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Addressing the ambiguity crisis in bioenergetics and thermodynamics

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Summary

Effective science communication is vital for research integrity. However, ambiguities in scientific language obscure comprehension and diminish publication quality**,** akin to irreproducibility. Established ambiguous terms misguide even graphical representations of scientific knowledge. For instance, 'electron transport chain' misrepresents the electron transfer system. Electron transfer to the Q-junction converges from branched pathways rather than following a *chain* of respiratory Complexes. Complex I catalyzes H^+-linked electron *transfer* from NADH, coupled to vectorial H^+-*transport* across the coupling membrane. Analogous to NADH for Complex I, succinate but not $FADH_2$ is the substrate of Complex II. Confusion between respiratory state 2 and LEAK states, or state 3 and OXPHOS capacity impedes accurate interpretation, as does imprecise usage of uncoupled, noncoupled, or dyscoupled respiration. In the context of the vague concepts of oxidative stress and normoxia, intracellular oxygen pressure warrants attention. Beyond gas pressure, force-pressure ambiguities penetrate thermodynamics. Protonmotive pressure builds bridges to kinetics and explains the enigmatic nonlinearity between protonmotive force and proton leak flux. Please, distinguish Gibbs force from Gibbs energy. Different meanings of entropy in closed and open systems fuel contentious debates surrounding negative entropy, involving a number of prominent figures such as Erwin Schrödinger, Linus Pauling, and Max Perutz. The fundamental terms number, count, and unit are intertwined even by the International System of Units (SI). Resolving ambiguities is crucial for scientific accuracy, to counter misinformation and enhance quality in publications. Clarifying terminology in bioenergetics and thermodynamics thus becomes pivotal towards advancing knowledge and fostering informed discourse within the scientific community and beyond.

1. Introduction

In the age of public disinformation, science communication is not exempt from false representations of research findings. Ambiguities spreading through exponentially increasing numbers of publications in scientific journals [1] indicate a contemporary crisis comparable to the credibility or reproducibility crisis in the biomedical sciences and beyond [2]. Scientific disinformation in the peer-reviewed literature infiltrates textbooks, educational platforms, and social media. Countering disinformation demands a governance strategy that raises awareness of and measures against the ambiguity crisis [1].

There is a continuum between (*1*) type 1 ambiguity in the sense of double meaning, lack of conceptual clarity, looking at things from both or more sides (Figure 1) or from multiple isomorphic points of view in constructive ambiguity [3]; and (*2*) type 2 ambiguity or rather erroneous statements due to neglect of published, amply tested and reproduced knowledge [1]. Seven types of ambiguity in the history of literary criticism [4, 5] indicate the complexity spanning from ambiguity as a mere fault to constructive ambiguity in the struggle towards conceptual innovation (type 1). Empson [4] defined ambiguity as '*any verbal nuance, however slight, which gives room for alternative reactions to the same piece of language*'. In contrast, outright misinformation and propagation of conceptual confusion stretches the term ambiguity from fault to sloppy neglect (type 2).

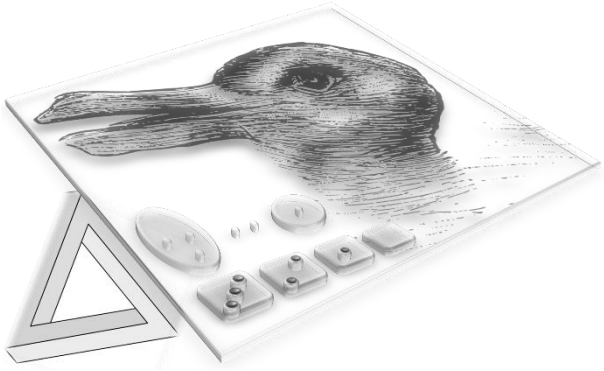

Figure 1. Graphical ambiguities. Images from *Fliegende Blätter* (1892-10-23), frames, to counting zero: Perception versus interpretation or showing versus saying (Ludwig Wittgenstein [66]), paradigm shift (Thomas S Kuhn [67]), and emotional versus logical (Daniel Kahnemann [68]).

2. Ambiguities in bioenergetics and thermodynamics

Ten case stories on ambiguities in the context of bioenergetics and thermodynamics take us to a journey of theoretical concerns linked to experimental discovery.

2.1. Electron transfer system

The commonly used term *electron transport chain* ETC contains two ambiguities. (*1*) In most contexts, the term *chain* connotates linearity. A chain typically refers to a series of sequentially connected items. Electron transfer through respiratory Complexes I and II (CI and CII), however, does not proceed through the linear sequence of a chain, but follows the convergent architecture of the electron transfer *system* ETS. In the mitochondrial ETS, multiple branches feed into the Q-junction, with downstream electron transfer through Complexes III and IV (CIII and CIV) or alternative oxidases to oxygen [6, 7]. Appreciation of the convergent architecture of the ETS paved the way towards application of physiologically relevant substrate combinations in studies of isolated mitochondria and other mitochondrial preparations [8]. With few exceptions, such substrate combinations

reveal higher respiratory capacities even if the additivity of separate pathways converging a the Q-junction is incomplete [7]. (*2*) Electron transfer is the fundamental step in redox reactions [9]. H^+-linked electron *transfer* 2{H^++e^-} in scalar chemical reactions is coupled by H^+-pumps to vectorial H^+ translocation across the mitochondrial inner membrane mtIM. Vectorial *transport* across membranes is either active with translocation through catalytic pumps or passive as diffusion, driven by the electrochemical pressure difference across cellular compartments [7]. In conclusion, the term *electron transport chain* should be replaced by *electron transfer system*.

2.2. $FADH_2$ and Complex II ambiguities

The ambiguous narrative that the reduced cofactors NADH and $FADH_2$ feed electrons from the TCA cycle into the ETS leads to erroneous graphical representations found in 312 publications (2001 to 2023), where $FADH_2$ appears as the substrate of respiratory CII [1]. In fact, succinate dehydrogenase – synonymous with CII – oxidizes succinate and reduces the covalently bound prosthetic group FAD to $FADH_2$ in the canonical forward tricarboxylic acid cycle at the entry to the membrane-bound ETS with further electron transfer to the Q-junction. When $FADH_2$ is shown free floating in the mt-matrix, a dubious role of CII in fatty acid oxidation is suggested as a consequence. In reality, the small redox protein electron transferring flavoprotein ETF mediates electron transfer between dehydrogenases of fatty acid oxidation, channeling electrons to the Q-junction but not through CII [1].

