SUPPORTING INFORMATION

Cell Culture Conditions. Single cells prepared from embryonic day (E) 9 chick embryo dorsal root ganglia (lumbar region) were cultivated on the indicated substrates at a low density (146 cell/mm^2) in DMEM/F12 medium (Sigma) supplemented with 1% N2 (Invitrogen), 10 ng/ml nerve growth factor (Invitrogen) and 0.1% Gentamicin (Invitrogen) for 24 h in an incubator providing 5% CO_2 and 95% humidity.

Supplementary Figure 1. Labeling of DM-GRASP (antiserum), neurites (β3-tubulin monoclonal antibody, Covance) and cell nuclei (chromatin-binding dye DAPI) and merged picture of the ventral (i.e. lower, substrate-attached) and dorsal (i.e. upper, "free") cell membrane region (Zeiss Axiovert 200M, 63x objective (NA 1.40), AxiocamRev2). Note the differences in cell shape (filopodia, neurite in ventral plane). The DM-GRASP signal in the cell membrane at the somata is about 5 times higher compared to the DM-GRASP signal of the substrate, a 54 nm-spaced DM-GRASP nanopattern. Immunofluorescence (IF) brightness levels were measured (n=50) on the substrate: 33.52±1.84, the ventral membrane: 147.77±5.04, and dorsal membrane: 165.26±5.83 (no significant difference between ventral and dorsal plasma membrane: p=0.348). These IF values correspond to DM-GRASP densities of 392±5 molecules/μm^2 in the nano-patterned substrate, 1636±149 molecules/μm^2 in the ventral membrane and 1849±169 molecules/μm^2 in the dorsal membrane. The plasma membrane thus provides sufficient numbers of DM-GRASP molecules for interactions with each DM-GRASP molecule offered by all nanopatterns used in this study.

Supplementary Figure 2. (a) - (c) Complete specification of number of experiments (n), evaluated optical fields (OF), and significance values (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$) of Figure 2 a - c.

Supplementary Figure 3. Immunofluorescence (IF) brightness levels (a.u.: arbitrary units, ranging from 0 to 512) of immunolabeled DM-GRASP as a function of DM-GRASP density, calculated on the basis of the known density (nanopatterns, red) or the known DM-GRASP concentration in the coating solution (coverslips, blue) under the assumption of a complete deposition of all DM-GRASP molecules on the glass. Re-use of the DM-GRASP solution (after incubation) for coating of a second coverslip did not result in detectable IF brightness levels, indicating a practically complete adsorption of DM-GRASP to the coverslip already during the first incubation period. Up to a density of 50000 molecules/μm^2 (corresponding to about 20 µg/mL coating solution), a strong and almost linear increase of the IFs is observed. The excellent match of the two curves in the low concentration range allows for the determination of DM-GRASP densities on coverslips and on cells, using the IFs measured on DM-GRASP nanopatterns as calibration curve. The high concentrations of DM-GRASP used in the coating solution only cause minor increases in IFs, indicating a saturation of the glass surface and/or increasing inaccessibility of the DM-GRASP molecules for the antibodies. Whereas incubation with 20 µg/mL doubles the amount of DM-GRASP bound to the coverslip compared to the 10 µg/mL solution, incubation with 50 µg/mL causes an only 1.4 fold increase (instead of expected 2.5) compared to the 20 µg/mL solution. DM-GRASP molecules would already cover about 19% and 75% of the glass surface coated with solutions containing 5 and 20 µg/mL, respectively, assuming a 100% coating efficiency and vertical orientation of the molecules. The cross section area of a DM-GRASP molecule is about 16 nm^2 (immunoglobulin domain diameter: 4.5 nm [1]; cross section about circular due to globular structure).

Supplementary Figure 4. (a) - (c) Complete specification of number of experiments (n), evaluated optical fields (OF), and significance values (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$) of Figure 3 a - c.

Supplementary Figure 5. Top views of the used nanopatterns displaying all possible distances and potential spectrin-DM-GRASP interaction sites.

(1) Positions and numbers of all possible distances between the gold dots within a 300 nm radius, in addition to the shortest hexagonal distance ("spacing"). From the 29 nm- to the 137 nm-spaced pattern (density decrease: 95%), the numbers of possible distances (i.e. putative anchorage site for cortical cytoskeleton proteins) decrease by 92%.

(2) Analysis of the potential spectrin (green) interactions with DM-GRASP (red) via merlin (blue) in the middle (merlin binding site: light green) and via F-actin (positioned exactly beneath the gold dots, black) and other FERM members (purple) at the ends of the tetramer on the nanopatterns used (see also supplementary Figure 6). Spectrin tetramers are flexible and vary in their length from 160 to 180 nm, as determined by electron microscopy of the inner side of the plasma membrane [2], rotary shadowing of purified spectrin [3], and zero force length prediction [4]. For each pattern, the spectrin lengths which allow for the maximum of spectrin-DM-GRASP interactions are displayed. On 29, 54, and 86 nm nanopatterns, spectrin heterotetramers are able to interact at three sites (both ends and middle) with DM-GRASP molecules of the nanopatterns. On 70 nm nanopatterns, in contrast, the spectrin heterotetramers can only interact at two sites (both ends or one end and middle), and on 137 nm nanopatterns only a single interaction per spectrin heterotetramer is possible, not allowing for the formation of a continuous spectrin lattice (see supplementary Figure 5.3). Note that dot diameters and all molecules are displayed in scale.

