Adenosine triphosphate

I. Preliminaries

1. The atomic number of phosphorus P is Z=15 and its most abundant natural isotope is ^{31}P. What is the composition in electrons, protons and neutrons of ^{31}P?
2. What is the ground state electronic configuration of P?

The orthophosphoric acid H_3PO_4 has P as the central atom, bonded to three OH groups and one O.

3. Draw the Lewis structure of H_3PO_4.
4. Does P satisfy the octet rule? Explain why this is possible.
5. What is the oxidation number of P in H_3PO_4?

The three pKa's for the orthophosphoric acid are pKa_1 = 2.2, pKa_2 = 7.2 and pKa_3 = 12.3.

6. Write the three deprotonation reactions corresponding to the three acidities of H_3PO_4 and express their equilibrium constant.
7. What is the most abundant form at neutral pH, pH=7. Calculate the ratio of concentrations of the other forms.
8. The low pKa for the first acidity of orthophosphoric acid is attributed to the stability of the $H_2PO_4^-$ anion. Explain this stability using the Lewis structure of that anion.

Orthophosphoric acid is used as a food additive, in sodas in particular. The concentration 0.6 g.L^{-1} is the limit value for food safety. We perform the titration of V = 25 mL of a soda from which CO_2 has been first extracted. The titration is performed using a NaOH solution at C_B=2.0x10^{-2} mol.L^{-1}. Only two equivalence points E1 and E2 are observed at V_1 = 5.5 mL and at V_2 = 14 mL.

9. Why is a third equivalence point not observed?
10. What would be the expected ration between V_1 and V_2? Can you find an explanation why this ratio is not found?
11. How would you then use the two observed values for the titration?
12. Calculate the concentration C_A of orthophosphoric acid initially present in the soda? Is it below the limit value?
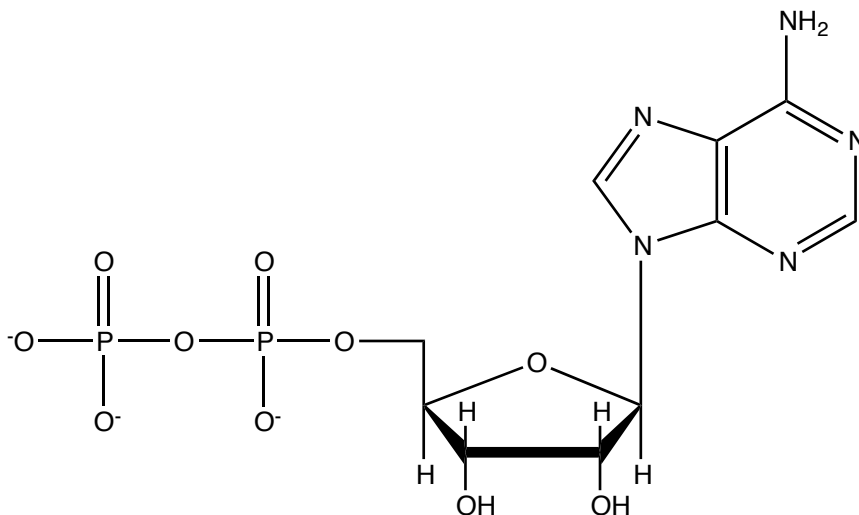
II. Hydrolysis of adenosine triphosphate

Adenosine triphosphate, denoted ATP and in the form ATP^{4-} at the pH considered, is considered the reservoir of energy in living system. The structure of ATP is given in Fig. 1.

