Supplemental Material

# Fenretinide binding to the lysosomal protein Saposin D alters ceramide solubilization and hydrolysis

Brandon T. Milliken,[a] Lindy Melegari,[a] Gideon L. Smith, Kris Grohn,[c] Aaron Wolfe,[c] Kelsey Moody,[c] Fadi Bou-Abdallah [b,*] and Robert P. Doyle[a,d,*]

[a.] *Department of Chemistry, Syracuse University, Syracuse, NY 13244, USA.*
[b.] *Department of Chemistry, State University of New York, Potsdam, NY 13676, USA.*
[c.] *Ichor Therapeutics, Inc. 2521 US-1, Lafayette, NY 13084, USA.*
[d.] *Department of Medicine, State University of New York, Upstate Medical University, 13210, USA.*

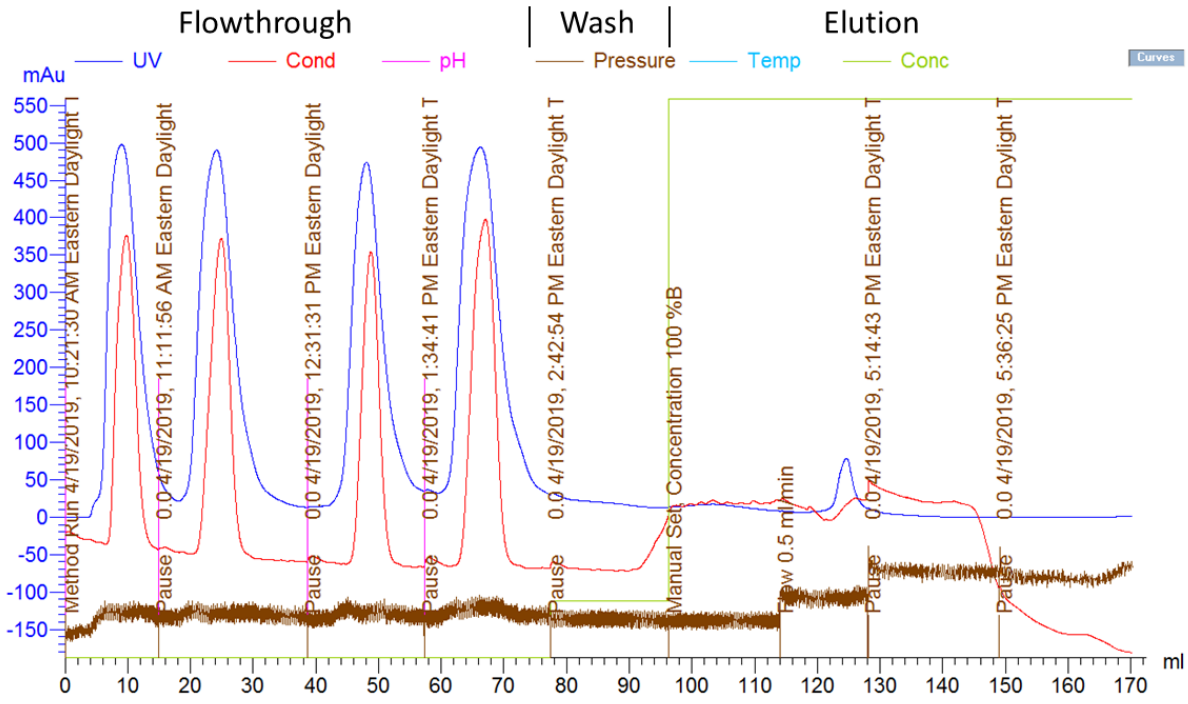


**Figure S1.** Purification of HIS-tagged SapD from concentrated lysate of E. *coli* by FPLC.

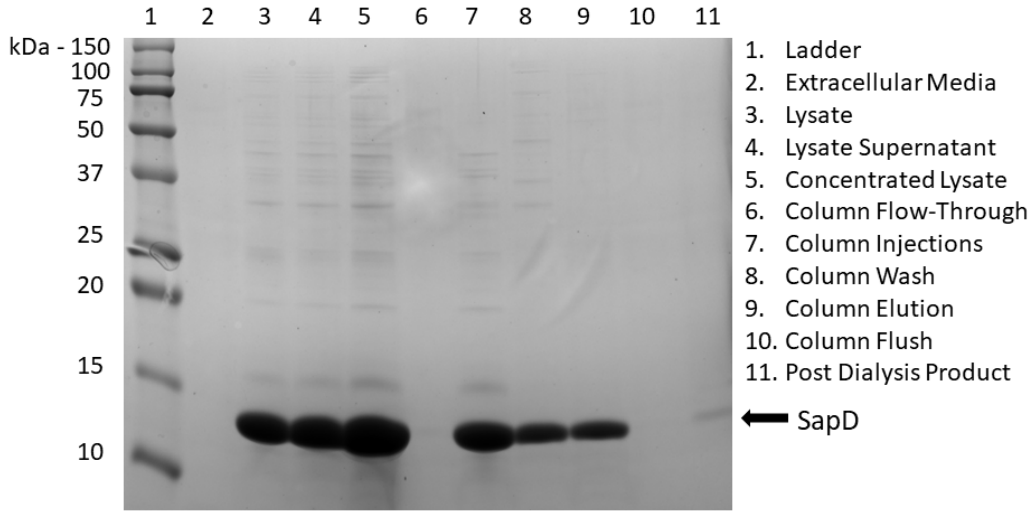


**Figure S2.** SDS-PAGE gel showing expression and purification of SapD induced with 400 µM IPGT.

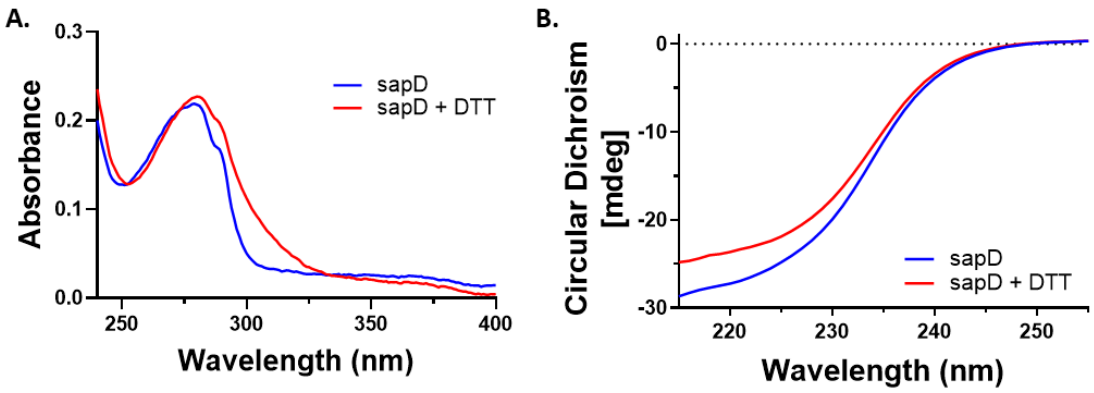


**Figure S3.** Evaluation for the presence of disulfide-bond formation. (a) Absorbance and (b) Circular Dichroism (CD) for 20 µM sapD initially evaluated. Sample was then spiked with >3 eq. of DTT and reevaluated.

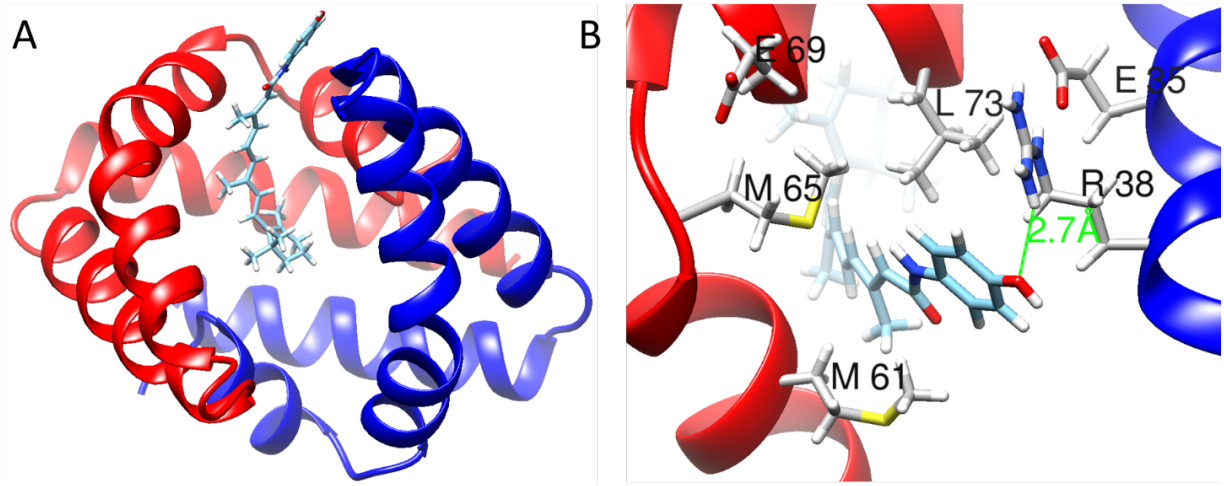


