Supplementary Information for

Inferring biochemical reactions and metabolite structures to cope with metabolic pathway drift

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Figures S1 to S6
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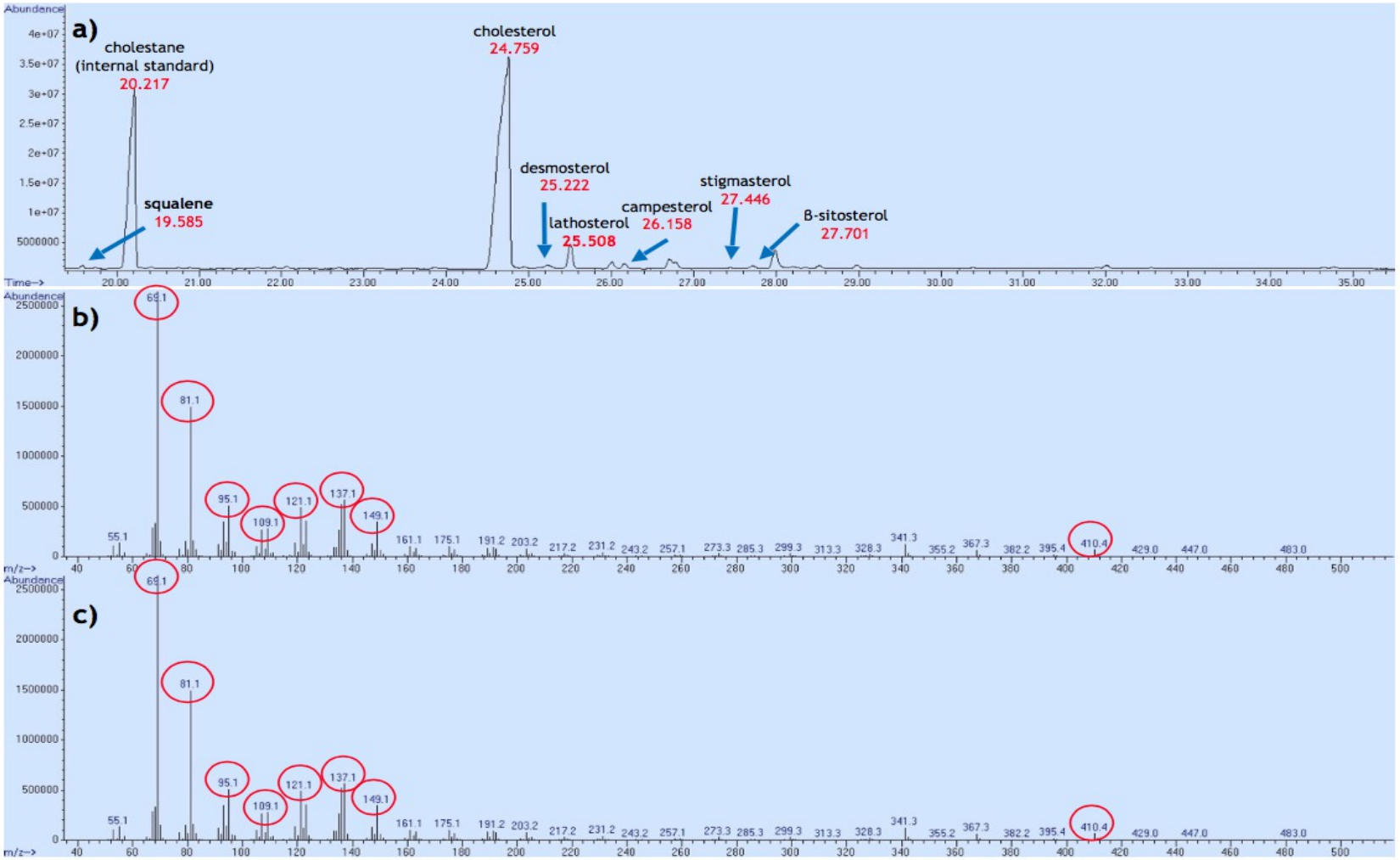
Supplementary dataset

