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LDRD PROJECT NUMBER: 195702
LDRD PROJECT TITLE: Fundamental Properties of Confined Enzymes
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ABSTRACT:

We recently developed an enzymatically active, ultra-thin, nano-stabilized liquid membrane for CO_2 separation from a mixture of gases, which was recognized by an international R&D 100 Award in 2015. The separation membrane is an approximately 18-nm thick water layer stabilized by capillary condensation within a hydrophilic mesoporous silica film and embedded with the metallo-enzyme carbonic anhydrase. The enzyme speeds CO_2 uptake and release from the membrane by catalyzing the rapid inter-conversion of carbon dioxide and water to bicarbonate and a proton. The membrane separates CO_2 from 1:1 gas mixtures at a rate of 2600 GPU with CO_2/N_2 and CO_2/H_2 selectivities exceeding 788 and 1500, the highest combined flux and selectivity yet reported. That membrane performance exceeds, for the first time, the U.S. Department of Energy standards for CO_2 capture technology. CO_2 flux depends sensitively on nanopore surface chemistry in the active region. To understand that dependence, we applied molecular simulations to interrogate enzyme behavior in the presence of varied surface chemistries. The results indicate that a polar surface chemistry within the membrane nanopores prevents aggregation of enzymes that would otherwise occur in both bulk liquid solution and non-polar nanopores. Additionally, the enzyme active site maintains a stable structure, even when the overall protein structure deforms within the nanopores. In summary, confinement in the ultra-thin layer of water within mesoporous silica nanopores facilitates a 15x higher enzyme concentration than in bulk conditions, without affecting the structure of the enzyme active site, when the nanopore surfaces are covered with polar functional groups. Thus, confinement of the carbonic enzymes in the membrane water-filled nanopores facilitates higher rates of CO_2 uptake and release than achievable in bulk solutions.

INTRODUCTION:

In his 2015 State-of-the-Union Address, President Obama said no challenge poses a greater threat to future generations than climate change. Governments worldwide have pledged to combat climate change by reducing carbon dioxide (CO_2) emissions from the power sector. Coal-fired and natural gas-fired power plants are the leading producers of CO_2, currently accounting for nearly half of the 40 billion tons/yr emitted by human activities worldwide [1]. Domestic and global CO_2 emissions are widely expected to increase over the next quarter century despite investments in renewable and low-carbon fuels for electricity [2, 3]. CO_2 is the most important of the greenhouse gases because it accounts for 80% of all GHG emissions and exerts its warming effect long after entering the atmosphere [4]. Earth's warming is linked to severe changes to the climate, such as rising sea levels, shrinking fresh water supplies, and altered ecosystems [5]. The Pentagon calls climate change a threat to national security.

The increasing global demand for cheap, dependable electricity to support economic well-being drives coal-based power and the consequent carbon pollution [6]. The US has called for 30% reduction in CO_2 emissions for new and existing power plants. Lack of an efficient CO_2 separation technology hinders compliance. Current technology based on reversible solvent absorption using liquid amines is prohibitively costly, almost doubling the cost of electricity generated. Those flaws threaten access to cheap, dependable electricity. A transformational CO_2-separation technology is critically needed.

A potential solution to separating CO_2 from industrial emissions can be found in separation membranes. The development of a membrane for the selective and efficient removal of CO_2 from a mixture of other gases, such as those contained in fuel gas (CO, H_2, H_2O, and H_2S), or flue gas (N_2, O_2, H_2O, SO_2, NO_x, and HCl) would have tremendous economic value and aid the effort to reduce carbon pollution.

An efficient membrane requires both high permeance (flux or permeability of a specific gas per unit of membrane area reported in gas permeation units, GPU) and high selectivity (ratio of gas permeances). Most existing membranes exhibit a sharp trade-off between permeance and selectivity [7-9]. As an example, dense membranes, typically made of polymers, may exhibit high selectivity (~100 GPU), but often exhibit low flux (< 30 GPU) because of the low solubility and diffusivity of CO_2 within the nominally dense membrane material. Therefore, an urgent need exists to develop a new membrane that avoids the limitations associated with polymeric membranes and achieves both high CO_2 permeance and high CO_2 selectivity.

