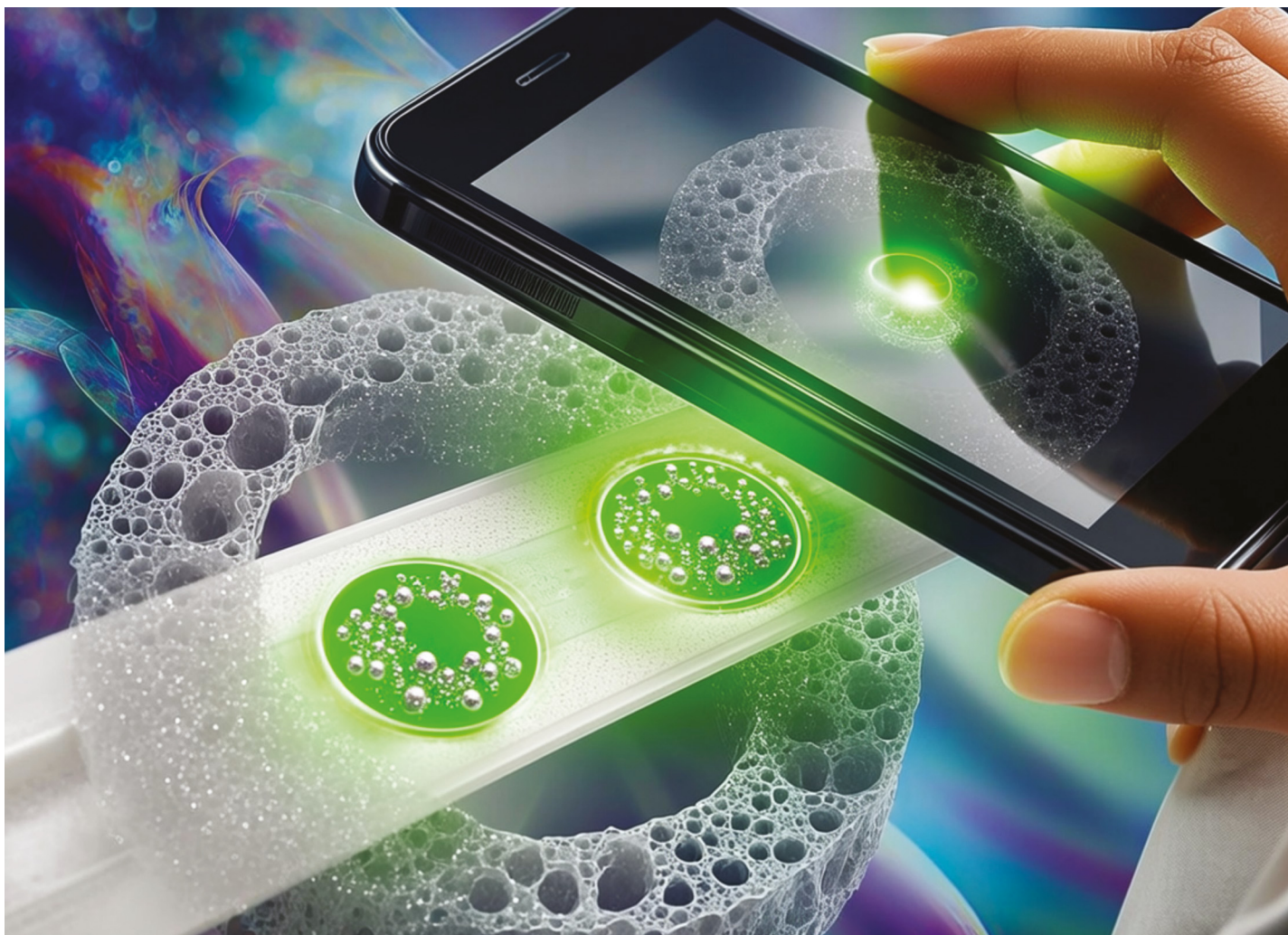




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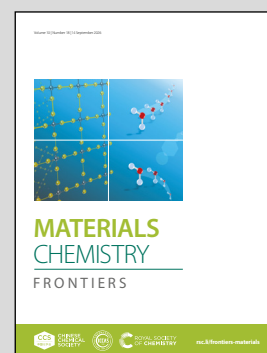
Paper-based immunoassay enabled by silver clusters embedded within zeolites

A paper-based lateral flow biosensor integrating luminescent silver cluster-zeolite composites enables direct antibody recognition and quantitative smartphone readout. The low-cost platform delivers sensitive, robust detection in serum while simplifying assay architecture for decentralized diagnostics.