Other supplementary material for this manuscript includes the following:

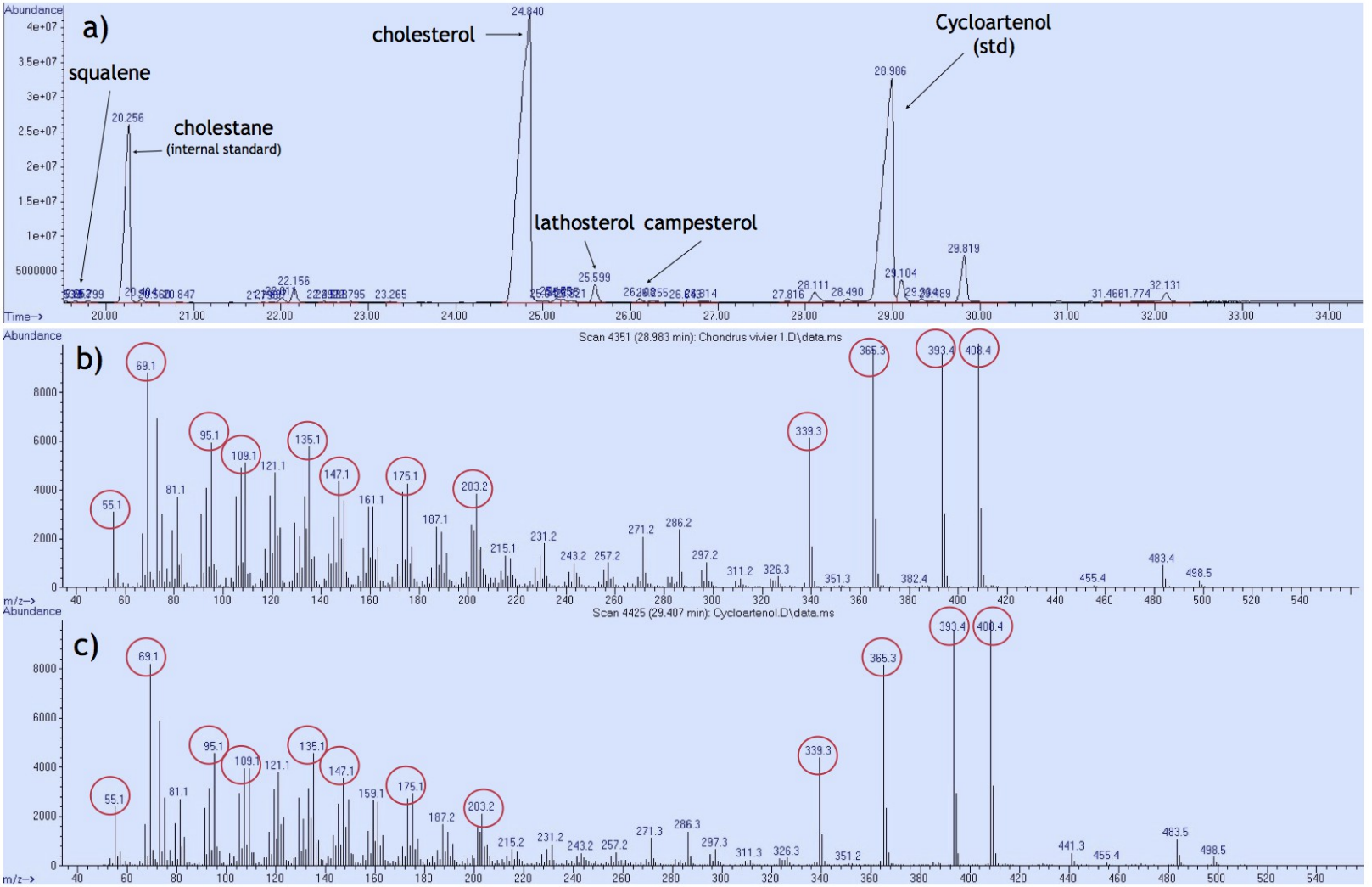
Tables S3 to S4 (separate pdf, content will be submitted to the Metabolights database)

