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Upconversion nanoparticle-based optical biosensor for early diagnosis of stroke

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ABSTRACT

Over 17 million people experience a stroke episode annually, with 5.9 million deaths. Stroke is diagnosed by physical tests and neuroimaging which need to be performed quickly to determine if the stroke is caused by ischaemia or haemorrhage. Neuroimaging can reliably confirm bleeding, but many patients with suspected ischaemic stroke (up to 40%) are subsequently confirmed to have alternative pathologies e.g., migraine or seizures (stroke mimics) delaying the transfer of stroke patients to an acute stroke unit for early intervention and treatment. Thus, a simple complimentary blood biomarker test to differentiate stroke patients from non-stroke patients with similar clinical symptoms is essential in prehospital and emergency settings for efficient stroke management and prompt treatment. The current 'Gold Standard' technique for detecting protein biomarkers is complex, time-consuming, and requires automated equipment. In this study, we have developed a proof-of-concept of lanthanide-doped upconversion nanoparticle (UCNP)-based optical biosensor platform for detecting glial fibrillary acidic protein (GFAP), a potential stroke biomarker, in human blood serum. The results show a linear response in photoluminescence quenching of UCNP conjugated GFAP antibody with the increasing concentration of GFAP biomarker in human blood serum. This approach can be used in the ambulance and Emergency Department to quickly diagnose a stroke. In the longer term, such techniques can be integrated into a self-assessment kit to monitor those patients who are at risk after strokes.

1. Introduction

Stroke is the most common cerebrovascular disease which can lead to long-term disability or death. It is caused by an interruption of cerebral blood flow due to ischaemia or haemorrhage. According to the Global Burden of Disease (2019) report, stroke is the third leading cause of death and physical disability worldwide (Feigin et al., 2021). In the last three decades, stroke cases and deaths from stroke have increased by 70% and 43%, respectively (Feigin et al., 2022). Apart from disability or death as a post-stroke outcome, stroke also has socio-economic impacts. Nearly two-thirds of stroke survivors discharged from the hospital with a disability are three times more likely to be unemployed within eight years of their stroke (Stroke, 2018). The estimated global cost of stroke is over 891 billion US dollars, equivalent to 1.12% of the global GDP (Owolabi et al., 2022). Thus, an early and accurate stroke diagnosis is essential for making fast clinical decisions, achieving good outcomes, and improving functional recovery.

Although recent advances in neuroimaging techniques have improved patient screening for diagnosis (Kakkar et al., 2021), these techniques cannot be used as a bedside diagnostic tool in emergency departments. Emerging research on blood-based biomarkers can be helpful in an early and rapid diagnosis of stroke, eventually leading to better clinical management of stroke patients. The current 'Gold Standard' technique, enzyme-linked immunosorbent assay (ELISA) for detecting and quantifying blood biomarkers is complex, time-consuming, expensive, and requires automated equipment. Therefore, there is a vital need to develop an easy-to-use and portable point-of-care (POC) diagnostic device for sensing blood-based biomarkers to shorten time-to-treatment, boost clinical decision-making, and improve patient outcomes (Harpaz et al., 2017). To meet this clinical need, we have developed an upconversion nanoparticle

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Abbreviations

EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
EG	Ethylene glycol
ELISA	Enzyme-linked immunosorbent assay
GFAP	Glial fibrillary acidic protein
NIR	Near-infrared
NHS	N-hydroxysuccinimidyl ester
POC	Point-of-care
PEI	Polyethleneimine
UCNP	Upconversion nanoparticle

(UCNP)-based sensor methodology to detect a potential stroke biomarker, glial fibrillary acidic protein (GFAP) in serum. GFAP is an intermediate filament protein present in the cytoskeleton of astrocytes in the brain (Hol and Pekny, 2015). Recently, numerous studies focused on the role of GFAP in differentiating intracerebral haemorrhage and ischaemic stroke in the early phase of stroke (Luger et al., 2017; Ren et al., 2016; Xiong et al., 2015). Serum GFAP levels increase within a few hours after the onset of haemorrhagic stroke, while in ischaemic stroke, serum GFAP levels reach their maximum at 48–72 h of onset (Amalia, 2021; Dvorak et al., 2009). Also, a study by Gaude et al. (2021) found that the combination of D-dimer and GFAP with stroke scale can help identify large vessel occlusion (Gaude et al., 2021), suggesting that GFAP may be a potential biomarker for the prediction of early stroke events.

In the last two decades, various diagnostic probes such as organic dyes (Zhang et al., 2002), fluorescent proteins (Betzig et al., 2006; Shaner et al., 2005), metal complexes (Chithrani et al., 2006; Huang et al., 2006), and semiconductor quantum dots (Wu et al., 2003) have been developed for bioimaging, sensing of proteins, and diagnosis of diseases such as cancer. Nevertheless, intense clinical research is still in progress for early stroke diagnosis for rapid intervention and treatment. A device (SMARTChip) to detect purines in blood is being developed by Sarissa Biomedical (UK) through electrochemical sensing. However, purines are not only released in acute stroke but also in other brain diseases, e.g., brain injuries (Tian et al., 2017), seizures and epilepsy (Beamer et al., 2021; Lemprière, 2021), angina and myocardial infarction, during systemic hypoxia, and peripheral arterial disease (Fisher et al., 2019). A magnetoimpedance biosensor platform to detect GFAP in blood for acute stroke classification is also under research at Monash University, Australia (Sayad et al., 2022). Similarly, another diagnostic platform based on enzymes tethered to nanoparticles is under research at Cornell University (USA) which detects neuron-specific enolase (NSE), a biomarker for multiple diseases ranging from acute brain injuries to lung cancer (Cohen et al., 2015). Upfront Diagnostics Ltd developed a test to identify D-dimer and GFAP for large vessel occlusion stroke in a retrospective collection of blood samples. However, the test has not yet been evaluated for market approval (Gaude et al., 2021).

