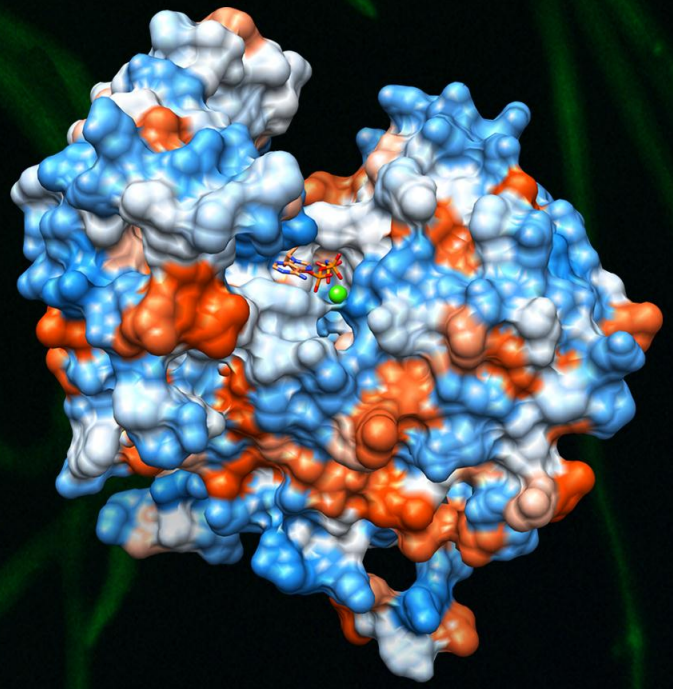
PAG XX
Engineering NUE Workshop
W248
January 14, 2012

Role of Soybean Ecto-apyrase in Nodulation



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(Gary Stacey Lab)

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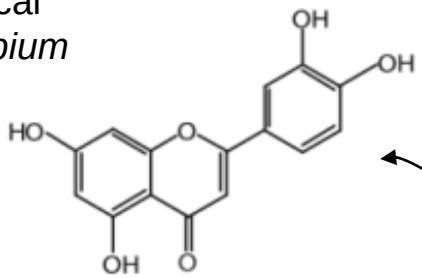
University of Missouri

Legume-*Rhizobium* symbiotic interaction

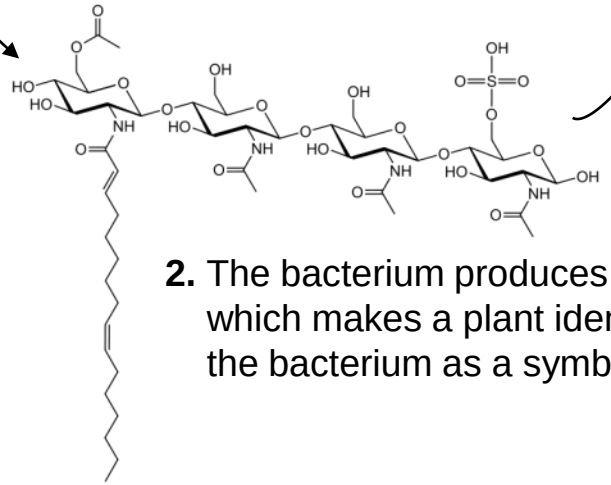
1. The plant root produces a flavonoid chemical that attracts *rhizobium*

3. The plant prepares to form a symbiotic nodule structure

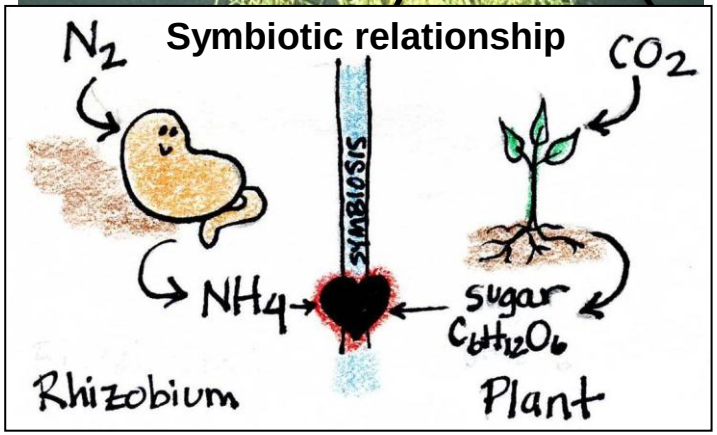


Rhizobium

Legume

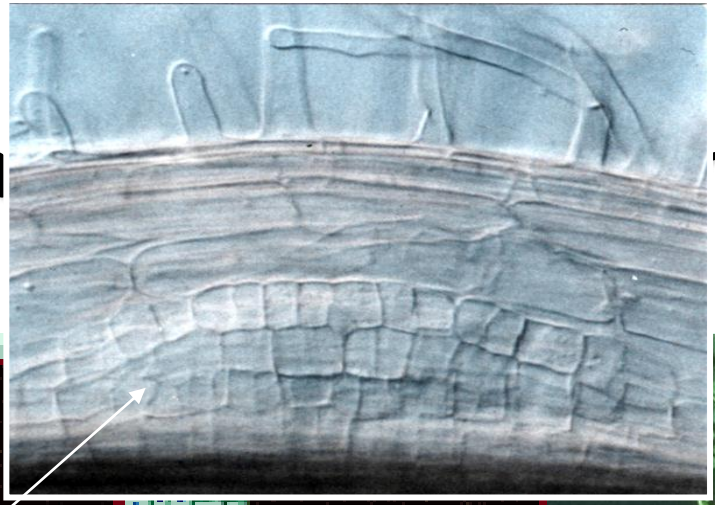


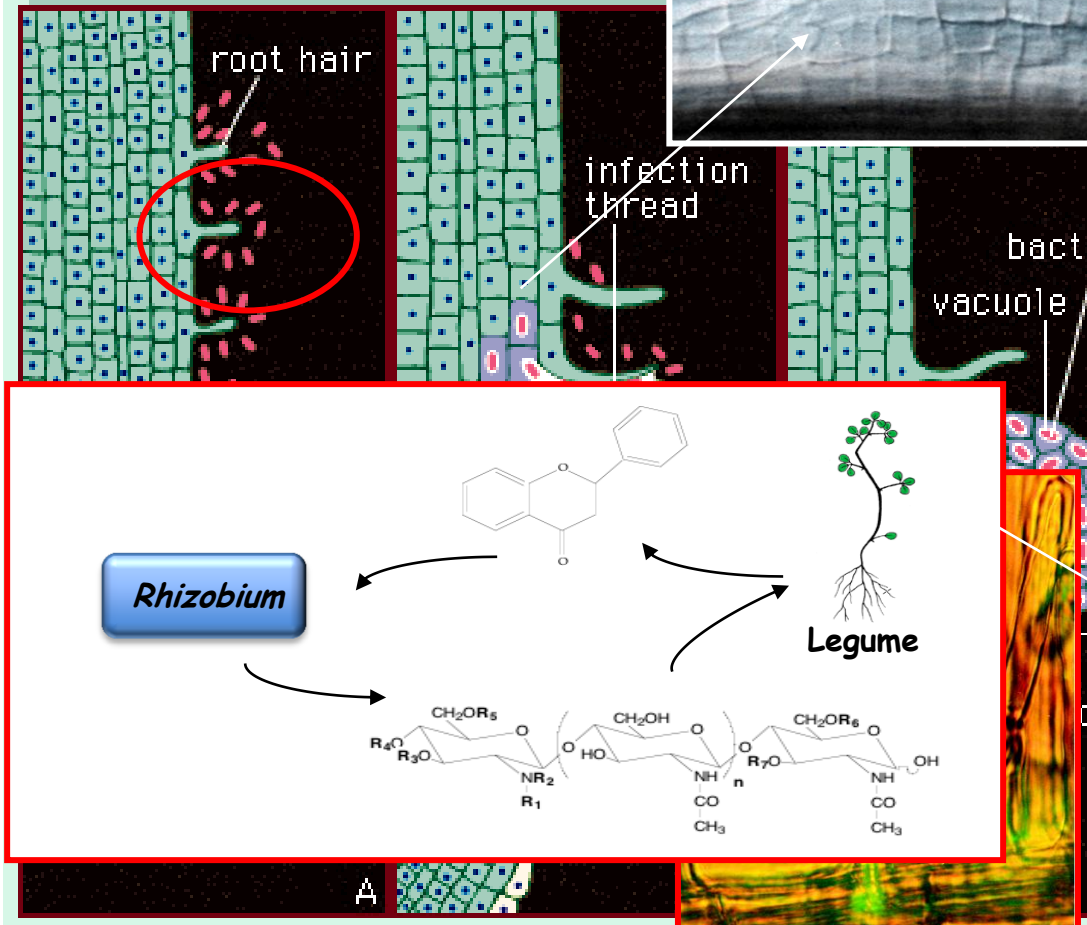
2. The bacterium produces a Nod factor, which makes a plant identify the bacterium as a symbiont

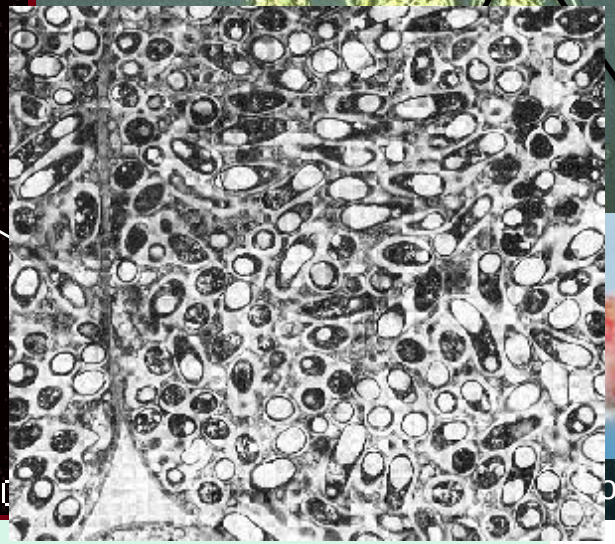


Illustrated by **Amanda K. Broz**
in Nodulation: A Love Story (2008)

Developm nodule







How is ecto-apyrase involved in the nodulation process?

