Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

The mechanism of catalase loading into porous vaterite $CaCO_3$ crystals by co-synthesis

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Supporting information

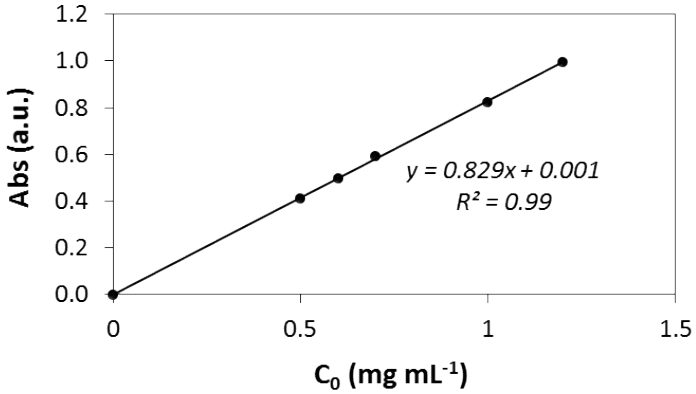

Figure S1. Calibration curve used for the determination of the concentration of catalase by absorbance at 280 nm.

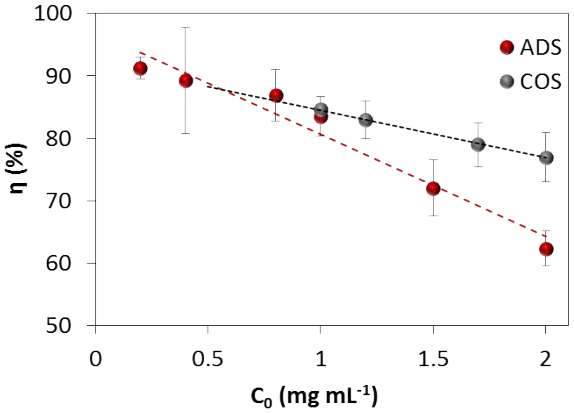

Figure S2. Concentration dependencies of the efficiency of catalase loading (η) by ADS and COS methods. To determine the efficiency of protein loading, the amount of protein incorporated into the crystals has been attributed to the initial amount of protein. Error bars are SD for n=3.

