Primary and secondary peptide binding to liposomes as seen by ITC, fluorescence, and MST

Anja Stulz[1], Michaela Breitsamer[2], Gerhard Winter[2], Heiko H. Heerklotz[1, 3, 4]

[1] *Institute of Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany*
[2] *Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-Universität München, Munich, Germany*
[3] *CIBBS and BIOSS Centers of Excellence on biological signalling, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany*
[4] *Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada*

The general motivation for studying the interactions of exenatide, a peptide drug used to treat diabetes, with liposomes had been the design of sustained release drug delivery systems. Peptide binding to lipid membranes can involve many different interactions and structural outcomes, possibly several in parallel or sequence. Typical experimental approaches reveal only part of this complexity and may therefore produce incomplete, potentially misleading results.

Isothermal titration calorimetry (ITC) is particularly sensitive to individual binding forces and mechanisms but sometimes not straightforward to interpret. To tackle potentially complicated systems and complex binding behaviors, it seems advantageous to combine ITC with other methods. A combination of ITC, microscale thermophoresis and tryptophan fluorescence allowed for a deep understanding and quantitative modelling of exenatide interacting with liposomes. Below its pKA, it shows primary, electrostatic binding to anionic lipids plus secondary binding to pre-bound peptide. At high concentrations of exenatide-covered liposomes, secondary binding may also proceed to liposomal aggregation as detected by dynamic light scattering. Not recognizing and taking into account this scheme gives rise to apparently conflicting results of different binding methods.