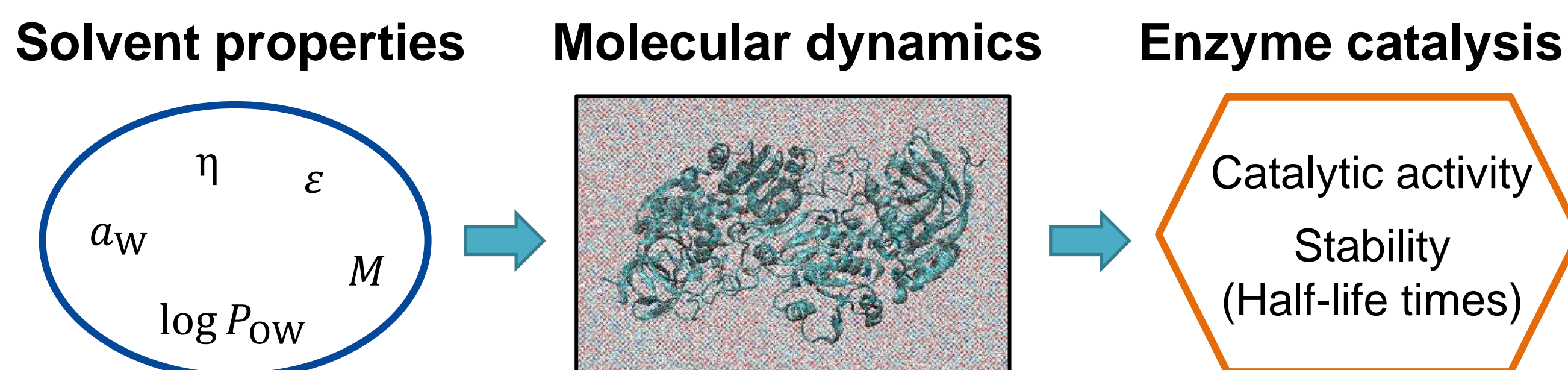


## Introduction

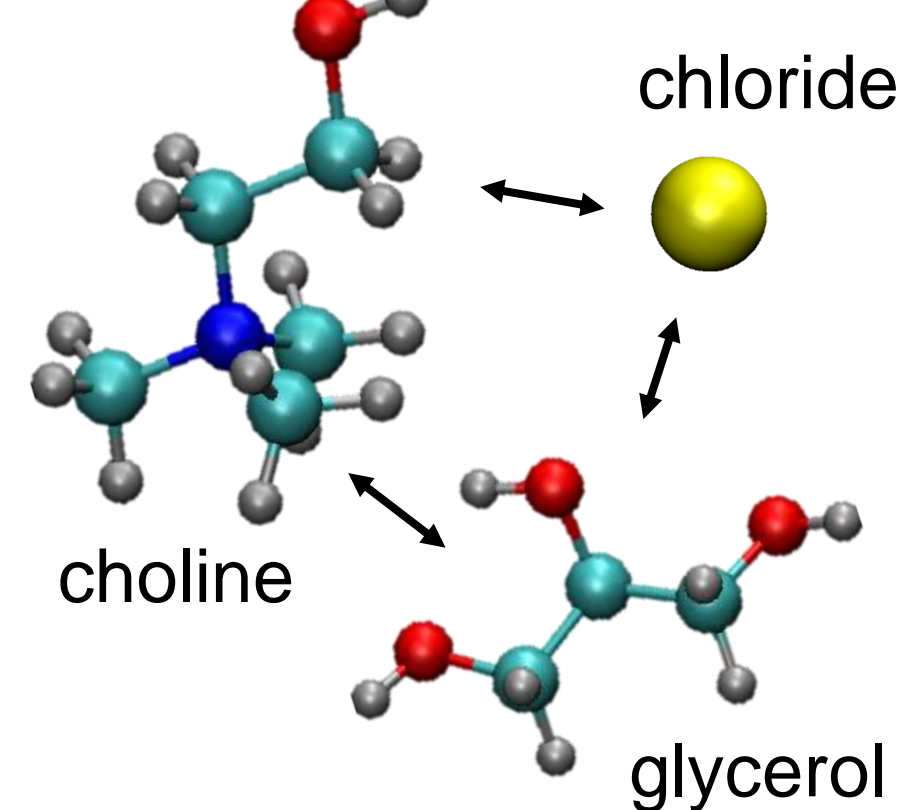
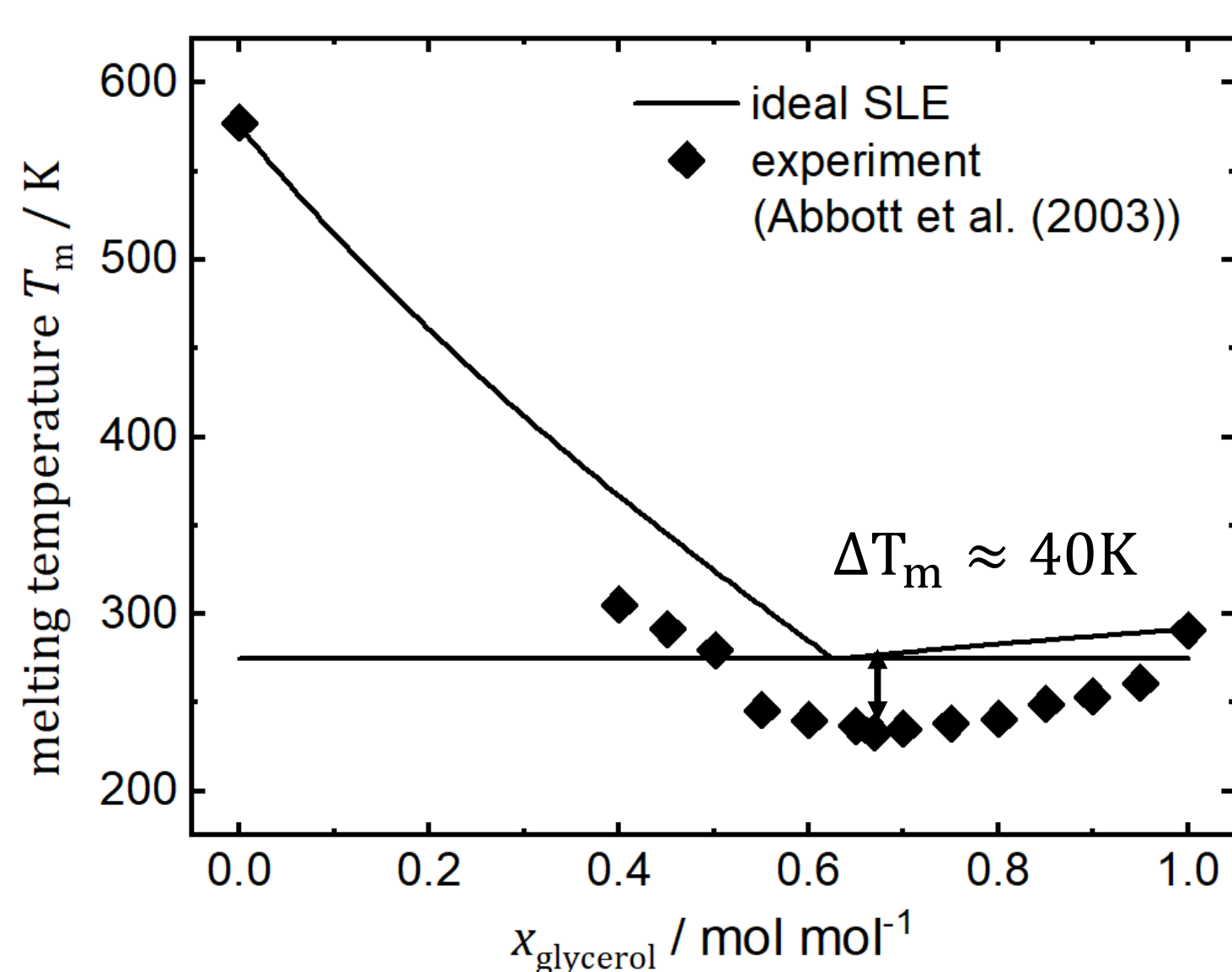


