## **Supporting Information**

## Neumann et al. 10.1073/pnas.1205307109

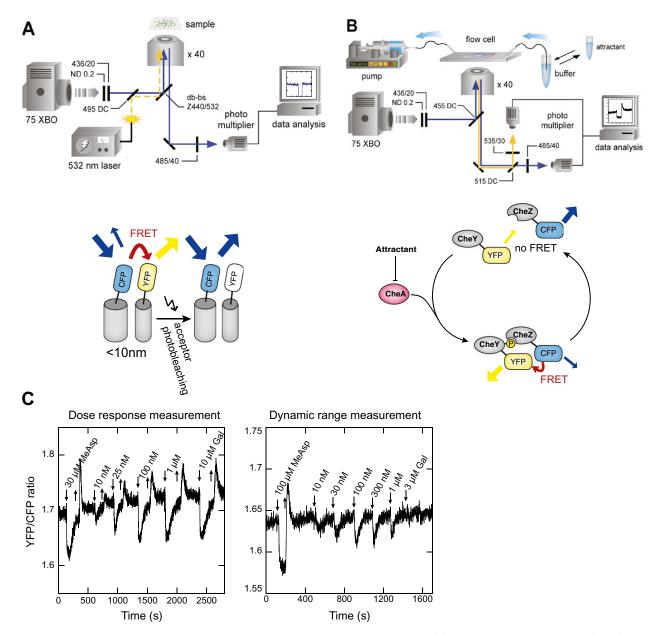


Fig. S1. FRET techniques used in this study to analyze protein interactions and pathway activity. (A) Acceptor photobleaching FRET. (*Upper*) Schematic drawing of the microscopy setup used for acceptor photobleaching measurements, adapted from ref. 1. (*Lower*) Application of acceptor photobleaching to detect steady-state interactions, for a positive FRET pair where bleaching of acceptor (YFP) results in donor (CFP) unquenching. Examples of positive and negative measurements are shown in Fig. 1B and in Fig. S2C. (B) Stimulation-dependent FRET. (*Upper*) Schematic drawing of the microscopy setup used for stimulation-dependence measurements, adapted from ref. 1. (*Lower*) Illustration of the kinase activity assay based on the CheY-YFP/CheZ-CFP FRET pair, where inhibition of kinase activity by attractant results in loss of FRET. (C) Examples of dose–response and dynamic range FRET measurements for galactose (Gal). The response was followed as a change in the ratio of YFP to CFP fluorescence due to FRET, with a higher ratio corresponding to higher FRET signal and therefore higher pathway activity. Addition and removal of α-methyl-DL-aspartate (MeAsp; control) and Gal are indicated by down and up arrows, respectively. Gradual drift of the YFP/CFP ratio base line arises from a relatively faster loss of the CFP fluorescence over the time course of the measurement. See main text for further details.

<sup>1.</sup> Kentner D, Sourjik V (2009) Dynamic map of protein interactions in the Escherichia coli chemotaxis pathway. Mol Syst Biol 5:238.

