An approach to identify potential KDEL receptor interaction partners

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Eukaryotic KDEL receptors represent seven trans-membrane proteins which are not only localized in the ER and Golgi apparatus, but also be found to a minor amount at the cell surface of mammalian cells. Until now, it was postulated that KDELRs are mainly responsible for the retention of KDEL bearing proteins from the Golgi back to the ER. In the last years, a wide range of new KDELR functions are discovered including their role in (i) A/B toxin endocytosis, (ii) extracellular matrix degradation, (iii) cell adhesion as well as (iv) maintenance of Golgi homeostasis. Despite the manifold functions and localizations, only a handful of KDELR interactors are experimentally identified at the moment. Here, we try to overcome this limitation by using an engineered ascorbate peroxidase (APEX2) to label potential KDELR interaction partners with biotin in the near proximity (20 nm). In our pilot study, we were able to express biologically active KDELR-APEX2 fusion proteins in HeLa cells. After successful biotin-labeling and enrichment of the biotinylated proteins, potential interaction partner datasets of KDELR1, KDELR2 and KDELR3 could be generated and bioinformatically analysed in more detail.