**Short Conceptual Overview**

Bernd Bufe* and Frank Zufall

# The sensing of bacteria: emerging principles for the detection of signal sequences by formyl peptide receptors

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**Abstract:** The ability to detect specific chemical signatures released by bacteria and other microorganisms is a fundamental feature of immune defense against pathogens. There is increasing evidence that chemodetection of such microorganism-associated molecular patterns (MAMPs) occurs at many places in the body including specific sets of chemosensory neurons in the mammalian nose. Formyl peptide receptors (FPRs) are a unique family of G protein-coupled receptors (GPCRs) that can detect the presence of bacteria and function as chemotactic receptors. Here, we highlight the recent discovery of a vast family of natural FPR agonists, the bacterial signal peptides (or signal sequences), thus providing new insight into the molecular mechanisms of bacterial sensing by human and mouse FPRs. Signal peptides in bacteria are formylated, N-terminal protein signatures required for directing the transfer of proteins through the plasma membrane. After their cleavage and release, signal peptides are available for FPR detection and thus provide a previously unrecognized MAMP. With over 170 000 predicted sequences, bacterial signal peptides represent one of the largest families of GPCR ligands and one of the most complex classes of natural activators of the innate immune system. By recognizing a conserved three-dimensional peptide motif, FPRs employ an unusual detection mechanism that combines structural promiscuity with high specificity and sensitivity, thus solving the problem of detecting thousands of distinct sequences yet maintaining selectivity. How signal peptides are released by bacteria and sensed by GPCRs and how these processes shape the responses of other cells and whole organisms represents an important topic for future research.

**Keywords:** bacterial signal peptides; formyl peptide receptor (FPR); pathogen-associated molecular pattern (PAMP); pattern recognition receptor (PRR); vomeronasal organ (VNO).

***Corresponding author: Bernd Bufe,** Center for Integrative Physiology and Molecular Medicine, Saarland University School of Medicine, Kirrbergerstr. Bldg. 48, D-66421 Homburg, Germany, e-mail: bernd.bufe@uks.eu
**Frank Zufall:** Center for Integrative Physiology and Molecular Medicine, Saarland University School of Medicine, Kirrbergerstr. Bldg. 48, D-66421 Homburg, Germany

## Introduction

The sensing of microorganisms by pattern recognition receptors (PRRs) is essential for many functions in the body (1–3), but, first and foremost, such receptors are required for the initial sensing of infection and the activation of immune defense (4, 5). PRRs recognize evolutionary conserved chemical signatures of microorganisms known as microorganism-associated molecular patterns (MAMPs) (4–6). It is well-known that this recognition process is not limited to immune cells (5, 7, 8). For example, there is increasing evidence suggesting that the sensing of pathogens is also a fundamental feature of social recognition and a prerequisite for the initiation of a wide range of social behaviors, at least in rodents (9–11). Rodents use chemical cues to distinguish between parasitized individuals, recognize infected conspecifics, and avoid and display aversive responses to infected individuals (9–11). Sensory neurons located in the mammalian nose, especially those of the vomeronasal organ (VNO) (12–14), seem to be capable of detecting a wide range of inflammation markers as well as bacterial and mitochondrial peptides (15, 16).

In this brief conceptual article, we will summarize the evidence for a recently emerged general and mutual mechanism that enables the sensing of bacteria by both cells of the innate immune system and sensory neurons of the mammalian nose. This detection mechanism consists essentially of two basic elements: (i) a vast family of

unsuspected ligands, the bacterial signal peptides; and (ii) a relatively small family of G protein-coupled receptors (GPCRs) expressed by both systems, the formyl peptide receptors (FPRs). We will summarize the evidence leading to the discovery of bacterial signal peptides as an agonist family for FPRs and, in turn, revealing that some FPRs function as highly sensitive signal-peptide-receptors. Furthermore, some of the principles for this recognition process are now emerging and are providing interesting structural solutions to the general problem as to how a few FPRs can recognize thousands of peptides with distinct amino acid sequences yet maintain high selectivity of discrimination. In retrospect, the discovery of this previously unrecognized concept is not as surprising as it sounds. Formylated peptides released by bacteria are among the first identified chemotactic stimuli for immune cells (17) and it has long been known that FPRs can detect formylated peptides (17, 18). However, the functional link between the signal peptide family and its detection by FPRs has not been recognized until very recently when formylated signal peptides were directly shown to activate FPR1, FPR2, and FPR3 and to trigger innate immune responses in human and mouse leukocytes in an FPR-dependent manner (19).

