Ligand Affinity and Kinase Activity are Independent of Bacterial Chemotaxis Receptor Concentration: Insight into Signaling Mechanisms

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Supporting Information

Receptor density in proteoliposomes is lower than in vesicle-templated CF study

The reconstituted Tsr.4E proteoliposomes with the highest receptor concentration contain 55 total lipids per total receptor, which also corresponds to 55 outer leaflet lipids surrounding each kinase-accessible receptor. Using a lipid area of 0.7 nm^2/lipid, the proteoliposomes contain 38.5 nm^2 of lipid area surrounding each accessible receptor. This is over 2-fold higher than the area surrounding each vesicle-templated CF at the density transition, which occurs at ≈ 18 nm^2/CF. Furthermore, in the templating vesicle system some of the lipids are covered by the CF binding: the transition occurs at 36 nm^2 total area/CF dimer with 4 nm^2 occupied by each CF dimer, so there are 32 nm^2 of lipid area surrounding each CF dimer or 16 nm^2/CF. The larger lipid area per receptor in the proteoliposomes (38.5 nm^2/receptor) would not be expected to drive the transition to a more compact state and create a locked-on state of Tsr.4E.

Figure S1. Coupled equilibria positioned for maximal ligand-induced change at physiological receptor concentrations predict negligible change in receptor activity vs concentration.

Data (replotted from Figure 6) were collected for receptor concentrations near the physiological range. Simulations of ligand-induced dissociation model (same parameters as in Figures 5-6) demonstrate that maximal ligand-induced change will occur if receptor association occurs below the physiological range in the absence of ligand (black line) and above the physiological range in the presence of receptor-saturating ligand concentrations (gray line). Under these conditions there would be negligible change in maximal activity but measurable change in ligand affinity (Fig 5).

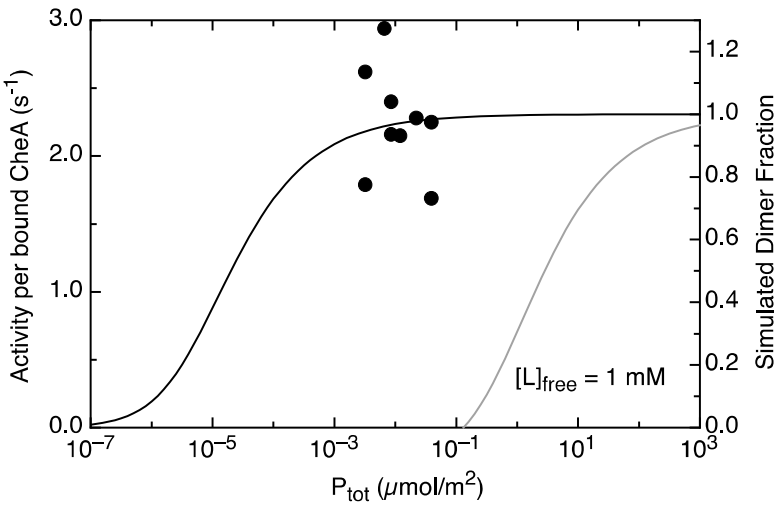

Table S1. Kinase activities[a] of reconstituted Tsr.4E.

	Lipid:Tsr (Target)	Actual Molar Lipid:Tsr	Activity per Total CheA (s^{-1})		Activity per Bound CheA[b] (s^{-1})	
			-Ser	+Ser	-Ser	+Ser
1	50:1	55:1	0.11 0.08	0.02	2.25 1.69	0.44
2	100:1	103:1	0.11	0.02	2.28	0.37
3	200:1	186:1	0.11	0.02	2.15	0.40
4	400:1	272:1	0.11 0.12	0.03	2.16 2.40	0.58
5	600:1	352:1	0.15	0.03	2.94	0.64
6	1000:1	603:1	0.13 0.09	0.04 0.02	2.62 1.79	0.79 0.43

[a]Activities were measured in the presence and absence of 10 mM Ser using [γ-^{32}P] ATP incorporation. Complex assembly conditions: 16 μM receptor, 7 μM CheA, 10 μM CheW incubated at 25˚C overnight.

[b]Bound CheA (0.35 μM) was measured by SDS-PAGE comparison of total and pellet on the 50:1 sample.

Table S2 Kinase activities[a] of Tsr.4Q[b].

	Lipid:Tsr (Target)	Actual Molar Lipid:Tsr	Activity per Total CheA (s^{-1})		Activity per Bound CheA[c] (s^{-1})	
			-Ser	+Ser	-Ser	+Ser
1	60:1	70:1	0.15 0.15	0.14 0.14	2.51 2.54	2.39 2.36
2	100:1	108:1	0.24 0.24	0.21 0.24	4.01 4.04	3.52 3.92
3	200:1	194:1	0.35 0.26	0.30 0.25	5.76 4.41	4.98 4.11
4	400:1	278:1	0.21	0.19	3.64	3.19
5	600:1	335:1	0.21 0.24	0.19	3.54 3.93	3.24
6	1000:1	557:1	0.18	0.16	2.93	2.63
2	**100:1 4E[b]**		**0.21**	**0.02**	**3.48**	**0.36**

[a]Activities were measured in the presence and absence of 10 mM Ser using [γ-^{32}P] ATP incorporation. Complex assembly conditions: 16 μM receptor, 7 μM CheA, 10 μM CheW incubated at 25˚C overnight.

[b]One sample of 100:1 Tsr.4E (bold) is shown for comparison.

[c]Bound CheA (0.44 μM) was measured by SDS-PAGE comparison of total and pellet on the 60:1 sample. Individual measurements of bound CheA on each sample of Tsr.4E and Tsr.4Q were not used because these showed a systematic increase in CheA in the pellet with increasing lipid-to-protein ratio. This was judged likely due to increasing entrapment of unbound CheA as the pellet became larger and less compact in the high lipid-to-protein ratio samples.