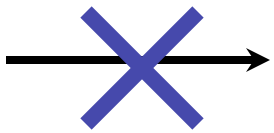
Design and Development of Superoxide Dismutase Mimetics as Therapeutics

Bio5476 November 25, 2008

Enzyme Mimetic

Traditional Approach

Block negative effects of enzymes



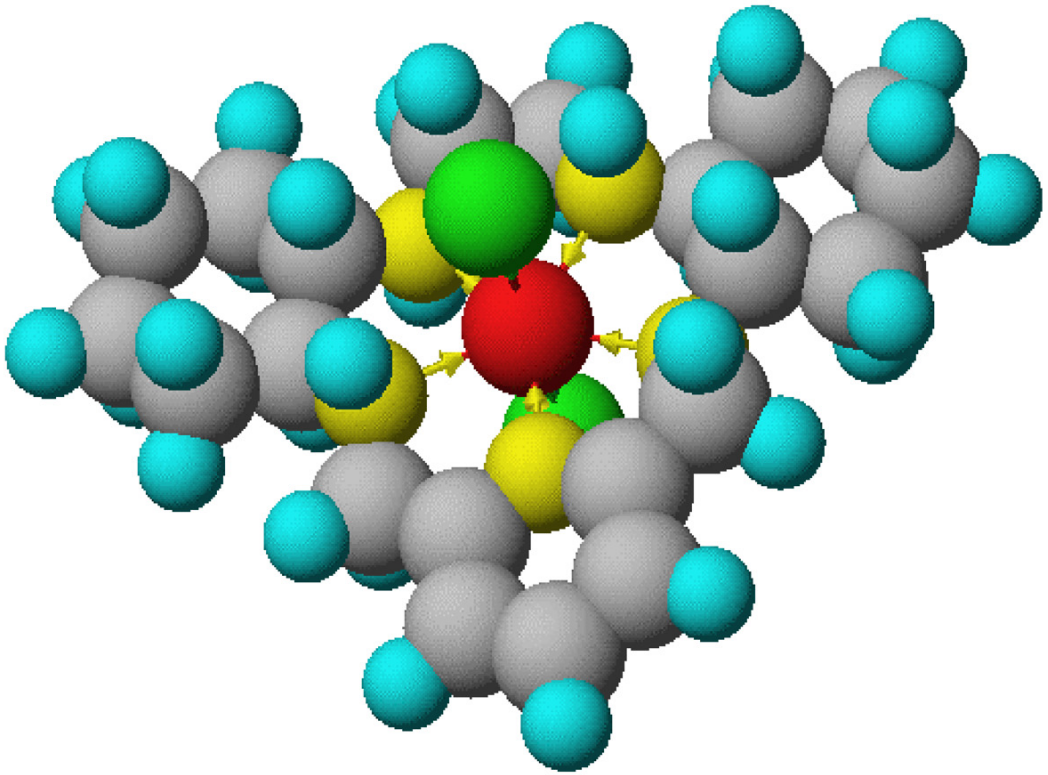
A B

MetaPhore's Approach

Amplify positive effects of enzymes

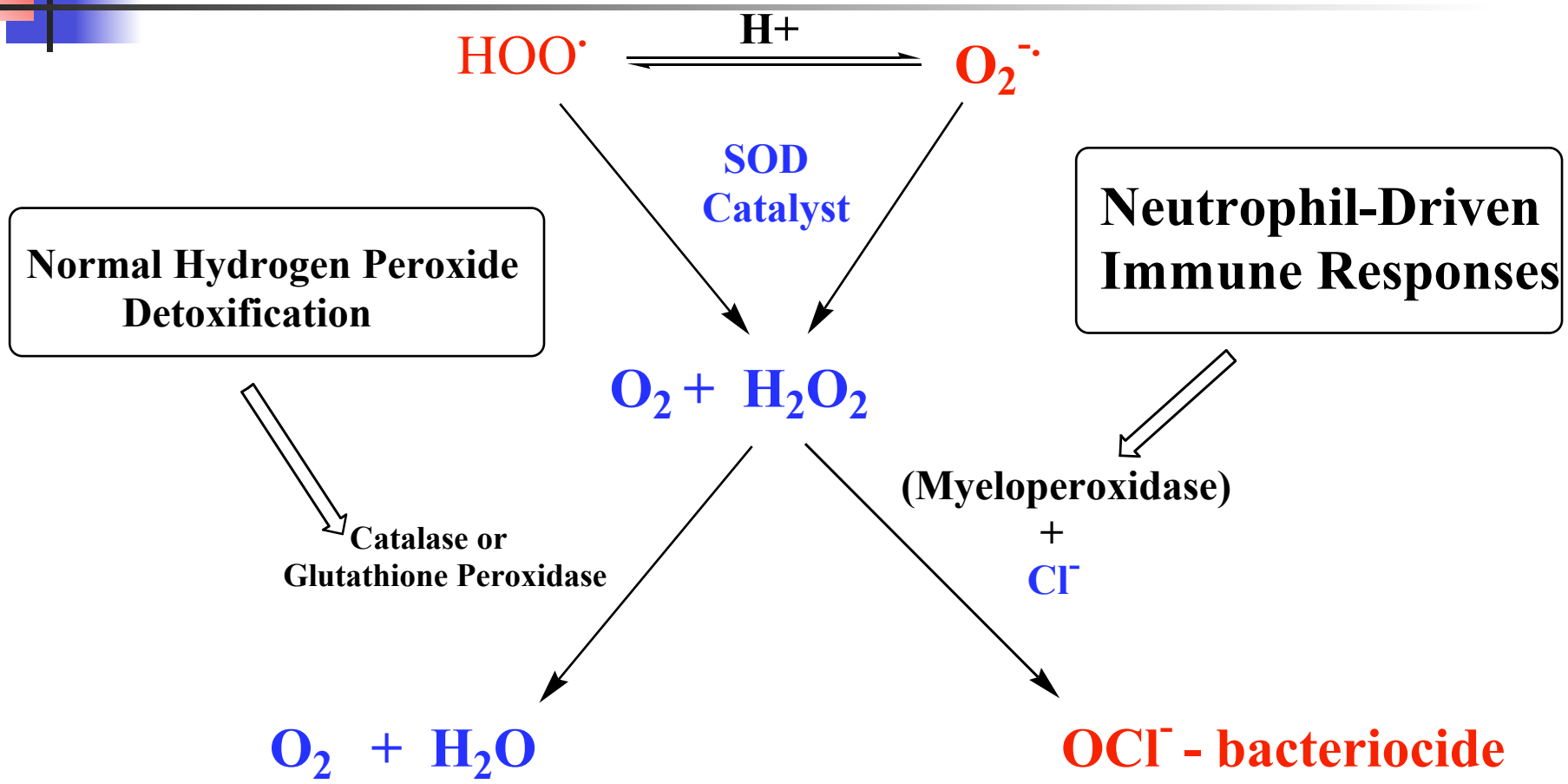
C D

SYNZYMES: A CASE STUDY

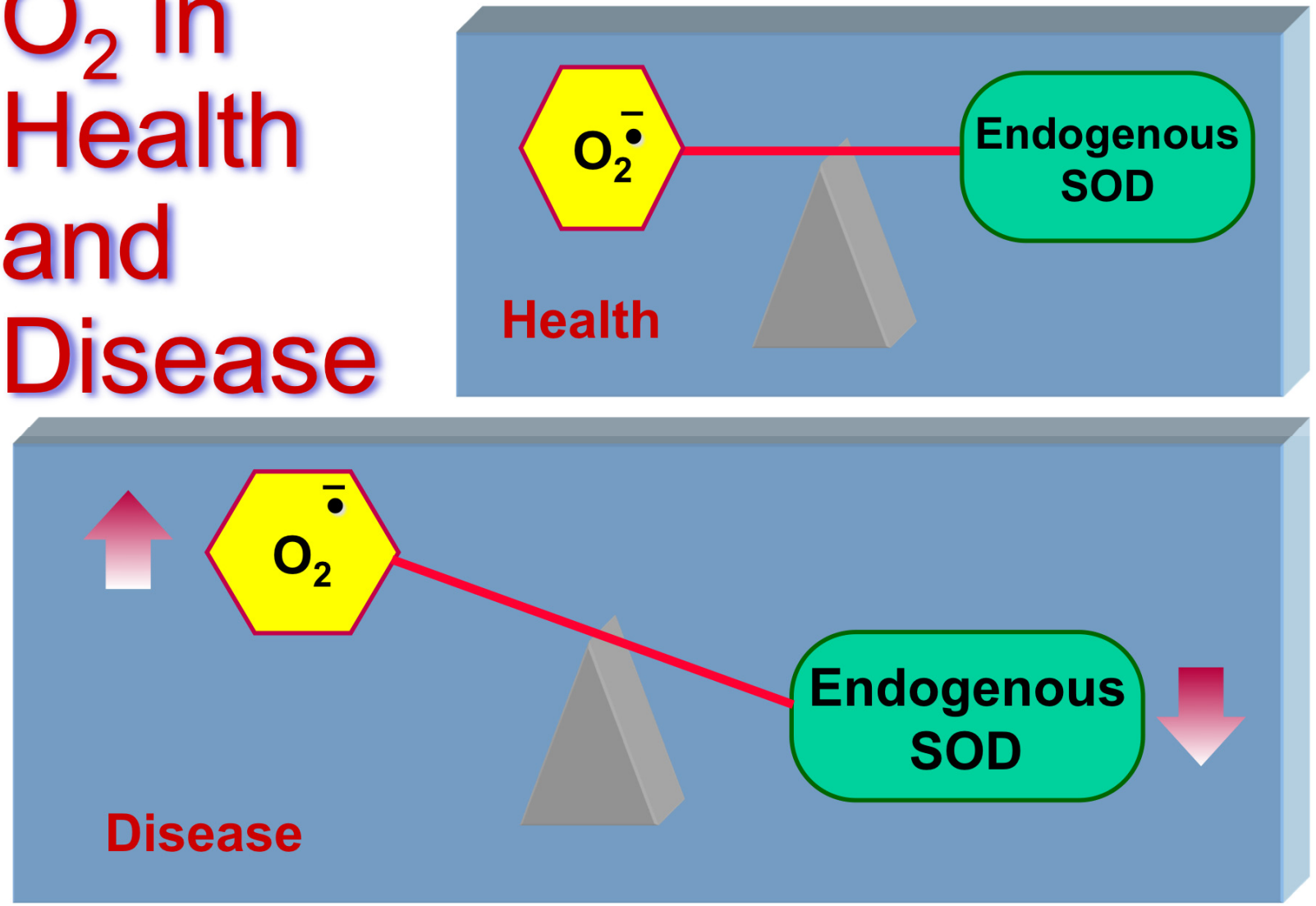


Mimics of Superoxide Dismutase : mSOD

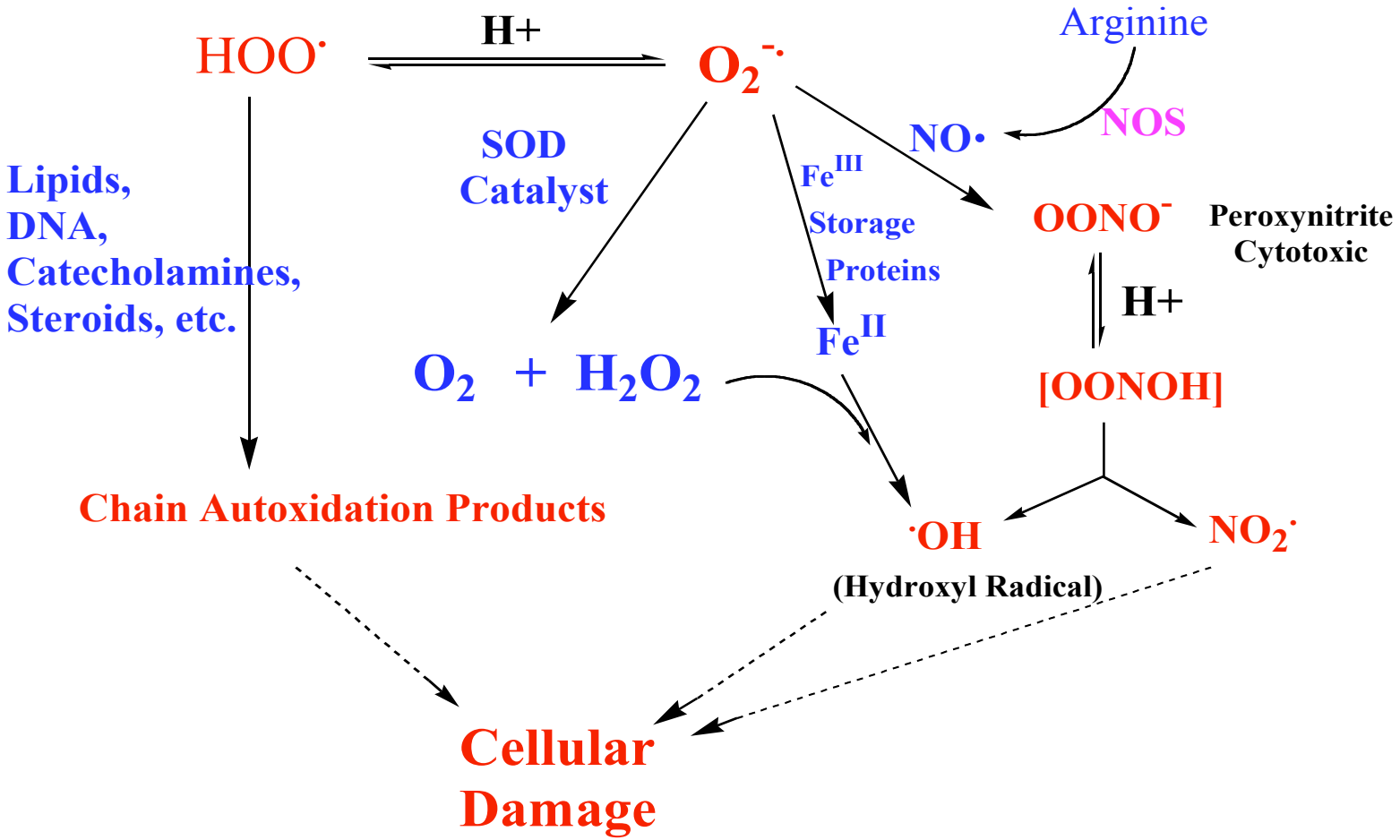
Superoxide Shunts



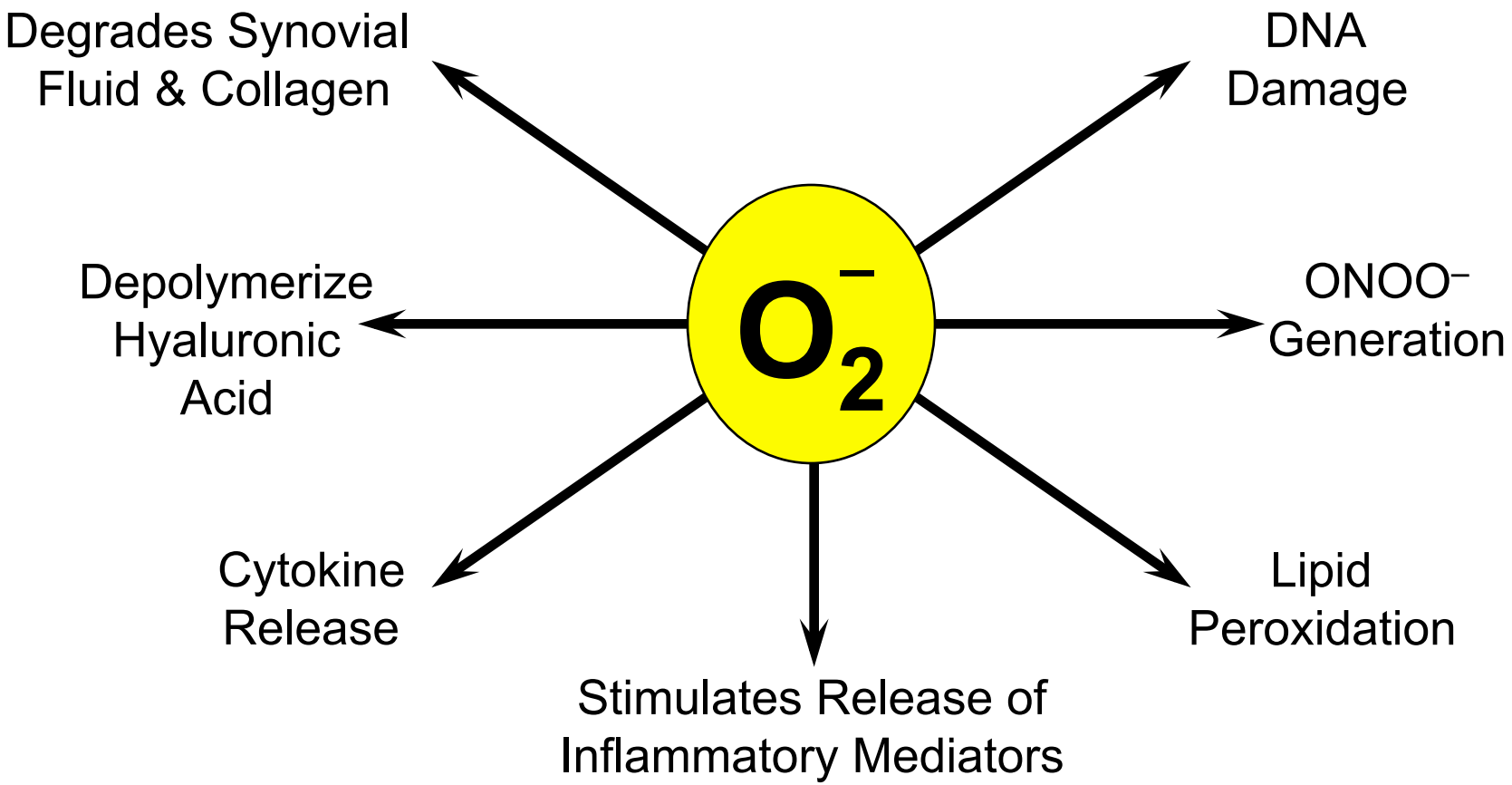
$O_2^{\bullet -}$ in Health and Disease



Reactive Oxygen Metabolites



Biochemical Impact of Excess Superoxide Anions



Elimination of Superoxide

- Superoxide is eliminated under physiological conditions by a set of superoxide dismutase enzymes that convert it to hydrogen peroxide which is then converted to water and oxygen by catalase.
- Mangenese superoxide dismutase is localized in mitochondria while Cu/Zn superoxide dismutases are in the cytoplasm and extracellular.
- Bovine Cu/Zn SOD (Orgatein®) was used as a pharmaceutical in Europe for many years for the treatment of osteoarthritis.
- In animal models, SOD enzymes have been shown efficacious in models of ischemia-reperfusion injury, inflammation, radiation damage, etc.

DISADVANTAGES OF PROTEIN SOD THERAPY

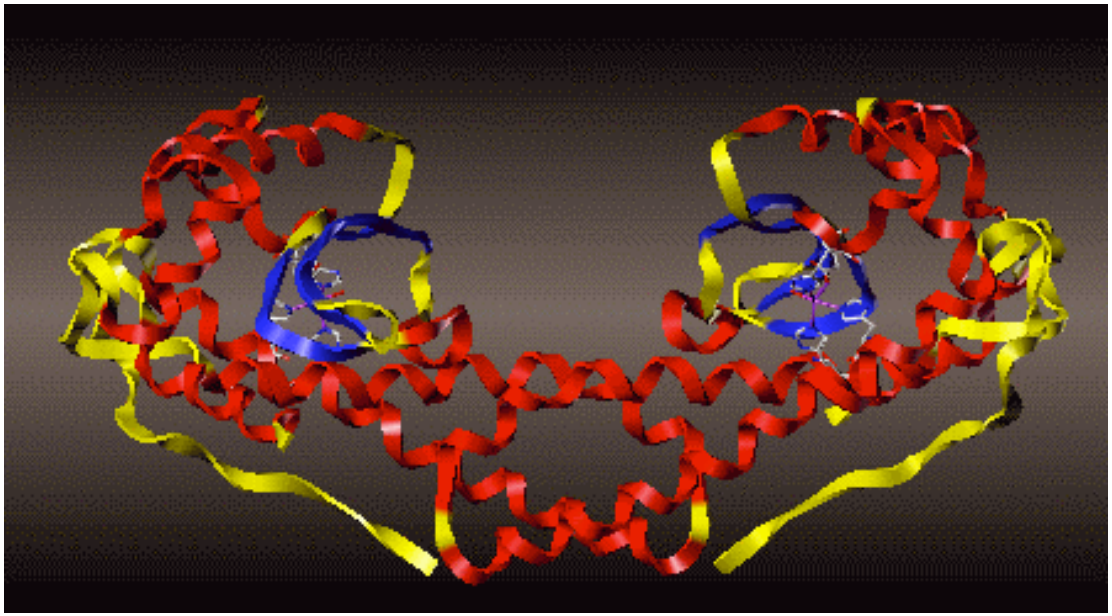
- Lack of Oral Activity
- Lack of Access to Intracellular Space where Superoxide is Produced
- Immunogenicity
- Short Half-Lives in Serum

Project Goal: SOD mimics

To develop a revolutionary advance in the small molecule approach to pharmacological intervention.

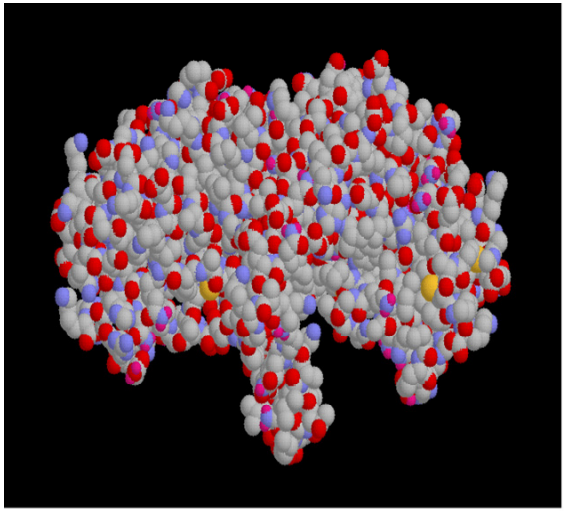
