Title:	MODIFIED INSULIN POLYPEPTIDES AND THEIR USES
Applicant:	AMBRX, INC.
Appl. No.:	Not Assigned
Atty:	John W. Wallen, III Telephone: (858) 875-2403
Docket No.:	AMBX-0146.00PCT

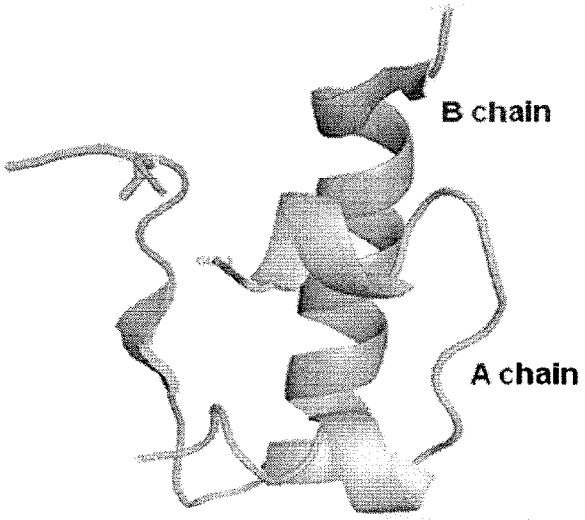
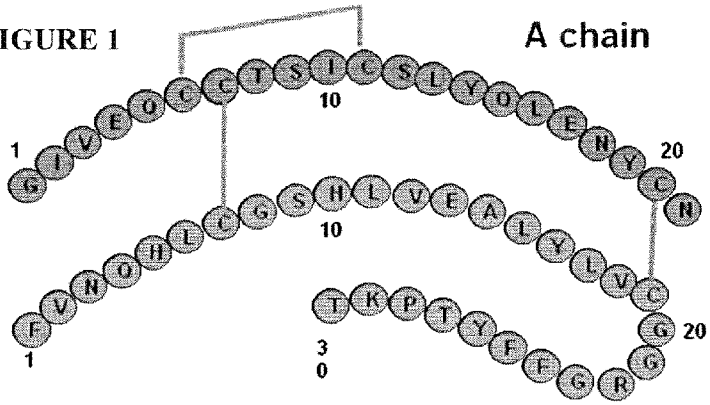


