

Supplement to “Isolation and Characterization of Mutant *Sinorhizobium meliloti* NodD1  
Proteins With Altered Responses to Luteolin” by Melicent C. Peck<sup>a</sup>, Robert F. Fisher<sup>b,#</sup>,  
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**Figure S1.** Locations of mutant residues based on structural and functional studies in LTTRs: *S. meliloti* NodD1 (1), *R. l. bv viciae* NodD (2, 3) (Peck and Long, unpublished data), *R. l. bv trifolii* NodD (4) (Peck and Long, unpublished data), *Burkholderia sp.* DntR (5), *P. putida* NahR (6) (7), *E.coli* XapR (8), *C. testosteroni* T-2 Tsar (9, 10), *A. tumefaciens* OccR (11) (12), *C. freundii* AmpR (13, 14), *E. coli* OxyR (15) (16, 17), *R. sphaeroides* CbbR (18), *A. baylyi* BenM (19) (20), *E.coli* Cbl (21) (22), and *S. typhimurium* CysB (23). Numbers to the right indicate amino acid residue. Residues shaded yellow are identical to *S. meliloti* NodD1. Outlined residues indicate the following mutant phenotypes based on mutations in LTTRs: green: activation-deficient except in Cbl in which mutants are unable to repress target transcription; red: constitutively-active; purple: enhanced inducer sensitivity. Mutant phenotypes based on LTTR crystal structures: orange: oligomerization; grey: ligand binding (i.e., lining the inducer binding pocket or contacting the ligand). Boxed areas indicate mutational hotspots at sites of mutant residues discussed in this study.

**TABLE S1. Strains and Plasmids**

<b>Strain or Plasmid</b>	<b>Relevant Characteristics</b>	<b>Source/Reference</b>
<i>Strains</i>		
DH5 $\alpha$	<i>Escherichia coli supE44 <math>\Delta</math>lacU169 (<math>\Phi</math>80lacZ<math>\Delta</math>M15) hsdR17 <i>recA1 endA1 gyrA96 thi-1 relA1</i></i>	(24)
XL1-Red	$\Delta$ [ <i>mcrA</i> ] <i>183</i> , $\Delta$ [ <i>mcrCB-hsdSMR-mrr</i> ] <i>173</i> , <i>endA1, supE44, thi-1,</i> <i>gyrA96, relA1, mutS, mutT, mutD5, lac Tn10 (Tet<sup>R</sup>)</i>	Stratagene
XL1-Red Tc <sup>S</sup>	$\Delta$ [ <i>mcrA</i> ] <i>183</i> , $\Delta$ [ <i>mcrCB-hsdSMR-mrr</i> ] <i>173</i> , <i>endA1, supE44, thi-1,</i> <i>gyrA96, relA1, mutS, mutT, mutD5, lac Tc<sup>S</sup></i>	This study
Rm 1021	<i>Sinorhizobium meliloti</i> SU47 str-21 Sm <sup>R</sup>	(25)
A2105	Rm1021 <i>nodD1::Tn5(9B7) nodD2::tm nodD3::sp/g-1 nodC'-lacZ</i>	(26)
BTH101	F', <i>cya-99, araD139, galE15, galK16, rpsL1(Str<sup>R</sup>), hsdR2, mcrA1,</i> <i>mcrB1</i>	(27)
<i>Plasmids</i>		
pASK75B	Vector encoding 10 amino-acid Strep-tag, Tc-inducible promoter	(28)
pUC119	Cloning vector, Ap <sup>R</sup>	(29)
pBluescript SK	Cloning vector, Ap <sup>R</sup>	Stratagene
pCRII-TOPO	TOPO Cloning vector, Ap <sup>R</sup>	Invitrogen
pGroESL	<i>E. coli groESL</i> in pTG10	(30)
pKT25	<i>B. pertussis cya</i> T25-expression plasmid, Kn <sup>R</sup>	(27)
pKT25-zip	<i>B. pertussis cya</i> T25-leucine zipper fusion, Kn <sup>R</sup>	(27)
pUT18	<i>B. pertussis cya</i> T18-expression plasmid, Ap <sup>R</sup>	(27)