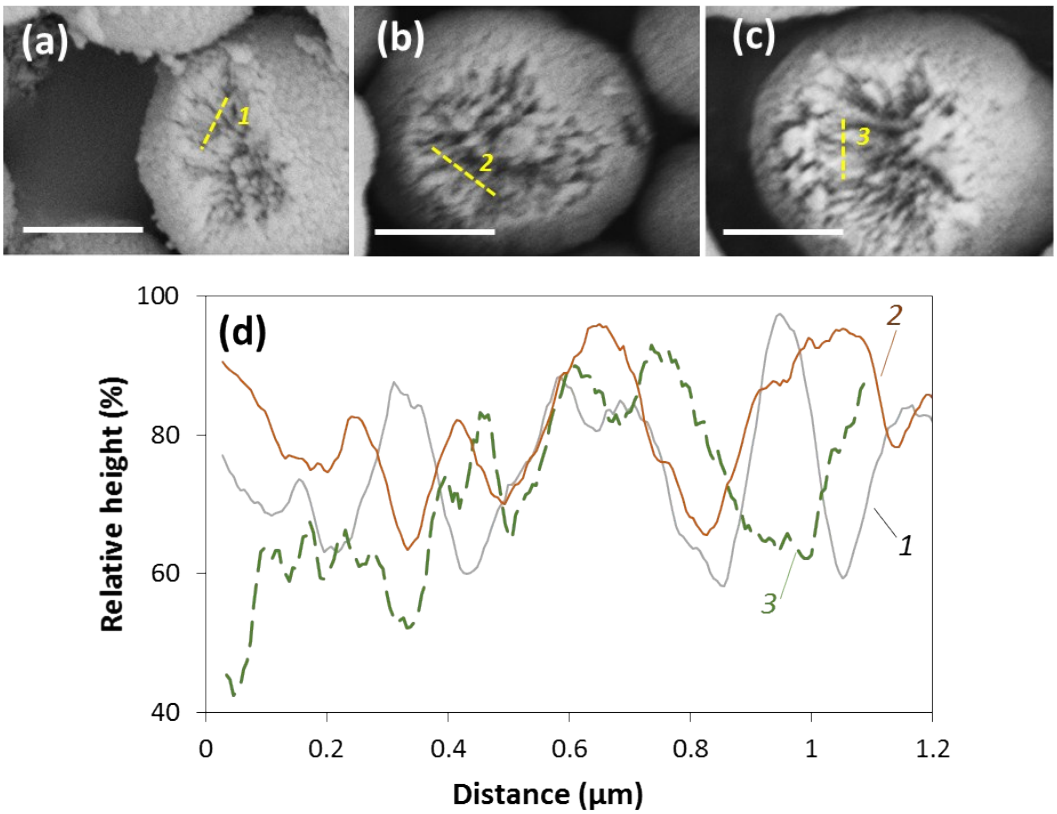

Figure S3. SEM images of $CaCO_3$ crystal prepared (a) in water, (b) in 0.05M glycine buffer pH 9.0 and (c) in 0.05M glycine buffer pH 9.0 in the presence of 2.0 mg ml^{-1} catalase. Scale bars are 2 µm. (d) Profiles taken along yellow lines 1-3 in (a)-(c), respectively and presented as relative height based on grey values.

Parameter	Value
BET slope	316.0
BET intercept	1.83
Coefficient of correlation	0.9967
BET constant (C)	18.3
BET surface area, $m^2\ g^{-1}$	10.4±0.3
Ave pore diameter, nm	5-25

Table S1. BET analysis of $CaCO_3$ crystals prepared in water at 22°C. Crystal size was found to be 5±1 µm.

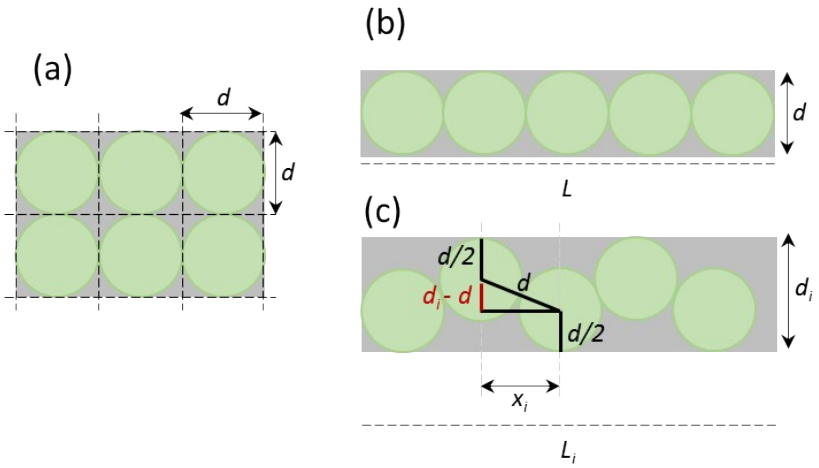

Figure S4. The schematic illustration for the derivation of the models A-C: projections of $CaCO_3$ surface area (grey) covered with catalase molecules (green) in 2D (top view for (a) and side view for (b) and (c)). Description is provided below.

Model A

We assume that catalase molecules (m_{Cat} $4.15 \cdot 10^{-19}$ g) cover the available surface of $CaCO_3$ (S_{CaCO_3} 10.4 $m^2\ g^{-1}$) as a homogeneous compact layer, each catalase molecule occupies an area of $1.10 \cdot 10^{-16}$ m^2 (the square with the side d 10.5 nm, Figure S2a). The number of catalase molecules adsorbed to 1 g of $CaCO_3$ (N_{Cat}):

$$N_{Cat} = {S_{CaCO_3}}/{d^2}$$

Maximum adsorption capacity:

$$q_{max} = N_{Cat} m_{Cat} = m_{Cat} \times {S_{CaCO_3}}/{d^2}$$

Model B

We assume that all pores of $CaCO_3$ crystal are of cylindrical shape and may host one layer of catalase (i.e. the diameter of the cylinder equals to the diameter of catalase molecule, Fig. S2b). In this case, the number of catalase molecules stored in the pores of the crystal is the number of spheres fitted into the cylinder with the length (L):

$$S_{CaCO_3} = S_{cylinder}^{lateral} = \pi d L$$

$$L = S_{CaCO_3}/\pi d$$

$$N_{Cat} = {L}/{d} = {S_{CaCO_3}}/{\pi d^2}$$

$$q_{max} = N_{Cat} m_{Cat} = m_{Cat} \times {S_{CaCO_3}}/{\pi d^2}$$

Model C

We assume that all pores of $CaCO_3$ crystal are of cylindrical shape and the diameters of the pores can be evaluated from the experimental distribution of differential pore volumes (Fig. 3).

