Supplementary Information

Coarse Grained Simulations Suggest the Epsin N-Terminal Homology Domain Can Sense Membrane Curvature without Its Terminal Amphipathic Helix

Alexis Belessiotis-Richards$^{1,2,3}$, Stuart G. Higgins$^{1,2,3}$, Mark S. P. Sansom$^{4}$, Alfredo Alexander-Katz$^{5}$* and Molly M. Stevens$^{1,2,3}$*

$^1$Department of Materials, Imperial College London, London SW7 2AZ, United Kingdom
$^2$Department of Bioengineering, Imperial College London, London SW7 2AZ, United Kingdom
$^3$Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, United Kingdom
$^4$Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom
$^5$Department of Materials Science & Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

*Corresponding Authors
E-mails: m.stevens@imperial.ac.uk (M.M.S.); aalexand@mit.edu (A.A.K.)

S1 Supplementary Discussion

Protein Localization

Figure 3 illustrates the protein localization behavior. Note that for the flexible ENTH (w/o H0)/PC case, the protein domain remained bound to the membrane in only half of the simulations, suggesting that H0 adds a significant amount of stability to the domains ability to bind neutral membranes.

We also observe that flexible ENTH domains seeded onto PIP2-containing membranes show an enhancement of localization between 0 and 5 nm. As well as an increase in fold change, this is also consistent with the observed concentration of PIP2 to concave membrane curvature in the interior of the pore (as shown in Figure 2b ii and in Figures S3e and S3g). This suggests that protein
binding to this region can be attributed to local PIP2 enrichment. Note that the scalloped features in Supplementary Figure S3 at 16 nm and 20 nm are artefacts due to the rectangular simulation cell.

Interestingly, when the domains are modelled with the rigid elastic network, while localization to convex membrane curvature is still observed, this behavior is more dependent upon membrane lipid density (as indicated by the lower peak fold changes at around 12 nm in Figures 3b, 3d, 3e and 3h). In the elastic network ENTH/PC/PIP2 case, we observe almost total dependence on PIP2 (indicated by a fold change of ~1 across the entire radius, as shown in Figure 3f). This suggests that restricting the flexibility of the domain increases the strength with which the domain can bind to PIP2, while hampering its ability to sense curvature.

We also find that the ENTH (w/o H0) domain consistently shows enhanced localization to all of the membranes studied here (PC-, PC/PIP2- and PC/PS-containing) irrespective of whether the domain is modelled as flexible or with an elastic network, see Supplementary Figure S4 for PC/PS data. The flexible ENTH (w/o H0) domain is so strongly localized to curvature that the domain remained bound to the curved region during our simulations. In order to confirm the consistency of this behavior, multiple simulations were made from two starting positions, one starting above the central pore and the other above curvature, with the combined results shown in Figures 3g and 3h (an illustration of this process is shown in Figure S5). We also replicated this profile by performing a “mutated” simulation case whereby a snapshot from the flexible ENTH/PC/PIP2
case was taken as a starting position and truncated, to remove H0, before running simulations, also shown in Figure S5.

Comparing the specific case of PIP2-containing membranes illustrates well the impact of modelling the ENTH domain either as flexible or with a rigid elastic network (Figures 3e-3f and Figures 3g-3h). For flexible ENTH domains, the presence or absence of H0 makes little difference to the fold change profile, suggesting H0 may not be the main actor for curvature localization. This is also observed for elastic network ENTH domains interacting with PC-only membranes, where the presence or absence of H0 also makes little difference to the fold change profile of the proteins (Figure 3b and 3d). When PIP2 is added, however, the localization of elastic network domains with H0 is mainly driven by PIP2 localization (again, as indicated by the broadly constant fold change of ~1 in Figure 3f). For the same system but without H0, a minor curvature sensing enhancement is observed, reinforcing the notion that H0 plays a relatively minor role. When interacting with PS-containing membranes, we observed similar behavior, flexible ENTH and ENTH (w/o H0) domains strongly sense curvature while the elastic network ENTH domain is more dependent on PIP2. In addition, on PS-membranes, ENTH (w/o H0) domains sense curvature very similarly both when flexible and with a rigid elastic network (Supplementary Figure S4).

Lipid Binding

We analyzed how the residues of each domain bind to PC-, PC/PIP2- and PC/PS-containing membranes. Figure 4 shows these contacts for the first 100 residues of the domains on PC and
PIP2-containing membranes (Figures S6 shows the full contact analysis for all 160 residues of the ENTH domain). Figures 4a shows the hydrophobic contacts between the ENTH domain and the different membranes. Interestingly, we find that in flexible domains helices H3 and H0 compete for hydrophobic access to the membrane. For example, on PC-only membranes (Figure 4a i), the flexible ENTH domain shows hydrophobic interactions at both H0 and H3 with the overall contacts at H3 being elevated. However, the activity of H3 is completely removed for ENTH domains with an elastic network. When H0 is removed (Figure 4a ii), we conserve the hydrophobic action of H3 in flexible domains. When an elastic network is added, we still observe hydrophobic contacts around the same region except that it is localized approximately to the edge of H3. On PC/PIP2 membranes, the activity of H3 is greatly reduced for flexible ENTH domains and is again not present with an elastic network (Figure 4a iii). For the ENTH (w/o H0) domains however, the hydrophobic activity of H3 is conserved both when flexible and with a rigid elastic network (Figure 4a iv). The consistent hydrophobic activity of H3 suggests innate membrane curvature sensitivity of ENTH enabling it to be guided to regions of membrane curvature even before the formation of H0. Combined with the localization behavior in Figure 3, these results suggest that H0 alone is insufficient to sustain membrane curvature sensitivity in the ENTH domain.

