HOKImag

Application in cell biology/ biochemistry:

Isolation of receptosomes and organelles from cells

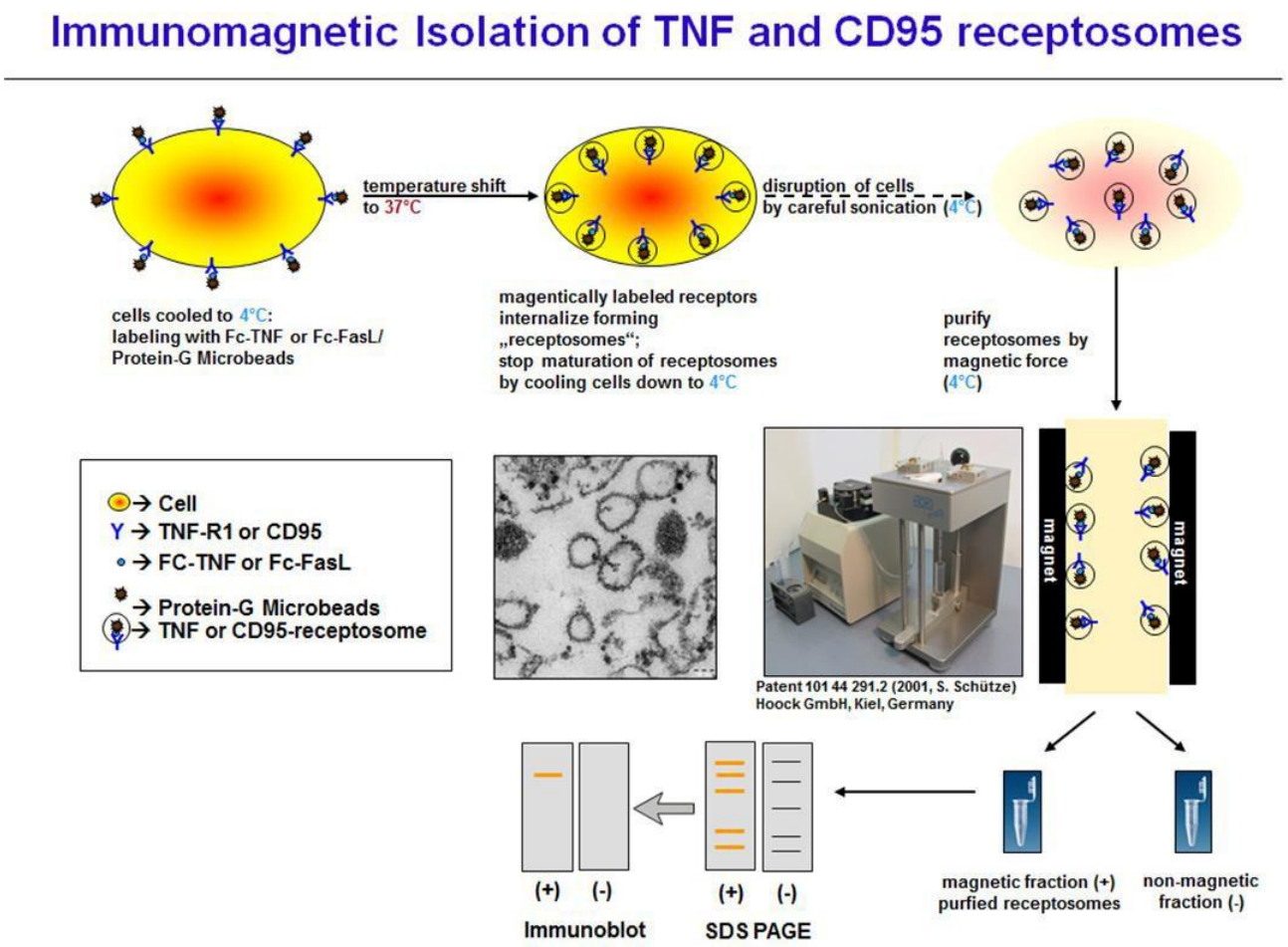
Analysis of the structure and function of intracellular **organelles, receptosomes, membrane complexes, and soluble protein complexes** from cells requires isolation of these compartments from whole cells. The HOKImag magnetic chamber system represents an ideal technology for selective immunomagnetic isolation.

