Table S1. Summary of Aer substitutions and possible roles of the native amino acids

Substitution and signaling phenotype[a]			Group[b]	Possible role based on phenotype[a], residue conservation[b], position in the *in silico* model[c], or function in other PAS domains[d]
OFF	ON	ND		
	T8W		1	N-cap structure. Substitution: (Watts et al., 2006)
	T12C		4	N-cap structure. Substitution: (Watts et al., 2006)
	L14A,H, N,P		4	In *Av*NifL, the equivalently positioned residue hydrogen bonds to Asn51 in the PAS core; may stabilize the N-cap to the PAS core. Substitutions: (Watts et al., 2006)
	*D17G**		4	N-cap structure. First residue in N-cap loop. Substitution: (Watts et al., 2006)
	T18I, N		4	Possible hinge in the N-cap loop. Substitution: this study
T19S	T19A *E,I,L,K*, P*,R**		4	Inverted response: K,P,R. Possible hinge in the N-cap loop. Substitutions: this study; (Watts et al., 2006)
L20K	L20P,Q *C,T**		2	Near N-cap loop; may be involved in signaling; Substitutions: (Watts et al., 2006)
M21K, *D,P*	M21T, *S*		4	Near N-cap loop; may be involved in signaling; Substitutions: (Watts et al., 2006)
S22P		*S22P,F*	2	In *Av*NifL, S39 is part of a network of bond donors and acceptors that facilitate uptake and release of protons at N5. Substitutions: this study; (Bibikov et al., 2000)
T24P			2	FAD-binding defective; likely important for structure. Substitution: this study
D25V			2	Low stability, rapid proteolysis; in PYP, D34 forms critical bonds for stabilizing fold; likely important for structure. Substitution: this study
	S28C		4	More often glycine in PAS_FAD1 and PAS_FAD2 subfamilies; may be critical for stability near FAD. Substitution: this study
I30N			2	Low stability; may be important for structure. Substitution: this study
T31A			1	Substitution: this study
H32R			4	FAD-binding defect; may be involved in structure. Substitution: this study
N34H, K	*N34C,D*		3	In *Av*NifL, N51 interacts with V31 (equivalent to Aer L14) to form a structurally continuous helix (N-cap and Cα helix) that is discontinuous in sequence; in PYP, N43 forms critical hydrogen bonds for stabilizing fold; likely important for structure. Substitutions: this study; C, (Repik et al., 2000); D, (Watts et al., 2004)
D35G, N, V			4	D35V has low stability with rapid proteolysis; likely important for structure. Substitutions: this study
F37I			3	In *Av*NifL, the side chain of F54 separates water cavities; likely important for structure. Substitution: this study
S41R			2	Low stability; in PYP, T50 binds the phenolic hydroxyl at the buried tip of the chromophore; likely important for structure. Substitution: this study
G42C			3	Substitution: (Repik et al., 2000)

