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Supplemental Information

Molecular Crowding and Diffusion-Capture in Synapses

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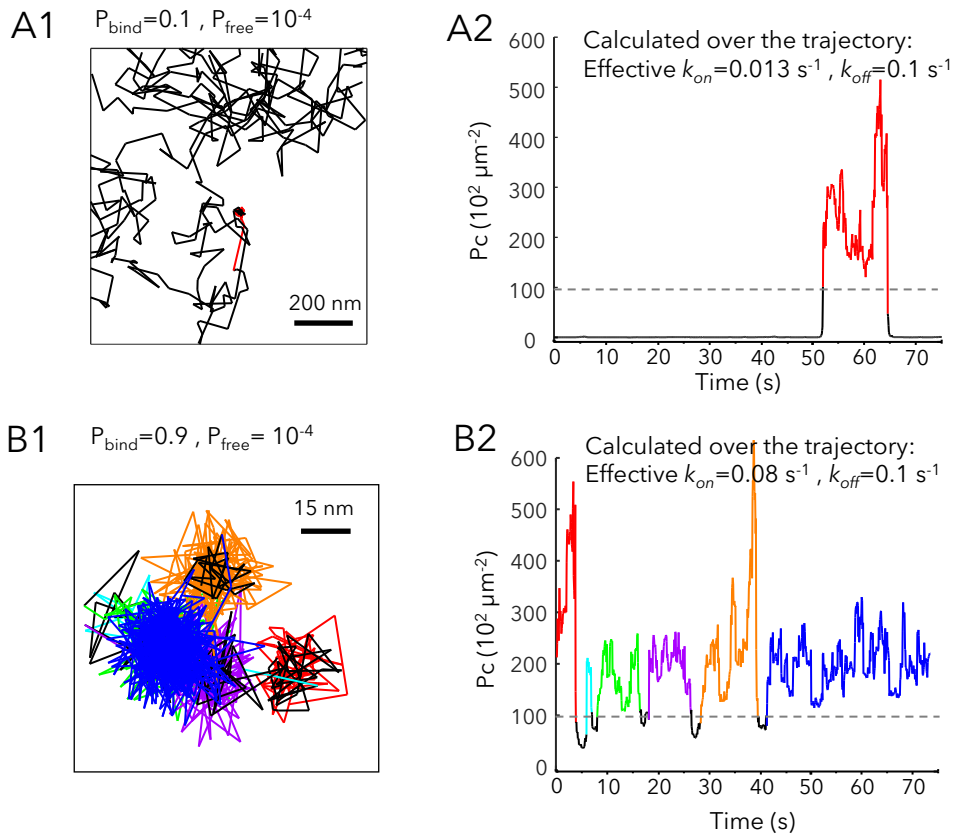

Figure S1: Analysis of capture on simulated trajectories. Related to Figure 1. Examples of simulated trajectories with the indicated P_{bind}, after conversion to SPT temporal and spatial resolutions (see Transparent methods). Binding periods were detected by Packing coefficient (Pc) analysis (see Transparent methods), and the calculated values of effective k_{on} and k_{off} on these trajectories are shown on the left panels. **A** : Trajectory with one short event of binding (red portion of the trajectory in A1, and on Pc plot in A2). **B**: Trajectory with several events of binding (color segments in B1 and B2).

