Published in final edited form as:

Methods. 2011 December ; 55(4): 415–420. doi:10.1016/j.ymeth.2011.11.001.

Membrane Proteins in Four Acts: Function Precedes Structure Determination

W. A. Cramer^{1,*}, S. D. Zakharov^{1,8}, S. Saif Hasan¹, H. Zhang³, D. Baniulis⁴, M. V. Zhalnina¹, G. M. Soriano¹, O. Sharma⁵, J. C. Rochet⁶, C. Ryan⁷, J. Whitelegge⁷, G. Kurisu⁹, and E. Yamashita²

¹Purdue University, Department of Biological Sciences, Hall of Structural Biology, 240 Hockmeyer Hall, West Lafayette, IN 47907-1354, sshasan@purdue.edu, zhalnina@purdue.edu, gsoriano@purdue.edu

²Institute for Protein Research, Department of Macromolecular Science, Osaka University, Yamada-oka Suita, 565-0871, Japan, eiki@protein.osaka-u.ac.jp

³Abtuit Inc., 3065 Kent Avenue, West Lafayette, IN 47906, huamin.zhang@abtuit.com

⁴Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno str. 30, Babtai, LT-5433, Kaunas reg., LITHUANIA, danas.baniulis@katalogas.net

⁵Microbiology and Molecular Genetics, Harvard Medical School, Warren Alpert Building, Room No. 353, 200 Longwood Ave., Boston, MA 02115, USA, Onkar_Sharma@hms.harvard.edu

⁶Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907, jrochet@purdue.edu

⁷The Pasarow Mass Spectrometry Laboratory, University of California, Los Angeles, Box 42, NPI – Semel Institute, Los Angeles, USA, jpw@chem.ucla.edu

⁸Institute of Basic Problems of Biology, Russian Academy of Science, Pushchino, Moscow Reg., 142290 Russian Federation, zakharos@purdue.edu

⁹Department of Macromolecular Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Osaka, Japan, gkurisu@protein.osaka-u.ac.jp

Abstract

Studies on four membrane protein systems, which combine information derived from crystal structures and biophysical studies have emphasized, as a precursor to crystallization, demonstration of functional activity. These assays have relied on sensitive spectrophotometric, electrophysiological, and microbiological assays of activity to select purification procedures that lead to functional complexes and with greater likelihood to successful crystallization: **(I)**, Hetero-oligomeric proteins involved in electron transport/ proton translocation). **(1)** Crystal structures of the eight subunit heterooligomeric trans-membrane dimeric cytochrome *b₆f* complex were obtained from cyanobacteria using a protocol that allowed an analysis of the structure and function of internal lipids at specific intra-membrane, intra-protein sites. Proteolysis and monomerization that inactivated the complex and prevented crystallization was minimized through the use of

© 2011 Elsevier Inc. All rights reserved

*to whom correspondence should be addressed (waclab@purdue.edu tel. 765-494-4956).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

filamentous cyanobacterial strains that seem to have a different set of membrane-active proteases. (2) An NADPH-quinone oxido-reductase isolated from cyanobacteria contains an expanded set of seventeen monotopic and polytopic hetero-subunits. (II) β -barrel outer membrane proteins (OMPs). High resolution structures of the vitamin B₁₂ binding protein, BtuB, solved *in meso* and *in surfo*, provide the best example of the differences in such structures that were anticipated in the first application of the lipid cubic phase to membrane proteins (1). A structure of the complex of BtuB with the colicin E3 and E2 receptor binding domain established a “fishing pole” model for outer membrane receptor function in cellular import of nuclease colicins. (III) A modified faster purification procedure contributed to significantly improved resolution (1.83 Å) of the universal porin, OmpF, the first membrane protein for which meaningful 3D crystals have been obtained (2). A crystal structure of the N-terminal translocation domain of colicin E3 complexed to OmpF established the role of OmpF as an import channel for colicin nuclease cytotoxins. (IV) α -synuclein, associated with the etiology of Parkinson's Disease, is an example of a protein, which is soluble and disordered in solution, but which can assume an ordered predominantly α -helical conformation upon binding to membranes. When subjected in its membrane-bound form to a trans-membrane electrical potential, α -synuclein can form voltage-gated ion channels.