13. How many asymmetric carbons are there in ATP^{4-}?
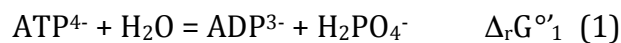14. What is the absolute configuration of the anomeric carbon in ATP?
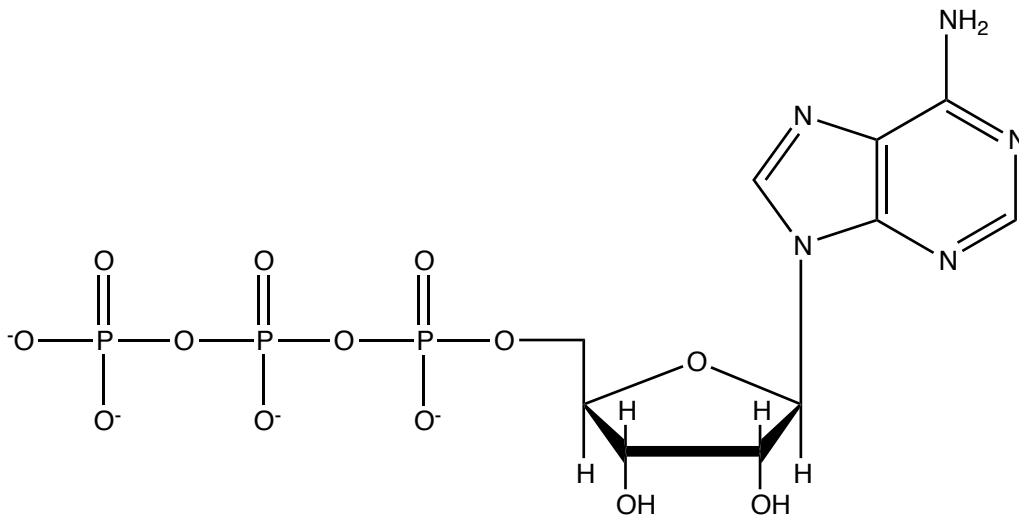
*Fig. 1: Structure of ATP^{4-}*

Fig. 2: Structure of ADP^{3-}

In living organisms, ATP undergoes hydrolysis into ADP, in the form ADP^{3-} at the pH considered, through the reaction

$$ATP^{4-} + H_2O = ADP^{3-} + H_2PO_4^- \qquad \Delta_rG°'_1 \quad (1)$$

15. Propose a mechanism for the hydrolysis of ATP^{4-} into ADP^{3-}.

Hydrolysis of ATP into ADP is thermodynamically favourable but kinetically unfavourable and in many organisms it is performed in a transmembrane enzyme that behaves as a molecular motor. We consider here the F1-ATPase enzyme that is an alternating assembly of 3 α subunits and 3 β subunits arranged in an hexagon with a γ subunit that passes through the center of the hexagon. The γ subunit can rotate and its rotation induces conformational changes of the three β subunits. The conformations of ATP/ADP binding site of the β subunits that have been identified are β_E, where the site is fully opened, β_{HO}, half-opened site, β_{HC}, half-closed site, and two binding conformations

β_{TP} and β_{DP}. Upon rotation of the γ subunit, one of the three β subunits goes through the following cycle:

$$\beta_E \rightarrow \beta_{TP} \rightarrow \beta_{DP} \rightarrow \beta_{HO} \rightarrow \beta_{HC} \rightarrow \beta_E$$
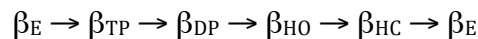
This is summarized in Fig. 3.

The reaction free energy $\Delta_r G°'_1$ for reaction (1) has been determined by W. Yang et al. [Yang, W., Gao, Y. Q., Cui, Q., Ma, J. & Karplus, M. PNAS 100, 874–879 (2003)] in aqueous solution and inside the binding site of the β subunit for its different conformations. The reaction free energy values are given in Table 1.

Site	$\Delta_r G°'_1$ (kJ/mol)
Aqueous solution (in presence of Mg^{2+})	-29
β_{TP}	6.2
β_{DP}	-37.7
β_{HC}	-53.1

