Cytochrome (cyt) bc$_1$, bcc and b$_{6f}$ complexes, collectively referred to as cyt bc complexes, are homologous isoprenoid quinol oxidising enzymes present in diverse phylogenetic lineages. Cyt bc$_1$ and bcc complexes are constituents of the electron transport chain (ETC) of cellular respiration, and cyt b$_{6f}$ complex is a component of the photosynthetic ETC. Cyt bc complexes share in general the same Mitchellian Q cycle mechanism, with which they accomplish proton translocation and thus contribute to the generation of proton motive force which drives ATP synthesis. They therefore require a quinol oxidation (Qo) and a quinone reduction (Qi) site. Yet, cyt bc complexes evolved to adapt to specific electrochemical properties of different quinone species and exhibit structural diversity. This review summarises structural information on native quinones and quinone-like inhibitors bound in cyt bc complexes resolved by X-ray crystallography and cryo-EM structures. Although the Qi site architecture of cyt bc$_1$ complex and cyt bcc complex differs considerably, quinone molecules were resolved at the respective Qi sites in very similar distance to haem b$_{1i}$. In contrast, more diverse positions of native quinone molecules were resolved at Qo sites, suggesting multiple quinone binding positions or captured snapshots of trajectories toward the catalytic site. A wide spectrum of inhibitors resolved at Qo or Qi site covers fungicides, antimalarial and antituberculosis medications and drug candidates. The impact of these structures for characterising the Q cycle mechanism, as well as their relevance for the development of medications and agrochemicals are discussed.

Introduction

Isoprenoid quinones are a family of natural electron and proton carriers present in prokaryotic cellular membranes, in the mitochondrial inner membrane and in the chloroplast thylakoid membrane [1–3]. The various isoprenoid quinone species differ in their water-soluble ring system and the length of the hydrophobic isoprenoid tails [4–6] (Figure 1A–E). The electrochemically active part of this family of molecules is the quinone ring system, which accepts two electrons and two protons to become the fully reduced quinol (Figure 1A), while the highly hydrophobic isoprenoid tail enhances its solubility in biological membranes. Isoprenoid quinone and quinol are substrates of respiratory chain and photosynthetic enzymes [7,8].

In the electron transport chain (ETC) of cellular respiration, NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) reduce quinone, harnessing the energy of redox equivalents obtained from metabolism while cytochrome bc$_1$ complex (cyt bc$_1$ complex, complex III) oxidises quinol and transfer the electrons to cytochrome c oxidase (cyt c oxidase, complex IV) via the electron carrier protein cytochrome (cyt) c. The cyt c oxidase catalyses the reduction in dioxygen to water. NADH dehydrogenase and cyt bc$_1$ complex couple quinone redox chemistry to proton translocation...
across the inner mitochondrial or bacterial cellular membrane to generate an electrochemical proton gradient and thereby power ATP synthesis [1,3]. In the ETC of photosynthesis, photosystem II utilises light energy to reduce quinone, and cyt b\textsubscript{6}f complex [9–11], a homologue of cyt bc\textsubscript{1} complex, oxidises quinol and passes electrons to photosystem I. Photosystem II and cyt b\textsubscript{6}f complex create a proton gradient across the chloroplast thylakoid membrane or the cyanobacterial plasma cellular membrane for ATP synthesis [12]. Therefore, cyt bc\textsubscript{1} and cyt b\textsubscript{6}f complex are substantial contributors to the driving forces of cellular energy conversion.

Cyt bc\textsubscript{1} and cyt b\textsubscript{6}f complexes form a large group of enzymes which all include a Rieske iron-sulfur protein (ISP), a b-type cytochrome (cyt b or cyt b\textsubscript{0},SUIV, ‘subunit four’) and a c-type cytochrome (cyt c\textsubscript{1}, cyt f or di-haem cyt cc) as the core catalytic module (Figure 1F,G) [2,13,14]. Cyt bc\textsubscript{1} and cyt b\textsubscript{6}f complexes are found in organisms from diverse phylogenetic clades [13], and they differ in composition in respect to number and types of peripheral subunits [10,15–17]. In actinobacteria, the catalytic Rieske ISP, cyt b, cyt cc and the cyt aa\textsubscript{3} oxidase plus peripheral subunits comprise the cyt bcc-aa\textsubscript{3} supercomplex [18,19]. Therefore, they are collectively referred to as cyt bc complexes in this mini-review.
In respiratory and photosynthetic ETCs, the overall forward reaction of cyt bc complexes is to oxidise quinol molecules and to reduce cytochrome c or plastocyanin, which will further transfer the electron to cyt c oxidase or photosystem I, respectively. Cyt bc complexes do not directly pump protons across the membrane such as for instance cyt c oxidases, instead, proton translocation is achieved through the Mitchellian Q cycle mechanism (Figure 2) [2,11,20–24]. As the first step in a Q cycle, a quinol molecule is oxidised at the quinol oxidation (Qo) site of cyt bc complex close to the positive side (P-side) of the membrane (Figure 1F). Next, using the mitochondrial cyt bc1 complex as an example, one electron of ubiquinol is transferred to the Rieske iron-sulfur cluster (FeS) and subsequently to haem c. The extrinsic domain of Rieske ISP undergoes a substantial conformational change [16,25–27] to bridge the 24 Å distance between the Qo site quinol and haem c (Figure 2).

