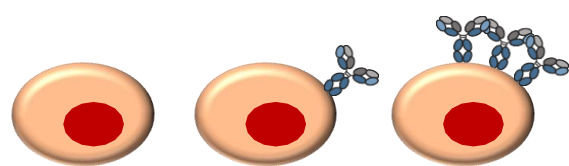


Introduction

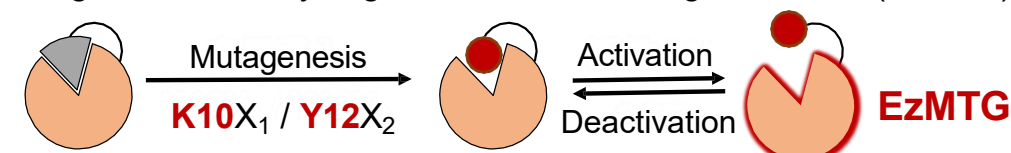
■ Features of target cells and enzymes

Target #1: Antibody-producing cells (hybridoma)



- ✓ Capable of producing large quantities.
 - × **Differential** antibody production among cell lines.
- It is essential to obtain cell lines with high antibody production capacity.

Target #2: Active zymogen of microbial transglutaminase (EzMTG)^{*1}

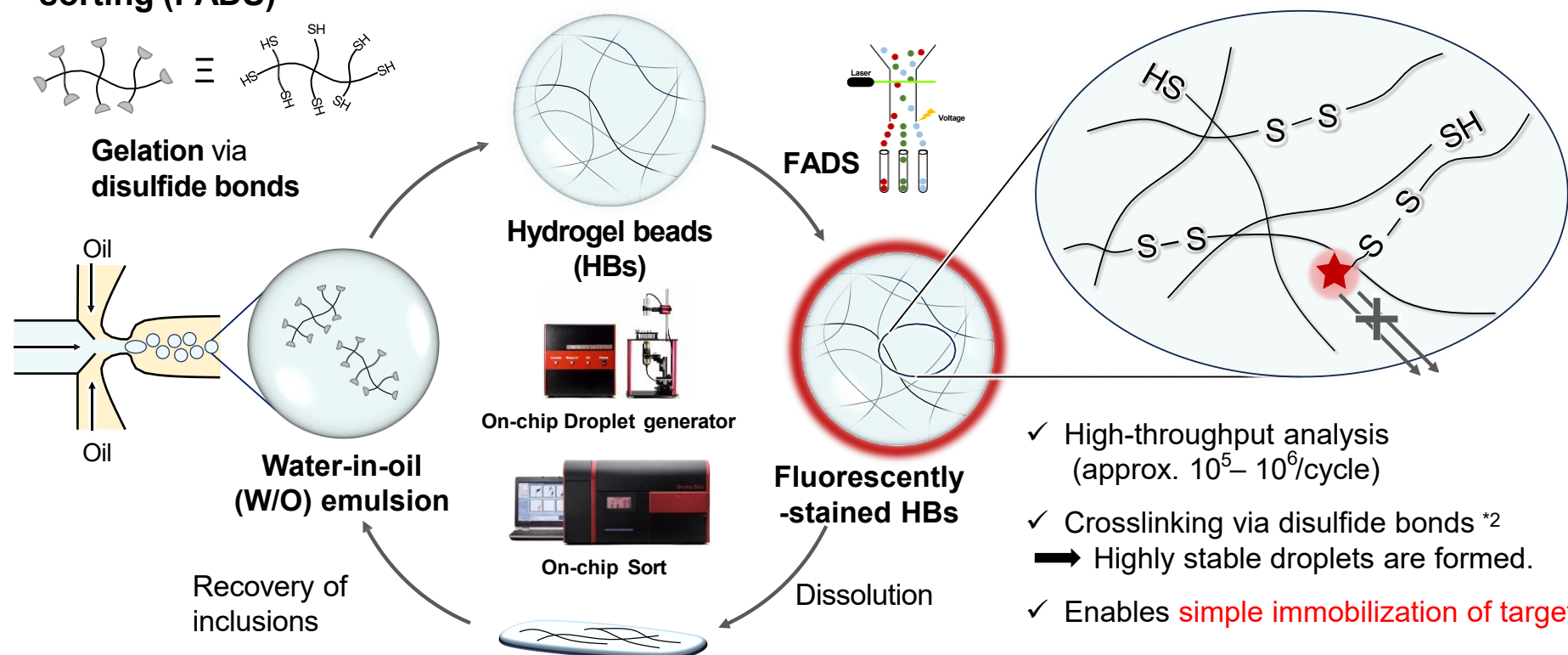


- ✓ Cross-links glutamine (Q) and lysine (K).
- × Difficult to identify mutation sites that contribute to activity.

