Supporting information

Routes of phosphoryl-group transfer during signal transmission and signal decay in the dimeric sensor histidine kinase ArcB

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Figures S1, S2 and S3, Table S1 and references

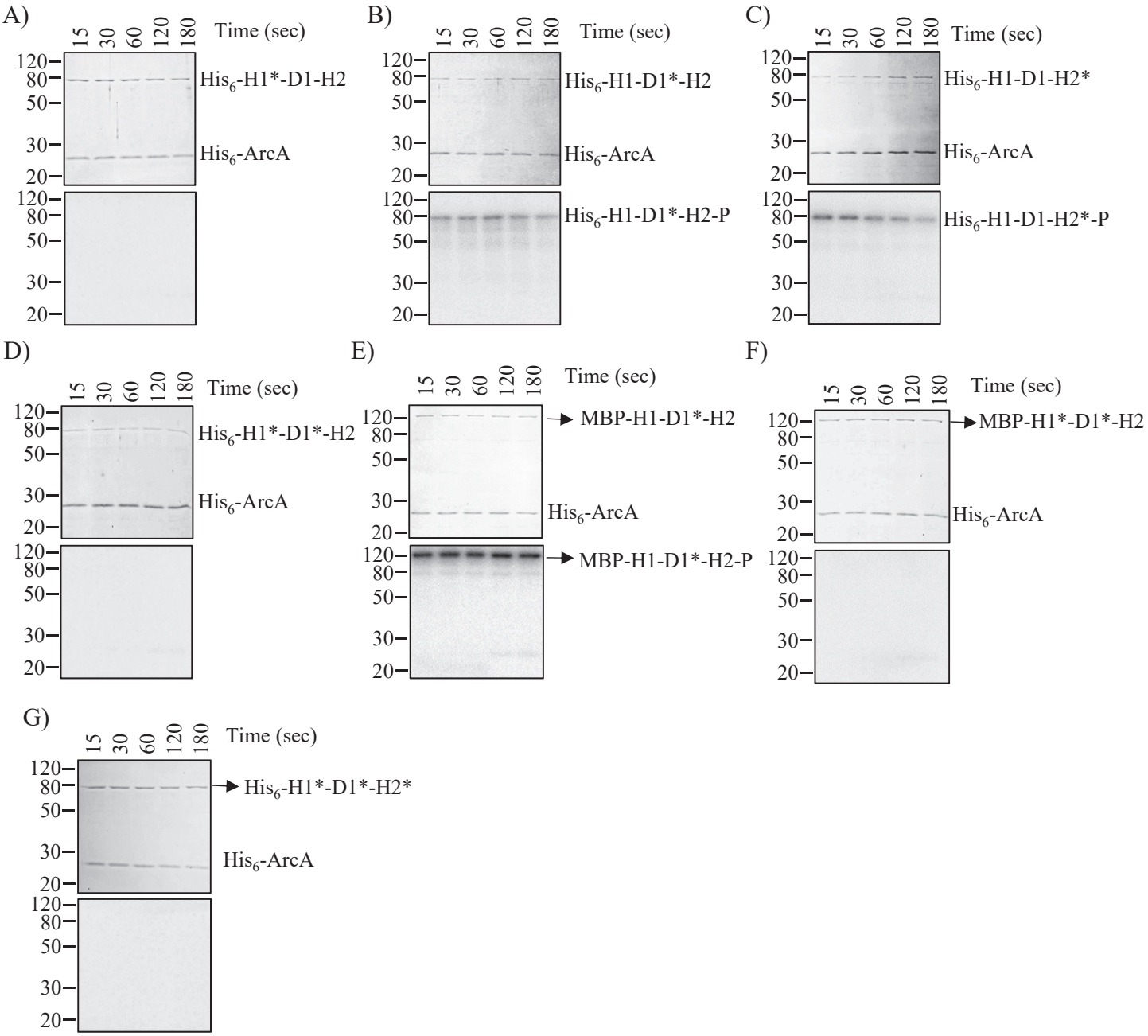

Figure S1. None of the His_6-$ArcB^{78-778}$ and MBP-$ArcB^{78-778}$ mutants are able to transphosphorylate ArcA. Purified ArcA was incubated in a 30 µl-reaction mixture, in the presence of [γ-^{32}P]ATP, with (A) His_6-H1*-D1-H2, (B) His_6-H1-D1*-H2, (C) His_6-H1-D1-H2*, (D) His_6-H1-D1*-H2*, (E) MBP-H1-D1*-H2, (F) MBP-H1*-D1*-H2, or (G) His_6-H1*-D1*-H2*, and 5-µl samples were withdrawn at the indicated time intervals for SDS-PAGE analysis. The Coomassie blue-stained gels revealing protein bands (upper panels), and the corresponding autoradiograms (bottom panels) are presented. The molecular mass standard values (kDa) are shown on the left, and the position of each polypeptide in the gel is indicated on the right side of each panel.

