Catalyst Design

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Tailoring the Active Site of Chemzymes by Using a Chemogenetic-Optimization Procedure: Towards Substrate-Specific Artificial Hydrogenases Based on the Biotin–Avidin Technology**

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Dedicated to Professor George M. Whitesides

Catalysis offers efficient means to produce enantiopure products. Traditionally, enzymatic and homogeneous catalysis have evolved independently to afford mild, robust, active, and highly selective catalysts.[1,2] Both systems are often considered complementary in terms of substrate and reaction scope, operating conditions, enantioselectivity mechanism, reaction medium, etc. For the optimization of activity and selectivity, directed-evolution methodologies (combined with an efficient selection or screening tool) outperform combinatorial ligand libraries.[3–13] With the hope of alleviating some of the inherent limitations of both enzymatic and organometallic catalysis, two approaches have recently witnessed a revival: 1) organocatalysis[14–19] and 2) artificial metalloenzymes based on either covalent[20,21] or supramolecular anchoring[22] of a catalytic moiety in a macromolecular host.[23–30]

Inspired by the early works of Whitesides and Wilson,[22] we recently reported artificial metalloenzymes based on the biotin–avidin technology.[31–35] Herein, we report our efforts to produce substrate-specific and *S*-selective artificial metalloenzymes based on the biotin–avidin technology for the hydrogenation of α-acetamidodehydroamino acids.

The starting point for the chemogenetic-optimization procedure presented herein is the identification of [Rh(cod)-(biot–**1**)]$^+$⊂S112G Sav (cod = 1,5-cyclooctadiene, biot =

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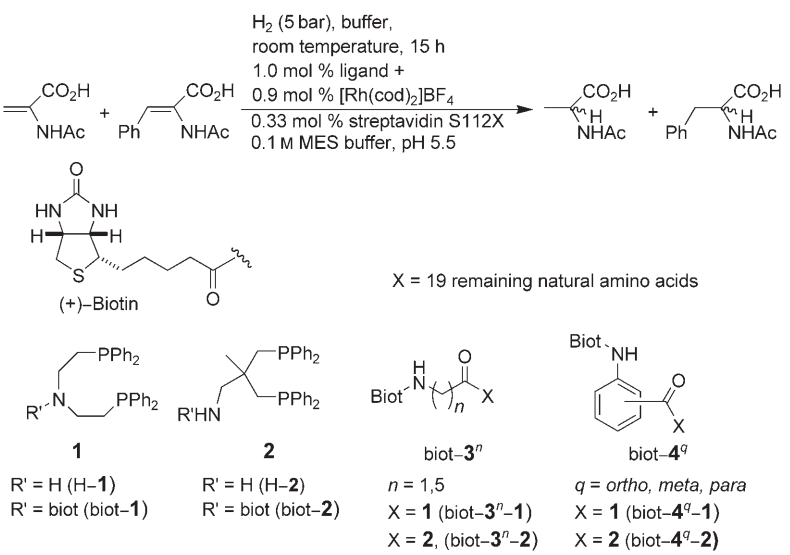
biotin, Sav = streptavidin, Scheme 1) as the best ligand–protein combination for the enantioselective reduction of α-acetamidoacrylic acid (up to 96 % *ee* in favor of (*R*)-acetamidoalanine (*N*-AcAla)).[32,33] These observations prompted us to perform saturation mutagenesis at position 112 of WT-Sav. The resultant 20 proteins were combined with the biotinylated catalyst precursors [Rh(biot-spacer-P_2)cod]BF_4 (P = diphenylphosphine donor; Scheme 1) to afford 360 artificial metalloenzymes. To gain broader insight into the substrate specificity, the catalytic runs were performed on both α-acetamidoacrylic acid and α-acetamidocinnamic acid simultaneously (both 50 equiv, Scheme 1). Control experiments established that the selectivity and conversion are identical to those obtained with a single substrate (i.e. no autoinduction).[6,36] The detailed experimental procedure is provided in the Supporting Information.



Scheme 1. Operating conditions used for the chemogenetic optimization of artificial hydrogenases in the reduction of α-acetamidoacrylic acid and α-acetamidocinnamic acid (50 equiv of each with respect to the ligand). MES buffer solution is composed of 4-morpholineethanesulfonic acid that is pH adjusted with sodium hydroxide.

