Deciphering the Mechanism of Thiol-Mediated Uptake: µMap Strategy for Labeling Transient TMU Partners

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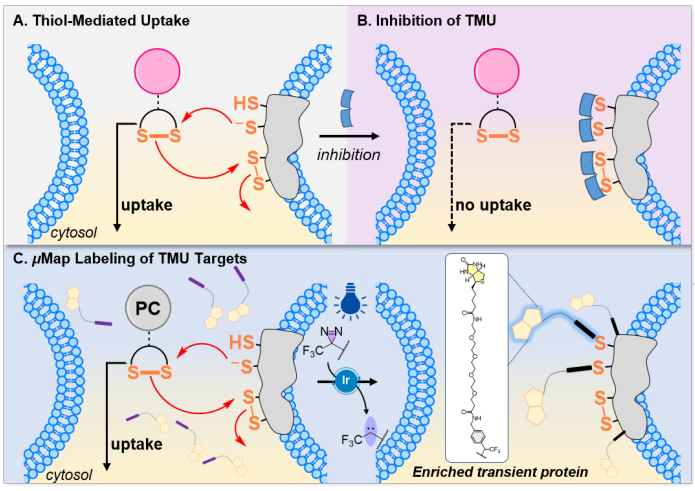
style="text-align: justify;">The cell membrane is the natural biological barrier of the cell that maintains its integrity, *i.e.* shape and size, and assures an exclusive exchange of molecules, from ions to proteins, with the outer matrix through several internalization processes. Thus deciphering its complete mechanism to exploit its full potential for drug delivery remains a challenging task with extremely high benefits in medicinal chemistry and pharmaceutics. Among the various studied internalization pathways, thiol-mediated uptake (TMU) has been recently given a lot attention due to its efficient transport of a wide range of substrates into the cytosol, from small molecules to HMW proteins. It is thought that this efficient method relies on a dynamic covalent cascade exchange (CAX) between a disulfide rich molecule, and exofacial thiols of transmembrane proteins on the cell surface. Despite the recent advances over the last years, the mechanism of uptake is still poorly understood.[1] This might be partially explained by the dynamic nature of the process that lacks of any exploitable steady state. Furthermore, recent studies validated TMU as a multi-target process,[2] thus adding a layer of complexity to the uptake mechanism. To elucidate this process, our toolkit was initially limited to classical chemical proteomics approaches, inefficient for labeling any transient interactions in this dynamic cascade exchange. To overcome this problem, the recently developed *µ*Map® strategy from the MacMillan group[3] appeared to be suitable for our project: the method relies on the use of a photocatalyst (PC) that upon blue light irradiation activates in a determined range, the UV-sensitive diazirines through a Dexter energy transfer (DET). The generated carbene reacts with its surrounding in a fast covalent manner, consequently, labeling the proximity of the photocatalyst. In our case, the CAX equipped with a PC could be a powerful tool to label the TMU pathway by highlighting all the transient interactions in the cell wall, thus, opening new perspectives on TMU mechanism.



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