

Dynamic Modeling of Catalysis using Rapid Prototyping

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Abstract

The objective of this research is to create educational models that explain concepts in catalysis. Three dimensional depictions of a combined catalytic reaction are created by the Z Corp Z406 Color 3D Printer and Selective Laser Sintering (SLS) Machine. This particular combination reaction illustrates the conversion of d-glucose to d-mannitol using an enzyme, glucose isomerase, and a copper catalyst. One model depicts substrate and transition state binding in an enzyme through the use of two different types of permanent magnets, which serve to simulate different binding strengths. A second model depicts the conversion of an intermediate product to an end product, d-mannitol, through metal catalysis. Two other models illustrate substrate-enzyme binding described by the lock and key and induced fit theories. Another concept, collision theory, is illustrated using models which highlight the importance of molecular orientation for initiating a reaction. This "catalysis teaching kit" illustrates concepts that are not typically depicted three dimensionally. The benefit of a kit which depicts combination catalysis projects to a variety of audiences. Researchers in the chemical industry can learn from models which depict a cost effective and efficient process. The models also serve as an instructional tool in education; students can use the tools to clarify subjects in chemical kinetics. Such a kit will benefit educators because it illustrates complex concepts in biology and chemistry in a manner that is both simple to teach and understand.

Keywords: catalysis, activation energy, binding affinity, transition intermediate, substrate, Rapid prototyping

1. Introduction

With advancements in the field of science and technology in the present day, it is becoming an increasing challenge to grasp basic scientific ideas. Published knowledge in the fields of biology and chemistry typically provide snapshots of seemingly invisible processes—cell division and carbon bonding for example—that cannot be witnessed with the unaided eye. This project aims to break down conceptual barriers regarding the subject of catalysis in a kinesthetic manner that not only clarifies important concepts dynamically, but also engages the audience into tactile exploration. The models created in this research are innovative in the way that they employ the use of electronic devices and magnet implantation methods freshly and creatively.

Catalysts are used in common chemical reactions, including the degradation of the ozone layer, the synthesis of sugars in the body, and the conversion of harmful car emissions to bio-friendly gases. Enzymes can speed up chemical reactions by more than 10 million times. Applications of the catalytic process appear in industrial circles, particularly in pharmaceuticals and chemical companies who can recreate catalysis to produce medical drugs for the health industry or products such as fuel and coolant for automobiles. The computer industry is able to produce PC

units using polymer-producing technology with the help of chemical catalysts.

Biological processes rely on catalysts for the conversion of digested materials to metabolically useful products. Enzymes are numerous in nature and critical for energy production for life forms such as bacteria, plants, and animals. Chemical catalysts are used industrially to create beneficial consumer products. Because of the importance of both, this research focused on depicting a biological catalyst in conjunction with a chemical catalyst. The models depict concepts of a catalytic process in a step-wise manner, so as to hopefully allow for a broader picture of otherwise abstract ideas. The benefit of such a model will also serve to help people understand the differences and similarities between two different types of catalysts.

1.1 catalysis

Catalysis is the acceleration of a reaction by means of a material, called a catalyst, which is itself not consumed by the reaction¹. Catalysts may be solids, liquids, or gases, and are never depleted during the reaction. Heterogeneous catalysis occurs between mediums in different states; for example, gas molecules reacting on metals. Homogeneous catalysis occurs between mediums in the same state. Activation energy is the minimum amount of heat or radiation required to activate molecules to a condition in which it is equally likely that they will undergo a chemical reaction as it is that they will return to their original state. The purpose of a catalyst is to provide an alternate path for a given reaction at a lower energy of activation, enabling the reaction to proceed at a much quicker rate. Activation energy is lowered as a function of the enzymatic reaction and products are formed quickly. That is, if the transition state is bound more tightly than the substrate, which may occur because of bond strain and destabilization, the effect will be catalysis.²

Common models that describe catalysis include the activation energy curve, illustrated in Figure 1, which shows starting materials as they proceed through a reaction. The energy needed for reactant activation is overcome with the help of the catalyst, and substrates peak at the highest energy state (transition state). Materials later reach product form and the total amount of energy released during the reaction is the net energy.

