De Novo Synthesizer

Cooper Union

iGEM 2014

What is De Novo Synthesis?

- Creation of DNA oligonucleotides
- Does not require a template strand

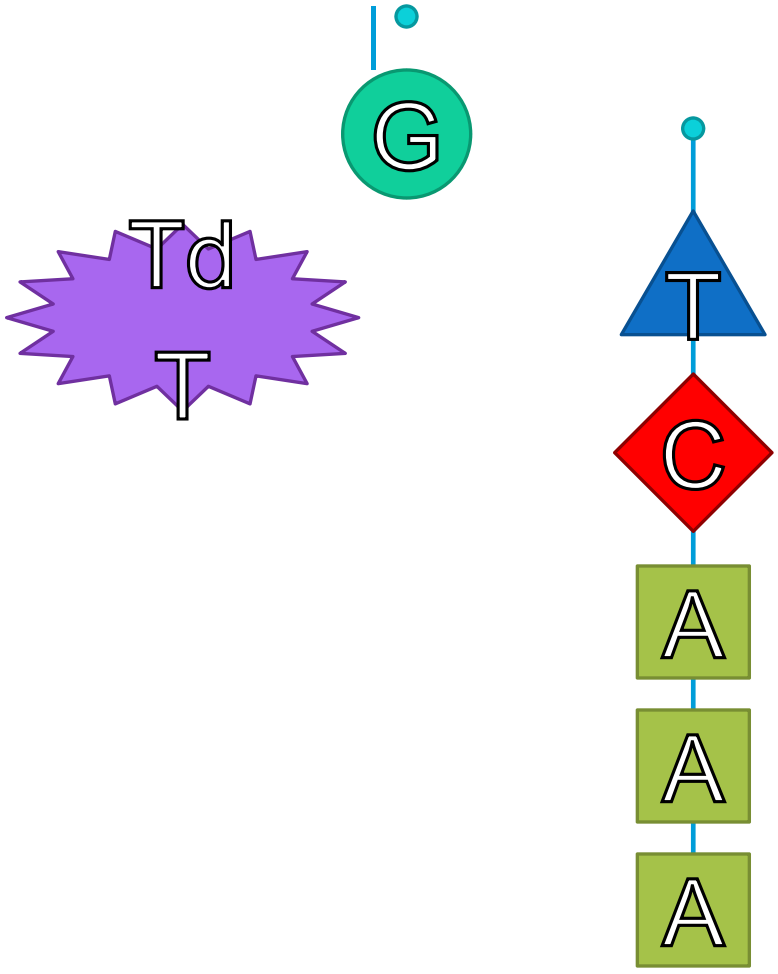
Problems

- Expensive
- Takes long time
- Complicated steps

Our Solution

- De novo synthesis on microfluidic platform
- Protected Nucleotides w/ TdT
- Enables labs to produce in-house oligos
- Saves time & money
- Increases efficiency of research process

De Novo Synthesizer

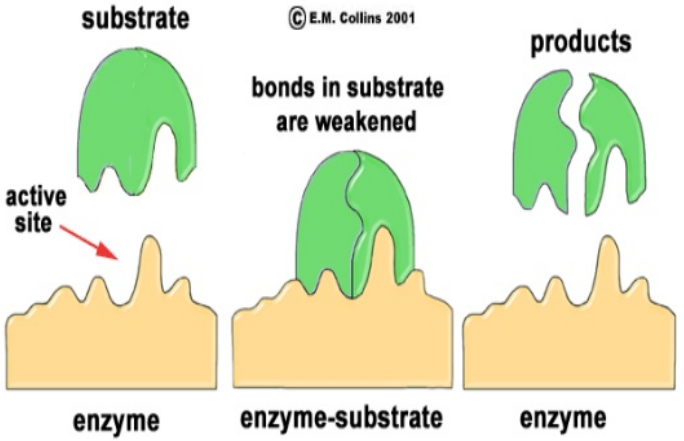


What is TdT?

