Supplement:

An infrared sensor analysing label-free the secondary structure of the Abeta peptide in presence of complex fluids

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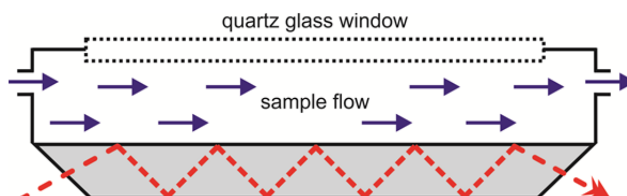
Schematics of the sensor



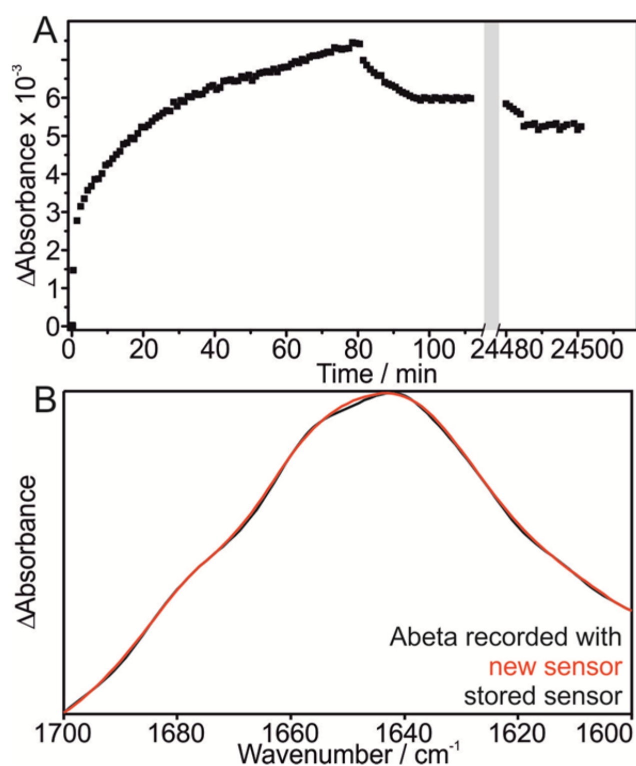
Supplementary Figure 1 Schematics of the sensor element. The germanium IRE (grey) is mounted in a flow-through cuvette. The casing features a quartz glass window for optional fluorescence analysis. The sample flow (blue arrows) through the cuvette is controlled with a peristaltic pump.

The signal to noise ratio (S/N) was calculated with a spectroscopic standard procedure: two single channel spectra I_0 and I were subsequently recorded of the receptive and blocked sensor element. The transmission spectrum $T=I/I_0$ was calculated. The root mean squared noise value (N_{RMS}) was calculated for the wavenumber range $\nu \in \{1700cm^{-1}..1600cm^{-1}\}$ with

$$N_{RMS} = \sqrt{1/_n \sum_{i=1}^{n} (1 - T(\nu_i))^2}$$

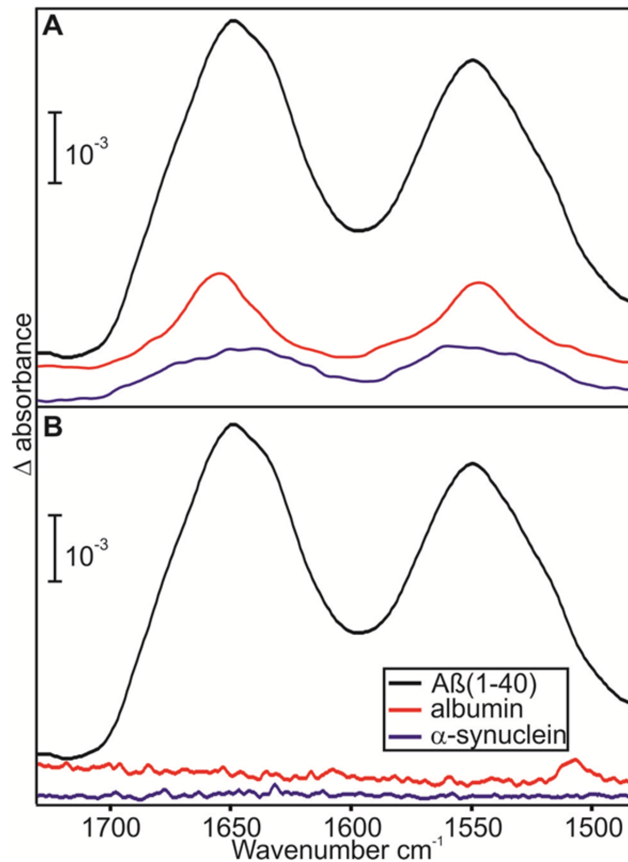
using the spectrometer software (Bruker OPUS) internal function. Then, S/N was calculated as maximum signal intensity of the absorbance spectrum divided by the noise level.

