Protonmotive force - from motive protons to membrane potential

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Advanced

Summary

The protonmotive force (pmF) is central to oxidative phosphorylation (OXPHOS), coupling oxygen consumption (OX) in cell respiration to phosphorylation of ADP to ATP (PHOS). Defined as an electrochemical potential difference, the pmF consists of two components: the electric part pmF_{el}, linked to the transmembrane potential difference ($\Delta\Psi$), and the diffusive part pmF_d, related to the pH difference (ΔpH) across the mitochondrial inner membrane. Although pmF_{el} is dominant in animal mitochondria, pmF_d — often overlooked — contributes significantly under physiological conditions.

Peter Mitchell's chemiosmotic theory defines four integrated coupling modules. **Module 1:** The ATP synthase utilizes the pmF producing ATP (PHOS). **Module 2:** The electron transfer system generates the pmF by redox-driven proton transport (OX). **Module 3:** Coupling of proton translocation to electroneutral ion exchange modulates the balance from pmF_d to pmF_{el}. **Module 4:** The coupling membrane integrates these structural and functional coupling modules.

A ΔpH of 0.5 units contributes approximately 15–20 % to the total pmF, emphasizing that pmF_d can provide a significant thermodynamic push. Oversimplified textbook conventions are challenged by rigorously incorporating stoichiometric numbers ν_{H^+} and the charge number z_{H^+} in the equations defining the advancement of proton translocation and the protonmotive force. A transparent theoretical framework bridges theory and experiment with an innovative conceptual drive.

- The pmF explains the mechanism of OXPHOS coupling.
- H⁺-linked electron *transfer* in redox reactions drives compartmental H⁺ *transport*.
- The pmF with units [J/mol] or [V = J/C] is isomorphic to physical forces with unit newton [N = J/m].
- Force times advancement of the motive quantity yields energy [J] in the form of exergy available for work.

Introduction: Coupling of phosphorylation of ADP to ATP (PHOS; module 1) to oxidation (OX; module 2) in oxidative phosphorylation (OXPHOS) is mediated by the protonmotive force (pmF) through the hydrogen ion H⁺ circuit [7] (Figure 1; Chapters 1 to 3). The term protonmotive force is derived from three elementary concepts: the electrochemical *proton* (p), the dynamic *motive* (m), and the thermodynamic (ergodynamic) *force* (F). Taken together, this yields the abbreviation pmF. The pmF consists of two physically inseparably bound but analytically distinct partial forces, measured with specific electrochemical sensors or fluorescent probes: The electric part pmF_{el} is linked to charge separation, while the diffusive part pmF_d is linked

Figure 1. Mitchell's modules 1 and 2 coupled by the protonmotive force pmF. Proton-linked electron transfer, 2{H⁺+e⁻}, through the ETS is coupled to proton translocation from the cathodic to the anodic phase, H_b^+ to H_a^+, and in turn to the phosphorylation of ADP to ATP.

to the pH difference between two compartments separated by the mitochondrial inner membrane (mtIM). To add the partial forces, $pmF_{el}+pmF_d$, both must be expressed interchangeably in the same units, whether in electrical (charge), chemical (amount), or molecular (count) formats (Chapter 4). Conversion between units is based on fundamental proportionality constants of nature (Chapter 5).
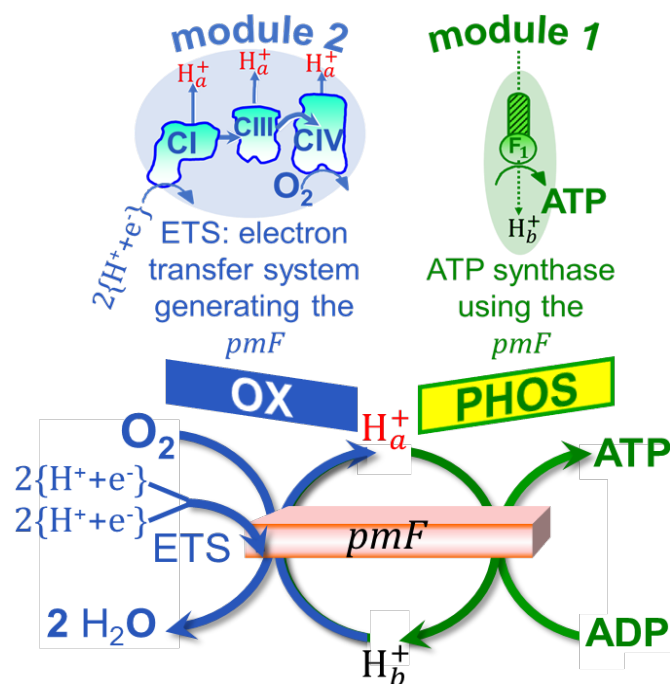
1. Proton

There are protons (p⁺) and hydrogen ions (H⁺). The superscript + indicates the positive electric charge when the electron (e⁻) with its negative charge is missing from

the hydrogen atom (H = H⁺ + e⁻). The proton is a positively charged subatomic particle in all atoms. However, the terms proton and hydrogen ion are used synonymously for the cation H⁺ or even collectively including the hydronium ion cluster formed with water, $H_2O + H^+ = H_3O^+$. It depends on the context, whether a distinction is needed or not.

The activity of H⁺ is of special relevance in water, aqueous solutions, and cellular compartments. Proton activity is frequently measured potentiometrically and expressed as the pH on a logarithmic scale. The pH indicates the acidity. A difference in pH of one unit (ΔpH = 1) implies a 10-fold difference in H⁺ activity. Water at pH 7 and 25 °C is said to be neutral. Below pH 7, aqueous solutions are acidic with a high activity of H⁺. Neutrality shifts to a lower pH at higher temperature. The H⁺ activity a_{H+} is related to the H⁺ concentration c_{H+} by the activity coefficient. Mitochondria are small, often elongated organelles with a tiny volume. At pH 8 the volume of the matrix enclosed by the mtIM contains two to six H⁺ per mitochondrion. The number of protons N_{H+} in the matrix is kept low by the action of molecular redox pumps which are large enzymes called respiratory Complexes, specifically CI, CIII, and CIV (Mitchell's module 2; Figure 2). These redox pumps push H⁺ out of the matrix space, generating a ΔpH across the mitochondrial inner membrane (mtIM). H⁺-linked electron
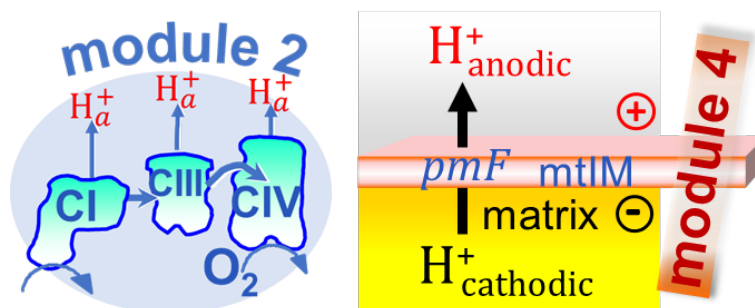