2.3. Respiratory States 2, 3, 4

The classical respiratory states 1 to 5 defined by Chance and Williams [10] refer to coupling control at an experimentally defined pathway control state, frequently restricted to NADH-linked pathways through CI, or succinate-linked pathways though CII. These respiratory states follow the ordinal numbering sequence of a specific titration protocol. To avoid ambiguities when considering alternative experimental designs, generalization is required towards a concept-driven terminology that addresses the bioenergetic meaning of respiratory states. "The focus of concept-driven nomenclature is primarily the theoretical *why*, along with clarification of the experimental *how*" [11].

State 2 – with endogenous or supplied substrates: Ambiguity persists in the meaning of State 2, which is defined originally as ADP-stimulated respiration limited by endogenous substrates [10], in contrast to a LEAK state, when respiration in the absence of phosphorylation compensates mainly for the proton leak in a set ET pathway state [11].

State 3 - high or saturating [ADP]: State 3 is originally defined as a state at 'high' ADP concentration [10], which may be kinetically limiting and thus underestimate OXPHOS capacity *P* defined at kinetically saturating ADP concentrations [11]. Failure of preventing ADP limitation of OXPHOS capacity leads to erroneous interpretations of *E-P* excess capacity. This is particularly relevant in evaluations of coupling efficiencies [12], replacing the bioenergetic use of statistically biased respiratory control ratios RCR [7] by RCR as responsible conduct of research (http://www.apa.org/research/responsible/).

2.4. Coupled and uncoupled respiration

'*Uncoupling was studied in stressed cells by measurement of coupled respiration, evaluation of uncoupled respiration after inhibition with oligomycin, and FCCP titration to quantify uncoupled respiration.*' This is not a quote from a single reference but summarizes

ambiguities in numerous publications. Uncoupling of mitochondrial respiration is a general term comprising diverse intrinsic and extrinsic mechanisms. Differences of terms — uncoupled vs. noncoupled — are easily overlooked, although they relate to different meanings of 'uncoupling'. To resolve this issue, the MitoEAGLE Task Group suggests the following definitions [11]. ***Uncoupled*** respiration is controlled by the intrinsic (physiological) conductivity of the mtIM. The intrinsic H^+ leak is the uncoupled H^+ leak current in which H^+ diffuses across the mtIM in the dissipative direction of the downhill protonmotive force *pmF* without coupling to phosphorylation. Another effective intrinsic uncoupling mechanism is Ca^{2+} influx into the mt-matrix balanced by exchange of Na^+/Ca^{2+} or H^+/Ca^{2+}, which is balanced by Na^+/H^+ or K^+/H^+ exchanges. In contrast, intrinsic ***decoupling*** is due to H^+ slip, when H^+ is only partially translocated across the mtIM and slips back to the original vesicular compartment. ***Loosely coupled*** respiration is caused by electron leak in the ETS leading to superoxide production and a bypass of redox H^+ pumps. Pathological or toxicological mitochondrial injuries may induce ***dyscoupling*** involving different mechanisms, e.g., opening the mtPT pore. Dyscoupled respiration is distinguished from experimentally induced ***noncoupled*** respiration, when a protonophore (uncoupler) is titrated to an optimum concentration to stimulate maximum respiration in the ET state. ***Acoupled*** respiration is caused by loss of mtIM integrity, when mitochondrial fragments maintain respiratory capacity without control by the *pmF*. The above sentence is then rephrased as: '*Dyscoupling was studied in stressed cells, compared to uncoupling in physiological controls, by measurement of OXPHOS capacity, evaluation of non-phosphorylating LEAK respiration after inhibition with oligomycin, and protonophore titration to quantify noncoupled respiration (ET capacity).*'

2.5. Oxidative stress

A prominent case of ambiguity in the grey zone between types 1 and 2 has been uniquely demonstrated by analysis of the popular notion of 'oxidative stress' - a term more frequently found in PubMed than 'mitochondria', widely used with vague definition and without expression by numerical values and corresponding units [13]. The production of reactive oxygen species, particularly H_2O_2, is a function of oxygen pressure [14].

2.6. Oxygen pressure in normoxia and hypoxia

Anthropocentric and clinical perspectives on hypoxia clash with an evolutionary view of life in environments of different oxygen regimes. Microenvironmental oxygenation in tissues is in stark contrast to the ambient oxygen pressure in our macroscopic environment, which we often apply uncritically in studies with isolated mitochondria or cultured cells, when ambient normoxia implies an effectively hyperoxic oxygen pressure [15-17].

2.7. The pressure-force ambiguity

Throughout the historical record of physical chemistry, pressure has been mixed up with force. Van't Hoff [18] switches between the terms osmotic pressure and osmotic force. Einstein [19] writes about "*pressure-forces*" of diffusion. Prigogine [20] concludes on "*linear relations between the rates and the affinities*" from Fick's law for diffusion, which is not a flux-force law but links diffusion flow of an uncharged substance X linearly to the concentration gradient dc_X/dz [$mol \cdot m^{-3} \cdot m^{-1}$] in direction z, and consequently to the pressure gradient which is $dp_X/dz = RT \cdot dc_X/dz$ [$J \cdot m^{-3} \cdot m^{-1}$]. As derived by Einstein [19], the

corresponding motive force is the chemical potential gradient $d\mu_X/dz$ [J·mol^{-1}·m^{-1}] [7]. Differences of gas pressure, osmotic pressure, diffusion pressure, and protonmotive pressure are isomorphic pressures $\Delta_{tr}\Pi$, uniformly expressed in the SI unit pascal [Pa = J·m^{-3}] and linearly related to the conjugated flows beyond near-equilibrium states. The corresponding isomorphic (generalized) motive forces $\Delta_{tr}F_X$ correlate linearly with flows only near equilibrium. They have varying units [J·MU^{-1}], depending on the motive quantity Q_X with motive unit [MU] that defines the flow or advancement per unit of time in the process of energy transformation tr. The motive quantity is volume in volume flow [MU = m^3], amount of substance [MU = mol] in osmotic and diffusional flow, or charge [MU = C] in electric flow. Protonmotive flow can be expressed equivalently in chemical terms of amount of substance [mol·s^{-1}] or electrical terms of charge, i.e. current [C·s^{-1} = A]. Ambiguities in the terms current, flow, or flux should and can be removed by rigorous definition [7, 11, 21]. Importantly, a chemiosmotic pressure difference is non-linearly related to the electrochemical potential difference [7], and they should not be considered as equal: "*.. the coupling membrane has a low permeability to ions generally and not only to protons, so that the electron transport and ATPase systems could be coupled through the sum of the electrical pressure difference and the osmotic pressure difference (i.e. the electrochemical potential difference) of protons that would thus be conserved across the membrane*" [22].

