Supporting Information for the paper:

**Investigating the Specific Interactions between Carbonic Anhydrase and a Sulfonamide Inhibitor by Single Molecule Force Spectroscopy**

Sarah Kamper,† Laura Porter-Peden,† Ronald Blankespoor,‡ Kumar Sinniah,*,† Dejian Zhou,‡ Chris Abell,§ and Trevor Rayment**

Department of Chemistry & Biochemistry, Calvin College, Grand Rapids, MI 49546, School of Chemistry, University of Leeds, Leeds, LS2 9JT, UK, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW UK, and Department of Chemistry, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

† Calvin College
‡ University of Leeds
§ University of Cambridge
** University of Birmingham
Scheme S1. Procedure for synthesis of N-(12-mercaptododecyl) pyridinium bromide from 1,12-dibromododecane.

**Synthesis of 12-bromododecyl thiol acetate.** To a solution of 2.77 g (24.3 mmol) of potassium thioacetate in 90 ml of THF was added 15.9 g (48.5 mmol) of 1,12-dibromododecane. The mixture was heated to reflux for 24 hours under N₂. After cooling, the resulting KBr precipitate was removed by vacuum filtration. Removal of solvent from the filtrate in a rotary evaporator under reduced pressure gave 7.30 g of a yellow oil, which was chromatographed on silica gel and eluted with hexane-CH₂Cl₂ ratios of 8:1, 6:1, and 4:1 to give 1.94 g (24%) of 12-bromododecylthiol acetate as a white solid. Low temperature recrystallization from hexane gave a pure sample: mp 39 °C; IR (KBr) 1132, 1468, 1687, 2852, 2920 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) @ 3.40 (t, 2H), 2.85 (t, 2H), 2.32 (s, 3H), 1.85 (m, 2H), 1.56 (m, 2H) 1.57-1.26 (m, 16H); ¹³C NMR (400 MHz, CDCl₃), @ 34.22, 33.05, 30.8, 29.7, 29.64, 29.61, 29.37, 29.31, 29.02, 28.96, 28.39.

**Synthesis of N-(12-acetylthioldodecyl)-pyridinium bromide.** To a solution of 1.96 g (24.8 mmol) of pyridine in 20 mL of THF was added 1.42 g (4.40 mmol) of 12-bromododecylthiol acetate. The solution was heated to reflux for 4 days under a CaCl₂ drying column. After cooling to room temperature, the mixture was place in an ice bath. The resulting precipitate of the bromide salt was collected by vacuum filtered and washed with ether (2x15 mL) and hexane (15mL). After drying in a vacuum desiccator,1.53 g (81%) of N-(12-acetylthioldodecyl) pyridinium bromide as a light yellow solid was obtained: mp 68 °C; IR (KBr) 3046, 3021, 2919, 2850, 1688, 1653, 1490, 1127, 1110, 681, 635 , cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.5 (2H, d, J = 5.65 Hz), 8.52 (1H, m), 8.14 (2H, t, J = 7.09 Hz), 4.96 (2H, t, J = 7.41 Hz), 2.81 (2H, t, J = 7.36 Hz), 2.26 (3H, s), 2.01 (2H, m), 1.51(2H, m), (16H, m); ¹³C NMR (100 MHz, CDCl₃) δ 196.32, 145.40, 128.71, 62.29, 32.20, 30.85, 29.70, 29.53, 29.48, 29.33, 29.23, 28.96, 26.23.

**Synthesis of N-(12-mercaptododecyl)-pyridinium bromide.** A solution of 1.00 g (2.49 mmol) of N-(12-acetylthioldodecyl)pyridinium bromide in 17 mL of 1.0 M HBr (1:1 methanol-water) was heated to reflux under N₂ for 24 hr. The solvent was removed in a rotary evaporator under reduced pressure leaving a white solid that was recrystallized from 30 ml of deionized H₂O. The crystals were collected by vacuum filtration and dried in a vacuum dessicator. To the filtrate was
added 2.5 g of NaBr resulting in the formation of more crystals that were collected and dried as before giving N-(12-mercaptododecyl)pyridinium bromide in a combined yield of 0.807 g (71%): mp 69-70 °C; IR (KBr) 3429, 3380, 3048, 2919, 2853, 1639, 1493, 1177 cm⁻¹; ¹H NMR (400 MHz, DMSO) δ 9.15 (2H, d, J = 6.95 Hz), 8.6 (1H, m), 8.15 (2H, t, J = 6.95), 4.62 (m, 2H), 2.44 (q, J = 4.43, 2H), 2.2 (d, J=7.81, 2H), 1.87-1.90 (m, 2H), 1.46-1.5 (m 2H), 1.27-1.19 (m, 20H); ¹³C NMR (400 MHz, DMSO) δ 146.18, 145.41, 128.77, 61.38, 34.01, 31.42, 29.59, 29.55, 29.44, 29.16, 29.06, 29.40, 26.04, 34.45.

**Calculation for the possible contact area of the AFM cantilever**

![Diagram](image)

\[
\cos(\theta) = \frac{(r - h)}{r} = \frac{(20 - 2.9)}{20} = 0.855
\]

\[
\theta = 31.24^\circ
\]

Possible contact area, \(A = 2\pi r^2 [1-\cos(\theta)]\)

\[
= 2 \times 3.14 \times 20^2 [1-\cos(31.24^\circ)]
\]

\[
= 364 \text{ nm}^2
\]

**Scheme S2.** Calculation for the possible contact area of the AFM cantilever assuming the gold coated AFM tip is spherical with a tip radius of 20 nm.

The flexible linker for (II) is ~ 2.9 nm long (excluding the C₃ chain which would be rigid when it forms the SAM). Assuming the end of the gold coated AFM tip is spherical with a tip radius of 20 nm, then the possible tip surface area where the sulfonamide inhibitor could come into contact with the enzyme surface (assuming a flat surface) is estimated to be ~ 360 nm². This contact area would then contain ~ 80 sulfonamide inhibitor molecules (II was diluted with MCU, 1:20), assuming each thiol molecule occupies a surface area of 0.22 nm². The tip shadow would cover a flat surface of ~330 nm², within which it would be possible to pack ~ 25 carbonic anhydrase molecules if the enzyme is closely packed on the surface (carbonic anhydrase has a diameter of ~4 nm). These are the theoretical maximum numbers, and in reality the packing density of the enzyme would be smaller.