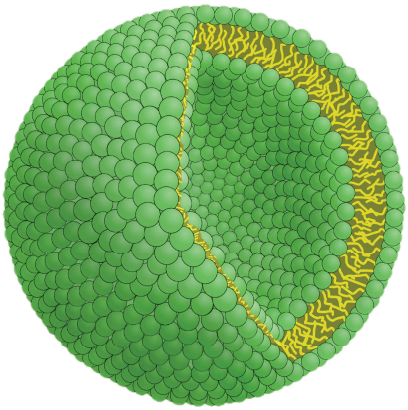
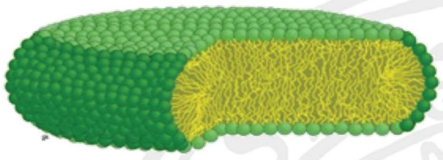
PEG-stabilized lipodisks –
from discovery to targeted drug delivery

Katarina Edwards
Department of Chemistry – Ångström Laboratory, Physical Chemistry

Liposomes vs lipodisks

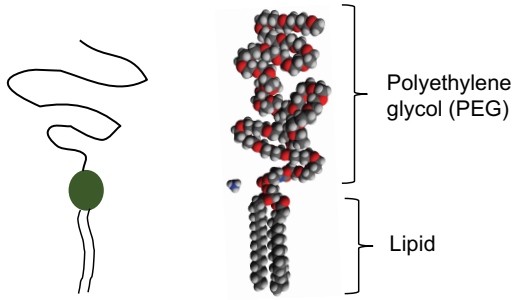


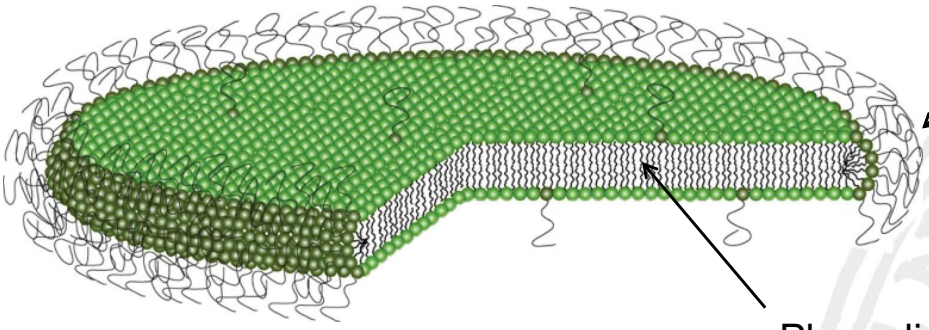
Liposome

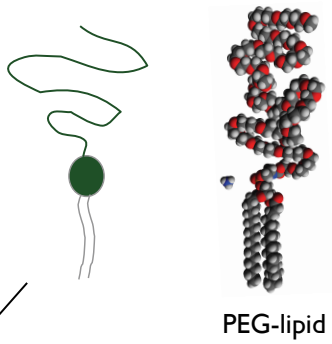


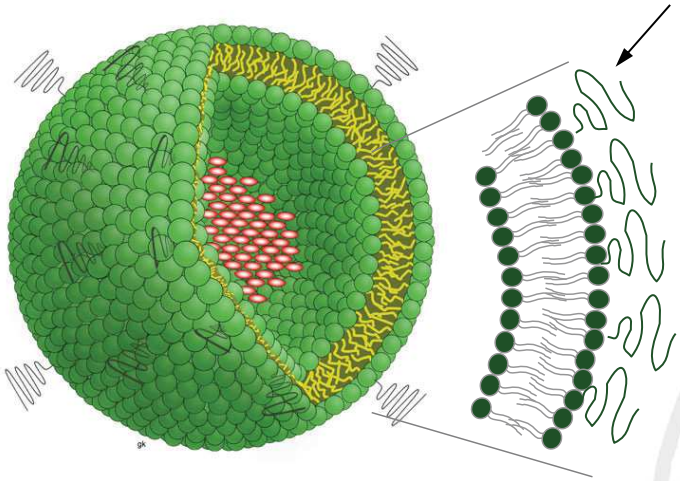
Lipodisk

Lipodisk structure



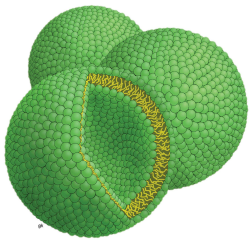




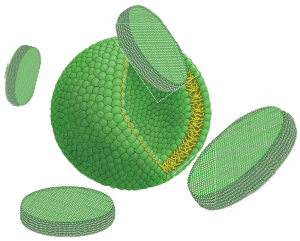


PEG-stabilized liposome

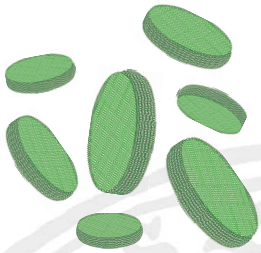
PEG-lipids induce structural transformation



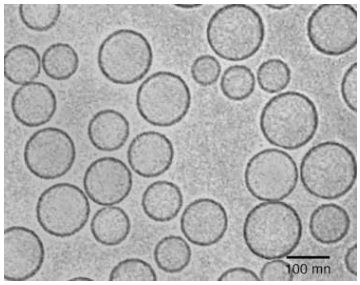
