Supplementary materials

Hydroxylated Fatty Acids: The Role of the Sphingomyelin Synthase and the Origin of Selectivity

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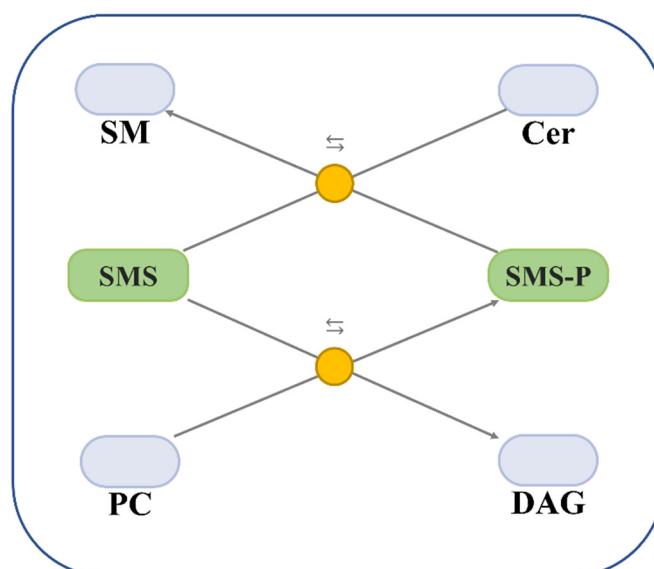**Reconstruction of the SMS pathway**

Figure S1. Graphical representation of modeled reactions. In green are the enzyme SMS and the enzyme state with the modified tyrosine (SMS-P). In gray are the substrates PC, DAG, Cr and SM.
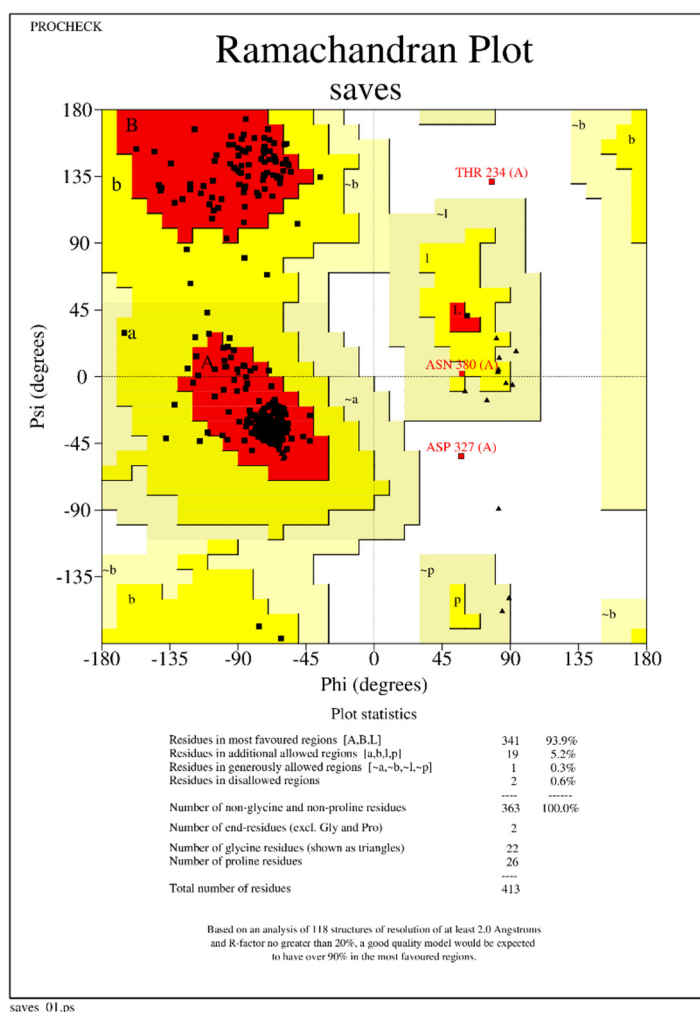
3D-structure validation

