SUPPORTING INFORMATION

Table S1. Strains used in this study.

Strain	Relevant genotype	Source
RP437	WT for chemotaxis	(*1*)
UU2619 ("EEEE")	tsr-Q297E/Q311E (EEEE) Δ(tar-cheB)4346	(*2*)
UU2564 ("QQQQ")	tsr-E304Q/E493Q [QQQQ] cheA$^+$ Δ(tar-cheB)4346	(*2*)
HCB326	Δtsr trg::TnO ΔcheA-cheZ	(*3*)
CO4	(cheAW)Δ2167 pPM25(CheA CheW) pCO3(tsr-A413T)	this study

Table S2. Summary of WMD-corrected PCA classification

Sample	# Subvolumes	Components used	Fraction Variance Explained	# Classes	AIC[1] improvement	BIC[2] improvement
Tsr-EEEE	181	1-5	0.18	2	**52.2862**	**23.4386**
Tsr-EEEE	181	1-5	0.18	4	7.1891	-79.3537
Tsr-QQQQ	60	1-5	0.16	2	-15.9904	-38.2131
Tsr, CheA, CheW overexpression	100	1-6	0.15	2	-47.0031	-72.2907
Tsr, CheA, CheW overexpression	100	1-6	0.15	4	**113.43**	**21.0942**
In vitro Tsr-CF, CheA, CheW, PEG	140	1-5	0.19	2	**121.278**	**93.971**
In vitro Tsr-CF, CheA, CheW, PEG	140	1-4	0.17	6	**346.906**	**238.707**

[1]Akaike Information Criterion, Value indicates improvement compared to one class
[2]Bayes Information Criterion, Value indicates improvement compared to one class
* Bold indicates significance

Figure S1. Classification of *E. coli* chemoreceptor array hexagons in cells expressing Tsr-QQQQ reveals ordered CheA occupancy.

Classification by principal components analysis and k-means clustering of hexagons results in two classes similar to those observed in Tsr-EEEE (Figure 1). Average of class 1 (n=39) is shown in green at left, class 2 (n=21) in turquoise in the middle. Organization of classes in the array patch is shown at right, with yellow representing particles not included in the clustering.

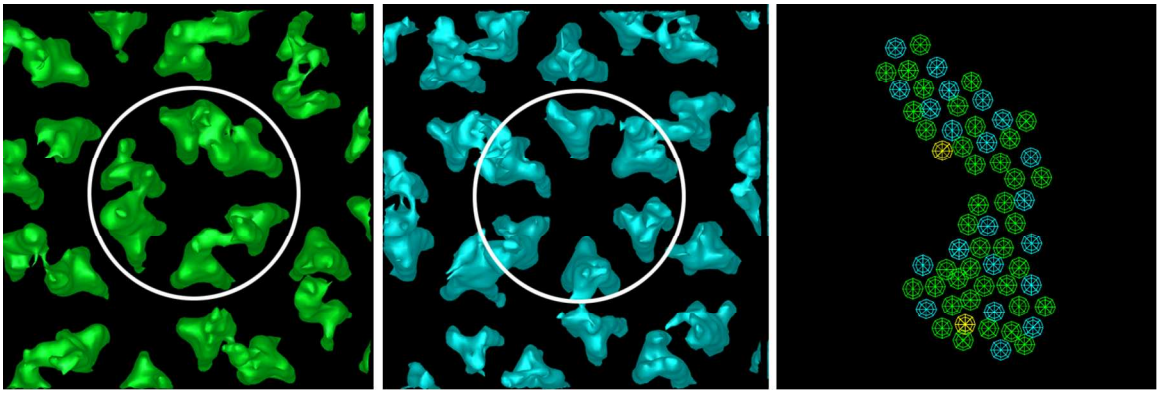


Figure S2. **Tomographic slice showing overview of *in vitro* complex assembly.** Scale bar 100nm.

