Electronic Supplementary Material (ESI) for Nanoscale.

Stimulation of GLP-1 secretion and delivery of GLP-1 agonists via nanostructured lipid carriers

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Materials and methods

S1 Materials

(Kolliphor 188 (Poloxamer 188) was gifted from BASF (Burgbernheim, DE), and precirol ATO®5 was kindly provided by Gattefossé (Saint-Priest, FR). Miglyol 812N/F provided by Cremer Oleo GmbH & Co. KG (Hamburg, DE). Tween®80 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (gradient HPLC grade) and trifluoroacetic acid (TFA) were obtained from VWR (Leuven, BE). Tetrahydrofuran (THF) was supplied by Alfa Aesar (Ward Hill, MA, USA). Exenatide (Exe) and Liraglutide (Lira) were kindly provided by Peptides and Elephants (Hennigsdorf, Germany). Ultrapure water was used throughout and obtained from a Milli-Q® Plus apparatus (Millipore).

Caco-2 cells were kindly provided by Dr Maria Rescigno (University of Milano-Bicocca, Milano, Italy) and used from passage *x* + 21 to *x* + 30, and the intestinal murine L cell line GLUTag was kindly provided by Dr. Daniel Drucker (University of Toronto, Toronto, CA). Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's modified Eagle's medium (DMEM)-GlutaMAX (5.5 mM glucose), Dulbecco's Phosphate-Buffered Saline (PBS), fetal bovine serum (FBS), Hank's Balanced Salt Solution (HBSS), HyClone™ Fetal Bovine Serum (H-FBS) and the lipophilic carbocyanine dye (DiD) were purchased from Thermo Fisher Scientific (MA, USA). Trypsin (0.05%) with EDTA, non-essential amino-acids (NEAA), L-Glutamine, Penicillin-Streptomycin (10,000 U/mL), Culture flasks and Transwell plates were obtained from Corning Incorporation (Corning, Lowell, MA, USA). 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) was purchased from Sigma-Aldrich (USA). Matrigel was obtained from BD Bioscience (BE).

The Caco-2 cell line was maintained in medium consisting of DMEM supplemented with 10% (v/v) H-FBS, 1% (v/v) L-Glutamine, 1% (v/v) non-essential amino acid (NEAA) and 1% (v/v) Penicillin-Streptomycin. Cells were grown in 162 cm² flasks (Corning, Lowell, MA, USA) in an atmosphere of 10% CO_2/90% air (v/v) at 37°C. For intestinal cell line GLUTag, media comprising (DMEM)-GlutaMAX (5.5 mM glucose) with 10% FBS and Penicillin-Streptomycin was used. Cells were grown in 75 cm² flasks in an atmosphere of 5% CO_2/95% air (v/v) at 37°C. The medium for both the cell lines were replaced every other day.

S2 HPLC quantification method:

Exenatide and Liraglutide was quantified using a gradient method using a Shimadzu HPLC (Shimadzu, Japan). A Kinetex® EVO C18 column (100Å, 2.6 µm, 150 x 4.6 mm) (Phenomenex, USA), with a security guard column (Phenomenex, USA) was used at room temperature. The aqueous mobile phase comprised of 0.05% (v/v) trifluoroacetic acid (TFA) in water and the organic mobile phase consisted of 0.05% (v/v) in acetonitrile. A gradient system was developed with an initial ratio of 10:90 (v/v, aqueous:organic phase) at flow rate of 1 mL/min, which was the linearly changed to 90:10 (v/v) over 8.5 min, and kept constant for the next minute. Then the ratio was linearly changed to initial composition in the next 1.5 minutes and was stabilized for the last minute. The injection volume used was 20 µL and the detection wavelength used was 220 nm. The retention time for Exenatide and Liraglutide was 5.9 min and 7.5 min, respectively. The limit of detection and limit of quantification for exenatide was 1.1 ± 0.4 µg/mL and 3.3 ± 1.1 µg/mL, respectively. Similarly, for Liraglutide the limit of detection and limit of quantification was 0.9 ± 0.4 µg/mL and 2.6 ± 1.1 µg/mL, respectively.

S3 Preparation of simulated gastrointestinal fluids

Simulated gastric fluid was composed of 0.2% (w/v) sodium chloride in 0.7% (v/v) hydrochloric acid with 3.2 mg/mL pepsin, with the pH maintained at 1.2. SGF solution was also prepared in the absence of pepsin [9]. For preparing fasted state simulated intestinal fluids (FaSSIF and FaSSIF-version 2) and in fed state simulated intestinal fluid (FeSSIF) (pH 6.8), the composition are shown in Table S2 [22].

