PHRM 836 September 10, 2015

Enzyme Catalysis: inhibition

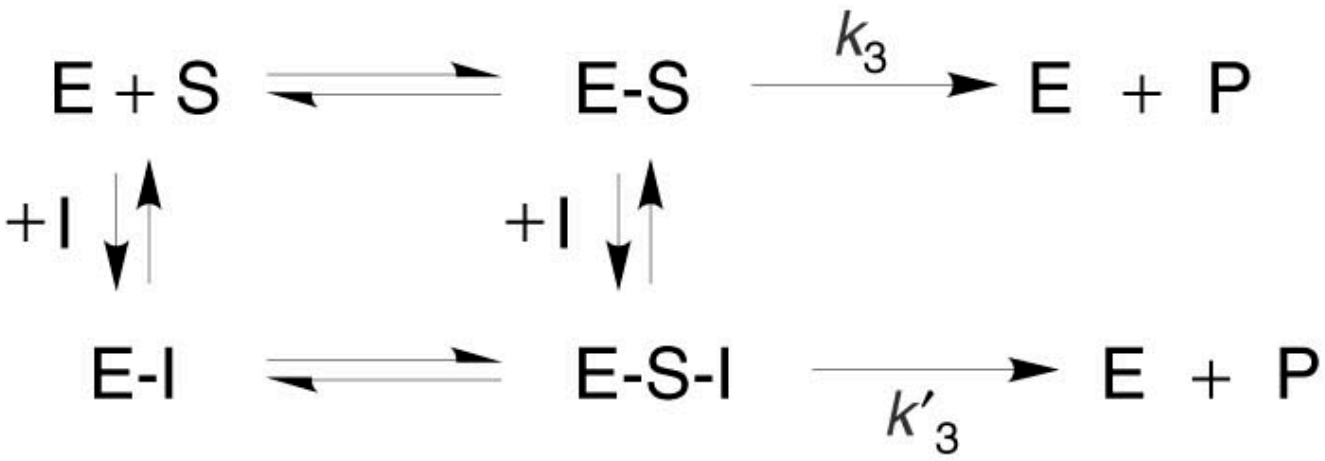
Devlin, section 10.10, 10.11, 10.9

1. Enzyme inhibition
   - Mechanisms
   - Changes in K_M and V_{max}
2. Enzyme inhibitors
   - Transition state analogues
   - Irreversible
   - Mechanism-based
3. Statins, structural insights

Enzymatic catalysis review topics

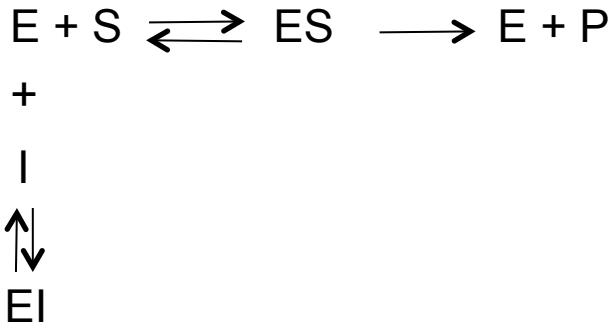
- Rate equations
- Michaelis-Menten equation
- V_{max}, K_m, k_{cat}, k_{cat}/K_m
- Lineweaver-Burk plot
- Basic ideas of enzyme inhibition and effect on kinetics
- Review Devlin 10.7

