



Enzyme cascade-induced optical sensing of hyaluronidase using an ultraviolet-modified liquid crystal interface

Duong Song Thai Duong¹ · Chang-Hyun Jang¹

Received: 12 June 2025 / Accepted: 15 August 2025 / Published online: 3 September 2025
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2025

Abstract

Hyaluronidase (HAase) is a clinically significant enzyme involved in tumor progression, extracellular matrix remodeling, and inflammation. Hence, HAase is an important biomarker for disease diagnostics. Here, a label-free, pH-responsive biosensor using ultraviolet (UV)-treated liquid crystals (LCs) for HAase detection is presented. The sensor utilizes a cascade enzymatic reaction where HAase hydrolyzes hyaluronic acid (HA) into oligosaccharides, which are sequentially degraded by β -glucuronidase, chitosanase, and amine oxidase into glucosaminic acid, hydrogen peroxide, and protons (H^+). This results in a significant decrease in pH. The pH change alters the protonation state of the 4-cyano-4'-biphenylcarboxylic acid UV-induced product of 4-cyano-4'-pentylbiphenyl, triggering a distinct optical reorientation of the LC at the aqueous interface. Reorientation is evident as a dark-to-bright transition in polarized optical microscopy. The sensor is highly sensitive with a detection limit of 0.0018 U/mL and a linear detection range from 0.3 to 40 U/mL. The sensor also demonstrates excellent selectivity and can detect HAase in dilute human serum and urine samples, highlighting its potential as a practical diagnostic platform.

Keywords Hyaluronidase · Liquid crystals · Biosensor · Enzyme cascade · pH sensing

Introduction

Hyaluronic acid (HA) is a high-molecular-weight linear polysaccharide composed of repeating disaccharide units of N-acetylglucosamine and D-glucuronic acid. As a major structural component of the extracellular matrix, HA plays essential roles in tissue hydration, cellular signaling, wound healing, and modulation of the tumor microenvironment [1, 2]. Its dynamic turnover in the human body is tightly regulated by hyaluronidases (HAases), a family of endoglycosidases that cleave β -1,4-glycosidic bonds in HA to yield smaller oligosaccharides. HAase is increasingly being recognized as a clinically relevant biomarker because of its elevated expression under various pathological conditions [3, 4]. The overexpression of HAase has been documented in several malignancies, including bladder, prostate, colorectal, and breast cancers, where it contributes to extracellular matrix degradation, tumor invasion, and metastatic

dissemination [5]. Sensitive and reliable detection of HAase is of paramount importance for the early diagnosis of cancer, monitoring disease progression and evaluating therapeutic efficacy. Numerous analytical approaches have been developed to detect HAase activity, including viscosimetry, turbidimetry, fluorometric assays, and surface-enhanced Raman scattering (SERS) [6–9]. However, these techniques often require complex instrumentation and labeled reagents or exhibit limited selectivity in biologically complex environments. As an alternative, liquid crystal (LC)-based biosensors have emerged as promising platforms for label-free, real-time, and optical transduction of molecular events.

LCs are a unique state of soft matter that combine the orientational order of crystalline solids with the fluidity of liquids [10, 11]. This hybrid nature gives LCs exceptional sensitivity to external stimuli, such as temperature, electric fields, surfactant interactions, and pH changes [12]. Among various types, nematic LCs—particularly 4-cyano-4'-pentylbiphenyl (5CB)—are widely used in sensing applications due to their optical anisotropy (birefringence), thermal stability, and responsiveness to interfacial molecular events. In the field of biosensing, LC-based sensors have emerged as powerful tools for label-free real-time detection of biomolecular interactions [13, 14]. These systems typically

✉ Chang-Hyun Jang
chjang4u@gachon.ac.kr

¹ Department of Chemistry, Gachon University, San 65, Bokjeong-Dong, Sujeong-Gu, Seongnam-City, Gyeonggi-Do 461-701, South Korea

rely on the orientational transition of LCs at the aqueous/LC interface, which is triggered by surface-bound events, such as enzymatic reactions [15, 16], protein adsorption [17], or chemical recognition [18, 19]. The reorientation of LCs leads to measurable changes in optical textures when observed under polarized optical microscopy (POM), often visualized as a shift between bright and dark states. Compared with conventional methods, LC-based sensors offer advantages that include high sensitivity, visual readout without labeling, low fabrication cost, and adaptability to point-of-care diagnostics [20, 21].

To enhance the responsiveness to biochemical reactions, 5CB can be photochemically modified via ultraviolet (UV) irradiation to form 4-cyano-4'-biphenylcarboxylic acid (CBA), an amphiphilic molecule with pH-sensitive properties [22–24]. This pH dependence forms the basis of numerous optical biosensing strategies. At alkaline pH (> 7.0), CBA is deprotonated, forming a negatively charged monolayer at the LC/aqueous interface [25]. This structured interface induces homeotropic alignment of the 5CB molecules, resulting in a dark optical image under POM. Conversely, at acidic pH, CBA becomes protonated, disrupting the monolayer and causing a transition to a planar or tilted LC alignment, which appears as a bright image under POM [26].

In this study, we introduce a novel pH-driven, LC-based biosensing platform for the detection of HAase, an enzyme that is overexpressed in several cancers and inflammatory conditions. The detection system is based on a multistep enzymatic cascade, in which HAase catalyzes the initial hydrolysis of HA into oligosaccharides. They are subsequently degraded by β -glucuronidase to D-glucuronic acid and N-acetylglucosamine (GlcNAc). GlcNAc is further cleaved by chitosanase to yield D-glucosamine, which is then oxidized by amine oxidase in the presence of oxygen to produce glucosaminic acid, hydrogen peroxide (H_2O_2), and protons (H^+) in the following sequence of reactions:

Hyaluronic acid $\rightarrow$ (HAase) $\rightarrow$ HA-oligosaccharides $\rightarrow$ (β -glucuronidase) $\rightarrow$ D-glucuronic acid + GlcNAc $\rightarrow$ (chitosanase) $\rightarrow$ D-glucosamine $\rightarrow$ (amine oxidase + O_2) $\rightarrow$ Glucosaminic acid + H_2O_2 + H^+

Each enzymatic transformation in the cascade is well-documented in literature. Specifically, HAase hydrolyzes hyaluronic acid into oligosaccharides [27–29], which are further cleaved by β -glucuronidase into D-glucuronic acid and N-acetylglucosamine [30]. Chitosanase subsequently converts N-acetylglucosamine into D-glucosamine [31, 32], which is finally oxidized by amine oxidase to yield glucosaminic acid, hydrogen peroxide, and protons [33]. These steps collectively result in a significant pH decrease at the LC interface, leading to the protonation of CBA. This protonation disrupts the interfacial alignment cues, resulting in the transition of 5CB from a homeotropic (dark) orientation to

a planar or tilted (bright) orientation. This optical change is readily observable under POM and is directly correlated with HAase activity. The platform is simple, label-free, and allows the highly sensitive, real-time, and visual detection of HAase. In addition, its performance was validated in complex biological matrices. Given the clinical importance of HAase as a biomarker for cancer progression, this LC-based sensor offers significant potential for biomedical diagnostics and demonstrates the practical utility of LC systems in enzyme-responsive biosensing technologies.