## What are signal peptides and how can they be employed for the sensing of bacteria?

We propose (19) that mammalian FPRs may have evolved originally as germ-line encoded PRRs that recognize structurally conserved export motifs of bacterial signal sequences as their cognate MAMP (see below). To appreciate this novel concept, it will first be necessary to gain some understanding on the general function and structure of bacterial signal peptides (also known as signal sequences).

Signal peptides play pivotal roles in the initiation of protein transport through cell membranes in all prokaryotes and eukaryotes (20, 21). They can be found at the N-terminus of most newly synthesized secretory and membrane proteins that are destined for export via a secretory protein translocation machinery (20, 21) (Figure 1A). Inside cells, signal peptides are recognized by signal recognition particles and used to guide the nascent protein chain to the translocation machinery (20, 21). In bacteria, signal peptides are N-terminal protein

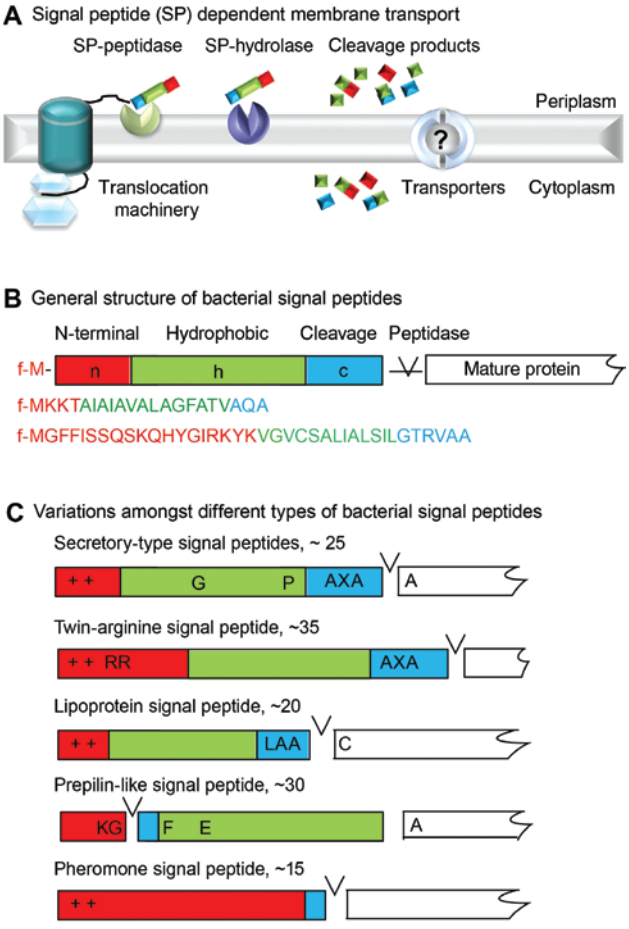


**Figure 1:** Metabolic pathway and structure of bacterial signal peptides.
(A) General mechanisms of N-terminal signal peptide-guided transport of secretory and membrane proteins by the translocation machinery. After transport initiation by signal peptides, the nascent peptides are first cotranslationally transported through a protein-conducting channel. In the periplasm, signal peptides are first cleaved off the nascent peptide chain by a signal peptidase and then subjected to further proteolysis by signal peptide hydrolases. The resulting fragments can be found in the periplasm and cytoplasm of bacteria. The molecular processes underlying the transport of signal peptides into the extra- and intracellular space are not yet well defined but likely involve peptide transporters. (B) Bacterial signal peptides have a characteristic structure. They consist of an n-region (red) starting with a formylated methionine (f-M), followed by an α-helical hydrophobic h-region (green), and a c-region (blue) containing a conserved signal peptidase recognition motif. The sequences below show the signal peptides of the outer-membrane protein A and hyaluronate lyase, comprising 22 and 37 residues, respectively. Letter colors indicate n-, c- and h-regions. (C) Bacterial signal peptides can be grouped into at least five different classes (20). The n-, h-, c-region are indicated by color, (+) denotes positively charged residues, letters indicate specific amino acid residues: (G) glycine, (P) proline, (A) alanine, (R) arginine, (L) leucine, (F) phenylalanine, (E) glutamate, (K) lysine, and (X) indicates a variable residue.

signatures that are required for directing the transfer of bacterial proteins through the plasma membrane during which they are cleaved off to give rise to the native form of membrane-associated or secreted proteins. Bacterial signal peptides are a complex family that consists of a vast number of peptides with highly variable structures. Currently, 175 542 bacterial signal peptides are annotated in the signal peptide database (http://www.signalpeptide.de), but their real number is likely to be much higher because current sequence information comprises only 29 395 different bacterial strains (http://bacteria.ensembl.org). Some estimates suggest that the total number of bacterial species on earth could be up to one billion (22).

