

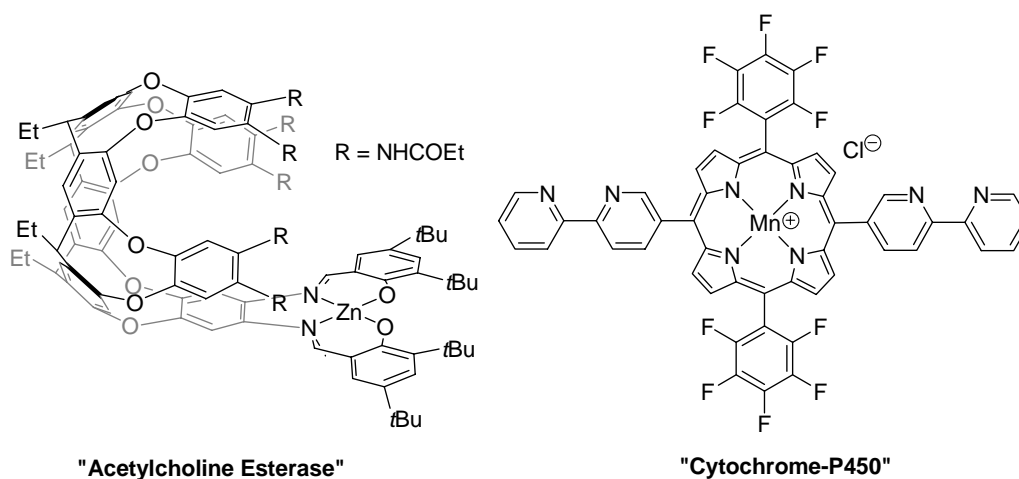
# Recent Advances in Enzyme Mimicry: Using Non-covalent Interactions to Define Reaction Geometries and Outcomes

Jason M. Nichols

Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742

[jnicholz@umd.edu](mailto:jnicholz@umd.edu)

## ABSTRACT



An enzyme has the ability to bind and define the relative geometry of reacting partners such that the intrinsic reactivity of a molecule can be overridden by the proximity of reaction centers. Recent advances in the field of enzyme mimicry and supramolecular chemistry have coalesced to provide mimics with similar levels of sophistication. Molecular systems designed to imitate the activity of acetylcholine esterase (AChE) and cytochrome-P450 mono-oxygenase (CP450) are presented as representative examples from the recent literature.

**Introduction.** In 1972, Professor Ronald Breslow introduced the term “biomimetic” to describe the selective halogenation of steroids for the synthesis of cortisone.<sup>1</sup> In the intervening 30 years, catalysts have been developed to mimic the essential properties of biological macromolecules.<sup>2</sup> *Structural mimics* contain an architectural element common to a particular biological moiety, while *functional mimics* are those that imitate a particular task performed by a biomolecule. Designing and synthesizing systems that incorporate one or more of these elements remains a considerable challenge for chemists.

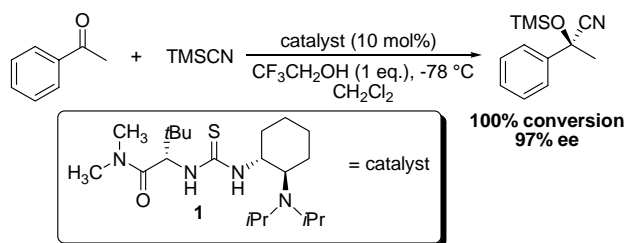
Specificity and molecular control in chemical processes are best found in Nature. The mechanisms associated with selectivity in enzymatic processes are explained classically by the “lock and key” binding of a substrate. More recently, according to the Pauling principle,<sup>3</sup> enzymatic selectivity is attributed to the selective binding of the transition state rather than complexation of a substrate. Regardless of interpretation, the interactions within an enzyme’s active site result from noncovalent forces such as dipole-dipole interactions, hydrogen-bonding,  $\pi$ -stacking, or solvophobic effects.<sup>4</sup>

Non-covalent interactions define the geometry of an enzyme-substrate complex. These interactions must be chemospecific and directional to control the geometry of a substrate within an active site. The spacial configuration of the enzyme-substrate complex can enhance or even override the substrate's natural reactivity as dictated by its functional groups. In essence, enzymes can literally hold a molecule in place so that *geometry* controls regio-, chemo-, and stereo-selectivity. The practitioners of enzyme mimicry seek to design catalysts that exploit non-covalent interactions to achieve similar levels of control.