pUT18c-zip	<i>B. pertussis cya</i> T18-leucine zipper fusion, Ap <sup>R</sup>	(27)
pNodD1-ST	C-terminal fusion of NodD1 to Strep-tag in pASK75B	(31)
pMP40	C-terminal fusion of NodD1 D284N to Strep-tag in pASK75B	This study
pMP41	C-terminal fusion of NodD1 K205N to Strep-tag in pASK75B	This study
pMP42	C-terminal fusion of NodD1 L280F to Strep-tag in pASK75B	This study
pMP43	C-terminal fusion of NodD1 L69F to Strep-tag in pASK75B	This study
pMP44	C-terminal fusion of NodD1 S104L to Strep-tag in pASK75B	This study
pMP45	C-terminal fusion of NodD1 D134N to Strep-tag in pASK75B	This study
pMP46	C-terminal fusion of NodD1 M193I to Strep-tag in pASK75B	This study
pMP50	<i>S. meliloti nodD1</i> in pCRII	This study
pMP60	<i>S. meliloti nodD1</i> D284N in pCRII	This study
pMP61	<i>S. meliloti nodD1</i> K205N in pCRII	This study
pMP62	<i>S. meliloti nodD1</i> L280F in pCRII	This study
pMP63	<i>S. meliloti nodD1</i> D284A in pCRII	This study
pMP64	<i>S. meliloti nodD1</i> L280A in pCRII	This study
pMP66	<i>S. meliloti nodD1</i> P285A in pCRII	This study
pMP67	<i>S. meliloti nodD1</i> P285Q in pCRII	This study
pMP68	<i>S. meliloti nodD1</i> G286A in pCRII	This study
pMP69	<i>S. meliloti nodD1</i> P285Q G286A in pCRII	This study
pMP70	<i>S. meliloti nodD1</i> K205A in pCRII	This study
pMP86	<i>S. meliloti nodD1</i> M193I in pCRII	This study
pAB 100	<i>S. meliloti nodD1</i> L69F in pCRII	This study
pAB 101	<i>S. meliloti nodD1</i> L103F in pCRII	This study
pAB 102	<i>S. meliloti nodD1</i> S104L in pCRII	This study
pAB 103	<i>S. meliloti nodD1</i> D134N in pCRII	This study
pAB 104	<i>S. meliloti nodD1</i> D135N in pCRII	This study
pRF796	<i>S. meliloti nodF nod</i> box in pCRII	This study
pRF1049	NodD1-T18 fusion in pUT18, Ap <sup>R</sup>	This study

pRF1050	T18-NodD1 fusion in pUT18c, Ap <sup>R</sup>	This study
pRF1051	T25-NodD1 fusion in pKT25, Kn <sup>R</sup>	This study
pRF1099	NodD1 L69F -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1101	NodD1 S104L -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1104	NodD1 M193I -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1105	NodD1 K205N -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1107	NodD1 D284N -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1108	T25-NodD1 S104L fusion in pKT25, Kn <sup>R</sup>	This study
pRF1113	T25-NodD1 L280F fusion in pKT25, Kn <sup>R</sup>	This study
pRF1114	T25-NodD1 D284N fusion in pKT25, Kn <sup>R</sup>	This study
pRF1115	NodD1 L103F -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1117	NodD1 L280F -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1119	T25-NodD1 L69F fusion in pKT25, Kn <sup>R</sup>	This study
pRF1120	T25-NodD1 K205N fusion in pKT25, Kn <sup>R</sup>	This study
pRF1134	T25-NodD1 M193I fusion in pKT25, Kn <sup>R</sup>	This study
pRF1138	T25-NodD1 L103F fusion in pKT25, Kn <sup>R</sup>	This study
pRF1145	NodD1 D134N/D135N -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1151	NodD1 L103F/S104L -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1153	T25-NodD1 L103F/S104L fusion in pKT25, Kn <sup>R</sup>	This study
pRF1155	T25-NodD1 D134N/D135N fusion in pKT25, Kn <sup>R</sup>	This study
pRF1151	NodD1 L103F/S104L -T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1161	NodD1 (BglII/BamHIA)-T18 fusion in pUT18, Ap <sup>R</sup>	This study
pRF1163	T25-NodD1 (BglII/BamHIA) fusion in pKT25, Kn <sup>R</sup>	This study
<i>Broad host-range plasmids</i>		
pRK2013	RK2 derivative helper plasmid, Kn <sup>R</sup>	(32)
pTE3	IncP cloning vector, Tc <sup>R</sup> , <i>S. typhimurium trp</i> promoter	(1)
pRF771	pTE3 with modified polylinker	(33)
pRmE43	<i>nodD1</i> in pTE3, Tc <sup>R</sup>	(34) (35)

