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Artificial plant cell walls as multi-catalyst systems for enzymatic cooperative asymmetric catalysis in non-aqueous media†

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The assembly of cellulose-based artificial plant cell wall (APCW) structures that contain different types of catalysts is a powerful strategy for the development of cascade reactions. Here we disclose an APCW catalytic system containing a lipase enzyme and nanopalladium particles that transform a racemic amine into the corresponding enantiomerically pure amide in high yield *via* a dynamic kinetic resolution.

The main structural building block of the plant cell wall is cellulose,[1,2] which forms interwoven microfibril networks that are embedded into a polysaccharide and protein matrix to constitute a multilayer architecture (Fig. S1, ESI†).

Advances in biocatalysis are a requirement for addressing the increasing need for sustainable chemistry in the preparation of materials, fine-chemicals and pharmaceutical synthesis.[3] The ability of biocatalysts to operate under unnatural conditions (*e.g.* in non-aqueous media and at high temperatures),[4–6] and to catalyze non-natural chemical reactions are important factors for future advancements of the "enzyme universe".[7] To date, the primary strategy for obtaining biocatalyst stability in non-aqueous media is through immobilization within, or on the surface of, solid supports such as silica or fossil-based polymer resins.[8] However, often increased stability leads to decrease in biocatalyst activity. Thus, there is a significant incentive for developing new (bio)technologies for the advancement of non-aqueous industrial biocatalysis. The development of homogeneous and heterogeneous multi-catalytic systems for cooperatively advancing chemical transformations by combining different types of catalysts (*e.g.* metal, enzyme or metal-free)[9] is another elegant way of expanding chemical synthesis and biocatalysis.

The construction of protein-derived artificial metalloenzymes for application in chemical synthesis in non-aqueous media is an important area of research that has attracted considerable attention.[10–21] Here the state-of-the-art involves directed evolution and/or introducing a transition metal into the enzyme. In the latter approach, the metal of a heme enzyme may be replaced by a transition metal[16–18] or a transition metal may be introduced into the active site of the enzyme.[10a,19,20] The development of heterogeneous artificial metalloenzyme systems based on dual enzyme/metal nanocatalysis was also recently disclosed.[21] However, partial deactivation of the enzyme component led to a limited recycling of the artificial metalloenzyme. This can also be the case for other types of heterogeneous enzyme–metal nanohybrid catalyst systems.[22–24]

To address this problem, we have focused on a multidisciplinary approach for the advancement of non-aqueous biocatalysis,[8,25] which is based on a synergistically combined platform of cellulose chemistry, nanocatalysis, and heterogeneous catalysis. Based on our research on cooperative heterogeneous catalysis[21,22,26–28] and construction/application of functional cellulose-based materials,[29,30] we envisioned a novel approach for setting up nature-inspired catalyst systems that are guided by the intriguing assembly of the primary cell wall[1,31] of plants. Specifically, self-assembled mixtures of cellulose, polysaccharides, enzymes, surfactants and metals can create an artificial plant cell wall (APCW)-like structure with an embedded multi catalyst system (**1**), which next could catalyze the stereoselective formation of product **D** from substrates **A** + **B** + **C** in non-aqueous media (Fig. 1). Herein, we disclose this concept by creation of an assembled PCW mimic, which is employed as a heterogeneous catalyst and a "deracemase" system for converting racemic mixtures of amines **2** to the corresponding chiral amides (*R*)-**3** by dynamic kinetic resolution (DKR).[32,33]

We began our search for catalytically active and synthetically useful APCW catalyst systems by a thorough study of the kinetic resolution of racemic amine **2a** using *Candida antarctica* Lipase B (CALB) as the enzyme of choice (Table S1 and Fig. S2, ESI†).

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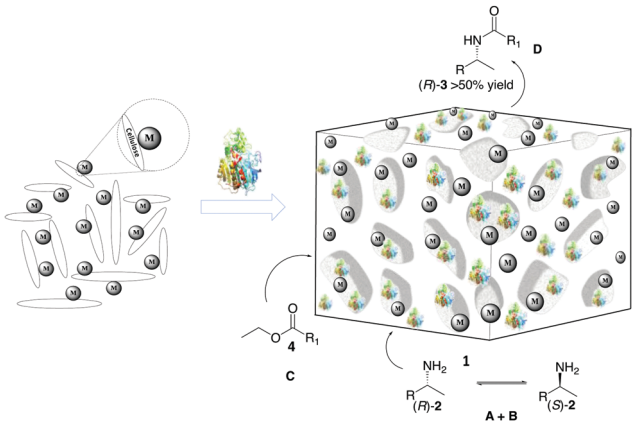

Fig. 1 Simplified scheme of self-assembly of components to create an artificial primary cell wall **1**, which simultaneously catalyzes racemization and amidation of racemic amines **2**. M = metal.