The structural and binding motifs found in Nature's catalysts provide a blueprint for engineering artificial enzymes that provide geometric control over the outcome of a reaction. The simplest example of binding or activation geometries controlling a reaction pathway is asymmetric catalysis. For example, chiral Lewis-acids confer geometric control over a substrate through metal-ligand interactions. As asymmetric technology begins to take advantage of binding technologies other than metal-ligation, non-covalent interactions are playing a prominent role in modern asymmetric catalysis.<sup>6</sup> Bifunctional asymmetric catalysts that bind and geometrically arrange both reaction partners in a transformation are an excellent preliminary example of geometric reaction control.

Recent work from the Jacobsen group provides an elegant demonstration of the state-of-the-art in bifunctional asymmetric catalysis. Thiourea based organo-catalyst **1** promotes the cyanosilylation of arylketones with TMS-cyanide (Scheme 1).<sup>7</sup> Thiourea **1** contains both a Lewis-basic tertiary amine tethered through a cyclohexyldiamine chiral backbone to a hydrogen-bond donating/Lewis acidic thiourea. The ability of **1** to bind both the arylketone, and TMS-cyanide leads to high yields and enantioselectivities reminiscent of enzymatic processes.

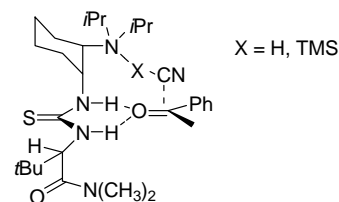
**Scheme 1.** Thiourea catalyzed cyanosilylation of arylketones.



While not a formal enzyme-mimic, **1** demonstrates how non-covalent interactions can be exploited to preorganize transition states in favor of an otherwise unfavorable outcome (Scheme 2). The unfavorable outcome in the thiourea catalyzed reaction is the purely geometrical phenomenon of asymmetry. The remaining

examples will focus on actual enzyme mimics that operate under the same fundamental principle: selectivity in a catalytic process can be achieved through the use of non-covalent interactions to define the geometry of reactions

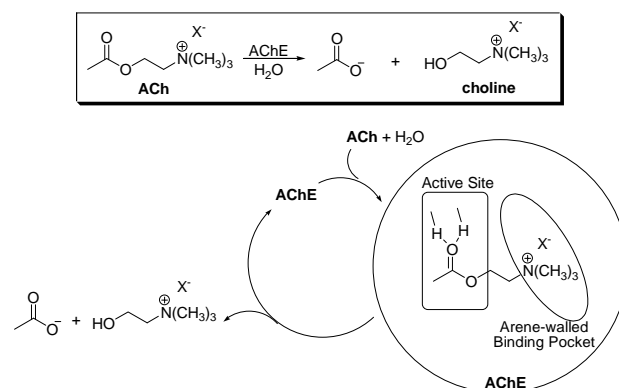
**Scheme 2.** Transition state organized by hydrogen bonding.



**Acetylcholine Esterase (AChE).** The neurotransmitter acetylcholine (ACh) is one of the oldest known biochemicals. The production of acetylcholine in a neuron is part of a process that generates a nerve impulse. When the impulse is no longer required AChE rapidly cleaves ACh into choline and acetate. AChE has been linked to memory function and is the prime therapeutic target in the treatment of Alzheimer's disease. The inhibition of AChE has led to viable therapies for Alzheimer's, making it a high-profile drug target.<sup>8</sup>

The mechanism<sup>9</sup> and structure<sup>10</sup> of AChE has been a topic of intense research. The macrostructure of AChE includes a 20 Å deep entrance gorge with a His-Ser-Glu active site triad responsible for substrate activation. Although hydrolysis of an ester may appear a simple matter, AChE operates as a near "perfect" catalyst with reaction rates near the diffusion control limit. Thus, mechanisms of transport along the narrow entrance channel, and active site-transition state geometries remain topics of current interest.

