FEMS Microbiology Reviews, 2025, **49**, fuaf019
DOI: 10.1093/femsre/fuaf019
Advance access publication date: 9 May 2025
Review Article

Exploring heme and iron acquisition strategies of *Porphyromonas gingivalis*—current facts and hypotheses

Michał Śmiga and Teresa Olczak

Laboratory of Medical Biology, Faculty of Biotechnology, University of Wrocław, 14A F. Joliot-Curie, 50-383 Wrocław, Poland
*Corresponding author. Laboratory of Medical Biology, Faculty of Biotechnology, University of Wrocław, 14A F. Joliot-Curie, 50-383 Wrocław, Poland. E-mail: michal.smiga@uwr.edu.pl
Editor: [Grzegorz Wegrzyn]

Abstract

Iron and heme are crucial for pathogenic bacteria living in the human host but are not available in free form due to their binding by iron- and heme-sequestering proteins. *Porphyromonas gingivalis* causes dysbiosis in the oral microbiome and is considered a keystone pathogen in the onset and progression of periodontal diseases. Its ability to infect and multiply in host cells and its presence in distant tissues and fluids highlights its pathogenic versatility and explains the relationship between periodontal diseases and systemic or neurodegenerative diseases. *Porphyromonas gingivalis* has evolved specialized mechanisms that allow it to thrive in the host under adverse nutrient-limited conditions. This review presents the updated summary of the mechanisms of iron and heme acquisition by *P. gingivalis*, with a central role played by gingipains and the unique Hmu system. The potential role of other iron and heme acquisition systems, such as Hus and Iht, indicates the importance of the partially conserved heme biosynthesis pathway, involving homologs of the HemN, HemG, and HemH proteins. In light of increasing antibiotic resistance, difficulties with diagnosis, and drug administration, targeting the mechanisms of heme and iron acquisition of *P. gingivalis* represents a promising target for developing diagnostic tests, preventive or therapeutic strategies.

Keywords: *Porphyromonas gingivalis*; periodontal disease; heme; iron; gingipain; Hmu

Abbreviations

ATP:	Adenosine triphosphate
FeoB:	Ferrous iron transport protein B
GCF:	Gingival crevicular fluid
GTP:	Guanosine triphosphate
HA:	Hemagglutinin/adhesin domain
Hb:	Hemoglobin
heme:	Fe^{2+}/Fe^{3+} protoporphyrin IX
K_d:	Dissociation constant
Kgp:	Lysine-specific gingipain
metHb:	Methemoglobin
OMVs:	Outer membrane vesicles
oxyHb:	Oxyhemoglobin
PPIX:	Protoporphyrin IX
RgpA and RgpB:	Arginine-specific gingipains
TDR:	TonB-dependent outer membrane receptor

Introduction

Porphyromonas gingivalis—a human opportunistic pathogen

The human body is inhabited by microbiota, creating multispecies consortia, with the oral microbiome being among the most diverse (Dewhirst et al. 2010). In healthy humans, the oral microbiome consists mainly of Gram-positive, aerobic bacteria, with the species of *Streptococcus* occupying a broad range of oral habitats (Verma et al. 2018, Baty et al. 2022). However, numerous factors such as poor oral hygiene, smoking, genetic predispositions, and comorbidities can disrupt the balance in the oral cavity and develop environmental conditions with reduced oxygen content within the periodontal pockets. The development of periodontal diseases is associated with an ecological shift in the oral microbiome and dysbiosis, resulting in the predominance of anaerobic, Gram-negative late colonizers over aerobic, commensal, Gram-positive early colonizers (reviewed in Socransky et al. 1998, Holt and Ebersole 2005, Cai et al. 2021, Boyapati et al. 2024, Lamont and Kuboniwa 2024). The most frequent late colonizers are *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* (Fig. 1). Other bacteria, mainly *Prevotella intermedia* and *Fusobacterium nucleatum*, serve as bridging species with late colonizers. Bacteria, especially those with pathogenic potential, cooperatively interact to establish anaerobic and reduced environments and exchange metabolic byproducts. This drives community maturation, dysbiosis, and subverting host immune defenses, resulting in periodontal diseases (reviewed in Hajishengallis et al. 2012, Hajishengallis 2015, Kuboniwa et al. 2017, Hajishengallis and Diaz 2020).

Periodontal diseases affect 20%–50% of the human population, with over 10% experiencing its most severe form, periodontitis (Hugoson et al. 2008, Kassebaum et al. 2014, Tonetti et al. 2018, Chen et al. 2021, Siddiqui et al. 2023, Nascimento et al. 2024). An inflammatory response in the adjacent gingiva is triggered in a suitable host environment, resulting in gingivitis, charac-

Received 9 March 2025; **revised** 2 May 2025; **accepted** 8 May 2025

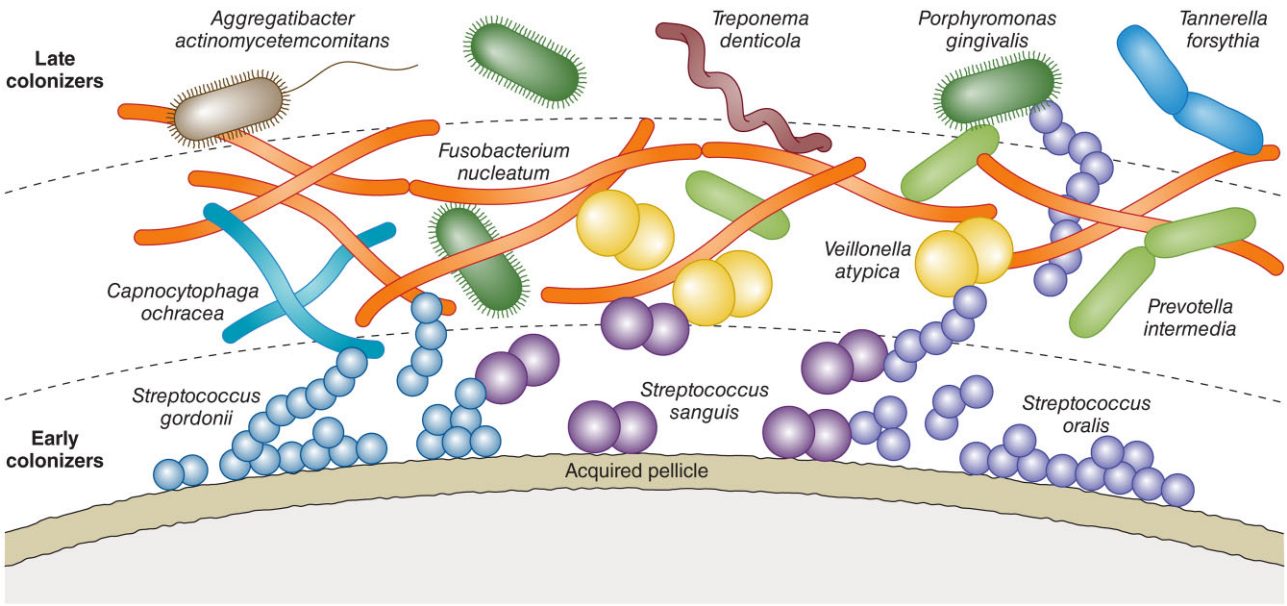

Figure 1. Schematic presentation of the oral biofilm with the key bacterial species involved in developing periodontal diseases. The formation of oral biofilm begins with the deposition of organic molecules on the surfaces of teeth and soft tissues, leading to the development of an acquired pellicle. This layer serves as a substrate for the initial adhesion of early bacterial colonizers, predominantly species from the *Streptococcus* genus, such as *S. oralis*, *S. sanguis*, and *S. gordonii*. These early colonizers help establish a multispecies microbial community through coaggregation and metabolic interactions. With environmental change and shift into more anaerobic and reduced conditions, colonization by late colonizers occurs, including anaerobic bacteria such as *P. gingivalis*, *T. forsythia*, and *T. denticola*. Other bacteria, including *P. intermedia* and *F. nucleatum*, are bridging species between early and late colonizers.

terized by redness, swelling, and bleeding of the gums. This mild, reversible periodontal disease is nondestructive to the tooth-supporting tissues (Philstrom et al. 2005, Schincaglia et al. 2017). When the microbial community undergoes further shifts in composition, resulting in the overgrowth of more pathogenic species, mainly *P. gingivalis*, *T. forsythia*, *T. denticola*, and *P. intermedia* (Fig. 1), irreversible periodontitis is developed (Flemming 1999, Kinane 2001, Philstrom et al. 2005, Hajishengallis and Diaz 2020). Periodontitis results in inflammation within tooth-supporting tissues, deepening of the periodontal pockets, gum bleeding, the loss of alveolar bone and connective tissue attachment to the tooth, and tooth loss. The severity of bleeding depends on the intensity of the gingival inflammation (Page and Schroeder 1976), partly caused by an exaggerated proinflammatory response of the host cells against bacterial virulence factors (Darveau et al. 2012, Olsen et al. 2017, Hajishengallis and Diaz 2020, Hajishengallis and Lamont 2021).

Porphyromonas gingivalis is recognized as the keystone pathogen responsible for dysbiosis in the oral microbiome and developing periodontitis in humans. It produces several virulence factors that participate in the destruction of tooth-supporting tissues. The main role in this process is played by proteases (Hocevar et al. 2018), described in more detail below. Some bacterial components induce macrophages to secrete proinflammatory cytokines and chemokines (Huang and Gibson 2014, Gmiterek et al. 2016), which recruit neutrophils and lymphocytes to infected sites, the latter producing additional proinflammatory mediators (Carvalho-Filho et al. 2016, Olsen and Yilmaz 2016, Suarez et al. 2020). As an outcome, these processes cause an exaggerated proinflammatory host immune response resulting in connective tissue destruction and alveolar bone loss. Importantly, as a part of the pathogenic process, *P. gingivalis* infects host cells, including gingival epithelial cells, endothelial cells, keratinocytes, fibroblasts, and cells of the immune system, multiplies within, and propagates between them, allowing spreading throughout the body and evading the host immune response (Dorn et al. 2001, Yilmaz et al. 2006, Mao et al. 2007, Kuboniwa et al. 2008, Wang and Hajishengallis 2008, Irshad et al. 2012, Olczak et al. 2015, Gmiterek et al. 2016, Sakanaka et al. 2016, Yang et al. 2020, de Jongh et al. 2023, Smiga et al. 2024a). The bacterium can also invade and modify the properties of periodontal ligament stem cells (Pan et al. 2017), which are used to differentiate into mature periodontal fibroblasts, cementoblasts, and osteoblasts, enabling proper regeneration and repair of the periodontium (Bartold et al. 2000).

The presence of *P. gingivalis*, its outer membrane vesicles (OMVs), or DNA was detected in host niches other than the oral cavity, such as plasma, synovial fluid, atherosclerotic plaque, or even the brain (Figuero et al. 2011, Dominy et al. 2019, Bregaint et al. 2022). Therefore it is not surprising that in addition to its role in the development of periodontal diseases, increasing evidence shows that *P. gingivalis* is considered one of the factors influencing the risk of development and progression of concomitant human diseases. Diabetes, osteoporosis, cardiovascular and respiratory diseases, rheumatoid arthritis, and cancer are among such comorbidities (Tunney et al. 2008, Benedyk et al. 2015, Mei et al. 2020, Hajishengallis and Chavakis 2021, Zhang et al. 2021b, Baima et al. 2024, Butler et al. 2024, Lu et al. 2024, Villoria et al. 2024). *Porphyromonas gingivalis* may also invade and colonize the gastrointestinal tract through mouth–gut transmission, resulting in participation in gut-related systemic diseases and gastrointestinal cancers (du Teil Espina et al. 2018, Baima et al. 2024). Growing evidence suggests that infection-based backgrounds, including periodontal diseases, may heighten the risk of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases (Dominy et al. 2019, Kanagasingam et al. 2020, Ermini et al. 2024, Li et al. 2024). Although *P. gingivalis* cells do not cross the

blood–brain barrier, OMVs with their highly proteolytic cargo (Veith et al. 2014) may invade microvascular endothelial cells and their components degrade tight junction proteins, leading to increased barrier permeability (Nonaka et al. 2022).

Porphyromonas gingivalis lifestyles and general growth requirements

The high bacterial density in the oral cavity causes intense competition among microbiota to acquire nutrients. Although direct interspecies cell-to-cell contact is not an absolute requirement for the interaction of *P. gingivalis* with other bacteria, close distance facilitates their communication and biofilm formation by delivering growth-promoting nutrients and signals (Kuboniwa and Lamont 2010, Marsh et al. 2011, Hoare et al. 2021). Although *P. gingivalis* is an anaerobic bacterium, it can also grow in microbiome regions exposed to aerobic conditions (Zijnge et al. 2010, Mark Welch et al. 2016). Therefore, colocalization with early colonizers, such as *Streptococcus gordonii* and *P. intermedia* (Fig. 1), benefits *P. gingivalis* under a range of higher oxygen levels (Brown et al. 2018, Bielecki et al. 2020, Slezak et al. 2020).

Bacteria prefer to live in biofilm structures rather than choose a planktonic lifestyle. The formation of oral biofilm is initiated by the salivary pellicle, which serves as a base for bacterial adhesion, colonization, and proliferation (Enax et al. 2023). Residents of oral biofilm differ depending on the niche they occupy (i.e. saliva, the surface of the tongue, dental enamel, and supra- and subgingival surfaces) and the biofilm layer they form (Marsh et al. 2011). Stages of biofilm formation comprise coaggregation, coadhesion, maturation, and dispersion, and require physical and metabolic relationships between bacteria (Wang et al. 2023, Zeineldin et al. 2023). Bacteria first attach reversibly to teeth through van der Waals and hydrophobic interactions and form colonies to stabilize attachment. They are surrounded by an extracellular polymeric matrix produced by bacteria, predominantly containing anionic bacterial exopolymers, such as polysaccharides, and by other bacterial or environmental components, including proteins, nucleic acids, lipids, teichoic acids, and organic molecules (Flemming et al. 2016, Dragos and Kovacs 2017). Channels and pores within the biofilm structure allow nutrient access and circulation. Living within a biofilm protects bacteria from environmental stresses, including mechanical and chemical forces and the host immune response (Takahashi 2015). It also limits the penetration of antibiotics and other antibacterial agents and reduces the metabolic activity of biofilm-embedded bacteria, resulting in their lower sensitivity to antibiotics (Marsh et al. 2011). Altogether, the biofilm lifestyle decreases the chance of their eradication and successful infection treatment. The maturation and accumulation of biofilm cause the formation of dental plaque (Rosan and Lamont 2000). If untreated, dental plaque undergoes mineralization, resulting in dental calculus, which consists of an organic matrix derived from saliva, gingival crevicular fluid (GCF), bacterial products, and inorganic components (D'Souza et al. 2023, Wei et al. 2024). Interestingly, dental calculus can persist even on ancient skeletal remains, allowing the identification of bacterial species. Among oral pathogens, *P. gingivalis* and *T. forsythia* were identified in ancient calcified dental plaque dating back even thousands of years (Adler et al. 2013, Bravo-Lopez et al. 2020).

Porphyromonas gingivalis is an asaccharolytic bacterium that acquires energy through the fermentation of amino acids (Ohara-Nemoto et al. 2011, Nemoto and Ohara-Nemoto 2016, Miller and Scott 2021). Since it cannot utilize free amino acids, it relies on peptides as its primary carbon and nitrogen source (Milner et al. 1996). *Porphyromonas gingivalis* is an extremely proteolytically active bacterium. Among the most important endopeptidases are gingipains, which are responsible for ~80% of its total proteolytic activity (Hocevar et al. 2018, Kadowaki 2021). By degrading host proteins, gingipains facilitate the acquisition of short peptides *via* the RagA/RagB transport system (Potempa et al. 2021). Peptides are then further degraded by the intracellular activity of oligopeptidases, and di- and tripeptidyl peptidases (Otogoto and Kuramitsu 1993, Lu and McBride 1998, Veillard et al. 2012, Nemoto and Ohara-Nemoto 2016, 2021, Shimoyama et al. 2023). *Porphyromonas gingivalis* becomes more proteolytic (mainly by increased gingipain expression) in response to increasing heme levels (Marsh et al. 1994). An increase in pH, which is correlated with inflammation, also causes higher gingipain expression (McDermid et al. 1998). Moreover, inflammation enhances endogenous proteolytic activity since neutrophils infiltrating into inflamed periodontal tissues deliver serine proteases (elastase, cathepsin G, and protease 3) and metalloproteases (MMP-8 and MMP-9) (Scott and Krauss 2012, Bondy-Carey et al. 2013, Benedyk et al. 2015, Bernaerts et al. 2024). In addition, the inactivation of protease inhibitors by gingipains increases *P. gingivalis* proteolytic activity and nutrient availability (Andrian et al. 2007, Plaza et al. 2016).

Porphyromonas gingivalis heme requirements

Bacteria synthesize heme from glutamate using the protoporphyrin IX (PPIX)-dependent pathway or may use the coproporphyrin III-dependent pathway (Fig. 2A), or other alternative pathways (Jacobs et al. 1971, Dailey et al. 2017, Layer 2021, Mingers et al. 2024). Like many members of the Bacteroidota (formerly Bacteroidetes) phylum, *P. gingivalis* is a heme auxotroph lacking the full heme biosynthesis pathway (Roper et al. 2000, Kusaba et al. 2002, Nelson et al. 2003, Rocha et al. 2019). *Porphyromonas gingivalis* encodes only four proteins of the final steps of the PPIX-dependent heme biosynthesis pathway: uroporphyrinogen III synthase (HemD), coproporphyrinogen III oxidase (HemN), protoporphyrinogen IX dehydrogenase (HemG), and ferrochelatase (HemH) (Fig. 2B). *Porphyromonas gingivalis* can grow in culture media without added heme but supplemented with PPIX and inorganic iron (Olczak et al. 2012, Gao et al. 2018, Smiga et al. 2024a). One of the explanations of this property is the hypothesis that heme can be formed from PPIX and iron due to preserved HemH ferrochelatase activity. *In vitro* studies showed that the *hemG* gene is not essential for *P. gingivalis*, since deletion of the *hemG* gene did not influence its phenotype (Szczesniak et al. 2023). However, the *P. gingivalis hemG* gene may be functional as it restored the phenotype of an *Escherichia coli hemG* deletion mutant strain (Kusaba et al. 2002). Nevertheless, studies on the partially preserved heme biosynthesis pathway in *P. gingivalis* are limited and its role should be elucidated.

Heme is a limiting growth factor for *P. gingivalis*, and its deficiency decreases its pathogenic potential (McKee et al. 1986, Guo et al. 2020). Heme requirements for *P. gingivalis* are also strain-specific (Ohya et al. 2016), with some strains more sensitive to fluctuations in heme concentration (Ohya et al. 2016, Smiga et al. 2024b). This may explain the presence of more invasive, encapsulated, poorly fimbriated strains (e.g. W83 and A7436) with greater resistance to high heme and hemoglobin (Hb) concentrations in periodontal pockets of patients with periodontitis, especially in advanced stages of the disease characterized by gum bleeding

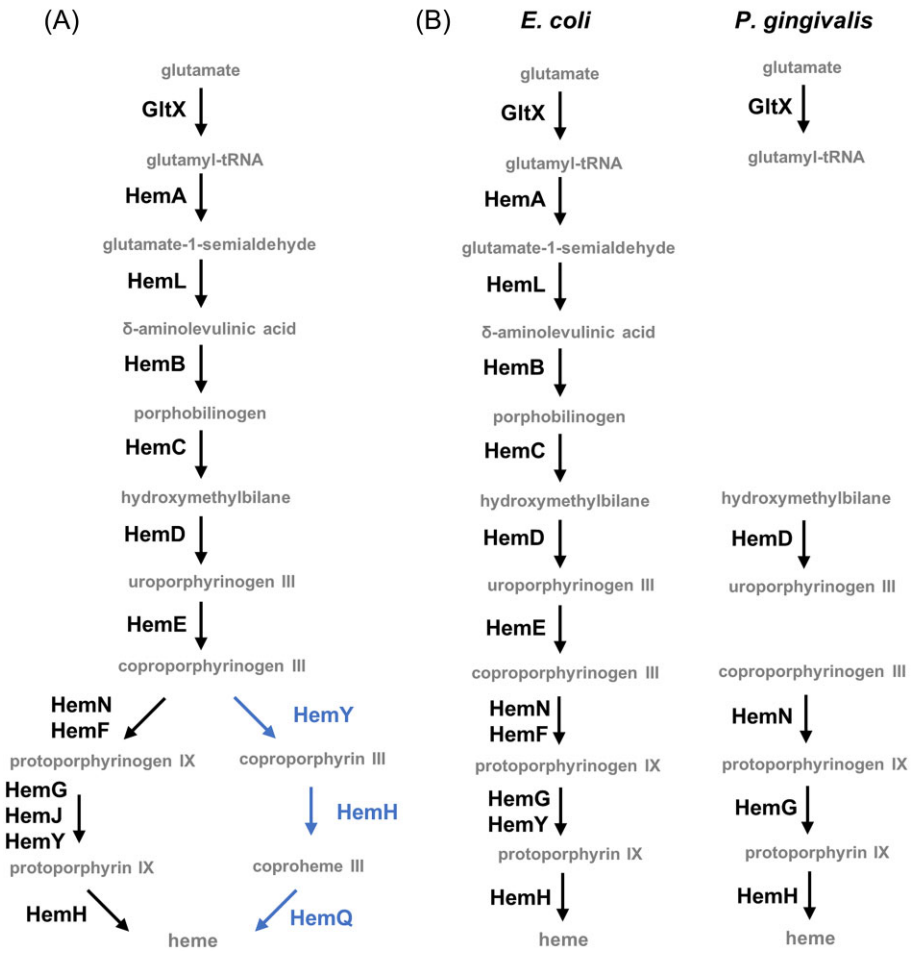

Figure 2. Heme biosynthesis pathways in bacteria. (A) Key proteins and reactions involved in bacterial heme biosynthesis. An alternative coproporphyrin-dependent heme biosynthesis pathway is shown in blue. (B) Comparison of the *E. coli* heme biosynthesis pathway and the residual heme biosynthesis pathway in *P. gingivalis*. Due to the preservation of the *hemN*, *hemG*, and *hemH* gene homologs, *P. gingivalis* theoretically can synthesize heme from coproporphyrinogen III. GltX—glutamyl-tRNA synthetase; HemA—glutamyl-tRNA reductase; HemL—glutamate-1-semialdehyde-2,1-aminomutase; HemB—porphobilinogen synthase; HemC—porphobilinogen deaminase; HemD—uroporphyrinogen III synthase; HemE—uroporphyrinogen decarboxylase; HemN—oxygen-independent coproporphyrinogen III oxidase; HemF—oxygen-dependent coproporphyrinogen III oxidase; HemG—oxygen-independent protoporphyrinogen IX dehydrogenase; HemY—oxygen-dependant coproporphyrinogen III oxidase (can convert protoporphyrinogen IX to protoporphyrin IX); HemJ—oxygen-independent protoporphyrinogen IX oxidase; HemH—ferrochelatase; and HemQ—coproheme decarboxylase.

(Griffen et al. 1998, Gmiterek et al. 2013). In contrast, less invasive, nonencapsulated, highly fimbriated strains (e.g. ATCC 33277), found mainly in healthy periodontium, are less resistant to high heme and Hb concentrations (Griffen et al. 1998, Gmiterek et al. 2013). Iron and heme availability influence the expression of several *P. gingivalis* genes through different mechanisms (Olczak et al. 2005, 2024, Lewis 2010, Ciuraszkiewicz et al. 2014, Smiga et al. 2019a), including DNA methylation (Costeira et al. 2023). Moreover, heme concentration in the growth medium modulates the lipopolysaccharide lipid A structural content (Champagne et al. 1996, Cutler et al. 1996, Al-Qutub et al. 2006). At low heme concentrations, one major penta-acylated lipid A structure is present, whereas at high heme concentrations, multiple tetra- and penta-acylated lipid A structures are found. Since these lipid A structures have opposite effects on TLR-4 activation, the alteration of its structure may differentially influence the host immune response to this bacterium (Wang and Ohura 2002, Darveau et al. 2004).

Iron and heme acquisition strategies used by Gram-negative bacteria

Iron is indispensable for life and only some lactic acid bacteria and *Borrelia burgdorferi* use manganese and cobalt instead of iron (Weinberg 1997, Posey and Gherardini 2000). The redox potential of Fe^{2+}/Fe^{3+} allows its versatility when bound to proteins as a catalytic center or electron carrier. Therefore, iron is required for many biological processes, including respiration, tricarboxylic acid cycle, oxygen transport, gene regulation, and DNA biosynthesis. However, ferric iron is insoluble under aerobic conditions, and ferrous iron is toxic due to hydroxyl radicals formation from hydrogen peroxide (Fenton reaction) or superoxide and hydrogen peroxide (Harber–Weiss reaction) (Halliwell and Gutteridge 1992, Kehrer 2000). Also heme, often used by pathogens as a source of iron, is essential for various cellular processes, including transport and storage of oxygen, electron transfer, aerobic respiration, or gas sensing (Choby and Skaar 2016). Similar to ferrous iron, heme is also toxic. Therefore, iron and heme acquisition mechanisms are

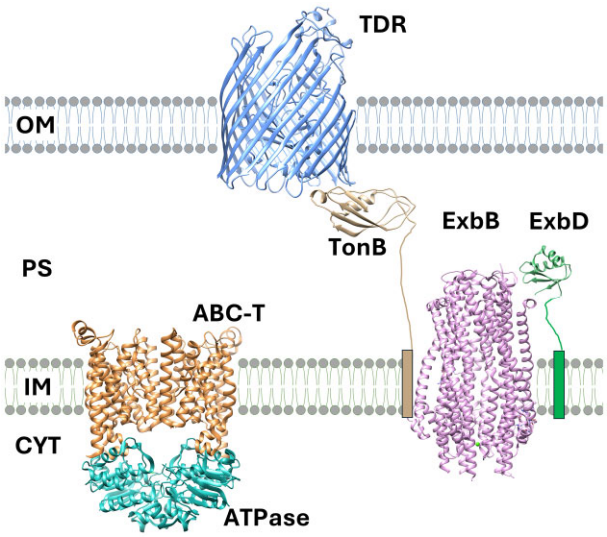

Figure 3. Classical iron or heme transport system of Gram-negative bacteria based on the TDR and the ABC transporter, delivering ligands into the cytoplasm. The transport of iron or heme through the outer membrane performed by the TDR is powered by the TonB–ExbB–ExbD complex. In the periplasmic space, the periplasmic binding protein delivers iron or heme to an ABC transporter. Further transport of ligands through the inner membrane is carried out by a typical ABC transporter, composed of a transmembrane domain (ABC-T) and powered by ATP hydrolysis occurring within the ATP-binding domain of the ATPase. Structures representing TDR (FhuA; PBD ID: 1BY3), TonB (AlphaFold ID: AF-P02929-F1), ExbB (PBD ID: 5SV0), ExbD (AlphaFold ID: AF-P0ABV2-F1), and ABC transporter (complex of *E. coli* $BtuC_2D_2$; PDB ID: 1L7V) were visualized with UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) (Pettersen et al. 2004). OM—outer membrane; PS –periplasmic space; IM—inner membrane; and CYT—cytoplasm.

precisely regulated (Clarke et al. 2001, Noinaj et al. 2010, Bradley et al. 2020).

