Supplementary information (ESI)

Self-assembly amphipathic peptides induce active enzyme aggregation that dramatically increases the operational stability of nitrilase

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Table S1. Residual nitrilase activity of the purified Nit and Nit-SEA after cross-linked by different reagents.

	Glutaraldehyde (4%)	poly-Ethyleneimine (5%)	Dextran polyaldehyde (5%)
Nit	0	0	8.2
Nit-SEA	0	9.6	29.4

Table S2 Half-lives of nitrilase of different forms (Unit: hours).

Temperature	Nit	Nit-i	Nit-SEA	Nit-iSEA
45 °C	23.8	58.7	161.0	237.4
50 °C	13.1	32.9	56.3	75.4

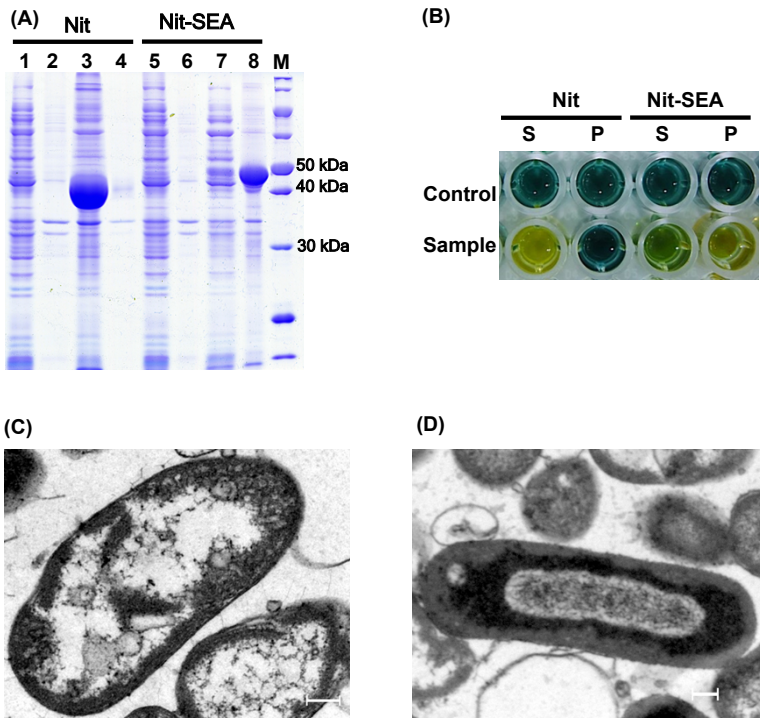

Figure S1. Active inclusion bodies formation in *E. coli* BL21(DE3) cells. (A) SDS-PAGE detection of native nitrilase (Nit) and fusion nitrilase (Nit-SEA) expression induced by 0.1 mM IPTG at 22 °C for 20 h. The supernatant of cell lysate of non-induced Nit (lane 1), IPTG induced Nit (lane 3), non-induced Nit-SEA (lane 5) and IPTG induced Nit-SEA (lane 7); and the pellets of cell lysates of non-induced Nit (lane 2), IPTG induced Nit (lane 4), non-induced Nit-SEA (lane 6) and IPTG induced Nit-SEA (lane 8); Lane M, protein molecular weight marker, Fermentas, Cat# 26630. (B) Fluorometric enzyme activity assay of the native nitrilase and the fusion nitrilase-18A using 30 mM mandelonitrile as substrate. S, supernatant fraction; P, precipitation fraction; control, recombinant cells were not induced by IPTG; sample, recombinant cells were induced by 0.1 mM IPTG at 22 °C for 22 h. Colors changed after 3 h incubation of cell lysis with the substrate in 96-well plates at 45 °C. (C) and (D) morphology of the thin-sectioned cells observed under transmission electron microscope (TEM). (C) Native nitrilase; (D) fusion nitrilase. Scale bars: 200 nm.

