Volume 6, Issue 1, 2026 ISSN: 2617-992X
DOI: https://doi.org/10.31058/j.ac.2026.61005

Electrochemiluminescence Biosensing for Neurodegenerative Biomarkers: Design and Translation

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Received: 11 September 2026**; Accepted:** 17 October 2026**; Published:** 3 November 2026

Abstract:

Electrochemiluminescence (ECL) has emerged as a powerful platform for ultrasensitive biomarker detection, but its translation to neurodegenerative disease testing is constrained by a mismatch between analytical sensitivity and clinical reliability. In this Review, we examine ECL biosensing across the full specimen-to-decision pathway, from biomarker definition and molecular recognition to luminophore and framework engineering, interfacial chemistry, microfluidic integration, smartphone imaging and clinical validation. Using α-synuclein and p-tau217 as contrasting examples, we show that molecular heterogeneity, matrix effects and pre-analytical variation can outweigh gains achieved by lower limits of detection (LODs). We further analyse how MOF/COF/HOF confinement, nucleic-acid amplification, magnetic enrichment and device miniaturization reshape not only signal intensity but also transport, background, workflow burden and calibration. These comparisons reveal a recurring trade-off: strategies that maximize photon output or minimize LOD often introduce new sources of uncertainty. We therefore propose a translation-oriented framework that prioritizes matrix robustness, reproducibility, calibrated imaging and clinically interpretable performance. Future progress will depend less on additional amplification than on preserving quantitative fidelity across the complete measurement chain.

Keywords:

Electrochemiluminescence, Neurodegenerative Biomarkers, α-synuclein, p-tau217, Porous Frameworks, Microfluidics, Smartphone Metrology, Point-of-care Testing

1. Introduction

Neurodegenerative biomarkers expose a central challenge for ultrasensitive biosensing: detecting smaller amounts does not necessarily mean measuring disease biology more faithfully. Relevant analytes are often scarce, distributed across biological compartments and present as multiple molecular species. In Parkinson's disease, α-synuclein may be measured as total protein, oligomers, aggregates or modified forms, while blood measurements are highly sensitive to erythrocyte contamination. In Alzheimer's disease, plasma p-tau217 has moved much closer to clinical use, shifting the analytical question from mere detectability toward precision

around validated cutoffs, agreement with reference methods and reproducibility across settings.

ECL is attractive in this context because it couples electrochemical control to low-background optical readout without external optical excitation. Emission can be localized near the electrode and integrated with molecular recognition, porous materials, catalytic chemistry, nucleic-acid circuits, magnetic enrichment and microfluidic transport. The same surface-confined emission also supports spatial imaging, enabling a transition from photomultiplier-based measurements to camera- and smartphone-based readout.

However, recent reviews have largely examined MOF-enhanced ECL, ECL imaging, smartphone-coupled CL/ECL and neurodegenerative biosensing as separate topics [1,2,3]. This leaves a more consequential question unresolved: how faithfully is molecular information preserved as it moves from specimen selection and target recognition to ECL generation, device handling, image acquisition and clinical interpretation?

This systems-level question matters because optimization at one layer can create failure at another. Higher luminophore loading can intensify emission while worsening self-quenching or transport; nucleic-acid cascades can lower the nominal LOD while adding leakage and timed enzymatic steps; magnetic enrichment changes spatial kinetics; and smartphone readout removes dedicated optics but introduces camera processing and device variability. The most sensitive assay can therefore remain biologically or clinically uninformative if target identity, matrix effects or calibration are inadequately controlled.

Here, we organize the field through a mechanism–design–translation framework (Figure 1). We first define the biological and matrix constraints, then examine reaction-zone chemistry, framework-engineered luminophores, molecular recognition and amplification, interfacial design, microfluidic integration and smartphone metrology, and finally benchmark these components against analytical and clinical evidence. Representative studies are used to extract general design rules rather than to catalogue every reported sensor; methodologically informative examples from adjacent biomarker fields are included when they clarify transferable principles. The central premise is that translation depends on preserving analytical fidelity across the complete specimen-to-decision chain.

