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**Supporting information for article:**

**The *BAM7* gene in *Zea mays* encodes a protein with structural and catalytic properties similar to *Arabidopsis* BAM2**

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**Table S1** Species and accession numbers for sequences used in Figure 1A-D and Supplementary Figure S2.

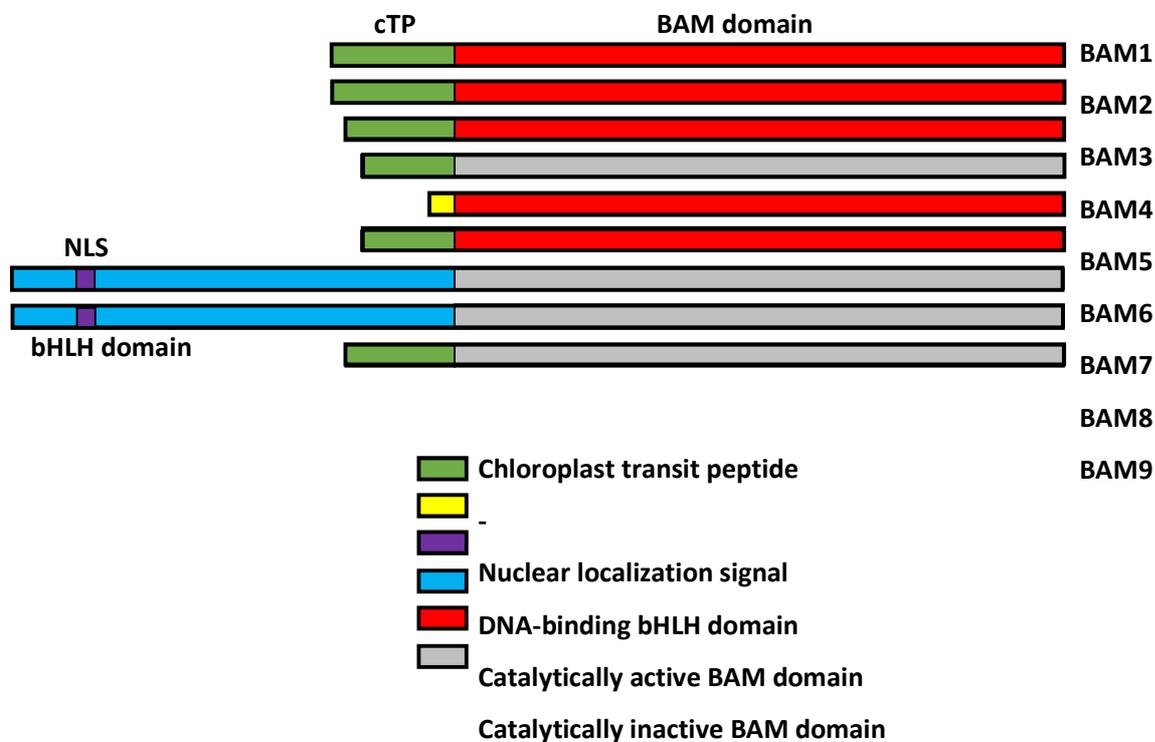
<b>Species</b>	<b>BAM2</b>	<b>BAM7</b>	<b>BAM1</b>
<i>Beta vulgaris</i>	XP_010670423.1	XP_010670436.1	XP_010666969.1
<i>Camellia sinensis</i>	XP_028124977.1	XP_028124979.1	XP_010488521.1
<i>Sesamum indicum</i>	XP_011092228.1	XP_011093139.1	XP_011091372.1
<i>Coffea arabica</i>	XP_027115181.1	XP_027115178.1	XP_027118940.1
<i>Helianthus annuus</i>	XP_022035754.1	XP_022035752.1	XP_022026724.1
<i>Daucus carota</i>	XP_017219681.1	XP_017218898.1	XP_017219233.1
<i>Ricinus communis</i>	XP_002511858.1	XP_002511857.1	XP_002518196.1
<i>Fragaria vesca</i>	XP_004306786.1	XP_011469023.1	XP_004296549.1
<i>Quercus lobata</i>	XP_030932199.1	XP_030929350.1	XP_030974613.1
<i>Citrus clementina</i>	XP_006445046.1	XP_006445048.2	XP_006420416.1
<i>Arabidopsis thaliana</i>	NP_191958.3	NP_182112.2	XP_010666969.1
<i>Theobroma cacao</i>	XP_017981267.1	XP_007051814.1	XP_007035340.2
<i>Eucalyptus grandis</i>	XP_010055131.2	XP_010055132.2	XP_010023784.2
<i>Vitis vinifera</i>	XP_002274612.2	XP_002273843.1	XP_002285569.1