Materials and methods

Materials

The 5CB nematic LC was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Glass microscope slides (76 $\times$ 26 mm, 1.0 mm thick) were obtained from Matsunami Glass Ind., Ltd. (Osaka, Japan). Copper transmission electron microscopy (TEM) grids (75 mesh, pitch = 340 μm , hole = 285 μm , bar = 55 μm) were purchased from Gilder Grids (Grantham, UK). Sulfuric acid (H_2SO_4 , $\geq 95\%$), hydrogen peroxide (H_2O_2 , 30%), methylene chloride (CH_2Cl_2 , $\geq 99.5\%$), octyltrichlorosilane (OTS, $\geq 97\%$), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Daejung Chemicals & Metals Co., Ltd. (Siheung, Korea). Ultrapure deionized (DI) water (resistivity 18.2 $\text{M}\Omega\cdot\text{cm}$) was obtained using a Milli-Q system (Millipore, Billerica, MA, USA). Human serum, HA, bovine testes HA, bovine liver β -glucuronidase, *Streptomyces griseus* chitosanase, and porcine kidney amine oxidase were obtained from Sigma-Aldrich (St. Louis, MO, USA). Additional enzymes used for the selectivity tests, including trypsin, lysozyme, α -amylase, urease, catalase, and lipase, were also purchased from Sigma-Aldrich. Adenine and glycine were used as small molecule controls. Phosphate-buffered saline (PBS; 10 mM phosphate, 138 mM NaCl, 2.7 mM KCl; pH 7.4) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Functionalization of glass slides with OTS

Glass slides were cleaned in freshly prepared piranha solution (7:3 v/v mixture of H_2SO_4 and H_2O_2) at 80 $^\circ\text{C}$ for 1 h. Slides were then rinsed thoroughly with DI water, dried under nitrogen, and heated at 120 $^\circ\text{C}$ overnight. The cleaned slides were immersed in 0.2% OTS solution in anhydrous n-heptane for 30 min at room temperature. After treatment, the slides were rinsed with CH (Cl) and dried under a nitrogen flow.

UV treatment of 5CB

Following literature protocols [22, 34], 5CB was UV-irradiated at 365 nm for 48 h to generate carboxylic acid-functionalized derivatives (CBA). This duration was selected based on prior work and our preliminary trials, where shorter exposure times yielded incomplete surface conversion and inconsistent pH sensitivity. Although the molar fraction of CBA was not directly measured, previous studies under identical conditions estimate the conversion to be approximately 0.42–0.48 mol%, which is sufficient to induce reliable anchoring transitions near pH = 7.

5CB is photochemically oxidized to generate pH-responsive CBA. Briefly, 600 μL of 5CB was placed in a clean glass vial and irradiated using a long-wavelength UV lamp (365 nm) for 48 h at a distance of 8 cm. UV-treated 5CB was protected from ambient light and used immediately after preparation.

Preparation of aqueous solutions

All enzyme and analyte solutions were prepared in DI water and adjusted to pH 7.0 using either 0.1 M HCl or 0.1 M NaOH. HA was dissolved at a final concentration of 0.2 μM , based on optimization results. β -glucuronidase, chitosanase, and amine oxidase were prepared at their respective optimized concentrations of 22, 15, and 18 $\mu\text{g/mL}$. For specificity studies, control enzymes including trypsin, lysozyme, urease, catalase, lipase, and α -amylase were individually prepared at 1 mg/mL to ensure sufficient enzymatic activity for interference evaluation.

Preparation of real samples

Human serum and urine samples were first filtered through a 0.2 μm membrane to remove particulates and then diluted tenfold with DI water to minimize background interference and reduce sample viscosity. HAase was subsequently added to the diluted samples at final concentrations of 1, 5, 10, 15, 20, and 30 U/mL, which corresponded to the validated detection range of the sensor. For each test, the enzyme cascade comprising β -glucuronidase (22 $\mu\text{g/mL}$), chitosanase (15 $\mu\text{g/mL}$), and amine oxidase (18 $\mu\text{g/mL}$) was added sequentially following HAase treatment to simulate the complete enzymatic conversion pathway leading to local acidification. The prepared samples were analyzed using an LC-based biosensor under standard assay conditions.

Assay procedure

OTS-treated glass slides were affixed to an 8-well chamber. A copper TEM grid was placed in each well, and CBA was added to the grid. Excess LC was removed using a capillary

tube. Analyte solutions (200 μL), including the full enzyme cascade, were added directly onto the grid, and the interface was allowed to equilibrate. POM images were captured, and the optical intensity was quantified using ImageJ software (NIH, Bethesda, MD, USA). Calibration curves were constructed using OriginPro software (OriginLab Corporation, Northampton, MA, USA).

POM

The optical textures of 5CB were characterized using a POM using an Eclipse LV100 POL instrument (Nikon, Tokyo, Japan) with a model DS-Fi3 digital camera (Nikon) at a gain of $1.0\times$, resolution of 2880×2048 pixels, and a shutter speed of 1/10 s using a $4\times$ objective lens. The focus was on the interface between the aqueous phase and 5CB. Gray-scale intensities of the optical images were measured using ImageJ software. Each step was repeated at least three times. Mean grayscale intensities and standard deviations (SDs) were computed.

Results and discussion

Mechanism of HAase detection

We developed a pH-responsive LC biosensor based on UV-treated 5CB for the detection of HAase. The detection strategy relies on a multistep enzyme cascade that generates acidic byproducts, ultimately lowering the pH of the aqueous phase in contact with the LC. The enzymatic cascade was (1) Hyaluronic acid + HAase $\rightarrow$ HA-oligosaccharides, (2) HA-oligosaccharides + β -glucuronidase $\rightarrow$ D-glucuronic acid + GlcNAc, (3) GlcNAc + chitosanase $\rightarrow$ D-glucosamine, and (4) D-glucosamine + amine oxidase $\rightarrow$ Glucosaminic acid + H_2O_2 + H^+ . This cascade significantly reduced pH due to the formation of glucosaminic acid and protons, along with H_2O_2 . To provide direct evidence of the acidification process, we monitored the pH variation of the reaction system over time following the addition of HAase and the full enzyme cascade. As shown in Fig. S1, the pH gradually decreased from neutral (~ 7.0) to approximately 6.1 within 30 min, reflecting the progressive generation of protons through the multistep enzymatic reactions. In contrast, control samples lacking HAase showed negligible pH changes, remaining around 7.0 throughout the same period. This confirms that pH modulation—and the resulting LC optical transition—is directly dependent on HAase activity.

