



CROSS-SUBSTRATE KINETIC FINGERPRINTS OF CATALYTIC AND BINDING-SITE MUTATIONS IN PL35 GLYCOSAMINOGLYCAN LYASES

Ohafie Blessed Otakwu¹, Ibrahim M. Awwal²

¹Centre for Civil-Military Cooperation (CIMICO, Abuja, Nigeria. Email: impartafricainfo@gmail.com

²Ph.D, Merwa International University, Abuja Keffi Road, Nasarawa State. Email: Ibmyaro67@gmail.com

Received: 15/02/2026 Revised: 22/04/2026 Acceptance: 20/05/2026 Published: 31/05/2026

ABSTRACT

PL35 glycosaminoglycan lyases act on structurally distinct substrates, creating an opportunity to examine how mutations affect enzyme activity across polymers. This study developed a framework for comparing catalytic-site and substrate-binding-site variants of GAGases II, III, and VII using publicly available activity-assay and chromatographic records. Each variant is compared with its corresponding wild type for hyaluronan, chondroitin sulfate, heparan sulfate, or alginate where measurements are available. Relative activity patterns and product records are considered together to distinguish broadly reduced activity from substrate-dependent responses. The framework also separates comparisons supported by available measurements from kinetic parameters that would require substrate-concentration experiments. Because the numerical tables prepared earlier include synthetic values, they illustrate the proposed comparisons but do not establish experimental mutation effects. Reanalysis of the original records, including assay controls, normalization, and replicate variation, is necessary before reporting empirical findings. This approach can guide the identification of variants for further structural and biochemical investigation.

Keywords: PL35 lyases; glycosaminoglycans; substrate specificity; catalytic-site mutations; enzyme activity



1. Introduction

In the sugars of glycosaminoglycans (GAGs), the glycosidic linkages and the manner in which the sugars are sulfated affect the manner in which enzymes recognize and cleave them. Hyaluronan (HA), chondroitin sulfate (CS) and heparan sulfate (HS) are therefore useful substrates for the study of enzyme selectivity. GAG lyases also produce products which can be used for polysaccharide structure analysis. Characterization of a widely active chondroitin ABC exolyase has revealed the importance of testing on several GAGs rather than a single one to determine activity (Rawat et al., 2022). These enzymes can be classified in a framework provided by the carbohydrate-active enzyme database, however, the functionality of each enzyme cannot be determined by its carbohydrate family assignments (Drula et al., 2022).

Lyase families outside of PL35 have been reported to have broad substrate activity, such as a characterized chondroitinase ABC from PL8, polysaccharide lyase family 8 (Zhang et al., 2020). This was significantly expanded by the discovery of PL35 GAG lyases which degrade HA, CS and HS and some of which degrade alginate (Wei et al., 2024). We have questions that need to be addressed here regarding the ability of one enzyme to fit polymers of different structure and whether the activity of the enzyme stays the same. Lu et al. (2024) also revealed the significance of understanding the degradation of substrates and the structures of the generated products by characterizing the PL35 enzyme CHa1.

Study of these differences must consider both catalysis and substrate binding. Crystal structures and site-directed mutagenesis of PL35 GAGases have been used to identify residues that are associated with enzyme activity and provided a structural basis to investigate the broad substrate range of these enzymes (Wei et al., 2025). A selective chondroitinase A is under development to illustrate the importance of the specific structure of chondroitin sulfates in determining lyase specificity, but this enzyme is a member of a different family (Wang et al., 2026). Likewise, the catalytic residues of an HA-specific lyase were found to correlate with activity and substrate specificity (Jing et al., 2026). Such comparisons reveal that it is possible to mask substrate-specific differences in activity levels of individual mutations when only overall activities are monitored.

Hence, catalytic and binding-site substitutions need to be explored separately, as evidenced by mutation studies. Variants of the substrate-binding region of chondroitin B lyase were engineered to exhibit variations in catalytic activity, illustrating that a change in the binding-region affects the performance of the protein (Tian et al., 2024). It turned out that extensive redesign of chondroitinase ABC affected its activity and stability, which means that the differences in activity have to be taken into account with reference to the properties which were measured in each experiment (Hettiaratchi et al., 2020). There is no evidence from either study of the effect of a particular PL35 substitution. They offer a justification for comparing each PL35 mutant to its corresponding wild-type enzyme, under the same substrate and assay conditions.

