

Critical Enzymes for Sustainable Biofuels from Cellulose

Project acronym: CESBIC

Name: Paul Walton

ERA-IB-2 final conference, Berlin, 16./17.02.2016

Project partners



- *Department of Chemistry, University of York, UK*
- *Department of Biochemistry, University of Cambridge, UK*
- *Department of Chemistry, University of Copenhagen, Denmark*
- *CNRS Marseilles, France*
- *Novozymes A/S, Denmark*
- *Total project budget: Euros 2.1 million*





Introduction

- *Project objectives (problem to be solved)*

Discovery of new 'LPMO' (GH61) enzymes—particularly on new substrates

Determination of mechanism of action

Population of CAZy database

Evaluation for industrial use

- *General project approach*

Genomics, activity testing, structure and spectroscopy, evaluation

Technical overview

1) Background

2) What was achieved:

Discovery of LPMO classes AA11 and AA13

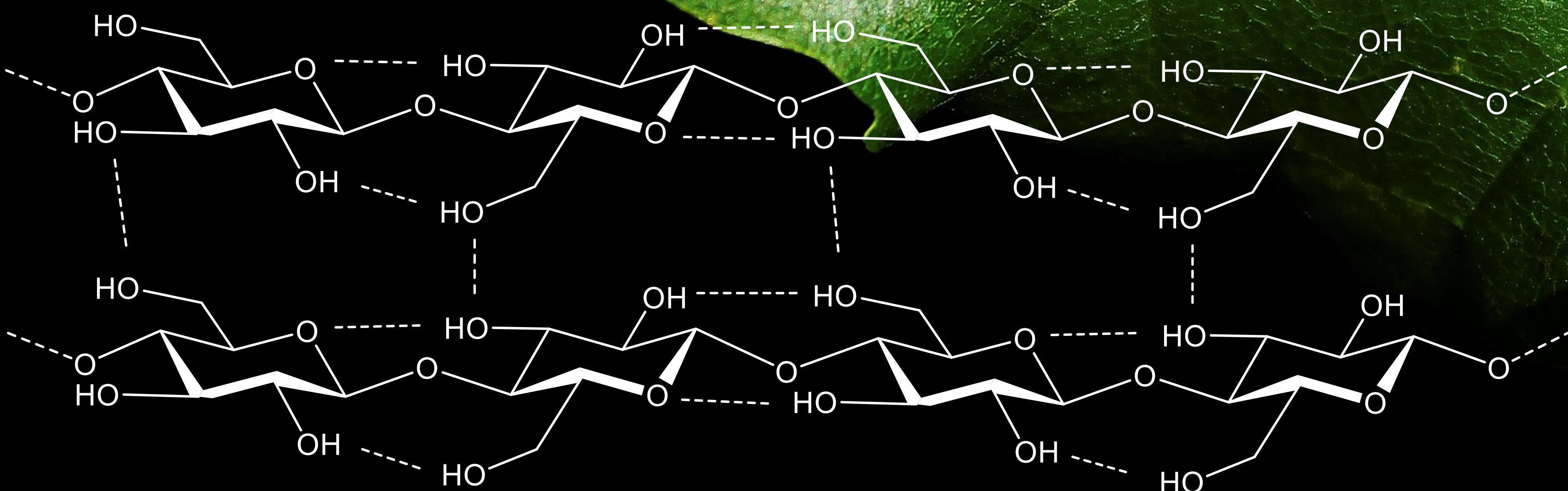
Overview of known LPMO

Mechanism insights

3) Future

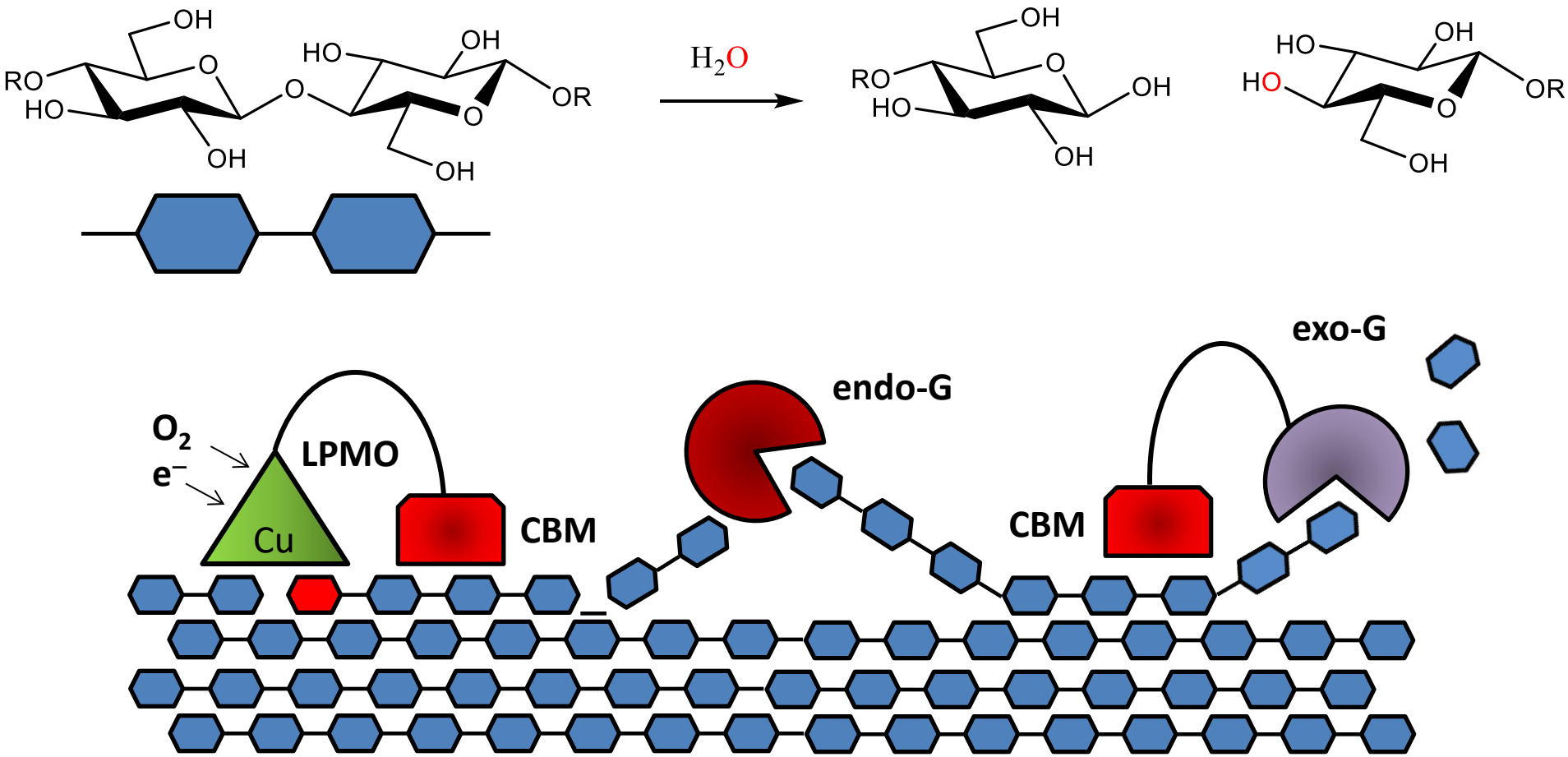
Biomass and Cellulose

~1,000,000,000 tonnes cellulose produced annually
368 million dry tonnes of dry biomass annually in US alone



Perlack, et al. 2005. *Biomass as feedstock for a bioenergy and bioproducts Industry: the technical feasibility of a billion-ton annual supply*.
Oak Ridge National Laboratory Report ORNL/TM-2005/66, US Dept. of Energy, Oak Ridge, TN

Fungal and bacterial biomass breakdown

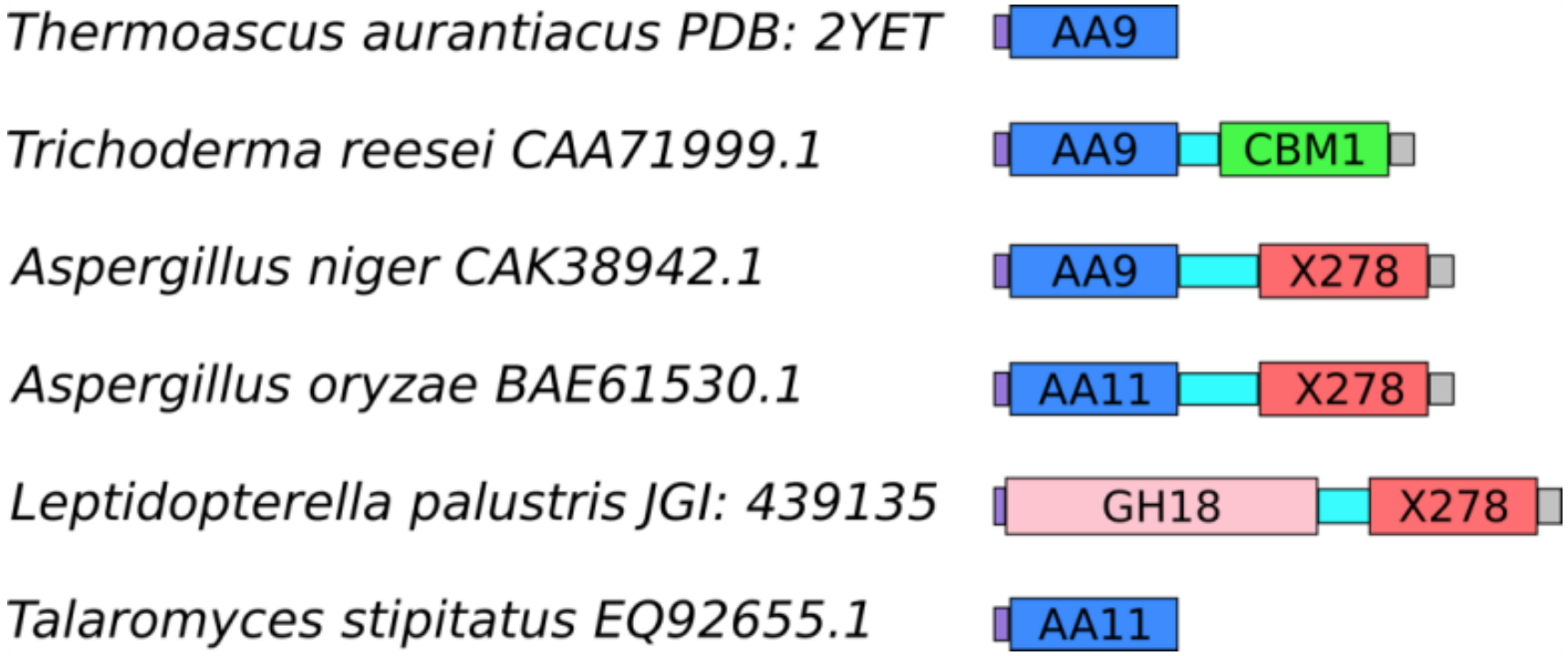


endo-GH endo-acting glycosidic hydrolase
exo-GH exo-acting glycosidic hydrolase
CBM carbohydrate binding module