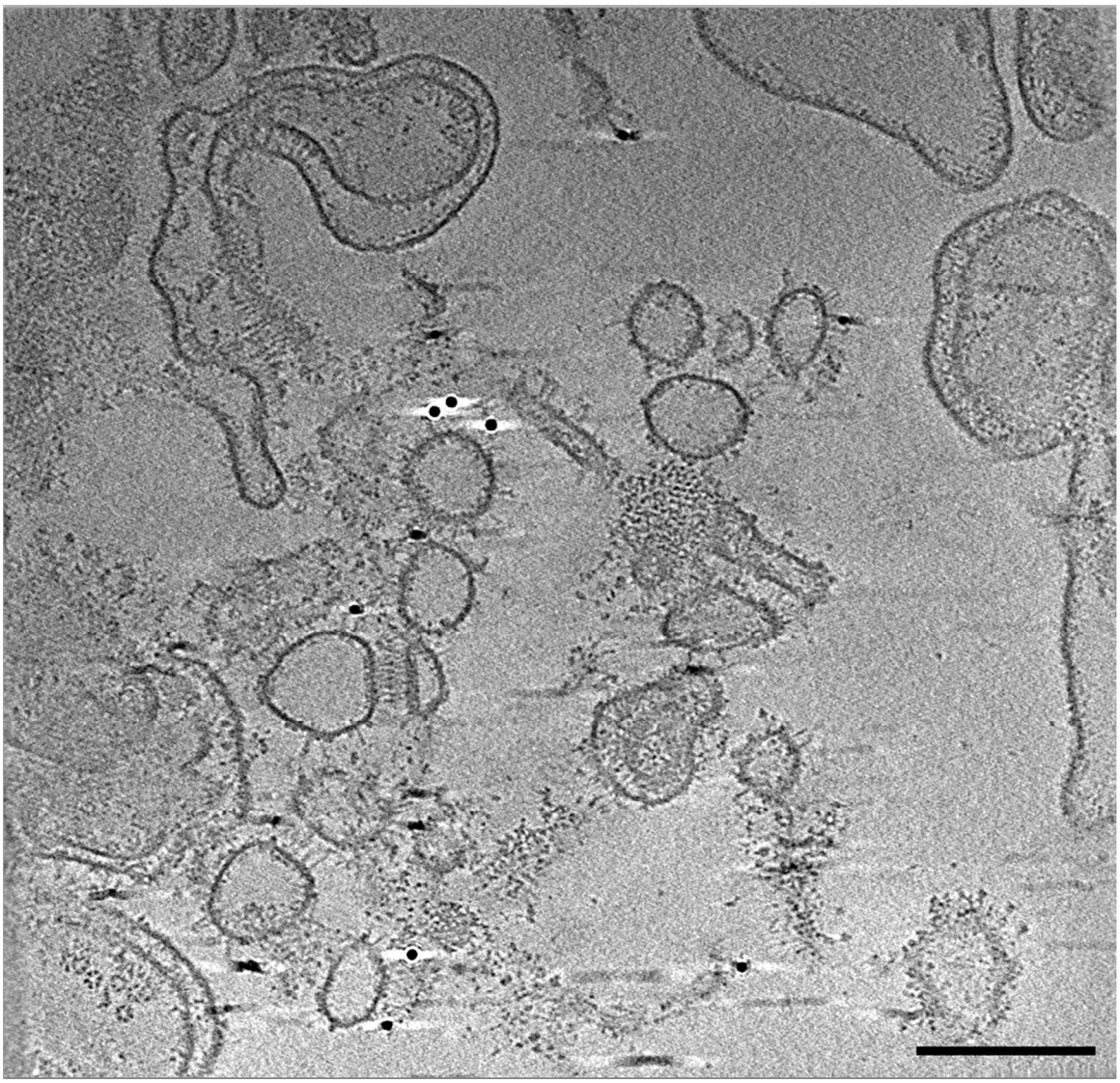


Figure S3. *In vitro* receptor hexagons are associated with three CheA dimers.

Tomographic slice of an *in vitro* preparation of Tsr in inner membrane vesicles following addition of CheA and CheW. Isosurface representation (left inset) of a cut-out section at the level of the CheA/W ring from the receptor hexagon (right inset) highlighted by the yellow circle. Yellow arrows indicate densities corresponding to CheA dimers.