Need to obtain highly active EzMTG and identify mutation sites.

*1: R. Ariyoshi, N. Kamiya et al., *Bioconjugate Chem.*, 35, 340-350 (2024)

■ A Selection system consisting of hydrogel beads (HBs) and fluorescence-activated droplet sorting (FADS)

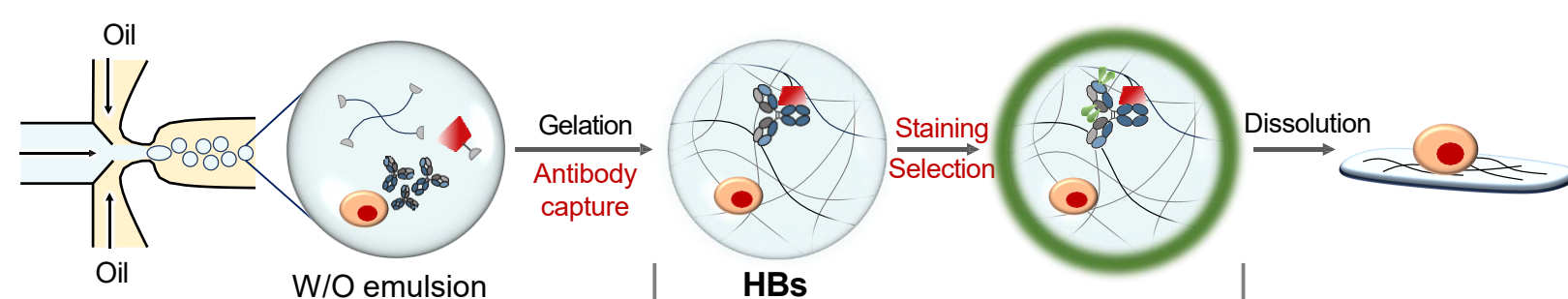


- ✓ High-throughput analysis (approx. 10^5 – 10^6 /cycle)
- ✓ Crosslinking via disulfide bonds^{*2}
- ➡ Highly stable droplets are formed.
- ✓ Enables **simple immobilization of targets**

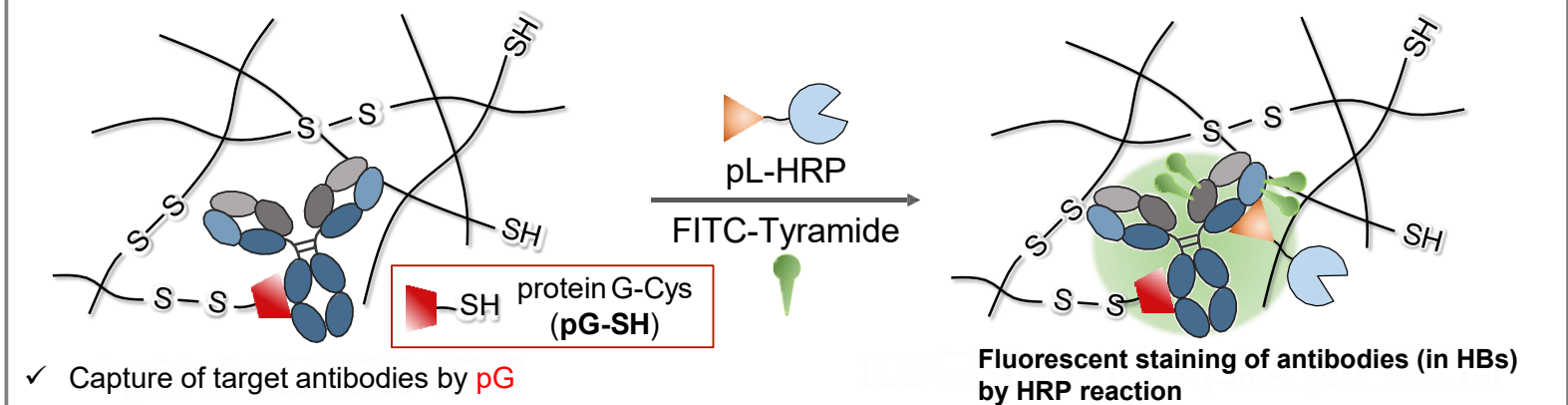
*2: K. Moriyama, N. Kamiya et al., *Chem. Commun.*, 50, 5895-5898 (2014).