www.cazy.org

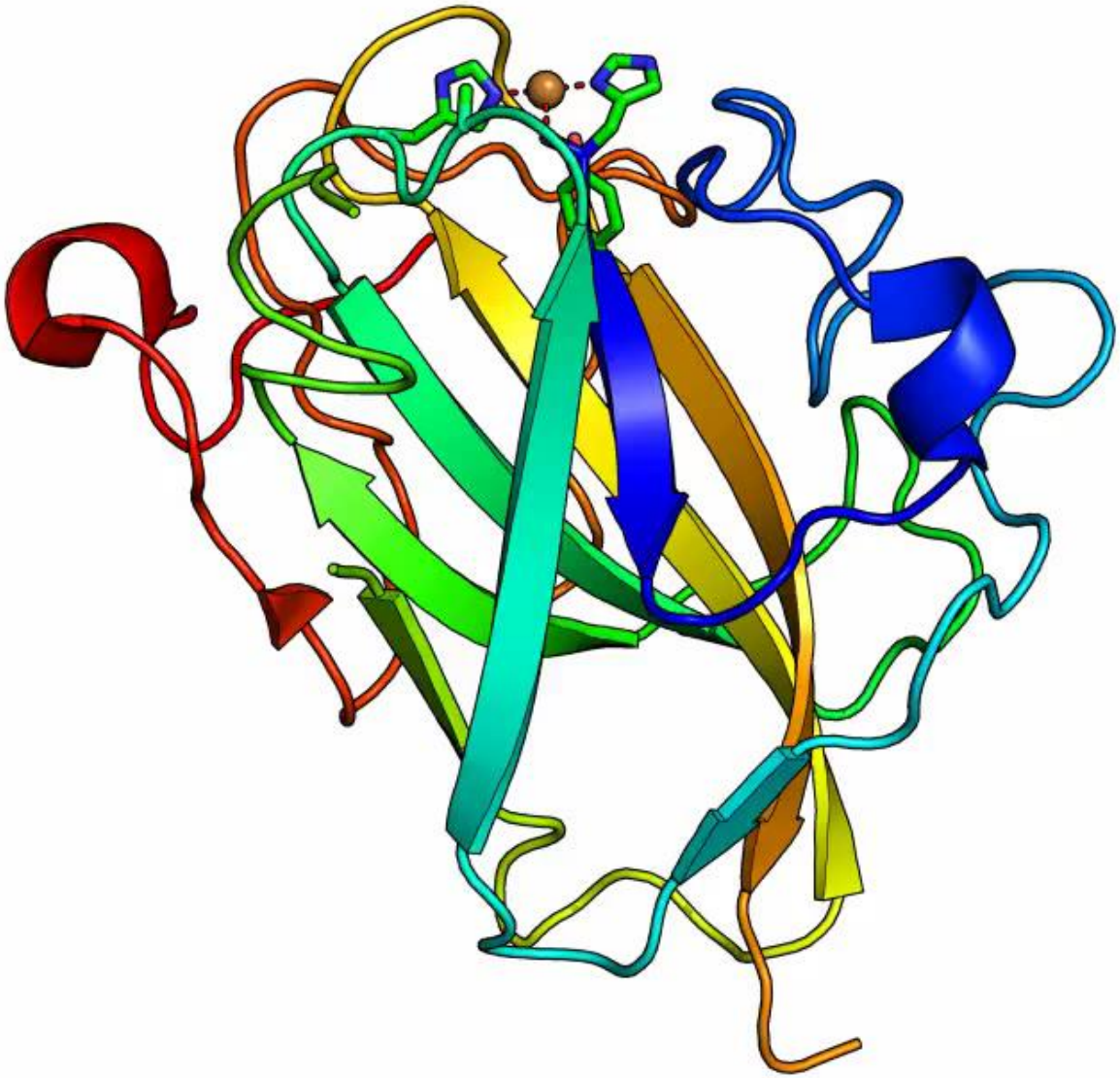
Discovery of LPMO class AA11

Module ‘walking’



Hemsworth, Henrissat, Davies, Walton, *Nature Chem Biol*, **2014**, *10*, 122

Structure



Hemsworth et al, *Nature Chem Biol*, **2014**, *10*, 122

Discovery and properties of LPMO class AA13

CAZy module walking

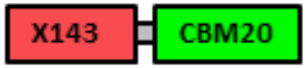
Arabidopsis thaliana 4-α-glucanotransferase
AAL91204.1

Neospora caninum trehalose synthase
CBZ50187.1

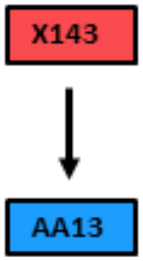
Bacillus circulans α-amylase
BAF37284.1

Bacillus cereus β-amylase
BAA34650.1 (PDB 1B90)

Arthrobacter globiformis 6-α-glucosyltransferase
BAD34980.1



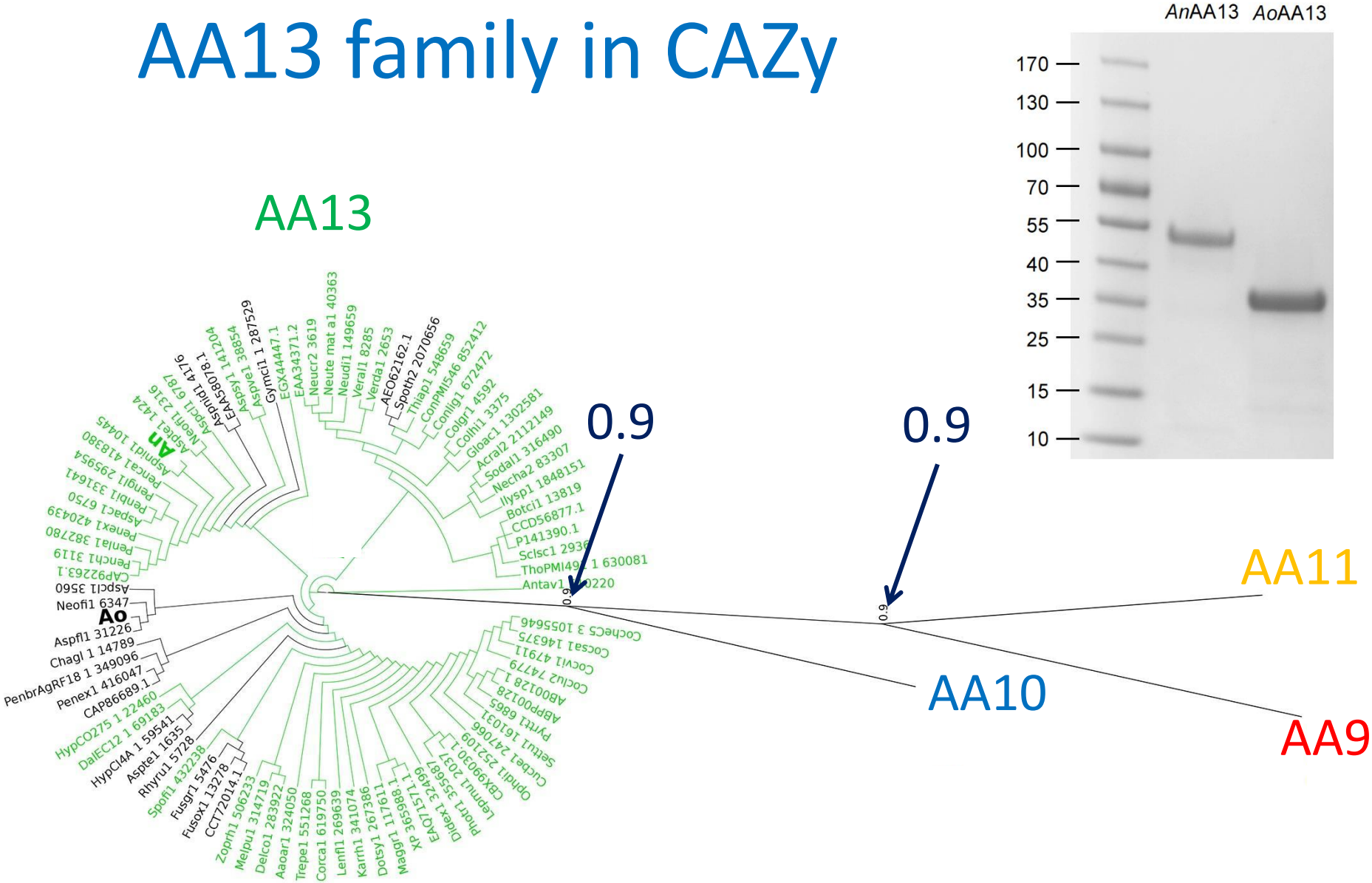
Aspergillus nidulans
EAA62623



Aspergillus oryzae
XP_001823115

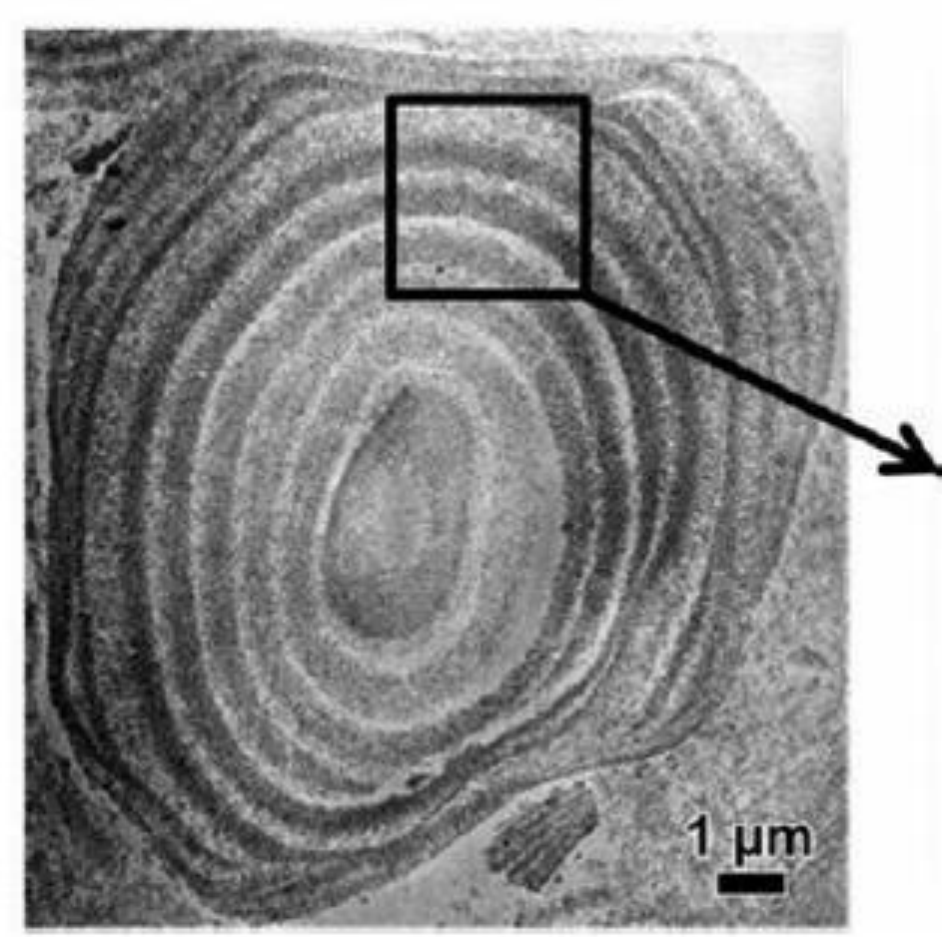
Horn *et al*, *Biotechnol. Biofuels* **2012**, *5(1)*, 45
Vu *et al*, *Proc. Nat. Acad. Sci*. **2014**, *111(38)*, 13822