Although the human body contains ∼4 g of iron, most of this element is bound to Hb and ferritin. As part of the innate immune response, iron-sequestering proteins such as transferrin in the serum and lactoferrin in mucous secretions are produced (Andrews 1999, Cherayil 2011, Sheldon et al. 2016). As a result, free iron concentration is about 10^{-18} M (Bullen et al. 1978, Cherayil 2011, Sheldon et al. 2016), which is far below the levels required to support bacterial growth (10^{-8}–10^{-6} M) (Guerinot 1994). Similarly, the concentration of free heme in the serum is at a negligible level (Khan and Quigley 2011), primarily due to the production of heme-sequestering proteins, albumin and hemopexin, and heme detoxification by the liver (Chiabrando et al. 2014). In addition, Hb released from erythrocytes is rapidly sequestered by haptoglobin (Kristiansen et al. 2001). Therefore, to obtain iron and heme from the host, bacteria have developed several sophisticated mechanisms.

Gram-negative bacteria rely on the TonB-dependent outer membrane receptor (TDR) and the TonB–ExbB–ExbD protein complex localized in the inner membrane and periplasmic space (Fig. 3). TDRs form the β-barrel structure composed of 22 antiparallel β strands and an N-terminal plug domain, regardless of the ligand transported (Ferguson and Deisenhofer 2002). Although their amino acid identity is relatively low, they exhibit high structural similarity. TDRs differ in the length and orientation of external loops, which are engaged in ligand recognition, and the length of the plug domain (Ferguson and Deisenhofer 2002, Noinaj et al. 2010). Substrate transport through TDR is powered by the electrochemical potential (proton motive force). The interaction of the TonB protein with TDR *via* a specific TonB box region of the TonB–ExbB–ExbD complex provides energy for substrate transport through TDR (Pawelek et al. 2006, Celia et al. 2016).

Iron acquisition mechanisms in Gram-negative bacteria

To acquire iron, many bacteria synthesize and secrete siderophores or utilize xenosiderophores produced by other microorganisms (Stinzi et al. 2000). Siderophores, belonging mainly to catecholates (e.g. enterobactin), hydroxycaboxylates (e.g. citrate), and hydroxymates (e.g. ferrichrome), chelate ferric iron (Fe^{3+}) with high affinity (K_d ∼10^{-10}–10^{-7} M) (Stinzi et al. 2000, Klebba et al. 2021). Siderophore–iron complexes are bound to and transported through the outer membrane by TDRs (Fig. 4) (Braun and Killmann 1999, Ferguson and Deisenhofer 2002, Krewulak and Vogel 2008). Some examples are diferric citrate receptor FecA from *E. coli* (PDB ID: 1KMO) (Ferguson et al. 2002), pyochelin receptor FptA from *Pseudomonas aeruginosa* (PDB ID: 1XKW) (Cobessi et al. 2005), enterobactin receptor FepA from *E. coli* (Buchanan et al. 1999), *E. coli* FhuA (1BY3) or FhuA receptor in complex with ferrichrome (1BY5) (Locher et al. 1998), as well as *E. coli* FhuA (1QJQ) (Ferguson et al. 2000) or FhuA in complex with albomycin (1QKC) (Ferguson et al. 2000) or rifamycin (PDB ID: 1FI1) (Ferguson et al. 2001). Determination of the structure of FhuA in complex with TonB (PDB ID: 2GRX) (Pawelek et al. 2006) shows the interaction between both partners. The ability of siderophore TDRs to transport antibiotics was employed to construct antibacterial drugs by conjugation of siderophores with antibiotics, resulting in higher treatment efficiency compared to antibiotics alone (Braun and Braun 2002, Luscher et al. 2018).

Other TDRs can recognize transferrin or lactoferrin and bind iron for subsequent transport into the periplasmic space (Fig. 4) (Perkins-Balding et al. 2004, Noinaj et al. 2013, Pogoutse and Moraes 2017, Ostan et al. 2021, Chan et al. 2023). Among them is TbpA from *Neisseria meningitidis* (PDB ID: 3V89) or TbpA from *Neisseria gonorrhoeae* (Noinaj et al. 2012, Chan et al. 2023). TbpA cooperates with outer membrane-associated lipoprotein TbpB (PDB ID: 3V8U) (Noinaj et al. 2012, Chan et al. 2023), which binds transferrin (K_d ∼10^{-8}–10^{-7} M) and delivers iron to TbpA, resulting in more efficient iron transport (Anderson et al. 1994, Moraes et al. 2009).

After transport of siderophore–iron complex or iron into the periplasmic space, they are shuttled by periplasmic-binding proteins and transported into the cytoplasm by inner membrane ABC (ATP-binding cassette) transporters, powered by adenosine triphosphate (ATP) (Velayudhan et al. 2000, Krewulak and Vogel 2008, Chu and Vogel 2011). After reduction, ferrous iron (Fe^{2+}) is transported from the periplasmic space into the cytoplasm by a FeoB (ferrous iron transport protein B) whose function is powered by guanosine triphosphate (GTP) (Fig. 4) (Lau et al. 2016). In the cytoplasm, the reduction of ferric iron by cytosolic or inner membrane-associated reductases facilitates its release from siderophores (Fischer et al. 1990, Josts et al. 2021). Apo-siderophores are then inactivated and excreted (Hartmann and Braun 1980). If not used, the iron excess is stored mainly in bacterioferritin, which, in contrast to mammalian ferritin, also binds heme (Ratnayake et al. 2000, Bradley et al. 2020).

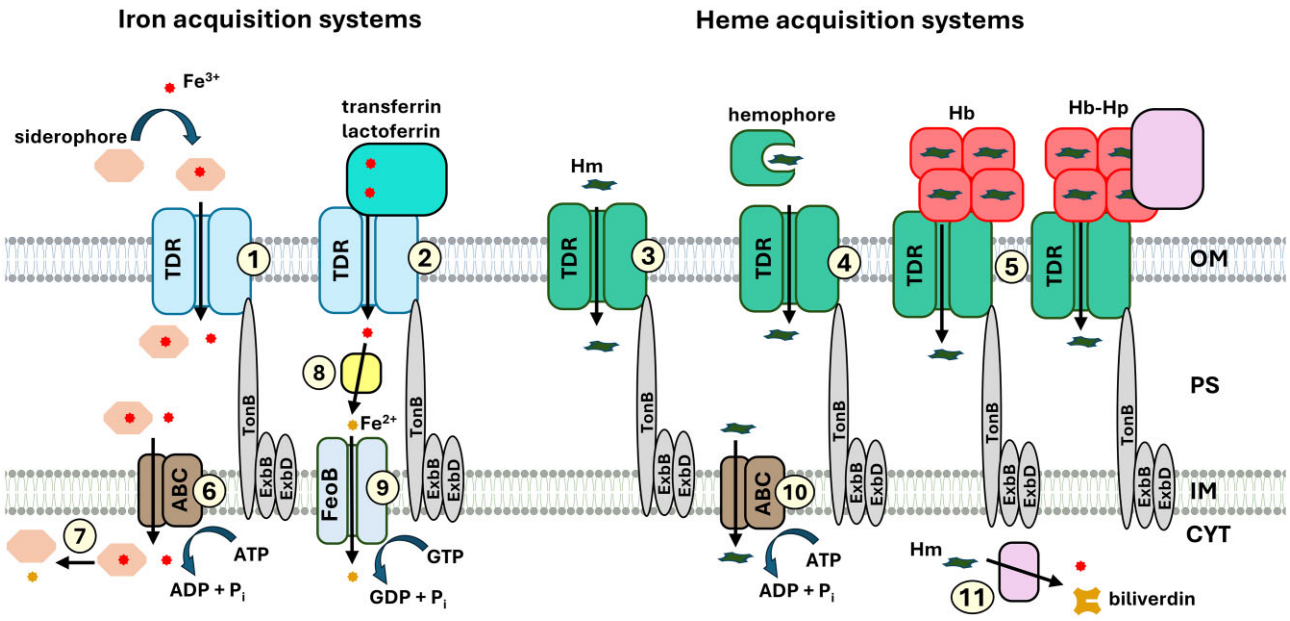

Figure 4. Iron and heme acquisition strategies used by Gram-negative bacteria. Iron (1 and 2) and heme (3–5) uptake occurs through TDRs, powered by the TonB–ExbB–ExbD complex. (1) In the siderophore-dependent mechanism, bacteria secrete and utilize siderophores or utilize xenosiderophores produced by other bacteria to chelate Fe^{3+} ions and deliver them to TDRs, which transport the iron–siderophore complex into the periplasm. (2) Some bacteria use transferrin or lactoferrin as an iron source. Iron-carrying proteins are recognized by TDRs or TDR-associated proteins. TDRs bind and transport iron into the periplasmic space. Iron or iron-siderophore complexes are transported from the periplasmic space to the cytoplasm *via* inner membrane ABC transporters powered by ATP hydrolysis (6). In the cytoplasm, ferric iron (Fe^{3+}) is reduced and released from the siderophores (7). Alternatively, after reduction in the periplasmic space (8), ferrous iron (Fe^{2+}) is transported by the FeoB protein, powered by GTP hydrolysis (9). Free heme (3) or heme delivered by hemophore or hemophore-like proteins (4) is bound to TDR and transported to the periplasmic space. Hb or hemoglobin–haptoglobin (Hb–Hp) complex may be a direct heme source from which heme is uptaken by TDRs (5). Heme is transported from the periplasmic space to the cytoplasm *via* inner membrane ABC transporters powered by ATP hydrolysis (10). Iron is released from heme by heme oxygenases or other iron-releasing mechanisms (11). Fe—iron; Hm—heme; ABC—ATP-binding cassette transporter; OM—outer membrane; PS—periplasmic space; IM—inner membrane; and CYT—cytoplasm.

Heme acquisition mechanisms in Gram-negative bacteria

A typical heme uptake system of Gram-negative bacteria is the Hmu system of *Yersinia pestis* (HmuRSTUV) (Hornung et al. 1996, Thompson et al. 1999) or the Hem system of *Yersinia enterocolitica* (Stojiljkovic and Hantke 1992). Heme is transported from the external environment across the outer membrane through TDRs (Fig. 4) (e.g. *Y. pestis* HemR or *Y. enterocolitica* HmuR) (Higgs et al. 2002, Ferguson et al. 2007, Contreras et al. 2014, Silale and van den Berg 2023). Depending on the bacterium, TDRs recognize Hb, Hb–haptoglobin complex, heme alone, or heme transferred by hemophores or hemophore-like proteins, and then transport released heme into the periplasmic space (Burkhard and Wilks 2007, Ascenzi et al. 2015). *Neisseria meningitidis* and *N. gonorrhoeae* utilize a two-component HpuA/HpuB system to acquire heme from Hb and Hb bound to haptoglobin (Lewis et al. 1998, Awate et al. 2024). HpuA is a typical TDR, and HpuB is an outer membrane-associated lipoprotein, different from hemophores or hemophore-like proteins, facilitating heme transfer to TDRs. Some bacteria possess multiple heme-transporting TDRs, including *Vibrio cholerae* (HutA, HutR, and HasR) (Mey and Payne 2001), *N. meningitidis* (HmbR and HpuB), *Serratia marcescens* (HemR and HasR), which are part of heme uptake systems utilized under different heme availability (Richardson and Stojiljkovic 1999, Benevides-Matos and Biville 2010). Heme binding and transport through TDR engage conserved histidine residues (e.g. His^{128} and His^{461} in *Y. enterocolitica* HemR, His^{86} and His^{420} in *S. dysenteriae* ShuA, or His^{189} and His^{603} in *S. marcescens* HasR) (Bracken et al. 1999, Burkhard and Wilks 2007, Brillet et al. 2009, Cobessi et al. 2010).

In the periplasmic space, heme is shuttled by periplasmic binding proteins (e.g. *Y. pestis* HmuT or *Y. enterocolitica* HemT) and transported into the cytoplasm by inner membrane ABC transporters (Fig. 4) (e.g. *Y. pestis* HmuU and HmuV or *Y. enterocolitica* HemU and HemV) powered by ATP (Wyckoff et al. 1998, Ho et al. 2007, Chu and Vogel 2011). In the cytoplasm, proteins such as HmuS and HemS transfer heme to enzymes that either utilize heme or break heme down to release iron from heme (e.g. heme oxygenases) (Schneider and Paoli 2005, Lansky et al. 2006, Schneider et al. 2006).

Primary sources of heme for periodontopathogens

In the primary niche, the periodontal pocket, heme availability changes during the progression of periodontitis. Heme is extremely limited in healthy individuals and at the early stages of gum inflammation. The main potential source of heme, aside from dietary intake, comes from the lysis of bacteria residing in the periodontal pocket and building the biofilm structures (Perry et al. 2009, Ibanez de Aldecoa et al 2017, Campoccia et al. 2021). For example, heme-synthesizing bacteria, such as *Veillonella atypica*, can support the growth of *P. gingivalis* (Zhou et al. 2016). Besides bacterial heme sources, at this stage of infection, periodontopathogens utilize proteins from the GCF, which is a complex mixture comprising substances derived from serum, leukocytes, structural cells of the periodontium, and oral bacteria (Khurshid et al. 2017). In the healthy periodontium, GCF volume in periodontal pockets is low and its flow rate is slow (Curtis et al. 1990, Hanioka et al. 2005). Although serum may contain free Hb at an

average level of 0.1 mg/ml (1.5 μM) and its concentration up to 0.25 mg/ml (3.85 μM) is considered normal (Lippi et al. 2014), in healthy individuals Hb level in GCF is much lower than in more advanced stages of gingival inflammation (Ito et al. 2016, Ito et al. 2021, Ito et al. 2024). Therefore, at the initial stage of gingival infection, albumin present in GCF is the main heme source, at concentrations comparable to those found in serum (~40 mg/ml; ~600 μM) (Bang and Cimasoni 1971, Muller-Eberhard and Morgan 1975, Morgan et al. 1976, Makela et al. 1991, Taketani et al. 1998, Miller and Shaklai 1999). Albumin possesses 1 high-affinity and at least 10 lower-affinity heme-binding sites for heme (K_d ~10^{-8} M) (Beaven et al. 1974, Ascenzi et al. 2005, Kamal and Behere 2005, Ascenzi and Fasano 2009, De Simone et al. 2023). However, under the physiological conditions, only ~0.018% of albumin molecules bind heme; therefore, albumin–heme complex concentration in the serum is about 7.3 μg/ml (~0.11 μM) (Miller and Shaklai 1999, Graca-Souza et al. 2002). As inflammation increases, the GCF flow and volume increase, and protein concentration, including albumin is higher (Bickel et al. 1985, Bostanci and Belibasakis 2018, Ito et al. 2021). Hemopexin, with 0.4–1.5 mg/ml (~6–24 μM) concentration in normal serum (Muller-Eberhard et al. 1968), begins to be delivered to the GCF at higher levels with the development of inflammation (Delanghe and Langlois 2001). It also has a much higher affinity for heme (K_d ~10^{-13}–10^{-14} M) than albumin (Morgan 1976, Paoli et al. 1999, Morgan et al. 1976, Tolosano and Altruda 2002, Ascenzi and Fasano 2007, Detzel et al. 2021). With the development of periodontal disease, Hb becomes the main source of heme for periodontopathogens, which is associated with the deepening of the periodontal pockets, weakening of the tooth-supporting tissues, and bleeding.

Heme acquisition strategies of *P. gingivalis*

Porphyromonas gingivalis gingipains facilitate heme acquisition from host hemoproteins

Due to the inability of the *de novo* heme biosynthesis, *P. gingivalis* is a heme auxotroph. Therefore, heme uptake fulfills both heme and iron requirements of this bacterium (Olczak et al. 2005, Smalley and Olczak 2017). In addition to heme uptake performed by TDRs, *P. gingivalis* developed specialized accessory mechanisms that facilitate heme acquisition from Hb present in erythrocytes and other hemoproteins. *Porphyromonas gingivalis* exhibits hemagglutinating and hemolytic activities due to the production of hemolysins and hemagglutinins (Chu et al. 1991, Lewis et al. 1999, Shi et al. 1999). The key role in this process is played by gingipains that belong to the peptidase family C25 (Eichinger et al. 1999). Interestingly, homologs of these cysteine proteases have been identified only in two other *Porphyromonas* species, *Porphyromonas gulae* and *Porphyromonas loveana*, which are canine and marsupial pathogens, respectively (Morales-Olavarria et al. 2023, Smiga and Olczak 2025). Therefore, in the human oral microbiome, gingipains are characteristic of *P. gingivalis* only.

Gingipains function in soluble and membrane-associated forms (Potempa et al. 2003, Guo et al. 2010). *Porphyromonas gingivalis* uses a type IX secretion system (T9SS) to transport gingipains to the surface of bacterial cells and secrete them. Proteins transported by this system (~30) are conjugated with an A-lipopolysaccharide (A-LPS) anchor, thus forming the electron-dense surface layer. At least 19 proteins have been identified to form T9SS system including PorE, PorF, PorG, PorK/GldK, PorL/GldL, PorM/GldM, PorN/GldN, PorP, PorQ, PorT/SprT, PorU, PorV, PorW/SprE, PorZ, Sov/SprA, and Plug, PorA, and PGN_1783, and 3 proteins involved in its regulation (PorX, PorY, and SigP) (Lasica et al. 2017, Veith et al. 2017, Gorasia et al. 2020, Paillat et al. 2023). Cargo proteins have an N-terminal signal peptide for transport across the inner membrane by the Sec system. They are targeted to the outer membrane translocon *via* their conserved C-terminal domain signal (CTD) composed of about 80 amino acid residues (Seers et al. 2006, Shoji et al. 2011, Veith et al. 2013, Mizgalska et al. 2024).

Porphyromonas gingivalis produces two types of gingipains: two arginine-specific (RgpA and RgpB) gingipains and one lysine-specific (Kgp) gingipain. Gingipains are multidomain protein complexes formed by proteolytic processing of the nascent translated polypeptides, resulting in noncovalently associated regions (Fig. 5A) (Han et al. 1996, Bhogal et al. 1997, Li and Collyer 2011). RgpB contains a catalytic domain, whereas Kgp and RgpA comprise a catalytic domain and carboxy-terminal hemagglutinin/adhesin (HA) domains (Potempa et al. 2003, Li and Collyer 2011, Dashper et al. 2017). Although RgpA and Kgp catalytic domains are divergent, their HA domains are similar, with high similarity also to the hemagglutinin A (HagA) (Fig. 5A) (Pavloff et al. 1995, 1997, Han et al. 1996, Nakayama et al. 1998, Shi et al. 1999, Sakai et al. 2007, Li and Collyer 2011). However, recent findings revealed strain-dependent differences in HA domains of Kgp (Li and Collyer 2011, Dashper et al. 2017).

Intact *P. gingivalis* cells and gingipains, including monomeric Kgp or RgpA and the heterodimeric Kgp/RgpA complex, purified from cell membranes or growth cultures, can agglutinate and lyse erythrocytes (Fig. 5B). Hemolytic and hemagglutinating activities of Kgp, RgpA, and HagA are performed mainly by their HA domains (Nakayama et al. 1998, Okamoto et al. 1998, DeCarlo et al. 1999, Shi et al. 1999, Olczak et al. 2001, Paramaesvaran et al. 2003, Sztukowska et al. 2004, Sakai et al. 2007, Nhien et al. 2010). The HA domains of Kgp and RgpA and their HA2 regions (RgpA15 and Kgp15 regions) (Fig. 5A) bind Hb. The determined affinity of the binding slightly differs depending on the particular region examined: RgpA (K_d ~10^{-9} M), Kgp (K_d ~10^{-9} M), and the RgpA/Kgp complex (K_d ~10^{-9} M) (Pike et al. 1994, Pathirana et al. 2006). Others showed that recombinant Kgp15 (K_d ~10^{-8} M) (Nakayama et al. 1998) and recombinant RgpA15 (K_d ~10^{-9} M) bind Hb (K_d ~10^{-8} M) and also heme, the latter with lower affinity (DeCarlo et al. 1999). In addition, other HA regions, such as Kgp44/HA3/HA4 (Kgp14, Kgp13, and Kgp20) or Rgp17/HA3 and Rgp27/HA4 can be engaged in Hb/heme binding (Fig. 5A) (Nakayama et al. 1998, DeCarlo et al. 1999, Nakayama 2010, Nhien et al. 2010).

More in-depth experiments showed that processed recombinant Rgp44 (aa 720–1081), but not unprocessed Rgp44 (aa 720–1138), is responsible for hemagglutinating activity due to the interaction of this domain with glycophorin A and more efficiently with asialoglycophorin A (K_d ~10^{-7} M) (Sakai et al. 2007). Degradation of glycophorin A by RgpB sensitizes erythrocytes to the hemolytic activity (Li et al. 2010). This causes a rapid local increase in the concentration of free Hb. Although free Hb is neutralized by binding to haptoglobin, gingipains can degrade haptoglobin and bind released Hb, resulting in *P. gingivalis* growth (Shizukuishi et al. 1995, Sroka et al. 2001).

In vivo, oxyhemoglobin (oxyHb) is resistant to degradation by Kgp (Smalley et al. 2008). Moreover, the heme in oxyHb is in the ferrous state (Fe^{2+}PPIX) (K_d ~10^{-15}–10^{-12} M) (Hargrove et al. 1996), making heme inaccessible to pathogens. However, the proteolytic activity of Rgps (mainly RgpA) significantly accelerates the process of Hb oxidation, resulting in the conversion of oxyHb to methemoglobin (metHb), rendering the Hb more susceptible to degradation by Kgp (Smalley et al. 2007). Hb monomers contain 11

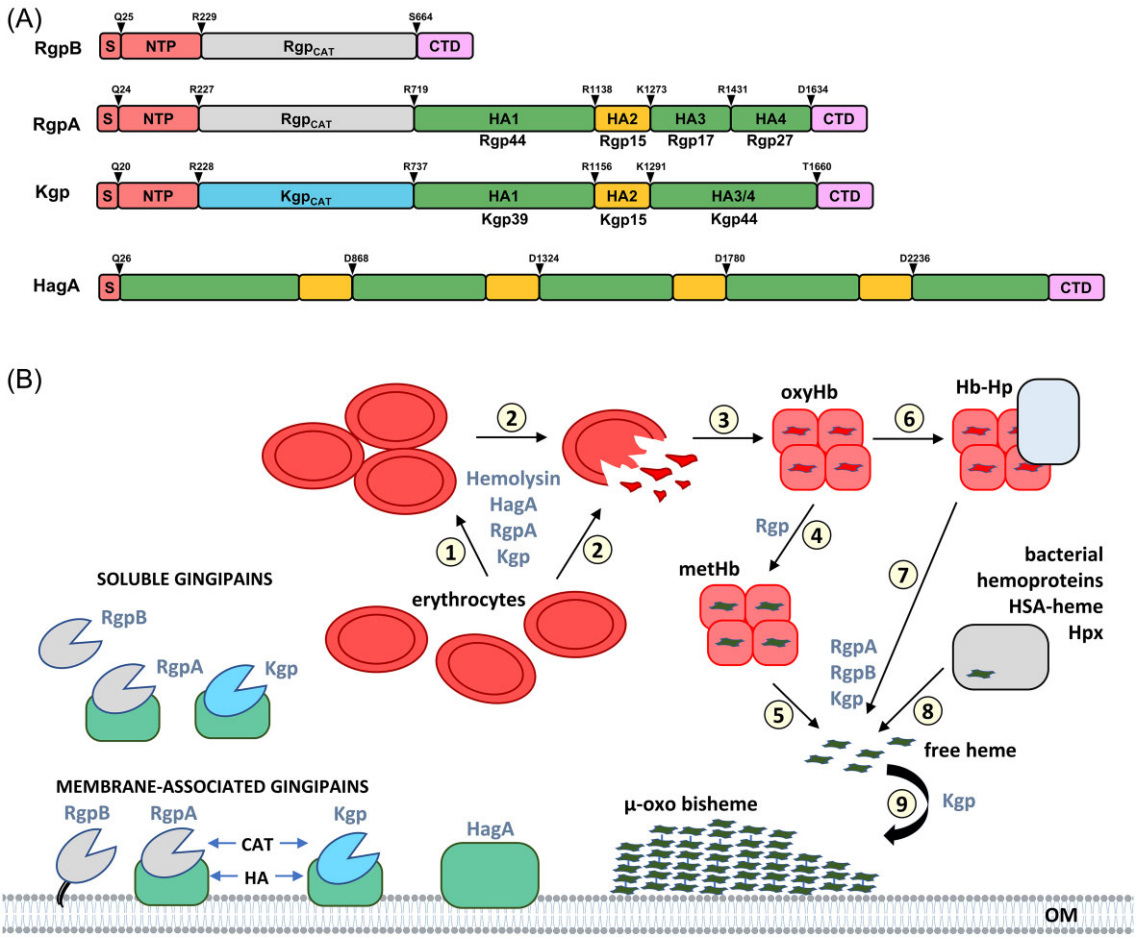

Figure 5. Involvement of gingipains in heme acquisition by *P. gingivalis*. (A) Schematic presentation of typical unprocessed preforms of RgpB, RgpA, Kgp gingipains, and hemagglutinin A (HagA) produced by the *P. gingivalis* W83 strain. RgpA and Kgp, in contrast to RgpB, besides the catalytic domain (CAT), possess the hemagglutinin/adhesin domains (HA) homologous to HagA. The arrows indicate the N-terminal amino acid residues of the processed regions formed during protein maturation. The HA2 domain binds Hb and heme. Domains showing a high degree of homology between gingipains and HagA are marked with the same color. S—signal peptide; NTP—N-terminal propeptide; Rgp_{CAT}—Rgp catalytic domain; Kgp_{CAT}—Kgp catalytic domain; HA1, HA2, HA3, and HA4—hemagglutinin/adhesin domains of gingipains; and CTD—C-terminal domain. (B) Gingipains are produced as membrane-associated proteins that can be spread in the host as associated with OMVs or in a secreted, soluble form. In cooperation with hemolysins and hemagglutinins (mainly HagA), RgpA and Kgp gingipains take part in the agglutination of erythrocytes (1) and their lysis (2), resulting in the release of Hb (3). Oxyhemoglobin (oxyHb) with the heme iron in the ferrous state (Fe^{2+}) is proteolytically processed by Rgp, resulting in Hb oxidation to metHb with heme iron in the ferric state (Fe^{3+}) (4). Due to the structural relaxation of metHb, lysine and arginine residues become more exposed and readily available, allowing gingipains to degrade metHb and release heme (5). Free Hb is bound to haptoglobin, forming the Hb–Hp complex (6), which can be degraded by gingipains, leading to heme release (7). Gingipains can also degrade albumin-heme (HSA-heme), hemopexin (Hpx), and various bacterial hemoproteins, releasing heme (8). Due to the Kgp activity, excess heme is deposited on the *P. gingivalis* surface, mainly in the form of μ-oxo bisheme (9), acting as its reservoir and protecting against the harmful effects of oxidative stress. OM—outer membrane.

lysine residues, which in metHb are more exposed and susceptible to degradation by Kgp (Smalley et al. 2008). The oxidation of oxyHb to metHb with iron in heme at a ferric state (Fe^{3+}PPIX) causes a decrease in the affinity of Hb for heme. Therefore, the synergism of Rgp and Kgp activity allows *P. gingivalis* to release heme from Hb. This cooperation is facilitated by the modification of host hemoproteins, an example of which is the glycation of Hb in diabetic patients, resulting in greater susceptibility of Hb to degradation and heme capture by the HmuY protein (Smiga et al. 2021), the process described below.

