Support Information

γ-Secretase Partitioning into Lipid Bilayers Remodels Membrane Microdomains after Direct Insertion

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Contents: Supplementary Figures.

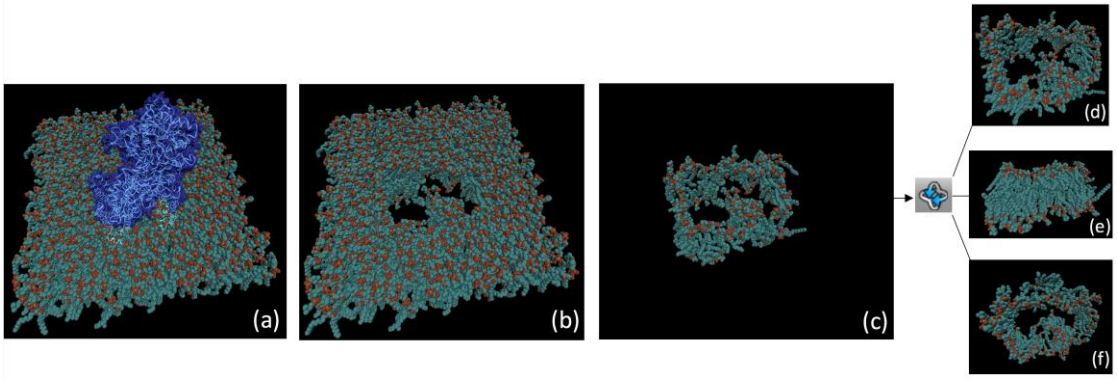

Figure S1: Estimation of the lipid shell surrounding γ–secretase Panel (a) displays the Bai et al structure of γ-secretase secretase embedded in a PC bilayer with the protruding subunit nicastrin is shown blue. In panel (b) the enzyme is hidden and panel (c) shows the lipid shell surrounding the hydrophobic region of the protein. Panels (d)-(f) show, different views of the lipid shell, (e) is the side view.

Note for Fig. S1. An estimate of the number of the lipids needed to form the protein-lipid interface was carried out using the MemprotMD database where an intact bilayer model of the enzyme was derived using the 3.4 Å cryo-EM structure 5a63 of Bai et al. embedded into an explicit phosphatidylcholine (PC) bilayer using an automated protocol.[1,2] Figure S1 (a) displays the Bai et al structure of γ-secretase embedded in a PC bilayer with the protruding subunit nicastrin is shown blue. For clarity, in panel (b) the enzyme is hidden and panel (c) shows the lipid shell surrounding the hydrophobic region of the protein. Panels (d)-(f) show, different views of the lipid shell, (e) is the side view. Inspection of this structure and the lipid-MP interface gives an estimate of ~68 PC lipids that would form a shell surrounding the hydrophobic region of the complex after insertion.

