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Suspension Bead Loading (SBL): An Economical Protein Delivery Platform to Study URM1's Behavior in Live Cells

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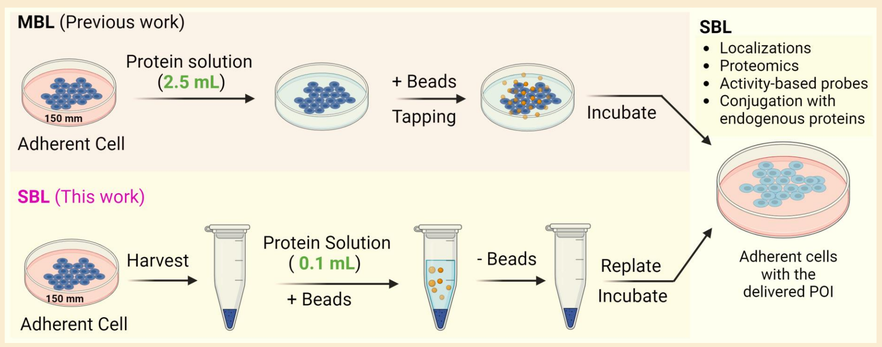
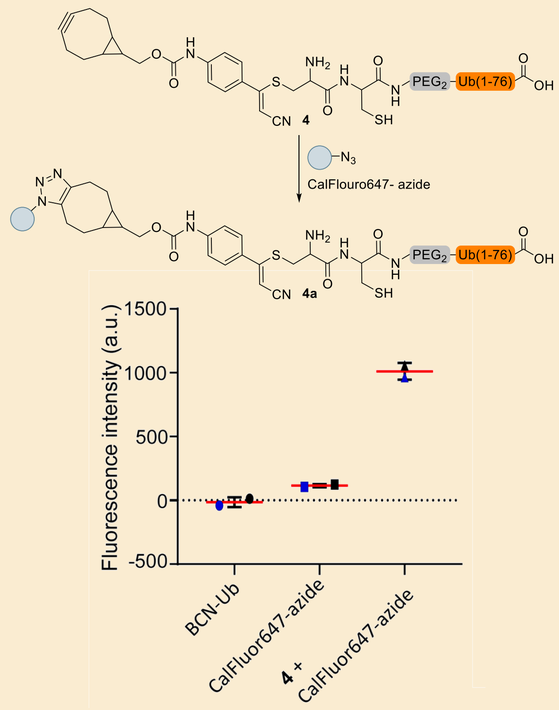
Abstract

Uniquely modified synthetic proteins are difficult to produce in large quantities, which could limit their use in various *in vitro* settings and in cellular studies. In this study, we developed a method named "suspension bead loading" (SBL), to deliver protein molecules into suspended living cells using glass beads, which significantly reduces the amount of protein required for effective delivery. We investigated the delivery efficiency of functionally different proteins and evaluated the cytotoxic effect of our method and the chemical and functional integrity of the delivered protein. We utilized SBL to address questions related to ubiquitin-related modifier 1 (URM1). Employing minimal protein quantities, SBL has enabled us to study its behavior within live cells under different redox conditions, including subcellular localization and conjugation patterns. We demonstrate that oxidative stress alters both the localization and conjugation pattern of URM1 in cells, highlighting its possible role in cellular response to such extreme conditions.



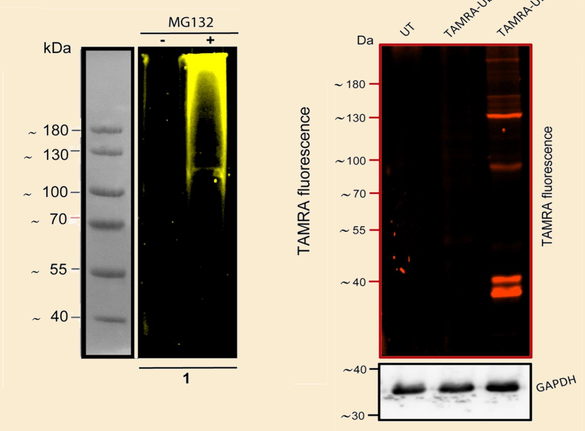
Chemical and functional charctrization of Ub cargos