Figure 2. Michell's module 2, electron transfer coupled to proton transport from the cathodic to the anodic compartment across the semipermeable mitochondrial inner membrane mtIM (module 4). The anodic and cathodic H⁺ are H_a^+ and H_b^+, respectively.

transfer in chemical reduction-oxidation (redox) reactions is coupled to compartmental transport (translocation) of protons (hydrogen ions H⁺) across the mtIM which has no or low pump-independent translocation capacity for ions. Since charges are involved, the matrix (low in H⁺) is the cathodic compartment with a negative charge. The outer space is the anodic compartment *a* with a positive charge (higher in H⁺, lower pH). Definition of the anodic compartment varies according to decisive experimental details determining where the anodic pH is measured: at the outer phase of the mtIM, in the intermembrane space between the mtIM and the mitochondrial outer membrane (mtOM), or – most frequently – in the extramitochondrial incubation medium as the outer space.

Notes on p _____

 – Symbol pH: stems from the term *potentia* (p) *hydrogenii* (H), not to be confused with symbol p which indicates *proton* but not *pressure* [2].
 – The terms hydrogen ion H⁺ and proton are used synonymously for the *hydron* H⁺.

- The charge number z_{H^+} of H^+ equals 1; the charge number z_{OH^-} of OH^- is -1.
- In dilute aqueous solutions, the hydrogen ion activity a_{H^+} is approximately (numerically) equal to the hydrogen ion concentration c_{H^+}.
- Activity a_{H^+} (dimensionless): concentration divided by the unit standard concentration (1 mol/L) and multiplied by the activity coefficient.
- Count concentration: number of H^+ per volume, $C_{H^+} = N_{H^+}/V$ [x/L].
- Amount concentration: moles of H^+ per volume, $c_{H^+} = n_{H^+}/V$ [mol/L].
- pH: negative base 10 logarithm of the activity of H^+, pH = $-\log_{10}(a_{H^+})$.
- At pH 8 in the mitochondrial matrix, the corresponding H^+ activity is 10^{-8} and the H^+ concentration is 0.01 µmol/L.
- ΔpH: pH difference between high pH on the cathodic matrix side b and lower pH on the anodic outer side a [5, 6], when the direction of transport is defined as $a{\rightarrow}b$.
- The *difference* in pH between the two compartments separated by a membrane, ΔpH, does not consider the thickness z of the membrane and must not be confused with a pH *gradient* ∂pH/∂z across the membrane.
- Compare cathode and anode (catabolism and anabolism): in ancient Greek hodos = way; 'cathodos' or 'cata' means *down*, 'anodos' means *up*. Figure 2 aligns with the literal meanings (down/up), visually reinforcing the concept.

2. Motive

The term *motive* (m) is familiar from the electromotive force in electrochemistry. It is helpful to distinguish (*1*) the *dynamic* or motive amount of protons in the process of translocation indicated by arrows ($b \rightarrow a$), versus (*2*) the amount of protons in a defined *state* of a compartment (Figure 3). Thereby, *what moves* between compartments is distinguished from *what is contained* in a compartment [2]. Even if the static number of H^+ is small in the cathodic matrix, the motive number of H^+ pumped across the membrane may be large in mitochondria respiring at a high rate.

Attention to the units of quantities helps explore the complex concept of the protonmotive force. In physics, the motive particles are expressed as a count, which is the number of particles involved in the process, with the elementary unit [x]. In chemistry,
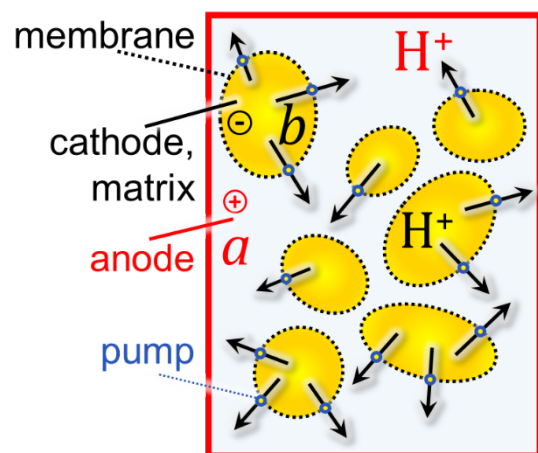

Figure 3. Translocation in a compartmental system. The motive amount of H^+ (arrows from compartment b to a) is distinguished from the concentration of H^+ within the compartments.

the motive particles are expressed as an amount, with unit mole [mol]. The charge number z_{H^+} of H^+ equals one. Therefore, the proton charges pumped are proportional to the motive amount of protons. In electrochemistry, the motive quantity of translocated H^+ is expressed as charge, with unit coulomb [C]. Both, one mole and one charge are fixed large numbers of particles. The familiar notation describing dissociation as $H_2O \rightarrow H^+ + OH^-$ illustrates, that the three motive terms in the transformation can be expressed in different units (x, mol, C) of the quantities count, amount, or charge. But in each case, the units must be chosen consistently for the transformation. Expressing H^+ in coulombs, therefore, implies that in this case the *uncharged* molecule H_2O is expressed in units of *charge*. This is not paradoxical if viewing the dissociation as $H^+OH^- \rightarrow H^+ + OH^-$.

Advancement of proton transfer across the mtIM between the anodic compartment a and the cathodic matrix b is a vectorial transformation $H_a^+ \rightarrow H_b^+$ (Figure 4). The stoichiometric numbers $\nu_{H_i^+}$ (with simplified symbol ν_i) relate the change of the *motive amount* of H^+ – that is, the amount associated with this transformation – to the change of advancement $d_m\xi_{H^+}$ (Eq. 1):

Figure 4. Translocation in a compartmental system with identical compartmental volumes ($V_a = V_b$) and volume fractions ($\Phi_i = V_i/V$). Stoichiometric numbers ν_i are defined as -1 in the exit compartment and +1 in the entry compartment.