(3) Analysis of the potential formation of a spectrin lattice on the DM-GRASP nanopatterns used. On 29, 54, and 86 nm nanopatterns, a continuous, typically hexagonal spectrin lattice [2] can form, with every spectrin heterotetramer stabilized at three DM-GRASP interaction sites. A spectrin lattice can form also on the 70 nm DM-GRASP nanopattern; the single heterotetramers, however, are only stabilized at two interaction sites and this is only achieved if the smallest/largest lengths (boundary values) are used. On the 137 nm DM-GRASP nanopattern, no spectrin lattice can be formed.

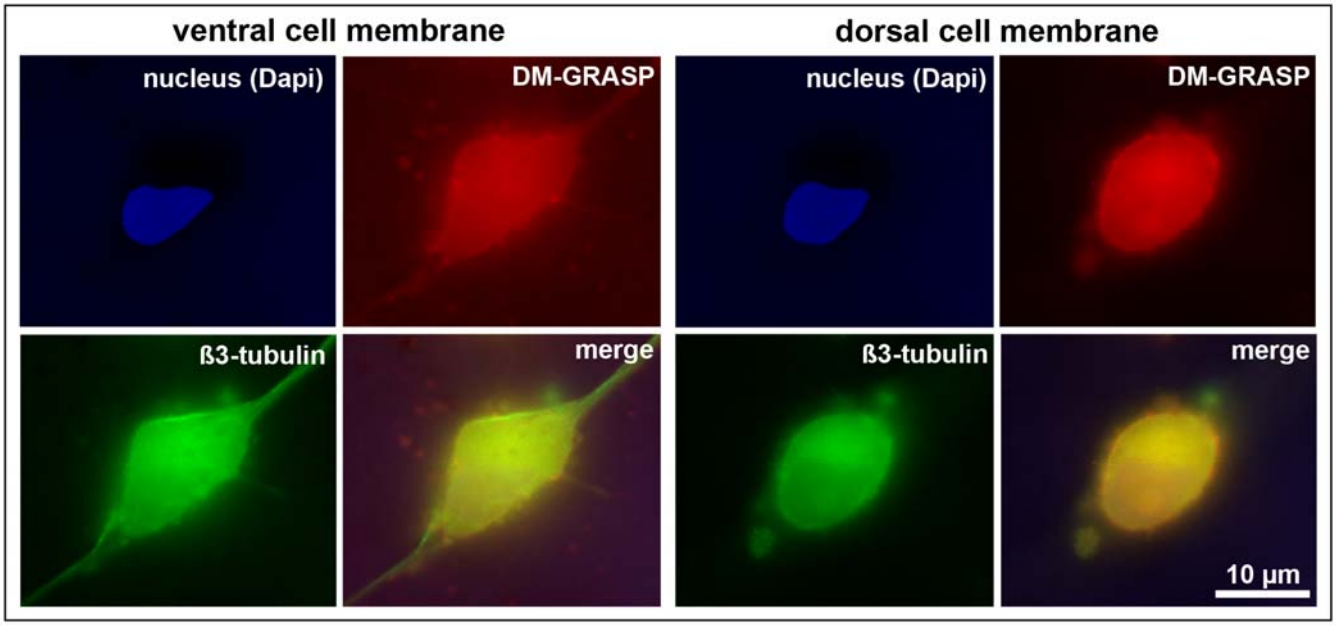
(4) Analysis of the occurrence of potential spectrin interactions with DM-GRASP on randomly distributed DM-GRASP, as e.g., present on conventionally coated glass coverslips; the density shown here corresponds to an 86 nm nanopattern, i.e. 148 molecules/μm^2. On the 1 μm^2 area displayed, only 17 spectrin heterotetramers are stabilized by 3 interaction sites which corresponds to 22% compared to the 86 nm nanopattern (78 heterotetramers). Since the spectrins which are stabilized at three sites are too sparse and uncoordinatedly located, the formation of a continuous spectrin lattice (as on the hexagonally arranged 86 nm spaced nanopatterns) is not possible. In addition, the number of spectrins actually stabilized at three sites by DM-GRASP trans-interactions is most likely much lower since - in contrast to nanopatterns - the N- to C-terminal orientation of DM-GRASP molecules is random and only a subpopulation might be accessible for interactions.

Supplementary Figure 6. (a), (b): Complete specification of number of experiments (n), evaluated optical fields (OF), and significance values (* = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$) of Figure 4 a, b.