Figure 2. The Q cycle mechanism.
Catalytic centres of dimeric cyt bc1 complex from S. cerevisiae are illustrated in two ways. The structure of the right half shows the inhibitor stigmatellin and the natural substrate UQ-6 in the X-ray structure (pdb 2ibz) as well as their hydrogen bonding partners. His181RIP1 is also a ligand of the iron-sulfur cluster (FeS). Stigmatellin represents the position of a transition state of ubiquinol (QH2) oxidation in the Qo site, and UQ-6 indicates the position of ubiquinone/semiubiquinone (Q/SQ) in the Qi site. The structure of the left schematically shows the Q cycle mechanism. The four highly conserved residues of cyt b (COB): Pro271COB (P), Glu272COB (E), Trp273COB (W) and Tyr274COB (Y) in the Qi site form the Qo motif [14]. Electron transfer in cyt bc1 complex can also cross the dimeric enzyme (pink arrow) [111]. Owing to the large-scale movement of the extrinsic domain (ED) of Rieske iron-sulfur protein (RIP1), the FeS is shown at two positions, the b-position (based on pdb 2ibz) close to the Qo site quinol and the c-position close to haem c (based on pdb 1be3). Whereas the Qo site ubiquinol releases two electrons and two protons upon oxidation, only one electron is transferred to Qi site, therefore the full reduction in the Qi site quinone requires oxidation of a second ubiquinol molecule at the Qo site and the uptake of two protons. The exact sequence of protonation steps at the Qi site is not differentiated in this simplified scheme. Iron atoms are depicted in brown, sulfur atoms are shown in yellow. The FeS and its ligand His181RIP1 at the c-position are shown in gray scale. Hydrogen bonds are depicted as dashed lines. Electron transfer pathways are shown in red, and proton release and uptake routes are in green.

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Physiological electron transfer rates typically require a maximal distance of 14 Å between electron donor and acceptor [28,29]. The other electron is routed through the low potential haem $b_1$, the high potential haem $b_1$, and reduces a quinone molecule in the $Q_i$ site to a semiquinone radical (SQ$^\cdot$). In this process, the $Q_o$ site quinol releases two protons to the P-side of the membrane and the complete reduction and protonation of a quinone molecule in the $Q_i$ site needs oxidation of a second quinol at the $Q_o$ site and proton uptake from the N-side of the membrane. Consequently, bifurcated electron transfer must be achieved upon quinol oxidation to enable the Q cycle, i.e. the highly reactive SQ$^\cdot$ at the $Q_o$ site must be controlled to avoid short circuits [29–34] which lead to futile bypass reactions which would lower the efficiency of cellular respiration and can generate reactive oxygen species [29] that can cause oxidative damage to the cell [35].

Experimental structures of cyt $bc$ complexes are essential to understand the molecular basis for efficient and safe electron and proton transfer mechanisms at $Q_o$ and $Q_i$ site. Position, geometry and distance of electron donors and acceptors, of substrate and analogous molecules as well as of prosthetic groups, are important to define electron transfer pathways [28]. Resolved positions of protonable amino acid side chains, hydronium ions (H$_3$O$^+$) or water molecules enable to identify proton transfer pathways [36]. Owing to the central role of cyt $bc$ complexes in cellular respiration and in photosynthesis, structural biology studies of these complexes based on X-ray crystallography and cryogenic electron microscopy (cryo-EM) have delivered, over the years, a great number of experimental structures of mitochondrial cyt $bc_1$ complexes [16,17,37–40] as well as of respiratory supercomplexes [41–48], alpha-proteobacterial cyt $b_1$ complexes [27,49,50], cyanobacterial [9] and chloroplast [10,51] cyt $b_6f$ complexes and actinobacterial cyt $bcc$–$aa_3$ supercomplexes [19,52–54]. One should note that, the electrochemical properties of the redox-active centres of cyt $bc$ complexes co-evolved with those of their native quinone substrates [13,55–57]. Hence, comparison of structures of cyt $bc$ complexes with bound substrates sampled from a wide spectrum of organisms sheds light on the conserved structural basis of the Q cycle’s quinone catalysis as well as on adaptations reflecting its molecular evolution, and may support development of medications precisely targeting different pathogens.

