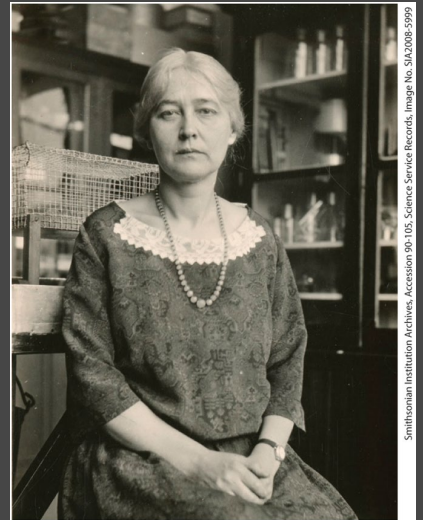
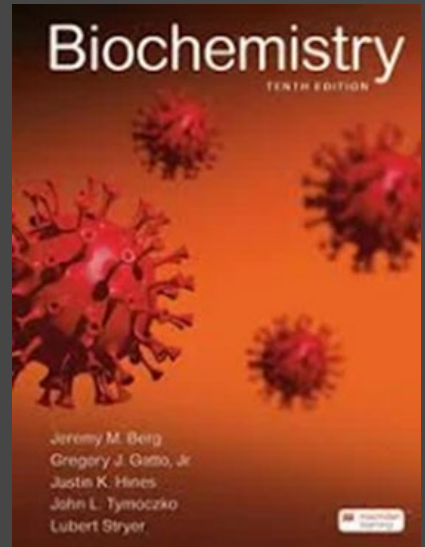


43W: HET RITME VAN ENZYMEN: MICHAELIS MENTEN KINETIEK

15 NOVEMBER 2024



Wie ben ik?

Mark Koren

mark.koren@hu.nl



Doelen vd workshop



Zicht krijgen op:

- ☐ Enzymkinetiek
- ☐ Werken met MM grafiek & Lineweaver Burk plot
- ☐ Discussie voor wie is dit leuk?

Zoals beloofd

Het ritme van enzymen – Michaelis Mentens grafieken

Mark Koren – Hogeschool Utrecht

43W

Enzymkinetiek wordt maar erg summier behandeld in het eerste-grads gebied en dat is erg jammer! Enzymkinetiek is het onderzoeksgebied dat zich bezighoudt met chemische reacties die door enzymen worden gekatalyseerd.

Werkvorm:

Workshop

Materiaal:

Je krijgt materiaal mee naar huis waarmee je zelf het practicum kan uitvoeren

We gaan aan de slag met een zeer eenvoudig practicum waarmee we samen data vergaren, om zo, met wat rekenwerk, zelf de grafieken van Michaelis Menten na te kunnen maken. Daarna bespreken we wat dit nu alles betekent. Dit alles is gebaseerd op het artikel van Hinckley. Met het materiaal van deze workshop kan je makkelijk een klein (vakoverstijgend) PO ontwikkelen.

Het meenemen van een laptop naar deze bijeenkomst is aan te raden!

JOURNAL OF
CHEMICAL EDUCATION

Communication

pubs.acs.org/jchemeduc

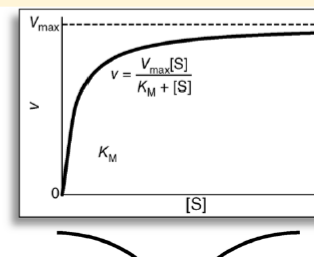
A Method for Teaching Enzyme Kinetics to Nonscience Majors

Glen Hinckley*

Department of Chemistry, Farmingdale State College, Farmingdale, New York 11735, United States

S Supporting Information

ABSTRACT: Enzyme kinetics is an essential part of any proper chemistry education, especially in health care fields such as nursing. Unfortunately, few quality methods for teaching the concepts to nonscience majors have yet been developed. Herein is described the modification of an existing active-learning teaching method with a conceptual basis for teaching enzyme kinetics to nonscience majors. The materials are readily available and the method is both facile and engaging.



Een alternatief

OPEN ACCESS Freely available online



Lesson

Breaking Bricks: A Hands-On Model of Enzyme Kinetics and Inhibition

Louise E.O. Darling, John W. Goss, and Julie A. Roden*

Department of Biological Sciences and Biochemistry Program, Wellesley College

Abstract

Enzyme kinetics and the impacts of inhibitors on the enzyme's maximal velocity and ability to bind substrates are important topics in cell biology and biochemistry. However, these topics can be difficult for students to grasp when instructed using a traditional lecture format. Teaching biological concepts using physical models has been shown to improve student comprehension and engagement with the topic. We have developed a pre-lab activity that uses plastic building bricks and student "enzymes" to expose students to these concepts prior to conducting enzyme assays at the bench. Small groups of students take turns acting as an enzyme that catalyzes a hydrolysis reaction with increasing substrate concentration in the presence and absence of a competitive inhibitor. Students graph brick breaking rate data and make observations about the effect of changing parameters on key metrics. We conclude the activity with a class discussion on their observations. According to survey data, our students show an increase in the ability to answer conceptual and graphical questions correctly after completing the activity and corresponding material. Moreover, the majority of students thought that the activity was moderately or greatly helpful at increasing their understanding of key concepts. This kinesthetic active learning approach provides an engaging and fun way to introduce students to modeling enzyme kinetics and is adaptable to any class or laboratory setting.

Citation: Darling LEO, Goss JW, Roden JA. 2021. Breaking bricks: A hands-on model of enzyme kinetics and inhibition. *CourseSource*. <https://doi.org/10.24918/cs.2021.16>

Editor: Leocadia Paliulis, Bucknell University

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Copyright: © 2021 Darling, Goss, and Roden. The authors affirm that they either own the copyright to or have received written permission to use the text, figures, tables, artwork, abstract, summaries, and supporting materials. This is an open-access article distributed under the Creative Commons Attribution-NonCommercial 4.0 International License. The authors retain ownership of the copyright to their article, but allow anyone to download, reuse, reprint, modify, distribute, and/or copy the article, as long as the original authors and source are cited and the intended use is not for commercial purposes.

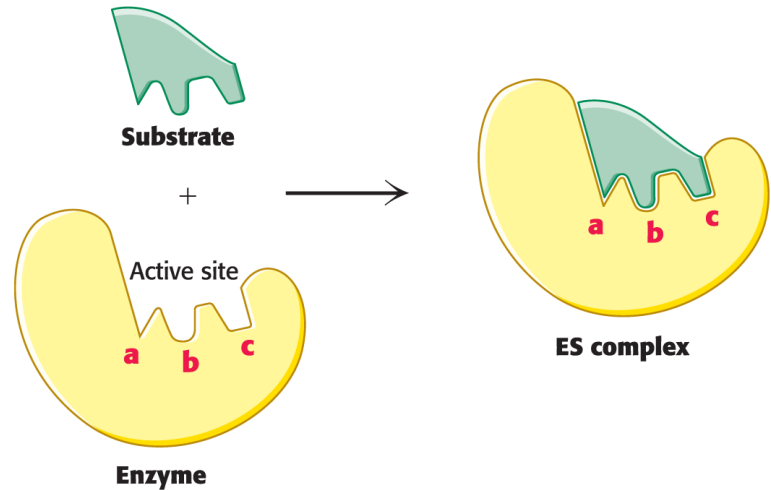
Even kort over enzymen

- ☐ Versnellen biologische reacties
- ☐ Hebben geen invloed op de reactieconstante K_{ev}
- ☐ Hebben geen invloed op ΔG
- ☐ Hebben geen invloed op de ligging van het evenwicht
- ☐ Hebben sterke specificiteit
- ☐ Hebben vrijwel allemaal een cofactor nodig
- ☐ Zijn in te delen in type reactie die ze versnellen