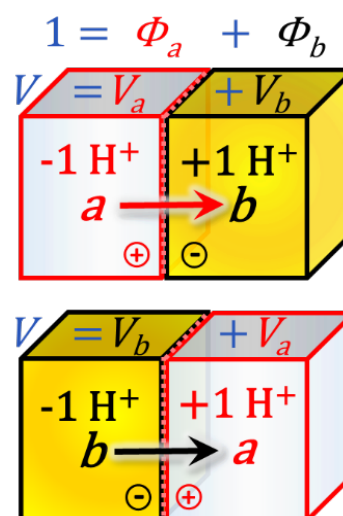$$d_m\xi_{H^+} = d_m n_{H_a^+} / \nu_a = d_m n_{H_b^+} / \nu_b \qquad [mol] \quad (Eq.\ 1)$$

This may appear formal, but the underlying concept is straightforward. Subscripts a and b refer to the two compartments i. The subscript m denotes the partial contribution of the motive process to the overall change. A motive amount $d_m n_{H_i^+}$ is associated with the *process* of the defined partial transformation, in contrast to the *state* quantity $dn_{H_i^+}$ referring to the change of the amount present in the indicated compartment i (Figure 3).

The stoichiometric numbers, ν_i ($\nu_{H_a^+}$ or $\nu_{H_b^+}$), define the motive directionality: for proton diffusion $a \rightarrow b$, the stoichiometric number $\nu_a = -1$ (-1 H^+: the entity is removed from compartment a) and $\nu_b = 1$. If the process $a \rightarrow b$ proceeds backwards, the advancement $d_m\xi_{H^+}$ assumes a negative value. This is different from defining the transformation in the opposite direction, $b \rightarrow a$, when the signs are reverted, and $\nu_a = 1$.

A simple stoichiometric elemental balance equation $\nu_b = -\nu_a$ (Eq. 2) is obtained under the condition that H^+ concentrations do not change in the path from the cathodic compartment b to the anodic compartment a,

$$0 = \nu_a\ H_a^+ + \nu_b\ H_b^+ \qquad\qquad (Eq.\ 2)$$

This path comprises the mtIM, intermembrane space, and the mtOM, if a is considered as the outer space.

External exchange of matter is a process prevented in closed systems. In such a system without simultaneous transformations other than those defined as 'm', there is an equality favored under experimentally simplified conditions, $\mathrm{d_m}n_{\mathrm{H}_a^+} = \mathrm{d}n_{\mathrm{H}_a^+}$. Then the rather elusive *motive* flow, $\mathrm{d_m}n_{\mathrm{H}_a^+}/\mathrm{d}t$, can actually be quantified as $\mathrm{d}n_{\mathrm{H}_a^+}/\mathrm{d}t$ in terms of a change over time monitored in compartment a in an overall *closed system*. Again, definition of compartment a depends on the experimental approach of measurements in the incubation medium outside the mtOM or in the intermembrane space.

pH measured in compartment a (at an activity coefficient of 1 in dilute solution) can be converted to the concentration $c_{\mathrm{H}_a^+}$ in the compartmental volume V_a,

$$\mathrm{d}c_{\mathrm{H}_a^+} = \mathrm{d}n_{\mathrm{H}_a^+}/V_a \qquad\qquad [\mathrm{mol/m^3}] \quad (\text{Eq. 3})$$

Advancement has been introduced originally for chemical reactions in a homogenous system without distinct compartments. When extending the concept of advancement in a homogeneous system to translocation in a compartmental system, it is important to clarify that volume-specific advancement (Eq. 4) is normalized for the total volume of the system, $V = V_a + V_b = \sum V_i$, where i indicates compartments a or b,

$$\mathrm{d_m}\xi_{\mathrm{H^+}}/V = \mathrm{d_m}n_{\mathrm{H}_i^+}/V \cdot \nu_i^{-1} \qquad\qquad [\mathrm{mol/m^3}] \quad (\text{Eq. 4})$$

Note that $\mathrm{d_m}n_{\mathrm{H}_i^+}/V$ equals a change of concentration only under restricted conditions of m being the only process, since it indicates the partial change of the volume-specific motive amount of H⁺ in transport. Importantly, compartmental volumes V_a and V_b may have different magnitudes (Figure 5). Substituting V in Eq.(4) by the volume fraction, $\Phi_i = V_i/V$, we obtain the volume-specific advancement as [2],

$$\mathrm{d_m}\xi_{\mathrm{H^+}}/V = \mathrm{d_m}c_{\mathrm{H}_i^+} \cdot \Phi_i \cdot \nu_i^{-1} \qquad\qquad [\mathrm{mol/m^3}] \quad (\text{Eq. 5})$$

Whereas $\mathrm{d_m}\xi_{\mathrm{H^+}}/V$ is defined theoretically in Eq.(4) in terms of the total volume of the system, its experimental determination requires consideration of compartmental volumes and extension of the stoichiometry from ν_i^{-1} to $\Phi_i \cdot \nu_i^{-1}$ (Eq. 5), to relate motive concentration changes $\mathrm{d_m}c_{\mathrm{H}_i^+}$ in compartments a and b.

Figure 5. Translocation in a compartmental system with different compartmental volumes ($V_a > V_b$ and volume fraction $\Phi_a > \Phi_b$).

Advancement per time is the generalized flow of a transformation. Advancement links the motive quantity to its conjugated force. This takes us from advancement to the protonmotive force. Motive quantities are expressed in different motive units. The product of advancement and the conjugated force, however, yields always energy in
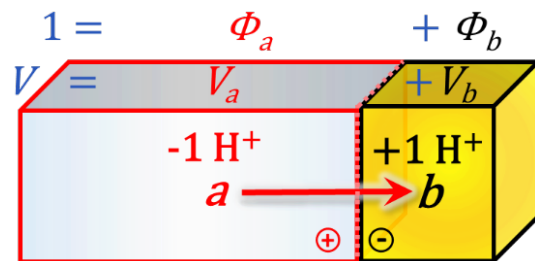
the unit joule [J], either in the form of exergy available for work in coupled energy transformations or in the form of exergy dissipated irreversibly and thus related to entropy production.