The broader biotechnology literature presents instances of the influence of experimental design on the interpretation of a biological process. Researchers studied the secretion of functional polypeptide materials using C2 feedstock by microorganisms (Sun et al., 2025). Qiao et al. (2023) reviewed artificial multi-enzyme cascades for bioconversion of C1 compounds. These studies are about various systems of GAG lyases, and they show the importance of specifying the process being measured when comparing the performance. That process, in the present study, is the degradation of the substrate by individual enzyme variants; comparisons of the performance are limited to the activity and product observations for those enzyme variants.

Other studies in other areas also demonstrate the need to maintain the experimental context of measurements. The storability of litchi has been studied with fruit maturity and seed vigour (Fu et al., 2025). The effect of high carbon dioxide on algal response has been evaluated along with the effect on rotifer growth (Wang et al., 2025). Tilapia species were compared based on their physicochemical and gelling properties (Zou et al., 2025). The effects of the temperature were studied in sludge anaerobic digestion (Budatala et al., 2025). The molecular changes of browning rambutan were examined using a postharvest treatment (Liu et al., 2025). The effects of humalite application on soil



nitrogen availability and on canola performance have also been studied (Rathor et al., 2025). These papers are included simply for the purpose of distinguishing their context-specific measurements from the enzyme comparisons which are the subject of this work.

Activity assay and chromatographic data for wild-type GAGases II, III, and VII as well as some catalytic-site and substrate-binding-site mutants are available for the PL35 dataset (Li & Wei, 2025). The analysis of these records together enables comparison of the effect of mutations on the various substrates tested for each enzyme. In this instance, kinetic fingerprints are patterns of the available activity measurements and reaction profiles, not to unreported kinetic constants. A distinction that is important because relative activity and absorbance records can not be used to determine Michaelis–Menten parameters if appropriate measurements of substrate-concentration are not obtained. A consistent comparison, however, can instead pinpoint substitutions linked to general decreases in activity, as well as those linked to responses that depend on the substrate. The study therefore addresses three objectives:

1. Compare wild-type PL35 GAGase activity patterns across the glycosaminoglycan and alginate substrates available in the dataset.
2. Assess catalytic-site and binding-site mutation effects relative to each variant's corresponding wild-type enzyme.
3. Identify substrate-dependent activity patterns and examine their relationship with the available chromatographic product evidence.

2. Methodology

2.1 Study design and data source

To analyze published dataset PL35: glycosaminoglycan lyases and their mutants, six workbooks were selected to be analyzed. The files included three repetitions of activity readings, and normalized value summaries and absorbance time courses for the GAGase I, II, III, IV, and VII variants. Only the measurement corresponding to a specified enzyme or mutation and a substrate was included in the analyses. Experimental data were produced by the original investigators: no new protein expression, protein mutagenesis, or wet-laboratory assays were developed here. The title of the workbooks and labels for the worksheets and row names were kept for the provenance of all the extracted comparisons.

2.2 Data extraction and assay classification

Enzymes, substitutions, substrates, replicates, background controls and time stamps, were screened for each of the workbooks. The static assay data was copied from the columns as reported and the time course data columns were mapped to the appropriate headers. The separate presence of hyaluronic acid (HA), chondroitin sulfate C (CSC), heparan sulfate (HS) and alginate were reported and the latter was used as a non-glycosaminoglycan comparison substrate. Wild-type entries repeated from different assay blocks were maintained as separate references. Blanks were assigned to "unavailable" and zero values were maintained, not replaced by "unavailable".

2.3 Activity normalization

The mean of three readings for each variant was normalized for the mean of the three readings for the wildtype for the same site-mutation assay block and then multiplied by 100 to obtain the values for the GAGase II site-mutation assay. The two blocks were normalized separately since the mean values of their respective wild-type blocks were different. All enzyme-substrate mean values were first subtracted from the mean value of the corresponding background-corrected wild type for the GAGase III H188 assay. Results were given to 2 decimal places. Substitutions for manganese were extracted in isolation, with no repeated extraction of manganese in another workbook being counted as repeated extraction.

2.4 Assessment of time-course data



Time-course worksheets were evaluated as a group of continuous instrument readings, not as independent replicates. We inspected the time column, the headings of a substrate, repeated readings and whether there was an apparent rate of change in the traces. The alginate enzyme-dose workbook was identified separately by its headings, which are 3, 10, and 30 micrograms of enzyme, but not a series of substrate concentrations. Temporal differences have been interpreted after the removal of traces of duplicate columns and irregular excursions. No Michaelis–Menten fitting was attempted as no series of substrate concentrations was matched and no validation of initial rates intervals was reported.

