Fluorescence based cost-effective rapid diagnostic kit for detection of small cell lung cancer

In the current scenario, the dominancy of cancer is becoming a disastrous threat to the whole of mankind. Among all, small cell lung cancer (SCLC) is known for the high tendency toward early metastasis that becomes worst because of its rapid doubling rate. The prognosis of SCLC is one of the lowest among the various cancer types due to the poor availability of diagnostic tools at an early stage. Therefore, an advanced analytical approach is desired as a need of the hour for the early diagnosis to curb the menace of SCLC cancer. In this direction, the quantitative detection of cancer biomarkers with higher accuracy and sensitivity could be utilized as a remedy towards the development of an efficient platform. In the view of foregoing, the present work illustrates the significance of nano surface energy transfer (NSET) based fluorescent biosensing platform for the early stage detection of SCLC, evading the limitations of traditional diagnostic techniques. To achieve the efficient NSET phenomenon in these fluorescent biosensors, the selection of appropriate donor-acceptor pair always plays a pivotal role. For which, continuous efforts have been made towards the exploration of suitable nanomaterials possessing excellent biocompatibility along with the desired optical properties.

The proposed diagnostic kit comprises of biofunctionalized graphene quantum dots (anti-NSE/amine-N-GQDs) which act as energy donors and gold nanoparticles (AuNPs) which act as energy acceptors for the quantitative detection of neuron-specific enolase (NSE); a well-known SCLC biomarker. The functionality of the kit relies on the fundamental principle of energy transferring capability of donor species (anti-NSE/amine-N-GQDs) to the nearby acceptor species (AuNPs), followed by the recovery of fluorescence intensity on the addition of target antigen. The efficient energy transfer process has been envisaged by incorporating the optimized anti-NSE/amine-N-GQDs donor with AuNPs acceptors. The addition of different NSE antigen concentrations to the optimized donor-acceptor mixture inhibits the energy transfer process that results in the restoration of amine-N-GQDs fluorescence. Whereas, the recovery of fluorescence intensity relies on the equivalent addition of NSE antigen concentration.

