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Aer on the Inside Looking Out:

Paradigm for a PAS-HAMP Role in Sensing Oxygen, Redox and Energy

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Abstract

Aer, the *Escherichia coli* aerotaxis (oxygen-sensing) receptor is representative of a small class of receptors that face the cytoplasm in bacteria. Instead of sensing oxygen directly, Aer detects redox changes in the electron transport system or cytoplasm when the bacteria enter or leave a hypoxic micro-niche. As a result, Aer sensing also enables bacteria to avoid environments where carbon deficiency, unfavourable reduction potential or other insults would limit energy production. An FAD-binding PAS domain is the sensor for Aer and a HAMP domain interacts with the PAS domain to form an input-output module for signal transduction. By analogy to the first solution structure of an isolated HAMP domain from *Archaeoglobus,* Aer HAMP is proposed to fold into a four-helix bundle that rotates between a signal-on and signal-off conformation. Aer is the first protein in which a PAS-HAMP input-output module has been investigated. The structure and signal transduction mechanism of Aer is providing important insights into signalling by PAS and HAMP domains.

Keywords

Aerotaxis; chemotaxis; PAS domains; HAMP domains; signal transduction; receptors

a. Introduction

The Aer (**aer**otaxis) receptor in *Escherichia coli* senses redox and oxygen inside the cell, enabling the bacteria to respond to changes in internal energy (Taylor *et al*., 1999). The sensing module is a PAS (Per-Arnt-Sim) domain, and the signal output is used by *E. coli* to navigate to microenvironments where the bacteria can support optimal energy production. The Aer aerotaxis (oxygen-sensing) response is a simple behavioural system that is being elucidated at the molecular level, and is also a model for how cells can sense their own internal energy. Aer has a HAMP (**h**istidine kinases, **a**denylyl cyclases, **m**ethyl-accepting chemotaxis proteins, **p**hosphatases) domain, and the PAS and HAMP domains function together as an input-output module for signal transduction (Buron-Barral *et al*., 2006; Ma *et al*., 2005)). This is important. More than 14,900 PAS domains and 8,300 HAMP domains have been identified in sensory proteins, which ranks them among the more frequently reported protein domains. However, there is limited knowledge of the role of these modules in sensory transduction systems, and almost nothing was known previously about how PAS and HAMP domains function together.

Typical receptors, such as the *E. coli* chemoreceptor, Tsr, have external (periplasmic) sensing domains, transmembrane segments that transmit a signal from the outside sensor to the inside of the cell, and cytoplasmic output domains (Fig. 1). In contrast, Aer has an FAD-binding PAS domain in the cytoplasm, joined to a membrane anchor by the F1 linker (Bibikov *et al*.,

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1997;Bibikov *et al*., 2000;Repik *et al*., 2000) (Fig. 1). The anchor consists of two transmembrane segments separated by four residues in the periplasm (Amin *et al*., 2006). A HAMP domain links the second transmembrane sequence to the C-terminal signalling domain (Ma, 2001). The signalling domains in Aer and the chemoreceptors are highly conserved, and regulate the same chemotaxis histidine kinase system [see (Wadhams and Armitage, 2004) for a review] (Fig. 1). The structural unit of Aer and chemoreceptors is a homodimer. The signalling domain of the Tsr chemoreceptor has an extended helix-turn-helix configuration that forms a four-helix bundle in the dimer (Fig. 1) (Kim *et al*., 1999). The HAMP domain was first projected to be a linear helix that extended from the proximal helix of the signalling domain to the transmembrane segment (Kim *et al*., 1999). Subsequent high resolution electron microscopy indicated that the intact Tsr receptor is shorter than predicted by the extended helix model (Weis *et al*., 2003). Figure 1 illustrates two possible structures for HAMP domains: an extended helix (Kim *et al*., 1999) or a four-helix bundle (Hulko *et al*., 2006).

b. Navigating with an energy/redox sensor

Some of the earliest studies of aerotaxis in bacteria demonstrated that, in a gradient of oxygen, microaerophilic bacteria always migrate to form a band where oxygen concentration is low, and aerobic bacteria accumulate where oxygen concentration is high (Beijerinck, 1893; Taylor *et al*., 1999). In some aquatic environments, bacteria accumulate in horizontal veils; each species navigates to the precise oxygen concentration, or reduction potential, that is optimal for growth (Taylor *et al*., 1999). Similarly, oxygen concentration is one of the ecological parameters that determine where commensal and virulent bacteria can establish colonies in mammals. As the mechanism of aerotaxis is investigated in different species, it is becoming clear that multiple strategies are utilized by bacteria to sense oxygen, but *E. coli* aerotaxis is the system that is understood best at the molecular level (Hou *et al*., 2000; Taylor *et al*., 1999).

E. coli does not sense oxygen directly; aerotaxis requires a functional electron transport system (Taylor *et al*., 1999). In response to an increase or decrease in oxygen, the respiratory components undergo oxidation or reduction, and the proton motive force changes accordingly. Aer and the serine chemoreceptor, Tsr, could sense the change in electron transport by sensing a change in redox potential, proton motive force, or possibly electron flux. These respiratory components are closely coupled, making it difficult to determine which parameter is the stimulus for *E. coli* aerotaxis. Recently, the parameters sensed by Aer and Tsr were successfully identified using electron transport system mutants that have different H^+/e^- ratios (Edwards *et al*., 2006). That is, each mutant differed in the number of H^+ atoms extruded by the electron transport system per electron that traverses the pathway. The flux of electrons through the pathway was similar for all mutants, indicating it was not the stimulus for aerotaxis. Tsr-mediated aerotaxis correlated directly with the jump in proton motive force when anaerobic *E. coli* were exposed to air (Fig. 2) (Edwards *et al*., 2006). On the other hand, Aer-mediated responses did not correlate with the jump in proton motive force. These and other findings indicate that Tsr senses changes in proton motive force and Aer directly, or indirectly, senses redox changes in the electron transport system. Further studies are needed to establish the precise sensing mechanism of Aer.