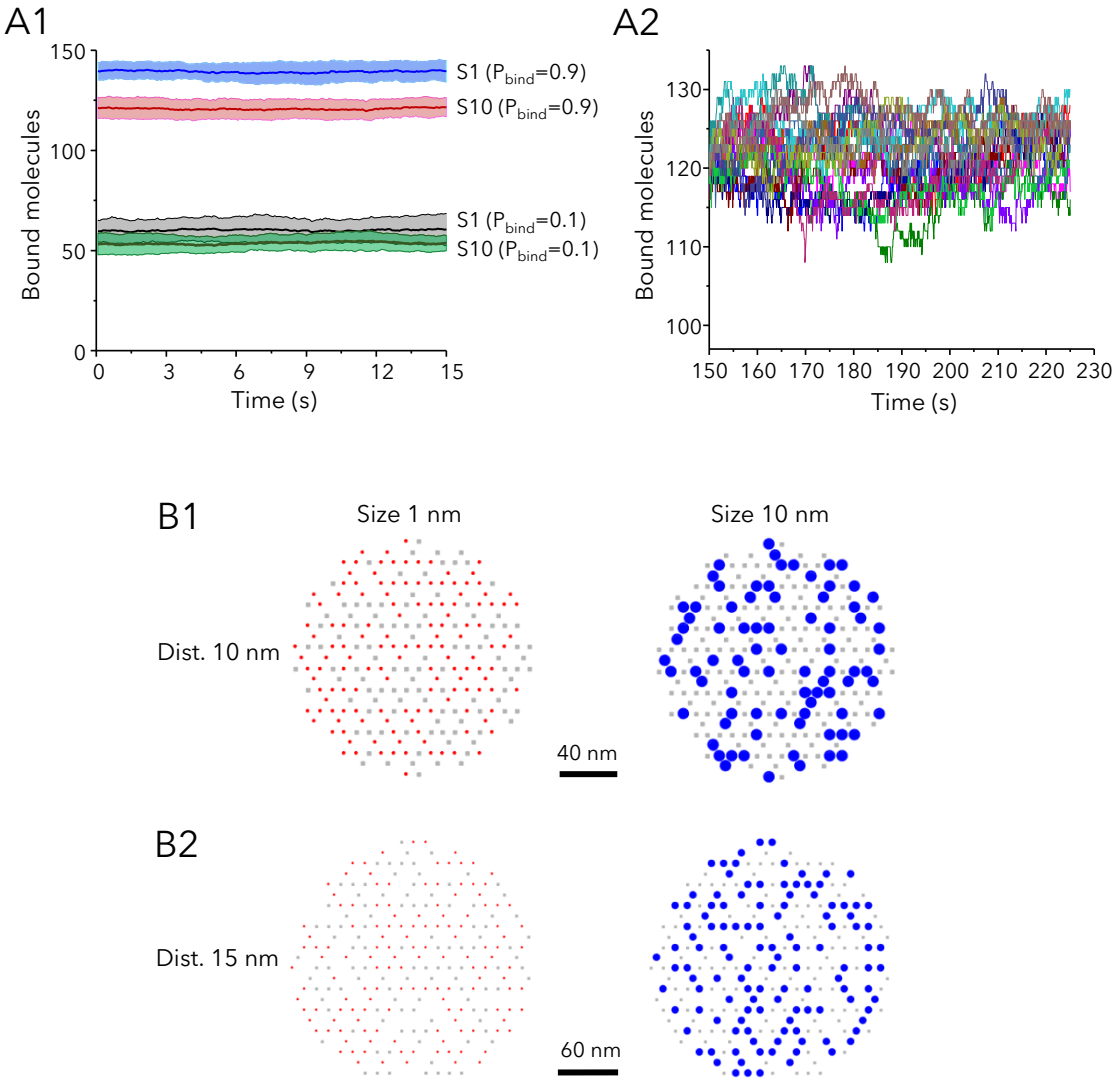

Figure S2: Simulation of capture of molecules in simulated synapses. Related to Figure 1. A: Number of bound molecules in time once the steady state was reached in synapses containing 250 sites. The distance between sites was 15 nm. **A1**: mean (central line) and SD (shaded areas) during a 15s-long period, for small (s1, size: 1nm in diameter) or large (s10, size: 10 nm) molecules with the indicated probability of binding P_{bind}. **A2**: window of 75s showing the number of bound molecules for 10 simulations (each simulation depicted in a different colors) for large molecules with P_{bind}=0.9. For the sake of clarity, only one every 75 time points are shown (one time point corresponds to 1ms). **B:** Examples of synaptic areas with bound molecules (snapshots), representative of the steady state in synapses containing 250 sites, distanced 10 nm (B1) or 15 nm (B2). Bound molecules are shown in red (small ones) or in blue (large ones). Empty sites are represented by grey dots.

