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# Iron assimilation and utilization in anaerobic ammonium oxidizing bacteria

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The most abundant transition metal in biological systems is iron. It is incorporated into protein cofactors and serves either catalytic, redox or regulatory purposes. Anaerobic ammonium oxidizing (anammox) bacteria rely heavily on iron-containing proteins – especially cytochromes – for their energy conservation, which occurs within a unique organelle, the anammoxosome. Both their anaerobic lifestyle and the presence of an additional cellular compartment challenge our understanding of iron processing. Here, we combine existing concepts of iron uptake, utilization and metabolism, and cellular fate with genomic and still limited biochemical and physiological data on anammox bacteria to propose pathways these bacteria may employ.

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## Introduction

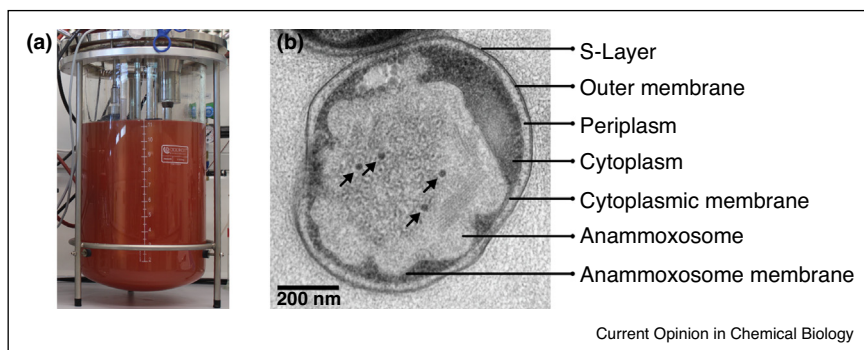
Iron is the most abundant transition metal in biological systems, where it is employed as an essential cofactor in redox chemistry, electron transfer reactions and regulatory processes. The capacity of iron to form complexes with carbon, oxygen, sulfur, and nitrogen, and the impressively wide range of redox potentials iron metalloproteins exhibit (–700 mV to +350 mV) [1] contributes to its involvement in all biogeochemical cycles.

The majority of iron-dependent redox proteins harbor iron within hemes and in the form of iron–sulfur (Fe–S) clusters [1]. Bacteria and Archaea show an enormous diversity and abundance of iron–sulfur and heme-containing proteins, which reflects their broad metabolic capacities [1,2]. Some of these organisms feature large amounts of iron-containing proteins. This is the case for anammox bacteria, which oxidize ammonium with nitrite as the terminal electron acceptor in the absence of oxygen [3,4\*]. The genomes of these microorganisms encode for about 60 *c*-type cytochromes [5–9], among which are key catabolic enzymes that constitute at least 50% of the total protein mass. Compared to an *Escherichia coli* cell that contains 10<sup>5</sup>–10<sup>6</sup> atoms of protein-bound iron [10,11], each anammox cell can be estimated to manage a pool of about 10<sup>7</sup> iron atoms. The bright red color of anammox enrichment cultures reflects this high content of heme-bound iron (Figure 1a).

Anammox bacteria are anaerobic Gram-negative microorganisms within the phylum of *Planctomycetes*, and have a compartmentalized cell plan. In addition to the peptidoglycan-containing cell wall [12\*] and the cytoplasmic membrane, they possess a third and innermost membrane that defines the anammoxosome, a unique energy-converting organelle that occupies a large volume of the cell (~70%) [13,14] (Figure 1b). This additional compartment adds a layer of complexity to intracellular trafficking of nutrients and co-substrates, as well as protein sorting.

The anammoxosome contains the vast majority of cellular iron in the form of cofactors within Fe–S proteins and multi-heme cytochromes [4\*], which are involved in the oxidation of ammonium to dinitrogen gas. In the current model (Figure 2) [3,4\*], nitrite is reduced to nitric oxide, and subsequently hydrazine synthase catalyzes the condensation of nitric oxide and ammonium to produce hydrazine [15,16], the most powerful chemical reductant in nature ( $E'_{0} = -700$  mV). This is followed by the oxidation of hydrazine to dinitrogen gas by hydrazine dehydrogenase [17]. The four low-potential electrons released in this reaction should pass through electrogenic respiratory complexes within the anammoxosome membrane via a sequence of electron transfer events to build up the membrane potential. Then, the electrons return to the anammoxosome to fuel the first two steps of the anammox reaction, thus closing the electron transfer cycle. Electron withdrawal from the cyclic anammox pathway during CO<sub>2</sub>

Figure 1



Anammox enrichment culture and cell plan. **(a)** Laboratory-scale membrane bioreactor enriched with 95% of the anammox bacterium *Kueneia stuttgartiensis*. **(b)** The cell plan of an anammox bacterium, illustrated with a transmission electron micrograph of *K. stuttgartiensis*. Cell compartments and membranes are indicated. Arrows indicate iron-rich nanoparticles. Courtesy, L. van Niftrik.

fixation is compensated through nitrite oxidation to nitrate catalyzed by nitrite oxidoreductase (NXR).

