3 A description of the main S&T results/foregrounds

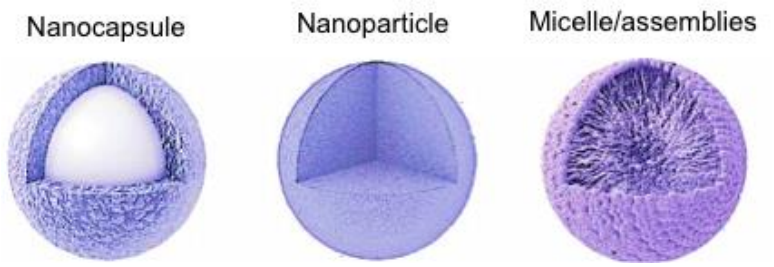

Figure 1. Schematic representation of the different nanostructures

Table 1. SOPs

Number	Authors	SOP title
1.1.	USC	Standard Operating Procedure for Size and ζ-Potential determination by Dynamic Light Scattering (DLS)
1.2.	USC	Standard Operating Procedure for Colloidal Stability in Simulated Intestinal Fluids
1.3	UCC	Standard Operating Procedure for Proteolytic study in Simulated Intestinal Fluids supplemented with enzymes
1.4	USC	Standard Operating Procedure for ICH Stability Testing of Blank Colloidal Nanosuspensions

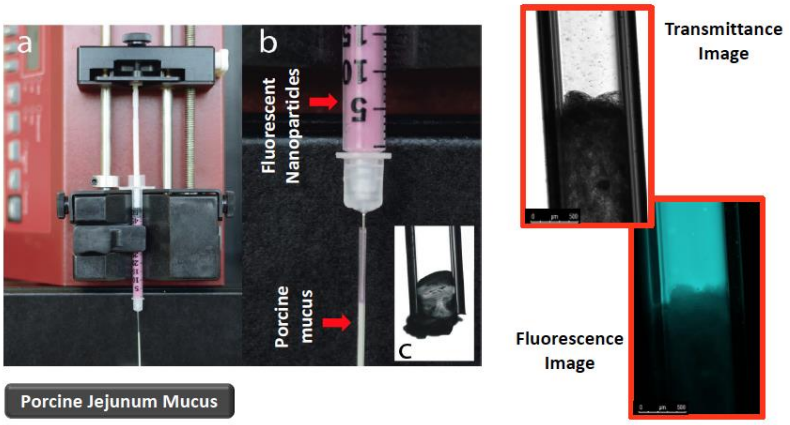

Figure 2. Microcapillary setup for measuring the mucodiffusion of fluorescent nanocarriers