**Quinone binding positions at the $Q_o$ site**

In cyt $bc_1$ and $b_6f$ complexes, the $Q_o$ site is embedded in subunit cyt $b$ and at the interface with the mobile extrinsic domain of Rieske ISP (Figs. 1F, 2). The native substrate at the $Q_o$ site is quinol, the reduced form of quinone, and the oxidised reaction product quinone has to leave the catalytic position at the $Q_o$ site. So far, native quinone or quinol molecules were not resolved at the catalytic $Q_o$ site position in X-ray crystallography studies (Table 1), in particular because crystal formation requires a defined conformation of the complex, and the unrestrained motion of the extrinsic domain of Rieske ISP may hinder this process. Consequently, the characterisation of the binding mode of the substrate in the $Q_o$ site was supported by the use of inhibitors, and three binding positions at the $Q_o$ site were suggested [58]. The proximal position (Figure 2) was assigned with myxothiazol, which is hydrogen-bonded solely to Glu272 of cyt $b$ (Glu272$^\text{COB}$, yeast numbering) and shows no interaction to Rieske ISP [37]. The distal binding position (Figure 2) is exemplified by HHDBT, which is hydrogen-bonded to the iron-sulfur-cluster (Fe$S_5$) ligand (His181$^{RIP_1}$) of the Rieske protein, and to Glu272$^\text{COB}$ with a water-mediated hydrogen bond [59]. The third binding position is characterised with stigmatellin, which is hydrogen bonded directly to both Glu272$^\text{COB}$ and His181$^{RIP_1}$ [17] (Figure 2). Stigmatellin also binds at the $Q_o$ site of the cyt $bcc$–$aa_3$ supercomplex of Corynebacterium glutamicum in a similar manner as in the mitochondrial cyt $bc_1$ complexes [19], therefore it exhibits a conserved binding pose in the $Q_o$ sites of cyt $bc$ complexes which oxidise respectively ubiquinone or menaquinone. The $Q_o$ site pocket is unlikely to accommodate two isoprenoid quinol molecules simultaneously due to spatial constraints, thus these aforementioned three inhibitor binding positions may reflect the locations of reaction intermediates in different oxidation or protonation states, as well as their interactions with potential proton acceptors [22,60,61]. One of the proton acceptors is His181$^{RIP_1}$, which undergoes a $pK_a$ change dependent on the Rieske protein redox state [62,63]. The other hypothetical proton acceptor is Glu272$^\text{COB}$. Its substitution with other residues by mutagenesis partially compromises the turnover of the enzyme [14] but its exact function remains elusive. Glu272$^\text{COB}$ is the second residue of the $Q_o$ motif of cyt $b$, a highly conserved motif of four consecutive amino acid residues (Figure 2) present in all cyt $bc$ complexes with systematic phylogenetic variations (PEWY in mitochondrial cyt $b$) [14]. The type of residue at the second position of the $Q_o$ motif is correlated with the redox midpoint potential of cyt $bc$ complex cofactors as well as with the quinone species [14]. Substrate binding positions in experimental structures of cyt $bc$ complexes from different organisms would be very important to derive the conserved structural basis of catalysis as well as species-specific adaptations.
Recently, native co-isolated quinone molecules at or in proximity to the Qo site were identified in several cryo-EM structures of respiratory chain supercomplexes (Table 1). In a mammalian respiratory I/III2 supercomplex [48], which is composed of a NADH dehydrogenase (complex I) and a dimeric cyt bc1 complex (complex III2), an ubiquinone molecule was identified in the Qo site which is distal to complex I, whereas the Qo site proximal to the quinone reduction tunnel of complex I was unoccupied (Figure 3A). The authors proposed that the Qo site close to complex I would accept ubiquinol reduced by complex I as they share the shortest diffusion distance [48]. The cryo-EM structure of cyt bc1 complex from Candida albicans contains a ubiquinone molecule in the Qo site of both protomers [40] (Figure 3B). By superimposition of the mammalian supercomplex I/III2 with Candida albicans complex III, and yeast cyt bc1 complex co-crystallised with stigmatellin, a trajectory of Qo site occupants can be deduced (Figure 3C). In comparison, stigmatellin reached deepest into the Qo site pocket. The ubiquinone molecules resolved in the cryo-EM structures only partially overlap with the stigmatellin binding position. Concomitantly, the FeS cluster of the cryo-EM structures are further apart from the Qo site. The FeS of the yeast cyt bc1 complex is located at the closest distance to the Qo site, as it is constrained by a hydrogen bond from its own ligand His181RIP1 to stigmatellin (Figure 2). In contrast, the FeS clusters of the mammalian supercomplex I/III2 and the Candida albicans complex III are more distant from the Qo site. The distances between ubiquinone and the FeS histidine ligand in these two complexes are larger than 4.5 Å, which is too long for a hydrogen bond. These two positions in the cryo-EM structures likely represent the states of ubiquinone, the product of ubiquinol-oxidation, exiting the catalytic Qo site position.

In prokaryotes, a co-isolated menaquinone at the Qo site was resolved in two cryo-EM structure of the actinobacterium Mycobacterium smegmatis [19,54] (Figure 4A). This menaquinone molecule is positioned in ∼6 Å distance to the closest possible H-bonding partners His355QcrA and Tyr153QcrB, respectively (QcrA and QcrB are homologous to mitochondrial Rieske ISP and cyt b), and is 9.4 and 13.7 Å apart from FeS and haem b1, respectively [19]. In a cryo-EM structure of the actinobacterial cyt bcc-aa3 supercomplex from Mycobacterium smegmatis, a menaquinone molecule was described in 14 Å and 16 Å distance from FeS and haem b1, respectively (Figure 4B) [52]. This binding position agrees with a menaquinone molecule resolved in another M. smegmatis cryo-EM structure [64], as well as a menaquinone molecule identified