The assembly of various APCWs containing this serine hydrolase revealed a significant enhancement in activity of the enzyme by the different self-assembled polysaccharide-based systems. In fact, CALB is nearly inactive without some type of activation. We also investigated the role of all components used when the APCW contained microcrystalline cellulose (MCC) as the component. The use of cellulose as a support material led to a significant activation as compared to just the use of CALB. Thus, the cellulose immobilization improved the performance of the enzyme in the organic solvent. This is a beneficial effect of immobilization.[34] The use of buffer[35] within the assembled network was also beneficial but is not necessary. Modifying CALB with a surfactant increased the rate of amidation, but this catalyst system cannot be recycled and used for multiple reactions.[36–40] The best self-assembly combination turned out to be non-covalent modification of CALB with surfactant polyethylene glycol hexadecyl ether (Brij) in phosphate buffer together with MCC forming APCW system 3 (**APCW3**).[41] We also successfully created assembled APCWs containing nanofibrillated cellulose (NFC, **APCW5**), chitosan (**APCW6**) and nanocrystalline cellulose (CNC, **APCW7**), respectively. However, in the latter cases (**APCW5-7**) the *E*-value (enantiomeric ratio)[42] for **2a** was lower than that obtained when MCC was used as the polysaccharide component. We next decided to further explore APCWs containing aminopropylsilane modified MCC (MCC-Amp) and Pd(0) nanoparticles (MCC-Amp-Pd(0)-NPs) for the kinetic resolution of rac-**2a**. Thus, self-assembled **APCW8** and **APCW9** (Fig. 2a) were evaluated as heterogeneous catalysts. The AmP surface modification as well as the presence of Pd(0)-NPs did not decrease the excellent enantioselectivity of the enzyme ($E > 400$). We also prepared and evaluated a hybrid catalyst containing CALB/Brij/Pd(0)/buffer salts. This catalyst did not exhibit good selectivity ($E = 21$). Thus, the presence of the MCC component is essential for reaching a high *E*-value under the evaluated reaction conditions. With these results in hand, the novel **APCW9** structure was chosen and further investigated as the nature-inspired multi-catalyst system. The assembled **APCW9** multi-catalyst structure (Fig. 2a) had a narrow distribution of Pd

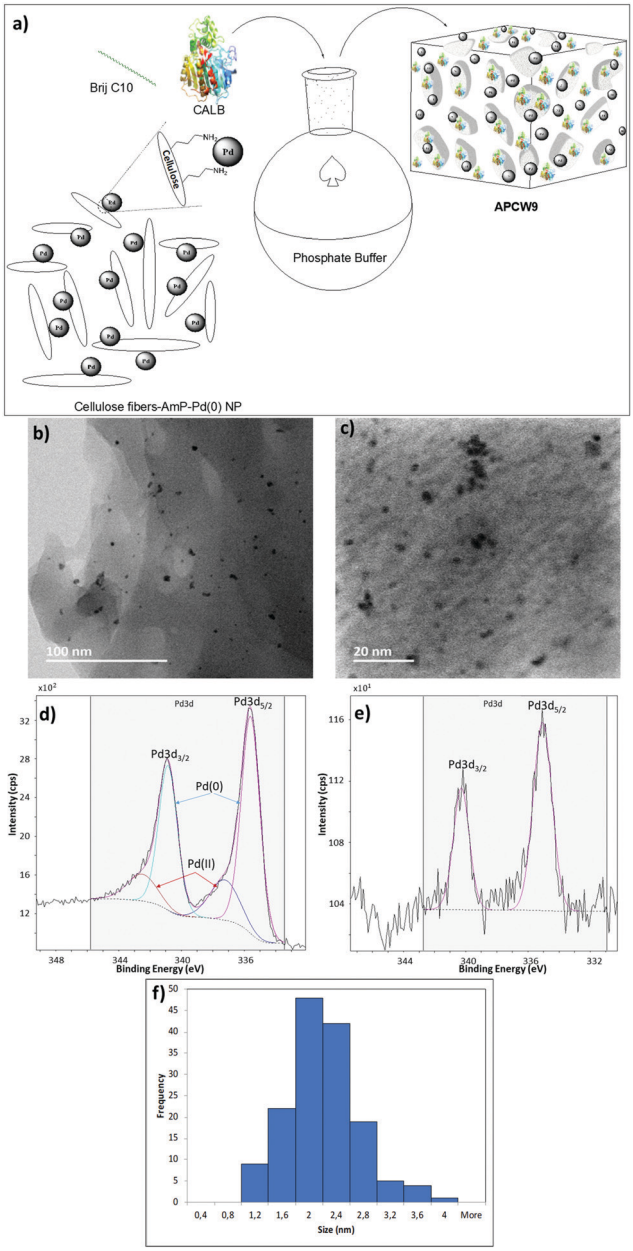

Fig. 2 (a) Fabrication of **APCW9**. STEM images of **APCW9**, (b) scale bar 100 nm and (c) scale bar 20 nm. XPS spectra of Pd 3d5/2 for (d) MCC-Amp-Pd^0 and (e) **APCW9** (that had been stirred for 24 h in toluene, at 90 °C, under H_2 atmosphere). (f) Size distribution of the Pd nanoparticles of **APCW9**.

