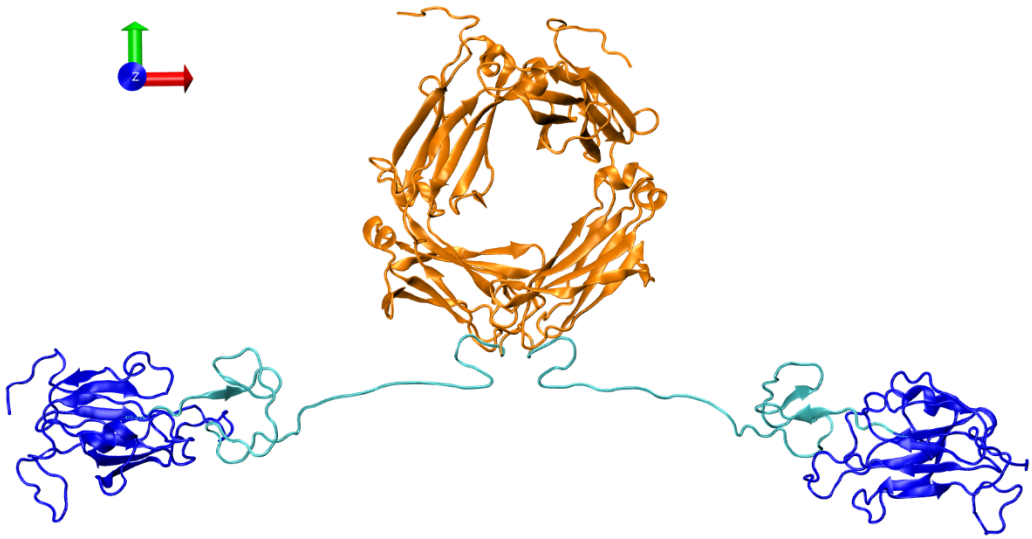
A. Rough estimation of length of Ephrin A5 construct

An atomic homology model of the recombinant human EphrinA5-Fc chimera (from info given at https://www.rndsystems.com/products/recombinant-human-ephrin-a5-fc-chimera-protein-cf_374-ea#product-details) was derived since a full-length crystallographic structure of recombinant EphrinA5-Fc chimera is lacking. The homology modelling with the Modeller 9.12 program (*A. Sali, T. L. Blundell, J. Mol. Biol., 1993, 234, 779*) was done on the basis of three templates. The N-terminal ephrin A5, parts of the linker between the domains, and the C-terminal Fc domain were generated by using the template structures of ephrin A5 (PDB: 2x11, chain B))(*E. Seiradake, K. Harlos, G. Sutton, A. R. Aricescu, E. Y. Jones, Nat. Struct. Mol. Biol., 17, 398*), the copper resistant protein K (PDB: 2km0, chain A) (*G. Sarret, A. Favier, J. L. Hazeman, M. Mergeay, B. Bersch, 2010, J. Am. Chem. Soc., 132, 3770*) and the immunoglobulin heavy chain (PDB: 1hzh, chain H) (*E. O. Saphire, P. W. Parren, R. Pantophlet, M. B. Zwick, G. M. Morris, P. M. Rudd, R. A. Dwek, R. L. Stanfield, D. R. Burton, I. A. Wilson, Science, 2001, 293, 1155*), respectively. The dimer was built by aligning the Fc domains of the monomers with the dimeric structure of 1hzh (*E. O. Saphire, P. W. Parren, R. Pantophlet, M. B. Zwick, G. M. Morris, P. M. Rudd, R. A. Dwek, R. L. Stanfield, D. R. Burton, I. A. Wilson, Science, 2001, 293, 1155*). The dimeric model was described with the CHARMM27 force field (*A. D. MacKerell, et al.. J. Phys. Chem. B, 1998, 102 (18), 3586*) and energy minimized (30.000 steps) with the conjugated gradient algorithm as implemented in NAMD 2.10 (*J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé, K. Schulten, J. Comput. Chem. 2005, 26, 1781*). The dimensions (X,Y,Z) of the dimer (ca. 200 x 110 x 85 Å3) were determined with VMD (*W. Humphrey, A. Dalke, K. Schulten, J. Mol. Graph. 1996, 14, 33*). These estimations are, however, biased by the badly resolved and very flexible spacer region.

