**Interaction of lysophosphatidic acid with signaling protein domains: affinity, stoichiometry and the site of binding.**

The SH2 domain of Nck1 selectively binds to LPA surfaces with high affinity. We showed via ITC the existence of two sites (KD1=480 nM, n1=50, KD2=2000 nM, n2=150). The high-affinity site represents the binding of the domain to LPA-surfaces, whereas the nature of the low-affinity site is still questionable (e.g. monomeric lipid site). This will be addressed by QCM measurements after the repair of our equipment.

The ATR-FTIR spectrum of the domain corresponds to the published -helical and -sheet elements. In the presence of LPA (lipid:protein ratio = 10:1) the -sheet band at 1639 cm-1 shifted to 1629 cm-1, probably by the re-arrangement of -sheets and -helical elements, favouring intermolecular interactions of the -sheets.

We demonstrated the specificity of the domain binding to its known phosphopeptide (ITC, QCM). In the presence of monomeric LPA the apparent affinity became weaker, while LPA micelles disrupted the binding, indicative of a competition mechanism.