De novo macrocyclic peptide modulators of protein-carbohydrate interactions

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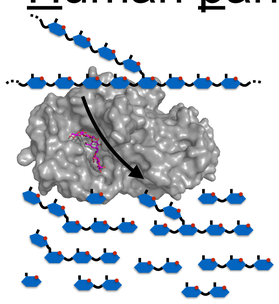
Overview

- Proteins play an important role in carbohydrate biology, particularly in their recognition and metabolism.
- Protein-carbohydrate interactions are often weak, taking place over a large interaction surface and/or with multivalency.
- Small molecules can have trouble binding tightly to such surfaces, while peptides are more well suited.
- The RaPID system is able to discover new peptide ligands, but has not yet been applied to carbohydrate binding sites.

➡ Proof-of-principle using human pancreatic α-amylase.

➡ Mimic MbA interactions in a new scaffold, make it more general.

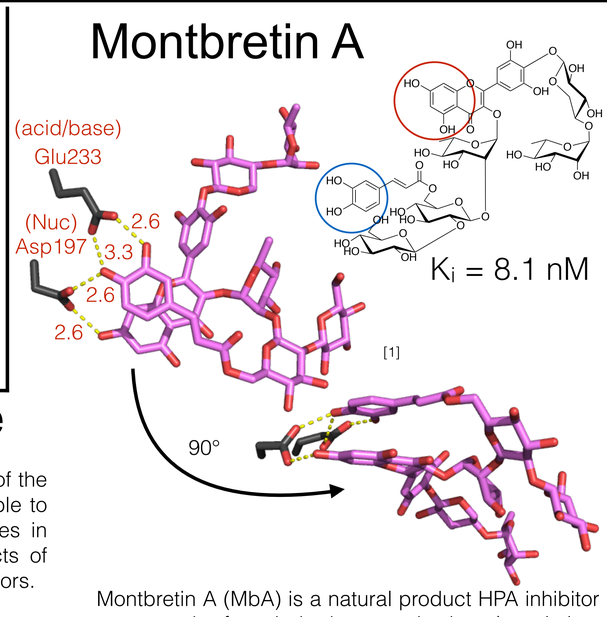
Human pancreatic α-amylase



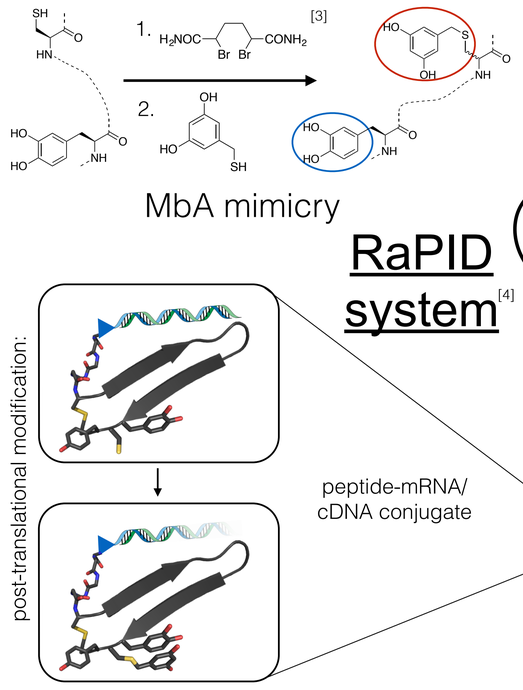
Selective inhibition of HPA, at the start of the starch digestion pathway, should be able to provide control of blood glucose spikes in type 2 diabetes without the side-effects of current-generation α-glucosidase inhibitors.

maltase-glucoamylase
sucrase-isomaltase
α-glucosidases

Montbretin A



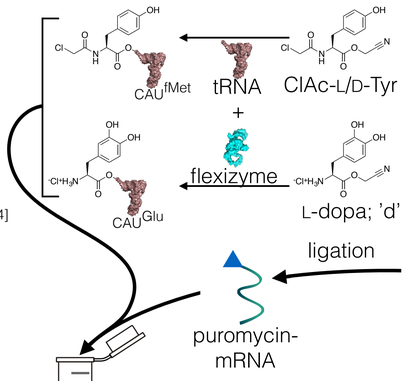
Montbretin A (MbA) is a natural product HPA inhibitor composed of carbohydrate and phenol moieties, identified from a high-throughput screen.[2] Crystallography revealed a new interaction motif with the catalytic residues, with caffeic acid and myricetin stacking to provide the majority of the inhibition.



RaPID system[4]

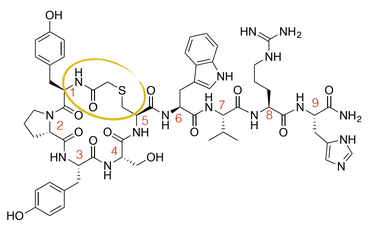
Genetic code reprogramming

(‘FIT system’)



‘Flexizymes’ are amino-acylating ribozymes. They recognise specific activated esters, with very little amino acid or tRNA specificity. This allows a lot of freedom in genetic code reprogramming.

