Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Identifying the sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) as a potential target for hypericin – A theoretical study

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Supporting Information

Fig. S1: Sequence alignment of human SERCA1A and rabbit SERCA1A with ClustalW2.

"*": identical residues; ":": conserved constitution; and ".": semiconserved residues.

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SERCA1A_human       MEAAHAKTTEECLAYFGVSETTGLTPDQVKRNLEKYGLNELPAEEGKTLWELVIEQFEDL 60
SERCA1A_rabbit      MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDL 60
                    *****:*:***********************:***** *********:************

SERCA1A_human       LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAIVGVWQERNAENAIEALK 120
SERCA1A_rabbit      LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAIVGVWQERNAENAIEALK 120
                    ************************************************************

SERCA1A_human       EYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILAIKSTTLRVDQSIL 180
SERCA1A_rabbit      EYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSIL 180
                    ************************************************:************

SERCA1A_human       TGESVSVIKHTEPVPDPRAVNQDKKNMLFSGTNIAAGKALGIVATTGVGTEIGKIRDQMA 240
SERCA1A_rabbit      TGESVSVIKHTEPVPDPRAVNQDKKNMLFSGTNIAAGKALGIVATTGVSTEIGKIRDQMA 240
                    ************************************************.************

SERCA1A_human       ATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGSWFRGAIYYFKIAV 300
SERCA1A_rabbit      ATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGSWIRGAIYYFKIAV 300
                    ************************************************:************

SERCA1A_human       ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ 360
SERCA1A_rabbit      ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ 360
                    ************************************************************

SERCA1A_human       MSVCKMFIIDKVDGDICLLNEFSITGSTYAPEGEVLKNDKPVRPGQYDGLVELATICALC 420
SERCA1A_rabbit      MSVCKMFIIDKVDGDFCSLNEFSITGSTYAPEGEVLKNDKPIRSGQFDGLVELATICALC 420
                    ***************:* ***********************:*.**:************

SERCA1A_human       NDSSLDFNEAKGVYEKVGEATETALTTLVEKMNVFNTDVRSLSKVERANACNSVIRQLMK 480
SERCA1A_rabbit      NDSSLDFNETKGVYEKVGEATETALTTLVEKMNVFNTEVRNLSKVERANACNSVIRQLMK 480
                    *********:****************************:**.******************

SERCA1A_human       KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTRVPLTGP 540
SERCA1A_rabbit      KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTRVPMTGP 540
                    ************************************************************:***

SERCA1A_human       VKEKIMAVIKEWGTGRDTLRCLALATRDTPPKREEMVLDDSARFLEYETDLTFVGVVGML 600
SERCA1A_rabbit      VKEKILSVIKEWGTGRDTLRCLALATRDTPPKREEMVLDDSSRFMEYETDLTFVGVVGML 600
                    *****::*************************************:**:***************

SERCA1A_human       DPPRKEVTGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFGENEEVADRAYTGREFDD 660
SERCA1A_rabbit      DPPRKEVMGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFGENEEVADRAYTGREFDD 660
                    ******* ****************************************************

SERCA1A_human       LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM 720
SERCA1A_rabbit      LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM 720
                    ************************************************************

SERCA1A_human       GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA 780
SERCA1A_rabbit      GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA 780
                    ************************************************************

SERCA1A_human       LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPKEPLISGWLFFRYMAI 840
SERCA1A_rabbit      LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPKEPLISGWLFFRYMAI 840
                    ************************************************************

SERCA1A_human       GGYVGAATVGAAAWWFLYAEDGPHVNYSQLTHFMQCTEDNTHFEGIDCEVFEAPEPMTMA 900
SERCA1A_rabbit      GGYVGAATVGAAAWWFMYAEDGPGVTYHQLTHFMQCTEDHPHFEGLDCEIFEAPEPMTMA 900
                    ***************:****** *.* ***********:.****:***:**********

SERCA1A_human       LSVLVTIEMCNALNSLSENQSLLRMPPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLR 960
SERCA1A_rabbit      LSVLVTIEMCNALNSLSENQSLMRMPPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLK 960
                    *********************:************************************:
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SERCA1A_human       ALDLTQWLMVLKISLPVIGLDEILKFVARNYLEG 994
SERCA1A_rabbit      ALDLTQWLMVLKISLPVIGLDEILKFIARNYLEG 994
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Section S2: Membrane embedding in YASARA

First, a cell is created around the protein. The cell was extended by 15 Å on each side of the protein in the x and y directions (the membrane plane), giving a box that is 30 Å larger than the protein. The cell was then extended by 10 Å on each side of the protein in the z direction (normal to the membrane plane), giving a water extension of 20 Å in total in this direction.

The protein was initially searched for exposed hydrophobic side-chains of amino acids in transmembrane helices and strands. After the transmembrane region in the protein was identified, a previously equilibrated POPE membrane fragment was loaded and replicated to reach the size of the simulation cell. The membrane of required size was constructed in the absence of the protein. The protein was then loaded and lipids deleted in order to fit the protein within the membrane. To ensure that not too many lipids were deleted, which would be the case if a single overlap was enough to delete a lipid, the protein was first compressed and lipids that overlapped with the compressed protein were deleted. The protein was then grown back to its original size during an energy minimization of the lipids to fill the membrane pore and generate a system where lipids smoothly cover the protein. The entire system was then energy minimized.

Next, the cell was filled with water of density 0.998 g/ml and a NaCl concentration of 0.9 % and pH 7.4. Water molecules inside the membrane region were deleted. The system was then minimized and equilibrated in a 250 ps MD simulation, during which waters were prevented from entering the membrane. A 38 ns MD simulation followed in which a time step of 2×1.25 fs was used. During the simulation the temperature was set to 298 K. The AMBER03 force field was applied throughout with a force cutoff of 7.86 Å. Periodic boundary conditions were applied and the PME algorithm was used to treat long-range electrostatics.

Section. S3: Energy minimization in YASARA.

A steepest descent minimization is first performed to remove bumps and correct the covalent geometry, followed by a simulated annealing minimization (time step 2 fs, atom velocities scaled down by 0.9 every 10th step) until convergence is reached, i.e. the energy improved by less than the convergence parameter per atom during 200 steps.

In a system containing solvent, the solvent alone is first subject to minimization. The convergence parameter for this minimization is set to 0.1 kJ/mol. The total system is then minimized, using a convergence parameter set to 0.05 kJ/mol.

Fig. S4: Sequence alignment of SERCA2A (human), SERCA2B (human), and SERCA1A (rabbit) with ClustalW2.

"*": identical residues; ":": conserved constitution; and ".": semiconserved residues.

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SERCA2A         MENAHTKTVEEVLGHFGVNESTGLSLEQVKKLKERWGSNELPAEEGKTLLELVIEQFEDL 60
SERCA2B         MENAHTKTVEEVLGHFGVNESTGLSLEQVKKLKERWGSNELPAEEGKTLLELVIEQFEDL 60
SERCA1A         MEAAHSKSTEECLAYFGVSETTGLTPDQVKRHLEKYGHNELPAEEGKSLWELVIEQFEDL 60
                ** **:*:.** *.:***.*:***: :***:  *::* *********:* **********

SERCA2A         LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILVANAIVGVWQERNAENAIEALK 120
SERCA2B         LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILVANAIVGVWQERNAENAIEALK 120
SERCA1A         LVRILLLAACISFVLAWFEEGEETITAFVEPFVILLILIANAIVGVWQERNAENAIEALK 120
                ************************************:*********************

SERCA2A         EYEPEMGKVYRQDRKSVQRIKAKDIVPGDIVEIAVGDKVPADIRLTSIKSTTLRVDQSIL 180
SERCA2B         EYEPEMGKVYRQDRKSVQRIKAKDIVPGDIVEIAVGDKVPADIRLTSIKSTTLRVDQSIL 180
SERCA1A         EYEPEMGKVYRADRKSVQRIKARDIVPGDIVEVAVGDKVPADIRILSIKSTTLRVDQSIL 180
                *********** **********:*********:***********: **************

SERCA2A         TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAMGVVVATGVNTEIGKIRDEMV 240
SERCA2B         TGESVSVIKHTDPVPDPRAVNQDKKNMLFSGTNIAAGKAMGVVVATGVNTEIGKIRDEMV 240
SERCA1A         TGESVSVIKHTEPVPDPRAVNQDKKNMLFSGTNIAAGKALGIVATTGVSTEIGKIRDQMA 240
                ***********:****************************:*:*.:***.********:*.

