pharmaceutics

MDPI

Supplementary Materials

Increased Carrier Peptide Stability Through pH Adjustment Improves Insulin and PTH(1-34) Delivery In Vitro And In Vivo Rather than by Enforced Penetratin-Cargo Complexation

Mie Kristensen[1], Ragna Guldsmed Diedrichsen[1,2], Valeria Vetri[3], Vito Foderà[1,2] and Hanne Mørck Nielsen[1,2]*

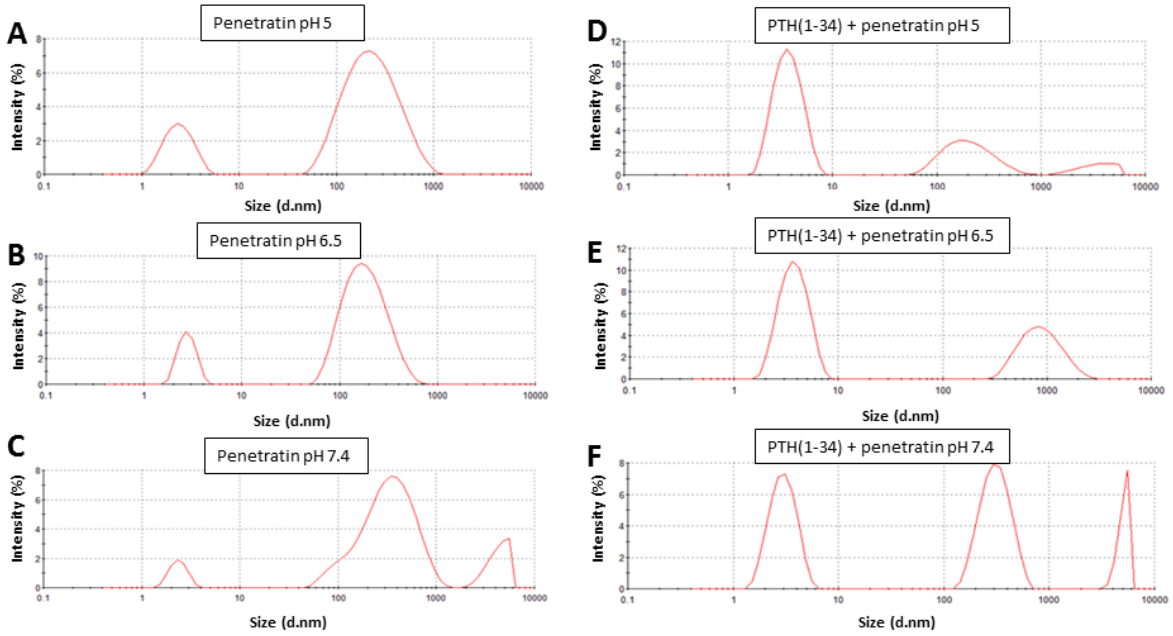

Figure S1. Representative size distributions by intensity of samples containing 720 μM penetratin (**A**-**C**) or 180 μM PTH and 720 μM penetratin (1-34) (**D**-**E**) at pH 5, 6.5, or 7.4 determined by dynamic light scattering.

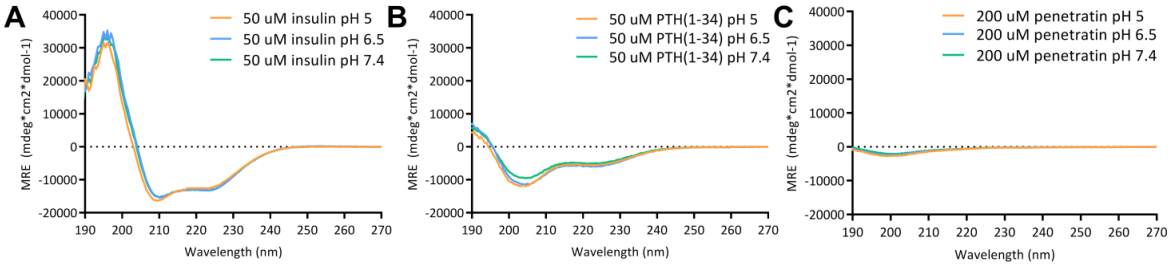

Figure S2. Circular dichroism spectra of 50 μM insulin (**A**), PTH(1-34) (**B**), and 200 μM penetratin (**C**) at pH 5, 6.5, or 7.4.