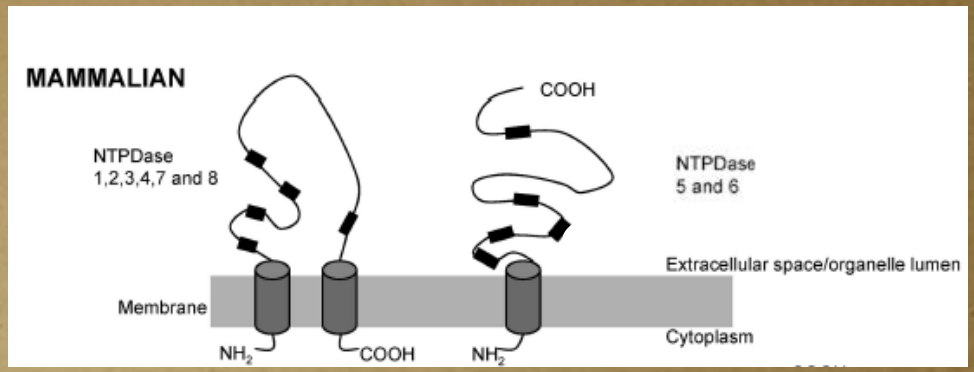
$N_2 \xrightarrow{\text{nitrogenase}} NH_3$

What is ecto-apyrase?

= ecto-NTPDase

(ecto-nucleoside triphosphate diphosphohydrolases)

Zimmermann (2001) Drug Dev. Res. 52: 44-56



*calcium-activated plasma membrane-bound enzyme

*Catalytic domain is exposed on the cell surface

*catalyses the hydrolysis of ATP to yield AMP and orthophosphates

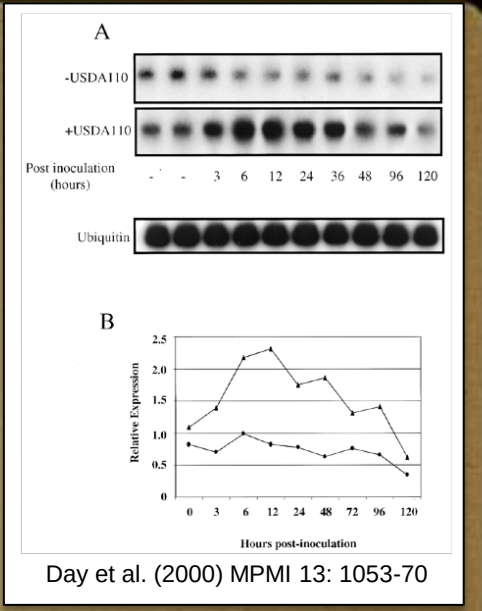
*also acts on ADP and other nucleoside triphosphates and diphosphates

NTP -> NDP + Pi

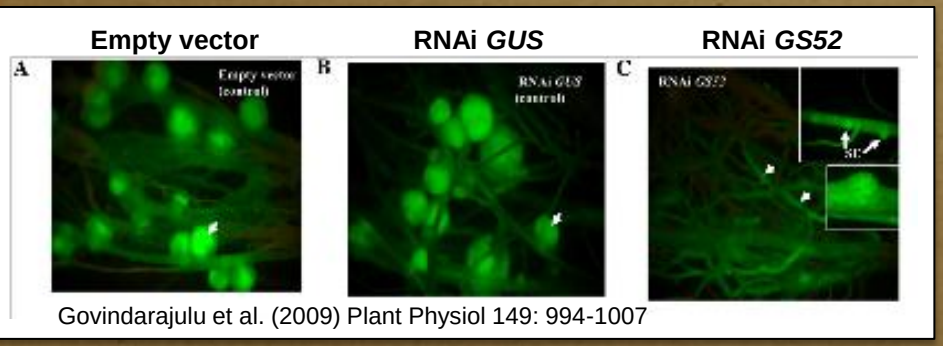
NDP -> NMP + Pi

Soybean ecto-apyrase GS52

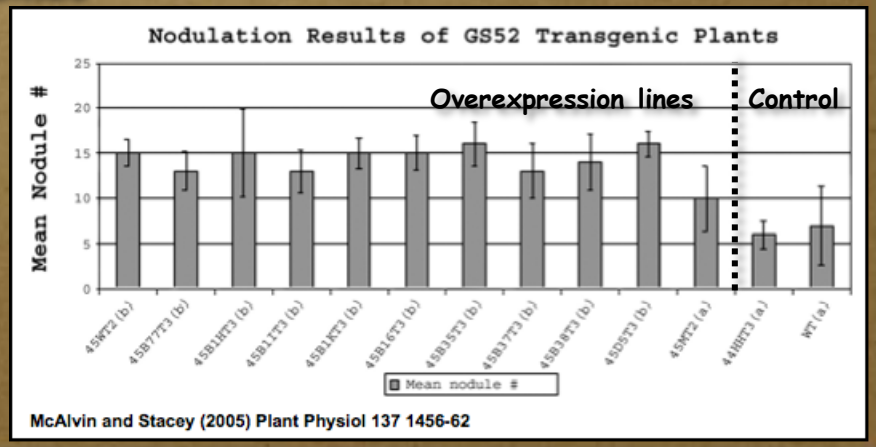
* Reported as an early nodulin gene
* 52 kDa protein from soybean (*Glycine soja*)
* Plasma membrane localization
* Upregulated upon infection by *Bradyrhizobium japonicum* (also during later stages of nodulation)
* Overexpression of GS52 in *Lotus japonicus*
  - increased numbers of nodules (infected by *Mesorhizobium loti*)
  - increased infection thread formation
* Anti-GS52 antibody inhibits nodule formation
* RNAi-mediated gene silencing of GS52 in soybean (*Glycine max*)
  - reduced numbers of mature nodules
  - devoid of bacteroid-containing symbiosomes



Day et al. (2000) MPMI 13: 1053-70



Govindarajulu et al. (2009) Plant Physiol 149: 994-1007



McAlvin and Stacey (2005) Plant Physiol 137 1456-62

Question:

Is catalytic activity of the GS52 ecto-apyrase required for stimulation of nodulation?

Q: What is Biochemical characteristics of GS52?

-> Characterize enzymatic properties of the GS52 protein

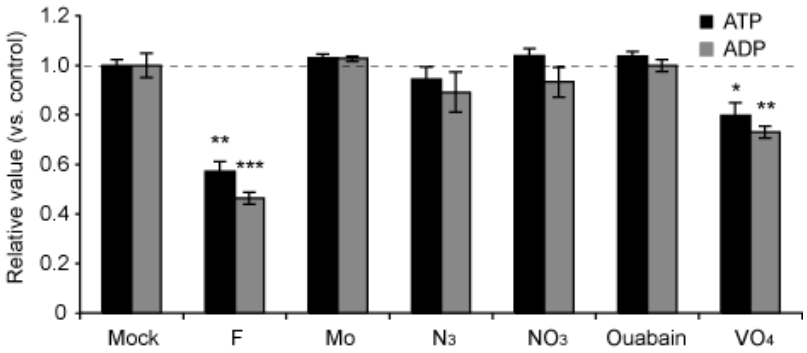
Q: Is apyrase activity of GS52 required for the enhanced nodulation phenotype?

