MitoFit Preprints 2023.3.v5
https://doi.org/10.26124/mitofit:2023-0003.v5
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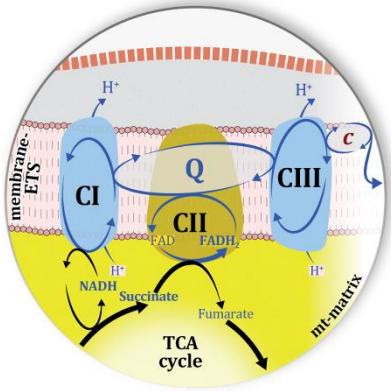
Theoretical Communication

Cite
Gnaiger E (2023) Complex II ambiguities — $FADH_2$ in the electron transfer system. MitoFit Preprints 2023.3.v5. https://doi.org/10.26124/mitofit:2023-0003.v5



Conflicts of interest
EG is editor-in-chief of *Bioenergetics Communications*.

Online v1 2023-03-24
Online v2 2023-04-04
Online v3 2023-05-04
Online v4 2023-05-12
Online v5 2023-05-31

Keywords
coenzyme Q junction, Q-junction
Complex II, CII
electron transfer system, ETS
fatty acid oxidation, FAO
flavin adenine dinucleotide, $FADH_2$/FAD
nicotinamide adenine dinucleotide, NADH/NAD^+
succinate dehydrogenase, SDH
tricarboxylic acid cycle, TCA

Complex II ambiguities — $FADH_2$ in the electron transfer system

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Summary

The prevailing notion that reduced cofactors NADH and $FADH_2$ transfer electrons from the tricarboxylic acid cycle to the mitochondrial electron transfer system creates ambiguities regarding respiratory Complex II (CII). The succinate dehydrogenase subunit SDHA of CII oxidizes succinate and reduces the covalently bound prosthetic group FAD to $FADH_2$ in the canonical forward tricarboxylic acid cycle. However, several graphical representations of the electron transfer system depict $FADH_2$ in the mitochondrial matrix to be oxidized by CII. This leads to the false conclusion that $FADH_2$ from the β-oxidation cycle in fatty acid oxidation feeds electrons into CII. In reality, dehydrogenases of fatty acid oxidation channel electrons to the coenzyme Q-junction but not through CII. The ambiguities surrounding Complex II in the literature and educational resources call for quality control, to secure scientific standards in current communications of bioenergetics, and ultimately support adequate clinical applications. This review aims to raise awareness of the inherent ambiguity crisis, complementing efforts to address the well-acknowledged issues of credibility and reproducibility.

1. Introduction

Current studies on cellular and mitochondrial bioenergetics sparked a new interest in the tricarboxylic acid (TCA) cycle — the citric acid cycle or Krebs cycle (Krebs, Eggleston 1940; Gnaiger et al 2020; Bénit et al 2022; Arnold, Finley 2023). TCA cycle metabolites are oxidized while reducing NAD^+ to NADH+H^+ in the forward cycle, or are transported into the cytosol (Murphy, O'Neill 2018). Respiratory Complex II (CII, succinate dehydrogenase SDH; succinate-ubiquinone oxidoreductase; EC 1.3.5.1) has a unique position in both the TCA cycle and the mitochondrial membrane-bound H^+-linked electron transfer system (membrane-ETS). All genes for CII are nuclear encoded, with exceptions in red algae and land plants (Huang et al 2019; Moosavi et al 2019). Succinate:quinone

oxidoreductases (SQRs, succinate dehydrogenases SDH) favour oxidation of succinate and reduction of quinone in the canonical forward direction of the TCA cycle and electron transfer into the Q-junction (Cecchini 2003). Operating in the reverse direction, quinol:fumarate reductases (QFRs, fumarate reductases, FRD) reduce fumarate and oxidize quinol (Iverson 2013; Maklashina et al 2022). The reversed TCA cycle has gained interest in studies ranging from metabolism in anaerobic animals (Hochachka, Somero 2002), thermodynamic efficiency of anaerobic and aerobic ATP production (Gnaiger 1993), reversed electron transfer and production of reactive oxygen species (Tretter et al 2016; Robb et al 2018; Spinelli et al 2021), hypoxia and ischemia-reperfusion injury (Couchani et al 2014), to evolution of metabolic pathways (Lane 2022). In cancer tissue CII plays a key role in metabolic remodeling (DeBerardinis, Chandel 2016; Schöpf et al 2020). Beyond its role in electron transfer in the TCA cycle and the membrane-ETS, CII serves multiple functions in metabolic signaling (Iverson et al 2023).

The coenzyme NAD^+ is reduced to $NADH+H^+$ during the oxidation of pyruvate and through redox reactions catalyzed by TCA cycle dehydrogenases (DH) including isocitrate DH, oxoglutarate (α-ketoglutarate) DH, and malate DH. In turn, coenzyme NADH is the substrate for the oxidation reaction catalyzed by CI which is linked to reduction of the prosthetic group FMN to $FMNH_2$. Likewise, the prosthetic group FAD is reduced to $FADH_2$ during oxidation of succinate by succinate DH (CII; footnotes on terminology: Supplement 1). Confusion emerges, however, when NADH and $FADH_2$ are considered as the reduced cofactors feeding electrons from the TCA cycle into the 'respiratory chain' — rather than NADH and succinate (Gnaiger 2020). This 'Complex II ambiguitiy' has deeply penetrated the scientific literature on bioenergetics without sufficient quality control. Therefore, a critical literature survey is needed to draw attention to widespread ambiguities, particularly in graphical representations of the mitochondrial electron transfer system, to ensure scientific standards in communications on bioenergetics.

2. Electron flow through CI and CII to the coenzyme Q junction

The reduced flavin groups $FMNH_2$ of flavin mononucleotide and $FADH_2$ of flavin adenine dinucleotide are at functionally comparable levels in the electron transfer through CI and CII, respectively (Figure 1a,b). $FMNH_2$ and $FADH_2$ are reoxidized downstream in CI and CII, respectively, by electron transfer converging at the Q-junction. The convergent architecture of the electron transfer system (ETS; in contrast to a linear electron transfer chain) is emphasized in Figures 1c and 1d (Hatefi 1962; Gnaiger 2020). Comparable to CII, several additional respiratory Complexes are localized in the mtIM which catalyze electron transfer converging at the Q-junction, including electron transferring flavoprotein Complex (CETF) in fatty acid oxidation, glycerophosphate DH Complex (CGpDH), sulfide-ubiquinone oxidoreductase, choline DH, dihydro-orotate DH, and proline DH (Gnaiger 2020; Bénit et al 2022; Pallag et al 2022).

Complex II is a flavoprotein with a covalently bound flavin adenine dinucleotide as documented in early reports (Kearney 1960) and summarized in classical textbooks (Lehninger 1970; Tzagoloff 1982). Microscopic detail on the structure and function of CII has expanded our knowledge on the mechanism of enzyme assembly (Maklashina et al 2022), enzyme structure (Vercellino, Sazanov 2022; Karavaeva, Sousa 2023), kinetic regulation of CII activity (Mills et al 2018; Fink et al 2022), and associated pathologies (Bénit et al 2022; Iverson et al 2023).

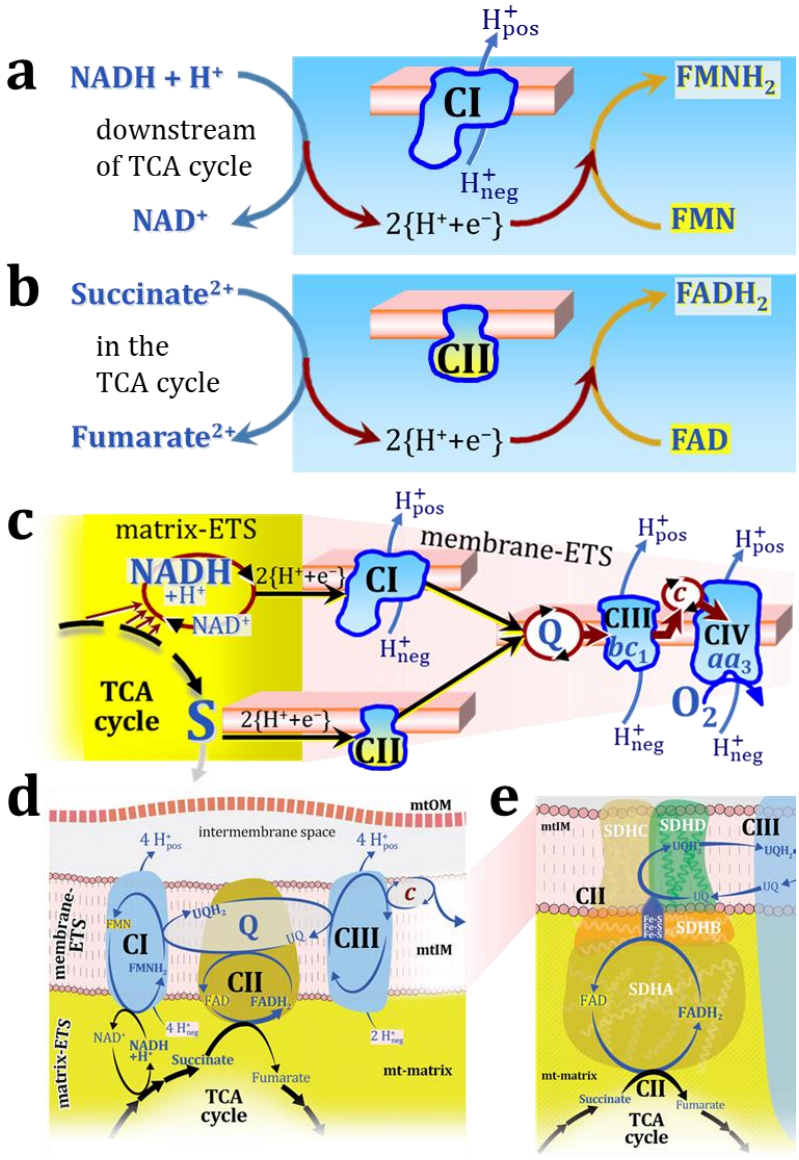

Figure 1. Complex II (SDH) bridges H⁺-linked electron transfer from the TCA cycle (matrix-ETS) to the electron transfer system (membrane-ETS) of the mt-inner membrane (mtIM). **(a)** NADH+H⁺ and **(b)** succinate are substrates of 2{H⁺+e⁻} transfer to CI and CII, respectively, with prosthetic groups FMN and FAD as the corresponding electron acceptors. **(c)** Symbolic representation of ETS pathway architecture. Electron flow converges at the N-junction (NAD⁺ → NADH+H⁺). Electron flow from NADH and succinate S converges through CI and CII at the Q-junction. CIII passes electrons to cytochrome *c* and in CIV to molecular O_2, 2{H⁺+e⁻}+0.5 O_2 ⇢ H_2O. **(d)** NADH+H⁺ and NAD⁺ cycle between matrix-dehydrogenases and CI, whereas FAD and $FADH_2$ cycle permanently bound within the same enzyme CII. Succinate and fumarate indicate the chemical entities irrespective of ionization, but charges are shown in NADH, NAD⁺, and H⁺. Joint pairs of half-circular arrows distinguish electron transfer 2{H⁺+e⁻} to CI and CII from vectorial H⁺ translocation across the mtIM (H^+_{neg} → H^+_{pos}). CI and CIII pump hydrogen ions from the negatively (neg) to the positively charged compartment (pos). **(e)** Iconic representation of SDH subunits. SDHA catalyzes the oxidation succinate → fumarate + 2{H⁺+e⁻} and reduction FAD + 2{H⁺+e⁻} → $FADH_2$ in the soluble domain of CII. The iron–sulfur protein SDHB transfers electrons through Fe-S clusters to the mtIM domain where ubiquinone UQ is reduced to ubiquinol UQH_2 in SDHC and SDHD.

H⁺-linked two-electron transfer from succinate to flavin adenoside dinucleotide FAD reduces the oxidized prosthetic group to $FADH_2$ with formation of fumarate. This H⁺-linked electron transfer through CII is not coupled to H⁺ translocation across the mitochondrial inner membrane (mtIM). Hence, CII is not a H⁺ pump in contrast to the respiratory Complexes CI, CIII and CIV through which electron transfer – more appropriately 2{H⁺+e⁻} transfer (Table 1) – drives and maintains the protonmotive force.

The reversible oxidoreduction of succinate and fumarate is catalyzed in the soluble domain of CII extending from the mtIM into the mt-matrix. Succinate donates electrons – i.e. two hydrogen ions and two electrons, $2\{H^+ + e^-\}$ – to FAD which is covalently bound to the subunit SDHA. SDHA contains the catalytically active dicarboxylate binding site where succinate is oxidized to fumarate. The oxidized yellow (450 nm) form FAD functions as hydrogen acceptor from succinate to the reduced internal product $FADH_2$ while fumarate is formed as the oxidized external product in the TCA cycle. Like in most flavin-linked dehydrogenases, the flavin nucleotide is a prosthetic group which remains permanently bound to the enzyme during the catalytic cycle when the redox state is regenerated in each enzymatic turnover. $FADH_2$ relays electrons further through a series of iron-sulfur redox centers in SDHB to ubiquinone in the membrane domain harboring SDHC and SDHD (Moosavi et al 2019) (Figure 1e).

