Supplementary Material for "A structure-based discovery platform for BACE2 and the development of selective BACE inhibitors"

*Yu-Chen Yen[1], Annalissa M. Kammeyer[1], Jagannadharao Tirlangi[2], Arun K. Ghosh[2,3], and Andrew D. Mesecar[1,2,4]**

[1]Department of Biological Sciences; [2]Department of Chemistry; [3]Department of Medicinal Chemistry, [4]Department of Biochemistry, Purdue University, West Lafayette IN 47907

*Corresponding author: amesecar@purdue.edu

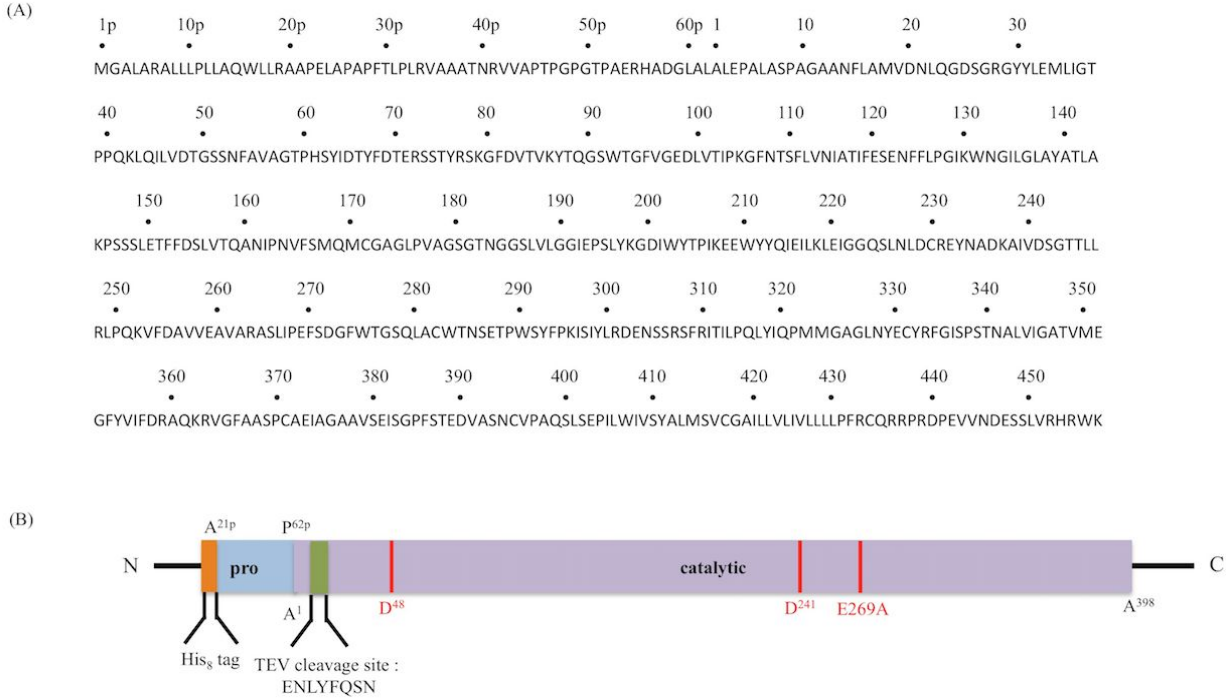

Figure S1. (A) The amino acid sequence and (B) expression construct of BACE2. The catalytic Asp residues and the mutation (E269A) made for crystallization are labelled in red.

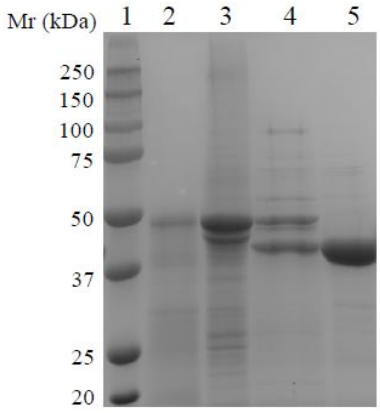

Figure S2. SDS-PAGE analysis throughout the purification of BACE2. Lanes: 1, Molecular weight ladder; 2, protein sample after solubilization; 3, BACE2 after refolding where the active protein starts the auto-cleaving process to remove its pro-domain and the molecular weight begins to drop; 4, BACE2 after ammonium sulfate precipitation with partial completion of the auto-catalytic cleaving process; 5, HIC pool showing complete processing to mature and active BACE2.