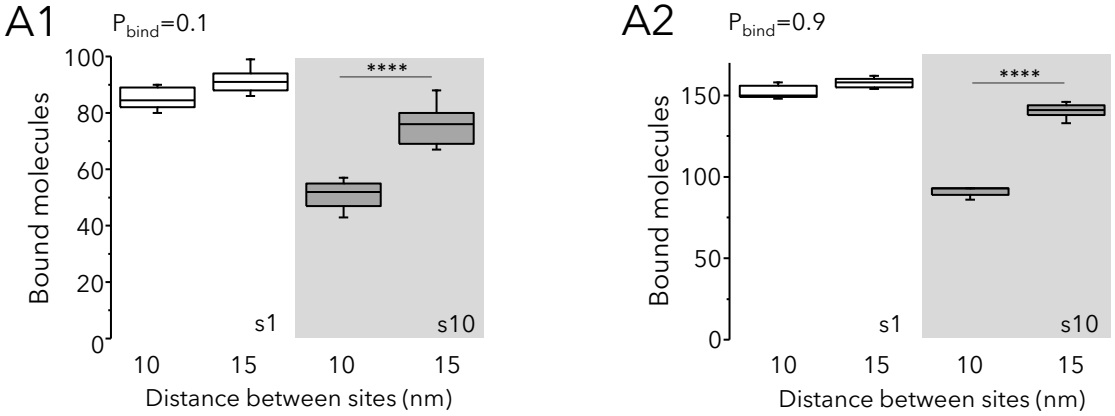

Figure S3: Capture of molecules in simulated synapses with reduced P_{free} ($0.5x10^{-4}$). Related to Figure 1. Number of bound molecules in synapses with 250 sites distanced 10 nm (A1) or 15 nm (A2), in steady state, for small (s1) or large (s10, gray area) molecules and low (B1) or high (B2) P_{bind} (median, 25-75 IQR and 5%-95% range, 10 independent simulations, unpaired t-test, ****: $p<0.0001$).

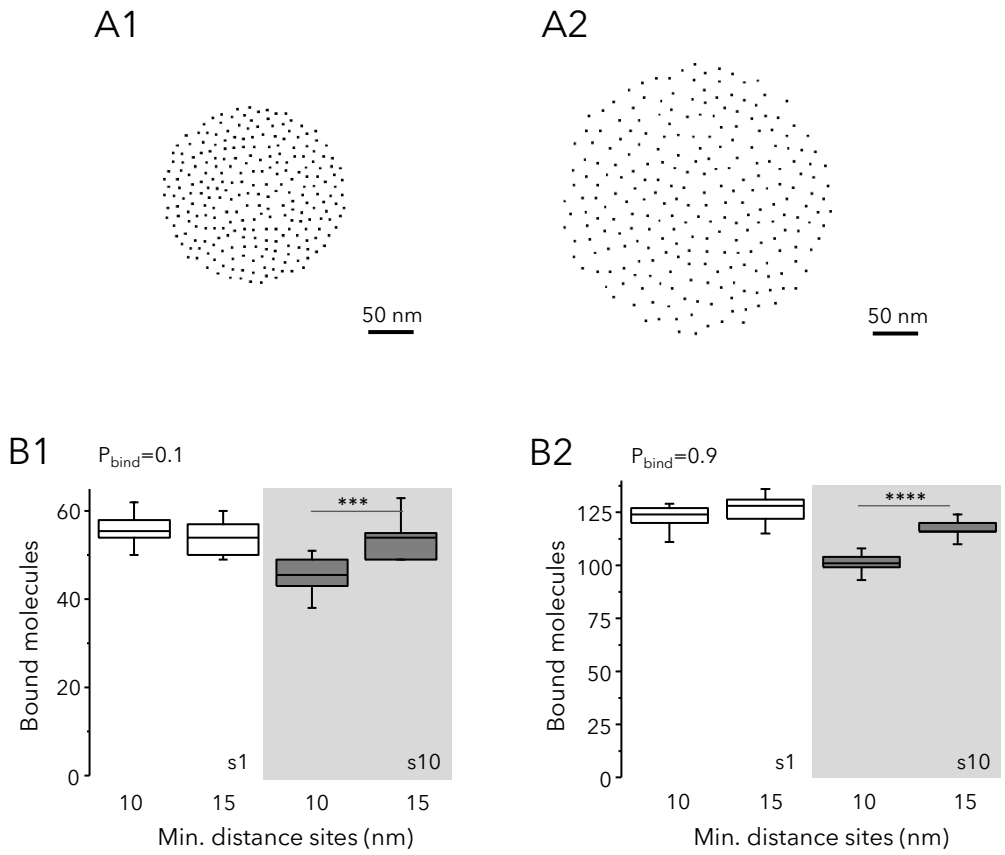

Figure S4: Capture of molecules in simulated synapses with random distribution of scaffolding sites. Related to Figure 1. A: Schemes of 250 randomly distributed binding sites, in a synapse of 210 nm in diameter and 10 nm as a minimum distance between sites (**A1**) or a synapse of 320 nm in diameter and 15 nm as the minimum distance between sites (**A2**). **B:** Number of bound molecules in synapses as in A, in steady state, for small (s1) or large (s10, gray area) molecules and low (B1) or high (B2) P_{bind} (median, 25-75 IQR and 5%-95% range, 10 independent simulations, unpaired t-test, ***: $p<0.001$, ****: $p<0.0001$).

