

1        Surface Engineering of Polypropylene Membranes with Carbonic  
2        Anhydrase-Loaded Mesoporous Silica Nanoparticles for Improved  
3        Carbon Dioxide Hydration

4        *Joel K. J. Yong,<sup>†</sup> Jiwei Cui,<sup>§</sup> Kwun Lun Cho,<sup>†</sup> Geoff W. Stevens,<sup>†</sup> Frank Caruso,<sup>§</sup> and Sandra*  
5        *E. Kentish<sup>†,\*</sup>*

6        <sup>†</sup> Department of Chemical and Biomolecular Engineering, The University of Melbourne,  
7        Parkville, VIC 3010, Australia

8        <sup>§</sup> ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the  
9        Department of Chemical and Biomolecular Engineering, The University of Melbourne,  
10        Parkville, Victoria 3010, Australia

11       Corresponding Author:

12       sandraek@unimelb.edu.au\*

13

1 **Abstract**

2 Carbonic anhydrase (CA) is a native enzyme that facilitates the hydration of carbon  
3 dioxide into bicarbonate ions. This study reports the fabrication of thin films of active CA  
4 enzyme onto a porous membrane substrate using the layer-by-layer (LbL) assembly.  
5 Deposition of multilayer films consisting of polyelectrolytes and CA was monitored by  
6 quartz crystal microgravimetry, while the enzymatic activity was assayed according to the  
7 rates of *p*-nitrophenylacetate (*p*-NPA) hydrolysis and CO<sub>2</sub> hydration. The fabrication of the  
8 films onto a nonporous glass substrate showed CO<sub>2</sub> hydration rates of  $0.52 \pm 0.09 \mu\text{mol cm}^{-2}$   
9  $\text{min}^{-1}$  per layer of bovine CA and  $2.6 \pm 0.7 \mu\text{mol cm}^{-2} \text{min}^{-1}$  per layer of a thermostable  
10 microbial CA. The fabrication of a multilayer film containing the microbial CA on a porous  
11 polypropylene membrane increased the hydration rate to  $5.3 \pm 0.8 \mu\text{mol cm}^{-2} \text{min}^{-1}$  per layer  
12 of microbial CA. The addition of mesoporous silica nanoparticles as a film layer prior to  
13 enzyme adsorption was found to increase the activity on the polypropylene membranes even  
14 further to a rate of  $19 \pm 4 \mu\text{mol cm}^{-2} \text{min}^{-1}$  per layer of microbial CA. The LbL treatment of  
15 these membranes increased the mass transfer resistance of the membrane but decreased the  
16 likelihood of membrane pore wetting. These results have potential application in the  
17 absorption of carbon dioxide from combustion flue gases into aqueous solvents using gas-  
18 liquid membrane contactors.

19

20 *Keywords:* Layer-by-layer assembly, enzymatic immobilization, carbon dioxide hydration,  
21 thin films, mesoporous silica nanoparticles

22

## Introduction

Carbon dioxide (CO<sub>2</sub>) emissions into the atmosphere are of great concern because of the environmental impact associated with climate change. The increase in CO<sub>2</sub> emissions is mainly derived from human activity and has followed an increasing trend over the past few decades.<sup>1, 2</sup> Hence, it is important to curb the rate of CO<sub>2</sub> emissions into the atmosphere through technologies such as carbon capture and sequestration operations.

The hydration of CO<sub>2</sub> into HCO<sub>3</sub><sup>-</sup> is known to be the rate-limiting step when absorbing carbon dioxide into an aqueous medium.<sup>3</sup> Hydration rates can be accelerated by a naturally occurring enzyme known as carbonic anhydrase (CA).<sup>4</sup> Therefore, efforts have been made to adsorb CA onto surfaces such as polymethylpentene hollow fiber membranes,<sup>5</sup> hydrogels,<sup>6</sup> activated carbon particles,<sup>7</sup> mesoporous silica,<sup>8</sup> gold nanoparticles conjugated with silica<sup>9</sup> and polyurethane films,<sup>10</sup> such that they can be used as immobilized solid-state catalysts in CO<sub>2</sub> capture operations.

Polymeric membranes are promising substitutes for absorbing gaseous CO<sub>2</sub> into an aqueous solution. In membrane-based CO<sub>2</sub> absorption operations, a feed gas containing CO<sub>2</sub> is pumped through one side of the membrane, while the other side of the membrane contains the solvent for absorbing the CO<sub>2</sub>.<sup>11</sup> The membrane is hydrophobic to prevent solvent leakage into the gas stream. Such membrane contactors are readily scalable<sup>11</sup> and are known to produce CO<sub>2</sub> streams of high purity even at high CO<sub>2</sub> removal rates.<sup>12</sup> The immobilization of CA onto these membranes would further enhance the rate of CO<sub>2</sub> absorption into the solvent. However, the CA layer that is immobilized onto the membrane will also add to the mass transfer resistance of CO<sub>2</sub> from the gas phase to the gas-liquid interface. It is preferable that the CA film layer be as thin as possible to reduce the mass transfer resistance,<sup>13</sup> which also allows the gas-liquid interface to be closer to the enzyme molecules for better reaction rates.<sup>14</sup>

Layer-by-layer (LbL) assembly is a well-established technique for the preparation of thin multilayered films of oppositely charged polyelectrolytes onto solid planar substrates<sup>15</sup> or particles.<sup>16</sup> The adsorption of proteins, such as immunoglobulin G,<sup>17</sup> glucose oxidase<sup>18</sup> and peroxidase<sup>19</sup> using LbL assembly have been investigated with the aim of developing immunosensors<sup>17</sup> or immobilized catalysts.<sup>20</sup> The construction of CA bilayers with polyethylene imine (PEI) by the LbL technique has also been reported, with a CA loading of 280 ng cm<sup>-2</sup>, as determined by quartz crystal microgravimetry (QCM).<sup>21</sup>

It has been shown that the immobilization of CA onto nanoparticles may further enhance the enzyme activity,<sup>9</sup> as there is a greater degree of freedom for the enzyme to attach

1 to the curved surfaces of the nanoparticles<sup>22</sup> and hence preserve the active sites.<sup>23</sup> For  
2 example, nonporous silica nanoparticles containing adsorbed CA have already been examined  
3 for their ability to hydrate CO<sub>2</sub>.<sup>24</sup> Mesoporous nanoparticles can further increase the  
4 enzymatic loading in comparison with nonporous nanoparticles by providing high surface  
5 areas with pores that are similar to the size of the target enzyme molecules.<sup>25, 28, 29</sup> Nanosized  
6 particles are selected in preference to micron sized particles to reduce the need for intra-  
7 particular mass transfer of CO<sub>2</sub>, as such transport limitations have been shown to cause a  
8 reduction in the overall kinetics.<sup>26</sup>

9 It has been shown that such nanoparticles can be dispersed within a polymer solution  
10 prior to casting as a flat sheet membrane, but that these nanoparticles are prone to leaching.<sup>23</sup>  
11 Alternatively, submicrometer hollow spheres that are resistant to leaching from aqueous  
12 solvents have been fabricated through the LbL technique using alternating layers of silica  
13 nanoparticles and polyelectrolytes.<sup>16</sup> Enzymes that are immobilized within such LbL films are  
14 known to exhibit enhanced stabilities relative to their free states and can maintain their  
15 activity levels within the film for a longer period of time.<sup>27</sup>

16 Herein, we report the fabrication of CA multilayer films both with and without the  
17 addition of mesoporous nanoparticles. We investigate the activity of the resulting films when  
18 deposited on both a smooth nonporous planar glass substrate and a commercial hydrophobic  
19 polypropylene porous membrane. The CA that was adsorbed within the films was found to  
20 increase the rate of CO<sub>2</sub> conversion into HCO<sub>3</sub><sup>-</sup> ions, with a linear correlation between the  
21 number of CA layers adsorbed and the quantity of CO<sub>2</sub> converted into HCO<sub>3</sub><sup>-</sup>. The specific  
22 enzyme activity was comparable to that of the free enzyme when it was immobilized within a  
23 polyelectrolyte film, but there was a significant decrease in the specific activity upon  
24 immobilization within the mesoporous nanoparticles. Enzyme activity also fell somewhat  
25 upon incubation at pH 12, typical of the conditions that might be experienced in a carbon  
26 capture operation. In addition, the films also provided increased resistance against water  
27 penetration into the membrane pores even though they caused the membrane surface to  
28 become more hydrophilic.

## 29 **Materials and Methods**

### 30 **Materials Used**

31 Polyethyleneimine (PEI, 25 kDa), polystyrene sulfonate (PSS, 70 kDa),  
32 polyallylamine hydrochloride (PAH, 10 kDa), *para*-nitrophenol (*p*-NP), *para*-

1 nitrophenylacetate (*p*-NPA) and trishydroxymethylaminomethane (Tris) were obtained from  
2 Sigma-Aldrich (Castle Hill, NSW, Australia). Concentrated hydrochloric acid (HCl, 37%)  
3 and concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>, 98%) were purchased from Scharlau (Gillman, SA,  
4 Australia). Compressed CO<sub>2</sub> was supplied by Coregas (Thomastown, VIC, Australia).  
5 Calcium chloride dihydrate (CaCl<sub>2</sub>·2H<sub>2</sub>O), sodium chloride (NaCl), sodium hydroxide  
6 (NaOH) and 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were obtained from ChemSupply (Gillman, SA,  
7 Australia). A suspension of silica particles (50 mg mL<sup>-1</sup>, 2.78 μm diameter) in water was  
8 obtained from Microparticles GmbH (Berlin, Germany). All chemicals were used as  
9 purchased unless otherwise stated. Ultrapure water was obtained from a Milli-Q purification  
10 system (Merck Millipore, Kilsyth, VIC, Australia) with a resistivity of more than 18.2 MΩ  
11 cm. Tris buffer (10 mM or 50 mM, pH 6.4 or pH 7.2) was prepared in Milli-Q water and the  
12 pH was adjusted by dropwise addition of hydrochloric acid (1 M). The polyelectrolytes (i.e.,  
13 PEI, PSS, and PAH) solutions were all prepared in Tris buffer (pH 7.2) at a concentration of  
14 1 mg mL<sup>-1</sup>.