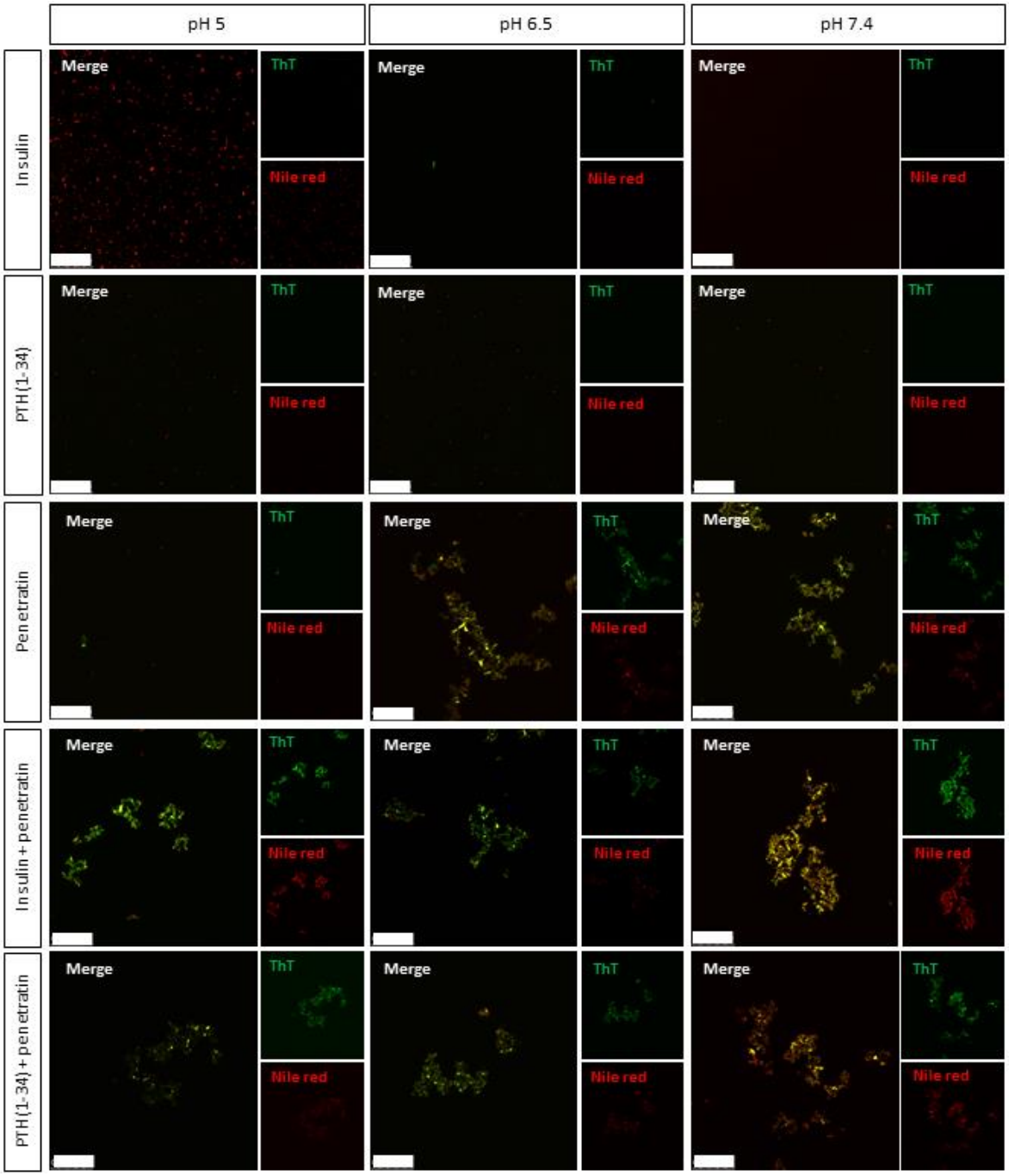

Figure S3. Confocal and two-photon excitation microscopy images of complexes obtained as a result of mixing 50 μM insulin or PTH(1-34) with 200 μM penetratin at pH 5, 6.5, or 7.4 in the presence of Thioflavin T (ThT) (green) and Nile Red (red). Scale bars: 50 μm.