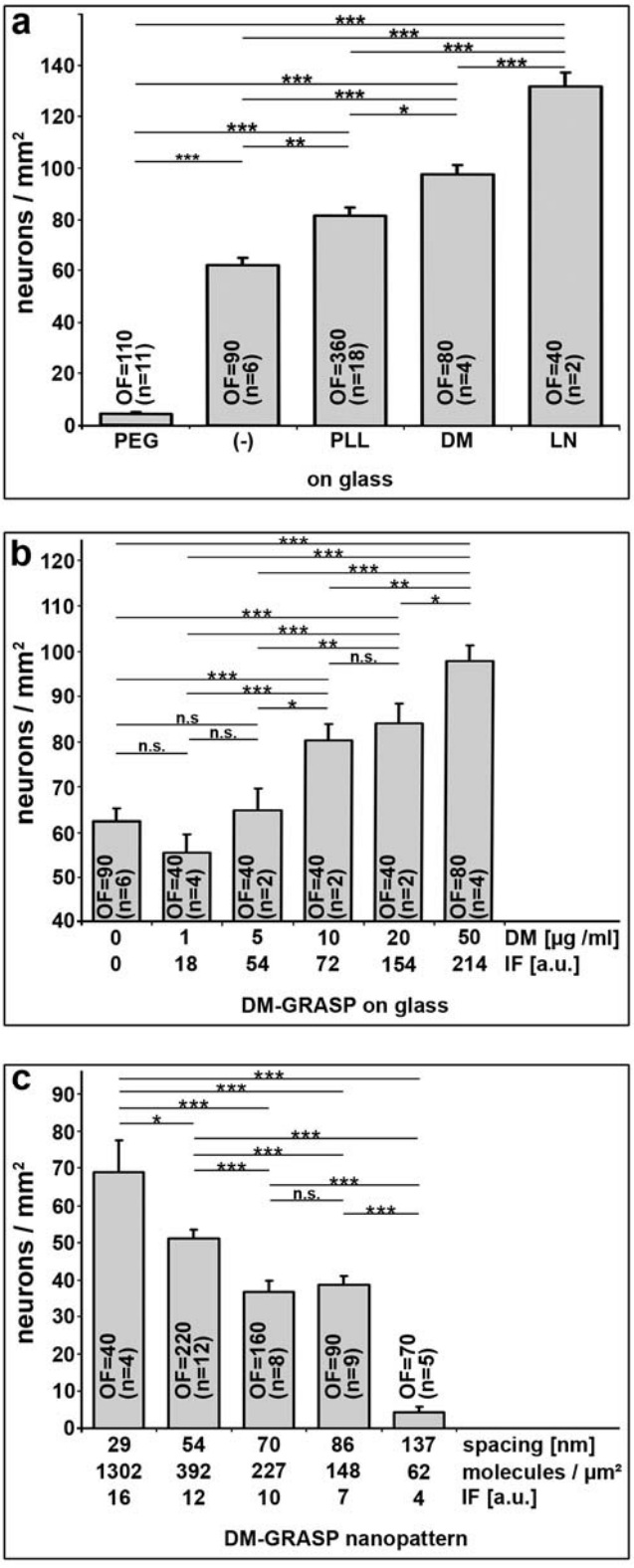
(c) - (e) Schematics of the side view (corresponding to top views in supplementary Figure 5.2 and 5.3) of DM-GRASP molecules in the plasma membrane (dark yellow) trans-interacting with DM-GRASP molecules on 70 nm (c, d) and 137 nm (e) nanopatterns. On 70 nm spaced patterns, spectrin can maximally interact with DM-GRASP molecules of the nanopattern at two sites (and only if smallest/largest lengths of 160 nm and 180 nm (boundary values) are assumed). On 137 nm-spaced nanopattern, spectrin is only anchored to the plasma membrane at one site (also if varying its length from 160 nm (depicted) to 180 nm). The merlin binding site on spectrin, which is identical with the ankyrin binding site (repeat 15) on spectrin, is located at a distance of 12 nm (two spectrin repeats (9 nm) and a pleckstrin homology domain (3 nm)) [5, 6] from the tetramerization site.

