Adiponectin Receptors crystal structures reveal an intrinsic ceramidase activity

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Supplementary Information:

Estimation of ACER kcat:

The activity (k_{cat}) of purified alkaline ceramidase is not known as all the published experiments were performed with microsomes, a crude preparation of reticulum endoplasmic membrane. The activity is thus dependent on the expression level of these proteins within these microsomes preparations. Using this approach, the human alkaline ceramidase 2 (ACER2) was shown to have V_{max} as low as 5 pmol/min/mg [1]. Assuming that microsomes used to perform this analysis contain between 10 to 100 pmoles of ACER2 enzyme per mg of protein, a typical expression level found in yeast overexpressing membrane proteins [2], then the k_{cat} can be roughly estimated to be between 8×10^{-3} and 0.8×10^{-3} s-1, hence in the same order of magnitude than the k_{cat} determined for ADIPOR.
The same does apply to ACER3 as the Vmax we estimated based on the double reciprocal plot described in the original characterization of ACER3 [3] is ~ 4 pmol/min/mg which would lead to a kcat as low as 0.66×10^{-3} per s using the same expression level as above (100 pmoles).
We also acknowledge that the measurements were made in detergent micelles that are notoriously known to destabilize membrane proteins and we cannot ascertain that the determined enzymatic parameters in a specific pH, ionic strength, and detergent condition are reflecting the optimal activity that would be attainable *in vivo* in a membrane environment.

REFERENCES

1 Sun, W. *et al.* Substrate specificity, membrane topology, and activity regulation of human alkaline ceramidase 2 (ACER2). *J Biol Chem* **285**, 8995-9007, doi:10.1074/jbc.M109.069203 (2010).
2 Lundstrom, K. Structural genomics and drug discovery. *J Cell Mol Med* **11**, 224-238, doi:10.1111/j.1582-4934.2007.00028.x (2007).
3 Mao, C. *et al.* Cloning and characterization of a novel human alkaline ceramidase. A mammalian enzyme that hydrolyzes phytoceramide. *J Biol Chem* **276**, 26577-26588, doi:10.1074/jbc.M102818200 (2001).

Table 2: Data collection and refinement statistics (molecular replacement)

	ADIPOR2-Fv[a] (before)	ADIPOR2-Fv (after)	ADIPOR1-Fv[a] (before)	ADIPOR1-Fv (after)
Data collection				
Space group	$P2_122_1$	$P2_122_1$	$C222_1$	$C222_1$
Cell dimensions				
a, *b*, *c* (Å)	74.6, 108.6, 101.0	74.58, 101.03, 108.63	92.3, 194.1, 74.3	92.31, 194.11, 74.33
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	19.5-2.4 (2.5-2.4)	101.03-2.40 (2.50-2.40)	20.0-2.9 (3.0-2.9)	97.06-2.73 (2.85-2.73)
R_{merge}	0.115 (1.297)[NA]	0.115 (1.297) [NA]	0.192 (0.930)	0.171 (NC)
$CC_{1/2}$	0.996 (0.506)	0.996 (0.506)	0.976 (0.517)	NC (NC)
I / σI	8.55 (1.19)	8.55 (1.19)	6.73 (2.03)	7.93 (NC)
Completeness (%)	98.3 (99.4)	98.3 (99.4)	99.7 (100.0)	99.7 (100)
Redundancy	4.5 (4.5)	4.5 (4.5)	7.4 (7.5)	7.4 (7.5)
Refinement				
Resolution (Å)	19.5-2.4	17.64-2.40	19.9-**2.9**	26.66-**2.73**
No. reflections	32141	32803	15098	18737
R_{work} / R_{free}	**24.8/29.0**	**19.51/22.00**	**23.9/30.0**	**21.36/22.83**
No. atoms				
Protein	4033	4082	4041	4041
Zn	1	1	1	1
Oleate	0	20	0	0
Mono-oleins	0	200	0	0
Water	52	313	0	164
B-factors				
Protein	66.4	81.6	62.1	74.5
Zn	56.3	64.1	49.5	62.5
Oleate	-	69.7	-	-
Mono-oleins	-	120.8	-	-
Water	61.5	67.4	-	63.0
R.m.s. deviations				
Bond lengths (Å)	0.003	0.008	0.004	0.011
Bond angles (°)	0.76	1.040	0.8	1.070

*Values in parentheses are for highest-resolution shell.
[a] Data collection statistics for the two revised structures were taken from the original pdb depositions 3WXW and 3WXV.
NA-not applicable, R_{merge} value over 1 is statistically meaningless.
NC-not communicated due to different resolutions reported in the publication (ref 2 in the main text) and pdb entry.

