Tracking enzyme activity with quantum light

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The importance of enzymes for biological activity can hardly be overestimated. Among all possible reactions, those leading to a change in optical activity of the products with respect to that of the substrate material can be effectively monitored by light. Measurements of circular dichroism and optical activity can be performed with good accuracy, precision, and time resolution at moderate illumination, allowing real-time tracking of how products are accumulated. Enzyme kinetics thus becomes accessible in the experiment.

Working in this intensity regime may come at a dear price: light can modify the properties of the sample, or even result in its permanent damage. Improving precision loses its point, if the response is driven far from that of the unperturbed system. This originates a necessary trade-off between the amount of modification and the quality of the measurement. The origin and details of the bound on the uncertainty are captured by inspecting the behaviour of light at the quantum level. Quantum metrology is the art of identifying how quantum properties need being controlled and measured, and provides clear guidelines on preparing the best possible probe for a given intensity [1,2]. Thanks to the careful control not only of the average intensity, but also of its quantum fluctuations, superior precision is possible.

We applied methods of quantum metrology to the hydrolysis of sucrose enabled by invertase, a test bed case for enzymatic reactions since the early days of biochemistry. In this reaction, sucrose, a right-handed optically active molecule, is hydrolysed into D-fructose and D-glucose, whose mixture has a left-handed activity.

Quantum light probes are generated by means of two-photon quantum interference, starting with a pair of photons in orthogonal linear polarisations. Since the photons are indistinguishable, in the circular polarizations the behaviour is that of a quantum superposition of the two photons being both in the same polarization state. By passing through the sample, the photon pair accumulates an optical phase ϕ, as a consequence of optical activity. This occurs twice as fast as for classical light, hence ensuring superior accuracy.

The experiment proceeds as follows: first, a solution containing sucrose (0.8 M) is prepared; then invertase is added to the sample and this sets the initial time for the time-tracking measurement. We record the kinetics at room temperature with two different invertase concentrations, 10 mg/ml and 20 mg/ml at a sampling rate of 37 s. In Fig. 1A we show how the phase evolves in time due to the catalyzing action of invertase. For each point, the choice of the measurement is optimized with an adaptive scheme to ensure each phase is estimated close to the ultimate precision (Fig. 1B).

The optical measurements have been validated employing a standard method based on dinitrosalicylic acid (DNS). When DNS binds to D-glucose and D-fructose, it shows a typical absorption peak at 540 nm proportional to the amount of these mono-saccharides. The same time scales for the completion of the process are observed with the two techniques, with minor discrepancies attributed to small variations in room temperature and/or invertase and sucrose batches concentration (Fig. 1D). To investigate possible effect of light on invertase activity, additional reactions were carried out with invertase samples illuminated for 1 h with laser at different frequencies and intensities. Comparison to untreated (*i.e.* not illuminated) invertase revealed that light exposure is detrimental to enzymatic activity (Fig. 1C), supporting the development of the quantum metrology approach for non-invasive measurements.

Optimizing the optical setup, the biological samples preparation, and the validation procedure has demanded in this study a multidisciplinary approach. As biological applications of quantum metrology are among the most ambitious and rewarding, it is crucial to develop tools and methods through which the communities can establish a common ground.

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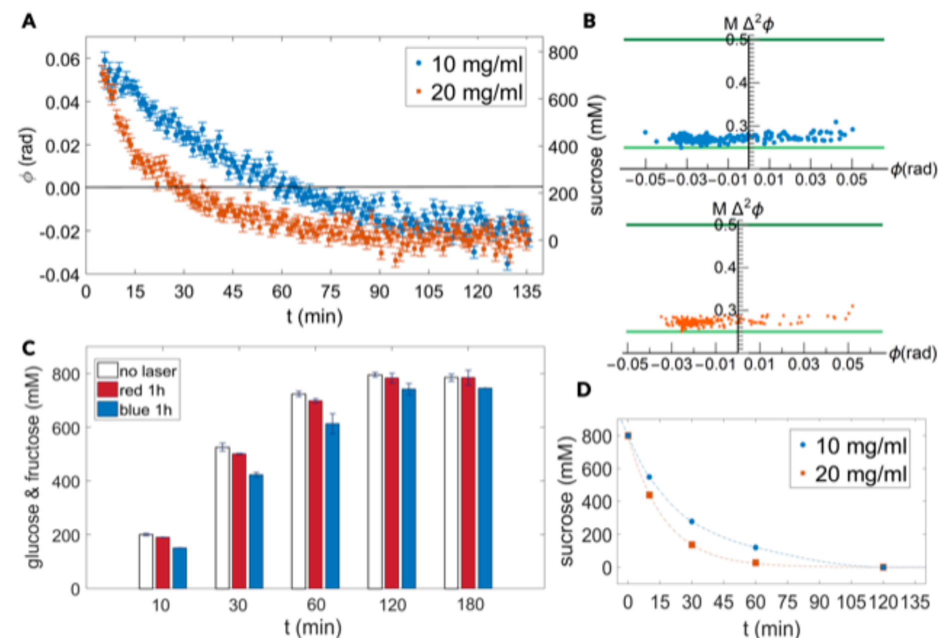

Figure 1. *Experimental results of optical tracking and DNS assays.* (A) Tracking of invertase activity using quantum light states, for two different enzyme concentrations: 10 mg/ml (blue dots) and 20 mg/ml (orange squares). The concentration of sucrose corresponding to each phase measurement is also shown on the right axis. The sampling interval is 37 s. (B) Errors obtained with the adaptive measurement strategy for the two concentrations (color scheme as panel A). The light green line corresponds to the optimal lower bound achievable, while the dark green line is the bound related to a classical probe with the same average intensity. (C) Products concentration measured with the DNS assay for samples of sucrose solution catalyzed by 10 mg/ml invertase undergone different illumination conditions. White: control sample; red: 1 h illumination with a 2.6 mW 800 nm CW laser; blue: 1 h illumination with 200 mW 405 nm CW laser. (D) Tracking with the DNS assay for two different invertase concentrations: 10 mg/ml (blue dots) and 20 mg/ml (orange squares).