≤ 5 mol% PEG-lipid

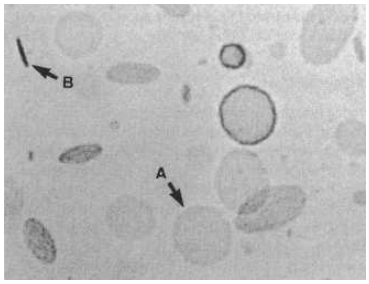


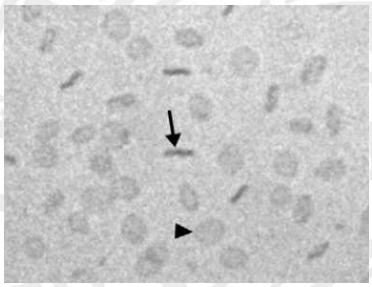
> 5 mol% PEG-lipid



≈ 20 mol% PEG-lipid





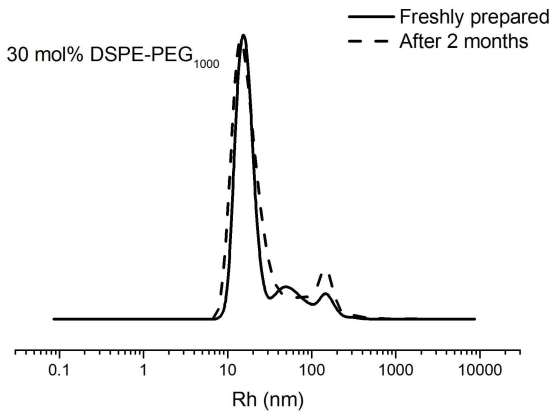


Edwards, K. et al. *Biophys. J.* **1997**, *73*, 258

Johnsson, M. and Edwards, K. *Biophys. J.* **2003**, *85*, 3839

Robust structure

- ✧ Insensitive to dilution and temperature changes
- ✧ Excellent long term stability
- ✧ Compatible with freeze- and spray-drying

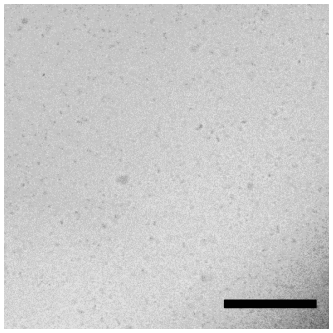


Zetterberg,M. et al. *J. Colloid Interf. Sci.* **2016**, *484*, 86

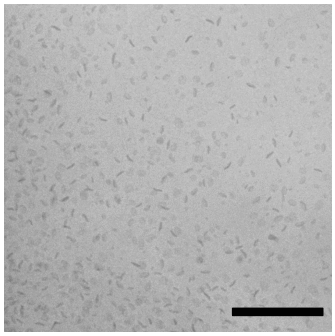
Wessman, P. Et al. et al. *J. Pharm. Sci.* **2010**, *99*, 2032