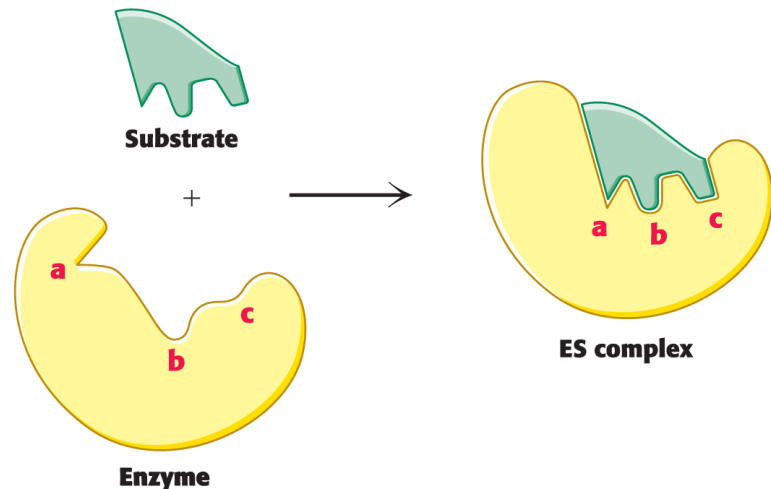
‘Nieuw’ model

- ☐ Sleutel slot model achterhaald
- ☐ ‘induced fit’ model:
- ☐ De fit (pasvorm) van E + S verandert E van vorm

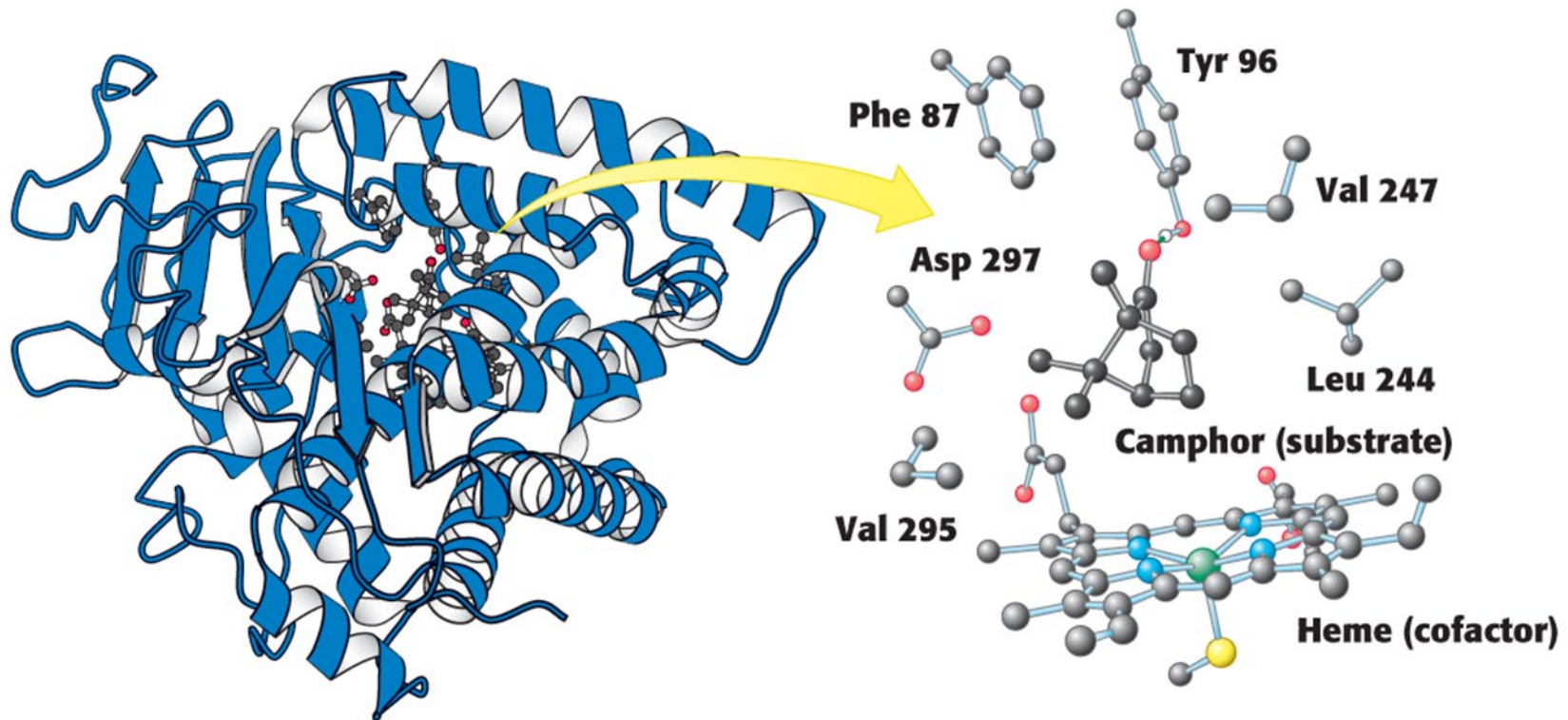
(A) Lock and Key



(B) Induced Fit

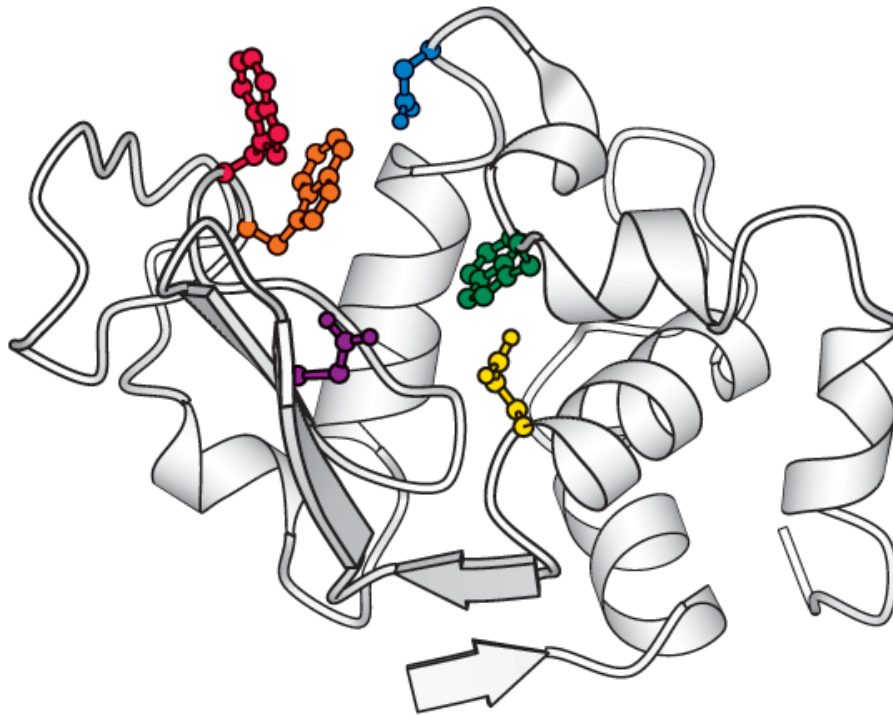


Actieve deel van enzyme wordt bepaald door een paar aminozuren en cofactoren

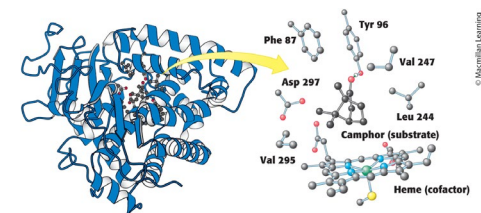


Aminozuren ver van elkaar verwijderd in primaire structuur
vormen de *active site*