Analysed compounds	Molecular weight (g.mol-1)	RT (min)	m/z $[M+H]^+$ (TMS)
brassicasterol	398.66	25.5	470
campesterol	400.68	26.6	472
5α-cholestane	372.67	20.3	372
cholesterol	386.65	24.7	458
cycloartanol	428.75	30.5	500
cycloartenol	426.72	29.0	498
cycloeucalenol	426.73	30.4	498
7-dehydrocholesterol	384.63	25.6	456
desmosterol	384.64	25.5	456
ergosterol	396.65	26.2	468
fucosterol	412.69	28.2	484
lanosterol	426.39	27.8	498
lathosterol	386.65	25.8	458
β-sitosterol	414.39	28.0	486
squalene	410.72	19.8	482
stigmasterol	412.69	27.0	484
Zymosterol	384.64	25.9	456

Supp. Table 3. Retention times and m/z ratio for analytical standards of sterols on a 7890-5975C Agilent GC-MS.



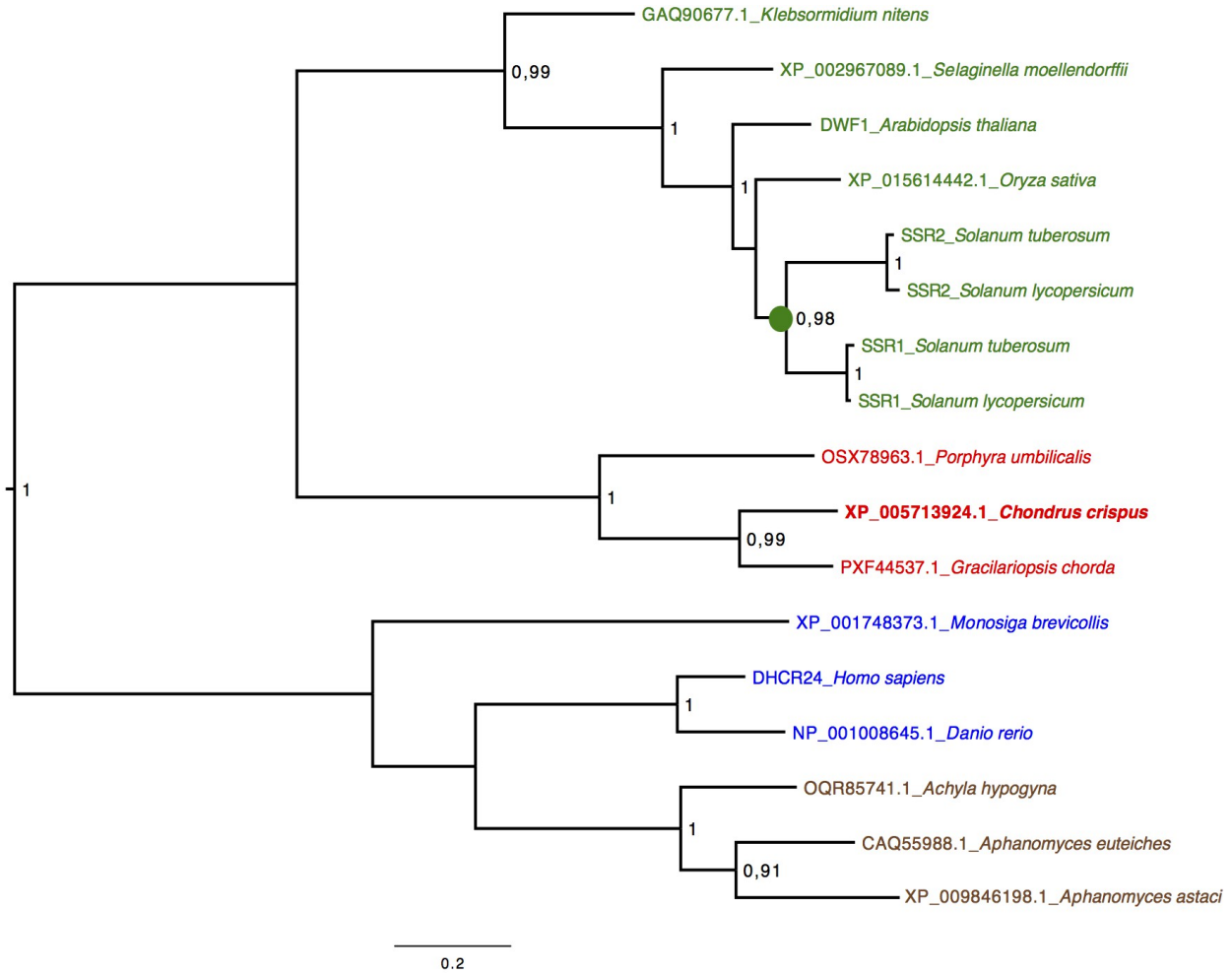
Supp. Figure 1. Identification of squalene in *C. crispus*. a) Total Ion Chromatogramm (TIC) from *C. crispus* extract. b) MS spectrum of squalene in *C. crispus* extract. c) MS spectrum of the squalene analytical standard. Main fragmentation peaks identical in both spectra are highlighted in red circles.