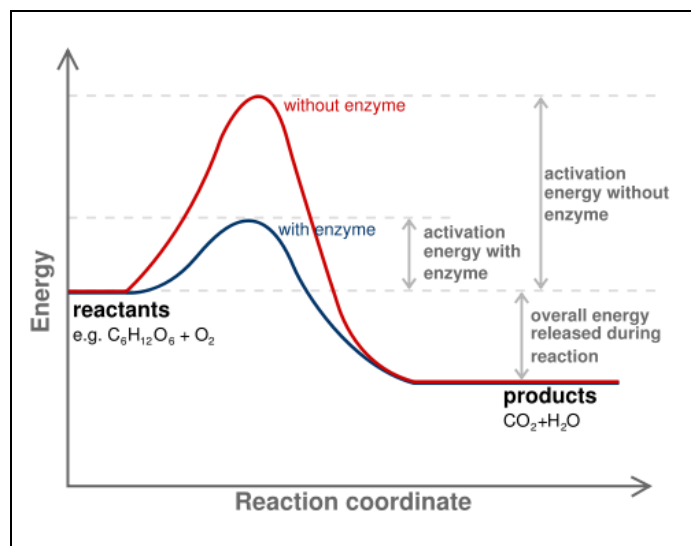


Figure 1. Activation energy curve¹

1.1.1 collision theory

The collision theory of chemical kinetics assumes that in order for a reaction to occur it is necessary for the reacting species (molecules or atoms) to crash into one another with enough force to enact a chemical reaction. The collision must have a sufficient amount of energy to overcome the activation energy needed to proceed to a product. A collision will only be effective, however, if the reactants can come together in a compatible orientation, so as to break bonds between the reacting species.

1.1.2 enzymes

Enzymes are biological catalysts. They are proteins that convert natural materials into expendable products to be used or disposed of by an organism. Most enzymes are substantially larger than the substrates that they catalyze, and only a very small part of the overall structure is actually involved in the process³. Typically buried within the enzyme is a pocket in which the changes to a substrate are made. This pocket is known as the active site, and contains specific molecules which act to break and build bonds of a substrate. Residues, or amino acid groups within the active site, are crucial for forming new conformations of the initial material, thereby changing the properties of that material and creating an end product. Substrate docking versus transition state binding involves the concept of affinity. The substrate in an enzymatic reaction binds into an active site, and changes within the enzyme are induced. The enzyme changes slightly to conform to the bound molecule with the appropriate affinity. The lock and key theory states that as the substrate undergoes changes approaching the transition state, the strength of which the changing complex is bound into the active site increases.

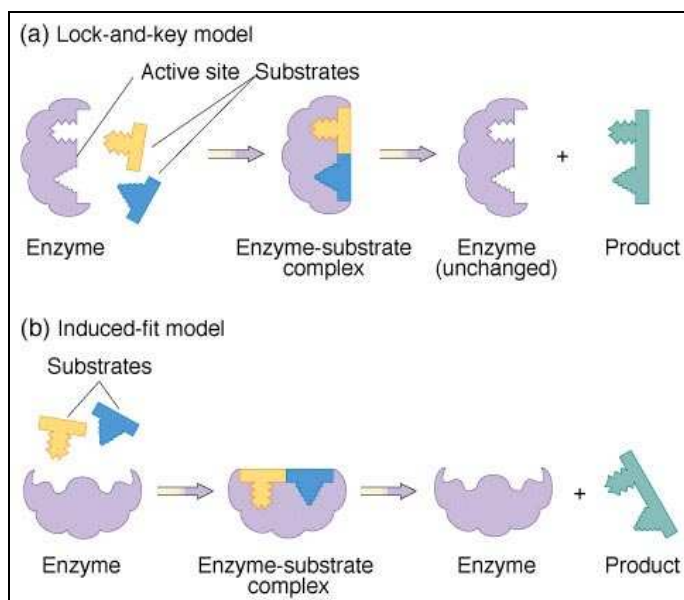


Figure 2. Enzyme-substrate binding¹

The lock and key theory, originated in 1894 by Emil Fischer, and the induced fit hypothesis, originated by Daniel Koshland in 1958, both state that a substrate binds within the active site of an enzyme that has a predisposed shape for that substrate. Figure 2 illustrates the differences between the two ideas. The lock and key theory is a more static depiction of enzyme specificity, and maintains that the site to which the reactant binds stays in the same conformation before, during, and after binding. Lock and key theory was replaced by Koshland's induced fit hypothesis, as it states that the enzyme active site is generally shaped for the substrate, but then changes conformations more specifically as the substrate is docked into the site.³