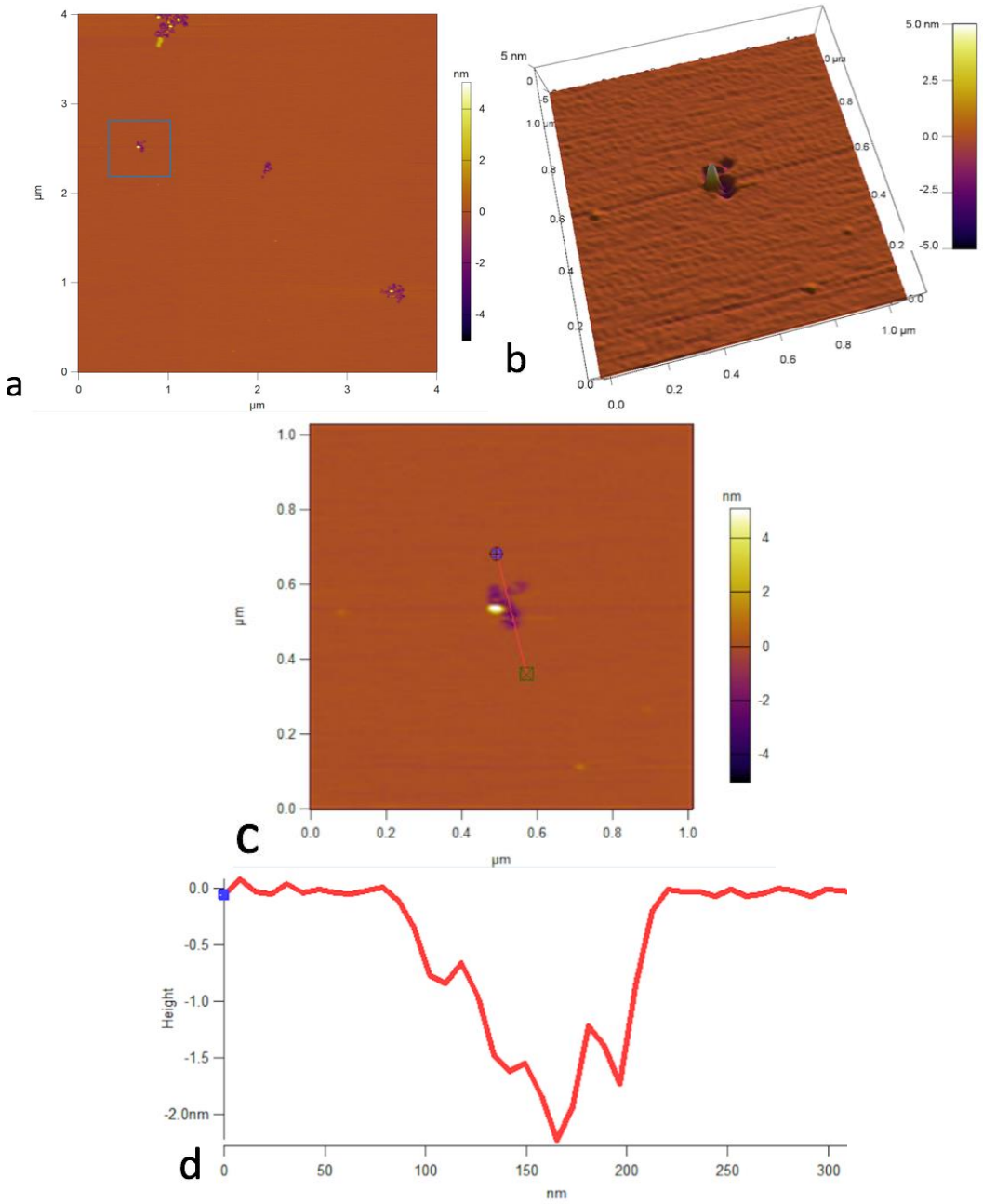

Figure S2: Closer Inspection of Direct Insertion Events for the Enzyme at 37ºC. Panel (a) is the zoomed out 10 x 10 micron image and panel (b) displays a 3D image of the result of the aftermath of a direct insertion event of solubilized γ–secretase, zoomed in from the larger area (inset in (a)), showing evidence for possible lipid loss adjacent to the inserted enzyme. Panel (c) is the 2D image with an indicated line profile, displayed in panel (d).

Note for Figure S2. Zoom in of direct insertion events for the enzyme at 37ºC. In Figure S2, Panel (a) is the zoomed out 10 x 10 micron image and panel (b) displays a 3D image of the result of the aftermath of a direct insertion event of solubilized γ–secretase, zoomed in from a larger area (inset in (a)), showing major lipid loss adjacent to the inserted enzyme. Panel (c) is the 2D image with an indicated line profile, displayed in panel (d). This lipid loss is evident in the dark regions at less than ~2.1 nm height level below the lipid interface, indicative of the loss of the top leaflet of the bilayer.

