

Description of Additional Supplementary Files

File name: Supplementary Movie 1

Description: Molecular dynamics trajectory of SPNS2 in the presence of S1P molecule (SPNS2::S1P system) showing S1P exploring the intracellular cavity and its upward movement in a time span of 1000 ns. S1P tail is shown in cyan and the polar head group in red, and the amino acid sidechains within 3Å distance of S1P are depicted.

File name: Supplementary Movie 2

Description: MD simulation of the SPNS2::S1P::glucose system (600 ns) showing inward movement of glucose through the central cavity of SPNS2 in the presence of upward movement of S1P in one replica run for 600 ns (terminated after glucose translocated into the solution). Two additional runs for 1000 ns each were performed. Across all three simulations, glucose consistently moved out of its initial binding cavity and interacted with S1P, demonstrating reproducible qualitative behavior. In two of the three replicas, glucose successfully passed within 600 ns, whereas in the third replica, glucose remained still interacting with S1P up to 1000 ns. These results indicate that the precise timing of glucose movement varies across replicas as expected for a stochastic process. Nevertheless, the qualitative behavior, namely glucose movement toward and interaction with S1P, is consistently observed.

File name: Supplementary Movie 3

Description: Molecular dynamics trajectory of outward-facing SPNS2 in the presence of glucose (SPNS2::glucose system) showing that glucose molecules remain in the extracellular cavity for the simulation duration of 600 ns.