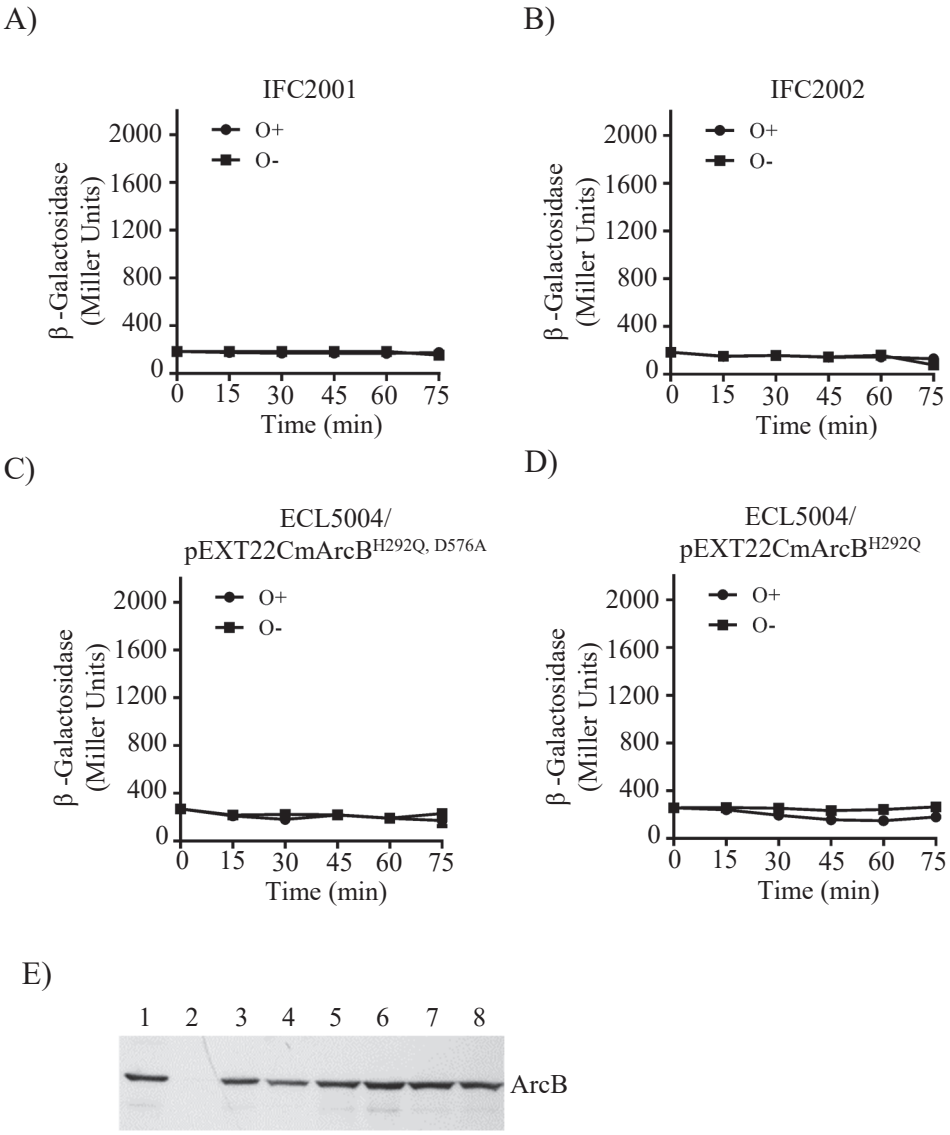

Figure S2. Mutant variants of ArcB do not activate the anaerobic expression of the *cydA'-lacZ* reporter. Cultures of strain (A) IFC2001 (*arcB*^{D576A}), (B) IFC5002 (*arcB*^{H717Q}), (C) ECL5004 (*arcB*$^{-}$) harboring plasmid pEXT22CmArcB$^{H292Q, D576A}$, and (D) ECL5004 (*arcB*$^{-}$) harboring plasmid pEXT22CmArcB^{H292Q}, all carrying the ArcA-P activatable λΦ(*cydA'-lacZ*) reporter, were grown aerobically in LB buffered with 0.1M MOPS (pH 7.4) and supplemented with 20 mM D-xylose. At an OD_{600} of 0.2, one aliquot was withdrawn, to measure the β-galactosidase activity (depicted as 0 min), and the rest of the culture was divided to two parts. One part was kept under aerobic conditions (circles), as a control, whereas the other was shifted to anaerobiosis (squares), and the time course of the β-galactosidase activity was followed. Data represent the averages from three independent experiments and the standard deviation values are indicated. (E) Equal number of bacteria of the above aerobic cultures and those used for Figure 3 were analyzed by Western blot analysis, using ArcB polyclonal antibodies. Lanes correspond to (1) ECL5003, (2) ECL5004, (3) IFC2001, (4) IFC2002, (5) IFC2001 carrying pEXT22CmArcB^{H292Q}, (6) IFC2002 carrying pEXT22CmArcB$^{H292Q, D576A}$, (7) ECL5004 carrying pEXT22CmArcB^{H292Q}, and (8) ECL5004 carrying pEXT22CmArcB$^{H292Q, D576A}$.