The primary structure of signal peptides is variable and fluctuates considerably in size and sequence (20). Small signal peptides can consist of only 16 amino acid residues (21) whereas complex signal peptides can comprise more than 100 residues. Signal peptides contain three typical domains that are evolutionarily conserved among all prokaryotes and eukaryotes (21, 23) (Figure 1B): an N-terminal (n) region starting with a methionine precedes a central α-helical, hydrophobic (h) region that is followed by a C-terminal (c) region. Only the sequence of the c-region is well defined. In eukaryotes, it consists of five and in bacteria of six sequence-related amino acid residues that contain a signal peptidase cleavage motif (21). The h-region seems to be more flexible. It has a minimal size of eight residues but can extend to more than 20 residues. Overall, hydrophobicity is a governing principle in this region. Its sequence shows little conservation but usually contains a serine, glycine, threonine, and proline as hydrophobic residues (21). The n-region is highly variable both in terms of length and amino acid composition. It consists of at least two residues but n-regions with 80 residues have also been observed (21). Maybe most importantly with respect to our concept, there is an essential difference in the n-region between bacterial and eukaryotic signal peptides; all bacterial peptides start with a formylated methionine (f-M), whereas eukaryotic peptides are unmodified (24). Hence, it is generally assumed that bacterial signal peptides are formylated peptides (25).

After successful transport initiation, signal peptides are usually cleaved off the maturing protein by signal peptidases (23, 26) and are subjected to rapid further proteolytic fragmentation by signal peptide hydrolases (26) (Figure 1A). This cleavage process is an important part of a quality control system that regulates the turnover of membrane proteins and adaptations to stressful environmental conditions (26). On the basis of specific signal peptidase recognition sequences, at least five distinct types of N-terminal signal peptides can be distinguished in bacteria (20) (Figure 1C). The first type comprises the classical secretory signal peptides and is used by most proteins that are secreted into the extracellular environment. The second type contains twin-arginine signal peptides, which guide proteins to the twin-arginin translocation pathway. The third type of signal peptides contains a well-conserved lipobox and is used for export of membrane-anchored lipoproteins. A fourth type is used for prepilin-like proteins. A fifth type lacking the h-region is used by bacteriocins and bacterial pheromones for ABC transporter-mediated export. Each of these pathways is known to employ different enzymes for the proteolytic signal peptide cleavage (23, 26).

Membrane proteins are critical for bacterial growth, division, and survival. Thus, it is not surprising that this proteolytic process is finely regulated in bacteria and can involve various subtypes of peptidases and hydrolases (26). The number and sequence of the proteolytic fragments can vary depending on several factors including the enzymes used, the bacterial species, the metabolic status, and the environmental conditions. Thus, any given receptor that is faced with the detection of peptides from different bacterial strains will encounter a complex universe of chemically divergent fragments. The cleavage products are subsequently released into the cytosol and the extracellular space (27, 28). Originally, these peptides were considered as biologically inactive degradation products. However, recent studies revealed that some signal peptides or their proteolytic fragments are still bioactive and seem to mediate various post-cleavage functions (28). For example, it has been shown that signal peptides contribute to glycoprotein maturation and infectivity of viruses can be presented via major histocompatibility complex I to inhibit natural killer cell-mediated immune surveillance, and even participate in regulating calcium signaling events through $Ca^{2+}$/calmodulin-dependent processes (28). The fact that signal peptides can be released from bacteria and subsequently be detected by other cells, for example by the host's immune system, provides a novel post-cleavage function for these molecules. In this case, bacterial signal peptides would represent a MAMP that can be employed for the detection of bacteria (19).

## Formyl peptide receptors are G protein-coupled receptors that can detect bacteria

Given that bacterial signal peptides are formylated peptides, it seemed obvious to provide a functional link

between these peptides and the FPR family. After all, the receptors in this family have been named according to their capability to detect formylated peptides. Yet, this connection has been overlooked for many years. Of note, one early report published before the identification of FPRs suggested that bacterial factors stimulating neutrophils may be derived from prokaryotic signal peptides (25).

