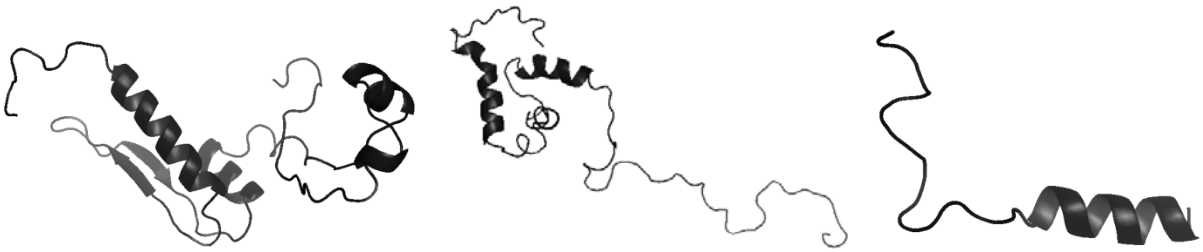
Supplementary Figure 1
Prediction(s) from PHYRE server

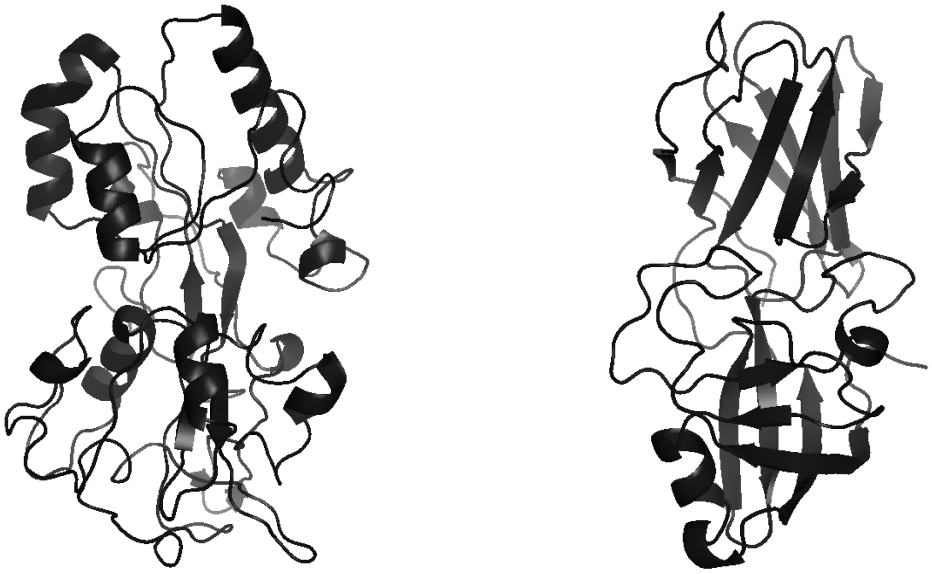


1ea6
R_G – 21.1 Å
D_{max} – 75.3 Å
Helix – 26 %
Sheet – 10 %
Residues – 60-173

1jvr
R_G – 30.3 Å
D_{max} – 108.6 Å
Helix – 23%
Sheet –6 %
Residues – 176-283

1cw5
R_G – 14.9 Å
D_{max} – 47 Å
Helix- 39 %
Sheet – 0%
Residues – 283-310

Prediction(s) from FUGUE server

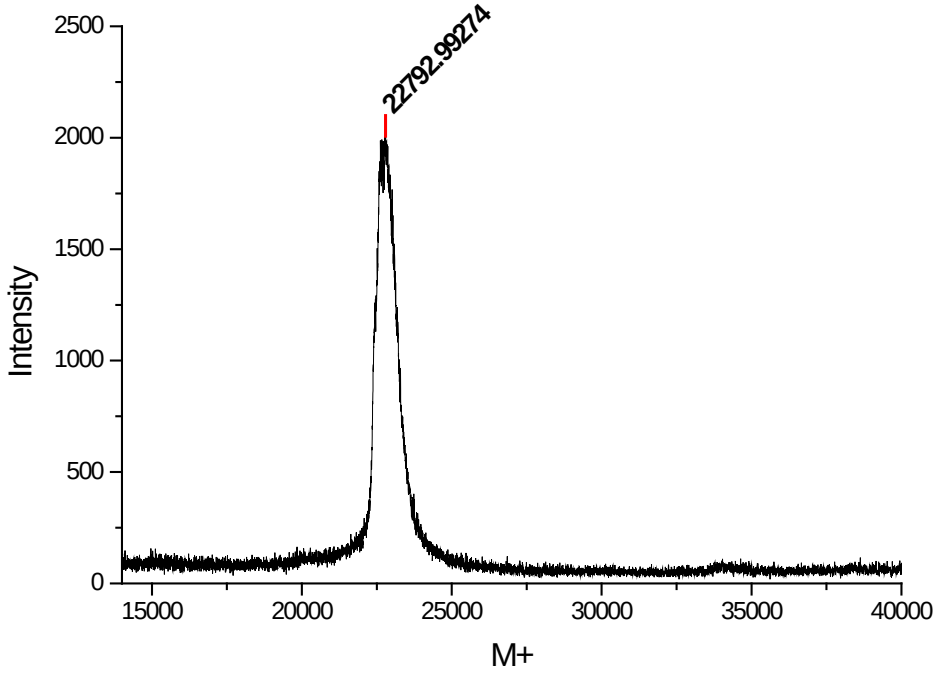


2v3q
D_{max} – 71 Å
R_G – 21 Å
Helix- 25 %
Sheet – 15 %

3d30
D_{max} – 61 Å
R_G – 18 Å
Helix- 5 %