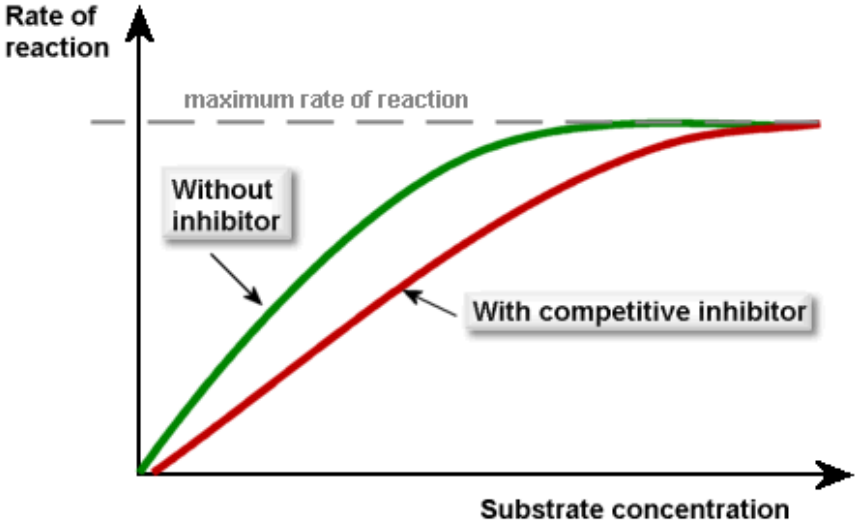
Enzyme inhibition



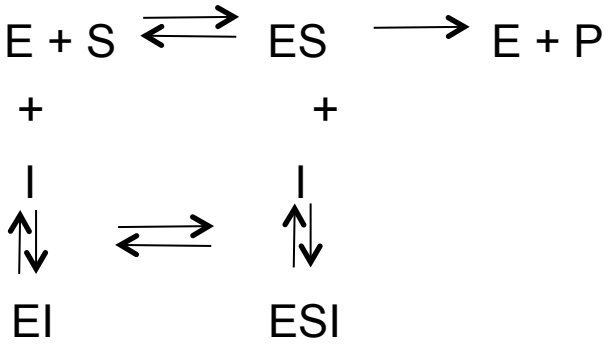
Enzyme inhibition

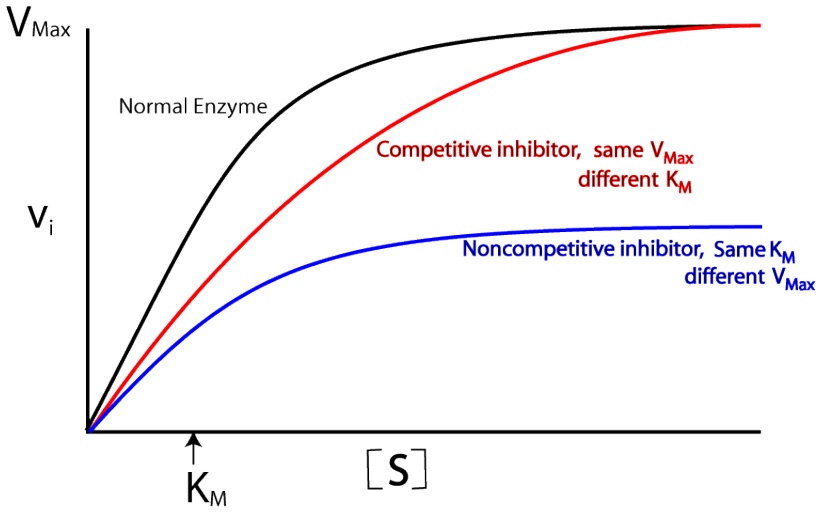
- Competitive inhibition





- Noncompetitive inhibition

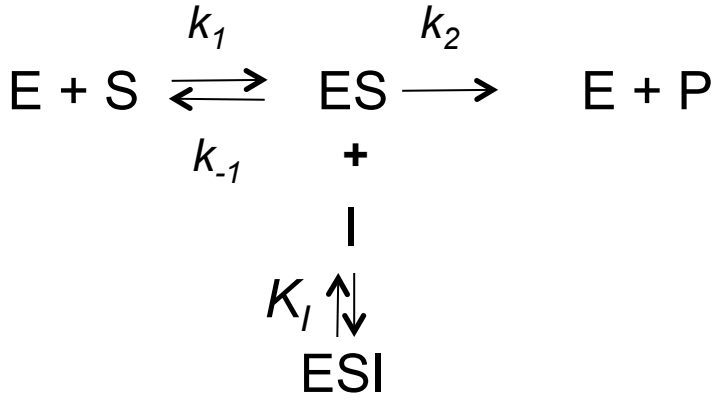


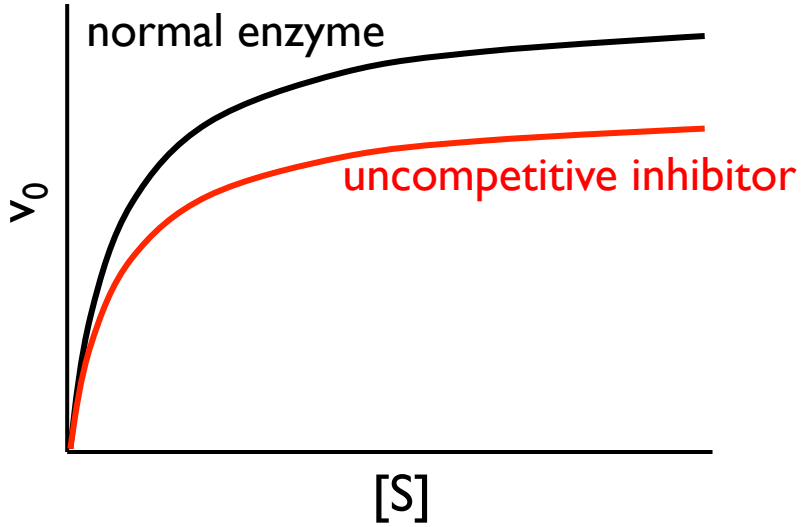


Plots from http://alevelnotes.com/Enzyme-Inhibitors/148

Enzyme inhibition

- Uncompetitive inhibition





V_{max} and K_M decrease

K_M/V_{max} unchanged

Effects via Lineweaver-Burk

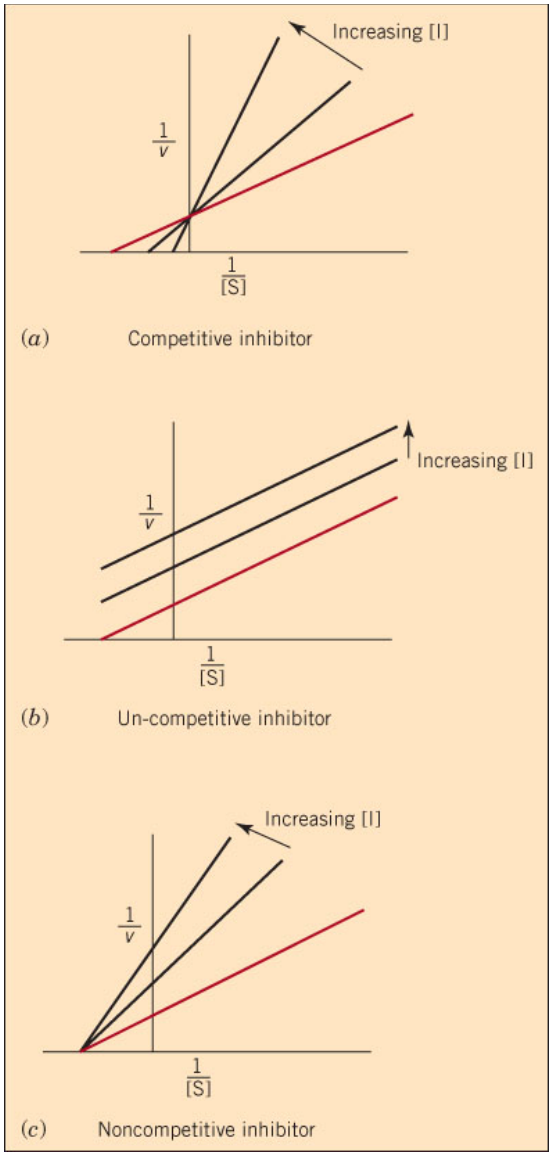
Uninhibited enzyme kinetics

$$v_0 = \frac{V_{\max}[S]}{K_M + [S]} \qquad \frac{1}{v_0} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{[S]}$$

Inhibited enzyme kinetics

intercepts; slope: give
<u>apparent</u> V_{max} and K_M

<u>apparent</u> V_{max} and K_M values
change by $(1+[I]/K_I)$

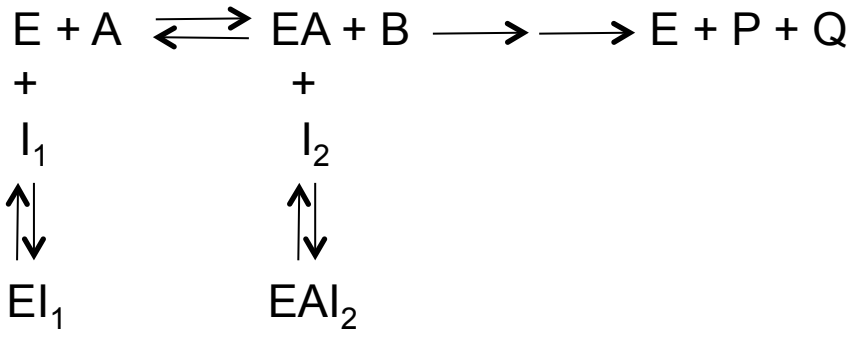


Apparent catalytic constants due to inhibition

Inhibition Type	K_M^{app}	V_{max}^{app}
No inhibitor	K_M	V_{max}
Competitive (inhibitor binds only free E)	$K_M\left(1+\frac{[I]}{K_I}\right)$	V_{max}
Non-competitive (inhibitor binds free E and ES complex with equal affinity)	K_M	$V_{max}\Big/\left(1+\frac{[I]}{K_I}\right)$
Uncompetitive (inhibitor only binds to ES complex)	$K_M\Big/\left(1+\frac{[I]}{K_I}\right)$	$V_{max}\Big/\left(1+\frac{[I]}{K_I}\right)$

$$K_I = \frac{[enz][I]}{[enz \cdot I]}$$

Inhibition of two-substrate reactions



- Inhibitors of multiple-substrate enzymes usually bind E or EA
  - Binds E: I is competitive against A.
  - Binds EA: I is competitive against B

Inhibitor Types: Transition-state analogues

- Enzymes stabilize the transition state more than substrate or product.
- A compound resembling the transition state (transition-state analogue) should bind more tightly to the enzyme than a compound resembling the substrate.
- Should be an excellent strategy for drug design… but isn't always successful.