SERCA2A         ATEQERTPLQQKLDEFGEQLSKVISLICIAVWIINIGHFNDPVHGGSWIRGAIYYFKIAV 300
SERCA2B         ATEQERTPLQQKLDEFGEQLSKVISLICIAVWIINIGHFNDPVHGGSWIRGAIYYFKIAV 300
SERCA1A         ATEQDKTPLQQKLDEFGEQLSKVISLICVAVWLINIGHFNDPVHGGSWIRGAIYYFKIAV 300
                ****::**********************:***:**************************

SERCA2A         ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ 360
SERCA2B         ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ 360
SERCA1A         ALAVAAIPEGLPAVITTCLALGTRRMAKKNAIVRSLPSVETLGCTSVICSDKTGTLTTNQ 360
                ************************************************************

SERCA2A         MSVCRMFILDRVEGDTCSLNEFTITGSTYAPIGEVHKDDKPVNCHQYDGLVELATICALC 420
SERCA2B         MSVCRMFILDRVEGDTCSLNEFTITGSTYAPIGEVHKDDKPVNCHQYDGLVELATICALC 420
SERCA1A         MSVCKMFIIDKVDGDFCSLNEFSITGSTYAPEGEVLKNDKPIRSGQFDGLVELATICALC 420
                ****:***:*:*:** ******:******** *** *:***:.. *:**************

SERCA2A         NDSALDYNEAKGVYEKVGEATETALTCLVEKMNVFDTELKGLSKIERANACNSVIKQLMK 480
SERCA2B         NDSALDYNEAKGVYEKVGEATETALTCLVEKMNVFDTELKGLSKIERANACNSVIKQLMK 480
SERCA1A         NDSSLDFNETKGVYEKVGEATETALTTLVEKMNVFNTEVRNLSKVERANACNSVIRQLMK 480
                ***:**:**:**************** ********:**::.***:**********:****

SERCA2A         KEFTLEFSRDRKSMSVYCTPNKPSRTSMS-KMFVKGAPEGVIDRCTHIRVGSTKVPMTSG 539
SERCA2B         KEFTLEFSRDRKSMSVYCTPNKPSRTSMS-KMFVKGAPEGVIDRCTHIRVGSTKVPMTSG 539
SERCA1A         KEFTLEFSRDRKSMSVYCSPAKSSRAAVGNKMFVKGAPEGVIDRCNYVRVGTTRVPMTGP 540
                ******************:* *.**:::. ***************.::***:*:****.

SERCA2A         VKQKIMSVIREWGSGSDTLRCLALATHDNPLRREEMHLEDSANFIKYETNLTFVGCVGML 599
SERCA2B         VKQKIMSVIREWGSGSDTLRCLALATHDNPLRREEMHLEDSANFIKYETNLTFVGCVGML 599
SERCA1A         VKEKILSVIKEWGTGRDTLRCLALATRDTPPKREEMVLDDSSRFMEYETDLTFVGVVGML 600
                **:**:***:***:* **********:*.* :**** *:**:.*::***:***** ****

SERCA2A         DPPRIEVASSVKLCRQAGIRVIMITGDNKGTAVAICRRIGIFGQDEDVTSKAFTGREFDE 659
SERCA2B         DPPRIEVASSVKLCRQAGIRVIMITGDNKGTAVAICRRIGIFGQDEDVTSKAFTGREFDE 659
SERCA1A         DPPRKEVMGSIQLCRDAGIRVIMITGDNKGTAIAICRRIGIFGENEEVADRAYTGREFDD 660
                **** ** .*::***:****************:**********::*:*:.:*:******:

SERCA2A         LNPSAQRDACLNARCFARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKAEIGIAM 719
SERCA2B         LNPSAQRDACLNARCFARVEPSHKSKIVEFLQSFDEITAMTGDGVNDAPALKKAEIGIAM 719
SERCA1A         LPLAEQREACRRACCFARVEPSHKSKIVEYLQSYDEITAMTGDGVNDAPALKKAEIGIAM 720
                *  : **:** .* ***************:***:**************************
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SERCA2A         GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA 779
SERCA2B         GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA 779
SERCA1A         GSGTAVAKTASEMVLADDNFSTIVAAVEEGRAIYNNMKQFIRYLISSNVGEVVCIFLTAA 780
                ************************************************************

SERCA2A         LGFPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMNKPPRNPKEPLISGWLFFRYLAI 839
SERCA2B         LGFPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMNKPPRNPKEPLISGWLFFRYLAI 839
SERCA1A         LGLPEALIPVQLLWVNLVTDGLPATALGFNPPDLDIMDRPPRSPKEPLISGWLFFRYMAI 840
                **:**********************************::***.**************:**

SERCA2A         GCYVGAATVGAAAWWFIAADGGPRVSFYQLSHFLQCKEDNPDFEGVDCAIFESPYPMTMA 899
SERCA2B         GCYVGAATVGAAAWWFIAADGGPRVSFYQLSHFLQCKEDNPDFEGVDCAIFESPYPMTMA 899
SERCA1A         GGYVGAATVGAAAWWFMYAEDGPGVTYHQLTHFMQCTEDHPHFEGLDCEIFEAPEPMTMA 900
                * **************: *:.** *:::**:**:**.**:*.***:** ***:* *****

SERCA2A         LSVLVTIEMCNALNSLSENQSLLRMPPWENIWLVGSICLSMSLHFLILYVEPLPLIFQIT 959
SERCA2B         LSVLVTIEMCNALNSLSENQSLLRMPPWENIWLVGSICLSMSLHFLILYVEPLPLIFQIT 959
SERCA1A         LSVLVTIEMCNALNSLSENQSLMRMPPWVNIWLLGSICLSMSLHFLILYVDPLPMIFKLK 960
                **********************:***** ****:****************:***:**::.

SERCA2A         PLNVTQWLMVLKISLPVILMDETLKFVARNYLEP-------------------------- 993
SERCA2B         PLNVTQWLMVLKISLPVILMDETLKFVARNYLEPGKECVQPATKSCSFSACTDGISWPFV 1019
SERCA1A         ALDLTQWLMVLKISLPVIGLDEILKFIARNYLEG-------------------------- 994
                .*::************** :** ***:******

