Design considerations of UV-visible Microspectroscopy at single crystal neutron diffractometers

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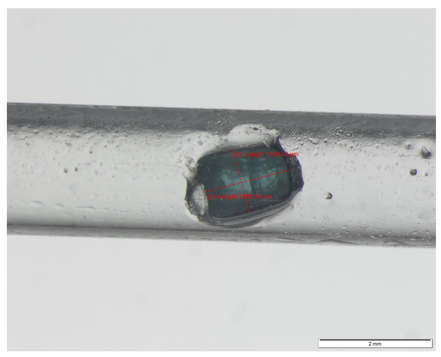
Scientific Motivation

Motivation for protein crystals Single crystal neutron diffraction experiments on protein crystals often require beamtimes of several days in order to measure a complete data set which leads to meaningful results on atom positions and occupancies. In case of room temperature measurements, the sample under investigation might change during that time. If one wants to study a radical intermediate state of the protein, often linked to a distinct UV-visible absorption of the crystal, one is often interested in the decay of the number of radicals in the crystal. Below a certain number, one would rather switch to another freshly prepared crystal. This would save precious neutron beamtime.

User example: Metal-free ribonucleotide reductase in pathogens



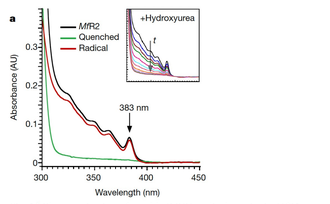


Fig. 3 | Characterization of a stable DOPA radical species in *Mf*R2. **a**, The UV–vis spectrum of the active blue-coloured protein shows a peak at 383 nm and additional structure at lower wavelengths (black trace). Incubation with 52 mM hydroxyurea for 20 min removed all features from the spectrum except the protein-related absorbance peak at 280 nm (green trace and inset). The red trace represents the spectrum of the active protein minus the spectrum of the quenched protein. The rate constants for the decay of the absorbance at 348, 364 and 383 nm were identical, which is consistent with all absorbance features arising from a single radical species.

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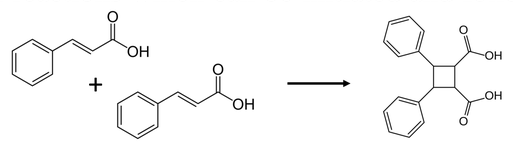
Motivation for small molecule crystals Here, one can think of photochemical reactions which can be initiated and followed in crystallo with UV-Visible light.



Photodimerisation of cinnamic acid compounds

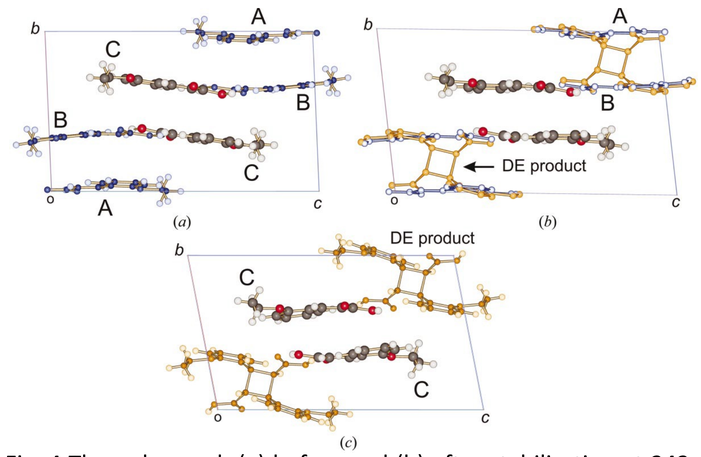


Fig. 4 The polymorph (a) before and (b) after stabilization at 343 K. Upon further exposure to UV light at 293 K a final product crystal containing only the C molecule and DE product is obtained. [3]