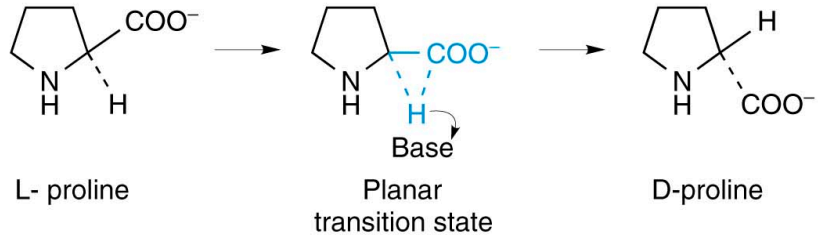
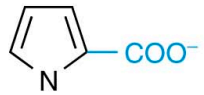


Figure 10.60

(*a*) Proline racemase reaction



High affinity inhibitor

(*b*) Pyrrole-2-carboxylate

Inhibitor Types: Irreversible inhibitors

- Compounds that chemically modify and inactivate an enzyme
- Usually bind competitively with substrate, and react with active-site surface residues, not necessarily catalytic residues
- Utilize the binding specificity of the target enzyme for selectivity

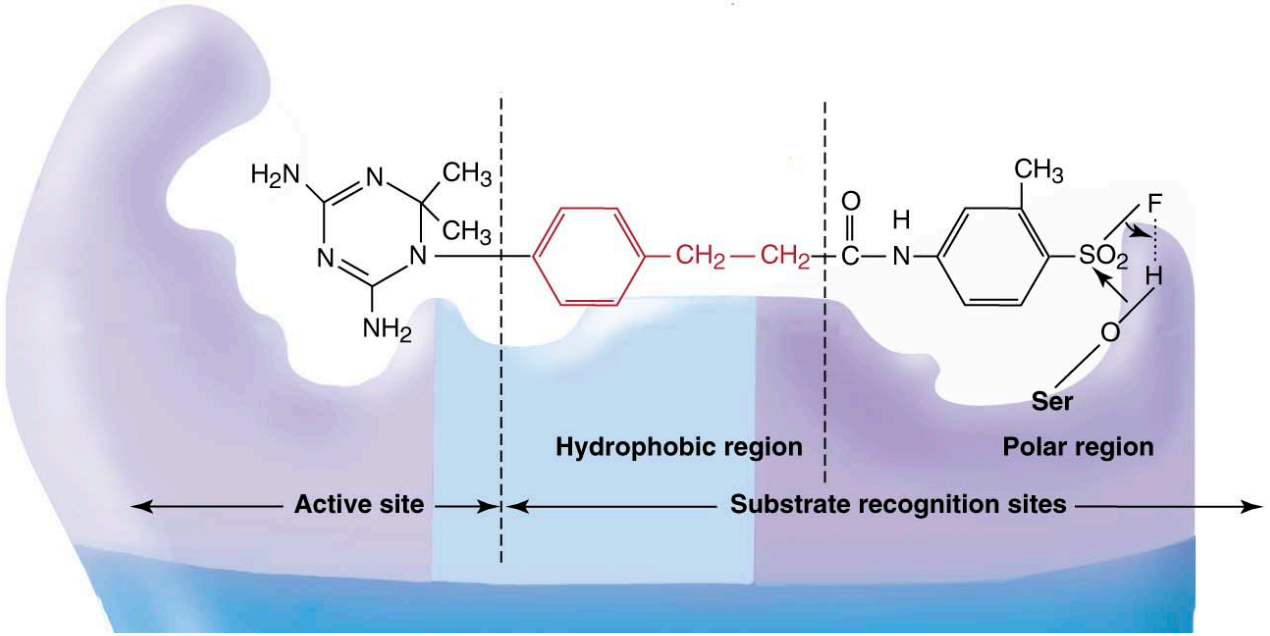
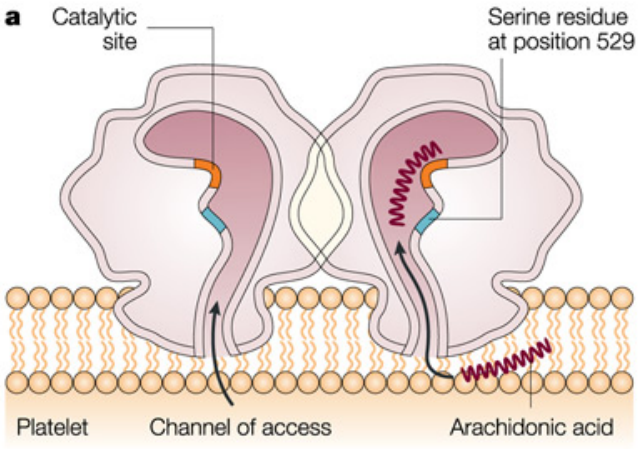
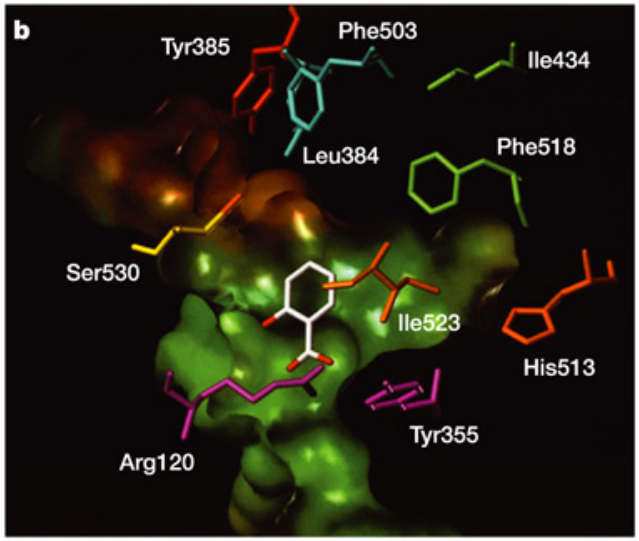


