# Nano-structure Development of Oral Pharmaceutical Formulations in Simulated Intestine – D-contrast SANS and DLS


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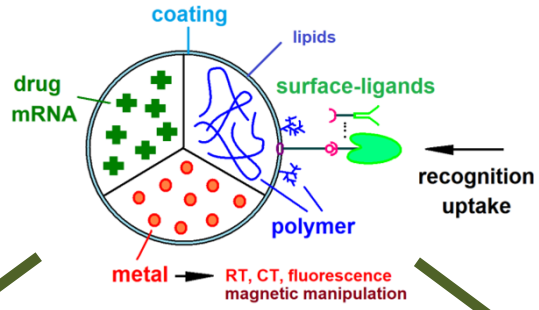
EU project OrBiTo

european innovative medicine initiative IMI


## Therapeutic Nanoparticles as Drug carriers:

**Mixed nanoparticles bearing material domains**




**Combination : DLS and SAXS and D-contrast Neutron scattering SANS**

**Therapy material development**

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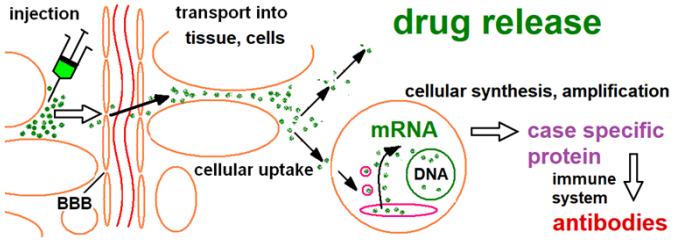
**(I)**

**Parenteral (injection) :**
**study of formulations and interactions**




**Therapy method development**
**Immuno-therapy (mRNA)**
**Vaccination**

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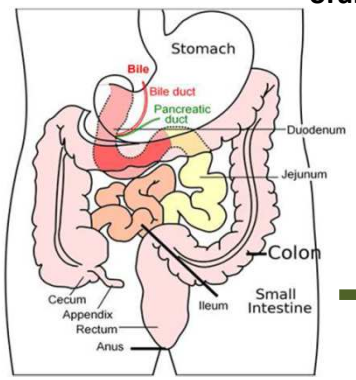
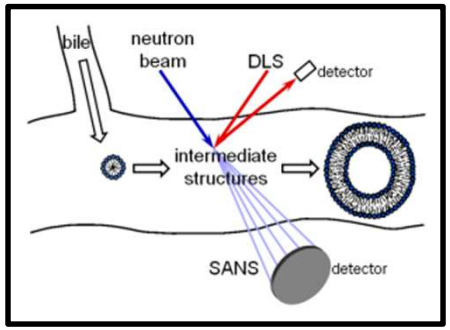
**(II)**

**oral (tablet, capsule,** solution**) :**




**study in a Gastro-Intestinal simulator models (GI-Sim)**




**(III)**

**tissue- and cell bio-targeting**

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# (I) Parenteral drugs in nanoparticles

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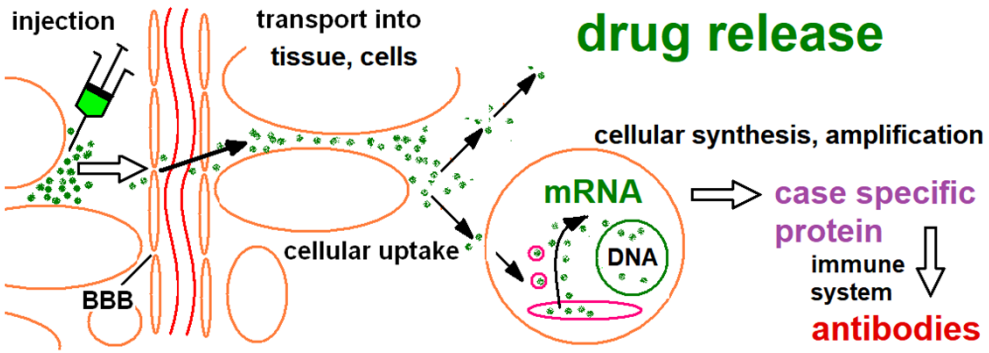
RNA Pharmaceuticals




**Drug nanoparticles for parenteral application** (injection): The drug (pharma agent, mRNA) in lipid, liposomes or polymer nanoparticles release the drug, **if the structure is sufficient**.

**A)** **Conventional parenteral drugs**: release the drug (pharma agent) from solution or nanoparticles **non-specific**.

**B)** **mRNA nano-drugs** : code for the cellular synthesis of a specific protein, which later induces antibody generation. This implies a double **amplification** (> 1000 x) by cell elements (ribosomes, T-Cells)

**mRNA is :**

- **not toxic**, as it is a native cellular intermediate (short-life work-copy of a gene)
- **sensitive**, as cells and tissue switch it off quickly by enzymatic **degradation** (RNAse) temperature and hydrolysis sensitve , need sterility and cooling, if **pure**
- **Nanoparticle structure** embedding can stabilize, and facilate cell uptake (activity)

**Analogy in a computer – CNC system:**

1 Harddisk (DNA) ⇒ 20 file-copies on USB-Sticks (mRNA) ⇒ 1000 products by 3D-printer set (ribosomes)

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# mRNA-Nanoparticles for immuno-therapy : two forms
## (1) mRNA polymer nanoparticles

SANS with Deuterium-contrast variation ⇒ component domains in drug nanoparticles

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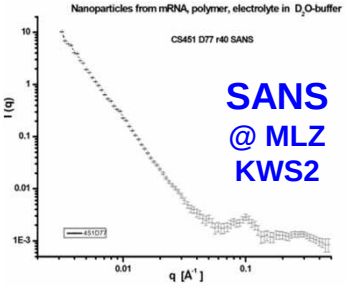
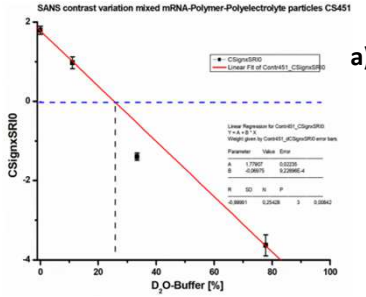
**mRNA in AminoDextrane NP's**

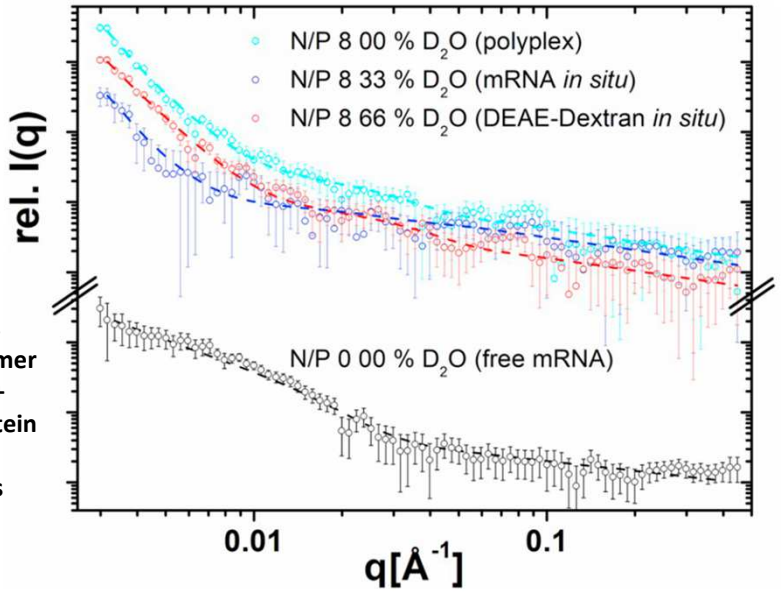


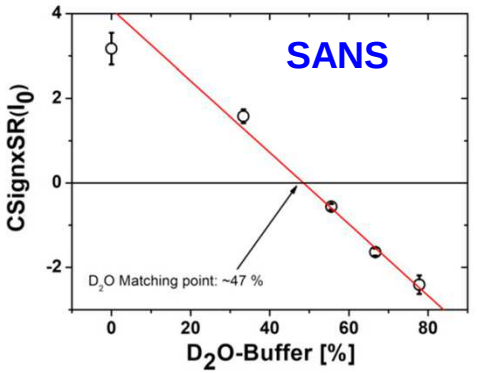

Improvements
a) charged polymer
b) basic lipid+ polymer, protein

Core-shell-NP's

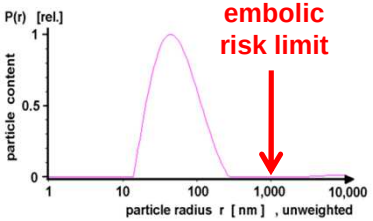
**mRNA in DEAE-Dextrane NP's**




Siewert C, Haas H, Nawroth T, Sahin U, Langguth P et al. (2019) **Biomaterials** 192, 612-20: **mRNA-DEAE-Dextran** polyplexes **SANS & DLS**