FPRs are intensely studied GPCRs with seven transmembrane domains that are involved in host defense against bacterial infections and in the clearance of damaged cells (17, 18). FPRs form a small multigene family that is present in many different cell types and detects a large variety of different ligands. Due to the versatile functions of FPRs many of their physiological roles are still incompletely understood (18). FPR expression was first observed in human cells of the innate and adaptive immune systems, such as monocytes and neutrophil granulocytes, dendritic cells, and T and B lymphocytes (18). But soon it became clear that their expression pattern was far more widespread. FPR expression has also been found in microglia and astroglia of the brain, neurons of the autonomic nervous system and the dorsal root ganglion, hepatocytes, fibroblast, and lung carcinoma cells. Interestingly, FPRs are not only present in humans and other primates, but are also found in chemosensory neurons of the mammalian nose (16, 29, 30). *FPR* genes have been found in the genomes of many other mammals ranging from mice to elephants, and they are even present far outside the mammalian clade, such as in fish. The total number of *FPR* genes is subjected to species-specific adaptations (31). Humans, like many other species, express three genes (*FPR1*, *FPR2*, and *FPR3*) whereas mice have seven genes (*Fpr1*, *Fpr2*, *Fpr3*, *Fpr-rs3*, *Fpr-rs4*, *Fpr-rs6*, and *Fpr-rs7*) (17, 29, 31). There is clear evidence that Fpr1, Fpr2, and Fpr3 are primarily serving as immune receptors and have maintained their specific roles in the immune system of humans, mice, and most other mammals (1, 17, 19). Recently, phylogenetic evidence (29) has suggested that the rodent-specific expansion of this gene family may coincide with the expression of *FPRs* in the olfactory system of these animals.

FPRs were first identified as sensitive chemotactic receptors for N-terminally formylated peptides such as N-formylmethionine-leucine-phenylalanine (f-MLF), a molecule that was found in bacterial supernatants of *Escherichia coli* (32, 33). Formylated peptides derived from other bacterial strains such as peptides from *Listeria monocytogenes* (34) and phenol soluble modulins from *Staphylococcus aureus* (1, 35) are also sensitive activators of FPR (Figure 2). The capability of FPRs to detect formylated peptides appears to be conserved among mammals (17, 19, 36, 37). However, formylated peptides do not always originate from invading bacteria, they can also originate from formylated mitochondrial proteins released by ruptured host cells (38). At significantly higher concentrations, FPRs can also detect a variety of other crucial, naturally occurring peptides, such as the cathelin-related antimicrobial peptide, the acute phase protein serum amyloid A, the Alzheimer disease-related β amyloid 42, and the envelope proteins of the human immune deficiency virus (17, 18, 39). Receptor activation by a number of non-peptide ligands such as lipopolysaccharide, resolvin, and lipoxin A4 has also been reported (17, 18, 39).

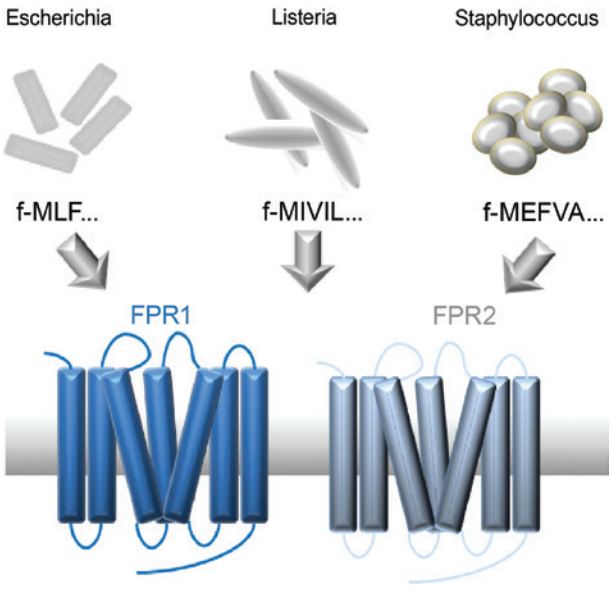


**Figure 2:** FPRs recognize formylated peptides from different bacteria.
FPRs are sensitive chemotactic receptors for N-terminally formylated (f) peptides from *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Representative peptides from potent FPR activators of each bacterial strain are shown.

