Lactose as a “Trojan Horse” for Quantum Dot Cell Transport**
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Electronic Supplementary Information

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Experimental Procedures

**General.** Chemicals were purchased and used without further purification. Dry solvents were obtained by distillation using standard procedures, or by passage through a column of anhydrous alumina using equipment from Anhydrous Engineering (University of Bristol) based on the Grubbs’ design. Reactions requiring anhydrous conditions were performed under N\textsubscript{2}; glassware and needles were either flame dried immediately prior to use, or placed in an oven (150 °C) for at least 2 h and allowed to cool in a desiccators or under reduced pressure. Liquid reagents, solutions or solvents were added \textit{via} syringe through rubber septa; solid reagents were added \textit{via} Schlenk type adapters. Teflon rings were used between the joints of the condensers and round bottom flasks. Reactions were monitored by TLC on Kieselgel 60 F\textsubscript{254} (Merck), with UV light (254 nm) detection and by charring with 10% sulfuric acid in ethanol. Flash column chromatography was performed using silica gel [Merck, 230–400 mesh (40–63 µm)]. Extracts were concentrated \textit{in vacuo} using both a Büchi rotary evaporator (bath temperatures up to 40 °C) at a pressure of 15 mmHg (diaphragm pump) or 0.1 mmHg (oil pump), as appropriate, and a high vacuum line at room temperature. Water soluble compounds were freeze dried on a Lytotrap Plus (LTE Scientific LTD). \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra were measured in the solvent stated at 400, 500 or 600 MHz. Chemical shifts are quoted in parts per million from residual solvent peak (CDCl\textsubscript{3}: \textsuperscript{1}H - 7.26 ppm and \textsuperscript{13}C - 77.16 ppm) and coupling constants (\textit{J}) given in Hertz. Multiplicities are abbreviated as: b (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or combinations thereof. Dynamic Light Scattering (DLS) and Zeta analysis are carried out using Malvern Instruments, Nano_S90 Red Laser Model ZEN1690 for DLS and Nano-Z ZEN 2600 for Zeta potential.

**Chemical Synthesis**

![Scheme S1](image)

\textbf{Scheme S1.} Reagents and conditions: (i) Ts\textsubscript{2}, Et\textsubscript{3}N; (ii) NaN\textsubscript{3}; (iii) H\textsubscript{2}, Pd/C, HCl

\textbf{2-(2-(2-Hydroxyethoxy)ethoxy)ethoxyethyl 4-methylbenzenesulfonate (S1).} To a stirred solution of tetraethyleneglycol (8.0 g, 41 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (82 mL), Et\textsubscript{3}N (5.7 mL, 41 mmol) and tosyl chloride (3.9 g, 21 mmol) were added, and the solution stirred at room temperature for 24 h. The mixture was then washed with H\textsubscript{2}O (3 x 30 mL), brine (1 x 30 mL), dried over MgSO\textsubscript{4}, filtered and concentrated \textit{in vacuo} to afford S\textsubscript{1} as a clear oil (8.6 g, 60%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \textit{d} 7.82-7.79 (m, 2H, Ar), 7.37-7.34 (m, 2H, Ar), 4.18-4.10 (m, 2H, CH\textsubscript{2}OTs), 3.74-3.56 (m, 14H, 7×CH\textsubscript{2}), 2.46 (s, 3H, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \textit{d} 145.0 (Ar), 133.1 (Ar), 130.0 (Ar), 128.08 (Ar), 128.07 (Ar), 72.6, 70.83, 70.81, 70.75, 70.64, 70.56, 69.4 (7×CH\textsubscript{2}), 68.8 (CH\textsubscript{2}OTs), 61.8 (CH\textsubscript{2}OH), 21.8 (CH\textsubscript{3}).
2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (S2). To a stirred solution of S1 (4.85 g, 13.9 mmol) in a mixture of H₂O/acetone (1/1, v/v, 70 mL), NaN₃ (4.52 g, 69.7 mmol) and NaI (0.21 g, 1.4 mmol) were added portionwise and the mixture refluxed for 17 h. The acetone was then removed under reduced pressure and the remaining aqueous phase was extracted with CH₂Cl₂ (3 x 30 mL), brine (1 x 30 mL), dried over MgSO₄, filtered and concentrated in vacuo to afford S2 as a clear oil (2.4 g, 80%).

1H NMR (400 MHz, CDCl₃) δ 3.61-3.54 (m, 12H, 6 × CH₂), 3.49-3.47 (m, 2H, CH₂OH), 3.27 (t, J = 5.1 Hz, CH₂N₃); 13C NMR (100 MHz, CDCl₃) δ 72.4, 70.54, 70.49, 70.4, 70.2, 69.9 (6 × CH₂), 61.5 (CH₂OH), 50.5 (CH₂N₃).

2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethanol (S3). To a stirred solution of S2 (2.4 g, 11 mmol) in a mixture of EtOH:HCl (95/5, v/v, 20 mL), Pd/C catalyst (10 mol%, 2.5 g) was added. The mixture was put under a N₂ atmosphere and hydrogen was then bubbled through the solution for 6 h. Upon completion of the reaction, as indicated by TLC, the solution was filtered through Celite™ and concentrated in vacuo to afford S3 as an brown oil (2.5 g, 97%).

1H NMR (400 MHz, CDCl₃) δ 3.67-3.61 (m, 12H, 6 × CH₂), 3.41-3.38 (m, 2H, CH₂OH), 2.88 (m, 2H, CH₂NH₂); 13C NMR (100 MHz, CDCl₃) δ 70.8, 70.6, 70.53, 70.49, 70.4, 70.2, 69.9 (6 × CH₂), 54.7 (CH₂OH), 50.8 (CH₂NH₂).

Scheme S2. Reagents and conditions: (i) NHS, DCC, THF; (ii) S3, DMF/H₂O; (iii) NaH, TBAI, DMF, 0°C.

Lipoic Acid NHS-Ester (LA-NHS) (S4). To a stirred solution of lipoic acid (3.0 g, 15 mmol) in THF (75 mL), N-hydroxysuccinimide (2.0 g, 17 mmol) was added, and the mixture was cooled to 4 °C. A solution of dicyclohexylcarbodiimide (3.6 g, 17 mmol) in THF (12 mL) was then added dropwise, and the reaction mixture was allowed to stir at room temperature overnight. The solution was then filtered through Celite™ and concentrated in vacuo to give S4 as a yellow solid (4.1 g, 94%).