Adjustable size

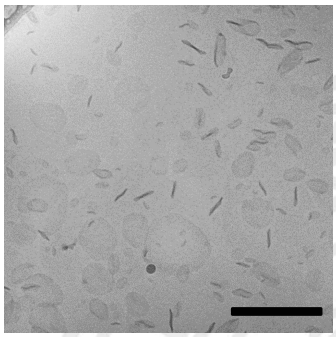
- Tunable diameter depending on PEG-lipid content and preparation method



25 mol% PEG
Detergent depletion
d_H ~ 20 nm

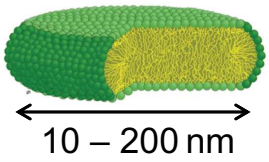


20 mol% PEG
Hydration
d_H ~ 45 nm

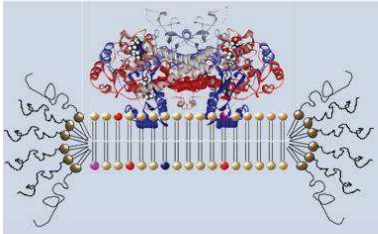
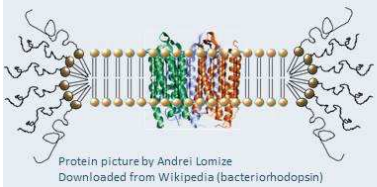
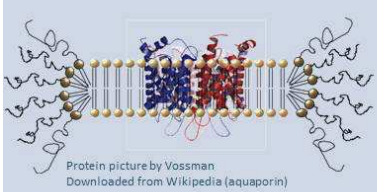


10 mol% PEG
Low temperature sonication
d_H ~ 150 nm

Bar = 200 nm

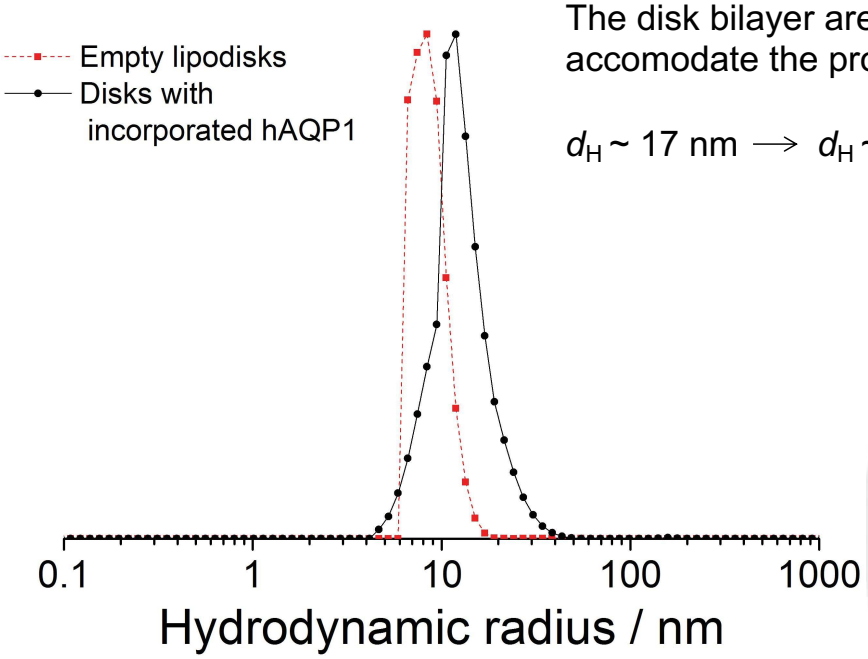


Proteodisks

	Protein	Lipid bilayer
	Cyclooxygenase-1 (COX-1) dimers	POPC:Soy-PE:cholesterol (40:37:23) Lundquist et al., 2010; Meiby et al., 2013
 Protein picture by Andrei Lomize Downloaded from Wikipedia (bacteriorhodopsin)	Bacteriorhodopsin (bR) trimers	DSPC:cholesterol (53:47) Johansson et al., 2007
 Protein picture by Vossman Downloaded from Wikipedia (aquaporin)	Human Aquaporin 1 (hAQP1) tetramers	DPPC:cholesterol (47:53) Duong-Thi et al., 2016

Protein picture by Vossman

Membrane spanning proteins



The disk bilayer area auto-adjusts to accomodate the protein!