Figure S2. Ramachandran plot for SMS1 showing the presence of amino acid residues in favored, allowed, and outlier regions

Ramachandran Plot

saves

Psi (degrees)

B
b
~b
~b
b
~l
l
~b
a
L
ASN 39 (A)
A
~a
HIS 270 (A)
GLU 271 (A)
ARS 59 (A)
~b
b
~p
p
~b

180
135
90
45
0
-45
-90
-135

-180 -135 -90 -45 0 45 90 135 180

Phi (degrees)

Plot statistics

Residues in most favoured regions [A,B,L]	289	90.3%
Residues in additional allowed regions [a,b,l,p]	26	8.1%
Residues in generously allowed regions [~a,~b,~l,~p]	2	0.6%
Residues in disallowed regions	3	0.9%
	----	------
Number of non-glycine and non-proline residues	320	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	21	
Number of proline residues	22	

Total number of residues	365	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms
and R-factor no greater than 20%, a good quality model would be expected
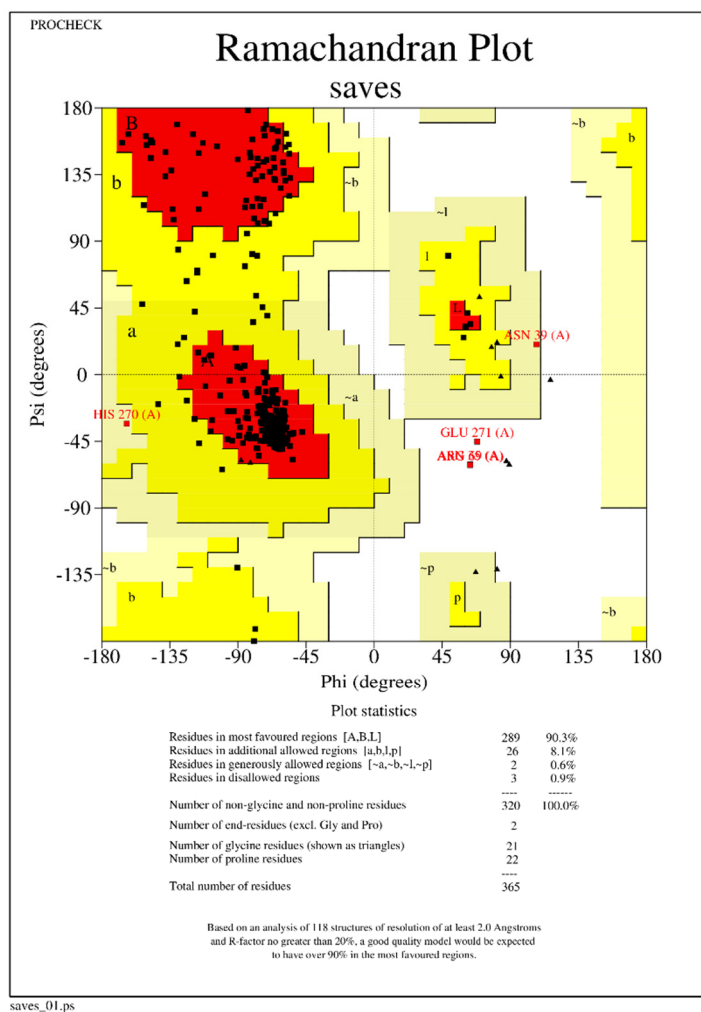to have over 90% in the most favoured regions.

saves_01.ps

Figure S3. Ramachandran plot for SMS2 showing the presence of amino acid residues in favored, allowed, and outlier regions

