Supplementary Information

Candida rugosa lipase covalent immobilization on facile synthesized carbon nitride nanosheets as novel biocatalysts

Ya Li,‡[a] Zhijun Ruan,‡[b] Mingming Zheng,[a] Qianchun Deng,[a] Shan Zhang,[a] Chang Zheng,[a] Hu Tang,[a] Fenghong Huang*[a] and Jie Shi*[a]

[a] *Hubei Key Laboratory of Lipid Chemistry and Nutrition, Oil Crops and Lipids Process Technology National & Local Joint Engineering Laboratory, Key Laboratory of Oilseeds Processing, Ministry of Agriculture, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China.*

[b] *Hubei Key Laboratory of Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China.*

‡These authors equally contributed to this study.

*Author for correspondence: huangfh@oilcrops.cn, shijie@caas.cn,

Table S1. Kinetic parameters for the free CRL and C_3N_4-NS@CRL.

Lipase	K_m (μM)	V_m (U/g)	specific activity (U/mg)
C_3N_4-NS@CRL	23.17 ± 3.59	6329.8 ± 636.4	141.7
free CRL	0.27 ± 0.02	41.15 ± 0.66	0.37

The Michaelis-Menten kinetic parameters of free CRL and C_3N_4-NS@CRL were determined by measuring the lipase activity using *p*-NPP as substrate in the initial concentration range from 0.19 to 19 μ mol/L at the 30 ℃ and 0.1M pH 7.0 PBS. The values of kinetic parameters K_m and V_m were obtained by fitting the experimental data to the Michaelis-Menten model using non-linear regression code.

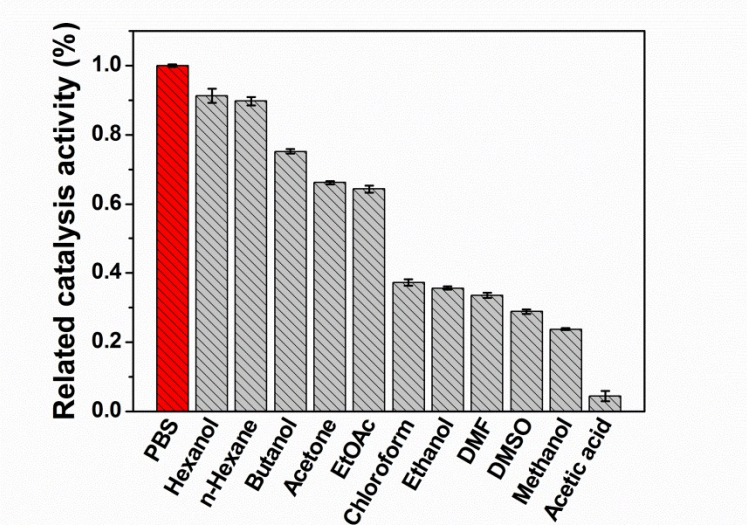

Figure S1 Effect of different organic solvents on relative activity of the C_3N_4-NS@CRL.

The organic solvent tolerant ability of this immobilized CRL was studied by incubating the biocatalyst in 0.1 M pH 7.0 PBS buffer and different organic solvents for 30 min at 37 °C, and collected by refrigerated centrifugation, dried with nitrogen purge, then their residual activities were assayed by the hydrolysis of *p*-NPP. Residual activity was expressed as percentage of the enzyme activity of C_3N_4-NS@CRL under PBS buffer (considered as 100%).

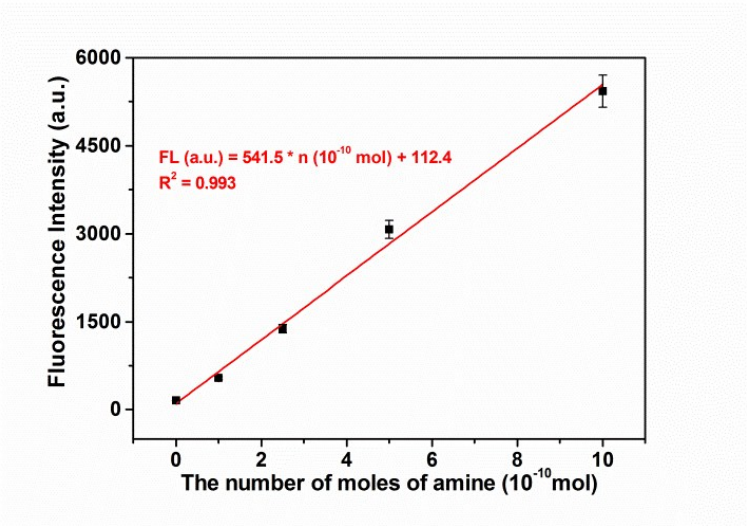

Figure S2 Fluorescence intensity of fluorescamine method plotted as a function of increasing amounts of amino groups of fresh APTES.

Borate buffer (0.1 mol/L, pH 8.0, 190 μL) and 20 μL of APTES solution were mixed. When detecting, 90 μL fluorescamine solution (1 mg/mL in acetonitrile) was added to the mixture. Excitation wavelength was set at 392 nm. For assay of C_3N_4-NS, 10 mg/mL nanoparticles was used as analyte.