International Journal of Chemistry Research

Online ISSN- 0976-5689 Vol 10, Issue 2, 2026

Review Article

A REVIEW ON THE ROLE OF KINETICS IN ELUCIDATING THE MECHANISM OF BIOLOGICALLY ACTIVE MOLECULES

FAKEHA MOHAMMED REHAN SHAIKH, ASHISH SAMBHAJI UZGARE*

Department of Chemistry, Faculty of Science, Wilson College (Autonomous), Mumbai-400007, Maharashtra, India
***Corresponding author: Ashish Sambhaji Uzgare; *Email: ashish.uzgare@wilsoncollege.edu**

Received: 25 Aug 2025 Revised and Accepted: 12 Dec 2025

ABSTRACT

Kinetic analysis plays a crucial role in elucidating the behavior of biologically active molecules, providing detailed insights into their interaction mechanisms, stability, and functional dynamics. This review underscores the importance of kinetic studies in enzyme catalysis, drug-target binding, and the behavior of lectins and glycoproteins. By examining kinetic parameters such as rate constants for association and dissociation, researchers can gain valuable information about the potency of inhibitors, the thermal stability of lectins, and the binding kinetics of glycoproteins. These parameters reveal essential aspects of molecular interactions, helping to assess the efficiency and specificity of therapeutic agents. Moreover, integrating kinetic data with genomic and proteomic information has propelled the field of systems biology, offering a more holistic understanding of molecular processes. Kinetic studies are also important for optimizing therapeutic strategies, as they provide insights into drug efficacy and resistance mechanisms. Additionally, they enhance the design of bioanalytical tools, leading to more accurate and efficient techniques for studying complex biological systems.

Keywords: Kinetics, Biologically active molecule, Reactions

DOI: http://dx.doi.org/10.22159/ijcr.2026v10i2.308 Journal homepage: https://ijcr.info/index.php/journal

INTRODUCTION

Kinetic studies focus on chemical steps designed to determine an appropriate mechanism model, correlating it with experimental data to find the best fit that closely represents the reaction rate and characterizes the chemical process [1]. Kinetic studies are essential for gaining a deeper understanding of the leaching process's nature and mechanism. Their purpose is to identify the most suitable kinetic model, extract relevant kinetic parameters, and provide insights that can aid in plant design, optimization of operating conditions, real-time control, and maximizing metal recovery [2]. The field of reaction kinetics focuses on understanding the rate dynamics in chemical and biological systems. By experimentally determining reaction rates, it is possible to estimate rate constants and enhance our comprehension of biological systems modeled by reaction-diffusion processes [3]. The exploration of kinetic principles in the study of biologically active molecules has been crucial for drug development, providing insights into the effectiveness, specificity, and binding affinity of compounds. Enzyme kinetics, in particular, has significantly enhanced our understanding of how enzymes catalyze biochemical reactions, revealing key aspects of enzyme-substrate interactions. This knowledge has been essential for designing inhibitors with therapeutic potential [4]. Kinetic studies of enzymes constitute a significant area of research and have greatly enhanced our understanding of enzyme mechanisms [5]. Moreover, kinetics can help predict the behavior of biologically active molecules under various physiological conditions, offering insights into their potential for therapeutic use. Techniques such as surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), and fluorescence anisotropy have become widely used to assess the kinetic parameters of drug-target interactions [6]. The combination of kinetic models with genomic and proteomic data has transformed systems biology, providing a more comprehensive understanding of biological processes at the molecular scale [7].

A review of the scientific literature on kinetics aimed to focus on research related to kinetic studies in elucidating the mechanisms of biologically active molecules. Data were gathered from various online scientific literature sources, including databases such as Google Scholar, Web of Science, Scopus, and Science Direct. Specific keywords related to kinetics studies on different biologically active molecules utilized in the search strategy. The study's inclusion criteria involved selecting research that contained one or more of these keywords in the article titles, focusing on articles published in English-language journals. Total 50 articles were selected for review from 1986 to 2025.

Role of kinetics

Table 1 indicates role of kinetics in biologically active molecule.

Table 1: Role of kinetics

Author(s)	Year	Study focus	References
Bachurin *et al.*	1993	Kinetic characteristics of reversible enzyme inhibition (AChE)	[8]
Weggen *et al.*	2023	Scale-up of ADC manufacturing using kinetics and CFD simulations	[9]
Yukawa *et al.*	1967	Kinetic study of Wolff rearrangement of α-diazoacetophenone	[10]
Hoebeke *et al.*	1978	Kinetics of Lens culinaris lectin binding to rabbit erythrocytes	[12]
Shnyrov *et al.*	1996	First-order kinetics on lentil lectin, temperature-dependent rate constants, activation energy calculated using the Arrhenius equation	[14]
Kitano *et al.*	2009	Binding kinetics of ConA to mannose residues in glycopolymer brushes	[15]
Ralin, D. W. *et al.*	2008	kinetics of glycoprotein–lectin interactions were studied using label-free internal reflection ellipsometry (LFIRE™)	[16]
Kato *et al.*	1997	Kinetic analysis of lectin-reactive α-fetoprotein (AFP) with different lectins	[18]