Strategy & Experimental Results

■ Antibody-secreting cell selection system using hydrogel beads (HBs)

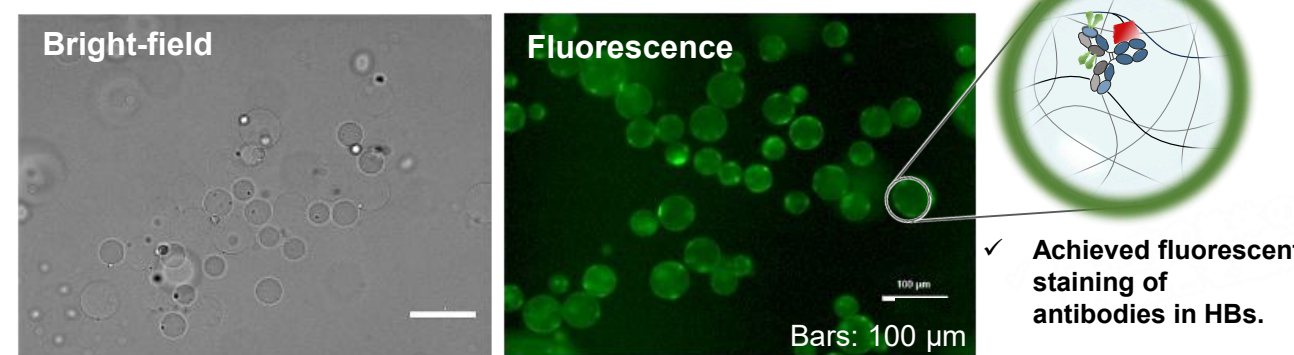


Strategy: Fluorescent staining of HBs based on antibody capture by antibody-binding molecules within HBs and TSA method^{*3,4}



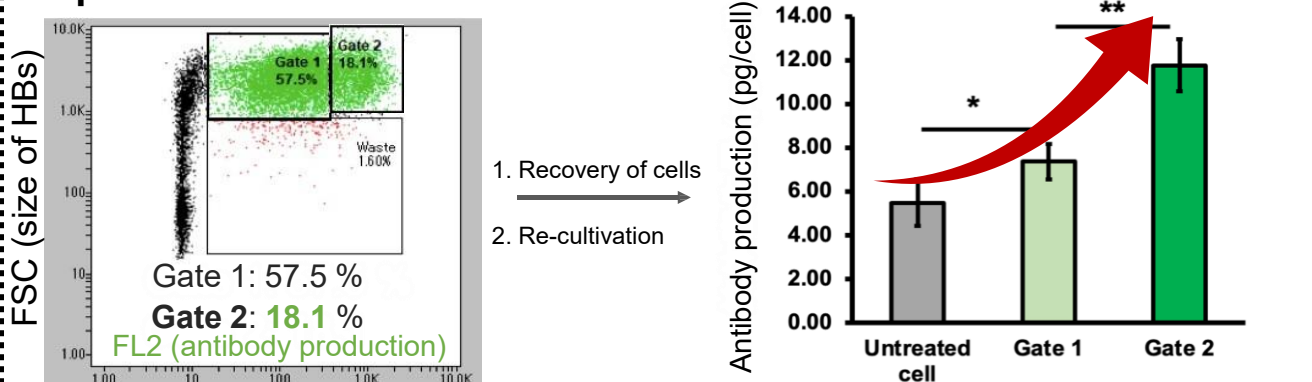
*3, 4: D. A. Wulandari, N. Kamiya et al., *ACS Biomater. Sci. Eng.*, 10, 628-636 (2024); *J. Biosci. Bioeng.*, 138, 89-95 (2024).