1H NMR (400 MHz, CDCl₃) δ 3.61-3.54 (m, 1H, H-3), 3.21-3.08 (m, 2H, 2 × H-1), 2.83 (s, 4H, 2 × H-10, 2 × H-11), 2.62 (t, 2H, J = 7.3 Hz, 2 × H-7) 2.50-2.42 (m, 1H, H-2a), 1.96-1.88 (m, 1H, H-2b), 1.82-1.75 (m, 2H, 2 × H-4), 1.74 (m, 2H, 2 × H-6), 1.60-
1.51 (m, 2H, 2×H-5); 13 C NMR (100 MHz, CDCl3) δ 169.1 (C-9, C-12), 168.4 (C-8), 56.1 (C-3), 40.1 (C-2), 38.5 (C-1), 34.4 (C-4), 30.7 (C-7), 28.3 (C-5), 25.6 (C-8, C-9), 24.4 (C-6).

**Lipoic Acid-PEG-OH (2).**

![Structure of Lipoic Acid-PEG-OH](image)

A solution of S3 (1.79 g, 5.92 mmol) was neutralised in aqueous NaHCO₃ (1 M, 28 mL) and the volume of the solution was reduced to 10 mL *in vacuo*. The solution was cooled to 4 °C and to it a solution of S4 (2.66 g, 9.23 mmol) was added dropwise, over 90 minutes. The reaction was then warmed to 20 °C and left to stir for 24 hours. The product was extracted with CHCl₃ (3 x 40 mL) and dried over MgSO₄. The product was evaporated to dryness and purified by flash silica gel chromatography (CH₂Cl₂/MeOH gradient: 1/0 to 9/1, v/v) to give 2 as a yellow oil (1.1 g, 70%).[^1] ¹H NMR (400 MHz, CDCl₃) δ 7.09 (s, 1H, NH), 3.75 – 3.49 (m, 15H, 6×OCH₂H-3), 3.52 – 3.44 (m, 2H, 2×H-9), 3.26 – 3.08 (m, 2H, 2×H-1), 2.49 – 2.52 (m, 1H, H-2a), 2.18 (t, J = 7.9 Hz, 2H, 2×H-7), 1.94 – 1.86 (m, 1, H-2b), 1.80 – 1.62 (m, 4H, 2×H-4, 2×H-6), 1.57-1.41 (m, 2H, 2×H-5); ^13C NMR (100 MHz, CDCl₃) δ 173.0 (C-8), 72.6, 70.6, 70.41, 70.35, 70.0 (6 x OCH₂), 61.5 (C-16), 56.5 (C-3), 40.2 (C-2), 39.1 (C-9), 38.4 (C-1), 36.2 (C-7), 34.7 (C-4), 29.0 (C-5), 25.4 (C-6); ESI-HRMS for C₁₈H₃₁NO₇NaS₂ (MNa⁺) Calculated: 404.1536; Found: 404.1528.

**Lipoic Acid-PEG-COOH (3).[^3]**

![Structure of Lipoic Acid-PEG-COOH](image)

Bromoacetic acid (0.11 g, 0.79 mmol) and TBAI (10 mol%) were added to a solution of 2 (0.1 g, 0.26 mmol) in DMF (2 mL) under a nitrogen atmosphere and the mixture cooled to 0°C. Sodium hydride (31 mg, 1.3 mmol) was then added and the mixture stirred at 0°C for 30 minutes before being allowed to warm to room temperature and stirred for 5 hours. The reaction was quenched using ammonium chloride solution and the solvent removed. The residue was then dissolved in chloroform (10 mL) and washed with brine (3 x 10 mL). The crude product was purified by flash column chromotography (gradient: DCM/MeOH: 1:0 to 9:1, v/v) to give 3 as a yellow syrup (41.6 mg, 0.09 mmol, 37%). ¹H NMR (400 MHz, CDCl3) δ 4.14 (s, 2H, 2×H-17), 3.73-3.53 8 (m, 15H, 7×OCH2, H-3), 3.55 (t, 2H, J = 5.5 Hz, 2×H-9), 3.21-3.03 (m, 2H, 2×H-1), 2.51-2.43 (m, 1H, H-2a), 2.22 (t, 2H, J = 7.4 Hz, 2×H-7), 1.94-1.86 (m, 1H, H-2b),
1.75-1.60 (m, 4H, 2×H-6, 2×H-4), 1.51-1.38 (m, 2H, 2×H-5), 13 C NMR (100 MHz, CDCl3) δ 172.9 (C-18), 169.4 (C-8), 72.3, 70.1, 69.9, 69.7, 69.6, 69.6, 69.5, 7×OCH2, 56.1 (C-3), 39.8 (C-2), 38.7 (C-9), 38.0 (C-1), 35.5 (C-7), 34.2 (C-4), 28.4 (C-5), 25.1 (C-6). C18H33NO7NaS2 (MNa+) Calculated: 462.1591; Found: 462.1585.

General Procedure for Synthesis of CdSe QDs. Tributylphosphine (TBP) (5 mL) was syringed into a vessel containing elemental selenium (Se) (39.5 mg, 0.50 mmol) under argon to give a 0.1M Se solution. Cadmium oxide (37 mg, 0.30 mmol), tetradecylphosphonic acid (TDPA) (334 mg, 1.2 mmol) and trioctylphosphine oxide (TOPO) (3.6 g, 1 mmol) were dried in vacuo at 40 °C for 15 min and then at 60 °C for 15 min. The atmosphere was exchanged for Ar and the solution heated to 300 °C. When the solution turned colourless the Se solution (2.5 mL, 0.25 mmol) was added. After 1-3 min*, the reaction was quenched by rapid cooling. Particles were precipitated by addition of MeOH (10 mL), centrifuged (4 min, 6000 rpm) and the supernatant discarded. The particles were then redissolved in CHCl3 (10 mL). (*Note: By varying the duration, different size QDs, i.e. different colors were obtained – with yellow-green for shorter times and orange-red for longer times)

General Procedure for ZnS Coating of CdSe QDs. CdSe QD solution (8 mL, 0.2 mmol) was added to TOPO (3.6 g, 1 mmol) and the CHCl3 evaporated under vacuum at 50 °C. The resultant material was then heated to 180 °C under Ar. In a separate flask, to a degassed (Ar for 30 min) solution of zinc stearate (198 mg, 0.313 mmol) in toluene (5 mL), was added hexamethyldisilathiane (0.05 mL, 0.2 mmol). The resulting ZnS solution (0.7 mL, 0.03 mmol) was added dropwise to the heated CdSe QD solution and mixed for 2 h at 100 °C. The mixture was then allowed to cool and the particles precipitated by the addition of MeOH (10 mL). The mixture was centrifuged (4 min, 6000 rpm) and the supernatant discarded before redissolving the CdSe/ZnS QDs in CHCl3.

