Non-Viral Gene Delivery Vectors Use Syndecan-dependent Transport Mechanisms in Filopodia to Reach the Cell Surface

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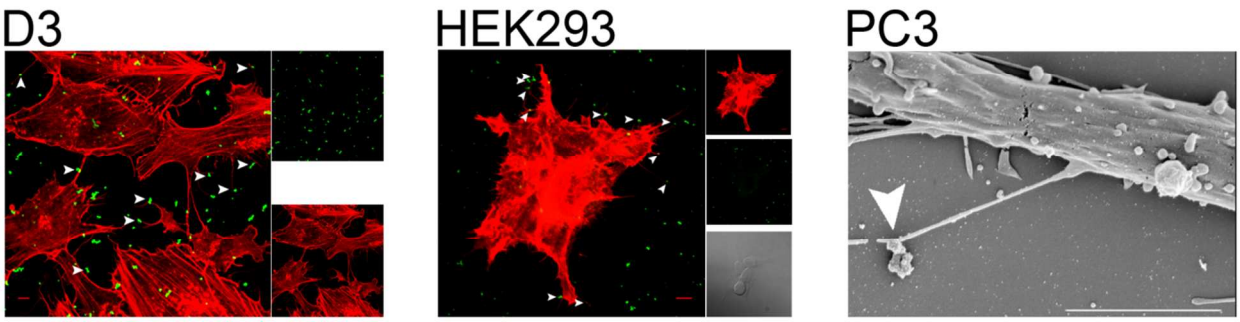

Supporting Figure S1: Binding of polyplexes to filopodia is not cell type dependent. Human vascular endothelial (D3) cells, HEK293 cells and PC3 cells were incubated with polyplexes for 90 minutes and subsequently processed for confocal or scanning electron microscopy. Actin was stained with alexa 488 (pseudocolored red). Scale bar, 3um.

Supporting Movie 1: PEI polyplexes surf along filopodia, facilitated by clustering of syndecan-1. HeLa cells expressing syndecan-1 GFP (SDC1-GFP) (green) were incubated with polyplexes (red) and monitored by time-lapse microscopy. An attached polyplex, induces a clustering of syndecans, and surfs along a filopodium, thus reaching the cell body for internalization. Images were taken at about every 30 seconds. The video is played at a speed of 3 frames/sec. For experimental details see the legend to Figure 3a.

Supporting Movie 2: Polyplexes surf along nanotubes between cells. Time-lapse microscopy of selected regions from HeLa cells connected by nanotubes, showing that polyplexes (red) surf along nanotubes, assisted by clusters of SDC1-GFP (green). Images were taken at about every 43 seconds and the video is played at 5 frames/sec. For details see the legend to Figure 3e.

Supporting Movie 3: Retraction of filopodia also mediates the transfer of polyplexes to the cell body. HeLa cells expressing SDC1-GFP (green) were incubated with polyplexes (red) and

monitored by live cell imaging. Binding of a polyplex to the filopodium triggers clustering of sydecans at the binding site of the polyplex. The filopodium subsequently retracts towards the cell body, assisted by a 'helper' filopodium. Images were acquired at about every 34 secs, and the video is played at a rate of 3 frames/sec.

Supporting Movie 4: Several filopodia cooperate in bringing a polyplex to the cell body. Cy3 labeled polyplexes (red) were added to HeLa cells expressing SDC1-GFP (green), and visualized by time-lapse microscopy. Initially contacted by one filopodium, the polyplex is subsequently captured by several filopodia to bring it to the cell body. Images were taken at about every 30 seconds and the video is played at a speed of 5 frames/sec. For details, see the text.

Supporting Movie 5a: Filopodia sense and capture polyplexes in the extracellular environment. SDC1-GFP expressing HeLa cells (green) were incubated with polyplexes (red) and monitered by live cell imaging. A filopodium extends towards a polyplex, located at a distinct distance from the cellular protrusion, indicating that the filopodium senses the polyplex prior to actual attachment. After the filopodium attaches to the polyplex, a rapid clustering of syndecans takes place, resulting in the surfing of the polyplex along the filopodium in a similar fashion as shown in Movie 1. Images were taken at about every 45 seconds and played with a speed of 3 frames/ sec.

Supporting Movie 5b: Filopodia sense and capture polyplexes in the extracellular environment: SDC1-GFP expressing HeLa cells (green) were incubated with polyplexes (red)

and monitered by live cell imaging. A filopodium changes direction and bends towards a nearing polyplex, prior to capturing the polyplex. Subsequently, instead of surfing along the same filopodium, the polyplex reaches the cell surface by moving along another one.

Supporting Movie 6: Blebbistatin, a myosin II inhibitor, impedes transfer of polyplexes along filopodia towards the cell body. Cells expressing SDC1-GFP were pretreated with blebbistatin (50uM) for 30 min, subsequently incubated with polyplexes, and monitered by time-lapse microscopy. Note that initially some clustering of syndecans still occurs upon treatment with the inhibitor, but the effect appears transient. However, surface transfer of the polyplex, either by surfing or retraction, is completely impeded. Images were taken at about every 48 secs and the video is played at a speed of 5 frames/sec.

Supporting Movie 7a: Jasplakinolide, an actin stabilizer, prevents clustering of syndecans and transport of polyplexes towards the cell surface. SDC1-GFP expressing cells were treated with jasplakinolide (100nM) at conditions otherwise similar to those in Movie 6. As can be seen no clustering of syndecans takes place and, hence, no movement of polyplexes takes place towards the cell body. Images were taken at about every 47 secs and the video is played at a speed of 5 frames/sec.

Supporting Movie 7b: Dissociation of polyplexes from filopodia in cells treated with jasplakinolide. Time-lapse microscopy of HeLa cells expressing SDC1-GFP (green) pretreated with jasplakinolide (100nM), followed by addition of polyplexes (red). Note that the polyplex that is initially attached to the filopodium subsequently dissociates, presumably due to a

weakened binding as a result of frustrated clustering of syndecan, which is presumably necessary for stabilization of polyplex-cell interaction (see text for details). Images were taken at about every 47 secs and the video is played at a speed of 5 frames/sec.

Supporting Movie 7c: 'Reverse surfing' of polyplexes along filopodia in cells treated with jasplakinolide, shows that attraction with the cell surface is not just simply a matter of electrostatic interaction. Cells expressing SDC1-GFP were treated in a similar fashion as mentioned in 7b. A polyplex (red) that is initially near to the cell body starts moving away from the cell body along a filopodium. Images are take every ~50 seconds and played with a speed of 5 frames/second.