FIGURE 1



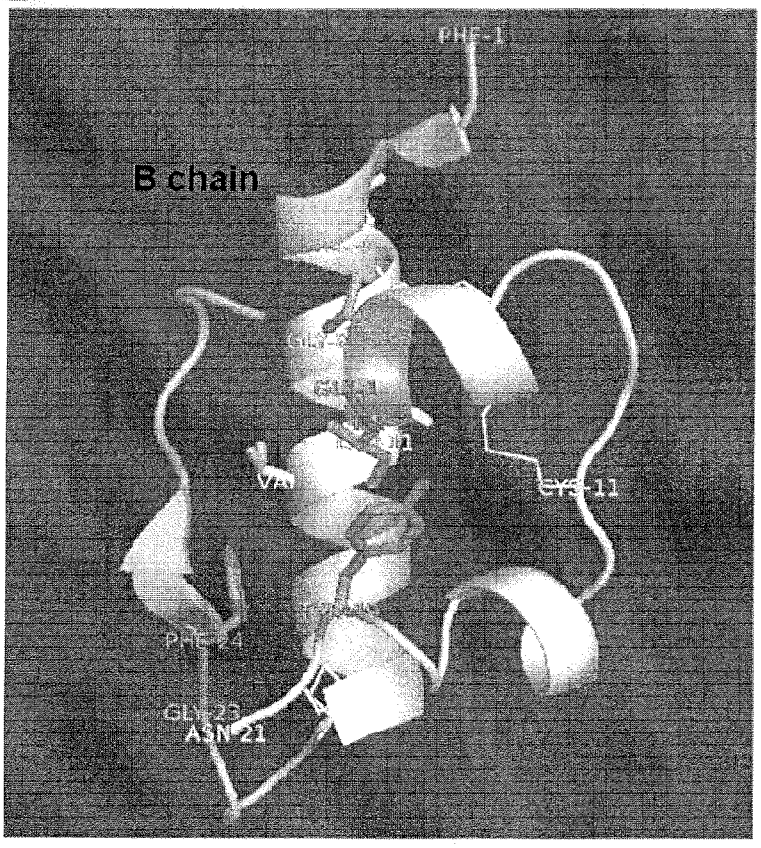
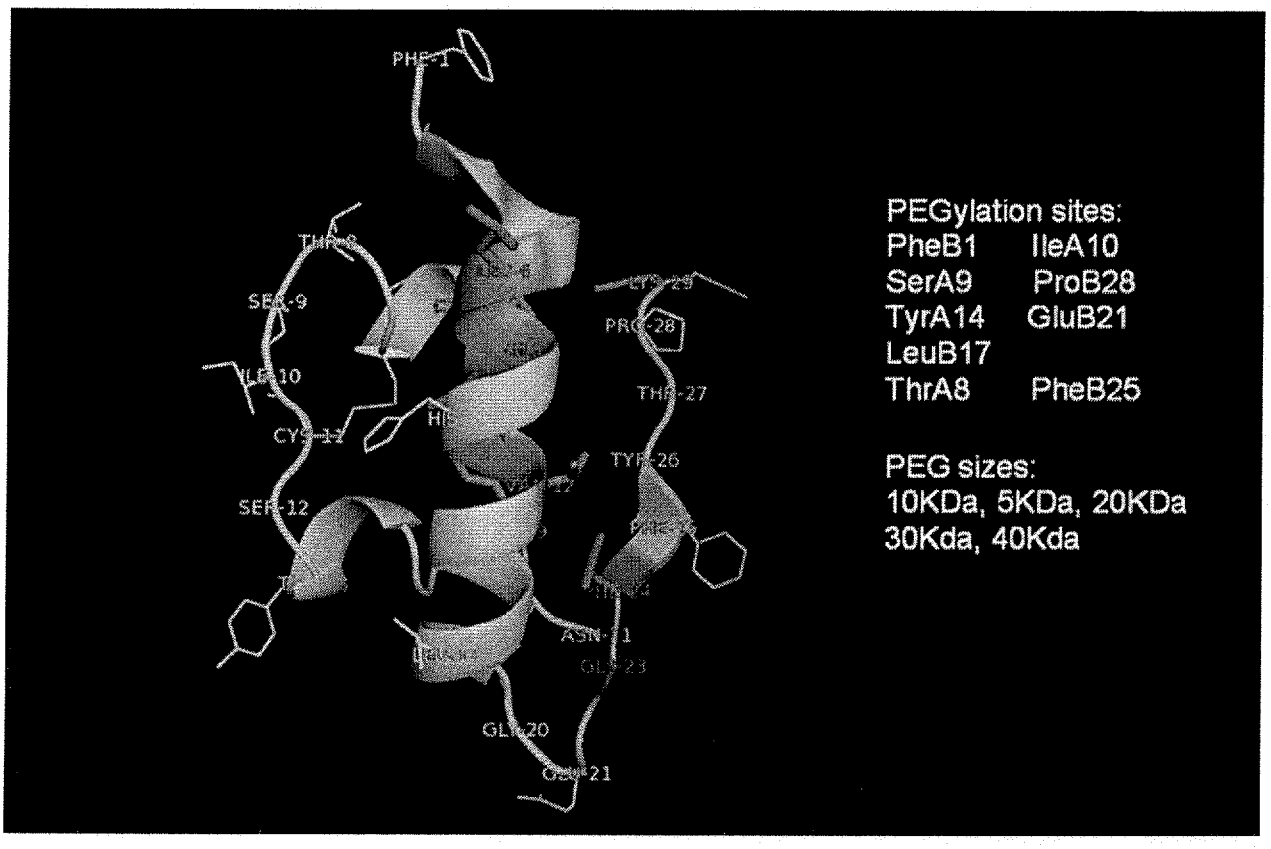


FIGURE 2


PEGylation sites:
PheB1 IleA10
SerA9 ProB28
TyrA14 GluB21
LeuB17
ThrA8 PheB25
PEG sizes:
10KDa, 5KDa, 20KDa
30Kda, 40Kda

