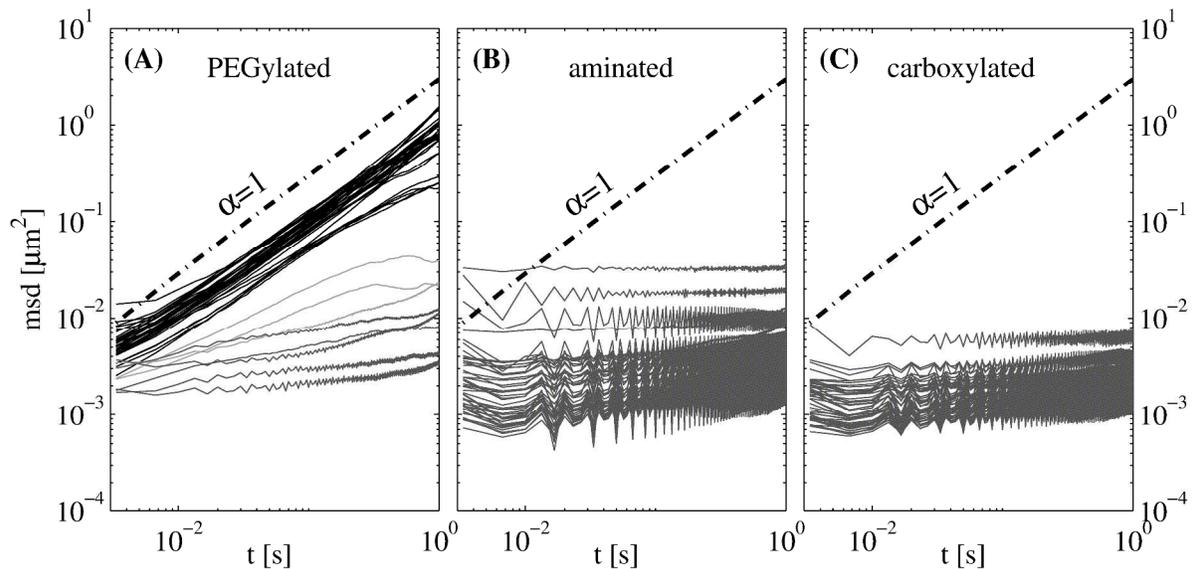


## Supplementary information for Arends et al.

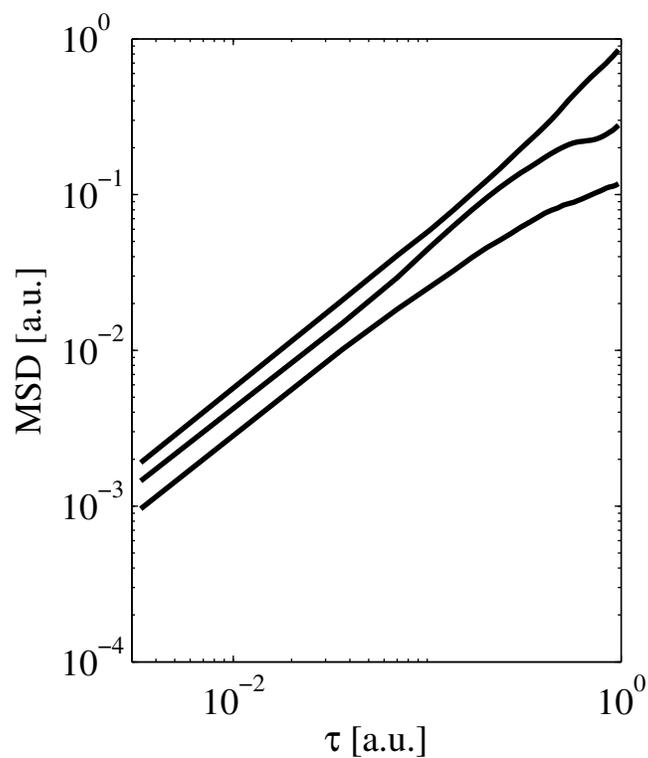
Protein	pI
Laminin subunit $\alpha$	6.26
Laminin subunit $\beta$	4.82
Laminin subunit $\gamma$	5.08
Heparan sulfate proteoglycan core protein	5.88
Collagen IV	8.85
Nidogen	5.22