pMP53	<i>S. meliloti nodd1</i> in pRF771	This study
pMP150	<i>S. meliloti nodd1</i> D284N in pRF771	This study
pMP151	<i>S. meliloti nodd1</i> K205N in pRF771	This study
pMP152	<i>S. meliloti nodd1</i> L280F in pRF771	This study
pMP153	<i>S. meliloti nodd1</i> D284A in pRF771	This study
pMP154	<i>S. meliloti nodd1</i> L280A in pRF771	This study
pMP156	<i>S. meliloti nodd1</i> P285A in pRF771	This study
pMP157	<i>S. meliloti nodd1</i> P285Q in pRF771	This study
pMP158	<i>S. meliloti nodd1</i> G286A in pRF771	This study
pMP159	<i>S. meliloti nodd1</i> P285Q/G286A in pRF771	This study
pMP160	<i>S. meliloti nodd1</i> K205A pRF771	This study
pMP173	<i>S. meliloti nodd1</i> M193I pRF771	This study
pAB 110	<i>S. meliloti nodd1</i> L69F pRF771	This study
pAB 111	<i>S. meliloti nodd1</i> L103F pRF771	This study
pAB 112	<i>S. meliloti nodd1</i> S104L pRF771	This study
pAB 113	<i>S. meliloti nodd1</i> D134N pRF771	This study
pAB 114	<i>S. meliloti nodd1</i> D135N pRF771	This study