15 Two sources of CA were used. The first source was a commercial form derived from  
16 bovine erythrocytes, referred to hereafter as BCA (Sigma-Aldrich product C-3934) and  
17 provided in a powder form, with an activity of 2500 Wilbur-Anderson units per mg of  
18 protein. BCA with a concentration of 0.2 mg mL<sup>-1</sup> was prepared in Tris buffer (pH 7.2) prior  
19 to use. The second source was an experimental thermostable microbial CA produced by  
20 Novozymes A/S (Bagsvaerd, Denmark), referred to hereafter as NCA. The enzyme was  
21 produced by microbial fermentation in a benign host organism, which was removed during  
22 recovery of the enzyme broth and is not present in the sample. The NCA in the solution had a  
23 CO<sub>2</sub> hydration activity that was equivalent to 320 g L<sup>-1</sup> of BCA. The NCA solution was  
24 filtered through 0.22 μm syringe filters to remove suspended solids for zeta potential and  
25 quartz crystal microgravimetry (QCM) analysis and then diluted with Tris buffer (pH 7.2) to  
26 2% of its original concentration prior to use.

27 Flat sheet polypropylene (PP) membrane coupons were obtained from Sterlitech  
28 Corporation (Kent, WA, USA). These membranes have a pore size of 0.10 μm, a thickness of  
29 75-110 μm and a diameter of 47 mm as per the information provided by the supplier.

30 Mesoporous silica (MS) nanoparticles with an average size of 110 nm were  
31 synthesized according to a previously reported method.<sup>28</sup> These particles were suspended in  
32 Tris buffer (pH 7.2) containing 0.1 M NaCl<sup>16</sup> to a particle concentration of 5 mg mL<sup>-1</sup> and  
33 sonicated for 5 min to ensure an even dispersion of the particles prior to layering.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

## **Zeta-Potential ( $\zeta$ ) Measurements of the Silica Particles**

Approximately 100  $\mu\text{L}$  of the silica particle suspension (50  $\text{mg mL}^{-1}$ ) was mixed with 1 mL of polyelectrolyte solution in Tris buffer for 5 min to deposit a polyelectrolyte layer. The particles were then spun down at 4000 g for 45 s and the supernatant was discarded. The particles were redispersed in 700  $\mu\text{L}$  of Tris buffer (10 mM, pH 7.2) via vortex and were centrifuged at 4000 g to remove free polyelectrolytes.

The washing step was repeated three times to remove all free polyelectrolyte before the deposition of the next polyelectrolyte layer. Approximately 1  $\mu\text{L}$  of the particle suspension was removed after the addition of each polyelectrolyte layer and mixed with 700  $\mu\text{L}$  of Tris buffer (10 mM, pH 7.2) for zeta-potential measurements on a Malvern Zetasizer Nano ZS instrument (Worcestershire, UK). A precursor PEI/[PSS/PAH]<sub>2</sub> film was deposited prior to introducing the CA enzyme, which was then either alternately layered with PAH or PSS onto the precursor film.

## **Quartz Crystal Microgravimetry**

QCM was used to determine the mass of enzyme adsorbed per unit area on AT cut 5 MHz QCM quartz crystals using a Q-Sense E4 Auto system to quantify the changes in the resonant frequency. Prior to QCM experiments, the crystal surfaces were cleaned with piranha solution (30%  $\text{H}_2\text{O}_2$  mixed with 70%  $\text{H}_2\text{SO}_4$ . *Caution! Piranha solution is extremely reactive and corrosive, only small volumes should be prepared freshly for use*) for 5 min, washed with Milli-Q water and ethanol, dried under a stream of nitrogen gas and irradiated with ultraviolet rays for 20 min. The changes in the resonance frequency of the crystals during multilayer deposition were monitored at the third overtone at room temperature. Three layers of CA were deposited for each crystal.

Deposition steps and washing steps were conducted for 3 min and 2 min, respectively, with 250  $\mu\text{L}$  aliquots of polyelectrolyte solution or Milli-Q water at a flow rate of 0.579  $\text{mL min}^{-1}$ . After the formation of a PEI/[PSS/PAH]<sub>2</sub> precursor film showing regular stepwise film growth,<sup>29</sup> CA was deposited onto the quartz crystals sequentially with the polyelectrolytes to form multilayer enzyme films. QCM was also used to investigate the construction of NCA films in a similar manner.

The Sauerbrey equation was used to determine the mass of material deposited onto the quartz crystal, which had an active area of 1 cm in diameter. This equation is valid when the ratio of the change in the dissipation factor  $\Delta D$  is less than  $10^{-7}$  times per change of Hz in

1 the resonant frequency ( $\Delta F$ ).<sup>30</sup> The change in  $\Delta F$  of the quartz crystals is linearly dependent  
2 on the mass of material ( $\Delta m$ ) deposited onto the crystal:

$$\Delta F = - \frac{n\Delta m}{C} \quad \text{Equation 1}$$

3 where  $n$  is the number of overtones and  $C$  is a constant ( $17.7 \text{ ng Hz}^{-1} \text{ cm}^{-2}$  for a 5 MHz AT  
4 cut crystal from Q-Sense). Equation 1 can be rewritten to relate the layer thickness ( $x$ ) to the  
5 change in the observed resonant frequency of the crystal ( $\Delta F$ ) and the density of the film  
6 ( $\rho$ ):<sup>29</sup>

$$x = -(2.12 \times 10^{-4}) \frac{\Delta F}{\rho} \quad \text{Equation 2}$$

7 The surface area of the crystal can be estimated to be approximately 20% larger after  
8 enzyme deposition.<sup>20</sup> The densities of protein and polyelectrolyte layers have been previously  
9 assumed to be approximately  $1.3 \pm 0.1 \text{ g cm}^{-3}$  and  $1.2 \pm 0.1 \text{ g cm}^{-3}$ , respectively.<sup>20</sup> For  
10 estimating the *maximum* theoretical film thickness of the hybrid polyelectrolyte/protein film,  
11 a film density of  $1.2 \pm 0.1 \text{ g cm}^{-3}$  was assumed.

12

### 13 **Enzyme Adsorption onto Smooth, Non-Porous Surfaces**

14 Glass slides ( $22 \text{ mm} \times 22 \text{ mm}$ ) were treated with piranha solution, Milli-Q water and  
15 ethanol in the same way as the QCM crystals prior to adsorption experiments. A  
16 PEI/[PSS/PAH]<sub>2</sub> precursor film was first deposited by immersing the glass slides or the  
17 silicon wafers into the respective polyelectrolyte solutions for 5 min. After the deposition of  
18 each layer, excess polyelectrolyte was removed by rinsing with excess Tris buffer (pH 7.2).

19 BCA or NCA was then adsorbed onto microscopic glass slides to form multilayered  
20 enzyme films similar to the films that were prepared on the QCM crystals. Both sides of the  
21 glass slides were exposed to the polyelectrolyte solutions.

22

### 23 **Enzyme Immobilization onto Flat Sheet Membranes**

24 PP membrane coupons were placed onto a glass substrate. Polyelectrolyte solutions  
25 were coated onto the active side of the membrane surface using a commercial paintbrush  
26 (Woolworths Ltd, Bella Vista, New South Wales, Australia) to spread the solution evenly  
27 across the membrane. Each polyelectrolyte solution was left on the surface of the membranes  
28 for 3 min before rinsing with excess Tris buffer (pH 7.2).

29

### 30 **Enzyme Immobilization with MS Nanoparticles**

1 To investigate the effect of the MS nanoparticles on the adsorption and the activity of  
2 the NCA, fresh glass slides or membrane coupons were coated sequentially as  
3 [MS/NCA/PAH]<sub>2</sub>/MS/NCA after the deposition of the precursor film. The membranes were  
4 contacted with the MS particles or the NCA solution for 20 min before washing with buffer  
5 and then contacted with PAH for 5 min before washing with buffer. The film that was formed  
6 is denoted as the MSNCA film. The quantity of enzyme that was adsorbed was determined by  
7 analyzing the supernatant using UV-Vis spectrophotometry on a Varian Cary 1E UV-Vis  
8 spectrophotometer (Agilent Technologies, Mulgrave, VIC, Australia) with 10 mm quartz  
9 cuvettes (Starna Pty Ltd, Baulkham Hills, NSW, Australia) at a wavelength of 280 nm.

### 11 **Electron Microscopy**

12 The MS nanoparticles were imaged by transmission electron microscopy (TEM) using  
13 a FEI Tecnai TF20 microscope at a voltage of 200 kV after being placed onto Formvar-  
14 coated copper grids and dried in air.

15 The glass slides and the membrane coupons containing adsorbed enzyme were dried  
16 under a nitrogen stream, gold sputter-coated and analyzed using a Phillips XL30 FEG field  
17 emission scanning electron microscope (FESEM) at a voltage of 2.0 kV to determine the  
18 surface morphologies of the glass slides before and after protein adsorption. A wetted  
19 membrane coupon containing the adsorbed MS nanoparticles was immersed in liquid  
20 nitrogen for 5 min and then fractured for an analysis of the membrane cross-section.

### 22 **Characterization of the Flat Sheet Membranes**

23 The liquid entry pressure (LEP) of the membranes was measured by placing a  
24 membrane coupon of 4.8 cm in diameter within a dead-end filtration cell (Sterlitech HP4750,  
25 Kent, WA, USA) that had an active filtration area of 3.2 cm in diameter. The cell was  
26 pressurized with air and increased in 50 kPa steps every 5 min to determine the pressure that  
27 was sufficient to force water through the membrane. Water flux tests were then conducted for  
28 the different membranes at a constant pressure (greater than the LEP) to determine the  
29 permeability of the membrane. The membranes were then air dried after the water flux tests  
30 for 72 h. Contact angle measurements were performed on the dry membranes using a  
31 FTÅ200 dynamic contact angle analyzer (First Ten Ångströms, Portsmouth, VA, USA).

