Supplemental Figure 1 Structural alignment of the NIP family with AQP1 and GlpF

The amino acid sequences of soybean nodulin 26 and the nine members of the Arabidopsis NIP family were aligned based on sequence and structural similarity with bovine AQP1 and *E. coli* GlpF by using the Multiple Alignment program of the MOE software as described in the Materials and Methods. The sequences are numbered as follows: **1** AQP1, **2** GlpF, **3** *Arabidopsis thaliana* (At)NIP1;1, **4** AtNIP1;2, **5** AtNIP2;1; **6** AtNIP3;1; **7** AtNIP4;1; **8** AtNIP4;2 **9** AtNIP5;1, **10** AtNIP6;1, **11** AtNIP7;1, **12** soybean nodulin 26. Also shown are the structurally aligned homology model of **13** soybean nodulin 26 and **14** AtNIP6;1. The NIP3;1 sequence used in this alignment is a composite cDNA based on the annotation of gene At1g31885 based on the transcriptome analysis of Yamada et al. (2003). The red bars above the sequences denote the positions of transmembrane and NPA α-helices (as outlined in Fig. 1 in the manuscript text) based on experimental structures (AQP1 and GlpF) and homology models (NIP6:1 and nodulin 26). The blue and green bars above sequences represent regions of loops that constitute turns. The aligned ar/R residues are highlighted in vertical blue boxes.

Multiple sequence alignment (residue positions 1–253).

Block 1 (positions 1–80):

```
      5    10   15   20   25   30   35   40   45   50   55   60   65   70   75   80
1   ..................................................................MASEFKKKLF
2   ..................................................................TL
3   M....ADISG.......NGY·GNAREEVVMVNLKDEVEHQQEM.........EDIHN·PRPLKKQD.......SLLSVSVPF
4   M....AEISG.......NGG·DARDGAVVVNLKEEDEQQQQQ.........QAIH···KPLKKQD.......SLLSISVPF
5   M....DDISV....SK..SNHGNVVVLNIKASSLADTSL.........PSNKHE....SSSPPLLSVHF
6   M....AEISDI.........TTQTQTVVLDIENYQSIDDS.........RSSD....LSAPLVSVSF
7   M....SSHSD.......EIEEEQISRIEKGKGKDCQG.........GIETVI.......CTSPSI·VCL
8   M....TSHGE.........EIEDEQISRIEKGNCKDSQG.........GMETAI.......CSSPSI·VCL
9   MAPPEAEVGAVMVAPPTPGTPGTPGGPLITGMRVDSMSFDHRKPTPR....CKCLPVMGSTWGQHD...TCFTDFPSPDVSL
10  M....DHEEIPSTPSTP·ATTPGTPGAPLFGGFEGKRNGHNGRY·TPKSLLKSCKCFSV·DNEWALEDGRLPPVTCSLPPPNVSL
11  M....NGEAR.........SRVVDQEAGSTPSTLRDEDH.........PSRQRL....FGCLPYDIDLNP
12  M....ADYSA.........GTESQEVVVNVTKNTSETIQR.........SDSLVSVPF
13  ..................................................................PF
14  ..................................................................CSLPPPNVSL
```