Table 1 Structures of cyt bc complexes with bound native quinone molecule resolved

| Position | Year | Complex type | PDB | Res (Å) | Method | Origin |
|----------|------|--------------|-----|---------|--------|--------|
| Qo       | 1998 | cyt bc1 complex | 1bcc | 3.16 | X-ray | Gallus gallus |
| Qo       | 2000 | cyt bc1 complex | 1ezv | 2.30 | X-ray | Saccharomyces cerevisiae |
| Qo       | 2003 | cyt bc1 complex | 1vf5 | 3.00 | X-ray | Mastigocladus laminosus |
| Qo       | 2005 | cyt bc1 complex | 1pp9 | 2.10 | X-ray | Bos taurus |
| Qo       | 2008 | cyt bc1 complex | 2qy  | 2.40 | X-ray | Rhodobacter sphaeroides |
| Qo       | 2018 | Supercomplex III2/IV2 | 6adq | 3.50 | cryo-EM | Mycobacterium smegmatis |
| Qo       | 2018 | Supercomplex III2/IV2 | 6hhw | 3.30 | cryo-EM | Mycobacterium smegmatis |
| Qo       | 2019 | Supercomplex III2/IV2 | 6q9e | 3.90 | cryo-EM | Ovis aries |
| Qo       | 2019 | cyt bc1 complex | 6qf  | 3.60 | cryo-EM | Spinacia oleracea |
| Qo       | 2019 | Supercomplex III2/IV2 | 6giq | 3.23 | cryo-EM | Saccharomyces cerevisiae |
| Qo       | 2019 | Supercomplex III2/IV2 | 6hu9 | 3.35 | cryo-EM | Saccharomyces cerevisiae |
| Qo       | 2020 | cyt bc1 complex | 6ks  | 3.30 | cryo-EM | Aquifex aeolicus |
| Qo       | 2021 | cyt bc1 complex | 7ja  | 3.00 | cryo-EM | Candida albicans |
| Qo       | 2021 | Supercomplex III2/IV2 | 7e1v | 2.68 | cryo-EM | Mycobacterium tuberculosis/smeagmatis |
| Qo       | 2021 | Supercomplex III2/IV2 | 7q21 | 2.90 | cryo-EM | Corynebacterium glutamicum |
| Qo       | 2022 | Supercomplex III2/IV2 | 7qhm | 2.80 | cryo-EM | Corynebacterium glutamicum |
| Qo       | 2022 | Supercomplex III2/IV2 | 7qho | 3.10 | cryo-EM | Corynebacterium glutamicum |

Binding position was assigned according to authors’ descriptions in the original publications (see text for references). The pdb code, resolution (Res) and experimental method of each entry are sourced from RCSB PDB (https://www.rcsb.org).
Figure 3. Positions of natural quinone molecules resolved in supercomplex I/III2, cyt bc1 complex and cyt bcc-aa3 supercomplex. Part 1 of 2
(A) cryo-EM structure of cyt bc1 complex as part of the supercomplex I/III2 from sheep. The cryo-EM map of cyt bc1 complex was reconstructed from focused-refinement of four supercomplex I/III2 maps representing different conformational states [48], therefore the outlines of all four corresponding supercomplex structures were all illustrated. Co-ordinates of supercomplex I/III2 were superposed on one cyt b of pdb 6q9e using secondary structure matching in Coot [112]. (B) cryo-EM structure of cyt bc1 complex from Candida albicans [40]. (C) Comparison of stigmatellin and ubiquinone-10 (UQ-10) binding positions by superimposition of the co-ordinates of the cyt bc1 complex structure. Cyt b of the yeast (S. cerevisiae) cyt bc1 complex (pdb 2ibz, with stigmatellin [67]) was used as reference, and cyt b of pdb 6q9e (O. aries), and pdb 7rja (C. albicans) were superposed using secondary structure matching in Coot [112]. The FeS of all structures are shown, whereas only the haem bL of pdb 2ibz is displayed for the sake of clarity. All distances are in Å. (D) Comparison of ubiquinone binding positions at the Qi site. Water molecule is labelled as W. Haem bH and the side chains of Asp229COB, His202COB are from pdb 2ibz. (E) cryo-EM structure of cyt bcc-aa3 supercomplex (supercomplex
in the hybrid supercomplex composed of the M. tuberculosis cyt bcc complex and M. smegmatis cyt aa₃ oxidase [65] (Figure 4C). By superimposition of the structures of the corynebacterial supercomplex with stigmatellin [19], with menaquinone [19,54], and the mycobacterial supercomplex structures with menaquinone [52,64,65], genus-specific consensus menaquinone binding positions can be deduced (Figure 4D). The locations of FeS in these structures are static. The menaquinone molecules in the two structures of the corynebacterial supercomplex both partially overlap with the stigmatellin binding position, whereas the menaquinone molecules of the three structures of the mycobacterial supercomplex were consistently located closer to the entrance of the quinone exchange cavity (Figure 4D). These experimentally resolved menaquinone molecules likely illustrate a migration path to the catalytic position of menaquinol, which is represented by the transition state analogue stigmatellin [66,67]. Interestingly, the Qₐ site menaquinone position assigned in a M. smegmatis supercomplex (pdb 6hw6, Figure 3D) [53] does not agree with the Qₐ site menaquinone positions shown in other four actinobacterial supercomplex structures and its naphthoquinone ring was resolved in 21 Å and 19 Å to FeS and the haem b₁ iron [53], therefore this model is not included in Figure 4D.

In addition to ubiquinone and menaquinone at the Qₐ site, a plastoquinone was described in the cryo-EM structure of cyt bₙf complex from spinach chloroplasts [51], with its benzoquinone ring 26.4 Å apart from FeS and 16.2 Å from haem b₁. It was described as in an approaching position to the Qₐ site (Figure 5A). Moreover, the entrance of the Qₐ site in this structure is partially blocked by the phytol tail of chlorophyll (Chl), which was suggested to gate the Qₐ site access [51].

