Filter Paper Based Fluorescence Sensor For Alkaline Phosphatase Detection Via Reversible Pyrene-Polymer Aggregation

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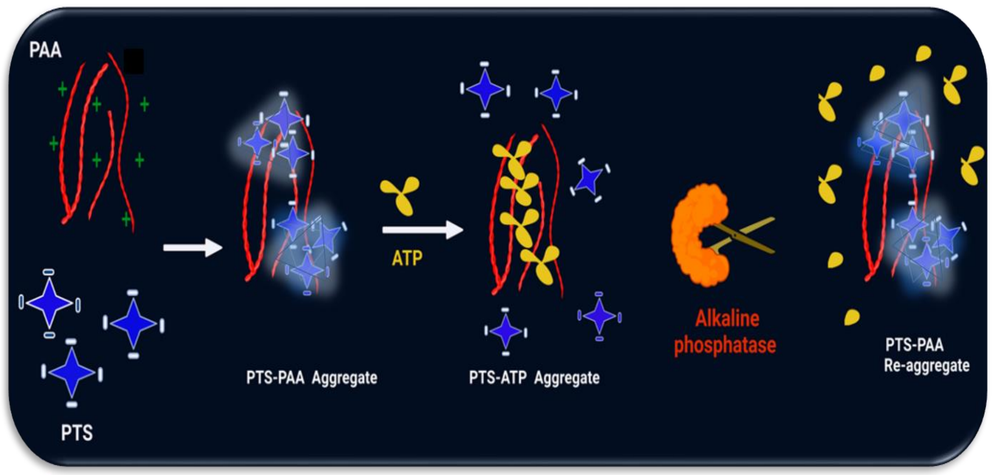
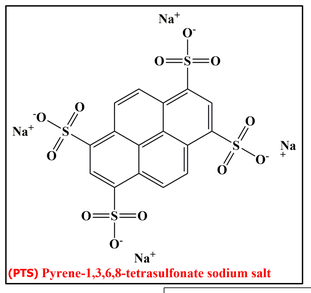
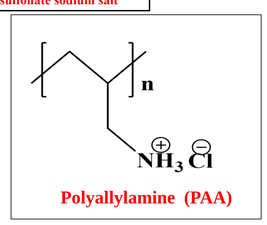
MOTIVATION: Alkaline phosphatase (ALP), an enzyme predominantly located in the liver, bones, intestines, and kidneys, serves as a biomarker for various disease conditions, including liver disease, bone disorders, and chronic kidney disease. This study presents the development of a ratiometric fluorescence-based sensor for ALP detection. The sensor operates through the modulation of monomer-aggregate equilibrium of PTS (Dye)-PAA (Polymer) system by ATP and subsequently by ALP. This provides a sensitive ratiometric fluorescence signal in response to ALP. This system offers high sensitivity and selectivity, positioning it as a promising tool for monitoring ALP-related health conditions. Among the various analytical methods, the exceptional features of fluorescence-based methods, such as quick response, high sensitivity, and selectivity, have enabled their wide adoption as a method of choice for devising sensors.