SERCA2A         -AILE------------------ 997
SERCA2B         LLIMPLVIWVYSTDTNFSDMFWS 1042
SERCA1A         ------------------------
```

Fig. S5: The transmembrane region of SERCA1A highlighted in cyan.
Amino acids included in the transmembrane region:
SERCA1A: 48-79, 83-106, 253-277, 291-314, 763-805, 832-862, 892-915, 933-987.
SERCA2A: 48-79, 83-106, 256-279, 290-314, 762-805, 833-861, 891-914, 932-984.

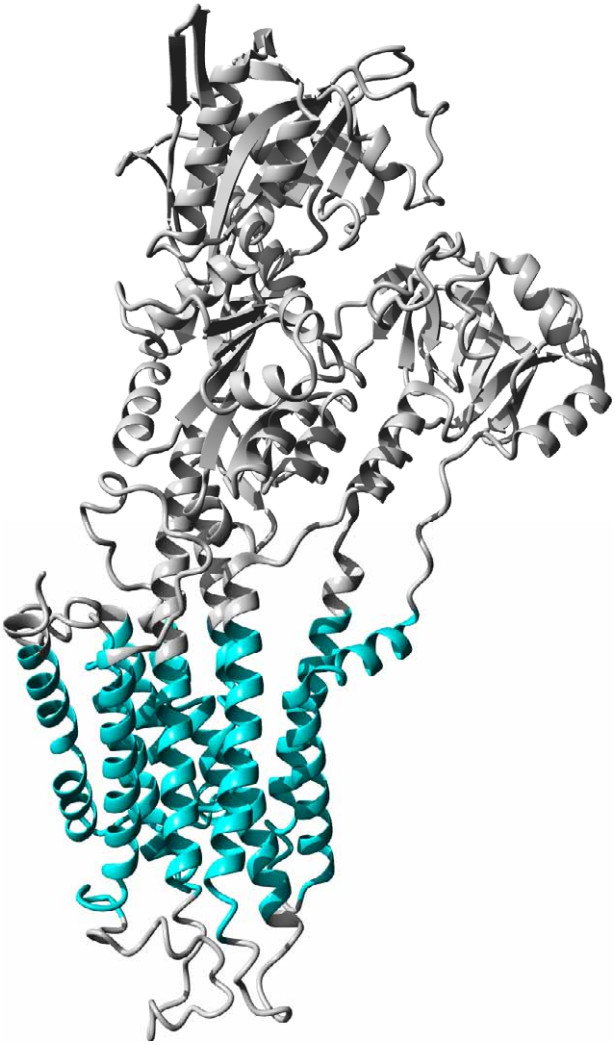


Fig. S6: Time evolution of the Cα RMSD of the transmembrane region of SERCA1A and SERCA2A during the MD simulations (800 snapshots) in the membranes, after alignment of these atoms (cf. S5).

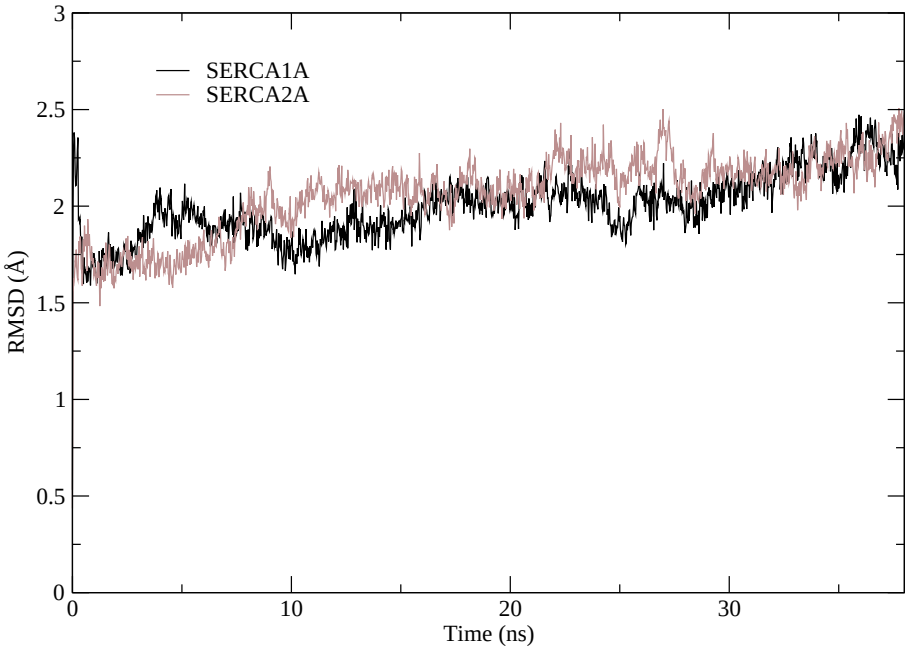


Fig. S7: Superposed backbones of (A) SERCA1A and (B) SERCA2A before (red) and after (blue) membrane embedding, MD simulation and energy minimization. Magnifications of (C and D) the TG binding pocket and (E and F) the BHQ binding pocket.

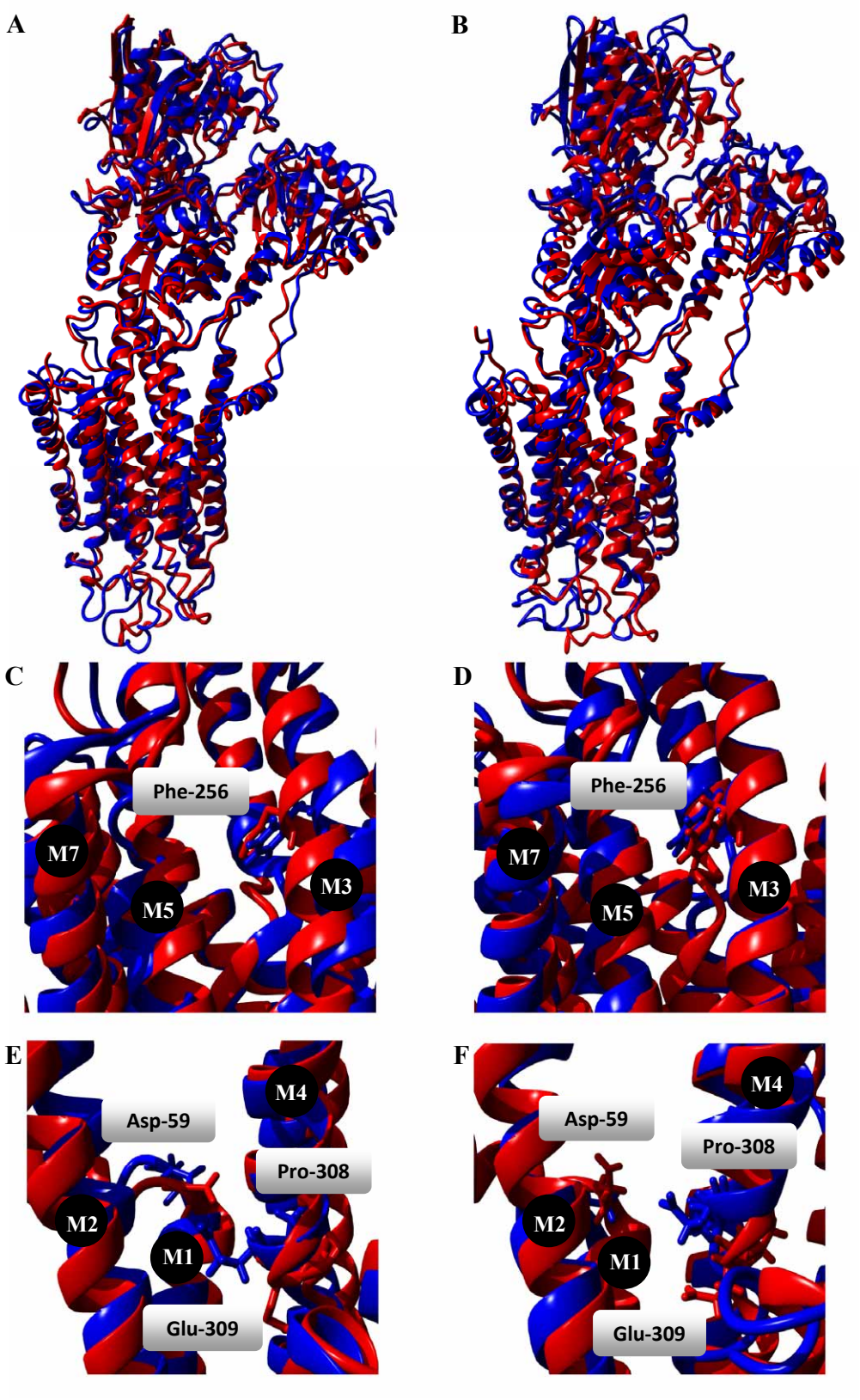
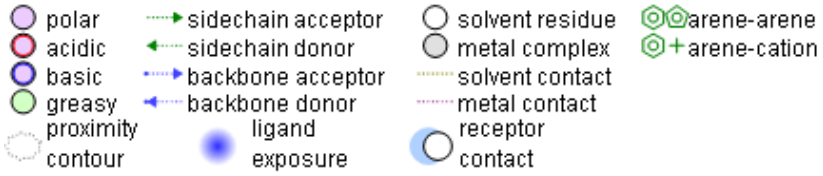
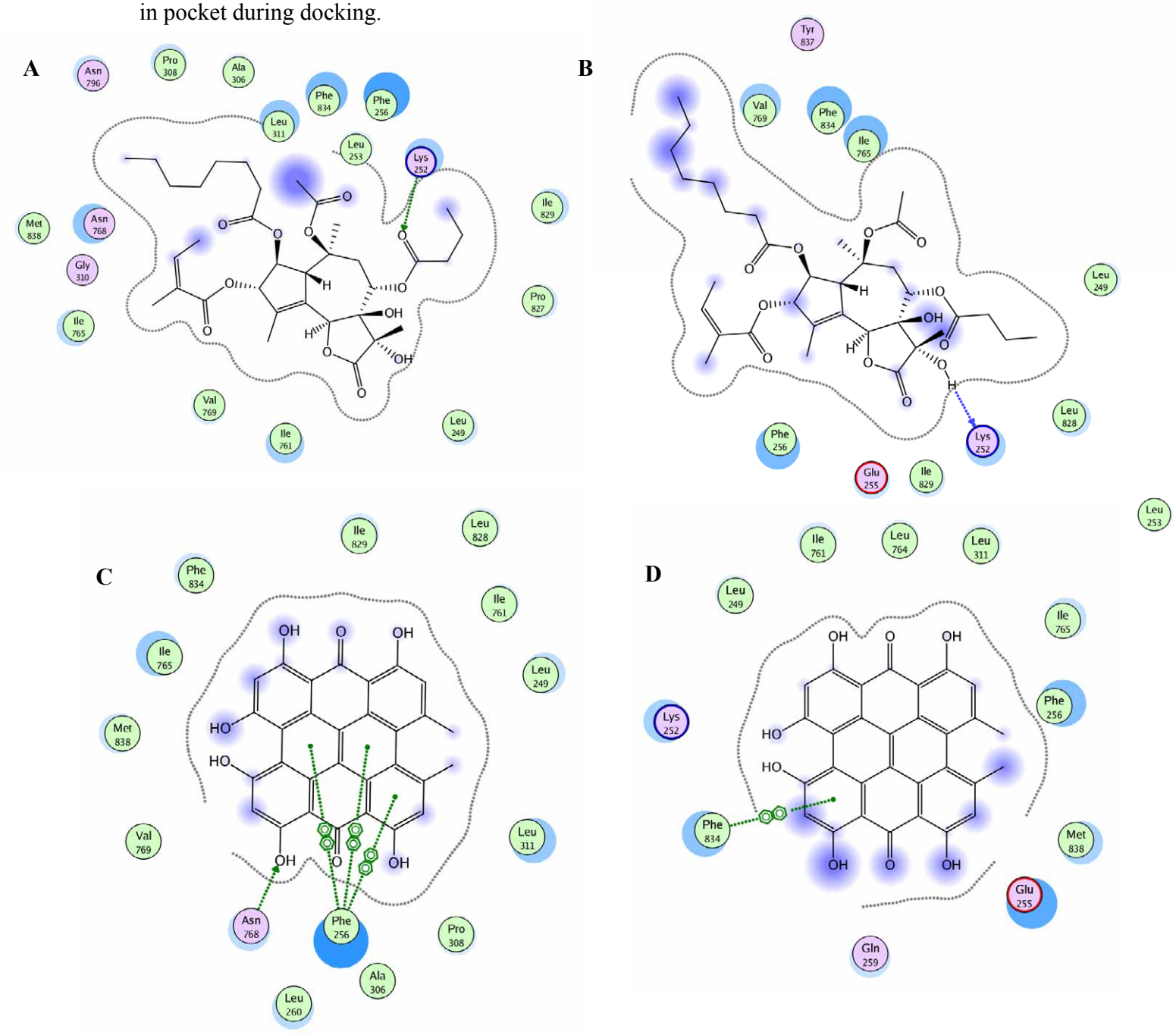


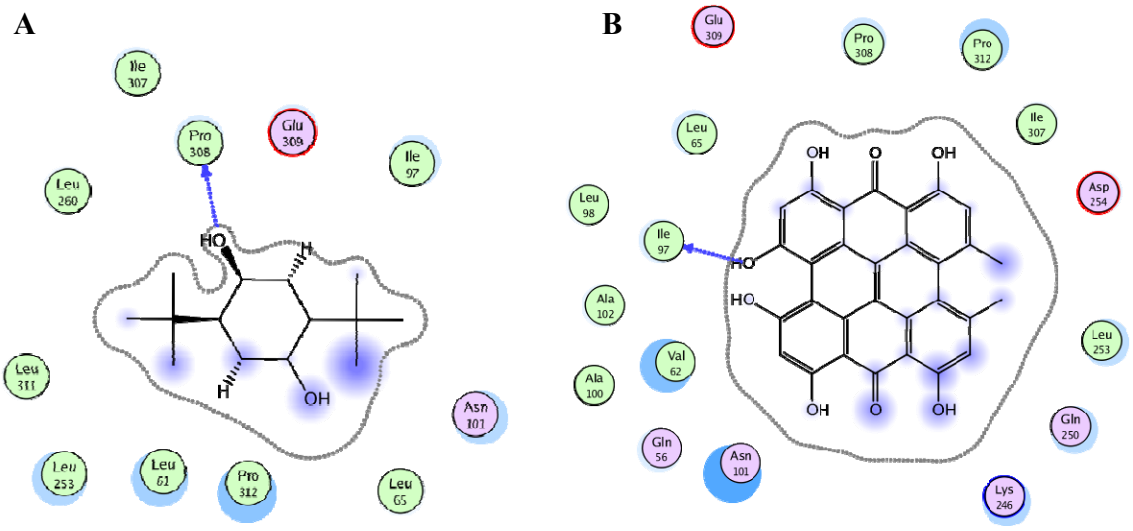
Fig. S8: Ligand interaction diagrams for ligands complexed with SERCA1A and SERCA2A.



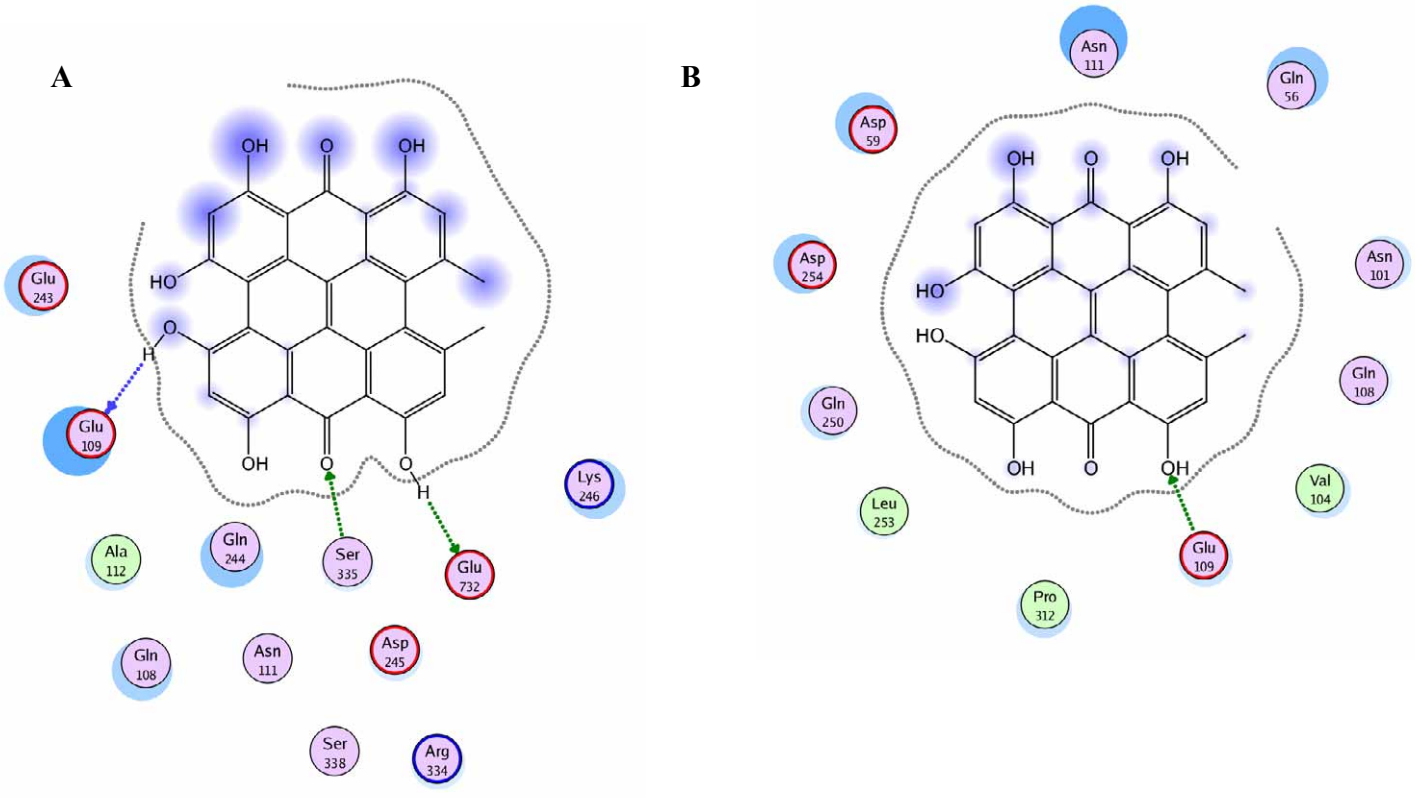
S8.1. (A) TG in the TG binding pocket in SERCA1A (A) with and (B) without lipid in pocket during docking. Hypericin in the TG binding pocket in SERCA1A (C) with and (D) without lipid in pocket during docking.