In the last decade, research on upconversion nanoparticles (UCNPs) has been rapidly developed and these advances have the potential to use UCNPs for next-generation POC diagnostic devices. UCNPs are a particular class of lanthanide-doped and optically active nanoparticles that convert near-infrared (NIR) light to visible light. The unique characteristics of UCNPs, anti-Stokes shifts, sharp emissions, non-photobleaching, and long lifetime have gained significant attention over the years for use in biosensing and bioimaging applications (Gerelkhuu et al., 2022). A low autofluorescence or photobleaching with NIR excitation enhances the sensitivity of biosensors by increasing the signal-to-noise ratio (Tan et al., 2016; Nampi et al., 2019). In addition, NIR light can penetrate biological tissues better than visible light and reduces damage to tissues, making them suitable for drug delivery, real-time imaging of cells/tissues, and photodynamic therapy of cancers. (Arai and de Camargo, 2021; Smith et al., 2009; Gerelkhuu et al., 2022). In recent years, UCNPs have made advancements in cancer diagnosis and treatment (Amri et al., 2021). In a study by Sharma et al., rare earth metal-doped UCNPs coated with D-glucose were synthesised for their evaluation for diagnosis and therapy in cancer (Sharma et al., 2020). Moreover, virus infections have emerged worldwide in recent years, threatening people's lives. So, UCNPs are also promising candidates for developing a rapid detection technology platform (Ma et al., 2022) for virus detection as virus infections have emerged worldwide in recent years, threatening people's lives. In 2021, Guo et al. developed a 5G-enabled ultra-sensitive fluorescence sensor by using silica-encapsulated UCNPs for the quantitative detection of spike protein and nucleocapsid protein of SARS-CoV-2 (Guo et al., 2021). In addition, UCNPs also have the potential to be a biosensing platform for food quality and safety monitoring (Peltomaa et al., 2021; Jiang et al., 2022). Recently, a study by Suresh et al. has shown that the efficacy of UCNPs as a pH probe can have various biomedical applications, especially in measuring intercellular pH values (Suresh et al., 2021).

In the present study, UCNPs with sodium yttrium fluoride (a host lattice) doped with Yb^{3+} (sensitiser) and Er^{3+} (activator) were surface-functionalised and conjugated with the antibody (receptor) to the potential stroke biomarker GFAP to detect and measure the concentration of GFAP in human blood serum samples. The results demonstrated a nearly linear response in photoluminescence quenching with an increasing concentration of GFAP in human blood serum in the range of 5 pg/ml-5000 pg/ml.

2. Materials and methods

2.1. Material required for nanoparticle synthesis

- Analytical grade branched Polyethyleneimine (PEI, Molecular weight: 25 kDa, Sigma-Aldrich, UK)
- Ethylene glycol (EG), Sodium chloride (NaCl), Yttrium nitrate (Y $(NO)_3.6H_2O$), Ytterbium nitrate (Yb $(NO)_3.5H_2O$), Erbium nitrate (Er $(NO)_3.5H_2O$), acetone, and Ammonium fluoride (NH_4F). All these reagents were also purchased from Sigma-Aldrich, UK.
- Ultrapure water ($R = 18M\Omega$) for washing the precipitate and resuspension of nanoparticles.

2.2. Nanoparticle synthesis

For this study, the experimental procedure used for UCNP synthesis is similar to the procedure followed by Nampi et al. (2019). For precursor preparation, NaCl (10 mmol), Y$(NO)_3.6H_2O$ (3.12 mmol), Yb $(NO)_3.5H2O$ (0.8 mmol), Er $(NO)_3.5H_2O$ (0.08 mmol), and 1.6 g of PEI were dissolved in 60 mL of EG with stirring for 2 h to form a rare-earth (RE) solution. NH_4F (16 mmol) dissolved in 40 mL of EG was prepared separately and was added to the prepared RE solution. The resultant solution was then stirred for a further 15 min. The whole mixture was then transferred to a 120 mL Teflon-lined Parr pressure vessel and hydrothermally heated at 200 °C for 2 h and the resultant solution was allowed to cool down to room temperature. The contents including a very fine precipitate consisting of UCNPs were transferred into a beaker before washing 3 to 4 times with acetone, followed by washing 4 to 5 times with ultrapure water by repeated ultracentrifugation at 80,000g for 30 min using a Beckman Avanti J20XP high-speed centrifuge (Fullerton, California, USA). The nanoparticle pellet obtained was redispersed by sonication using an ultrasonic probe for a maximum of 30 s (Bandelin GM2070 with 100% power; cycle 0.7 s). The purified nanoparticles were then resuspended in 4–5 mL of water and passed through a desalting column (PD10; GE Healthcare Life Sciences, Pharmacia Biotech Inc., UK) to separate the finer UCNPs.

