Supporting Information For:

Amyloidogenicity, Cytotoxicity and Receptor Activity of Bovine Amylin; Implications for Xenobiotic Transplantation and the Design of Non-toxic Amylin Variants

Rehana Akter, Rebekah L. Bower, Andisheh Abedini, Ann Marie Schmidt, Debbie L. Hay

and Daniel P. Raleigh

Figure S1: Comparison of the sequence of amylins from different species. Residues that differ from human amylin are colored in blue. Human, other primates and cats are reported to form islet amyloid *in vivo* and develop diabetes, but rodents do not. Porcine and ferret amylin are less amyloidogenic *in vitro* and less cytotoxic than human amylin. Only partial sequences have been reported for tamarin, rabbit, hedgehog, sheep, hare, kangaroo, salmon and zebrafish amylin.

```
                                 1         10         20         30     37
Human:                           KCNTATCAT QRLANFLVHS SNNFGAILSS TNVGSNTY
Bovine:                          KCGTATCET QRLANFLAPS SNKLGAIFSP TKMGSNTY
Wild Yak:                        KCGTATCET QRLANFLAPS SNKLGAISSP TKMGSNTY
Rat:                             KCNTATCAT QRLANFLVRS SNNLGPVLPP TNVGSNTY
Naked Mole Rat:                  KCNTATCTI QRLTNFLVRS SHNLGAVLLP TDVGSNTY
Mouse:                           KCNTATCAT QRLANFLVRS SNNLGPVLPP TNVGSNTY
Garnett's Greater Bushbaby:      KCNTATCAT QRLANFLVRS SNNFGAVHSP TNVGSNTY
Gray Short-tailed Opossum:       KCNTATCVT QRLADFLIRS SNNIGAVFSP TNVGSNTY
Golden Hamster:                  KCNTATCAT QRLANFLVHS NNNLGPVLSP TNVGSNTY
Chinese Hamster:                 KCNTATCAT QRLANFLVHS NNNLGPVLSP TNVGSNTY
Degu:                            KCNTATCAT QRLTNFLVRS SHNLGAALPP TKVGSNTY
Ferret:                          KCNTATCVT QRLANFLVRS SNNLGAILLP TDVGSNTY
Thirteen-lined Ground Squirrel:  KCNTATCAT QRLANFLVRS SHNLGAVLST TNVGSNTY
Chinese Tree Shrew:              KCNTATCAT QRLANFLVRS SNNLGAVLPP TNVGSNTY
Horse:                           KCDTATCVT QRLANFLVHS SNNLGAILSP TSVGSNTY
Porcine:                         KCNMATCAT QHLANFLDRS RNNLGTIFSP TKVGSNTY
Guinea Pig:                      KCNTATCAT QRLTNFLVRS SHNLGAALLP TDVGSNTY
Green Monkey:                    KCNTATCAT QRLANFLVRS SNNFGTILSS TDVGSNTY
Rhesus Macaque:                  KCNTATCAT QRLANFLVRS SNNFGTILSS TNVGSNTY
Crab-eating Macaque:             KCNTATCAT QRLANFLVRS SNNFGTILSS TNVGSNTY
Hamadryas Baboon:                ICNTATCAT QRLANFLVRS SNNFGTILSS TNVGSNTY
Olive Baboon:                    ICNTATCAT QRLANFLVRS SNNFGTILSS TNVGSNTY
Chimpanzee:                      KCNTATCAT QRLANFLVRS SNNFGAILSS TNVGSNTY
Gorilla:                         KCNTVTCAT QRLANFLVRS SNNFGAILSS TNVGSNTY
Northern White-cheeked gibbon:   KCNTATCAT QRLANFLVRS SNNFGAILSS TNVGSNTY
Cat:                             KCNTATCAT QRLANFLIRS SNNLGAILSP TNVGSNTY
Dog:                             KCNTATCAT QRLANFLVRT SNNLGAILSP TNVGSNTY
Bears:                           KCNTATCAT QRLANFLVRS GNNLGAILSP TNVGSNTY
Spectacled Bear:                 KCNTATCAT QRLANFLVRS SNNLGAILSP TNVGSNTY
Giant Panda:                     KCNTATCAT QRLANFLVRS SNNLGAILSP TNVGSNTY
Puffer Fish:                     KCNTATCVT QRLADFLVRS SNTIGTVYAP TNVGSTTY
Spotted Green Pufferfish:        KCNTATCVT QRLADFLVRS SNTIGTVYAP TNVGSATY
Gold Fish:                       KCNTATCVT QRLADFLVRS SNTRGTVYAP TNVGANTY
Nile Tilapia:                    KCNTATCVT QRLADFLVRS SNTIGTVYAP TNVGSATY
Beira Killifish:                 KCNTATCVT QRLADFLVRS SNTIGTVYVP TNVGSSTY
Chicken:                         KCNTATCVT QRLADFLVRS SSNIGAIYSP TNVGSNTY
Common Turkey:                   KCNTATCVT QRLADFLVRS SSNIGAIYSP TNVGSNTY
Common Cuckoo:                   KCNTATCVT QRLADFLVRS SNSIGAIYPP TNVGSNTY
Common Ostrich:                  KCNTATCVT QRLADFLVRS SNNIGAIYSP TNVGSNTY
Emperor Penguin:                 KCNTATCVT QRLADFLVRS SNNIGAIYSP TNVGSNTY
Zebra Finch:                     KCNTATCVT QRLADFLVRS SSSLGALYPP TNVGSNTY
Bengalese Finch:                 KCNTATCVT QRLADFLVRS SSSLGALYPP TNVGSNTY
Rifleman Bird:                   KCNTATCVT QRLADFLVRS SSHIGAIYSP TNVGSNTY
Mallard:                         KCNTATCVT QRLADFLVRS SSNIGAIYSP TNVGSNTY
Crested Crane:                   KCNTATCVT QRLADFLVRS SNNIGAIYSP TNVGSNTY
Chinese Softshell Turtle:        KCNTATCVT QRLADFLVRS SNNIGAIYSP TNVGSNTY
Green Anole:                     RCNTATCVT QRLADFLVRS SNTIGAIYSP TNVGSNTY
American alligator:              KCNTATCVT QRLADFLVRS SNHIGAIYSP TNVGSNTY
King Cobra:                      KCNTATCVT QRLADFLVRS SNTIGTIYAP TNVGSNTY
Rabbit:                          KCNTVTCAT QRLANFLIHS SNNFGAIFSP PSVGS
Western European Hedgehog:       RCNTATCAT QRLVNFLSRS SNNLGAILSP TDVG
Sheep:                             GTATCET QRLANFLAPS SNKLGAIFSP TKMGS
Cotton-top Tamarin:                NTATCSM HRLADFLGRS SNNFGAILSP TNVGS
Hare:                                    T QRLANFLIHS SNNFGAFLPP T
Red Kangaroo:                            T QRLADFLVRS NNNMGAIFSP TNVG
Salmon:                             TCAT QRLADFLTRS SNTIGTVYAP TNVGS
Zebrafish:                                  TRS SSPIGTVNAP TNVGS
```