Molecular dynamics (MD) simulations offer a deep understanding of protein behavior and interactions with non-conventional solvents.

- Transfer of oxidoreductase catalysis from aqueous to **non-conventional media**
- **Advantages:** (1) high solubility of reagents, (2) less water-induced side reactions and (3) less water-induced enzyme denaturation
- **Deep eutectic solvents (DESs)** emerged as novel solvent class for biocatalysis
- **Aim:** Development of rules for guiding **solvent selections** for horse-liver alcohol dehydrogenase (HLADH) catalysis as model enzyme

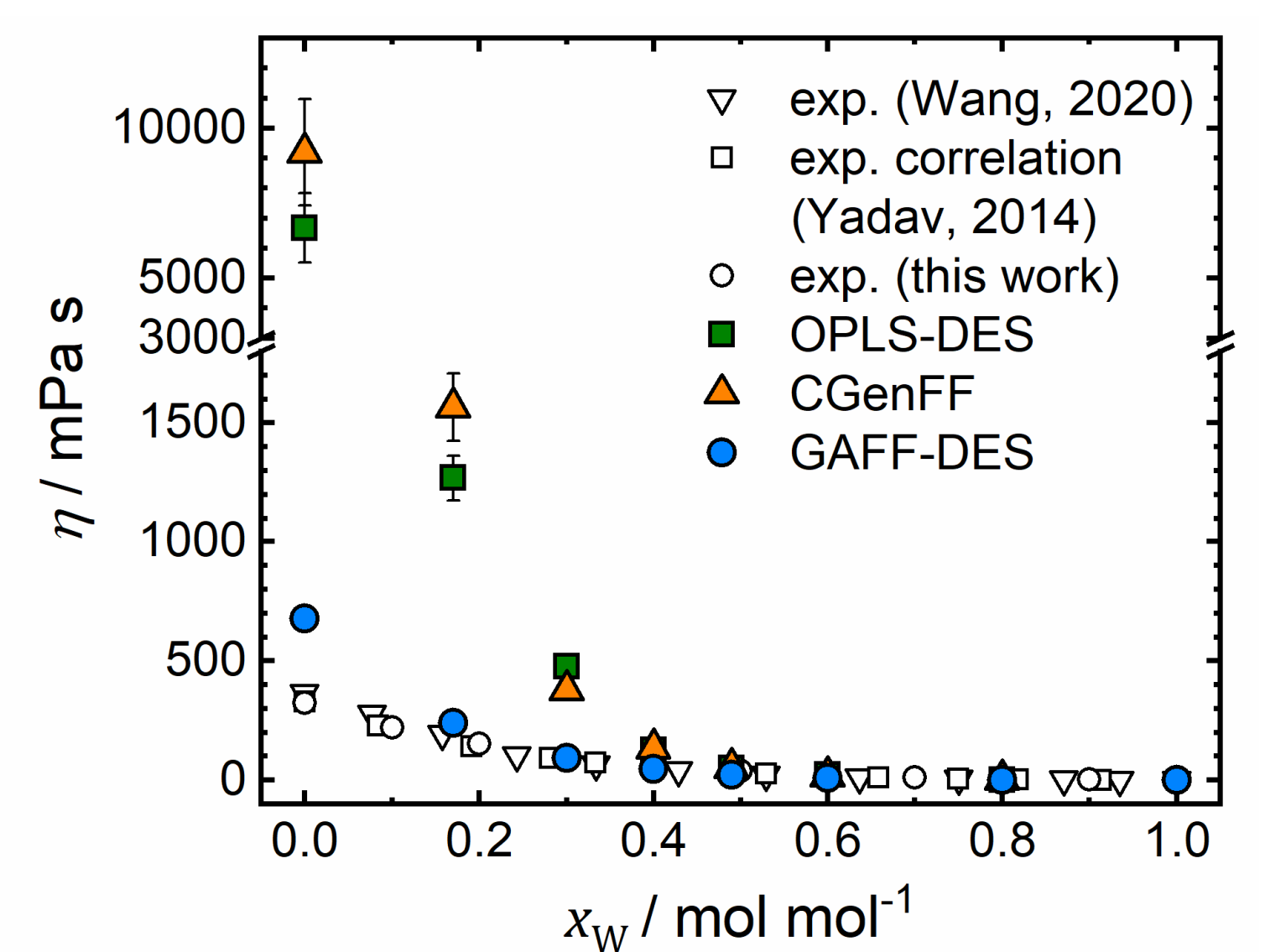
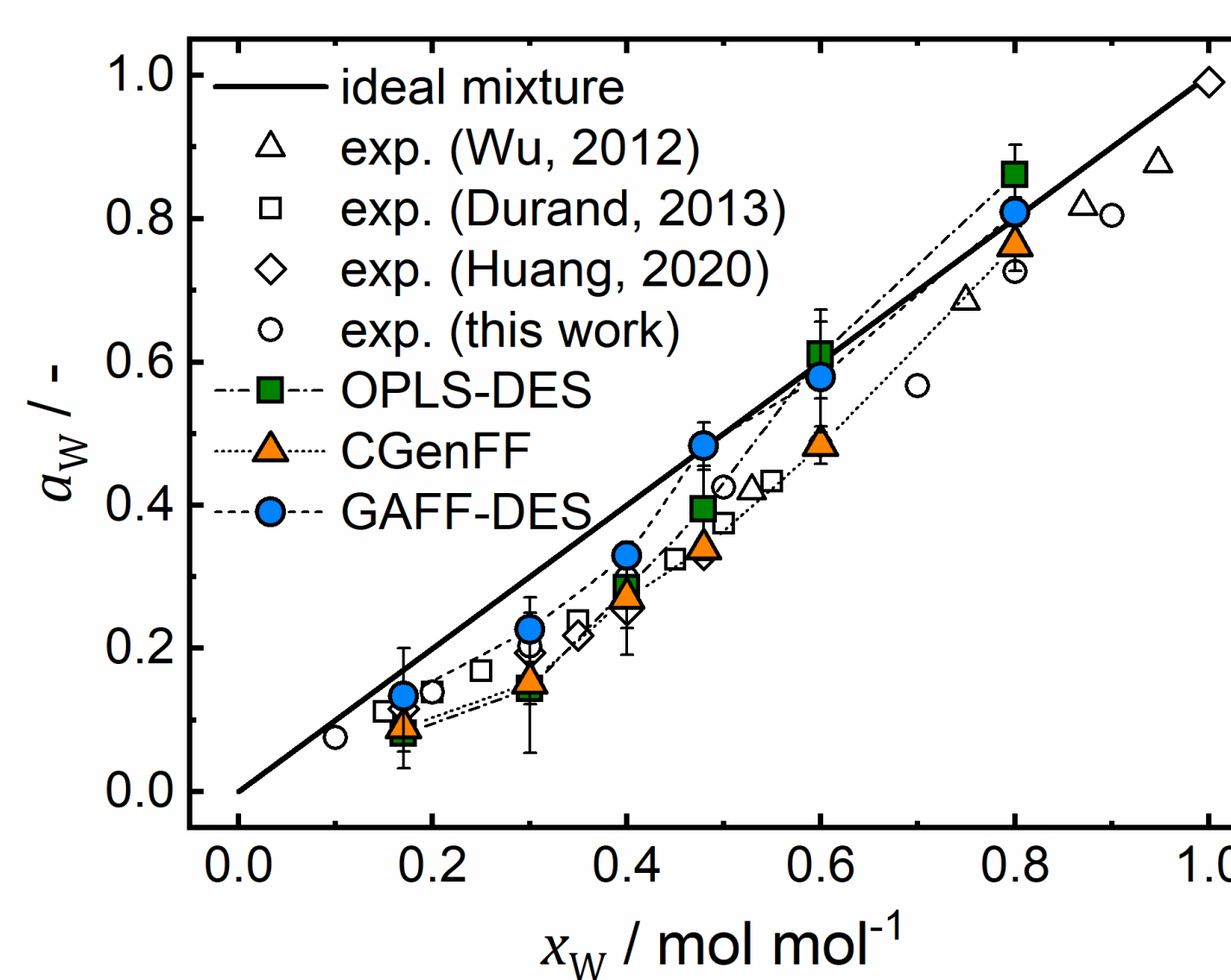
## Results