REFERENCES

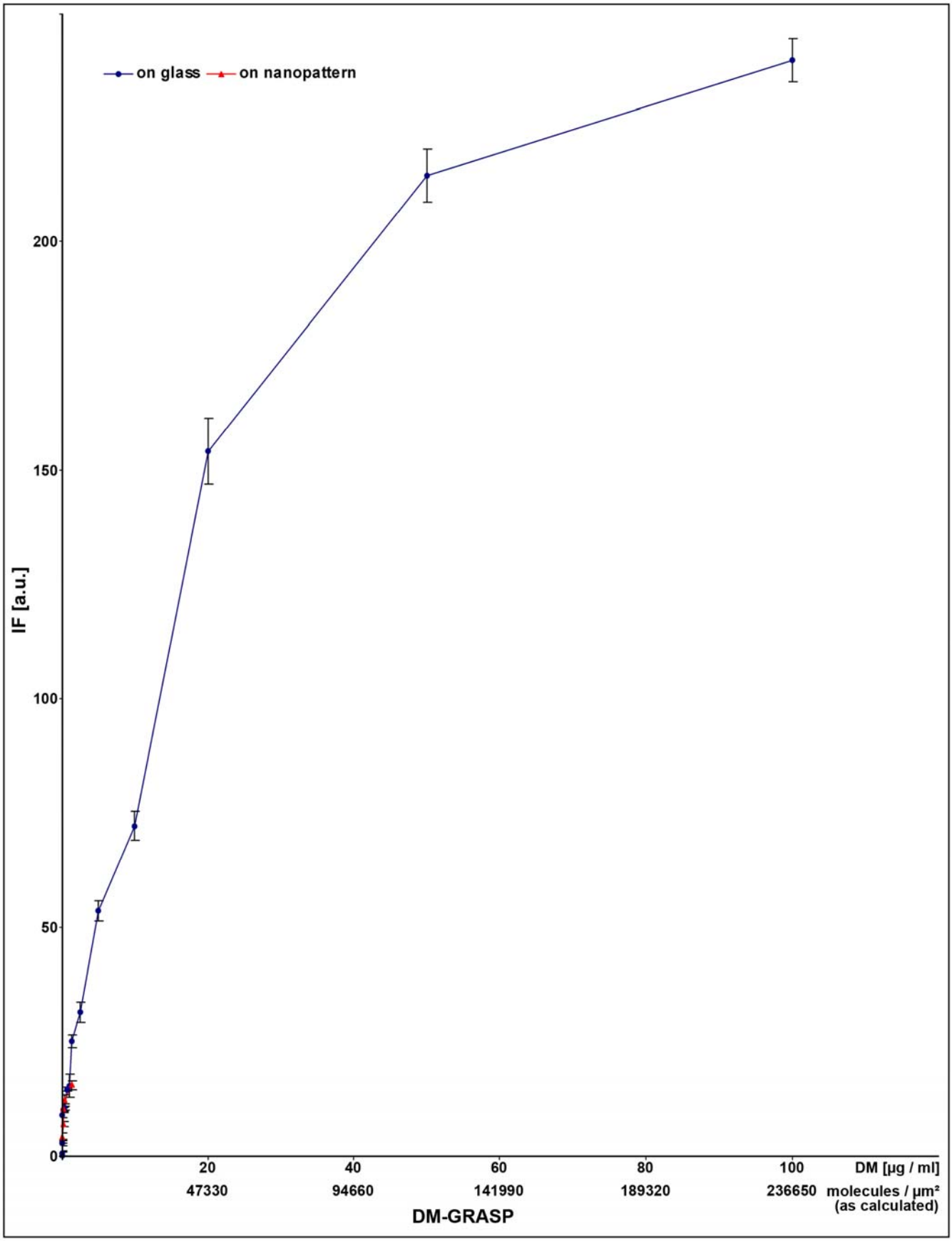
1. Chothia, C.; Jones, E. Y. *Annu Rev Biochem* **1997,** 66, 823-62.
2. McGough, A. M.; Josephs, R. *Proc Natl Acad Sci U S A* **1990,** 87, (13), 5208-12.
3. Shotton, D. M.; Burke, B. E.; Branton, D. *J Mol Biol* **1979,** 131, (2), 303-29.
4. Mirijanian, D. T.; Voth, G. A. *Proc Natl Acad Sci U S A* **2008,** 105, (4), 1204-8.
5. Grum, V. L.; Li, D.; MacDonald, R. I.; Mondragon, A. *Cell* **1999,** 98, (4), 523-35.
6. Davis, L.; Abdi, K.; Machius, M.; Brautigam, C.; Tomchick, D. R.; Bennett, V.; Michaely, P. *J Biol Chem* **2009,** 284, (11), 6982-7.