Figure 10.62: inhibition of tetrahydrofolate reductase

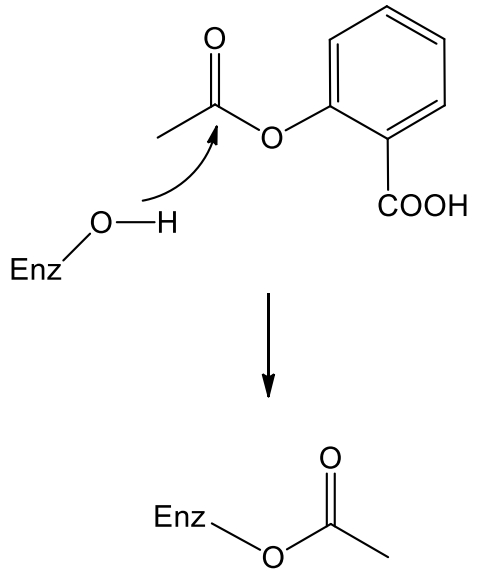
Example of an irreversible inhibitor:

Aspirin inhibition of cyclo-oxygenases (COX)





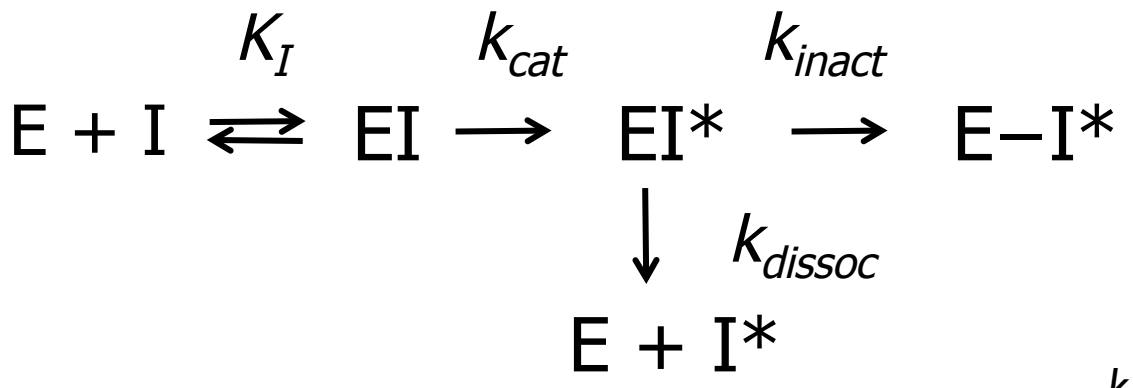
Aspirin acetylates a serine residue (S530) near (not at) active site and blocks substrate access



FitzGerald, Nat Rev Drug Discovery **2**, 879 (2003)

Inhibitor Types: Mechanism-based irreversible inhibitors

- Irreversible inhibitors that utilize the enzyme catalytic properties to generate a chemically active species.
  - Effective drug molecules: an innocuous reversible inhibitor is converted to an irreversible inhibitor
- Avoids side effects of highly reactive chemical compounds
- Also called suicide inhibitors, trojan-horses, and enzyme-activated substrate inhibitors (EASI)