In addition to participating in the acquisition of essential nutrients, gingipains contribute to the maturation of proteins, including autoprocessing (Kadowaki et al. 1998). Their important role is also ascribed to pathogenicity by degrading or inactivating proteins critical to the host's immune defense (e.g. complement system proteins, cytokines, and integrins), deregulating signaling pathways, destroying connective tissue integrity due to the degradation of components of cell-to-cell contacts and detachment of epithelial cells from connective tissues of the gingiva. This aspect has been reported or reviewed by others (e.g. Baba et al. 2001, O'Brien-Simpson et al. 2001, Takii et al. 2005, Hocevar et al. 2018, Widziolek et al. 2025).

Hmu system as the main *P. gingivalis* heme uptake strategy

The main and best-characterized heme acquisition mechanism in *P. gingivalis* is the Hmu system encoded on the *hmu* operon (Fig. 6). It is a novel heme uptake mechanism characteristic of the Bacteroidota phylum (Olczak et al. 2024, Smiga and Olczak 2024, 2025). In *P. gingivalis*, the Hmu system consists of six proteins: HmuY, HmuR, and four additional proteins (HmuS, HmuT, HmuU, and HmuV) (Fig. 7) (Lewis et al. 2006, Olczak et al. 2008, 2024). In contrast to typical Hmu systems in other bacteria, this

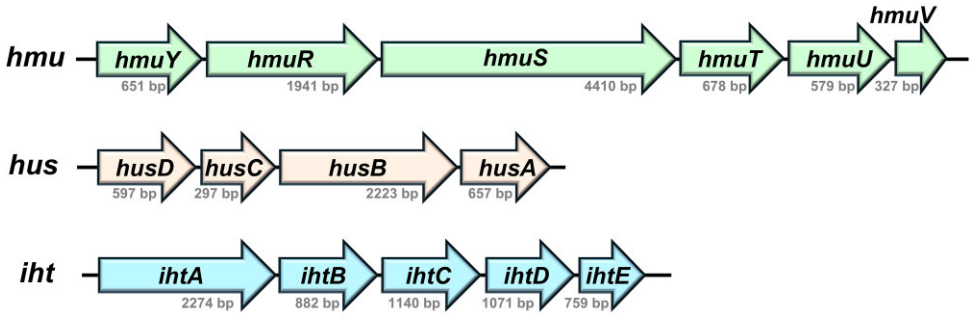

Figure 6. Schematic presentation of operons encoding Hmu, Hus, and Iht systems of *P. gingivalis*.

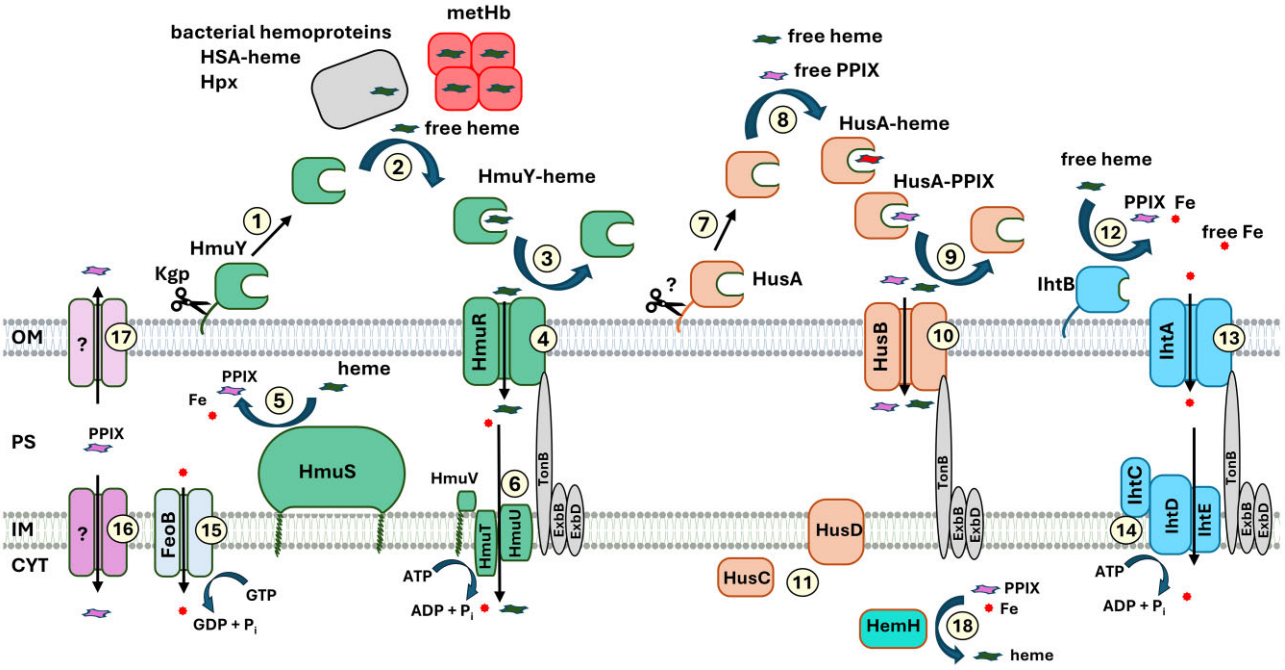

Figure 7. Heme and iron acquisition strategies used by *P. gingivalis*. The Hmu system is the main heme uptake mechanism used by *P. gingivalis*. HmuY protein is a hemophore-like protein produced as a lipoprotein anchored to the outer membrane, which can be released to the extracellular environment by limited proteolysis with Kgp (1). HmuY binds free heme and sequesters heme from methemoglobin (metHb), serum albumin-heme (HSA-heme), hemopexin (Hpx), and bacterial hemoproteins (2). Heme is transferred from HmuY to HmuR (3), a TDR (driven by TonB/ExbB/ExbD proteins), responsible for heme transfer into the periplasmic space (4). HmuS protein is a putative reverse ferrochelatase/dechelatase that removes iron from heme (5). HmuT, HmuU, and HmuV proteins most likely play a role in the transport of heme or iron through the inner membrane into the cytoplasm (6). Hus system may be involved in the acquisition of heme, noniron metalloporphyrins, or PPIX. HusA is a hemophore-like protein associated with the outer membrane, also found in the external environment (7). HusA binds free heme or PPIX (8) and transfers it to HusB, a TDR (9). HusB transfers heme/noniron metalloporphyrin/PPIX into the periplasmic space (10). HusC is a putative transcription factor, whereas HusD is a membrane protein with an unknown function (11). The putative role of the Iht system is connected with iron acquisition from heme. IhtB is a putative reverse ferrochelatase associated with the outer membrane and exposed to the external environment (12). Iron removed from heme by IhtB may be transferred into the periplasmic space by IhtA, a TDR (13). From the periplasmic space, iron can be transferred into the cytoplasm by IhtC, IhtD, and IhtE proteins (14). *Porphyromonas gingivalis* expresses a homolog of FeoB protein, which can transport iron from the periplasmic space into the cytoplasm (15). PPIX transported by the Hus system or derived from heme after iron removal performed by HmuS may be further transferred into the cytoplasm (16). PPIX excess could be exported from the bacterial cell (17), but the proteins responsible for this process have not been identified (potential exporters are listed in Table 1). Under heme-depleted conditions, HemH could synthesize heme using the intracellular pool of PPIX and iron (18). OM—outer membrane; PS—periplasmic space; IM—inner membrane; and CYT—cytoplasm.

system is slightly different in *P. gingivalis*. HmuR is a typical TDR, enabling heme transport across the outer membrane. HmuY is a unique hemophore-like heme-binding protein that transfers heme from the external environment to HmuR. The third gene in the *hmu* operon encodes HmuS, an inner membrane-associated protein homologous to CobN chelatase with putative reverse ferrochelatase/dechelatase activity. The following two genes encode proteins homologous to ATPase (HmuT) and proton channel MotA/TolQ/ExbB domain-containing permease (HmuU). The sixth gene of the *hmu* operon encodes a HmuV protein with no homology to any known protein family. While the roles of HmuY and HmuR are well-defined, the functions of the other proteins encoded on the *hmu* operon remain unclear. However, it is hypothesized that they may play a role in heme transport through the inner membrane to the cytoplasm and/or in iron release from heme (Fig. 7).

HmuY is a lipoprotein associated with the outer membrane, which can be released into the external environment as a component of OMV or as a soluble protein after specific shedding from the cell surface due to the limited proteolytic activity of Kgp (Wojtowicz et al. 2009a, Olczak et al. 2010). HmuY binds free heme released from hemoproteins by gingipains, but importantly, sequesters heme directly from albumin-heme and hemopexin in both oxidative and reducing environments. Moreover, in contrast to other members of the HmuY family, it can directly sequester heme from metHb (Smalley et al. 2011, Smiga and Olczak 2024). HmuY protein production significantly increases under iron- and heme-limited conditions (Bielecki et al. 2020, Smiga and Olczak 2024, Smiga et al. 2024a). Additionally, elevated expression of the *hmuY* gene occurs when *P. gingivalis* grows within a biofilm or in co-cultures with other bacteria (Olczak et al. 2010, Slezak et al. 2020). The expression of HmuY is also correlated with the expression of Kgp and RgpA (Liu et al. 2006, Smiga et al. 2023a). Metatranscriptomic analyses have further revealed that genes encoding the Hmu system and gingipains are highly expressed in individuals with periodontitis (Szafranski et al. 2015, Deng et al. 2018). Hence, gingipains and the HmuY protein are expressed and function synergistically to optimize heme acquisition for *P. gingivalis*. Moreover, their presence in the soluble forms and OMVs carrying HmuY and gingipains as the main cargo facilitates spreading in the host and reaching niches other than the oral cavity (Veith et al. 2014, Dominy et al. 2019).

HmuY protein is the first representative of a novel family of hemophore-like proteins with a unique all-β-fold structure (Wojtowicz et al. 2009a, Olczak et al. 2024). In *P. gingivalis* HmuY, heme is bound by the coordination of heme-iron by two histidines, His^{134} and His^{166}. The structure of the HmuY protein in both heme-bound (PDB ID: 3H8T) and apo-form (PDB ID: 6EWM) shows that the apo-HmuY has a relaxed heme-binding pocket, which closes upon heme binding (Fig. 8A and B) (Wojtowicz et al. 2009a, Bielecki et al. 2018). Heme-iron coordination is a two-step process employing His^{134} and His^{166}(Fig. 8C). This process is supported by other amino acids, including Tyr^{48}, Arg^{79}, Tyr^{89}, Thr^{124}, Met^{136}, Tyr^{127}, and Tyr^{173}, which facilitate heme binding, mostly by hydrophobic interactions with PPIX side chains (Wojtowicz et al. 2009a,b). *Porphyromonas gingivalis* HmuY binds heme in a 1:1 molar ratio under oxidative and reducing conditions with K_d ~10^{-8}–10^{-9} M for ferrous and $K_d < 10^{-9}$ M for ferric heme-iron forms (Bielecki et al. 2018, Olczak et al. 2024). The coordination of heme-iron and heme sequestration ability of proteins belonging to the HmuY family differ (Bielecki et al. 2018, 2020, Sieminska et al. 2021, Antonyuk et al. 2023, Smiga and Olczak 2024). Importantly, *P. gingivalis* HmuY exhibits a superior ability to gain heme regardless of environmental conditions, resulting in the *P. gingivalis* advantage over cohabitating oral pathogens. A detailed description of HmuY family proteins is presented in the recent review (Olczak et al. 2024).

Porphyromonas gingivalis HmuR is structurally similar to vitamin B_{12}/cyanocobalamin receptor BtuB from *E. coli* (PDB ID: 1NQE) (Chimento et al. 2003) and other TDRs (Antonyuk et al. 2023). To bind heme, HmuR uses two histidines, His^{434} located in the extracellular loop, and His^{95} located in the plug domain (Fig. 8D) (Liu et al. 2006). Additionally, two motifs, Tyr^{420}–Arg^{421}–Ala^{422}–Pro^{423} and Asn^{442}–Pro^{443}–Asp^{444}–Leu^{445}, and conserved glutamic acids are involved in heme transport (Liu et al. 2006, Olczak 2006). HmuR delivers heme to *P. gingivalis* directly, but cooperation with HmuY facilitates heme uptake, which is particularly important under conditions where heme is present at very low levels or bound to host or bacterial hemoproteins. Although the transfer of heme from HmuY to HmuR occurs, there is a dissonance because the affinity of HmuR for heme is much lower (K_d ~10^{-5}–10^{-6} M) (Olczak et al. 2001) than that of HmuY ($K_d < 10^{-9}$ M) (Bielecki et al. 2018). It is believed that the HmuY–heme complex interacts specifically with the HmuR region exposed to the external environment, which results in conformational changes in the HmuY heme-binding pocket. A similar mechanism has been documented for HasA (a classical hemophore) and HasR (typical TDR) from *S. marcescens* (Izadi-Pruneyre et al. 2006, Caillet-Saguy et al. 2009, Krieg et al. 2009). Therefore, physical contact of HmuY with HmuR may disrupt the heme–iron coordinating bond with His^{166} of the HmuY, heme transfer to His^{434} of the HmuR, followed by dissociation of the heme–iron coordinating bond from His^{134} of the HmuY, and heme transfer to His^{95} of the HmuR. This step-by-step process may enable the sequential transfer of heme from HmuY to HmuR (Fig. 8E). Preliminary studies of binding of gaseous ligands, nitric oxide (NO) and carbon monoxide (CO), to the HmuY–heme complex (Exertier et al. 2025), as well as crystallographic data of apo- and holo-HmuY protein structures (Wojtowicz et al. 2009a, Bielecki et al. 2018) suggest the possibility of heme release from HmuY following conformational changes of the heme-binding pocket. From the theoretical perspective, the influence of gaseous ligands demonstrates potential bases for the step-by-step mechanism of heme release from HmuY (Exertier et al. 2025). Taking into account biological implications, higher levels of NO and CO in periodontitis caused by inflammation and smoking may improve heme release from HmuY for subsequent transfer to HmuR (Reher et al. 2007, Wadhwa et al. 2013, Parwani and Parwani 2015).

Hus system as a second heme or metal-free porphyrin uptake mechanism

Hus system consists of 4 proteins (HusA, HusB, HusC, and HusD) and is encoded on the *hus* operon (Fig. 6). HusA is a hemophore-like protein not belonging to the HmuY family or classical hemophores, and HusB is a typical TDR. Both proteins, like HmuY and HmuR, form a two-component heme/PPIX uptake system (Fig. 7) (Gao et al. 2010, 2018). HusC has a winged-helix DNA-binding domain and is a putative transcription regulator from the MarR family, whereas HusD is a putative membrane protein (Gao et al. 2010).

HusA is a lipoprotein associated with the outer membrane and can be found extracellularly as a soluble protein or associated with OMV; however, its detailed localization is unclear (Gao et al. 2010, 2018b). Veith et al. (2014) showed that HusA is present in the OMV lumen, suggesting that it could be associated with the outer membrane and exposed to the periplasmic space, as its contents are enclosed in the lumen during the formation of OMVs (Zhang et al. 2021a). It could also be hypothesized that this protein may be secreted in an atypical way *via* OMVs. However, this remains speculative, and determining the exact localization of HusA is essential to fully understand its role and the function of the Hus system.

HusA exhibits a hemophore-like function; however, differences exist compared to HmuY (Gao et al. 2018, Smiga et al. 2023a). HusA binds free heme or heme bound in the ferrous form to albumin only. Following the dissociation constant of the HusA–heme complex (K_d ~10^{-6} M), HmuY extracts heme bound to HusA (Smiga et al. 2024a). Moreover, gingipains can degrade the soluble form of HusA, releasing heme (Smiga et al. 2023a), which is in contrast to the high resistance to proteolysis of HmuY (Wojtowicz et al. 2009a, Byrne et al. 2013, Benedyk et al. 2015). Similar to HmuY (Wojaczynski et al. 2011, Wojtowicz et al. 2013), HusA binds

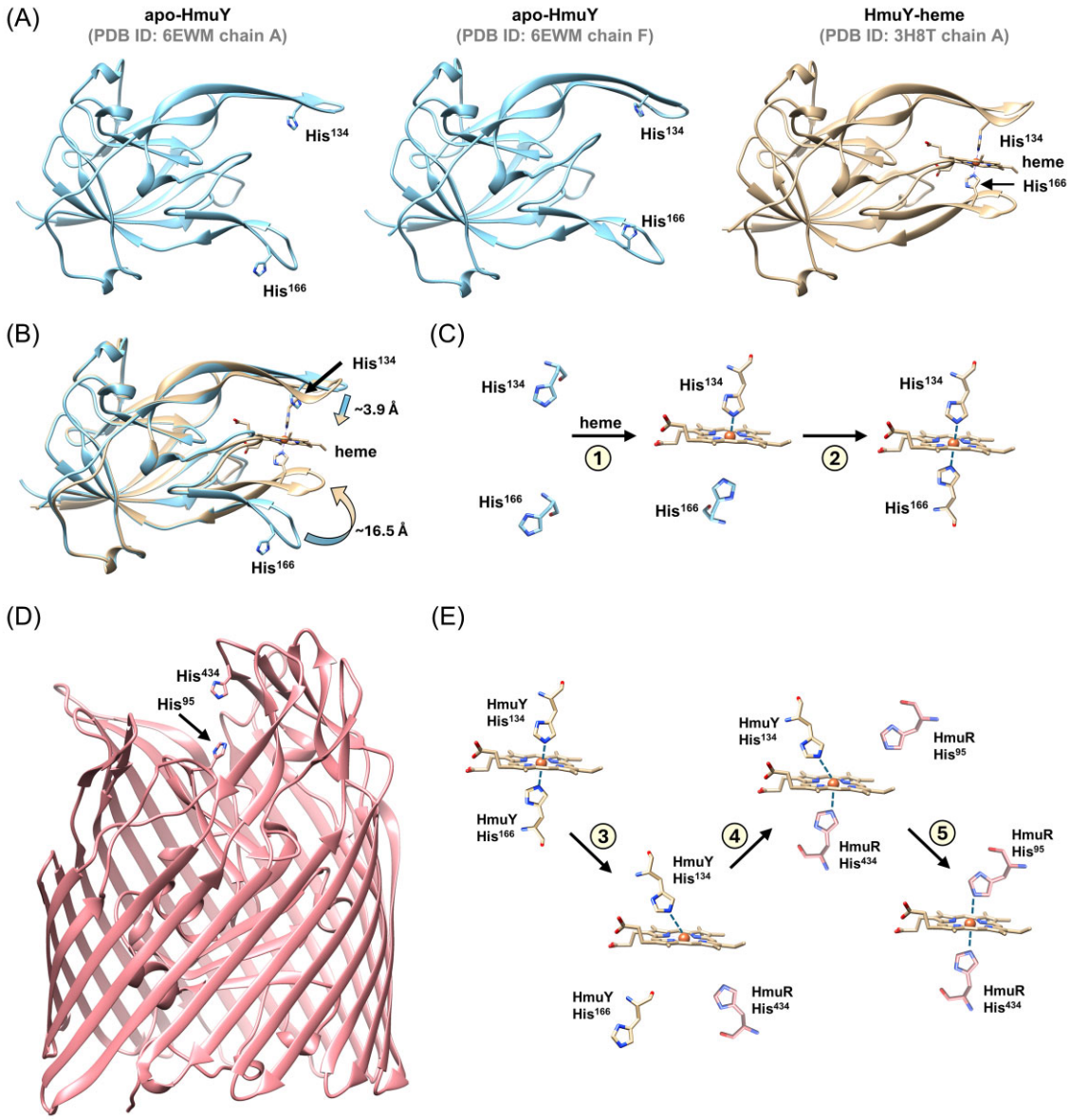

Figure 8. Heme binding and release in the *P. gingivalis* Hmu system. (A) Analysis of the three-dimensional HmuY structures reveals its differences in the spatial position of the heme iron-coordinating histidines. The apo-HmuY has a more open heme-binding pocket compared to the HmuY–heme complex and (B) heme binding by HmuY results in the closing of the ligand-binding pocket and movement of the His^{134}NE2 and His^{166}NE2 atoms approximately by 3.9 Å and 16.5 Å, respectively. (C) Heme binding by HmuY likely occurs in a two-step process: (1) heme iron is first coordinated by His^{134}, which results in (2) conformational changes and movement of the heme-binding pocket loop containing His^{166}. (D) HmuR (AlphaFold ID: AF-Q7MUG9-F1) is a β-barrel, TonB-dependent heme receptor that binds heme with His^{95} and His^{434}. (E) The exact mechanism of heme transfer from HmuY to HmuR remains to be specified, but it is hypothesized that it involves at least three steps: The proximity of HmuY and HmuR induces conformational changes in both proteins, leading to the dissociation of HmuY His^{166} from heme iron (3). This allows His^{434} to associate with the heme iron (4). Further conformational changes facilitate the exchange of HmuY His^{134} with HmuR His^{95}(5). HmuY and HmuR proteins structures were visualized with UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) (Pettersen et al. 2004).

Fe(III)deuteroporphyrin IX (K_d ~10^{-7} M) and noniron metalloporphyrins, including Zn^{2+}PPIX (K_d ~10^{-6} M). However, in contrast to HmuY, HusA more efficiently binds PPIX (K_d ~10^{-6} M) and deuteroporphyrin IX (K_d ~10^{-7} M) (Gao et al. 2018, Smiga and Olczak 2025) and binds noniron metalloporphyrins regardless of the oxidation state (Smiga and Olczak 2025). The HusA properties can be attributed to its structure. The protein is built of 9 α-helices that form a binding pocket predominantly made up of neutral or hydrophobic amino acid residues. Its mutational studies have identified Leu^{123}, Val^{124}, Trp^{130}, and Tyr^{164} as potentially involved in the binding of heme and other porphyrins, likely through interactions with the porphyrin ring rather than direct metal coordination (Gao et al. 2018, Smiga and Olczak 2025).

The analysis of the *husA* gene (Δ*husA*) or *husA* and *husB* genes (Δ*husA*Δ*husB*) *P. gingivalis* deletion mutant strains did not reveal a major role for the Hus system in heme uptake or heme and iron homeostasis (Smiga et al. 2023a,b). However, a significant reduction in infection of host cells by Δ*husA* and Δ*husA*Δ*husB* mutant strains was observed, with a significantly more pronounced effect in the case of the double deletion mutant strain (Gao et al. 2018, Smiga et al. 2023a, 5). Unlike HmuY, the production of HusA is not strongly dependent on the concentration of iron and heme

in the external environment (Smiga et al. 2023a, b). Its production increases when *P. gingivalis* is a biofilm constituent or enters host cells (Zainal-Abidin et al. 2012, Gao et al. 2018). This may indicate that the Hus system functions under different conditions than the Hmu system, where alternative heme sources are available, as mentioned below.

As suggested by Gao et al. (2018) and taking into account the properties of the HusA protein and the effect of *husA* and *husB* genes deletion on the phenotype of *P. gingivalis*, it can be assumed that the Hus system may be involved in the acquisition of heme precursors, including coproporphyrinogen III or PPIX. These compounds are present in the cytoplasm (Sachar et al. 2016), and their amount may be elevated in some human disorders (Kiening and Lange 2022), leading to the disruption of homeostasis of heme precursors. Excess PPIX may be accumulated inside erythrocytes or released into the intercellular space, and therefore present in the serum. Additionally, under iron deficiency, PPIX can be converted to Zn^{2+}PPIX (Sachar et al. 2016). In periods of heme deficiency, coproporphyrinogen III, PPIX, or Zn^{2+}PPIX could be acquired by the Hus system and used as substrates for the proteins of the final steps of the heme biosynthesis pathway preserved in *P. gingivalis* (HemN, HemG, and HemH), thus constituting an alternative strategy for obtaining heme by this bacterium. However, this remains a theoretical hypothesis and requires experimental validation.

Heme pirating or synergism between periodontopathogens—*P. gingivalis* alternative heme sources

In the oral cavity, *P. gingivalis* must compete for heme with other members of the multispecies oral biofilm. *In vitro* studies showed that *P. gingivalis* HmuY utilizes hemoproteins produced by other periodontopathogens to acquire heme. *Streptococcus gordonii* is an early colonizer of the oral plaque, and by direct interactions, it is one of the factors involved in recruiting *P. gingivalis* to the biofilm structures (Kuboniwa and Lamont 2010). *Streptococcus gordonii* exhibits α-hemolytic activity by production of hydrogen peroxide and oxidizes oxyHb to metHb, which allows heme sequestration by *P. gingivalis* HmuY even in the presence of hydrogen peroxide (Brown et al. 2018, Slezak et al. 2020). Bacteria belonging to the *Streptococcus* genus secrete the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) into the external environment. Interestingly, GAPDH produced by many bacterial species, including *S. gordonii*, bind heme, and *P. gingivalis* and other periodontopathogens sequester heme from GAPDH using HmuY proteins (Slezak et al. 2020). This mechanism represents a novel adaptive strategy employed by *P. gingivalis* to obtain heme during the initial stages of infection.

Porphyromonas gingivalis utilizes heme uptake strategies to compete for heme also with other colonizers of the oral microbiome. *Prevotella intermedia* produces interpain A (InpA), a cysteine protease that is unrelated to *P. gingivalis* gingipains. Through its proteolytic activity, similar to *P. gingivalis* RgpA (Smalley et al. 2011), InpA facilitates the conversion of oxyHb to metHb, enabling HmuY to extract heme from metHb (Byrne et al. 2013). Notably, *P. intermedia* produces two HmuY homologs, ($HmuY^{Pi\text{-}1}$, former name PinO and $HmuY^{Pi\text{-}2}$, former name PinA), and *P. gingivalis* HmuY sequesters heme from both proteins (Bielecki et al. 2020), as well as from HmuY homologs produced by other periodontopathogens, such as *T. forsythia* ($HmuY^{Tf}$, former name Tfo) and *Porphyromonas endodontalis* ($HmuY^{Pe}$) (Bielecki et al. 2018, Smiga and Olczak 2024). *Porphyromonas gingivalis* HmuY is characterized by high resistance to proteases produced by many periodontopathogens, such as *P. gingivalis* (including gingipains), *T. forsythia*, *P. intermedia* (including InpA), *P endodontalis*, and host immune cells (Wojtowicz et al. 2009a, Smalley et al. 2011, Byrne et al. 2013, Benedyk et al. 2015). Moreover, *P. gingivalis* can degrade hemophore-like proteins produced by cohabitating bacteria (Bielecki et al. 2018, 2020, Slezak et al. 2020, Smiga and Olczak 2024), which gives it an additional advantage in obtaining heme over other bacteria residing in the oral microbiome.