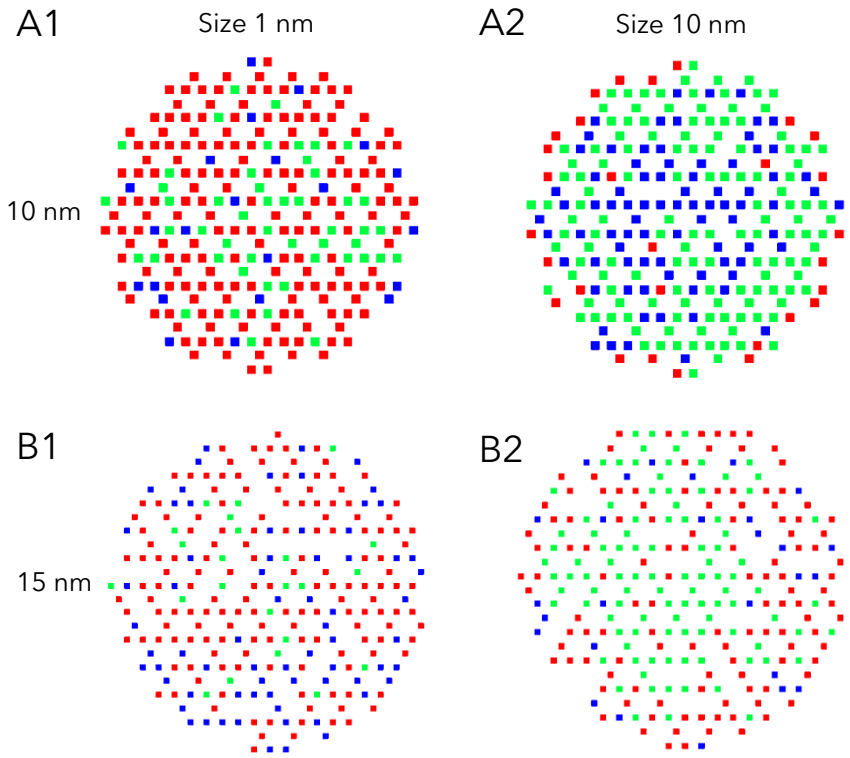

Figure S5: Availability of sites. Related to Figure 1. Examples of synaptic areas containing 250 sites, distanced 10 nm (A) or 15 nm (B). Sites are shown in color (size of sites not in scale) depending on the relative number of molecules that were bound to them, for small (Size 1nm; A1 and B1) or large (Size 10 nm; A2 and B2) molecules. Results correspond to molecules with P_{bind}=0.9. In red: sites that were often occupied (more than 66% of bindings); in green: sites that collected 33 to 66% of bindings; in blue: sites that were occasionally occupied (less than 33% of bindings). All the sites were visited at least once during the simulation run (225s). Note that the sites in the center of synapses are less efficient to capture large molecules (A2 and B2).

