

New Insights into the Photophysical Properties and Interaction Mechanisms of Janus Green Blue Dye with Polyanions and Its Applications in Colorimetric Visualization of Loop-mediated Isothermal Amplification and Polymerase Chain Reaction

Arumugam Selva Sharma^a, Sung Min Ryou^b, Ji Hyeok Lee^b, and Nae Yoon Lee^{b*}

^aDepartment of Nanoscience and Technology, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do 13120, South Korea

^bDepartment of BioNano Technology, Gachon University, 1342 Seongnam-daero, Sujeong-gu, Seongnam-si, Gyeonggi-do 13120, South Korea

*Corresponding author: nylee@gachon.ac.kr.

Supplementary Information

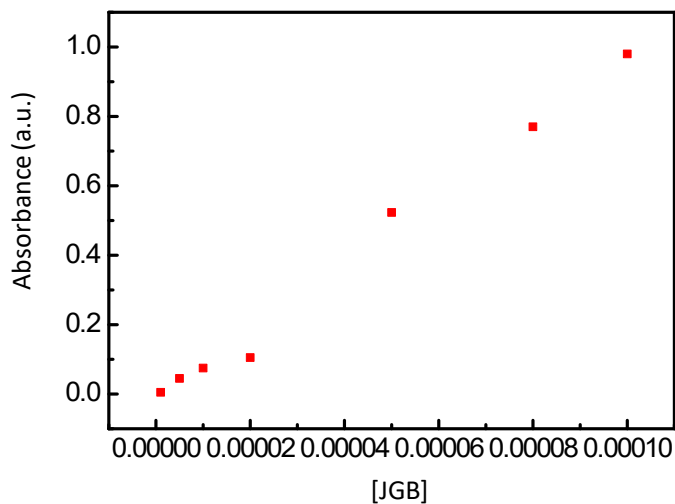


Figure S1. Maximum absorption intensity of JGB dye at various concentrations (1) 1, (2) 5, (3) 10, (4) 20, (5) 50, (6) 80, and (7) 100 μ M.

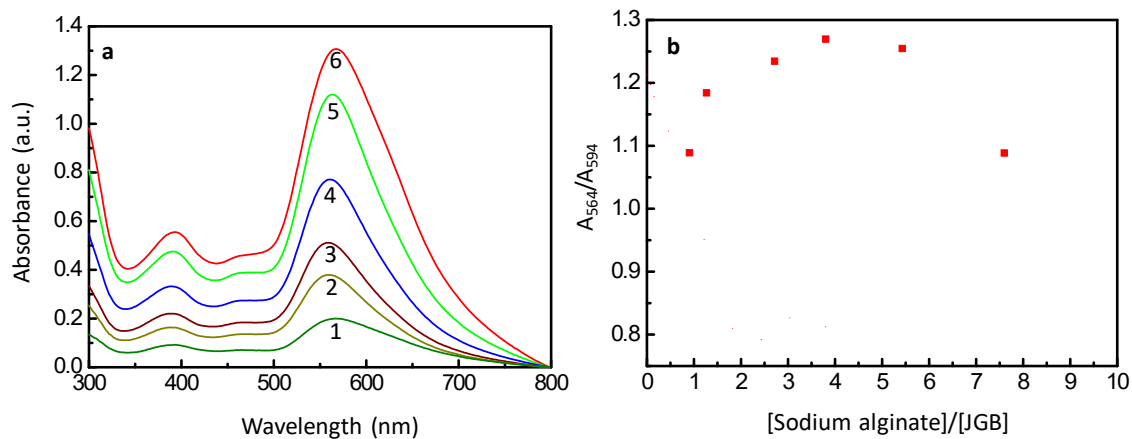


Figure S2. (a) Absorption spectra of JGB dye at various concentrations (1) 50, (2) 70 (3) 100 (4) 200, (5) 300, and (6) 420 μM in the presence of sodium alginate (380 μM). (b) Change in A_{564}/A_{594} with respect to different [sodium alginate]/[JGB] ratio.