Although quinone molecules were resolved in the Qₐ site of cyt bc complexes in several positions, structural information of the natural substrate in the catalytic relevant position in the Qₐ site with close distance to electron and proton acceptors is still lacking. So far, only the structures with inhibitors bound at the Qₐ site suggest the potential proton acceptors for quinol oxidation. Taken together, the cryo-EM structure of the bovine supercomplex I/III₂ provided a first hint of a co-isolated quinone in the Qₐ pocket in the context of substrate exchange between complexes I and III. The diverse binding positions of native co-purified ubiquinone, menaquinone and plastoquinone molecules resolved in structures of cyt bc complexes, most likely exemplify snapshots of their migration paths in and out of the active site and stand-by positions.

### Quinone binding positions at the Qₐ site

In contrast with the Qₐ site characterisation, many X-ray and cryo-EM structures of cyt bc complexes described co-purified quinone molecules in the Qₐ site. A plausible explanation is that the Qₐ site substrate has to be stabilised within the cyt b pocket to ensure a full Q cycle turnover with the two-step reduction to semiquinone and quinol, which is strictly coupled to the oxidation of two quinol molecules in the Qₐ site (Figure 2). Binding poses of Qₐ site ubiquinone including ordered water molecules were obtained with high resolution X-ray structures of bovine [38], chicken [39,68], yeast cyt bc₁ complexes (Figure 5D) [17] and that from Rhodobacter sphaeroides (Figure 5B) [69]. In brief, the Qₐ site ubiquinone is consistently located within ca. 5 Å distance to the porphyrin ring of haem b₁ (Figure 3D) in the different structures. Two proposed proton transfer pathways were assigned from the protein surface on the mitochondrial matrix side (the electro-negative side) to Asp229COB and His202COB (yeast numbering, Figs. 2, 3D). Each residue is connected via hydrogen bonds to a carbonyl group of the Qₐ site ubiquinone. The exact hydrogen bond pattern, whether it is a direct interaction or mediated by water molecules, varies in X-ray structures of the complex from different species [21]. That the binding of the Qₐ site inhibitor antimycin A replaced the natively occupied ubiquinone with Asp229COB as its direct interaction partner (in the bovine structure, pdb 1ppj) [38].

X-ray crystallographic analysis resolved highly ordered quinone molecules in the Qₐ site of crystallised cyt bc₁ complex. The power of cryo-EM to better cope with global or local protein dynamics brought forward a higher
Figure 4. Positions of natural quinone molecules resolved in cyt bcc-aa₃ supercomplex. Part 1 of 2

(A) cryo-EM structure of cyt bcc-aa₃ supercomplex (supercomplex III₂/IV₂) from Corynebacterium glutamicum [19]. (B) cryo-EM structure of cyt bcc-aa₃ supercomplex (supercomplex III₂/IV₂/SOD₂) from Mycobacterium smegmatis [52]. (C) cryo-EM structure
of a hybrid cyt bcc-aa₃ supercomplex (supercomplex III₂/IVₑ) with complex III₂ from M. tuberculosis and complex IVₑ from M. smegmatis [65]. (D) Comparison of stigmatellin and menaquinone-9 (MK-9) binding positions by superimposing the co-ordinates of cyt bcc-aa₃ supercomplex. The QcrB of the C. glutamicum cyt bcc-aa₃ supercomplex (pdb 7qhm, with stigmatellin) was used as the reference, and the QcrB of pdb 7qho (C. glutamicum), pdb 7q21 (C. glutamicum), pdb 6adq (M. smegmatis), pdb 7rh5 (M. smegmatis), and pdb 7e1v (M. tuberculosis) were superposed using secondary structure matching in Coot [112]. The FeS of all structures are shown, whereas only the haem b₁ of pdb 7q21 is displayed for the sake of clarity. Cor. and Myc, respectively, indicate the consensus position of the naphthoquinone ring of MK-9 in the corynebacterial and mycobacterial structures. (E) Comparison of MK-9 resolved in the Q₁ site. The Glu38QcrB side chain and haem b₁ are from pdb 7qhm. All distances are in Å.

Figure 4. Positions of natural quinone molecules resolved in cyt bcc-aa₃ supercomplex. Part 2 of 2

variety of quinone binding modes at the Q₁ site. In cryo-EM structures of mitochondrial respiratory chain supercomplexes, of the yeast supercomplex III₂/IVₑ [47] (Figure 5E) and the ovine supercomplex I/III₂ [48] (Figure 3A) one ubiquinone molecule was resolved in each Q₁ site, in a position consistent to the known binding poses in X-ray structures of mitochondrial cyt bc₁ complexes (Figs. 3D, 5D). In contrast, in the cryo-EM structure of yeast supercomplex III₂/IVₑ [46], an ubiquinone ring was modelled on the internal two-fold symmetry axis of the dimeric cyt bc₁ complex with two alternate conformations (Figure 5F). The distance from the quinone ring to haem b₁ of each protomer is 15.3 Å.

