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Hydrolysis rate constants of ATP up to 120 °C and 1.6 GPa: Implications for life at extreme conditions

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Abstract

Extreme environments are habitats for a diverse array of microorganisms, namely extremophiles, that have evolved unique biochemical adaptations to their geological setting. Some examples of extremophiles can be found in the seafloor or near hydrothermal vents on the ocean floor. Cultivated strains of extremophiles have demonstrated the ability to tolerate temperatures (T) up to 122 °C and pressures (P) up to 125 MPa. Organisms depend on the stability of key metabolites, such as adenosine triphosphate (ATP), to survive and reproduce under these conditions. In order to maintain their intracellular ATP levels, living cells must compensate for the abiotic hydrolysis of ATP, which occurs at a particularly rapid rate at high temperatures. The role of high pressures and high temperatures in abiotic hydrolysis of ATP has been rarely investigated despite the potential for this phenomenon to contribute to limit the adaptation of microorganisms to simultaneous extreme temperatures and pressures. This study presents new data on the effect of pressure on the abiotic hydrolysis of ATP to adenosine diphosphate (ADP) at elevated temperatures. *In situ* Raman spectra were measured at high pressure and high temperature of the hydrolysis of aqueous disodium ATP solutions in two experimental systems: a hydrothermal diamond anvil cell (HDAC) and a gas-pressurized autoclave. These two systems permitted the determination of hydrolysis rate constants of ATP into ADP up to 1670 MPa at 80 °C, 100 °C, and 120 °C. The data exhibited Arrhenian behavior with a slight decrease in activation energy from 0.5 MPa to 140 MPa. The effect of pressure on ATP hydrolysis rate constants was found to be vanishingly low in the so far known vital range up to 125 MPa. Abiotic hydrolysis rates of ATP showed a pronounced increase at higher pressures. For example, at 100 °C, a rise in pressure from 365 MPa to approximately 1670 MPa results in a nearly tenfold increase in the ATP hydrolysis rate constant. When compared with the typical ATP turnover times reported in living cells, the abiotic ATP hydrolysis rates determined in this study provide insights into the pressure and temperature conditions that could be consistent with living microorganisms.

Keywords: Raman spectroscopy, adenosine triphosphate (ATP), adenosine diphosphate (ADP), hydrothermal diamond anvil cell (HDAC), autoclave, sapphire cell, high pressure, high temperature

44 1. Introduction

45 The study of extremophiles, microorganisms that thrive under extreme temperature (T) and
46 pressure (P) conditions (Macelroy, 1974), has broadened our understanding of the potential for
47 life beyond previously known boundaries. In the 1970s, the discovery of biological communi-
48 ties living near hydrothermal vents on the ocean floor, an environment that seemed inhospita-
49 ble, came as a surprise and challenged conventional perspectives of habitability (Corliss and
50 Ballard, 1977). In these conditions, organisms are capable of growth and reproduction at tem-
51 peratures up to 110 °C to 120 °C and at hydrostatic pressures ranging from 20 MPa to 40 MPa
52 (Delaney et al., 1992; Glickson et al., 2007; Blöchl et al., 1997; Edwards et al., 2011). More
53 recent findings in the deep seafloor have demonstrated significant microbial activity up to
54 120 °C (Heuer et al., 2020). Currently, two cultivated strains of hyperthermophilic archaea
55 isolated from ocean floor hydrothermal vents tentatively set the known P-T limits for life.
56 *Methanopyrus kandleri sp.* exhibits cell growth up to 122 °C at 20 MPa (Takai et al., 2008),
57 while *Thermococcus piezophilus sp.* is capable of surviving up to 125 MPa at 65 °C to 95 °C
58 (Dalmasso et al., 2016). Raman spectroscopic investigations of *Shewanella oneidensis sp.* in a
59 diamond anvil cell have suggested the possibility of life at pressures up to the gigapascal range
60 (Sharma et al., 2002). However, the criterion for a viable state, namely formate oxidation, has
61 been heavily criticized and is indeed very ambiguous (e.g., Yayanos, 2002).

62 In directed evolution studies, the mesophilic bacterium *Escherichia coli sp.* demonstrated the
63 ability to develop resistance to high pressure within a short period of time (Vanlint et al., 2011).
64 Traditional cultivation experiments on piezophiles from the deep biosphere are extremely chal-
65 lenging due to their incredibly slow growth rates with a cell division down to once per 1000
66 years (e.g., Jørgensen, 2011). Therefore, it would not come to a surprise that organisms may
67 live beyond currently defined pressure boundaries. In order to live under these conditions, or-
68 ganisms would have to maintain an active metabolism. Consequently, a study of the physico-
69 chemical properties of key metabolites is essential to comprehend potential survival strategies
70 of life. Up to 2000 MPa, pressure can alter intermolecular distances and conformational states
71 of molecules in cells, whereas the covalent bond distances and bond angles of a molecule re-
72 main unchanged (Jebbar and Oger, 2010). These changes can result in alterations to kinetic
73 parameters, thermodynamic equilibrium constants, folding profiles and ligand bindings of pro-
74 teins and organic compounds (Bridgman, 1914; Kauzmann, 1987; Urayama et al., 2002).
75 Among essential metabolites are adenosine triphosphate (ATP), adenosine diphosphate (ADP),
76 and adenosine monophosphate (AMP).

77 In living cells, enzymes catalyze the endergonic phosphorylation of ADP to ATP through var-
78 ious pathways including oxidative phosphorylation catalyzed by membrane-bound ATP syn-
79 thase or substrate-level phosphorylation. The exergonic hydrolysis of ATP is then coupled with
80 other vital endergonic biochemical reactions. In other words, ATP carries chemical energy by
81 transferring a phosphoryl group from a donor to an acceptor (Burgot, 2019). This mechanism
82 is universal in all metabolic systems; thus, ATP was coined the universal biological energy
83 currency (Lipmann, 1941). To ensure their vital functioning, living cells use redox reactions to
84 maintain a steady state of ATP to ADP ratios far from thermodynamic equilibrium. Rapid abi-
85 otic ATP hydrolysis kinetics thus implies higher maintenance energy costs for cells, and it has

86 been suggested that this is an important factor in setting the limits to the functioning of living
87 organisms (Bains et al., 2015).

88 A number of studies have been conducted to ascertain the kinetic effects of temperature and
89 chemical environment on ATP hydrolysis. The increase in hydrolysis rate with temperature is
90 well described by an Arrhenius relation (Hulett, 1970; Leibrock et al., 1995; Daniel et al., 2004;
91 Moeller et al., 2022). The addition of metal cations can result in a deceleration of the hydrolysis
92 rate as observed for Ca or Mg (Ramirez et al., 1980; Leibrock et al., 1995), or an acceleration
93 as demonstrated for Cu (II) (Buisson and Sigel, 1974) or Co (III) complexes (Suzuki et al.,
94 1978). Moeller et al. (2022) estimated a temperature of about 200 °C at which abiotic ATP
95 hydrolysis would not be compensated by normal cellular regeneration time. This suggests that
96 the abiotic hydrolysis of ATP is unlikely to be the main factor determining the upper tempera-
97 tures recorded for microbial life. Nevertheless, the question of the cross-effect of pressure on
98 this temperature limit remains unanswered, despite its importance, given that the subsurface or
99 ocean floor deep biospheres are indeed subjected to high pressures. The effect of pressure on
100 abiotic ATP hydrolysis has so far been studied only by Leibrock et al. (1995). The authors
101 observed that the hydrolysis of ATP-D₂O at 80 °C was accelerated from 10 MPa to 120 MPa
102 and then decelerated from 120 MPa to 220 MPa.

103 Moeller et al. (2022) introduced a protocol to directly study ATP hydrolysis at high temperature
104 using *in situ* Raman spectroscopy. *In situ* Raman spectroscopy is an efficient technique utilized
105 across scientific disciplines, including high pressure biology, to monitor the effects of different
106 pressures, temperatures, and chemical environments on the kinetics of biochemical reactions.
107 In the present study, Raman spectroscopy combined with a hydrothermal diamond anvil cell
108 (HDAC) was employed to obtain *in situ* measurements of abiotic ATP hydrolysis under pres-
109 sures up to 2000 MPa. To improve the pressure resolution, particularly below 140 MPa, an *in*
110 *situ* Raman setup based on an autoclave fitted with optical high-pressure windows was utilized.

111 2. Materials and methods

112 2.1. Sample preparation

113 Ultrapure adenosine triphosphate disodium salt C₁₀H₁₄N₅Na₂O₁₃P₃Na₂ · 3 H₂O with a purity of
114 98%, hereafter referred to as ATP, was purchased from VWR (VWR, Germany). This com-
115 pound was chosen to be comparable to previous data (e.g., Leibrock et al., 1995; Moeller et al.,
116 2022). In the hydrothermal diamond anvil cell, Milli-Q water purification system water was
117 used for experiments, while high-performance liquid chromatography (HPLC) water was used
118 in the autoclave due to availability. Pure deuterated water (D₂O) was also utilized as a solvent.
119 Solutions were prepared by weighing and dissolving 0.1 mol/l ATP in water by manually stir-
120 ring and gently shaking the sample container. This concentration of Na₂H₂ATP corresponds to
121 the solubility product. A notable increase in concentration due to evaporation would result in
122 the precipitation of Na₂H₂ATP, which was not observed. The error of weighing was less than
123 0.003 g. Each solution was prepared with a minimum volume of 3 ml. The resulting concen-
124 tration of ATP was 0.1 mol/l ± 0.009 mol/l. All solutions were transparent without visible
125 precipitates. The initial pH was measured with Endress and Hauser electrodes at room temper-
126 ature and repeatedly controlled with indicator paper. The pH of the ATP-H₂O solutions varied
127 from 2.8 to 3.0, while the pD of ATP-D₂O solutions was 3.3 to 3.5. This difference is within

128 the error of the general pD/pH relationship of $pD = pH + 0.41$ (Covington et al., 1968). Note
129 that the pH was not buffered during the experiments.

130 2.2. Isobaric experiments using an autoclave

131 For isobaric experiments, samples were loaded into a sapphire reaction cell, which was in-
132 stalled within a high-pressure autoclave (Testemale et al., 2005; Louvel et al., 2015). The au-
133 toclave was pressurized by helium (He) and was fitted with high-pressure optical windows
134 made of sapphire. A double wall system of the autoclave enabled cooling by water. The sap-
135 phire cell was 100 mm in length and had an inner diameter of 5 mm. It was manufactured by
136 R.S.A. le Rubis SA, France. The sample was contained within the tube between two moving
137 pistons of vitreous carbon, which transmitted the pressure of the helium to the solution. Two
138 silicone O-rings were used to seal the cell, between pistons and tube. The sample volume was
139 about 0.2 ml.

140 Pressure and temperature were controlled independently by Eurotherm® controllers. The pres-
141 sure regulation had a precision of ± 0.2 MPa (Bruyere et al., 2008). Heating was provided by a
142 resistive furnace made of 0.4 mm diameter molybdenum (Mo) wire wrapped around a cylin-
143 drical Mo heat distributor in which the sample cell was placed. K-type thermocouples were
144 placed inside the distributor. The temperature was measured with a precision of ± 0.1 °C. The
145 discrepancy between the thermocouples and the sample was 10 °C. The overall uncertainty on
146 temperature was estimated to be ± 5 °C. The target temperature and pressure were reached
147 within two to three minutes.

148 The apparatus was attached to a Princeton SpectraPro SP2758 Raman spectrometer. A diode-
149 pumped solid-state laser (Cobolt Fandango 100) of 515 nm was used for excitation. The laser
150 power was 80 mW without objective before the laser beam entered the autoclave. A diffraction
151 grating of 1800 lines/mm and edge filters were installed. Only the 1800 lines/mm grating had
152 a sufficient resolution of 1 cm^{-1} for the peak-fitting-model applied later. A CCD Pylon detector
153 with 1340 pixels measured the Raman scattering. It was cooled with liquid nitrogen at -120 °C.
154 Spectra were acquired every 30 seconds as an average of three accumulations of nine seconds
155 each. Each data set was acquired sequentially under isothermal and isobaric conditions. The
156 temperatures of the experiments were 80 °C, 100 °C and 120 °C. The pressure of the experi-
157 ments ranged from 0.5 MPa to 140 MPa. Figure 1 illustrates exemplary timeseries of each ATP
158 solution and sample holder.

159 2.3. Experiments using a hydrothermal diamond anvil cell

160 A Bassett-type hydrothermal diamond anvil cell, HDAC (Bassett et al. 1993; Schmidt and
161 Chou, 2021), was utilized for experiments ranging from vapor-saturated pressure (P_{sat}) to 2000
162 MPa at 80 °C, 100 °C and 120 °C. The cell was equipped with colorless ultra-low fluorescence
163 and ultra-low birefringence diamond anvils with a diameter of the culet faces of 0.9 mm. The
164 solution was loaded into the sample chamber, which was formed by a cylindrical hole in an
165 iridium (Ir) gasket. The initial diameter of the sample chamber was 300 μm with a height of
166 125 μm . A quartz grain was added into the cell as a Raman spectroscopic pressure sensor. The
167 pressure was determined by measuring the shift of the Raman band at 465 cm^{-1} (Schmidt and
168 Ziemann, 2000). The position of the mercury (Hg) line at 546 nm from a fluorescent lamp was
169 simultaneously monitored to correct the Raman band position if required. The Raman spectrum
170 of the quartz grain was recorded before the experiment at room temperature and after the

171 experiment at target temperature to minimize delays in measuring the reaction kinetics. The Ir
 172 gasket may shrink upon heating leading to a slight volume change. This change is known to
 173 occur rapidly after reaching the temperature with no further change over run time (Schmidt and
 174 Ziemann, 2000). Gasket failure due to expansion resulting in a larger sample chamber diameter
 175 or deviations from a circular shape has not been observed. The temperature effect on the peak
 176 position was corrected using the equation provided by Schmidt and Ziemann (2000).
 177 The pressure accuracy is approximately ± 30 MPa (Schmidt, 2009). The temperature was meas-
 178 ured by two K-type thermocouples attached to the anvils. The power input to two resistive
 179 heaters, Ni-Cr coils around the seats, was controlled using Eurotherm[®] temperature control-
 180 lers. The target temperature was reached within one minute. The overall uncertainty in the
 181 temperature was ± 1 °C.

