Understanding structure-activity relationships of polymeric nanoparticles in biological applications

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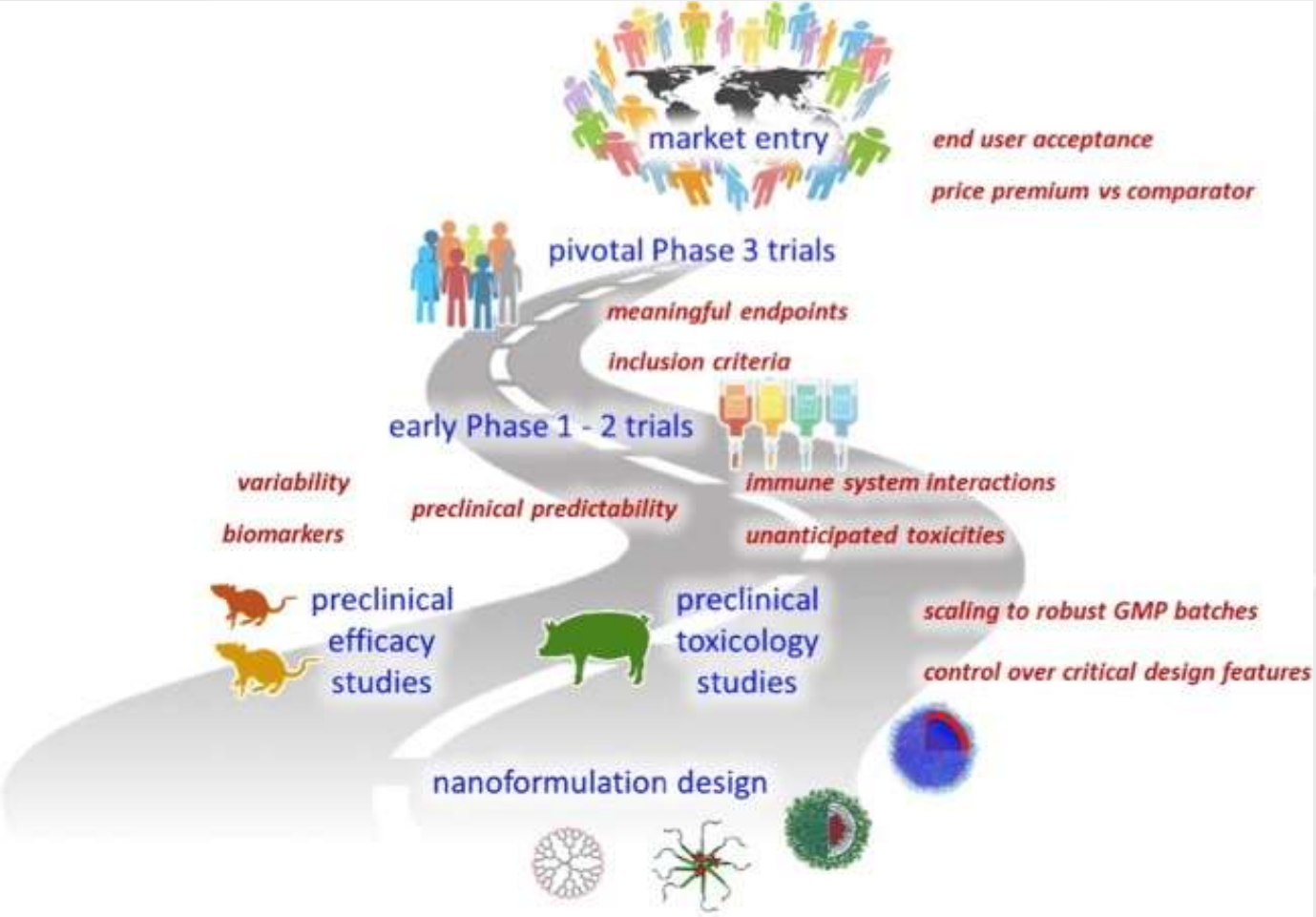
(Luff-bruh)

Polymer nanomedicines – why?

- Many small molecule drugs suffer poor solubility and rapid clearance after administration in the body
- Researchers have explored nanoparticles as drug carriers
  - Enhanced, site-specific drug delivery while minimising off-target toxicity
- Nanomedicines are a highly diverse group of drug products
  - Polymer-drug conjugates, polymer-protein conjugates, protein-based nanoparticles, polymeric micelles, inorganic nanoparticles, lipid-based etc
- Polymers are attractive here – they are highly tuneable, can target limitless chemistries, topologies etc
- **Aim: improve the stability & solubility of encapsulated cargos, promote transport across membranes and prolong circulation times to increase safety and efficacy**

Why is new research needed?

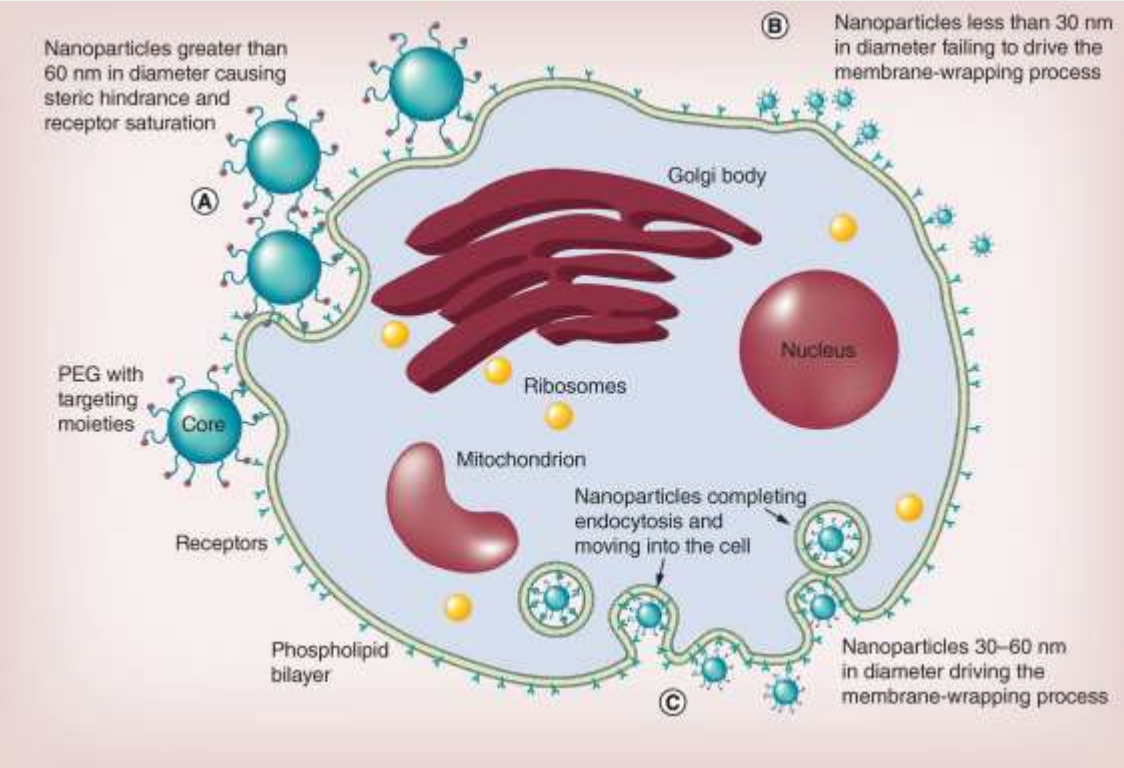
- We are by no means there yet!
- Thousands of different nanomedicine formulations have been designed and evaluated over the years
- Approximately fifty of these formulations are currently approved for clinical use – roughly equates to less than 10% success rate!
- Major issue of a translational gap between animal and human studies
- **Mainly, we don't fully understand the behaviour and functionality of nanomedicines in the body**

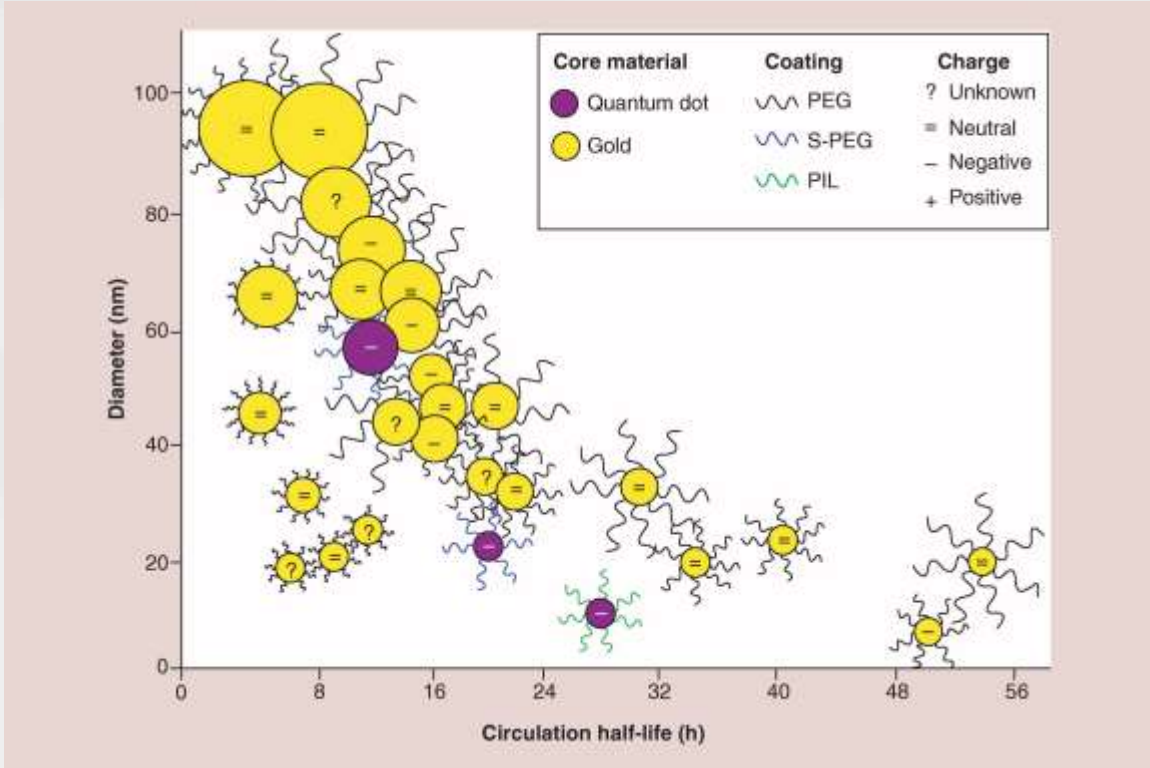


Drug Deliv Transl Res. 2020; 10(3): 721–725

Challenges in polymer nanomedicine: understanding key nanoparticle properties

In vivo biological systems are complicated, and many factors can impact nanoparticle behaviours