- **Synzymes: Small molecules that achieve the destruction of superoxide at a rate of tens of millions of times per second per molecule, mechanistically similar to SOD, for acute and chronic therapy.**

OBJECTIVE "SYNZYME" – A SYNTHETIC ENZYME THAT CATALYZED CONVERSION OF SUPEROXIDE TO HYDROGEN PEROXIDE



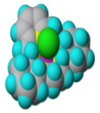
Ribbon diagram of crystal structure of human superoxide dismutase dimer; active sites are within blue ribbons

TECHNOLOGY: mSODs



10 years of research
>$50 Million

Monsanto/ Searle



SOD

- 88kDA
- k_{cat} = 10^{8}-10^{9} $M^{-1}s^{-1}$
- superoxide ⇨ hydrogen peroxide

mSOD

- ca. 500Da
- k_{cat} = 10^{7}-10^{9} $M^{-1}s^{-1}$
- superoxide ⇨ hydrogen peroxide

Monsanto/Searle Synzyme Project

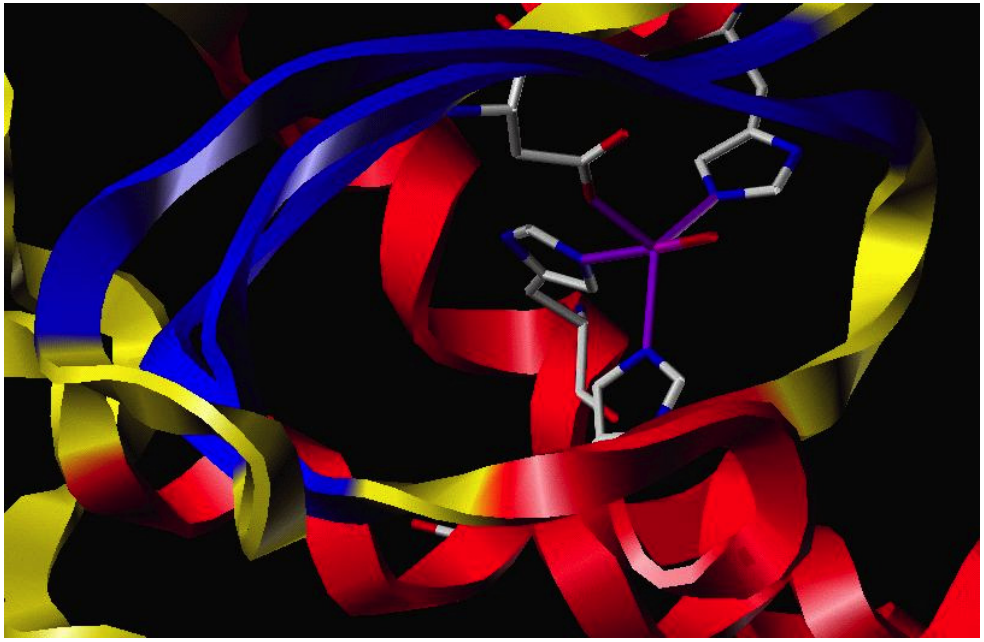
I did an evaluation of a synthetic enzyme project at Monsanto as a consultant.

I was extremely excited about the scientific achievements and therapeutic potential.

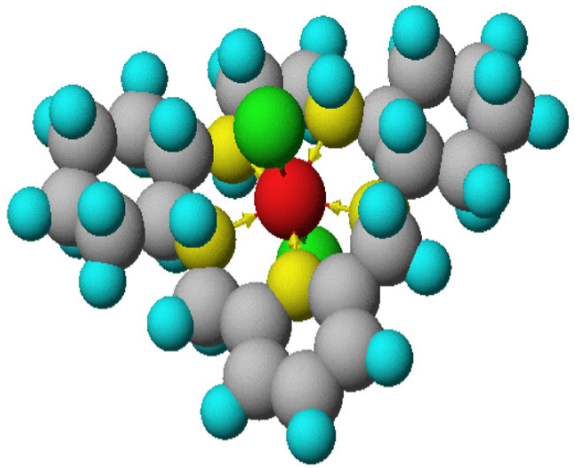
Due to external events (Monsanto purchased $6 billion worth of seed companies and COX-2 inhibitors were still in clinical trials), the decision was made to shelf the synzyme project.

I offered to out-license the project and finance it (over $90 million so far).

M40403 IS NOT A COPY OF ACTIVE SITE OF MN SOD, BUT A MIMIC OF CATALYTIC MECHANISM

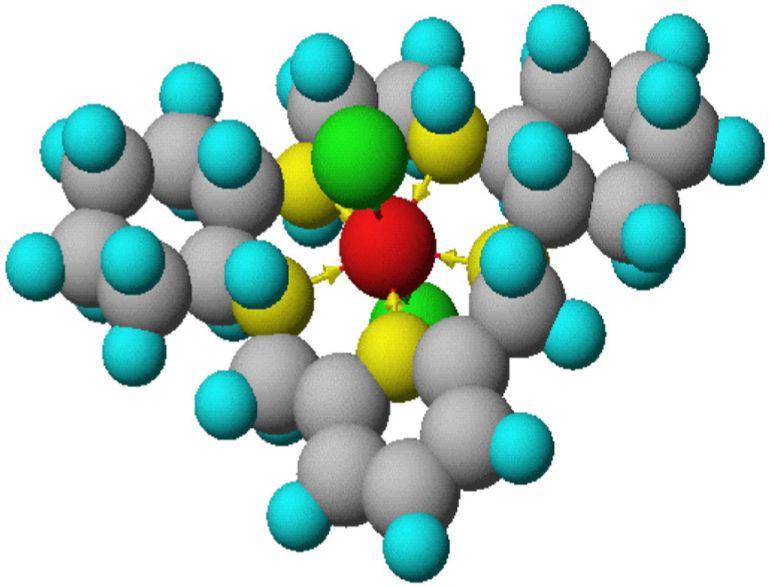


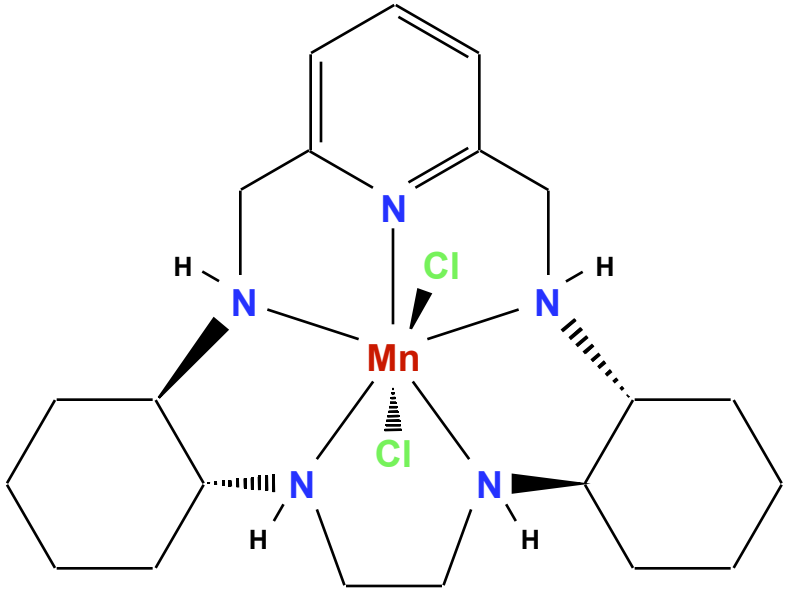
Active Site of human SOD – 3 His and 1 Asp coordinate with Mn



M40403 has 5 nitrogen ligands to Mn from its pentaazacrown crown scaffold

HOW WAS TECHNOLOGY DEVELOPED THAT GENERATED CLINICAL CANDIDATE?





M40403

SEVERAL CHOICES WERE MADE:

Choice of metal – Mn, Cu or Fe?