Simple arrows (Figure 1a-c) or a pair of rounded arrows – an external arrow touching the enzyme and an internal arrow within the enzyme – indicate H^+-linked electron transfer (Hsu et al 2022) in terms of $2\{H^+ + e^-\}$ (Figure 1d,e). Caution is warranted to distinguish three types of transformation with hydrogen ions (Table 1).

Table 1. Three distinct types of transformation with hydrogen ions H^+.

Transformation		Equation		Type
1.	acid-base equilibrium	$H_3O^+ \leftrightarrow H_2O + H^+$ $H_2CO_3 \leftrightarrow HCO_3^- + H^+$	(*a*) (*b*)	scalar, chemical
2a.	H^+-linked electron transfer, oxidation	$Malate^{2-} \longrightarrow Oxaloacetate^{2-} + 2\{H^+ + e^-\}$ $Succinate^{2-} \longrightarrow Fumarate^{2-} + 2\{H^+ + e^-\}$	(*c*) (*d*)	scalar, chemical
2b.	H^+-linked electron transfer, reduction	$2\{H^+ + e^-\} + NAD^+ \longrightarrow NADH + H^+$ $2\{H^+ + e^-\} + E\text{-}FAD \longrightarrow E\text{-}FADH_2$	(*e*) (*f*)	scalar, chemical
3.	transport, translocation	pumping: $H^+_{neg} \longrightarrow H^+_{pos}$ diffusion: $H^+_{pos} \longrightarrow H^+_{neg}$	(*g*) (*h*)	vectorial, compartmental, transmembrane

3. The source and consequence of Complex II ambiguities

> '*No representation is ever perfectly expressive, for if it were it would not be a representation but the thing itself*' (Grosholz 2007).

Ambiguities emerge if the representation of a concept is vague to an extent that allows for equivocal interpretations. As a consequence, even a basically clear concept (Figure 1) may be communicated as a divergence from an established 'truth'. The comparison between NADH linked to CI and $FADH_2$ (instead of succinate) linked to CII leads us astray, as illustrated by the following textbook-quotes (Cooper 2000) (Figure 2).

(*1*) '*Electrons from NADH enter the electron transport chain in complex I, .. A distinct protein complex (complex II), which consists of four polypeptides, receives electrons from the citric acid cycle intermediate, succinate (Figure 10.9). These electrons are transferred to* $FADH_2$*, rather than to NADH, and then to coenzyme Q.*'

(*2*) '*In contrast to the transfer of electrons from NADH to coenzyme Q at complex I, the transfer of electrons from FADH₂ to coenzyme Q is not associated with a significant decrease in free energy and, therefore, is not coupled to ATP synthesis.*' Note that CI is in the path of electron transfer from NADH to coenzyme Q. In contrast, electron transfer from $FADH_2$ to coenzyme Q is downstream of succinate oxidation by CII. Thus instead of the Gibbs force ('*decrease in free energy*') in $FADH_2 \rightarrow Q$ potentially driving the coupled process of proton translocation through CII, the total Gibbs force (Gnaiger 2020) in $S \rightarrow FADH_2 \rightarrow Q$ must be accounted for. (In parentheses: None of these steps are directly coupled to ATP synthesis. Redox-driven proton translocation must be distinguished from phosphorylation of ADP driven by the protonmotive force).

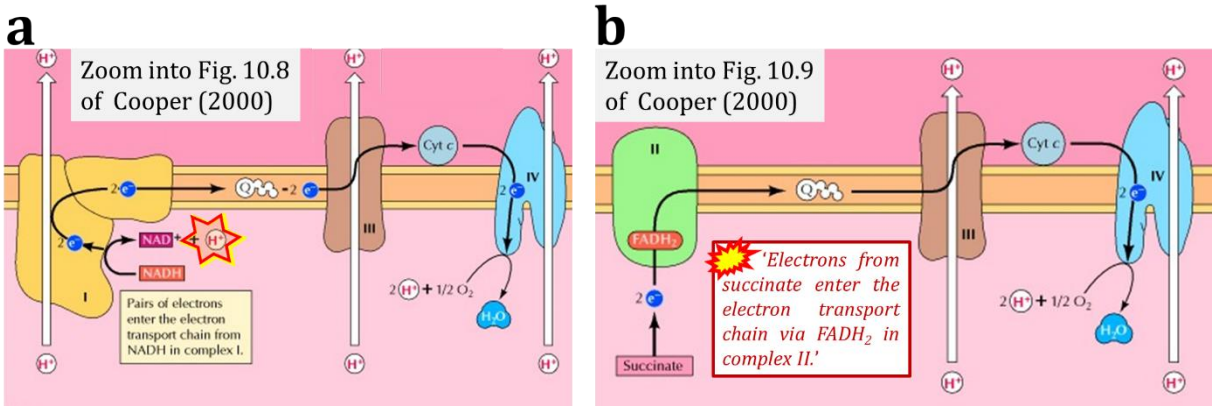

Figure 2. Electron transfer to CI and CII. Zoom into figures of Cooper (2000). **(a)** The marked H^+ is consumed in H^+-linked electron transfer instead of being produced. **(b)** Marked quote inserted from the legend to Fig. 10.9.

(*3*) CII receives electrons from succinate, yet it is suggested that '*electrons from succinate enter the electron transport chain via FADH₂ in complex II.*' The ambiguity is caused by a lack of unequivocal definition of the electron transfer system ('*electron transport chain*'; Supplement 1). Two contrasting definitions are implied of the '*electron transport chain*' or ETS. (*a*) CII is part of the ETS. Hence electrons enter the ETS in the succinate branch from succinate but not from $FADH_2$ – from the matrix-ETS to the membrane-ETS (Figure 1c,d). (*b*) If electrons enter the '*electron transport chain* via *FADH₂ in complex II*', then subunit SDHA would be upstream and hence not part of the ETS (to which conclusion obviously nobody would agree). There remains the ambiguity of electron entry into CII from succinate (Figure 1) or from $FADH_2$ as the 'product' of succinate dehydrogenase in the TCA cycle (Figure 3a,b).

4. The $FADH_2$ - FAD confusion in the succinate-pathway

'*Like drops of water on stone, one drop will do no harm, but over time, grooves are cut deep*' (Wardle 2023).

The narrative that the reduced cofactors NADH and $FADH_2$ feed electrons from the TCA cycle into the mitochondrial electron transfer system causes confusion. As a consequence, the prosthetic group $FADH_2$ appears erroneously as the substrate of CII in the ETS linked to succinate oxidation. This error is widely propagated in 99 publications found from 2001 to 2023 (Supplements 2 to 6) and numerous educational websites (Supplement 7). Clarification is required (Gnaiger 2020; page 48). The following examples illustrate the transition from ambiguity to misunderstanding.

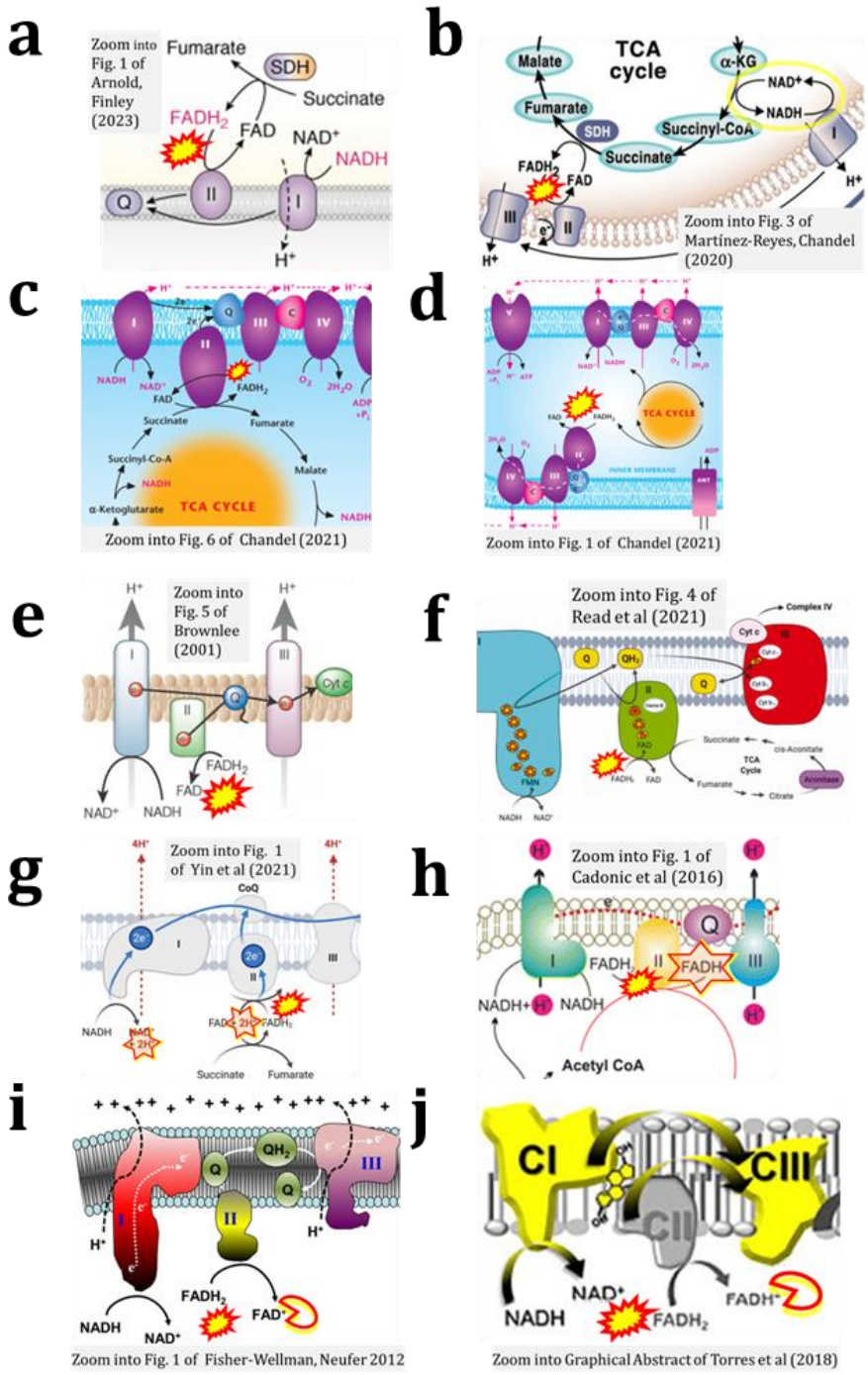

Figure 3. Complex II ambiguities. (a) $FADH_2$ depicted as product and substrate of Complex II by Arnold, Finley (2023), **(b)** Martínez-Reyes, Chandel (2020). NADH and NAD^+ cycle between different types of enzymes (yellow circle), in contrast to the $FADH_2$-FAD cycle located within the same enzyme (SDH and CII are synonyms). **(c)** From ambiguity to **(d)** graphical misconception (Chandel 2021). **(e)** $FADH_2$ shown as substrate of CII (Brownlee 2001). **(f)** FAD shown as product in the mt-matrix and inside CII (Read et al 2021). **(g)** Unjustified indication of $2H^+$ formation in the mt-matrix (Yin et al 2021), not trivial considering the concept of the protonmotive force. **(h)** $FADH_2$ as substrate of CII and FADH (?) as product (Cadonic et al 2016). **(i)** The NADH→NAD^+ analogy is taken to the level of copying a charge to FAD^+ (Fisher-Wellman, Neufer 2012) or **(j)** $FADH^+$ (Torres et al 2018).

(1) Ambiguities appear in graphical representations, where $FADH_2$ is the product of SDH and the substrate of CII – synonymous with SDH (Figure 3a,b; Suppl Figure S2).

(2) Ambiguity evolved to misconception in graphical representations (Figure 3c,d).

(*3*) Graphical errors on electron entry from $FADH_2$ into CII show up without comment (Figure 3e,f; Suppl Figures S3).

(*4*) Instead of $NADH+H^+ \rightarrow NAD^+$ there appears $NADH \rightarrow NAD^+ + H^+$ (or $+2H^+$) and by analogy $FADH_2 \rightarrow FAD + 2H^+$ (Figure 3g; Suppl Figure S4) or $FADH_2 \rightarrow FADH$ (Figure 3h). The analogy $NADH \rightarrow NAD^+$ is taken further to include a charge for FAD or even writing $FADH^+$ (Figure 3i,j; Suppl Figures S5 and S6). Disturbing patterns are shown in various figures with analogous representations of oxidation of NADH and $FADH_2$ (Table 2).