d_H ~ 17 nm ⟶ d_H ~ 23 nm

Lipodisks as model membranes

Drug partition, peptide binding and ligand-protein interactions studies employing

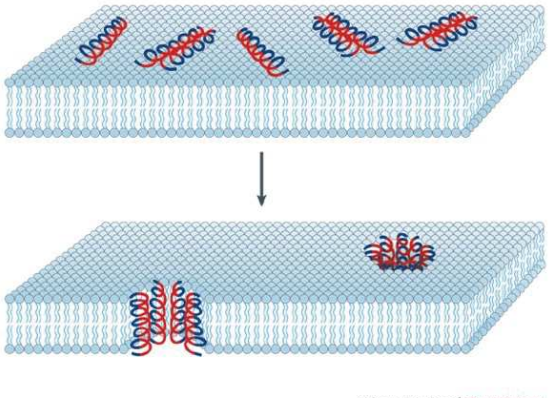
- capillary electrophoresis
- chromatographic methods
- surface plasmon resonance (SPR)
- quarts crystal microbalance (QCM)
- fluorescence spectroscopy
- …

Lipodisks for drug delivery

- Protein/peptide drugs
- Hydrophobic drugs, conventional chemotherapeutics
- Genetic material (oligonucleotides, siRNA)
- Radiotherapeutic drugs

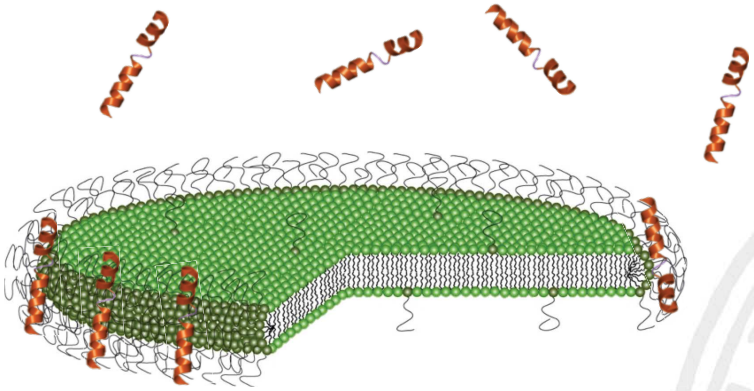
Cationic amphiphilic peptides (CAPs)

- ✧ Derived from, or inspired by, natural antimicrobial peptides (AMPs)
- ✧ Membranolytic action; creates membrane pores
- ✧ Potential novel antibiotics
- ✧ Promising anticancer peptides



… but concerns regarding poor aqueous solubility, fast proteolytic degradation and lack of specificity!

CAPs bind with high affinity to the rim of lipodisks



- Melittin
- Mastoparan
- Mastoparan X
- Magainin 2
- Alamethicin
- LL37

Wessman, P. et al., *BBA* **(2008)** *1778*, 2210, Agmo Hernández, V. et al. *Anal Chem* **(2013)** *85*, 7377, Reijmar, K. et al. *Langmuir* **(2016)** *32*, 12091

Melittin

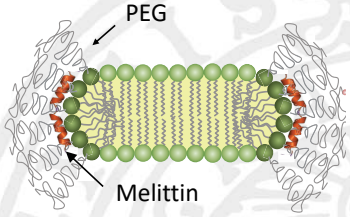
✧ active component in bee venom

✧ strong antibacterial effect

✧ potent anticancer peptide

✧ fast proteolytic degradation

✧ affects also healthy cells (hemolytic)

