PNAS

Supplementary Information for

On the formation of ordered protein assemblies in cell-cell interfaces

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- Figures S1 to S5
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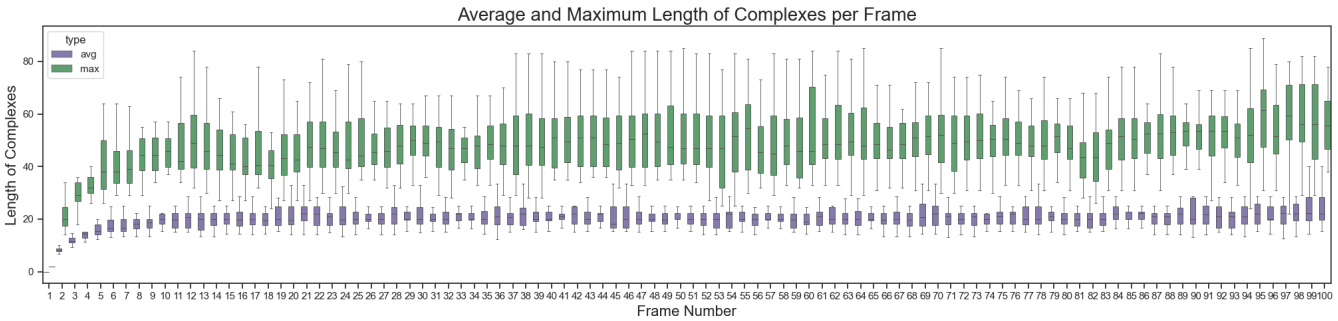
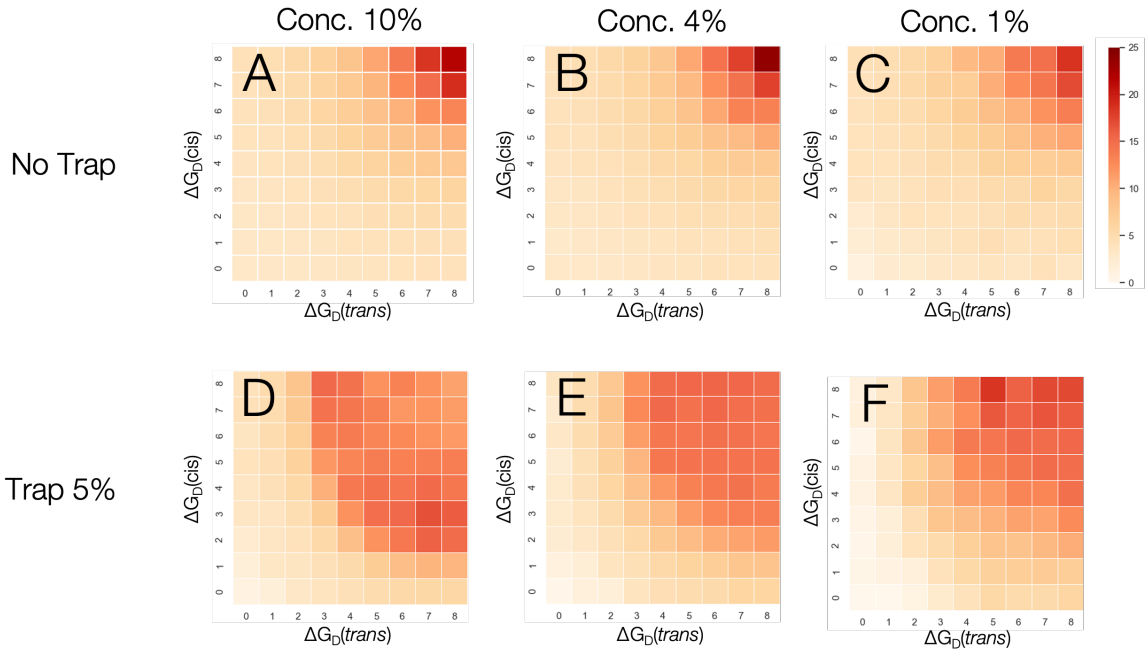
SI Figure 1