■ Proof of concept using purified IgG



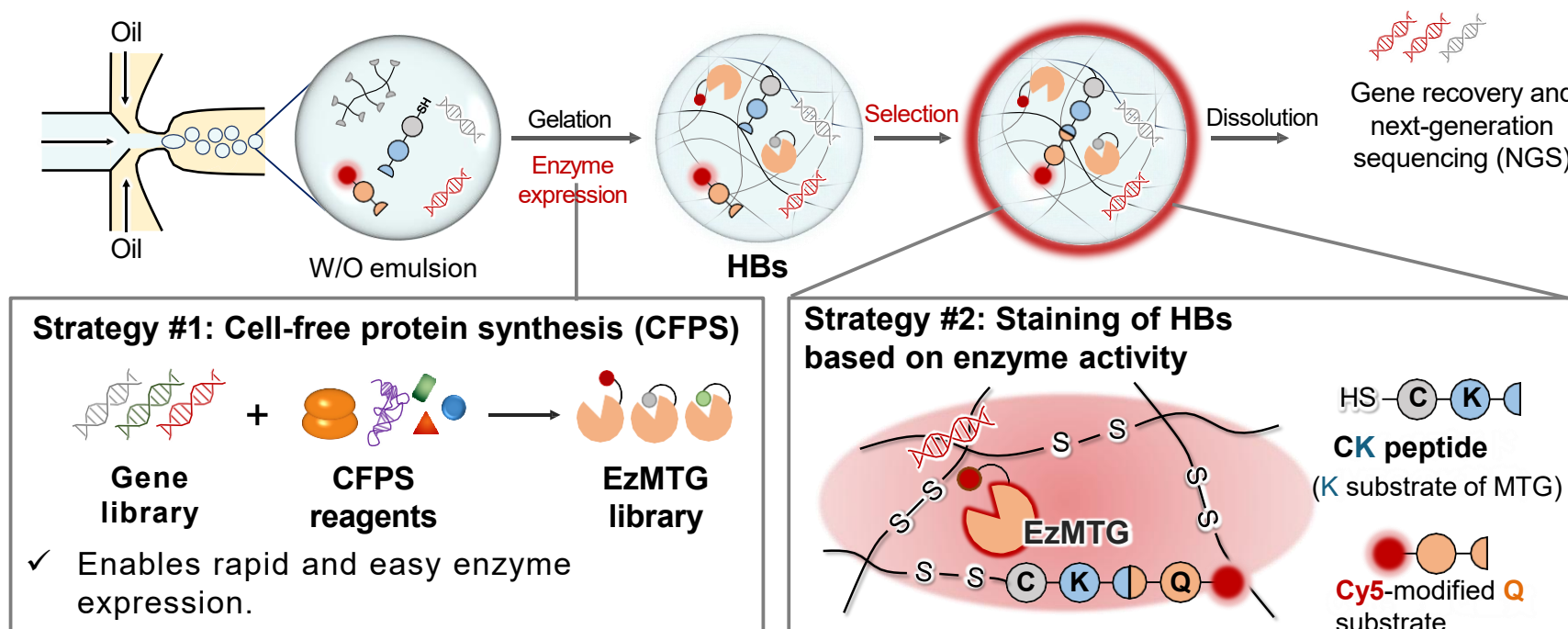
✓ Achieved fluorescent staining of antibodies in HBs.

