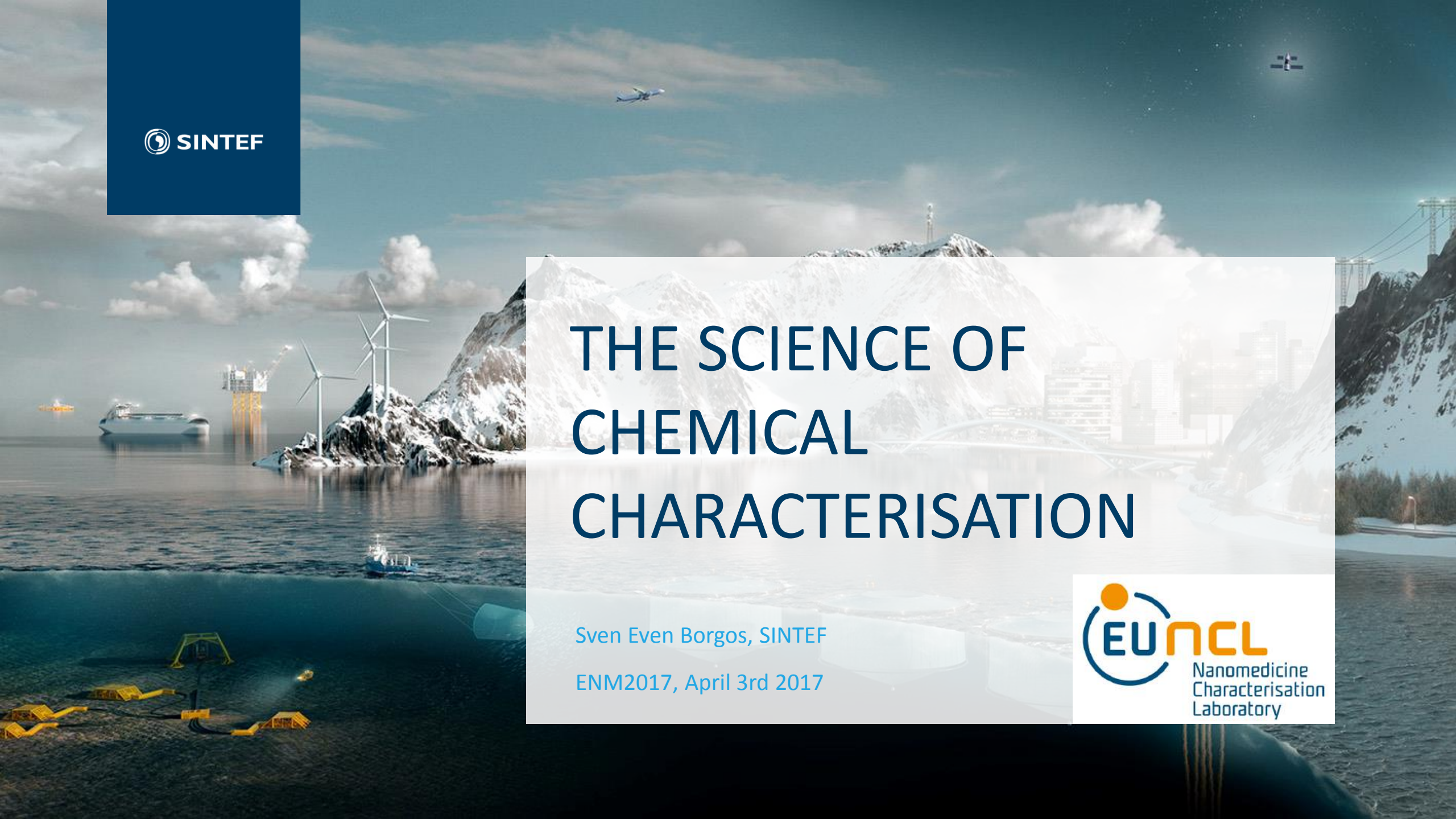


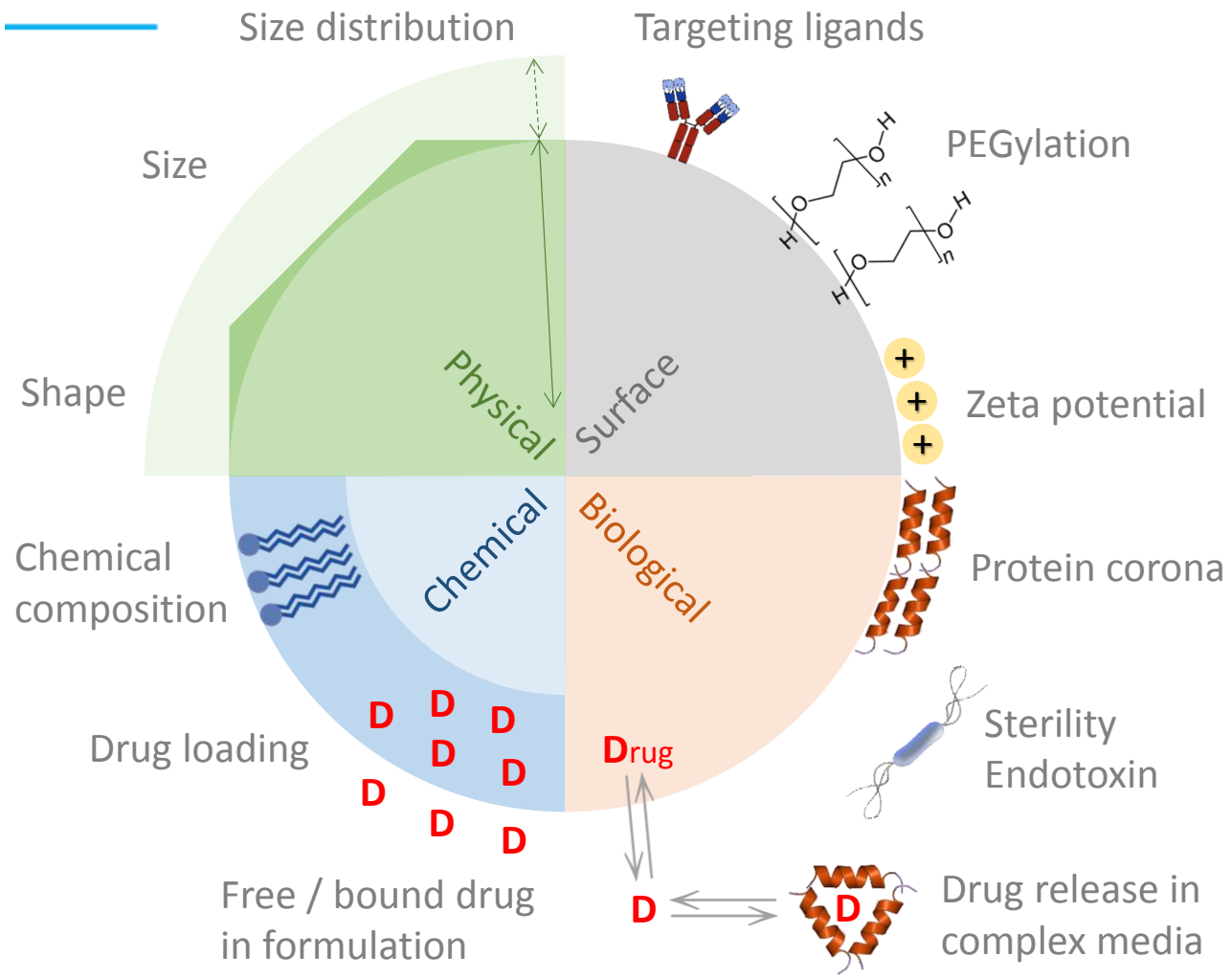
THE SCIENCE OF CHEMICAL CHARACTERISATION

Sven Even Borgos, SINTEF

ENM2017, April 3rd 2017

EUNCL Nanomedicine Characterisation Laboratory

Analytical aspects of nanomedicines



… and a lot of this is chemistry, really

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Borgos, S.E.F., *Characterization Methods: Physical and Chemical Characterization Techniques.* Pharmaceutical Nanotechnology: Innovation and Production, 2 Volumes, 2016.

Mass spectrometry – a good tool

Sensitivity + Specificity + Selectivity (+ Wide Applicability !)

- Low- or sub-ng/ml LOD for most compounds
- Can quantify even in very complex samples
- Can verify molecular integrity
- Can monitor closely related compounds simultaneously
- Can see most organic molecules (and inorganic elements)

NPs we've worked on include:

- Polymers (PACA – PU – PLGA – chitosans)
- Lipid NPs
- Liposomes
- Inorganic NPs