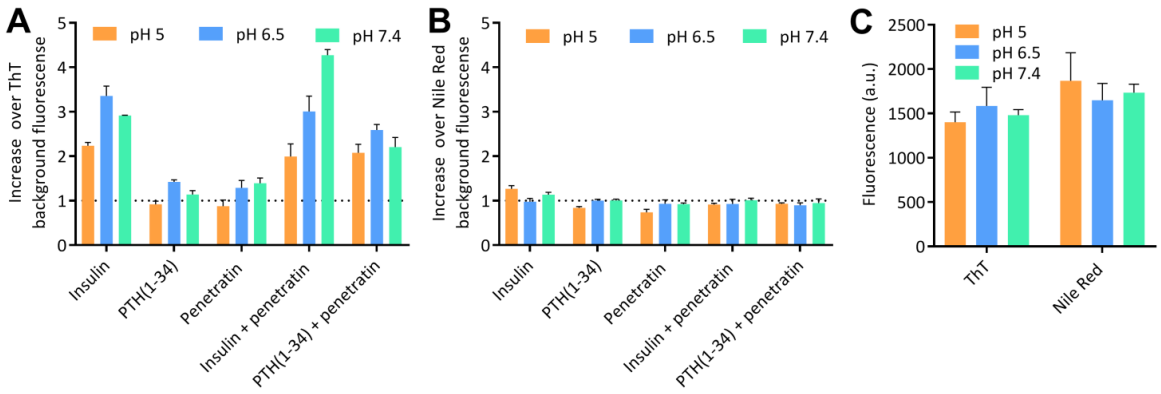

Figure S4. Thioflavin T (ThT) (**A**) and Nile Red (**B**) increase over ThT or Nile Red background fluorescence (**C**) of samples containing 50 μM insulin or PTH(1-34) or 200 μM penetratin alone or as insulin/PTH(1-34) + penetratin mixtures prepared at pH 5, 6.5, or 7.4. (N = 3, mean ± SD).