### 33 **Characterization of Enzyme Activity**

1 The enzymatic activity of the adsorbed BCA on the glass slides was initially  
2 determined based on the rate that the adsorbed BCA converted *p*-NPA to *p*-NP. Solutions of  
3 *p*-NPA in acetonitrile (2.5 mg mL<sup>-1</sup>) were mixed with Tris buffer (pH 7.2) at a volumetric  
4 ratio of 1:9 for the activity assays.<sup>10, 31</sup> CA-coated glass slides were incubated within a 20 mL  
5 aliquot of *p*-NPA solution, and a small volume of the mixture was extracted at set timepoints  
6 and analyzed by UV-Vis spectrophotometry at 348 nm to determine the total concentration of  
7 *p*-NP and *p*-nitrophenoxide in solution<sup>32</sup> for calculating the rate of *p*-NPA hydrolysis.

8 The activity of the immobilized enzymes on the glass slides and the membranes were  
9 also quantified for their ability to hydrate CO<sub>2</sub> into HCO<sub>3</sub><sup>-</sup>, based on the method provided by  
10 Vinoba and co-workers.<sup>8, 9</sup> A saturated CO<sub>2</sub> solution was prepared by passing a CO<sub>2</sub> gas  
11 stream into a bottle of Milli-Q water at 25°C for 1 h. Approximately 40 mL of Tris buffer  
12 (pH 6.4) was added to 100 mL of the saturated CO<sub>2</sub> solution before the enzyme-coated glass  
13 slide or the membrane coupon was immersed into the mixture. The mixture was stirred for 1  
14 min followed by the removal of the glass slide or the membrane coupon before the addition  
15 of 20 mL of CaCl<sub>2</sub>·2H<sub>2</sub>O solution (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench  
16 the reaction and precipitate the Ca(HCO<sub>3</sub>)<sub>2</sub> as CaCO<sub>3</sub>. The precipitated CaCO<sub>3</sub> was then  
17 filtered, dried in an oven at 80°C overnight and weighed to determine the quantity of CO<sub>2</sub>  
18 that had been hydrated based on the quantity of CaCO<sub>3</sub> produced.

19 A separate activity test was set up for the determination of the free NCA activity in  
20 solution. A sample of 2% NCA in Tris buffer (pH 7.2) was sequentially diluted to various  
21 levels of dilution, and 1 mL aliquots of diluted NCA was added to different batches of 100  
22 mL saturated CO<sub>2</sub> solution and stirred for 1 min before adding 20 mL of CaCl<sub>2</sub>·2H<sub>2</sub>O solution  
23 (5.3% w/v) and 10 mL of NaOH solution (1 M) to quench the reaction. The precipitated  
24 CaCO<sub>3</sub> was then filtered and dried before determining the quantity of CaCO<sub>3</sub> that was  
25 produced.

26 Control experiments were conducted to determine the quantity of CaCO<sub>3</sub> that was  
27 produced in the absence of any enzyme. Any incremental CaCO<sub>3</sub> that was produced in the  
28 presence of CA was then attributed to the hydration activity of the enzyme.

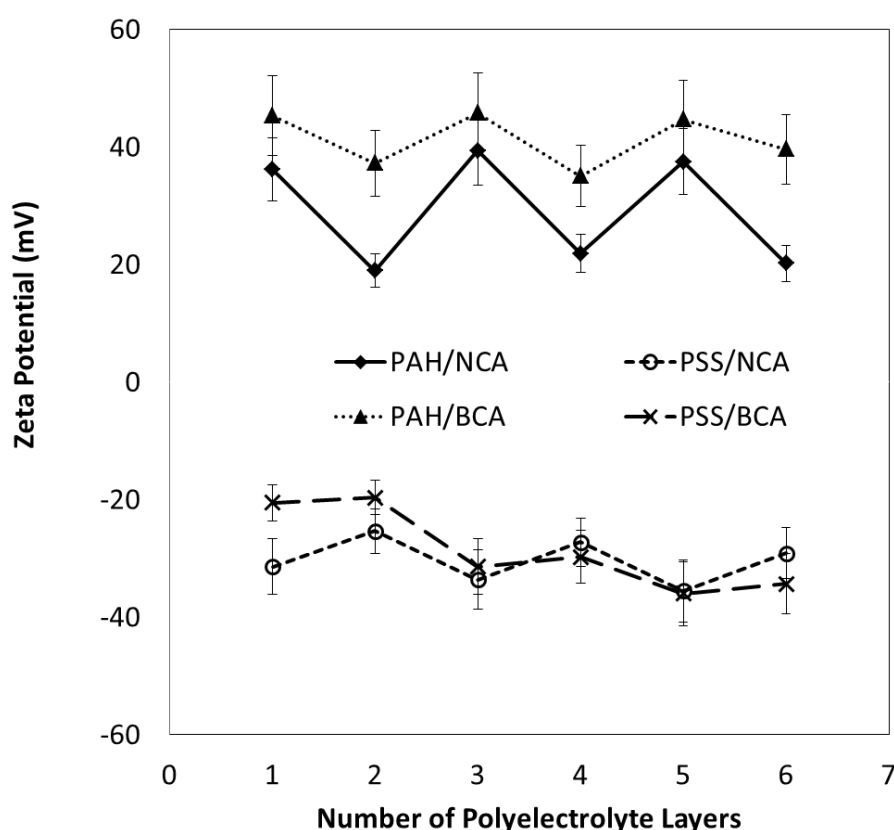
29 Free NCA samples and membrane samples containing adsorbed NCA were also  
30 directly compared for their pH stability via incubation either in a 0.2 M KCl solution  
31 (adjusted to pH 12 with NaOH) or in Tris buffer (pH 7.2) for 72 h at 4°C. This stability is  
32 important as most carbon dioxide absorption systems operate at pH greater than 10.

33

## Results and Discussion

### Enzyme Adsorption and Multilayer Film Formation

Zeta-potential measurements of PAH/CA and PSS/CA layers deposited onto silica spheres at pH 7.2 confirmed that both BCA and NCA are negatively charged (Figure 1). However, the inability of either enzyme to reverse the surface zeta potential when applied after the positively charged PAH layer indicates that these proteins possess only a weak net negative charge at the measurement pH of 7.2. This weak net negative charge is consistent with the reported isoelectric points for commercial BCA, which range from pH 4.9 to pH 6.7.<sup>33</sup>

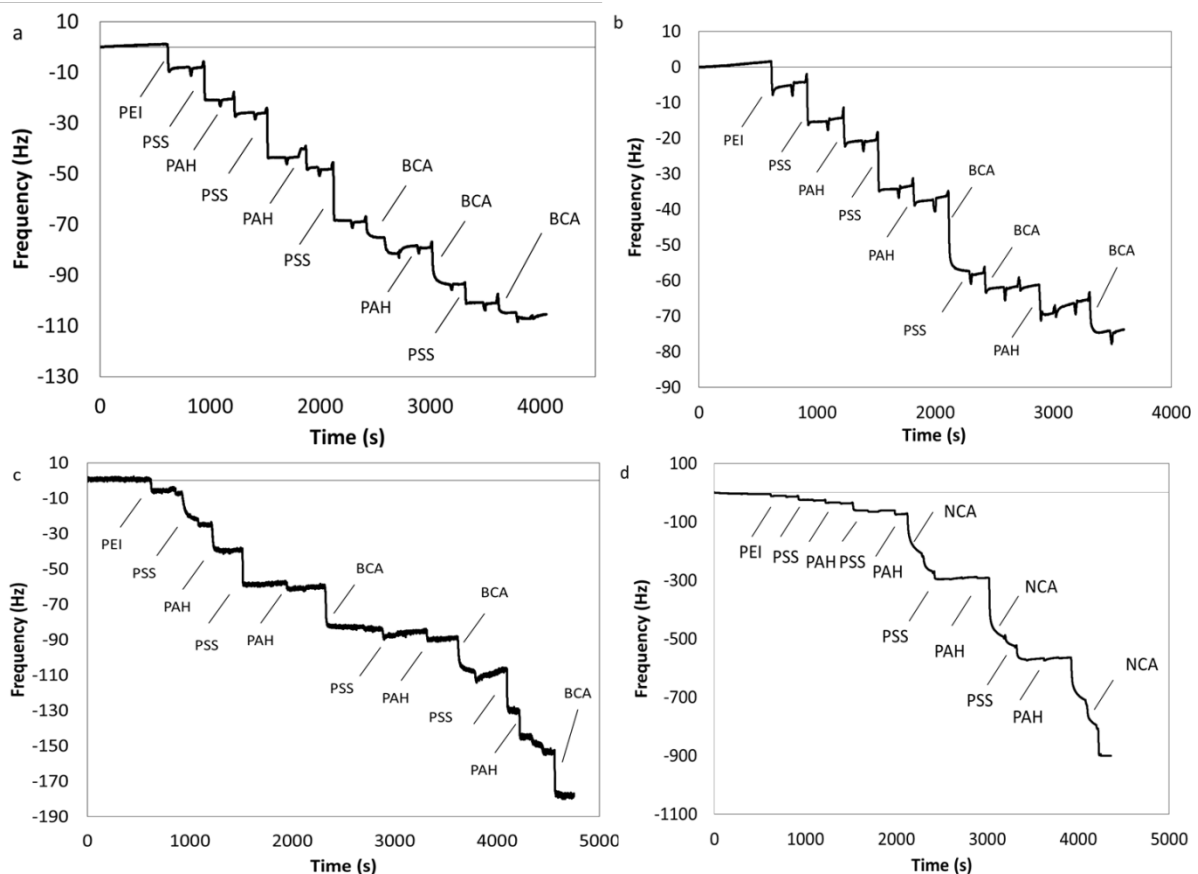


**Figure 1.** Zeta-potential measurements for PAH/BCA, PSS/BCA, PAH/NCA and PSS/NCA bilayers prepared in a Tris buffer solution at pH 7.2. The odd numbered layers (1, 3 and 5) represent PAH or PSS layers, while the even numbered layers (2, 4 and 6) represent BCA or NCA layers.