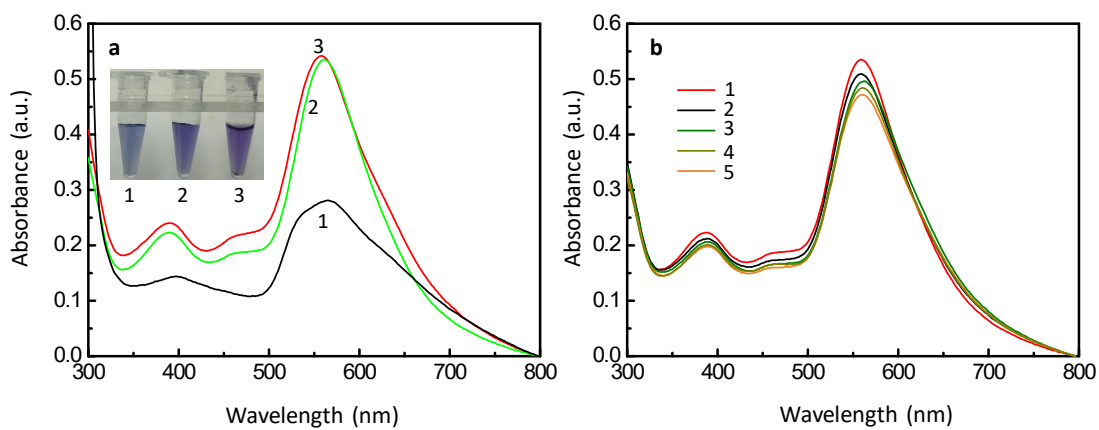


Figure S3. Absorptions spectral profile of (a) JGB-Alginate complex (1:1) under different pH conditions (1) 4, (2) 7 and (3) 9. (b) JGB-Alginate complex (1:1) in the absence and presence of varying concentrations of NaCl: (1) 0, (2) 5, (3) 10, (4) 20 and (5) 30 mM. (Concentration of JGB dye: 1.5×10^{-4} , Sodium alginate: 1.5×10^{-4}).

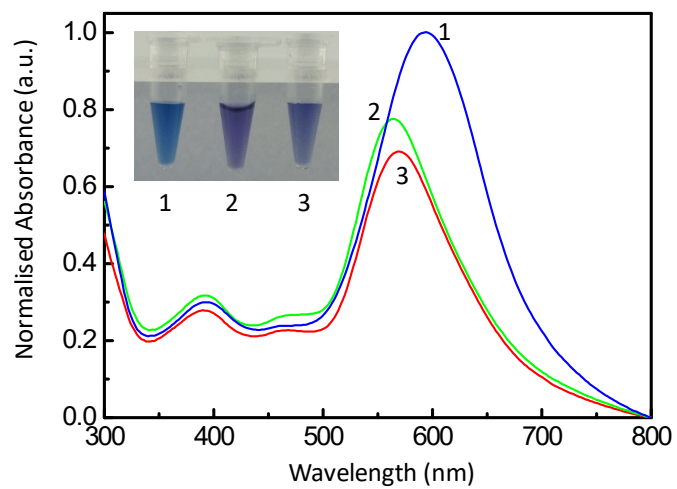


Figure S4. Absorption spectra of (1) JGB dye, (2) JGB dye in the presence of sodium alginate and (3) JGB dye-alginate complex in the presence of DNA. Inset shows the reversal of metachromasia upon the addition of DNA (Concentration of JGB dye: 1×10^{-4} , Sodium alginate: $380 \mu\text{M}$ and DNA: 1.15×10^{-4}).