2.3. Characterisation of nanoparticles

The α-$NaYF_4$:Yb^{3+}, Er^{3+} (cubic) UCNPs are one of the best host materials of the upconversion process considering their low energy phonon modes. When excited at 980 nm these nanoparticles emit characteristic photoluminescent upconversion emission in green and red wavelength regions corresponding to the transitions $^2H_{11/2,}$ $^4S_{3/2} \rightarrow {}^4I_{15/2}$ and $^4F_{9/2} \rightarrow {}^4I_{15/2}$ respectively with red being more prominent in the alpha phase as shown in Fig. 1A. The average size of UCNPs is found to be 34.5 nm from the TEM image (Fig. 1B) of α-$NaYF_4$:Yb^{3+}, Er^{3+} UCNPs. The UCNPs are coated with PEI during the hydrothermal synthesis procedure which gives surface functional amine groups that have been exploited for the crosslinking strategy used in the current study as detailed in the next section.

2.4. Material used for conjugation of blood biomarker with UCNP

- Streptavidin (BioLegend, San Diego, UK)
- EDC (ThermoScientific, UK)
- Sulfo-NHS (ThermoScientific, UK)
- Biotinylated antibody for GFAP (Bt-GFAP Ab, R&D Systems, Minneapolis, USA)
- StartingBlock blocking buffer (ThermoScientific, UK)
- β-mercaptoethanol (Promega, Madison, USA)
- 0.5M Hydroxylamine (Merck, UK)
- Upconversion nanoparticles (5 mg/ml)
- 2-(N-morpholino) ethane sulfonic acid (MES) buffer: 0.1M MES + 0.5M NaCl (pH 6.0)

Additional material required.

- Silicone chambers (Grace Bio-Labs Inc., Oregon, USA)
- Polysine glass slides (ThermoScientific), 12 mm cover glasses
- 980 nm NIR laser (BL976-PAG300; Thorlabs, New Jersey, USA)
- Eppendorf tubes
- A centrifuge
- A table-top shaker, and a test-tube rotator/mixer

2.5. Conjugation of biomarker-specific antibodies with nanoparticles

2.5.1. Development of crosslinking methodology

EDC/EDC-NHS crosslinking was assessed to conjugate the carboxylic group present in the residual chain of streptavidin with amine groups present on the surface of UCNPs. This step aims to develop a custom platform that would utilise strong streptavidin-biotin interaction to bind target receptors/antibodies specific to biomolecules such as proteins/antigens (Dundas et al., 2013). Streptavidin-Alexa 488 (or Strep-488) was used as an identifier to evaluate which cross-linking strategy could bind streptavidin with amine-modified UCNPs. Five samples were prepared with Strep-488, UCNPs, and a cross-linker. In sample 1, there is no cross-linker. In sample 2, EDC was first added followed by NHS. In samples 3 and 4, only EDC and NHS have been added, respectively. In sample 5, EDC and NHS together have been added to conjugate Strep-488 with UCNPs. These samples were incubated overnight and fluorescence emission of Strep-488 was measured in each sample post-washing steps (to remove unbound Strep-488) to correlate which cross-linking strategy could bind streptavidin on UCNPs as shown in Fig. 2A. It can be inferred from Fig. 2A that streptavidin conjugated more with UCNPs in sample 3 (EDC-NHS cross-linking) and sample 2 (only EDC cross-linker) from their higher fluorescence intensity compared to other samples.

2.5.2. Assessment of conjugation of biotin with streptavidin modified UCNPs

These two cross-linking methods (samples 2 & 3) were further evaluated to assess their biotin-capturing ability on the streptavidin binding site. Fluorescein biotin (Biotin-488) was added to sample 2 (EDC-NHS cross-linking) and sample 3 (only EDC cross-linker). The photoluminescence of Biotin-488 from two samples is shown in Fig. 2B. Sample 3 cross-linking strategy depicts higher fluorescence indicating biotin-fluorescein is more bound with streptavidin-conjugated UCNPs in sample 3 than sample 2. However, when the Bt-GFAP antibody captured UCNPs (Bt-GFAP-UCNPs) sample was prepared using the crosslinking strategy used in sample 3, a nonlinear response was observed for the detection of the GFAP biomarker whilst a linear response was observed when the cross-linking strategy of sample 2 was employed (shown in supplementary data). Hence, strategy 2 was adopted for the biosensing method described hereafter (Fig. 2C).

2.5.3. Protocol for preparing Bt-GFAP antibody-captured UCNPs

In this study, GFAP antibody was captured on each UCNP using EDC-NHS crosslinking chemistry depicted in Fig. 2 (C). The procedure starts with adding 100 μl of streptavidin (1 mg/ml) to 900 μl of EDC solution (8 mg/ml, prepared in MES buffer) in a 1.5 ml Eppendorf tube. It is then incubated on a test-tube rotator for 2 h. Further, 5.8 μl of β-mercaptoethanol (final concentration of 20 mM) is added to the solution and kept for 30 min to quench the unconjugated EDC. After that, 22 mg of sulfo-NHS powder and 400 μl of UCNP (final concentration of 2 mg/ml) are mixed and incubated on a test-tube rotator overnight. The next day, 40 μl of hydroxylamine (final concentration of 10 mM) is added for 30 min to quench the unconjugated sulfo-NHS. The streptavidin functionalised UCNPs are separated using a centrifuge at 4500 rpm for 2 min and resuspended in 1 ml of MES buffer. To conjugate the antibody, 2 μl of Bt-GFAP Ab (100 μg/ml) is added and incubated on a test-tube rotator