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FIGURE 3

PRIMARY PROINSULIN STRUCTURE

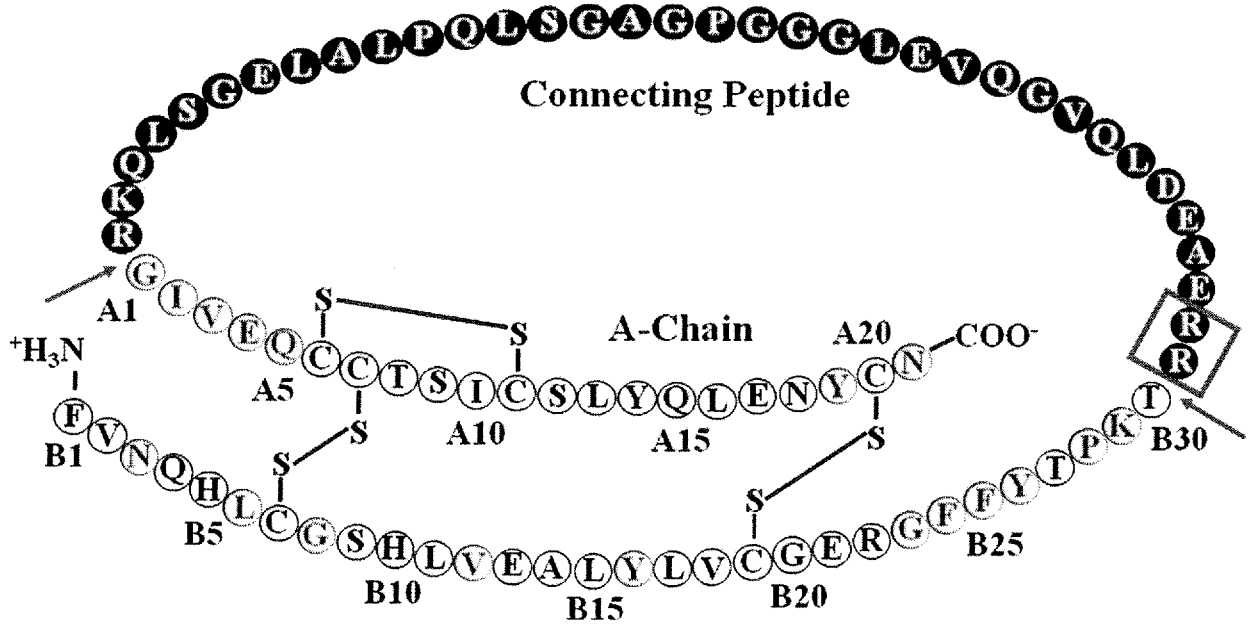


