



UNIVERSITY OF  
LIVERPOOL



# Novel Approaches to Theranostic Nanomedicine Design

Dr Marco Giardiello

Nano2Clinic, Zagreb, Croatia

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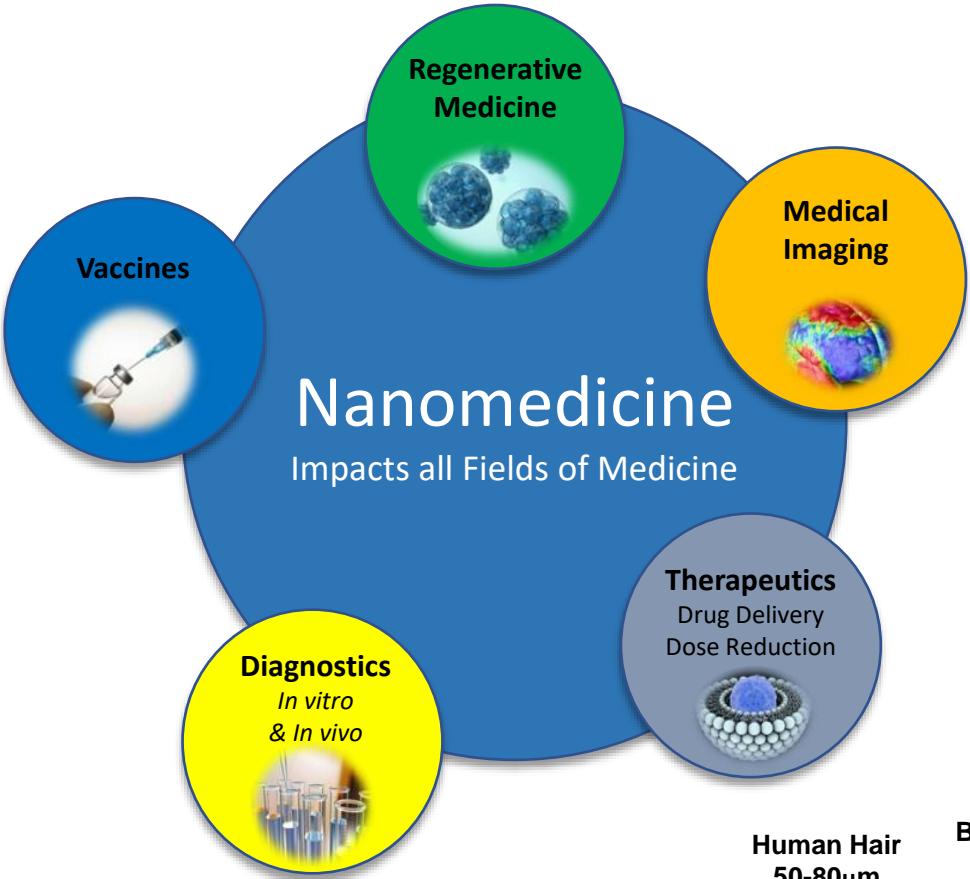


UK Research  
and Innovation

# Introduction

- Brief overview of Nanomedicine and Theranostics
- Strategies to Theranostic Nanomedicine Design:
  - Inorganic/Organic Nanocomposite Particles (I/O-NP)
    - Solid Drug Nanoparticle (SDN)
    - Polymer “Cargo” Encapsulation

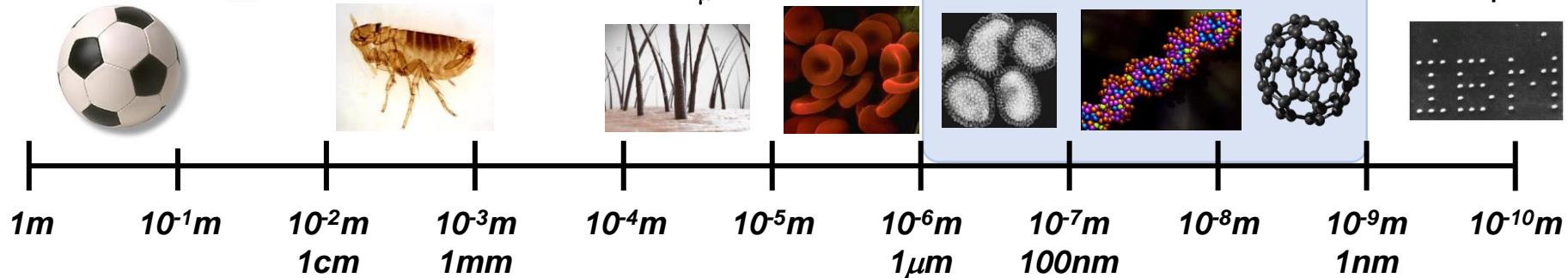
# Nanomedicine



*“The Application of Nanotechnology to Medicine”*

1 nm = 1 billionth of a metre

## Nano Range 1 nm – 1 μm

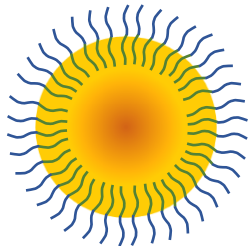


# Nanotechnology in Medicine

All advances in nanomedicine are borne out of material development on the nanoscale

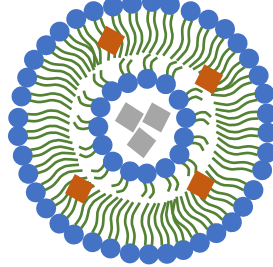
Lipid Nanocarriers

Solid Lipid Nanoparticles



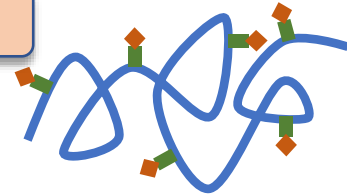
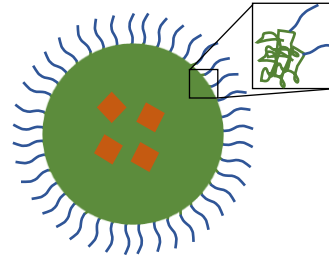
Nanoemulsion

Liposome

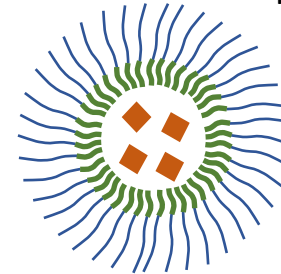


Polymer based

Polymer Nanoparticles



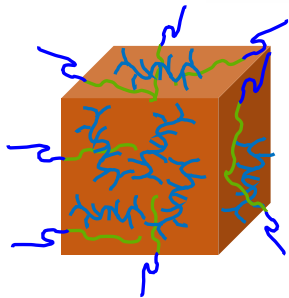
Polymer-Drug Conjugate



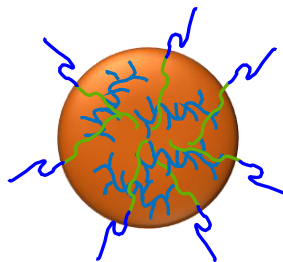
Polymer Micelle

Nanomedicine

Solid Drug Nanoparticles



Nanocrystal

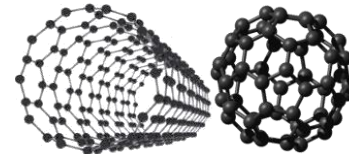


Nanosuspension

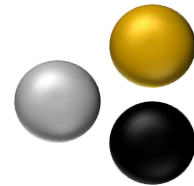
Inorganic Nanoparticles



Silica



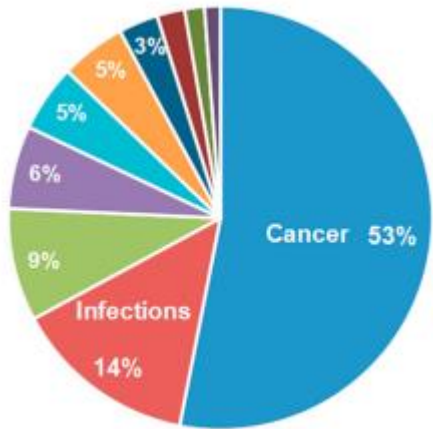
Nanocages/tubes



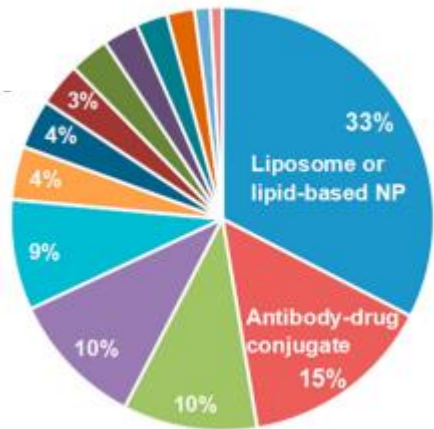
Metallic

# Current state of Nanomedicine

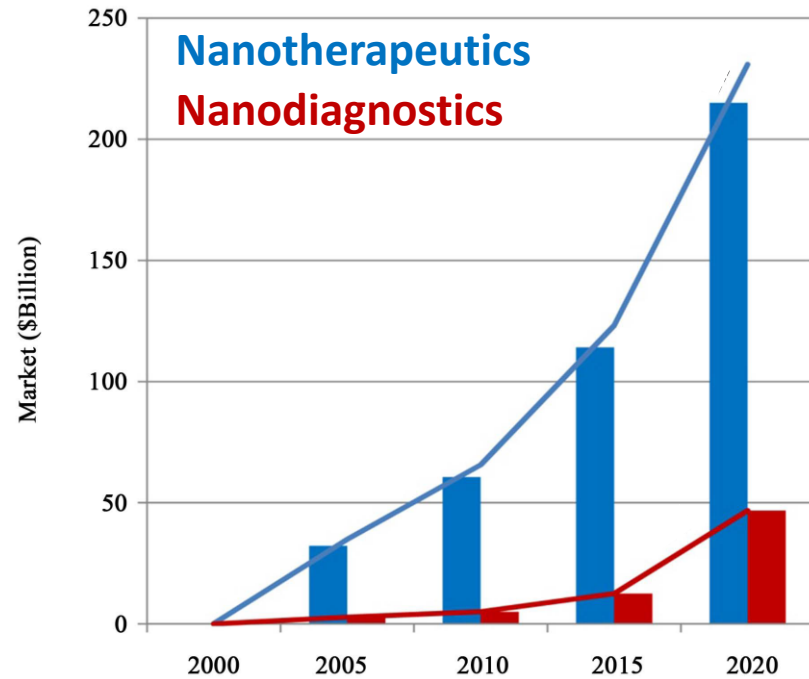
Around 100 nanomedicines on the market and around 550 in clinical process or other stages



- Cancer
- Infections
- Blood disorders, endocrine and metabolic diseases
- Other indications
- Nervous system and mental health diseases
- Immunological disease and inflammation
- Cardiovascular disease
- Cancer treatment-related indications
- Ocular diseases
- Skin diseases



- Liposome or lipid based
- Antibody-drug conjugate
- Polymer/protein drug conjugate
- Polymer
- Viral vector
- Cell-derived vehicle
- Others
- Inorganic NP
- Emulsion
- NP
- Protein based
- Micelle
- Nanocrystal
- Dendrimer