■ Selection of HBs and evaluation of antibody production after culture

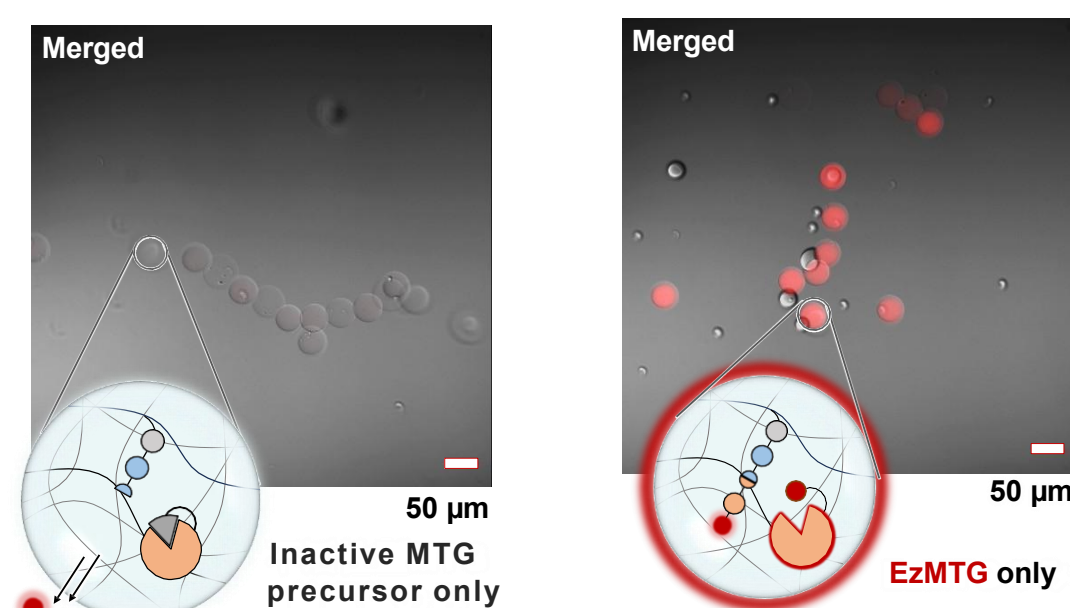


Successful selection and recovery of hybridoma with high antibody-producing capacity.

■ Construction of an enzyme selection system using hydrogel beads (HBs)