Notes on m _____

- Count N_{H^+}: H⁺ is countable and the number of H⁺ is a count, with the elementary unit [x]. In electrochemistry, the count format is replaced by amount or charge.
- Amount n_{H^+}: expressed in the unit mole [mol].
- Charge Q_{H^+}: expressed in the unit coulomb [C].
- Motive versus state entities: The *motive* count, amount, or charge translocated between compartments is distinguished from the *state* entities which describe the system's current condition.
- Advancement $d_m\xi_{H^+}$: As motive entities are removed from an exit compartment and moved into an entry compartment, the advancement describes this process independent of an exit or entry point of view.

3. Force

The protonmotive force is a thermodynamic force different from the fundamental forces of physics. To be alert of the code-switching between physics and chemistry, it is emphasized that the thermodynamic forces are implicitly isomorphic *motive* forces and statistical (entropic). In contrast, the forces of physics are vectors with spatial direction, in units of newton [N], where force [N = J/m] is energy per distance, with units joule [J] and meter [m], respectively. In compartmental (discontinuous) systems, forces such as the electromotive force *emF* and *pmF* are related to potential differences, hence the symbol Δp used by Peter Mitchell [7]. The covered distance is replaced by advancement $d_m\xi_{H^+}$ of the motive entity H⁺ expressed in various units. Force and advancement are conjugated quantities with identical formats: their product always yields energy with the unique unit joule. Motive forces are known as the 'generalized' forces of the thermodynamics of irreversible processes [2; 4].

Work is performed when protons H⁺ are actively transported from the cathodic matrix 'uphill' to the anodic compartment (Figure 6). The chemical work is supplied by oxidation of fuel substrates. 'Available energy' — potentially transformed into work — is a distinct form of energy defined as Gibbs energy or *exergy* under typical experimental conditions of constant pressure and temperature, expressed in the general energy unit joule [J]. The
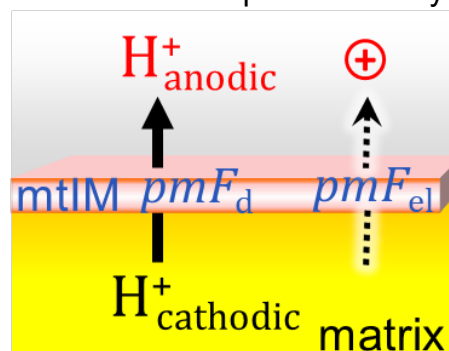

Figure 6. H⁺ translocation generates a chemical diffusion potential difference and an electric potential difference.

pmF is the potential energy *per* advancement of H⁺. When the *pmF* is expressed in its electrical format, the *pmF* unit is joule per coulomb [J/C]. The unit J/C has the name volt with symbol V; V = J/C.

The *pmF* of functional mitochondria is typically 160 to 240 mV (0.16 to 0.24 V), depending on the respiratory state. When oxygen is consumed and ATP produced at maximum OXPHOS capacity *P*, then the phosphorylation system exerts a load and thus lowers the *pmF*. In contrast, when ATP production is entirely blocked, then the *pmF* increases to a maximum and the oxygen consumption rate drops. Under this condition, the oxidation system – the electron transfer system driving the redox proton pumps – pushes protons slowly out of the matrix space, only to compensate for the back-diffusion of protons through the rather tight mtIM. Back-diffusion of protons is higher when the mtIM is leaky, and then requires a higher pumping rate of protons to compensate for the leak, which in turn stimulates respiration at a lower coupling efficiency. Therefore, this respiratory state is identified as the leak state. The corresponding respiration rate is the leak respiration *L* (Figure 7) [3].

A defective mtIM may become increasingly leaky, causing at the limit a complete short-circuit of the proton cycle across the mtIM. This leads to a collapse of the *pmF*. Experimentally, a short-circuit can be chemically induced by adding so-called protonophores which are lipid-soluble substances that shuttle protons with their charge through the membrane. Then, OXPHOS-coupled ATP production cannot proceed; therefore, protonophores are called uncouplers. However, 'coupling' remains between redox proton pumps and proton translocation in module 2.

When the redox proton pumps do not face a *pmF* against which to push, oxygen consumption increases to a maximum rate in the decoupled state. This rate in the decoupled state provides a
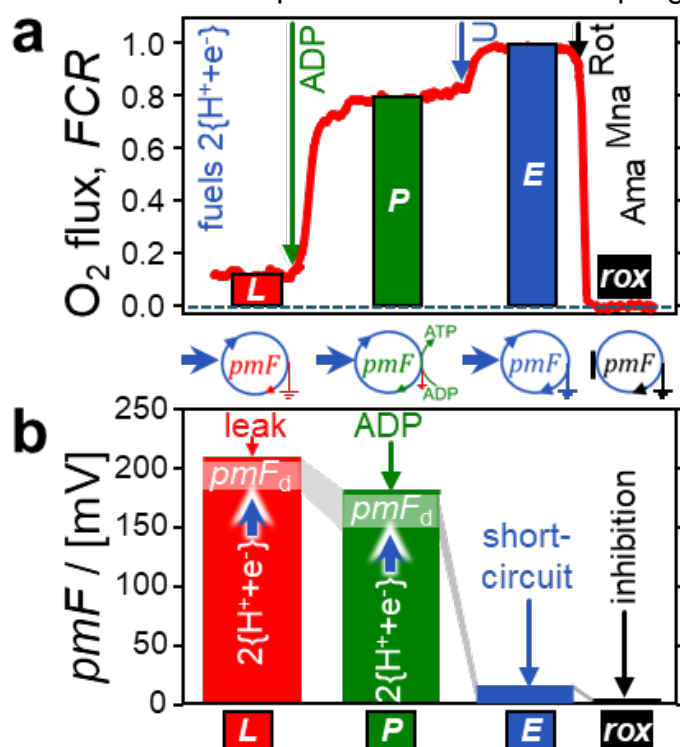
Figure 7. Respiratory coupling control in isolated mitochondria. **(a)** Respiration (*L*, *P*, *E*; flux control ratios *FCR* relative to *E*) corrected for *rox*. **(b)** Protonmotive force as a function of electron pressure (2{H⁺+e⁻}; blue upwards arrows) generating the *pmF* (see Figure 1), counteracted by mtIM leaks (*L*) or backpressure by ATP production (*P*). *pmF*el and *pmF*d are distinguished as full- and light-colored bars. Collapse of the *pmF* in decoupled respiration *E*, and further by inhibition of the ETS (*rox*).

measure of mitochondrial oxidative (electron transfer) capacity E. If the capacity of the PHOS system to utilize the pmF limits OXPHOS capacity P, then E is higher than OXPHOS capacity (Figure 7). P cannot be higher than E, but $P = E$ in several types of mitochondria. In well-coupled mitochondria, leak respiration L is as low as 5 to 15 % of E. Coupling, uncoupling, and decoupling are key components of mitochondrial respiratory control [3].