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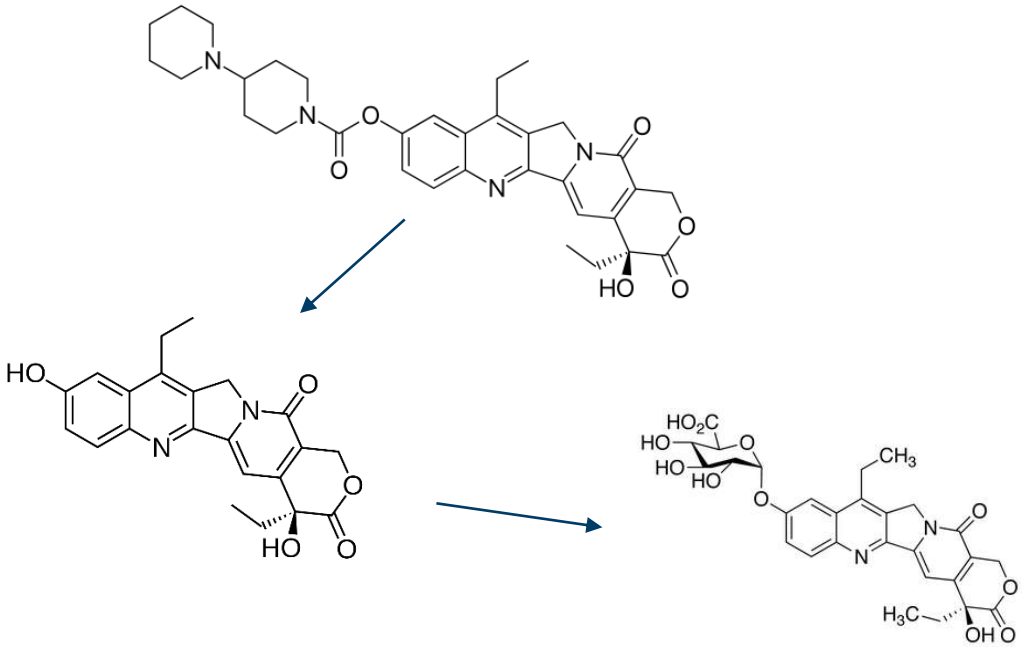
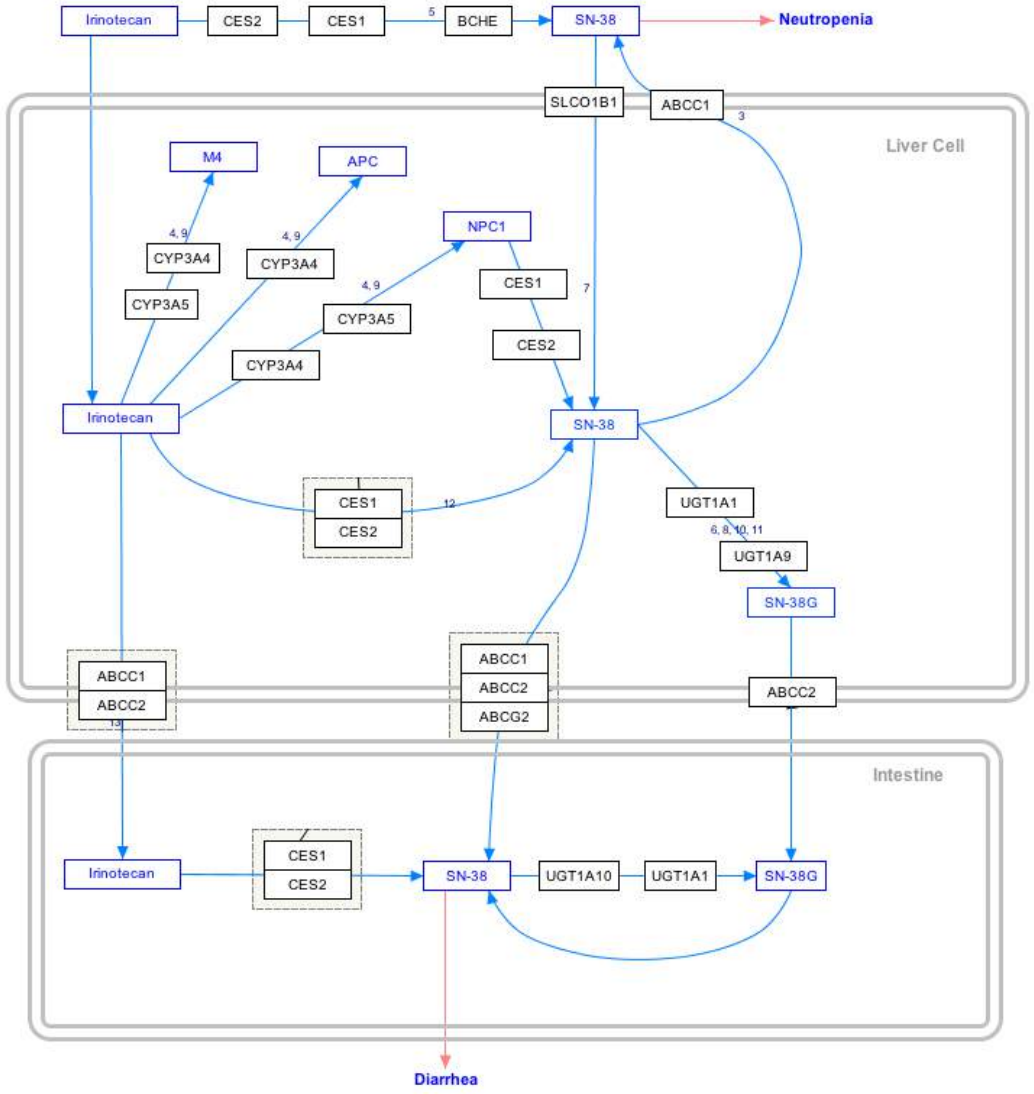
Example: EUNCL-011