**Scheme 3.** General Reaction and Catalytic Cycle for AChE.

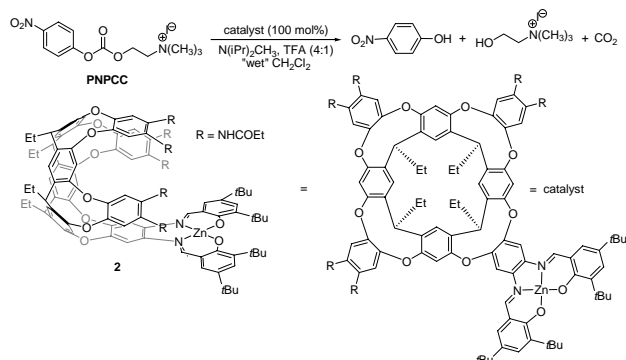


Two general characteristics provide the basis for building a functional mimic (Scheme 3). In general,

AChE catalyzes the simple hydrolysis of ACh through binding and activation of the ester carbonyl. Upon nucleophilic attack, the same interactions serve to bind and stabilize the subsequent tetrahedral intermediate. Secondly, in addition to binding the ester of ACh, an arene walled “pocket” binds the quaternary ammonium moiety via cation- $\pi$  interactions. The cation- $\pi$  interaction imparts a directional, chemospecific binding of the quaternary ammonium group making AChE selective for ACh. Overall, the arrangement of the two binding domains provides a geometrically specific interaction between AChE and ACh as the quaternary ammonium head group directs the ester of ACh into the active site.

A novel receptor developed in the Rebek laboratory recently demonstrated the catalytic cleavage of a choline derived carbonate in organic solvent (Scheme 4). The receptor **2** combined the general characteristics of AChE into a single supramolecular host. A deep-cavity cavitant with an upper wall held rigid through hydrogen-bonded amides provides the quaternary ammonium receptor. A Zn(II)-salen complex provides the carbonyl binding/activating moiety. Cation- $\pi$  interactions between the quaternary ammonium of choline derivative p-nitrophenylcholinecarbonate (PNPCC) and the deep-cavity cavitant of receptor **2** direct the carbonate towards the tethered Zn(II)-salen Lewis acid in fulfillment of the template provided by AChE.

**Scheme 4.** Deep-cavity cavitant as an AChE mimic.



The enzyme-like activity of **2** was observed as a 50 fold rate enhancement over the background reaction, and a 5 fold enhancement over the untethered Zn(II)-salen complex. Acetylcholine was an inhibitor of **2** under the reaction conditions as can be expected from its structural similarity to PNPCC.

In addition to demonstrating rate enhancements, the cavitant receptor **2** also selectively bound PNPCC over p-nitrophenylbenzylcarbonate (PNPBC). Without the quaternary ammonium moiety, no binding interaction could be measured for PNPBC with **2**. The loss of binding corresponded to loss in catalytic competency as **2** catalyzed the hydrolysis of PNPBC at just half the rate of unmodified Zn(II)-salen complex. This implied the

catalytic hydrolysis of PNPBC by **2** must occur on the opposite face of the binding cavity. Selectivity was attributed to binding affinities and their geometries based on a relative rate of approximately 2:1 in the hydrolysis of PNPBC by unmodified Zn(II)-salen complex with two reactive sites and **2** with a single accessible reactive site.