All found in SOD enzymes; only Mn not involved in Fenton chemistry that produces hydroxyl radicals

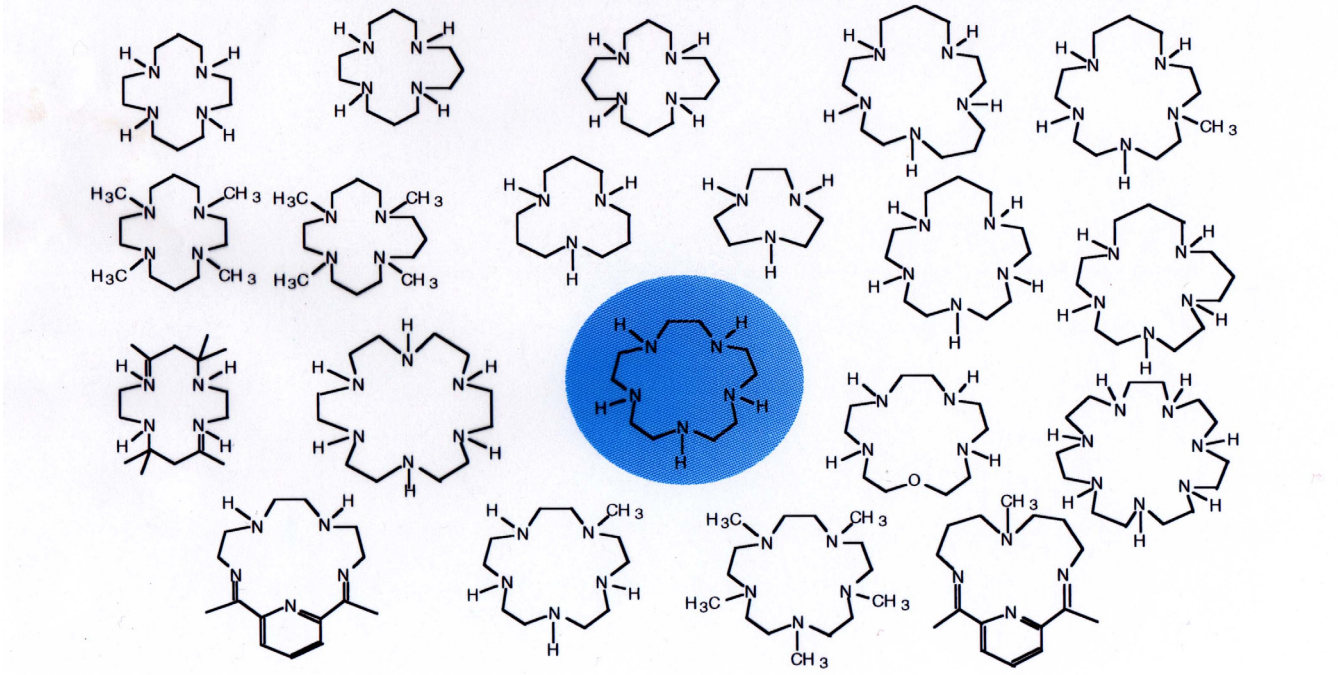
Choice of ligand – stability of complex a crucial feature; cyclization was known to enhance complex stability

Redox potential had to match that of both superoxide and anion and not that of other potential substrates; nitrogen coordination was appropriate

Screen Mn complexes of azacrownethers

Initial SAR

1. $MnCl_2$ complex prepared for each macrocycle
2. The $MnCl_2$ complex of one ligand, **SC-52608**, showed substantial SOD activity



D. P. Riley and R. H. Weiss, J. Am. Chem. Soc. 116:387-388, 1994

Stopped-Flow UV Assay for SOD Mimetics

D. P. Riley and R. H. Weiss, J. Am. Che Soc. 116:387-388, 1994

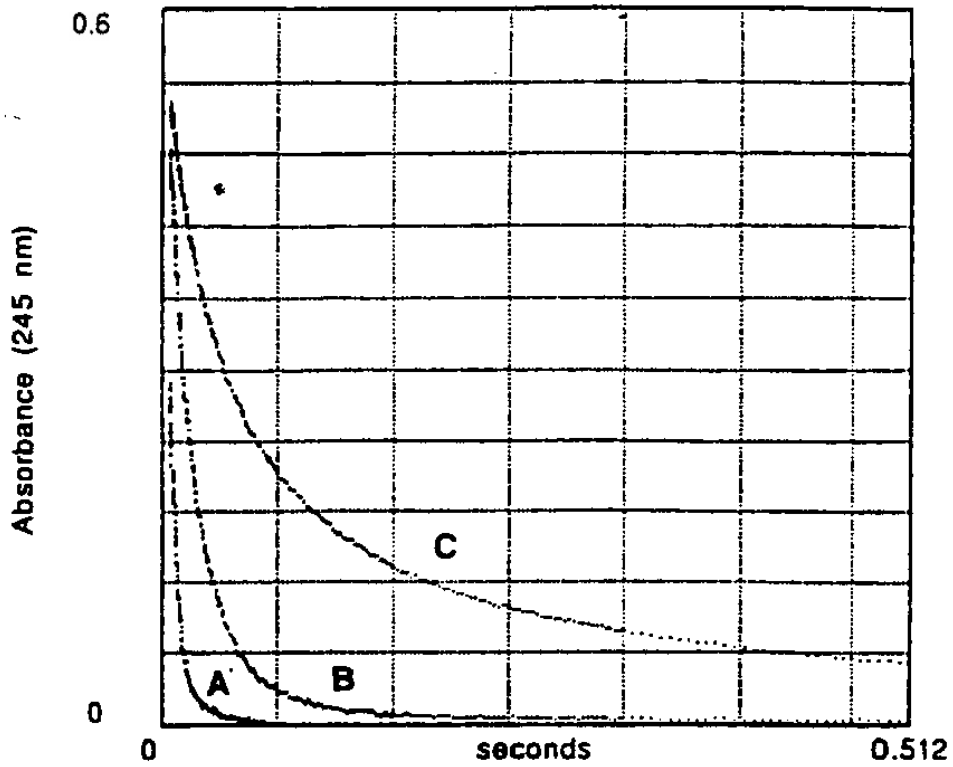
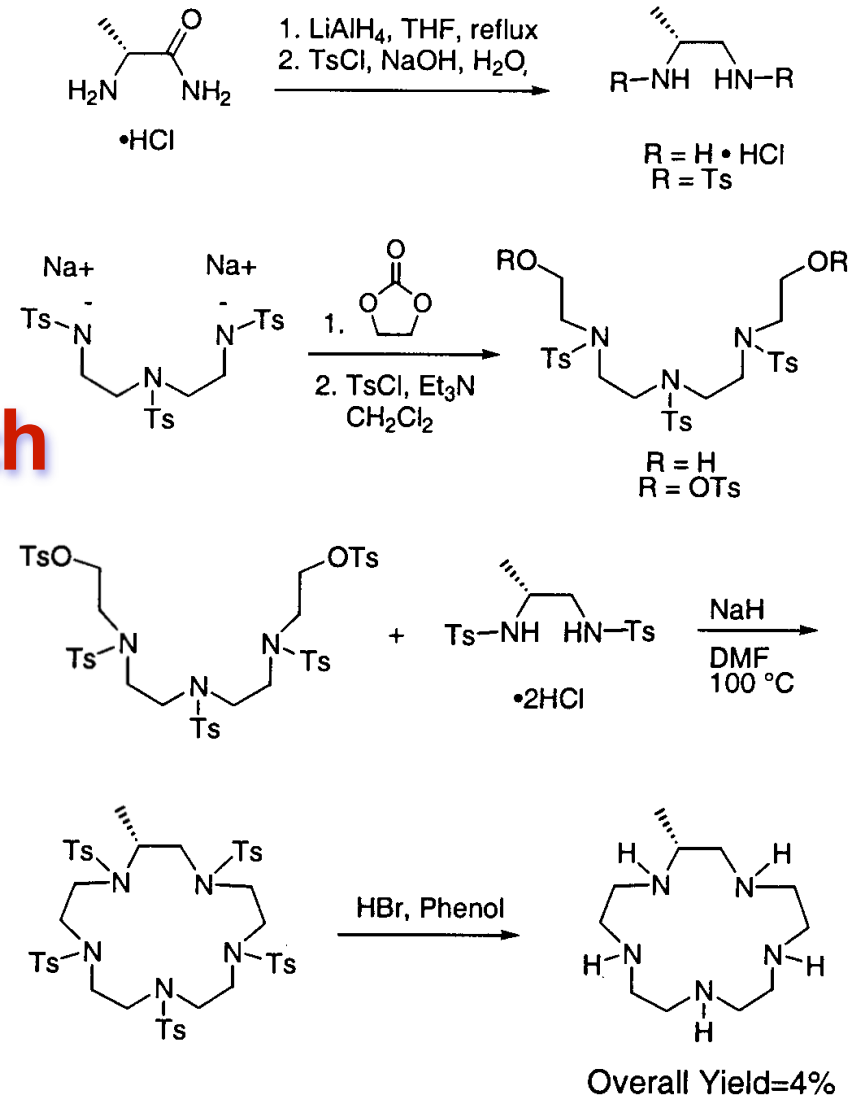


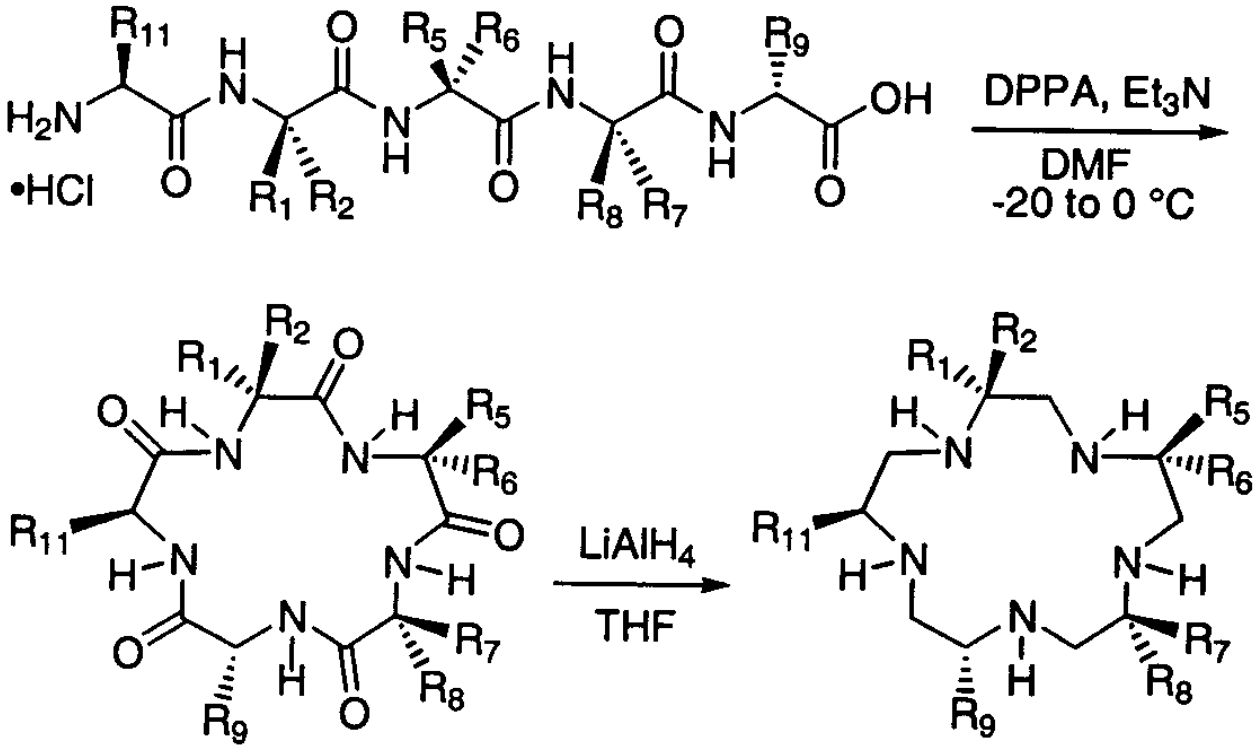
Figure 2. Stopped-flow decays of superoxide measured at 245 nm at 21 °C, where $[SO]_i \sim 100\ \mu M$, in the presence and absence of complex **1**. All reactions were carried out using 80 mmol of HEPES buffer at pH = 7.75. A: 8.80×10^{-6} M [Mn([15]aneN$_5$)Cl]Cl. B: 2.20×10^{-6} M [Mn([15]aneN$_5$)Cl]Cl. C: uncatalyzed.

Traditional Approach to Synthesis of Pentaazacrowns Gave Poor Yields



Scheme 1. Richman–Atkins type synthesis of the monomethyl-substituted ligand **16** accomplished in an overall yield of ~4%.

Cyclic Peptide Approach to Cyclopentaazacrowns



Aston et al. Tetrahedron Lett. 35:3687, 1994

SOD Mimetics Used to Develop Models of Mechanisms

Changes in Chirality of Substituents and Introduction of Cyclic Constraints Modified Complex Geometry

D. P. Riley, Susan L. Henke, Patrick J. Lennon and Karl Aston, Inorg. Chem. 38:1908-1917, 1999

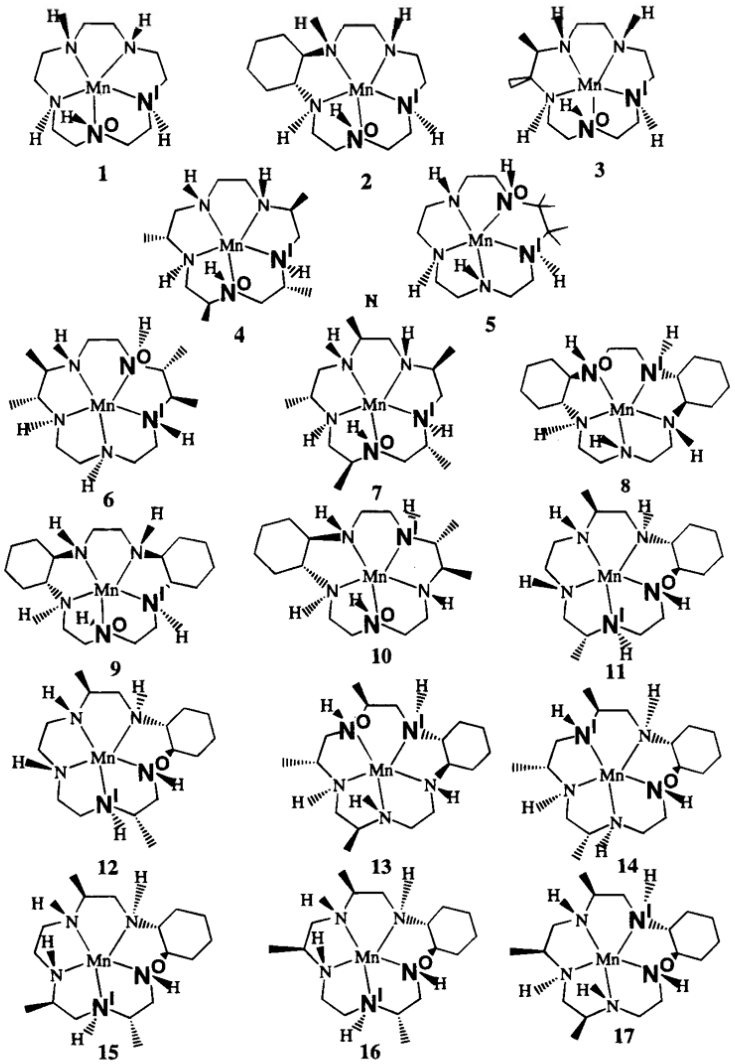
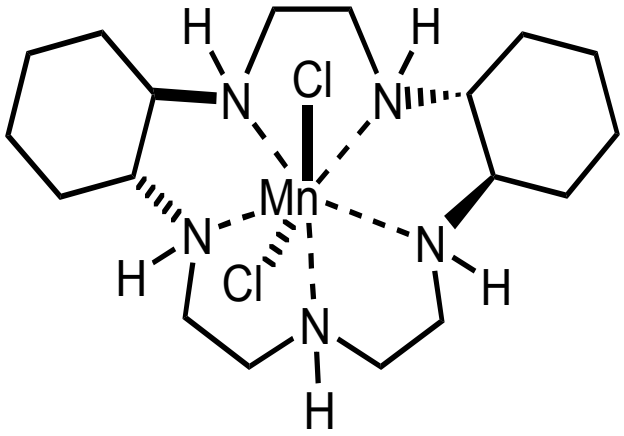
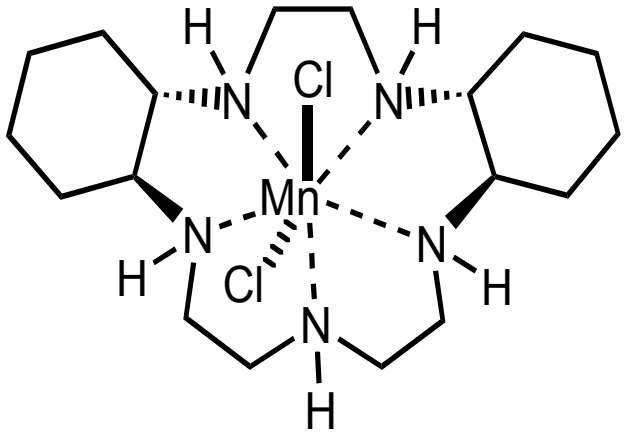


Figure 1. Structures of Mn(II) complexes (axial ligands omitted for clarity) with the macrocyclic ligands utilized for generating a modeling database. The stereochemistry of substituents and NH's is included along with the nitrogen labels: N^O (outer-sphere NH fold) and N^I (inner-sphere NH fold).

