Sparse matrix sampling of anti-fibrillation sequence mutations yields a library of novel solution stable glucagon analogs suitable for an artificial pancreas

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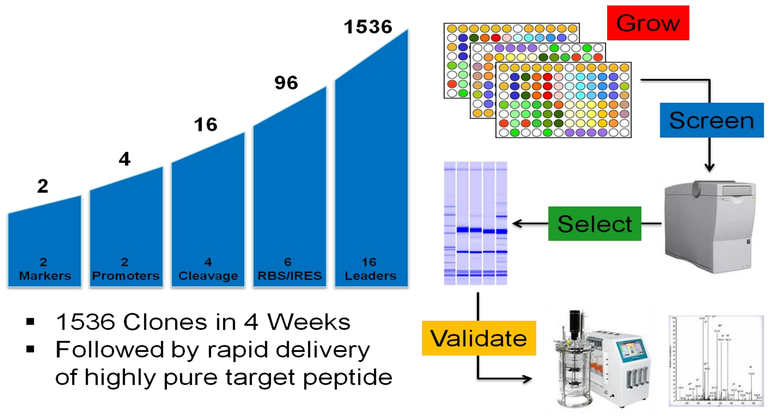
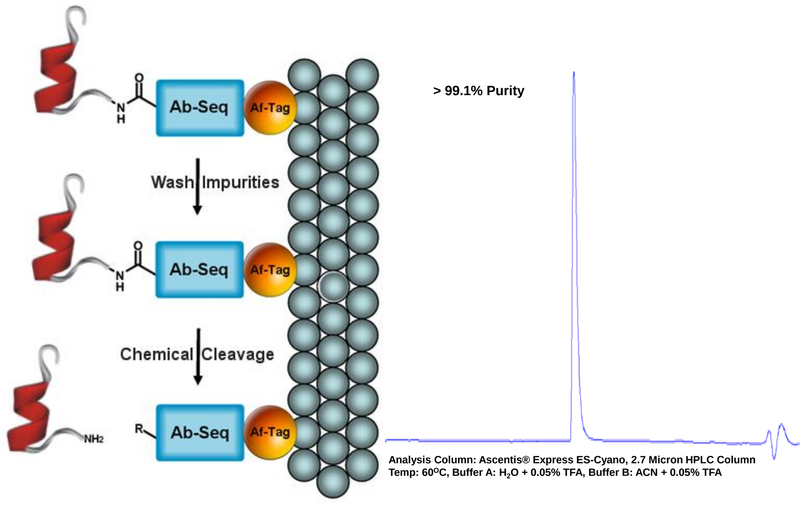
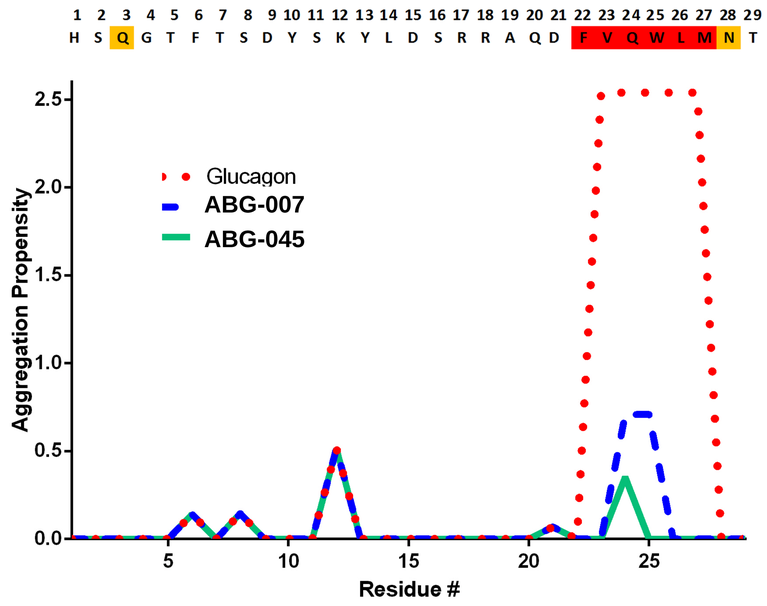
INTRODUCTION. Glucagon is commonly used to treat severe episodes of hypoglycemia diabetics and is a required hormone for a fully functional artificial pancreas. However, native glucagon has physicochemical properties that prevent solution formulation primarily due to fibrillation. Attempts to mitigate the fibrillation properties of glucagon have been varied and fall into two general classes; formulation strategies and sequence modifications to enhance solubility at physiological pH. These strategies have been met with modest success and to date there are no solution stable glucagon analogues or formulations approved by the FDA. Here we describe the implementation of a sparse matrix sampling of anti-fibrillation mutations to discover novel solution stable glucagon analogues. All of the analogues demonstrate superior solution stability at physiological pH. Surprisingly, some of the most solution stable analogues have only a modest isoelectric point shift in contraindication to the expected solubility requirement at physiological pH. Several of the analogues were tested for glucagon receptor activation and found to have in vitro activities ranging from 10% to 60% of native glucagon.

