Physikalisch-Technische Bundesanstalt
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Development of peptide-based IDMS for quantification of cell-surface markers

Background

The measurement of a blood count, via flow cytometry, is a routinely requested measurement in the field of laboratory medicine. Here the diagnostic measurand is the antibody binding capacity for a specific antibody with a cell surface antigen. This is indicative of a cell's state or its response to certain stimuli. It enables rapid screening and thus the initial diagnosis of many diseases. Accurate and comparable measurement results are essential to support the diagnosis and treatment of patients. Standardization, the linking of measurement results to standards of known quantities, is a recognized approach to improving the comparability of measurements. Therefore, an unbroken calibration chain for antibody binding capacity with traceability to the SI would help achieve comparable measurement results. To realize this for flow cytometry applications, it is necessary to understand the amount of cell surface markers/antigens on the surface of a cell. In this project, we intend to quantify the amount of cell-surface markers per cell via peptide-based isotope dilution mass spectrometry.

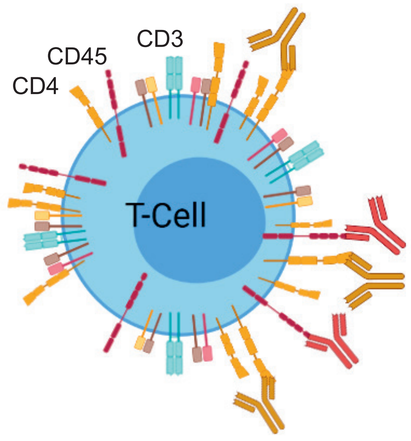
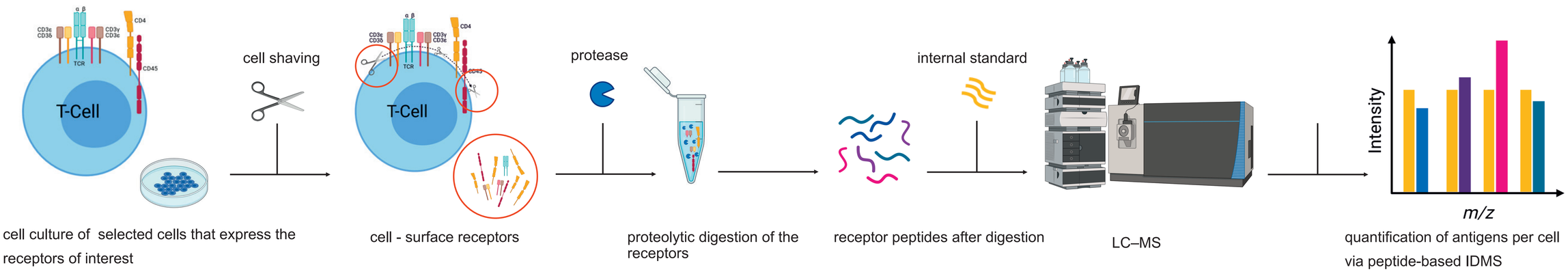


Figure 1: T- cell with CD3, CD4 and CD45 antigens, bound antibodies cover neighbouring receptors/ antigens → prevent binding of further antibodies. In this project T-cells serve as a model system to quantify the cell surface proteins.

Principle of experiments



cell culture of selected cells that express the receptors of interest

cell - surface receptors

proteolytic digestion of the receptors

receptor peptides after digestion

LC–MS

quantification of antigens per cell via peptide-based IDMS

Figure 2: Experimental workflow. After cell culture, the surface receptors/antigens are cut off from the cell. Then the preconcentrated and purified antigens go through proteolytic digestion to release unique peptides for quantification. These peptides are analysed by LC-MS/MS. The intensity ratio between cell-surface peptides and internal standards enables the absolute protein quantification of cell-surface proteins to be determined. To ensure SI-traceability, the internal peptide standards were verified by amino acid analysis.

All figures created with BioRender.com.