Hooper *et al.*	2022	Kinetics of multivalent lectin-glycan interactions using glycan-QDs	[19]
Nantwi *et al.*	1997	Kinetics of lectin binding to human buccal cells	[20]
Shinohara *et al.*	1997	Kinetics of lectin-oligosaccharide interactions	[21]
Lebed *et al.*	2007	Kinetics of ConA-Carboxypeptidase Y binding	[22]
Tholen *et al.*	2025	Lectin-PAINT for multiplexed visualization of the glycocalyx	[23]
Walter *et al.*	1999	Kinetics of lectin levels in Penaeus vanammei after antigen exposure	[24]
Goumenou *et al.*	2021	Glycosylation's impact on protein activity and stability	[25]
Swamy *et al.*	1986	Kinetics of N-dansylgalactosamine binding to SBA using stopped-flow spectrofluorimetry	[26]
Roy *et al.*,	2011	Kinetics of lectin binding influenced by hydrodynamic shear forces	[27]
Fernandez-Poza *et al.*	2021	Kinetics of recombinant prokaryotic lectins (RPLs) binding to glycan residues	[28]
Damian *et al.*	2005	Binding kinetics of Xerocomus chrysenteron lectin (XCL)	[31]
Sakonwan *et al.*	2020	LecA/PA-IL galactose-binding lectin kinetics	[33]
Khan *et al.*	2013	Kinetic studies, temperature and pH analysis of Bananaa lectin	[34]
Bhangare *et al.*	2022	Degradation kinetics of pharmaceuticals	[35]
Abebrese *et al.*	2011	Kinetics of oxygen atom transfer from trans-dioxoruthenium(VI)	[36]
Wakelin *et al.*	1987	Dissociation kinetics of DNA complexes with antitumor drugs	[37]
AlRashidi *et al.*	2024	Kinetic studies, molecular docking simulations	[38]
Weerawardhana *et al.*	2023	Kinetic studies, activation parameters	[39]
Habibi-Khorassani *et al.*	2008	Kinetics of reaction between triphenylphosphine and acetylenedicarboxylates	[40]
Grunwald and Krueger	2008	Bioinformatics in enzyme kinetics	[41]
Sadutto *et al.*	2023	HPLC, pseudo-first-order rate constant measurements	[42]
Mustafa *et al.*	2023	Kinetic analysis, molecular dynamics and docking simulations	[43]
Banerjee *et al.*	2014	Kinetic properties of adenylosuccinate lyase (ASL) from Mycobacteria	[44]
Sigmundsson *et al.*	2002	Kinetics of biomolecular interactions using SPR	[45]
Basha *et al.*	2013	Kinetics of oxyhemoglobin oxidation by thiosemicarbazone complexes	[46]
Tarhouchi *et al.*	2022	Kinetic and thermodynamic analysis, diffusion studies	[47]
Sung and Nesbitt	2020	DNA hybridization kinetics under extreme pressures	[48]
Van Oijen	2011	Single-molecule fluorescence, real-time binding event tracking	[49]

The study by Bachurin *et al.* assesses the kinetic characteristics of various mechanisms of reversible enzyme inhibition, focusing on their efficacy in relation to substrate concentration. The research examines the inhibition of acetylcholinesterase (AChE) by phenylpyridinium derivatives and their effects on animals. Key findings include the significant impact of inhibition mechanisms on enzyme efficacy and the differential toxic effects of MPP, EPP, and BPP. The study highlights the importance of kinetic parameters in understanding and optimizing the physiological activity of these compounds [8].

Weggen, *et al.* A study investigates the scale-up of antibody-drug conjugate (ADC) manufacturing using experimental kinetics and CFD simulations. It shows that internal stirrer mixing provides more robust kinetics than external mixing devices. Simulations of large-scale reactors reveal that mixing is sufficient at all scales, with deviations occurring mainly during payload addition. The findings highlight the use of kinetic models to optimize ADC production without large-scale experiments [9].

Kinetic studies of the Wolff rearrangement of α-diazoacetophenone were conducted by Yukawa Y *et al.* in t-butyl alcohol, measuring nitrogen evolution using silver benzoate in triethylamine as a catalyst. The reaction followed pseudo-first-order kinetics with an induction period. The effects of silver benzoate and triethylamine concentrations on the reaction rate were examined, with silver ions being consumed only in the reaction's initial stage. Based on these findings, a new mechanism was proposed, suggesting that the active catalyst entity is a silver ion-triethylamine complex [10]. I He *et al.* study the thermal stability of lectin from black turtle bean was analyzed using first-order reaction kinetics. Kinetic data showed that the inactivation of lectin followed first-order kinetics, with an activation energy of 78.80 kJ/mol. This indicates the lectin's thermal sensitivity and hydrophobic stability. The results underscore the importance of temperature and treatment time in determining the thermal stability of the lectin [11].

Hoebeke, *et al.*, in conducted Kinetic studies on *Lens culinaris* lectin binding to rabbit erythrocytes yielded association and dissociation rate constants of 3–10 x 10^3 M^{-1} s^{-1} and 3–33 x 10^{-4} M^{-1} s^{-1}, respectively, over a temperature range of 5 to 37 °C. The activation energies were 7 kcal/mol for the forward reaction and 13 kcal/mol for the reverse. These kinetic parameters were consistent with equilibrium findings, showing the binding process to be exothermic (ΔH =-4.6 kcal/mol) and associated with positive entropy (ΔS =+15 eu) [12].

Dam *et al.* examined the binding and cross-linking of concanavalin A (ConA) and *Dioclea grandiflora* lectin (DGL) with synthetic divalent carbohydrates. Results showed that carbohydrates with flexible spacers between terminal Man residues had higher affinities for both lectins. Kinetic and electron microscopy studies revealed differences in cross-linking behavior, with increased spacer length leading to the loss of organized cross-linked lattices. These findings highlight the impact of spacer flexibility on lectin-carbohydrate interactions [13].