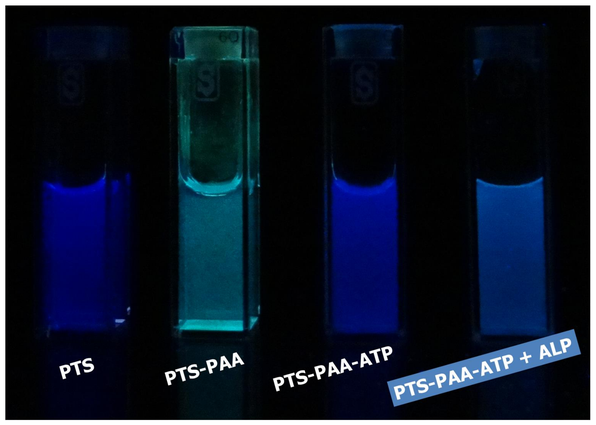
Scheme of Sensor operation





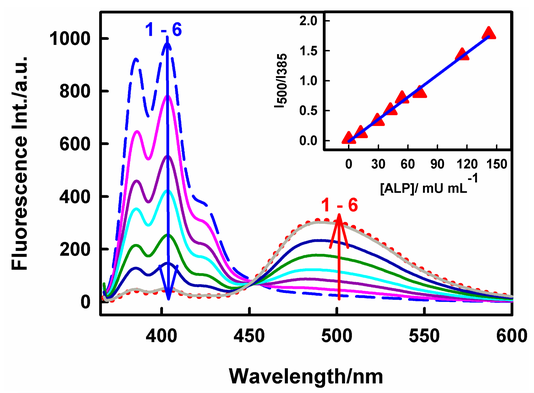


Cuvette images



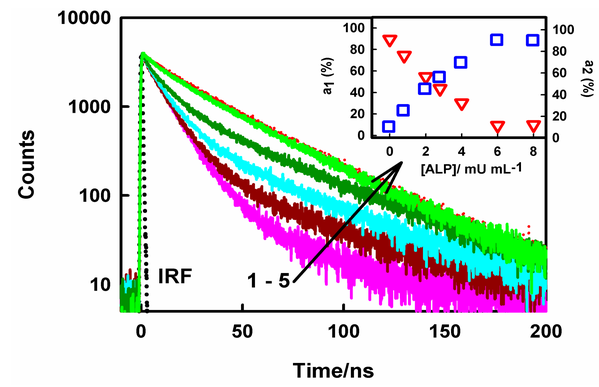
Detection of Alkaline phosphatase

Fluorescence



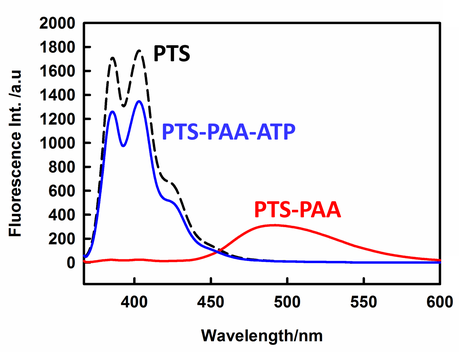
- Addition of ALP to PTS-PAA-ATP system causes gradual recovery of the PTS-PAA aggregate band at 500 nm.
- Ratiometric signal increases linearly, LOD = 63.3 μU mL^{-1}
- ❖ ALP hydrolyses ATP and paves the way for reformation of PTS-PAA aggregate.

Lifetime

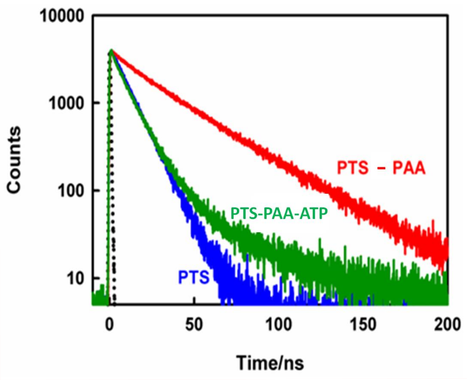


- Amplitude of slower component (Excimer) increases with increase in the ALP concentration to PTS-PAA-ATP system.
- Suggests the cleavage of ATP by ALP, reformation of PTS-PAA complex.

Interaction of PTS with PAA

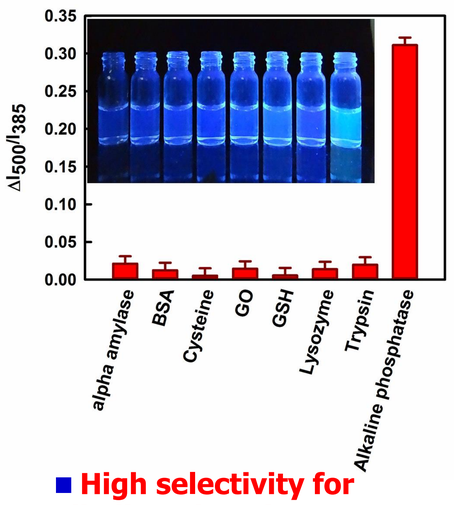


- Fluorescence bands:
  385 nm (monomeric PTS)
  500 nm (PTS-PAA aggregate)
- Polyallylamine (PAA) induces aggregation of PTS due to charge neutralization of anionic PTS.
- ATP addition reverses the emission to the PTS monomer state, suggesting competitive binding of ATP to PAA.



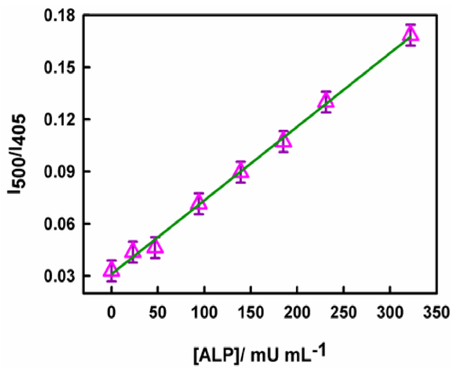
- The excimer of PTS displays a longer lifetime (τ=36.3ns) as compared to PTS monomer (τ= 10.5 ns) .
- ATP addition to PTS-PAA brings it to the monomer state, indicating the breakage of PTS aggregates from PAA .

Selectivity



- High selectivity for Alkaline phosphatase

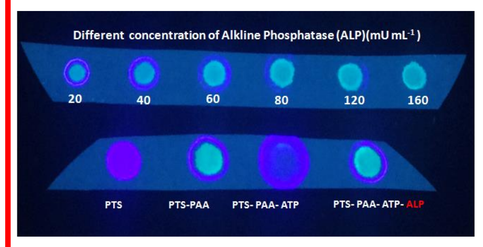
Human Serum matrix



- Good response in Human serum matrix

LOD=936 μU mL^{-1}

Filter Paper Based ALP Detection



- UV Exposure- 365nm
- Whatman filter paper used.

CONCLUSIONS:

- The reversible switching between the monomeric and excimer emission states of PTS-PAA system, upon ATP addition and subsequent ALP hydrolysis serves as the core sensing mechanism for ALP detection.
- High sensitivity and selectivity, commercially available probe molecule, operation in aqueous medium.
- Good response in Human serum matrix .

REFERENCES:

1. J. Kaur, P. K. Singh, Sens. Actuators, B: Chem., 346(2021), 130517