AA13 family in CAZy

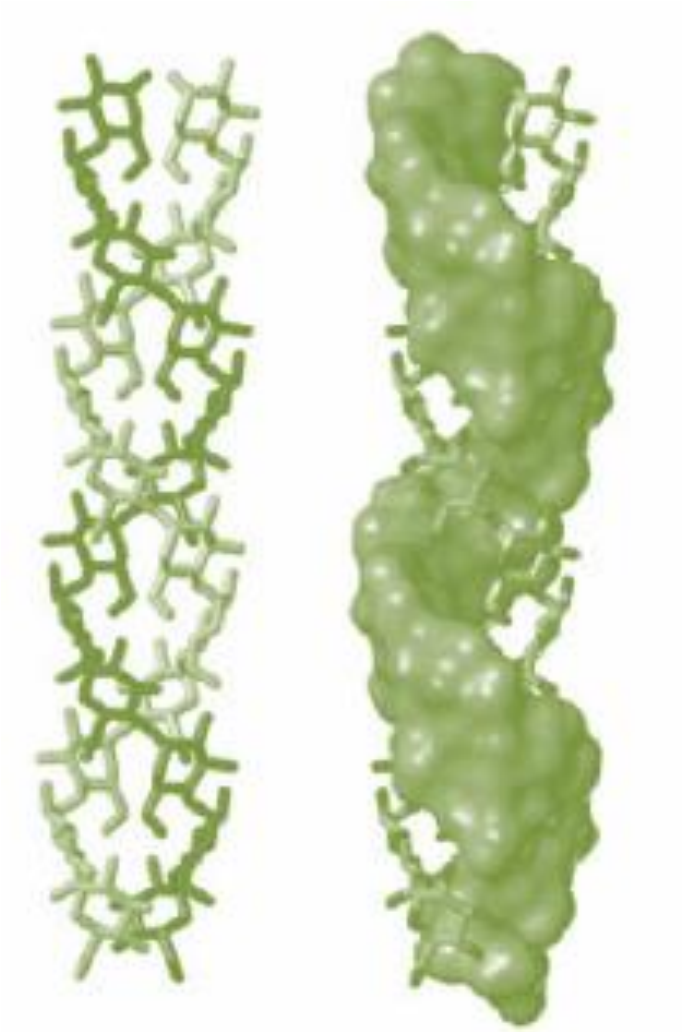


Lo Leggio *et al*, *Nature Communications,* **2015**, *6*, #5961

Crystalline and retrograded starch

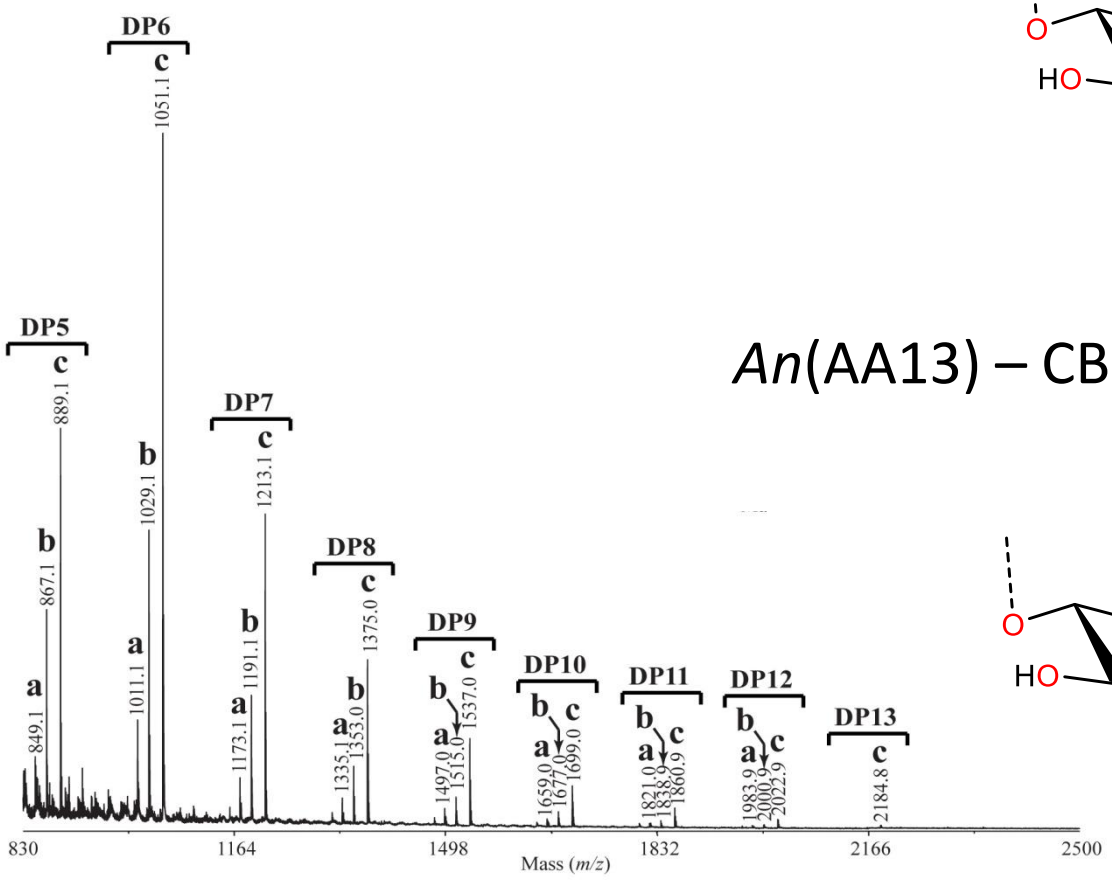


Maize grain starch granule

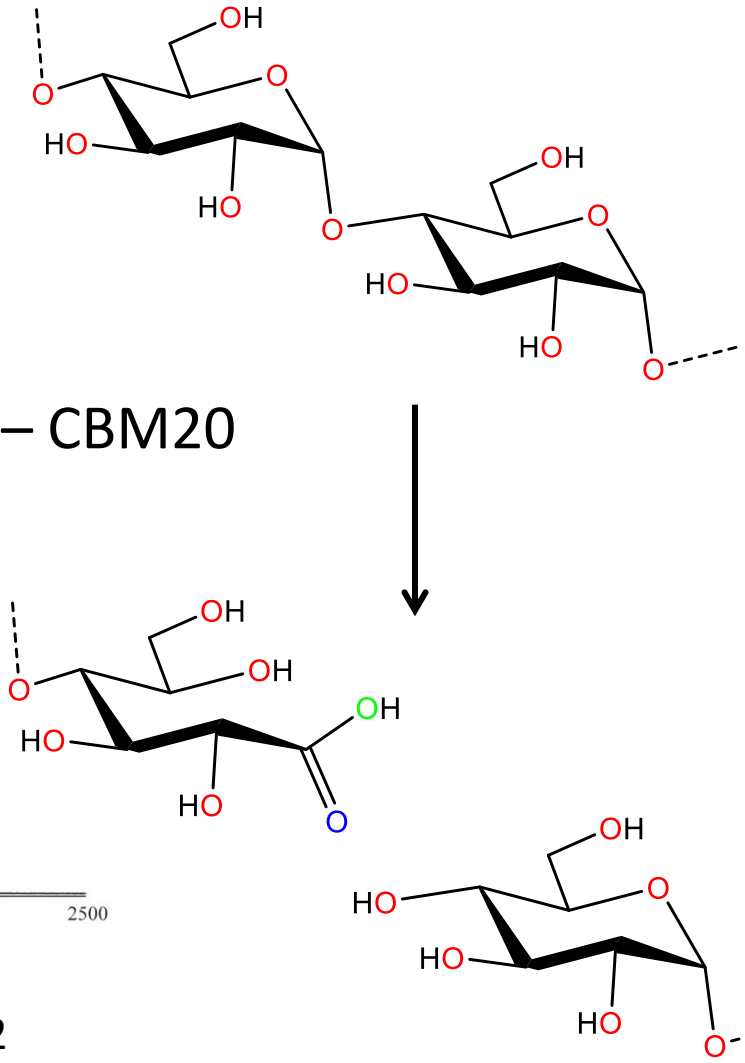


Perez, Bertoft, *Starch*, **2010**, *62*, 389

Activity on retrograded starch



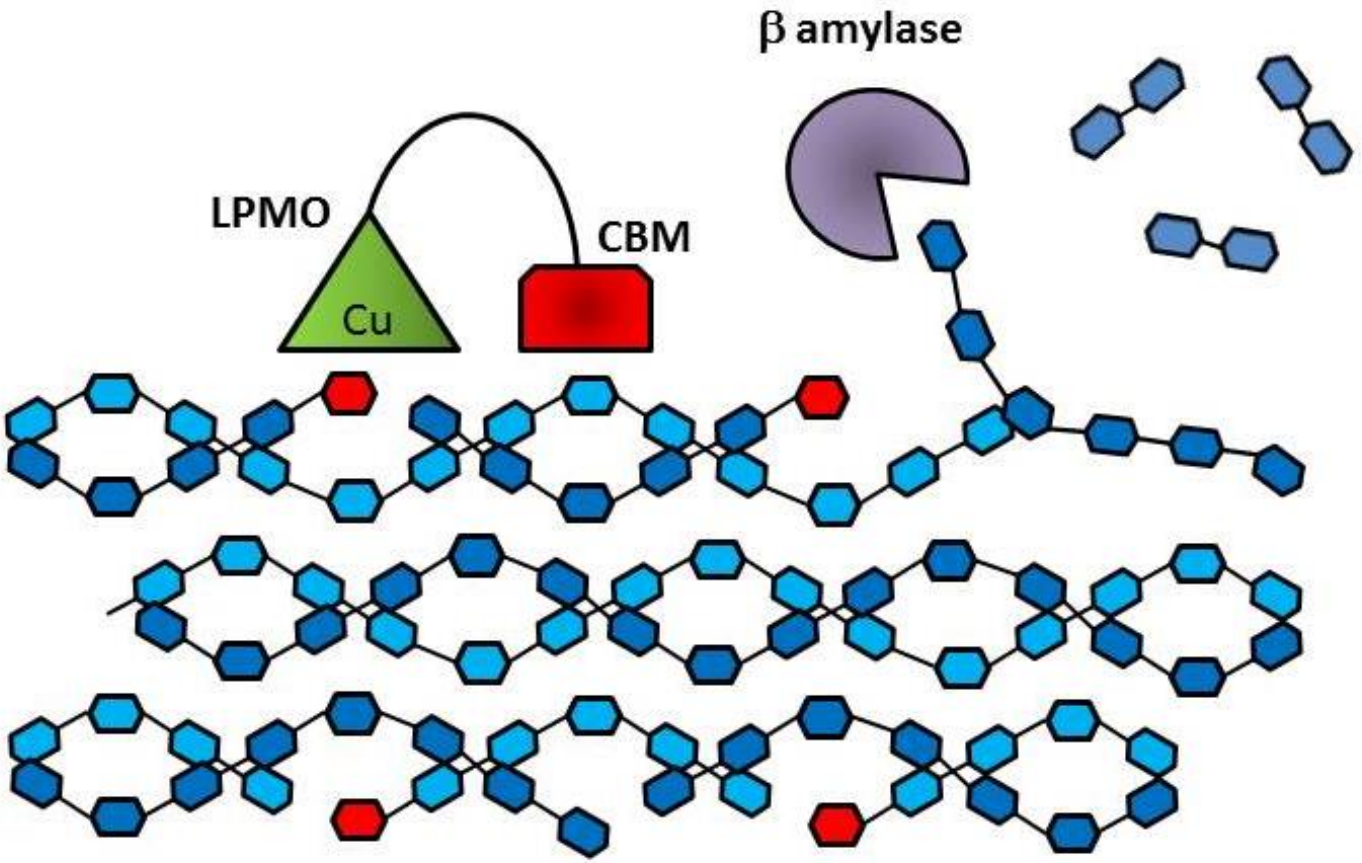
An(AA13) – CBM20



Vu *et al*, *Proc. Nat. Acad. Sci*. **2014**, *111(38)*, 13822

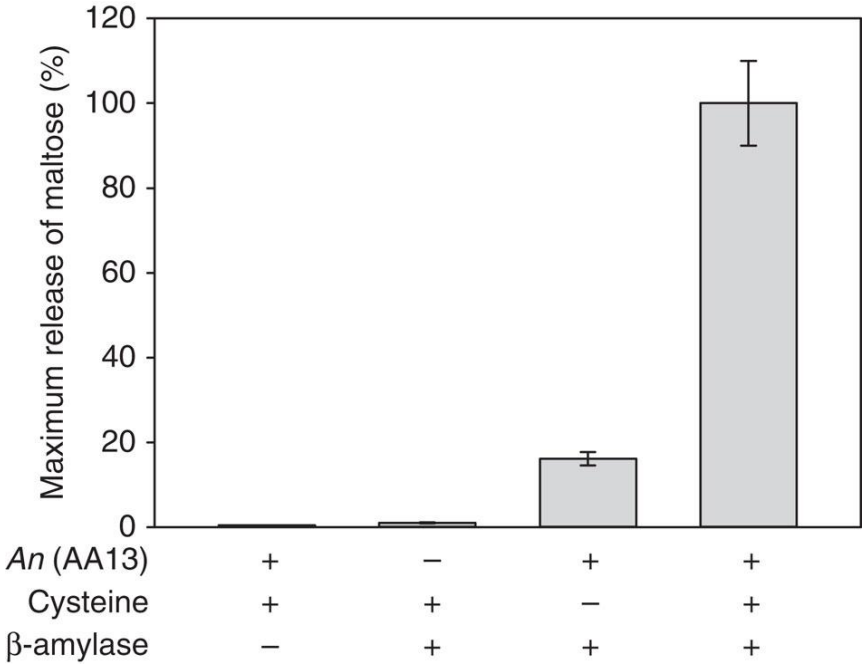
