CatalyticAntibodies (Abzymes)

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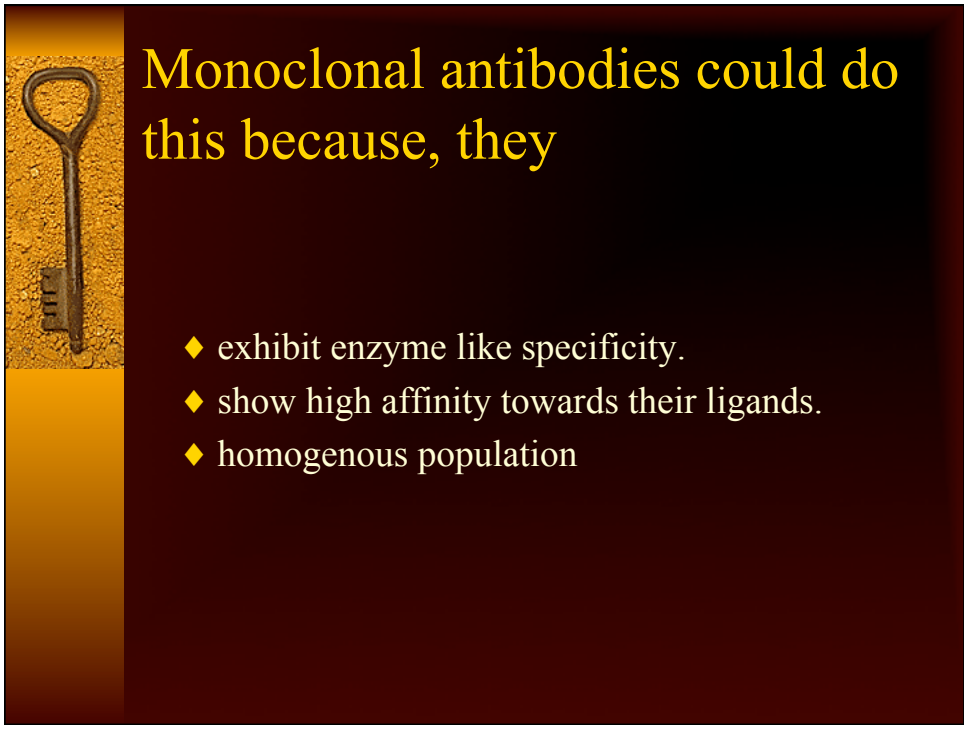
Catalytic antibodies/ Abzymes

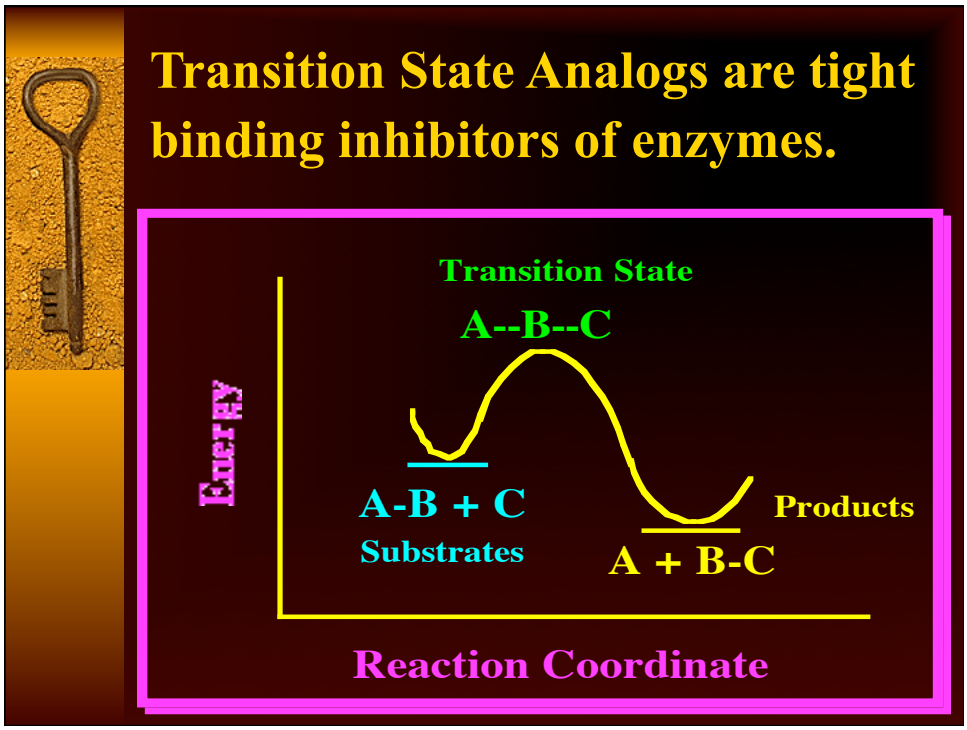
- **In 1969, W. P. Jencks predicted that antibodies specific to transition state of a chemical reaction should act like an enzyme and catalyze that particular reaction.**
- **In 1986, Lerner and Schultz generated catalytic antibodies.**

- "If complementarity between the active site and the transition state contributes significantly to enzymatic catalysis, it should be possible to synthesize an enzyme by constructing such an active site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining sites of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrates to resemble the transition state".
  - W.P. Jencks in Catalysis and Chemistry in Enzymology (McGraw Hill, 1969; p. 288)

Key to selective design of a desired catalyst

- **Generate a specific binding site**


Monoclonal antibodies could do this because, they
♦ exhibit enzyme like specificity.
♦ show high affinity towards their ligands.
♦ homogenous population

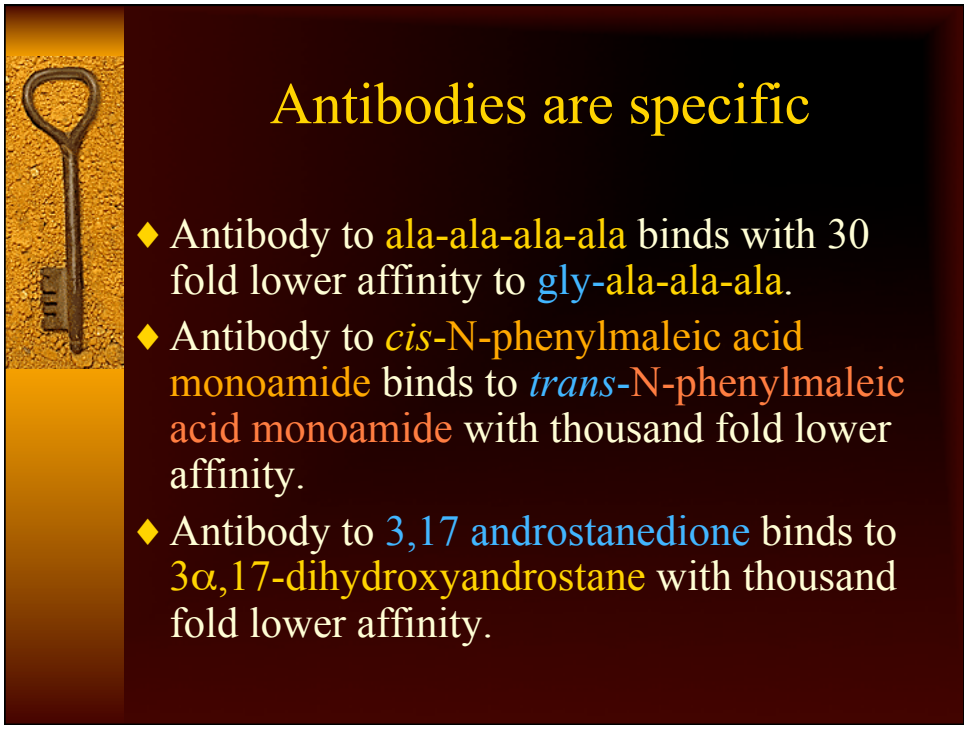

Transition State Analogs are tight binding inhibitors of enzymes.
Transition State
A--B--C
Energy
A-B + C
Substrates
Products
A + B-C
Reaction Coordinate

