Nanoscale Functionalities for Targeted Delivery of Biopharmaceutics NANO(BIOPHARMACEUTICS)

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General Assembly ETP Nanomedicine

16 January, 2008, UCB Center, Brussels, Belgium

Consortium

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- ✓ Johannes Gutenberg University Mainz, Germany
- ✓ Medical University Graz, Austria
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European Dimension of Partnership

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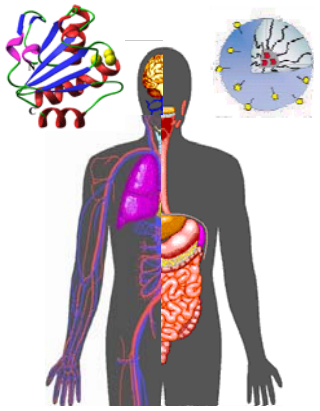
Our Mission

- Diabetes, cancer, AIDS, Alzheimer's disease, and other neurodegenerative diseases represent major challenges for today's society, especially in combination with the increased age of the average population.
- "NanoBioPharmaceutics" aims at breakthrough advances in novel biopharmaceutics delivery systems for the treatment of these and other diseases.
- Nanocarrier-based protein/peptide (P/P) delivery systems for pulmonary and oral delivery and blood brain barrier (BBB) crossing applications are developed within this project allowing a targeted and controlled release of the drugs.



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Objectives

- Design, synthesis and functionalization of novel nanocarriers and nanoparticle-based microcarriers for targeted delivery of P/P drugs via oral, pulmonary and BBB crossing administration routes.
- Toxicological screening of the nanocarriers, investigation of the release profile of P/P drugs under various environmental conditions and assessment of the biocompatibility and biodegradability of the new formulations.
- Novel pulmonary P/P carriers with improved delivery features to overcome the administration difficulties and increase efficiency of protein delivery to the deep lung.
- Oral nanoparticulate P/P carrier systems capable of adhering to the gastrointestinal mucosa and also displaying protective and permeation enhancing properties.
- Development of an in vitro model for the assessment of nanocarriers' permeability through the Blood Brain Barrier (BBB).

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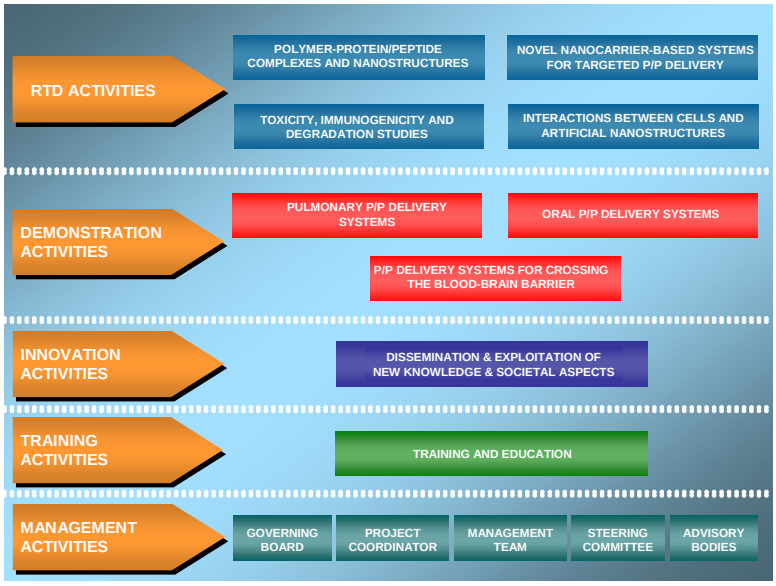
Project Structure



RTD Activities

WP1: Polymer-Protein/Peptide Complexes and Nanostructures

Objective: Modification and direct functionalization of therapeutic proteins and their analogues (peptides) for the enhancement of their biological stability

Tasks:

1.1: *Modification and Direct Functionalization of P/P Biopharmaceutics*
1.2: *Polymer-Protein/Peptide Complexes*
1.3: *Bioconjugate-Protein/Peptide Nanostructures*

WP2: Novel Nanocarrier-based Systems for Targeted P/P Delivery

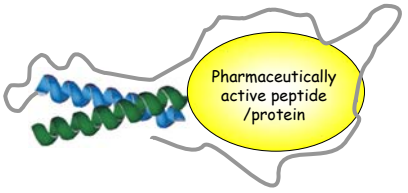
Objective: Design and synthesis of functionalized nanocarriers for P/P delivery in the form of nanoparticles, micelles, dendritic polymers, pegylated liposomes, organic/inorganic nanocomposites and nanogels

Tasks:

2.1: *Synthesis of Functionalized Nanocarriers*
2.2: *Functionalization for Active Targeting*
2.3: *Development of Nano-scale based Microcarriers*

Delivery of Proteins/Peptides

- Critical issues associated with P/P delivery:
  - ✓ Due to their large molecular weight and size, they show poor permeability characteristics through the various mucosal surfaces and biological membranes.
  - ✓ P/P tertiary structure can be lost under various physical and chemical environments with consequent loss of their biological activity.
  - ✓ P/P drugs have very short biological half-lives in vivo due to their rapid clearance in the liver and other body tissues by proteolytic enzymes.
  - ✓ P/P drugs have very specific actions and are highly potent, their precise clinical dosing is of utmost importance.



