SUPPORTING INFORMATION: SUPPLEMENTARY FIGURES S1-S6

Slivka & Falke (2012) "The Isolated Bacterial Chemosensory Array Possesses Quasi- and Ultra-Stable Components: Functional Links between Array Stability, Cooperativity, and Order"

METHODS FOR SUPPLEMENTARY FIGURES

Testing Array Formation and Aging Conditions

Arrays were formed and aged according to the protocol outlined in Materials and Methods with a variety of modifications. **(S1)** To test the effect of attractant on stability 1 mM serine was added to arrays during reconstitution (and subsequently washed out before aging) or immediately after reconstitution, then arrays were aged and analyzed as in Methods. (See Fig. S1). **(S2)** In another experiment this procedure was repeated with 10% glycerol instead of serine. (See Fig. S2). **(S3)** The effect of formation time on array assembly was investigated by combining Tsr, CheA, and CheW and incubating them for 15, 30, 45 or 90 minutes. After the incubation time, arrays were immediately pelleted and washed to ensure that unbound components were rapidly removed, then were aged and analyzed as in Methods. (See Fig. S3). **(S4, S5)** The effect of temperature on array decay was tested by incubating Tsr, CheA and CheW at 15°C, 22°C, or 30°C either during the array formation step or during the aging process. Aging and analysis were otherwise performed as in Methods. Arrays reconstituted at 15°C, 22°C, or 30°C were aged under the standard condition at 22°C. (See Fig. S4). Arrays reconstituted at 22°C were aged at 15°C, 22°C, or 30°C. (See Fig. S5). **(S6)** The effect of varying the array assembly context was tested by comparing (i) arrays formed *in vitro* on Tsr inner membranes, (ii) arrays formed *in vitro* on Tar sonicated membranes, and (iii) *ex vivo* arrays formed in live cells then isolated in bacterial membranes. The Tar sonicated membrane-bound arrays and the *ex vivo* arrays were prepared as previously described[1,2].

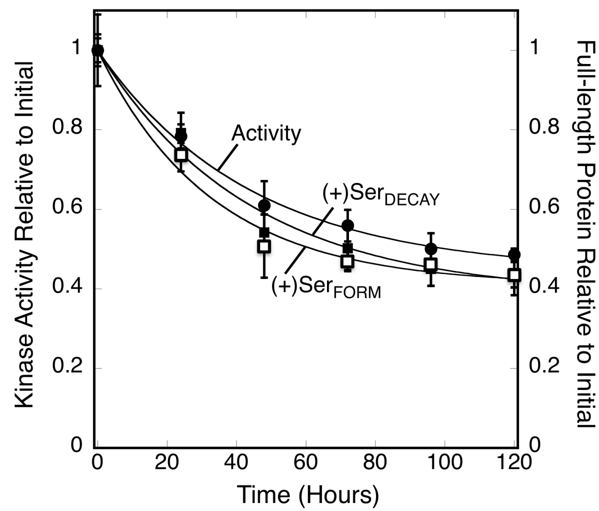

Figure S1. Effect of Serine on Array Decay. Triplicate samples of membrane-bound arrays were reconstituted in the presence (open squares) or absence (circles, squares) of 1 mM serine. After reconstitution arrays were washed and incubated at 22°C for 120 hrs (5 days) in the presence (squares) or absence (open squares, circles) of 1 mM serine. At the indicated timepoints a sample was removed from each triplicate and its kinase activity or full-length CheA retention was determined. Error bars indicate the standard deviation of each triplicate mean. Each data set is best fit by Eq. 5.