As a special case among all flow-force and force-pressure relationships, force and pressure are identical in Maxwell's gas equation. Pressure-volume work (exergy, $d_V W$) equals $p \cdot dV$ [Pa·m^3 = J]. The motive force of volume expansion/compression equals exergy per advancement, $\Delta_V F = \partial G/\partial_V \xi$. "*This force is called the pressure of the gas*", p [J·m^{-3} = Pa] [23]. In physics, work [J] is the product of force [N] and distance [m]. Therefore, force [N = J·m^{-1}] (compare electric force [V = J·C^{-1}]) is clearly distinguished from mechanical pressure [Pa = N·m^{-2} = J·m^{-3}]. The forces of physics are vectors in continuous systems described by gradients. Instead of *vectoral* potential *gradients*, *vectorial* potential *differences* are the isomorphic motive forces in compartmental or discontinuous systems, in which subsystems are separated by a membrane without consideration of membrane thickness. Diffusion d across a membrane of an uncharged dissolved substance X at high dilution is a linear function of the chemical pressure difference, $\Delta_d \Pi_X = RT \cdot \Delta_d c_X$ [Pa]. Therefore, the corresponding flow-force relationship cannot be linear, except in the trivial near-equilibrium range. In turn, the chemiosmotic or protonmotive force pmF cannot theoretically be expected to be linearly related to proton leak flow. The concept of protonmotive pressure is required instead, recognizing any motive pressure difference as the product of a vectorial motive force (based on concentration ratios) and a concentration or free activity term [7].

The ambiguous use of the terms force and pressure has deep consequences on the enigmatic concept of non-ohmic flux-force relationships in the context of mitochondrial membrane potential and the protonmotive force (non-linear) versus protonmotive pressure (linear). Flow-pressure relations provide the theoretical link between nonequilibrium thermodynamics adhering to flow-force relations and kinetics [7, 24].

2.8. Gibbs energy or Gibbs force?

> *The remarks on* negative entropy *have met with doubt and opposition from physicist colleagues. Let me say first, that if I had been catering for them alone I should have let the discussion turn on* free energy *instead. It is the more familiar notion in this context. But this highly technical term seemed*

linguistically too near to energy *for making the average reader alive to the contrast between the two things.* Erwin Schrödinger (1967) [25]

The ambiguous use of the term Gibbs (free) *energy* rather than Gibbs *force* [J·mol^{-1}] is solidly fixed in textbooks of physical chemistry [26]. In contrast to the extensive quantity energy [J], the Gibbs force of reaction, $\Delta_r F_X = \partial G/\partial_r \xi_X$, is an intensive quantity. The partial derivative of Gibbs energy ∂G [J] per advancement of reaction $\partial_r \xi_X$ [mol] is an isomorphic motive force of thermodynamics (Figure 2) [7, 20, 21].

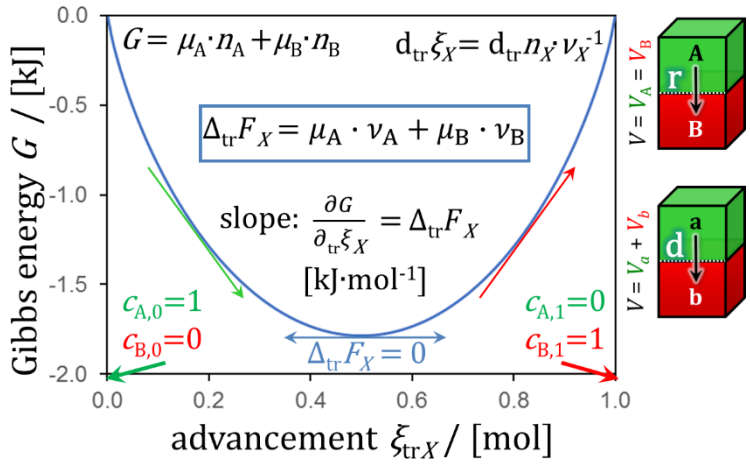

Figure 2. Gibbs energy as a function of advancement of transformation in a closed isothermal system at constant pressure. Isomorphic forces $\Delta_{tr}F_X = \partial G/\partial_{tr}\xi_X$ are the slope of Gibbs energy (exergy) of the system per advancement. For transformation tr=r: advancement of reaction in a homogenous volume V=1 L. For tr=d: advancement of diffusion of an uncharged substance between homogenous compartments, a→b, of equal volumes, V_a=V_b=1 L. Equilibrium constants are K_r = 1 and K_d = 1. At a negative slope, the force $\Delta_{tr}F_X$ is negative such that the transformation is exergonic and proceeds spontaneously in the forward direction, A→B. Equilibrium is obtained at the minimum of Gibbs energy, when $\Delta_{tr}F_X$=0. The stoichiometric numbers ν_X are ν_A=-1 and ν_B=1. Concentrations c_X are considered to be numerically equal to activities $a_X \equiv c_X/c°$, where $c°$ is the standard concentration of 1 mol·L^{-1}. Modified after Gnaiger (2020) [7].

According to the IUPAC definition, the affinity of reaction, A [J·mol^{-1}], equals the negative molar Gibbs energy of reaction [27]. The concept of *affinity* and hence chemical force is deeply rooted in the notion of *attraction* (and repulsion) of alchemy which was the foundation of chemistry originally, but diverted away from laboratory experiments towards the occult [28]. Newton's extensive experimental alchemical work and his substantial track record on alchemy is recognized today as a key inspiration for his development of the concept of the gravitational force [29-31]. This marks a transition of the meaning of affinity, from the descriptive 'adjacent' (proximity) to the causative 'attractive' (force) [32]. Correspondingly, Lavoisier [33] equates affinity and force: "*... the degree of force or affinity with which the acid adheres to the base*". By discussing the influence of electricity and gravity on chemical affinity, Liebig [34] considers affinity as a force. This leads to Guldberg and Waage's mass action ratio ('Studies concerning affinity', 1864; see [32], the free energy and chemical affinity of Helmholtz [35], and chemical thermodynamics of irreversible processes [36, 37], where flux-force relations and the dissipation function are center stage [20, 38].