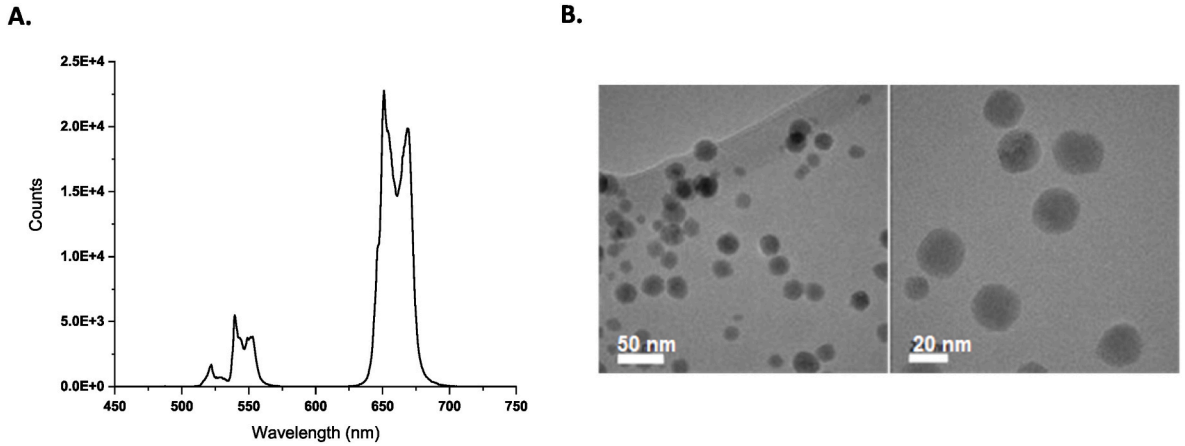

Fig. 1. A) Photoluminescence emission of cubic nanoparticles when excited at 980 nm. B) TEM micrograph of cubic nanoparticles at two different magnifications.

