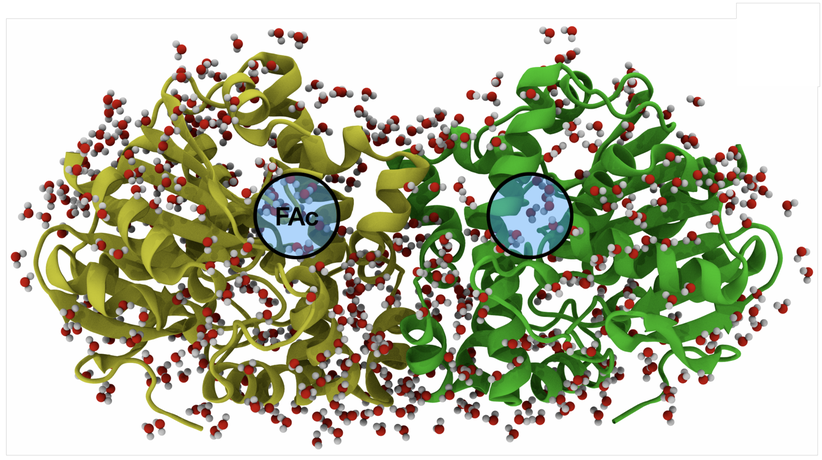
Describing Allosteric Water Networks In Enzymes

See Method

MOTIVATION

- Fluoracetate dehalogenase (FAcD) hydrolyses FAc via a cleavage of C-F bond.
- Half-of-site reactivity: catalysis happens only when one protomer is occupied by substrate. Requires strong coupling between protomers, i.e. allosteric network.



Experiments highlight the importance of water in the function of FAcD

Experiments are conducted in D_2O and H_2O: hydrogen bonds are stronger in D_2O.
1. The rate of catalysis increases in D_2O (^{19}F NMR).
2. Inter-protomer dynamics is enhanced in D_2O (^{19}F NMR).
3. Crystal structure (protein + water) and fold-stability does not change (NMR, X-ray).

Hypothesis: an allosteric water network contributes to the signal propagation between binding sites.

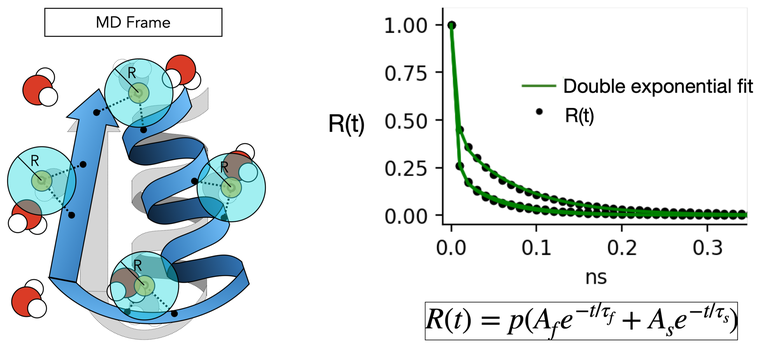
Objective: to identify water molecules that might be involved in the allosteric regulation of enzymatic activity

METHODS

Protein conformational ensembles are similar between H_2O and D_2O - consistent with experiments

MD Simulations	H_2O	D_2O
Water model	TIP3P	TIP3P-HW*
Force field	Amber ff14SB	
Simulation length	3 × 600 ns @ T=300K of **substrate-bound** structure 3 × 1500 ns @ T=300K of **apo** structure	

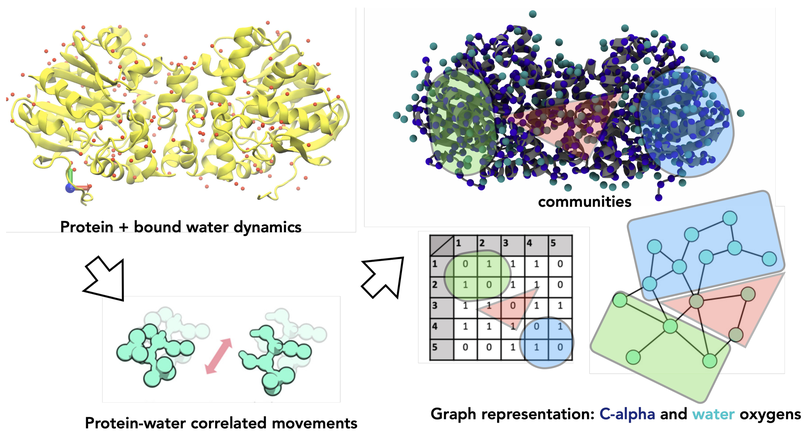
Determining occupancy and residence times of water molecules