Living organisms are open systems, exchanging energy and matter with the environment across their boundaries [39]. How can concepts of classical thermodynamics of closed systems (Figure 2) be applied to the bioenergetics of open systems and irreversible processes? Classical treatments of thermodynamic principles benefit from the

simplifications of describing reversible and irreversible processes in closed systems (Figure 2). However, when consistent applications of these concepts to open systems are ignored and combined with erroneous terminology, false conclusions emerge in bioenergetics when purporting that "classical equilibrium thermodynamics cannot be applied precisely to open systems because the flow of matter across their boundaries precludes the establishment of a true equilibrium" [40]. Consider advancement per time as metabolic flow in a defined process (internal transformation tr) or of all internal processes related to compound X (internal transformations i), $d_i\xi_X/dt = (d_i n_X \cdot \nu_X^{-1})/dt$. Then a closed system allows for measurement of metabolic flow by monitoring $d_i n_X$ in terms of changes of the system variable dn_X (Figure 2). Exchange of matter across the system boundaries, $d_e n_X$, is zero in a closed system. In summary,

closed system	$dn_X = d_i n_X$	$d_e n_X = 0$ mol	Eq. 1
open system, general	$dn_X = d_i n_X + d_e n_X$		Eq. 2
open system, steady state	$d_i n_X = -d_e n_X$	$dn_X = 0$ mol	Eq. 3

When a transformation tr is the single process in a closed system (at constant temperature and pressure), there is a continuous trajectory of Gibbs energy G towards equilibrium reached at $\Delta_r F_X = \partial G/\partial_r \xi_X = 0$ kJ (Figure 2). Under these conditions, an uphill progression to increasing G is impossible, which is a specific form of the entropy law of thermodynamics (Section 2.9). In an open system studied at steady state, a single data point is obtained on the function plotted as a continuous curve for the closed system. Then it is necessary to perturb the system, transitioning to a different steady state along the axis of advancement (Figure 2). Thus distinct steady-states are analyzed to obtain multiple data points along the trajectory. It is easy to apply this concept to design experimental protocols supported by appropriately designed instruments [17, 41]. Different styles of thinking, however, separate minds with a focus on equilibrium states in closed systems from pioneers of the dynamic view of open systems maintained away from and even evolving further away from equilibrium, as discussed in the next section.

2.9. Negative entropy – 80 years after Erwin Schrödinger's *What is Life?*

> *Every process, event, happening - call it what you will; in a word, everything that is going on in Nature means an increase of the entropy of the part of the world where it is going on. ... What an organism feeds upon is negative entropy. Or, to put it less paradoxically, the essential thing in metabolism is that the organism succeeds in freeing itself from all the entropy it cannot help producing while alive.* Erwin Schrödinger (1944) [39]

A number of scientists with profound background in thermodynamics – including Nobel laureates in chemistry and physics – disagree with Erwin Schrödinger expanding on the negative entropy concept of Ludwig Boltzmann [42], that the living organism *"feeds upon negative entropy, attracting, as it were, a stream of negative entropy upon itself, to compensate the entropy increase it produces by living and thus to maintain itself on a stationary and fairly low entropy level"* [39]. Schrödinger was attacked by Linus Pauling [43] and Max Perutz [44] in that his "*thermodynamics is vague and superficial to an extent that should not be tolerated even in a popular lecture*". This is probably the most prominent case of ambiguity in the 'hard science' of physical chemistry, resulting not merely from trivial sloppy terminology on account of Schrödinger's opponents, but resonating different styles of thinking in the thermodynamics of irreversible processes [45] and classical thermodynamics [26, 27]. It would be ridiculous to assume that the 'negentropy' ambiguity reflects an incompleteness of

the fundamental concept of entropy, or that these proponents on either side of the controversy lack understanding of the concept. The actual disparity is not specifically rooted in irreversibility, but in the complexity encountered when describing processes in open systems [46].

(*1*) Entropy production: *".. increase of the entropy of the part of the world where it is going on"* – This is the internal entropy production in irreversible processes, $d_iS > 0$ J·K^{-1}, a general expression of the Second Law of Thermodynamics [20]. Locally ("*the part of the world where it is going on*"), entropy increases and energy available for work (exergy; Gibbs energy at constant temperature and pressure) is dissipated as $-T \cdot d_iS$ [J] (Figure 3). This is simple at constant temperature T (isothermal conditions; $dT=0$). In general, T is replaced by the lowest temperature of the external heat sink, T_e [K], relevant for heat exchange [21]. The local increase (production) of entropy can only be compensated by external entropy exchange in open systems.

(*2*) External entropy exchange in open systems: ".. *the organism succeeds in freeing itself from all the entropy it cannot help producing while alive*" – External entropy d_eS is transmitted across the boundaries of the system 'organism'. In an open system at steady state (when the entropy change of the system is $dS = 0$ J·K^{-1}), $d_iS = -d_eS$ (compare Eq. 3). "*What an organism feeds upon is negative entropy*" – this includes the exchange in the form of matter, $d_{mat}S$. The dissipated energy is compensated by $-T \cdot d_eS$, the sum of heat d_eQ and the entropic term $T \cdot d_{mat}S$ reversibly exchanged across the system boundaries (Figure 3).

(*3*) Entropy change of a closed isolated system: "*Simon pointed out .. that 'The reactions in the living body are only partly reversible and consequently heat is developed of which we have to get rid to the surroundings. With this irreversibly produced heat also flow small amounts (either + or -) of reversibly produced heat ($T\,\Delta S$), but they are quite insignificant and therefore cannot have the important effects on life processes which you assign to them'*" [44]; see [25]. The "*reversibly produced heat*" ($T\,\Delta S$) refers to the chemist's favourite thermodynamics of closed isothermal systems (ci; Figure 3). Strictly, *heat* in thermodynamics is exclusively an external term, d_eQ, hence the subscript e is merely written for clarification and can be omitted without change of meaning (except when temperature gradients are considered in continuous systems).