Table S1. Summary of the quality of SMS1 structure

SMS1 structure built in this work	Previous SMS1 structure
Structure Z-scores, positive is better than average:	Structure Z-scores, positive is better than average:
1st generation packing quality : -1.538	1st generation packing quality : -3.250
2nd generation packing quality : -1.675	2nd generation packing quality : -3.718 (poor)
Ramachandran plot appearance : -0.541	Ramachandran plot appearance : -3.698 (poor)
chi-1/chi-2 rotamer normality : 1.311	chi-1/chi-2 rotamer normality : -3.618 (poor)
Backbone conformation : -1.966	Backbone conformation : -3.838 (poor)
RMS Z-scores, should be close to 1.0:	RMS Z-scores, should be close to 1.0:
Bond lengths : 1.127	Bond lengths : 1.174
Bond angles : 0.442 (tight)	Bond angles : 0.675
Omega angle restraints : 1.469 (loose)	Omega angle restraints : 1.655 (loose)
Side chain planarity : 0.943	Side chain planarity : 1.513
Improper dihedral distribution : 0.572	Improper dihedral distribution : 1.156
Inside/Outside distribution : 1.237 (unusual)	Inside/Outside distribution : 1.276 (unusual)

We compared the 3D structure of SMS1 built in this work with our previous work [1]. The new model is a much more accurate three-dimensional structure

Sequence alignment

Table S2. Multiple Sequence alignment for the whole sequences of hSMS1 and hSMS2

CLUSTAL O(Version 1.2.4) multiple sequence alignment

```
SMS1    MKEVVYWSPKKVADWLLENAMPEYCEPLEHFTGQDLINLTQEDFKKPPLCRVSSDNGQRL          60

SMS2    ------------------------------------------------------------           0

SMS1    LDMIETLKMEHHLEAHKNGHANGHLNIGVDIPTPDGSFSIKIKPNGMPNGYR------KE         114

SMS2    MDIIETAKLEEHLENQPSDPTNTYARPAE--PVEEENKNGNGKPKSLSSGLRKGTKKYPD          58

        :*:*** *:*.*** : .. :* : . .  *. : . . : **:.: .* *      :

SMS1    MIKIPMPELERSQYPMEWGKTFLAFLYALSCFVLTTVMISVVHERVPPKEVQPPLPDTFF         174

SMS2    YIQIAMPTESRNKFPLEWWKTGIAFIYAVFNLVLTTVMITVVHERVPPKELSPPLPDKFF         118

        *:* **  .*.::*:** ** :**:**:  :*******:**********:.*****.**

SMS1    DHFNRVQWAFSICEINGMILVGLWLIQWLLLKYKSIISRRFFCIVGTLYLYRCITMYVTT         234

SMS2    DYIDRVKWAFSVSEINGIILVGLWITQWLFLRYKSIVGRRFCFIIGTLYLYRCITMYVTT         178

        *::.**:****:.****:******: ***:*:****:.*** *:***************

SMS1    LPVPGMHFNCSPKLFGDWEAQLRRIMKLIAGGGLSITGSHNMCGDYLYSGHTVMLTLTYL         294

SMS2    LPVPGMHFQCAPKLNGDSQAKVQRILRLISGGGLSITGSHILCGDFLFSGHTVTLTLTYL         238

        ********:*:*** ** :*.::**:.**:**.********** :***:*:***** ******

SMS1    FIKEYSPRRLWWYHWICWLLSVVGIFCILLAHDHYTVDVVVAYYITTRLFWWYHTMANQQ         354

SMS2    FIKEYSPRHFWWYHLICWLLSAAGIICILVAHEHYTIDVIIAYYITTRLFWWYHSMANEK         298

        ********::**** ******..**:***:**:***:**::**************:***::

SMS1    VLKEASQMNLLARVWWYRPFQYFEKNVQGIVPRSYHWPFPWPVVHLSRQV-KYSRLVNDT         413

SMS2    NLKVSSQTNFLSRAWWFPIFYFFEKNVQGSIPCCFSWPLSWPPGCFKSSCKKYSRVQKIG         358
```

```
            ** :** *:*:*.**:  * :******* :* .: **: **   :. . ****: :

SMS1      -------        413

SMS2      EDNEKST        365
```