**DLS**




**DLS as med. security test**

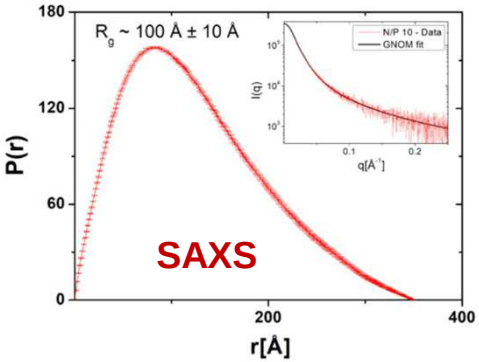
**mRNA immuno Nanoparticles:**

**Medical use for :**
a) vaccination, e.g. Covid-19
b) cancer immuno therapy

**Structure based development :**
variant coding for luciferase
SANS, SAXS, DLS, animal tests

**mRNA nanoparticles**
for
**immuno-therapy** :

MLZ FRM-II, KWS2
DESY-Petra III , P12




Siewert CD, Haas H, Cornet V, Nogueira S, Nawroth T, Uebbing L, Ziller, A, Al-Gousous J, Radulescu A, Schroer MA, Blanchet CE, Svergun DI, Radsak MP, Sahin U, Langguth P (2020) **Cells** Doi 10.3390/cells9092034 **mRNA-Lipid-Polym**er complexes **SANS, SAXS, DLS**

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with
**M. Schroer,**
**D. Svergun**
**C. Blanchet**

# mRNA-Nanoparticles for immuno-therapy : two forms
## (2) mRNA lipid-layer nanoparticles

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AK Langguth

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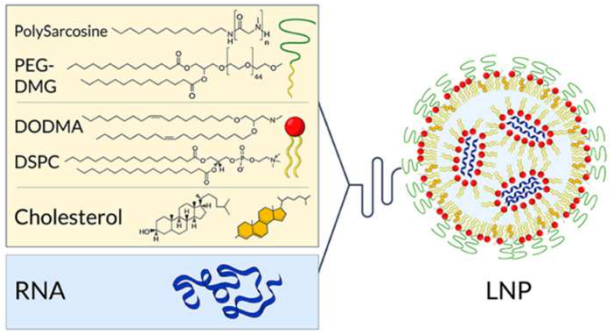
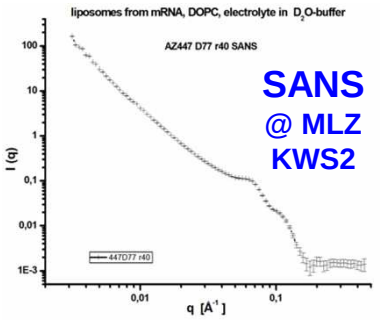


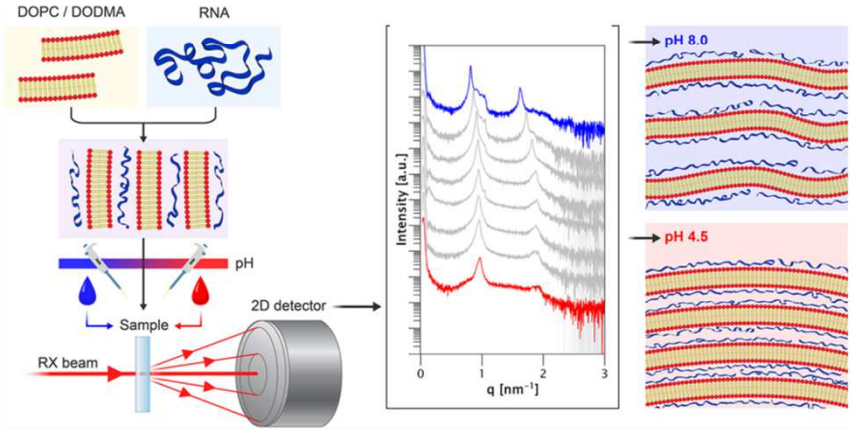

**mRNA** is assembled with **lipids**: charged, neutral and extended hydrophilic headgroups. Sequential mixing, shearing in a pH-regime results in spherical solid **lipid nucleo particles** (LNP), or **multilayer nanoparticles** (below), depending on the charge-lipid-head ratio.

Uebbing L, Schroer MA, Svergun D, Sahin U, Haas H, Langguth P et al. (2020) **Langmuir** 10.1021/acs.langmuir.0c02446
**pH-response** of **mRNA** NP's




**SANS**
**@ MLZ**
**KWS2**

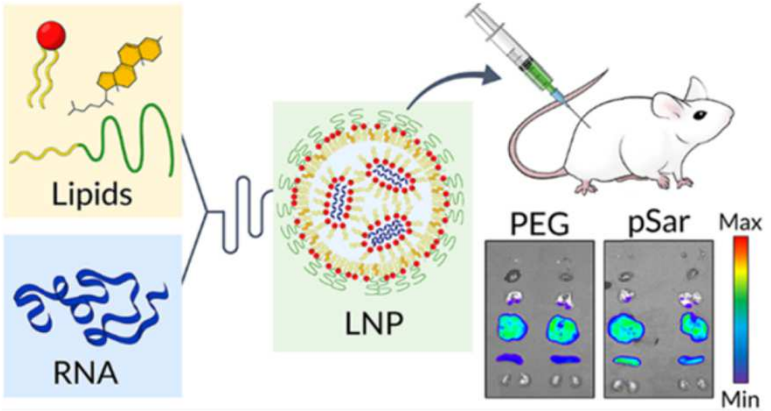
**SANS** of mRNA in non-charged lipsomes (DOPC)




**DESY-Petra III , P12**

Nogueira SS, Svergun D, Langguth P, Sahin U, Haas H et al. (2020) **Applied Nano Materials**
doi 10.1021/acsanm.0c1834
Polysarcosine-**mRNA** NP's, SAXS, DLS, **animal tests**

further
**SANS**
**beamtime is applied**




For **structure based nanoparticle development** a mRNA variant is choosen, which codes for luciferase (the light generator of fire fly). Thus the medical success of a mRNA nanodrug treatment (cell uptake and induced protein synthesis) can be proven in an animal test (mice) by light generation: the active regions get luminescent.

Membrane Pharmacy Structure Dynamics (MPSD) project

# Nanoparticle & Therapy – Licencing and Patents

## no medical use without patent and licence possible

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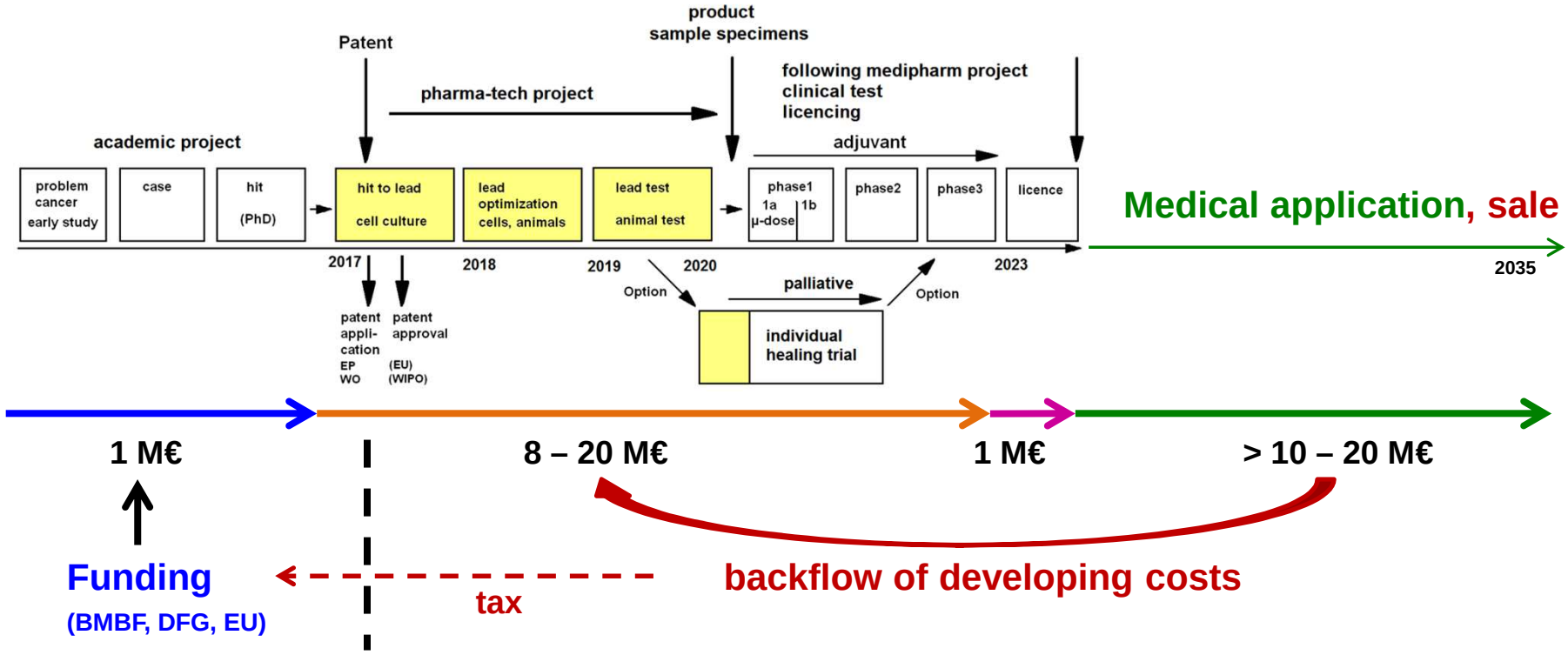