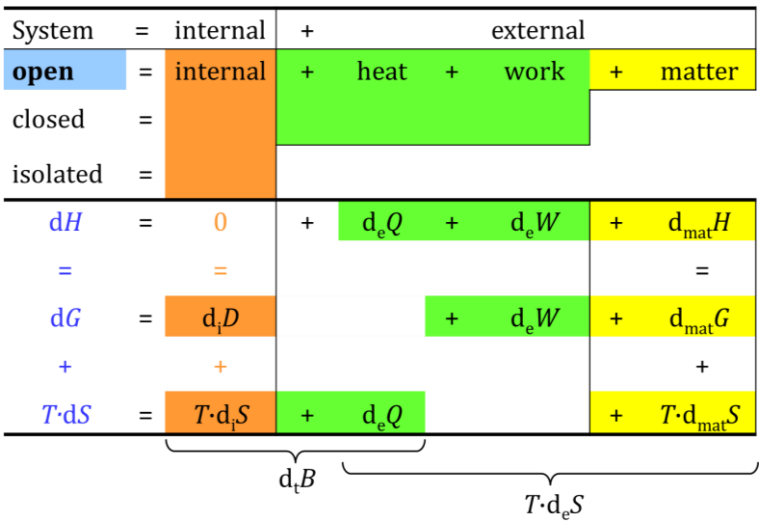

Figure 3. Thermodynamic balance equations for enthalpy, Gibbs energy and entropy, arranged according to the Gibbs equation ($dT=0$; $dp=0$). The changes of the system, $dH = dG + T \cdot dS$, are zero at steady state. The internal terms are the sources, with $d_iH = 0$ kJ (conservation law) and $d_iD = 0$ kJ at equilibrium. The external terms are zero in isolated systems. Changes due to translocation of matter are zero in closed and isolated systems. Modified after Gnaiger (1994) [45].

Confusion arises from the ambiguous use of the term of entropy without sufficient distinction in different contexts of open versus closed systems and irreversible versus reversible transformations (Figure 3). Notably, this contrasts with the introduction of different terms and symbols which help to distinguish between Helmholtz and Gibbs energy change, dA and dG = dA-d$_V W$, and internal energy and enthalpy change, dU and dH = dU-d$_V W$, under isovolumetric (dV = 0 m^3) and isobaric (dp = 0 kPa) conditions, respectively. An analogous linguistic discrimination between bound energy dB and dissipated energy dD helps to resolve the confusion between opposite meanings of various forms of entropy [21, 45].

(*1*) Bound energy change of a closed isothermal system, dB = T·dS. The heat exchanged reversibly between a closed system and the heat sink at temperature T is the *bound energy transformation* d$_t B$ not available for work. At equilibrium, d$_t B$ = d$_e Q$. The term d$_t B$ = T·d$_i S$+d$_e Q$ [21] combines the internal dissipative term T·d$_i S$ and the external term d$_e Q$ (Figure 3). The subscript t indicates the sum total of all simultaneous energy transformations. But the fundamental equation for energy transformations is applicable for each partial transformation tr [21],

$$\mathrm{d_{tr}}H = \mathrm{d_{tr}}G + T\cdot\mathrm{d_{tr}}S \stackrel{\text{def}}{=} \mathrm{d_{tr}}G + \mathrm{d_{tr}}B \qquad \text{Eq. 4}$$

The beauty of closed isothermal systems is that the changes of system variables (state variables) equal the energy transformations, T·ΔS(ci) $\stackrel{\text{def}}{=}$ ΔB = Δ$_t B$. Thus dB = T·dS(ci) is fundamentally different from the negative entropy exchange T·d$_e S$ in the context of open systems considered by Boltzmann and Schrödinger (Figure 3).

(*2*) Dissipative exergy change of a closed isothermal system, dD = dG in the absence of external work, d$_e W$ = 0 kJ. Under reversible conditions at 100 % efficiency, d$_i D$ = 0 kJ and the Gibbs energy change dG is fully converted to external work d$_e W$, which indicates the maximum obtainable work, dG = d$_e W_{max}$. Irreversible internal exergy dissipation, d$_i G$ = -T·d$_i S$ $\stackrel{\text{def}}{=}$ d$_i D$, is integrated over all energy transformations at constant temperature and pressure. Then we find the concept of 'negentropy' together with Schrödinger's comment to "*let the discussion turn on* free energy *instead*", in the equation (Figure 3),

$$\text{steady state, } \mathrm{d_e}W = 0\ \mathrm{J} \qquad \mathrm{d_{mat}}G = -T\cdot\mathrm{d_i}S \stackrel{\text{def}}{=} \mathrm{d_i}D \qquad \text{Eq. 5}$$

The dynamic style of thinking considers entropy flows across the open system boundaries, including exchanges of matter: *"How does the living organism avoid decay? The obvious answer is: By eating, drinking, breathing and (in the case of plants) assimilating"* [39]. The negative entropy ambiguities are resolved [45] by explanation of different meanings of entropy changes pertaining to open and closed systems, internal transformations, and external transfer (Figure 3). Recognition of clarity in Schrödinger's communication even in a popular science book complements the large number of reviews on the science and life of Erwin Schrödinger, which consider particularly his contributions to quantum biology and molecular biology [47-54].

2.10. Count and number – the elementary unit of the count

Terms for quantities with a number of different meanings require explanation, taking into account the benefit of disambiguous scientific language and explicit units of quantities. We count on the value of accuracy. 'We number on the value of ..' - uuups, this does not make sense. We have an apparently intuitive understanding with a long historical record [55] of the difference between *count* and *number* (German *Anzahl* and *Zahl*). However, the International System of Units (SI) suggests that "quantities relating to

counting .. are just numbers" (SI p.151). Why does the SI give to a *count* the *unit* of a *number*? References to Bureau International des Poids et Mesures (2019) [56] are abbreviated here as (SI p.*N*), showing the page number *N*, not the count.

Grounding abstract units in fundamental constants of the SI marks the revolutionary departure from the previous reliance on material artefacts. For instance, the SI unit kilogram [kg] has been defined by the International Prototype of the Kilogram held in Paris, but is now fixed by numerical values of the Planck constant, the speed of light in vacuum, and the hyperfine transition frequency of Cs (SI p.131). However, there remains a fundamental ambiguity to be resolved in defining the unit of the quantity *count* in the SI. The notion that some quantities *Q* "cannot be described in terms of the seven base quantities of the SI, but have the nature of a count .. with the associated unit one" (SI p.136) causes a mix-up between count, number, and unit. Such incoherence is unexpected, considering that the revision of the SI is probably the most fundamental and formal publication in the entire scientific literature.