<b>Species</b>	<b>DF-BAM7</b>
<i>Amborella trichopoda</i>	XP_006827627.2
<i>Nymphaea colorata</i>	XP_031483343.1
<i>Zea mays</i>	NP_001337631.1
<i>Sorghum bicolor</i>	XP_002459849.2
<i>Panicum hallii</i>	XP_025804467.1
<i>Setaria italica</i>	XP_012699441.2
<i>Aegilops tauschii</i>	XP_020176205.1
<i>Brachypodium distachyon</i>	XP_003576871.1
<i>Oryza sativa</i>	XP_015611670.2
<i>Macleaya cordata</i>	OVA14036.1
<i>Aquilegia coerulea</i>	PIA49404.1
<i>Nelumbo nucifera</i>	XP_010255372.1

**Table S2** Functionally important residues in AtBAM8 and AtBAM2 as determined by mutagenesis. Both AtBAM7 and -8 are nuclear localized and have the same putative NLS sequence (Reinhold et al., 2011). Only BAM8 was mutated to test function of the NLS.

Protein	Mutation	General location, and Rationale	Phenotype	Citation
AtBAM8	66-67RRQQ	b-HLH domain, Removal of positive charges to test bipartite NLS function	Partial disruption of nuclear localization	Reinhold et al., 2011
AtBAM8	84-87RRRRQQQQ	b-HLH domain, Removal of positive charge to test bipartite NLS function	Disruption of nuclear localization	Reinhold et al., 2011
AtBAM8	66-67RRQQ and 84-87RRRRQQQQ	b-HLH domain, Removal of positive charge to test bipartite NLS function	Disruption of nuclear localization	Reinhold et al., 2011
AtBAM2	W456A	Tetramer interface A, Disrupt tetramer structure and determine effect on activity	Tetramer→Dimer Catalytic activity ~2% of WT	Monroe et al., 2018
AtBAM2	D490R	Tetramer interface A, Disrupt tetramer structure and determine effect on activity	Tetramer→Monomer Catalytic activity ~5% of WT	Monroe et al., 2018
AtBAM2	F238A	Tetramer interface B, Disrupt tetramer structure and determine effect on activity	Tetramer→Dimer Catalytically active	Monroe et al., 2018

AtBAM2	S464G	Adjacent to catalytic Glu465 with side chain facing the SBS, Ser is replaced with Gly in all other monomeric BAMs	Lowers $K_M$ for the substrate by ~10 fold	Monroe et al., 2018
AtBAM2	G335M	SBS, top of groove, Replace Gly with bulky Met to disrupt starch binding in the groove	Catalytic activity is ~5% of WT	Monroe et al., 2017
AtBAM2	G446M	SBS, middle of groove, Replace Gly with bulky Met to disrupt starch binding in the groove	Catalytic activity is ~30% of WT	Monroe et al., 2017
AtBAM2	W449A	SBS, middle of groove between subunits, Remove putative sugar-binding sidechain	Catalytic activity is ~7% of WT	Monroe et al., 2018

*Arabidopsis thaliana*  $\beta$ -amylase proteins

**Figure S1** Maps of the nine BAM proteins in *Arabidopsis* illustrating the positions of the BAM domain that is either catalytically active in BAM1, -2, -3, -5 and -6 (red) or catalytically inactive in BAM4, -7, -8, and -9 (grey), and the bHLH domains in BAM7 and -8 (blue). Nuclear localization signals (purple) and chloroplast transit peptides (green) are also shown. For citations see Monroe and Storm (2020).