FIGURE 4

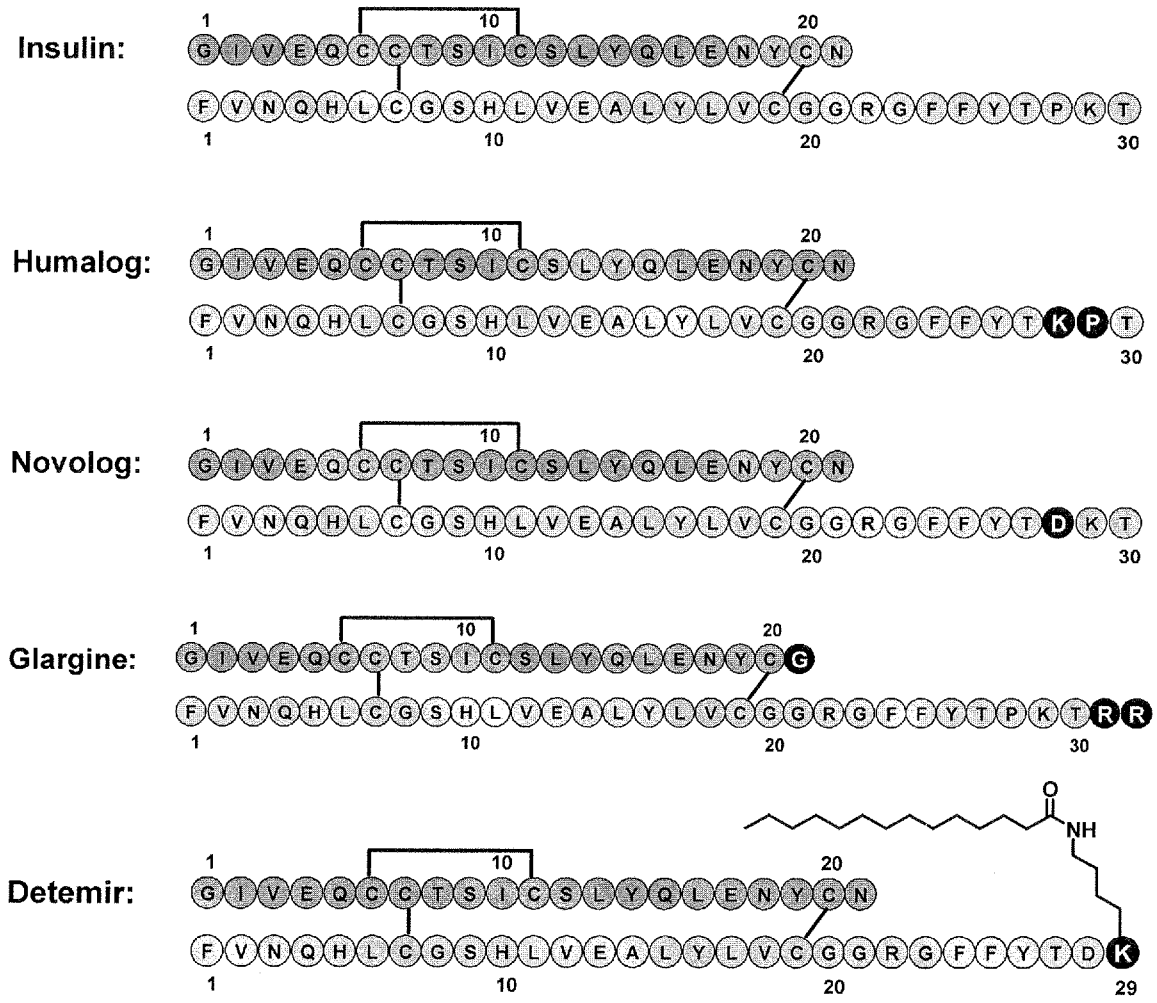
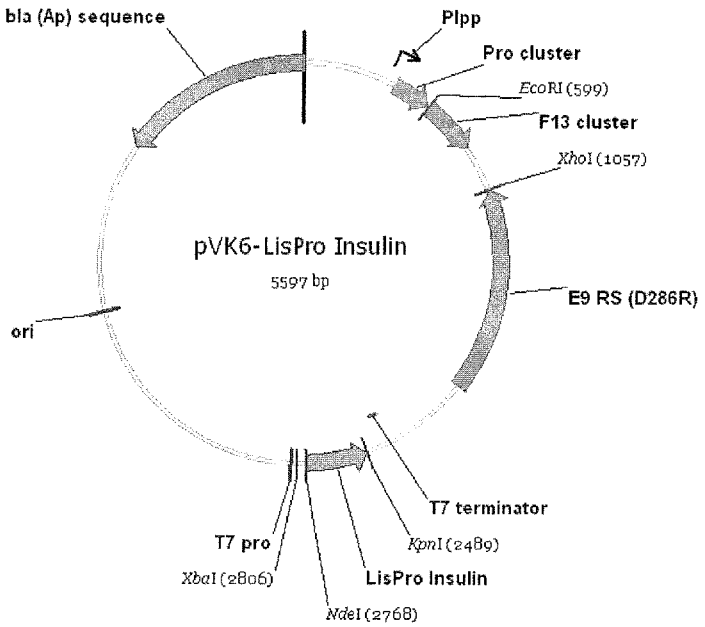