Notes on F _____

- The protonmotive force pmF is exergy per advancement, $\partial G / \partial_m \xi_{H^+}$, indicated as a partial derivative (Table A1, row 1).
- The joule [J] is the unit of all forms of energy, including heat, work, enthalpy and exergy.
- $d_m \xi_{H^+} / dt$ is a motive flow. Always, the product of a flow times the conjugated force yields power with unit watt [W = J/s].
- The pmF of functional mitochondria ranges from 160 to 240 mV (= mJ/C).
- Physiological *uncoupling* and pathological *dyscoupling* diminish the pmF [3].
- The pmF is a physiological parameter of mitochondrial respiratory control.

4. Electric and diffusive nature of the pmF

The two partial forces add up to $pmF = pmF_d + pmF_{el}$. A unit pH difference corresponds to a pmF_d of 5.9 kJ/mol (62 mV) plus an equal partial contribution pmF_{el,H^+} at 37 °C. At ΔpH = 1, $pmF = pmF_d + pmF_{el,H^+}$ = 12 kJ/mol = 123 mV if only protons without any other cations nor an external electric field are present.

Here comes a challenge: In mammalian mitochondria, pmF_{el} reaches typically up to 200 mV, higher than 62 mV although ΔpH is <1. This apparent discrepancy is resolved by Mitchell's module 3 [7]: Consider the generation of the pmF from zero mV, not the proton cycling at a steady-state pmF in leak respiration or OXPHOS. While the proton pumps build up a ΔpH, the H$^+$ ions extruded from the matrix are neutralized by negatively charged counterions Z$^-$ (Figure 8). Z$^-$

Figure 8. Mitchell's module 3: Electroneutral diffusion of HZ across the mtIM to the cathodic matrix and exchange diffusion of H$^+$ against Y$^+$ reduce the pH difference while maintaining the electric potential difference.

cannot pass the mtIM. While the negatively charged Z$^-$ (e.g. OH$^-$) combines with H$^+$ in the anodic phase, the uncharged molecule HZ equilibrates readily across the mtIM by electroneutral diffusion, reducing the pH difference but maintaining the pmF_{el}. HZ dissociates in the cathodic matrix to Z$^-$ + H$^+$, maintaining the number of protons
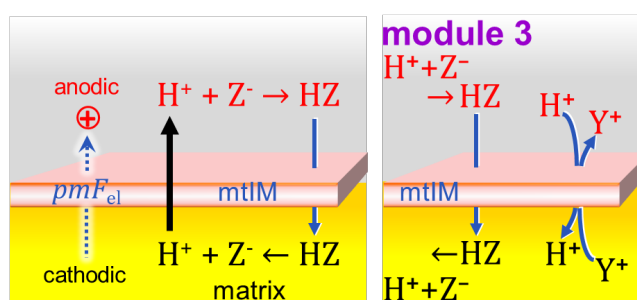
constant in the cathodic and anodic phases while building up the electric potential difference by adding negative charges to the matrix. A similar scheme applies to Z^{2-} (e.g. inorganic phosphate). Additionally, exchange diffusion of H^+ against Y^+ (e.g. K^+) counteracts the pH difference across the mtIM, adding positive charges to anodic phase. Although the primary mechanism of generating the pmF is the translocation of H^+ by the proton pumps, ΔpH remains low, and the predominant contribution to the pmF is the electric potential difference maintained by the counterions (Figure 8).

Methods for measurement of pmF_{el} are more commonly applied compared to determination of ΔpH and thus pmF_d. The electroneutral K^+/H^+ antiporter nigericin may be applied to use the increase of pmF_{el} as an indirect measure of pmF_d [6]. Changes in pmF_{el} may be accompanied by compensatory shifts in pmF_d. If measurements are restricted to pmF_{el}, the limitations of interpretation should always be considered to avoid ambiguities arising when pmF_{el} is presented as a substitute of the actual pmF.

pmF_d is the partial derivative of exergy G [J] per advancement of proton translocation irrespective of charge (Table A1, row 1). pmF_d is expressed in different formats: count with elementary unit [x], amount with unit mole [mol], or charge with unit coulomb [C]. The partial protonmotive force of translocation ($b{\rightarrow}a$) is the sum of *stoichiometric chemical potentials* (Eq. 6; Table A1, row 2),

$$pmF_d = \mu_{H_a^+}\cdot\nu_a + \mu_{H_b^+}\cdot\nu_b = RT \cdot \Delta \ln a_{H^+} \qquad \text{[kJ/mol]} \quad \text{(Eq. 6)}$$

where $\mu_{H_i^+}$ is the chemical potential of H^+ in compartment i,

$$\mu_{H_i^+} = \mu_{H_i^+}^o + RT\cdot\ln a_{H_i^+} \qquad \text{[kJ/mol]} \quad \text{(Eq. 7)}$$

The standard chemical potential $\mu_{H_i^+}^o$ (Eq. 7) cancels in (Eq. 6) when the activity coefficient of H^+ is identical on both sides of the membrane. Thus pmF_d can be calculated from ΔpH at a given temperature T [K] (37 °C in the numerical examples below; T = 310 K). This requires (*1*) selection of the motive unit for the formats count ($\underline{N}$), amount ($\underline{n}$), or charge ($\underline{e}$), using the corresponding proportionality constants k, R, or f (Chapter 5); (*2*) conversion of the base 10 logarithm of pH to the base 2 logarithm, $\ln a_{H^+}$ = -ln(10)·pH, where ln(10) = 2.303; and (*3*) definition of the direction of the translocation across the mt-membrane, either passive diffusion of H^+ to the cathodic matrix ($a{\rightarrow}b$) when the force has a negative sign, or active pumping from the cathodic matrix (b) to the anodic compartment (a) when the force has a positive sign (Eq. 8, $b{\rightarrow}a$; Table A1, row 4),

$$\underline{N}: \quad pmF_{d\underline{N}} = -kT\cdot\ln(10)\cdot\Delta\text{pH} \quad = -9.86\cdot10^{-21}\cdot\Delta\text{pH} \quad \text{[J/x]} \quad \text{(Eq. 8a)}$$
$$\underline{n}: \quad pmF_{d\underline{n}} = -RT\cdot\ln(10)\cdot\Delta\text{pH} \quad = -5.94\cdot\Delta\text{pH} \quad \text{[kJ/mol]} \quad \text{(Eq. 8b)}$$
$$\underline{e}: \quad pmF_{d\underline{e}} = -fT\cdot z_{H^+}^{-1}\cdot\ln(10)\cdot\Delta\text{pH} = -61.5\cdot\Delta\text{pH} \quad \text{[mJ/C]} \quad \text{(Eq. 8c)}$$