In human and mouse neutrophils, FPR activation by f-MLF or other formylated peptides induces directional migration of neutrophils, transient increase of intracellular calcium levels, generation of reactive oxygen species, and release of granule constituents from neutrophils (17, 18, 39). The FPR-induced granule release results in a proteolytic cleavage of membrane-localized adhesion molecules and the liberation of proinflammatory matrix proteins and enzymes that can cause tissue degradation and killing of bacteria (17). Outside the immune system, FPRs have been implicated in various other functions, such as pain perception (40), wound healing (41), and olfactory pathogen sensing (16, 29).

Due to these versatile functions, it has been difficult to determine whether FPRs are primarily promiscuous receptors for diverse cellular dysfunctions (18), danger receptors that use host-endogenous formylated mitochondrial peptides as a danger-associated molecular pattern to detect ruptured host cells (38), or PRRs that detect bacterial infections through exogenous peptides that are released by bacteria (39). However, several well-known features of FPRs suggest that they may primarily serve as PRRs for the detection of bacteria. First, FPRs were initially identified as sensors for formyl peptides that are released by bacteria (32, 33). Second, they are expressed in immune cells and the activation of these receptors triggers various cellular defense mechanisms that are typical for PRRs (39). Third, FPR-deficient mice are more susceptible to bacterial infections than wild-type mice (42, 43). Fourth, the function to detect formyl peptides is conserved among mammalian FPRs, and they are expressed in various regions of the body where the capability to detect bacteria is essential (17).

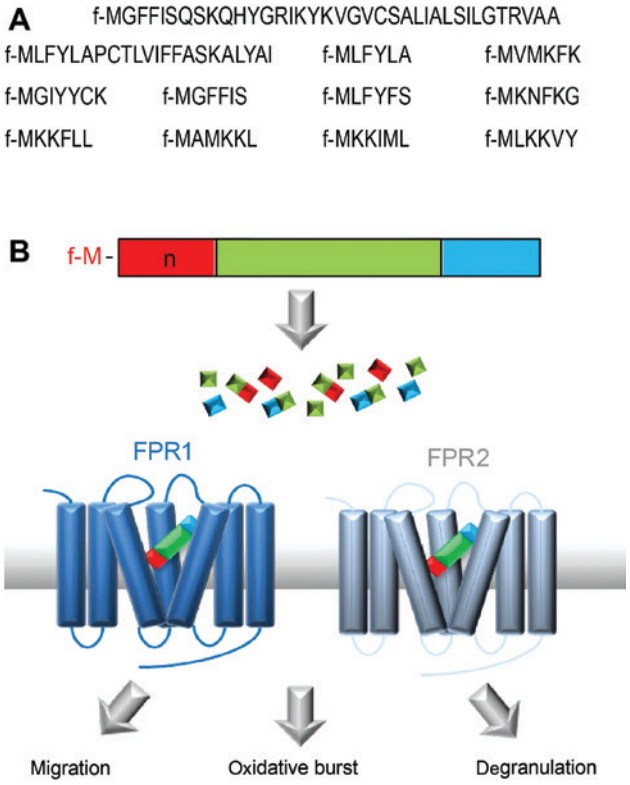


**Figure 3:** Schematic indicating that a large number of formylated bacterial signal peptides are capable of triggering immune responses by activation of FPR1 and FPR2.
(A) Sequences of a few representative bacterial signal peptide activators of FPR1 and FPR2 are shown. Note that the activators comprise a large variety of sequence divergent peptides ranging from short N-terminal hexamer fragments to full-length peptides with up to 37 amino acids. (B) Stimulation of human neutrophils with these peptides leads to robust cell migration, radical oxygen species production, and degranulation. Based on the results from Ref. (19).

## FPRs as pattern recognition receptors for formylated bacterial signal peptides

On the basis of several lines of evidence, we have proposed that FPRs may have evolved originally as PRRs that utilize formylated bacterial signal peptides as their genuine MAMP (19). This evidence will be detailed in the following section.