In actinobacterial respiratory supercomplexes, a menaquinone molecule was identified in the Q₁ site of the cyt bcc-aa₃ supercomplex of C. glutamicum, M. smegmatis, and M. tuberculosis (Figs. 3E, 4A–C) [19,52–54,65]. The interaction mode between haem b₁ and menaquinone is very similar to that of ubiquinone in cyt bc₁ complexes (Figure 4F). In contrast with mitochondrial cyt bc₁ complexes, in which protons could be delivered to the Q₁ site ubiquinone via a histidine and an aspartate residue, of which the side chains have direct or water-mediated hydrogen bonds to both carbonyl groups of the quinone, the menaquinone molecule resolved in the Q₁ site of the cyt bcc complex from C. glutamicum is single hydrogen-bonded directly to a glutamate side chain (Figure 4E) [19]. Interestingly, a second menaquinone was identified near the Q₁ site of the bcc complex from M. smegmatis [52], with its naphthoquinone ring in 3.6 Å distance to that of the other menaquinone in the Q₁ site (Figure 4B). This short distance between the two menaquinone molecules in and close to the Q₁ site would allow a consecutive reduction from one to the other. Menaquinone and ubiquinone are quinone species of low (−78 mV) and high (+90 mV) redox midpoint potential, respectively [13,14,55–57]. The hyperthermophilic Aquifex aeolicus uses demethylmenaquinone (DMK) which has a potential of +36 mV [70], giving it a transitional position in the evolution of cyt bc complexes from low to high potentials [71]. The cryo-EM structure of the A. aeolicus cyt bc₁ complex with bound DMK molecules at the Q₁ site (Figure 5C) revealed a 6.1 Å distance from the naphthoquinone ring to haem b₁ [72], which is in good agreement with the binding mode of the Q₁ site ubiquinone in yeast and Rhodobacter homologues as well as the Q₁ site menaquinone of the actinobacterial cyt bcc-aa₃ supercomplex.

The most unique Q₁ site architecture of cyt bc complexes is found in cyt bcf complexes. The position equivalent to the aforementioned ubiquinone and menaquinone ring plane in the Q₁ site is replaced by a high spin c-type haem (haem c₁), which is attached via a single thioether bond to cyt b₄, which has no amino acid axial ligand [9,10]. A recent cryo-EM structure of spinach cyt bcf complex revealed the position of a plastoquinone molecule at the Q₁ site (Figure 5A) [51]. The benzoquinone ring of this plastoquinone molecule is 4.4 Å apart from the haem c₁ porphyrin ring. In addition, one of its carbonyl groups is hydrogen-bonded to a propionate carboxylate of haem c₁ in 3.2 Å. Notably, the Q₁ site plastoquinone breaks the internal two-fold symmetry of cyt bcf complex (Figure 5A). The isoprenoid tail of the Q₁ site plastoquinone extends into the entrance of the unoccupied Q₁ site of the other protomer while a second plastoquinone was modelled in a diagonal position with respect to the Q₁ site plastoquinone, in a position approaching the Q₃ site of the other protomer [51]. In addition, the Q₁ site occupancy of plastoquinone seems to be correlated to the orientation of the propionate group of haem c₁, which may control access to a potential proton transfer pathway from the stromal side (the electronegative side) via Asp20 and Arg207 [51]. It was therefore hypothesised that both Q₁ sites are not simultaneously functional [51].

Whereas high-resolution X-ray structures revealed detailed binding modes of the Q₁ site ubiquinone in mitochondrial cyt bc₁ complexes, cryo-EM structures more recently provided additional information of ubiquinone positions in the context of supercomplexes, and previously unavailable structures of plastoquinone and
Figure 5. Positions of natural quinone molecules resolved in cyt $b_6f$ and bc$_1$ complexes. Part 1 of 2

(A) cryo-EM structure of cyt $b_6f$ complex (pdb 6rql) from spinach [51]. (B) X-ray structure of cyt bc$_1$ complex (pdb 2qjy) from Rhodobacter sphaeroides [69]; (C) cryo-EM structure of cyt bc$_1$ complex (pdb 6klk) from Aquifex aeolicus [72]; (D) X-ray structure of cyt bc$_1$ complex (pdb 2ibz) from baker’s yeast [67]. The deposited structure contains only one protomer which belongs to the crystallographic asymmetric unit. Here the dimeric structure shown was generated by applying symmetry operation; (E) cryo-EM structure of a supercomplex containing a dimeric cyt bc$_1$ complex and a monomeric cyt c oxidase.
Inhibitors bound at $Q_0$ or $Q_1$ site of cyt $bc$ complexes