## &gt;AtBAM2

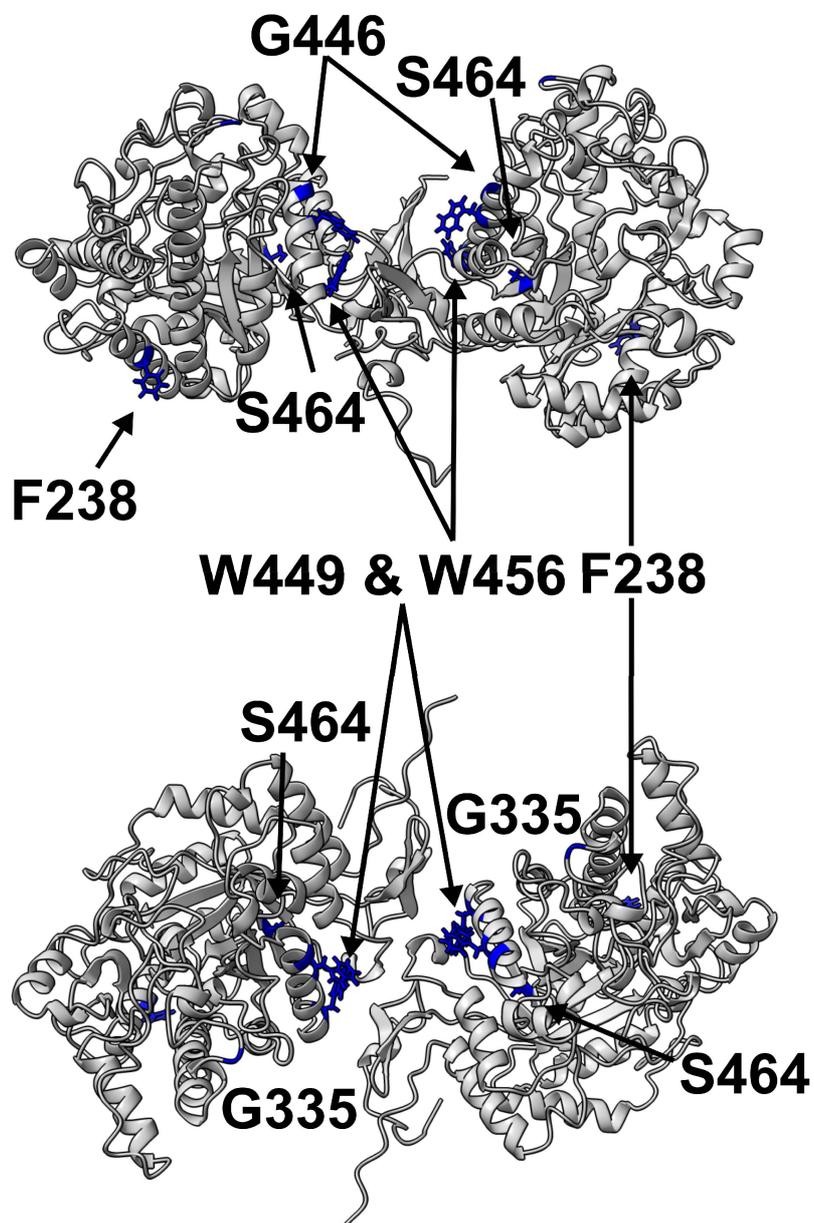
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## &gt;ZmBAM7-S