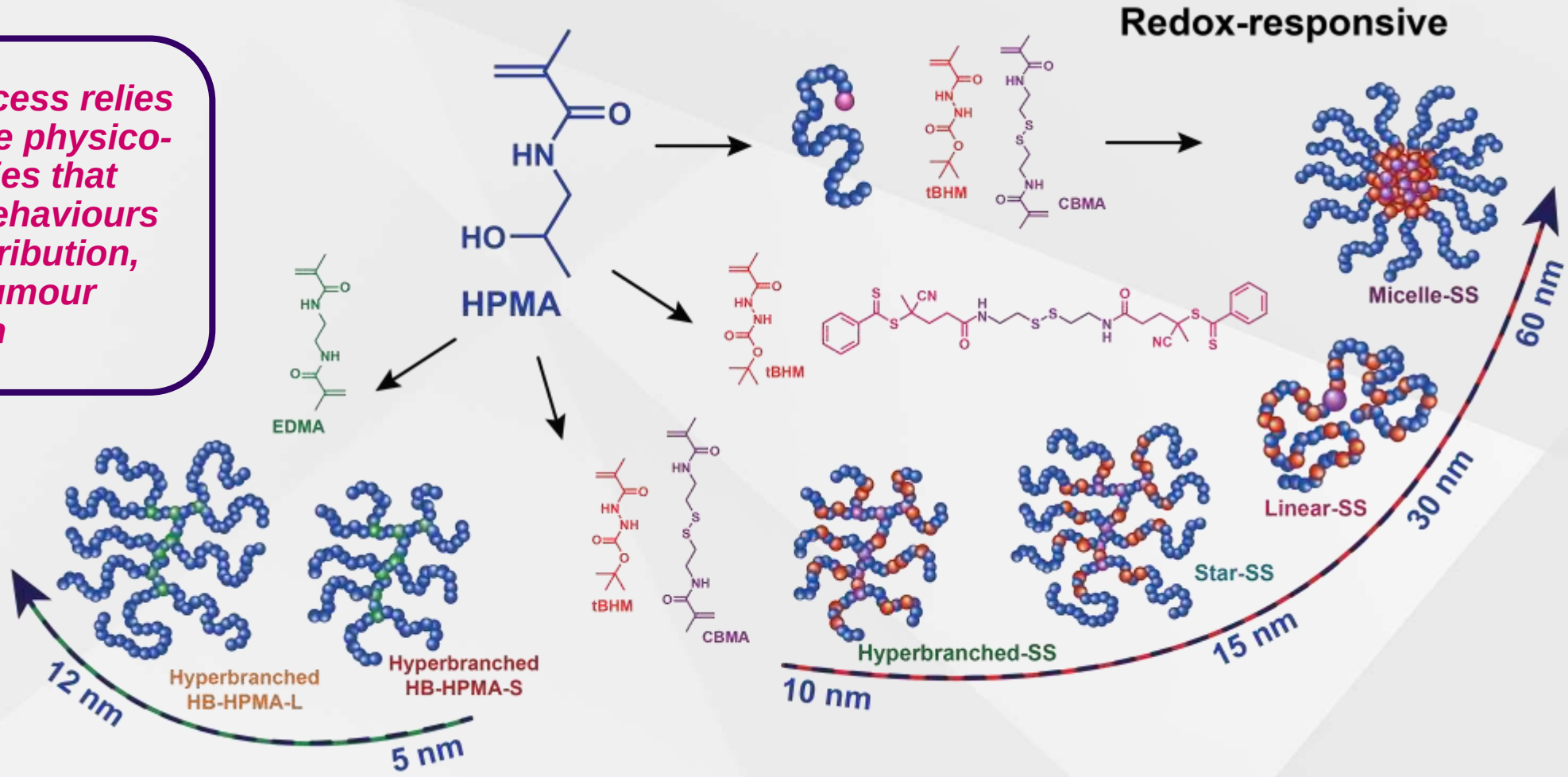




Study limitations: narrow scope of nanoparticle systems, lack of biodegradability

Design and synthesis of varying polymer architectures with the same underlying chemistries

Ultimate clinical success relies on understanding the physico-chemical properties that govern biological behaviours such as organ distribution, clearance and tumour penetration



EPSRC
Engineering and Physical Sciences Research Council

Biomaterials Discovery

Loughborough University

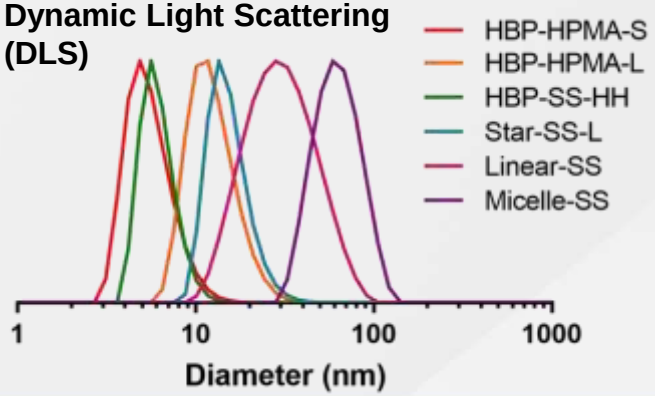
A. K. Pearce*, et al. *Bioconj. Chem.* 2019, *30* (9), 2300-2311.
A. K. Pearce*, et al., *Adv. Healthc. Mater*. 2020, 9 (22), 2000892.

#InspiringWinners since 1909

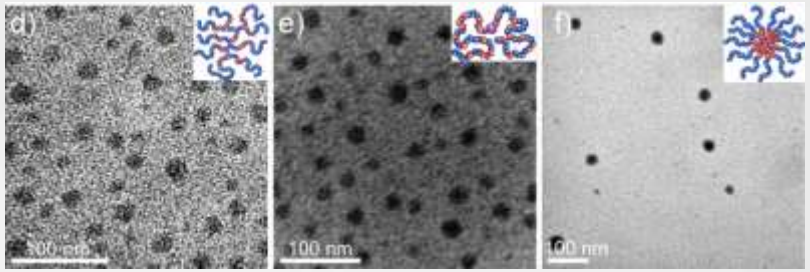
Polymers form nanoparticles with varying sizes and architectures from the same fundamental chemistries

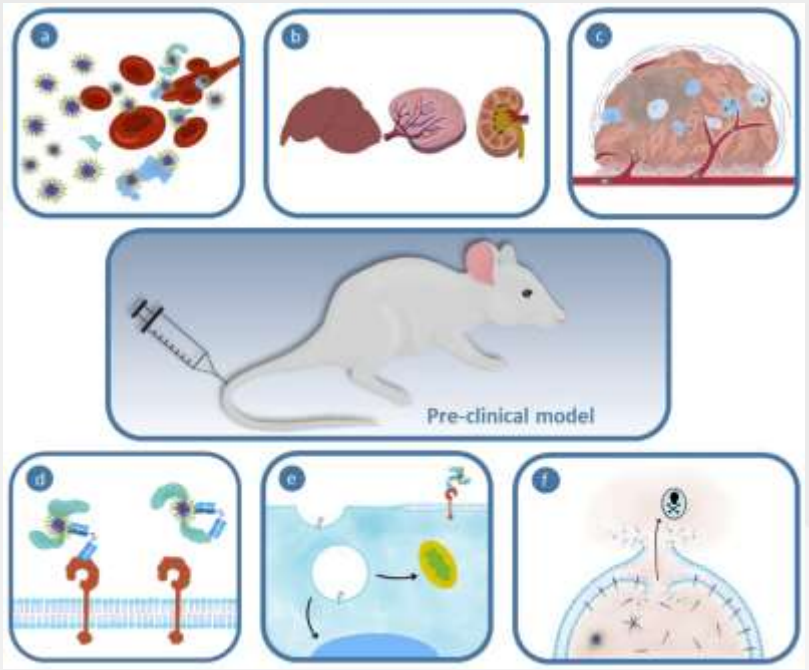
	Mn (SEC-MALLS)	SS per polymer (mol%)	D_h (DLS)	Size (TEM)	Zeta Potential (mV)
HBP-HPMA-S	15 kDa	0	5 nm	5 ± 1 nm	-21
HBP-HPMA-L	22 kDa	0	12 nm	11 ± 3 nm	-12
HBP-SS-LH	50 kDa	10	8 nm	-	-19
HBP-SS-HH	58 kDa	10	10 nm	10 ± 1 nm	-24
Star-SS-S	86 kDa	7	12 nm	-	-16
Star-SS-L	122 kDa	5	15 nm	13 ± 2 nm	-15
Linear-SS	257 kDa	1	30 nm	22 ± 2 nm	-5.5
Micelle-SS	3,000 kDa	5	60 nm	36 ± 3 nm	-21

Dynamic Light Scattering (DLS)

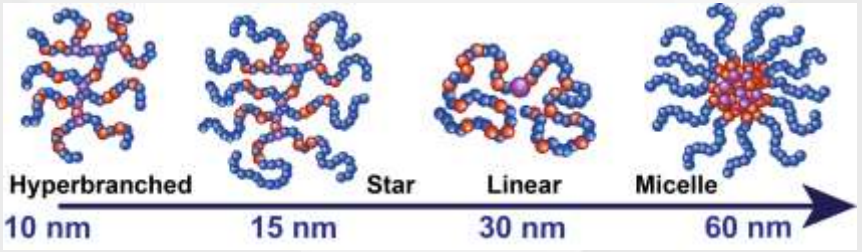


Transmission Electron Microscopy (TEM)



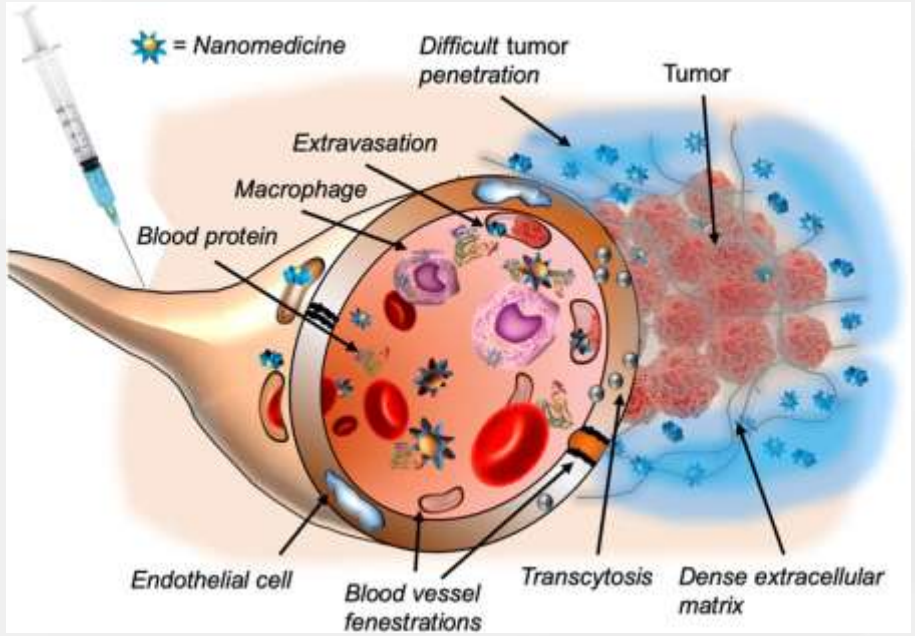


Polymers, **2019**



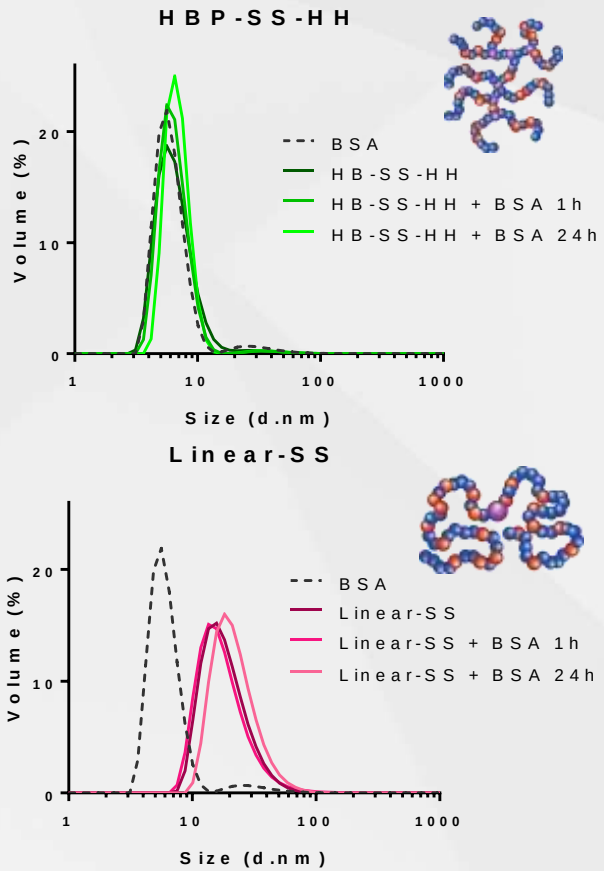
A. K. Pearce*, et al., *Adv. Healthc. Mater*. 2020, 9 (22), 2000892.