Table S1- FaSSIF, FaSSIF-V2 and FeSSIF compositions

Composition	FaSSIF	FaSSIF-V2	FeSSIF
Sodium taurocholate	3mM	3 mM	15 mM
Lecithin	0.75 mM	0.2 mM	3.75 mM
Sodium monobasic phosphate	28.65 mM	-	-
Acetic acid	-	-	144.04 mM
Maleic acid	-	19.12 mM	-
Sodium hydroxide	8.71 mM	34.8 mM	101.02 mM
Sodium chloride	105.85 mM	68.62 mM	203.18 mM
pH	6.5	6.5	5

S4 LC/MS/MS method for quantification of Exenatide and Liraglutide:

The peptide from cellular transport studies were quantified using LC/MSMS system from ThermoFisher (San Jose, California) equipped with an Accela 1250 pump and a Accela autosampler. The separation of peptides was performed on a C18 Kinetex column (150 x 2.0 mm i.d., 3 µm; Phenomenex) using a linear gradient from 97% H_2O (with 1% CH_3CN and 0.1% HCOOH)/3% CH_3CN to 5% H_2O in 20 min. The flow rate was 0.2 ml min-1; the column was maintained at 30°C and the autosampler at 7°C. Mass spectra were acquired with a Q-Exactive mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source in the positive mode. ESI inlet conditions were: spray voltage 5 kV, capillary temperature 350°C, sheath gas 20 PSI, and auxiliary gas 5 PSI. MS2 spectra were taken with at a relative collision energy of 10%. The limit of quantification for the peptides using this method was 1 ng/ml.

The apparent permeability (Papp, cm/s) was calculated using the following equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{AC^0}$$

Where dQ/dt is the transport rate (µg/s), C^0 is the initial drug concentration on the apical side (µg/mL) and A is the surface area of the membrane filter (cm2).

Results

S5 In vivo effects on blood glucose levels

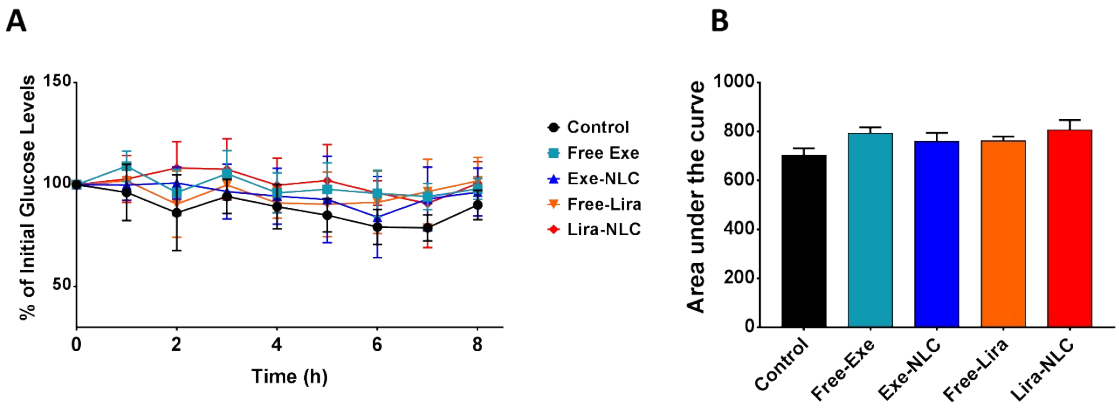

Figure S1: (A) Change in blood glucose levels (as percentage of the initial value) in non-diabetic mice after intestinal instillation of different treatment groups. (B) Area under the curve of the blood glucose profile of different treatment groups. Controls used were the solution of exenatide and liraglutide and empty NLC. Data shown as mean ± SEM (n=5).

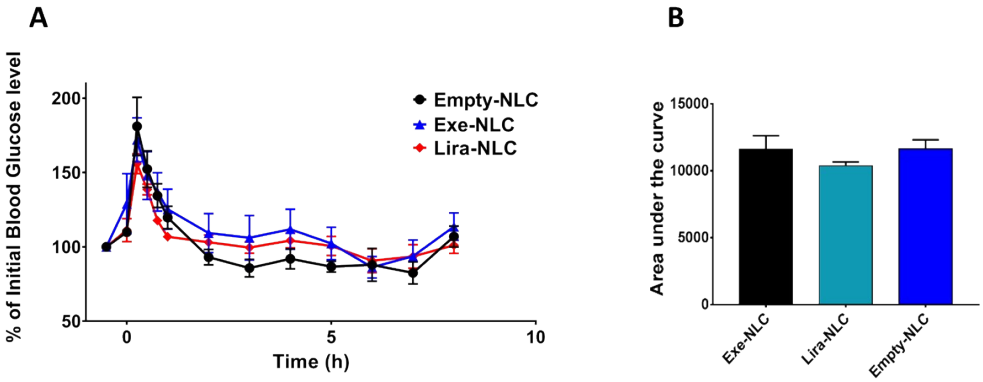

Figure S2: (A) Change in blood glucose levels (as percentage of the initial value) in non-diabetic mice after intestinal instillation of different treatment groups. (B) Area under the curve of the blood glucose profile of different treatment groups. Data shown as mean ± SEM (n=4-7).