FIGURE 5

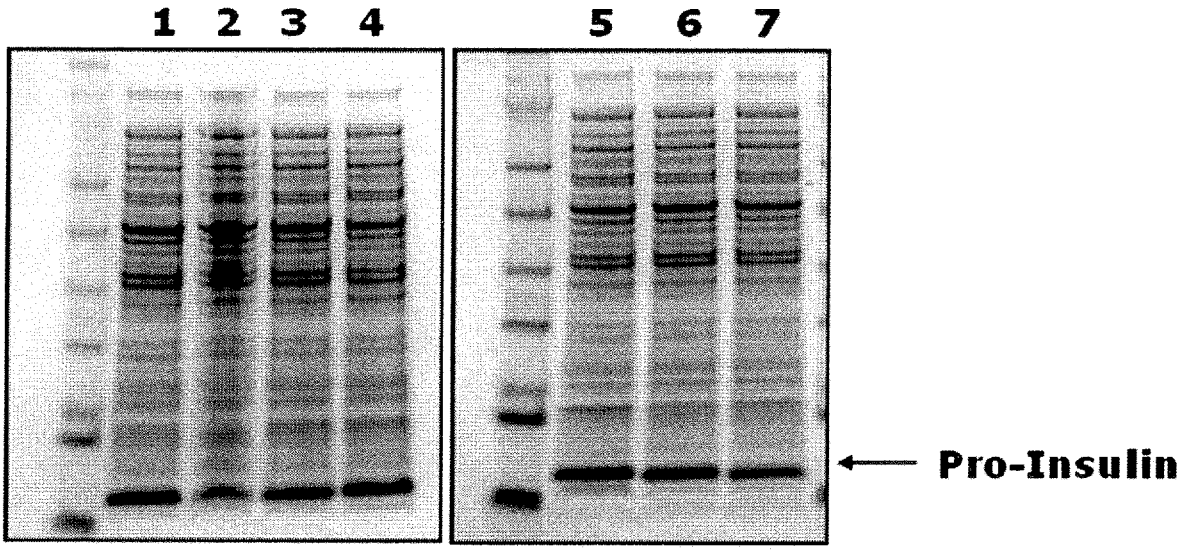
Pro-insulin expression and TAG-suppression in *E.coli*




```
atgattgaaggtggtcgtttcgttaaccagcacctgtgcggctcccacctggtggaagct
 M  I  E  G  G  R  F  V  N  Q  H  L  C  G  S  H  L  V  E  A
ctgtacctggtgtgcggtgaacgtggcttcttctacaccaagccgacccgccgtgaggca
 L  Y  L  V  C  G  E  R  G  F  F  Y  T  K  P  T  R  R  E  A
gaggacctgcaggtgggtcaggtggagctgggcggtggcccgggtgcaggcagcctgcag
 E  D  L  Q  V  G  Q  V  E  L  G  G  G  P  G  A  G  S  L  Q
ccgctggccctggagggttccctgcagaagcgtggcattgtggaacaatgctgtaccagc
 P  L  A  L  E  G  S  L  Q  K  R  G  I  V  E  Q  C  C  T  S
atctgctccctgtaccagctggaaaactactgcggc
 I  C  S  L  Y  Q  L  E  N  Y  C  G
```

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FIGURE 6



1. Pro-INS-A(Q15pAF)
2. Pro-INS-A(Y14pAF)
3. Pro-INS-B(R22pAF)
4. Pro-INS-B(F1pAF)
5. Pro-INS-A(G1pAF)
6. Pro-INS-A(S9pAF)
7. Pro-INS-B(K28pAF)

Total lysate samples of LB shake flask cultures, 5 hrs post induction (W3110-B2 cells, transformed with pVK6-LisPro derivatives and induced with 0.2% L-arabinose at 37 C)