Table 1: standard reaction free energy of reaction (1) in different environments

16. From Table 1, identify the β subunits conformations that are favourable for ATP binding and those that are favourable to ADP binding.
17. Summarize and rationalize the coupling between ATP hydrolysis and rotation of the γ subunit.
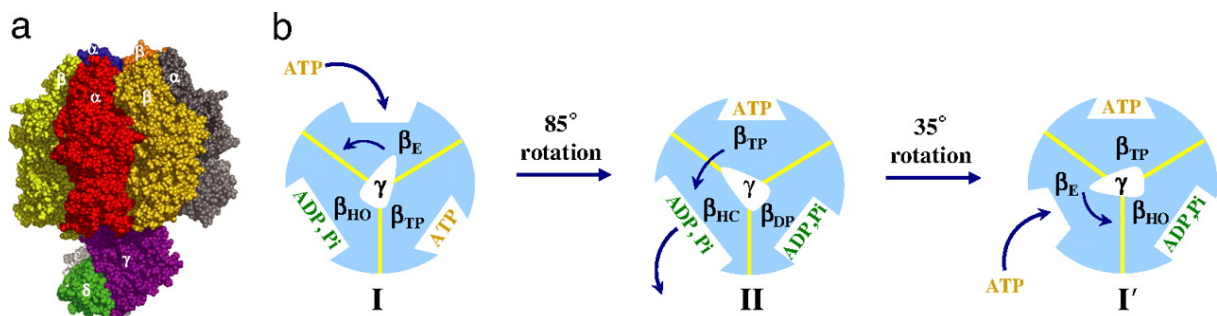
Fig. 3 : Hydrolysis cycle of F1-ATPase. (a) Molecular representation of the mitochondrial F1-ATPase (α3β3γδε). (b) The proposed cycle of the tri-site ATP hydrolysis mechanism. The cycle starts from an ATP waiting state, with one β-subunit fully open (βE), the second β-subunit half-open (βHO), and the third β-subunit closed (βTP); βTP contains the ATP bound in the previous cycle. The calculated 85° rotation is caused by the ATP binding to βE and a cooperative opening of the βHO-subunit to adopt a "half-closed" (HC) conformation in preparation for product release. The subsequent calculated 35° rotation is related to hydrolysis product release from the βHC-subunit and a concomitant expansion of the βDP binding pocket to form βHO. [From Jingzhi Pu, and Martin Karplus PNAS 2008;105:1192-1197]

18. Figure 4 shows the atomic arrangement of ATP or ADP + $H_2PO_4^-$ in the β_{TP} subunit. It can be seen that a Mg^{2+} cation is located close to the phosphodiester groups of ATP. Suggest a possible role for this cation.
19. What other kind of interactions can stabilize the ligands, ADP or ATP, in the binding pockets?
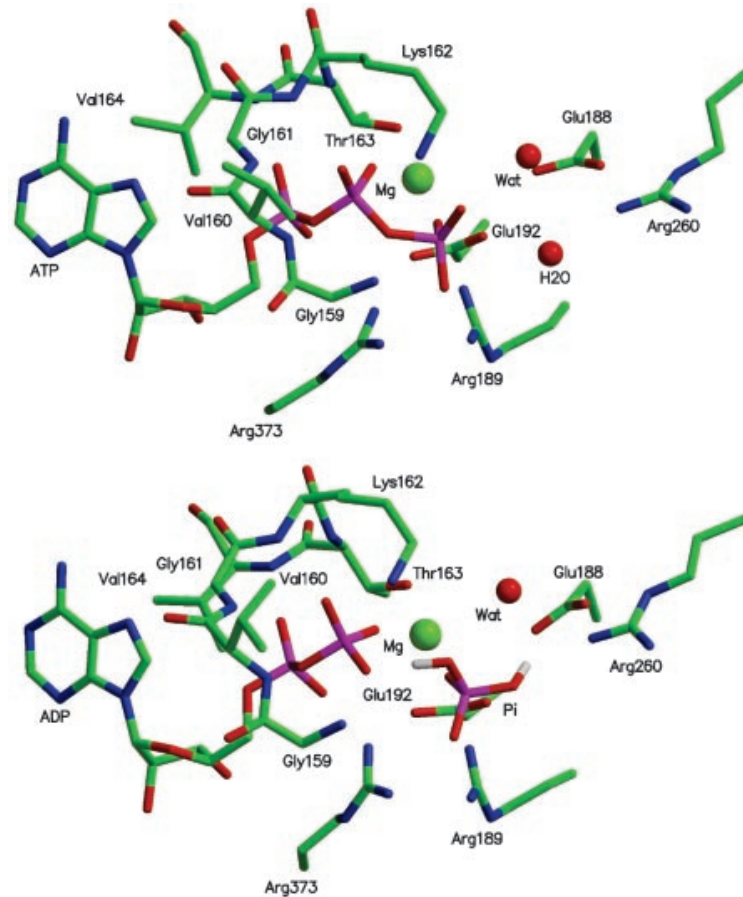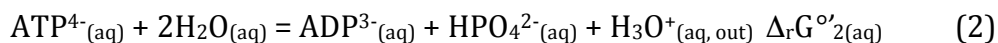
Fig 4: Atomistic representation of the β_{TP} site [From Yang, W., Gao, Y. Q., Cui, Q., Ma, J. & Karplus, M. PNAS 100, 874–879 (2003)]

