Supplementary data

Dynamic map of protein interactions in the *Escherichia coli* chemotaxis pathway

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Table of content:

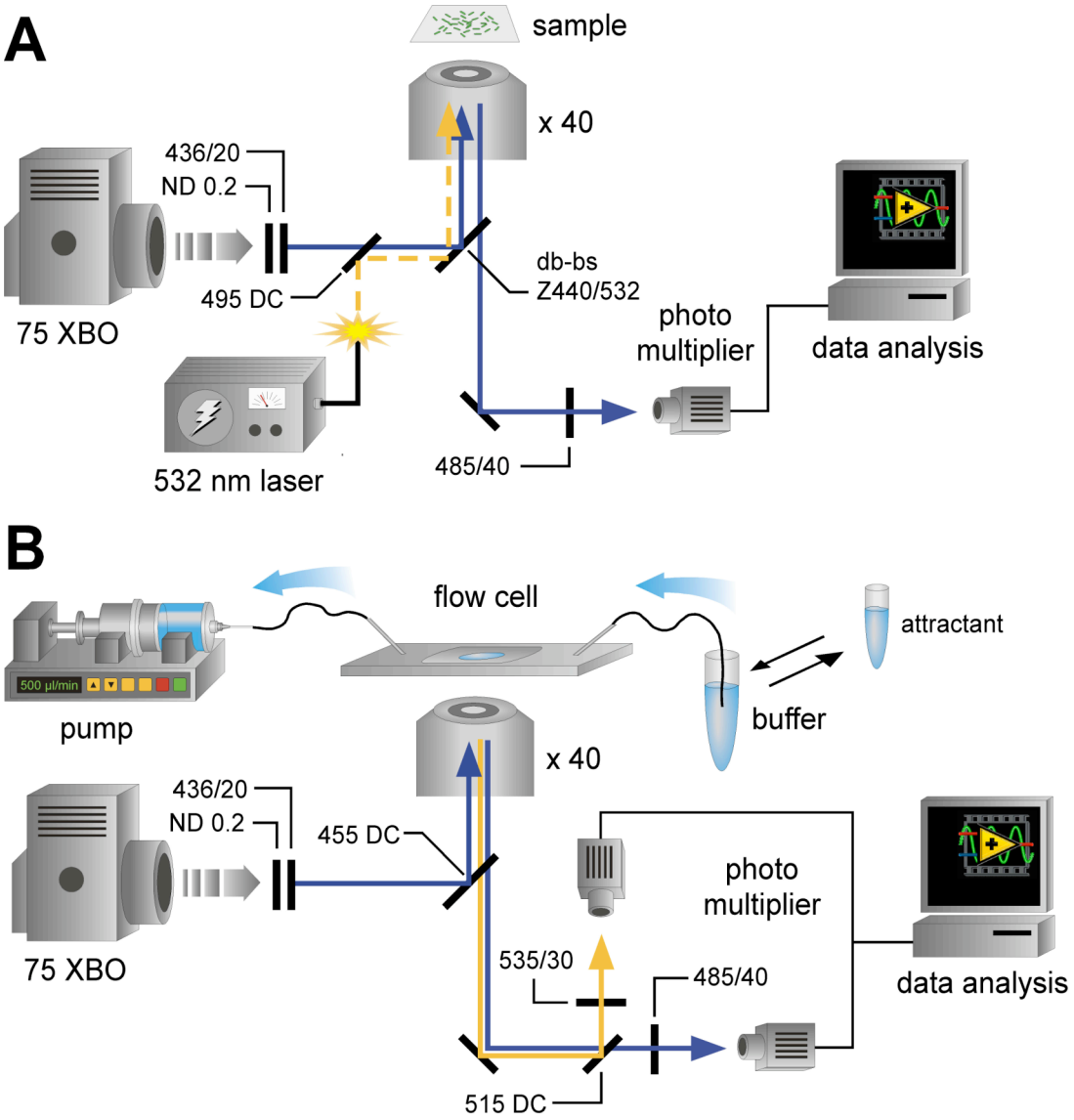

Figure S1 Microscopy setups used to measure FRET. (**A**) Acceptor photobleaching assay. (**B**) Flow assay used to test stimulation dependence of protein interactions.