- Terminal Deoxynucleotidyl Transferase
- Enzyme found in bovine
- Can add nucleotides to single stranded oligos
  - All other DNA enzymes can only add to double stranded DNA

Enzymes

- 99% proteins
- Biological catalysts
- increase chemical reaction
- Not consumed in reaction
- Both fwd/rev reaction
- Highly selective



http://www.google.com/imgres?imgurl=&imgrefurl=http%3A%2F%2Fwww.slideshare.net%2Fmzsanders%2Fhow-enzymes-work&h=0&w=0&tbnid=Bx5MMOXZVpb5YM&zoom=1&tbnh=194&tbnw=259&docid=rH30GWBhamRTYM&tbm=isch&ei=V0rRU8jtJqOi0QWHwoDIBA&ved=0CAgQsCUoAg

Substrate

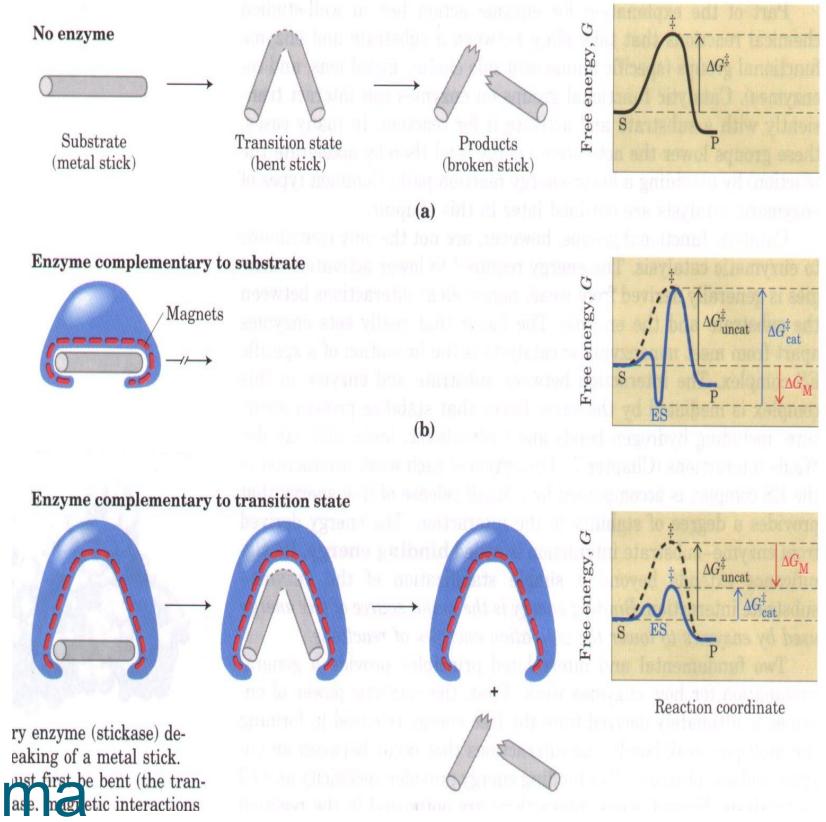
- Molecule upon which an enzyme acts

Active Site

- Area in which the enzyme binds to the substrate

Free Energy

- Amount of energy system can work
  - ATP in the body
- Enzymes decrease free energy increase reaction rates