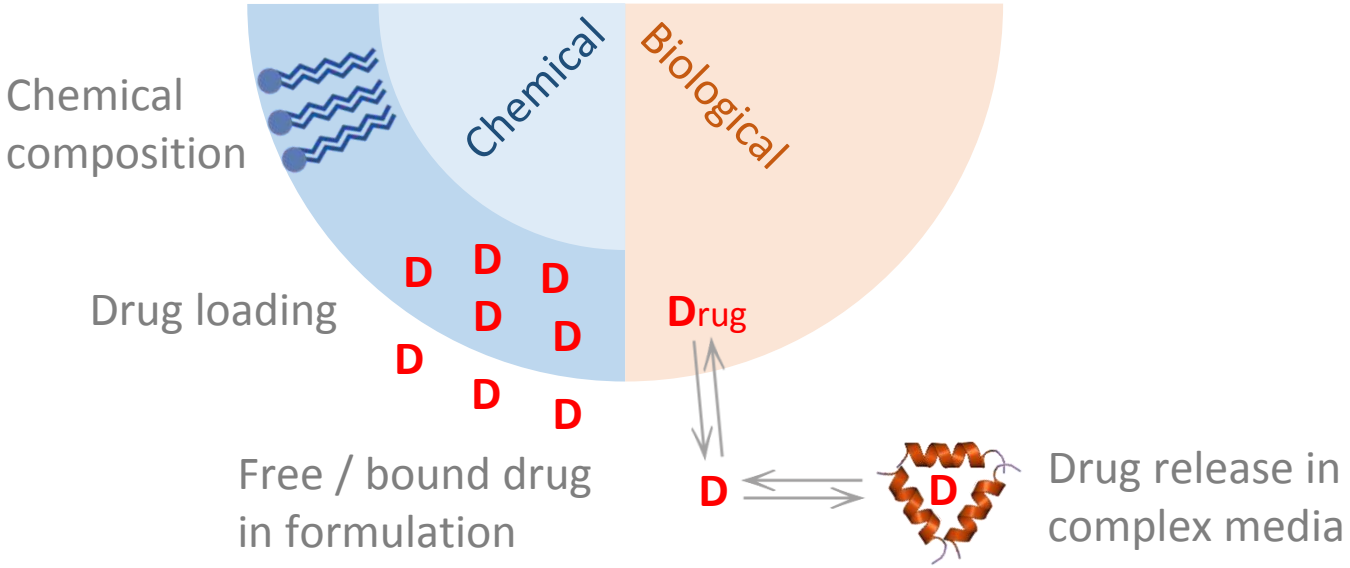
- Liposomal irinotecan (prodrug)
- Irinotecan → SN-38 → SN-38-glucuronide ('SN-38-G') (mainly)



Model human liver cell showing blood, bile and intestinal compartments, indicating tissue involvment of genes in the irinotecan pathway.



Assays to run



The following assays were chosen to run for EUNCL-011 sample:

- Drug loading (EUNCL_PCC_30)
- Free/bound drug (EUNCL_PCC_31)
- Lipid composition (EUNCL_PCC_32)
- Drug release in complex media