By using a high-throughput calcium imaging methodology with heterologously expressed FPRs as developed earlier (36), we showed that human FPR1 and FPR2 both function as broad signal peptide receptors that detect a wide range of structurally divergent bacterial signal peptides, although with various sensitivities and specificities. All 21 bacterial signal peptides that we tested so far were, without exemption, activators of at least one of either receptor subtype. The size and sequence of these ligands could vary considerably ranging from short N-terminal hexamer fragments to full-length peptides with up to 37 amino acids (Figure 3). The core agonist motif of these 21 signal peptides can be found in 4293 bacterial signal peptides. Statistical calculations for the total amount of detected signal peptides predict that FPR1 and FPR2 should be capable to detect at least 78% of the 175 542 sequences in the signal peptide database. Thus, FPR1 and FPR2 are capable of detecting a wide range of bacterial signal peptides. Nonetheless, these receptors are quite selective for specific amino acid sequences because fragments from other signal peptide regions, such as the h- and c-regions, were inactive, even when used at 1 000 000-fold higher concentration. Moreover, we observed that formylated signal peptides were nearly 1000-fold more effective than the non-formylated ones in activating these FPRs. Thus, these FPRs strongly prefer a formylated signal peptide N-terminus. Interestingly, the N-terminus is the only structural key element of signal peptides that can be used for the reliable discrimination of bacterial signal peptides from corresponding non-formylated peptides produced by mammalian cells.

Our experiments (19) also showed that FPR1 and FPR2 detect bacterial signal peptides with extremely high sensitivity. Most bacterial signal peptides activated at least one of these receptors in the nano- to picomolar range. Thus,

these ligands belong to the most effective natural FPR agonists known to date. Interestingly, we found that the sequence of the classical FPR agonist f-MLF is contained in the N-terminal domain of signal peptides of several hundred distinct bacterial strains including the highly pathogenic *Yersinia pestis*, *Clostridium botulinum*, and *Bacillus cereus*. These signal peptides likely constitute a natural source for f-MLF and its derivatives. We, therefore, tested three f-MLF containing signal peptides and found all three of them to be very sensitive FPR agonists, with two of these being 5- to 10-fold more potent than f-MLF itself.

The general activation pattern of FPRs by specific bacterial signal peptides was highly conserved among species (19). All peptides that activated a specific human FPR were always also activators of the corresponding mouse Fpr. In fact, various peptides showed nearly identical concentration responses for the mouse and human receptors.

Calcium imaging experiments (19) with primary isolated human monocytes and granulocytes equipped with native FPR1 and FPR2 receptors demonstrated that these cells can detect signal peptides in an almost identical manner as heterologous cells expressing these receptors. Single cell calcium imaging in combination with *post hoc* immunocytochemistry for FPRs gave a clear correlation between calcium responses and receptor expression. Moreover, bacterial signal peptides activated not only human but also mouse leukocytes and these responses were absent in Fpr1-deficient cells. These experiments provided the first evidence that the capability to detect signal peptides is conserved in immune cells among two different species, humans and mice.

We also showed (19) that bacterial signal peptides can mobilize the innate immune defense. Stimulation of human monocytes and neutrophil granulocytes with these peptides induced production of reactive oxygen species, matrix metallopeptidase-9 release, and chemotactic cell migration. Monocytes and neutrophil granulocytes can detect a wide range of structurally divergent signal peptides. Consistent with the results obtained by heterologous expression of FPRs, the size and sequence of activating signal peptides could vary tremendously by ranging from full-length peptides with up to 37 amino acids to short N-terminal hexamer fragments, demonstrating that many N-terminal cleavage products of signal peptides are activators of these immune cells.

These experimental observations are consistent with the biological and structural requirements necessary for a specific and reliable detection mechanism for bacterial signal peptides. Our hypothesis that FPR1 and FPR2 function primarily as sensors for bacterial signal peptides is fully consistent with many published FPR features. The structural promiscuity of FPRs (18) is required to recognize a large amount of structurally diverse signal peptides. The strong preference of FPRs for formylated peptides (17) is explained by the necessity of FPRs to focus on the recognition of the bacteria-specific formylated N-terminus. The robust responses of FPRs to the N-terminally formylated parts of mitochondrial membrane proteins (38) are explained by the fact that these organelles originated from endosymbiotic bacteria. The capability of FPRs to serve as broad bacterial detectors explains their expression in many other cell types (17) where they could be involved in the general management of the bacterial microbiome.

## Bacterial signal peptides exhibit a unique set of structural features

The ability of FPR1 and FPR2 to recognize thousands of bacterial signal peptides with distinct amino acid sequences yet maintaining selectivity requires a compromise between broad specificity and high affinity. Several key features of this recognition process have already emerged (19). Taken together, these results provide a remarkable solution to this general problem.