Polymer size and architecture control stability to protein attachment and macrophage association

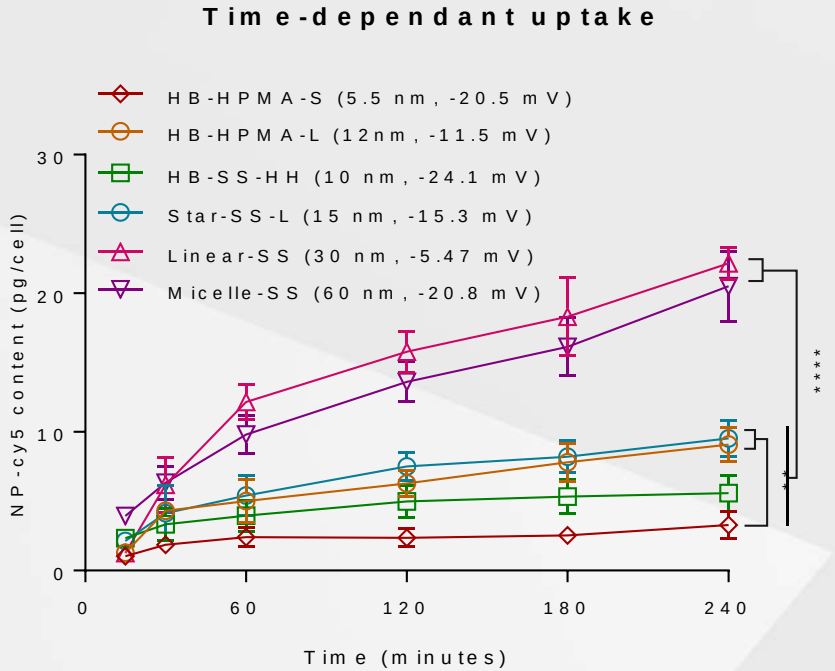


WIREs Nanomed Nanobiotechnol. 2021

Stability to protein binding (BSA)



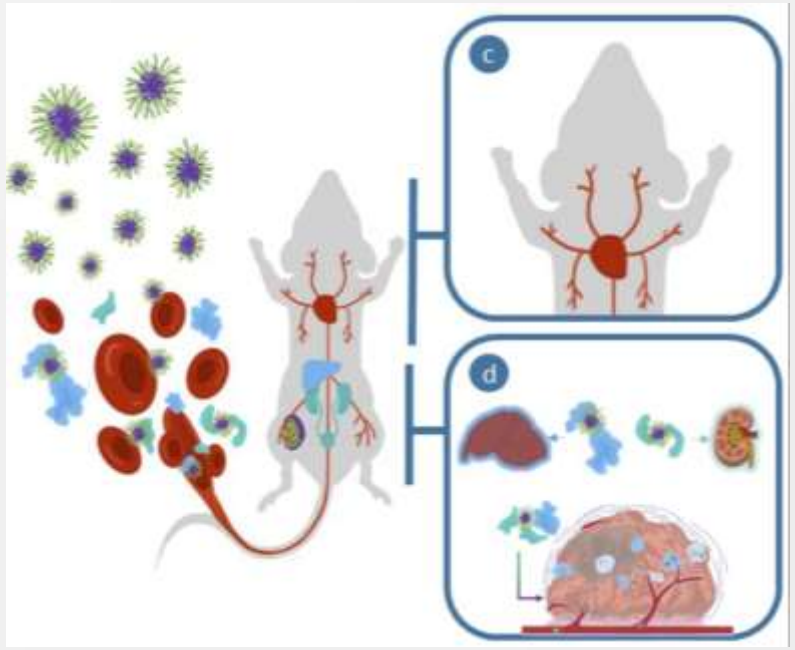
Uptake by macrophages (RAW264.7 cells)



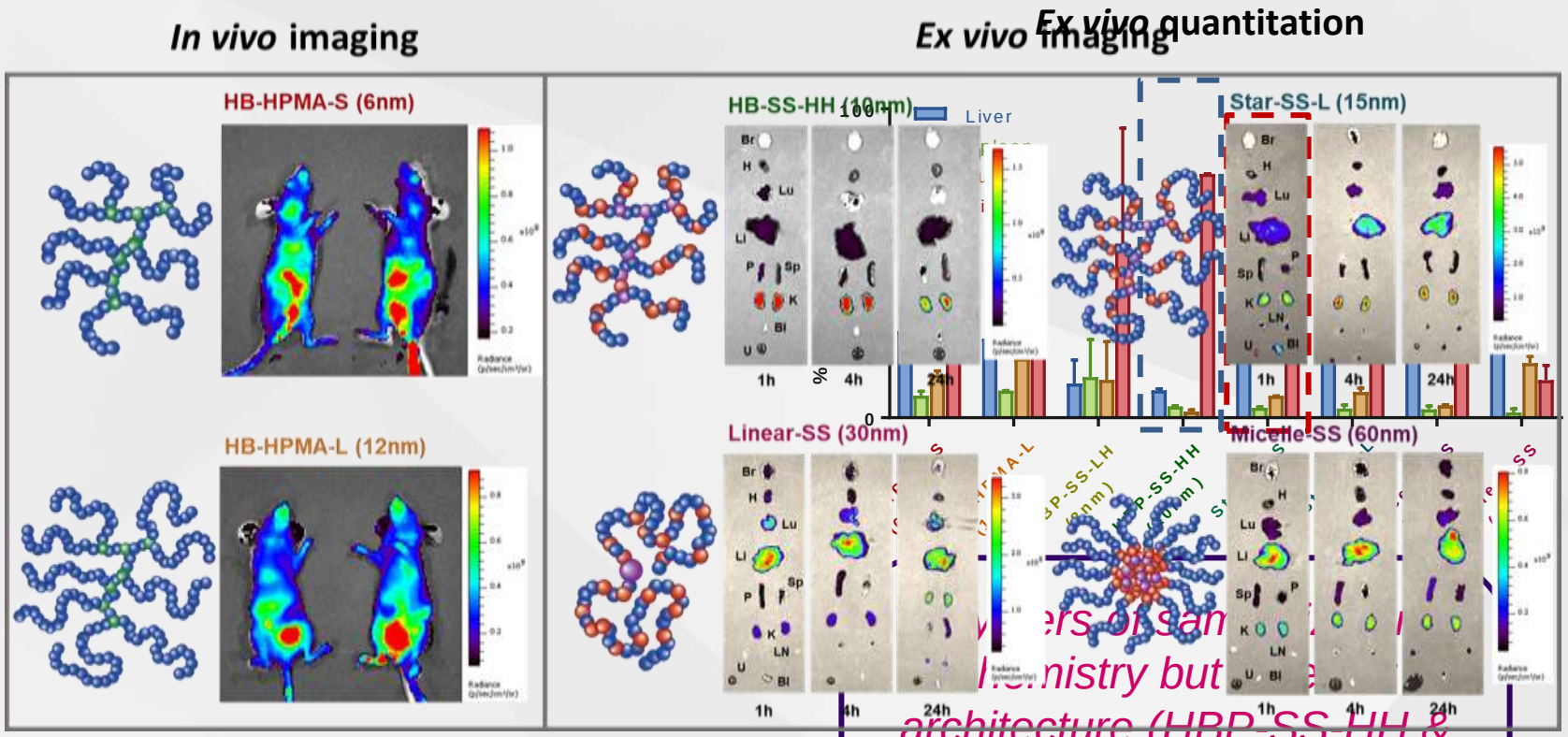
Less protein/macrophage association for small branched particles in comparison to self-assembled copolymers

Protein interactions

How does nanoparticle architecture influence end fate in healthy mice following systemic injection?



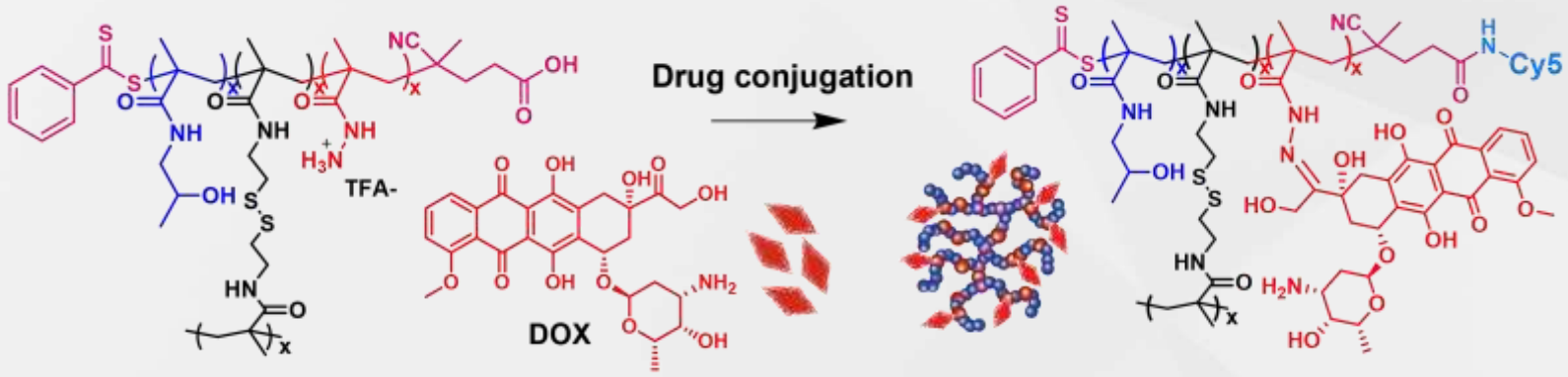
From systemic circulation, polymer properties and biological interactions lead to clearance from the system or accumulation within organs or tumour tissue. *Polymers*, **2019**

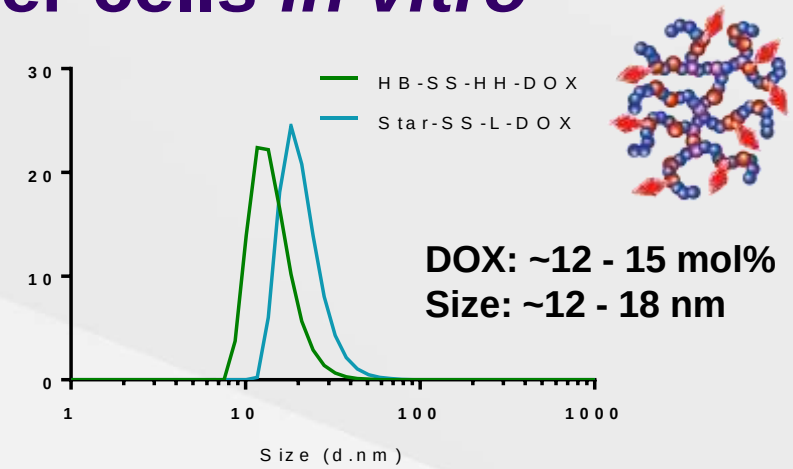


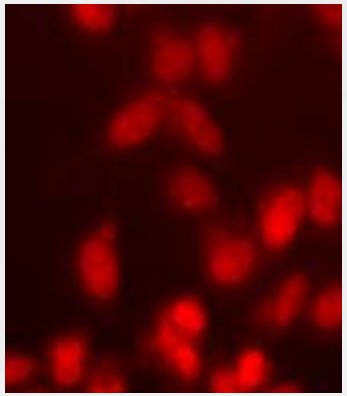
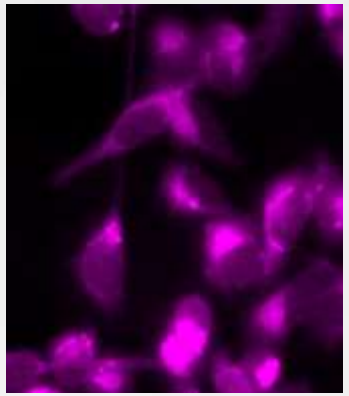
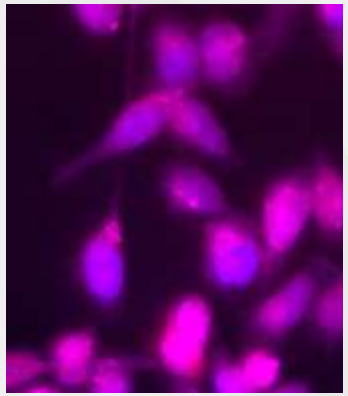
- **Architecture-dependence on clearance route (renal vs. MPS) in agreement with *in vitro* data**