Method: LC-MS/MS

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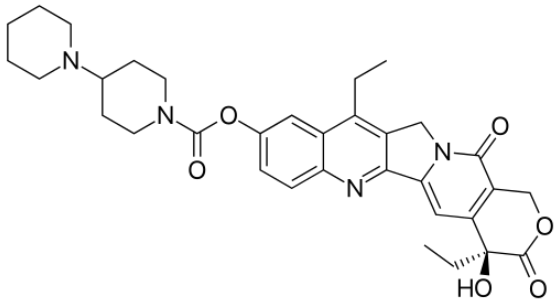
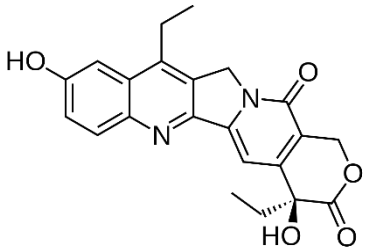
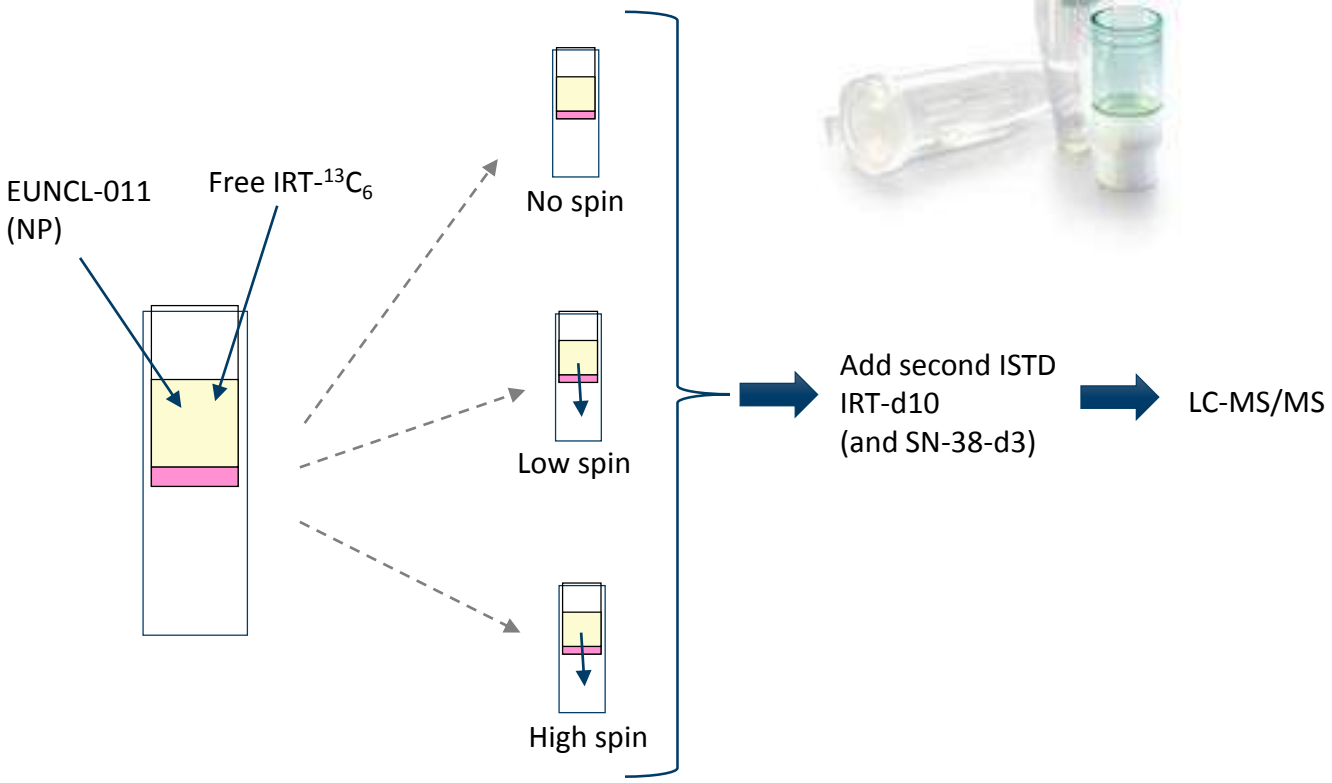
Total drug loading



- Dissolve/dilute NPs in methanol (triplicate)
- Isotope ISTDs: IRT-d10 and SN-38-d3
- Quantified IRT: **4.33** mg/ml, std.dev. 0.06 mg/ml (spec.: **4.3** mg/ml)
- Also: Found SN-38 in EUNCL-011; 1.47 µg/ml (i.e. < 0.4% of IRT)
  - Spontaneous decarboxylation?
  - Note much higher potency of SN-38