**Table S2. Dimerization of NodD1 mutants**

	T18 fusion	T25 fusion	$\beta$ -gal <sup>a</sup>	SD
1	D1(L69F)-T18	T25-zip	26.2	9.2
2	D1(L69F)-T18	T25-D1	540.3	59.5
3	D1(L69F)-T18	T25-D1(L69F)	657.7	118.6
4	D1-T18	T25-D1(L69F)	356.1	125.2
5	T18-zip	T25-D1(L69F)	27.6	9.5
6	T18 vector	T25-D1(L69F)	27.5	18.9
7	D1(L103F/S104L)-T18	T25-zip	29.1	7.7
8	D1(L103F/S104L)-T18	T25-D1	413.8	122.9
9	D1(L103F/S104L)-T18	T25-D1(L103F/S104L)	728.7	90.8
10	D1-T18	T25-D1(L103F/S104L)	302.6	105.3
11	T18-zip	T25-D1(L103F/S104L)	27.6	4.3
12	T18 vector	T25-D1(L103F/S104L)	29.5	4.8
13	D1(D134N/D135N)-T18	T25-zip	27.0	8.6
14	D1(D134N/D135N)-T18	T25-D1	712.9	103.3
15	D1(D134N/D135N)-T18	T25-D1(D134N/D135N)	818.0	104.2
16	D1-T18	T25-D1(D134N/D135N)	396.9	40.0
17	T18-zip	T25-D1(D134N/D135N)	34.1	8.8
18	T18 vector	T25-D1(D134N/D135N)	23.1	9.5
19	D1(M193I)-T18	T25-zip	25.1	10.2
20	D1(M193I)-T18	T25-D1	570.7	47.9
21	D1(M193I)-T18	T25-D1(M193I)	534.0	40.7
22	D1-T18	T25-D1(M193I)	387.3	56.6
23	T18-zip	T25-D1(M193I)	51.4	28.5
24	T18 vector	T25-D1(M193I)	14.1	7.5
25	D1(K205N)-T18	T25-zip	26.0	32.5
26	D1(K205N)-T18	T25-D1	562.4	180.8
27	D1(K205N)-T18	T25-D1(K205N)	709.1	138.5
28	D1-T18	T25-D1(K205N)	459.2	227.7
29	T18-zip	T25-D1(K205N)	108.5	59.0
30	T18 vector	T25-D1(K205N)	12.1	11.6
31	D1(L280F)-T18	T25-zip	16.6	22.5
32	D1(L280F)-T18	T25-D1	627.0	154.5
33	D1(L280F)-T18	T25-D1(L280F)	774.9	143.0
34	D1-T18	T25-D1(L280F)	263.7	116.0
35	T18-zip	T25-D1(L280F)	30.4	21.1
36	T18 vector	T25-D1(L280F)	20.1	8.5
37	D1(D284N)-T18	T25-zip	26.3	16.4
38	D1(D284N)-T18	T25-D1	579.2	110.2
39	D1(D284N)-T18	T25-D1(D284N)	764.4	164.3
40	D1-T18	T25-D1(D284N)	361.4	163.4
41	T18-zip	T25-D1(D284N)	44.0	21.8
42	T18 vector	T25-D1(D284N)	17.1	5.5
43	D1(L103F)-T18	T25-zip	15.7	7.9
44	D1(L103F)-T18	T25-D1	460.8	266.6
45	D1(L103F)-T18	T25-D1(L103F)	557.7	105.8
46	D1-T18	T25-D1(L103F)	659.4	128.5
47	T18-zip	T25-D1(L103F)	51.5	36.6
48	T18 vector	T25-D1(L103F)	52.7	12.7
49	D1(S104L)-T18	T25-zip	13.1	14.8
50	D1(S104L)-T18	T25-D1	553.9	99.9
51	D1(S104L)-T18	T25-D1(S104L)	767.4	177.6
52	D1-T18	T25-D1(S104L)	369.5	49.0
53	T18-zip	T25-D1(S104L)	20.8	24.3
54	T18 vector	T25-D1(S104L)	14.1	13.4

<sup>a</sup> $\beta$ -galactosidase activity (Miller units) of wild-type *lacZ* in *cyA E.coli*



**Table S2.** Dimerization of NodD1 mutants. *cya E. coli* BTH101 was co-transformed with plasmids expressing the indicated T18- and T25-NodD1 fusions. Six colonies from each co-transformation were patched onto minimal maltose medium (left) and assayed for cAMP-dependent  $\beta$ -galactosidase activity (right). D1 is wild-type NodD1; mutations in NodD1 give rise to the residues in parentheses. Data is presented as mean +/- SD from a representative assay.