Fluorecent intensity analysis of cargo (4) using SPAAc reaction confirms the intigerity of our synthetic Ub in live cells

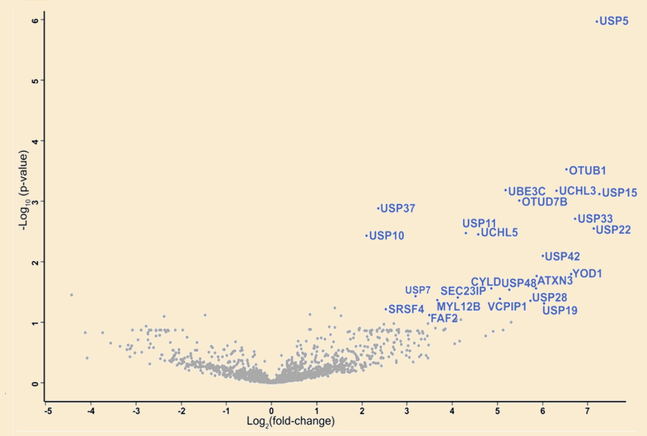


All functional analyses confirm that SBL didn't affect the integrity and functioanlity of our delivered proteins

Florecent gel analysis of the conjugation pattern of synthetic Ub (1), and for Ub-PA (5) caputring DUBs in live cells

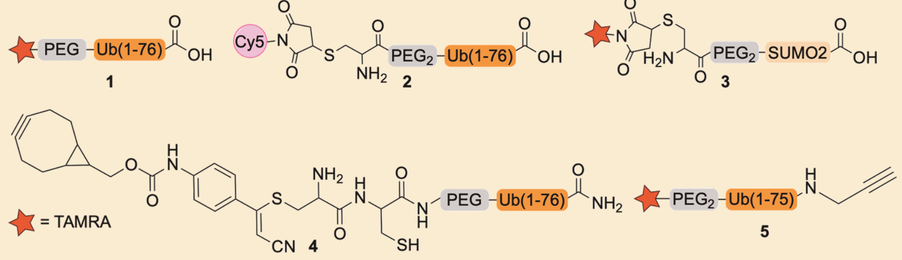


Volcano plot of enriched DUBs captured by Biotin-Ub-PA



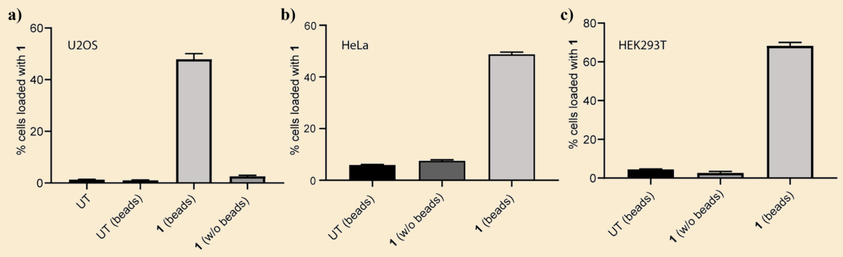
Results

The synthetic proteins emplyoed in SBL development



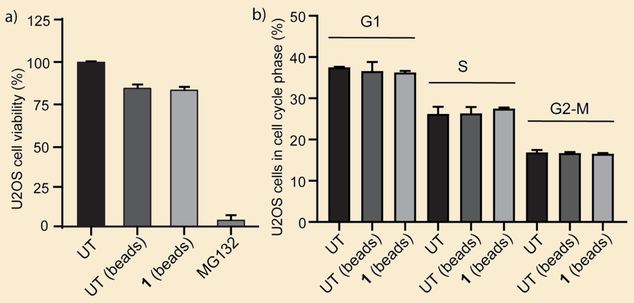
All protein were prepared chemically by Fmoc-SPPS

Cellular uptake of TAMRA-Ub (1)