Advantages of the "Free-Flow" method with HOKImag:

The up to 3 Tesla strong inhomogeneous magnetic field allows the use of smallest superparamagnetic beads (50nm) for labeling membrane receptors, organelle-specific surface proteins as well as soluble proteins. The small size of the magnetic particles is a prerequisite for undisturbed uptake of internalized receptor complexes (receptosomes), thus allowing physiological behavior of the compartments in the context of vesicular transport and maturation and fusion processes with other intracellular membrane complexes. The isolated structures are morphologically and functionally intact.

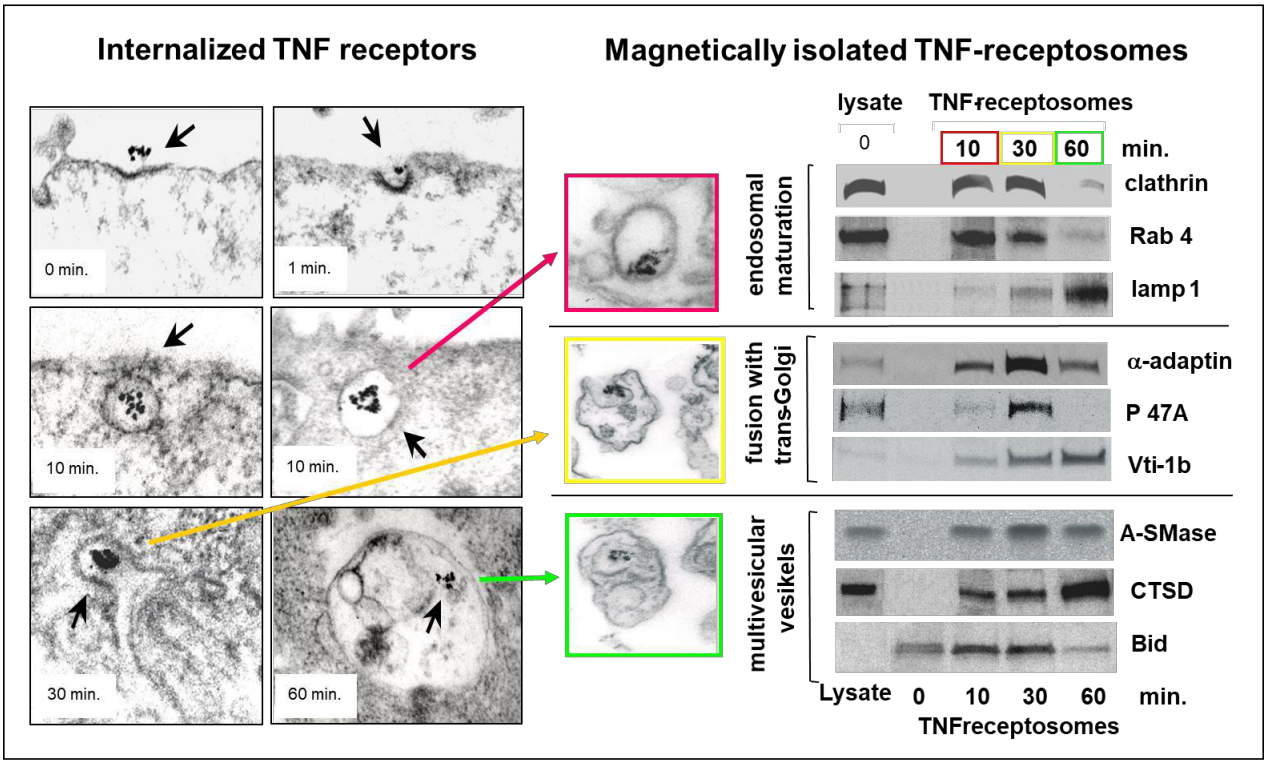
Examples for the application of the HOKImag magnetic chamber:

Labeling of the TNF receptors with FC-TNF and protein-G magnetic microbeads (alternatively biotinylated TNF and streptavidin-magnetic beads), followed by internalization of the receptor complex, homogenization of the cells and isolation of the magnetized receptosomes with HOKImag.