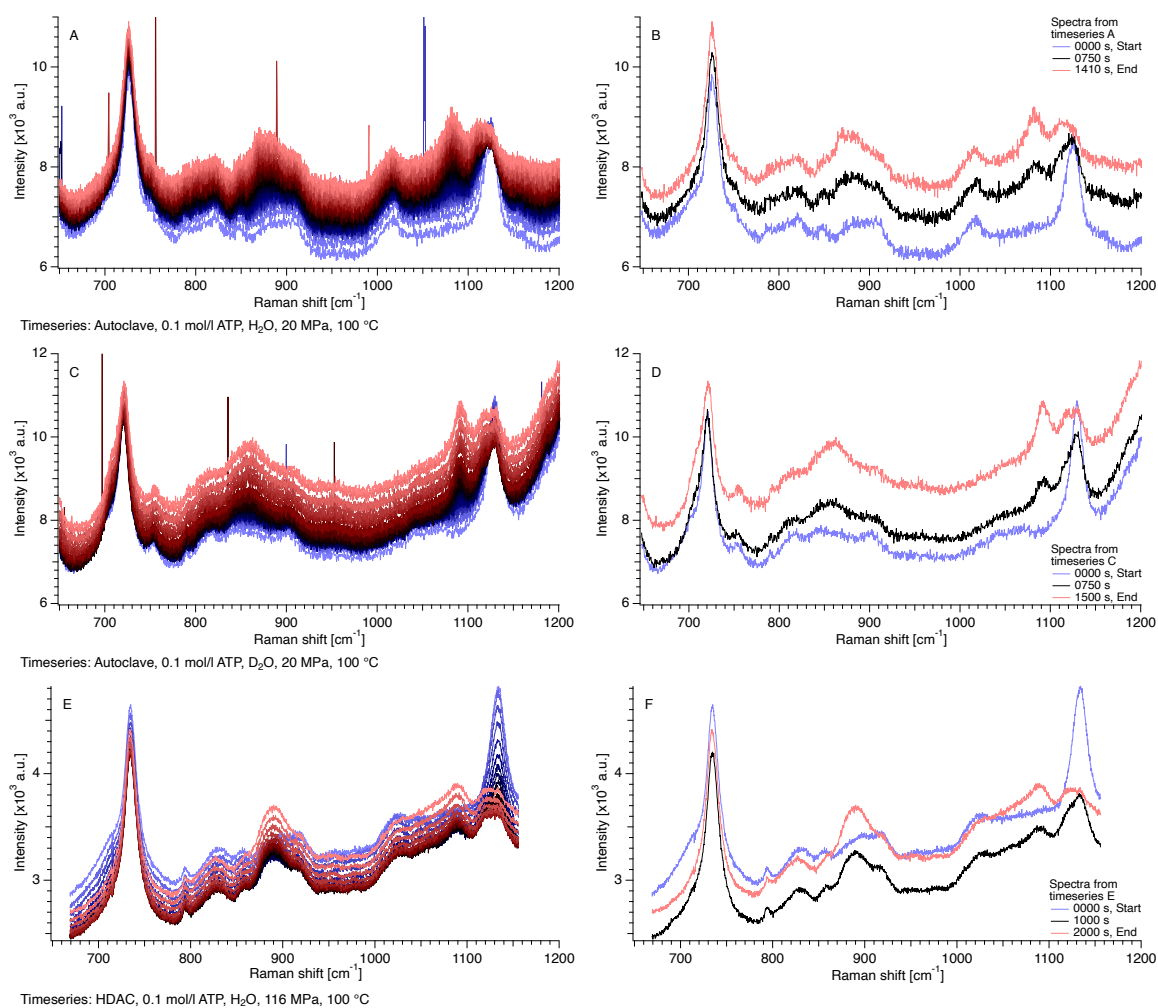


Figure 1: Exemplary Raman spectra as a function of time (on the left) and Raman spectra showing the first, an intermediate and the final Raman spectrum of the timeseries (on the right). A and B: Timeseries of 0.1 mol/l ATP-H₂O obtained in the autoclave at 100 °C and 20 MPa. C and D: Timeseries of 0.1 mol/l ATP-D₂O obtained in the autoclave at 100 °C and 20 MPa. E and F: Timeseries of 0.1 mol/l ATP-H₂O obtained in the HDAC at 100 °C and 116 MPa.

182 The Raman spectrometer was a HORIBA Jobin Yvon LabRAM HR800 VIS equipped with an
 183 Olympus BXFM confocal microscope and a motorized XYZ stage. A frequency-doubled
 184 Nd:YAG laser with a wavelength of 532 nm was used for excitation. The laser power was 32
 185 mW without objective before the laser beam entered the HDAC. The objective was an Olympus
 186 SLMPlanN 20 \times with a working distance of 25 mm. A diffraction grating with 1800 lines/mm,

187 a Peltier-cooled CCD detector with 1024 x 256-pixels, and edge filters were installed. The
188 Raman spectra were acquired as an average of five accumulations of 20 seconds each.

189 2.4. Experimental data treatment

190 The recorded ranges of the Raman spectra were 647 cm^{-1} to 1200 cm^{-1} in the autoclave and 669
191 cm^{-1} to 1155 cm^{-1} in the HDAC. The intensity of the Raman scattering in the autoclave was
192 approximately two to three times higher than that collected in the HDAC. An increasing lumi-
193 nescence effect was visible in the spectra when AMP formed in the solution. All raw spectra
194 are available in the Data Availability section ([dataset] Moeller et al., 2024).

195 The symmetric stretching mode ν_s of the terminal phosphate group $\text{PO}_2(\text{OH})$ at 1080 cm^{-1} for
196 AMP, at 1110 cm^{-1} for ADP, and at 1120 cm^{-1} for ATP was used to detect the relative change
197 in the respective species concentration. The ring vibration at 728 cm^{-1} of the adenosine moiety
198 ν_{ring} (C–C, N–C) was exclusively assigned to the adenosine moiety (Mathlouthi et al., 1984;
199 Rimai et al., 1969). Therefore, it could be used as an internal standard for Raman intensity
200 (Moeller et al., 2022). Comparative measurements on solutions of 0.1 mol/l ATP and ADP
201 demonstrate that the ring vibration is identical within the usual measurement inaccuracies (Fig-
202 ure 2).

203 The spectra of the solutions were divided into two sections to allow a linear baseline subtrac-
204 tion. This was done by dividing the spectra into two sections, one ranging from 666 cm^{-1} to
205 785 cm^{-1} and the other from 978 cm^{-1} to 1155 cm^{-1} for spectra obtained using the HDAC (Figure
206 3, A-B). Similarly, the spectra were divided into two sections, one ranging from 662 cm^{-1} to
207 778 cm^{-1} and the other from 965 cm^{-1} to 1198 cm^{-1} for spectra acquired using the autoclave in
208 the presence of water (Figure 3, C-D). For spectra obtained in the autoclave in the presence of
209 deuterated water (Figure 3, E-F), the ranges were 662 cm^{-1} to 778 cm^{-1} and 1067 cm^{-1} to 1152
210 cm^{-1} . The addition of peaks to the peak-fitting model ensured the stability and consistency of
211 the procedure throughout the entire series. The peak assignments are provided in Table 1, along
212 with the initial peak positions of the peak-fitting model. It is notable the position of the Raman
213 band of the terminal phosphate group $\text{PO}_2(\text{OH})$ at 1080 cm^{-1} (AMP), at 1110 cm^{-1} (ADP) and
214 at 1120 cm^{-1} (ATP) at ambient conditions shifts considerably with pressure. To compensate for
215 this effect in the high-pressure (HP) experiments in the HDAC, and to ensure a robust fit, the
216 initial peak positions of these modes were shifted. The maximum shift was 10 cm^{-1} for the
217 adenosine polyphosphate bands in H_2O solutions.

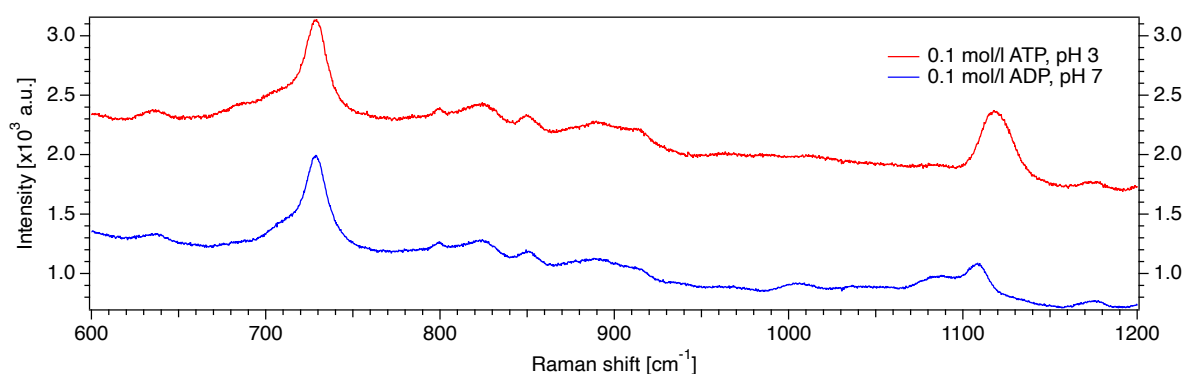


Figure 2: Raman spectra of 0.1 mol/l ATP and ADP solutions measured in a quartz cuvette at ambient conditions.

Table 1: Assignment of the vibrational modes of the measured Raman spectra to literature values for the spectra of ATP- H_2O/D_2O solutions in the HDAC and the autoclave (n.a. = not assigned).

Peak position [cm^{-1}] – peak-fitting-model					Reference
Reference	H_2O^H	H_2O^S	D_2O^S		
713	720	700	720	v_s (POP)	Eysel and Lim (1988)
727-731	729	727 ^L	729 ^L	v_{ring} (C-C, N-C)	Mathlouthi et al. (1984), Rimai et al. (1969)
755		752	755	Sapphire signal	n.a.
984	990			v_{as} POP (Fermi level)	Eysel and Lim (1988), Marshall and Begun (1989)
1008	1010	1015		v_{as} P(OH) ₃	Rudolph (2010)
1045	1045	1035		n.a.	n.a.
1065	1065	1060		n.a.	n.a.
1080	1080	1083	1090	v_s PO ₂ (OH), AMP	Rimai et al. (1969)
1097	1096	1096		v_e PO ₃	Eysel and Lim (1988)
1110	1111	1111	1110	v_s PO ₂ (OH), ADP	Rimai et al. (1969)
1120	1123-1130	1124-1130	1125 ^L	v_s PO ₂ (OH), ATP	Rimai et al. (1969), Heyde and Rimai (1971)
1128	1140		1130	v_e PO ₃	Eysel and Lim (1988), Preston and Adams (1979)
1150		1160		v_{as} PO ₂	Eysel and Lim (1988), Marshall and Begun (1989)

^HHDAC; ^SSapphire cell; ^LLorentzian distribution

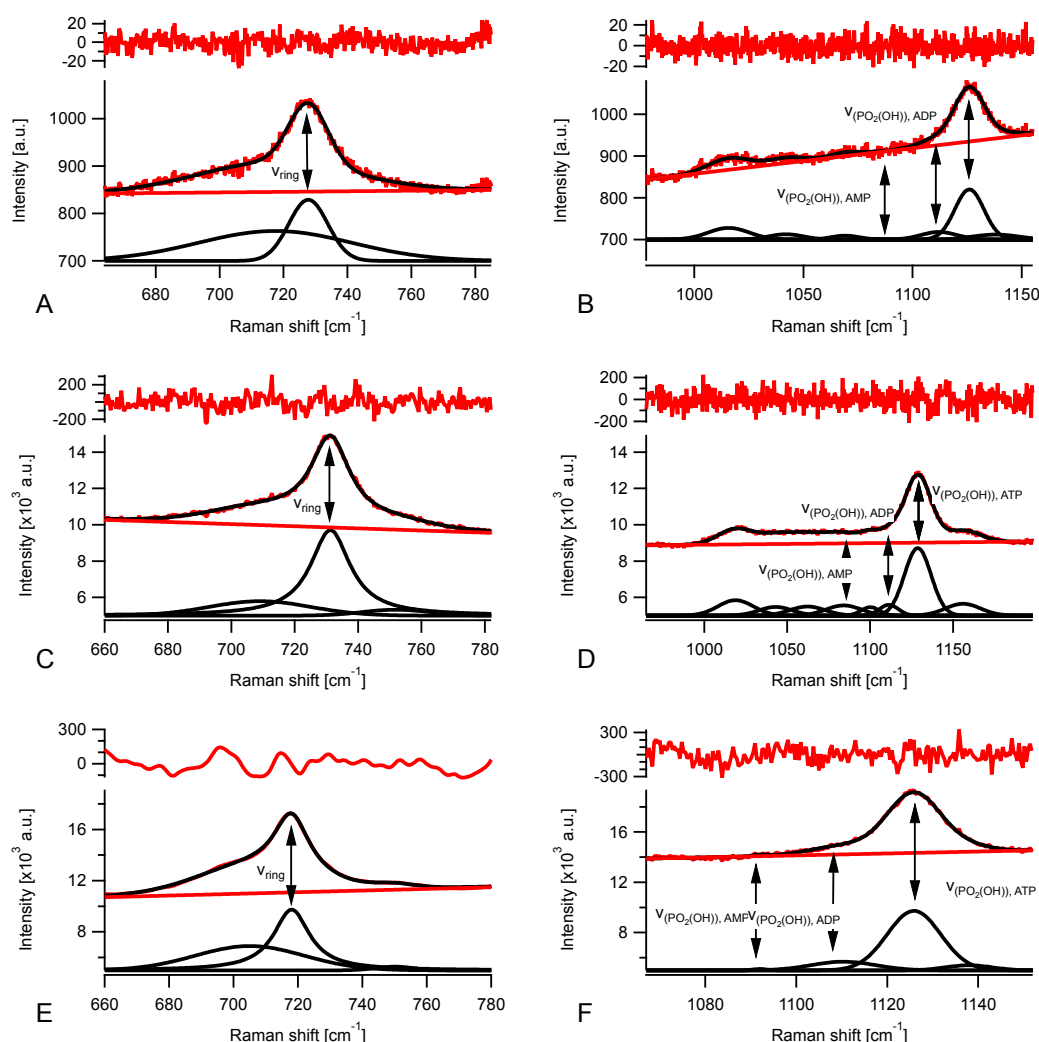


Figure 3: Peak-fitting-model after Moeller et al. (2022), were applied to the measured timeseries. The main peaks are marked and further described in the text. A) ATP + H_2O obtained in a HDAC in the range from 660 v to 785 cm^{-1} . B) ATP + H_2O obtained in a HDAC in the range from 978 cm^{-1} to 1155 cm^{-1} . C) ATP + H_2O obtained in the autoclave in the range from 660 cm^{-1} to 785 cm^{-1} . D) ATP + H_2O obtained in the autoclave in the range from 965 cm^{-1} to 1198 cm^{-1} . E) ATP + D_2O obtained in the autoclave in the range from 662 cm^{-1} to 778 cm^{-1} . F) ATP + D_2O obtained in the autoclave in the range from 1067 cm^{-1} to 1152 cm^{-1} .