FIGURE 7

SEC-HPLC of Pegylated and Unpegylated INS

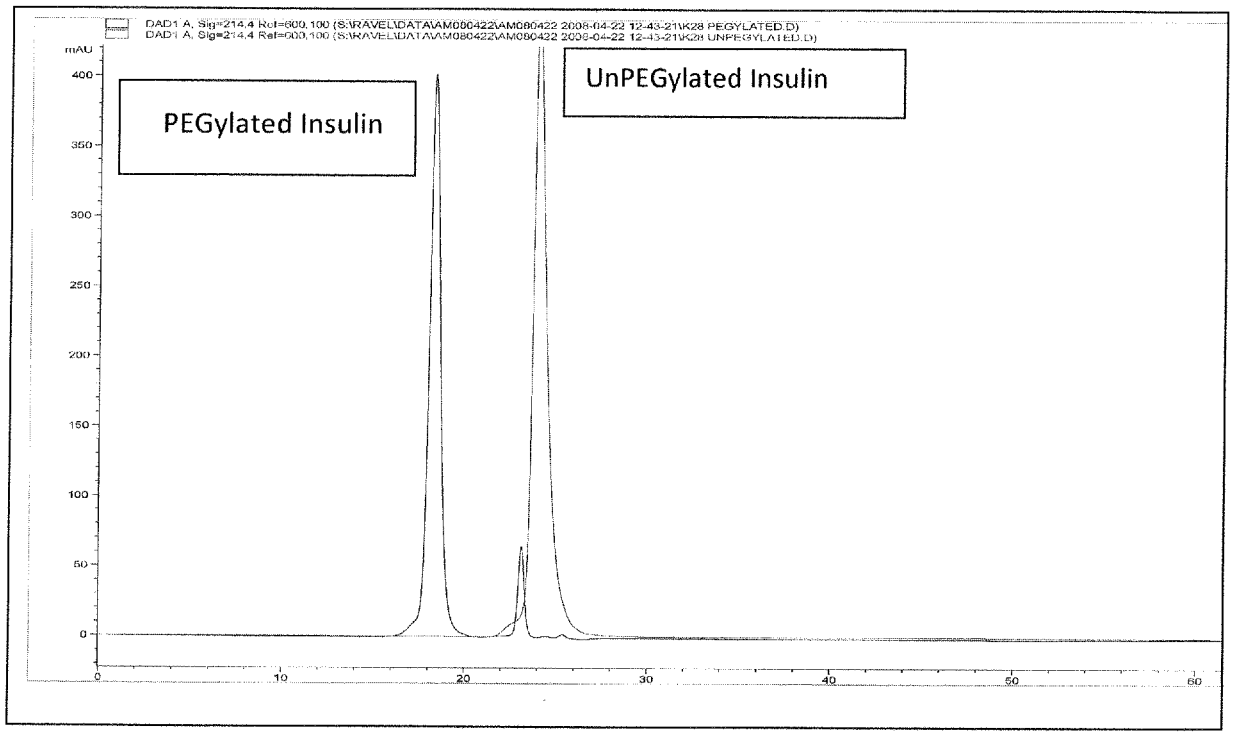


FIGURE 8

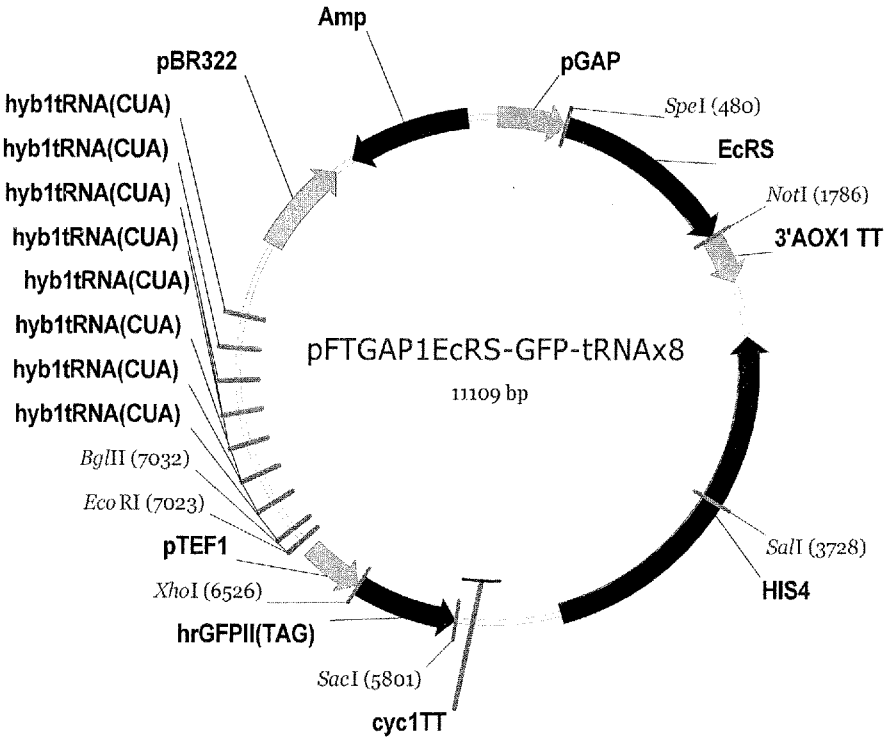

Amp
pBR322
pGAP
SpeI (480)
EcRS
NotI (1786)
3'AOX1 TT
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
hyb1tRNA(CUA)
pFTGAP1EcRS-GFP-tRNAx8
11109 bp
BglII (7032)
EcoRI (7023)
pTEF1
XhoI (6526)
hrGFPII(TAG)
SacI (5801)
cyc1TT
SalI (3728)
HIS4