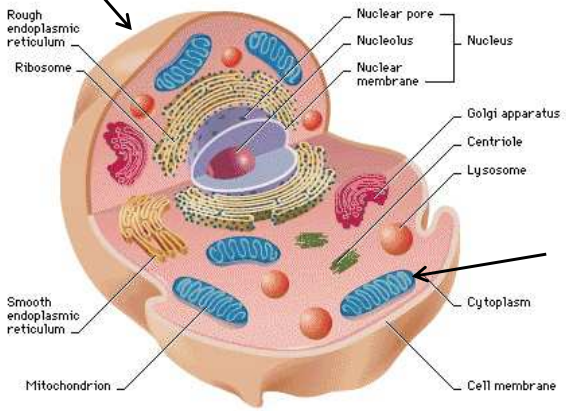


Zetterberg, M et al., J Control Release (2011) 156, 323-328

Gao, J. et al., *Nanoscale* (2016) *8*, 7209

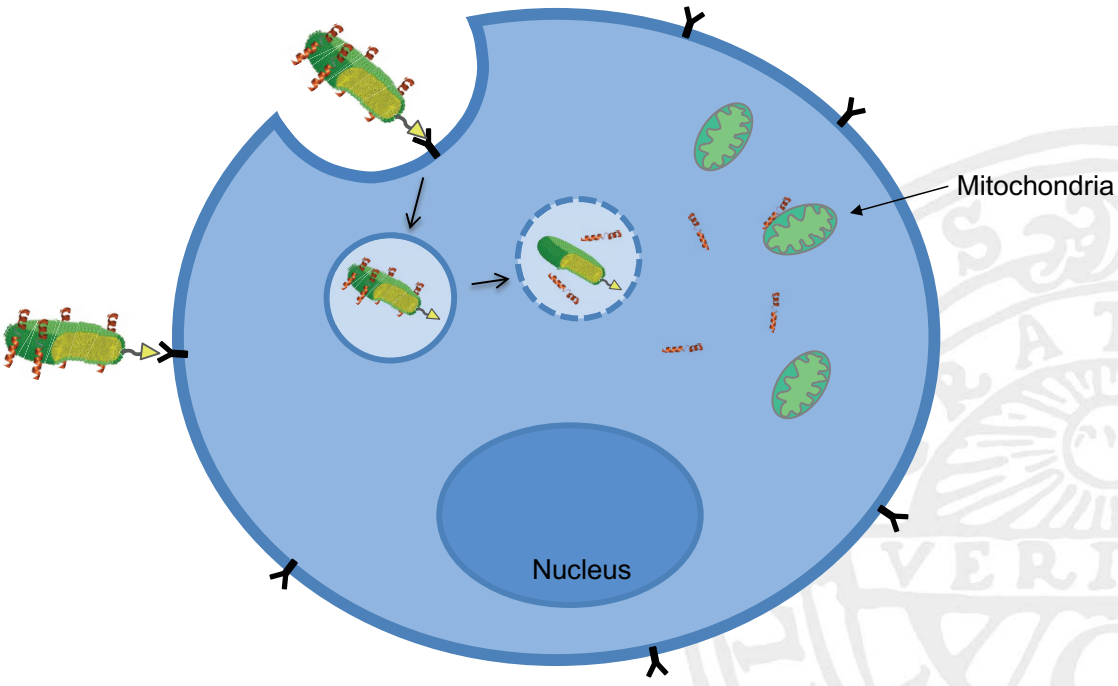
Melittin - a potent anticancer peptide

Permeabilizes cell membrane

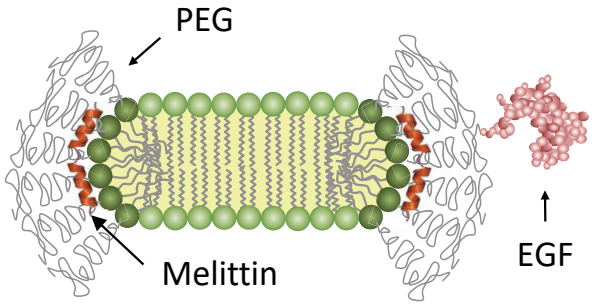


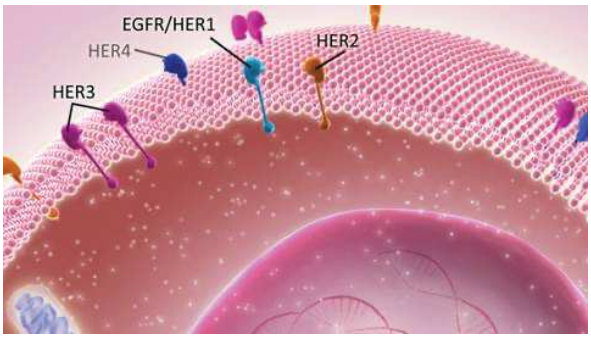
Causes apoptosis by disrupting mitochondria

