Quantitative Study of Unsaturated Transport of Glycerol through Aquaglyceroporin That Has High Affinity for Glycerol

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Abstract

The structures of several aquaglyceroporins have been resolved to atomic resolution showing two or more glycerols bound inside a channel and confirming a glycerol-facilitator's affinity for its substrate glycerol. However, the kinetics data of glycerol transport experiments all point to unsaturated transport that is characteristic of low substrate affinity in terms of the Michaelis-Menten kinetics. In this article, we present an *in silico-in vitro* research focused on AQP3, one of the human aquaglyceroporins that is natively expressed in the abundantly available erythrocytes. We conducted 2.1 μs *in silico* simulations of AQP3 embedded in a model erythrocyte membrane with intracellular-extracellular asymmetries in leaflet lipid compositions and compartment salt ions. From the equilibrium molecular dynamics (MD), we elucidated the mechanism of glycerol transport at high substrate concentrations. From the steered MD simulations, we computed the Gibbs free-energy profile throughout the AQP3 channel. From the free energy profile, we quantified the kinetics of glycerol transport that is unsaturated due to glycerol-glycerol interaction mediated by AQP3 resulting in the concerted movement of two glycerol molecules for the transport of one glycerol molecule across the cell membrane. We conducted *in vitro* experiments on glycerol uptake into human erythrocytes for a wide range of substrate concentrations and various temperatures. The experimental data quantitatively validated our theoretical-computational conclusions on the unsaturated glycerol transport through AQP3 that has high affinity for glycerol.

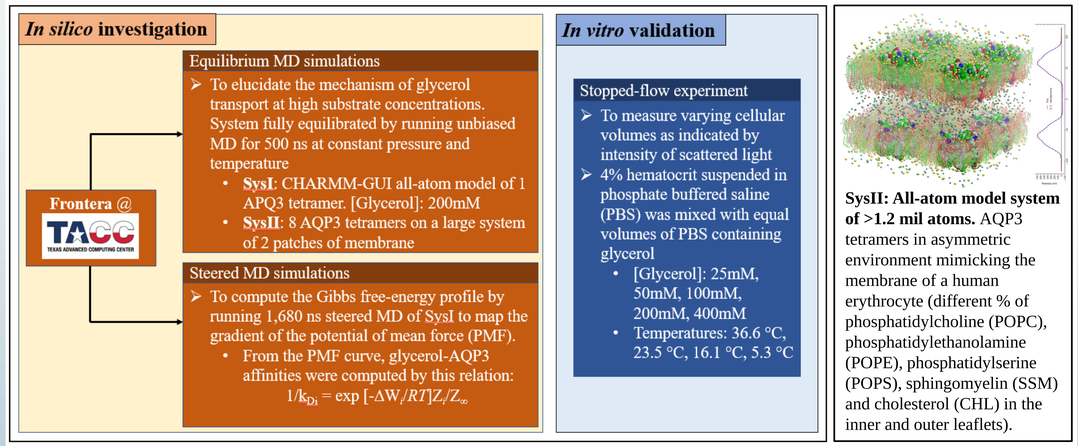
Introduction

- Aquaglyceroporins (AQPs) facilitate glycerol diffusion across the cell membrane down the concentration gradient. In the human body, AQP3, AQP7, AQP9, and AQP10 are responsible for lipid homeostasis and other physiological functions.
- Understanding the transport mechanism of glycerol by AQPs is fundamental in human physiology, biology and biotechnology, and has important applications in cancer therapy, obesity research etc.
- To date, high-resolution X-ray structures have been obtained, and numerous kinetic experiments conducted. However, gaps remain, and a key paradox will be addressed in this paper.
- We present an *in silico-in vitro* investigation of AQP3 in the erythrocyte membrane.
- Microsecond molecular dynamics simulation on Frontera provided by the Texas Advanced Computing Center (TACC) was used as the molecular dynamics (MD) simulation engine.
- Key simulation parameters are AQP3 copy number on erythrocytes, experimentally-validated lipid compositions, quantitatively accurate water model (TIP3P), and CHARMM36 parameters for inter- and intra-molecular interactions.

Motivation

- X-ray structures showed glycerols bound inside the channels, demonstrating that AQPs have affinity for their substrate, glycerol.
- However, many kinetics experiments of glycerol transport showed unsaturable characteristics for glycerol concentrations up to ~1 M, which suggest that AQPs are simple channels without affinity for their substrate.
- This presents a paradox: **how can an aquaglyceroporin have high affinity for glycerol but also transport the substrate in an unsaturable manner?**

Methods



SysII: All-atom model system of >1.2 mil atoms. AQP3 tetramers in asymmetric environment mimicking the membrane of a human erythrocyte (different % of phosphatidylcholine (POPC), phosphatidylethanolamine (POPE), phosphatidylserine (POPS), sphingomyelin (SSM) and cholesterol (CHL) in the inner and outer leaflets).