1.1.3 combined catalysis

Knowledge of the concept of combined catalysis became prevalent in the 1980s. Dr. Tom Kieboom of the Dutch State Mines (DSM) company, an active leader in nutritional and pharmaceutical ingredients, worked extensively in the 1990s with a process which produced sugars in solution suspended with enzymes and metal to effectively produce synthetic sugars used in food.⁴

The process combines two or more different catalysts to produce specialty chemicals and food ingredients in an effective and cost-efficient manner. Classified by scientists as one pot multi-catalytic syntheses, these processes can feature an enzyme as a starting catalyst, which then simultaneously converts a starting reactant to a product without the physical separation of intermediate products. The result of the end enzymatic synthesis can then be treated by a chemical catalyst—either a metal or acid-base combination—to create a final product. Such products are then utilized, for example, by the health care industry in pharmaceuticals and especially in agriculture as fertilizers and

pesticides. Combined catalytic processes are projected by Kieboom to be especially useful in the future because they will ultimately increase product yields and lessen the exposure of toxic chemicals to industry workers⁵.

The reaction depicted is the conversion of alpha d-glucose to d-mannitol, which is a nutritive sweetener typically used in “sugar-free” gum and for pharmaceutical purposes. First developed by Michiel Makkee in the 1980s, the conversion of glucose to mannitol was chosen as a model for the combined catalysis concept because of its widespread usage in medical and consumer industries. The starting solution of glucose is typically obtained by the hydrolysis of sucrose and then treated through isomerization using an enzymatic catalyst. Alpha d-fructose is produced in mixture form as a by-product, along with d-xylitol, and is then simultaneously converted to d-mannitol in the presence of a copper catalyst, suspended on silica.

1.2 glucose isomerase

The synthesis of glucose into xylitol and fructose is provided by glucose (xylose) isomerase. The common practice is to use bacterial species of the protein, the most common forms being *Streptomyces olivochromogenes*, *Streptomyces violaciniger* and *Streptomyces rubiginosus*. All of the proteins catalyze in virtually the same way in nature, differing by one or two amino acid sequences for each corresponding residue in the active site. This project focused on *Streptomyces rubiginosus*, as the combined reaction modeled by Kieboom and associates used the species.

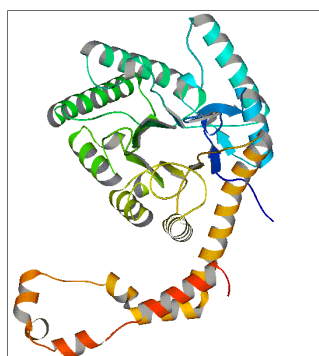


Figure 3. *Streptomyces Rubiginosus*⁵

1.3 copper hydrogenation

Copper, a common metal used in industry, is used in the conversion of glucose to mannose because it yields the most optimal levels of the product in comparison to other commonly used metals, including Rainey-Nickel, and nickel on silica. The selectivity of the d-mannitol enantiomer is also preferred at a 30% increase when copper is used as a catalyst. Hydrogen atoms are introduced onto the surface of the catalyst, which is suspended in a solution, and then act to attach themselves to the d-fructose species via the formation of hydrogen bonds. The end result is the formation of d-mannitol in a linear conformation.

2. Methodology

An electronic device and over 10 digital files were employed to produce the three dimensional depictions for this research. The use of computer technology allowed the conversion of files to compatible programs, and the implantation of electrical devices in the models made them dynamic and kinesthetically illustrative.