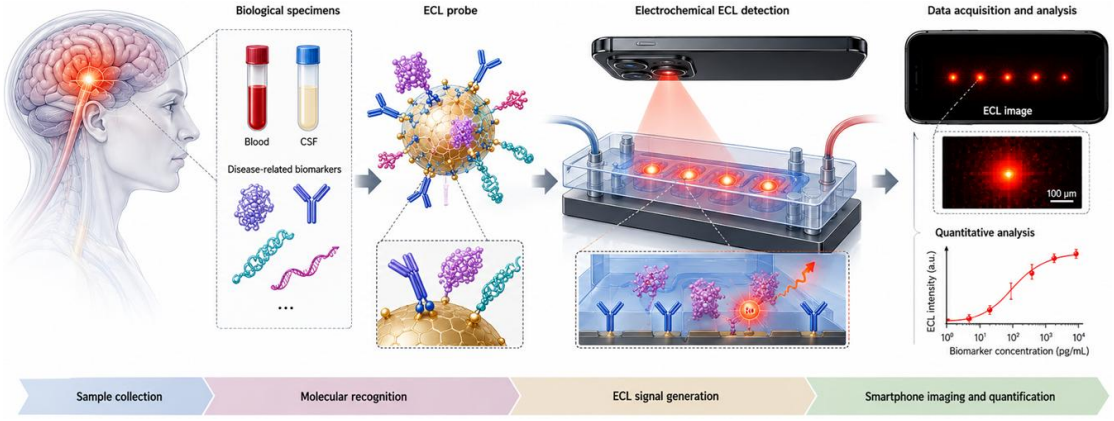

Figure 1. *From biological specimen to clinical decision in ECL biosensing for neurodegenerative-disease biomarkers. The proposed review framework follows the analytical-information chain from clinical need and specimen selection through molecular recognition, ECL signal generation, device-based imaging, and quantitative interpretation.*

2. Biomarkers and Biological Matrices: Defining What Is Measured

Analytical sensitivity is meaningful only after the molecular species, specimen and pre-analytical confounders have been defined.

Neurodegenerative biomarkers differ from many conventional analytes because disease relevance often depends on molecular form rather than total protein abundance. This is particularly evident for α-synuclein (α-syn), which exists as monomeric, oligomeric, aggregated and post-translationally modified species. ECL studies have already targeted both total and oligomeric α-syn, yet these analytes should not be treated as interchangeable. Recognition elements must therefore be described by the species they bind, and analytical performance should be interpreted in relation to that species rather than reported generically as "α-syn detection".

Blood α-syn also illustrates why the matrix is part of the analytical problem. A 2025 targeted mass-spectrometry study found that more than 99% of blood α-syn is associated with erythrocytes and showed that erythrocytic α-syn, although elevated in idiopathic Parkinson's disease, provided only modest stand-alone discrimination (AUC approximately 0.62) [4]. Because erythrocytes contain orders of magnitude more α-syn than plasma, hemolysis can strongly bias plasma or serum measurements. Consequently, a highly sensitive ECL platform can still produce biologically misleading results if red-cell contamination, sample processing and freeze–thaw history are not controlled.

For Alzheimer's disease, phosphorylated tau has a different translational profile. Plasma p-tau217 has now undergone extensive analytical and clinical validation, including studies in independent clinical cohorts and comparisons with PET or CSF reference standards. Recent work has also shown that plasma and serum can yield comparable classification performance for several p-tau217 assays, although matrix-specific calibration remains important [5]. A 2025 multicohort study evaluated plasma p-tau217 with predefined cutoffs in 1,767 symptomatic participants across primary- and secondary-care cohorts, illustrating the level of clinical evidence against which emerging ECL assays should ultimately be benchmarked [29]. For p-tau217, therefore, the key analytical question is no longer merely whether sub-pg/mL concentrations can be detected, but whether the assay is precise around clinically meaningful decision thresholds and agrees with validated reference methods.

Amyloid-β markers, particularly Aβ42 and the Aβ42/Aβ40 relationship, remain relevant comparators because they represent an upstream component of Alzheimer's pathology. However, their clinical interpretation is increasingly integrated with tau and other markers rather than treated as a single isolated concentration. This reinforces a broader point for multiplex ECL design: combining biomarkers is useful only when the biological interpretation of each marker and the statistical rule for combining them are prespecified.

Neurofilament light chain (NfL) provides a complementary example. NfL is a sensitive body-fluid marker of neuroaxonal injury and has growing value for prognosis, disease activity and treatment monitoring across multiple neurological disorders. Its limitation is disease specificity: increased NfL does not uniquely identify Parkinson's or Alzheimer's disease. Age, body composition and other physiological or disease-related factors also influence interpretation, and cross-platform standardization remains an important translational requirement [6].

These examples support a matrix-first reporting rule for ECL biosensors. Each study should specify: (i) exact biomarker species; (ii) specimen type and processing; (iii) dilution and pretreatment; (iv) known matrix confounders; and (v) the biological or clinical reference against which the signal is interpreted. “Detection in serum” should not be used as a surrogate for clinical validation. Accordingly, an ultrasensitive assay should target a molecular species that can be measured reproducibly in a defined matrix and linked to a meaningful biological or clinical interpretation.

3. ECL Fundamentals: the Reaction–transport Basis of Sensitivity

ECL sensitivity is a property of the coupled electrochemical–photophysical reaction zone, not of emitter brightness alone.

Electrochemiluminescence generates electronically excited luminophores through electrode-driven redox chemistry rather than optical excitation. Two mechanistic classes are particularly important for biosensing: annihilation ECL, in which electrochemically generated oxidized and reduced species react to form an excited state, and coreactant ECL, in which a luminophore and sacrificial coreactant generate highly reactive intermediates that undergo subsequent electron-transfer reactions. Classical Ru(bpy)32+ chemistry remains a central model for both mechanistic understanding and analytical implementation [7].

For practical biosensors, coreactant ECL is especially important because emission is generated within a finite reaction zone near the electrode. The thickness and efficiency of this zone depend on electron-transfer rates, coreactant oxidation or reduction, radical lifetime, diffusion and the spatial position of the luminophore. Mechanistic work by Zanut and co-workers demonstrated that improved understanding and control of coreactant pathways can directly enhance bioanalytical performance [8]. This is why porous frameworks, catalytic nodes and magnetic localization can increase ECL without changing the intrinsic molecular identity of the emitter: they modify the probability that the required reactive species meet within the active region.