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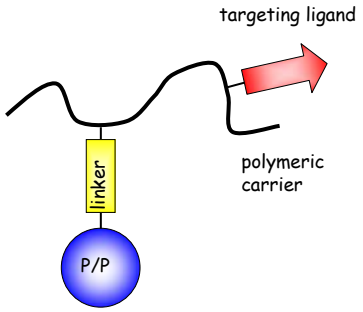
Polymer- P/P Drug Complexes

- Covalent attachment of P/P Drugs to polymer chains via specific linkers.



• **P/P**	**: peptide / protein**
• **Linker**	**: enzymatically or pH sensitive**
• **Targeting ligand**	**: peptide / saccharide**
• **Polymer carrier**	**: Hydrophilic polymers, polyelectrolytes**

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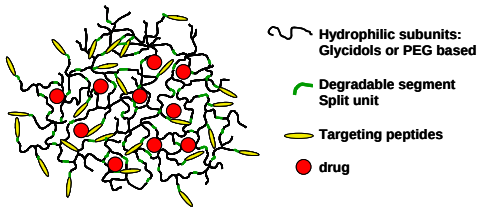
WP2: Novel Nanocarrier-based Systems for Targeted P/P Delivery

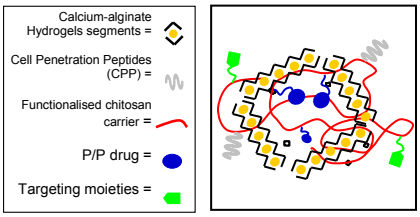
Carrier Systems

- Thiomer NPs
- Nanoaggregates based on Glycidol Copolymers
- Nanogels based on Tailored Glycidols
- PABOL / Insulin Nanoaggregates
- Alginate/Chitosan NPs
- Vesicles
- Functionalized gold NPs
- Biodegradable polymeric NPs

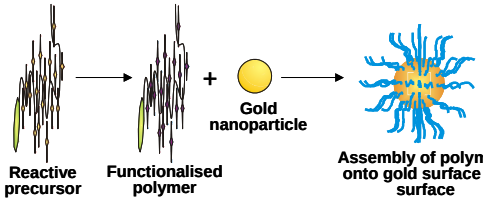
Nanogels based on Tailored Glycidols



Alginate/chitosan NPs



Functionalized gold NPs



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WP1 & WP2

	Technology Platforms	Applications: Pulmonary delivery	Applications: Oral delivery	Applications: Blood-brain barrier
WP 1	Peptides/Proteins (P/P)			
WP 1	P/P modification			
WP 1	Self-assembling P/P complexes			
WP 2	P/P loaded polymersomes/liposomes			
WP 2	Geldepots and microgel systems			
WP 2	Surface functionalized nanoparticles			
WP 2	Nanoparticle aggregates			
WP 2	Functionalization for active targeting			

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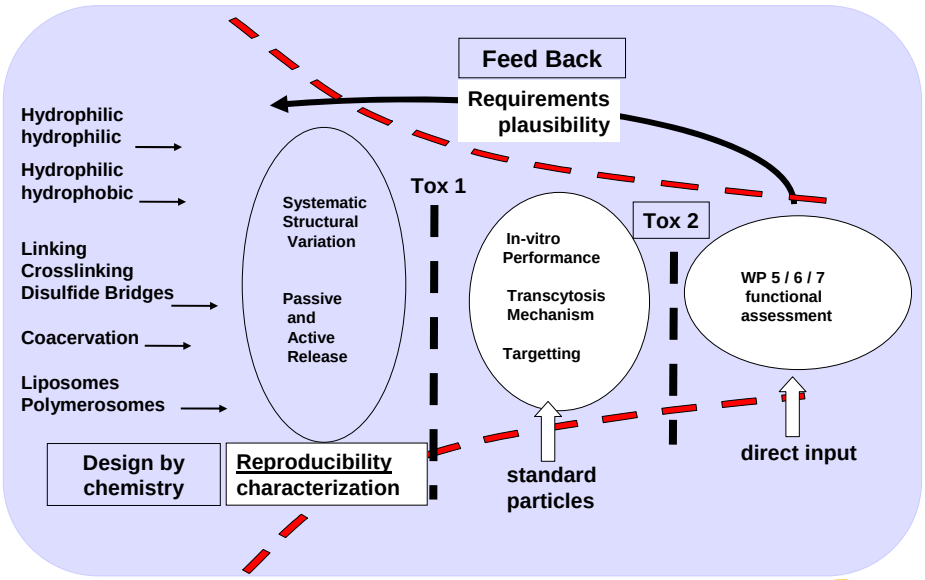
WP1 & WP2



