



# Conjugation of peptide therapeutics using small, mono-dispersed amphiphilic oligomers for oral delivery.



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## ABSTRACT

**PURPOSE:** To develop orally deliverable peptide therapeutics by conjugating peptides of known efficacy with small molecular weight (typically less than 500 daltons), mono-dispersed amphiphilic oligomers to enhance oral bioavailability, improve stability toward proteases, and increase circulating half life, while retaining the biological activity of the natural peptide.

**METHODS:** Two therapeutic peptides (insulin and BNP) were coupled to an activated amphiphilic oligomer in either aqueous or organic medium. The extent of conjugation (mono-, di-, tri-conjugates, etc) to a specific peptide was controlled by the number of equivalents of activated oligomer. The conjugates were analyzed and purified using reversed HPLC and characterized by mass spectral analyses. Stabilities of conjugates were evaluated by incubation with pancreatic proteases. BNP conjugates were assayed *in vitro* to measure agonist activity in cells expressing the human natriuretic peptide receptor A (NPR-A). Conjugates showing activity were then assayed for bioavailability after oral dosing in rats. The activities of insulin conjugates were ascertained by measuring blood glucose levels after oral dosing in fasted mice. The solubility's of insulin conjugates were assayed by HPLC during titrations with NaOH in the range from pH 2 – 8.

**RESULTS:** Nobex Corporation's proprietary amphiphilic oligomers comprising of PEG and alkyl moieties have been successfully conjugated to various peptide therapeutics, thus improving desirable characteristics of the peptides including: oral absorption, PK/PD profile, stability to proteases, aqueous solubility, and duration of action.

**CONCLUSION:** Oral delivery of therapeutic peptides (e.g. insulin, BNP) can be accomplished by covalent attachment of small, mono-dispersed amphiphilic oligomers that substantially contribute to overcoming the challenges of oral peptide delivery.

## INTRODUCTION

The modification of proteins with PEG is now a well established technique, with numerous reports in the literature of this modification having been applied to a range of molecules, from proteins both large and small, through to liposomes and viruses (1,2). Protein therapeutics often have very poor pharmacokinetic profiles and are metabolized and cleared rapidly, thus preventing their oral delivery. Moreover, clearance depends on molecular size, charge, and the presence of specific cellular receptors for the proteins of interest. Nobex corporation's technology of Alkyl-PEGylation involves attachment of amphiphilic oligomers comprising of small monodispersed polyethylene glycol and fatty acid moieties to a protein, which is in contrast to general PEGylation involving attachment of large polydispersed, high molecular weight PEGs. Alkyl-PEGylation affects a protein's molecular size, charge, and receptor-binding capabilities, which can serve to increase its oral absorption and decrease the conjugate's overall rate of clearance. Second, the metabolism of proteins by enzymes leads to the rapid loss of biological activity of therapeutic proteins. By sterically shielding the protein domains susceptible to proteolytic attack, amphiphilic oligomers decreases the protein degradation that renders it biologically inactive.

Two therapeutic proteins were identified for the study: insulin and B-type natriuretic peptide. Protein conjugates thus obtained with these peptides had the properties of increased stability towards proteases, higher aqueous solubility and retention of bioactivity. The conjugation of amphiphilic oligomers with the protein therapeutics is accomplished mainly by reaction with free amino groups.