2.1 protein data bank and CAD programs

The RCSB Protein Data Bank is a server that compiles files of biological macromolecules for the study of their uses as they relate to structure and function.⁶ The files are converted into digital form from information atomic sequencing in proteins generated by X-ray crystallography, which is a process that determines atom arrangements within a crystal using X-rays scattered by electron. The protein data bank currently features over 44,000 atomic-resolution structures of proteins, including 5 models of *Streptomyces rubiginosus*. *S. rubiginosus* was downloaded as a PDB file and then formatted using RasMol, a Computer Aided Design (CAD) program which allows proteins to be

manipulated three dimensionally. The active site was isolated from the rest of the protein, and interactive molecule groups, or residues, were highlighted with respect to substrate and transition state analogue interactions. Magics software was used to enlarge and duplicate a space-filled view of the active site cut. Magnet holes for binding of the molecules in the active site were also created using Magics software.

2.2 rapid prototyping

Rapid prototyping is a process which takes a computerized, three dimensional format of an object and converts it into free solid form. The Z Corp Z406 Color 3D Printer and the Selective Laser Sintering machine were the two types of Rapid Prototyping machines used to create the models for this research.

The Z Corp Z406 is a printer which uses Computer Aided Design (CAD) files to produce colored, physical parts in 3D. The machine lays colored slices of a three-dimensional optimization layer by layer, eventually producing a solid, opaque part. The SLS printer melts plastics and powders using a built in laser powered by carbon dioxide gases. A CAD file is sent to be processed by the machine and layers of plastic or powder are fused according the dimensions specified by the file⁷.

2.2.1 enzyme modeling: biological catalyst

The enzyme model was created using RasMol and the Z Corp Z406 Color 3D Printer. Substrate and transition state complexes (intermediates) will be featured. The model itself will have permanent magnets implanted into the active site on four residues. These residues, Histamine 54, Glutamic acid 81, Aspartic acid 245 and Tryptophan 137 bind compatibly with the starting molecule, alpha d-glucose^{8,9}. This substrate is implanted with a characteristically weak magnet, aluminum-nickel-cobalt (alnico), which docks into the active site and binds loosely. Neodymium weak permanent magnets were implanted into the transition state species, which then binds into the active site with a noticeable stronger attraction. Each magnet holds a very different degree of magnet pull. The interactions correspond to the idea that the transition state species is of higher affinity than the starting molecule.