Keywords

cytochrome *b₆f*; NAD(P)H dehydrogenase (NDH-1); BtuB; OmpF; α -synuclein

Introduction

I. The **cytochrome *b₆f* complex** of oxygenic photosynthesis and the NAD(P)H dehydrogenase (complex I in the mitochondrial respiratory chain) are hetero-oligomeric membrane proteins, the most complex group of integral membrane proteins for which the smallest number of crystal structures have thus far been solved. The *b₆f* complex (3), a plastoquinone-plastocyanin oxidoreductase, a member of the family of cytochrome *bc* complexes (4), provides the link in the photosynthetic electron transport chain between plastoquinol (PQH₂), reduced by the photosystem II reaction center (PSII), and the photosystem I reaction center (PSI) (Fig. 1A). Crystal structures of the *b₆f* complex, in the native state and with different bound inhibitors, have been solved from cyanobacteria (5–8) and the green alga, *C. reinhardtii* (9).

II. The mitochondrial and bacterial NADH dehydrogenase, complex I (NDH), which accepts electrons from NADH ($E_{m7} = -0.32$ V) via a bound flavin mono-nucleotide, is the most reducing electron transport/proton translocation integral membrane protein complex in the respiratory chain. Thus, it has the potential to generate reactive oxygen species, a property that may be related to the correlation between mutations in this protein complex and neurodegenerative diseases (10). NDH complexes are among the largest membrane proteins in terms of total size, number of subunits, and of TMH, for which crystal structures have been obtained. Structures have separately been solved of (i) the soluble domain of the 14 subunit *E. coli* bacterial NDH-1 complex (11), (ii) the membrane-bound sector of this complex (12), (iii) the entire 15 subunit complex from *Thermus thermophilus* (12), and (iv) a 40 subunit, 947 kDa NDH complex from the aerobic yeast, *Yarrowia lipolytica* (13). The **cyanobacterial NDH-1** has properties distinct from the NDH-1 in other bacteria (14–16), including complexes of different size and subunit composition some of which are defined as NDH-1L, NDH-1M, and NDH-1MS (17, 16), the structure dependent on the ambient CO₂ concentration. The NDH-1L from *Thermosynechococcus elongatus* was proposed to contain 15 subunits (16).

III. Omp Proteins: BtuB and OmpF; the Rosenbusch Hypothesis (1). The integral outer membrane (Omp) proteins have the special property that they are predominately β - barrel proteins. The vitamin B₁₂ Omp translocator, BtuB, and the universal porin, OmpF, function in the import mechanisms of the cytotoxic colicins (18). Present structures of BtuB provide a test of the Rosenbusch Hypothesis, which provided the original impetus and concept behind the development of the lipid cubic phase methodology for crystallization of membrane proteins. The Hypothesis was based on the inference that preservation of the lateral pressure imposed on the intra-membrane proteins by lipids, the native physiological environment, would lead to a more highly stabilized and native structure (1). The outer membrane vitamin B₁₂ binding/translocation protein (BtuB), for which crystal structures have been obtained to a very similar resolution *in surfo* (2.00 Å, pdb 1NQE (19)) and *in surfo* (pdb 1GUF) (20) provides the best test of the question of whether crystallization in a lipid environment, in the lipid cubic phase (*in meso*) or in detergent (*in surfo*) makes a difference in the determined structure.

α -synuclein is a 140 residue protein implicated in the etiology of Parkinson's Disease (21). Upon binding to membranes containing phospholipids that are anionic and have negative curvature, its secondary structure which is disordered in solution, becomes predominantly α -helical (22).

Results and Discussion

I. The cytochrome *b₆f* complex; a system for studies on structure-function of integrally bound lipid. Utilization of a hydrophobic (propyl-agarose) column (23) during the purification of the cytochrome *b₆f* complex (plastoquinone-plastocyanin oxidoreductase), shown in the context of the electron transport and proton translocating chain of oxygenic photosynthesis (Fig. 1A) required a hydrophobic (propyl-agarose) column (23) during the purification of the cyanobacterial complex in order to separate the cytochrome complex from the light-harvesting phycobiliproteins (24) that are unique to this photosynthetic system. It was impossible to obtain meaningful crystals from this purified protein until it was realized that the protein complex had been delipidated, containing < 1 lipid molecule per monomer compared to the 7–8 lipids or lipid binding sites per monomer, some with unique functions, now known to be present in the complex (25). Crystals diffracting to 3.0 Å could, however, be obtained if lipid was added back to the cytochrome complex prior to crystallization. The lipid, DOPC, that was arbitrarily chosen for the first experiments allowed overnight formation of well-defined orthogonal crystals. Crystal structures for the cytochrome *b₆f* complex in two cyanobacteria, the filamentous *Mastigocladus laminosus* (5, 7) and *Nostoc* sp. PCC 7120 (8), could be obtained. A dependence on the presence of lipid for crystal quality and the details of the crystal structure was also found in early studies of the K⁺ (KcsA) channel (26) and the lactose permease (27).