## BACKGROUND AND DESIGN

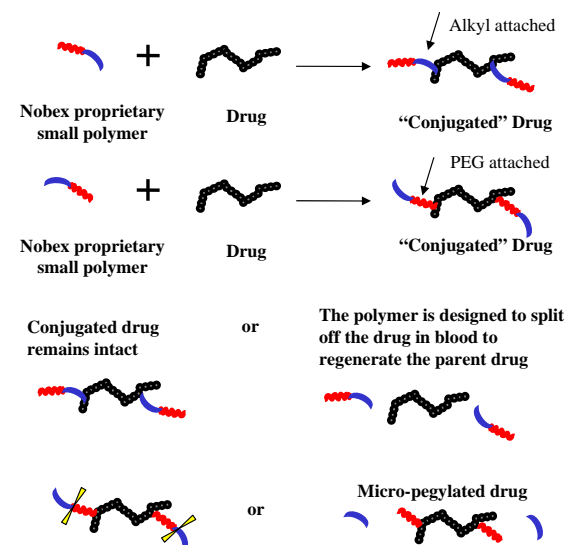


Figure 1. NOBEX Technology Platform

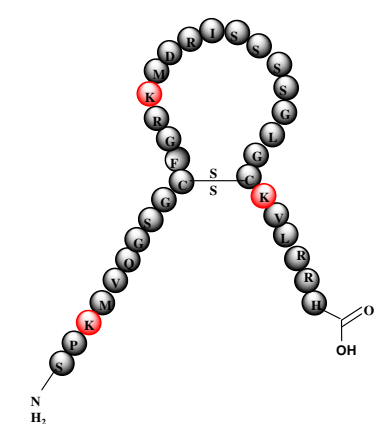


Figure 2. Primary structure of mature hBNP. It is a 32-amino acid peptide with a loop region comprised of 17 residues, which is the characteristic loop size for the various natriuretic peptides. hBNP has four sites suitable for conjugation with our oligomers: the free N-terminus and three lysine residues (shown in red).

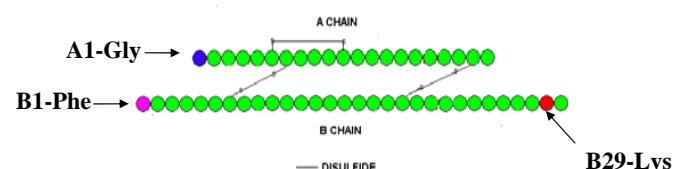


Figure 3. Insulin is a small helical protein hormone consisting of two polypeptides, chain A (21 residues) and chain B (30 residues), linked together by two interchain disulfide bridges.

## RESULTS

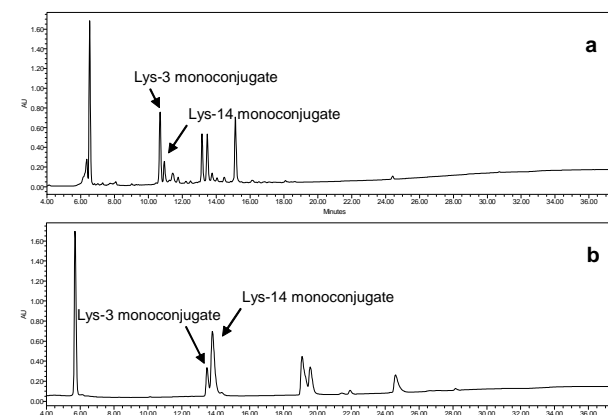


Figure 4. Chromatograms illustrating differing selectivity based on the amphiphilic balance of the oligomer conjugating to hBNP. Detection was set at 220 nm. a. Hydrophilic oligomers react first at Lys3. b. Hydrophobic oligomers react preferentially at Lys14.

Table 1. Observed half-lives of insulin compounds in the presence of chymotrypsin.

| Conjugate <sup>s</sup> | t <sub>1/2</sub> (min)<br>Chymotrypsin | n = |
|------------------------|--|-----|
| Insulin                | 3.3 – 5.3                              | 17  |
| IN-058                 | 7.4 – 9.1                              | 4   |
| IN-075                 | 3.7 – 4.6                              | 4   |
| IN-076                 | 8.2 – 9.8                              | 4   |
| IN-084                 | 14.0 – 16.0                            | 5   |
| IN-093                 | 11.1 – 12.6                            | 4   |
| IN-094                 | ND                                     | 0   |
| IN-095                 | 8.9 – 12.5                             | 4   |
| IN-096                 | 8.5 – 10.5                             | 4   |
| IN-098                 | 9.0 – 9.7                              | 4   |
| IN-101                 | 7.6 – 10.1                             | 4   |
| IN-103                 | 9.4 – 11.1                             | 4   |
| IN-104                 | 7.7 – 9.0                              | 4   |
| IN-106                 | 14.0 – 16.9                            | 4   |
| IN-107                 | ND                                     | 0   |

