Occurrence and impact of the inhibitor of starch debranching enzyme in barley seed and malt

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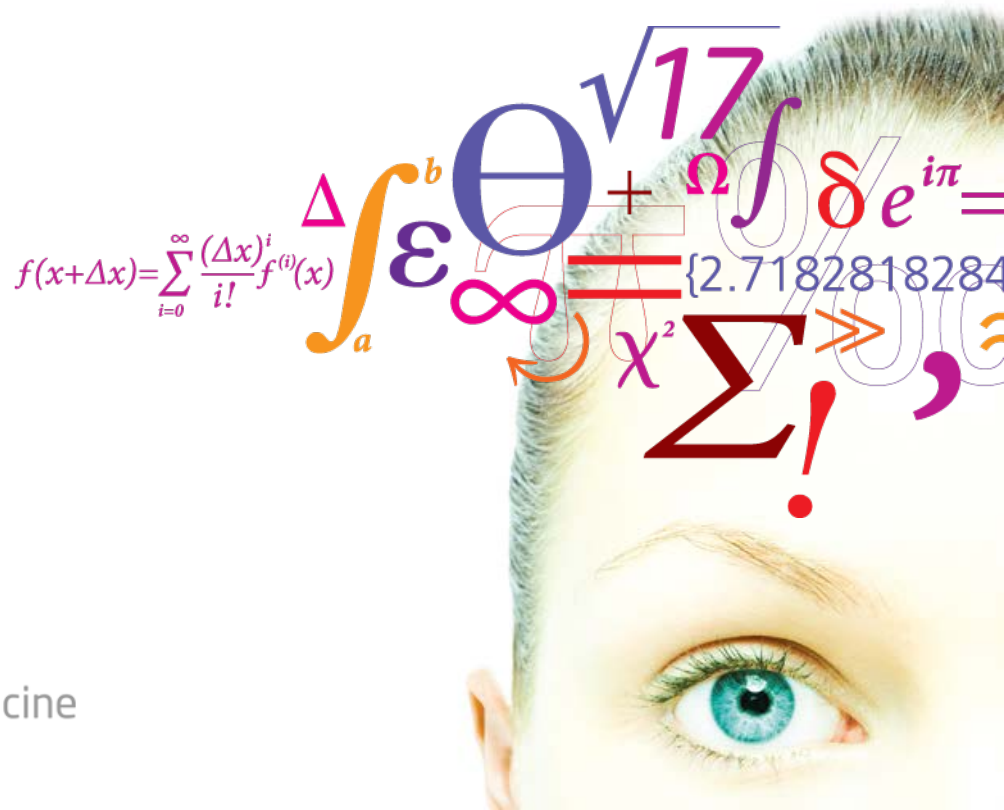
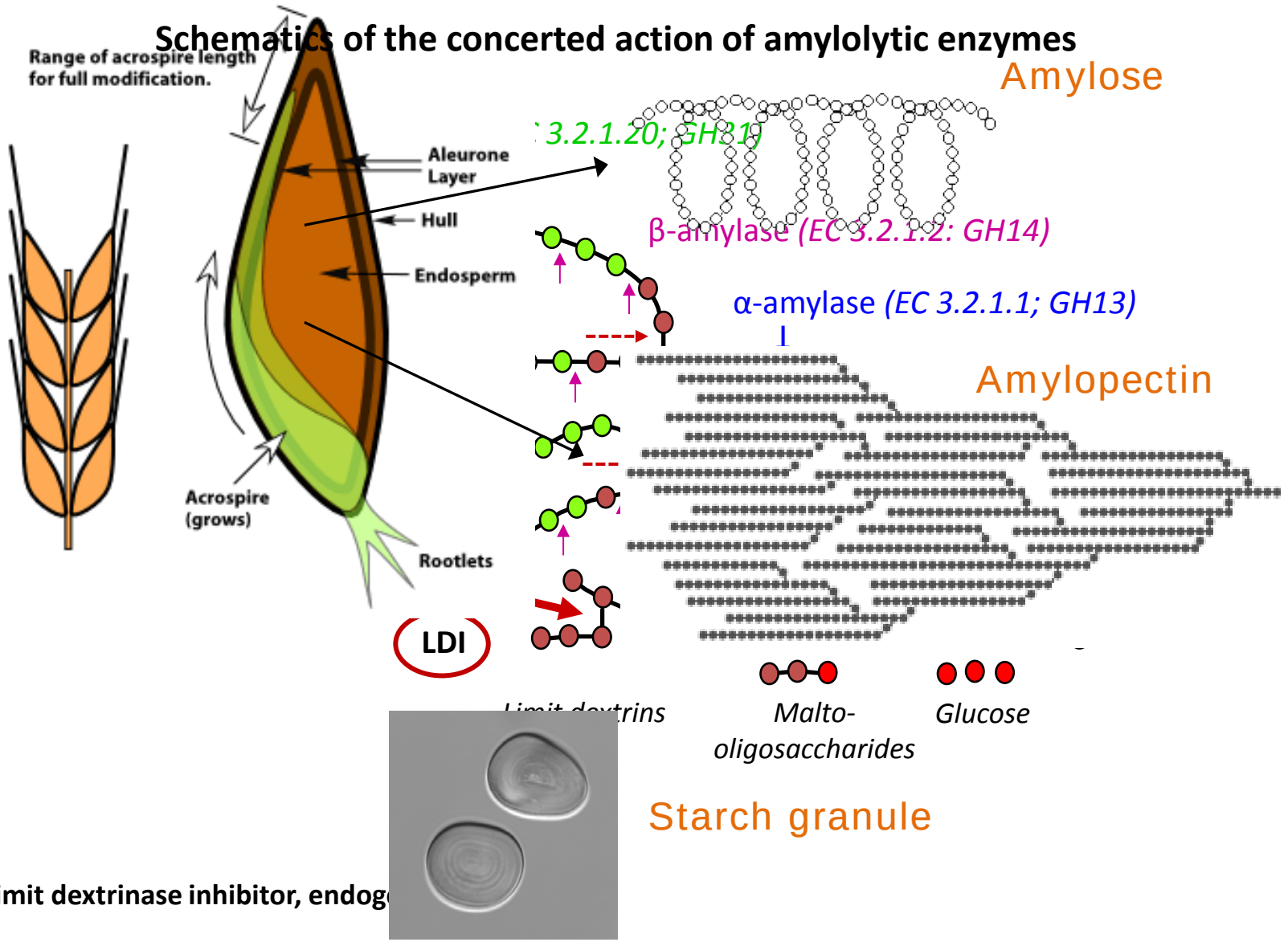