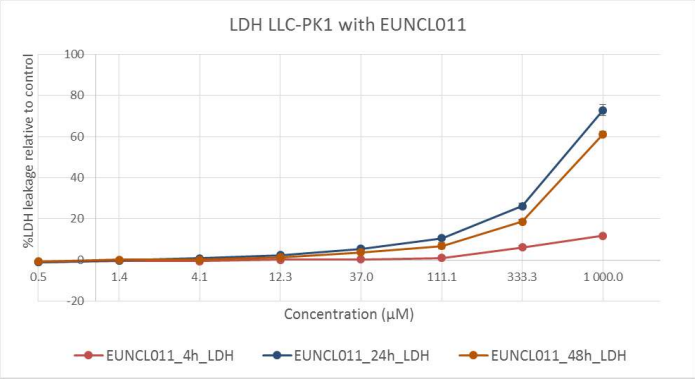
Free/bound drug

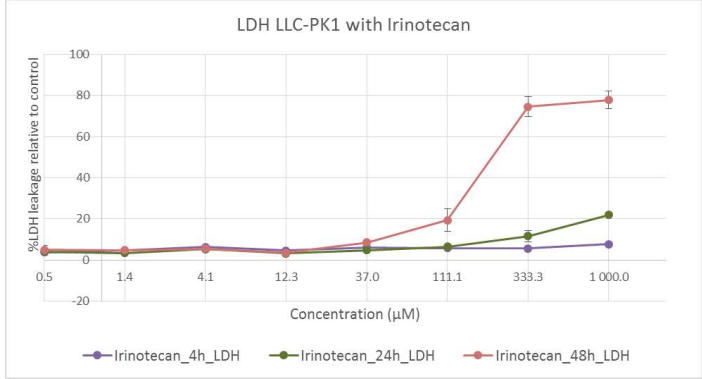


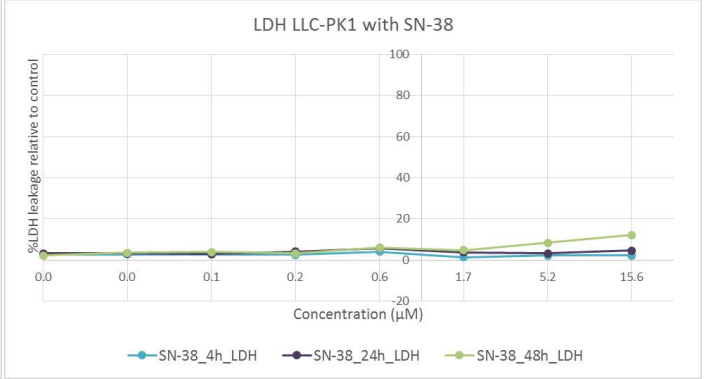
- Quantification of IRT in filtrate gives concentration of
  - 39.7 µg/ml (low speed), std.dev. 2.48 µg/ml
  - 40.7µg/ml (high speed), std.dev. 0.78 µg/ml
- Concentration in total drug sample: 4.33 mg/ml, std.dev. 0.059 mg/ml

- Provided we can assume equilibrium unchanged upon dilution: **0.94 % of IRT** (rel. to high speed spin) exists as free drug in EUNCL-011.

Actual drug conc. experienced *in vitro*







- Irinotecan added at 333µM (NP or free)
- Only ~ 3 % irinotecan is observed as in solution after 48 hours
- Metabolism to SN-38 to SN-38G is observed in LLC-PK1 cells (not HepG2)

		Irinotecan HCl (µM)		SN-38 (µM)		SN-38G (nM)	
Sample	**Exposed to**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
Analysis of distribution of irinotecan, SN-38 and SN-38G in assay wells with LLC-PK1 cells							
Unfiltered aqueous phase	EUNCL-011	329.9	335.0 →	0.820	1.899 →	3.1	2.9
	Irinotecan	191.7	188.9	0.526	0.696	NA	NA
	SN-38	0.0071	NA	9.448	10.806 →	336.7	458.2
Filtered aqueous phase	EUNCL-011	13.4	9.9	0.092	0.111 →	1.7	1.9
	Irinotecan	159.0	174.4	0.222	0.237	NA	NA
	SN-38	0.0022	0.0026	2.980	2.827 →	195.9	232.9
Extracted cell layer	EUNCL-011	1,1	13,3	0,000	0,005	NA	NA
	Irinotecan	36,4	36,9	0,002	0,006	NA	NA
	SN-38	0,0018	0,0034	0,057	0,147 →	3,2	7,7