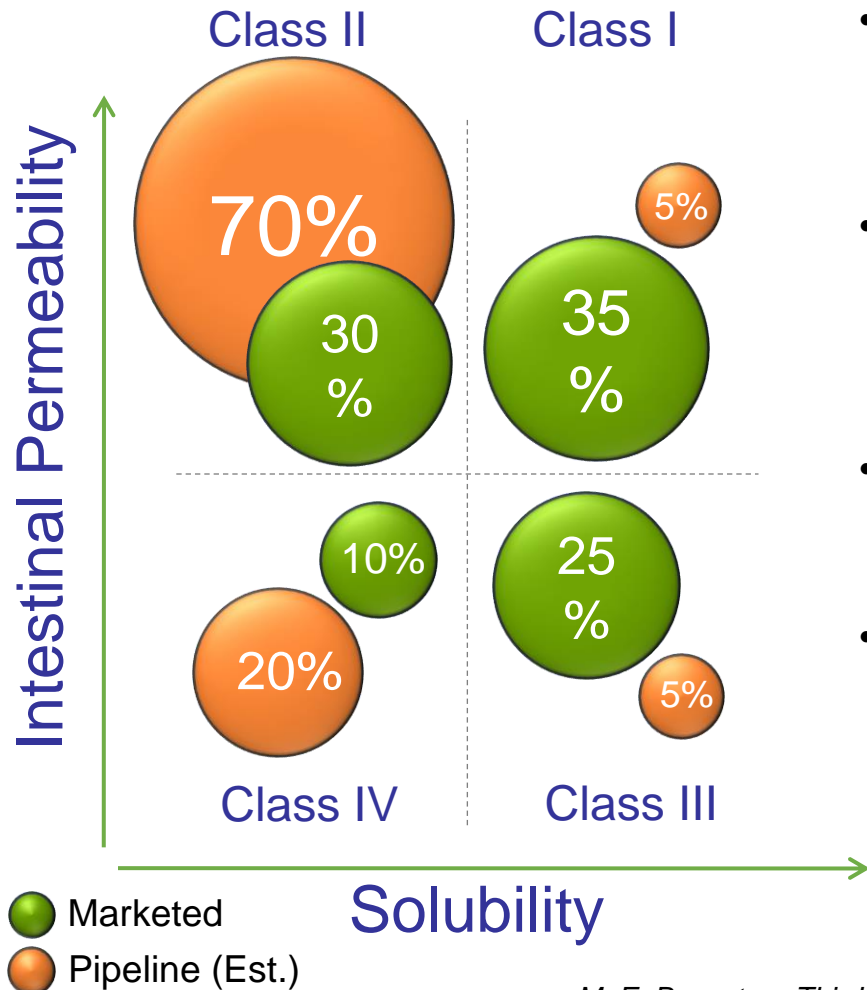
Graphical representation of historical and projected records of **nanotherapeutics** and **nanodiagnostics**. *Advances in Nanoparticles, 2017, 6, 93-102*

Nanomedicines in the market or in clinical translation showing indications (above) and formulation (below)

*Acta Pharmaceutica Sinica B, 2022, 12, 3028-3048*

# Why combine therapy with diagnostics?

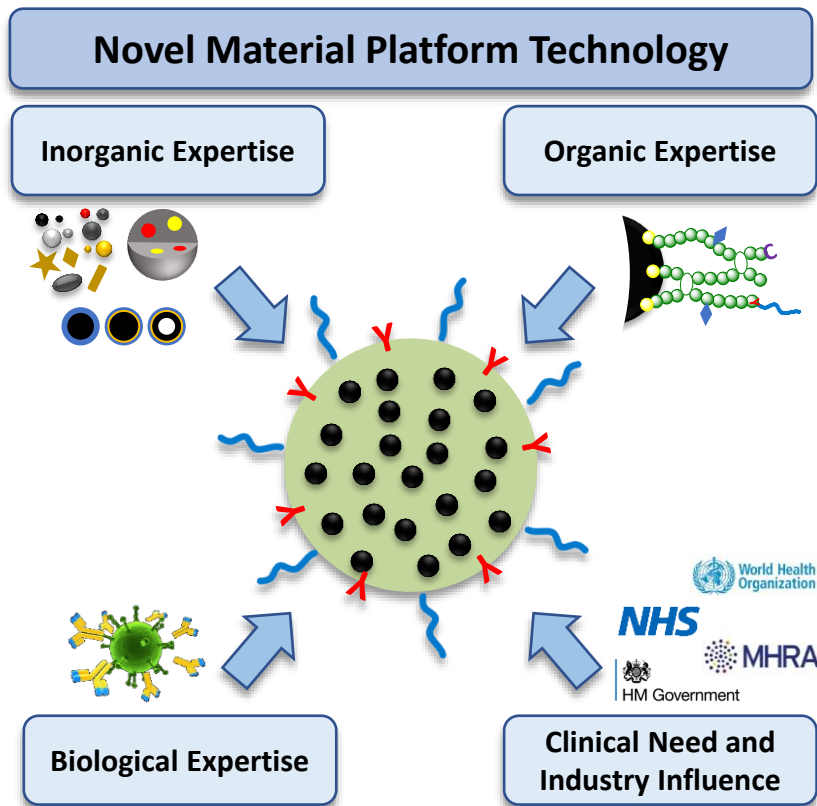
## Biopharmaceutics Classification System



- Motive for therapeutic nanomedicine
  - Majority of pipeline drugs compounds have low water-solubility and low bioavailability
- Reformulation of existing drugs offers:
  - Varied biodistribution
  - Reduced off target toxicity
  - “Payload” delivery
- Regulatory issues with regards Inorganic Nanoparticles progression to clinical trial
- Theranostic development aims to combine the benefits of both diagnostic and therapeutic nanomedicine
  - Personalised/precision medicine
  - Biodistribution monitoring
  - Reduction in Inorganic Particle Toxicity

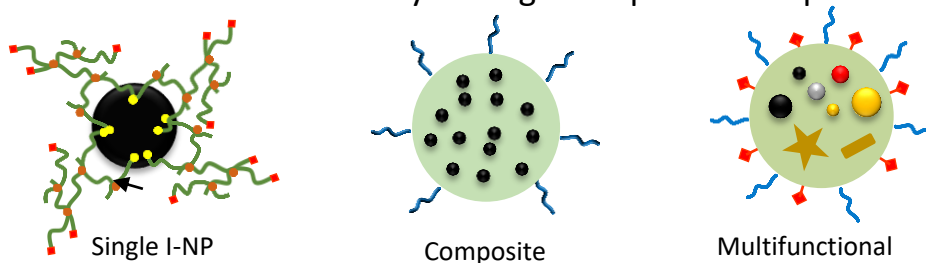
# Inorganic/Organic Nanocomposite Particles (I/O-NP)

A Platform Technology for Next Generation Healthcare Applications

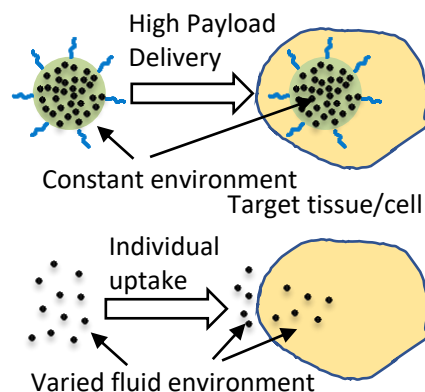


## Composite Structure Variability

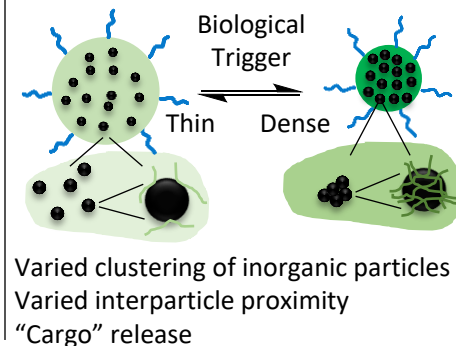
Reduction in I-NP toxicity through composite encapsulation



## Amplified Delivery



## Biologically Responsive Structures

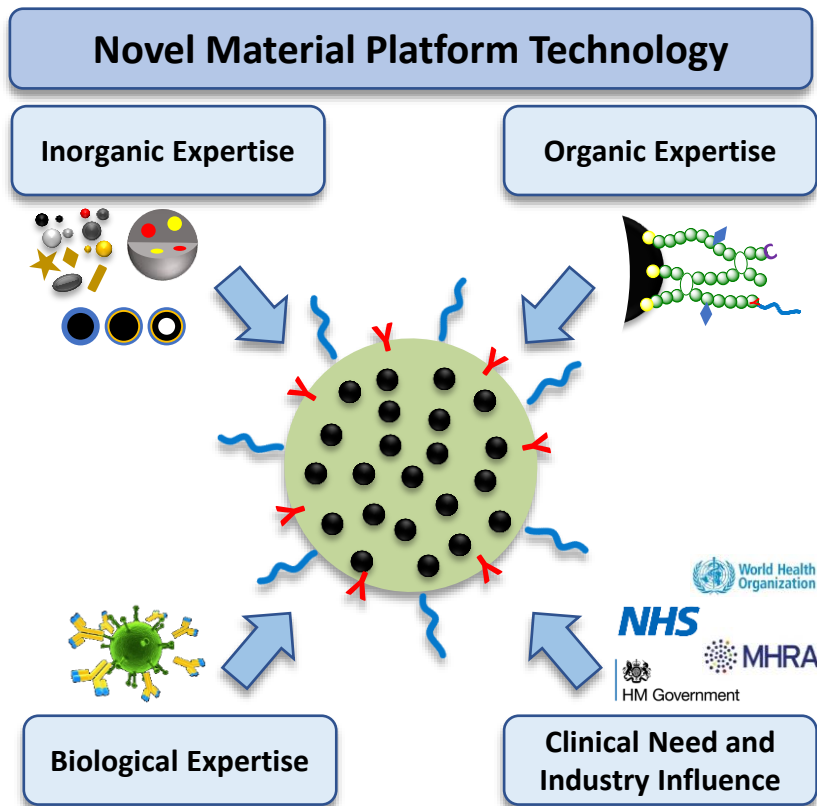


Giardiello et al., *Nanoscale*, 8, 7224 (2016) <https://pubs.rsc.org/en/content/articlepdf/2016/nr/c6nr00788k>

Giardiello et al. *J. Mater. Chem.*, 22(47), 24744 (2012) <https://pubs.rsc.org/en/content/articlepdf/2012/jm/c2jm34974d>

# Inorganic/Organic Nanocomposite Particles (I/O-NP)

A Platform Technology for Next Generation Healthcare Applications



Strategies to I/O-NP Design:

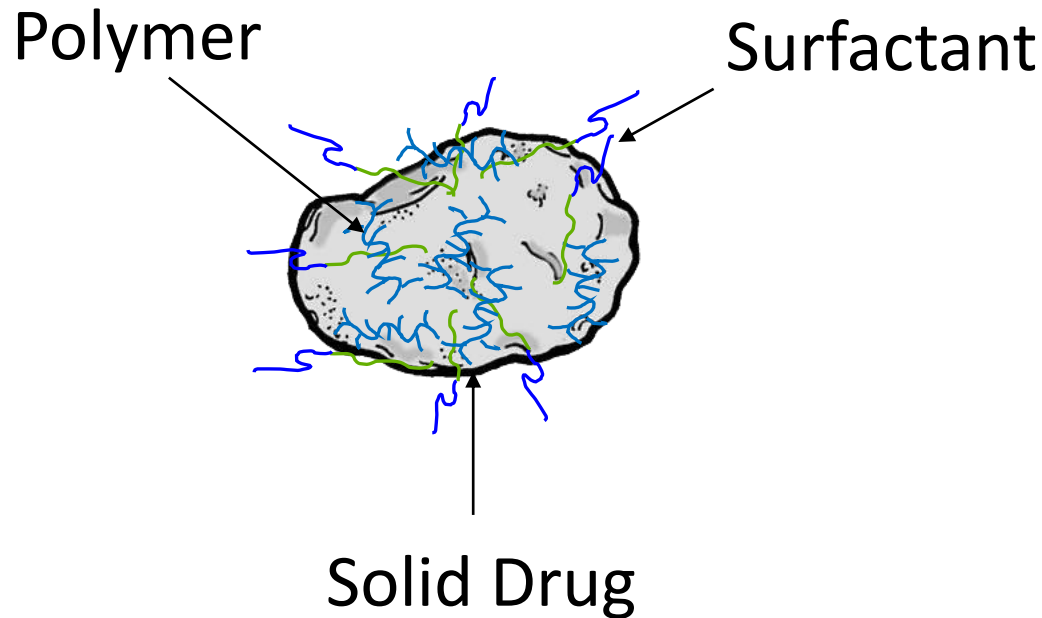
- Solid Drug Nanoparticle (SDN)
- Polymer “Cargo” Encapsulation

Giardiello et al., *Nanoscale*, 8, 7224 (2016) <https://pubs.rsc.org/en/content/articlepdf/2016/nr/c6nr00788k>

Giardiello et al. *J. Mater. Chem.*, 22(47), 24744 (2012) <https://pubs.rsc.org/en/content/articlepdf/2012/jm/c2jm34974d>



