SUPPORTING INFORMATION

Asymmetric synthesis of (R)-cyanohydrins using enzymes entrapped in lens-shaped gels

by

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Supporting Information to Scheme 1:
Preparation of (*R*)-oxynitrilase-containing, lens-shaped PVAL-hydrogels

The cross-linking step of the (*R*)-oxynitrilase was carried out using chitosan and glutardialdehyde. At first 1.5 g of chitosan are dissolved in 98.5 g acetic acid solution (0.5%), and 1 M NaOH are added until a pH 5.5 has been obtained. Subsequently 7.89 g of a non-purified oxynitrilase solution (60 mg protein; purchased from ASA Spezialenzyme GmbH, Braunschweig; specific activity: 13.6 U/mg proteine; amount of proteine: 7.6 mg/mL) are added to 4 g of the chitosan solution. The resulting mixture was treated with 200 µL of a glutardialdehyde solution (50%; pH 5.5). After stirring for 16h at 4 °C the crosslinked (*R*)-oxynitrilase (after centrifugation) is entrapped in LentiKat® by addition of 2.07 g of the chitosan/glutardialdehyde-crosslinked enzyme solution and 7.9 g of water to 74 g of LentiKat®Liquid (a polyvinylalcohol-containing aqueous solution which is commercially available from geniaLab®, see also reference [8]). The lens-shaped gels are obtained after dropping the polymeric suspension on a plate using a LentiKat®-printer. For further details for the step of preparation of the lenses, see references [7] and [8] in the article. The entrapped (*R*)-oxynitrilases have been on obtained as highly elastic, lense-shaped gels with a defined diameter of 3 to 5 mm. The activities of the lenses have been varied between 8 and 40 U per g lenses.

Supporting Information to Scheme 2:
Recycling experiments (in total 21 reactions) using (*R*)-oxynitrilases entrapped in PVAL-hydrogels as biocatalysts

CAUTION:
HCN IS VERY TOXIC AND MUST BE HANDLED WITH HIGH CAUTION. Safety instructions are given in the material safety data sheet (MSDS) of HCN. For further safety

information, see the international chemical safety card of HCN (ICSC0492) which is available from the internet: http://www.cdc.gov/niosh/ipcsneng/neng0492.html).

First reaction (1) of this recycling experiment:

To a mixture of 12 mL of a 50 mM citrate buffer (pH 4,5) and 11,0 g of cross-linked and PVAL-hydrogel entrapped (*R*)-oxynitrilases (40 U/g; hydrogels prepared as lens-shaped LentiKats) are subsequently added an organic solvent mixture consisting of 4,8 mL of methyl-tert.-butylether and 7,2 mL of *n*-hexane, 318 mg of freshly distilled benzaldehyde (3 mmol), and 1,4 g of an aqueous HCN solution (20%; 3,5 equiv.). The reaction mixture is stirred for 2 h at room temperature. After addition of 10 mL of MTBE the organic layer is separated, and the aqueous phase is washed with 2 x 15 mL of MTBE (subsequently, the aqueous layer is treated with NaOCl solution in order to decompose HCN which has been used in excess amount). The collected organic phases are dried over sodium sulfate, and the volatile components are evaporated in vacuum. The desired product (*R*)-mandelonitril is obtained in 74% yield and with an enantioselectivity of 91% ee.

Subsequent recycling reactions (2)-(21) of this recycling experiment:

The buffer solution and the LentiKats are re-used in the next reaction cycle. Thus, in the reaction (2) – (21), to the buffer solution with the lense-shaped LentiKats (containing the oxynitrilase) which were recycled from the previous experiment are added an organic solvent mixture which consists of 4.8 mL of methyl-tert.-butylether and 7.2 mL of *n*-hexane, 318 mg of a freshly distilled benzaldehyde (3 mmol), and 1.4 g of an aqueous HCN solution (20%; 3,5 equiv.). The reaction mixture is stirred for 2 h at room temperature. After addition of 10 mL of MTBE the organic layer is separated, and the aqueous phase is washed with 2 x 15 mL of MTBE (subsequently, the aqueous layer is treated with NaOCl solution in order to decompose HCN which has been used in excess amount). The collected organic phases are dried over sodium sulfate, and the volatile components are evaporated in vacuum. The resulting product (*R*)-mandelonitril is obtained in the yields and with the enantioselectivities described in Table S.I.1 below.

Table S.I.1: Yields and enantioselectivities in the recycling experiments

reaction-no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Yield (%)	74	76	80	81	58	81	80	73	88
ee (%)	91	91	90	93	92	92	91	90	92

reaction-no.	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Yield (%)	78	87	83	72	81	83	86	73	87
ee (%)	93	93	93	93	94	93	94	95	94
reaction-no.	**19**	**20**	**21**						
Yield (%)	86	85	84						
ee (%)	94	94	95						

Supporting information to Scheme 3:

Investigation about a leaching of the (*R*)-oxynitrilases entrapped in LentiKat-hydrogels in the dehydrocyanation reaction of *rac*-mandelonitril (at pH 3.75):

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To 100 mL of a 50 mM citrate buffer are added 0.6 g of (*R*)-oxynitrilases entrapped in LentiKat-hydrogels (8,16 U/g). After stirring this mixture for 143 h the LentiKat-hydrogels are separated, and the separated LentiKat-hydrogels as well as the resulting supernatant are investigated with respect to their enzymatic activity (results see in Tables S.I.,2b and S.I.,2c, respectively). For a comparison, a freshly prepared sample of (*R*)-oxynitrilases entrapped in LentiKat-hydrogels is also investigated with respect to its enzymatic activity (results see in Table S.I.,2a).

The investigation of the enzymatic activity is carried out as follows (exemplified by an experiment using LentiKats): To 100 mL of a solution of *rac*-mandelonitrile (1 mmol/L) in citrate buffer (50 mM; pH 3.75) are added 0,6 g of (*R*)-oxynitrilases entrapped in LentiKat-hydrogels. Subsequently the reaction course is observed at a reaction temperature of 20 °C. The cleavage of the mandelonitrile is determined according to the increase of the concentration of benzaldehyde (extinction E) which was detected with a photometer (at 250 nm).

Tab. S.I.2a: Extinction of the freshly prepared LentiKats with oxynitrilases

Time (min.)	0	1	2	4	6	8	10	12	14
Extinction	.22	.243	.254	.265	.274	.291	.302	.317	.313
Time (min.)	16	18	20	30	40	50	61	72	80
Extinction	.356	.332	.346	.386	.456	.501	.559	.578	.603
Time (min.)	90	104	110	120	135	150	165	180	240
Extinction	.641	.651	.665	.687	.704	.737	.748	.769	.771

Tab. S.I.2b: Extinction of the LentiKats with oxynitrilases after stirring for 143 h

Time (min.)	0	1	2	5	10	15	20	31	45
Extinction	.205	.191	.176	.190	.220	.242	.262	.302	.356
Time (min.)	60	75	90	105	120	150	180	210	241
Extinction	.402	.446	.490	.536	.559	.660	.682	.686	.752
Time (min.)	265	424							
Extinction	.750	.761							

Tab. S.I.2c: Extinction of the supernatant after stirring for 143 h

Time (min.)	0	1	2	4	6	8	10	20	30
Extinction	.249	.227	.229	.206	.202	.202	.207	.204	.219
Time (min.)	40	50	60	90	120	200	245	270	300
Extinction	.211	.210	.229	.239	.230	.232	.237	.217	.207
Time (min.)	332	360	390	420					
Extinction	.225	.213	.231	.235					