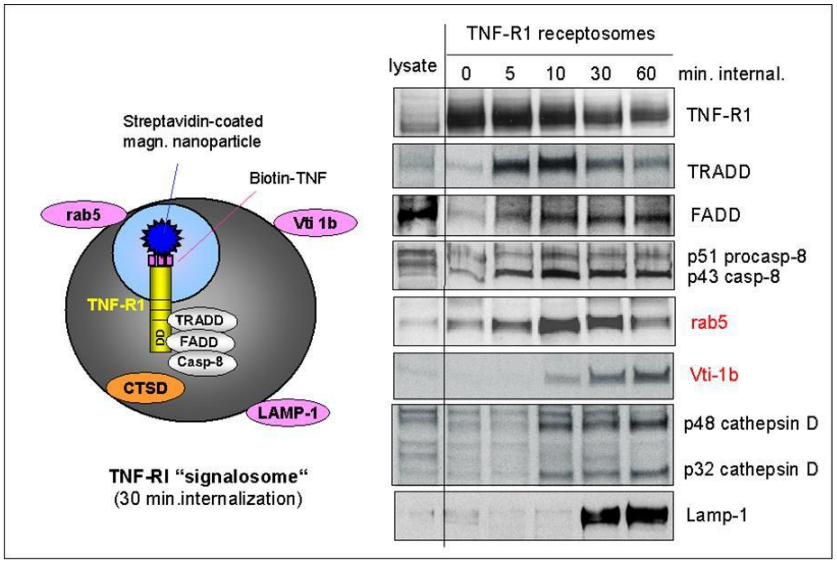
1. Isolation and characterization of TNF receptosomes

Electron microscopic visualization of internalization of TNF receptors labeled with streptavidin microbeads and isolation of receptosomes at different time points of intracellular transport by HOKImag, characterization of the different magnetic fractions based on the signature proteins vesicular transport by Western blot.



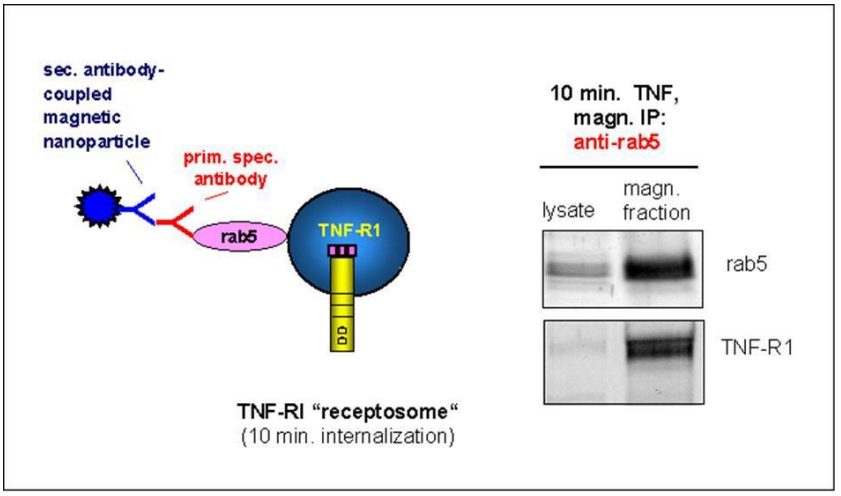
Source: Tchikov, V., Fritsch, J., Kabelitz, D., Schütze, S. (2010) Immunomagnetic isolation of subcellular compartments. ***Meth. Microbiol.*** 37, 21-34

II. Isolation of TNF receptosomes with streptavidin magnetic particles / biotin-TNF treated cells



Western blot analysis with HOKImag of isolated TNF receptosomes: association of the TNF receptor with the recruited adaptor proteins TRADD, FADD, and caspase-8, as well as the endosomal signature protein rab5, the MVB fusion protein Vti-1b, and the lysosomal proteins cathepsin D and Lamp-1.