Selection results

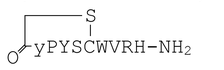


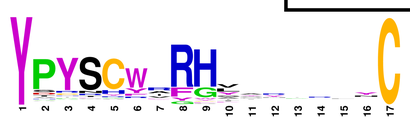
Selectivity

Enzyme	Inhibition (IC_{50}, nM)
Sus domesticus (porcine) pancreatic α-amylase	8.5 ± 0.9
Homo sapiens (human) salivary α-amylase	34 ± 6
Butyrivibrio fibrisolvens α-amylase	NI
Aspergillus oryzae α-amylase	NI
Homo sapiens (human) maltase-glucoamylase	520 ± 10
Mus musculus (mouse) maltase-glucoamylase	990 ± 40
Saccharomyces cerevisiae (yeast) α-glucosidase	NI
Agrobacterium faecalis β-glucosidase	NI
Bos taurus (bovine) liver β-galactosidase	NI
Caffea arabica (green coffee bean) α-galactosidase	NI
Canavalia ensiformis (Jack bean) α-mannosidase	NI

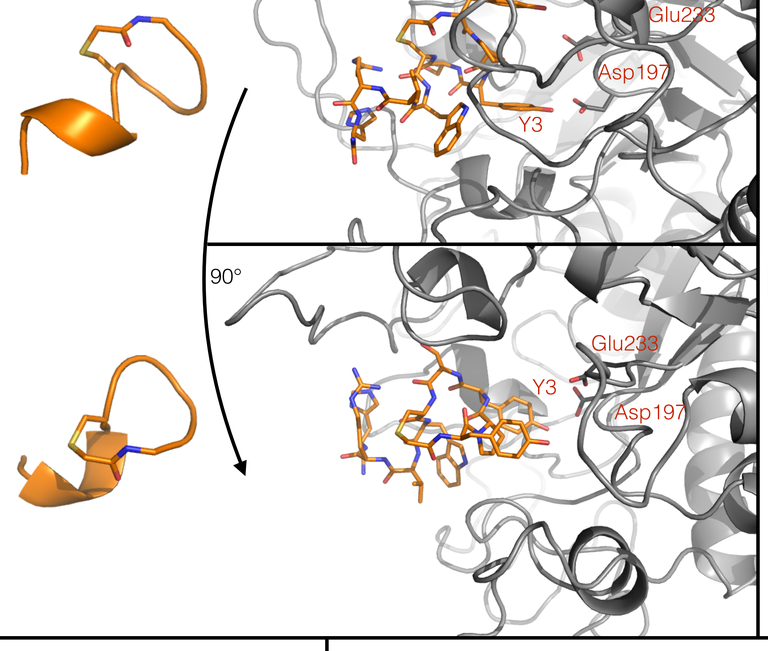
piHA-Dm[5]

(K_i = 7.0 nM)



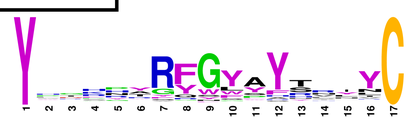


Consensus motif, D-Tyr initiated library

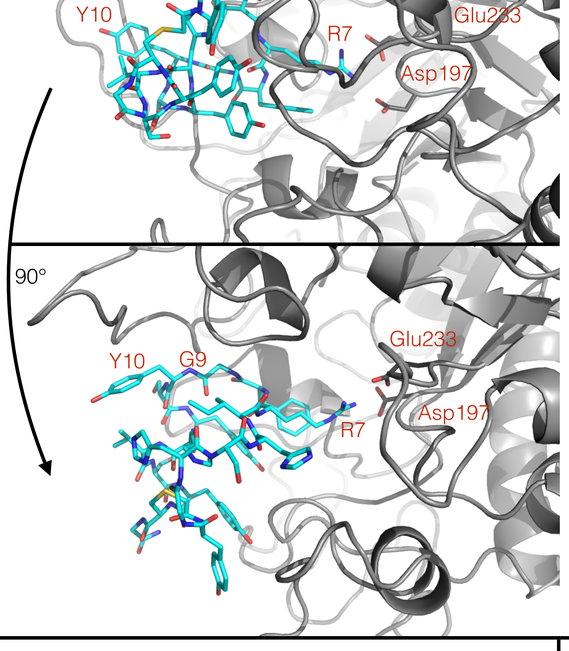


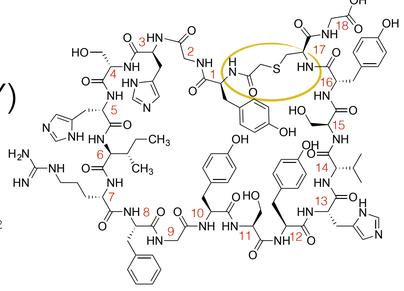
piHA-L5 (d10Y)

(K_i = 9.2 nM)