On PC/PS-containing membranes, we see similar binding behavior, with H3 being hydrophobically engaged for ENTH (w/o H0) when flexible and with an elastic network, see Supplementary Figure S7.
We performed the same binding analysis of our protein domains to flat membranes as a control shown in Supplementary Figure S8. On PC-only flat membranes, we observed H3 activation only for the ENTH (w/o H0) domain and not ENTH. On PC/PIP2 membranes, neither ENTH domain, flexible or with elastic network, strongly engages H3. This suggests that H3 is curvature specific for PIP2-containing membranes. On flat PC/PS membranes, however, we see strong activation of H3 for both ENTH and ENTH (w/o H0) domains. This can be explained by the smaller headgroup size of PC and PS which allows (see Figure 1b) the domains to flatten onto the membrane as it binds, exposing more hydrophobic residues.

S2 Supplementary Figures

Figure S1: (a) Structural comparison of the two ENTH domain structures used in this study (1EDU.pdb and 1H0A.pdb) colored by similarity between the structure. (b) Q values and radial mean squared distance (RMSD) difference between two ENTH domain structures used in this study as a function of residue number.
Figure S2: Snapshots of the systems studied for this work. (a) Curved membrane showing box size. (b) Flat membranes of different compositions and (c) a slice through the middle of the system shown in (a) highlighting membrane curvature.
Figure S3: Protein and lipid density profiles normalized over 100 bins used to calculate fold change in ENTH domain localization behavior in Figure 3 of the main manuscript. Each histogram was normalized to the total number of counts recorded for each simulation case. Note that the scalloped features at 16 nm and 20 nm are artefacts due to the rectangular simulation cell.
**Figure S4:** Statistical frequency heat maps showing center-of-mass positions of ENTH domains and density profiles normalized to lipid density for (a,b) ENTH domain and (c,d) ENTH (w/o H0) domain interacting with a curved PS-containing membrane. Dashed lines represent the start and stop of tapered region of underlying nanoporous wafer approximating the region of membrane curvature.
Figure S5: Comparison of simulations for the flexible ENTH (w/o H0) PC/PIP2 case showing similar fold change behavior both for a mixed 2-start simulation and for a “mutated” run.
Figure S6: Contact analysis of all residues (0 to 160) of the ENTH domain. (a) Average number of hydrophobic contacts of the ENTH domains in contact with PC- or PC/PIP2-containing membranes. A schematic of the ENTH domains secondary structure as a function of residue number is represented above the figures, each block represents an alpha helix. (b) Distance between PIP2 headgroup center-of-mass and each residue of ENTH computed from the crystal structure (PDB code: 1H0A.pdb). Black asterisks represent binding pockets of PIP2. (c) Average number of contacts with PIP2 headgroup of the ENTH domains in contact with PIP2 containing membranes.
Figure S7: Contact analysis of all residues (0 to 160) of the ENTH domain on curved PS-containing membranes. (a) Average hydrophobic contacts per residue and (b) average PS headgroup contacts per residue.
**Figure S8:** (a) Sequence and secondary structure of the ENTH domain highlighting helices H0 and H3 in pink and orange respectively. Residues 48, 50 and 51 are identified with yellow asterisks. (b) Crystal structure of the ENTH domain with hydrophobic residues of helices H0 and H3 highlighted in yellow and hydrophobic surfaces in transparent yellow along with helical wheels for helices H3 and H0 showing hydrophobic moment of each helix produced by HELIQUEST.1 Residues 48, 50 and 51 are marked on the wheel and structure as these residues are hydrophobically active in the elastic network ENTH (w/o H0) case (see Figure 4a ii and iv).
Figure S9: Snapshots of flexible and elastic network proteins interacting with curvature for (a) ENTH/PC/PIP2 and (b) ENTH (w/o H0)/PC/PIP2 case. Black arrows have been added to show the elastic network proteins strong orientation away from curvature.
**Figure S10:** Contact analysis of all residues (0 to 160) of the ENTH domain on each simulated case on flat membranes of different composition. (a) Average hydrophobic contacts per residue, (b) distance from PIP2 headgroup to each residue in ENTH domain, (c) average PIP2 headgroup contacts and (d) average PS headgroup contacts per residue.
Figure S11: (a) Backbone snapshots of the average structures of 8 simulations of the flexible ENTH domain (blue) and a single averaged elastic network ENTH domain (green). Note that the first 35 residues of the flexible domains are not represented in the snapshots for clarity. (b) Root Mean Squared Fluctuations (RMSF) and (c) Root Mean Squared Deviations (RMSD) of protein residues of the ENTH domains.
Figure S12: Snapshots of the ENTH domain showing flexibility of its N-terminal region (residues 0 to 35). Shown in green is the average elastic network structure and overlaid are three random snapshots (colored randomly) of the flexible ENTH domains.

Supplementary References

(1) Gautier, R.; Douguet, D.; Antonny, B.; Drin, G. HELIQUEST: A Web Server to Screen Sequences with Specific α-Helical Properties. *Bioinformatics* **2008**, *24* (18), 2101–2102. https://doi.org/10.1093/bioinformatics/btn392.