$$R(t) = p(A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s})$$

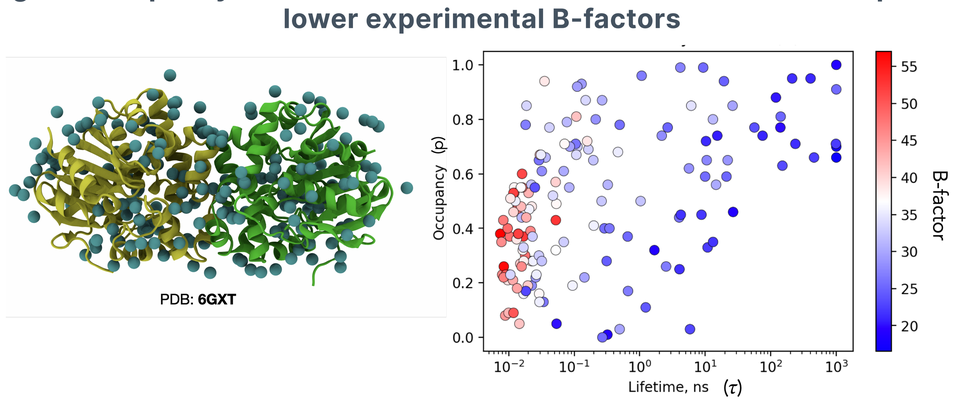
- Check how often water occupies a sphere of R=1.4 Å around a water site to determine occupancy (p).
- Compute autocorrelation function R(t) for each WS to determine the average residence time via a double exponential fit.

Analysis of correlated dynamics of protein and water using graph theory

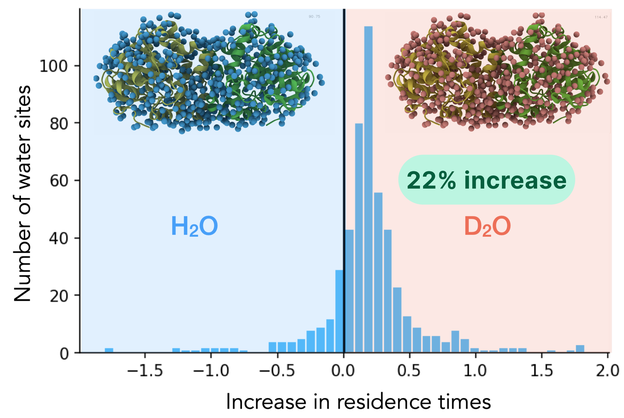


VALIDATION

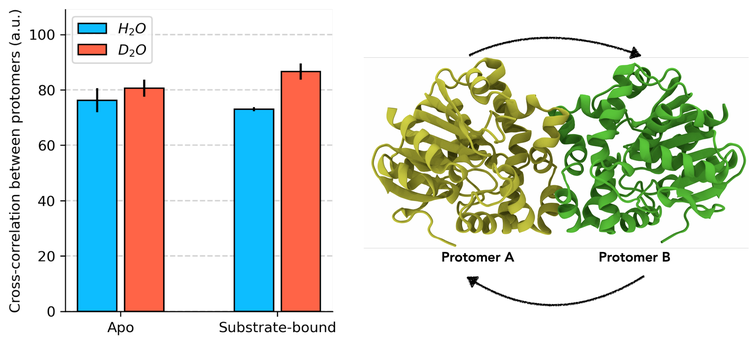
Higher occupancy and residence times of water molecules correspond to lower experimental B-factors



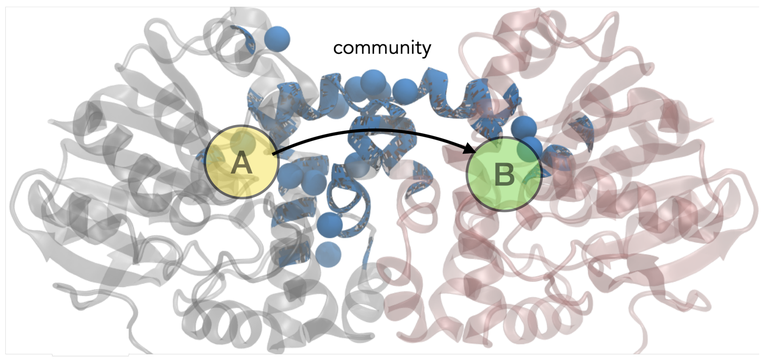
STIFFENING OF THE WATER NETWORK IN D_2O



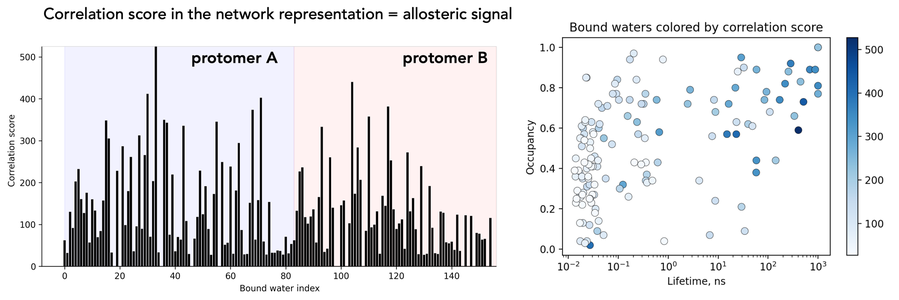
HIGHER CORRELATION BETWEEN PROTOMERS IN D_2O



ALLOSTERIC PATHWAY CONNECTING BINDING SITES



Correlation score in the network representation = allosteric signal



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Acknowlegments

UNIVERSITY OF TORONTO · NSERC CRSNG · compute canada · CONNAUGHT FUND