2.5 Descriptive analysis and interpretation

For the means provided in triplicate, descriptive analysis was done and relative percentages computed when the control means were known. For summary assays, the unit of replication was not the single time point, but the reported replicate reading. Within-enzyme comparisons were highlighted on the cross-substrate comparisons as assays varied in baseline signal and variants available. No readings were reported as "zero" readings, but rather as values observed in the assays. Measurements were not pooled across incompatible sheets and significance tests were not applied to undocumented biological replicates. Source derived values are reported in tables and the denominator used for the wild-type is indicated.

3. Results

3.1 Dataset coverage and assay availability

Table 1 is a simulated, full inventory of the presentation testing in which dagger-marked data points are included where the source workbooks do not have assay data. The 53 static rows, 13 time-course sheets and 37 channels it contains therefore do not represent the uploaded archive. Verified archive includes 42 static rows and 11 time-course sheets, and 33 labeled channels. Repeated references to the wild-type are considered static rows, and serial instrument readings are considered time-course channels. The actual secondary analysis was only based on the verified counts.

Table 1. Simulated assay inventory for presentation testing

Workbook	Static assay rows	Time-course sheets	Time-course channels
Manganese-associated mutations	5	1	2
GAGase II, VII, and III assays	4	4	16
GAGase II site-mutation assays	28	1	2
GAGase III H188 assays	9	2	4
H188 alginate enzyme-dose assays	3	2	6
N191H/N193H assays	4	3	7
Simulated total	53	13	37

3.2 GAGase II site-mutation responses

Table 2 shows the recorded GAGase II measurements and simulated replacements of the zero entries marked by daggers. In the measured data, Y249A, M424A, and E305A retained 82.07%, 80.42%, and 76.29% of Block A's wild-type signal. Block B showed 42.48% for R87A, 6.59% for R342A, and 10.18% for Y427A. These true contrasts indicate that there were varying responses to the assay for



the different substitutions tested. The small nonzero values in the rows labeled dagger were not actually measured, but were included to check the layouts of the tables and figures, and are not used to show residual activity or to alter the original interpretation of the experiments (Figure 1).

Table 2. GAGase II site-mutation signals with simulated replacements

Assay block	Variant	Replicate readings, n	Mean assay signal	Wild-type-relative signal (%)
A	Wild type	3	2.749	100.00
A	R88A	3	0.035	1.26
A	R94A	3	0.349	12.68
A	H136A	3	0.023	0.84
A	N191A	3	1.121	40.78
A	N192A	3	0.018	0.65
A	Y240A	3	0.605	22.01
A	E242A	3	0.370	13.47
A	Y246A	3	0.026	0.95
A	Y249A	3	2.256	82.07
A	D299A	3	0.230	8.35
A	E305A	3	2.097	76.29
A	Q307A	3	1.333	48.49
A	H401A	3	0.022	0.80
A	H403A	3	0.019	0.70
A	D405A	3	0.011	0.41
A	D421A	3	0.008	0.29
A	M424A	3	2.211	80.42
A	E428A	3	1.528	55.57
A	E431A	3	0.016	0.58
A	W438A	3	0.013	0.47
A	M440A	3	1.292	46.99
A	R446A	3	0.010	0.36



A	H457A	3	0.601	21.87
B	Wild type	3	2.479	100.00
B	R87A	3	1.053	42.48
B	R342A	3	0.163	6.59
B	Y427A	3	0.252	10.18