# Solid Drug Nanoparticles (SDN)

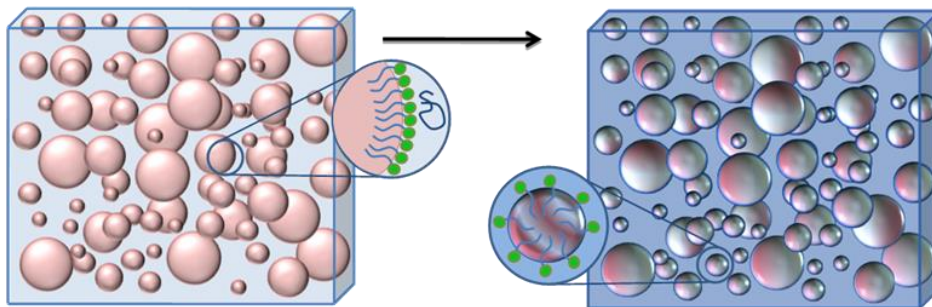


Solid nanosized particles of hydrophobic drug stabilised by polymer/surfactant excipients

Aim: to develop orally dose medicines on low bioavailable drugs

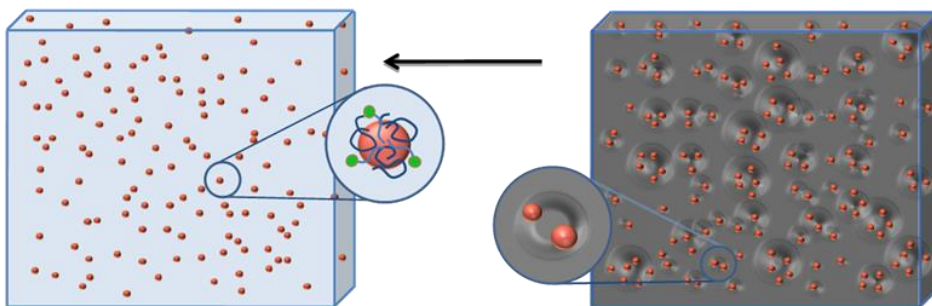
# Novel Emulsion Processing Route Emulsion-Templated Freeze Drying (ETFD)

- Generates **Solid Drug Nanoparticle (SDN)** dispersions in one step
  - SDNs are solid nanosized particles of hydrophobic drug stabilised by polymer/surfactant excipients

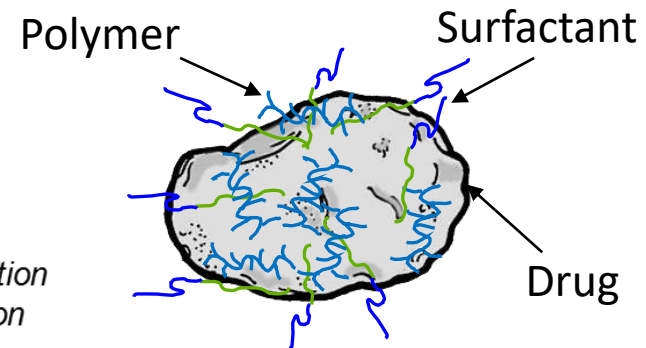


Two solvents  
APIs and excipients dissolved

*Dessication  
Supersaturation  
Local Concentration  
Particle Formation*



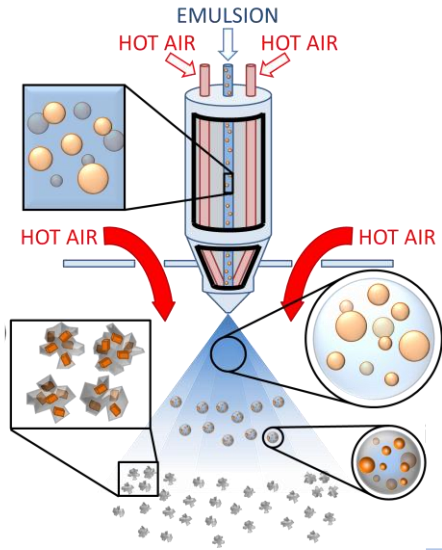
*Dry Porous Structure  
"Zones" of Organic  
Compound*



Solid Drug Nanoparticle  
(SDN)



# Translation to Emulsion Spray Dry (ESD)



Small laboratory  
spray drying

Industrial cGMP  
spray drying

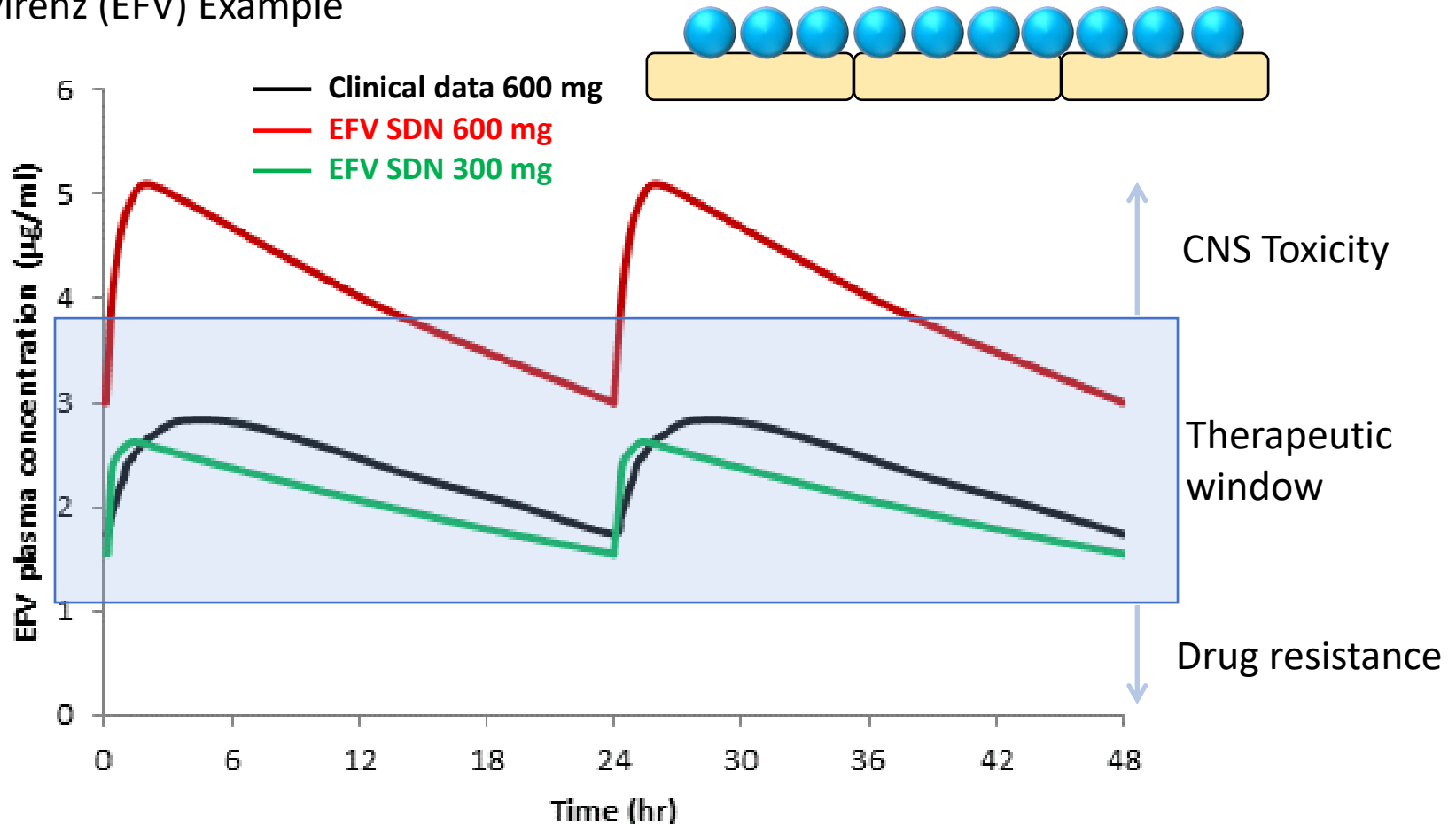
Clinical product

Achieved direct translation to spray dry

- 50 wt% loading
- Matched particle properties
- Matched cellular accumulation
- Matched cytotoxicity
- High recovery
- Parameters are optimised to be industrially relevant
  - **8 wt% solids in feed**
- Capsule loading and powder storage stability studies carried out successfully at 25°C/60%RH and 40°C/75%RH
- **Drug product complied with European Pharmacopoeia standard**

# Increased Surface Area through Nanoformulation

Efavirenz (EFV) Example

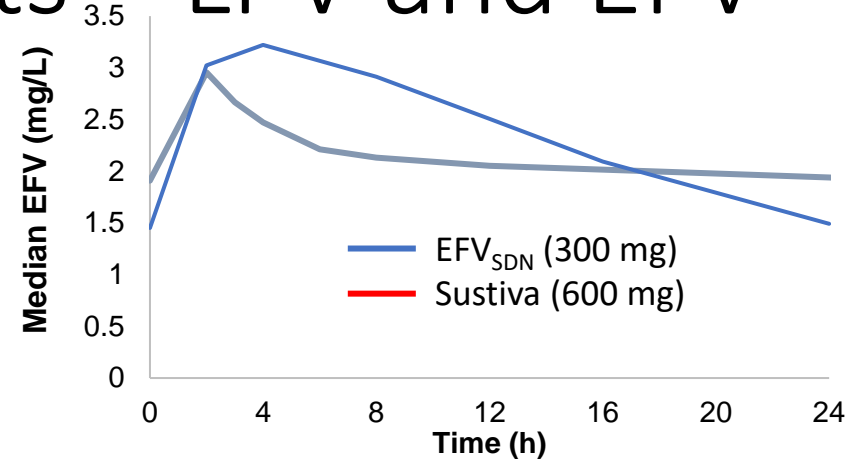
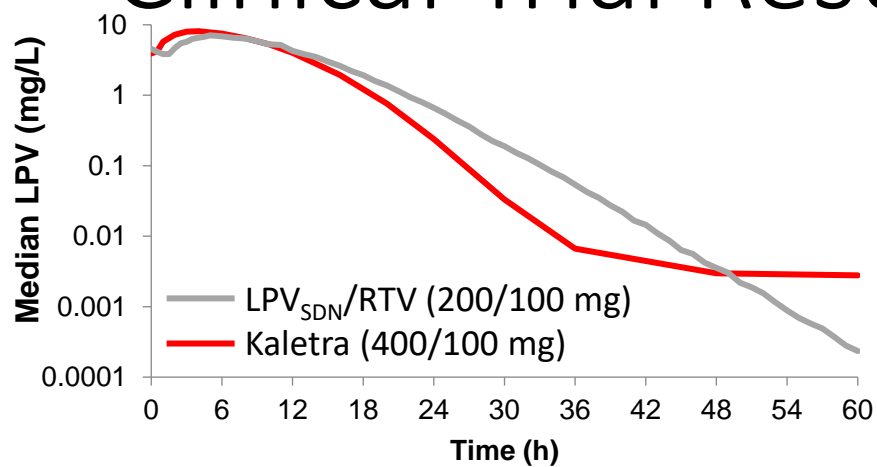


**Potential 50% dose reduction**

- **Greater patient access to therapy**
- **Reduced manufacturing cost with capacity able to meet demand**

EudraCT number 2013-004913-41

# Clinical Trial Results – LPV and EFV



	Geometric mean		Geometric mean ratio
<b>LOPINAVIR</b>	LPV SDN 200 mg	Kaletra 400 mg	GMR (90% CI)
C <sub>12</sub> (mg/L)	4.16	4.02	1.04 (0.99-1.08)
AUC <sub>0-12</sub> (mg.h/L)	72.35	79.07	0.92 (0.89-0.94)
C <sub>max</sub> (mg/L)	10.69	9.97	1.07 (1.05-1.10)
<b>EFAVIRENZ</b>	EFV SDN 300 mg	Sustiva 600 mg	GMR (90% CI)
C <sub>12</sub> (mg/L)	2.03	2.51	0.81 (0.78-0.83)
C <sub>24</sub> (mg/L)	1.90	1.44	1.32 (1.26-1.37)
AUC <sub>0-12</sub> (mg.h/L)	51.56	58.61	0.88 (0.86-0.90)
C <sub>max</sub> (mg/L)	2.99	3.36	0.89 (0.87-0.91)