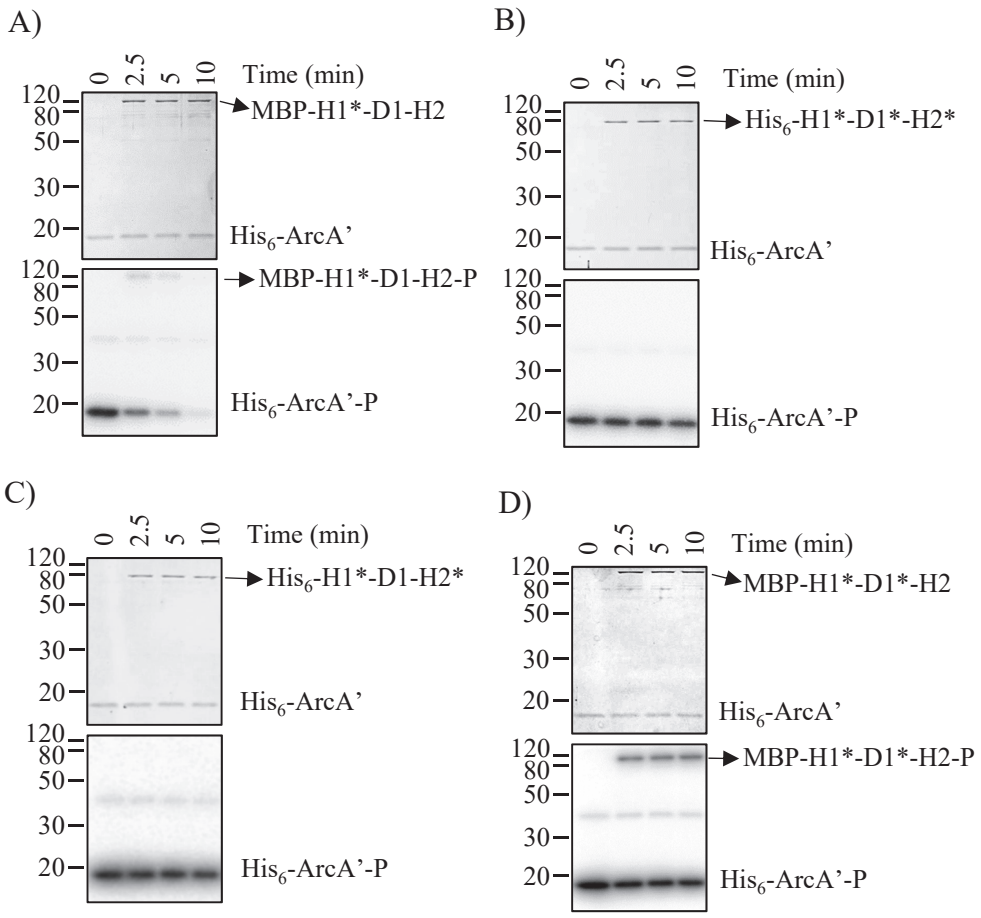

Figure S3. ArcA´-P dephosphorylation by His_6-$ArcB^{78-778}$ and MBP-$ArcB^{78-778}$ mutant variants. Purified ArcA´-P was incubated with (A) MBP-H1*-D1-H2, (B) His_6-H1*-D1*-H2*, (C) His6-H1*-D1-H2*, or (D) MBP-H1*-D1*-H2, in 25 µl-reaction mixtures. At the indicated time points, 5-µl samples were withdrawn for SDS-PAGE analysis. The Coomassie blue-stained gels revealing protein bands (upper panels), and the corresponding autoradiograms (bottom panels) are presented. The molecular mass standard values (kDa) are shown on the left, and the position of each polypeptide in the gel is indicated on the right side of each panel.

Table S1. *E. coli* strain and plasmids used in this work

Strain	Relevant characteristics	Source
MC4100	*F⁻ araD139* (*argF-lac*) *U169 rpsL150 relA1 flbB5301 deoC ptsF25 rbsR*	(1)
ECL5002	MC4100 λΦ(*lldP'-lacZ*)	(2)
ECL5003	MC4100 Δ*fnr*::Tn*9*(Cm^r) λΦ(*cydA'-lacZ*)	(2)
ECL5004	MC4100 Δ*arcB*::Tet^r Δ*fnr*::Tn*9*(Cm^r) λΦ(*cydA'-lacZ*)	(2)
ECL5012	MC4100 Δ*arcB*::Tet^r λΦ(*lldP'-lacZ*)	(2)
ECL5023	MC4100 *arcB^{D576A}*Kan^r λΦ(*cydA'-lacZ*) Δ*fnr*::Tn*9*(Cm^r)	(3)
ECL5024	MC4100 *arcB^{H717Q}* Kan^r λΦ(*cydA'-lacZ*) Δ*fnr*::Tn*9*(Cm^r)	(3)
ECL5032	MC4100 *arcB^{H717Q}* Kan^{r}λΦ(*lldP'-lacZ*)	(3)
IFC2001	MC4100 *arcB^{D576A}*Kan^r λΦ(*cydA'-lacZ*) Δ*fnr*::Tet^r	This study
IFC2002	MC4100 *arcB^{H717Q}* Kan^r λΦ(*cydA'-lacZ*) Δ*fnr*::Tet^r	This study
Plasmid		
pEXT22	Low copy number vector, Kan^r	(4)