Finally, error propagation from graphical representation (Figure 3) leads to misconception in the text: '*SDH reduces FAD to $FADH_2$, which donates its electrons to complex II*'; '*each complete turn of the TCA cycle generates three NADH and one $FADH_2$ molecules, which donate their electrons to complex I and complex II, respectively*'; '*complex I and complex II oxidize NADH and $FADH_2$, respectively*' (Arnold, Finley 2023).

Table 2. Misconceptions in graphical representations of electron entry into CII.

Analogy with NADH			Suppl Figure	$FADH_2$			Suppl Figure
$NADH + H^+$	⟶	NAD^+					
NADH	⟶	$NAD^+ + H^+$	S3d,q, κ, o, υ	$FADH_2$	⟶	FAD	S2, S3
NADH	⟶	$NAD^+ + H^+$	S4a,e,g	$FADH_2$	⟶	$FAD + 2H^+$	S4a-i
NADH	⟶	$NAD^+ + 2H^+$	S4c,f,h,i				
				$FADH_2$	⟶	FAD^+	S5a-i
$NADH + H^+$	⟶	NADH	S6a	$FADH_2$	⟶	FADH	S6a-d
				$FADH_2$	⟶	$FADH^+$	S6e
				FADH	⟶		S6f
NADH	⟶	$NAD + H^+$	S4b	FADH	⟶	FAD^+	S6g
NADH	⟶	$NAD^+ + H^+$	S6h	FADH	⟶	$FAD^+ + H^+$	S6h
NADH	⟶	$NAD^+ + H^+$	S6i	FADH	⟶	$FAD^+ + 2H^+$	S6i

Electron transfer from succinate in the TCA cycle to the prosthetic group FAD is a redox reaction, where oxidation (ox) of succinate yields $2\{H^+ + e^-\}$ – two hydrogen ions and two electrons – which are donated in the reduction (red) of FAD to $FADH_2$ (Table 1),

ox: $Succinate^{2-} \longrightarrow Fumarate^{2-} + 2\{H^+ + e^-\}$ (Eq. 1a)

red: $2\{H^+ + e^-\} + SDHA\text{-}FAD \longrightarrow SDHA\text{-}FADH_2$ (Eq. 1b)

The net redox reaction equation is

redox: $Succinate^{2-} + SDHA\text{-}FAD \longrightarrow SDHA\text{-}FADH_2 + Fumarate^{2-}$ (Eq. 1)

Commonly the charges of succinate, fumarate (Eq. 1), and other metabolites are not shown explicitly to simplify graphical representations of metabolic pathways. But NAD^+ is clearly distinguished from FAD (Figure 1). H^+ in $NADH+H^+ \longrightarrow NAD^+$ is frequently omitted (Figure 3). The equilibrium (Eq. *e* in Table 1) depends on pH, whereas Eq. 1b (Eq. *f* in Table 1) is independent of pH. The fundamental difference between H^+ and $2\{H^+ + e^-\}$ in Eq. *e* (Table 1) is lost in representations such as Figure 3.

In summary, two-electron oxidation of succinate is redox-linked to reduction of FAD to $FADH_2$. In terms of electron entry into CII many publications show it in the wrong direction, i.e. $FADH_2$ as electron donor from the TCA cycle to CII (Figure 3; Suppl Figures

S2 to S6). This erroneous presentation has a logical consequence. β-oxidation generates $FADH_2$ (Figure 4). If $FADH_2$ would donate electrons to CII, then CII can be seen as an enzyme involved downstream of $FADH_2$ in FAO. This error requires clarification.

5. Complex II and fatty acid oxidation

Electron transferring flavoprotein CETF and CI are the respiratory Complexes involved in convergent electron entry into the Q-junction during FAO (Figure 4).

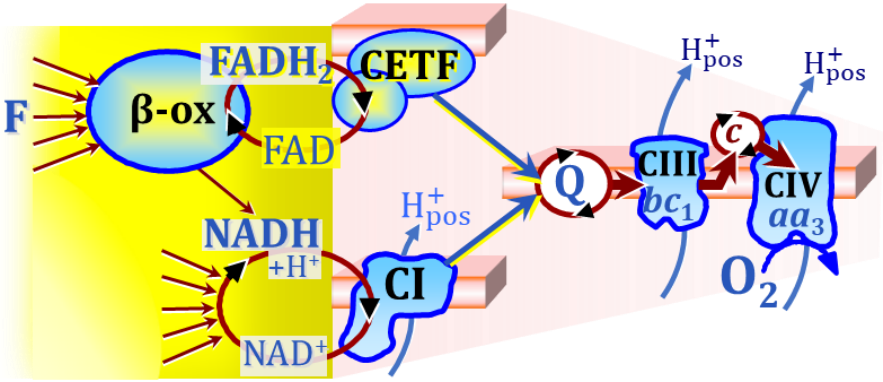

Figure 4. Fatty acid oxidation through the β-oxidation cycle (β-ox), the multi-enzyme electron transferring flavoprotein Complex (CETF, ETF:ETFDH; see text), and Complex I (CI) with convergent electron transfer into the Q-junction.

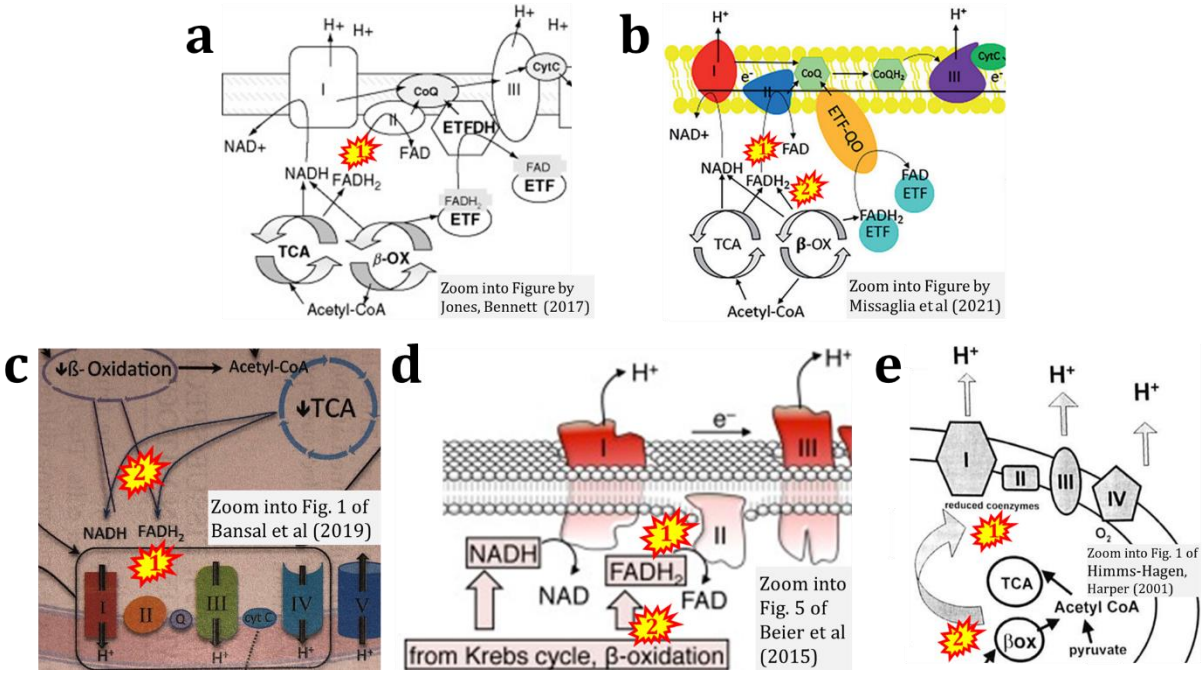

Figure 5. When $FADH_2$ is erroneously shown as a substrate of CII (1), a role of CII in oxidation of $FADH_2$ from fatty acid oxidation is suggested as a consequence (2). Zoom into figures from **(a)** Jones, Bennett (2017); **(b)** Missaglia et al (2021); **(c)** Bansal et al (2019); **(d)** Beier et al (2015); **(e)** Himms-Hagen, Harper (2001).

In the β-oxidation cycle of FAO, acetyl-CoA and the reducing equivalents $FADH_2$ and NADH are formed in reactions catalyzed by acyl-CoA dehydrogenases and hydroxyacyl-CoA dehydrogenases, respectively, in the mitochondrial matrix (Houten et al 2016). When $FADH_2$ is erroneously shown free floating in the mt-matrix as a substrate of CII, a dubious role of CII in FAO is suggested as a consequence (Figure 5; Supplement 8).

Lemmi et al (1990) noted: '*mitochondrial Complex II also participates in the oxidation of fatty acids*'. This holds for the oxidation of acetyl-Co in the TCA cycle, forming NADH and succinate with downstream electron flow through CI and CII, respectively, into the Q-junction (Figure 1). In contrast, electron transfer from $FADH_2$ formed during β-oxidation proceeds through electron transferring flavoprotein ETF. Fatty acylCoA dehydrogenases in the mitochondrial matrix reduce FAD to $FADH_2$. The $FADH_2$ of the fatty acyl-CoA DHs is reoxidized by the FAD-containing ETF (Crane, Beinert 1956).

ETF and ETFDH (Wang et al 2019; or electron transfer flavoprotein:ubiqionone oxidoreductase ETF-QO, Watmough, Frerman 2010) comprise the ETF Complex (CETF), i.e. the ETF:ETFDH or ETF:ETF-QO system. CETF links electron transfer in β-oxidation to electron entry into the Q-junction independent of CII (Figure 4). Thus $FADH_2$ can be seen as an internal substrate of CETF, comparable to the external substrates NADH for CI, succinate for CII, and glycerophosphate for CGpDH.

6. Conclusions

There is currently ambiguity surrounding the precise role of Complex II in fatty acid oxidation. While Complex II is not essential for fatty acid oxidation, it plays a regulatory role by sensing changes in metabolic demand and activating the TCA cycle for oxidation of acetyl-Co depending on the metabolic conditions. This regulatory function may be particularly important during periods of low oxygen availability or high energy demand. The integration of FAO with the membrane-bound ETS (Wang et al 2019) has significant implications for understanding and treating disorders related to β-oxidation and oxidative phosphorylation. Using precisely defined terminology can prevent misunderstandings (Gnaiger et al 2020; footnotes in Supplement 1). Do misinformed diagrams – from ambiguous electron transfer (Suppl Figures S2 to S8) to presentation of CII as a H^+ pump (Suppl Figure S9) – cast any doubts on the quality of the publication? Whether using iconic or symbolic elements in graphical representations, incorporating complementary text not only enhances the communication of intended meaning but diagrams will be improved in the process. When peer review provides insufficient help for corrections, post-peer review by editors and critical readers is required for revisions of articles which may be re-published as living communications (Gnaiger 2021). The present review aims to raise awareness in the scientific community about the inherent ambiguity crisis, complementary to addressing the widely recognized issues of the reproducibility and credibility crisis (Gall et al 2017). Clarification instead of perpetuation of Complex II ambiguities leads to a better representation of fundamental concepts of bioenergetics and helps to maintain the high scientific standards required for translating knowledge on metabolism into clinical solutions for mitochondrial diseases.

Acknowledgements

I thank Luiza H Cardoso and Sabine Schmitt for stimulating discussions, and Paolo Cocco for expert help on the graphical abstract and Figures 1d and e. The constructive

comments of an anonymous reviewer (J Biol Chem) are explicitly acknowledged. Contribution to the European Union's Horizon 2020 research and innovation program Grant 857394 (FAT4BRAIN).

Abbreviations

$2\{H^+ + e^-\}$	redox equivalents in H^+-linked electron transfer	mt-matrix	mitochondrial matrix
		mtIM	mitochondrial inner membrane
CI	Complex I	$NADH_2$	reduced nicotinamide adenine dinucleotide
CII	Complex II		
CETF	electron transferring flavoprotein Complex (ETF:ETFDH)	NAD^+	oxidized nicotinamide adenine dinucleotide
CGpDH	mt-glycerophosphate dehydrogenase Complex	Q	ETS-reactive coenzyme Q, oxidation state is not implied
DH	dehydrogenase		mena-quinol-fumarate
$FADH_2$	reduced flavin adenoside dinucleotide	QFR	oxidoreductase
			succinate-ubiquinone
FAD	oxidized flavin adenoside dinucleotide	SQR	oxidoreductase
			succinate dehydrogenase, CII
FAO	fatty acid oxidation	SDH, SDHABCD	tricarboxylic acid cycle
$FMNH_2$	reduced flavin mononucleotide	TCA cycle	

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Supplement 1. Footnotes on terminology

Coenzyme: A coenzyme or cosubstrate is a cofactor that is attached loosely and transiently to an enzyme (IUPAC definition).

Cofactor: A cofactor is 'an organic molecule or ion (usually a metal ion) that is required by an enzyme for its activity. It may be attached either loosely (coenzyme) or tightly (prosthetic group)' (IUPAC definition).

Electron transfer system ETS: The *convergent* architecture of the electron transfer *system* is emphasized in contrast to *linear* electron transfer *chains* ETCs within segments of the ETS.