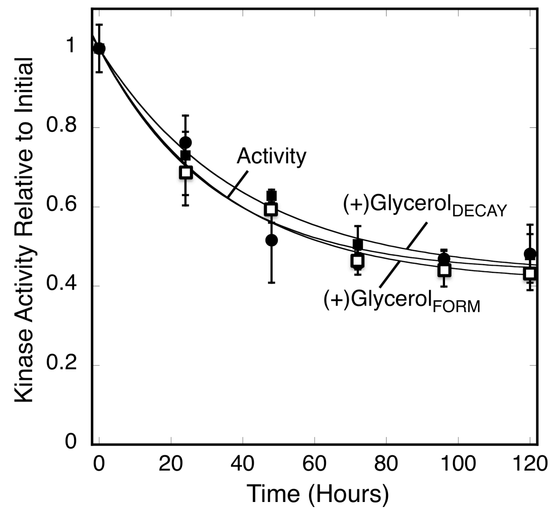

Figure S2. Effect of Glycerol on Array Decay. Triplicate samples of membrane-bound arrays were reconstituted in the presence (open squares) or absence (circles, squares) of 10% glycerol. After reconstitution arrays were washed and incubated at 22°C for 120 hrs (5 days) in the presence (squares) or absence (open squares, circles) of 10% glycerol. At the indicated timepoints a sample was removed from each triplicate and its kinase activity was determined. Error bars indicate the standard deviation of each triplicate mean. Each data set is best fit by Eq. 5.

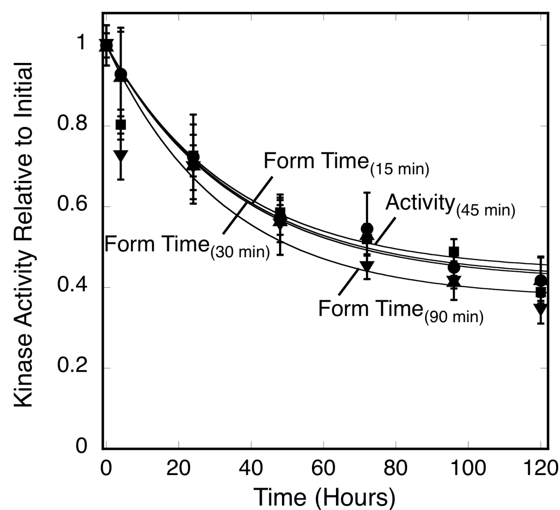

Figure S3. Effect of Formation Time on Array Stability. Triplicate samples of membrane-bound arrays were reconstituted for 15, 30, 45, or 90 minutes. After the allotted reconstitution time, arrays were immediately washed and incubated at 22°C for 120 hrs (5 days). At the indicated timepoints a sample was removed from each triplicate and its kinase activity was measured. Error bars indicate the standard deviation of each triplicate mean. Each data set is best fit by Eq. 5.

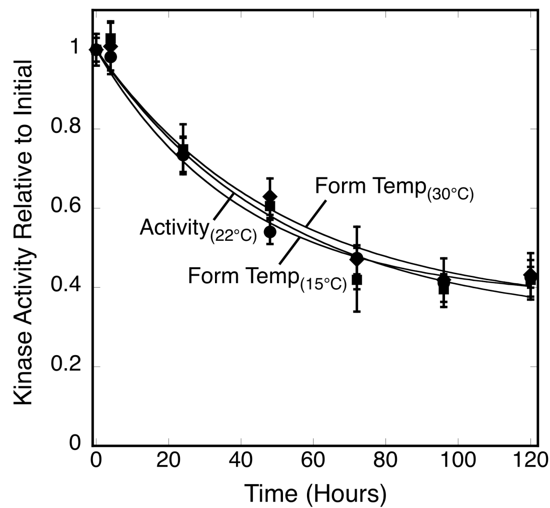

Figure S4. Effect of Formation Temperature on Array Stability. Triplicate samples of membrane-bound arrays were reconstituted at 15°C, 22°C, or 30°C for 45 minutes. Once reconstituted, arrays were immediately washed and incubated at 22°C for 120 hrs (5 days). At the indicated timepoints a sample was removed from each triplicate and its kinase activity was measured. Error bars indicate the standard deviation of each triplicate mean. Each data set is best fit by Eq. 5.

