TABLE I

Affinities of CheW mutant proteins for CheA and Tar

Affinities were determined using fluorescence anisotropy (to define Kd^{CheA}) and competition pull-down assays (to define Kd^{Tar}) as detailed under "Experimental Procedures." Each value represents the average of at least two independent titration experiments. ± values represent S.E.

CheW variant	Mutant category	Kd^{CheA}	Kd^{Tar}
		μM	μM
Wild type		6.0 ± 0.2	11.0 ± 0.5
G57D	Loss-of-function	NDB[a]	33 ± 6
V36M	Loss-of-function	47 ± 2	NDB[b]
R62H	Loss-of-function	14.7 ± 0.5	23 ± 4
G133E	Weak titrator	75 ± 5	45 ± 6
G41D	Weak titrator	20 ± 1	37 ± 3
154ocr	Strong titrator	43 ± 4	3.6 ± 0.2[c]

[a] This mutant exhibited no detectable binding (NDB) to CheA. Titrations with this mutant demonstrated anisotropy changes that did not differ significantly from those observed in comparable titrations in which BSA was used instead of CheA. Based on these results, we can place a lower limit value of 130 μM for Kd^{CheA} for this mutant.

[b] This mutant exhibited no detectable binding to Tar. Competition titrations with this mutant generated signals that did not differ significantly from those observed with comparable titrations in which BSA was substituted for CheW. Based on these results, we can place a lower limit value of 230 μM for Kd^{Tar} with this mutant.

[c] The affinity of this mutant for Tar was determined by a direct pull-down approach, rather than by competition titrations, for reasons discussed in the text.