Protein mass, cell mass, body mass are extensive quantities expressed in the SI unit [kg]. Cell mass is wet or dry *mass*, but not *weight*, since "the weight of a body is the product of its mass and the acceleration due to gravity" (SI p.159). Apart from "the necessity to put an end to the ambiguity which in current practice still exists on the meaning of the word *weight*, used sometimes for *mass*, sometimes for *mechanical force*" (SI p.159), a distinction must be made between the mass m_X 'of' a sample of type *X* and the mass M_X 'per' unit entity U_X. If cell mass m_{ce} [kg] is the mass *of cells*, ce, in an experimental chamber containing a number of cells N_{ce} [x], then the mass *per cell* is $M_{ce} = m_{ce} \cdot N_{ce}^{-1}$ [kg·x^{-1}]. The number of cells is the cell count N_{ce} [x].

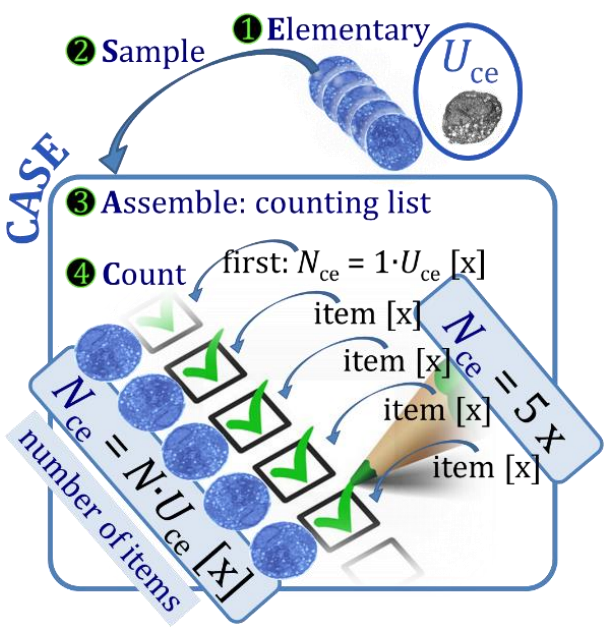

Figure 4. Counting: count, assembly, sample, elementary (CASE). The elementary entity cell, U_{ce}, is the material unit of the cell count N_{ce}, expressed in the abstract unit [x] with the meaning 'one item'. Cells are a subset of entities *X* which are countable objects. A sample of cells can be expressed as a *count*, the number of single individual items. The elementary entity U_{ce} is the specific *material* unit (Euclidean unit) of a cell count. The single individual cell defines the elementary U_{ce}. A count $N_X = N \cdot U_X$ equals the number of U_X. *X* must represent the same entity in both occurrences. An elementary entity U_X is not a count (U_X is not a number of U_X). Both quantities N_X and U_X have the same abstract unit, the 'elementary unit' [x]. The cell count N_{ce} is an elementary quantity obtained by counting the number *N* of cells U_{ce}, item after item. From Gnaiger (2020) [7].

Counting involves multiple steps (Figure 4). (*1*) Before counting can begin, the **elementary entity** U_X is specified as the *entetic* unit (Euclidean unit), representing the type of event or object. A unit is defined as 'a single individual thing' in Euclid's *Elements, Book*

VII [57]. The *real unit* U_X is a single countable elementary entity of entity-type *X* (thing) with numerical value one. If *X* is 'cells' ce, then U_{ce} is the unit cell. (*2*) **Sampling** is required to collect items for transfer to the 'counting table'. This may be the pipetting of cells onto a microscope slide to be used for cell counting. (*3*) The counting list must be prepared for **assembling** items on the counting table. The microscope slide is fixed on the microscope stage. (*4*) Finally, **counting** entails adding sequentially item after item into the counting list yielding the count $N_X = N \cdot U_X$ [x]. Count is the number of items (likewise entities). A count N_X = 5 x can be spelled out as 'five times' (Figure 4). In common language, a cell count concentration $N_{ce} \cdot V^{-1}$ of $5 \cdot 10^6$ x·mL^{-1} is said to be 'five million cells per milliliter'. This divides, however, a real thing (cells) by an abstract unit [mL]. The inconsistency is solved by introducing the 'elementary unit' [x] as the *abstract unit* of any count, which is also the abstract unit of the real unit U_x. Then a cell count concentration of $5 \cdot 10^6$ x·mL^{-1} can be expressed as 5 Mx·mL^{-1}, using the prefix mega for 10^6.

The number of different types of specified events and types of countable objects U_X is practically unbounded, as is the vast number of entity types that can be expressed in the unit mole [mol]. In contrast, there is a single abstract unit either for the count [x] or amount [mol]. The *abstract unit* of a count is the exclusive elementary unit [x] – applicable to all kinds of count and missing in the SI [11, 58]. The unit [x] does not specify if an event is simultaneous, random or periodic in time, or if the entity oxygen is atomic O or molecular O_2. Consistent with the fact that it "is important to give a precise definition of the entity involved" (SI p.134) for amount of substance (with unit 'mole'), the specific entity of a count (with unit 'elementary unit') must be defined separately from the unit [x]. Count concentration C_X (with the ambiguous IUPAC term 'number concentration' [27], amount of substance concentration c_X, charge density ρ_{el}, mass density ρ_X, and volume fraction Φ_X of entity *X* in a total volume *V* are then expressed in consistent units of [x·m^{-3}], [mol·m^{-3}], [C·m^{-3}], [kg·m^{-3}], and [m^3·m^{-3}], respectively. The quantity count is unique, dependent on quantization U_X of entities *X*, with a minimum value of N_X = 1 x. However, if one accepts a *zero* count without item to be counted (Figures 1 and 4), then *counting* numbers are equivalent to *natural* or *whole* numbers, represented by the numerals 0, 1, 2, 3, 4, 5, ..

The International System of Units defines: "The value of a quantity is generally expressed as the product of a number and a unit. The unit is simply a particular example of the quantity concerned which is used as a reference, and the number is the ratio of the value of the quantity to the unit" (SI p.127). Applying this definition to the count, the value of the count (with abstract unit x) is divided by the entetic unit U_X (with abstract unit x), such that the abstract unit [x] cancels in the definition of a number $N = N_X \cdot U_X^{-1}$. The SI definition suggests that a *number* cannot be the *unit* of a quantity, since a number – such as the number with name *one*, symbol 1 – is not a "particular example of the quantity concerned". Neither can the value of a count be expressed simply as a number, but "as the product of a number and a unit". The symbol of a count or "number of specified elementary entities" is N_X, defined for entity *X* as $N_X = N \cdot U_X$, with dimensionless "number" *N* (without subscript) and elementary unit [x] [58]. Unfortunately, the SI contradicts its own definition: "Counting quantities are also quantities with the associated unit one" (SI p.136) and ".. values of quantities with unit one, are expressed simply as numbers. The unit symbol 1 or unit name "one" are not explicitly shown (SI p.151).