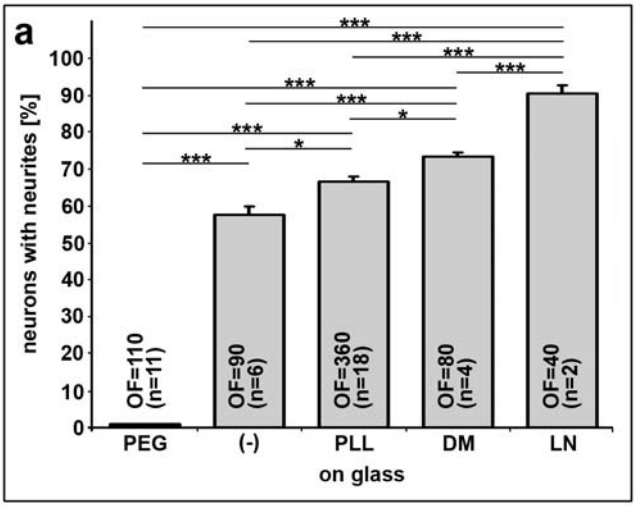
Supplementary Figure 1

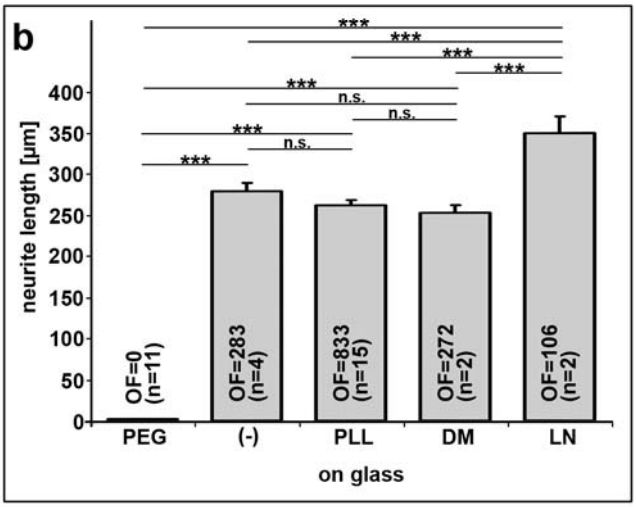


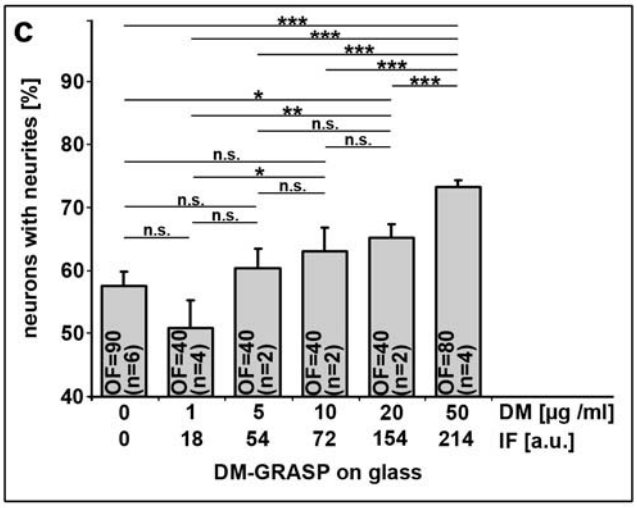