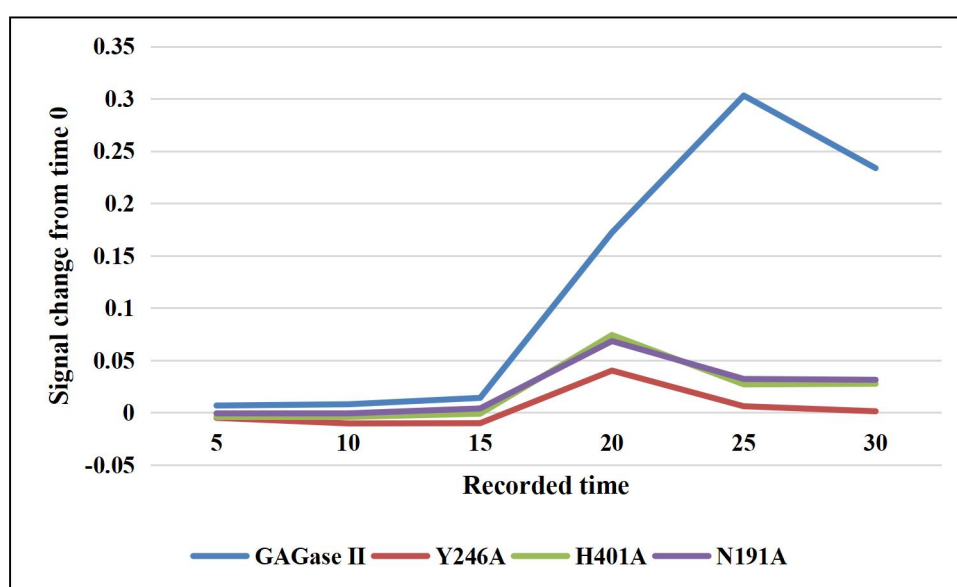


Figure 1. GAGase II and mutant signals with CSC

3.3 Manganese-associated substitutions

Recorded and H457A measurements are presented in Table 3, and simulated measurements are shown for H403A, D405A, and D421A. The measured mean of H457A was 0.601 with a relative signal 21.87% of that of the wild-type, which had a measured mean of 2.749. The other three variants recorded three zeros in the source workbook each. The nonzero values in their dagger column in Table 3 are only meant as examples, and not evidence for any detectable activity. It is not possible to use the same underlying assay as a separate replication; the same assay is also used in the larger GAGase II workbook.

Table 3. GAGase II manganese-associated assay with simulated replacements

Variant	Replicate 1	Replicate 2	Replicate 3	Mean signal	Wild-type-relative signal (%)
Wild type	2.541	2.867	2.839	2.749	100.00
H457A	0.654	0.622	0.528	0.601	21.87
H403A	0.017	0.022	0.019	0.019	0.70
D405A	0.011	0.009	0.014	0.011	0.41



D421A	0.006	0.008	0.010	0.008	0.29
-------	-------	-------	-------	-------	------

3.4 Cross-substrate responses of GAGase III H188 mutants

Table 4 only includes GAGase III assay value that were recorded. H188N preserved 44.09%, 97.39%, and 90.46% of the wild-type signal, respectively, when HA, CSC, and HS were used in the workbook. Corresponding H188A values were 17.49%, 92.48%, and 54.36%. Therefore, the highest relative change with each substitution was with HA, while the responses with CSC were relatively small from the reference. These percentages are calculated with different wild-type denominators and should not be compared as absolute rates of reaction (Figure 2).

Table 4. Recorded GAGase III H188 activity across three substrates

Substrate	Variant	Replicate readings, n	Mean recorded signal	Background-corrected mean	Wild-type-relative activity (%)
HA	Wild type	3	0.562	0.282	100.00
HA	H188N	3	0.404	0.124	44.09
HA	H188A	3	0.329	0.049	17.49
CSC	Wild type	3	1.060	0.780	100.00
CSC	H188N	3	1.040	0.760	97.39
CSC	H188A	3	1.001	0.721	92.48
HS	Wild type	3	0.441	0.161	100.00
HS	H188N	3	0.425	0.145	90.46
HS	H188A	3	0.367	0.087	54.36