Block 2 (positions 85–170):

```
      90   95   100  105  110  115  120  125  130  135  140  145  150  155  160  165
1   WRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQDNVKVSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISVLR
2   KGQCIAEFLGTGLLIFFGVGCVAALKVAG......ASFGQWEISVIWGLGVAMAIYLTAGVSGAHLNPAVTIALWLFACFDKRK
3   LQKLIAEFLGTYFLVFTGCASVVVNMQND......NVVTLPGIAIVWGLTIMVLIYSLGHISGAHINPAVTIAFASCGRFPLKQ
4   LQKLMAEVLGTYFLIFAGCAAVAVNTQHD......KAVTLPGIAIVWGLTVMVLVYSLGHISGAHFNPAVTIAFASCGRFPLKQ
5   LQKLLAELVGTYYLIFAGCAAIAVNAQHN......HVVTLVGIAVVWGIVIMVLVYCLGHLS·AHFNPAVTLALASSQRFPLNQ
6   VQKLIGEFVGTFTMIFAGCSAIVVNETYG......KPVTLPGIALVWGLVVTVMIYSIGHVSGAHFNPAVSIAFASSKKFPFNQ
7   TQKLIAEMIGTYFIVFSGCGVVVVNVLYG......GTITFPGICVTWGLIVMVMIYSTGHISGAHFNPAVTVTFAIFRRFPWHQ
8   TQKLIAEMIGTYFIIFSGCGVVVVNVLYG......GTITFPGICVTWGLIVMVMIYSTGHISGAHFNPAVTVTFAVFRRFPWYQ
9   TRKLGAEFVGTFILIFTATAGPIVNQKYD......GAETLIGNAACAGLAVMIIILSTGHISGAHLNPSLTIAFAALRHFPWAH
10  YRKLGAEFVGTLILIFAGTATAIVNQKTD......GAETLIGCAASAGLAVMIVILSTGHISGAHLNPAVTIAFAALKHFPWKH
11  LRIVMAELVGTFILMFSVCGVISSTQLSG......GHVGLLEYAVTAGLSVVVVVYSIGHISGAHLNPSITIAFAVFGGFPWSQ
12  LQKLVAEAVGTYFLIFAGCASLVVNENYY......NMITFPGIAIVWGLVLTVLVYTVGHISGGHFNPAVTIAFASTRRFPLIQ
13  LQKLVAEAVGTYFLIFAGCASLVVNENYY......NMLTFPGIAIVWGLVLTVLVYTVGHISGGHFNPAVTIAFASTRRFPLIQ
14  YRKLGAEFVGTLILIFAGTAT····AIVNQ···KTDGAETLIGCAASAGLAVMIVILSTGHISGAHLNPAVTIAFAALKHFPWKH
```

Helix1   *Helix2*   *NPA Loop B*

Block 3 (positions 170–253):

```
      175  180  185  190  195  200  205  210  215  220  225  230  235  240  245  250
1   AIMYIIAQCVGAIVATAILSGITSSLP...............DNSLG·LNALAPGVNSGQGLGIEIIGTLQLVLCVLATTDRR
2   VIPFIVSQVAGAFCAAALVYGLYYNLFFDFEQTHHIVRGSVESVDLAGTFSTYPNPHINFVQAFAVEMVITAILMGLILALTDDG
3   VPAYVISQVIGSTLAAATLRLLFGLDH............DVCS·GKHDVFIGSSPVGSDL·QAFTMEFIVTFYLMFIISGVATDN
4   VPAYVISQVIGSTLAAATLRLLFGLDQ............DVCS·GKHDVFVGTLPSGSNL·QSFVIEFIITFYLMFVISGVATDN
5   VPAYITVQVIGSTLASATLRLLFDLNN............DVCS·KKHDVFLGSSPSGSDL·QAFVMEFIITGFLMLVVCAVTTTK
6   VPGYIAAQLLGSTLAAAVLRLVFHLDD............DVCS·LKGDVYVGTYPSNSNT·TSFVMEFIATFNLMFVISAVATDK
7   VPLYIGAQFAGSLLASLTLRLMFKV.............TPEAFFGTTPADSPA·RALVAEIIISFLLMFVISGVATDN
8   VPLYIGAQLTGSLLASLTLRLMFNV.............TPKAFFGTTPTDSSG·QALVAEIIISFLLMFVISGVATDS
9   VPAYIAAQVSASICASFALKGVF...............HPFMSGGVTIPSVSLGQAFALEFIITFILLFVVTAVATDT
10  VPVYIGAQVMASVSAAFALKAVF...............EPTMSGGVTVPTVGLSQAFALEFIISFNLMFVVTAVATDT
11  VPLYITAQTLGATAATLVGVSVYGV.............NADI·MATKPALSCV·SAFFVELIATSIVVFLASALHCDF
12  VPAYVVAQLLGSILASGTLRLLFMG.............NHDQFSGTVPNGTNL·QAFVEFEFIMTFFLMFVICGVATDN
13  VPAYVVAQLLGSILASGTLRLLFMGNH............DQFSGTVPNGTN·LQAFVFEFIMTFFLMFVICGVATDN
14  VPVYIGAQVMASVSAAFALKAVF...............EPTMS·GGVTVPTVGLSQAFALEFIISFNLMFVVTAVATDT
```