CBA is amphiphilic, and its deprotonated form (CB^-) exhibits partial solubility in aqueous environments, whereas the neutral CBA is preferentially retained within the hydrophobic LC phase. This distribution is driven by hydrophobicity and ionization state. Literature reports

UV-treated 5CB forming carboxyl-functionalized layers at the interface that respond sharply to pH variation [22, 34].

At pH values below 7.0, CBA becomes protonated, disrupting its self-assembled monolayer at the LC–aqueous interface. This transition causes the LC alignment to shift from homeotropic (dark image under POM) to planar or tilted (bright image). The resulting optical texture change provides a label-free visual signal that is directly correlated with HAase activity.

Our sensor system consists of a UV-treated 5CB layer confined between an OTS-treated glass slide and a copper TEM grid (Fig. 1). The LC layer directly interacts with an aqueous solution containing enzymatic components. Under neutral or alkaline pH conditions ($\text{pH} \geq 7.0$), CBA is deprotonated and self-assembles at the interface, inducing vertical (homeotropic) orientation of 5CB. This alignment blocks the transmission of polarized light, yielding a dark texture (Fig. 1b). However, in the presence of HAase, the enzymatic cascade lowers the pH of the solution. The resulting protonation of CBA weakens the interfacial alignment forces, causing the 5CB molecules to adopt a planar or tilted orientation. As a result, the POM image becomes bright because of light transmission through the analyzer (Fig. 1c). This transition from dark-to-bright optical signals is directly correlated with the HAase concentration and is readily quantifiable using image analysis tools. Thus, the sensor can translate the enzymatic activity into

a macroscopic optical response with high sensitivity and spatial resolution.

pH-dependent optical behavior of UV-treated 5CB

To validate the pH responsiveness of UV-treated 5CB and establish its role as a transduction element in the HAase detection platform, we systematically investigated the optical behavior of CBA-doped 5CB under various pH conditions.

Upon UV exposure, 5CB undergoes photochemical oxidation that generates CBA at its terminal alkyl chain. CBA acts as a pH-sensitive amphiphile capable of deprotonation in basic conditions, forming 4-cyano-4'-biphenylcarboxylate (CB^-), which is hydrophilic and thus tends to associate at or near the LC/aqueous interface and self-assembles. The neutral, protonated form of CBA is more hydrophobic and partitions preferentially into the LC bulk. This differential partitioning is driven by the hydrophobicity of the biphenyl core and the ionization state of the carboxyl group. These molecular events at the LC–aqueous interface are sufficient to induce pH-responsive anchoring transitions, even in the absence of full aqueous solubility.

In alkaline environments ($\text{pH} \geq 7.0$, Fig. 2), CBA is largely deprotonated, forming anionic CB^- that assembles at the interface and induces vertical (homeotropic) alignment of the LC molecules. This results in a dark optical appearance under POM. Conversely, at an acidic pH ($\text{pH} < 7.0$, Fig. 2), CBA

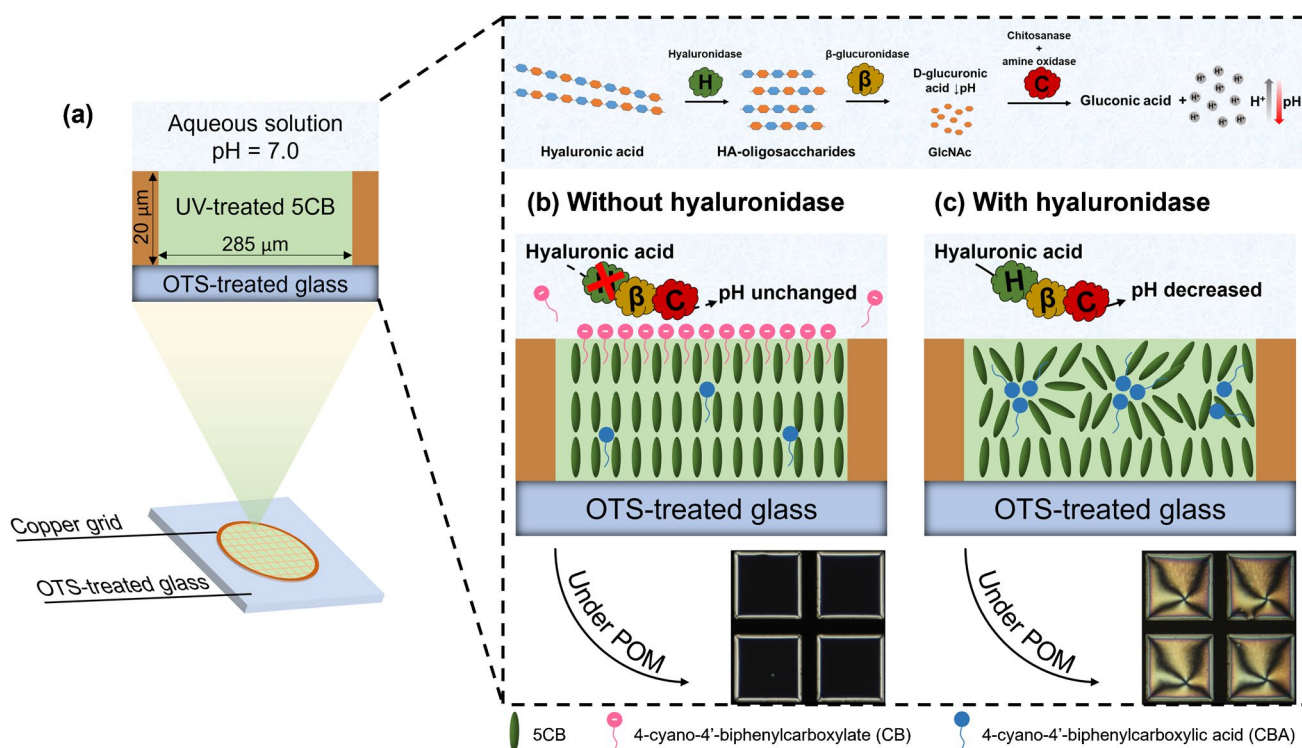


Fig. 1 Schematic illustration of LC sensor design and HAase-induced enzyme cascade mechanism. Mesh size = 285 μm

