pharmaceutics

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Supplementary Materials

Internalization and Transport of PEGylated Lipid-Based Mixed Micelles Across Caco-2 Cells Mediated by Scavenger Receptor B1

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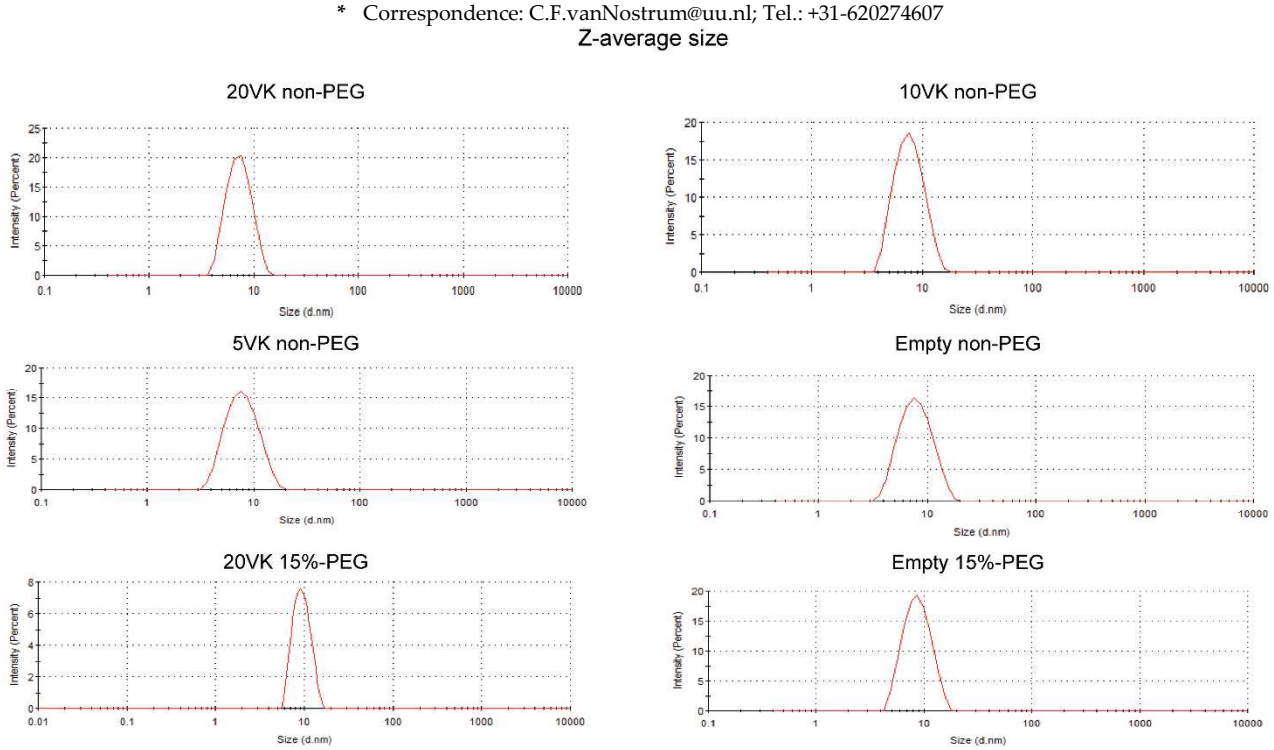
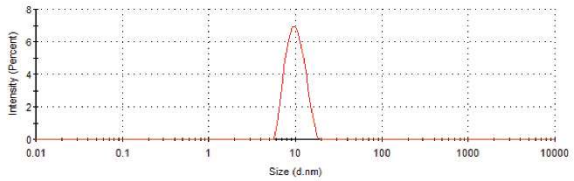


20VK 30%-PEG



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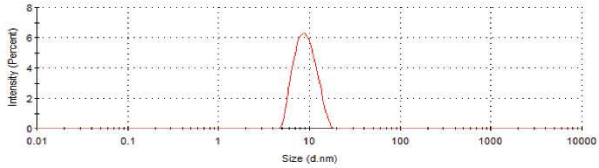

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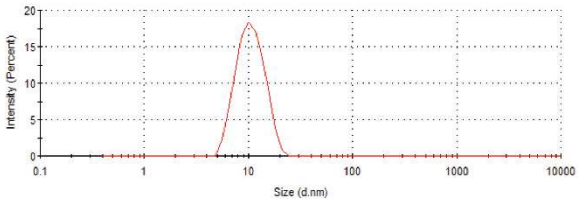