Sheet – 35 %

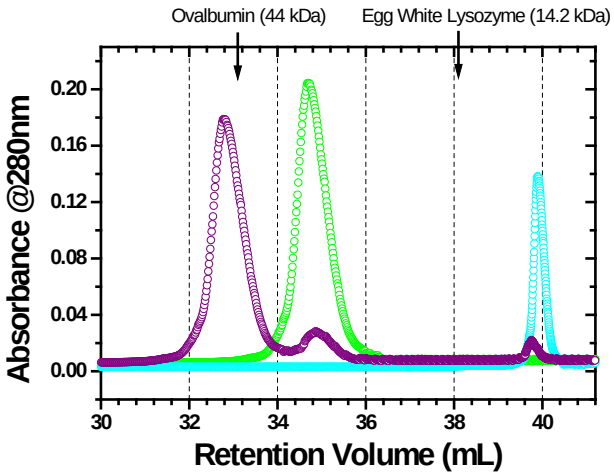
Predictions from PHYRE and FUGUE servers (with their templates) are shown. The calculated Dmax, RG and secondary structure content is listed below the respective models.

Supplementary Figure 2
Mass spectra of tagless Neph1-CD protein

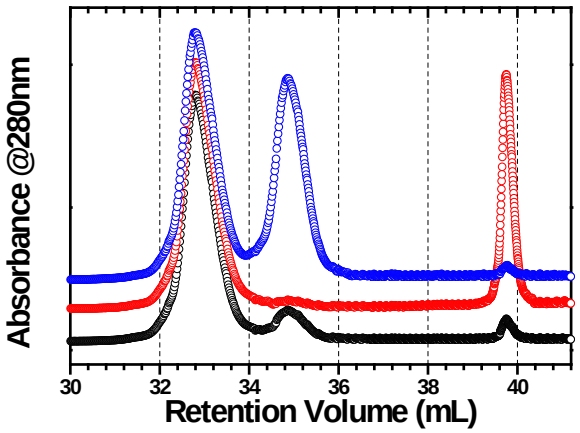


MALDI-TOF result from the sample that was obtained from the proteolysis of His-Neph1-CD is shown. A single peak around 22.7 kDa was observed and the 35 kDa peak corresponding to the parent protein was absent.