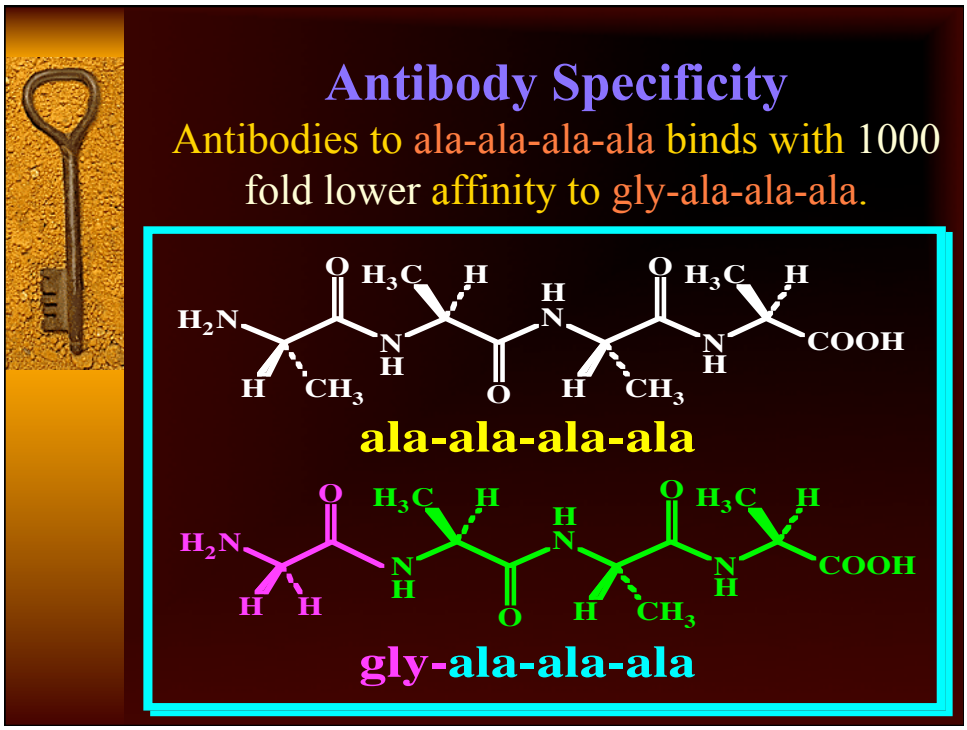
Active site of enzymes are complementary to the transition state.

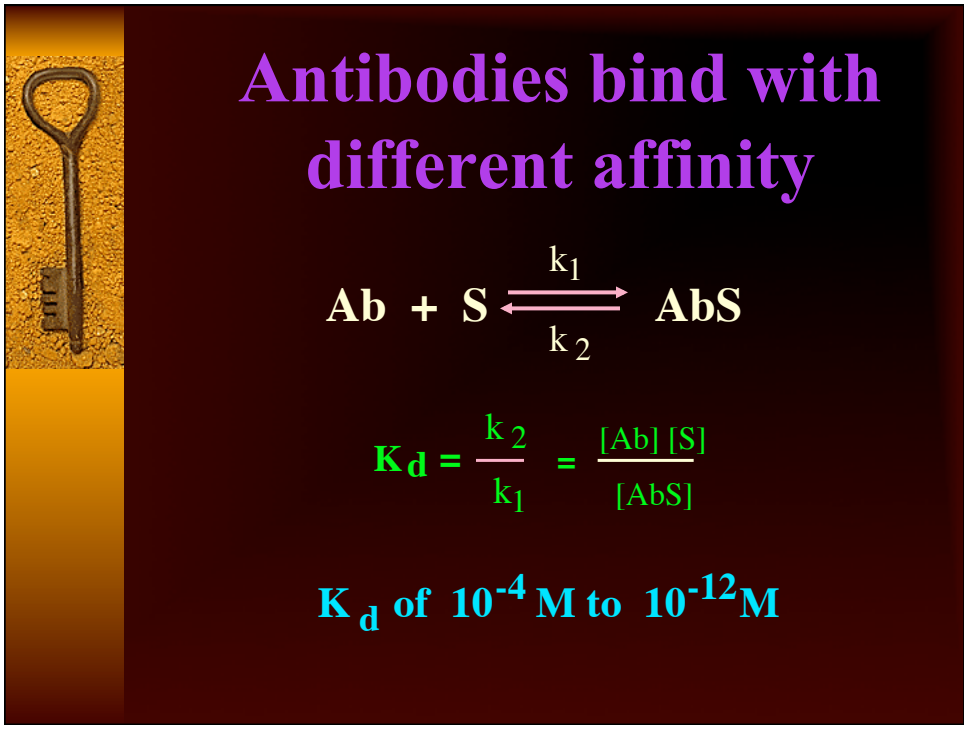
- Evidence:

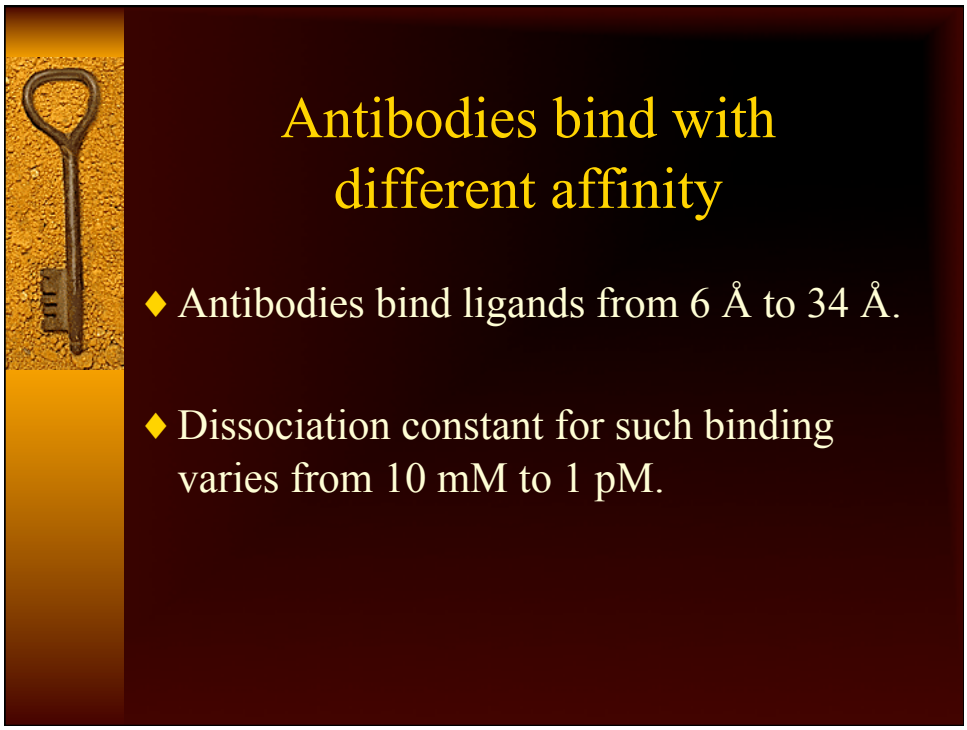
1. Three dimensional structure of enzyme-inhibitor complexes.
2. Transition state inhibitors are tight binding inhibitors of enzymes.

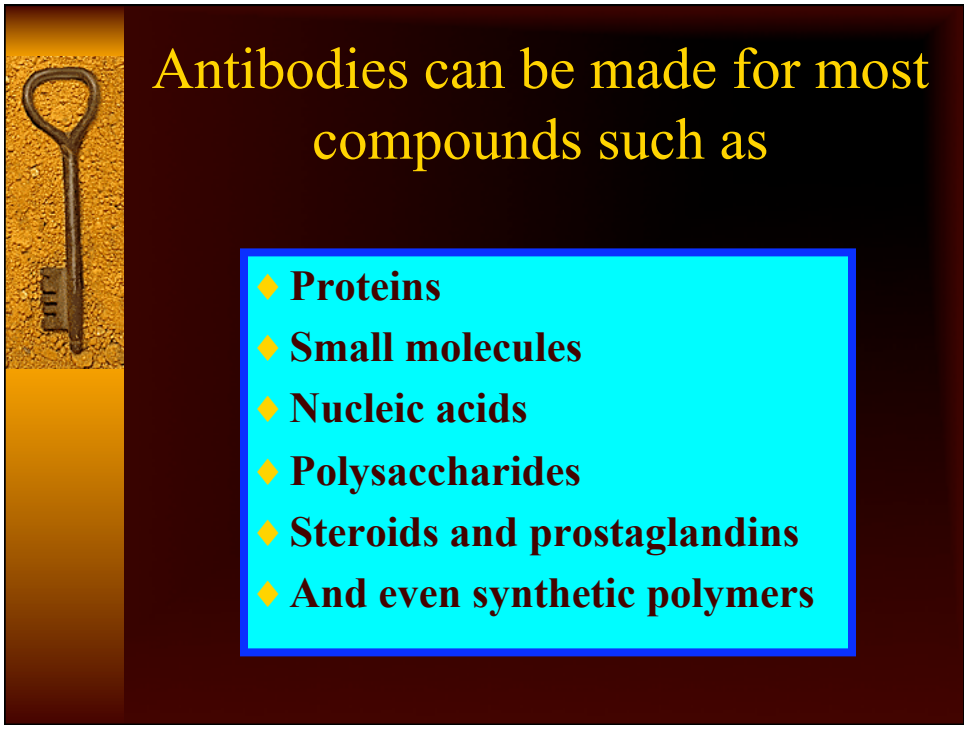
What is special about antibodies?