The selection of different preparations of the cytochrome *b₆f* complex for crystallization utilized sensitive difference spectrophotometry to assay electron transfer activity. This assay of function was a selection tool in the development of the purification of the cytochrome complex for crystallization. With a rate-limiting step for photosynthetic electron transport of 1–10 msec, acceptable turnover rates of purified *b₆f* complex are 150–200 electrons/cytochrome *f*-sec. Hydrophobic chromatography (23, 24) is used to study the lipid dependence of structure-function of the *b₆f* complex.

The presence of four small single TMH, Pet G, L, M, and N in the *b₆f* complex form a hydrophobic “picket fence” at the periphery of each monomer (Fig. 1B) provides an interesting comparison with the bovine and yeast NDH structures, in which the presence of an abundance of such single TMH subunits has been noted, but not in this kind of array (28).

It has been hypothesized that this picket fence that surrounds each monomer has a role in the assembly of the *b₆f* complex, as summarized in the ‘hors d'oeuvres’ hypothesis for its assembly (29). It was proposed that the subunits in each picket fence, each of which has a net positive charge on the electrochemically negative side of the complex, insert spontaneously with favorable thermodynamics. The β -carotene molecule that is seen to be inserted through the picket fence, and which is separated by an unusually large (14 Å) distance for triplet energy transfer from the single chlorophyll *a* molecule in the complex (5, 30), could function as a “toothpick” in the assembly of the *b₆f* hors d'oeuvres, guiding the insertion of the polytopic subunits (e. g., subunit IV with 3 TMH) into the membrane bilayer (29). A special role has been proposed for the Pet G and Pet N subunits, which are essential for function (31), in stabilizing sub-domain interactions (25).

II. Cyanobacterial NAD(P)H Dehydrogenase (NDH-1). Using liquid chromatography coupled to electrospray-ionization mass spectrometry, with special attention paid to the low molecular weight region of the mass spectrum (32), two additional small subunits, NdhP and NdhQ, were found to be part of the cyanobacterial NAD(P)H dehydrogenase NDH-1L. The NDH-1 in *Thermosynechococcus elongatus* (33) has a total of 17 subunits, compared to 14 in *E. coli* and 15 in *T. thermophilus* bacterial complexes. The two additional subunits NdhP and NdhQ each code for one predicted TMH, contributing to a total of 3 such TMH in the smaller *T. elongatus* complex (Table I). It has been suggested that membrane protein subunits in hetero-oligomeric proteins with a single TMH have a special role in energy-transducing that define the binding site for the FMN electron acceptor of NADH membrane proteins (28). (It is of interest to compare these ideas with those suggested for the *b₆f* complex (29, 34, 25)). The 40-subunit yeast complex I codes for eight single TMH subunits, and the bovine NDH for fourteen. However, the crystal structure of the *E. coli* NDH-1 (12) does not indicate the presence of a subunit containing a single TMH (Table 1). A major conundrum is that the cyanobacterial NDH-1 does not contain the 3 subunits, NuoE, NuoF, and NuoG, which define the binding site for the FMN electron acceptor of NADH in the *E. coli* NDH-1 complex (15, 16) (Table I).