Supplementary Figure 3

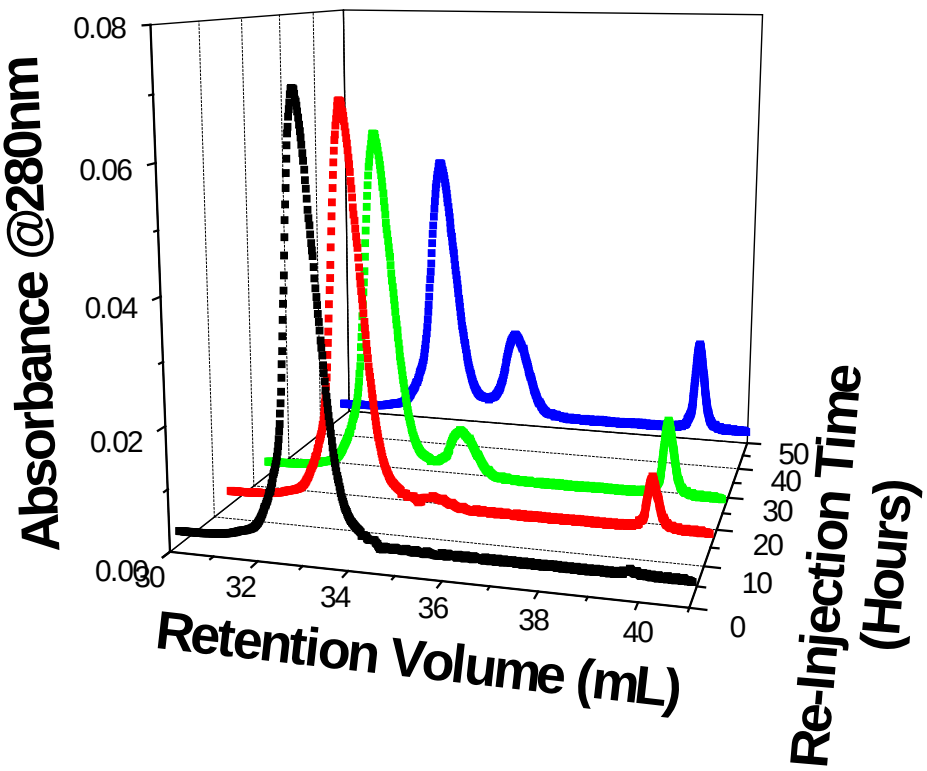


Gel filtration profiles of 0.5 mg/ml samples of His-Neph1-CD (green), His-ZO-1-PDZ1 (cyan) and their 1:1 equimolar mixture (purple) obtained using two Superdex200 columns connected in tandem. The UV-Vis detector was connected at the exit point (within 50 µL) of the second column.



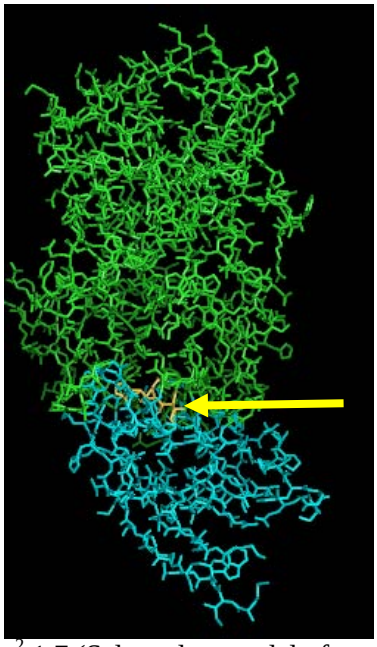
Gel filtration profile of 1:1 (black), 1:2 (red) and 2:1 (blue) molar mixtures of His-Neph1-CD-ZO-1-PDZ1 using the FPLC set-up described above.

Supplementary Figure 4

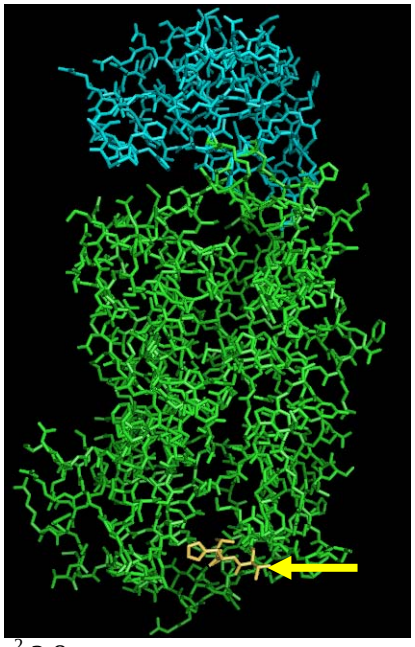


The gel filtration profile of 1:1 equimolar His-Neph1-CD-ZO-1-PDZ1 complex purified from FPLC and re-injected on the columns after 2 hours (black), 14 hours (red), 24 hours (green) and 48 hours (blue). The FPLC was carried out using the set-up described in Supplementary Figure 3.