Pharma-product development and licensing steps: 8 -10 years , costs ~ 20 mio €

- Fast licencensing, emergency licence ~ individual healing trial ; e.g. in case of Covid-19
- Development and licensing can be accelerated by fast bioequivalent lab methods, e.g. a **drug application simulator device** ⇒⇒ second part of the presentation

EU project
OrBiTo

# Pharma-Nanoparticles II : Drug application simulation

## Transient nanoparticles from oral forms in the human digestion system (GI)

## FaSSIF-C : GI-model fluid with cholesterol nanoparticles

Membrane Pharmacy Structure Dynamics (MPSD) project

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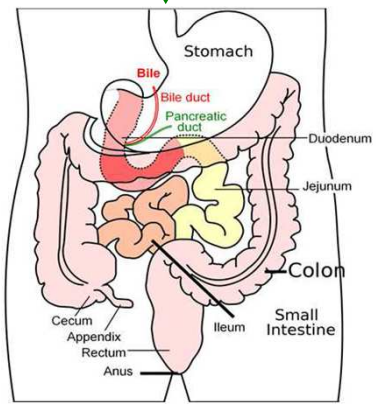
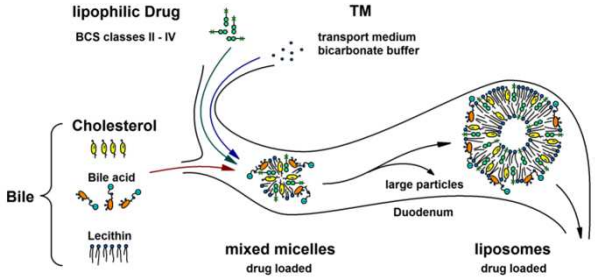
**Drug ( tablets, capsules ) :**

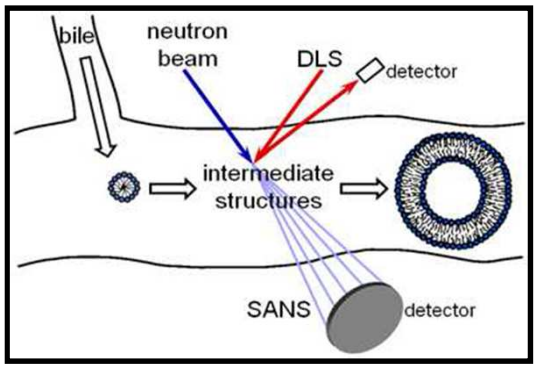
stomach




**Gastro-Intestinal –Simulator device (GI – Sim)**

**20 min.**




**Particle size scale: 5 nm – 200 µm !**

⇒ **TR-SANS + DLS**

The micelle to liposome **structure development** in simulated intestinal fluid (FaSSIF) was detected by **time resolved SANS + DLS**. (ILL-D11, and @ FRM-II KWS2)
Nawroth T, Buch P, Buch K, Langguth P, Schweins R (2011) Mol Pharmaceutics 8, 2162-72

At **FRM-II KWS2** a set of cholesterol based **bioequivalent model fluids "FaSSIF-C"** for the *in vitro* study of intestinal drug resolution and uptake in the duodenum was developed. The drug solubilization capacity was related to the nanostructure estimated by DLS and neutron scattering SANS; bio-equivalent location: **2nd half of duodenum**

Khoshakhlagh P, Johnson R, Langguth P, Nawroth T, Szekely N.K., et al. (2015) J Pharm Sci 104, 2213-24
.. FaSSIF-C **with Cholesterol** ⇒ **Study with 8 drugs** (2015-2020: R. Johnson, P. Thumarati, V. Stahl)

- Interaction of BCS2/4-drugs in nanoparticles, with intestinal model fluids and transient nanoparticles therein (intermediates)
- 8 Drugs:
  - **BCS2:** Fenofibrate, Danazol, Griseofulvin, Carbamazepin, Paracetamol, Gemcitabin, Curcumin; in **nanoparticles**
  - **BCS4:** Amphtericin B, Amphtericin B - Cholesterol (**SL Dr. R. Johnson**, Ghana)
- Methods combined (time resolved):
  - Drug solubility (in FaSSIF, FaSSIF-C)
  - DLS (time resolved): micelles, liposomes, large NP's
  - SANS (time resolved) at FRM-II, Muni-Garching
- Intestinal model fluids, physiological relevant:
  - $FaSSIF_{mod6.5}$: pH-stabilized (BiCarbonate+HEPES)
  - FaSSIF-C, pH-stabilized, containing Cholesterol (as bile)

# Pharma-Nanoparticles II : Nano-drug-structure in intestine

## Gastro-Intestinal Simulator devices (GI Sim) with SANS + DLS

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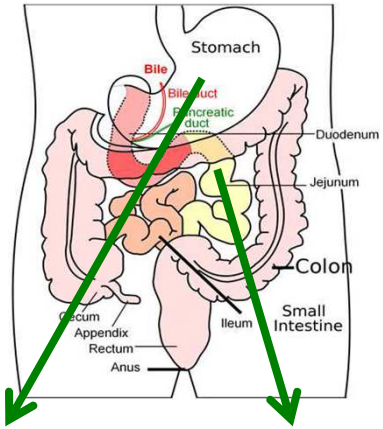
Johannes Gutenberg Universität Mainz — Pharma-Technology AK Langguth

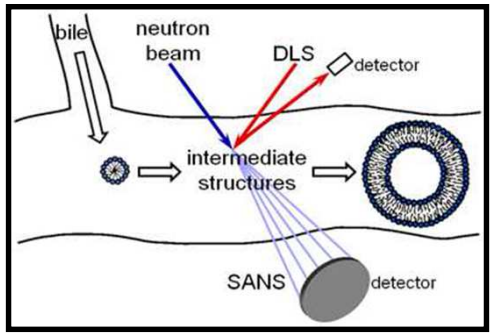
**Solid form ⇒ Fragments mm ⇒ Microparticles µm ⇒ Nanoparticle development : Liposomes ⇒ Micelles ⇒ Solution**

**Drug** ( tablets, capsules)

**Particle size scale: 5 nm – 200 µm !**

**Solid form**
⇓
**Disintegration**
⇓
**Dissolution**
⇓
**Solution**
⇓
**uptake**




**Gastro-Intestinal –Simulator device (GI – Sim)**

⇒ **TR-SANS + DLS**

**Oral drug formulation development**
for difficult drugs : BCS 2, 4 , (3)

| GI model | stomach | intestine | Technology, particle | drug form development |
|---|---|---|---|---|
| **GiSim 1** | mixing, pH-jump, stiring | vessel, cuvette, **static** („pot“) | Stopped flow, fast mixing, **100 nm** NP | Solid to disintegration and dissolution, nanoparticles, time |
| **GISim 2** | mixing, pH-jump, stiring | **flow through**, closed channel | Continuous flow, syringe pumps, **100 nm** | Solid to dissolution to nanoparticles, localized |
| **GISim 3** | stiring, pH-shift, pressure, flow | flow through, series of vessels | GI-Batch flow (n x 50 ml), peristaltic pumps for micro-particles (**50 µm**) | Stepwise tablet (solid) **nano-disintegration** and dissolution to nanoparticles |
| **GISim 4** | stiring, pH-shift, pressure, flow | flow through, series of vessels | GI-Batch flow, spiral pumps for large particles ( **2 mm** ) | Stepwise **coated tablet** disintegration to **µm particles**, later intestine dissolution to NP |
| **GISim 5** | stiring, pH-shift, pressure, flow | flow through, open channel, then vessels | GI-Batch flow, spiral pumps, solid form / tablet transport ( **2 cm** ) | **Enteric coated tablet** (solid) disintegration in intestine, dissolution to nanoparticles |

**Gastro-Intestinal simulator designs** for the *in vitro* study of stepwise drug form disintegration, dissolution (resolution): The technology is related to the micro- and nanostructure estimated by **projecting DLS** (10 nm – 200 µm) and neutron scattering **SANS** (1-200 nm), **SAXS** option.