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Paper-based immunoassay enabled by silver clusters embedded within zeolites

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Portable biosensing technologies are crucial for decentralized diagnostics and point-of-care applications. Conventional lateral flow assays rely on colorimetric labels, limiting sensitivity and quantification. Here, we integrate optical responsive silver cluster–zeolite composites directly functionalized with antibodies into paper-based lateral flow devices, enabling simultaneous analyte recognition and luminescent signal transduction without conjugate pads. Using human immunoglobulin G as a model analyte, the device achieves quantitative detection with high sensitivity and robustness against interfering proteins and human serum. Luminescence changes upon immunocomplex formation are captured via smartphone-based fluorescence imaging and analyzed with custom algorithms. This simplified, low-cost platform demonstrates the potential of silver cluster–zeolite composites as versatile luminescent probes for scalable, smartphone-readable, and decentralized biosensing technologies suitable for real-world diagnostics.

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Introduction

Given the need for decentralized healthcare, real-time environmental monitoring and food safety assessments, the demand for rapid, reliable and portable biosensing technologies has significantly increased over the past two decades.^{1,2} Traditional laboratory-based analytical methods such as enzyme-linked immunosorbent assays (ELISA) remain the gold standard in terms of sensitivity and specificity in immunoassays. However, this well-established immunoassay requires sophisticated instrumentation, time-consuming procedures, highly trained personnel and centralized laboratory facilities, which limit their accessibility and suitability for point-of-care applications.^{3,4}

Portable biosensors have emerged as powerful alternatives to bridge this gap, enabling bioanalysis at the point-of-care, such as hospitals among other medical facilities, without the need to send samples to specialized laboratories.⁵ These systems offer rapid results (within minutes) and do not require sophisticated instrumentation, accelerating medical decision-making processes.^{5,6} Furthermore, the integration of data analysis methods, such as machine learning and image analysis,

enhances the accuracy of readouts, delivering reliable results quickly.⁷

Among portable biosensing formats, paper-based biosensors have attracted special attention due to their affordability, disposability, and (bio)compatibility with small sample volumes.⁸ In this field, lateral flow assays (LFAs) represent the most successful commercial platform, popularized by over-the-counter pregnancy test and, more recently, rapid antigen test for COVID-19 and other infectious diseases such as malaria and HIV.^{9,10} Their success lies in their simplicity, low-cost production, user friendly operation, and ability to deliver rapid results without laboratory infrastructure, positioning LFAs as ideal point-of-care diagnostic tools.¹¹

Despite these advantages, conventional LFAs rely predominantly on colorimetric labels, most commonly gold nanoparticles, which limit sensitivity and often provide only qualitative or semi-quantitative results.¹² In response, fluorescent nanomaterials, such as quantum dots, upconverting nanoparticles, and organic dyes have been employed to achieve higher sensitivity and semi-quantitative readouts.^{13–15} However, the search for luminescent probes that combine capture and detection of the analyte in tandem, high signal stability, low toxicity, and facile integration into solid supports remains ongoing.

In this context, silver clusters confined within zeolites emerge as a promising luminescent material for biosensing.¹⁶ These composites combine the quantum-confined optical properties of few-atom silver clusters such as discrete electronic states and size-dependent emission, tuning their luminescence through the visible to near-infrared region. Besides, these

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composites offer exceptionally large Stokes shifts with the structural and chemical robustness of zeolite frameworks. The rigid, porous environment of the zeolite not only stabilizes the clusters against aggregation and photobleaching but also ensures reproducible emission properties and long-term photostability.^{17,18}