(A)

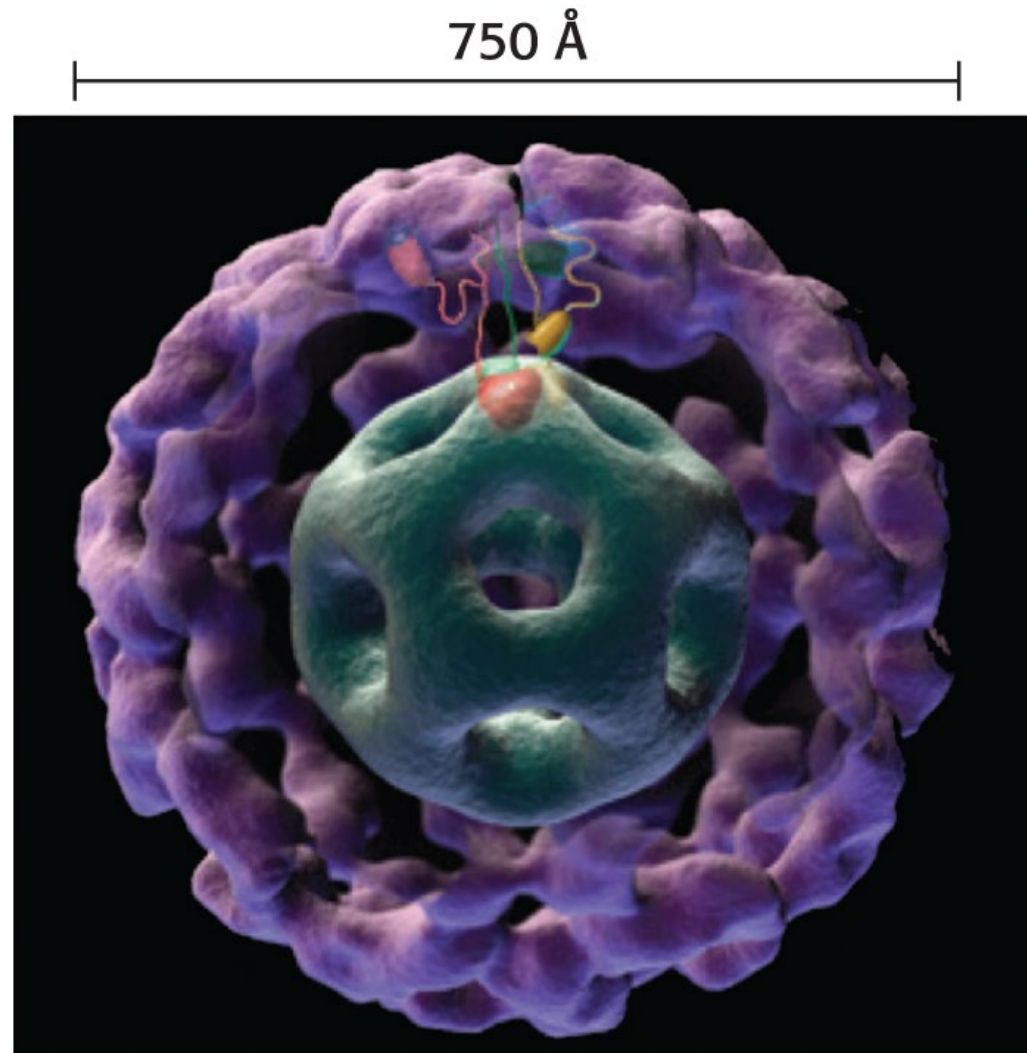


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pyruvaatdehydrogenase



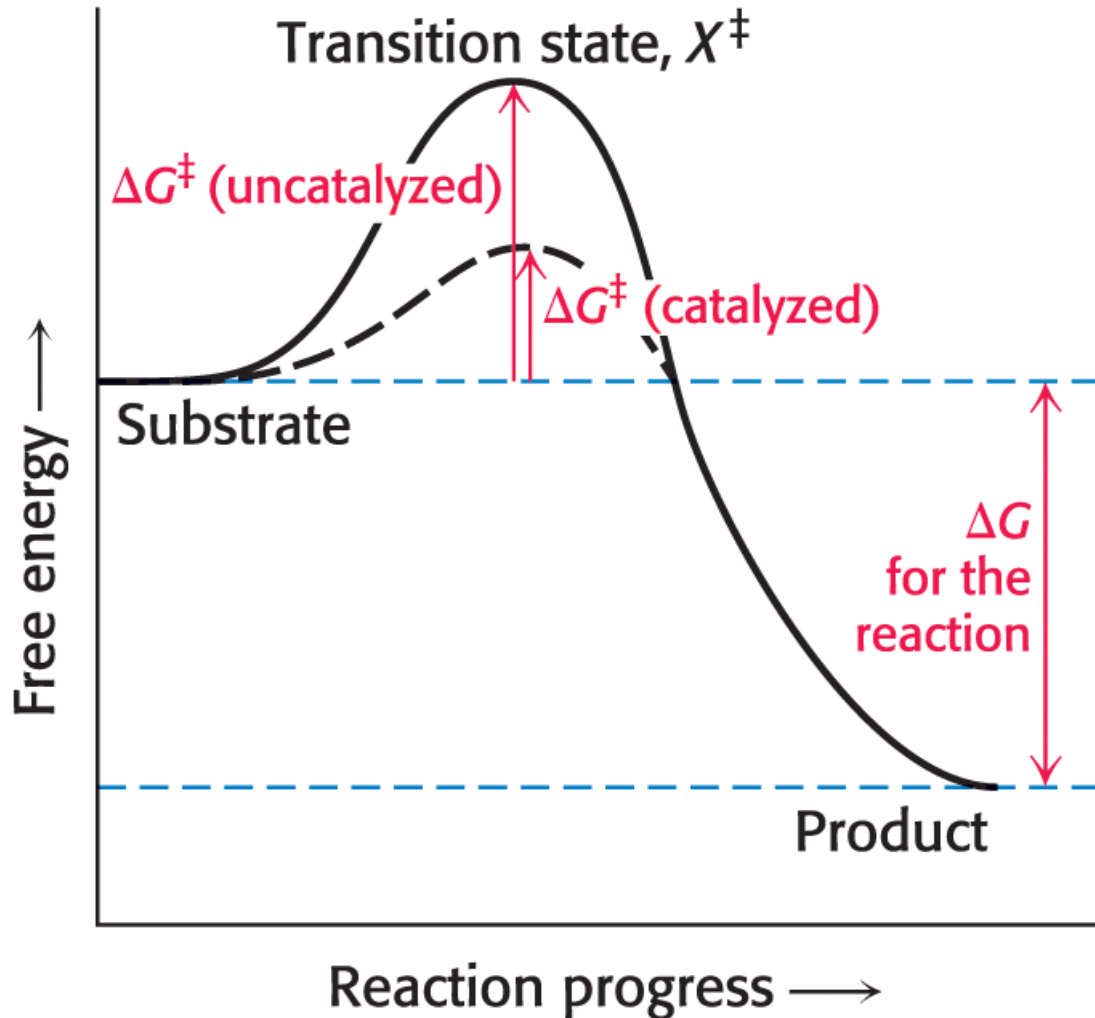
Donald Bliss, National Library of Medicine.



