Artificial Metalloenzymes for Enantioselective Catalysis

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Chiral Environments





Proteins as Host for Enantioselective Catalysis: Artificial Metalloenzymes?

EFE

Anchoring of the Catalyst: Two Alternatives to Ensure Localization

Covalent Anchoring

Produce a proteín with a síngle accessible cysteine

Link the catalyst via a thioether or a disulfide

Test for enantioselective catalysis Supramolecular Anchoring

Exploit the affinity of Biotin for Avidin

Línk the catalyst vía an amíde to the valeríc acíd síde chain of biotin

Test for enantioselective catalysis

Kaiser et. al. *Science* **1984**, *226*, 505. Distefano et. al. *Chem. Rev.* **2001**, *101*, 3081. Ward et. al. *Helv. Chim. Acta.* **2001**, *84*, 3148. Reetz el. al. *Chimia* **2002**, *56*, 721. de Vries et. al. *Chem. Commun.* **2005**, 5656. Whitesides et. al. J. Am. Chem. Soc. 1978, 100, 306.
Chan et. al. Tetrahedron Asym.1999, 10, 1887.
Thomas et. al. Chem. Soc. Rev. 2005, 34, 337 (Review).
Ward Chem. Eur. J. 2005, 11, 3798 (Concept).
Letondor et. al. ChemBioChem 2006, 7, 1845 (Review).

Biotin-Avidin Technology: Molecular Velcro



Avidin and Streptavidin Binding Sites



Trp 70, Phe 72, Phe 79, Trp 97, Trp 110 Biotin \subset Avidin Trp 79, Trp 92, Trp 108, Trp 120 Biotin ⊂ Streptavidin Chemical Diversity



Typical Hydrogenation Catalyst Precursor



Genetic Diversity

Protein Production

Streptavídín (E. colí, 230 mg/L, soluble ín cytoplasm)

Avidín (P. pastorís, 330 mg/L, ín culture medíum)

Protein Purification
Affinity chromatography on iminobiotin

 Diversity Generation
 Site-directed or randommutagenesis



Zocchi et. al. *Prot. Expr. & Purif.* **2003**, 32, 167. Humbert et. al. *Electrophoresis* **2005**, 26, 47. Humbert et. al. *Methods in Molecular Biology, Vol 418: Biotin-Avidin Interactions, in press.*

Hydrogenation with Streptavidin



Chemogenetic Optimization



Identifying "Hot-Spots" on the Host Protein



Skander et. al. J. Am. Chem. Soc. 2004, 126, 14411.

Substrate Specificity: Enzyme-Like or Homogeneous Catalyst-Like?





R = H 94 % ee (R) quant. R = Ph 93 % ee (R) quant.

Chemo-Genetic Optimization: Fingerprints



Genetic diversity

Compare with directed evolution approach: Reetz et al. *Chem. Commun.* **2006**, 4318.

Second Generation Ligands: Enantiopure Spacers



Do Artíficial Metalloenzymes Follow Michaelís Menten Kínetics?





Allylic Alkylation: Add Surfactant!



1 eq.

Dimethylmalonate: 5 eq. [Pd(Ph₂allyl)Cl]₂ cat.: 0.02 eq. Ligand: 0.048 eq.

> Protein : 0.013 eq. K_2CO_3 : 5 eq. DMB: 2 eq. $H_2O / DMSO 10/1$ $V_{tot} = 390 \,\mu L$ [AllyIOAc] = 5 mM



RT, 16 h





Allylic Alkylation: Summary



Sav: 32 % ee (S), 21 % y S112K: 66 % ee (S), 21 % y No protein: 5% ee (S), 62 % y



Sav: 79 % ee (S), 95 % y S112A: 90 % ee (R), 96 % y S112A: 93 % ee (R), 20 % y no surfactant S112A: 95 % ee (R), quant. (45% DMSO) S112Q: 31 % ee (S), 96 % y No protein: 0 % ee, 83 % y



Pierron and Malan et. al. Angew. Chem. Ind. Ed., 2008, 48, 701.