The Boltzmann constant k [J·x^{-1}/K] is a defining constant of the SI [1]. The gas constant R [J·mol^{-1}/K] is related to k by the Avogadro constant, $R = k\cdot N_A$. Consistently, the electromotive constant f [J·C^{-1}/K] is related to k by the elementary charge, $f = k/e$

[2; 4]. These proportionality constants relate temperature $[\mathrm{K}]$ to the concept of isomorphic forces $[\mathrm{J/MU}]$, expressed in different motive units $[\mathrm{MU}]$. The explicit symbol x is introduced for the elementary SI unit 'one', such that the unit of force $[\mathrm{J/x}]$ in kT is distinguished from the unit of energy $[\mathrm{J}]$. Table A2 illustrates the variation of pmF_d as a function of $\Delta\mathrm{pH}$ in the chemical and electrical format.

Notes on *pmF* _____

- Mitchell's module 3 modulates the *pmF*, shifting pmF_d to pmF_el by coupling of proton translocation to electroneutral and reversible proton charge exchange of cations against $\mathrm{H^+}$ and anions against $\mathrm{OH^-}$ or electroneutral counterion exchange [7].
- The *physical nature* of the two additive motive forces (electric and diffusive) must be distinguished from the *formats* of the protonmotive force — specified by their units.
- The *pmF* can be represented in formats of count, amount, or charge. Correspondingly, the *pmF* is expressed in units $[\mathrm{J/x}]$, $[\mathrm{J/mol}]$, or $[\mathrm{J/C}]$.
- The unit of charge is the coulomb $[\mathrm{C}]$. Joule per coulomb defines the unit volt $[\mathrm{V}]$.
- The unit of the fundamental forces of physics $[\mathrm{N = J/m}]$ can be compared with the isomorphic electrical and chemical units $[\mathrm{V = J/C}]$ and $[\mathrm{J/mol}]$.
- The unit of motive force is V or $\mathrm{J/mol}$ in the electrical or chemical format, respectively. A comparable unit for the chemical force $[\mathrm{Jol} \stackrel{\mathrm{def}}{=} \mathrm{J/mol}]$ is not available but would be as practical as N and V.
- The *pmF* does not express the pH gradient and electric potential gradient, but compartmental differences. This fundamental concept is explicitly considered in the isomorphic symbol $\Delta_\mathrm{m}F_\mathrm{H^+}$ for the *pmF*, and the corresponding electric and diffusive parts, $\Delta_\mathrm{el}F_\mathrm{p^+}$ and $\Delta_\mathrm{d}F_\mathrm{H^+}$, respectively [2]. In contrast to a gradient with direction in space, the protonmotive force is related to differences of electrochemical potentials between compartments.

5. Conversions

The partial protonmotive forces cannot be added if pmF_el is expressed in the electrical unit $[\mathrm{mV}]$ and pmF_d is expressed in the chemical unit $[\mathrm{kJ/mol}]$. The partial forces must be aligned by conversion between chemical and electrical formats and corresponding motive units. The *pmF* is expressed in different motive formats depending on the electrical or chemical motive unit (C or mol). Conversion of units changes the numerical value without change of meaning (Eq. 9). Historically, different symbols have been assigned to the *pmF* in the *e* and *n* format,

$$e: \qquad pmF_{\underline{e}} \stackrel{\mathrm{def}}{=} \Delta\mathrm{p} \qquad\qquad [\mathrm{mV = mJ/C}] \quad (\text{Eq. 9a})$$

$$n: \qquad pmF_{\underline{n}} \stackrel{\mathrm{def}}{=} \Delta\tilde{\mu}_\mathrm{H^+} \qquad\qquad [\mathrm{kJ/mol}] \quad (\text{Eq. 9b})$$

$N \rightarrow \underline{n}$ and $N \rightarrow \underline{e}$ format conversion from count to amount and count to charge (Eq. 10): The number of H^+, N_{H^+} [x] (count format $\underline{N}$), divided by the Avogadro constant N_A [x/mol] is the molar amount of H^+, $n_{H^+} = N_{H^+}{\cdot}N_A^{-1}$ [mol] (molar format $\underline{n}$). Multiplication by the elementary charge e [C/x] converts the quantity count N_X [x] to the quantity charge, $Q_{H^+} = N_{H^+}{\cdot}e$ [C] (molar format $\underline{e}$).

$\underline{N}$ [x] $\rightarrow$ $\underline{n}$ [mol]:	$pmF_{\underline{n}} = pmF_{\underline{N}} \cdot N_A$	[J/mol]	(Eq. 10a)
$\underline{N}$ [x] $\rightarrow$ $\underline{e}$ [C]:	$pmF_{\underline{e}} = pmF_{\underline{N}} \cdot e^{-1}$	[J/C]	(Eq. 10b)

$\underline{e} \leftrightarrow \underline{n}$ format interconversion between charge and amount (Eq. 11): The product of the two fundamental constants e and N_A is the Faraday constant $F = e{\cdot}N_A$ [C/mol] which is the proportionality constant between charge [C] and amount [mol]. The electrical charge of H^+, Q_{H^+} [C] (format $\underline{e}$), divided by the charge number of the proton ($z_{H^+} = 1$) and by the Faraday constant is the amount, $n_{H^+} = Q_{H^+}{\cdot}(z_{H^+}{\cdot}F)^{-1}$ [mol].