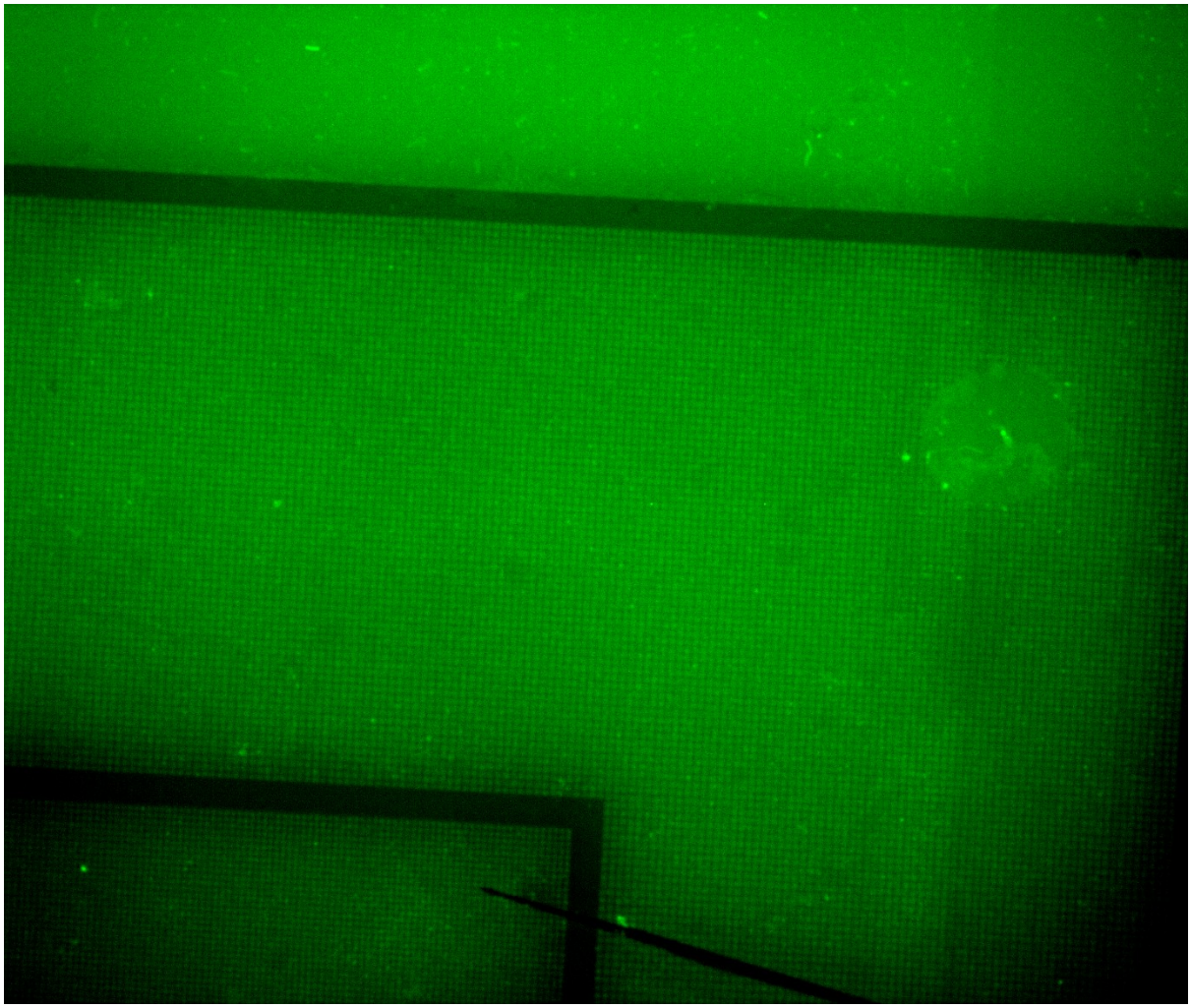


Supplementary FIG. 1 Schematic of the EphrinA5 construct derived from atomic homology modeling

B. Visualization of Lipid Bilayer within the nanostructures

Liposomes were prepared with 5% NBD-PE (1, 2-diphytanoyl-sn -glycerol-3-phosphoethanolamine-N- (7– nitro-2-1, 3-benzoxadiazol-4-yl) (ammonium salt)) mixed with POPC. The patterned substrate were thoroughly cleaned before liposome fusion. Lipid