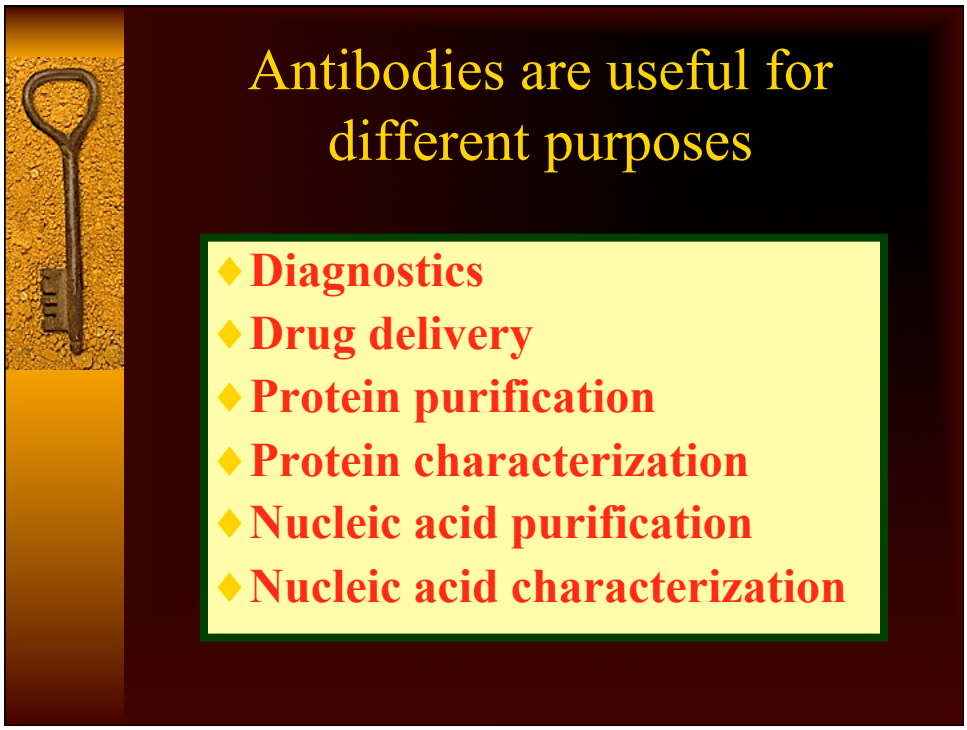

Antibodies are specific
♦ Antibody to ala-ala-ala-ala binds with 30 fold lower affinity to gly-ala-ala-ala.
♦ Antibody to *cis*-N-phenylmaleic acid monoamide binds to *trans*-N-phenylmaleic acid monoamide with thousand fold lower affinity.
♦ Antibody to 3,17 androstanedione binds to 3α,17-dihydroxyandrostane with thousand fold lower affinity.

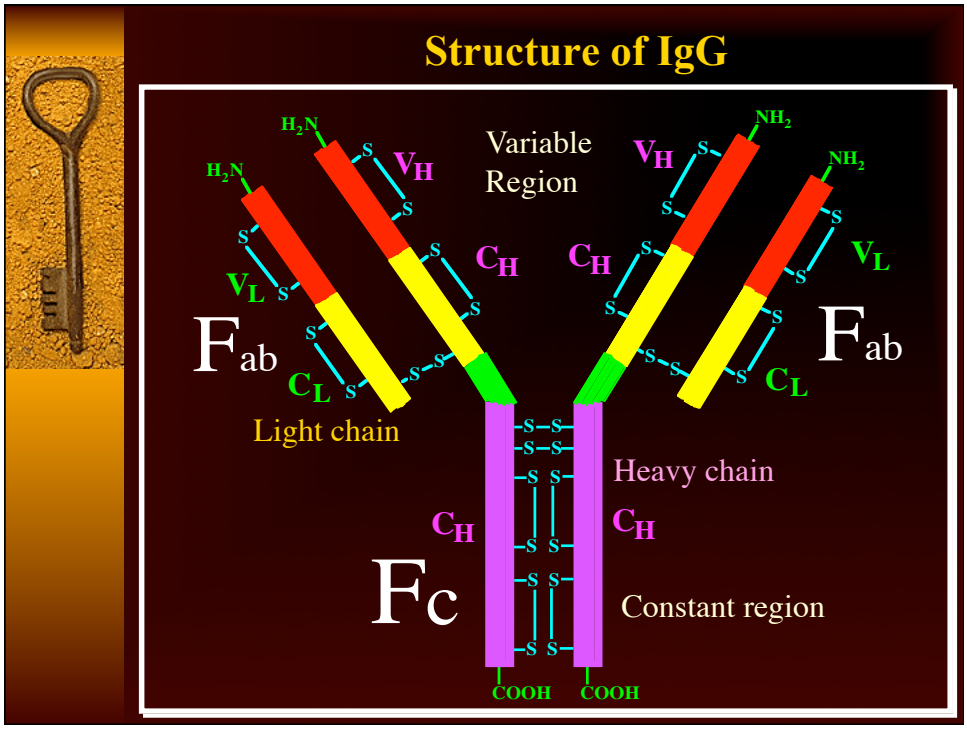

Antibody Specificity
Antibodies to ala-ala-ala-ala binds with 1000 fold lower affinity to gly-ala-ala-ala.
ala-ala-ala-ala
gly-ala-ala-ala


Antibodies bind with different affinity
Ab + S
k1
k2
AbS
Kd = k2/k1 = [Ab] [S] / [AbS]
Kd of 10-4 M to 10-12M


Antibodies bind with different affinity
♦ Antibodies bind ligands from 6 Å to 34 Å.
♦ Dissociation constant for such binding varies from 10 mM to 1 pM.

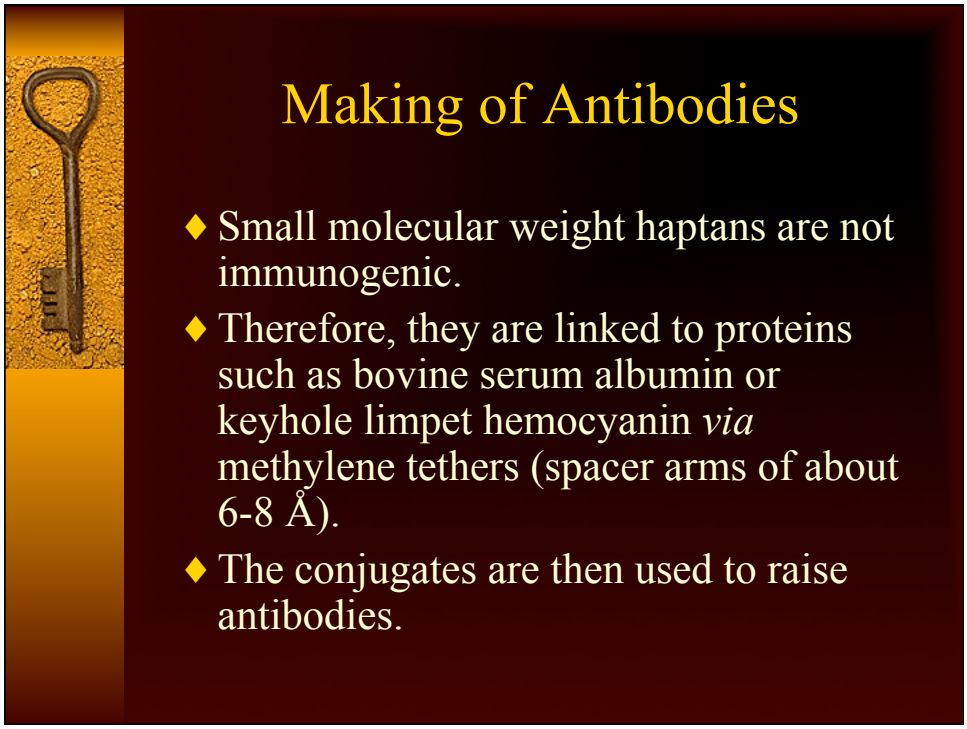

Antibodies can be made for most compounds such as
Proteins
Small molecules
Nucleic acids
Polysaccharides
Steroids and prostaglandins
And even synthetic polymers