Supplementary Figure 2



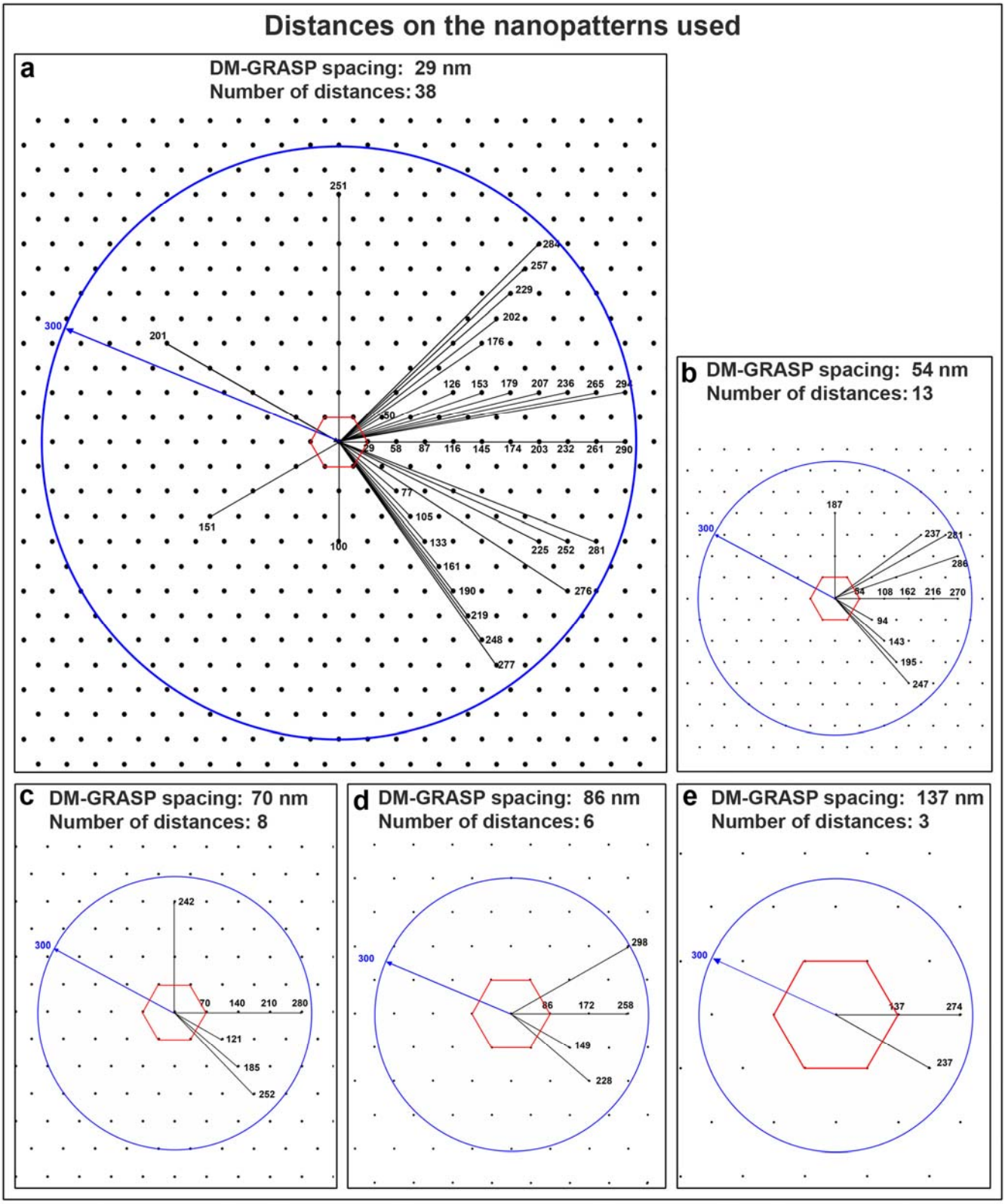
Supplementary Figure 3



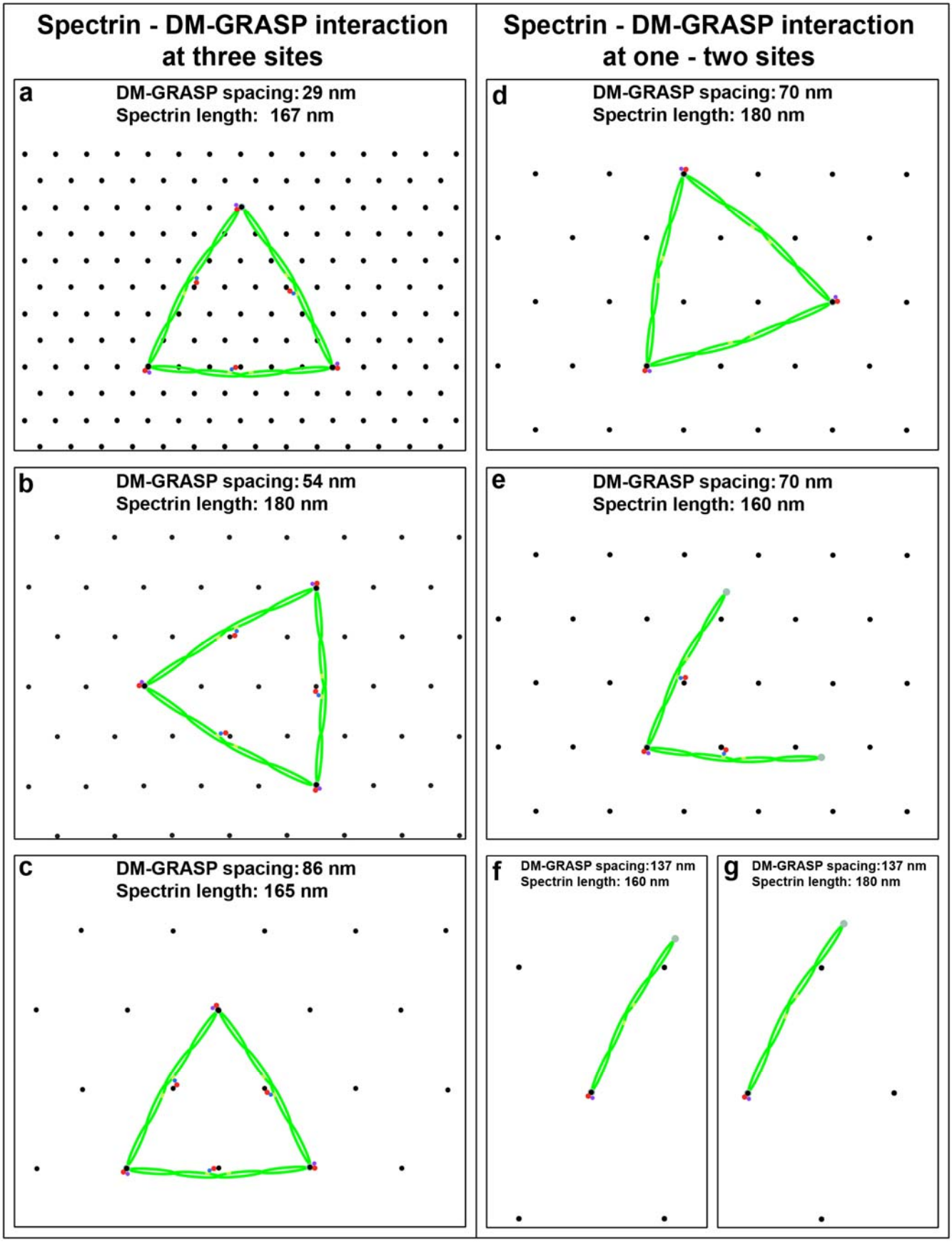