The lack of surface charge inversion prevents the uniform addition of extra PSS/CA or PAH/CA bilayers sequentially beyond the first bilayer, as stepwise growth in multilayer film formation requires a complete reversal in the surface charge.<sup>29</sup> This is demonstrated by the limiting changes in zeta-potential for successive PSS/CA and PAH/CA bilayers in Figure 1.

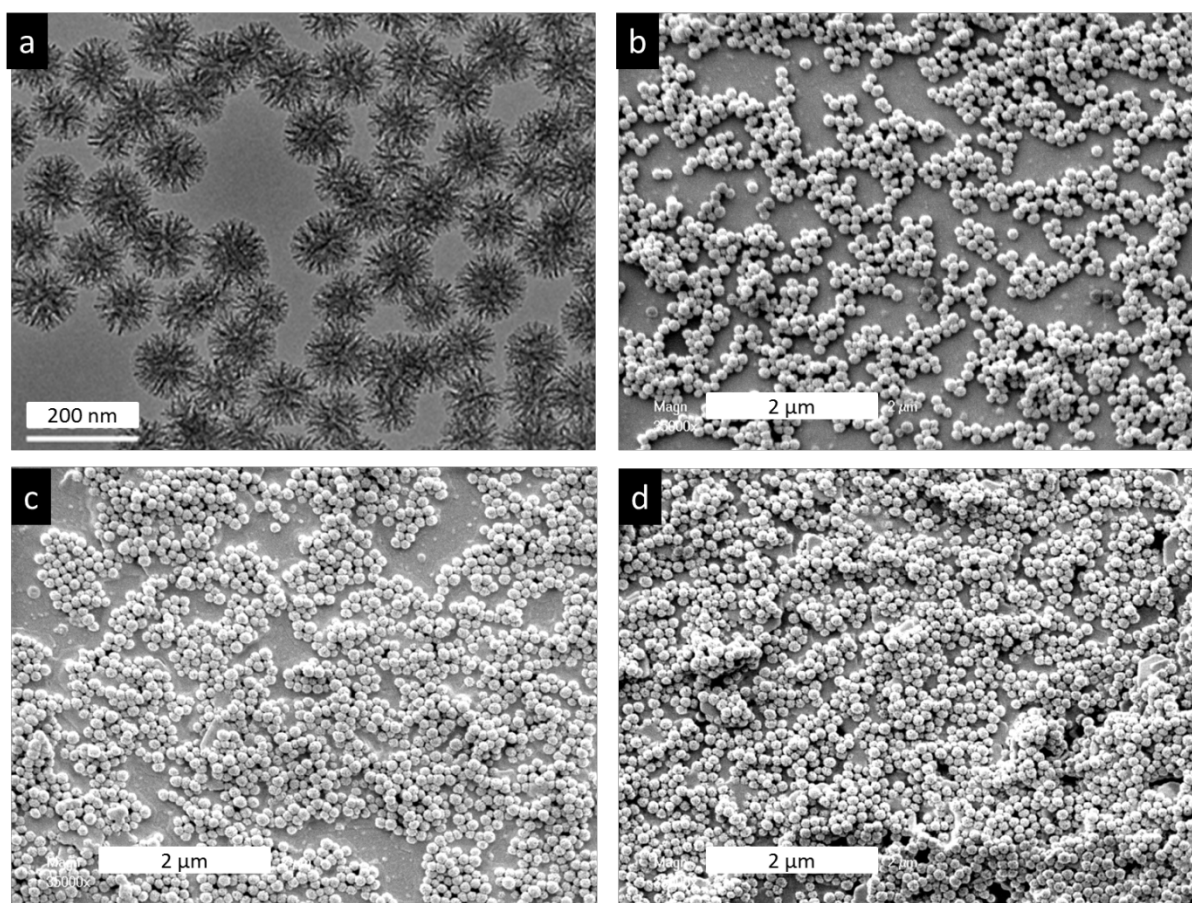
1 Consistent with these data, QCM investigations showed that CA was readily adsorbed onto  
 2 PAH or PSS, but the subsequent deposition of PAH onto a PAH/CA film or PSS onto a  
 3 PSS/CA film resulted in no further mass being added onto the quartz crystal, similar to what  
 4 was observed elsewhere for an invertase/poly(dimethyldiallylammonium chloride) system.<sup>34</sup>  
 5

6 CA films were also prepared on a QCM crystal to determine the adsorption of BCA  
 7 onto alternating PAH and PSS layers, as a BCA/PSS/BCA/PAH/BCA film (Figure 2a) and a  
 8 PSS/BCA/PAH/BCA/PSS/BCA film (Figure 2b). However, for both cases, the rate of decrease  
 9 in frequency slowed after repeated depositions of CA, which was again consistent with the  $\zeta$ -  
 10 potential results. The quantity of BCA deposited onto PAH layers was greater than that  
 11 deposited onto PSS layers, reflecting the stronger electrostatic attraction forces between the  
 12 negatively charged BCA and the positively charged PAH. The  $\Delta D:\Delta F$  ratios were consistently  
 13 of the order  $10^{-8} \text{ Hz}^{-1}$ , which reflects that Equation 1 is valid for estimating the mass of  
 14 polyelectrolyte being deposited onto the quartz crystal surface.



15 **Figure 2.** Real-time QCM investigations of the deposition of (a)  
 16 PSS/BCA/PAH/BCA/PSS/BCA, (b) PAH/BCA/PSS/BCA/PAH/BCA, (c)  
 17 [BCA/PSS/PAH]<sub>2</sub>/BCA, and (d) [NCA/PSS/PAH]<sub>2</sub>/NCA multilayers.  
 18  
 19

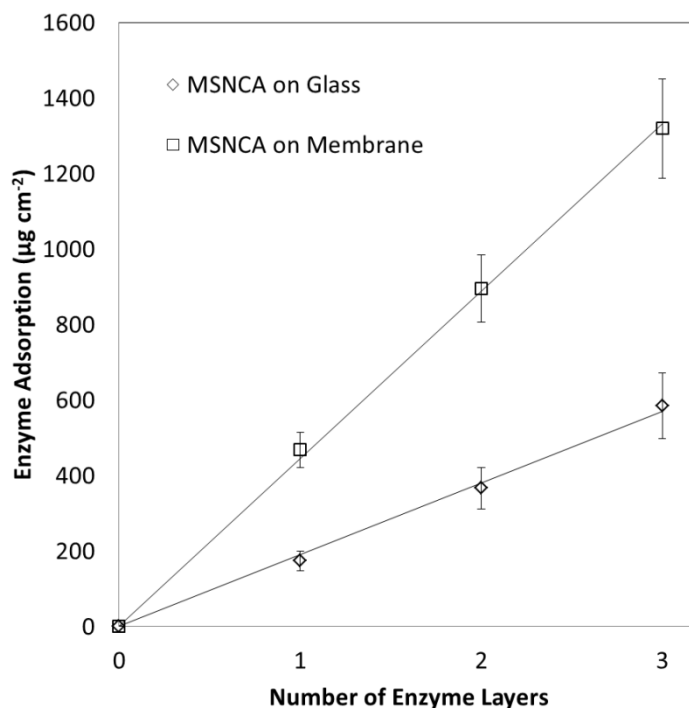
1            However, the deposition of BCA/PSS/PAH (Figure 2c) or NCA/PSS/PAH (Figure 2d)  
2 in repeating trilayers gave a better indication of consistent film formation in comparison to the  
3 films that were fabricated in Figures 2a or 2b. A frequency decrease of  $226 \pm 11$  Hz per layer of  
4 NCA was observed for a  $[\text{NCA}/\text{PSS}/\text{PAH}]_2/\text{NCA}$  film (Figure 2d), which translates into an  
5 NCA coverage of  $790 \pm 30$  ng cm<sup>-2</sup>. This value is significantly greater than the single layer  
6 enzyme loading obtained from a covalent coupling of the same enzyme to a surface (less than  
7 100 ng cm<sup>-2</sup>).<sup>7, 35</sup> This value was also higher than the 280 ng cm<sup>-2</sup> of CA that was deposited as  
8 PEI/CA bilayers in prior work (the type of CA that was used was unspecified).<sup>21</sup>



9  
10 **Figure 3.** (a) TEM image of the MS nanoparticles before deposition. SEM images of MS  
11 nanoparticles on silicon wafers after (b) one layer, (c) two layers, (d) three layers of coating.  
12 Scale bars in (b-d) are 2 μm.  
13

14            To increase the quantity of enzyme being immobilized onto the films, MS particles with  
15 an average size of 115 nm (Figure 3a) were synthesized and used to load NCA in films of  
16  $[\text{MS}/\text{NCA}/\text{PAH}]_2/\text{MS}/\text{NCA}$  onto either a glass plate or a silicon wafer (Figures 3 b, c, d). The  
17 quantity of NCA adsorbed in this case was determined from the difference in the UV  
18 absorbance of the NCA solution before and after contact with the glass substrate. Each coating

1 of NCA was found to add  $190 \pm 20 \mu\text{g cm}^{-2}$  of NCA to the MS layer on the film (Figure 4),  
2 which is approximately 140 times greater than the adsorption of NCA onto PAH.



3  
4 **Figure 4.** Quantity of NCA adsorbed onto each MS nanoparticle layer deposited onto the film.

5  
6 These significantly increased enzyme loadings are indicative of the mesoporous  
7 nanoparticles with their high surface areas allowing for large polymer/protein loadings.<sup>25</sup> In  
8 comparison, the concentration of NCA in the supernatant did not change significantly during  
9 the fabrication of the  $[\text{NCA}/\text{PSS}/\text{PAH}]_2\text{NCA}$  film, which is consistent with the lower quantities  
10 of enzyme adsorbed onto PAH as compared to the MS nanoparticles.