$\Delta G_D(cis) = 8kT$, $\Delta G_D(trans) = 8kT$

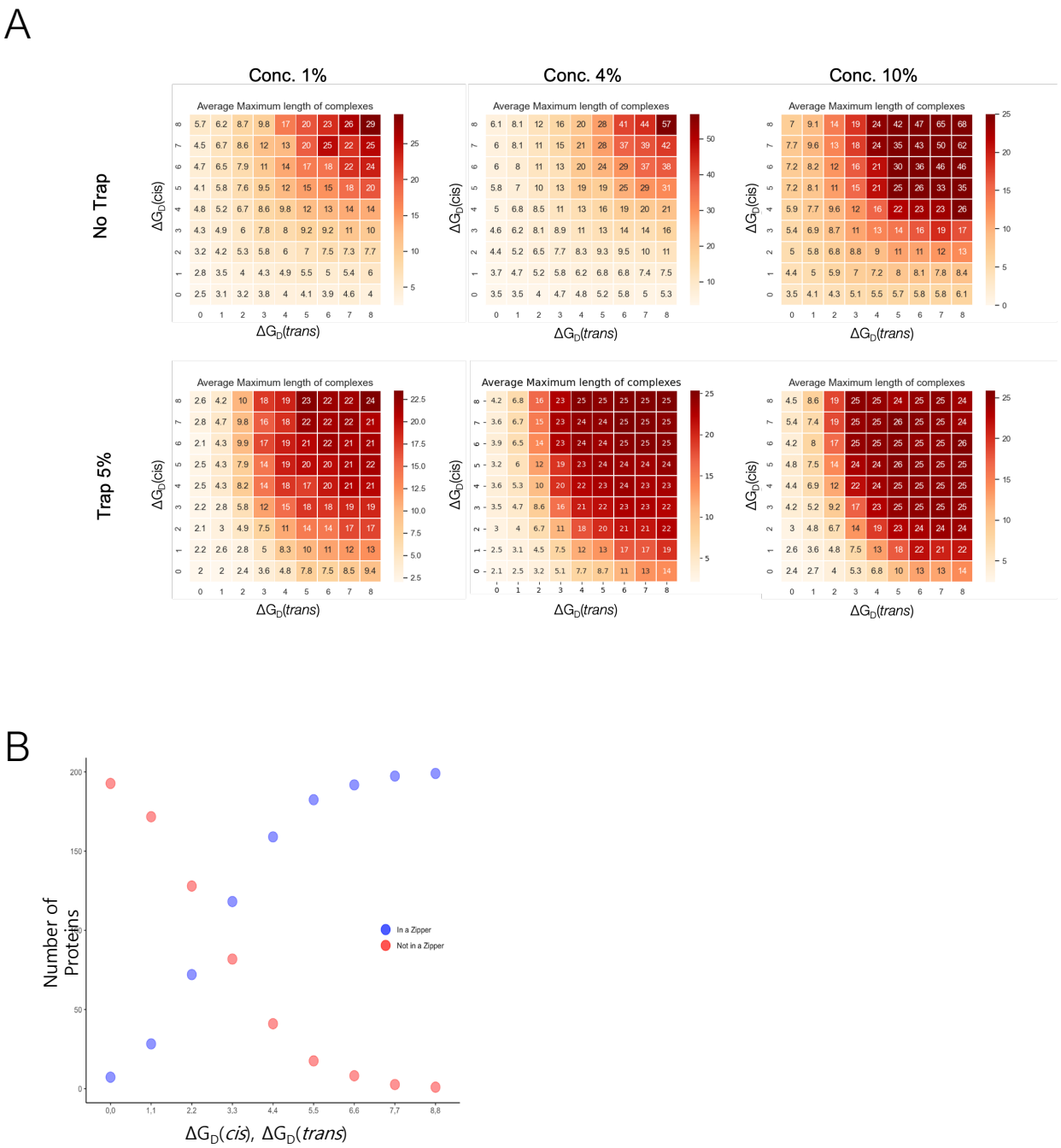
SI Figure 1. The simulations reach equilibrium. All simulations reached equilibrium as tested by ploting the average (purple) and maximum zipper length (green). *In this case the average and maximum are calculated for 20 simulations with the same parameters (concentration = 4%,* ΔG_D*-cis = 8kT,* ΔG_D*-trans = 8kT*)

