Detection of Acetylcholinesterase Inhibitors using Back-scattering Interferometry

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Introduction

Acetylcholinesterase (AChE) plays an important enzymatic role in neurotransmission, as well as a pivotal role in Alzheimer's Disease (AD).[1] The enzyme contains two binding sites, the active site, located at the bottom of a 20 Å deep gorge; and a peripheral anionic site (PAS) at the top of the gorge. The aggregation of plaque precursors, amyloid-beta (Aβ) peptides, is accelerated by the PAS.[2] Therefore, inhibitors of AChE, particularly targeting the PAS, are of great importance. Equally important are fast, effective screening techniques for identification of new novel AChE PAS inhibitors. Traditional methods used to detect interactions with AChE focus on the indirect measurement of substrate hydrolysis and often require specialized probes and laborious procedures.[3]

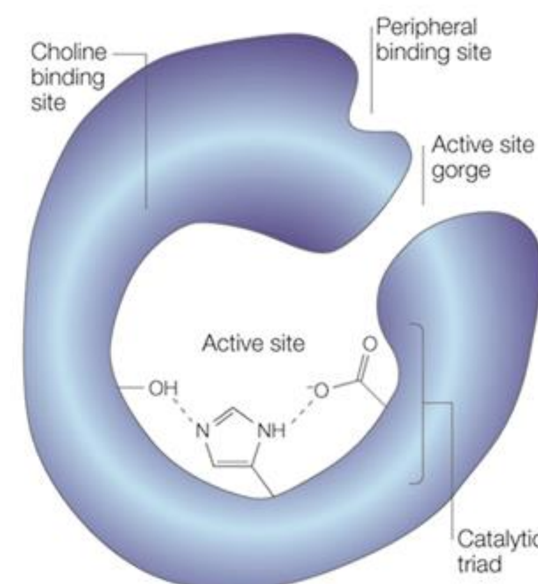

Figure 1: Acetylcholinesterase[4]

Back-scattering Interferometry (BSI)[5]