DTU Bioengineering
Department of Biotechnology and Biomedicine

Setting the scene – The germinating seed



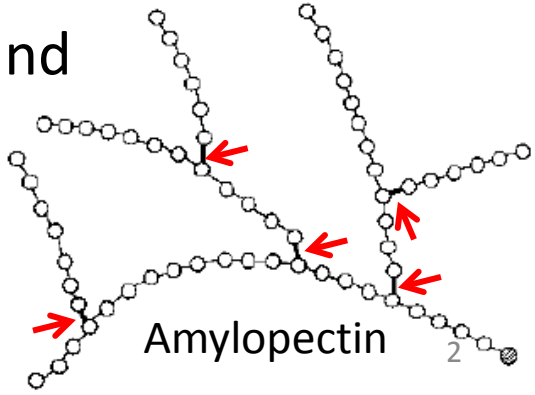
LDI = limit dextrinase inhibitor, endog

Fundamental and applied relevance of limit dextrinase (LD) and limit dextrinase inhibitor (LDI)

- **LD** is the sole starch-debranching enzyme present in germinating barley seeds, *i.e.* also in malting and mashing

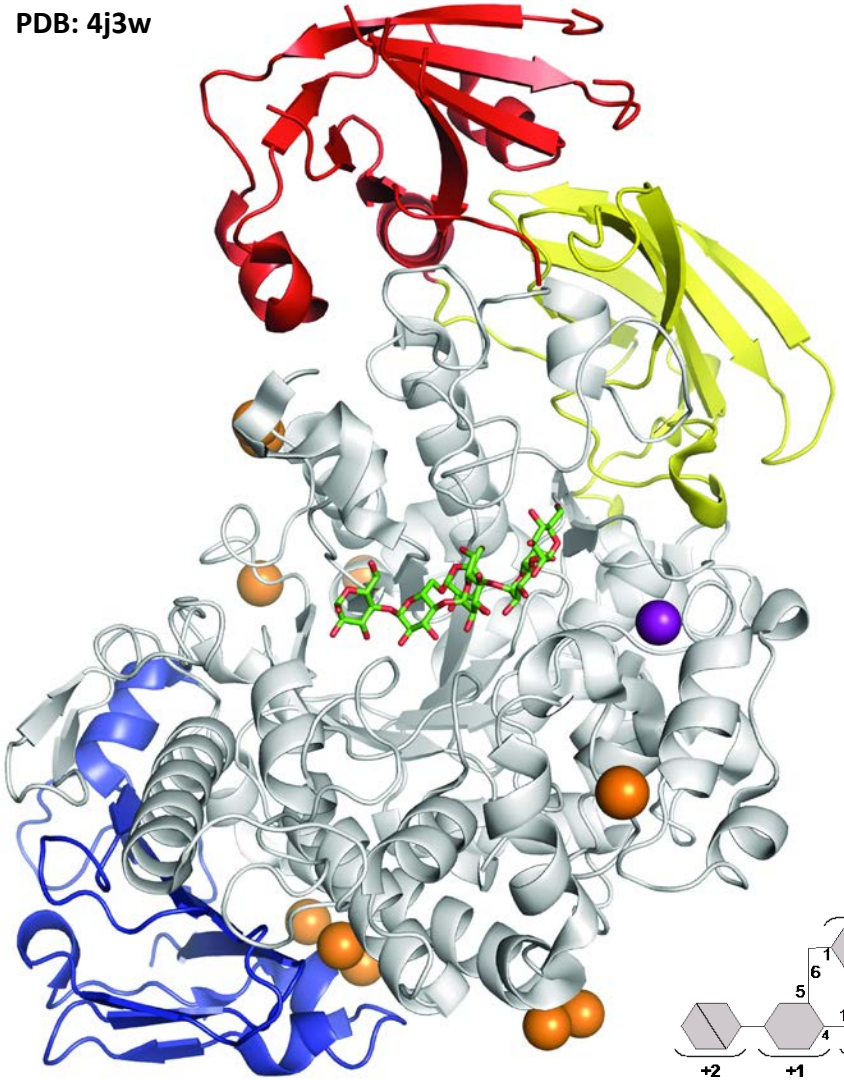
- **LD** and **LDI** are limiting and central in beer production

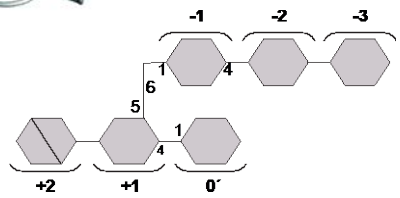
- **LD** is from a large family of starch degrading enzymes (GH13), which have industrial important members as well as enzymes being a part of organisms energy metabolism.
- Knowledge is limited on the structural basis and mechanism of proteinaceous inhibitors of carbohydrate active enzymes



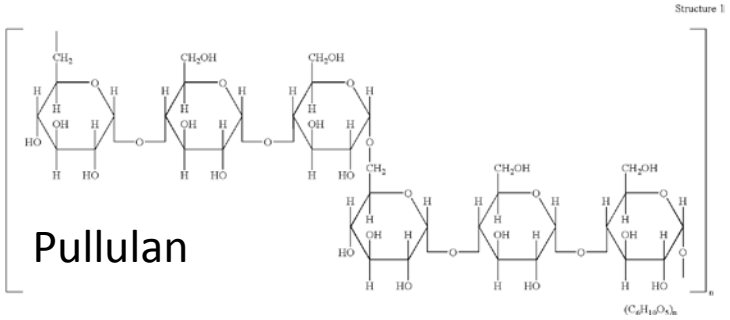
Limit dextrinase (LD)