-> Examine the effects of inactive GS52 mutant enzymes on nodulation

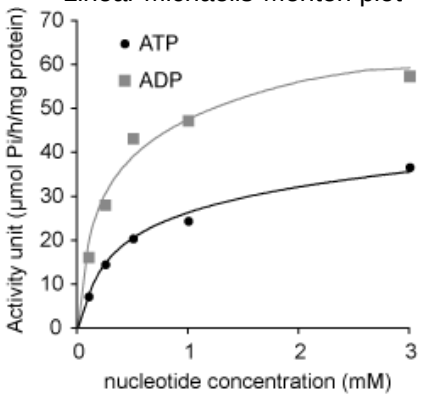
Biochemical characterization of recombinant the GS52 apyrase

ATPase activity
K_m = 424 µM
V_{max} = 38.2 umol Pi/h/mg
K_{cat}/K_m = 1.28 x 10^3/M/s

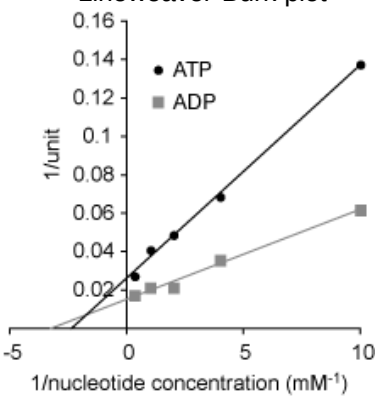
ADPase activity
K_m = 309 µM
V_{max} = 65.8 umol Pi/h/mg
K_{cat}/K_m = 3.02 x 10^3/M/s

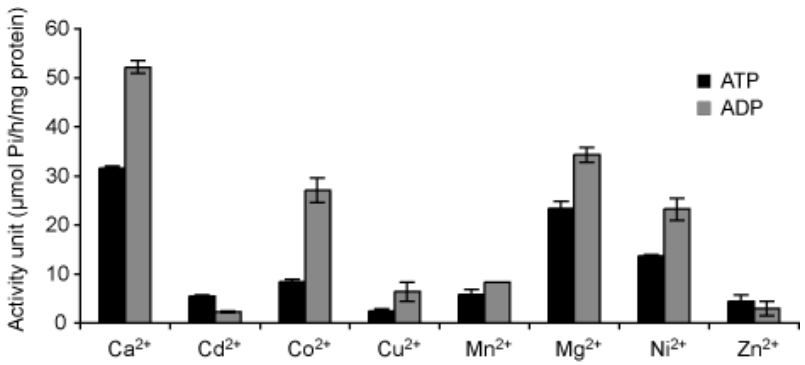


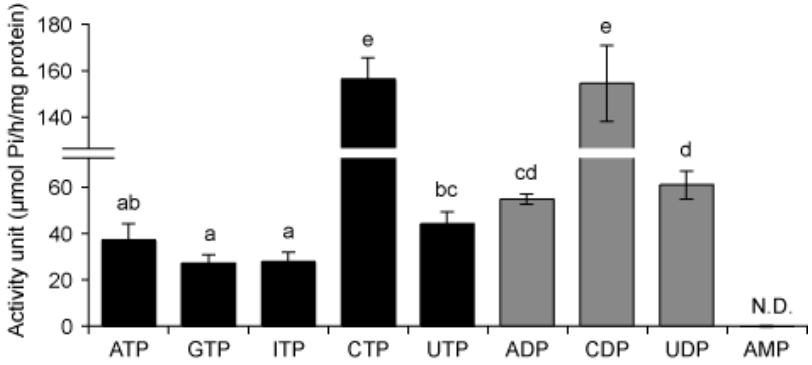
Linear Michaelis-Menten plot



Lineweaver-Burk plot







☑ **The GS52 enzyme possesses typical plant apyrase properties**

☑ **The GS52 enzyme showed broad substrate specificity**
- Di-phosphate nucleotides > Tri-phosphate nucleotides
- Pyrimidine nucleotides > Purine nucleotides

Tanaka et al. (2011) Plant Physiol 155: 1988-98

Zebisch and Strater 2008 PNAS 105, 6882-7

Structural insight into signal conversion and inactivation by NTPDase2 in purinergic signaling

Matthias Zebisch and Norbert Sträter*

Center for Biotechnology and Biomedicine, Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, University of Leipzig,

GS52 vs. RnNTPDase2

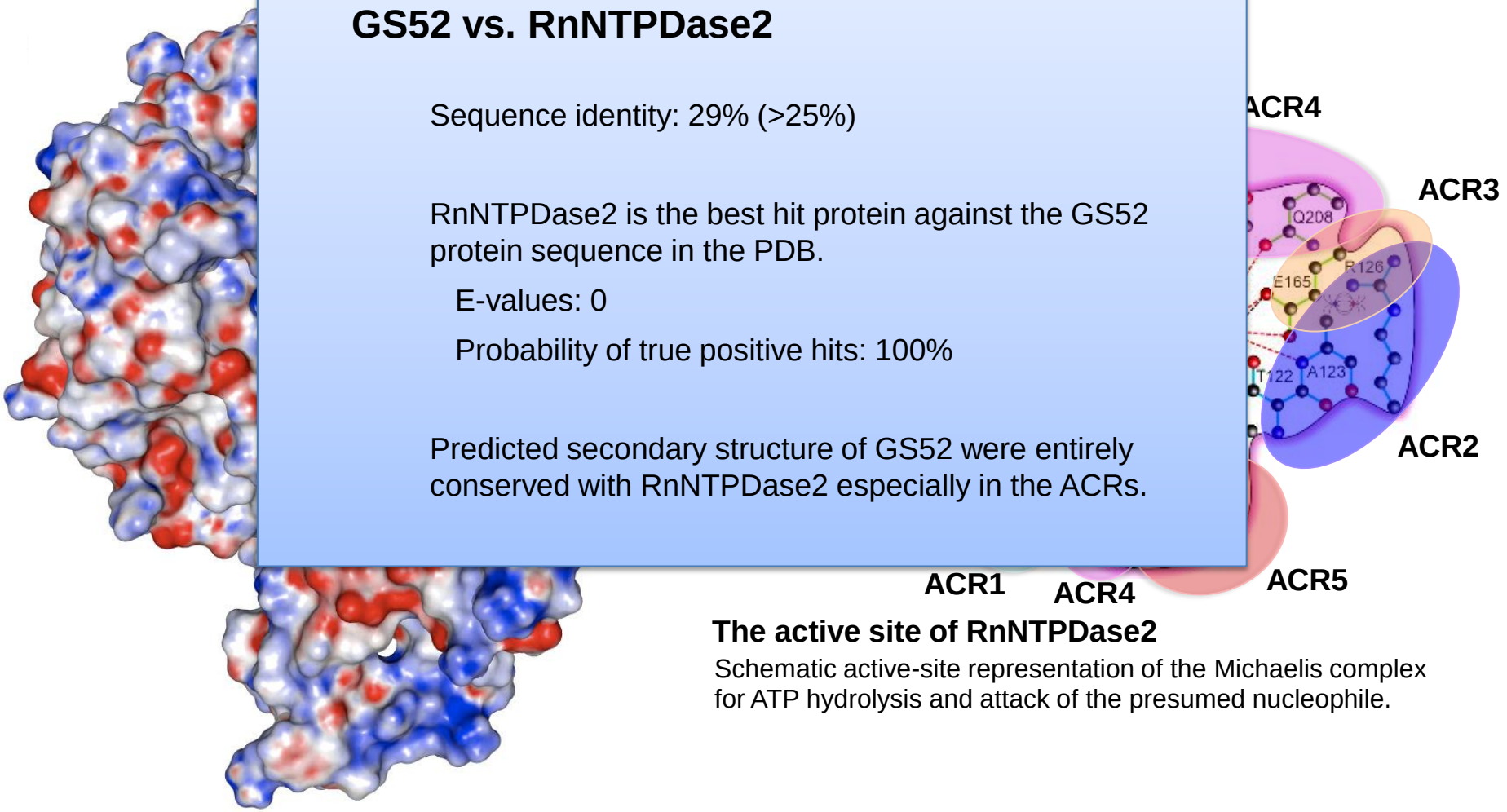
Sequence identity: 29% (>25%)

RnNTPDase2 is the best hit protein against the GS52 protein sequence in the PDB.