nanoparticles (main distribution of 1.6 to 2.8 nm in size, Fig. 2f) as confirmed by TEM analyses (Fig. 2b and c). The distribution between Pd(0) and Pd(II) in the Pd NPs of **APCW9** was difficult to determine due to the presence of the salts of the phosphate buffer; however, it was similar to that of its MCC-AmP-Pd(0) component as determined by XPS (Fig. 2d and e). Thus, we had accomplished the assembly of an APCW structure containing Pd-nanoparticles and enzyme units as well-defined catalysts. Next, the APCW-catalyzed deracemization of amine **2a** was investigated (Table 1). We found that **APCW9** is an excellent catalyst system, which is able to catalyze the formation of amide **3a** under DKR

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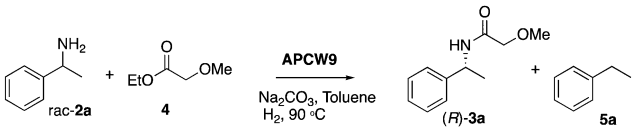
Table 1 Screening of APCW-catalyzed dynamic kinetic resolution[a]



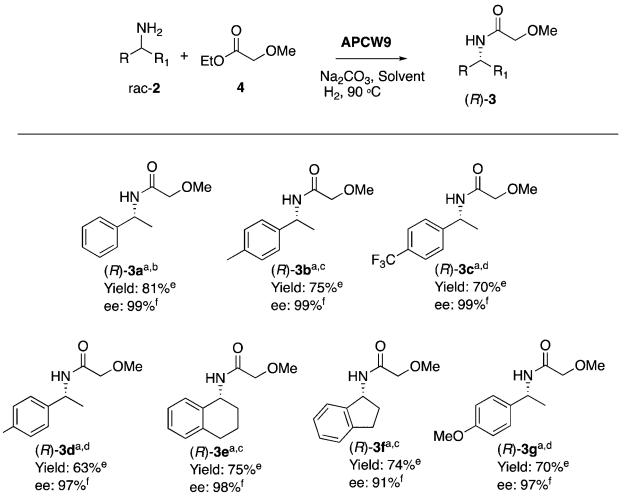
Entry	Solvent	Time [h]	3a : 5a : 2a[b]	3a Yield [%][c]	Ee 3a [%][d]
1	Toluene	23	87 : 13 : 0	81	99
2[e]	Toluene	23	67 : 33 : 0	67	48
3[f]	Toluene	23	72 : 28 : 0	58	63
4	1,4-Dioxane	70	70 : 21 : 9	70	99
5[g]	1,4-Dioxane	24	46 : 54 : 0	44	99
6	3-methyl3-pentanol	70	63 : 23 : 7	62	99
7[h]	Toluene	22	46 : 54 : 0	49	84
8[h]	1,4-Dioxane	69	32 : 56 : 12	33	99
9[i]	Toluene	23	0 : 0 : 100	ND	ND
10[j]	Toluene	44	74 : 26 : 0	70	99
11[k]	Toluene	23	76 : 24 : 0	77	99
12[l]	Toluene	117	65 : 30 : 5	64	99
13[m]	Toluene	22	52 : 48 : 0	54	57
14[n]	Toluene	27	50 : 0 : 50	46	99

[a] Reaction conditions: **2a** (0.25 mmol, 1 equiv.), **4** (2.5 mmol, 10 equiv.), **APCW9** (MCC-Amp-Pd^0/CALB/Brij/buffer) (9 mg, 1.1 mol% Pd), Na_2CO_3 (0.75 mmol, 3 equiv.), toluene (1 mL), 90 °C, H_2. [b] Determined by ^{1}H NMR. [c] Isolated yield. [d] Determined by chiral HPLC. [e] Molecular sieves 4 Å (125 mg) were used. [f] Reaction performed at 70 °C. [g] **APCW9** (18 mg, 2.2 mol% Pd) was used. [h] **APCW10** (CNC-Amp-Pd^0/CALB/Brij/buffer) (9 mg, 1.5 mol% Pd). [i] No catalyst used. ND stands for not determined. [j] **APCW9** (18 mg, 2.2 mol% Pd) was stirred with Na_2CO_3 under H_2 at 90 °C for 23 hours and next **2a** and **4** were added. [k] MCC-Amp-Pd^0 (16.5 mg, 12 mol% Pd) and **APCW3** (MCC/CALB/Brij/buffer) (9 mg) were used as catalysts. [l] CALB/Brij/Pd^0/buffer (9 mg) was used as a catalyst. [m] **APCW11** (MCC-Amp-Pd^{II}/CALB/Brij/buffer) was used as a catalyst. [n] **APCW3** was used as a catalyst.