SI Figure 2



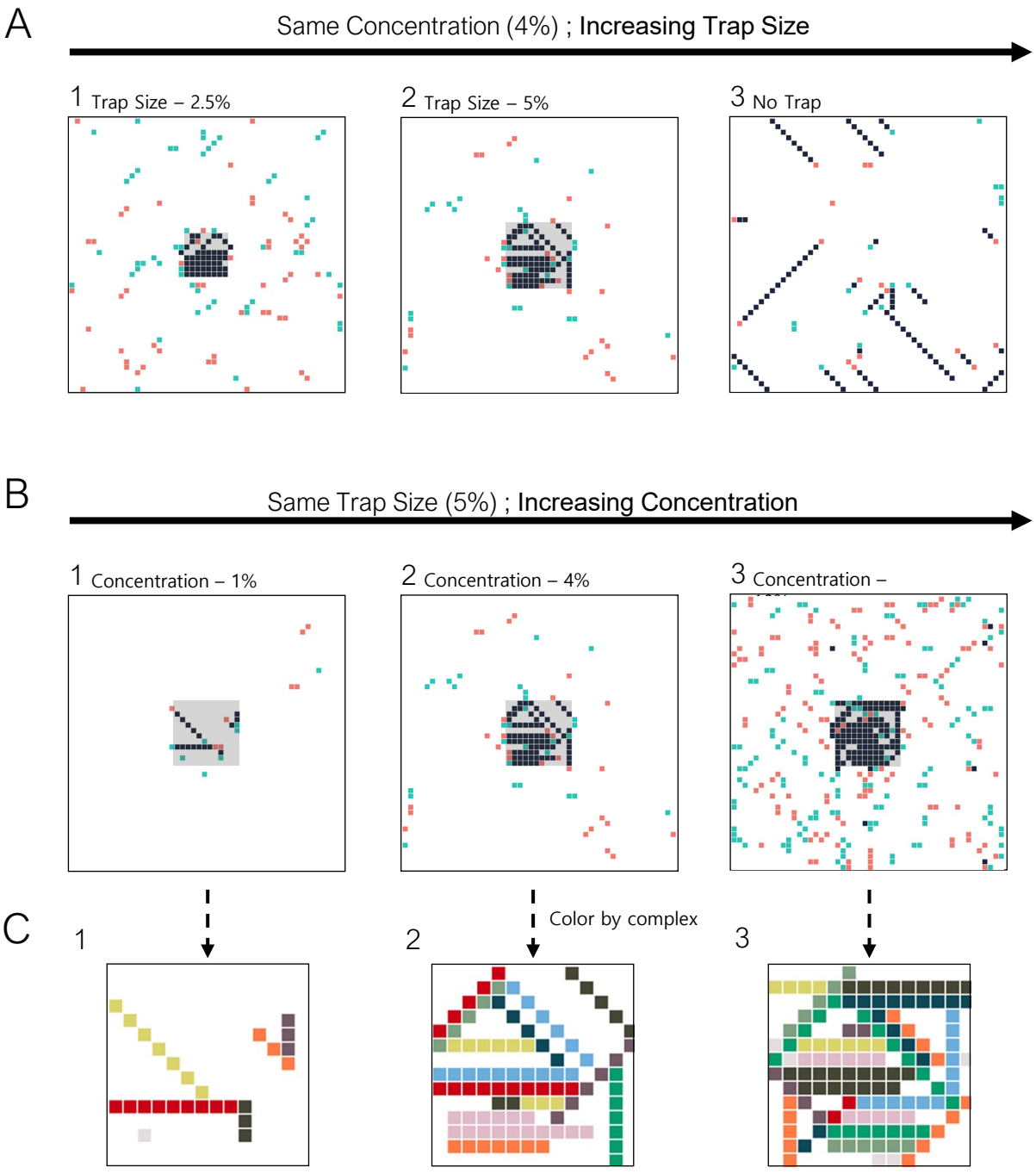
SI 2: ***cPcdhs form long zippers whose length is affinity and concentration dependent.***
Heatmaps showing the average size of zipper-like arrays at the last step of simulation using different combinations of *trans* and *cis* affinities (X and Y axis respectively), three different protein concentrations (1%, 4%, and 10%), and two different cell-cell contact areas (no trap and 5% diffusion trap) . For each set of parameters, the values of the average zipper size are averaged over 20 independent simulations. The size of the zipper-like array increases with increase of affinities and protein concentration.