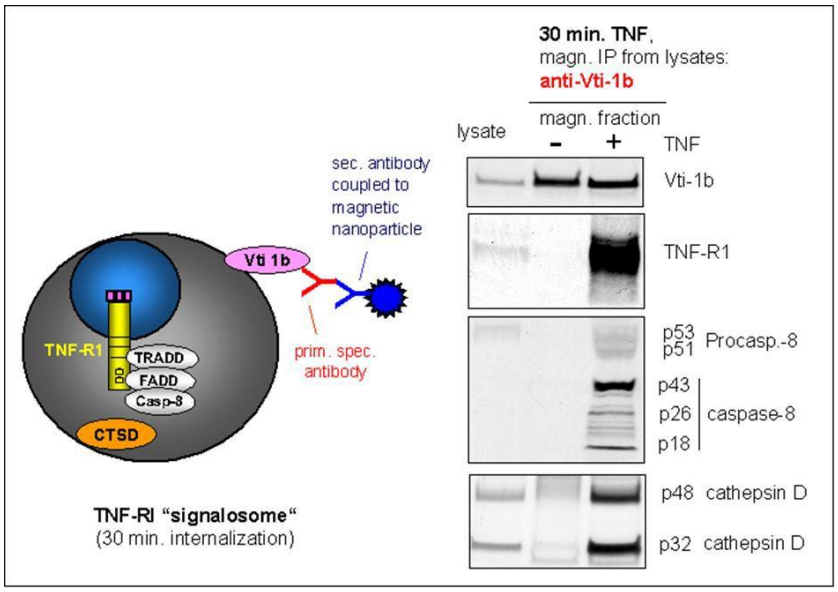
III. Isolation of endosomes with anti-rab5 specific antibody complexes from cells treated with TNF



Early endosomes containing the activated TNF receptor isolated from cell lysates using anti-rab 5 magnetic antibodies in the HOKImag chamber.

Source: Tchikov, V., Fritsch, J., Kabelitz, D., Schütze, S. (2010) Immunomagnetic isolation of subcellular compartments. ***Meth. Microbiol.*** 37, 21-34

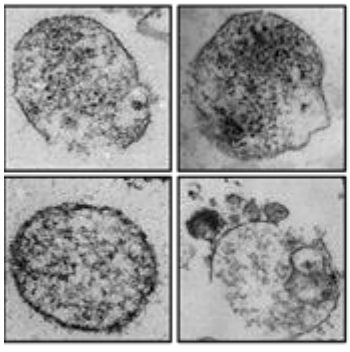
IV. Isolation of multivesicular compartments with anti-Vti-1b specific antibody complexes from cells treated with TNF

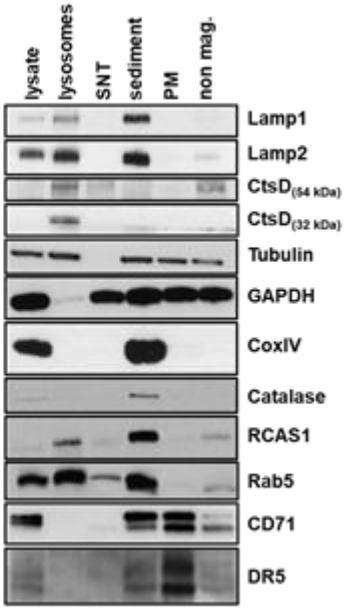


Late multivesicular vesicles containing activated TNF receptor isolated from cell lysates using anti-VTi-Ib magnetic antibodies in HOKImag magnetic chamber.

Source: Tchikov, V., Fritsch, J., Kabelitz, D., Schütze, S. (2010) Immunomagnetic isolation of subcellular compartments. ***Meth. Microbiol.*** 37, 21-34

V. Isolation of lysosomes with Lamp-1 specific antibody complexes from cell homogenates





Lysosomes isolated from cell lysates using magnetic anti-Lamp-1 specific antibodies in the HOKImag chamber.

