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PURIFICATION OF MEMBRANE PROTEINS FROM ENDOTHELIAL CELLS OF THE BLOOD BRAIN BARRIER FOR THEIR ANALYSIS BY LC-MS/MS

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The central nervous system (CNS) is a therapeutic target for many pharmacologically active compounds. To reach their target, these molecules must cross the blood-brain barrier (BBB). The BBB is a selective interface between the bloodstream (BS) and the CNS, whose role is to maintain brain homeostasis. Thus, in order to pass from the bloodstream to the CNS or vice versa, endogenous and exogenous compounds must pass through the endothelial cells of the BBB. These cells regulate the penetration of molecules through their membrane transporters [1]. To model the passage of drugs through the BBB using Physiologically Based Pharmacokinetic models, it is necessary to quantify the transport proteins there. The tandem LC MS / MS quantification method, which we currently use, shows high LOQs for some of these proteins. The goal of our study is to develop a specific extraction method for membrane proteins from BBB endothelial cells in order to reduce these LOQ. The approach used is the biotinylation of surface proteins by sulfo-NHS-SS-biotin and their extraction using magnetic beads coupled to streptavidin, a protein with a very high affinity for biotin ($K_d = 10^{14}$ - 10^{15} mol.L⁻¹) [2]. A cellular model of BBB endothelial cells is used to perform these studies: the hCMEC/D3 cell [3]. First, we ensured the localization of the proteins bound to sulfo-NHS-SS-biotin by cell labelling and mass spectrometry analysis. We found that the reagent binds to different amino acids (K, T, Y, S, N, Q) of proteins located at the plasma membrane (Fig. 1). We also developed the rinsing and elution buffers for biotinylated proteins. For this purpose, we biotinylated BSA and introduced it into a lysate of hCMEC/D3 cells.

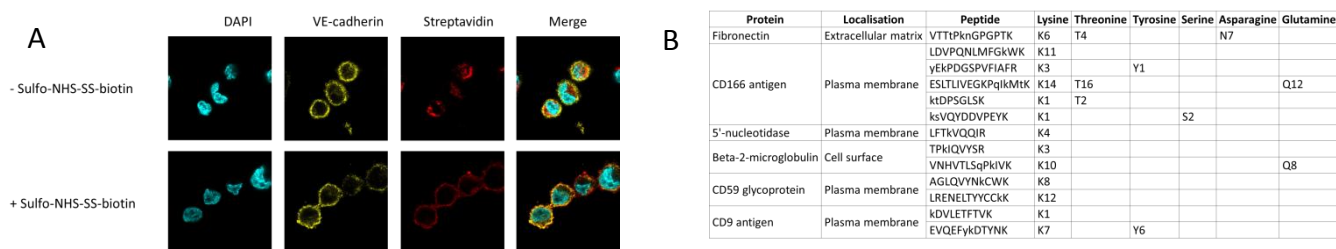


Fig. : Localization of biotinylated proteins by cell labelling (A) and mass spectrometry analysis (B).

[1] Abbott N.J. *et al.* (2010). Structure and function of the blood-brain barrier. **Neurobiol Dis.** 37(1):13–25

[2] Ohtsuki S. *et al.* (2013). Quantitative Targeted Absolute Proteomic Analysis of Transporters, Receptors and Junction Proteins for Validation of Human Cerebral Microvascular Endothelial Cell Line hCMEC/D3 as a Human Blood–Brain Barrier Model. **Mol Pharm.** 10:289-296

[3] Hirsch J.D. *et al.* (2002). Easily reversible desthiobiotin binding to streptavidin, avidin, and other biotin-binding proteins: uses for protein labeling, detection, and isolation. **Anal Biochem.** 308(2):343-57

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