## REFERENCES

1. **Egelhoff TT, Fisher RF, Jacobs TW, Mulligan JT, Long SR.** 1985. Nucleotide sequence of *Rhizobium meliloti* 1021 nodulation genes: *nodD* is read divergently from *nodABC*. *DNA* **4**:241-248.
2. **Burn J, Rossen L, Johnston AWB.** 1987. Four classes of mutations in the *nodD* gene of *Rhizobium leguminosarum* biovar *viciae* that affect its ability to autoregulate and/or activate other *nod* genes in the presence of flavonoid inducers. *Genes Dev.* **1**:456-464.
3. **Burn JE, Hamilton WD, Wootton JC, Johnston AWB.** 1989. Single and multiple mutations affecting properties of the regulatory gene *nodD* of *Rhizobium*. *Mol. Microbiol.* **3**:1567-1577.
4. **McIver J, Djordjevic MA, Weinman JJ, Bender GL, Rolfe BG.** 1989. Extension of host range of *Rhizobium leguminosarum* bv. *trifolii* caused by point mutations in *nodD* that result in alterations in regulatory function and recognition of inducer molecules. *Mol. Plant-Microbe Interact.* **2**:97-106.
5. **Smirnova IA, Dian C, Leonard GA, McSweeney S, Birse D, Brzezinski P.** 2004. Development of a bacterial biosensor for nitrotoluenes: the crystal structure of the transcriptional regulator DntR. *J Mol Biol* **340**:405-418.
6. **Schell MA, Brown PH, Raju S.** 1990. Use of saturation mutagenesis to localize probable functional domains in the NahR protein, a LysR-type transcription activator. *J. Biol. Chem.* **265**:3844-3850.
7. **Cebolla A, Sousa C, de Lorenzo V.** 1997. Effector specificity mutants of the transcriptional activator NahR of naphthalene degrading *Pseudomonas* define protein sites involved in binding of aromatic inducers. *J. Biol. Chem.* **272**:3986-3992.
8. **Jorgensen C, Dandanell G.** 1999. Isolation and characterization of mutations in the Escherichia coli regulatory protein XapR. *J Bacteriol* **181**:4397-4403.
9. **Monferrer D, Tralau T, Kertesz MA, Dix I, Sola M, Uson I.** 2010. Structural studies on the full-length LysR-type regulator TsaR from Comamonas

- testosteroni T-2 reveal a novel open conformation of the tetrameric LTTR fold. *Mol Microbiol* **75**:1199-1214.
10. **Monferrer D, Tralau T, Kertesz MA, Panjekar S, Uson I.** 2008. High crystallizability under air-exclusion conditions of the full-length LysR-type transcriptional regulator TsaR from *Comamonas testosteroni* T-2 and dataset analysis for a MIRAS structure-solution approach. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **64**:764-769.
  11. **Cho K, Winans SC.** 1993. Altered-function mutations in the *Agrobacterium tumefaciens* OccR protein and in an OccR-regulated promoter. *J. Bacteriol.* **175**:7715-7719.
  12. **Akakura R, Winans SC.** 2002. Constitutive mutations of the OccR regulatory protein affect DNA bending in response to metabolites released from plant tumors. *J. Biol. Chem.* **277**:5866-5874.
  13. **Bartowsky E, Normark S.** 1991. Purification and mutant analysis of *Citrobacter freundii* AmpR, the regulator for chromosomal AmpC beta-lactamase. *Mol. Microbiol.* **5**:1715-1725.
  14. **Bartowsky E, Normark S.** 1993. Interactions of wild-type and mutant AmpR of *Citrobacter freundii* with target DNA. *Mol. Microbiol.* **10**:555-565.
  15. **Choi H, Kim S, Mukhopadhyay P, Cho S, Woo J, Storz G, Ryu S.** 2001. Structural basis of the redox switch in the OxyR transcription factor. *Cell* **105**:103-113.
  16. **Kullik I, Stevens J, Toledano MB, Storz G.** 1995. Mutational analysis of the redox-sensitive transcriptional regulator OxyR: regions important for DNA binding and multimerization. *J. Bacteriol.* **177**:1285-1291.
  17. **Kullik I, Toledano MB, Tartaglia LA, Storz G.** 1995. Mutational analysis of the redox-sensitive transcriptional regulator OxyR: regions important for oxidation and transcriptional activation. *J. Bacteriol.* **177**:1275-1284.
  18. **Dangel AW, Gibson JL, Janssen AP, Tabita FR.** 2005. Residues that influence *in vivo* and *in vitro* CbbR function in *Rhodobacter sphaeroides* and identification of a specific region critical for co-inducer recognition. *Mol Microbiol* **57**:1397-1414.
  19. **Craven SH, Ezezika OC, Haddad S, Hall RA, Momany C, Neidle EL.** 2009. Inducer responses of BenM, a LysR-type transcriptional regulator from *Acinetobacter baylyi* ADP1. *Mol Microbiol* **72**:881-894.
  20. **Ezezika OC, Haddad S, Neidle EL, Momany C.** 2007. Oligomerization of BenM, a LysR-type transcriptional regulator: structural basis for the aggregation of proteins in this family. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **63**:361-368.
  21. **Bykowski T, van der Ploeg JR, Iwanicka-Nowicka R, Hryniewicz MM.** 2002. The switch from inorganic to organic sulphur assimilation in *Escherichia coli*: adenosine 5'-phosphosulphate (APS) as a signalling molecule for sulphate excess. *Mol Microbiol* **43**:1347-1358.
  22. **Stec E, Witkowska-Zimny M, Hryniewicz MM, Neumann P, Wilkinson AJ, Brzozowski AM, Verma CS, Zaim J, Wysocki S, Bujacz GD.** 2006. Structural basis of the sulphate starvation response in *E. coli*: crystal structure and

