Pattern Generation to Decode Thiol-Mediated Uptake

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Thiol-mediated uptake (TMU) is the process of cellular internalization facilitated by the continuous exchange (CAX) between thiol-reacting groups and exofacial thiols on transmembrane proteins. This process can be inhibited by using hydrophilic surface-thiol-reactive agents preventing any other molecule to participate in any CAX. TMU cascades are complex mechanisms that are not yet well understood due to their dynamic nature and fleeting intermediates. Recently, we demonstrated that TMU is not a single target process but rather involves a network of transmembrane proteins (TPs) meshed in a dynamic fashion. The most relevant example is the direct dependence of asparagusic acid to transferrin receptor for TMU.[1] These results drove our attention to thiol/disulfide rich transmembrane proteins that might be potentially involved in TMU. Integrins are TPs responsible for cell adhesion and motility, which contain a well-distributed thiol-rich β-subunit.[2] PDIs, more specifically PDIA3, are redox cell chaperones that are responsible for redox dependent processes both at the membrane surface, e.g. integrin activation, but also in the ER, e.g. protein folding.[3] To evaluate such relationships, we developed general protocols to decode dynamic covalent networks between CAXs and TPs. On a first instance, scratch assay was developed to assess how TMU inhibitors perform in motility-based studies. To further solidify our hypothesis, uptake inhibition patterns were obtained from removal of key integrin β-subunits by protein knockdown or by using specific inhibitors to inhibit PDI function. These inclusive TMU patterns reveal that the best three CAXs known today enter cells by almost orthogonal pathways. The bioinspired epidithiodiketopiperazines (ETP) exchange preferably with integrins, benzopolysulfanes (BPS) with protein disulfide isomerases (PDI), and asparagusic acid (AspA) with the transferrin receptor.

References

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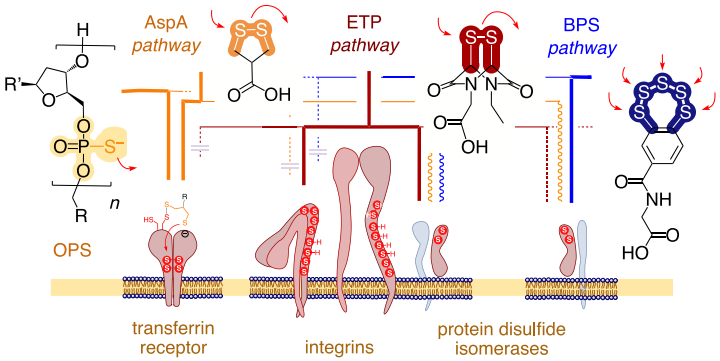

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