conditions in several solvents (Table 1). The deracemization (DKR process) involves a synergistic interplay between Pd(0)-catalyzed racemization and CALB-catalyzed enantioselective amidation of **2a** within the cellulose network of **APCW9**.[43] Another competing catalytic side-reaction that the APCW can catalyze, is deamination of **2a** to give the corresponding ethylbenzene (**5a**). It is noteworthy that the integrated heterogeneous **APCW9** catalyst system is more efficient and chemoselective than when using mixed separate Pd-NPs on MCC and CALB on MCC or when using a hybrid-catalyst made of CALB, Brij, Pd(0)-NP and buffer (Table 1, entries 1, 11 and 12).

In addition, performing the reaction with **APCW11**, which contains Pd(II), gave a low ee (entry 13). The reaction with **APCW3**, which do not contain Pd, led to a kinetic resolution (**3a**, 46% yield, entry 14). Moreover, the Pd-nanoparticle catalysts are not able to racemize the product (*R*)-**3a** just the amine **2a** (see ESI†). The heterogeneous **APCW9** multi-catalyst system catalyzed the asymmetric synthesis of a variety of amides **3**, which were isolated in good to high yields with 91–99% enantiomeric excess (Table 2). We further investigated the sustainable APCW multi-catalyst systems by probing their recyclability. The recycling studies revealed that the APCW catalytic systems, which contained a hydrolytic enzyme can be recycled multiple times when catalyzing the KR of amine **2a** maintaining their high enantioselectivity in the organic solvent.

Table 2 **APCW9**-catalyzed deracemization of chiral amines **2**[a]



[a] Reaction conditions: **2** (0.25 mmol, 1 equiv.), **4** (2.5 mmol, 10 equiv.), **APCW9** (9 mg, 1.1 mol% Pd), Na_2CO_3 (0.75 mmol, 3 equiv.), solvent (1 mL), 90 °C, H_2 balloon. [b] Reaction performed in toluene for 23 hours. [c] Reaction performed in 1,4-dioxane for 69 hours. [d] Reaction performed in 3-methyl 3-pentanol for 69 hours. [e] Isolated yield. [f] Determined by chiral HPLC.

For example, the **APCW3** system catalyzed the amidation of **2a** in high enantioselectivity and the corresponding amide (*R*)-**3a** was isolated in 48% yield (50% is the maximum theoretical yield) in >99% ee even after 9 recycles (Fig. S2a, ESI†). It is remarkable that the sustainable **APCW9** multi-catalyst system can catalyze the DKR of **2a** over several cycles (Fig. S2b, ESI†),[44] since most of the previous recycling studies of artificial "deracemases" and hybrid enzyme catalyst systems containing CALB displayed a limited recycling due to significant deactivation of the enzyme catalyst.[21] Thus, the disclosed artificial plant-cell wall concept enables several important features of accomplishing effective biocatalysis in non-aqueous media such as maintaining activity and stability in organic solvents, high stereoselectivity, recyclability, asymmetric synthesis, high temperature tolerance (90 °C), and efficient cooperation with chemical catalysts. No leaching of the metal catalyst was observed during the recycling studies.

We have demonstrated that sustainable artificial plant cell-wall (APCW) structures, which contain a multi-catalytic system of both enzyme and metal nanoparticle catalysts, can be used to accomplish asymmetric catalysis and cascade synthesis in non-aqueous media. A key feature of the disclosed APCW concept is its intrinsic ability to activate the enzyme protein for catalysis in organic solvents. The heterogeneous polysaccharide-based APCW network having both enzyme and metal nanoparticles in its structure, catalyzed DKR of primary amines by cooperative catalysis. Thus, the assembled sustainable APCW structure acts as a "deracemase". It is also possible to reuse the bioinspired APCW structures in multiple-reaction cycles. In comparison, previous attempts of constructing artificial "deracemases" using silica-based or cross-linked enzyme aggregate systems had limitations in their recycling due to deactivation of the enzyme

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component. The concept of introducing catalysts with different functions within an artificial primary cell-wall structure holds great promise in creating other types of APCW catalysts with novel reactivity. Thus, various enzymes and chemical catalysts can be combined within nature-inspired plant cell wall structures to create heterogeneous catalysts for cooperative chemoenzymatic cascade reactions.

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Conflicts of interest

There are no conflicts to declare.

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