Biological systems exhibit the combined characteristics necessary for high flux of specific molecules. First, separation processes take place in a liquid medium, where molecular diffusivity is 3 orders of magnitude higher than in dense or solid media. Second, transport pathways are typically short. For example, transport across a cellular membrane takes place over a distance of 10 nm. That distance is 2-3 orders of magnitude shorter than in synthetic separation membranes. Finally, biological systems utilize enzymes to catalyze uptake and release of molecules from the separation system. Red blood cells, for example, use the enzyme carbonic anhydrase to rapidly and selectively dissolve CO_2 produced by tissue and regenerate CO_2 exhaled by the lung. Carbonic anhydrase, a metalloprotein, catalyzes the rapid interconversion of CO_2 and water to bicarbonate ion and a proton. As the fastest enzymes in nature, one molecule of carbonic anhydrase can catalyze the hydration/dissolution of 1,000,000 CO_2 molecules per second [10-11].

Prior works have reported incorporation of carbonic anhydrase enzymes into separation membranes. While the enzyme improved membrane performance, overall performance was still poor due in part to thick membranes with long transport pathways. For instance, carbonic anhydrase incorporated in cellulose acetate films produced a factor of 6 increase in CO_2 permeability relative to plain acetate films, but the 70 micrometer effective thickness of the "immobilized liquid membrane" limited CO_2 permeance to only 30 GPU [12]. Similarly, Carbozyme, Inc. developed a "contained liquid membrane" by encapsulating an aqueous carbonic

anhydrase solution layer within a microporous polypropylene hollow fiber membrane mat [13]. Although 5 times higher CO_2 permeability was observed in the Carbozyme membrane compared with the earlier immobilized liquid membrane, the CO_2 flux still fell far short of that needed for practical CO_2 capture as defined by DOE (permeance ~3000 GPU, selectivity ~50). Inherent limitations to these early enzyme-loaded membranes include membrane thickness in the 10-100 micrometer range, which establishes the diffusion length and governs CO_2 flux, and the enzyme concentration of < 0.01 mM characteristic of bulk solutions, which limits the rates of CO_2 uptake and release into the membrane.

To overcome the limitations of current membranes for CO_2 separation, we developed an ultra-thin, enzyme-catalyzed, nano-stabilized liquid membrane called the Memzyme^{TM}. Due to the exceptional thinness (~18 nm) and the high concentration of enzymes in the membrane (~1 M), the Memzyme demonstrates unprecedented values of combined CO_2 flux (2600 GPU) and CO_2/N_2 selectivity (greater than 788). Thus, the Memzyme provides a potential environmentally-friendly solution for cost-effective CO_2 separation that can be adapted to other separation tasks. To help realize this potential, a molecular-level understanding is needed to permit optimization of membrane design. CO_2 flux depends sensitively on nanopore surface chemistry in the Memzyme^{TM} active region. To understand that dependence, we applied molecular simulations to interrogate enzyme behavior in the presence of varied membrane surface chemistries.

DETAILED DESCRIPTION OF EXPERIMENT/METHOD:

Molecular dynamics simulations were performed with the GROMACS software package [14]. The CHARMM36 force field [15-16] was used to model the bovine carbonic anhydrase (CA) enzyme (Protein Data Bank accession number PDB 1V9E) under different conditions relevant to CO_2 separation including interaction with silica nanopores. The silica nanopore atoms were modeled with the CHARMM36-compatible INTERFACE force field [17]. First, we simulated free enzyme in aqueous solution at various concentrations and with amino acid protonation states expected for solution pH values of 7 and 4. Acidic solution conditions mimic the environment resulting from dissolution of CO_2 at 1 atm pressure or higher, when CO_2 spontaneously converts to bicarbonate ion and a proton. Second, we simulated silica nanopores occupied by one (2.5 mM concentration) or two (5 mM) enzymes in the liquid water layer at pH 7 and 4. The latter studies were designed to mimic experimental conditions during CO_2 separation.

We constructed a model (FIG. 1) of the experimental membrane, which is composed of mesoporous silica. Using the alpha-cristobalite unit cell, we first built a rectangular silica nanopore (8 x 8 x 10 nm^3). The average surface silanol density of 5.9 SiOH/nm^2 provides a reasonable model of the amorphous silica surface used in the experimental membranes. For simulations at pH 4, all silanol groups were protonated. For simulations at pH 7, 16.5% of silanols were ionized. Sodium (Na^+) ions were added to counter the negative charge of the silanol groups. No additional molecules (e.g., buffer) were added other than water and sodium (Na^+) or chloride (Cl^-) to produce an overall neutral charge simulation system.