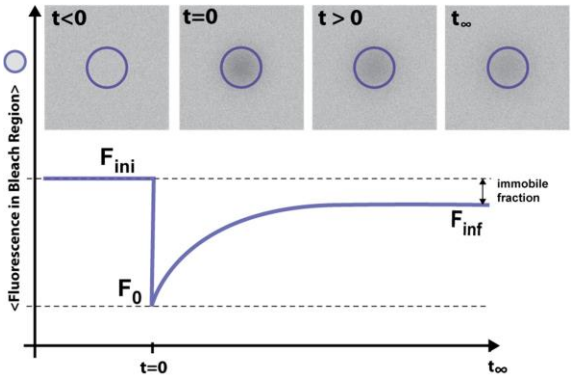

Figure 3. The FRAP experiment

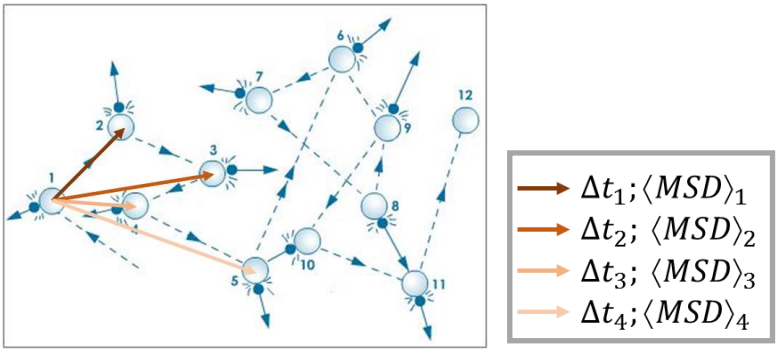

Figure 4. Measurement of nanparticle diffusion by multiple particle tracking

Table 2. The prototype families analysed using the different techniques

Method	Advantages	Critical factors, limitations	Prototypes tested
DLS in mucin solution	Fast, simple, no fluorescent labeling	Mucin cc. << physiological	6
Interaction with mucin film	Fast, simple	Mucin characterization and rheology	5
Mucus-filled microcapillar	Fast, quantitative	Type, concentration of mucin/mucus Particle concentration, Injection method	4
Microfluidics			3
FRAP	Highly specific, quantitative	Complex equipment Type of fluorescent dye	5
Particle tracking	Highly specific, quantitative	Complex equipment, time consuming	2 (in progress)

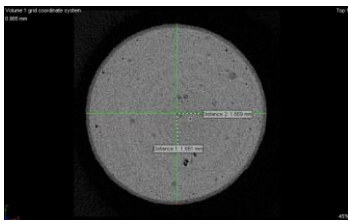

Figure 5. The SmPill technology

WP2: Formulation of peptide drug candidates

Table 3. Peptides used in each Prototype Family

Prototype Family	Nanocarrier (Partner)	Peptides supplied by Sanofi R&D
1	Chitosan nanocapsules/nanoemulsions (USC)	Laby, modified insulin, liraglutide
2	Polyarginine nanocapsules (USC)	Insuman®, Laby
3	Protamine nanocapsules (USC)	Apidra®, Insuman®
4	Aqueous core nanocapsules (UA)	Apidra®
5	Chitosan/cyclodextrin nanoparticles (USC)	Insuman®
6	PGA-PEG coated(R8)-INS nanocomplexes (USC)	Apidra®
7	Polysaccharide-based complexes (UCD)	Insuman®
8	Cyclodextrin-based nanocomplexes (UCC)	Insuman®, Apidra®, modified insulin
9	Quaternary ammonium palmitoyl glycol chitosan (GPCQ) micelles (UCLondon)	Insuman®, Laby, liraglutide
10	Eudragit® nanocomplexes (UA)	Insuman®, Apidra®
11	Lipid nanocapsules (UCLovain)	Exenatide®, liraglutide
12	Insulin core-shell nanocomplexes (UCD)	Insuman®, Apidra®
13	Insulin-Nanocomplexes (USC)	Insuman®

Table 4. SOPs

Number	Authors	SOP title
1	UA, USC	Standard Operating Procedure for Size and ζ-Potential determination by Dynamic Light Scattering (DLS)
2	USC	Standard Operating Procedure for mucoadhesion to mucin film
3	USC	Standard Operating Procedure for ICH Stability Testing of Blank Colloidal Nanosuspensions
4	UA	Standard Operating Procedure for Measurement of the Diffusion of the Nanoparticles in the Mucus
5	UCC	Proteolytic study in Simulated Intestinal Fluids supplemented with enzymes
6	IRB Barcelona	Standard Operating Procedure for validated analytical HPLC technique for Apidra, Insuman and Laby peptides
7	IRB Barcelona	Standard Operating Procedure for stability testing of therapeutic peptides
8	IRB Barcelona	Standard Operating Procedure for peptide Stability in Simulated Intestinal Fluids
9	IRB Barcelona	Standard Operating Procedure circular dichroism measures for insulin peptides
10	UCLondon	Standard Operating Procedure for quantification of Labyrinthopeptin

WP3: In vitro studies of toxicity and mechanism of action

Table 5. SOPs

Number	Authors	SOP title
1	Veneto Group	Standard Operating Procedure for MTS: cell viability
2	Veneto Group	Standard Operating Procedure for ROS assay (flow cytometer protocol)
3	Veneto Group	Standard Operating Procedure Neutral Red
4	Veneto Group	Standard Operating Procedure for Lactate dehydrogenase
5	Veneto Group	Standard Operating Procedure for Caspase 3/7 apoptosis detection assay
6	Veneto Group	Standard Operating Procedure for thawing, freezing and subculturing Caco2 cells
7	Veneto Group	Standard operating procedure for Apoptosis/Necrosis cytometric assay
8	Veneto Group	Standard Operating Procedure ATP cell viability assay
9	CEA	Standard Operating Procedure Assay of Insuman and Apidra by LC-MS/MS in CACO-2 cells and culture media