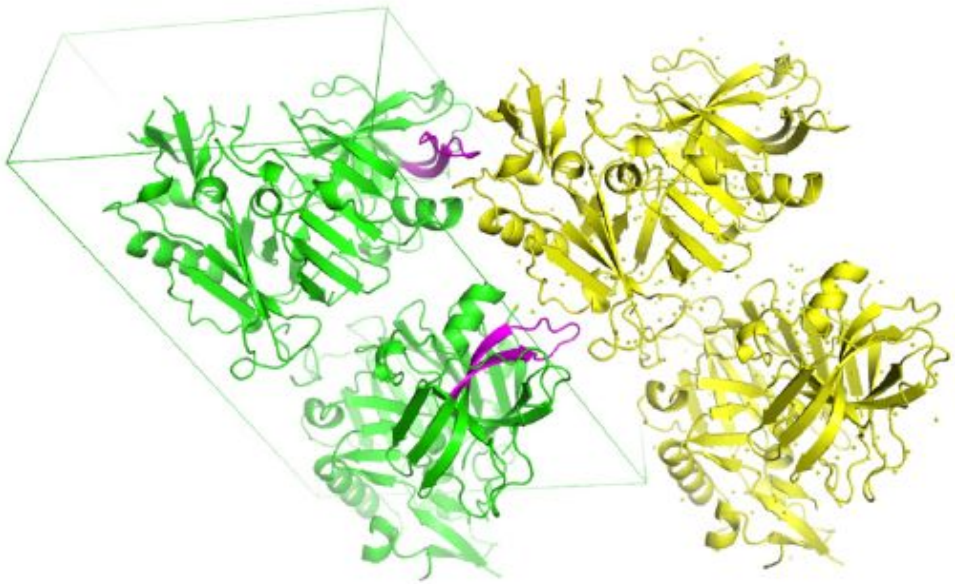

Figure S3. Crystal packing of unbound BACE2 (PDB:6UJ0). Molecules colored in green and yellow represent a non-biological, crystallographic dimer in an asymmetric unit. Loop$^{102\text{-}110}$ is highlighted in magenta in one of the dimer.