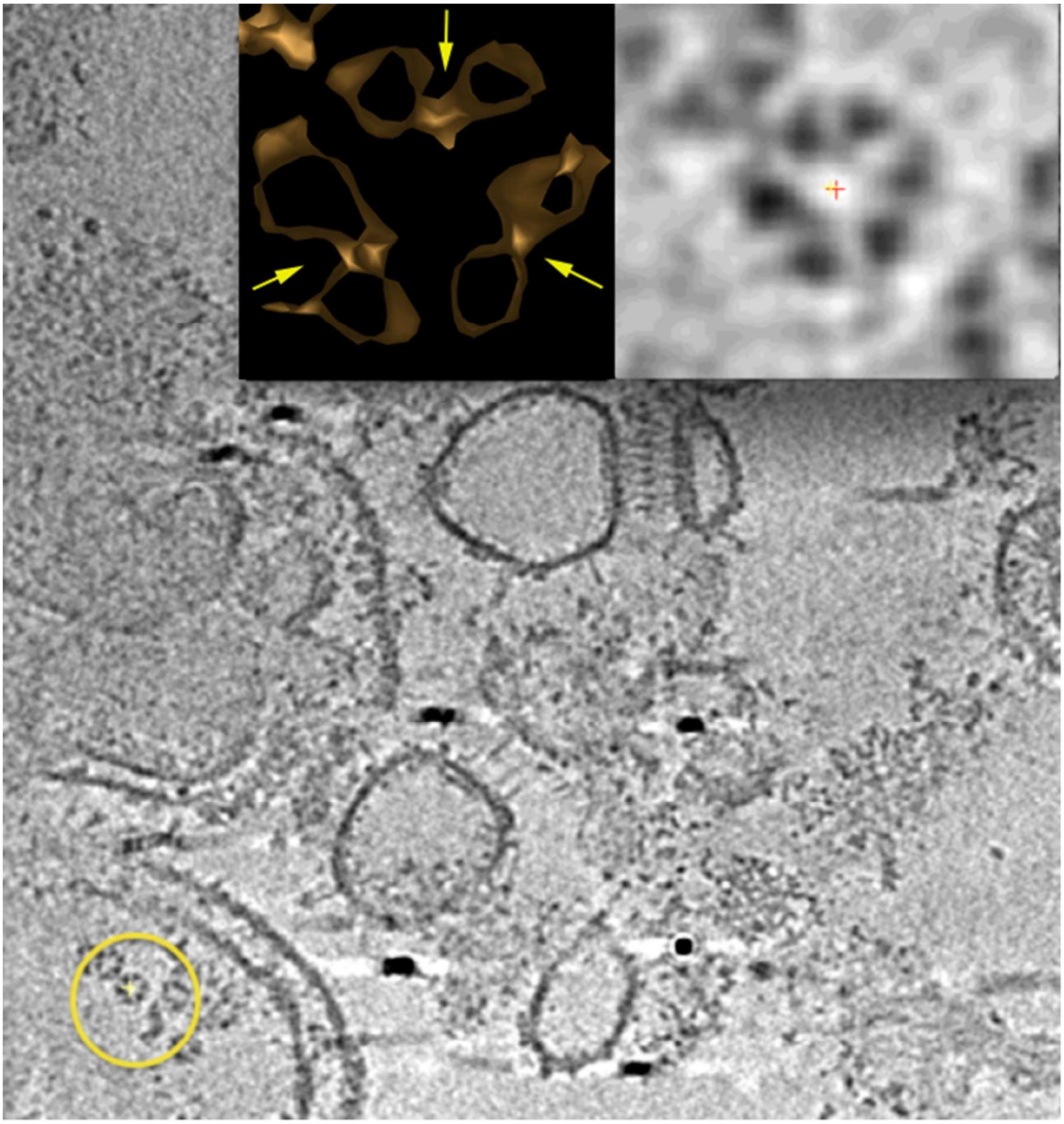


Figure S4. Relative protein levels of CheA and Tsr overexpression. Western blot showing levels of CheA (top left), Tsr (top right), and a loading control (bottom; unknown protein detected by α-β-lactamase) in RP437, UU2619, and CO4. Quantification by densitometry gives the following overexpression levels:

CO4/RP437 CheA 25.5, Tsr 26

CO4/UU2619 CheA 33.7, Tsr 15.5.