The ability to sense proton motive force or redox potential provides *E. coli* with a measure of internal energy. In aerobic bacteria, proton motive force is the major source of energy for ATP synthesis, active transport, and motility (Harold and Maloney, 1996). Homeostasis in cells ceases in the absence of a membrane potential. By sensing proton motive force or redox, rather than oxygen *per se, E. coli* is able to navigate away from any environmental insults that threaten energy production (Taylor *et al*., 1999). The threats include carbon starvation, respiratory poisons, hypoxia, and extremes in pH and redox. A universal receptor that can measure internal

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redox potential or proton motive force in all cells has long been hypothesized (Glagolev, 1980; Taylor *et al*., 1979), but Aer and Tsr were the first such receptors shown to sense internal energy (Bibikov *et al*., 1997; Rebbapragada *et al*., 1997). If there is a decline in internal energy, aerotaxis guides the bacteria to a more favourable environment where higher energy levels can be supported.

c. Signal excitation in the PAS domain

The PAS domain is the sensory input site for Aer (Bibikov *et al*., 2000; Rebbapragada *et al*., 1997; Repik *et al*., 2000). In the PAS superfamily, PAS domains are cytoplasmic sensing modules of signal transduction proteins in archaea, bacteria, and eukarya (Taylor and Zhulin, 1999). Stimuli recognized by PAS domains include light, oxygen, redox and small molecules. Biological processes that utilize PAS domains include global regulation of metabolism, behaviour and development in prokaryotes, and circadian rhythms, response to hypoxia, ion channel function, and development in eukaryotes. The specificity in sensing is determined, in part, by cofactors that are associated with PAS domains: 4-hydroxycinnamic acid in photoactive yellow protein (PYP)(Pellequer *et al*., 1998); a heme in the oxygen sensor, FixL (Gong *et al*., 1998); FAD in Aer and NifL (Bibikov *et al*., 1997; Crosson and Moffat, 2001; Repik *et al*., 2000); and flavin mononucleotide (FMN) in the plant NPH1 photoreceptor that also senses redox (Christie *et al*., 1999). Some PAS domains may not have a cofactor: the crystal structure of the PAS domain from the HERG cardiac K^+ channel lacks a cofactor (Morais Cabral *et al*., 1998).

The PAS structure, resolved for more than 15 proteins, including PYP, FixL, HERG, NifL and NPH1, has a distinctive αβ fold with a five-stranded antiparallel β-sheet core (Pellequer *et al*., 1998). The structure is also described as a left-handed glove in which the fingers enclose a pocket that binds the cofactor. The fingers of the glove are formed primarily from the β-sheet, the palm from α-helical loops and the thumb from β-strands (Gong *et al*., 1998). The common structure of PAS domains could be associated with similar mechanisms for signalling, but different cofactors in the PAS fold are likely to introduce significant variation in the signalling pathways. The *in silico* modelling of the Aer structure using the coordinates of resolved PAS structures has been very successful in advancing investigations of the Aer PAS domain. The *Azotobacter vinelandii* NifL and *Neurospora crassa* Vivid PAS domains were resolved recently with FAD cofactors (Key *et al*., 2007; Zoltowski *et al*., 2007) and the NifL coordinates were used to further refine the Aer model to include a putative position of the FAD cofactor (Fig. 3).

Critical residues for signalling by the Aer PAS domain have been identified (Bibikov *et al*., 2000; Buron-Barral *et al*., 2006; Repik *et al*., 2000; Watts *et al*., 2006b). Null Aer mutants have a signal-off conformation that produces a counterclockwise (CCW) rotational bias of the flagellar motors. The signal from Aer PAS enhances the signal-on conformation of the signalling domain (Fig. 1), imposing a clockwise (CW) bias on the motors. Thirteen cysteine PAS mutants had defective input-output control and were not rescued by simultaneous production of the Tar, Trg, and Tap chemoreceptors (Repik *et al*., 2000; Watts *et al*., 2006b). Cysteine replacements at Arg57, His58, and Asp60 abolished FAD binding to Aer. These residues surround the pocket in which FAD is predicted to bind (Fig 3). Residues Arg57, His58 and Asp60 are unique to the Aer _ PAS (FAD-binding) subfamily and are conserved in members of the subfamily, but not in other PAS domains (L. Ulrich, W. Black and I. Zhulin, personal communication). This suggests that they bind to FAD or are structural residues that determine the shape of the binding site. Although these particular mutants are stable, some null Aer mutants are unstable and give a false-negative result for FAD-binding. The lesions in those mutants are usually not associated with the FAD-binding site (Buron-Barral *et al*., 2006) Tyr111 also projects into the putative FAD pocket (Fig. 3) and the Aer-Y111C mutant has an

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inverse response in a temporal oxygen gradient (Repik *et al*., 2000). *E. coli* cells producing wild-type Aer give a positive (CCW) response to an increase in oxygen, whereas cells producing Aer-Y111C display a negative (CW) response. A putative signalling pathway in Aer is proposed to include residues in contact with the isoallaxazine ring of FAD that transduce redox changes in FAD into conformational changes in the PAS domain. The latter are then transmitted to downstream components of the pathway. The inverted aerotaxis response caused by the Y111C substitution can be explained by a 3-state model, where fully oxidized and reduced forms of FAD generate a CW signal, and the semiquinone generates a CCW signal. Normally, aerobic/anaerobic changes produce the semiquinone/quinol forms, but the mutation might shift the redox potential of the FAD cofactor so that it is fully oxidized during maximal rates of electron transport (Repik *et al*., 2000; Watts *et al*., 2006b).

The Aer PAS domain has a hot spot for mutations at the junction of the N-terminal cap (N-cap) and the PAS core, suggesting residues Thr19, Leu20, Met21, and Ser22 are critical for Aer structure and/or signalling (Watts *et al*., 2006b). The resulting null mutant Aer proteins did not bind FAD, suggesting that the N-cap stabilizes FAD binding to the PAS domain. Substitutions at Leu20 and Met21 are dominant, indicating that the defective receptors disrupt both signalling within the Aer dimer and interactions with neighbouring dimers. The PAS N-cap from HERG and PYP also have an important role in signalling. Deleting part of the N-cap from the PAS domain of the PYP photoreceptor prolonged the lifetime of the active form of PYP (Imamoto *et al*., 2002; van der Horst *et al*., 2001). Deleting the PAS N-cap from the HERG voltage-dependent K^+ channel had a similar effect on the kinetics of channel deactivation as removing the entire PAS domain (Morais Cabral *et al*., 1998).

Two classes of mutations in the PAS domain might interrupt the aerotaxis pathway from FAD to the downstream components (Buron-Barral *et al*., 2006; Repik *et al*., 2000). The first class results in proteins that bind FAD but have an extreme CCW bias (e.g., M21D, L20K, G42C, W79C, G90C in Fig 3). The second class includes those with a gain-of-function mutation that results in a strong signal-on (CW) bias (e.g., L20C, M34C, F66C, N85C). These variant proteins bind FAD, are not rescued by chemoreceptors, and are present at normal levels (Fig. 3). The CW-biased mutants probably augment an interaction between the PAS and HAMP domains that promotes CW signal output [(Buron-Barral *et al*., 2006); see section d below].

PAS domains are present as dimers in other sensory proteins, but it is not known whether the Aer PAS domain forms stable dimers. The *A. vinelandii* NifL PAS-FAD domain crystallized as a dimer, in part due to a N-cap dimerization motif (A'α), which is also conserved in Aer (Key *et al*., 2007). In NifL, the A'α helix packs against the hydrophobic surface of the β-sheet on the opposing monomer. The Aer-PAS domain is predicted to have a similar PAS/PAS' dimerization interface. The putative contact surfaces would be asymmetric and would not have been detectable by our earlier study of symmetric crosslinking surfaces in the Aer N-cap (Watts *et al*., 2006b).