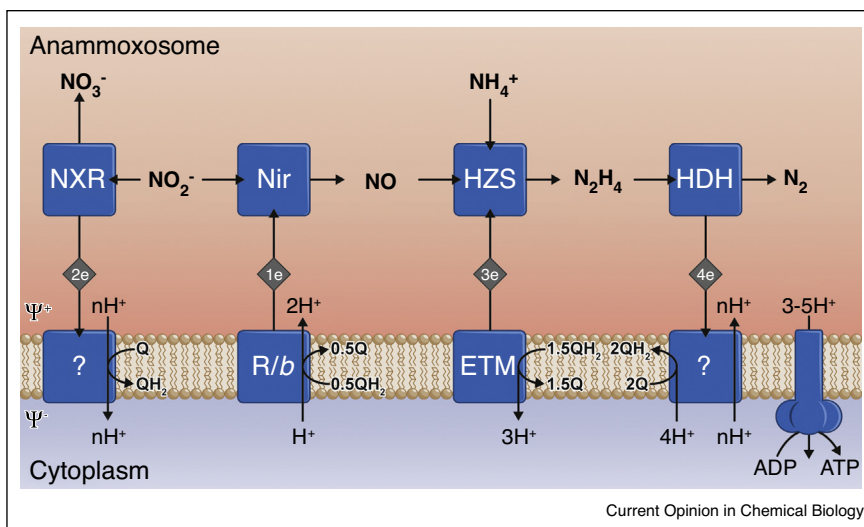
Despite the central role of iron in the anammox process, most aspects of iron in anammox bacteria have remained unexplored to date. In this review we will briefly discuss iron metabolism and protein systems that these microorganisms may use to assimilate and utilize iron. We thereby follow the ions from outside the cell through

the cytoplasm to the anammoxosome interior (Figure 3), with a special emphasis on the peculiar cell plan and a final discussion of iron-rich nanoparticles inside the anammoxosome.

### Iron metabolism

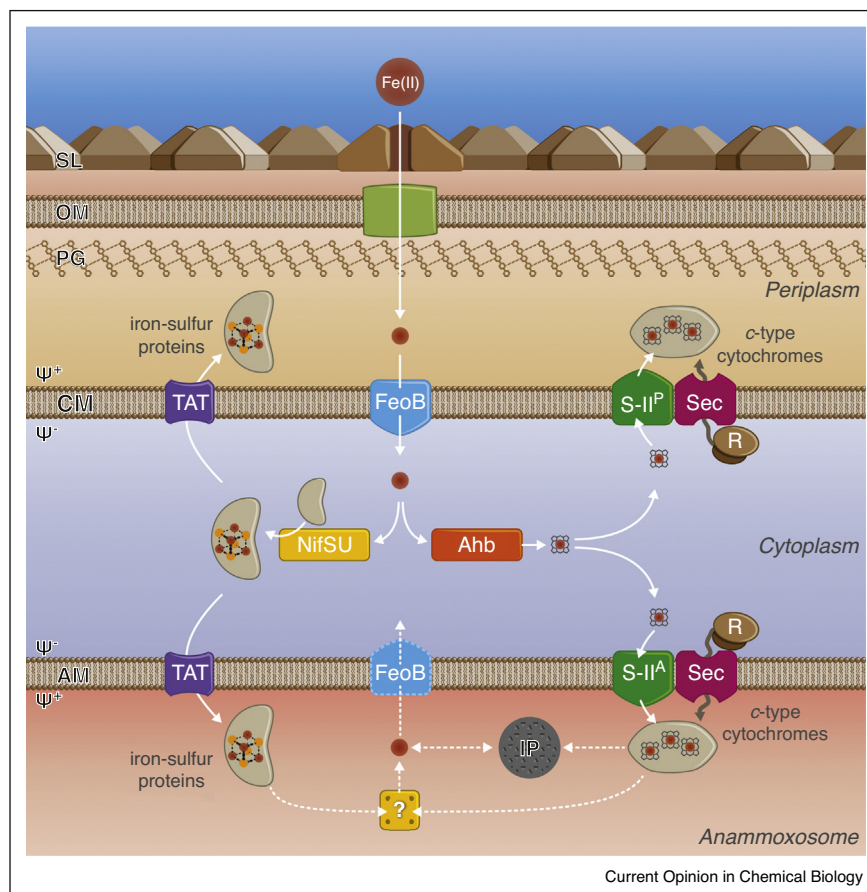
The natural abundance of iron combined with its redox properties renders it a prevalent substrate for both heterotrophic respiration and autotrophic growth. In the

Figure 2



Current model of the anammox pathway. Anaerobic oxidation of ammonium ( $\text{NH}_4^+$ ) to dinitrogen gas ( $\text{N}_2$ ) with nitrite ( $\text{NO}_2^-$ ) as terminal electron acceptor proceeds via nitric oxide (NO) and hydrazine ( $\text{N}_2\text{H}_4$ ) as free intermediates. The available free energy of the anammox process is utilized to maintain an electrochemical potential gradient across the anammoxosome membrane, which drives ATP synthesis. Electron withdrawal from the cyclic anammox pathway during carbon dioxide ( $\text{CO}_2$ ) fixation (not shown) is compensated by nitrite oxidation to nitrate ( $\text{NO}_3^-$ ) catalyzed by nitrite oxidoreductase (NXR). Diamonds represent putative electron carriers and indicate the number of electrons that are transferred in each reaction. Question marks represent the hypothetical cytochrome:quinone oxidoreductases that feed the quinone pool (Q/QH<sub>2</sub>) with electrons yielded from the oxidation of hydrazine and nitrite. Nir: putative nitrite reductase; HZS: hydrazine synthase; HDH: hydrazine dehydrogenase; R/b: Rieske/cytochrome *b* complex, ETM: electron transfer module for hydrazine synthesis;  $\Psi^+$ ,  $\Psi^-$ : positive and negative sides of the membrane, respectively.