SI Figure 3



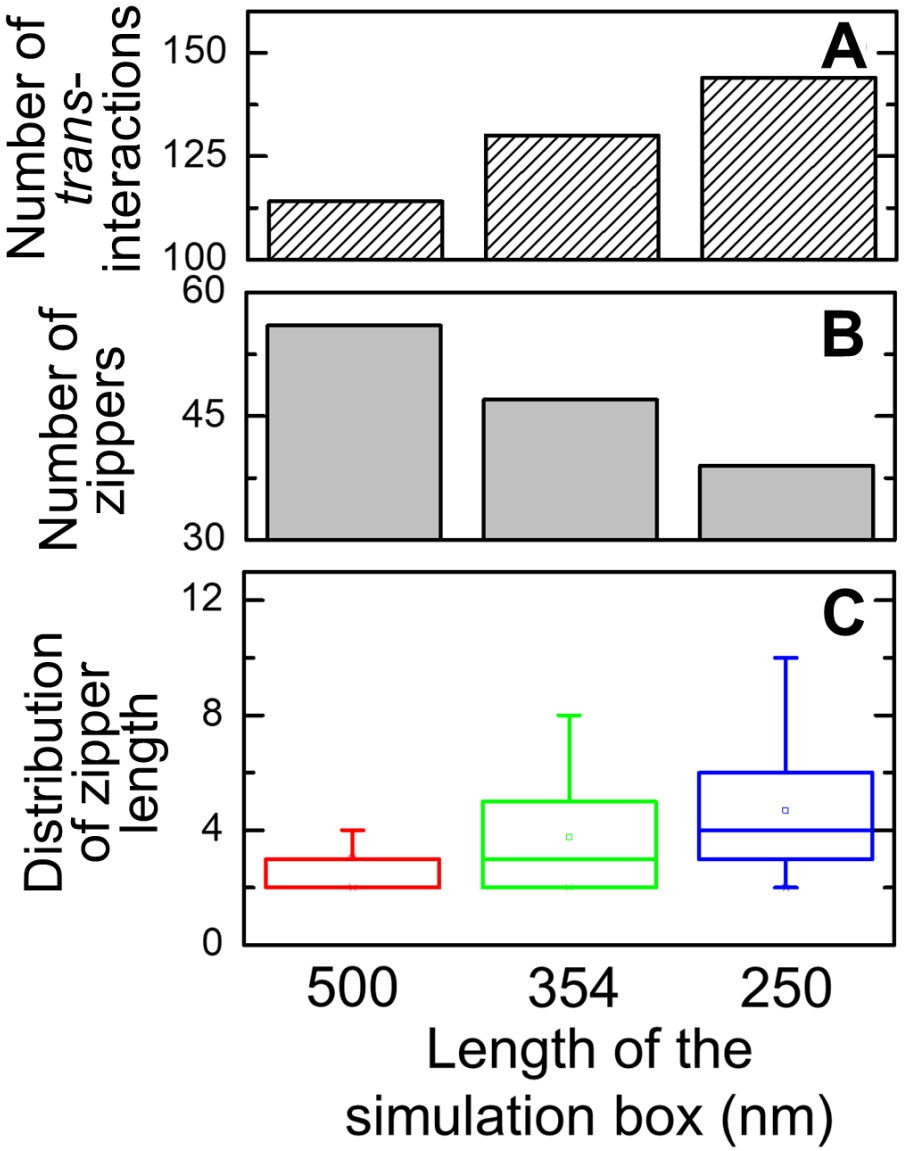
SI Figure 3: c*Pcdhs form long zippers whose length is affinity and concentration dependent.* A) Heatmaps showing the maximum size of zipper-like arrays at the last step of simulation using different combinations of *trans* and *cis* affinities (X and Y axis respectively), three different protein concentrations (1%, 4%, and 10%), and two different cell-cell contact area (no trap and 5% diffusion trap). For each set of parameters, the values of the maximum zipper size are averaged over 20 independent simulations. B) The Number of cPcdhs that are in zippers (blue) and are not in zippers (red) was counted at the last step of simulations with nine different combinations of *cis* and *trans* affinities (x-axis). Protein concentration in each simulation was 4% of the total grid size (200 proteins in total). When *cis and trans* affinities are both at least moderate ($\Delta G_D \geq 3kT$ for both) the majority of cPcdhs are part of zipper like assemblies.