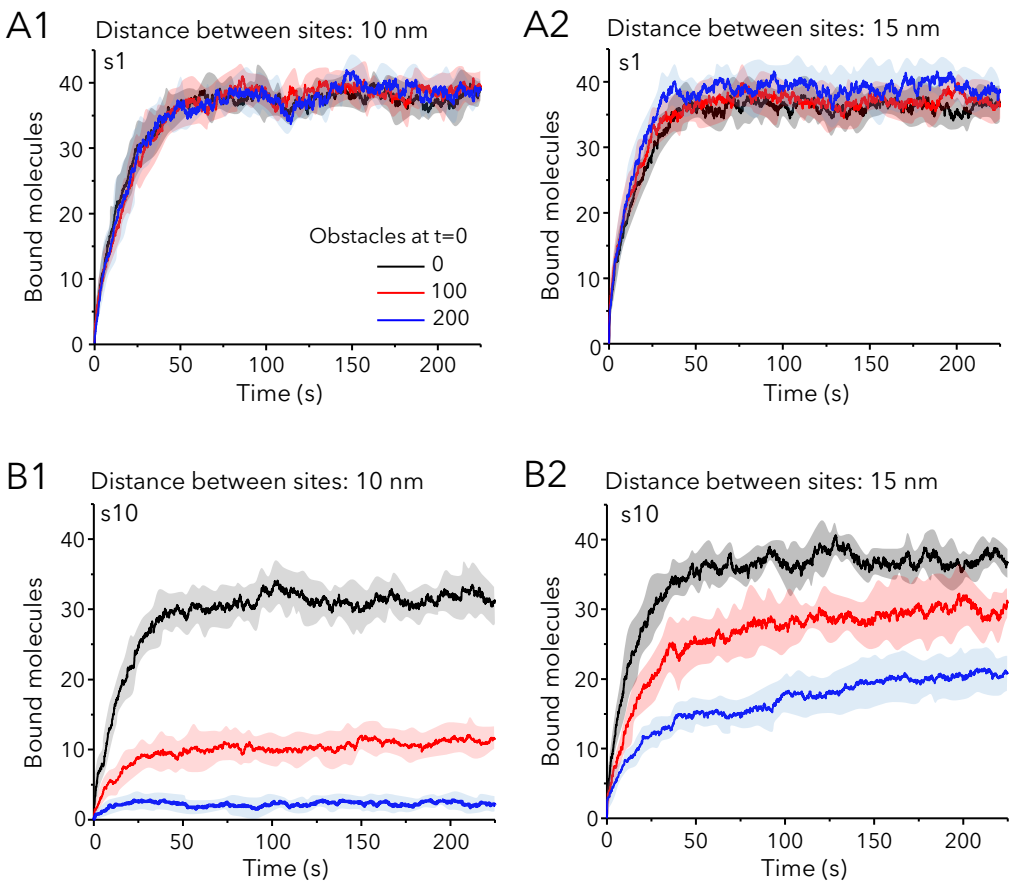

Figure S6: Molecular crowding slows down the capture of new molecules. Related to Figure 3. Number of small (A1, A2) or large (B1,B2) molecules bound (P_{bind} =0.9) vs time during the whole simulation run (225s) in synapses with initially 50 free sites and with 0, 100 or 200 extra obstacles at t=0 as indicated (colors in A1). Sites were separated 10 nm (A1, B1) or 15 nm (A2, B2). Mean (line) and SD (shaded areas) (10 independent simulations).