Number	Authors	SOP title
1	USC	Detection and Quantification of Gram Negative Bacterial Endotoxin Contamination in Nanoparticle Formulations by Kinetic Turbidity LAL Assay

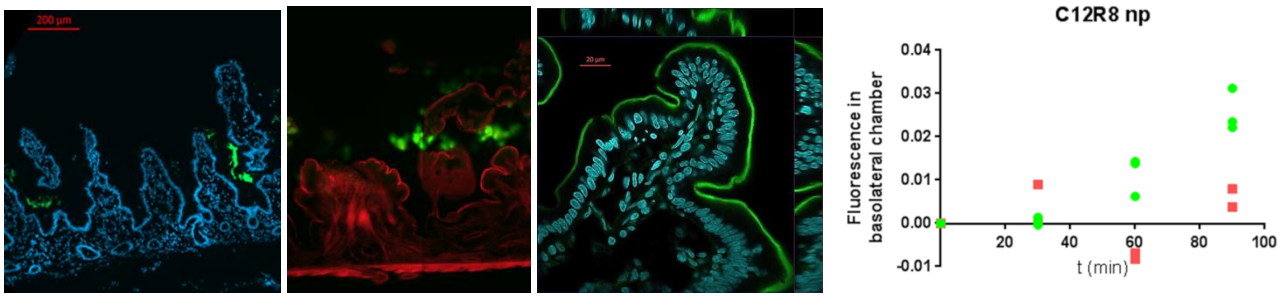

Figure 6. Interactions of tested nanoparticle prototypes with human jejunal tissue. ***A:*** *Prototype families 2a, 10, and 11 showed pronounced mucus binding. No nanoparticles could be detected in close proximity with the tissue. These nanoparticles showed zero permeability. (Nanoparticles from family 10 in green, nuclei in blue)* ***B:*** *Prototype family3 nanoparticles showed pronounced mucus binding but could also be detected at low levels in the tissue and exhibited some permeability. Nanoparticles in tissue were always found beneath defects in the epithelium (blue arrow). (Nanoparticles in green, actin stained in red to visualize an epithelial defect in vicinity of blue arrow)* ***C:*** *Prototype family 6a nanoparticles were highly mucodiffusive and no binding to mucus could be detected. The nanoparticles were endocytosed to a high degree by the enterocytes of the intestinal epithelium and showed a small but measurable permeability. (Nanoparticles from family 6a in green, nuclei in blue)* ***D:*** *Permeability of prototype family 6a nanoparticles could be inhibited by endocytosis inhibitors such as the dynamin inhibitor Dynasore indicating that active endocytosis is the permeation mechanism*