### 12 **Multilayer Film Characterization**

13 The total thickness of the multilayered protein films can significantly influence the mass  
14 transfer performance in membrane contactor applications. Ideally, the deposited polyelectrolyte  
15 layers will cover the pores of the top surface (typically 100 nm in diameter) to reduce pore  
16 wetting, but their thickness should not result in a significant resistance to the flow of carbon  
17 dioxide across the membrane. The total film thickness of the three enzyme layers in addition to  
18 the precursor film can be calculated based on Equation 2. These film thicknesses are shown in  
19 Table 1:

1

**Table 1.** Maximum Polyelectrolyte Film Thicknesses

Polyelectrolyte Film	Maximum Film Thickness (nm)
PSS/BCA/PAH/BCA/PSS/BCA <sup>a</sup>	19 ± 1
BCA/PSS/BCA/PAH/BCA <sup>a</sup>	14 ± 1
[BCA/PSS/PAH] <sub>2</sub> /BCA <sup>a</sup>	38 ± 4
[NCA/PSS/PAH] <sub>2</sub> /NCA <sup>a</sup>	154 ± 8
[MS/NCA/PAH] <sub>2</sub> /MS/NCA <sup>b</sup>	337 ± 67

2

<sup>a</sup>These results were obtained from QCM measurements. <sup>b</sup>This result was obtained from a cross-sectional SEM analysis of a fractured silicon wafer.

3

4

5

The thickness of the PSS/BCA/PAH/BCA/PSS/BCA and the BCA/PSS/BCA/PAH/BCA films are similar to the protein bilayer thicknesses and the PAH/PSS bilayer thicknesses that have been described elsewhere.<sup>21, 29</sup> The [BCA/PSS/PAH]<sub>2</sub>/BCA films were two to three times thicker than the PSS/BCA/PAH/BCA/PSS/BCA or the BCA/PSS/BCA/PAH/BCA films, which can be attributed to more BCA adsorbing onto a PAH precursor layer than onto a PSS precursor layer, as evidenced in the QCM analyses from Figure 2. The average frequency change was 9.2 ± 2.5 Hz when BCA was adsorbed onto PSS, which is approximately half of the 17 ± 5 Hz exhibited by the adsorption of BCA onto PAH.

14

The thickness of the [NCA/PSS/PAH]<sub>2</sub>/NCA films was four times greater than the equivalent thickness of the [BCA/PSS/PAH]<sub>2</sub>/BCA films. This reflects the greater mass of NCA and other proteins that adsorb onto the quartz crystal at every deposition step (Figure 2). The [MS/NCA/PAH]<sub>2</sub>/MS/NCA film is thicker again as a result of the addition of the MS nanoparticles, which are approximately 115 nm in diameter. A film thickness of approximately 337 ± 67 nm indicates that there is multilayer coverage of the nanoparticles on the surface of the silicon wafer.

21

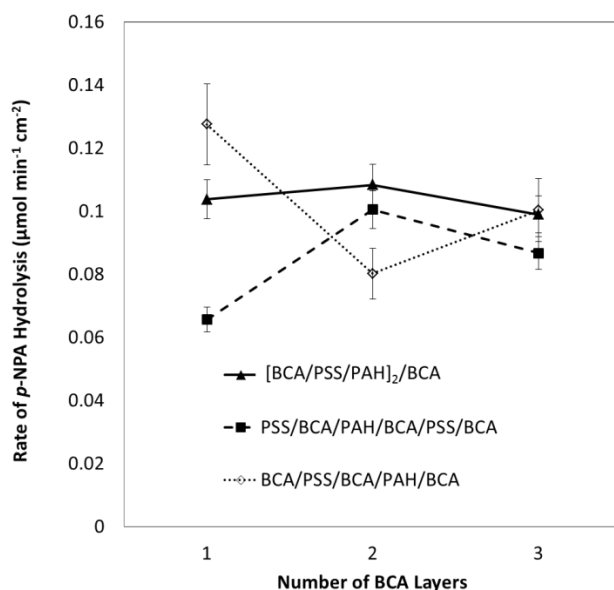
## 22 Enzyme Activity Assays

23

Within experimental error, the resulting [BCA/PSS/PAH]<sub>2</sub>/BCA films exhibited a uniform *p*-NP production rate of 0.10 ± 0.01 μmol min<sup>-1</sup> cm<sup>-2</sup>. This *p*-NP production rate was irrespective of the number of BCA layers that were adsorbed (Figure 5). However, the PSS/BCA/PAH/BCA/PSS/BCA and the BCA/PSS/BCA/PAH/BCA films showed that the BCA adsorbed preferentially more onto PAH than PSS. The similar *p*-NPA hydrolysis rates (instead of an increased enzymatic activity upon the deposition of more enzyme layers<sup>19</sup>)

28

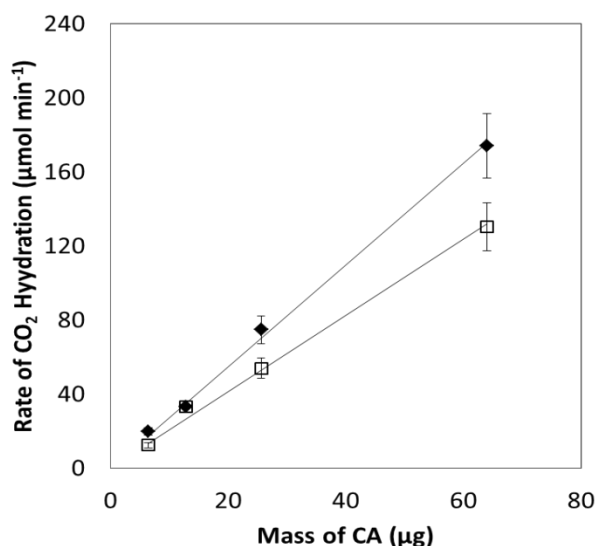
1 suggest that the hydrophilic PAH and PSS layers were sufficiently non-porous to limit the  
2 diffusion of the large hydrophobic *p*-NPA molecules to the lower layers of immobilized  
3 BCA.



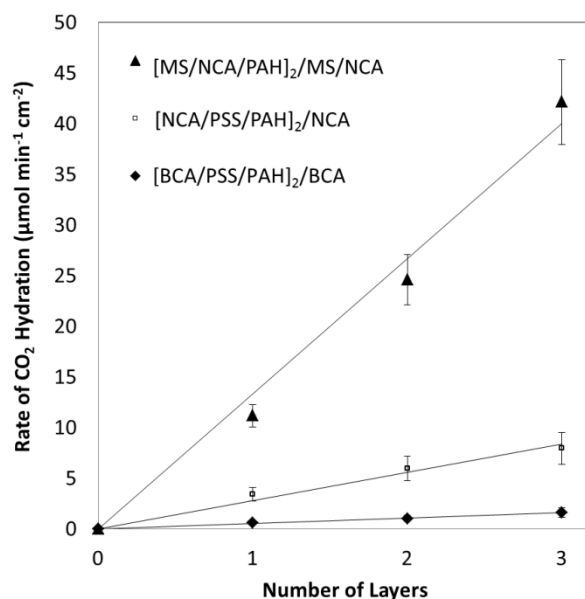
4  
5 **Figure 5.** Enzymatic activity of [BCA/PSS/PAH]<sub>2</sub>/BCA, PSS/BCA/PAH/BCA/PSS/BCA  
6 and BCA/PSS/BCA/PAH/BCA films in the hydrolysis of *p*-NPA.

7  
8 However, CO<sub>2</sub> is a small uncharged molecule that will be able to penetrate the  
9 polyelectrolyte multilayers more readily. Thus, it is necessary to evaluate the contribution of  
10 each immobilized CA layer to the overall total enzymatic activity using CO<sub>2</sub> hydration  
11 assays. Glass slides were prepared with up to three adsorbed layers of CA as  
12 [CA/PSS/PAH]<sub>2</sub>/CA films and assayed for their CO<sub>2</sub> hydration activities.

13 A calibration curve was first prepared for determining the CO<sub>2</sub> hydration activity of  
14 the free NCA in a saturated CO<sub>2</sub> solution. The activity of the free NCA is shown to be  
15 approximately 2.7 µmol min<sup>-1</sup> µg<sup>-1</sup> of NCA (Figure 6). However, the specific activity of the  
16 NCA decreased to 2.1 µmol min<sup>-1</sup> µg<sup>-1</sup> after it was stored at pH 12 over a period of 72 h at  
17 4°C (Figure 6). A number of other researchers have also shown that CA loses activity upon  
18 exposure to high pH.<sup>1, 24</sup> However, the residual activity recorded here (78%) is significantly  
19 greater than that observed in this earlier work. Sharma and Bhattacharya<sup>1</sup> observe residual  
20 activities of between 24 and 57% across four CA varieties after exposure for only 6 h at pH  
21 10, presumably at ambient temperature. Similarly, Zhang *et al.*<sup>24</sup> observe a residual activity of  
22 63% for a commercial enzyme when tested at pH 10.5 and 4°C.



1  
 2 **Figure 6.** CO<sub>2</sub> hydration rate as a function of the quantity of free NCA added to solution. The  
 3 filled diamond symbols (◆) indicate the rate for fresh NCA solution, while the open  
 4 squares(□) indicate the rate of hydration for NCA that was incubated for 72 hours at pH 12  
 5 and 4°C.



6  
 7 **Figure 7.** CO<sub>2</sub> hydration activity as a function of the number of CA layers on glass.