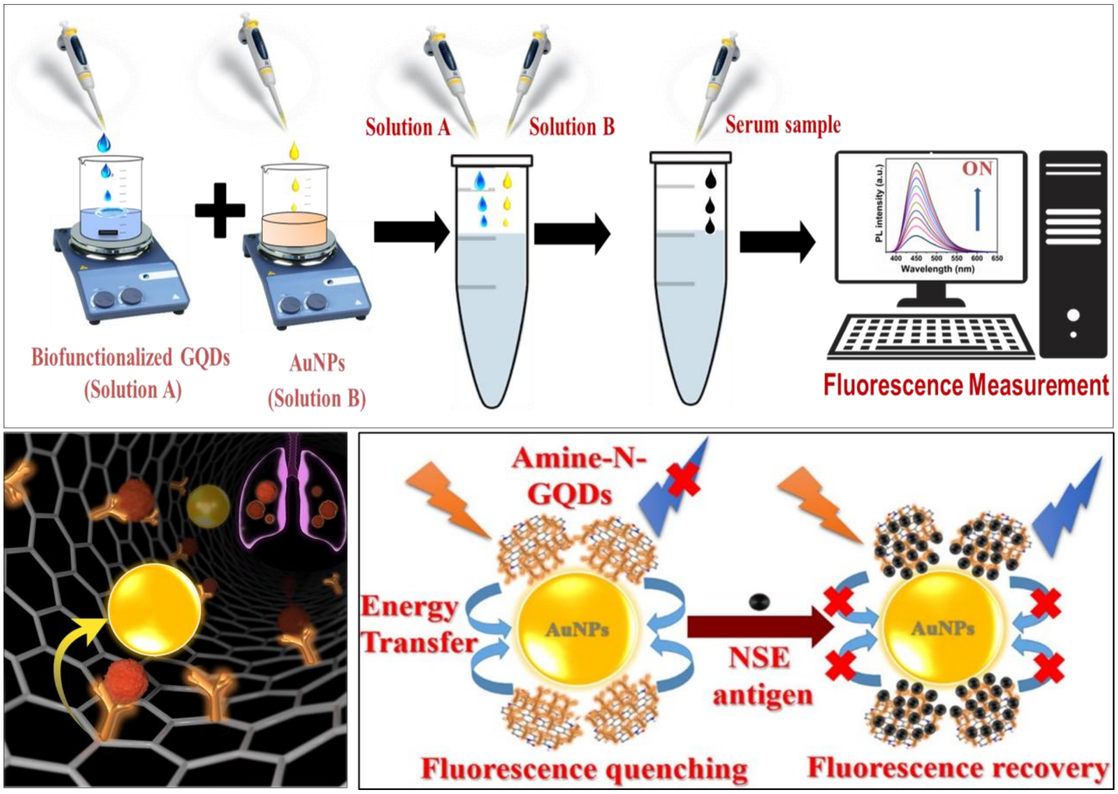

Figure 1: Schematic representation of fluorescent biosensor for small cell lung cancer detection.

In this context, the proposed fluorescent diagnostic kit successfully detected NSE biomarkers with notable biosensing parameters including a wider linear detection range (0.1 pg mL^{-1} to 1000 ng mL^{-1}), a fast response time (16 min), and a remarkable low detection limit (0.09 pg mL^{-1}). Additionally, excellent performance in real samples, with an average recovery of 94.69%.the has also been obtained.

Publication:

A. Kalkal, R. Pradhan, S. Kadian, G. Manik, and **P.Gopinath***. Biofunctionalized graphene quantum dots based fluorescent biosensor towards efficient detection of small cell lung cancer. *ACS Applied Bio Materials*, **2020** ,3, 4922–4932 featured in "**NATURE INDIA**"

Patent granted:

P.Gopinath, Ashish and Rangadhar Pradhan. Patent granted on 11 April 2023 for "Fluorescence based cost-effective rapid diagnostic kit for detection of small-cell lung cancer biomarker" Indian Patent Application number 202011010110.

Ag@Ti_3C_2-MXene nanohybrid as dual energy acceptor for highly efficient detection of small cell lung cancer

Over the last decade, 0D and 2D nanomaterials, including metal nanoparticles, graphene and its derivatives delivered an extraordinary impact as energy acceptor species in the field of fluorescent biosensors. However, it has been reported that these OD/2D materials have a tendency to agglomerate/restack, forming larger clusters that may influence the energy transfer efficiency and biosensing characteristics. This problem might be overcome by preparing a 0D-2D nanohybrid wherein 2D layered nanomaterial can help in inhibiting the Brownian motion of OD by acting as a support matrix and can generate the interlayer spacing by decorating with 0D nanoparticles. In the case of graphene-based traditional 2D nanomaterials, the decoration of 0D materials can occur only on the edge and defect sites of graphene nanosheets. Besides, chemically synthesized graphene requires an additional reducing agent for in-situ decoration or an additional step for ex-situ decoration of 0D nanomaterial. Moreover, the conventional graphene-based 2D layered nanomaterials possess low-hydrophilicity, inadequate surface terminated functionality, and difficulty in functionalization that may influence fluorescent biosensing applications.

In the view of foregoing, a new and emerging material named MXene having the general formula of $M_{n+1}X_nT_x$ (M represents a transition metal, X can be nitrogen and/or carbon, and T_x denotes surface functionalization) has attracted enormous consideration for biosensing applications. The ultrathin Ti_3C_2-MXene sheets terminated with oxygen and hydroxyl groups facilitate the material to communicate with a large number of biomolecules via hydrogen bonds, van der Waals forces, and electrostatic interactions. Unlike graphene, Ti_3C_2-MXene offers excellent aqueous solubility, superior surface terminated functionality, and better biocompatibility, preferable in the fabrication of efficient fluorescent biosensors. Besides, the self-reducing capability of Ti_3C_2-MXene exempts the requirement of an additional reducing agent for decorating 0D nanomaterial on Ti_3C_2-MXene nanosheets.

In the present work, Ti_3C_2-MXene nanosheets are decorated with silver nanoparticles (AgNPs) to obsolete the agglomeration and restacking through a one-pot direct reduction method wherein the 2D Ti_3C_2-MXene nanosheets acted both as a reducing agent and support matrix for AgNPs. The Ag@Ti_3C_2-MXene nanohybrid acts as a dual-quencher in a single system for ultrahigh fluorescence quenching of donor species owing to their higher surface-to-volume ratio that enables effective energy transfer utilizing the concept of single-acceptor multiple-donor. The Ag@Ti_3C_2-MXene nanohybrid as a dual-energy acceptor exhibits higher quenching efficiency (~94%) compared to bare 2D Ti_3C_2-MXene (~87%), 0D AgNPs (~84%) and 0D AuNPs (~81%).