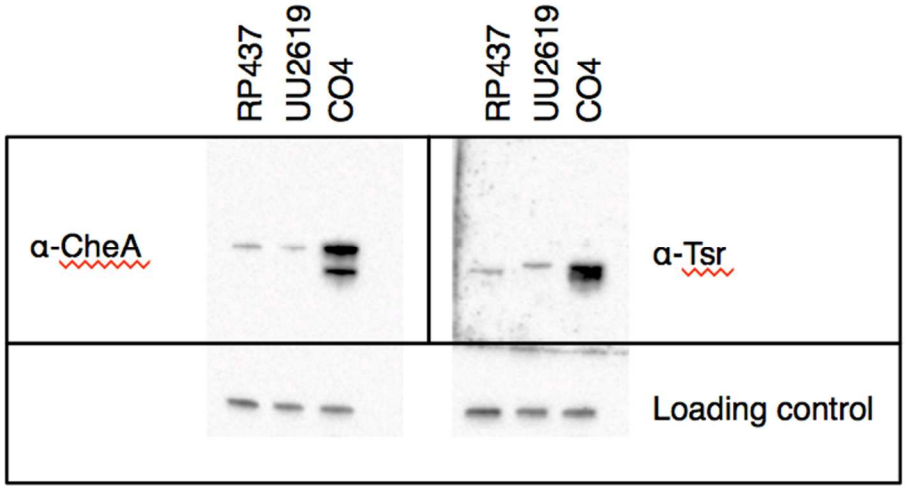


Figure S5. Classification of array hexagons formed by co-overexpression of Tsr, CheA, and CheW. Classification by principal components analysis and k-means clustering of hexagons in the array patch shown in Figure 3 results in 4 classes. Average of class 1 (n=54) is shown in green at left, class 2 (n=40) in turquoise in the middle. Class 3 (purple, n=4) and Class 4 (yellow, n=2) represented bad particles. Organization of classes in the array patch is shown at right, with red representing particles not included in the clustering. Note that colors denote the relative abundance of the classes and do not correspond to the classes in Figure 1.

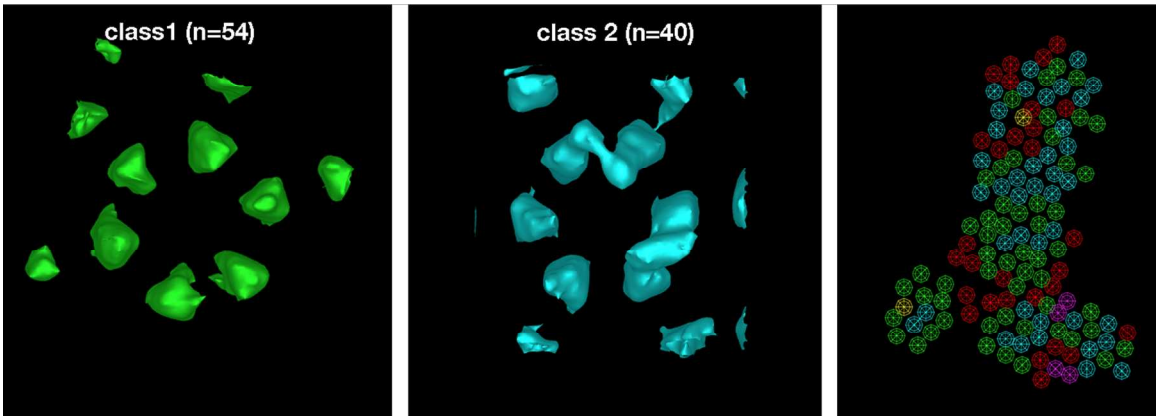


Figure S6. Cytoplasmic chemoreceptor array in *Vibrio cholerae*. Side view of a cytoplasmic chemoreceptor array (CA). PA: polar chemoreceptor array, IM: inner membrane, OM: outer membrane.