The rotation of the γ subunit is coupled to another unit of the ATPase transmembrane complex that generates a flux of protons across the membrane, creating a pH gradient across the surface. An important quantity is therefore the number of protons that are transferred through the membrane per ATP molecule that is hydrolysed in the F1-ATPase complex. To determine that quantity Peter Gräber and collaborators [P. Turina, D. Samoray and P. Gräber EMBO **22**, pp. 418-426, 2003] reconstituted ATPase complexes into liposomes separating an inside region from an outside environment, for which they can control the pH difference between the two regions, see Fig. 5.

Given the high pH of the outside region, the hydrolysis reaction is here considered to be

$$\text{ATP}^{4-}_{(aq)} + 2\text{H}_2\text{O}_{(aq)} = \text{ADP}^{3-}_{(aq)} + \text{HPO}_4^{2-}_{(aq)} + \text{H}_3\text{O}^{+}_{(aq,\,out)} \quad \Delta_r G^{\circ\prime}_{2(aq)} \qquad (2)$$

where $\text{H}_3\text{O}^{+}_{(aq,\,out)}$ designates an aqueous $\text{H}_3\text{O}^{+}_{(aq)}$ cation in the outside environment. Reaction (2) is then coupled to the transfer of n protons from the outside to the inside:

$$n\text{H}_3\text{O}^{+}_{(aq,\,out)} = n\text{H}_3\text{O}^{+}_{(aq,\,in)} \quad \Delta_r G^{\circ\prime}_3 \quad (3)$$
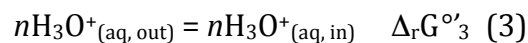
The standard reaction free energy for the proton transfer reaction (3) is given by

$$\Delta_r G°'_3 = nF\Delta\varphi \qquad (4)$$

where F is the Faraday constant and $\Delta\varphi$ is the transmembrane electric potential difference. In the experiment by Gräber et al., $\Delta\varphi$ is kept constant equal to $\Delta\varphi = 15$ mV.

20. Write the global reaction for the ATP hydrolysis coupled to the proton transfer across the membrane.
21. We will denote $\Delta_r G°'_{global}$ its standard reaction free energy. Determine $\Delta_r G°'_{global}$ as a function of $\Delta_r G°'_{2(aq)}$ and $\Delta\varphi$.
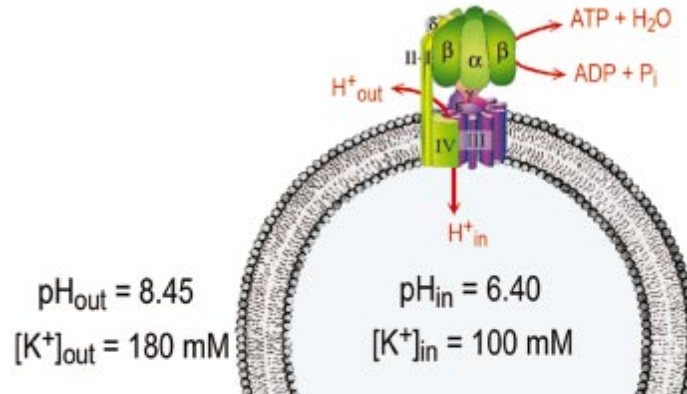
Fig 5: an ATPase complex embedded inside a liposome membrane separating an inner region from an outside environment. [from P. Turina, D. Samoray and P. Gräber EMBO 22, pp. 418-426, 2003]