**Duties of a GI-Simulator :**

- Interaction of **BCS2/4-drugs** in nanoparticles, with intestinal model fluids and **transient nanoparticles** therein (intermediates)
- **Licensing process** of drug formulations : kinetics
- **Food effect**, viscosity
- **Comparison** of pharmaceutical products, generic products
- **Methods** combined in GI-Sim:
  - **Drug solubility and uptake** (time resolved, in FaSSIF-C)
  - **DLS** (time resolved): **size and kinetics** of micelles, liposomes, large NP‘s
  - **SANS** (time resolved) **structure and dynamics** of nanoparticles

# Gastro-Intestinal –Simulator devices (GI – Sim)

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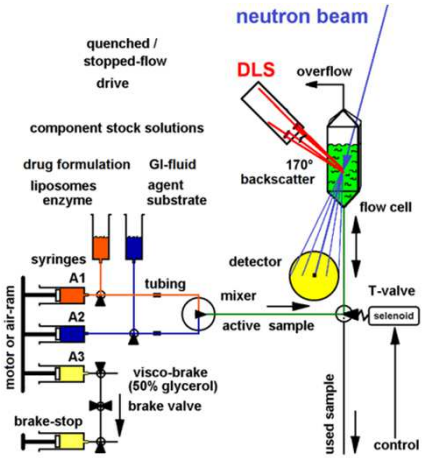
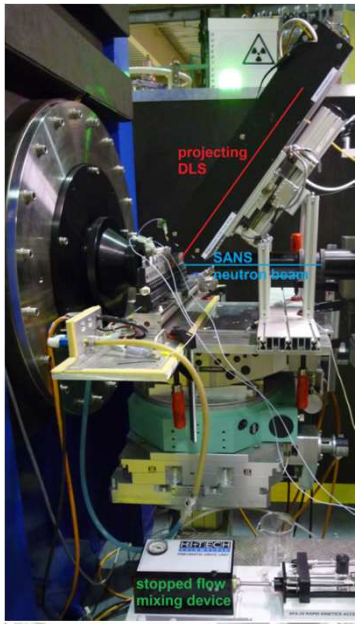
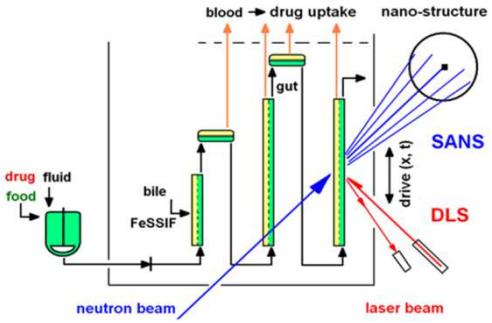
GISim 1 :
one shot mix



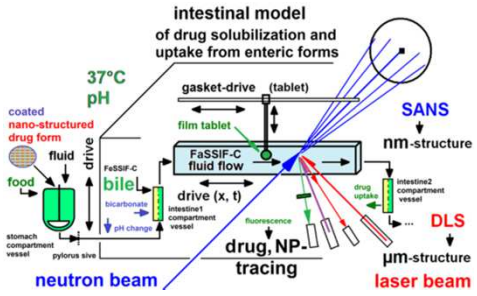




**GISim 1** setup for **SANS+DLS** with a stopped-flow mixer at ILL D11

- GISim 2: flow-through
- GISim 3, 4: batch flow (n x 50ml) multi-vessel
- GISim 5: coated solid form transport




The frame with the flow-chanel (Quarz, 200x12x2 mm) is moved in the beam




**GISim 2** setup for **SANS+DLS** with flow-through pumps at MLZ/JCNS KWS2

**DLS as q-range extension for large drug-particles**

**SANS for nanoparticles**

# Stopped-flow Simulator GISim1 with combined SANS+DLS

## Nanoparticle development in Intestinal model fluid

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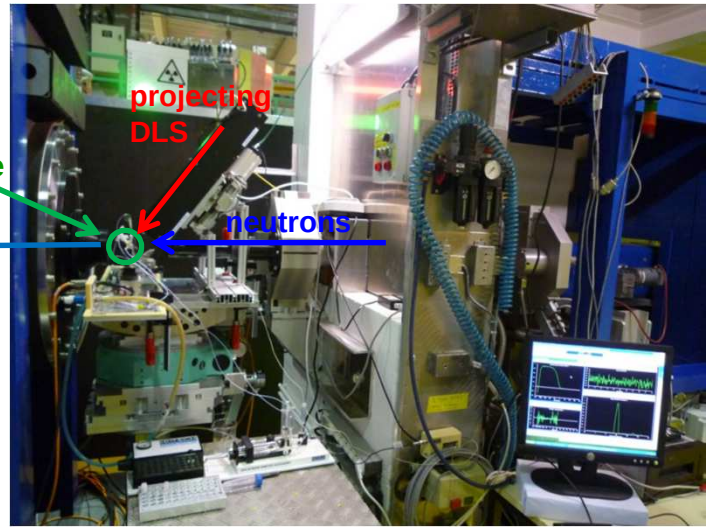
**D11**

**ILL-D11 :**



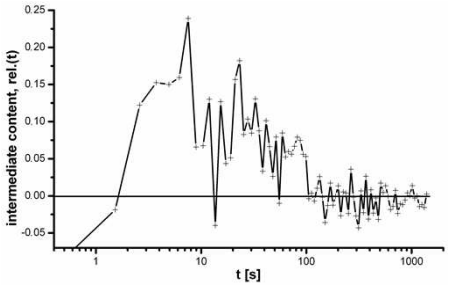

**ILL-D11 : setup**

**SANS:**

sample

SANS

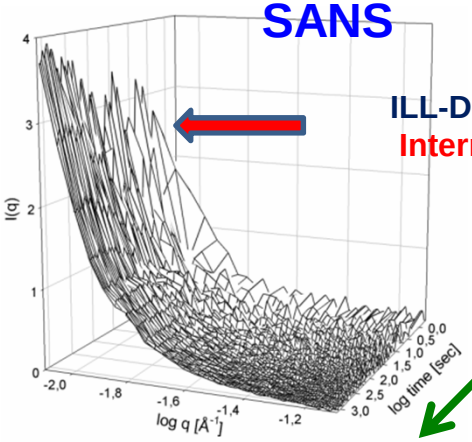
**Intermediate 1**
**in intestinal fluid**




- FaSSIF$_{mod6.5}$: pH-stabilized **intestinal model fluid**
  - HEPES as pH stabilizer
  - physiological osmolality
- Nanostructures develop in time after dilution (FeSSIF to FaSSIF, **bile influx in gut**)
  - time resolved **DLS (@university-lab and with SANS)**: large structures <50µm, but limited precision
  - time resolved neutron scattering **SANS**: high precision, component resolution by partial deuteration, 71% $D_2O$

**time resolved :**

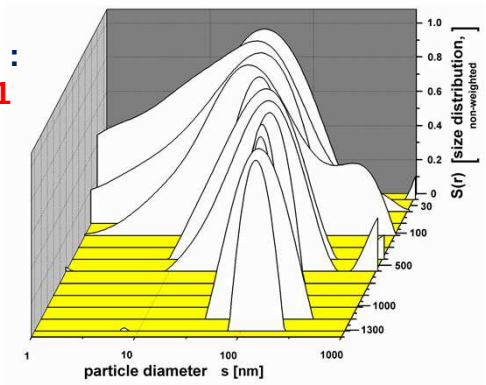
**SANS**




**ILL-D11 (2011) :**
**Intermediate 1**

**1h**

**+ DLS:**




- SANS-Facilities :
  1. ILL, Grenoble (D11)
  2. FRM2, Munich-Garching (KWS2)
- Results:
  - Intermediate nanostructures deteced at ILL (Grenoble) and FRM-II (Munich-Garching)
  - Components resolution by deuerium matching with $D_2O$-solvent possible (see <2> HST)
  - Drug contribution: Fenofibrate, Danazol, Griseofulvin,Curcumin (FRM-II KWS2, GI-simulator)

**Nanoparticle development : Micelles (bile) ⇒ Liposomes ⇒ Micelles ⇒ Solution**

Cooperation: **Ralf Schweins**
**ILL Grenoble,** LSS group

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Forschungs-Neutronenquelle
Heinz Maier-Leibnitz