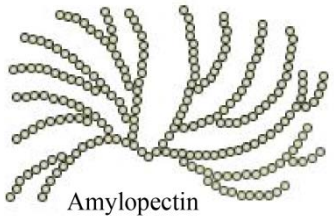
PDB: 4j3w





	K_m (mg/ml)	k_{cat} (s^{-1})	k_{cat}/K_m (ml/(mg s))
Pullulan	0.16±0.02	78±10	488
Potato amylopectin	6.9±1.0	15.6±1.2	2.3





LD-E510A; Solved by MR; Resolution: 1.67 Å; R_{cryst}/R_{free}=16.4%/19.6%

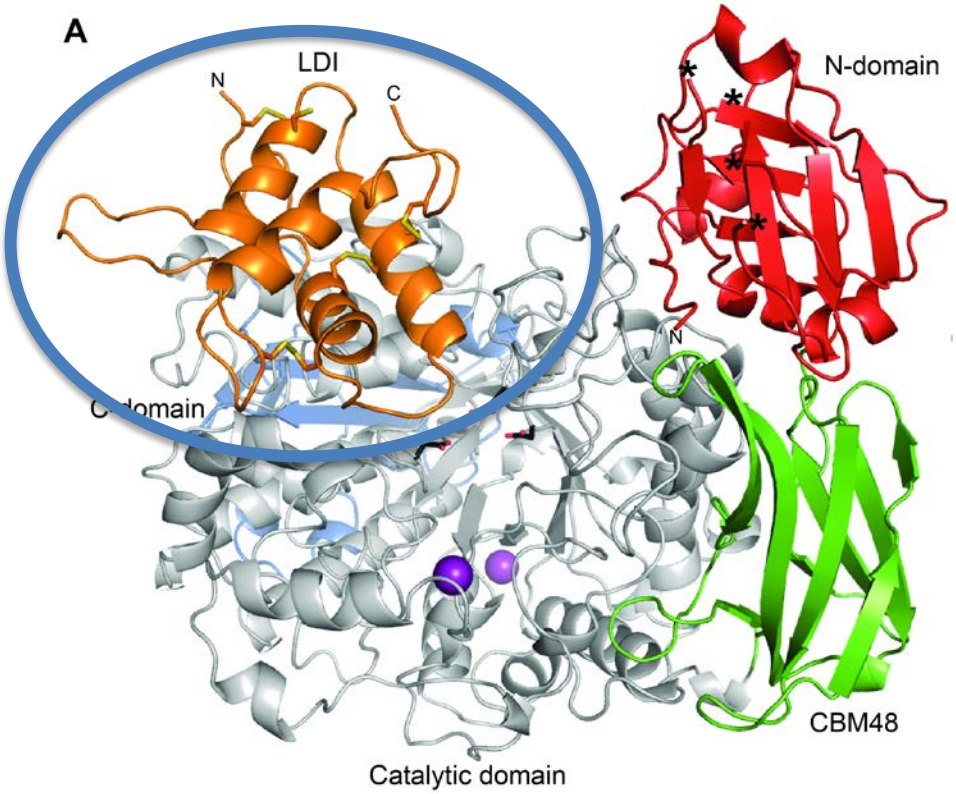
Møller et al. (2015), *J. Mol. Biol.* 427, 1263-1277

LD:LDI structure

Barley limit dextrinase (starch debranching enzyme)

+

its endogenous inhibitor (LDI)

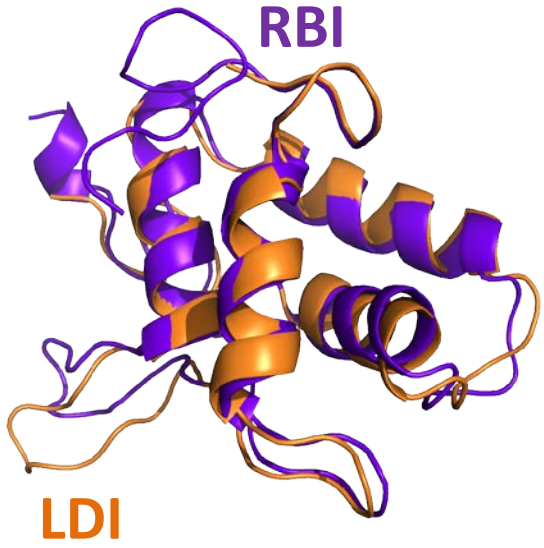


PDB: 4CVW

Møller et al., (2015) *J. Biol. Chem.,* 290: 12614–12629

Limit dextrinase inhibitor (LDI)