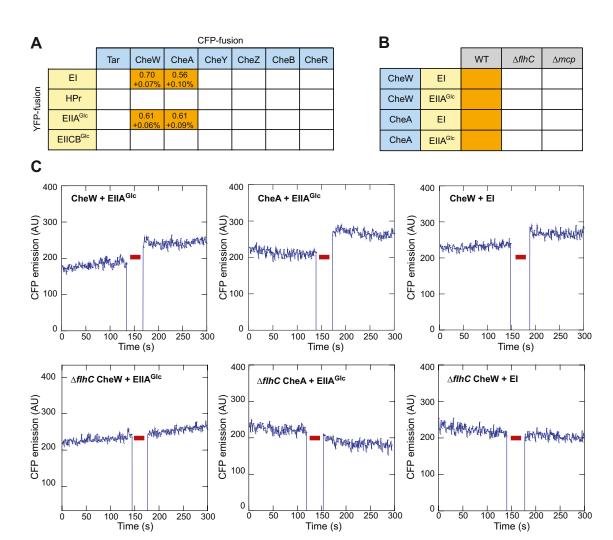


Fig. S2. Summary of interactions between PTS and chemotaxis proteins. (A) Interaction pairs of general (El and HPr) and glucose-specific PTS components (ElIAGlic and ElIBCGlic) and chemotaxis proteins, tested using acceptor photobleaching FRET in LJ110 wild-type cells. Positive interactions are marked in orange, with numbers indicating the average value of the increase in the CFP signal due to unquenching of donor fluorescence upon photoinactivation of the acceptor (FRET signal; ref. 1). Errors are SEs. Negative pairs showed <0.5% FRET signal. Notably, the FRET signal measured in vivo is substantially lower than the theoretical efficiency of energy transfer within a corresponding complex, because only a fraction of donor molecules are in a FRET complex at any given moment of time and also because specific signal is lowered by the contribution of the autofluorescence background to fluorescence in the CFP channel. Because the experiments were performed in wild-type cells, the FRET signal was further lowered due to the competition by the untagged native proteins. (B) Background dependence of positive PTS-chemotaxis interactions. Whereas all interactions were reproducibly observed in LJ110 wild-type cells, none could be detected in a ΔflhC strain (KG28) that expresses no chemotaxis and flagellar proteins or in a receptorless (Δmcp) strain (UU2612). (C) Representative measurements of interactions for CheW-ElIAGlic, CheA-ElIAGlic, and CheW-El pairs in the wild-type and a ΔflhC background. Measurements for the CheA-El pair are shown in Fig. 1B. Red bar indicates a period of acceptor (YFP) photobleaching. AU, arbitrary units of fluorescence.

1. Kentner D, Sourjik V (2009) Dynamic map of protein interactions in the Escherichia coli chemotaxis pathway. Mol Syst Biol 5:238.

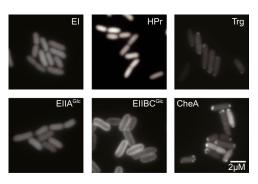


Fig. S3. Cellular localization of PTS proteins. Images of wild-type cells expressing YFP fusions to indicated PTS or chemotaxis proteins from plasmid constructs (Table S1).

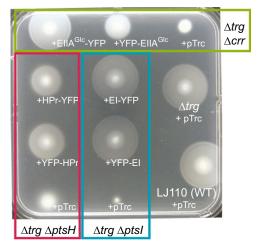


Fig. S4. Functionality of fluorescent protein fusions to PTS components. Functionality of indicated fusions was confirmed by their ability to complement chemotaxis on minimal medium soft agar plates supplemented with 1 mM glucose (*Methods*). Cells were inoculated with single clones from fresh overnight LB plates and incubated for 16 h. pTrc, empty pTrc99a vector.

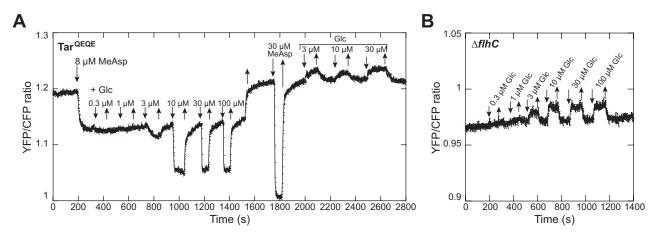


Fig. 55. PTS-mediated response to glucose depends on receptors and on their activity. (A) CheY/CheZ FRET measurement of the response to glucose for VS274 cells expressing half-modified  $Tar^{QEQE}$  from a plasmid as a sole receptor. Cells responded to glucose only when receptor activity was partly lowered by a subsaturating amount of the Tar ligand MeAsp. Corresponding dose–response curves at different levels of MeAsp are shown in Fig. 3*D.* (*B*) CheY/CheZ FRET measurement of the glucose response in  $\Delta flhC$  (KG28) cells that do not express any other chemotaxis proteins, showing a weak increase in FRET upon glucose stimulation, similar to that observed in *A* in absence of MeAsp.

