SUPPLEMENTARY INFORMATION

SUPPLEMENTARY Figure S1

METHODS FOR THIS FIGURE:

Determination of Attractant Regulated CheA Kinase Activity in the Presence of 1 mM Cu(II). Serine receptor and Cysless CheW adaptor protein were combined with the indicated CheA kinase single-Cys mutant, then arrays were formed as described in Materials and Methods. The kinase assay was performed as described with only one change: $CuCl_2$ was added to the arrays 10 seconds before the addition of ATP to mimic the crosslinking reactions.

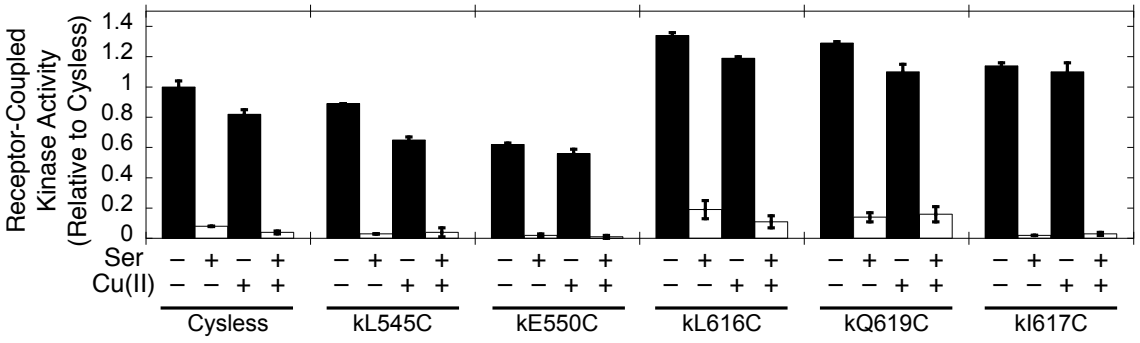

Figure S1: Kinase activity of arrays formed with CheA kinase single Cys mutants. WT Serine receptor-containing *E. coli* membranes were reconstituted *in vitro* with Cysless or single-Cys mutant CheA kinase, and Cysless CheW adaptor protein. The resulting arrays were mixed with response regulator CheY and ATP to measure the kinase activity in the presence and absence of 1 mM Cu(II) (black bars). An identical sample was mixed with serine to measure attractant regulation (white bars). Error bars indicate the standard error of each triplicate mean. Cu(II) has only small effects on the array-associated kinase activity, likely due to competitive displacement of Mg^{2+} from the catalytic site. These data demonstrate that native array structure and dynamics are largely retained during disulfide mapping under mild oxidation conditions, and that perturbations of the array by interactions with Cu(II) are negligible.

SUPPLEMENTARY Figure S2

METHODS FOR THIS FIGURE:

Quantification of CheA Kinase-Receptor S-S Heterodimer Formation in the Presence of 0.5 mM Cu(II). Single Cys CheA kinase was mixed with single Cys Serine receptor and Cysless CheW adaptor protein and arrays were formed as described in Materials and Methods. The oxidation reaction was performed as described except that redox catalyst was lowered to 0.5 mM Cu(II) + 3 mM EDTA to further decrease the oxidation strength.