### 1. Deep eutectic solvent ChCl-Gly



Due to tailored characteristics, DESs have recently emerged as novel solvent alternatives for biocatalytic applications. They are mostly formed by mixing quaternary ammonium salts (e.g., choline chloride (ChCl)) with a hydrogen bond donor (e.g., glycerol). Strong interactions within the DESs result in significantly lower melting points of the mixture compared to an ideal system. This is shown for the widely used DES composed of ChCl and glycerol (ChCl-Gly).<sup>1</sup>

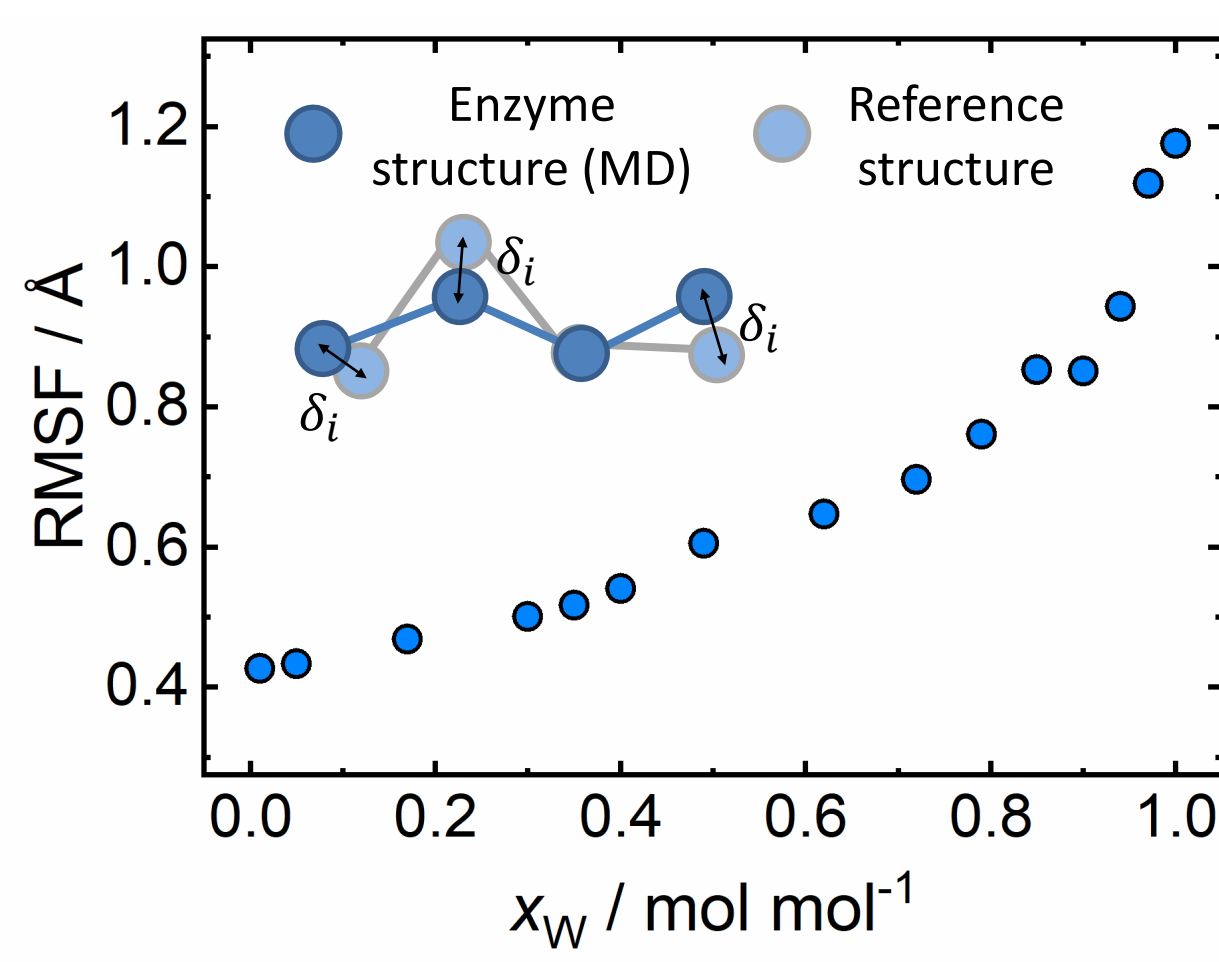
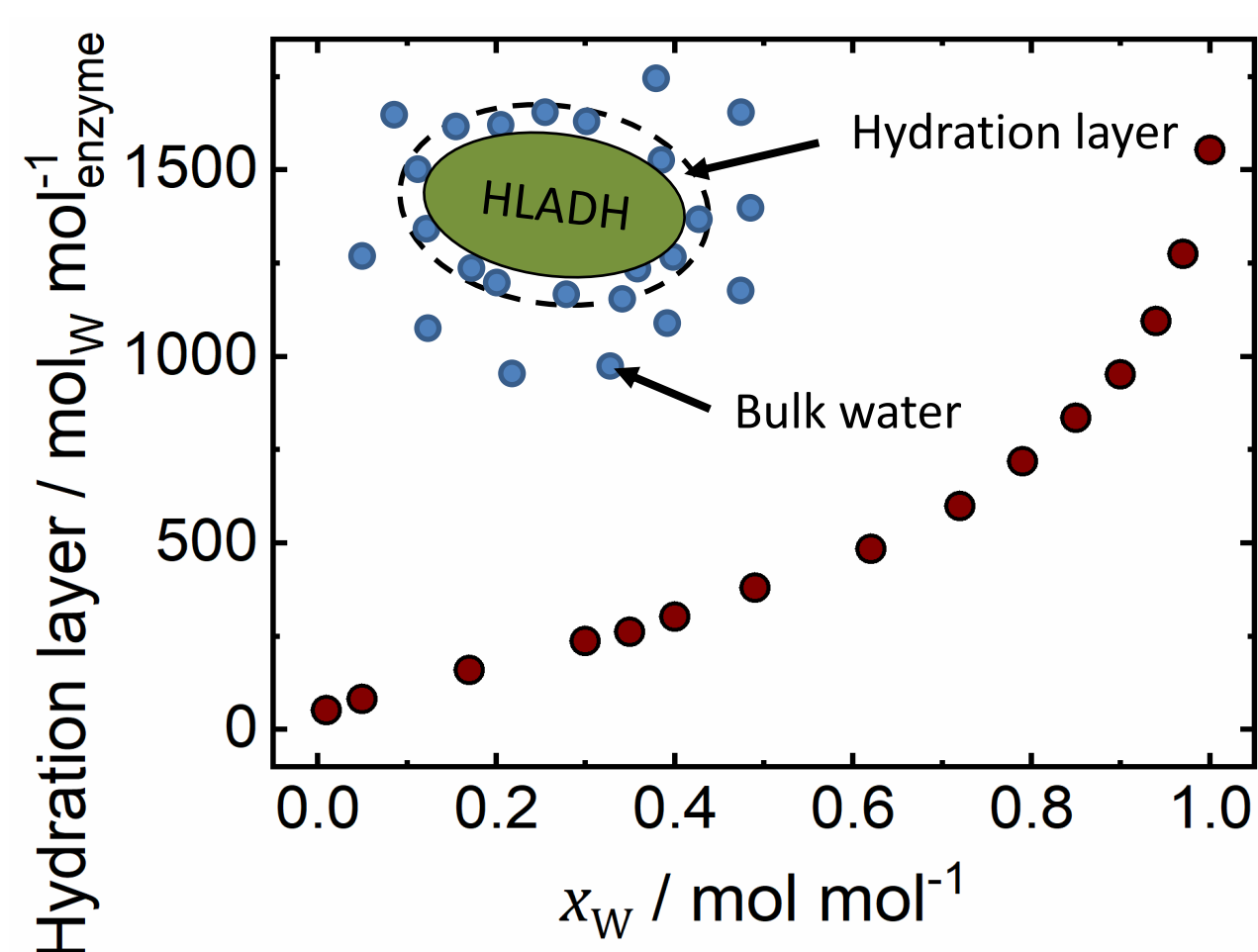
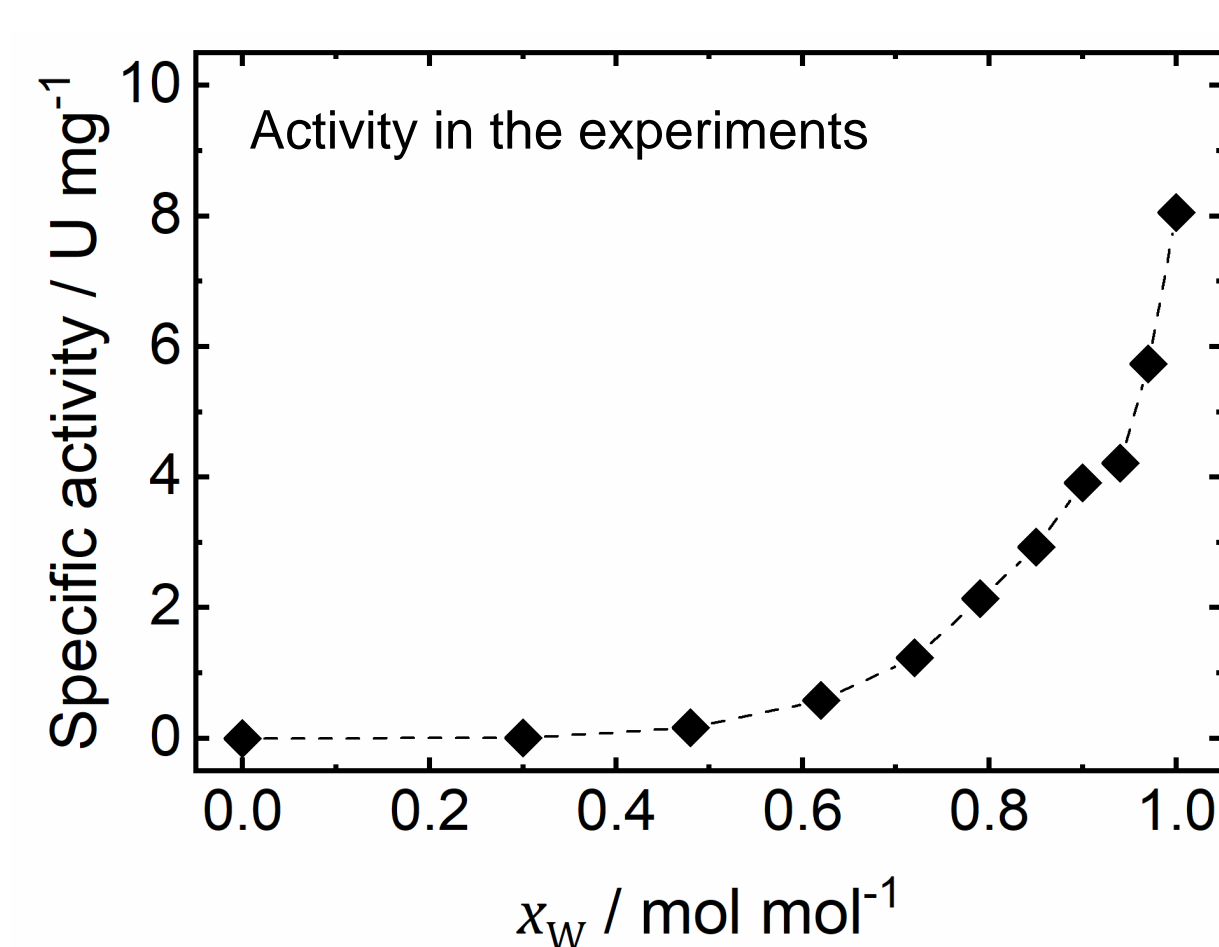
### 2. Validation of force fields for MD simulations