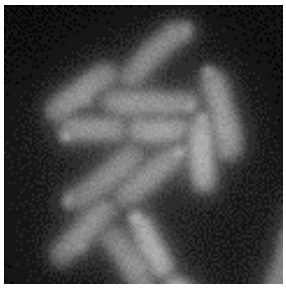

Figure S2 Localization of CheZ in *cheA*^{M98I} cells. CheZ-YFP is expressed at 5 μM IPTG induction in strain VS176 (*ΔcheY-cheZ cheA*^{M98I}), which contains $CheA_L$, but not $CheA_S$.

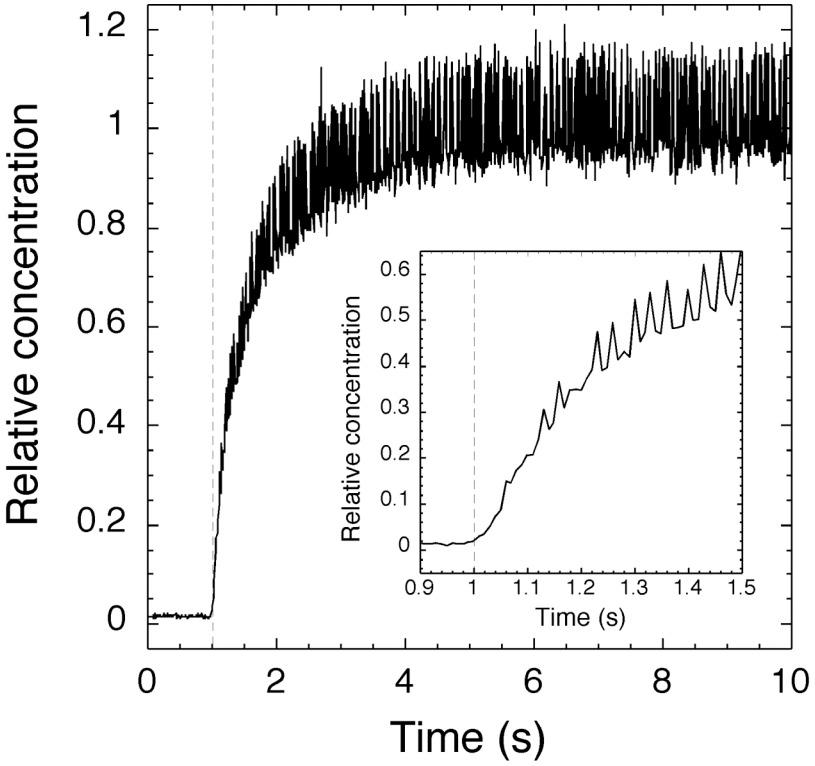

Figure S3 Attractant exchange profile during kinetics measurements. Exchange profile was measured as fluorescence in yellow channel using 100 nM solution of fluorescein as a tracer, and normalized to the final level of fluorescence. Signal integration time was 0.01 s. *Inset* shows profile during first 0.5 s of the exchange. Dashed line marks beginning of the exchange at 1 s. With the final concentration of aspartate used in these experiments being 10 mM, the saturating stimulation was reached in < 0.1 s. Signal fluctuations are due to the shott noise of the measurement.

Table S1 Strains and plasmids used in this study.