Stereochemical Effects



all-*R*-



R,R,S,S-

k_{cat} (pH=7.4)= 1.2 x 10^{+8} M^{-1} s^{-1}

k_{cat} (pH=7.4): Inactive

***R,R,S,S,*-isomer is rigidly planar; i.e., will not fold, thus no catalytic activity**

Ligands Used to Test Predictive Ability of Model

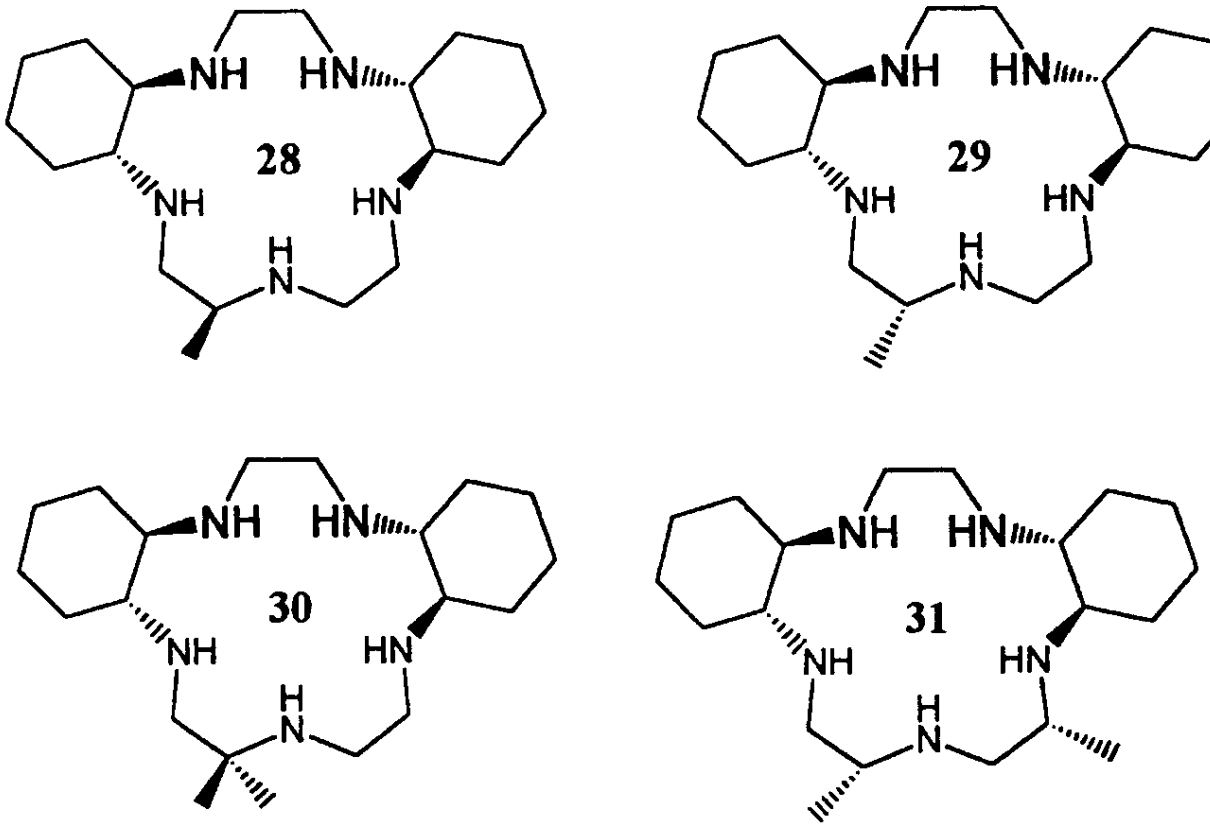
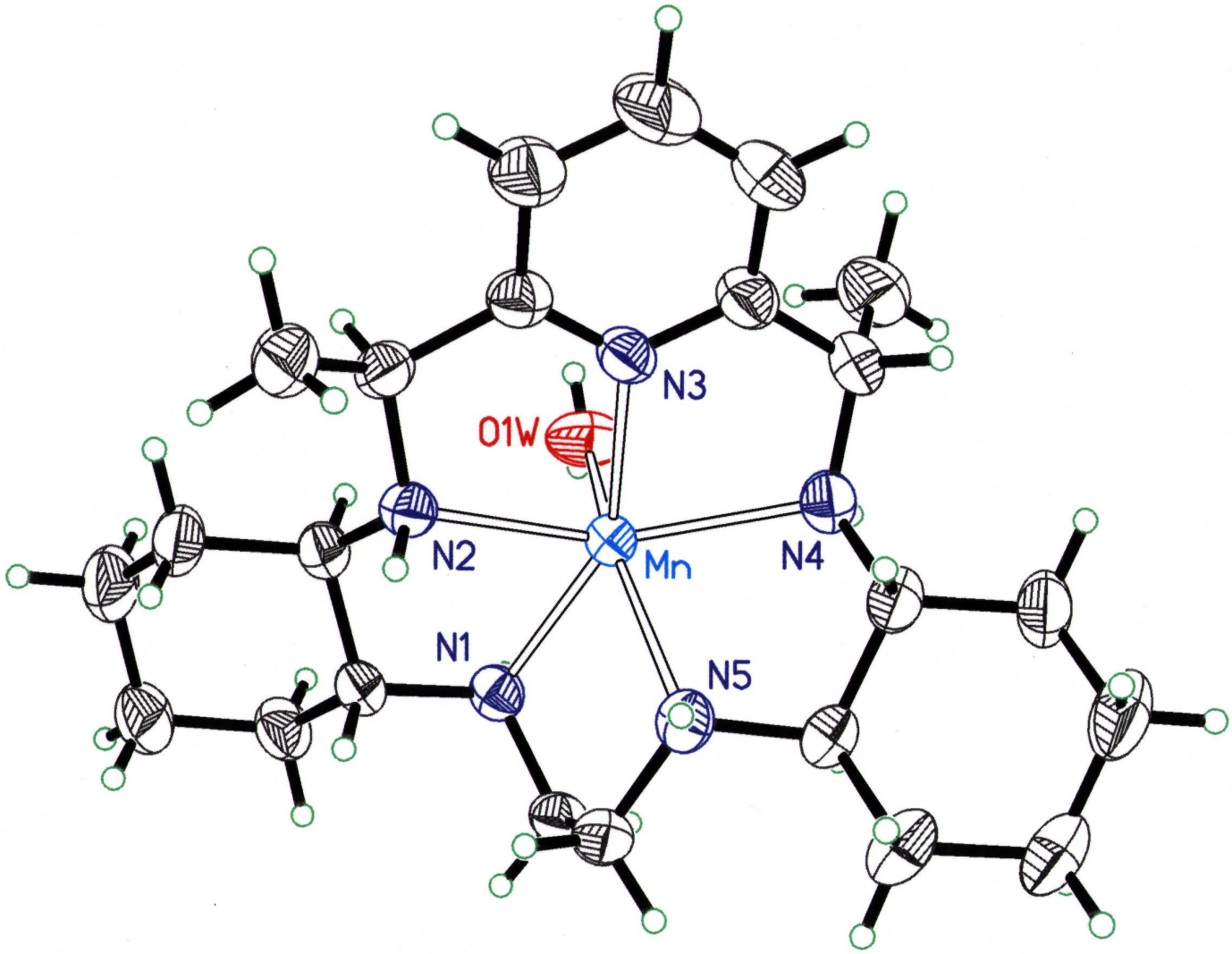
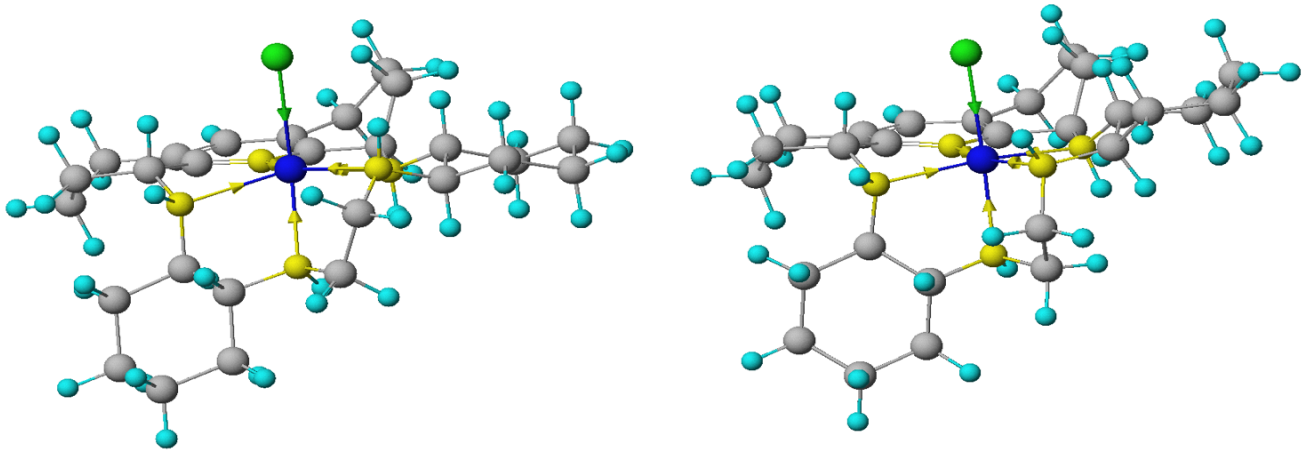


Figure 5. Ligands utilized for testing the predictive ability of the folding paradigm for MM calculations.

Crystal Structure of M40403



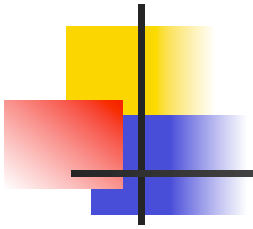
Energy Minimized Structures for M40403



Mn(II): E = 32.2 kcal **Mn(III): 31.0 kcal**

The folded six-coordinate Mn(II) structure is very close to that of the six-coordinate Mn(III) indicating a favorable geometry for facile electron transfer & hence fast SOD catalysis. (*Science, 286*, 304 (1999)).

RATIONALIZATION FOR CATALYSIS



Marcus – lower reorganizational energy correlates with lower activation energy and higher rates of catalysis

Pauling – enzyme catalysis is based on stabilization of the transition state

MECHANISMS FOR CONVERSION OF SUPER-OXIDE TO HYDROGEN PEROXIDE VIA INNER SPHERE OR OUTER SHERE OXIDATION OF MN(II)

D. P. Riley, Susan L. Henke, Patrick J. Lennon and Karl Aston, Inorg. Chem. 38:1908-1917, 1999

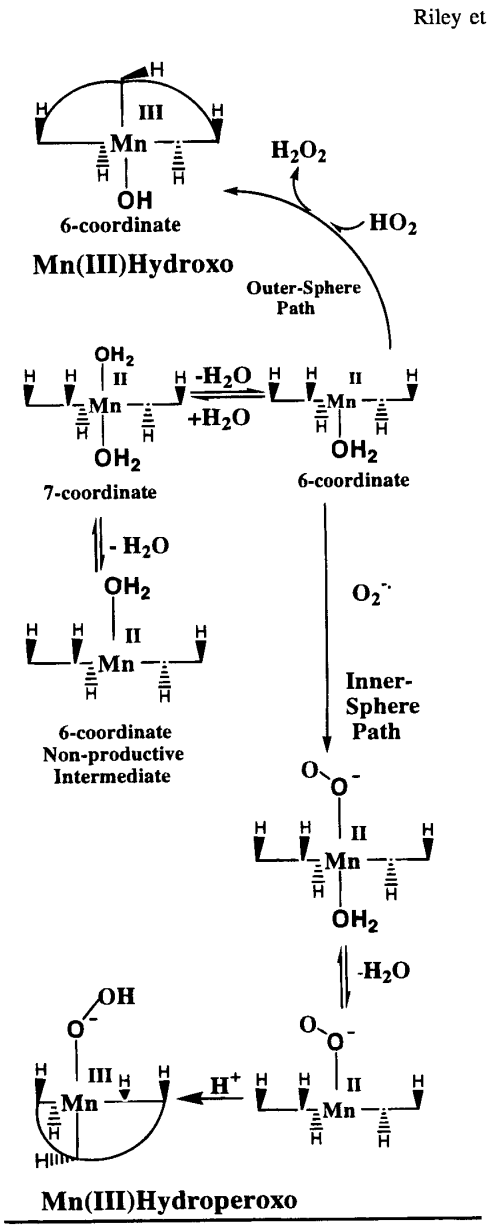
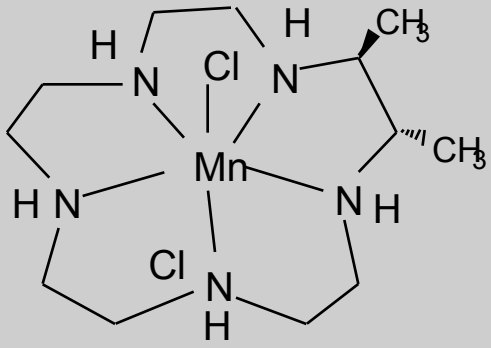


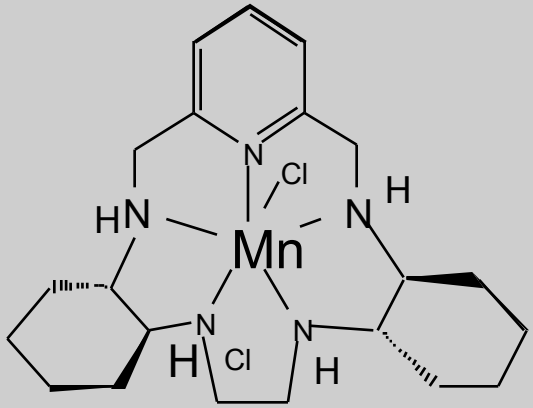
Figure 5. Mechanistic scheme depiction for the formation of a unique six-coordinate intermediate complex which gives rise to both the inner-sphere and outer-sphere pathways for oxidation of Mn(II).