General Procedure for Ligand Exchange. To a flask containing a solution of the Linker (0.29 mmol) in a mixture of MeOH/H2O (1/1, v/v, 3 mL) was added NaBH4 (21.9 mg, 0.58 mmol) under a N2 atmosphere and the mixture stirred for 1 h. The pH of the solution was then adjusted to neutral by the dropwise addition of HCl (1 M) before transferring it to a glass vial. The CdSe/ZnS QD solution in CHCl3 (2 mL) was then added to the vial and the biphasic mixture stirred vigorously for 16 h. Once the coloured QDs had transferred to the aqueous layer, the CHCl3 phase was removed and the aqueous layer dialyzed for 16 h to before the QDs solution was freeze dried.

NMR spectra of Lipoic Acid-PEG-coated QDs.

A) Compound 5a (Linker = 100 % of 3)
B) Compound 5b (Linker = 60 % of 3 and 40 % of 2)
C) Compound 5c (Linker = 40 % of 3 and 60 % of 2)
D) Compound 5d (Linker = 100 % of 2)
A) $^1$H NMR spectra of compound 5a

B) $^1$H NMR spectra of compound 5b

C) $^1$H NMR spectra of compound 5c

D) $^1$H NMR spectra of compound 5d
General Procedure for Functionalisation with Aminated Carbohydrates. Glycosylamine carbohydrates were prepared using the microwave assisted Kochetkov amination protocol of the corresponding unprotected glycosides with ammonium carbonate (5 fold excess w/w over sugar). Water soluble QDs (4 and 5) were added to a PBS buffer solution (pH 7.4, 3 mL) and 1 mL transferred to a glass vial. Glycosylamine (0.04 mmol) and EDC (34 mg, 0.22 mmol) were then added and the mixture stirred vigorously for 18 h at room temperature. The QD solution was then dialysed against water for 16 h and the QDs collected and freeze dried.

β-galactosylamine

$^1$H NMR (500 MHz, D$_2$O) δ 3.90 (d, 1H, $J$ = 8.7 Hz, H-1) ppm

β-lactosylamine

$^1$H NMR (500 MHz, D$_2$O) δ 4.32 (d, 1H, $J$ = 7.8 Hz, H-1’), 3.99 (d, 1H, $J$ = 8.8 Hz, H-1) ppm

β-maltotriosylamine

$^1$H NMR (500 MHz, D$_2$O) δ 5.27 (d, 2H, $J$ = 3.8 Hz, H-1’, H-1’’), 3.98 (d, 1H, $J$ = 8.8 Hz, H-1) ppm
β-mannosamine

\[^1\text{H} \text{NMR (500 MHz, D}_2\text{O)} \delta 4.40 \text{ (bs, H-1) ppm}\]

NMR spectra of carbohydrate coated QDs

A) Compound 13 (Glycosylamine = Dextran amine 10,000 MW (Invitrogen) conjugated onto 4)
B) Compound 14b (Glycosylamine = β-galactosylamine conjugated onto 5b)
C) Compound 18b (Glycosylamine = β-lactosylamine conjugated onto 5b)
D) Compound 20b (Glycosylamine = β-lactosylamine/β-mannosamine (1/1) conjugated onto 5b)
E) Compound 21b (Glycosylamine = β-lactosylamine/β-maltotriosylamine (1/1) conjugated onto 5b)

A) \[^1\text{H} \text{NMR spectra of compound Dextran-QD 13}\]

\[^1\text{H} \text{NMR (500 MHz, D}_2\text{O)} \text{ anomeric peaks: } \delta 5.21, 4.26 \text{ ppm (H}_2\text{O peak suppressed at 4.65 ppm)}\]

B) \[^1\text{H} \text{NMR spectra of compound Galactose 14b}\]

\[^1\text{H} \text{NMR (500 MHz, D}_2\text{O)} \text{ anomeric peaks: } \delta 4.46 \text{ ppm}\]
C) $^1$H NMR spectra of compound Lactose-QD 17b

$^1$H NMR (500 MHz, D$_2$O) anomeric peaks: $\delta$ 4.58 ppm

D) $^1$H NMR spectra of compound Lactose/maltotriose-QD 21b

$^1$H NMR (500 MHz, D$_2$O) anomeric peaks: $\delta$ 5.24, 4.29, 4.14, 3.96 ppm

E) $^1$H NMR spectra of compound Lactose/mannose-QD 20b

$^1$H NMR (500 MHz, D$_2$O) anomeric peaks: $\delta$ 4.55, 4.33 ppm
QD Characterization data

Figure S1. Room temperature absorption and luminescence spectra of the two different CdSe QDs used in the study (2.7±0.2nm and 4.0±0.4 nm).

Figure S2. (a) Characteristic TEM of CdSe/ZnS (2.7±0.2nm). Scale bar = 5nm; (b) EDX analysis of CdSe/ZnS (2.7±0.2nm); (c) uv-vis (solid line) and Photoluminescence analysis (dotted line) of CdSe QDs before (red) and after ZnS coating (blue). The TEM analysis was performed on a JEOL 200 kV Hi Resolution TEM 2011 fitted with an EDX Oxford Instruments ISIS 300 system.
Figure S3. TEM images of a) PEG-QDs; b) 40% lactosamine-PEG-QDs 16c; c) 60% lactosamine-PEG-QDs 17b; d) 100% lactosamine-PEG-QDs 17a (scale bar = 200 nm); e) Emission spectra of 60% Carboxy-PEG-QDs (blue line) 5b, and 60% lactosamine-capped PEG-QDs (red line) 17b in PBS. Note peak emission at 569 nm.
Cell culture

HeLa cells were maintained in Minimal Essential Medium (MEM) with GlutaMAX™ and 10% fetal bovine serum (FBS); AS cells were grown in Dulbecco’s MEM with Glutamax and 10% FBS. Both media were supplemented with antibiotic-antimycotic (Anti-Anti). Confluent cultures were detached from the surface using trypsin (Tryp LE Express) and plated at $10^4$ cells/well in either petri dishes (Mat-Tek 35 mm, with 14mm glass microwell) for imaging, or 96-well plates for toxicity tests. Tests were carried out the next day as specified below. All cell culture media and additives were purchased from Invitrogen, Life Technologies.