The most significant gap in our understanding of the Aer signalling pathway is the mechanism by which PAS-FAD detects changes in the electron transport system when the concentration of oxygen increases or decreases. Two hybrid studies designed to identify direct interaction of PAS-FAD with a respiratory component, such as NADH dehydrogenase 1 (NDH-1), were unsuccessful. NDH-1 has an important role in Aer signalling but is not essential (Edwards *et al*., 2006). Another possibility is that PAS-FAD interacts with a quinone, NADH or free FAD/FADH from the electron transport system (Fig. 4). The purified Aer protein has a low affinity for FAD and it is possible that the FAD cofactor in the PAS domain readily exchanges with the cytoplasmic FAD/FADH pool.

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In *E. coli,* the NADH-dependent flavin reductase (Fre) reduces approximately 80% of all free cytosolic FAD (Woodmansee and Imlay, 2002). Purified FAD-bound Aer is reduced by Fre and an *E. coli fre* mutant is only weakly aerotactic in succinate soft agar (Webster and Xun, 2003), suggesting that Fre might be involved in the aerotaxis signalling pathway (Fig. 4). Expression of Fre from a plasmid or slight overproduction of Aer, however, restores normal aerotaxis (L. Xun and M.S. Johnson, unpublished observation). Higher Aer levels inhibited swarming in *fre* and wild-type cells. These results suggest that Fre contributes to the parameters sensed by Aer on succinate soft agar, but Aer does not require Fre expression to function. The effects of Fre are similar in a true oxygen gradient. In an open-ended capillary tube, *E. coli* respiration generates an oxygen gradient and the cells form a band near the meniscus at a preferred oxygen concentration (Taylor *et al*., 2001). Cells with Aer-mediated aerotaxis consistently banded further from the meniscus in the absence of Fre than when Fre was present. This indicated that the *fre* strain preferred a lower oxygen concentration. These results can be accommodated by the following hypothesis. Fre maintains the intracellular reducing potential within a range favourable to growth and aerotaxis. In the absence of Fre, the reduction potential of FAD/FADH is lower and the critical number of Aer receptors in a reduced, signal-on state is reached at a lower concentration of environmental oxygen. The number of reduced Aer receptors in the *fre* strain is less than the critical number for signalling at normal oxygen levels but can be increased by overproduction of Aer. This hypothesis can also explain the observation that, in a capillary, the band of *E. coli* cells moves closer to the meniscus when Aer is overproduced (Ma *et al*., 2005). In Tsr-mediated aerotaxis, which is not dependent on reduced flavin for signalling, the band position did not reproducibly shift in *fre* strains.

d. HAMP domain structure and function

The HAMP domain in *E. coli* chemoreceptors was initially considered a linker that relayed a mechanical displacement in the periplasmic sensor domain to the signalling domain (compare Fig. 1). The linker was shown to consist of two amphipathic helices (AS-1 and AS-2) connected by a sequence of unknown structure (Butler and Falke, 1998). The HAMP domain was recognized as a sensory transduction module after *in silico* analyses showed that the domain is widely conserved in sensory proteins that include **h**istidine kinases, **a**denyl cyclases, **m**ethyl-accepting chemotaxis proteins and **p**hosphatases (Aravind and Ponting, 1999;Williams and Stewart, 1999). The AS-2 helix, as defined, ends at residue C253 in Aer but the sequence is conserved in HAMP proteins through Aer residue 271, forming a proximal signalling domain (Ma, 2001;Ma *et al*., 2005) (Fig. 5A).

Recently the tertiary fold of an isolated HAMP domain from the *Archaeoglobus fulgidus* Af1503 protein was resolved (Hulko *et al*., 2006). The AS-1 and AS-2 helices from the dimeric protein are folded into a parallel four-helix bundle with the connectors tightly packed into a groove between AS-1 and AS-2 (Fig. 5B). The packing of HAMP core residues deviates from the knobs-into-holes packing that defines a coiled-coil structure. The latter geometry specifies the packing of a core residue (knob) from one helix into a space surrounded by four residues from the facing helix (hole). In the Af1503 HAMP domain, the residues of the hydrophobic core have knobs-to-knobs packing with alternating layers in which hydrophobic side chains point at the central supercoil axis in one layer, and point sideways to form an interacting ring of residues enclosing the central cavity in the alternating layer (Fig. 5C) (Hulko *et al*., 2006).

The solution structure of the Af1503 HAMP domain is of great interest, but there is some concern that the structure of an Af1503 HAMP domain may be atypical and not resemble the conformation of other HAMP domains.The Af1503 HAMP domain is normally tethered to a transmembrane domain *in vivo* but not to a signalling domain, as are most HAMP domains, and it has no known signalling function. However, there are now some indications that the structure of other HAMP domains may be similar to the Af1503 HAMP domain. Models based

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on the coordinates of the Af1503 HAMP structure have been developed for the HAMP domains from Aer (K. Watts, unpublished data) and the *Salmonella* Tar chemoreceptor (K. Swain and J. Falke, personal communication). Investigations are currently underway to test these *in silico* models. In crosslinking studies, the cysteine replacements with the most extensive crosslinking are those that collide most often and are therefore expected to be at the dimer interface. The residues on the AS-1 and AS-2 dimer interface of the Aer model were similar to the residues predicted by *in vivo* crosslinking to be on the interface (K. Watts, unpublished observation), and mutations that disrupt Aer stability and maturation would be predicted to disrupt the four-helix structure (K. Watts, unpublished finding).

One of the properties that makes HAMP domains so interesting is their versatility. The ligand-binding domains of *E. coli* chemoreceptors generate a mechanical signal, an inward displacement of the second transmembrane segment that is perpendicular to the plane of the membrane (Falke and Hazelbauer, 2001; Milburn *et al*., 1991). On the other hand, in Aer, the PAS domain interacts directly with the HAMP domain so that the signal is transmitted through the cytoplasm and parallel to the plane of the membrane. The strongest evidence for a direct PAS-HAMP interaction is from allele-specific suppression in intragenic pseudoreversion analysis (Watts *et al*., 2004). Specifically, a missense mutation in the HAMP domain (C253R) is exclusively suppressed by a missense mutation in the PAS domain (N34D), indicating that these residues are in close proximity in the Aer protein (Watts *et al*., 2004). It remains to be determined how a HAMP domain can transform different signals from chemoreceptors and Aer into a common output signal for the similar signalling domains in these proteins. Considering that there are more than 8,300 HAMP domains, it is likely that HAMP domains have adapted to process a variety of different transduction signals.