Three distinct quantities should therefore be separated. ECL intensity is the measured optical output under specified conditions; ECL efficiency reflects how effectively electrochemical input generates photons; and analytical sensitivity describes how strongly the measured signal changes with analyte concentration. These quantities can move in different directions. A brighter emitter may increase baseline intensity but provide little analytical gain if target binding changes only a small fraction of the active interface.

Mass transport provides another key constraint. Coreactant availability, pore diffusion and access of target-bound labels to the electrode can become rate-limiting, particularly in microfluidic or highly porous architectures. Similarly, radical-generating catalysts may strengthen ECL but also increase nonspecific background if reactive intermediates are produced independently of target binding. The analytically useful signal is therefore a target-dependent perturbation of a reaction–transport system, not simply the maximum attainable luminescence.

For the remainder of this review, ECL design is consequently analyzed through four coupled variables: excited-state generation, interfacial electron transfer, radical/coreactant transport and photon collection. This framework is sufficient to interpret the effects of Ru-MOF confinement, catalytic amplification, magnetic

enrichment, microfluidic flow and smartphone imaging without reproducing a general ECL textbook. The practical objective is to maximize the fraction of ECL that is both chemically efficient and specifically modulated by target recognition. (Figure 2)

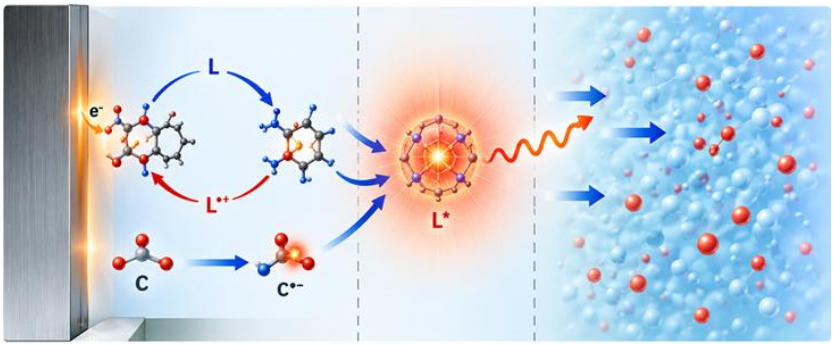

Figure 2. *Fundamental reaction–transport processes governing Ru(II)-based ECL. At the electrode interface, Ru(II) luminophores and coreactants undergo electrochemical reactions that generate reactive intermediates and the excited Ru(II)* state. The measured ECL response depends on electron transfer, radical lifetime, diffusion, and the spatial overlap of the reaction and diffusion layers.*

4. Framework-engineered Luminophores: Controlling the ECL Reaction Space

Porous frameworks can enhance ECL by reorganizing the reaction space—not simply by increasing luminophore loading.

Ruthenium(II) polypyridyl complexes remain among the most established ECL luminophores because of their reversible redox chemistry and well-defined excited-state emission. Their direct use in biosensing, however, creates recurring constraints, including emitter loss from the interface, uncontrolled intermolecular association and limited control over the local reaction environment. Incorporating Ru-based luminophores into metal–organic frameworks (MOFs) addresses these limitations by converting molecular emitters into spatially organized architectures. Early 2D Ru-MOF nanosheets built from Ru(dcbpy)32+ demonstrated that the luminophore itself can function as a framework ligand, providing high local Ru content while reducing emitter leakage [9]. Later Ru-MOF systems retained this principle but increasingly separated three functions: emitter immobilization, interfacial electron transfer and biological recognition.

Higher luminophore loading does not necessarily yield a proportionally larger analytical signal. In a 2D hexagonal Ru-MOF immunosensor, the framework increased $Ru(dcbpy)_3^{2+}$ loading and limited luminophore leakage, whereas a separate MoS_2@GO layer was introduced to improve electron transfer and antigen loading [10]. The architecture illustrates a general rule: signal generation and interfacial transport can be limited at different physical locations. Framework design should therefore be evaluated together with the electrode interface rather than assuming that an emitter-rich material also solves charge-transfer limitations.

Self-luminous frameworks illustrate a second principle: structural rigidity and pore architecture can alter the photophysical and chemical efficiency of ECL itself. RuIn-MOF, formed by coordinating $Ru(dcbpy)_3^{2+}$ with In^{3+}, combines high chromophore incorporation with a rigid three-dimensional porous structure. The reported ECL enhancement was attributed not only to luminophore loading but also to increased structural rigidity and enrichment of the coreactant tripropylamine within the pores

[11]. Here, the MOF functions simultaneously as an emitter scaffold and a local reaction environment, shifting the design concept from passive carrier to engineered reaction space.