Shnyrov *et al.* studied the irreversible thermal denaturation of lentil lectin at pH 7.4 was analyzed using a first-order kinetic model. The temperature-dependent rate constant, described by the Arrhenius equation, was calculated along with the activation energy. Results from differential scanning calorimetry, fluorescence, and hemagglutination assays supported the two-state kinetic mechanism [14].

Kitano H. *et al.*, focus on the binding kinetics of Concanavalin A (Con A) to mannose residues in a disulfide-carrying glycopolymer brush (DT-PMEMan) were studied using UV–vis spectroscopy and localized surface plasmon resonance. Con a exhibited concentration-dependent binding, with a significantly higher apparent association constant compared to small sugars, attributed to the cluster effect. The glycopolymer brush demonstrated a detection limit below 5 nM, highlighting the enhanced binding kinetics and sensitivity of the system [15].

Ralin *et al.* study, kinetics of glycoprotein–lectin interactions were studied using label-free internal reflection ellipsometry (LFIRE™), showing improved binding with multi-lectin strategies. LFIRE™ offers a cost-effective, high-throughput solution for real-time kinetic screening, enabling the rapid identification of glycoprotein biomarkers and providing insights into glycan functionality in cellular health and disease [16].

Sanadi, A. R., and Surolia, A. Study Coccinia indica agglutinin (CIA) is a chitooligosaccharide-specific lectin with two binding sites. Tryptophan involvement in binding was confirmed through quenching and chemical modification studies Thermodynamic analysis revealed that binding is

entropically driven, with the nonreducing sugar protruding from the binding pocket. Stopped-flow kinetic studies indicated that the binding process follows a one-step mechanism [17].

The study by Kato *et al.* focused on the affinity and kinetic analyses of lectin-reactive α-fetoprotein (AFP) using a surface plasmon resonance (SPR) biosensor. The researchers immobilized human AFP from hepatocellular carcinoma (HCC) patients onto a sensor chip and monitored its interaction with three lectins: Lens culinaris agglutinin (LCA), concanavalin A (Con-A), and erythroagglutinating phytohemagglutinin (E-PHA). Real-time SPR monitoring revealed distinct variations in the association (k_ass) and dissociation (k_diss) rate constants for each lectin-AFP interaction. This SPR-based method offers a rapid and efficient technique for evaluating lectin-AFP interactions and provides detailed kinetic insights into their binding behavior [18].

Hooper *et al.* introduces glycan-functionalized quantum dots (glycan-QDs) to probe multivalent lectin-glycan interactions (MLGIs) and their kinetics. Using the lectins DC-SIGN and DC-SIGNR, the researchers observed that DC-SIGN binds a single glycan-QD, while DC-SIGNR cross-links multiple QDs. Kinetic analysis revealed that DC-SIGN binding follows a single, fast second-order association rate, while DC-SIGNR exhibits two distinct association phases: a rapid initial binding followed by a slower secondary interaction. This highlights the potential of glycan-QDs for studying the kinetics of complex, multivalent interactions [19].

Nantwi *et al.* investigated the kinetics of lectin binding to human buccal cells, showing significant binding within 20 seconds, particularly for lectins from *Pisum sativum* and *Arachis hypogaea*. The study found that lectin binding was reduced when buccal cells were pre-treated with secretor or non-secretor saliva, although once bound, lectins were not displaced by saliva. These findings highlight the rapid kinetics of lectin binding to buccal cells and the influence of saliva on binding strength [20].

Shinohara *et al.* examined the kinetics of lectin-oligosaccharide interactions using a solid-phase assay. They found that increasing oligosaccharide density decreased both the association (k_ass) and dissociation (k_diss) rate constants. Lectin oligomerization enhanced binding by reducing k_diss. These results highlight the role of mass transport and rebinding in modulating lectin kinetics [21].

Lebed *et al.* used atomic force microscopy (AFM) and quartz crystal microbalance (QCM) to study the kinetics of concanavalin A-carboxypeptidase Y binding. Both methods yielded similar dissociation rate constants (k_diss), with QCM also providing the association rate (k_ass = 5.6±0.1 × 10^4 M^{-1} s^{-1}) and equilibrium constant (K_a = 0.59±0.01 × 10^6 M^{-1}). AFM revealed the energy barrier for dissociation (r = 2.29±0.04 Å). The study highlights the complementary use of AFM and QCM for kinetic analysis of molecular interactions [22].

Tholen *et al.* introduced Lectin-PAINT, a super-resolution imaging technique for multiplexed, live-cell visualization of the glycocalyx at the single-cell and single-molecule levels. By leveraging lectin binding to specific carbohydrates, Lectin-PAINT enables nanoscale mapping of glycans with high resolution. Using an extensive lectin library, the method allows for the extraction of over 350 glycosylation parameters per cell, creating a "glycotype." This approach was applied to classify cancer cell types, revealing significant glycocalyx heterogeneity and variability, with potential for advancing understanding of glycocalyx changes in disease [23].

Walter *et al.* studied lectin level kinetics in *Penaeus vanammei* after antigen exposure. They observed that lectin levels recovered more quickly after a second challenge, indicating an adaptive immune response. This pattern could serve as a potential marker for evaluating disease resistance in shrimp strains for aquaculture [24].