Strain or plasmid	Relevant genotype	Reference
Strains		
RP437	wild type	Parkinson and Houts, 1982
RP1076	*Δ(cheW tar tap cheR cheB cheY)*	Sandy Parkinson, personal gift; Sourjik and Berg, 2004
RP2893	*Δ(tap cheR cheB cheY cheZ)*	Sandy Parkinson, personal gift; Sourjik and Berg, 2002
RP4972	*ΔcheB*	Sandy Parkinson, personal gift; Lovdok *et al*, 2007
RBB1041	*Δ(cheA cheW tar tap cheR cheB cheY cheZ)::Zeo*^{R}	Robert B. Bourret, personal gift; Kentner *et al*, 2006; Sourjik *et al*, 2004
UU1250	*tsr tar tap trg aer*	Ames *et al*, 2002
VS100	*ΔcheY*	Sourjik and Berg, 2000
VS116	*ΔflhC*	Kentner *et al*, 2006
VS124	*Δ(cheB cheY cheZ)*	Sourjik and Berg, 2002
VS126	*ΔcheR*	Lovdok *et al*, 2007
VS149	*Δ(cheR cheB cheY cheZ)*	Sourjik and Berg, 2004
VS153	*Δ (cheR cheB cheY cheZ) Δtsr*	Sourjik and Berg, 2004
VS161	*ΔcheZ*	Lovdok *et al*, 2007; Vaknin and Berg, 2004
VS166	*ΔcheA*	this study
VS167	*ΔcheA Δ(tap cheR cheB cheY cheZ)*	this study
VS176	*Δ(cheY cheZ) cheA*^{M98I}	Ady Vaknin, personal gift
VS177	*Δ(cheY cheZ) cheAΔP2*	Ady Vaknin, personal gift
DK1	*Δ (cheR cheB cheY cheZ) Δtsr tarΔpp*	this study
Plasmids		
pTrc99a	Expression vector. pBR ori, pTrc promotor, Amp^{R}.	Amann *et al*, 1988
pBAD33	Expression vector. pACYC ori, pBAD promotor; Cm^{R}.	Guzman *et al*, 1995
pDK2[a]	Expression vector for cloning of N-terminal CFP^{A206K} fusions; pTrc99a derivative.	Kentner *et al*, 2006
pDK4[a]	Expression vector for cloning of N-terminal YFP^{A206K} fusions; pTrc99a derivative.	Kentner *et al*, 2006
pDK66[a]	Expression vector for cloning of C-terminal YFP^{A206K} fusions; pTrc99a derivative.	Kentner *et al*, 2006
pDK85[a]	Expression vector for cloning of C-terminal CFP^{A206K} fusions; pTrc99a derivative.	Kentner *et al*, 2006
pDK79	Expression vector. pACYC ori, pBAD promotor; Kan^{R}.	Kentner *et al*, 2006
pDK53	Tar-CFP expression plasmid; pDK79 derivative.	Kentner *et al*, 2006
pDK58	Tar-YFP expression plasmid; pDK66 derivative.	Kentner *et al*, 2006
pDK80	Tsr-YFP expression plasmid; pDK66 derivative.	this study
pDK198	Tar-CFP and Tar-YFP expression plasmid; pDK66 derivative.	this study
pDK203	Tar-CFP and Tsr-YFP expression plasmid; pDK66 derivative.	this study
pDK158	YFP-CheA^{M98I} expression plasmid; pDK4 derivative.	this study
pDK173	CheA^{M98I}-YFP expression plasmid; pDK66 derivative.	this study
pDK165	CFP-CheA^{M98I} expression plasmid; pDK79 derivative.	this study
pDK168	CheA^{M98I}-CFP expression plasmid; pDK79 derivative.	this study
pDK36	YFP-CheA$^{98-655}$ expression plasmid; pDK4 derivative.	this study
pDK57	CheA$^{98-655}$-YFP expression plasmid; pDK66 derivative.	this study
pDK38	CFP-CheA$^{98-655}$ expression plasmid; pDK79 derivative.	Kentner *et al*, 2006
pDK52	CheA$^{98-655}$-CFP expression plasmid; pDK79 derivative.	this study
pDK12	YFP-CheW expression plasmid; pDK4 derivative.	Kentner *et al*, 2006
pDK54	CheW-YFP expression plasmid; pDK66 derivative.	this study
pDK14	CFP-CheW expression plasmid; pDK79 derivative.	Kentner *et al*, 2006
pDK49	CheW-CFP expression plasmid; pDK79 derivative.	this study
pDK20	YFP-CheR expression plasmid; pDK4 derivative.	Kentner *et al*, 2006
pDK19	CheR-YFP expression plasmid; pDK66 derivative.	this study
pDK22	CFP-CheR expression plasmid; pDK79 derivative.	this study
pDK21	CheR-CFP expression plasmid; pDK79 derivative.	this study
pDK116	YFP-CheR^{D154A} expression plasmid; pDK4 derivative.	this study
pVS19	YFP-CheY expression plasmid; pTrc99a derivative.	this study
pVS18	CheY-YFP expression plasmid; pTrc99a derivative.	Sourjik and Berg, 2002

pVS74	CFP-CheY expression plasmid; pBAD33 derivative.	this study
pVS73	CheY-CFP expression plasmid; pBAD33 derivative.	this study
pVS63	YFP-CheZ expression plasmid; pTrc99a derivative.	this study
pVS64	CheZ-YFP expression plasmid; pTrc99a derivative.	Liberman *et al*, 2004
pVS51	CFP-CheZ expression plasmid; pBAD33 derivative.	this study
pVS54	CheZ-CFP expression plasmid; pBAD33 derivative.	Sourjik *et al*, 2002
pVS102	YFP-CheR expression plasmid; pBAD33 derivative.	Kentner *et al*, 2006
pDK135	CheB-YFP expression plasmid; pDK79 derivative.	this study
pDK159	$CheB^{S164C}$-YFP expression plasmid; pDK79 derivative.	this study

[a]pDK expression vectors, and derivatives thereof, used A206K versions of YFP and CFP with abolished weak dimerization (Zacharias *et al*, 2002).