## UoL owned Spin-Out Company

- Contract licencing of proprietary SDN technology
- IP is well established and comprises 19 patent families

## Validated Technology:

- Pharmaceuticals and Agrochemicals
- Two Human Clinical (EudraCT number 2013-004913-41)

<https://www.tandemnano.com/>



## Centre of Excellence for Long Acting Therapeutics

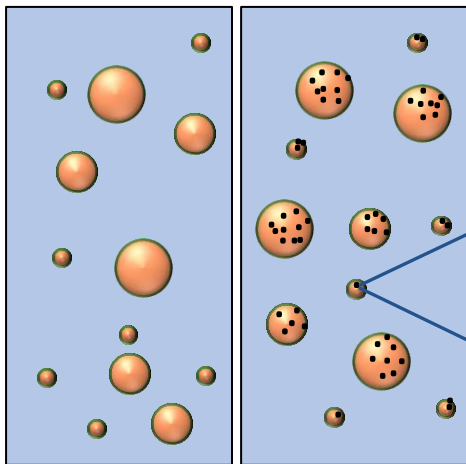
- Synergistic approach, combines expertise in pharmacology and materials chemistry
- £40m ongoing research funding in diverse project portfolio

<https://www.liverpool.ac.uk/centre-of-excellence-for-long-acting-therapeutics/>

# Adapt SDN to form I/O-NP

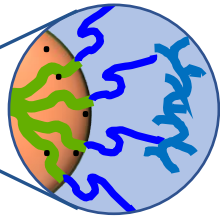
Blended hydrophobic Polymer (**P**), Organic Molecule (**O**) and **SPION** into emulsion oil phase to create triple component hydrophobic inorganic–organic nanocomposite particles

## Emulsion

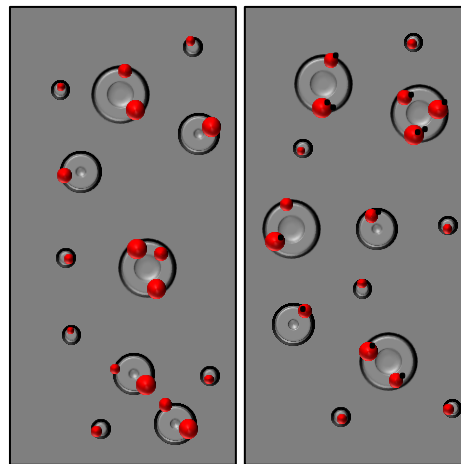


Organic Inorganic/Organic

Supersaturation  
Freeze dry

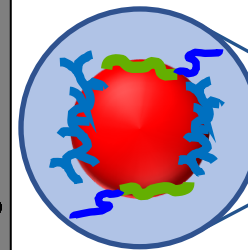


## Porous Freeze Dried Material

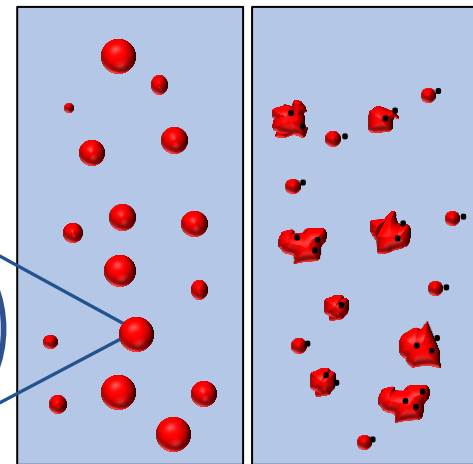


Organic Inorganic/Organic

Addition  
of water



## Nanodispersion




Organic Inorganic/Organic


 Solutol HS® 15

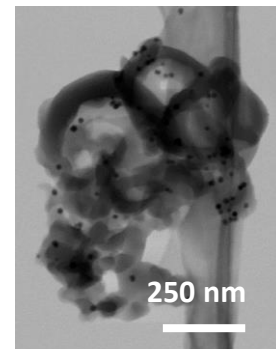
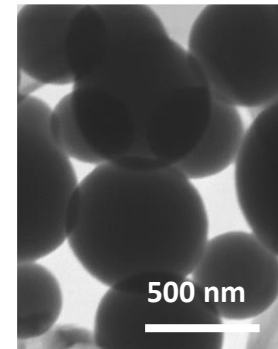
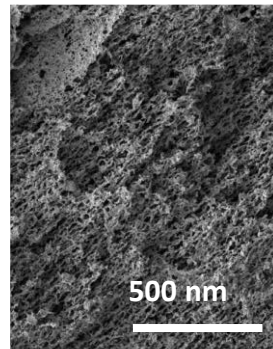
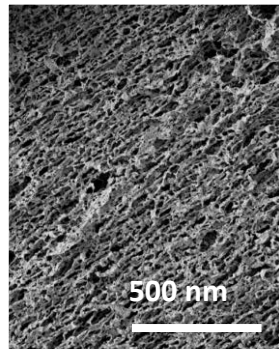
 Kollicoat Protect®

 Oil droplet containing **P+O**

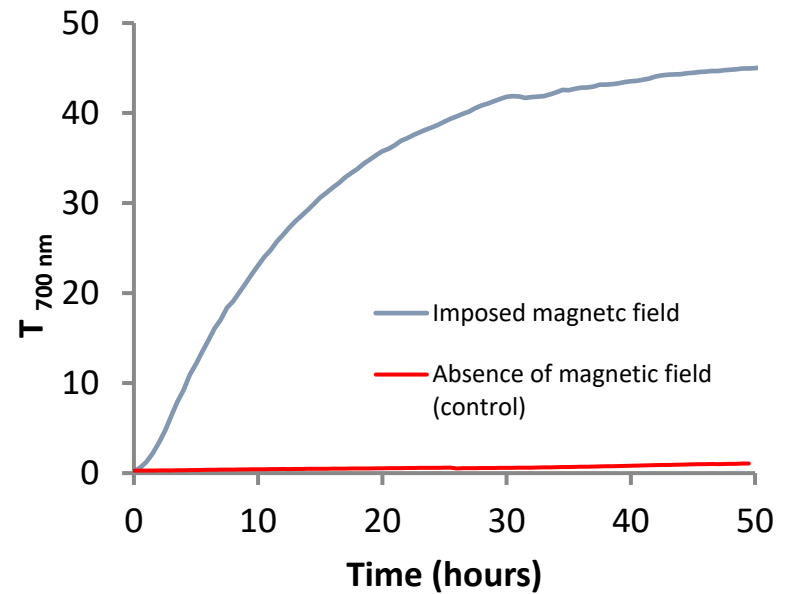
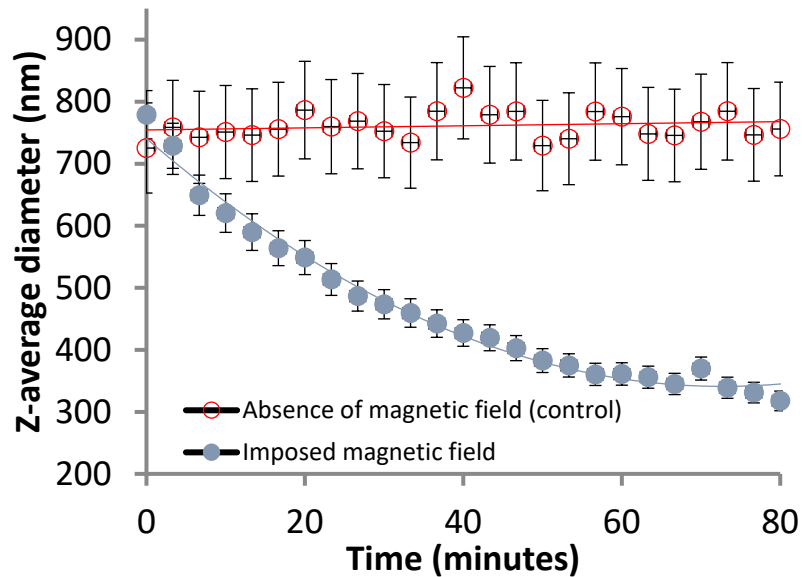
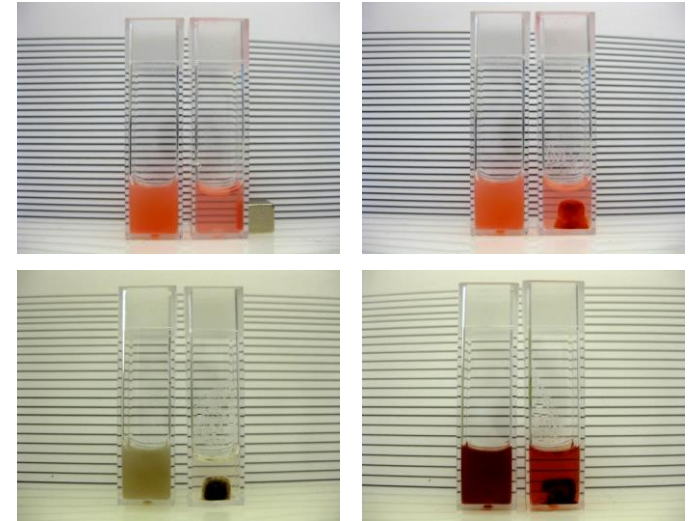
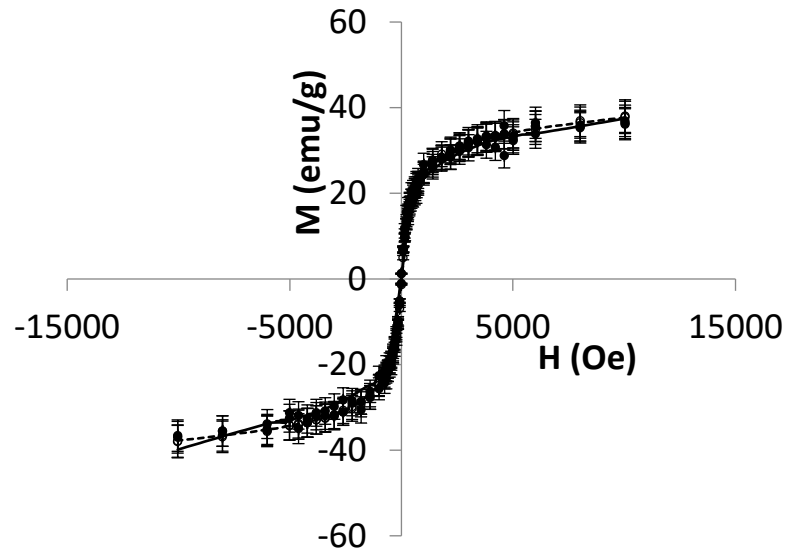
 Oil droplet containing **P+O+ SPION**