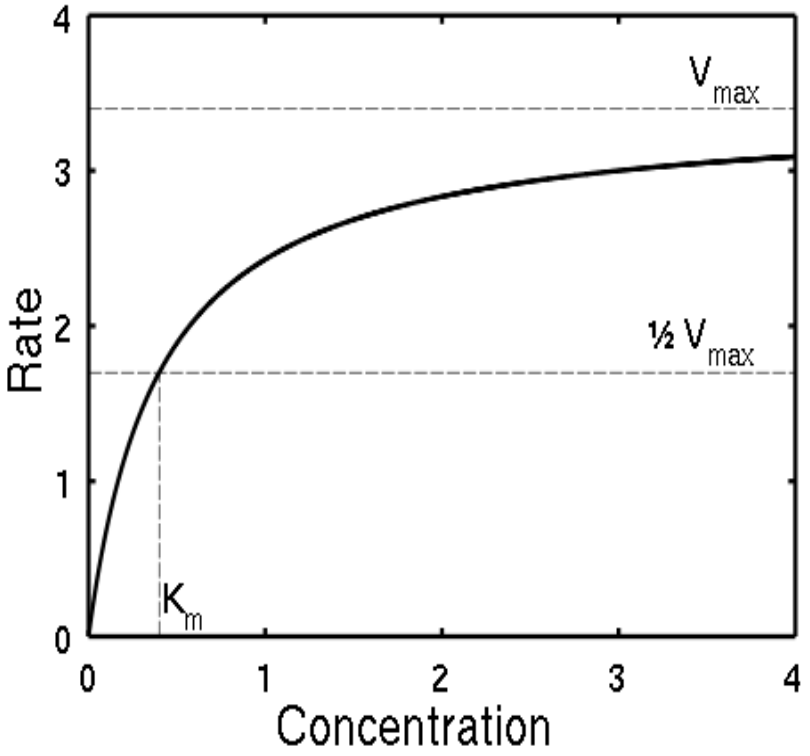
Transition State

- Occurs at an energy maxima
- Exists for a fleeting moment
  - cannot be isolated or directly observed.

http://www.bioinfo.org.cn/book/biochemistry/chapt08/sim1.htm

Michaelis-Menten Equation

- Named after German biochemist Leonor Michaelis and Canadi
- Models enzyme kineti
  - v = reaction rate
  - [S] = concentration of
  - [P]=concentration of
  - V_{max} = maximum rate
  - K_m = substrate conce



http://en.wikipedia.org/wiki/Michaelis%E2%80%93Menten_kinetics

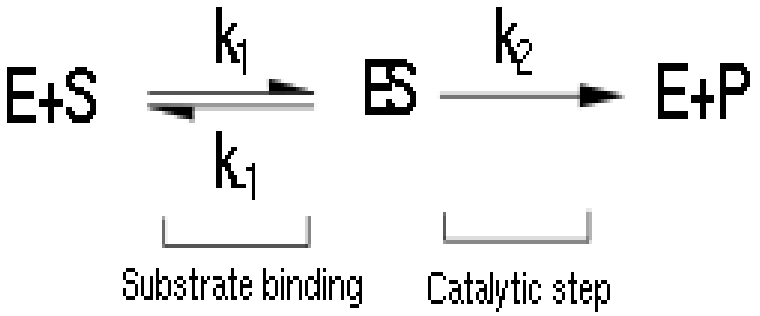
Enzyme Kinematics

- K_M and V_{max} are dependent on the rate constants
- Because ES is a transition state, it is in equilibrium with E+S
- Because enzymes are proteins, their kinetics are also effected by environmental factors such as salt concentrations, pH, temperature, and cofactors

$$K_M \stackrel{\text{def}}{=} \frac{k_2 + k_{-1}}{k_1} \approx K_D$$

$$V_{\max} \stackrel{\text{def}}{=} k_{cat}[E]_{tot}$$

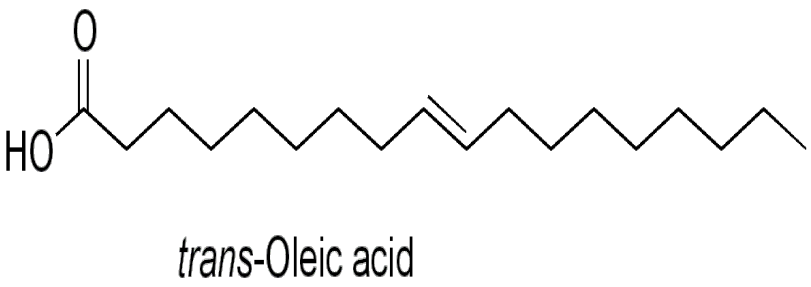
$$[E]_{\text{tot}} = [E] + [ES] \stackrel{!}{=} \text{const}$$