A unique feature of these composites is their environmental responsiveness, observed by tuning their emission intensity and wavelength in response to changes in their surrounding media.^{17,19} This property has enabled their usage as sensitive probes for humidity,²⁰ oxygen²¹ and volatile amines²² analysis. Moreover, zeolite surfaces and pores can be further functionalized with biomolecules, endowing the composites with specific recognition capabilities.²³ By combining intrinsic luminescence with tunable surface chemistry, silver cluster-zeolite composites emerge as multifunctional sensing platforms capable of both analyte recognition and optical signal transduction in tandem. Existing literature provides a critical overview on how these composites are contributing to the advancement of the measurement science.²⁴

The biosensing potential of these composites has been demonstrated by functionalizing colloidal silver cluster-zeolite composites with antibodies.²⁵ Immunocomplex formation was shown to modulate the luminescence of the composites with high specificity, effectively converting molecular recognition into measurable optical signals. This proof-of-concept highlighted the versatility of silver-cluster zeolite composites for molecular recognition and opened opportunities for their translation into portable biosensing platforms.

Herein, we developed a portable luminescent biosensor based on antibody-functionalized silver cluster-zeolite composites immobilized within lateral flow strips and coupled to smartphone-based fluorescence readout. Control zone consisted of silver cluster-zeolite composites without the recognition antibody and blocked with bovine serum albumin (BSA) and casein to provide an internal fluorescence reference. Unlike conventional fluorescent lateral flow assays, the proposed platform eliminates the need for a conjugate pad by enabling the antibody-functionalized silver cluster-zeolite composites to simultaneously perform analyte recognition and optical signal transduction.

Results and discussion

Immobilizing silver cluster-zeolite composites into paper-based substrates represents an attractive strategy for the development of robust and portable biosensors, as paper-based devices enhance material stability, minimize aggregation, and facilitate device fabrication in scalable formats.²⁶ Among the available paper-based supports, nitrocellulose membranes are particularly suitable due to their high protein-binding capacity and well-controlled capillary flow properties.^{1,27} The Experimental section (reagents, materials, procedures and equipment) of our overall approach is described in the SI. The successful incorporation of silver cluster-zeolite composites into nitrocellulose-based biosensing platforms was confirmed by scanning electron microscopy (SEM). As shown in Fig. 1a, pristine nitrocellulose membranes exhibit a characteristic fibrous network composed of cellulose