...ers of sam... ...l chemistry but ... architecture (HBP-SS-HH & Star-SS-S) accumulate in different organs

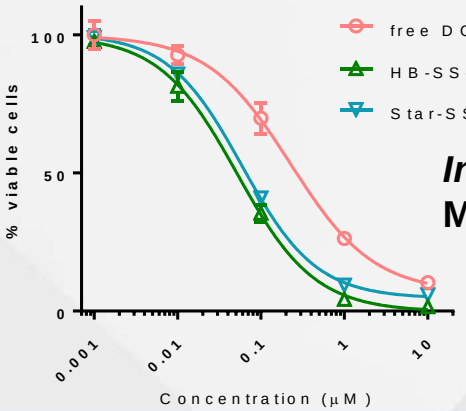
Bioreducible polymer-dox conjugate NPs are more effective than free drug to triple negative breast cancer cells *in vitro*





	Brightfield	Doxorubicin	Cy5	Merge
2h uptake				

	Free DOX	HB-SS-HH-DOX	Star-SS-L-DOX
IC_{50} (μM)	0.226	0.0516	0.0592

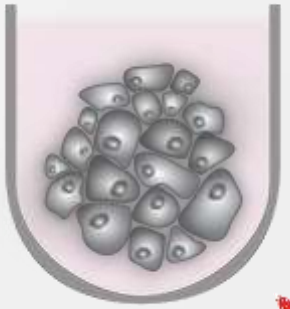


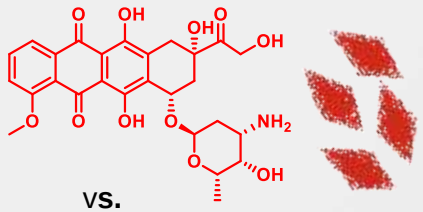
***In vitro* evaluation in MDA-MB-231 cells**

Reduction of IC_{50} values through drug conjugation

Bioreducible polymer-dox conjugate NPs are more effective than free drug to triple negative breast cancer cells *in vitro*

3D spheroids





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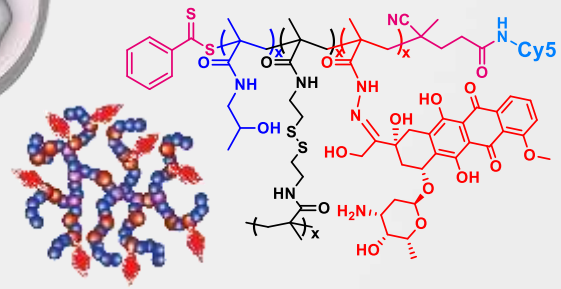
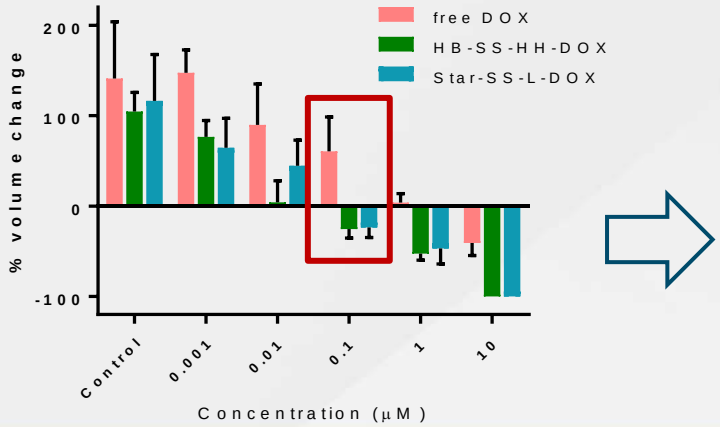
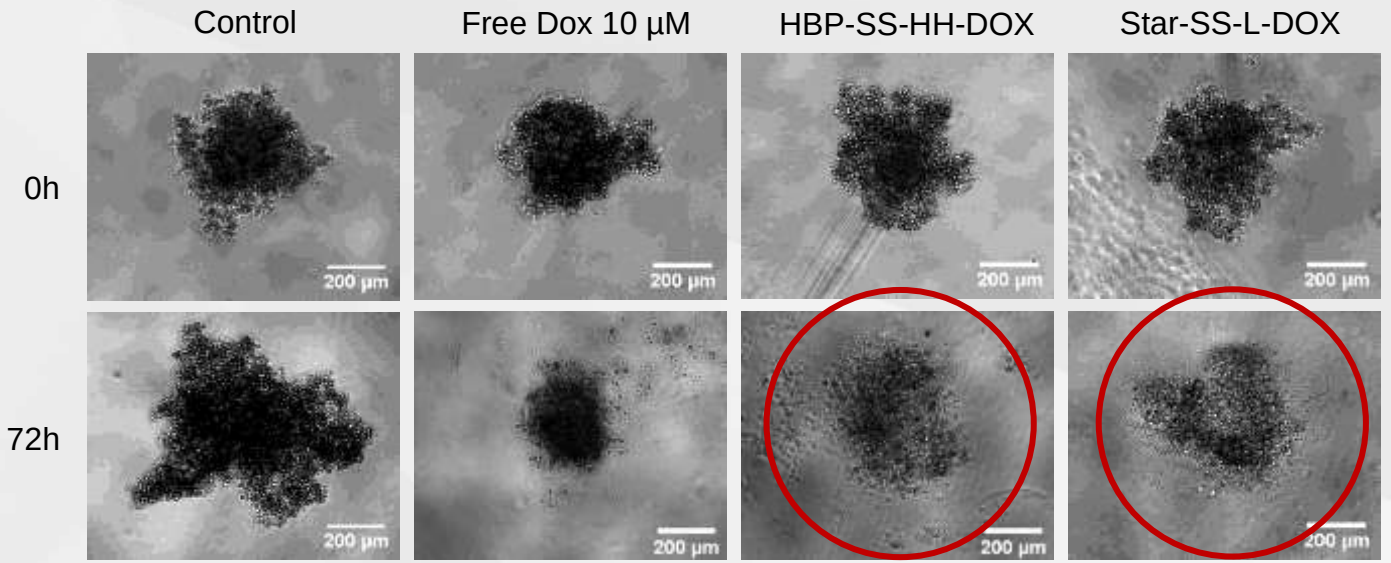
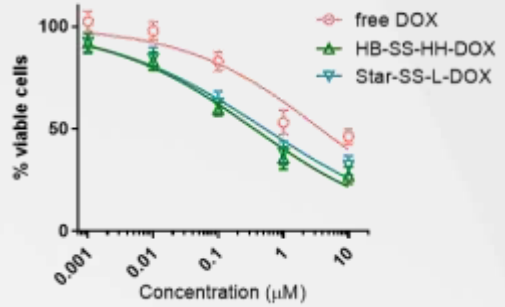




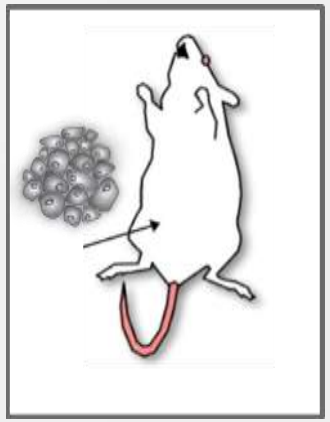


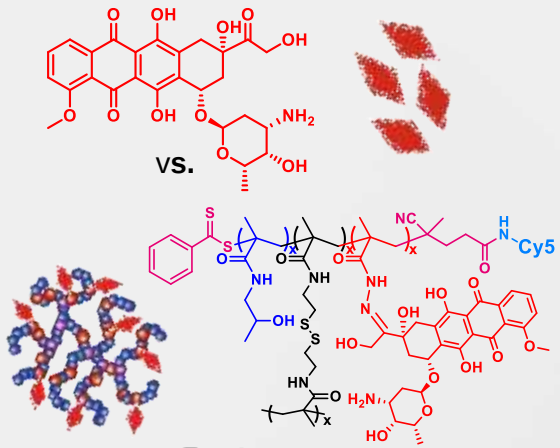
	Free DOX	HB-SS-HH-DOX	Star-SS-L-DOX
IC_{50} (μM)	3.69	0.356	0.524

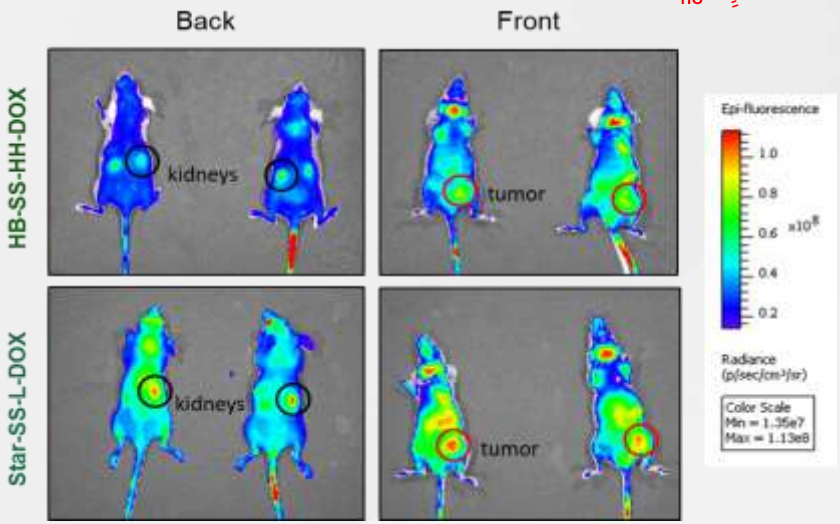


Destruction of spheroid structure for polymer-drug NPs – improved drug penetration through tissues?