Iron acquisition strategies of *P. gingivalis*

It is worth mentioning that in contrast to *P. gingivalis*, some Bacteroidota members utilize siderophores for iron acquisition. For example, *Bacteroides fragilis* utilizes hydroxymate Fe(III)-ferrichrome, whereas *Bacteroides vulgatus* and *Bacteroides thetaiotaomicron* utilize catecholates enterobactin and salmochelin S4 (Rocha and Krykunivsky 2017). *Porphyromonas gingivalis* does not produce/utilize siderophores/xenosiderophores (Nelson et al. 2003); therefore, other iron acquisition mechanisms may fulfill its requirements for this element during periods of heme deficiency, or when PPIX is not needed. *Porphyromonas gingivalis* may utilize iron released by degradation of host transferrin by gingipains (Goulet et al. 2004). *Porphyromonas gingivalis* encodes two FeoB protein homologs participating in iron and manganese transport (Fig. 7). They are homologous to *E. coli* and *B. fragilis* FeoB proteins (Nelson et al. 2003, Dashper et al. 2005, He et al. 2006, Anaya-Bergman et al. 2010, Lewis 2010, Zhang et al 2016, Rocha et al. 2019). Expression of both *P. gingivalis feoB* genes increases under heme- and iron-limited conditions (Smiga et al. 2024a, b), suggesting their role in the acquisition of iron/manganese to support bacterial survival and growth in heme-restricted environments.

Although *P. gingivalis* does not encode classical proteins involved in iron transport through the outer membrane (Nelson et al. 2003, Klebba et al. 2021), it is postulated that the Iht system may be involved in this process. This system consists of five proteins (IhtA-E) encoded on the *iht* operon (Fig. 6). IhtB (also known as FetB) is a heme-binding lipoprotein with homology to cobaltochelatase CbiK (Dashper et al. 2000). IhtB most likely acts as a reverse ferrochelatase/dechelatase, which can remove iron from heme and potentially other metals from noniron metalloporphyrins (Yukitake et al. 2011). Once released from heme, iron can be transferred to the periplasmic space through IhtA, a typical TDR. Then, using IhtC (putative lipoprotein), IhtD (permease, iron compound ABC transporter), and IhtE (ATP-binding protein), iron can be transported into the cytoplasm (Fig. 7). Global transcriptomic analyses revealed that the expression of the *iht* operon genes remains unchanged in *P. gingivalis* under iron and heme starvation (Dashper et al. 2009, Anaya-Bergman et al. 2015, Smiga et al. 2024a, b). However, it has been shown that IhtB production increases when the wild-type A7436 strain is grown in media depleted of iron and heme (Smiga et al. 2024b). Interestingly, in the wild-type ATCC 33277 strain, IhtB production remains unchanged regardless of iron and heme availability (Smiga et al. 2024b). This phenomenon can be explained by differences in maintaining iron and heme homeostasis between both strains (Smiga et al. 2024b).

In most aerobic bacteria, after heme internalization, ferric iron is released from heme by cleavage of the PPIX ring with heme oxygenases, producing biliverdin and carbon monoxide (Frankenberg-Dinkel 2004, Unno et al. 2007, Matsui et al. 2010, Wilks and Heinzl 2014, Wilks and Ikeda-Saito 2014, Lyles and Eichenbaum 2018, Richard et al. 2019). An example is HemO from *N. meningitidis*. Another group of enzymes comprises heme-binding, noncanonical heme-degrading proteins, including HemS

from *Y. enterocolitica*, HmuS from *Yersinia pseudotuberculosis*, and ChuS from pathogenic enterohemorrhagic *E. coli* O157:H7 strain (Onzuka et al. 2017, Lyles and Eichenbaum 2018, Matthew et al. 2019, Keith et al. 2024). These proteins function as heme-degrading and iron-releasing enzymes under aerobic iron-deplete conditions but also participate in intracellular heme shuttling or protecting from oxidative stress as heme chaperones under anaerobic iron-replete conditions (Mathew et al. 2019). Some bacteria remove iron and preserve the PPIX ring intact, examples being *E. coli* K-12 YfeX and EfeB (Letoffe et al. 2009) or *Staphylococcus aureus* FepB (Turlin et al. 2013). However, it was later shown that YfeX functions as a porphyrinogen oxidase, not a heme dechelatase (Dailey et al. 2011).

The knowledge of iron release from heme under anaerobic conditions is limited. Data gained from research on *E. coli* O157:H7 strain demonstrated engagement of proteins encoded on the *chu* operon in anaerobic iron release and heme degradation (ChuS in cooperation with ChuXWY), resulting in the production of anaerobilin (Suits et al. 2005, 2006, LaMattina et al. 2016, 2017, Mathew et al. 2019). ChuW, an *S*-adenosylmethionine methyltransferase, degrades heme in the absence of oxygen using alternate electron donors (e.g. flavodoxin) and produces anaerobilin instead of biliverdin (LaMattina et al. 2016, 2017, Mathew et al. 2019). Anaerobilin is then shuttled by ChuX from ChuW to ChuY for further breakdown. Similar to *E. coli* ChuS, also HemS from *Y. pestis* and HmuS from *Y. enterocolitica* can degrade heme under anaerobic conditions using a NADH-dependent hydride transfer mechanism (Schneider and Paoli 2005, Suits et al. 2005, Schneider et al. 2006, Keith et al. 2024). Interestingly, both ChuS and HmuS degrade heme under low heme availability, but at higher heme concentrations serve as heme chaperones/heme storage proteins.

Porphyromonas gingivalis does not express proteins belonging to the heme oxygenase classes (Nelson et al. 2003), and the exact mechanism of iron extraction from heme is not yet understood. Among proteins potentially responsible for iron release from heme is the HmuS (Fig. 7) (our unpublished data), a protein similar to its close homolog BtuS2 from *B. fragilis* (Rocha et al. 2019). Since this protein is anchored to the inner membrane and localized in the periplasmic space (our unpublished data), it may function differently to cytosolic heme oxygenases, ChuS, or HemS/HmuS proteins, but similarly to BtuS2.

Heme and iron homeostasis in *P. gingivalis*

Iron and heme starvation of *P. gingivalis* induces changes in the expression of up to 500 genes, constituting ∼23% of the total genes (Smiga et al. 2024a). Among them are genes responsible for heme and iron uptake, their homeostasis, energy metabolism, virulence, and response to oxidative stress, which indicates a global and diverse response to iron and heme starvation (Dashper et al. 2009, Anaya-Bergman et al. 2015, Smiga et al. 2024a, b). Efficient regulation of heme homeostasis is crucial for all organisms and is maintained through heme synthesis, heme uptake, and heme degradation, processes described above. *Porphyromonas gingivalis* utilizes several regulatory proteins to control the expression of the Hmu system, such as the Fur protein homolog (PgFur) (Butler et al. 2014, 2015, Ciuraszkiewicz et al. 2014, Smiga et al. 2019a, b), LuxR/CdhR (Wu et al. 2009, Boutrin et al. 2023), PgRsp (Smiga and Olczak 2019), the HaeSR two-component system (Scott et al. 2013), and others which were summed up in more detail in the recent review (Olczak et al. 2024).

From the other side, high heme levels, occurring in various pathophysiological conditions, can be toxic *via* prooxidant, proinflammatory, and cytotoxic effects, mainly by triggering radical chain reactions to generate reactive oxygen species, resulting in oxidative injury, inflammation, and immune dysfunction (Choby and Skaar 2016, Vallelian et al. 2022). Due to a lipophilic nature, heme intercalates and aggregates in the cell membrane, leading to lipid oxidation, causing increased membrane permeability and cell lysis (Chou and Fitch 1980, 1981, Fitch et al. 1983, Aft and Mueller 1984, Liu et al. 1985, Vincent 1989). Free heme also oxidizes proteins, triggering their cross-linking, aggregation, and degradation (Vincent 1989). Free heme concentration above 50 μM inhibits the growth of *P. gingivalis* (Smalley et al. 2000). Therefore, bacteria have developed efficient strategies to neutralize the toxic effect of an excess of free heme.

Bacteria can use systems capable of exporting iron and heme out of the cell. Heme efflux systems were identified in Gram-positive (e.g. *S. aureus* HrtAB system composed of ATPase and permease) and Gram-negative (e.g. *Neisseria* MtrCDE system) bacteria (Stauff et al. 2008, Fernandez et al. 2010, Turlin et al. 2014). Some bacteria can also export PPIX, an example being *E. coli* MacAB-TolC pump (Turlin et al. 2014), or can export both heme and PPIX, such as *Staphylococcus agalactiae* which uses PefAB and PefCD, respectively (Fernandez et al. 2010). Although such mechanisms are not characterized in *P. gingivalis*, this bacterium encodes potential efflux systems summed up in Table 1. Moreover, two systems (locus IDs in W83 strain: PG0280–PG0283/PG_RS01255–PG_RS01270 and PG1662–PG1667/PG_RS07305–PG_RS07330) show high similarity to *E. coli* MacAB-TolC system (Fig. 9) but their involvement in heme/PPIX export is only hypothetical. Some bacteria also utilize iron efflux systems, such as *E. coli* FieF and *E. coli* FetA/FetB iron exporters (Grass et al. 2005, Nicolau et al. 2013). However, no such mechanisms have been identified *in P. gingivalis*.

Nevertheless, there are known mechanisms used by *P. gingivalis* for heme detoxification. One of them is performed by gingipains (Smalley et al. 1999, 2004). Gingipains function as a receptor for heme and Hb and serve as a template to transiently bind monomeric heme, which facilitates reacting with other heme monomers either free or bound to proteins (Nakayama et al. 1998, DeCarlo et al. 1999, Smalley et al. 2006, Nhien et al. 2010). This process leads to the formation of μ-oxo bisheme, the major component of the green–black pigment deposited on the bacterial cell surface (Smalley et al. 1998, 2000, 2004, 2006, Smalley and Olczak 2017). Heme monomers are released proteolytically (mainly by Kgp) from deoxyhemoglobin containing Fe^{2+}PPIX but more efficiently from metHb containing Fe^{3+}PPIX. In the generation of μ-oxo bisheme and its aggregation through weak π-bonding interactions and porphyrin stacking participate Kgp, RgpA, and isolated HA domains (Smalley et al. 2006). A detailed description of this process was reported in the recent review (Smalley and Olczak 2017). Deleting the *kgp* gene or constructing the mutant with reduced Kgp activity results in lower pigmentation (Ishida et al. 2008, Smiga et al. 2023a). The accumulated heme pigment functions as heme storage but also as an oxidative buffer that can catalytically degrade hydrogen peroxide, thus protecting from reactive oxygen species generated by neutrophils and by-products of the breakdown of molecular oxygen (Smalley et al. 1998, 2000, 2004).

Porphyromonas gingivalis also uses another mechanism to protect against excess heme, involving a homolog of DNA-protective protein from starved cells (Dps) (Ueshima et al. 2003, Gao et al. 2012). Dps, a cytoplasmic ferritin-like protein, is composed of 12 identical monomeric subunits that assemble into a spherical

Table 1. Potential efflux systems encoded in *P. gingivalis* W83 strain.

Gene cluster locus ID (alternative cluster locus ID)	Gene locus ID	Description	Homologous protein (% amino acid sequence identity/% of sequence coverage)
PG0679–PG0685 (PG_RS02985–PG_RS03015)	PG0679	TolC family protein	MtrE (25/36)[e]
	PG0680	Efflux RND transporter periplasmic adaptor subunit	MacA (22/87)[f]
	PG0681	Hypothetical protein	
	PG0682	ABC transporter permease	
	PG0683	ABC transporter permease	
	PG0684	ABC transporter permease	
	PG0685	ABC transporter ATP-binding protein	HrtA (39/91)[a]; MacB (42/34)[g]
PG1662–PG1667 (PG_RS07305–PG_RS07330)	PG1662	DUF6249 domain-containing protein	
	PG1663	ABC transporter ATP-binding protein	HrtA (37/93)[a]; MacB (50/34)[g]
	PG1664	ABC transporter permease	MacB (25/59)[g]
	PG1665	ABC transporter permease	MacB (24/48)[g]
	PG1666	Efflux RND transporter periplasmic adaptor subunit	MtrC (24/72)[c]; MacA (22/94)[f]
	PG1667	TolC family protein	MtrE (24/61)[e]
PG0280–PG0283 (PG_RS01255–PG_RS01270)	PG0280	ABC transporter permease	HrtB (22/33)[b]
	PG0281	ABC transporter permease	MacB (22/46)[g]
	PG0282	ABC transporter ATP-binding protein	HrtA (37/98)[a]; MacB (42/34)[g]
	PG0283	Efflux RND transporter periplasmic adaptor subunit	MacA (26/51)[f]
PG0538–PG0541 (PG_RS02405–PG_RS02420)	PG0538	TolC family protein	
	PG0539	Efflux RND transporter periplasmic adaptor subunit	MtrC (24/88)[c]; MacA (24/88)[f]
	PG0540	Efflux RND transporter permease subunit	MtrD (27/97)[d]
	PG0541	Transporter-associated protein	
PG0063–PG0065 (PG_RS00290–PG_RS00300)	PG0063	TolC family protein	
	PG0064	Efflux RND transporter permease subunit	MtrD (26/87)[d]
	PG0065	Efflux RND transporter periplasmic adaptor subunit	MtrC (24/83)[c]; MacA (24/48)[f]
PG0091–PG0094 (PG_RS00410–PG_RS00425)	PG0091	ABC transporter permease	
	PG0092	ABC transporter permease	
	PG0093	HlyD family secretion protein	MacA (25/43)[f]
	PG0094	TolC family protein	MtrE (24/40)[e]

[a]Homology to *S. aureus* HrtA protein (NCBI accession ID: AIU86285.1).
[b]Homology to *S. aureus* HrtB protein (NCBI accession ID: AIU86642.1).
[c]Homology to *N. gonorrhoeae* MtrC protein (NCBI accession ID: XCC31532.1).
[d]Homology to *N. gonorrhoeae* MtrD protein (NCBI accession ID: WP_404523989.1).
[e]Homology to *N. gonorrhoeae* MtrE protein (NCBI accession ID: WP_371360591.1).
[f]Homology to *E. coli* MacA protein (NCBI accession ID: QPD58655.1).
[g]Homology to *E. coli* MacB protein (NCBI accession ID: QPD58656.1).

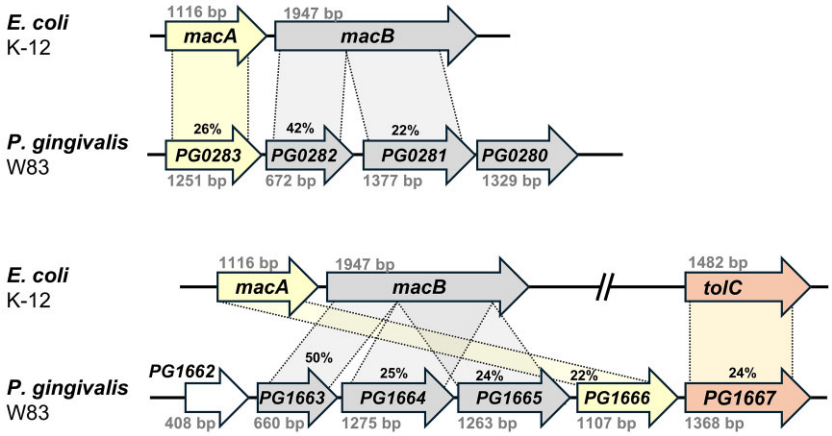

Figure 9. Schematic presentation of *P. gingivalis PG0280–PG0283* and *PG1662–PG1667* operons encoding potential efflux systems similar to the *E. coli* MacAB–TolC efflux system (listed in Table 1). Genes encoding homologous proteins are marked with the same color. The amino acid sequence identity of proteins or protein fragments is shown in %.

shape with a hollow core exhibiting iron sequestration and ferroxidase activity of ferritins. It also exhibits properties of bacterioferritin due to ferric heme binding (K_d ~10^{-8} M). A single surface-located cysteine (Cys101) coordinates heme–iron at the fifth axial ligand. Dps improves the efficiency of heme utilization at low heme concentrations, and at high heme concentrations, it prevents heme toxicity.

Porphyromonas gingivalis heme uptake mechanisms as targets for developing diagnostic tests, preventive or therapeutic strategies

Diagnosing periodontal diseases is based mainly on clinical measurements and radiographic methods (Tonetti et al. 2018, Heitz-Mayfield 2024, Jacobs et al. 2024, Ramseier 2024). However, there is a need for supplemental diagnostic methods not only to diagnose periodontitis but also to monitor its treatment progress. Tests based on microbial analytical methods include high-throughput sequencing of *16S rRNA* genes, shotgun metagenomic sequencing, or quantitative PCR (qPCR), which allow the identification and determination of periodontopathogens' load (e.g. Hortz and Conrads 2007, Hyvarinen et al. 2009, Arweiler et al. 2020, Claesson et al. 2022, Manoil et al. 2024). Using qPCR, the detection of unique *P. gingivalis* genes in the oral microbiome or other host niches (e.g. the brain and cerebrospinal fluid), such as the *hmuY* gene, may be more specific than the employment of the *16S rRNA* gene (Gmiterek et al. 2013, Dominy et al. 2019). Therapy of periodontal diseases is mostly based on scaling and root planning with adjunctive therapies. However, rising concerns highlight the inefficiency of the antiseptics and antibiotics applied to treat *P. gingivalis*-related infections, especially in light of increasing antibiotic resistance (Conrads et al. 2021, Abe et al. 2022, Ng et al. 2023, Rams et al. 2023).

Detection of *P. gingivalis* is not a specific biomarker of periodontitis because the bacterium is found in low numbers in healthy individuals (Griffen et al. 1998). However, the determination of serum antibodies against *P. gingivalis* can be used as a general biomarker of periodontitis, reflecting bacterial load because the serum anti-*P. gingivalis* IgG antibody titer correlates with the detection frequency of *P. gingivalis* (Kojima et al. 1997, Franca et al. 2007, Dye et al. 2009, Kudo et al. 2012, Ebersole et al. 2020, Massarenti et al. 2024). Screening periodontitis by the determination of serum IgG antibodies against total *P. gingivalis* antigens derived from the crude bacterial extract or heat-killed *P. gingivalis* cells using an enzyme-linked immunosorbent assay showed a diagnostic potential (Kudo et al. 2012, Trindade et al. 2012, Nobre dos Santos-Lima et al. 2020, Massarenti et al. 2024). However, different immunogenicity of particular *P. gingivalis* strains causes low specificity of such tests (Ebersole et al. 2020, Seers et al. 2020). Nevertheless, immunization with killed *P. gingivalis* reduced the progression of periodontitis in a nonhuman primate model (Persson et al. 1994). Therefore, vaccination using *P. gingivalis* virulence factors as antigens has been proposed (Grover et al. 2014, Wilensky et al. 2017, Zhu et al. 2018, Huang et al. 2019, Liao et al. 2024, Wang et al. 2024).

Response to *P. gingivalis* gingipains and their hemagglutinin/adhesin domains results in the production of serum IgG antibodies (O'Brien-Simpson et al. 2000a, Inagaki et al. 2003), and patients with periodontitis produce more IgG antibodies reacting with the HA2 domain than individuals with healthy periodontium (DeCarlo et al. 2004, Nguyen et al. 2004). Modified recombinant gingipains with decreased proteolytic activity, mainly RgpA and its N-terminal region, showed promise as a specific antigen to determine serum IgG levels (Hirai et al. 2020). Recombinant HA2 domains of RgpA and Kgp or synthetic peptides derived from HA2 domains protected against *P. gingivalis*-induced alveolar bone loss and attenuated *P. gingivalis* infection in murine and rat periodontitis models, suggesting a potential for vaccine development (O'Brien-Simpson et al. 2000b, Rajapakse et al. 2002, Frazer et al. 2006). The DHYAVMISK peptide derived from the HA2 domain inhibits heme binding (Yang et al. 2015, Zhu et al. 2018) and the vaccination with DGFPG-DHYAVMISK peptide in a rat infection model resulted in higher serum anti-peptide IgG and saliva IgA antibody levels, demonstrating a potential protective effect of the immunization (Zhu et al. 2018). The treatment with monoclonal antibodies raised against synthetic DGFPG–DHYAVMISK peptide conjugated with KLH reduced bone loss in mice (An et al. 2021). Also, immunization of mice with HagA domain in fusion with maltose-binding protein resulted in higher levels of IgG and IgA antibodies in serum and higher IgA secretion in saliva eliciting a protective immune effect against *P. gingivalis* (Yuzawa et al. 2012). An example of a potential therapeutic target is also nicotinamide, a vitamin B_3 derivative, which inhibits *P. gingivalis* growth due to decreased gingipain activity manifested by decreased hemagglutination and hemolysis capacity (Lei et al. 2024). Treatment with this compound caused decreased heme acquisition,

preventing alveolar bone loss and reducing inflammatory cell infiltration.

On the contrary, while levels of antibodies against recombinant RgpA and RgpB are elevated in periodontitis patients, they have proven to be poor indicators of disease severity or *P. gingivalis* load (Massarenti et al. 2024). Therefore, there is still a need to search for specific biomarkers. Such a possibility for a diagnostic test is the determination of serum anti-HmuY IgG antibodies, which specifically recognize *P. gingivalis* HmuY protein, exhibit high levels in periodontitis patients, and do not cross-react with HmuY homologs produced by other bacteria (Trindade et al. 2012, Smiga et al. 2015, 2023b, Smiga and Olczak 2024, Nobre dos Santos-Lima et al. 2020).

Increased *P. gingivalis* resistance to antibiotics (Conrads et al. 2021, Abe et al. 2022, Ng et al. 2023, Rams et al. 2023) forces the search for alternative periodontitis treatment methods. Antibiotics such as metronidazole act against anaerobic bacteria and are used to treat periodontal diseases (Lofmark et al. 2010). *Porphyromonas gingivalis* susceptibility to metronidazole depends on heme availability since some strains displayed differential expression of iron and heme uptake systems (Li et al. 2018, Seers et al. 2020). Therefore, elaborating diagnostic and treatment methods using components of heme uptake as a target appears promising (Grover et al. 2014, Wang et al. 2024). For example, noniron metalloporphyrins, exploiting heme uptake systems due to the Trojan horse strategy, exhibit potent antibacterial activity (Stojiljkovic et al. 1999, Wojaczynski et al. 2011, Yukitake et al. 2011, Olczak et al. 2012, Wojtowicz et al. 2013). Porphyrin–antibiotic conjugates, such as deuteroporphyrin–metronidazole or deuteroporphyrin–nitroimidazole, targeting HA2 domains reduce *P. gingivalis* growth more efficiently, including intracellular bacteria (Yap et al. 2009, Dingsdag et al. 2015, Ye et al. 2017, Gao et al. 2018). Assuming the ability of erythrocyte binding to *P. gingivalis* and the Trojan horse strategy, erythrocyte-mimicking nanovesicles loaded with gallium porphyrins were constructed (Tang et al. 2024). Their utilization by *P. gingivalis* reduced its growth and invasion of epithelial cells, resulting in a decreased proportion of *P. gingivalis* in subgingival plaque, alleviating periodontitis progression in a rat experimental model.

Porphyrin- or metalloporphyrin-based photothermal and photodynamic therapies can be used as alternative antibacterial methods (Soukos et al. 2005, Imran et al. 2018). Therapy based on endogenous porphyrins and applying antimicrobial blue light seems to be a promising tool since it dysregulates the expression of genes engaged in *P. gingivalis* in heme acquisition (mainly those encoding gingipains and HmuR) and causes oxidative damage in bacterial cells (Yuan et al. 2023). However, these methods are not specific and are less effective under anaerobic conditions. Therefore, modified nanophotosensitizers were constructed, oxyHb@IR820 being an example (Bai et al. 2023). A natural oxygen-binding protein, oxyHb, is a better carrier for photosensitizers. Due to the binding to the HA2 domain of Kgp, RgpA, and HagA, oxyHb@IR820 after laser irradiation augmented antibacterial therapies in an experimental hamster model (Bai et al. 2023).

A summary of studies exploring microbiome-derived biomarkers showed that *P. gingivalis* and its virulence factors, including components of heme acquisition mechanisms as targets, could be considered reliable for differentiating between healthy individuals and periodontitis patients (Dong et al. 2025). As a result of research in this area, a growing number of patents propose new strategies to combat *P. gingivalis*-mediated infections (Bernardoni et al. 2024). Many attempts have been made to develop successful inhibitors of gingipain proteolytic activity. This aspect has been reported or reviewed extensively by others (e.g. Travis and Potempa 2000, Kataoka et al. 2014, Ho et al. 2018, Dominy et al. 2019, Guevara et al. 2019, Sabbagh and Decourt 2022). The compound COR388 was designed to block the activity of Kgp gingipain (Dominy et al. 2019). It effectively reduced the oral *P. gingivalis* load and improved gum conditions (Arastu-Kapur et al. 2020). Importantly, it reduced neuroinflammation, blocked Aβ accumulation, and rescued hippocampal neurons in an Alzheimer's disease mouse model (Dominy et al. 2019). Since preclinical studies were promising (Costa et al. 2021), the safety, tolerance, and efficiency of orally administered COR388 (Atuzaginstat) were examined under study in Phase II/III clinical trials (NCT03823404). Although this drug and its derivative (COR588) reduced the oral load of *P. gingivalis* and improved cognitive functions, they caused liver abnormalities. Therefore, no treatment method based on them has been introduced so far.

Concluding remarks

Unraveling the fundamental mechanisms within pathogenic bacteria is essential for understanding how they influence host health and cause disease. Besides classical strategies of pathogens used to avoid the host immune response (Hitzler et al. 2025), *P. gingivalis* developed additional mechanisms to persist in host cells and evade host immune response, with gingipains being unique for this bacterium (e.g. Bostanci and Belibasakis 2012, Hocevar et al. 2018, Hajishengallis and Diaz 2020, Hajishengallis and Lamont 2021, Widziolek et al. 2025). Although like other Gram-negative bacteria, *P. gingivalis* relies on TDRs for heme acquisition, it has developed sophisticated strategies allowing more efficient utilization of various heme sources in hostile environments, and efficient competition with cohabitating bacteria. This includes a unique accessory gingipain-Hmu system-based mechanism that facilitates heme acquisition from erythrocytes and hemoproteins. While many aspects of *P. gingivalis* heme uptake and homeostasis remain unclear, in recent years, significant progress has been made in uncovering its heme and iron acquisition strategies, which can be leveraged to combat this pathogen. Ongoing research could pave the way for groundbreaking therapies against *P. gingivalis* and innovative diagnostic tools to assess the severity of periodontal diseases.

Acknowledgment

We thank Patrick Lane (ScEYEnce Studios) for the creation of Fig. 1.

Conflict of interest: The authors have no conflict of interest to declare.

Funding

Part of the findings presented in this review have been gained from studies financed by grants 2021/41/B/NZ6/00702 and 2023/51/D/NZ6/00324 (to M.Ś.), and 2015/17/B/NZ6/01969, 2016/23/B/NZ6/00080, 2019/33/B/NZ6/00292, and 2023/49/B/NZ6/00129 (to T.O.) from the National Science Center (Narodowe Centrum Nauki, NCN, Krakow, Poland).