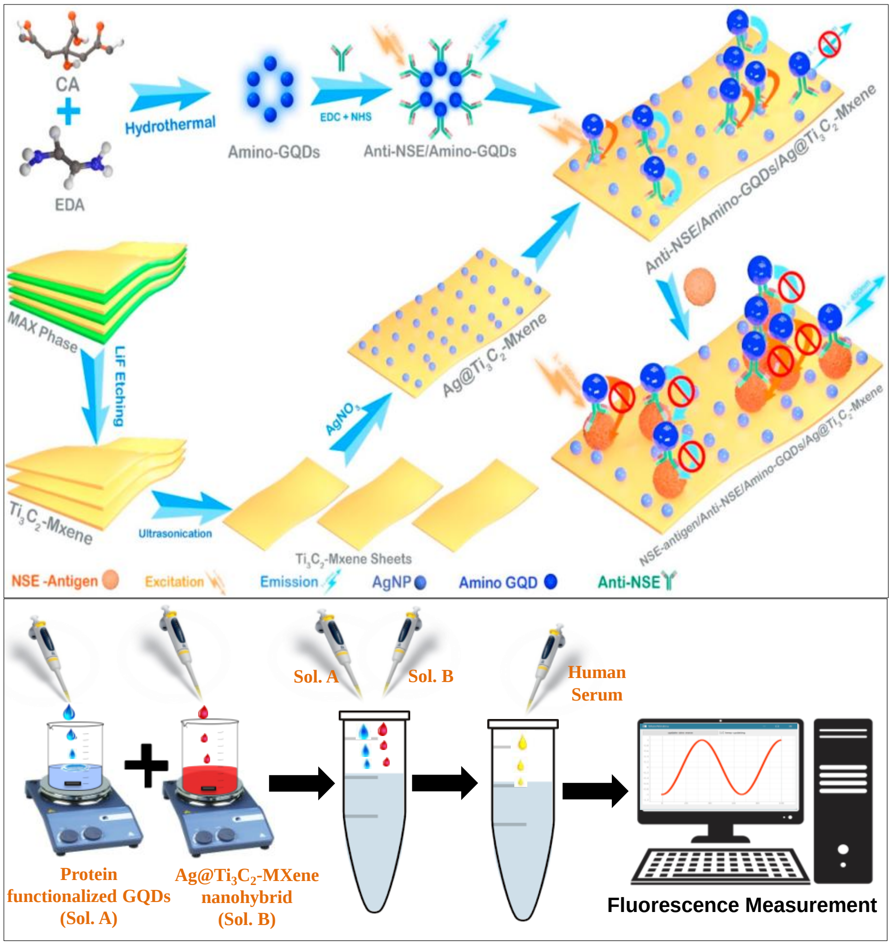

Figure 1: Schematic representation of Ag@Ti_3C_2-MXene nanohybrid based fluorescent biosensor for Neuron Specific Enolase detection.

Further, a potential fluorescent biosensor comprising of specific protein functionalized graphene quantum dots (anti-NSE/amino-GQDs) and Ag@Ti_3C_2-MXene nanohybrid as donor-acceptor pair is developed for the quantitative Neuron specific enolase (NSE) detection. The functionality of this selective, rapid, label-free, and highly sensitive biosensor relies on the fluorescence quenching of donor species (anti-NSE/amino-GQDs) by the acceptor species (Ag@Ti_3C_2-MXene), followed by the restoration of quenched fluorescence upon the addition of NSE antigen. The developed biosensor exhibit improved biosensing parameters such as broader linear detection range (0.0001–1500 ng mL^{-1}), better LOD (0.05 pg mL^{-1}), higher sensitivity (~771 mL ng^{-1}), and faster response time (12 min). It is worth mentioning that the sensitivity of the Ag@Ti_3C_2-MXene based biosensing platform is exceeding two times in contrast to 2D classic graphene (~352 mL ng^{-1}) and 0D AuNPs (~333 mL ng^{-1}) based platforms.

Publication:

A.Kalkal, S.Kadian, S. Kumar, G.Manik, P.Sen, S. Kumar* *and* **P.Gopinath***.Ti_3C_2-MXene decorated with nanostructured silver as a dual-energy acceptor for the fluorometric cancer biomarker detection. *Biosensors and Bioelectronics*, **2022, *(IF=12.6)***

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