Confocal microscopy

Cell trackers used were purchased from Life Technologies: CellLight® Reagents *BacMam 2.0: early Endosomes (Rab5a), late endosomes (Rab7a), ER-Tracker™ for the endoplasmic reticulum, BODIPY TR ceramide for the Golgi reticulum, mitochondria and LysoTracker™ for lysosomes, carbocyanine-based MitoTracker® (not dependent on oxidation status), and NucBlue™ Live Cell Stain (Hoechst 33342) for nuclei. These markers were used in line with manufacturer’s protocols. All images were acquired on a Leica SP5 confocal system equipped with a Leica DMI 6000 inverted microscope. For the excitation of the QDs, either the blue laser or the blue lines of the argon ion laser (405 and 488nm respectively) were used. For the excitation of cellular manufacturers specifications were followed. The images were analysed using Velocity software (PerkinElmer). See full list of images at the end of ESI.

Toxicity assays

The influence of quantum dots on cell metabolism was assessed using AlamarBlue, a cytosolic substrate for reductive metabolism (resazurin to resorufin) whose fluorescence spectrum changes on reduction (Invitrogen Life Technologies). Cell numbers and proliferation were assessed by total protein, bicinchoninic acid assay (BCA, Pierce, ThermoScientific). Cells were exposed to quantum dots for 4, 24 and 48h in medium without foetal bovine serum. Results are expressed as changes in metabolism per µg protein. Figure S4 shows that toxic effects depend on oligosaccharide ligand concentration on QDs. The effects of a 24h exposure are illustrated in Figure S5a for Araki Sasaki cells. Figure S5b shows the effect of a 48h exposure on the metabolic compatibility of HeLa cells. After testing for homogeneity of variance, ANOVA or Kruskal-Wallis analysis of variance with post-hoc tests were used to assess the statistical significance of quantum dot effects.
Figure S4 Effects of QDs on reductive metabolism is dependant on the concentration of ligand. Galactose (14b), glucose (15b), mannose (16b), lactose (17b), maltose (18b), maltotriose (19b), lactose/mannose (20b), lactose/maltotriose (21b) and dextran (13) functionalized QDs. Repeated measures ANOVA indicated that there were significant effects of glyco-QD dilution for every sugar density p < 0.0001. Differences between sugar densities were significant at p < 0.0001.

HeLa cells were exposed to Lactose-functionalised QDs for 24h. Foetal bovine serum was omitted from the medium to avoid masking of toxic effects on metabolism.

Figure S5 Reductive metabolism relative to cell numbers after exposure to equal doses of differently functionalized Quantum dots. 

a. AS cells exposed for 24 hours; cells exposed to dextran-, galactose- and lactose-functionalised QDs are not significantly different from the controls, while cells exposed to capped quantum dots have significantly lower metabolic activity than the controls (p<0.001).

b. HeLa cells exposed for 28 hours; dextran- and lactose-QDs have no effect on HeLa cell metabolism. Capped QDs and galactose-QDs are more toxic than no treatment (p<0.05).
c. Colocalization Analysis

**Figure S6.** Manders’ overlap coefficient \((R)\)\(^{6,7}\) for Dextran QD 13, Galactose QD 14b, Lactose 17b, Lactose/Mannose QD 20b and Lactose/Maltotriose QD 21b: Manders’ \((R)\) coefficients for internalization of Galactose, Maltotriose, Lactose and Dextran in A) HeLa cells and B) AS cells. Note that most galactose-QDs were located in the early and Late endosomes in HeLa, while just over half colocalised with early endosomes in AS. The low level of colocalization of other sugar-QDs with intracellular organelles is supported by their presence in the cytosol. Images and regions of interest were analyzed for each experimental condition. The data were analyzed by Student’s \(t\) test or one-way analysis of variance; \(P < 0.05\) was considered significant.
CLEM Experimental Protocol

The Correlative Light Electron Microscopy (CLEM) approach is described in detail in van Weering et al., 2010. In brief, cells were grown on special gridded glass bottom dishes. QDs were added to the cells for 2h and analysed under a Leica SP5 Confocal microscope registering both the fluorescence from the QDs as well as the bright field image with the finder pattern and outline of the cells. After fixation and processing for Electron Microscopy, ultrathin sections of the cells were made. Some sections were cut at 70 nm and stained for conventional TEM and Dark Field STEM imaging, and some specimens were cut at 300 nm, left unstained and imaged by TEM, STEM and Electron Tomography. The electron microscopy was done on a FEI Tecnai 20 TEM at 200kV fitted with a Fischione High Angle Anular Dark Field (HAADF) detector. This latter technique is extremely sensitive to atomic number contrast so that the QDs show up as particularly bright spots. The electron tomography has been used to confirm that the QDs are truly inside the cell and to further investigate the nature of some of the cellular structures enclosing them. This data is not presented here. The images were TEM images were recorded on an FEI 4k x 4k Eagle CCD camera.
Figure S7. Correlative microscopy of AS cell with Lactose (17b) Qds

a. Confocal image of the cell with most Lactose-QDs (Particle size ~ 15nm) bound to ER membranes
b. General view
c. STEM of QDs in Golgi vesicles
d. ER and Golgi: QDs on ER not in Golgi (BF)
e. STEM image of d.
Figure S8. Correlative microscopy of AS cells with Lactose-Maltotriose (21b) bifunctional QDs

a. Confocal image. Note the large vesicles, less ER localization than with Lactose (17b) QD’s, and worm-like aggregates in the cytoplasm.
b. B. General view of a cell (HAADF). The three inserts are magnified, top right to bottom left in the next panels.
c. QDs in Golgi (BF)
d. QDs in the cytosol (BF)
e. QDs in Golgi
Figure S9. Correlative microscopy of HeLa cells with Lactose (17b) QDs

a. confocal image QDs are more localized in specific areas than in AS. Note the perinuclear and ER localization
b. general view (HAADF); inserts are magnified top to bottom in the following panels
c. QDs in ER (BF)
d. QDs in Golgi (BF)
e. QDs in ER (BF)
**Figure S10** Correlative microscopy of HeLa cells with bifunctional Lactose-Maltotriose QDs

- a. confocal image
- b. general view (HAADF)
- c. perinuclear QDs
- d. QDs on ER
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Confocal Images
Colocalization Experiments HeLa

Early Endosomes

|  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
| Pearson Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson Correlation |
| 0.195 | 1 | 1 | 0.474 | 1.633 | 0.139 | 1 | 255 | 1 | 255 | 1 | 0.195 |

Dextran-QD (13)
### Colocalization Experiments HeLa

**Late Endosomes**

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|-----------------------|-----------------------------|-----------------------------|------------------------|------------------------|------------------------|-----------------|-----------------|-----------------|-----------------|----------------------|-----------------------------|
| 0.299                 | 1                           | 1                           | 0.533                  | 1.58                   | 0.185                  | 1               | 201             | 1               | 201             | 1                    | 0.299                        |

**Dextran-QD (13)**

![Image of HeLa cells with late endosomes and Dextran-QD](image)
## Colocalization Experiments HeLa