Artificial Transfer-Hydrogenase Acetophone as Model Substrate



3 Lígands, 3 Metals, 6 η⁶-Arenes 20 Proteíns (Saturatíon Mutagenesís at S112)

Letondor et al. *Proc. Natl. Acad. Sci*, **2005**, *102*, 4683. Letondor et al. *J. Am. Chem. Soc*, **2006**, *128*, 8320. Acetophenone Reduction by Artificial Transfer Hydrogenase: Conversion and Selectivity Profiles



 $B(OH)_3 \cdot HCO_2 Na + MOPS buffer$ $pH = 0.5 \cdot (pK_{a1} + pK_{a2})$

Chemical Optimization: ee vs. $\{\eta^n - (C_n R_n)M\}$





Enantiopure @ Ru 20% occupancy

Towards "Higher Throughput" Screening: Overcoming the Purification Bottleneck

Biotinylated Sepharose

Biotinylated Catalyst

Crude Streptavidin from 250 mL Baffled Flask

p-Bromoacetophenone with [Rn(p-cymene) (Biot-pL)] ⊂ P64G Sav © pure 94 % ee quant. conversion © extract 92 % ee, 95 % conversion

Designed Evolution: Selected Examples



 $[Ru(cymene)(Biot-p-L)] \subset L124V : 96 \% (R)$

OH



 $[Ru(benzene)(Biot-p-L)] \subset T114G: 84 \% (S)$



 $[Ru(cymene)(Biot-p-L)] \subset P64G : 91 \% (R), 25 \% conv.$ $[Ru(cymene)(Biot-p-L)] \subset P64G : 84 \% (R), 93 \% conv.$



 $[Ru(cymene)(Biot-p-L)] \subset S112A-K121T : 90 \% (R)$



 $[Ru(cymene)(Biot-p-L)] \subset S112A-K121T : 88 \% (R)$ $[Ru(benzene)(Biot-p-L)] \subset S112A-K121N : 72 \% (S)$



 $[Ru(benzene)(Biot-p-L)] \subset S112A-K121N : 91 \% (S)$

Creus, Pordea, Rossel et al. Angew. Chem. 2008, 47, 1400.

Could Streptavidín Accomodate a Non-Biotinylated Polar Coordination Compound?



Pordea

Sulfoxidation Results

t-BuOOH, pH 2.2, RT

2 mol% artificial metalloenzyme

artificial metalloenzyme $[(H_2O)_5V=O]^{2+} \subset streptavidin$



46 % ee (*R*)





О

93 % ee (*R*)

 \bigcirc



Kinetic Aspects



Is Vanadium Inside Streptavidin: Wishful Thinking?





$$\begin{split} & [(H_2O)_5V=O]^{2+} \subset WT \text{ Sav} \\ & [(H_2O)_5V=O]^{2+} + \text{Biotin} \subset WT \text{ Sav} \\ & [(H_2O)_5V=O]^{2+} \subset \text{D128A Sav} \\ & [(H_2O)_5V=O]^{2+} \subset \text{BSA} \end{split}$$

90 % ee (*R*) [(H₂O)₅ V=O]²⁺ ⊂ WT Sav 87 % ee (*R*) racemic [(H₂O)₅ V=O]²⁺ ⊂ Aviloop 90 % ee (*R*) racemic racemic

48 52 WT Sav SAVGN---AESRYVLT WT Avi TAVTATSNE-IKESPLH

38

45

Artificial Metalloenzymes: Extending Small Molecule Recognition to Macromolecule Recognition



Creus, Rossel, Sardo and Wohlschlager

Homogeneous vs. Enzymatic Catalysis: Pro and Con Enzymatic Homogeneous Enantiomers Both enantioners Single Enantioner Solvent Tolerance Organic Aqueous Substrate Specificity Broad Scope Narrow Scope Optimization chemical Genetic Catalyst Lifetime Limited Extended

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