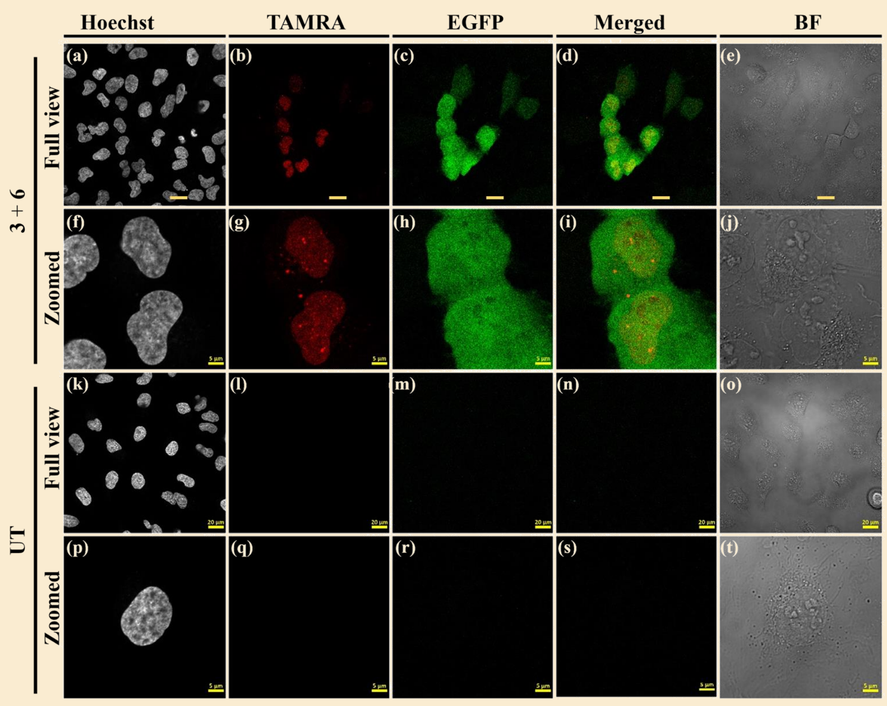
Our developed SBL method produces a considerable number of cells containing the protein cargo

Cytotoxcicity studies of SBL



a) **MTT cell proliferation assay on U2OS cells showed more than 85% of the cells remained viable after SBL**
b) **Propidium iodide (PI) based cell cycle assay shows no impact on the cell cycle phases**

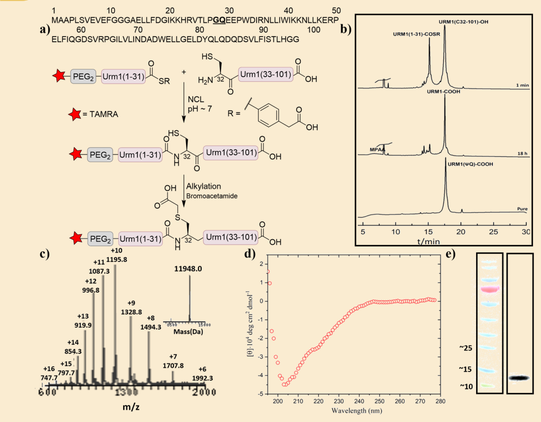
Live cells LCSM images of U2OS cells loaded with SUMO2 and EGFP



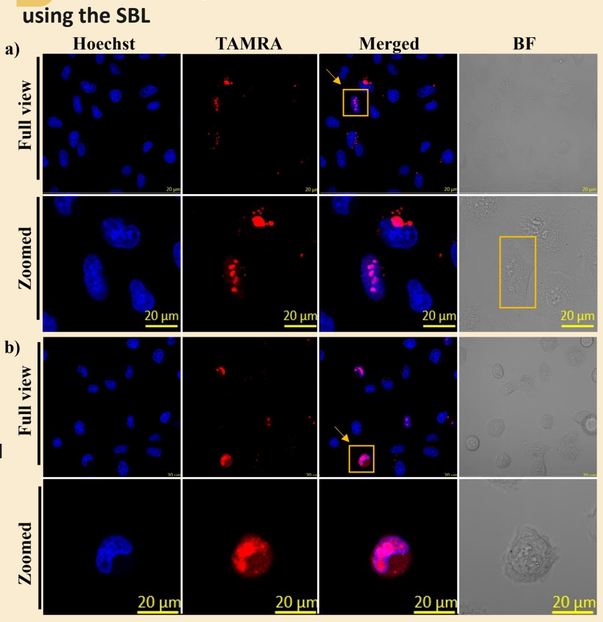
- **SUMO2 (3) localized in both the cystosol nucleus, and EGFP (6) is distributed all over the cell.**
- **Multiplexed bead loading in suspention showed also reproducible results.**