III. High Resolution Structures of the *E. coli* BtuB and OmpF Outer Membrane Proteins: Receptor-Mediated Colicin Import. (A) Crystal structures of BtuB provide a test of the Rosenbusch Hypothesis (1), the founding concept behind the development of the lipid cubic phase methodology. It was hypothesized that preservation of the lateral pressure imposed on the intra-membrane proteins by the lipids would lead to a more highly stabilized and native structure (1). Crystal structures of the outer membrane vitamin B₁₂ binding/translocation protein (BtuB), for which crystal structures have been obtained to a similar resolution, in detergent *in surfo* (2.00 Å resolution, PDB 1NQE (19)), and in the lipid cubic phase *in meso* (1.95 Å, PDB 1GUF (20)) currently provide the best test in the literature of whether the two crystallization methods make a significant difference in the resulting crystal structure. In fact, a notable difference between the *in meso* and *in surfo* structures of the apo-BtuB protein is seen in the N-terminal TonB box of BtuB. The N-terminal five residues of the TonB box are seen in the *in meso* structure, but not *in surfo*. The C α backbones diverge in opposite directions for the first two residues, Asp6-Thr7. This difference was proposed to be the conformational change associated with substrate binding and signal transduction across the membrane (19), but appears as well to be associated with the method of crystallization. Crystal structures were obtained for the complex of BtuB, which is the high affinity (< 1 nM) receptor for E-type colicins (37), and the receptor binding domains of the nuclease colicins E3 (38) (Fig. 2A, the “fishing pole” model that describes, along with lateral diffusion (39), the search mechanism of the receptor-bound colicin for the OmpF import pore) and E2 (40), and the conformation change of BtuB Asp6-Thr7 has been noted for the former complex (38, 20). The selection of BtuB complex for crystallization was based on a

microbiological assay for function (37), i. e., the ability of BtuB in detergent to neutralize the cytotoxic activity of nuclease colicins.

(B) OmpF. Approximately 30 years after the first meaningful 3-D diffraction images of OmpF were obtained, a 1.83 Å crystal structure was obtained (41) (Fig. 3B, ribbon diagram of trimer). The methods used to obtain this improved structure were not esoteric, but involved (i) the use of different detergents, obtained by screening, that resulted in a major decrease in the time required for isolation and purification of pure OmpF, almost free of LPS, from about 7 to about 2 days, and (ii) crystallization in a high Mg^{2+} concentration, which resulted in lower B factors, perhaps because of neutralization of the carboxylates in the narrow domain of the pore.

Assay of Function Electrophysiological screening (Fig. 3) of the ability of colicin peptides to occlude the nS single channel currents of OmpF incorporated into planar lipid bilayers (42) led to co-crystallization screens of OmpF. A crystal structure was obtained of the complex between OmpF and the colicin E3 N-terminal disordered domain (41, 43), which points to the pathway of transfer of the colicin toxicity domain across the outer membrane.

IV. Monomeric α -synuclein was isolated, with which structure studies are potentially possible. Studies were focused on α S as a membrane protein. Far-UV circular dichroism spectra described a predominately helical structure formed upon the binding of α -synuclein to liposomes containing a mixture of anionic (DOPG) and high curvature (DOPE) lipid (22). Well-defined conductance states are associated with voltage-gated specific ion channels of α -synuclein bound to planar lipid bilayers, measured as in Fig. 4, consisting of a mixture of anionic and high curvature lipid, DOPG/DOPE (22). In the vast literature on α S, this appears to be a unique approach to the function of α S.

Acknowledgments

The studies reported here have been supported by NIH grants GM038323, GM018457, 1 P50 GM088499, and the Michael J. Fox Foundation. Diffraction measurements associated with crystal structures of the cytochrome *b₆f* complex, the Omp BtuB and OmpF structures, complexes of BtuB with the R135 receptor binding domain of colicins E2 and E3, and that of OmpF with the N-83 fragment of colicin E3 were carried out with advice from S. Ginell, J. Lanarz, and F. Rotella at Beam Line 19-ID of the Structural Biology Center, Advanced Photon Source, Argonne National Laboratory, operated by the University of Chicago (contract DE-AC02-06CH11357, U.S. Department of Energy, Office of Biological and Environmental Research).