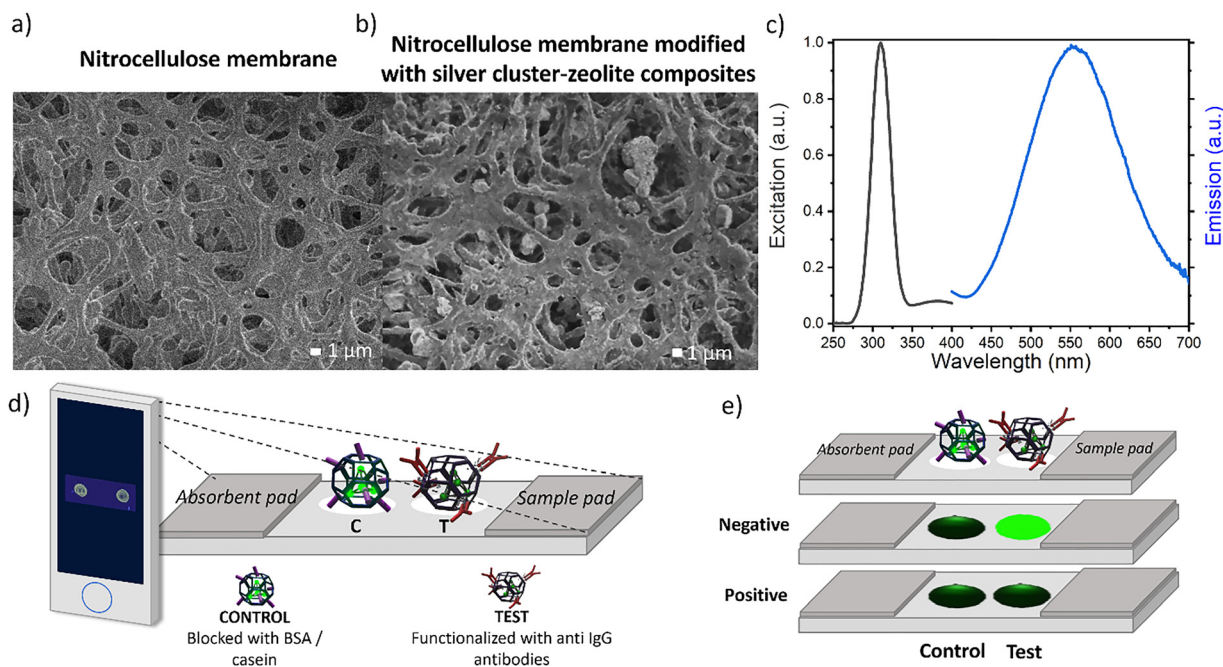


Fig. 1 Characterization and operational principle of the proposed device. SEM micrography of pristine nitrocellulose membrane (a) and nitrocellulose membrane modified with silver clusters embedded in faujasite X zeolites (b). Excitation and emission spectra of silver cluster embedded within zeolite composites deposited onto the lateral flow nitrocellulose membrane (c). Schematic representation of the smartphone-based portable fluorescent readout (d) and the negative and positive results (e).

nitrate fibers, forming a porous structure with interconnected channels that support capillary-driven flow. After deposition (Fig. 1b), the composites were observed within the fibrous matrix, displaying a semi-homogeneous distribution across multiple depths of the membrane. This penetration into the porous structure promotes stable immobilization, while deposition along and between the nitrocellulose fibers provides mechanical entrapment that minimizes particle displacement during sample flow. In addition, the intrinsic protein-binding affinity of nitrocellulose facilitates passive adsorption of the protein-decorated zeolite composites, further enhancing their retention and stability within the membrane. Compared to colloidal suspensions, where silver cluster-zeolite composites may sediment or aggregate over time, immobilization within nitrocellulose confers improved robustness and positional stability, which are critical for reliable performance during lateral flow operation.

Complementary energy-dispersive X-ray spectroscopy (EDS) analysis further confirmed that silver clusters remained confined within the zeolite framework after immobilization (Fig. S2 in the SI). The absence of signals corresponding to bulk silver nanoparticles indicates that the clusters preserved their encapsulated, subnanometric nature, as the unique luminescent properties of silver clusters arise from their dimensions and defined electronic states, which would be lost upon nanoparticle growth.

Lateral flow strips were fabricated using cellulose as the sample and absorbent pads and nitrocellulose as the test membrane. Unlike conventional LFAs, where pre-labeled particles migrate with the sample and are captured at the test line, the proposed device does not require a conjugate pad because the silver cluster-zeolite composites act simultaneously as the recognition and signal transduction elements. The test zone

was prepared with silver cluster-zeolite composites functionalized with anti-human IgG antibodies, whereas the control zone contained silver cluster-zeolite composites without the recognition antibody and subsequently blocked with bovine serum albumin (BSA) and casein (Fig. 1d). Consequently, specific immunocomplex formation cannot occur in the control zone. Rather than operating as a conventional control zone, this zone serves as an internal fluorescence reference whose reference signal enables normalization of the test response, compensating for variations associated with membrane wetting, composite deposition, and image acquisition. This design allows fluorescence changes observed in the test zone to be predominantly attributed to specific immunocomplex formation.

The compatibility of paper-based platforms with smartphone imaging was exploited for signal readout. As shown in the excitation and emission spectra of Fig. 1c, the silver cluster-zeolite composites deposited on the nitrocellulose membrane exhibit a characteristic luminescent emission band centered at 550 nm, with optimal excitation at 310 nm. These results confirm that the luminescent properties of the composites are preserved after immobilization onto the paper substrate. Although the handheld UV lamp used in this work is nominally specified as a 254 nm mercury lamp, its emission spectrum includes a characteristic mercury emission band near 310 nm that overlaps with the excitation band of the composites, see Fig. S3 in the SI. Therefore, the lateral flow strips could be efficiently excited by this type of UV illumination, and fluorescence images were captured with a smartphone camera under controlled conditions. Images were acquired before sample addition and after 10 and 20 minutes of analyte interaction, enabling direct comparison of luminescence variations. Because silver cluster-zeolite composites exhibit measurable fluorescence prior to analyte exposure, the analyte