Supp Figure 2. Control for technical detectability of cycloartenol in spiked *Chondrus crispus* extract.
a) TIC from *Chondrus crispus* extract incubated with cycloartenol. b) MS spectrum of cycloartenol standard incorporated in *C. crispus* extract. c) MS spectrum of cycloartenol standard alone. Main fragmentation peaks identical in both spectra are highlighted in red circles.

MAAs	Palythine	Mycosporine-glycine	MAA1	Isujirene/Palythene	Asterina-330	Palythinol or MAA2	Shinorine	Porphyra-334
Rt (min.)	8.3	20.0	10.8	19.3	8.7	10.1	18.5	19.5
m/z [M+H]+ observed	245.1090	246.0932	271.1241	285.1401	289.1349	303.1497	333.1245	347.1399
m/z calculated	245.1132	246.0972	271.1288	285.1445	289.1394	303.1551	333.1292	347.1449
EIC (Intens. x108)								
C. crispus (April)	16118542	707375	3254803	209911	5116637	26945	3129533	353130
C. crispus (July)	12600749	85700	928894	36714	3788544	18560	394887	11021
C. crispus (August)	16469850	219296	857212	238033	5653618	32998	1063642	83569
C. crispus (Sept.)	11230824	56477	2546286	77636	2917730	< LOD	5199737	33580
UV (mAU)								
C. crispus (April)	20420	31,525	1541	< LOD	6996	< LOD	2299	117
C. crispus (July)	14578	171,83	1487	< LOD	7106	327,43	335	< LOD
C. crispus (August)	19005	242,7	2143	< LOD	9927	707,57	1245	248
C. crispus (Sept.)	12768	< LOD	989	< LOD	6367	< LOD	5128	136

Supp. Table 4. MAAs composition in *Chondrus crispus* by LC-UV-HRMS. Extracted Ion Chromatogramm (EIC) of selected MMAs were obtained in positive mode; UV Absorbance was recorded at 330 nm (LOD = Limit Of Detection).



Supp. Figure 3. Maximum-likelihood tree of eukaryotic side-chain reductases. In green: sequences from green plants (streptophytes). The green dot indicates lineage-specific duplication in solanaceans. In red: sequences from red algae. In blue: sequences from opistokonts (vertebrates + choanoflagellates). In brown: sequences from oomycete stramenopiles. Likelihood-ratio test values above 0.90 are indicated. Those above 0.97 are considered significant.