Figure 3



Proposed pathways for iron assimilation and utilization in anammox bacteria. Soluble ferrous iron (Fe(II)) freely diffuses into the periplasm through pores in the S-Layer (SL) and pores in the outer membrane (OM) (green). Transport across the cytoplasmic membrane (CM) occurs via the Fe(II)-specific FeoB system. The alternative heme biosynthesis (Ahb) pathway synthesizes *b*-type hemes. Both a Sec-translocon (Sec) and cytochrome *c* maturation systems S-II<sup>A</sup> and S-II<sup>P</sup> are required for maturation of *c*-type cytochromes in the anammoxosome and periplasm, respectively. The NifSU machinery assembles mature Fe-S proteins, which are translocated into the periplasm and anammoxosome via the TAT pathway. The yellow box inside the anammoxosome denotes unknown pathways for the disassembly of iron-sulfur proteins and cytochromes and release of iron ions. FeoB at the anammoxosome membrane (AM) represents a possible route for free iron back into the cytoplasm for recycling. Iron-rich nanoparticles (IP) were hypothesized to be iron storage sites, but may alternatively be cytochrome-containing encapsulin nanocompartments. Red spheres represent iron ions. Dotted lines represent uncertain pathways. R: ribosome; SL: S-layer; PG: peptidoglycan;  $\Psi^+$ ,  $\Psi^-$ : positive and negative sides of the membrane, respectively.

former case, oxidation of organic compounds is coupled to reduction of extracellular ferric iron, whereas in the latter case oxidation of iron donates electrons to oxygen, bicarbonate or nitrate. These processes involve *c*-type cytochromes and are performed by phylogenetically diverse microorganisms. Anammox bacteria have been observed to couple formate oxidation to iron reduction [5,18,19] and perform nitrate-dependent iron oxidation [5,20\*].

### Fe(III) reduction

*Shewanella* and *Geobacter* are the best-studied iron-reducing organisms and provide us with general templates for the identification and understanding of extracellular electron transfer [21–23]. They employ different, but

functionally similar, systems to enable electron transfer from the quinone (Q) pool at the cytoplasmic membrane through the outer membrane to the iron mineral outside the cell. The first component is a membrane quinol (QH<sub>2</sub>) dehydrogenase that is located on the cytoplasmic membrane and extends into the periplasm with its cytochrome-rich domain (CymA in *Shewanella* and ImcH or CbcL in *Geobacter*) [21]. Electron transfer toward a multi-heme protein that is channeled through an outer membrane beta barrel occurs either via soluble periplasmic cytochromes [24] or direct interaction [25]. Ultimately, electrons reach the insoluble extracellular mineral either via soluble shuttles such as flavins [26], appendages like nanowires [27] or direct contact [21].

Reduction of Fe(III) coupled to formate oxidation has been reported for anammox bacteria [5,19], and seems to adhere to the same design for electron transfer to extracellular iron. They express a membrane QH<sub>2</sub> dehydrogenase homologous to CymA but with eleven instead of four heme binding sites, two periplasmic multiheme proteins homologous to *Shewanella*-MtrA and at least one outer membrane beta barrel porin-like protein [28\*\*]. Although the electron transfer through the periplasm could occur via direct contact between anammox-CymA and anammox-MtrA, electron transfer via soluble *c*-type cytochromes cannot be excluded. Once outside the cell, it is unclear how the reduction of particulate iron proceeds. Furthermore, how the oxidation of organic acids inside the cell and the reduction of iron outside the cell are coupled to energy conservation remains elusive.

### Fe(II) oxidation

In contrast to iron reduction, the molecular mechanism of biotic iron oxidation is less well understood. In addition to oxygen in microaerophilic and bicarbonate in phototrophic iron oxidation, nitrate is identified as another possible terminal electron acceptor of iron oxidation [29,30]. Formation of insoluble ferric oxides in the cell and possible interference of abiotic and biotic iron redox transitions are potential complications [31\*\*].

Anammox bacteria were shown to perform nitrate-dependent iron oxidation [5,20\*]. The only candidate for catalyzing nitrate reduction (NXR) is exclusively localized in the anammoxosome [32], which requires either Fe(II) to be imported into the anammoxosome or electrons from Fe(II) to cross the cytoplasmic membrane, the cytoplasm and the anammoxosome membrane. In the first case, export of Fe(III) might be problematic (see Section ‘Degradation of iron-containing proteins’), whereas in the second case the free energy available from the reaction seems insufficient to drive electron transfer over two membranes.