Electron transfer: A distinction is necessary between electron *transfer* in redox reactions and electron *transport* (translocation) in the diffusion of charged ionic species within or between cellular compartments. The symbol $2\{H^+ + e^-\}$ is introduced to indicate H^+-linked electron transfer of two hydrogen ions and two electrons in a redox reaction.

H^+-linked electron transfer: The term H^+-coupled electron transfer (Hsu et al 2022) is replaced by H^+-*linked* electron transfer, to avoid confusion with *coupled* H^+ translocation.

Matrix-ETS: Electron transfer and corresponding OXPHOS capacities are classically studied in mitochondrial preparations as oxygen consumption supported by various fuel substrates undergoing partial oxidation in the mt-matrix, such as pyruvate, malate, succinate, and others. Therefore, the *matrix* component of ETS (matrix-ETS) is distinguished from the ETS *bound to the mt-inner membrane* (membrane-ETS; Gnaiger et al 2020).

Membrane-ETS: Electron transfer is frequently considered as the segment of redox reactions linked to the mtIM. However, the *membrane*-ETS is only part of the total ETS, which includes the upstream *matrix*-ETS.

Misinformation: Misinformation is the mistaken sharing of the same content (Wardle 2023).

Prosthetic group: A prosthetic group is cofactor that is attached permanently and tightly or even covalently to an enzyme and that is regenerated in each enzymatic turnover.

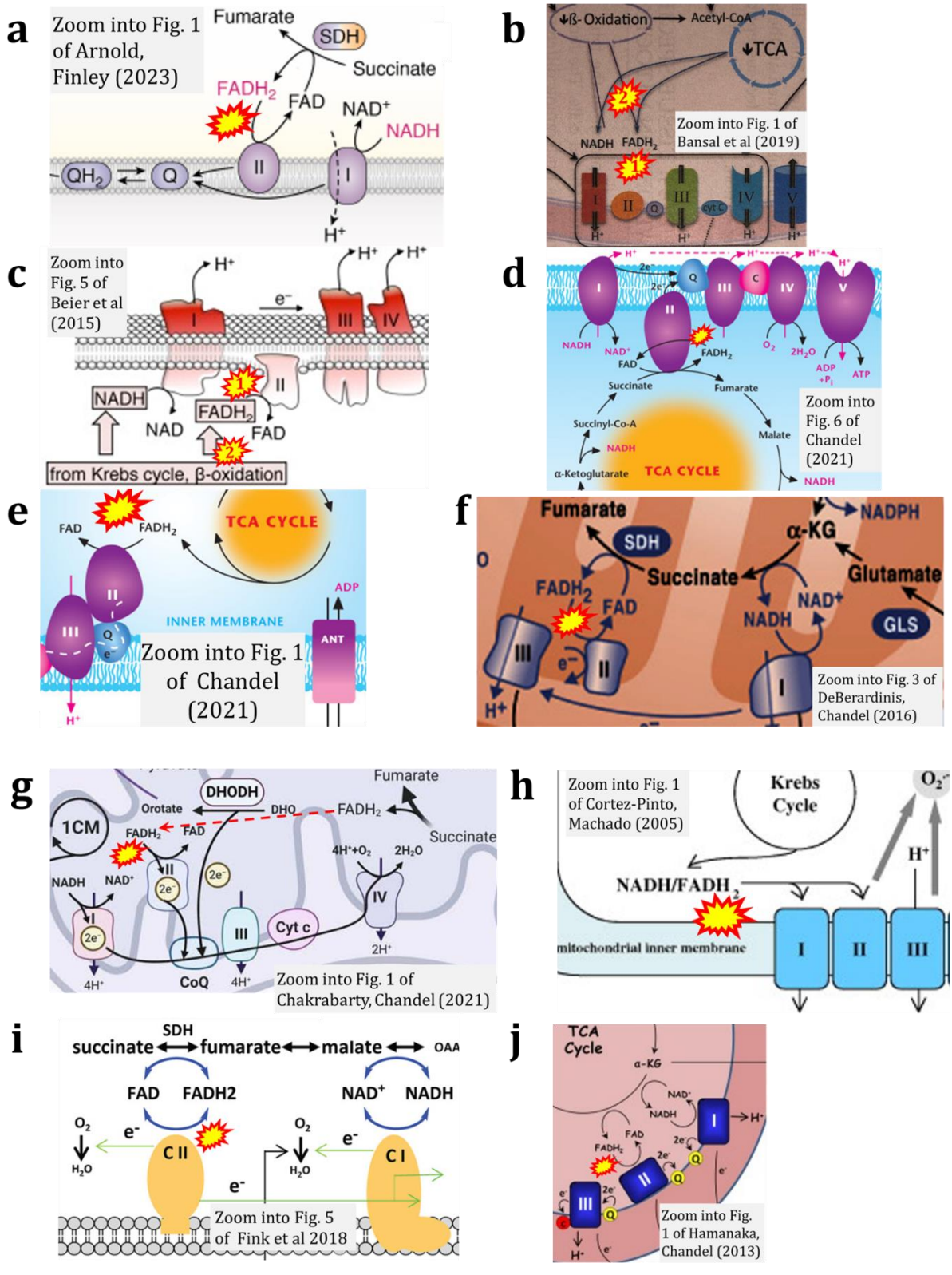
Substrate: A substrate in a chemical reaction has a negative stoichiometric number since it is consumed, whereas a product has a positive stoichiometric number since it is produced. The general definition of a substrate in an enzyme-catalized reaction relies on the definition of the chemical reaction, without restriction to the nature of the substrate, i.e. independent of the substrate being a chemical entity in solution or a loosely bound cosubstrate (coenzyme) or even a tightly bound prosthetic group. The latter may be explicitly distinguished as a bound (internal) substrate from a free (external) substrate. Even different substrate pools may coexist (CoQ).

$2\{H^+ + e-\}$: The symbol [2 H] is frequently used to indicate redox equivalents in the transfer from hydrogen donors to hydrogen acceptors. However, 2[H] does not explicitly express that it applies to both *electron* and *hydrogen ion* transfer. Brackets are avoided to exclude the confusion with their frequent application to indicate amount-of-substance concentrations. Two-electron transfer $2\{H^+ + e-\}$ is distinguished from single-electron transfer $\{H^+\}+\{e-\}$.

Supplement 2

FAD a substrate of SDH and $FADH_2$ a substrate of CII (Figure S2)

SDH: FAD ⟶ $FADH_2$; TCA cycle: ⟶ $FADH_2$
CII: $FADH_2$ ⟶ FAD ; $FADH_2$ ⟶ (*1*)



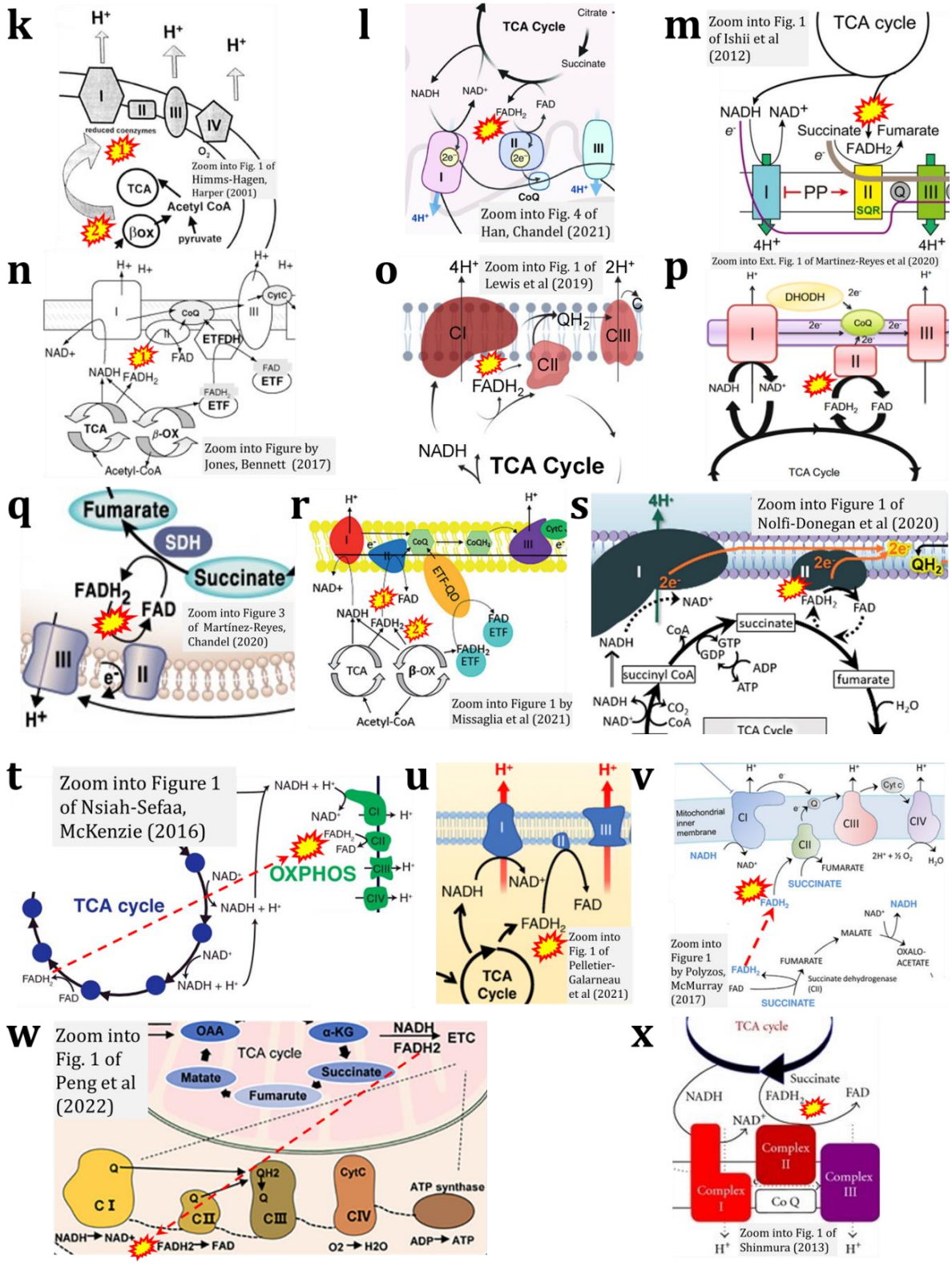


Figure S2. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. The TCA cycle reduces FAD to $FADH_2$ – in several cases shown to be catalyzed by SDH. Then $FADH_2$ is erroneously shown to feed electrons into CII. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S2.

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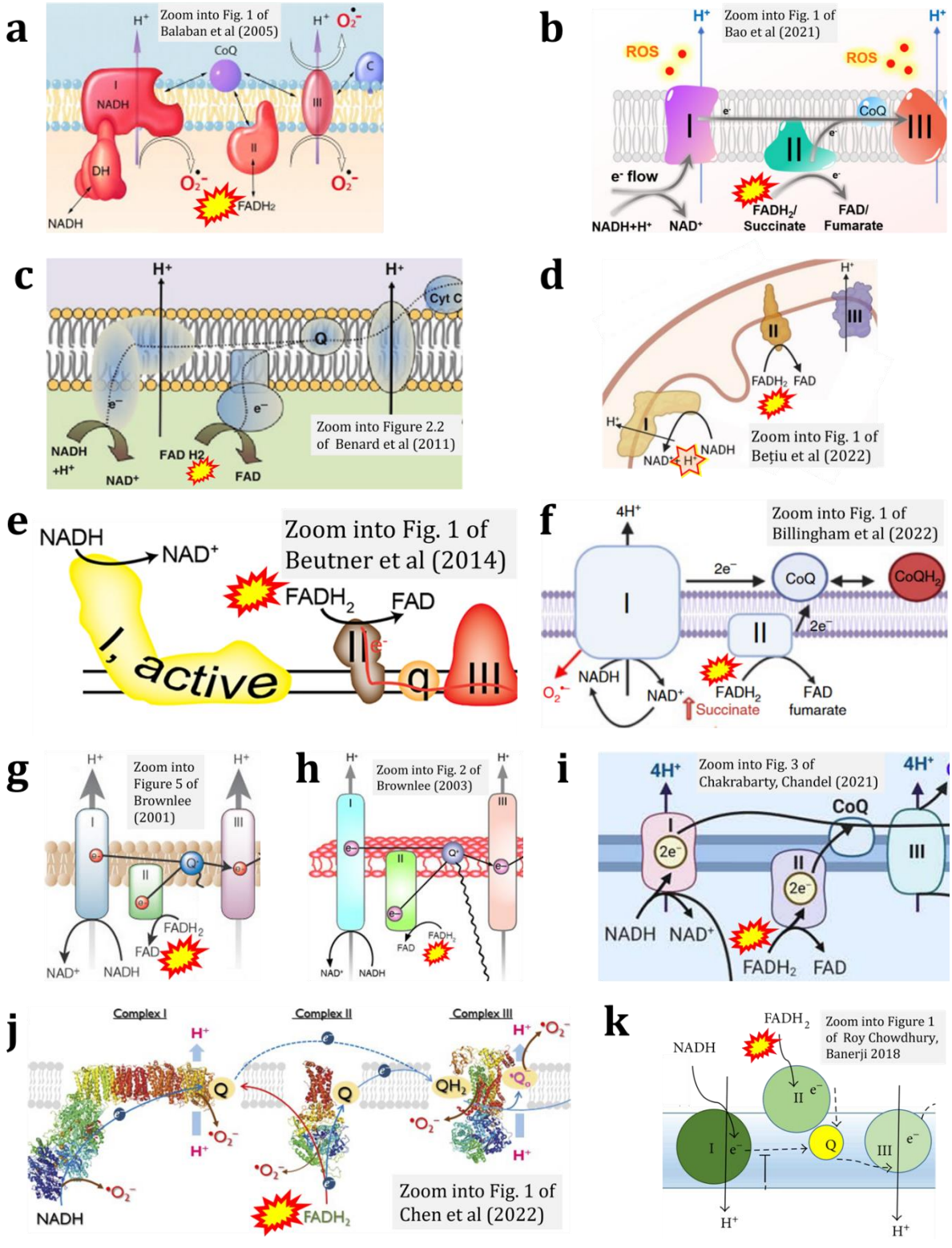
Supplement 3