Targeting lipodisks

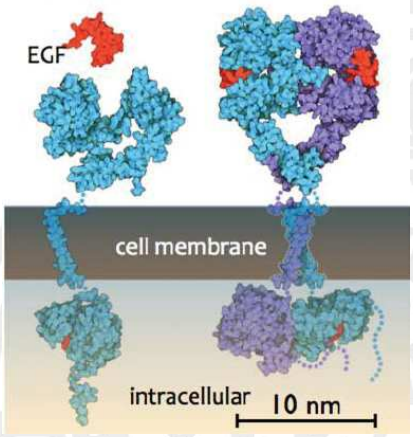


Targeting melittin-loaded lipodisks





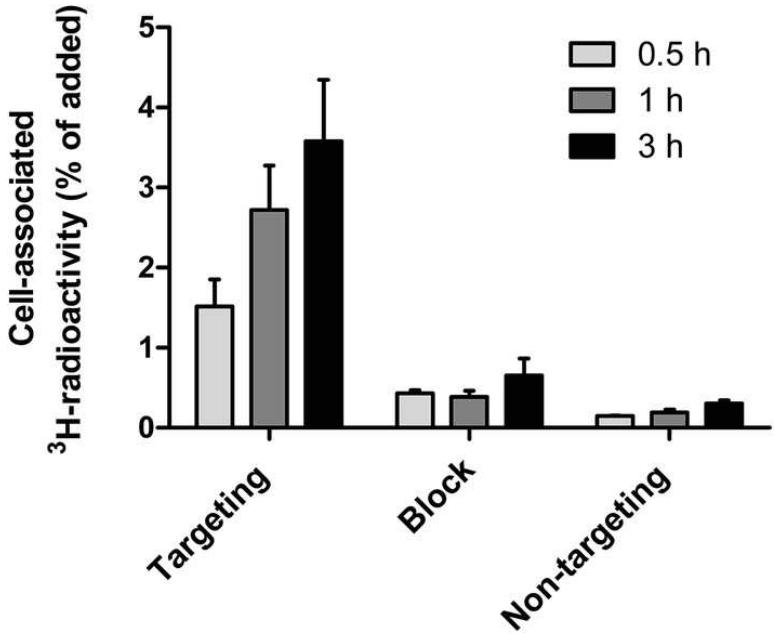
EGF-family receptors



D.B. Pecky and N. de Jonge, Microscopy and Microanalysis (2014) 20, 346-365

EGFR-specific targeting

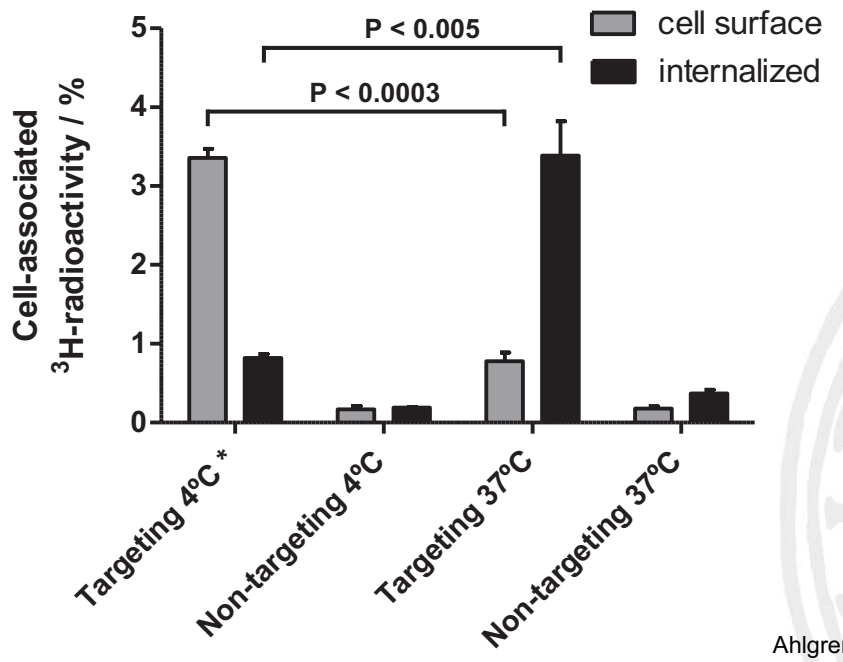
Cellular binding of ^{3}H-labelled lipodisks to A-431 tumour cells