### Lysosomes

| Pearson Correlation | Mx | My | Overlap Coefficient R | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson Correlation |
|---------------------|----|----|------------------------|------------------------|-----------------|-----------------|-----------------|-----------------|-------------------------|---------------------------|
| 0.116               | 0.391 | 0.225 | 0.227                  | 0.596                  | 5              | 170             | 18              | 170             | 0.6                     | 0.137                      |

Dextran-QD (13)
Colocalization Experiments HeLa

Dextran-QD (13)

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|------------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|----------------|----------------|----------------|----------------|------------------------|-----------------------------|
| -0.064                 | 0.382                         | 0.056                         | 0.122                 | 1.235                  | 0.012                  | 12            | 255            | 41             | 255            | 0.144                  | 0.063                       |
Colocalization Experiments HeLa

Golgi

Dextran-QD (13)

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|------------------------|------------------------|------------------------|------------------|-----------------|------------------|------------------|---------------------------|-----------------------------|
| -0.147                | 0.121                         | 0.123                         | 0.08                   | 0.139                  | 0.058                  | 11               | 255             | 27               | 255              | 0.857                     | -0.04                       |
### Colocalization Experiments HeLa

**Mitochondria**

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-----------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------------------|----------------------------|
| -0.152                | 0.046                       | 0.029                       | 0.023                 | 0.16                  | 0.005                 | 12              | 255             | 70              | 255             | 0.517                       | -0.055                       |

**Dextran-QD (13)**

![Mitochondria Images](image-url)
Colocalization Experiments AS

Early Endosomes

| Pearson's Correlation | Colocalization Coefficient (Mx) | Colocalization Coefficient (My) | Overlap Coefficient (R) | Overlap Coefficient (Kx) | Overlap Coefficient (Ky) | X Min | X Max | Y Min | Y Max | Voxel Ratio (Ch.X/Ch.Y) | Global Pearson's Correlation |
|-----------------------|---------------------------------|---------------------------------|-------------------------|-------------------------|-------------------------|-------|-------|-------|-------|-------------------------|-----------------------------|
| 0.4365                | 0.479                           | 0.824                           | 0.577                   | 0.438                   | 0.773                   | 6     | 255   | 5     | 255   | 2.83                    | 0.504                       |
# Colocalization Experiments AS

## Late Endosomes

| Pearson Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio | Global Pearson Correlation |
|---------------------|-----------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-------------|---------------------------|
| 0.1525              | 1                           | 1                           | 0.197                 | 0.7165                | 0.0542                | 0               | 255             | 0               | 255             | 1           | 0.1525                    |

Dextran-QD (13)
Colocalization Experiments AS

Dextran-QD (13)

Late Endosomes
Colocalization Experiments AS

Dextrans-QD (13)

Lysosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-----------------------------|-------------------------------|------------------------|------------------------|------------------------|----------------|----------------|----------------|----------------|------------------------|----------------------------|
| -0.044                | 0.031                       | 0.234                         | 0.06                   | 0.025                  | 0.149                  | 29             | 255            | 22             | 255            | 6.865                  | 0.018                      |
Colocalization Experiments AS

Dextran-QD (13)

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|-----------------------|-----------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|----------------|----------------|----------------|----------------|------------------------|-----------------------------|
| -0.272                | 0.116                       | 0.213                       | 0.081                 | 0.151                 | 0.073                 | 18            | 255            | 96             | 255            | 1.263                  | -0.068                      |
| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient Rx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|------------------------|------------------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------------------|
| -0.271                | 0.401                         | 0.266                         | 0.199                  | 0.479                  | 13              | 255             | 68              | 255             | 0.801                  | -0.157                      |
Colocalization Experiments AS

Mitochondria

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-----------------------------|-----------------------------|----------------------|----------------------|----------------------|------------------|----------------|------------------|------------------|-------------------------|-----------------------------|
| -0.134               | 0.214                       | 0.109                       | 0.099                | 0.469                | 0.024                | 14               | 255            | 78               | 255              | 545                    | -0.059                     |
## Colocalization Experiments HeLa

### Early Endosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|
| 0.753                 | 1                             | 1                             | 0.952                 | 0.896                 | 1.012                 | 6               | 91              | 6               | 91              | 1                        | 0.753                      |

Gal-QD (14b)
Colocalization Experiments HeLa

Late Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-----------------------------|-----------------------------|---------------------|----------------------|----------------------|------------------|------------------|------------------|------------------|----------------------|-----------------------------|
| 0.9027               | 1                           | 1                           | 0.91                | 1.697                | 0.505                | 5                | 203              | 5                | 203              | 1                    | 0.9027                      |
### Colocalization Experiments HeLa

#### Lysosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|------------------------------|-----------------------|-----------------------|-----------------------|------------------|----------------|----------------|----------------|----------------|--------------------------|---------------------------|
| 0.095                 | 0.3195                        | 0.444                        | 0.339                 | 0.135                 | 0.899                 | 26               | 227            | 9              | 227            | 1.42                      | 0.204                     |
Colocalization Experiments HeLa

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------------------|
| -0.124               | 0.6635                        | 1                             | 0.578                 | 0.774                 | 0.448                 | 7               | 68              | 5               | 68              | 2.075                  | -0.0725                     |
Colocalization Experiments HeLa

Gal-QD (14b)

Golgi

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------|----------------|----------------|----------------|------------------|-----------------------------|
| -0.0115              | 0.9875                        | 1                             | 0.694                 | 3.06                  | 0.1605               | 8               | 156            | 3              | 255            | 1.015            | -0.011                     |

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### Colocalization Experiments HeLa

**Mitochondria**

| Pearson Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson Correlation |
|---------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|------------------|-----------------|-----------------|-----------------|------------------------|---------------------------|
| 0.382               | 0.887                         | 1                             | 0.861                 | 0.789                  | 0.982                  | 6                | 91              | 7               | 91              | 1.207                  | 0.386                     |

**Gal-QD (14b)**
Colocalization Experiments AS

Early Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|------------------------------|------------------------------|-----------------------|------------------------|------------------------|-------------------|-----------------|-----------------|-----------------|------------------------|-----------------------------|
| 0.1825               | 1                            | 1                            | 0.629                 | 1.2675                 | 0.345                  | 6                 | 255             | 6               | 255             | 1                      | 0.1825                     |

Gal-QD (14b)
Colocalization Experiments AS
Late Endosomes

| Pearson's Correlation  | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R  | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|------------------------|------------------------|------------------------|------------------|------------------|------------------|------------------|--------------------------|-------------------------------|
| 0.283                 | 0.993                         | 0.3855                        | 0.4765                 | 0.7335                 | 0.328                  | 8                | 255              | 3                | 255              | 4.66                     | 0.281                        |