# Flow-through GI-Simulator GISim 2 with Nano-analysis

## combination of stucture estimation with uptake/ dissolution

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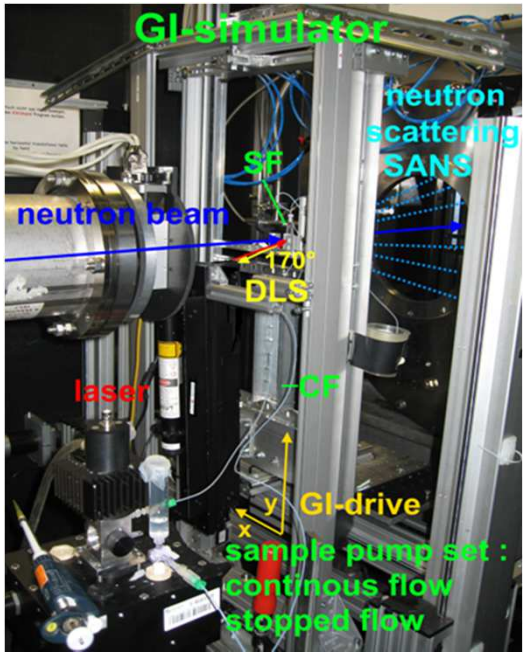
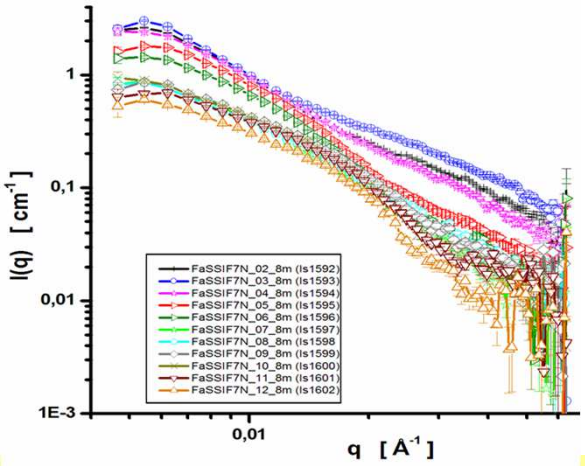
**Pharma-Technology**
**AK Langguth**

**xtr-SANS + DLS**
**flow-trough pumps**
**⇒ steady-state**
**Quarz-channel**
**200x12x2 mm**

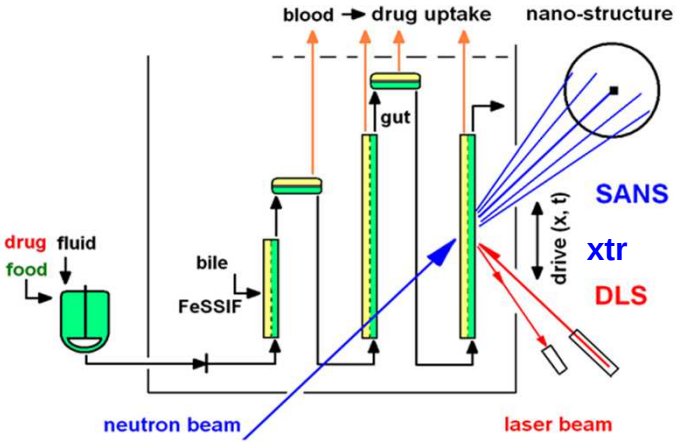



**GISim 2 simulator,**
drug uptake and digestion simulator concept,
NP structure analysis:
**continous flow CF**

**Drug analysis:**
- gut & blood side (layers)
- membrane separator - gut
- drug nano-dissolution




**Detection:**
- **drug-transfer (dialysis cells)**
- **DLS (x,t resolved)**
- **SANS (x,t resolved)**




- **Parallel tracing** of :
  - drug solubilization
  - nanoparticle structure
  - model uptake/ transport
- **Time- and local resolved drug & nanostructure profiles (2D)**
  $C_{drug}(x,t)$ for gut and blood side
- **SF: Stopped flow (duodenum-2)**
  for fast events (<20 cm, <20 min after bile influx into the Duodenum)
- **CF: Continous flow** (flow through) model device for **full simulaton**
  - module concept (flexible)
  - two compartment/ channels
  - long modules: uptake-dialysis
  - short modules: cell layer, gut
- Drug x-t-profiles: time-local resolved sampling
- **Nanostructure estimation**:
  - x-t profiles
  - DLS as overview (+-20%), in lab
  - neutron scattering SANS for precision and deuterium contrast
- **Resolution** of nanocarrier and drug:
  - for neutrons by deuteration (lipid, solvent contrast $D_2O$-matching)
  - option for improved tracing: FCPS (DLS with fluorescence)


T. Nawroth, K. Buch, P. Langguth, R. Schweins; (2011) Molecular Pharmaceutics / 8, 2162-72
Khoshakhlagh P., Johnson R., Nawroth T., Langguth P., Szekely N.K., et al. (2014) Eur J Lipid Sci Techn. 116, 1155-66
Johnson R., Nawroth T., Khoshakhlagh P., Langguth P., Szekely N.K., et al. (2014) Eur J Lipid Sci Techn. 116, 1167-73
Khoshakhlagh P, Johnson R, Langguth P., Nawroth T., Szekely N.K., et al. (2015) J Pharm Sci 104, 2213-24 .. FaSSIF-C

Membrane Pharmacy Structure Dynamics (MPSD) project

# Continous Flow (GSIM 2): time-space Resolution xtr

## long Quartz-cell with continous sample-mix supply

FRM II Forschungs-Neutronenquelle Heinz Maier-Leibnitz

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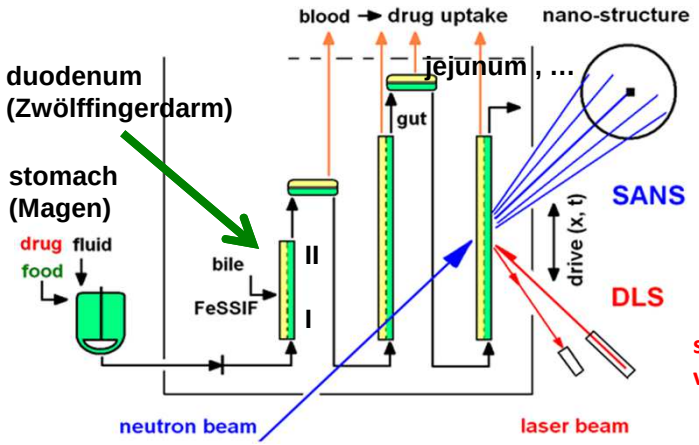
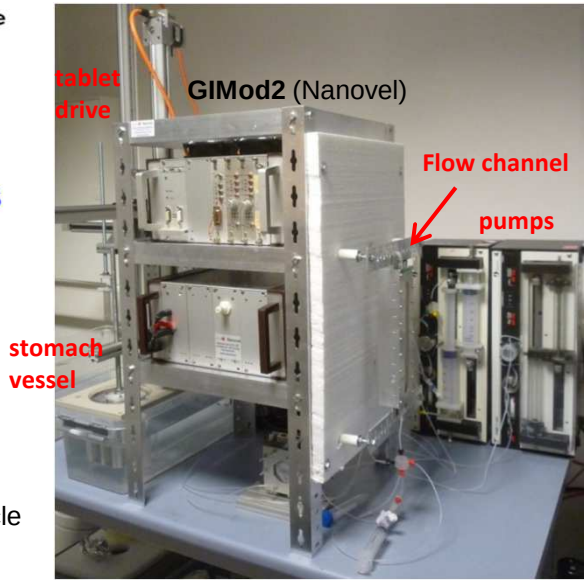
**Pharma-Technology**
AK Langguth

**V1 @ FRM-II_KWS2 : SANS + DLS**




**V2 @ Pharma-Lab : DLS+sampling**



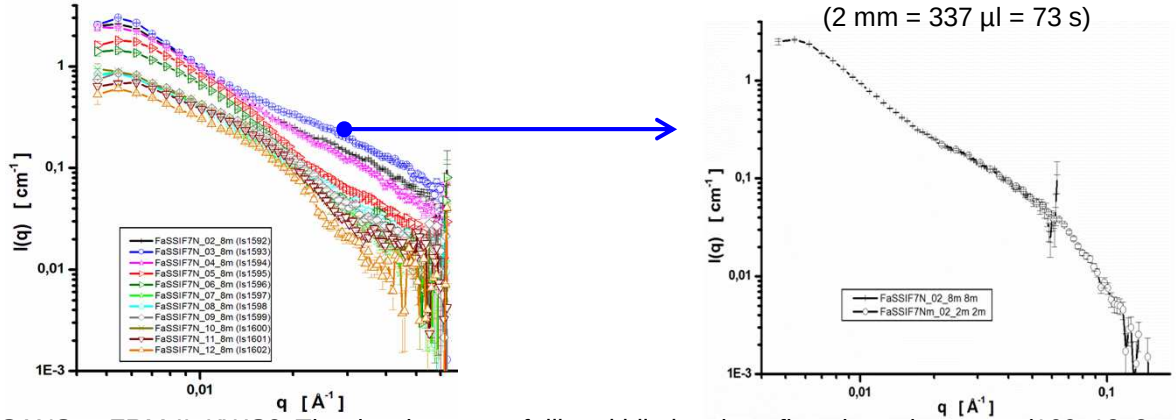

The continous flow (CF) modules simulate the gut segments (total: 6m; ~12h). In the channel nanoparticle structure develops in time and space (dx = dt).