e. PAS-HAMP as input-output module

There is increasing evidence that the PAS and HAMP domains function together as the input-output module for Aer. Chimeric receptors were constructed by fusing the PAS domain of Aer to the signalling domain of the Tar or Tsr chemoreceptors (Bibikov *et al*., 2000; Repik *et al*., 2000). The chimeric receptors were competent for signalling only if the PAS and HAMP domains were both derived from Aer. This suggests that specific contact surfaces are required for signalling between the domains. Mutations in the HAMP domain can affect FAD binding to the PAS domain and maturation of the Aer protein (Bibikov *et al*., 2000; Buron-Barral *et al*., 2006; Ma *et al*., 2005). Folding of the nascent Aer peptide into the native protein is a complex process. The nascent peptide is sequestered in the GroEL chaperone protein until critical structural determinants are correctly assembled (Herrmann *et al*., 2004). The determinants include the transmembrane sequences and AS-1. To complete folding of the PAS domain, AS-2 is also required and interaction of the HAMP and PAS domains is postulated. Aer folding has been investigated by comparing proteolytic degradation of variant Aer proteins that have residue replacements in the HAMP domain. Some variants exist as a mixture of native and partially unfolded forms (Buron-Barral *et al*., 2006). There are varying degrees of stability of the mature protein, which presumably depend on how far the folding process has progressed. A critical step in maturation is FAD binding. FAD deficiency could result from failure of the Aer protein to complete the folding process, or alternatively, FAD binding might be required before folding can be completed.

Most HAMP mutations that prevent FAD binding are suppressed by three non-specific suppressor mutations that map in the PAS domain (Watts *et al*., 2004). These suppressors restore FAD binding, signalling and stability to null HAMP mutants (Buron-Barral *et al*., 2006; Watts *et al*., 2004). By analogy to the NifL PAS domain, the suppressors (S28G, A65V, A99V) are predicted to surround the FAD binding pocket and might increase the affinity of the PAS domain for FAD (Fig. 6). The clustering of critical residues and suppressor mutations

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around the putative FAD binding site (Fig. 6) supports a role for FAD binding in Aer maturation. We propose that interactions between HAMP AS-2 and the PAS domain stabilize folding of the PAS domain into the FAD-binding conformation and that FAD binding is necessary for completion of the maturation process. Once the native fold is formed, it is relatively stable and FAD can be removed by dialysis and replaced without significant degradation of the protein (M.S. Johnson and S. L. Fry, unpublished observation).

The lesions that affect Aer maturation are distributed throughout the HAMP domain (Fig. 5A) (Buron-Barral *et al*., 2006). However, it is likely that interactions required for Aer maturation are between PAS and HAMP-AS-2. In addition to allele-specific suppression between AS-2 and PAS (Watts *et al*., 2004), there are conserved residues in all AS-2 sequences of Aer-like proteins, more so than in the HAMP AS-1 and connector. Our current hypothesis is that maturation requires a four-helix HAMP structure to interact with the nascent PAS domain. Lesions in AS-1 and the connector that interrupt maturation are proposed to disrupt the four-helix HAMP structure.

In addition to PAS-HAMP contacts that are required for maturation, there is a second class of interaction in which the PAS domain promotes formation of the kinase-on (CW) conformation of the HAMP domain (Fig. 5A) (Buron-Barral *et al*., 2006;Ma *et al*., 2005). Gain-of-function mutants with a CW-bias in signalling have lesions in the PAS or HAMP domain that augment the PAS-HAMP interaction that is responsible for promoting CW signalling output. Such lesions in the HAMP domain are focused at the AS-1/connector junction and AS-2/proximal signalling junction. In the folded four-helix HAMP structure, the CW lesions from AS-1 and AS-2 are grouped around the distal end of the HAMP domain and likely define the contact domain where the PAS domain promotes CW signalling by the HAMP domain. The PAS domain appears to interact with the HAMP domain from the cognate subunit in the Aer homodimer (Watts *et al*., 2006a).

Null mutants with defects in the proximal signalling domain differ from those with defects in the HAMP domain. The proximal signalling mutants have higher levels of Aer, bind FAD and are dominant over wild-type Aer (Buron-Barral *et al*., 2006; Ma *et al*., 2005). Some defects are rescued in the presence of the Tar or Tsr chemoreceptors, and these defects might have less severe structural effects on maturation. Other lesions are not rescued and are more likely to affect determinants or structures for PAS/HAMP signalling directly.

There are further clues to the signal transduction mechanism in the PAS-HAMP module. The knobs-into-holes and knobs-to-knobs packing geometries predicted for the Af1503 HAMP domain are nearly isoenergetic and could be interconverted by a concerted rotation of 26° in all four helices (Fig. 5C, D) (Hulko *et al*., 2006). The interdigitation of side chains gives this HAMP rotation the characteristics of a gear box with four cogwheels, where neighbouring helices must rotate in opposite directions. The four helices of the Aer HAMP domain might also rotate between the kinase-on and kinase-off state. If so, changes in the redox state of the isoalloxazine ring of the FAD co-factor in the PAS domain must trigger an appropriate HAMP rotation, presumably through contact of the PAS domain with the distal end of the four-helix HAMP domain (Fig. 5A) (Ma *et al*., 2005; Watts *et al*., 2004). However, it is unlikely that the final output from the signalling domain is a rotation. In a fluorescence polarization study of signalling by *E. coli* chemoreceptors, the motion of a yellow-fluorescent-protein probe was interpreted to be consistent with displacement but not rotation of the signalling domain (Vaknin and Berg, 2007). Signalling changed the spacing between chemoreceptor dimers within a trimer-of-dimers: attractants increased the spacing by 10% and repellents decreased the spacing. Flexing of the signalling domain is made possible by a glycine-hinge (Gly330 and Gly331 in Aer) within the flexible-bundle subdomain of the signalling domain (Coleman *et al*., 2005). Aer presumably uses a similar signalling mechanism because the signalling domains

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of chemoreceptors are interchangeable with the Aer signalling domain (Bibikov *et al*., 2000; Repik *et al*., 2000), and Aer can signal in mixed trimers-of-dimer teams with the other chemoreceptors (Gosink *et al*., 2006).