RTD Activities

WP3: Toxicity, Immunogenicity and Degradation Studies

Objectives:

- ✓ Prevention of the use of toxic nanocarriers in in-vivo experiments
- ✓ Assessment of different aspects of toxicity for nanoparticles

Tasks:

3.1: *In vitro Toxicological Screening*

3.2: *In vitro Screening of Targeting Ability*

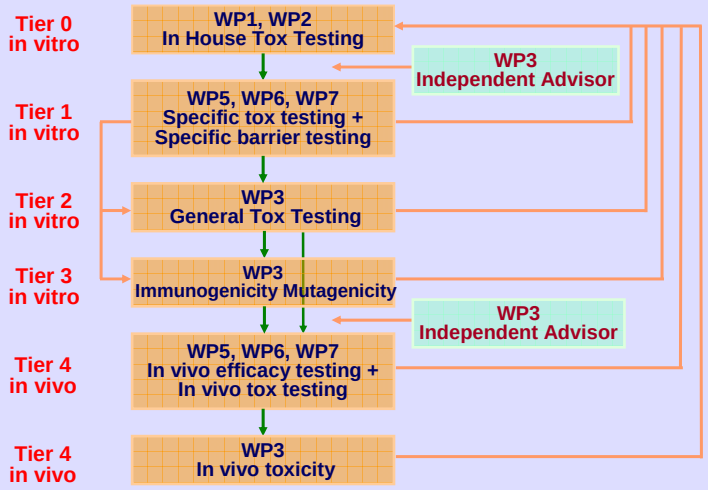
3.3: *In vitro Cytotoxicity, Hemocompatibility, Immunogenicity and Genotoxicity Testing*

3.4: *In vivo Cytotoxicity, Immunogenicity and Genotoxicity Testing*

3.5: *Traced Model Drug and Carrier Biodistribution and Degradation*

WP3: Toxicity, Immunogenicity and Degradation Studies

General Tox Strategy

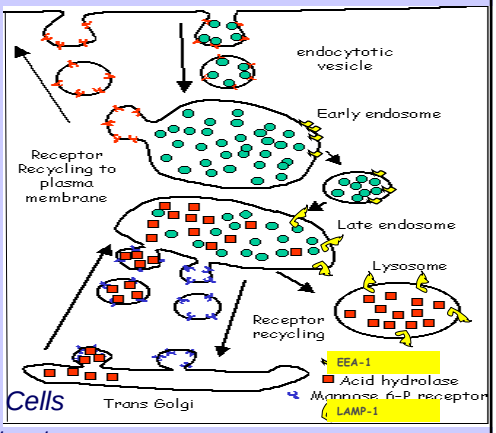


RTD Activities

WP4: Interaction between Cells and Artificial Nanostructures

Objectives:

- ✓ Elucidate the mechanisms of adsorption, uptake and exocytosis / transcytosis of specific artificially engineered nanoparticles (in terms of size, shape, charge and surface makeup) by cells using in vitro cell / barrier model systems.



Tasks:

4.1: *Absorption of Nanostructures to Cells*

4.2: *Uptake and Recycling of Nanostructures*

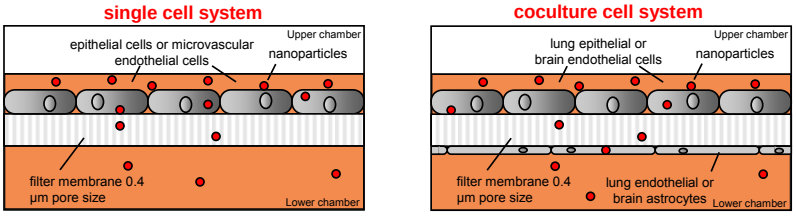
4.3: *Trans-endocytosis of Nanostructures*

4.4: *Endosomal Escape*

WP4: Interaction between Cells and Artificial Nanostructures

In vitro Cell Culture Methods

- Development of in vitro cell model systems to examine how nanocarriers react with the relevant cells that make up the barrier systems of WP5-7.
- Use of cell lines as well as primary human cells cultured on cell culture plastic and in transwell systems.
  - ✓ Human epithelial cells from the lung and intestine cell lines.
  - ✓ Human microvascular endothelial cells: from skin, lung and brain; pig brain tissues.
  - ✓ Co-culture cell models: Alveolar+endothelial cells; enterocytes+goblet cells.



