Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Pushing the detection limit of infrared spectroscopy for structural analysis of dilute protein samples

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Polydimethylsiloxane and glycerol as infrared filters

Siloxanes are compounds consisting of silicon bound to oxygen and carbon. The stretching vibration (ν) of the Si—O bond gives rise to an intense, broad band between 1150 and 1000 cm^{-1}, while the Si—C vibration gives rise to a sharp vibration at about 1260 cm^{-1}.[1,2] In (poly)dimethylsiloxanes, the presence of the methyl group results in strong, sharp bands between 3000 and 2900 cm^{-1}, and medium, sharp bands between 1450 and 1375 cm^{-1}. These arise from the stretching (ν) and bending (δ) vibrations of the C—H bonds, respectively. Figure S1A shows the transmission spectrum of vacuum grease, a common and inexpensive lubricant mainly composed of polydimethylsiloxanes.
Alcohols are additional compounds with strong, noteworthy absorptivities both above and below the amide I region. These include the strong ν(C—O) band between 1300 and 1000 cm^{-1}, and the strong ν(O—H) band between 3650 and 3600 cm^{-1}.[3] Hydrogen bonding broadens and shifts this band to ~3500--3200 cm^{-1}, thereby increasing its usefulness as a filter. The presence of CH and CH_2 groups leads to further absorptivities between 3000 and 2900 cm^{-1}, as well as below 1450 cm^{-1}. The transmission spectrum of glycerol, a polyalcohol, is shown in Figure S1B.

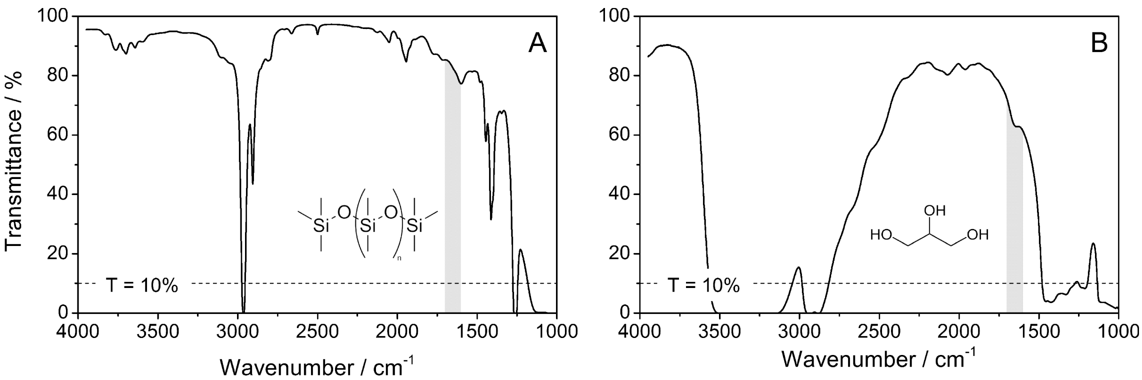

Figure (S1) Infrared transmission spectra of polydimethylsiloxane (A) and glycerol (B). The chemical structure of each compound is shown under the corresponding spectrum. The area highlighted in grey denotes the region where the amide I band is observed. The spectra of each compound were recorded using a CaF_2 cell with a trough of 25-μm.

Absorption spectrum of a metal grid

Figure S1 shows the absorption spectrum of the mashed metal screen used in our background attenuation measurements. It transmits ~60% of the infrared light that shines through it, resulting in a constant optical density of –log(0.6) = 0.23 in the spectral window used in our studies.