Results

Protein	Peptide sequence	Peptide length	Peptide mass [M+H]2+ av.
T-cell surface glycoprotein CD4	ILGNQGSFLTK	11	589.5
	IDIVVLAFQK	10	573.5
	SWITFDLK	8	505.1
	GDTVELTCTASQK	13	676.9
T-cell surface glycoprotein CD3 epsilon chain	NIGSDEDHLSLK	12	664.4
	EFSELEQSGYYVCYPR	16	985.5
	GSKPEDANFYLYLR	14	837.0
Receptor-type-tyrosine-protein phosphatase C (CD45)	TLILDVPPGVEK	12	641.0
	LENLEPEHEYK	11	701.0
	DLQYSTDYTFK	11	690.9
	YVLSLHAYIIAK	12	696.1

Table 1: Peptide selection based in mass spectrometry results. Unique peptides were selected according to the guidelines of Ludwig et al. 2014.

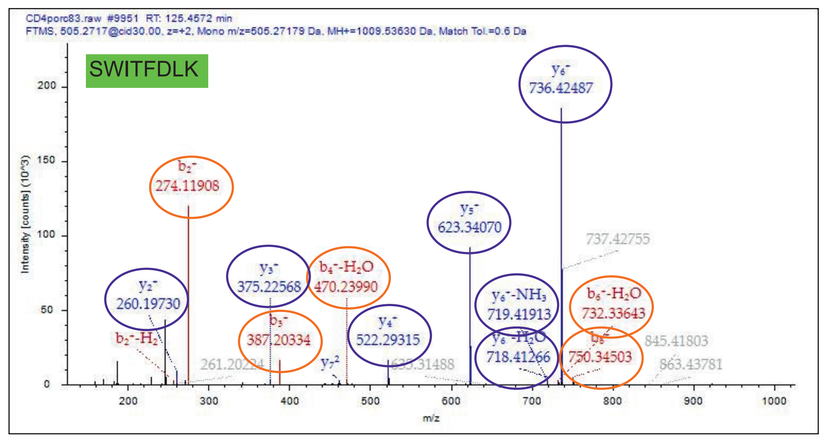


Figure 3: Example of a CD4 peptide product ion spectrum. Both ion series are consistent with the amino acid sequence.

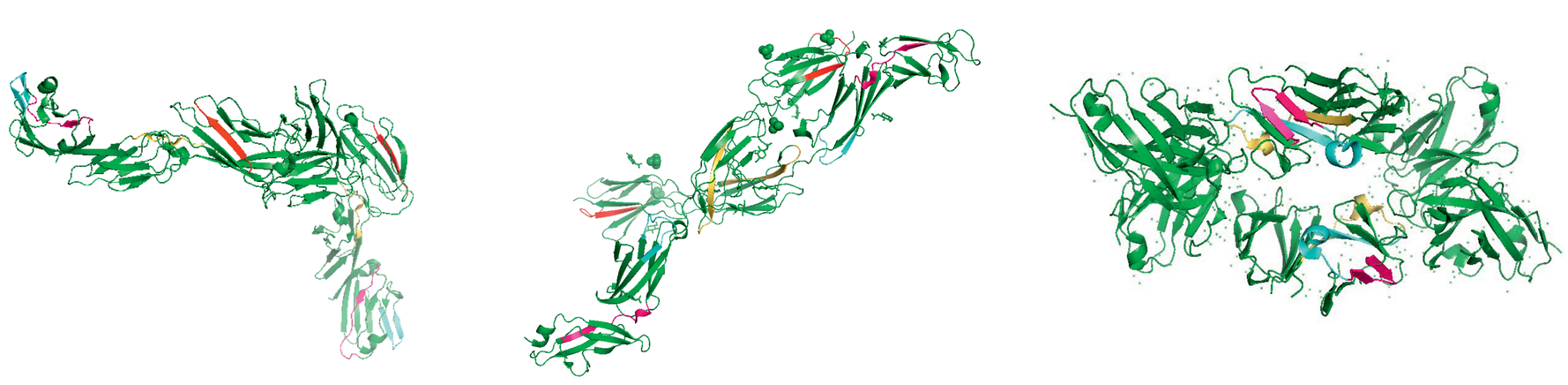


Figure 4: 3D-Model of cell-surface proteins CD3, CD4 and CD45. The unique peptides for quantification that were found after LC-MS/MS are shown in magenta, cyan, yellow and red.

T-cell surface glycoprotein CD4 (A/B chain)

T-cell surface glycoprotein CD45 extracellular region, domains d1-d4 (A/B chain)

T-cell surface glycoprotein CD3-E/D dimer

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