Supplementary dataset S1. New or edited protein sequences for the sterol synthesis pathway in *Chondrus crispus*.

>scaffold90:7511-6165(-) candidate squalene epoxidase
RDGRRVLCVERQLYAPSGALCAPPRIVGELLQPGGYDALCRLGLADALLDIDAQVIRGYA
LFLGPRAERLPYHQPGGPDPDPDPAARPQPEGRAFHNGRFLKRLREIARAHPNV
TLVEGNVLALLERDGAVVGVRYATRGNKAATAHAGLTIAC
DGCGSALRKRAAAHHHVTVYSNFHGLVLHVPALPFPNHGHVVLADPCPVLFYPISATEVR
CLVDIPSTYAGDAAEYILHTVVPQVPPPLRAPLATAVRERRSKMMPNRVMPAPA
HVVPGAVLLGDAFNMRHPLTGGGMTVALTDVELLRELLAPVPDLSDAPAVAAKLQLFYER
RKPMSTTINILANALYTLFCATDDPALRDMRAACLDYLAKGGRMTHDPIAMLGGLKPQRH
LLLAHFFAVALYGCGKALMPFPTPARLVRAWSIFRASFNIIKPLANAEGFWPLSWLPLNSL

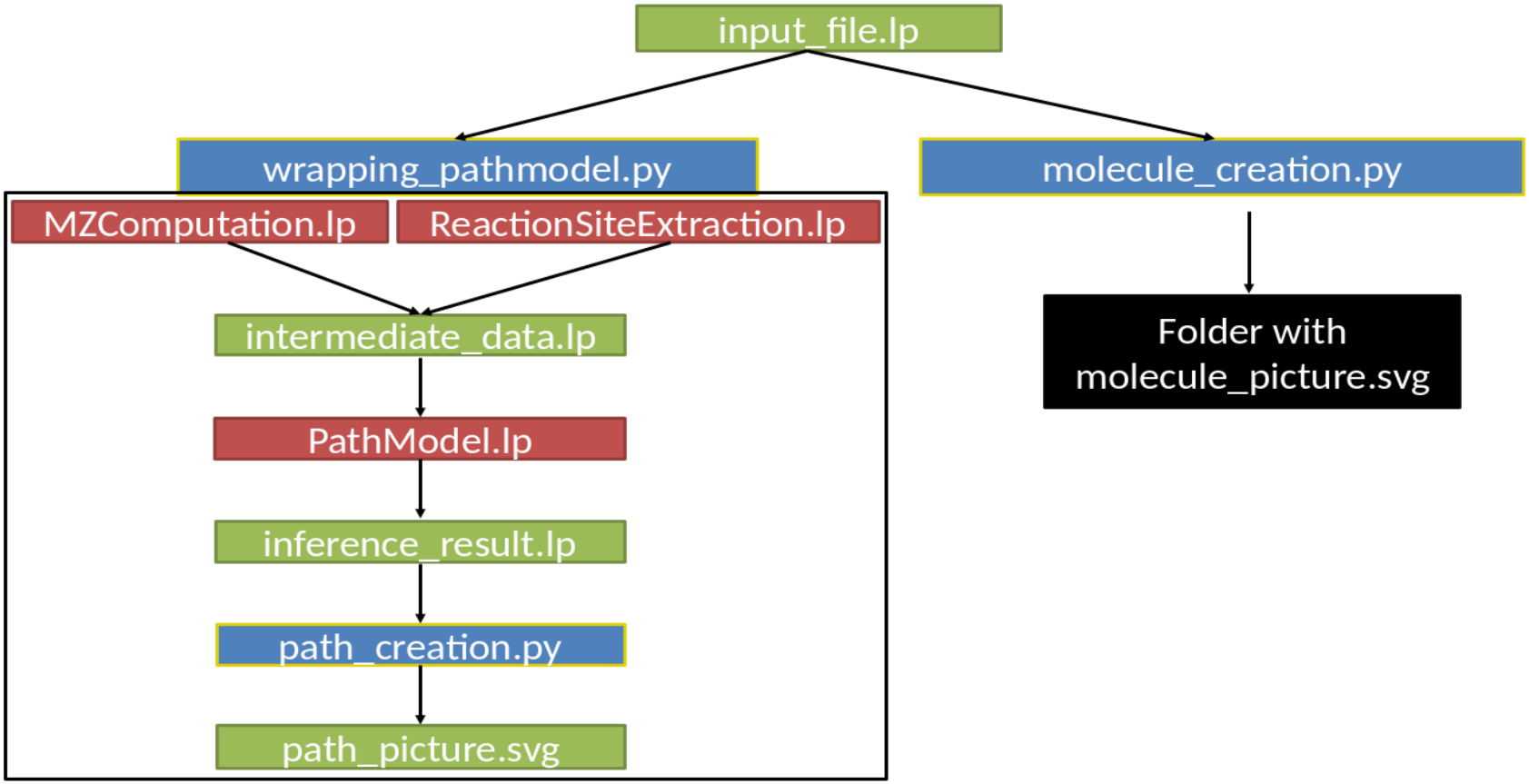
>scaffold20:461442-460650(-) candidate squalene epoxidase
LCRLGLADALLHIDAQVIRGYALFLGPRAERLPYHQPGEPDPDPAARPQPEG
RAFHNGRFLKRLREIARAHPNVTLIEGNVLALLERDGAVV
GVRYATRGNKAATAHAGLTIACDGCGSALRKRAAAHHHVTVYSNFHGLVLHVPALPFPNH
GHVVLAHPCPVLFYPISATEVRCLVDL
YILHTVVPQVPPSLRAPLATTVRERRSKMMPNRVMPAPAHVVPGAVLLGDAFNMRHPLTG
GGMTVALTDVELLRGLLAP