220 The symmetric stretching mode ν_s of the terminal phosphate group $\text{PO}_2(\text{OH})$ of ADP at 1110
221 cm^{-1} and ATP at 1120 cm^{-1} are partly overlapping (Figure 2) which resulted in less accurate
222 results in unconstrained fits. To achieve maximum flexibility and to maintain the fit approach
223 stable throughout the reaction, a minimum of fitting constraints was chosen. The peak position
224 was allowed to vary within 3 cm^{-1} to 5 cm^{-1} . To prevent the occurrence of large, flat peaks, the
225 width of ν_s ($\text{PO}_2(\text{OH})$) of ATP from the very first spectra in the timeseries was employed as a
226 constraint for the peak width of all peaks. Peak height and width were constrained to be posi-
227 tive.

228 The integrated intensity of the ν_s ($\text{PO}_2(\text{OH})$) peak was normalized to that of ν_{ring} (C–C, N–C).
229 Both values were corrected for temperature (Brooker et al., 1988) and excitation efficiency
230 (Schmidt et al., 2009). The result was a measure of the concentration of [ATP] without further
231 calibration as only the relative changes are of interest for the determination of the rate constant.
232 Rate constants for the reaction of ATP to ADP were calculated assuming a first order reaction
233 (Khan and Mohan, 1974). The formation of diphosphate from the reaction of ATP to AMP has
234 not been reported in experimental studies (Leibroek et al., 1995). Therefore, the concentration
235 of the reactant as a function of time t can be described by

$$236 \quad \ln \left(\frac{[\text{ATP}]_t}{[\text{ATP}]_0} \right) = -kt \quad \text{Eq. 1}$$

237 and the corresponding half-life $t_{1/2}$ can be determined by

$$238 \quad t_{1/2} = \frac{\ln 2}{k} \quad \text{Eq. 2}$$

240 where $[\text{ATP}]_t$ is the concentration of ATP at time t , $[\text{ATP}]_0$ the concentration at $t = 0$, and the
241 rate constant k . The constant can be determined by substituting the reduced intensity for the
242 concentration in Eq. 1. The slope of a linear fit is the rate constant k . By convention, k is
243 positive. During the fit the y-intercept was set to 0. Representative fits for datasets from the
244 autoclave are shown in Figure 4. The lowest values of $\ln([\text{ATP}]_{i,t}/[\text{ATP}]_{i,0})$ included in the fits
245 were -1 for data in the autoclave and -1.6 for data in the HDAC. All fitted lines consisted of at
246 least three points, except for one data set at 120 °C and 176 MPa in the HDAC due to the
247 insufficient temporal resolution of 100 s/spectrum of the HDAC; therefore, it has a presumably
248 much higher uncertainty. The qualitative detection limits of the setups are around 0.005 mol/l,
249 which is equivalent to 5% of the initial ATP concentration. The limit of the peak-fit-model was
250 approximately 0.025 mol/l, which is 25% of the initial ATP concentration. Exemplary peak-
251 fitting-models, the assignment of the peaks, and all intermediate results are available in the
252 Data Availability section ([dataset] Moeller et al., 2024).

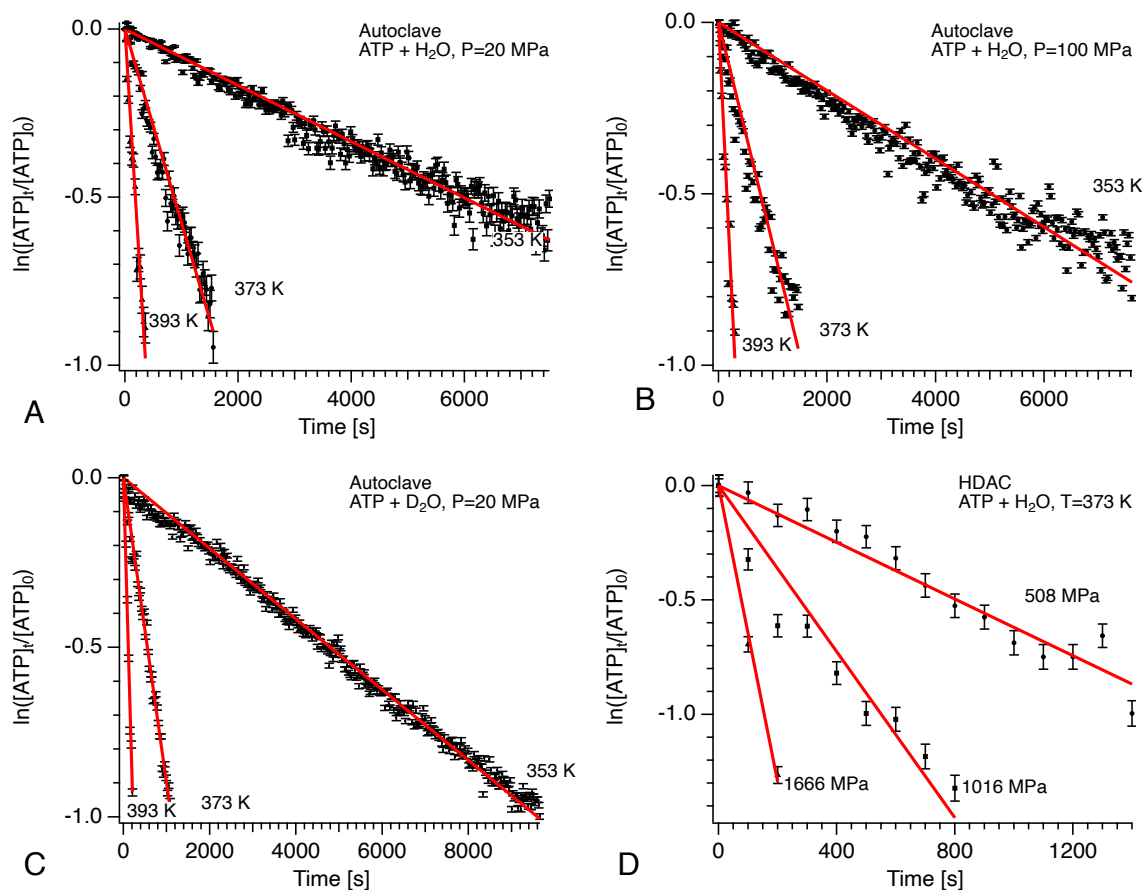


Figure 4: Linear fits of $\ln([ATP]_t/[ATP]_0)$ vs Time, where the slope is k . Datasets A-C were determined in the autoclave. Dataset D was obtained from the HDAC. A) Linear fits of datasets at 20 MPa and H_2O . B) Linear fits of datasets at 100 MPa. C) Linear fits of datasets of ATP- D_2O solutions at 20 MPa. D) The linear fits of the datasets obtained at high pressure in the HDAC at 100 °C are presented below.

253

254 2.5. Thermodynamic calculations of pH during ATP hydrolysis

255 For the thermodynamic properties of ATP, a distinction is made between Mg-Ca and Mg-Ca-
 256 free systems, as Mg^{2+} and Ca^{2+} are known to form complexes with ATP (Alberty, 1969; Gajew-
 257 ski et al., 1986; Phillips et al., 1966). In the present study, it is assumed that Na^+ ions do not
 258 form complexes with ATP and its hydrolysis components. The system components chosen for
 259 the thermodynamic modeling are listed in Table 2 and include water, dissolved organic and
 260 inorganic aqueous species, and real gases. The provided data are only applicable for the P-T
 261 conditions of our experiments. The Haar-Gallagher-Kell and Marshall and Franck models
 262 (Marshall and Franck, 1981; Kestin et al., 1984) were employed to determine the thermody-
 263 namic properties and dissociation constant of H_2O for the experimental conditions. The Helge-
 264 son-Kirkham-Flowers (HKF) equation of state (Helgeson et al., 1981) was employed for aque-
 265 ous species, while the Peng-Robinson equation of state (Peng and Robinson, 1978) was utilized
 266 for aqueous vapor and solid phases. The HKF equation of state is applicable up to 500 MPa.

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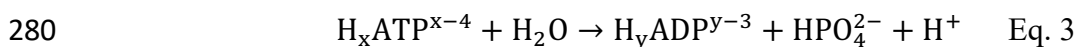
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Table 2: The system components chosen for the thermodynamic modeling and the corresponding thermodynamic data sources.

Species	Charge	Chemical Formula of organic aqueous species	Reference
<i>Solid phase</i>			
Quartz (SiO ₂)	0		Robie and Hemingway (1978)
<i>Aqueous solution</i>			
H ₂ O	0		Marshall and Franck 1981; Kestin et al. (1984)
OH ⁻	1-		Shock et al. (1997), zero by definition
H ⁺	1+		Shock and Helgeson (1988)
PO ₄ ³⁻	3-		Shock et al. (1997)
HPO ₄ ²⁻	2-		Shock et al. (1997)
H ₂ PO ₄ ²⁻	2-		Shock et al. (1997)
H ₃ PO ₄ (aq)	0		Shock et al. (1989)
Na ⁺	1+		Shock et al. (1997)
NaOH (aq)	0		Shock et al. (1997)
SiO ₂ (aq)	0		Sverjensky et al. (2014)
Si ₂ O ₄ (aq)	0		Sverjensky et al. (2014)
N ₂ (aq), O ₂ (aq), H ₂ (aq)	0		Robie and Hemingway (1978)
AMP ²⁻	2-	C ₁₀ H ₁₂ N ₅ O ₇ P	LaRowe and Helgeson (2006)
HAMP ¹⁻	1-	C ₁₀ H ₁₃ N ₅ O ₇ P	LaRowe and Helgeson (2006)
H ₂ AMP (aq)	0	C ₁₀ H ₁₄ N ₅ O ₇ P	LaRowe and Helgeson (2006)
ADP ³⁻	3-	C ₁₀ H ₁₂ N ₅ O ₁₀ P ₂	LaRowe and Helgeson (2006)
HADP ²⁻	2-	C ₁₀ H ₁₃ N ₅ O ₁₀ P ₂	LaRowe and Helgeson (2006)
H ₂ ADP ¹⁻	1-	C ₁₀ H ₁₄ N ₅ O ₁₀ P ₂	LaRowe and Helgeson (2006)
H ₃ ADP (aq)	0	C ₁₀ H ₁₅ N ₅ O ₁₀ P ₂	LaRowe and Helgeson (2006)
ATP ⁴⁻	4-	C ₁₀ H ₁₂ N ₅ O ₁₃ P ₃	LaRowe and Helgeson (2006)
HATP ³⁻	3-	C ₁₀ H ₁₃ N ₅ O ₁₃ P ₃	LaRowe and Helgeson (2006)
H ₂ ATP ²⁻	2-	C ₁₀ H ₁₄ N ₅ O ₁₃ P ₃	LaRowe and Helgeson (2006)
H ₃ ATP ¹⁻	1-	C ₁₀ H ₁₅ N ₅ O ₁₃ P ₃	LaRowe and Helgeson (2006)
H ₄ ATP (aq)	0	C ₁₀ H ₁₆ N ₅ O ₁₃ P ₃	LaRowe and Helgeson (2006)
<i>Gaseous mixture</i>			
H ₂ O (g)	0		Robie and Hemingway (1978)
N ₂ (g)	0		Robie and Hemingway (1978)

272 The HCh software (Shvarov, 1999) was used to calculate the pH of the experimental solutions
 273 under study at specified P-T conditions. HCh minimizes the total Gibbs free energy of the
 274 system, thereby providing the concentrations of the system components, their activity coeffi-
 275 cients, the ionic strength of an aqueous solution, and its pH and Eh. In order to model the pH
 276 at elevated temperatures, pH_T, in our experiments, it was assumed that 0.1 mol of Na₂H₂ATP
 277 dissociates completely in 1 kg of H₂O, forming H₂ATP²⁻ and 2 Na⁺. The reactions involved in
 278 the hydrolysis of ATP to ADP can be summarized by the following equation (Eq. 3):

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281

282 with values of x between 0 and 4 and values of y between 0 and 3. To simulate the effect of the
 283 additional phases in our system, the partition of H₂O from the liquid phase to the vapor phase
 284 at P_{sat} was calculated. The effect of quartz dissolution at equilibrium conditions was simulated
 285 by the addition of one mole of quartz to the solution. To calculate the metastable equilibrium
 286 conditions, where the ATP hydrolysis progresses step-by-step, the following steps were calcu-
 287 lated: first, forming ATP species in a calculated system with one liquid phase containing ATP
 288 species (I₁), and then, dissociating further to form ADP and AMP species in calculated systems
 289 with one liquid phase containing ATP and ADP species (I₂) and one liquid phase containing
 290 ATP, ADP and AMP species (I₃), respectively. These system compositions served as the basis
 291 for the thermodynamic simulations. To simulate a potential exchange reaction of H₂O from the
 292 liquid to the vapor phase, a nitrogen phase was added to the system and gaseous H₂O (g) was

293 allowed to fractionate to the vapor phase which is designated as “+v”. To simulate the role of
 294 quartz in the system, quartz was included in the thermodynamic model which is designated as
 295 “+q”.