Goumenou, A., Delaunay, N., and Pichon, V. Glycosylation significantly impacts protein activity and stability. Despite advances, fully characterizing glycosylation is challenging due to complex mixtures in biological samples. Lectin-based affinity sorbents are commonly used for selective enrichment of glycosylated proteins or peptides, aiding in their analysis. The review highlights recent progress in lectin affinity techniques, enabling faster and more automated glycosylation analysis, with a focus on kinetic aspects of protein-enrichment interactions [25].

Swamy *et al.* studied the kinetics of N-dansylgalactosamine binding to soy-bean agglutinin (SBA) using stopped-flow spectrofluorimetry. The binding followed a single-step mechanism with association and dissociation rate constants of k+1 = 2.4 × 10^5 $M^{-1}{\cdot}s^{-1}$ and k−1 = 0.2 s^{-1} at 20 °C. The kinetic data indicated an enthalpy-driven process [26].

Roy *et al.* demonstrate that the reaction kinetics of lectins, such as Concanavalin A and Abrus Agglutinin, are influenced by hydrodynamic shear forces. Binding efficiency declines beyond a critical shear rate, indicating that association and dissociation constants depend on hydrodynamic conditions. This suggests that strong shear forces can distort lectin structure, altering their kinetics. The study emphasizes the importance of considering hydrodynamics in microfluidics-based assays [27].

In their 2021 study, Fernandez-Poza *et al.* examine the development of novel recombinant prokaryotic lectins (RPLs) through directed evolution, focusing on their binding kinetics to specific glycan residues (α-mannose, β-galactose, fucose, and sialic acid). Using bio-layer interferometry (BLI), the study compares the binding activity and specificity of RPLs to plant-derived lectins. RPLs showed superior binding in most cases, with significantly better limits of detection (LoDs) for glycoprotein interactions [28].

Murthy and Jayaraman performed a kinetic analysis of the T-antigen's interaction with peanut agglutinin lectin, employing surface plasmon resonance. Their findings showed that the initial antigen-lectin binding step had a fast association rate and a slow dissociation rate. In contrast, subsequent binding events exhibited slower kinetics. The study also compared these kinetics with other glycoside derivatives, highlighting differences in the interaction rates [29].

Vico *et al.* investigated the kinetics of vesicle aggregation in a system with β-cyclodextrin vesicles decorated with maltose-and lactose-adamantane conjugates interacting with lectins. They found that aggregation rates were proportional to lectin and vesicle concentrations, with each lectin requiring a specific carbohydrate density for optimal binding. This study provides insights into multivalent interactions and ligand-receptor kinetics at interfaces [30].

Damian *et al.* studied the binding kinetics of *Xerocomus chrysenteron* lectin (XCL) using isothermal titration calorimetry. They found that XCL binds N-acetylgalactosamine with a dissociation constant (Kd) of 5.2 μM at 27 °C, and the binding affinity increases at lower temperatures. The dissociation constant for the Thomsen-Friedenreich antigen was 500 times higher (Kd = 0.94 μM), suggesting XCL's important role in recognizing specific glycan motifs [31].

Zaree *et al.* investigated the binding kinetics of mono-and divalent ligands to *Pseudomonas aeruginosa* LecA using multiple techniques. They found that the divalent ligand had a low-nanomolar affinity and a residence time of about 7 h, with no strong binding to related lectin tetramers. These findings provide key insights into the binding kinetics and chelation-based interactions of the ligand with LecA [32].

Sakonwan *et al.* study LecA/PA-IL is a galactose-binding lectin from *Pseudomonas aeruginosa* that binds α-galactose on glycosphingolipids, playing a role in cell adhesion and bacterial internalization. It also exhibits direct toxic activity. Kinetic studies, including surface plasmon resonance assays and structural analyses, can be used to identify high-affinity inhibitors for this lectin [33].

Khan *et al.* studied the pH-induced monomerization of banana lectin (BL), showing that at pH 2.0, BL transitions to a molten globule-like state. The monomeric form exhibited greater resistance to chemical denaturation than the native dimer, with higher Cm and ΔG values. Additionally, the monomer retained secondary structure up to 95 °C, while the dimer's Tm was 77 °C, indicating superior stability of the monomer under denaturing conditions [34].

Bhangare *et al.* highlight the importance of degradation kinetic studies in determining the shelf life of pharmaceuticals under various conditions. The article discusses the lack of systematic strategies and regulatory guidelines for such studies and emphasizes the need for proper selection of variables to ensure accurate kinetic data. It provides a comprehensive approach to degradation kinetics, including advanced models, offering valuable insights for researchers in stability analysis to establish optimal storage conditions for drugs [35].

Abebrese *et al.* studied the kinetics of oxygen atom transfer from three trans-dioxoruthenium(VI) porphyrins to organic sulfides, focusing on sulfoxidation reactions with thioanisoles. The second-order rate constants for these reactions ranged from 8 to 60 M^{-1} s^{-1}, significantly faster than those for alkene epoxidations or C–H bond oxidations. Reactivity followed the order 2a<2b<2c, consistent with the electron-withdrawing and steric effects of the porphyrin groups. Kinetic analysis suggested a concerted oxygen transfer mechanism, and competition reactions indicated a multiple oxidant model for sulfoxidation [36].

Wakelin *et al.* studied the dissociation kinetics of DNA complexes with the antitumor drug N-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide and its derivatives. They found a fourth, stable binding mode in derivatives with a 4-CONH(CH2)2NR1R2 side chain, which correlated with better antitumor activity and selective binding to GC-rich DNA. The results support a molecular model where the drug intercalates into the narrow groove of DNA, with key hydrogen bonds contributing to its biological activity [37].