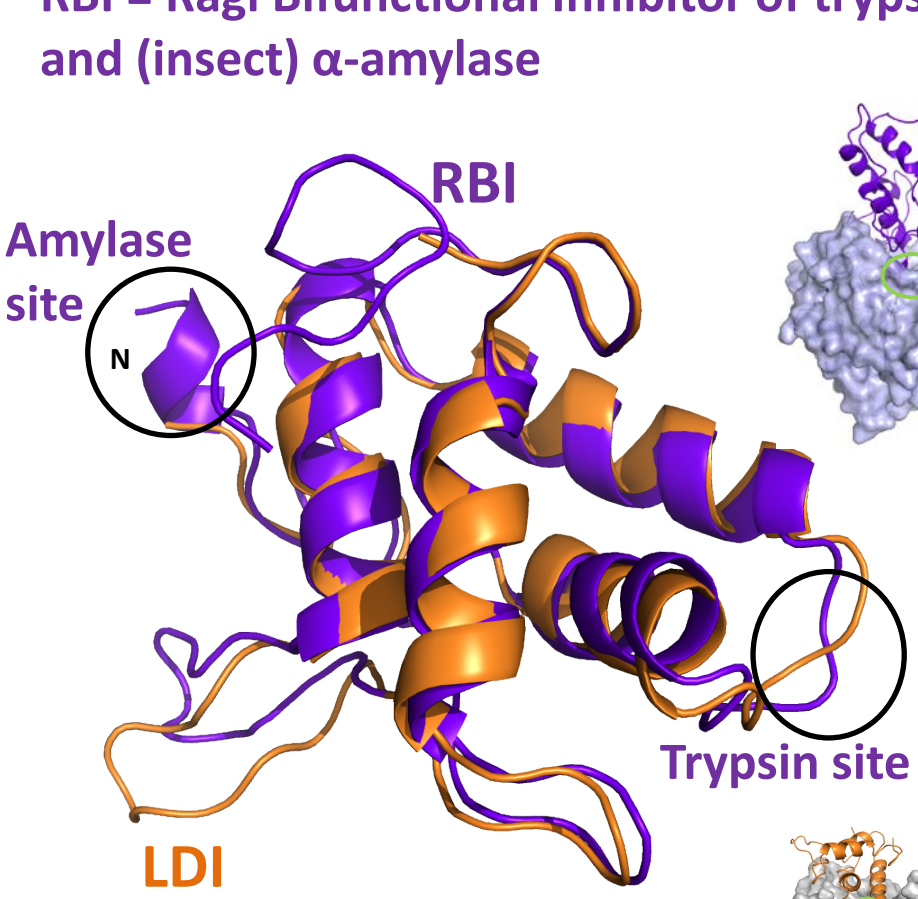
- **LDI** is a small inhibitor (13.4 kDa) with 4 disulfide bonds and one glutathionylated cysteine and belongs to the cereal-type inhibitors
- Antisense down-regulation of **LDI** reduces starch biosynthesis and alters the amylopectin:amylose ratio
- **LDI** is the sole characterised inhibitor of a debranching enzyme
- **LDI** can be recombinantly produced in *P. pastoris* resulting in 200 mg/L
- **LDI** has a T_m of 97°C and bind to LD with pM affinity
- Crystal structures of 3 other cereal-type inhibitors are solved:
  - **Bifunctional inhibitor of trypsin and α-amylase from ragi (RBI), 1B1U**
  - Corn Hageman factor inhibitor (**CHFI**), 1BEA
  - 0.19 α-amylase inhibitor from wheat kernel (**0.19 AI**), 1HSS
- All known cereal type inhibitors except LDI are acting against pests, i.e. not inhibiting the plants own enzymes



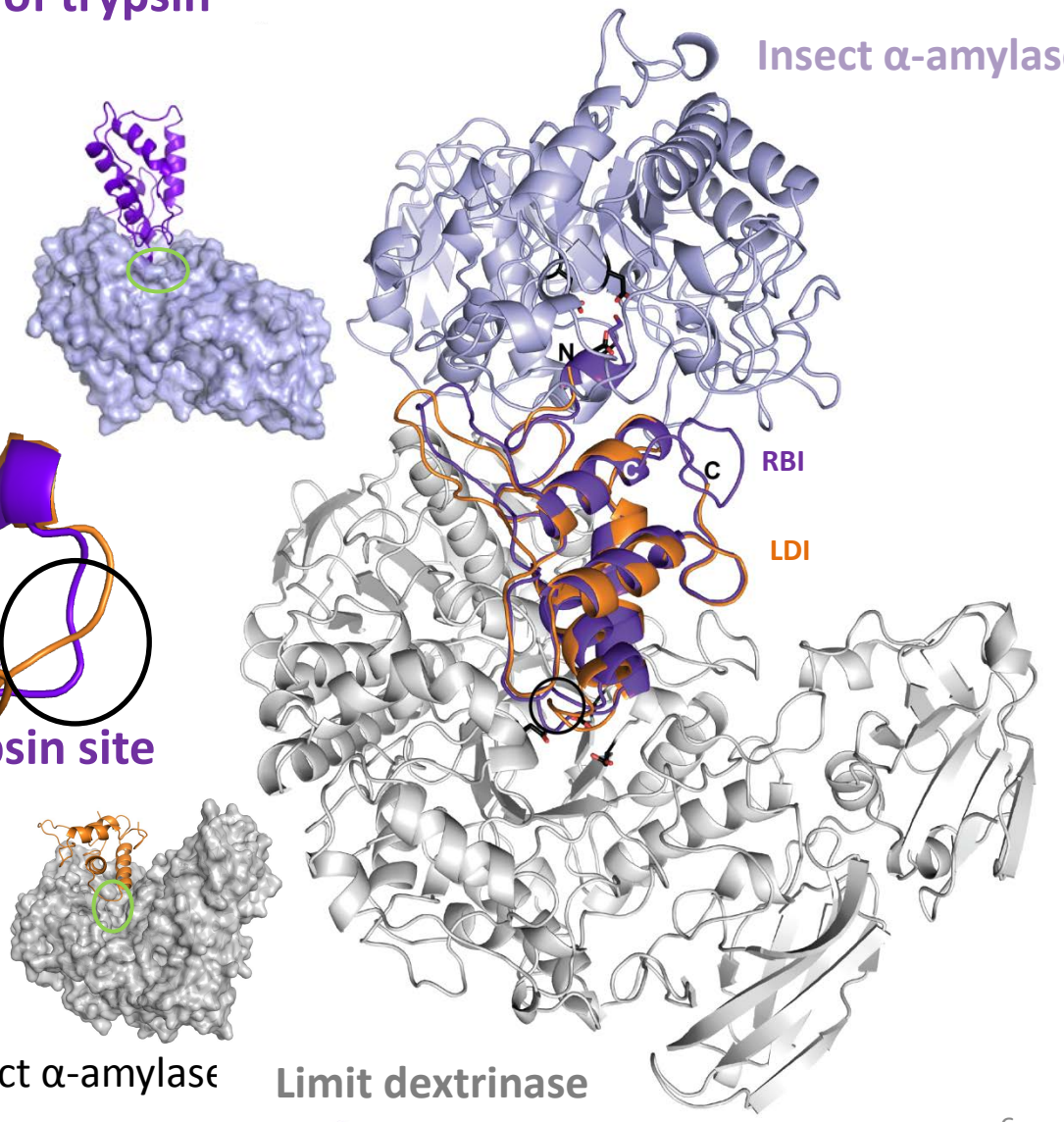
	Id. to LDI (%)	Sim. to LDI (%)
RBI	53.3	68.9
CHFI	56.0	71.6
0.19 AI	32.7	54.8