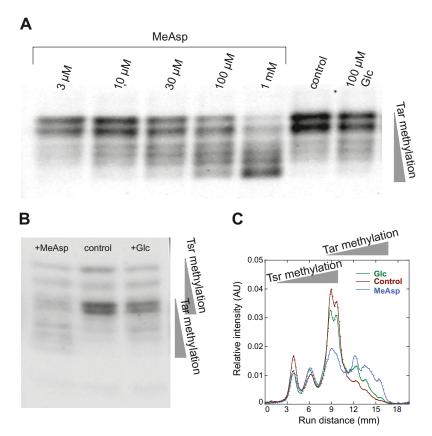


Fig. S6. Change of receptor methylation pattern upon PTS-mediated stimulation with glucose. (A) Immunoblot with anti-Tar antibody for trg and tsr deleted cells (SN74). Cell cultures were concentrated about fivefold and preadapted in buffer (control), and aliquots were subsequently stimulated with indicated concentrations of MeAsp or with a saturating concentration of glucose for 2 min. Because glucose was assumed to be metabolized rapidly, to ensure that its level did not drop below saturation of the PTS-mediated response it was added to a final concentration of 100 μM at the beginning and again after 1 min of incubation. The reaction was stopped by boiling 200 μL of cells with 100 μL 3x Laemmli buffer preheated to 95 °C for 10 min. The distribution of Tar methylation levels was measured using immunoblotting as described in Methods. Note that higher receptor mobility on the SDS/PAGE gel corresponds to higher methylation, as indicated. Intensity profiles of the lanes corresponding to control, 10 μM MeAsp and glucose are shown in Fig. 3E. (B) Immunoblot made as in A but for LJ110  $\Delta trg$  (SN71) cells that were preadapted in buffer (control) or stimulated with 100 μM MeAsp or glucose for 10 min. As in A, 100 μM final concentration of glucose was added every minute of incubation. (C) Intensity profiles of the lanes from B. For better comparison, each profile curve was normalized to the integral intensity of all bands within the respective lane after subtraction of the background. Although in the middle part of the profile bands corresponding to high-methylation states of Tsr and low-methylation states of Tar overlap, glucose stimulation apparently decreases the fraction of low-methylated Tsr and increases the fraction of high-methylated Tar, suggesting that adaptation to PTS-mediated stimuli is mediated by methylation of both receptors.

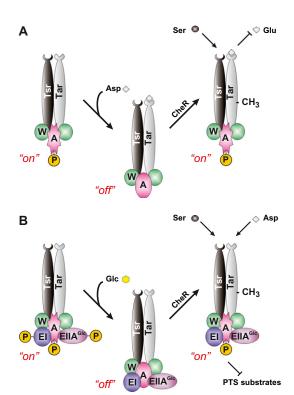


Fig. 57. Mechanisms of signaling by receptor- and PTS-specific ligands. (A) Binding of attractant ligand asparate (Asp) to the periplasmic sensory domain of Tar promotes inactive ("off") state of the entire sensory complex consisting of receptors, CheA and CheW. Subsequent methylation-dependent adaptation restores complex activity and enables signaling by ligands of other receptors, such as Tsr-specific ligand serine, whereas signaling by glutamate (Glu) that directly competes with Asp for binding remains inhibited. (B) PTS-mediated uptake of glucose lowers phosphorylation of El and ElIA<sup>GIc</sup>, inhibiting activity of sensory complexes from the cytoplasmic side and leading to methylation-dependent adaptation as in A. Adaptation restores receptor-mediated responses but not the level of El and ElIA<sup>GIc</sup> phosphorylation. As a consequence, response to other PTS substrates remains inhibited.