 Organic **P+O**

 Inorganic/organic SDN **P+O+SPION**



# Exposure to Magnetic Field



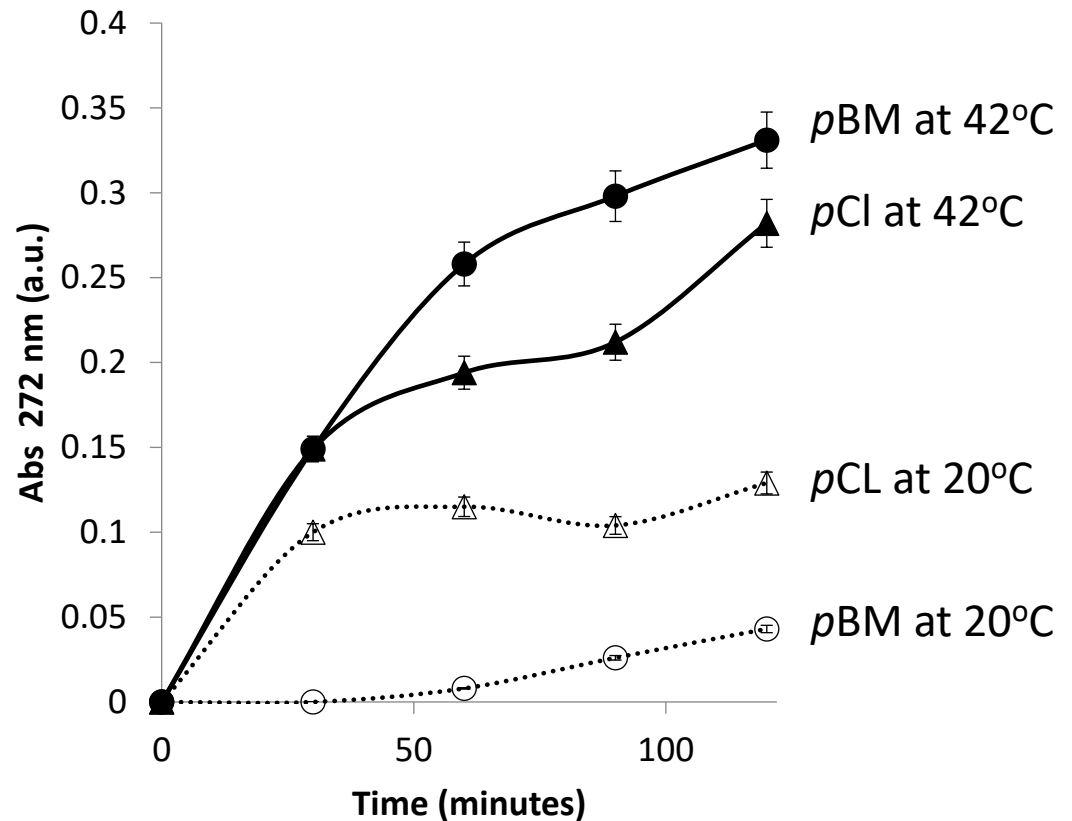


# Temperature Dependant Drug Release

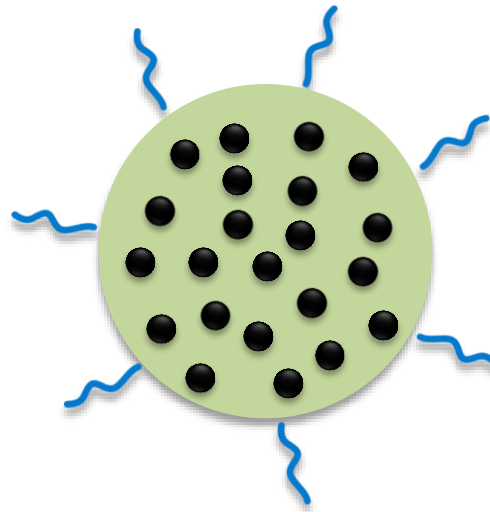
poly(n-butyl methacrylate) (pBM)  $T_g = 20^\circ\text{C}$   
 Polycaprolactone (pCL)  $T_g = -60^\circ\text{C}$

Prepared composite particles containing Ibuprofen drug with polymers of varying glass transition temperature  $T_g$

- **Magnetic Fluid Hyperthermia** – cell death occurs between  $42\text{--}45^\circ\text{C}$
- Saw variation of drug release at  $42^\circ\text{C}$  and  $20^\circ\text{C}$ , attributed to polymer  $T_g$



# Polymer “Cargo” Encapsulation

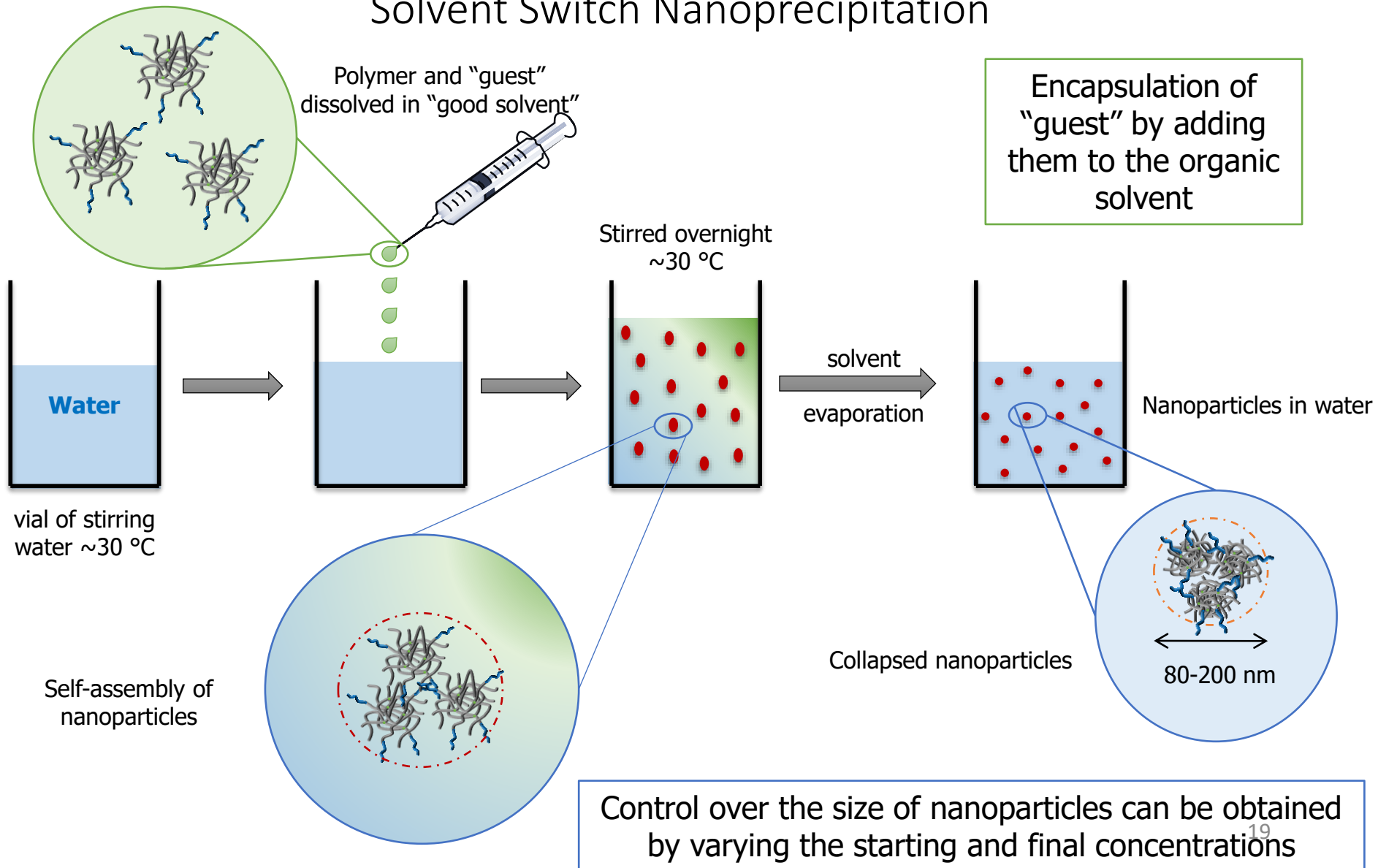


Encapsulation of Drug Molecules and/or Inorganic Nanoparticles within or on the surface of Polymeric Nanoparticles

Aim: high payload delivery

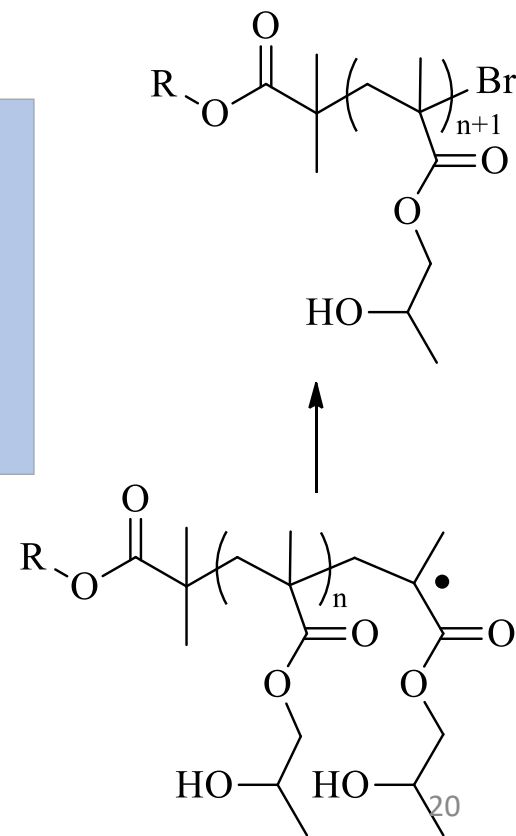
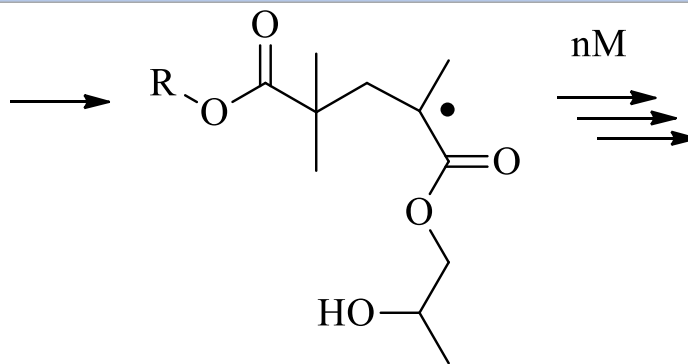
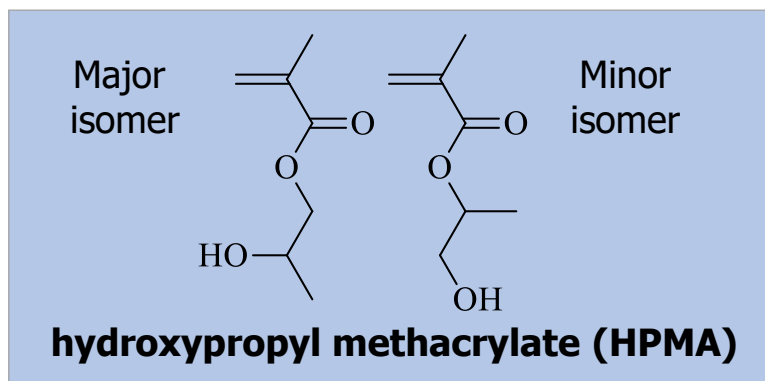
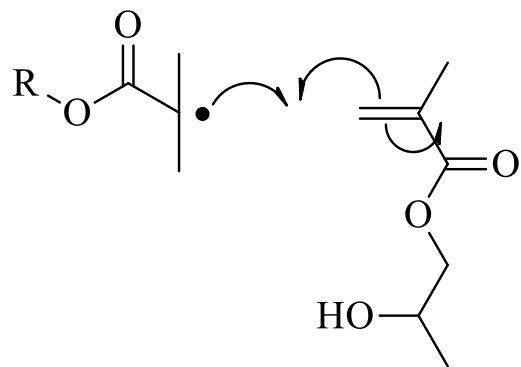
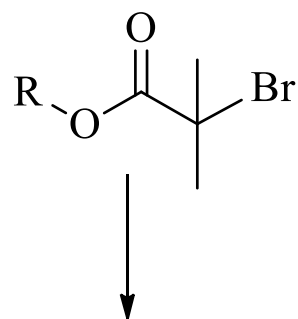
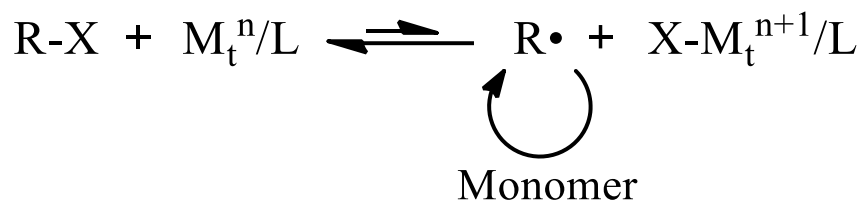
# Polymer "Cargo" Encapsulation