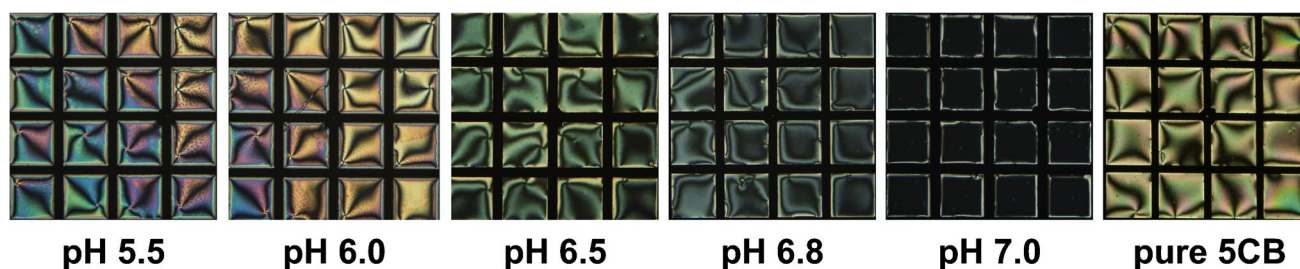


Fig. 2 Optical response of UV-treated 5CB under various pH conditions using a mesh size of 285 μm

remains protonated and is partially solubilized in the hydrophobic LC bulk phase, disrupting the interfacial ordering and resulting in a tilted or planar alignment of the 5CB molecules, which appears as a bright optical texture under POM.

The transition from bright to dark was clearly observed as the pH increased from 6.5 to 7.0, confirming that the critical threshold of the CBA-based pH response lies near physiological pH. Pure 5CB (without UV treatment) did not exhibit such sensitivity to pH variation, verifying that the observed anchoring transition arose solely from the presence of CBA. Thus, we selected UV treatment for 48 h to generate the optimal amount of CBA with a critical pH transition point of approximately 7.0.

Optimization of HA and enzyme concentration

To ensure reliable detection and avoid perturbation of LC alignment caused by excessive enzymatic reaction products, we optimized the concentrations of HA, β -glucuronidase, chitosanase, and amine oxidase involved in the cascade reaction. These enzymes act sequentially following HAase-mediated hydrolysis of HA, contributing to a gradual and cumulative pH reduction through the production of D-glucuronic acid, D-glucosamine, and ultimately glucosaminic acid and protons.

To determine the appropriate concentration of HA that does not independently perturb LC alignment in the absence of HAase, a series of HA solutions (0.1, 0.2, 0.5, and 1.0 μM) were applied to UV-treated LC films. As shown in Fig. 3a, HA concentrations of 0.1 and 0.2 μM maintained the characteristic dark texture of homeotropically aligned LCs, indicating minimal pH alteration or interfacial disruption. At 0.5 μM , a slight increase in brightness was observed, which we attribute to partial tilting of LC molecules caused by localized acidification from the HA solution. This suggests that higher HA concentrations can lower the local pH enough to affect interfacial anchoring. At 1.0 μM , the LC texture appeared significantly brighter, indicating a disruption of homeotropic alignment and a clear pH-driven anchoring transition. Based on these observations, 0.2 μM was selected as the optimal HA concentration

for subsequent assays, as it preserves LC orientation in the absence of enzymatic activity while allowing sufficient substrate availability for HAase-catalyzed reactions.

β -Glucuronidase concentration was optimized by fixing HA at 0.2 μM and using excess amounts of the other enzymes: HAase (100 U/mL), chitosanase (100 $\mu\text{g/mL}$), and amine oxidase (100 $\mu\text{g/mL}$). Enzyme titration revealed that at concentrations < 15 $\mu\text{g/mL}$, β -glucuronidase had no noticeable effect on the LC optical response (Fig. 3b). Between 15 and 22 $\mu\text{g/mL}$, the brightness of LC films increased linearly, confirming progressive hydrolysis of HA-derived oligosaccharides into D-glucuronic acid. At concentrations > 22 $\mu\text{g/mL}$, the optical intensity plateaued. Thus, 22 $\mu\text{g/mL}$ was selected as the optimal concentration of β -glucuronidase.

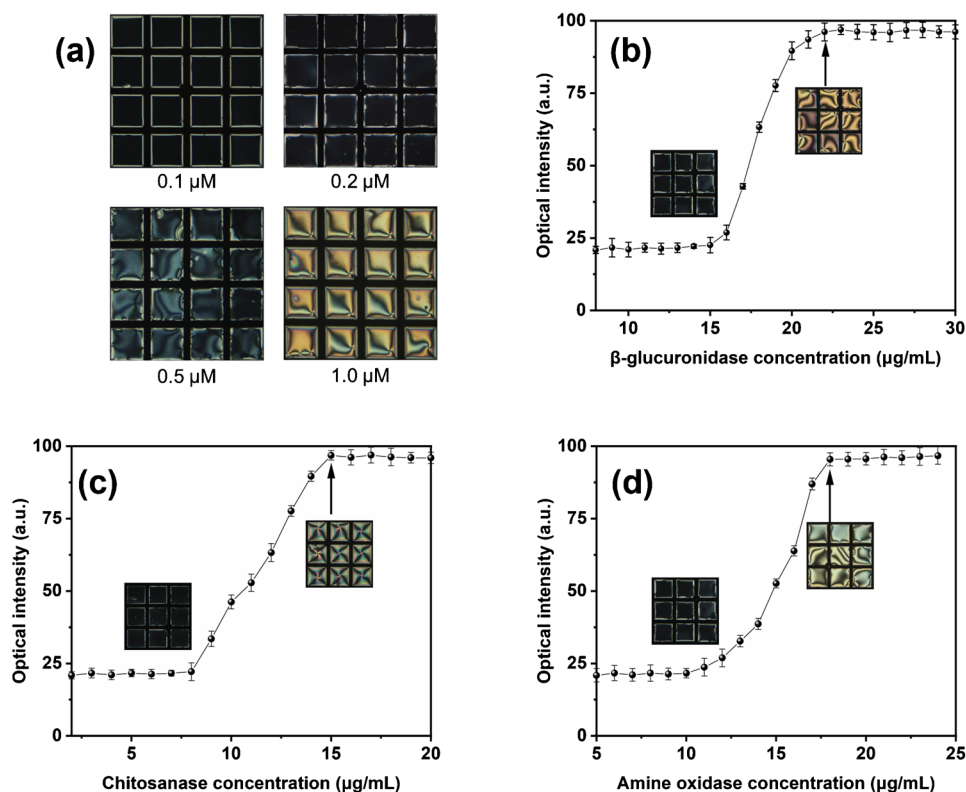
The optimization of chitosanase concentration was then assessed under similar cascade conditions of 0.2 μM HA, 100 U/mL HAase, 22 $\mu\text{g/mL}$ β -glucuronidase, and 100 $\mu\text{g/mL}$ amine oxidase. Chitosanase concentrations from 2 to 20 $\mu\text{g/mL}$ were examined (Fig. 3c). No optical change occurred below 8 $\mu\text{g/mL}$. A progressive increase in brightness was observed from 8 to 15 $\mu\text{g/mL}$, corresponding to effective cleavage of N-acetylglucosamine into D-glucosamine. Above 15 $\mu\text{g/mL}$, signal saturation was reached. Therefore, 15 $\mu\text{g/mL}$ chitosanase was chosen as the optimal concentration.