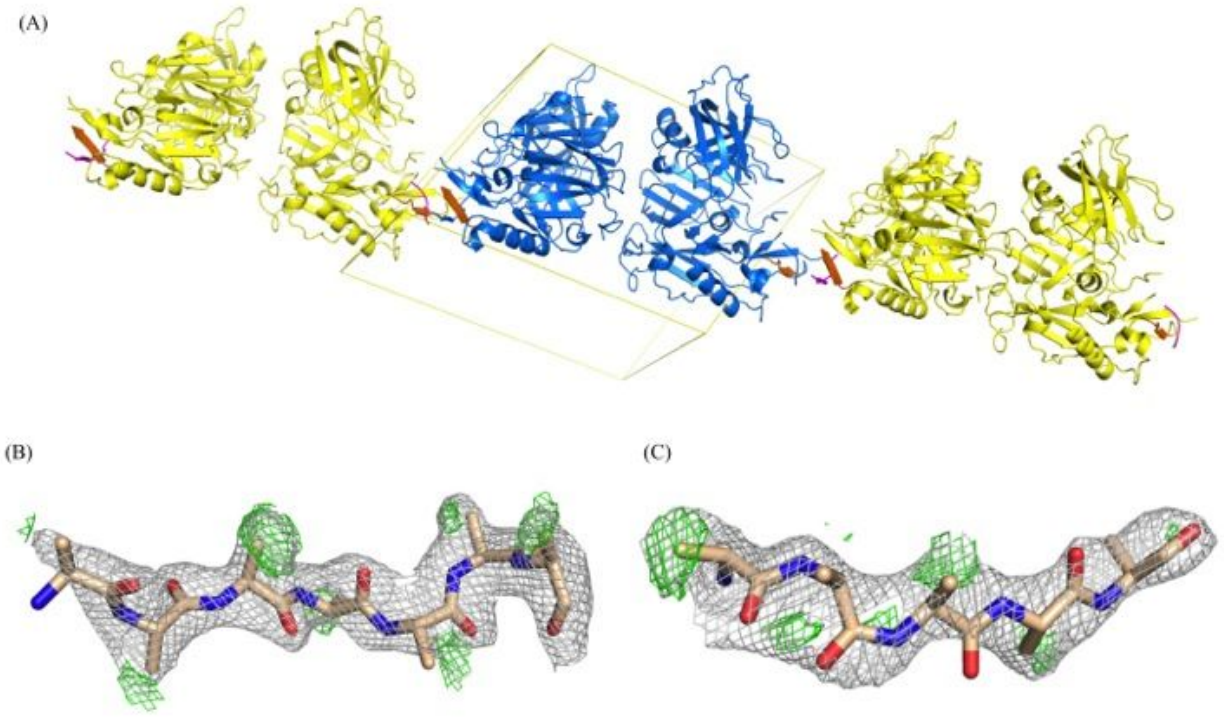

Figure S4. Residual electron density is observed in unbound BACE2 structure (PDB:6UJ0) near residues ^{278}SQLACW^{283}. (A) The crystal packing interfaces of unbound BACE2 are shown near BACE2 residues ^{278}SQLACW^{283} on each side of the unit cell . Molecules colored in blue and yellow represent non-biological, crystallographic dimers in an asymmetric unit. The residual electron density that was fit with a poly-alanine peptide is highlighted in orange. (B) and (C) The Fo-Fc residual electron density was fit with a seven (B) or five (C) -residue poly-alanine peptide in separate monomers of the asymmetric unit and the resulting 2Fo-Fc map is show in grey mesh and contoured to 1.0 σ whereas the final resulting Fo-Fc map is shown in green mesh and contoured to 3.0 σ.

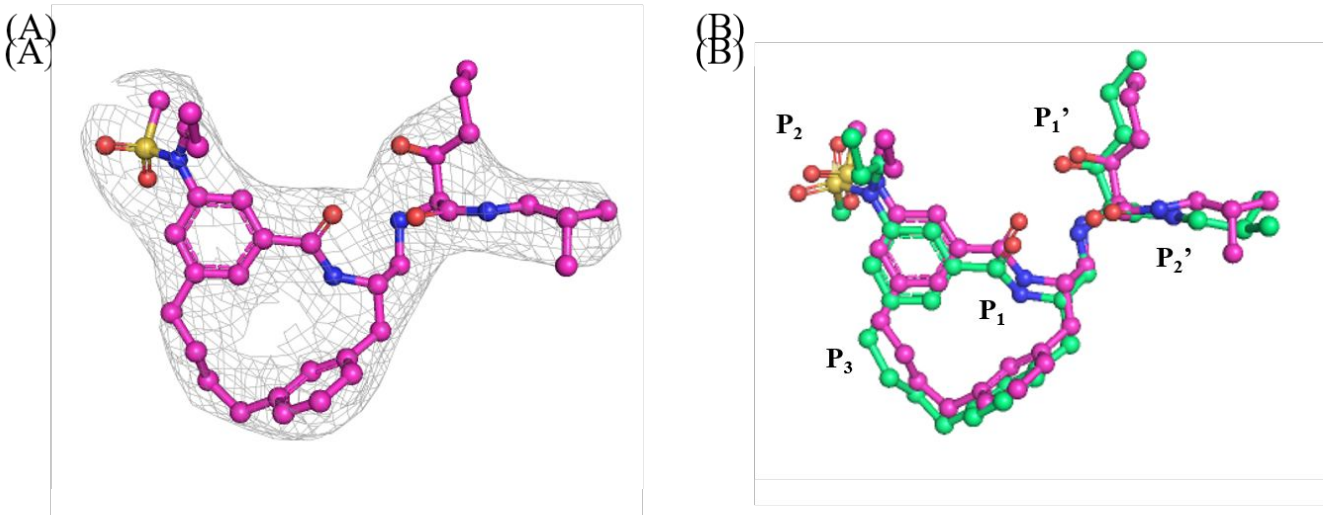

Figure S5. (A) Electron density map of inhibitor **3** (shown in stick and colored according to atom types) in the BACE2 active site. The Fo-Fc electron density omit map is contoured to 3.0 σ and shown in grey mesh. (B) Overlay of inhibitor **3** in the active site of BACE1 (green sticks, PDB : 6NV7) and BACE2 (magenta sticks, PDB : 6UJ1).