Al Rashidi *et al.* synthesized imidazo-isoxazole derivatives and evaluated their α-amylase and α-glucosidase inhibitory activity. Compound 5f exhibited the best inhibitory potency (IC50 of 26.67±1.25 μM and 39.12±1.83 μM, respectively) and was identified as a competitive inhibitor for both enzymes, similar to acarbose. Kinetic studies indicated that substitutions on the R1 and R2 groups of the thiourea/urea moiety influenced activity, with 5f showing significant binding affinity in molecular docking and stability in molecular dynamics simulations, supporting its potential as a lead compound for diabetes management [38].

Weerawardhana *et al.* synthesized [2Fe–2E] complexes (E = S, Se) with an N-alkyl, N′-aryl-β-diketiminate ligand and studied their electron transfer properties. The [2Fe–2S] complex exhibited an unusually long Fe···Fe distance, while the [2Fe–2Se] complex had a more typical Fe···Fe distance. Kinetic studies and activation parameters provided insights into the formation mechanism of the [2Fe–2S] compound. The study also explored the atom transfer reactivity of the [2Fe–2S] cluster to investigate its sulfur-transfer mechanism [39].

Habibi-Khorassani *et al.* investigated the kinetics of the reaction between triphenylphosphine and dialkyl acetylenedicarboxylates in the presence of NH-acid (benzoxazoline), using UV spectrophotometry to monitor the reaction. The reaction followed second-order kinetics, and the second-order rate constants (k_2) were calculated, with Arrhenius analysis yielding activation energy values. The study also examined the effects of solvent, reactant structure, and reactant concentration on the reaction rate. The proposed mechanism was confirmed, with the first step (k_2) identified as the rate-determining step. Additionally, isomer stability (Z or E) was analyzed using AIM theory [40].

Grunwald and Krueger developed a bioinformatics laboratory exercise that complements traditional alkaline phosphatase kinetics experiments in biochemistry courses. In this exercise, students use the Protein Explorer program to analyze the enzyme's structure, specifically its active-site residues, and propose their functions. By linking kinetic data to the enzyme's mechanism, students identify the roles of active-site residues in catalysis. A paired t-test of pre-and post-exercise assessments demonstrated that the exercise significantly improved students' ability to use kinetic data to determine the catalytic function of amino acids in the active site [41].

Sadutto *et al.* provided valuable kinetic data for understanding the factors influencing the stability and conversion of imine and enamine tautomers. These findings could help optimize conditions for maintaining the stereochemical integrity of similar bioactive compounds during synthesis [42].

Mustafa *et al.* conducted kinetic studies to evaluate the inhibitory effect of novel quinolinyl-iminothiazolines on alkaline phosphatase. The kinetic analysis revealed that the compound N-benzamide quinolinyl iminothiazoline (6g) exhibited the most potent inhibition, with an IC50 value of 0.337 ± 0.015 μM, significantly outperforming the standard KH2PO4 (IC50 = 5.245 ± 0.477 μM). The kinetic data, supported by molecular dynamics and docking studies, confirmed the strong inhibitory potential of 6g against alkaline phosphatase, indicating its promising role as a lead molecule for further drug development [43].

Banerjee *et al.* in their study on adenylosuccinate lyase (ASL) from *Mycobacterium smegmatis* (MsASL) and *Mycobacterium tuberculosis* (MtbASL), the enzyme's kinetic properties were evaluated. X-ray crystallography revealed that MsASL has a partially ordered C3 loop, with His149 and either Lys285 or Ser279 likely functioning as the catalytic acid and base. The enzymes from mycobacteria showed lower catalytic activity compared to other organisms' ASLs, which could be attributed to the Arg319Gly substitution and reduced flexibility in the C3 loop. These findings align with the slow growth rate of Mycobacteria and their reliance on alternative purine salvage pathways [44].

Sigmundsson *et al.* developed a method for accurately determining kinetic rate constants of biomolecular interactions using surface plasmon resonance (SPR) technology. The approach addresses mass transport limitations and does not require standards of known concentrations, relying instead on molecular mass. By analyzing binding curves obtained at varying flow rates, they introduced an analytical solution to the differential equations for 1:1 bimolecular complex formation. This method allows the precise determination of association and dissociation rate constants, as demonstrated by experiments with tyrosine-phosphorylated recombinant proteins and anti-phosphotyrosine antibodies, achieving picomolar sensitivity. The results showed strong agreement with traditional methods, highlighting the effectiveness of SPR for kinetic studies [45].

Basha *et al.* studied the kinetics of oxyhemoglobin oxidation by iron(III) thiosemicarbazone complexes, observing a two-step process involving the sequential oxidation of oxyheme cofactors in the α and β chains. Kinetic differences across complexes were attributed to steric and hydrogen-bonding effects in the thiosemicarbazone ligand series (HDpxxT) influencing the reaction rate [46].

Tarhouchi *et al.* investigated the kinetics of paracetamol diffusion across polymer membranes, specifically polymer inclusion membranes (PIM) and grafted polymer membranes (GPM) containing gluconic acid. The study showed that the PVA–GA membrane outperformed GPM–GA, with diffusion coefficients of 41.807 and 31.211 cm^2/s, respectively. Kinetic and thermodynamic analysis indicated that the process was controlled by kinetic factors rather than energy, as evidenced by low activation energy and enthalpy values. The extraction process was most efficient at pH 1, providing a sustainable method for recovering paracetamol from aqueous solutions [47].