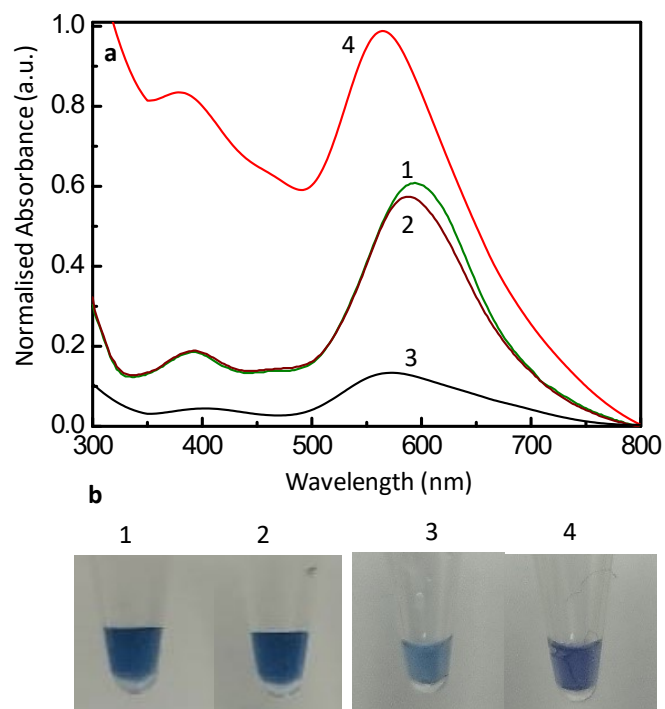


Figure S5. (a) Absorption spectra of (1) JGB dye, (2) JGB dye in LAMP buffer, (3) JB dye in the presence of NC and (4) LAMP amplicon. (b) Photographs of (1) JGB dye, (2) JGB dye in LAMP buffer, (3) JGB dye in the presence of NC and (4) LAMP amplicon.

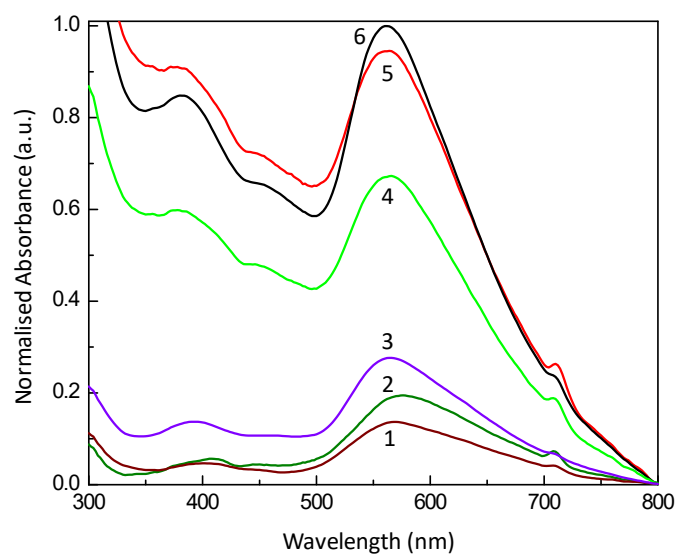


Figure S6. Absorption spectral profile of JGB dye in the presence of (1) NC and LAMP amplicons obtained from (2) 0.1, (3) 1, (4) 10, (5) 100 and (6) 1000 $\text{pg}/\mu\text{L}$ of template DNA.