296 The pH was calculated in 12 distinct model systems, including single aqueous solutions (l_1 , l_2 ,
 297 l_3), two-phase systems with an aqueous solution and a vapor phase (l_1+v , l_2+v , l_3+v , l_1+v_c ,
 298 l_2+v_c , l_3+v_c) and two-phase systems with an aqueous solution and a quartz grain (l_1+q , l_2+q ,
 299 l_3+q) (Table 3). Thus, it was possible to simulate the pH at equilibrium conditions in three types
 300 of experimental systems: a single-phase isothermal-isobaric aqueous system applicable to our
 301 experiments in the autoclave, a single-phase isothermal-isochoric aqueous system with a quartz
 302 grain as a pressure sensor applicable to our experiments in the HDAC and a two-phase aqueous
 303 system applicable in the HDAC that were performed at P_{sat} .

304 *Table 3: Initial chemical phase composition of the thermodynamic calculations.*

System name	System components
l_1	H_2O , H^+ , OH^- , Na^+ , NaOH (aq), O_2 (aq), H_2 (aq), N_2 (aq), PO_4^{3-} , HPO_4^{2-} , H_2PO_4^- , H_3PO_4 (aq), ATP^4 , HATP^3 , H_2ATP^2 , H_3ATP^1 , H_4ATP (aq)
l_2	aqueous fluid of system l_1 + ADP^{3-} , HADP^{2-} , H_2ADP^- , H_3ADP (aq)
l_3	aqueous fluid of system l_2 + AMP^{2-} , HAM^1 , H_2AMP (aq)
$l_1 + v$	aqueous fluid of system l_1 + N_2 (g), H_2O (g)
$l_2 + v$	aqueous fluid of system l_2 + N_2 (g), H_2O (g)
$l_3 + v$	aqueous fluid of system l_3 + N_2 (g), H_2O (g)
$l_1 + q$	aqueous fluid of system l_1 + SiO_2 (aq), Si_2O_4 (aq), quartz
$l_2 + q$	aqueous fluid of system l_2 + SiO_2 (aq), Si_2O_4 (aq), quartz
$l_3 + q$	aqueous fluid of system l_3 + SiO_2 (aq), Si_2O_4 (aq), quartz

305 3. Results

306 Table 4 and Figure 5 summarizes all 49 hydrolysis rate constants and their respective half-
 307 lives. These include 40 for the ATP- H_2O system and 9 for the ATP- D_2O system. Of these, 38
 308 were determined in the autoclave, while 11 were measured in the HDAC. In addition to the
 309 experimental kinetic data, thermodynamically calculated pH values at these P-T conditions are
 310 provided at run conditions (Table 4). It should be noted that the calculation of ion concentra-
 311 tions and thus a pH above 500 MPa is limited by the HKF equation of state. Figure 5 depicts
 312 the distribution of these rate constants as a function of pressure. A standard deviation (σ_1) for
 313 the rate constant was determined by performing three experiments at 20 MPa in the autoclave,
 314 each at temperatures of 80 °C, 100 °C, and 120 °C. The standard deviations ranged from 4%
 315 to 9.5% relative to the value of the rate constant. To be conservative, a standard deviation of
 316 10% was assumed for data sets in the autoclave. It is not possible to reproduce single fluid
 317 aqueous experiments in the isochoric HDAC at the exact same conditions due to technical
 318 limitations. Nevertheless, a replication of the experiment at P_{sat} of Moeller et al. (2022)
 319 demonstrated a standard deviation of 5.5%, which is within the estimated standard deviation
 320 of 10%. It can therefore be reasonably assumed that the overall precision of both apparatuses
 321 and the fitting procedure is 10%. The data encompass a pressure range from P_{sat} to 1670 MPa
 322 and temperatures of 80 °C, 100 °C, and 120 °C (Table 4). Additionally, datasets from Leibrock
 323 et al. (1995) and Moeller et al. (2022) are provided for comparison.

324 The results are in satisfactory agreement with previous studies in the overlapping pressure and
 325 temperature domains as illustrated in Figure 5. There is also satisfactory agreement between
 326 the hydrolysis constants measured in the autoclave and in HDAC when pressures and temper-
 327 atures overlap. Finally, ATP hydrolysis rates exhibited a significant increase with pressure

328 when the latter exceeds 800 MPa. Each temperature series of the constants obtained in the
 329 autoclave for the hydrolysis in H₂O follows a similar trend with pressure. The ATP hydrolysis
 330 rate exhibits an average increase of 1.14 ± 0.11 at 80 °C, 1.32 ± 0.05 at 100 °C and 1.14 ± 0.07
 331 at 120 °C between 0.5 and 20 MPa. The rate constants fluctuate around an average value which
 332 is $10.7 \times 10^{-5} \text{ s}^{-1} \pm 2.5 \times 10^{-5} \text{ s}^{-1}$ at 80 °C, $7.5 \times 10^{-4} \text{ s}^{-1} \pm 1.6 \times 10^{-4} \text{ s}^{-1}$ at 100 °C and 3.8×10^{-3}
 333 $\text{ s}^{-1} \pm 0.85 \times 10^{-4} \text{ s}^{-1}$ 120 °C for the pressure range of 20 MPa to 100 MPa. The rate constants
 334 show a slight increase from 100 MPa to 140 MPa with a factor of 1.32 ± 0.02 . The overall
 335 increase from 0.5 MPa to 140 MPa is by a maximum factor of 1.7 ± 0.08 . In the autoclave, the
 336 lowest achievable pressure for all three temperatures was 0.5 MPa. The data are generally con-
 337 sistent with the P_{sat} data of Moeller et al. (2022), although some minor systematic differences
 338 can be observed. Autoclave values appear to be larger by a factor of approximately 1.3 at 80
 339 °C, identical within the σ_1 of 10% at 100 °C, and smaller by a factor of approximately 0.7 at
 340 120 °C. The hydrolysis rate constants obtained in the HDAC in the overlapping pressure range
 341 plot slightly below the autoclave data. The HDAC at 80 °C and 90 MPa and at 120 °C and 176
 342 MPa data are lower than the autoclave data at 80 °C and 120 °C and at 100 MPa by a factor of
 343 1.13 ± 0.01 . At 100 °C, the ratio of the HDAC data at 116 MPa to the autoclave data at 100
 344 MPa is approximately 1.6. However, at 80 °C, both datasets demonstrate a moderate increase
 345 in the rate constants within the pressure ranges of 0.1-90 MPa for the HDAC and 0.5-100 MPa
 346 for the autoclave, with a moderate increase of the rate constants by a factor of 1.34 ± 0.07 .

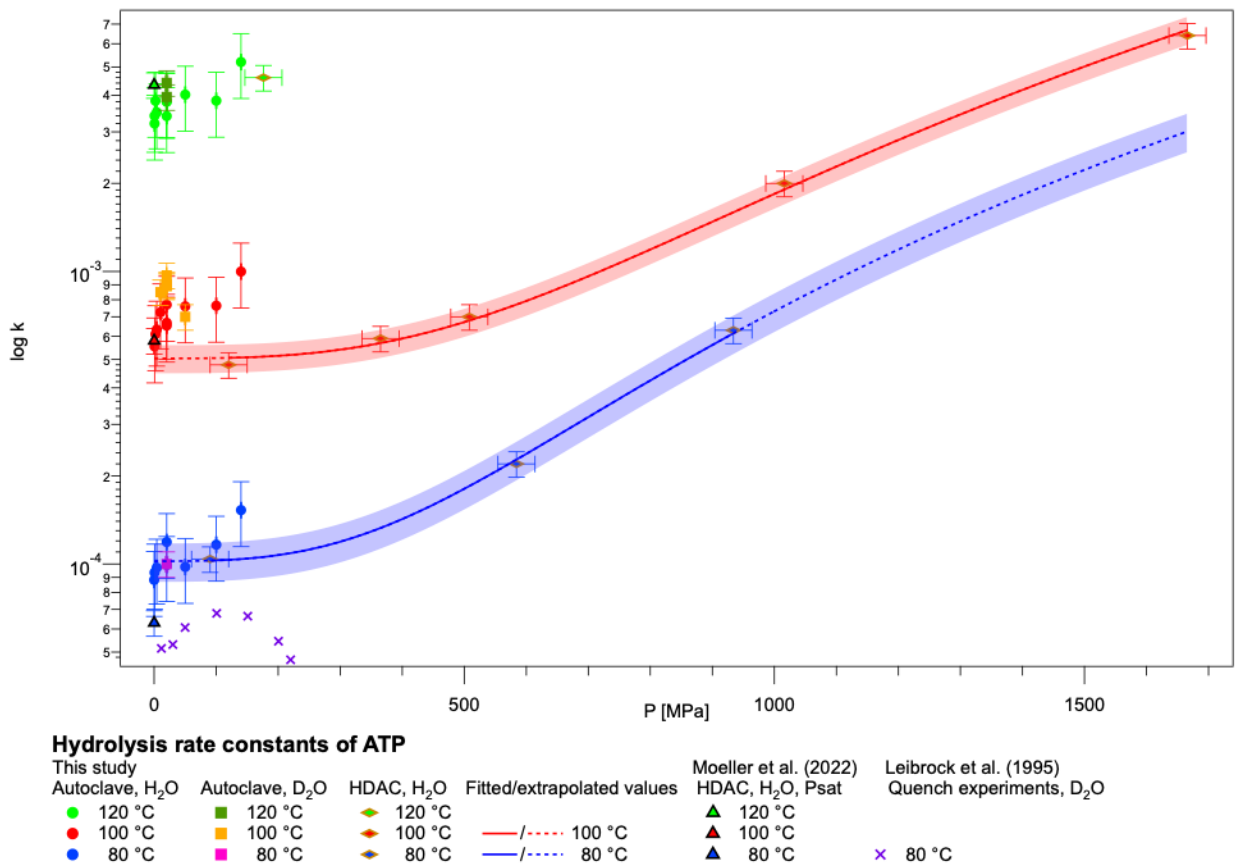


Figure 5: Hydrolysis rate constants of ATP in H₂O/D₂O taken in the course of this study. It is shown the range of 0-1700 MPa. The data from Moeller et al. (2022) and Leibrock et al. (1995) are plotted for comparison.

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Table 4: Hydrolysis rate constants of ATP for solutions with H₂O and D₂O with corresponding half-lives determined in the HDAC and autoclave. A pH at run conditions was thermodynamically calculated based on the phase composition l_1 , l_2 , l_3 , l_1+q , l_2+q , l_3+q , l_1+v , l_2+v , l_3+v described in Table 3. The calculation of pH values above 500 MPa is limited by the HKF equation of state.

Hydrolysis rate constants obtained in the autoclave				Calculated pH			
T [°C] ± 1 [°C]	P [MPa] ± 0.2 [MPa]	k [$\times 10^{-3} \text{ s}^{-1}$] ± 10%	t _{1/2} [s] ± 10%	l ₁	l ₂	l ₃	
80	0.1	9.09×10^{-2}	7625	2.81	2.70	2.47	
	0.5	9.35×10^{-2}	7413	2.81	2.70	2.47	
	4	9.72×10^{-2}	7131	2.81	2.70	2.46	
	20	9.93×10^{-2}	6980	2.81	2.70	2.45	
	20	10.4×10^{-2}	6665	2.81	2.70	2.45	
	20	11.9×10^{-2}	5825	2.81	2.70	2.45	
	50	9.78×10^{-2}	7087	2.82	2.70	2.42	
	100	11.7×10^{-2}	5924	2.83	2.70	2.39	
	140	15.3×10^{-2}	4530	2.84	2.70	2.36	
	100	0.5	5.55×10^{-1}	1249	2.83	2.68	2.46
		2	6.11×10^{-1}	1134	2.83	2.68	2.46
		4	6.33×10^{-1}	1095	2.83	2.68	2.46
		10	7.26×10^{-1}	955	2.83	2.68	2.45
		20	7.10×10^{-1}	976	2.84	2.68	2.44
20		7.33×10^{-1}	946	2.84	2.68	2.44	
20		7.70×10^{-1}	900	2.84	2.68	2.44	
50		7.60×10^{-1}	912	2.85	2.68	2.41	
100		7.64×10^{-1}	907	2.86	2.68	2.37	
140		1.0	693	2.87	2.68	2.34	
120	0.5	3.2	217	2.88	2.67	2.47	
	0.5	3.4	204	2.88	2.67	2.47	
	2	3.8	181	2.88	2.67	2.47	
	4	3.5	198	2.88	2.67	2.46	
	20	3.4	204	2.89	2.67	2.44	
	20	3.8	181	2.89	2.67	2.44	
	20	3.8	182	2.89	2.67	2.44	
	50	4.0	172	2.90	2.66	2.41	
	100	4.0	181	2.91	2.66	2.36	
	140	5.2	133	2.92	2.66	2.33	
	Hydrolysis rate constants obtained in the HDAC						
	T [°C] ± 1 [°C]	P [MPa] ± 20 [MPa]	k [$\times 10^{-3} \text{ s}^{-1}$] ± 10%	t _{1/2} [s] ± 10%	l _{1+q}	l _{2+q}	l _{3+q}
80	0.1	7.51×10^{-2}	9762	2.81	2.70	2.47	
	90	1.04×10^{-1}	6664	2.81	2.70	2.39	
	584	2.2×10^{-1}	3150	n.a.	n.a.	n.a.	
	934	6.3×10^{-1}	1100	n.a.	n.a.	n.a.	
100	116	4.8×10^{-1}	1444	2.83	2.68	2.35	
	365	5.9×10^{-1}	1175	2.84	2.68	2.22	
	508	7×10^{-1}	990	n.a.	n.a.	n.a.	
	1016	2	347	n.a.	n.a.	n.a.	
	1666	6.4	108	n.a.	n.a.	n.a.	
120	176	4.6	126	2.88	2.67	2.31	
Hydrolysis rate constants at Psat obtained in HDAC							
T [°C] ± 1 [°C]	P [MPa]	k [$\times 10^{-3} \text{ s}^{-1}$]	t _{1/2} [s]	l _{1+v}	l _{2+v}	l _{3+v}	
100	0.1	$5.5 \times 10^{-1} \pm 10\%$	$1260 \pm 10\%$	2.83	2.61	2.46	
Moeller et al. (2022)							
80	0.05	$6.1 \times 10^{-2} \pm 0.0036$	11304 ± 63	2.81	2.63	2.47	
100	0.1	$5.8 \times 10^{-1} \pm 0.089$	1164 ± 0.29	2.83	2.61	2.46	
120	0.2	4.3 ± 0.012	143 ± 0.01	2.88	2.61	2.47	
Hydrolysis rate constants of D ₂ O solutions obtained in the autoclave							

T [°C] ± 5 [°C]	P [MPa] ± 0.2 [MPa]	k [$\times 10^{-3} \text{ s}^{-1}$] ± 10%	t _{1/2} [s] ± 10%			
80	20	0.10	6931	n.a.	n.a.	n.a.
100	10	0.85	815	n.a.	n.a.	n.a.
	20	0.97	714	n.a.	n.a.	n.a.
	20	0.89	778	n.a.	n.a.	n.a.
	20	0.90	770	n.a.	n.a.	n.a.
	20	0.90	770	n.a.	n.a.	n.a.
	50	0.70	990	n.a.	n.a.	n.a.
120	20	3.95	175	n.a.	n.a.	n.a.
	20	4.41	157	n.a.	n.a.	n.a.