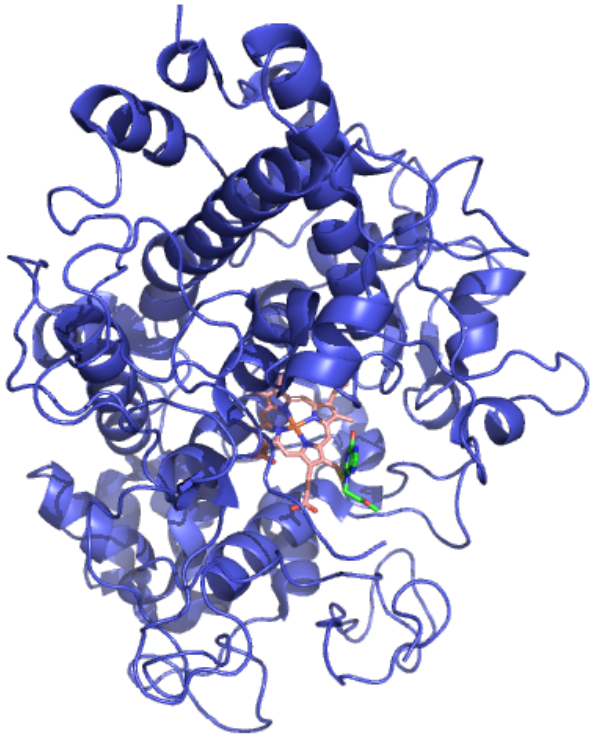
k_{dissoc} slower than k_{inact}

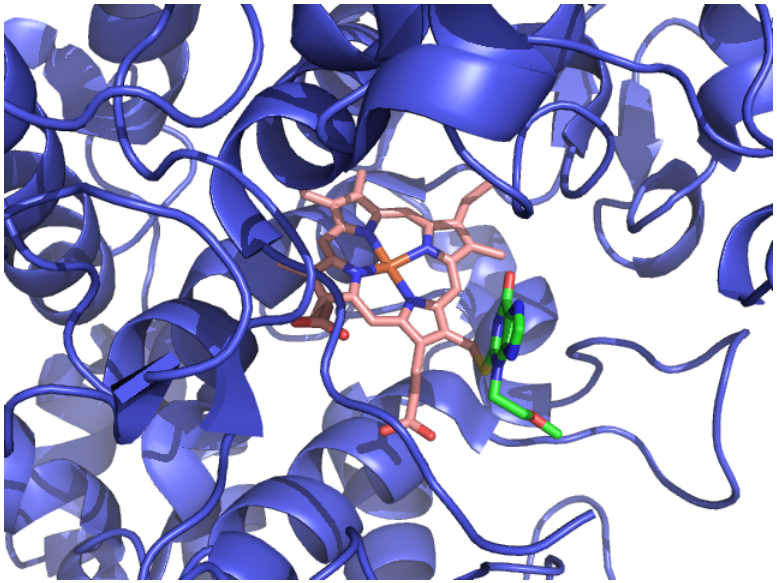
Inhibitor Types: Mechanism-based irreversible inhibitors

- Myeloperoxidase (MPO) promotes oxidative stress in inflammation
- MPO (in neutrophils) uses H_2O_2 to form reactive species (e.g. oxidizes chlorine) that cause oxidative damage to lipids, DNA, etc.
- MPO is a therapeutic target
  - Proposed inhibitor: 2-thioxanthines
  - Crystallographic structure of complex was determined

Crystal structure of MPO after inactivation by a thioxanthine TX2

- TX2 is covalently attached to the heme via a thioether bond between the exocyclic sulfur of the 2-thioxanthine ring and one of the heme methyl groups