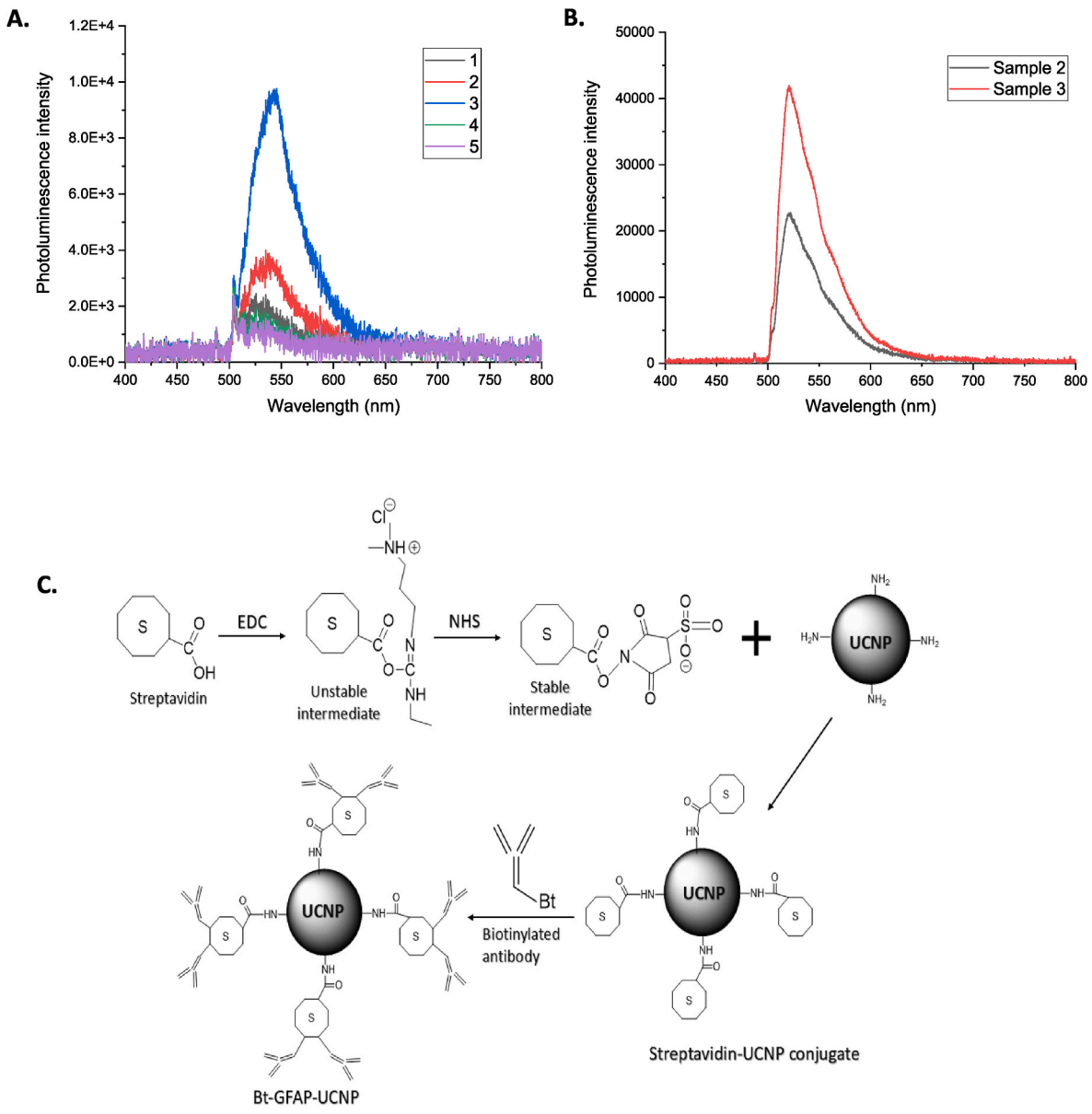

Fig. 2. Cross-linking evaluation: Photoluminescence of A) Samples 1–5 in Table 1 cross-linked with Strep-488 B) Biotin-488 conjugated with upconversion nanoparticles when excited at 488 nm. Sample 3 emits higher intensity as compared to sample 2 excited at 488 nm indicating streptavidin is more bound to UCNPs using the crosslinking strategy used in sample 3 than sample 2. C) Schematic representation steps involved in the UCNP conjugation with biomarker-specific antibodies for stroke sensing.

Table 1
Samples prepared for UCNP crosslinking assessment.

Sample	Crosslinkers
1	**No cross-linker**
2	**EDC incubation followed by Sulfo-NHS**
3	**EDC**
4	**Sulfo-NHS**
5	**EDC - Sulfo NHS mixture**

overnight. The Bt-GFAP Ab captured UCNPs (Bt-GFAP-UCNPs) are washed 2 times using a centrifuge at 4500 rpm for 2 min with MES buffer and resuspended in 1 ml of MES buffer. After the second wash 100 μl of blocking buffer is added and 900 μl of MES buffer to resuspend them. Bt-GFAP UCNPs are rewashed with MES buffer using a centrifuge at 4500 rpm for 2 min and resuspended in 1 ml of MES buffer. GFAP biomarker dilutions are prepared in 20% human serum (collected from the Stroke Unit at Leeds Teaching Hospitals NHS Trust, NHS REC reference 19/YH/0232, IRAS reference No: 50831) and Bt-GFAP-UCNPs. Bt-GFAP-UCNPs were incubated with GFAP biomarker dilutions for 2 h on the table-top shaker. Photoluminescence measurements were carried out for each sample. To assess the sensitivity of the Bt-GFAP-UCNPs, myoglobin, and S100B samples were prepared in 20% human serum and followed by incubation with Bt-GFAP-UCNPs for 2 h. For specificity assessment, dilutions of GFAP & myoglobin (5–5000 pg/ml), GFAP & S100B (5–5000 pg/ml) in 20% human serum were prepared followed by incubation with Bt-GFAP-UCNPs for 2 h.

2.6. Photoluminescence measurement

To measure the photoluminescence, the sample was loaded into silicone chambers mounted on glass slides and then covered with cover glass. For upconversion photoluminescence measurement, samples were illuminated with a NIR laser with a wavelength of 980 nm (BL976-

PAG900; Thorlabs, New Jersey, USA), operating at 350 mW. UCNP emission was recorded using a high-performance spectrometer (QE-PRO, Ocean Optics, Florida, USA) with an integration time of 4000 ms.

3. Results and discussion

3.1. GFAP biomarker sensing in MES buffer

Bt-GFAP-UCNPs prepared through an EDC-NHS cross-linking strategy as shown in Fig. 2C were then incubated with increasing concentrations of GFAP. Linear quenching of photoluminescence of UCNPs is observed with increasing concentration of GFAP in MES buffer (as shown in Fig. S1 from supplementary data). To further optimise the linearity of the photoluminescence quenching of UCNPs with altering GFAP concentration, Bt-GFAP-UCNPs were treated with different concentrations of blocking buffer as shown in Fig. 3. A blocking buffer was added to occupy the non-specific binding sites available on the surface of the UCNP-GFAP sensor. The blocking buffer helps improve the signal-to-noise ratio and reduce the non-specific binding of biomarkers on the sensor/assay surface (Sun et al.). UCNP-GFAP sensor treated with a 10% blocking buffer shows a better linear response (Fig. 3B) with increasing GFAP concentration (5 pg/ml-5000 pg/ml).