Abbreviations

α S	alpha-synuclein
DOPE, DOPG	dioleoyl-phosphatylethanolamine, - phosphatidylglycerol
E_{m7}	midpoint oxidation-reduction potential at pH 7
ETC	electron transport chain
LPS	lipopolysaccharide
NDH	NAD(P)H dehydrogenase
Omp	outer membrane protein
p, n	electrochemically positive and negative sides of the membrane
Pet	photosynthetic electron transport
PS	photosystem
TMH	trans-membrane helices

TMHMM	trans-membrane hidden Markov model
V	volts

References

- Landau EM, Rosenbusch JP. Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:14532–14535. [PubMed: 8962086]
- Garavito RM, Rosenbusch JP. Three-dimensional crystals of an integral membrane protein: an initial x-ray analysis. *J Cell Biol*. 1980; 86:327–329. 3. [PubMed: 6252213]
- Cramer WA, Zhang H, Yan J, Kurisu G, Smith JL. Trans-membrane traffic in the cytochrome *b₆f* complex. *Ann. Rev. Biochem*. 2006; 75:769–790. [PubMed: 16756511]
- Cramer WA, Yamashita E, Hasan SS. The Q cycle of cytochrome bc complexes: a structure perspective. *Biochim. Biophys Acta/Bioenergetics*. 2011; 1807:788–802.
- Kurisu G, Zhang H, Smith JL, Cramer WA. Structure of the cytochrome *b₆f* complex of oxygenic photosynthesis: tuning the cavity. *Science (New York, N.Y.)*. 2003; 302:1009–1014.
- Yan J, Kurisu G, Cramer WA. Structure of the cytochrome *b₆f* complex: Binding site and intraprotein transfer of the quinone analogue inhibitor 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone. *Proc. Nat. Acad. Sci. USA*. 2006; 103:67–74.
- Yamashita E, Zhang H, Cramer WA. Structure of the cytochrome *b₆f* complex: quinone analogue inhibitors as ligands of heme *c_H*. *J. Mol. Biol*. 2007; 370:39–52. [PubMed: 17498743]
- Baniulis D, Yamashita E, Whitelegge JP, Zatsman AI, Hendrich MP, Hasan SS, Ryan CM, Cramer WA. Structure-Function, Stability, and Chemical Modification of the Cyanobacterial Cytochrome *b₆f* Complex from *Nostoc* sp. PCC 7120. *J. Biol. Chem*. 2009; 284:9861–9869. [PubMed: 19189962]
- Stroebel D, Choquet Y, Popot J-L, Picot D. An atypical heme in the cytochrome *b₆f* complex. *Nature*. 2003; 426:413–418. [PubMed: 14647374]
- Brandt U. Energy converting NADH:quinone oxidoreductase (complex I). *Annu Rev Biochem*. 2006; 75:69–92. [PubMed: 16756485]
- Sazanov LA, Hinchcliffe P. Structure of the hydrophilic domain of the respiratory complex I from *Thermus thermophilus*. *Science (New York, N.Y.)*. 2006; 311:1430–1436.
- Efremov RG, Baradaran R, Sazanov LA. The architecture of respiratory complex I. *Nature*. 2010; 465:441–445. [PubMed: 20505720]
- Hunte C, Zickermann V, Brandt U. Functional modules and structural basis of conformational coupling in mitochondrial complex I. *Science (New York, N.Y.)*. 2010; 329:448–451.
- Battchikova N, Aro EM. Cyanobacterial NDH-1 complexes: multiplicity in function and subunit composition. *Physiol Plant*. 2007; 131:22–32. [PubMed: 18251921]
- Battchikova N, Aro EM, Nixon P. Structure and Physiological Function of NDH-1 Complexes in Cyanobacteria. In: al., G. A. P. e., editor. *Bioenergetic Processes of Cyanobacteria*. Springer Science; 2010. p. 445–467.
- Battchikova N, Eisenhut M, Aro EM. Cyanobacterial NDH-1 complexes: Novel insights and remaining puzzles. *Biochimica et biophysica acta*. 2011; 1807:935–944. [PubMed: 21035426]
- Battchikova N, Zhang P, Rudd S, Ogawa T, Aro EM. Identification of NdhL and Ss11690 (NdhO) in NDH-1L and NDH-1M complexes of *Synechocystis* sp. PCC 6803. *Journal of Biological Chemistry*. 2005; 280:2587–2596. [PubMed: 15548534]
- Sharma, O.; Zakharov, SD.; Cramer, WA. Colicins. In: Hancock, REW.; Kastin, AJ., editors. *Handbook of Biologically Active Peptides*. Elsevier; San Diego: 2006.
- Chimento DP, Mohanty AK, Kadner RJ, Wiener MC. Substrate-induced transmembrane signaling in the cobalamin transporter BtuB. *Nature Structural Biology*. 2003; 10:394–401.
- Cherezov V, Yamashita E, Liu W, Zhaltina M, Cramer WA, Caffrey M. In meso structure of the cobalamin transporter, BtuB, at 1.95 Å resolution. *J Mol Biol*. 2006; 364:716–734. [PubMed: 17028020]