E-values: 0

Probability of true positive hits: 100%

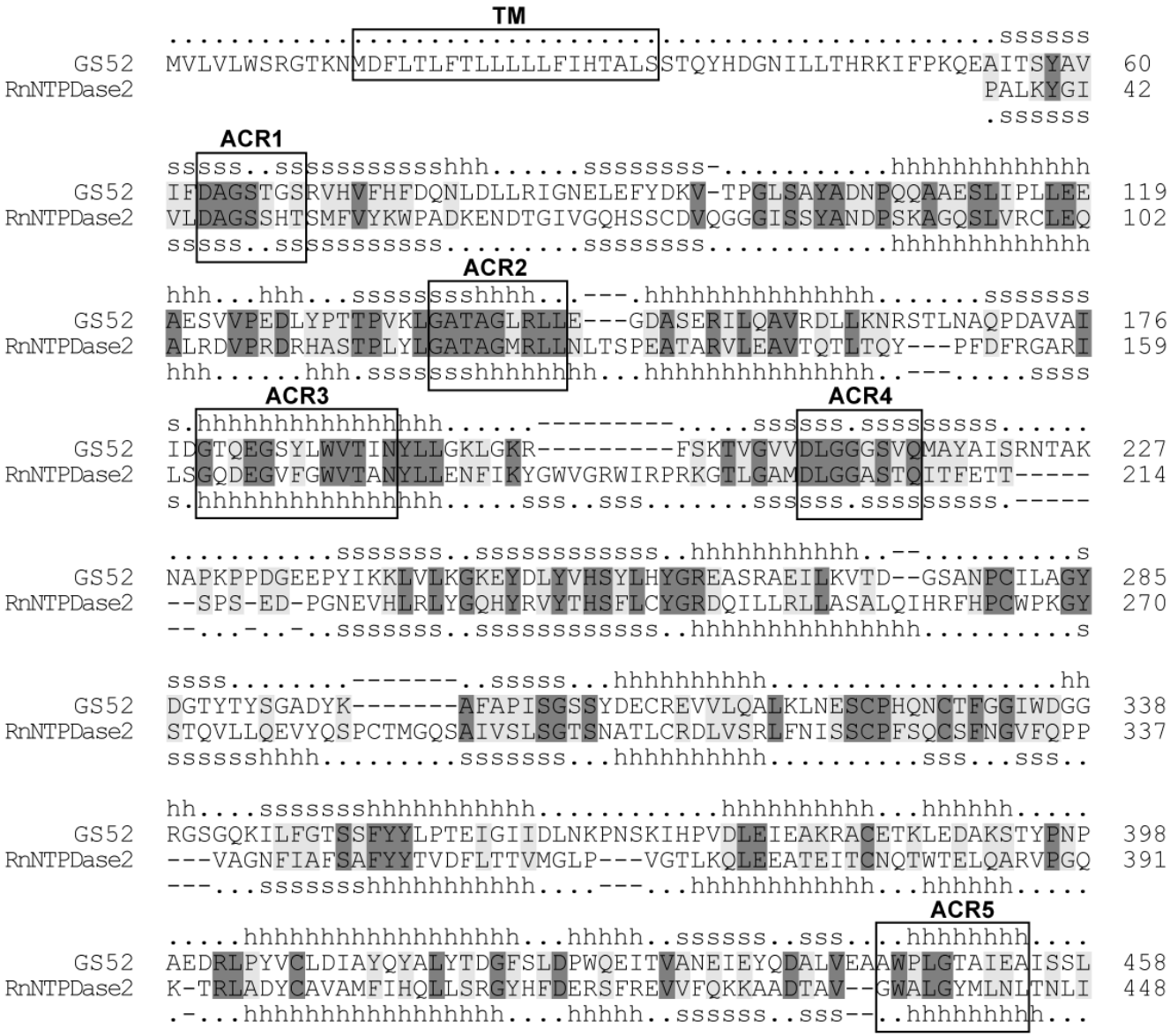
Predicted secondary structure of GS52 were entirely conserved with RnNTPDase2 especially in the ACRs.



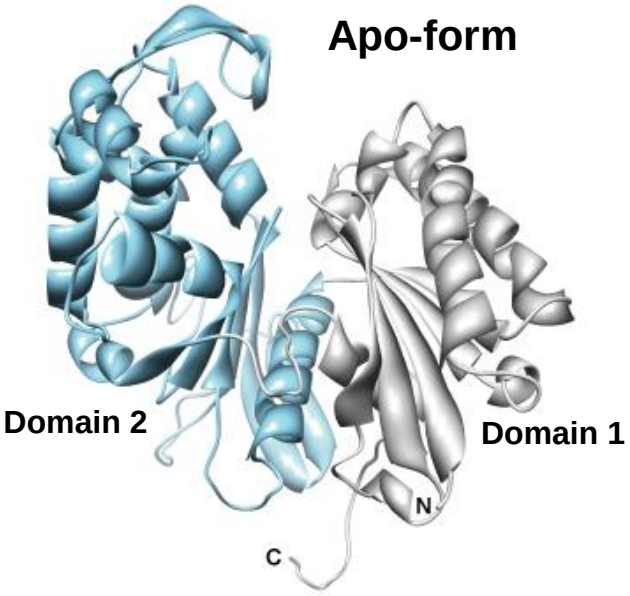
The active site of RnNTPDase2

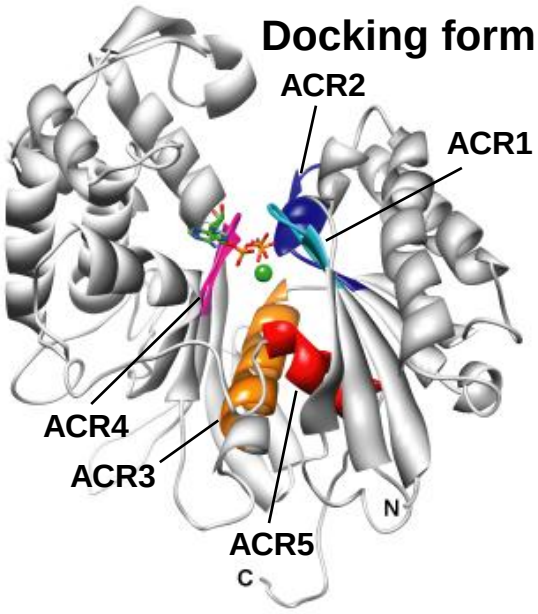
Schematic active-site representation of the Michaelis complex for ATP hydrolysis and attack of the presumed nucleophile.

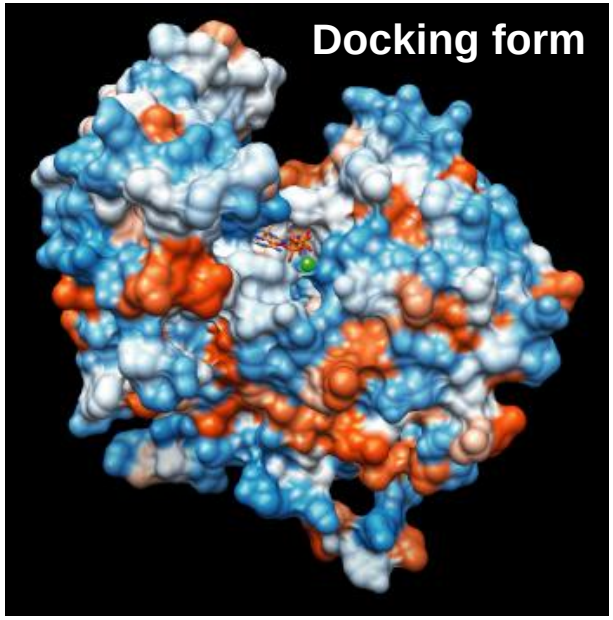
Structure-based sequence alignment between GS52 and RnNTPDase2



GS52 protein model







Molecular surface colored by electrostatic surface potential

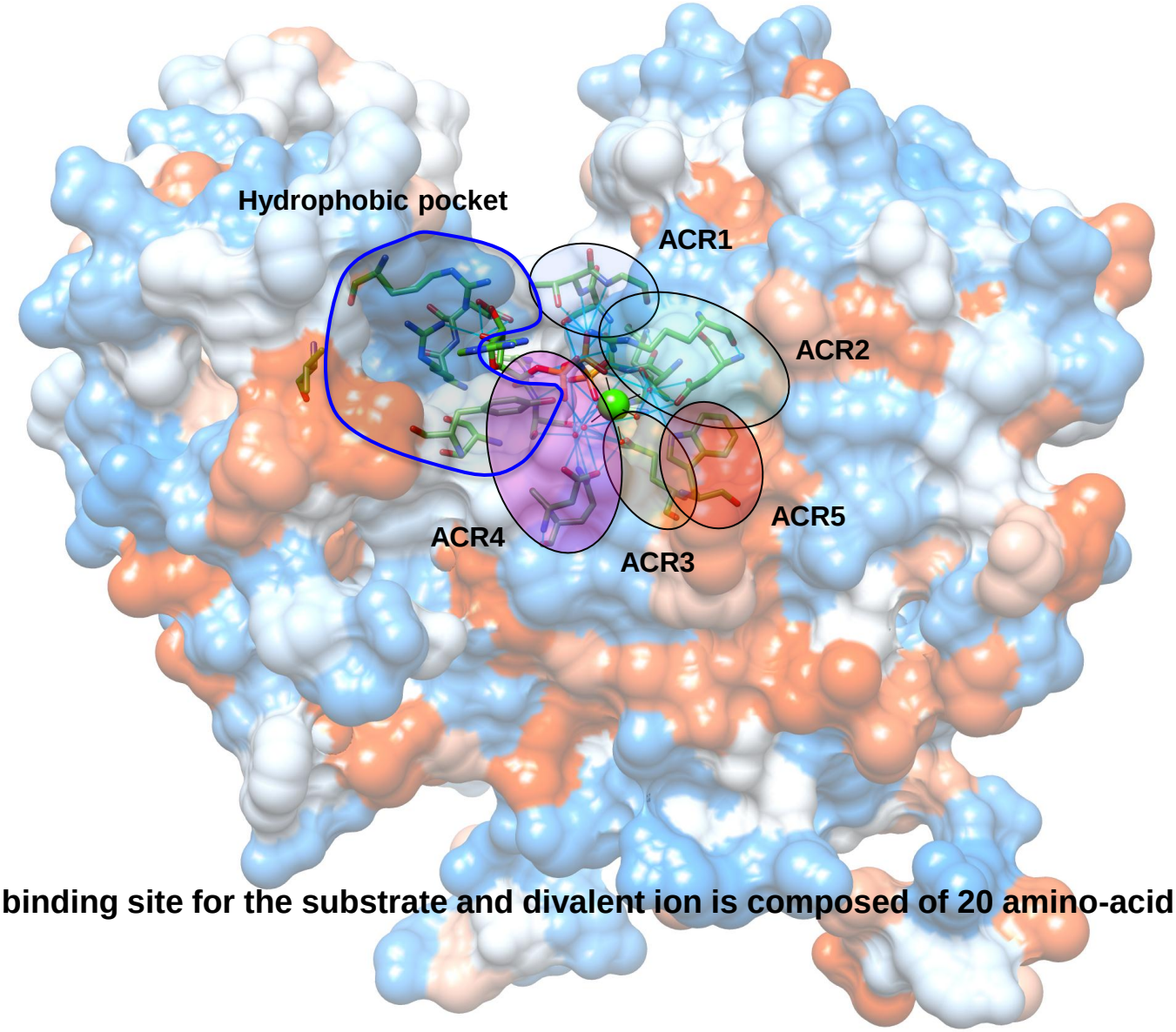
RMSD in 389 atom pairs: 0.514 Å
Quality score: 0.849