FIGURE 9

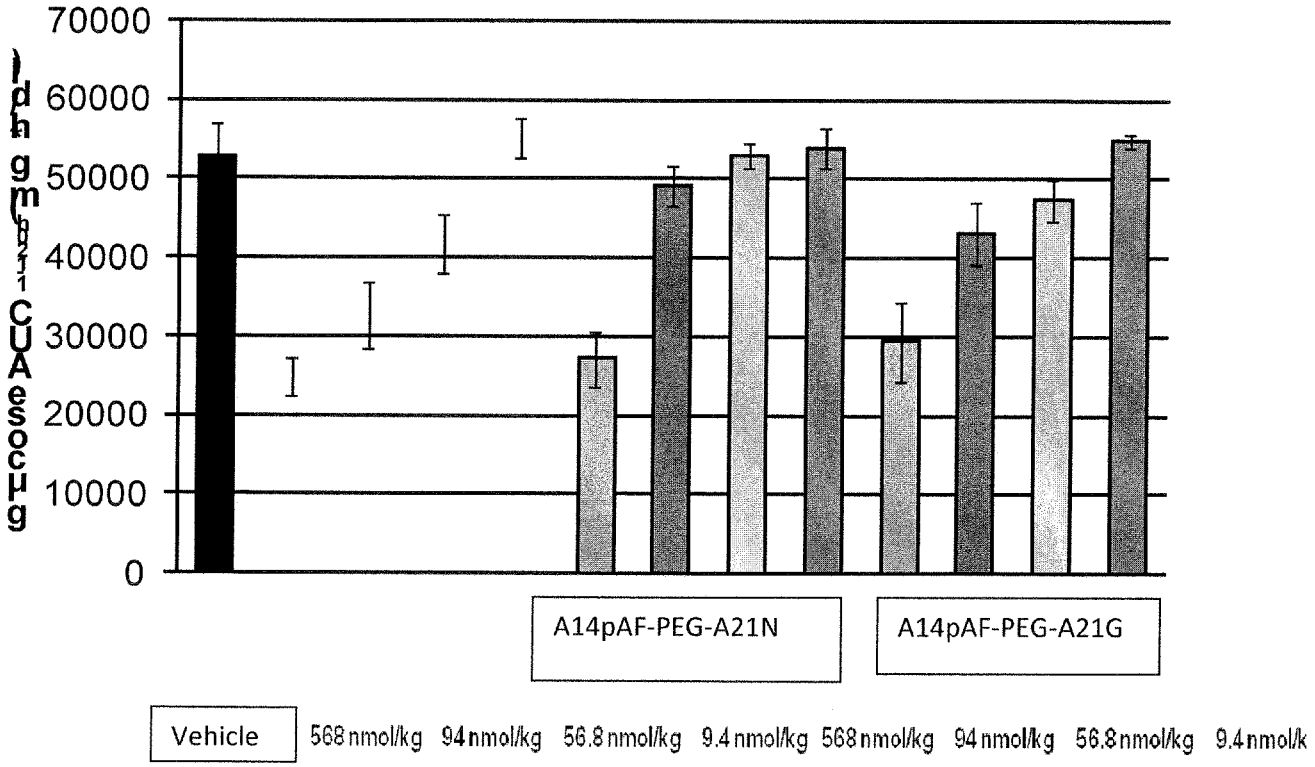

70000
60000
50000
40000
30000
20000
10000
0
A14pAF-PEG-A21N
A14pAF-PEG-A21G
Vehicle
568 nmol/kg
94 nmol/kg
56.8 nmol/kg
9.4 nmol/kg
568 nmol/kg
94 nmol/kg
56.8 nmol/kg
9.4 nmol/k