Gal-QD (14b)
Colocalization Experiments AS

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|-----------------|-----------------|-----------------|------------------------|-----------------------------|
| 0.412                | 0.676                         | 1                             | 0.698                 | 1.163                  | 0.42                   | 5                 | 184             | 8               | 184             | 1.718                  | 0.412                       |

Gal-QD (14b)
Colocalization Experiments AS

**ER**

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-----------------------------|-------------------------------|------------------------|------------------------|------------------------|-----------------|-----------------|------------------|------------------|---------------------------|-----------------------------|
| -0.0795              | 0.4175                      | 0.13275                       | 0.2025                 | 1.02                   | 0.04525                | 24              | 250             | 48               | 250              | 0.3095                    | -0.11395                   |
### Colocalization Experiments AS

#### Gal-QD (14b)

#### Golgi

| Parameters | Values |
|------------|--------|
| Pearson's Coefficient Mx | -0.0035 |
| Pearson's Coefficient My | 1 |
| Overlap Coefficient R | 1 |
| Overlap Coefficient Kx | 1 |
| Overlap Coefficient Ky | 0.47 |
| X Min Threshold | 0.258 |
| X Max Threshold | 2 |
| Y Min Threshold | 2 |
| Y Max Threshold | 255 |
| Voxel Ratio Ch.X/Ch.Y | 1 |
| Global Pearson's Coefficient | -0.0035 |
Colocalization Experiments AS
Mitochondria

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-----------------------------|-----------------------------|----------------------|----------------------|----------------------|------------------|------------------|------------------|------------------|------------------------|-----------------------------|
| 0.2565               | 0.5285                      | 0.08635                     | 0.2095               | 0.852                | 0.0518               | 10               | 91               | 10               | 91               | 10.166                 | 0.16945                     |
Colocalization Experiments HeLa

Early Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|------------------|------------------|------------------|------------------|------------------------|-----------------------------|
| -0.05205             | 0.1095                        | 0.0995                        | 0.0668                | 0.05155               | 0.0867                | 17               | 255              | 11               | 255              | 1.405                  | 0.05075                     |
Colocalization Experiments HeLa

Late Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-----------------------------|-----------------------------|----------------------|----------------------|----------------------|----------------|----------------|----------------|----------------|------------------------|-----------------------------|
| 0.4465               | 0.03995                     | 0.4445                      | 0.1785               | 0.0698               | 0.474                | 17             | 255            | 15             | 255            | 9.85                   | 0.1581                      |
Colocalization Experiments HeLa

Late Endosomes

![Image of HeLa cells showing colocalization of Lactose-QD (17b)]

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|------------------|------------------|------------------|--------------------------|-----------------------------|
| 0.695                | 0.176                         | 0.0389                        | 0.146                 | 0.566                  | 0.0377                 | 11                | 255              | 23               | 255              | 0.246                    | 0.129                       |
**Colocalization Experiments HeLa**

**Late Endosomes 4 C**

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|----------------------|--------------------------|
| 0.693                | 1                             | 1                             | 0.745                 | 2.393                 | 0.241                 | 0               | 255             | 0               | 255             | 1                    | 0.693                    |
## Colocalization Experiments HeLa

### Lysosomes

- **Pearsons Correlation**: -0.1609
- **Colocalization Coefficient Mx**: 0.2665
- **Colocalization Coefficient My**: 0.10015
- **Overlap Coefficient R**: 0.08825
- **Overlap Coefficient Kx**: 0.454
- **Overlap Coefficient Ky**: 0.01715
- **X Min Threshold**: 9
- **X Max Threshold**: 255
- **Y Min Threshold**: 38
- **Y Max Threshold**: 255
- **Voxel Ratio Ch.X/Ch.Y**: 0.319
- **Global Pearsons Correlation**: 0.06

### Lactose-QD (17b)
Colocalization Experiments HeLa

Lactose-QD (17b)

Nucleus
Colocalization Experiments HeLa

Lactose-QD (17b)

ER 37C

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|------------------|------------------|-----------------|------------------|--------------------------|----------------------------|
| 0.838                | 0.777                         | 1                             | 0.892                 | 1.35                   | 0.633                  | 1.333            | 255              | 1               | 255              | 1.963                    | 0.87                       |
### Colocalization Experiments HeLa

Lactose-QD (17b)

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-----------------------------|-----------------------|------------------------|------------------------|-----------------|-----------------|-----------------|-----------------|---------------------------|-----------------------------|
| 0.309                 | 0.74                          | 0.643                       | 0.616                 | 5.227                  | 0.074                  | 2               | 171             | 13              | 171             | 0.833                     | 0.387                       |
Colocalization Experiments HeLa

Lactose-QD (17b)

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|----------------------|----------------------|----------------------|-----------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|
| 0.8735                | 0.6345                        | 0.7565                        | 0.7625               | 2.755                | 0.217                | 10              | 255             | 8               | 255             | 1.1605                  | 0.7345                     |
Colocalization Experiments HeLa

Lactose-QD (17b)

Golgi

| Pearson Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson Correlation |
|---------------------|-----------------------------|-----------------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-------------------------|-----------------------------|
| 0.335               | 1                           | 1                           | 0.642                 | 3.87                  | 0.106           | 2               | 255             | 2               | 255                     | 0.335                       |
Colocalization Experiments HeLa

Golgi

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|------------------------------|------------------------------|-----------------------|-----------------------|-----------------------|-----------------|----------------|----------------|----------------|-------------------|-------------------------|
| 0.463                | 1                            | 1                            | 0.676                 | 2.543                 | 0.213                 | 2               | 255            | 2              | 255            | 1                 | 0.463                   |

Lactose-QD (17b)

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Colocalization Experiments AS

**Early Endosomes**

| Pearsons Correlation | Colocalization Coefficient | Colocalization Coefficient R | Overlap Coefficient | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons |
|----------------------|----------------------------|------------------------------|---------------------|------------------------|------------------------|------------------|-----------------|-----------------|-----------------|-----------------------|----------------|
| 0.6575               | 0.834                      | 0.649                        | 0.6165              | 0.5335                 | 0.739                  | 19               | 251             | 11              | 251             | 509.8075              | 0.5095         |
### Colocalization Experiments AS

**Late Endosomes**

![Immunofluorescence images](image)

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|-----------------|-------------------|-------------------|--------------------------|-----------------------------|
| 0.5165               | 1                             | 1                             | 0.5965                | 1.955                  | 0.1815                 | 0                 | 255             | 0                 | 255               | 1                        | 0.5165                     |

