Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary material

EGF-targeting Lipodisks for Specific Delivery of Poorly Water-Soluble Anticancer Agents to Tumour cells

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Quantification of lipodisk components

Table S1 Recovered proportions of lipodisk components

production method	phospholipid	cholesterol	curcumin
starting material	1	1	1
sonication	1	1	0.74[a]
SEC	0.75[a, b, c]	0.75[b]	0.5[a, b]
BioBeads	0.97[c]	0.87[b]	0.07[a]

[a] Calculated using absorbance measurements.
[b] Calculated using a ^{3}H-labelled fraction of the DPPC or cholesterol component.
[c] Calculated from measurements of phosphorous content

Table S2 Final molar ratio of lipodisk components. Based on the data presented in Table S1

production method	phospholipid	cholesterol	curcumin
starting material	1	0.67	0.33
sonication	1	0.67	0.25
SEC	1	0.67	0.22
BioBeads	1	0.60	0.02

Size distribution of lipodisks as assessed by Dynamic light scattering (DLS)

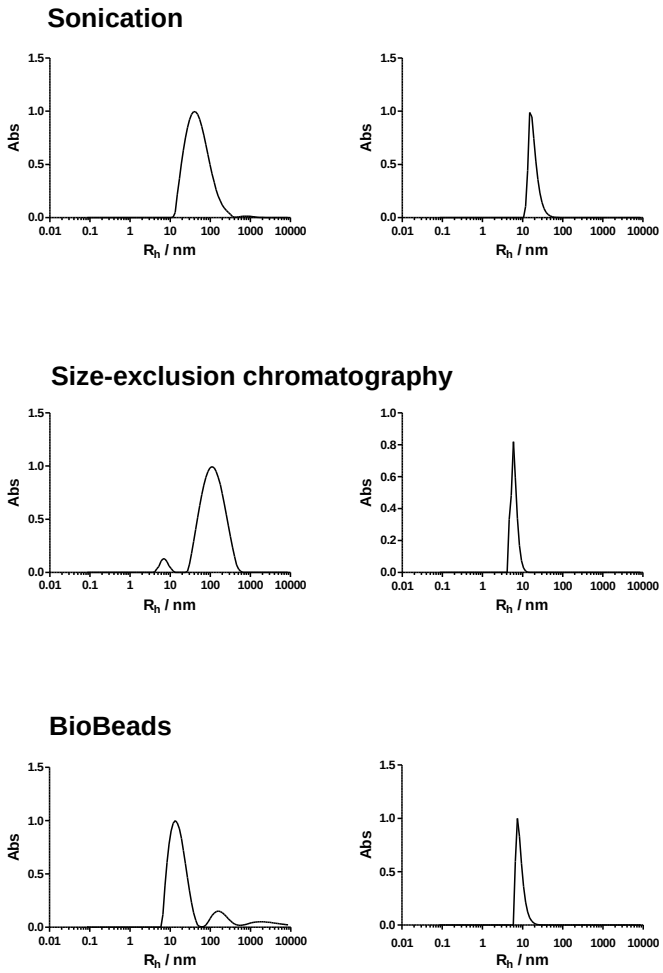

Fig. S1 Apparent hydrodynamic radii (R_h) distribution obtained for targeting lipodisks (DPPC/cholesterol/curcumin/EGF-NHS-PEG_{3400}-DSPE) produced by sonication, and detergent depletion using size-exclusion chromatography or BioBeads. Calculations were made using both unweighted data (left) and number weighted data (right), n = 3.

Influence of freeze-thawing on EGF binding affinity

^{125}I labelled EGF in PBS was freeze-thawed five times in liquid nitrogen alternated with hydration at 60 °C. Aliquots were collected after each freeze-thawing. The binding affinity of each aliquot was compared to that of non-freeze-thawed ^{125}I-EGF using LigandTracer Gray (Ridgeview Instruments, Uppsala, Sweden). For each sample 3 nM of ^{125}I-EGF was added to a petri dish containing A-431 cells and 3 mL of complete cell media. After 3.5 h of incubation the ^{125}I-EGF concentration of the solution was increased to 9 nM, followed by another 3.5 h of incubation. Finally, the incubation solution was removed and 7 mL of complete media was added to study the retention of the ^{125}I-EGF overnight.

Fig. S2 shows that freeze-thawing has a negative influence on the binding affinity of EGF to its receptor. After only one freeze-thawing cycle the signal due to cell-associated ^{125}I-EGF decreased to approximately half of that obtained with non-freeze-thawed EGF. A similar reduction was seen for the samples freeze-thawed two to five times.