**Table S1.** The main constituents of Matrigel and their corresponding pI values calculated from expasy.

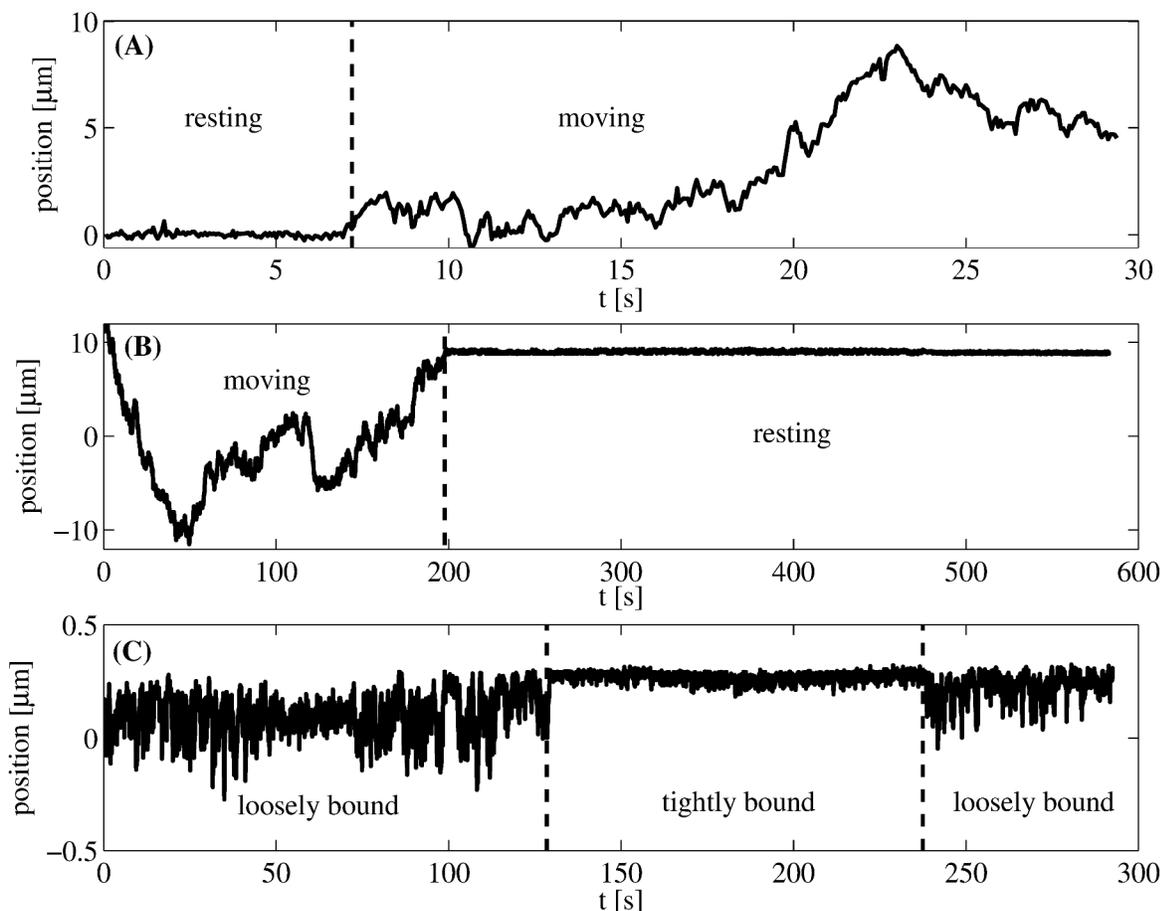


**Figure S1.** Diffusion behavior of 1  $\mu\text{m}$  polystyrene particles in Matrigel diluted with HEPES buffer (pH 7.5) at an ionic strength of 100 mM KCl. From the particle trajectories, mean square

displacement curves are calculated for (A) PEGylated, (B) amine-terminated and (C) carboxyl-terminated polystyrene particles.

