Supplementary Information

Simulation of bound protein gradients created by diffusive printing

We simulated the diffusive printing process in a 2-dimensional model as shown in Fig. M1. At time 0, a solution of a finite amount of protein is added to the source rectangle of 350 x 100 µm in size, and the protein molecules were allowed to diffuse into the gel, which was simulated by a matrix of unit cells of 5 x 5 µm each, with concurrent adsorption of the protein molecules to the adsorbing surface, which was simulated by a row of unit cells of the same size at the bottom of the gel matrix. For each time step of 0.01 sec, the concentration of the protein inside each cell was calculated according to the governing equations. The diffusion process is governed by the standard diffusion equation (Dantzig and Tucker, 2001):
\[ \frac{\partial \phi}{\partial t} = D \nabla^2 \phi(\rho, t); \]

Where \( \phi \) is the concentration of the diffusing material at location \( \rho \) and time \( t \), and \( D \) is the constant diffusion coefficient of the diffusing protein. The adsorption of the protein to the substratum is modeled as a protein adsorption/desorption process using the following equation:

\[ \frac{d\Gamma(t)}{dt} = k_{on}[\Gamma_{\text{max}} - \Gamma(t)] \cdot c(0, t) - k_{off} \Gamma(t); \]

Where \( k_{on} \) and \( k_{off} \) are the protein adsorption and desorption rate constant, respectively, \( \Gamma(t) \) is the amount of protein adsorbed per unit surface area at time \( t \), \( \Gamma_{\text{max}} \) is the maximum density of adsorbed protein, \( [\Gamma_{\text{max}} - \Gamma(t)] \) is the density of the remaining unoccupied protein binding sites at time \( t \), and \( c(0, t) \) is the protein concentration right next to the adsorbing surface. The density of covalently bound protein at time \( t \) is assumed to be the same as \( \Gamma(t) \). This assumption is based on the fact that after the printing process, the patterned coverslips were kept undisturbed for another 30 min before rinsing to ensure the completion of all the covalent reactions between the adsorbed proteins and the epoxy surface. Simulations with different source protein concentrations and printing times were performed with the parameters that fell within the range reported by previous studies (Tie et al., 2003; Gutenwik et al., 2004; He and Niemeyer, 2003). To produce the profiles shown in Fig. 1b and Fig. 6c & 6d, we used the following parameters:

\[ D_{iG} = 3.0 \times 10^{-11} \text{ m}^2/\text{s}, \quad D_{BDNF} = 5.1 \times 10^{-11} \text{ m}^2/\text{s}, \]

\[ k_{on} = 8 \times 10^{-8} \text{ m}^3/\text{molecules} \cdot \text{s}, \quad k_{off} = 1 \times 10^{-2} /\text{s}, \quad \text{and} \quad \Gamma_{\text{max}} = 2 \times 10^{14} \text{ molecules/m}^2. \]
References:


Supplementary Figure S1
Supplementary Figure S2
axon initiation and turning on netrin-1 gradient

Supplementary Figure S3