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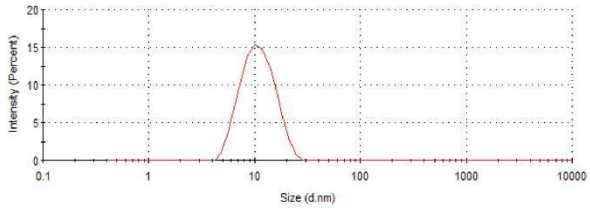
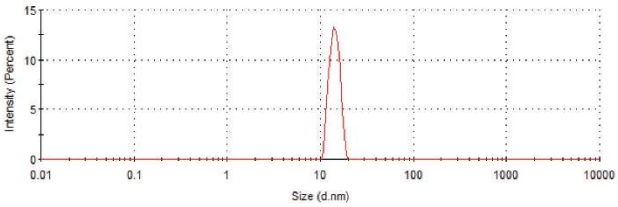
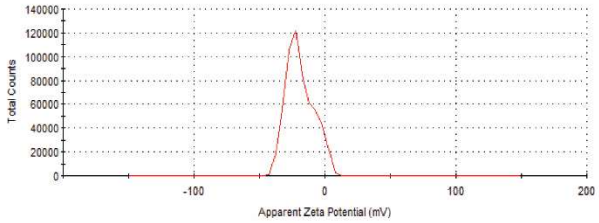


DSPE-PEG

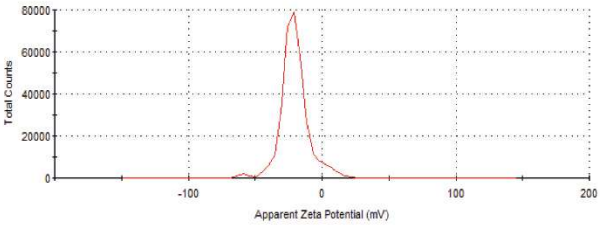


Zeta potential

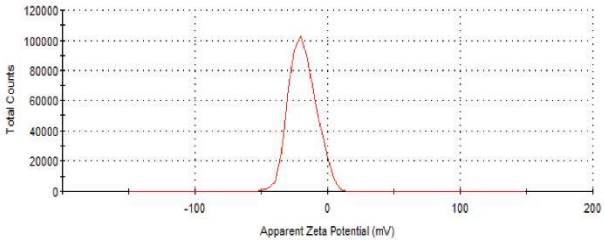
20VK non-PEG



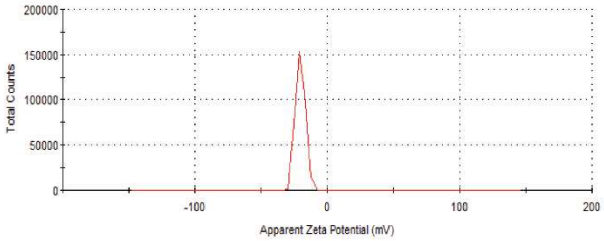
10VK non-PEG



5VK non-PEG



Epmty non-PEG



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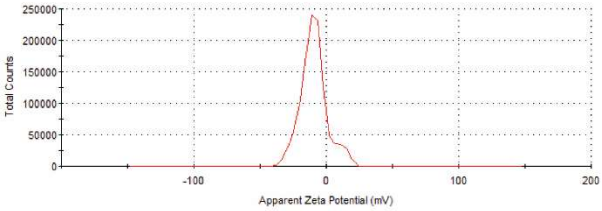
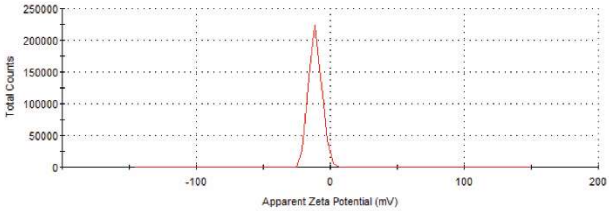


