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**Supporting information for article:**

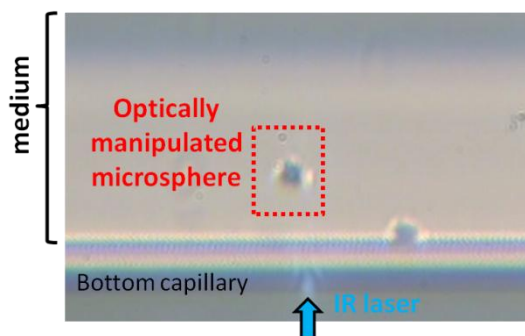
**Methodological challenges of optical tweezers based X-ray  
fluorescence imaging of biological model organisms at  
synchrotron facilities**

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## Supporting information

### S1. Optical tweezers alignment

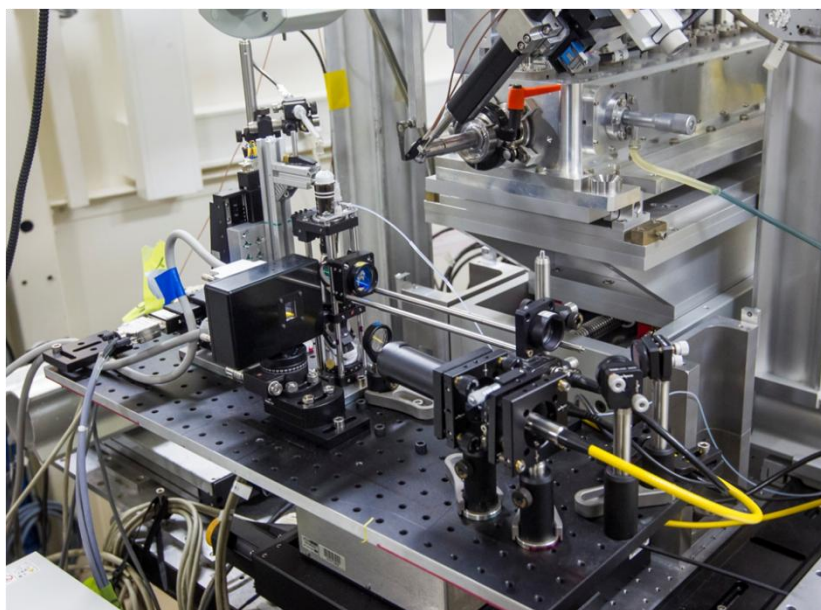
In case of proper OT setup alignment, optical trapping of micron-sized polystyrene beads (Bangs Laboratories, Inc., 3.5  $\mu\text{m}$  diameter) is perfectly feasible (Fig. S1). The polystyrene beads are contained in a horizontally mounted quartz capillary (QGCT 0.2, Capillary Tube Supplies Ltd, UK, 200  $\mu\text{m}$  diameter, 10  $\mu\text{m}$  wall thickness) filled with Milli-Q water and sealed with putty.



**Figure S1** Optical manipulation of silica microspheres in aqueous environment.

### S2. OT setup installed at ESRF-ID13

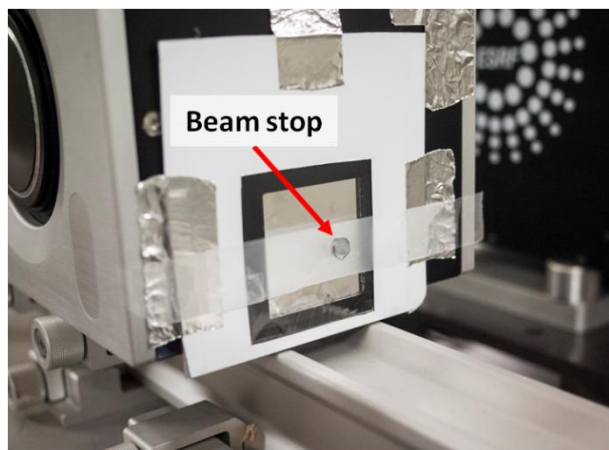
For SR *in vivo* OT XRF imaging experiments, the compact OT setup is mounted on the beamline scanning stages of ESRF-ID13 and a raster scan is performed by translating the complete OT setup (Fig. S2).