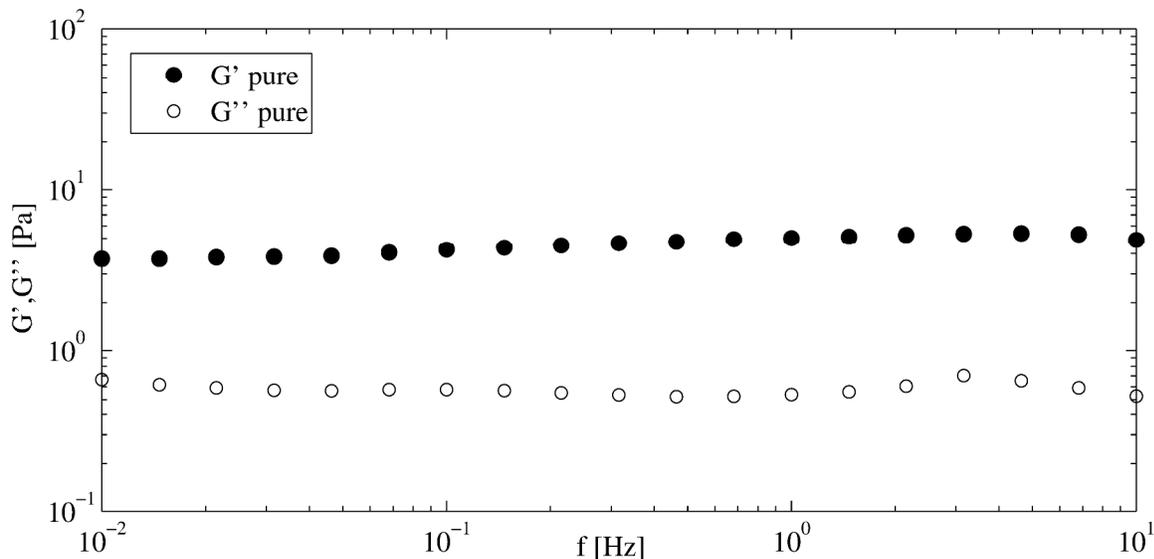


**Figure S2.** Three exemplary MSD curves with an exponent  $\alpha$  ranging from 0.7 to 1.2 obtained from simulations of free diffusion (curves are shifted for clarity). The exponent  $\alpha$  is defined by  $\langle x^2 \rangle \propto \tau^\alpha$  as described in the manuscript. The amount of data points matches the number of data points collected in our single particle tracking experiments.



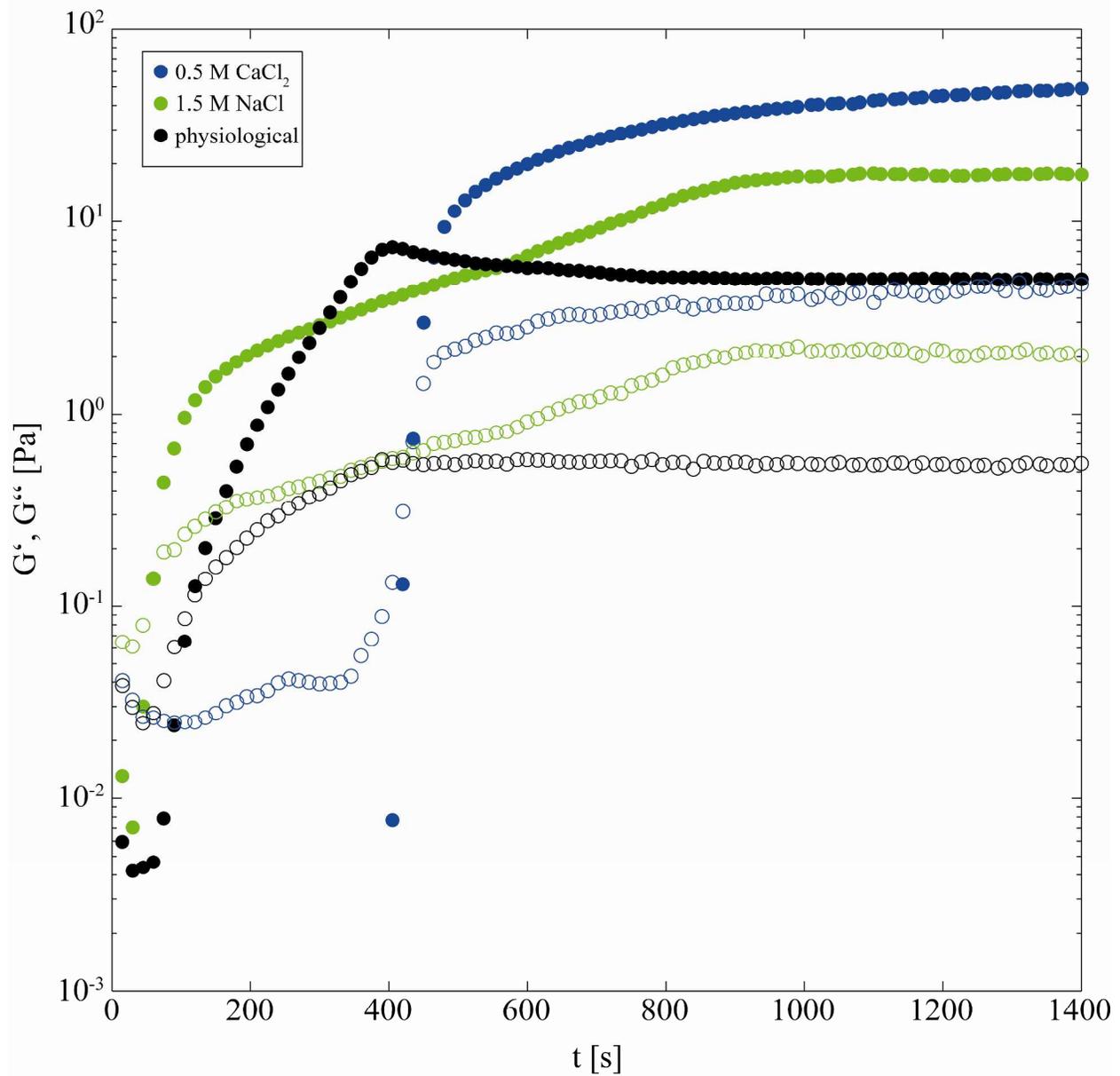
**Figure S3.** Further examples for trapping/release events observed for 300 nm-sized particles in Matrigel. **(A)** shows the x-coordinate of a particle trajectory obtained for a 300 nm carboxyl-terminated polystyrene particle in Matrigel at a salt concentration of 0.5 M KCl. At first, the particle is bound to the hydrogel but starts diffusing after  $\sim 7$  s. **(B)** The particle diffuses for the first 3 min of the observation but then it becomes trapped as it binds to the hydrogel. **(C)** Two types of binding can be observed: at the beginning and at the end of the observation, the particle is loosely bound as reflected the high fluctuation amplitude of the particle displacement. In between those loosely bound states, the particle is tightly bound as indicated by the smaller

