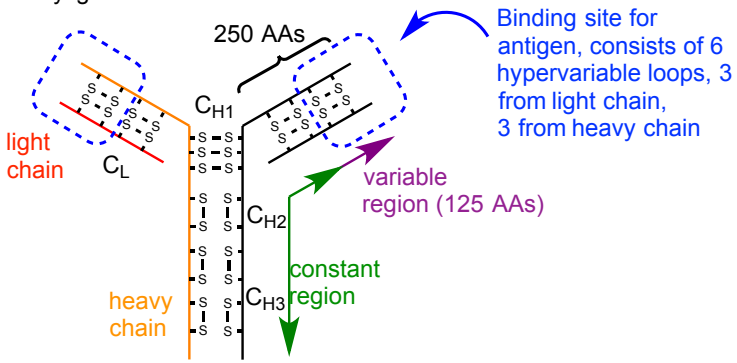
Lecture 14 -Enzymes Cont.

Enzymes - preferentially stabilize transitions states versus ground states
-Site directed mutagenesis (Tyr tRNA synthetase)
-Transition state analogue inhibitors
-Design of protein active sites that stabilize the transition state

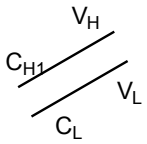
Antibodies

Antibody IgG

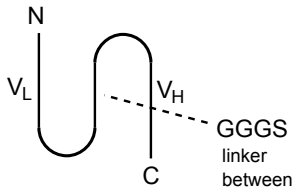


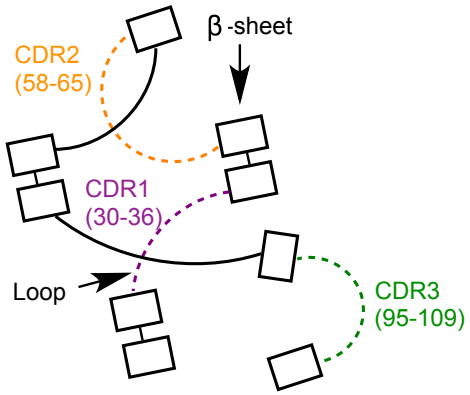
Often use smaller fragments:

-Fab fragment



-scFv fragment





CDRs have large sequence variation (protypic diversity system)

1. multiple germline V genes
- light chain V, J; heavychain V, D, J

In mice:

V_H ~250 | **D** ~15 | **J** ~5

V_H L_1-V_{H1}-L_2-V_{H2}-L_3-V_{H3}---L_n-V_{Hn}----D_1-D_2-D_3---D_n----J_1-J_2----J_n

⇓ -splice together in different combinations
-in mice ~20,000 different combinations (combinatorial diversity)

L-V_{H1}-D_2-J_1

V_L ~250 | **J** ~4

V_L L-V_{L1}---L-V_{Hn}----J_1-J_2----J_n

2. recombination inaccuracy
-When you combine D genes insertions and deletions can occur

CCC CCC TGG joining region
Pro Pro Trp

gives:

CCC CGG near joining region
Pro Arg

3. somatic mutation, 3-20 point mutations into the germline variable regnion to increase affinity.
-Germiline diversity of ~10^8 variable regions
-V region - point mutations, affinity maturation 10^9-10^{11} M^{-1}

4. multiple constant regions

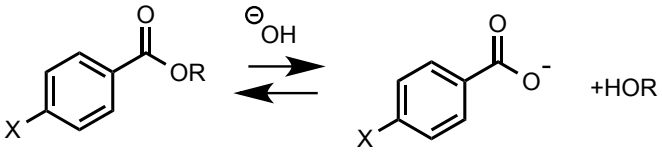
μ δ γ_3 γ_2 γ_{2b} γ_{2a} ε α
IgM IgD IgG IgE IgA

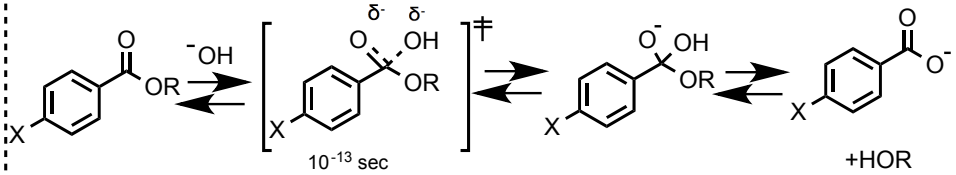
Antibodies are expressed at the surface of pre-B cells. When an antigen binds it crosslinks two antibodies on the surface, sending signals to the B cell to proliferate & undergo somatic mutation.