Sung and Nesbit, studied DNA hybridization kinetics under extreme pressures, finding that pressure slowed folding and accelerated unfolding. Van't Hoff analysis showed an increase in the DNA-solvent volume during folding. Sodium ions ([Na+]) stabilized DNA and reduced pressure dependence on folding kinetics, with higher [Na+] decreasing activation volumes for dehybridization, suggesting a shift to a more folded transition state [48].

Van Oijen discusses the use of single-molecule fluorescence techniques to explore the kinetics of biomolecular interactions. These methods enable real-time tracking of binding events by labeling molecules with fluorescent tags. While significant advancements have been made, the requirement for low, nanomolar concentrations of molecules remains a challenge for studying intermolecular interactions [49].

CONCLUSION

Kinetic studies are essential for advancing our understanding of biologically active molecules and their interactions. By examining kinetic parameters, such as rate constants for association and dissociation, researchers gain valuable insights into the potency and specificity of inhibitors. Kinetic analysis is an indispensable tool in both basic and applied biomedical research, providing a detailed understanding of molecular interactions. It plays a key role in optimizing therapeutic strategies and refining bioanalytical techniques. As the field evolves, the integration of kinetic studies with advanced technologies and interdisciplinary approaches will drive further breakthroughs in our understanding of biological systems. This, in turn, will facilitate the development of novel therapeutics and enhance the precision of biomedical interventions.

ACKNOWLEDGEMENT

The authors would like to acknowledge the valuable contributions of fellow researchers, whose work is cited in the references section, for their insightful publications that have informed and enriched this review paper. The table made by the authors themselves doesn't copy from any sources.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Manuscript written by Fakeha Shaikh under the guidance of Ashish Uzgare. Fig. and table made by author themselves doesn't copy from any sources.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

REFERENCES

1. Klippenstein SJ, Pande VS, Truhlar DG. Chemical kinetics and mechanisms of complex systems: a perspective on recent theoretical advances. J Am Chem Soc. 2014;136(2):528-46. doi: 10.1021/ja408723a, PMID 24283502.
2. Baniasadi M, Vakilchap F, Bahaloo-Horeh N, Mousavi SM, Farnaud S. Advances in bioleaching as a sustainable method for metal recovery from e-waste: a review. J Ind Eng Chem. 2019;76:75-90. doi: 10.1016/j.jiec.2019.03.047. jiec.2019.03.047.
3. Kapcan E, Krygier K, da Luz M, Serniuck NJ, Zhang A, Bramson J et al. Mimicry of molecular glues using dual covalent chimeras. Nat Commun. 2025;16(1):2855. doi: 10.1038/s41467-025-58083-z, PMID 40128528.
4. Berg JM, Tymoczko JL, Gatto GJ. Biochemistry. 8th ed. New York: W. H. Freeman and Company; 2015.
5. Hammes GG, Hammes-Schiffer S. Applications of kinetics to biological systems. Physical chemistry for the biological sciences. Chichester: John Wiley & Sons; 2015. p. 121-52. doi: 10.1002/9781118859148.ch.
6. Rich RL, Myszka DG. Survey of the 2000-2001 literature on biomolecular interaction analysis: a critical review. J Mol Recognit. 2000;13(6):258-72.
7. Alon U. An introduction to systems biology: design principles of biological circuits. 1st ed. New York: Chapman & Hall/CRC; 2006. doi: 10.1201/9781420011432.
8. Bachurin SO, Dunaevetskii AA, Lermontova NN, Serkova TP. Kinetic characteristics of the action of biologically active compounds. II. Effect of the inhibition mechanism on the efficacy of reversible enzyme inhibition. Pharm Chem J. 1993;27(11):723-30. doi: 10.1007/BF00780397.
9. Weggen JT, Seidel J, Bean R, Wendeler M, Hubbuch J. Corrigendum: kinetic studies and CFD-based reaction modeling for insights into the scalability of ADC conjugation reactions. Front Bioeng Biotechnol. 2023;11:1229416. doi: 10.3389/fbioe.2023.1229416, PMID 37362209.
10. Yukawa Y, Tsuno Y, Ibata T. The Wolff rearrangement. I. Kinetic studies of the decomposition of α-diazoacetophenone. Bull Chem Soc Jpn. 1967;40(11):2613-7. doi: 10.1246/bcsj.40.2613.
11. He S, Shi J, Ma Y, Xue SJ, Zhang H, Zhao S. Kinetics for the thermal stability of lectin from black turtle bean. J Food Eng. 2014;142:132-7. doi: 10.1016/j.jfoodeng.2014.06.008.
12. Hoebeke J, Foriers A, Schreiber AB, Strosberg AD. Equilibrium and kinetic studies of the binding of Lens culinaris lectin to rabbit erythrocytes by a quantitative fluorometric method. Biochemistry. 1978;17(23):5000-5. doi: 10.1021/bi00616a022, PMID 718870.
13. Dam TK, Oscarson S, Roy R, Das SK, Pagé D, Macaluso F et al. Thermodynamic, kinetic, and electron microscopy studies of concanavalin A and Dioclea grandiflora lectin cross-linked with synthetic divalent carbohydrates. J Biol Chem. 2005;280(10):8640-6. doi: 10.1074/jbc.M412827200. PMID 15632152.
14. Shnyrov VL, Marcos MJ, Villar E. Kinetic study on the irreversible thermal denaturation of lentil lectin. Biochem Mol Biol Int. 1996;39(4):647-56. doi: 10.1080/15216549600201711, PMID 8843331.
15. Kitano H, Takahashi Y, Mizukami K, Matsuura K. Kinetic study on the binding of lectin to mannose residues in a polymer brush. Colloids Surf B Biointerfaces. 2009;70(1):91-7. doi: 10.1016/j.colsurfb.2008.12.016, PMID 19152782. colsurfb.2008.12.016.
16. Ralin DW, Dultz SC, Silver JE, Travis JC, Kullolli M, Hancock WS et al. Kinetic analysis of glycoprotein–lectin interactions by label-free internal reflection ellipsometry. Clin Proteom. 2008;4(1-2):37-46. doi: 10.1007/s12014-008-9007-y.
17. Sanadi AR, Surolia A. Studies on a chitooligosaccharide-specific lectin from Coccinia indica. Thermodynamics and kinetics of umbelliferyl glycoside binding. J Biol Chem. 1994;269(7):5072-7. doi: 10.1016/S0021-9258(17)37656-1, PMID 8106485.
18. Kato K. Affinity and kinetic studies for the evaluation of lectin-reactive α-fetoprotein with a biosensor based on surface plasmon resonance. Hepatology Research. 1997;8(2):95-105. doi: 10.1016/S1386-6346(97)00055-7.
19. Hooper J, Liu Y, Budhadev D, Zhou D, Guo Y. Probing thermodynamics, kinetics and structural details of multivalent lectin-glycan interactions by quantum dot-FRET. version 1 [working paper]; 2022.
20. Nantwi PK, Cook DJ, Rogers DJ, Smart JD. Lectins for drug delivery within the oral cavity—investigation of lectin binding to oral mucosa. J Drug Target. 1997;5(1):45-55. doi: 10.3109/10611869708995857, PMID 9524313.
21. Shinohara Y, Hasegawa Y, Kaku H, Shibuya N. Elucidation of the mechanism enhancing the avidity of lectin with oligosaccharides on the solid phase surface. Glycobiology. 1997;7(8):1201-8. doi: 10.1093/glycob/7.8.1201, PMID 9455921.