Long-term-stability



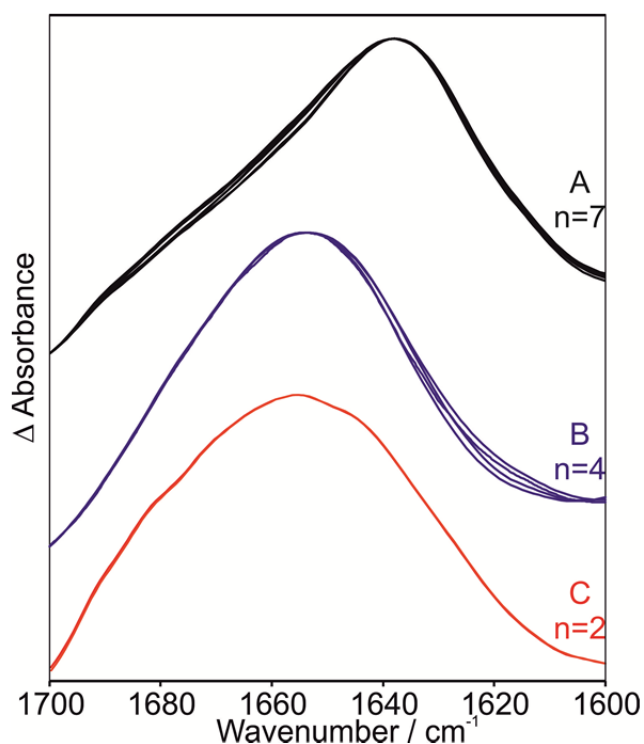
Supplementary Figure 2 Long term stability of the prepared sensor element was evaluated with one antibody coated sensor stored outside the spectrometer at 4°C for 17 days. The amide II band at 1550 cm^{-1} was followed over time (A). After storage, the spectrum recording was resumed at room temperature. Only minimal dissociation from the surface was observed with prolonged rinsing. The scaled Abeta peptide absorbance spectra recorded with the stored and a freshly prepared sensor demonstrate extreme reproducibility (B).

Cross-reactivity



Supplementary Figure 3 Cross-reactivity of the sensor surface with albumin and α-synuclein as two selected blood components. Albumin is the most abundant blood protein, whereas α-synuclein represents another amyloidogenic protein. An unblocked sensor surface is receptive for α-synuclein incubated at 20 ng/ml (blue), albumin incubated at 25 µg/ml concentration (red), and Abeta1-40 peptide incubated at 15 ng/ml concentration (A). The blocked sensor was not receptive except for Abeta1-40 peptide (B, black). Spectra have been offset for comparability.

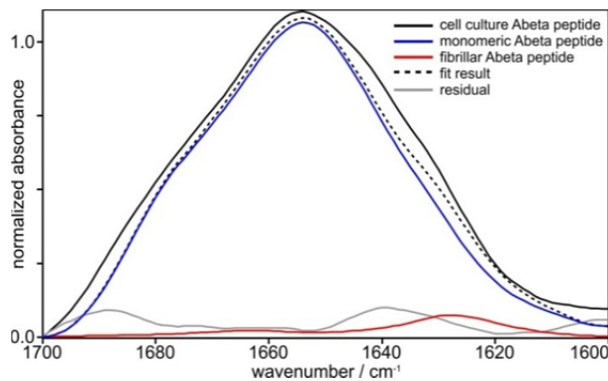
Reproducibility



Supplementary Figure 4 The amide I bands recorded from n separately prepared sensors binding antibody 1E8 onto silane (A), casein binding onto antibody loaded sensors (B), and Abeta peptide captured from chicken neuroblast cell culture medium (C, sample taken from another cell culture as analysed in the manuscript and suppl. Fig. 5) indicate an excellent spectral reproducibility. Spectra were obtained as differences from a buffer rinsed reference state before and a buffer rinsed sample state after substance application into the flow system. The antibody secondary structure is preserved, although the covalent antibody binding is not limited to a particular epitope besides accessible primary amines. The Abeta peptide absorbance spectra reproducibility is excellent.

Determination of the dominating Abeta conformation as excreted out of neuronal cells

The amide I band of Abeta extracted out of neuronal cell culture media, Fig. 4 C, was decomposed as a linear combination of monomeric (Fig. 4 A) and fibrillar Abeta proteins (Fig. 4 B). There is no oligomeric Abeta spectrum available as reference. The secondary structure analysis of these two Abeta conformations by IR was compared to NMR analysis- of Abeta monomers (PDB 1Z0Q) and fibrils (PDB 2BEG) and showed good agreement. 96 % of the cell culture amide I band agrees with synthetic Abeta proteins, with 92 % monomeric and 4 % fibrillar fold, respectively. The remaining fraction of 4 % covered by the residual spectrum indicated the possible presence of other, maybe oligomeric conformations or Abeta related proteins that also can crossreact with the 1E8 antibody.



Supplementary Figure 5 The absorbance spectrum obtained from chicken neuronal cell culture (Fig. 4 C) as a linear combination of monomeric and fibrillar synthetic Abeta protein (Fig. 4 A, B) results in a distribution of 92 % monomeric, and 4 % fibrillar protein. A residual of 4% indicates at least a third conformation present in the spectrum.