## "Cell fractionation and organelle isolation"

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Eukaryotic cells are composed of different compartments that comprise all the closed parts within the cytosol surrounded by a single or double lipid layer membrane. These include mitochondria, peroxisomes, endosomes, lysosomes, the endoplasmic reticulum, the nucleus and the Golgi apparatus. The major role of these compartments is to establish physical boarders for biological processes that enable the cell to carry out different metabolic activities at the same time.

In our course we want to teach how cellular compartmentalization can be observed by different techniques. First we will visualize different cellular compartments using immunofluorescence techniques with compartment specific antibodies. To gain an insight into the dynamics of these organelles we will perform life cell imaging of secretory proteins and focal adhesion markers fused to GFP. Finally we will biochemically isolate Golgi membranes and endosomes by sucrose density gradient fractionation. Thus this course will provide a basis of classical biochemical and cell biology approaches.

## Literature:

- Balch WE, Dunphy WG, Braell WA and Rothman JE, Reconstitution of the transport of protein between successive compartments of the golgi measured by the coupled incorporation of N-acetylglucosamine, 1984, Cell, 39 (2), 405-416
- von Blume J, Alleaume AM, Kienzle C, Carreras-Sureda M, Valverde M, and Malhotra V. Cab45 is required for Ca2+-dependent secretory cargo sorting at the trans-Golgi network. J. Cell Biol. 2012 199 (7)
- Böttcher, R.T., Stremmel, C., Meves, A., Meyer, H., Widmaier, M., Tseng, H.-Y., Fässler, R. (2012) Sorting nexin 17 prevents lysosomal degradation of ß1 integrins by binding to the ß1-integrin tail.Nature Cell Biology 14:584–592
- Tseng HY, Thorausch N, Ziegler T, Meves A, Fässler R, Böttcher RT. (2014) Sorting Nexin 31 Binds Multiple ß Integrin Cytoplasmic Domains and Regulates ß1 Integrin Surface Levels and Stability.J Mol Biol. 426(18):3180-94