RMSD in 389 atom pairs: 0.466 Å
Quality score: 0.851

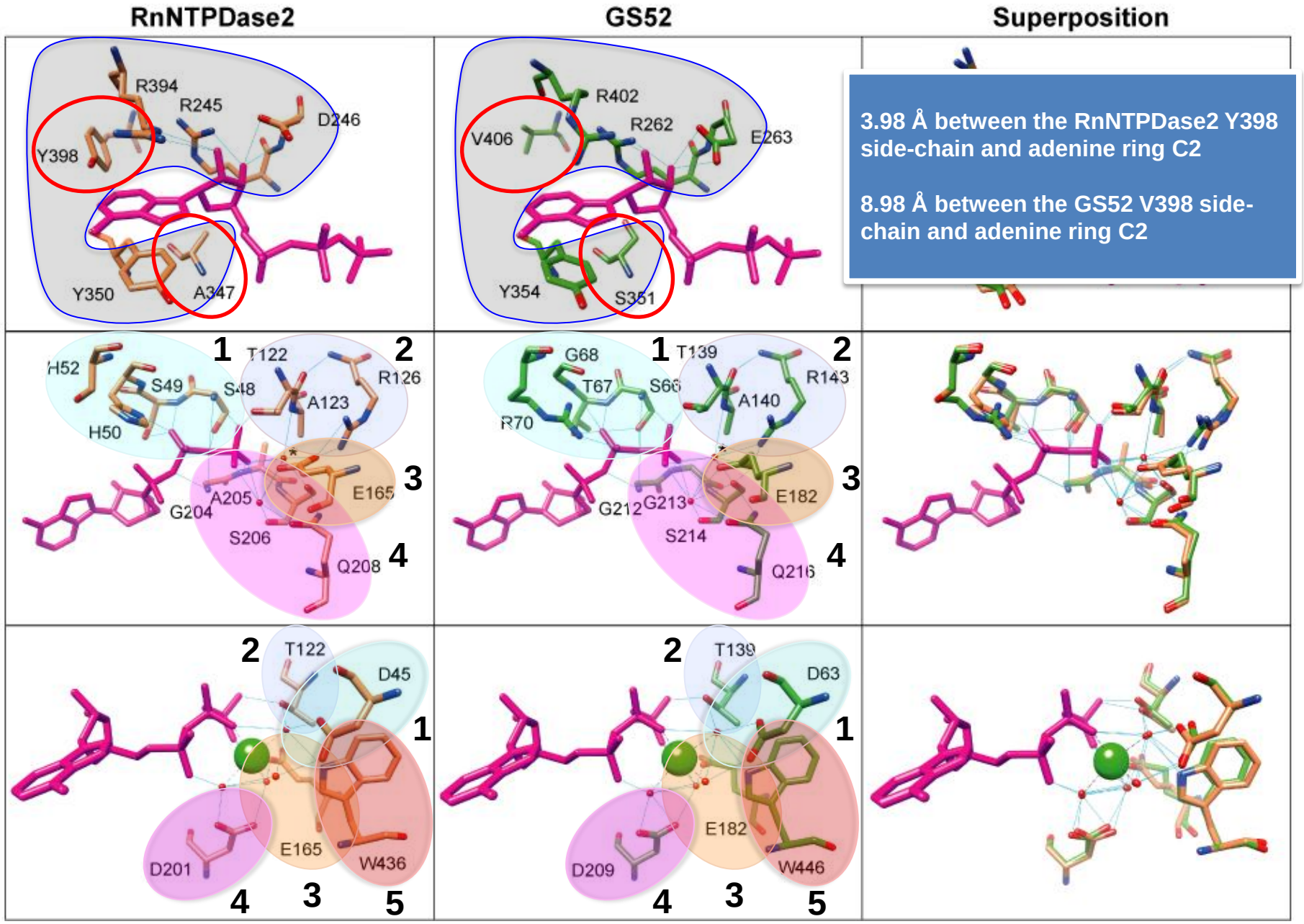
*Quality score was assessed by ModelEvaluator [Wang et al. (2009) Protiens 75: 638-47]

The binding site, accessible from the surface, is located in the cleft between the two domains

Tanaka et al. (2011) Plant Physiol 155: 1988-98

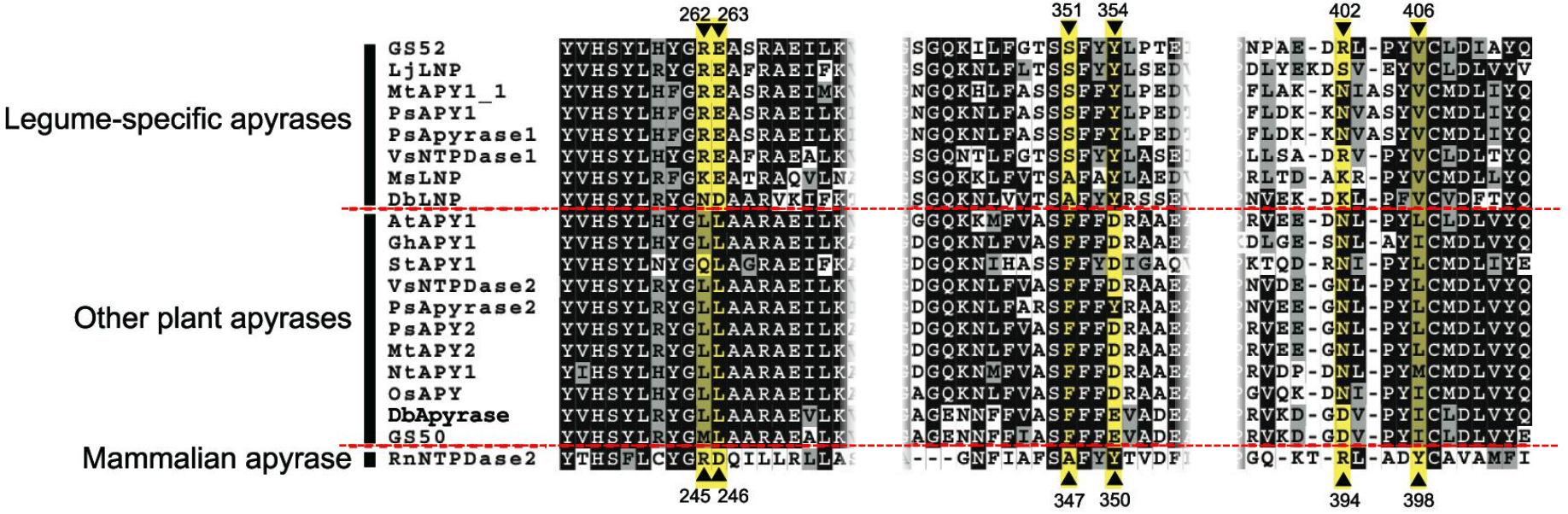


The binding site for the substrate and divalent ion is composed of 20 amino-acid residues



Tanaka et al. (2011) Plant Physiol 155: 1988-98

Multiple sequence alignment of the region around amino-acid residues for the hydrophobic pocket



The legume-specific apyrases and the other apyrases can be classified by the usage of amino-acid residues in the hydrophobic pocket

Question:

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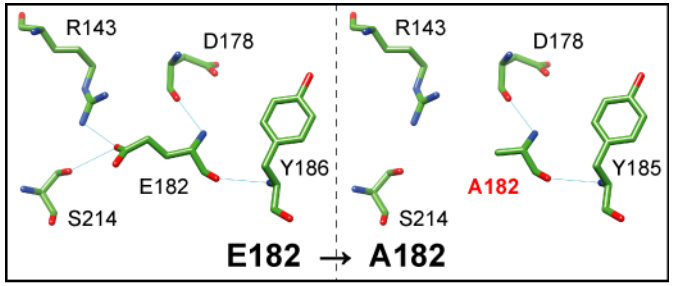
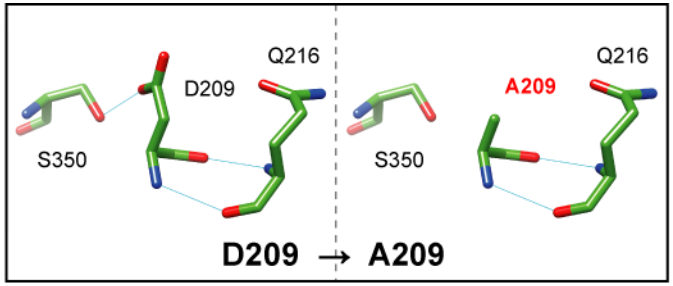
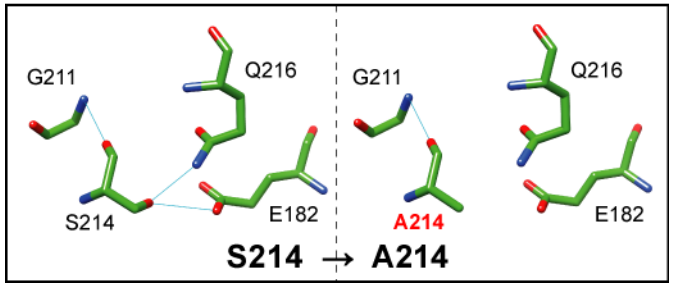
Q: Is apyrase activity of GS52 required for the enhanced nodulation phenotype?