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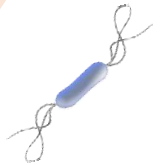
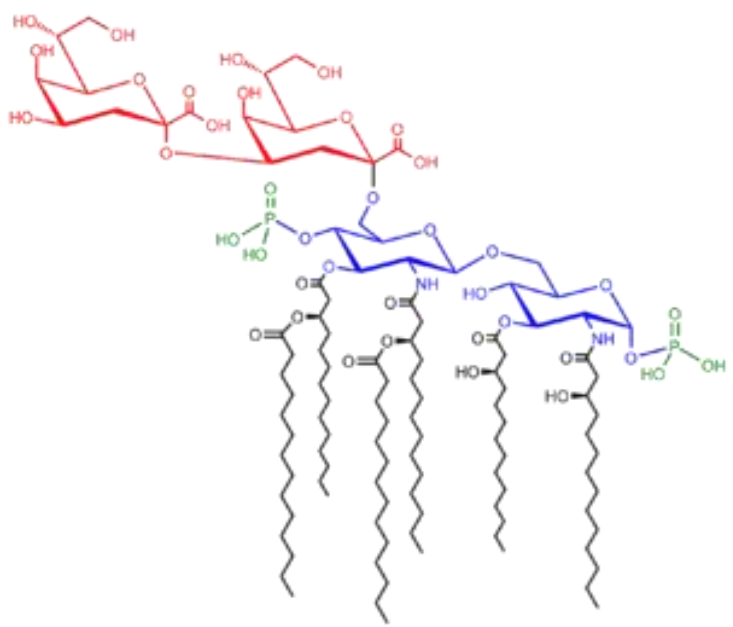
Challenge: Endotoxin (LPS)

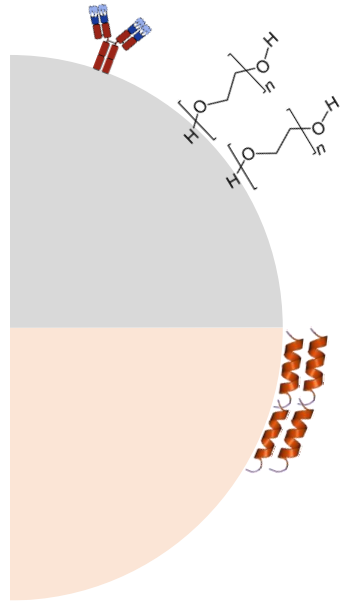


- Major issue with candidate nanomedicines
- An unconditional showstopper
- Current assays (LAL) are enzyme-based
  - Technically demanding
  - Prone to nanoparticle-specific problems (e.g. encapsulated endotoxin)
- Direct detection would be ideal
  - …at least to complement functional assays like LAL



Challenge: Surface functionalities

- PEGylation degree and distribution
- Hydrophobicity
- Protein corona – and its use as a tool for targeting (?)



- Protein ligands and corona can be analysed as peptides digested/released from the surface
- PEGylation analysis is hard – but important!

- NMR
- TOF-SIMS / MALDI-MS
- Chromatography (?)

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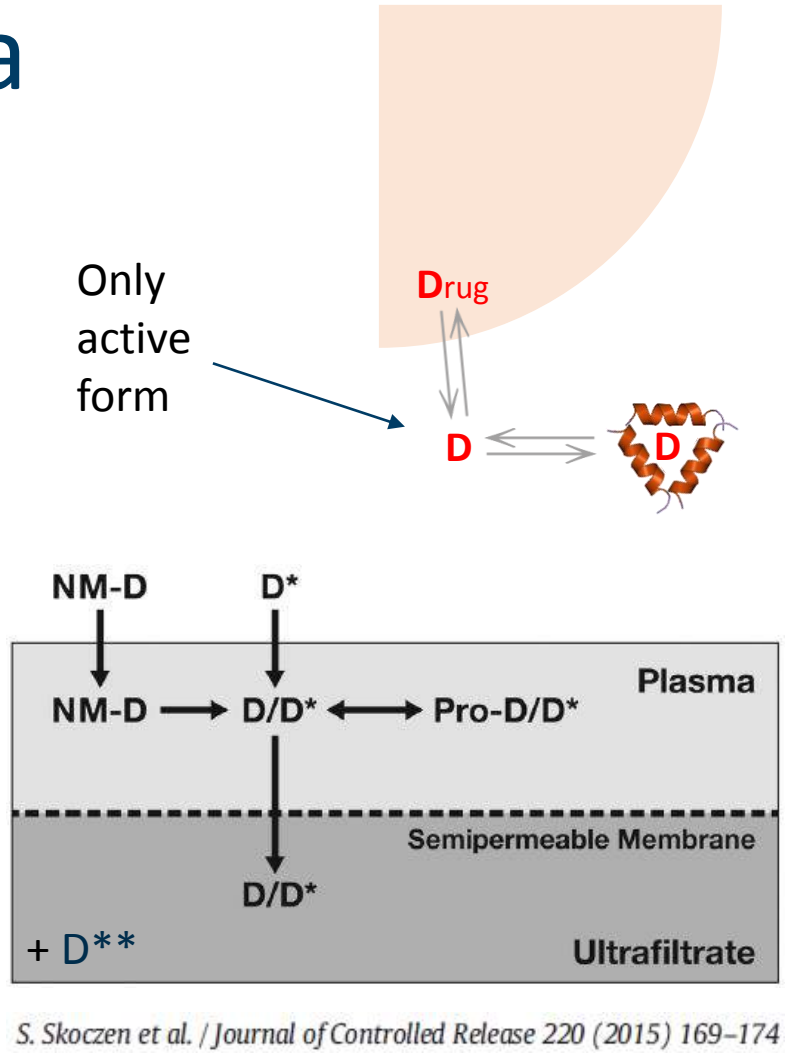
Drug release in complex media