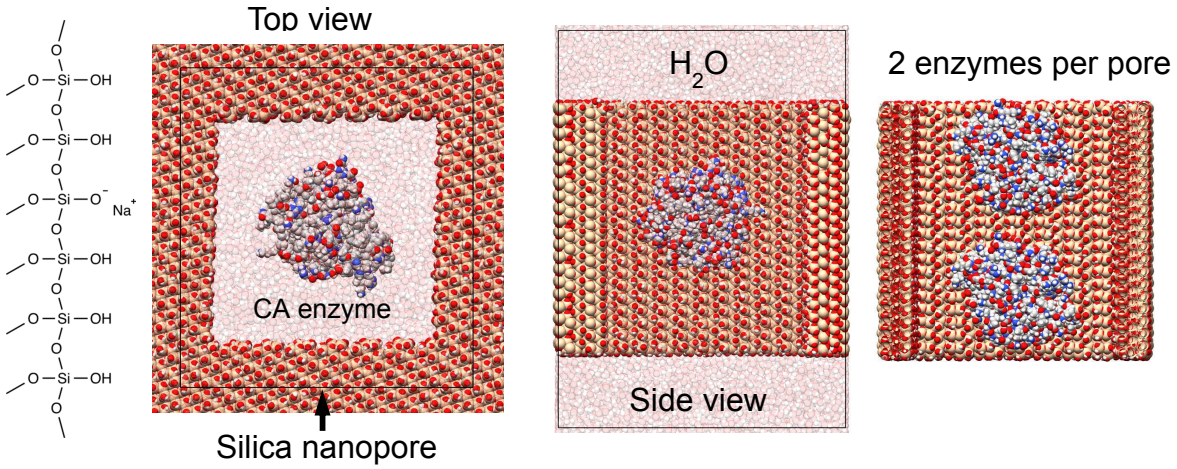

Figure 1. Computational model of the mesoporous silica membrane filled with water and one or more CA enzymes. The internal dimensions of the silica nanopore are approximately 8x8 nm (as seen from the top view, and 10 nm long (as seen from the side view).

A single nanopore was created in the simulation box by evacuating a rectangular region of 4 nm diameter and 10 nm length to mimic the experimental system. Water, ions, and CA enzymes were added and the system was simulated with periodic boundary conditions to mimic bulk conditions (no surfaces in contact with a vacuum). A vertical water-filled space exists between periodic images of the simulation cell of height 5 nm, giving the CA enzyme space to exit the nanopore.

The systems were simulated at room temperature (300 K) for 350 ns, which permitted ample sampling of enzyme binding and releasing from the nanopore walls in an equilibrated system.

RESULTS:

At pH 7, 16.5% of the nanopore surfaces are ionized. Following equilibration of the system, ions in solution bind to the charged SiO- surfaces and to charged groups on the enzyme surface. Nevertheless, the enzyme rapidly displaces ions bound to the surfaces and sticks and 'walks' along the nanopore surfaces. The active site remains accessible to the solution, permitting substrate and product molecules to diffuse in and out, and the active site structure remains robust, as determined by root-mean-squared deviation from the initial crystal structure (FIG. 2). Closely packed enzymes in the silica nanopore intermittently interact with each other, but do not aggregate as they prefer to interact with nanopore's walls (FIG. 3). Similar behavior is observed at pH 4, when all silanol surfaces and most enzyme surfaces are protonated.