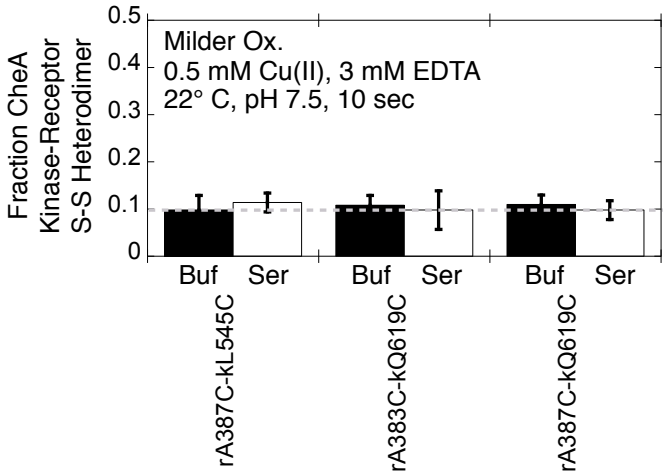

Figure S2: Effects of receptor-triggered on-off switching on disulfide formation rates under milder oxidation conditions. Under normal mild conditions (1 mM Cu(II) + 3 mM EDTA) the indicated three of the six fast Cys pairs did not show attractant effects, but yielded $\geq$ 30% CheA kinase-receptor S-S heterodimer formation (Fig. 8), and thus might be leaving the linear initial rate limit. An even milder oxidation condition (0.5 mM Cu(II) + 3 mM EDTA) was utilized to test whether an attractant effect could be detected earlier in the linear rate regime. Error bars indicate the standard error of each triplicate mean. While the milder oxidation condition does lower the fraction CheA kinase-receptor S-S heterodimer formed approximately 2-fold, an attractant effect on the rate is still not observed for rA387C-kL545C, rA383C-kQ619C, or rA387C-kQ619C.

SUPPLEMENTARY Figure S3

METHODS FOR THIS FIGURE:

Dependence of CheA Kinase Stable Array Incorporation on CheW Adaptor Protein. CheA Kinase array incorporation was determined as described in the Materials and Methods in the presence or absence of CheW adaptor protein.

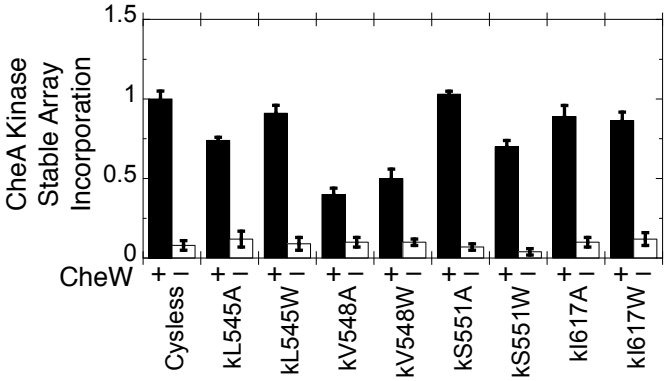

Figure S3: CheW adaptor protein dependent CheA Kinase incorporation into stable arrays. Upon standard array formation and washing (see Methods), Ala and Trp mutant CheA kinase proteins are incorporated into arrays at normal levels ranging from 40 to 105% of the Cysless CheA kinase lacking these mutations (black bars). Moreover, in the absence of CheW adaptor protein, incorporation of mutant and Cysless CheA proteins is greatly reduced (white bars), demonstrating that the bulk of stable CheA incorporation into the array is strongly CheW-dependent as expected for native array formation. The observed minimal level of nonspecific CheA binding also provides evidence these CheA mutants are well-folded, since denatured protein would be expected to yield nonspecific membrane binding. Error bars indicate the standard error of each triplicate mean.

SUPPLEMENTARY Table S1

$CheA_s$	$CheA_e$	$CheA_t$	$CheW_s$	$CheW_e$	$CheW_t$
L545	L528	I560	I33	I33	V27
V548	V531	I563	V36	V36	I30
E550	E533	T565	E38	E38	M32
S551	S534	I566	I39	I39	V33
L616	L599	L629	V105	V105	V98
I617	I600	L630	S106	S106	L99
Q619	Q602	Q632	V108	V108	V101

Tsr_e	Tar_s	$TM0014_t$
A383	A381	T141
A387	A385	A145
V398	V396	I156
G401	G399	N159

Table S1. Upper: Shows the structurally homologous positions between *S. typhimurium* (s), *E. coli* (e), and *T. maritima* (t) CheA kinase and CheW adaptor protein. Lower: Shows the structurally homologous positions between *E. coli* Tsr, *S. typhimurium* Tar, and *T. maritima* TM00014.