>scaffold57:152407-364140(+) candidate squalene epoxidase
RFAGPEHPSCGLKPQRHLLLAHFFAVALYGCGKALMPFPTPARPVRAWSIFRASFNFIK

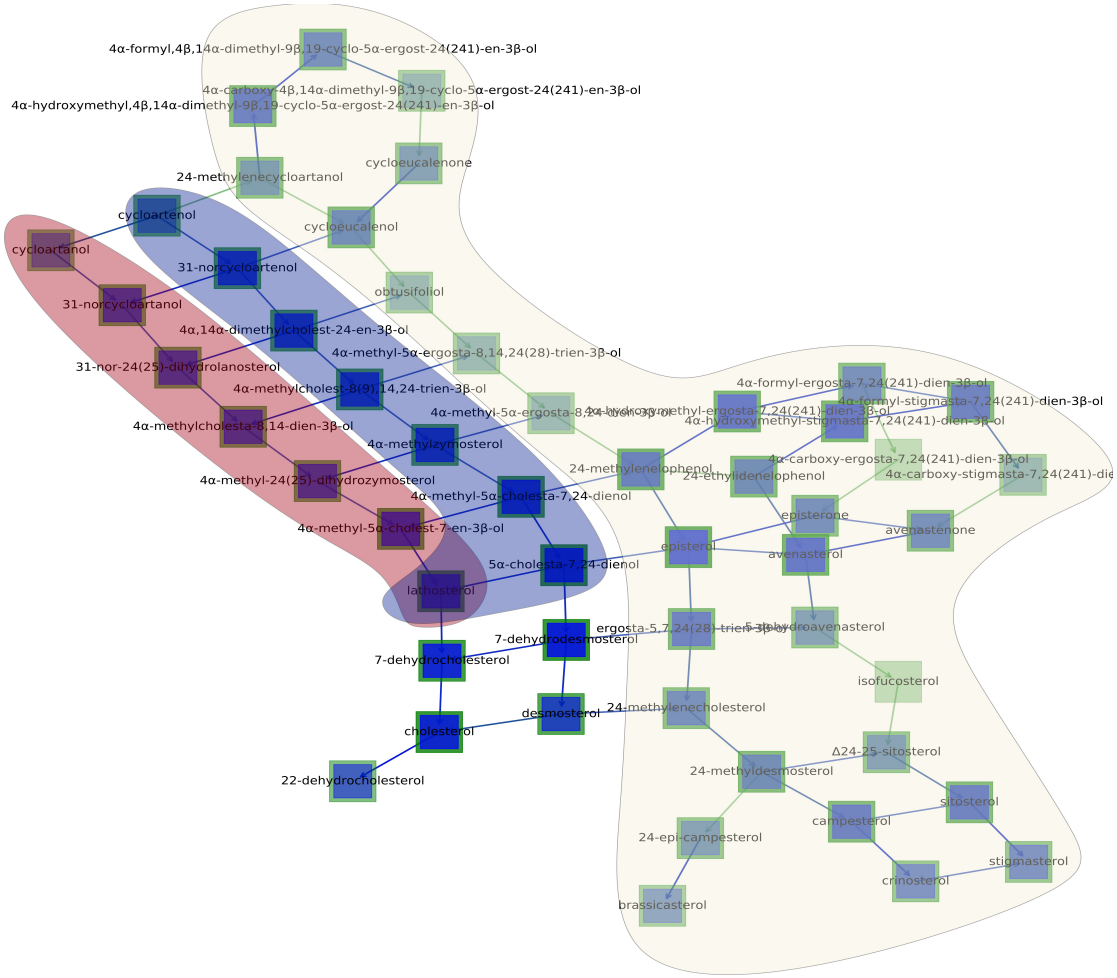
PLANAEGFWPLSWLPLN
LCRLGLADALLDIDAQVIRGYALFLGPRAERLPY
LCRLGLADALLHIDAQVIRGYALFLGPRAERLPYHQPGGPDPDPAARPQPEG
RAFHNCRFLKRLREIARAHPNVTLIEGNVLALLERDGAVV
GVRYATRGNKAATAHAGLTIACDGCGSALRKRAAAHHHVTVYSNFHGLVLHVPALPFPNH
GHVVLAHPCPVLFYPISATEVRCLVDL
WSTYAGDAAEYILHTVVPQVPPSLRAPLATAVRERRSKMMPNRVMPAPAHVVPGAVLLGD
AFNMRHPLTGGGMTVALTDVELLRGLLAP

>scaffold212:177405-176674(-) candidate C-4 sterol methyl oxidase
WDLLCRHTRAYPMFVVGCFASQLAGYFLGCAPFVLLDALRARSTPFRKIQPGKYAPRRAV
FAAAAAMLRSFATVVLPLLAAGGLFIERVGISRDAPFPSPRVVLLQVAYFFLVEDFLNYW
VHRALHLPWLYTRVHSVHHEYDAPFAVVAAYAHPVEVVLQALPTFAGPLMLGPHLYTLCV
WQLFRNWEAIDIHSGYDHAWGLASVLPWYAGPEHHDFHHFLHSGNFASVFTWCDWAYGTD
LAYE