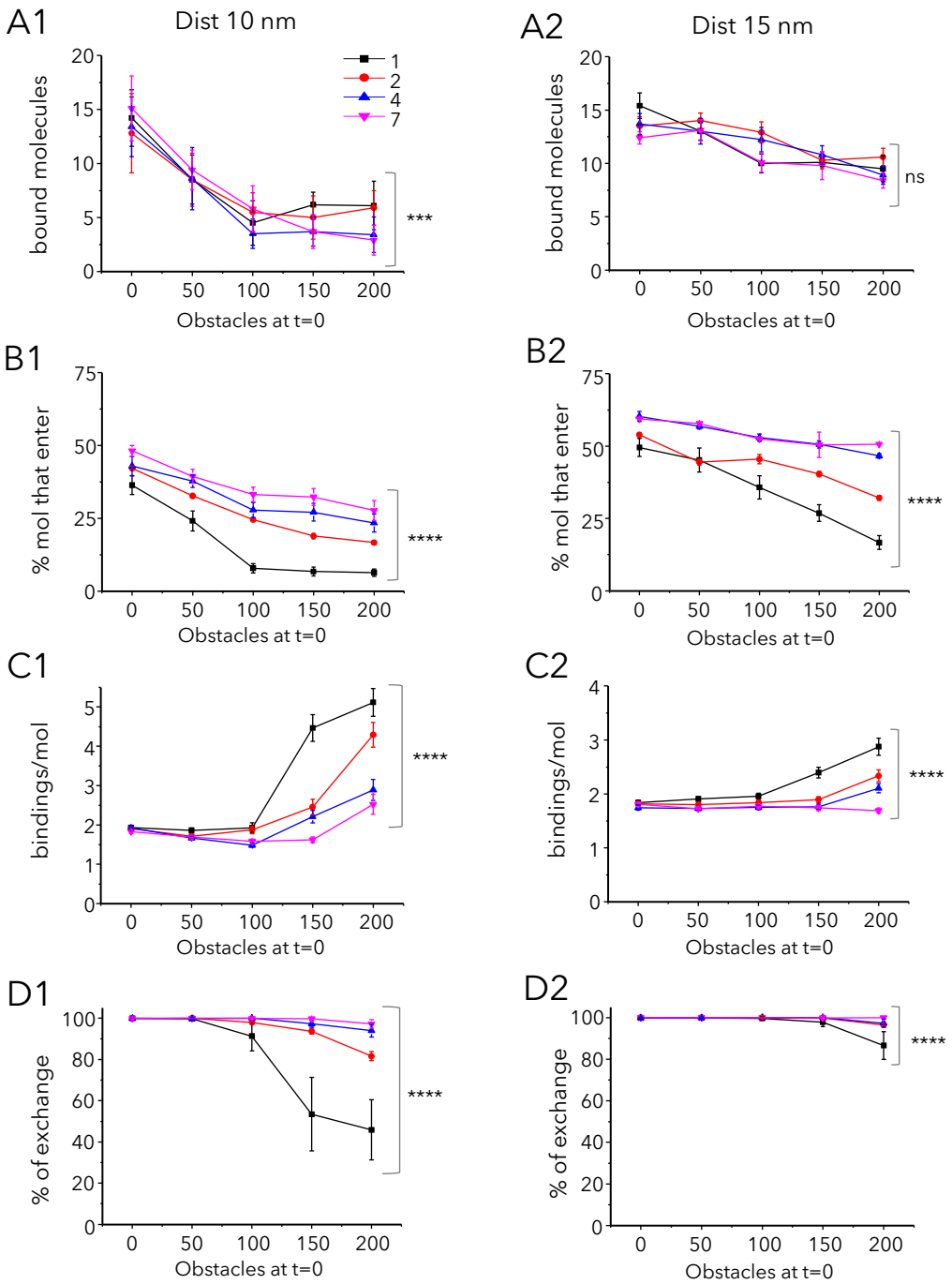

Figure S7: Molecular crowding favors multiple bindings and reduces the exchange of molecules. Related to Figure 5. Binding and exchange of molecules (size=10 nm and P_{bind} =0.1) in and out synapses at steady state. Synaptic areas had 50 binding sites and the indicated number of obstacles at t=0. Sites were distanced 10 nm (A1,B1,C1,D1) or 15 nm (A2,B2,C2,D2) and distributed in 1,2,4 or 7 clusters (color code in A1). Values are the mean ± s.e.m. of 10 independent simulations (statistical comparisons in case of 200 obstacles: one-way ANOVA, ns: not significant, ****:p<0.0001). **A:** Number of bound molecules at the end of the simulation period (225s). **B:** Percentage of simulated molecules that enter at least once in the synaptic area. **C:** Number of bindings (to the same or a different site) per molecule during the whole simulation run. **D:** Percentage of exchange (proportion of molecules that enter and exit the synaptic area at least once).