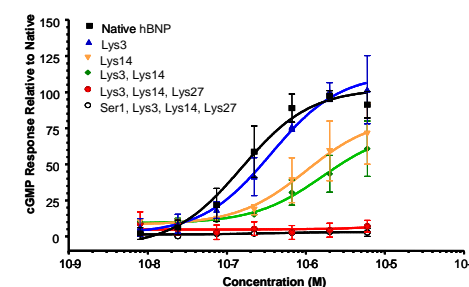


Figure 5. Activity curves depicting cGMP production from HAEC after exposure to hBNP conjugates utilizing amphiphilic oligomer. The conjugates differ in site and/or extent of conjugation.

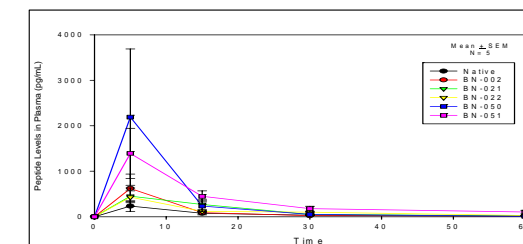
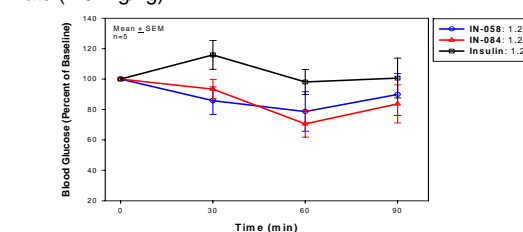
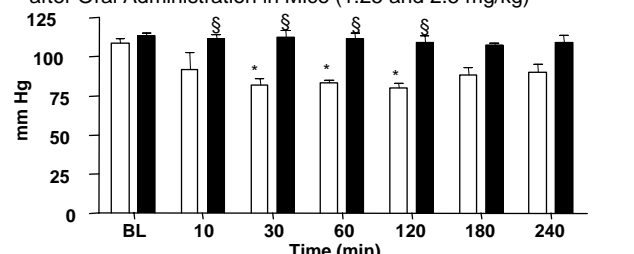


Figure 6. Oral Bioavailability hBNP Conjugates after oral dosing in Rats (2.5 mg/kg).



\* All data is relative to IN-058 which is run as a standard in all oral administrations

Figure 7. Effect of Insulin, IN-058, and IN-084 on Blood Glucose after Oral Administration in Mice (1.25 and 2.5 mg/kg)



\*p<0.05 vs BL; §p<0.05 vs BN-050; p<0.0001

Figure 8. Mean Arterial Pressure in Healthy Dogs after Oral Dosing (350 µg/kg).

## CONCLUSIONS

Alkyl-PEGylation increases the stability towards proteases and solubility several fold over native peptide.

In a few cases, Alky-PEGylation was detrimental to biological activity, but site specific conjugation helps retain the biological activity.

The attachment of amphiphilic oligomers comprising of small monodispersed polyethylene glycol (PEG) and fatty acid moieties to these peptides therapeutics has enabled their oral delivery.

## REFERENCES

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- (2) G.E. Francis, D. Fisher, C. Delgado, F. Malik, A. Gardiner, D. Neale, PEGylation of cytokines and other therapeutic proteins and peptides: the importance of biological optimisation of coupling techniques, *Int. J. Haematol.* 68 (1998) 1–18.