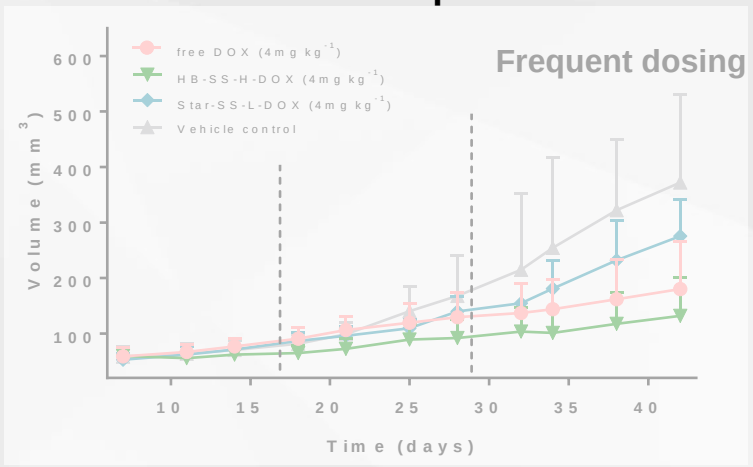
Dosing schedule plays an important role in polymer-drug conjugate efficacy in orthoptic *in vivo* mouse models

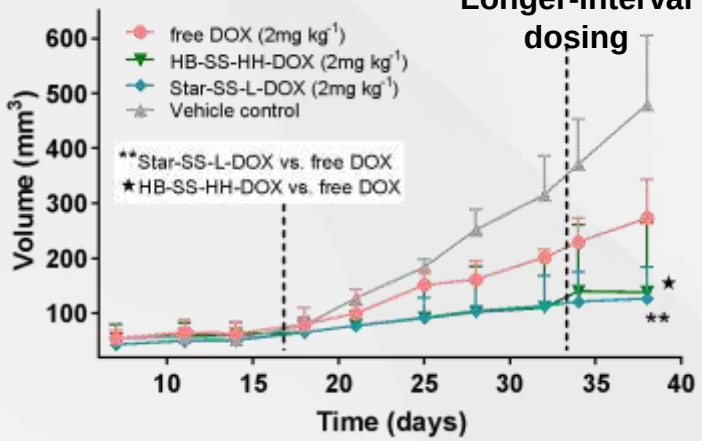


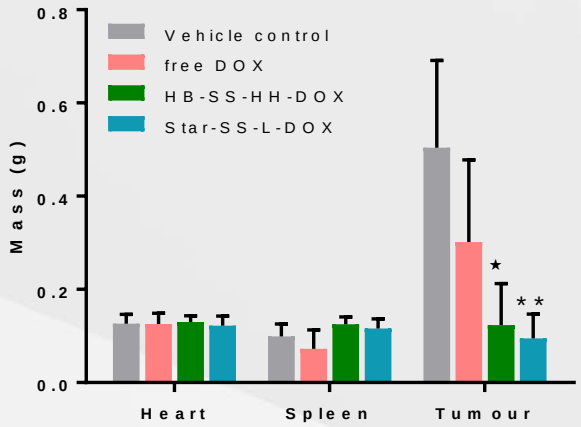




***In vivo* efficacy in MDA-MB-231-fLuc orthotopic model**







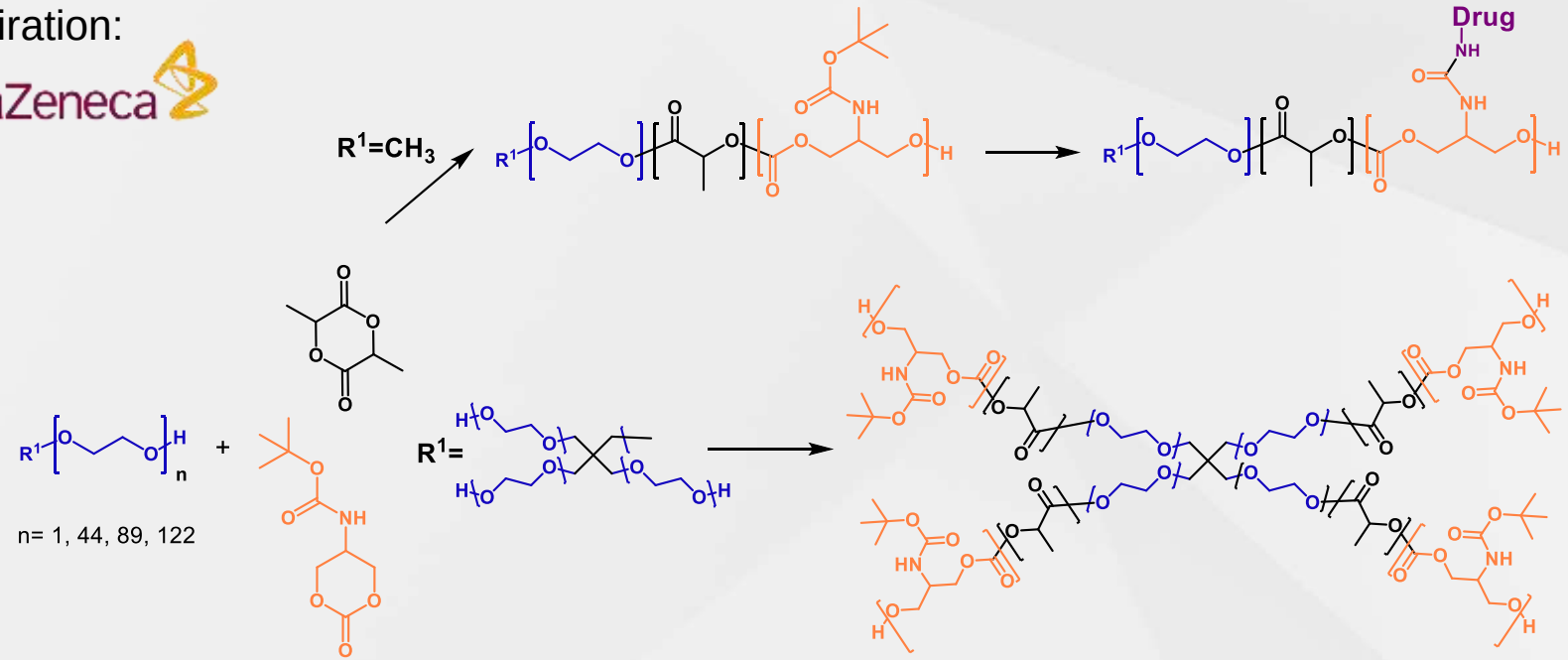
Over long dosing periods, polymer-drug conjugates have greater efficacy – prolonged circulation lifetimes? Complicated question to answer from high-level in vivo studies!

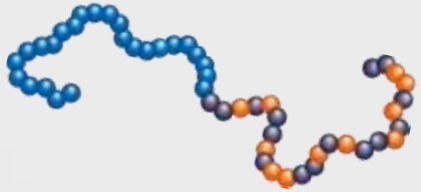
A. K. Pearce*, et al., *Adv. Healthc. Mater*. 2020, 9 (22), 2000892.

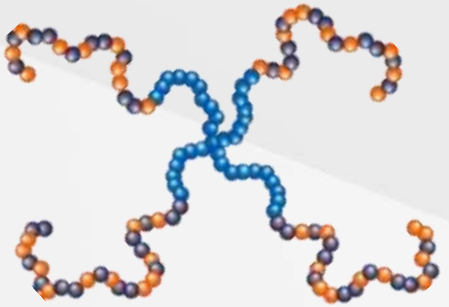
Moving these materials towards clinical relevance: biodegradability matters!

The inspiration:

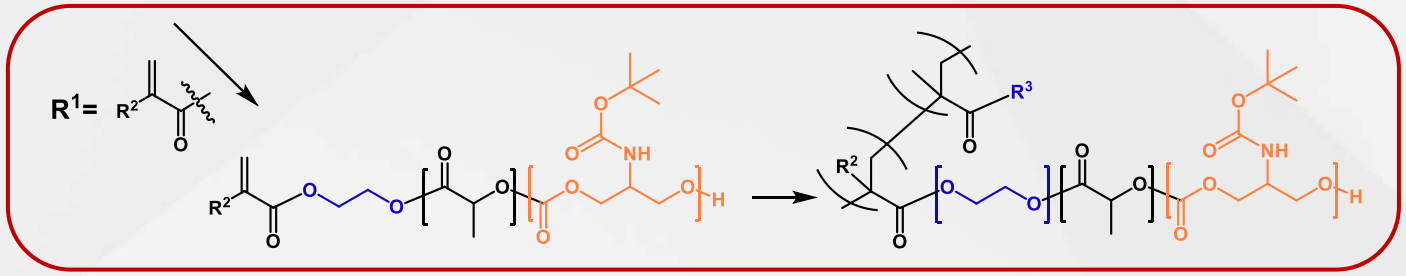
AstraZeneca

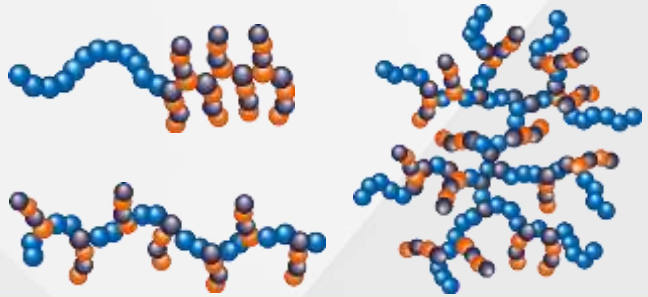




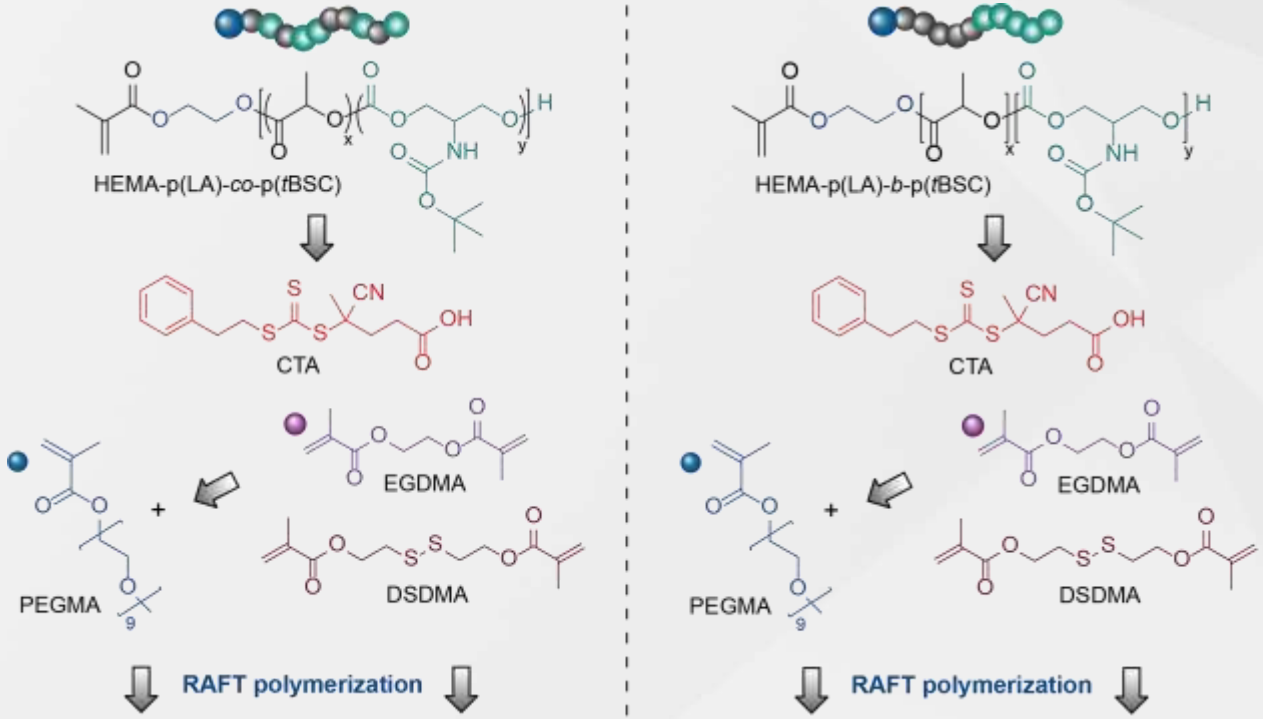


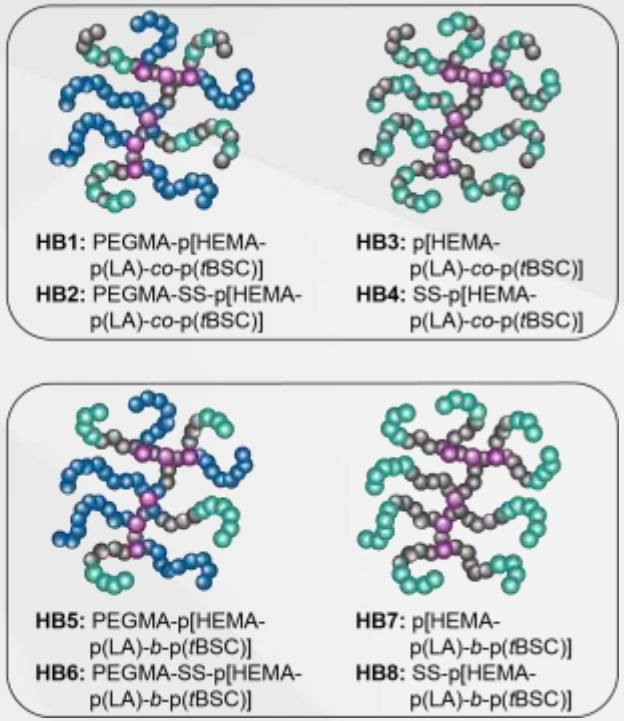
The idea:



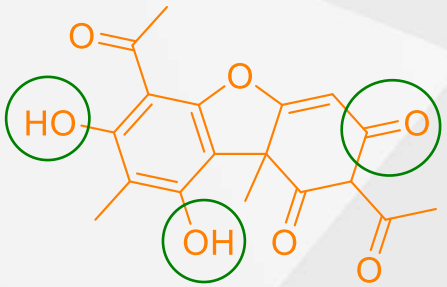


Block spacing of cationic monomers enhances drug loading and antimicrobial efficacy





Demonstrate a simple approach to drug-functionalised particles by exploiting electrostatic interactions with NH_3^+ groups

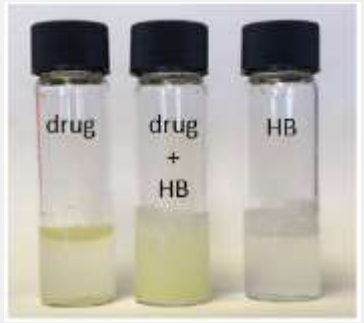


How does structure, chemistry and monomer arrangement affect polymer properties?

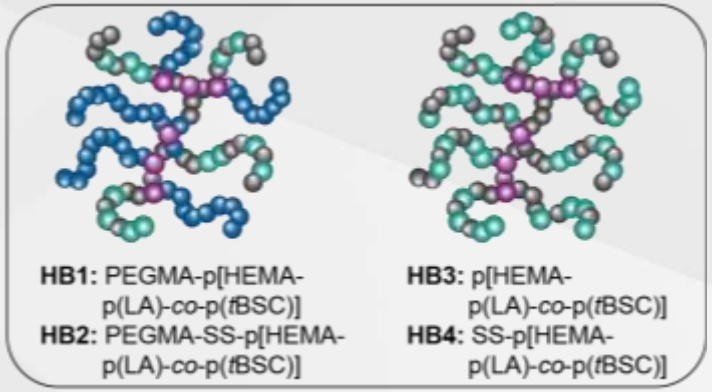
Copolymer vs homopolymer? Block vs random? Redox-responsive vs non-responsive?

M. Rauschenbach, S. B. Lawrenson, V. Taresco, A. K. Pearce*, R. K. O'Reilly*, *Macromol. Rapid Commun.* 2020, 2000190.

Block spacing of cationic monomers enhances drug loading and antimicrobial efficacy



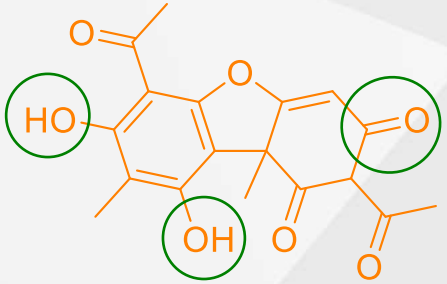
Block copolymers outperformed their random analogues regardless of amphiphilicity (PEGMA)



Drug loading and antimicrobial activity

Sample	Structure of macromonomer	Usnic acid complexation		MIC (μg/mL)	
		Drug content (wt%)	Encapsulation efficiency (%EE)	SA01	SA02
Usnic acid*	-	-		250	125
HB1-UA	Random	2	19	125	63
HB2-UA	Random	4	33	125	63
HB3-UA	Random	33	27	31	16
HB4-UA	Random	37	30	31	16
HB5-UA	Block	4	29	63	31
HB6-UA	Block	6	50	63	31
HB7-UA	Block	46	37	8	4
HB8-UA	Block	51	41	8	4

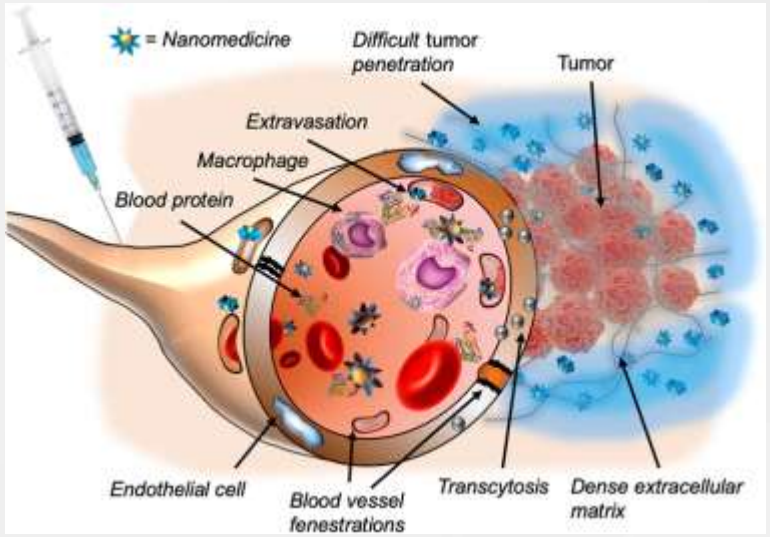
Demonstrate a simple approach to drug-functionalised particles by exploiting electrostatic interactions with NH_3^+ groups



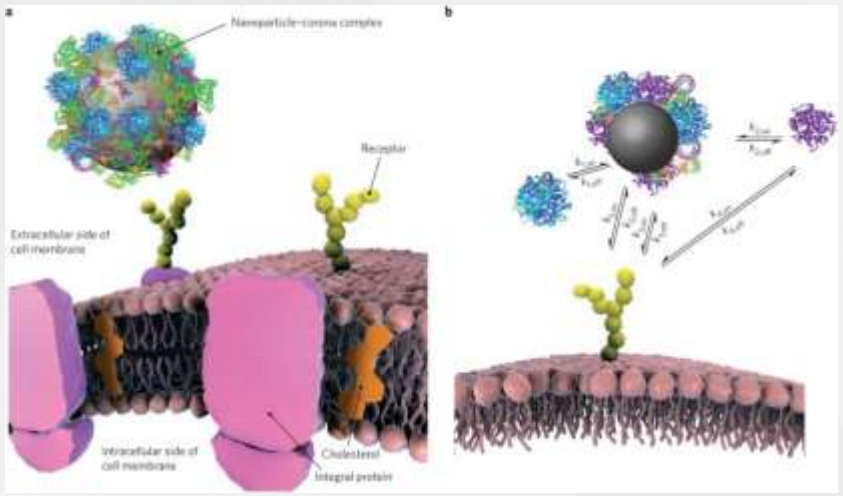
M. Rauschenbach, S. B. Lawrenson, V. Taresco, A. K. Pearce*, R. K. O'Reilly*, *Macromol. Rapid Commun.* 2020, 2000190.

Getting back to fundamentals – the protein corona

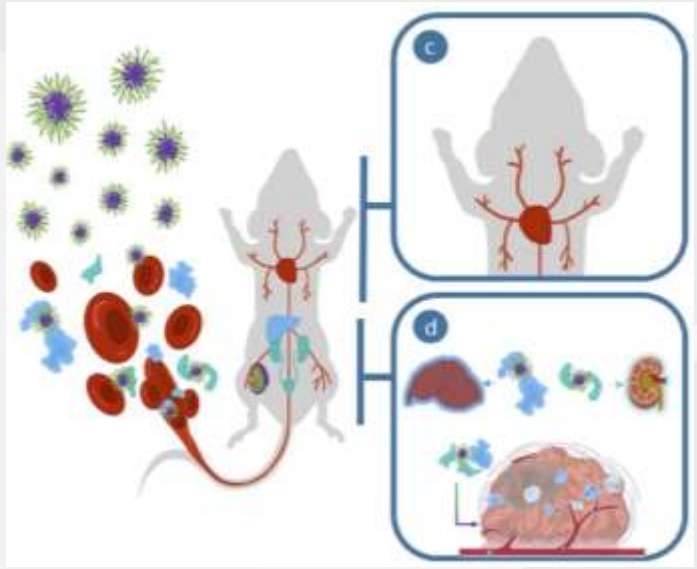
- We have a lot of synthetic tools to create nanoparticles with virtually any chemistry
- Exploit this to gain a more complete understanding of protein corona formation and downstream impacts on circulation, clearance, cell uptake, endosomal escape.



From systemic circulation, polymer properties direct biological interactions with serum proteins and macrophages. WIREs Nanomed Nanobiotechnol. 2021



Biomolecular coronas provide the biological identity of nanosized materials, Nat. Nanotechnol. 2012

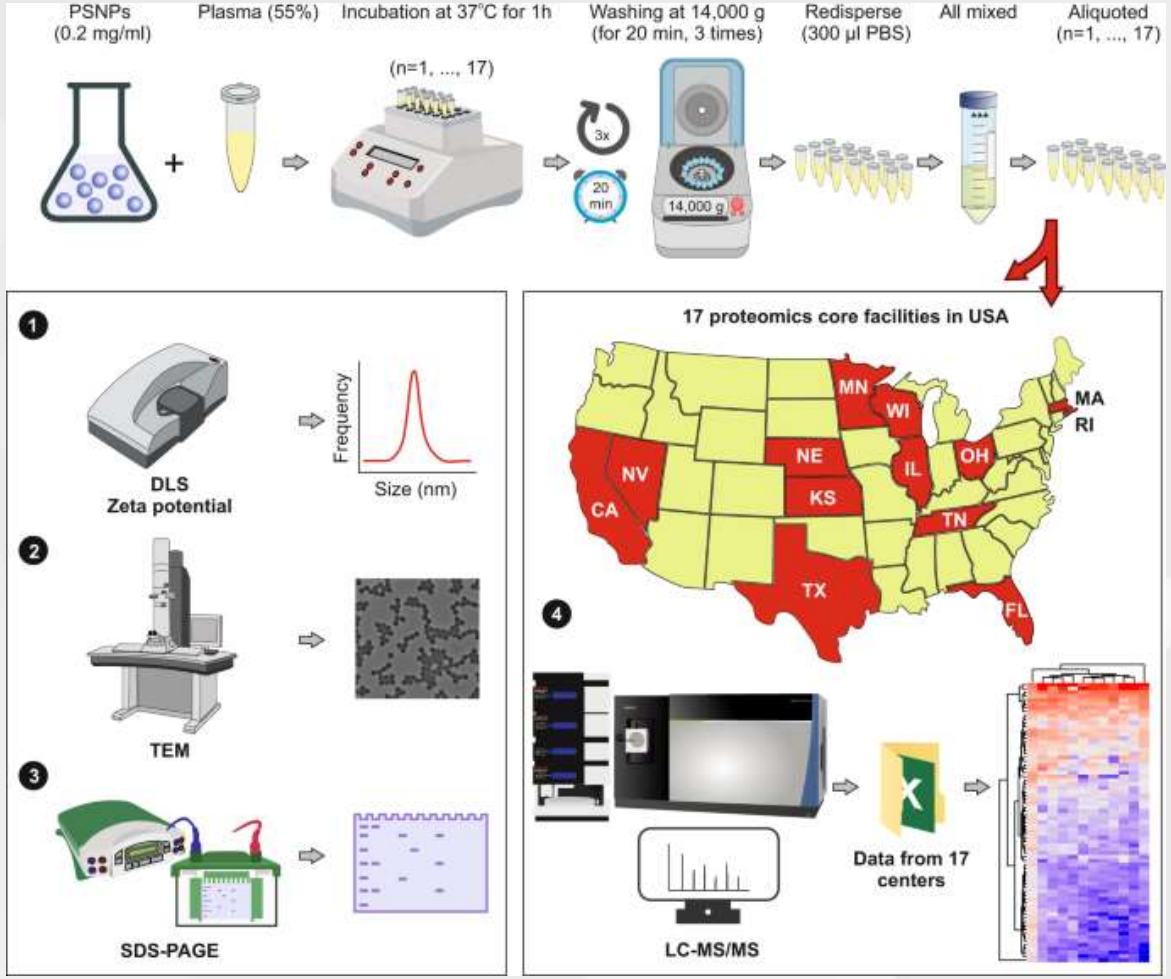


Polymer properties and biological interactions lead to clearance from the system or accumulation within organs or tumour tissue. Polymers, ***2019***