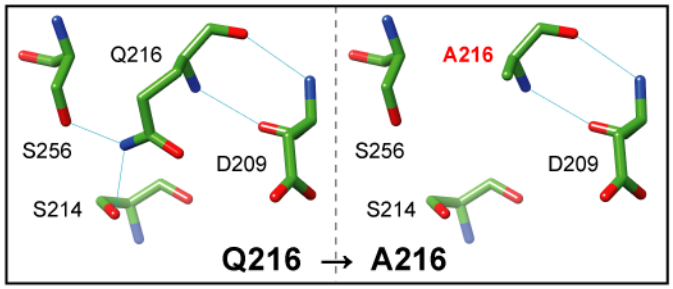
-> Examine the effects of inactive GS52 mutant enzymes on nodulation

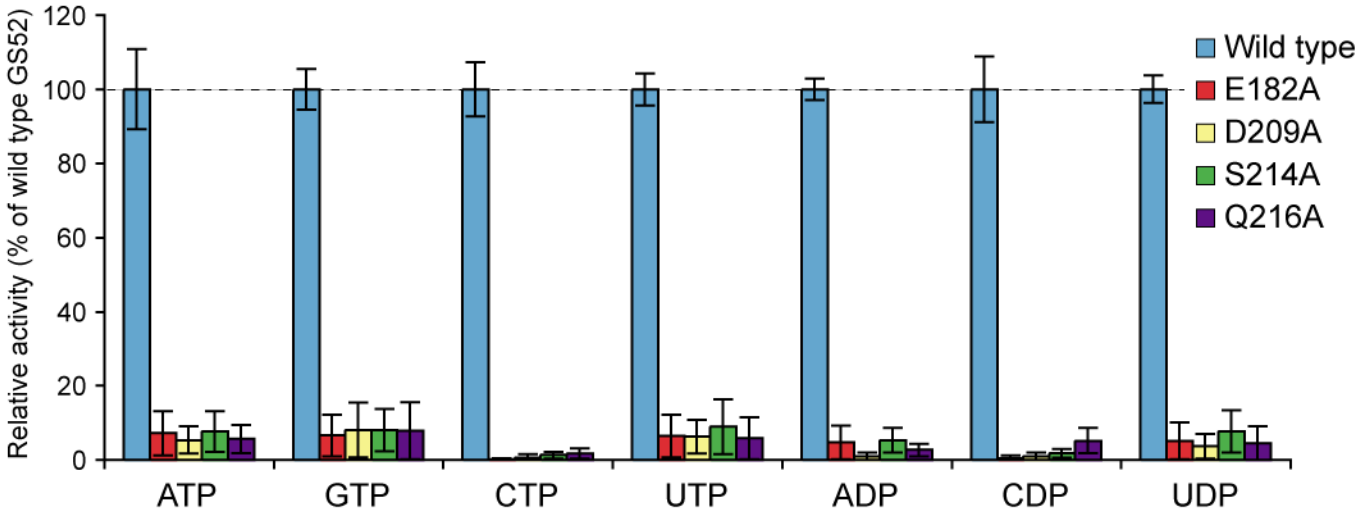
Inactivation of enzymatic activity by site-directed mutagenesis





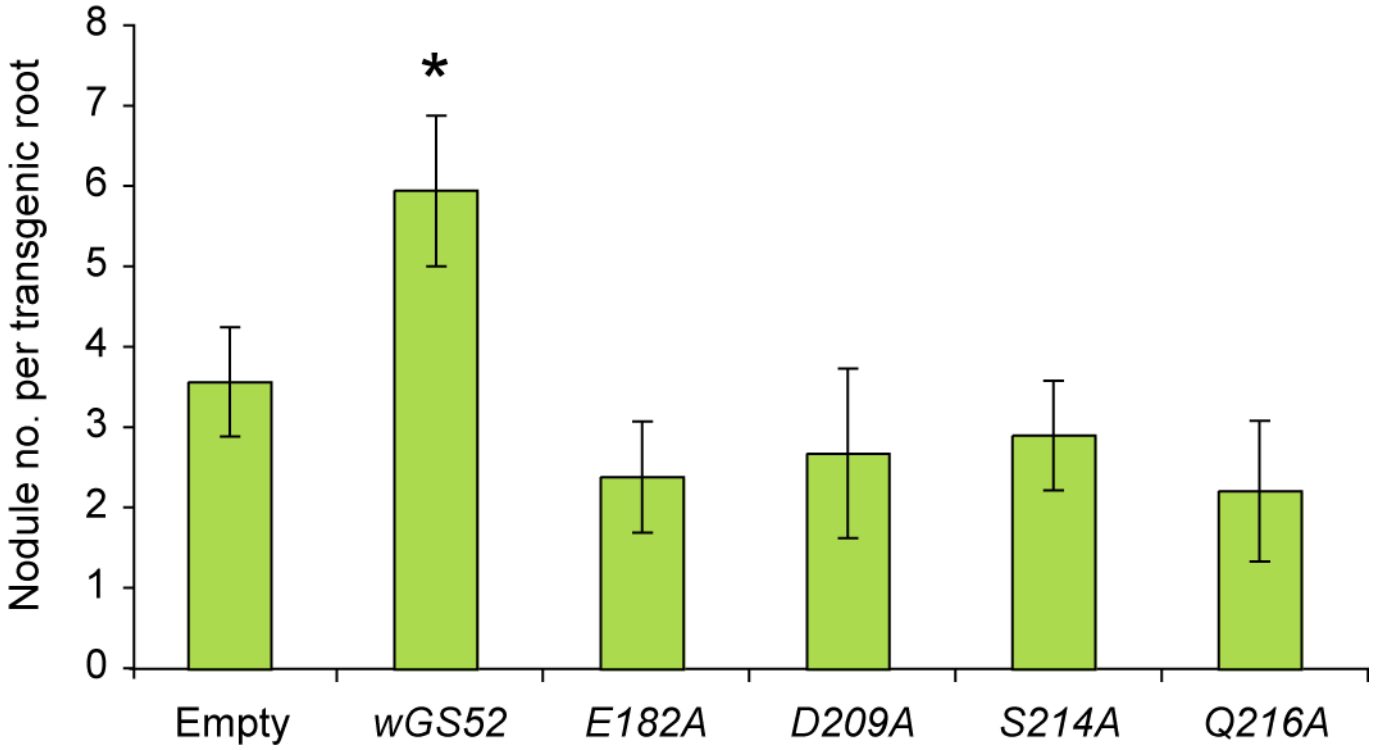






Tanaka et al. (2011) Plant Physiol 155: 1988-98

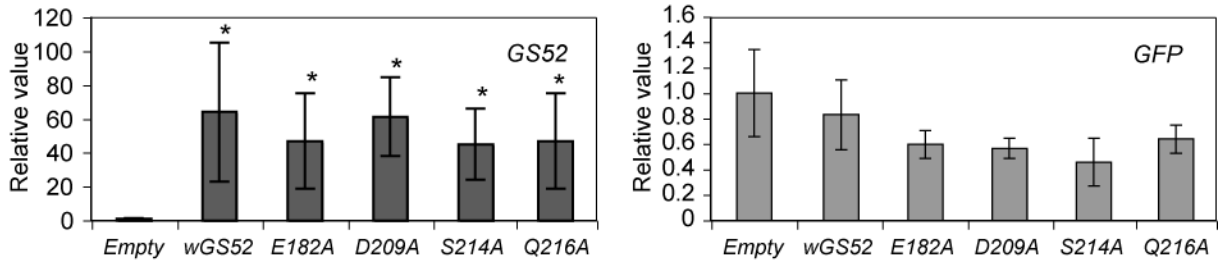
Ectopic expressions of the GS52 apyrase and its mutants in soybean roots