The same principle extends beyond MOFs. HOF-14 supports both annihilation and coreactant-type ECL, and the HOF-14/triethylamine system showed a higher ECL quantum efficiency than the corresponding $Ru(bpy)_3^{2+}$/triethylamine reference under the reported conditions [12]. Subsequent confinement of isoluminol within HOF-14 produced a 33-fold ECL increase relative to the unconstrained isoluminol/H_2O_2 system; the proposed mechanism combined restricted radical diffusion with a higher effective collision probability, while an added nanozyme generated additional reactive oxygen species [13]. A related 2026 COF@luminol system attributed a 60.14-fold gain in ECL efficiency to nanoconfinement, followed by a further 6.6-fold signal enhancement after electrocatalytic regulation of H_2O_2-derived reactive species [14]. Together, these studies show that porous frameworks can enhance ECL by controlling where reactive intermediates are generated, how far they diffuse and how often they encounter the emitter.

A third design axis is the competition between radiative and nonradiative excited-state decay. β-Keto-enamine covalent organic frameworks (COFs) illustrate this shift in emphasis: a planar donor–acceptor COF showed stronger ECL than a less favorable reference framework, with the improvement attributed to enhanced charge transfer and suppression of nonradiative decay by a rigid planar keto-enamine structure [15]. The broader implication is that ECL materials should not be judged only by how many excited states are generated, but also by how efficiently those states undergo photon-emitting relaxation. Excited-state utilization should therefore be analysed alongside radical generation and electron-transfer kinetics.

For biological samples, materials design must also address interface stability. A 2025 Zn/Ru-MOF study combined coordination-induced ECL enhancement with a zwitterionic antifouling modification. The framework promoted $Ru(dcbpy)_3^{2+}$ aggregation in a controlled coordination environment, suppressed nonradiative relaxation and was proposed to support energy transfer involving Zn^{2+} and Ru centers; the surface modification reduced nonspecific adsorption in serum [16]. Although the target was α-fetoprotein rather than a neurodegenerative biomarker, the transferable principle is clear: a bright luminophore has limited practical value if its signal is unstable in a protein-rich matrix.

These observations support a more useful classification of framework ECL materials than grouping them simply as MOFs, COFs or HOFs. Framework engineering can instead be organized by the physical variable it controls: (i) emitter immobilization and spacing; (ii) conformational rigidity and nonradiative decay; (iii) coreactant and radical transport; (iv) electrocatalytic generation of reactive intermediates; (v) interfacial electron transfer; and (vi) surface accessibility and antifouling. This mechanism-based classification also exposes the main trade-offs. Increasing emitter density may eventually promote self-quenching or impede transport; stronger confinement can increase local collision probability but slow molecular exchange; highly multicomponent architectures may improve signal intensity while compromising batch reproducibility and manufacturing simplicity. For neurodegenerative assays, framework performance should therefore be judged by stable photon generation, reproducible transport, accessible bioconjugation and matrix compatibility—not by buffer intensity alone. (Figure 3)

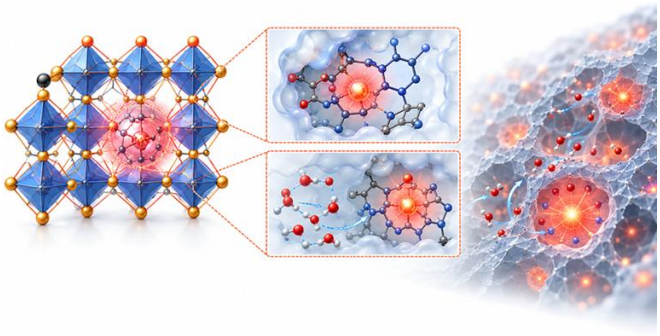

Figure 3. *Framework-engineering mechanisms for high-performance ECL luminophores. Ru-containing porous frameworks can enhance ECL by restricting luminophore motion, organizing emitter spacing, regulating solvent and coreactant access, facilitating charge transport, and stabilizing the emissive environment. These functions should be balanced against synthetic complexity, accessibility of recognition sites, and matrix compatibility.*

5. Molecular Recognition and Amplification: Analytical Gain Versus Workflow Burden

Recognition and amplification should be optimized together because signal gain is useful only when molecular specificity and workflow reliability are preserved.

For neurodegenerative biomarkers, molecular recognition is unusually consequential because the clinically relevant analyte is often a specific molecular species rather than the total abundance of a protein family. α-Synuclein may be measured as total, oligomeric, aggregated or modified forms, while tau assays increasingly distinguish phosphorylation sites such as p-tau181 and p-tau217. Antibodies remain attractive where validated epitopes and clinical comparators already exist, whereas aptamers provide compact, sequence-defined recognition elements that are readily integrated with nucleic-acid signal conversion. Their analytical roles are therefore different: antibodies often connect more naturally to clinically established protein assays, while aptamers facilitate programmable signal amplification and structure-switching designs.

A simple recognition event can itself generate an ECL response without a large amplification network. The p-tau217 immunosensor reported in 2024 provides a useful example: antigen binding increased steric hindrance at an antibody-modified gold electrode and reduced access of $Ru@SiO_2$ ECL indicators to the interface, enabling serum quantification over 1 pg/mL–100 ng/mL with a reported LOD of 0.178 pg/mL [18]. Such an architecture is analytically less spectacular than multi-stage nucleic-acid amplification, but it converts target binding directly into an interfacial transport change and minimizes biochemical processing. For biomarkers whose clinically relevant concentration range is already accessible, this type of direct transduction may offer greater translational value than further lowering the LOD.