21. Cookson MR. The biochemistry of Parkinson's disease. *Annu Rev Biochem.* 2005; 74:29–52. [PubMed: 15952880]
22. Zakharov SD, Hulleman JD, Dutseva EA, Antonenko YN, Rochet JC, Cramer WA. Helical alpha-synuclein forms highly conductive ion channels. *Biochemistry-Us.* 2007; 46:14369–14379.
23. Schmidt C, Malkin R. Low molecular weight subunits associated with the cytochrome *b₆f* complexes from spinach and *Chlamydomonas reinhardtii*. *Photosynthesis research.* 1993; 38:73–81.
24. Zhang H, Kurisu G, Smith JL, Cramer WA. A defined protein-detergent-lipid complex for crystallization of integral membrane proteins: The cytochrome *b₆f* complex of oxygenic photosynthesis. *Proc. Nat. Acad. Sci. USA.* 2003; 100:5160–5163. [PubMed: 12702760]
25. Hasan SS, Yamashita E, Ryan CM, Whitelegge JP, Cramer WA. Conservation of Lipid Functions in Cytochrome bc Complexes. *J. Mol. Biol.* 2011 in press.
26. Valiyaveetil FI, Zhou Y, MacKinnon R. Lipids in the structure, folding, and function of the KcsA K⁺ channel. *Biochemistry-Us.* 2002; 41:10771–10777.
27. Guan L, Smirnova IN, Verner G, Nagamori S, Kaback HR. Manipulating phospholipids for crystallization of a membrane transport protein. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:1723–1726. [PubMed: 16446422]
28. Zickermann V, Angerer H, Ding MG, Nubel E, Brandt U. Small single transmembrane domain (STMD) proteins organize the hydrophobic subunits of large membrane protein complexes. *FEBS letters.* 2010; 584:2516–2525. [PubMed: 20398659]
29. Cramer WA, Yan J, Zhang H, Kurisu G, Smith JL. Structure of the cytochrome *b₆f* complex: new prosthetic groups, Q-space, and the 'hors d'oeuvres hypothesis' for assembly of the complex. *Photosynth. Res.* 2005; 85:133–143. [PubMed: 15977064]
30. Kim H, Dashdorj N, Zhang H, Yan J, Cramer WA, Savikhin S. An Anomalous Distance Dependence of Intra-Protein Chlorophyll-Carotenoid Triplet Energy Transfer. *Biophysical journal.* 2005; 89:28–30.
31. Schneider D, Volkmer T, Rogner M. PetG and PetN, but not PetL, are essential subunits of the cytochrome *b₆f* complex from *Synechocystis* PCC 6803. *Research in microbiology.* 2007; 158:45–50. [PubMed: 17224258]
32. Whitelegge JP, Zhang H, Taylor R, Cramer WA. Full subunit coverage liquid chromatography electrospray-ionization mass spectrometry (LCMS⁺) of an oligomeric membrane protein complex: the cytochrome *b₆f* complex from spinach and the cyanobacterium. *M. laminosus*, *Mol. Cell Prot.* 2002; 1:816–827.
33. Nowaczyk MM, Wulforth H, Ryan CM, Souda P, Zhang H, Cramer WA, Whitelegge JP. NdhP and NdhQ: two novel small subunits of the cyanobacterial NDH-1 complex. *Biochemistry-Us.* 2011; 50:1121–1124.
34. Schneider D, Volkmer T, Rogner M. PetG and PetN, but not PetL, are essential subunits of the cytochrome *b₆f* complex from *Synechocystis* PCC 6803. *Research in microbiology.* 2007; 158:45–50. [PubMed: 17224258]
35. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol.* 2001; 305:567–580. [PubMed: 11152613]
36. Nakao M, Okamoto S, Kohara M, Fujishiro T, Fujisawa T, Sato S, Tabata S, Kaneko T, Nakamura Y. CyanoBase: the cyanobacteria genome database update 2010. *Nucleic Acids Res.* 2010; 38:D379–381. [PubMed: 19880388]
37. Taylor R, Burgner JW, Clifton J, Cramer WA. Purification and characterization of monomeric *Escherichia coli* vitamin B12 receptor with high affinity for colicin E3. *The Journal of biological chemistry.* 1998; 273:31113–31118. [PubMed: 9813013]
38. Kurisu G, Zakharov SD, Zhalnina MV, Bano S, Eroukova VY, Rokitskaya TI, Antonenko YN, Wiener MC, Cramer WA. The structure of BtuB with bound colicin E3 R-domain implies a translocon. *Nat Struct Biol.* 2003; 10:948–954. [PubMed: 14528295]
39. Spector J, Zakharov S, Lill Y, Sharma O, Cramer WA, Ritchie K. Mobility of BtuB and OmpF in the *Escherichia coli* outer membrane: implications for dynamic formation of a translocon complex. *Biophysical journal.* 2010; 99:3880–3886. [PubMed: 21156129]