SI Figure 4



SI Figure 4: cPcdhs zippers form 2D arrays that cluster at high protein concentration. Simulation snapshots of the 2D lattice model with the same conditions as in Figure 3 except zippers assemblies cannot rotate. The snapshot represents how diffusion trap size (panel A) and concentration (panel B) influence zipper formation and clustering. C) Magnification of the diffusion trap area (grey area) in B. Each zipper-like assembly is depicted by a different color.

SI Figure 5



SI Figure 5: The kinetic MC simulations were carried out under different surface concentrations. The dependence of total number of *trans* interactions and total number of zippers on the length of simulation box are plotted in A and B, respectively. The distributions of zipper length under different concentrations are further shown in C as a box-whisker plot. The box of each distribution in the plot includes the 25–75 percentile range for the zipper length formed in each system, while their average number is marked in the middle of the corresponding box. The whisker indicates the outlier of the distribution with the coefficient equal to 1.5.

SI Table 1

	Simulation	Variable	Values used	Explanation
1	Lattice-based	Concentration	1%, 4%, 10%	From previous studies on classical cadherins, the number of classical cadherins cell surfaces ranges between 25,000– 250,000 (7, 42). Since no such information is available regarding the expression level of cPcdhs we used 1%, 4% and 10% concentration corresponding approximately to this range.
2		ΔG_D(*trans*)	0kT – 8kT	*Trans* binding free energies in solution of classical cadherins range from 20-170µM (41). These values correspond to ΔG_D values of around 6.7-7.7kT in 2D (7, 41). Since cPcdhs in solution have *trans* binding free energies of 2-150µM (24, 26, 30, 31), we used a similar range of ΔG_D values.
3		ΔG_D(*cis*)	0kT – 8kT	cPcdhs *cis* binding affinities are similar to the *trans* binding affinities and are around 9-80µM (24, 27, 30) in solution. We therefore use similar values as used for trans interactions.
4		Contact area	100%, 5%, 2.5%	Since the contact area between two membranes is limited and leads to a high concentration of interacting proteins, we tested both full-membrane contact and two limited areas to understand the effect of this phenomenon.
5	Domain-based	Concentration	200 *cis*-dimers on a surface of dimension 500nm×500nm	This is equivalent to 800 *cis*-dimers per μm^2 corresponding roughly to concentrations between 1% and 4% used in the lattice-based simulations.
6		ΔG_D(*trans*)	The association rate of the *trans* interactions was fixed at 10 ns^{-1}, while the dissociation rate varied from 0.003ns^{-1} to 0.000001ns^{-1}	These values correspond to 2D K_Ds ranging from $3x10^{-4}$ to 10^{-7} corresponding to 2D affinities, ΔG_D(*trans*), of 3.5kT – 7kT.
7		ΔG_D(*cis*)	NA	For computational efficiency we assumed that all cPcdhs are in *cis* dimers.
8		Contact area	Grow dynamically over the course of simulations	We created a circular zone in the center of the simulation box. The radius of the contact area was initially set to 0 and increased linearly with the simulation time, mimicking the growth of the contact area between two cells driven by the formation of *trans* interactions