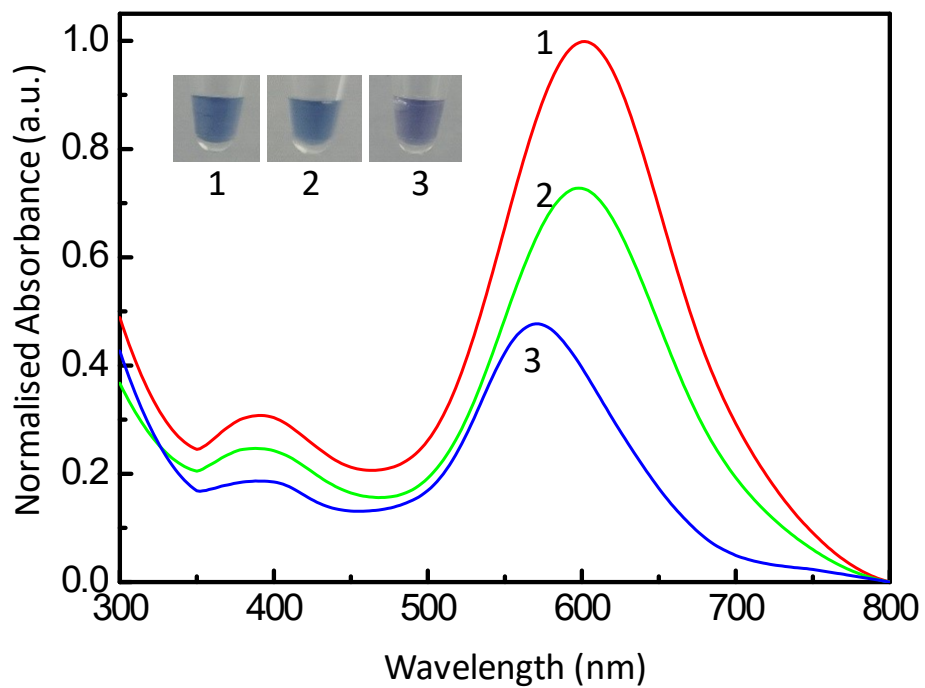


Figure S7. Absorption spectral profile of (1) JGB dye, (2) JGB dye in the presence of PCR reagents and primers and (3) PCR amplicons (Inset shows the photographs of (1) JGB dye, (2) JGB dye in the presence of PCR reagents and primers and (3) PCR amplicons).

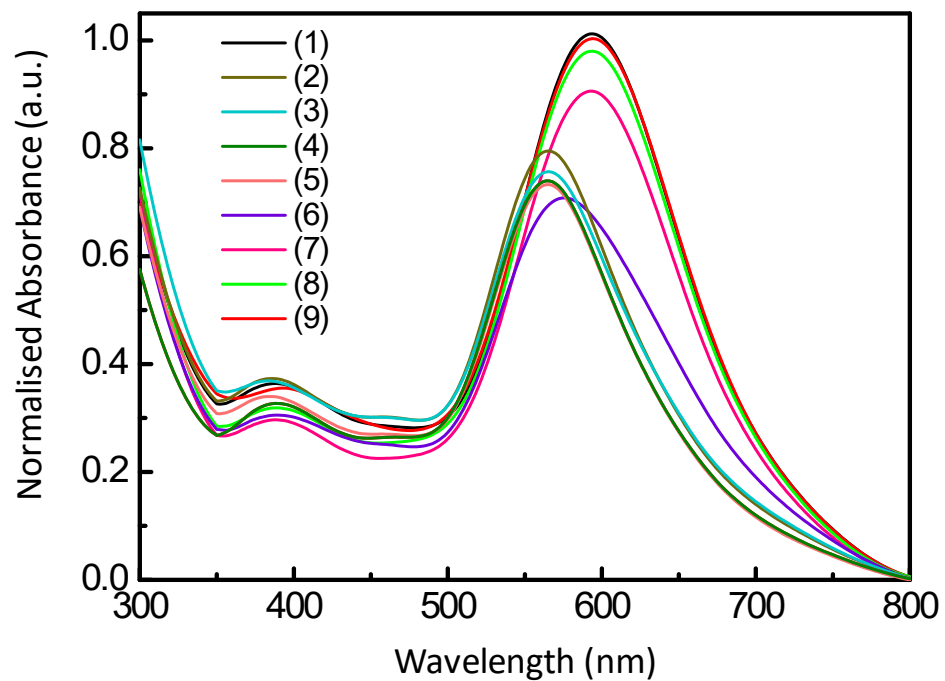


Figure S8. Absorption spectral profile of JGB dye in the presence of (1) NC and PCR amplicons obtained from (2) 1 ng/ μ L, (3) 0.1 ng/ μ L, (4) 10 pg/ μ L, (5) 1 pg/ μ L, (6) 0.1 pg/ μ L, (7) 10 fg/ μ L, (8) 1 fg/ μ L, and (9) 0.1 fg/ μ L of template DNA (MTF-1).