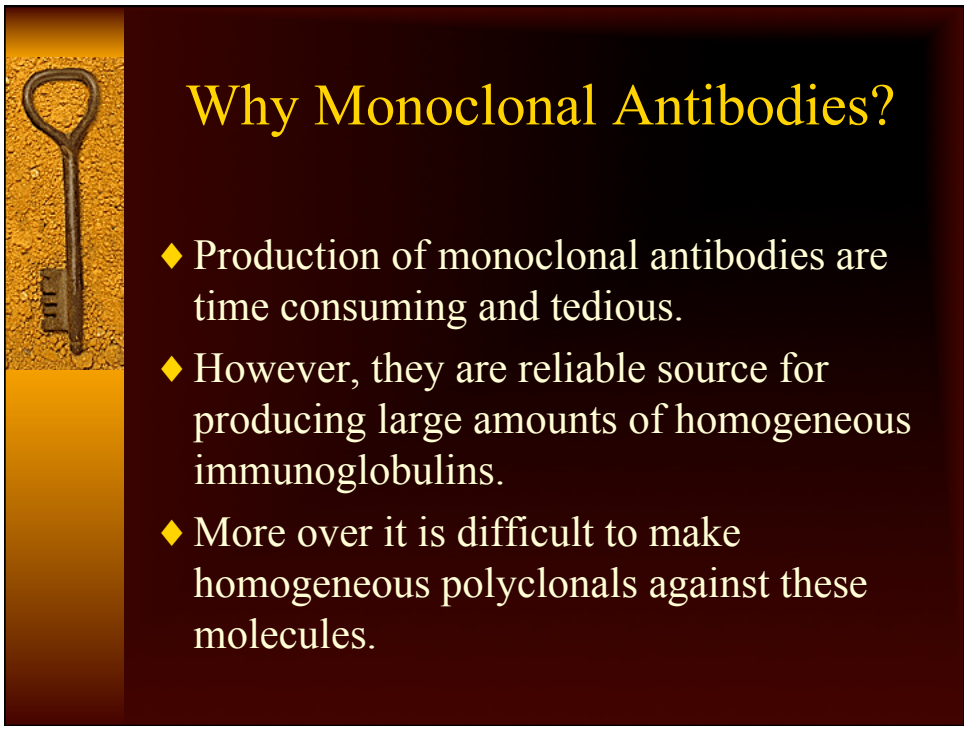

Antibodies are useful for different purposes
Diagnostics
Drug delivery
Protein purification
Protein characterization
Nucleic acid purification
Nucleic acid characterization



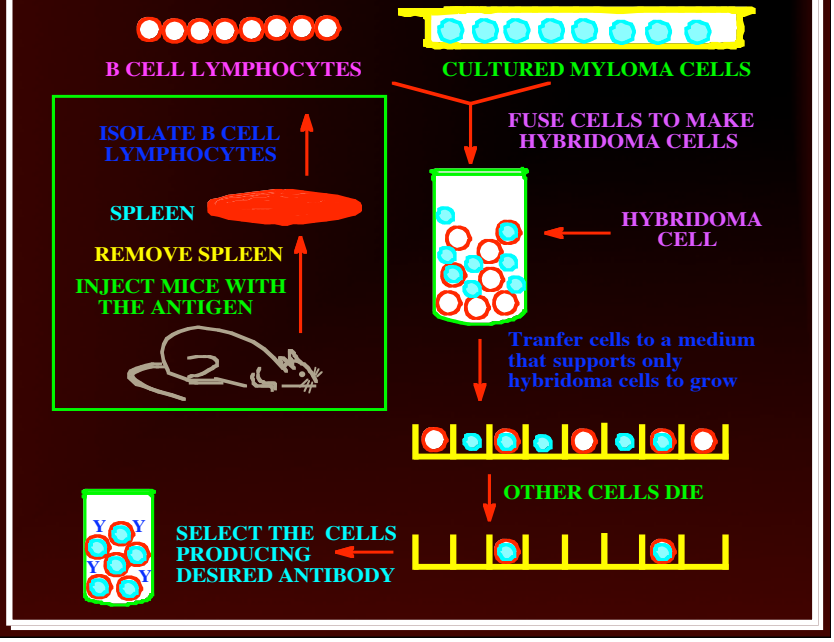
Antibody number

- The combinatorial joining of the genes corresponding to variable region light chains and heavy chains (V_H and V_L) in combination with the combinatorial linkage of different constant region light chains and heavy chains (C_H and C_L) can generate as much as 10^8 antibody molecules.
- Mutations will further increase the available antibodies to a larger number.


Making of Antibodies
♦ Small molecular weight haptans are not immunogenic.
♦ Therefore, they are linked to proteins such as bovine serum albumin or keyhole limpet hemocyanin *via* methylene tethers (spacer arms of about 6-8 Å).
♦ The conjugates are then used to raise antibodies.


Why Monoclonal Antibodies?
♦ Production of monoclonal antibodies are time consuming and tedious.
♦ However, they are reliable source for producing large amounts of homogeneous immunoglobulins.
♦ More over it is difficult to make homogeneous polyclonals against these molecules.

How monoclonal antibodies are made?



Catalytic Antibodies

Antibodies can be practically made for any molecules. So if one makes an antibody to a transition state analog, that antibody has the potential to catalyze a particular reaction whose transition state mimics the transition state analog used to make antibody.

Catalytic antibodies can be made by two general approaches.

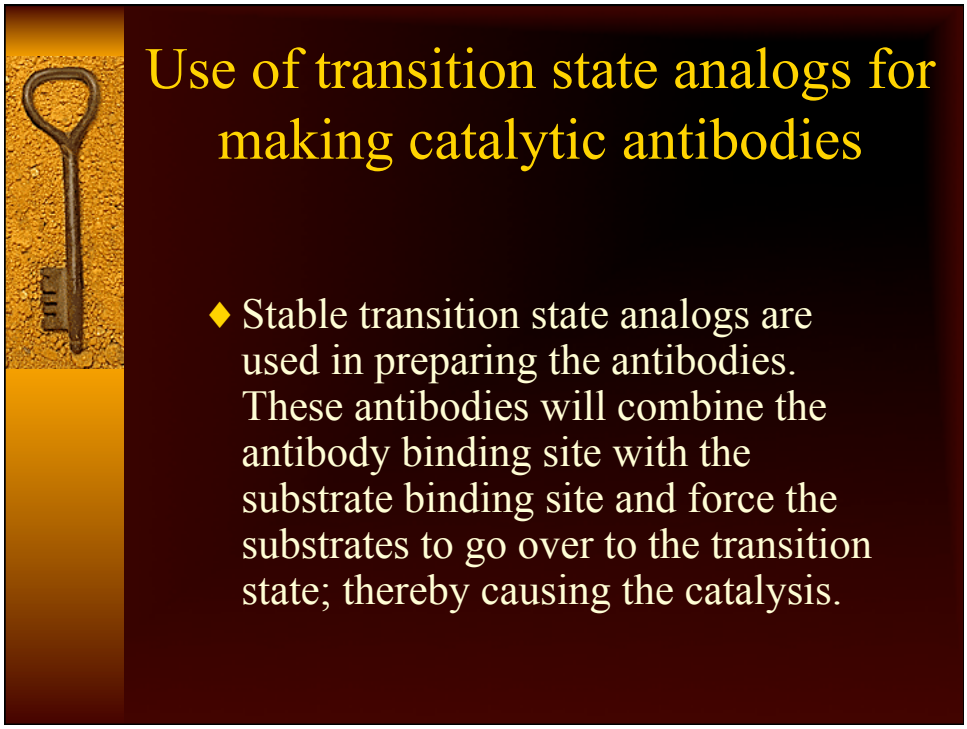
One method:
Exploit the steric and electronic complementarity of antibody to a hapten to generate abzymes having