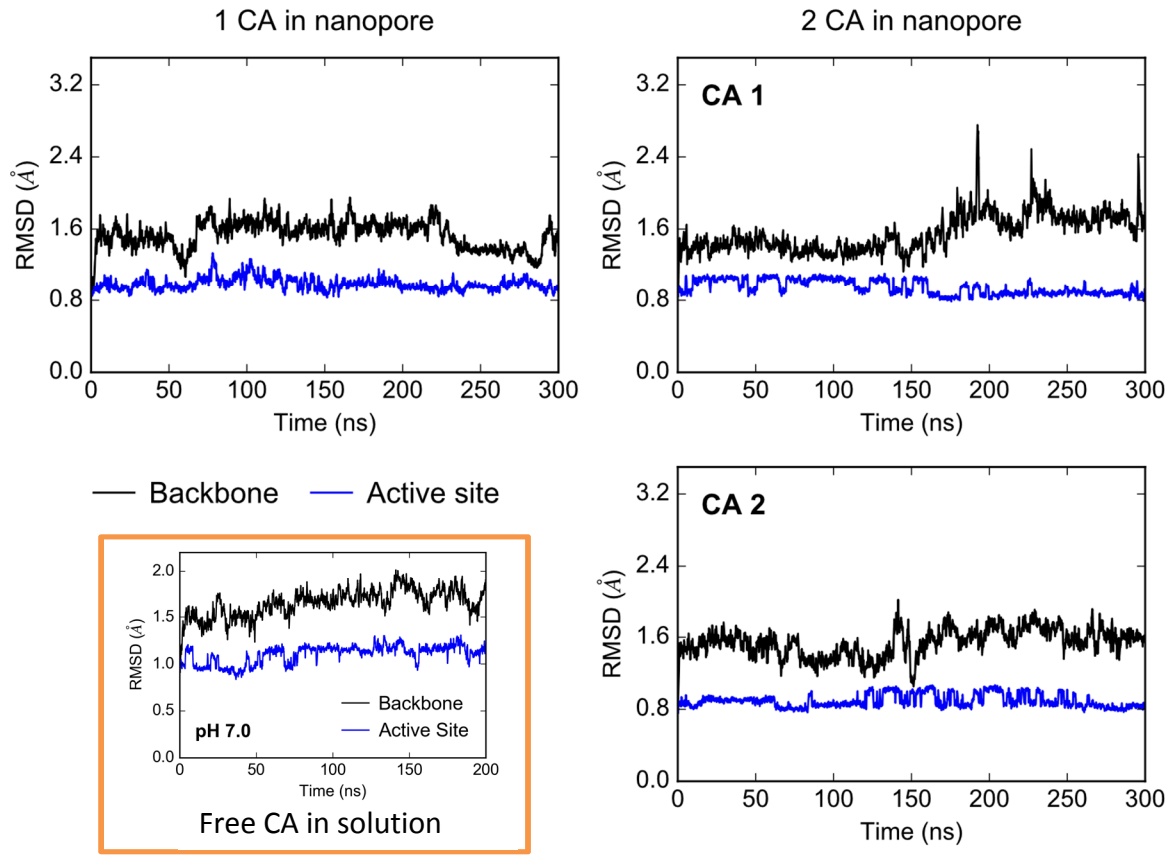

Figure 2. Root mean squared deviations (RMSD) of the enzyme's backbone or active site atoms compared to the CA crystal structure. The CA structure shows similar deviations inside the nanopore compared to what is observed for the free enzyme in solution (inset on the bottom left).

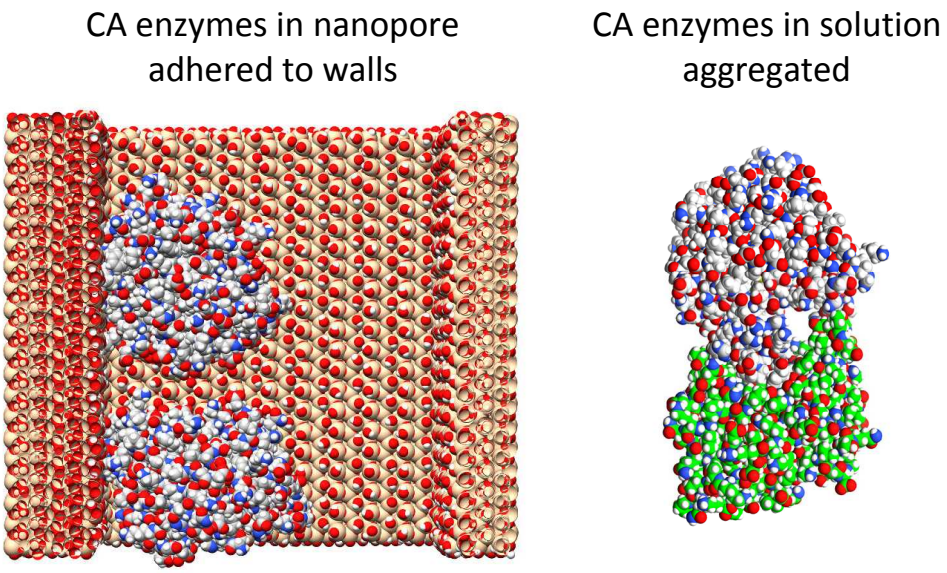

Figure 3. CA enzymes simulated inside the silica nanopore (left) intermittently interact with each other, but do not aggregate due to favorable interactions with the pore walls. In contrast, free CA enzymes in solution at a similar concentration rapidly aggregate (right).