- **Contiuous flow (CF) study of simulated intestinal colloids:**
  - Nano-development in **time & distance** : x, t
  - Long Quartz flow-through channel (cuvette)
  - Mixer in front (fluids + bile; by pumps)
  - SANS + DLS investigation (online or offline)
  - Large structure investigation *in situ* by projecting DLS: 50 nm – 200 µm (ProSpecD: Nanovel)

- **CF results at FRM-II_KWS2:**
  - Modules with melt-drawn Quartz ok.
  - Duodenum-II : segment after **bile** influx, **71%$D_2O$**
  - Shortest time (first frame @ 2mm) ~337 µl after mixing of model bile with fluid from stomach (TM)
  - Inspection by: **a) DLS, and b) neutrons, SANS**, CF-module on a CNC-drive) : well statistics
  - novel **bio-equivalent model intestinal medium** FaSSIF-7C, **containing 7% cholesterol**
  - Excipients (detergents+ targeting lipids)
  - Core-shell drug polymer nanoparticles and lipid particles

**Duodenum-II: a) CF-distance evolution during 30min.**

**b) shortest frame – distance**
(2 mm = 337 µl = 73 s)




CF SANS at FRM-II_KWS2: The development of diluted bile in a long flow-through cuvette (160x18x2 mm) from micelles to liposomes and large particles, with cholesterol containing model fluid FaSSIF-C (7% cholesterol)


Khoshakhlagh P, Johnson R, Langguth P., Nawroth T., Szekely N.K., et al. **(2015) J Pharm Sci** 104, 2213-24 .. **FaSSIF-C with Cholesterol**


Cooperation:
**Aurel Radulescu, Noemi Szekely**
**FRM-II, JCNS,** KWS2

# (III) Bio-Targeting and Bio-availability :

## targeting by ligands/ proteins at the particle surface

Membrane Pharmacy Structure Dynamics (MPSD) project

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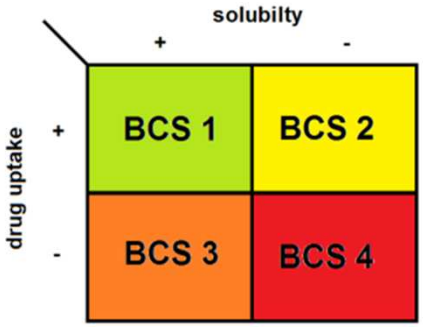
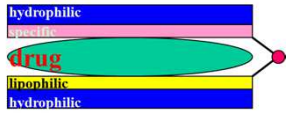
Pharma-Technology

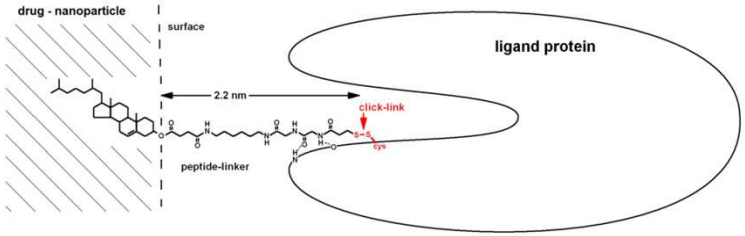
**Oral drug formulation development**
for difficult drugs : BCS 2, 4 , (3)



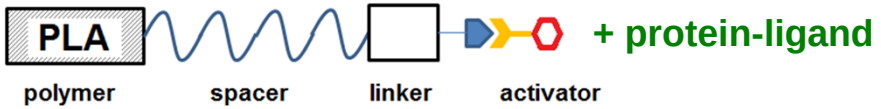





**principle:**
**4 domain targeting-excipient**




**Cholesterol-targeting construct**




**Polymer-targeting construct**

- **Cancer problem: in 95% of the cases, the disease is individual different at cell level**
- **=> Concept** of **individual therapeutics** by **case selective surface modification**
  - transport of anti-cancer drug by nanoparticles (10,000,000 drug molecules / particle of 100 nm size); for radiotherapy: Lanthanides, Isotopes (**Er, Gd, Lu, $^{10}$B, Pt, Bi**); or/and chemo-therapeutics
  - Individual modification of pre-manufactured Nano-carriers (coated) as very last step (1 day, „click-link“)
  - The surface ligand(protein) is recognized by over-expressed receptors of the cancer cell => endocytosis
- **Drug carrier and linker development:**
  - Domain structure and linker conformation (ligand exposition) **SANS + DLS** : **Deuterium-contrast SANS**
- **Product Quality control** : **DLS + SANS** for **medical security** (embolic risk exclosure)

Membrane Pharmacy Structure Dynamics (MPSD) project

# Target Polymer Nanoparticles as drug carriers

## PLGA triple component NP for modification by Ligands (Proteins)

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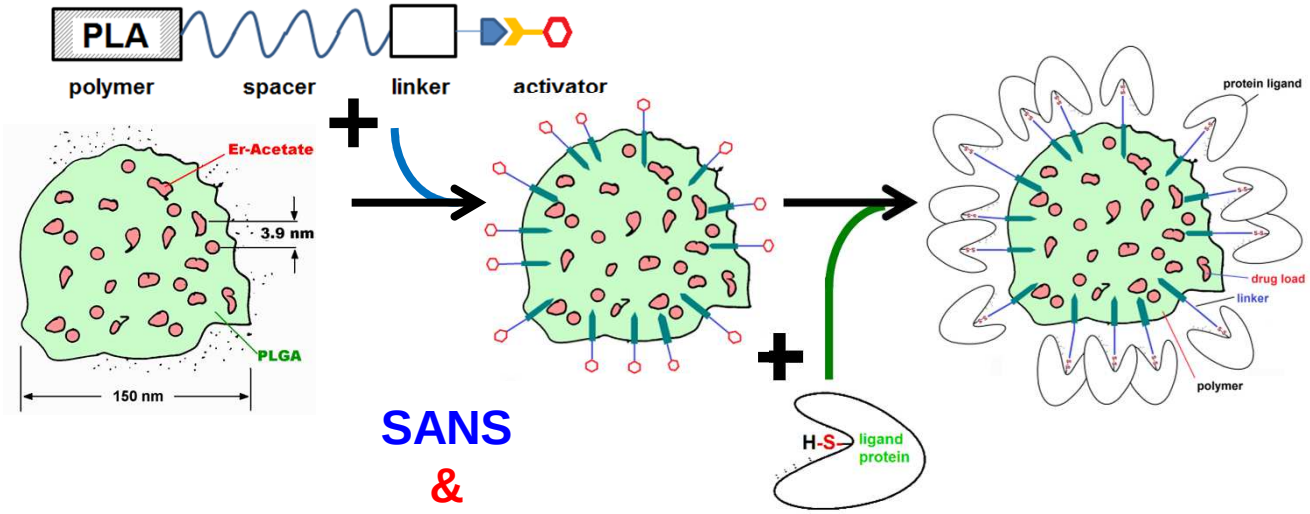
**PLGA** w/o/w nanoparticles (10% heavy metal) => **cell target nanoparticles**




**SANS**
**&**
**DLS** (offline)

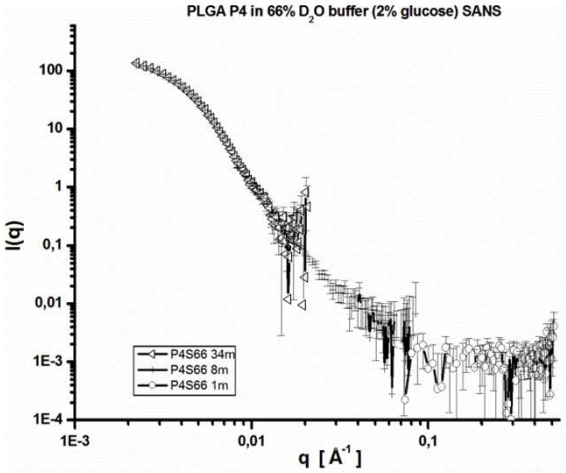
Cooperations:
**Ralf Schweins**
**ILL Grenoble,** LSS group

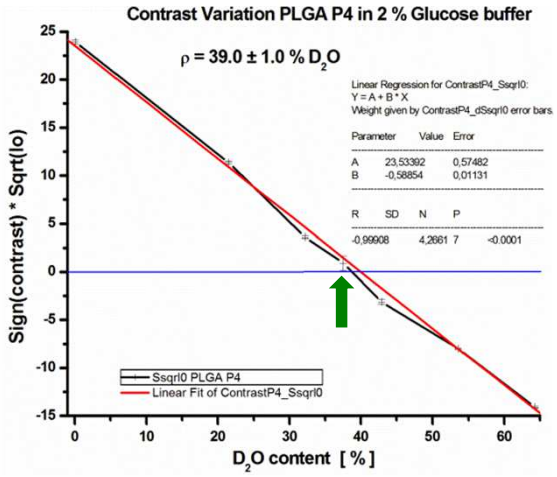
FRM-II, MEDAPP

**PLGA w/o/w triple NP's**
double emulsion + protein prepared from activated target-polymer: 2% PLA, 98% PLGA (Resomer 502H)