22. Lebed K, Kulik AJ, Forró L, Lekka M. Atomic force microscopy and quartz crystal microbalance study of the lectin–carbohydrate interaction kinetics. Acta Phys Pol A. 2007;111(2):273-86. doi: 10.12693/APhysPolA.111.273.
23. Tholen MM, Riera R, Izquierdo-Lozano C, Albertazzi L. Multiplexed Lectin-PAINT super-resolution microscopy enables cell glycotyping. Commun Biol. 2025;8(1):267. doi: 10.1038/s42003-025-07626-7, PMID 39979385.
24. Walter FL, Patterson RA, Middlebrooks BL. Preliminary evaluation of the kinetics of changes in lectin levels following antigen exposure as a measure of potential disease resistance in strains of Penaeus vanammei. IAAAM. 1999.
25. Goumenou A, Delaunay N, Pichon V. Recent advances in lectin-based affinity sorbents for protein glycosylation studies. Front Mol Biosci. 2021;8:746822. doi: 10.3389/fmolb.2021.746822, PMID 34778373.
26. Swamy MJ, Krishna Sastry MV, Khan MI, Surolia A. Thermodynamic and kinetic studies on saccharide binding to soya-bean agglutinin. Biochem J. 1986;234(3):515-22. doi: 10.1042/bj2340515, PMID 3755041.
27. Roy B, Das T, Maiti TK, Chakraborty S. Effect of fluidic transport on the reaction kinetics in lectin microarrays. Anal Chim Acta. 2011;701(1):6-14. doi: 10.1016/j.aca.2011.05.049. PMID 21763802.
28. Fernandez-Poza S, Padros A, Thompson R, Butler L, Islam M, Mosely JA, et al. Tailor-made recombinant prokaryotic lectins for characterisation of glycoproteins. Anal Chim Acta. 2021;1155:338352. doi: 10.1016/j.aca.2021.338352. PMID 33766322.
29. Murthy BN, Jayaraman N. A kinetic analysis of the tumor-associated galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-d-galactopyranoside antigen—lectin interaction. J Chem Sci. 2008;120(1):195-203. doi: 10.1007/s12039-008-0023-2.
30. Vico RV, Voskuhl J, Ravoo BJ. Multivalent interaction of cyclodextrin vesicles, carbohydrate guests, and lectins: a kinetic investigation. Langmuir. 2010;27(4):1391-7. doi: 10.1021/la1038975, PMID 21090662.
31. Damian L, Fournier D, Winterhalter M, Paquereau L. Determination of thermodynamic parameters of Xerocomus chrysenteron lectin interactions with N-acetylgalactosamine and Thomsen-Friedenreich antigen by isothermal titration calorimetry. BMC Biochem. 2005;6:11. doi: 10.1186/1471-2091-6-11, PMID 15929788.
32. Zaree P, Sastre Torano J, de Haan CA, Scheltema RA, Barendregt A, Thijssen V, et al. The assessment of Pseudomonas aeruginosa lectin LecA binding characteristics of divalent galactosides using multiple techniques. Glycobiology. 2021;31(11):1490-9. doi: 10.1093/glycob/cwab074, PMID 34255029.
33. Kuhaudomlarp S, Gillon E, Varrot A, Imberty A. LecA (PA-IL): A galactose-binding lectin from Pseudomonas aeruginosa. Methods Mol Biol. 2020;2132:257-66. doi: 10.1007/978-1-0716-0430-4_25, PMID 32306333.
34. Khan JM, Qadeer A, Ahmad E, Ashraf R, Bhushan B, Chaturvedi SK, et al. Monomeric banana lectin at acidic pH overrules conformational stability of its native dimeric form. PLOS One. 2013;8(4):e62428. doi: 10.1371/journal.pone.0062428. PMID 23638080.
35. Bhangare D, Rajput N, Jadav T, Sahu AK, Tekade RK, Sengupta P. Systematic strategies for degradation kinetic study of pharmaceuticals: an issue of utmost importance concerning current stability analysis practices. J Anal Sci Technol. 2022;13(1):7. doi: 10.1186/s40543-022-00317-6.
36. Abebrese C, Huang Y, Pan A, Yuan Z, Zhang R. Kinetic studies of oxygen atom transfer reactions from trans-dioxoruthenium(VI) porphyrins to sulfides. J Inorg Biochem. 2011;105(12):1555-61. doi: 10.1016/j.jinorgbio.2011.08.003. PMID 22071078.