## Solvent Switch Nanoprecipitation

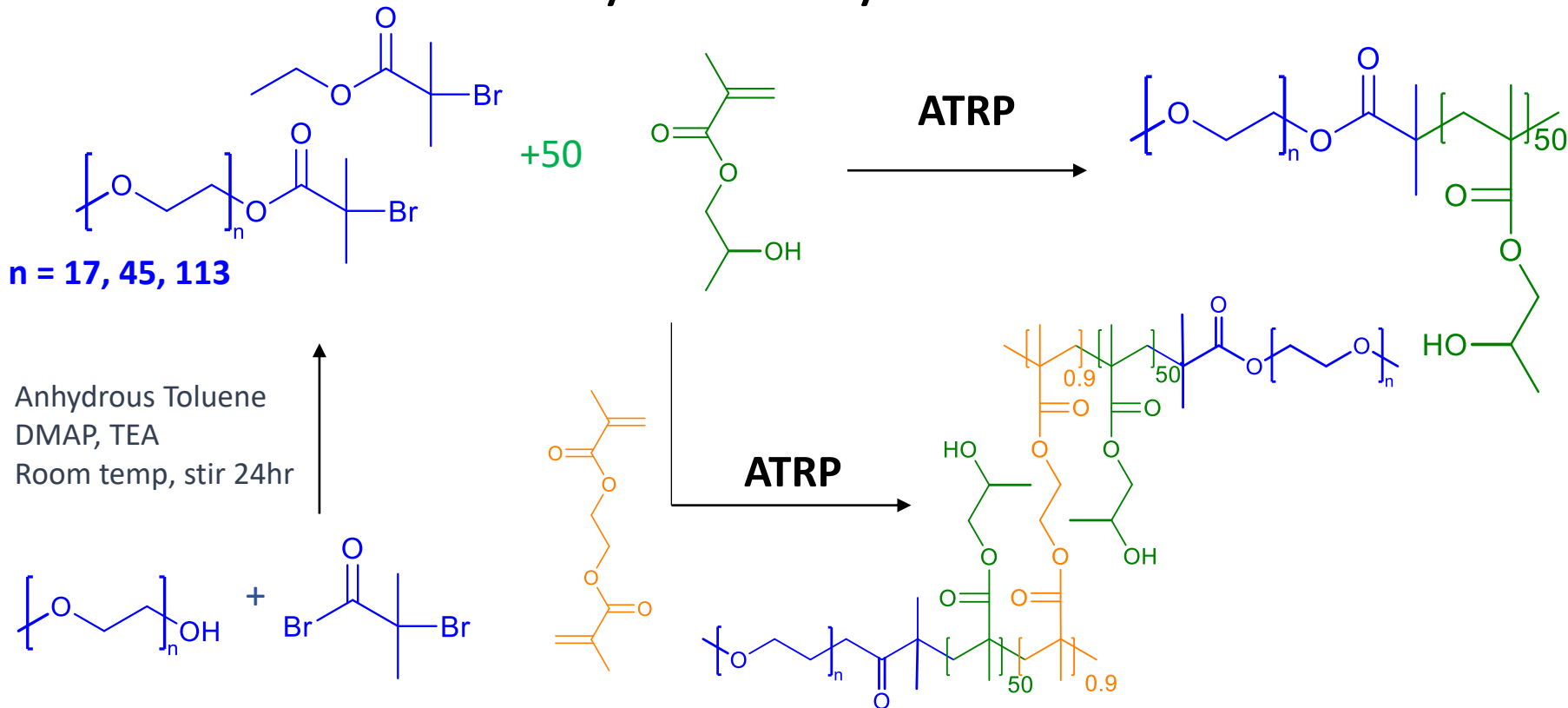


# Polymer Synthesis

- **A**tom **T**ransfer **R**adical **P**olymerisation is a controlled radical polymerisation technique
- The dynamic equilibrium between dormant and reactive radical species maintains control

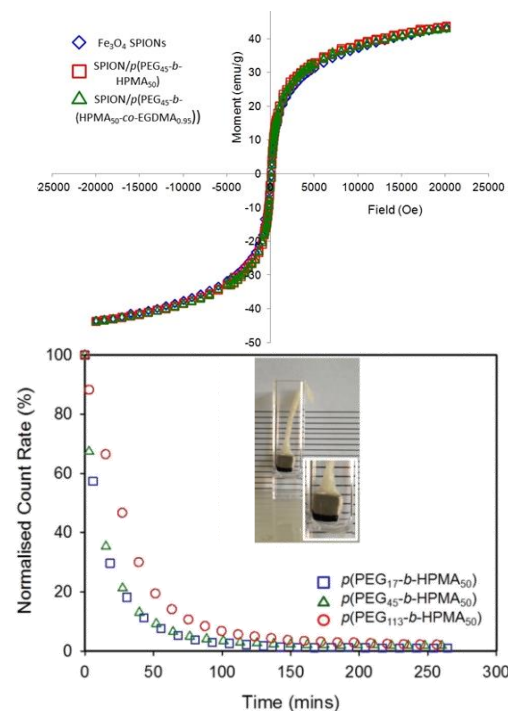
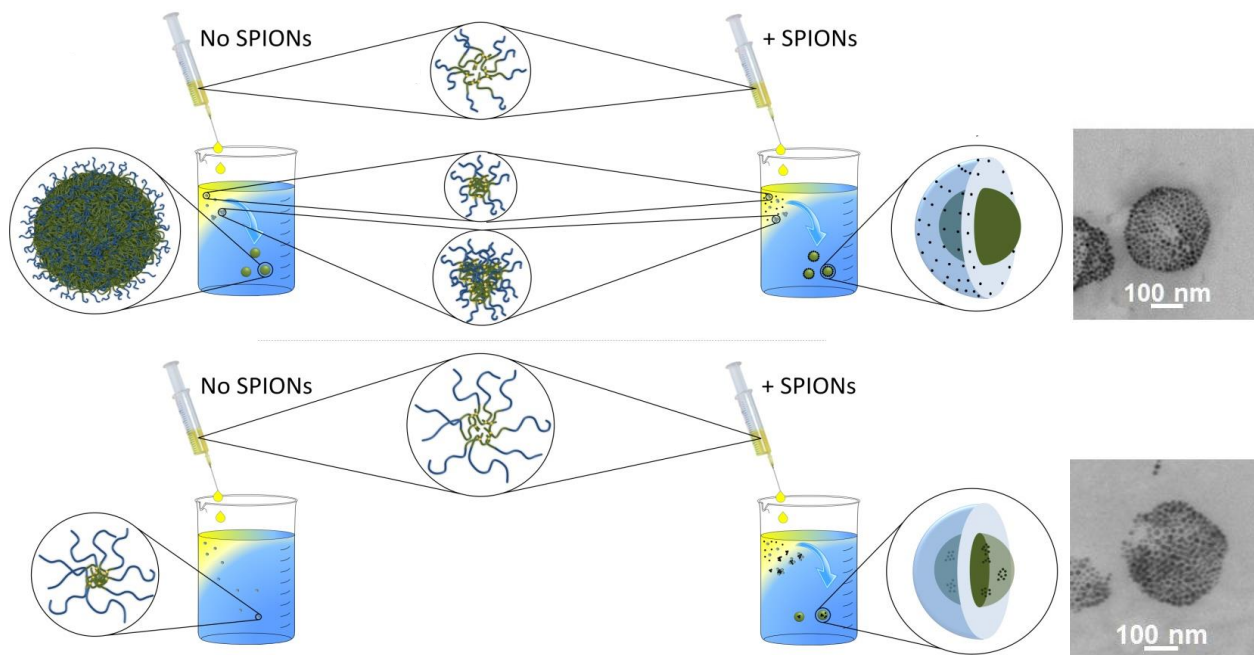


# Polymer Synthesis



Polymer	$M_n$ g/mol (Theory)	$M_n$ g/mol (SEC)	$M_w$ g/mol (SEC)	$\bar{D}$ (SEC)	Weight Av. # of chains
$p(\text{HPMA}_{50})$	7200	11250	13950	1.24	1
$p(\text{PEG}_{17}\text{-}b\text{-}(\text{HPMA}_{50}))$	8000	8100	9800	1.21	1
$p(\text{PEG}_{45}\text{-}b\text{-}(\text{HPMA}_{50}))$	9200	10000	12000	1.20	1
$p(\text{PEG}_{113}\text{-}b\text{-}(\text{HPMA}_{50}))$	12200	13300	16800	1.26	1
$p(\text{HPMA}_{50}\text{-}co\text{-}(\text{EGDMA}_{0.95}))$	-	147,100	928,500	6.31	67
$p(\text{PEG}_{17}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-}(\text{EGDMA}_{0.95})))$	-	57200	150,400	2.63	15
$p(\text{PEG}_{45}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-}(\text{EGDMA}_{0.95})))$	-	43300	110,400	2.55	9
$p(\text{PEG}_{113}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-}(\text{EGDMA}_{0.95})))$	-	45400	118,500	2.61	7

# Polymer Directed I/O-NP Formation



Polymer/Sample	-SPION			+SPION		
	$D_z$ (nm)	$D_n$ (nm)	PdI	$D_z$ (nm)	$D_n$ (nm)	PdI
$p(\text{HPMA}_{50})$	510	360	0.26	280	240	0.21
$p(\text{PEG}_{17}\text{-}b\text{-HPMA}_{50})$	670	520	0.22	365	220	0.25
$p(\text{PEG}_{45}\text{-}b\text{-HPMA}_{50})$	-	-	-	225	185	0.13
$p(\text{PEG}_{113}\text{-}b\text{-HPMA}_{50})$	-	-	-	175	145	0.09
$p(\text{HPMA}_{50}\text{-}co\text{-EGDMA}_{0.8})$	110	85	0.08	310	270	0.14
$p(\text{PEG}_{17}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-EGDMA}_{0.95}))$	49	30	0.17	195	70	0.24
$p(\text{PEG}_{45}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-EGDMA}_{0.95}))$	-	-	-	180	140	0.12
$p(\text{PEG}_{113}\text{-}b\text{-}(\text{HPMA}_{50}\text{-}co\text{-EGDMA}_{0.95}))$	-	-	-	165	135	0.07
Oleic acid –stabilised SPIONs <sup>a</sup>	-	-	-	310	265	0.17

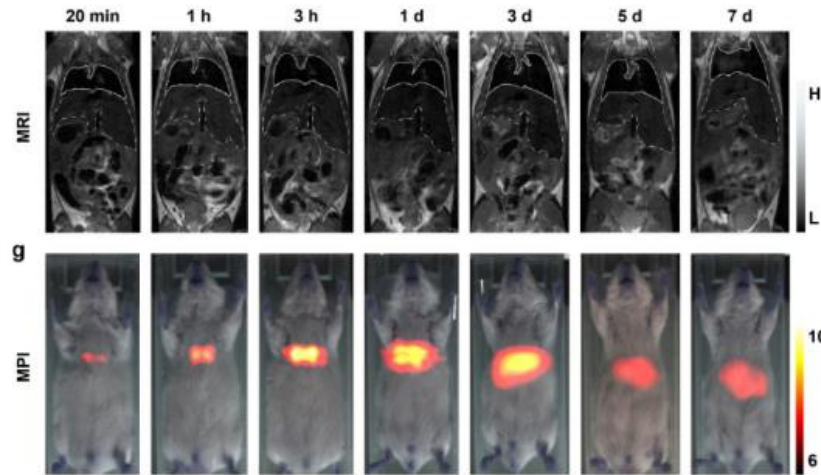
# Pre-Clinical Magnetic Particle Imaging (MPI) at UoL



- MOMENTUM MPI scanner
  - First in the UK
  - Expected – Spring/Summer 2023
- RELAX
  - Tracer Optimization & Relaxometry software
- MOMENTUM SDK
  - Image reconstruction software



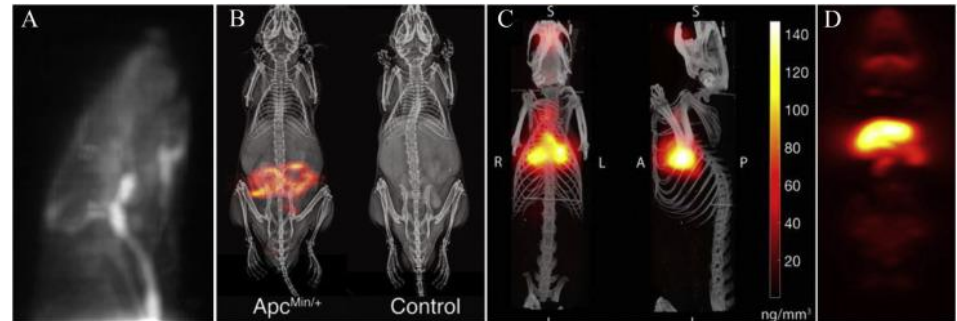
# Magnetic Particle Imaging (MPI)



MRI & MPI monitoring of labelled Mesenchymal Stem Cells biodistribution.