Counting and measuring are the two fundamental methods to quantify anything – any entity *X* or sample type *X*. Counting and measuring may be considered as the most basic concepts in experimental science. The *size* of something is quantified by *measuring*

extensive quantities such as mass m_S [kg] or volume V_S [m³] of the sample S (Figure 5a). The *value* of a quantity Q_u is the product of a number N and an *abstract* unit u_Q. Similarly, the *value* of a count N_X is the product of a number N and the *abstract* unit x. A *count* N_X is the number of individual items U_X (Figure 5b). Thus counting is equivalent to enumeration [59]. A count of *events* results from monitoring repetitions of identical occurrences observed simultaneously or sequentially in time. Countable *objects* are discrete individual items in space, in contrast to continuous quantities that cannot be counted but are measured. Count N_X, amount n_X, and charge Q_{el} are the three elementary quantities Q_X of countable, discrete entities. Irrespective of the fact that amount and charge are practically obtained by conversion of primary measurements rather than by direct counting, the value of an elementary quantity Q_X is a count N_X multiplied by a defining SI constant d_{SI} (Figure 5b). Subscripts Q and X for 'quantity-type' and 'entity-type', respectively, point to the contrasting meanings of the two fundamental definitions of an *abstract* versus *entetic* 'unit'. The ambivalent term 'unit' with its dual meanings is used and confused in practical language and the scientific literature. In the elementary entity U_X, the unit (the 'one') relates to the entity-type X, to the single individual thing (individual or undivided; the root of the word *thing* has the meaning of 'assembly'). In contrast to counting, a unit u_Q is linked to the measurement of quantities $Q_u = N \cdot u_Q$, such as volume, mass, energy. These quantities — and hence the units u_Q — are abstracted from entity-types, pulled away from the world of real things. This should resolve the confusion regarding the distinction between entities and units [52, 59]. The new SI [56] has completed the total abstraction of units, from the previous necessity to not only provide a quantitative definition but also a physical realization of a unit in the form of an 'artefact', such as the International Prototype of the Kilogram. The new definitions of the base SI units are independent of any physical realization: u_Q is separate from X. In agreement, the unit x is separate from the nature of countable entities X.

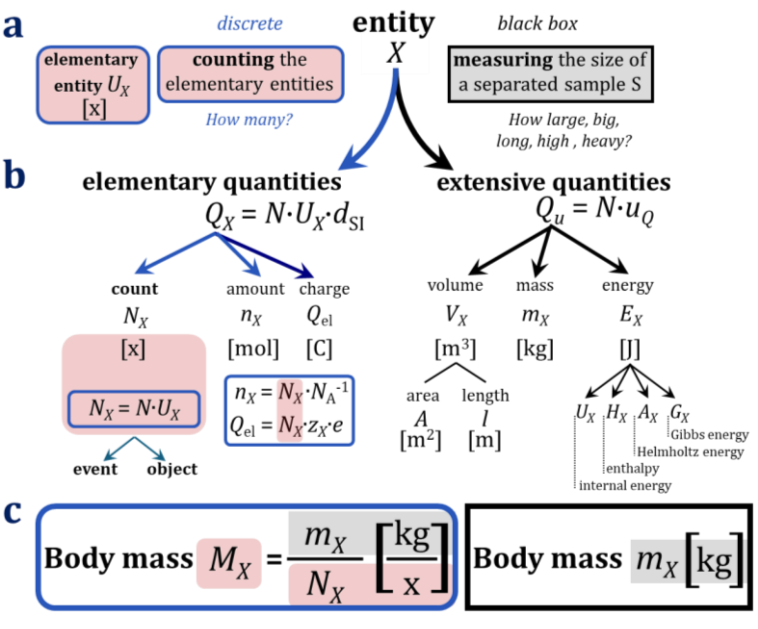

Figure 5. Elementary quantities linked to counts by defining SI constants (left) and extensive quantities based on measurements expressed in SI units (right). (**a**) The elementary entity U_X is the specific material unit (Euclidean unit) of a count. The single individual occurrence of a discrete entity X defines the U_X. Measuring the size of a sample is a black-box approach without counting. (**b**) All elementary quantities Q_X are defined as a count N_X multiplied by a defining SI constant d_{SI}. d_{SI} equals one for the count, $d_{SI} = N_A^{-1}$ for amount, and $d_{SI} = z_X \cdot e$ for charge. Quantities Q_u are expressed in *abstract* units u_Q, applicable to any kind of sample of objects X. (**c**) Elementary mass M_X is the extensive quantity mass m_X normalized for the count N_X. Modified after Gnaiger (2021) [58].

In addition to measuring the mass m_{ce} of a pure sample of cells, cell counting is required to obtain information on the individual elementary cell mass M_{ce} (Figure 5c). When measuring the body mass m_B *of an individual*, the black box of a mass balance (Figure 5a) must be opened to obtain the additional information on the count N_B = 1 x. Then the elementary body mass or the mass *per individual* is $M_B = m_B \cdot N_B^{-1}$ [kg·x^{-1}]. m_B may refer to a group of individuals. Therefore, the elementary unit [x] does not imply that N_B = 1 x. However, if the abstract unit of the count is taken as "one, symbol 1" (SI p.129), and "is rarely explicitly written" (SI p.136), then m_X and $m_X \cdot N_X^{-1}$ have the same unit [kg] in the SI. This prevents an explicit distinction between the units [kg] and [kg·x^{-1}] of the extensive and elementary quantities, m_X and M_X, respectively (Figure 5c). Confusion with the symbol M for molar mass [kg·mol^{-1}] [27] must be carefully avoided by using – if necessary – the more explicit symbol $M_{U_{ce}}$ for the elementary mass [58].

Two of the seven fundamental SI constants, N_A and e, relate the count to the other elementary quantities, amount and charge: (*1*) "The Avogadro constant N_A is a proportionality constant between the quantity amount of substance (with unit mole) and the quantity for counting entities (with unit one, symbol 1)" (SI p.129), and N_A is "expressed in the SI unit mol^{-1}" (SI p.191). (*2*) The elementary charge e relates charge to count (Table 1). In the SI, charge Q_{el} and elementary charge e are expressed in the same unit coulomb [C] despite the fact that e is Q_{el} per proton [C·x^{-1}] (Table 1). The SI units for N_A [mol^{-1}] and e [C] obscure entirely the distinction between *division* by [x] in e [C·x^{-1}] in contrast to *multiplication* by [x] in N_A [x·mol^{-1}].