Development of the SOD Mimic: Advances in Chemistry (from SC-68328 to M40403)



SC-68328

6000-fold stability increase, while retaining catalytic activity

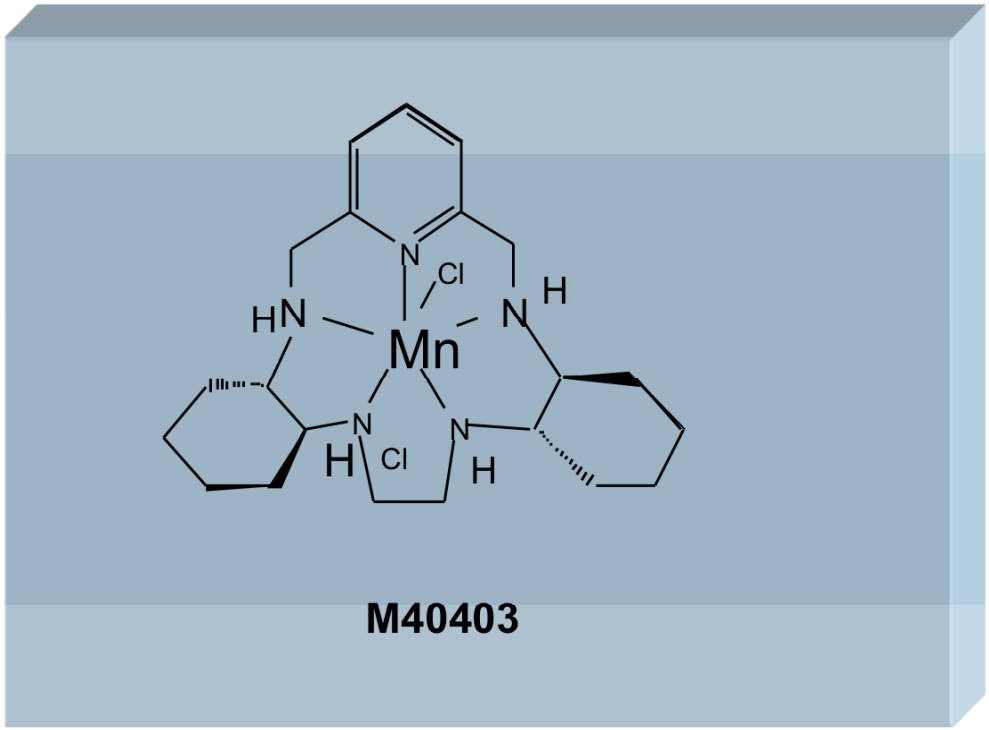


M40403

M40403 – Lead Candidate Properties

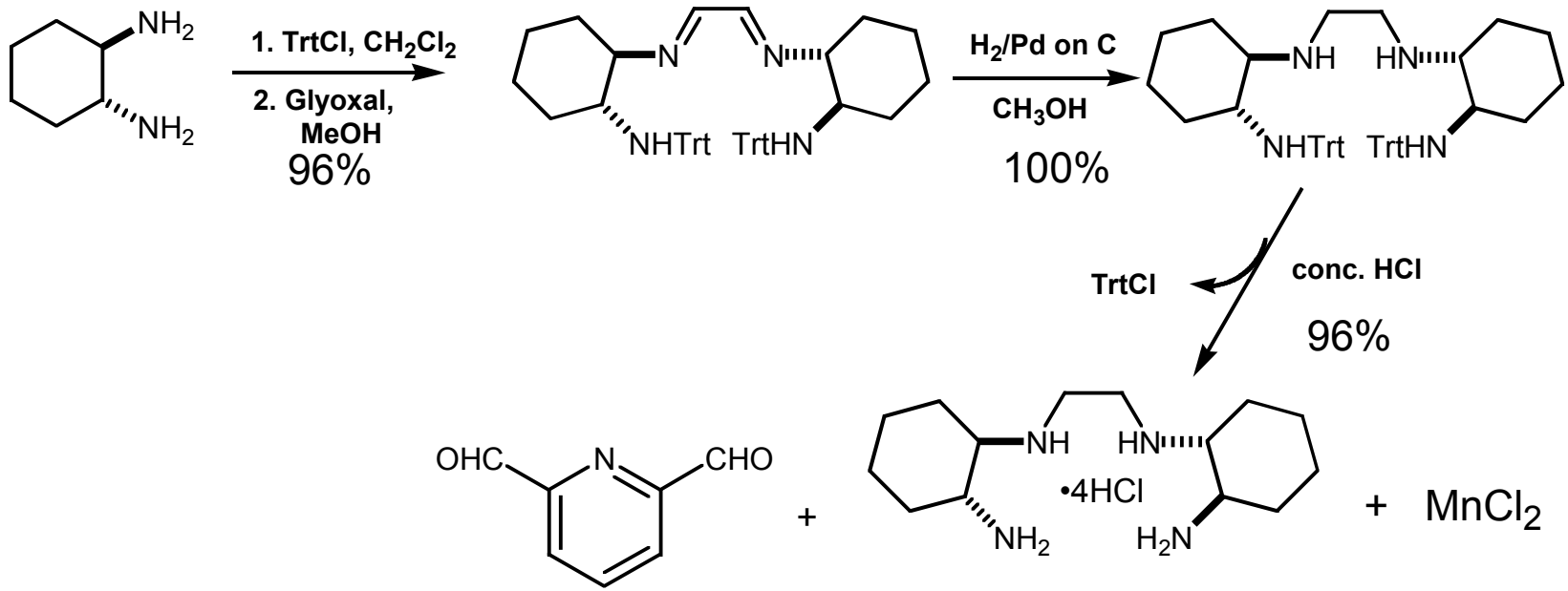
Manganese-containing biscyclohexylpyridine mSOD

- **Catalytic activity equivalent to that of the native enzyme**
- **Very stable in vivo: extracted intact (97%) from plasma,urine, bile after iv dosing**
- **Protective in various models of acute and chronic inflammation**

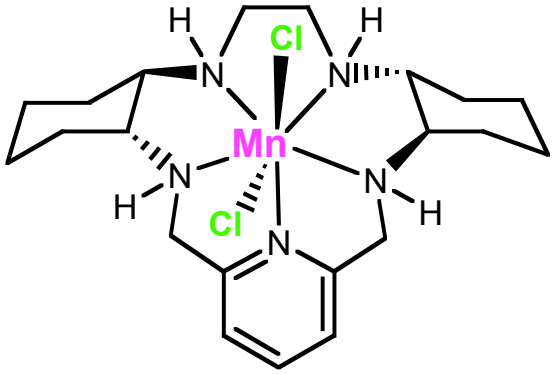


M40403

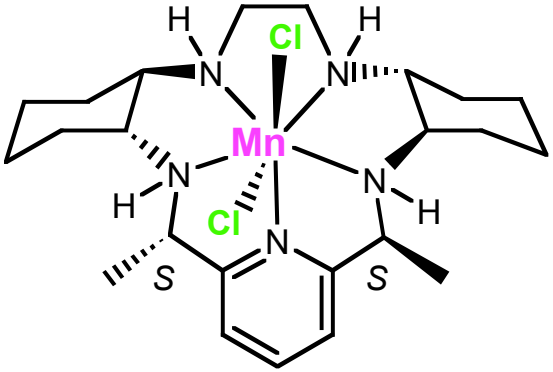
Synthesis of M40403



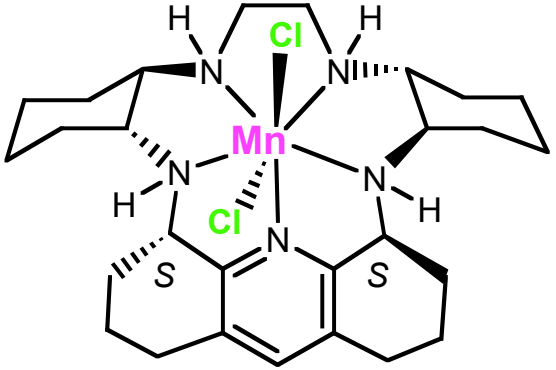
Molecular Modeling has made possible the design of more stable compounds



M40403



M40401



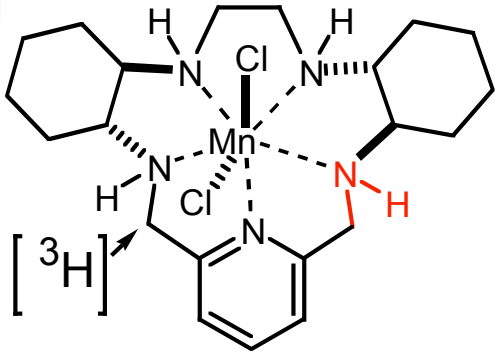
M40484

*Riley, et.al, *Inorg. Chem.*, **2001**, *40(8), 1779.*

Chemical History of SODm

- SAR studies utilizing the [Mn([15]aneN$_5$)Cl$_2$] lead reveals that extremely high chemical stability is achieved with added substituents to carbon centers of the macrocyclic ligand (*Inorg.Chem.,35,* 5213, 1996)
- SAR studies reveal that catalytic activity can be dramatically affected with the stereochemistry, number, and position of such substituents (JACS, 119, 995 (1997)).
- Mechanistic understanding developed that led to a molecular modeling paradigm (MM) allowing prediction of catalytic activity (*Inorg. Chem., 38*, 1908 (1999); and *ibid., 40*(8), 1779 (2000).)
- *Bis*-cyclohexylpyridine class of SOD mimic affords high activity and high stability with excellent *in vivo* efficacy (*Science, 286*, 304 (1999)).

Metabolism of M40403



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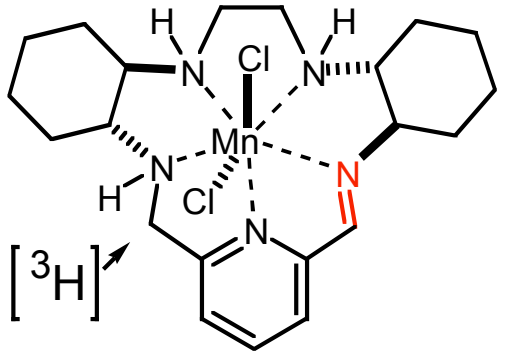
k_{cat} (pH=7.4) = 1.6 x 10^{+7}

log P = -0.3

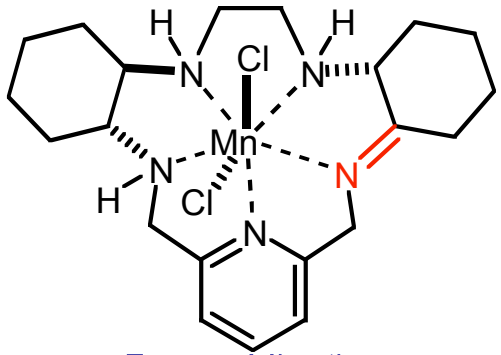
Liver Oxidases



M40414

k_{cat} (pH=7.4) = 1.35 x 10^{+7}

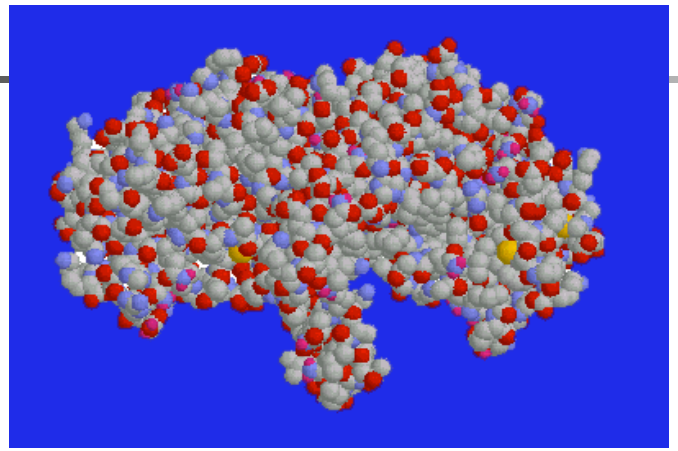
log P = -0.9



Enzyme Mimetic

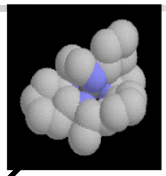
isomer

Native Enzyme



One of two subunits.

M40403



$O_2^{\bullet -}$

Inflammation

- Pain
- IBD/Crohn's
- Osteoarthritis
- Rheumatoid arthritis
- Psoriasis

Cardiovascular/CNS

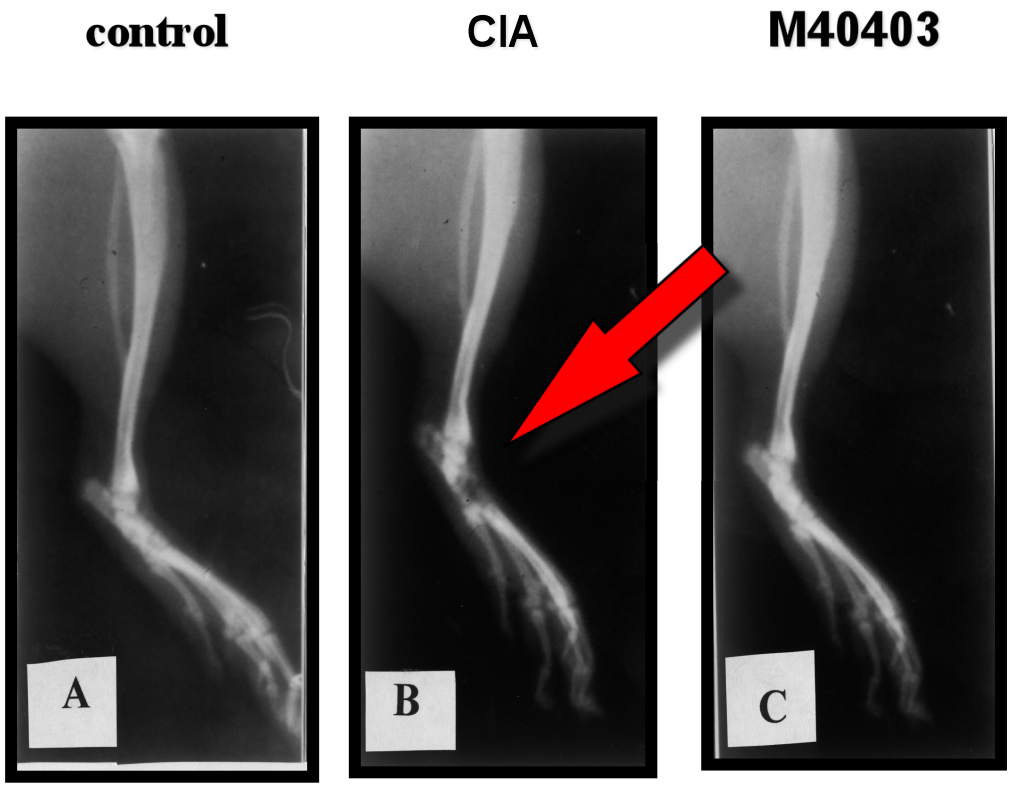
- Ischemia-reperfusion injury
- Septic shock
- Organ transplantation
- Stroke

Oncology

- Pain
- Inhibition of tumor growth
- Reduction of side effects associated with chemotherapy and radiation therapy

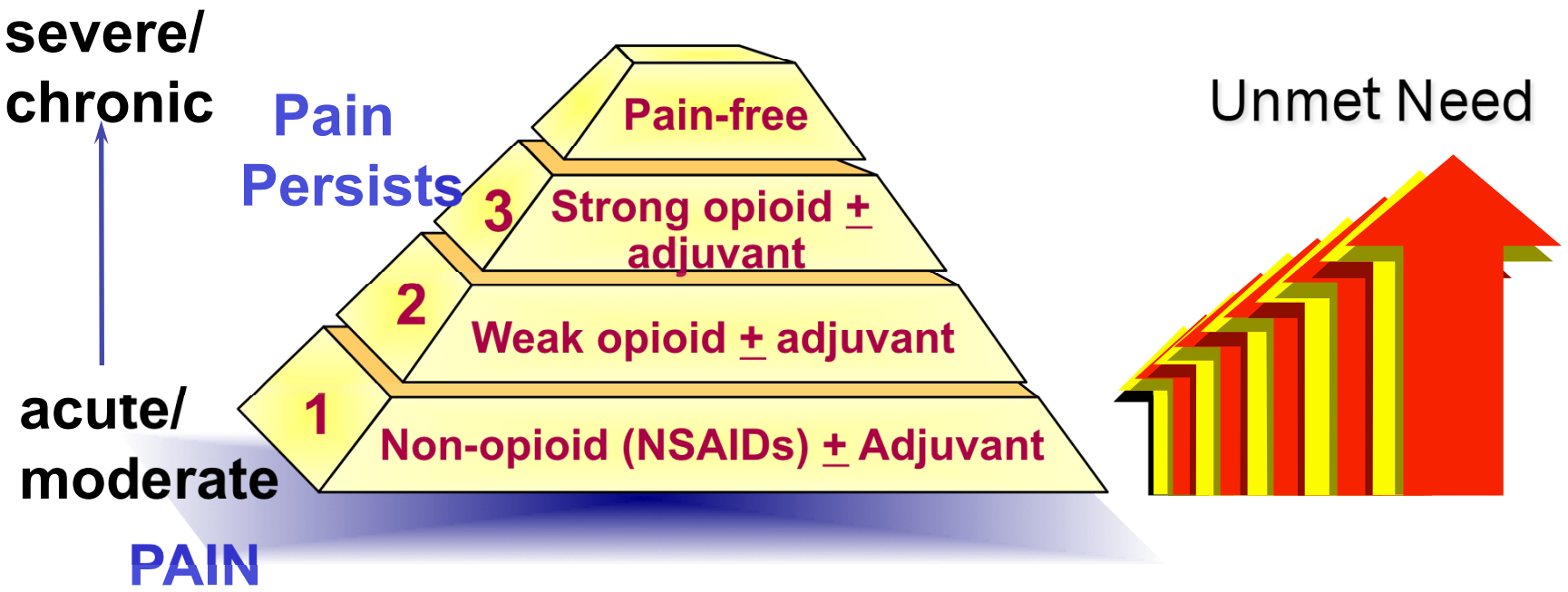
Inflammation

- Superoxide – central role
- SOD mimetics effective in multiple models
  - Rheumatoid arthritis
    - Blocks TNFα release
  - Asthma
- Many diseases



