

Supporting Material for Nanoscale spatially resolved infrared spectra from single microdroplets

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1 Morphology of the dried droplets

Several conditions for drying and analysing the droplets were investigated. Namely, we have deposited microdroplets on pure ZnSe prisms and SiO₂-coated ZnSe prisms. Drying was effected by either placing the prisms under vacuum at room temperature or in an oven at 65 °C at ambient pressure for a time span of 15 h. The resulting morphologies varied significantly, and are summarised in Fig. 1. Most importantly, droplets that have been heated overnight (g-j) exhibit a smaller size which may well be caused by the protein becoming more attached to the pdms stamp and therefore being removed when detaching the stamp. Note that in the main work, only data from vacuum-dried droplets is presented.

2 Spectra from droplets under different conditions

To demonstrate the repeatability of our approach, we present spectra of droplets containing monomeric and aggregated lysozyme dried under different conditions in Figs. 2(a) and (b), respectively. Specifically, we have investigated two different types of infrared-transparent prisms (ZnSe and SiO₂-coated ZnSe) and dried the droplets either at room temperature under vacuum or at 65 °C at ambient pressure.

For the monomeric protein, the vacuum-dried droplets on either substrate exhibit a distinct peak around 1655 cm⁻¹ as well as a sharp feature in the amide III band which is pronounced more strongly for the plain ZnSe prism. Heating the droplets for 15 h to evaporate all solvents lead to a drastic increase of the absorption in the amide I band (see black ellipse in Fig. 2(a)) that corresponds very well to the spectra observed for aggregated protein. Furthermore, in the case of the heated, SiO₂-coated prism the peak in the amide III band also van-

ishes.

Considering the original approach for forming the aggregates, it is highly plausible that the conditions for drying the droplets at elevated temperatures are sufficient to cause at least partial aggregation of the initially monomeric protein - in particular since the evaporation of water continuously increases the protein concentration.

The spectra of the droplets containing aggregated protein presented in Fig. 2(b) are very similar for all conditions and demonstrate the reliability of our approach. The most notable difference is that with the additional layer of SiO₂ on the surface of the prism the absorption at the high-energy side of the amide I band is increased relatively to the rest of the spectrum. The peak positions, however, are unchanged. Also, the spectra from the three droplets on the same substrate (solid, dashed and dotted blue lines) indicate the quantitative variability between individual droplets with nominally the same contents.

In both cases - that is monomeric and aggregated protein - some of the differences between the spectra from ZnSe prisms and SiO₂-coated ZnSe prisms may be explained by the different absorption behaviour on different substrates. Indeed, in all instances of the coated prisms we observe stronger absorption towards higher wavenumbers.

Therefore, if protein aggregation from microdroplets is investigated, it is highly recommended to effect the drying under vacuum instead of at elevated temperature. Furthermore, we find that our spectra exhibit sharper features on the pure ZnSe prisms.

3 Sensitivity and throughput of the method

Given a droplet diameter of 25 μm, the molar weight of lysozyme (14.3 kDa) and the concentration we have used for the monomers (6 mg/ml ~ 400 μM), each droplet contains as little as 50 pg or 3 fmol of protein.

We can estimate the sensitivity of this approach via the minimal sample thickness (recommended is 0.1 μm, but we have obtained spectra from samples of $h \approx 60$ nm height) of our Anasys nanoIR platform

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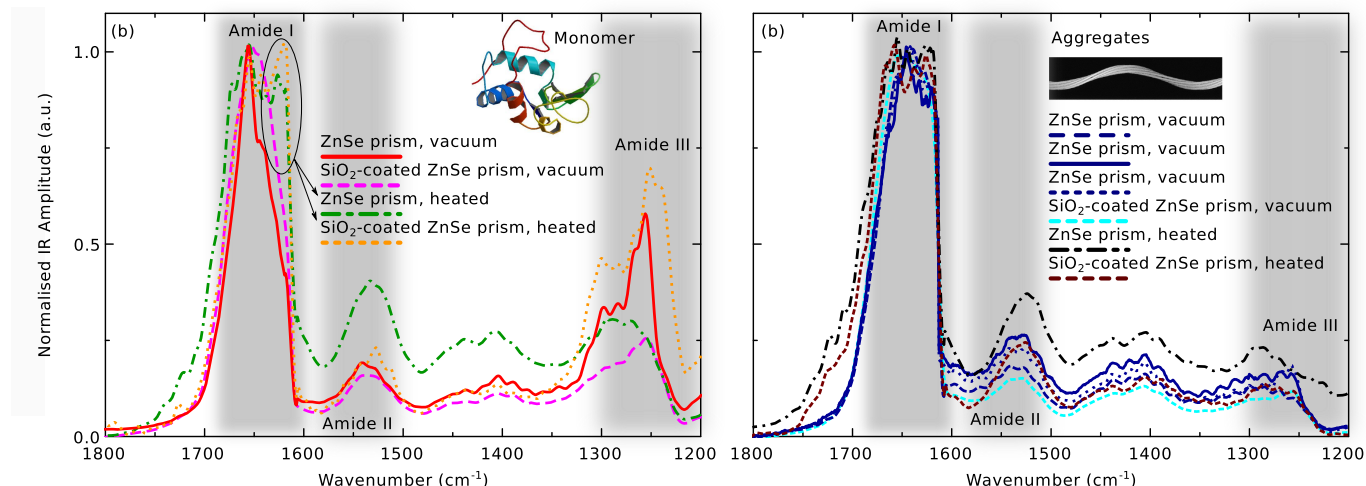


Fig. 2 (a) Spectra of dried droplets containing initially monomeric protein. Shown are droplets dried in vacuum at room temperature during 15 h, deposited on a ZnSe prism (solid red line; same curve as in main text) or a SiO₂-coated ZnSe prism (dotted orange line), as well as dried at ambient pressure at a temperature of 65 °C on a ZnSe prism (dash-dotted green line; same curve as in main text) or on a SiO₂-coated ZnSe prism (dashed magenta line). The black ellipse marks the appearance of a shoulder in the amide I band for the heated droplets. (b) Same as in (a) but for aggregated protein. For the vacuum-dried ZnSe prism spectra of three different droplets are presented (dashed, solid and dotted blue lines). Insets for the structures of monomer and lysozyme amyloid are adapted from Refs. ¹ and ², respectively.