Table 2: Summary of molecular dynamics simulations

M.D. system	Number of trajectories	Total simulation time (μs)	Primary zinc coordination sphere*	Membrane composition
ADIPOR2 + CER18 (PLANTS best score)	3	0.8	H202, S198, H352, D219 (monodentate), 2HOH	100% POPC
ADIPOR2 + CER18 (PLANTS best score)	10	10 x 0.01	H202, S198, H352, D219 (monodentate), 2HOH**	100% POPC
ADIPOR2 + CER18 (PLANTS best score)	1	0.15	H202, S198, H352, D219 (monodentate), 2HOH***	50% POPC, 20% CER16, 20% CER18, 10% CHL
ADIPOR2 + CER16 (PLANTS best score)	1	1.1	H202, S198, H352, D219 (monodentate), 2HOH	50% POPC, 40% CER16, 10% CHL
ADIPOR2 + CER18 (GLIDE best score)	2	0.3	H202, H348, H352, D219 (mono- or bidentate), 1 or 2 HOH	50% POPC, 20% CER16, 20% CER18, 10% CHL
ADIPOR2 + CER16 (PLANTS 2nd best score – amide flipped towards ZN)	1	0.1	H202, H348, H352, D219 (bidentate), CER16 carbonyl	50% POPC, 40% CER16, 10% CHL
ADIPOR2 + CER16 (SWISSDOCK best score – upside down orientation with sphingosine moiety in the FFA binding pocket)	1	1.3	H202, H348, H352, D219 (monodentate), CER16 carbonyl and secondary alcohol hydroxyl	75% POPC, 25% CER16
ADIPOR2 + OLE + SPH	3	2.8	H202, H348, H352, S198 or D219 (monodentate), 1HOH, unstable after ~ 500 ns in 2 trajectories****	100% POPC
ADIPOR2 + PAL + SPH	1	1.0	H202, H348, H352, 3HOH	50% POPC, 40% CER16, 10% CHL
ADIPOR2	1	1.0	H202, H348, H352, D219 (bidentate), 1HOH	60% POPC, 40% CER16
ADIPOR2 + Fv	1	0.6	H202, H348, H352, D219 (monodentate), 2HOH	60% POPC, 40% CER16

*The primary Zn coordination sphere is defined by any atom positioned less than 2.6 Å from the metal ion. Unless noted otherwise, the zinc coordination sphere corresponds to the configuration observed at the end of the trajectories. Classically, catalysis requires a His or Asp/Glu residue within the active site to be available (i.e. not directly coordinating the zinc), in addition to at least one water molecule in the zinc coordination sphere. It can be seen that these conditions are met only in the case of the systems that start from the top scoring PLANTS docking pose for CER16/18, whereas the other systems (GLIDE, SWISSDOCK and PLANTS 2nd best score) display zinc coordination spheres that do not appear to support catalysis. These data indicate that the conformations of the active site residues are highly sensitive to the CER16/18 docking poses.

**This Zn coordination sphere was observed in nine out of ten trajectories.

***In this trajectory, we observed that Ser198 started interacting with the zinc during equilibration, but the movements of His348 and Asp219 occurred only after about 7 ns of simulation time. This behavior differs from the system containing 100% POPC where all three residues rearranged at the start of equilibration (Fig. 3e), which suggests that the timescale of the observed conformational changes are sensitive to lipid composition.

****The observed instability of the active site at longer time scales suggests that the presence of ceramide and/or cholesterol in the lipid bilayer might play an important stabilizing role on ADIPOR2 structure.

Table 3 : Data collection and refinement statistics (molecular replacement)

	ADIPOR2-scFv	ADIPOR2-scFv
Data collection		
No. of crystals (No. of wedges)	3 (6)	4 (10)
Space group	$P2_122_1$	$P2_122_1$
Cell dimensions		
a, *b*, *c* (Å)	74.56, 101.06, 111.6	74.86, 101.14, 110.08
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	74.91-2.50 (2.60-2.50) *	101.14-3.30 (3.62-3.30)
R_{merge}	0.156 (NA)	0.242 (0.711)
R_{pim}	0.094 (0.739)	0.139 (0.403)
$CC_{1/2}$	0.995 (0.579)	0.989 (0.709)
I / σI	7.2 (1.4)	3.9 (1.7)
Completeness (%)	98.8 (98.4)	90.8 (91.6)
Redundancy	3.9 (3.9)	3.9 (4.0)
Refinement		
Resolution (Å)	31.38-2.50	61.90-3.30
No. reflections	29330	13088
R_{work} / R_{free}	18.80/19.71	21.84/23.30
No. atoms		
Protein	4038	4087
Zn	1	1
Oleate	20	20
Mono-oleins	300	25
Water	331	94
B-factors		
Protein	46.5	56.1
Zn	38.5	47.4
Oleate	45.3	47.7
Mono-oleins	78.8	66.7
Water	52.8	41.9
R.m.s. deviations		
Bond lengths (Å)	0.009	0.008
Bond angles (°)	1.040	1.010

*Values in parentheses are for highest-resolution shell.
NA-not applicable, R_{merge} value over 1 is statistically meaningless.