Supporting Information

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Nanoparticle Transport in Epithelial Cells: Pathway Switching Through Bioconjugation

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Figure S1. $^1$H-NMR (MeOD, 600 MHz) of $\alpha$-$\omega$-aminohexylcarbamate vitamin B$_{12}$ (top) and cyanocobalamin with HOD solvent suppression (bottom).

Figure S2. 1D selective TOCSY $^1$H-NMR spectrum (MeOD, 600 MHz) of $\alpha$-$\omega$-aminohexylcarbamate vitamin B$_{12}$ (irradiated at 6.24 ppm with 200 ms TOCSY mixing time) (top) and cyanocobalamin (irradiated at 4.7 ppm with 60 ms TOCSY mixing time (bottom).
Figure S3. COSY (MeOD, 600 MHz) of α-ω-aminohexylcarbamate vitamin B$_{12}$ (2 scans per increment 2048 x 400 points).
Figure S4. Liquid chromatographs of crude (bottom; labelled ‘before purification’) and purified α-ω-aminohexylcarbamate vitamin B₁₂ (top; labelled ‘after purification’).
Figure S5. TOF-MS of purified α-ω-aminohexylcarbamate vitamin B_{12}
Figure S6. Fluorescence characterization of Yelow Orange nanoparticles. A) Wavelength scan (530-650 nm) showing unmodified and B$_{12}$-conjugated nanoparticles for 50, 100 and 200 nm particle sizes. B) Wavelength scan of 50 nm unmodified nanoparticles with increasing percentage amounts (w/w relative to nanoparticles) of soluble B$_{12}$ in suspending medium and of B$_{12}$-conjugated nanoparticles (shown in black line). C) Dialysis experiment showing restored fluorescence intensity of unmodified nanoparticles following removal of free B$_{12}$ and comparison with B$_{12}$-conjugated nanoparticles. D) Exponential decay model (R$^2 \geq 0.997$) for determination of the weight percentage of B$_{12}$ in B$_{12}$-conjugated particles (Data was fitted using Prism5 v 5.0 from GraphPad Software, Inc, using an in built model).

Comparison

![Comparison](image)

Figure S7. Potentiometric titration of unmodified YO carboxy polystyrene nanoparticles (50, 100, 200 nm) with 0.05M NaOH.

Figure S8. Characterization of unconjugated and vitamin B$_{12}$-conjugated nanoparticles by Dynamic Light Scattering (DLS). A) DLS intensity distribution of unmodified YO nanoparticles with nominal diameters of 50 nm, 100 nm and 200 nm. B) DLS intensity distribution of vitamin B$_{12}$-conjugated nanoparticles. Measurements were taken at 25°C in Hank’s Balanced Salt Solution (biological solution in which nanoparticles were applied to the cells). The data represent a mean of 10 measurements. The DLS data is expressed as average hydrodynamic radii, which for unmodified nanoparticles were 21, 40 and 70 nm for nanoparticles with a nominal diameter of 50, 100 and 200 nm, respectively. For B$_{12}$-conjugated nanoparticles, the measured hydrodynamic radii were 39, 100 and 139 nm for nanoparticles with a nominal, pre-conjugation diameter of 50, 100 and 200 nm, respectively.
Table S1. Zeta potential of unmodified and vitamin B12-conjugated nanoparticles. The values represent the mean of three measurements taken in Hank’s Balanced Salt Solution (biological solution used for nanoparticle application to the cells).

<table>
<thead>
<tr>
<th>Zeta Potential ± SD (mV)</th>
<th>Unmodified 50 nm</th>
<th>B12-conjugated 50 nm</th>
<th>Unmodified 100 nm</th>
<th>B12-conjugated 100 nm</th>
<th>Unmodified 200 nm</th>
<th>B12-conjugated 200 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-34.6 ± 0.1</td>
<td>-26.3 ± 1.7</td>
<td>-32.1 ± 0.6</td>
<td>-27.8 ± 0.7</td>
<td>-33.1 ± 0.2</td>
<td>-27.4 ± 2.6</td>
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</table>
Table S9. Control Lysosomal studies using Lysotracker™ Green DND-26 with unmodified nanoparticles (50 nm) in Caco-2 monolayers.

Experiments: A. Cells treated with both unlabelled (non-fluorescent) 50 nm nanoparticles and YO fluorescent 50 nm nanoparticles and imaged under Lysotracker™ settings: i) blue channel (Hoechst stain) for cell nuclei; ii) green channel, iii) overlay image of blue and green channels. B. Cells treated with unlabelled nanoparticles and the Lysotracker™ probe and imaged under YO nanoparticle settings: i) blue channel (Hoechst stain) for cell nuclei; ii) red channel, iii) overlay image of blue and red channels. C. Cells treated with YO 50 nm nanoparticles and imaged under Lysotracker™ settings: i) blue channel (Hoechst stain) for cell nuclei, ii) green channel, iii) overlay image of blue and green channels. D. Cells treated with the Lysotracker™ probe and imaged under YO nanoparticle settings: i) blue channel (Hoechst stain) for cell nuclei, ii) red channel, iii) overlay image of blue and red channels. E) Cells treated with unlabelled (non-fluorescent) 50 nm nanoparticles and stained with the Lysotracker™ probe, followed by imaging under Lysotracker™ settings: i) blue channel (Hoechst stain) for cell nuclei, ii) green channel, iii) overlay image of blue and green channels.

F. Tabulated summary of experimental conditions, where ‘+’ denotes ‘present’ in each sample. Confocal scan settings for Lysotracker™ Green DND-26: excitation 504 nm, emission 511 nm. Scan settings for nanoparticles: excitation 529 nm, emission 546 nm.
Figure S10. Inhibition of cell trafficking of FITC-transferrin and Cholera-toxin B subunit. A) Effect of chlorpromazine on cell uptake of FITC-transferrin. B) Effect of chlorpromazine on transport of FITC-transferrin. C) Effect of filipin on cell uptake of cholera-toxin B subunit (‘Ch-T-B’). D) Effect of filipin on transport of cholera-toxin B subunit. Chlorpromazine was applied at 10 µg/ml and filipin at 5 µg/ml.
Figure S11. Effect of pathway inhibitors, chlorpromazine (applied at 10 µg/ml) and filipin (applied at 5 µg/ml) on (A) cell uptake and (B) transport of unmodified nanoparticles (50 nm). Statistical comparison between ‘Chlorpromazine (10µg/ml) treated’ and ‘untreated 50nm nanoparticles’ and ‘Filipin (5µg/ml) treated’ and ‘untreated 50nm nanoparticles’. Statistical comparison between chlorpromazine or filipin treated with ‘untreated 50nm nanoparticles’; ‘ns’ = non significant.