The results of the chemogenetic-screening experiments with both substrates are summarized in Figure 1 by using a fingerprint display for each substrate–protein–ligand combination.[37] The selectivity is color coded: pink for *S*-selective and green for *R*-selective ligand–protein combinations. The intensity of the color reflects the percentage of conversion.[38] Both substrates are displayed as two hypotenuse-sharing triangles (HyShaTri) for each ligand–protein matrix element. This convenient display allows rapid identification of interesting ligand–protein combinations. Selected results are collated in Table 1 and a summary, including all catalytic runs (as well as multiple reproduction of selected experiments), is provided in the Supporting Information.

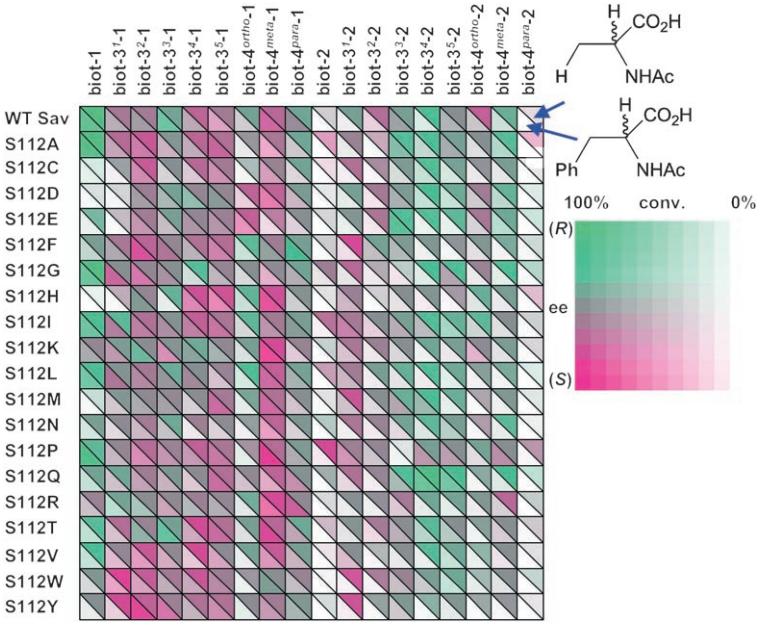


Figure 1. Fingerprint display of the results for the chemogenetic optimization for the reduction of α-acetamidoacrylic acid (top triangle) and α-acetamidocinnamic acid (bottom triangle) in the presence of 18 biotinylated ligands and 20 streptavidin isoforms obtained by saturation mutagenesis at position 112.

Analysis of the results that are displayed graphically in Figure 1 reveals several noteworthy general features:

1) Most ligand–protein combinations yield distinctively different results for both substrates. This trend is reflected by the differences in color (selectivity) and/or intensity (activity) for two HyShaTri (Figure 1). In general, enantioselectivity for *N*-AcPhe was higher than for *N*-AcAla; however, the reverse trend was observed for conversion as the smaller substrates systematically display higher yields.
2) The chemical optimization brings more diversity than the genetic counterpart. This is best illustrated through the comparison of line vectors (i.e. chemical optimization) with column vectors (i.e. genetic optimization). The line vector S112P yields reduction products with both *R* and *S* configurations in respectable *ee* values for *N*-AcPhe (Table 1, entries 5 and 8).
3) Overall, the ligand scaffold **1** outperformed the ligand scaffold **2** both in terms of enantioselectivity and conversion. The best spacer–ligand combinations are biot–**1** (which yields *R* products, Table 1, entries 1–5) and biot–**4**^{meta}–**1**, in combination with cationic amino acid residues in position 112 (which yields *S* products, Table 1, entries 6–9). We speculated that the cationic side chains interact through ionic hydrogen bonds with the carboxylate functionality of the substrate, thus favoring coordination of one of the prochiral faces of the substrate.
4) The most pronounced differences in substrate selectivity (in terms of conversion) are observed with biot–**3**^{1}–**2** in combination with aromatic residues in position 112 (Table 1, entries 10–12).
5) For the biot-**1** vector, the lowest conversions were obtained with mutants that had a potentially coordinating amino acid side chain in position 112 and included S112C (Table 1, entry 3), S112D, S112H, and S112M (to a lesser extent S112E and S112Y as well, Figure 1). We speculated

Table 1: Numerical summary of selected results of the catalytic experiments.

