe have been proven by line-scan analysis of EDX spectroscopy (inset in Fig. 3B). The magnetic behaviour of functionalized cells is observed without any assistance of a microscope (Fig. 3B (b)). The encapsulated cells can be easily and quickly separated from solution by an external magnetic force in 5 seconds. On the basis of their magnetic properties, these encapsulated cells can be easily separated from the reaction mixture and beneficial for orientation in biosensors and microchips. It should be pointed out that the pure iron oxide nanoparticles are difficult to be directly post-functionalized around the cells surface due to the lack of the hydroxyl groups (Fig. S8B). The additional of silica nanoparticles is necessary in this synthesis. These results further indicate that the hydrogen bond might be the main interaction between the amino acid-based biohybrid shell and the hydroxyl of nanoparticles. This is also in good agreement with the formation process our proposed (Fig.1).

The successful encapsulation of secondary nanoshell should be directly attributed to the present of biohybrid shell. The comparison experiments in absence of the biohybrids show that TiO_2 nanoparticles are deposited around cell, but a small amount of nanoparticles can be found on cell surface (Fig. S8A). Similarly, very few magnetic nanoparticles are coated on cell surface (Fig. S8B). It can also be found that magnetic nanoparticles have been easily separated from the solution, while the solution is still light brown and turbid, indicating that cells are still dispersed in the solution (inset of Fig. S8B). The result further proves that most of cells are not encapsulated by magnetic nanoparticles.

In summary, the amino acid-based biohybrids have been successfully used to encapsulate individual desulfurizing bacteria surfaces by shellization process. The shells present the nanothin and nanoporous structure and endow the encapsulated cells with reusability. More importantly, the biohybrid shell provides a platform for cell post-functionalization to expand cell applicability in enhancement of desulfurizing activity and behaviour of easy separation. It is believed that our strategy developed here would increase the number of tools available for more functionalization in cell-based nanobiotechnology.

This work was supported by a Chinese Ministry of Education (NCET-11-0688, IRT1169, RFDP-20110143120006, 2012-YB-04, 2014-CL-A2-08), NFSC-51101115, and NFSC-51472190.

Notes and references

[a] *State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, and School of Materials Science and Engineering, Wuhan University of Technology, Wuhan,430070 China. E-mail: xyyang@whut.edu.cn; tian.ge@yahoo.com*
[b] *Laboratory of Inorganic Materials Chemistry, The University of Namur (FUNDP), Namur B-5000, Belgium. E-mail: bao-lian.su@fundp.ac.be;*
[c] *School of Material Science and Engineering, Wuhan Institute of Technology, Wuhan 430073, China.*

† Electronic Supplementary Information (ESI) available: [Experimental section and other details]. See DOI: 10.1039/b000000x/

1 V. Berry and R. F. Saraf, *Angew. Chem.*, 2005, **117**, 6826.
2 M. T. Stephan and D. J. Irvine, *Nano Today*, 2011, **6**, 309.
3 X. Y. Yang, G. Tian, N. Jiang and B. L. Su, *Energ. Environ. Sci.*, 2012, **5**, 5540.
4 B. Wang, P. Liu and R. K. Tang, *Bioessays*, 2010, **32**, 698.
5 S. H. Yang, K.-B. Lee, B. Kong, J.-H. Kim, H.-S. Kim and I. S. Choi, *Angew. Chem. Int. Ed.*, 2009, **48**, 9160.
6 W. Xiong, Z. Yang, H. Zhai, G. Wang, X. Xu, W. Ma and R. Tang, *Chem. Commun.*, 2013, **49**, 7525.
7 R. Kempaiah, A. Chung and V. Maheshwari, *ACS Nano*, 2011, **5**, 6025.
8 I. Drachuk, M. K. Gupta and V. V. Tsukruk, *Adv. Funct. Mater.*, 2013, **23**, 4437.
9 S. R. Shin, H. Bae, J. M. Cha, J. Y. Mun, Y.-C. Chen, H. Tekin, H. Shin, S. Farshchi, M. R. Dokmeci, S. Tang and A. Khademhosseini, *ACS Nano*, 2012, **6**, 362.
10 S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee and I. S. Choi, *J. Am. Chem. Soc.*, 2011, **133**, 2795.
11 A. Diaspro, D. Silvano, S. Krol, O. Cavalleri and A. Gliozzi, *Langmuir*, 2002, **18**, 5047.
12 I. Drachuk, O. Shchepelina, M. Lisunova, S. Harbaugh, N. Kelley-Loughnane, M. Stone and V. V. Tsukruk, *ACS Nano*, 2012, **6**, 4226.
13 E. H. Ko, Y. Yoon, J. H. Park, S. H. Yang, D. Hong, K.-B. Lee, H. K. Shon, T. G. Lee and I. S. Choi, *Angew. Chem.*, 2013, **125**, 12505.
14 S. A. Konnova, I. R. Sharipova, T. A. Demina, Y. N. Osin, D. R. Yarullina, O. N. Ilinskaya, Y. M. Lvov and R. F. Fakhrullin, *Chem. Comm.*, 2013, **49**, 4208.

15 I. Drachuk, O. Shchepelina, S. Harbaugh, N. Kelley-Loughnane, M. Stone and V. V. Tsukruk, *Small*, 2013, **9**, 3128.
16 A. Matsuzawa, M. Matsusaki and M. Akashi, *Langmuir*, 2013, **29**, 7362.
17 X. Q. Jia, J. P. Wen, Z. P. Sun, Q. Caiyin and S. P. Xie, *Chem. Eng. Sci.*, 2006, **61**, 1987.
18 G. B. Shan, J. M. Xing, H. Y. Zhang and H. Z. Liu, *Appl. Environ. Microbiol.*, 2005, **71**, 4497.
19 X. Y. Yang, Z. -Q. Li, B. Liu, A. Klein-Hofmann, G. Tian, Y.-F. Feng, Y. Ding, D. S. Su and F. -S. Xiao, *Adv. Mater.*, 2006, **18**, 410.
20 H. J. Butt, K. Graf and M. Kappl, *Physics and chemistry of interfaces.* John Wiley & Sons, 2006.
21 B. Heurtault, P.Saulnier, B. Pech, J. E. Proust and J. P. Benoit, *Biomaterials*, 2003, **24**, 4283.
22 L. Reijnders, *J. Hazard. Mater.*, 2008, **152**, 440.
23 M. Martín , F. Carmona , R. Cuesta , D. Rondón, N. Gálvez and J. M. Domínguez-Vera, *Adv. Funct. Mater.*, 2014, **24**, 3489.