351 To the best of our knowledge, only Leibrock et al. (1995) have determined the hydrolysis rate
352 constants of ATP at elevated pressures up to 220 MPa. A systematic difference by a factor of
353 approximately two to our data is clearly visible (Figure 5) even though the rate constants of our
354 study are in the same order of magnitude as those of Leibrock et al. (1995). Leibrock et al.
355 (1995) used nuclear magnetic resonance (NMR) to measure ATP concentrations in quenched
356 D₂O solutions derived from quenched experiments. To ensure comparability with Leibrock et
357 al. (1995), we carried out 9 hydrolysis experiments in the autoclave in D₂O at 80 °C, 100 °C
358 and 120 °C at 10 MPa, 20 MPa and 50 MPa (Figure 5, Table 4). Eight rate constants as a
359 function of pressure were reported by Leibrock et al. (1995) in Figure 5 (in reference) in a
360 relative manner normalized to the value at 10 MPa. These relative values were taken from the
361 graphs and converted to absolute values based on the rate constant at 100 MPa and pD 5 in
362 Leibrock et al. (1995) Figure 2 (in reference). The pH was corrected to pD 3.38 using the
363 relation shown in Leibrock et al. (1995), Figure 4 therein (in reference). Figure 5 summarizes
364 the comparison of these two studies. The rate constants from Leibrock et al. (1995) show an
365 increase in pressure by a factor of ca. 1.4 with a maximum between 120 MPa and 140 MPa. At
366 higher pressures up to 220 MPa, the rate constants measured by Leibrock et al. (1995) decrease
367 to a value at up to the pressure of 220 MPa that is below the initial value at 10 MPa. Our values
368 for both the D₂O and H₂O systems also demonstrate an increasing trend up to 140 MPa by a
369 similar factor of roughly 1.4. We obtained only few measurements of the ATP hydrolysis rate
370 constants between 140 and 220 MPa and thus could not compare our data well with those of
371 Leibrock et al (1995) in this pressure range.

372 We note that our data show that the ATP hydrolysis rate constants determined in D₂O are in-
373 distinguishable within error from those obtained in H₂O and that the difference in solvent is
374 unlikely to be the explanation for the differences between our data and those of Leibrock et al
375 (1995). However, our data at higher pressure from the HDAC do not indicate any decrease or
376 a minimum in the pressure dependence. The rapid increase of the rate constant at pressures
377 above 500 MPa raises the intriguing question of whether there is a progressive change or two
378 not yet identified abrupt changes between 500 MPa and 1000 MPa, and between 1000 MPa
379 and 1600 MPa. Currently, the data are consistent with both possibilities, therefore, more data
380 on rate constants at high pressures are needed to address this question.

381 Figure 5 compares three sets of *in situ* rate constants for the hydrolysis of ATP under three
382 different conditions: at vapor-saturated conditions in the HDAC, at isochoric conditions in the
383 HDAC, and at isobaric conditions in the autoclave. The investigated pressure range of 0.5 MPa
384 to 140 MPa in the autoclave is too narrow and scattered to derive a distinct trend. In the HDAC
385 at isochoric conditions, the rate constants exhibit similar progressive trends at 80 °C and 100
386 °C. Assuming that the hydrolysis rate changes continuously with pressure, the data were fitted

387 with an empirical power law allowing only positive integers in the exponent. The data were
 388 best fitted by a power law of the following format (Eq. 4):

$$389 \quad k(P) = k_0 + a * P^3 \quad \text{Eq. 4}$$

391
 392 where $k(P)$ is the rate constant as a function of pressure (P), k_0 is the calculated rate constant
 393 at $P=0$ MPa and a is a parameter. The results of the fits and their respective standard deviations
 394 are as follows: $k_{0,80^\circ\text{C}}=1.0 \times 10^{-4} \text{ s}^{-1} \pm 0.1 \times 10^{-4} \text{ s}^{-1}$ and $a_{0,80^\circ\text{C}}=6.3 \times 10^{-13} \pm 0.69 \times 10^{-13}$ for the
 395 dataset at 80°C and $k_{0,100^\circ\text{C}}=5.0 \times 10^{-4} \text{ s}^{-1} \pm 0.34 \times 10^{-4} \text{ s}^{-1}$ and $a_{0,100^\circ\text{C}}=1.34 \times 10^{-12} \pm 0.12 \times 10^{-12}$
 396 for the dataset at 100°C . The chi-square values (χ^2) are 0.19 for the fit of the power laws at
 397 80°C and 0.93 for the fit at 100°C (Eq. 5):

$$398 \quad \chi^2 = \sum \left(\frac{k_{\text{expected}} - k_{\text{measured}}}{\sigma_{1,\text{measured}}} \right)^2 \quad \text{Eq. 5}$$

400
 401 where k_{expected} is the rate constant from the fitted line, k_{measured} are the measured rate constants
 402 and $\sigma_{1,\text{measured}}$ is the first standard deviation of the measured rate constants. Ideally, a chi-square
 403 value equal to the number of fitted points would indicate a perfect fit for the weighted coeffi-
 404 cients of the data. A chi-square value lower than the number of fitted points suggests potential
 405 overfitting of the data or too large uncertainties. However, in a first instance, these fits ade-
 406 quately capture the trends, given the limited number of data points. Th error propagation of the
 407 fit parameter results in a standard deviation of 15% for the fitted line at 80°C and 11% at 100°C .
 408 $^\circ\text{C}$.

409 The highest experimental pressure at which a rate constant could be obtained was 1670 MPa
 410 at 100°C . This rate constant is about $6.4 \times 10^{-3} \text{ s}^{-1}$, which corresponds to a half-life of 108 s.
 411 That half-life at 1670 MPa and 100°C is 25 s shorter than the value reported by Moeller et al.
 412 (2022) at P_{sat} and 120°C . In experiments at 100°C and pressures above 2000 MPa, only AMP
 413 in solution was detected. Thus, (near) complete hydrolysis of ATP to AMP took place within
 414 about 30 s, after heating was stopped and before the very Raman spectrum of the solution was
 415 acquired. In these experiments, we observed nucleation and growth of crystals (Figure 6).

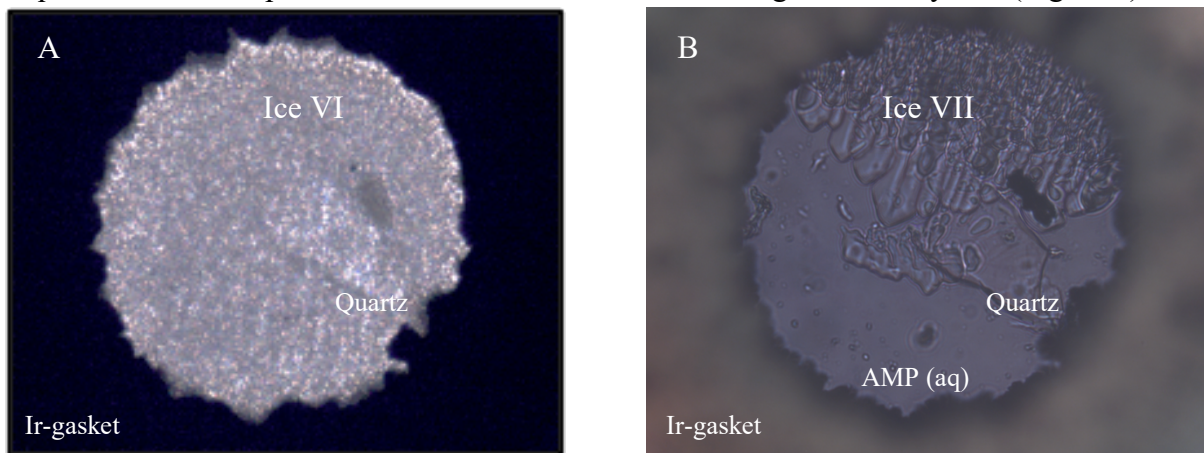


Figure 6: View of the sample chamber of the HDAC showing the experiment at the highest pressure. A: Ice VI and quartz at 2.06 GPa and 26°C . B: Ice VII, AMP solution, and quartz at 2.3 GPa and 100°C . Quartz was used as a Raman spectroscopic pressure sensor. The initial diameter of the sample chamber was $300 \mu\text{m}$.

416 A Raman spectrum of the solid phase indicated Ice VII at 100 °C and 2.3 GPa (Figure 7). The
417 identification is based on the OH stretching band shape (Walrafen et al., 1984). At 26 °C and
418 2.06 GPa, the Raman spectrum of the solid phase was superimposed by luminescence of the
419 AMP solution, preventing unambiguous identification. Upon heating, this solid phase trans-
420 formed to Ice VII at about 92 °C, which suggests that it was most likely Ice VI.

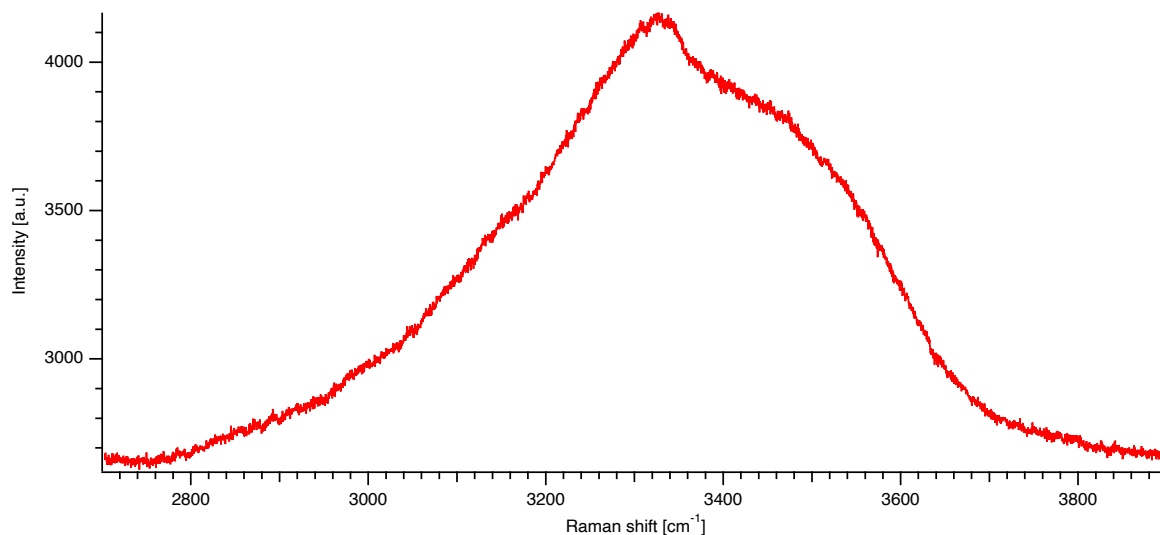


Figure 7: Raman spectra of the solid phase at 100 °C and 2.3 GPa identified as Ice VII after Walrafen et al. (1984). The raw data are available in the Data Availability section ([dataset] Moeller et al., 2024).

421 4. Discussion

422 4.1. Data quality

423 The complementary application of the HDAC and an autoclave has yielded insights into the
424 kinetic properties of ATP hydrolysis at elevated temperatures and pressures up to 1670 MPa
425 that were previously unattainable. In the overlapping pressure range, the hydrolysis rate con-
426 stants of both setups deviate by an average factor of 1.2 ± 0.3 . However, in both datasets, a
427 similar relative increase by a factor of 1.34 ± 0.07 is shown between 0.1 MPa to 100 MPa and
428 0.1 MPa to 90 MPa, respectively.

429 The observed discrepancy between the two methods may be attributed to the applied laser in-
430 tensities of 32 mW and 80 mW in the HDAC and autoclave, respectively. It is improbable that
431 visible light absorption occurs in colorless solutions. Moreover, any minor heating induced by
432 the laser would likely be homogenized by convection in the reaction cells. Nevertheless, it is
433 not possible to completely rule out the possibility of heating nor of direct photon-molecule
434 reactions induced by the laser. Therefore, measurements of ATP-H₂O sample solutions were
435 repeated at 20 MPa and 100 °C in the autoclave, utilizing both laser intensities of 20 mW and
436 80 mW for comparison. A reduction in laser intensity by 60 mW resulted in a 20% to 25%
437 decrease in the fitted values of the hydrolysis rate constants. The considerably lower signal-to-
438 noise ratio at 20 mW laser power resulted in poorer counting statistics of the spectra, which
439 then led to a larger error in the ATP/ADP estimates, as documented by the larger scatter of the
440 data points (Figure 8). The poorer quality of the data likely contributed to the apparent decrease
441 of the hydrolysis rate by 20% to 25%.