*   asterisk   positions that have a single and fully conserved residue
:   colon conservation between groups of strongly similar properties
with a score greater than .5 on the PAM 250 matrix

.   period   conservation between groups of weakly similar properties
with a score less than or equal to .5 on the PAM 250 matrix

Table S3: Prediction of sp|Q86VZ5|SMS1_HUMAN

```
ID    sp|Q86VZ5|SMS1_HUMAN
FT    TOPO_DOM      1    134      CYTOPLASMIC.
FT    TRANSMEM    135    156
FT    TOPO_DOM    157    181      NON CYTOPLASMIC.
FT    TRANSMEM    182    203
FT    TOPO_DOM    204    214      CYTOPLASMIC.
FT    TRANSMEM    215    233
FT    TOPO_DOM    234    279      NON CYTOPLASMIC.
FT    TRANSMEM    280    296
FT    TOPO_DOM    297    304      CYTOPLASMIC.
FT    TRANSMEM    305    325
FT    TOPO_DOM    326    330      NON CYTOPLASMIC.
FT    TRANSMEM    331    347
FT    TOPO_DOM    348    413      CYTOPLASMIC.
```

Table S4: Prediction of sp|Q8NHU3|SMS2_HUMAN

```
ID    sp|Q8NHU3|SMS2_HUMAN
FT    TOPO_DOM      1     78      CYTOPLASMIC.
FT    TRANSMEM     79    100
FT    TOPO_DOM    101    125      NON CYTOPLASMIC.
FT    TRANSMEM    126    147
FT    TOPO_DOM    148    158      CYTOPLASMIC.
FT    TRANSMEM    159    177
FT    TOPO_DOM    178    216      NON CYTOPLASMIC.
FT    TRANSMEM    217    240
FT    TOPO_DOM    241    248      CYTOPLASMIC.
FT    TRANSMEM    249    269
FT    TOPO_DOM    270    274      NON CYTOPLASMIC.
FT    TRANSMEM    275    291
FT    TOPO_DOM    292    365      CYTOPLASMIC.
```

Table S5. Multiple Sequence alignment   for the transmembrane portion sequences of hSMS1 (residues from 131 to 353)   and hSMS2 (residues from 75 to 297)

CLUSTAL O(1.2.4) multiple sequence alignment

```
SMS1_TM    ---EWGKTFLAFLYALSCFVLTTVMISVVHERVPPKEVQPPLPDTFFDHFNRVQWAFSIC    57

SMS2_TM    SMSEWWKTGIAFIYAVFNLVLTTVMITVVHERVPPKELSPPLPDKFFDYIDRVKWAFSVS    60

             ** **  :**:**:   :*******:**********:.*****.***::*:**:****:.

SMS1_TM    EINGMILVGLWLIQWLLLKYKSIISRRFFCIVGTLYLYRCITMYVTTLPVPGMHFNCSPK    117

SMS2_TM    EINGIILVGLWITQWLFLRYKSIVGRRFCFIIGTLYLYRCITMYVTTLPVPGMHFQCAPK    120

           ****:******: ***:*:****:.***  *:********************:*:**

SMS1_TM    LFGDWEAQLRRIMKLIAGGGLSITGSHNMCGDYLYSGHTVMLTLTYLFIKEYSPRRLWWY    177

SMS2_TM    LNGDSQAKVQRILRLISGGGLSITGSHILCGDFLFSGHTVTLTLTYLFIKEYSPRHFWWY    180

           * **  :*::*:::**:*:**:**********  :***:*:***** **************::***

SMS1_TM    HWICWLLSVVGIFCILLAHDHYTVDVVVAYYITTRLFWWYHTMANQ         223

SMS2_TM    HLICWLLSAAGIICILVAHEHYTIDVIIAYYITTRLFWWYHSMANE         226

           * ******..**:***:**:***:**::**************:***:
```