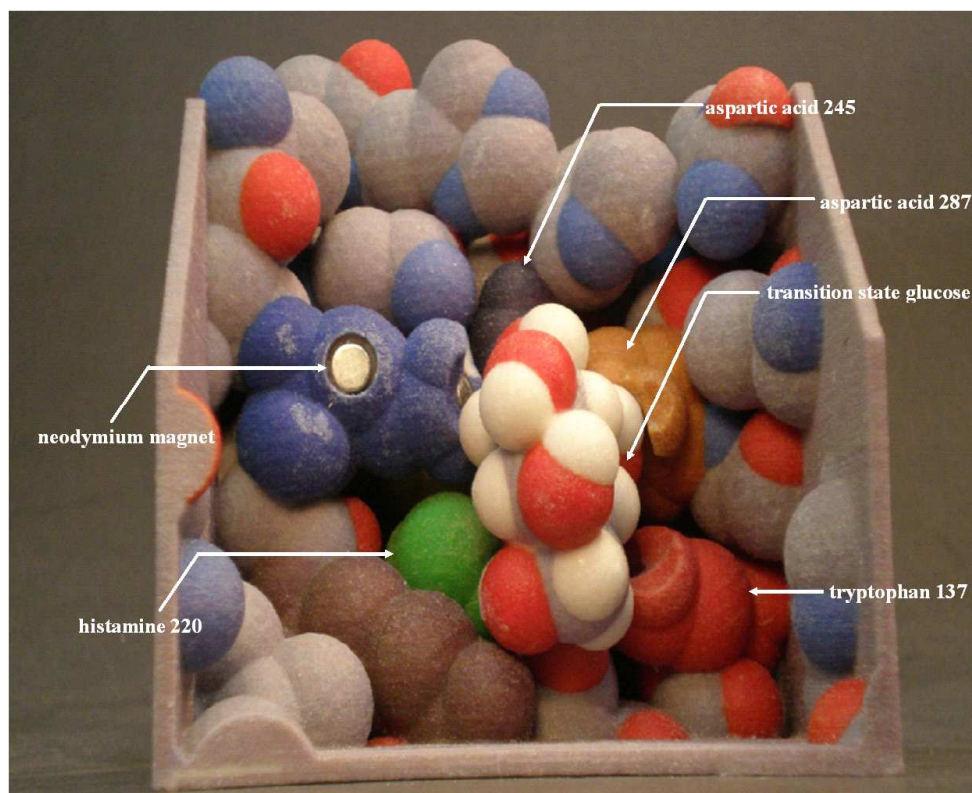


Figure 4. Active site cut of glucose isomerase with transition state

2.2.1.1 magnets

Permanent magnets were implanted into the substrate and transition state analogues of the enzyme model. The enzyme active site houses four sites for magnet implantation. A maximum of four neodymium magnets make contact with the substrates in the active site of the enzyme model at a time. The initial substrate model houses two alnico magnets. The transition state molecule houses three permanent neodymium magnets. All neodymium magnets used were disk shaped and possess a .125" diameter. The alnico magnets have .2" diameters and are cylindrical. Neodymium is 20 to 40 times stronger than sintered alnico¹⁰. The use of different magnets to compare concepts in chemical kinetics such as binding affinity has not been depicted in molecular models; this approach is innovative and interactive.

2.2.2 metal catalyst model- heterogeneous catalyst

The metal catalyst model was created using the SLS Machine. The catalyst is a layered "sheet" of molecules. Copper atoms were represented by 1" spheres, mounted on a platform which provides stability and also houses the linear voice actuator. The heterogeneous catalytic process is depicted through a progression of four steps:

- *Adsorption*, or the attachment of substrate to the surface of the catalyst,
- *Diffusion*, or the interaction between the adhering molecules and the substance,
- *Reaction*, or breaking and connecting of bonds between molecules present in a solution and the substrate
- *Desorption*, or the release of a product from the surface of the catalyst into the solution.

Each of the first three steps is depicted using molecules produced by the Z-Corp machine. Both the substrate and model were implanted with magnets to simulate binding action on different activity sites on the surface of the model. The launching of the product by the voice coil depicts the release of the end product from the surface. The model proves to be an interactive tool for students, as it simulates the behavior of molecules in nature.



Figure 5. Heterogeneous catalysis of d-fructose on a copper surface

2.2.3 lock and key and induced fit models

The lock and key theory and induced fit hypothesis were illustrated three dimensionally using SLS technology. The lock and key depiction features corresponding parts as which fit together similar to the manner in which actual key fits a lock. The induced fit model borrows the same base shape as the lock and key model, however, the binding is depicted in screw and lock form. Threads were extruded out on the substrate model and extruded in the enzyme; the action of fitting the models together represents the gradual conformity of substrate settling into enzyme, which possesses a general shape for the substrate.

2.2.4 collision theory model

Collision theory was depicted using SLS technology. The model highlights the orientation rule using the same 4" blocks as used in the induced fit model. Using the same models to illustrate different concepts allows the kit to be more portable. Two pairs of the induced fit models depict the collision of molecule pairings "A" and "B" in an orientation that is most desired for a chemical reaction to occur. Collision theory model uses basic shapes to illustrate fruitful and unfruitful collisions.