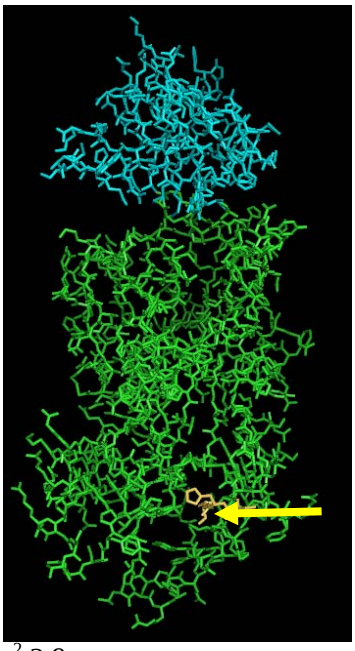
Supplementary Figure 5



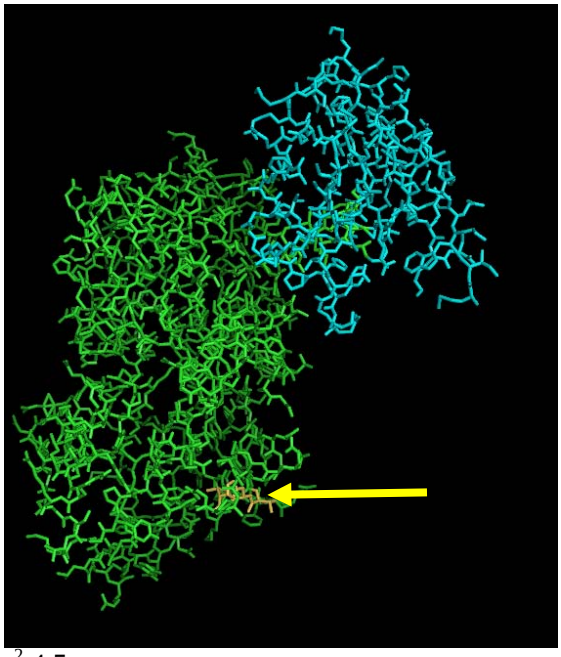
χ^2 1.7 (Selected as model of complex in this work)



χ^2 2.8



χ^2 2.9



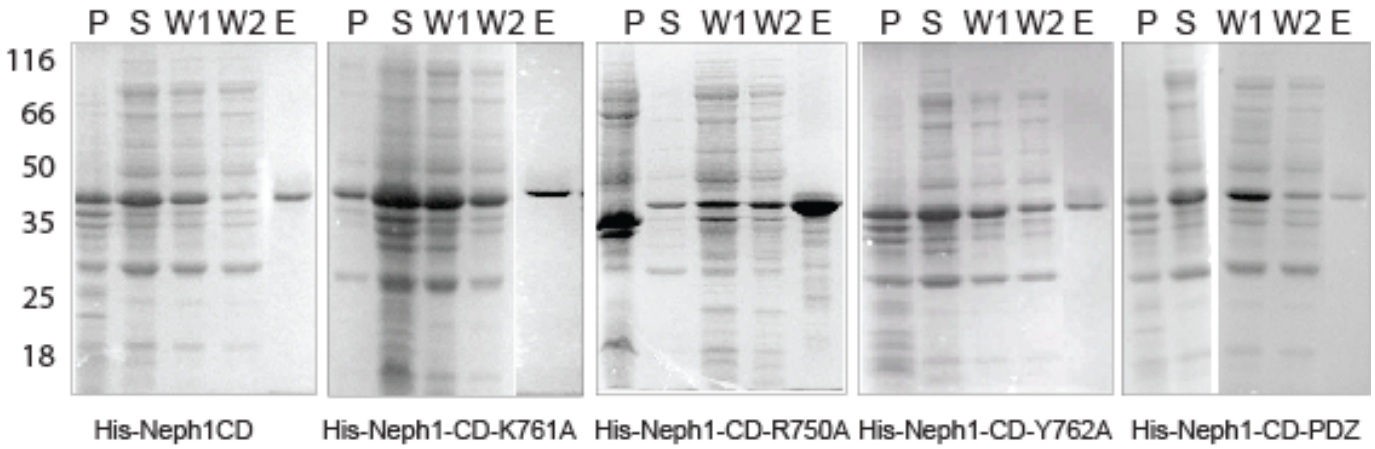
χ^2 4.5

The various relative orientations of His-Neph1-CD and ZO-1-PDZ1, computed by SASREF program are shown. The similarity of theoretical SAXS profile of the respective model to the measured data is reflected in the computed χ^2 value (listed below each figure). The coordinates for the His-Neph1-CD (modeled structure) and ZO-1-PDZ1 (Crystal Structure) are represented in green and cyan respectively. The C-terminal residues "THV" in Neph1 are highlighted in yellow (also indicated by the arrow).

