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A cryostat for low temperature resonance Raman measurements on *operando* oxygenated bioinorganic model complexes

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ABSTRACT

Raman spectroscopy is a useful tool for the elucidation of bioinorganic mechanisms. To investigate charge-transfer processes in *operando* oxygenated bioinorganic samples, we present a new cryostat for liquid samples at low temperature. We reach temperatures below −80 °C inside a Suprasil glass cuvette which is prerequisite for the formation of the oxo species and long-term stability of the sample. Under *operando* conditions we demonstrate resonance Raman as well as isotope substitution measurements on a bis(μ-oxo) dicopper(III) complex. Future applications of our setup are envisioned to be transient Raman, steady-state and time-resolved fluorescence as well as transient absorption spectroscopy of bioinorganic or other charge-transfer complexes.

1. Introduction

The copper containing enzyme tyrosinase is part of the melanin production and catalyzes the biological oxidation of phenols such as tyrosine. Most model complexes for tyrosinase are not stable at room temperature when oxygenation occurs but usually stable for several hours when the temperature is below −60 °C [1]. Tyrosinase serves as an archetype for nature's solution to activate molecular oxygen and use it for mild and selective hydroxylation of phenols. In the last 30 years, a plethora of ligand systems have been used for the stabilization of model complexes [2].

To investigate direct hydroxylation of phenolic substrates bis(μ-oxo) dicopper(III) and peroxo dicopper(II) [3] complexes have been used. For the synthesis of bis(μ-oxo) dicopper(III) complexes, guanidine ligands have been utilized due to their good N-donor properties and UV/Vis and X-ray absorption spectroscopy confirmed the Cu_2O_2 species [4–10]. Guanidine ligands offer a facile variation of the substitution pattern [11] and allow the steering of formation and decay rates [12,13]. The complex used here is [Cu(btmgp)]I together with the corresponding bis(μ-oxo) dicopper(III) complex [14,15]. At 20 °C, the bis(μ-oxo) species decays with a rate constant of 1 s^{-1} whereas it is stable for days at −80 °C [16,12].

Since the early 1950s low temperature Raman measurements have been sought after [17–20] as they open up pathways to emerging properties of materials [21–25] at e.g. phase transitions. A longer stability of the sample can also be observed as damage by the incident laser beam is reduced [26]. This is especially valuable for understanding charge-transfer processes in bioinorganic chemistry where temperature-sensitive metal complexes are investigated and resonance Raman spectroscopy unveils bonding modes, crucial vibrations of the active sites and insights into the electronic structure [27]. However, when tuning the incident photon energies through the absorption bands, the sample decays much faster. Other groups use cooled nitrogen gas [28], liquid nitrogen [4], liquid-helium flow cryostats [29], or cooled spinning cells [30] to keep the samples stable for longer time periods.

While our previous Raman measurements on similar model complexes have been successful at room temperature [24] and low temperatures as well, leaving the cuvette with the studied complex taken from the cold bath uncooled resulted in a stability of the complex of approximately 90 s until the Cu_2O_2 species disappeared due to the warm up process, thus making resonance Raman or other time-consuming measurements impossible [31].

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2. Experimental

To overcome these experimental shortcomings of short-time stability while keeping the flexibility of the UT-3 Raman spectrometer [32] a new low-temperature cryostat was developed (see Fig. 1.). To avoid the usage of liquid gases and minimize vibrations, the cryostat uses a two-way cooling system to reach low temperatures. It consists of a liquid ethanol based closed-cycle Proline RP890 chiller (Lauda, Lauda-Königshofen, Germany) and a multistage Peltier module (TEC4-97-49-17-7-05, Thermonamic, China). The chiller flushes the copper block behind the Peltier module with cooled liquid ethanol. The Peltier module was glued to this copper block with a thin layer of Ceramique 2 heat paste (Arctic Silver, USA). On top of the Peltier module another small copper block was used for better heat transport. The cuvette is pressed against this small copper block with a holder for best thermal conductivity. The cap for the cuvette inlet has two pipe inlets which are used for *operando* oxygenation and the corresponding overpressure reduction inside the cuvette. With a vacuum pump a pressure of $\approx 2 \cdot 10^{-2}$ mbar inside the cryo was reached.

A temperature of around −70 °C on the warm side of the Peltier module was reached when using the ethanol chiller only. When the Peltier module was turned on, the temperature behind the cuvette further decreases to around −150 °C which yields a temperature inside the cuvette below −60 °C after ≈15 min and below −80 °C after ≈55 min (see Fig. 2.) measured with a Pt100 temperature sensor inside the solution. This makes the cryostat suitable for many complexes.

For all wavelengths two Tsunami Ti:Sapphire laser systems, model 3960C-15HP and model 3950-X1BB (both Spectra Physics Lasers Inc., California) were used. They were synchronized using a Lok-to-clock (LTC) system. The fundamental laser line was frequency doubled and tripled with a flexible harmonic generation (FHG) unit, model GWU2 23-PS (GWU-Lasertechnik Vertriebsges.