40. Sharma O, Yamashita E, Zhalnina MV, Zakharov SD, Datsenko KA, Wanner BL, Cramer WA. Structure of the complex of the colicin E2 R-domain and its BtuB receptor. The outer membrane colicin translocon. The Journal of biological chemistry. 2007; 282:23163–23170. [PubMed: 17548346]
41. Yamashita E, Zhalnina MV, Zakharov SD, Sharma O, Cramer WA. Crystal structures of the OmpF porin: function in a colicin translocon. The EMBO journal. 2008; 27:2171–2180. [PubMed: 18636093]
42. Zakharov SD, Eroukova VY, Rokitskaya TI, Zhalnina MV, Sharma O, Loll PJ, Zgurskaya HI, Antonenko YN, Cramer WA. Colicin occlusion of OmpF and TolC channels: outer membrane translocons for colicin import. Biophysical journal. 2004; 87:3901–3911. [PubMed: 15465872]
43. Housden NG, Wojdyla JA, Korczynska J, Grishkovskaya I, Kirkpatrick N, Brzozowski AM, Kleanthous C. Directed epitope delivery across the Escherichia coli outer membrane through the porin OmpF. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:21412–21417. [PubMed: 21098297]

Summary of methods to assay functions/activities

(i) sensitive spectrophotometric assay to measure electron transfer activities; (ii) hydrophobic chromatography to deplete lipids, allowing reconstitution with specific lipids for studies on lipid-protein interactions; (iii) microbiological screen to assay high affinity binding of colicin receptor domains to *E. coli* outer membrane receptors; (iv) electrophysiology/channel analysis (a) to select channel-occluding ligands for co-crystallization with ion channels of OmpF, and (b) to provide a unique description of voltage-gated ion channels of α -synuclein.

Figs. 1A, B.

(A) The electron transport chain of oxygenic photosynthesis, extending from water as the p-side ultimate electron and proton donor to NADP^+ as the n-side electron acceptor. A suggested role of NDH-1 as an electron donor to plastoquinone (pathway dashed, red) is shown. Its connection to the photosystem I reaction center I, which has not been documented, in a possible pathway of cyclic electron transport is not shown. (B) Ribbon structure of the 220 kDa dimeric structure of the cytochrome b_6f complex, which functions in the middle of the electron transport chain (panel A) is shown in a view parallel to the membrane plane: the seven prosthetic groups and the arrangement of the 13 TMH per monomer in which the domain-swapping TMH of the Rieske [2Fe-2S] protein and the small subunits, PetG, L, M, and N, that form a picket fence at the periphery of each monomer are of particular interest. The seven prosthetic groups are: four hemes, the covalently-bound c-type heme of the cytochrome f subunit on the p-side aqueous phase, the heme c_n covalently bound to the cytochrome b subunit on the n-side, and the two non-covalently bound hemes b_p and b_n on the electrochemically positive and negative sides of the membrane, one [2Fe-2S] iron-sulfur cluster, one chlorophyll a , and one β -carotene. Color code: (a) polypeptides: Cyt f , dark green; cyt b_6 , yellow; Rieske ISP, light orange; Sub IV, pink; Pet G, blue; Pet L, red; Pet M, bright orange; PetN, gray; (b) prosthetic groups: Heme f , orange; heme b_p and b_n , red; heme c_n , blue; Rieske [2Fe-2S] cluster, brown and yellow; chlorophyll- a , green; β -carotene, yellow.

Fig. 2.