Epmty 15%-PEG



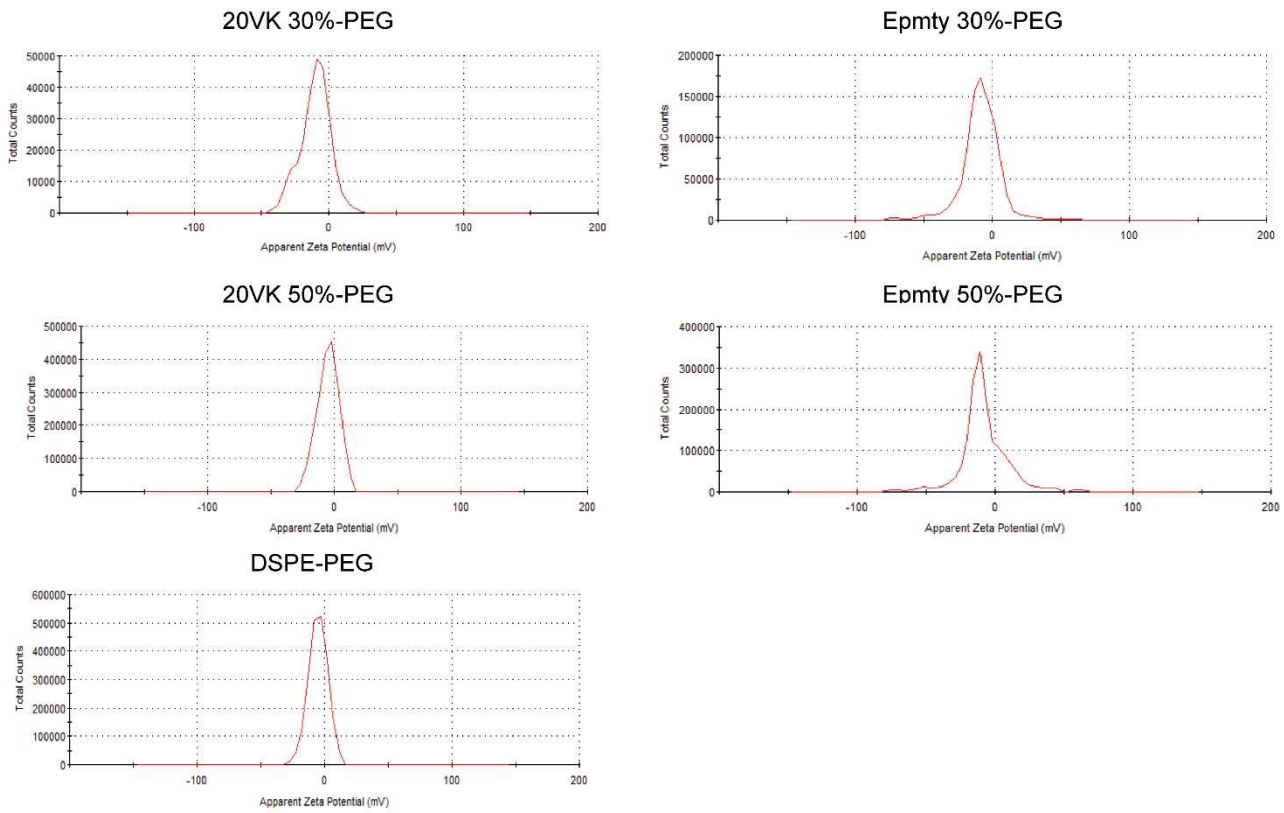


Figure S1. Representative Z-average size and zeta potential of mixed micelles with varying vitamin K loading and PEG content

Table S1. Calculated distance between neighboring chains (*D*) and *Rf*/*D* of micellar PEG layer

Formulations	Radius of Micelle* (nm)	wt% PEG	*D*(nm)	R_f/D
20VK-15%-PEG	4.55	17.5%	2.61	1.32
Empty 15%-PEG	4.20	18.5%	2.86	1.20
20VK-30%-PEG	4.85	29.7%	1.94	1.78
Empty 30%-PEG	4.35	31.2%	2.09	1.65
20VK-50%-PEG	5.35	41.2%	1.33	2.58
Empty 50%-PEG	5.25	42.9%	1.34	2.56
DSPE-PEG	7.05	71.3%	0.67	5.13

* Radius of micelle as determined by DLS, Rf = 3.44.

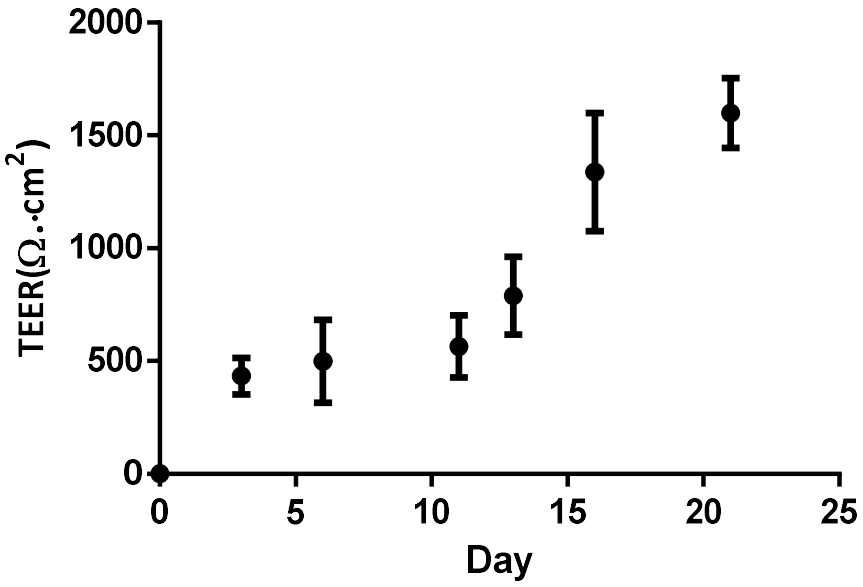


Figure S2. Trans-epithelial electric resistance (TEER) values (mean ± SD from 24 wells) of Caco-2 monolayers on transwell inserts as a function of the culture time.