Ahlgren, S. et al., *RSC Adv* **(2017)** *7*, 22178

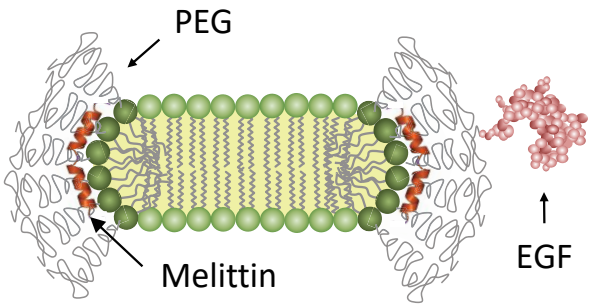
Cellular internalization

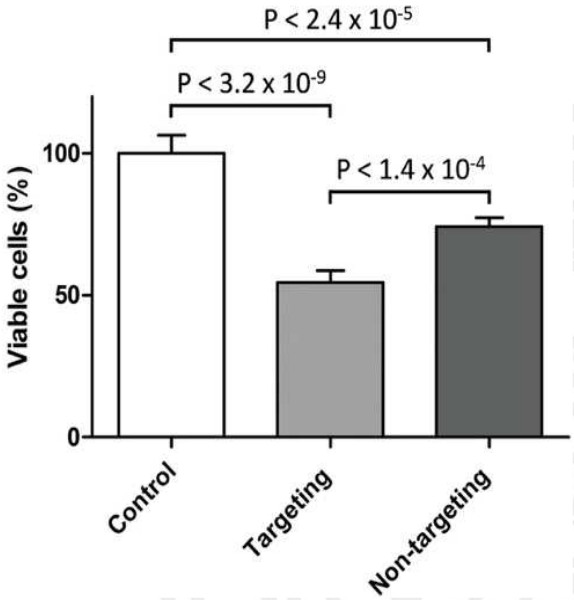
Acid wash analysis after 3h incubation with A-431 cells



Ahlgren, S. et al., *RSC Adv* **(2017)** *7*, 22178

Targeting melittin-loaded lipodisks





Ahlgren, S. et al., *Nanomedicine:NBM* **(2017)** *7*, 2325

Small size and non-spherical shape of lipodisks potentially

- reduces uptake in liver and spleen, longer circulation time
- facilitates passage over leaky blood vessel wall
- increases opportunities for deep tumour penetration
- reduces immunological response

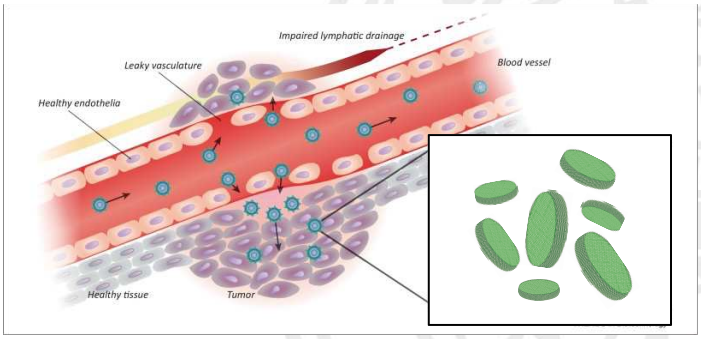
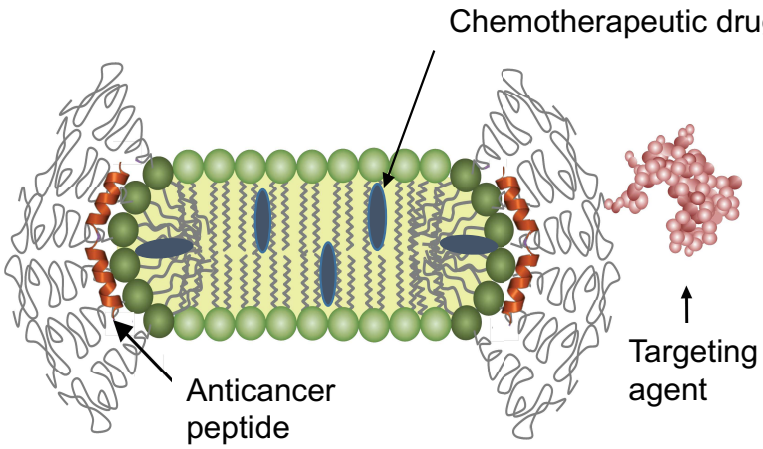