Finally, amine oxidase was optimized using the same approach with 0.2 μM HA, 100 U/mL HAase, 22 $\mu\text{g/mL}$ β -glucuronidase, and 15 $\mu\text{g/mL}$ chitosanase. Below 10 $\mu\text{g/mL}$, no significant changes in LC alignment were detected (Fig. 3d). Optical intensity increased gradually from 10 to 18 $\mu\text{g/mL}$, reflecting the enzymatic oxidation of D-glucosamine to glucosaminic acid and H^+ . At concentrations > 18 $\mu\text{g/mL}$, no further increase in signal was observed. Consequently, 18 $\mu\text{g/mL}$ amine oxidase was established as the optimal level.

Quantitative detection of HAase

To evaluate the sensing performance of the LC-based platform for HAase, polarized optical images were recorded at

Fig. 3 **a** Optical image of UV-treated 5CB in response with HA concentrations. **b–d** Optimization of enzyme condition for enzyme cascade-induced LC reorientation. Mesh size = 285 μm



increasing enzyme concentrations. As depicted in Fig. 4a, LC films underwent a distinct optical transition from dark-to-bright with increasing HAase concentration. At low concentrations (e.g., 0.3–1 U/mL), the optical appearance began to show slight brightening in the isolated LC microdomains, suggesting the initiation of pH-induced disruption. At concentrations > 5 U/mL, the brightness became more uniform and intensified across the entire field of view, indicating progressive acid generation via the cascade reaction. At the highest concentrations (20–40 U/mL), the LC alignment fully transitioned to a bright planar state, confirming that the accumulation of protons effectively protonated the carboxylic acid headgroups and disrupted homeotropic anchoring. The collective results demonstrate the high sensitivity of the system to HAase activity through visual changes in LC alignment.

The corresponding calibration curve revealed a strong linear relationship between HAase concentrations of 0.3 to 40 U/mL and optical intensity (Fig. 4b). Representative LC images corresponding to each HAase concentration in Fig. 4b are provided in the Supporting Information (Fig. S2) for visual reference. The calculated regression equation of $y = 1.818x + 23.288$ had an excellent correlation coefficient of $R^2 = 0.999$, demonstrating quantitative reliability. The limit of detection (LOD) calculated as $3\sigma/\text{slope}$ was 0.0018 U/mL, reflecting the sensor's superior sensitivity, allowing detection of HAase levels well below

the physiological concentrations reported in clinical studies (Table 1). Notably, HAase levels in normal human serum are 0.256 ± 0.198 U/mL, while normal human urine contains 0.0042 ± 0.0012 U/mL [35]. This low detection threshold highlights the sensor's suitability for real-time monitoring of HAase in biologically relevant samples and its potential for clinical application in early diagnosis of HAase-associated diseases, such as cancer and inflammation.

Selectivity test

To evaluate the specificity of the HAase-responsive LC sensor, we investigated its response to structurally unrelated enzymes and common biological interferents. Solutions containing 100 $\mu\text{g/mL}$ of trypsin, lysozyme, urease, catalase, lipase, and α -amylase were individually tested under the same experimental conditions as HAase. As shown in Fig. 5, none of the nontarget enzymes induced a significant change in the optical signal of the LC film. The POM images remained predominantly dark, indicating that these enzymes did not trigger the acid-generating cascade necessary to alter the LC orientation. In contrast, the presence of HAase resulted in a noticeable transition to a bright texture. Furthermore, small molecules, such as adenine and glycine (10 μM), were tested. They had no effect on the optical texture of UV-treated 5CB. These

Fig. 4 **a** Optical texture of enzyme cascade-induced UV-treated 5CB variation with increasing HAase concentration. **b** Calibration curve for quantitative HAase detection in range of 0.3–40 U/mL. Mesh size = 285 μm

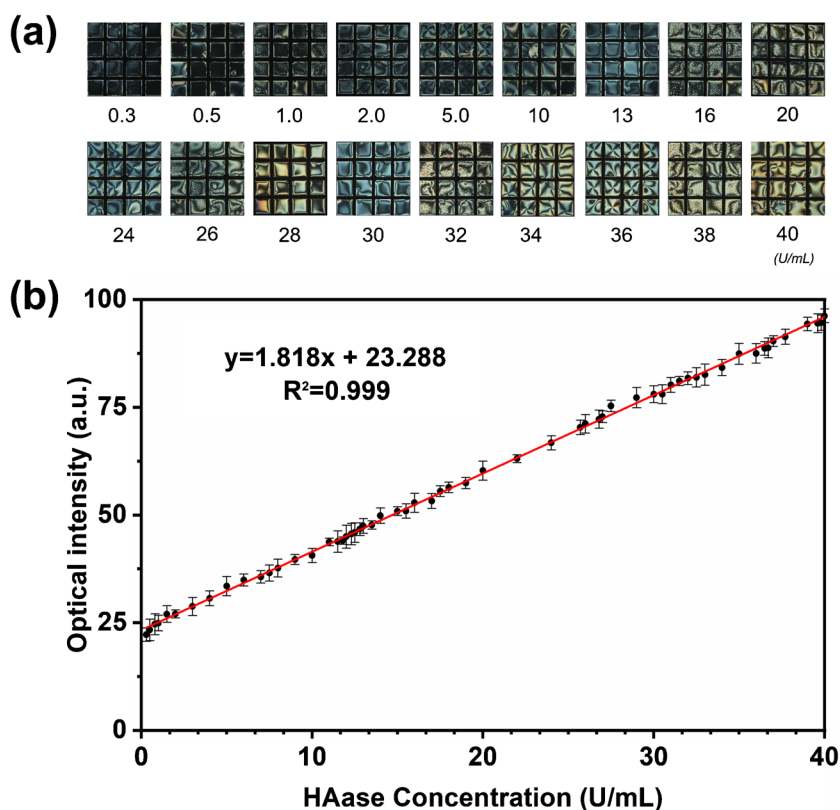


Table 1 Comparison of recent HAase detection methods based on sensing platform, LOD, and sample matrix