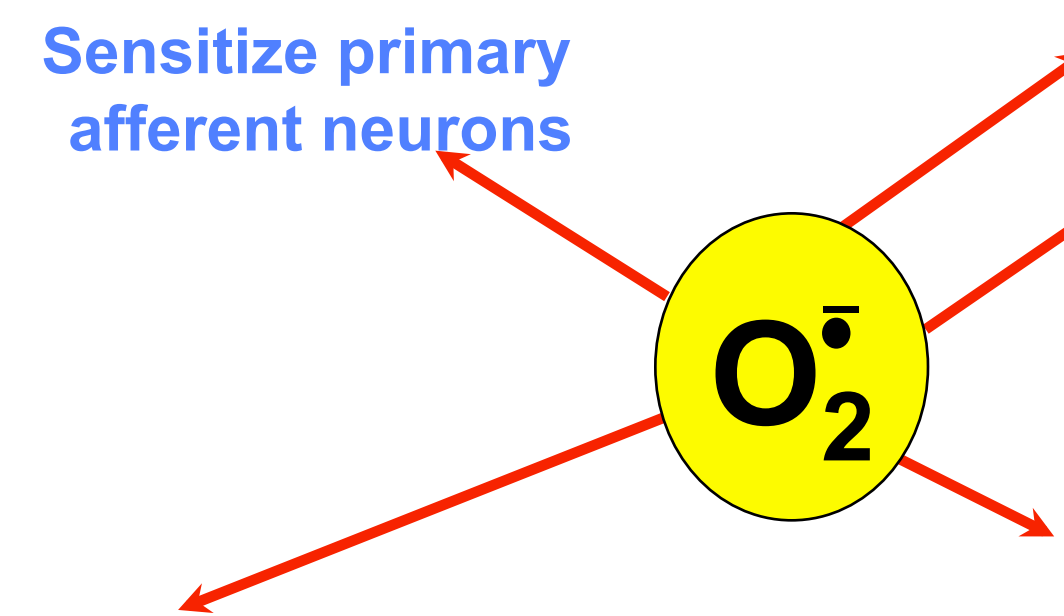
Collagen-induced Arthritis

The WHO Analgesic Pyramid



- Traditional NSAIDs provide unsatisfactory relief in models of severe chronic pain (neuropathic pain, visceral pain, cancer pain)
- Opportunities exist beyond opioids and NSAIDs for the treatment of acute and severe pain

Roles of Superoxide Anions ($O_2^{\bullet -}$) in Pain



Sensitize primary afferent neurons

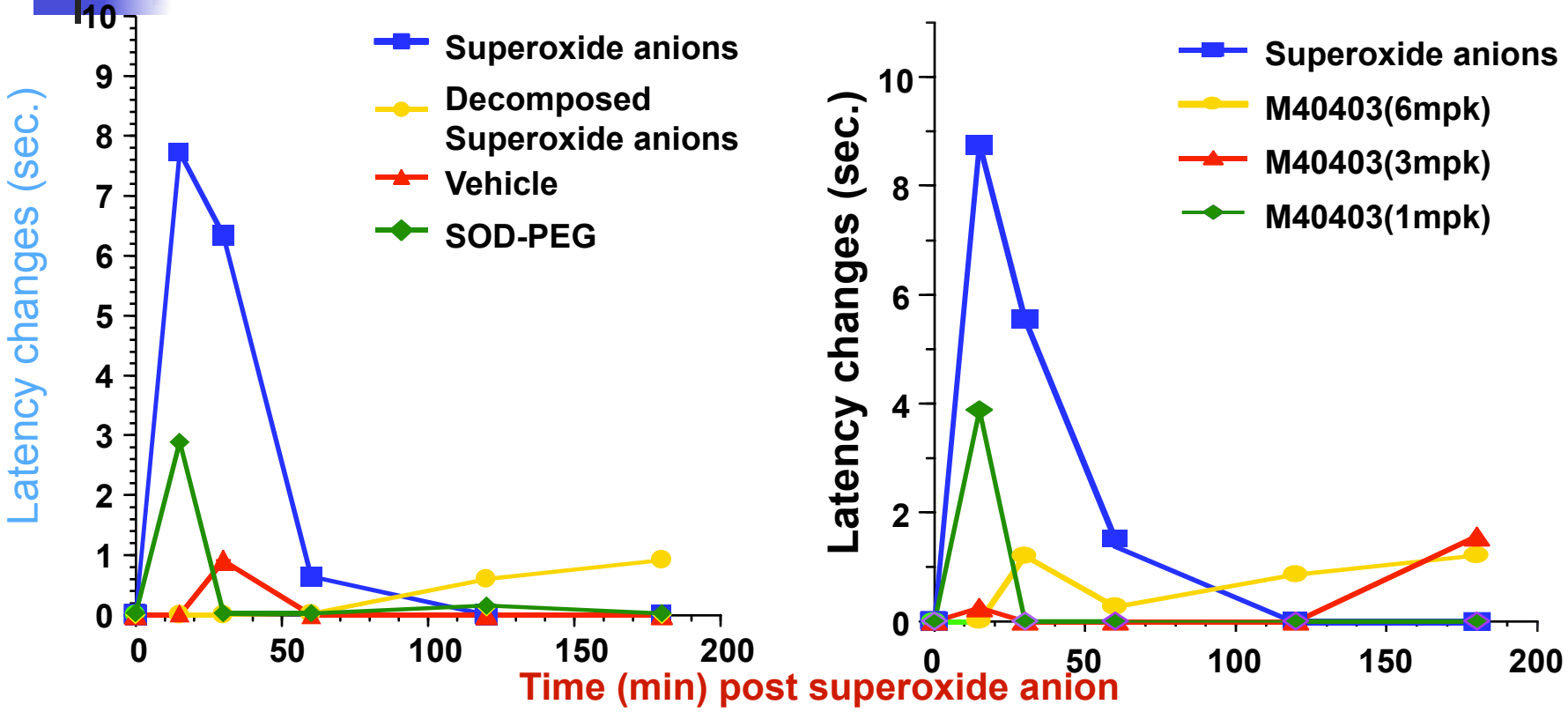
Damage nerve endings

Direct algesic action

Increase spinal release of excitatory amino acids (NMDA)

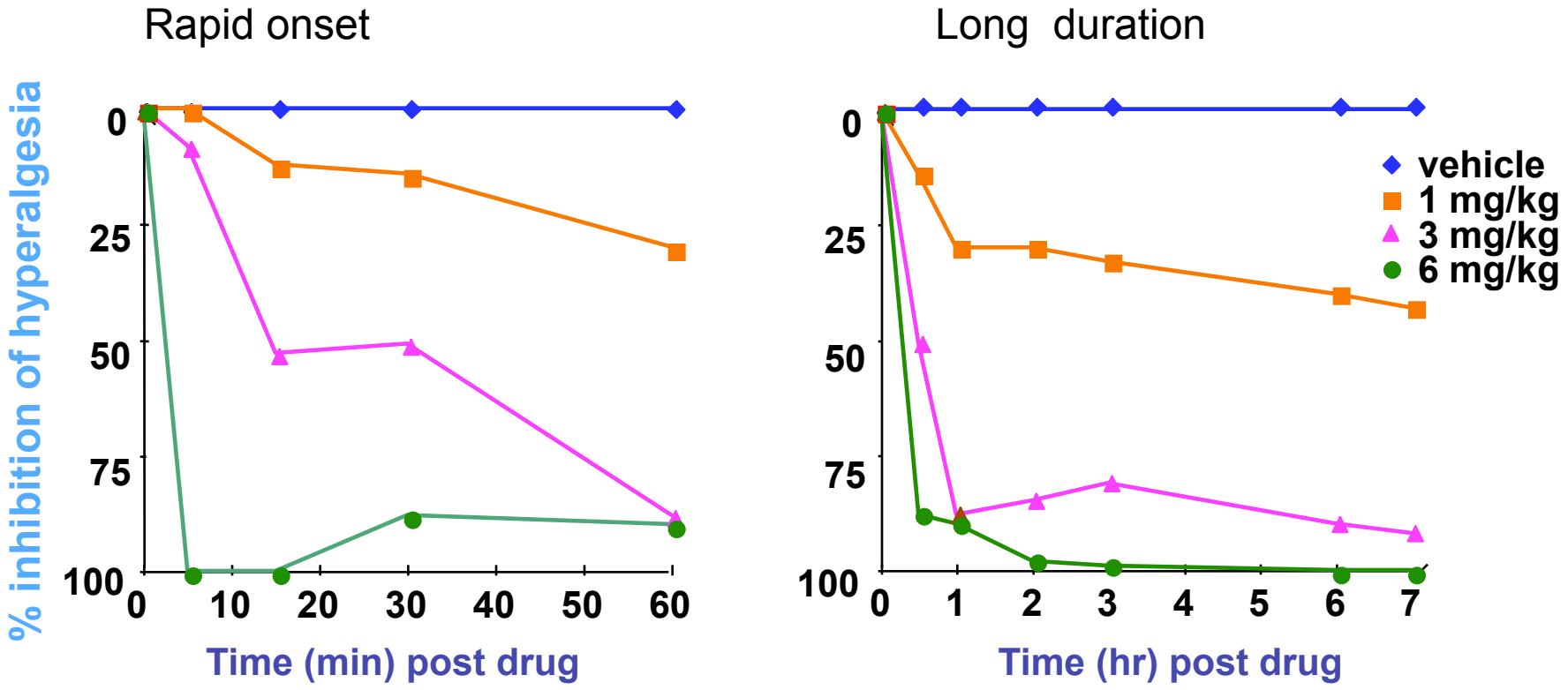
Deactivate NE, a potent endogenous analgesic

Intraplantar Injection of Superoxide Anion Evokes Pain in Rats



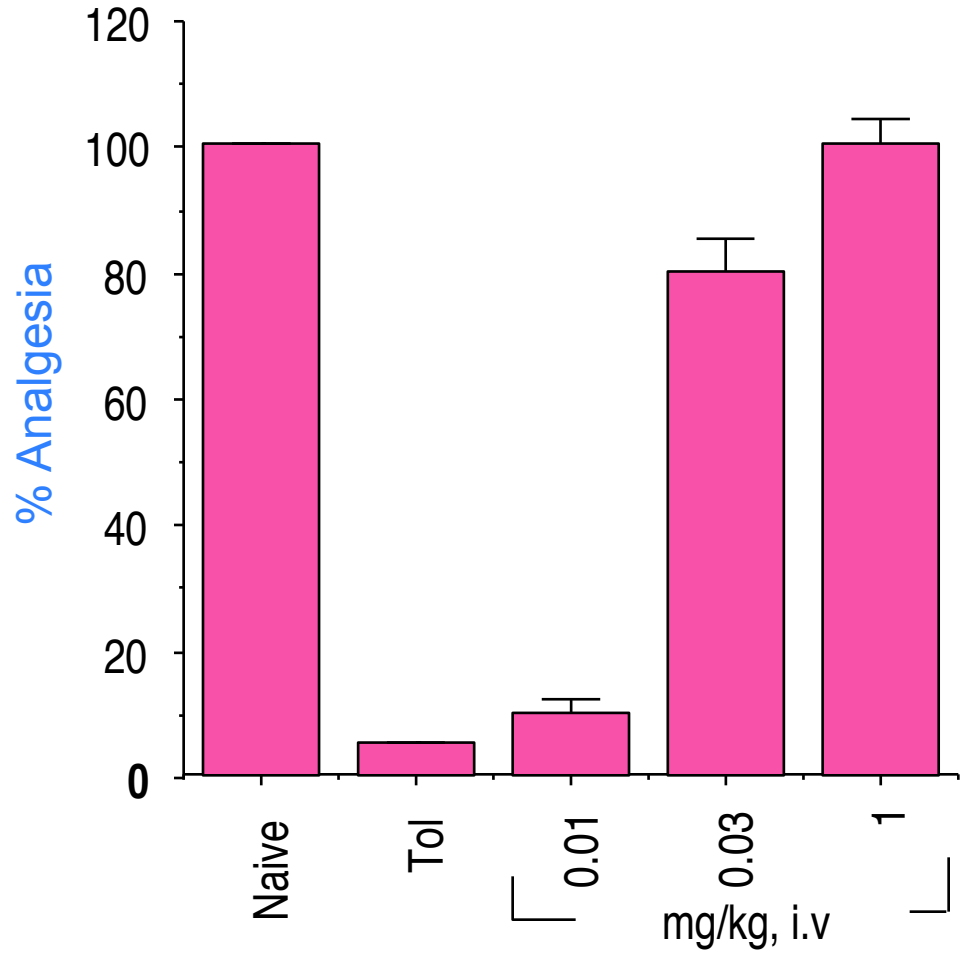
M40404 which lacks SODm activity does not block this response.
Drugs given i.v. 15min before challenge

Reversal of Acute Hyperalgesia by M40403 in Rat Footpad Carrageenan Model



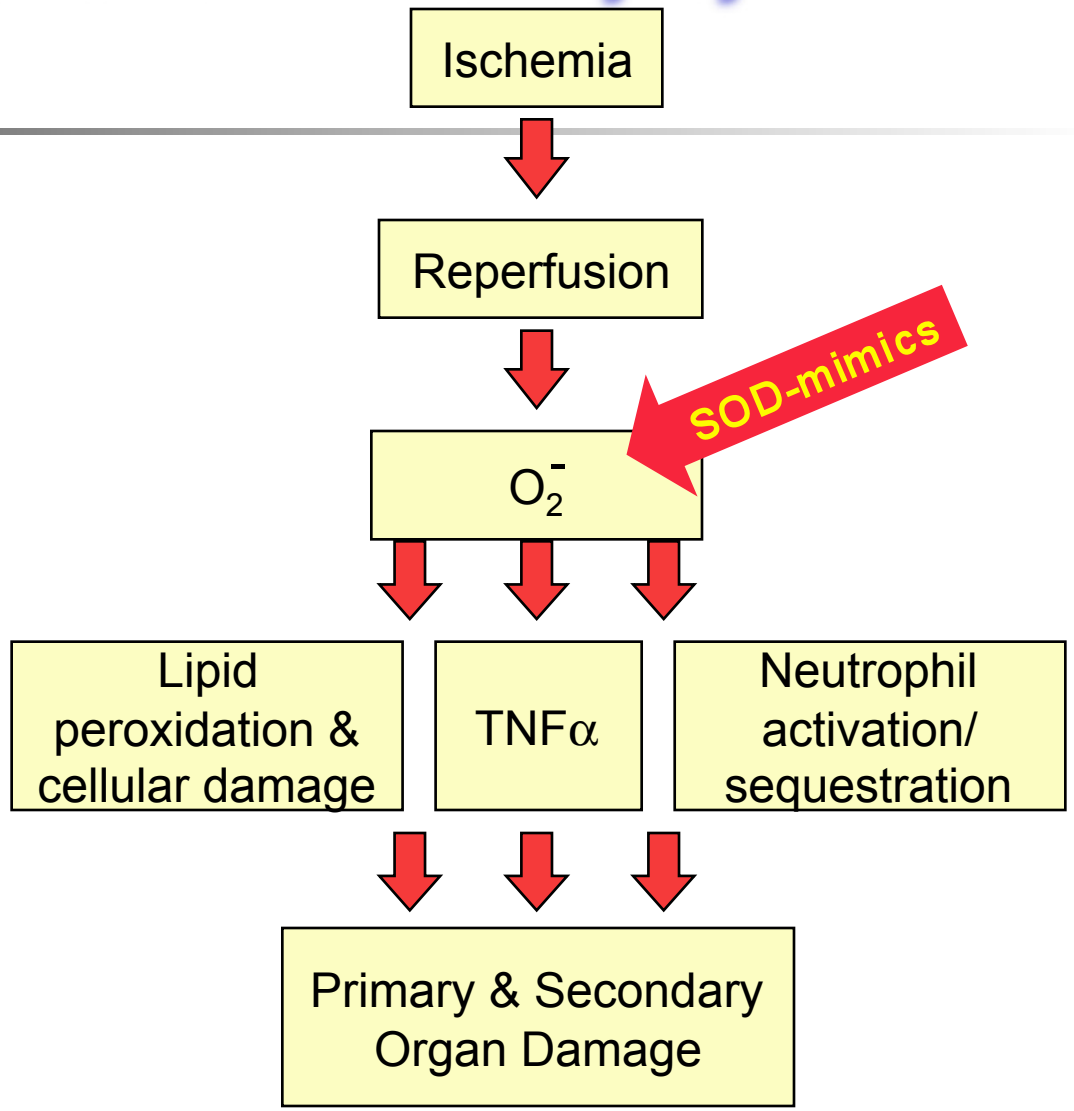
drug administered iv 3 hr after carrageenan