Source: Fritsch, J., Tchikov, V., Hennig, L., Lucius, R., Schütze, S. (2019) A toolbox for the immunomagnetic purification of signaling organelles. ***Traffic.*** Mar;20(3):246-258.

Some of the results in which the HOKImag magnetic chamber system has found substantial use:

Organelle isolation (receptosomes, endosomes, lysosomes), methodology:

- Fritsch, J., Tchikov, V., Hennig, L., Lucius, R., Schütze, S. (2019) A toolbox for the immunomagnetic purification of signaling organelles. ***Traffic***. Mar;20(3):246-258. doi: 10.1111/tra. 12631. Epub 2019 Jan 15.
- Schütze, S., Tchikov, V., Fritsch, J. (2014). Separation of magnetically isolated TNF-receptosomes from mitochondria. ***Meth. Enzymol***. Vol. 535, Chapter 19, 327-349
- Schütze, S., Tchikov, V. (2008) Immunomagnetic isolation of TNF-receptosomes. ***Meth. Enzymol***. 442, 101-123
- Tchikov, V., Fritsch, J., Kabelitz, D., Schütze, S. (2010) Immunomagnetic isolation of subcellular compartments. ***Meth. Microbiol***. 37, 21-34
- Melum, E., Jiang, X., Baker, K.D., Macedo, M.F., Fritsch, J., Dowds, C.M., Wang, J., Pharo, A., Kaser, A., Tan, C., Pereira, C.S., Kelly, S.L., Duan, J., Karlsen, T.H., Exley, M.A., Schütze, S., Zajonc, D.M., Merrill, A.H., Schuchman, E.H., Zeissig, S., Blumberg, R.S. (2020) Control of CD1d-restricted antigen presentation and inflammation by sphingomyelin. ***Nature Immunol***. 2019 Dec;20(12):1644-1655

Soluble protein complexes:

- Marischen, L., Wesch, D., Oberg, H.H., Rosenstiel, P., Trad, A., Shomali, M., Grötzinger, J., Janssen, O., Tchikov, V., Schütze, S., Kabelitz, D. (2011) Functional expression of NOD2 in human peripheral blood γδT-cells.***Scand. J. Immunol***. 74, 126-134

TNF receptosomes:

- Zingler, P., Särchen, V., Glatter, T., Caning, L., Saggau, C., Kathayat, R.S., Dickinson, B.C., Adam, D., Schneider-Brachert, W., Schütze, S., Fritsch, J. (2019) Palmitoylation is required for TNF-R1 signaling. ***Cell Commun. Signal.*** Aug 5;17(1):90. doi: 10.1186/s12964-019-0405-8.