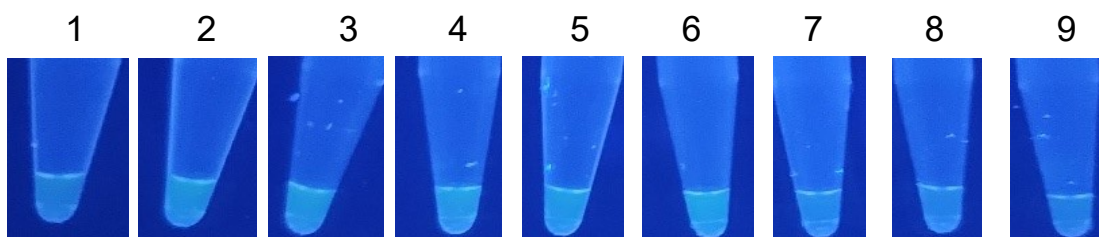


Figure S9. Photograph showing the fluorescence response of SYBR green for (1) NC and PCR amplicons obtained from (2) 1 ng/ μ L, (3) 0.1 ng/ μ L, (4) 10 pg/ μ L, (5) 1 pg/ μ L, (6) 0.1 pg/ μ L, (7) 10 fg/ μ L, (8) 1 fg/ μ L, and (9) 0.1 fg/ μ L of template DNA (MTF-1) under UV irradiation.

Table S1. Primer sequences used in LAMP to amplify plasmid containing *S. pneumoniae*.

F3	AAAGAAGCGGAGCTTGTC
B3	TCCAATTGGAGAAAGCTATC
FIP	ACTACGAGAAGTGCTCCAGGTGATAT TTCTGTAACAGCTACCAA
BIP	AATCCCACTCTTCTTGCGGTGCTACTT GCCAAACCAGG
LB	CGATCGTGCTCCGATGACTT

Table S2. Comparison of the JGB based colorimetric visualisation of LAMP with other strategies for the detection of *S. pneumoniae*.

Detection method	Probe	Readout	Limit of Detection	Assay time	Reference
LAMP	Leuco crystal violet	Colorimetric	10 ² genome copies	60 min	1
LAMP	Fuchsin	Colorimetric	1 pg/μL	60 min	2
LAMP	SYBR Green I	Fluorescence	0.01 pg/μL	62 min	3
Lateral flow detection	Fe ₃ O ₄ @DQDs	Fluorescence	10 cells/mL	45 min	4
LAMP	Phenol red	Electrochemical	2 × 10 ⁵ copies/μL	90 min	5
LAMP	JGB	Colorimetric	1 pg/μL	50 min	This work

References

1. C. Takano, Y. Kuramochi, M. Seki, D. W. Kim, D. Omagari, M. Sasano, B. Chang, M. Ohnishi, E. J. Kim, K. Fuwa, P. E. Kilgore, T. Hoshino and S. Hayakawa, *Scientific Reports*, 2019, **9**, 19823.
2. H. M. Kim and N. Y. Lee, *Chemosensors*, 2024, **12**, 75.
3. M. D. Cima-Cabal, E. Vázquez-Espinosa, F. Vazquez and M. d. M. García-Suárez, *J Pediatr Infect Dis*, 2020, **16**, 018-025.
4. C. Wang, W. Shen, Z. Rong, X. Liu, B. Gu, R. Xiao and S. Wang, *Nanoscale*, 2020, **12**, 795-807.
5. A. González-López, M. D. Cima-Cabal, P. Rioboó-Legaspi, E. Costa-Rama, M. d. M. García-Suárez and M. T. Fernández-Abedul, *Anal. Chem.*, 2022, **94**, 13061-13067.