### Iron uptake

Utilization of iron as metal cofactor of redox enzymes requires translocation of iron across membranes into the cell interior/cytoplasm, where cofactor assembly occurs. Because the availability of reduced and oxidized iron differs dramatically between ecosystems, microorganisms employ different strategies to assimilate iron in their respective habitats. Prokaryotes that thrive in pH-neutral aerobic environments where iron mainly exists in the poorly soluble ferric form use a wide range of mechanisms to assimilate iron from minerals [33]. A prominent example is secretion of siderophores: organic iron-chelators that solubilize iron and facilitate its uptake [33,34].

Anammox bacteria live in oxygen-limited and anoxic habitats where soluble ferrous iron is the predominant iron species and do not possess genes required for

siderophore synthesis [5–9]. Soluble ferrous iron is believed to freely diffuse into the periplasm through outer membrane porins [28\*\*]. Although several non-specific divalent metal ion transporters exist, only two machineries are known to exclusively transport Fe(II) into the cytoplasm (*i.e.*, EfeUOB and FeoABC) [35\*\*,36]. The FeoABC system exhibits broad phylogenetic distribution and it is the only system for iron uptake found in anammox genomes (Figure 3). Although FeoA and FeoC have been speculated to enhance iron uptake [35\*\*], anammox bacteria only carry the gene for the iron transporter FeoB. The universal transcriptional regulator related to anaerobic metabolism Fnr (fumarate and nitrate reductase) [37], as well as the metal-specific regulator Fur (ferric uptake regulator) [38] can be assumed to participate in regulation of iron uptake and homeostasis in anammox bacteria, but a detailed analysis is currently missing.

### Biosynthesis of iron cofactors

The most common way for biological systems to exploit the redox properties of iron is its assembly into metalloprothetic groups that are incorporated into protein complexes, which serve either catalytic, redox or regulatory purposes. Among iron-containing cofactors, *c*-type hemes and Fe–S clusters are omnipresent throughout all domains of life, and anammox bacteria rely heavily on these two classes of cofactors for their energy metabolism [3]. Below we discuss heme *b* biosynthesis, cytochrome *c* maturation and Fe–S cluster biogenesis in anammox bacteria.

#### Heme *b* biosynthesis

Hemes belong to a broad class of organic cofactors that use a tetrapyrrole macrocyclic template to accommodate the chelation of a metal center. Tetrapyrrole formation proceeds via four or six universally conserved steps, the C-4 and C-5 pathway, respectively, and leads to formation of uroporphyrinogen III [39]. From this intermediate on, three different pathways for heme *b* biosynthesis have been described. The ‘classic pathway’ is conserved among *Eukaryotes* and *Proteobacteria* [40] and the ‘HemQ-based route’ is only present in Gram-positive bacteria [41]. Remarkably, unlike the other known members of the phylum *Planctomycetes*, which employ the ‘classic pathway’, anammox genomes encode for the complete machinery of the so-called ‘alternative heme biosynthesis’ (Ahb) pathway [42,43\*] that is possibly phylogenetically older than its classic counterpart [44,45].

#### Cytochrome *c* maturation

Enzymatic modification of heme *b* yields chemically distinct heme cofactors (*a*, *b*, *c*, *d* and *o*-type). In *c*-type cytochromes, which are the most abundant heme proteins in anammox, heme *b* molecules are covalently attached via thioether bonds of their vinyl groups to the sulfhydryls of two, or in rare cases one, cysteine residue(s) [46].

Regardless of the heme *b* biosynthesis pathway, Sec-based protein translocation machinery and cytochrome *c* maturation systems are required for the production of *c*-type cytochromes [47]. All three studied cytochrome *c* maturation systems (I–III) comprise membrane complexes that transport *b* heme across the membrane and catalyze its covalent attachment to the apoprotein at the extra-cytoplasmic side [47]. Although, *c*-type cytochromes are likely to be present in the periplasm (see Section ‘Iron metabolism’), the main site for cytochromes is the anammoxosome [48]. Since anammox bacteria express two highly similar copies of maturation system II [49], we propose that both cytoplasmic and anammoxosome membranes possess their individual maturation machinery (Figure 3). How proteins destined for the periplasm or anammoxosome are targeted to the respective translocation and maturation systems remains an intriguing open question.

### Iron–sulfur cluster biosynthesis

Iron–sulfur clusters in anammox bacteria are found not only in ferredoxins, Rieske/cytochrome *b* complexes, complex I, and hydrogenases, but also as electron-transferring cofactors of the highly abundant NXR protein complex residing inside the anammoxosome [3,32]. Biosynthesis of iron–sulfur clusters in prokaryotes is catalyzed by cytoplasmic proteins and involves donation of iron and sulfur to the assembly scaffold, maturation of the cluster, and delivery to the apoprotein [50]. Three iron–sulfur synthesis systems are known: *Isc*, *Suf* and *Nif* [51]. The *Isc* system appears to be the generic pathway for Fe–S cluster assembly whereas the *Suf* pathway has been associated with iron limitation and oxygen stress. The *Nif* system, on the contrary, was initially associated exclusively with the assembly of the nitrogenase enzyme in nitrogen-fixing bacteria [52]. However, its recent identification as the sole Fe–S maturation system in two diverse non-nitrogen fixing organisms has changed this view [53,54\*]. Interestingly, also in anammox bacteria the *Nif* system is seemingly the only Fe–S assembly machinery, supporting the notion that the *Nif* system is more versatile than previously assumed. Matured iron–sulfur proteins destined for either the anammoxosome or the periplasm are transported across the membranes via the twin-arginine translocation system (Figure 3).