Methods	Materials	Samples	LOD	Detection range	Reference
Fluorescence sensor	Carbon dots and naphthalimide	PBS buffer	0.09 U/mL	0.1–16 U/mL	[36]
Fluorescence sensor	Hyaluronan/tetraphenylethylene	Urine	0.0017 U/mL	-	[7]
Fluorescence sensor	Si QDs/HA- δ -FeOOH nanoassembly	Urine	0.02 ng/mL	0.1–12 ng/mL	[37]
Ratiometric fluorescence sensor	Positively-charged pyrene analog	Urine	0.007 U/mL	-	[8]
Electrochemiluminescence sensor	$\text{Ru}(\text{bpy})_3^{2+}$	Urine	0.33 U/mL	2.0–40 U/mL	[38]
Electrochemiluminescence sensor	$\text{Fe}_3\text{O}_4@\text{Ru}@\text{SiO}_2\text{-HA}$	Human serum and urine	1.8 U/mL	5.0 to 60 U/mL	[39]
Electrochemiluminescence sensor	MC- γ -Fe 2O_3 /AAO	Fetal bovine serum	0.348 U/mL	1–50 U/mL	[9]
Viscosity-based slippery sensor	Slippery substrate, time-based readout	Urine	0.23 U/mL	-	[6]
Multicolor hydrogel-AuNRs sensor	Target-responsive hydrogel + gold nanorods	Human serum	3.8 U/mL	10–60 U/mL	[40]
Liquid crystal sensor	UV-treated 5CB/enzyme cascade	Human serum and urine	0.0018 U/mL	0.3–40 U/mL	This work

results confirm the high specificity of the sensor platform for HAase and minimal cross-reactivity with nontarget biological substances.

To evaluate the possibility of false-positive signals arising from endogenous enzymes in serum or urine, we performed negative control experiments in which diluted biological samples were spiked with the cascade enzymes (β -glucuronidase, chitosanase, and amine oxidase) but without HAase.

Real sample analysis

To evaluate the practical applicability of the developed LC-based biosensor, recovery tests were performed using human serum and urine samples spiked with known HAase concentrations. Prior to analysis, each biological sample was filtered through a 0.2 μm membrane to remove particulates and subsequently diluted tenfold in DI water to minimize matrix interference and reduce viscosity. Due to the naturally low levels

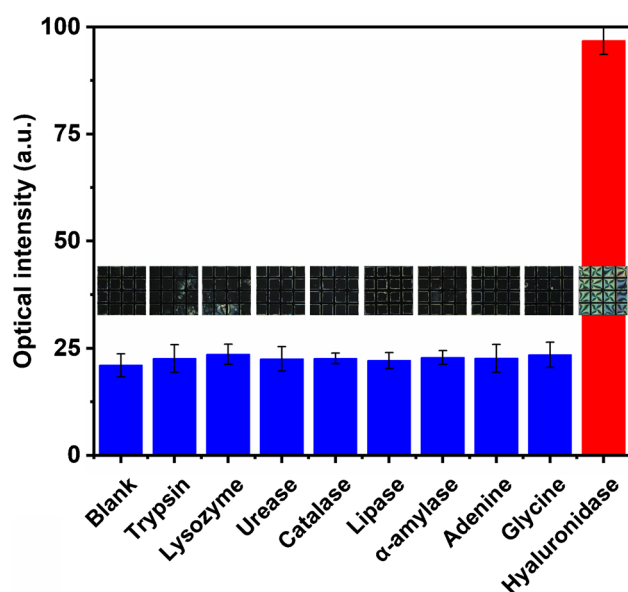


Fig. 5 Comparison of optical intensity and corresponding POM images responses induced by 20 U/mL HAase and 100 µg/mL of various control enzymes. Mesh size = 285 µm

of HAase in human serum ($\sim 0.256 \pm 0.198$ U/mL) and urine ($\sim 0.0042 \pm 0.0012$ U/mL) [35], the blank samples yielded no detectable optical signal, as their concentrations fell below the established detection range of the sensor (0.3–40 U/mL).

The diluted samples were spiked with HAase at final concentrations of 1, 5, 10, 15, 20, and 30 U/mL. As shown in Fig. 6, POM images of the LC interface reveal a concentration-dependent transition from dark-to-bright textures, consistent with the enzymatic generation of protons that alter LC alignment. The calculated recovery rates ranged from 96.47% to 106.10%, with relative standard deviations (RSD) between 1.68% and 5.35%, demonstrating excellent analytical accuracy

and repeatability (Table 2). Notably, even in complex biological matrices, the biosensor maintained its optical responsiveness without requiring sophisticated sample pretreatment or signal amplification. These results validate the robustness and reliability of the sensing platform under realistic conditions and support its potential deployment in clinical settings for monitoring HAase-associated diseases, such as cancer and inflammation. The ability of the system to function effectively in both serum and urine matrices further highlights its versatility for noninvasive diagnostics and point-of-care applications.

To evaluate the potential for false-positive signals arising from endogenous enzymes in biological samples, a series of negative control experiments was conducted using tenfold diluted human serum and urine. Each sample was individually treated with β -glucuronidase, chitosanase, or amine oxidase in the absence of HAase. A combined treatment with all three cascade enzymes but without HAase was also tested. As shown in Fig. S3 (panels a–d), the optical textures remained uniformly dark in all cases, indicating that neither individual nor combined enzyme treatments generated sufficient protons to disrupt LC alignment. In contrast, when HAase was added alongside the cascade enzymes (panel e), a distinct bright transition was observed. These findings confirm that endogenous enzyme activity in serum or urine is insufficient to initiate the cascade reaction and does not interfere with the specificity of the sensor.

Furthermore, pH measurements were performed for each condition. As shown in Fig. S1, the pH values of samples containing HA and the cascade enzymes—without HAase—remained stable ($\text{pH} \approx 7.0$), consistent with the HA-only control. A notable pH drop (~ 6.1) was observed only when HAase was present, aligning with the observed optical brightening. These combined optical and pH results confirm that the enzymatic cascade—and the resulting LC reorientation—is specifically triggered by HAase activity.