**Validation of interaction models (force fields) for MD simulations of the novel solvent class DESs.** The activity of water  $a_w$  was determined from free energy calculations and compared to experimental values<sup>2-4</sup> (left). The viscosity  $\eta$  was determined by the periodic perturbation method and compared to experimental data<sup>5,6</sup> (right). CGenFF<sup>7</sup> reproduces the thermodynamic water activity  $a_w$  best for the entire water concentration range, whereas the GAFF-DES<sup>8</sup> performs better for low water contents.<sup>9</sup> On the other side, GAFF-DES<sup>8</sup> outperforms OPLS-DES<sup>10</sup> and CGenFF<sup>7</sup> in reflecting the dynamic viscosity  $\eta$  of ChCl-Gly/water mixtures in MD simulations.<sup>9</sup>

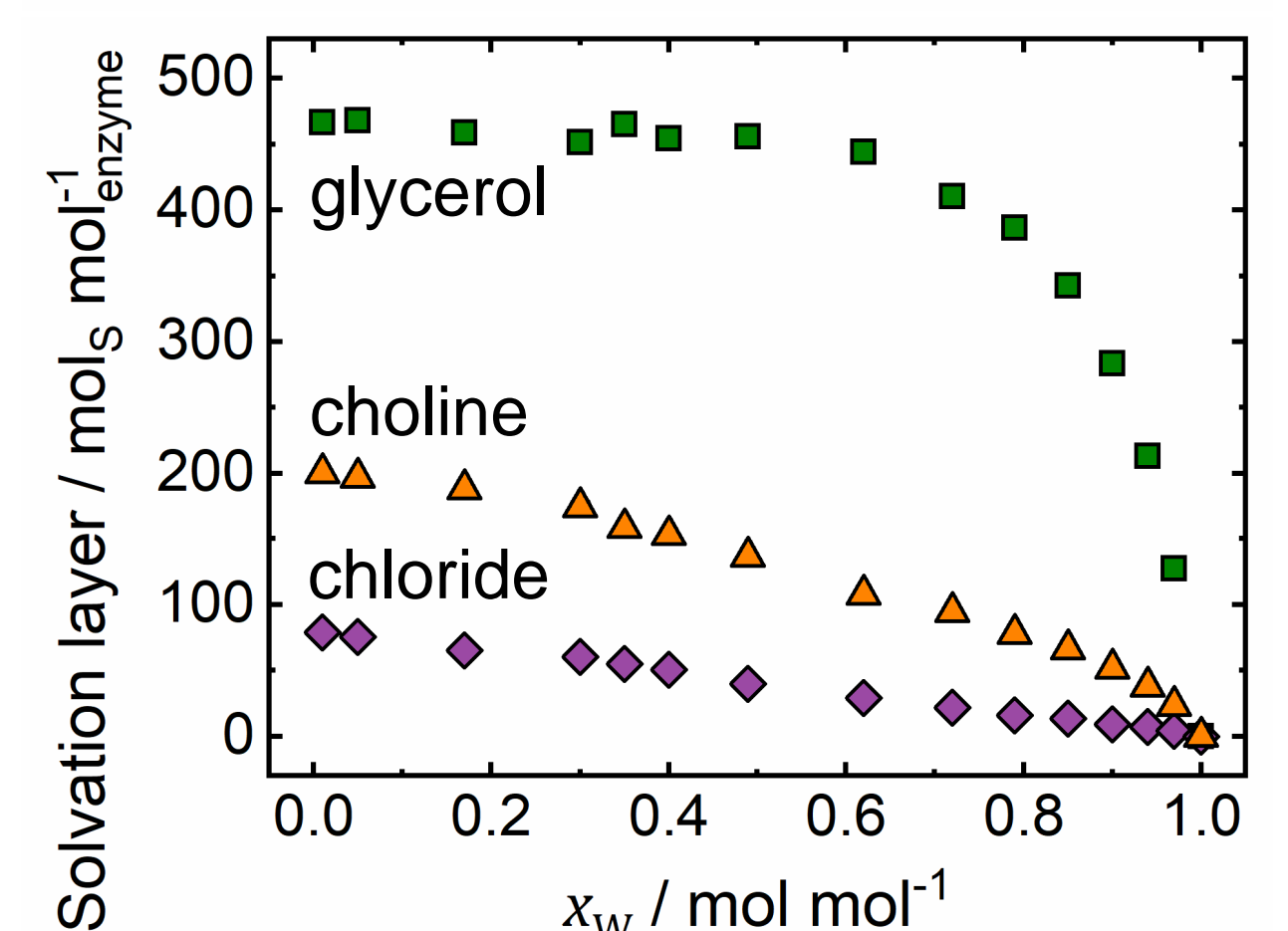
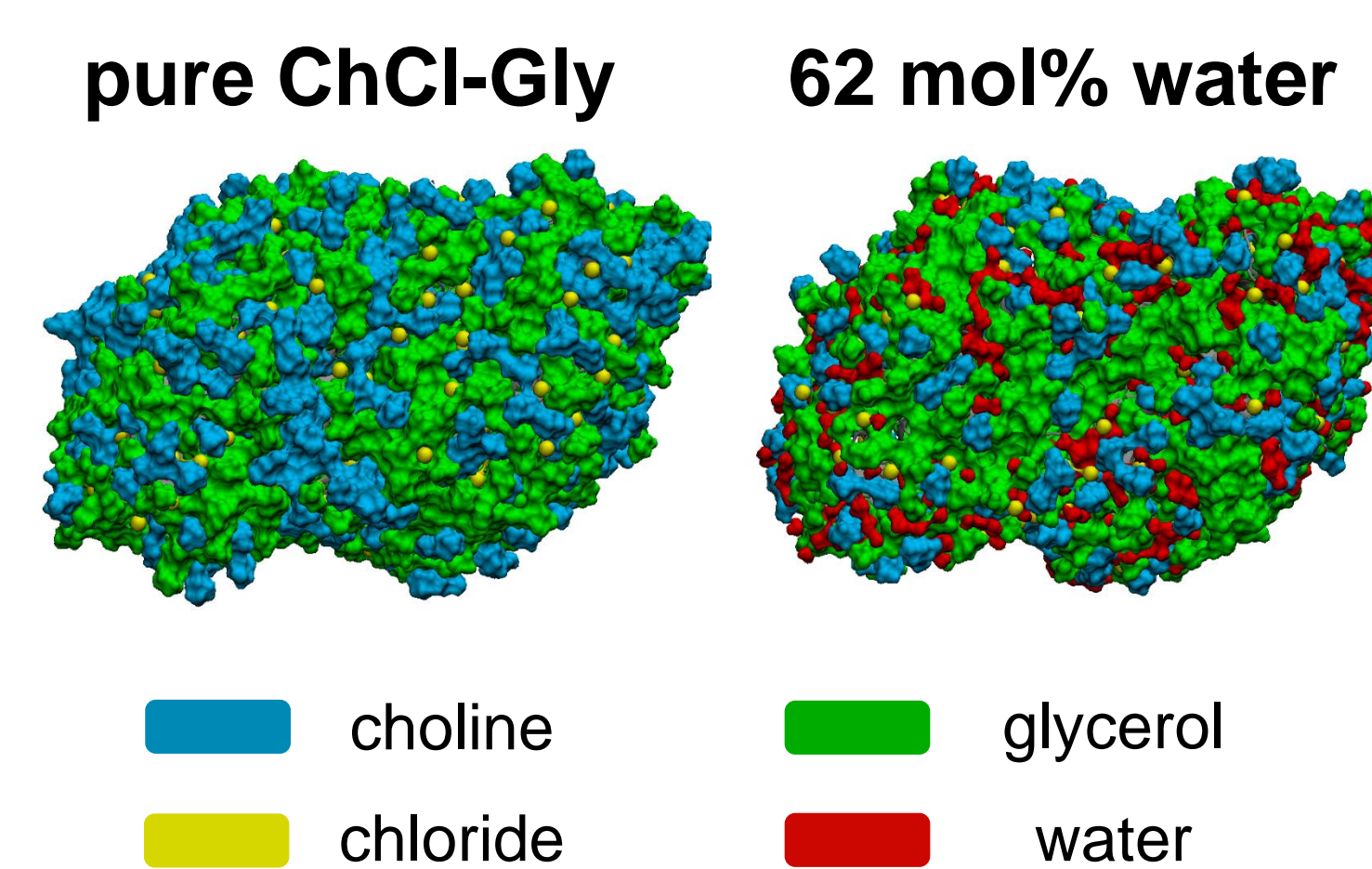
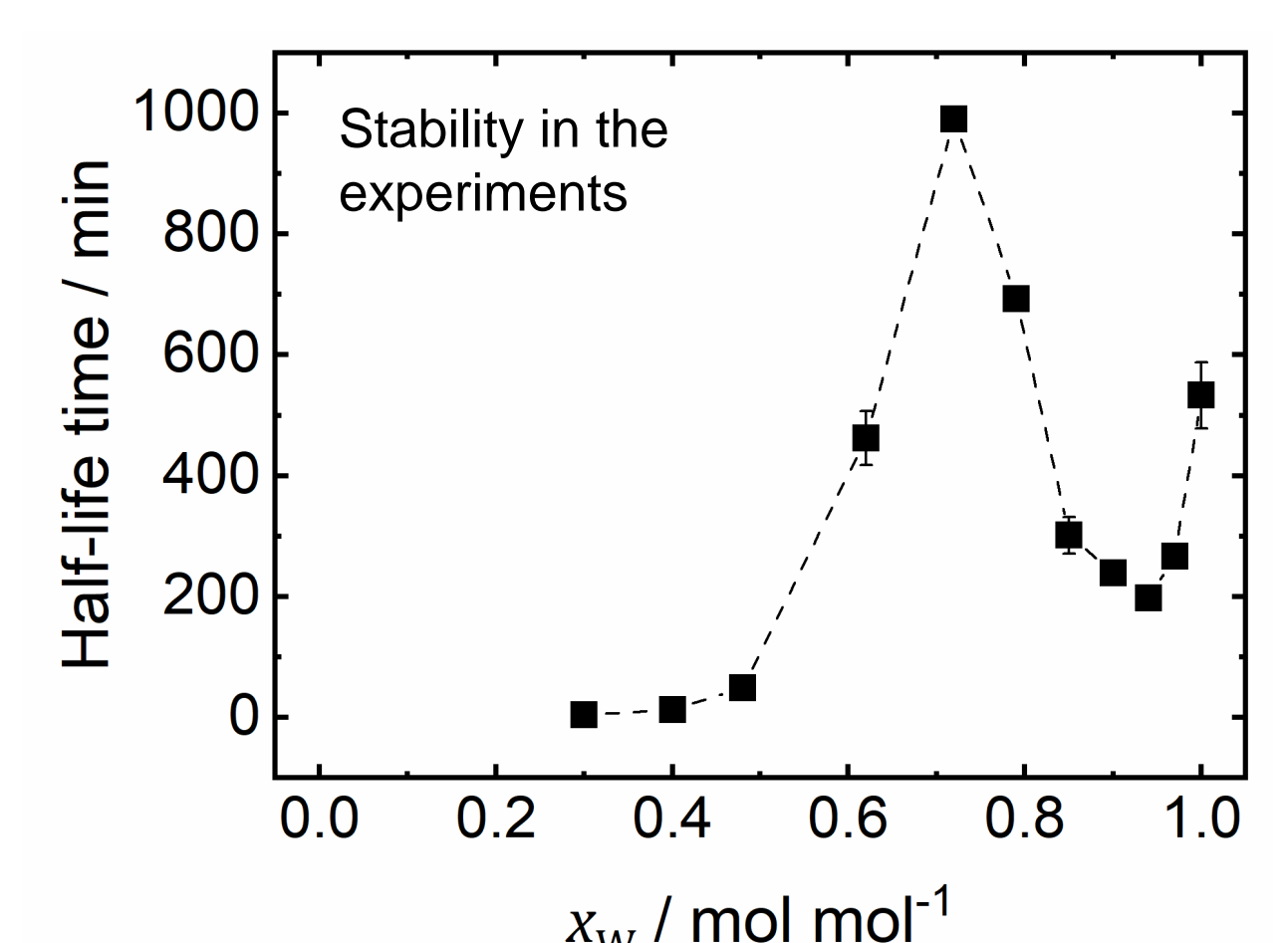
### 3. Activity of HLADH in ChCl-Gly/water