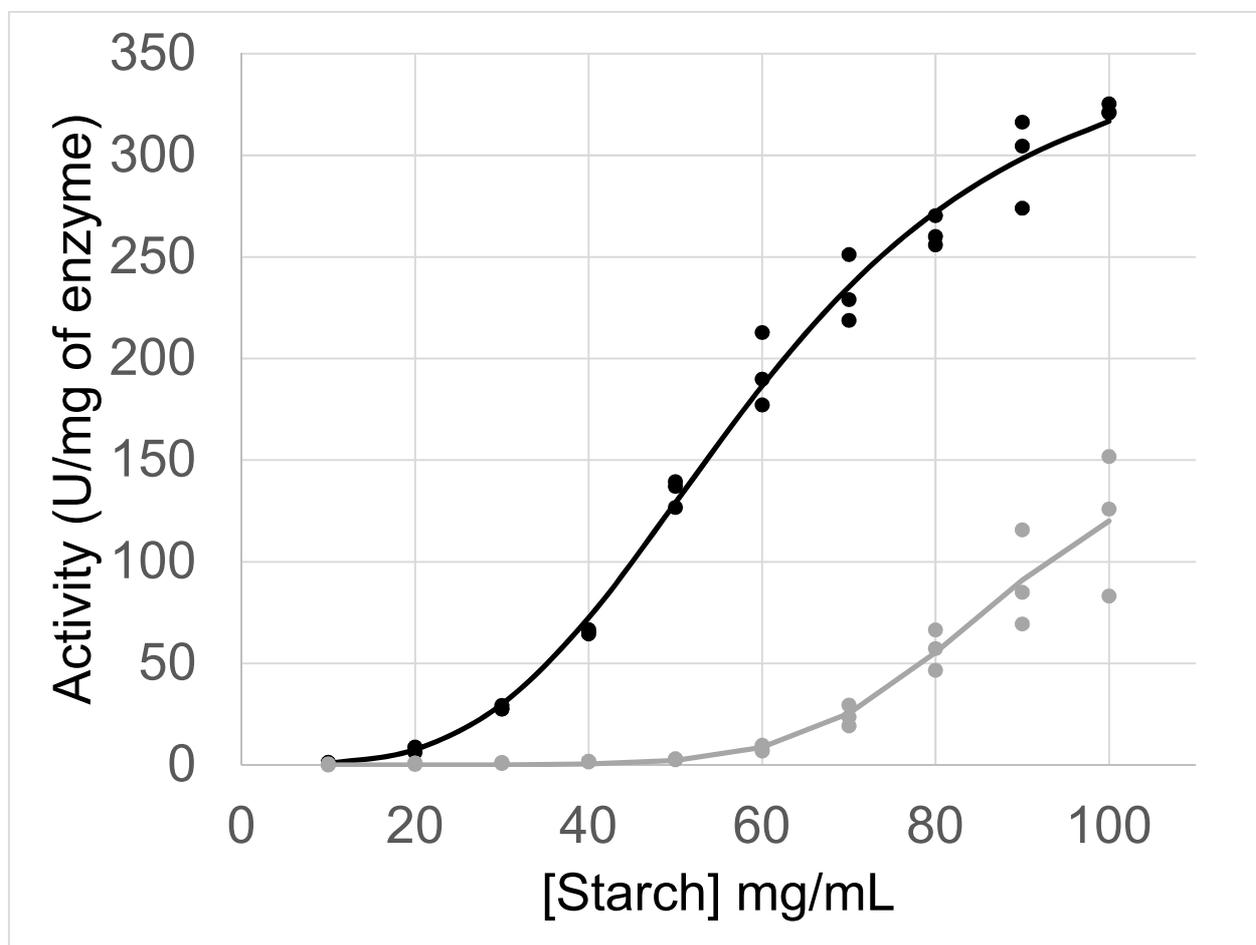
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CTTCTTTGAGTTCGAACGTTTTATCAAACGTATGCACGGTGAGGCGGTGCTGGATCTGCAA  
GTTTAA

**Figure S2** Sequences of AtBAM2 and ZmBAM7-S synthesized by GenScript and cloned into pET15a for expression in *E. coli*.



**Figure S3** Structure of an Arabidopsis BAM2 dimer rendered with ChimeraX showing residues that appear to be unique to BAM2-like proteins for tetramer formation and sigmoidal kinetic curves (Monroe, 2017, 2018; Pettersen, 2021).



**Figure S4** Effect of substrate concentration on ZmBAM7-S (grey) and AtBAM2 (black) activity on a per mg protein basis. Points are shown for each of three replicate assays. Data were fitted to the Michaelis-Menten equation for cooperative enzymes. For AtBAM2 the  $V_{\max}$  was calculated to be 367 U/mg of enzyme (95% C.I. 353 – 381 U/mg), the  $K_M$  was 59.4 mg/mL (95% C.I. 58 – 61 mg/mL), and the  $n$  value was 3.5 (95% C.I. 3.2 – 3.9). For ZmBAM7-S the  $V_{\max}$  was calculated to be 160.7 U/mg of enzyme (95% C.I. 137 – 185 U/mg), the  $K_M$  was 86.9 mg/mL (95% C.I. 83 – 91 mg/mL), and the  $n$  value was 7.7 (95% C.I. 5.2 – 11.6). Because we were unable to saturate ZmBAM7-S with substrate, these kinetic values, particularly the  $n$  value, are estimates.

Monroe, J. D., Breault, J. S., Pope, L. E., Torres, C. E., Gebrejesus, T. B., Berndsen, C. E. & Storm, A. R. (2017). *Plant Physiol.* **175**, 1525–1535.

Monroe, J. D., Pope, L. E., Breault, J. S., Berndsen, C. E. & Storm, A. R. (2018). *Front. Plant Sci.* **9**, 1176.

Monroe, J. D. & Storm, A. R. (2018). *Plant Sci.* **276**, 163–170.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., Morris, J. H. & Ferrin, T. E. (2021). *Protein Sci.* **30**, 70–82.