New binding orientation of a cereal-type inhibitor

RBI = Ragi Bifunctional Inhibitor of trypsin and (insect) α-amylase



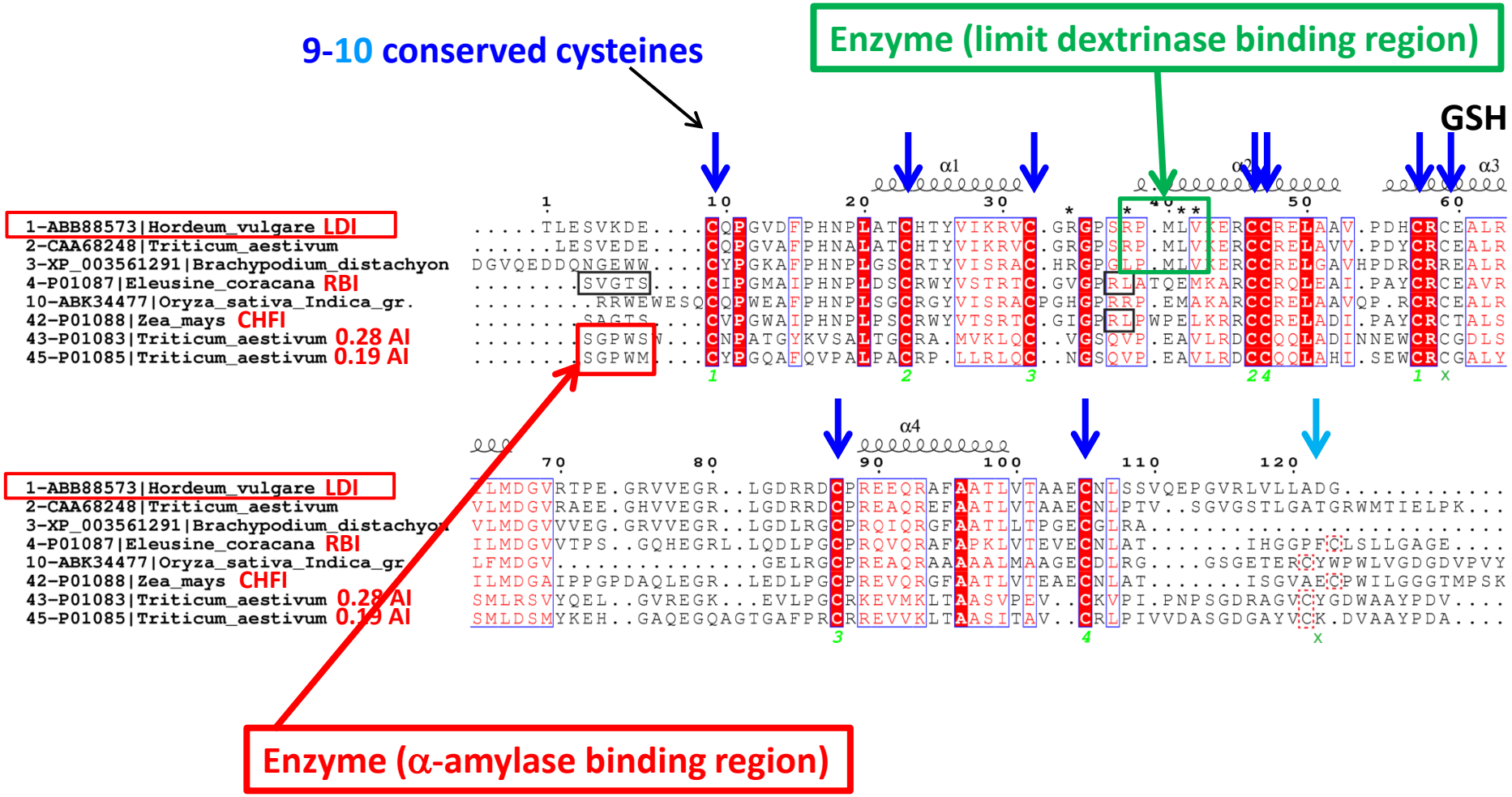
Sequence identity:	53.3%
Sequence similarity:	68.9%
RMSD:	0.83



LDI has no inhibitory effect towards insect α-amylase bacterial pullulanases, or bovine trypsin

Møller et al., (2015) *J. Biol. Chem.,* 290: 12614–12629

Conserved features of cereal-type inhibitors



LD/LDI in relation to malt quality

Limit dextrinase

Limit dextrinase inhibitor

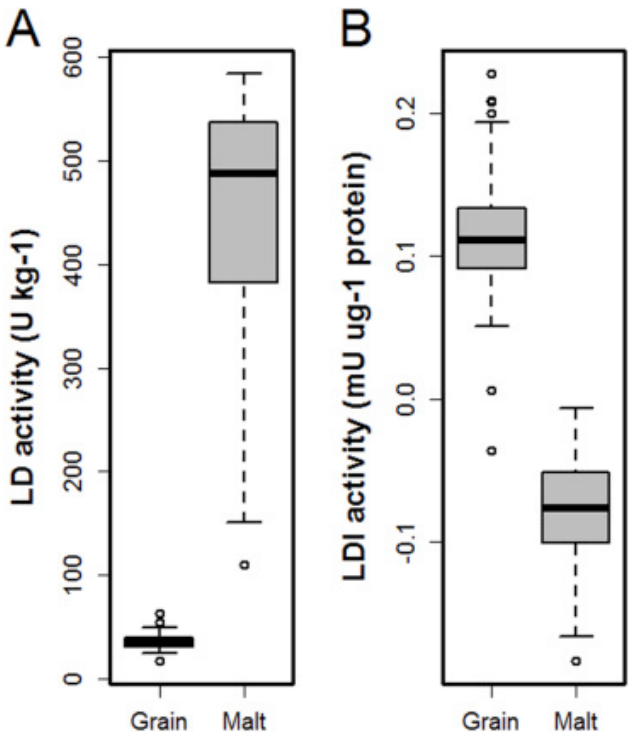


Fig. S2. Boxplot of LD and LDI activity in barley grain and malt. A, LD activity; B, LDI activity.