M40403 Restores Analgesia in Morphine-tolerant Mice

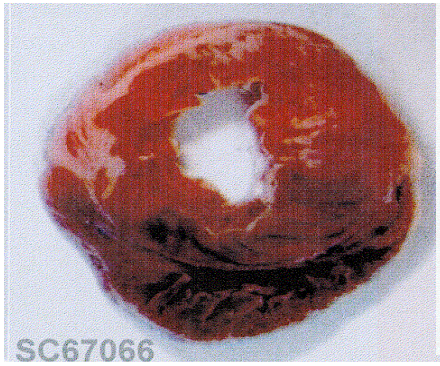


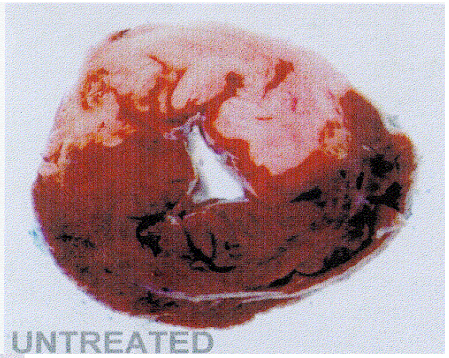
Compound given 5 min prior to morphine

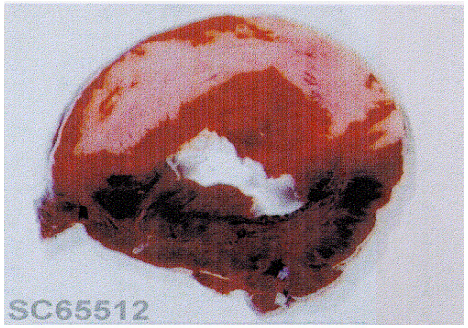
Mechanism: SOD-mimics in Ischemia – Reperfusion-Mediated Injury



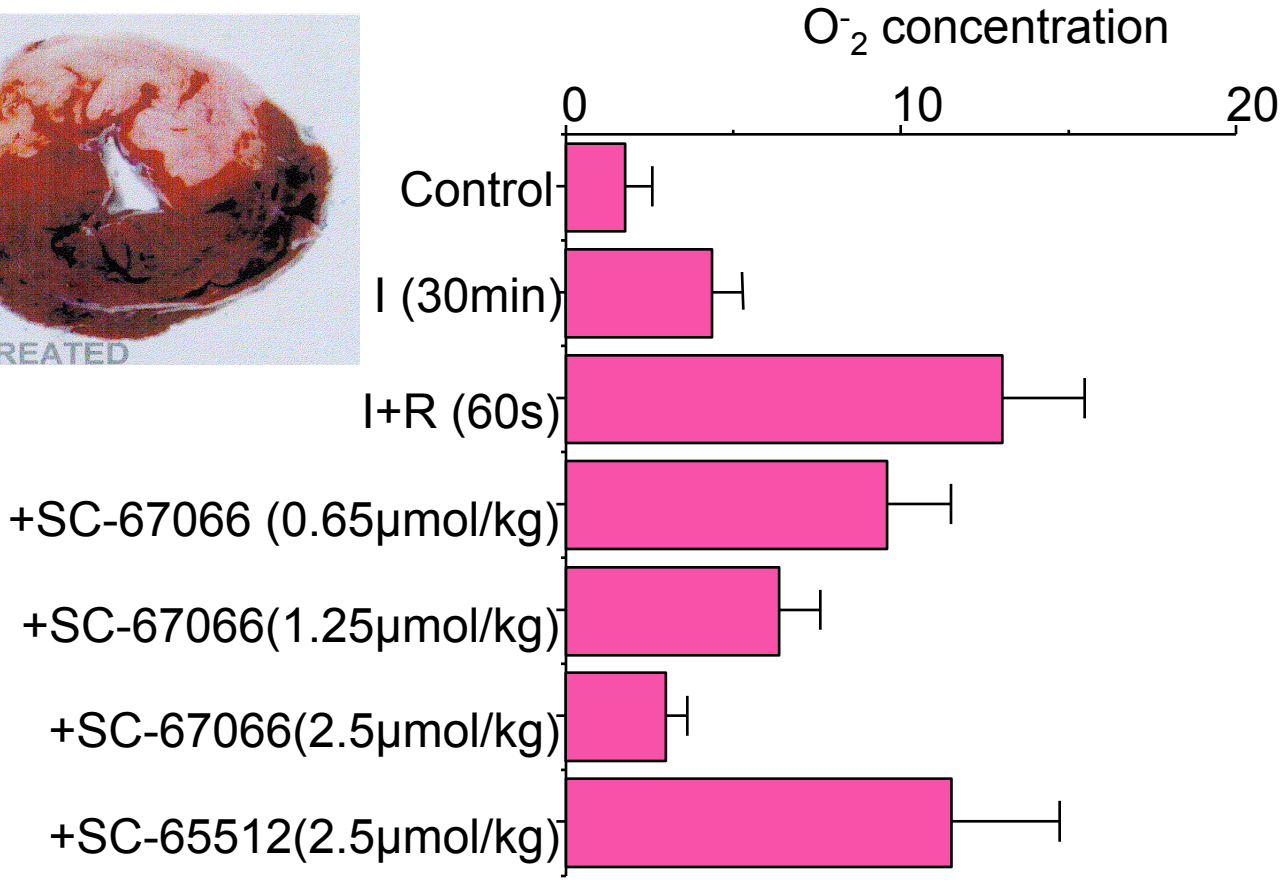
SC-67066 Inhibits the Burst of O_2 Release and Reduces Myocardial Damage upon Reperfusion of the Ischemic Rabbit Heart



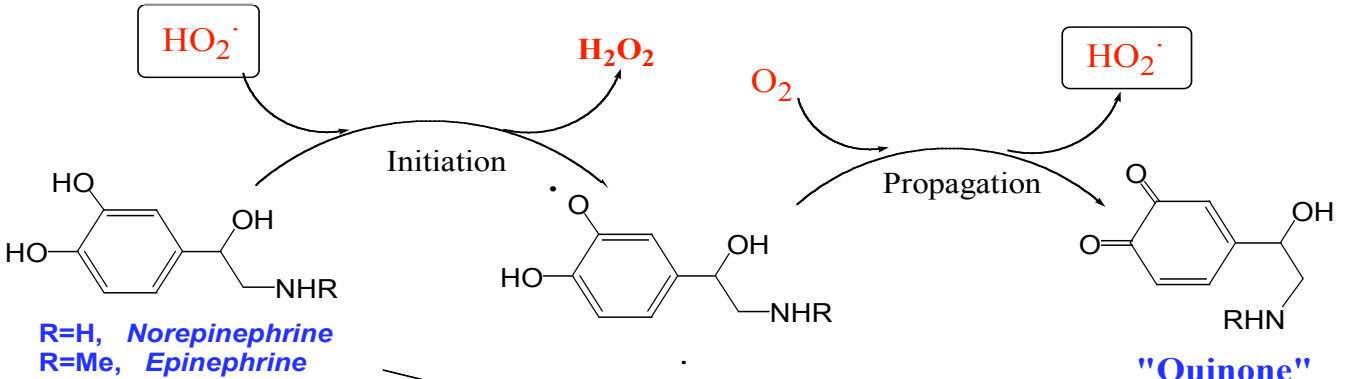




Inactive SODm

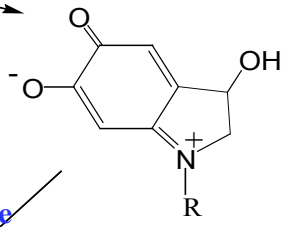


Catecholamine Autoxidation



OONO⁻

Stoichiometric oxidation



A Single Reaction with $HO_2^{\cdot}$ Initiates a "Chain Autoxidation" Affording Multiple Conversions to "Adrenochromes"!

Polymerize

"Adrenochromes"
Cytotoxic

Crosslinked Proteins

Melanin

Salvemini, MacArthur, Riley, ***PNAS*, 2000, 97(17), 9753**

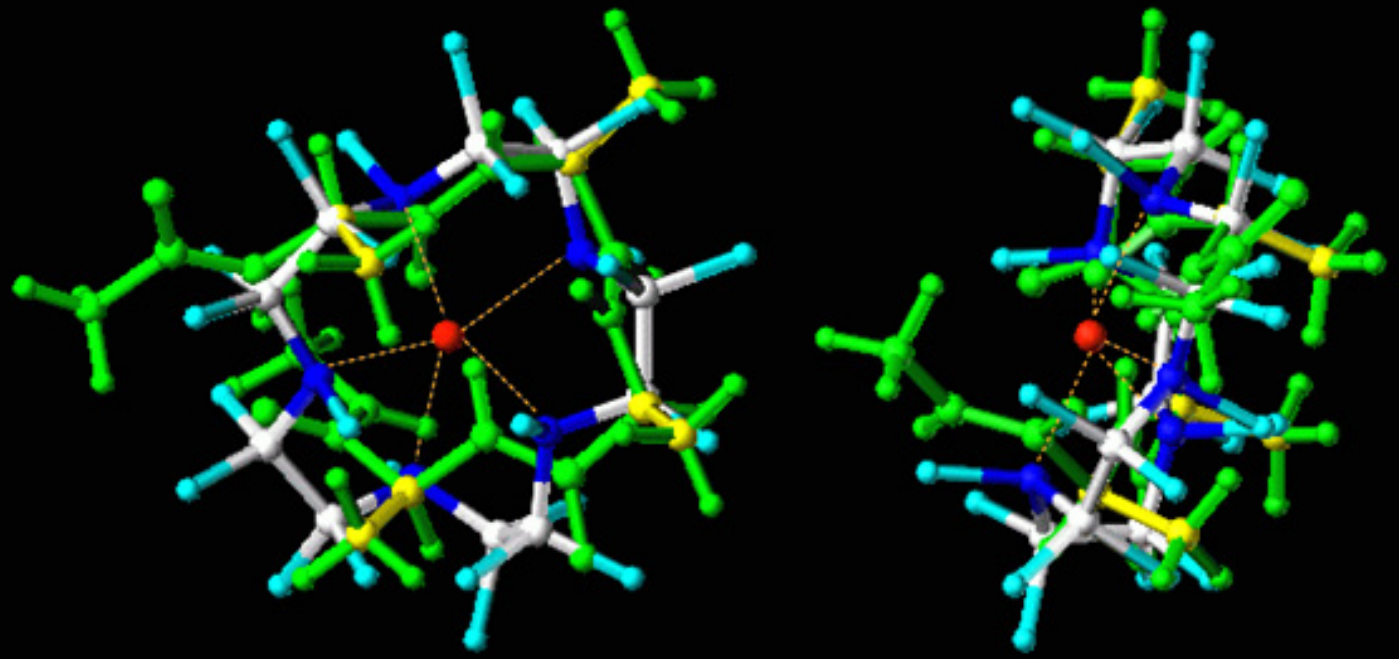
Targeting SOD mimetics to Receptors

Use of Chiral Metal-Azacrowns as Peptidomimetics

Marshall et al., CCB, WUMS, St. Louis

Reaka et al. J. Comput.-Aided Mol. Des. (2003)

PAC Conformational Template vs. Type I β-Turn

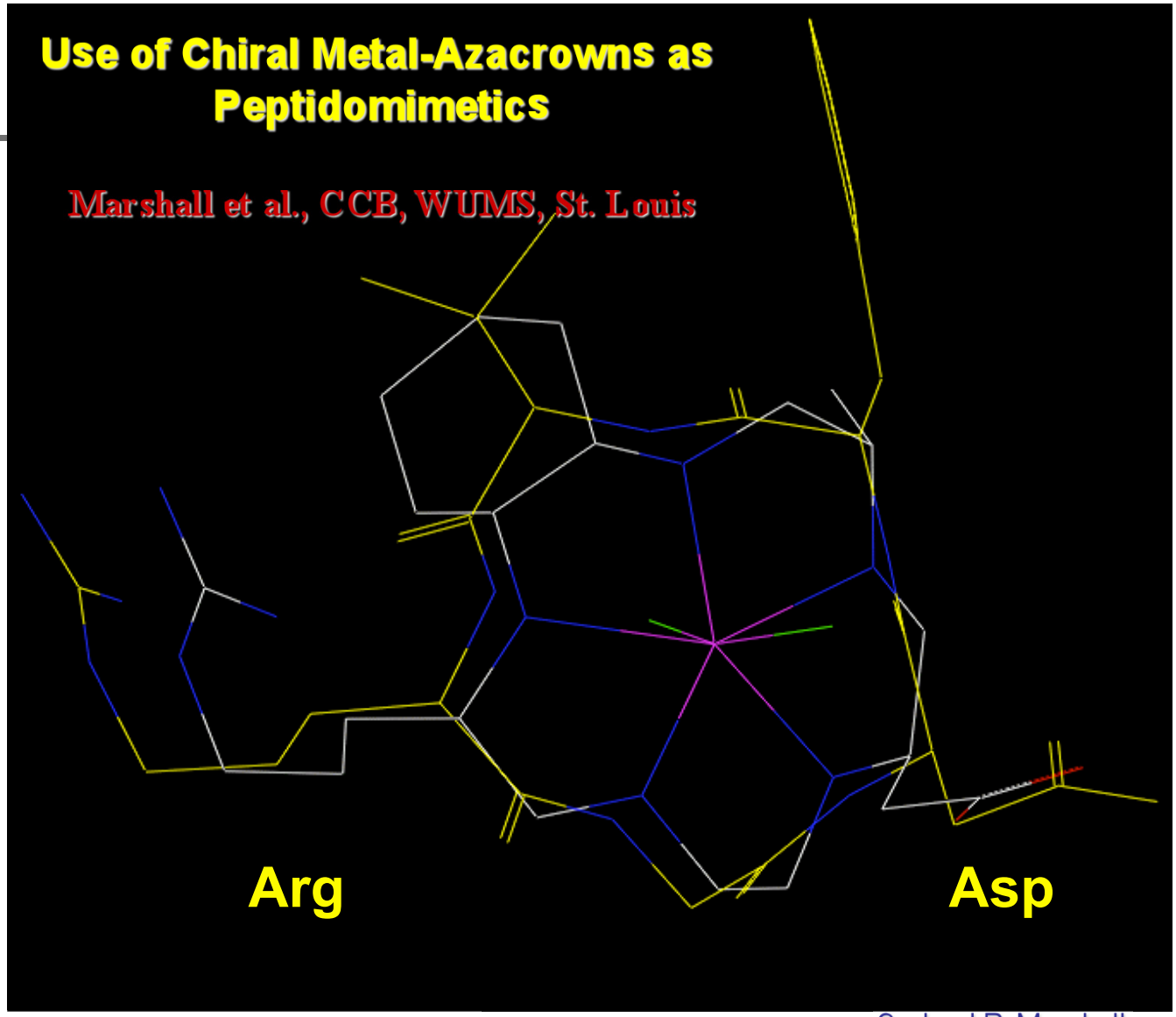


Pentaazacrown complexed with Mn(II, red ball) showing overlap of i, i+1, and i+2 side-chain orientations (yellow)