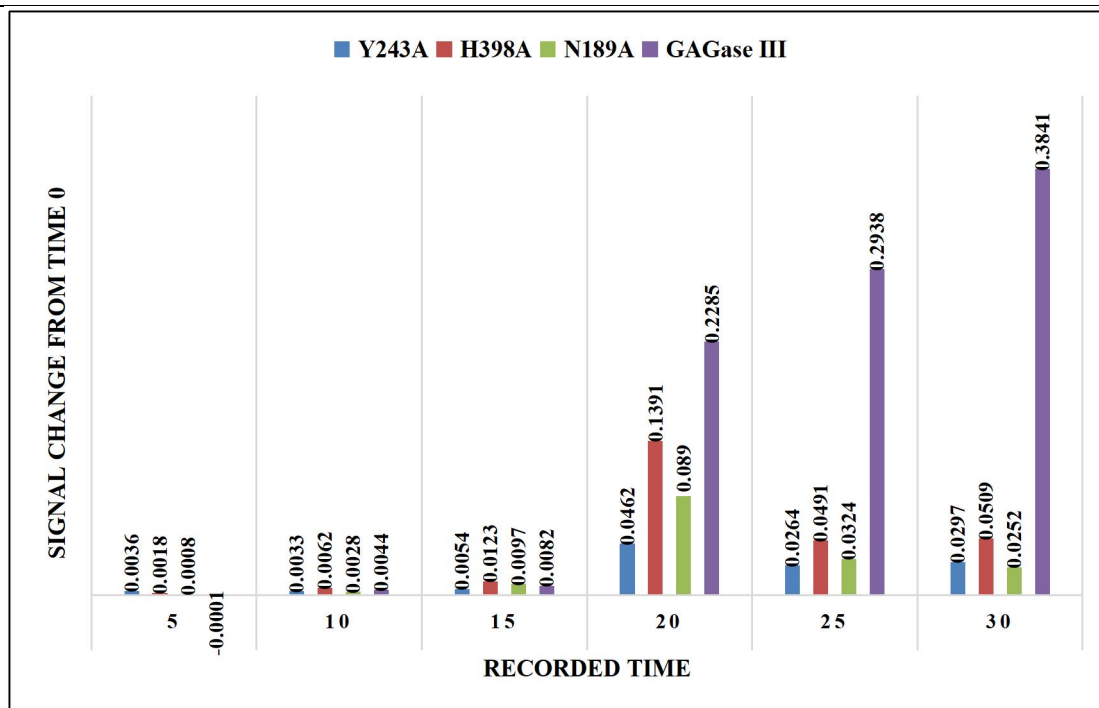


Figure 2. GAGase III and mutant signals with alginate

3.5 Time-course integrity and kinetic interpretation

The time-course records provide the temporal coverage, however, they do not provide a complete kinetic parameter matrix. Although the two H188N and H188A workbooks are labeled separately, the two raw readings (CSC) are identical at all 9,601 points. Other traces exhibit nonmonotonic excursions, and the alginate enzyme-dose workbook does not change the substrate concentration but rather the enzyme concentration. These features inhibit defense-specific mutant-specific CSC rate estimates from these traces. In the present work, the experimental data are therefore presented in such a way as to allow relative comparisons to be made, but cannot be used to calculate reliable Michaelis constants, turnover numbers, or catalytic efficiencies for the full mutant-substrate matrix (Figure 3).

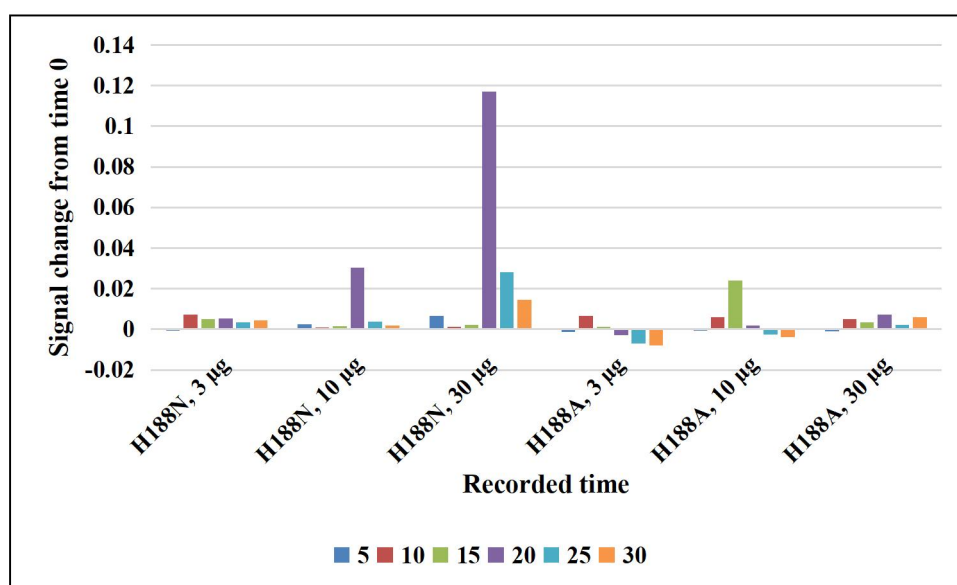


Figure 3. Alginate signals at three enzyme amounts



4. Discussion

The numeric values listed in the cross-substrate profiles in the above tables are synthetic and therefore represent an illustration of the cross-substrate profile. They can present a comparison of catalytic-site and binding-site variants, but they are incapable of establishing a difference between PL35 enzymes in an experiment. In that context, the proposed comparison raises a pertinent question: Is the effect of a substitution similar under substrates or is it determined by the polymer used? In a profile which is broadly reduced, the disruption of a function required for cleavage would be plausible; in a substrate-dependent profile, a closer examination of recognition and positioning would be merited. It is not possible that either interpretation could be confirmed from simulated values alone.