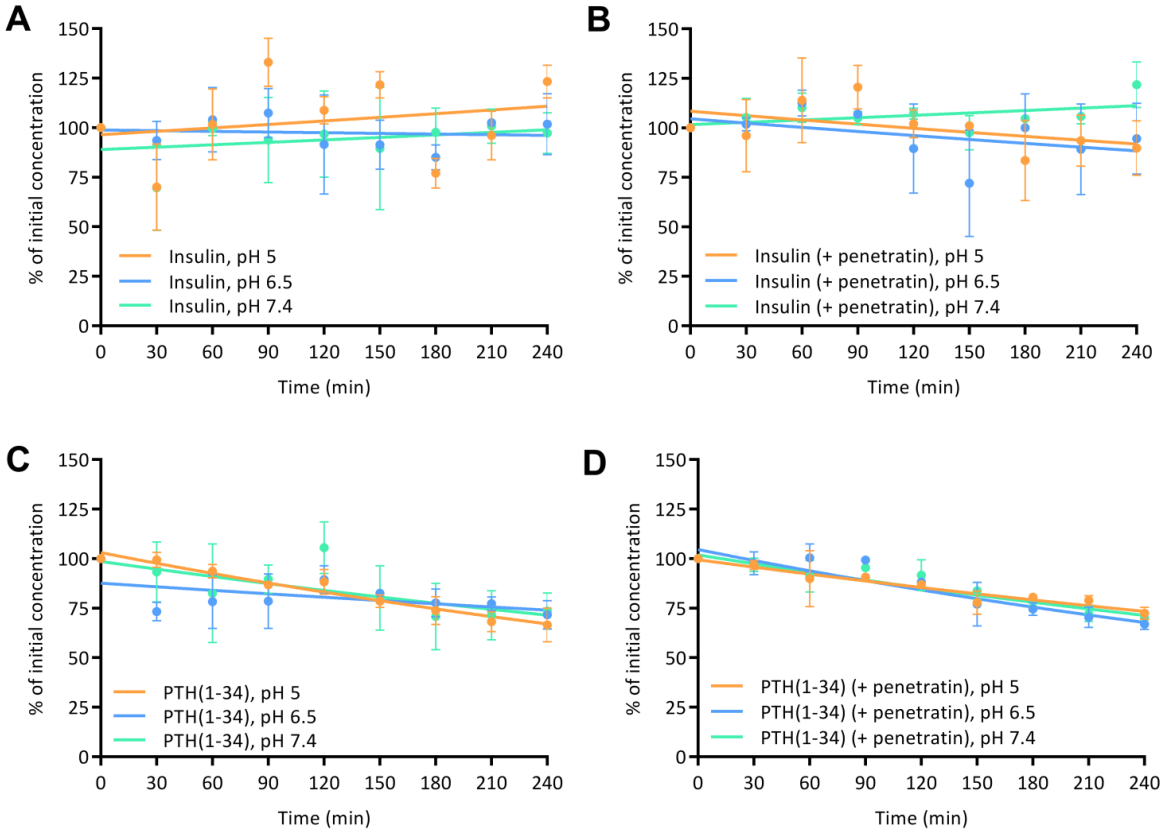

Figure S5. Stability of 5 μM insulin (**A**), 5 μM insulin in the presence of 20 μM penetratin (**B**), 5 μM PTH(1-34) (**C**), or 5 μM PTH(1-34) in the presence of 20 μM penetratin (**D**) during apical incubation on Caco-2 cell monolayers at pH 5, 6.5, or 7.4 over 4 hours. Data are presented as % of initial concentration (N = 3, mean ± SD).

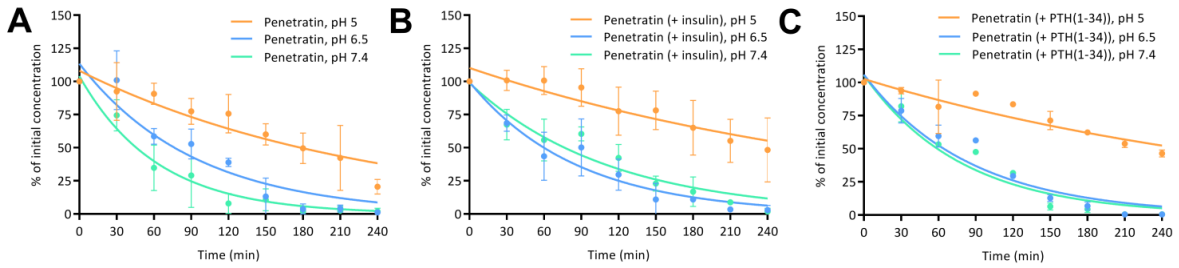

Figure S6. Stability of 20 μM penetratin (**A**), 20 μM penetratin in the presence of 5 μM insulin (**B**), or 20 μM penetratin in the presence of 5 μM PTH(1-34) (**C**) during apical incubation with Caco-2 cell monolayers at pH 5, 6.5, or 7.4 over 4 hours. Data are presented as % of initial concentration ± SD (N = 3, mean ± SD).