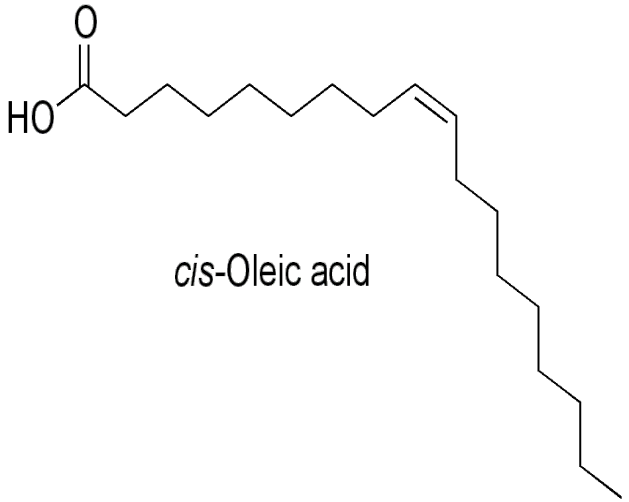
http://en.wikipedia.org/wiki/Enzyme_kinetics

Conformational Change

- When a molecule (proteins, enzymes, fats, etc.) changes its shape



trans-Oleic acid

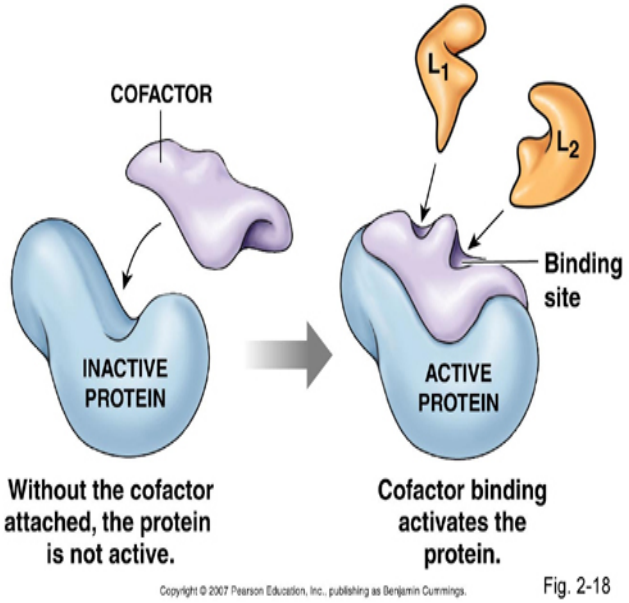


cis-Oleic acid

http://upload.wikimedia.org/wikipedia/commons/a/a9/Isomers_of_oleic_acid.png

Cofactor

- Non-protein
- Required for enzymatic activity
- Induces conformational change
- Not required by all enzymes