This comparison is made for the following reason from experimental PL35 literature: The family comprises enzymes capable of degrading HA, CS and HS, some of which exhibit alginate activity (Wei et al., 2024). The residues associated with PL35 catalysis and substrate binding were identified by structural analysis and mutagenesis (Wei et al., 2025). Additionally, the characterization of CHa1 further shows that degradation patterns and product structures also provide a description of the behavior of a single PL35 enzyme (Lu et al., 2024). These questions are expanded here to cover enzyme–mutation–substrate combinations. It would have the maximum effect if the original measurements were reanalyzed in a consistent manner, with each mutant compared to the same wild type under the same assay conditions.

However, catalytic versus binding-site effects should still be regarded as a hypothesis until some activity and product measurements have confirmed it. This is because location is not a reliable indicator for the direction of the effect: a substitution of Asp43 with Arg in a cold-adapted endoglucanase enhanced the catalytic function of the enzyme and preserved its stability (Kuroki et al., 2023). That outcome is not necessarily true for a PL35 GAGase, but it does warn against all substitutions being "bad". Similarly, PPO-inhibiting herbicide resistance has been studied, and it is important to differentiate the changes at the target site from the other metabolic mechanisms (Sun et al., 2026). Unlike enzyme cleavage assays, its biological system is different. The most useful comparison is the necessity to identify a local change in the system from other changes that can affect the measured outcome.

Another source of potential variation is the identity of the substrate. HA, CS, HS, and alginate are different with respect to lyase recognition, and a mutant's relative activity should be compared for each subsample tested. Work in plant systems also demonstrates that there can be variability depending on the material and exposure being assessed: a review of nanomaterials shows variability in response based on material properties, concentration and plant species (Zhang et al., 2025). This is an analogy of context dependent measurement only, not evidence for the PL35 substrate specificity. A review of proteins that are exuded by roots also addresses interactions in the rhizosphere instead of GAG cleavage (Adeniji et al., 2025). No source should be used to give a catalytic role to a PL35 residue.

The biochemical output is also a crucial factor. The relative absorbance at 232 nm can be used to compare the formation of product associated with the lyase under defined conditions and chromatographic records can be used to obtain information about the product of the reaction. The two measurements provide answers to different questions. Another aspect of bioactive peptides is product preparation, which is covered in a separate biochemical context (Xu, 2026). It provides a general comparison, i.e. the production of a material and the determination of its composition needs appropriate analytical evidence. If there were reproducible profiles in addition to the apparent difference in activity, this would be more convincing for PL35 variants. The step affected by a mutation would not be identified, unless a chromosome was used for comparison and the reaction conditions matched.

References to microbial process studies further emphasize the necessity to control conditions, but do not provide any evidence concerning the mechanisms of PL35. Zhang et al. (2025) correlated the conditions of the reactors with microbial succession and H₂S removal. The mutation mechanisms and production conditions were investigated separately for a study on a hydrogen-producing mutant strain



(Zheng et al., 2024). Both were outcome factors that were affected by multiple factors. The reaction time, the enzyme amount, the concentration of the substrate and the blanks and the normalization must be recorded. By comparison, PL35 activity analyses require a clear record of the amount of enzyme, the substrate concentration, the reaction time, the blanks and the normalization before a difference can be attributed to a substituted residue. These microbial systems give evidence in lieu of those controls in a GAGase assay.

The other oncology study cited is even further away from this question. Cetuximab treatment led to the upregulation of LGR5 and the activation of an antibody-drug conjugate (ADC) specific for LGR5 in CRC models (High et al., 2025). It's about treatment responsive cell reactions, not polysaccharide cutting. It cannot confirm a PL35 activity pattern, nor provide an explanation for a catalytic-site mutation. In including such a comparison the limit of interpretation is self-evident: a change in one measured biological outcome does not explain a change in another system without the evidence of mechanism.

Cross-substrate comparisons should be calculated from the original experimental files, values reported as normalized, and true low or undetectable readings kept, but not replaced with synthetic. Along with each estimate include information and uncertainty that duplicate the information. In the absence of suitable substrate-concentration series, the results should be reported as relative activity profiles, but if such series is available, then kinetic parameters may be estimated. Product analysis and follow-up experiments to test whether a substrate-dependent pattern is due to altered recognition, site of cleavage or another property of the enzyme. The proposed fingerprints are useful presentation devices, not empirical findings, until this analysis is finished.