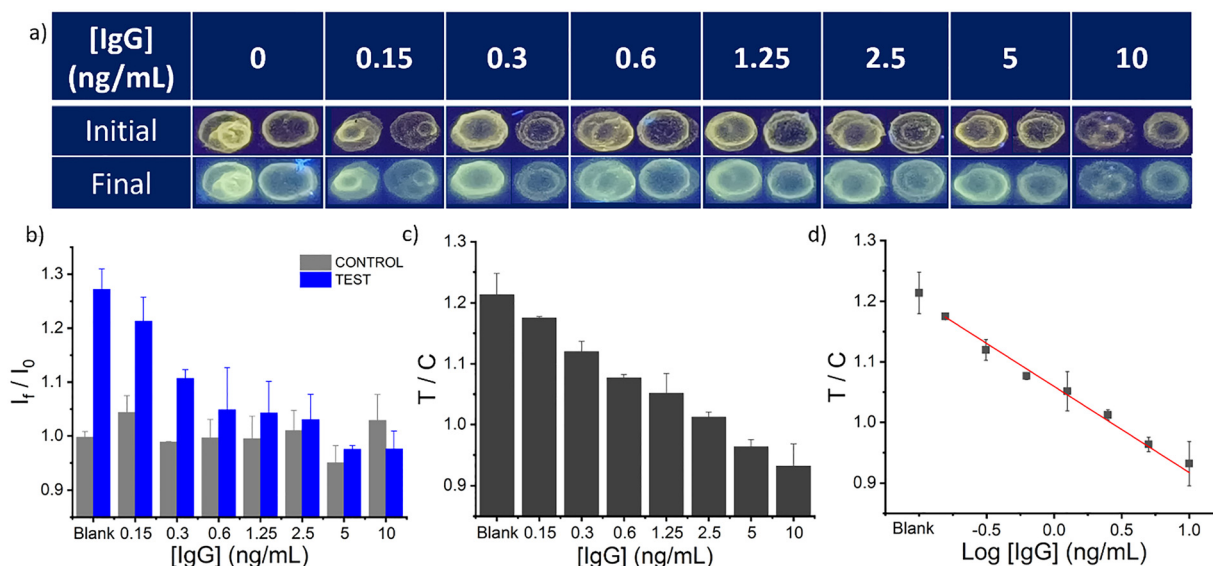


Fig. 2 Analytical performance of the proposed single-antibody lateral flow immunoassay targeting human IgG as a model analyte. Smartphone images of the Control (left side) and Test (right side) (a). Intensity of the control and test signals (b). Normalized test/control ratio (c). The resulting Calibration curve (d). Error bars represent the standard deviation of three independent measurements ($n = 3$). The blank sample is shown for comparison but was not included in the linear regression used for calibration.