WP4: Preliminary in vivo mechanistic and toxicity studies

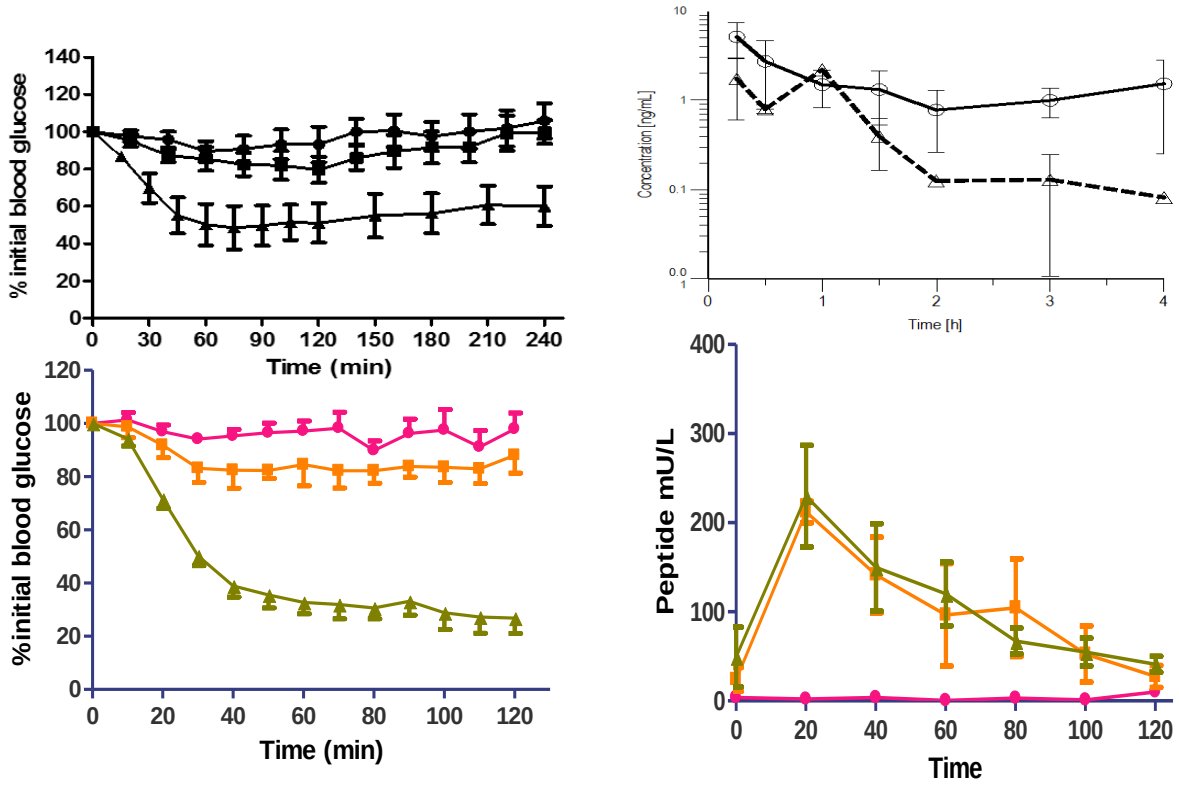

Figure 7. Instillations in rat jejunal loops by UCD. Upper left: Glucose levels achieved for Prototype 8 (PD, n=4). ● PBS; ■ insulin solution; ▲ insulin in nanocarrier. Upper right: Plasma levels achieved for Prototype 8 (PK, n=4): o insulin in nanocarrier; Δ insulin solution (s.c.). Lower left: Prototype 12 (PD, n=6). ● PBS; ■ insulin solution; ▲ insulin in nanocarrier. Lower right: Prototype 12 (PK, n=6): symbols as for lower left. Dose was 50 IU/kg (instillations), 1 IU.kg (s.c.)

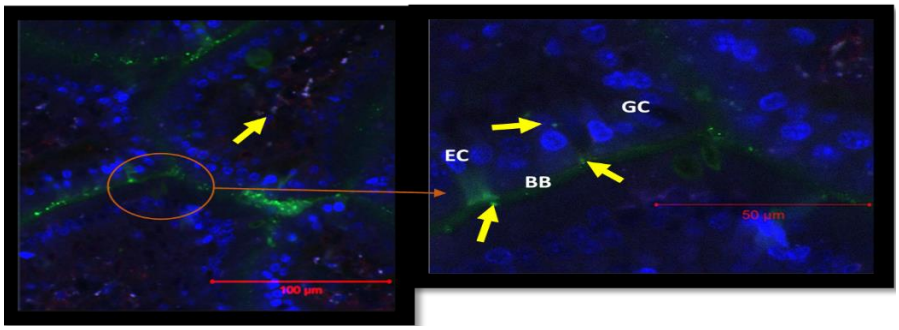

Figure 8. Labelled PF 9 adhering very closely to the brush border of the mouse small intestine following gavage appears to be taken up by the epithelial cells and into the lamina propria. Co-localisation with the Dylight 488 tomato lectin suggests some uptake into circulation