*   asterisk   positions that have a single and fully conserved residue
:   colon conservation between groups of strongly similar properties
with a score greater than .5 on the PAM 250 matrix

.   period   conservation between groups of weakly similar properties
with a score less than or equal to .5 on the PAM 250 matrix
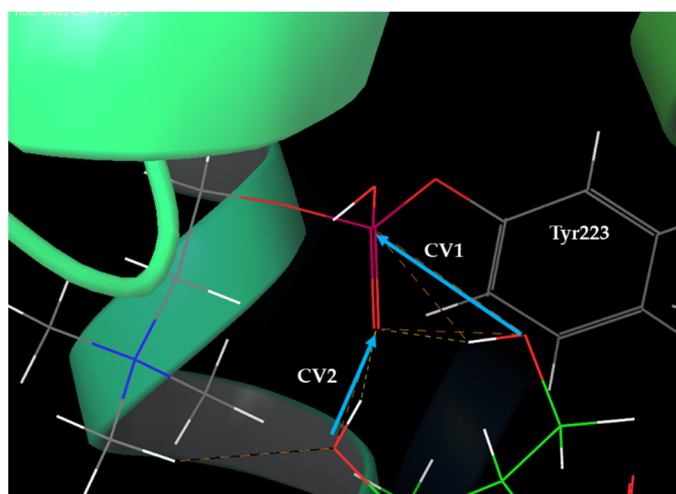
Metadynamics Simulations

Figure S4. Atoms chosen as CV for metadynamics simulations for the SMS1 system.

The CV1 is the distance between the atom O5 of the Ceramide and the P1 of the modified tyrosine residue. The CV2 is the distance between the oxygen atom of the phosphate group and the hydroxyl group of the sphingosine chain.
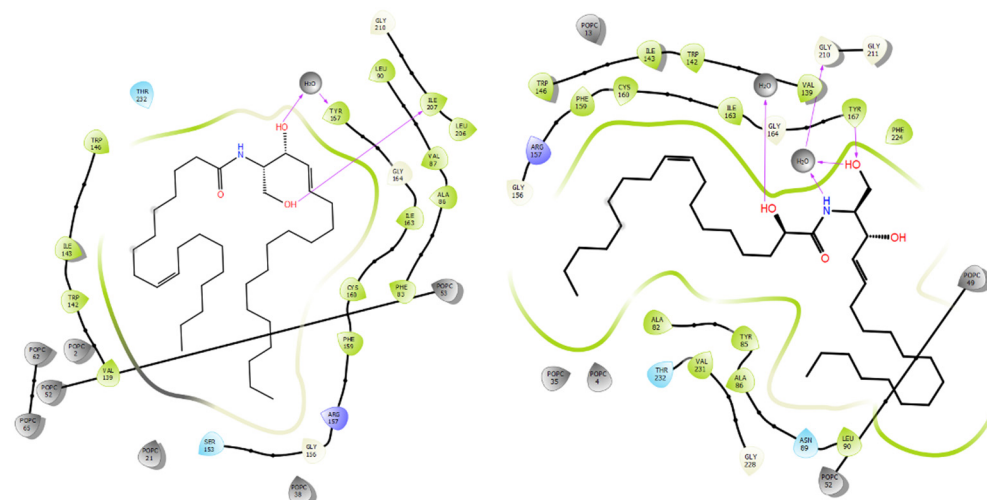

Figure S5. Ligand interaction diagram of Ceramide (on the left) and 2ROHCer (on the right) in complex with SMS2P. The pink arrows indicate the hydrogen bond that is established between the oxygen atoms of the ligand and the residues of the SMS2 protein.

Reference

1.   Piotto, S.; Sessa, L.; Iannelli, P.; Concilio, S. Computational study on human sphingomyelin synthase 1 (hSMS1). *Biochimica et biophysica acta. Biomembranes* **2017**, *1859*, 1517-1525, doi:10.1016/j.bbamem.2017.04.004.