

Supporting Information

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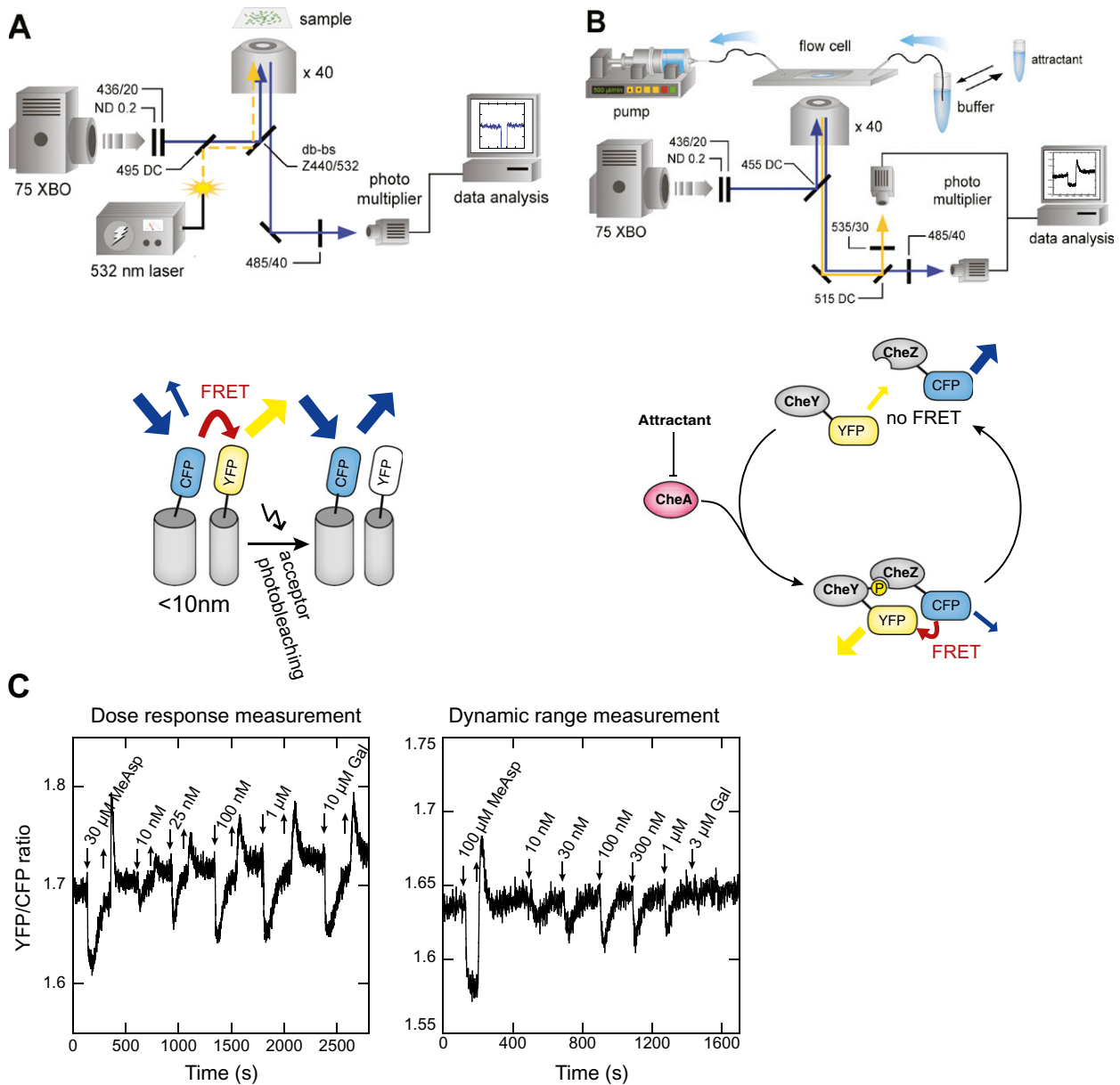


Fig. S1. FRET techniques used in this study to analyze protein interactions and pathway activity. (A) Acceptor photobleaching FRET. (Upper) Schematic drawing of the microscopy setup used for acceptor photobleaching measurements, adapted from ref. 1. (Lower) Application of acceptor photobleaching to detect 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.