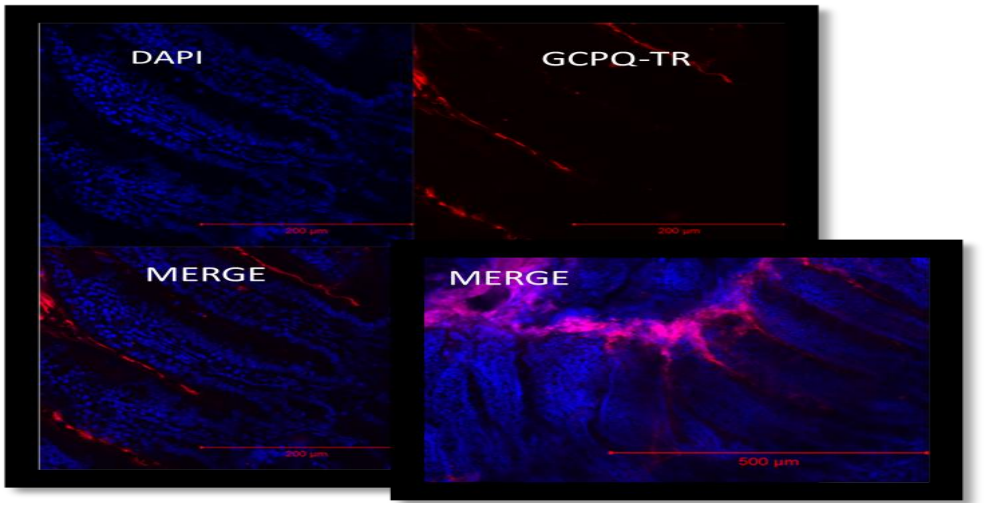

Figure 9. Texas Red (TR)-labelled PF 9 (GPCQ-TR) adheres to mucus and is abundant between the villi of mice

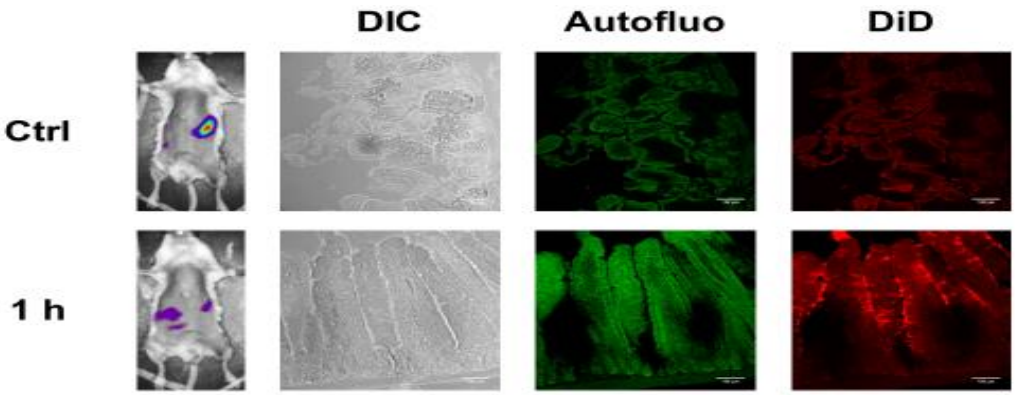

Figure 10. IOV/ECAMRICERT evidence of labelled PF 2 along the side of duodenal villi in rats following oral gavage

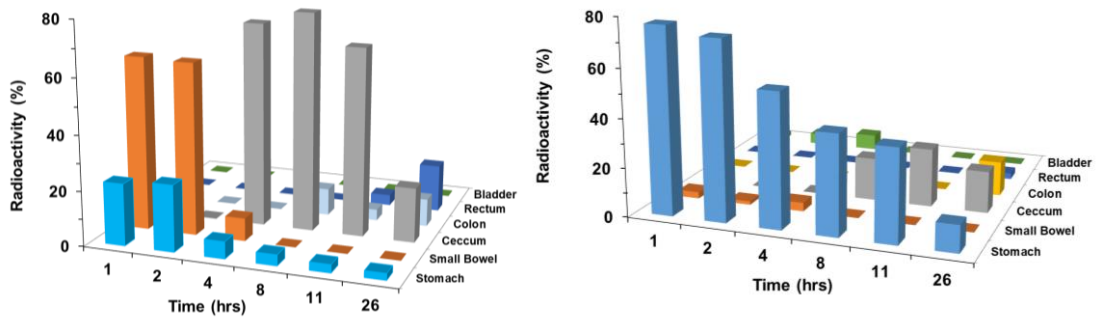

Figure 11. In vivo tissue distribution of the orally administered ^{99m}Tc- PGA-PEG/R8-glulisine (ENCPs) (A) and the free ^{99m}Tc control (B)