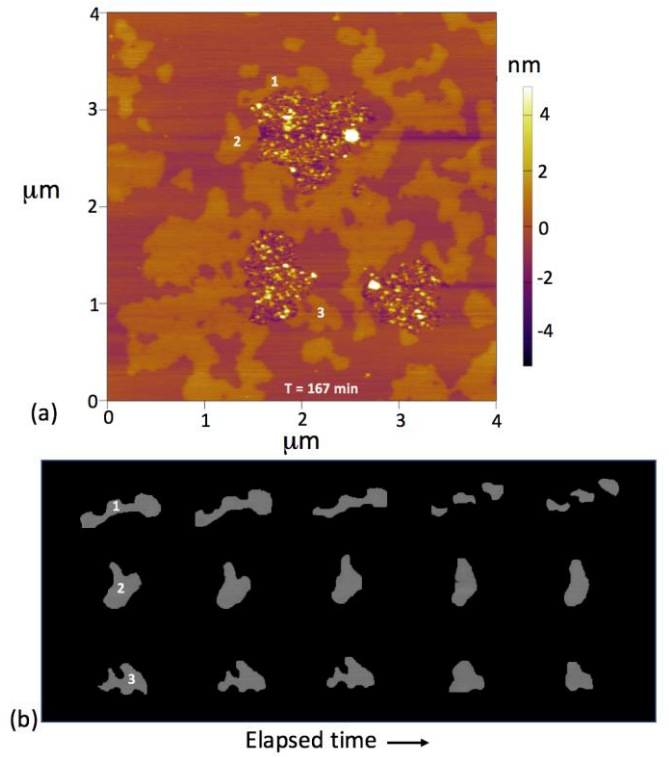

Figure S3: Direct Observation of Enzyme-Adjacent Lo Domain Reorganization by AFM. Panel S3a shows the locations of three relevant adjacent L_o domains that exhibit considerable reorganization during the equilibration (t = 167 min after loading). The changes in adjacent L_o domain area over elapsed incubation time are shown in panel S2b, obtained from thresholding in Igor Pro.

Note for Fig. S3 During the 25ºC incubation over the course of ~2 hrs, Lo domains adjacent to concentrated regions of embedded enzyme under observation by AFM were shown to undergo dramatic reorganization. Panel S3a shows the locations of three relevant adjacent L_o domains that exhibit considerable reorganization during the equilibration (starting at t = 167 min after loading). The changes in adjacent L_o domain area over elapsed incubation time are shown in panel S2b, obtained from thresholding in Igor Pro.