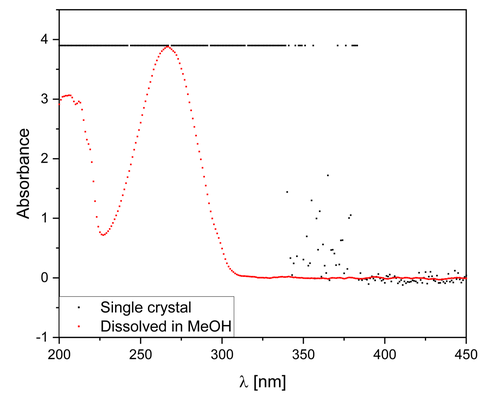
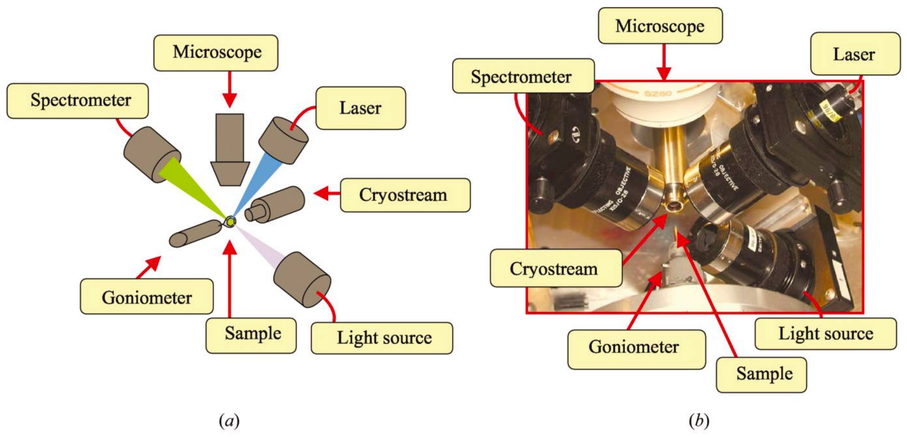


Fig. 5 Uv/Vis of the trans-4-trifluoromethyl cinnamic acid of a single crystal (black) and dissolved in MeOH. The data were scaled.

UV-light as starter for the reaction

Realisations on other instruments/ x-ray beamlines

Off beamline, stand alone:



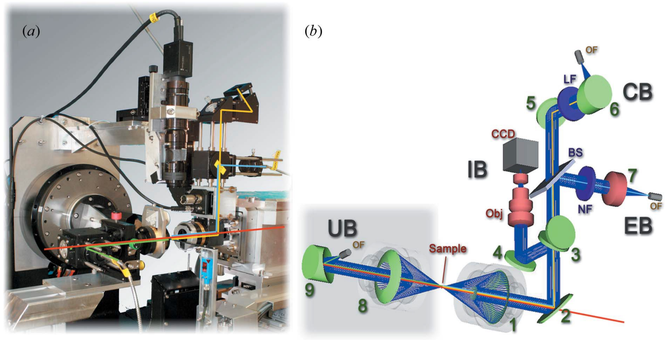
(a) Schematic view of the absorption and ¯uorescence spectrophotometer. (b) Corresponding picture of the device.

Figure taken from J. Appl. Cryst. (2002). 35, 319-326

On axis at beamline X10SA at SLS:

Realized techniques:

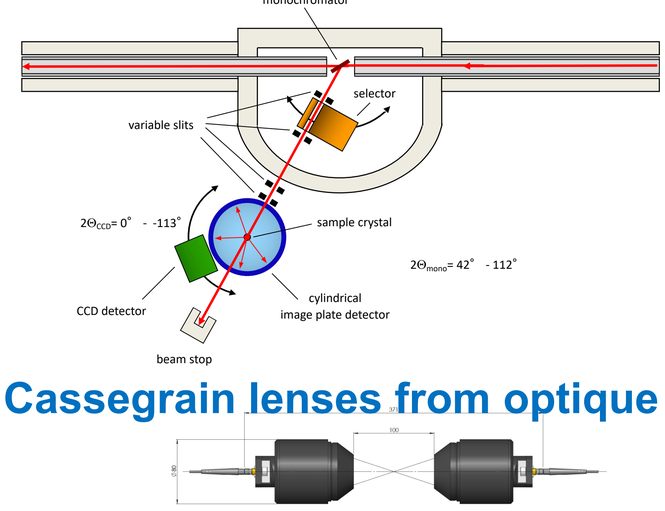
1. Raman
2. UV-Vis absorption
3. Fluorescence
4. IR absorption