The use of $Q_0$ and $Q_1$ site inhibitors was instrumental in studies of cyt $bc_1$ complexes in order to explore the molecular basis of the Q cycle mechanism and to elucidate electron transfer pathways [58]. Their binding positions in $Q_0$ and $Q_1$ site, in particular that of stigmatellin [16,17], myxothiazol, UHDBT, NQNO and antimycin A [37] were all analyzed as early as the first X-ray structures of cyt $bc_1$ complexes were determined (Table 2). Stigmatellin is a semiquinone analogue, i.e. it mimics a transition state of quinol oxidation and reduction [66,67], which is difficult to be captured in protein crystals or cryo-EM specimens with natural substrates. Therefore, its binding poses in the $Q_0$ site of cyt $bc_1$ complex [17] and cyt $bcc$ complex [19] provide insights in the catalytic position from which the protons and electrons are released to their respective acceptors. Parallel to fundamental research, cyt $bc_1$ complex inhibitors are also of great agricultural and medical importance: Azoxystrobin [37,73] belongs to the strobilurins [74], a group of chemically similar compounds [75] which accounted for 27% of the total fungicide worldwide sales in year 2015 [76]. The $Q_0$ site inhibitor Famoxadone is a fungicide for crops [77]. Atovaquone [78] is used in a fixed-dose combination with proguanil as antimalarial drug [79–81], and is also used for treating pneumocystis infection [82]. Note that both, atovaquone and strobilurin inhibitors target the $Q_0$ site, however, resistances were identified soon after these compounds were made commercially available [81,83–85]. Consequently, development of cyt $bc_1$ complex inhibitors targeting the $Q_1$ site could provide a chance to bypass this issue [86,87]. Interestingly, in the past 5 years, almost all new antimalarial drug candidates resolved in structures of cyt $bc_1$ complexes published in the RCSB protein data bank (PDB, www.rcsb.org) are $Q_1$ site inhibitors (Table 2). This includes the X-ray structures of cyt $bc_1$ complex inhibited by the antimalarial 4(1H)-pyridones GSK 932121 and GW844520 [88], MJM170 [89], and a 2-pyrazolyl quinolone WDH2G7 [90]. Although X-ray structures can deliver information on protein–ligand interaction with atomic detail, structure-based drug discovery is often hindered by the amount of protein available, time required for crystallisation trials, and conformational heterogeneity or dynamic properties of proteins. The cryo-EM structures of cyt $bc_1$ complex with bound compounds SCR0911 and GSK 932121 [91] exemplified the scope of cryo-EM structures to characterise binding of drug candidates to target proteins with dynamic properties. Cryo-EM structures of the Mycobacterium cyt $bcc$-aa$_3$ complex with the tuberculosis drug candidate telacebec (Q203) [92] and with TB47 bound at the $Q_0$, site demonstrated this approach for bacterial cyt $bc$ complexes and supercomplexes [64,65]. Cryo-EM has the advantage of lower sample consumption for single particle analysis as compared with X-ray crystallography. This is especially important for proteins isolated from scarce sources such as patient tissue [93], or from pathogens which are difficult or dangerous to cultivate [94]. In this respect, cryo-EM also opens new possibilities in obtaining structural information of cyt $bc$ complexes to develop novel human medications as well as agrochemicals [95–98].

Detergent, lipids and the native membrane

Owing to the nature that membrane proteins are located in the lipidic compartments of the cell [99], structural biology studies of membrane proteins have greatly benefited from the use of detergents to solubilise them from their native environment into aqueous solution. Detergent molecules bind to hydrophobic surfaces of membrane proteins and increase their solubility in aqueous environment. Detergents differ in their chemical and physical properties and the selection of the type of detergent is key to prepare well-diffracting membrane protein crystals [100] as well as cryo-EM grids with good contrast and particle distributions [101]. However, detergents compete with the binding of lipids and lipidic compounds such as quinone thus delipidation is unavoidable. Severe delipidation compromises the stability and eventually the integrity of isolated membrane proteins, which may cause artificial structural disorder and may account for poor resolution of X-ray and cryo-EM structures. Reintroducing the detergent solubilised membrane protein back into lipidic cubic phase
Table 2. Structures of cyt bc complexes with bound non-native compounds and their application  