### Degradation of iron-containing proteins

Controlled degradation of anammoxosomal iron-containing proteins as a mechanism of metabolic regulation or in the context of protein quality control has not been studied, but is likely to occur. While the anammox genomes provide candidate proteases that could cleave the protein backbone, the processing of iron cofactors, and especially heme moieties, is intriguingly more elusive. Heme degradation, for example, requires the oxidative cleavage of the porphyrin ring by the action of heme oxygenases [55]; a process that is oxygen-dependent and not compatible

with the anaerobic lifestyle of anammox bacteria. An alternative oxygen-independent system, similar to the recently discovered radical *S*-adenosyl-L-methionine (SAM)-based pathway [56], is conceivable but difficult to identify on the genomic level and requires dedicated genetic and biochemical studies. Furthermore, the fate of iron released from disassembled iron–sulfur proteins and cytochromes is unknown. Whether the anammoxosome membrane provides a route for iron into the cytoplasm is unclear, but we might hypothesize that, if present in the anammoxosome membrane, *FeoB* could play this role (Figure 3). However, in the absence of such a mechanism iron would accumulate and could only be diluted through cell – and anammoxosome – division.

### Iron-rich nanoparticles in anammox

Anammox bacteria cultured under laboratory conditions possess nanosized (diameter 16–25 nm) iron-rich particles inside the anammoxosome [13] (Figure 1). These particles were speculated to be iron storage sites, possibly formed by bacterioferritins; spherical, hollow protein complexes that contain large amounts of iron oxides (~2000 Fe atoms per complex) [57]. However, anammox bacterioferritins lack signal peptides that would target them into the anammoxosome. The recently discovered encapsulins [58,59] may provide an alternative explanation for the observed particles. Encapsulins form nanocompartments that store cargo proteins with different functions [60]. Indeed, one encapsulin homologue that is potentially targeted to the anammoxosome via a signal sequence, and its heme-rich cargo protein were hypothesized for anammox bacteria [61]. The nature of the iron-rich particles in anammox bacteria and the proposed existence of functional encapsulins should be the focus of future investigations.

### Conclusions

Although we have gained considerable insights into the physiology, cell architecture and energy metabolism of anammox bacteria, our knowledge on their iron uptake and incorporation is rather limited. In this review we present our current views on the various protein systems that anammox bacteria likely employ to support their iron-based lifestyle.

In addition to nitrite-dependent ammonium oxidation, a limited number of studies show that anammox bacteria utilize extracellular iron (Fe(II) and Fe(III)) as respiratory substrates. The pathways and bioenergetics involved in iron reduction and oxidation are poorly understood, and this interesting topic clearly deserves more attention and experimental efforts.

Even though substantial amounts of iron are present in *c*-type cytochromes and Fe–S proteins, anammox bacteria rely on common assimilation systems. Interestingly, they seem to be dependent on the presence of Fe(II), which is

taken up by the core component of the Feo system (FeoB). Assembly of Fe–S clusters is performed by the compact NifSU system, and the alternative heme biosynthesis (Ahb) pathway produces *b*-type hemes. Maturation system II completes the assembly of *c*-type cytochromes in the anammoxosome, and we propose that a copy of this maturation system also provides the periplasm with *c*-type cytochromes.