FIGURE 10

Insulin A21G Refold Reaction

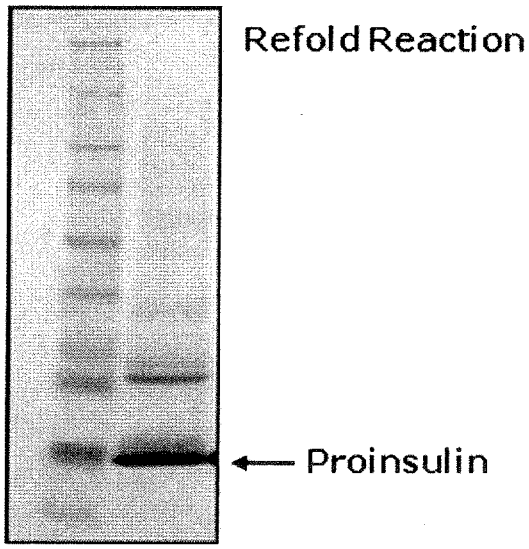
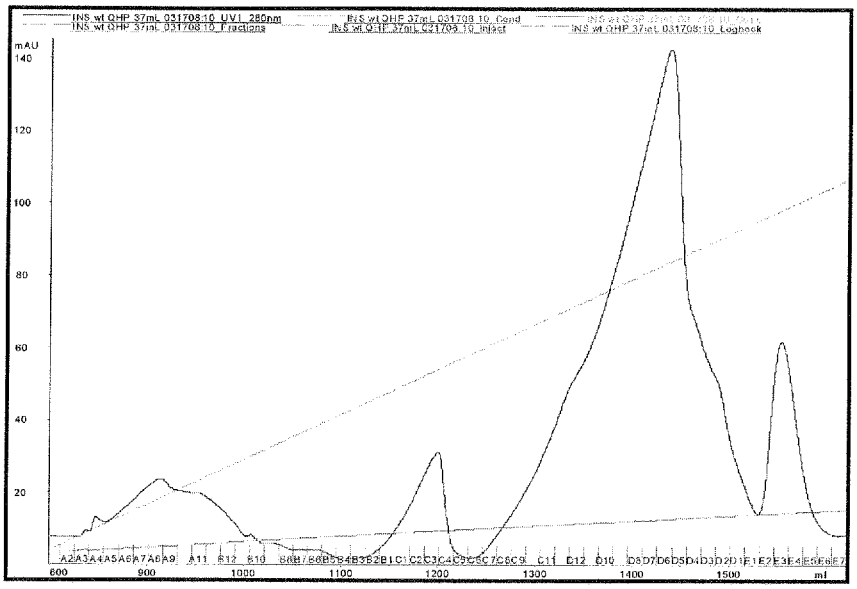


FIGURE 11

QHP Purification of Refolded Proinsulin



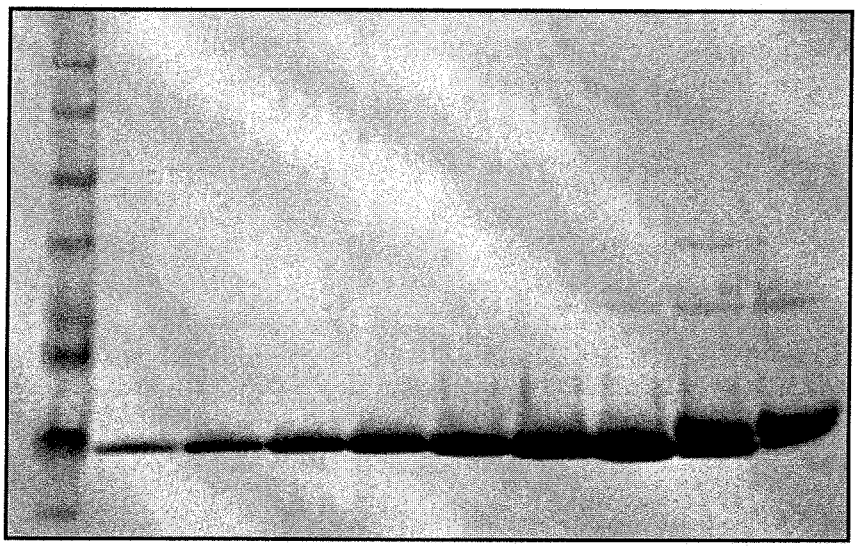


FIGURE 12

PEGylation Reaction and Purification