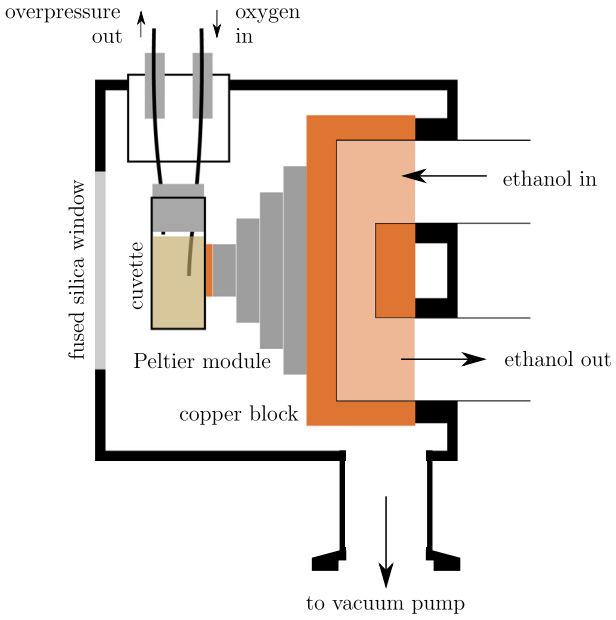

Fig. 1. Schematic setup of the cryostat. The cuvette is cooled by a small copper block (brown) on top of a multistage Peltier module (grey). The copper block (brown) on the backside of the Peltier module is cooled by liquid ethanol. During operation a pressure inside the cryostat of around $2 \cdot 10^{-2}$ mbar is reached. Lower pressures could be achieved but those are not suitable for cuvette operation. The oxygen is supplied via a pipe which leads directly into the cuvette. Overpressure is regulated by a one-way exhaust valve. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

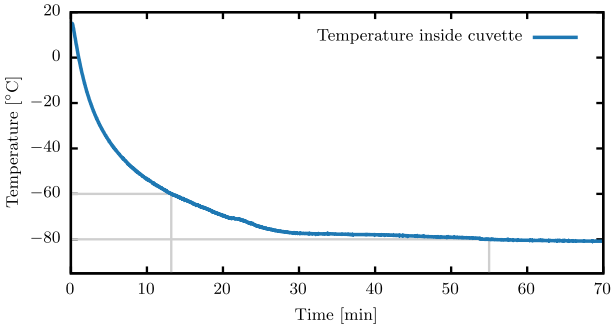

Fig. 2. Temperature measured inside the solution while cooling. With the setup a temperature below −60 °C was reached after ≈15 mins and below −80 °C after ≈55 mins.

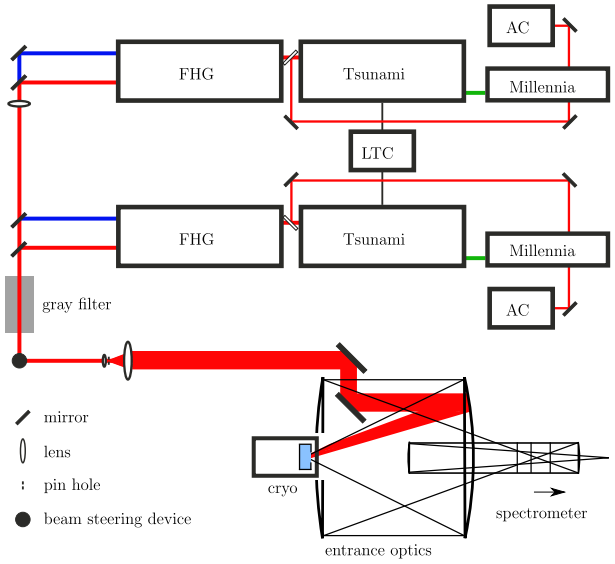

Fig. 3. Two Tsunami Ti:Sa lasers were used in the resonance Raman study. The beam was widened by a spatial filter and then focused on to the cuvette inside the cryostat. Raman scattered light was captured with the entrance optics of the UT-3 triple monochromator spectrometer and recorded with a liquid nitrogen cooled CCD camera.

mbH, Erfstadt, Germany). The laser beam was widened with a spatial filter and then focused on the cuvette inside the cryostat (see Fig. 3.). The focus spot size was around 20 µm in diameter.

The used laser power in front of the entrance optics was, depending on the wavelength, between 8.6 mW and 23.7 mW. Pulse width was between 1.5 ps and 2.5 ps. All spectra were normalized to 1 s integration time and 1 mW laser power. Background was subtracted and the resulting spectra were corrected for the spectrometer sensitivity in the respective wavelength regions. The experiments were conducted in a clean room with constant temperature (20.0 °C ± 0.5 °C) and humidity (45% ± 3%). To determine the pulse width of the laser, a small part of the Tsunami fundamental was mirrored out using a glass plate and the reflex then coupled into an autocorrelator (AC) (APE GmbH, Berlin, Germany). For the measurements a custom made half-height Suprasil glass cuvette (Hellma Analytics, Müllheim, Germany) with 1.7 ml sample volume was used.

3. Sample preparation

The copper complexes were prepared in an oxygen and water free atmosphere (<0.5 ppm) inside a LABstar glovebox (MBraun,