fluctuation amplitude. **(B)** and **(C)** were both obtained for 300 nm carboxyl-terminated polystyrene particles in Matrigel supplemented with 1.5 M KCl.



**Figure S4.** Viscoelastic frequency spectrum obtained for Matrigel which has been diluted 1:1 with MEM- $\alpha$  cell media. The storage modulus  $G'$  (filled circles) as well as the loss modulus  $G''$  (open circles) are nearly constant over three decades of frequency. This allows us to define a plateau modulus  $G_0 = G' \sim 5$  Pa. It is tempting to calculate a mesh size from this plateau value, however, the plateau modulus of a biopolymer network depends on a range of parameters: First, the bending stiffness of the biopolymer is a crucial parameter: scaling relations for the plateau modulus are markedly different for networks of flexible, semi-flexible or stiff polymers. Second, it is important to distinguish between an entangled solution and a cross-linked polymer network, respectively. For a cross-linked polymer network, as it is the case for Matrigel, the concentration

of cross-linking points mainly sets the value of the plateau modulus. Tharmann et al (PRL 98, 088103 (2007)) have shown that the plateau modulus of an actin network can be increased by two orders of magnitude while the mesh size is kept constant. The weak frequency dependence of the loss modulus  $G''$  is an indication for suppressed macromolecule reptation which is probably due to cross-linking interactions of laminin with other laminin macromolecules. The measurement was performed on a stress-controlled macrorheometer (MCR 102, Anton Paar, Graz, Austria) with a 25 mm plate-plate geometry at a plate separation of 175  $\mu\text{m}$  after 30 min gelation time at 37°C.



**Figure S5.** Gelation curve for Matrigel which has been diluted 1:1 with MEM- $\alpha$  cell media without further addition of ions (black) and with either additional 0.5 M  $\text{CaCl}_2$  (blue) or 1.5 M  $\text{NaCl}$  (green). The storage modulus  $G'$  (filled circles) as well as the loss modulus  $G''$  (open circles) approach a plateau for all conditions. The final storage modulus of Matrigel with additional  $\text{CaCl}_2$  is the highest of the three conditions tested. This finding supports the idea of deswelling of the gel at higher divalent ion concentrations. The measurement was performed on

a stress-controlled macrorheometer (MCR 102, Anton Paar, Graz, Austria) with a 25 mm plate-plate geometry at a plate separation of 175  $\mu\text{m}$  and at 37°C.