Supplementary references for Table SI.

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Table SII FRET mapping of protein interactions by acceptor photobleaching.

CFP fusion[a]	YFP fusion[a]	strain	strain genotype[b]	protein-CFP/ protein-YFP FRET (%)[c]	protein-CFP/ YFP-protein FRET (%)	CFP-protein/ protein-YFP FRET (%)	CFP-protein/ YFP-protein FRET (%)	Comment
Tar	Tar	RP437 VS116	wild type *flhC*	3.0 6.6 ± 2.1	N/A	N/A	N/A	Direct interaction.
Tar	Tsr	RP437 VS116	wild type *flhC*	6 15	N/A	N/A	N/A	Direct interaction.
Tar	$CheA_L$	RP437 VS166	wild type *A*	- -	- -	N/A	N/A	CheA and CheW have previously been shown to bind receptors. The absence of FRET between Tar-CFP and CheA or CheW fusions is probably due to the large distance between the fluorophores. Using a Tar^{1-425}-CFP fusion, we detected FRET with CheW-YFP, but not with YFP-CheW or CheA fusions.
Tar	$CheA_S$	RP437 VS166	wild type *A*	- -	- -	N/A	N/A	
Tar	CheW	RP437 RP1076	wild type *WRBY*	- -	- -	N/A	N/A	
Tar^{1-425}	CheW	RP1041 VS116	*AW tar tap RBYZ* *flhC*	1.4 0.8	- -	N/A	N/A	
Tar^{1-425}	$CheA_S$	RP437 RP1041	wild type *AW tar tap RBYZ*	- -	- -	N/A	N/A	
Tar	CheY	RP437 VS100	wild type *Y*	- -	- -	N/A	N/A	No interaction.
Tar	CheZ	RP437 VS161	wild type *Z*	- -	- -	N/A	N/A	No interaction.
Tar	CheR	RP437 VS116	*wild type* *flhC*	- -	2.3 1.5	N/A	N/A	Direct interaction. FRET was only seen with the N-terminal CheR fusion, consistent with its better localization to clusters in fluorescence images.
Tar	$CheB^{S164C}$	VS153 DK1 VS177	*RBYZ tsr* *RBYZ tsr tarΔpp YZ AΔP2*	8.5 - 1.2[d]	N/A	N/A	N/A	Direct interaction. FRET was also detected with $Tar^{425-551}$/$CheB^{S164C}$, demonstrating that the interaction determined lies in the C-terminal receptor region.
$CheA_L$	$CheA_L$	RP437 VS116	*wild type* *flhC*	2.0 1.8	2.3 2.2	1.9 1.4	2.1 2.3	Direct interaction (dimerization).
$CheA_L$	$CheA_S$	RP437 VS116	*wild type* *flhC*	2.2 1.8	2.1 2.1	1,7 1.8	1.7 2.2	Direct interaction (dimerization).
$CheA_L$	CheW	RP437 VS116	*wild type* *flhC*	4.1 5.7	1.2 2.0	3.5 4.1	1.2 1.6	Direct interaction.
$CheA_L$	CheY	RP437 VS116	*wild type* *flhC*	2.0 4.6	1.1 3.5	1.2 2.4	1.3 2.6	Direct interaction.
$CheA_L$	CheZ	RP437 VS116	*wild type* *flhC*	2.0 2.4	0.7 0.8	3.3 2.4	2.1 -	Direct interaction. In contrast to previous reports, CheZ is observed to bind $CheA_L$.
$CheA_L$	CheR	RP437 VS126 VS166 UU1250	wild type *R* *A* *tsr tar tap trg aer*	- - - -	- 1.0 - -	- - - -	- 0.6 - -	Presumably indirect FRET through binding of CheA and CheR to receptors.
$CheA_L$	CheB	RP437 VS116	wild type *flhC*	1.7 1.9	N/A	4.2 2.6	N/A	Direct interaction.
$CheA_S$	$CheA_S$	RP437 VS116	wild type *flhC*	1.1 1.3	1.2 0.9	2.5 3.5	2.6 2.5	Direct interaction (dimerization).
$CheA_S$	CheW	RP437 VS116	wild type *flhC*	6.3 3.9	2.2 0.6	4.2 5.0	3.2 3.1	Direct interaction.
$CheA_S$	CheY	RP437 VS116	wild type *flhC*	1.0 1.2	0.9 0.6	2.1 2.5	2.0 3.5	Direct interaction.
$CheA_S$	CheZ	RP437 VS116	wild type *flhC*	2.1 ± 0.1 -	- -	7.1 3.4	4.4 2.8	Direct interaction.
$CheA_S$	CheR	RP437 VS126 VS166	wild type *R* *A*	- - -	1 ± 0.3 1.2 ± 0.1 -	- - -	- - -	See $CheA_L$/CheR.