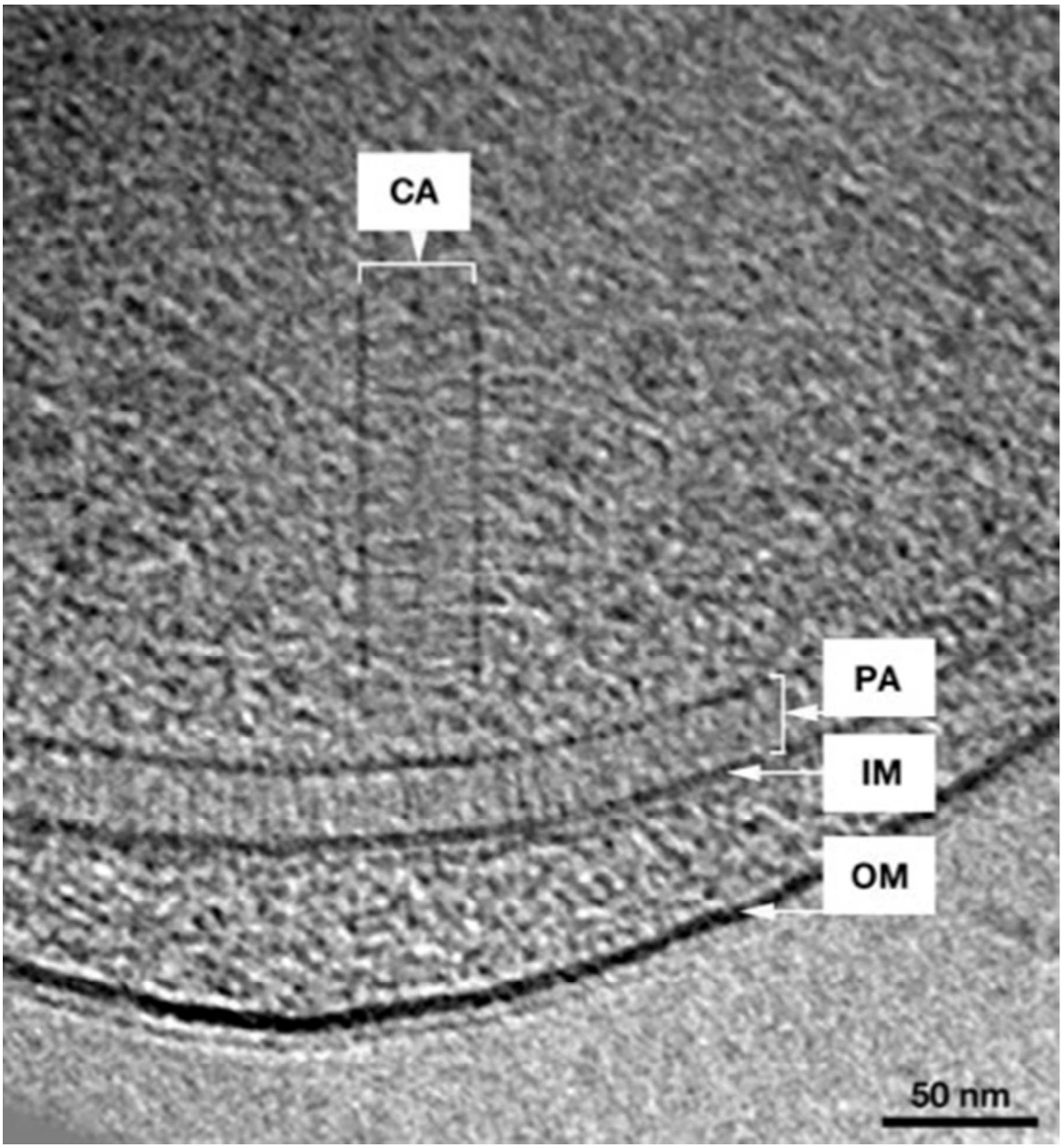


Figure S7. Arrangement of cytoplasmic receptor fragments assembled *in vitro* with CheA, CheW, and molecular crowding agents. Three tomographic slices through a 12-nm array show ordered chemoreceptor lattices at the top (left) and bottom (right), with loss of order at their interacting tips (middle). Bar 100nm.