3.2. GFAP biomarker sensing in human blood serum

GFAP maintains the structural integrity of the astrocytes. The range of detection of GFAP using our detection method with surface-functionalised UCNPs is from 5 pg/ml-5000 pg/ml. In healthy humans, the concentration of GFAP is 0.03–0.07 ng/ml (Kawata et al., 2018). However exact concentration has not been well established yet. According to a study by Ren et al. (2016), GFAP can aid in the diagnosis of stroke and depict the severity of stroke with a threshold prediction of 0.34 ng/ml (Ren et al., 2016). Another study by Puspitasari et al. (2019) has reported a GFAP serum level of 0.112 ng/mL as a cut-off for high-level GFAP serum concentration which might predict stroke severity until day 7 (Puspitasari et al., 2019). The GFAP biomarker sensing using our surface-functionalised UCNPs depicted linear photoluminescence quenching with increasing concentration of the biomarker in MES buffer with 10% blocking buffer (Fig. 3B). In this experiment, biomarker sensing was carried out in the presence of 20% human blood serum in MES buffer. The sensitivity of the Bt-GFAP-UCNPs was tested against three biomarkers, GFAP, myoglobin, and S100B. Myoglobin is a protein found in cardiac and skeletal muscle tissue often found elevated in people with cardiac ischaemia (Gondal et al., 2023). GFAP and S100B are glial-specific proteins expressed by astrocytes that are directly related to neuronal dysfunction during cerebrovascular diseases (Dassan et al., 2009; Amalia, 2021). The nonlinear response was observed in photoluminescence quenching of the Bt-GFAP-UCNPs in the presence of S100B with Pearson's r coefficient showing a poor positive correlation of 0.14, Adj. R^2 -0.12, R^2 (COD) 0.02. Furthermore, poor non-linear response was also observed in myoglobin with Pearson's r coefficient showing a poor positive correlation of 0.44, Adj. R^2 -0.08, R^2 (COD) 0.19 (Fig. 4 (B, C, D)). A linear response in photoluminescence quenching was observed with increasing concentration of GFAP (Fig. 4(A–D)) with a strong negative correlation (Pearson's $r = -0.95$) and linear regression model (Fig. 4 (E)) depicting strong sensitivity of UCNP sensor to the logarithmic increase in the concentration of GFAP (Adj. R^2 0.89, R^2 (COD) 0.90).

However, it was also important to ascertain the specificity of the Bt-GFAP-UCNPs to GFAP in the presence of other biomarkers. Therefore, the specificity of the GFAP biomarker sensor was assessed in the presence of S100B and myoglobin. Linear photoluminescence quenching was observed with increasing concentrations of GFAP in the presence of myoglobin (Fig. 5(A–C)) and S100B (Fig. 5(B–D)). A strong negative

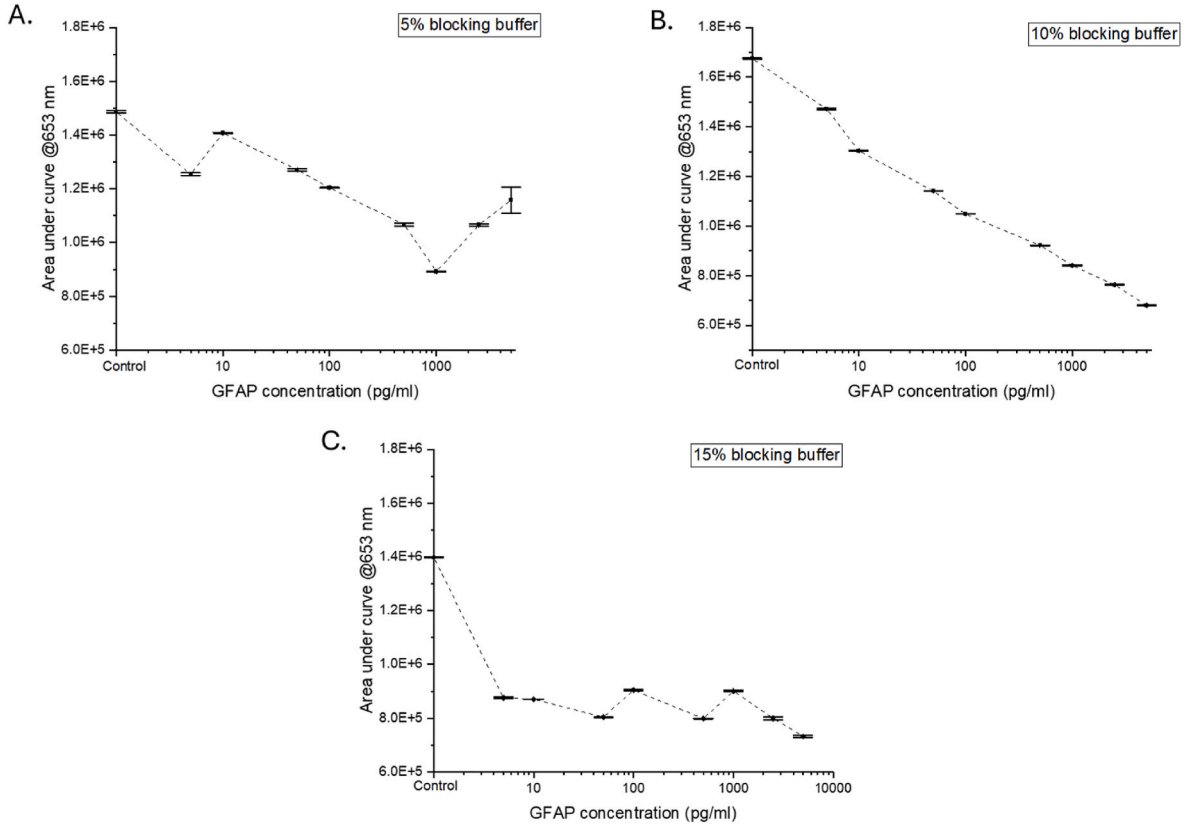

Fig. 3. Assessment of blocking buffer concentrations. The area under the curve at 653 nm peak versus GFAP concentration with A) 5%, B) 10%, and C) 15% blocking buffer concentrations.