The overview presented in this review is based on annotated systems and inferred genetic homology. We identified pathways for iron, as part of metalloproteins, into the anammoxosome, but mechanisms of heme degradation and iron export from anammoxosome remain elusive. In this context, the origin and role of the observed iron-rich particles are particularly intriguing. Slow growth and the lack of molecular tools make biochemical studies on these fascinating organisms very challenging, but investigation of iron-related effects on anammox proteomes and transcriptomes certainly promises to yield valuable insights.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Liu J, Chakraborty S, Hosseinzadeh P, Yu Y, Tian S, Petrik I, Bhagi A, Lu Y: **Metalloproteins containing cytochrome, iron-sulfur, or copper redox centers.** *Chem Rev* 2014, **114**:4366-4469.
2. Bertini I, Cavallaro G, Rosato A: **Cytochrome c: occurrence and functions.** *Chem Rev* 2006, **106**:90-115.
3. Kartal B, Almeida NM, Maalcke WJ, Camp HJ, Jetten MS, Keltjens JT: **How to make a living from anaerobic ammonium oxidation.** *FEMS Microbiol Rev* 2013, **37**:428-461.
4. Kartal B, Keltjens JT: **Anammox biochemistry: a tale of heme c proteins.** *Trends Biochem Sci* 2016, **41**:998-1011.  
The authors of this review provide a comprehensive view on the advancements and current status of anammox biochemistry and structural biology. Central catabolic reactions are discussed in light of the recently solved crystal structures of multiheme *c*-type cytochrome complexes.
5. Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P *et al.*: **Deciphering the evolution and metabolism of an anammox bacterium from a community genome.** *Nature* 2006, **440**:790-794.
6. Hira D, Toh H, Migita CT, Okubo H, Nishiyama T, Hattori M, Furukawa K, Fujii T: **Anammox organism KSU-1 expresses a NirK-type copper-containing nitrite reductase instead of a NirS-type with cytochrome *cd*<sub>1</sub>.** *FEBS Lett* 2012, **586**:1658-1663.
7. van de Vossenberg J, Woebken D, Maalcke WJ, Wessels HJ, Dutilh BE, Janssen-Megens EM, Roeselers G, Yan J, Speth D *et al.*: **The metagenome of the marine anammox bacterium 'Candidatus Scalindua profunda' illustrates the versatility of this globally important nitrogen cycle bacterium.** *Environ Microbiol* 2013, **15**:1275-1289.
8. Speth DR, Russ L, Kartal B, Op den Camp HJ, Dutilh BE, Jetten MS: **Draft genome sequence of anammox bacterium 'Candidatus Scalindua brodae' obtained using differential coverage binning of sequencing data from two reactor enrichments.** *Genome Announc* 2015, **3**.
9. Oshiki M, Shinyako-Hata K, Satoh H, Okabe S: **Draft genome sequence of an anaerobic ammonium-oxidizing bacterium, 'Candidatus Brocadia sinica'.** *Genome Announc* 2015, **3**.
10. Outten CE, O'Halloran TV: **Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis.** *Science* 2001, **292**:2488-2492.
11. Posey JE, Gherardini FC: **Lack of a role for iron in the Lyme disease pathogen.** *Science* 2000, **288**:1651-1653.
12. van Teeseling MC, Mesman RJ, Kuru E, Espaillet A, Cava F, Brun YV, VanNieuwenhze MS, Kartal B, van Niftrik L: **Anammox Planctomycetes have a peptidoglycan cell wall.** *Nat Commun* 2015, **6**:6878.  
This study unambiguously shows that anammox bacteria possess a peptidoglycan-containing cell wall, which resolves the controversy on the cell envelope composition of anammox bacteria.
13. van Niftrik L, Geerts WJ, van Donselaar EG, Humbel BM, Yakushevskaya A, Verkleij AJ, Jetten MS, Strous M: **Combined structural and chemical analysis of the anammoxosome: a membrane-bounded intracytoplasmic compartment in anammox bacteria.** *J Struct Biol* 2008, **161**:401-410.
14. Neumann S, Wessels HJ, Rijpstra WI, Sinninghe Damste JS, Kartal B, Jetten MS, van Niftrik L: **Isolation and characterization of a prokaryotic cell organelle from the anammox bacterium *Kueneenia stuttgartiensis*.** *Mol Microbiol* 2014, **94**:794-802.
15. Kartal B, Maalcke WJ, de Almeida NM, Cirpus I, Gloerich J, Geerts W, Op den Camp HJM, Harhangi HR, Janssen-Megens EM, Francoijs K-J *et al.*: **Molecular mechanism of anaerobic ammonium oxidation.** *Nature* 2011, **479**:127-130.
16. Dietl A, Ferousi C, Maalcke WJ, Menzel A, de Vries S, Keltjens JT, Jetten MS, Kartal B, Barends TR: **The inner workings of the hydrazine synthase multiprotein complex.** *Nature* 2015, **527**:394-397.
17. Maalcke WJ, Reimann J, de Vries S, Butt JN, Dietl A, Kip N, Mersdorf U, Barends TR, Jetten MS, Keltjens JT *et al.*: **Characterization of anammox hydrazine dehydrogenase, a key N<sub>2</sub>-producing enzyme in the global nitrogen cycle.** *J Biol Chem* 2016, **291**:17077-17092.
18. Zhao R, Zhang H, Li Y, Jiang T, Yang F: **Research of iron reduction and the iron reductase localization of anammox bacteria.** *Curr Microbiol* 2014, **69**:880-887.
19. van de Vossenberg J, Rattray JE, Geerts W, Kartal B, van Niftrik L, van Donselaar EG, Sinninghe Damste JS, Strous M, Jetten MS: **Enrichment and characterization of marine anammox bacteria associated with global nitrogen gas production.** *Environ Microbiol* 2008, **10**:3120-3129.
20. Oshiki M, Ishii S, Yoshida K, Fujii N, Ishiguro M, Satoh H, Okabe S: **Nitrate-dependent ferrous iron oxidation by anaerobic ammonium oxidation (anammox) bacteria.** *Appl Environ Microbiol* 2013, **79**:4087-4093.  
This is the first detailed physiological study of iron oxidation coupled to nitrate reduction mediated by two anammox species. Stable isotope labelling was utilized to determine activity rates for nitrate-dependent iron oxidation and its combination with anaerobic ammonium oxidation activity.
21. Shi L, Dong H, Reguera G, Beyenal H, Lu A, Liu J, Yu HQ, Fredrickson JK: **Extracellular electron transfer mechanisms between microorganisms and minerals.** *Nat Rev Microbiol* 2016, **14**:651-662.
22. Richardson DJ, Butt JN, Fredrickson JK, Zachara JM, Shi L, Edwards MJ, White G, Baiden N, Gates AJ, Marritt SJ *et al.*: **The 'porin-cytochrome' model for microbe-to-mineral electron transfer.** *Mol Microbiol* 2012, **85**:201-212.