Helix3   *Helix4*

Alignment — positions 260–335:

```
        260   265    270   275    280   285    290   295    300   305    310   315    320   325    330   335
 1 RRDLGG·SGPLAIGFSVALGHLLAIDYTGCGINPARSFGSSVITH······NFQ·····DHWIFWVGPFIGAALAVLIYDFI
 2 NGVPRGPLAPLLIGLLIAVIGASMGPLTGFAMNPARDFGPKVFAWLAGWGNVAFTGGRDIPYFLVPLFGPIVGAIVGAFAYRKL·
 3 RAIGE··LAGLAIGSTVLLNVLIAAPVSSASMNPGRSLGPALVYG·······CYK······GIWIYLVAPTLGAIAGAWVYNTVR
 4 RAIGE··LAGLAVGSTVLLNVIIAGPVSGASMNPGRSLGPAMVYS·······CYR······GLWIYIVSPIVGAVSGAWVYNMVR
 5 RTTEE··LEGLIIGATVTLNVIFAGEVSGASMNPARSIGPALVWG·······CYK······GIWIYLLAPTLGAVSGALIHKMLP
 6 RATGS··FAGIAIGATIVLDILFSGPISGASMNPARSLGPALIWG·······CYK······DLWLYIVSPVIGALSGAWTYGLLR
 7 RAVGE··LAGIAVGMTIMVNVFVAGPISGASMNPARSLGPALVMG·······VYK······HIWVYIVGPVLGVISGGFVYNLIR
 8 RATGE··LAGIAVGMTIILNVFVAGPISGASMNPARSLGPAIVMG·······RYK······GIWVYIVGPFVGIFAGGFVYNFMR
 9 RAVGE··LAGIAVGATVMLNILVAGPSTGGSMNPVRTLGPAVASG·······NYR······SLWVYLVAPTLGAISGAAVYTGVK
10 RAVGE··LAGIAVGATVMLNILIAGPATSASMNPVRTLGPAIAAN······NYR······AIWVYLTAPILGALIGAGTYTIVK
11 VQLGN··LTGFVIGTVISLGVLITGPISGGSMNPARSLGPAVVAW······DFE······DLWIYMTAPVIGAIIGVLTYRSIS
12 RAVGE··FAGIAIGSTLLLNVIIGGPVTGASMNPARSLGPAFVHG······EYE·····GIWIYLLAPVVGAIAGAWVYNIVR
13 RAVGE··FAGLAIGSTLLLNVIIGGPVTGASMNPARSLGPAFVHG······EY······EGIWIYLLAPVVGAIAGAWVYNIVR
14 RAVGE··LAGIAVGATVMLNILIAGPATSASMNPVRTLGPAIAAN······NYR······AIWVYLTAPILGALIGAGTYTIV·
```

Helix5   *NPA Loop E*   *Helix6*

Alignment — positions 345–420:

```
      345   350    355   360    365   370    375   380    385   390    395   400    405   410    415   420
 1 LAPRSSDLTDRVK··VWTS
 2 IGRHL
 3 YTDKPLREITKSG··SFLKTVRIGST
 4 YTDKPLREITKSG··SFLKTVRNGSSR
 5 SIQNAEPEFSKTG··SSHKRVTDLPL
 6 STKKSYSEIIRPN··CNKVSSRDRQEASQDEICVLRVVDPANQNYFICSSPTDINGKCNVTCKLA
 7 FTDKPLRELTKSA··SFLRAVSPSHKGSSSKT
 8 FTDKPLRELTKSA··SFLRSVAQKDNASKSDG
 9 LNDSVTDPPRPVR··SFRR
10 LPEED·EAPKERR··SFRR
11 LKTRPCPSPVSPSVSSLLR
12 YTDKPLSEITKSA··SFLKGRAASK
13 YTDKPL
14 KLPEEDEAPKERR··SFRR
```