**Lactose-QD (17b)**
Colocalization Experiments AS

Lysosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|
| 0.2225               | 0.0221                        | 0.653                         | 0.06685               | 0.01685               | 0.2665               | 13              | 255             | 5               | 255             | 17.63                    | 0.03725                    |
Colocalization Experiments AS

Lysosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min | X Max | Y Min | Y Max | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------|-------|-------|-------|------------------------|-----------------------------|
| 0.0417               | 0.04895                       | 0.683                        | 0.087                 | 0.0445                 | 0.4515                 | 12    | 255   | 5     | 255   | 13.86                  | 0.11405                     |
Colocalization Experiments AS

Nucleus
### Colocalization Experiments AS

#### Lactose-QD (17b)

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|----------------|----------------|----------------|----------------|---------------------------|-----------------------------|
| 0.56                  | 0.787                         | 1                             | 0.763                 | 1.248                  | 0.467                  | 12                | 255            | 15             | 255            | 1.64                      | 0.543                      |
Colocalization Experiments AS

Lactose-QD (17b)

| Pearson's Correlation | X Min | X Max | Y Min | Y Max |
|-----------------------|-------|-------|-------|-------|
| 0.176                 | 1     | 173   | 173   | 8     |
| 0.711                 | 5     | 8     | 173   |      | 1.475 | 0.2155 |

Golgi

| X Overlap Coefficient | Y Overlap Coefficient | X Min Threshold | Y Min Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-----------------------|-----------------|-----------------|-----------------------|-----------------------------|
| 0.7055                | 0.3215                | 5               | 8               | 1.475                 | 0.2155                      |
Colocalization Experiments AS
Late Endosomes

Lactose/Mann-QD (20b)

| Pearsons Correlation | Colocalization Coefficient M1 | Colocalization Coefficient M2 | Overlap Coefficient R | Overlap Coefficient k1 | Overlap Coefficient k2 | Ch.1 Threshold | Ch.2 Threshold | Voxel Ratio Ch.1 / Ch.2 | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|-----------------------|---------------|---------------|-------------------------|-----------------------------|
| -0.074               | 0.209                         | 0.047                         | 0.074                 | 0.351                  | 0.019                 | 9.333         | 11.000        | 0.169                   | 0.068                       |
### Colocalization Experiments AS

| Parameter                  | Value          |
|---------------------------|----------------|
| Pearsons Correlation      | -0.17152       |
| Colocalization Coefficient M1 | 0.15422       |
| Colocalization Coefficient M2 | 0.007432      |
| Colocalization Coefficient R | 0.02652       |
| Overlap Coefficient k1    | 0.4868         |
| Overlap Coefficient k2    | 0.0016668      |
| Ch.1 Threshold            | 11.6           |
| Ch.2 Threshold            | 39             |
| Voxel Ratio Ch.1 / Ch.2   | 0.04928        |
| Global Pearsons Correlation | 0.02222      |
Colocalization Experiments AS

Lyso

Lactose/Mann-QD (20b)

| Metric                      | Value   |
|-----------------------------|---------|
| Pearson's Correlation       | 0.003   |
| Colocalization Coefficient M1 | 0.224   |
| Colocalization Coefficient M2 | 0.223   |
| Coefficient R               | 0.136   |
| Coefficient k1              | 0.155   |
| Coefficient k2              | 0.154   |
| Ch.1 Threshold              | 7.667   |
| Ch.2 Threshold              | 9.833   |
| Voxel Ratio Ch.1 / Ch.2     | 3.537   |
| Global Pearson's Correlation| 0.081   |
Colocalization Experiments AS

Golgi

Lactose/Mann-QD (20b)

| Pearson Correlation | Colocalization Coefficient M1 | Colocalization Coefficient M2 | Overlap Coefficient R | Overlap Coefficient K1 | Overlap Coefficient K2 | Ch.1 Threshold | Ch.2 Threshold | Voxel Ratio Ch.1 / Ch.2 | Global Pearson Correlation |
|---------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|-------------------|---------------------------|-----------------------------|
| 0.085               | 0.266                         | 0.064                         | 0.121                 | 0.554                  | 0.041                  | 10.167            | 19.000           | 0.241                     | 0.119                       |
Colocalization Experiments HeLa
Early Endosomes

Lactose/Mann-QD (20b)

| Pearson's Correlation | Colocalization Coefficient M1 | Colocalization Coefficient M2 | Overlap Coefficient R | Overlap Coefficient k1 | Overlap Coefficient k2 | Ch.1 Threshold | Ch.2 Threshold | Voxel Ratio Ch.1/Ch.2 | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-----------------|-----------------|-----------------------|-----------------------------|
| 0.410                 | 0.589                         | 0.666                         | 0.578                 | 3.190                  | 0.106                  | 4.500           | 12.000         | 0.894                 | 0.565                       |
Colocalization Experiments HeLa

Late Endosomes

|                  | Pearson Correlation | Colocalization Coefficient M1 | Colocalization Coefficient M2 | Overlap Coefficient R | Overlap Coefficient k1 | Overlap Coefficient k2 | Ch.1 Threshold | Ch.2 Threshold | Voxel Ratio Ch.1 / Ch.2 | Global Pearson Correlation |
|------------------|---------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-----------------|-----------------|-----------------------------|---------------------------|
|                  | -0.082              | 0.436                         | 0.448                         | 0.304                 | 0.731                  | 0.792                  | 8.000           | 10.750          | 1.058                       | 0.250                     |
Colocalization Experiments HeLa

Lactose/Mann-QD (20b)

Pearsos Correlation: 0.227
Colocalization Coefficient M1: 0.253
Colocalization Coefficient M2: 0.070
Overlap Coefficient R: 0.158
Overlap Coefficient k1: 1.005
Overlap Coefficient k2: 0.028
Ch.1 Threshold: 6.500
Ch.2 Threshold: 24.000
Voxel Ratio Ch.1 / Ch.2: 0.270
Global Pearsos Correlation: 0.156
Colocalization Experiments HeLa

Golgi

Lactose/Mann-QD (20b)

| Pearsons Correlation | Colocalization Coefficient M1 | Colocalization Coefficient M2 | Overlap Coefficient R | Overlap Coefficient k1 | Overlap Coefficient k2 | Ch.1 Threshold | Ch.2 Threshold | Voxel Ratio Ch.1 / Ch.2 | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|-----------------|--------------------------|-----------------------------|
| 0.031                | 0.785                         | 0.760                         | 0.447                 | 2.390                  | 0.146                  | 4.000             | 7.500          | 0.921                    | 0.107                       |
Colocalization Experiments HeLa