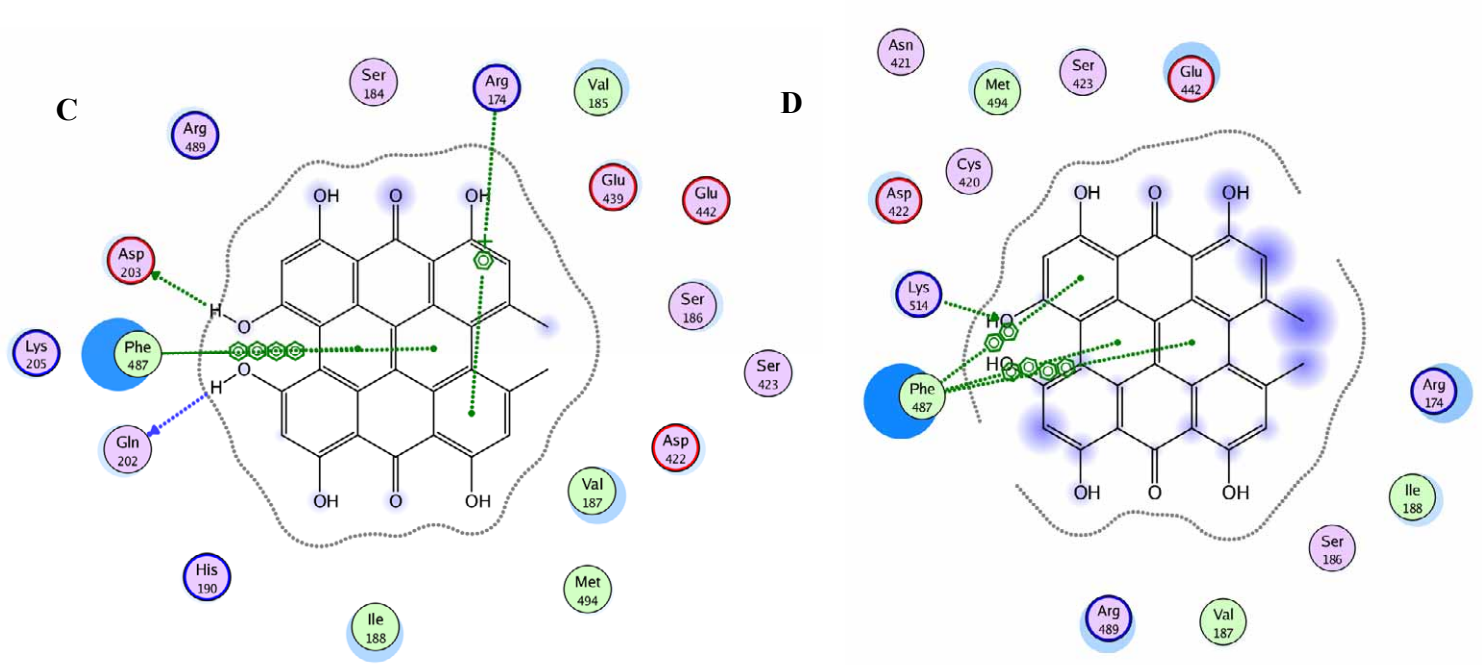


S8.2. (A) BHQ and (B) hypericin in the BHQ binding pocket in SERCA1A.

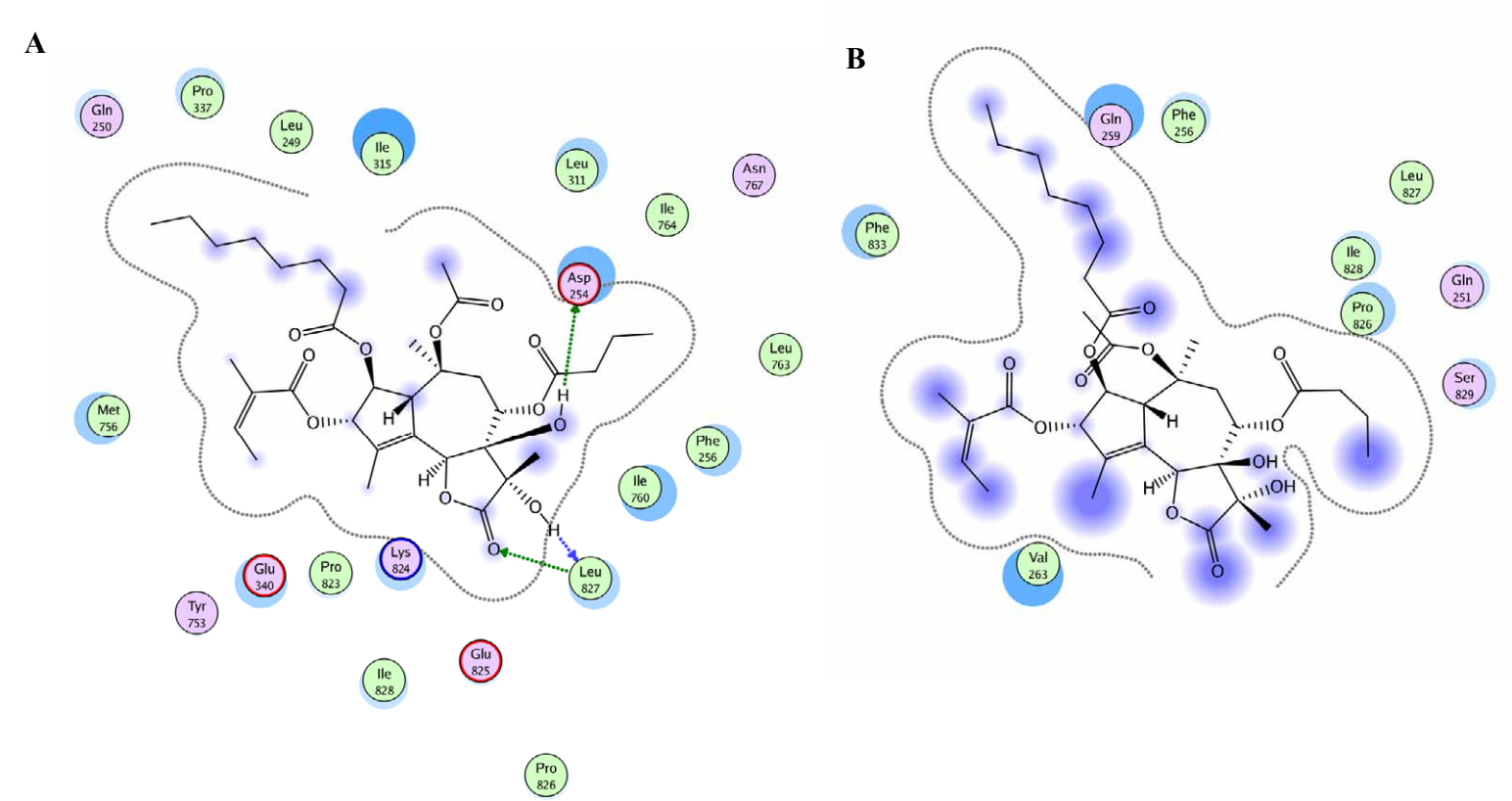


S8.3: Hypericin in site 2 in (A) SERCA1A and (B) SERCA2A. Hypericin in the cytosolic region of (C) SERCA1A and (D) SERCA2A.

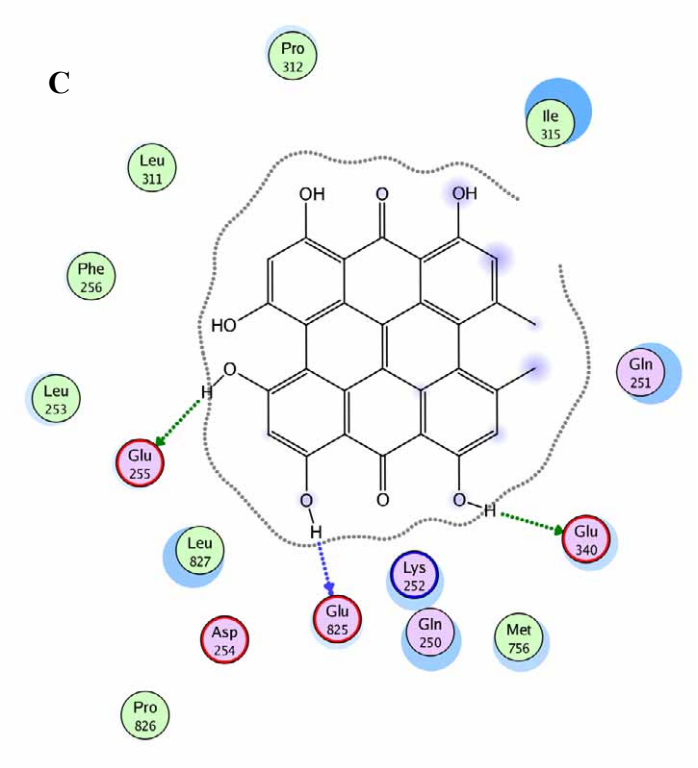




S8.4: TG in the TG binding pocket in SERCA2A (A) with and (B) without lipid in pocket during docking. Hypericin in the TG binding pocket in SERCA2A (C) with and (D) without lipid in pocket during docking.



C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Leu 311
Phe 256
Leu 253
Gln 251
Glu 255
Leu 827
Lys 252
Glu 825
Asp 254
Gln 250
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Pro 826
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O
O
O
H
H
Glu 340

D

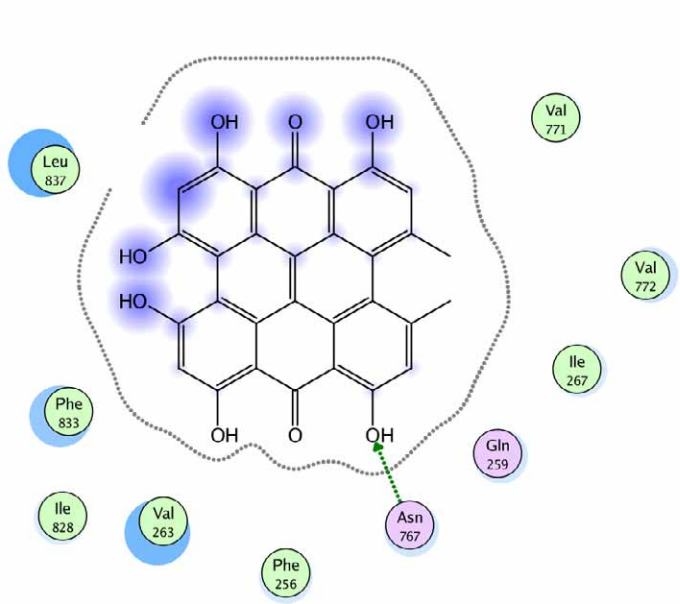

Leu 837
Val 771
Val 772
Ile 267
Phe 833
Gln 259
Ile 828
Val 263
Asn 767
Phe 256
OH
O
OH
HO
HO
OH
O
OH

Fig. S9: Time evolution of the ligand heavy atom RMSD during the MD simulations (800 snapshots per simulation) after superposition of the protein transmembrane Cα's of SERCA1A.

(A) TG in the TG pocket with a lipid present
(B) TG in the TG pocket without a lipid present
(C) Hypericin in the TG pocket with a lipid present
(D) Hypericin in the TG pocket without a lipid present
(E) BHQ in the BHQ pocket
(F) Hypericin in the BHQ pocket
(G) Hypericin in Site 2

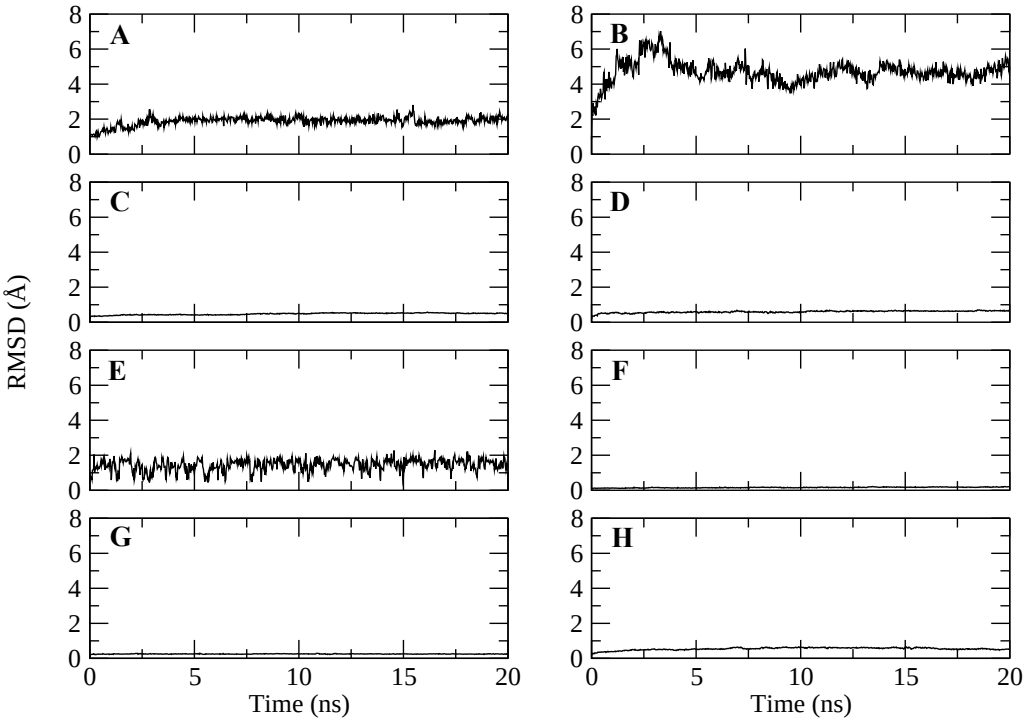