No activity on PASC, chitin, polygalacturonan, pectin, Arabidopsis stem walls

Boosting of β amylase activity



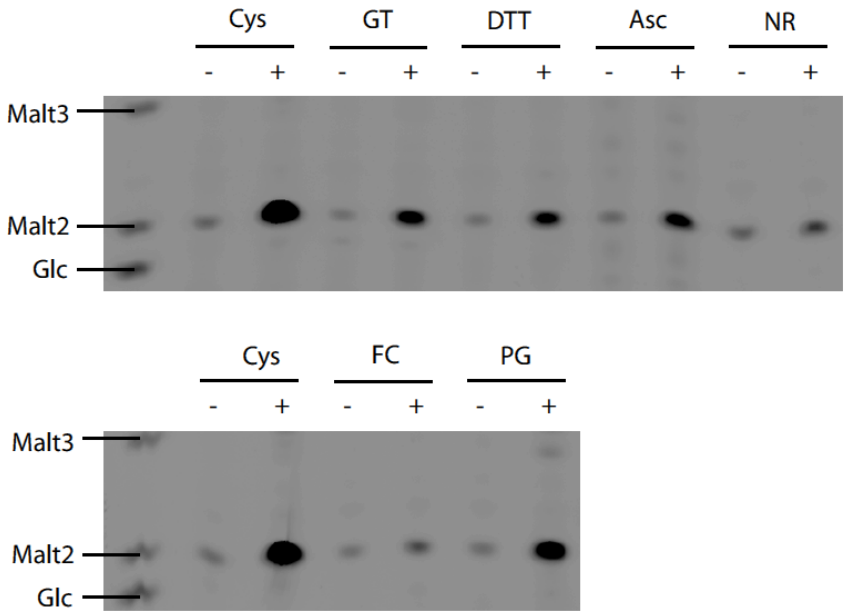
Boosting of β amylase activity

Release of maltose



4 h, 25 °C,
2.5% of total starch release

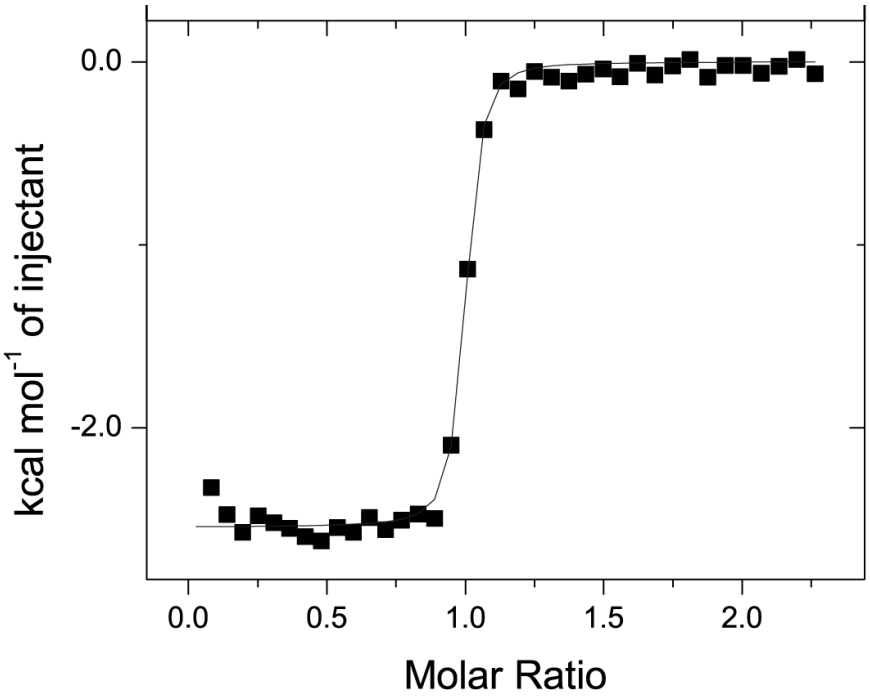
PACE gel reducing agent assay



+/-, with and without AnAA12; Cys, cysteine; GT, glutathione; DTT, dithiothreitol; Asc, ascorbate; NR, no reductant; FC, ferrocyanide; PG, pyrogallol

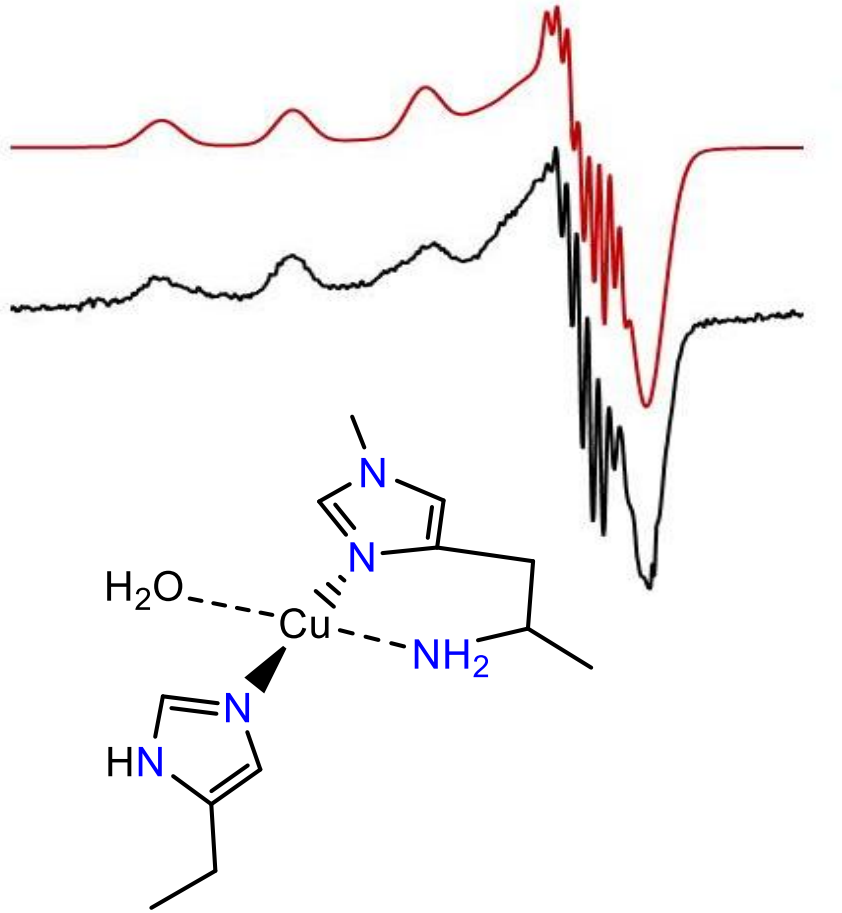
Physico chemical properties of An(AA13)–CBM20 and Ao(AA13)