(<http://www.anasysinstruments.com>). To do so we evaluate the amount of analyte deposited on a square with length l to a height h using the inverse density of lysozyme ($v = 0.7$ ml/g). This yields

$$C_{min} = \frac{V_{sample}/v}{V_{droplet}} \sim \frac{h/v}{l}, \quad (1)$$

and with l being maximally of the order of 300 μ m the minimal concentration is around 0.2 mg/ml \sim 20 μ M. While standard optical techniques may detect much smaller concentrations,^{3,4} nanoIR provides a quite different set of information - namely nanometer spatially resolved IR spectra from picograms or sub-femtomoles of protein material.

The time required to obtain a spectrum of a single droplet is typically of the order of a few minutes. However, performing spatially resolved measurements may take much longer. Therefore, nanoIR can by no means be considered a high-throughput technique. However, nanoIR may provide information on the contents of the droplets that is not accessible using traditional techniques and should rather be regarded as a method complementary to on-line high-throughput techniques where measurements can be taken at kHz rates at concentrations as low as tens of nM.^{3,4}

References

- 1 Image from the RCSB PDB (www.pdb.org) of PDB ID 2LYZ (Diamond, R. (1974) Real-space refinement of the structure of hen egg-white lysozyme *J. Mol. Biol.* 82: 371-391).

- 2 J. L. Jiménez, G. Tennent, M. Pepys and H. R. Saibil, *J Mol Biol*, 2001, **311**, 241–247.
- 3 M. Srisa-Art, A. J. deMello and J. B. Edel, *Anal Chem*, 2007, **79**, 6682–6689.
- 4 F. Guo, M. I. Lapsley, A. A. Nawaz, Y. Zhao, S.-C. S. Lin, Y. Chen, S. Yang, X.-Z. Zhao and T. J. Huang, *Anal Chem*, 2012, **84**, 10745–10749.

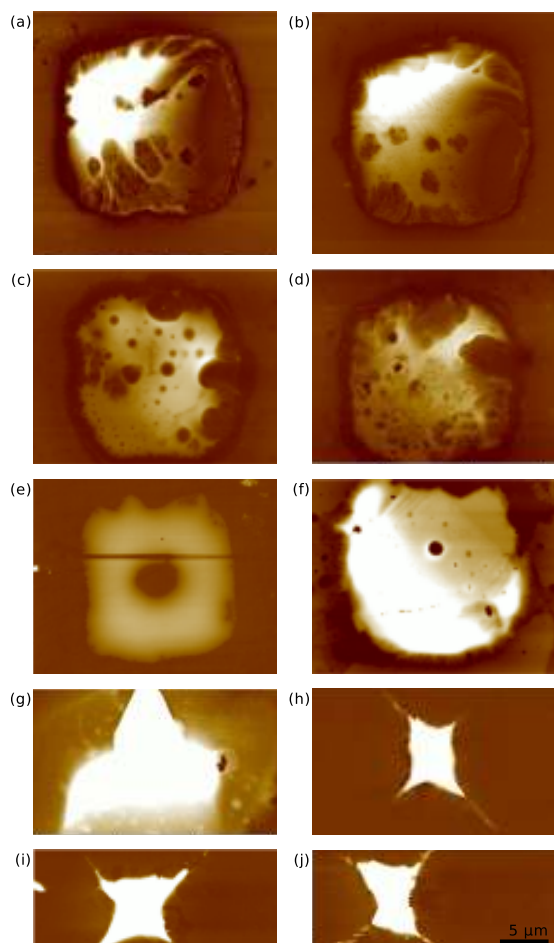


Fig. 1 Atomic force microscopy images of droplets dried under different conditions. (a) Droplet containing aggregated lysozyme, deposited on a ZnSe prism and dried for 15 h at room temperature under vacuum. The full scale height is ± 250 nm. (b) Same as (a), for a different droplet. The full scale height is ± 500 nm. (c) Droplet containing aggregates, deposited on a SiO₂-coated ZnSe prism and dried for 15 h at room temperature under vacuum. The full scale height is ± 500 nm. (d) Same as (c) but for a different droplet. The full scale height is ± 500 nm. (e) Droplet containing monomeric lysozyme, deposited on a ZnSe prism and dried for 15 h at room temperature under vacuum. The full scale height is ± 150 nm. (f) Droplet containing monomers, deposited on a SiO₂-coated ZnSe prism and dried for 15 h at room temperature under vacuum. The full scale height is ± 250 nm. (g) Droplet containing aggregated lysozyme, deposited on a ZnSe prism and dried for 15 h at 65 °C at ambient pressure. The full scale height is ± 300 nm. (h) Droplet containing monomeric lysozyme, deposited on a ZnSe prism and dried for 15 h at 65 °C at ambient pressure. The full scale height is ± 300 nm. (i) Same as (h), but for a different droplet. The full scale height is ± 300 nm. (j) Same as (h), but for a different droplet. The full scale height is ± 300 nm.