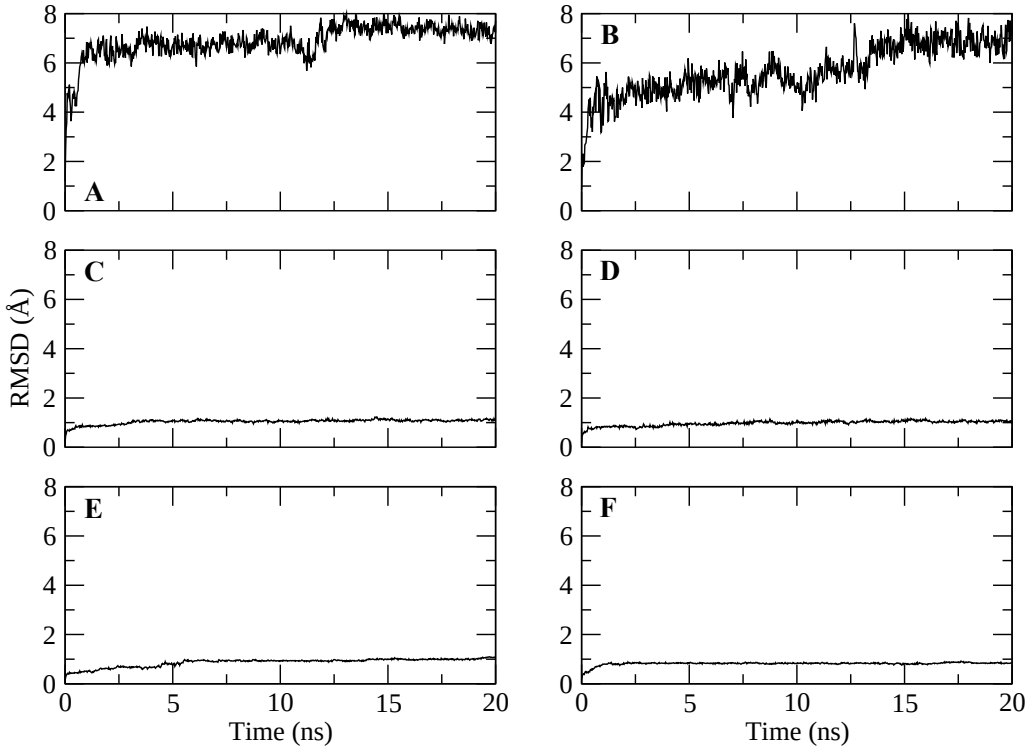


Fig. S10: Time evolution of the ligand heavy atom RMSD during the MD simulations (800 snapshots per simulation) after superposition of the protein transmembrane Cα's of SERCA2A.

(A) TG in the TG pocket with a lipid present
(B) TG in the TG pocket without a lipid present
(C) Hypericin in the TG pocket with a lipid present
(D) Hypericin in the TG pocket without a lipid present
(E) Hypericin in Site 2
(F) Hypericin in the cytosolic pocket



Section S11: TG and BHQ in SERCA1A and SERCA2A

TG in SERCA1A

In SERCA1A, TG is docked deeper into the binding pocket when a lipid occupies a major part thereof, compared to the crystal structure. The three rings of the TG skeleton are positioned approximately the same as in the crystal structure, i.e. close to the interface between the lipids and the cytosole, however, the long tail of TG is positioned in the direction of the interior of the pocket instead of 'down' due to the presence of the lipid tail (Fig. S11A). During the MD simulation there is no significant change in the location of neither TG nor the lipid, and it is clear that the TG molecule is not able to push the lipid out of the active site.

When TG is docked without the lipid occupying the binding pocket, the binding mode reproduces that of the crystal structure very well, and the similarity remains fairly well during the MD simulation (Fig. S11B). The flexibility of the TG tail contributes