Rapid mixing time resolved Fourier transform infrared spectroscopy to study bio-ligand interactions

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We used a previously presented micromixer[1] with time resolved Fourier transform infrared (FT-IR) spectroscopy to investigate the binding of vancomycin – a glycopeptide antibiotic – to a cell wall precursor peptide with a time resolution in the low millisecond range. The used micromixer is based on lamination of two liquid flow sheets of ~7 µm thickness and ~1 mm width on top of each other and is operated in the stopped-flow mode. The microfluidic pattern is made on IR transmitting CaF_2 discs and built up with two epoxy negative photoresist layers (~7 µm) and one separating silver layer (~2 µm) in between (Fig. 2). Due to the reduced inter-stream distances the reactant solutions are rapidly mixed by diffusion within approximately 50 ms and reactions can be directly followed by time resolved infrared spectroscopy.
Vancomycin is of high clinical relevance as it represents one of the last lines of defence against bacteria that have developed resistance to other antibiotics. The binding mechanism is based on hydrogen bonding and hydrophobic interactions between the peptide and the antibiotic as revealed by NMR and crystallographic data. The micromachined mixing chip together with time resolved FT-IR spectroscopy enabled us to elucidate the course and the kinetics of the binding process with high structural information, indicating that different kinetics occur for different binding centres involved (Fig. 1). Most likely there is a two stage binding process where in the first stage the ionic interactions between the carboxylate of the peptide and the NH groups of vancomycin are the driving force. In addition to this fast interaction one can identify a second one taking place at lower rate. This interaction can be assigned to hydrophobic interaction taking place between the methyl groups of the alanin residues of the peptide and the aromatic rings of the antibiotic.

Figure 1: difference spectra (0-2 s, 45 ms each)

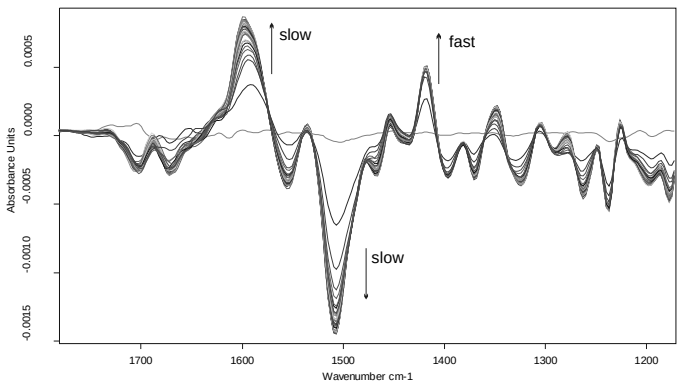
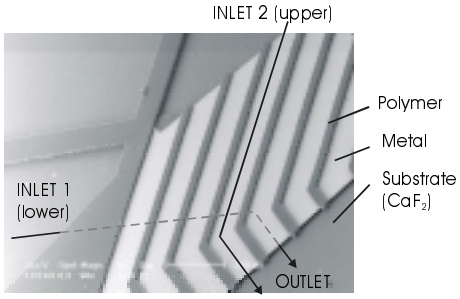


Figure 2: microfluidic pattern



[1] P. Hinsmann, J. Frank, P. Svasek, M. Harasek and B. Lendl, *Lab chip* 2001, 1, 16-21