Crystal structures of (A) BtuB with bound 135 residue receptor binding (R) -domain of colicin E2 (38, 40); (B) ribbon diagram of OmpF porin, *in surfo*, 1.6 Å resolution (R-values, 0.182, 0.218), 0.5 M Mg^{2+} ; 2 molecules of detergent bound/monomer; no bound LPS (41); figure modified from (41). (C) “Fishing Pole” model of outer membrane stages of cellular import of nuclease colicins: (i) high affinity binding by BtuB (32, 33), (ii) lateral diffusion ($D \approx 10^{-9} \text{ cm}^2\text{-sec}^{-1}$) of colicin bound to BtuB (39), and (iii) binding/insertion through the OM via the OmpF porin (35, 36).

Fig. 3. Schematic of planar bilayer apparatus used to screen occlusion of OmpF ion channels by colicin peptides (42).

Fig. 4.

Model for ion channel formation by monomeric α -synuclein. Unordered in aqueous solution α -synuclein (left) becomes α -helical upon binding to membrane containing anionic lipids (center). Application of negative trans-membrane potential to the side of membrane opposite to one where α -synuclein was added, result in formation of ion channels (right).

Table 1

Summary of the molecular weights and predicted (TMHMM algorithm, (35)) trans-membrane helices present in the structure of the Ndh-1 complex of the moderately thermophilic cyanobacterium, *Thermosynechococcus elongatus* (33) and *E. coli* (11, 12). Only *E. coli* subunits that correspond to subunits in cyanobacteria are shown; the three soluble proteins that contain redox centers and cofactor-binding sites (14, 16) are omitted.

Subunits and Properties of NDH-1						
T. elongatus				E. coli		
Subunit ¹ (gene ID)	prediction from genome		subunits present in NDH-1L	Predicted TMH	Subunit	TMH measured in xtal structure
	No of aa	MW (Da)				
ndhA (tlr0667)	329	41347	+	6	nuoH	absent
ndhB (tlr0045)	515	55144	+	14	nuoN	14
ndhC (tlr1429)	132	15003	+	3	nuoA	3 ²
ndhD1 (tlr0719)	514	56079	-	12	-	-
ndhD2 (tlr1819)	533	58069	+	13	-	-
ndhD3 (tlr0905)	501	53875	-	14	nuoM	14
ndhD4 (tlr2125)	518	53376	-	14	-	-
ndhD5 (tlr11817)	478	51474	-	15	-	-
ndhE (tlr0670)	101	11133	+	3	nuoK	3 ¹
ndhF1 (tlr0720)	656	71973	+	16	-	-
ndhF3 (tlr0904)	611	66251	-	16	nuoL	16
ndhF4 (tlr2121)	609	66138	-	16	-	-
ndhG (tlr0669)	200	21570	+	5	nuoJ	5 ¹
ndhH (tlr1288)	394	45216	+	0	nuoDa	0
ndhI (tlr0668)	196	22416	+	0	nuoI	0
ndhJ (tlr1430)	168	19344	+	0	nuoCa	0
ndhK (tlr0705)	237	25742	+	1 ³	nuoB	0
ndhL (tlr0706)	76	8571	+	2	-	-
ndhM (tlr0447)	111	12567	+	0	-	-
ndhN (tlr1130)	150	16636	+	0	-	-
ndhO (tlr0017)	70	7867	+	0	-	-

Subunits and Properties of NDH-1							
T. elongatus				E. coli			
Subunit ¹ (gene ID)	prediction from genome		subunits present in NDH-1L	Predicted TMH	Subunit	TMH measured in xtal structure	
	No of aa	MW (Da)					
ndhP	44	4875	+	1	–	–	
ndhQ	45	4842	+	1	–	–	
CupA (Tlr0906)	437	50944	–	0	–	–	
CupB (Tlr2126)	380	43275	–	0	–	–	
MW(Complex)			442,315		–	–	

¹ Cyanobase: <http://genome.kazusa.or.jp/cyanobase> (36).

² predicted; +, present; – absent, 0, soluble

³ TMHMM predicts one TMH for ndhK but 0 for nuoB. Only 6 TMH were observed in the crystal structure of the membrane portion of the NDH-1 complex in *E. coli* (12). The crystal structure of the soluble domain of NDH-1 in *Thermus thermophilus* (12) includes the subunit *nqo6*, which corresponds to *nuoB* in *E. coli*.