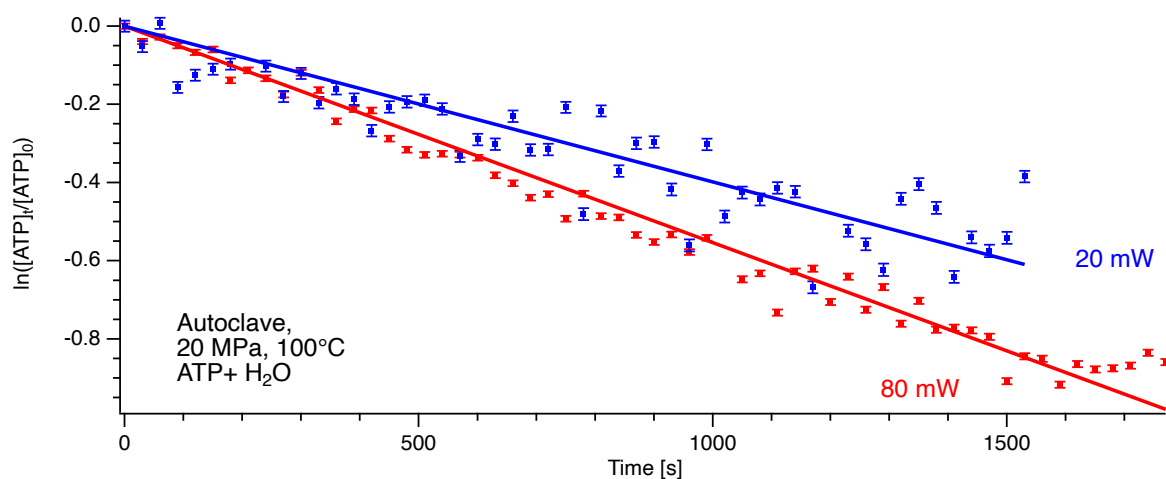


Figure 8: Linear fits of $\ln([ATP]_t/[ATP]_0)$ vs. Time for measurements with laser intensities of 20 mW and 80 mW. The measurements were taken in the autoclave at 20 MPa and 100 °C for solutions of ATP-H₂O. The raw data are available in the Data Availability section ([dataset] Moeller et al., 2024).

442 This potential photo-stimulatory effect presents an intriguing phenomenon. In the field of bio-
 443 chemistry, a multitude of experiments involve the use of "caged" molecules. These molecules
 444 are rendered inactive through chemical modification (Kaplan and Somlyo, 1989) until they are
 445 activated by UV light with a wavelength greater than 300 nm (Mayer and Heckel, 2006). It is
 446 noteworthy that the term "caged molecules" was initially coined in the context of experiments
 447 concerning ATP hydrolysis (Kaplan et al., 1978). Although this technique is well described in
 448 the literature, a photo-stimulation of the ATP hydrolysis has not yet been reported, and further
 449 investigation may be needed.

450 The discrepancy between the rate constants determined in the HDAC and in the autoclave ob-
 451 served at 80 °C may also be attributed to the precision of the temperature measurement in the
 452 autoclave itself. Temperature measurements in the HDAC are accurate to within 1 °C (Schmidt
 453 and Chou, 2012). Temperature measurements of the autoclave are more complex due to the
 454 large sample chamber, which results in the existence of temperature gradients between the
 455 heater and the sample chamber. Heat transfer is ensured by convecting helium at high pressure.
 456 Currently, the estimated accuracy of the setup is approximately 5 °C. This 5 °C deviation could
 457 plausibly contribute to the observed difference in the datasets.

458 Moreover, the minor discrepancies between the HDAC data, HDAC at Psat data (Moeller et al
 459 2022) and the autoclave experiments may be attributed to the three distinct chemical systems
 460 involved. The following chemical systems were considered: ATP in aqueous solution (l_1 , l_2 and
 461 l_3 , in table 3), ATP in aqueous solution coexisting with a vapor phase (l_1+v , l_2+v and l_3+v , in
 462 table 3) and ATP in aqueous solution and a quartz grain in the HDAC (l_1+q , l_2+q and l_3+q , in
 463 table 3). In order to test possible effects, the concentrations of the chemical species were cal-
 464 culated and the pH was utilized as an indicator for changes in the solution in the three cases
 465 (Table 4). For a single aqueous phase, a progressive decrease in pH from 2.8 to 2.7 and 2.47
 466 was observed as ATP hydrolysis progressed. The calculated differences in pH due to the pres-
 467 ence of an additional vapor phase or a quartz grain are within the error of calculations and thus
 468 considered insignificant.

469 In conclusion, the disparity in hydrolysis rates of ATP observed between the HDAC and auto-
 470oclave experiments is not attributable to differences in system composition. A 20% to 25%

471 reduction in the fitted value of ATP hydrolysis rate constants was observed in the autoclave
472 when laser intensity was reduced from 80 mW to 20 mW. A number of potential explanations
473 for this discrepancy exist, including a lower signal-to-noise ratio, laser illumination effects or
474 systematic temperature differences between the setups. It is important to note that this differ-
475 ence falls within the estimated 5 °C uncertainty of temperature calibration within the autoclave.
476 Moreover, the small offsets between HDAC data and low-pressure data in the presence of a
477 vapor phase (Moeller et al., 2022) are also likely due to subtle temperature calibration or laser
478 illumination effects, which would be interesting to determine in future studies.

479 4.2. Effect of temperature on rates of ATP hydrolysis at isobaric conditions

480 The temperature dependence of the kinetics of a chemical reaction is evaluated using the Arrhenius
481 equation (Eq. 6) where $R=8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ is the ideal gas constant, T the temperature
482 [K] and A is the pre-exponential or frequency factor [s^{-1}]. An Arrhenius plot relates the tem-
483 perature, apparent activation energy and rate constants graphically (Figure 9). The term appar-
484 ent activation energy is used since the solutions were unbuffered in pH which causes pH vari-
485 ations between given temperatures due to different repartition of ATP species e.g., ATP^{4-} ,
486 HATP^{3-} , $\text{H}_2\text{ATP}^{2-}$ (Moeller et al., 2022).

487

$$488 \ln k = \frac{E_a}{R} * \frac{1}{T} + A \quad \text{Eq. 6}$$

489

490 The data at pressures between 0.5 MPa and 140 MPa plot on a straight line, indicating a con-
491 stant reaction mechanism between 80 °C and 120 °C. The apparent activation energy, E_a , is not
492 significantly different at 4 MPa and 140 MPa (respective values $104 \text{ kJ mol}^{-1} \pm 30 \text{ kJ mol}^{-1}$ at
493 0.5 MPa to $101 \text{ kJ mol}^{-1} \pm 30 \text{ kJ mol}^{-1}$ at 140 MPa). The pre-exponential factor, A , ranges
494 between 25.6 ± 9.4 and 28.5 ± 9.6 . The average activation energy is $104.3 \text{ kJ/mol} \pm 30 \text{ kJ/mol}$
495 and the pre-exponential factor is 26.5 ± 9.5 . A potential effect within this pressure range on the
496 hydrolysis of ATP is insignificant given these uncertainties. Moeller et al. (2022) reported an
497 activation energy of $122.7 \text{ kJ mol}^{-1} \pm 4.4 \text{ kJ mol}^{-1}$ at P_{sat} . The uncertainty in activation energy
498 was based on the square root of the initially fitted integral, which was propagated throughout
499 their study. A new fit of the data of Moeller et al. (2022) with a standard deviation of 10%
500 yielded an activation energy of $122 \text{ kJ mol}^{-1} \pm 30 \text{ kJ mol}^{-1}$ and a pre-exponential factor of 32.1
501 ± 9.8 for their data at pH 3 and P_{sat} . Additionally, Moeller et al. (2022) reported rate constants
502 at pH 7. If we re-fit also these data with uncertainties of 10%, the activation energy is 124 kJ
503 $\text{mol}^{-1} \pm 32 \text{ kJ mol}^{-1}$ and the pre-exponential factor is 32.2 ± 10.3 . The discrepancy in the acti-
504 vation energies reported by Moeller et al. (2022) and the refitted values is likely due to a mis-
505 take, i.e., transposed numbers in the gas constant, 8.13, which was used instead of 8.31. The
506 effect of this number transposition appears to be relatively minor, given the inherent uncertain-
507 ties. However, at vapor saturation and 80 °C, ATP hydrolysis rates are found to be slower than
508 those without vapor phase, respectively. At 100 °C the rate constants are almost equal and at
509 120 °C the constants at P_{sat} are larger than those at elevated pressure as discussed above. This

510 suggests a stronger temperature dependency for the hydrolysis rates in the presence of vapor
 511 than in a single liquid.

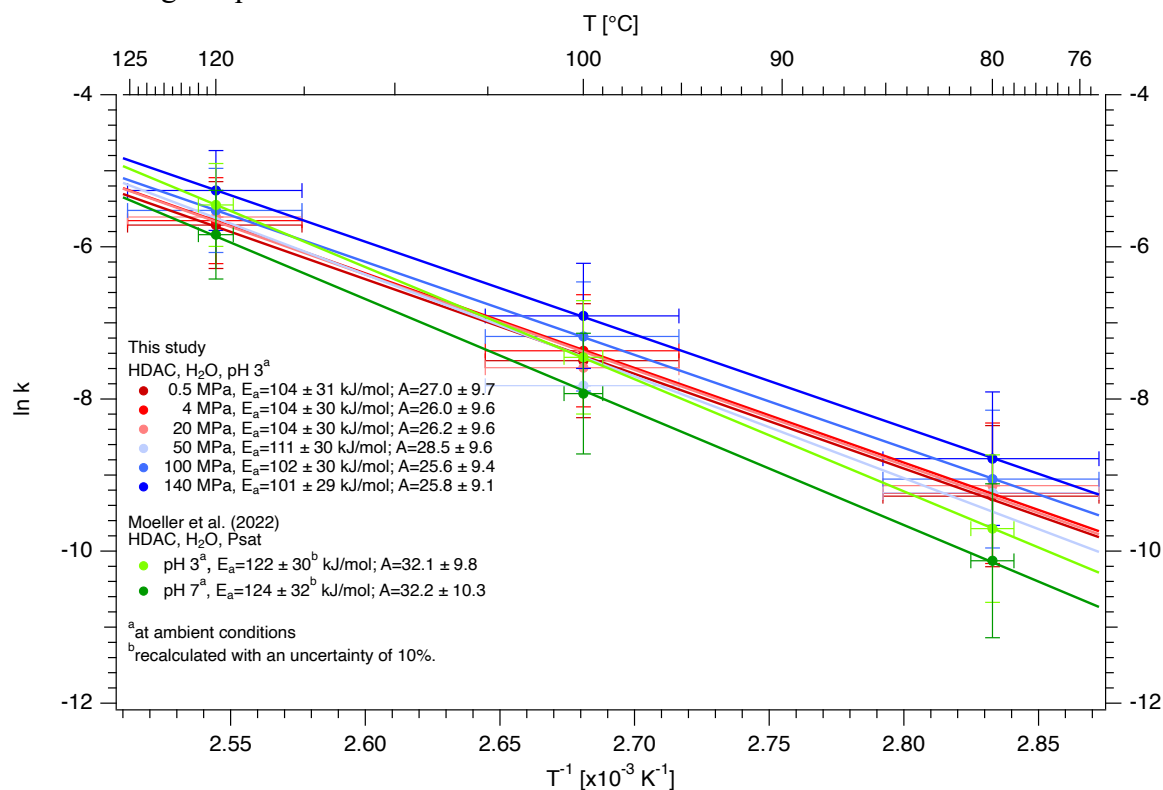


Figure 9: Arrhenius plot of the isobaric hydrolysis rate constants in comparison to kinetic data of Moeller et al. (2022) and Daniel et al. (2004).

512 At pressures above 140 MPa, data on ATP hydrolysis rates are available only at two tempera-
 513 tures, 80 °C and 100 °C, for each pressure. In this case, the results from the fitted power law
 514 functions (Eq. 3, 4) can be used in a first approximation to provide an estimate of isobaric
 515 activation energies ($E_{a,extrapolated}$), which have been extrapolated in [J] according to the follow-
 516 ing equation:

517

$$518 \quad E_{a,extrapolated} = \frac{\ln k(P)_{100\text{ }^{\circ}\text{C}} - \ln k(P)_{80\text{ }^{\circ}\text{C}}}{\left(\frac{1}{373} - \frac{1}{353}\right)} * R \quad \text{Eq. 7}$$

519

520 where $k(P)$ is the calculated rate constant from Eq. 4 at 80 °C and 100 °C at a given pressure
 521 and R is the universal gas constant. This estimation yields an activation energy of 88 kJ mol^{-1}
 522 $\pm 16.4 \text{ kJ mol}^{-1}$ at 1 MPa (Figure 10), which differs by approximately 20% from the calculated
 523 values determined in the autoclave. This discrepancy may be acceptable given that only two
 524 isotherms were available from the HDAC runs, which are based on eight individual experi-
 525 mental loadings. Only above 150 MPa to 200 MPa is there a substantial decrease in the extrap-
 526 olated activation energy, which reaches a value of about 42 kJ mol^{-1} at 1700 MPa. This obser-
 527 vation lend support to the observation that the changes in activation energy and thus, in the
 528 reaction mechanism between 0.5 MPa and 140 MPa are insignificant.