Targeting SOD mimetics to Receptors

RGD Bioactive Conformation Mimicked by Chiral MAC

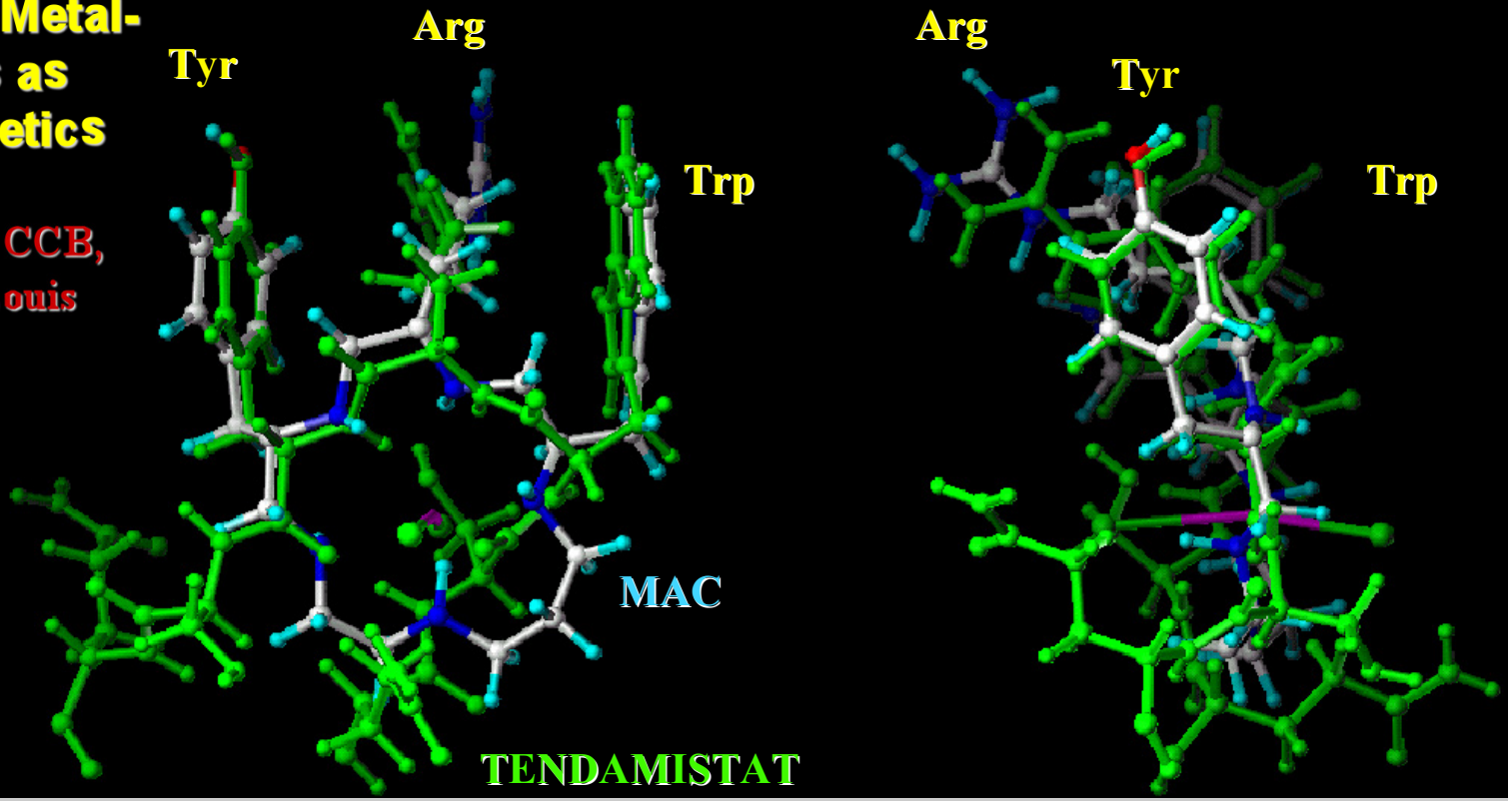
Peptide Backbone (yellow): cyclo(Arg-Gly-Asp-D-Val-Phe) Overlay: PAC - cyclo(Arg-Gly-Asp-cyclohex-Ala)



Targeting Metal Complexes to Receptors

Use of Chiral Metal-Azacrowns as Peptidomimetics

Marshall et al., CCB, WUMS, St. Louis



MIMICRY OF CHIRAL MAC WITH WRY SEQUENCE OF TENDAMISTAT THAT INHIBITS α-AMYLASE

ACKNOWLEDGMENTS

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Dr. Daniela Salvemini, Chief Pharmacologist

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Dr. Todd Johnson, Development

Dr. Otto F. Schall, Chemistry

Dr. Bobby N. Trawick, Chemistry

MetaPhore Pharmaceuticals – http://www.metaphore.com
For further information – contact Alan Dunton, M. D., President and CEO, adunton@metaphore.com

Entropy and Solvation

1. Lafont, V., A.A. Armstrong, H. Ohtaka, Y. Kiso, L. Mario Amzel, and E. Freire, *Compensating enthalpic and entropic changes hinder binding affinity optimization.* Chem Biol Drug Des, 2007. **69**(6): p. 413-22.

2. Young, T., R. Abel, B. Kim, B.J. Berne, and R.A. Friesner, *Motifs for molecular recognition exploiting hydrophobic enclosure in protein-ligand binding.* Proc Natl Acad Sci U S A, 2007. **104**(3): p. 808-13.

Structured Waters

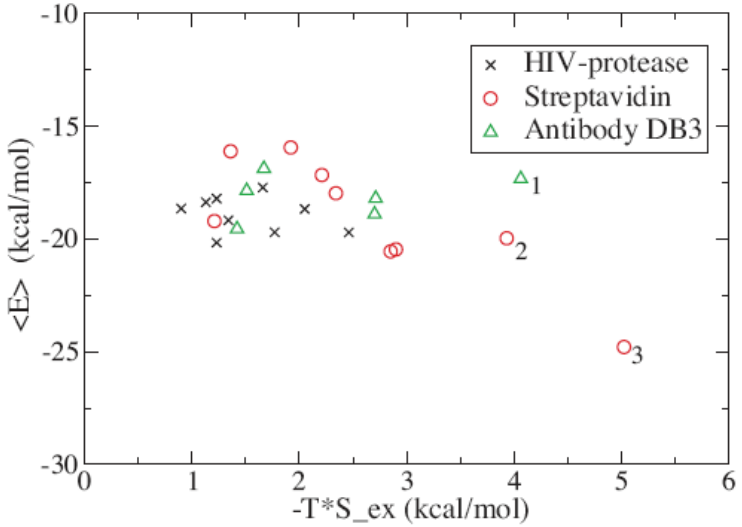

Fig. 1. World energies and excess entropies of water molecules in the principal hydration sites of the binding cavities. The world energy is the energy of interaction of the water molecules with the entire system. Shown are data for principle hydration sites that are proximal to hydrophylic protein groups. The points labeled 1, 2, and 3 represent data for hydration sites with unusually high ordering.

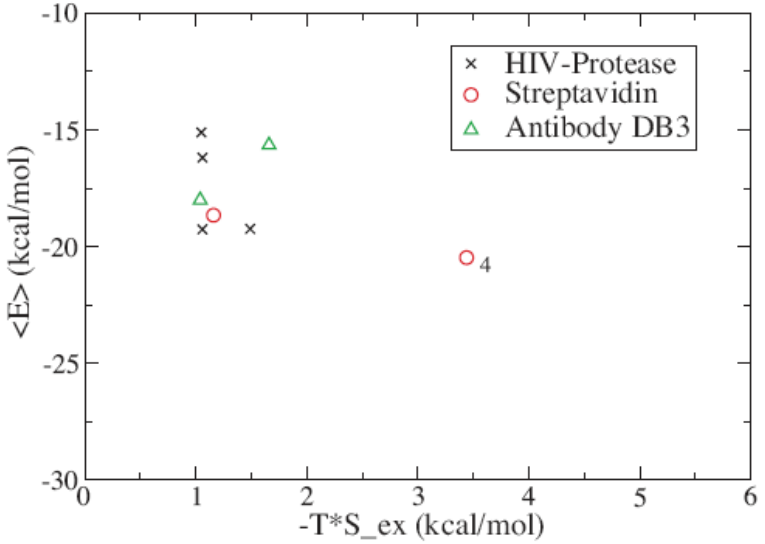

Fig. 2. World energies and excess entropies of water molecules in the principal hydration sites of the binding cavities. The world energy is the energy of interaction of the water molecules with the entire system. Shown are data for principle hydration sites that are proximal to hydrophobic protein groups. The point labeled 4 represents data for a hydration site with unusually high ordering.

Waters in Strepavidin

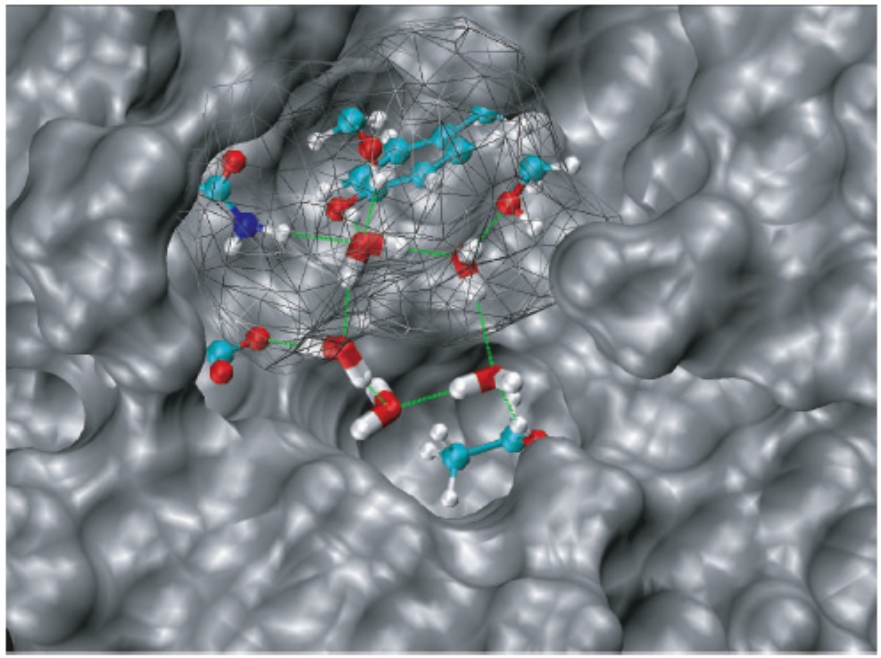

Fig. 3. The binding cavity of streptavidin and a typical solvating water configuration. Also shown is the protein structure that stabilizes the ring. The green lines represent hydrogen bonds. The hydrogen bonds between the ring water molecules and the protein are the correlated hydrogen bonds referred to in the text. The gray scaffolding is the protein that encloses the ring from above.

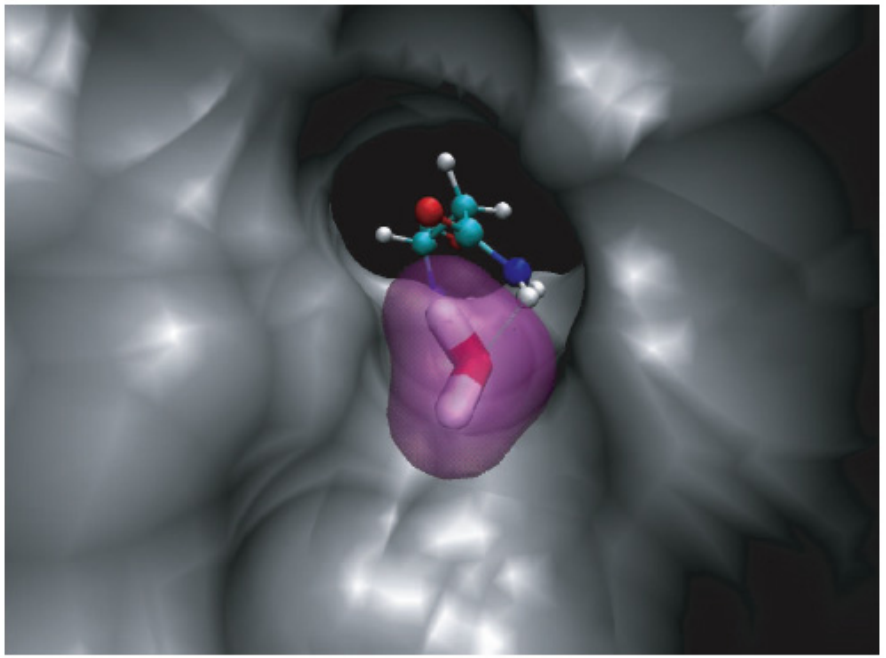

Fig. 5. A typical configuration for a water molecule in principle hydration site 1 from Fig. 1 in the 1DBJ binding cavity. The molecule is orientationally constrained such that its oxygen atom maintains a hydrogen bond with Asn-35. It is also flanked on three sides (to the left, right, and below) by hydrophobic groups. The two hydrogen bond vectors point toward additional solvent with which the water molecule can hydrogen bond. The purple shading is to the scale of a Van der Waals radius for a water molecule.

Waters in Strepavidin

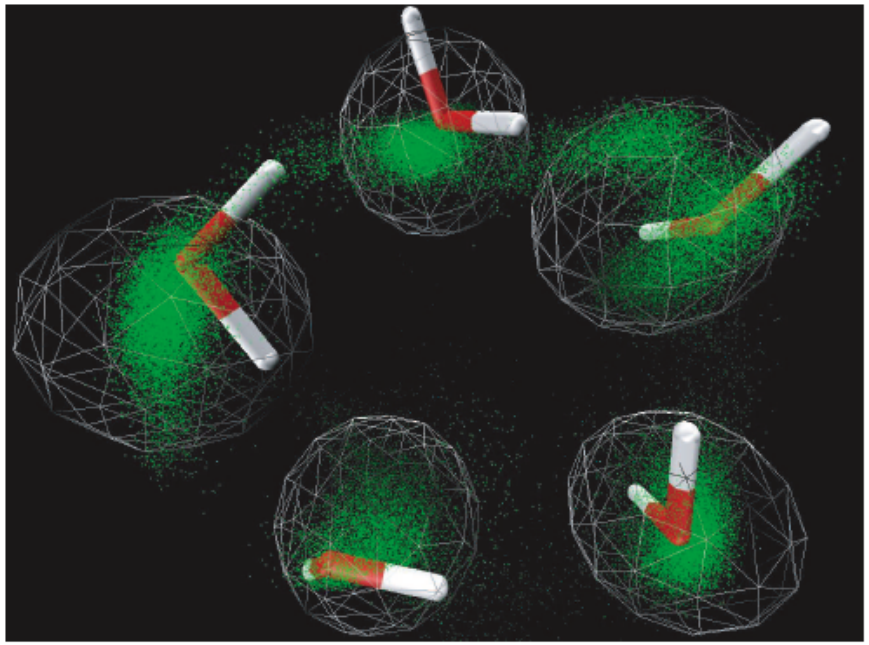

Fig. 4. Shown is the same conformation of the water molecules as is shown in Fig. 3 (from a different perspective) with the solvent density averaged over all simulations in green. The hydration sites determined by the clustering of this density are shown in wireframe. Note the near absence of water molecules in the inner part of the five-membered ring and in between the ring sites.

"Mutating Trp-79 to Phe was found to enthalpically stabilize biotin binding by 1.5 kcal/mol but entropically destabilize it by 2.4 kcal/mol (10). This mutation effectively enlarges the cavity and partially removes the hydrophobic enclosure, resulting in more entropically favorable binding-cavity solvation. This result explains why Poisson–Boltzmann-based methods, which cannot capture molecular-length scale solvation physics, underestimate the binding affinity, as measured by the disassociation constant, of the streptavidin–biotin complex by three to six orders of magnitude, whereas explicit solvent simulations predict the binding affinity within chemical accuracy (11–13)."