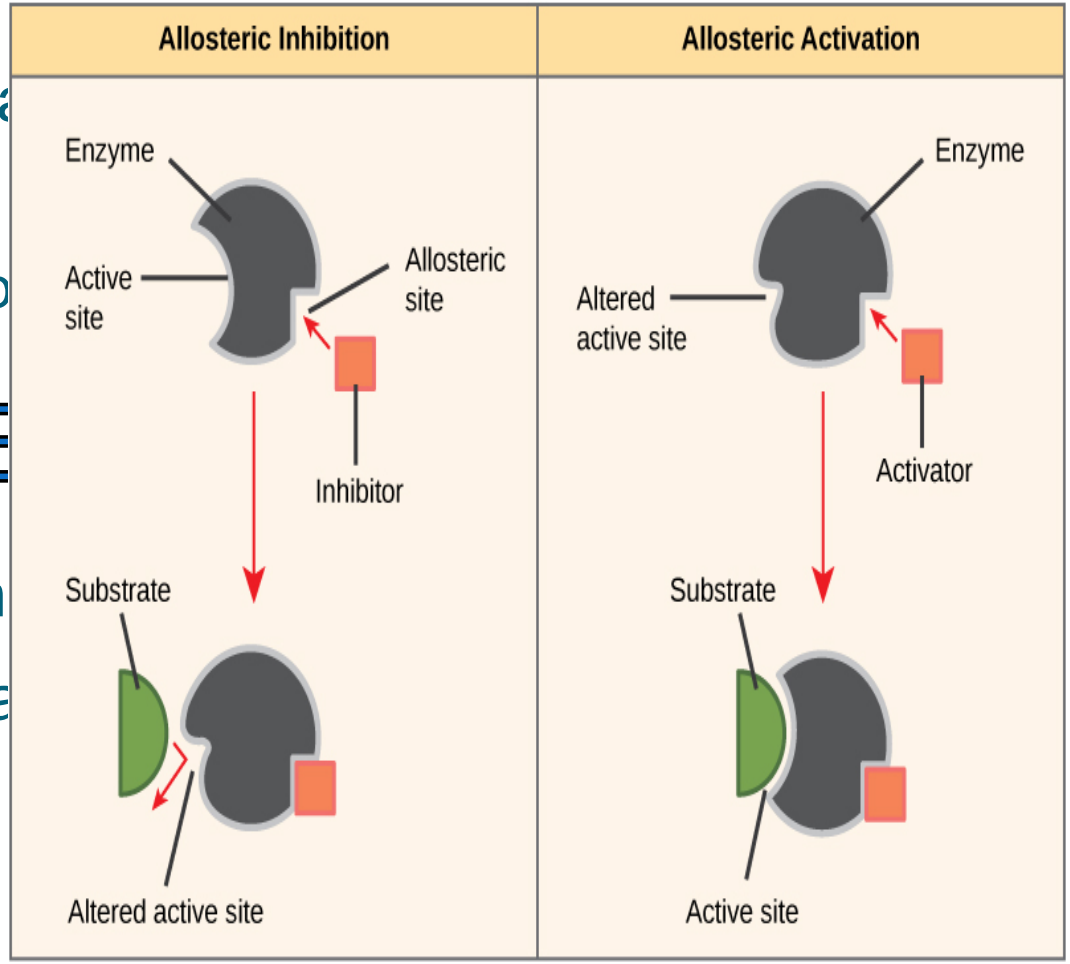
http://2.bp.blogspot.com/_guSOnFRs_Ks/TQXyKW1jWNI/AAAAAAAAAQ4/P_PhLCJshO0/s1600/cofactor.jpg

Effector Molecule

- Molecule that ca
  activity
  - Activator, inhib

Allosteric E

- Enzyme that ch
- Switches from a
- Cofactors used
  changes



http://cnx.org/content/m48610/latest/Figure_06_05_05.jpg

Progress so far

- Ligated pET28b^{+}_TdT
- Verified that TdT was cloned into the pET28b^{+} vector’
- Tested TdT’s functionality
- Attempted to clone TdT into pSB1C3

For the Future

- Express TdT in E. Coli
- Purify TdT from E. Coli
- Optimize synthesis protocol
- Create microfluidic platform

Q&A

Activity—Directions

- Pick the roles:
  Counter, Reaction, Inhibitor, Activator

- Reaction person will get a small spoon to transfer candies to the other cup
- Try bigger spoon
- Inhibitors will use tape to prevent transferring, and try transferring
- Activators will step in and help to aid transfer
- Counters will take time and count the candies

Questions to Consider

- Which reaction was the hardest?
  - Took the most time
  - Had least number of candies transferred during the same time period
- What other factors influence your reaction?
- What happened to the total number of candies in the containers?