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## A new in-situ method to determine the hydrolysis rate constant of adenosine triphosphate (ATP) by application of Raman spectroscopy in a hydrothermal diamond anvil cell

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In recent decades increasing evidence was found for life under extreme conditions, e.g., near black smokers on the ocean floor. The synthesis and stability of vital molecules like adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are essential to maintain the metabolism of all known organisms. The lifetime of these molecules in water is limited by the non-enzymatic hydrolysis reaction that becomes dominant at elevated temperatures. A better understanding of this mechanism will provide us insights of life at extreme conditions.

Previous studies determined the hydrolysis rate constants of ATP for several compositions, temperatures and pressures using quench experiments and subsequent analysis. So far, it was not tested whether quench artefacts might have affected those results. Therefore, the current study was performed to develop a method to follow the reaction in-situ with a high sampling rate at elevated temperatures. A confocal micro-Raman spectrometer and a hydrothermal diamond anvil cell were used to perform experiments at elevated temperatures and vapour pressure. Spectra were obtained in the range of 660  $\text{cm}^{-1}$  to 1157  $\text{cm}^{-1}$  as a function of time. Different solutions of ATP and ADP were measured at 353 K, 373 K, and 393 K, at starting pH values of 3 and 7. First findings are consistent with previous studies and show that with decreasing pH value the hydrolysis rate increases. The data indicate hydrolysis rate constants in the magnitude of  $10^{-3} \text{ s}^{-1}$  by 393 K,  $10^{-4} \text{ s}^{-1}$  by 373 K and  $10^{-5} \text{ s}^{-1}$  by 353 K. These initial observations show that this technique produces reliable kinetic data on this reaction. It also provides much better sampling statistics than quench experiments.

The high reaction rates suggest that a mechanism exists to regulate this reaction at higher temperatures, which is necessary to allow metabolism under extreme conditions. Moreover, it is commonly known that ATP interacts with various metal ions with different effects on the reaction rate. An application of this method would be the quantification of the hydrolysis rate constant in chemically more complex systems.