Studies on the structure and function of Na^+-pumping NADH-quinone oxidoreductase from *Vibrio cholerae*

Moe Ishikawa

The Na^+-pumping NADH-ubiquinone (UQ) oxidoreductase (Na^+-NQR) is an integral membrane protein complex composed of six subunits (NqrA–F), encoded by *nqrA-F* on the *nqr* operon, with a total molecular mass of about 200 kDa. Na^+-NQR couples electron transfer from NADH to UQ with Na^+-pumping, generating an electrochemical Na^+ gradient across the inner bacterial membrane, which is indispensable for many biological functions. Since Na^+-NQR is exclusively found in prokaryotes and structurally unrelated to mitochondrial H^+-pumping NADH-UQ oxidoreductase (respiratory complex I), it is a promising target for highly selective antibiotics. However, the molecular mechanisms are not well-understood for lack of atomic structural information such as an inhibitor-bound state. The author's approach based on the chemical and structural biology provided a definite foundation for understanding the function of Na^+-NQR and the molecular mechanism of its specific inhibitors. This knowledge would significantly boost the molecular design of novel antibiotics aimed at targeting this enzyme.

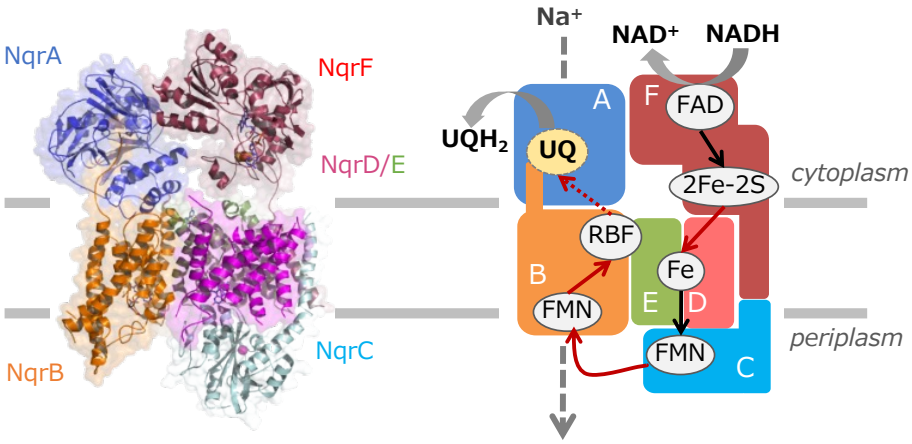

Fig. 1. The X-ray crystallographic structure of Na^+-NQR from *V. cholerae*. The positions of the six cofactors are schematically presented. The electron pathway from NADH to UQ is shown by arrows. The several pairs of redox cofactors, which are so far for effective electron transfer, are shown as red arrows. The distance from riboflavin (RBF) to UQ is shown as a dashed arrow since the correct distance is unknown due to lack of bound UQ in the crystallographic structure.

Ref: **1.** J. Steuber et al. (2014) *Nature* **516**, 62–67.

Chapter 1

Using korormicin-based high affinity ligands NAS-K1 and NAS-K2, the author succeeded in the pinpoint modification of Lys22 of the NqrB subunit, which resides in the unmodeled region of the N-terminal stretch in the X-ray crystallographic structure[33]. The fact that hydrophobic NAS-Ks interact with NqrB-Lys22 suggests that the nonmodeled region likely orient itself toward the membrane phase rather than protruding into cytoplasmic medium. This conformation may be essential for regulating the ubiquinone reaction in the adjacent NqrA subunit.

Chapter 2

The author determined high-resolution cryo-EM structures of Na^+-NQR with or without a bound inhibitors (korormicin A and aurachin D-42) at 2.5−2.9 Å. These structures elucidate the arrangement of all six redox cofactors including identification of a 2Fe-2S cluster located between the NqrD and NqrE subunits. As hypothesized in Chapter 1, the N-terminal region of NqrB orients itself toward the membrane phase by folding up at the cytoplasmic membrane surface, rather than protruding into cytoplasmic medium along the surface of NqrA. The structural works provide new insights into the structure/function of Na^+-NQR and the binding manner of specific inhibitors.

Chapter 3

The author investigated the relation between reduction of UQ and Na^+ translocation coupled with this reduction using a series of UQs with Na^+-NQR reconstituted into liposomes. The results revealed that the final UQ reduction step makes an important contribution to completing Na^+ translocation through the critical role of the UQ side chain. The author hypothesizes that the bound UQs with long side chains would prevent Na^+ once taken up from moving back to the cytoplasm.