The positive contrast MPI data clearly shows a shift of tracer biodistribution from lungs to liver over 7 days.

Wang et al. ACS Nano (2020) 14, 2, 2053-2062



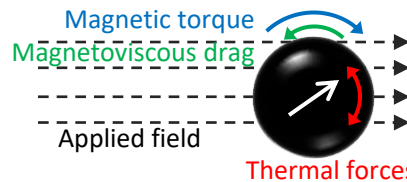
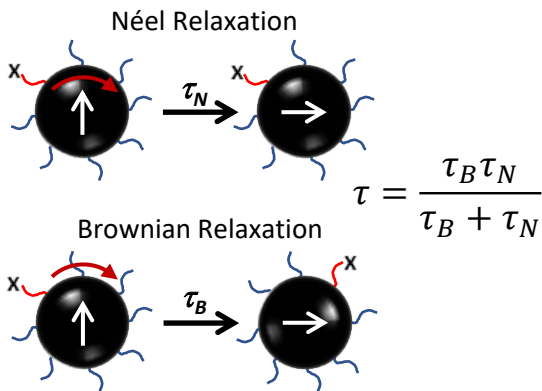
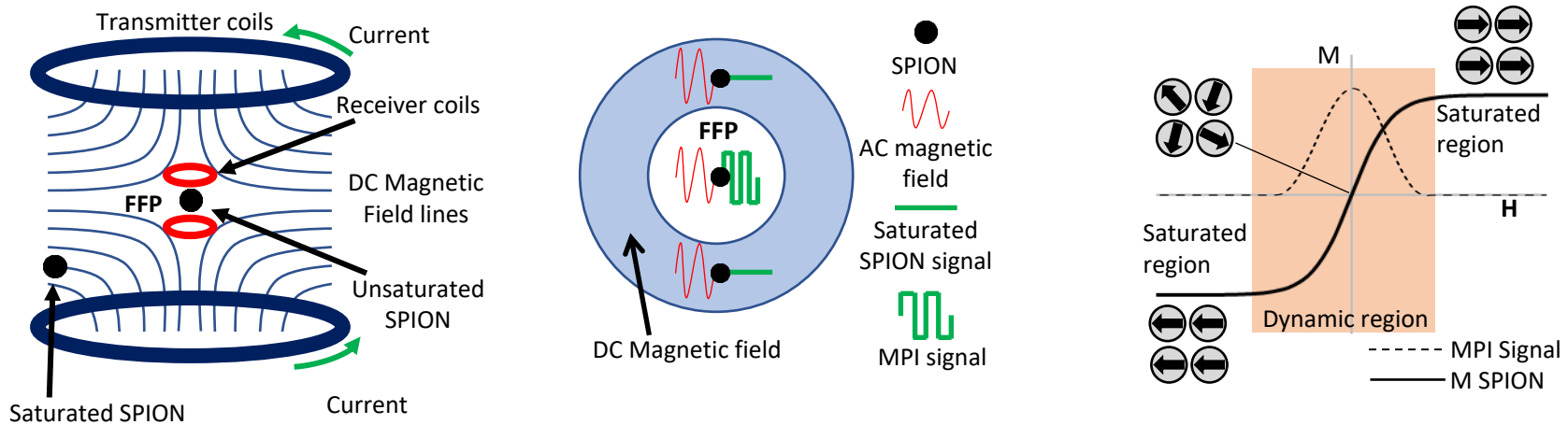
- (A) Cerebral blood volume (note the direct imaging of capillary-level perfusion in tissue). (
- (B) Sensitive detection of gastrointestinal bleed.
- (C) Evaluation of inhaled drug therapeutics (MPI is not affected by air-tissue susceptibility interfaces and can even image in lungs).
- (D) MPI images of in situ labelled neutrophil distribution in bone marrow and the organs of the reticuloendothelial system

Chandrasekharan et al. Molecular Imaging (2<sup>nd</sup> Ed); Magnetic Particle Imaging for Vascular, Cellular and Molecular Imaging (2021)

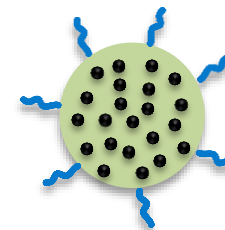


# Magnetic Particle Imaging (MPI)

- Diagnostic Imaging technique which detects nanomolar concentrations of Superparamagnetic Iron Oxide Nanoparticle (SPION) tracers
- MPI is the first imaging modality to be driven by tracer technology
- Offers quantification and real-time monitoring of biological systems with no background signal



## Fluid Environment effects rotation

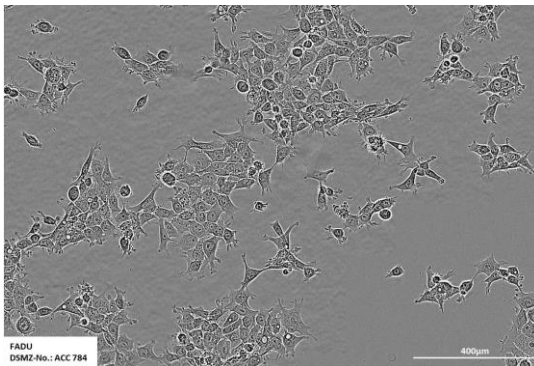


- Tracking Drug Nanocarriers
- Effect of SPION clustering on MPI signal

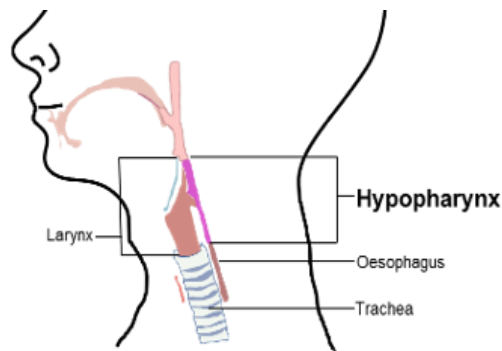
# AuNP Radiotherapy Enhancement

- There are many cancer cells that show high resistance to radiotherapy
- Gold nanoparticles have been shown to enhance radiosensitisation of such cancer cells
  - up to 20 fold increase in some cancer cell lines
- Investigation of AuNP radiotherapy (x-ray and proton) enhancement:
  - AuNP size, shape and surface stabiliser effects
  - Mechanistic studies into radiotherapy enhancement mode of action

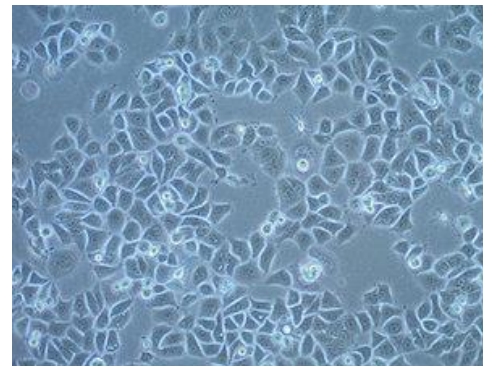
## FaDu



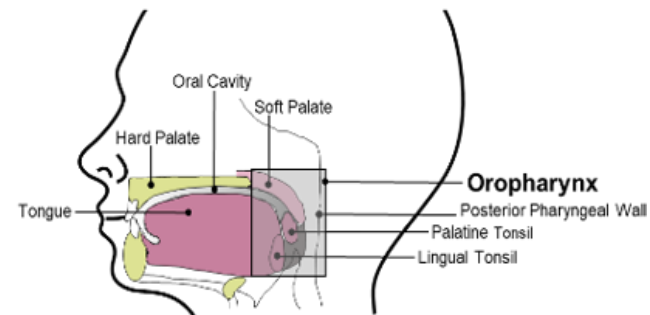
Squamous cell carcinoma of the hypopharynx (throat)



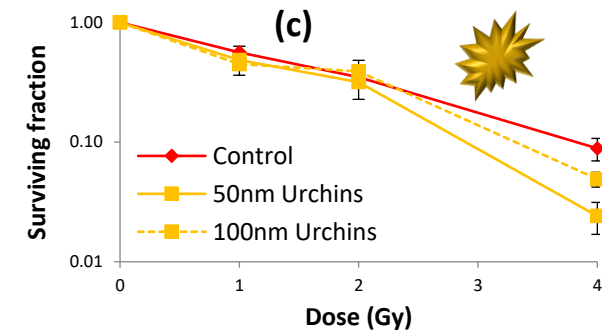
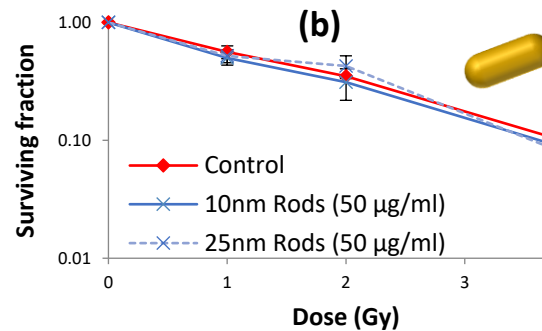
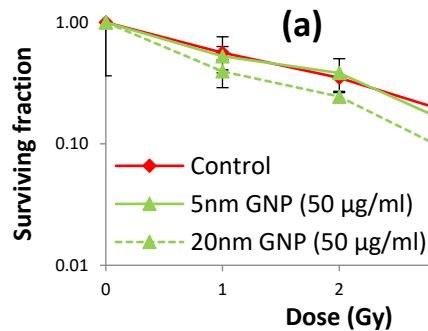
## UMSCC6



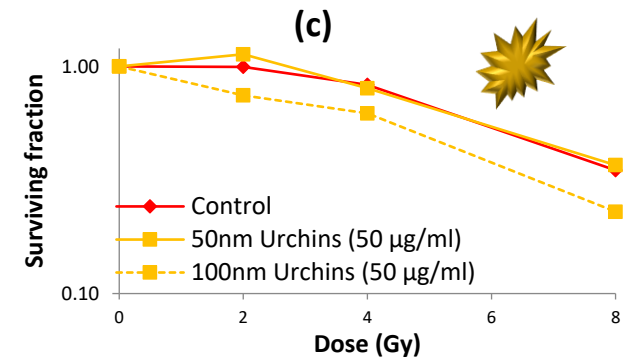
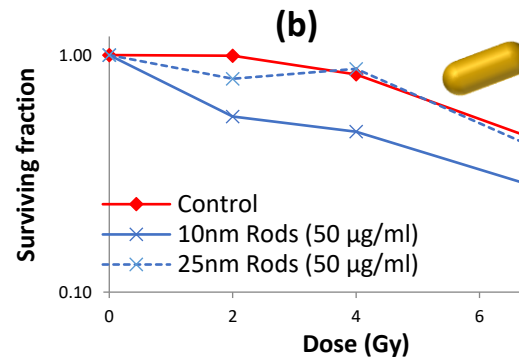
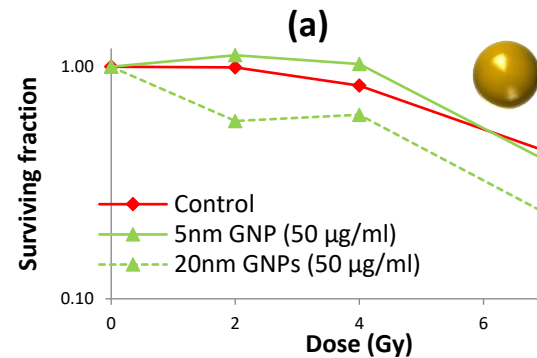
Oropharyngeal squamous cell carcinoma (tongue)



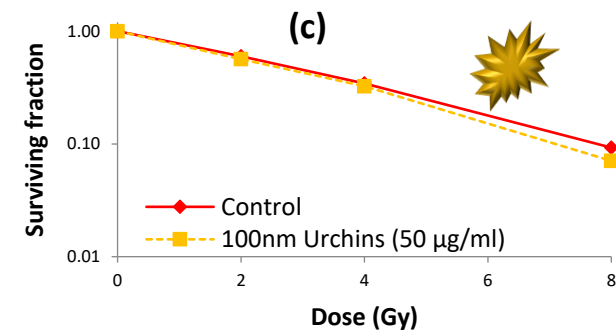
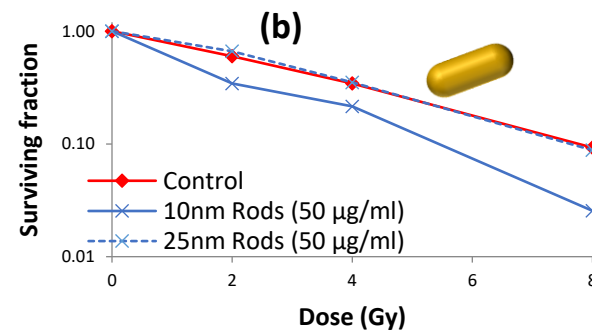
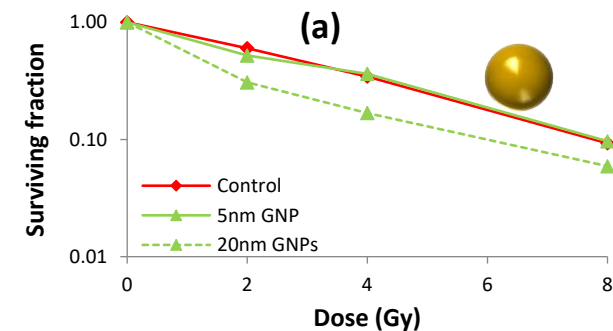
# AuNPs and Radiotherapy



X-ray irradiation (100 kV) of UMSCC74A cells (human papilloma virus type-16 (HPV)-negative oropharyngeal squamous cell carcinoma cells) treated with (a) spherical GNPs, (b) gold nanorods and (c) gold nanourchins.