TABLE 5.1 Rate enhancement by selected enzymes

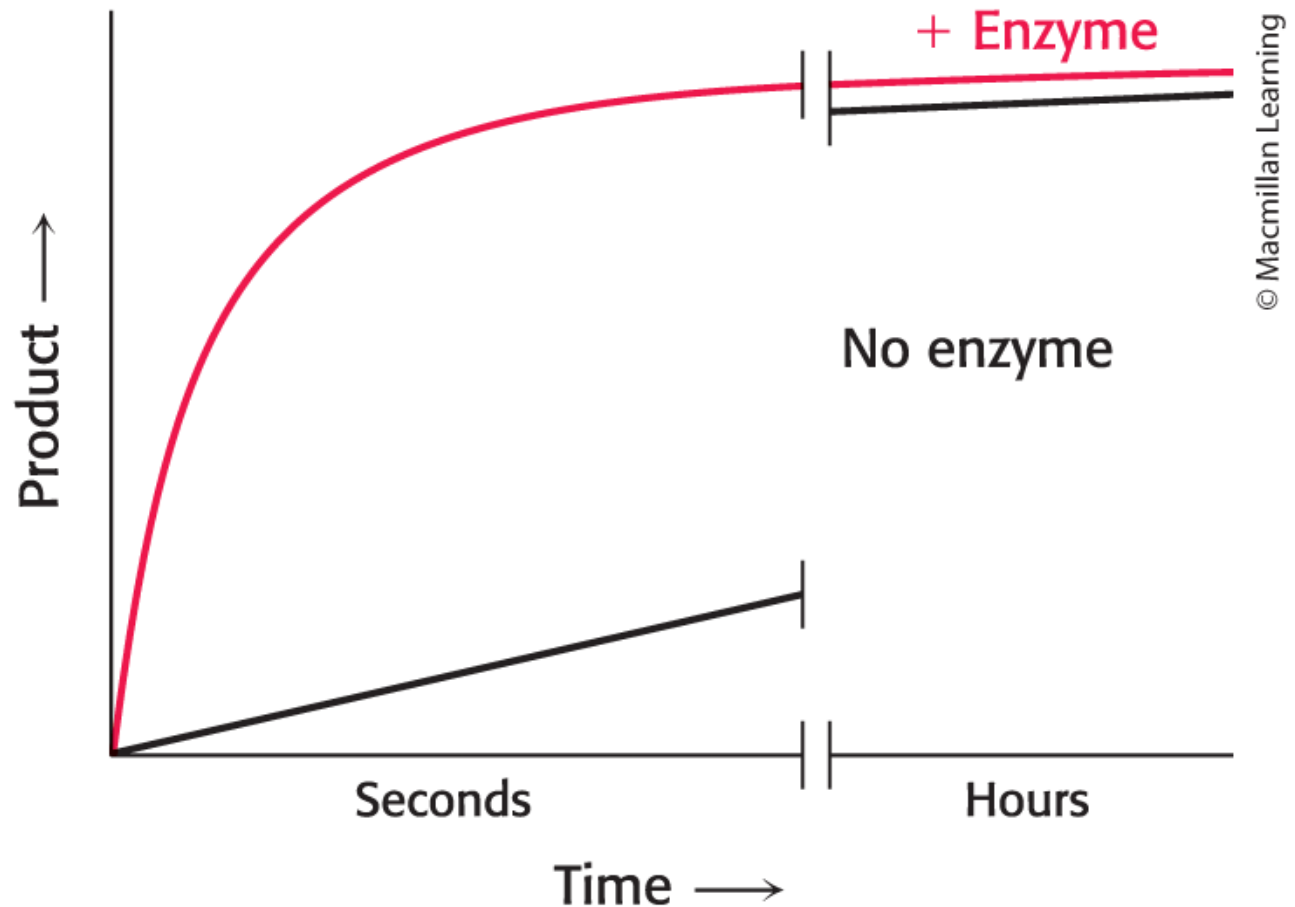
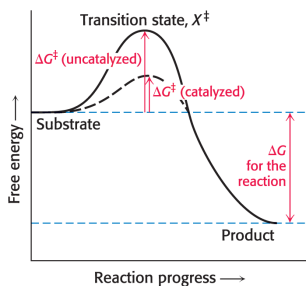
Enzyme	Nonenzymatic half-life	Uncatalyzed rate ($k_{\text{un}} \text{ s}^{-1}$)	Catalyzed rate ($k_{\text{cat}} \text{ s}^{-1}$)	Rate enhancement ($k_{\text{cat}} \text{ s}^{-1} / k_{\text{un}} \text{ s}^{-1}$)
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4300	1.0×10^9
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^6
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^6	7.7×10^6

Enzymen verlaging activeringsenergie



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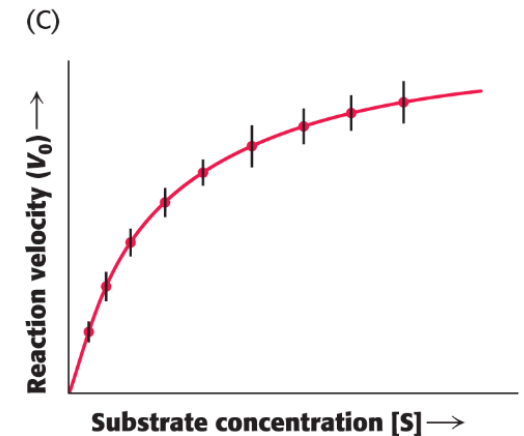
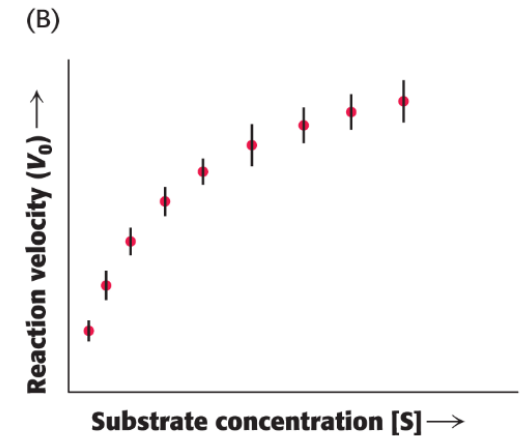
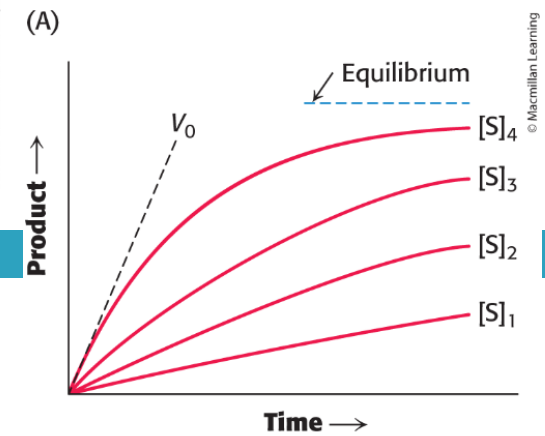
Enzymen hebben geen effect op thermodynamica