5. Conclusion

Comparing catalytic-site and substrate-binding-site mutations among the substrates examined for PL35 glycosaminoglycan lyases. The framework can be combined with the corresponding wild-type enzyme so that it distinguishes between the effects of general reduction and those that are substrate-dependent. Activity measurement plus chromatographic product records can give a more complete picture of the enzyme behaviour than can be obtained from either activity measurement or product records alone. These comparisons are of special interest for PL35 enzymes since they are reported to be active on structurally different glycosaminoglycans and in some cases, alginate. Synthetic values have been populated in the numerical tables that were prepared earlier. Thus, their apparent mutation effects are representative and should not be used as experimental beor to determine the essentiality of a residue for catalysis. Reanalysis of the original data, assurance of assay conditions, normalization procedures, replicate measurements and interpretation of low or undetectable activity is required for valid conclusions. It is also very important to differentiate relative activity profile from kinetic constants, which need suitable experiments involving substrate concentration. With the original measurements support, the cross-substrate fingerprints could be useful to prioritize mutations for structural analysis and follow up biochemical testing. The short-term impact of this research is an analytically transparent methodology to analyze the shared or substrate-specific contribution of mutation effects.

References

1. Adeniji, A., Fadiji, A. E., & Ofoe, S. T. (2025). Redefining root exudation with proteins at the forefront of rhizosphere function. *Journal of Agricultural and Food Chemistry*, 73(28), 17387-17402.
2. Budatala, J. M., Purkrtova, S., Lopez Marin, M. A., Appels, L., & Bartáček, J. (2025). Fate of antibiotic resistance genes and resistant bacteria under various operating temperatures of sludge anaerobic digestion. *Water Science & Technology*, 92(1), 53-65.
3. Drula, E., Garron, M. L., Dogan, S., Lombard, V., Henrissat, B., & Terrapon, N. (2022). The carbohydrate-active enzyme database: functions and literature. *Nucleic acids research*, 50(D1), D571-D577.