One key finding seems to be a detection mechanism in which FPRs focus on the recognition of a conserved spatial (i.e. 3D) structure rather than on a linear peptide sequence with conserved residues. Structural variation analysis of FPR agonists, combined with molecular modeling, predicted that effective FPR activators comprise a three-dimensional, spiral-shaped core motif that can vary considerably in length, but should contain a minimum of three amino acids. Several critical features of this claw-like agonist structure can now be defined. In our model (19), the first amino acid residue of a given peptide represents an element of special importance. Our studies of peptide derivatives show that this residue has the most stringent spatial and chemical limitations (19). For FPR1 activation, this residue essentially has to be a methionine. A given chemical modification of this group will strongly influence the agonist potency. Studies with chemical substitutes other than an N-terminal formylation show that the carbonyl group (C=O) in the formyl moiety is a crucial determinant for agonist affinity. Most likely this C=O group will be used to form a hydrogen bond with residues in the receptor. Models for different peptides (Figure 4) predict that amino acid residues following the f-M form a distinct spiral-shaped symmetrical core structure. This structure is oriented in an α-turn around the

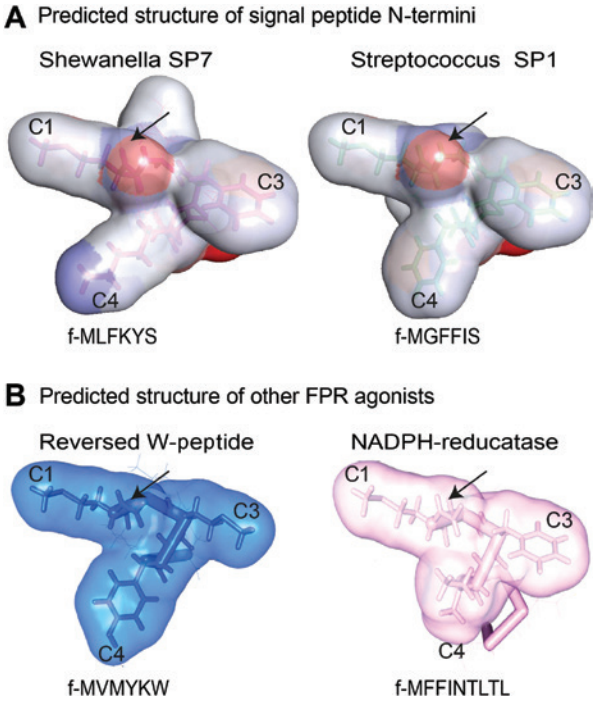


**Figure 4:** Comparison of the structural features of bacterial signal peptides with other FPR agonists.
(A) Molecular models of the charge distribution and molecular surface of two similarly potent bacterial signal peptides, SP7 from the TonB-dependent siderophore receptor of *Shewanella baltica* and SP1 from the hyaluronate lyase of *Streptococcus suis*. Corresponding amino acid sequences are depicted below each model. Black arrows indicate the position of the formyl group that is attached to the N-terminal α-C-atom (C1) of the first amino acid. C3 and C4 indicate functional groups attached to α-C-atoms of the third and fourth amino acid. Polar regions with a positive charge are depicted in blue, negative charges are shown in red, hydrophobic regions are shown in gray. Note that both sequence-divergent bacterial signal peptides display a similar claw-like 3D structure with a conserved hydrophobic surface. The only conserved polar position is the formyl group. Charge distributions in the amino acid side chains differ, which argues against a prominent role of polar interactions with these residues. (B) The 3D structure of the synthetic reversed W-peptide (blue) and the mitochondrial NADH reductase subunit 1 (pink), which are also potent FPR activators (19). Note that the shape of both structures shows obvious similarities to the bacterial signal peptides shown in panel (A).

f-M and comprises at least two to three further residues. The chemical structure of the amino acid side chains is far too variable for further site-specific hydrogen bonds. However, the side chains also influence agonist potency in a shape-oriented fashion. In FPR1, they will most likely be used to form flexible van der Waals interactions. Most likely, additional hydrogen bonds can only be formed with the C=O or N-H groups of the peptide backbone because the amino acid side chains are too variable. The spiral-shaped symmetrical core structure with an α-turn would permit a certain degree of specificity for the formation of such bonds.

The symmetrical organization of this agonist motif, together with the observation that a similar carbonyl group exists in N-terminal formyl and C-terminal amidated methionines, can explain why FPRs can recognize N-terminally formylated and C-terminally amidated peptides with similar sensitivities: These peptides behave like chemical mirror images. Our models predict that a C-terminally amidated peptide would first interact with the receptor binding pocket via its C-terminal methionine, whereas an N-terminally formylated peptide would bind first through its N-terminus. Experimental evidence for the validity of this mechanism has been provided by our demonstration that C-terminally amidated peptides are equally potent FPR agonists as corresponding peptides in which the amino acid sequence has been reversed and now comprises an N-terminal formylation (19).