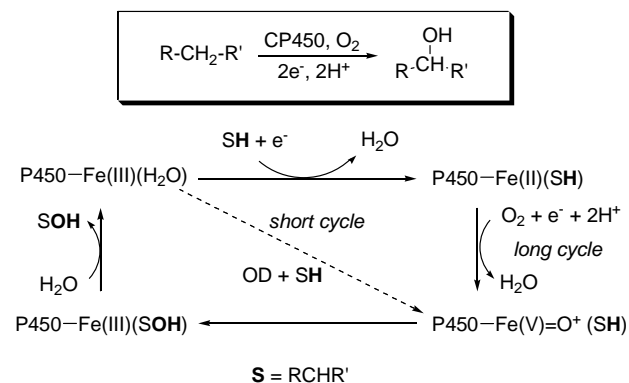
Catalysis by receptor **2** is an example of enhancing substrate reactivity through geometrically specific binding. Binding of PNPCC by **2** was selective for a quaternary ammonium over an uncharged hydrocarbon. The interaction between PNPCC and the deep-cavity receptor of **2** was directional, arranging the ester to interact with the tethered Zn(II) active site. The result was a chemoselective and geometrically specific binding event that enhanced catalytic ability of the active site. The modest 50-fold rate enhancement in the hydrolysis of PNPCC provided by **2** is a successful demonstration of an esterase mimic.

### Cytochrome-P450 mono-oxygenase (CP450).

Exciting developments over the last 20 years in biomimetic oxidations have provided some of the most advanced examples of enzyme mimics.<sup>11</sup> Cytochrome-P450 is a superfamily of mono-oxygenases that catalyze the hydroxylation of hydrocarbons.<sup>12</sup> The functional moiety within CP-450 is an iron-porphyrin core that activates dioxygen in xenobiotic oxidation processes.

A great deal of research has revealed many aspects of the enzymatic mechanism for CP450 including the major catalytic intermediates (Scheme 5).<sup>13</sup> The full catalytic cycle (*long cycle*) reductively activates oxygen. The cycle commences with substrate complexation, which activates a prosthetic iron-heme complex that then goes on to reduce molecular oxygen to water and an iron-oxenoid complex (Fe(V)=O<sup>+</sup>(porph), porph = porphyrin). Work from the Groves laboratory first demonstrated a *short cycle* that bypasses the activation of oxygen with an oxygen-atom donor (OD = PhI=O) to generate the active oxidant directly.<sup>14</sup>

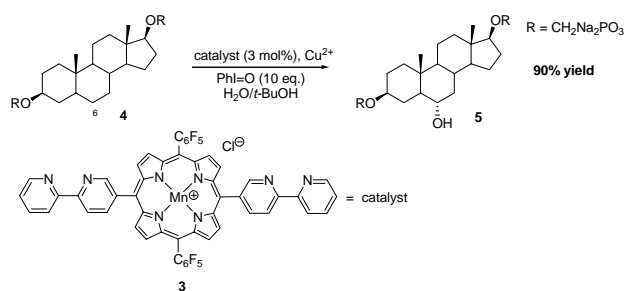
**Scheme 5.** General reaction and catalytic cycle for CP450.



An enzyme mimic developed in the Breslow laboratories has demonstrated remarkable selectivity in the hydroxylation of cholesterols (Scheme 6).<sup>15</sup> A Mn(III)(porph) was modified with 2,2' bipyridyl and perfluorophenyl groups at the trans *meso*-positions (**3**). Complex **3** provides both the metal-heme based oxidation center and a substrate recognition element. Cholesterol **4** derivatized with simple  $\alpha$ -phosphonoacetyl groups was bound to **3** with  $\text{Cu}^{2+}$  ions.

Upon treatment of **4** with  $\text{PhI}=\text{O}$  in the presence of **3** as a catalyst,  $\alpha$ -hydroxy cholesterol **5** was formed in 90% yield. Good turnover catalysis was observed with a turnover number of 32. The observed regioselectivity confirmed that  $\text{Cu}^{2+}$  binding bridges **4** over the oxidant,  $\text{Mn(V)}=\text{O}^+(\text{porph})$ , and directs hydroxylation to occur at the unactivated C6 position. The oxidation at C6 was stereospecific for the trans- $\alpha$ -hydroxy cholesterol **5**.