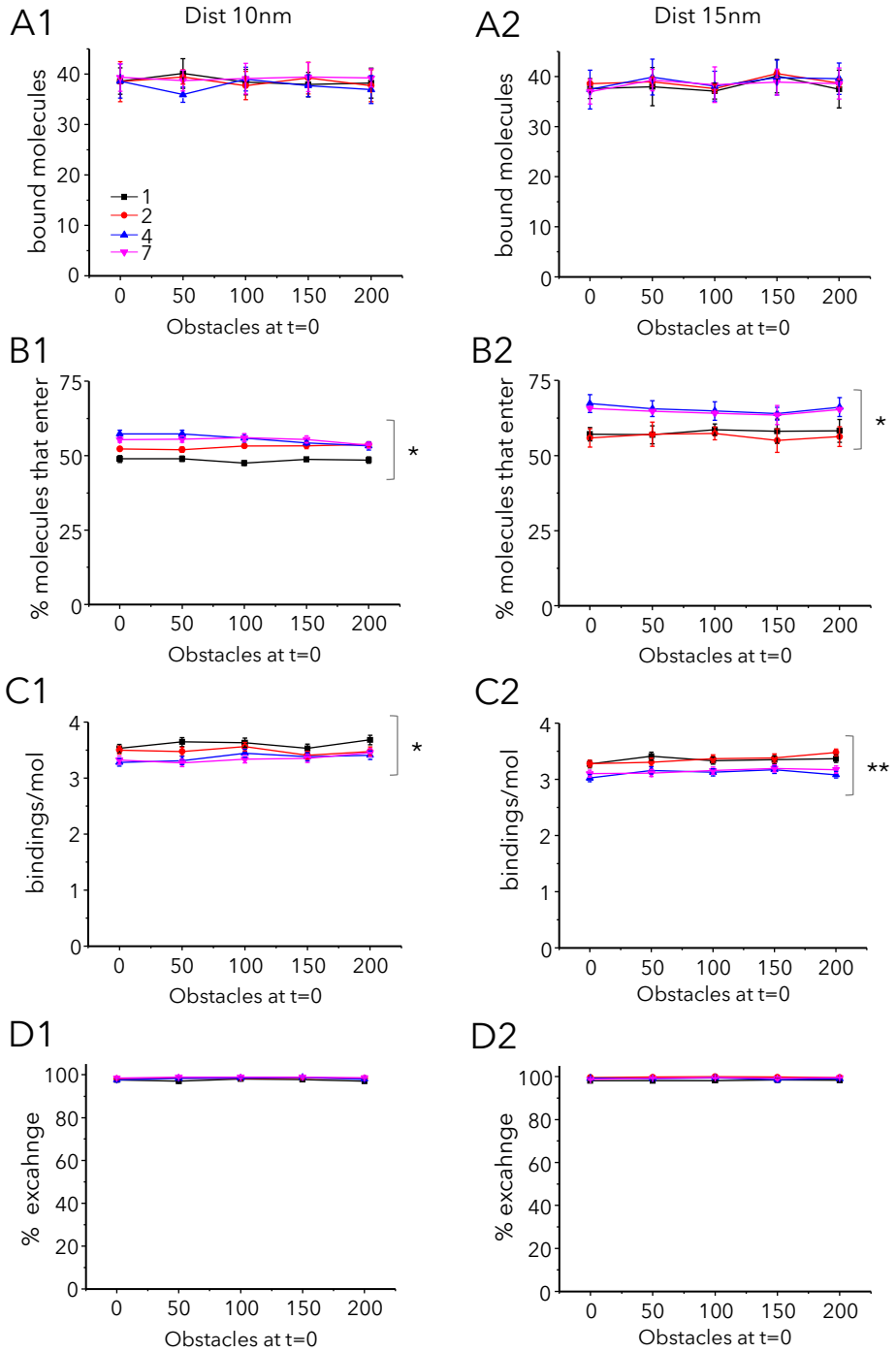

Figure S8: In absence of crowding, the distribution of sites in multiple clusters promotes the exchange of molecules. Related to Figure 5. Binding and exchange of molecules in absence of crowding (size=1 nm and P_{bind} =0.9) in and out synapses at steady state. Synaptic areas had 50 binding sites and the indicated number of obstacles at t=0. Sites were distanced 10 nm (A1,B1,C1,D1) or 15 nm (A2,B2,C2,D2) and distributed in 1,2,4 or 7 clusters (color code in A1). Values are the mean ± s.e.m. of 10 independent simulations (statistical comparisons in case of 200 obstacles: one-way ANOVA, ns: not significant, *: p<0.05, **: p<0,01). **A:** Number of bound molecules at the end of the simulation period (225s). **B:** Percentage of simulated molecules that enter at least once in the synaptic area. **C:** Number of bindings (to the same or a different site) per molecule during the whole simulation run. **D:** Percentage of exchange (proportion of molecules that enter and exit the synaptic area at least once).

Transparent methods

Monte Carlo simulations

The simulation script was coded in Matlab (The MathWorks) and run in a personal computer. Trajectories were simulated in a 2D space (square of 15 x 15 μm) introducing rebound conditions on each side to keep all the molecules (200 in total) in the area and reach equilibrium. In the center of this square, the binding sites area was simulated as a circle with the minimum diameter needed to hold at least 250 binding sites. Binding sites (squares of 3 nm in size) were distributed at randomly selected nodes of a hexagonal grid (Fig.1A). Nodes were separated 10 (cercle of 190 nm) or 15 nm (cercle of 300 nm). Alternatively, sites were distributed randomly on the entire cercle, respecting a minimum distance of 10 nm or 15 nm. In this case, cercles were somehow larger (210 nm or 320 nm in diameter, respectively).

In case of synapses with several clusters of sites (Fig. 2A), the diameters of individual clusters were 140-210 nm in case of two clusters, 100-150 nm in case of four clusters, 75-115 nm for seven clusters (sizes corresponding to sites distanced 10 and 15 nm, respectively). Clusters were distributed in a cercle of 250-550 nm in diameter, compatible with the reported sizes of the post-synaptic density (Specht et al., 2013; reviewed in Choquet and Triller, 2014). They were positioned so to leave at least 30 nm of free space between them.