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

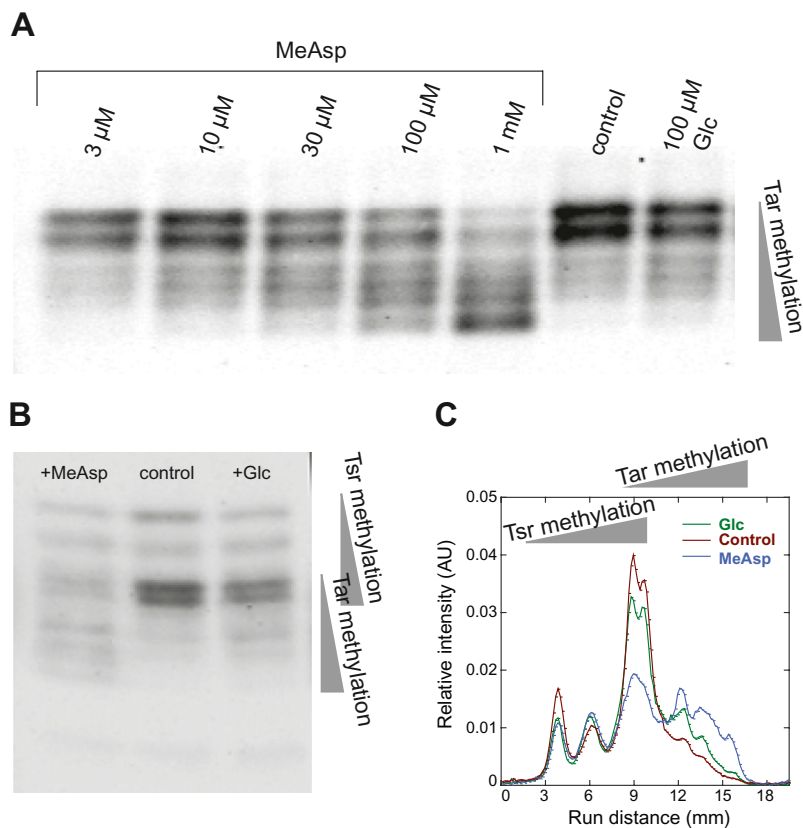


Fig. 56. Change of receptor methylation pattern upon PTS-mediated stimulation with glucose. (A) Immunoblot with anti-Tar antibody for *trg* and *tsr* deleted cells (SN74). Cell cultures were concentrated about fivefold and preadapted in buffer (control), and aliquots were subsequently stimulated with 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 μ M at the beginning and again after 1 min of incubation. The reaction was stopped by boiling 200 μ L of cells with 100 μ L 3 \times Laemmli buffer preheated to 95 $^{\circ}$ 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 μ M MeAsp and glucose are shown in Fig. 3E. (B) Immunoblot made as in A but for LJ110 Δ *trg* (SN71) cells that were preadapted in buffer (control) or stimulated with 100 μ M MeAsp or glucose for 10 min. As in A, 100 μ M 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.