23. Lovley DR, Holmes DE, Nevin KP: **Dissimilatory Fe(III) and Mn(IV) reduction.** *Adv Microb Physiol* 2004, **49**:219-286.
24. Sturm G, Richter K, Doetsch A, Heide H, Louro RO, Gescher J: **A dynamic periplasmic electron transfer network enables respiratory flexibility beyond a thermodynamic regulatory regime.** *ISME J* 2015, **9**:1802-1811.
25. Shi L, Fredrickson JK, Zachara JM: **Genomic analyses of bacterial porin-cytochrome gene clusters.** *Front Microbiol* 2014, **5**.
26. Kotloski NJ, Gralnick JA: **Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis*.** *mBio* 2013, **4**.
27. Lovley DR, Malvankar NS: **Seeing is believing: novel imaging techniques help clarify microbial nanowire structure and function.** *Environ Microbiol* 2015, **17**:2209-2215.
28. de Almeida NM, Wessels HJCT, de Graaf RM, Ferousi C, Jetten MSM, Keltjens JT, Kartal B: **Membrane-bound electron transport systems of an anammox bacterium: a complexome analysis.** *Biochim Biophys Acta (BBA) Bioenerg* 2016, **1857**:1694-1704.
- Here, membrane protein complexes of the anammox bacterium *Kuenenia stuttgartiensis* were analyzed by a proteomics-based complexome profiling technique. The results confirm and expand the model of energy conservation in anammox bacteria, and demonstrate the strength of this approach as a complementary method to study metabolic capacities of an organism.
29. Straub KL, Benz M, Schink B, Widdel F: **Anaerobic, nitrate-dependent microbial oxidation of ferrous iron.** *Appl Environ Microbiol* 1996, **62**:1458-1460.
30. Klueglein N, Kappler A: **Abiotic oxidation of Fe(II) by reactive nitrogen species in cultures of the nitrate-reducing Fe(II) oxidizer *Acidovorax* sp. BoFeN1—questioning the existence of enzymatic Fe(II) oxidation.** *Geobiology* 2013, **11**:180-190.
31. Melton ED, Swanner ED, Behrens S, Schmidt C, Kappler A: **The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle.** *Nat Rev Microbiol* 2014, **12**:797-808.
- In this review, the authors summarize all known processes governing the biogeochemical iron cycle and critically discuss the fine intersection between biotic and chemically mediated iron redox transformations. Furthermore, general experimental designs for estimating relative contributions of biotic and abiotic processes in physiological investigations are proposed.
32. de Almeida NM, Neumann S, Mesman RJ, Ferousi C, Keltjens JT, Jetten MS, Kartal B, van Niftrik L: **Immunogold localization of key metabolic enzymes in the anammoxosome and on the tubule-like structures of *Kuenenia stuttgartiensis*.** *J Bacteriol* 2015, **197**:2432-2441.
33. Miethke M: **Molecular strategies of microbial iron assimilation: from high-affinity complexes to cofactor assembly systems.** *Metalomics* 2013, **5**:15-28.
34. Barry SM, Challis GL: **Recent advances in siderophore biosynthesis.** *Curr Opin Chem Biol* 2009, **13**:205-215.
35. Lau CK, Krewulak KD, Vogel HJ: **Bacterial ferrous iron transport: the Feo system.** *FEMS Microbiol Rev* 2016, **40**:273-298.
- This is the most extensive and up-to-date review on the ferrous iron uptake system Feo. The authors present recent insights from structural and functional studies and discuss the molecular details of iron uptake by the Feo protein complex.
36. Roy EM, Griffith KL: **Characterization of a novel iron acquisition activity that coordinates the iron response with population density during iron replete conditions in *Bacillus subtilis*.** *J Bacteriol* 2017, **199**:1-23.
37. Carpenter C, Payne SM: **Regulation of iron transport systems in *Enterobacteriaceae* in response to oxygen and iron availability.** *J Inorg Biochem* 2014, **133**:110-117.
38. Fillat MF: **The FUR (ferric uptake regulator) superfamily: diversity and versatility of key transcriptional regulators.** *Arch Biochem Biophys* 2014, **546**:41-52.
39. Frankenberg N, Moser J, Jahn D: **Bacterial heme biosynthesis and its biotechnological application.** *Appl Microbiol Biotechnol* 2003, **63**:115-127.
40. Layer G, Reichelt J, Jahn D, Heinz DW: **Structure and function of enzymes in heme biosynthesis.** *Protein Sci* 2010, **19**:1137-1161.
41. Dailey HA, Gerdes S, Dailey TA, Burch JS, Phillips JD: **Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin.** *Proc Natl Acad Sci U S A* 2015, **112**:2210-2215.
42. Ishida T, Yu L, Akutsu H, Ozawa K, Kawanishi S, Seto A, Inubushi T, Sano S: **A primitive pathway of porphyrin biosynthesis and enzymology in *Desulfovibrio vulgaris*.** *Proc Natl Acad Sci U S A* 1998, **95**:4853-4858.
43. Bali S, Lawrence AD, Lobo SA, Saraiva LM, Golding BT, Palmer DJ, Howard MJ, Ferguson SJ, Warren MJ: **Molecular hijacking of siroheme for the synthesis of heme and *d*<sub>1</sub> heme.** *Proc Natl Acad Sci U S A* 2011, **108**:18260-18265.
- In this study a combination of heterologous protein expression, UV-visible spectroscopy and mass spectrometry was applied to identify the intermediates of both heme and heme *d*<sub>1</sub> biosynthesis pathways in divergent bacterial species. The authors show that precorrin-2, a previously identified intermediate of siroheme biosynthesis, is also an intermediate in these two pathways. This is the first detailed description of the so-called alternative heme biosynthesis pathway.
44. Bali S, Palmer DJ, Schroeder S, Ferguson SJ, Warren MJ: **Recent advances in the biosynthesis of modified tetrapyrroles: the discovery of an alternative pathway for the formation of heme and heme *d*<sub>1</sub>.** *Cell Mol Life Sci* 2014, **71**:2837-2863.
45. Ducluzeau A-L, Nitschke W: **When did hemes enter the scene of life? On the natural history of heme cofactors and heme-containing enzymes.** *Adv Photosynth Respir* 2016, **41**:13-24.
46. de Vitry C: **Cytochrome *c* maturation system on the negative side of bioenergetic membranes: CCB or System IV.** *FEBS J* 2011, **278**:4189-4197.
47. Kranz RG, Richard-Fogal C, Taylor JS, Frawley ER: **Cytochrome *c* biogenesis: mechanisms for covalent modifications and trafficking of heme and for heme-iron redox control.** *Microbiol Mol Biol Rev* 2009, **73**:510-528.
48. van Niftrik L, Geerts WJC, van Donselaar EG, Humbel BM, Webb RI, Fuerst JA, Verkleij AJ, Jetten MSM, Strous M: **Linking ultrastructure and function in four genera of anaerobic ammonium-oxidizing bacteria: cell plan, glycogen storage, and localization of cytochrome *c* proteins.** *J Bacteriol* 2008, **190**:708-717.
49. Ferousi C, Speth DR, Reimann J, Op den Camp HJ, Allen JW, Keltjens JT, Jetten MS: **Identification of the type II cytochrome *c* maturation pathway in anammox bacteria by comparative genomics.** *BMC Microbiol* 2013, **13**:265.
50. Roche B, Aussel L, Ezraty B, Mandin P, Py B, Barras F: **Iron/sulfur proteins biogenesis in prokaryotes: formation, regulation and diversity.** *Biochim Biophys Acta* 2013, **1827**:455-469.
51. Bandyopadhyay S, Chandramouli K, Johnson MK: **Iron-sulfur cluster biosynthesis.** *Biochem Soc Trans* 2008, **36**:1112-1119.
52. Frazzon J, Dean DR: **Formation of iron-sulfur clusters in bacteria: an emerging field in bioinorganic chemistry.** *Curr Opin Chem Biol* 2003, **7**:166-173.
53. Olson JW, Agar JN, Johnson MK, Maier RJ: **Characterization of the NifU and NifS Fe-S cluster formation proteins essential for viability in *Helicobacter pylori*.** *Biochemistry* 2000, **39**:16213-16219.
54. Nyvltova E, Sutak R, Harant K, Sednova M, Hrdy I, Paces J, Vlcek C, Tachezy J: **NIF-type iron-sulfur cluster assembly system is duplicated and distributed in the mitochondria and cytosol of *Mastigamoeba balamuthi*.** *Proc Natl Acad Sci U S A* 2013, **110**:7371-7376.
- Here, the biogenesis of iron-sulfur proteins in the eukaryotic unicellular free-living *Mastigamoeba balamuthi* was studied. Unlike all eukaryotes known to date, this organism does not code for an isc machinery, but uses the nif system instead. Interestingly, *M. balamuthi* was found to encode and express two copies of the nif components, which are differentially targeted to the cytoplasm and mitochondria.
55. Wilks A: **Heme oxygenase: evolution, structure, and mechanism.** *Antioxid Redox Signal* 2002, **4**:603-614.