to slightly larger fluctuations in ligand RMSD compared to the other ligands (Fig. S9). A hydrogen bond is formed between one of the hydroxyl groups and the backbone of Lys-252 on the M3 helix, and close contacts are found with Phe-256, which constitutes a key residue in TG binding,[1-4] and Phe-834. During the MD simulation the neighboring lipids are closing in and tightly surround the TG molecule. Despite the highly different binding modes of TG when a lipid is present or absent, the calculated binding energies are the same (ensemble average and MM/GBVI). In both TG binding modes the molecule is found to reside relatively close to Ile-829 in the upper part of the binding pocket, a residue that is believed to form a hydrogen bond with TG.[5]

BHQ in SERCA1A

Docking of BHQ in this site generated the molecule in a position very similar to the one in the crystal structure and the binding was maintained during the MD simulation (Fig. S11E). Asp-59 and Pro-308, amino acids that are known to be important for BHQ binding,[4] are positioned to enable interaction. Due to the presence of BHQ that occupies the space on this side of the M4 helix, the side-chain of Glu-309 is positioned on the opposite side of the M4 helix such that it blocks the entrance of Ca^{2+}, as observed in the crystal structure,[4] cf. Fig. S11E. The binding energies for BHQ are lower than that for TG, in agreement with experimental inhibition constants (cf. Table 1).[6] The binding energies are also lower than those for hypericin in all binding sites.