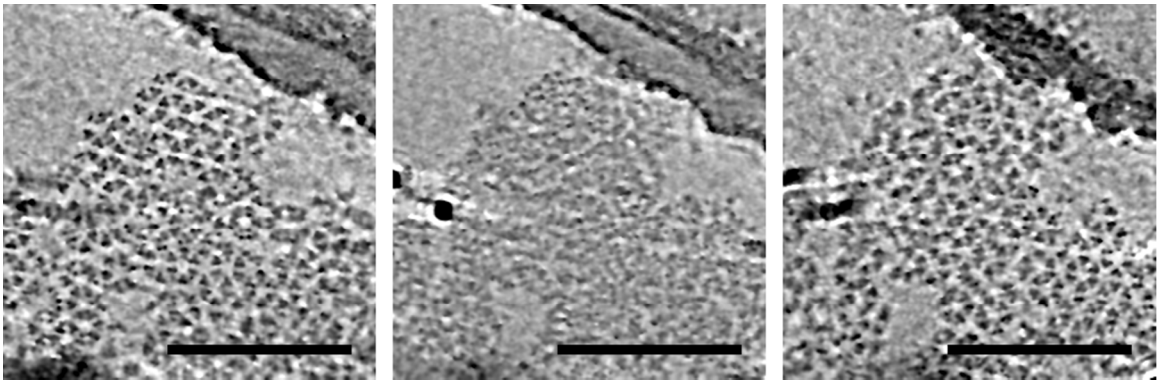
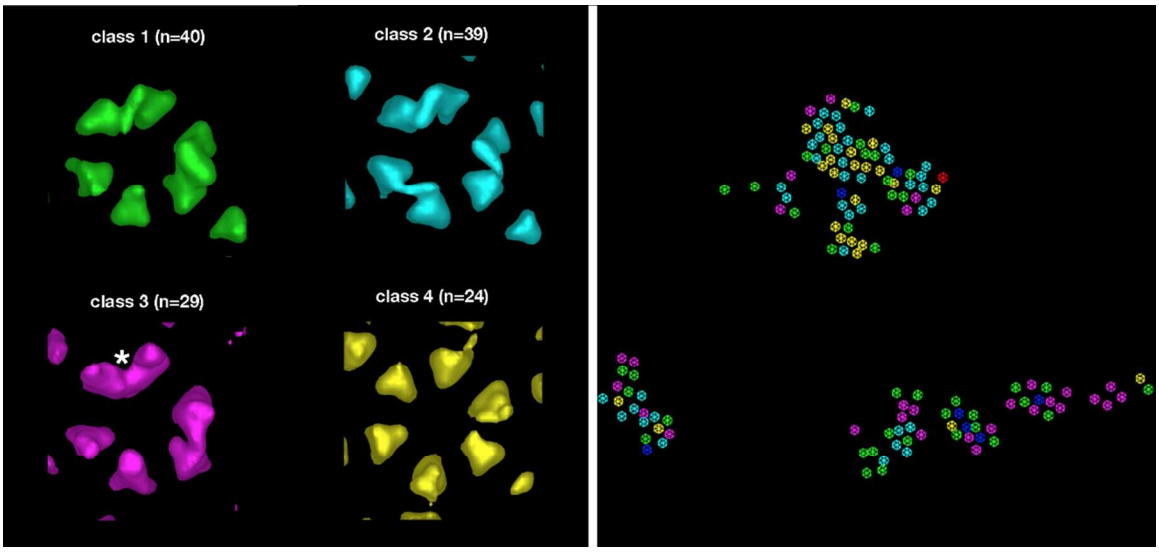


Figure S8. Classification of array hexagons formed by *in vitro* assembly of Tsr-CF, CheA, CheW, and a molecular crowding agent. Classification by principal components analysis and k-means clustering of hexagons in the array patch shown in Figure 6 results in 6 classes. Average of class 1 (2 CheA dimers; n=40) is shown in green, class 2 (3 CheA dimers; n=39) in turquoise, class 3 (1 CheA dimer; n=29) in purple, and class 4 (no CheA; n=24) in yellow. Class 5 (blue, n=7) and Class 6 (red, n=1) represented bad particles. Organization of classes in the array patch is shown at right. Asterisk in class 3 indicates a connecting density at the receptor tips, not CheA. Note that colors correspond to the relative abundance of the classes and do not correspond to classes in previous figures.



References:

1. Parkinson, J. S., and Revello, P. T. (1978) Sensory adaptation mutants of *E. coli*, *Cell 15*, 1221-1230.

2. Briegel, A., Ames, P., Gumbart, J., Oikonomou, C. M., Parkinson, J. S., and Jensen, G. J. (2013) The mobility of two kinase domains in the Escherichia coli chemoreceptor array varies with signaling state, *Mol Microbiol 89*, 831-841.

3. Wolfe, A. J., Conley, P., Kramer, T. J., and Berg, H. C. (1987) Reconstitution of signaling in bacterial chemotaxis, *J Bacteriol 169*, 1878-1885.