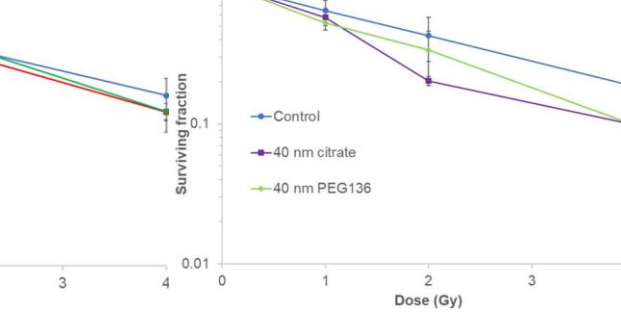
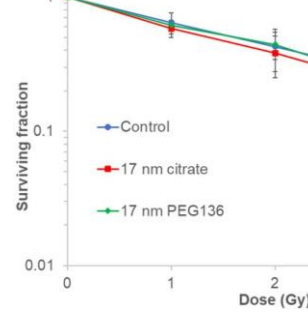
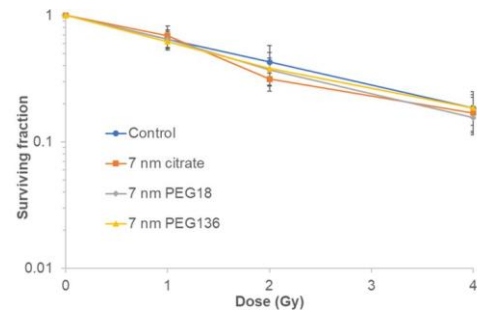
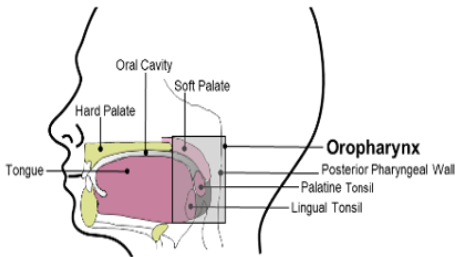
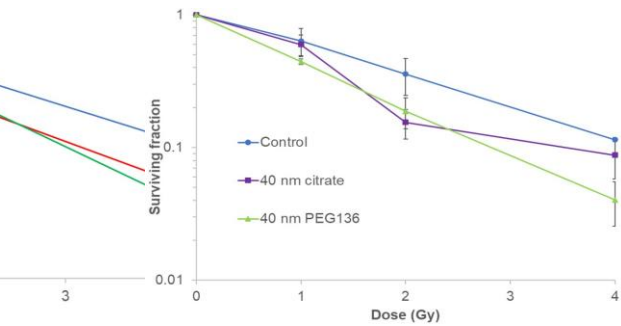
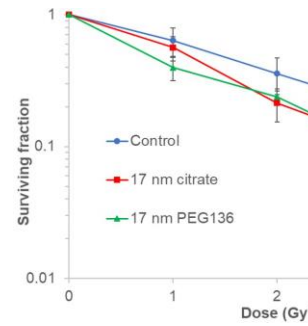
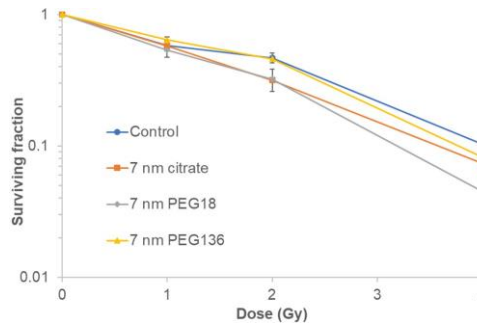
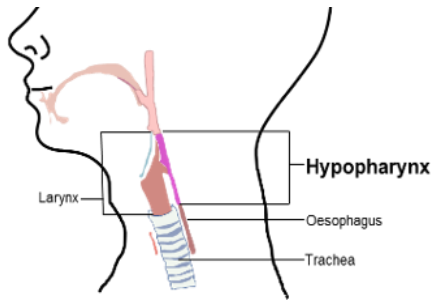


Proton irradiation (58 MeV) of UMSCC74A cells (human papilloma virus type-16 (HPV)-negative oropharyngeal squamous cell carcinoma cells) treated with (a) spherical GNPs, (b) gold nanorods and (c) gold nanourchins.



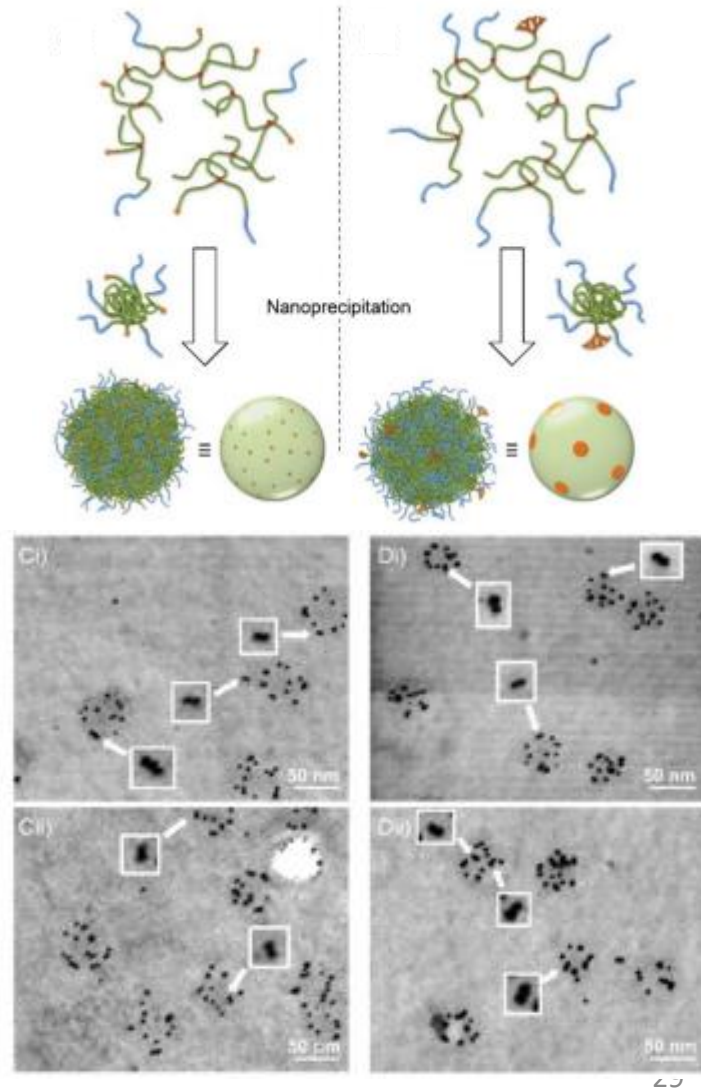
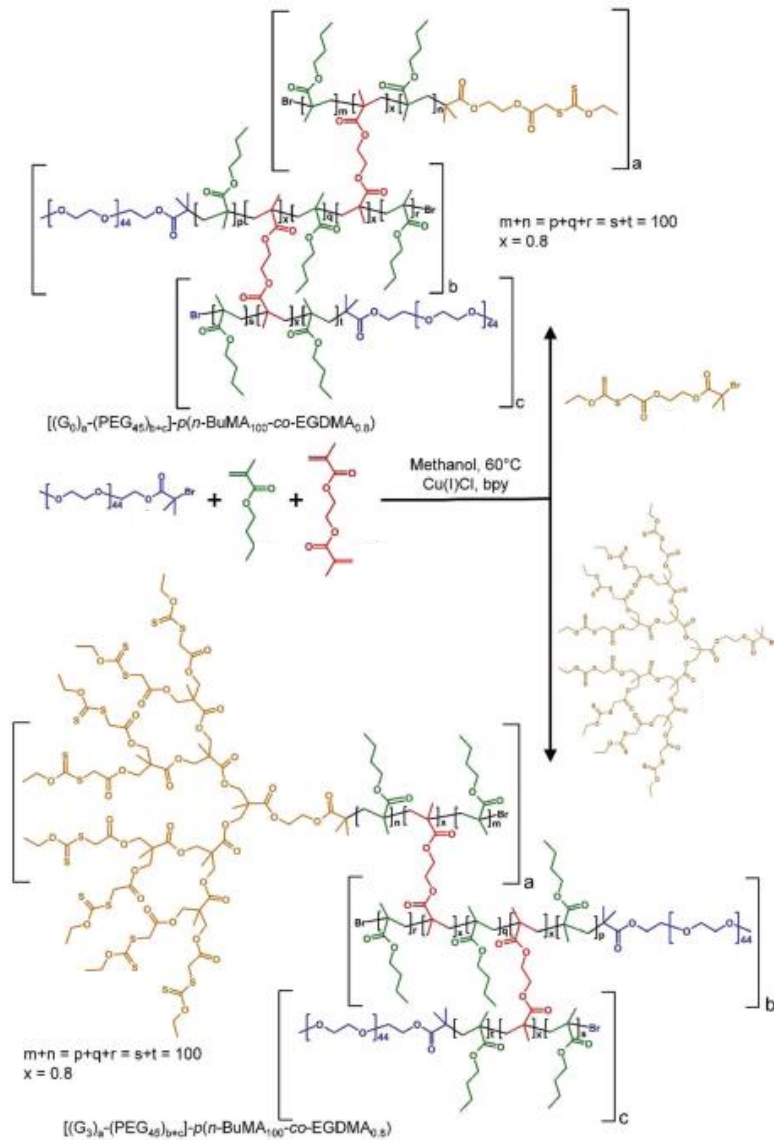
Proton irradiation (58 MeV) of HeLa cells (cervical carcinoma cells) treated with (a) spherical GNPs, (b) gold nanorods and (c) gold nanourchins.

# AuNPs and Radiotherapy



AuNP size and surface chemistry have an effect on X-ray radiotherapy enhancement on Hypopharyngeal squamous cell carcinoma (FaDu) and Oropharyngeal squamous cell carcinoma (UMSCC6) cell lines.

# AuNP Polymer Composites



# Summary

- Brief overview of Nanomedicine and Theranostics
  - Takes advantage of both therapeutic and diagnostic nanomedicine towards personalised/precision medicine
- Strategies to Theranostic Nanomedicine Design:
  - Inorganic/Organic Nanocomposite Particles (I/O-NP)
    - Solid Drug Nanoparticle (SDN)
    - Polymer “Cargo” Encapsulation
  - Applications for magnetic particles (MPI)
    - Novel imaging technology – monitor drug carrier biodistribution
    - Payload Delivery of AuNPs for radiotherapy

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*Thank you!*



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