A mechanical (Lego©) model that simulates a four-helix domain is able to convert a gear-box rotation of the 'helices' into bending of an attached helix, if a flexible hinge is inserted at the distal end (R. Alexander and I. Zhulin, personal communication). It remains to be seen whether the mechanical model has relevance for HAMP proteins. The proximal signalling domain that adjoins the HAMP domain is a candidate for a hinge in Aer (Fig. 5A). Crosslinking data indicate that the helical structure of the proximal signalling domain is interrupted by a four-residue loop. The concept of bending is conjecture at this time but torque conversion of a HAMP signal into displacement of the signalling domain is a fruitful area for further investigation in Aer, and in chemoreceptors, to determine whether torque conversion is a general principle in these sensory systems..

f. Organization of the Aer receptor

The basic building block of the Aer protein is a homodimer (Ma *et al*., 2004). Domain interactions that transduce the aerotaxis signal could occur in *cis* (within one subunit), or in *trans* (between cognate subunits). To address this question, two Aer proteins with amino acid substitutions in different domains were expressed from compatible plasmids and intragenic complementation was examined (Watts *et al*., 2006a). Aer heterodimers can function with one wild-type PAS and HAMP sequence per dimer, provided the functional PAS and HAMP sequences are on different subunits. However, Aer signals from the PAS to the signalling domain of the same subunit. To confirm an intrasubunit signalling pathway, truncated Aer proteins were produced together with a full-length Aer-HAMP missense variant. Truncations that deleted most of the signalling domain ($Aer_{1\text{-}285}$), or the PAS domain ($Aer_{120\text{-}506}$) from the non-signalling subunit, did not disrupt aerotaxis of heterodimers (Fig. 7). However, two complete HAMP sequences were required for aerotaxis, even where one sequence had an amino acid substitution. This suggests that a four-helix HAMP structure is essential for signalling. Aer can therefore sense and signal with one PAS and one signalling domain per dimer, provided they are on the signalling subunit. However, as stated above, the AS-2 helix interacts with the cognate PAS domain to stabilize the native fold of Aer. These findings do not exclude the likelihood that signalling domains, and possibly PAS domains from heterodimers, dimerize by interacting with a second heterodimer.

Aer, like chemoreceptors (Kim *et al*., 2002), forms trimers-of-dimers, the functional signalling team for signal transduction (Gosink *et al*., 2006). Aer is a low abundance receptor, comprising only about 2.5% of the total receptor pool (D. Salcedo and M. S. Johnson, unpublished observation), but Aer apparently enhances signalling by forming mixed trimers-of-dimers with the high abundance receptors Tsr and Tar (Gosink *et al*., 2006). An important unknown is whether the abundant receptors have a role in adaptation of Aer to aerotaxis stimuli. Aer lacks the glutamyl-methylation system that enables Tar and Tsr to adapt to stimuli (Bibikov *et al*., 2004; Niwano and Taylor, 1982). However, Aer can function in *E. coli* without chemoreceptor assistance, provided that it is produced at high enough levels to establish a suitable prestimulus swimming pattern.

g. Summary

Aer and the aerotaxis system enable *E. coli* to navigate to environments that support optimal energy production. Aer is a cytoplasmic receptor that senses changes in the redox state of the electron transport system via an FAD-containing PAS domain. The PAS and HAMP domains constitute an input-output module that transduces FAD reduction into a signal that promotes a signal-on conformational change in the HAMP domain. The emerging paradigm for Aer

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includes a four-helix HAMP domain which is a versatile signal-transduction module that might respond to diverse stimuli by rotating interlocking helices. The HAMP output is probably not a rotation but might flex the signalling domains of Aer and chemoreceptor proteins.

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Abbreviations

CW, clockwise; CCW, counterclockwise.

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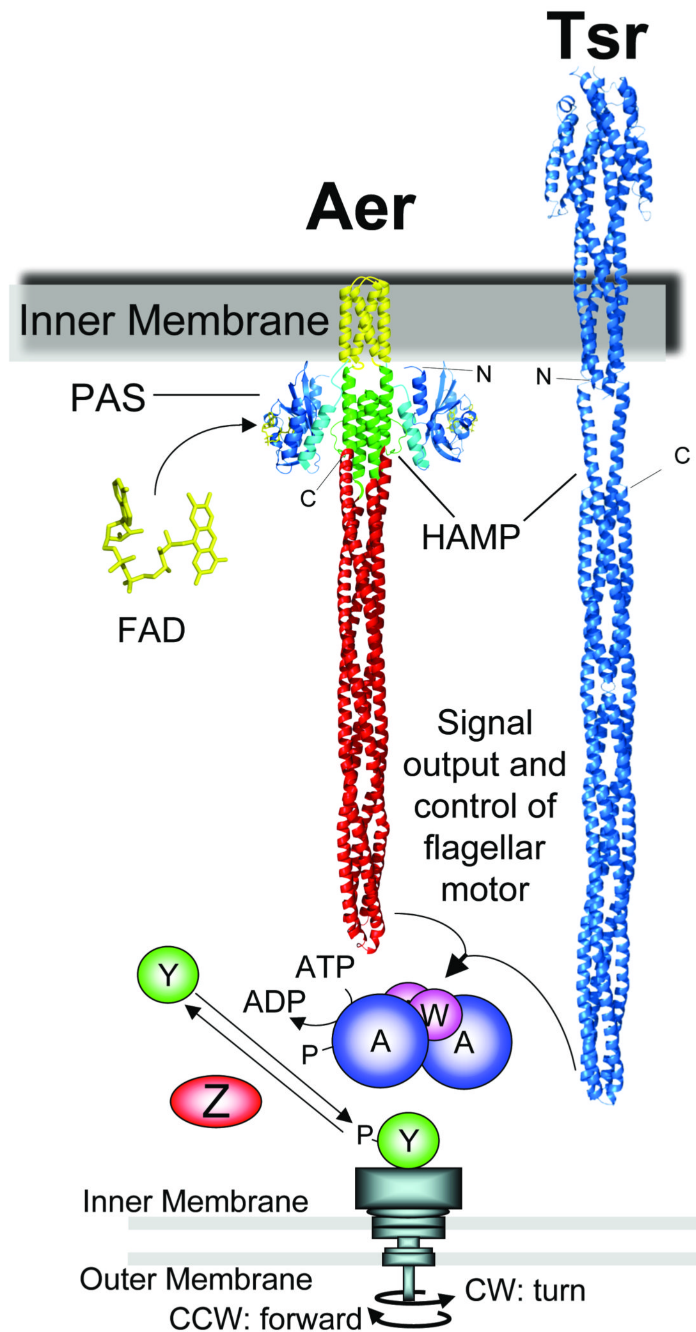

Fig. 1.

Comparison of the *E. coli* aerotaxis receptor, Aer, and the Tsr chemoreceptor, an alternative receptor for aerotaxis. The HAMP domain is shown in Tsr as an extended helix and in Aer as a four-helix bundle to illustrate different models for HAMP structure. Receptors shown are *in silico* constructions modeled from resolved structures and disulfide crosslinking studies: Aer-PAS [blue, (Key *et al*., 2007)]; F-1 (cyan); Aer-transmembrane [yellow, (Amin *et al*., 2006)]; Aer-HAMP [green, (Hulko *et al*., 2006)]; Aer-signalling [red, (Park *et al*., 2006)]. Tsr coordinates are from S-H. Kim as described in (Chi *et al*., 1997; Kim *et al*., 1999). The chemotaxis signalling pathway that controls flagellar rotation is also shown. Abbreviations:

A, CheA protein; W, CheW; Y, CheY, Z, CheZ; P, phosphate; IM, inner membrane; OM, outer membrane.