Applying SBL to study ubiqutin like modifier-1 (URM1)

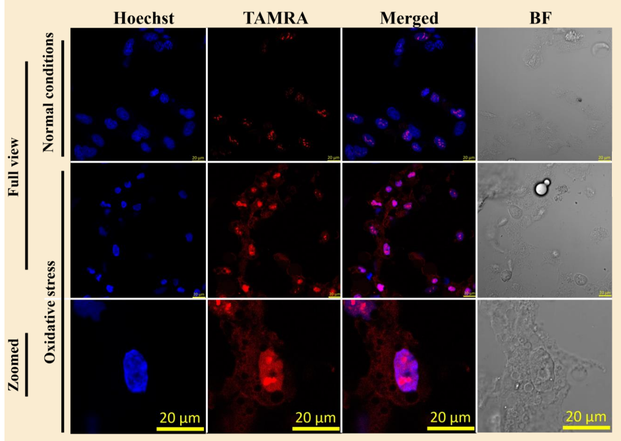
Chemical protein synthesis of URM1



Live cell LSCM images of U2OS cells loaded with URM1-COOH using the SBL

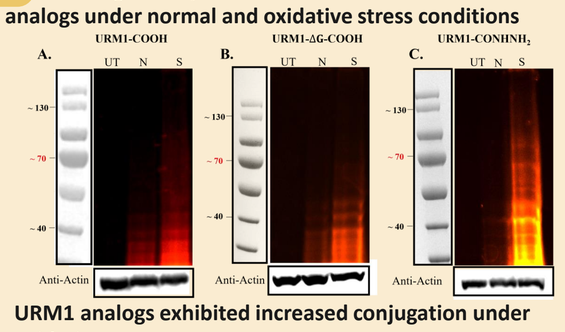


Live cell LSCM images of U2OS cells loaded with URM1-COOH using bead loading



All URM1 analogs localize in the nucleoles under normal condition and diffuse out under oxidative stress.

Fluorecent gel analysis of U2OS cells loaded with URM1 analogs under normal and oxidative stress conditions



URM1 analogs exhibited increased conjugation under oxidative stress

Conclusions

- **We developed a new general approach for delivering different functional proteins, in suspension phase, applying small amounts of protein(s).**
- **SBL is also suitable for multiplexed loading of proteins without requiring any special machinery or equipment.**
- **SBL allowed accessing for the first time URM1, and study some of its cellular properties. Despite challenges in URM1 synthesis, SBL enabled applying minimal quantities (0.1 ml vs. 1ml of protein solution).**
- **URM1 localizes in the nucleoli under normal conditions and diffuses out under oxidative stress, emphasizing its role and involvement in stress damage response.**
- **Regardless of its C-terminal functionality, URM1 forms fewer conjugates under normal conditions compared to oxidative stress.**

1. Abhishek Saha‡, Reem Mousa ‡, *et.al.* Suspension Bead Loading (SBL): An Economical Protein Delivery Platform to Study URM1's Behavior in Live Cells, *Angew. Chem. Int. Ed. Under revision* .
2. Mann, G.; Sadhu, P.; Brik, A. Multiplexed Delivery of Synthetic (Un) Conjugatable Ubiquitin and SUMO2 Enables Simultaneous Monitoring of Their Localization and Function in Live Cells. *ChemBioChem* 2022, *23*, e202200122.
3. Mann, G.; Sadhu, P.; Brik, A. Synthetic Proteins behind the Plasma Barrier: Molecular Spies. *Acc. Chem. Res.* 2022, *55*, 2055–2067.

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