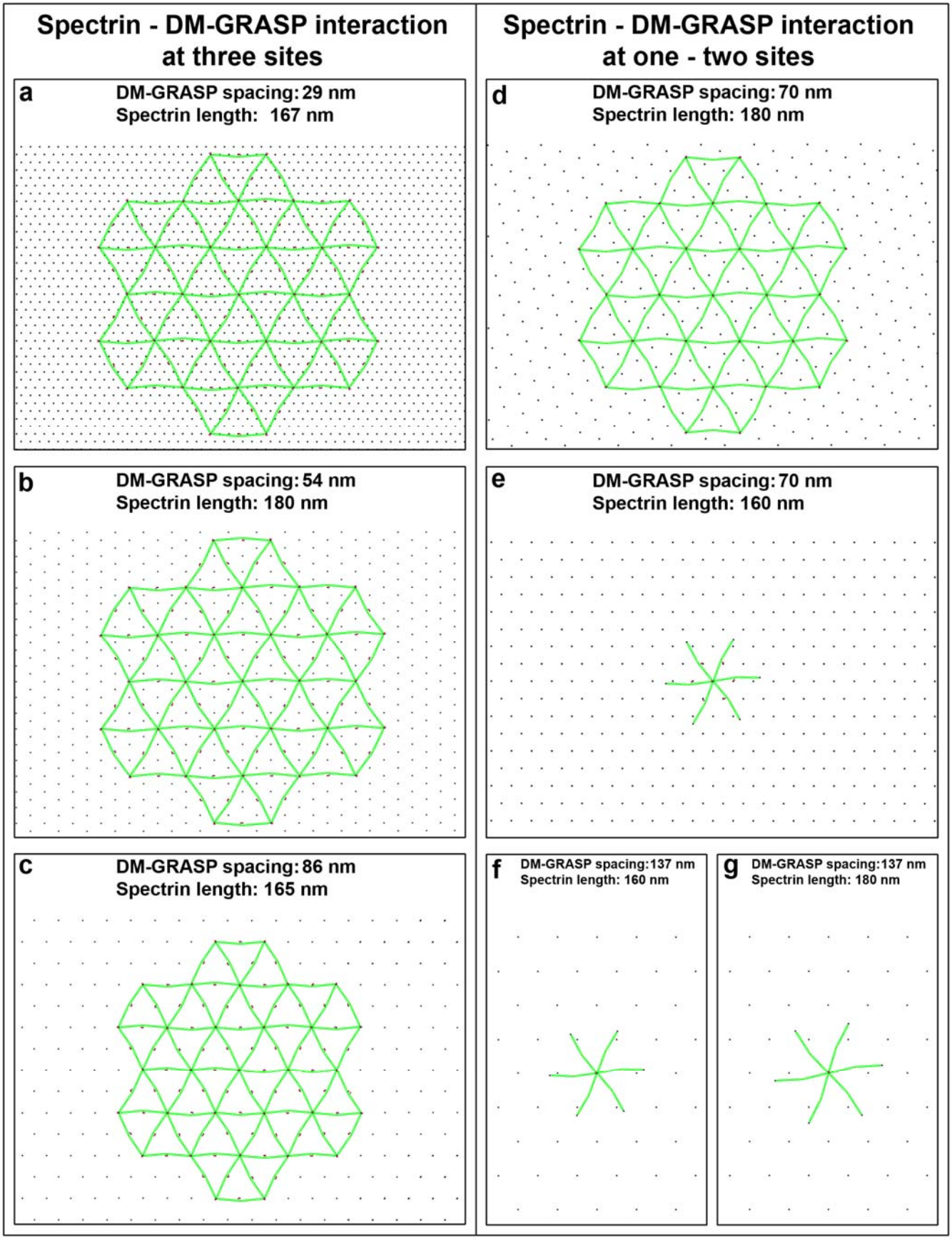
Supplementary Figure 4



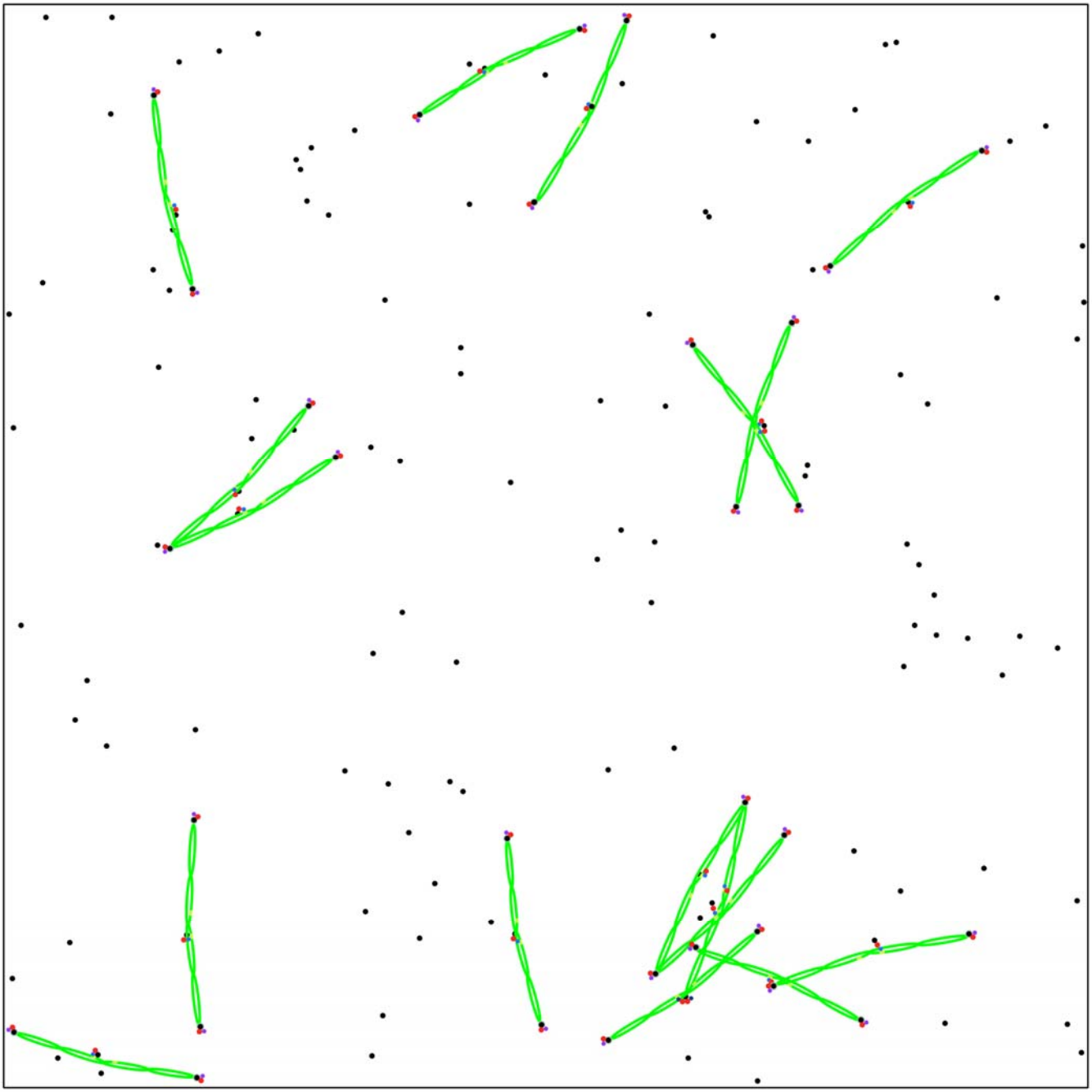
Supplementary Figure 5.1