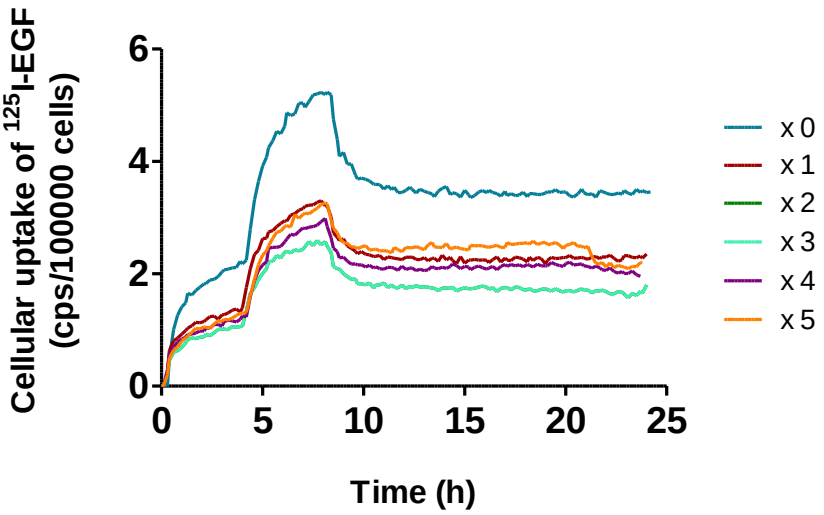

Fig. S2 The influence of freeze-thawing (0-5 times) on binding affinity of EGF to EGFR on A-431 cells.

Effect of free EGF on cellular retention of lipodisks

Real-time analysis of uptake and retention of targeting lipodisks (DPPC/cholesterol/^{125}I-EGF-NHS-PEG$_{3400}$-DSPE) on A-431 cells was performed as described in the experimental section, with the exception that the kinetics were studied during a three-step, three-fold increase of the concentration: 1, 3 and 9 µM with respect to total lipid concentration. At 11 h the incubation medium was removed and 7 mL fresh cell medium was added to study the lipodisk retention. After a total of 24 hours, 100 nM of free EGF was added to study if the bound EGF-targeting lipodisks could be displaced from the cells.

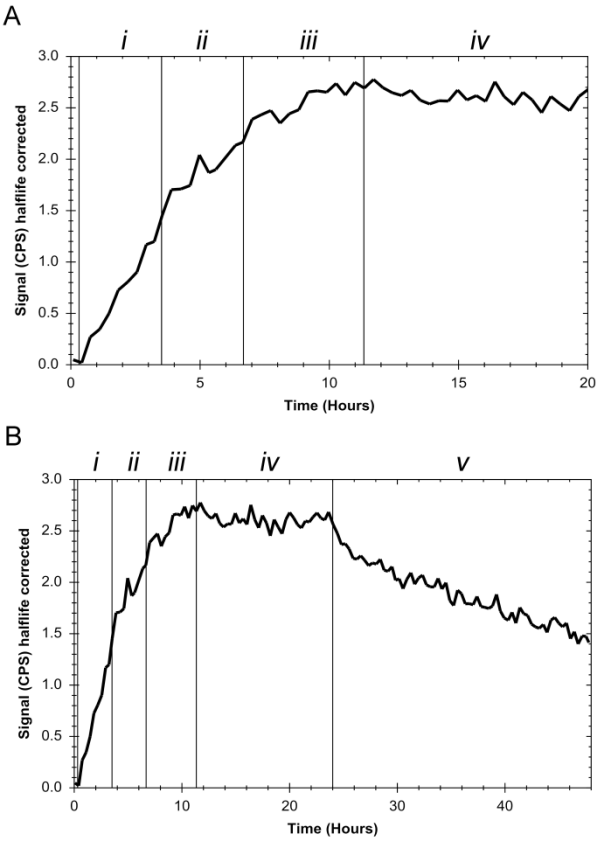

Fig. S3 Real-time analysis of the interaction between targeting lipodisks and A-431 cells. Increasing concentrations of lipodisks (1 µM, 3 µM and 9 µM with respect to total lipid concentration) were added at time points 0.5, 3.5 and 6.5 h (i, ii and iii) followed by removal of the lipodisk solution and observation of the retention of cell bound targeting lipodisks in cell medium (iv). Thereafter, 100 nM of free EGF was added to the medium and the signal due to cell-associated radioactivity was followed over an additional 24 h (v). To avoid internalization of the receptor the experiments were performed at 4 °C.

The decreasing signal observed in section v in Fig. S3B shows that the EGF-targeting lipodisks are displaced from the cells upon addition of free EGF, and thus proves that the binding of the lipodisks is receptor-specific.