One-STrEP-tag for protein interactome analysis

Neumann et al., 2009

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate identification and characterization is a major challenge in life sciences. The extremely efficient and fast One-STrEP system is a reliable tool for the isolation of protein complexes in one purification step at high purity. Subsequent mass spectrometry analysis allows the identification of protein complex components (Junttila et al., 2005).

To elucidate the interactome of a protein of interest Neumann et al., 2009, employed stable isotope labeling by amino acids in cell culture (SILAC) in combination with high-end mass spectrometry analysis of One-STrEP-purified protein complexes. In their study they introduced this proteomic approach to analyse the interactome of the growth factor receptor-bound protein 2 (Grb2) in B cells *in vivo*, an adapter protein possessing a vast number of described interaction partners for the regulation of signal transduction in non B-lymphoid cells. For this purpose they reconstituted murine B-cells, lacking endogenous Grb2 expression, with either wildtype Grb2 or with One-STrEP-tagged Grb2. To unambiguously identify a protein as a Grb2 ligand by mass spectrometry, transfectants expressing wildtype Grb2 were metabolically labelled in SILAC medium containing `light´ forms of lysine and arginine, whereas transfectants expressing the One-STrEP-tagged version of Grb2 were labelled with `heavy´ forms of these two amino acids. The proteins of the corresponding B-cell lysates were affinity-purified via *Strep*- Tactin® columns and analysed by SDS-PAGE and MS/MS (Figure 1).

This quantitative proteomic approach, combining MS analysis of protein complexes purified with the efficient and specific One-STrEP system and SILAC, revealed a comprehensive overview of the Grb2 interactome in B cells.

The same authors published in Oellerich et al., 2009, another application example of SILAC in combination with the purification of One-STrEP-tagged proteins and LC-MS/MS. In this case they employed this method to quantify phosphorylation sites of the B cell receptor-proximal effector SLP-65.

References:

1. Junttila MR, Saarinen S, Schmidt T, Kast J, Westermarck J, 2005: Proteomics 5: 1199-1203. Single-step Strep-tag® purification for the isolation and identification of protein complexes from mammalian cells.
2. Neumann K, Oellerich T, Urlaub H, Wienands J, 2009: Immunol Rev 232: 135-149. The B-lymphoid Grb2 interaction code.
3. Oellerich T, Grønborg M, Neumann K, Hsiao HH, Urlaub H, Wienands J, 2009: Mol Cell Proteomics 8: 1738-1750. SLP-65 Phosphorylation dynamics reveals a functional basis for Signal integration by receptor-proximal adaptor proteins.

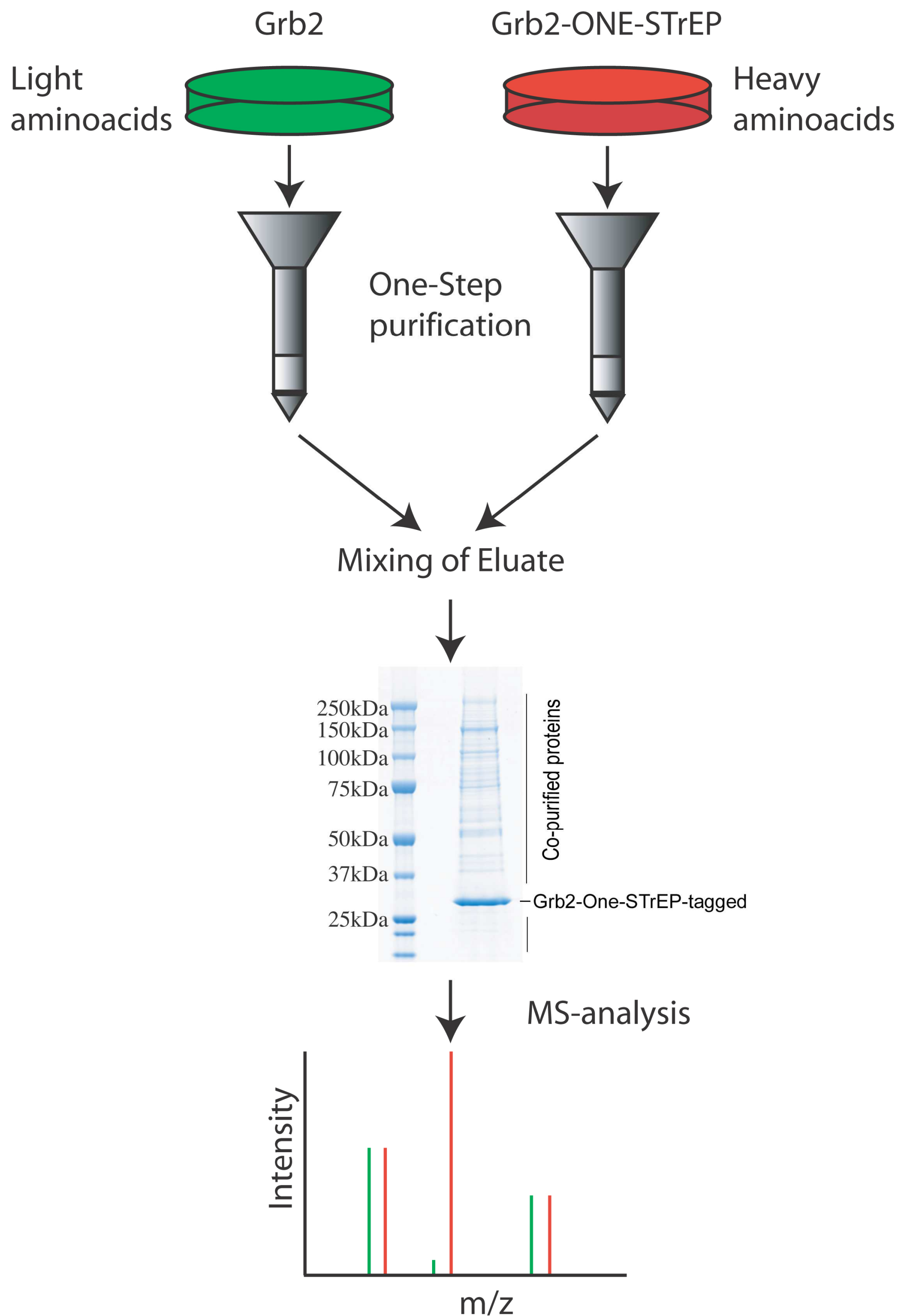

Figure 1: Workflow of SILAC combined with MS analysis of One-STrEP-tag purified proteins. One-STrEP-tagged Grb2 and untagged Grb2 were expressed in murine Bal17.TR cells metabolically labelled with \`heavy´ and \`light´ forms of lysine and arginine, respectively. Cells were stimulated through their IgM-BCR and proteins were affinity purified via Strep-Tactin® columns. Protein fractions were pooled at a 1:1 ratio, separated by one-dimensional gel electrophoresis, and in-gel digested. In MS analysis proteins non-specifically adhering to the matrix can be detected by their appearance in the \`heavy´ and \`light´ version at a ratio of approximately 1:1. Whereas the peptide peak intensities of specifically affinity-purified Grb2 ligands are much higher in the preparation from cells expressing tagged Grb2 (in red) than in the negative control from cells expressing untagged Grb2 (in green). (*Kindly provided by Dr. Konstantin Neumann*)