$\underline{e}$ [C] $\rightarrow$ $\underline{n}$ [mol]:	$pmF_{\underline{n}} = pmF_{\underline{e}} \cdot (z_{H^+}{\cdot}e{\cdot}N_A)$	$= pmF_{\underline{e}} \cdot (z_{H^+}{\cdot}F)$	[J/mol]	(Eq. 11a)
$\underline{n}$ [mol] $\rightarrow$ $\underline{e}$ [C]:	$pmF_{\underline{e}} = pmF_{\underline{n}} \cdot (z_{H^+}{\cdot}e{\cdot}N_A)^{-1}$	$= pmF_{\underline{n}} \cdot (z_{H^+}{\cdot}F)^{-1}$	[J/C]	(Eq. 11b)

Notes on constants and units _____

- $\underline{N}$ and the Boltzmann constant $k = 1.380\ 649{\cdot}10^{-23}$ J·x^{-1}/K; a defining SI constant [1; 4].
- $\underline{n}$ and the gas constant $R = 8.314462618$ J·mol^{-1}/K; related to k by the Avogadro constant, $R = k{\cdot}N_A = f{\cdot}F$.
- $\underline{e}$ and the electromotive constant $f = 8.617333262{\cdot}10^{-5}$ J·C^{-1}/K; related to k by the elementary charge, $f = k/e = R/F$ [2; 4].
- $\underline{n} \rightarrow \underline{N}$ and the Avogadro constant $N_A = 6.022\ 140\ 76{\cdot}10^{23}$ x/mol; a defining SI constant [1], the number of elementary entities [x] per mole [mol].
- $\underline{N} \rightarrow \underline{e}$ and the elementary charge $e = 1.602\ 176\ 634{\cdot}10^{-19}$ C/x; a defining SI constant [1], relating the electric unit [C] to the elementary unit [x].
- $\underline{n} \rightarrow \underline{e}$ and the Faraday constant $F = 96\ 485.332\ 12$ C/mol; relating the electric unit [C] to the chemical unit [mol], $F = e \cdot N_A = R/f$. The Faraday constant is the ratio of chemical and electrical units $\left[\frac{J/mol}{V} = \frac{C}{mol}\right]$.

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Conclusions: pmF_{el} and pmF_d are distinct but complementary components of OXPHOS coupling mediated by the **proton** circuit. The term **motive** expresses a dynamic concept. A motive amount [mol] or charge [C] in a *process* is distinguished from the corresponding *state* quantities amount or charge in a system or compartment. The advancement of H^+ across a semipermeable membrane times the conjugated electrochemical **force** *pmF* yields the electrochemical exergy available for work. *Protonmotive* indicates a potential for the movement of H^+; *force* is a measure of the potential for motion [2].

The positive endergonic protonmotive force is generated by the electron transfer system ETS during oxidation of reduced fuel substrates (OX) and counteracts the negative exergonic driving force of the ETS. As the *pmF* approaches its maximum under conditions blocking phosphorylation, leak respiration *L* is suppressed and primarily compensates for proton back-diffusion across the coupling membrane. In contrast, respiration *P* in the OXPHOS state is stimulated as the *pmF* is lowered by driving ATP synthesis (PHOS). Uncouplers collapse the *pmF*, abolishing the counterforce and fully stimulating electron transfer capacity *E*.

Under typical physiological conditions, the *pmF* ranges from 160 to 240 mV (= 0.16 to 0.24 J·C^{-1}), or 16 to 23 kJ·mol^{-1} in the chemical format. When the cytosolic pH is 7.2 and the mitochondrial matrix is only 0.5 pH units more alkaline (pH 7.7), this small pH difference (ΔpH) contributes about 31 mV or 3 kJ·mol^{-1} to the total *pmF* at 37 °C. While the electric component pmF_{el} predominates in animal mitochondria, the diffusive component pmF_d can account for a physiologically relevant 15 % of the total *pmF* or 20 % of pmF_{el}. A small ΔpH generates a significant thermodynamic push. The *pmF* is not merely an electric potential difference across a membrane: it is a finely tuned balance of electric and diffusive components, each playing a critical role in the bioenergetics of the cell.

The electric and diffusive nature of the two components of the *pmF* are distinguished from the electrical and chemical formats of the *pmF*. For conversion of formats with different units, the Boltzmann constant k and elementary charge e, $kT = fT·e$ [J/x], provide the link between the electromotive constant, $f = R/F$ [J/C], the gas constant, $R = f·F$ [J/mol], and the Faraday constant, $F = R/f$ [C/mol],

$$\Delta\tilde{\mu}_{H^+}: \quad pmF_{\underline{n}} = -RT \cdot \ln(10)\cdot\Delta pH + \Delta\Psi_{p^+}\cdot(z_{H^+}\cdot F) \qquad [kJ/mol] \quad (12a)$$

$$\Delta p: \quad pmF_{\underline{e}} = fT/z_{H^+} \cdot \left(\ln a_{H_a^+} \cdot v_a + \ln a_{H_b^+} \cdot v_b\right) + \Delta\Psi_{p^+} \qquad [V] \quad (12b)$$

For $v_b = -1$, the endergonic direction of proton transport is $b{\to}a$. In most textbook representations, neither the charge number z_{H^+} nor the stoichiometric numbers $v_{H_i^+}$ are shown explicitly in the equations defining the protonmotive force. Eq. 12b uses the sum of stoichiometric chemical potentials defining the diffusive partial protonmotive force pmF_d (Eq. 6), related to -ΔpH as shown in Eq. 12a (Table A1). While omitting the terms $v_{H_i^+}$ or z_{H^+} with values 1 or -1 may merely change the sign without other mathematical consequences, neglect of the fundamental meaning of such quantities obscures the conceptual foundations and hinders proper understanding. In contrast, applying a rigorous and transparent theoretical framework improves our experimental design and fosters interpretation of results with an innovative conceptual drive.