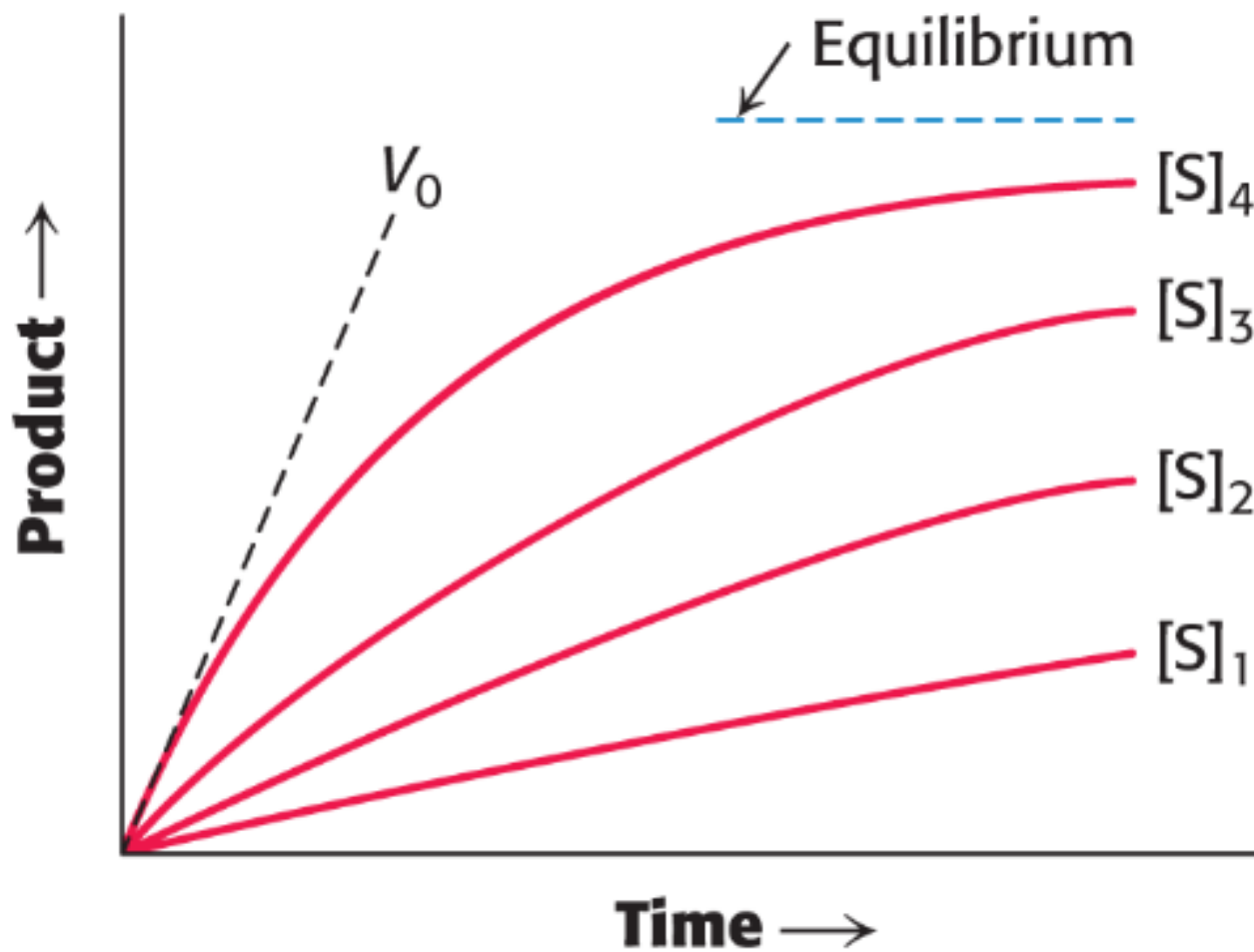
Michaelis–Menten

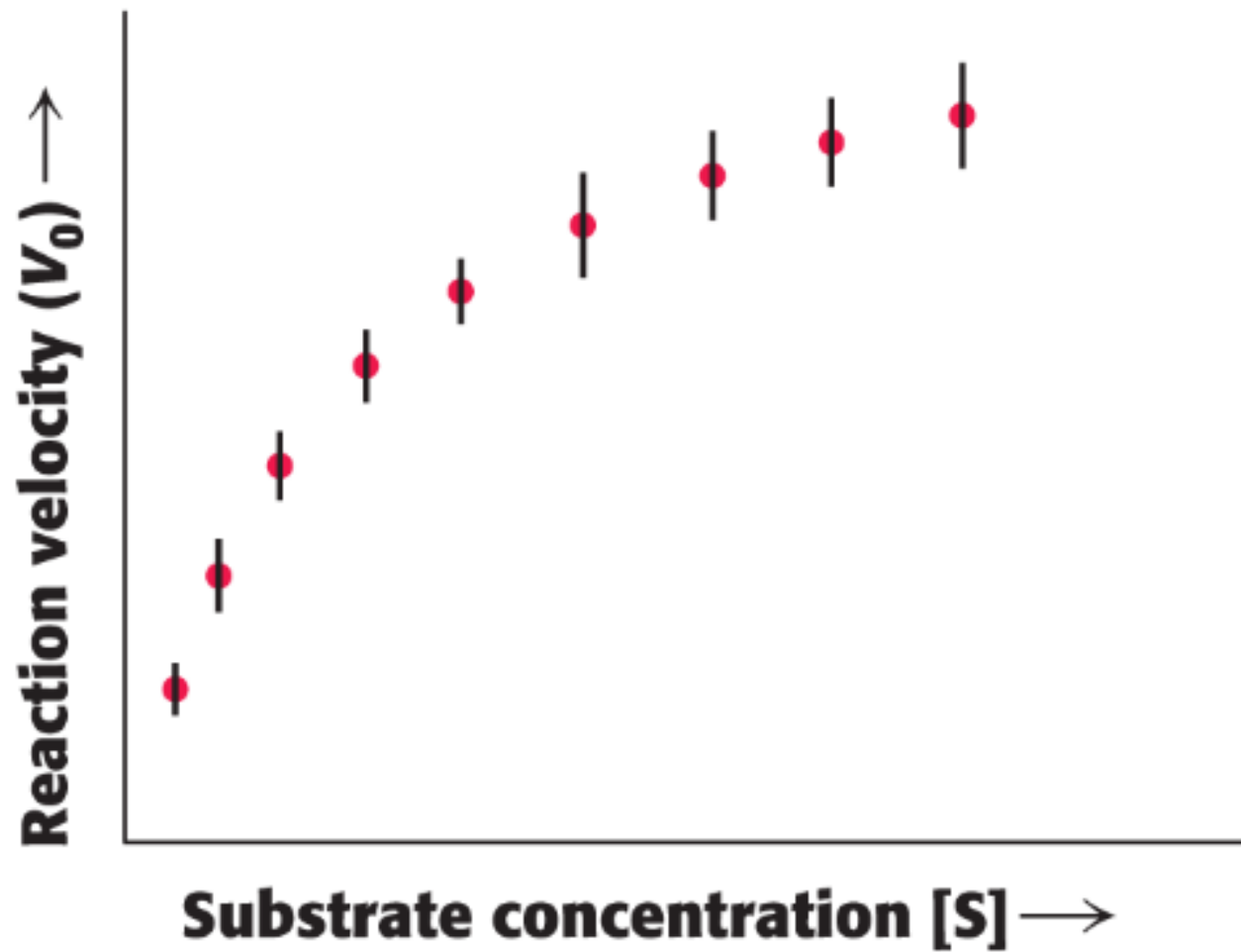


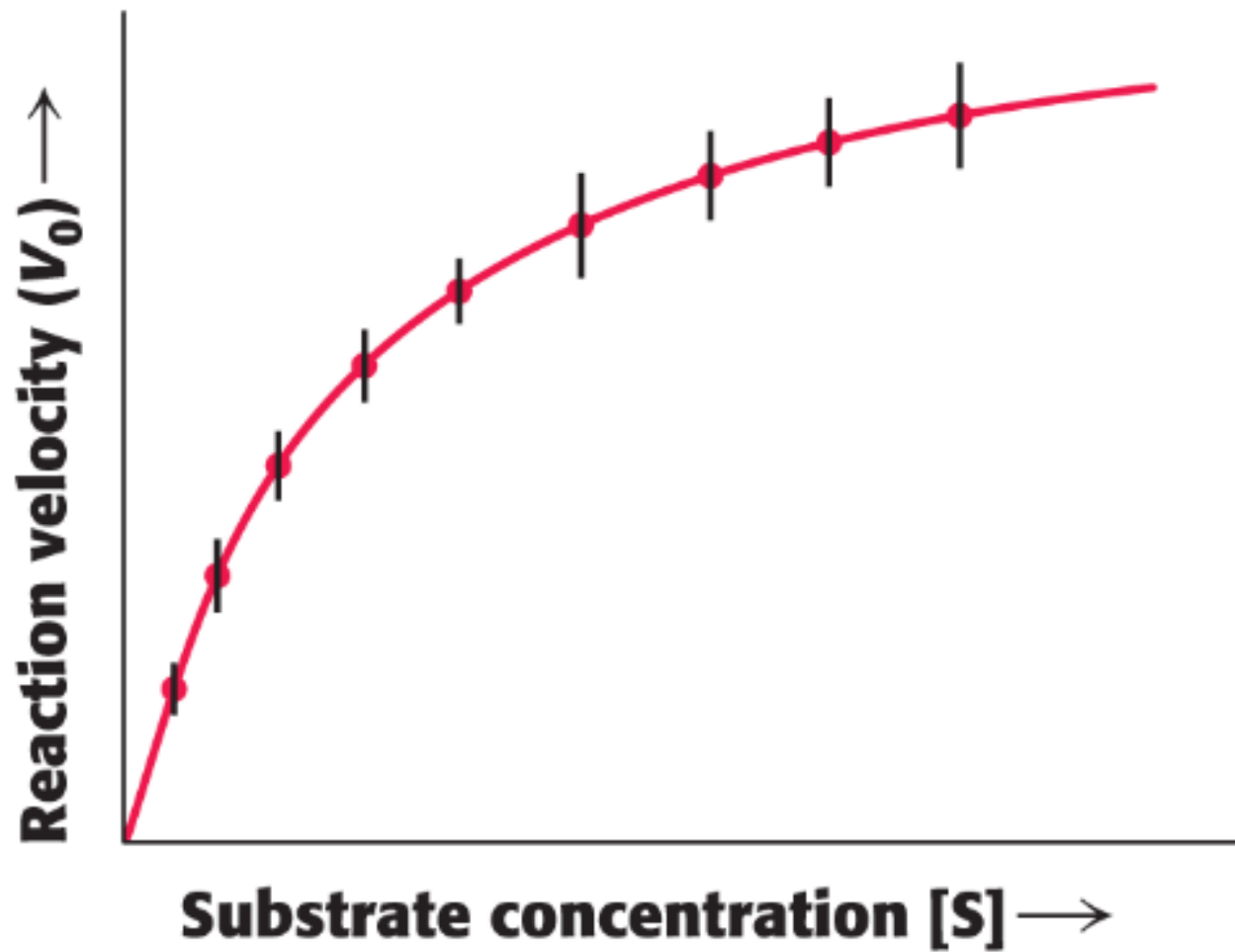
1. Enzym + Substraat worden een ES-complex met bepaalde snelheid k_1
2. Deze kan 'terugvallen in $\text{E} + \text{S}$ met snelheid k_{-1}
