

Traceless Delivery Strategy for Proteins

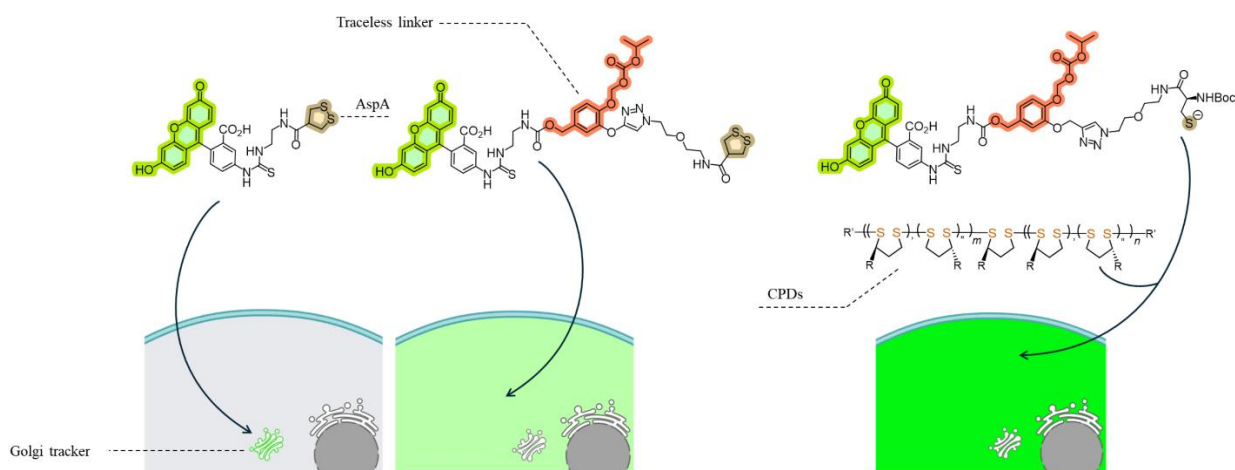
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The cell membrane is a key biological barrier that protects the cell and strictly regulates molecular exchange with the outer environment. While this control is essential for cellular integrity, it also prevents many functional large biomolecules, especially proteins, from entering the cytosol due to their size and polarity. Therefore, developing strategies that enable proteins to reach the cytosol without altering their structure or activity remains a major challenge.

Among the internalization mechanisms studied in recent years, thiol-mediated uptake (TMU) has emerged as a highly efficient pathway.^[1] TMU operates through dynamic covalent exchange between disulfide-rich transporters and exofacial thiols on the cell surface, allowing a broad range of substrates to enter cells.

In this work, we developed a traceless delivery strategy that combines TMU with an esterase-cleavable self-immolative linker.^[2] We first established a traceable model system based on an AspA scaffold, previously reported as a Golgi tracker, and demonstrated traceless release through the loss of this characteristic Golgi localization.^[3] Building on this result, we designed a second traceless probe in which AspA was replaced by cysteine, enabling compatibility with the “grafting-to” strategy using cell-penetrating poly(disulfide)s (CPDs). This approach retains the efficiency of TMU-mediated delivery at nanomolar concentration and provides an effective traceless system for the intracellular delivery of large cargos.^[4]



References:

- [1] Q. Laurent, R. Martinent, B. Lim, A.-T. Pham, T. Kato, J. López-Andarias, N. Sakai, S. Matile, *JACS Au* **2021**, *1*, 710–728.
- [2] J. V. Jun, Y. D. Petri, L. W. Erickson, R. T. Raines, *J. Am. Chem. Soc.* **2023**, *145*, 6615–6621.
- [3] S. Saidjalolov, X.-X. Chen, J. Moreno, M. Cognet, L. Wong-Dilworth, F. Bottanelli, N. Sakai, S. Matile, *JACS Au* **2024**, *4*, 3759–3765.
- [4] M. Cognet, G. Renno, F. Coelho, N. Sakai, S. Matile, *Angew. Chem. Int. Ed.* **2025**, e17229.