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Demonstration Activities

WP5: Pulmonary P/P Delivery Systems

Objectives:

- ✓ Development of novel pulmonary P/P formulations with good flow and dispersability properties, based on controlled aggregation of functionalized nanoparticles.
- ✓ In vitro and in vivo examination of nanocarriers / formulations ability to cross the air-blood barrier and their interaction with lung epithelial and endothelial cells (establishment of in vitro and in vivo models).

Tasks:

5.1: *In vitro Model Prediction of Pulmonary Drug Uptake*
5.2: *In vivo Assessment of Bioavailability and Biofeedback Studies*
5.3: *Aerodynamic Characterization and Modeling*
5.4: *Nasal Delivery of Vaccines*

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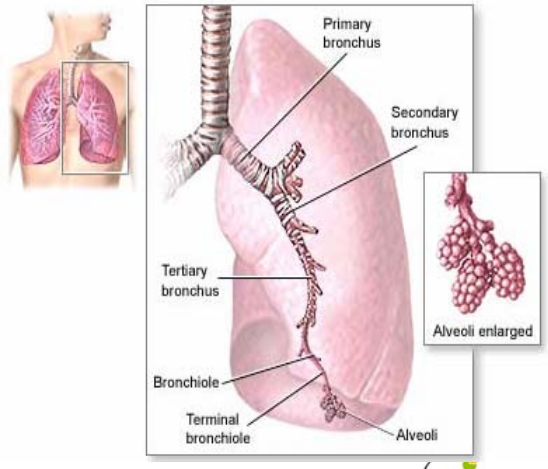
WP5: Pulmonary P/P Delivery Systems

Pulmonary Administration Route :

- Local delivery
- Systemic delivery

Main Barriers for Pulmonary Administration Route:

- Air-way geometry
- Humidity
- Clearance mechanisms
  - ✓ *mucociliary clearance*
  - ✓ *alveolar macrophages*
  - ✓ *metabolic activity of enzyme in the lung*
- Lung diseases
  - ✓ *mucus accumulation*
  - ✓ *alterations in lung architectu*



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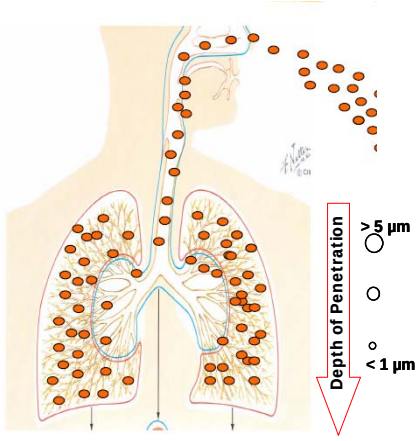
WP5: Pulmonary P/P Delivery Systems

Problems to be Addressed

- Carrier Development
  - ✓ *Carrier specifications*
  - ✓ *Synthesis methods*
  - ✓ *Loading of P/P drug*
- Functionalization / Targeting
- Aerosolization / Nebulization
- Aerodynamic Characterization
  - ✓ *Modeling*
  - ✓ *CFD simulations*
- Deposition



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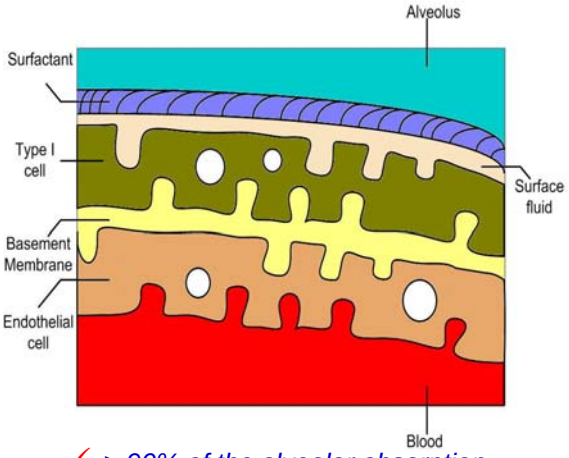
WP5: Pulmonary P/P Delivery Systems

Air-Blood Barrier



- Lung Surfactant: Molecular monolayer spreading at the air/water interface.
- Surface lining fluid: i) mucus containing airway fluid (5-10 μm) & ii) alveolar fluid (0.05-0.08 μm).
- Epithelium cell monolayer.
- Interstitium and basement membrane: extracellular space inside tissues
- Vascular endothelium cell monolayer.

✓ *> 90% of the alveolar absorption 'barrier' is in the epithelium*

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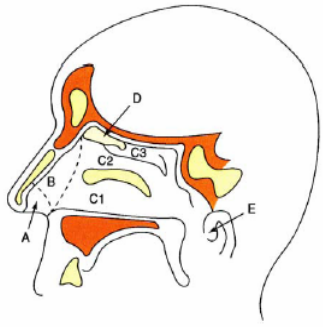
WP5: Pulmonary P/P Delivery Systems

Nasal Vaccination

- Viral infections can be acquired through the nasal associated lymphoid tissue (NALT).
- Both mucosal and systemic immune responses can be induced, in contrast to injected vaccines, which only induce a systemic immune response.
- Intranasal immunization is straightforward (i.e., administration via drops or sprays) and in general lower doses are required to elicit comparable antibody titers than those elicited by other mucosal routes of immunization.