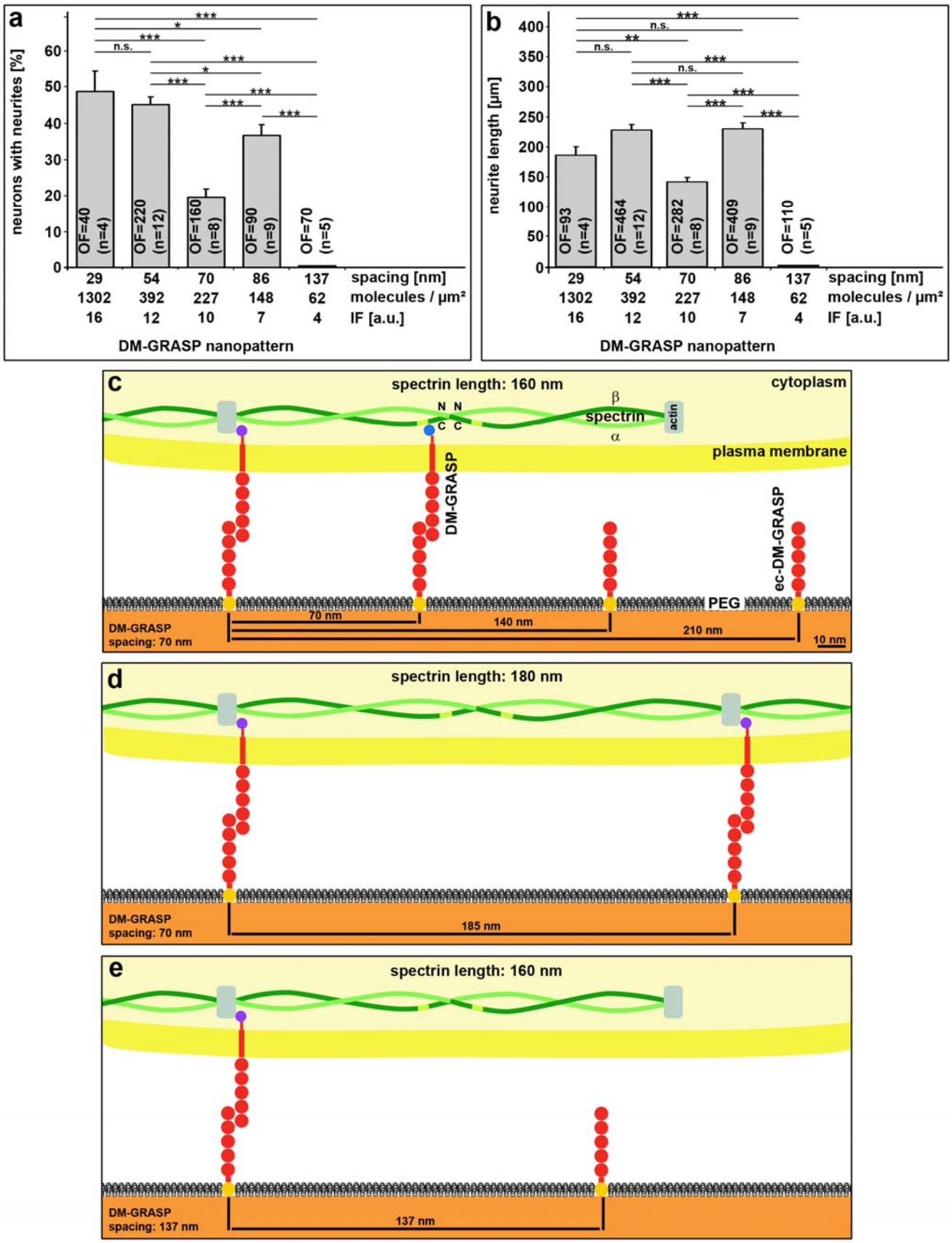
Supplementary Figure 5.2



Supplementary Figure 5.3



Supplementary Figure 5.4



Supplementary Figure 6