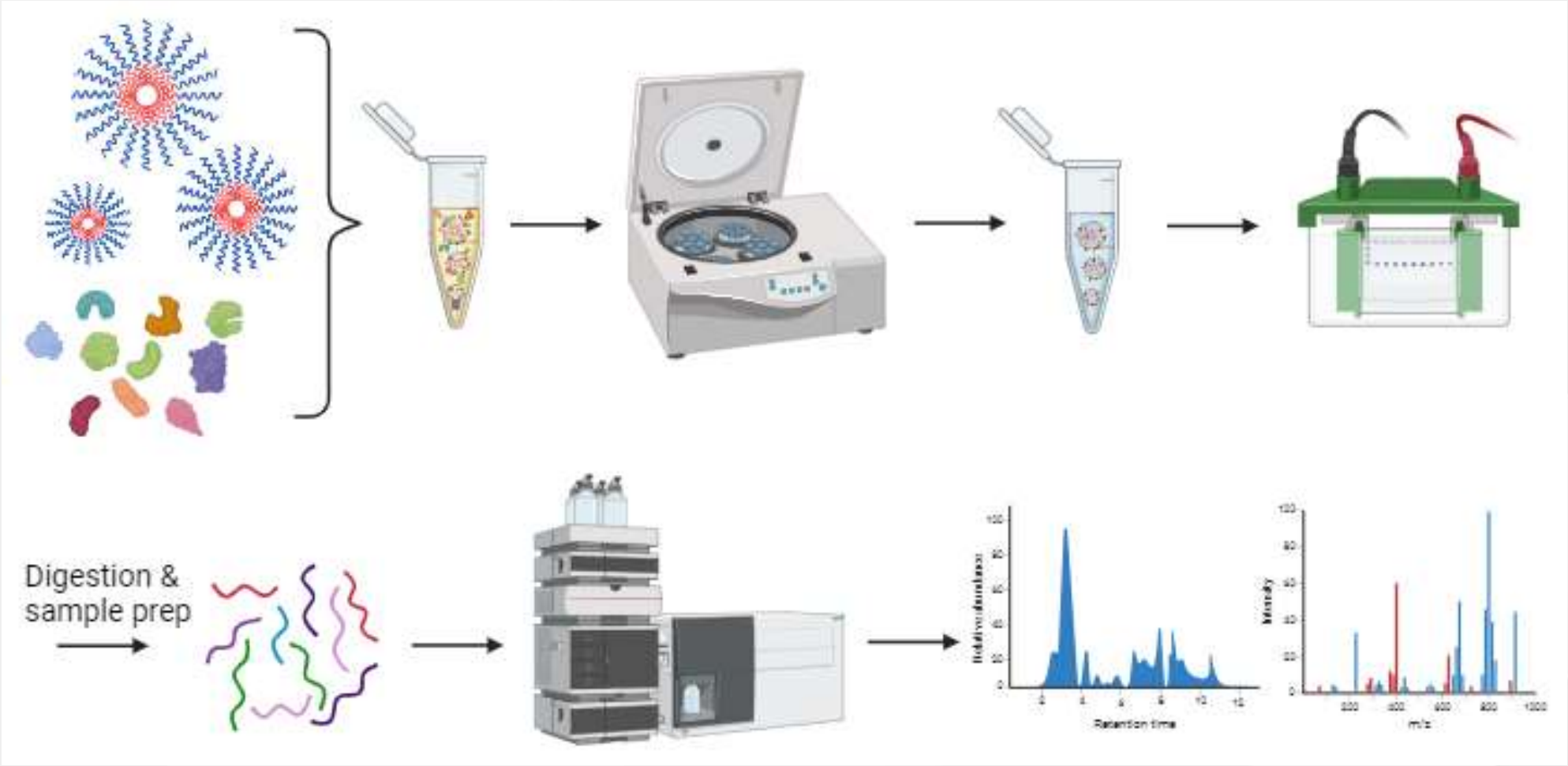
Challenges in studying the protein corona

- **Not only do we need to analyse the PC, but we need to ensure methods are reproducible, reliable, and accessible in a wide range of chemistry/pharmacy research labs**
- Need to standardise everything from the PC experiments through to database searching and analysis

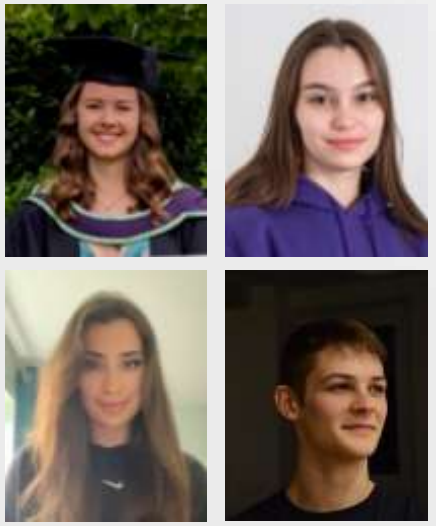
Significant data variability, with only 1.8% of proteins consistently identified across these centres!



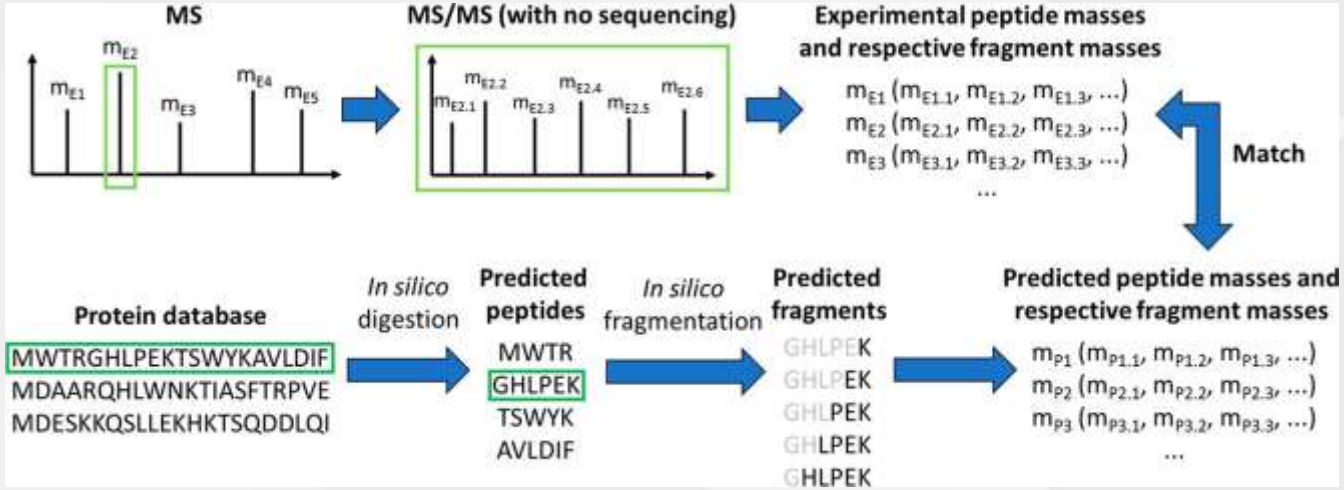
Peptide LC-MS for studying the protein corona



An automated solution



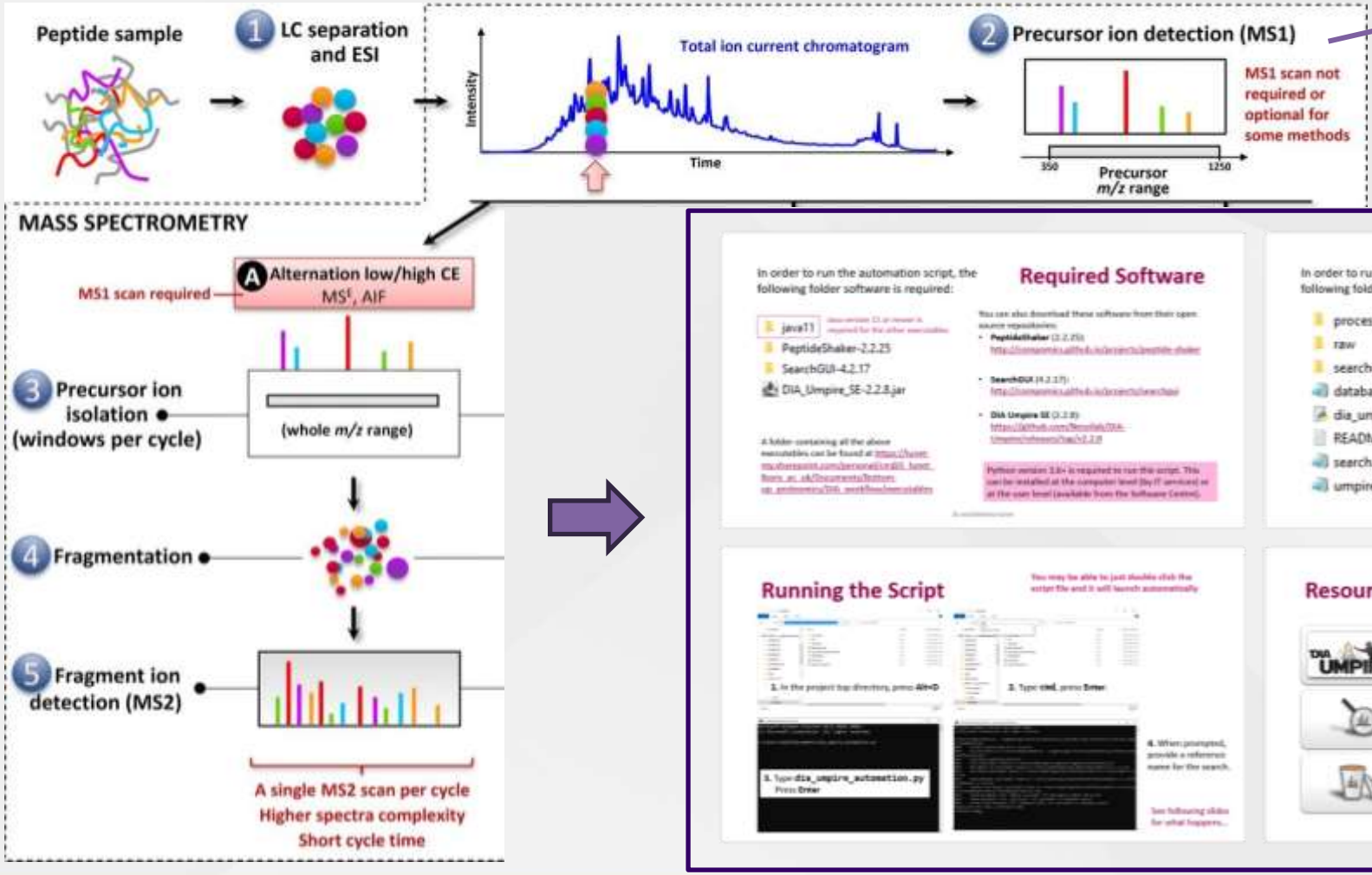
- Pseudo LC-MS/MS with Data Independent Acquisition (DIA)
- Data Dependent Acquisition (DDA)
  - Isolate a specific precursor ion and fragment, fragments directly linked to peptide precursor
  - Requires at least two MS steps (normally two different mass analysers)
- Data Independent Acquisition
  - Window with many precursors
  - Fragments from multiple precursors need to be demultiplexed
  - Can be done with one MS step
  - Alternate low/high collision energy