**Scheme 6.** Modified porphyrin as a CP450 mimic.



The Breslow system is a very elegant example of geometrically enforced selectivity through non-covalent binding interactions. The configurationally specific binding of cholesterol **4** activates an otherwise inert methylene unit for oxidation. In addition to activating the C6 position, the binding interaction is stereospecific forcing the formation of  $\alpha$ -hydroxy cholesterol **5**. The Mn(III)(porph) system has been modified both with cyclodextrins<sup>16</sup> and other substrate binding moieties<sup>17</sup> to achieve selective oxidations of cholesterols that demonstrate the generality of this approach.

**Conclusion.** The growing field of enzyme mimicry continues to provide examples of powerful applications of non-covalent interactions that fulfill the rigorous binding requirements of selective catalysis. An enzyme requires chemospecific, directional non-covalent interactions to define an enzyme-substrate geometry that enhances or overrides a molecule's intrinsic reactivity. Careful analysis of enzymatic binding geometries and mechanisms provide general specifications that can be incorporated into the design of a simple catalyst. As supramolecular chemistry develops more advanced receptors, and enzymology decodes the actions of Nature's catalysts, there is certain to be additional exciting contributions in the field of enzyme mimicry and catalysis.

**Acknowledgment:** I thank Michael P. Doyle, Lyle Isaacs, and Heinz Koch for their guidance and support.

## References

- For geometric free radical halogenation of steroids: (a) Breslow, R., *Advances in Chemistry Series*, **1971**, No. 100, 21-43; (b) Breslow, R.; Dale, J. A.; Kalicky, P.; Liu, S. Y.; Washburn, W. N., *J. Am. Chem. Soc.*, **1972**, *94*, 3276-3278; (c) Breslow, R.; Corcoran, R. J.; Snider, B. B., *J. Am. Chem. Soc.*, **1974**, *96*, 6791-6792; (d) Breslow, R.; Snider, B. B.; Corcoran, R. J., *J. Am. Chem. Soc.*, **1974**, *96*, 6792-6794; For early reviews on "biomimetic processes": (e) Breslow, R., *Chem. Soc. Rev.*, **1972**, *1*, 553-580; (f) Breslow, R., *Acc. Chem. Res.*, **1980**, *13*, 170-177.
- For reviews on the concept of an enzyme mimic: (a) Breslow, R., *Pure Appl. Chem.*, **1990**, *62*, 1859-1866; (b) Kirby, A. J., *Stimulating Concepts in Chemistry*, **2000**, 341-353; For a review of small molecule enzyme mimics: Groger, H.; Wilken, J., *Angew. Chem., Int. Ed.*, **2001**, *40*, 529-532; For reviews on supramolecular enzyme mimics: (a) Newkome, G. R.; He, E.; Moorefield, C. N., *Chem. Rev.*, **1999**, *99*, 1689-1746; (b) Zimmerman, S. C.; Zharov, I.; Wendland, M. S.; Rakow, N. A.; Suslick, K. S., *J. Am. Chem. Soc.*, **2003**, *125*, 13504-13518; (c) Thordarson, P.; Nolte, R. J. M.; Rowan, A. E., *Aust. J. Chem.*, **2004**, *57*, 323-327.
- A discussion of "induced-fit" models of binding that contradicts traditional "lock and key" models: Jorgensen, W. L., *Science*, **1991**, *254*, 954-955; A review of molecular imprinted polymers with an excellent discussion of transition state binding: Wulff, G., *Chem. Rev.*, **2002**, *102*, 1-27.