**Figure S4**. SwissDock molecular modeling for sapB (PDB:4V2O)[13] and Fen. Docking images were generated using the SwissDock modeling in Chimera 1.14. SwissDock Estimated ΔG (kcal/mol) -8.12.

| Ligand | Residue | Bond | Distance (Å) |
|---|---|---|---|
| 18:1 ceramide-1-phosphate | Tyr54 | Van der Waals | 3.8 |
| | Pro56 | Van der Waals | 4.1 |
| | Val57 | Van der Waals | 3.8 |
| | Glu60 | Van der Waals | 4.3 |
| | Ile61 | Van der Waals | 4.2 |
| | Lys74 | Van der Waals | 4.4 |
| | | | |
| Fenretinide | Glu53 | Van der Waals | 5.2 |
| | Tyr54 | Van der Waals | 3.4 |
| | Pro56 | Van der Waals | 5.3 |
| | Val57 | Van der Waals | 3.9 |
| | Iue73 | Van der Waals | 3.9 |
| | Lys74 | Van der Waals | 4.0 |
| | Lys74 | Hydrogen | 3.8 |
| | Ile75 | Van der Waals | 5.6 |
| | | | |
| Tamoxifen | Glu53 | Van der Waals | 4.4 |
| | Tyr54 | Van der Waals | 3.9 |
| | Pro56 | Van der Waals | 4.4 |
| | Val57 | Van der Waals | 3.7 |
| | Iue75 | Van der Waals | 4.6 |
| | Ser80 | Hydrogen | 3.7 |

**Table S1.** Summary of SwissDock modeling interactions for sapD and ligands.