

# [Upon 2 unique peptide matches to prevent false](https://assignbuster.com/upon-2-unique-peptide-matches-to-prevent-false/)

Upon EGF stimulation, EGFR in Her14 cells will be activated by theformation of dimers, tetramers, or higher-order oligomers. This could eitherinduce several downstream signal transduction pathways or it may induceendocytosis. According to Seedham et al, higher-order oligomerization starts bythe formation of dimers by a back-to-back dimerization interface located ondomain II, followed by repeated incorporation of additional dimers using theface-to-face interface at EGFR ectodomain I and III. The clustering of EGFRoligomers leads to a negative membrane curvature organized by the dimertransmembrane helices, which will function as hydrophobic wedges in the plasmamembrane. This EGFR clustering-induced membrane invagination will recruitcurvature sensitive proteins, such as F-BAR domain-containing FCHOproteins.  By isolating these activatedEGFR oligomer clusters using SMA copolymer, we are able to determine the relativeabundance of proteins recruited in the first steps of endocytosis.

Aspreviously mentioned, the EGF stimulated EGFR-enriched nanodiscs showedenhanced Tyr-1068 phosphorylation indicating trans-phosphorylation of kinasedomains (figure 3A). Because EGFR was used as bait during theimmunoprecipitation, the NSAF of each protein was normalized by the NSAF ofEGFR (see methods for further details). The relative protein abundance in thestimulated EGFR interactome was based on the ratio between the EGF-induced NSAFof proteins against the control NSAF of proteins (supplementary table 2 and 3). Table 1 provides an overview for a total of 72 proteins with an enrichmentfactor of ³ 1. 5 containing at least 2 unique peptidematches to prevent false positives in the EGF stimulated sample. Here, MYB-binding proteinP160 1a (Mybbp1a) shows the highest enrichmentvalue of 4. 375.

The enrichment value could not be calculated for twentyproteins due to the absence of unique peptide matches in the control sample. Nonetheless, they are taken into account based on the presence of ³2 unique peptide matches in the EGF-stimulated sample.