Y43N			3	FAD-binding defective; in PYP, R52 binds the phenolic hydroxyl at the buried tip of the chromophore; may be important for structure. Substitution: this study
E47C			3	Substitution: (Repik et al., 2000)
L48S			2	FAD-binding defective; may be important for structure. Substitution: this study
H53R	H53Y		3	In *Av*NifL, E70 lies directly over the N5 atom of the isoalloxazine ring and is poised to accept a proton from the reduced form of the FAD; In Vivid, C108 forms a light-induced, covalent flavin-cysteinyl adduct; In MmoS, the side chain of H132 is 3.2 Å from N5 and proposed to play a role in protonation and deprotonation of N1 and N5. Substitution: this study
	N54D		1	In MmoS, the side chain of R133 stabilizes the FAD ribityl chain. Substitution: this study
R57C$^{\Delta}$	R57C, H	*R57H*	1	Highly conserved RHPDMP motif; important for structure/function; In MmoS, the side chain of N136 binds a common water molecule with O2 of FAD. Substitutions: this study; *C,* (Repik et al., 2000); *H*, (Bibikov et al., 2000)
H58R,*C*			1	Highly conserved RHPDMP motif; likely important for structure/function. Substitutions: this study; (Repik et al., 2000)
D60V, *C*		*D60N*	1	Highly conserved RHPDMP motif; likely important for structure/function. Substitutions: this study; C, (Repik et al., 2000); D, (Bibikov et al., 2000)
M61K			1	Highly conserved RHPDMP motif; likely important for structure/function; Substitution: this study
P62Q		*P62T*	1	Highly conserved RHPDMP motif; likely important for structure/function. Substitutions: this study; (Bibikov et al., 2000)
	F66L		1	May be important for signaling
D68G, N, V, *C*			2	D68G has an FAD-binding defect; D68N, V have low stability; may be important for structure. Substitutions: this study; (Repik et al., 2000)
M69T	M69I, K	*M69V*	2	In MmoS, M148 is 6 Å from the FAD isoalloxazine ring; posited to mediate redox potential. Substitutions: this study; (Bibikov et al., 2000)
	W70R		3	In *Av*NifL, the side chain of W87 stacks with the adenine base and H-bonds to the FAD ribityl; In MmoS, W149 stacks with the adenine ring and may mediate electron transfer from membrane and W158 (Aer-W79) to FAD. Substitution: this study
T72I			2	Low stability; may be important for structure. Substitution: this study
G76E			2	This was the only leaky mutant protein that recovered normal aerotaxis function in a temporal assay at 200 μM IPTG induction. Substitution: this study
E77G			4	FAD-binding defect; partial loss of function. Substitution: this study
W79R,S, *C*			2	In MmoS, W158 was postulated to facilitate electron transfer from the membrane redox component to W149 (Aer-W70), then FAD. Substitution: this study; (Repik et al., 2000)
S80I			4	Substitution: this study
G81D			2	FAD-binding defective; may be important for structure. Substitution: this study

	N85D, S			In *Av*NifL, N102, and in MmoS, N164 binds to the N3 and O4 of FAD. Substitution: this study
R87H, P			4	Substitution: this study
G90C			3	On a loop between Gβ and Hβ. Substitution: (Repik et al., 2000)
Y93C, H		*Y93H*	2	In *Av*NifL, Y110 is part of a network of bond donors and acceptors that facilitate uptake and release of protons at N5. Substitutions: this study; (Bibikov et al., 2000)
W94G, L, R			3	W94G has an FAD-binding defect; W94L,R have low stability; may be important for structure. Substitution: this study
V95A			2	Low stability, rapid proteolysis; likely important for structure. Substitution: this study
	A97D		3	May be involved in signaling. Substitution: this study
	N98I		4	May be involved in signaling. Substitution: this study
	V100L		4	May be involved in signaling. Substitution: this study
P101L			3	High conservation in all PAS subfamilies; likely important for structure. Substitution: this study
	V103G		4	Substitution: (Burón-Barral et al., 2006).
R104H, L,*C*			4	Substitutions: this study; (Bibikov et al., 2000)
G110D		*G110S*	1	Low stability, rapid proteolysis; may be important for structure. Substitutions: this study; (Bibikov et al., 2000)
Y111N	*Y111C**		2	C, inverted response. May be involved in signaling. Substitutions: this study; (Repik et al., 2000)
M112K	M112I, T,V,*V,I,*		4	In *Av*NifL, L130 in the Iβ-strand packs against the N-cap of the opposite monomer. Substitutions: this study; (Burón-Barral et al., 2006)
S113L, P, *P*	S113T		2	In MmoS, the S193 side chain binds a common water molecule with the N5 of FAD. Substitutions: this study; (Burón-Barral et al., 2006)
	I114C		4	Usually valine in PAS_FAD1 subfamily. Rescued by Tar. May contact HAMP domain (K. Watts, unpublished). Substitution: (Campbell, 2010)

*, inverted response, where cells were constantly tumbly in air, and smooth in nitrogen.