- Steed, J. W.; Atwood, J. L., *Supramolecular Chemistry: A Concise Introduction*. Wiley: **2000**.
- Breslow, R.; Yang, J.; Yan, J., *Tetrahedron*, **2002**, *58*, 653-659.
- Ma, J.-A.; Cahard, D., *Angew. Chem., Int. Ed.*, **2004**, *43*, 4566-4583.
- Fuerst, D. E.; Jacobsen, E. N., *J. Am. Chem. Soc.*, **2005**, *127*, 8964-8965.
- Lahiri, D. K.; Farlow, M. R.; Sambamurti, K.; Greig, N. H.; Giacobini, E.; Schneider, L. S., *Current Drug Targets*, **2003**, *4*, 97-112.
- Gilson, M. K.; Straatsma, T. P.; McCammon, J. A.; Ripoll, D. R.; Faerman, C. H.; Axelsen, P. H.; Silman, I.; Sussman, J. L., *Science*, **1994**, *263*, 1276-1278; Kaplan, D.; Barak, D.; Ordentlich, A.; Kronman, C.; Velan, B.; Shafferman, A., *Biochemistry*, **2004**, *43*, 3129-3136.
- Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Tokar, L.; Silman, I., *Science*, **1991**, *253*, 872-879; Bartolucci, C.; Perola, E.; Cellai, L.; Brufani, M.; Lamba, D., *Biochemistry*, **1999**, *38*, 5714-5719; Luo, W.; Yu, Q.-s.; Zhan, M.; Parrish, D.; Deschamps, J. R.; Kulkarni, S. S.; Holloway, H. W.; Alley, G. M.; Lahiri, D. K.; Brossi, A.; Greig, N. H., *J. Med. Chem.*, **2005**, *48*, 986-994.
- For reviews and selected examples of cytochrome-P450 mimics: (a) Meunier, B., *Chem. Rev.*, **1992**, *92*, 1411-1456; (b) Feiters, M. C.; Rowan, A. E.; Nolte, R. J. M., *Chem. Soc. Rev.*, **2000**, *29*, 375-384; (c) Groves, J. T.; Neumann, R., *J. Am. Chem. Soc.*, **1989**, *111*, 2900-2909; (d) Parton, R. F.; Vankelecom, I. F. J.; Casselman, M. J. A.; Bezoukhanova, C. P.; Uytterhoeven, J. B.; Jacobs, P. A., *Nature*, **1994**, *370*, 541-544; (e) Schenning, A. P. H. J.; Spelberg, J. H. L.; Hubert, D. H. W.; Feiters, M. C.; Molte, R. J. M., *Chemistry--A European Journal*, **1998**, *4*, 871-880.
- Lewis, D. F. V., *Cytochromes P450: Structure, Function and Mechanism*. Taylor & Francis: **1996**.
- A comprehensive review of cytochrome-P450 enzymatic mechanisms: (a) Meunier, B.; de Visser, S. P.; Shaik, S., *Chem. Rev.*, **2004**, *104*, 3947-3980; (b) McLain, J. L.; Lee, J.; Groves, J. T., *Biomimetic Oxidations Catalyzed by Transition Metal Complexes*, **2000**, 91-169.
- Groves, J. T.; Nemo, T. E.; Myers, R. S., *J. Am. Chem. Soc.*, **1979**, *101*, 1032-1033.
- Fang, Z.; Breslow, R., *Organic Letters*, **2006**, *8*, 251-254.
- For metalloporphyrin cholesterol oxidations: (a) Breslow, R.; Zhang, X.; Xu, R.; Maletic, M.; Merger, R., *J. Am. Chem. Soc.*, **1996**, *118*, 11678-11679; (b) Breslow, R.; Zhang, X.; Huang, Y., *J. Am. Chem. Soc.*, **1997**, *119*, 4535-4536; (c) Yang, J.; Breslow, R., *Angew. Chem., Int. Ed.*, **2000**, *39*, 2692-2694; (d) Yang, J.; Gabriele, B.; Belvedere, S.; Huang, Y.; Breslow, R., *J. Org. Chem.*, **2002**, *67*, 5057-5067; (e) Fang, Z.; Breslow, R., *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 5463-5466.
- Initial report using metal-ligand binding: Belvedere, S.; Breslow, R., *Bioorg. Chem.*, **2001**, *29*, 321-331.