Table S1. Plasmids and strains used in this study

	Relevant genotype	Induction	Source
Plasmids			
pBAD33	Arabinose (Ara) regulation (P <sub>BAD</sub> ), pACYC ori, Cm <sup>R</sup>	*	(1)
pDK79	Arabinose regulation (P <sub>BAD</sub> ), pACYC ori, Kan <sup>R</sup>	_	(2)
pTrc99a	IPTG regulation (P <sub>trc</sub> ), pBR ori, Amp <sup>R</sup>	_	(3)
pKG110	Salicylate (Sal) regulation (P <sub>nahG</sub> ), pACYC ori, Cm <sup>R</sup>	_	J. S. Parkinson, University of Utah, Salt Lake City
pCP20	Yeast Flp recombinase, Cm <sup>R</sup> , Amp <sup>R</sup>	_	(4)
pPA114	tsr, pKG116 derivative, Cm <sup>R</sup>	0.7 μM Sal	(5)
pSN1	eyfp-ptsH, pTrc99a derivative	10 μM IPTG	This work
pSN2	eyfp-ptsI, pTrc99a derivative	5 μM IPTG	This work
pSN8	trg-eyfp, pTrc99a derivative	100 μM IPTG	This work
pSN24	crr-eyfp, pTrc99a derivative	5 μM IPTG	This work
pSN25	ptsH-eyfp, pTrc99a derivative	10 μM IPTG	This work
pVS54	cheZ-ecfp, pBAD33 derivative	0.001% Ara	(6)
pVS73	cheY-ecfp, pBAD33 derivative	0.001% Ara	(7)
pVS88	cheY-eyfp cheZ-ecfp, pTrc99a derivative	50 μM IPTG	(8)
pVS91	cheB, pBAD33 derivative	0.0003% Ara	(9)
pVS103	cheB-ecfp, pBAD33 derivative	0.001% Ara	This work
pVS112	cheB <sub>G</sub> pBAD33 derivative	0.0008% Ara	This work
pVS123	tar <sup>QEQE</sup> , pKG110 derivative	2 μM Sal	(8)
•	cheR-ecfp, pDK79 derivative	0.001% Ara	(7)
pVS226 = pDK21			(7)
pVS233 = pDK28	eyfp-cheA, pTrc99a derivative	30 μM IPTG	This work
pVS235	ecfp-cheA, pDK79 derivative	0.001% Ara	
pVS254 = pDK49	cheW-ecfp, pDK79 derivative	0.001% Ara	(7)
pVS258 = pDK53	tar-ecfp, pDK79 derivative	0.001% Ara	(7)
pVS344	ptsl-eyfp, pTrc99a derivative	5 μM IPTG	This work
pVS346	ptsG-eyfp, pTrc99a derivative	5 μM IPTG	This work
pV\$347	eyfp-crr, pTrc99a derivative	5 μM IPTG	This work
pVS1087	tar <sup>QEEE</sup> , pKG110 derivative	2 μM Sal	This work
Strains	Well I		(4.0)
RP437	Wild type	_	(10)
LJ110	W3110 Fnr <sup>+</sup> (wild type)	_	(11)
RP1131	RP437 ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> )	_	(12)
KG22	RP437 $\triangle$ (cheB cheY cheZ) $\triangle$ trg::kan	_	This work
KG28	LJ110 ∆flhC	_	This work
SN1	LJ110 Δ(cheY cheZ)	_	(13)
SN3	LJ110 ∆( <i>cheY cheZ</i> ) ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> )	_	This work
SN70	LJ110 ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> ) ∆ <i>ptsl</i> ::kan	_	This work
SN71	LJ110 ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> )	_	This work
SN72	LJ110 ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> ) ∆ <i>ptsH</i> ::kan	_	This work
SN73	LJ110 ∆trg::Tn10 (Tet <sup>R</sup> ) ∆ <i>crr</i> ::kan	_	This work
SN74	LJ110 ∆ <i>trg</i> ::Tn10 (Tet <sup>R</sup> ) ∆ <i>tsr</i> ::kan	_	This work
VS116	RP437 ∆flhC	_	(2)
VS181	RP437 $\Delta$ (cheY cheZ) $\Delta$ (tsr, tar, tap, trg, aer)	_	(8)
VS274 = VH1	RP437 $\triangle$ (cheR cheB cheY cheZ) $\triangle$ (tsr, tar, tap, trg, aer)	_	(14)
UU2612	RP437 $\Delta$ (tsr, tar, tap, trg, aer)	_	(15)
JW1417	MG1655 ∆ <i>trg</i> ::kan	_	(16)
JW1880	MG1655 ∆ <i>flhC</i> ::kan	_	(16)
JW2408	MG1655 ∆ <i>ptsH</i> ::kan	_	(16)
JW2409	MG1655 Δ <i>ptsl</i> ::kan	_	(16)
JW2410	MG1655 ∆ <i>crr</i> ::kan	_	(16)
JW4318	MG1655 ∆ <i>tsr</i> ::kan	_	(16)