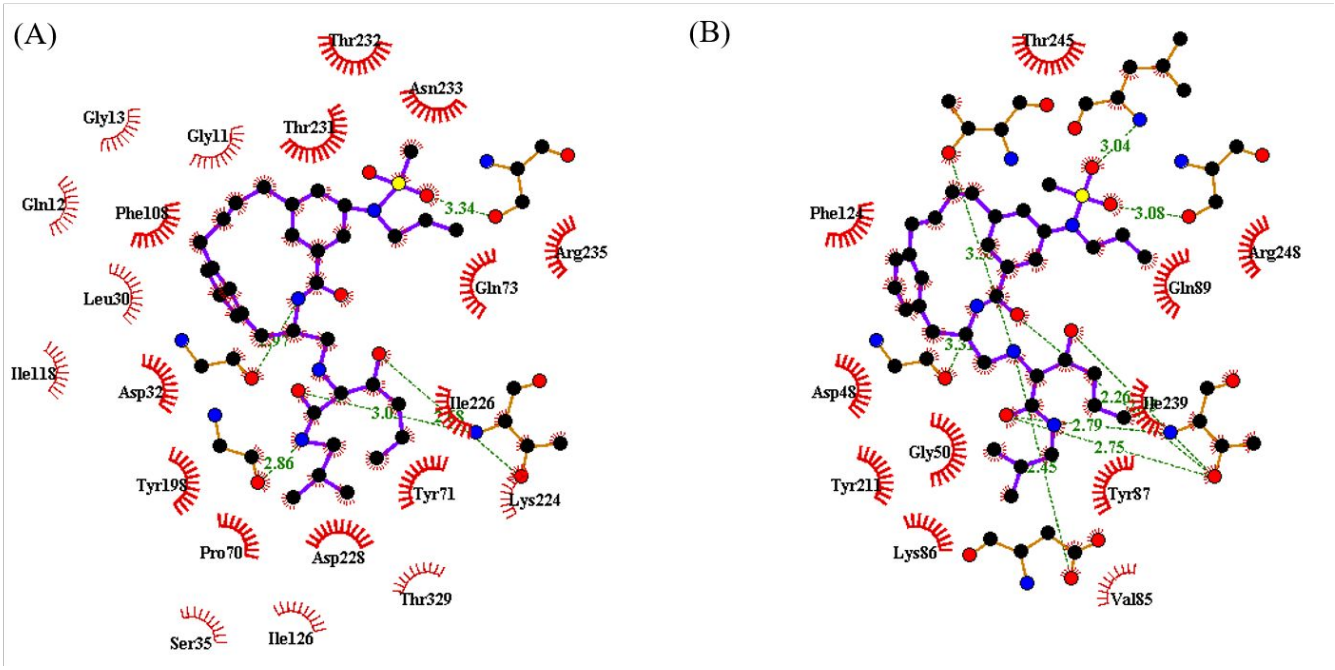

Figure S6. Ligand interaction plot of inhibitor **3** with (C) BACE1 and (D) BACE2 generating using LIGPLOT. Inhibitor **3** is shown in blue line and the atoms are colored according to the atom types. The polar contacts are indicated by the dash lines with distances shown in angstroms and the interacting residues are represented in orange line. The residues involved in the hydrophobic interactions are labeled and shown in red hashes.

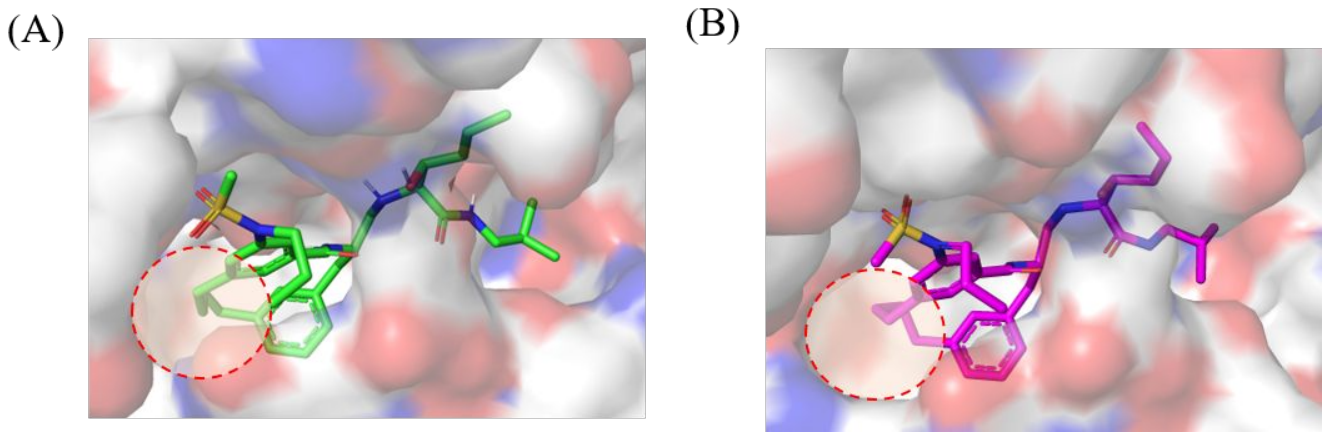

Figure S7. Inhibitor **3** in the active site of (A) BACE1 and (B) BACE2. Inhibitor **3** is shown in green and magenta stick and the atoms are colored according to the atom types. BACE1 and BACE2 are shown in surface presentation and colored in grey. The P_1-P_3 linkage in the S_1-S_3 pocket is highlighted in the pink circle.