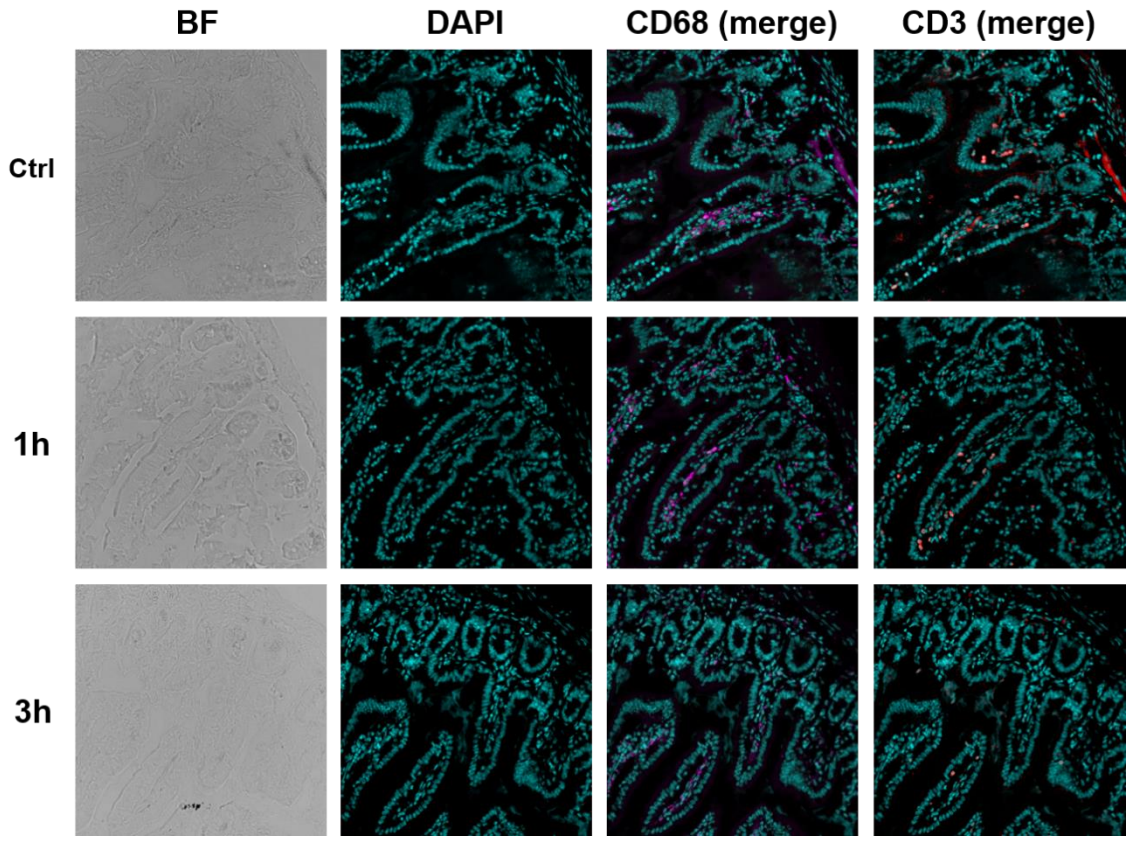

Figure 12. Immunohistochemistry of the small intestine after Octa NCPs administration with CD3 and CD68. In the figure co-localization with DAPI is reported (magnification 40X with dry objective). (Ctrl=untreated)