	0 U/mL	1 U/mL	5 U/mL	10 U/mL	15 U/mL	20 U/mL	30 U/mL
Human serum							
Urine							

Fig. 6 Brightening of LC textures observed via POM with increasing HAase concentrations (1–30 U/mL) spiked into tenfold diluted human serum and urine. Mesh size = 285 µm

Table 2 Recovery and precision of HAase detection in serum and urine samples

Sample	Amount added (U/mL)	Amount detected (U/mL)	Recovery (% <i>n</i> = 3)	RSD (%)
Human serum	0	Non-detectable	–	–
	1	0.97	97.00	3.42
	5	4.96	99.20	4.62
	10	10.61	106.10	5.23
	15	14.87	99.13	3.74
	20	19.62	98.10	4.62
	30	28.94	96.47	1.68
Urine	0	Non-detectable	–	–
	1	0.98	98.00	2.96
	5	5.04	100.80	3.64
	10	9.96	99.60	4.32
	15	14.87	99.13	5.35
	20	20.11	100.55	4.62
	30	29.97	99.90	3.88

Conclusion

In this study, we developed a label-free pH-responsive LC-based biosensor for the sensitive detection of HAase. The sensing strategy integrates a multistep enzymatic cascade comprising HAase, β -glucuronidase, chitosanase, and amine oxidase that collectively generate glucosaminic acid and protons, thereby markedly lowering the local pH. This pH variation induces a distinct optical transition in UV-treated 5CB due to the protonation-dependent orientation of CBA at the LC/aqueous interface. The resulting change in optical texture observed via POM enabled direct visualization and quantification of HAase activity. The calculated LOD of 0.0018 U/mL is significantly lower than the physiological levels of HAase reported for normal human serum and urine, confirming the sensitivity of the sensor. The sensor demonstrated high sensitivity and selectivity toward HAase with minimal cross-reactivity against other enzymes and small biomolecules. Furthermore, the successful application of the sensor to diluted human serum and urine samples confirmed its robustness and potential for practical bioanalytical use. This work highlights the potential of LC-based systems as simple, low-cost, and powerful tools for enzyme detection and noninvasive diagnostics.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00604-025-07499-x>.

Author contribution D.S.T.D. conceptualized the study and contributed to the writing, review, and editing of the manuscript. C.-H.J. provided resources, supervised the project, and contributed to the writing, review, and editing of the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT) (RS-2024-00450413).

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

- Skarmoutsos I, Skarmoutsos A, Katafigiotis I, Tataki E, Giagini A, Adamakis I, Alamanis C, Duvdevani M, Sitaras N, Constantinides C (2018) Hyaluronic acid and hyaluronidase as possible novel urine biomarkers for the diagnosis of prostate cancer. *Med Oncol* 35:1–6. <https://doi.org/10.1007/s12032-018-1157-9>
- Jayadev C, Rout R, Price A, Hulley P, Mahoney D (2012) Hyaluronidase treatment of synovial fluid to improve assay precision for biomarker research using multiplex immunoassay platforms. *J Immunol Methods* 386:22–30. <https://doi.org/10.1016/j.jim.2012.08.012>
- Posey JT, Soloway MS, Ekici S, Sofer M, Civantos F, Duncan RC, Lokeshwar VB (2003) Evaluation of the prognostic potential of hyaluronic acid and hyaluronidase (HYAL1) for prostate cancer. *Cancer Res* 63:2638–2644
- Meszaros M, Kis A, Kunos L, Tarnoki AD, Tarnoki DL, Lazar Z, Bikov A (2020) The role of hyaluronic acid and hyaluronidase-1 in obstructive sleep apnoea. *Sci Rep* 10:19484. <https://doi.org/10.1038/s41598-020-74769-4>
- Morera DS, Hennig MS, Talukder A, Lokeshwar SD, Wang J, Garcia-Roig M, Ortiz N, Yates TJ, Lopez LE, Kallifatidis G (2017) Hyaluronic acid family in bladder cancer: potential prognostic biomarkers and therapeutic targets. *Br J Cancer* 117:1507–1517. <https://doi.org/10.1038/bjc.2017.318>
- Bi Y, Khan M, Liu J, Ping J, Zhu J, Wang Y, Ma Y, Yu L, Lin J-M, Hu Q (2023) Slippery viscosity-sensing platform with time