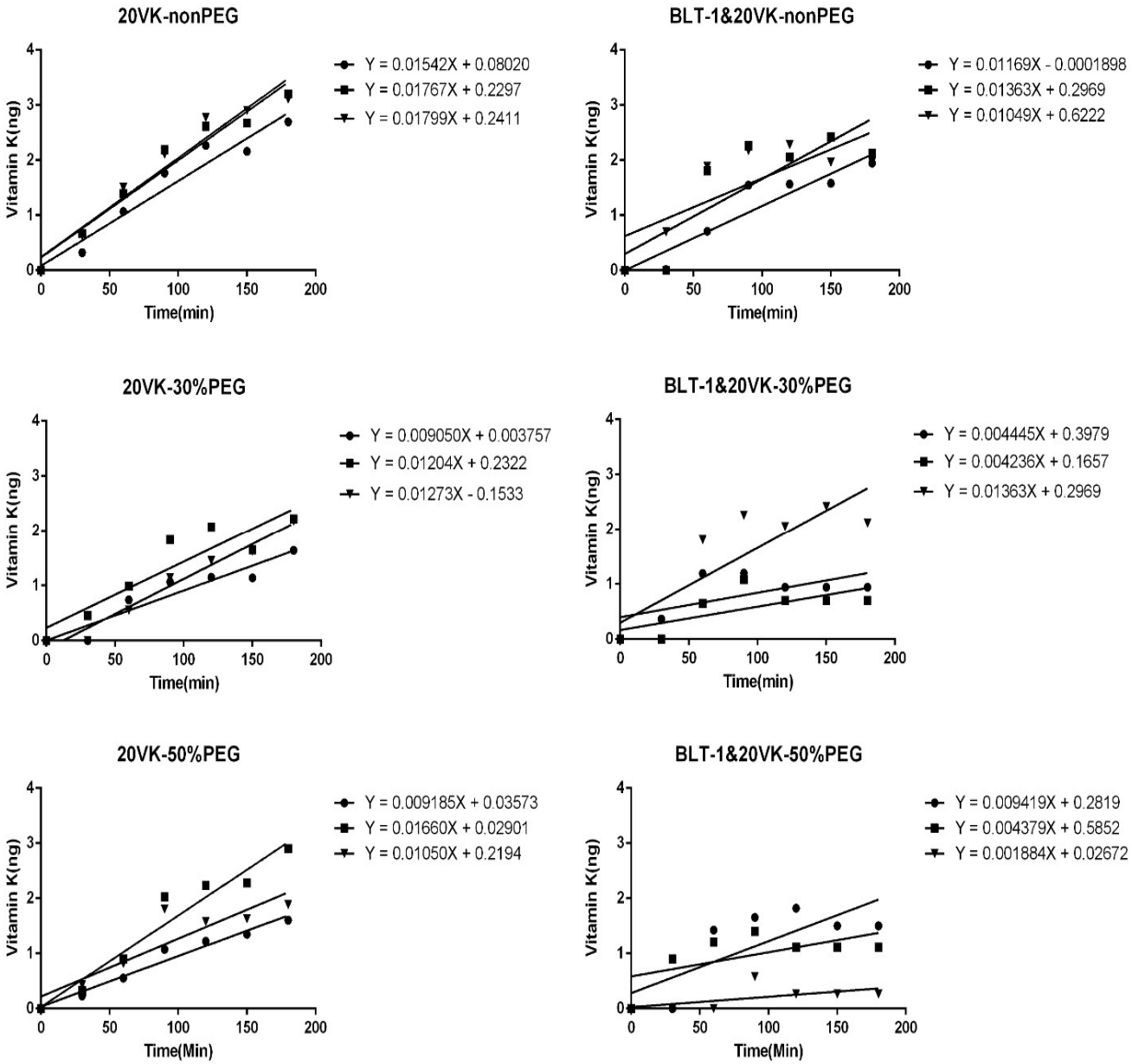


Figure S3. Cumulative amounts of transported vitamin K across Caco-2 monolayers. From top to bottom: non-PEGylated micelles, 30%-PEGylated micelles and 50%-PEGylated micelles; without (left) and with 10 μM BLT-1 inhibitor (right) (triplicates for each group).