pEXT22Cm	Low copy number vector, Kan^r, Cm^r	This study
pACT3	Low copy number vector, Cm^r	(4)
pMX712	*arcB* under native promoter in pBluescript KS II (+), Amp^r	(5)
pMX517	*arcB* in pBAD30 under control of l-arabinose inducible promoter, Amp^r	(5)
pQE30ArcB$^{78\text{-}778}$	His$_6$-ArcB$^{78\text{-}778}$ in pQE30 under IPTG inducible promoter, Amp^r	(6)
pMX028	His$_6$-ArcB$^{78\text{-}778,\ H292Q}$ in pQE30 under IPTG inducible promoter, Amp^r	(7)
pQE30ArcB$^{78\text{-}778,\ D576A,\ H717Q}$	His$_6$-ArcB$^{78\text{-}778,\ D576A,\ H717Q}$ in pQE30 under IPTG inducible promoter, Amp^r	(8)
pQE30ArcB$^{78\text{-}661,\ D576A}$	His$_6$-ArcB$^{78\text{-}661,\ D576A}$ in pQE30 under IPTG inducible promoter, Amp^r	(8)
pQE30ArcB$^{521\text{-}778,\ H717Q}$	His$_6$-ArcB$^{521\text{-}778,\ H717Q}$ in pQE30 under IPTG inducible promoter, Amp^r	(9)
pQE30ArcA	His$_6$-ArcA in pQE30 under IPTG inducible promoter, Amp^r	(10)
pQE30ArcA$^{1\text{-}136}$	His$_6$-ArcA$^{1\text{-}136}$ (His$_6$ArcA') in pQE30 under IPTG inducible promoter, Amp^r	(9)
pQE30ArcB$^{78\text{-}778,\ D576A}$	His$_6$-ArcB$^{78\text{-}778,\ D576A}$ in pQE30 under IPTG inducible promoter, Amp^r	This study
pQE30ArcB$^{78\text{-}778,\ H717Q}$	His$_6$-ArcB$^{78\text{-}778,\ H717Q}$ in pQE30 under IPTG inducible promoter, Amp^r	This study
pQE30ArcB$^{78\text{-}778,\ H292Q,\ H717Q}$	His$_6$-ArcB$^{78\text{-}778,\ H292Q,\ H717Q}$ in pQE30 under IPTG inducible promoter, Amp^r	This study