8  
 9 Figure 7 shows that the overall CO<sub>2</sub> hydration activity of the [BCA/PSS/PAH]<sub>2</sub>/BCA  
 10 film was approximately  $0.52 \pm 0.09 \mu\text{mol min}^{-1} \text{cm}^{-2}$  per layer of adsorbed BCA. This CO<sub>2</sub>  
 11 hydration activity is approximately 5 times greater than the *p*-NPA hydrolysis activity, which  
 12 is consistent with the CO<sub>2</sub> hydration to *p*-NPA hydrolysis activity ratios that have been  
 13 reported elsewhere.<sup>32</sup> This activity ratio reflects the limitations of the *p*-NPA diffusion within  
 14 the film. Moreover, in contrast with the *p*-NPA analyses, the CA activity increases linearly

1 with the deposition of further layers when hydrating CO<sub>2</sub>, regardless of the type of CA that  
2 was used (Figure 7). This linear trend indicates that the dissolved CO<sub>2</sub> is able to readily  
3 penetrate through the PAH/PSS bilayers to the lower levels of the film for reaction. It is  
4 probable that the activity eventually plateaus with increasing CA layers because of diffusion  
5 rate limitations, as was observed in activity assays of immobilized peroxidase enzymes.<sup>19</sup>

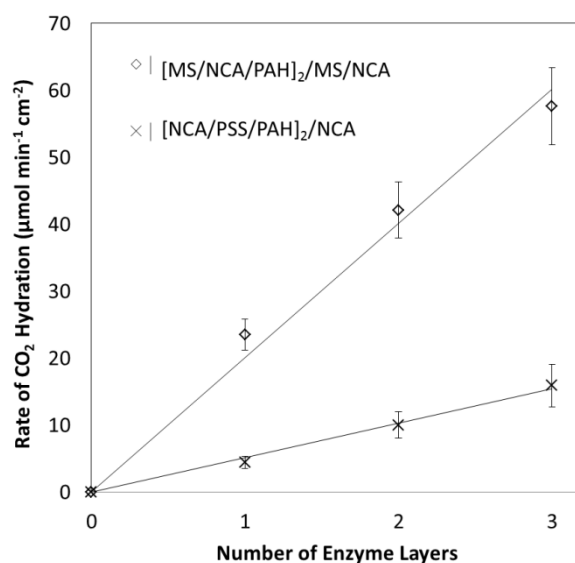
6 The CO<sub>2</sub> hydration activity of the film prepared with NCA was observed to be  $2.6 \pm$   
7  $0.7 \mu\text{mol min}^{-1} \text{cm}^{-2}$  per layer of adsorbed NCA, which was approximately 5 times greater  
8 than that of the BCA film. The specific activity of the NCA in terms of the mass of adsorbed  
9 NCA was  $3.5 \mu\text{mol min}^{-1} \mu\text{g}^{-1}$ , which is comparable within experimental error to the specific  
10 activity of the free NCA at  $2.7 \mu\text{mol min}^{-1} \mu\text{g}^{-1}$ . This indicates that there is no significant loss  
11 of activity upon immobilisation onto the plate.

12 The addition of the MS nanoparticles onto the glass plate further boosts the CO<sub>2</sub>  
13 hydration activity to  $13 \pm 4 \mu\text{mol min}^{-1} \text{cm}^{-2}$  per layer of adsorbed NCA. However, given that  
14 the adsorption of NCA was as high as  $190 \mu\text{g cm}^{-2}$  NCA (Figure 4), this CO<sub>2</sub> hydration  
15 activity translates to a specific NCA activity of  $0.067 \mu\text{mol min}^{-1} \mu\text{g}^{-1}$ . This specific activity  
16 is a decrease of 97.5% in comparison with the specific activity of the free enzyme at  $2.7 \mu\text{mol}$   
17  $\text{min}^{-1} \mu\text{g}^{-1}$  NCA. Therefore, there is a loss of enzymatic activity when the NCA is  
18 immobilized onto the nanoparticles, which was not observed for immobilization upon the  
19 glass plate. Similar losses in enzymatic activity have been reported previously for both  
20 nonporous nanoparticles<sup>24</sup> and porous nanoparticles.<sup>39</sup> In the present case, the decline in the  
21 specific enzyme activity is likely to be the result of the added diffusional resistance for the  
22 CO<sub>2</sub> molecules<sup>39</sup> when entering the pores of the MS nanoparticles.

## 24 Membrane Characterization

25 When coated on the surface of a porous polypropylene membrane, the MS particles  
26 continue to outperform a simple multilayer film, in this case by a factor of four (Figure 8) at  
27 an average rate of  $19 \pm 4 \mu\text{mol cm}^{-2} \text{min}^{-1}$  per layer in comparison with the multilayer film  
28 performance at  $5.3 \pm 0.8 \mu\text{mol cm}^{-2} \text{min}^{-1}$  per layer. The enzymatic activity on the membrane  
29 is approximately double that of the activity on the flat glass plate (Figure 7). This increased  
30 enzymatic activity is consistent with the data in Figure 4, which also shows a doubling in  
31 NCA adsorption from 190 to  $440 \mu\text{g cm}^{-2}$  when the plate is replaced with a membrane. This  
32 increase in enzyme activity probably reflects some penetration of the enzyme layers into the  
33 porous structure.

1            These hydration rates compare well to the flux rates determined for CO<sub>2</sub> absorption  
2 within membrane contactors described in the literature. The flux rates for the absorption of  
3 CO<sub>2</sub> into monoethanolamine through a porous PP contactor range from 1.5 to 30 μmol cm<sup>-2</sup>  
4 min<sup>-1</sup>.<sup>40,41</sup> The CO<sub>2</sub> flux is generally lower for solvents with slower reaction kinetics such as  
5 potassium carbonate, where promotion with CA would be of value. Capannelli *et al.*<sup>42</sup> report  
6 a flux of approximately 4 μmol cm<sup>-2</sup> min<sup>-1</sup> for a 1 M K<sub>2</sub>CO<sub>3</sub> solution, versus 50 μmol cm<sup>-2</sup>  
7 min<sup>-1</sup> for the same concentration of piperazine and 30 μmol cm<sup>-2</sup> min<sup>-1</sup> for monoethanolamine.



8

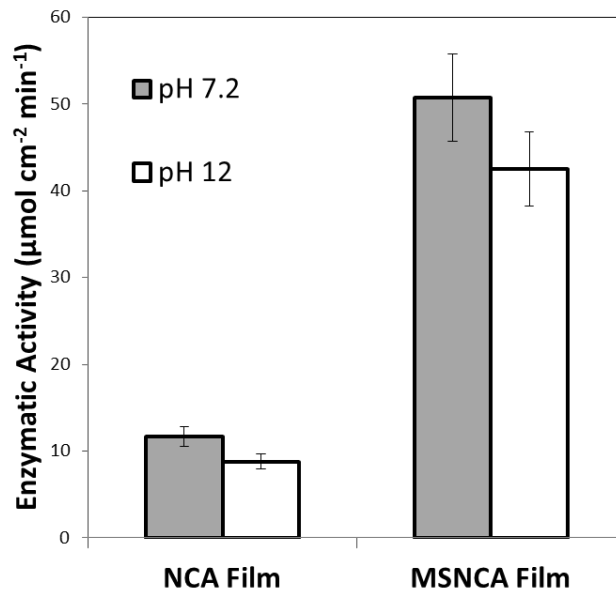
9 **Figure 8.** CO<sub>2</sub> hydration activity as a function of the number of NCA layers on the PP  
10 membranes.

11

12            Incubation of the membrane films for 72 h at pH 12 leads to a loss of activity, but 70  
13 to 80% of the residual activity is retained, relative to films incubated in Tris Buffer at pH 7.2  
14 for the same period (Figure 9). This is consistent with the data recorded for the free enzyme  
15 (Figure 6), indicating that the enzyme is more tolerant of high pH conditions than other  
16 strains that have been presented in the literature.<sup>1,24</sup>

17

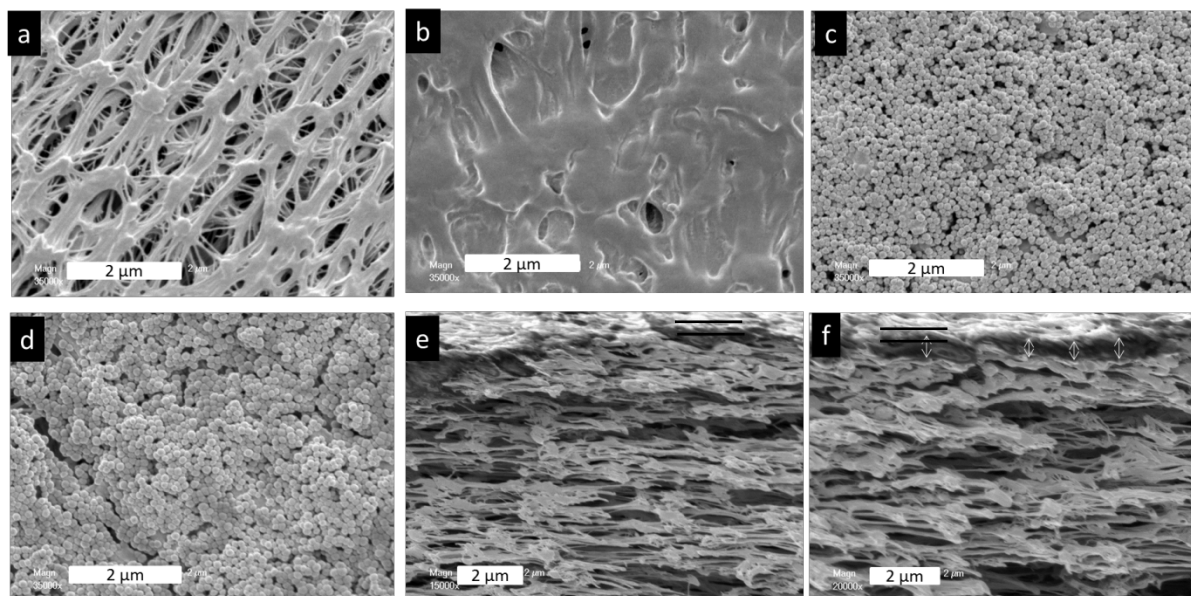
18



1 **Figure 9.** The enzymatic activity of the immobilized membrane films both after incubation  
 2 for 72h at pH 7.2 (grey bars) and at pH 12 (white bars) at 4°C. These films were prepared as  
 3 [NCA/PSS/PAH]<sub>2</sub>NCA films (denoted as NCA) and [MS/NCA/PAH]<sub>2</sub>MS/NCA (denoted as  
 4 MSNCA), respectively.  
 5

6  
 7 SEM images of the PP membrane surfaces and cross sections before and after LbL  
 8 treatment (Figure 10) show clear evidence of the multilayer films and MS spheres on the  
 9 surface. Figure 10b shows that the [NCA/PSS/PAH]<sub>2</sub>NCA layers form a flat dense film<sup>43</sup> that  
 10 is akin to the formation of nanofiltration-type barriers caused by the interactions between  
 11 PAH and PSS.<sup>44</sup> Figures 10c and 10d also show that the MS spheres assemble uniformly onto  
 12 the membrane surface. However, there is little evidence of deep penetration into the porous  
 13 substructure. (Figures 10e and 10f). The multilayer film thicknesses on the membrane  
 14 surface are of the order of 300-500 nm, consistent with that observed for the flat plate (Table  
 15 1). This means that the mass transfer resistance through the membrane itself should not be  
 16 significantly compromised.