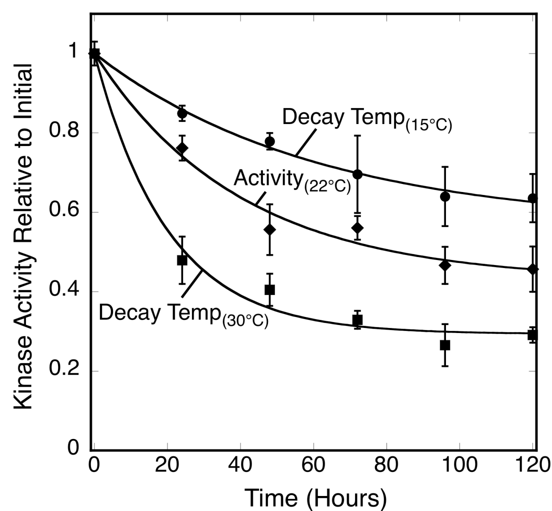

Figure S5. Effect of Aging Temperature on Array Stability. Triplicate samples of membrane-bound arrays were reconstituted at 22°C for 45 minutes. Once reconstituted, arrays were immediately washed and incubated at 15°C, 22°C, or 30°C for 120 hrs (5 days). At the indicated timepoints a sample was removed from each triplicate and its kinase activity was measured. Error bars indicate the standard deviation of each triplicate mean. Each data set is best fit by Eq. 5.

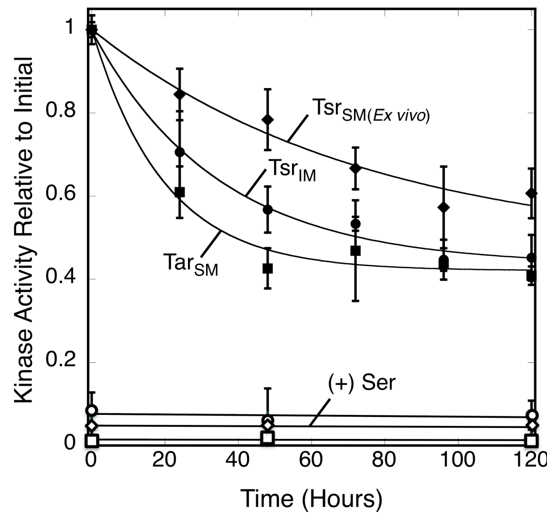

Figure S6. Comparison of Arrays Formed *In Vitro and* in Live Cells. Tsr inner-membrane-bound *in vitro* arrays (IM) or Tar sonicated-membrane-bound *in vitro* arrays (SM) were prepared by reconstitution at 22°C for 45 minutes. Once reconstituted, these *in vitro* arrays were immediately washed to remove unbound components. *Ex vivo* arrays were formed in live cells and isolated in bacterial membranes, then diluted prior to array aging in fresh buffer. Triplicate samples of all three arrays were aged simultaneously at 22°C for 120 hrs (5 days). At the indicated timepoints a sample was removed from each triplicate and its kinase activity was measured, either in the absence (filled symbols) or presence (open symbols) of 1 mM attractant. Error bars indicate the standard deviation of each triplicate mean. The curves are best fit by Eq. 5 yielding the following parameters: IM prep (n = 10) quasi-stable τ = 33 ± 10 hrs and amplitude 0.5 ± 0.1, ultra-stable amplitude 0.5 ± 0.1; SM prep (n = 3) quasi-stable τ = 33 ± 7 hrs and amplitude 0.57 ± 0.06, ultra-stable amplitude 0.43 ± 0.07; and *Ex vivo* prep (n = 6) quasi-stable τ = 70 ± 20 hrs and amplitude 0.5 ± 0.1, ultra-stable amplitude 0.5 ± 0.2. The parameter "n" indicates the number of independent trials.

REFERENCES FOR SUPPORTING INFORMATION

1. Chervitz, S. A.; Lin, C. M.; Falke, J. J., Transmembrane Signaling by the Aspartate Receptor - Engineered Disulfides Reveal Static Regions of the Subunit Interface. *Biochemistry* **1995,** *34* (30), 9722-9733.

2. Erbse, A. H.; Falke, J. J., The core signaling proteins of bacterial chemotaxis assemble to form an ultrastable complex. *Biochemistry* **2009,** *48* (29), 6975-87.