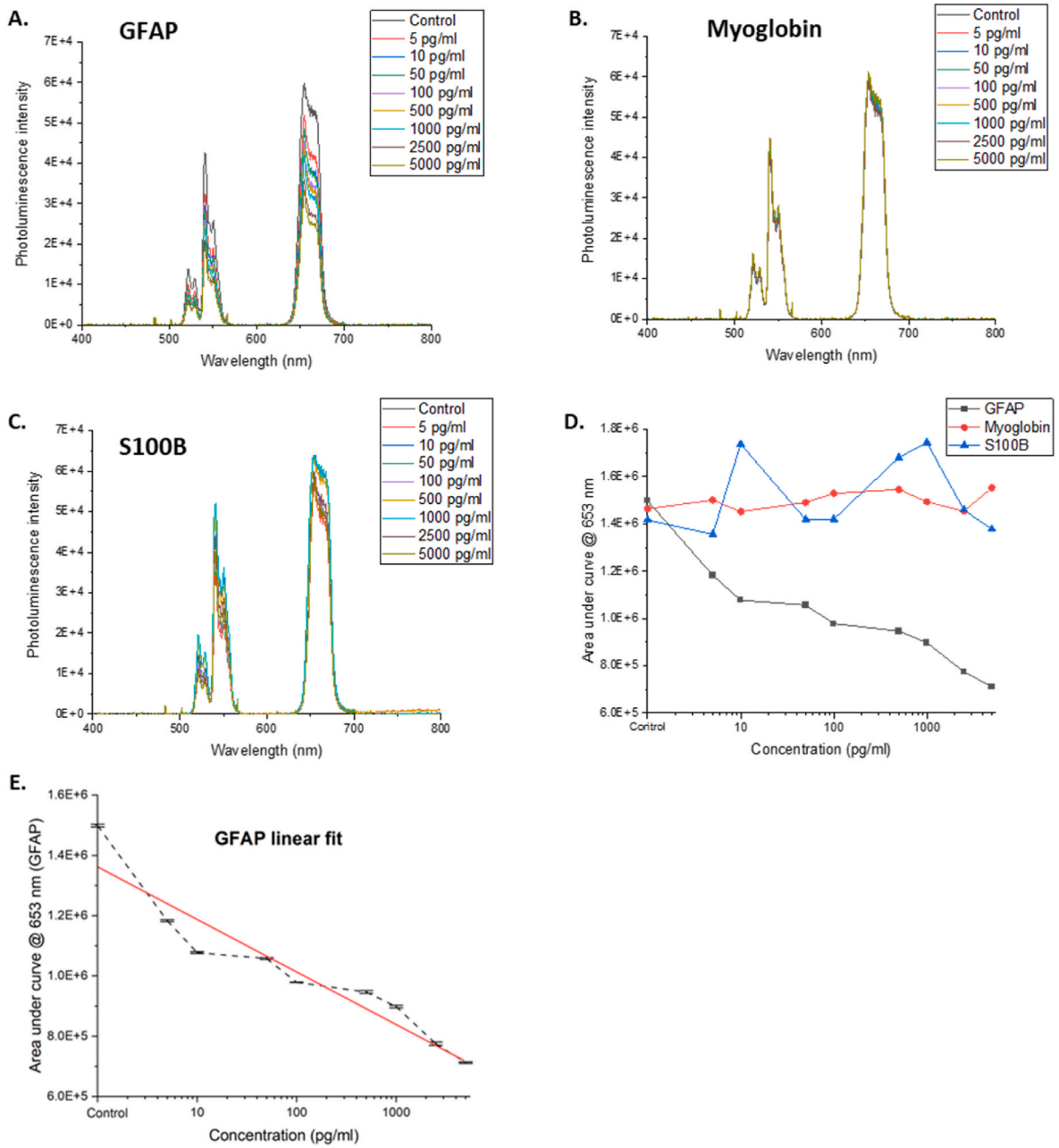

Fig. 4. Biomarker sensing. Photoluminescence spectrum of Bt-GFAP-UCNPs when excited at 980 nm with different concentrations of A) GFAP, B) Myoglobin, and C) S100B. D) Area under the curve at 653 nm peak with GFAP, Myoglobin, and S100B concentrations in 20% human blood serum. E) Linear fit curve for GFAP.

correlation was observed in detecting the GFAP using the UCNP sensor with Pearson r's coefficient −0.96 (GFAP + S100B), −0.98 (GFAP + myoglobin). The linear regression model predicts strong specificity of logarithmic change in concentrations of GFAP to photoluminescence quenching of UCNP sensor with Adj. R^2 0.90, R^2 (COD) 0.92 for GFAP in the presence of S100B and Adj. R^2 0.96, R^2 (COD) 0.97 with GFAP in the presence of myoglobin.

One of the possible mechanisms for fluorescence quenching can be the saturation of epitopes on antibodies by the capture of biomarkers resulting in higher electronic transition due to probable Columbic interaction leading to surface quenching of PEI-coated UCNPs. The overall linear fluorescence quenching is only observed between the concentration range of 10 pg/ml to 5000 pg/ml of GFAP. No linear response is observed above 5000 pg/ml suggesting the available epitopes on the antibody sites have been saturated by GFAP capture (Vyshnava and Dowlathabad, 2024).

From our results, the limit of detection (LOD) value of GFAP without the presence of any other biomarker has come out to be 0.082 pg/ml. In the presence of myoglobin and S100B, LOD is 0.210 pg/ml and 0.04 pg/ml, respectively. LOD values have been calculated according to the formula mentioned in (Ma et al., 2022).