| Position | Year | Non-native compound | PDB  | Res (Å) | Method | Origin | Applications |
|----------|------|----------------------|------|---------|--------|--------|--------------|
| Qi       | 1998 | Antimycin            | 3bcc | 3.70    | X-ray  | Gallus gallus | Research     |
| Qo       | 1998 | Stigmatellin         | 3h1j | 3.00    | X-ray  | Gallus gallus | Research     |
| Qo       | 2000 | Stigmatellin         | 1ezv | 2.30    | X-ray  | Saccharomyces cerevisiae | Research |
| Qo       | 2003 | Famoxadone           | 10l  | 2.35    | X-ray  | Bos taurus    | Fungicide    |
| Qo       | 2003 | NOQ                  | 1nu1 | 3.20    | X-ray  | Bos taurus    | Research     |
| Qo       | 2003 | Tridecylstigmatellin | 1v5f | 3.00    | X-ray  | Mastigocladus laminosus | Research |
| Qo       | 2004 | Azoxystrobin         | 1sqb | 2.69    | X-ray  | Bos taurus    | Fungicide    |
| Qo       | 2004 | HHDBT                | 1p84 | 2.50    | X-ray  | Saccharomyces cerevisiae | Research |
| Qo       | 2004 | MOAS                 | 1sqq | 3.00    | X-ray  | Bos taurus    | Fungicide    |
| Qo       | 2004 | Myxothizol           | 1sqp | 2.70    | X-ray  | Bos taurus    | Research     |
| Qo       | 2004 | HHDBT                | 1sqv | 2.85    | X-ray  | Bos taurus    | Research     |
| Qi       | 2005 | Antimycin A          | 1ppj | 2.10    | X-ray  | Bos taurus    | Research     |
| Qo       | 2005 | Stigmatellin         | 1pp9 | 2.10    | X-ray  | Bos taurus    | Research     |
| Qo       | 2006 | JG144                | 2hyu | 2.26    | X-ray  | Bos taurus    | Fungicide    |
| Qo       | 2006 | Stigmatellin         | 2hyn | 3.20    | X-ray  | Rhodobacter sphaeroides | Research |
| Qo       | 2007 | NOQ                  | 2e75 | 3.55    | X-ray  | Mastigocladus laminosus | Research |
| Qo       | 2008 | Croccacin-D iodinated analogue | 3cwb | 3.51 | X-ray | Gallus gallus | Fungicide |
| Qo       | 2008 | Stigmatellin         | 2qy  | 2.40    | X-ray  | Rhodobacter sphaeroides | Research |
| Qo       | 2010 | Ascochlorin          | 3h1l | 3.21    | X-ray  | Gallus gallus | Anti-Trypanosomiasis |
| Qo       | 2010 | Azoxystrobin         | 3i71 | 2.84    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2010 | Famoxadone           | 3i74 | 2.76    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2010 | Fenamidone           | 3i75 | 2.79    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2010 | Kresoxim-methyl      | 3i72 | 3.06    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2010 | Kresoxim-methyl iodinated derivative | 3h1k | 3.48 | X-ray | Gallus gallus | Fungicide |
| Qo       | 2010 | Triazalone           | 3i73 | 3.04    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2010 | Trioxystrobin        | 3i70 | 2.75    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2011 | Stigmatellin         | 2yu  | 2.70    | X-ray  | Paracoccus denitrificans | Research |
| Qo       | 2012 | MOA-like (WF3)       | 3tgw | 2.70    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2014 | Atovaquone           | 4pd4 | 3.04    | X-ray  | Saccharomyces cerevisiae | Antimalarial |
| Qo       | 2015 | 4(1H)-pyridone GSK932121 | 4d6u | 4.09 | X-ray | Bos taurus | Antimalarial |
| Qo       | 2015 | 4(1H)-pyridone GWB44520 | 4d6t | 3.57 | X-ray | Bos taurus | Antimalarial |
| Qo       | 2015 | Famoxadone           | 5kz  | 2.97    | X-ray  | Rhodobacter sphaeroides | Fungicide |
| Qo       | 2015 | MOA-like (Y52)       | 4u3f | 3.23    | X-ray  | Gallus gallus | Fungicide |
| Qo       | 2016 | Fenamidone           | 5klv | 2.65    | X-ray  | Bos taurus    | Fungicide    |
| Qo       | 2016 | MMJ170               | 5mn  | 3.50    | X-ray  | Bos taurus    | Anti-Apicomplexan |
| Qo       | 2018 | 2-pyrazolyl quinolone WDH2G7 | 6hav | 3.45 | X-ray | Bos taurus | Antimalarial |
| Qo       | 2018 | 4(1H)-pyridone GSK932121 | 6fo0 | 4.10 | cryo-EM | Bos taurus | Antimalarial |
| Qo       | 2018 | SCR0911              | 5kcd | 3.10    | X-ray  | Bos taurus    | Antimalarial |
| Qo       | 2018 | SCR0911              | 6fo6 | 4.10    | cryo-EM | Bos taurus | Antimalarial |
| Qo       | 2019 | Azoxystrobin        | 6nhh | 3.00    | X-ray  | Rhodobacter sphaeroides | Fungicide |
| Qo       | 2020 | Antimycin A          | 6klv | 3.20    | cryo-EM | Aquilex aeolicus | Research |
(L.C.P.) for crystallisation [102,103] and the application of lipidic nanodiscs in solubilisation or reconstitution of isolated membrane protein complexes for cryo-EM specimen preparation [104,105] have shown superior stabilisation effect so as to improve resolution. This can be exemplified by the X-ray structure of *Thermus thermophilus* cyt *caa*₃ oxidase (2.36 Å resolution, L.C.P. [106]), cryo-EM structures of *Escherichia coli* cyt bd oxidase (2.68 Å resolution, nanodiscs [107]) and the cryo-EM structure of *Paracoccus denitrificans* cyt *c* oxidase (2.37 Å resolution, nanodiscs [108]; all resolution of cryo-EM data refer to the FSC = 0.143 criteria for the same basis of comparison). Respiratory chain complexes and supercomplexes in nanodiscs may provide additional information about partitioning of co-purified quinone molecules and their trajectories to fully reflect the native electron transport chain in the hydrophobic environment. Finally, structural studies using *in situ* cryogenic electron tomography (cryo-ET) permits the determination of higher order assemblies of protein complexes as well as structural dynamics directly in cells [109]. Although many technical limitations, such as to resolve small molecules with sufficient resolution still need to be overcome, the rapid and intensive development of cryo-ET [110] will eventually allow to visualise the respiratory chain and photosynthesis complexes in cellular context and maybe in action.

### Perspectives

- Structural biology research of cyt bc complexes will contribute to an in-depth understanding of redox-driven proton translocation via the Q cycle and its regulation as well as support the design of fungicides, anti-malarial and anti-tuberculosis drugs.

- Structural characterisation of cyt bc complexes from a wide spectrum of species as well as in different types of supercomplexes is important to expand the knowledge on conserved and species-specific binding modes of native substrates, drugs, and inhibitors at the quinone binding sites.

- Structural information on the enzyme-substrate complex and defined catalytic states of cyt bc complexes is still lacking. We encourage that the cryo-EM specimens or crystals should be prepared in lipid environment.

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations
DMK, demethylmenaquinone; ETC, electron transport chain; ISP, iron-sulfur protein.

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