		UU1250	*tsr tar tap trg aer*	-	-	-	-	
$CheA_S$	CheB	VS153	*RBYZ tsr*		N/A	3.9	N/A	Direct interaction. FRET was also detected for $CheA^{98-257}$/$CheB^{1-134}$, consistent with an association of the CheA response regulator binding domain with the N-terminal CheB domain.
CheW	CheW	RP437 RBB1041 VS116	wild type *AW tar tap RBYZ* *flhC*	4.5 2.1 -	2.3 1.5 -	3.2 2.4 -	2.7 1.3 -	Indirect FRET through binding of CheW to receptor and possibly CheA oligomers.
CheW	CheY	RP437 RP1076 RBB1041 VS177	wild type *W tar tap RBY* *AW tar tap RBYZ* *YZ cheAΔP2*	1.2 1.5 - -	- - - -	2.8 4.1 - -	- - - -	Presumably indirect FRET through binding of CheW and CheY to CheA.
CheW	CheZ	RP437 VS161	wild type *Z*	- -	- -	- -	- -	No interaction.
CheW	CheR	RP437 VS126	wild type *R*	- -	- -	- -	- -	No interaction.
CheW	CheB	RP437 RP1076 RBB1041	wild type *W tar tap RBY* *AW tar tap RBYZ*	2.0 3.0 -	N/A	1.7 2.5 ± 0.8 -	N/A	Presumably indirect FRET through binding of CheW and CheB to CheA.
CheY	CheY	RP437 VS100 RP1076 VS167 VS116	wild type *Y* *W tar tap RBY* *tap RBYZ A* *flhC*	0.7 +/- +/- - -	- +/- - - -	- 1.3± 0.6 - - -	- - - - -	Two CheY fusions probably come into proximity when CheY interacts with CheA, CheZ and/or FliM. Our measurements of FRET responses to chemotactic stimulation showed that these interactions are phosphorylation-dependent, and we assume that variations in CheY phosphorylation account for variations in acceptor bleaching measurements of CheY/CheY FRET.
CheZ	CheY	RP437 RP1076 VS166	wild type *W tar tap RBY* *A*	3.0 2.8 -	2.0 2.7 -	1.9 1.5 -	2.2 1.5 -	Direct interaction between CheZ and phospho-CheY. FRET in RP1076, where CheA should be largely inactive by lack of CheW and CheY should therefore be non-phosphorylated, is probably due to binding of CheZ and dephosphorylated CheY to CheA. Stimulation-dependent FRET between CheY and CheZ was only observed for the C-terminal CheZ fusions.
CheY	CheR	RP437 VS100 VS126	wild type *Y* *R*	- - -	- - -	- - -	- - -	No interaction.
CheY	CheB	RP437 RP1076 RBB1041	wild type *W tar tap RBY* *AW tar tap RBYZ*	- +/- -	N/A	- - -	N/A	FRET was only detected in some experiments in strain RP1076, probably as a result of CheY and CheB both binding to CheA. Competetion of