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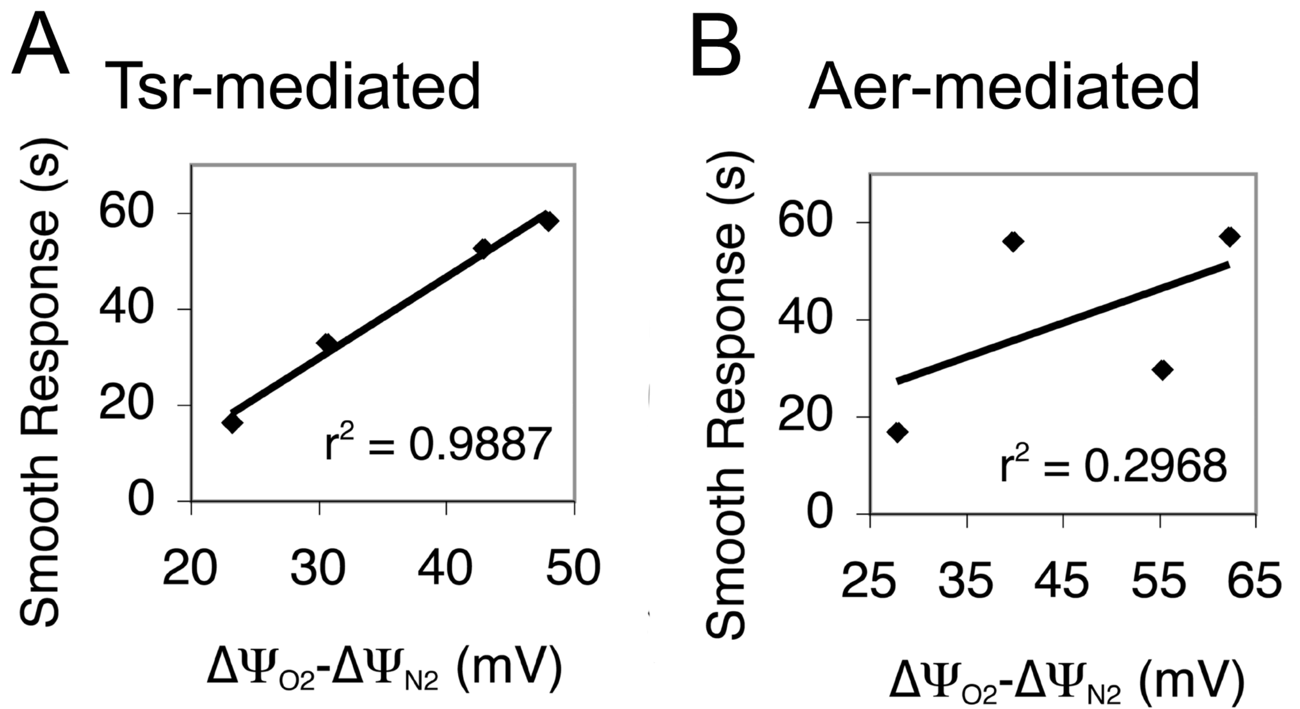

Fig. 2.

Comparison of the aerotaxis response of Aer- and Tsr- expressing strains as a function of the step increase in $\Delta\psi$ ($\Delta\psi_{O2}$ - $\Delta\psi_{N2}$, the jump in proton motive force) when anaerobic bacteria are exposed to air [modified from (Edwards *et al*., 2006)]. Abbreviation: r^2, coefficient of determination

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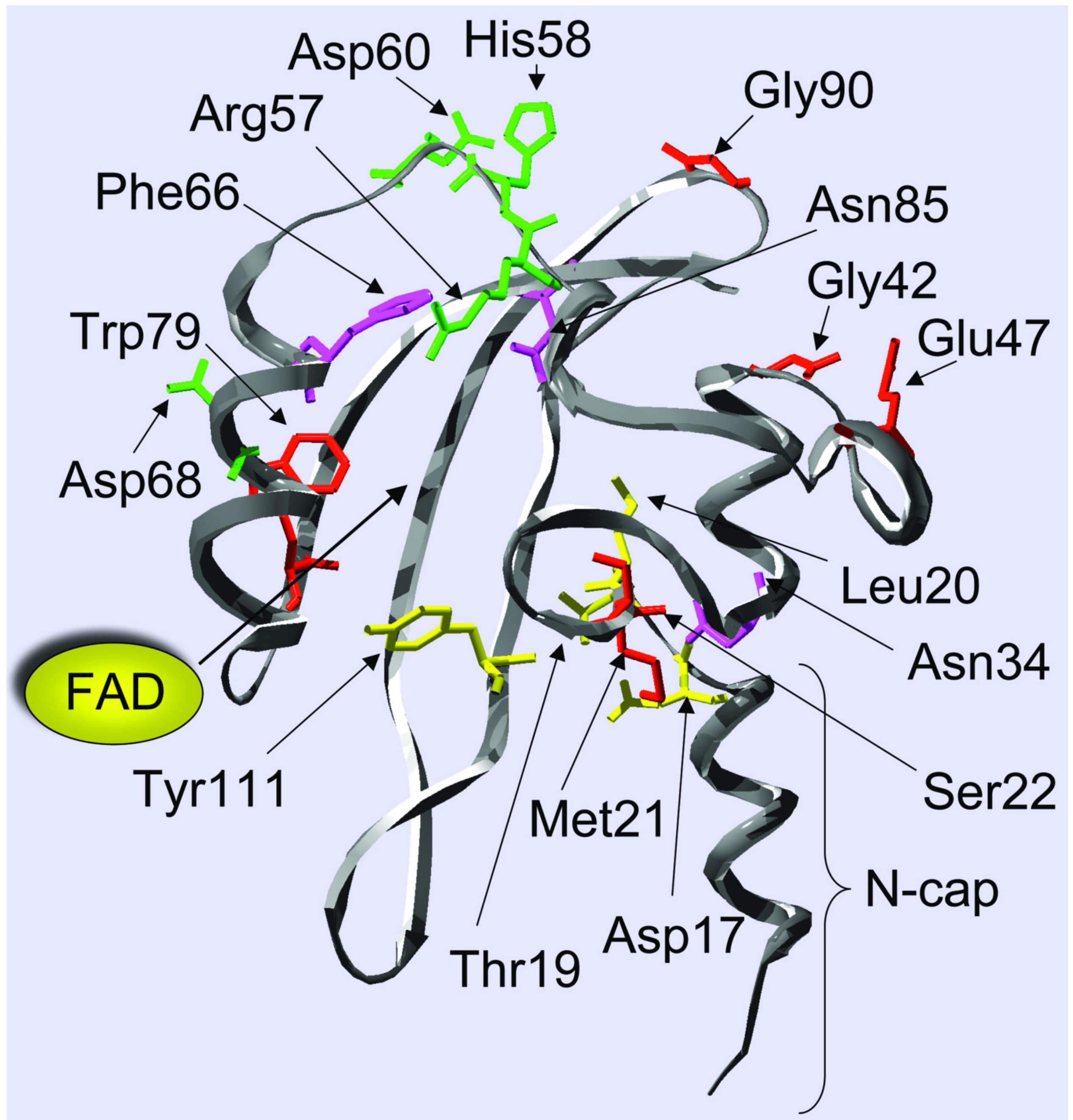

Fig. 3.
In silico model of the Aer PAS domain, highlighting residues involved in aerotaxis. The cleft in which FAD binds is shown. Replacement of the residues shown produced a null aerotaxis phenotype (red), a loss of FAD binding and a null phenotype (green), an inverted response (yellow), and a CW-signalling bias (magenta).