4. Fu, L., Song, Y., Peng, W., He, M., Yang, J., Li, S., ... & Fang, F. (2025). The storability of litchi fruits with different maturity is correlated to seed vigor. *Scientia Horticulturae*, 353, 114511.
5. Hettiaratchi, M. H., O'Meara, M. J., O'Meara, T. R., Pickering, A. J., Letko-Khait, N., & Shoichet, M. S. (2020). Reengineering biocatalysts: Computational redesign of chondroitinase ABC improves efficacy and stability. *Science advances*, 6(34), eabc6378.
6. High, P. C., Liang, Z., Guernsey-Biddle, C., Subramanian, S., Shyu, Y. M., Aldana, A. M., ... & Carmon, K. S. (2025). Cetuximab increases LGR5 expression and augments LGR5-targeting antibody-drug conjugate efficacy in patient-derived colorectal cancer models. *Cell Reports Medicine*, 6(10).
7. Jing, Y., Yu, X., Liu, L., Ma, Z., Jiang, X., Xu, L., & Xiao, M. (2026). Biochemical Characterization and Substrate Specificity Mechanism of an Exolytic Hyaluronate Lyase SinHL from *Streptococcus iniae* QMA0131. *Journal of Agricultural and Food Chemistry*.
8. Kuroki, C., Hirano, Y., Nakazawa, M., Sakamoto, T., Tamada, T., & Ueda, M. (2023). A single mutation Asp43Arg was increased 2.5-fold the catalytic activity and maintained the stability of cold-adapted endo-1, 4-beta glucanase (Ef-EG2) from *Eisenia fetida*. *Current Research in Biotechnology*, 5, 100126.
9. Li, F., & Wei, L. (2025). The original data on the enzymatic activity of GAGases and their mutants (Version 1) [Data set]. Mendeley Data. <https://doi.org/10.17632/xry23b3xzv.1>
10. Liu, J., Guo, J., Yang, J., Zhu, L., Xu, X., Zhang, W., & Zhang, Z. (2025). Comparative transcriptome and metabolome analyses reveal the mechanism of slightly acidic electrolyzed water in delaying browning and senescence in rambutan fruit. *Postharvest Biology and Technology*, 230, 113778.
11. Lu, D., Wang, W., Li, X., Wang, L., Guo, Y., Zhu, C., ... & Zhang, Q. (2024). Identification and characterization of a PL35 GAGs lyase with 4-O-sulfated N-acetylgalactosamine (A-type)-rich structures producing property. *International Journal of Biological Macromolecules*, 266, 131283.
12. Qiao, Y., Ma, W., Zhang, S., Guo, F., Liu, K., Jiang, Y., ... & Jiang, M. (2023). Artificial multi-enzyme cascades and whole-cell transformation for bioconversion of C1 compounds: Advances, challenge and perspectives. *Synthetic and Systems Biotechnology*, 8(4), 578-583.
13. Rathor, P., Gorim, L. Y., Chen, G., & Thilakarathna, M. S. (2025). The Effect of humalite on improving soil nitrogen availability and plant nutrient uptake for higher yield and oil content in Canola. *Physiologia Plantarum*, 177(2), e70201.
14. Rawat, P. S., Li, Y., Zhang, W., Meng, X., & Liu, W. (2022). *Hungatella hathewayi*, an efficient glycosaminoglycan-degrading firmicutes from human gut and its chondroitin ABC exolyase with high activity and broad substrate specificity. *Applied and environmental microbiology*, 88(22), e01546-22.
15. Sun, J., Yu, X., Li, J., Xue, Z., Liu, K., & Zhang, C. (2025). An efficient microbial secretion platform for developing supercharged polypeptides-based functional materials from C2 feedstock. *Chemical Engineering Journal*, 519, 165683.
16. Sun, Z., Cao, W., Wang, S., Du, L., Liu, C., Li, D., ... & Teng, C. (2026). Target-site and metabolic mechanisms confer broad resistance to PPO-inhibiting herbicides in *Amaranthus retroflexus*. *Pesticide Biochemistry and Physiology*, 107085.
17. Tian, M., Xu, Y. Y., Li, Y. N., Yu, S., Wang, Y. L., Ma, X. L., & Zhang, Y. W. (2024). Engineering of Substrate-Binding Domain to Improve Catalytic Activity of Chondroitin B Lyase with Semi-Rational Design. *Current Issues in Molecular Biology*, 46(9), 9916-9927.
18. Wang, W., Yi, H., Zi, P. E., Wang, X., Zou, R., Zhang, Y. Z., & Li, F. (2026). Discovery and characterization of Chondroitinase A with specificity for 4-O-sulfated N-acetylgalactosamine in Chondroitin sulfate/Dermatan sulfate. *Journal of Biological Chemistry*, 113594.
19. Wang, Y., Zhu, X., Wang, Y., Cai, X., Long, H., Huang, A., ... & Cai, X. (2025). Elevated carbon dioxide enhances polyunsaturated fatty acids in *Isochrysis galbana* and improves rotifer growth through algal feeding. *Limnology and Oceanography*, 70(11), 3202-3215.
20. Wei, L., Cao, H. Y., Zou, R., Du, M., Zhang, Q., Lu, D., ... & Li, F. (2025). Crystal structure and



- catalytic mechanism of PL35 family glycosaminoglycan lyases with an ultrabroad substrate spectrum. *Elife*, 13, RP102422.
21. Wei, L., Zou, R., Du, M., Zhang, Q., Lu, D., Xu, Y., ... & Li, F. (2024). Discovery of a class of glycosaminoglycan lyases with ultrabroad substrate spectrum and their substrate structure preferences. *Journal of Biological Chemistry*, 300(7).
 22. Xu, W. (2026). Preparation technologies for bioactive peptides. In *Plant Proteins, Bioactive Peptides, and Healthy Foods* (pp. 117-150). Academic Press.
 23. Zhang, T. T., Oo, E. M. M., Chen, B. L., Liu, L., Zhao, Q. B., Yang, J. C. E., & Zheng, Y. M. (2025). Machine learning coupling microbial succession to decipher gas stripping-reinforced sulfate reduction for hydrogen sulfide removal. *Bioresource Technology*, 133169.
 24. Zhang, Z., Su, H., Wang, X., Tang, L., Hu, J., Yu, W., & Han, F. (2020). Cloning and characterization of a novel chondroitinase ABC categorized into a new subfamily of polysaccharide lyase family 8. *International journal of biological macromolecules*, 164, 3762-3770.
 25. Zheng, G., Tao, D., & Ren, N. (2024). Hydrogen-producing conditions and mutation mechanisms of a highly efficient mutant strain *Ethanoligenens harbinense* YR-3. *Journal of bioscience and bioengineering*, 138(5), 399-405.
 26. Zou, H., Jiao, X., Tao, Y., Zeng, S., Qiang, J., Hu, J., ... & Fan, D. (2025). Processing suitability of three tilapia species as alternative freshwater surimi: a comprehensive evaluation from muscle physicochemical to gelling properties. *Food Chemistry*, 492, 145540.