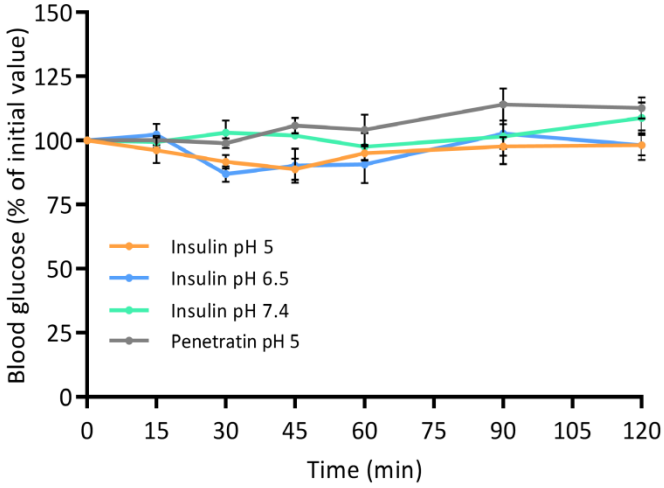

Figure S7. Blood glucose following intraintestinal administration of insulin (50 IU/kg) at pH 5, 6.5, or 7.4 or 720 μM penetratin at pH 5. Data are presented as % of initial value (N = 6, mean ±SD).

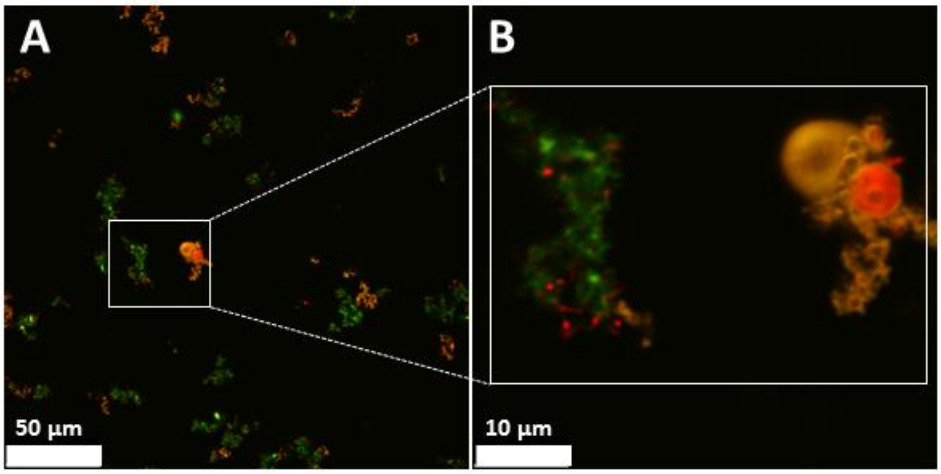

Figure S8. Confocal and two-photon excitation microscopy images of complexes obtained as a result of mixing 50 μM insulin with 200 μM penetratin at pH 6.5 in the presence of POPC:POPG (80:20 molar ratio) liposomes with addition of Thioflavin T (ThT) (green) and Nile Red (red).

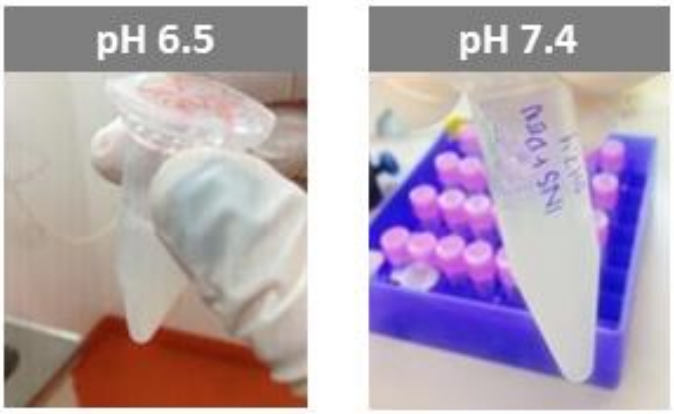

Figure S9. Visual inspection of pH 6.5 (**left**) and pH 7.4 (**right**) samples containing 180 μM insulin in physical mixture with 720 μM penetratin prior intraintestinal administration in rats.