- Multiple equilibria exist
- *Existing:* Dialysis, SEC/IEX/SPE, ultracentrifugation, Liq-liq extraction, ultrafiltration
- Process induced drug release a general concern
- Dual stable isotope method – the gold standard?
- Based on *(bio)chemical equivalence* of isotopes (^{2}H vs ^{1}H, ^{13}C vs ^{12}C)



S. Skoczen et al. / Journal of Controlled Release 220 (2015) 169–174

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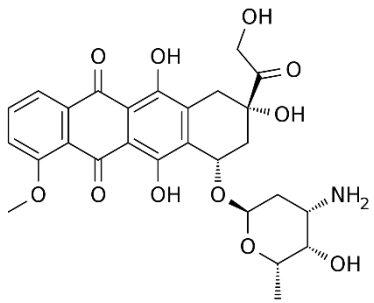
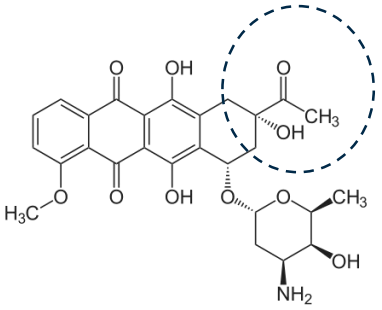
And suddenly…

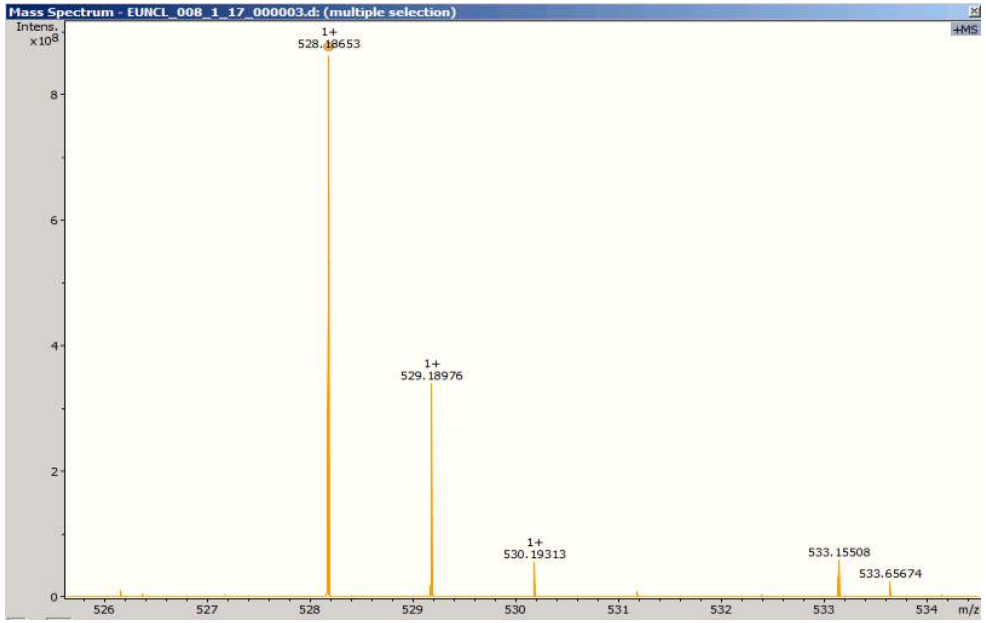


Doxorubicin



Daunorubicin

- EUNCL-008 – liposomal doxorubicin (target 2 mg/ml)
- Drug loading analyses by LC-MS/MS gave slight shift in retention cmp. to doxorubicin standard
- …and small shift in MS/MS fragmentation pattern (although parent ion is actually present)
- Turns out it contains wrong API!



Meas. m/z	#	Ion Formula	Score	m/z	err [mDa]	err [ppm]	e⁻ Conf	N-Rule	Adduct
528,186531	1	C27H30NO10	100	528,1864	-0,1	-0,2	even	ok	M+H

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