3. Het ES-complex kan ook 'z'n werk doen en $\text{E} + \text{Product}$ worden met snelheid k_2
4. In veel biologische situaties is er geen k_{-2}



(A)

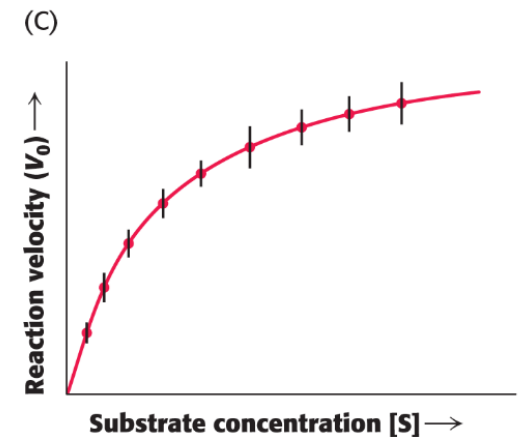
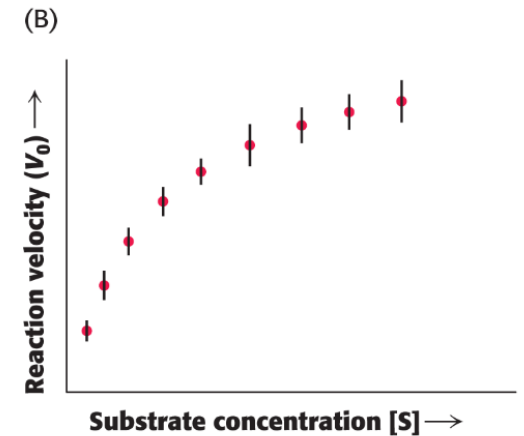
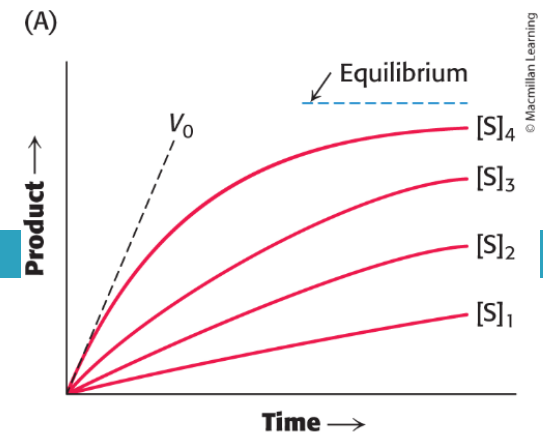
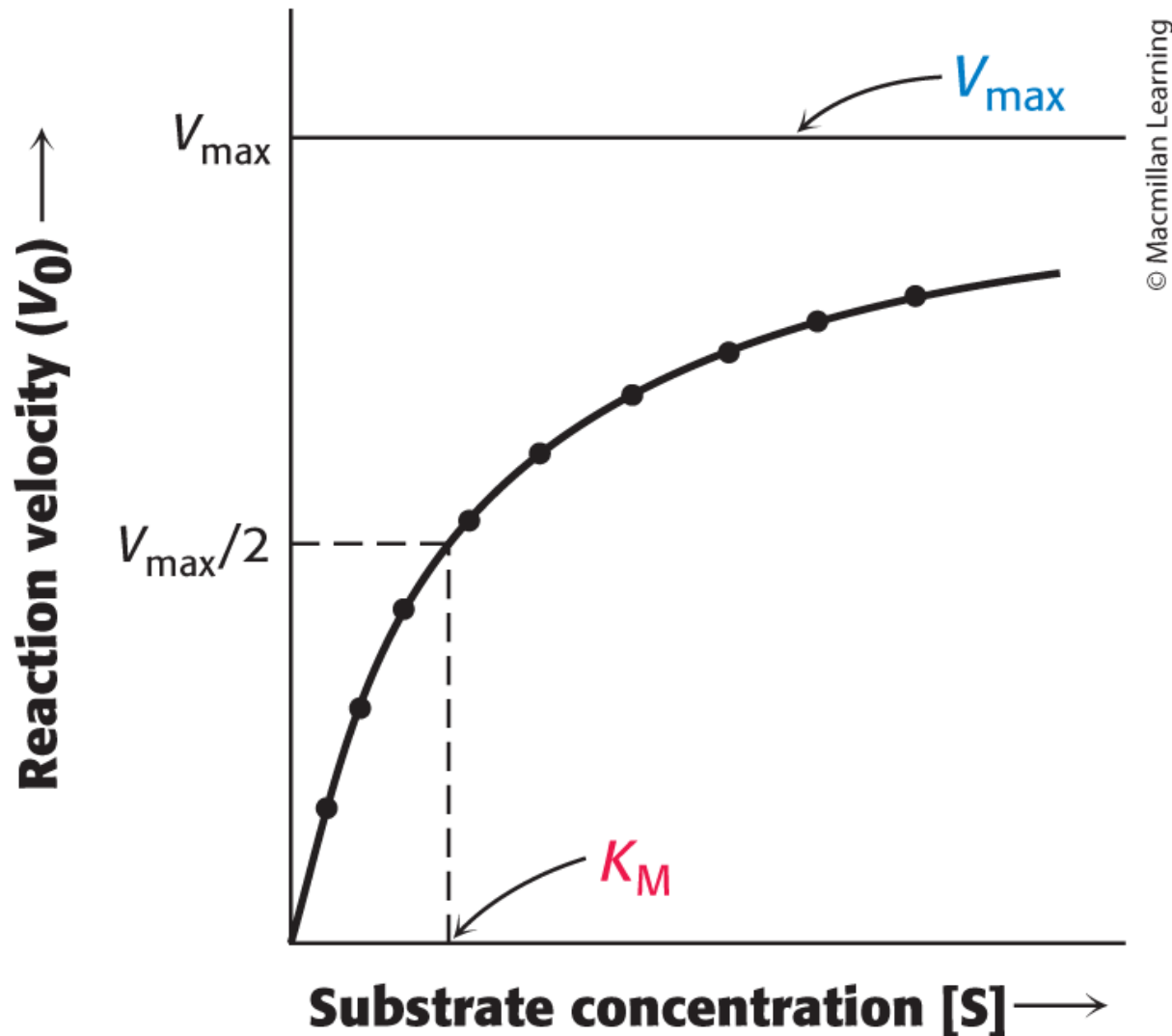