References

Abe FC, Kodaira K, Motta CCB *et al.* Antimicrobial resistance of microorganisms present in periodontal diseases: a systematic review and meta-analysis. *Front Microbiol* 2022;**13**:961986. https://doi.org/10.3389/fmicb.2022.961986.

Adler CJ, Dobney K, Weyrich LS *et al.* Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 2013;**45**:450–455e1. https://doi.org/10.1038/ng.2536.

Aft RL, Mueller GC Hemin-mediated oxidative degradation of proteins. *J Biol Chem* 1984;**59**:301–5. https://doi.org/10.1016/S0021-9258(17)43657-X.

Al-Qutub MN., Braham PH, Karimi-Naser LM *et al.* Hemin-dependent modulation of the lipid A structure of *Porphyromonas gingivalis* lipopolysaccharide. *Infect Immun* 2006;**74**:4474–85. https://doi.org/10.1128/IAI.01924-05.

An T, Chen Y, Li M *et al.* Inhibition of experimental periodontitis by a monoclonal antibody against *Porphyromonas gingivalis* HA2. *Microb Pathog* 2021;**54**:104633. https://doi.org/10.1016/j.micpath.2020.104633.

Anaya-Bergma C, He J, Jones K *et al. Porphyromonas gingivalis* ferrous iron transporter FeoB1 influences sensitivity to oxidative stress. *Infect Immun* 2010;**78**:688–96. https://doi.org/10.1128/IAI.00108-09.

Anaya-Bergman C, Rosato A, Lewis JP Iron- and hemin-dependent gene expression of *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2015;**30**:39–61. https://doi.org/10.1111/omi.12066.

Anderson JE, Sparling PF, Cornelissen CN Gonococcal transferrin-binding protein 2 facilitates but is not essential for transferrin utilization. *J Bacteriol* 1994;**176**:3162–70. https://doi.org/10.1128/jb.176.11.3162-3170.1994.

Andrews NC Disorders of iron metabolism. *N Engl J Med* 1999;**23**:1986–95. https://doi.org/10.1056/NEJM199912233412607.

Andrian E, Mostefaoui Y, Rouabhia M *et al.* Regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by *Porphyromonas gingivalis* in an engineered human oral mucosa model. *J Cell Physiol* 2007;**211**:56–62. https://doi.org/10.1002/jcp.20894.

Antonyuk SV, Sieminska K, Smiga M *et al. Bacteroides fragilis* expresses three proteins similar to *Poprhyromonas gingivalis* HmuY: hemophore-like proteins differentially evolved to participate in heme acquisition in oral and gut microbiomes. *FASEB J* 2023;**37**:e22981. https://doi.org/10.1096/fj.202300366R.

Arastu-Kapur S, Nguyen M, Raha D *et al.* Treatment of *Porphyromonas gulae* infection and downstream pathology in the aged dog by lysine-gingipain inhibitor COR388. *Pharmacol Res Perspect* 2020;**8**:e00562. https://doi.org/10.1002/prp2.562.

Arweiler NB, Marx VK, Laugisch O *et al.* Clinical evaluation of a newly developed chairside test to determine periodontal pathogens. *J Periodontol* 2020;**91**:387–95. https://doi.org/10.1002/JPER.19-0180.

Ascenzi P, Fasano M Heme-hemopexin: A 'chronosteric' heme-protein. *IUBMB Life* 2007;**59**:700–8. https://doi.org/10.1080/15216540701689666.

Ascenzi P, Fasano M Serum heme-albumin: an allosteric protein. *IUBMB Life* 2009;**61**:1118–22. https://doi.org/10.1002/iub.263.

Ascenzi P, Bocedi A, Visca P *et al.* Hemoglobin and heme scavenging. *IUBMB Life* 2005;**57**:749–59. https://doi.org/10.1080/15216540500380871.

Ascenzi P, di Masi A, Leboffe L *et al.* Structural biology of bacterial haemophores. *Adv Microb Physiol* 2015;**67**:127–76. https://doi.org/10.1016/bs.ampbs.2015.09.002.

Awate OA, Ng D, Stoudenmire JL *et al.* Investigating the importance of selected surface-exposed loops in HpuB for hemoglobin binding and utilization *by Neisseria gonorrhoeae*. *Infect Immun* 2024;**92**:e0021124. https://doi.org/10.1128/iai.00211-24.

Baba A, Abe N, Kadowaki T *et al.* Arg-gingipain is responsible for the degradation of cell adhesion molecules of human gingival fibroblasts and their death induced by *Porphyromonas gingivalis*. *Biol Chem* 2001;**382**:817–24. https://doi.org/10.1515/BC.2001.099.

Bai L, Shi E, Li Y *et al.* Oxyhemoglobin-based nanophotosensitizer for specific and synergistic photothermal and photodynamic therapies against *Porphyromonas gingivalis* oral infection. *ACS Biomater Sci Eng* 2023;**9**:485–97. https://doi.org/10.1021/acsbiomaterials.2c01034.

Baima G, Minoli M, Michaud DS *et al.* Periodontitis and risk of cancer: mechanistic evidence. *Periodontol 2000* 2024;**96**:83–94. https://doi.org/10.1111/prd.12540.

Bang J, Cimasoni G Total protein in human crevicular fluid. *J Dent Res* 1971;**50**:1683. https://doi.org/10.1177/00220345710500065701.

Bartold PM, McCulloch CA, Narayanan AS *et al.* Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. *Periodontol 2000* 2000;**24**:253–69. https://doi.org/10.1034/j.1600-0757.2000.2240113.x.

Baty JJ, Stoner SN, Scoffield JA Oral commensal streptococci: gatekeepers of the oral cavity. *J Bacteriol* 2022;**204**:e0025722. https://doi.org/10.1128/jb.00257-22.

Beaven GH, Chen SH, D'Albis G *et al.* A spectroscopic study of the haemin-human-serum-albumin system. *Eur J Biochem* 1974;**41**:539–46. https://doi.org/10.1111/j.1432-1033.1974.tb03295.x.

Benedyk M, Byrne DP, Glowczyk I *et al.* Pyocyanin, a contributory factor in haem acquisition and virulence enhancement of *Porphyromonas gingivalis* in the lung. *PLoS One* 2015;**10**:e0118319. https://doi.org/10.1371/journal.pone.0118319.

Benevides-Matos BF. The Hem and Has haem uptake systems in *Serratia marcescens*. *Microbiology* 2010;**156**:1749–57. https://doi.org/10.1099/mic.0.034405-0.

Bernaerts E, Ahmadzadeh K, De Visscher A *et al.* Human monocyte-derived macrophages shift subcellular metalloprotease activity depending on their activation site. *iScience* 2024;**27**:111171. https://doi.org/10.1016/j.isci.2024.111171.

Bernardoni BL, D'Agostino I, La Motta C *et al.* An insight into the last 5-year patents on *Porphyromonas gingivalis* and *Streptococcus mutans*, the pivotal pathogens in the oral cavity. *Expert Opin Ther Pat* 2024;**34**:433–63. https://doi.org/10.1080/13543776.2024.2349739.

Bhogal PS, Slakeski N, Reynolds EC A cell-associated protein complex of *Porphyromonas gingivalis* W50 composed of Arg- and Lys-specific cysteine proteinases and adhesins. *Microbiology* 1997;**143**:2485–95. https://doi.org/10.1099/00221287-143-7-2485.

Bickel M, Cimasoni G, Andersen E Flow and albumin content of early (pre-inflammatory) gingival crevicular fluid from human subjects. *Arch Oral Biol* 1985;**30**:599–602. https://doi.org/10.1016/0003-9969(85)90079-2.

Bielecki M, Antonyuk S, Strange RW *et al. Tannerella forsythia* Tfo belongs to *Porphyromonas gingivalis* HmuY-like family of proteins but differs in heme-binding properties. *Biosci Rep* 2018;**38**:BSR20181325. https://doi.org/10.1042/BSR20181325.

Bielecki M, Antonyuk S, Strange RW *et al. Prevotella intermedia* produces two proteins homologous to *Porphyromonas gingivalis* HmuY but with different heme coordination mode. *Biochem J* 2020;**477**:381–405. https://doi.org/10.1042/BCJ20190607.

Bondy-Carey JL, Galicia J, Bagaitkar J *et al.* Neutrophils alter epithelial response to *Porphyromonas gingivalis* in a gingival crevice model. *Mol Oral Microbiol* 2013;**28**:102–13. https://doi.org/10.1111/omi.12008.

Bostanci N, Belibasakis GN *Porphyromonas gingivalis*: an invasive and evasive opportunistic pathogen. *FEMS Microbiol Lett* 2012;**333**:1–9. https://doi.org/10.1111/j.1574-6968.2012.02579.x.

Bostanci N, Belibasakis GN Gingival crevicular fluid and its immune mediators in the proteomic era. *Periodontol 2000* 2018;**76**:68–84. https://doi.org/10.1111/prd.12154.

Boutrin MC, Mishra A, Wang C *et al.* The involvement of CdhR in *Porphyromonas gingivalis* during nitric oxide stress. *Mol Oral Microbiol* 2023;**38**:289–308. https://doi.org/10.1111/omi.12414.

Boyapati R, Lanke RB, Mudaliyar MC *et al.* Exploring the microbiome landscape of dental plaque: a cross-sectional analysis in periodontal health and disease. *Cureus* 2024;**16**:e57334. https://doi.org/10.7759/cureus.57334.

Bracken CS, Baer MT, Abdur-Rashid A *et al.* Use of heme-protein complexes by the *Yersinia enterocolitica* HemR receptor: histidine residues are essential for receptor function. *J Bacteriol* 1999;**181**:6063. https://doi.org/10.1128/JB.181.19.6063-6072.1999.

Bradley JM, Svistunenko DA, Wilson MT *et al.* Bacterial iron detoxification at the molecular level. *J Biol Chem* 2020;**295**:17602–23. https://doi.org/10.1074/jbc.REV120.007746.

Braun V, Braun M Active transport of iron and siderophore antibiotics. *Curr Opin Microbiol* 2002;**5**:194–201. https://doi.org/10.1016/S1369-5274(02)00298-9.

Braun V, Killmann H Bacterial solutions to the iron-supply problem. *Trends Biochem Sci* 1999;**24**:104–9. https://doi.org/10.1016/S0968-0004(99)01359-6.

Bravo-Lopez M, Villa-Islas V, Rocha Arriaga C *et al.* Paleogenomic insights into the red complex bacteria *Tannerella forsythia* in Pre-Hispanic and Colonial individuals from Mexico. *Philos Trans R Soc Lond B Biol Sci* 2020;**375**:20190580. https://doi.org/10.1098/rstb.2019.0580.

Bregaint S, Boyer E, Fong SB *et al. Porphyromonas gingivalis* outside the oral cavity. *Odontology* 2022;**110**:1–19. https://doi.org/10.1007/s10266-021-00647-8.

Brillet K, Meksem A, Thompson A *et al.* Expression, purification, crystallization and preliminary X-ray diffraction analysis of the TonB-dependent haem outer membrane transporter ShuA from *Shigella dysentariae*. *Acta Crystallogr Sec F Struct Biol Cryst Commun* 2009;**65**:402–5. https://doi.org/10.1107/S1744309109008148.

Brown JL, Yates EA, Bielecki M *et al.* Potential role for *Streptococcus gordonii*-derived hydrogen peroxide in heme acquisition by *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2018;**33**:322–35. https://doi.org/10.1111/omi.12229.

Buchanan SK, Smith BS, Venkatramani L *et al.* Crystal structure of the outer membrane active transporter FepA from *Escherichia coli*. *Nat Struct Biol* 1999;**6**:56–63. https://doi.org/10.1038/4931.

Bullen JJ, Rogers HJ, Griffiths E Role of iron in bacterial infection. *Curr Top Microbiol Immunol* 1978;**80**:1–35. https://doi.org/10.1007/978-3-642-66956-9_1.

Burkhard KA, Wilks A Characterization of the outer membrane receptor ShuA from the heme uptake system of *Shigella dysenteriae*. Substrate specificity and identification of the heme protein ligands. *J Biol Chem* 2007;**282**:15126–36. https://doi.org/10.1074/jbc.M611121200.

Butler CA, Dashper SG, Zhang L *et al.* The *Porphyromonas gingivalis* ferric uptake regulator orthologue binds hemin and regulates hemin-responsive biofilm development. *PLoS One* 2014;**9**:e111168. https://doi.org/10.1371/journal.pone.0111168.

Butler C, Mitchell H, Dashper S *et al.* The *Porphyromonas gingivalis* ferric uptake regulator orthologue does not regulate iron homeostasis. *Genom Data* 2015;**5**:167–8. https://doi.org/10.1016/j.gdata.2015.05.042.

Butler CA, Ciccotosto GD, Rygh N *et al.* Bacterial membrane vesicles: the missing link between bacterial infection and Alzheimer disease. *J Infect Dis* 2024;**230**:S87–94. https://doi.org/10.1093/infdis/jiae228.

Byrne DP, Potempa J, Olczak T *et al.* Evidence of mutualism between two periodontal pathogens: co-operative haem acquisition by the HmuY hemophore of *Porphyromonas gingivalis* and the cysteine protease interpain A (InpA) of *Prevotella intermedia*. *Mol Oral Microbiol* 2013;**28**:219–29. https://doi.org/10.1111/omi.12018.

Cai Z, Lin S, Hu S *et al.* Structure and function of oral microbial community in periodontitis based on integrated data. *Front Cell Infect Microbiol* 2021;**11**:663756. https://doi.org/10.3389/fcimb.2021.663756.

Caillet-Saguy C, Piccioli M, Turano P *et al.* Mapping the interaction between the hemophore HasA and its outer membrane receptor HasR using CRINEPT-TROSY NMR spectroscopy. *J Am Chem Soc* 2009;**131**:1736–44. https://doi.org/10.1021/ja804783x.

Campoccia D, Montanaro L, Arciola CR Tracing the origins of extracellular DNA in bacterial biofilms: story of death and predation to community benefit. *Biofouling* 2021;**37**:1022–39. https://doi.org/10.1080/08927014.2021.2002987.

Carvalho-Filho PC, Gomes-Filho IS, meyer R *et al.* Role of *Porphyromonas gingivalis* HmuY in immunopathogenesis of chronic periodontitis. *Mediat Inflamm* 2016;**2016**:7465852. https://doi.org/10.1155/2016/7465852.

Celia H, Noinaj N, Zakharov SD *et al.* Structural insight into the role of the Ton complex in energy transduction. *Nature* 2016;**538**:60–65. https://doi.org/10.1038/nature19757.

Champagne CM, Holt SC, Van Dyke TE *et al.* Lipopolysaccharide isolated from *Porphyromonas gingivalis* grown in hemin-limited chemostat conditions has a reduced capacity for human neutrophil priming. *Oral Microbiol Immunol* 1996;**11**:319–25. https://doi.org/10.1111/j.1399-302X.1996.tb00188.x.

Chan C, Ng D, Fraser ME *et al.* Structural and functional insights into iron acquisition from lactoferrin and transferrin in Gram-negative bacterial pathogens. *Biometals* 2023;**36**:683–702. https://doi.org/10.1007/s10534-022-00466-6.

Chen MX, Zhong YJ, Dong QQ *et al.* Global, regional, and national burden of severe periodontitis, 1990-2019: an analysis of the Global Burden of Disease Study 2019. *J Clin Periodontol* 2021;**48**:1165–88. https://doi.org/10.1111/jcpe.13506.

Cherayil BJ The role of iron in the immune response to bacterial infection. *Immunol Res* 2011;**50**:1–9. https://doi.org/10.1007/s12026-010-8199-1.

Chiabrando D, Mercurio S, Tolosano E Heme and erythropoiesis: more than a structural role. *Haematologica* 2014;**99**:973–83. https://doi.org/10.3324/haematol.2013.091991.

Chimento DP, Mohanty AK, Kadner RJ *et al.* Substrate-induced transmembrane signaling in the cobalamin transporter BtuB. *Nat Struct Biol* 2003;**10**:394–401. https://doi.org/10.1038/nsb914.

Choby JE, Skaar EP Heme synthesis and acquisition in bacterial pathogens. *J Mol Biol* 2016;**428**:3408–28. https://doi.org/10.1016/j.jmb.2016.03.018.

Chou AC, Fitch CD Hemolysis of mouse erythrocytes by ferriprotoporphyrin IX and chloroquine. Chemotherapeutic implications. *J Clin Invest* 1980;**6**:856–8. https://doi.org/10.1172/JCI109925.

Chou AC, Fitch CD Mechanism of hemolysis induced by ferriprotoporphyrin IX. *J Clin Invest* 1981;**68**:672–7. https://doi.org/10.1172/JCI110302.

Chu L, Bramanti TE, Ebersole JL *et al.* Haemolytic activity in the periodontopathogen *Porphyromonas gingivalis*: kinetics of enzyme release and localization. *Infect Immun* 1991;**59**:1932–40. https://doi.org/10.1128/iai.59.6.1932-1940.1991.

Chu BCH, Vogel HJ A structural and functional analysis of type III periplasmic and substrate binding proteins: their role in bacterial siderophore and heme transport. *Biol Chem* 2011;**392**:39–52. https://doi.org/10.1515/bc.2011.012.

Ciuraszkiewicz J, Smiga M, Mackiewicz P *et al.* Fur homolog regulates *Porphyromonas gingivalis* virulence under low-iron/heme conditions through a complex regulatory network. *Mol Oral Microbiol* 2014;**29**:333–53. `.

Claesson R, Johansson A, Belibasakis GN Clinical laboratory diagnostics in dentistry: application of microbiological methods. *Front Oral Health* 2022;**3**:983991. https://doi.org/10.3389/froh.2022.983991.

Clarke TE, Tari LW, Vogel HJ Structural biology of bacterial iron uptake systems. *Curr Top Med Chem* 2001;**1**:7–30. https://doi.org/10.2174/1568026013395623.

Cobessi D, Celia H, Pattus F Crystal structure at high resolution of ferric-pyochelin and its membrane receptor FptA from *Pseudomonas aeruginosa*. *J Mol Biol* 2005;**352**:893–904. https://doi.org/10.1016/j.jmb.2005.08.004.

Cobessi D, Meksem A, Brillet K Structure of the heme/hemoglobin outer membrane receptor ShuA from *Shigella dysenteriae*: heme binding by an induced fit mechanism. *Proteins* 2010;**78**:286–94. https://doi.org/10.1002/prot.22539.

Conrads G, KlompT DD *et al.* The antimicrobial susceptibility of *Porphyromonas gingivalis*: genetic repertoire, global phenotype, and review of the literature. *Antibiotics* 2021;**10**:1438. https://doi.org/10.3390/antibiotics10121438.

Contreras H, Chim N, Credali A *et al.* Heme uptake in bacterial pathogens. *Curr Opin Chem Biol* 2014;**19**:34–41. https://doi.org/10.1016/j.cbpa.2013.12.014.

Costa MJF, de Araujo ID, da Rocha Alves L *et al.* Relationship of *Porphyromonas gingivalis* and Alzheimer's disease: a systematic review of pre-clinical studies. *Clin Oral Invest* 2021;**25**:797–806. https://doi.org/10.1007/s00784-020-03764-w.

Costeira R, Aduse-Opoku J, Vernon JJ *et al.* Hemin availability induces coordinated DNA methylation and gene expression changes in *Porphyromonas gingivalis*. *mSystems* 2023;**8**:e0119322. https://doi.org/10.1128/msystems.01193-22.

Curtis MA, Sterne JA, Price SJ *et al.* The protein composition of gingival crevicular fluid sampled from male adolescents with no destructive periodontitis: baseline data of a longitudinal study. *J Periodontal Res* 1990;**25**:6–16. https://doi.org/10.1111/j.1600-0765.1990.tb01202.x.

Cutler CW, Eke PI, Genco CA *et al.* Hemin-induced modifications of the antigenicity and hemin-binding capacity of *Porphyromonas gingivalis* lipopolysaccharide. *Infect Immun* 1996;**64**:2282–7. https://doi.org/10.1128/iai.64.6.2282-2287.1996.

D'Souza LL, Lawande SA, Samuel J *et al.* Effect of salivary urea, pH and ureolytic microflora on dental calculus formation and its correlation with periodontal status. *J Oral Biol Craniofac Res* 2023;**13**:8–12. https://doi.org/10.1016/j.jobcr.2022.10.004.

Dailey HA, Septer AN, Daugherty L *et al.* The *Escherichia coli* protein YfeX functions as a porphyrinogen oxidase, not a heme dechelatase. *mBio* 2011;**2**:e00248–11. https://doi.org/10.1128/mBio.00248-11.

Dailey HA, Dailey TA, Gerdes S *et al.* Prokaryotic heme biosynthesis: multiple pathways to a common essential product. *Microbiol Mol Biol Rev* 2017;**81**:e00048–16. https://doi.org/10.1128/MMBR.00048-16.

Darveau RP, Pham TT, Lemley K *et al.* *Porphyromonas gingivalis* lipopolysaccharide contains multiple lipid A species that functionally interact with both Toll-like receptors 2 and 4. *Infect Immun* 2004;**72**:5041–51. https://doi.org/10.1128/IAI.72.9.5041-5051.2004.

Darveau RP, Hajishengallis G, Curtis MA *Porphyromonas gingivalis* as a potential community activist for disease. *J Dent Res* 2012;**91**:816–20. https://doi.org/10.1177/0022034512453589.

Dashper SG, Hendtlass A, Slakeski N *et al.* Characterization of a novel outer membrane hemin-binding protein of *Porphyromonas gingivalis*. *J Bacteriol* 2000;**182**:6456–62. https://doi.org/10.1128/JB.182.22.6456-6462.2000.

Dashper SG, Butler CA, Lissel JP *et al.* A novel *Porphyromonas gingivalis* FeoB plays a role in manganese accumulation. *J Biol Chem* 2005;**280**:28095–102. https://doi.org/10.1074/jbc.M503896200.

Dashper SG, Ang CS, Veith PD *et al.* Response of *Porphyromonas gingivalis* to heme limitation in continuous culture. *J Bacteriol* 2009;**191**:1044–55. https://doi.org/10.1128/JB.01270-08.

Dashper SG, Mitchell HK, Seers CA *et al.* *Porphyromonas gingivalis* uses specific domain rearrangements and allelic exchange to generate diversity in surface virulence factors. *Front Microbiol* 2017;**8**:48. https://doi.org/10.3389/fmicb.2017.00048.

de Jongh CA, de Vries TJ, Bikker FJ *et al.* Mechanisms of *Porphyromonas gingivalis* to translocate over the oral mucosa and other tissue barriers. *J Oral Microbiol* 2023;**15**:2205291. https://doi.org/10.1080/20002297.2023.2205291.

De Simone G, Varricchio R, Ruberto TF *et al.* Heme scavenging and delivery: the role of human serum albumin. *Biomolecules* 2023;**13**:575. https://doi.org/10.3390/biom13030575.

DeCarlo AA, Nadkarni M, Paramaesvaran M *et al.* Serum antibodies against the hemoglobin-binding domain (HA2) of *Porphyromonas gingivalis*. *J Periodontal Res* 2004;**39**:228–35. https://doi.org/10.1111/j.1600-0765.2004.00730.x.

DeCarlo AA, Paramaesvaran M, Yun PL *et al.* Porphyrin-mediated binding to hemoglobin by the HA2 domain of cysteine proteinases (gingipains) and hemagglutinins from the periodontal pathogen *Porphyromonas gingivalis*. *J Bacteriol* 1999;**181**:3784–91. https://doi.org/10.1128/JB.181.12.3784-3791.1999.

Delanghe JR, Langlois MR Hemopexin: a review of biological aspects and the role in laboratory medicine. *Clin Chim Acta* 2001;**312**:13–23. https://doi.org/10.1016/S0009-8981(01)00586-1.

Deng ZL, Sztajer H, Jarek M *et al.* Worlds apart—transcriptome profiles of key oral microbes in the periodontal pocket compared to single laboratory culture reflect synergistic interactions. *Front Microbiol* 2018;**9**:124. https://doi.org/10.3389/fmicb.2018.00124.

Detzel MS, Schmalohr BF, Steinbock F *et al.* Revisiting the interaction of heme hemopexin. *Biol Chem* 2021;**402**:675–91. https://doi.org/10.1515/hsz-2020-0347.

Dewhirst FE, Chen T, Izard J *et al.* The human oral microbiome. *J Bacteriol* 2010;**192**:5002–17. https://doi.org/10.1128/JB.00542-10.

Dingsdag SA, Yap BCM, Hunter N *et al.* Amino acid-linked porphyrin-nitroimidazole antibiotics targeting *Porphyromonas gingivalis*. *Org Biomol Chem* 2015;**13**:98–109. https://doi.org/10.1039/C4OB01841A.

Dominy SS, Lynch C, Ermini F *et al.* *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Sci Adv* 2019;**5**:eaau3333. https://doi.org/10.1126/sciadv.aau3333.

Dong A, Proctor G, Zaric S Diagnostic accuracy of microbiome-derived biomarkers in periodontitis: systematic review and meta-analysis. *J Periodontal Res* 2025;**0**:1–14. https://doi.org/10.1111/jre.13377.

Dorn BR, Dunn WAJ *et al.* *Porphyromonas gingivalis* traffics to autophagosomes in human coronary artery endothelial cells. *Infect Immun* 2001;**69**:5698–708. https://doi.org/10.1128/IAI.69.9.5698-5708.2001.

Dragos A, Kovacs A The peculiar functions of the bacterial extracellular matrix. *Trends Microbiol* 2017;**25**:257–66. https://doi.org/10.1016/j.tim.2016.12.010.

du Teil Espina M, Gabarrini G, Harmsen HJM Talk to your gut: the oral-gut microbiome axis and its immunomodulatory role in the

etiology of rheumatoid arthritis. *FEMS Microbiol Rev* 2018;**43**:1–18. https://doi.org/10.1093/femsre/fuy035.

Dye BA, Herrera-Abreu M, Lerche-Sehm J *et al.* Serum antibodies to periodontal bacteria as diagnostic markers of periodontitis. *J Periodontol* 2009;**80**:634–7. https://doi.org/10.1902/jop.2009.080474.

Ebersole JL, Hamzeh R, Nguyen L *et al.* Variations in IgG antibody subclass responses to oral bacteria: effects of periodontal disease and modifying factors. *J Periodontal Res* 2020;**56**:863–76. https://doi.org/10.1111/jre.12882.

Eichinger A, Beisel HG, Jacob U *et al.* Crystal structure of gingipain R: an Arg-specific bacterial cysteine proteinase with a caspase-like fold. *EMBO J* 1999;**18**:5453–62. https://doi.org/10.1093/emboj/18.20.5453.

Enax J, Ganss B, Amaechi BT *et al.* The composition of the dental pellicle: an updated literature review. *Front Oral Health* 2023;**4**:1260442. https://doi.org/10.3389/froh.2023.1260442.

Ermini F, Low VF, Song JJ *et al.* Ultrastructural localization of *Porphyromonas gingivalis* gingipains in the substantia nigra of Parkinson's disease brains. *NPJ Parkinsons Dis* 2024;**10**:90. https://doi.org/10.1038/s41531-024-00705-2.