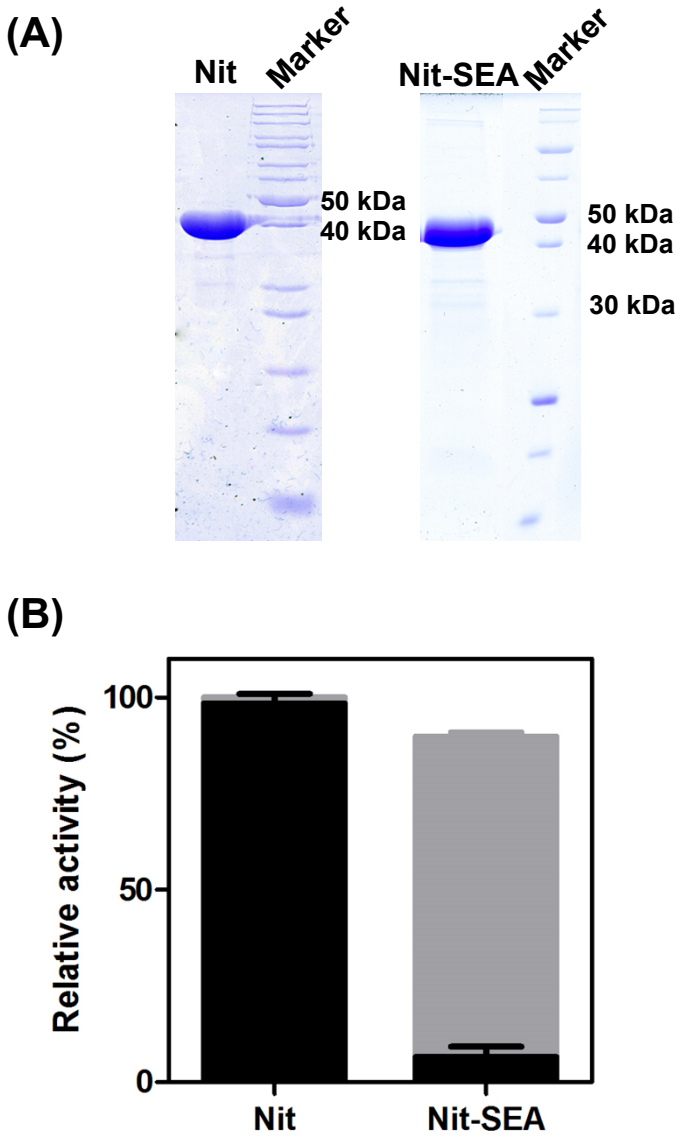

Figure S2. (A) SDS-PAGE analysis of the purified native nitrilase (Nit) and the purified fusion form (Nit-SEA). (B) Distributions of nitrilase activities in the supernatant (■) and precipitation (■) fractions of cell lysates. The activities calculated and normalized to the total activities of the native nitrilase. All enzymes were extracted from the same amount of cells (OD_{600}) and performed in triplicate.

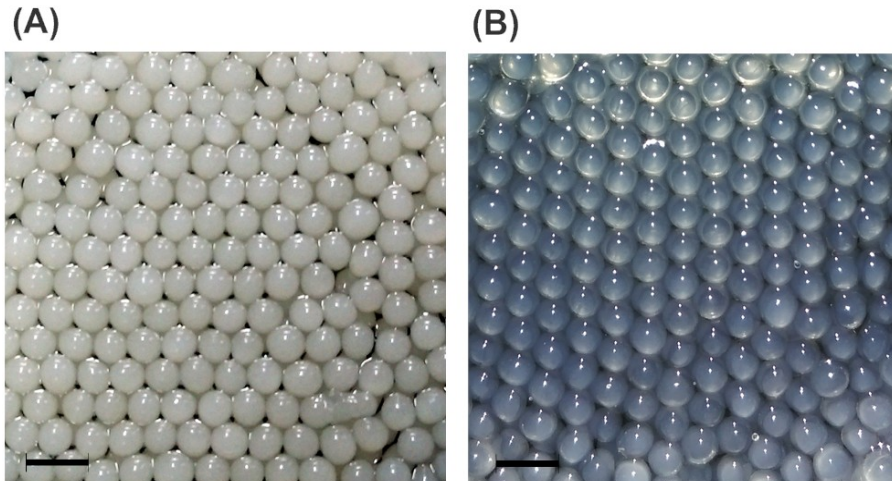

Figure S3. (A) Nit-iSEA particles and and (B) Nit-i particles. Scale bars: 2 mm.