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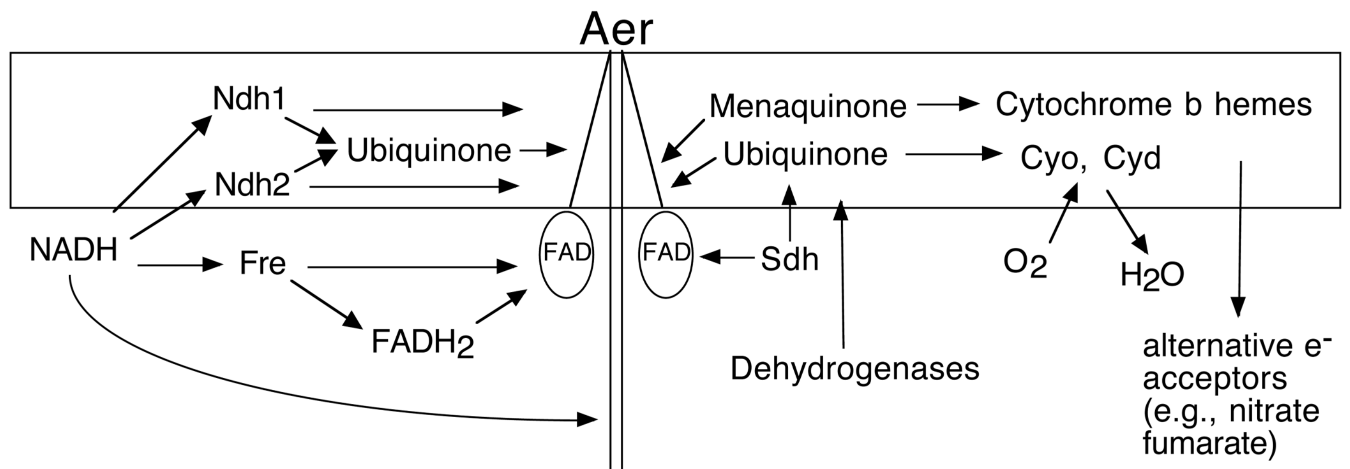

Fig. 4.

Components that might influence Aer signalling activity. Abbreviations: Cyo, cytochrome *bo*; Cyd, cytochrome *bd*; Ndh1, NADH dehydrogenase 1; Ndh2, NADH dehydrogenase 2; Sdh, succinate dehydrogenase.

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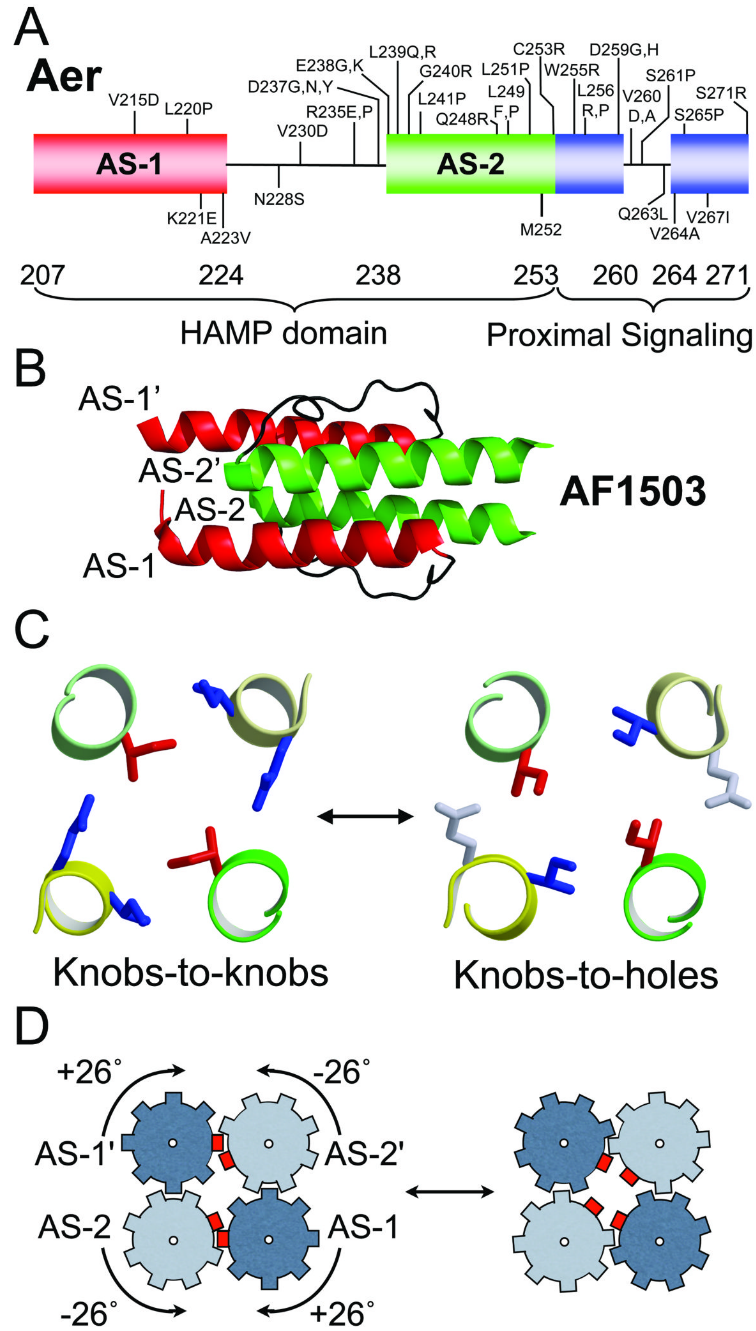

Fig. 5.

The Af1503 HAMP domain as a model for the Aer HAMP domain. A. Domain sub-structure of the Aer HAMP and proximal signalling domains showing gain-of-function residue substitutions (vertical lines below the HAMP diagram) that resulted in Aer proteins with a CW-biased signal output and bound FAD. Vertical lines above the HAMP diagram represent substitutions that resulted in a null aerotaxis phenotype, loss of FAD binding and defective maturation of Aer [Data from (Buron-Barral *et al*., 2006; Ma *et al*., 2005)]. B. Solution structure of the isolated Af1503 HAMP domain from *A. fulgidus*. C. The knobs-to-knobs packing geometry of the Af1503 HAMP domain is converted to a knobs-to-holes geometry by a 26° rotation of the HAMP helices. Compare the orientation of Ile residues (red). D. Proposed

direction of rotation of interlocking helices in the Af1503 HAMP domain. (B, C, and D are modified from (Hulko *et al*., 2006). Used with permission).