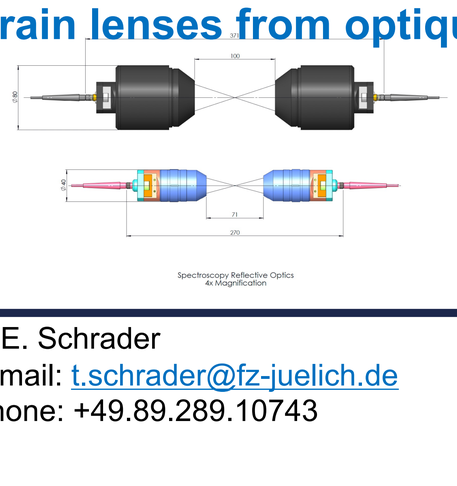
(a) The MS2 mounted on beamline X10SA, shown in UV/Vis absorption mode with the illumination Schwarzschild objective moved in for measurement. Red: X-ray beam path; green: illumination light path; blue: laser excitation path; yellow: signal detection light path. (b) Schematic representation of the branches, light path and optical components in the MS2. Naming as discussed in the text: IB, imaging branch; CB, collection branch; EB, excitation branch; UB, UV/Vis illumination branch; 1–9, optical elements; OF, light guide fiber coupling; BS, beamsplitter; LF, laser line filter; NF, notch filter.

Possible realisations on BIODIFF and on the Rigaku Synergy-S Single Crystal Diffractometer

Detector radius of BIODIFF is 200 mm



Cassegrain lenses from optique peter

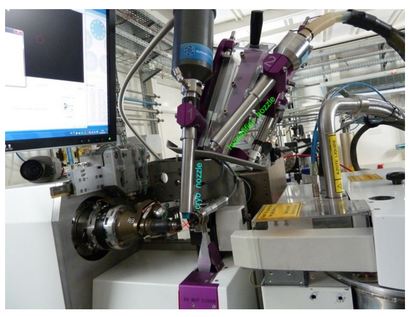


In combination with the following sample environment:

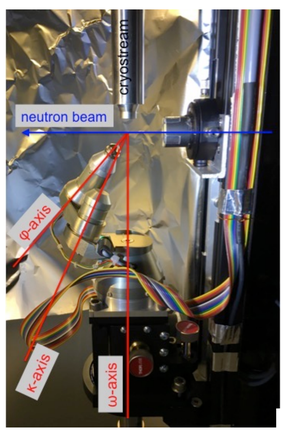
Croystream 100 K

Mini-kappa-goniometer
➞ optimizing data collection strategy

Standard Oxford cryostream 700+
➞ temperature range 90 – 500 K

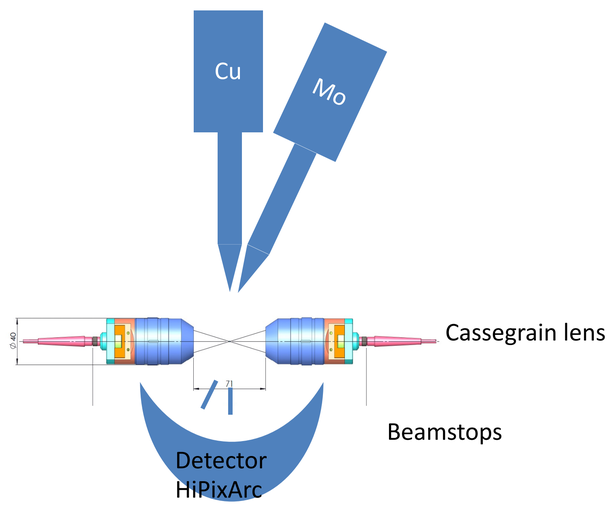


Humiditiy gas stream



+Room temperature capillaries with Mini-Kappa goniometer

Realisation at Synergy-S x-ray Diffractometer



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