BIOPURE™ PROCESS. AmideBio has developed a low cost peptide production platform combining recombinant and chemical methods for rapid SAR of complex or difficult to manufacture peptides[2]. The process implements a library of expression vectors optimized for bacterial or yeast expression combined with an on column chemical cleavage process which provides a highly orthogonal platform enabling the rapid high purity production of a variety of peptides and proteins for drug discovery. Here we describe the BioPure™ method and its application to the discovery or novel solution stable glucagon analogues.

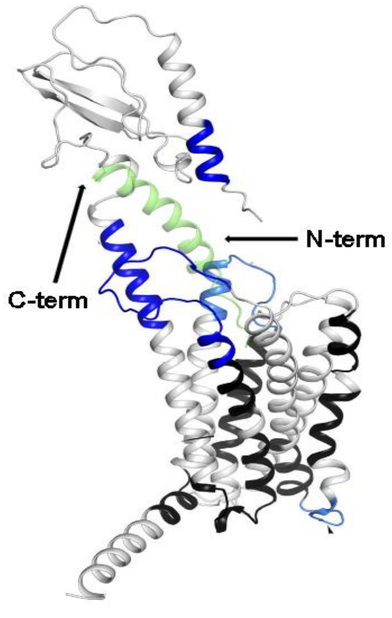


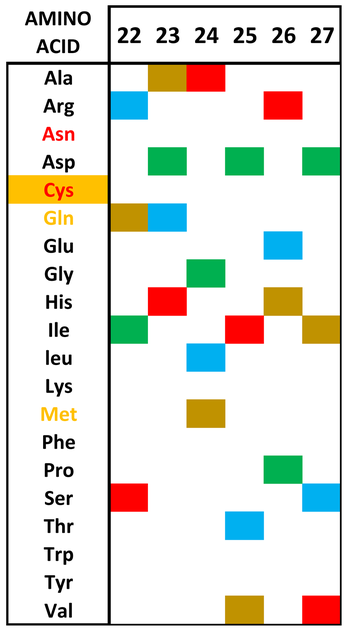


GLUCAGON DESIGN. AmideBio has implemented the BioPure™ process for the discovery of solution stable glucagon suitable for use in an artificial pancreas and as emergency treatment of hypoglycemia. Glucagon is inherently unstable in solution and currently is only available in a lyophilized powder that must be reconstituted prior to use in the case of hypoglycemia. Using a structure based sparse matrix design approach, we targeted a library of more than 100 potential candidates for testing[3].