Exertier C, Montemiglio LC, Tognaccini L *et al.* Gaseous ligand binding to *Porphyromonas gingivalis* HmuY hemophore-like protein: insights into the mechanism of heme release. *J Inorg Biochem* 2025;**269**:11879. https://doi.org/10.1016/j.jinorgbio.2025.112879.

Ferguson AD, Amezcua CA, Halabi NM *et al.* Signal transduction pathway of TonB-dependent transporters. *Proc Natl Acad Sci USA* 2007;**104**:513–8. https://doi.org/10.1073/pnas.0609887104.

Ferguson AD, Braun V, Fiedler HP *et al.* Crystal structure of the antibiotic albomycin in complex with the outer membrane transporter FhuA. *Protein Sci* 2000;**9**:956–63. https://doi.org/10.1110/ps.9.5.956.

Ferguson AD, Chakraborty R, Smith BS *et al.* Structural basis of gating by the outer membrane transporter FecA. *Science* 2002;**295**:1715–9. https://doi.org/10.1126/science.1067313.

Ferguson AD, Deisenhofer J TonB-dependent receptors—structural perspectives. *Biochim Biophys Acta Biomembr* 2002;**1565**:318–32. https://doi.org/10.1016/S0005-2736(02)00578-3.

Ferguson AD, Koedding J, Walker G *et al.* Active transport of an antibiotic rifamycin derivative by the outer-membrane protein FhuA. *Structure* 2001;**9**:707–16. https://doi.org/10.1016/S0969-2126(01)00631-1.

Fernandez A, Lechar Deur D, Derre-Bobillot A *et al.* Two coregulated efflux transporters modulate intracellular heme and protoporphyrin IX availability in *Streptococcus agalactiae*. *PLoS Pathog* 2010;**6**:e1000860. https://doi.org/10.1371/journal.ppat.1000860.

Figuero E, Sanchez-Beltran M, Cuesta-Frechoso S *et al.* Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011;**82**:1469–77. https://doi.org/10.1902/jop.2011.100719.

Fischer E, Strehlow B, Hartz D *et al.* Soluble and membrane-bound ferrisiderophore reductase of *Escherichia coli* K-12. *Arch Microbiol* 1990;**153**:329–36. https://doi.org/10.1007/BF00249001.

Fitch CD, Chewli R, Kanjananggulpan P *et al.* Intracellular ferriprotoporphyrin IX is a lytic agent. *Blood* 1983;**62**:1165–8. https://doi.org/10.1182/blood.V62.6.1165.1165.

Flemming HC, Wingender J, Szewzyk U *et al.* Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol* 2016;**14**:563–75. https://doi.org/10.1038/nrmicro.2016.94.

Flemming TF Periodontitis. *Ann Periodontol* 1999;**4**:32–38. https://doi.org/10.1902/annals.1999.4.1.32.

Franca M, Moura-Costa L, Meyer RJ *et al.* Humoral immune response to antigens of *Porphyromonas gingivalis* ATCC 33277 in chronic periodontitis. *J Appl Oral Sci* 2007;**15**:213–9. https://doi.org/10.1590/S1678-77572007000300011.

Frankenberg-Dinkel N Bacterial heme oxygenases. *Antioxid Redox Signal* 2004;**5**:825–34. https://doi.org/10.1089/ars.2004.6.825.

Frazer LT, O'Brien-Simpson NM, Slakeski N *et al.* Vaccination with recombinant adhesins from RgpA-Kgp proteinase complex protects against *Porphyromonas gingivalis* infection. *Vaccine* 2006;**24**:6542–54. https://doi.org/10.1016/j.vaccine.2006.06.013.

Gao JL, Nguyen KA, Hunter N Characterization of a hemophore-like protein from *Porphyromonas gingivalis*. *J Biol Chem* 2010;**285**:40028–38. https://doi.org/10.1074/jbc.M110.163535.

Gao JL, Lu Y, Browne G *et al.* The role of heme binding by DNA-protective protein from starved cells (Dps) in the tolerance of *Porphyromonas gingivalis* to heme toxicity. *J Biol Chem* 2012;**287**:42243–58. https://doi.org/10.1074/jbc.M112.392787.

Gao JL, Kwan AH, Yammine A *et al.* Structural properties of a haemophore facilitate targeted elimination of the pathogen *Porphyromonas gingivalis*. *Nat Commun* 2018;**9**:4097. https://doi.org/10.1038/s41467-018-06470-0.

Gmiterek A, Wojtowicz H, Mackiewicz P *et al.* The unique hmuY gene sequence as a specific marker of *Porphyromonas gingivalis*. *PLoS One* 2013;**8**:e67719. https://doi.org/10.1371/journal.pone.0067719.

Gmiterek A, Kłopot A, Wojtowicz H *et al.* Immune response of macrophages induced by *Porphyromonas gingivalis* requires HmuY protein. *Immunobiology* 2016;**221**:1382–94. https://doi.org/10.1016/j.imbio.2016.07.007.

Gorasia DG, Veith PD, Reynolds EC The type IX secretion system: advances in structure, function and organisation. *Microorganisms* 2020;**8**:1173. https://doi.org/10.3390/microorganisms8081173.

Goulet V, Britigan B, Nakayama K *et al.* Cleavage of human transferrin by *Porphyromonas gingivalis* gingipains promotes growth and formation of hydroxyl radicals. *Infect Immun* 2004;**72**:4351–6. 4356.

Graca-Souza AV, Arruda MA, De Freitas MS *et al.* Neutrophil activation by heme: implications for inflammatory processes. *Blood* 2002;**99**:4160–5. https://doi.org/10.1182/blood.V99.11.4160.

Grass G, Otto M, Fricke B *et al.* Fief (YiiP) from *Escherichia coli* mediates decreased cellular accumulation of iron and relieves iron stress. *Arch Microbiol* 2005;**183**:9–18. https://doi.org/10.1007/s00203-004-0739-4.

Griffen AL, Becker MR, Lyons SR *et al.* Prevalence of *Porphyromonas gingivalis* and periodontal health status. *J Clin Microbiol* 1998;**36**:3239–42. https://doi.org/10.1128/JCM.36.11.3239-3242.1998.

Grover V, Kapoor A, Malhotra R *et al.* *Porphyromonas gingivalis* antigenic determinants—potential targets for the vaccine development against periodontitis. *Infect Disord Drug Targets* 2014;**14**:1–13. https://doi.org/10.2174/1871526514666140827100930.

Guerinot ML Microbial iron transport. *Annu Rev Microbiol* 1994;**48**:743–72. https://doi.org/10.1146/annurev.mi.48.100194.003523.

Guevara T, Rodriguez-Banqueri A, Lasica AM *et al.* Structural determinants of inhibition of *Porphyromonas gingivalis* gingipain K by KYT-36, a potent, selective, and bioavailable peptidase inhibitor. *Sci Rep* 2019;**9**:4935. https://doi.org/10.1038/s41598-019-41354-3.

Guo Y, Nguyen KA, Potempa J Dichotomy of gingipains action as virulence factors: from cleaving substrates with the precision of a surgeon's knife to a meat chopper-like brutal degradation of proteins. *Periodontol 2000* 2010;**54**:15–44. https://doi.org/10.1111/j.1600-0757.2010.00377.x.

Guo Y, Wang Y, Wang Y *et al.* Heme competition triggers an increase in the pathogenic potential of *Porphyromonas gingivalis* in *Porphyromonas gingivalis-Candida albicans* mixed biofilm. *Front Microbiol* 2020;**11**:596459. https://doi.org/10.3389/fmicb.2020.596459.

Hajishengallis G Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 2015;**15**:30–44. https://doi.org/10.1038/nri3785.

Hajishengallis G, Chavakis T Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nat Rev Immunol* 2021;**28**:1–15. https://doi.org/10.1038/s41577-020-00488-6.

Hajishengallis G, Darveau RP, Curtis MA The keystone pathogen hypothesis. *Nat Rev Microbiol* 2012;**10**:717–25. https://doi.org/10.1038/nrmicro2873.

Hajishengallis G, Diaz PI *Porphyromonas gingivalis*: immune subversion activities and role in periodontal dysbiosis. *Curr Oral Health Rep* 2020;**7**:12–21. https://doi.org/10.1007/s40496-020-00249-3.

Hajishengallis G, Lamont RJ Polymicrobial communities in periodontal disease: their quasi-organismal nature and dialogue with the host. *Periodontol 2000* 2021;**86**:210–30. https://doi.org/10.1111/prd.12371.

Halliwell B, Gutteridge JM Biologically relevant metal ion-dependent hydroxyl radical generation. An update. *FEBS Lett* 1992;**307**:108–12. https://doi.org/10.1016/0014-5793(92)80911-Y.

Han N, Whitlock J, Progulske-Fox A. The hemagglutinin gene A (*hagA*) of *Porphyromonas gingivalis* 381 contains four large, contiguous, direct repeats. *Infect Immun* 1996;**64**:4000–7. https://doi.org/10.1128/iai.64.10.4000-4007.1996.

Hanioka T, Matsuse R, Shigemoto Y *et al.* Relationship between periodontal disease status and combination of biochemical assays of gingival crevicular fluid. *J Periodontal Res* 2005;**40**:331–8. https://doi.org/10.1111/j.1600-0765.2005.00807.x.

Hargrove MS, Barrick D, Olson JS The association rate constant for heme binding to globin is independent of protein structure. *Biochemistry* 1996;**35**:11293–9. https://doi.org/10.1021/bi9603711.

Hartmann A, Braun V Iron transport in *Escherichia coli*: uptake and modification of ferrichrome. *J Bacteriol* 1980;**143**:246–55. https://doi.org/10.1128/jb.143.1.246-255.1980.

He J, Miyazaki H, Anaya C *et al.* Role of *Porphyromonas gingivalis* FeoB2 in metal uptake and oxidative stress protection. *Infect Immun* 2006;**74**:4214–23. https://doi.org/10.1128/IAI.00014-06.

Heitz-Mayfield LJA Conventional diagnostic criteria for periodontal diseases (plaque-induced gingivitis and periodontitis). *Periodontol 2000* 2024;**95**:10–19. https://doi.org/10.1111/prd.12579.

Higgs PI, Larsen RA, Postle K Quantification of known components of the *Escherichia coli* TonB energy transduction system: tonB, ExbB, ExbD and FepA. *Mol Microbiol* 2002;**44**:271–8. https://doi.org/10.1046/j.1365-2958.2002.02880.x.

Hirai K, Yamaguchi-Tomikawa T, Eguchi T *et al.* Identification and modification of *Porphyromonas gingivalis* cysteine protease, gingipain, ideal for screening periodontitis. *Front Immunol* 2020;**11**:1017. https://doi.org/10.3389/fimmu.2020.01017.

Hitzler SUJ, Fernandez-Fernandez C, Montano DE *et al.* Microbial adaptive pathogenicity strategies to the host inflammatory environment. *FEMS Microbiol Rev* 2025;**49**:fuae032. https://doi.org/10.1093/femsre/fuae032.

Ho MH, Lamont RJ, Chazin WJ *et al.* Characterization and development of SAAP as a specific peptidic inhibitor that targets *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2018;**33**:430–9. https://doi.org/10.1111/omi.12246.

Ho WW, Li H, Eakanunkul S *et al.* Holo- and apo-bound structures of bacterial periplasmic heme-binding proteins. *J Biol Chem* 2007;**282**:35796–802. https://doi.org/10.1074/jbc.M706761200.

Hoare A, Wang H, Meethil A *et al.* A cross-species interaction with a symbiotic commensal enables cell-density-dependent growth and in vivo virulence of an oral pathogen. *ISME J* 2021;**15**:1490–504. https://doi.org/10.1038/s41396-020-00865-y.

Hocevar K, Potempa J, Turk B Host cell-surface proteins as substrates of gingipains, the main proteases of *Porphyromonas gingivalis*. *Biol Chem* 2018;**399**:1353–61. https://doi.org/10.1515/hsz-2018-0215.

Holt SC, Ebersole JL *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the "red complex", a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol 2000* 2005;**38**:72–122. https://doi.org/10.1111/j.1600-0757.2005.00113.x.

Hornung JM, Jones HA, Perry RD The *hmu* locus of *Yersinia pestis* is essential for utilization of free haemin and haem-protein complexes as iron sources. *Mol Microbiol* 1996;**20**:725–39. https://doi.org/10.1111/j.1365-2958.1996.tb02512.x.

Horz HP, Conrads G Diagnosis and anti-infective therapy for periodontitis. *Expert Rev Anti Infect Ther* 2007;**5**:703–15. https://doi.org/10.1586/14787210.5.4.703.

Huang N, Gibson FC 3rd. Immuno-pathogenesis of periodontal disease: current and emerging paradigms. *Curr Oral Health Rep* 2014;**1**:124–32. https://doi.org/10.1007/s40496-014-0017-8.

Huang N, Shimomura E, Yin G *et al.* Immunization with cell-free-generated vaccine protects from *Porphyromonas gingivalis*-induced alveolar bone loss. *J Clin Periodontol* 2019;**46**:197–205. https://doi.org/10.1111/jcpe.13047.

Hugoson A, Sjodin B, Norderyd O Trends over 30 years, 1973-2003, in the prevalence and severity of periodontal disease. *J Clin Periodontol* 2008;**35**:405–14. https://doi.org/10.1111/j.1600-051X.2008.01225.x.

Hyvarinen K, Laitinen S, Paju S *et al.* Detection and quantification of five major periodontal pathogens by single copy gene-based real-time PCR. *Innate Immun* 2009;**15**:195–204. https://doi.org/10.1177/1753425908101920.

Ibanez de Aldecoa AL, Zafra O, Gonzalez-Pastor JE. Mechanisms and regulation of extracellular DNA release and its biological roles in microbial communities. *Front Microbiol* 2017;**8**:1390. https://doi.org/10.3389/fmicb.2017.01390.

Imran M, Ramzan M, Qureshi AK *et al.* Emerging applications of porphyrins and metalloporphyrins in biomedicine and diagnostic magnetic resonance imaging. *Biosensors* 2018;**8**:95. https://doi.org/10.3390/bios8040095.

Inagaki S, Ishihara K, Yasaki Y *et al.* Antibody responses of periodontitis patients to gingipains of *Porphyromonas gingivalis*. *J Periodontol* 2003;**74**:1432–9. https://doi.org/10.1902/jop.2003.74.10.1432.

Irshad M, van der Reijden WA, Crielaard W *et al.* *In vitro* invasion and survival of *Porphyromonas gingivalis* in gingival fibroblasts: role of the capsule. *Arch Immun Ther Exp* 2012;**60**:469–76. https://doi.org/10.1007/s00005-012-0196-8.

Ishida Y, Hu J, Sakai E *et al.* Determination of active site of lysine-specific cysteine proteinase (Lys-gingipain) by use of a *Porphyromonas gingivalis* plasmid system. *Arch Oral Biol* 2008;**53**:538–44. https://doi.org/10.1016/j.archoralbio.2008.01.004.

Ito H, Numabe Y, Hashimoto S *et al.* Correlation between gingival crevicular fluid hemoglobin content and periodontal clinical parameters. *J Periodontol* 2016;**87**:1314–9. https://doi.org/10.1902/jop.2016.160092.

Ito H, Numabe Y, Hashimoto S *et al.* Usefulness of hemoglobin examination in gingival crevicular fluid during supportive periodontal therapy to diagnose the pre-symptomatic state in periodontal disease. *Clin Oral Investig* 2021;**25**:487–95. https://doi.org/10.1007/s00784-020-03396-0.

Ito H, Numabe Y, Hashimoto S *et al.* Utility of a haemoglobin test of gingival crevicular fluid: a multicentre, observational study. *Oral Dis* 2024;**30**:1533–42. https://doi.org/10.1111/odi.14536.

Izadi-Pruneyre N, Huche F, Lukat-Rodgers GS *et al.* The heme transfer from the soluble HasA hemophore to its membrane-bound re-

ceptor HasR is driven by protein-protein interaction from a high to a lower affinity binding site. *J Biol Chem* 2006;**281**:25541–50. https://doi.org/10.1074/jbc.M603698200.

Jacobs NJ, Jacobs JM, Brent P Characterization of the late steps of microbial heme synthesis: conversion of coproporphyrinogen to protoporphyrin. *J Bacteriol* 1971;**107**:203–9. https://doi.org/10.1128/jb.107.1.203-209.1971.

Jacobs R, Fontenele RC, Lahoud P *et al.* Radiographic diagnosis of periodontal diseases—current evidence versus innovations. *Periodontol 2000* 2024;**95**:51–69. https://doi.org/10.1111/prd.12580.

Josts I, Veith K, Normant V *et al.* Structural insights into a novel family of integral membrane siderophores reductases. *Proc Natl Acad Sci USA* 2021;**118**:e2101952118. https://doi.org/10.1073/pnas.2101952118.

Kadowaki T Enzymatic characteristics and activities of gingipains from *Porphyromonas gingivalis*. *Methods Mol Biol* 2021;**2210**:97–12. https://doi.org/10.1007/978-1-0716-0939-2_10.

Kadowaki T, Nakayama K, Yoshimura F *et al.* Arg-gingipain acts as a major processing enzyme for various cell surface proteins in *Porphyromonas gingivalis*. *J Biol Chem* 1998;**273**:29072–6. https://doi.org/10.1074/jbc.273.44.29072.

Kamal JA, Behere DV Binding of heme to human serum albumin: steady-state fluorescence, circular dichroism and optical difference spectroscopic studies. *Indian J Biochem Biophys* 2005;**42**:7–12.

Kanagasingam S, Chukkapalli SS, Welbury R *et al.* *Porphyromonas gingivalis* is a strong risk factor for Alzheimer's disease. *J Alzheimers Dis Rep* 2020;**4**:501–11. https://doi.org/10.3233/ADR-200250.

Kassebaum NJ, Bernabe E, Dahiya M *et al.* Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. *J Dent Res* 2014;**93**:1045–53. https://doi.org/10.1177/0022034514552491.

Kataoka S, Baba A, Suda Y *et al.* A novel, potent dual inhibitor of Arg-gingipain and Lys-gingipain as a promising agent for periodontal disease therapy. *FASEB J* 2014;**28**:3564–78.

Kehrer JP The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* 2000;**149**:43–50. https://doi.org/10.1016/S0300-483X(00)00231-6.

Keith AD, Sawyer EB, Choy DCY *et al.* Combining experiment and energy landscapes to explore anaerobic heme breakdown in multifunctional hemoproteins. *Phys Chem Chem Phys* 2024;**26**:695–712. https://doi.org/10.1039/D3CP03897A.

Khan AA, Quigley JG Control of intracellular heme levels: heme transporters and Heme oxygenases. *Biochim Biophys Acta* 2011;**1813**:668–82. https://doi.org/10.1016/j.bbamcr.2011.01.008.

Khurshid Z, Mali M, Naseem M *et al.* Human gingival crevicular fluids (GCF) proteomics: an overview. *Dent J* 2017;**5**:12. https://doi.org/10.3390/dj5010012.

Kiening M, Lange N A recap of heme metabolism towards understanding protoporphyrin IX selectivity in cancer cells. *Int J Mol Sci* 2022;**23**:7974. https://doi.org/10.3390/ijms23147974.

Kinane DF Causation and pathogenesis of periodontal disease. *Periodontol 2000* 2001;**25**:8–20. https://doi.org/10.1034/j.1600-0757.2001.22250102.x.

Klebba PE, Newton SMC, Six DA *et al.* Iron acquisition systems of Gram-negative bacterial pathogens define TonB-dependent pathways to novel antibiotics. *Chem Rev* 2021;**121**:5193–239. https://doi.org/10.1021/acs.chemrev.0c01005.

Kojima T, Yano K, Ishikawa I Relationship between serum antibody levels and subgingival colonization of *Porphyromonas gingivalis* in patients with various types of periodontitis. *J Periodontol* 1997;**68**:618–25. https://doi.org/10.1902/jop.1997.68.7.618.

Krewulak KD, Vogel HJ Structural biology of bacterial iron uptake. *Biochim Biophys Acta Biomembr* 2008;**1778**:1781–804. https://doi.org/10.1016/j.bbamem.2007.07.026.

Krieg S, Huche F, Diederichs K *et al.* Heme uptake across the outer membrane as revealed by crystal structures of the receptor-hemophore complex. *Proc Natl Acad Sci USA* 2009;**106**:1045–50. https://doi.org/10.1073/pnas.0809406106.

Kristiansen M, Graversen JH, Jacobsen C *et al.* Identification of the haemoglobin scavenger receptor. *Nature* 2001;**409**:198–201. https://doi.org/10.1038/35051594.

Kuboniwa M, Hasegawa Y, Mao S *et al.* *P. gingivalis* accelerates gingival epithelial cell progression through the cell cycle. *Microbes Infect* 2008;**10**:122–8. https://doi.org/10.1016/j.micinf.2007.10.011.

Kuboniwa M, Houser JR, Hendrickson EL *et al.* Metabolic crosstalk regulates *Porphyromonas gingivalis* colonization and virulence during oral polymicrobial infection. *Nat Microbiol* 2017;**2**:1493–9. https://doi.org/10.1038/s41564-017-0021-6.

Kuboniwa M, Lamont RJ Subgingival biofilm formation. *Periodontol 2000* 2010;**52**:38–52. https://doi.org/10.1111/j.1600-0757.2009.00311.x.

Kudo C, Naruishi K, Maeda H *et al.* Assessment of the plasma/serum IgG test to screen for periodontitis. *J Dent Res* 2012;**91**:1190–5. https://doi.org/10.1177/0022034512461796.

Kusaba A, Ansai T, Akifusa S *et al.* Cloning and expression of a *Porphyromonas gingivalis* gene for protoporphyrinogen oxidase by complementation of a HemG mutant of *Escherichia coli*. *Oral Microbiol Immunol* 2002;**17**:290–5. https://doi.org/10.1034/j.1399-302X.2002.170505.x.

LaMattina JW, Delrossi M, Uy KG *et al.* Anaerobic heme degradation: ChuY is an anaerobilin reductase that exhibits kinetic cooperativity. *Biochemistry* 2017;**56**:845–55. https://doi.org/10.1021/acs.biochem.6b01099.

LaMattina JW, Nix DB, Lanzilotta WN Radical new paradigm for heme degradation in *Escherichia coli* O157:H7. *Proc Natl Acad Sci USA* 2016;**113**:12138–43. https://doi.org/10.1073/pnas.1603209113.

Lamont RJ, Kuboniwa M The polymicrobial pathogenicity of *Porphyromonas gingivalis*. *Front Oral Health* 2024;**5**:1404917. https://doi.org/10.3389/froh.2024.1404917.

Lansky IB, Lukat-Rodgers GS, Block D *et al.* The cytoplasmic heme-binding protein (PhuS) from the heme uptake system of *Pseudomonas aeruginosa* is an intracellular heme-trafficking protein to the delta-regioselective heme oxygenase. *J Biol Chem* 2006;**281**:13652–62. https://doi.org/10.1074/jbc.M600824200.

Lasica AM, Ksiazek M, Madej M *et al.* The type IX secretion system (T9SS): highlights and recent insights into its structure and function. *Front Cell Infect Microbiol* 2017;**7**:215. https://doi.org/10.3389/fcimb.2017.00215.

Lau CKY, Krewulak KD, Vogel H Bacterial ferrous iron transport: the Feo system. *FEMS Microbiol Rev* 2016;**40**:273–98. https://doi.org/10.1093/femsre/fuv049.

Layer G Heme biosynthesis in prokaryotes. *Biochim Biophys Acta Mol Cell Res* 2021;**1868**:118861. https://doi.org/10.1016/j.bbamcr.2020.118861.

Lei Z, Ma Q, Tu Y *et al.* Nicotinamide employs a starvation strategy against *Porphyromonas gingivalis* virulence by inhibiting the heme uptake system and gingipain activities. *Mol Oral Microbiol* 2024;**39**:321–33. https://doi.org/10.1111/omi.12448.

Letoffe S, Heuck G, Delepelaire P *et al.* Bacteria capture iron from heme by keeping tetrapyrrole skeleton intact. *Proc Natl Acad Sci USA* 2009;**106**:11719–24. https://doi.org/10.1073/pnas.0903842106.

Lewis JP Metal uptake in host-pathogen interactions: role of iron in *Porphyromonas gingivalis* interactions with host organisms. *Periodontol 2000* 2010;**52**:94–116. https://doi.org/10.1111/j.1600-0757.2009.00329.x.

Lewis JP, Dawson JA, Hannis JC *et al.* Hemoglobinase activity of the lysine gingipain protease (Kgp) of *Porphyromonas gingivalis* W83. *J Bacteriol* 1999;**181**:4905–13. https://doi.org/10.1128/JB.181.16.4905-4913.1999.

Lewis JP, Plata K, Yu F *et al.* Transcriptional organization, regulation, and role of the *Porphyromonas gingivalis* W83 *hmu* haemin-uptake locus. *Microbiology* 2006;**152**:3367–82. https://doi.org/10.1099/mic.0.29011-0.

Lewis LA, Sung MH, Gipson M *et al.* Transport of intact porphyrin by HpuAB, the hemoglobin-haptoglobin utilization system of *Neisseria meningitidis*. *J Bacteriol* 1998;**180**:6043–7. https://doi.org/10.1128/JB.180.22.6043-6047.1998.

Li F, Ma C, Lei S *et al.* Gingipains may be one of the key virulence factors of *Porphyromonas gingivalis* to impair cognition and enhance blood-brain barrier permeability: an animal study. *J Clin Periodontol* 2024;**51**:818–39. https://doi.org/10.1111/jcpe.13966.

Li N, Collyer CA Gingipains from *Porphyromonas gingivalis*—complex domain structures confer diverse functions. *Eur J Microbiol Immunol* 2011;**1**:41–58. https://doi.org/10.1556/EuJMI.1.2011.1.7.

Li N, Yun P, Nadkarni MA *et al.* Structure determination and analysis of a hemolytic gingipain adhesin domain from *Porphyromonas gingivalis*. *Mol Microbiol* 2010;**76**:861–73. https://doi.org/10.1111/j.1365-2958.2010.07123.x.

Li P, Fung YME, Y X, Seneviratne CJ *et al.* Controlled cellular redox, repressive hemin utilization and adaptive stress responses are crucial to metronidazole tolerance of *Porphyromonas gingivalis* persisters. *J Clin Periodontol* 2018;**45**:1211–21. https://doi.org/10.1111/jcpe.13002.

Liao L, Wang Q, Feng Y *et al.* Advances and challenges in the development of periodontitis vaccine: a comprehensive review. *Int Immunopharmacol* 2024;**140**:112650. https://doi.org/10.1016/j.intimp.2024.112650.

Lippi G, Giavarina D, Gelati M *et al.* Reference range of hemolysis index in serum and lithium-heparin plasma measured with two analytical platforms in a population of unselected outpatients. *Clin Chim Acta* 2014;**429**:143–6. https://doi.org/10.1016/j.cca.2013.12.010.

Liu SC, Zhai S, Lawler J *et al.* Hemin-mediated dissociation of erythrocyte membrane skeletal proteins. *J Biol Chem* 1985;**260**:12234–9. https://doi.org/10.1016/S0021-9258(17)39015-4.