$^{\Delta}$, a previous result in which His-tagged Aer-R57C was identified as a signal-off mutant that lacked FAD binding (Repik et al., 2000). This result was likely due to the low stability of the His-tagged construct.

a. Phenotypes: Signal-on, signal-off, ND (not determined; these are from previous studies), FAD-binding defect, low stability. Substitutions identified in previous studies are in italics.

b. Group 1: Substitutions at residues that are conserved in the PAS_FAD1 (Aer) subfamily only; Group 2: Substitutions at residues that are conserved in the PAS_FAD1 subfamily and at least one FAD-binding (non-Aer) PAS domain; Group 3: Substitutions at residues that are conserved in PAS_FAD1, PAS_FAD2 (AerC) and at least one FAD-binding (non-Aer) PAS domain; Group 4: Substitutions at residues that are not conserved within any group. Sequence conservation and domain architecture are from (Xie *et al.*, 2010).

c. The Aer-PAS domain was modeled from the resolved structure of *Av*NifL [2GJ3; (Key *et al.*, 2007)].

d. Analyses of PYP, Mmos, AvNifL and Vivid are from (Pellequer *et al.*, 1998), (Ukaegbu & Rosenzweig, 2009), (Key et al., 2007) and (Zoltowski *et al.*, 2007), respectively.

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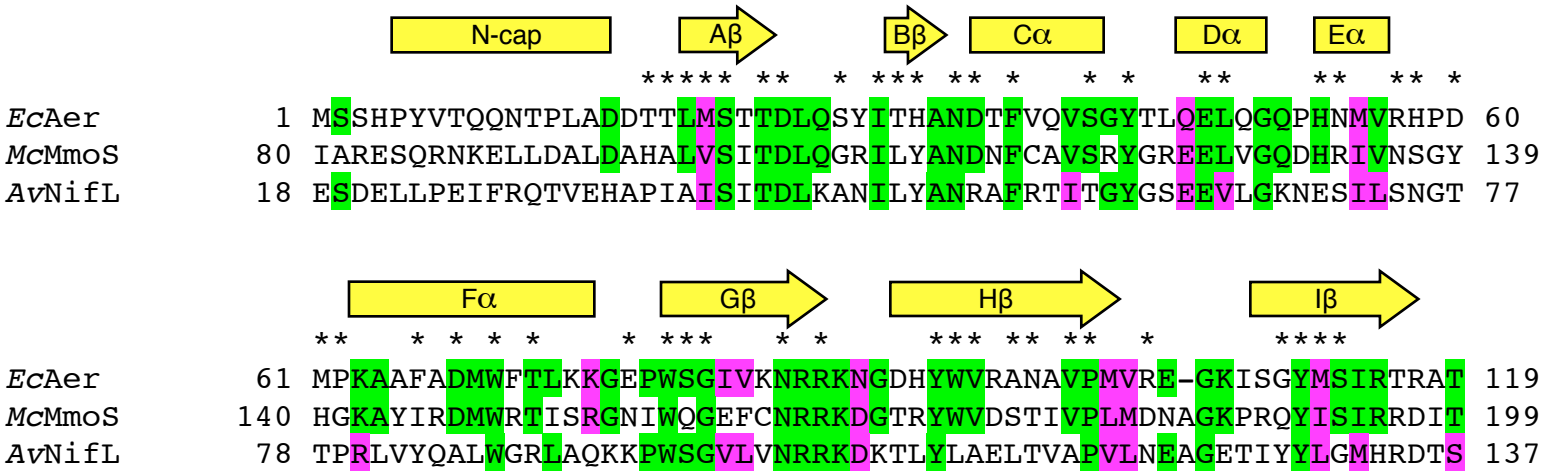

Fig. S1. Sequence alignment of the PAS domains of Aer from *E. coli,* MmoS (PAS-A) from *Methylococcus capsulatus* (Ukaegbu & Rosenzweig, 2009), and NifL from *Azotobacter vinelandii* (Key *et al.*, 2007). Residues identical to those of Aer are highlighted in green, while those similar to Aer are highlighted in magenta. The predicted secondary structural elements for Aer are shown as yellow rectangles for helices and as arrows for β-strands. Positions where amino acid substitutions altered Aer function are marked with an asterisk (*). The alignment was made with ClustalW (Larkin *et al.*, 2007), after which residue Aer-E105 was moved one position to the left to align with NifL-E122.

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