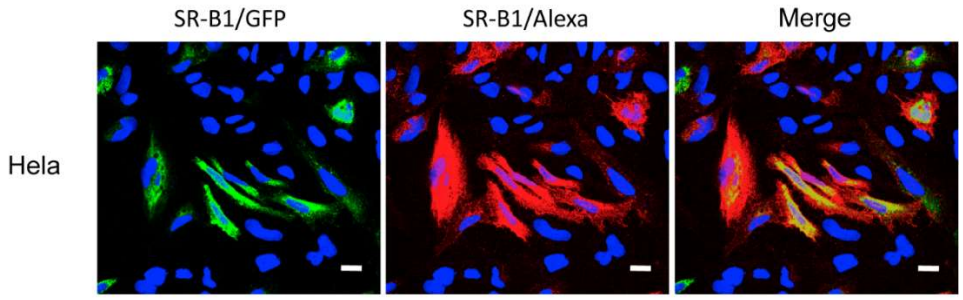
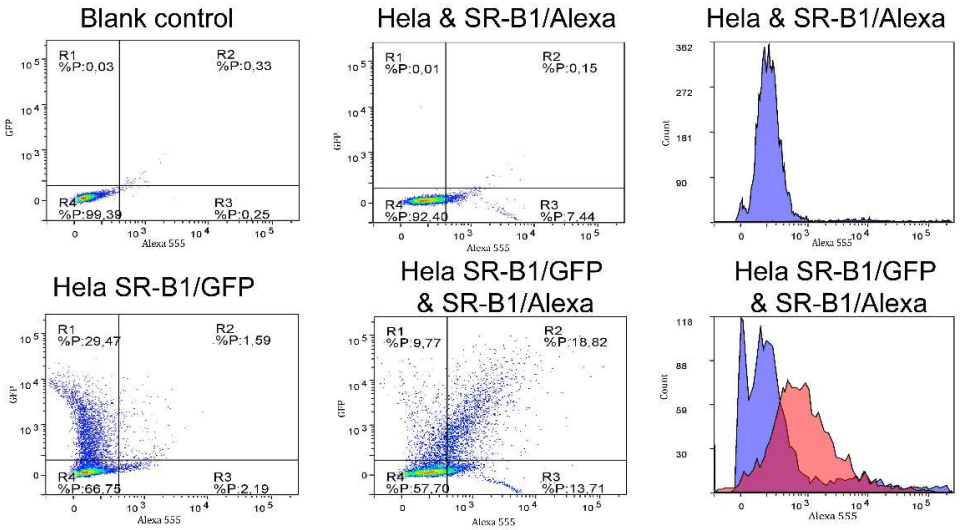


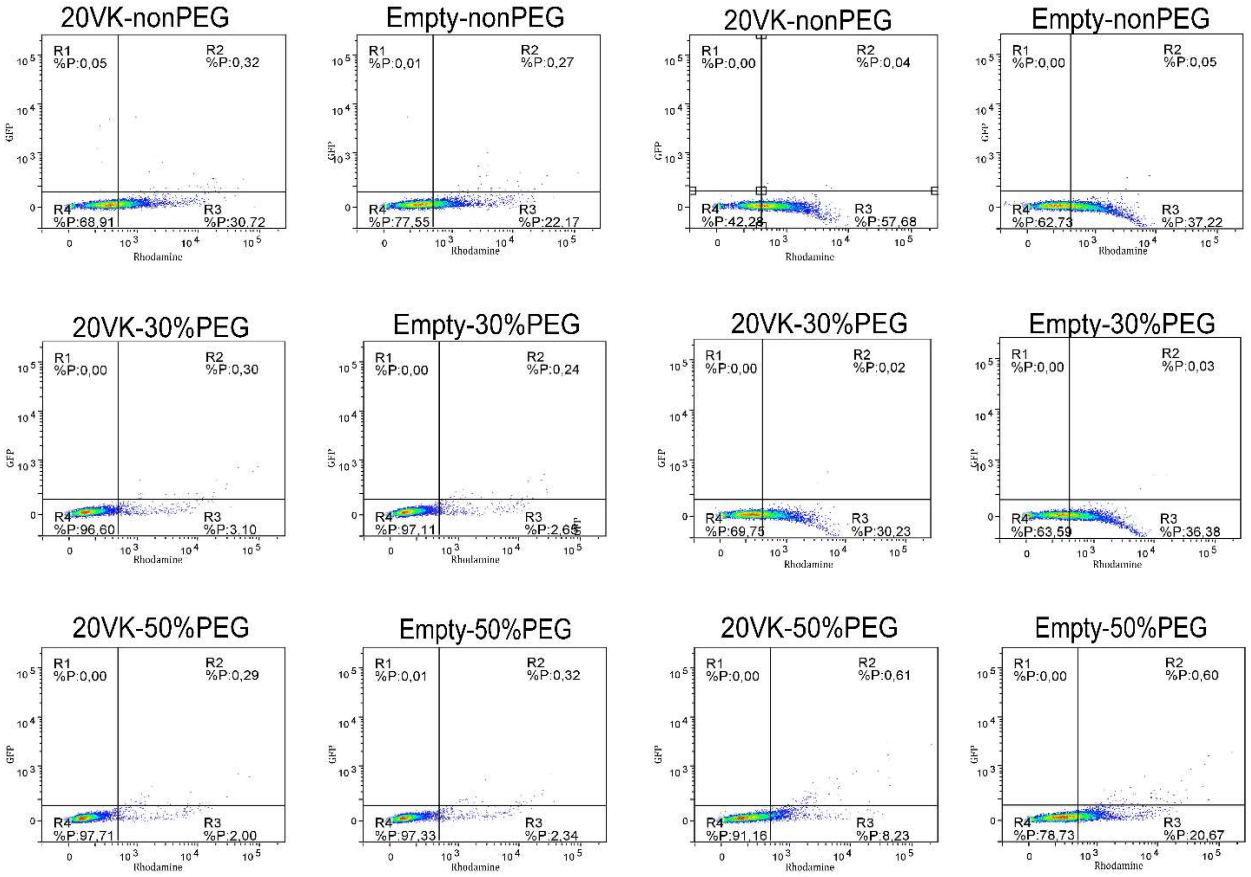
Figure S4. Fluorescence of the SR-B1/GFP fusion protein and immunofluorescence staining of SR-B1/Alexa in transfected HELA cells (the scale bar is 20 μm).