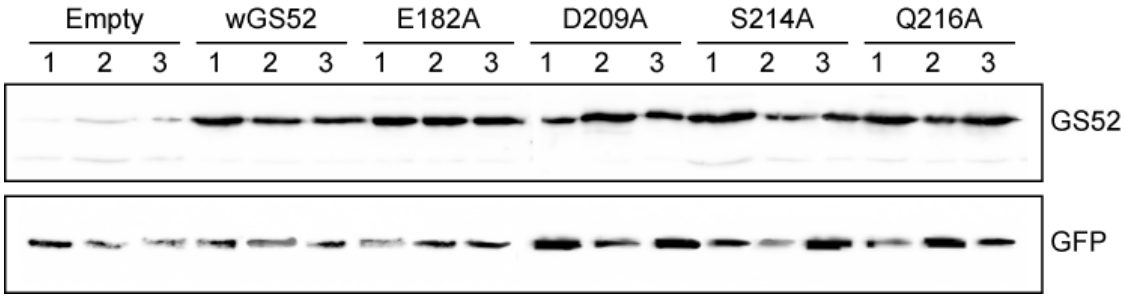
Hairy root transformation of *mGS52s* in *Glycine max*
Host cultivar: Williams 82
Rhizobium strain: *B. japonicum* USDA110

Tanaka et al. (2011) Plant Physiol 155: 1988-98

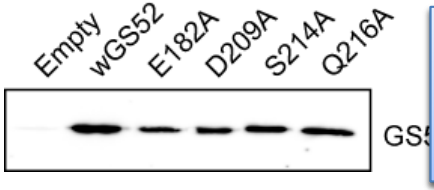
qRT-PCR



Western blotting (S10 fraction)



Western blotting (P100 fraction)

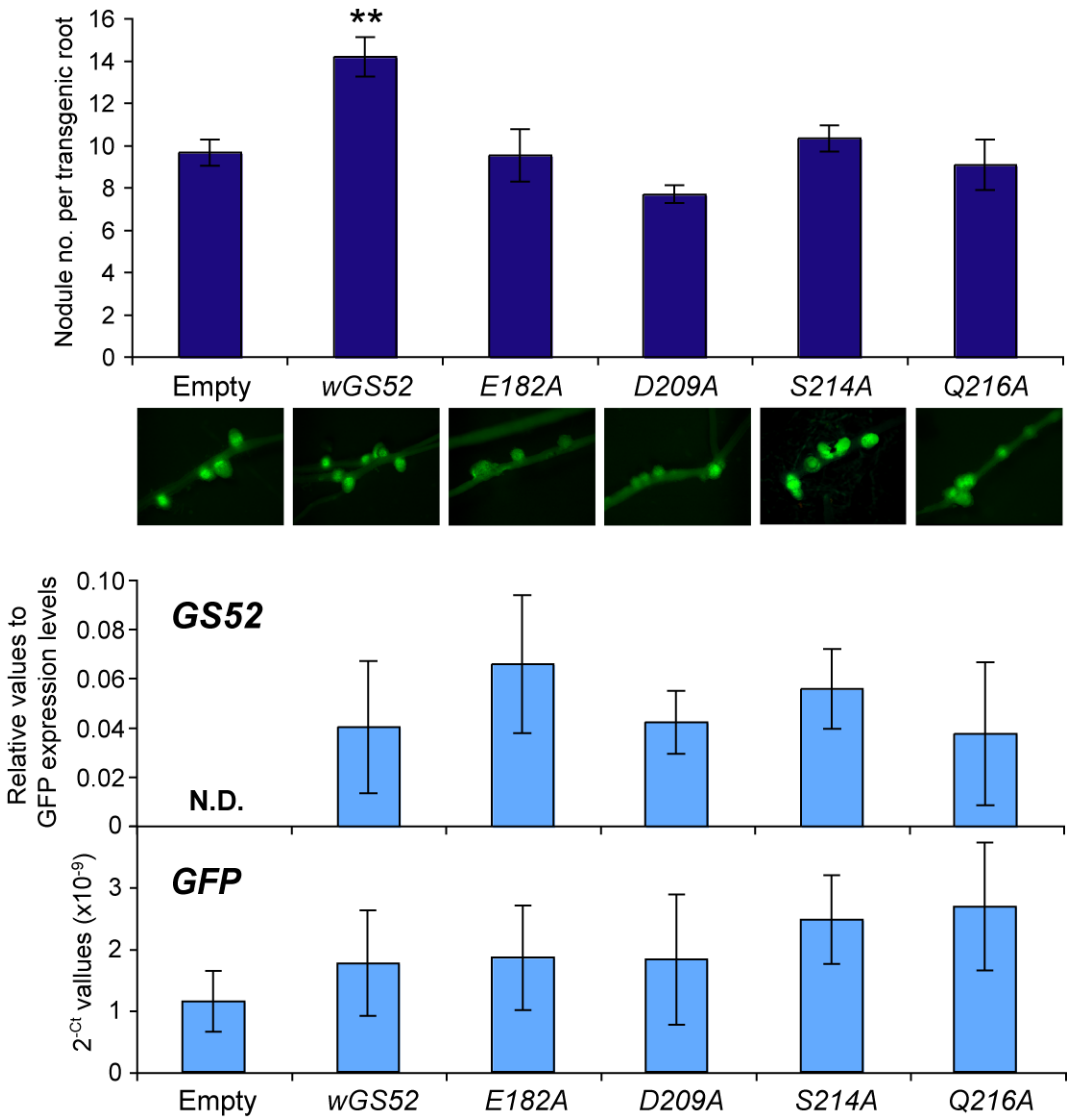


The increase in expression of nodulation-specific genes is due to the stronger nodulation response in the wild-type GS52 transformant.

Tanaka et al. (2011) Plant Physiol 155: 1988-98

Hairy root transformation of *mGS52s* in *Medicago truncatula*

Host cultivar: Jemalong A17
Rhizobium strain: *S. meliloti* ABS7M



Summary

- The GS52 enzyme exhibited broad substrate specificity.
- Structural modeling of GS52 predicted a low specificity for the adenine base within the substrate-binding pocket of the enzyme.

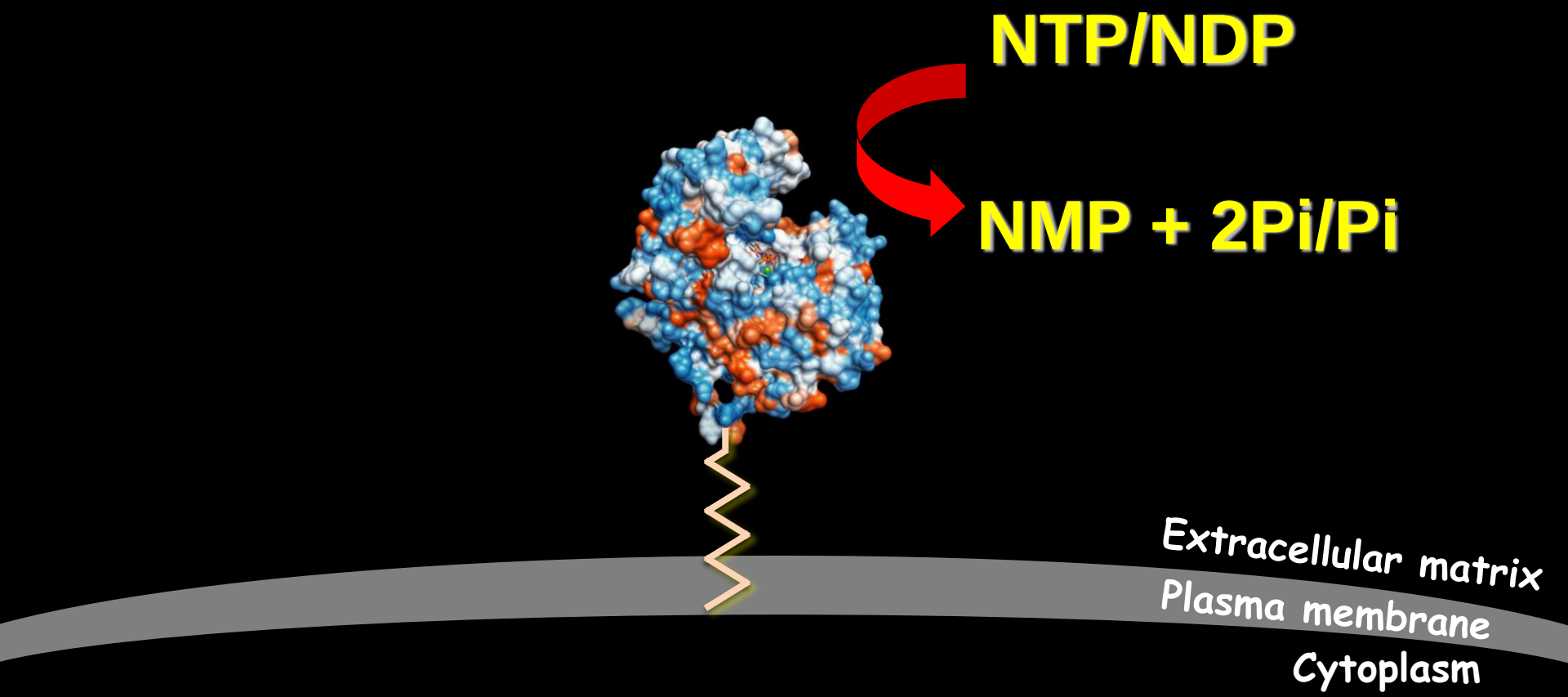
  -> These characteristics are likely conserved in the legume-specific apyrases.