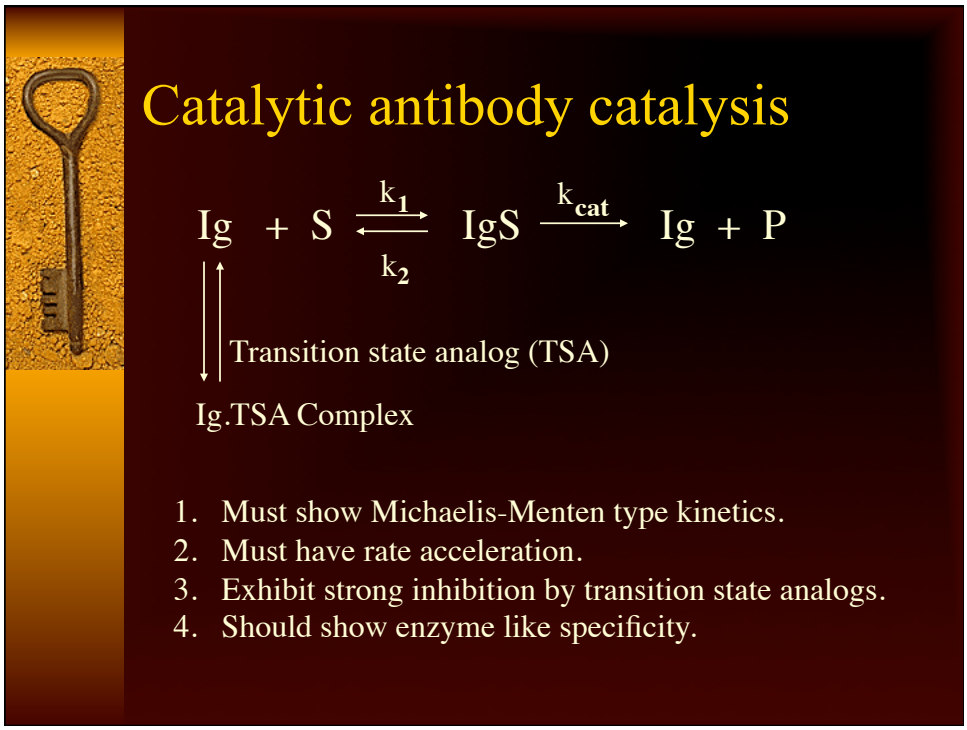
a) Complementary binding site to transition state analogs
b) Overcome entropy barriers in orienting reactants.
c) Appropriate catalytic amino acid at antibody site.
d) Cofactor binding sites.

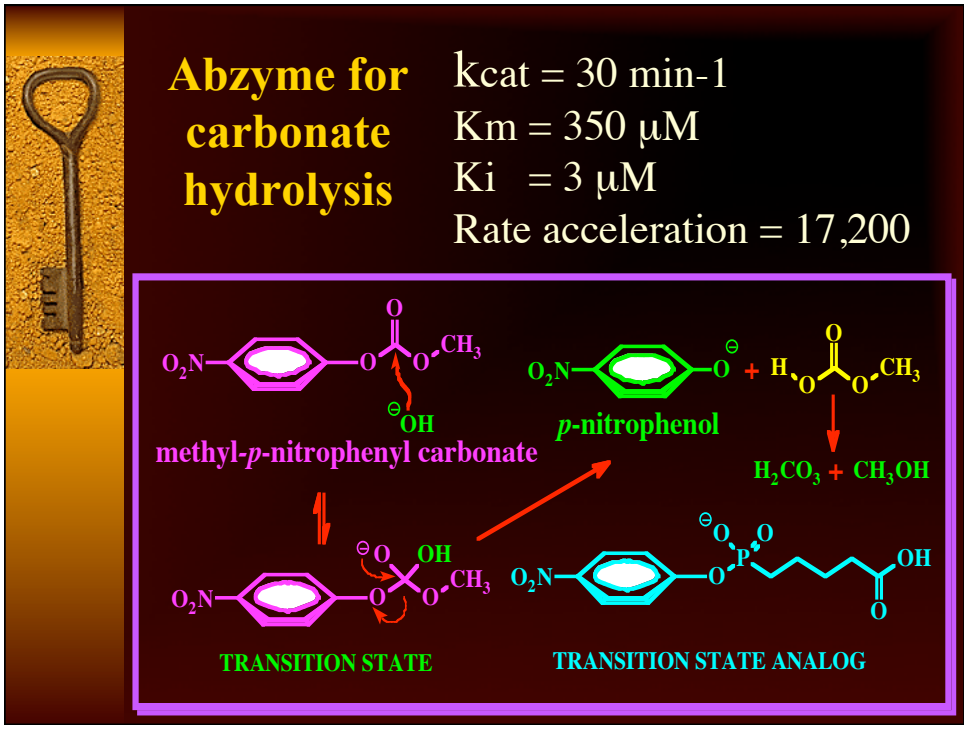
Second Method:
Introduce catalytic groups directly into the antibody combining site by chemical modification studies or site directed mutagenesis or genetic selection.

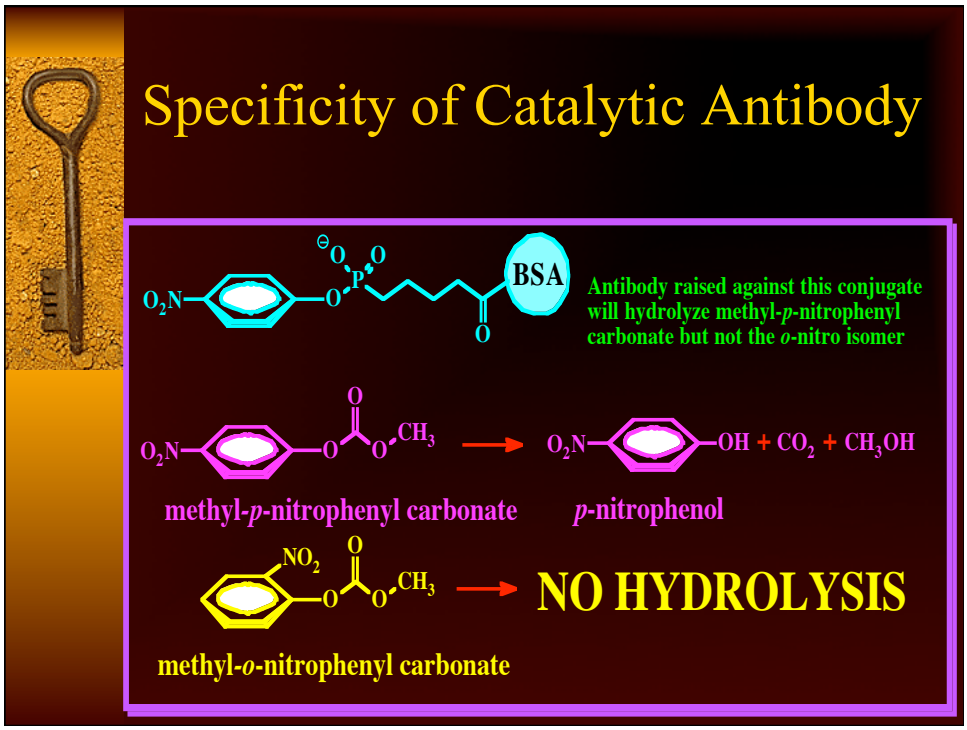
Strategy I:
Transition State Stabilization

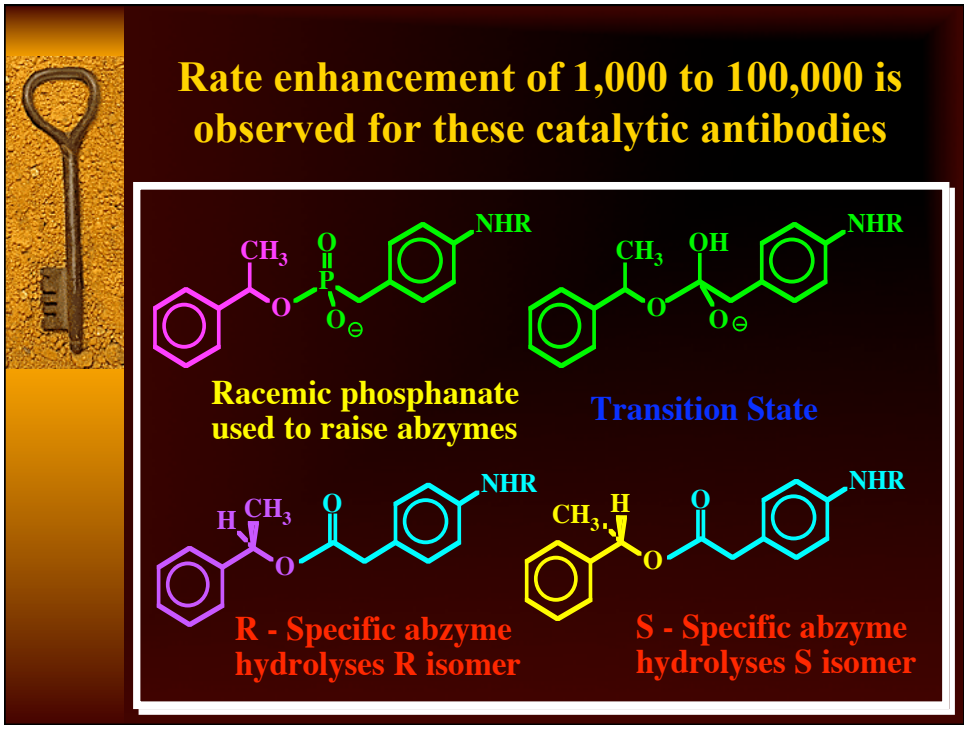
1. Active site of several enzymes are complementary to the transition state of the reaction they catalyze.
2. Transition state analogs are potent inhibitors of enzymes.
3. Therefore, antibody specificity could be used to stabilize the transition state and make catalytic antibodies