$FADH_2$ as substrate of CII (Figure S3)

$FADH_2 \longrightarrow FAD$ (*1*)



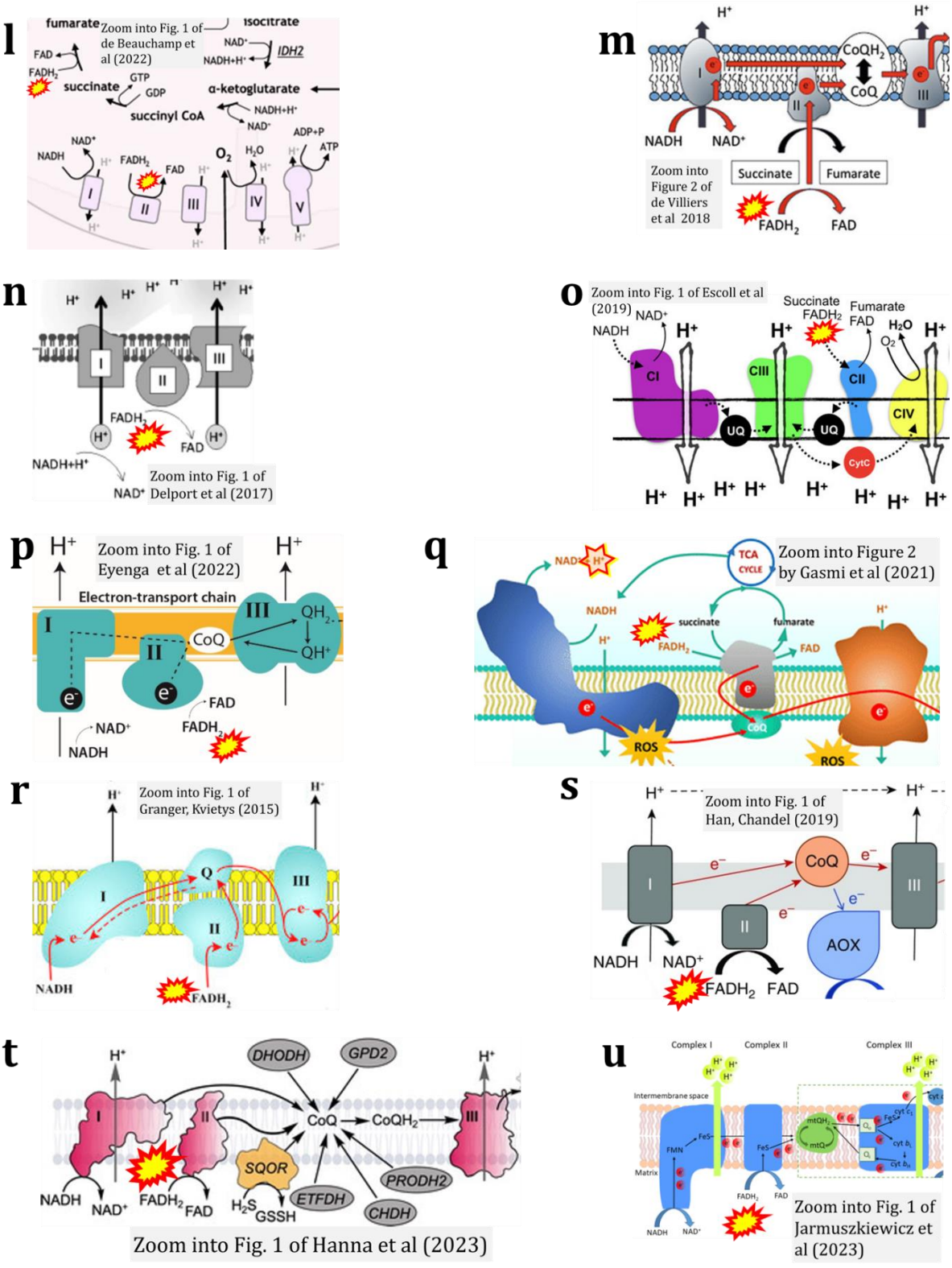


Figure S3. *Continued*

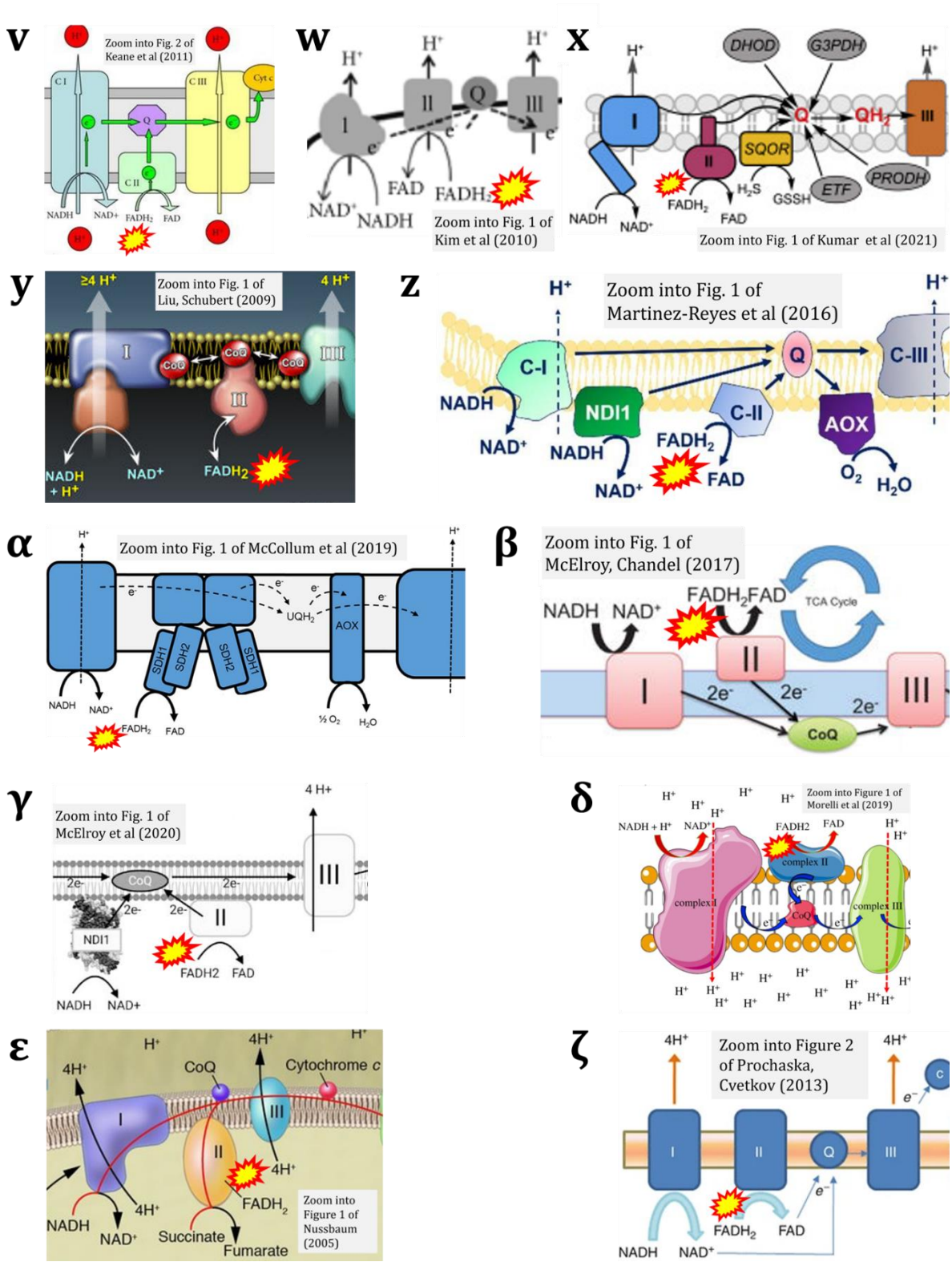


Figure S3. *Continued*

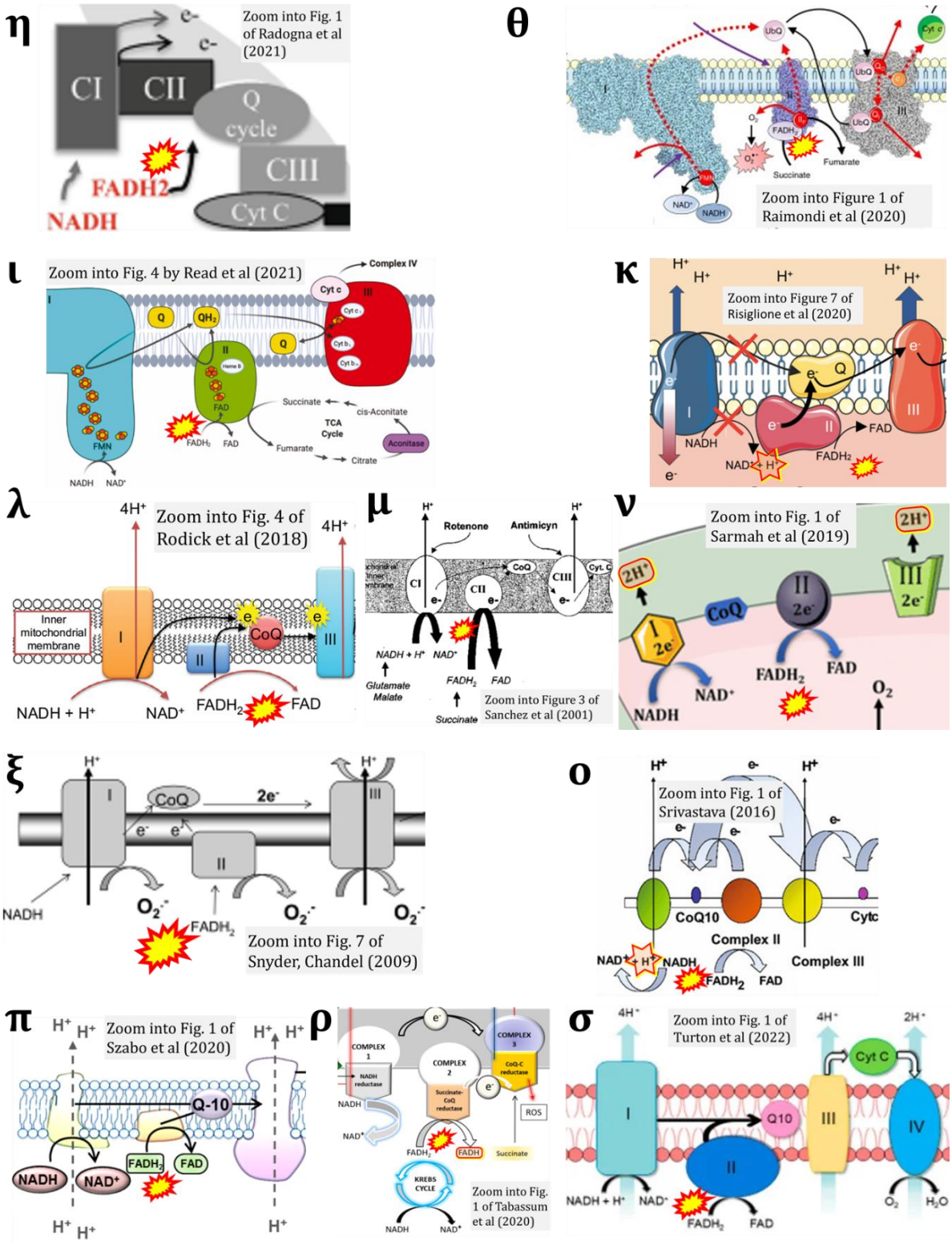


Figure S3. *Continued*

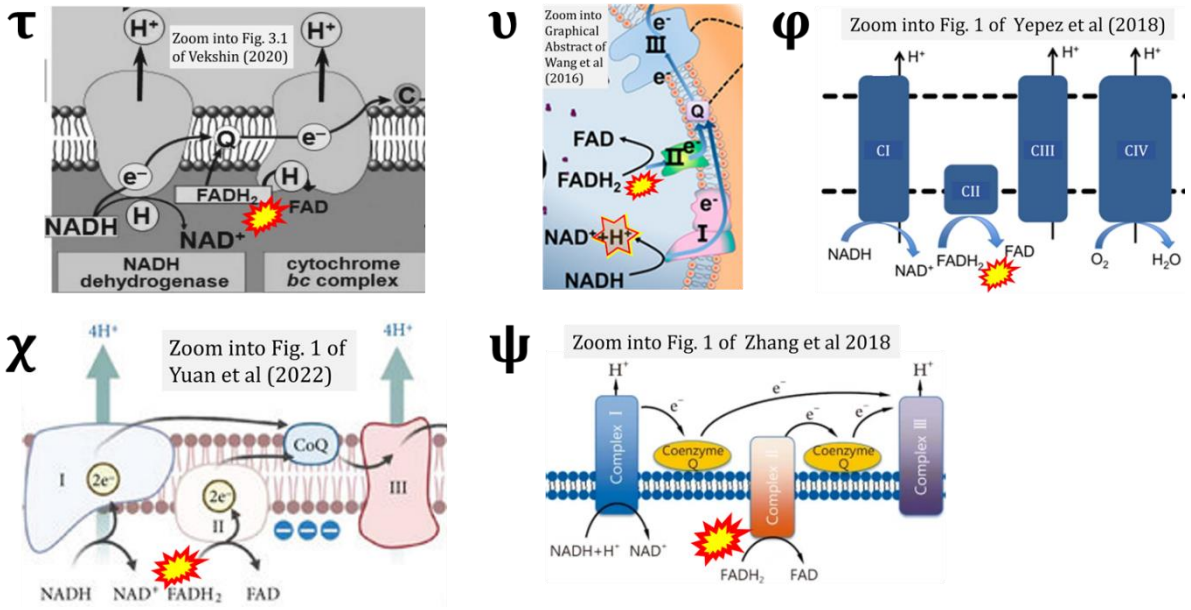


Figure S3. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S3.

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Supplement 4

$FADH_2$ as substrate of CII and FAD + $2H^+$ as products (Figure S4)

$FADH_2 \longrightarrow FAD + 2H^+$ (*2*)

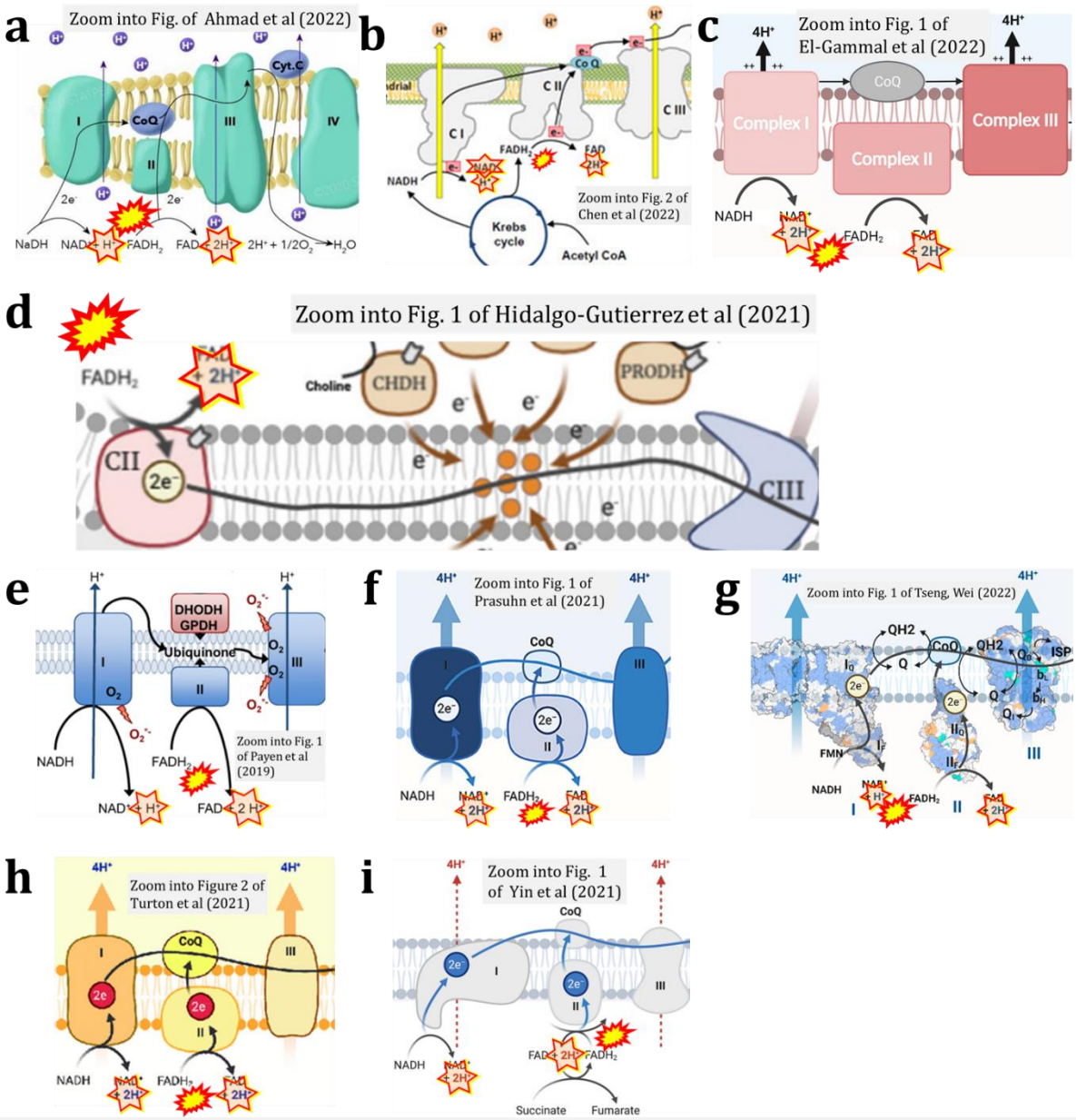

Figure S4. Complex II ambiguities: $FADH_2$ as substrate of CII and FAD + $2H^+$ as products. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S4.

References for Figure S4

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Supplement 5

$FADH_2$ as substrate of CII and FAD^+ as product (Figure S5)