- The number of nodules were increased by ectopic expression of the GS52 protein in soybean roots, but not by its inactive mutant proteins.

  -> The catalytic activity of the GS52 ecto-apyrase, likely acting on extracellular nucleotides, is critical for stimulation of nodulation.

So… GS52 is an ecto-apyrase…

This predicts the presence of extracellular ATP in plants…



How is ecto-apyrase involved in nodulation by controlling extracellular ATP?

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 5856–5861, May 1999

A nod factor binding lectin with apyrase activity from legume roots

MARILYNN E. ETZLER*†, GURPREET KALSI*, NICHOLAS N. EWING*‡, NICHOLAS J. ROBERTS*, R. BRADLEY DAY§, AND JUDITH B. MURPHY*

*Section of Molecular and Cellular Biology, University of California, Davis, CA 95616; and §Center for Legume Research, University of Tennessee, Knoxville, TN 37996

Communicated by Sharon R. Long, Stanford University, Stanford, CA, March 22, 1999 (received for review November 10, 1998)

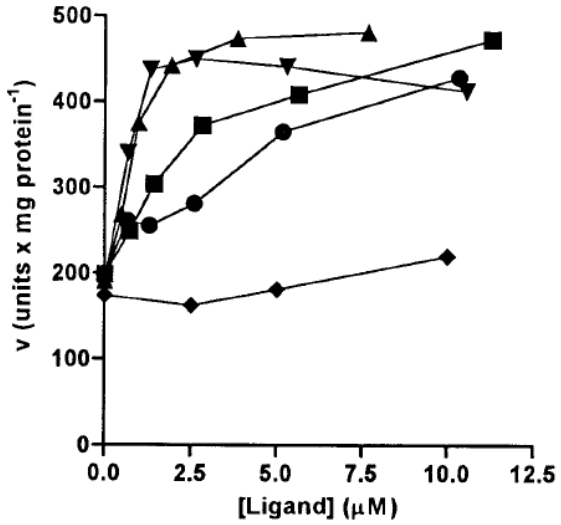
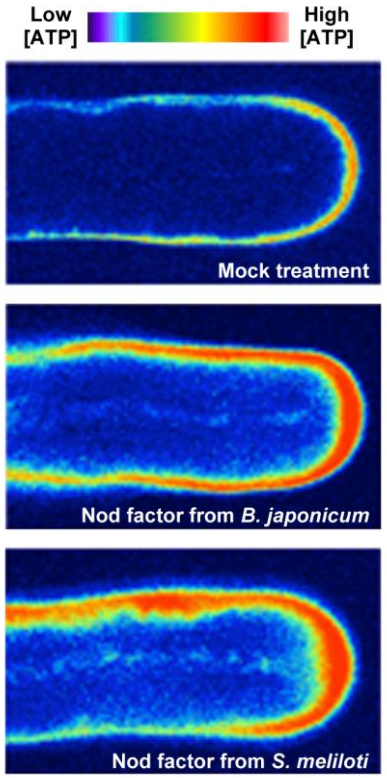
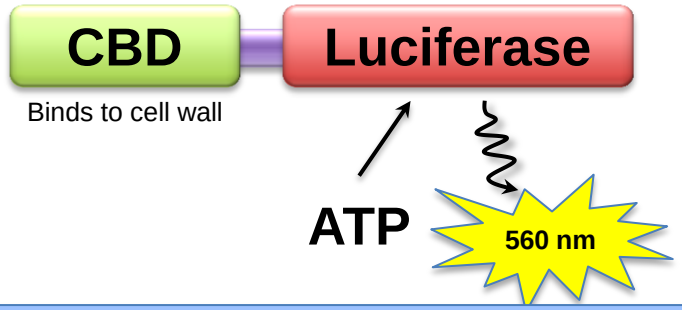


FIG. 3. Effect of carbohydrate ligands on phosphatase activity of LNP isolated from *D. biflorus* roots. LNP (402 ng/ml) was preincubated for 1 hour in 10 mM MOPS buffer, pH 7.2, containing various concentrations of *B. japonicum* USDA110 Nod factor (■), *Rhizobium* sp. NGR234(NGR_A) Nod factor (▲), *Rhizobium* sp. NGR234(NGR_B) Nod factor (▼), *R. meliloti* Nod factor (●), or *cis*-vaccenic acid (◆) and then assayed for phosphatase activity by using a final concentration of 3 mM Mg-ADP. Points are averages of duplicate determinations.

Procedure:
DhLNP (apyrase) was incubated with nod factors for 1 h

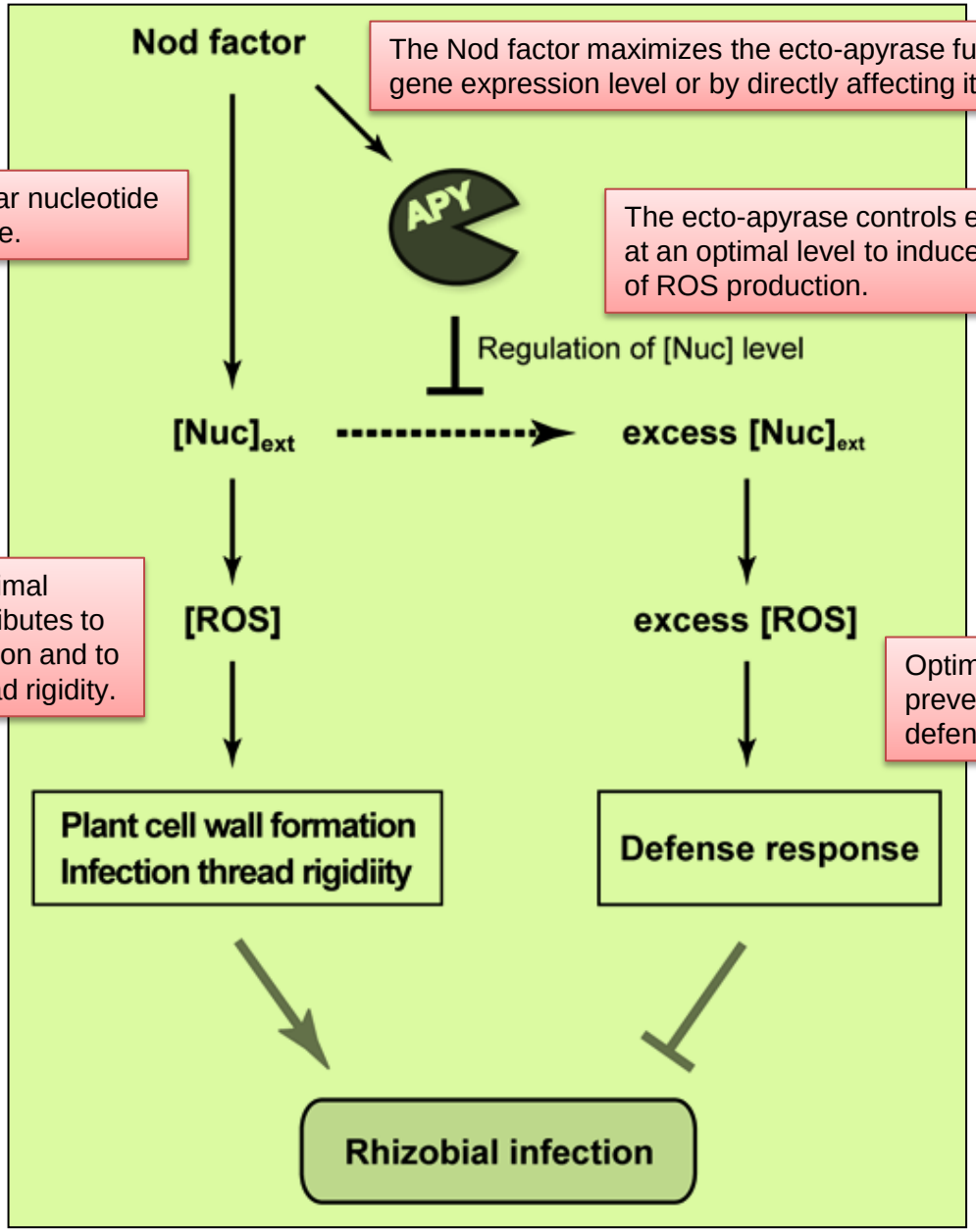
☑ Nod factors directly enhance enzymatic activity of legume apyrase.





☑ Nod factors induce release of extracellular nucleotides.

The hypothesized role of the ecto-apyrases in maintaining an optimal extracellular nucleotide concentration to allow rhizobial infection at the root hair surface



The Nod factor maximizes the ecto-apyrase function by increased its gene expression level or by directly affecting its enzymatic activity.

Nod factor increases the extracellular nucleotide concentration at the root hair surface.

The ecto-apyrase controls extracellular nucleotides at an optimal level to induce a proper concentration of ROS production.

Optimal ROS controlled by optimal extracellular nucleotides contributes to changing plant cell wall formation and to regulation of the infection thread rigidity.

Optimizing ROS levels may prevent the induction of a plant defense response.

Tanaka et al. (2011) Plant Signal Behav 6: 1034-6

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University of Texas

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- Department of Energy (DOE)