Aptamer-based α-synuclein ECL illustrates the opposite end of the design space. Early MOF/aptamer systems already reached sub-femtomolar detection [17], and later nanocomposite and photodynamic strategies pushed sensitivity further. In 2025, a CRISPR/Cas13a ECL assay coupled structure-switching aptamer recognition to T7 transcription and EXPAR, reaching a reported LOD of 1.025 aM in diluted human serum [19]. The analytical gain is substantial, but it is achieved through multiple enzymatic conversions and an assay lasting approximately two hours. A related Cas13a/HCR strategy similarly converts α-syn oligomer recognition into a large

nucleic-acid output. These systems demonstrate that modern ECL can detect vanishingly small amounts of target, but they also expose a central trade-off: molecular amplification can reduce concentration uncertainty while increasing operational uncertainty.

DNA circuits and DNA walkers occupy an intermediate position. Catalytic hairpin assembly, hybridization chain reaction and DNAzyme walkers can provide enzyme-free or partially enzyme-assisted amplification while preserving sequence programmability. Recent ECL work has improved these systems by controlling track geometry and reducing signal leakage. For example, self-replicating catalytic hybridization assembly of bipedal DNAzyme walkers was designed specifically to deepen amplification while suppressing leakage [20], whereas self-protected and in-situ-generated walker architectures have addressed nuclease degradation or unintended activation. These advances are mechanistically important because they show that the relevant question is no longer merely "how many amplification cycles occur?" but "how much target-dependent signal is gained relative to leakage, nuclease sensitivity and workflow burden?"

Magnetic beads provide a different form of amplification: physical enrichment rather than biochemical multiplication. They can increase local analyte concentration, separate target-bound complexes from matrix components and spatially localize ECL labels near an electrode. Their value is especially high in complex samples because they combine recognition and sample preparation. However, magnetic ECL systems are sensitive to bead–electrode distance, magnetic-field geometry and radical diffusion. Recent microbead-resolved ECL studies have shown [25] that the luminescent reaction zone is spatially structured rather than uniform, supporting the need to optimize bead localization rather than treating magnetic capture as a purely biochemical step.

For this review, amplification strategies are therefore classified by where information gain occurs: (i) direct recognition-to-interface conversion; (ii) molecular replication through HCR/CHA/EXPAR/CRISPR; (iii) mechanical or DNA-machine amplification; and (iv) physical enrichment by magnetic particles. Their comparison should include not only LOD but also amplification time, leakage/background, reagent count, temperature control, washing burden, nuclease susceptibility and compatibility with automated cartridges. Once the required concentration range is reached, additional amplification is justified only when the gain in diagnostic reliability exceeds the added processing, failure and calibration burden. (Figure 4)

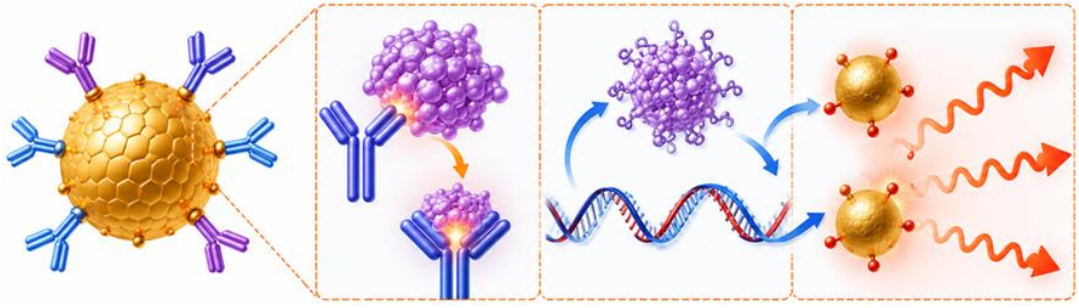

Figure 4. *Coupling molecular recognition with ECL signal amplification. Antibodies and aptamers provide target-selective capture, while particle enrichment and programmable nucleic-acid processes can increase target-dependent signal. A practical architecture must balance selectivity, resistance to nonspecific interference, amplification gain, and assay burden.*

6. Electrode Interfaces: Where Recognition Becomes Signal

At the electrode interface, molecular recognition, electron transfer, radical chemistry and matrix interference converge.

ECL is generated within an electrochemically defined reaction zone near the electrode, making interfacial architecture a primary determinant of analytical performance. Immobilization chemistry must position recognition elements close enough to preserve efficient signal coupling while maintaining sufficient conformational freedom for target binding. Dense probe layers may increase nominal capture capacity but can also introduce steric exclusion and slow mass transfer. DNA nanostructures provide one strategy for controlling this geometry. In a 2023 Ru-MOF immunosensor, tetrahedral DNA probes were used to improve target accessibility while Ru-MOF@AuNPs served as the signal probe [21]. The broader lesson is that ordered probe spacing can be as important as total probe density.