Table S1: Comparison of the predicted amyloidogenicity of human amylin and bovine amylin as deduced by different amyloid prediction programs. A red downward arrow, ↓, indicates that the sequence is predicted to be less amyloidogenic.

Methods	**Properties comparing**	**Human amylin**	**Bovine amylin**
3D Profile method (ZipperDB)	**Steric zipper segments** (six residues segments predicted to form steric zipper)	9	2 ↓
AGGRESCAN	**Total hot spot area**	5.77	4.98 ↓
AmylPred	**Amyloidogenic segments and number of residues**	3 segments, 12residues	1 segment, 2 residues ↓
FoldAmyloid	**Amyloidogenic segment**	Residue 13 to 18(length 6)	Residue 13 to 17(length 5) ↓
PASTA	**Top ranked energetics segments**	-4.9	-1.9 ↓
TANGO	**Aggregation propensity**	43.78	7.56 ↓
Waltz	**Amyloidogenic segment**	Residue 22 to 29	Non amyloidogenic ↓
Zyggregator	**Intrinsic aggregation propensity**	-4.40	-5.43 ↓

Figure S2: Bovine amylin is non amyloidogenic in phosphate buffered saline. (a) Thioflavin-T fluorescence experiments comparing the kinetics of amyloid formation by human amylin (black) and bovine amylin (blue). (b) TEM images recorded at the end of the experiments for samples of human and bovine amylin. Scale bar represent 100 nm. Experiments were conducted using 16 μM peptide, 32 μM thioflavin-T at pH 7.4, 25 °C, in 10 mM phosphate with 140 mM KCl.