TG in SERCA2A

When TG is docked into SERCA2A with a lipid present in the binding pocket, the TG molecule is positioned at an almost 90 degree angle compared to the crystal structure of SERCA1A, with the long tail pointing in the direction of the interior of the protein. Initially during the MD simulation the ring system of the molecule becomes even more rotated, almost 180 degrees, with respect to the TG pose in the SERCA1A crystal structure, but with the long tail still pointing in the direction of the interior of the protein (Fig. S11C). The lipid in the pocket did not move during the MD simulation. The location of the molecule is even higher up in the pocket compared to in SERCA1A and approximately half of the molecule is located in the cytosolic region of the protein. Despite the fact that this binding mode differs significantly from TG in the crystal structure of SERCA1A, the binding energies are higher than the corresponding binding in SERCA1A and it is the overall highest binding energy found for any ligand in the present study.

For TG docked into the protein without the lipid occupying the pocket, the binding mode is more similar to the binding mode of TG in the crystal structure of SERCA1A. However, during the MD simulation the molecule moves out from the pocket and is after the simulation positioned on the surface of the pocket, a location that offers very few interactions with amino acids (Fig. S11D). The binding energies are considerably lower compared to the previous system with TG, and lower than that of both binding modes of TG in SERCA1A.

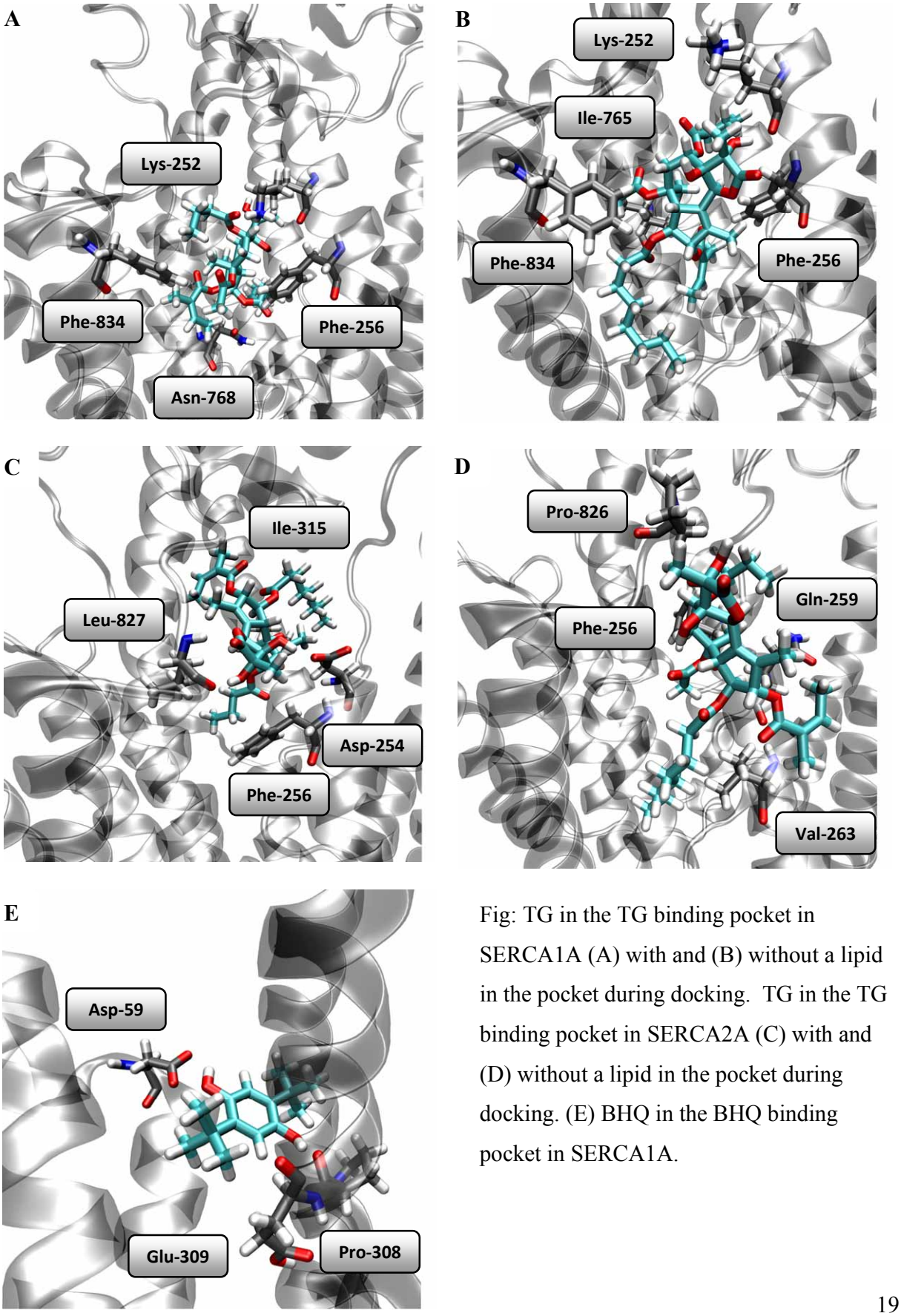


Fig: TG in the TG binding pocket in SERCA1A (A) with and (B) without a lipid in the pocket during docking. TG in the TG binding pocket in SERCA2A (C) with and (D) without a lipid in the pocket during docking. (E) BHQ in the BHQ binding pocket in SERCA1A.

Fig. S12: Hypericin in the TG binding pocket in (A) SERCA1A and (B) SERCA2A with a lipid in the pocket during docking.

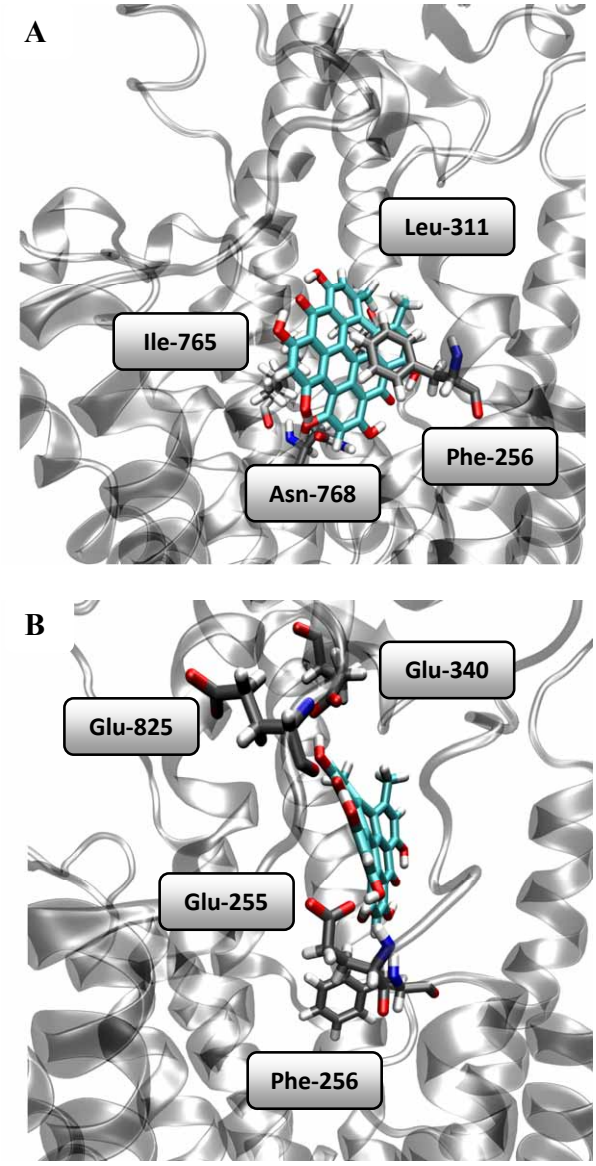


Fig. S13: The two possible conformers of the hypericin molecule; (A) propeller and (B) double-butterfly. Geometries obtained from B3LYP/6-31G(d,p) optimizations.

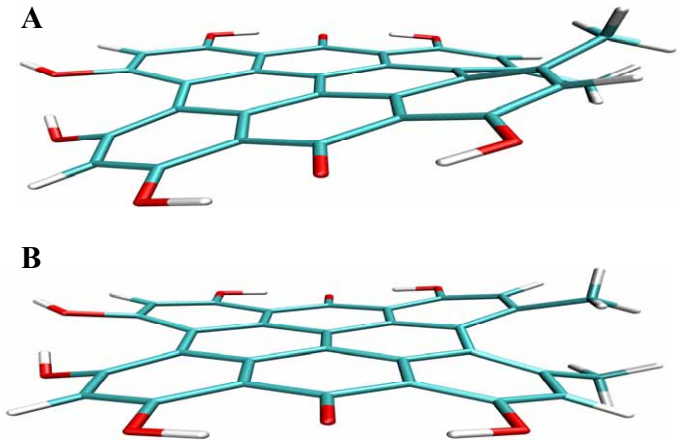
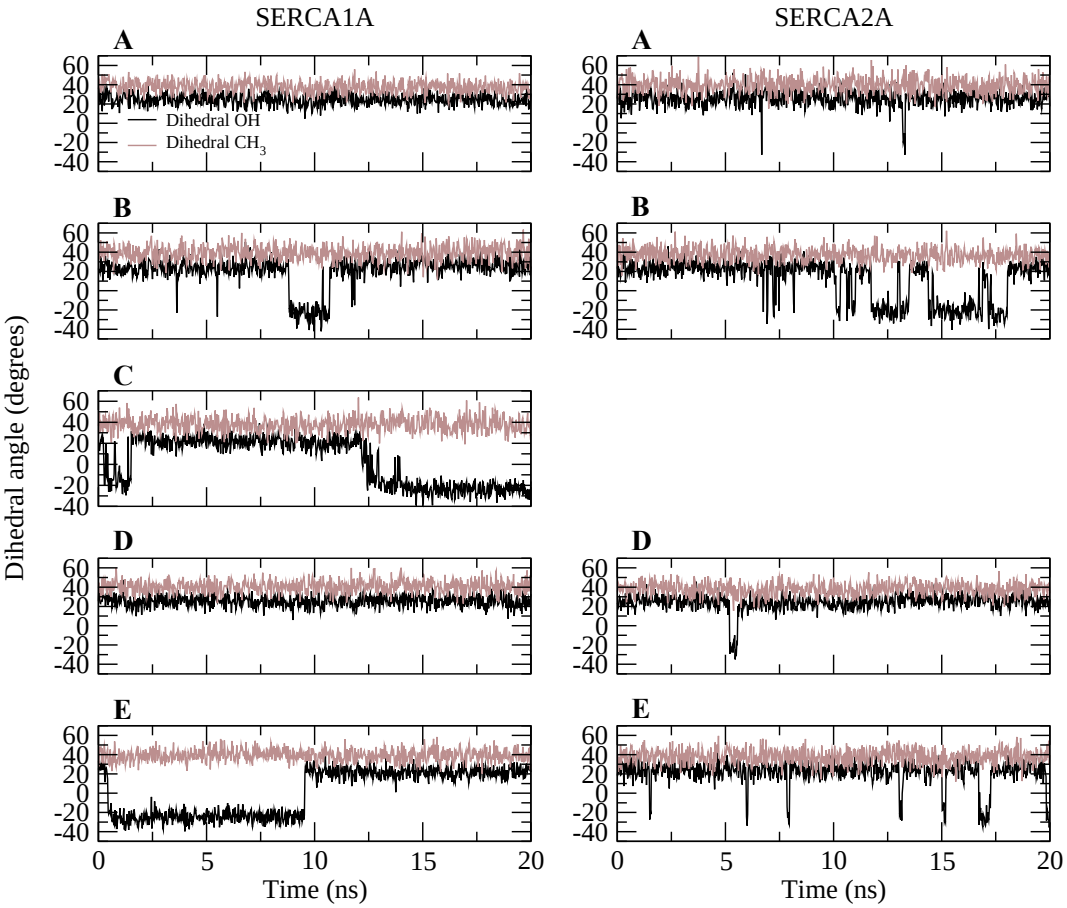


Fig. S14: Dihedral angles of hypericin as function of time during the MD simulations (800 snapshots) in SERCA1A and SERCA2A.

(A) Hypericin in the TG pocket with a lipid present
(B) Hypericin in the TG pocket without a lipid present
(C) Hypericin in the BHQ pocket
(D) Hypericin in Site2
(E) Hypericin in the cytosolic pocket

Propeller conformer: both dihedral angles positive.
Double-butterfly conformer: one dihedral angle positive, one negative.



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