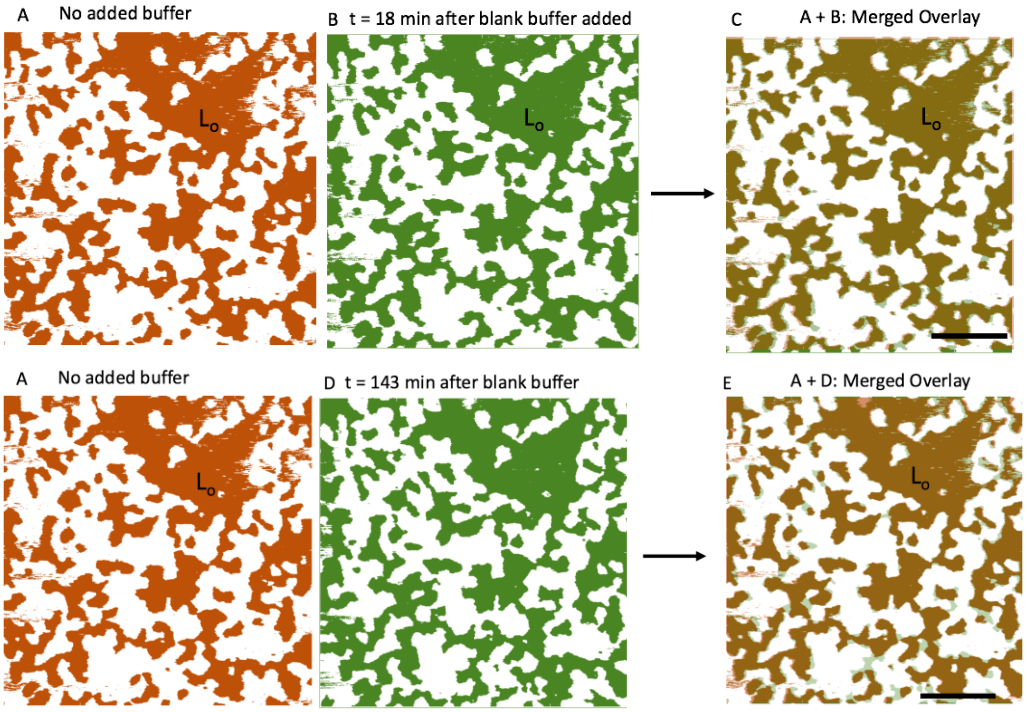

Figure S4 AFM imagery of the impact of blank buffer without γ-secretase on an existing L_o/L_d SLB. Image A is a 4 x 4 micron image of canonical Lo/Ld SLB (DOPC:SM:Chol 2:2:1). Image B was taken 18 minutes after the addition of blank γ-secretase buffer to a L_o/L_d SLB. The merged overlay of A + B is shown in C. Image D was taken 143 minutes after the addition of blank γ-secretase buffer. The merged overlay of A + D is shown in C. The minimal differences in domain structure are indicated by the green regions adjacent to the brown overlay regions, shown in images C and E. Scale bar 1 µm, T = 25ºC.

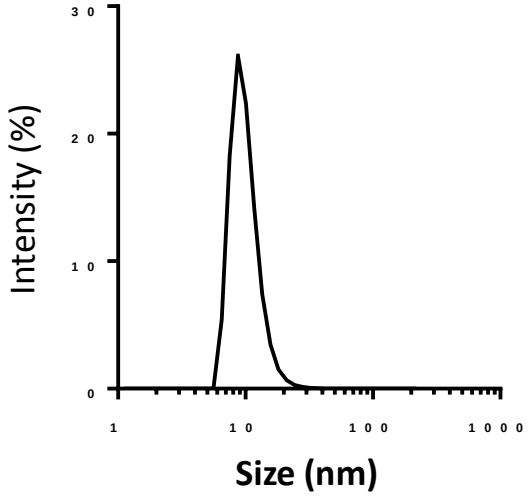

Figure S5: Dynamic Light Scattering of solubilized γ-secretase in solution. Shown above is the size distribution intensity histogram for the enzyme in the detergent dodecylmaltoside (DDM). All measurements were carried out at 0.2 mg/ml in 0.01% DDM. Data was collected using a Malvern Instruments Zetasizer DLS molecular size analyser.

Note for Fig. S5. The majority of the complexes have diameter of 10nm consistent with monomeric size of the γ-secretase complex [3].

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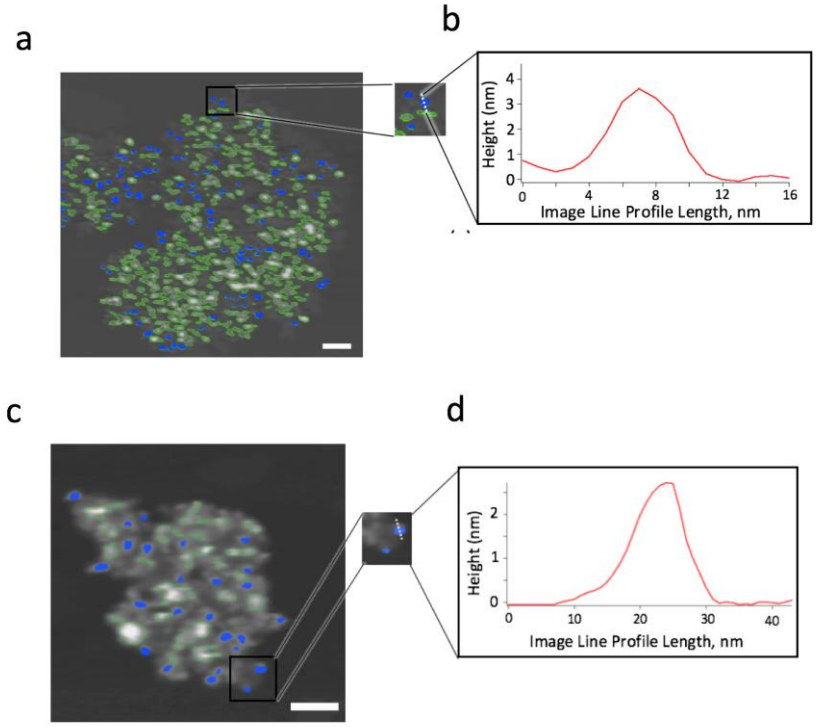