A



B 2 h incubation @ 4°C

C 2 h incubation @ 37 °C



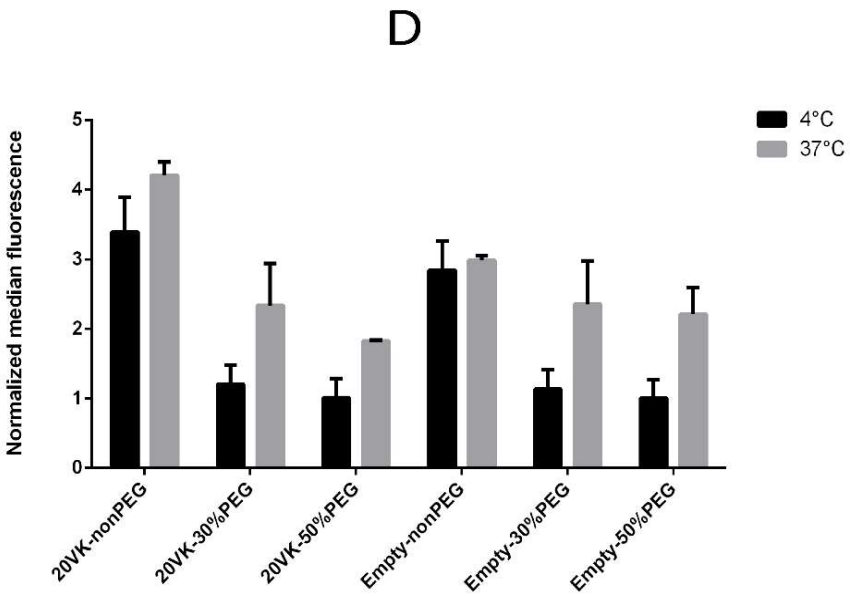


Figure S5. (**A**) FACS analysis of Hela cells showing SR-B1/GFP fluorescence (Y-axis) and/or SR-B1 expression as determined by immunofluorescence staining (X-axis) (Top: native Hela cells, Bottom: transfected Hela cells). GFP/SR-B1 fluorescence(Y-axis) and rhodamine fluorescence (X-axis) upon incubation at 4 °C (**B**) and at 37 °C (**C**) with native Hela cells of, from top to bottom: non-PEGylated, 30%-PEGylated and 50%-PEGylated mixed micelles (1.58 mM lipid) labeled with rhodamine without (empty) or loaded with vitamin K (350 μM). (**D**) Quantification of corresponding fluorescence of rhodamine for native HeLa cells incubated with micellar formulations, shown as mean ± SD. The quantified fluorescence intensity was normalized by the intensity observed for cells incubated with 20VK-50%PEG micelles.