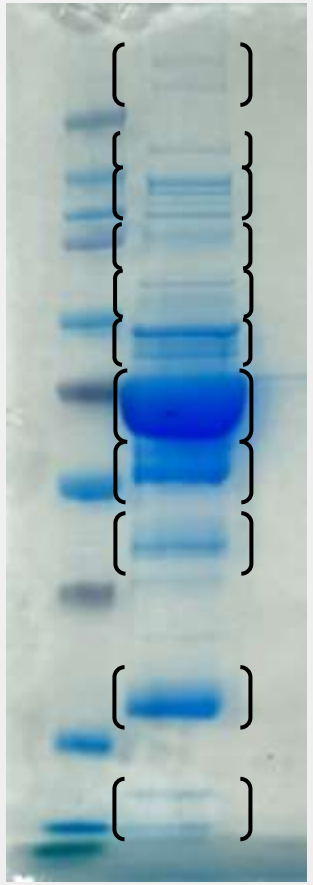
An automated solution

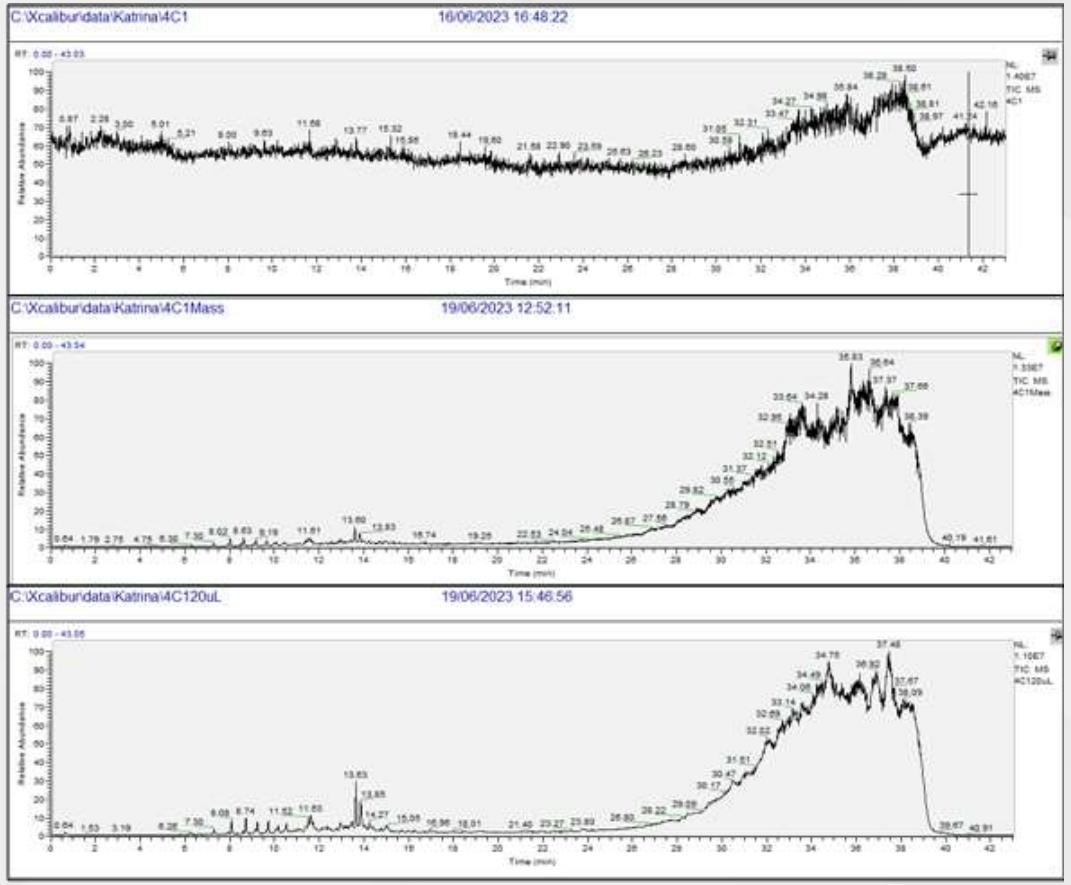
Restek C18 column

Thermo-Exactive Orbitrap Mass Spectrometer with an ESI source



Method development using human serum



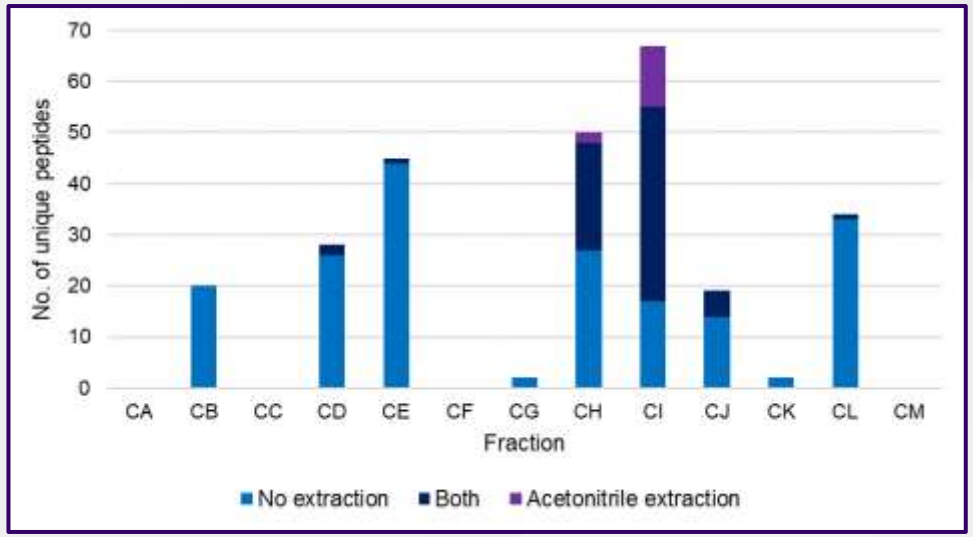


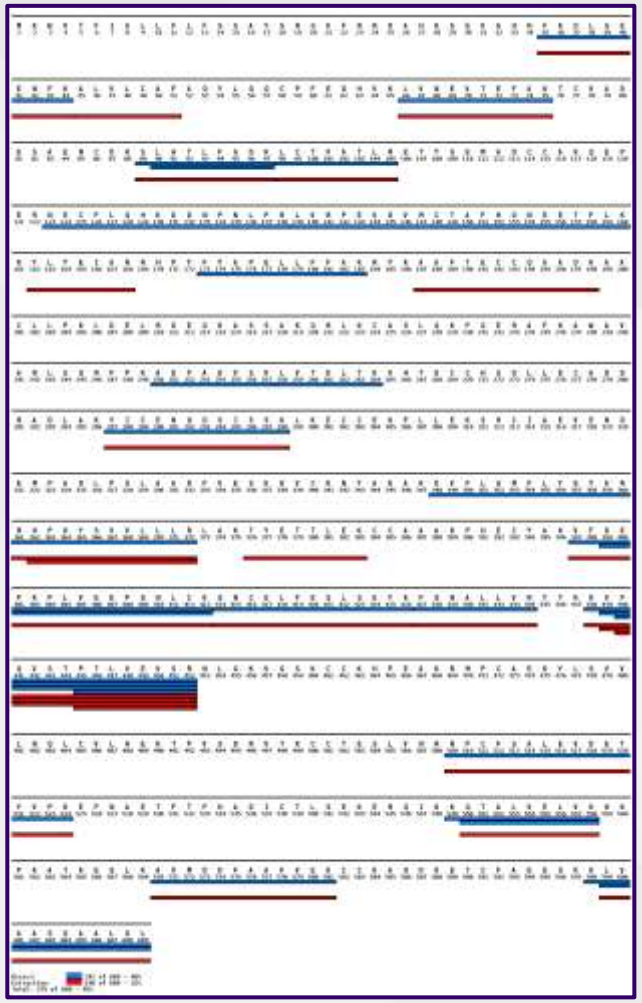
Fraction	UniProt code	Protein name	No extraction	Acetonitrile extraction
			Unique peptide count	
CA				
CB	P01023	Alpha-2-macroglobulin (A2MG_HUMAN)	16	
	P00738	Haptoglobin (HPT_HUMAN)	1*	
CC				
CD	P01857	Immunoglobulin heavy constant gamma 1 (IGHG1_HUMAN)	9	2
	A0M8Q6	Immunoglobulin lambda constant 7 (IGLC7_HUMAN)	2	
	B9A064	Immunoglobulin lambda-like polypeptide 5 (IGLL5_HUMAN)	1*	
	A0M8Q6;B9A064	Immunoglobulin lambda constant 7 (IGLC7_HUMAN);Immunoglobulin lambda-like polypeptide 5 (IGLL5_HUMAN)	1*	
	P01834	Immunoglobulin kappa constant (IGKC_HUMAN)	3	
	P01859	Immunoglobulin heavy constant gamma 2 (IGHG2_HUMAN)	2	
CE	P01857	Immunoglobulin heavy constant gamma 1 (IGHG1_HUMAN)	4	1*
	P00450	Ceruloplasmin (CERU_HUMAN)	1*	
	P01024	Complement C3 (CO3_HUMAN)	14	
	P01834	Immunoglobulin kappa constant (IGKC_HUMAN)	2	
	P01876	Immunoglobulin heavy constant alpha 1 (IGHA1_HUMAN)	4	
	P02768	Albumin (ALBU_HUMAN)	5	
	P0C0L4	Complement C4-A (CO4A_HUMAN)	3	
	P01859	Immunoglobulin heavy constant gamma 2 (IGHG2_HUMAN)	2	
CF				
CG	P02768	Albumin (ALBU_HUMAN)	2	
CH	P02787	Serotransferrin (TRFE_HUMAN)	26	15
	P01011	Alpha-1-antichymotrypsin (AACT_HUMAN)	1*	
	P04217	Alpha-1B-glycoprotein (A1BG_HUMAN)	1*	
CI	P01009	Alpha-1-antitrypsin (A1AT_HUMAN)	5	4
	P02768	Albumin (ALBU_HUMAN)	27	22
	P02790	Hemopexin (HEMO_HUMAN)	1*	1*
	Q00610	Clathrin heavy chain 1 (CLH1_HUMAN)		1*
	Q96IU4	Putative protein-lysine deacylase ABHD14B (ABHEB_HUMAN)		1*
	P01011	Alpha-1-antichymotrypsin (AACT_HUMAN)	1*	
CJ	P02768	Albumin (ALBU_HUMAN)	12	4
	P01024	Complement C3 (CO3_HUMAN)	1*	
CK	P02768	Albumin (ALBU_HUMAN)	2	
CL	P02647	Apolipoprotein A-I (APOA1_HUMAN)	16	1*
	P02768	Albumin (ALBU_HUMAN)	5	
CM				

Method development using human serum

Numbers of unique peptides in each analysed sample

Some samples not yielding peptides – sensitivity issue?





Additional extraction steps improve peptide recovery and aid in mapping more of the protein sequence → higher accuracy for database searching!

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