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Proteomic analysis of primary cells, mouse organs and whole embryos by 18 O-labeling and mass spectrometric analysis

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Analysis of tissues and organs by quantitative mass spectrometry is hampered by the inability to culture these materials for longer times. Metabolic labeling in these cases is not straightforward and therefore inexpensive and simple alternative protocols are desirable. We have used tryptic digest performed either in H216O or H218O to pursue comparative proteomics of primary cells, individual organs or whole embryos. In conjunction with peptide-based affinity selection a direct comparison with SILAC-based data showed that a very similar set of interaction partners for the T cell protein ADAP was obtained from Jurkat T cells when using the 18O method. Extension of the protocol to protein domain-based pulldown experiments and human primary T cells allowed us to delineate novel protein complexes in the context of proximal T cell receptor signaling events.

Biography

Christian Freund has completed his Ph.D at the Max-Planck-Institute of Biochemistry in Martinsried/Germany. After two post-docs in the labs of Andreas Plückthun (Zuerich University) and Gerhard Wagner and Ellis Reinherz (Harvard Medical School) he became independent group leader at the FMP and now holds a position as Professor of Biochemistry at Freie Universitaet Berlin, Germany.

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