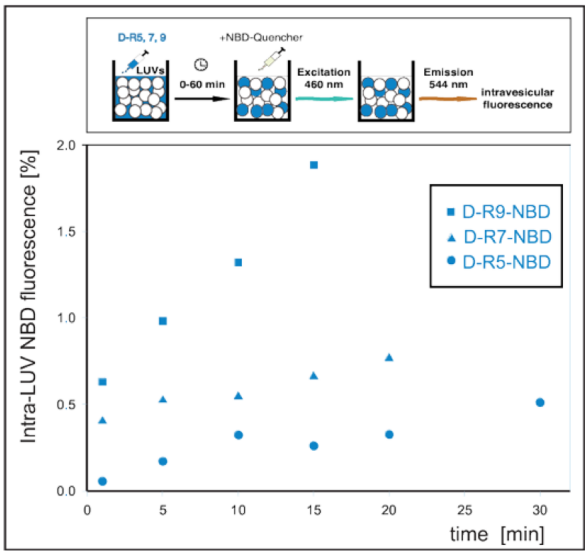
Supplementary figure 1



Supplemental figure legend:

Supplementary Figure 1 Penetration of artificial membranes by oligo-arginines. LUVs (Large Unilamellar Vesicles) were prepared from a mixture from 70 mol% of DOPC (dioleoylphosphatidylcholine) and 30 mol% of DOPS (dioleoylphosphatidylserine). In total 1 µmol of lipids were mixed in chloroform. A dry lipid film was formed by solvent evaporation under a nitrogen stream. The dried lipids were resolubilized in 2 ml of PBS (pH 7.4) by 5 min of vortexing. To yield LUVs the lipid suspension was processed by freeze/thaw-cycles (5x) and extrusion through a 0.1 µm filter (10x). Consecutive arginines (R5, R7, R9) as D-isomers were synthesized and coupled directly to the NBD (7-nitro-2-1,3-benzoxadiazol-4-yl)-group at the N-terminus by Peptide Specialty Laboratories GmbH (Heidelberg, Germany). For the quenching assay 740 µl of PBS were mixed with 60 µl of LUV suspension and incubated with the NBD-labeled peptides at 5 µM for different time spans. NBD fluorescence from peptides remaining in the exterior of the LUVs was then quenched by adding 25 mM of the non membrane permeable sodium dithionite. Fluorescence was detected with a FluoroMax-4-spectrofluorometer (Horiba Jobin Yvon, Edison, USA). NBD was excited at 460 nm and the fluorescence was recorded at 544 nm. For measuring the maximal quenchable fluorescence of the peptides present in the exterior and also in the interior of the LUVs, 0.5 % Triton X-100 was added afterwards to dissolve the vesicles. Counts for total fluorescence and fluorescence after quenching were corrected by subtracting this non-quenchable fraction. The intravesicular peptide in LUVs was displayed as percentage of total fluorescence after dithionate quenching for the different NBD-peptides (●) R5-NBD, (▲) R7-NBD and (■) R9-NBD. At a first glance the percentage of transduction as measured by the non-quenched intra-LUV peptide fluorescence seemed to be low in comparison to the experiments in living cells. However, under our experimental conditions the total volume of LUVs corresponded to about 0.2 % of the suspension volume assuming a LUV diameter of 100 nm and a surface area of lipids of 0.6 nm2. In the light of this estimate the results indicate an enrichment of peptides in the lumen of LUVs at least for R9.