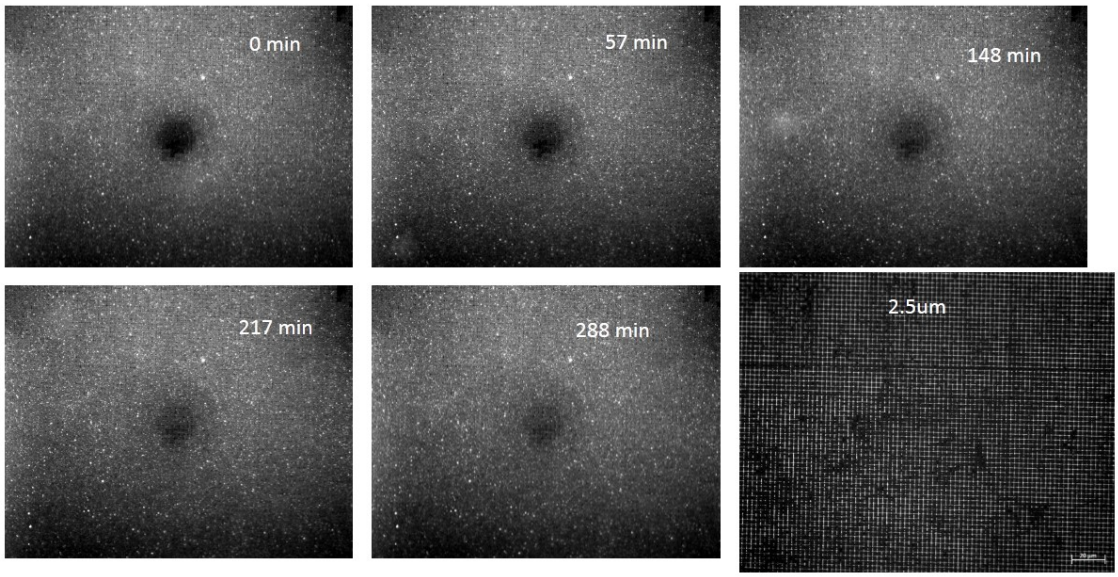
bilayer was prepared on such grid structures. The green fluorescence image of the bilayer is shown in Supplementary Fig. 2. The thick black lines represents the barrier of 10μm in width.



Supplementary FIG. 2 The fluorescence image of lipid bilayer prepared on the patterned substrate.

C. Movements of lipid molecules through Nanogaps

It was noted that during FRAP measurements the lipid molecules in some cases do move within the grid structures which could be accounted for the nano gaps within the grids through which lipids can move from one grid to another because there is always a possibility that during lift off or cleaning process chunks of gold are removed, which affect the homogeneity of the structure and create the nano gaps in the grid wall.