Early Endosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|------------------|-----------------|------------------|------------------|--------------------------|-----------------------------|
| 0.675                 | 0.698                         | 0.319                         | 1.53                  | 2                     | 255                   | 2                | 255             | 3.167            | 0.661            |                          |                             |
### Colocalization Experiments HeLa

#### Late Endosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|----------------|----------------|----------------|----------------|------------------------|-------------------------------|
| 0.427                 | 1                             | 1                             | 0.647                 | 0.93                   | 0.864                  | 3              | 255            | 3              | 255            | 1                      | 0.427                        |

Lact/Malt3-QD (21b)
Colocalization Experiments HeLa

Nucleus

Lact/Malt3-QD (21b)
Colocalization Experiments HeLa

Lysosomes

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|----------------|----------------|----------------|----------------|----------------|--------------------------|
| 0.2695                | 0.2435                        | 0.6635                        | 0.407                 | 0.07955                | 2.3155                 | 6             | 255            | 5              | 255            | 7.575                    | 0.3755                    |

Lact/Malt3-QD (21b)

S78
Colocalization Experiments HeLa

Lact/Malt3-QD (21b)

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|-------------------|----------------|----------------|----------------|----------------|---------------------------|---------------------------|
| -0.06925             | 0.3435                        | 0.10945                     | 0.13                  | 1.56                  | 0.01115               | 5                 | 255            | 26             | 255            | 0.2755                    | 0.0426                    |

ER
| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|------------------|------------------|------------------|------------------|--------------------------|-----------------------------|
| 0.0144                | 0.6575                        | 0.2775                        | 0.329                 | 6.07                  | 0.01885              | 3                | 255              | 41               | 255              | 0.372                    | 0.211                      |
### Colocalization Experiments HeLa

#### Lact/Malt3-QD (21b)

**Golgi**

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|------------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|------------------|------------------|------------------|------------------|--------------------------|----------------------------|
| -0.0911                | 0.638                         | 0.15                          | 0.239                 | 0.658                 | 0.0866                | 12               | 167              | 19               | 167              | 0.238                    | 0.212                      |
Colocalization Experiments HeLa

Golgi

|                | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------|-----------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|-----------------|----------------|-----------------|-----------------|-------------------------|-----------------------------|
| Pearson's Coefficient | 0.0144                     | 0.6575                     | 0.2775                | 0.329                 | 6.07                  | 0.01885         | 3              | 255             | 41              | 255                     | 0.372                       | 0.211                       |

Lact/Malt3-QD (21b)
Colocalization Experiments HeLa

Mitochondria

Lact/Malt3-QD (21b)
Colocalization Experiments AS

Early Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-----------------|-----------------|-----------------|-----------------|--------------------------|----------------------------|
| 0.416                | 1                             | 1                             | 0.53                  | 1.2745                 | 0.2395                 | 1               | 255             | 1               | 255             | 1                       | 0.416                     |

Lact/Malt3-QD (21b)
Colocalization Experiments AS

Late Endosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|
| 0.022                | 0.2512                        | 0.0602                        | 0.127                 | 0.5                    | 0.035                 | 20              | 255             | 21              | 255             | 0.227                    | 0.1                         |
### Colocalization Experiments AS

#### Lysosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|------------------------|------------------------|------------------------|------------------|------------------|------------------|------------------|--------------------------|-----------------------------|
| -0.0947              | 0.08675                       | 0.689                         | 0.265                  | 0.0548                | 1.307                  | 51               | 255              | 57               | 255              | 11.01                    | 0.2445                      |

Lact/Malt3-QD (21b)
### Lysosomes

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|-------------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|
| -0.2175              | 0.00862                       | 0.16045                       | 0.1374                | 0.00372                | 0.3205                 | 41                | 255             | 41              | 255             | 18.75                    | 0.0205                      |

**LactMalt3-QD (21b)**
### Colocalization Experiments AS

#### Lact/Malt3-QD (21b)

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|------------------------------|------------------------------|------------------------|------------------------|------------------------|------------------|------------------|------------------|------------------|--------------------------|-----------------------------|
| -0.102               | 0.05625                      | 0.133                        | 0.0708                 | 0.15385                | 0.04125                | 8                | 128              | 27               | 128              | 3.015                    | 0.0034                      |

**ER**

![Image of ER](image1)

![Image of Lact/Malt3-QD (21b)](image2)
Colocalization Experiments AS

**ER**

| Pearson's Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson's Correlation |
|-----------------------|-------------------------------|-------------------------------|------------------------|------------------------|-----------------|----------------|-----------------|-----------------|------------------------|-----------------------------|
| -0.07375              | 0.5245                        | 0.2195                        | 0.2155                 | 1.0725                 | 5               | 255            | 12              | 255             | 0.52                   | 0.1121                      |
### Colocalization Experiments AS

#### Lact/Malt3-QD (21b)

**Golgi**

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|------------------|----------------|------------------|------------------|---------------------------|----------------------------|
| 0.167                | 0.805                         | 1                             | 0.4705                | 0.433                  | 0.514                  | 5                | 255            | 7                | 255              | 1.63                      | 0.2113                     |
Colocalization Experiments AS

Golgi

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|------------------|-----------------|------------------|------------------|------------------------|-----------------------------|
| 0.1097               | 0.25                          | 0.193                         | 0.2015                | 1.5225                 | 0.0281                 | 9                | 255             | 58               | 255              | 0.8                    | 0.12315                    |
### Colocalization Experiments AS

**Mitochondria**

| Pearsons Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearsons Correlation |
|----------------------|-------------------------------|-------------------------------|-----------------------|-----------------------|-----------------------|------------------|------------------|------------------|------------------|--------------------------|-----------------------------|
| -0.02715             | 0.3595                        | 0.10215                       | 0.15515               | 0.3035               | 0.0924              | 11               | 134              | 13               | 134              | 0.267                    | 0.05645                     |

Lact/Malt3-QD (21b)
## Colocalization Experiments AS

### Mitochondria

| Pearson Correlation | Colocalization Coefficient Mx | Colocalization Coefficient My | Overlap Coefficient R | Overlap Coefficient Kx | Overlap Coefficient Ky | X Min Threshold | X Max Threshold | Y Min Threshold | Y Max Threshold | Voxel Ratio Ch.X/Ch.Y | Global Pearson Correlation |
|---------------------|-------------------------------|-------------------------------|-----------------------|------------------------|------------------------|------------------|-----------------|------------------|------------------|--------------------------|-----------------------------|
| 0.0553              | 0.421666667                   | 0.158866667                   | 0.2167                | 0.353                  | 0.1503                 | 10               | 117             | 12               | 117              | 0.351                    | 0.061233333                      |