pQE30ArcB$^{78\text{-}778,\ H292Q,\ D576A}$	His_6-ArcB$^{78\text{-}778,\ H292Q,\ D576A}$ in pQE30 under IPTG inducible promoter, Amp^r	This study
pQE30ArcB$^{78\text{-}778,\ H292Q,\ D576A,\ H717Q}$	His_6-ArcB$^{78\text{-}778,\ H292Q,\ D576A,\ H717Q}$ in pQE30 under IPTG inducible promoter, Amp^r	This study
pBADHis-ArcB$^{78\text{-}778,\ H292Q}$	His_6-ArcB$^{78\text{-}778,\ H292Q}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pBADHis-ArcB$^{78\text{-}778,\ H292Q,\ D576A}$	His_6-ArcB$^{78\text{-}778,\ H292Q,\ D576A}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pBADHis-ArcB$^{78\text{-}778,\ H292Q,\ D576A,\ H717Q}$	His_6-ArcB$^{78\text{-}778,\ H292Q,\ D576A,\ H717Q}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pBADHis-ArcB$^{78\text{-}778,\ H717Q}$	His_6-ArcB$^{78\text{-}778,\ H717Q}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pBADHis-ArcB$^{78\text{-}778,\ H292Q,\ H717Q}$	His_6-ArcB$^{78\text{-}778,\ H292Q,\ H717Q}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pMAL-ArcB$^{78\text{-}778}$	MBP-ArcB$^{78\text{-}778}$ in pMALc2x under IPTG inducible promoter, Amp^r	This study
pACT3MBP-ArcB$^{78\text{-}778}$	MBP-ArcB$^{78\text{-}778}$ in pACT3 under IPTG inducible promoter, Cm^r	This study
pACT3MBP-ArcB$^{78\text{-}778,\ H292Q}$	MBP-ArcB$^{78\text{-}778,\ H292Q}$ in pACT3 under IPTG inducible promoter, Cm^r	This study
pACT3MBP-ArcB$^{78\text{-}778,\ D576A}$	MBP-ArcB$^{78\text{-}778,\ D576A}$ in pACT3 under IPTG inducible promoter, Cm^r	This study
pACT3MBP-ArcB$^{78\text{-}778,\ H292Q,\ D576A}$	MBP-ArcB$^{78\text{-}778,\ H292Q,\ D576A}$ in pACT3 under IPTG inducible promoter, Cm^r	This study
pMX546	$arcB^{H292Q}$ under native promoter in pBluescript KS II (+), Amp^r	This study
pMX547	$arcB^{H292Q,\ D576A}$ under native promoter in pBluescript KS II (+), Amp^r	This study
pMX548	$arcB^{D576A,\ H717Q}$ under native promoter in pBluescript KS II (+), Amp^r	This study
pEXT22CmArcB^{wt}	*arcB* under native promoter in pEXT22Cm, Kan^r, Cm^r	This study
pEXT22CmArcB^{H292Q}	$arcB^{H292Q}$ under native promoter in pEXT22Cm, Kan^r, Cm^r	This study
pEXT22CmArcB$^{H292Q,\ D576A}$	$arcB^{H292Q,\ D576A}$ under native promoter in pEXT22Cm, Kan^r, Cm^r	This study
pBADArcB$^{H292Q,\ D576A}$	$arcB^{H292Q,\ D576A}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study
pBADArcB$^{D576D,\ H717A}$	$arcB^{D576A,\ H717Q}$ in pBAD30 under l-arabinose inducible promoter, Amp^r	This study

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