Liu X, Olczak T, Guo HC *et al.* Identification of amino acid residues involved in heme binding and hemoprotein utilization in the *Porphyromonas gingivalis* heme receptor HmuR. *Infect Immun* 2006;**74**:1222–32. https://doi.org/10.1128/IAI.74.2.1222-1232.2006.

Locher KP, Rees B, Koebnik R *et al.* Transmembrane signaling across the ligand-gated FhuA receptor: crystal structures of free and ferrichrome-bound states reveal allosteric changes. *Cell* 1998;**95**:771–8. https://doi.org/10.1016/S0092-8674(00)81700-6.

Lofmark S, Edlund C, Nord CE Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis* 2010;**50**:S16–23. https://doi.org/10.1086/647939.

Lu B, McBride BC Expression of the *tpr* protease gene of *Porphyromonas gingivalis* is regulated by peptide nutrients. *Infect Immun* 1998;**66**:5147–56. https://doi.org/10.1128/IAI.66.11.5147-5156.1998.

Lu Z, Cao R, Geng F *et al.* Persistent infection with *Porphyromonas gingivalis* increases the tumorigenic potential of human immortalized oral epithelial cells through ZFP36 inhibition. *Cell Prolif* 2024;**57**:e13609. https://doi.org/10.1111/cpr.13609.

Luscher A, Moynie L, Saint AP *et al.* TonB-dependent receptor repertoire of *Pseudomonas aeruginosa* for uptake of siderophore-drug conjugates. *Antimicrob Agents Chemother* 2018;**62**:e00097–18. https://doi.org/10.1128/AAC.00097-18.

Lyles KV, Eichenbaum Z From host heme to iron: the expanding spectrum of heme degrading enzymes used by pathogenic bacteria. *Front Cell Infect Microbiol* 2018;**8**:198. https://doi.org/10.3389/fcimb.2018.00198.

Makela M, Soderling E, Paunio K *et al.* Protein composition of crevicular fluid before and after treatment. *Scand J Dent Res* 1991;**99**:413–23. https://doi.org/10.1111/j.1600-0722.1991.tb01049.x.

Manoil D, Parga A, Bostanci N *et al.* Microbial diagnostics in periodontal diseases. *Periodontol 2000* 2024;**95**:176–93. https://doi.org/10.1111/prd.12571.

Mao S, Park Y, Hasegawa Y *et al.* Intrinsic apoptotic pathways of gingival epithelial cells modulated by *Porphyromonas gingivalis*. *Cell Microbiol* 2007;**9**:1997–2007. https://doi.org/10.1111/j.1462-5822.2007.00931.x.

Mark Welch JL, Rossetti BJ, Rieken CW *et al.* Biogeography of a human oral microbiome at the micron scale. *Proc Natl Acad Sci USA* 2016;**113**:E791–800. https://doi.org/10.1073/pnas.1522149113.

Marsh PD, McDermid AS, McKee AS *et al.* The effect of growth rate and haemin on the virulence and proteolytic activity of *Porphyromonas gingivalis* W50. *Microbiology* 1994;**140**:861–5. https://doi.org/10.1099/00221287-140-4-861.

Marsh PD, Moter A, Devine DA Dental plaque biofilms: communities, conflict and control. *Periodontol 2000* 2011;**55**:16–35. https://doi.org/10.1111/j.1600-0757.2009.00339.x.

Massarenti L, Nielsen CH, Danielsen AK *et al.* Evaluation of circulating IgG antibodies against *Porphyromonas gingivalis* or its gingipains as serological markers of periodontitis and carriage of the bacterium. *J Periodontol* 2024;**96**:119–28. https://doi.org/10.1002/JPER.23-0766.

Mathew LG, Beattie NR, Pritchett C *et al.* New insight into the mechanism of anaerobic heme degradation. *Biochemistry* 2019;**58**:4641–54. https://doi.org/10.1021/acs.biochem.9b00841.

Matsui T, Unno M, Ikeda-Saito M Heme oxygenase reveals its strategy for catalyzing three successive oxygenation reactions. *Acc Chem Res* 2010;**43**:240–7. https://doi.org/10.1021/ar9001685.

McDermid AS, McKee AS, Marsh PD Effect of environmental pH on enzyme activity and growth of *Bacteroides gingivalis* W50. *Infect Immun* 1998;**56**:1096–100. https://doi.org/10.1128/iai.56.5.1096-1100.1988.

McKee AS, McDermid AS, Baskerville A *et al.* Effect of hemin on the physiology and virulence of *Bacteroides gingivalis* W50. *Infect Immun* 1986;**52**:349–55. https://doi.org/10.1128/iai.52.2.349-355.1986.

Mei F, Xie M, Huang X *et al.* *Porphyromonas gingivalis* and its systemic impact: current status. *Pathogens* 2020;**9**:944. https://doi.org/10.3390/pathogens9110944.

Mey AR, Payne SM Haem utilization in *Vibrio cholerae* involves multiple TonB-dependent haem receptors. *Mol Microbiol* 2001;**42**:835–49. https://doi.org/10.1046/j.1365-2958.2001.02683.x.

Miller DP, Scott DA Inherently and conditionally essential protein catabolism genes of *Porphyromonas gingivalis*. *Trends Microbiol* 2021;**29**:54–64. https://doi.org/10.1016/j.tim.2020.09.002.

Miller YI, Shaklai N Kinetics of hemin distribution in plasma reveals its role in lipoprotein oxidation. *Biochim Biophys Acta Mol Bases of Dis* 1999;**1454**:153–64. https://doi.org/10.1016/S0925-4439(99)00027-7.

Milner P, Batten JE, Curtis MA Development of a simple chemically defined medium for *Porphyromonas gingivalis*: requirement for alpha ketoglutarate. *FEMS Microbiol Lett* 1996;**140**:125–30. https://doi.org/10.1016/0378-1097(96)00159-0.

Mingers T, Barthels S, Mass V *et al.* The alternative coproporphyrinogen III oxidase (CgoN) catalyzes the oxygen-independent conversion of coproporphyrinogen III into coproporphyrin III. *Front Microbiol* 2024;**15**:1378989. https://doi.org/10.3389/fmicb.2024.1378989.

Mizgalska D, Rodriguez-Banqueri A, Veillard F *et al.* Structural and functional insights into the C-terminal signal domain of the Bacteroidetes type-IX secretion system. *Open Biol* 2024;**14**:230448. https://doi.org/10.1098/rsob.230448.

Molares-Olavarria M, Nunez-Belmar J, Gonzalez D *et al.* Phylogenomic analysis of the *Porphyromonas gingivalis-Porphyromonas gulae* duo: approaches to the origin of periodontitis. *Front Microbiol* 2023;**14**:1226166. https://doi.org/10.3389/fmicb.2023.1226166.

Moraes TF, Yu RH, Strynnadka NC, Schryvers AB Insights into the bacterial transferrin receptor: the structure of transferrin-binding protein B from *Actinobacillus pleuropneumoniae*. *Mol Cell* 2009;**35**:523–33. https://doi.org/10.1016/j.molcel.2009.06.029.

Morgan WT The binding and transport of heme by hemopexin. *Ann Clin Res* 1976;**8**:223–32.

Morgan WT, Liem HH, Sutor RP *et al.* Transfer of heme from heme-albumin to hemopexin. *Biochim Biophys Acta* 1976;**444**:435–45. https://doi.org/10.1016/0304-4165(76)90387-1.

Muller-Eberhard U, Javid J, Liem HH *et al.* Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. *Blood* 1968;**32**:811–5. https://doi.org/10.1182/blood.V32.5.811.811.

Muller-Eberhard U, Morgan WT Porphyrin-binding proteins in serum. *Ann N Y Acad Sci* 1975;**244**:624–49. https://doi.org/10.1111/j.1749-6632.1975.tb41558.x.

Nakayama K *Porphyromonas gingivalis* cell-induced hemagglutinaion and platelet aggregation. *Periodontol 2000* 2010;**54**:45–52. https://doi.org/10.1111/j.1600-0757.2010.00351.x.

Nakayama K, Ratnayake DB, Tsukuba T *et al.* Haemoglobin receptor protein is intragenically encoded by the cysteine proteinase-encoding genes and the haemagglutinin-encoding gene of *Porphyromonas gingivalis*. *Mol Microbiol* 1998;**27**:51–61. https://doi.org/10.1046/j.1365-2958.1998.00656.x.

Nascimento GG, Alves-Costa S, Romandini M Burden of severe periodontitis and edentulism in 2021, with projections up to 2050: the Global Burden of Disease 2021 Study. *J Periodontal Res* 2024;**59**:823–67. https://doi.org/10.1111/jre.13337.

Nelson KE, Fleischmann RD, DeBoy RT *et al.* Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J Bacteriol* 2003;**185**:5591–601. https://doi.org/10.1128/JB.185.18.5591-5601.2003.

Nemoto TK, Ohara-Nemoto Y Dipeptidyl-peptidases: key enzymes producing entry forms of extracellular proteins in asaccharolytic periodontopathic bacterium *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2021;**36**:145–56. https://doi.org/10.1111/omi.12317.

Nemoto TK, Ohara-Nemoto Y Exopeptidases and gingipains in *Porphyromonas gingivalis* as prerequisites for its amino acid metabolism. *Jpn Dent Sci Rev* 2016;**52**:22–29. https://doi.org/10.1016/j.jdsr.2015.08.002.

Ng E, Tay JRH, Boey SK *et al.* Antibiotic resistance in the microbiota of periodontitis patients: an update of current findings. *Crit Rev Microbiol* 2023;**50**:329–40. https://doi.org/10.1080/1040841X.2023.2197481.

Nguyen KA, DeCarlo AA, Paramaesvaran M *et al.* Human responses to *Porphyromonas gingivalis* gingipain adhesin domains in subjects with chronic periodontitis. *Infect Immun* 2004;**72**:1374–82. https://doi.org/10.1128/IAI.72.3.1374-1382.2004.

Nhien NTT, Huy NT, Naito M *et al.* Neutralization of toxic haem by *Porphyromonas gingivalis* haemoglobin receptor. *J Biochem* 2010;**147**:317–25. https://doi.org/10.1093/jb/mvp164.

Nicolau SA, Fast AG, Nakamaru-Ogiso E *et al.* Overexpression of *fetA* (*ybbL*) and *fetB* (*ybbM*), encoding an iron exporter, enhances resistance to oxidative stress in *Escherichia coli*. *Appl Environ Microbiol* 2013;**79**:7210–9. https://doi.org/10.1128/AEM.02322-13.

Nobre dos Santos-Lima EK, Cardoso KAPA, de Miranda PM *et al.* Novel synthetic peptide derived from *Porphyromonas gingivalis* Lys-gingipain detects IgG-mediated host response in periodontitis. *Anaerobe* 2020;**61**:102140. https://doi.org/10.1016/j.anaerobe.2019.102140.

Noinaj N, Buchanan SK, Cornelissen CN The transferrin-iron import system from pathogenic *Neisseria* species. *Mol Microbiol* 2012;**86**:246–57. https://doi.org/10.1111/mmi.12002.

Noinaj N, Cornelissen CN, Buchanan SK Structural insight into the lactoferrin receptors from pathogenic *Neisseria*. *J Struct Biol* 2013;**184**:83–92. https://doi.org/10.1016/j.jsb.2013.02.009.

Noinaj N, Guiller M, Barnard TJ *et al.* TonB-dependent transporters: regulation, structure, and function. *Annu Rev Microbiol* 2010;**64**:43–60. https://doi.org/10.1146/annurev.micro.112408.134247.

Nonaka S, Kadowaki T, Nakanishi H Secreted gingipains from *Porphyromonas gingivalis* increase permeability in human cerebral microvascular endothelial cells through intracellular degradation of tight junction proteins. *Neurochem Int* 2022;**154**:105282. https://doi.org/10.1016/j.neuint.2022.105282.

O'Brien-Simpson NM, Black CL, Bhogal PS *et al.* Serum IgG and IgG subclass responses to the RgpA-Kgp proteinase-adhesin complex of *P. gingivalis* in adult periodontitis. *Infect Immun* 2000a;**68**:2704–12. https://doi.org/10.1128/IAI.68.5.2704-2712.2000.

O'Brien-Simpson NM, Paolini R, Reynolds EC RgpA-Kgp peptide-base immunogens provide protection against *Porphyromonas gingivalis* challenge in a murine lesion model. *Infect Immun* 2000b;**68**:4055–63. https://doi.org/10.1128/IAI.68.7.4055-4063.2000.

O'Brien-Simpson NM, Paolini RA, Hoffmann B *et al.* Role of RgpA, RgpB, and Kgp proteinases in virulence of *Porphyromonas gingivalis* W50 in a murine lesion model. *Infect Immun* 2001;**69**:7527–34. https://doi.org/10.1128/IAI.69.12.7527-7534.2001.

Ohara-Nemoto Y, Shinoyama Y, Kimura S *et al.* Asp- and Glu-specific novel dipeptidyl peptidase 11 of *Porphyromonas gingivalis* that ensures utilization of proteinaceous energy sources. *J Biol Chem* 2011;**286**:38115–27. https://doi.org/10.1074/jbc.M111.278572.

Ohya M, Cueno ME, Tamura M *et al.* Varying hemin concentrations affect *Porphyromonas gingivalis* strains differently. *Microb Pathog* 2016;**94**:54–9. https://doi.org/10.1016/j.micpath.2015.10.016.

Okamoto K, Nakayama K, Kadowaki T *et al.* Involvement of a lysine-specific cysteine proteinase in hemoglobin adsorption and heme accumulation by *Porphyromonas gingivalis*. *J Biol Chem* 1998;**273**:21225–31. https://doi.org/10.1074/jbc.273.33.21225.

Olczak T Analysis of conserved glutamate residues in *Porphyromonas gingivalis* outer membrane receptor HmuR: toward a further understanding of heme uptake. *Arch Microbiol* 2006;**186**:393–402. https://doi.org/10.1007/s00203-006-0151-3.

Olczak T, Dixon DW, Genco CA Binding specificity of the *Porphyromonas gingivalis* heme and hemoglobin receptor HmuR, gingipain K, and gingipain R1 for heme, porphyrins, and metalloporphyrins. *J Bacteriol* 2001;**183**:5599–608. https://doi.org/10.1128/JB.183.19.5599-5608.2001.

Olczak T, Maszczak-Seneczko D, Smalley JW *et al.* Gallium(III), cobalt(III) and copper(II) protoporphyrin IX exhibit antimicro-

bial activity against *Porphyromonas gingivalis* by reducing planktonic and biofilm growth and invasion of host epithelial cells. *Arch Microbiol* 2012;**194**:719–24. https://doi.org/10.1007/s00203-012-0804-3.

Olczak T, Simpson W, Liu X *et al.* Iron and heme utilization in *Porphyromonas gingivalis*. *FEMS Microbiol Rev* 2005;**29**:119–44. https://doi.org/10.1016/j.femsre.2004.09.001.

Olczak T, Smiga M, Antonyuk SV *et al.* Hemophore-like proteins of the HmuY family in the oral and gut microbiome: unraveling the mystery of their evolution. *Microbiol Mol Biol Rev* 2024;**88**:e0013123. https://doi.org/10.1128/mmbr.00131-23.

Olczak T, Sosicka P, Olczak M HmuY is an important virulence factor for *Porphyromonas gingivalis* growth in the heme-limited host environment and infection of macrophages. *Biochem Biophys Res Commun* 2015;**467**:748–53. https://doi.org/10.1016/j.bbrc.2015.10.070.

Olczak T, Sroka A, Potempa J *et al.* *Porphyromonas gingivalis* HmuY and HmuR: further characterization of a novel mechanism of heme utilization. *Arch Microbiol* 2008;**189**:197–210. https://doi.org/10.1007/s00203-007-0309-7.

Olczak T, Wojtowicz H, Ciuraszkiewicz J *et al.* Species specificity, surface exposure, protein expression, immunogenicity, and participation in biofilm formation of *Porphyromonas gingivalis* HmuY. *BMC Microbiol* 2010;**10**:134. https://doi.org/10.1186/1471-2180-10-134.

Olsen I, Lambris JD, Hajishengallis G *Porphyromonas gingivalis* disturbs host–commensal homeostasis by changing complement function. *J Oral Microbiol* 2017;**9**:1340085. https://doi.org/10.1080/20002297.2017.1340085.

Olsen I, Yilmaz O Modulation of inflammasome activity by *Porphyromonas gingivalis* in periodontitis and associated systemic diseases. *J Oral Microbiol* 2016;**8**:30385. https://doi.org/10.3402/jom.v8.30385.

Onzuka M, Sekine Y, Uchida T *et al.* HmuS from *Yersinia pseudotuberculosis* is a non-canonical heme-degrading enzyme to acquire iron from heme. *Biochim Biophys Acta* 2017;**1861**:1870–978. https://doi.org/10.1016/j.bbagen.2017.04.003.

Ostan NKH, Moraes TF, Schryvers AB Lactoferrin receptors in Gram-negative bacteria: an evolutionary perspective. *Biochem Cell Biol* 2021;**99**:102–8. https://doi.org/10.1139/bcb-2020-0079.

Otogoto J, Kuramitsu HK Isolation and characterization of the *Porphyromonas gingivalis prtT* gene, coding for protease activity. *Infect Immun* 1993;**61**:117–23. https://doi.org/10.1128/iai.61.1.117-123.1993.

Page RC, Schroeder HE Pathogenesis of inflammatory periodontal disease: a summary of current work. *Lab Invest* 1976;**34**:235–49.

Paillat M, Silva IL, Cacales E *et al.* A journey with type IX secretion system effectors: selection, transport, processing and activities. *Microbiology* 2023;**169**:0011320. https://doi.org/10.1099/mic.0.001320.

Pan C, Liu J, Wang H *et al.* *Porphyromonas gingivalis* can invade periodontal ligament stem cells. *BMC Microbiol* 2017;**17**:38. https://doi.org/10.1186/s12866-017-0950-5.

Paoli M, Anderson BF, Baker HM *et al.* Crystal structure of hemopexin reveals a novel high-affinity heme site formed between two β propeller domains. *Nat Struct Biol* 1999;**6**:926–31. https://doi.org/10.1038/13294.

Paramaesvaran M, Nguyen KA, Caldon E *et al.* Porphyrin-mediated cell surface heme capture from hemoglobin by *Porphyromonas gingivalis*. *J Bacteriol* 2003;**185**:2528–37. https://doi.org/10.1128/JB.185.8.2528-2537.2003.

Parwani SR, Parwani RN Nitric oxide and inflammatory periodontal disease. *Gen Dent* 2015;**63**:34–40.

Pathirana RD, O'Brien-Simpson NM, Veith PD *et al.* Characterization of proteinase-adhesin complexes of *Porphyromonas gingivalis*. *Microbiology* 2006;**152**:2381–94. https://doi.org/10.1099/mic.0.28787-0.

Pavloff N, Pemberton PA, Potempa J *et al.* Molecular cloning and characterization of *Porphyromonas gingivalis* lysine-specific gingipain. A new member of an emerging family of pathogenic bacterial cysteine proteinases. *J Biol Chem* 1997;**272**:1595–600. https://doi.org/10.1074/jbc.272.3.1595.

Pavloff N, Potempa J, Pike RN *et al.* Molecular cloning and structural characterization of the Arg-gingipain proteinase of *Porphyromonas gingivalis*. Biosynthesis as a proteinase-adhesin polyprotein. *J Biol Chem* 1995;**270**:1007–10. https://doi.org/10.1074/jbc.270.3.1007.

Pawelek PD, Croteau N, Ng-Thow-Hing C *et al.* Structure of TonB in complex with FhuA, *E. coli* outer membrane receptor. *Science* 2006;**312**:1399–402. https://doi.org/10.1126/science.1128057.

Perkins-Balding D, Ratliff-Griffin M, Stojiljkovic I Iron transport systems in *Neisseria meningitidis*. *Microbiol Mol Biol Rev* 2004;**68**:154–71. https://doi.org/10.1128/MMBR.68.1.154-171.2004.

Perry JA, Jones MB, Peterson SN *et al.* Peptide alarmone signalling triggers an auto-active bacteriocin necessary for genetic competence. *Mol Microbiol* 2009;**72**:905–17. https://doi.org/10.1111/j.1365-2958.2009.06693.x.

Persson GR, Engel D, Whitney G *et al.* Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in non-humate primates. *Infect Immun* 1994;**62**:1026–31. https://doi.org/10.1128/iai.62.3.1026-1031.1994.

Pettersen EF, Goddard TD, Huang CC *et al.* UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 2004;**25**:1605–12. https://doi.org/10.1002/jcc.20084.

Philstrom BL, Michalowicz BS, Johnson NW Periodontal diseases. *Lancet* 2005;**366**:809–1820. https://doi.org/10.1016/S0140-6736(05)67728-8.

Pike R, McGraw W, Potempa J *et al.* Lysine- and arginine-specific proteinases from *Porphyromonas gingivalis*. Isolation, characterization, and evidence for the existence of complexes with haemagglutinins. *J Biol Chem* 1994;**269**:406–11. https://doi.org/10.1016/S0021-9258(17)42365-9.

Plaza K, Kalinska M, Bochenska O *et al.* Gingipains of *Porphyromonas gingivalis* affect the stability and function of serine protease inhibitor of Kazal-type 6 (SPINK6), a tissue inhibitor of human kallikreins. *J Biol Chem* 2016;**291**:18753–64. https://doi.org/10.1074/jbc.M116.722942.

Pogoutse AK, Moraes T Iron acquisition through the bacterial transferrin receptor. *Crit Rev Biochem Mol Biol* 2017;**52**:314–26. https://doi.org/10.1080/10409238.2017.1293606.

Posey JE, Gherardini FC Lack of a role for iron in the Lyme disease pathogen. *Science* 2000;**288**:1651–3. https://doi.org/10.1126/science.288.5471.1651.

Potempa J, Madej M, Scott DA The RagA and RagB proteins of *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2021;**36**:225–32. https://doi.org/10.1111/omi.12345.

Potempa J, Sroka A, Imamura T *et al.* Gingipains, the major cysteine proteinases and virulence factors of *Porphyromonas gingivalis*: structure, function and assembly of multidomain protein complexes. *Curr Protein Pept Sci* 2003;**4**:397–407. https://doi.org/10.2174/1389203033487036.

Rajapakse PS, O'Brien-Simpson NM, Slakeski N *et al.* Immunization with the RgpA-Kgp proteinase-adhesin complexes of *Porphyromonas gingivalis* protects against periodontal bone loss in the rat periodontitis model. *Infect Immun* 2002;**70**:2480–6. https://doi.org/10.1128/IAI.70.5.2480-2486.2002.

Rams TE, Sautter JD, van Winkelhoff AJ Emergence of antibiotic-resistant *Porphyromonas gingivalis* in United States periodontitis patients. *Antibiotics* 2023;**12**:1584. https://doi.org/10.3390/antibiotics12111584.

Ramseier CA Diagnostic measures for monitoring and follow-up in periodontology and implant dentistry. *Periodontol 2000* 2024;**95**:129–55. https://doi.org/10.1111/prd.12588.

Ratnayake DB, Wai SN, Shi Y *et al.* Ferritin from the obligate anaerobe *Porphyromonas gingivalis*: purification, gene cloning and mutant studies. *Microbiology* 2000;**146**:1119–27. https://doi.org/10.1099/00221287-146-5-1119.

Reher VGS, Zenobio EG, Costa FO *et al.* Nitric oxide levels in saliva increase with severity of chronic periodontitis. *J Oral Sci* 2007;**49**:271–6. https://doi.org/10.2334/josnusd.49.271.

Richard KL, Kelley BR, Johnson JG Heme uptake and utilization by Gram-negative bacterial pathogens. *Front Cell Infect Microbiol* 2019;**9**:81. https://doi.org/10.3389/fcimb.2019.00081.

Richardson AR, Stojiljkovic I HmbR, a hemoglobin-binding outer membrane protein of *Neisseria meningitidis*, undergoes phase variation. *J Bacteriol* 1999;**181**:2067–74. https://doi.org/10.1128/JB.181.7.2067-2074.1999.

Rocha ER, Bergonia HA, Gerdes S *et al. Bacteroides fragilis* requires the ferrous-iron transporter FeoAB and the CobN-like proteins BtuS1 and BtuS2 for assimilation of iron released from heme. *Microbiologyopen* 2019;**8**:e00669. https://doi.org/10.1002/mbo3.669.

Rocha ER, Krykunivsky AS Anaerobic utilization of Fe(III)-xenosiderophores among *Bacteroides* species and the distinct assimilation of Fe(III)-ferrichrome by *Bacteroides fragilis* within the genus. *MicrobiologyOpen* 2017;**l6**:e00479. https://doi.org/10.1002/mbo3.479.

Roper JM, Raux E, Brindley AA *et al.* The enigma of cobalamin (Vitamin B12) biosynthesis in *Porphyromonas gingvalis*. Identification and characterization of a functional corrin pathway. *J Biol Chem* 2000;**275**:40316–23. https://doi.org/10.1074/jbc.M007146200.

Rosan B, Lamont RJ Dental plaque formation. *Microbes Infect* 2000;**2**:1599–607. https://doi.org/10.1016/S1286-4579(00)01316-2.

Sabbagh MN, Decourt B COR388 (atuzaginstat): an investigational gingipain inhibitor for the treatment of Alzheimer's disease. *Expert Opin Investig Drugs* 2022;**31**:987–93. https://doi.org/10.1080/13543784.2022.2117605.

Sachar M, Anderson KE, Ma X Protoporphyrin IX: the good, the bad, and the ugly. *J Pharmacol Exp Ther* 2016;**356**:267–75. https://doi.org/10.1124/jpet.115.228130.

Sakai E, Naito M, Sato K *et al.* Construction of recombinant hemagglutinin derived from the gingipain-encoding gene of *Porphyromonas gingivalis*, identification of its target protein on erythrocytes, and inhibition of hemagglutination by an interdomain regional peptide. *J Bacterial* 2007;**189**:3977–86. https://doi.org/10.1128/JB.01691-06.

Sakanaka A, Takeuchi H, Kuboniwa M *et al.* Dual lifestyle of *Porphyromonas gingivalis* in biofilm and gingival cells. *Microb Pathog* 2016;**94**:42–47. https://doi.org/10.1016/j.micpath.2015.10.003.

Schincaglia GP, Hong BY, Rosania A *et al.* Clinical, immune, and microbiome traits of gingivitis and peri-implant mucositis. *J Dent Res* 2017;**96**:47–55. https://doi.org/10.1177/0022034516668847.

Schneider S, Paoli M Crystallization and preliminary X-ray diffraction analysis of the haem-binding protein HemS from *Yersinia enterocolitica*. *Acta Crystallogr Sec F Struct Biol Cryst Commun* 2005;**61**:802–5. https://doi.org/10.1107/S1744309105023523.

Schneider S, Sharp KH, Barker PD *et al.* An induced fit conformational change underlies the binding mechanism of the heme transport proteobacteria-protein HemS. *J Biol Chem* 2006;**281**:32606–10. https://doi.org/10.1074/jbc.M607516200.