<sup>\*—,</sup> not applicable.

<sup>1.</sup> Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol 177:4121–4130.

<sup>2.</sup> Kentner D, Thiem S, Hildenbeutel M, Sourjik V (2006) Determinants of chemoreceptor cluster formation in Escherichia coli. Mol Microbiol 61:407–417.

<sup>3.</sup> Amann E, Ochs B, Abel KJ (1988) Tightly regulated tac promoter vectors useful for the expression of unfused and fused proteins in Escherichia coli. Gene 69:301–315.

<sup>4.</sup> Cherepanov PP, Wackernagel W (1995) Gene disruption in *Escherichia coli*: Tc<sup>R</sup> and Km<sup>R</sup> cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* 158:9–14.

<sup>5.</sup> Ames P, Studdert CA, Reiser RH, Parkinson JS (2002) Collaborative signaling by mixed chemoreceptor teams in Escherichia coli. Proc Natl Acad Sci USA 99:7060–7065.

<sup>6.</sup> Sourjik V, Berg HC (2002) Receptor sensitivity in bacterial chemotaxis. Proc Natl Acad Sci USA 99:123–127.

<sup>7.</sup> Kentner D, Sourjik V (2009) Dynamic map of protein interactions in the *Escherichia coli* chemotaxis pathway. *Mol Syst Biol* 5:238.

<sup>8.</sup> Sourjik V (2004) Receptor clustering and signal processing in E. coli chemotaxis. Trends Microbiol 12:569–576.

<sup>9.</sup> Liberman L, Berg HC, Sourjik V (2004) Effect of chemoreceptor modification on assembly and activity of the receptor-kinase complex in Escherichia coli. J Bacteriol 186:6643–6646.

<sup>10.</sup> Parkinson JS, Houts SE (1982) Isolation and behavior of Escherichia coli deletion mutants lacking chemotaxis functions. J Bacteriol 151:106–113.

<sup>11.</sup> Zeppenfeld T, Larisch C, Lengeler JW, Jahreis K (2000) Glucose transporter mutants of Escherichia coli K-12 with changes in substrate recognition of IICB(GIc) and induction behavior of the ptsG gene. J Bacteriol 182:4443–4452.

- Abouhamad WN, et al. (1998) Computer-aided resolution of an experimental paradox in bacterial chemotaxis. *J Bacteriol* 180:3757–3764.
  Neumann S, Hansen CH, Wingreen NS, Sourjik V (2010) Differences in signalling by directly and indirectly binding ligands in bacterial chemotaxis. *EMBO J* 29:3484–3495.
  Endres RG, et al. (2008) Variable sizes of *Escherichia coli* chemoreceptor signaling teams. *Mol Syst Biol* 4:211.
- 15. Zhou Q, Ames P, Parkinson JS (2011) Biphasic control logic of HAMP domain signalling in the Escherichia coli serine chemoreceptor. Mol Microbiol 80:596–611.
- 16. Baba T, et al. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: The Keio collection. Mol Syst Biol 2:2006.0008.