Sagittal section of human nasal cavity

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Nasal Vaccination Requirements

Three major parts should constitute the nanostructure-based vaccines: the carrier, the antigen and danger signals (e.g., lipopolysaccharides or their derivatives, ssRNA, etc.)

Carrier Properties

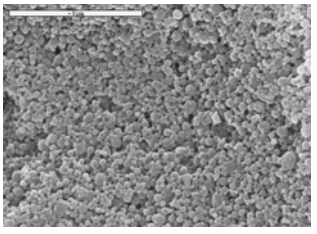
- Size in the range of 100 nm would be ideal for both sterile filtration and optimal in vivo efficacy.
- NPs should preferentially be mucoadhesive with negative zeta potential.
- Positively charged NPs may aggregate due to the presence of negatively charged substances in the mucus.
- A minimum 3-4 weeks stability (at least with respect to size and antigen integrity) is required.
- Injectable, isotonic excipient/buffer solutions can be used.

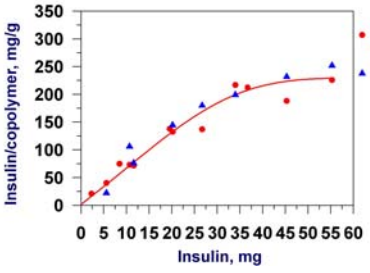
WP5: Pulmonary P/P Delivery Systems

Nanocarriers

- Insulin and ovalbumin (OVA) loaded PLGA NPs.
- Insulin and OVA loaded PGly-b-PLLA and PGly-b-PDLA NPs.
- Nanogels crosslinked with disulfide linkages.
- SS-poly(amidoamine)-protein NPs
- Liposomes containing protein/peptide vaccines composed of a variety of lipids.

OVA loaded NPs





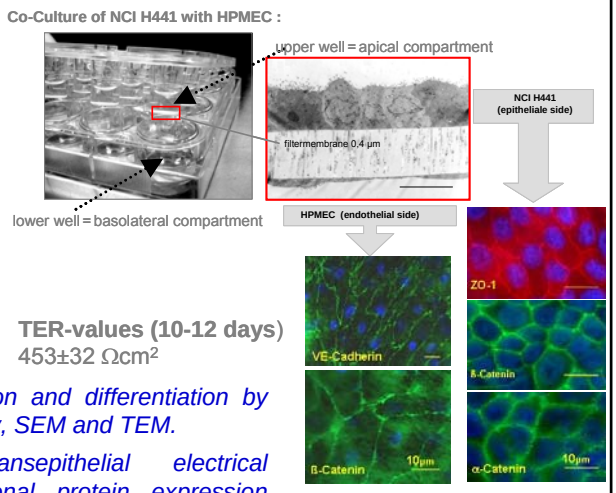
Functionalization of NPs with targeting peptides, cell penetration peptides, functionalized sugars, lectins (e.g., TL and WGA) as well as the Fc region fragment of the IgG

✓ *Insulin loading: 5->20%wt*

WP5: Pulmonary P/P Delivery Systems

Co-culture Model

- In vitro co-culture model of a human distal lung epithelial cell line (NCI H441) and pulmonary microvascular endo-thelial cells (HPMEC) on opposite sides of transwell filter units.



TER-values (10-12 days)
453±32 Ωcm²

- ✓ *Morphology, cell orientation and differentiation by light & confocal microscopy, SEM and TEM.*
- ✓ *Barrier properties: transepithelial electrical resistance (TER), junctional protein expression (immunofluorescence).*

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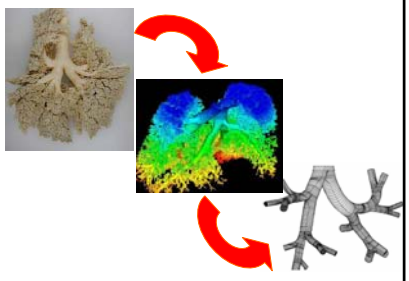
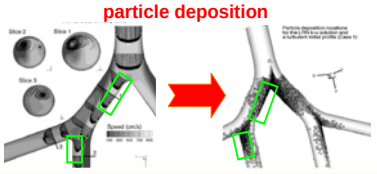
WP5: Pulmonary P/P Delivery Systems

CFD Simulation of Pulmonary Delivery

- Modeling of particulate fluid flow in lungs (global)
  - ✓ *Physiological modeling of lungs, CFD simulations of flow based on lung models, particle tracking simulations based on CFD results.*
- Modeling of particle / cell-layer interaction (local)
  - ✓ *Modeling of particle motion proximal to cellular or mucosal surface and mechanics of particle collision with surface.*
- CFD Simulations
- Particle Deposition Simulations
  - ✓ *Particle Motion based on Newtons Law*
  - ✓ *Identify deposition areas*
  - ✓ *Determine Local Particle fluxes*



Lagrangian /Eulerian simulation of particle deposition



Particles deposit at regions where the main flow is in proximity to the wall

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Demonstration Activities

WP6: Oral P/P Delivery Systems

Objectives:

- ✓ Assess the potential of nano- and micro-carriers (consisting of nanoparticles) having been prepared in WPs 1&2 as delivery systems for oral peptide/protein drug administration.