**Figure S2** Compact OT setup mounted on the beamline scanning stages of ESRF-ID13.

### S3. MAXIPIX beam stop

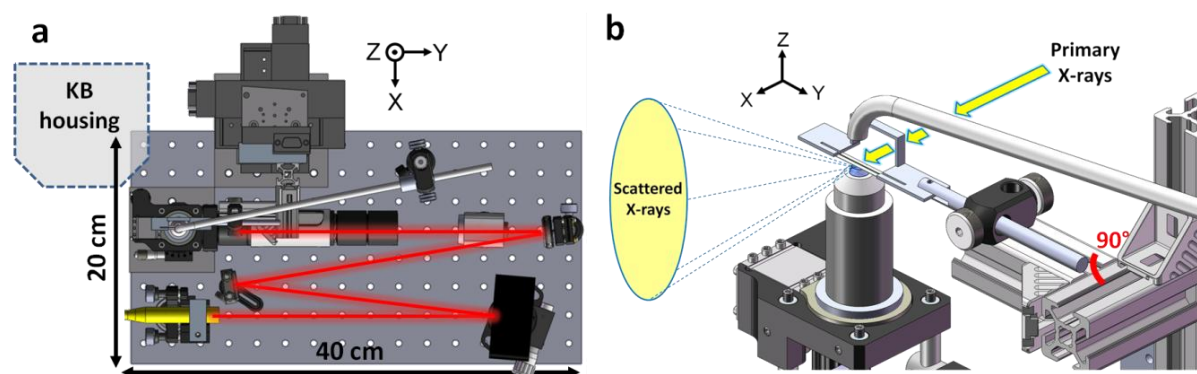
Due to the relatively long exposure time per point ( $> 0.2$  s), an extra beam stop was positioned in front of the MAXIPIX detector for protective purposes (Fig. S3), resulting in a significant reduction of the detected scattered signal and therefore requiring a dedicated data treatment strategy.



**Figure S3** An extra lead beam stop was positioned in front of the MAXIPIX detector for protective purposes.

### S4. OT XRD setup

Santucci *et al.* (2011) designed a dedicated OT setup for SR XRD experiments of protein crystals in their natural aqueous environments at ESRF-ID13. Previously, Microfocus beamline ID13 was equipped with a KB focussing system (Fig. S4a) which strongly limited the spatial flexibility for OT setup integration. Due to the housing of the KB-system, the motorized sample stages and light guide were positioned on the right-hand side of the trapping objective, resulting in an extra  $90^\circ$  angle for sample mounting (Fig. S4b). Note that the OT XRD sample holder is characterized by a reversed bridging system (Fig. S4b) as opposed to the OT XRF holder.

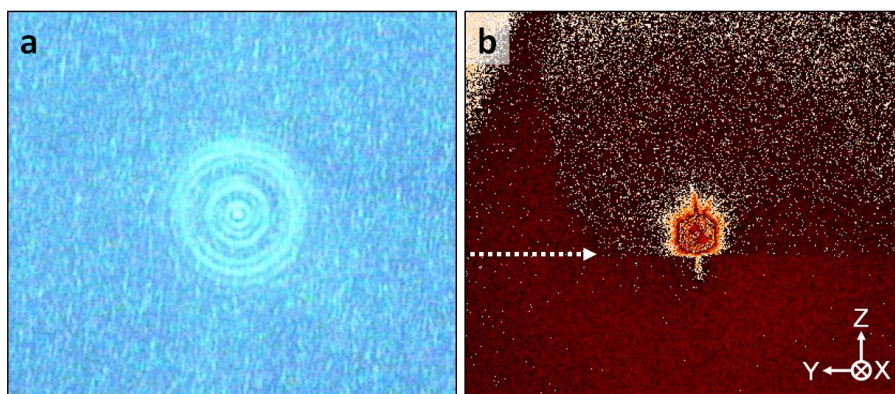


**Figure S4** OT XRD technical overview, adapted from Santucci *et al.* (2011). (a) CAD representation with an indication of the IR laser path in red. (b) Detail of the sample area with an

indication of the primary X-ray beam and the direction of the scattered photons. Note that the sample mounting structure includes an extra  $90^\circ$  angle compared to the current OT setup adapted for XRF imaging.

### S5. Coverslip implications

When the trapping laser is properly aligned and focussed onto the coverslip, a concentric, circular pattern should be observed (Fig. S5a). One coverslip related issue involves the absorption of a substantial amount of photons from the lower half of the scattering cone. The latter can be observed at the dotted arrow that indicates the dividing line between coverslip and the sample in Fig. S5b. Note that the scattering pattern in Fig. S5b was obtained using a 16 bit readout FReLoN charge-coupled device (CCD) detector available from ESRF-ID13 (0.5 s/pattern).



**Figure S5** Coverslip implications. (a) Laser beam focussed on the coverslip, the concentric profile indicates a well-aligned OT setup. (b) A single scattering pattern obtained from a sample scanned under the OT XRF geometry, the primary beam was focussed on the upper capillary wall. The dotted arrow indicates the boundary between the sample and the absorbing coverslip.