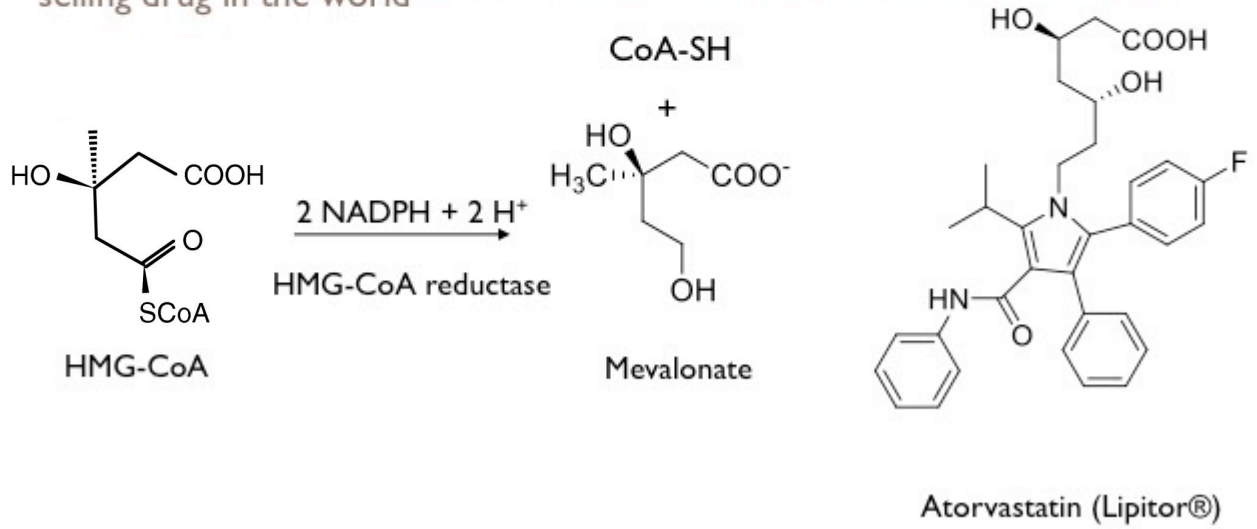


E–I*

Tidén A et al. J. Biol. Chem. 2011;286:37578-37589

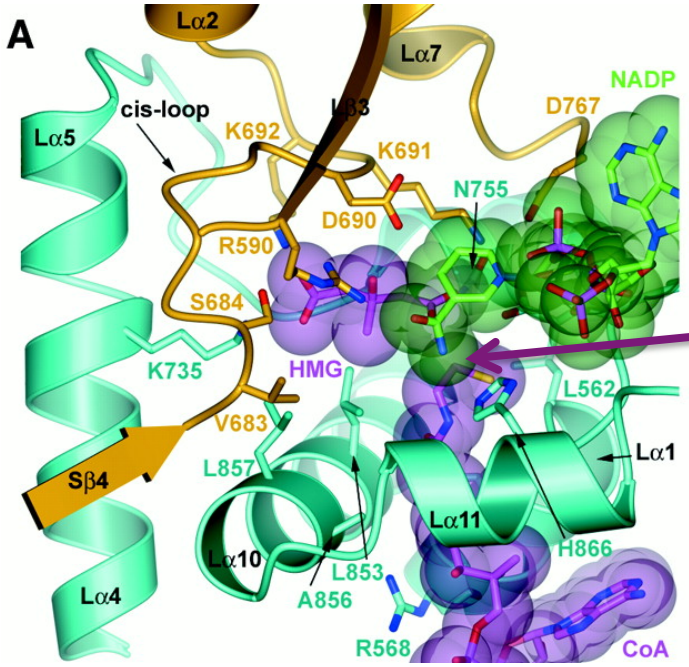
Statins: inhibitors of HMG-CoA reductase

- Atorvastatin (Lipitor®, Pfizer): 2009 sales of $13.2 billion made it the best selling drug in the world



- HMG-CoA reductase catalyzes the deacylation of HMG-CoA to form mevalonate and CoA.
- Mevalonate
  - precursor to cholesterol
  - formation is committed step in cholesterol biosynthesis
- K_M for HMG-CoA and NADPH are μM
- Merck Research Laboratories discovered potent HMG-CoA R inhibitor (1987)
  - Fermentation broth of *Aspergillus terreus*
  - Competitive with HMG-CoA
  - Later named lovastatin
  - Now a large number of statins are FDA approved, including atorvastatin

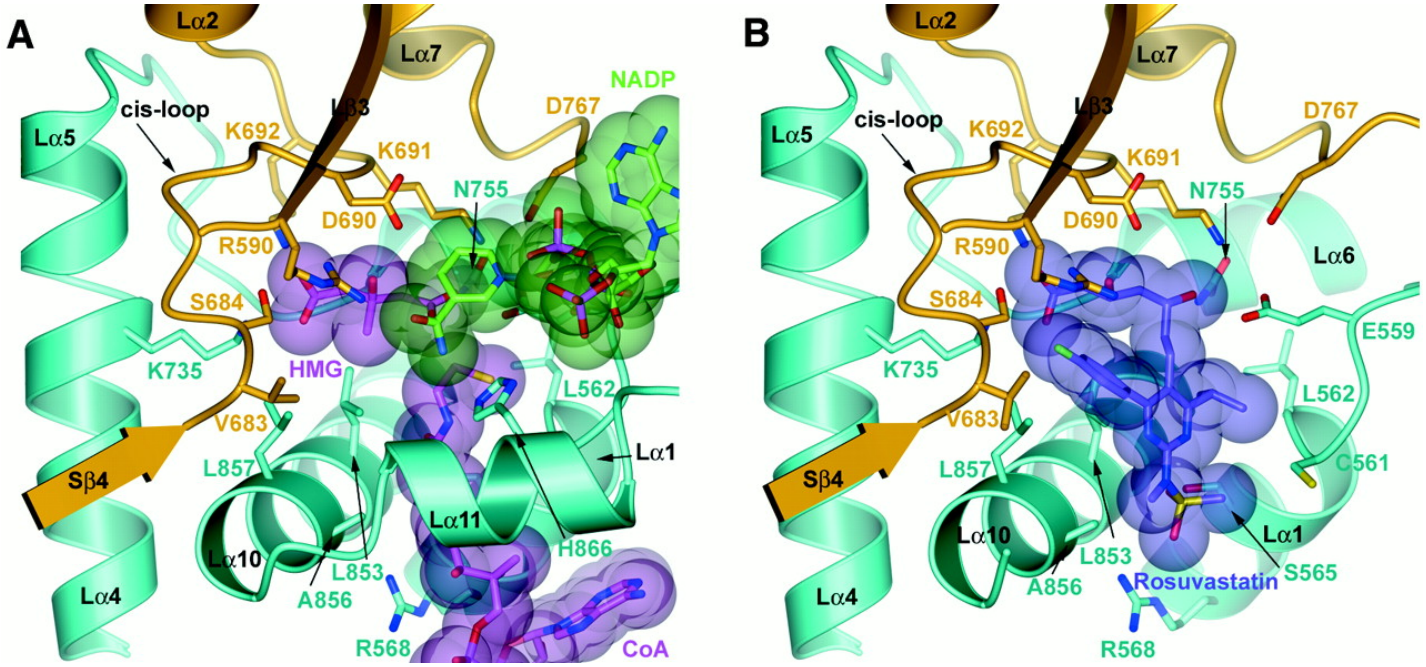
Statins: inhibitors of HMG-CoA reductase