- **D-contrast variation SANS**
  **SANS + DLS**
- **Ligand-protein** loading on D-matched target-PLGA NP upon time-resolved SANS of D-contrast matched polymer nanoparticles (PLGA w/o/w)
- **Cell targeting** nanoparticles with surface-protein ligand as final product: non-toxic, bio-degradable; for **RT & CT**

**ILL-D11** (2015) **: SANS + DLS** of **PLGA** (w/o/w) NP with activated targeting polymer



# Target Nanoparticles Polymer for Cancer Therapy

## PLGA triple component NP : modification by Ligands (Proteins)

Membrane Pharmacy Structure Dynamics (MPSD) project

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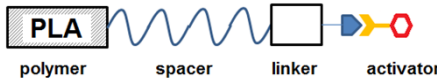
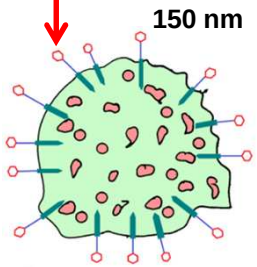
Pharma-Technology

**PLGA** w/o/w nanoparticles loading by **SH-protein** => **cell targeting nanoparticles**

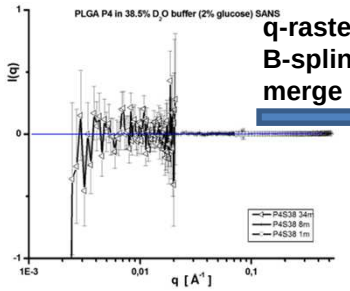
**ILL-D11 : TR-SANS + DLS** of **PLGA** (w/o/w) NP with activated targeting polymer + protein

Cooperation: **Ralf Schweins, ILL Grenoble,** LSS group

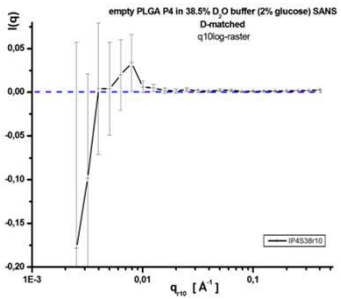


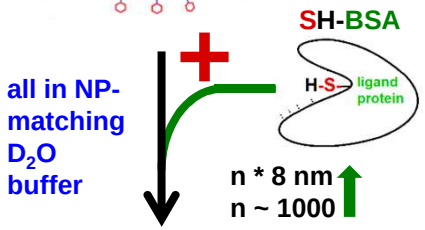




empty PLGA nanoparticles before loading



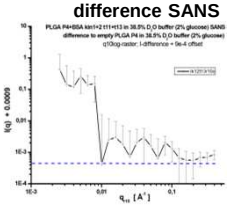

q-raster
B-spline
merge

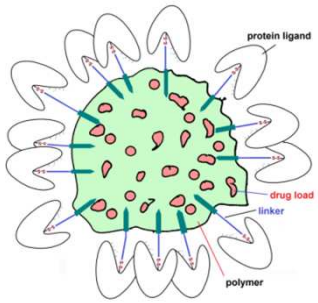


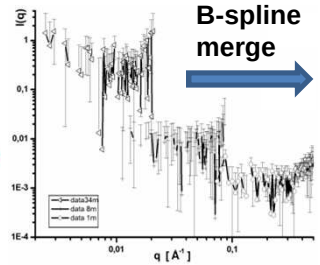




**Protein coupling of D-contrast matched Polymer nanoparticles for individual therapy**

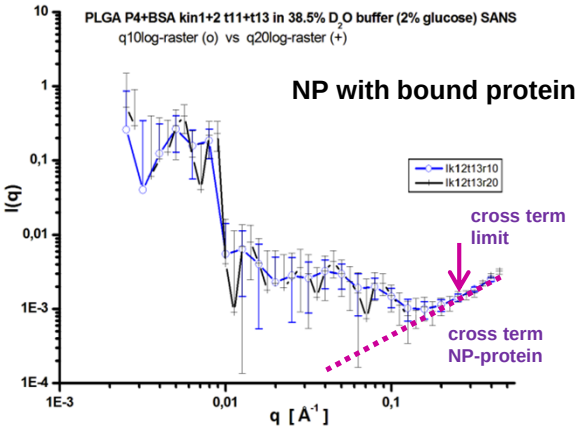
difference SANS



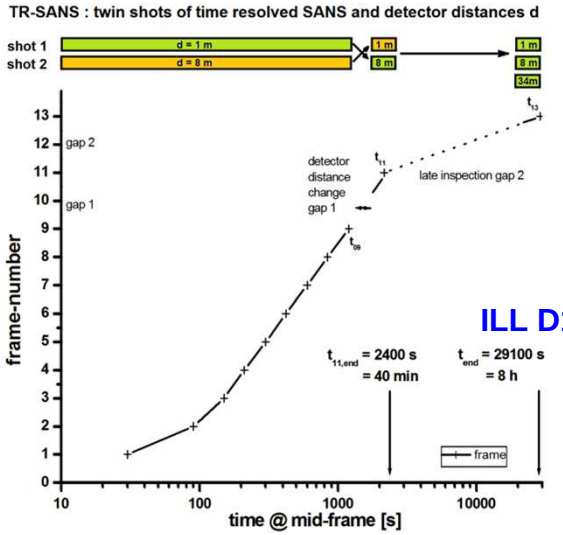




q-raster
B-spline
merge




SANS of protein-loaded polymer nanoparticles (PLGA w/o/w) in polymer-matching $D_2O$ buffer: logarithmic q-rastering for noise reduction




ILL D11

- **D-contrast variation SANS**
  **TR-SANS + DLS**
- **Ligand-protein** loading on **D-matched target-PLGA NP** upon time-resolved SANS of **D-contrast matched** polymer nanoparticles (PLGA w/o/w)
- noise reduction by q-rastering
- current work: improvements for stabilization by surface coatings

Membrane Pharmacy Structure Dynamics (MPSD) project

# Target Nanoparticles : Stabilization by Coating

## mixed shell of Polymer, Lipids and Ligands (target-Proteins)

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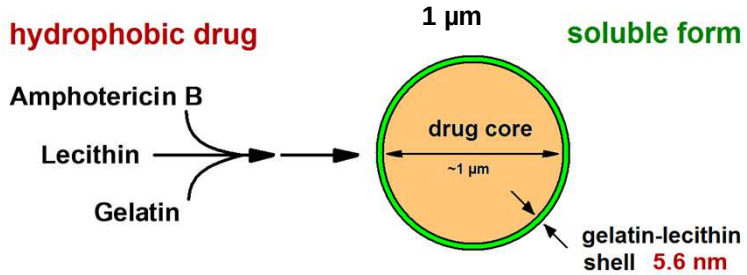
Pharma-Technology

JCNS KWS2

FRM II Forschungs-Neutronenquelle Heinz Maier-Leibnitz

### 1) Hydrophobic drug nanoparticles coating by lipid+gelatin => stable nanoparticles

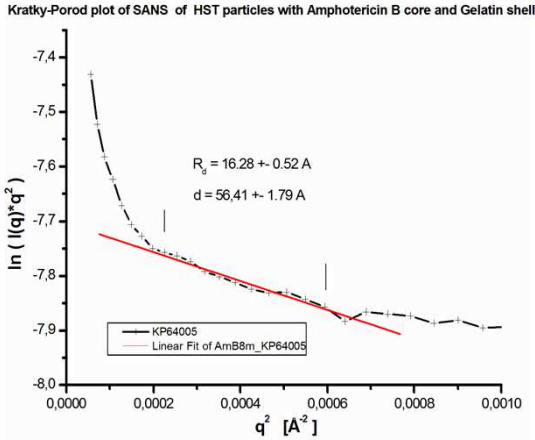
Cooperation: **Aurel Radulescu, Noemi Szekely, FRM-II, JCNS,** KWS2




AmB-entrapping **core shell nanoparticles :** estimation of the shell span by a Kratky-Porod plot evaluation of SANS yields a span radius $R_d$ = 1.63 +- 0.052 nm; i.e a shell span of d = 5.64 +- 0.18 nm, similar to biomembranes (lecithin bilayers ~ 5 nm).

a) R.Johnson, P.Khoshakhlagh, T. Nawroth, P. Langguth, N. Szekely **(2014) Eur J Lipid Sci Techn**. 116, 1167-73

b) Improvement : AmB in Cholesterol-DOPC (manuscript)