$FADH_2 \longrightarrow FAD^+$ (*3*)

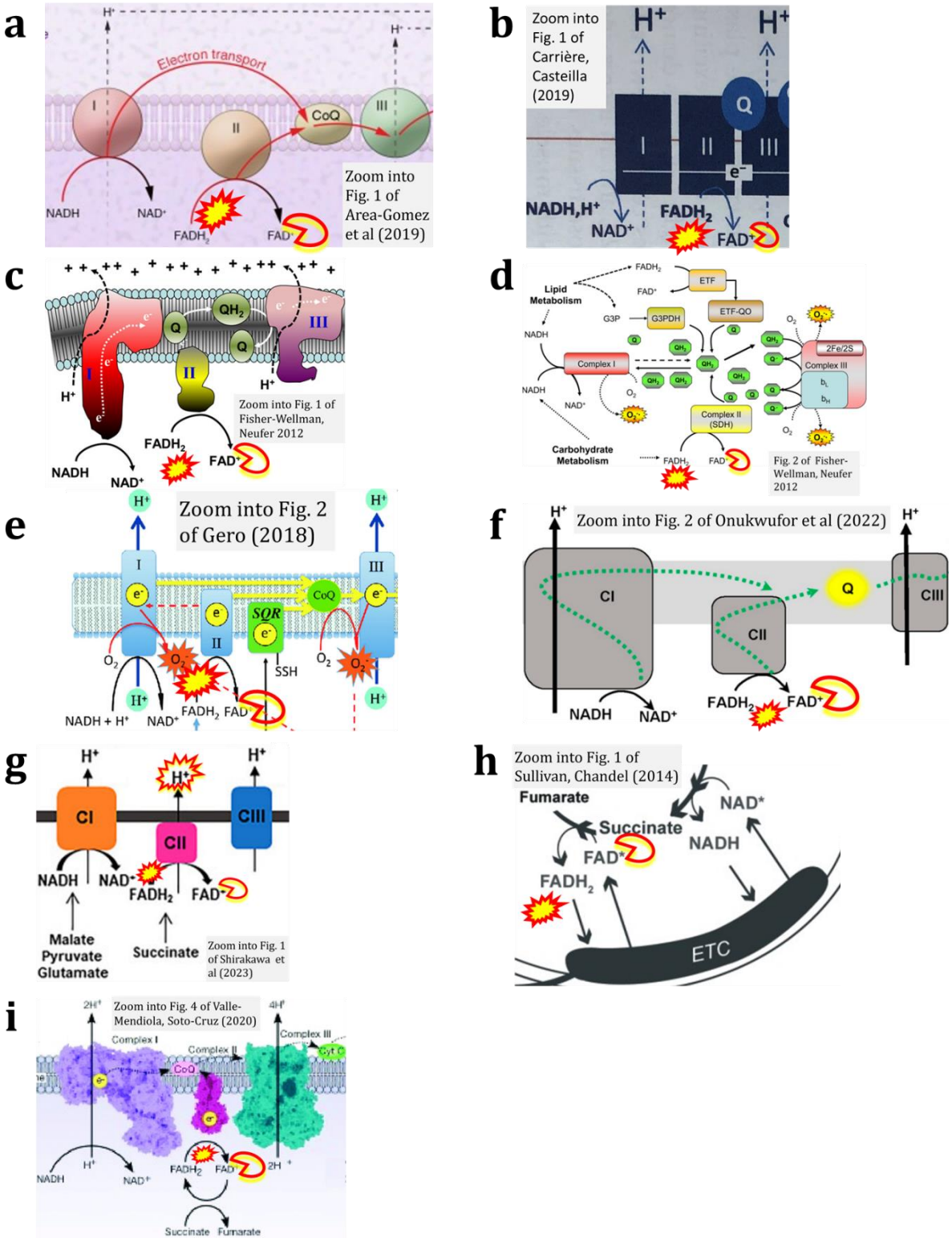

Figure S5. Complex II ambiguities: $FADH_2$ as substrate of CII and FAD^+ as product. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S5.

References for Figure S5

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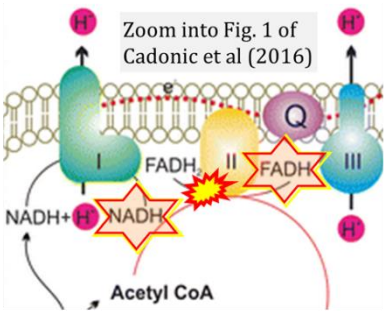
Supplement 6

$FADH_2$ or FADH as substrate of CII and FADH, $FADH^+$, or FAD^+ as product (Figure S6)

a-d	$FADH_2$	⟶ FADH	(*4*)
e	$FADH_2$	⟶ $FADH^+$	(*5*)
f	FADH	⟶	(*6*)
g	FADH	⟶ FAD^+	(*7*)
h	FADH	⟶ FAD^+ + H^+	(*8*)
i	FADH	⟶ FAD^+ + $2H^+$	(*9*)

a



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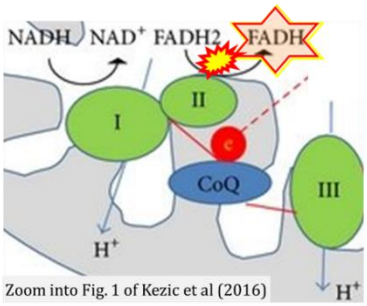

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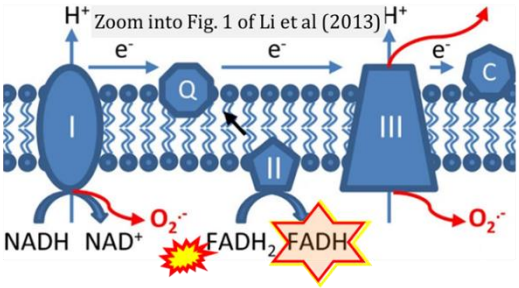

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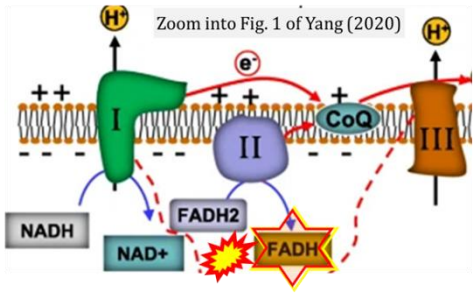