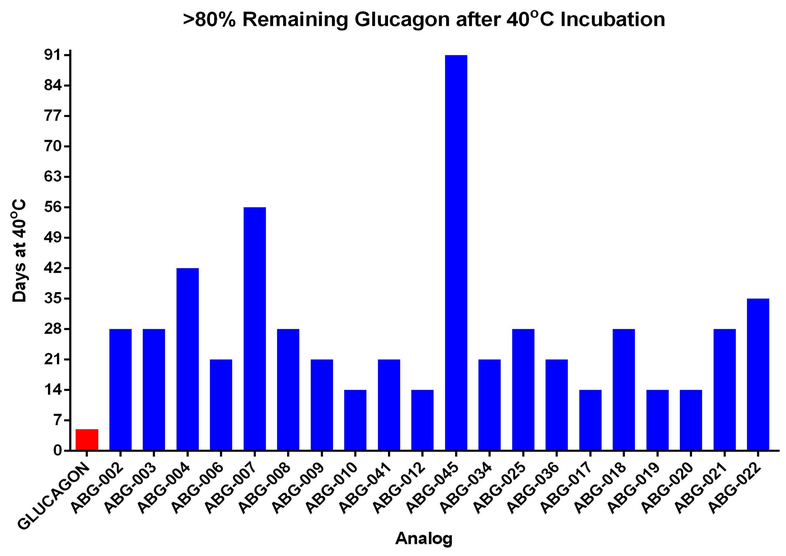


SPARSE MATRIX APROACH. With 18 amino acids (n) (excluding Cys and Asn) being substituted in random order at 6 (r) positions from residue 22 to 27 there would be 18^6 (n^r) or 34,012,224 possible glucagon sequences. Producing and testing each of these possible candidates is clearly impractical. In order to approach this problem we implemented an iterative computational method to minimize predicted fibrillation combined with sparse matrix sampling. Sparse matric sampling has been successfully implemented to sample complex multi-dimensional space such as protein crystallization. The initial matrix consisted of 100 potential target candidates of which 40 were chosen for production and testing.

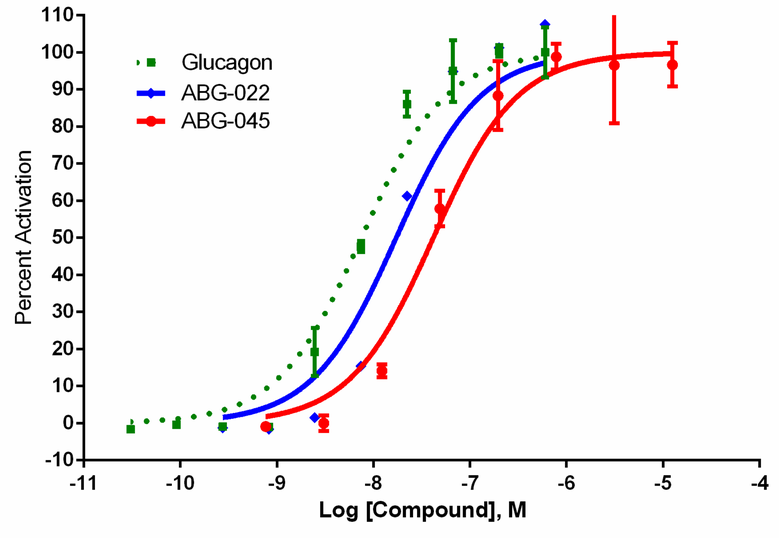




GLUCAGON PRODUCTION AND STABILITY. Using the BioPure™ process we manufactured 40 candidates from the initial 100 targets. We were able to produce the targets with >99% purity using the BioPure™ process. Because solution stability is the most critical aspect of this program our initial screen was for long term stability in PBS and 40°C using quantitative HPLC. Gratifyingly, all analogues demonstrated enhanced stability relative to native glucagon with the most stable analogue showing > 3 month solution stability.



GLUCAGON ACTIVITY. Several of the most stable glucagon analogues were tested for in-vitro activity using a Ca^{++} FLIPR assay using a FLIPRTETRA instrument. Samples were compared to native glucagon as control and E_{max} values determined ranged from 2 to 10 times that of native glucagon.



CONCLUSIONS. AmideBio has combined a sparse matrix sampling method and the BioPure™ process for the discovery of novel solution stable glucagon analogues with unexpected properties. Several of these analogues are potentially suitable for implementation in an artificial pancreas.

NOTES

1. **Presenter, E: stowellm@amidebio.com**
2. **The BioPure™ Process is covered under US Patent 8,796,431.**
3. **The design and discover principles as well as composition of matter for the novel glucagons are patent pending.**

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