4. Conclusion and future perspective

The benefits of surface-functionalised UCNPs adopted in the present detection technology include their low nonspecific binding, minimal scattering, and high quantum yield. Compared to conventional fluorophores, including organic dyes and quantum dots, UCNPs are excited in the NIR region, thereby eliminating any auto-fluorescence from biological samples that are inherent in ultraviolet or visible wavelength excitation. The present study demonstrated the feasibility of a UCNP-based detection methodology, which is customisable to detect

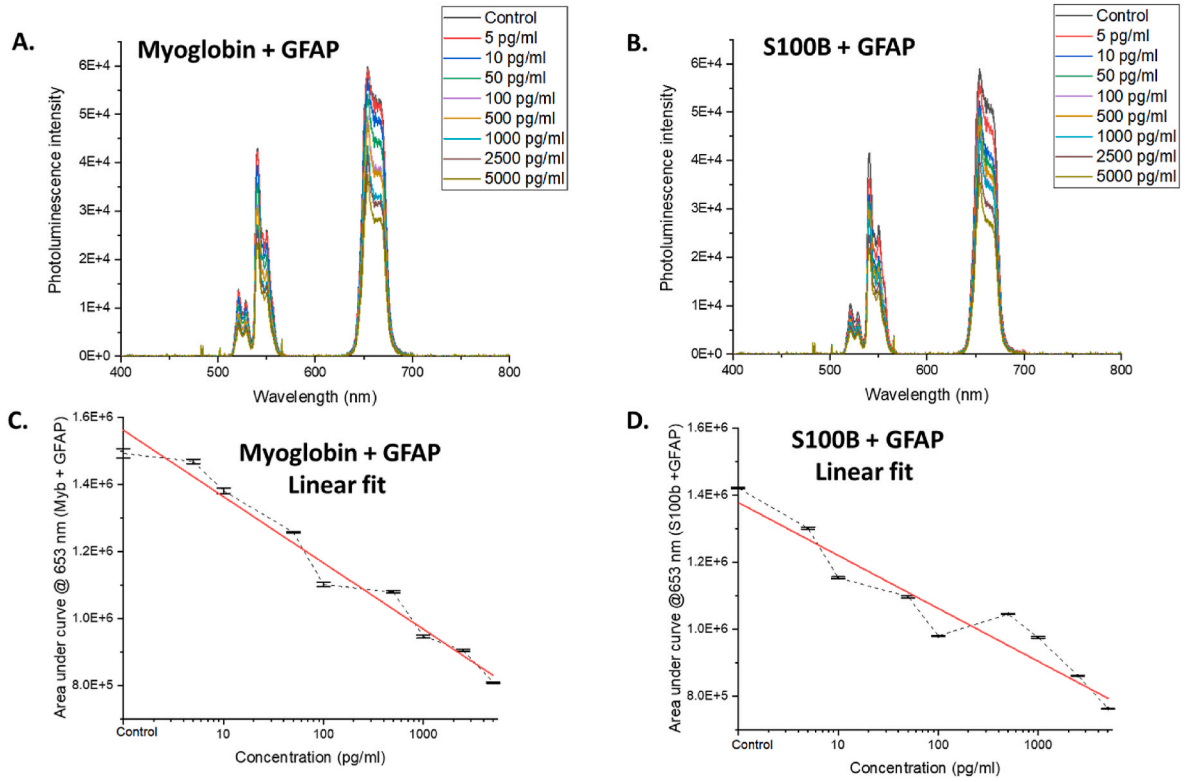

Fig. 5. Biomarker sensing: Photoluminescence spectrum of Bt-GFAP-UCNPs when excited at 980 nm with different concentrations of GFAP in the presence of A) Myoglobin and B) S100B. C) Area under the curve at 653 nm peak in the presence of different concentrations of GFAP and Myoglobin, (D) GFAP and S100B, in 20% human blood serum.

biomarkers specific to stroke. We have proven specific detection of GFAP, a potential stroke biomarker using UCNPs surface functionalised and bioconjugated with antibodies targeted to GFAP biomarker. The photoluminescence is characterised using the custom-built NIR laser-based setup coupled with a QE-PRO spectrometer. The bioconjugated UCNP shows a linear response in photoluminescence quenching with an increasing concentration of GFAP biomarker. The detection range of 5 pg/ml-5000 pg/ml GFAP was achieved using the present detection methodology adopting UCNP technology. The specificity of the GFAP sensing technique was tested against other protein markers such as myoglobin and S100B.

Our future aim is to develop and validate a portable, easy, and specific in-vitro diagnostic device for rapid detection of multiple potential brain damage-specific circulating biomarkers in blood obtained from stroke patients and non-stroke patients, which can be used for preliminary assessment of the levels of circulating biomarkers by paramedics and emergency departments. The rapid, accurate, simultaneous detection of multiple biomarkers with neurological scores performed in ambulances and emergency departments to differentiate between stroke and non-stroke patients will accelerate the treatment and management of stroke patients but this is not possible with current immunoassay methods, which measure one biomarker at a time and takes about 4 h.

CRediT authorship contribution statement

Pragati Kakkar: Writing – original draft, Validation, Methodology, Investigation, Data curation. **Tarun Kakkar:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Padmaja Parameswaran Nampi:** Writing – review & editing, Supervision, Methodology. **Gin Jose:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Sikha Saha:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2025.117227.

Data availability

Data will be made available on request.

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