Supplementary Figure 6

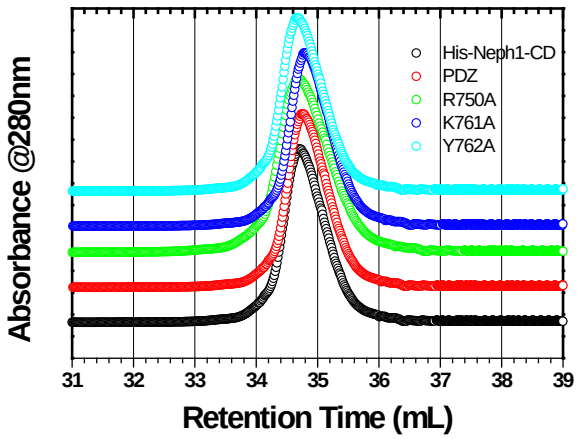
A.

Purification profile of Neph1CD and its mutants.



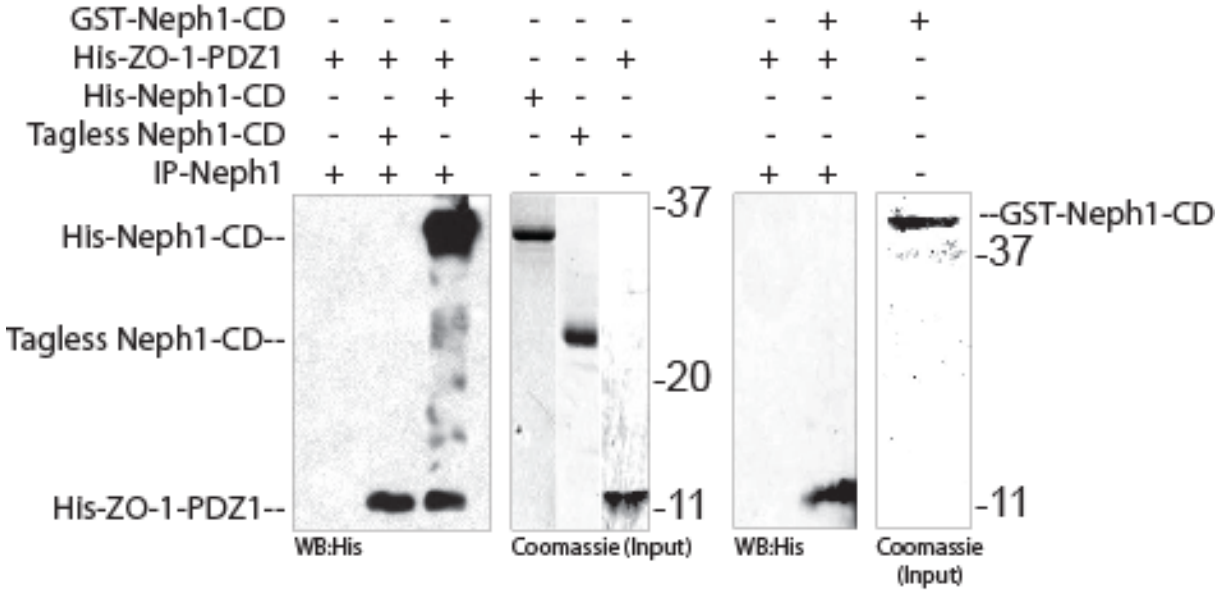
Cytoplasmic domain of Neph1 including wild type and its various mutants were recombinantly expressed in bacteria and purified using Ni-NTA column. P=pellet, S=supernatant, W1=first wash, W2=second wash, E=elute from the column.

B.



The Gel filtration profile of His-Neph1-CD and its mutants (concentration 0.4-0.7 mg/ml) obtained using the set-up described in Supplementary Figures 3 and 4.

Supplementary Figure 7



Tagless Neph1 binds His-ZO-1-PDZ1. Equimolar amounts of purified tagless Neph1 protein (cytoplasmic domain) or His-Neph1CD (as positive control) were incubated with purified His-ZO-1-PDZ1 protein and the pull down was performed using Neph1 rabbit polyclonal antibody and the complex was analyzed for the presence of His-ZO-1-PDZ1 using His monoclonal antibody. His-ZO-1-PDZ1 pull down is shown in the left panel (pull down is observed with untagged and His-tagged Neph1CD) and the right panel shows coommassie staining of the input proteins.