ITC of An(AA13) -- CBM20 with Cu^{2+}

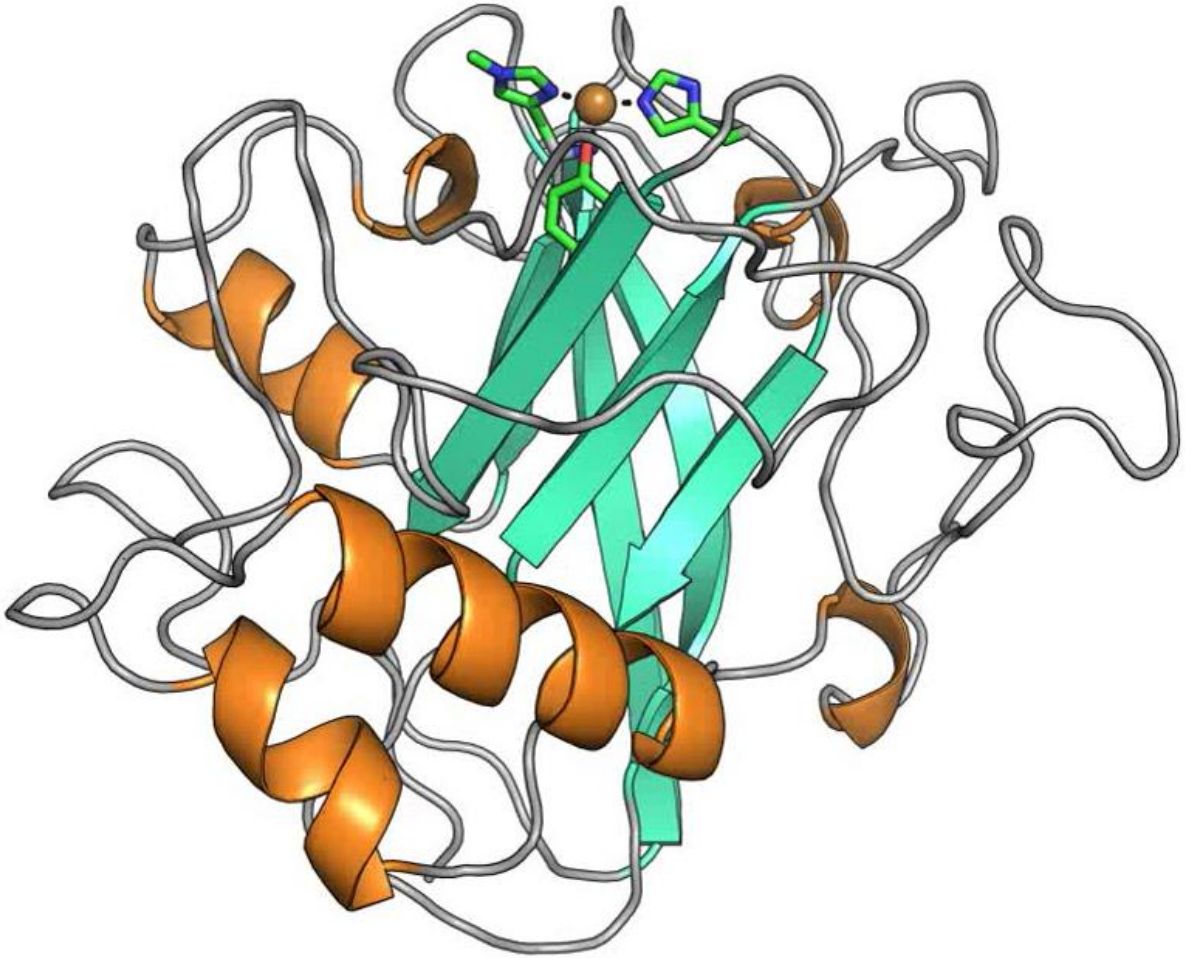


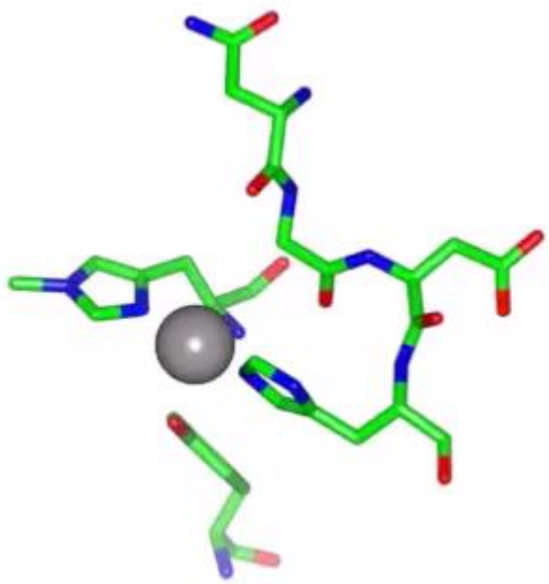
Copper binding is tight, ~ 20 nM for both

CW X-band EPR (150 K)



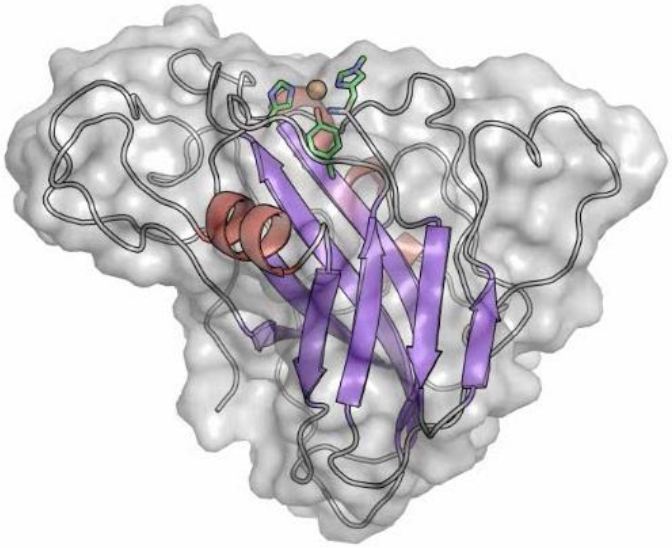
Structure of *Ao*(AA13)