Table S1. BACE2 purification from 2 L *E.coli* culture

	Volume (ml)	Total (mg)	Specific activity (μM/min/mg)	Total unit
Inclusion Body Solubilized Fraction	150	525	Not applicable	Not applicable
Refold	40	277	3	837
Ammonium sulfate precipitation	40	28	64	1789
HIC	4.2	5	330	2916

Table S2. Data collection and refinement statistics

Data Collection	***Apo-BACE2***	***BACE2 in complex with Inhibitor-3***
PDB ID	6UJ0	6UJ1
X-ray source and detector	LRL-CAT Sector 31 ID-D	LRL-CAT Sector 31 ID-D
Wavelength (Å)	0.9793	0.9793
Space Group	P1	$P3_121$
Unit Cell dimensions:		
a, b, c (Å)	46.6, 59.0, 84.6	123.07, 123.07, 118.06
α, β, γ (°)	113.4, 93.7, 96.4	90, 90, 120
Data Processing Statistics	Overall [Last Shell]	
Resolution range (Å)	50-2.15 [2.20-2.15]	29-3.03 [3.14-3.03]
No. reflection recorded	405,337	40,834
No. averaged reflections	44,589	20,418
Average Redundancy	2.2	2.0
$CC_{1/2}$ (%)	[82.5]	[62.1]
Rpim[1]	[37.2]	[47.1]
I/σI	17.7 [2.5]	11 [1.1]
% Completeness	97.6 [97.1]	100 [99.9]
Refinement		
Resolution Range (Å)	50-2.15 [2.20-2.15]	29-3.03 [3.14-3.03]
No. Reflections in Working Set	43,480	20,418
No. Reflections in Test Set	2,198	1,016
R_{work} (%) [2]	21.9	24.0
R_{free} (%)	25.7	29.0
Average B-factor (Å²)	46.0	114.7
RMSD from ideal geometry:		
Bond Lengths (Å)	0.008	0.019
Bond Angles (degrees)	0.846	1.564
Ramachandran Plot		
Most Favored (%)	97.78	94.30
Allowed (%)	2.08	5.27
Disallowed (%)	0.14	0.43

[1] $R_{pim} = \sum_{hkl}\sqrt{1/n-1}\sum_i|I_i(hkl) - \langle I(hkl)\rangle| \,/\, \sum_{hkl}\sum_i I_i(hkl)$, where n is the multiplicity.
[2] $R_{work} = \sum_{hkl}||F_{obs}| - |F_{calc}|| \,/\, \sum_{hkl}|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively.

Table S3. Comparison of BACE2 structures crystallized under different conditions and space group

PDB	Construct	Crystallization Chaperone Protein	inhibitor	Resolution (Å)	Space group	Flap orientation[4]
2EWY	Wild type	No	Yes	3.1	H3	Closed
6UJ1	E269A	No	Yes	3.0	$P3_12\ 1$	Closed
3ZKI	E269A	No	Yes	2.4	$P2_1$	Open
3ZKG	E269A	No	No	1.9	$P2_1$	Open
6UJ0	E269A	No	No	2.1	P1	Closed
3ZKN	Wild type	Fab[1]	Yes	2.0	$P2_1$	Open
3ZKM	Wild type	Fab	No	1.8	$P2_12_12_1$	Closed
3ZKQ	Wild type	Xaperone[2]	No	1.5	$P2_12_12_1$	Open
3ZKS	Wild type	Xaperone	Yes	2.1	$P2_12_12_1$	Open
4BEL	Wild type	Xaperone	No	1.8	$P2_1$	Open
4BFB	Wild type	Xaperone	No	2.2	$P2_12_12_1$	Closed
6JSZ	Wild type	Xaperone	Yes	1.5	$P2_12_12_1$	Open
3ZKX	E269A	Xaperone	No	2.4	I222	Closed
3ZLQ	E269A	Xaperone	Yes	2.1	$P2_1$	Open
3ZL7	E269A	Fynomer[3]	No	3.2	$P4_32_12$	Closed

[1] BACE2-binding antibody Fab fragments

[2] Single domain camelid antibody V_HH fragments

[3] Fyn-kinase-derived SH3 domains

[4] Structures were superimposed to the unbound BACE2 (PDB: 2EWY) using pymol and the movement of the flap loop is determined by measuring the distance of Thr88 Cα atoms between the structures and unbound BACE2. Structures that move more than 2 Å in the flap loop are defined as open conformations.