Antibodies as catalysts

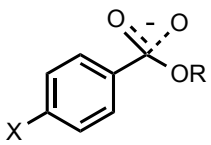
-Enzyme have evolved maximal affinity towards TS‡

How would you make an enzyme-like catalyst for the reaction:

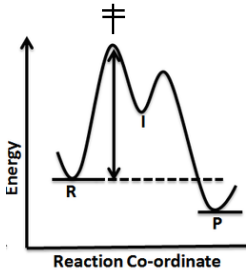


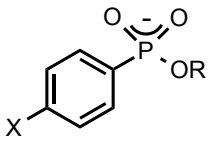


Want an immunogen like:



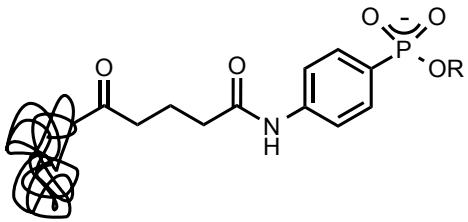
-Hammond's postulate: for high energy intermediates, the structure of the transition state is similar to that of the intermediate





-transition state analogue: tetrahedral, negatively-charged, stable

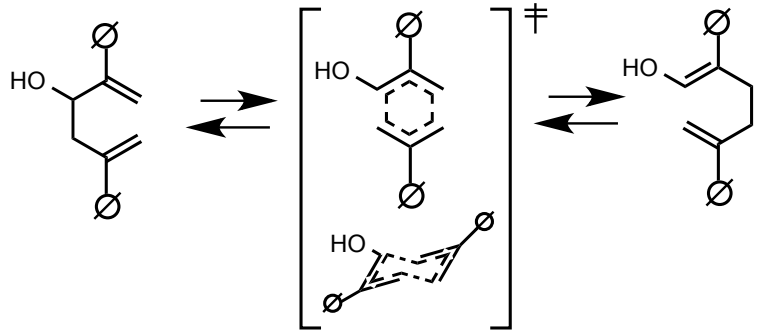
Take albumin, covalently attach the ligand



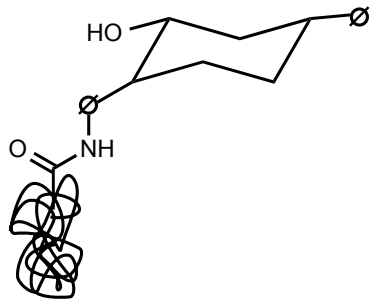
k_{cat}/k_{uncat}=~10^3-10^4

For more complex reactions:

Ex. **Cope rearrangement**

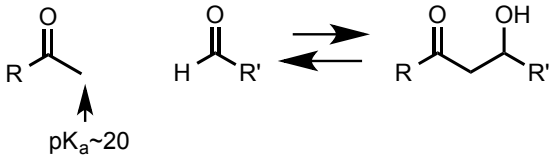


Make antibodies to:

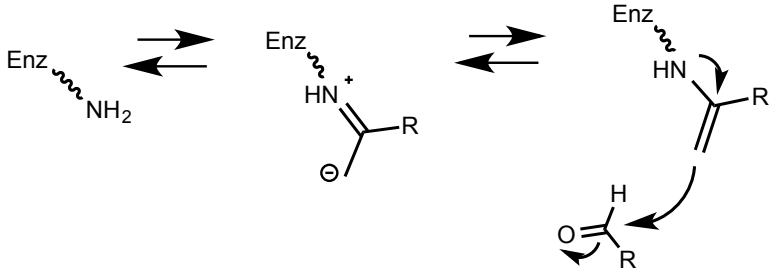


k_{cat}/k_{uncat}~10^4

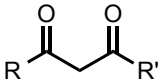
Ex. **Aldol Reaction:**



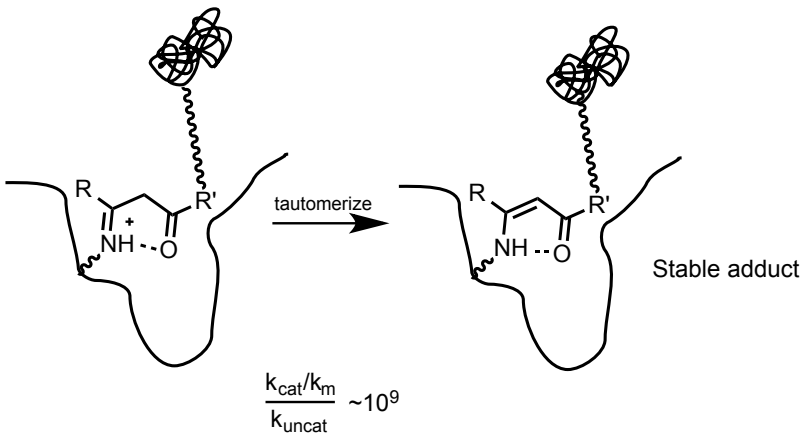
Enzymes make a Schiff-base:



Make an immunogen:



If any antibodies have a Lysine in the binding pocket:



Enzyme catalysis:

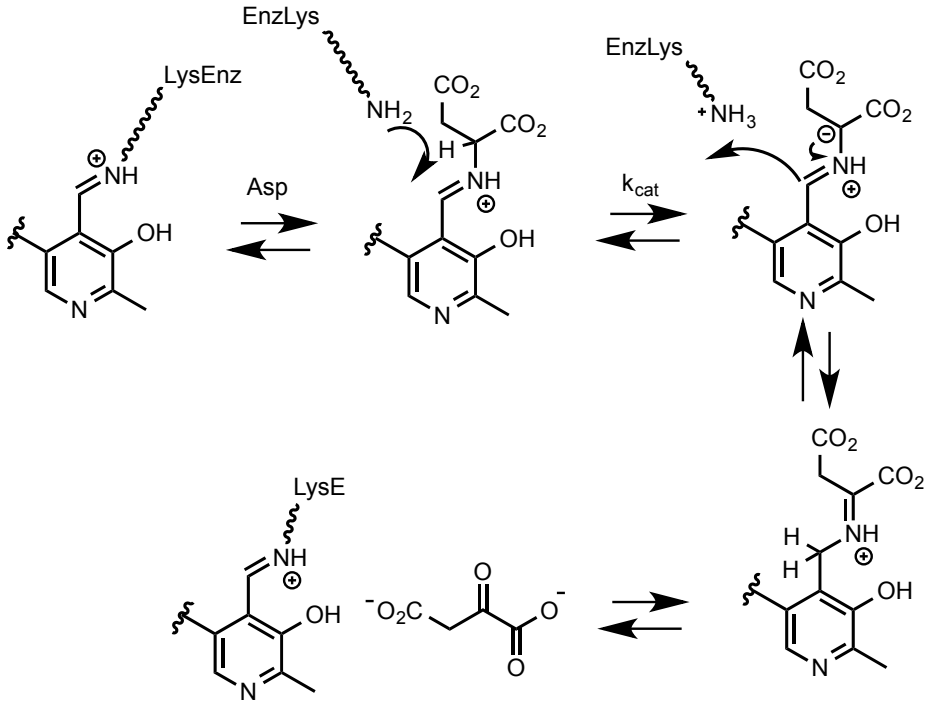
1. $TS^{\ddagger}$ stabilization
2. proximity effects
3. covalent catalysis
4. general acid base catalysis
5. cofactors

4. General base catalysis

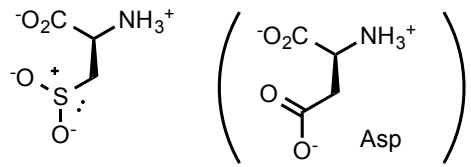
$\log k = \beta (pK_a) + C$ -Note pK_a His ~6-7, Lys ~10

Bronsted coefficient

PLP catalyzed reactions:



Strategy - mutate K258A - inactive (with respect to H^+ transfer) $<10^{-6}$ of wt activity
- no large changes observed in crystal structure
-"rescue" activity with exogenous amines
Use L-cysteine sulfinate as substrate (Asp analog with greater reactivity)