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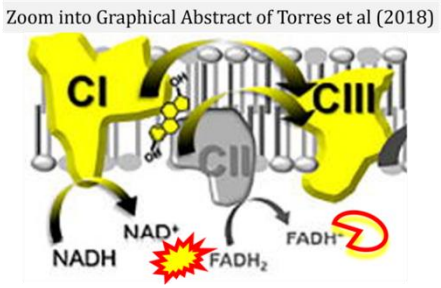

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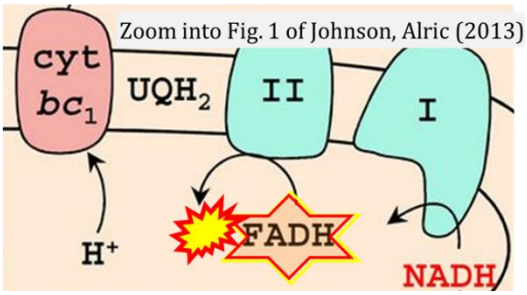

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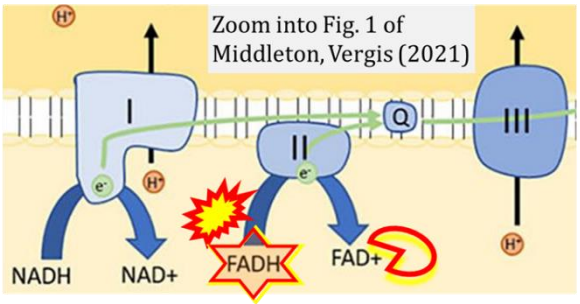

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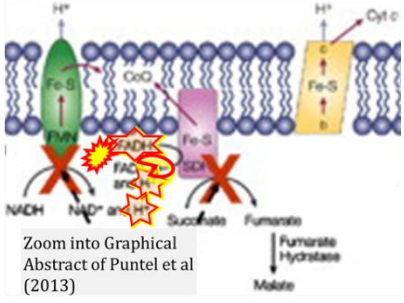

i

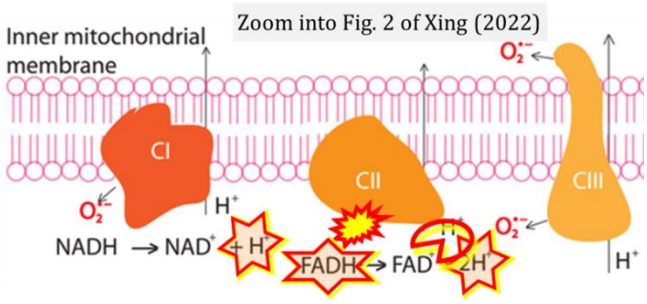

Figure S6. Complex II ambiguities: $FADH_2$ as substrate of CII and FADH or $FADH^+$ as product. Sequence of publications from 2001 to 2023 according to (*4*) to (*9*). See References for Figure S6.

References for Figure S6

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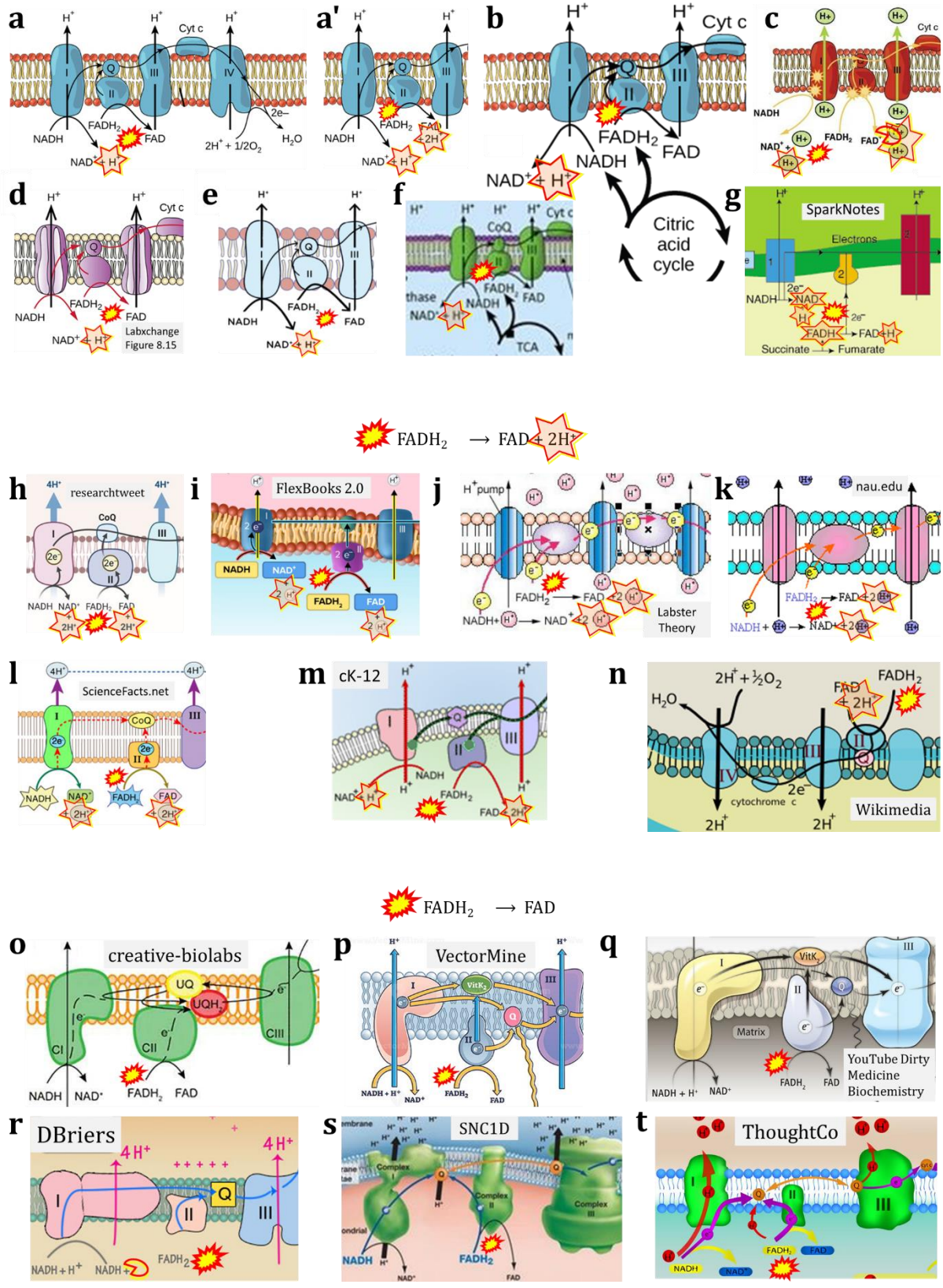
Supplement 7

$FADH_2$ or FADH as substrate of CII in websites (Figure S7)



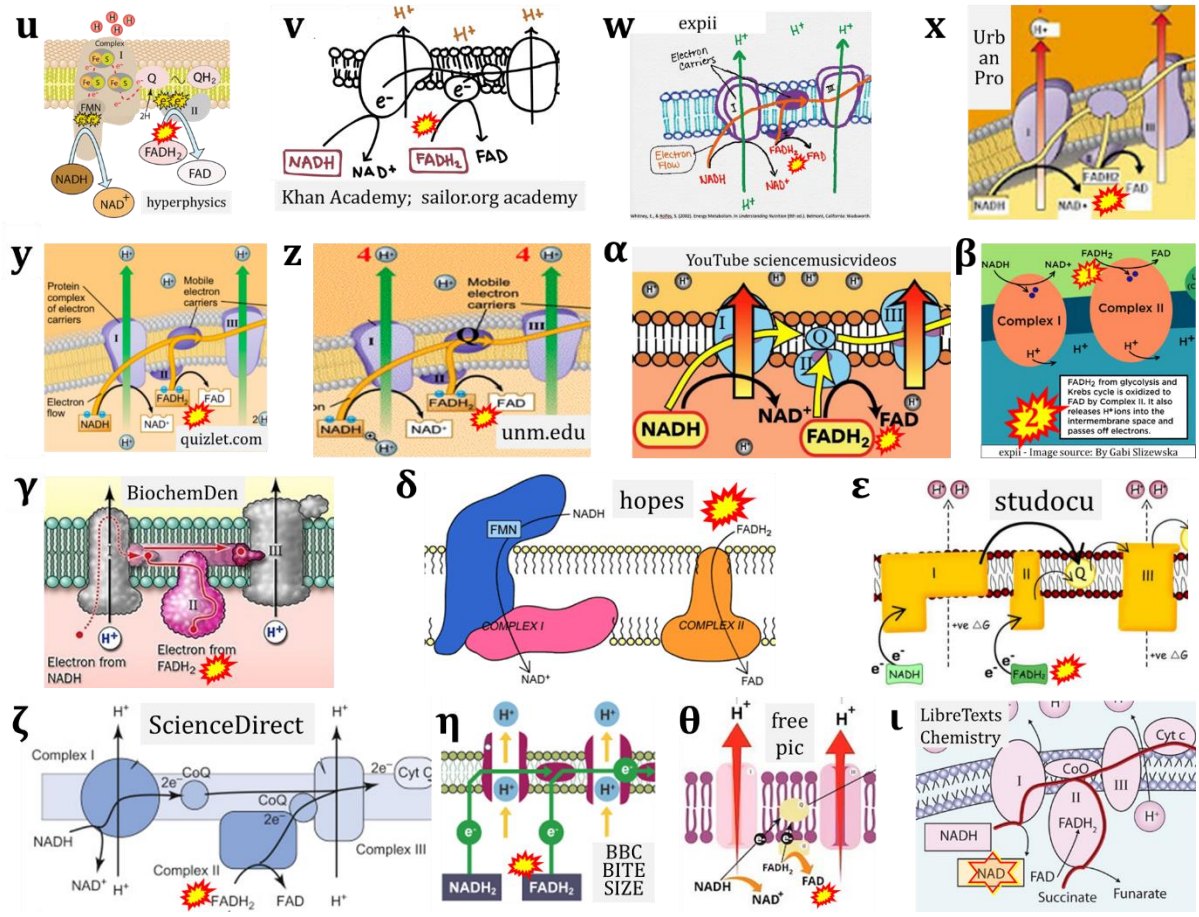


Figure S7. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. (**a**, **b**, **d-f**, **o-θ**) $FADH_2 \rightarrow FAD$; (**a'**, **h-n**) $FADH_2 \rightarrow FAD+2H^+$; (**c**) $FADH_2 \rightarrow FAD^+ + 2H^+$; and (**g**) $FADH \rightarrow FAD+H$; should be corrected to $FAD \rightarrow FADH_2$. $NADH \rightarrow NAD^+$ is frequently written in graphs without showing the H^+ on the left side of the arrow, except for (**p-r**). (**a-f**, **m**) $NADH \rightarrow NAD^+ + H^+$; (**g**) $NADH \rightarrow NAD+H$; (**h**, **i**, **l**) $NADH \rightarrow NAD^+ + 2H^+$; (**j**, **k**) $NADH+H^+ \rightarrow NAD^+ + 2H^+$; and (**ι**) $NADH \rightarrow NAD$ should be corrected to $NADH+H^+ \rightarrow NAD^+$ (Eq. 3a). Weblinks #: (**a**) 1-8; (**a'**) 9-10; (**b**) 1-6,9-11; (**c**) 11-17; (**d**) 18; (**e**) 19; (**f**) 20; (**g**) 21; (**h**) 22-23; (**i**) 24; (**j**) 25; (**k**) 26; (**l**) 27; (**m**) 28; (**n**) 11,29; (**o**) 30; (**p**) 31-32; (**q**) 33; (**r**) 34; (**s**) 35; (**t**) 12,22,36; (**u**) 37; (**v**) 9,10; (**w**) 11; (**x**) 38; (**y**) 39; (**z**) 40; (**α**) 41; (**β**) 11; (**γ**) 42; (**δ**) 43; (**ε**) 44; (**ζ**) 45; (**η**) 46; (**θ**) 47; (**ι**) 48.

Weblinks for Figure S7 (retrieved 2023-03-21 to 2023-05-04)

1 (**a,b**) https://openstax.org/books/biology/pages/7-4-oxidative-phosphorylation - OpenStax Biology (CC BY 3.0) - Fig. 7.10, Fig. 7.12.

2 (**a,b**) https://opentextbc.ca/biology/chapter/4-3-citric-acid-cycle-and-oxidative-phosphorylation/ - Charles Molnar, Jane Gair, Concepts of Biology - 1st Canadian Edition, BCcampus - Fig. 4.19.

3 (**a,b**) https://www.pharmaguideline.com/2022/01/electron-transport-chain.html - Pharmaguideline

4 (**a,b**) https://www.texasgateway.org/resource/74-oxidative-phosphorylation - Texas Gateway - Fig. 7.11, Fig. 7.13.

5 (**a,b**) https://opened.cuny.edu/courseware/lesson/639/overview - CUNY

6 (**a,b**) https://courses.lumenlearning.com/wm-biology1/chapter/reading-electron-transport-chain/ - lumen Biology for Majors I - Fig. 1, Fig. 3.