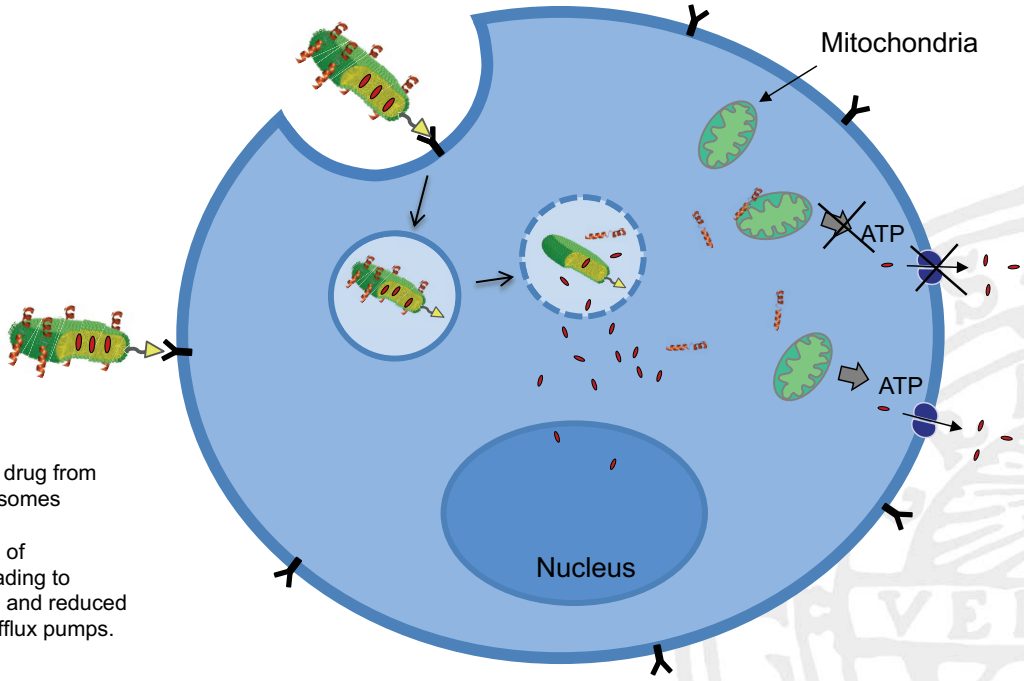
Figure adapted from Noble at al., *Trends in Biotechnology* (2014), *32*, 32-45

Lipodisks as nanocarriers for conventional chemotherapeutic agents

Lipodisk composition	Drug	Targeting	Reference
DSPC	Doxorubicin	NA	Zhang et al., 2014
DPPC:Chol	Curcumin	Tumour cells	Ahlgren et al., 2017
DSPC	Doxorubicin/Paclitaxel	Tumour tissue	Feng et al., 2019
POPC:Chol	Paclitaxel	Vasculature	Wang H. et al, 2018
POPC:Chol	Paclitaxel/ Melittin	Vasculature	Wang H. et al. 2019
POPC:Chol	Paclitaxel	Vasculature	Wang L. et al., 2019

Lipodisks for targeted dual delivery





1) Rapid release of drug from endosomes/lysosomes

2) Permeabilization of mitochondria, leading to depletion of ATP and reduced activity of drug efflux pumps.

Unpublished results

Planned and ongoing …

- *In vitro/in vivo* studies to explore cytotoxicity and potential synergistic effects
- Comparative studies using targeting and non-tageting lipodisks
- Alternative chemotherapeutic agents and anticancer peptides

Thank You!

Luís Silva, Lars Gedda, Víctor Agmo Hernández, Sara Ahlgren, Amelie Fondell, Malin Morin Zetterberg, Karin Reijmar, Per Wessman, Anna Lundquist, Emma Johansson, Maria Sandström, Markus Johnsson…

Phospholipid Research Center

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Swedish Research Council

Lipoid