WP5: In vivo pharmacokinetic/pharmacological evaluation of nanomedicines

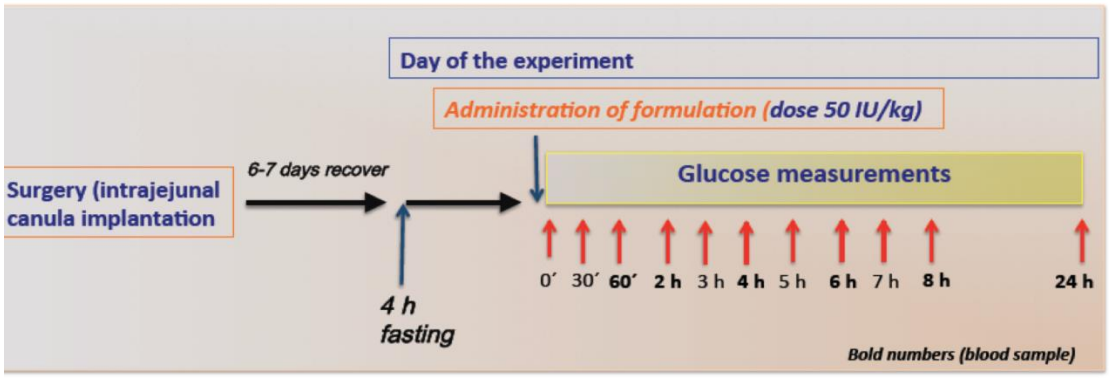

Figure 13: Protocol for the administration of formulation using normal animals (i.e. non-obese and non-diabetic animals)

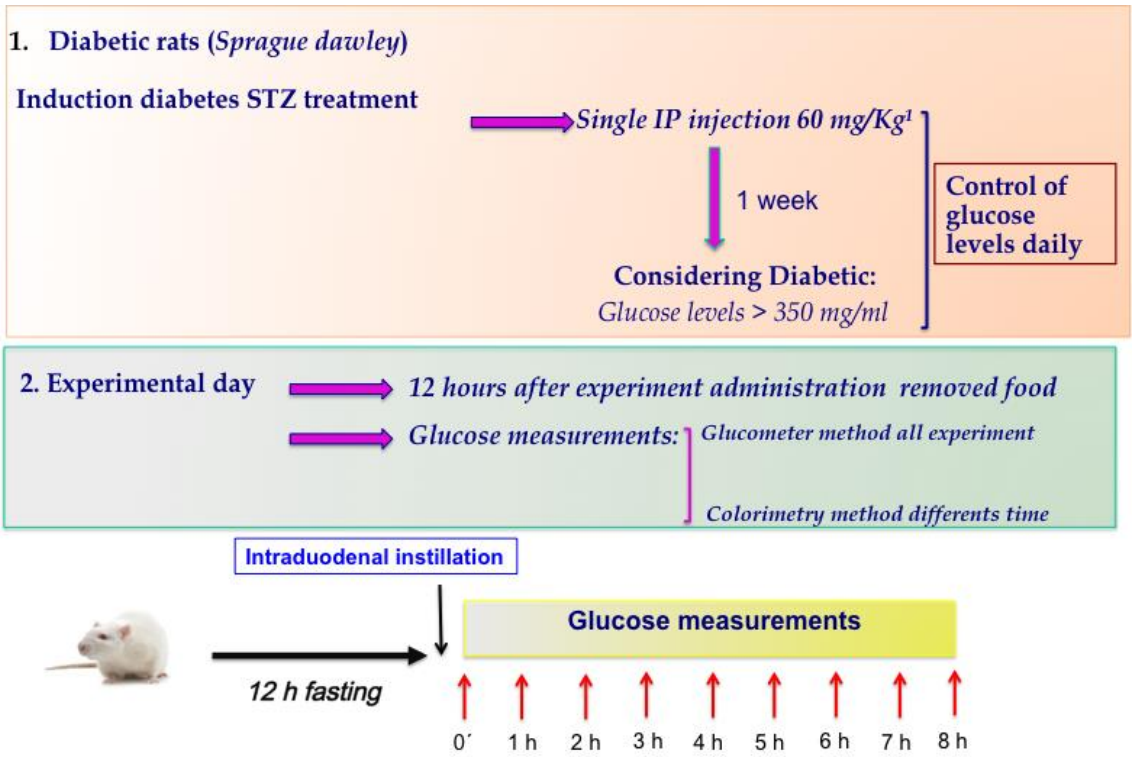

Figure 14: Protocol for the administration of formulation using diabetic rodents (diabetes induced by streptozotocin administration during 1 week)