7 (**a**) https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/07%3A_Cellular_Respiration/7.11%3A_Oxidative_Phosphorylation_-_Electron_Transport_Chain - LibreTexts Biology – Fig. 7.11.1

8 (**a**) https://brainbrooder.com/lesson/254/7-4-1-electron-transport-chain - Brain Brooder

9 (**a',b,v**) https://www.khanacademy.org/science/ap-biology/cellular-energetics/cellular-respiration-ap/a/oxidative-phosphorylation-etc - Khan Academy - Image modified from "Oxidative phosphorylation: Fig. 1", by OpenStax College, Biology (CC BY 3.0) / Image modified from "Oxidative phosphorylation: Fig. 3," by Openstax College, Biology (CC BY 3.0)

10 (**a',b,v**) https://learn.saylor.org/mod/page/view.php?id=32815 -Saylor Academy

11 (**b**,**c,n,w,β**) https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139 - expii - Image source: By CNX OpenStax / By OpenStax College CC BY 3.0, via Wikimedia Commons / Whitney, Rolfes 2002 / By User:Rozzychan CC BY-SA 2.5, via Wikimedia Commons

12 (**c,t**) https://www.thoughtco.com/electron-transport-chain-and-energy-production-4136143 - ThoughtCo / extender01 / iStock / Getty Images Plus

13 (**c**) https://commons.wikimedia.org/w/index.php?curid=30148497 - wikimedia 30148497 - Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/, 2013-06-19

14 (**c**) https://biologydictionary.net/electron-transport-chain-and-oxidative-phosphorylation/ - biologydictionary.net 2018-08-21

15 (**c**) https://www.quora.com/Why-does-FADH2-form-2-ATP - Quora

16 (**c**) https://teachmephysiology.com/biochemistry/atp-production/electron-transport-chain/ - TeachMePhysiology - Fig. 1. 2023-03-13

17 (**c**) https://www.toppr.com/ask/question/short-long-answer-types-whatis-the-electron-transport-system-and-what-are-its-functions/ - toppr

18 (**d**) https://www.labxchange.org/library/items/lb:LabXchange:005ad47f-7556-3887-b4a6-66e74198fbcf:html:1 - Labxchange - Figure 8.15 credit: modification of work by Klaus Hoffmeier

19 (**e**) https://jackwestin.com/resources/mcat-content/oxidative-phosphorylation/electron-transfer-in-mitochondria - Jack Westin MCAT Courses

20 (**f**) https://videodelivery.net/79e91c40bf96f9692560fa378c5086b6/thumbnails/thumbnail.jpg - videodelivery

21 (**g**) https://www.sparknotes.com/biology/cellrespiration/oxidativephosphorylation/section2/ - SparkNotes

22 (**h,t**) https://researchtweet.com/mitochondrial-electron-transport-chain-2/ - researchtweet

23 (**h**) https://microbenotes.com/electron-transport-chain/ - Microbe Notes

24 (**i**) https://flexbooks.ck12.org/cbook/ck-12-biology-flexbook-2.0/section/2.28/primary/lesson/electron-transport-bio/ - FlexBooks - CK-12 Biology for High School- 2.28 Electron Transport, Fig. 2

25 (**j**) https://theory.labster.com/Electron_Transport_Chain/ - Labster Theory

26 (**k**) https://www2.nau.edu/~fpm/bio205/u4fg36.html - nau.edu

27 (**l**) https://www.sciencefacts.net/electron-transport-chain.html - ScienceFacts

28 (**m**) https://www.ck12.org/biology/electron-transport/lesson/The-Electron-Transport-Chain-Advanced-BIO-ADV/ - cK-12

29 (**n**) https://commons.wikimedia.org/wiki/File:Mitochondrial_electron_transport_chain.png - Wikimedia

30 (**o**) https://www.creative-biolabs.com/drug-discovery/therapeutics/electron-transport-chain.htm - creative-biolabs
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32 (**p**) https://vectormine.com/item/electron-transport-chain-as-respiratory-embedded-transporters-outline-diagram/ - VectorMine
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34 (**r**) http://www.dbriers.com/tutorials/2012/04/the-electron-transport-chain-simplified/ - DBriers
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42 (**γ**) https://biochemden.com/electron-transport-chain-mechanism/ - BiochemDen.com
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46 (**η**) https://www.bbc.co.uk/bitesize/guides/zdq9382/revision/5 - BBC BITESIZE
47 (**θ**) https://www.freepik.com/premium-vector/oxidative-phosphorylation-process-electron-transport-chain-final-step-cellular-respiration_29211885.htm - freepik
48 (**ι**) https://chem.libretexts.org/Courses/Saint_Marys_College_Notre_Dame_IN/CHEM_118_(Under_Construction)/CHEM_118_Textbook/12%3A_Metabolism_(Biological_Energy)/12.4

%3A_The_Citric_Acid_Cycle_and_Electron_Transport - LibreTexts Chemistry - The Citric Acid Cycle and Electron Transport – Fig. 12.4.3

Supplement 8

Weblinks on FAO and CII (retrieved 2023-03-21 to 2023-05-02)

49 https://conductscience.com/electron-transport-chain/ - Conduct Science: "*In Complex II, the enzyme succinate dehydrogenase in the inner mitochondrial membrane reduce $FADH_2$ to FAD^+. Simultaneously, succinate, an intermediate in the Krebs cycle, is oxidized to fumarate.*" - Comments: FAD does not have a postive charge. $FADH_2$ is the reduced form, it is not reduced. And again: *In CII, FAD is reduced to $FADH_2$.*

50 https://themedicalbiochemistrypage.org/oxidative-phosphorylation-related-mitochondrial-functions/ - The Medical Biochemistry Page: '*In addition to transferring electrons from the $FADH_2$ generated by SDH, complex II also accepts electrons from the $FADH_2$ generated during fatty acid oxidation via the fatty acyl-CoA dehydrogenases and from mitochondrial glycerol-3-phosphate dehydrogenase (GPD2) of the glycerol phosphate shuttle*' (Figure S8).

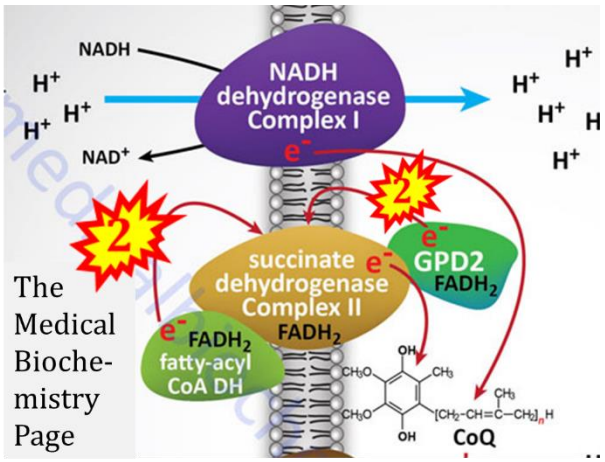

Figure S8. Fatty acid oxidation and glycerophosphate dehydrogenase erroneously shown to feed FADH2 into Complex II. Weblink #50.

51 https://www.chem.purdue.edu/courses/chm333/Spring%202013/Lectures/Spring%202013%20Lecture%2037%20-%2038.pdf - CHM333 LECTURES 37 & 38: 4/27 – 29/13 SPRING 2013 Professor Christine Hrycyna - Acyl-CoA dehydrogenase is listed under 'Electron transfer in Complex II'.

Supplement 9

CII as a proton pump (Figure S9)

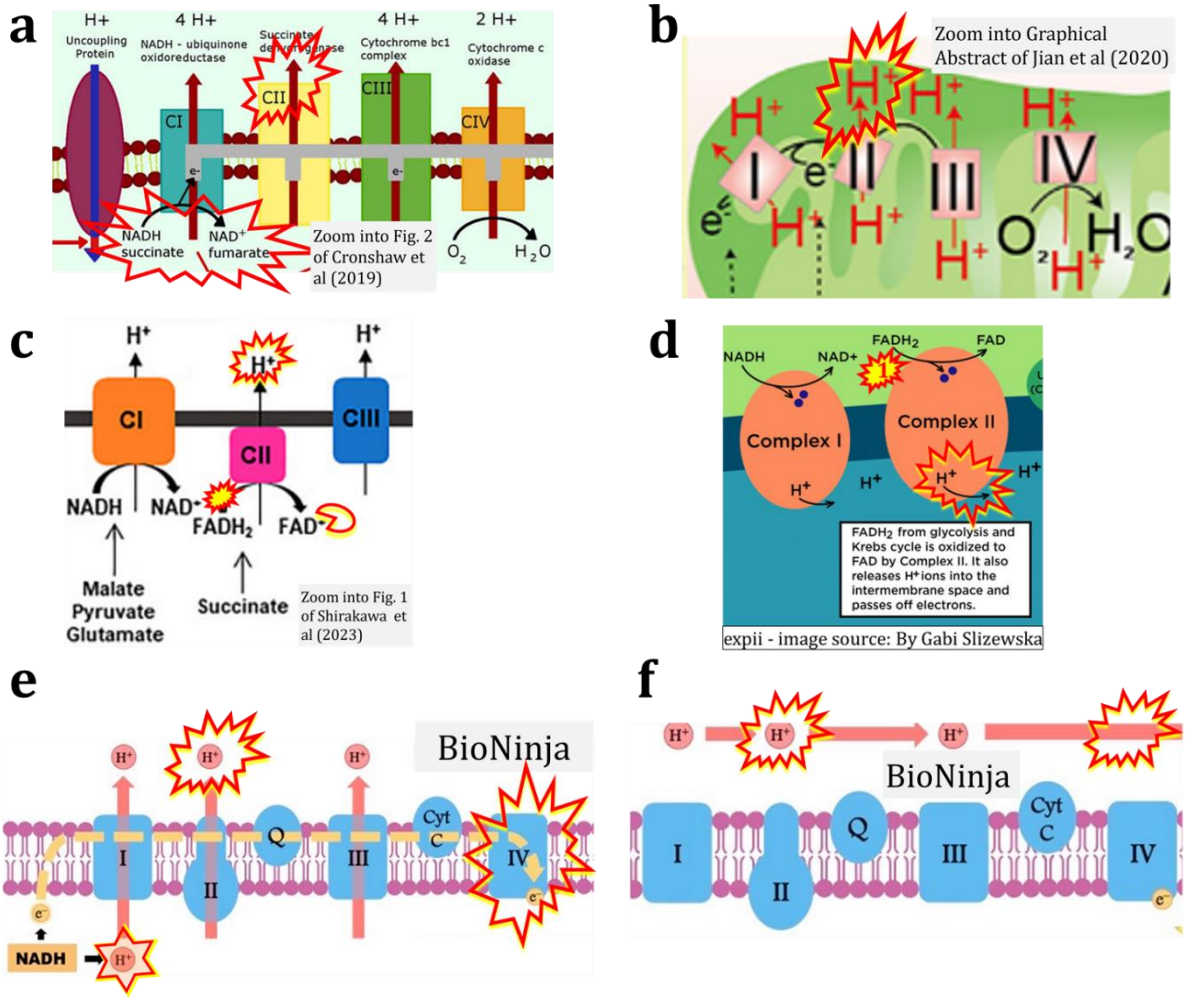

Figure S9. Complex II as a proton pump.

a Cronshaw M, Parker S, Arany P (2019) Feeling the heat: evolutionary and microbial basis for the analgesic mechanisms of photobiomodulation therapy. **Photobiomodul Photomed Laser Surg** 37:517-26. https://doi.org/10.1089/photob.2019.4684

b Jian C, Fu J, Cheng X, Shen LJ, Ji YX, Wang X, Pan S, Tian H, Tian S, Liao R, Song K, Wang HP, Zhang X, Wang Y, Huang Z, She ZG, Zhang XJ, Zhu L, Li H (2020) Low-dose sorafenib acts as a mitochondrial uncoupler and ameliorates nonalcoholic steatohepatitis. **Cell Metab** 31:892-908. https://doi.org/10.1016/j.cmet.2020.04.011

c Shirakawa R, Nakajima T, Yoshimura A, Kawahara Y, Orito C, Yamane M, Handa H, Takada S, Furihata T, Fukushima A, Ishimori N, Nakagawa M, Yokota I, Sabe H, Hashino S, Kinugawa S, Yokota T (2023) Enhanced mitochondrial oxidative metabolism in peripheral blood mononuclear cells is associated with fatty liver in obese young adults. **Sci Rep** 13:5203. https://doi.org/10.1038/s41598-023-32549-w

d https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139 - expii - Image source: By Gabi Slizewska: '*$FADH_2$ from glycolysis and Krebs cycle is oxidized to FAD by Complex II. It also releases H+ ions into the intermembrane space and passes off electrons*' (retrieved 2023-05-04).

e,f https://ib.bioninja.com.au/higher-level/topic-8-metabolism-cell/untitled/electron-transport-chain.html - BioNinja (retrieved 2023-05-04).