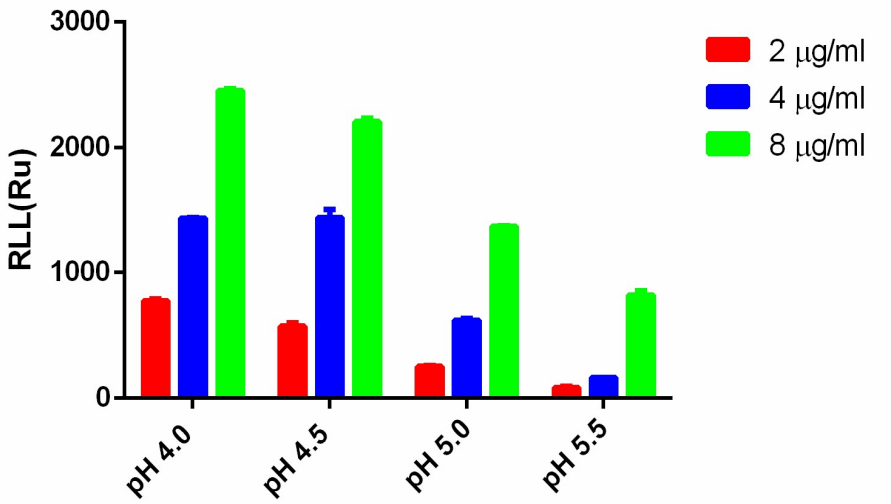


Figure S6. RLL (response of local ligand) values of SR-B1 spots immobilized on the chip using different SR-B1 solutions in PBS (2, 4 and 8 μg/mL) at four different pHs (4.0, 4.5, 5.0, 5.5).

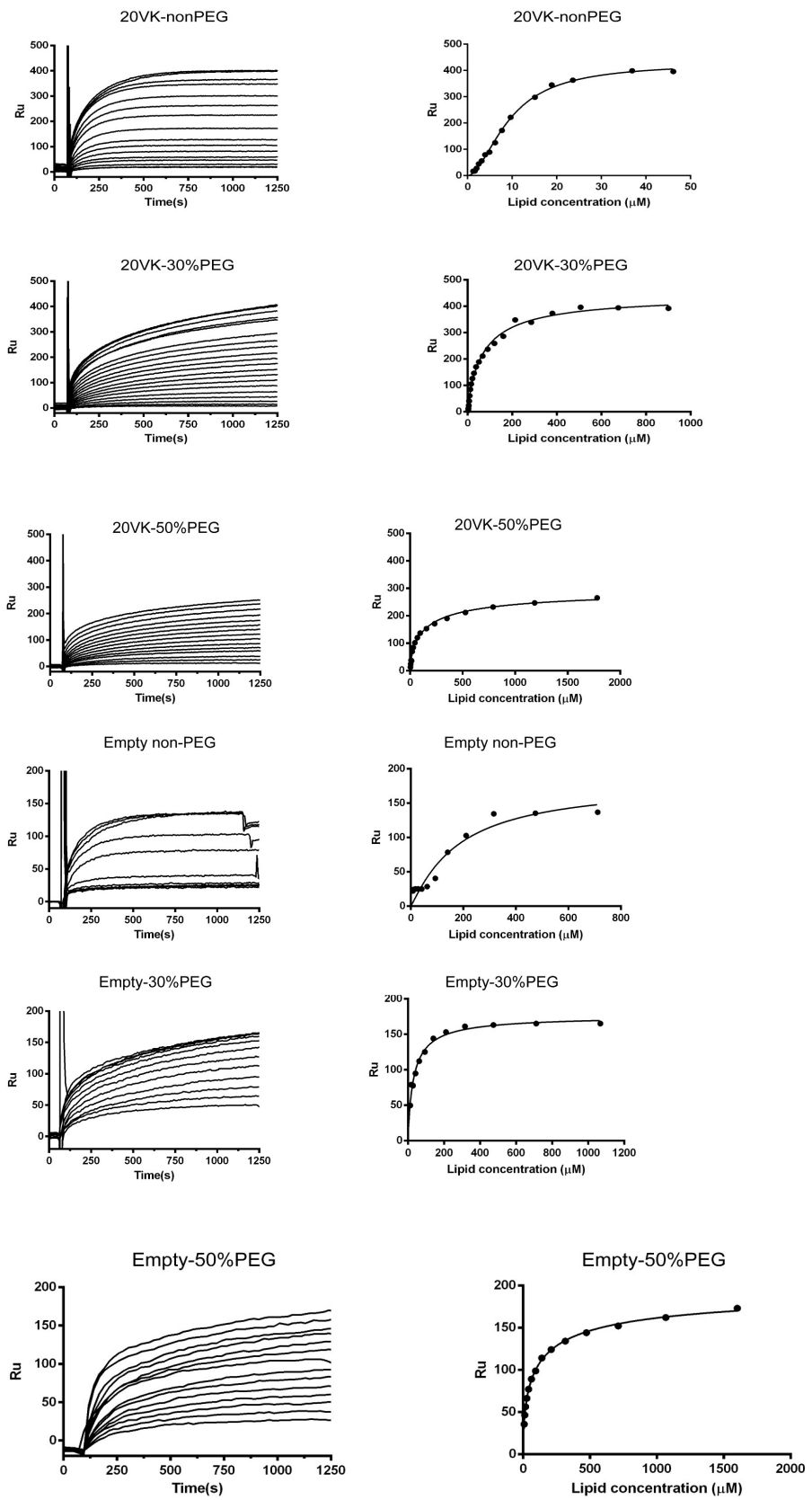


Figure S7. The representative binding responses of serial dilutions of mixed micelles (non-PEG, 30%-PEG and 50%-PEG without (empty) and with 20 mg vitamin K) interacting with SR-B1immobilized on the SPR chip (left) and fitting of SPR data to one site specific model (right). The response was recorded at spots with RLL 2452 ± 16.