- mutational analysis of the cofactor-binding domain of the Cbl transcriptional regulator. *J Mol Biol* **364**:309-322.
23. **Colyer TE, Kredich NM.** 1996. *In vitro* characterization of constitutive CysB proteins from *Salmonella typhimurium*. *Mol. Microbiol.* **21**:247-256.
  24. **Hanahan D.** 1985. Techniques for transformation of *E. coli*, p. 109-114. *In* Glover D (ed.), *DNA Cloning, A Practical Approach*, vol. 1. IRL Press, Oxford.
  25. **Meade HM, Long SR, Ruvkun GB, Brown SE, Ausubel FM.** 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn5 mutagenesis. *J. Bacteriol.* **149**:114-122.
  26. **Honma M, Ausubel F.** 1987. *Rhizobium meliloti* has three functional copies of the *nodD* symbiotic regulatory gene. *Proc. Natl. Acad. Sci USA* **84**:8558-8562.
  27. **Karimova G, Pidoux J, Ullmann A, Ladant D.** 1998. A bacterial two-hybrid system based on a reconstituted signal transduction pathway. *Proc Natl Acad Sci U S A* **95**:5752-5756.
  28. **Yeh K, Wu S, Murphy J, Lagarias J.** 1997. A cyanobacterial phytochrome two-component light sensory system. *Science* **277**:1505-1508.
  29. **Vieira J, Messing J.** 1987. Production of single stranded plasmid DNA. *Meth. Enzymol.* **153**:3-11.
  30. **Ogawa J, Long SR.** 1995. The *Rhizobium meliloti groELc* locus is required for regulation of early *nod* genes by the transcription activator NodD. *Genes Dev.* **9**:714-729.
  31. **Yeh KC, Peck MC, Long SR.** 2002. Luteolin and GroESL modulate *in vitro* activity of NodD. *J. Bacteriol.* **184**:525-530.
  32. **Figurski DH, Helinski DR.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in trans. *Proc Natl Acad Sci U S A* **76**:1648-1652.
  33. **Wells DH, Long SR.** 2002. The *Sinorhizobium meliloti* stringent response affects multiple aspects of symbiosis. *Mol Microbiol* **43**:1115-1127.
  34. **Fisher RF, Egelhoff TT, Mulligan JT, Yelton MM, Long SR.** 1988. *Rhizobium meliloti* NodD binds to DNA sequences upstream of inducible nodulation genes., p. 391-398. *In* Boethe H, de Bruijn FJ, Newton WE (ed.), *Nitrogen fixation: hundred years after*. Gustav Fischer, New York.
  35. **Egelhoff TT.** 1987. Molecular analysis of *Rhizobium meliloti* nodulation genes and gene products. Ph. D. thesis. Stanford University, Stanford, CA.