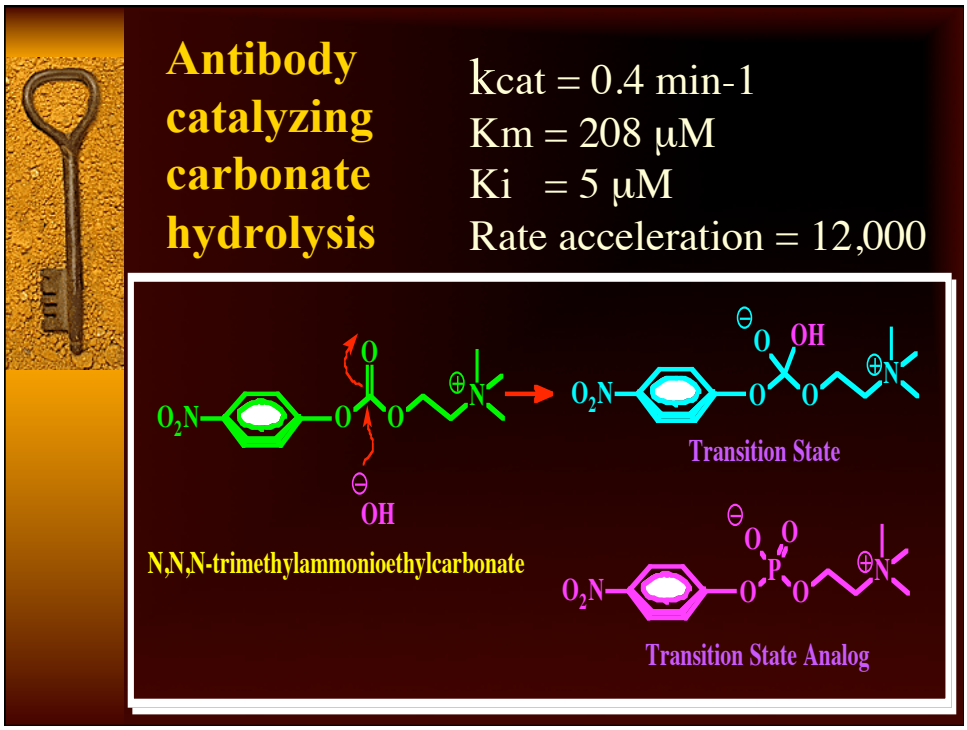

Use of transition state analogs for making catalytic antibodies
Stable transition state analogs are used in preparing the antibodies. These antibodies will combine the antibody binding site with the substrate binding site and force the substrates to go over to the transition state; thereby causing the catalysis.


Catalytic antibody catalysis
Ig + S
k1
k2
IgS
kcat
Ig + P
Transition state analog (TSA)
Ig.TSA Complex
1. Must show Michaelis-Menten type kinetics.
2. Must have rate acceleration.
3. Exhibit strong inhibition by transition state analogs.
4. Should show enzyme like specificity.


Abzyme for carbonate hydrolysis
kcat = 30 min-1
Km = 350 μM
Ki = 3 μM
Rate acceleration = 17,200
methyl-p-nitrophenyl carbonate
p-nitrophenol
H2CO3 + CH3OH
TRANSITION STATE
TRANSITION STATE ANALOG

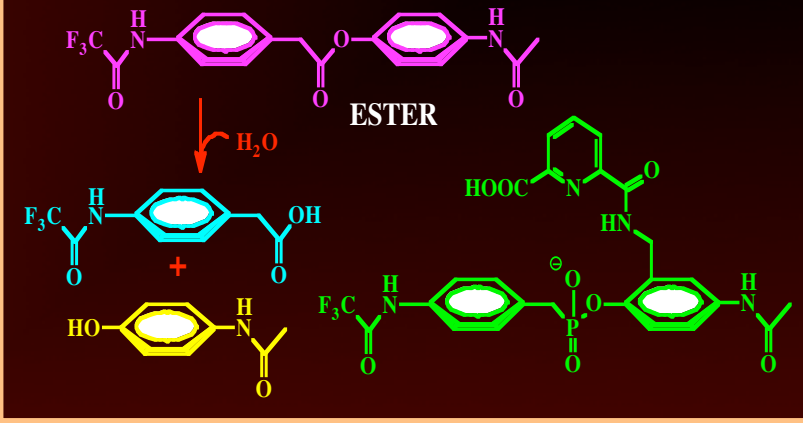

Specificity of Catalytic Antibody
BSA
Antibody raised against this conjugate will hydrolyze methyl-p-nitrophenyl carbonate but not the o-nitro isomer
methyl-p-nitrophenyl carbonate
p-nitrophenol
NO HYDROLYSIS
methyl-o-nitrophenyl carbonate


Rate enhancement of 1,000 to 100,000 is observed for these catalytic antibodies
NHR
CH3
O
P
Racemic phosphanate used to raise abzymes
OH
Transition State
H
R - Specific abzyme hydrolyses R isomer
S - Specific abzyme hydrolyses S isomer


Antibody catalyzing carbonate hydrolysis
kcat = 0.4 min-1
Km = 208 μM
Ki = 5 μM
Rate acceleration = 12,000
O2N
OH
N,N,N-trimethylammonioethylcarbonate
Transition State
Transition State Analog

Antibody performing ester hydrolysis

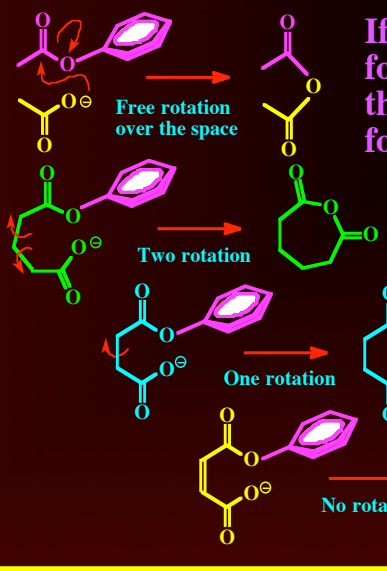
kcat = 1.6 min-1
Km = 1.9 μM
Ki = 0.16 μM



Strategy 2: Use of Proximity Effects

- To increase the reaction rate, use antibody binding affinity to over come the entropy barriers.
- The binding at the active site should reduce the rotational and translational motion of substrates and orient them appropriately at the antibody combining site. This will allow the reaction to occur.

Proximity effects



If the rate of this anhydride formation is taken as one the relative rates for the following reaction are

1×10^3

2×10^5

1×10^7

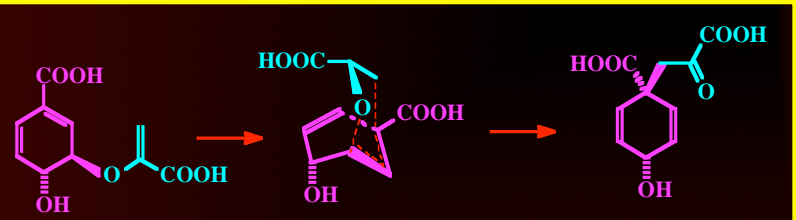
Restricting the free rotations

- Therefore, restricting the free rotation around C-C bonds (and other crucial bonds) during catalysis can result in great catalytic potentials. Antibody combining site can make use of this fact and force some reactions to occur.

Chorismate mutase reaction

- Chorismate mutase catalyzes the conversion of chorismic acid to prephenic acid.
- It is a 3,3-sigmotropic rearrangement.
- Occurs *via* a boat like transition state.
- Entropy of activation : -12.85 entropy units.
- Enthalpy of the reaction 20.7 kcal/mol.
- Unimolecular rearrangement catalyzed by the enzyme is 1,000,000 times more than the nonenzymatic reaction.