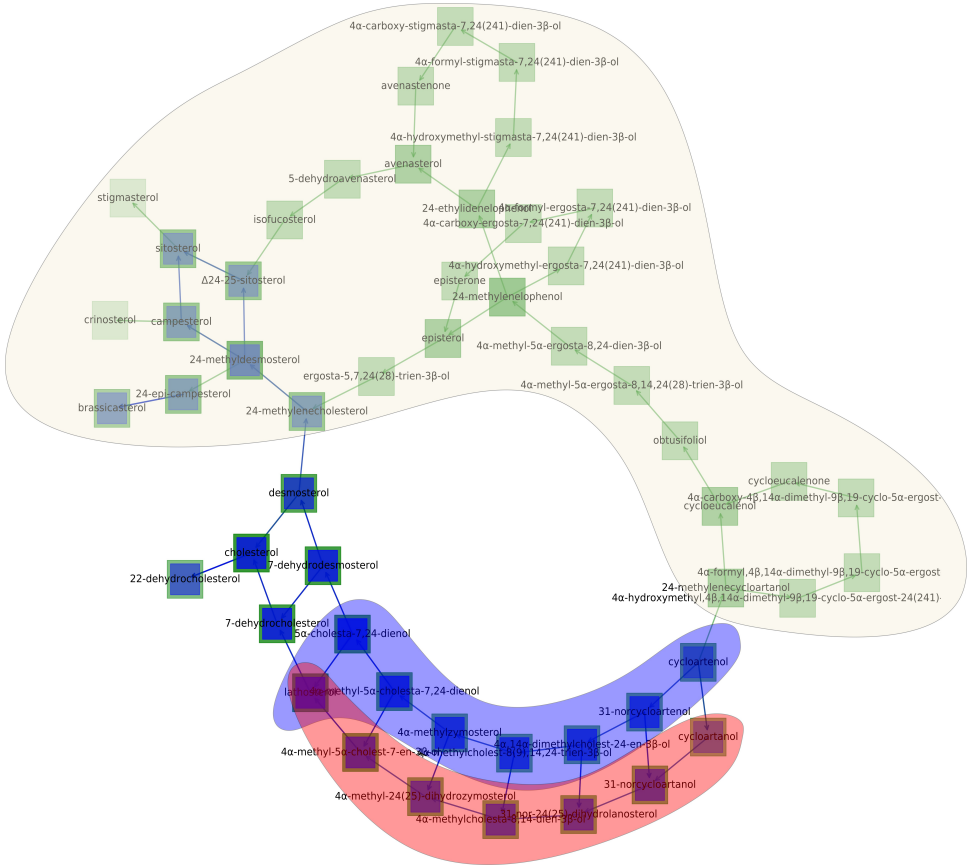
>CHC_T00006492-3001 fusion of adjacent protein predictions CHC_T00006492001 and CHC_T00006493001; candidate sterol delta-7 reductase
MLGIAAWKGFIRYGLLYDHFGEVLAFLGKFALVVTVLLYFRGIYFPTNSDSGTTSFGIVWDMWHGTELHP
EIFGVSLKQLVNCRFALMGWSVAIVAFACKQREQYGYVSNSMLVSVVLQLVYIFKFFVWEAGYFNSVSLD
HSHVCLFWIYLRPLY
MVGVGAICCNYWTDKQREVFRATNGQVTIWGQKPVSIEAQYVTGDGKKRRSLLLASGWWGVSRHVNYVFE
IALTFCWSVPAGGTGVIPYVYVMFLTILLTDRAYRDEVRCSEKYGKYYEEYCRLVPYKMIPGVY



Supp. Figure 4. Architecture of Pathmodel scripts. In green: Data files, either input or result files. In red: ASP scripts. In blue: Python scripts. In black: folder containing results from molecule_creation.py (molecule pictures). The black line shows the wrapping of all the scripts inside by wrapping_pathmodel.py.



Supp. Figure 5. Result of Pathmodel for sterol by path_creation.py. In white: reactions and metabolites from PWY-2541 (Metacyc). In red: Early SSR pathways. In blue: Late SSR pathways. Arrows in green are from PWY-2541 and arrows in blue are inferred by Pathmodel.



Supp. Figure 6. Result of Pathmodel for sterol with path_creation.py and absent molecules. In white: reactions and metabolites from PWY-2541 (Metacyc). In red: Early SSR pathways. In blue: Late SSR pathways. Arrows in green are from PWY-2541 and arrows in blue are inferred by Pathmodel. In this example, we put all the metabolites of the PWY-2541 as absent molecules, so no inference was made on them.