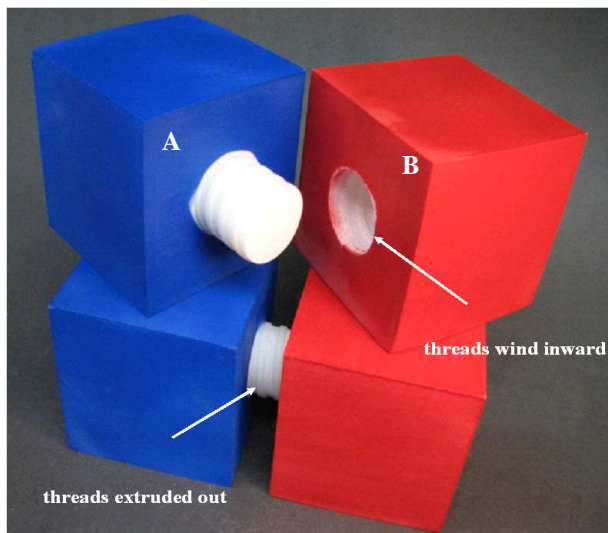


Figure 6. Collision Theory Model

2.2.5 linear voice coil actuator

A linear voice actuator is a two wire device used in rotary-motion machines using linear forces and high acceleration. It is comprised of a coil which sits within a cylinder that possesses a magnetic field when powered. The coil, referred to as the bobbin, sits upon an extruded shaft and inserts loosely into a groove that circles the inner diameter of the actuator piece.¹¹ When current ran through the device, a magnetic field runs around the groove, enabling the coil to move upward. The actuator was implanted into the model to simulate the movement of molecules off of the surface of a metal catalyst. The piece used had a 1.56" diameter and allowed a maximum of 25 volts run over it. The actuator was run at a 15 volt maximum however, and produced the desired level of elevation. The molecular model was shot up a distance of 5" when mounted onto the surface of the bobbin. The actuator extends upward quickly as a current is passed through it, releasing the final product. The linear voice coil used in this research was produced by the BEI Kimco Company and stands 1.2" tall with a 1.56" diameter.



Figure 7. Linear Voice Coil Actuator¹¹

3. Conclusion

The end result of this research provides both an educational tool and resource for educators and students. Studying the combined catalytic reactions will enable researchers to perhaps develop methods of producing industrial materials, in which chemical and biological reactions can be joined to provide resource and cost effective ways of

producing a given product. The concept of chemical kinetics can be more clearly illustrated with the use of such a catalysis tool kit. As science students learn kinesthetically, they gain from learning abstract processes along with the ideas and definitions behind them. Such tools may be the subject of research in the future; the three dimensional depiction of a concept and its associations in a holistic manner.

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5. References

1. Search Database, “Enzymes.”<http://www.search.com/reference/Enzyme>
2. Gregory A. Petsko and Dagmar Ringe, *Protein Structure and Function* (London: New Sciences Press Ltd, (2004): 52-69.
3. Alex Gutteridge and Janet Thornton, “Conformational Changes Observed in Enzyme Crystal Structures upon Substrate Binding,” *Journal of Molecular Biology* 346 (2005): 21-28.
4. A.P.G. Kieboom, “Integration of Biocatalysis with Chemocatalysts: Cascade Catalysis and Multi-step Conversions in Concert,” *Catalysis for renewables: From Feedstock to Energy Production* (2007): 273-298.
5. Michiel Makkee, A.P.G. Kieboom and H.van Bekkum. Production Methods of D-Mannitol,” *Starke (Starch)* 37: (1985): 136-141.
6. RSCB Protein Data Bank, “Glucose Isomerase” <http://www.rcsb.org/pdb/home>
7. MSOE Website. “Rapid Prototyping” <http://www.msOE.edu/>
8. Richard D. Whitaker and others, eds., “Probing the Roles of Active Site residues in D-Xylose Isomerase,” *Journal of Biological Chemistry* 270 no. 39 (1995): 22895-22906.
9. Amy K. Katz and others, eds., “Locating active site hydrogen atoms in D-xylose isomerase: Time of flight neutron diffraction,” *PNAS* 103 no. 22 (May 2006): 8342-8347.
10. Stanford Magnets Company, “Permanent Magnets” www.stanfordmagnets.com/
11. BEI Kimco Magnetics. “Linear Voice Coil Actuator” <http://www.beikimco.com/>
12. Ossi Pastinen, “Xylose isomerase from *Streptomyces rubiginosus*: Stability, novel reactions and applications,” Espoo2000, Helsinki University of Technology, www.tkk.fi/Units/BioprocessEngineering/
13. Abdel M. Akher, “Factors Affecting the Catalytic Hydrogenation of D-Glucose,” *Starke (Starch)* 9: (1974): 307-312.
14. A.P.G. Kieboom and Rob Shoevaart, “Combined Catalytic Reactions—Nature’s Way,” *Chemical Innovation* 31 (December 2001): 33-39.