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Project Abstract
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Phospholipases A2 conjugated to Concavalin A as a tool for the determination of annular lipid composition of Band 3 at the intact human erythrocyte

Due to its simplicity, the human erythrocyte membrane has been studied from differing perspectives. The general composition of its lipids, as well as their protein content, has not been an exception. However, despite the existence of microdomains or lipid rafts, and segregation of lipid species in different cells, the lipid composition at the boundaries of membrane proteins has not been studied.

In our laboratory, we have studied the hydrolytic capacity of phospholipases A2 (sPLA2) extracted, isolated and purified from snake venoms against intact human erythrocytes. The sPLA2 are characterized by the hydrolytic catalysis of phospholipids at the sn-2 position of glycerolipids. This reaction is very stereospecific. Although, the vast majority of these enzymes do not present specificity towards some species of fatty acids in the position Sn-2. The objective of this project is the construction of a covalent complex between a sPLA2 extracted from the venom of the cobra *Naja nigricollis* and another protein with high specificity towards the membrane protein Band 3, which is found in a high number of copies in the human erythrocyte surface. The conjugate will consist of sPLA2 at one end followed by a spacer of about 16-18 Armstrong in length and finally coupled to Concavalin A (ConA). ConA binds specifically α-D-mannosyl and α-D-glucosyl residues. This specificity will be used as a hook to bind the whole conjugate to the mannose residues present in the extracellular part of Band 3 in the human erythrocyte. The hydrolytic activity of this conjugate will be assayed against the intact human erythrocyte cells at different periods of hydrolysis and at low and high levels of sPLA2-ConA conjugate. The hydrolysis product will be extracted, separated by thin layer chromatography, and the fatty acids at the sn-2 position will be analyzed by gas chromatography coupled to mass spectrometry.