- Fritsch, J., Zingler, P., Särchen, V., Heck, A.-L., Schütze, S. Role of ubiqutination and proteolysis in the regulation of pro- and anti-apoptotic TNF-R1 signaling. (2017) ***BBA – Mol. Cell Res.*** 2017 Jul 29. pii: S0167-4889(17)30203-3. doi: 10.1016/j.bbamcr.2017.07.017. [Epub ahead of print]
- Fritsch, J., Fickers, R., Klawitter, J., Särchen, V., Zingler, P., Adam, D., Janssen, O., Krause, E., Schütze, S. (2016) TNF induced cleavage of HSP90 by Cathepsin D potentiates apoptotic cell death. ***Oncotarget***, 2016. doi: 10.18632/oncotarget.12411. [Epub ahead of print]
- Fritsch,J., Stephan, M., Winoto-Morbach, S., Tchikov, V., Gubkina, S., Kabelitz, D., Schütze, S. (2014) Cell-fate decisions to TNF regulated by K63 ubiquitination of TNF-receptor 1. ***Mol. Cell. Biol***. 34 (17), 3214-3228
- Sosna, J., Voigt, S., Mathieu, S., Kabelitz, D., Trad, A., Tholey, A., Janssen, O., Meyer-Schwesinger, C., Schütze, S., Adam, D. (2013) The protease Htr2/Omi and UCH-L1 regulate TNF-induced necroptosis. ***Cell. Signal. Commun***., 2013, Oct 3;11:76. doi: 10.1186/1478-811X-11-76
- Sosna, J., Voigt, S., Mathieu, S., Lange, A., Thon, L., Davarnia, P., Herdegen, T., Linkermann, A., Rittger, A., Ka-Ming Chan, F., Kabelitz, D., Schütze, S., Adam, D. (2013) TNF-induced necroptosis and PARP-1-mediated necrosis represent two distinct and independent routes to programmed cell death. ***Cell. Mol. Life Sci***. . (published online 13. June 2013; DOI 10.1007/s00018-013-1381-6)
- Klingeisen, L., Ehrenschwender, M., Heigl, U., Wajant, H., Helgans, T., Schütze, S., Schneider-Brachert, W. (2012) E3-14.7K is recruited to TNF-Receptor 1 and blocks TNF cytolysis independent from interaction with optineurin. ***PLoS ONE*** 7(6):e38348. doi: 10.1371/journal.pone.0038348. Epub 2012 Jun 4.7
- Edelmann, B., Bertsch, U., Tchikov, V., Winoto-Morbach, S., Jakob, M., Adam-Klages, S., Kabelitz, D., Schütze, S. (2011). Caspase-8 and caspase-7 sequentially mediate proteolytic activation of acid sphingomyelinase in TNF-R1-receptosomes. ***EMBO-J***. 30. 379-394
- Bertsch, U., Edelmann, B., Tchikov, V., Winoto-Morbach, S., Schütze, S. (2011) Compartmentalization of TNF-receptor 1 signaling: TNF-R 1 - associated caspase-8 mediates activation of acid sphingomyelinase in late endosomes. ***Adv. Exp. Med. Biol***. 691, 605-16
- Tchikov, V., Bertsch, U., Fritsch, J., Edelmann, B., Schütze, S. (2011) Subcellular compartmentalization of TNF receptor-1 and CD95 signaling pathways. ***Eur. J. Cell Biol.*** 90, 467-475

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- Liao, W., Fujita, K., Tchikov, V., Yang, W., Gunsor, M., Garfield, S., Goldsmith, P., El-Deiry, W.S., Schütze, S., Srinivasula, S. (2009) CARP1 regulates induction of NF−κB by TNFα. ***Curr. Biol***. 19, R17-R19
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- Schneider-Brachert, W., Tchikov, V., Held-Feindt, J., Winoto-Morbach, S., Neumeyer, J., Jakob, M., Merkel, O., Ehrenschwender, M., Heinrich, D., Adam, D., Kabelitz, D., and Schütze, S. (2004) Compartmentalization of TNF Receptor-1 Signaling: Internalized TNF Receptosomes as Death Signaling Vesicles***. Immunity*** 21, 415-428

TRAIL receptosomes:

- Voigt, S, Philipp, S, Davarnia, S, Winoto-Morbach, S, Röder, C, Arenz, C, Trauzold, A, Kabelitz, D, Schütze, S, Kalthoff, H, Adam, D. (2014) TRAIL-induced programmed necrosis as a novel approach to eliminate tumour cells. ***BMC Cancer*** 14(1):74

- Lemke J, Noack A, Adam D, Tchikov V, Bertsch U, Röder C, Schütze S, Wajant H, Kalthoff H, Trauzold A. (2010) TRAIL signaling is mediated by DR4 in pancreatic tumor cells despite the expression of functional DR5. ***J. Mol. Med***. 88, 729-740

CD95 receptosomes:

- Stephan, M., Edelmann, B., Janssen, O., Schütze, S. Fritsch, J. (2017) Biphasic activation of acid sphingomyelinase by CD95 Ligand stimulation. ***Oncotarget***, Mar 21;8(12):20067-20085. doi: 10.18632/oncotarget.15379.
- Feig, C., Tchikov, V., Schütze, S., Peter, M.E. (2007). Palmitoylation of CD95 facilitates formation of SDS-stable receptor aggregates that initiate apoptosis signaling. ***EMBO J.*** 26:221-231
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