Abzyme catalyzing chorismate mutase reaction



Chorismic acid Transition State Prephenic acid

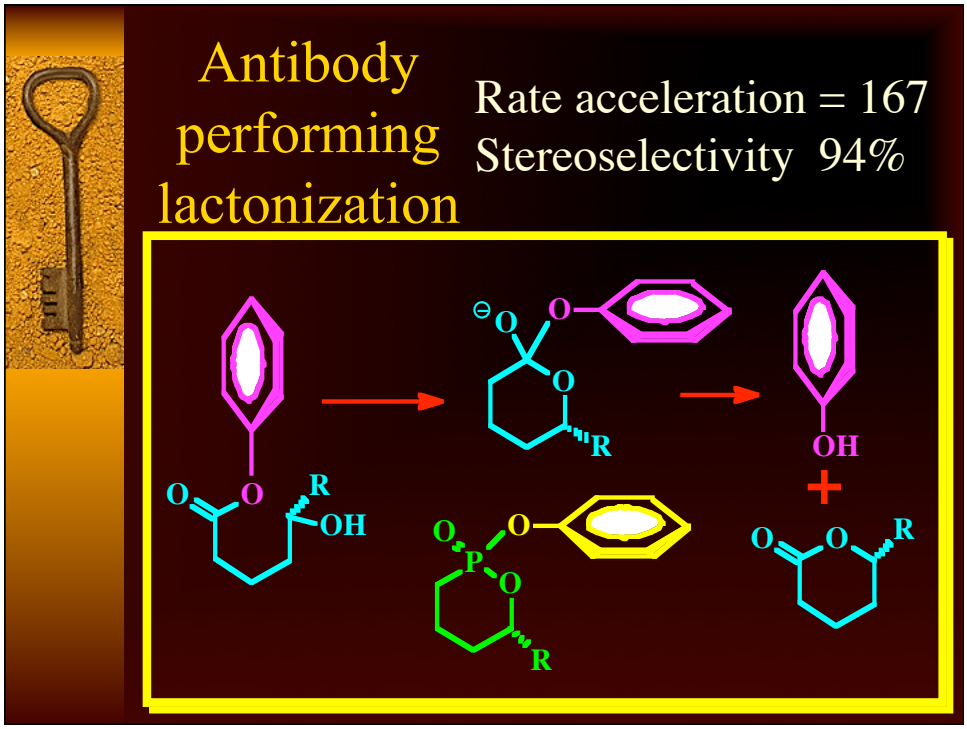
Properties of abzyme

k_{Cat} = 2.7 min^{-1}

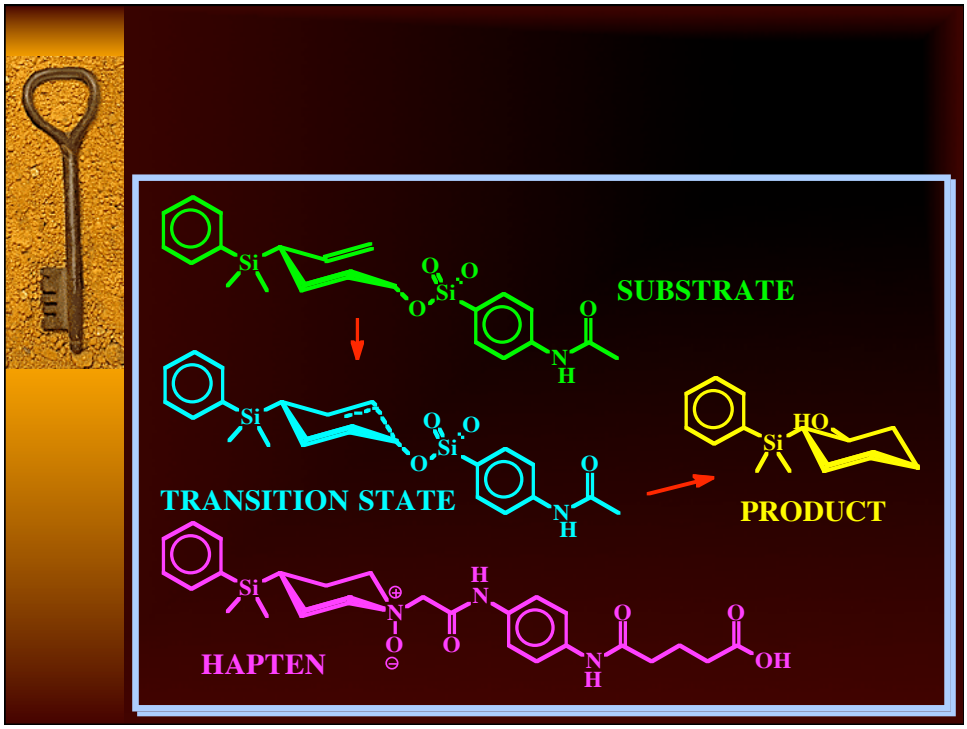
K_m = 260 μM

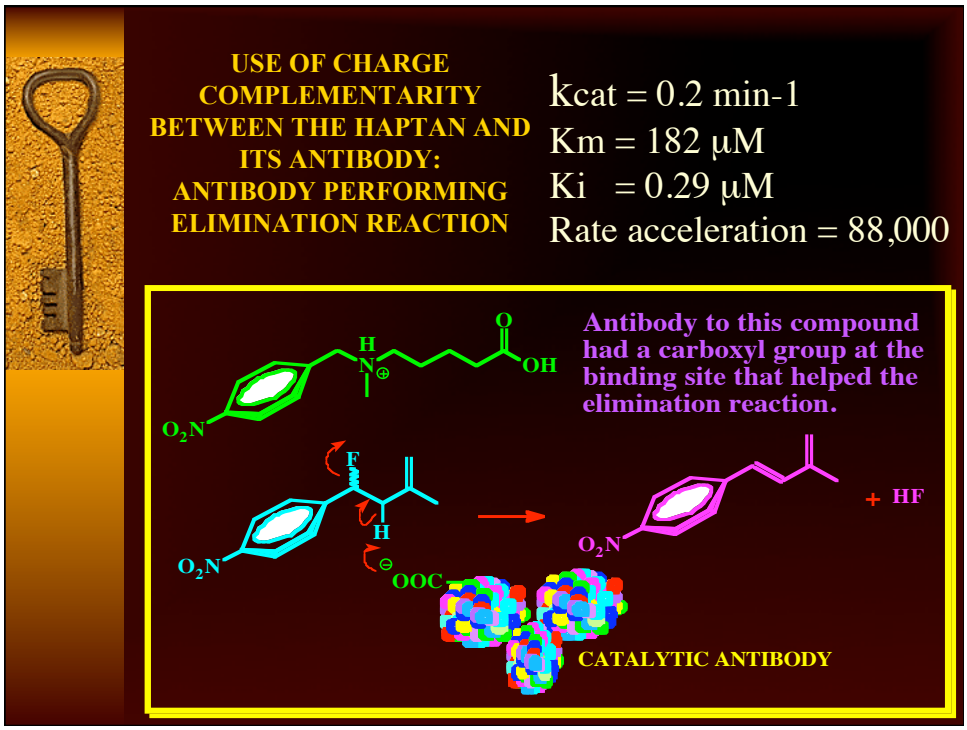
K_i = 9 μM

Rate acceleration = 10,000

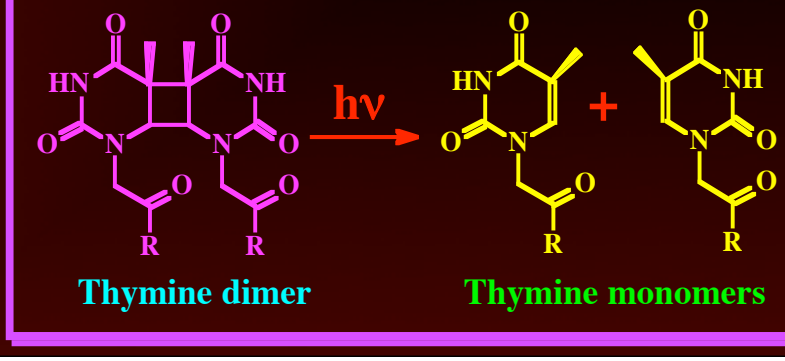

Antibody performing lactonization
Rate acceleration = 167
Stereoselectivity 94%
O
O
R
OH
O
O
O
O
R
O
O
P
O
R
OH
+
O
O
R


Strategy 3:
Introducing catalytic groups at the antibody combining site.
By specifically designing the haptan one can introduce catalytic groups at the antibody combining site.
Alternately, one can add a synthetic catalyst also near the antibody combining site to make catalytic antibodies.


