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Supplemental Information

Lipid-Mediated Regulation of Embedded Receptor Kinases via Parallel Allosteric Relays

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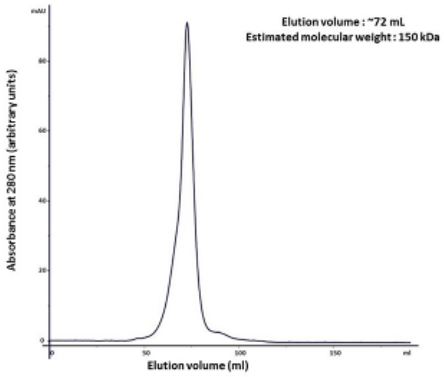
SUPPORTING MATERIAL



Supplementary figure 1: Reconstitution of EnvZ in *E. coli* total lipid nanodisc

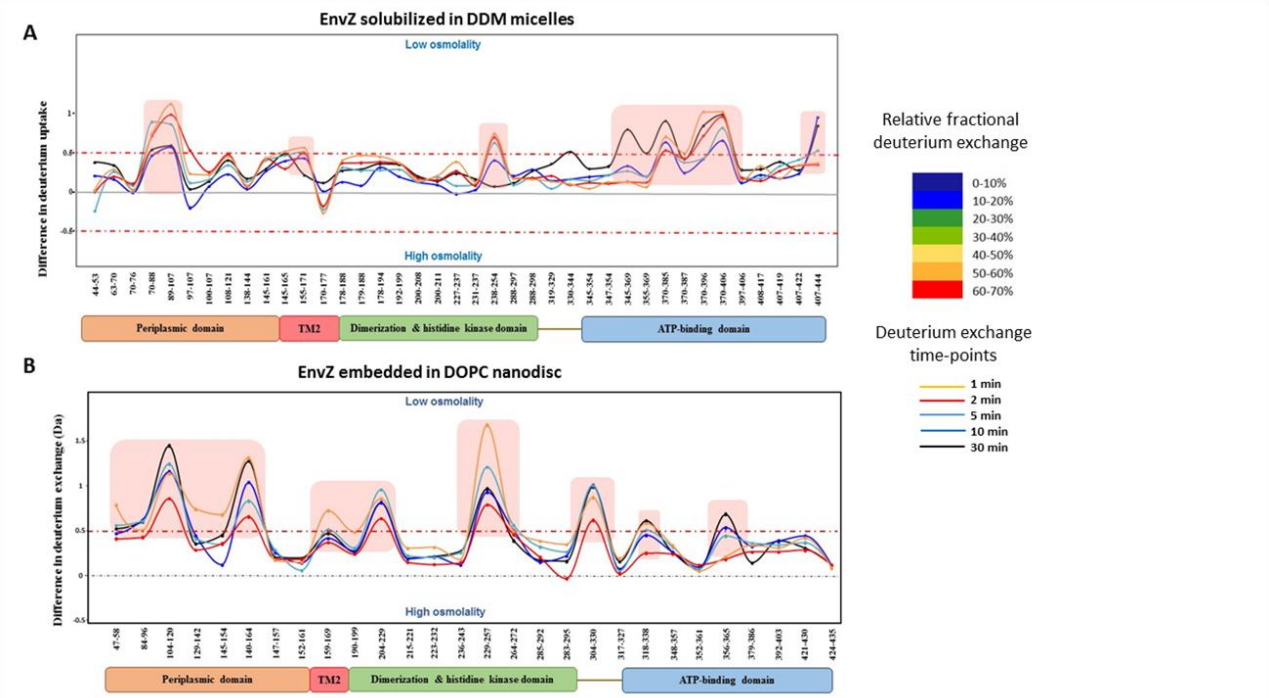
Full length EnvZ was embedded into an *E. coli* total lipid nanodisc and subjected to size-exclusion chromatography in a HiLoad 16/60 Superdex 200 prep grade column. The chromatogram shows a single monodisperse peak corresponding to an estimated molecular weight of ~ 150 kDa which is equivalent to the theoretical molecular weight of an EnvZ dimer (~100kDa) embedded in a nanodisc encircled by a MSP1D1 dimer (~50 kDa).

MSP1D1 peptides ($[M+H]^+$ m/z; charge state)	***E. coli* total lipid**	**DOPC**
19-25 (849.378; +1)	**2.0**	**1.4**
26-38 (1484.807; +2)	**6.0**	**6.0**
44-59 (1921.917; +3)	**6.9**	**6.9**
72-79 (1122.558; +2)	**2.1**	**2.2**
83-92 (1259.722; +2)	**3.2**	**3.2**
116-126 (1297.654; +3)	**1.9**	**1.8**
127-138 (1414.733; +2)	**2.5**	**2.2**
147-157 (1157.627; +2)	**4.0**	**4.1**

180-187 (943.521; +2)	**2.8**	**3.5**
204-211 (995.55; +2)	**3.4**	**2.6**

Supplementary table 1 : Reproducibility in deuterium exchange of MSP1D1 in nanodiscs of different lipid compositions

Deuterium exchange for select pepsin digest fragments for the membrane scaffold protein MSP1D1 encircling the phospholipid bilayer have been tabulated here. Comparison of deuterium exchange of MSP1D1 in E. coli total lipid and DOPC nanodiscs shows that the lipid composition only has a minor impact on the deuterium exchange of MSP1D1. This underscores the reproducibility and integrity of receptor-embedded nanodiscs composed of different lipid compositions. The values reported here are an average of duplicate measurements and lie within a standard deviation of ± 0.1 Da.



Supplementary figure 2: Osmolyte-induced stabilization of EnvZ in non-physiological membrane environments

Comparison of deuterium exchange between low and high osmolality in EnvZ solubilized in DDM micelles and (B) embedded in DOPC nanodisc is demonstrated by a difference plot. A threshold difference of 0.5 Da is considered to be a significant difference and is indicated by the red dashed line on the difference plot. Positive changes denote decreased deuterium exchange while negative changes denote increased exchange in the presence of high osmolality. Regions boxed in red undergo decreased deuterium exchange under high osmolality. Under both conditions, stabilization of the His^{243} region is observed. Multiple regions of the protein respond to osmolality in both DDM micelles and DOPC nanodisc, including the transmembrane, periplasmic and kinase domains besides the dimerization and autophosphorylation domain..