Results

Human aquaglyceroporin AQP3 has ~500/M affinity for glycerol and transports glycerol in an unsaturable manner for glycerol concentrations <1M.

- At low [glycerol]: A transport pathway with a single glycerol occupancy in AQP3 which is transported to either the extracellular (EC) or intracellular (IC) side.
- At high [glycerol] of ≥200mM: A second pathway predominates due to the lower energy requirements, with double glycerol occupancies inside the channel. The molecules move collectively to transport one from the cytoplasm to the EC fluid or vice versa.

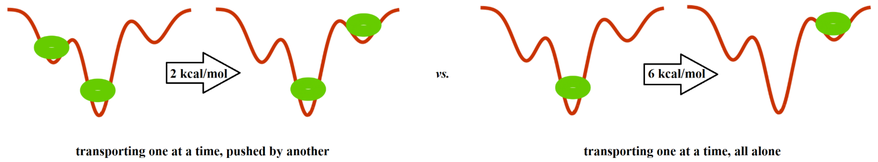
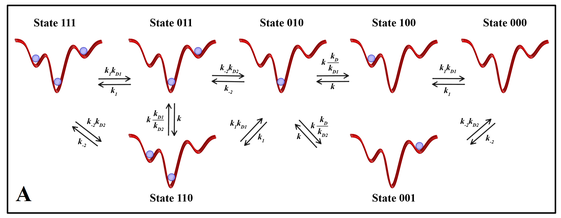


Fig 3 A. Seven states of the glycerol-AQP3 complex and rate constants of transitions between them.

- State 010 was always occupied
- States 000, 100, 111, 001 were never nearly occupied
- In the 0.2 μs timeframe, transitions between States 010 and 110, and States 010 and 011 occurred multiple times
- Transitions also occurred between States 011 and 110, indicating they have similar free energies



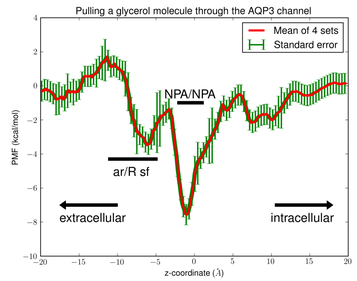


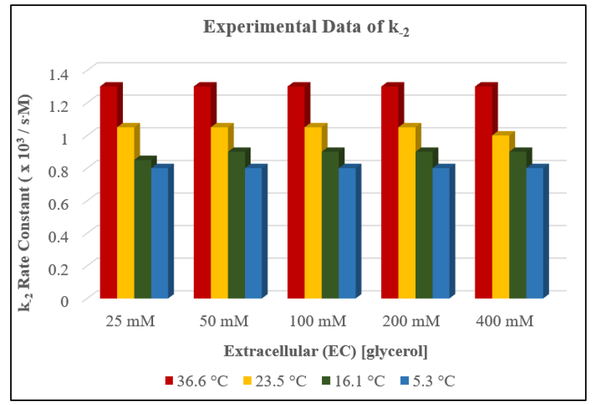
Fig. 4. PMF of glycerol throughout the AQP3 channel

- The PMF curve dictates that the rate constants for transitions between the states are related to one another
- Site 0: central binding site near NPA motifs (ΔW_0 = -7.5 kcal/mol), z ~ 0Å
- Site 1: on the EC side of the NPA (ΔW_1 = -3.5 kcal/mol), z ~ -6Å
- Site 2: on the IC side of the NPA (ΔW_2 = -2.2 kcal/mol), z ~ 8Å
- From the PMF curve and the fluctuations at the three binding sites inside the AQP3 channel, we computed glycerol-AQP3 affinities showing high affinity ~ 500/M at Site 0, and low affinities ~ 1/M at Sites 1 and 2

Results Continued

Graph of Table IV. Experimental data vs. computational predictions.

- In order to validate our PMF-based theoretical-computational study, we performed 20 sets of *in vitro* experiments on human erythrocytes at 4 different temperatures between 5°C and 37°C for a wide range of glycerol concentrations.
- The fitted values of the single parameter k_{-2} was weakly dependent on temperature and almost independent of glycerol concentration
- The experimental results was in perfect agreement with the kinetics theory based on the PMF curve, and glycerol transport was clearly unsaturated in the concentration range up to ~1M.



Acknowledgements

We would like to thank Roberto A. Rodriguez and Huiyun Liang for their assistance in the theoretical-computational work and experimental measurements, respectively. This work was funded by the NIH grant (GM121275). Computational time was done on Frontera at the Texas Advanced Computing Center at the University of Texas at Austin. Frontera is made possible by the National Science Foundation award OAC-1818253.

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