17 Figure 10d indicates that there has been little degradation of the MS spheres after  
 18 storage at pH 12 for 72 h. However, it should be noted that silica materials will dissolve in an  
 19 alkaline environment over a long period of time. The instability of silica materials at high pH  
 20 could limit the use of the MS nanoparticles in carbon capture applications operating under  
 21 high pH conditions.  
 22  
 23  
 24



**Figure 10.** The PP membrane surface (a) before LbL treatment, (b) after LbL treatment with [NCA/PSS/PAH]<sub>2</sub>NCA, (c) after LbL treatment with [MS/NCA/PAH]<sub>2</sub>MS/NCA and storage at pH 7.2 for 72 h, (d) after LbL treatment with [MS/NCA/PAH]<sub>2</sub>MS/NCA and storage at pH 12 for 72 h. The PP membrane cross-section (e) after LbL treatment with [NCA/PSS/PAH]<sub>2</sub>NCA, (f) after LbL treatment with [MS/NCA/PAH]<sub>2</sub>MS/NCA. The black bars in (e) and (f) indicate the approximate thickness of the LBL films.

It is well known that the pores of the membrane must remain filled with gas to maintain effective mass transfer rates in a membrane contactor,<sup>45</sup> and a reduction of the local pore diameter could assist in preventing the wetting of the pore volume with solvent.<sup>46, 47</sup> Conversely, the deposition of CA and other polyelectrolytes on the membrane surface could reduce the contact angle and so enhance wetting. In the present case, the pressure required to force water through the membrane (the liquid entry pressure) was recorded experimentally, as an indication of these competing effects (Table 2):

**Table 2.** Characterization of the PP membrane before and after LbL treatment

Membrane	Contact Angle (°)	Liquid Entry Pressure (kPa)	Water Flux (kg m <sup>-2</sup> h <sup>-1</sup> ) at 575 kPa
PP (clean)	96 ± 2	< 525	9.4 ± 0.6
PP (LbL with NCA)	60 ± 2	< 550	2.25 ± 0.08
PP (LbL with MSNCA)	52 ± 1	< 575	0.53 ± 0.02

Table 2 indicates that the LbL treatment caused the membrane surface to become more hydrophilic, as evidenced by the contact angle measurements. However, an increased pressure was required to force water through the thin film that was fabricated on the membrane, and the water flux was also observed to decline, particularly after LbL treatment with the MSNCA film. This confirms that the average pore size has been reduced significantly, as is evident from the images in Figure 9. The tighter pore size will reduce pore

1 wetting, but may also increase the resistance to the mass transfer of carbon dioxide across the  
2 membrane. A compromise between these two competing effects will be required.

3

#### 4 **Conclusions**

5 This work has shown that carbonic anhydrase can be readily applied to the surface of  
6 a porous polymeric membrane as a uniform thin film. The use of LbL techniques leads to  
7 enzyme loadings that are significantly greater than those previously obtained using covalent  
8 coupling. The diffusion of CO<sub>2</sub> through these multilayers was unhindered, as evidenced by  
9 the uniform increase in CO<sub>2</sub> hydration activity with each successive CA layer. The use of a  
10 thermostable microbial enzyme (NCA) leads to higher loadings and to greater enzymatic  
11 efficiency. The addition of MS nanoparticles to the polyelectrolyte film prior to enzyme  
12 adsorption increases the CO<sub>2</sub> hydration rates by eightfold relative to the bovine enzyme film.  
13 However, the specific activity of the NCA decreased sharply upon immobilization onto the  
14 MS nanoparticles, which may be indicative of diffusional resistances for CO<sub>2</sub> molecules  
15 entering the pores of the nanoparticles for reaction.

16 While it can be observed that the overall CO<sub>2</sub> hydration rates increased when the  
17 enzyme was adsorbed onto a membrane, the flux through the membrane declined, which may  
18 lead to additional mass transfer resistance in a full scale membrane module. These results  
19 show potential towards the development of new membrane materials for use as membrane  
20 contactors in CO<sub>2</sub> absorption processes. However, the activity of these membranes was only  
21 quantified in a stirred solution pre-saturated with carbon dioxide, where mass transfer  
22 limitations are avoided. The results need to be validated by work at a larger scale in a gas-  
23 liquid membrane contactor format at realistic pH levels and over a longer period of time. This  
24 is the focus of our ongoing work.

25

#### 26 **Acknowledgements**

27 The work was supported by the Australian Research Council Centre of Excellence in  
28 Convergent Bio-Nano Science and Technology (Project number CE140100036) and the Particulate  
29 Fluids Processing Centre (PFPC), a Special Research Centre of the Australian Research Council. KLC  
30 is the recipient of a John Stocker Postdoctoral Fellowship from the Science and Industry Endowment  
31 Fund. Novozymes A/S kindly supplied the enzyme, NCA. These sources of support are gratefully  
32 acknowledged.

33

## 1 References

- 2 1. Sharma, A.; Bhattacharya, A., Enhanced Biomimetic Sequestration of CO<sub>2</sub> into CaCO<sub>3</sub> using  
3 Purified Carbonic Anhydrase from Indigenous Bacterial Strains. *J. Mol. Catal. B* **2010**, *67*,  
4 122-128.
- 5 2. Samanta, A.; Zhao, A.; Shimizu, G. K. H.; Sarkar, P.; Gupta, R., Post-Combustion CO<sub>2</sub>  
6 Capture Using Solid Sorbents: A Review. *Ind. Eng. Chem. Res.* **2012**, *2012*, 1438-1463.
- 7 3. Yong, J. K. J.; Stevens, G. W.; Caruso, F.; Kentish, S. E., The Use of Carbonic Anhydrase to  
8 Accelerate Carbon Dioxide Capture Processes. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 3-10.
- 9 4. Shekh, A. Y.; Krishnamurthi, K.; Mudliar, S. N.; Yadav, R. R.; Fulke, A. B.; Devi, S. S.;  
10 Chakrabarti, T., Recent Advancements in Carbonic Anhydrase-Driven Processes for CO<sub>2</sub>  
11 Sequestration: Minireview. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 1419-1440.
- 12 5. Arazawa, D. T.; Oh, H. I.; Ye, S. H.; Johnson, C. A., Jr.; Woolley, J. R.; Wagner, W. R.;  
13 Federspiel, W. J., Immobilized Carbonic Anhydrase on Hollow Fiber Membranes Accelerates  
14 CO<sub>2</sub> Removal from Blood. *J. Membr. Sci.* **2012**, *404*, 25-31.
- 15 6. Zhang, Y.-T.; Zhang, L.; Chen, H.-L.; Zhang, H.-M., Selective Separation of Low  
16 Concentration CO<sub>2</sub> using Hydrogel Immobilized CA Enzyme Based Hollow Fiber Membrane  
17 Reactors. *Chem. Eng. Sci.* **2010**, *65*, 3199-3207.
- 18 7. Zhang, S.; Zhang, Z.; Lu, Y.; Rostam-Abadi, M.; Jones, A., Activity and Stability of  
19 Immobilized Carbonic Anhydrase for Promoting CO<sub>2</sub> Absorption into a Carbonate Solution  
20 for Post-Combustion CO<sub>2</sub> Capture. *Bioresour. Technol.* **2011**, *102*, 10194-201.
- 21 8. Vinoba, M.; Bhagiyalakshmi, M.; Jeong, S. K.; Yoon, Y. I.; Nam, S. C., Immobilization of  
22 Carbonic Anhydrase on Spherical SBA-15 for Hydration and Sequestration of CO<sub>2</sub>. *Colloid*  
23 *Surface B* **2012**, *90*, 91-96.
- 24 9. Vinoba, M.; Lim, K. S.; Lee, S. H.; Jeong, S. K.; Alagar, M., Immobilization of Human  
25 Carbonic Anhydrase on Gold Nanoparticles Assembled onto Amine/Thiol-Functionalized  
26 Mesoporous SBA-15 for Biomimetic Sequestration of CO<sub>2</sub>. *Langmuir* **2011**, *27*, 6227-6234.
- 27 10. Ozdemir, E., Biomimetic CO<sub>2</sub> Sequestration: 1. Immobilization of Carbonic Anhydrase  
28 within Polyurethane Foam. *Energ. Fuel* **2009**, *23*, 5725-5730.
- 29 11. Gabelman, A.; Hwang, S.-T., Hollow Fiber Membrane Contactors. *J. Membr. Sci.* **1999**, *159*,  
30 61-106.
- 31 12. Li, S.; Rocha, D. J.; James Zhou, S.; Meyer, H. S.; Bikson, B.; Ding, Y., Post-Combustion  
32 CO<sub>2</sub> Capture Using Super-Hydrophobic, Polyether Ether Ketone, Hollow Fiber Membrane  
33 Contactors. *J. Membr. Sci.* **2013**, *430*, 79-86.
- 34 13. Wilcox, J., *Carbon Capture*. Springer: New York, 2012.
- 35 14. Larachi, F.; Lacroix, O.; Grandjean, B. P. A., CO<sub>2</sub> Hydration by Immobilized Carbonic  
36 Anhydrase in Robinson-Mahoney and Packed-Bed Scrubbers—Role of Mass Transfer and  
37 Inhibitor Removal. *Chem. Eng. Sci.* **2012**, *73*, 99-115.
- 38 15. Decher, G., Fuzzy Nanoassemblies: Toward Layered Polymeric Multicomposites. *Science*  
39 **1997**, *277*, 1232-1237.
- 40 16. Caruso, F.; Caruso, R. A.; Möhwald, H., Nanoengineering of Inorganic and Hybrid Hollow  
41 Spheres by Colloidal Templating. *Science* **1998**, *282*, 1111-1114.
- 42 17. Caruso, F.; Niikura, K.; Furlong, D. N.; Okahata, Y., 2. Assembly of Alternating  
43 Polyelectrolyte and Protein Multilayer Films for Immunosensing. *Langmuir* **1997**, *13*, 3427-  
44 3433.
- 45 18. Onda, M.; Lvov, Y.; Ariga, K.; Kunitake, T., Sequential Reaction and Product Separation on  
46 Molecular Films of Glucoamylase and Glucose Oxidase Assembled on an Ultrafilter. *J.*  
47 *Ferment. Bioeng.* **1996**, *82*, 502-506.