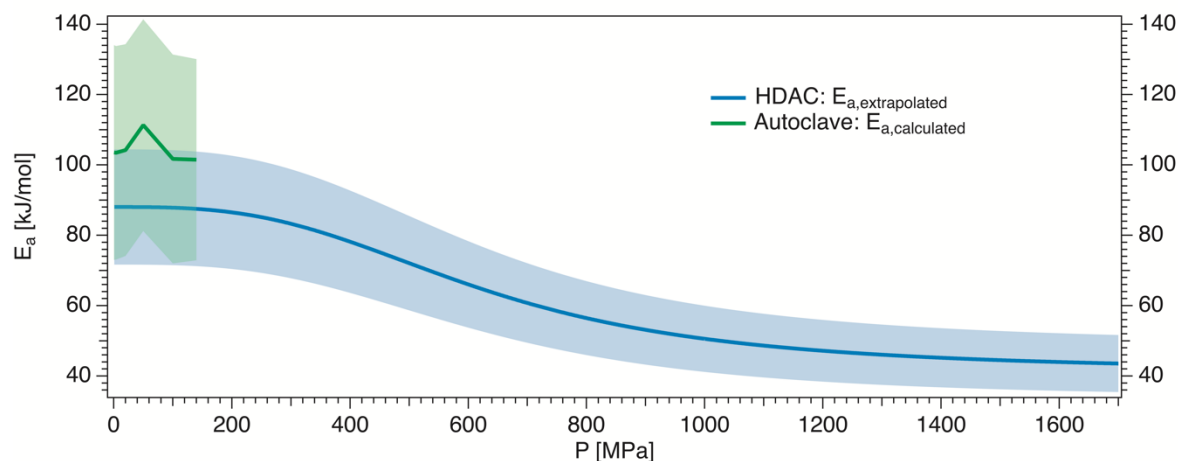


Figure 10: Calculated activation energies from autoclave experiments and extrapolated activation energies from fitted power laws of HDAC data as functions of pressure.

529 4.3. Effect of pressure on rates of ATP hydrolysis at isothermal conditions

530 To our knowledge the only other study investigating the pressure effect on the hydrolysis rate
 531 constant of ATP is that of Leibrock et al. (1995). They analyzed samples quenched from high
 532 temperatures and pressures. The concentration was determined with subsequent NMR analysis,
 533 which is a highly accurate and precise analytical tool. Replacement of H₂O by D₂O may result
 534 in slowing down any reaction involving breaking of hydrogen bonds (Westheimer, 1961). As
 535 illustrated in Figure 4, the rate constants determined by Leibrock et al. (1995) in D₂O are lower
 536 by roughly 50 % lower than those measured in H₂O in the present study. However, the rate
 537 constants obtained here for the ATP-D₂O system (Figure 5 and 6) are within the uncertainty of
 538 the data for H₂O. Therefore, a kinetic isotope effect alone, as proposed by Moeller et al. (2022),
 539 would not explain the slightly slower ATP hydrolysis rates of Leibrock et al 1995 (Figure 6).
 540 The enhanced decomposition of ATP under high laser power could account for an increase in
 541 the ATP hydrolysis rate constant of up to 25% in some of our experiments as previously dis-
 542 cussed. Furthermore, it is important to consider the potential impact of artifacts due to quen-
 543 ching and subsequent analysis in *ex situ* studies. Quenching may lead to artifacts due to back
 544 reactions and other alterations of the species' concentrations after quenching and depressuriza-
 545 tion. It is possible that the data of Leibrock et al. (1995) may have been affected by such effects.
 546 However, tests with solutions at ambient conditions as well as extrapolation of data here indi-
 547 cate that any reaction at ambient conditions will be extremely sluggish, making it unlikely that
 548 a change in concentration between the quench of the run and the NMR measurement would
 549 occur. The only significant differences between the two studies are the total concentrations of
 550 ATP, which were approximately ten times higher in this study, and the sample volume, which
 551 was approximately twenty-five times larger in Leibrock et al. (1995). However, the two da-
 552 taset agree in terms of a relative increase in the rate constants up to 120 MPa to 140 MPa by
 553 a factor of approximately 1.4. A decrease in the ATP hydrolysis rate constants was observed
 554 by Leibrock et al. (1995) between 140 MPa and 220 MPa. However, rate constants in this
 555 pressure range could not be determined, as the autoclave was unable to reach these pressures.
 556 The application of the HDAC between 140 MPa and 220 MPa is challenging and results in
 557 uncertainties of 30 MPa due to the low resolution and sensitivity of quartz as a pressure indi-
 558 cator in this range. Beyond the pressure range studied by Leibrock et al. (1995), we observed

559 a continuous increase of the ATP hydrolysis constant with pressure above 400 MPa. In future
 560 studies, it would be beneficial to connect those observations by obtaining additional data points
 561 between 150 MPa and 400 MPa.

562 The effect of pressure on reaction rates depends on the reaction and the activation volume (V^\ddagger).
 563 Le Châtelier's principle postulates that an increase in pressure will result in a shift of the chem-
 564 ical equilibrium towards the state with a smaller volume. Transferred to kinetic para-meters
 565 according to transition state theory, it implies that processes for which the transition states have
 566 the smallest volumes are the fastest (e.g., Mohana-Borges et al., 1999; Jebbar and Oger, 2010).
 567 The activation volume, is defined by (e.g., Kelm and Palmer, 1977):

568

$$569 \quad V^\ddagger = -RT \frac{d \ln k}{dP} \quad \text{Eq. 8}$$

570

571 where R is the universal gas constant. The activation volume of ATP hydrolysis can be deter-
 572 mined for the dataset of single-phase aqueous high-pressure experiments in and the autoclave
 573 and the HDAC by using the Eq. 8. In the autoclave, the determined values of V^\ddagger for the pressure
 574 range of 0.5 MPa to 140 MPa are $-8.4 \text{ cm}^3 \text{ mol}^{-1} \pm 2 \text{ cm}^3 \text{ mol}^{-1}$ at 80 °C, $-8.7 \text{ cm}^3 \text{ mol}^{-1} \pm 2 \text{ cm}^3$
 575 mol^{-1} at 100 °C and $-8.1 \text{ cm}^3 \text{ mol}^{-1} \pm 2 \text{ cm}^3 \text{ mol}^{-1}$ at 120 °C. In the HDAC, V^\ddagger is $-5.5 \text{ cm}^3 \text{ mol}^{-1}$
 576 $\pm 1.5 \text{ cm}^3 \text{ mol}^{-1}$ for 90 MPa to 934 MPa at 80 °C and $-6.3 \text{ cm}^3 \text{ mol}^{-1} \pm 4.1 \text{ cm}^3 \text{ mol}^{-1}$ for 116
 577 MPa to 1166 MPa at 100 °C. On average, V^\ddagger is $7.4 \pm 0.82 \text{ cm}^3 \text{ mol}^{-1}$. Leibrock et al. (1995)
 578 reported a value of $-15 \pm 4 \text{ cm}^3 \text{ mol}^{-1}$ over a pressure range between ca. 10 MPa and 120 MPa.
 579 Based on the literature cited therein, Leibrock et al (1995) suggested that values between -8
 580 $\text{cm}^3 \text{ mol}^{-1}$ and $-15 \text{ cm}^3 \text{ mol}^{-1}$ are consistent with a mechanism of anhydride and ester hydrolysis
 581 (A_{AC} 2-mechanism). Asano and Noble (1978) distinguished the activation volume for ester
 582 hydrolysis between $-10 \text{ cm}^3 \text{ mol}^{-1}$ and $-15 \text{ cm}^3 \text{ mol}^{-1}$ for basic solutions and greater than -10
 583 $\text{cm}^3 \text{ mol}^{-1}$ for acidic solutions, which aligns with our findings. In the context of ester hydrolysis,
 584 protonation of the phosphate group by H^+ precedes a nucleophilic attack of H_2O (Figure 11).
 585

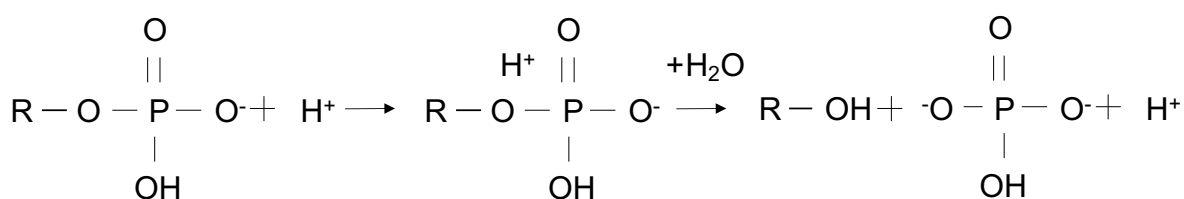


Figure 11: Reaction scheme of the anhydride and ester hydrolysis after Leibrock et al. (1995).

586 Leibrock et al. (1995) proposed that the nucleophilic attack of water represents the limiting
 587 factor of hydrolysis at low pressures and the protonation reaction becomes rate limiting at pres-
 588 sures above 120 MPa. It can be postulated that the negative activation volumes and thus en-
 589 hancement of ATP hydrolysis rates with pressure may be related, at least in part, to the fact
 590 that the ionization product of water increases with pressure. This implies that the neutral pH of
 591 water decreases as pressure increases. For instance, isothermal compression of pure water at
 592 100 °C would result in a shift of the pK_w from 12.25 at 0 MPa to 11.94 at 100 MPa and to 11.02
 593 at 500 MPa. This would consequently lead to a shift in the neutral pH of water from 6.1 to 6
 594 and to 5.5. To test the hypothesis that an increased autoprotolysis of water with increasing

595 pressure affects ATP hydrolysis, the pH was calculated in thermodynamic equilibrium of ATP
 596 solutions containing ATP, ADP, AMP, and inorganic phosphate groups (Table 4). The calcu-
 597 lated pH_p values for solutions of 0.1 mol/l ATP at equilibrium conditions for the three species
 598 are shown in Figure 12. The calculations predict a significant decrease in the pH value with
 599 increasing pressure for a given temperature. The changes in pH for solutions of 0.01 mol/l ATP
 600 are within 0.1 to 0.3 per 500 MPa. Therefore, the general decrease with increasing pressure is
 601 consistent with the enhancement of the hydrolysis rate with decreasing pH, as reported by Lei-
 602 brock et al. (1995).

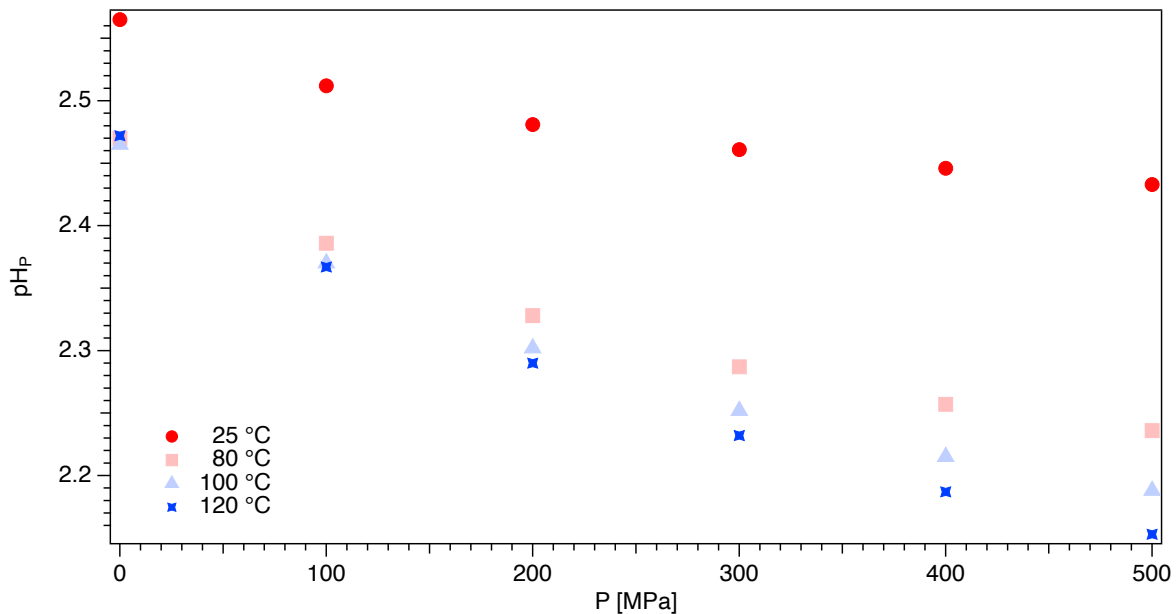


Figure 12: The modelled pH of 0.1 mol/l ATP solutions without gaseous species at equilibrium conditions, here solution l_1 (see Table 3), as a function of pressure at 25 °C, 80 °C, 100 °C, and 120 °C. The absolute pH values are available in the Data Availability section ([dataset] Moeller et al., 2024).

603 It can be concluded that elevated pressure may increase the catalyzing effect of hydronium ions
 604 on the hydrolysis reaction. However, this model does not explain the decrease in ATP hydroly-
 605 sis rate observed by Leibrock et al. (1995) above 120 MPa and up to 220 MPa. To resolve
 606 this discrepancy, it is necessary to obtain more *in situ* data of ATP hydrolysis in this pressure
 607 range.

608 Above 2000 MPa and 100 °C, no rate constants were determined as the hydrolysis rate was
 609 below the temporal resolution of our experimental setup with the hydrothermal diamond anvil
 610 cell. However, it is evident that the complete abiotic hydrolysis of ATP to AMP must have
 611 occurred within the initial 30 s of heating and 20 s of the very first spectrum acquisition, as
 612 only AMP was detectable. To evaluate this observation, we can extrapolate with Eq. 4 the
 613 hydrolysis rate of ATP to 2000 MPa and 100 °C. The rate is approximately 0.1 s^{-1} with a cor-
 614 responding half-life of few seconds. Thus, our minimum measurement time of the first spectra
 615 of 50 s would encompass several half-lives under such conditions. At this point in time, the
 616 remaining concentration of ATP was below 0.001 mol/l, which is below the detection limit of
 617 approximately 0.005 mol/l.

618 4.4. Implications for the limits of metabolic pathways

619 In order to contextualize the abiotic hydrolysis of ATP data within the biological realm, it is
 620 interesting to compare them with ATP turnover times that have been measured in

621 microorganisms (see Bains et al., 2015). The average pool turnover rate is defined as the quan-
 622 tity of material that is turned over per unit of time in a biological system (Zilversmit, 1955).
 623 The average pool turnover rate of ATP for *Escherichia coli sp.* is 0.05 s^{-1} , while for *Bdellovib-*
 624 *rio bacteriovorus sp.* it is 0.13 s^{-1} at $28 \text{ }^\circ\text{C}$ (Gadkari et al., 1976). For some *Streptococcus*
 625 *mutans sp.*, these rates are up to 1.5 s^{-1} at $37 \text{ }^\circ\text{C}$ (Fukui et al., 1988). It is important to note that
 626 these microorganisms are mesophiles and are not exposed to any extreme conditions, such as
 627 temperature or pressure, which might strongly affect the turnover times. To our best
 628 knowledge, these have not yet been measured in extremophiles.