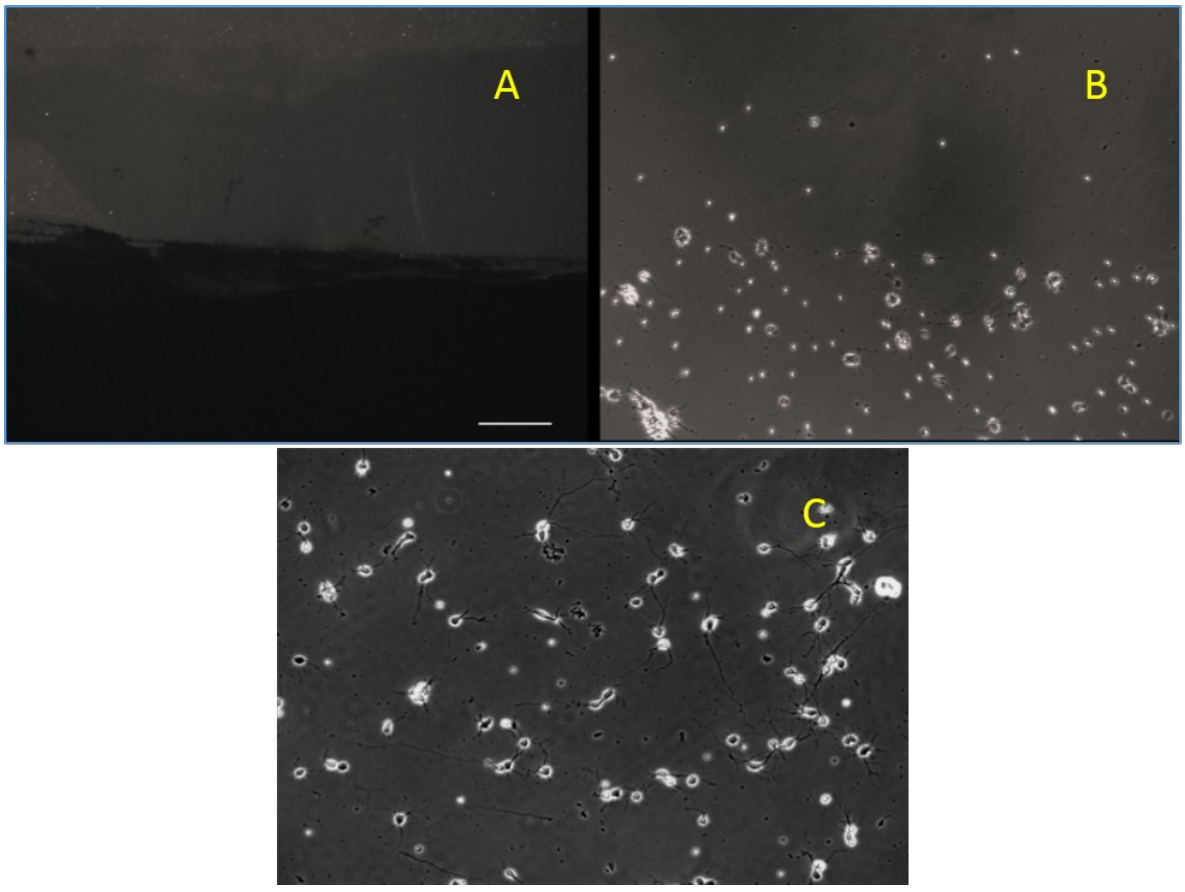


Supplementary FIG. 3 The FRAP images of lipid molecles moving within the grid structure of 2.5 μm.

D. NOG mediated incorporation

Earlier we found that when liposomes were prepared without the detergent (NOG) and bilayer was prepared with these liposomes, no Ephrin A5-Fc could be detected by antibody binding experiment in QCM-D[10]. Indicating protein Ephrin A5 could not be inserted

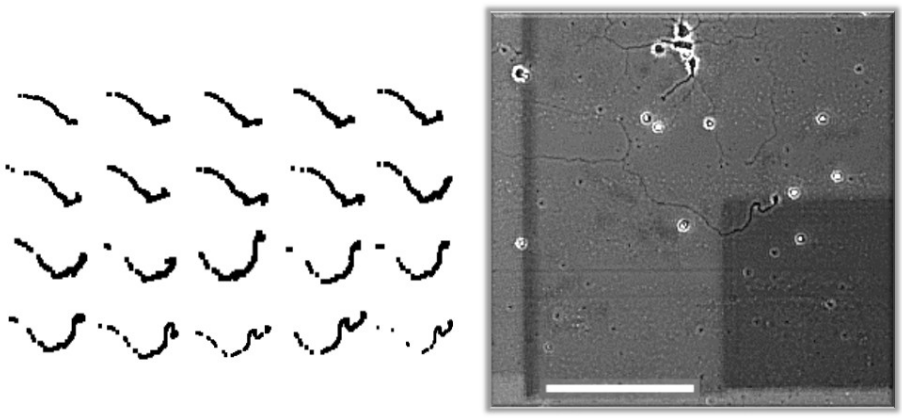
without NOG. In this paper we show that when planar bilayer was prepared without NOG and neurons were cultured on them. It was found that the surface behaved similarly to pure POPC[8] and cells did not adhere on them. The Supplementary FIG. 4(A) shows fluorescence image of a Texas Red DHPE doped lipid bilayer prepared without NOG and Supplementary FIG. 4(B) shows DIV 3 image of the same section of the bilayer after neuronal culture which remains vacant. Whereas Supplementary FIG. 4(C) shows cells on bilayer when prepared with NOG indicating Ephrin A5 insertion requires NOG.



Supplementary Fig. 4 shows images of Texas Red DHPE bilayer when prepared without NOG (A), cultured neurons on them (B) and cultured neurons on bilayer when prepared with NOG. The scalebar represents 100μm.