Huang et al. (2016) The relationship of limit dextrinase, limit dextrinase inhibitor and malt quality parameters in barley and their genetic analysis. *J. Cereal Science,* 70, 140–145

LD/LDI in relation to malt quality

Limit dextrinase

Limit dextrinase inhibitor

Table 2
The correlation between LD activity, LDI content and malting quality parameters.

	LD_M	LDI_M	LD_G	LDI_G
DP	0.344**	0.289*	0.346**	0.000
KI	0.575**	−0.564**	−0.374**	−0.018
ME	−0.013	−0.116	−0.402**	0.122
VC	−0.504**	0.168	0.315**	−0.145
SN	0.552**	0.166	0.124	0.225
TN	0.178	0.573**	0.435**	0.235

Note: LD_G: LD activity in grains; LD_M: LD activity in malt; LDI_G: LDI content in grains; LDI_M: LDI content in malt; DP: diastatic power; KI: Kolbach index; ME: malt extract; VC: Viscosity; TN: total nitrogen content in malt. * and ** Represents significant at $P < 0.05$ and $P < 0.01$ level, respectively.

Huang et al. (2016) The relationship of limit dextrinase, limit dextrinase inhibitor and malt quality parameters in barley and their genetic analysis. *J. Cereal Science,* 70, 140–145

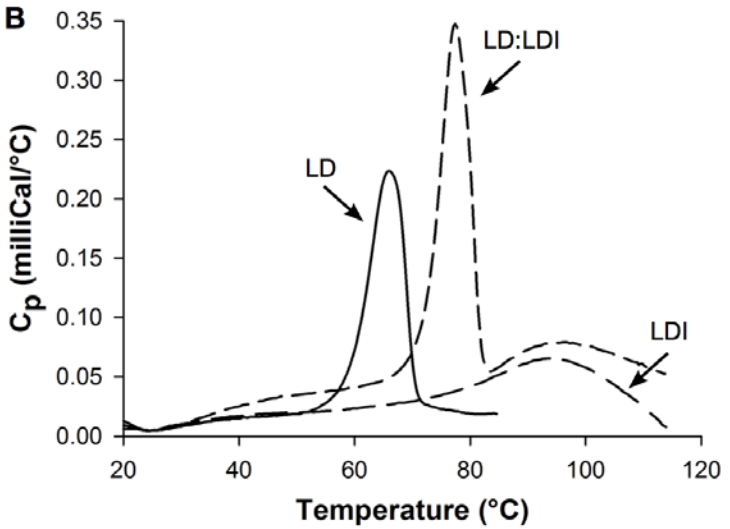
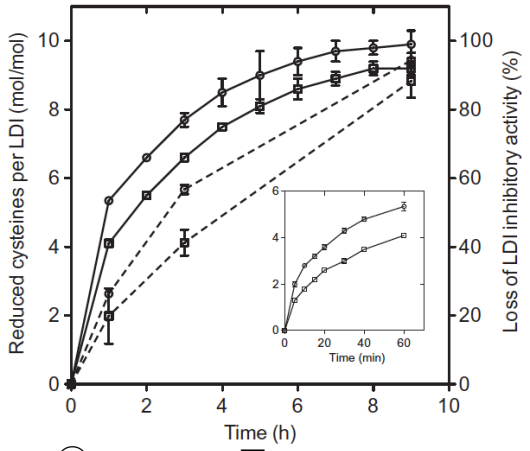


Fig. 8B. Differential scanning calorimetry thermograms of LD (5 µM), LDI (25 µM), and LD-LDI (5 µM:25 µM) unfolding at pH 6.5.

Møller et al. (2015) Crystal structure of barley limit dextrinase-limit dextrinase inhibitor (LD-LDI) complex reveals insights into mechanism and diversity of cereal-type inhibitors. *J. Biol. Chem.,* 290, 12614–12629