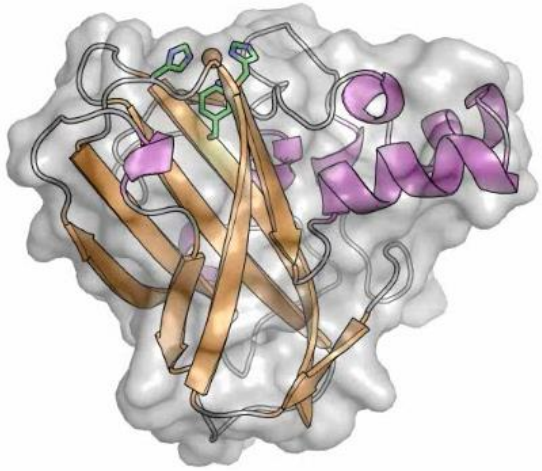


Lo Leggio et al
1.5 Å resolution
R(free) 17%
PDB, 4OPB

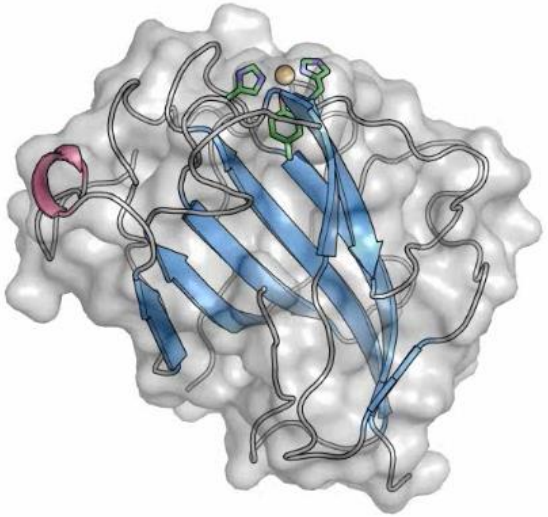
AA9



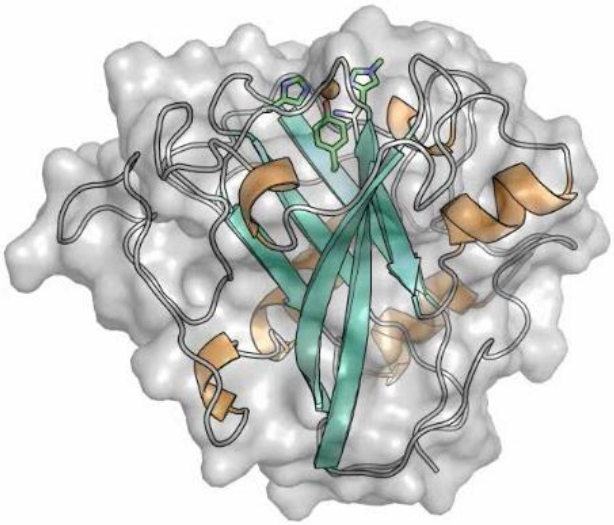
AA10



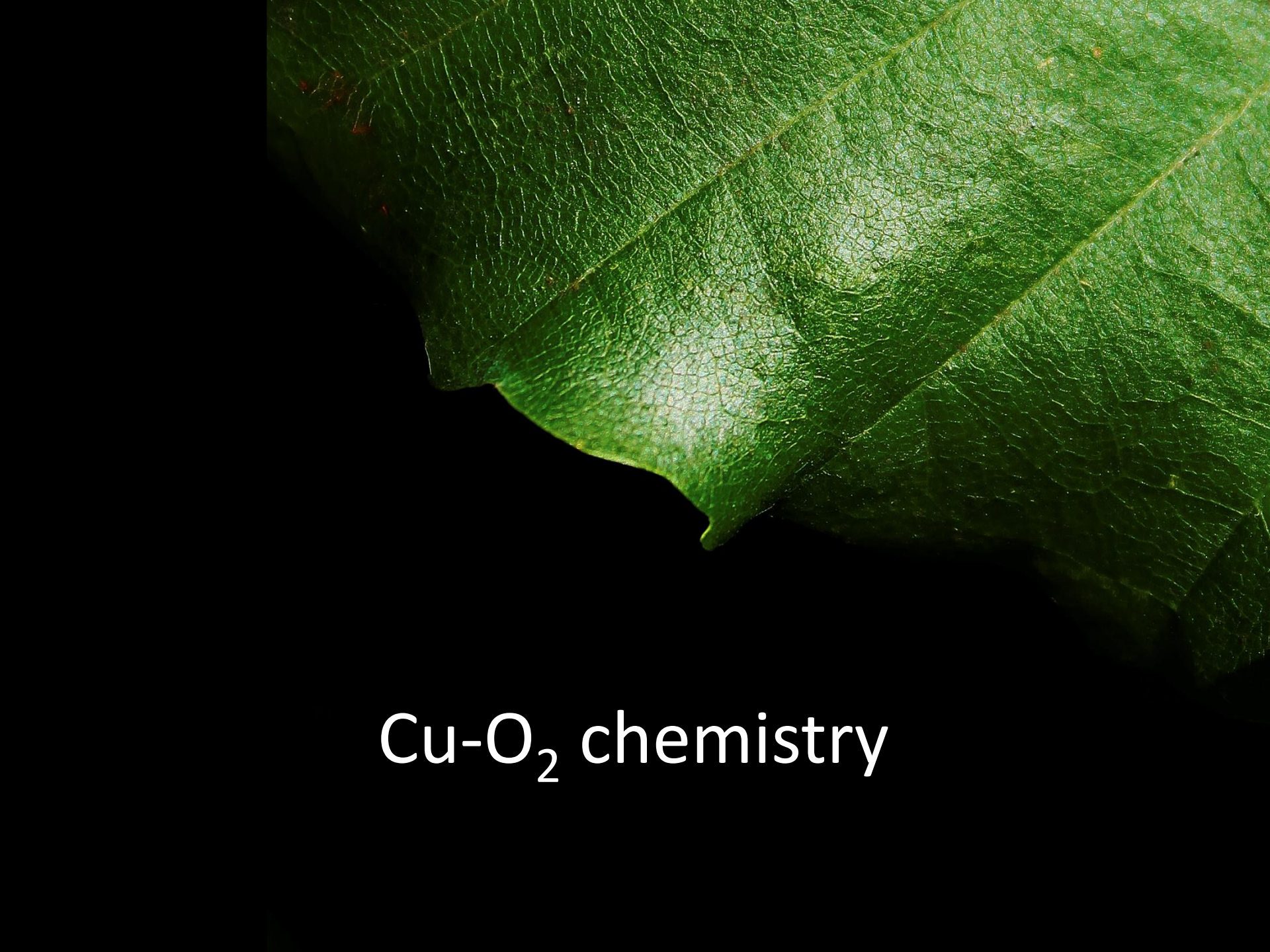
AA11



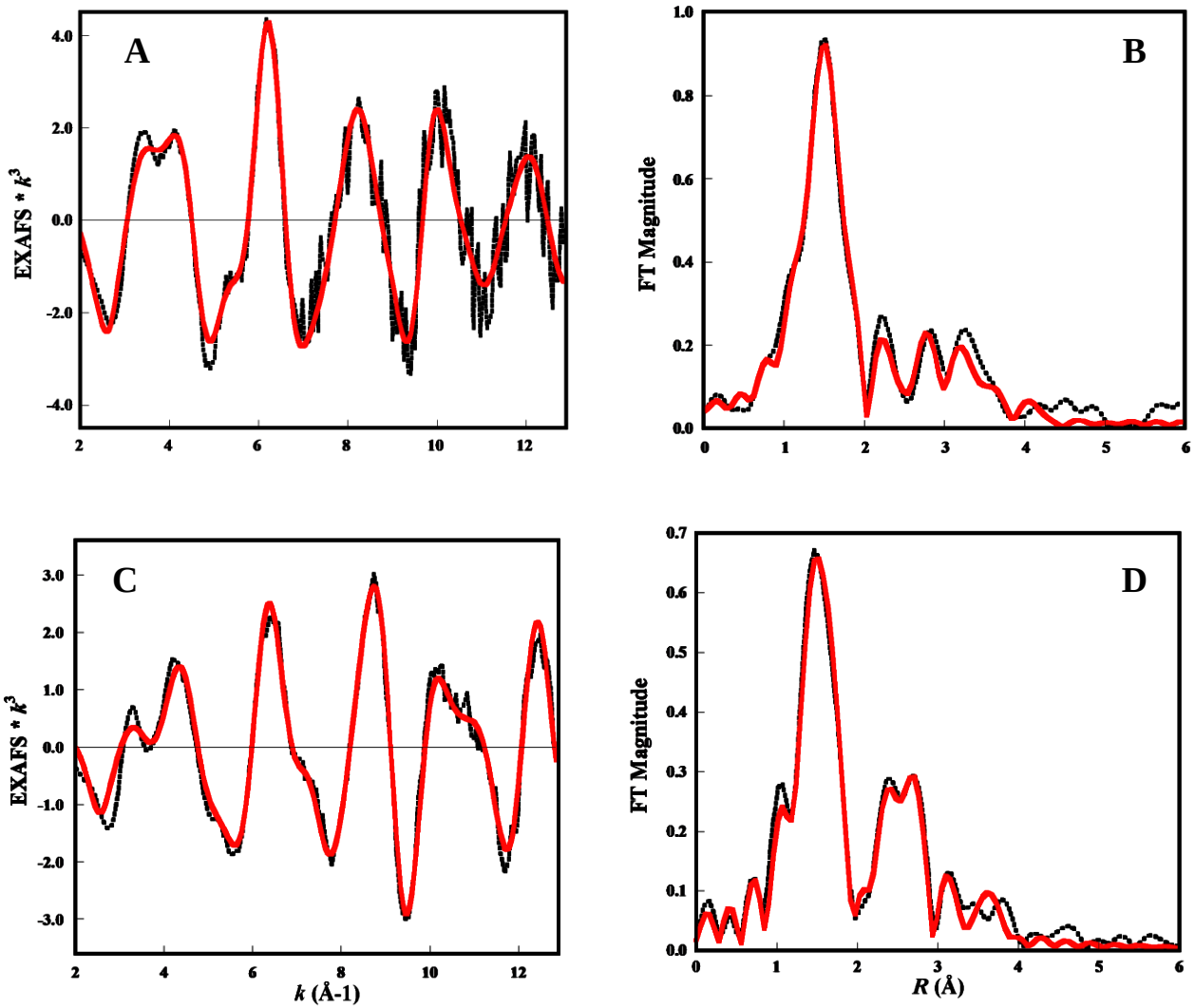
AA13



3zud, 2yoy, 4mai, 4opb


Cu-O_2 chemistry

EXAFS on Cu-AA9 (ex substrate)

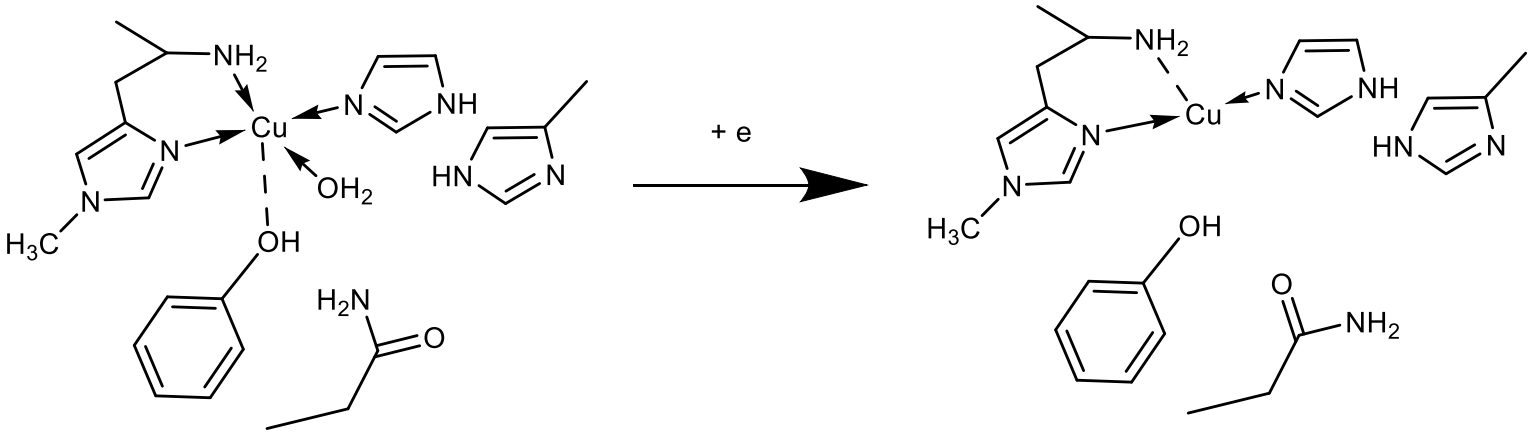


Cu(II)

Cu(I)

C Kjaergaard, Solomon, Davies, Walton et al *Proc. Nat. Acad. Sci.* **2014**, 8797

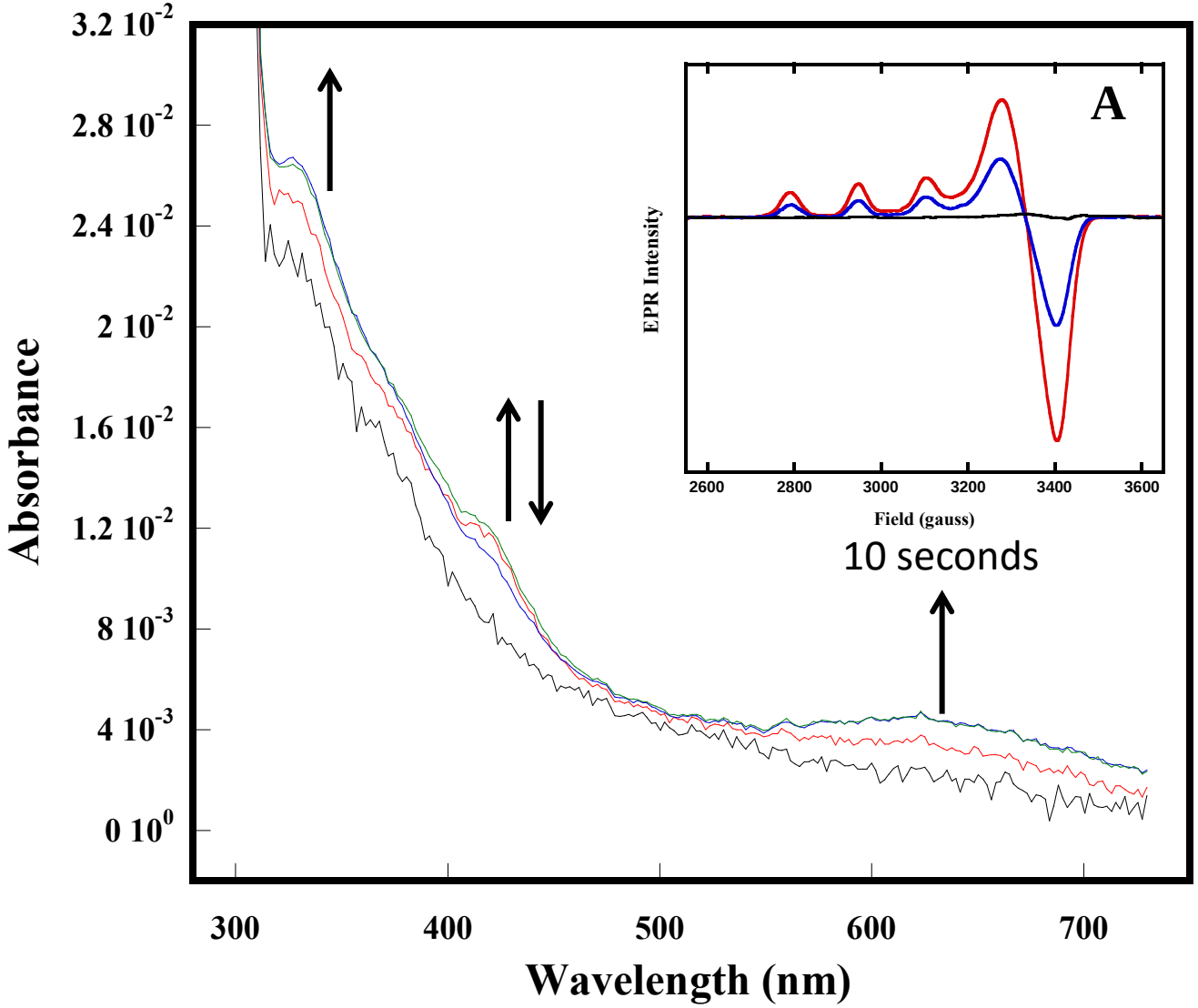
Reduction of Cu(II) to Cu(I)



Kjaergaard, Solomon, Davies, Walton et al *Proc. Nat. Acad. Sci.* **2014**, 8797.