E. Growth of neuritis on nanostructures

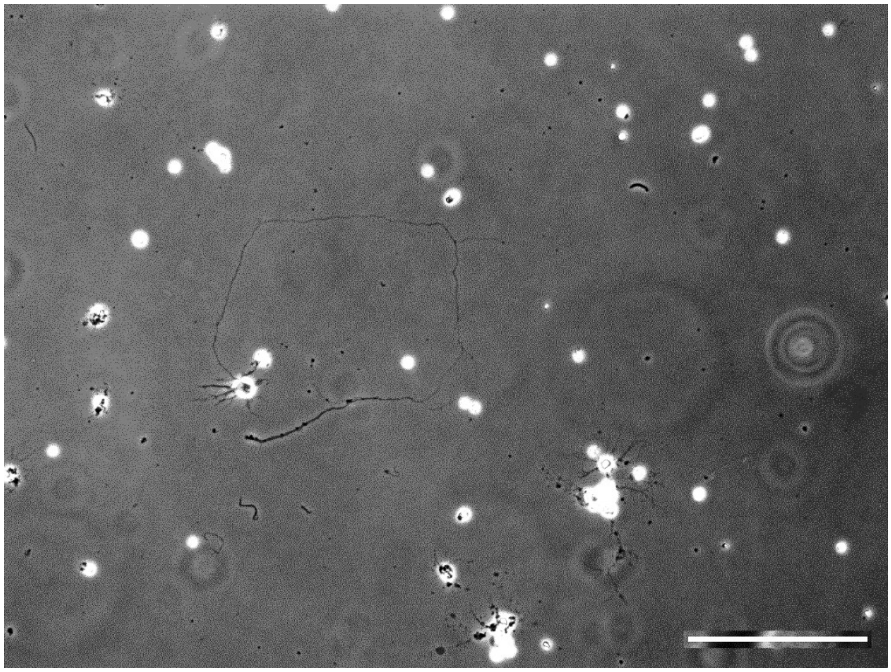
Supplementary Fig. 5 gives details of neurite progression through 500nm grids. The snapshots were recorded in between DIV 3 to DIV 5 of cultured neurons. It is found that the neurites easily cross the gold barriers in search of its neighbours. The small fragments of membrane does not hinder its movements but favours in taking turns and rounds. The progress of axon after image analysis is shown in the left panel of Supplementary Fig. 5. The images were recorded for 10 hours at an interval of half an hour. On the right handside of the figure the original image with the axon is shown.



Supplementary Fig. 5 shows advancement of an axon through 500nm grid. The scalebar is of 100μm.

F. Long Axon

Neurons undergo several stages of development and forms networks. Formation of long neurites in search of neighbours for network formation was observed on nonpatterned synthetic EA5-POPC membrane when very low number of neurons (only 20k) were plated on them. The propagation of axon can be visualized in the supplementary Fig. 6

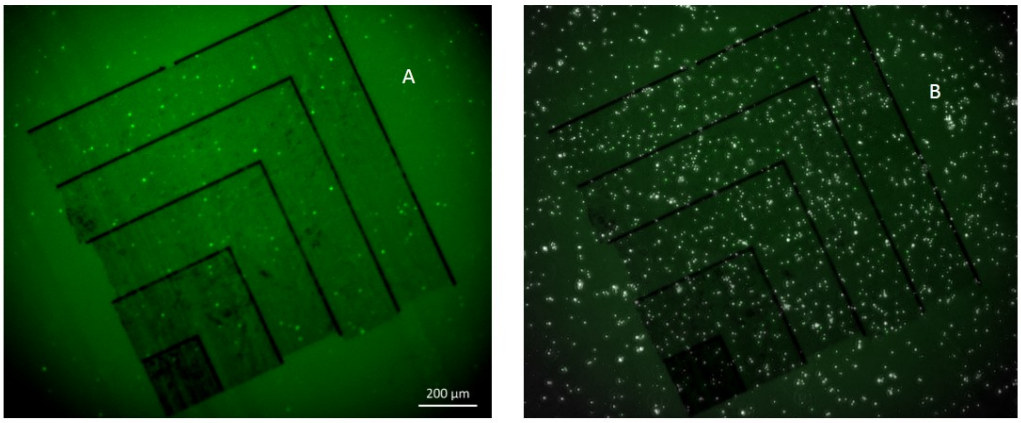


Supplementary Fig. 6: Formation of a long axon on EA5-POPC membrane. The scalebar corresponds to 200μm.

G. Lipid clustering

An unusual phenomenon of accumulation of lipids by the cellbody was observed during the experiment. It was noted that the neurons do not only accumulate the protein ephrin A5s but also clusters the lipid. The accumulated lipids can be seen as bright spots in the Supplementary Fig. 7 (A). It represents the fluorescence image of the NBD-PE doped

Ephrin A5 containing lipid bilayer. The merged image of the artificial bilayer with the cells is shown in Supplementary Fig. 7 (B). The image was taken a day after cell seeding (DIV1).



Supplementary Fig. 7: The accumulated lipids are seen as small bright spots on the artificial bilayer (A). And the neurons on the same are shown in B.