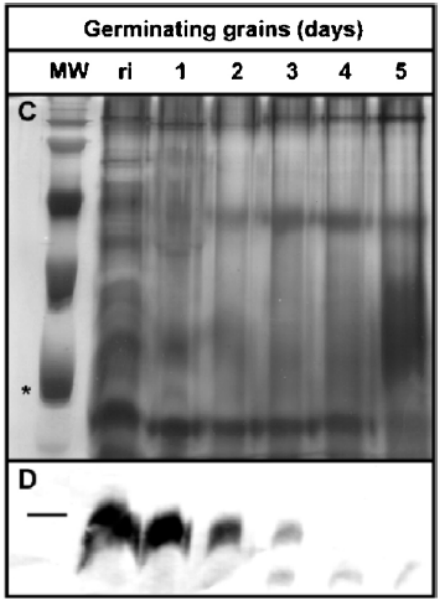
How LDI "disappear" during germination

Reduction by thioredoxin

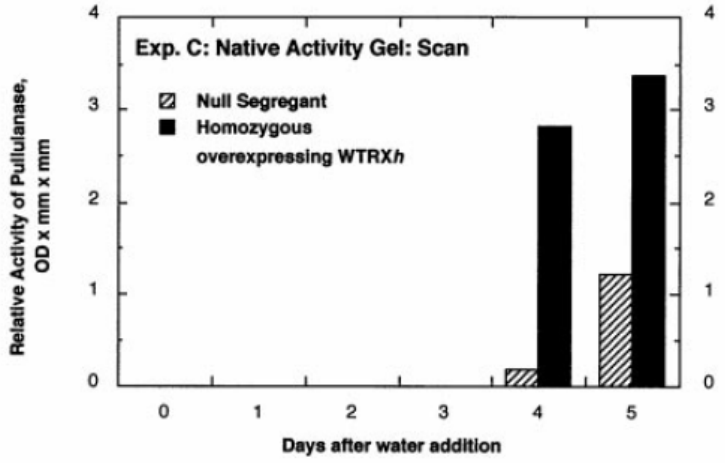


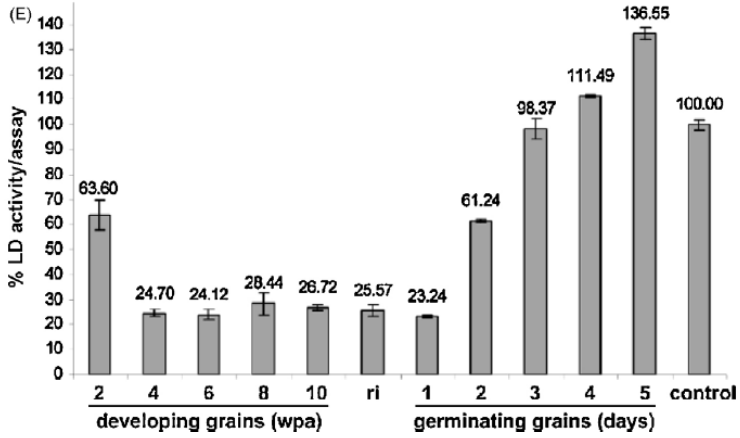
HvTrxh1 (◯), HvTrxh2 (☐)
Release of thiol groups (—), loss of LDI activity (---)

Degradation by proteases (?)



Effect of overexpression of Trx in barley grains



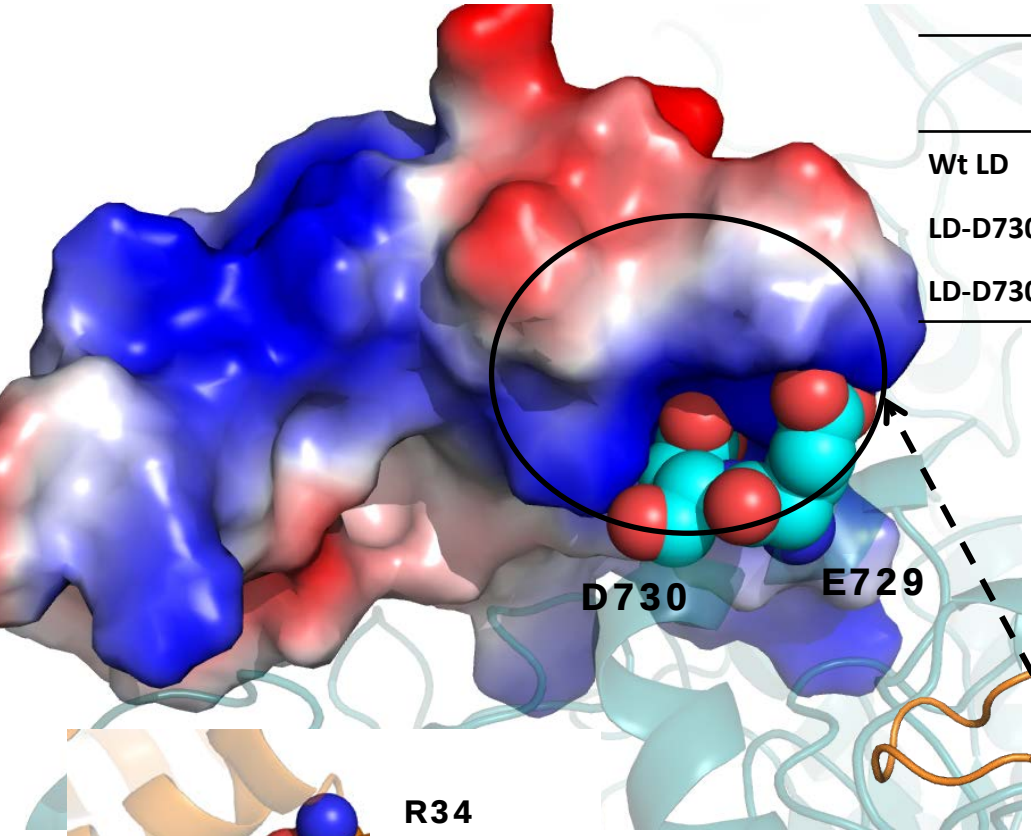


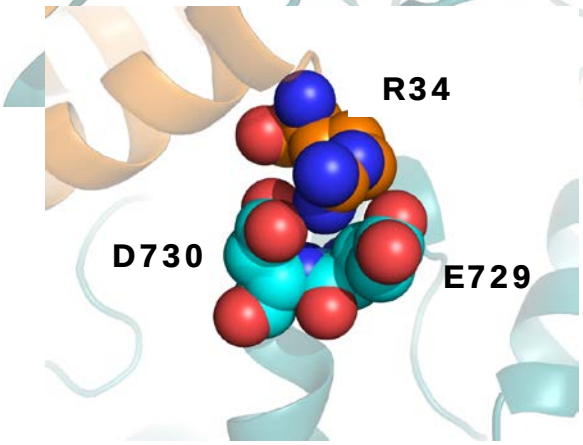
Jensen et al. (2012) *FEBS let*. 586, 2479–2482; Stahl et al. (2007) *Plant Sci.* 172, 452–461; Cho et al. (1999), *PNAS*, 96, 14641–14646