629 In a first approximation and neglecting other potentially very important biological effects,
 630 Moeller et al. (2022) used the Arrhenius relation to determine the temperature at which the
 631 hydrolysis rate equals to the pool turnover rate of 0.05 s^{-1} of *Escherichia coli sp.* and of 1.5 s^{-1}
 632 of *Streptococcus mutans sp.* by inserting them into the rearranged Arrhenius equation (Eq. 9):
 633

$$634 \quad T = \frac{\frac{E_a}{R}}{(\ln k_{\text{average pool turnover rate}} - A)}. \quad \text{Eq. 9}$$

635
 636 Moeller et al. (2022) estimated that the maximum temperatures allowing cells to maintain their
 637 ATP pools were on average $145 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$ for *Escherichia coli sp.* and $195 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$ for
 638 *Streptococcus mutans sp.* For pH 3 and pH 7, the authors estimated an average tentative lower
 639 and upper temperature limit for ATP-supported metabolism to be $145 \text{ }^\circ\text{C} \pm 3 \text{ }^\circ\text{C}$ and $195 \text{ }^\circ\text{C} \pm$
 640 $3 \text{ }^\circ\text{C}$ at P_{sat} for pH 3 to 7. The authors' conclusion was that, with the exception of measuring
 641 ATP turnover times in hyperthermophilic organisms, abiotic destabilization of ATP does not
 642 appear to be the primary factor determining the currently observed temperature limit of life.
 643 The lower temperature limit of ATP is $144 \text{ }^\circ\text{C} \pm 57 \text{ }^\circ\text{C}$ and the upper temperature limit is 189
 644 $^\circ\text{C} \pm 75 \text{ }^\circ\text{C}$ at pH 3 and P_{sat} , when using the refitted parameters with an uncertainty of 10%
 645 for the data reported above. In the present study, the average temperature limits for the single
 646 fluid phase are $152 \text{ }^\circ\text{C} \pm 69 \text{ }^\circ\text{C}$ and $207 \text{ }^\circ\text{C} \pm 95 \text{ }^\circ\text{C}$ at pH 3, based on a similar comparison
 647 with the parameters.

648 Similarly, by rewriting Eq. 4 as Eq. 10 and inserting the pool turnover rates of *Escherichia coli*
 649 *sp.* and of these *Streptococcus mutans sp.*, a lower and an upper pressure limit can be estimated
 650 at $80 \text{ }^\circ\text{C}$ (Equation 10) and at $100 \text{ }^\circ\text{C}$, respectively.
 651

$$652 \quad P = \sqrt[3]{\frac{k_{\text{average pool turnover rate}} - k_{0,80^\circ\text{C}}}{a_{80^\circ\text{C}}}} \quad \text{Eq. 10}$$

653
 654 The estimated lower pressure limits are of $4.3 \text{ GPa} \pm 0.7 \text{ GPa}$ at $80 \text{ }^\circ\text{C}$ and $3.3 \text{ GPa} \pm 0.3 \text{ GPa}$
 655 at $100 \text{ }^\circ\text{C}$, while the upper pressure limits are $13 \text{ GPa} \pm 2 \text{ GPa}$ at $80 \text{ }^\circ\text{C}$ and $10 \text{ GPa} \pm 1 \text{ GPa}$ at
 656 $100 \text{ }^\circ\text{C}$. Remarkably, the upper pressure limits are almost a magnitude higher than the maxi-
 657 mum pressure of 1.6 GPa reached in our experiments. Such estimates are far beyond the phase
 658 boundary of liquid water and Ice VII, which is between 2.2 GPa and 2.8 GPa at temperatures
 659 from $80 \text{ }^\circ\text{C}$ to $120 \text{ }^\circ\text{C}$ (Bridgman, 1937). Figure 15 shows the phase diagram of water as a
 660 function of pressure and temperature (after Eisenberg and Kauzmann 1969; Guildner et al.,
 661 1976; Petrenko and Whitworth, 1999; IAPWS, 2011; Journaux et al., 2020), including the
 662 lower and the upper temperature limit of the ATP hydrolysis obtained in this study and by

663 Moeller et al. (2022). Interestingly, the comparison to pool turnover rates suggests that ATP is
664 kinetically stable enough to sustain known metabolic pathways across the entire pressure range
665 of liquid water, at least up to 100 °C.

666 These lower and upper P-T-limits can help to understand the P-T-conditions compatible with
667 ATP-involving metabolic pathways in extreme environments, both on Earth and on other plan-
668 ets. Figure 15 provides information about extreme conditions in terrestrial settings adapted
669 from Table 2 and 5 in Merino et al. (2019) and the tolerance ranges of *Methanopyrus kandleri*
670 *sp.* (Takai et al., 2008) and *Thermococcus piezophilus sp.* (Dalmasso et al., 2016). This graphic
671 compilation suggests that, from the necessarily limited point of view of comparing the pool
672 turnover rate with the kinetic stability of ATP, metabolic pathways may be possible up to pres-
673 sures of a few GPa.

674 Earth's subsurface is subject to high-pressure and temperature environments (Merino et al.,
675 2019; Figure 13). The known deep biosphere is estimated to span only an average of the first
676 5 km of the Earth's crust, based on a geothermal gradient of 25°C/km to 30 °C/km and a crustal
677 pressure gradient of 30 MPa/km with temperature and pressure limits of 122 °C and 130 MPa
678 to 150 MPa (Oger and Jebbar, 2010). This environment is believed to host 15% Earth's total
679 biota (Bar-On et al., 2018). Notably, these thresholds are considerably lower than the estimated
680 kinetic stability range of ATP (Figure 13), suggesting that abiotic hydrolysis of ATP is not a
681 limiting factor for known life forms. Finally, this raises the intriguing question of whether life
682 could in principle exist deeper into the subsurface. Plümper et al. (2017) and Sharma et al.
683 (2002), attempted to address this issue. Plümper et al. (2017) discovered organic matter encap-
684 sulated in rock clasts in an oceanic serpentinite mud volcano in the vicinity of a subduction
685 zone forearc. In diamond anvil cell experiments, Sharma et al. (2002) reported the viability of
686 *Shewanella oneidensis sp.* at 1.6 GPa and 25 °C (Figure 13). While both studies attracted con-
687 siderable attention, they lacked definitive evidence to support their hypotheses. In particular,
688 Sharma et al. (2002) was merely measuring an abiotic enzymatic reaction (Yayanos, 2002).
689 We may conclude from the limited perspective of kinetic stability of ATP, should life exist
690 beyond the notion of life, ATP hydrolysis could still be a suitable energy system in living cells.
691 It is important to note that physical extremes, such as temperature and pressure, can affect the
692 intra- and extracellular environment of a microorganism. In contrast, chemical extremes, such
693 as pH and salinity, primarily affect the habitat of an organism. Extremophiles invest a signifi-
694 cant quantity of metabolic resources to maintain their intracellular environment at a neutral pH
695 (e.g., Golyshina et al., 2006). Self-regulatory mechanisms, known as homeostasis, ensure in-
696 ternal stability and optimal functioning of vital processes (Cannon, 1929). Nevertheless, to
697 avoid chemical crossover effects by bases and buffers, ATP solutions with only Na₂H₂ATP as
698 an ion source were selected for this study. The pH-relation shown by Leibrock et al. (1995),
699 which is supported by measurements of Moeller et al. (2022), indicates the rate constants at pH
700 3 should be halved in order to obtain an estimate for rate constants at pH 7. Based on the re-
701 fitted Arrhenius parameter at pH 7 for the data of Moeller et al. (2022), the tentative

702 temperature limits shift consistently by 7 °C to higher temperatures. This shift is negligible,
 703 given that the estimated limits are already beyond the common notions of life, as discussed
 704 earlier.

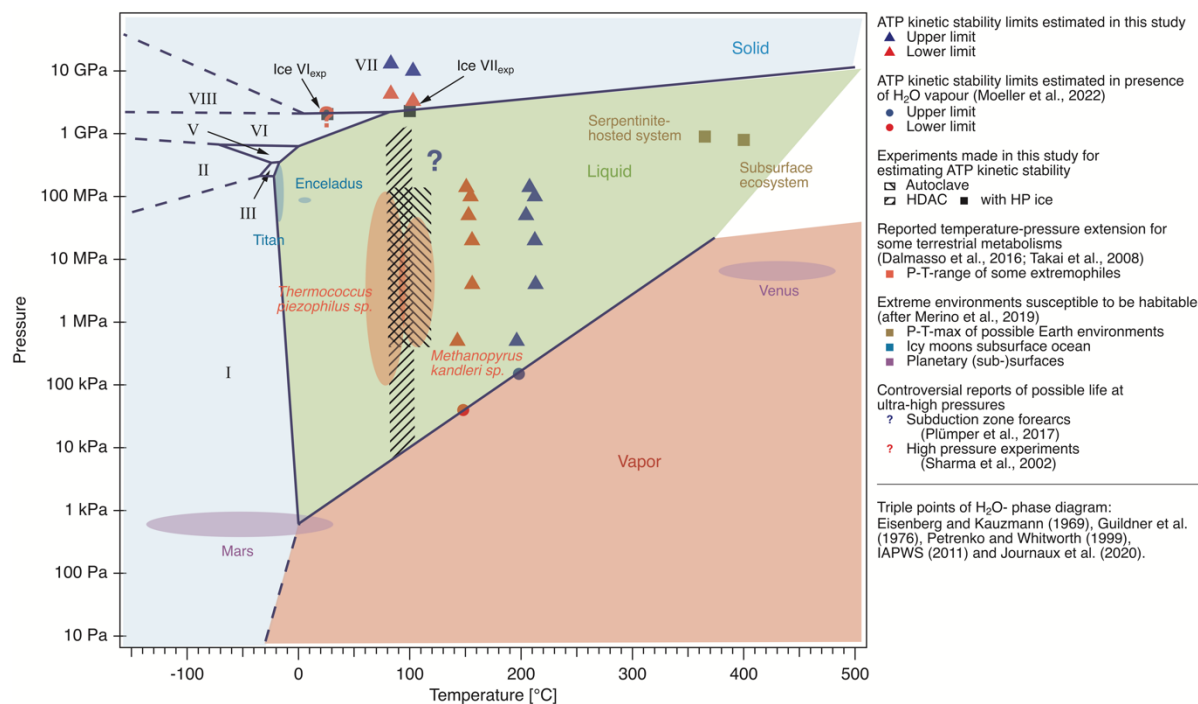


Figure 13: Estimated limits of ATP kinetic stability temperature-pressure field superimposed to the H₂O phase diagram, to possible environments for life and to experimental reports on living microorganisms.

705 The use of ATP’s kinetic stability in aqueous solution as a proxy for the possibility of metabo-
 706 lism is an oversimplified approach. Metabolic pathways involve various interrelated pro-
 707 cesses, and ATP-involving reactions are just a few of them. In addition, extremophiles have
 708 developed mechanisms such as substrate channeling (e.g., Legrain et al., 1995) or metabolite
 709 substitution, in which ATP is replaced by ADP in certain metabolic processes (Kengen et al.,
 710 1995). The upper and lower P-T limits proposed here for the kinetic stability of ATP are based
 711 on the average ATP pool turnover rates measured in mesophilic organisms. Therefore, the
 712 model does not consider the necessity for extremophiles to maintain normal cell functioning
 713 while utilizing substantial quantities of metabolic resources to compensate for thermal damage
 714 and cell integrity (Beulig et al., 2019). Additionally, this approach on the abiotic side also en-
 715 tails certain simplifications that warrant further investigation. For instance, the kinetic stability
 716 of ATP that were measured is based on chemical systems with only Na⁺ and H⁺ ions as coun-
 717 terions. It is well established that other ions, in particular Mg²⁺, exert a significant influence on
 718 the reaction kinetics of ATP hydrolysis.
 719 Furthermore, the approach could be greatly enhanced by a comprehensive investigation of the
 720 photostimulatory effect on ATP hydrolysis and the determination of rate constants at a neutral
 721 pH. Further insights could involve expanding our understanding of the biological turnover rates
 722 of ATP, particularly in extremophiles, and by considering the kinetic effects of abiotic ATP
 723 hydrolysis in the presence of ions such as Mg²⁺. In light of these complexities, superimposing
 724 the kinetic stability field of ATP onto the phase diagram of water and correlating it with con-
 725 ditions under which life could potentially thrive will offer another perspective for discussing
 726 the potential distribution of terrestrial-like life forms in extreme environments.

727 5. Conclusion and outlook for the future

728 This study presents the first *in situ* determination of rate constants for the abiotic/non-enzymatic hydrolysis of ATP at high pressure and temperature. Thermodynamic calculations provide insights into the pH evolution of the ATP system under elevated temperature and pressure conditions. A first comparison of the abiotic hydrolysis rates to the biotic average pool turnover rates of ATP, as measured in mesophilic organisms, indicates that ATP hydrolysis may still be a viable energy system in living organisms. Furthermore, this comparison enables us to estimate lower and upper temperature and pressure limits for the kinetic stability of ATP. The result is a tentative kinetic stability field of ATP offering an interesting perspective on the potential extension of terrestrial and extraterrestrial life.

737 The application of different laser intensities may suggest that the hydrolysis of ATP is photo stimulated. This effect, which has not been yet reported in the literature, may require further quantification and consideration in future studies. Furthermore, the data currently available concern the kinetic properties of ATP in the presence of Na⁺ and H⁺ ions as counterions. However, divalent metal ions, in particular Mg²⁺, form complexes with ATP that exert a significant influence on ATP hydrolysis kinetics. Further investigations aimed at quantifying the chemical effects of such complexes on the rates of abiotic hydrolysis of ATP would enhance our understanding of the kinetic stability field of ATP. In this context, the acquisition of new microbiological data on ATP turnover rates in extremophile microorganisms is of great importance.

746

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758 Data Availability

759 Data are available through GFZ Data Services at <https://doi.org/10.5880/figeo.2023.031>.

760 Declaration of Competing Interest

761 The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

762

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