Scott DA, Krauss J Neutrophils in periodontal inflammation. *Front Oral Biol* 2012;**15**:56–83. https://doi.org/10.1159/000329672.

Scott JC, Klein BA, Duran-Pinedo A *et al.* A two-component system regulates hemin acquisition in *Porphyromonas gingivalis*. *PLoS One* 2013;**8**:e73351. https://doi.org/10.1371/journal.pone.0073351.

Seers CA, Mahmud ASM, Huq NL *et al. Porphyromonas gingivalis* laboratory strains and clinical isolates exhibit different distribution of cell surface and secreted gingipains. *J Oral Microbiol* 2020;**13**:1858001. https://doi.org/10.1080/20002297.2020.1858001.

Seers CA, Slakeski N, Veith PD *et al.* The RgpB C-terminal domain has a role in attachment of RgpB to the outer membrane and belongs to a novel C-terminal-domain family found in *Porphyromonas gingivalis*. *J Bacteriol* 2006;**188**:6376–86. https://doi.org/10.1128/JB.00731-06.

Sheldon JR, Laakso HA, Heinrichs DE Iron acquisition strategies of bacterial pathogens. *Microbiol Spectr* 2016;**4**. https://doi.org/10.1128/microbiolspec.VMBF-0010-2015.

Shi Y, Ratnayake DB, Okamoto K *et al.* Genetic analyses of proteolysis, hemoglobin binding, and hemagglutination of *Porphyromonas gingivalis*. Construction of mutants with a combination of *rgpA*, *rgpB*, *kgp*, and *hagA*. *J Biol Chem* 1999;**274**:17955–60. https://doi.org/10.1074/jbc.274.25.17955.

Shimoyama Y, Sasaki D, Ohara-Nemoto Y *et al.* Immunoelectron microscopic analysis of dipeptidyl-peptidases and dipeptide transporter involved in nutrient acquisition in *Porphyromonas gingivalis*. *Curr Microbiol* 2023;**80**:106. https://doi.org/10.1007/s00284-023-03212-4.

Shizukuishi S, Tazaki K, Inoshita E *et al.* Effect of concentration of compounds containing iron on the growth of *Porphyromonas gingivalis*. *FEMS Microbiol Lett* 1995;**131**:313–7. https://doi.org/10.1111/j.1574-6968.1995.tb07793.x.

Shoji M, Sato K, Yukitake H *et al.* Por secretion system-dependent secretion and glycosylation of *Porphyromonas gingivalis* hemin-binding protein 35. *PLoS One* 2011;**6**:e21372. https://doi.org/10.1371/journal.pone.0021372.

Siddiqui R, Badran Z, Boghossian A *et al.* The increasing importance of the oral microbiome in periodontal health and disease. *Future Sci OA* 2023;**9**:FSO856. https://doi.org/10.2144/fsoa-2023-0062.

Sieminska K, Cierpisz P, Smiga M *et al. Porphyromonas gingivalis* HmuY and *Bacteroides vulgatus* Bvu—a novel competitive heme acquisition strategy. *Int J Mol Sci* 2021;**22**:2237. https://doi.org/10.3390/ijms22052237.

Silale A, van den Berg B TonB-dependent transport across the bacterial outer membrane. *Annu Rev Microbiol* 2023;**77**:67–88. https://doi.org/10.1146/annurev-micro-032421-111116.

Slezak P, Smiga M, Smalley JW *et al. Porphyromonas gingivalis* HmuY and *Streptococcus gordonii* GAPDH—novel heme acquisition strategy in the oral microbiome. *Int J Mol Sci* 2020;**21**:4150. https://doi.org/10.3390/ijms21114150.

Smalley JW, Birss AJ Iron protoporphyrin IX-albumin complexing increases the capacity and avidity of its binding to the periodontopathogen *Porphyromonas gingivalis*. *Microb Pathog* 1999;**26**:131–7. https://doi.org/10.1006/mpat.1998.0259.

Smalley JW, Birss AJ, Silver J The periodontal pathogen *Porphyromonas gingivalis* harnesses the chemistry of the mu-oxo bishaem of iron protoporphyrin IX to protect against hydrogen peroxide. *FEMS Microbiol Lett* 2000;**183**:159–64. https://doi.org/10.1111/j.1574-6968.2000.tb08951.x.

Smalley JW, Birss AJ, Szmigielski B *et al.* Mechanism of methaemoglobin breakdown by the lysine-specific gingipain of the periodontal pathogen *Porphyromonas gingivalis*. *Biol Chem* 2008;**389**:1235–8. https://doi.org/10.1515/BC.2008.140.

Smalley JW, Birss AJ, Szmigielski B *et al.* Sequential action of R and K-specific gingipains of *Porphyromonas gingivalis* in the generation of the haem containing pigment from oxyhaemoglobin. *Arch Biochem Biophys* 2007;**465**:44–49. https://doi.org/10.1016/j.abb.2007.05.011.

Smalley JW, Birss AJ, Szmigielski B *et al.* The HA2 haemagglutinin domain of the lysine-specific gingipain (Kgp) of *Porphyromonas gingivalis* promotes μ-oxo bishaem formation from monomers iron(III) protoporphyrin IX. *Microbiology* 2006;**152**:1839–45. https://doi.org/10.1099/mic.0.28835-0.

Smalley JW, Byrne DP, Birss AJ *et al.* HmuY haemophore and gingipain proteases constitute a unique synthrophic system of haem acquisition by *Porphyromonas gingivalis*. *PLoS One* 2011;**6**:e17182. https://doi.org/10.1371/journal.pone.0017182.

Smalley JW, Olczak T Heme acquisition mechanisms of *Porphyromonas gingivalis*—strategies used in polymicrobial community in a heme-limited host environment. *Mol Oral Microbiol* 2017;**32**:1–23. https://doi.org/10.1111/omi.12149.

Smalley JW, Silver J, Marsh PJ *et al.* The periodontopathogen *Porphyromonas gingivalis* binds iron protoporphyrin IX in the mu-oxo dimeric form: an oxidative buffer and possible pathogenic mechanism. *Biochem J* 1998;**331**:681–5. https://doi.org/10.1042/bj3310681.

Smalley JW, Thomas MF, Birss AJ *et al.* A combination of both arginine- and lysine-specific gingipain activity of *Porphyromonas gingivalis* is necessary for the generation of the micro-oxo bishaem-containing pigment from haemoglobin. *Biochem J* 2004;**379**:833–40. https://doi.org/10.1042/bj20031221.

Smiga M, Bielecki M, Olczak M *et al.* Anti-HmuY antibodies specifically recognize *Porphyromonas gingivalis* HmuY protein but not homologous proteins in other periodontopathogens. *PLoS One* 2015;**10**:e0117508. https://doi.org/10.1371/journal.pone.0117508.

Smiga M, Stepien P, Olczak M *et al.* PgFur participates differentially in expression of virulence factors in more virulent A7436 and less virulent ATCC 33277 *Porphyromonas gingivalis* strains. *BMC Microbiol* 2019a;**19**:127. https://doi.org/10.1186/s12866-019-1511-x.

Smiga M, Bielecki M, Olczak M *et al. Porphyromonas gingivalis* PgFur is a member of a novel Fur subfamily with non-canonical function. *Front Cell Infect Microbiol* 2019b;**9**:233. https://doi.org/10.3389/fcimb.2019.00233.

Smiga M, Olczak T HmuY proteins of the *Porphyromonas* genus show diversity in heme-binding properties. *Front Cell Infect Microbiol* 2025;**15**:1560779. https://doi.org/10.3389/fcimb.2025.1560779

Smiga M, Olczak T PgRsp is a novel redox-sensing transcription regulator essential for *Porphyromonas gingivalis* virulence. *Microorganisms* 2019;**7**:623. https://doi.org/10.3390/microorganisms7120623.

Smiga M, Olczak T *Porphyromonas endodontalis* HmuY differentially participates in heme acquisition compared to the *Porphyromonas gingivalis* and *Tannerella forsythia* hemophore-like proteins. *Front Cell Infect Microbiol* 2024;**14**:1421018. https://doi.org/10.3389/fcimb.2024.1421018.

Smiga M, Slezak P, Wagner M *et al.* Interplay between *Porphyromonas gingivalis* hemophore-like protein HmuY and Kgp/RgpA gingipains plays a superior role in heme supply. *Microbiol Spectr* 2023a;**11**:e0459322. https://doi.org/10.1128/spectrum.04593-22.

Smiga M, Sieminska K, Trindade SC *et al.* Hemophore-like proteins produced by periodontopathogens are recognized by the host immune system and react differentially with IgG antibodies. *J Oral Microbiol* 2023b;**15**:2214455. https://doi.org/10.1080/20002297.2023.2214455.

Smiga M, Slezak P, Tracz M *et al.* Defining the role of Hmu and Hus systems in *Porphyromonas gingivalis* heme and iron homeostasis and virulence. *Sci Rep* 2024a;**14**:31156. https://doi.org/10.1038/s41598-024-82326-6.

Smiga M, Slezak P, Olczak T Comparative analysis of *Porphyromonas gingivalis* A7436 and ATCC 33277 strains reveals differences in the expression of heme acquisition systems. *Microbiol Spectr* 2024b;**12**:e0286523. https://doi.org/10.1128/spectrum.02865-23.

Smiga M, Smalley JW, Slezak P *et al.* Glycation of host proteins increases pathogenic potential of *Porphyromonas gingivalis*. *Int J Mol Sci* 2021;**22**:12084. https://doi.org/10.3390/ijms222112084.

Socransky SS, Haffajee AD, Cugini MA *et al.* Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;**25**:134–44. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x.

Soukos NS, Soma S, Abernethy AD *et al.* Phototargeting oral black-pigmented bacteria. *Antimicrob Agents Chemother* 2005;**49**:1391–6. https://doi.org/10.1128/AAC.49.4.1391-1396.2005.

Sroka A, Sztukowska M, Potempa J *et al.* Degradation of host heme proteins by lysine- and arginine-specific cysteine proteinases (gingipains) of *Porphyromonas gingivalis J Bacteriol* 2001;**183**:5609–16. https://doi.org/10.1128/JB.183.19.5609-5616.2001.

Stauff DL, Bagaley D, Torres VJ *et al. Staphylococcus aureus* HrtA is an ATPase required for protection against heme toxicity and prevention of a transcriptional heme stress response. *J Bacteriol* 2008;**190**:3588–96. https://doi.org/10.1128/JB.01921-07.

Stinzi A, Barnes C, Xu J *et al.* Microbial iron transport via a siderophore shuttle: a membrane ion transport paradigm. *Proc Natl Acad Sci USA* 2000;**97**:10691–6. https://doi.org/10.1073/pnas.200318797.

Stojiljkovic I, Hantke K Hemin uptake system of *Yersinia enterocolitica*: similarities with other TonB-dependent systems in Gram-negative bacteria. *EMBO J* 1992;**11**:4359–67. https://doi.org/10.1002/j.1460-2075.1992.tb05535.x.

Stojiljkovic I, Kumar V, Srinivasan N Non-iron metalloporphyrins: potent antibacterial compounds that exploit haem/Hb uptake systems of pathogenic bacteria. *Mol Microbiol* 1999;**31**:429–42. https://doi.org/10.1046/j.1365-2958.1999.01175.x.

Suarez LJ, Garzon H, Arboleda S *et al.* Oral dysbiosis and autoimmunity: from local periodontal responses to an imbalanced systemic immunity. A review. *Front Immunol* 2020;**11**:591255. https://doi.org/10.3389/fimmu.2020.591255.

Suits MD, Jaffer N, Jia Z Structure of the *Escherichia coli* O157:H7 heme oxygenase ChuS in complex with heme and enzymatic inactivation by mutation of the heme coordinating residues His-193. *J Biol Chem* 2006;**281**:36776–82. https://doi.org/10.1074/jbc.M607684200.

Suits MD, Pal GP, Nakatsu K *et al.* Identification of an *Escherichia coli* O157:H7 heme oxygenase with tandem functional repeats. *Proc Natl Acad Sci USA* 2005;**102**:16955–60. https://doi.org/10.1073/pnas.0504289102.

Szafranski SP, Deng ZL, Tomasch J *et al.* Functional biomarkers for chronic periodontitis and insights into the roles of *Prevotella nigrescens* and *Fusobacterium nucleatum*; a metatranscriptome analysis. *npj Biofilms Microbiomes* 2015;**1**:15017. https://doi.org/10.1038/npjbiofilms.2015.17.

Szczesniak K, Veillard F, Scavenius C *et al.* The Bacteroidetes Q-rule and glutaminyl cyclase activity increase the stability of extracytoplasmic proteins. *mBio* 2023;**14**:e0098023. https://doi.org/10.1128/mbio.00980-23.

Sztukowska M, Sroka A, Bugno M *et al.* The C-terminal domains of the gingipain K polyprotein are necessary for assembly of the active enzyme and expression of associated activities. *Mol Microbiol* 2004;**54**:1393–408. https://doi.org/10.1111/j.1365-2958.2004.04357.x.

Takahashi N Oral microbiome metabolism: from "Who are they?" to "What are they doing?". *J Dent Res* 2015;**94**:1628–37. https://doi.org/10.1177/0022034515606045.

Taketani S, Immenschuh S, Go S *et al.* Hemopexin from four species inhibits the association of heme with cultured hepatoma cells or primary rat hepatocytes exhibiting a small number of species specific hemopexin receptors. *Hepatology* 1998;**27**:808–14. https://doi.org/10.1002/hep.510270324.

Takii R, Kadowaki T, Baba A *et al.* A functional virulence complex composed of gingipains, adhesins, and lipopolysaccharide shows high affinity to host cells and matrix proteins and escapes recognition by host immune systems. *Infect Immun* 2005;**73**:883–93. https://doi.org/10.1128/IAI.73.2.883-893.2005.

Tang Y, Qi Y, Chen Y *et al.* Erythrocyte-mimicking nanovesicle targeting *Porphyromonas gingivalis* for periodontitis. *ACS Nano* 2024;**18**:21077–90. https://doi.org/10.1021/acsnano.4c02316.

Thompson JM, Jones HA, Perry RD Molecular characterization of the hemin uptake locus (hmu) from *Yersinia pestis* and analysis of hmu mutants for hemin and hemoprotein utilization. *Infect Immun* 1999;**67**:3879–92. https://doi.org/10.1128/IAI.67.8.3879-3892.1999.

Tolosano E, Altruda F Hemopexin: structure, function, and regulation. *DNA Cell Biol* 2002;**21**:297–306. https://doi.org/10.1089/104454902753759717.

Tonetti MS, Greenwell H, Kornman KS Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol* 2018;**89**:S159–72. https://doi.org/10.1002/JPER.18-0006.

Travis J, Potempa J Bacterial proteinases as targets for the development of second-generation antibiotics. *Biochim Biophys Acta Protein Struct Mol Enzymol* 2000;**1477**:35–50. https://doi.org/10.1016/S0167-4838(99)00278-2.

Trindade SC, Olczak T, Gomes-Filho IS *et al.* Induction of interleukin (IL)-1β, IL-10, IL-8 and immunoglobulin G by *Porphyromonas gingivalis* in humans. *J Periodontal Res* 2012;**47**:27–32. https://doi.org/10.1111/j.1600-0765.2011.01401.x.

Tunney MM, Field TR, Moriarty TF *et al.* Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am J Respir Crit Care Med* 2008;**177**:995–1001. https://doi.org/10.1164/rccm.200708-1151OC.

Turlin E, Debarbouille M, Augustyniak K *et al. Staphylococcus aureus* FepA and FepB proteins drive heme iron utilization in *Escherichia coli*. *PLoS One* 2013;**8**:56529. https://doi.org/10.1371/journal.pone.0056529.

Turlin E, Heuck G, Simoes Brandao MI *et al.* Protoporphyrin IX (PPIX) efflux by the MacAB-TolC pump in *Escherichia coli*. *Microbiologyopen* 2014;**3**:849–59. https://doi.org/10.1002/mbo3.203.

Ueshima J, Shoji M, Ratnayake DB *et al.* Purification, gene cloning, gene expression, and mutants of Dps from the obligate anaerobe *Porphyromonas gingivalis*. *Infect Immun* 2003;**71**:1170–8. https://doi.org/10.1128/IAI.71.3.1170-1178.2003.

Unno M, Matsui T, Ikeda-Saito M Structure and catalytic mechanism of heme oxygenase. *Nat Prod Rep* 2007;**24**:553–70. https://doi.org/10.1039/b604180a.

Vallelian F, Buehler PW, Schaer DJ Hemolysin, free hemoglobin toxicity, and scavenger protein therapeutics. *Blood* 2022;**140**:1837–44. https://doi.org/10.1182/blood.2022015596.

Veillard F, Potempa B, Poreba M *et al.* Gingipain aminopeptidase activities in *Porphyromonas gingivalis*. *Biol Chem* 2012;**393**:1471–6. https://doi.org/10.1515/hsz-2012-0222.

Veith PD, Chen YY, Gorasia DG *et al. Porphyromonas gingivalis* outer membrane vesicles exclusively contain outer membrane and periplasmic proteins and carry a cargo enriched with virulence factors. *J Proteome Res* 2014;**13**:2420–32. https://doi.org/10.1021/pr401227e.

Veith PD, Glew MD, Gorasia DG *et al.* Type IX secretion: the generation of bacterial cell surface coatings involved in virulence, gliding motility and the degradation of complex polymers. *Mol Microbiol* 2017;**106**:35–53. https://doi.org/10.1111/mmi.13752.

Veith PD, Nor Muhammad NA, Dashper SG *et al.* Protein substrates of a novel secretion system are numerous in the Bacteroidetes phylum and have in common a cleavable C-terminal secretion signal, extensive post-translational modification, and cell-surface attachment. *J Proteome Res* 2013;**12**:4449–61. https://doi.org/10.1021/pr400487b.

Velayudhan J, Hughes NJ, McColm AA *et al.* Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high affinity ferrous iron transporter. *Mol Microbiol* 2000;**37**:274–86. https://doi.org/10.1046/j.1365-2958.2000.01987.x.

Verma D, Garg PK, Dubey AK Insights into the human oral microbiome. *Arch Microbiol* 2018;**200**:525–40. https://doi.org/10.1007/s00203-018-1505-3.

Villoria GEM, Fischer RG, Tinoco EMB *et al.* Periodontal disease: a systemic condition. *Periodontol 2000* 2024;**96**:7–19. https://doi.org/10.1111/prd.12616.

Vincent SH Oxidative effects of heme and porphyrins on proteins and lipids. *Semin Hematol* 1989;**26**:105–13.

Wadhwa D, Bey A, Hasija M *et al.* Determination of levels of nitric oxide in smoker and nonsmoker patients with chronic periodontitis. *J Periodontal Implant Sci* 2013;**43**:215–20. https://doi.org/10.5051/jpis.2013.43.5.215.

Wang M, Hajishengallis G Lipid raft-dependent uptake, signalling and intracellular fate of *Porphyromonas gingivalis* in mouse macrophages. *Cell Microbiol* 2008;**10**:2029–42. https://doi.org/10.1111/j.1462-5822.2008.01185.x.

Wang PL, Ohura K *Porphyromonas gingivalis* lipopolysaccharide signaling in gingival fibroblasts-CD14 and Toll-like receptors. *Crit Rev Oral Biol Med* 2002;**13**:132–42. https://doi.org/10.1177/154411130201300204.

Wang S, Yan T, Zhang B *et al. Porphyromonas gingivalis* vaccine: antigens and mucosal adjuvants. *Vaccines* 2024;**12**:619. https://doi.org/10.3390/vaccines12060619.

Wang X, Liu M, Yu C *et al.* Biofilm formation: mechanistic insights and therapeutic targets. *Mol Biomed* 2023;**4**:49. https://doi.org/10.1186/s43556-023-00164-w.

Wei Y, Dang GP, Ren ZY *et al.* Recent advances in the pathogenesis and prevention strategies of dental calculus. *npj Biofilms Microbiomes* 2024;**10**:56. https://doi.org/10.1038/s41522-024-00529-1.

Weinberg ED The *Lactobacillus* anomaly: total iron abstinence. *Perspect Biol Med* 1997;**40**:578–83. https://doi.org/10.1353/pbm.1997.0072.

Widziolek M, Mieszkowska A, Marcinkowska M *et al.* Gingipains protect *Porphyromonas gingivalis* from macrophage-mediated phagocytic clearance. *PLoS Pathog* 2025;**21**:e1012821. https://doi.org/10.1371/journal.ppat.1012821.

Wilensky A, Potempa J, Houri-Haddad Y *et al.* Vaccination with recombinant RgpA peptide protects against *Porphyromonas gingivalis*-induced bone loss. *J Periodontal Res* 2017;**52**:285–91. https://doi.org/10.1111/jre.12393.

Wilks A, Heinzl G Heme oxygenation and the widening paradigm of heme degradation. *Arch Biochem Biophys* 2014;**544**:87–95. https://doi.org/10.1016/j.abb.2013.10.013.

Wilks A, Ikeda-Saito M Heme utilization by pathogenic bacteria: not all pathways lead to biliverdin. *Acc Chem Res* 2014;**47**:2291–8. https://doi.org/10.1021/ar500028n.

Wojaczynski J, Wojtowicz H, Bielecki M *et al.* Iron(III) mesoporphyrin IX and iron(III) deuteroporphyrin IX bind to the *Porphyromonas gingivalis* HmuY hemophore. *Biochem Biophys Res Commun* 2011;**411**:299–304. https://doi.org/10.1016/j.bbrc.2011.06.129.

Wojtowicz H, Bielecki M, Wojaczynski J *et al.* *Porphyromonas gingivalis* HmuY haemophore binds gallium(III), zinc(II), cobalt(III), manganese(III), nickel(II), and copper(II) protoporphyrin IX but in a manner different to iron(III) protoporphyrin IX. *Metallomics* 2013;**5**:343–51. https://doi.org/10.1039/c3mt20215a.

Wojtowicz H, Guevara T, Tallant C *et al.* Unique structure and stability of HmuY, a novel heme-binding protein of *Porphyromonas gingivalis*. *PLoS Pathog* 2009a;**5**:e1000419. https://doi.org/10.1371/journal.ppat.1000419.

Wojtowicz H, Wojaczynski J, Olczak M *et al.* Heme environment in HmuY, the heme-binding protein of *Porphyromonas gingivalis*. *Biochem Biophys Res Commun* 2009b;**383**:178–82. https://doi.org/10.1016/j.bbrc.2009.03.148.

Wu J, Lin X, Xie H Regulation of hemin binding proteins by a novel transcriptional activator in *Porphyromonas gingivalis*. *J Bacteriol* 2009;**191**:115–22. https://doi.org/10.1128/JB.00841-08.

Wyckoff EE, Duncan D, Torres AG *et al.* Structure of the *Shigella dysenteriae* haem transport locus and its phylogenetic distribution in enteric bacteria. *Mol Microbiol* 1998;**28**:11139–1152. https://doi.org/10.1046/j.1365-2958.1998.00873.x.

Yang QB, Yu FY, Sun L *et al.* Identification of amino acid residues involved in hemin binding in *Porphyromonas gingivalis* hemagglutinin 2. *Mol Oral Microbiol* 2015;**30**:337–46. https://doi.org/10.1111/omi.12097.

Yang X, Niu L, Pan Y *et al.* LL-37-induced autophagy contributed to the elimination of live *Porphyromonas gingivalis* internalized in keratinocytes. *Front Cell Infect Microbiol* 2020;**10**:561761. https://doi.org/10.3389/fcimb.2020.561761.

Yap BCM, Simpkins GL, Collyer CA *et al.* Porphyrin-linked nitroimidazole antibiotics targeting *Porphyromonas gingivalis*. *Org Biomol Chem* 2009;**7**:2855–63. https://doi.org/10.1039/b904340c.

Ye P, Chang J, Foo LF *et al.* An early report: a modified porphyrin-linked metronidazole targeting intracellular *Porphyromonas gingivalis* in cultured oral epithelial cells. *Int J Oral Sci* 2017;**9**:167–73. https://doi.org/10.1038/ijos.2017.31.

Yilmaz O, Verbeke P, Lamont RJ *et al.* Intercellular spreading of *Porphyromonas gingivalis* infection in primary gingival epithelial cells. *Infect Immun* 2006;**74**:703–10. https://doi.org/10.1128/IAI.74.1.703-710.2006.

Yuan L, Wang Y, Zong Y *et al.* Response of genes related to iron and porphyrin transport in *Porphyromonas gingivalis* to blue light. *J Photochem Photobiol B* 2023;**241**:112670. https://doi.org/10.1016/j.jphotobiol.2023.112670.

Yukitake H, Naito M, Sato K *et al.* Effects of non-iron metalloporphyrins on growth and gene expression of *Porphyromonas gingivalis*. *Microbiol Immunol* 2011;**55**:141–53. https://doi.org/10.1111/j.1348-0421.2010.00299.x.

Yuzawa S, Kurita-Ochiai T, Hashizume T *et al.* Sublingual vaccination with fusion protein consisting of the functional domain of hemagglutinin A of *Porphyromonas gingivalis* and *Escherichia coli* maltose-binding protein elicits protective immunity in the oral cavity. *FEMS Immunol Med Microbiol* 2012;**64**:265–72. https://doi.org/10.1111/j.1574-695X.2011.00895.x.

Zainal-Abidin Z, Veith PD, Dashper SG *et al.* Differential proteomic analysis of a polymicrobial biofilm. *J Proteome Res* 2012;**11**:4449–64. https://doi.org/10.1021/pr300201c.

Zeineldin M, Esmael A, Al-Hindi RR *et al.* Beyond the risk of biofilms: an up-and-coming battleground of bacterial life and potential antibiofilm agents. *Life* 2023;**13**:503. https://doi.org/10.3390/life13020503.

Zhang L, Butler C, Khan HSG *et al.* Characterisation of the *Porphyromonas gingivalis* manganese transport regulator orthologue. *PLoS One* 2016;**11**:e0151407. https://doi.org/10.1371/journal.pone.0151407.

Zhang J, Yu J, Dou J *et al.* The impact of smoking on subgingival plaque and the development of periodontitis: a literature review. *Front Oral Health* 2021a;**2**:751099. https://doi.org/10.3389/froh.2021.751099.

Zhang Z, Liu D, Liu S *et al.* The role of *Porphyromonas gingivalis* outer membrane vesicles in periodontal disease and related systemic diseases. *Front Cell Infect Microbiol* 2021b;**10**:585917. https://doi.org/10.3389/fcimb.2020.585917.

Zhou P, Li X, Qi F Identification and characterization of a haem biosynthesis locus in *Veillonella*. *Microbiology* 2016;**162**:1735–43. https://doi.org/10.1099/mic.0.000366.

Zhu YC, An T, Zhang ZL *et al.* Immunoprotective effects of a hemin-binding peptide derived from hemagglutinin-2 against infection with *Porphyromonas gingivalis*. *Mol Oral Microbiol* 2018;**33**:81–88. https://doi.org/10.1111/omi.12202.

Zijnge V, van Leeuwen MB, Degner JE Oral biofilm architecture on natural teeth. *PLoS One* 2010;**5**:e9321. https://doi.org/10.1371/journal.pone.0009321.

Received 9 March 2025; **revised** 2 May 2025; **accepted** 8 May 2025