Trajectories were simulated as in Renner et al. (2017), with some modifications. The x and y components of the i-th displacement step in the trajectory were randomly selected from two independent normal distributions with the mean of zero and the variance equal to 2 D_{sim} Δt. D_{sim} was 0.02 $\mu m^2/s$. The difficulty of particle-based Brownian dynamics simulations is to choose a time step Δt small enough to accurately describe reaction-diffusion processes without sacrificing computation efficiency, taking into account the period of time relevant for the system. As a trade-off, two time steps Δt were used: Δt = 1 ms was used in regions far from binding sites (no binding site in a distance that could be travelled in one time step) and Δt = 0.1 ms in the vicinity of a binding site. The presence of binding sites within this distance was analyzed before any movement was done, to choose Δt for the next step. With D_{sim} = 0.02 $\mu m^2/s$ the typical displacement of free molecules in one time step was 8.9 nm with Δt = 1 ms and 2.8 nm with Δt = 0.1 ms.

Molecules and obstacles were simulated as circles of 1 or 10 nm in diameter. Obstacles behave as a separate type of molecules that occupied nodes of the hexagonal grid. They were kept unreactive and immobile during all the simulation run. When one molecule hit another (mobile or immobile) or obstacles, it bounced back. When the molecule passed on top of a binding site, it remained quasi-immobile ($D_{sim}=10^{-4}$ μm2/s, the mobility of scaffolding sites in synapses, see in Supplemental references Hanus et al., 2006) on top of it if its probability of interaction P_{bind} was above a number *R* randomly generated from a uniform distribution. The stabilization lasted until the probability for detachment, P_{free}, exceeded another random number. P_{free}, which represents k_{off}, was set to 0.5 or $1x10^{-4}$ to obtain similar effective k_{off} that those calculated from experimental data (see Packing coefficient analysis below). P_{bind}, which represents k_{on}, was also chosen (0.1 or 0.9) regarding experimental values of effective k_{on}.

Plasticity-like changes were simulated by modifying P_{bind} after 15s of simulation at steady state. The total length of the simulation run was 225s. For LTP simulations, P_{bind} changed from 0.1 to 0.9. For LTD, P_{bind} changed from 0.9 to 0.1.

10-20 independent simulation rounds were run for each case. The random generator was seeded on the current time to produce a different sequence of numbers each time.

Bindings were registered during the simulation. The number of bound molecules was evaluated at given time points of the run. To calculate the percentage of molecules entering the synaptic region and the percentage of exchange, the synaptic area was defined as the convex hull containing all the binding sites. The position of the molecules was then evaluated with respect to this area. The percentage of molecules that enter the synaptic area at steady state was calculated with respect to the number of simulated molecules, during the last 75 s of the run. The percentage of exchange corresponded to the proportion of molecules that enter and exit the synapse at least once during the last 75 s of the run (100% means that all the molecules that enter the synaptic area did not remain in it during the whole the run).

"SPT-like" simulated trajectories and Packing coefficient analysis

To verify whether simulated trajectories had similar characteristics than experimental ones, they were converted to the temporal and spatial resolutions of trajectories obtained previously with SPT in the laboratory. Only one every 75 time points was kept (to simulate the acquisition frequency of 18 Hz) and a gaussian noise was added to each position of the molecule (mean zero and σ=20 nm) that corresponds to the localization accuracy of our SPT set up.

On these "SPT-like" trajectories, we applied the packing coefficient (Pc) analysis (Renner et al., 2017) as for experimental ones. Briefly, *Pc* was calculated at each time point *i* as

$$Pc_i = \sum_i^{i+n-1} \frac{(x_{i+1}-x_i)^2+(y_{i+1}-y_i)^2}{S_i^2}$$

where x_i, y_i are the coordinates at time *i*; x_{i+1}, y_{i+1} are the coordinates at time *i+1*, *n* is the length of the time window (*n*=30 time points) and S_i is the surface area of the convex hull of the trajectory segment between time points *i* and *i+n*. S_i was calculated using the *convhull* function in Matlab. Binding events were detected using $Pc_{thresh}=10^4\ \mu m^{-2}$, corresponding to a confinement in an area with a diameter of ~20 nm. This value was chosen considering the noise introduced into simulated trajectories. Periods of the trajectory avec higher *Pc* were considered as binding events. Effective k_{on} was calculated as the frequency of these events; and effective k_{off}, as the inverse of their duration (Renner et al., 2017).

Statistical analyses

Statistical analyses were done using two-tailed Student's t or one way ANOVA with Turkey's multiple comparison tests using Prism (GraphPad software, USA). Normality of distributions was checked with the Kolmogorov-Smirnov test. Images were prepared using Photoshop (Adobe Systems, USA).

Supplemental References

Hanus C., Ehrensperger M.V., and Triller A. (2006) Activity-dependent movements of postsynaptic scaffolds at inhibitory synapses. J Neurosci 26:4586–4595.