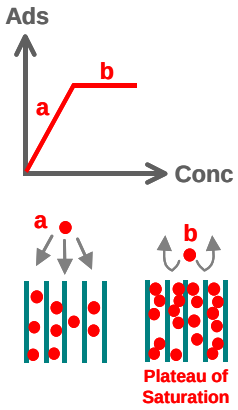
Tasks:

6.1: *In vitro Model Prediction of Oral Drug Uptake*
6.2: *In vivo Assessment of Bioavailability and Biofeedback Studies*
6.3: *Evaluation of Gastrointestinal Transit times of DDS*

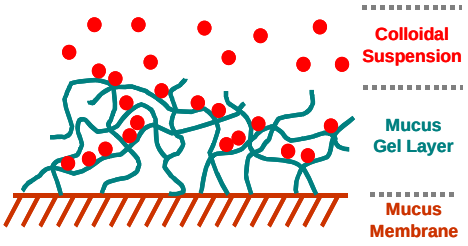
WP6: Oral P/P Delivery Systems

- ➢ Advantages of Oral Delivery:
  - ✓ Ease of administration
  - ✓ Patient acceptability and compliance
  - ✓ Large surface area for systemic absorption
- ➢ Barriers to Oral Delivery of P/P drugs:
  - ✓ Acid-induced hydrolysis in the stomach.
  - ✓ Enzymatic degradation through the gastrointestinal track (GIT).
  - ✓ Bacterial fermentation in the colon.
  - ✓ Unstirred aqueous boundary layer and viscous mucus layer covering the surface of the GI epithelium.
  - ✓ Intercellular spaces gated by closely fitting tight junctions.

WP6: Oral P/P Delivery Systems



For particles <1µm



- ✓ *The linear increase in the isotherm corresponds to the creation of new adsorption sites when the bulk particle concentration is increased.*
- ✓ *These sites are available for further adsorption up to the isotherm plateau which corresponds to a saturation of the available sites.*

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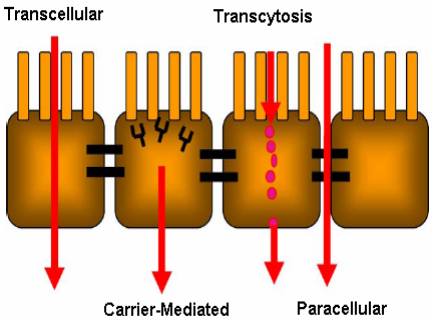
WP6: Oral P/P Delivery Systems

- Transcellular passive transport
  - ✓ Drug lipophilicity is the most important parameter for the drug crossing through this route.
- Transcellular carrier - mediated active or facilitated transport
  - ✓ Carrier size and its surface functionalization are crucial parameters.
- Paracellular passive transport
  - ✓ Drug hydrophilicity and molecular size are the most important parameters for the paracellular drug crossing. This route takes advantage of the leakiness of cell to cell junction.

Transepithelial pathways available for transport from intestinal lumen to the bloodstream:



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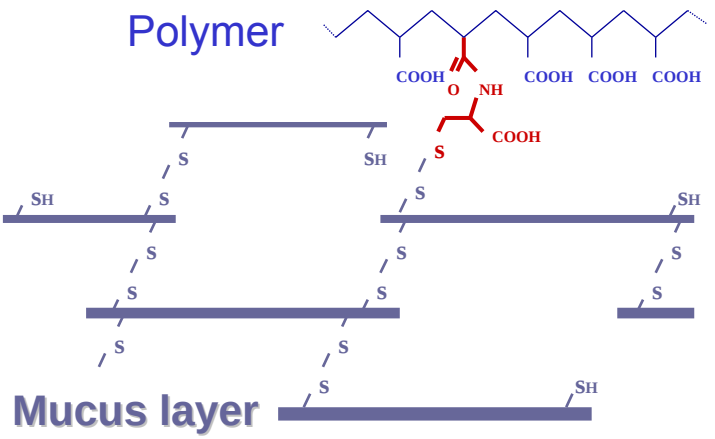
Carrier Requirements

➢ Systemic Delivery:

- ✓ Size: 100nm – 10μm in artificial intestinal fluid
- ✓ Strong mucoadhesive properties (e.g., chitosan)
- ✓ Permeation enhancing properties (polymeric permeation enhancers like medium chain fatty acids – a steeper concentration gradient as driving force for passive drug uptake can be achieved)
- ✓ Protective effect towards pepsin (unless enteric coated) & trypsin
- ✓ No aggregation (surface area available for carrier – mucus gel layer interactions is reduced ⇒ particle penetration is reduced)
- ✓ Insulin release: 2hrs (50% per hour)

✓ **Thiomer-Nanoparticles:**

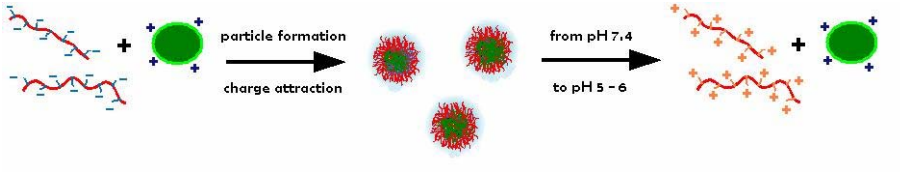


WP6: Oral P/P Delivery Systems

Nanocarriers

- Thiolated Polyacrylic Acid NPs
- Thiolated chitosan NPs
- Modified Alginate Micro- /NPs
- SS-functionalized Poly(amido amine) NPs
- Polyglycidol-b-polylactide copolymer NPs
- Modified PLGA NPs

SS-Poly(amido amine)-protein nanoparticles



✓ *pH-Triggered protein release from SS-PAA nanoparticles by charge reversal of the polymer.*

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Demonstration Activities

WP7: P/P Delivery Systems for Crossing the Blood-Brain Barrier

Objectives:

- ✓ Examination of the functionalized nanocarriers prepared in WPs1&2 for targeted peptide/protein delivery over the blood-brain barrier (BBB).
- ✓ Establishment and use of in vitro and in vivo methods for the estimation of brain uptake index and brain permeation ability of the carriers.

Tasks:

7.1: *Analysis of Carrier Interaction with an In vitro Model of the BBB*

7.2: *Peptide/Protein Distribution in the Brain*

7.3: *Peptide/Protein Action in the Brain*

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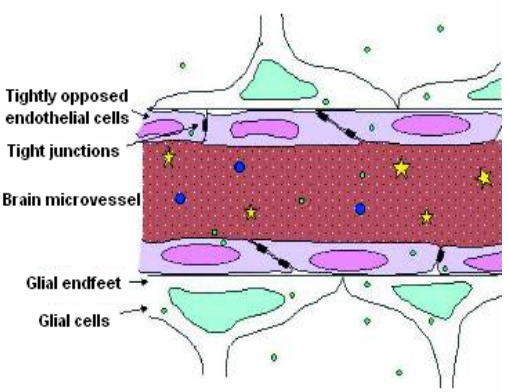
Blood Brain Barrier (BBB)

- The BBB is a very specialized barrier system of endothelial cells.
- It separates the blood from the underlying brain cells.
- It provides protection to brain cells and preserving brain homeostasis (stability).
- The brain endothelium has a complex arrangement of tight junctions between the cells that restricts the passage of molecules.

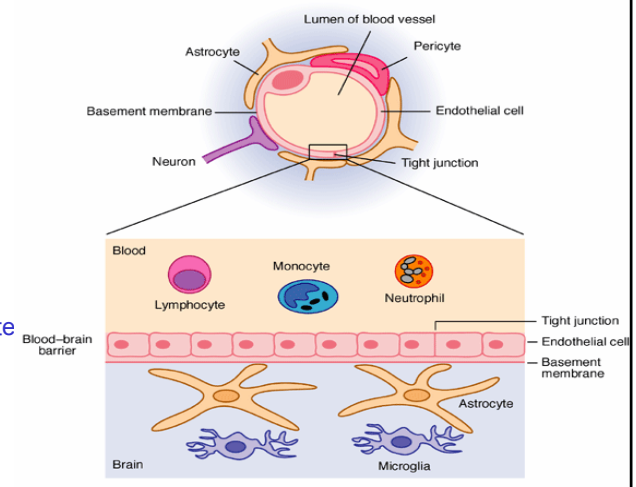
Morphology of the Blood-Brain Barrier



Delivery to the Brain

- Ideal Properties of Polymeric-Based Nanoparticles for Drug Delivery to the Brain:

- ✓ Stable in blood
- ✓ Non-toxic
- ✓ Non-thrombogenic
- ✓ Non-immunogenic
- ✓ Non-inflammatory
- ✓ Biodegradable
- ✓ Avoidance of the reticuloendothelial syste