Figure S6: Feature volume analysis of AFM height images of γ-secretase substrates. A) Direct Overlaid image with the results of a particle localization analysis tool in Igor Pro (blue and green overlay; scale bar 100 nm). The inset is zoomed in to a smaller ROI, and shown in b) and an image line profile of the dotted line in the inset. The feature shown has an approximate volume of ~66 nm^3. c) Overlaid image with the results of a particle localization analysis tool in Igor Pro (blue and green overlay; scale bar 50 nm). The inset is zoomed in to a smaller ROI, and shown in d) and an image line profile of the dotted line in the inset. The feature shown has an approximate volume of ~77 nm^3.

Note for Fig. S6 Particle distribution analysis was performed for the SB4 and NTM2 fragments (S6a and S2c), with representative profiles shown in Fig. 4b and 4d for SB4 and NTM2, respectively. The single structures observed have an approximate volume of 66 nm^3 for MBP-SB4 and 77nm^3 for MBP-NTM2, approximately consistent with the volume of MBP.
The molecular volume was calculated using

$$V_m = (\pi h/6)(3r^2 + h^2)$$

where h is the particle height and r is the radius at half-maximum. The calculation of molecular volume based on molecular weight was done using

$$V_c = (M_0/N_0)(V_1 + dV_2)$$

In this equation, M_0 is the molecular mass of the protein, N_0 is Avogadro's number, V_1 and V_2 are the partial specific volumes of protein and water (0.74 and 1 cm^3/g, respectively), and d is the protein hydration (0.4 mol water/mol protein)[Schneider,1998;Saslowsky,2002] Considering Nicastrin has a length of 709 residues and (mass 78.4 kDa:UniProt Database entry: NCSTN) subtracting residues 670-709 – α-helix single pass of 5.2 kDa that is buried in the lipid bilayer region gives a globular size of Nicastrin that would protrude from the membrane as a 73.2 kDa protein ($V_c = (M_0/N_0)(V_1 + dV_2)$ = 147 nm^3/molecule). Likewise for maltose-binding protein, (MBP: 42.4 kDa) would protrude from the membrane as a 73.2 kDa protein ($V_c = (M_0/N_0)(V_1 + dV_2)$ = 85 nm^3/molecule)

References

(1) Bai, X. C.; Yan, C. Y.; Yang, G. H.; Lu, P. L.; Ma, D.; Sun, L. F.; Zhou, R.; Scheres, S. H. W.; Shi, Y. G. *Nature* **2015**, *525*, 212.

(2) Stansfeld, P. J.; Goose, J. E.; Caffrey, M.; Carpenter, E. P.; Parker, J. L.; Newstead, S.; Sansom, M. S. P. *Structure* **2015**, *23*, 1350.

(3) Sun, L.; Zhao, L.; Yang, G.; Yan, C.; Zhou, R.; Zhou, X.; Xie, T.; Zhao, Y.; Wu, S.; Li, X.; Shi, Y. *Proc Natl Acad Sci U S A* **2015**, *112*, 6003.