**Limited flexibility and hydration of HLADH in concentrated DES mixtures reduces its catalytic activity.**<sup>4,11</sup> Specific activity of HLADH-catalyzed reduction of cyclohexanone (top), hydration layer of HLADH (left) and root mean square fluctuations (RMSF) of HLADH (right). Strong attractive interactions between ChCl-Gly (see  $a_w$  estimates) and water cause a stripping of water from the enzyme surface and a rigid enzyme structure.



### 4. Stability of HLADH in ChCl-Gly/water

**Optimum of enzyme stability at ~70 mol% results from a preferential solvation of glycerol.**<sup>11</sup> Half-life time of HLADH in ChCl-Gly mixtures (top), solvation layer of choline, chloride and glycerol in the MD simulations (right) and snapshots of solvation layers at 0 mol% and 62 mol% of water (left). Up to ~70 mol%, choline and chloride are replaced by water on HLADH's surface, while the interaction sites of glycerol remain.



## Conclusions & Outlook

- **GAFF-DES** among the best force fields to model ChCl-Gly/water mixtures<sup>7</sup>
- **First evaluation** of the catalytic performance of HLADH in the DES ChCl-Gly by experiment and MD simulation<sup>4</sup>
- Preferential solvation of water in the DES bulk phase **inhibits hydration** of HLADH
- **Reduced flexibility and hydration** of HLADH limits its catalytic activity
- Maximum stability at ~70mol% of water results from **preferential solvation of glycerol**<sup>11</sup>
- **In the future:** Screening of further DESs and organic solvents for HLADH catalysis