In free solution at similar concentrations as in the nanopores, CA enzymes rapidly aggregate at pH 7 (FIG. 3). Experimentally, CA enzyme solubility in bulk aqueous solution is limited to ~0.3 mM (10 g/L). In past work, an enzyme concentration of 0.16 mM (5 g/L) was achieved in the Carbozyme membrane [13], and 0.06 mM (2 g/L) in the liquid immobilized membrane [12]. In contrast, concentrations of 5 mM (145 g/L) are achievable in the nanopores due to polar walls that inhibit enzyme aggregation. Measurements based on amide signals in the FTIR spectrum indicate that ~2 carbonic enzymes occupy each pore, consistent with the ~5 mM concentration predicted by the simulation studies.

DISCUSSION:

Membrane surfaces can be tuned by atomic layer deposition to have polar or hydrophobic chemistries. Experimental measurements on the Memzyme^{TM} reveal that surface chemistry affects the membrane's ability to separate CO_2 from nitrogen at high rates of CO_2 flux. Molecular simulation studies carried out here provide atomic insight into this behavior. The simulation results show that interactions between the protein and polar groups on the membrane walls inhibit protein-protein aggregation without affecting enzyme active site structure. Inhibition of protein aggregation permits a far higher (15x) enzyme concentration in the water-filled active layer of the membrane than occurs in bulk liquid water conditions. That higher enzyme concentration increases the rate of CO_2 uptake and release from the membrane, and underlies the remarkable permeance and selectivity properties of the Memzyme^{TM}.

Molecular simulations can be used in future work to gain molecular insights that aid in optimizing surface chemistry and pore dimensions for other membrane separation applications. One near-term goal involves optimizing the Memzyme^{TM} active layer to separate CO_2 from real industrial emissions that contain contaminants. Prior studies confirm that bovine carbonic anhydrase II, the enzyme under study here, retains full activity in aqueous solutions exposed to gas mixtures with industrially relevant amounts of SO_x and NO_x [18]. Although the carbonic anhydrase enzyme is well-known for its robustness, some conditions may cause loss in activity. In that case, synthetic CO_2 catalysts with higher tolerance to industrial conditions may act as substitutes [19-22]. Then, molecular simulations would play an important role in understanding the behavior of the synthetic catalysts and designing the membrane active layer to optimize catalytic activity and CO_2 separation.

ANTICIPATED IMPACT:

The computational work described here, in collaboration with experimental work, has led to a membrane for CO_2 separation that is characterized by biomimetic features that underlie the highest combined performance yet reported for CO_2 flux (2600 GPU) and selectivity (788). The biomimetic features include an active layer that is ultra-thin, liquid, and filled with a highly concentrated enzyme solution. The membrane is tunable for specific applications, such as separating CO_2 from gas mixtures produced from fracking, fossil-fuel electricity production, and cement production. The membrane can be used in small-scale applications such as air purification needed on submarines and space shuttles. The membrane can facilitate recycling of CO_2 from power-plant or cement emissions to commercial applications in enhanced oil recovery, algae cultivation for biofuels, chemical synthesis, carbonated beverage production, and microbrewery alcohol production. The membrane can also be tuned to separate different gases by replacing the enzyme. The next steps involve carrying-out the tuning and scaling for specific customers, such as Occidental Petroleum Company, the world's largest user of CO_2 for enhanced oil recovery, and NRG Energy, one of the world's largest energy companies. The DOE Technology Commercialization program provides a funding mechanism to pursue membrane tuning and scaling.

CONCLUSION:

CO_2 flux depends sensitively on nanopore surface chemistry in the active region of a novel membrane, called the Memzyme^{TM}, recently developed by us for gas separation. To understand that dependence, we applied molecular simulations to interrogate enzyme behavior in the presence of varied surface chemistries. The results indicate that a polar surface chemistry within the membrane nanopores prevents aggregation of enzymes that would otherwise occur in both bulk liquid solution and non-polar nanopores. Additionally, the enzyme active site maintains a stable structure, even when the overall protein structure deforms within the nanopores. In summary,

confinement in the ultra-thin layer of water within mesoporous silica nanopores facilitates a 15x higher enzyme concentration than in bulk conditions, without affecting the structure of the enzyme active site, when the nanopore surfaces are covered with polar functional groups. Thus, confinement of the carbonic enzymes in the membrane water-filled nanopores facilitates higher rates of CO_2 uptake and release than achievable in bulk solutions.

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