								the two fusions and, in wild type cells, of native CheY/CheB for CheA could explain poor FRET signals. As for CheY/CheY FRET, variations between experiments can be explained by variations in the response regulator phosphorylation level.
CheZ	CheZ	RP437 VS116	wild type *flhC*	3.4 3.5	1.9 2.2	3.2 4.2	3.1 3.8	Direct interaction (dimerization).
CheZ	CheR	RP437 VS161 VS126	wild type *Z* *R*	- - -	- - -	- - -	- - -	No interaction.
CheZ	CheB	RP437 VS124 VS149 VS167	wild type *BYZ* *RBYZ* *tap RBYZ A*	- 3.2 0.8 -	N/A	- 3.8 1.3 -	N/A	Interaction presumably results from CheZ and CheB both binding to CheA. The absence of FRET in wild type cells could be due to competetive binding of native proteins.
CheR	CheR	RP437 VS126 RBB1041 VS116	wild type *R* *AW tar tap RBYZ* *flhC*	- - - -	- 0.9 1.2 -	- - - -	1.6 2.1 ± 0.4 2.4 -	CheR proteins probably meet when binding to receptors. FRET is more efficient for N-terminal CheR fusions, which localize better to clusters.
CheR	CheB	RP437 VS149	wild type *RBYZ*	- -	N/A	- -	N/A	Although CheR and CheB both bind to receptors, no FRET was detected, probably due to mutual displacement from their overlapping binding sites and the distance between fluorescent protein fusions bound to different receptors being too large.
CheB	CheB	RP437 RP4972	wild type *B*	- -	N/A	- -	N/A	No interaction.

[a]Expression of CFP and YFP fusions was induced with 0.01% arabinose and 50 μM IPTG, respectively, except for pDK58, pDK138, pDK159, pDK203 (20 μM IPTG) and pDK198 (100 μM IPTG).

[b]Deletions of chemotaxis genes are abbreviated by single letters, i.e. *Y* for *cheY*.

[c]FRET efficiency was defined as a percentage change in CFP fluorescence upon bleaching (see Figure 2), $(C_{post}-C_{pre})/C_{post} \times 100\%$, where C_{pre} is CFP fluorescence before YFP bleaching, and C_{post} is CFP fluorescence after YFP bleaching.

[d]Measured as a percentage change in CFP fluorescence upon saturating attractant stimulation in the flow cell.

Table SIII Total and stimulation-dependent FRET values

Fusion pair[a]	Total FRET (%)[b]	Stimulus-dependent FRET (%)	Stimulus-dependent FRET/ Total FRET (%)
Tar-CFP/Tar-YFP	13.6 ±1.0	2.1 ±0.1	15.5 ±1.5
Tar-CFP/Tsr-YFP	20.8 ±0.9	2.8 ± 0.4	13.2 ±1.4
Tar-CFP/CheB^{S164C}-YFP	8.9 ±1.1	4.2 ±0.4	47.8 ± 4.3
CFP-CheA$_S$/CheY-YFP	5.5 ±0.8	2.7 ±0.4	48.5 ±0.5
CFP-CheA$_S$/CheB^{S164C}-YFP	3.6 ±0.3	1.1 ±0.1	32.3 ±1.0
CheZ-CFP/CheY-YFP	8.6 ±0.3	6.5 ±0.4	76 ±2.0
FliM-CFP/CheY-YFP	2.2 ±0.1	1.9 ±0.1	85 ±6

[a]FRET amplitude was determined in the same strains that have been used in Figures 3 and 4: Tar/Tar and Tar/Tsr pairs were measured in strain VS116 (*flhC*) and all other FRET pairs in strain VS153 (*tsr cheR cheB cheY cheZ*).
[b]FRET efficiency was determined as in Table SII.

Table SIV Expression levels of FRET pairs used for attractant stimulation experiments

Fusion pair	CFP[a] (×10^3 molecules/cell)	YFP (×10^3 molecules/cell)	CFP (μM)	YFP (μM)
Tar-CFP/Tar-YFP	10.1 ±1.3	18.7 ±1.8	9.3 ± 1.2	17.3 ± 1.7
Tar-CFP/Tsr-YFP	9.7 ±1.8	58.5 ±4.6	9.0 ± 1.7	54.0 ± 4.3
Tar-CFP/CheB^{S164C}-YFP	2.4 ±0.2	46.1 ±5.5	2.3 ± 0.2	42.5 ± 5.1
CFP-CheA$_S$/CheY-YFP	18.7 ±2.2	37.9 ±3.0	17.2 ± 2.0	34.9 ± 2.8
CFP-CheA$_S$/CheB^{S164C}-YFP	14.9 ±1.3	45.3 ±7.2	13.8 ± 1.2	41.8 ± 6.1
CheZ-CFP/CheY-YFP	12.7 ±0.5	24.0 ±3.0	11.7 ± 0.5	22.1 ± 3.0
FliM-CFP/CheY-YFP	32.5 ±1.6	36.3 ±6.1	30.0 ± 1.5	33.4 ± 5.6

[a]Expression levels were quantified as described in Materials and methods.