56. LaMattina JW, Nix DB, Lanzilotta WN: **Radical new paradigm for heme degradation in *Escherichia coli* O157:H7**. *Proc Natl Acad Sci U S A* 2016, **113**:12138-12143.
57. Andrews SC: **Iron storage in bacteria**. *Adv Microb Physiol* 1998, **40**:281-351.
58. Sutter M, Boehringer D, Gutmann S, Gunther S, Prangishvili D, Loessner MJ, Stetter KO, Weber-Ban E, Ban N: **Structural basis of enzyme encapsulation into a bacterial nanocompartment**. *Nat Struct Mol Biol* 2008, **15**:939-947.
59. McHugh CA, Fontana J, Nemecek D, Cheng N, Aksyuk AA, Heymann JB, Winkler DC, Lam AS, Wall JS, Steven AC *et al.*: **A virus capsid-like nanocompartment that stores iron and protects bacteria from oxidative stress**. *EMBO J* 2014, **33**:1896-1911.
60. Giessen TW: **Encapsulins: microbial nanocompartments with applications in biomedicine, nanobiotechnology and materials science**. *Curr Opin Chem Biol* 2016, **34**:1-10.
61. Giessen TW, Silver PA: **Widespread distribution of encapsulin nanocompartments reveals functional diversity**. *Nat Microbiol* 2017, **2**:1-11.