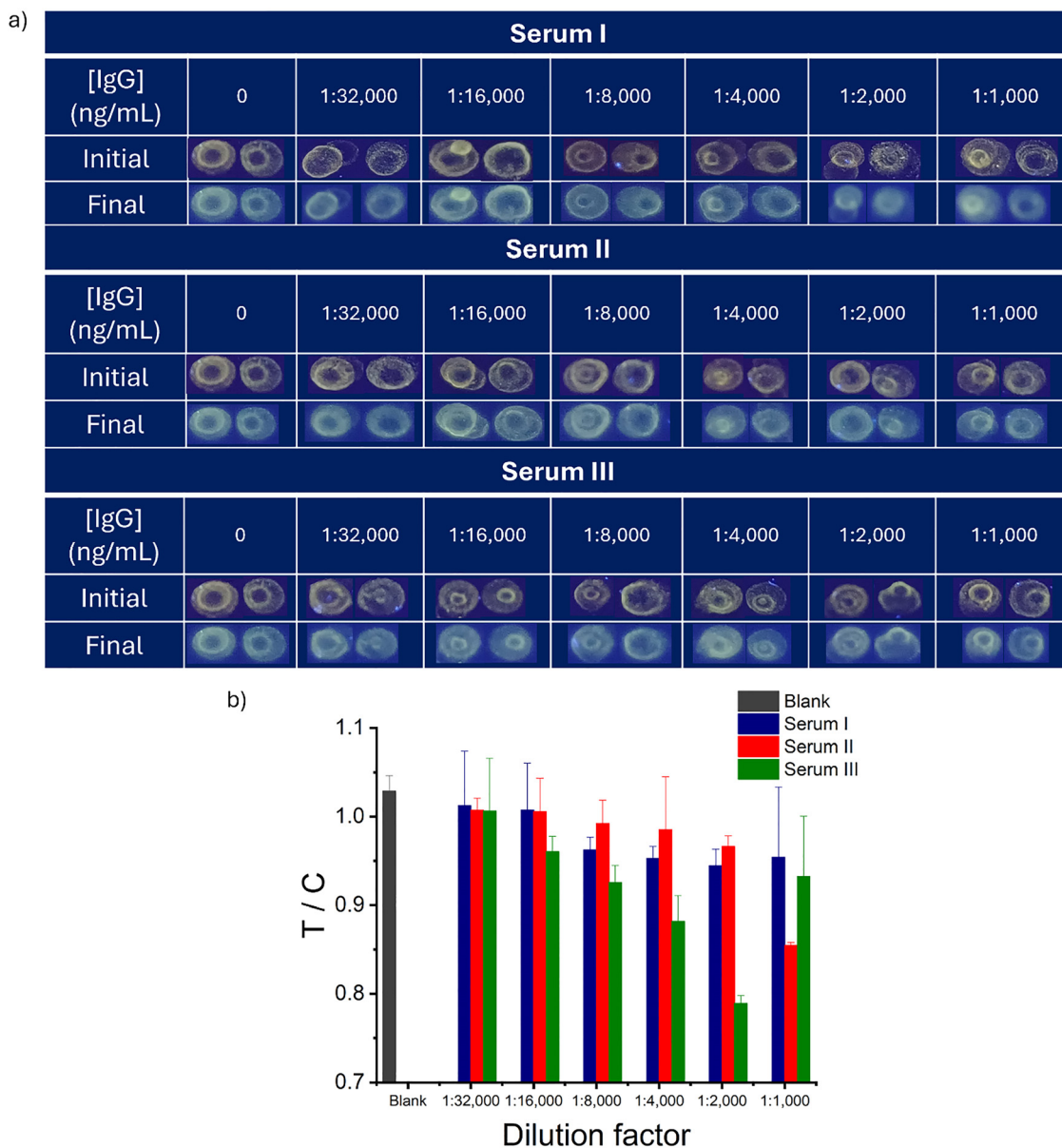


Fig. 3 Serum analysis from three volunteers *via* the resulting single-antibody lateral flow immunoassay. Smartphone images of the Control (left side) and Test (right side) (a). Normalized test/control ratio (b). Error bars represent the standard deviation of three independent measurements ($n = 3$).

recognition was reflected as changes in fluorescence intensity rather than the appearance of a visually distinguishable fluorescent band. Therefore, quantitative discrimination between positive and negative samples requires smartphone image acquisition and digital image analysis. The recorded images were subsequently processed using a custom image-analysis code that automatically identified the test and control zones and extracted their corresponding pixel intensities, see the respective workflow in Fig. S1 in the SI.

Upon analyte exposure, an increase in emission intensity was initially observed in both test and control zones, primarily associated with membrane wetting effects, see Fig. 2a. Consequently, background subtraction and signal normalization were required to isolate analyte-specific responses. Following

background correction, fluorescence intensities from the test zone were normalized against the corresponding control zone intensities in both the initial and final images. The analytical response was then calculated by comparing the resulting test-to-control ratios before and after sample addition, as described in eqn (1).

$$\frac{T}{C} = \frac{\frac{I_{\text{Final test}}}{I_{\text{Final control}}}}{\frac{I_{\text{Initial test}}}{I_{\text{Initial control}}}} \quad (1)$$

Silver cluster-zeolite composites are highly sensitive to changes in their electronic environment, a property that underpins their sensing performance but may also introduce

nonspecific signal variations due to close particle proximity and nonspecific interactions. To mitigate these effects, the influence of the PBS eluent medium applied after sample flow was evaluated. Devices operated without the PBS eluent medium exhibited nonspecific luminescence change in both test and control zones, likely arising from transient electronic perturbations induced by interfering species present in the sample (Fig. S4 and Table S3 in the SI). In contrast, the application of the PBS eluent medium stabilized the luminescence response of the control zone while preserving analyte-specific modulation in the test zone, resulting in improved signal reliability, see Fig. 2a and b.