For this, the experimental distribution was fitted with normal and lognormal functions as follows:

$$Y = a + (b - a) \cdot \exp\left(-\frac{(X - c)^2}{2d^2}\right)$$

where Y is the differential pore volume, X is the diameter of the pore or $\ln d$ for normal and lognormal functions, respectively. *a*, *b*, *c* and *d* are the fitted parameters.

The coefficients of determination (R^2) for normal and lognormal functions were found to be 0.935 and 0.955, respectively. Lognormal distribution was used for further calculations.

Based on the experimental distribution fitted with lognormal function (Fig. 3), the pores with the diameter less than 10.5 nm represent about 37% (by volume) from all the pores. These pores are assumed to be empty because $d_{Cat} > d_{pore}$.

The pores with the diameter in a range from 10.5 to 20.5 nm can host catalase molecules closely packed into the cylindrical pores (Fig. S2c). These pores represent a fraction of about 59% of all pores by volume.

To estimate a number of molecules stored in a pore with diameter d_i, one needs to know its length L_i (Fig. S2c). It can be calculated from the known pore volume:

$$V_i = V_{cylinder} = \frac{\pi d_i^2}{4} \cdot L_i$$

$$L_i = \frac{4V_i}{\pi d_i^2}$$

The projection of the distance between two centers of the spheres (x_i) can be calculated as follows (Fig. S2c):

$$x_i = \sqrt{d^2 - (d_i - d)^2}$$

Finally, the number of catalase molecules in the pores with diameter d_i in 1 g of $CaCO_3$ crystals:

$$N_i = {L_i}/{x_i} = \frac{4V_i}{\pi d_i^2 \sqrt{d_i^2 - 2d \cdot d_i}}$$

The maximum adsorption capacity

$$q_{max} = \sum_i N_i \times m_{Cat}$$

The pores with diameter more than 21 nm represent less than 5% of all the pores and their impact to the adsorption capacity was neglected.

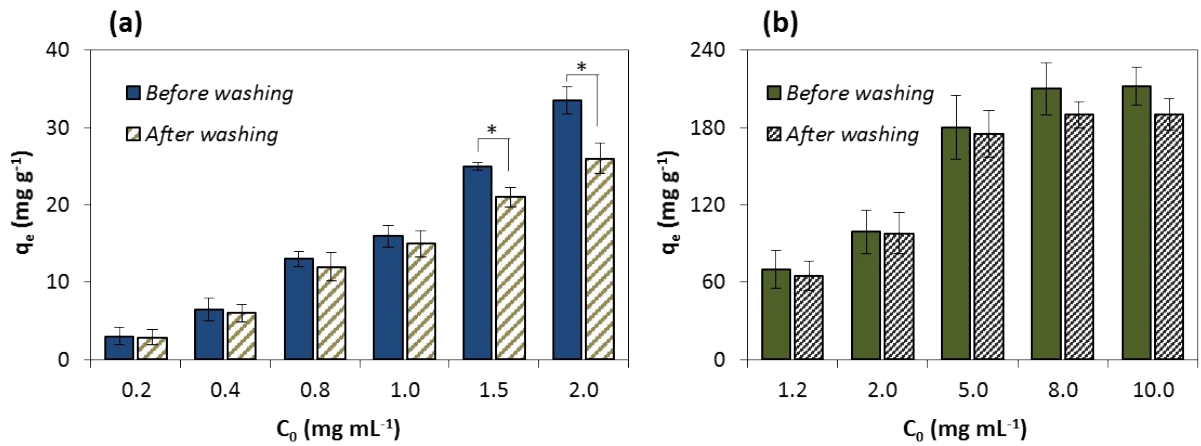

Figure S5. Effect of washing with 0.05 M glycine buffer pH 9.0 on adsorbed amount of catalase (q_e) at different initial concentrations (c_0) used for catalase loading by means of **(a)** adsorption and **(b)** co-synthesis. Values are means ± SD, n=3; *significance level $p<0.01$ versus adsorbed amount of catalase before washing.