Terms and symbols

a   anodic compartment (positive)

a_{H^+} activity of H$^+$

b	cathodic compartment (negative)
C	coulomb, SI unit for charge
C_{H^+}	count concentration of H$^+$ [x/m^3]
c_{H^+}	amount concentration of H$^+$ [mol/m^3]
e	elementary charge, 1.602 176 634·10^{-19} C/x
$\underline{e}$	format for charge
e$^-$	electron, negative charge
el	electric
E	electron transfer capacity
ETS	electron transfer system
f	electromotive constant, 8.617333262·10^{-5} J·C^{-1}/K
F	Faraday constant, 96 485.332 12 C/mol
G	Gibbs energy, exergy [J]
H$^+$	hydrogen ion, positively charged, proton is used synonymously in bioenergetics
J	joule, SI unit for any form of energy, heat, work, exergy [kg·m^2·s^{-2}]
k	Boltzmann constant, 1.380 649·10^{-23} J·x^{-1}/K
L	leak respiration
m	motive
mol	mole, SI base unit for the amount of substance, one mole contains exactly 6.022 140 76 × 10^{23} elementary entities
mtIM	mitochondrial inner membrane
mtOM	mitochondrial outer membrane
$\underline{n}$	format for amount
N	newton, SI unit for force [J/m]
$\underline{N}$	format for count
N_A	Avogadro constant, 6.022 140 76·10^{23} x/mol
N_{H^+}	count of H$^+$ [x]
n_{H^+}	amount of H$^+$ [mol]
$d_m n_{H_i^+}$	partial change of the motive amount of H$^+$ in compartment i
OX	oxidation of reduced fuel substrates
OXPHOS	oxidative phosphorylation
P	OXPHOS capacity
PHOS	phosphorylation of ADP to ATP
pH	negative of the base 10 logarithm of the activity of H$^+$, pH = $-\log_{10}(a_{H^+})$
pmF	protonmotive force, other symbols are Δp, $\Delta\tilde{\mu}_{H^+}$, or $\Delta_m F_{H^+}$
pmF_d	diffusive part of the pmF, other symbols are $\Delta\mu_{H^+}$ or $\Delta_d F_{H^+}$
pmF_{el}	electric part of the pmF, other symbols are $\Delta\Psi_{mt}$, $\Delta\Psi_{p^+}$, or $\Delta_{el}F_{p^+}$
Q_{H^+}	charge per H$^+$ [C/x], equivalent to elementary charge e
rox	residual oxygen consumption

R	gas constant, 8.314462618 J·mol^{-1}/K
T	absolute temperature [K]
V	volt, SI unit for electromotive force [J/C]
V	volume, total volume [m^3] or [L] (1 m^3 = 1000 L)
V_i	volume of compartment i
x	elementary unit
z_{H^+}	charge number of H$^+$; z_{H^+} = 1
$\mu_{H_i^+}$	chemical potential of H$^+$ in compartment a or b
$\nu_i, \nu_{H_i^+}$	stoichiometric number of H$^+$ on the exit or entry side i, simplified as ν_i (ν_a or ν_b)
$d_m\xi_{H^+}$	advancement of transformation m
Φ_i	volume fraction of compartment i
$\Delta\Psi_{p^+}$	electric potential difference, electric part of the pmF, where subscript p$^+$ instead of H$^+$ emphasizes that charge is not specific to H$^+$ [2]

Acknowledgements

Further reading

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Appendix

Table A1. Equivalent expressions of the diffusive partial protonmotive force. For $\nu_b = -1$ ($b{\to}a$) at constant temperature and barometric pressure. Subscript d indicates diffusion of the particle irrespective of its charge. General equations (=) of physical chemistry are compared with simplified expressions (equivalence ≡). For details, see [2].

	pmF_d / [kJ·mol^{-1}]		
1. Force	$\Delta_d F_{H^+}$	$= \dfrac{\partial G}{\partial_d n_{H_i^+}} \cdot \nu_i$	$= \dfrac{\partial G}{\partial_d \xi_{H^+}}$
2. Chem. potential	$\mu_{H_a^+} \cdot \nu_a + \mu_{H_b^+} \cdot \nu_b$	$= \sum\limits_i \mu_{H_i^+} \cdot \nu_i$	
		$\equiv \mu_{H_a^+} - \mu_{H_b^+}$	$\equiv \Delta\mu_{H^+}$
3. Activity	$RT \cdot \left(\ln a_{H_a^+} \cdot \nu_a + \ln a_{H_b^+} \cdot \nu_b \right)$	$= RT \cdot \sum\limits_i \ln a_{H_i^+} \cdot \nu_i$	
		$= RT \cdot \ln \prod\limits_i a_{H_i^+}^{\nu_i}$	
	$\equiv RT \cdot \left(\ln a_{H_a^+} - \ln a_{H_b^+} \right)$	$\equiv RT \cdot \ln \dfrac{a_{H_a^+}}{a_{H_b^+}}$	$\equiv RT \cdot \Delta \ln a_{H^+}$
4. pH	$-RT \cdot 2.3 \cdot (pH_a \cdot \nu_a + pH_b \cdot \nu_b)$	$= -RT \cdot 2.3 \cdot \sum\limits_i pH_i \cdot \nu_i$	
	$\equiv -RT \cdot 2.3 \cdot (pH_a - pH_b)$	$\equiv RT \cdot 2.3 \cdot (pH_b - pH_a)$	$\equiv -RT \cdot 2.3 \cdot \Delta pH$

Table A2. The diffusive partial protonmotive force. Variation of the cathodic matrix pH (pH_b) at constant anodic pH_a = 7.2. ΔpH ($b{\to}a$) calculated for $\nu_b = -1$. pmF_d calculated from ΔpH (Eq. 8). The total protonmotive force is pmF = 210 mV (endergonic), and the electric part is pmF_{el} = 210 mV - pmF_d.

pH_b	ΔpH	$a_{H_b^+}$	activity ratio		pmF_d		pmF_d/pmF	pmF_d/pmF_{el}
		·10^6	b/a	a/b	[kJ/mol]	[mV]	%	%
7.2	0.0	0.063	1.00	1.0	0.0	0	0	0
7.3	-0.1	0.050	0.79	1.3	0.6	6	3	4
7.4	-0.2	0.040	0.63	1.6	1.2	12	7	7
7.5	-0.3	0.032	0.50	2.0	1.8	18	10	11
7.6	-0.4	0.025	0.40	2.5	2.4	25	14	16
7.7	-0.5	0.020	0.32	3.2	3.0	31	17	21
7.8	-0.6	0.016	0.25	4.0	3.6	37	21	26
7.9	-0.7	0.013	0.20	5.0	4.2	43	24	31
8.0	-0.8	0.010	0.16	6.3	4.8	49	27	38
8.1	-0.9	0.008	0.13	7.9	5.3	55	31	44
8.2	-1.0	0.006	0.10	10.0	5.9	62	34	52