WP7: P/P Delivery Systems for Crossing the Blood-Brain Barrier

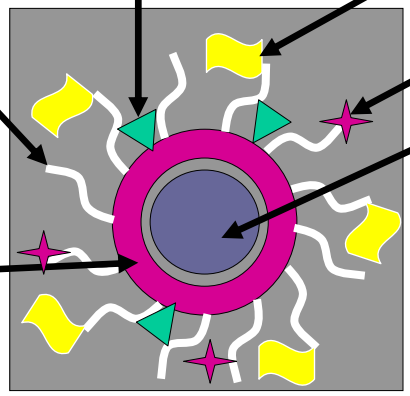
Nanoparticle Technology

Surface stabilizers:
- lecithin
- tween 80
- polyprpoylene oxide (poloxamer 188)
- poloxamine 908
- PEG
- polyacrilamide (PAA)
- polyvinyl pyrrolidone (PVP)
- sodium glycocholate
- polyvinylpyrrolidone (PVP)
- OX26 MAb

Polymer composition:
- dextran
- starch
- chitosan
- gelatin
- albumin
- sodium alginate
- polylactic acid (PLA)
- polyglycolic acid (PLGA)
- polymethyl methacrylate (PMMA)

Mask for macrophage system:
- albumin
- apoliprotein A-I, A-IV, C-III, E and H

Receptor targeting molecules:
- anti-transferrin receptor MAb
- anti-insulin and insulin-like growth factor receptor MAb
- anti-leptin receptor MAb
- anti-lectin receptor MAb
- recombinant human melanotransferrin (P97)



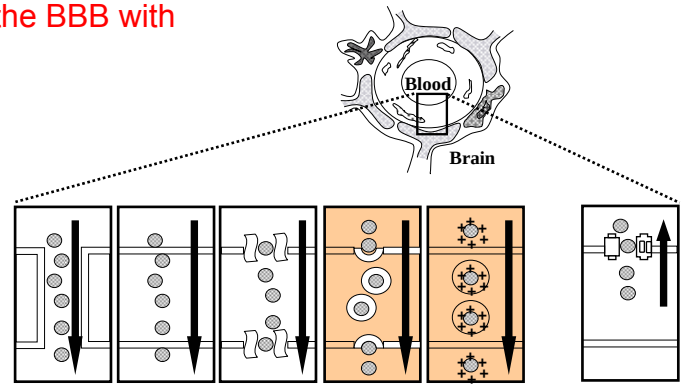
Labeling molecules:
- fluorescein isothiocyanate (FITC)
- rhodamine isothiocyanate (RITC)
- quantum dots

P/P drugs:
- neuroprotective activity-dependent protein (NAP)
- vasoactive intestinal peptide (VIP)
- nerve growth factor
- activity-dependent neurotrophic factor (ADNF)
- erythropoietin (EPO)
- insulin
- enfuvirtide

Nano BioPharmaceutics

WP7: P/P Delivery Systems for Crossing the Blood-Brain Barrier

How to cross the BBB with nanoparticles



A Paracellular aqueous pathway

B Transcellular lipophilic pathway

C Active carrier-mediated pathway

D Receptor-mediated endocytosis

E Adsorptive endocytosis

F Efflux transport pathway

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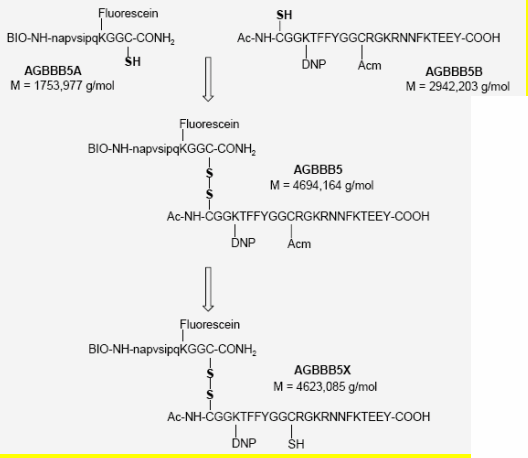
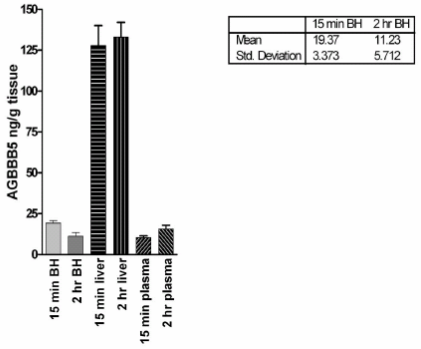
WP7: P/P Delivery Systems for Crossing the Blood-Brain Barrier



Use of AGBBB5B to deliver AGBBB5A over the BBB



	15 min BH	2 hr BH
Mean	19.37	11.23
Std. Deviation	3.373	5.712

✓ *Detection of AGBBB5 in brain homogenate, liver homogenate and plasma*

WP8: Dissemination and Exploitation of New Knowledge and Societal Aspects

Web-based internal communication and management platform