Interfacial steric effects can also be used deliberately as the transduction mechanism. The p-tau217 sensor discussed above converts antibody–antigen binding into reduced access of Ru@SiO_2 particles to the electrode. This design avoids an additional labeled secondary antibody and illustrates a useful principle: target-induced obstruction can be analytically productive when the ECL reporter is transport-limited in a controlled way. Conversely, uncontrolled obstruction from serum proteins is detrimental, making antifouling one of the most important interface requirements for clinical matrices.

Recent ECL work increasingly integrates signal generation and antifouling in the same interface. A 2025 Zn/Ru-MOF system combined coordination-enhanced ECL with a zwitterionic surface designed to resist nonspecific adsorption in serum [16]. These approaches demonstrate that antifouling should not be treated as a late-stage validation coating. It changes local hydration, target accessibility, electron transfer and nonspecific signal simultaneously.

Low-potential ECL is analytically attractive because it can reduce electrochemical side reactions and cross-talk in complex media. This principle is transferable to serum and plasma sensing, where lowering electrochemical demand can reduce the burden placed on antifouling and selectivity mechanisms.

For practical biosensors, four interface variables should therefore be reported separately: recognition-site density/accessibility, nonspecific adsorption, interfacial electron-transfer resistance and batch-to-batch fabrication variability. A sensor optimized only by selecting the modification sequence that gives the highest ECL intensity may inadvertently favor an unstable or irreproducible interface. In particular, multilayer architectures involving nanoparticles, polymers, DNA assemblies and antibodies should be evaluated across electrode batches rather than only through repeated scans of the same electrode. The practical target is therefore a minimally sufficient, reproducible interface rather than a maximally modified one.

7. Microfluidic And Device Integration: Toward Sample-to-Answer Testing

Miniaturization is translationally useful only when integration reduces user operations while controlling transport, excitation and readout.

Moving ECL from a benchtop analyzer to point-of-care testing requires simultaneous control of electrochemistry, fluid handling and photon detection. Simply

placing a modified electrode inside a small housing does not achieve this goal. The device must define sample volume, reagent delivery, washing, electrical excitation and optical geometry while minimizing operator-dependent steps.

Integrated microfluidics provides a direct way to control these variables. A 2023 microfluidic ECL device for heart-type fatty-acid-binding protein combined an ITO-based immunoassay, microfluidic liquid handling, integrated voltage supply and silicon-photomultiplier detection in a compact instrument. Importantly, it measured clinical serum samples without pretreatment and produced results consistent with ELISA [22]. Although the biomarker is cardiovascular rather than neurological, the study establishes a translational benchmark: integration should be evaluated by performance in authentic samples and comparison with a reference method, not only by device dimensions.

Microfluidics can also alter the ECL reaction itself. Alternating-current electroosmotic flow has been used to accelerate transport in an ECL microfluidic POCT platform for proteins and miRNAs in unprocessed samples [23]. Likewise, recent experiments with functionalized microbeads trapped in microfluidic channels showed that confinement and convective transport can enhance bead-associated ECL [25]. These studies support a broader principle: channel geometry and flow are part of the reaction mechanism because they determine analyte capture, coreactant delivery and the distance between labeled particles and the electrode.

The most clinically relevant direction is automation. Kumar and Goel reported a fully automated standalone microfluidic ECL platform for sample-to-answer detection of diabetes-related markers in 2025 [24]. The important advance is not simply smaller instrumentation, but the reduction of manual fluid-handling operations. For neurodegenerative biomarkers, this distinction is critical because multistep aptamer, magnetic-bead or nucleic-acid amplification protocols can lose their analytical advantage if users must perform repeated timed incubations and washes.

Power delivery and optical detection are also being decentralized. A 2024 wireless ECL system used electromagnetic induction with printed electrodes and smartphone-based signal quantification, and demonstrated operation in human serum and artificial sweat [26]. A 2025 high-throughput platform combined a position-resolved ITO chip with a smartphone analyzer and cloud computing [27]. These systems demonstrate that a portable ECL device can distribute functions across the disposable chip, phone and remote computation. The trade-off is that wireless coupling, cloud processing and phone-specific imaging each become additional sources of measurement uncertainty.

For the present review, device maturity is therefore judged by five questions: How many user operations remain? How is fluid transport controlled? How reproducible is electrical excitation? How is the optical path standardized? Can the device be fabricated and calibrated across independent cartridges? These criteria distinguish a compact laboratory prototype from a credible point-of-care architecture. A credible point-of-care architecture is defined by reproducible automation and controlled transport, excitation and readout—not by size alone. (Figure 5)

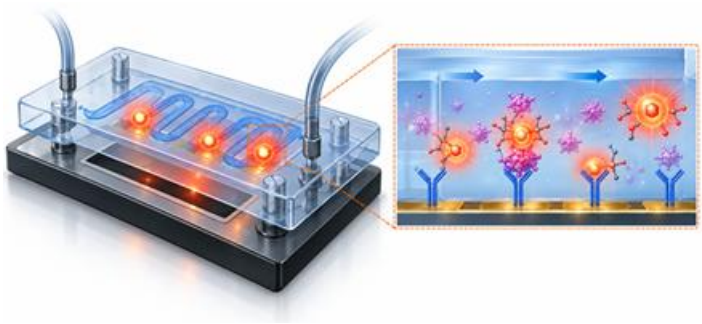

Figure 5. *Integrated microfluidic ECL architecture for controlled sample handling and signal generation. Microchannels guide analytes and coreactants through the detection chamber, where surface-bound recognition complexes localize Ru-based emitters near the working electrode. Controlled flow shortens transport distances and supports low-volume, potentially automated sample-to-answer detection.*

8. Smartphone Imaging As Quantitative Metrology

A smartphone is a quantitative ECL detector only when acquisition and image processing are controlled and calibrated.