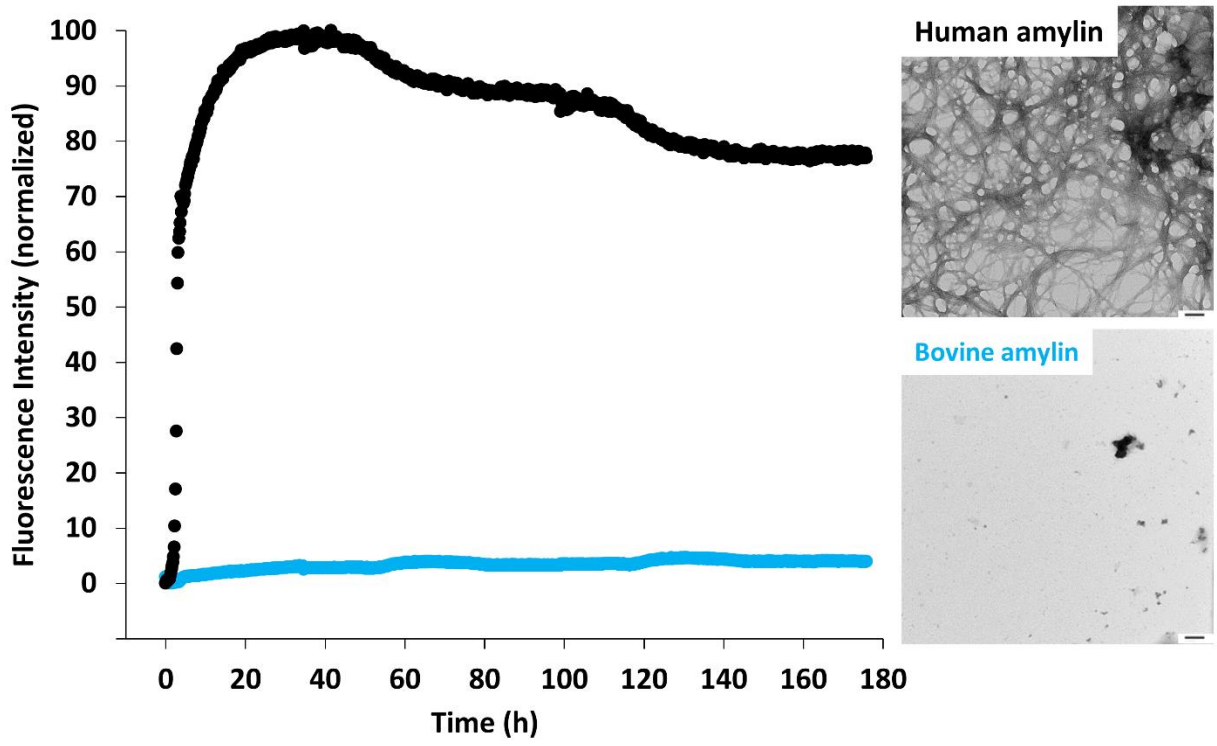

Figure S3: Bovine amylin is not toxic to β-cells. The effects of incubating different concentrations of human amylin (black) and bovine amylin (blue) on rat INS-1 β-cells for 48 hours and 84 hours. Final peptide concentrations on cells were 15 μM, 22 μM and 30 μM. Data is normalized relative to cells treated with media (gray).

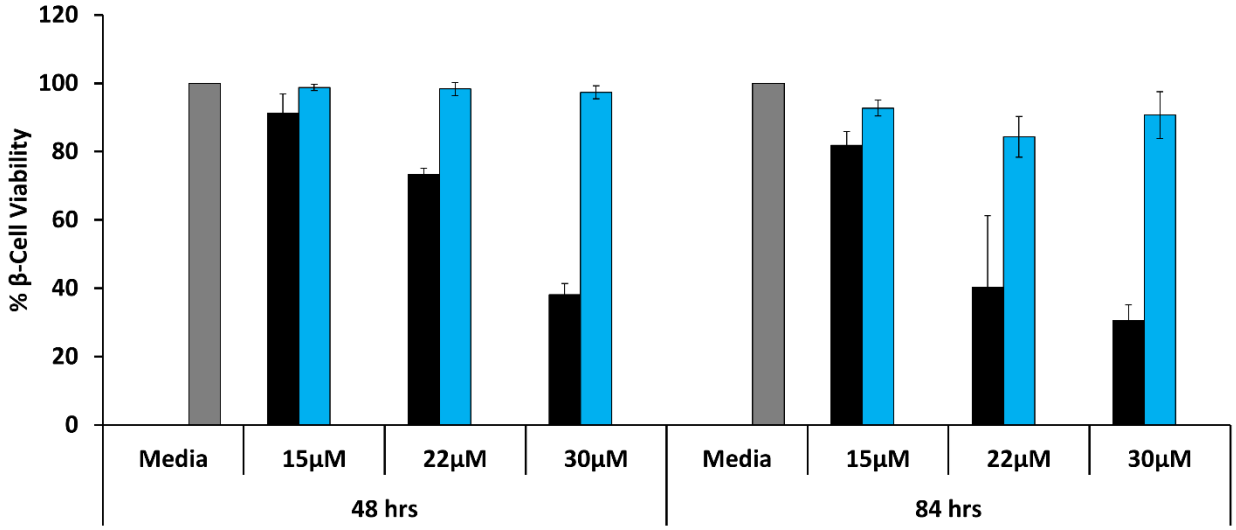

Figure S4:

Bar graph comparing the value of T_{50} for wild type h-amylin, H18P h-amylin, N22K h-amylin, S29P h-amylin and N31K h-amylin. Experiments were conducted using 16 μM peptide, 32 μM thioflavin-T in 20 mM Tris at pH 7.4 and 25°C. Data represent the mean and the uncertainties are the standard deviation.