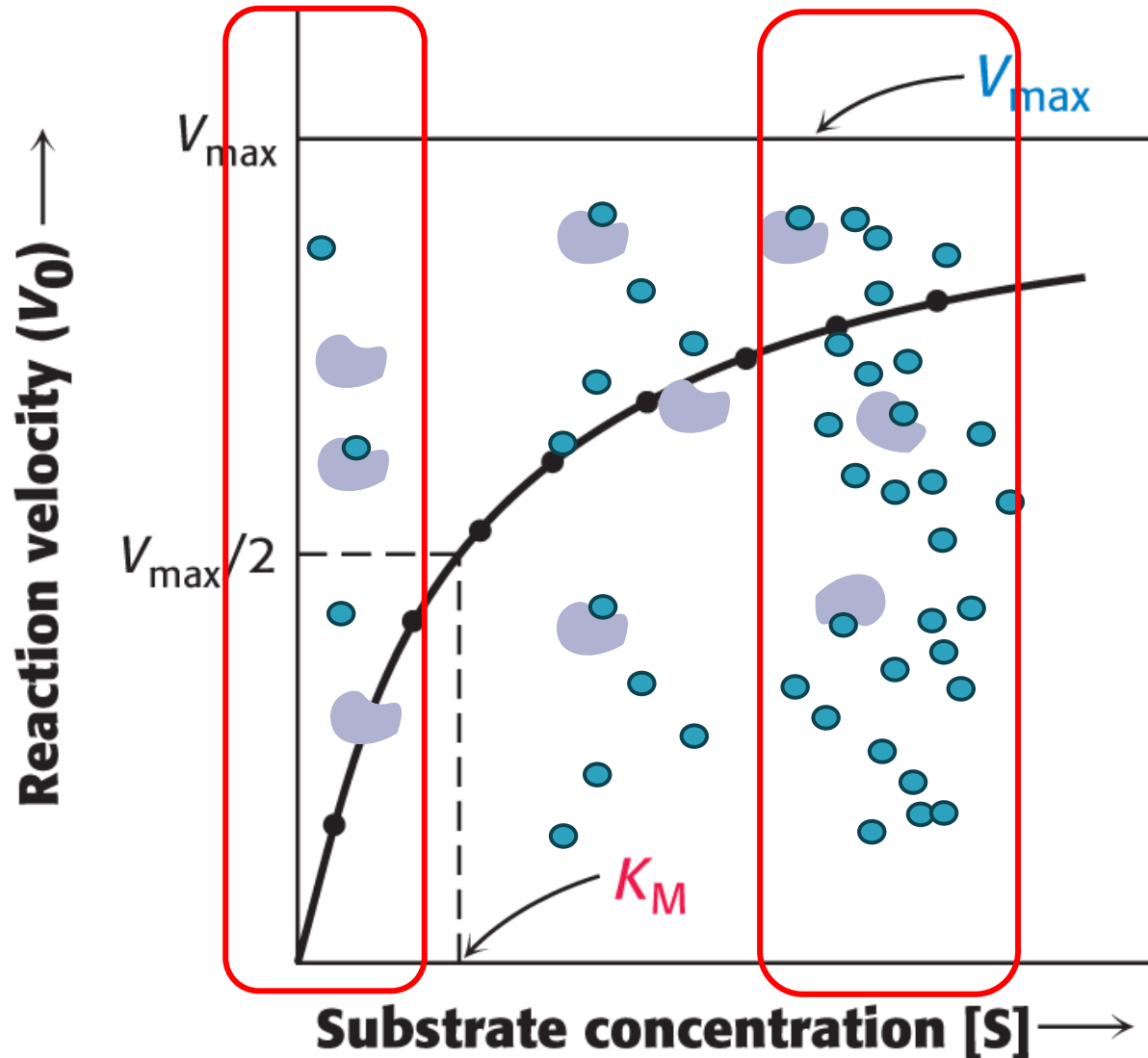


Michaelis–Menten





Michaelis–Menten



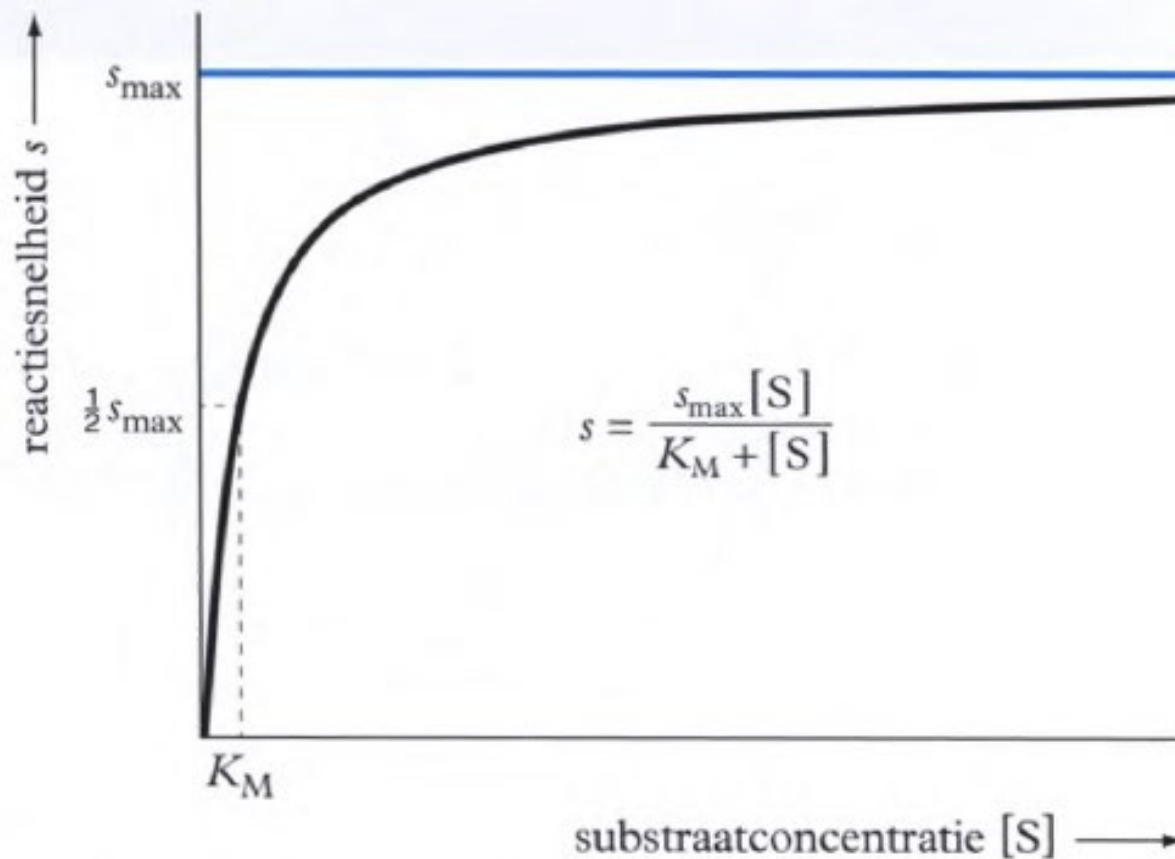
37

Scheikundeformules

A

Reactiesnelheid

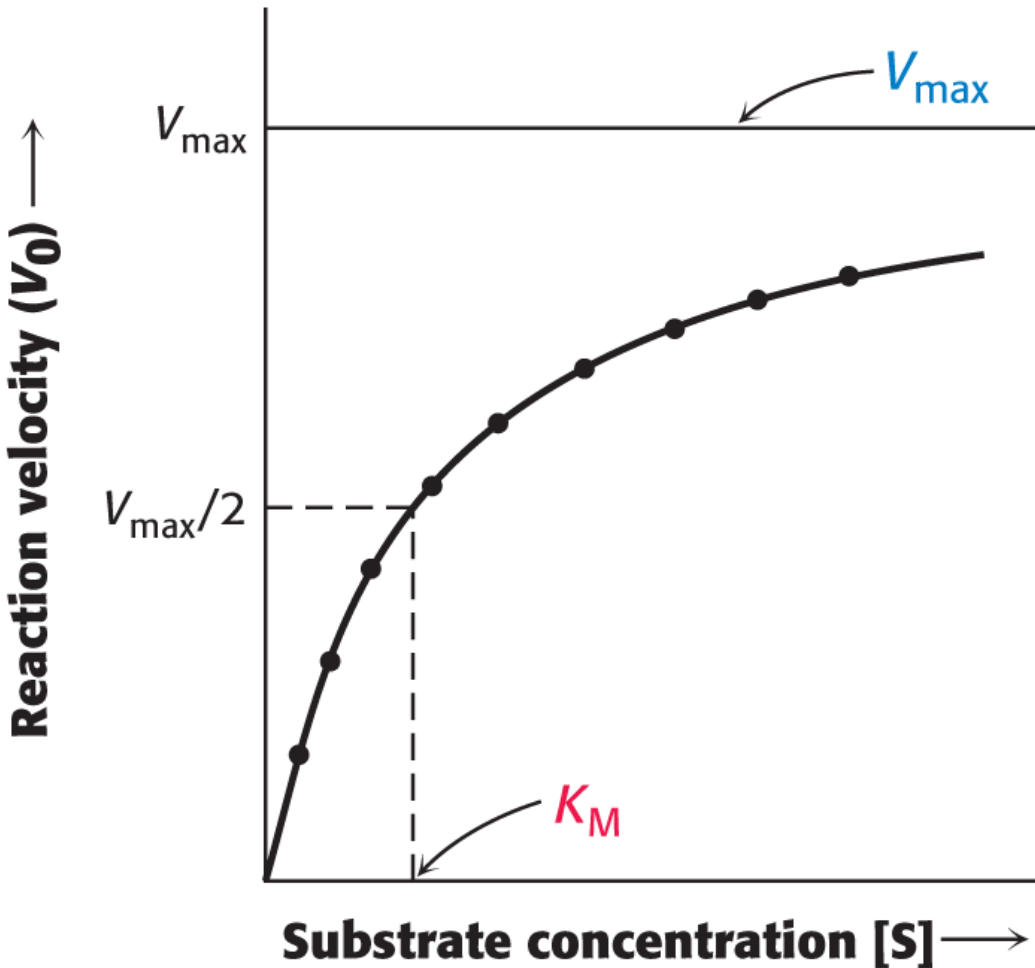
enzymatische reactie (Michaelis-Menten)



Affiniteit hoogoflaag

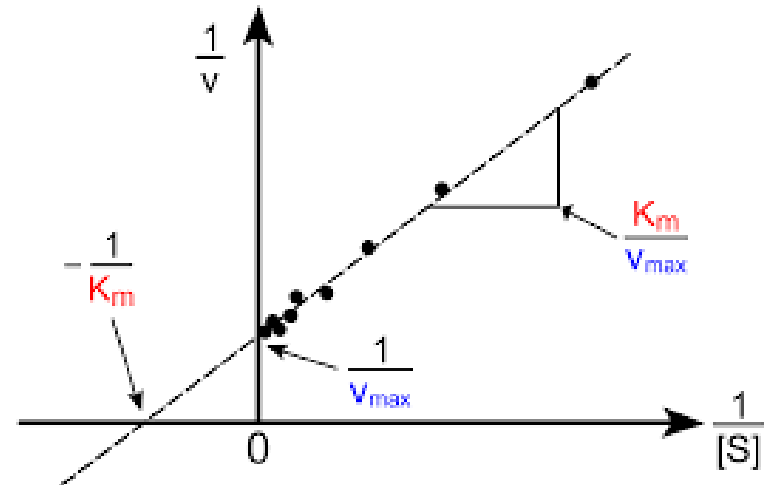


$$V_0 = V_{\max} [S] / [S] + K_m$$



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- Lastig te meten dus V_{\max} en K_m ?
- Wiskundig op te lossen door inverse grafieken te maken in Excel: Lineweaver-Burk plot!



Aan de slag

- Handout met instructies
- Download het EXCEL van
 - ▣ <https://shorturl.at/xHMf7>
- Minstens 1 laptop per groepje
 - ▣ Met daar de geopende Excel
- Idealiter 4 mensen per groep
- Gebruik een stopwatch

Aanwijzingen

- Met 1 hand 1 blokje pakken!
- Breken met 2 handen



- Product terug in de zak
- 5, 12, 20, 40 & 60 Legos in de zak
- 15 remmers in tweede sessie



Lesdoelen Check



- ☐ Chemisch inzicht krijgen
- ☐ Rol en werking van enzymen krijgen
- ☐ Basale concepten van celmetabolisme krijgen
- ☐ Overzicht van de cursus krijgen

Implicaties



- Geen CE stof, dus nutteloos?
- Waar kunnen we dit in de les gebruiken?
- Andere vragen?