- 1 19. Yu, A.; Liang, Z.; Caruso, F., Enzyme Multilayer-Modified Porous Membranes as  
2 Biocatalysts. *Chem. Mater.* **2005**, *17*, 171-175.
- 3 20. Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T., Assembly of Multicomponent Protein Films  
4 by Means of Electrostatic Layer-by-Layer Adsorption. *J. Am. Chem. Soc.* **1995**, *117*, 6117-  
5 6123.
- 6 21. Ichinose, I.; Kuroiwa, K.; Lvov, Y.; Kunitake, T., Recent Progress in the Surface Sol-Gel  
7 Process and Protein Multilayers. In *Multilayer Thin Films*, Decher, G.; Schlenoff, J. B., Eds.  
8 Wiley-VCH: Weinheim, 2003; pp 155-175.
- 9 22. Asuri, P.; Karajanagi, S. S.; Vertegel, A. A.; Dordick, J. S.; Kane, R. S., Enhanced Stability of  
10 Enzymes Adsorbed onto Nanoparticles. *J. Nanosci. Nanotechnol.* **2007**, *7*, 1675-1678.
- 11 23. Hou, J.; Dong, G.; Ye, Y.; Chen, V., Laccase Immobilization on Titania Nanoparticles and  
12 Titania-Functionalized Membranes. *J. Membr. Sci.* **2014**, *452*, 229-240.
- 13 24. Zhang, S.; Lu, Y.; Ye, X., Catalytic Behavior of Carbonic Anhydrase Enzyme Immobilized  
14 onto Nonporous Silica Nanoparticles for Enhancing CO<sub>2</sub> Absorption into a Carbonate  
15 Solution. *Int. J. Greenh. Gas Con.* **2013**, *13*, 17-25.
- 16 25. Wang, Y.; Caruso, F., Mesoporous Silica Spheres as Supports for Enzyme Immobilization  
17 and Encapsulation. *Chem. Mater.* **2005**, *17*, 953-961.
- 18 26. Penders-van Elk, N. J. M. C.; Hamborg, E. S.; Huttenhuis, P. J. G.; Fradette, S.; Carley, J. A.;  
19 Versteeg, G. F., Kinetics of Absorption of Carbon Dioxide in Aqueous Amine and Carbonate  
20 Solutions with Carbonic Anhydrase. *Int. J. Greenh. Gas Con.* **2013**, *12*, 259-268.
- 21 27. Onda, M.; Ariga, K.; Kunitake, T., Activity and Stability of Glucose Oxidase in Molecular  
22 Films Assembled Alternately with Polyions. *J. Biosci. Bioeng.* **1999**, *87*, 69-75.
- 23 28. Zhang, K.; Xu, L. L.; Jiang, J. G.; Calin, N.; Lam, K. F.; Zhang, S. J.; Wu, H. H.; Wu, G. D.;  
24 Albela, B.; Bonneviot, L.; Wu, P., Facile Large-Scale Synthesis of Monodisperse Mesoporous  
25 Silica Nanospheres with Tunable Pore Structure. *J. Am. Chem. Soc.* **2013**, *135*, 2427-2430.
- 26 29. Caruso, F.; Niikura, K.; Furlong, D. N.; Okahata, Y., 1. Ultrathin Multilayer Polyelectrolyte  
27 Films on Gold: Construction and Thickness Determination. *Langmuir* **1997**, *13*, 3422-3426.
- 28 30. Olsson, A. L. J.; Quevedo, I. R.; He, D.; Basnet, M.; Tufenkji, N., Using the Quartz Crystal  
29 Microbalance with Dissipation Monitoring to Evaluate the Size of Nanoparticles Deposited  
30 on Surfaces. *ACS Nano* **2013**, *7*, 7833-7843.
- 31 31. Bond, G. M.; Stringer, J.; Brandvold, D. K.; Simsek, F. A.; Medina, M.-G.; Egeland, G.,  
32 Development of Integrated System for Biomimetic CO<sub>2</sub> Sequestration Using the Enzyme  
33 Carbonic Anhydrase. *Energ. Fuel* **2001**, *15*, 309-316.
- 34 32. Garg, L. C., Catalytic Activity and Inhibition of Carbonic Anhydrase of Rat Tissues.  
35 *Biochem. Pharmacol.* **1974**, *23*, 3153-3161.
- 36 33. IsoPrime IEF Purification Unit User Manual.  
37 [https://www.gelifesciences.com/gehcls\\_images/GELS/Related%20Content/Files/1314729545](https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314729545976/litdocIsoPRIMEum_20110830211647.pdf)  
38 [976/litdocIsoPRIMEum\\_20110830211647.pdf](https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314729545976/litdocIsoPRIMEum_20110830211647.pdf) (accessed 8 July).
- 39 34. Lvov, Y.; Ariga, K.; Ichinose, I.; Kunitake, T., Molecular Film Assembly via Layer-by-Layer  
40 Adsorption of Oppositely Charged Macromolecules (Linear Polymer, Protein and Clay) and  
41 Concanavalin A and Glycogen. *Thin Solid Films* **1996**, *284-285*, 797-801.
- 42 35. Crumbliss, A. L.; McLachlan, K. L.; O'Daly, J. P.; Henkens, R. W., Preparation and Activity  
43 of Carbonic Anhydrase Immobilized on Porous Silica Beads and Graphite Rods. *Biotechnol.*  
44 *Bioeng.* **1988**, *31*, 796-801.
- 45 36. Thee, H.; Smith, K. H.; da Silva, G.; Kentish, S. E.; Stevens, G. W., Carbonic Anhydrase  
46 Promoted Absorption of CO<sub>2</sub> into Potassium Carbonate Solutions. *Greenh. Gas Sci. Technol.*  
47 **2015**, *5*, 108-114.

- 1 37. Borchert, M.; Saunders, P., Heat-Stable Carbonic Anhydrases and Their Use. US Patent  
2 20130203156: 2013.
- 3 38. Lindskog, S., Structure and Mechanism of Carbonic Anhydrase. *Pharmacol. Ther.* **1997**, *74*,  
4 1-20.
- 5 39. Vertegel, A. A.; Siegel, R. W.; Dordick, J. S., Silica Nanoparticle Size Influences the  
6 Structure and Enzymatic Activity of Adsorbed Lysozyme. *Langmuir* **2004**, *20*, 6800-6807.
- 7 40. Franco, J. A.; Montigny, D. D.; Kentish, S. E.; Perera, J. M.; Stevens, G. W., Effect of Amine  
8 Degradation Products on the Membrane Gas Absorption Process. *Chem. Eng. Sci.* **2009**, *64*,  
9 4016-4023.
- 10 41. Hoff, K.; Juliussen, O.; Falk-Pedersen, O.; Svendsen, H. F., Modeling and Experimental  
11 Study of Carbon Dioxide Absorption in Aqueous Alkanolamine Solutions Using a Membrane  
12 Contactor. *Ind. Eng. Chem. Res.* **2004**, *43*, 4908-4921.
- 13 42. Capannelli, G.; Comite, A.; Costa, C.; di Felice, R. Effect of Absorbent Type and  
14 Concentration on CO<sub>2</sub> Capture from a Gas Stream into a Liquid Phase. *Ind. Eng. Chem. Res.*  
15 **2013**, *52*, 13128-13136.
- 16 43. Decher, G.; Schmitt, J., Fine-Tuning of the Film Thickness of Ultrathin Multilayer Films  
17 Composed of Consecutively Alternating Layers of Anionic and Cationic Polyelectrolytes  
18 *Progr. Colloid Polym. Sci.* **1992**, *89*, 160-164.
- 19 44. Malaisamy, R.; Bruening, M. L., High-Flux Nanofiltration Membranes Prepared by  
20 Adsorption of Multilayer Polyelectrolyte Membranes on Polymeric Supports. *Langmuir* **2005**,  
21 *21*, 10587-10592.
- 22 45. Franco, J. A.; deMontigny, D. D.; Kentish, S. E.; Perera, J. M.; Stevens, G. W.,  
23 Polytetrafluoroethylene (PTFE)-Sputtered Polypropylene Membranes for Carbon Dioxide  
24 Separation in Membrane Gas Absorption: Hollow Fiber Configuration. *Ind. Eng. Chem. Res.*  
25 **2012**, *51*, 1376-1382.
- 26 46. Franken, A. C. M.; Nolten, J. A. M.; Mulder, M. H. V.; Bargeman, D.; Smolders, C. A.,  
27 Wetting Criteria for the Applicability of Membrane Distillation. *J. Membr. Sci.* **1987**, *33*, 315-  
28 328.
- 29 47. García-Payo, M. C.; Izquierdo-Gil, M. A.; Fernández-Pineda, C., Wetting Study of  
30 Hydrophobic Membranes via Liquid Entry Pressure Measurements with Aqueous Alcohol  
31 Solutions. *J. Colloid Interf. Sci.* **2000**, *230*, 420-431  
32