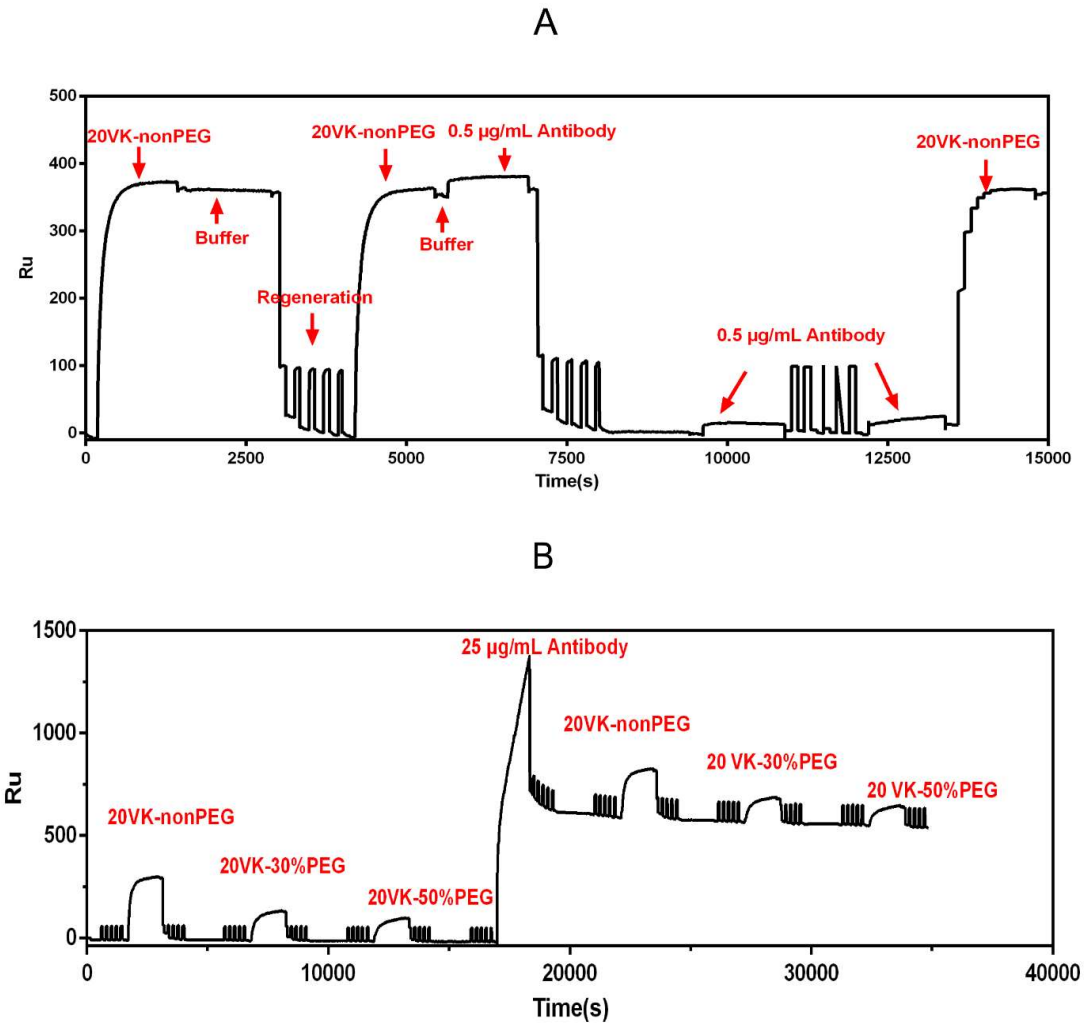


Figure S8. Competition between vitamin K loaded mixed micelles and a matching antibody for their interaction with the SR-B1. (**A**) Subsequent injections of 20VK-nonPEG formulations (8 μM vitamin K, 36 μM lipid) and 0.5 μg/mL antibody: antibody was first exposed to the occupied receptor (after second injection of the micelles), then twice to the regenerated receptor, after which the micelles were injected again. (**B**) SPR response of micelles with different degrees of PEGylation (20VK-nonPEG, 20VK-30PEG, 20VK-50PEG, with 8 μM vitamin K and 36 μM lipid) before and after the SR-B1 was blocked by 25μg/mL antibody.