22. We call Q the stoichiometric ratio of the ATP hydrolysis:

$$Q = \frac{[\mathrm{ADP}^{3-}][\mathrm{HPO}_4^{2-}]}{[\mathrm{ATP}^{4-}]c^0} \qquad (5)$$

where c^0 is the standard concentration. Show that this ratio Q_{eq} at equilibrium satisfies the relation

$$RT \ln Q_{eq} = -\Delta_r G^{o'}_{global} + nRT \ln 10 \, \mathrm{pH}_{in} - (n-1)RT \ln 10 \, \mathrm{pH}_{out} \qquad (6)$$

The outside pH is maintained at $\mathrm{pH}_{out} = 8.45$ and the system is prepared at a given value of Q. The system is at temperature $T = 298$ K and the solution contains $MgCl_2$. pH_{in} is then given different values and the evolution of ATP concentration is followed in time through a luciferin/luciferase assay. The value of $\mathrm{pH}_{in}(eq)$ for which the initial value of Q corresponds to equilibrium, $Q = Q_{eq}$ (no evolution of the ATP concentration), is then reported. Table 2 reports the obtained values of $\mathrm{pH}_{in}(eq)$ for different values of Q.

ln Q	pH_{in}(eq)
0.217	7.07
-1.54	6.91
-3.36	6.68
-5.44	6.46

Table 2: values of the internal pH for which equilibrium is found for different initial values of stoichiometric ratio Q

23. Why is it important to keep the outside pH fixed in this experiment and vary only the inside pH?

24. Determine n, the number of proton transferred per hydrolysed ATP molecule, from the results reported in Table 2.

25. Determine from these results the standard reaction free energy $\Delta_r G^{\circ\prime}_{2(aq)}$ for reaction (2). Is it compatible with the standard reaction free energy $\Delta_r G^{\circ\prime}_{1(aq)}$ for reaction (1) that is reported in Table 1?

Numerical constants

Faraday's constant: F = 96500 C/mol

Universal gas constant: R = 8.314 J/K

ln 10 = 2.303

CLASSIFICATION PERIODIQUE DES ELEMENTS

MÉTAUX DE TRANSITION

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 H 1.0079																	2 He 4.0026
3 Li 6.941	4 Be 9.0122											5 B 10.811	6 C 12.011	7 N 14.007	8 O 15.999	9 F 18.998	10 Ne 20.180
11 Na 22.990	12 Mg 24.305											13 Al 26.982	14 Si 28.086	15 P 30.974	16 S 32.066	17 Cl 35.453	18 Ar 39.948
19 K 39.098	20 Ca 40.078	21 Sc 44.956	22 Ti 47.88	23 V 50.942	24 Cr 51.996	25 Mn 54.938	26 Fe 55.847	27 Co 58.933	28 Ni 58.69	29 Cu 63.546	30 Zn 65.39	31 Ga 69.723	32 Ge 72.61	33 As 74.922	34 Se 78.96	35 Br 79.904	36 Kr 83.80
37 Rb 85.468	38 Sr 87.62	39 Y 88.906	40 Zr 91.224	41 Nb 92.906	42 Mo 95.94	43 Tc [98]	44 Ru 101.07	45 Rh 102.91	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.75	52 Te 127.60	53 I 126.90	54 Xe 131.29
55 Cs 132.91	56 Ba 137.33	57 *La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.85	75 Re 186.21	76 Os 190.2	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po [209]	85 At [210]	86 Rn [222]
87 Fr [223]	88 Ra 226.03	89 **Ac 227.03	104 Unq [261]	105 Unp [262]	106 Unh [263]												

* LANTHANIDES

58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm [145]	62 Sm 150.36	63 Eu 151.97	64 Gd 157.25	65 Tb 158.93	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97

** ACTINIDES

90 Th 232.04	91 Pa 231.04	92 U 238.03	93 Np 237.05	94 Pu [244]	95 Am [243]	96 Cm [247]	97 Bk [247]	98 Cf [251]	99 Es [252]	100 Fm [257]	101 Md [258]	102 No [259]	103 Lr [260]

(masses atomiques basées sur $^{12}C = 12$)