Smartphones reduce the hardware barrier for ECL detection and have already been integrated with 3D-printed cells, wireless platforms, machine-learning interfaces and multiplex chips. Their main analytical limitation is that the reported pixel intensity is not a direct measurement of incident photon flux. Exposure time, sensor gain, optical distance, focus, automatic image enhancement, gamma encoding and JPEG compression can all alter the calibration slope.

A 2026 Biosensors and Bioelectronics study directly demonstrated that unconstrained acquisition and encoded image processing distort smartphone ECL quantification, whereas fixed settings and linearized RAW-based analysis more faithfully preserve the relation between emitted light and digital response [28]. The implication is fundamental: image processing belongs inside the transduction model, not after it.

For quantitative ECL, exposure and gain should be fixed across standards and unknowns, saturation must be avoided, and the optical geometry should remain constant. RAW data are preferable when available because they retain sensor-proximal information; JPEG values require characterization of gamma and compression effects. The analysis metric should also be specified. Mean ROI intensity, peak intensity and integrated signal are not interchangeable, and background subtraction becomes particularly important when light is spatially heterogeneous.

Inter-device variability is the next barrier. A calibration generated on one phone model cannot automatically be transferred to another because spectral response, optics and computational processing differ. Internal optical standards, ratiometric readouts or device-specific calibration may reduce this bias, but their performance must be demonstrated experimentally. The minimum reporting set for smartphone ECL should therefore include phone model, exposure/gain, image format, optical geometry, ROI rule, background correction, calibration function and, when translation is claimed, an inter-device reproducibility test. Accordingly, “smartphone-readable” should denote a validated metrological workflow rather than the ability to photograph ECL emission. (Figure 6)

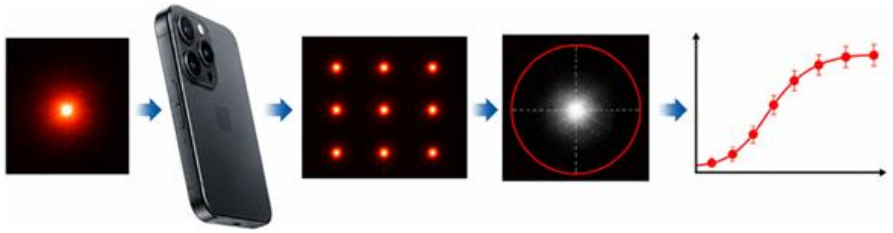

Figure 6. *Smartphone ECL metrology from photon emission to reported concentration. Quantitative smartphone readout requires control of the complete imaging chain, including photon capture, camera response, image processing, region-of-interest selection, background correction, and calibration.*

9. Benchmarking Analytical and Clinical Performance

Clinical value depends on reproducibility in representative specimens, not on the lowest concentration detectable in optimized buffer.

LOD remains useful for demonstrating analytical capability, but it becomes misleading when used as the primary ranking metric. Recent α-synuclein ECL assays range from sub-femtomolar aptasensors to attomolar CRISPR-coupled systems, yet these methods differ substantially in sample dilution, enzymatic steps, total assay time and device requirements. A lower LOD therefore does not necessarily represent a more deployable assay.

Evidence should instead be separated into four levels: buffer proof-of-concept, spiked matrix, authentic biological specimens and clinically characterized cohorts. These stages answer different questions and should not be merged in comparison tables. A sensor that demonstrates 95–105% recovery in diluted serum confirms matrix compatibility under controlled addition; it does not establish diagnostic discrimination.

This distinction is particularly important for blood α-synuclein, where erythrocyte-derived protein and hemolysis can dominate measured concentrations. Analytical sensitivity cannot compensate for poorly controlled pre-analytical bias. Similarly, p-tau217 has reached a level of clinical maturity where assay standardization, cut-points and comparison against validated reference methods matter more than pushing the LOD far below the biological decision range. NfL provides a complementary lesson: high molecular specificity does not create disease specificity because NfL rises in multiple forms of neuroaxonal injury.

Precision should therefore be decomposed into within-run, between-day, between-electrode and between-batch variability. For framework ECL sensors, inter-batch material variation is especially important because morphology, defect density and luminophore loading can alter signal efficiency. Operational burden should also be quantified through total turnaround time, hands-on time, reagent additions, wash steps and temperature-controlled operations.

Clinical claims require a further evidence layer: characterized cohorts, standardized sample handling, appropriate comparator methods, confidence intervals for sensitivity/specificity and preferably independent or blinded validation. Exploratory ROC analysis in the same cohort used to define a threshold should be distinguished from external diagnostic validation. Once analytical sensitivity is sufficient, matrix robustness, reproducibility, operational simplicity and external validation usually provide greater translational value than another order-of-magnitude reduction in LOD.