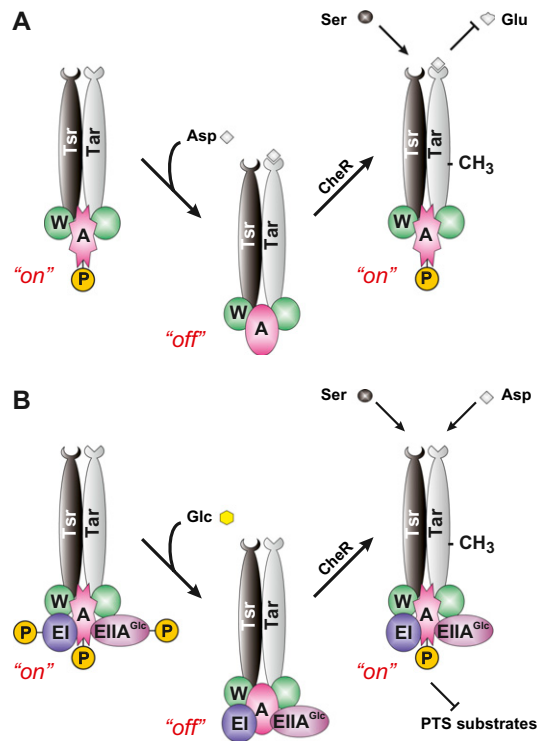


Fig. S7. Mechanisms of signaling by receptor- and PTS-specific ligands. (A) Binding of attractant ligand aspartate (Asp) to the periplasmic sensory domain of Tar promotes inactive ("off") state of the entire sensory complex consisting of receptors, CheA and CheW. Subsequent methylation-dependent adaptation 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 EI and EIIA^{Glc}, 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 EI and EIIA^{Glc} 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_{BAD}), pACYC ori, Cm ^R | —* | (1) |
| pDK79 | Arabinose regulation (P_{BAD}), pACYC ori, Kan ^R | — | (2) |
| pTrc99a | IPTG regulation (P_{trc}), pBR ori, Amp ^R | — | (3) |
| pKG110 | Salicylate (Sal) regulation (P_{nahG}), pACYC ori, Cm ^R | — | J. S. Parkinson, University of Utah, Salt Lake City |
| pCP20 | Yeast Flp recombinase, Cm ^R , Amp ^R | — | (4) |
| pPA114 | <i>tsr</i> , pKG116 derivative, Cm ^R | 0.7 μ M Sal | (5) |
| pSN1 | <i>eyfp-ptsH</i> , pTrc99a derivative | 10 μ M IPTG | This work |
| pSN2 | <i>eyfp-ptsI</i> , pTrc99a derivative | 5 μ M IPTG | This work |
| pSN8 | <i>trg-eyfp</i> , pTrc99a derivative | 100 μ M IPTG | This work |
| pSN24 | <i>crr-eyfp</i> , pTrc99a derivative | 5 μ M IPTG | This work |
| pSN25 | <i>ptsH-eyfp</i> , pTrc99a derivative | 10 μ M IPTG | This work |
| pVS54 | <i>cheZ-ecfp</i> , pBAD33 derivative | 0.001% Ara | (6) |
| pVS73 | <i>cheY-ecfp</i> , pBAD33 derivative | 0.001% Ara | (7) |
| pVS88 | <i>cheY-eyfp cheZ-ecfp</i> , pTrc99a derivative | 50 μ M IPTG | (8) |
| pVS91 | <i>cheB</i> , pBAD33 derivative | 0.0003% Ara | (9) |
| pVS103 | <i>cheB-ecfp</i> , pBAD33 derivative | 0.001% Ara | This work |
| pVS112 | <i>cheB_C</i> , pBAD33 derivative | 0.0008% Ara | This work |
| pVS123 | <i>tar^{OEQE}</i> , pKG110 derivative | 2 μ M Sal | (8) |
| pVS226 = pDK21 | <i>cheR-ecfp</i> , pDK79 derivative | 0.001% Ara | (7) |
| pVS233 = pDK28 | <i>eyfp-cheA</i> , pTrc99a derivative | 30 μ M IPTG | (2) |
| pVS235 | <i>ecfp-cheA</i> , pDK79 derivative | 0.001% Ara | This work |
| pVS254 = pDK49 | <i>cheW-ecfp</i> , pDK79 derivative | 0.001% Ara | (7) |
| pVS258 = pDK53 | <i>tar-ecfp</i> , pDK79 derivative | 0.001% Ara | (7) |
| pVS344 | <i>ptsI-eyfp</i> , pTrc99a derivative | 5 μ M IPTG | This work |
| pVS346 | <i>ptsG-eyfp</i> , pTrc99a derivative | 5 μ M IPTG | This work |
| pVS347 | <i>eyfp-crr</i> , pTrc99a derivative | 5 μ M IPTG | This work |
| pVS1087 | <i>tar^{OESE}</i> , pKG110 derivative | 2 μ M Sal | This work |
| Strains | | | |
| RP437 | Wild type | — | (10) |
| LJ110 | W3110 Fnr ⁺ (wild type) | — | (11) |
| RP1131 | RP437 Δ <i>trg::Tn10</i> (Tet ^R) | — | (12) |
| KG22 | RP437 Δ (<i>cheB cheY cheZ</i>) Δ <i>trg::kan</i> | — | This work |
| KG28 | LJ110 Δ <i>flhC</i> | — | This work |
| SN1 | LJ110 Δ (<i>cheY cheZ</i>) | — | (13) |
| SN3 | LJ110 Δ (<i>cheY cheZ</i>) Δ <i>trg::Tn10</i> (Tet ^R) | — | This work |
| SN70 | LJ110 Δ <i>trg::Tn10</i> (Tet ^R) Δ <i>ptsI::kan</i> | — | This work |
| SN71 | LJ110 Δ <i>trg::Tn10</i> (Tet ^R) | — | This work |
| SN72 | LJ110 Δ <i>trg::Tn10</i> (Tet ^R) Δ <i>ptsH::kan</i> | — | This work |
| SN73 | LJ110 Δ <i>trg::Tn10</i> (Tet ^R) Δ <i>crr::kan</i> | — | This work |
| SN74 | LJ110 Δ <i>trg::Tn10</i> (Tet ^R) Δ <i>tsr::kan</i> | — | This work |
| VS116 | RP437 Δ <i>flhC</i> | — | (2) |
| VS181 | RP437 Δ (<i>cheY cheZ</i>) Δ (<i>tsr, tar, tap, trg, aer</i>) | — | (8) |
| VS274 = VH1 | RP437 Δ (<i>cheR cheB cheY cheZ</i>) Δ (<i>tsr, tar, tap, trg, aer</i>) | — | (14) |
| UU2612 | RP437 Δ (<i>tsr, tar, tap, trg, aer</i>) | — | (15) |
| JW1417 | MG1655 Δ <i>trg::kan</i> | — | (16) |
| JW1880 | MG1655 Δ <i>flhC::kan</i> | — | (16) |
| JW2408 | MG1655 Δ <i>ptsH::kan</i> | — | (16) |
| JW2409 | MG1655 Δ <i>ptsI::kan</i> | — | (16) |
| JW2410 | MG1655 Δ <i>crr::kan</i> | — | (16) |
| JW4318 | MG1655 Δ <i>tsr::kan</i> | — | (16) |

*—, not applicable.

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