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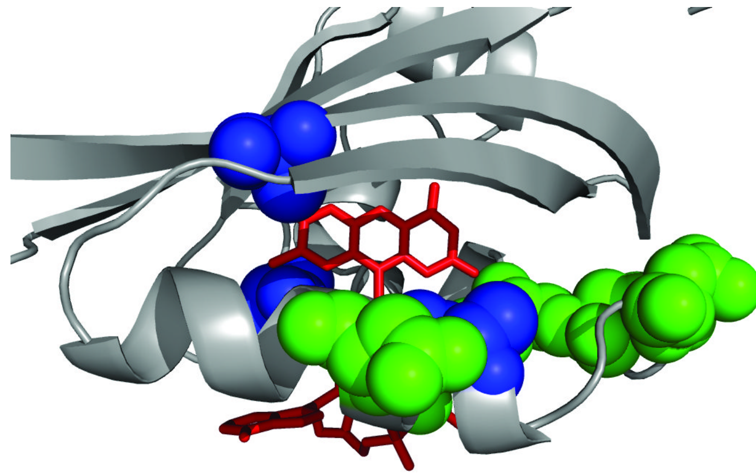

Fig. 6.
A view of the FAD-binding cavity in Aer PAS showing residues critical for FAD binding (green), and location of general suppressors (blue) of HAMP[AS-2] null mutants. FAD coordinates are modeled on the crystal structure of the NifL protein.

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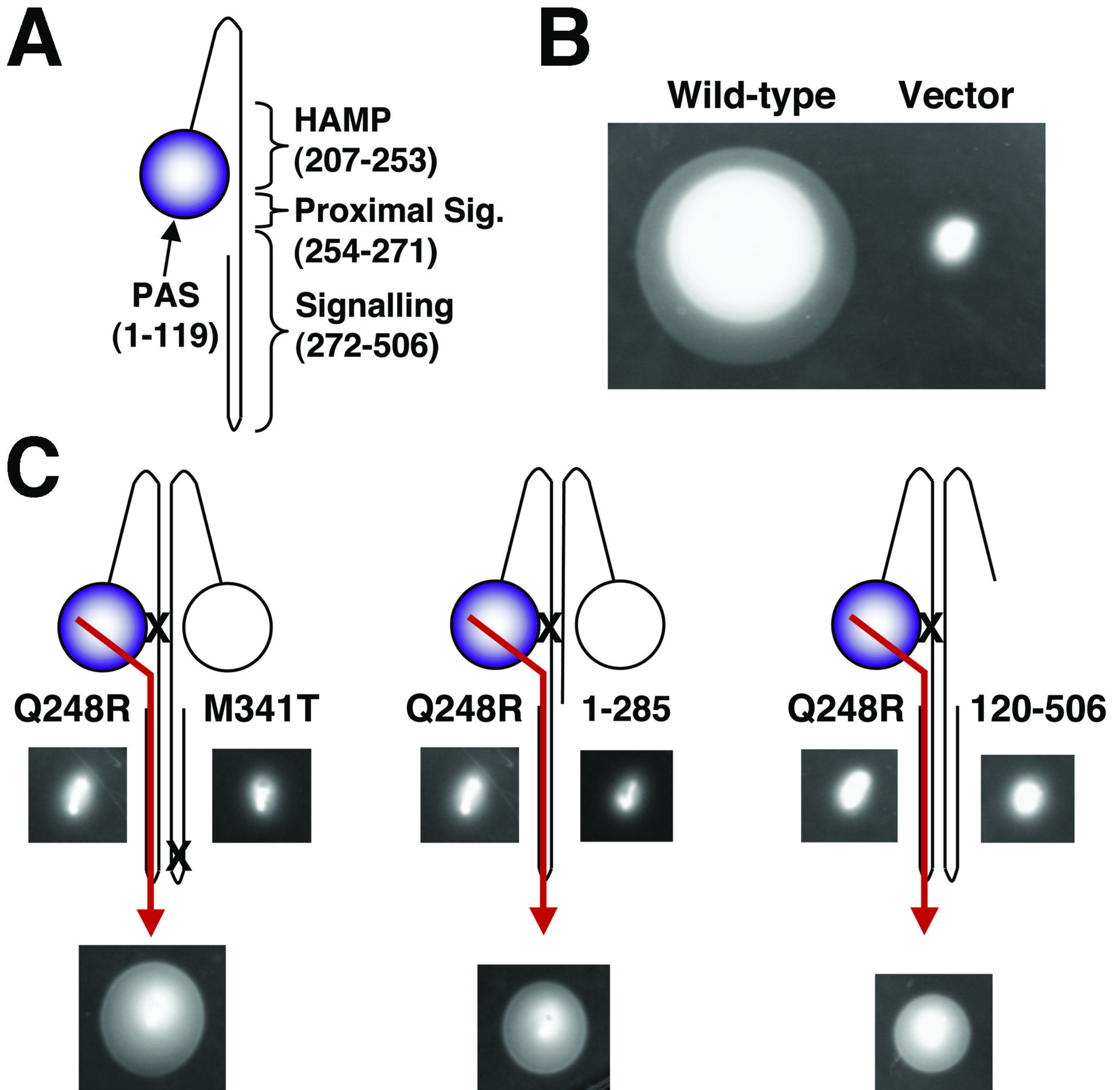

Fig. 7.

The minimal sequence required for aerotaxis in an Aer heterodimer. A. The organisation and boundaries of domains relevant to the experiments described in B and C. B. For heterodimer experiments, mutant Aer constructs were expressed from compatible plasmids in BT3400 (*aer*, *tsr*, *recA*), an aerotaxis- and recombination-deficient *E. coli* strain. In succinate soft agar, aerotactic colonies, such as BT3400 expressing wild-type Aer, expand rapidly and form a defined ring at their edge. In contrast, non-aerotactic colonies (e.g., BT3400 expressing vector alone) stay trapped near the inoculation site. C. In succinate soft agar, colonies formed by cells expressing the homodimers of each mutant Aer protein were non-aerotactic (as shown on both sides of each cartoon), whereas colonies formed by cells expressing the heterodimers shown were aerotactic (as shown beneath each cartoon). Arrows indicate the proposed signalling

pathway within each heterodimer. Heterodimer experiments, such as those shown, indicate that Aer requires one PAS and one signalling domain, but two HAMP domains, in order to support aerotaxis. Modified from (Watts *et al*., 2006a). Used with permission. Abbreviation: Sig., signalling.

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