See also Wu et al, *J. Biol. Chem.* **2013**, *288*, 1282 for Cu(I)-AA9 structure

Reaction of O_2 with Cu(I)-AA9, pH 5

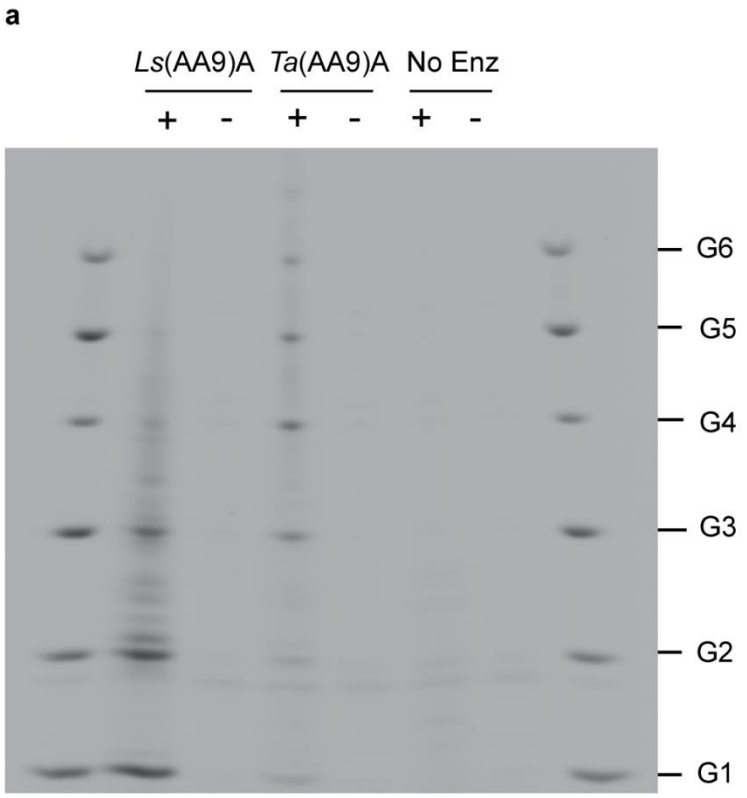


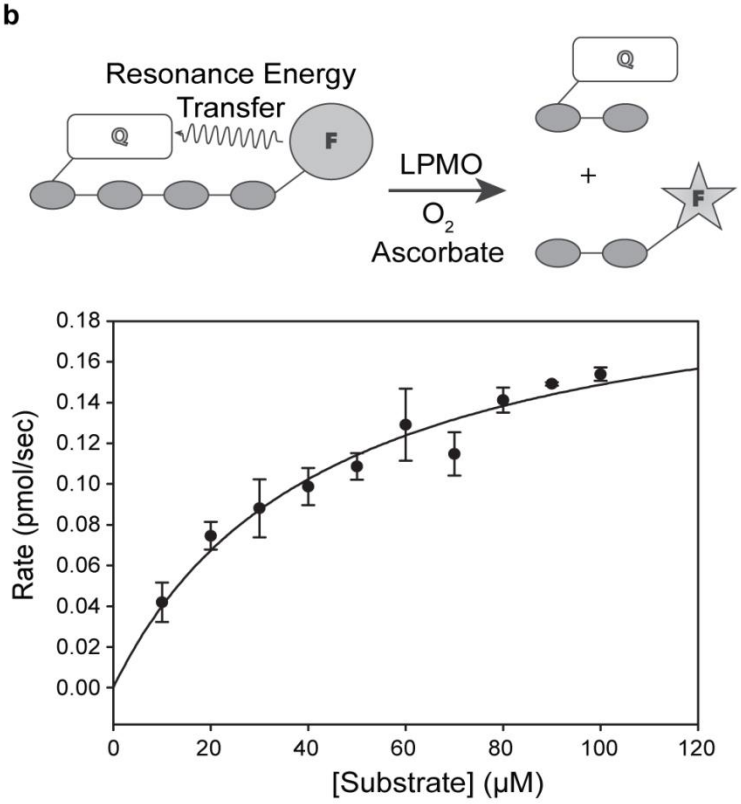
Reformation of Cu(II)—AA9

0.15 s^{-1}

C Kjaergaard, Solomon, Davies, Walton et al *Proc. Nat. Acad. Sci.* **2014**, 8797.

Structure with substrate: new LPMO activity on oligosaccharides

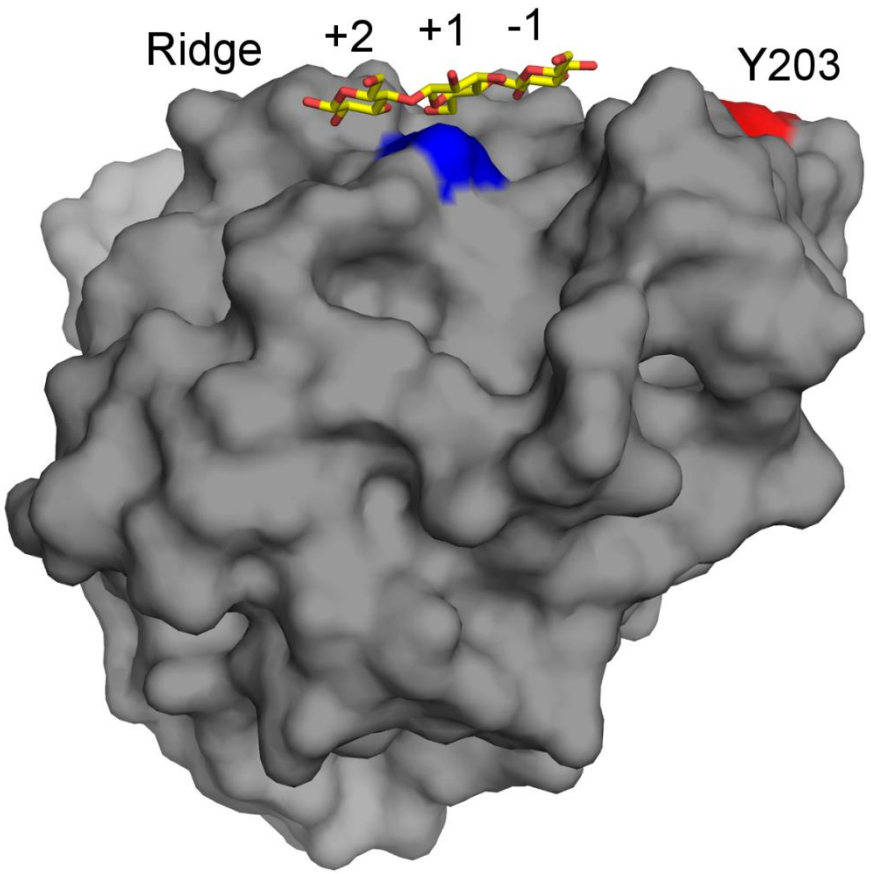


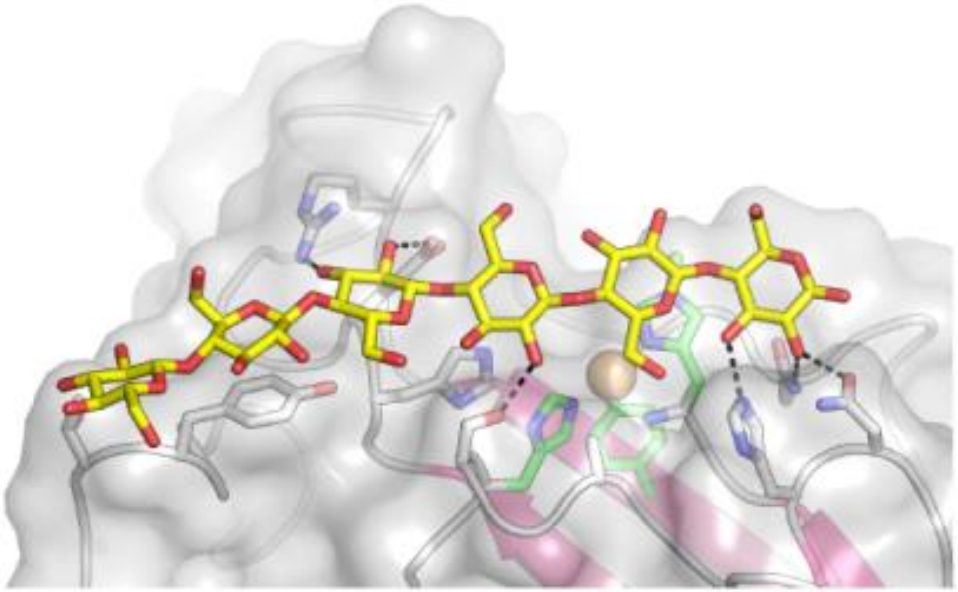


K_m = 43(9) μM, k_{cat} = 0.11(1) s^{-1}
300 K, pH 7.0

Lo Leggio,…Walton et al *Nature Chem. Biol.* **2016**.

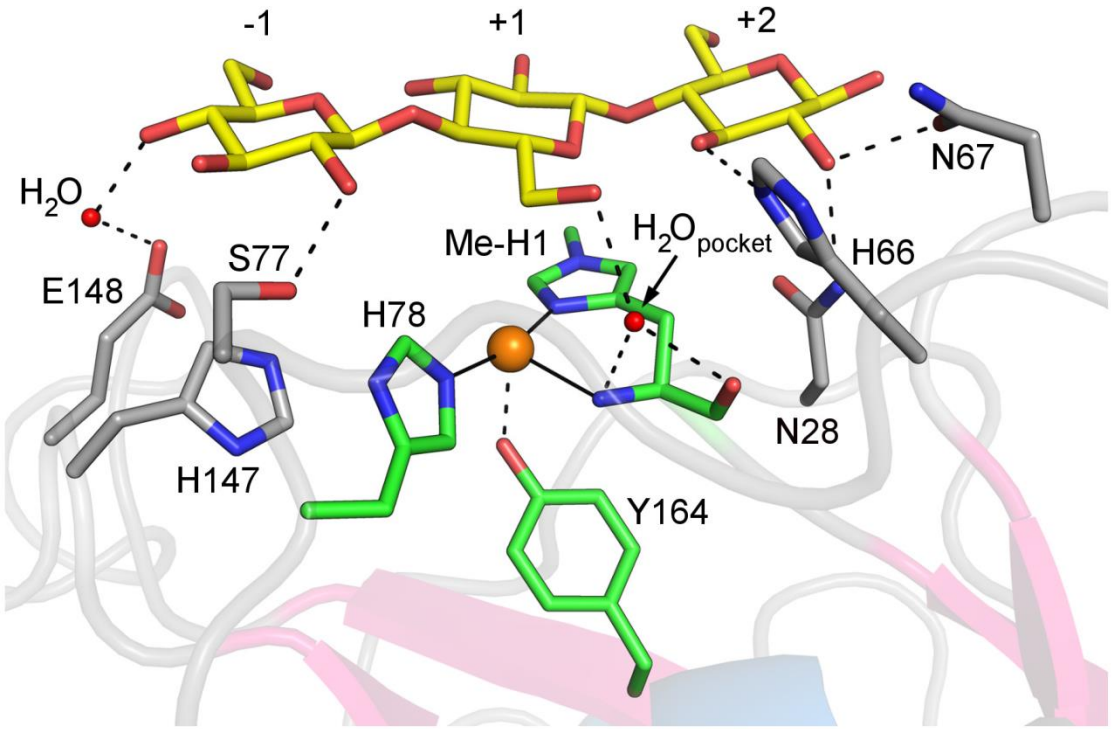
Structure with substrate

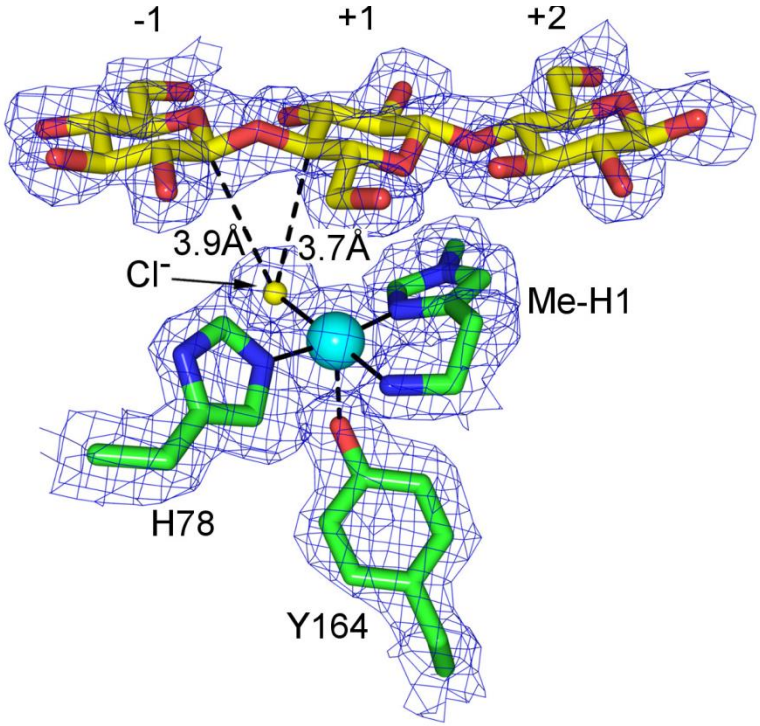




Lo Leggio,…Walton et al *Nature Chem. Biol.* **2016**.