37. Wakelin LP, Atwell GJ, Rewcastle GW, Denny WA. Relationships between DNA-binding kinetics and biological activity for the 9-aminoacridine-4-carboxamide class of antitumor agents. J Med Chem. 1987;30(5):855-61. doi: 10.1021/jm00388a019, PMID 3572974.
38. AlRashidi E, Ghannay S, Albadri AE, Abid M, Kadri A, Aouadi K. Design, synthesis, biological evaluation, kinetic studies and molecular modeling of imidazo-isoxazole derivatives targeting both α-amylase and α-glucosidase inhibitors. Heliyon. 2024;10(20):e38376. doi: 10.1016/j.heliyon.2024.e38376, PMID 39640664.
39. Weerawardhana EA, Zeller M, Lee WT. Synthesis, kinetic studies, and atom transfer reactivity of [2Fe–2E] model compounds. Inorg Chem Front. 2023;10(13):3940-6. doi: 10.1039/D3QI00728F.
40. Habibi-Khorassani SM, Ebrahimi A, Maghsoodlou MT, Kazemian MA. Biologically active benzoxazolinone in reaction with triphenylphosphine, dialkyl acetylenedicarboxilates and theoretical study on the kinetic and mechanism investigation of the reactions. Biomed Pharmacol J. 2008;1(1).
41. Grunwald SK, Krueger KJ. Improvement of student understanding of how kinetic data facilitates the determination of amino acid catalytic function through an alkaline phosphatase structure/mechanism bioinformatics exercise. Biochem Mol Biol Educ. 2008;36(1):9-15. doi: 10.1002/bmb.120, PMID 21591153.
42. Sadutto D, Guglielmi P, Carradori S, Secci D, Cirilli R. Kinetic study on the base-catalyzed imine-enamine tautomerism of a chiral biologically active isoxazoline derivative by HPLC on amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase. Molecules. 2023;28(18):6518. doi: 10.3390/molecules28186518, PMID 37764294.
43. Mustafa MN, Channar PA, Sarfraz M, Saeed A, Ejaz SA, Aziz M et al. Synthesis, kinetic studies and in-silico investigations of novel quinolinyl-iminothiazolines as alkaline phosphatase inhibitors. J Enzyme Inhib Med Chem. 2023;38(1):2163394. doi: 10.1080/14756366.2022.2163394, PMID 36629454.
44. Banerjee S, Agrawal MJ, Mishra D, Sharan S, Balaram H, Savithri HS, et al. Structural and kinetic studies on adenylosuccinate lyase from Mycobacterium smegmatis and Mycobacterium tuberculosis provide new insights on the catalytic residues of the enzyme. FEBS Journal. 2014;281(6):1642-58. doi: 10.1111/febs.12730, PMID 24479855.
45. Sigmundsson K, Masson G, Rice R, Beauchemin N, Obrink B. Determination of active concentrations and association and dissociation rate constants of interacting biomolecules: an analytical solution to the theory for kinetic and mass transport limitations in biosensor technology and its experimental verification. Biochemistry. 2002;41(26):8263-76. doi: 10.1021/bi020099h, PMID 12081475.
46. Basha MT, Rodríguez C, Richardson DR, Martínez M, Bernhardt PV. Kinetic studies on the oxidation of oxyhemoglobin by biologically active iron thiosemicarbazone complexes: relevance to iron-chelator-induced methemoglobinemia. J Biol Inorg Chem. 2013;19(3):349-57. doi: 10.1007/s00775-013-1070-9, PMID 24317633.
47. Tarhouchi S, Louafy R, El Atmani EH, Hlaïbi M. Kinetic control concept for the diffusion processes of paracetamol active molecules across affinity polymer membranes from acidic solutions. BMC Chem. 2022;16(1):2. doi: 10.1186/s13065-021-00794-7, PMID 35027092.
48. Sung HL, Nesbitt DJ. Single-molecule kinetic studies of DNA hybridization under extreme pressures. Phys Chem Chem Phys. 2020;22(41):23491-501. doi: 10.1039/D0CP04035E, PMID 33078795.
49. Van Oijen AM. Single-molecule approaches to characterizing kinetics of biomolecular interactions. Curr Opin Biotechnol. 2011;22(1):75-80. doi: 10.1016/j.copbio.2010.10.002. PMID 21036593.