Consensus motif, L-Tyr initiated library





Diversity

‘L1’ (1.9 nM):
YPTKRYGQWLPYRNNNCG-NH2

‘L3’ (2.6 nM):
YWDRPTRFGYAYSVIYCG-NH2

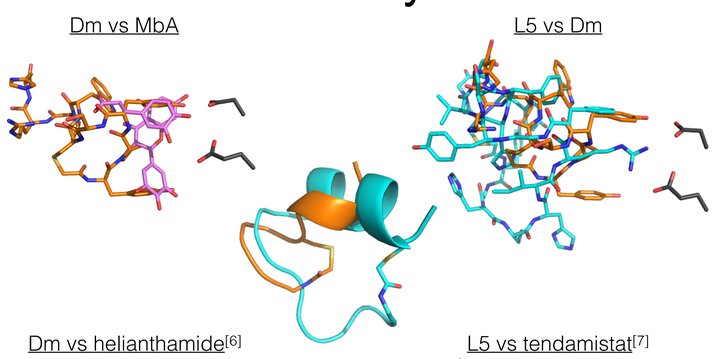
‘L5’ (2.8 nM)
YGHSHIRFGdSYHVSYCG-NH2

‘L12’ (2.5 nM):
YTFRDWRRSYGGITVRCG-NH2

‘L26’ (1.0 nM):
YGQSHSAWCRWINYNP-NH2
(YGQSHSAWCRWINdNPCG)

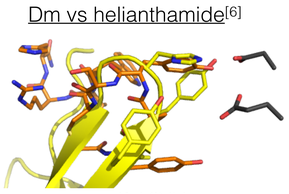
Overlays

Dm vs MbA



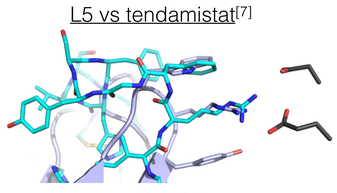
L5 vs Dm

Dm vs helianthamide[6]



motif: YIYH

L5 vs tendamistat[7]



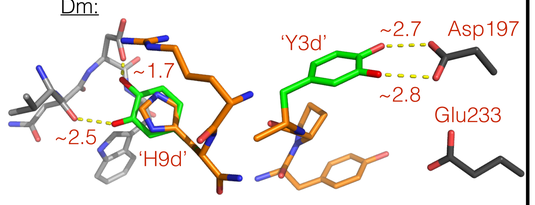
motif: WRY

Mutagenesis

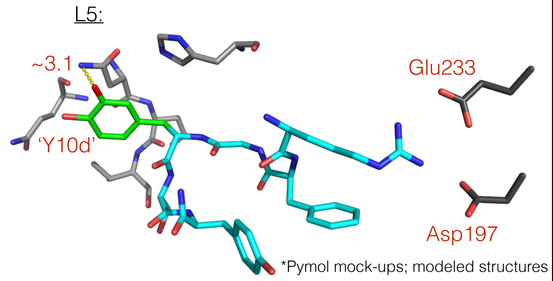
Code	Sequence	K_i (nM)
D1	yPYS**C**WARHVRIREN	1
D3	yPYS**C**WVRHSDPHKF	2.7
Dm (D3Δ10)	yPYS**C**WVRH	7
Dm-lin	yPYSAWVRH (linear)	200
Dm-V7A	yPYS**C**WARH	2
D1-D11	yPYS**C**WARHV	1.3
Dm-H9d	yPYS**C**WVRd	3.7
Dm-Y3d	yPdS**C**WVRH	0.5
L1	YPTKRYGQWLPYRNNN**C**G	1.9
L3	YWDRPTRFGYAYSVIY**C**G	2.6
L3-F8Y	YWDRPTRYGYAYSVIY**C**G	10
L3-Y10d	YWDRPTRYGdAYSVIY**C**G	31
L5	YGHSHIRFGdSYHVSY**C**G	2.8
L5-d10Y	YGHSHIRFGYSYHVSY**C**G	9.2
L12	YTFRDWRRSYGGITVR**C**G	2.5
L26-Δ17	YGQSHSAW**C**RWINdNP	12
L26-Δ17-d14Y	YGQSHSAW**C**RWINYNP	1
L26-Δ14	YGQSHSAW**C**RWIN	2.9
L26-Δ10	YGQSHSAW**C**	>10000

Possible L-dopa interactions

Dm:



L5:



*Pymol mock-ups; modeled structures

Conclusions

- ★ The first use of the RaPID system with a carbohydrate-active enzyme was successful.
- ★ Potent macrocyclic peptide inhibitors of human pancreatic α-amylase discovered.
- ★ Most promising hit is piHA-Dm, a nonapeptide with 7 nM K_i.
- ★ Selective for pancreatic α-amylase over all other enzymes tested.
- ★ Forms a compact fold, with the ‘tail’ forming a 3_{10} helix.
- ★ L-Dopa reintroduced based on structure and high throughput sequencing data; 500 pM inhibitor.
- ★ L-Tyr initiated motif less conserved, but equally potent.
- ★ Also helical, but different arrangement and interactions.
- ★ No MbA-like motif, despite both moieties in library.
- ★ Interactions with human pancreatic α-amylase converge on known as well as new motifs, in a new scaffold.

References

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GlycoNet