SUBSTRATE
TRANSITION STATE
PRODUCT
HAPTEN

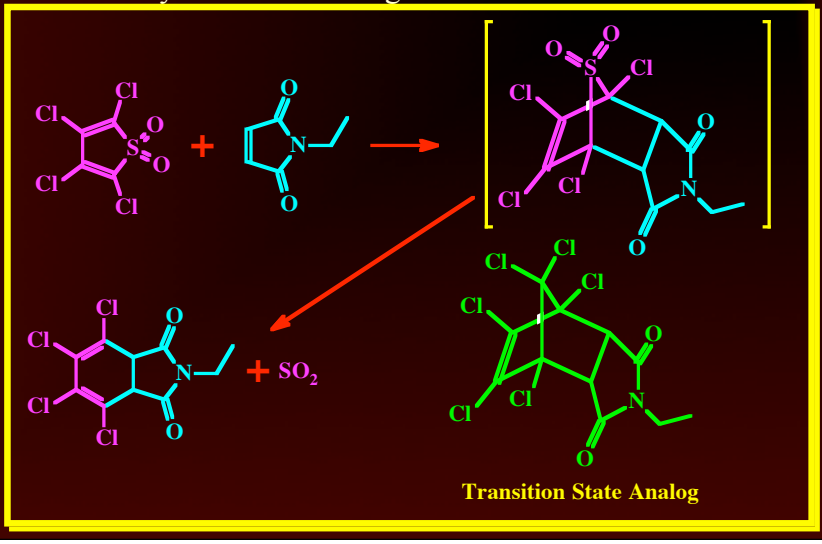

USE OF CHARGE COMPLEMENTARITY BETWEEN THE HAPTAN AND ITS ANTIBODY: ANTIBODY PERFORMING ELIMINATION REACTION
kcat = 0.2 min-1
Km = 182 μM
Ki = 0.29 μM
Rate acceleration = 88,000
Antibody to this compound had a carboxyl group at the binding site that helped the elimination reaction.
+ HF
CATALYTIC ANTIBODY

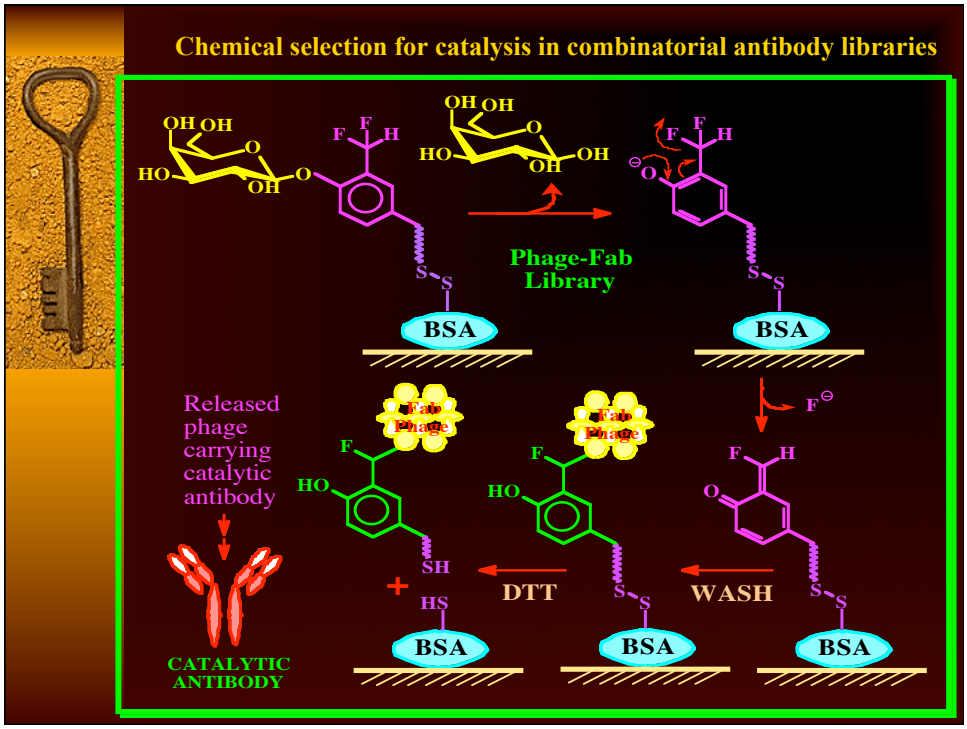
Example 2: **Thymine dimer cleavage**



Diels-Alder Reaction

The transition state analog (green) for the following Diels Alder reaction was used to make the abzyme. The abzyme catalyzes the nonbiological Diels-Alder reaction.





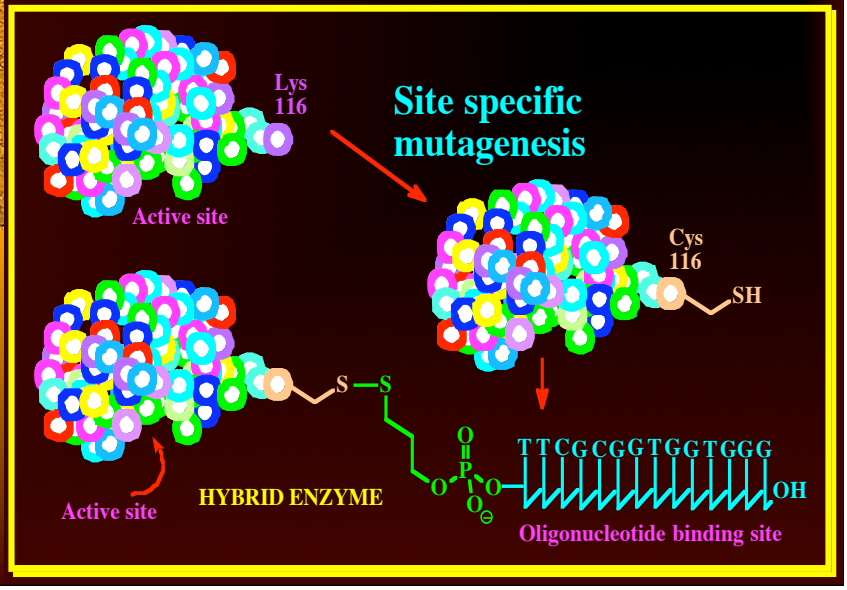
Hybrid Enzymes

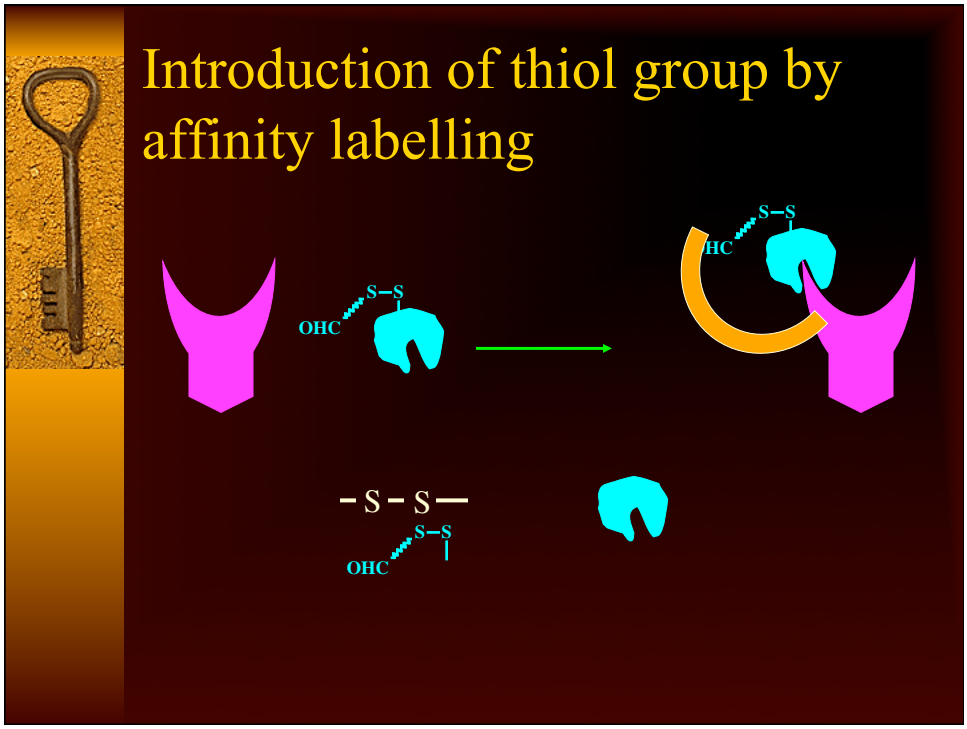
- Non specific enzymes can be made into specific enzymes.
- New catalytic potentials can be generated for known enzymes.

Staphyloccoccal nuclease

- Highly non specific - hydrolysis single stranded RNA, single stranded DNA and duplex DNA at A-U or A-Y rich regions.
- Rate acceleration of 10^{12} over hydroxide ion catalyzed reaction.
- 149 amino acids long single polypeptide.
- Ca^{2+} ions are needed for catalysis.
- Enzyme mechanism known.
- X-ray date at 1.5 Å resolution is available.

Construction of a hybrid enzyme - Staphylococcal nuclease




Introduction of thiol group by affinity labelling
OHC
S–S
HC
S–S
– S – S —
S–S
OHC