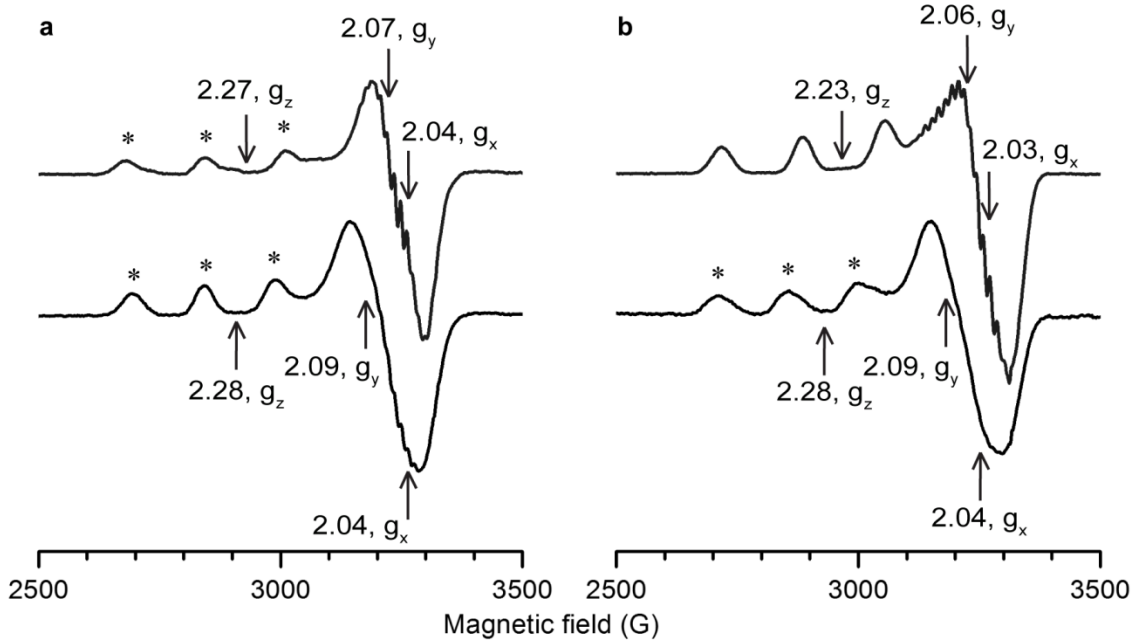
Structure with substrate

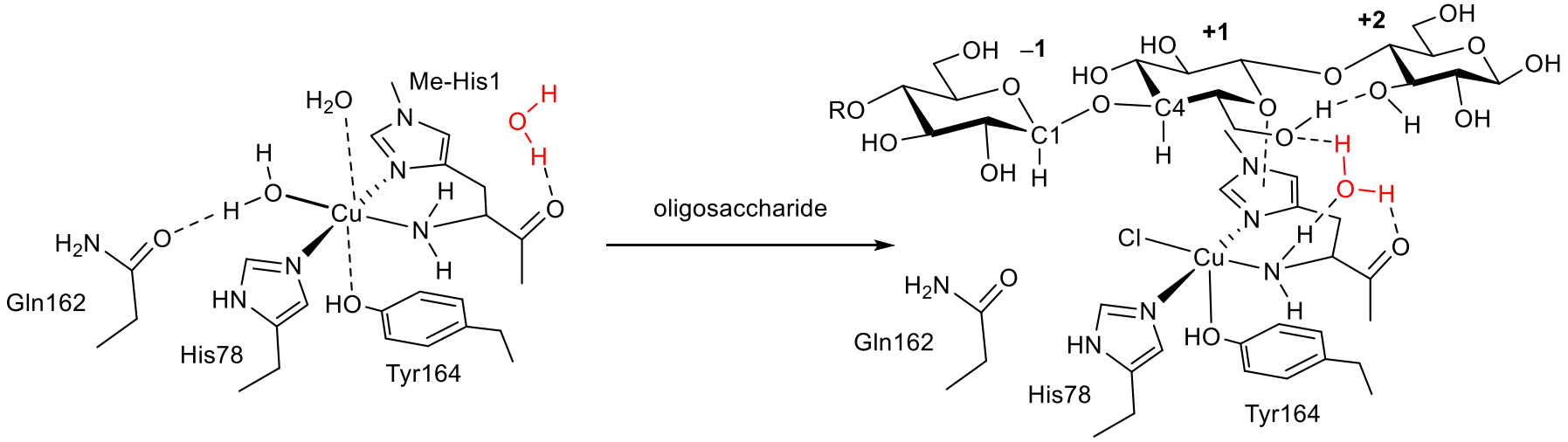




Lo Leggio,…Walton et al *Nature Chem. Biol.* **2016**.

EPR spectroscopy



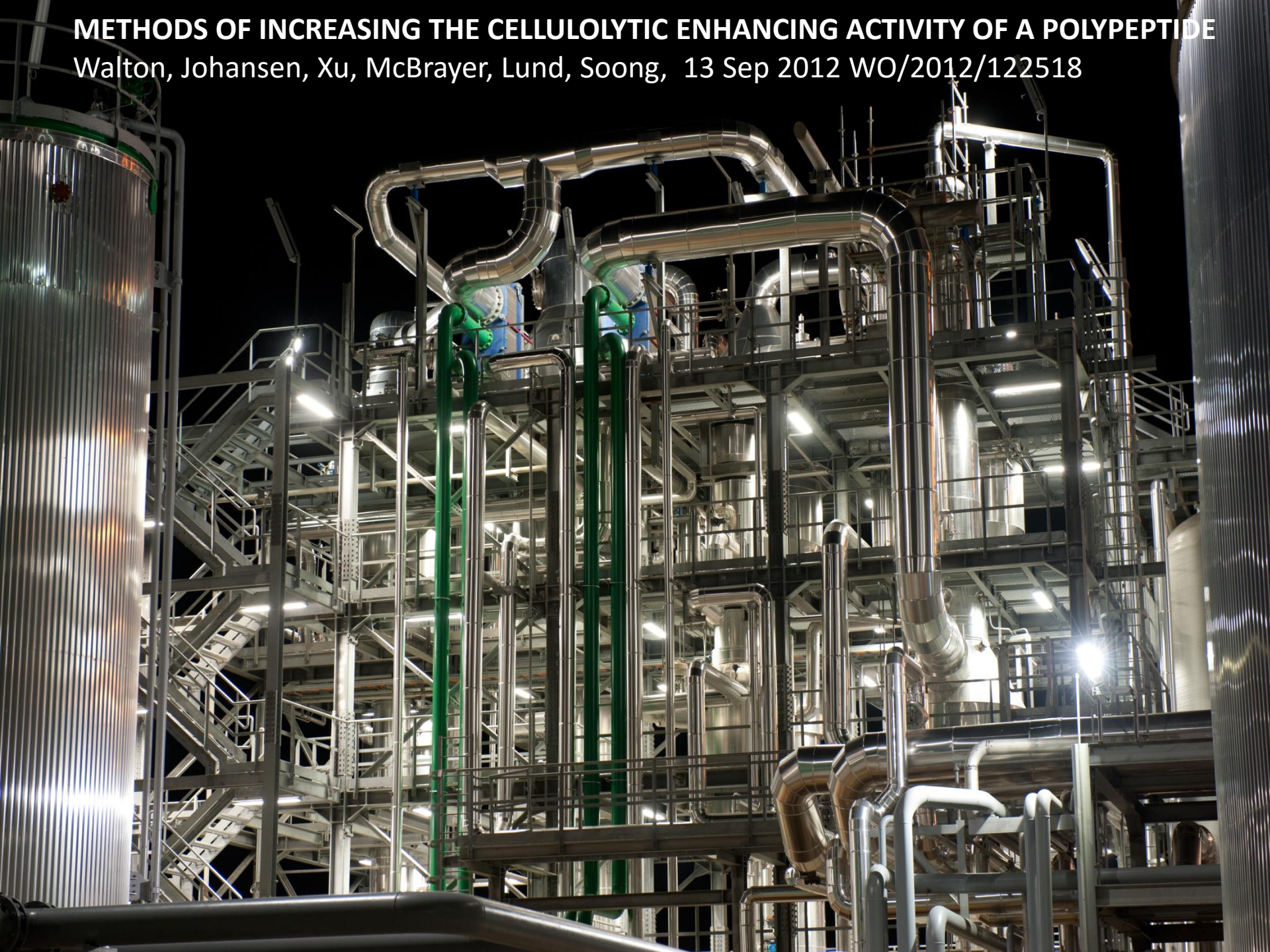


Lo Leggio,…Walton et al *Nature Chem. Biol.* **2016**.

Cellulosic bioethanol plant,
Crescentino Italy

METHODS OF INCREASING THE CELLULOLYTIC ENHANCING ACTIVITY OF A POLYPEPTIDE

Walton, Johansen, Xu, McBrayer, Lund, Soong, 13 Sep 2012 WO/2012/122518




MERLO P40.16 KS
TORINOLEGGI

Summary



- *Proposal: discovery, characterisation and use of new LPMOs*
- *New LPMO classes: AA11 and AA13*

  Nature Chem. Biol. **2014**, 122.

  Nature Commun. **2015**, #5961
- *New mechanistic insights*

  Proc. Nat. Acad. Sci., **2014**, 8797.

  Nature Chem. Biol. **2016**, in press
- *New entries on CAZy database.*
- *More understanding of biomass degradation by LPMOs*

General Evaluation



- *Collaboration has allowed us to compete on an international stage. Unique combination of genomics, biochemistry, molecular biology, spectroscopy*

Publications:

Nature Chem. Biol. **2014**, 122.

Nature Commun. **2015**, #5961

Proc. Nat. Acad. Sci., **2014**, 8797.

Nature Chem. Biol. **2016**.

Trends in Biotechnology, **2015**, 747 (review).

- *Feedback to ERA-IB:* more funding?

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