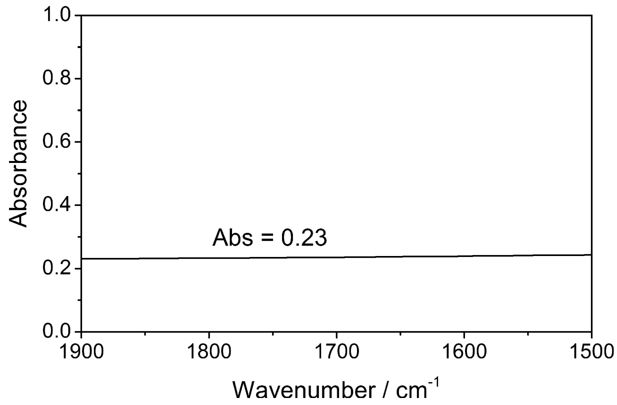

Figure (S2) Absorption spectrum of the metal grid used in our studies.

Experimental layout

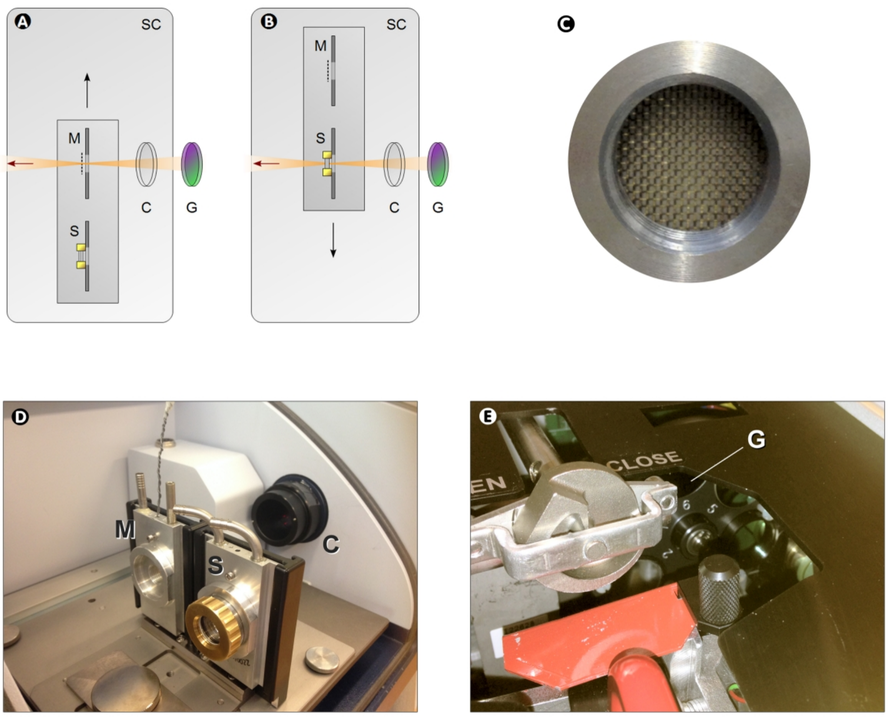

Figure (S3) Layout and pictures of our experimental setup. Layout of the proposed setup with the sample shuttle set to record the background (A) and sample (B) spectra.

Symbols: M = metal grid; S = sample; SC = sample compartment; C = cellulose filter; G = germanium filter. The vertical arrows denote the movement of the sample shuttle. The horizontal arrow denotes the direction of the infrared beam from the source (right) to the detector (left). The germanium filter is mounted on a software-controlled filter wheel, while the cellulose filter is fitted, via a plastic cylinder, to the beam entrance hole. (B) Close-up of the metal grid. (D) Picture of the sample compartment, showing the metal grid (M) and sample (M) mounted on the sample shuttle. The cylinder holding the cellulose filter (C) is visible. (E) Picture of the filter wheel within the spectrometer.

References

[1] Romanenko, E. A. and Tkachuk, B. V. Journal of Applied Spectroscopy 18 (2), 188–192 (1973).

[2] Socrates, G. Infrared and Raman Characteristic Group Frequencies: Tables and Charts, chapter Organic Silicon Compounds, 241–246. Wiley (2004).

[3] Socrates, G. Infrared and Raman Characteristic Group Frequencies: Tables and Charts, chapter Hydroxyl Group Compounds: O-H Group, 241–246. Wiley (2004).