The fact that this mechanistic concept enabled us to identify bacterial signal peptides as a novel class of FPR agonists provides direct support for its validity. Bacterial signal peptides have structural features that fit particularly well to our model predictions (Figure 4A), including a highly variable primary structure that contains a largely hydrophobic α-helical domain close to a conserved N-terminus starting with an f-M. Importantly, key features derived from our analyses can also be found in other previously identified FPR agonists (19), such as mitochondrial peptides, suggesting that these results could be of general significance. Consistent with this idea, the NADH-reductase N-terminus reveals clear structural similarities to our agonist model (Figure 4B). Further structural comparisons demonstrate that other known FPR ligands, such as humanin and f-MLF, also display striking similarities with our agonist model (19). Independent support for our concept came from recent work using non-peptide agonists to identify a potential binding motif in FPR2 (44). Moreover, other FPR agonists, such as phenol-soluble modulin peptide toxins (35), amyloid-β (1–42, 45), or urokinase receptor (46), also fit into this scheme. In essence, our data argue that a universal motif for bacterial signal peptides exists that can possibly be extended to many other peptide activators of FPRs.

## Expert opinion

The fundamental concept of PRRs and MAMPs has originally been developed for the fields of innate immunity and inflammation to explain the unique role of Toll-like

receptors (4–6) in chemosensory recognition of large groups of microorganisms. However, soon it became clear that this concept can also be applied beyond the immune system. The detection of MAMPs by PRRs is generally useful to monitor and manage the microbiome (1–3). Our results demonstrated that signal peptides constitute a valid molecular signature that is shared by virtually all bacteria (19). Hence, the basic concept of MAMP recognition by PRRs should be extended to the FPR-mediated recognition of bacterial signal peptides. Many important biological functions of FPRs beyond the immune system still await their discovery. This sets the stage for new studies including those directed at solving the agonist recognition site of FPRs, and to develop a better understanding of the role of FPRs in various human diseases, such as inflammation, cancer, wound healing, and neurodegenerative disorders.

## Outlook

In this review, we have summarized a new model for the detection of bacteria in which FPRs function as PRRs that focus on a pathogen-associated molecular pattern (PAMP) that is defined by the key structural features of bacterial signal peptides. We predict that this general capability will be employed at many places in the body where FPRs are expressed, particularly for the sensing and management of the microbiome. One such place is likely to be the nose where some of the nasal sensory epithelia are known to express FPRs in specific sets of sensory neurons (16, 29). There is currently strong interest in the question whether 'olfactory' FPRs are used for pathogen sensing mechanisms by the mammalian nose. Indeed, a recent study provided evidence that the VNO is involved in the recognition and avoidance of sick conspecifics (10). Sensory neurons in the VNO can detect inflammation markers and as well as bacterial and mitochondrial peptides (15, 16), which raises the possibility that the immune and olfactory systems employ similar mechanisms to detect these illness-associated chemical cues.

Inflammation markers, as well as bacterial and mitochondrial peptides, are typical FPR activators (17, 18, 39) for several members of the *Fpr* gene family (16, 19, 36). Our most recent finding (30) that Fpr3 is expressed in both vomeronasal sensory neurons and mouse neutrophils and the discovery that a large number of inbreed mouse strains carry a non-functional Fpr3 variant provides an important foundation to address these questions.

Should the hypothesis that FPRs mediate pathogen sensing in the olfactory system turn out to be correct, this would probably implicate that vomeronasal FPRs have evolved some functional properties that differ from their relatives in the immune system. The vomeronasal sensory epithelium represents a body surface on which both commensal and symbiotic bacteria are present. Thus, a large number of formylated bacterial peptides from harmless bacteria should always be available for detection. Hence, broad and sensitive detection of any type of formylated peptide will not be useful here. Instead, one may expect much more specific tuning and a lower sensitivity of receptors responding only to relevant concentrations of a few specific molecular markers that possibly indicate the presence of highly pathogenic bacteria. Indeed our functional studies already provide some evidence indicating that this could be the case (19, 30, 36). It will be interesting to see how olfactory FPR function has adapted evolutionary to this challenge.

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