10.Translational Bottlenecks and Roadmap

The next stage of neurodegenerative-biomarker ECL should be judged against measurable translational bottlenecks, not progressively lower LODs.

The first bottleneck is biomarker definition. α-Synuclein assays remain difficult to compare because different studies target total, oligomeric or modified species and use different blood compartments. Progress requires species-defined standards, explicit hemolysis control and orthogonal comparison with established biochemical methods. For Alzheimer's biomarkers, the challenge is increasingly one of standardization around clinically validated cut-points rather than analytical detectability alone.

The second bottleneck is matrix robustness. Protein fouling, blood-cell contamination, ionic-strength differences and matrix-dependent recovery can alter both recognition and ECL kinetics. Antifouling interfaces should therefore be validated using representative interferent panels and authentic specimens, and performance should be reported across independently prepared electrode/material batches.

The third bottleneck is workflow complexity. CRISPR, DNA circuits and multistage amplification can produce exceptional sensitivity but often require sequential incubation, enzymes, temperature control and washing. Translation requires either removing nonessential amplification or integrating reagents and fluid handling into a closed cartridge. The relevant metrics are total turnaround time, hands-on time, number of user interventions and assay failure rate.

The fourth bottleneck is quantitative imaging standardization. Smartphone readout should progress toward fixed acquisition, linearized image processing and inter-device calibration. Internal references or ratiometric strategies are particularly attractive when they compensate for differences in illumination collection, sensor response or electrode-to-electrode variation.

The fifth bottleneck is clinical evidence. A proof-of-concept cohort can demonstrate feasibility but cannot establish diagnostic performance. Studies intended to support clinical claims should progress toward blinded testing, prespecified decision rules, reference-method comparison and independent validation. The maturation of plasma p-tau217 testing shows the expected trajectory: analytical validation across lots and operators, clinically representative cohorts, predefined cutoffs and comparison with PET or CSF biomarkers.

Finally, manufacturability should enter ECL design earlier. Framework materials, nanocomposites and multilayer electrodes are often optimized for peak signal rather than process control. A translation-ready platform should specify synthesis yield, inter-lot variability, storage stability, electrode fabrication tolerance and cost per test. Once adequate sensitivity has been reached, these metrics may be more important than another increment in signal amplification. The optimization target should therefore shift from "more ECL" to reliable information transfer from specimen to decision. (Table 1; Figure 7)

Table 1. *Translational bottlenecks and minimum evidence requirements for neurodegenerative-biomarker ECL systems.*

Bottleneck	Why it fails	Priority action	Minimum evidence
Biomarker ambiguity	Different molecular species or biological compartments	Use species-defined recognition and traceable reference standards	Cross-reactivity assessment; agreement with an orthogonal method
Hemolysis / matrix bias	Erythrocyte-derived α-syn, fouling and matrix chemistry	Standardize collection; control hemolysis; incorporate antifouling	Matrix effect and recovery in authentic specimens
Material variability	Variation in morphology, loading and defect density	Control batch synthesis and electrode quality	Inter-lot coefficient of variation
Amplification burden	Multiple enzymes, incubations and washing steps	Integrate into a closed cartridge or simplify transduction	Hands-on time; total assay time; failure rate
Phone variability	Nonlinear and device-specific imaging pipelines	Fix acquisition settings; linearize response; use an internal reference	Inter-device bias and calibration transferability
Weak clinical evidence	Small, single-center or non-independent cohorts	Use blinded, independent validation with prespecified rules	Confidence intervals for sensitivity/specificity or externally validated AUC
Manufacturing gap	Bespoke multicomponent fabrication	Standardize/print components and perform stability studies	Yield; shelf life; cost per test

Figure 7. *Translational pathway from analytical proof-of-concept to real-world point-of-care use. The schematic links assay validation and biomarker testing with clinical deployment, smartphone-based interpretation and decentralized/home use; each step requires progressively stronger evidence for reproducibility, standardization and implementation readiness.*

11.Conclusions

ECL biosensing for neurodegenerative biomarkers is approaching a change in optimization target. Signal generation is no longer the sole bottleneck: Ru-based frameworks, catalytic nanomaterials, nucleic-acid circuits and CRISPR-assisted schemes can already deliver extreme analytical sensitivity. The more difficult task is to preserve the biological meaning of that sensitivity across target definition, specimen handling, interfacial transport, device operation and quantitative readout.

A translation-oriented design hierarchy follows from this shift. Frameworks should be engineered as reaction spaces rather than high-capacity luminophore carriers; amplification should be judged by target-dependent information gained per added operation; interfaces by matrix resistance and batch reproducibility; devices by

automation and transport control; and smartphone readout by calibrated metrology. Clinical claims, in turn, require authentic specimens, appropriate reference methods, prespecified decision rules and independent validation.

The practical endpoint is not the brightest ECL material or the smallest nominal LOD, but a measurement system whose uncertainty is sufficiently low, characterized and reproducible to preserve molecular information from specimen to decision.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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