- readout for the detection of hyaluronidase and its inhibitor. *ACS sensors* 8:4071–4078. <https://doi.org/10.1021/acssensors.3c01190>
7. Xie H, Zeng F, Wu S (2014) Ratiometric fluorescent biosensor for hyaluronidase with hyaluronan as both nanoparticle scaffold and substrate for enzymatic reaction. *Biomacromol* 15:3383–3389. <https://doi.org/10.1021/bm500890d>
 8. Hu Q, Zeng F, Wu S (2016) A ratiometric fluorescent probe for hyaluronidase detection via hyaluronan-induced formation of red-light emitting excimers. *Biosens Bioelectron* 79:776–783. <https://doi.org/10.1016/j.bios.2016.01.019>
 9. Zhang X, Zhou S, He Y, Zeng H, Hu X, Liang Q, Huang Y, Xu Y, Guo Y, Liu T (2024) Superassembled mesoporous carbon-Fe₂O₃ heterochannels for photothermal-enhanced hyaluronidase sensing. *Anal Chem* 96:13971–13979. <https://doi.org/10.1021/acs.analchem.4c02753>
 10. Woltman SJ, Jay GD, Crawford GP (2007) Liquid-crystal materials find a new order in biomedical applications. *Nat Mater* 6:929–938. <https://doi.org/10.1038/nmat2010>
 11. Ren H, Jang C-H (2019) A simple liquid crystal-based aptasensor using a hairpin-shaped aptamer for the bare-eye detection of carcinoembryonic antigen. *BioChip J* 13:352–361. <https://doi.org/10.1007/s13206-019-3406-1>
 12. Esteves C, Ramou E, Porteira ARP, Moura Barbosa AJ, Roque ACA (2020) Seeing the unseen: the role of liquid crystals in gas-sensing technologies. *Adv Opt Mater* 8:1902117. <https://doi.org/10.1002/adom.201902117>
 13. Ryu J-J, Jang C-H (2024) Label-free, liquid crystal-based aptasensor for detecting carbendazim at picomolar levels. *Food Chem* 445:138789. <https://doi.org/10.1016/j.foodchem.2024.138789>
 14. Hussain Z, Qazi F, Ahmed MI, Usman A, Riaz A, Abbasi AD (2016) Liquid crystals based sensing platform-technological aspects. *Biosens Bioelectron* 85:110–127. <https://doi.org/10.1016/j.bios.2016.04.069>
 15. Chen J, Liu Z, Yang R, Liu M, Feng H, Li N, Jin M, Zhang M, Shui L (2022) A liquid crystal-based biosensor for detection of insulin driven by conformational change of an aptamer at aqueous-liquid crystal interface. *J Colloid Interface Sci* 628:215–222. <https://doi.org/10.1016/j.jcis.2022.07.051>
 16. Bi X, Hartono D, Yang KL (2009) Real-time liquid crystal pH sensor for monitoring enzymatic activities of penicillinase. *Adv Funct Mater* 19:3760–3765. <https://doi.org/10.1002/adfm.200900823>
 17. Ryu JJ, Jang CH (2023) A liquid crystal-based sensor exploiting the aptamer-mediated recognition at the aqueous/liquid crystal interface for sensitive detection of serotonin. *Biotechnol Appl Biochem* 70:1972–1982. <https://doi.org/10.1002/bab.2503>
 18. Duong DST, Jang C-H (2023) Liquid crystal-based sensor for real-time detection of paraoxon pesticides based on acetylcholinesterase enzyme inhibition. *Microchim Acta* 190:122. <https://doi.org/10.1007/s00604-023-05716-z>
 19. Du X, Liu Y, Wang F, Zhao D, Gleeson HF, Luo D (2021) A fluorescence sensor for Pb²⁺ detection based on liquid crystals and aggregation-induced emission luminogens. *ACS Appl Mater Interfaces* 13:22361–22367. <https://doi.org/10.1021/acsami.1c02585>
 20. Ryu J-J, Jang C-H (2024) A liquid crystal-based biosensor for sensitive detection of tumor necrosis factor- α . *Microchim Acta* 191:55. <https://doi.org/10.1007/s00604-023-06125-y>
 21. Hong PTK, Yun K, Jang C-H (2021) Liquid crystal-based droplet sensor for the detection of Hg (II) ions using an aptamer as the recognition element. *BioChip J* 15:152–161. <https://doi.org/10.1007/s13206-021-00010-7>
 22. Kim C, Jang C-H (2022) A pH-dependent optical sensor based on ultraviolet-treated liquid crystals to detect xanthine. *Sens Actuators B Chem* 372:132652. <https://doi.org/10.1016/j.snb.2022.132652>
 23. Bao P, Phillips K, Raval R (2024) Membrane proteins in action monitored by pH-responsive liquid crystal biosensors. *ACS Appl Mater Interfaces* 16:31843–31850. <https://doi.org/10.1021/acsami.4c06614>
 24. Zhong S, Jang C-H (2014) Highly sensitive and selective glucose sensor based on ultraviolet-treated nematic liquid crystals. *Biosens Bioelectron* 59:293–299. <https://doi.org/10.1016/j.bios.2014.03.070>
 25. Oladepo SA (2022) Development and application of liquid crystals as stimuli-responsive sensors. *Molecules* 27:1453. <https://doi.org/10.3390/molecules27041453>
 26. Hu Q-Z, Jang C-H (2012) Using liquid crystals for the real-time detection of urease at aqueous/liquid crystal interfaces. *J Mater Sci* 47:969–975. <https://doi.org/10.1007/s10853-011-5876-y>
 27. Hayashi S (1977) Study on the degradation of glycosaminoglycans by canine liver lysosomal enzymes: I. The mode of contribution of hyaluronidase, β -glucuronidase, and β -N-acetylhexosaminidase on hyaluronic acid. *J Biochem* 82:1287–1295. <https://doi.org/10.1093/oxfordjournals.jbchem.a131816>
 28. Kobayashi T, Chanmee T, Itano N (2020) Hyaluronan: metabolism and function. *Biomolecules* 10:1525. <https://doi.org/10.3390/biom10111525>
 29. Lenormand H, Deschrevel B, Vincent J-C (2010) pH effects on the hyaluronan hydrolysis catalysed by hyaluronidase in the presence of proteins: part I. Dual aspect of the pH-dependence. *Matrix Biol* 29:330–337. <https://doi.org/10.1016/j.matbio.2009.12.007>
 30. Meyer K, Linker A, Rapport MM (1951) The production of monosaccharides from hyaluronic acid by β -glucuronidase. *J Biol Chem* 192:275–281. [https://doi.org/10.1016/S0021-9258\(18\)55931-7](https://doi.org/10.1016/S0021-9258(18)55931-7)
 31. Kumar M, Rajput M, Soni T, Vivekanand V, Pareek N (2020) Chemoenzymatic production and engineering of chitooligosaccharides and N-acetyl glucosamine for refining biological activities. *Front Chem* 8:469. <https://doi.org/10.3389/fchem.2020.00469>
 32. Jiang Z, Lv X, Liu Y, Shin H-d, Li J, Du G, Liu L (2018) Biocatalytic production of glucosamine from N-acetylglucosamine by diacetylchitobiose deacetylase. *J Microbiol Biotechnol*. <https://doi.org/10.4014/jmb.1805.05061>
 33. Lin S-F, Yang T-Y, Inukai T, Yamasaki M, Tsai Y-C (1991) Purification and characterization of a novel glucosaminoglycan oxidase from *Acremonium strictum* T1. *BBA Prot Struct Mol Enzymol* 1118:41–47. [https://doi.org/10.1016/0167-4838\(91\)90439-7](https://doi.org/10.1016/0167-4838(91)90439-7)
 34. Park J-S, Jang C-H, Tingey ML, Lowe AM, Abbott NL (2006) Influence of 4-cyano-4'-biphenylcarboxylic acid on the orientational ordering of cyanobiphenyl liquid crystals at chemically functionalized surfaces. *J Colloid Interface Sci*. 304:459–473. *BBA Prot Struct Mol Enzymol*
 35. Marei WF, Salavati M, Fouladi-Nashta AA (2013) Critical role of hyaluronidase-2 during preimplantation embryo development. *Mol Hum Reprod* 19:590–599. <https://doi.org/10.1093/molehr/gat032>
 36. Raj P, Lee S-y, Lee TY (2021) Carbon dot/naphthalimide based ratiometric fluorescence biosensor for hyaluronidase detection. *Materials* 14:1313. <https://doi.org/10.3390/ma14051313>
 37. Li X, Wu T, Fu Y, Ding X, Li Z, Zhu G, Fan J (2020) A high sensitivity background eliminated fluorescence sensing platform for hyaluronidase activity detection based on Si QDs/HA- δ -FeOOH nanoassembly. *Biosens Bioelectron* 150:111928. <https://doi.org/10.1016/j.bios.2019.111928>
 38. Li Z, Chen H, Zhuo Z, Huang D, Luo F, Chen L, Wang J, Guo L, Qiu B, Lin Z (2018) Electrochemiluminescence biosensor for hyaluronidase activity detection and inhibitor assay based on the electrostatic interaction between hyaluronic acid and Ru (bpy)₃²⁺. *Sens Actuators B Chem* 275:409–414. <https://doi.org/10.1016/j.snb.2018.08.069>

39. Wang W, Luo P, Wang J, Hong M, Lu F, Zeng R, Lan T, Yuan Y, Lin Y, Liu J (2024) Homogeneous electrochemiluminescence biosensor for hyaluronidase based on the regulation of hyaluronic acid coating on magnetic nanoparticles. *Microchem J* 207:112131. <https://doi.org/10.1016/j.microc.2024.112131>
40. Wang W, Xu Y, Hou J, Li Z, Luo F, He D, Lin Z (2023) Multicolor biosensor for hyaluronidase based on target-responsive hydrogel and etching of gold nanorods by H₂O₂. *Talanta* 257:124367. <https://doi.org/10.1016/j.talanta.2023.124367>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.