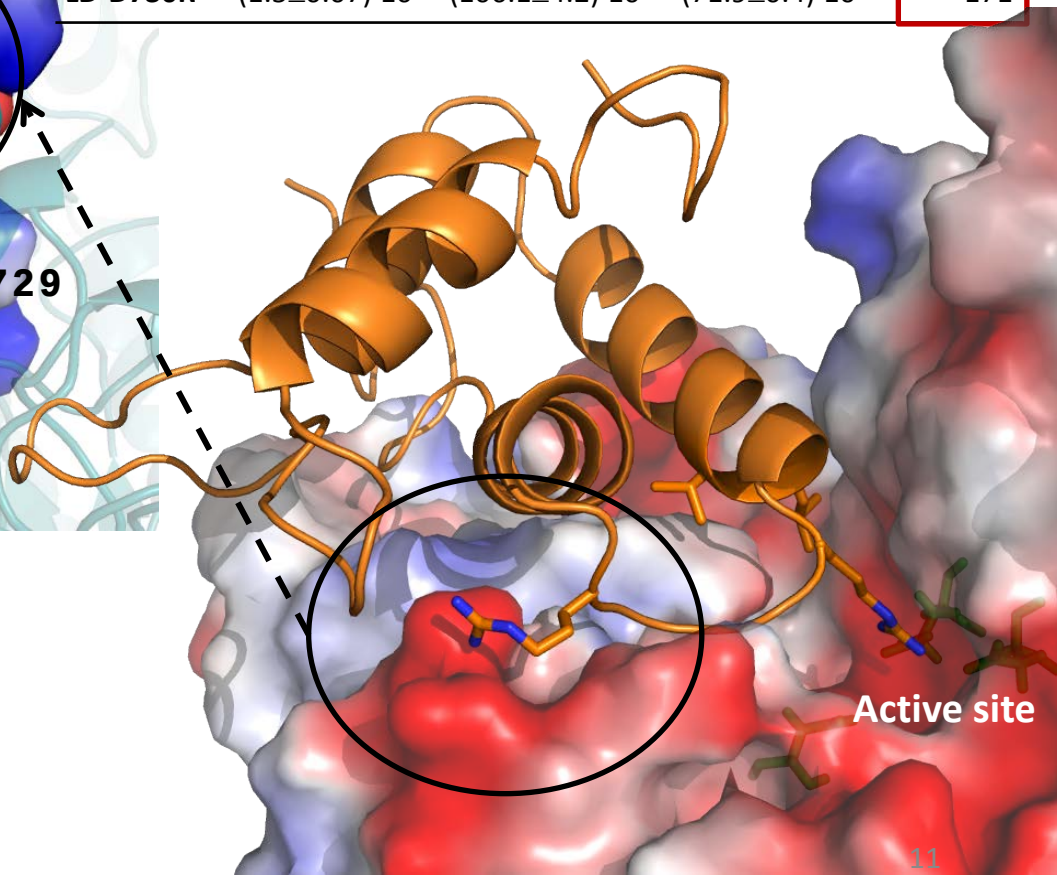
Engineering of LDI-insensitive limit dextrinase?

SPR analysis

	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (M)	Relative K_D
Wt LD	$(15.4\pm0.2)\cdot10^{5}$	$(6.4\pm0.2)\cdot10^{-5}$	$(42.0\pm2.0)\cdot10^{-12}$	1
LD-D730W	$(10.6\pm0.4)\cdot10^{5}$	$(35.1\pm3.6)\cdot10^{-5}$	$(33.0\pm2.2)\cdot10^{-11}$	≈8
LD-D730R	$(1.5\pm0.07)\cdot10^{5}$	$(106.1\pm4.2)\cdot10^{-5}$	$(71.9\pm0.4)\cdot10^{-10}$	≈171







Møller et al., (2015) *J. Biol. Chem.*, 290: 12614–12629

Present research activity on LD

nature COMMUNICATIONS

Sorghum starch digestibility ↔ LD-activity

ARTICLE

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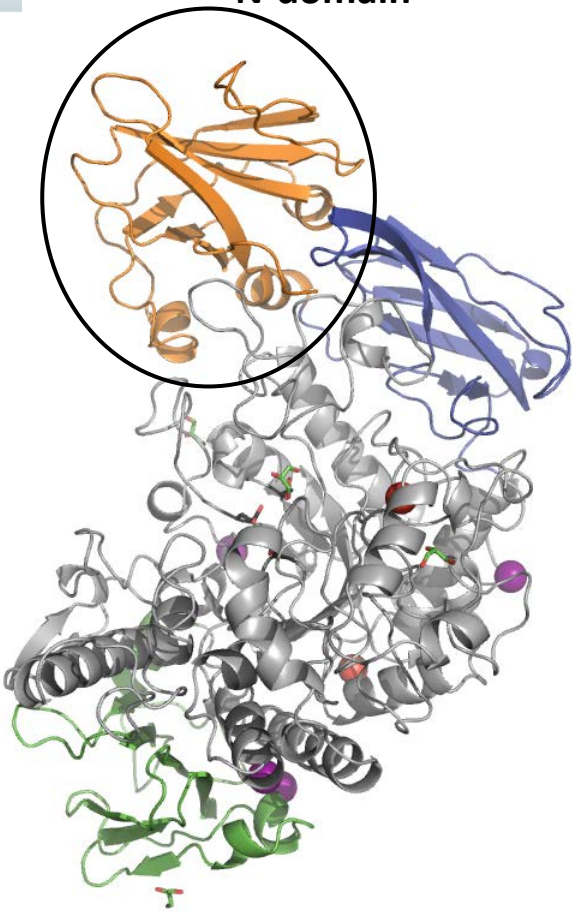
Allelic variation at a single gene increases food value in a drought-tolerant staple cereal

Edward K. Gilding[1,*], Celine H. Frère[2,*], Alan Cruickshank[3], Anna K. Rada[4], Peter J. Prentis[5], Agnieszka M. Mudge[1], Emma S. Mace[3], David R. Jordan[6] & Ian D. Godwin[1]

The production of adequate agricultural outputs to support the growing human population places great demands on agriculture, especially in light of ever-greater restrictions on input resources. Sorghum is a drought-adapted cereal capable of reliable production where other cereals fail, and thus represents a good candidate to address food security as agricultural inputs of water and arable land grow scarce. A long-standing issue with sorghum grain is that it has an inherently lower digestibility. Here we show that a low-frequency allele type in the starch metabolic gene, pullulanase, is associated with increased digestibility, regardless of genotypic background. We also provide evidence that the beneficial allele type is not associated with deleterious pleiotropic effects in the modern field environment. We argue that increasing the digestibility of an adapted crop is a viable way forward towards addressing food security while maximizing water and land-use efficiency.

CBM21-like N-domain



Structure of free barley limit dextrinase

Møller et al. (2012) *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.,* F68, 1008-1012

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