### 2) Polymer nanoparticles coating by SH-protein + polymer => stable cell targeting nanoparticles

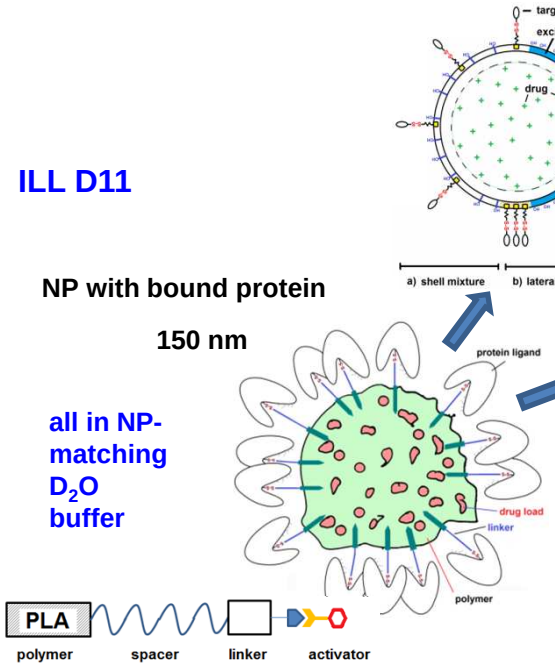
Cooperation: **Ralf Schweins ILL Grenoble,** LSS group

**ILL D11**

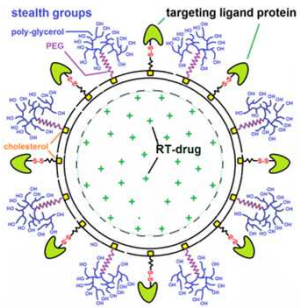
**NP with bound protein**

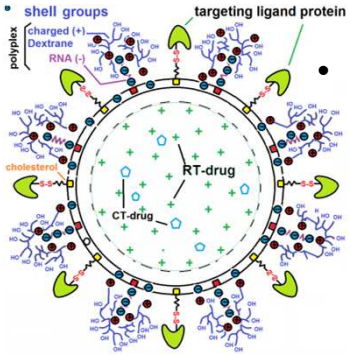
**150 nm**

**all in NP-matching $D_2O$ buffer**




**PLGA target nanoparticles with mixed coatings**



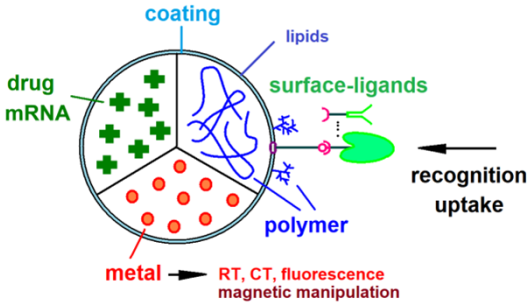

**Twin coating of D-contrast matched Polymer nanoparticles with protein targeting**

**ILL-D11: TR-SANS + DLS** of **PLGA** NP with activated targeting polymer + protein + lipid-coat

- **D-contrast variation SANS TR-SANS + DLS**
- **D-contrast matched** polymer nanoparticles (PLGA w/o/w) with protein ltargeting load
- current work: improvements for stabilization by surface coatings
  - **Lipid based coats, domains**
  - **Cholesterol-stealth coat**
  - **C-C-chain polymer coat (PVA)**
  - **Charge coulpled ionic coating (polyplexes with dextranes, mRNA / DEAE-Dextrane)**
  - **Hydrophobic drugs in NP**

Membrane Pharmacy Structure Dynamics (MPSD) project

# Conclusion

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**Pharma-Technology**
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NEUTRONS FOR SCIENCE AND HEALTH

JCNS
FRM II
Forschungs-Neutronenquelle Heinz Maier-Leibnitz

HZB BESSY

ESRF

DESY PETRA III

- Bio-Medical and Pharmaceutical Nanoparticles are **multicomponent systems** consisting of therapeutic drugs (10%), polymers, lipids, metal oxides (filler) and proteins, which can be **distinguished by neutron scattering with D-contrast variation**

**SANS Deuterium-contrast variation**

**ASAXS energy contrast variation**

- The **wide size scale** varies from 1 nm (drugs), over ~5 nm (proteins) to the upper 100 nm scale, in several cases up to 200µm. This requires a combination of complementary methods: **SANS + DLS (projecting dual optics, long focal length)**

**SANS**
**+ DLS / FCPS**

- The **SANS + DLS combination** can be applied **online** (dual beam) or **offline** (DLS immediately after SANS)
- Metal drug domains can be distinguished by **ASAXS** at the **L-edge**, eg. Erbium+Gd for **cancer radiotherapy**.

**SANS / SAXS**
**&**
**DLS** (online)
**DLS** (offline)

- For systems developing in **structure in a process**, e.g. digestion, binding, uptake, SANS and DLS have to be applied as **time resolved methods : TR-SANS + TR-DLS**

**TR-SANS / -SAXS**
**+ TR-DLS**

# Thanks

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**Pharma-Technology**
**AK Langguth**

Membrane Pharmacy Structure Dynamics (MPSD) project

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# Beam-Target Nanoparticles for Cancer Radio-Therapy

## Liposomes and PLGA NP for Radiotherapy by Photons and Neutrons

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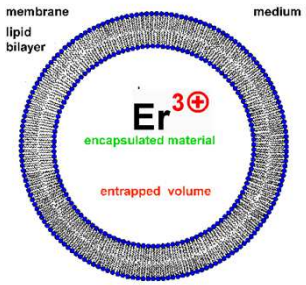
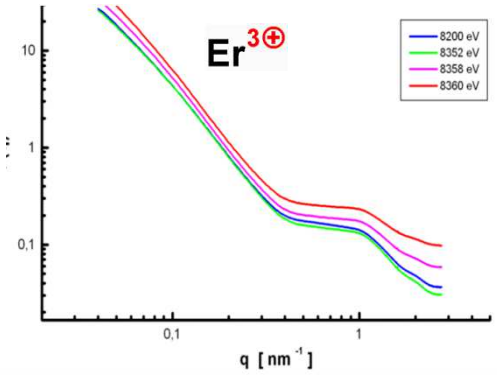
Pharma-Technology

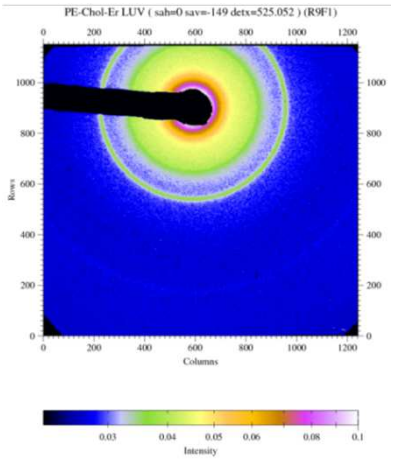
**Liposome** nanoparticles with entrapped Erbium




HZB / BESSY




ESRF




Cooperation:
**Peter Boesecke**
ESRF
**ID01**

Cooperation:
**Guenther Goerigk**
**HZB / BESSY**
M. Ballauf group

- **Liposomes** from lecithin (DOPC) with entrapped **metal salt** solution (0.5 M)
- L-edge contrast variation SAXS = ASAXS (5 energies)
- Bio-targeting by protein ligand surface modification

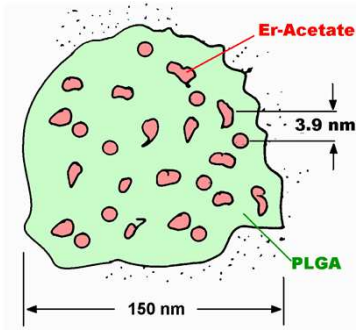
**ASAXS & SAXS + DLS** (offline) **: done at BESSY, option for DESY – PETRA III , P12**

**PLGA** w/o/w nanoparticles (foam-like) with Erbium in domains

2x ultrasound treatment, wash

**PLGA** in $CH_2Cl_2$ → **w/o emulsion** in $CH_2Cl_2$ → **1% PVA** in $H_2O$

**Er-Acetate** in $H_2O$




ASAXS @ HZB BESSY

- **PLGA NP's 10% metal** prepared as w/o/w double emulsion from polymer (PLGA) and metal-salt solution (Erbium-Acetate)
- L-edge contrast variation SAXS = ASAXS (5 energies)

Cooperation:
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**HZB / BESSY**
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**JGU Mainz** / inventors: Buch K, Nawroth T, Langguth P, Schmidberger H:
**European Patent** **WO 2013/037487** / **EP 2 567 702 A1**
(2013/12) „Diminishing Cell Growth with Metal-Polymer Nanoparticles upon Radiotherapy" … **Metal-PLGA** as example

**BMBF project 05KS7UMA**

**w/o/w double emulsion NP**
**8 - 10% metal , in $H_2O$**

**SANS** : @ **ILL-D11**, Ralf Schweins