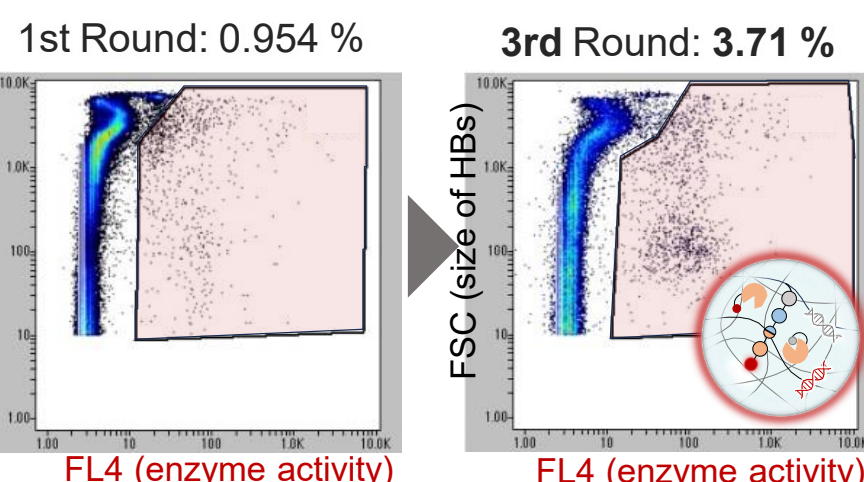


■ Staining of HBs according to MTG activity



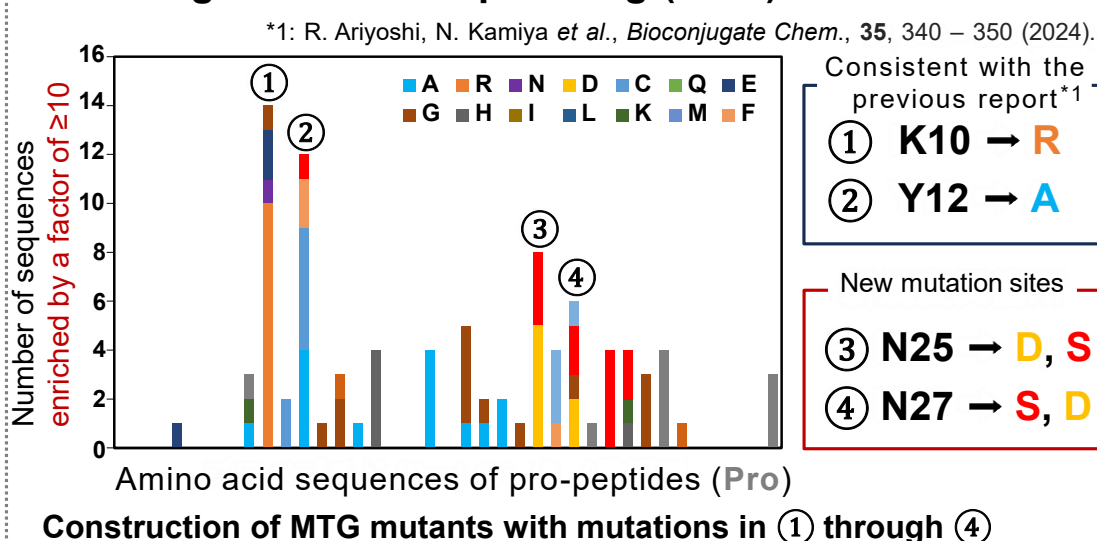
✓ Achieved staining of HBs according to enzyme activity.

■ Selection of HBs using On-chip Sort



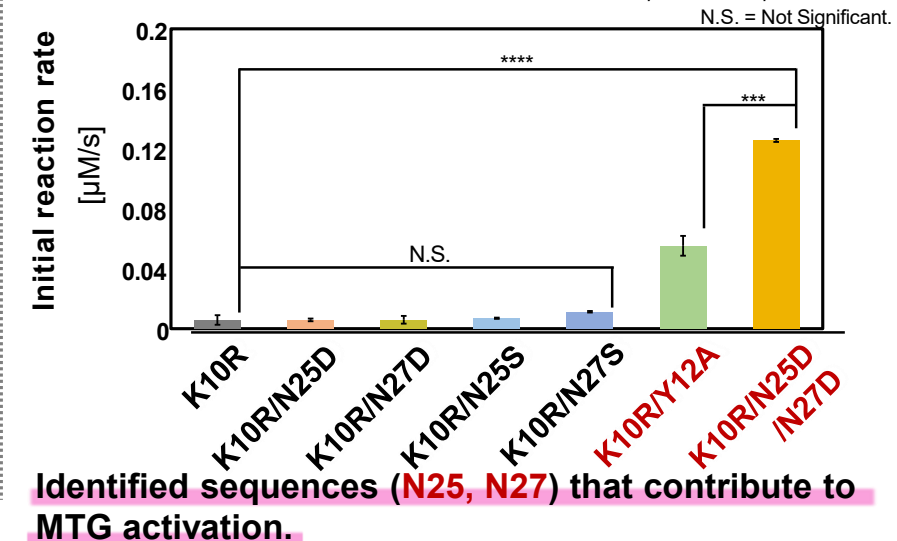
Redox-responsive HBs: Function as a site for selecting target cells and enzymes

■ Next-generation sequencing (NGS)



Construction of MTG mutants with mutations in ① through ④

■ Activity evaluation of the obtained mutants



Identified sequences (N25, N27) that contribute to MTG activation.

The establishment of foundational technology for the FADS-based selection system will be our next goal.

Conclusion

- ✓ We successfully obtained a system for detecting antibodies in HBs and cells with superior antibody production.
- ✓ A novel EzMTG mutant with excellent cross-linking activity was obtained using an enzyme selection system consisting of HBs and CFPS.

Acknowledgement

This study was supported by NEDO Biomufacturing Project and JSPS Grants-in-Aid for Scientific Research (JP23H00247).