Table 1: Fundamental physical constants defining relationships between the units of the elementary quantities count [x], amount [mol] and charge [C]. From Gnaiger (2020) [7].

Fundamental constant	Definition	Numerical value	Unit
Boltzmann constant*	$k = f \cdot e = R/N_A$	= 1.380 649·10^{-23}	J·x^{-1} ·K^{-1}
gas constant	$R = f \cdot F = k \cdot N_A$	= 8.314 462 618	J·mol^{-1} ·K^{-1}
electromotive constant	$f = k/e = R/F$	= 8.617 333 262·10^{-5}	J·C^{-1} ·K^{-1}
Avogadro constant*	$N_A = R/k = F/e$	= 6.022 140 76·10^{23}	x·mol^{-1}
elementary charge*	$e = k/f = F/N_A$	= 1.602 176 634·10^{-19}	C·x^{-1}
Faraday constant	$F = R/f = e \cdot N_A$	= 96 485.332 12	C·mol^{-1}

* Redefinition came into force on 2019-05-20; Bureau International des Poids et Mesures (2019) The International System of Units (SI). 9th edition [56].

The Boltzmann constant times absolute temperature is the exergy quantum per particle, kT [J·x^{-1}], linked to the gas constant by N_A, $RT = kT \cdot N_A$ [J·mol^{-1}]. The electromotive constant times T, $fT = RT/F$ [J·C^{-1}], links exergy to charge, where F is the Faraday constant (Table 1). Thus k, R, and f are the three fundamental constants in the formats of count, amount, and charge with the associated units [J·x^{-1}·K^{-1}], [J·mol^{-1}·K^{-1}], and [J·C^{-1}·K^{-1}] (Gnaiger 2020). They are related as $k/f = e$ and $R/f = F$ (Table 1). Surprisingly, the electromotive constant $f = R/F$ remained unnoticed in thermodynamics. This means that f is written explicitly as the ratio of two constants R/F, as in the Nernst equation applied to the partial electric potential difference $\Delta\Psi_{H^+}$ generated by the distribution of H^+ (related to ΔpH) between the positive anodic and negative cathodic compartments a and b,

$$\Delta\Psi_{H^+} = \frac{1}{z_{H^+}} \cdot \frac{RT}{F} \cdot \ln \frac{c_{H^+}{}_b}{c_{H^+}{}_a} = \frac{1}{z_{H^+}} \cdot fT \cdot \ln \frac{c_{H^+}{}_b}{c_{H^+}{}_a} \quad \text{Eq. 6}$$

Count N_X and number N are confused in the SI: "Amount of substance, symbol n, is defined to be proportional to the number of specified elementary entities N in a sample" (SI p.151). "The mole, symbol mol, is the SI unit of amount of substance. One mole contains exactly 6.022 140 76 × 10^{23} elementary entities. This number is the fixed numerical value of the Avogadro constant, N_A, when expressed in the unit mol^{-1} and is called the Avogadro number" (SI p.134). A number N of elementary entities X is a count N_X. Hence reference to the count as this 'number' is ambiguous, linked to the lack of distinguishing the dimensionless number N (without unit) from the count N_X (with unit [x]).

Concerns about incoherence of the SI due to the missing unit of the count are not new [60]. The proposal to apply distinct 'unit symbols' for events [evt] (number of counts [cnt], number of decays [dcy]) and entities [ent] (number of molecules [mcl], number of atoms [atm], number of particles [pcl] [61] does not appreciate the concept of an *abstract* elementary unit [x] in contrast to a *realized* elementary entity U_X as a representative real example. Frequency is a number of periodic or random events per unit of time. The corresponding unit hertz is defined here in terms of the number of specified events per second, Hz = x·s^{-1}. According to Mohr and Phillips [61], the SI-definition Hz = s^{-1} should be replaced by 1 Hz = 1 cnt·s^{-1} = 1 cyl·s^{-1} = 2 π·rad·s^{-1}. Counts (cnt), cycles (cyl), or 2π radians (2 π·rad), however, are not abstract units, but are types of elementary entities. Such a mixture of description of an entity with abstract SI units should be avoided [62]. Therefore, O_2 consumption per cell is expressed in units [amol·s^{-1}·x^{-1}] but not as amol O_2·s^{-1}·$cell^{-1}$ [11].

3. Reproducibility and credibility

> *The integrity of knowledge that emerges from research is based on individual and collective adherence to core values of objectivity, honesty, openness, fairness, accountability, and stewardship. Integrity in science means that the organizations in which research is conducted encourage those involved to exemplify these values in every step of the research process. Understanding the dynamics that support – or distort – practices that uphold the integrity of research by all participants ensures that the research enterprise advances knowledge.*
>
> National Academies of Sciences, Engineering, and Medicine (2017) [63]

Reproducibility assumes an undesirable meaning in the context of the ambiguity crisis. The more frequently an ambiguous concept is uncritically reproduced (copied) in vast numbers of publications, the more firmly such ambiguous or even false narratives become established. Once ambiguities are imprinted as scientific fashion, we may either follow the crowd or raise fundamental arguments.

The erosion of public trust in science intensifies when even scientists find it challenging to rely on the scientific literature. To combat this escalating issue, scientists must unite to spotlight ambiguities within their own field. Beyond physical disease outbreaks, a scientific infodemic spreads with the exponential increase of insufficiently quality-controlled publications [64]. Institutions – such as National Academies and International Scientific Societies – spearhead initiatives to combat the ambiguity crisis [65]. Committees ought to be established to identify ambiguity hotspots, publish guidelines to address specific ambiguities, and prompt journal editors to rectify ambiguities post-publication when peer review falls short. Adhering to the principles of

'*Addressing the ambiguity crisis*' can bestow a quality management label on scientific journals. This serves as an effective measure to safeguard the integrity of the invaluable work of the scientific community, wielding awareness, and implementing a proactive deterrent against the uncontrolled spread of ambiguities or disinformation into educational materials and social media.

Abbreviations

CI to CIV	respiratory Complex I to IV	OXPHOS	oxidative phosphorylation
ETS	electron transfer system	*pmF*	protonmotive force
mtIM	mitochondrial inner membrane	Q	ETS-reactive coenzyme Q

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