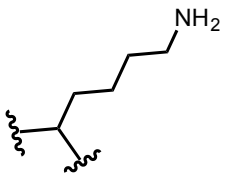
Results -linear dependence of rate versus [amine]
-plot of logk vs pK_a was poor
-plot of log k vs "A^3" - Linear (Me/Et/Pr/Bu) with same pK_a (~10.6)
-therefore use double linear plot:

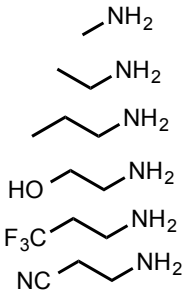
$\log k_B = \beta (pK_a) + V + C$

$\beta = 0.39 \pm 0.05$ ← 40% of full + charge on Lys 258 in TS

$V = (-0.055 \pm 0.005)$ Å $^{-3}$

$c = -0.7 \pm 0.5$

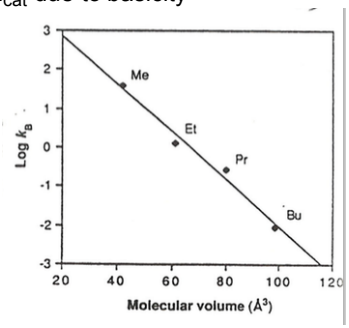




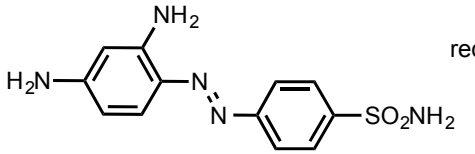
-total variation: 10x greater due to volume than k_{cat} due to basicity
-points to the effect of cavities/steric effects

Amine	pK_a	Molecular volume (Å^3)	k_B* ($M^{-1}s^{-1}$)
Methyl-	10.6	42.1	40 (2)
Ethyl-	10.6	60.9	1.30 (0.03)
Propyl-	10.5	79.8	0.27 (0.02)
Butyl-	10.6	98.7	0.009 (0.002)
Ethylenedi-	10.0	74.6	0.084 (0.002)†
Ethanol-	9.5	71.5	0.078 (0.003)
Ammonia	9.2	23.2	21 (2)
2-Fluoroethyl-	9.0	64.4	0.257 (0.009)
2-Cyanoethyl-	7.7	70.5	0.0108 (0.0003)
2,2,2-Trifluoroethyl-	5.7	71.6	0.0073 (0.0002)
Cyanomethyl-	5.3	51.1	0.050 (0.001)

* The k_B values are for the free base. Standard errors are given in parentheses. † k_B has been divided by 2 to correct for statistical effects. The neutral species is active.

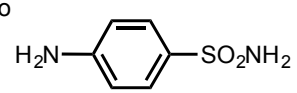


Enzyme Inhibitors: antibiotics
Ex. Sulfa-drugs



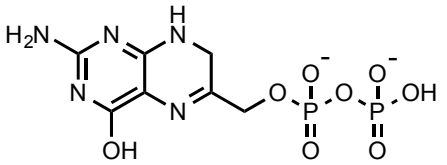
Prodrug

reduced in vivo
e^-/H^+

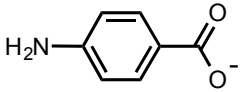


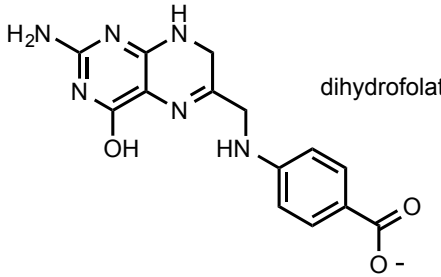
para-amino-sulfonamide

Mechanism of action:



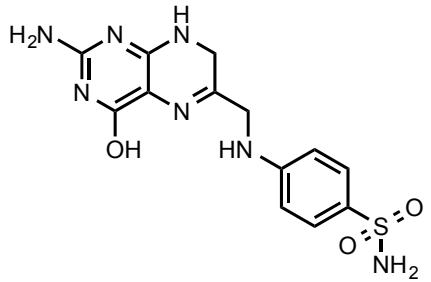
dihydropteroate synthesis





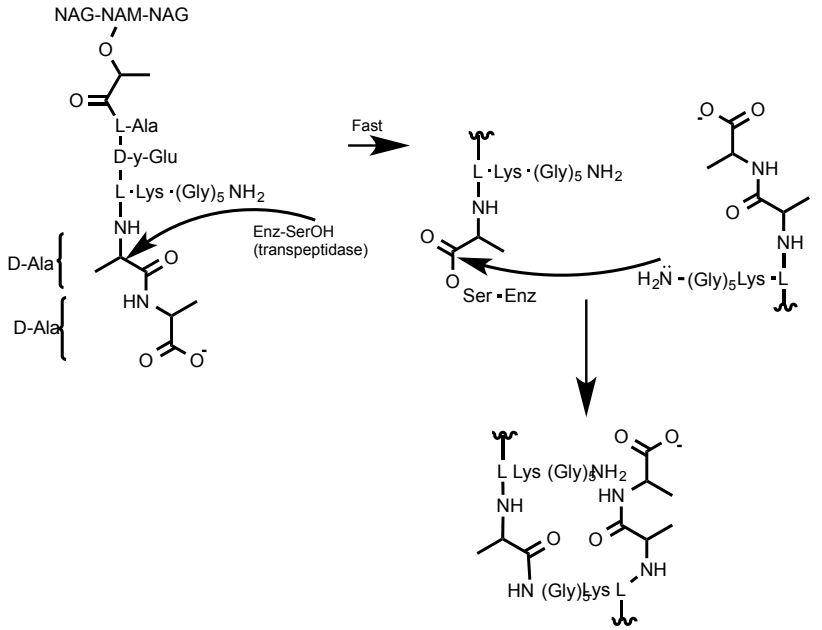
dihydrofolate

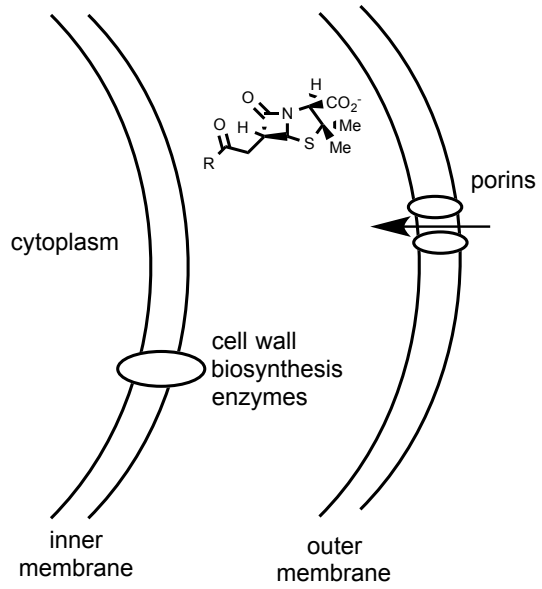
Bacteria utilize para-amino-sulfonamide and substitute it into dihydrofolate synthesis, resulting in an inactive cofacter.



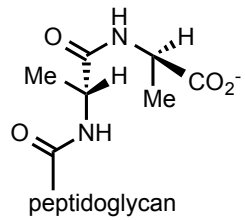
Ex. β-lactam penicillins

Cell wall biosynthesis:

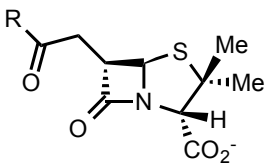


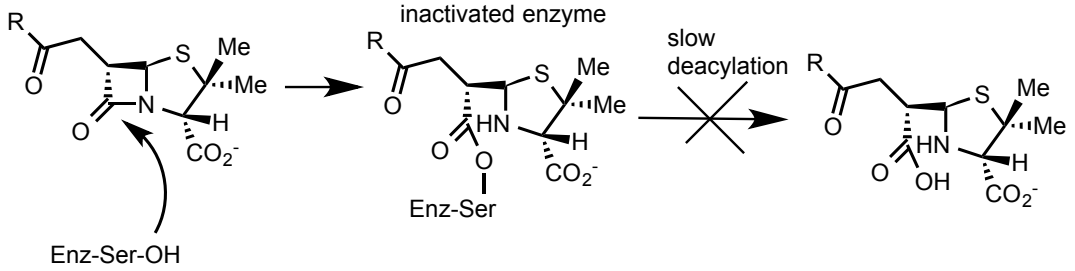


mimic :



with:





For resistance make a trans| β otidase -lactamase