Entry	Ligand	Protein	*ee* *N*-AcPhe[1]	*ee* *N*-AcAla[2]	Conv. *N*-AcPhe	Conv. *N*-AcAla
1	biot–**1**	WT Sav	93(*R*)	94(*R*)	84	quant.[3]
2	biot–**1**	S112A	94(*R*)	93(*R*)	94	quant.[3]
3	biot–**1**	S112C	90(*R*)	76(*R*)	10	19
4	biot–**1**	S112G	94(*R*)	93(*R*)	77	quant.[3]
5	biot–**1**	S112P	87(*R*)	31(*R*)	96	quant.[3]
6	biot–**4**^{meta}–**1**	S112H	81(*S*)	58(*S*)	88	quant.[3]
7	biot–**4**^{meta}–**1**	S112K	88(*S*)	63(*S*)	89	quant.[3]
8	biot–**4**^{meta}–**1**	S112P	78(*S*)	36(*S*)	quant.[3]	quant.[3]
9	biot–**4**^{meta}–**1**	S112R	86(*S*)	63(*S*)	71	quant.[3]
10	biot–**3**^{1}–**2**	S112F	36(*S*)	64(*S*)	20	quant.[3]
11	biot–**3**^{1}–**2**	S112W	33(*S*)	59(*S*)	8	96
12	biot–**3**^{1}–**2**	S112Y	42(*S*)	55(*S*)	10	quant.[3]
13	biot–**3**^{4}–**2**	S112Q	92(*R*)	87(*R*)	77	quant.[3]

[1] *N*-AcPhe = α-acetamidophenylalanine; [2] *N*-AcAla = α-acetamidoalanine; [3] quant. = quantitative conversion. Reproducibility: *ee* ± 1.5% (when *ee* > 60%; up to *ee* ± 5% with low *ee* values) and conversion ± 10%. All listed experiments were performed at least in duplicate.

that these Lewis basic side chains coordinate to the rhodium center, thus interfering with the catalytic cycle.

6) Introduction of a glutamine in position 112 yields *R*-selective artificial metalloenzymes when combined with biot-**3**^{4}-**2** (92% *ee* (*R*), Table 1, entry 13).

This study thus demonstrates the potential of saturation mutagenesis at position 112 coupled with chemical optimization to yield both *R* and *S* reduction products as well as substrate-specific artificial metalloenzymes. Although general trends in enantioselectivity are mostly dictated by the biot–spacer–ligand scaffold (chemical optimization), saturation mutagenesis (genetic optimization) provides the critical second-coordination-sphere interactions between the host protein and the prochiral substrate. It is precisely such crucial weak interactions between a catalyst and its substrate that distinguish enzymatic from homogeneous systems.[41]

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[38] To generate the RGB codes for a catalytic experiment yielding one of the products in *x*% yield, *y*%(*R*), and *z*%(*S*) (100% − *y*% = *z*% (*S*)), the following formulas were imple-

mented in an Excel macro: RGB1 = [100−(*y***x*)/100]*2.55; RGB2 = [100−(*z***x*)/100]*2.55; RGB3 = 0.5*(RGB1+RGB2).

[39] To solubilize both substrates, MES buffer solution (0.1M, pH 5.5) was used. Compared with the buffer solution traditionally used for α-acetamidoacrylic acid (0.1M acetate, pH 4.0), slightly lower conversions (up to −5%) and lower enantioselectivities (up to −3%) were occasionally encountered. For the sake of coherence, all results presented herein are based on the dual substrate screening which must be performed in 0.1M MES (pH 5.5). This is because of the low solubility of α-acetamidocinnamic acid in 0.1M acetate buffer at pH 4.0. The results reported previously for *N*-AcAla (96% *ee* (R) with the biot–**1**–S112G) can be attained only with an 0.1M acetate buffer (pH 4.0).[32,33]

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