Bulky aromatic groups would fall in this region and clash with helices of HMG-CoA reductase

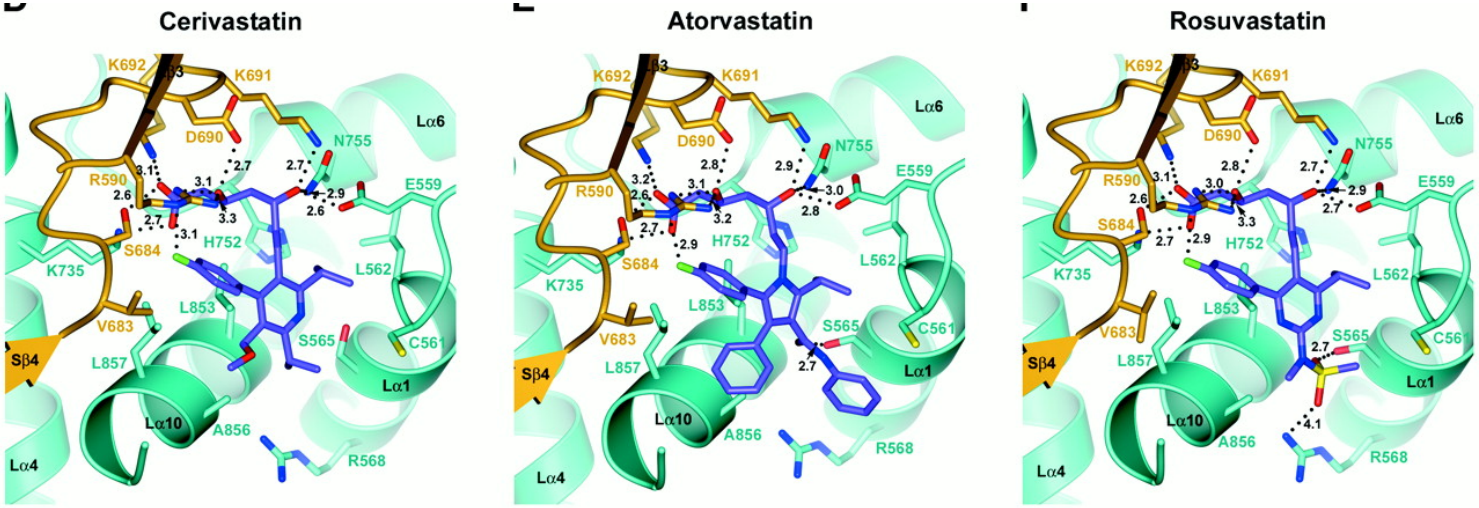
- Crystallographic structure determined for HMG-CoA reductase + HMG-CoA + NADP (2000)
  - Mevalonate moiety of HMG-CoA interacts with a loop of HMG-CoA R, helix La10 and La11 fold over substrate
  - Structure doesn't explain though how the statins inhibit the enzyme! If statins are assumed to bind as the mevalonate moiety of HMG-CoA, there would be no room for the bulky aromatic groups.

Statins: inhibitors of HMG-CoA reductase



- Subsequent crystallographic structures determined for HMG-CoA reductase with various statins (Istvan, Deisenhofer, *Science* 2001 **292**, 1160) solved the puzzle
  - Statins do bind similarly to mevalonate moiety of HMG-CoA
  - In the statin-bound structure, residues near the C-terminus of helix L10 and all of helix L11 are disordered (and not observable by crystallography) and allow a shallow groove to accommodate the statin bulky aromatic groups

Statins: inhibitors of HMG-CoA reductase



- Structures of three statins show that interactions with the mevalonate-like moiety are essentially identical for all three complexes and the binding modes are highly similar

Statins: inhibitors of HMG-CoA reductase [Answer before next class]

1. Why are statins effective at lowering cholesterol?
2. Why is it advantageous that statins have nanomolar affinity?
3. Explain the observation that statins are competitive with HMG-CoA.
4. Is it likely that statins are competitive with NADPH?
5. If HMG-CoA reductase was a fully rigid molecule, would Pfizer be marketing Lipitor?

Summary of Enzyme Regulation and Inhibition

- Small molecule inhibitors can alter either K_M or V_{max} or both.
- K_I is the inhibitory equilibrium binding constant that defines the efficacy of the inhibitor.
- Many drug molecules are small molecule inhibitors. These are characterized in terms of competitive binding as any other inhibitors and their structures are designed with similar concepts such as transition-state/substrate/product analogues, irreversible inhibitors, mechanism-based inhibitors.