The effect of analyte incubation time was subsequently investigated by comparing reaction times of 10 and 20 minutes (Fig. 2a, b and Fig. S5, Table S4 in the SI). Although luminescence changes were detectable after 10 minutes, the response exhibited higher variability and lacked a clear concentration-dependent trend. Extending the sample incubation time to 20 minutes resulted in a consistent decrease in emission intensity with increasing analyte concentration, yielding improved correlation and reduced signal dispersion, see Table S4.

Calibration curves representing the normalized intensity ratio (I/C) as a function of the logarithm of the IgG concentration showed a linear response over the range of 0.156–10 ng mL⁻¹ (Fig. 2d), with a slope of -0.1423 , an R^2 value of 0.9908. The linear regression was performed using only the non-zero IgG concentrations. All the measurements were performed by triplicate, yielding an average coefficient of variation as low as 1%, see Table S4 in the SI, showing acceptable repeatability of the assay. The limit of detection (LOD) was calculated from the mean blank response plus three times the standard deviation of the blank and interpolated using the linear calibration equation, yielding a value of 0.4294 ng mL⁻¹. This analytical performance falls within the range reported for fluorescent lateral flow immunoassays employing different luminescent labels and detection strategies.^{28,29} Although some fluorescence-based lateral flow platforms have reported lower detection limits,³⁰ the present approach offers a simplified assay architecture by integrating analyte recognition and optical signal transduction into a single antibody-functionalized silver cluster–zeolite composite, thereby eliminating the need for a conventional conjugate pad while maintaining compatibility with smartphone-based fluorescence imaging.

Specificity was further evaluated in the presence of 0.5 mg mL⁻¹ BSA as an interfering protein. Although matrix effects were observed, the device maintained clear concentration-dependent emission changes (Fig. S6 and Table S5 in the SI), demonstrating resilience against nonspecific protein interference. To further assess real-world applicability, human serum samples from three healthy volunteers were analyzed after serial dilution. As shown in Fig. 3, emission intensity decreased proportionally with IgG concentration across different dilution factors. Together, the interference and serum studies demonstrate that the proposed assay provides specificity and reliable analyte-dependent fluorescence modulation, even in the presence of interfering proteins and complex biological matrices. Although these experiments demonstrate the feasibility of operating the device in diluted human

serum, the determination of absolute IgG concentrations was beyond the scope of the present proof-of-concept study. Future work will focus on quantitative validation through spike-recovery experiments and comparison with established analytical methods such as ELISA.

Overall, the results demonstrate that the immobilization of silver cluster–zeolite composites within paper-based supports preserves their luminescent properties, enables stable biofunctionalization, and allows specific biorecognition events to be transduced into measurable optical sensing signals. The absence of a conventional conjugate pad, combined with the direct functionalization of the composites, simplifies the device design while maintaining sensitivity and selectivity and compatibility with smartphone-based readout.

Conclusion

Silver cluster–zeolite composites were successfully translated from colloidal systems into paper-based lateral flow devices, preserving their luminescent responsiveness and biorecognition capability in a portable format. Direct antibody functionalization enables the composites to act simultaneously as recognition and signal transduction elements, simplifying lateral flow architectures by eliminating the need for conventional conjugate pads.

The resulting luminescent LFA provides a low-cost diagnostic platform ($\approx$ \$0.6 per strip at laboratory scale, see Table S6 in the SI) that is compatible with nitrocellulose membranes and smartphone-based fluorescence readout. This approach positions silver cluster–zeolite composites as versatile luminescent building blocks for scalable, smartphone-readable, and decentralized point-of-care biosensing technologies.

Author contributions

Cecilia García-Guzmán: conceptualization, investigation, formal analysis, data curation, writing – original draft. Roberto García-Guzmán: software, methodology, formal analysis. Amauri Horta-Velázquez: software. Eduardo Coutino-Gonzalez: supervision, writing – review and editing. Eden Morales-Narváez: conceptualization, supervision, funding acquisition, writing – review and editing.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

The data supporting the findings of this study are available within the article and its supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d6qm00338a>.

Additional data are available from the corresponding authors upon reasonable request.

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