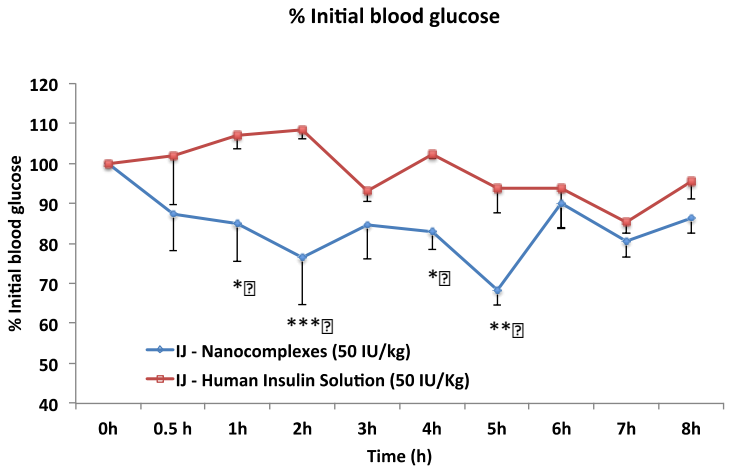

Figure 15. Intrajejunal (i.j.) administration of Prototype 13. The graphic presents the % of initial glucose values vs. time. Holm-Sidak's post-hoc test (black dots) and Fisher's LSD post-hoc test (red dots) were applied ($p < 0.05$); ($p < 0.05$; ** $p < 0.01$; *** < 0.001001*

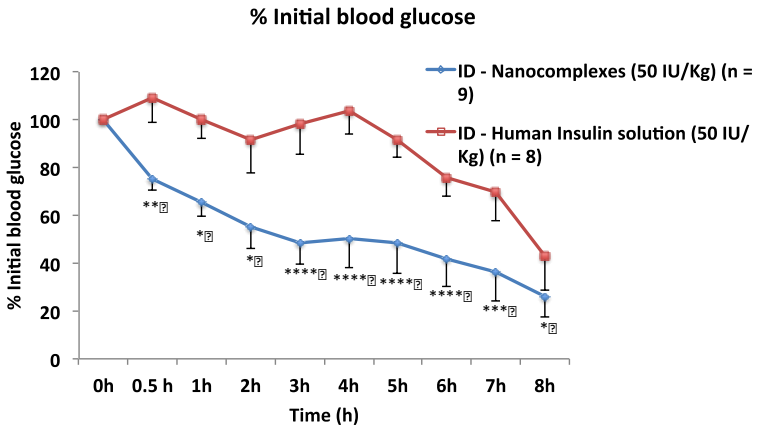

Figure 16. Blood Glucose levels represented in % in relation with basal levels after intraduodenal administration of Prototype 13 to STZ-induced diabetic rats. The graphic presents the % of initial glucose values vs. time. Fisher's LSD post-hoc test (red dots) were applied ($p < 0.05$); ($p < 0.05$; ** $p < 0.01$; *** < 0.001*

4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

Table 6. Dissemination actions/tools vs. audiences and purposes

Action/Tool	Audience	Purpose
Project website	All targeted audiences	Awareness Inform Engage Promote
Press releases	All targeted audiences	Awareness
Scientific publications	Scientific community Pharmaceutical and healthcare industry	Inform
Participations in events	Scientific community Pharmaceutical and healthcare industry Clinicians	Awareness Inform Engage Promote
Use of scientific networks	Scientific community Pharmaceutical and healthcare industry	Awareness Inform Engage Promote
Use of social networks	All targeted audiences	Awareness Inform Engage Promote
Debates, round tables and easy disclosure of case studies	Clinicians Policy makers and governmental bodies Regulatory bodies Healthcare and patient's organizations General public	Inform Engage
Reports	Scientific community Policy makers and governmental bodies	Inform
Training	Scientific community	Inform Engage

Table 7. List of patent applications generated within TRANS-INT

Application reference	Actual/Expected publication date	Title	Applicant
EP143058998	17 Dec 2015	Nanocapsular formulation of active pharmaceutical ingredients	USC/Sanofi
P201631221	19 Feb 2016	Nanoparticles with protected interiors and method of use thereof	USC
GB1605740.8	17 May 2017 (lapsed). New application with more robust data will be filed by UCD during Q3, 2017	An oral delivery system	UCD
In preparation	In preparation	Nanocompositions made of metallic ions for drug delivery	USC