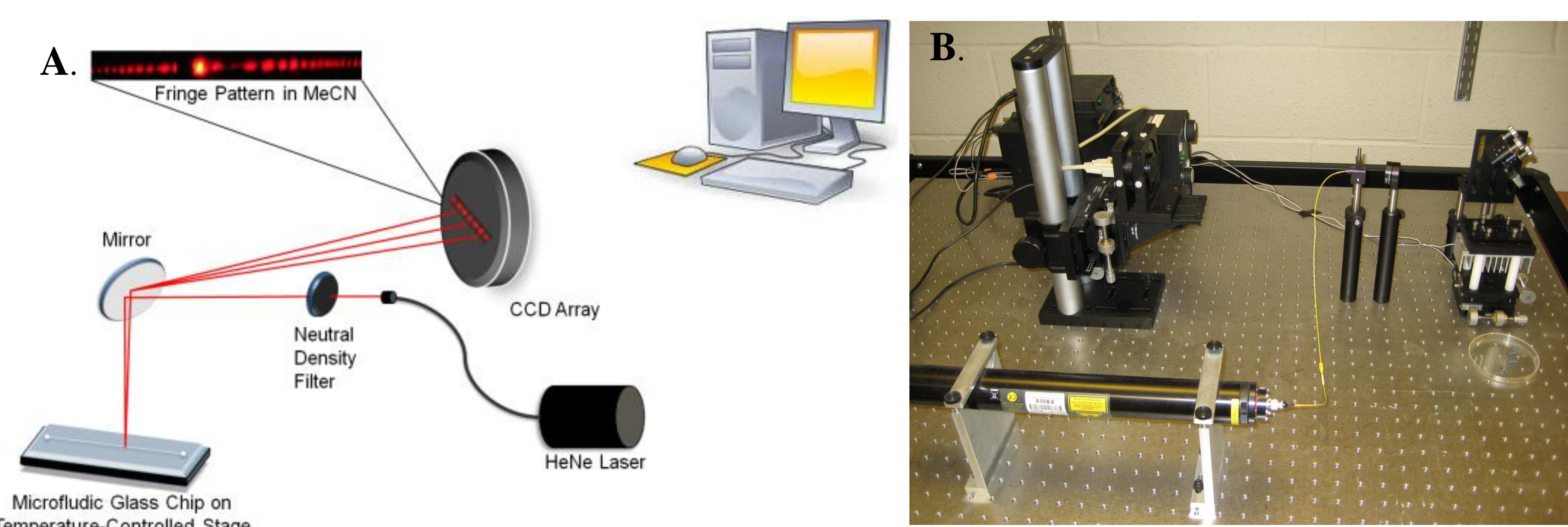

Figure 2. A) Diagram of BSI instrument set-up.[5] **B)** Photograph of instrument.

BSI measures binding affinity by detecting changes in the refractive index (RI) of solutions. A red laser beam is directed using a fiber optic cable onto a glass microfluidic chip containing the sample solution. The monochromatic light is refracted in the chip, backscattered, and reflected with a mirror to the detector. The chip is placed on a temperature-controlled stage, as RI is temperature-dependent. BSI utilizes a multipass configuration to greatly increase the path length and lower the detection limit, allowing for the study of pM to µM concentrations of samples.[5]

BSI experiments are straightforward, require only microliters of sample, and can be run in free solution without the need for surface immobilization or fluorescent probes, providing a strong advantage over other, more common techniques such as isothermal calorimetry (ITC),[6] surface plasmon resonance (SPR),[7] and fluorometric assays.[8] BSI measures end-point assays in which the two binding species are pre-mixed and equilibrated.

Data is collected in real time from a CCD camera using a fast Fourier Transform (FFT). Each data point is plotted as phase shift as a function of concentration to obtain a binding curve, which can be fitted to a rise to max equation (1) to obtain a K_D value, a measure of binding affinity.

$$y = \frac{B_{max} * x}{K_D + x} \qquad (1)$$

Data & Results

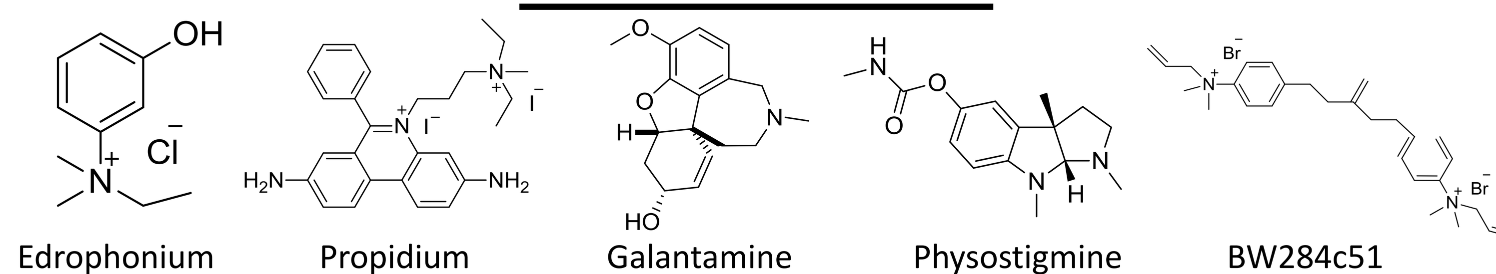

Figure 3: Known AChEIs

Table 1: Literature and Experimental K_D values for AChEIs.

Ligand	Type	BSI K_D (µM)	Lit. K_i (µM)[9]
Edrophonium	Competitive	1.27 ± 0.4	1.5-3.8
Propidium	Noncompetitive	0.64 ± 0.09	0.63-1.5
Galantamine	Mixed	1.23 ± 0.5	0.20-0.61
Physostigmine	Pseudo-irreversible	0.020 ± 0.009	0.013[a]-0.11
BW284c51	Mixed	0.0077 ± 0.0016	0.0032-0.008

[a] IC_{50} value

Detection Limit: 100 pM AChE with BW284c51 ⟶ 22,000 molecules

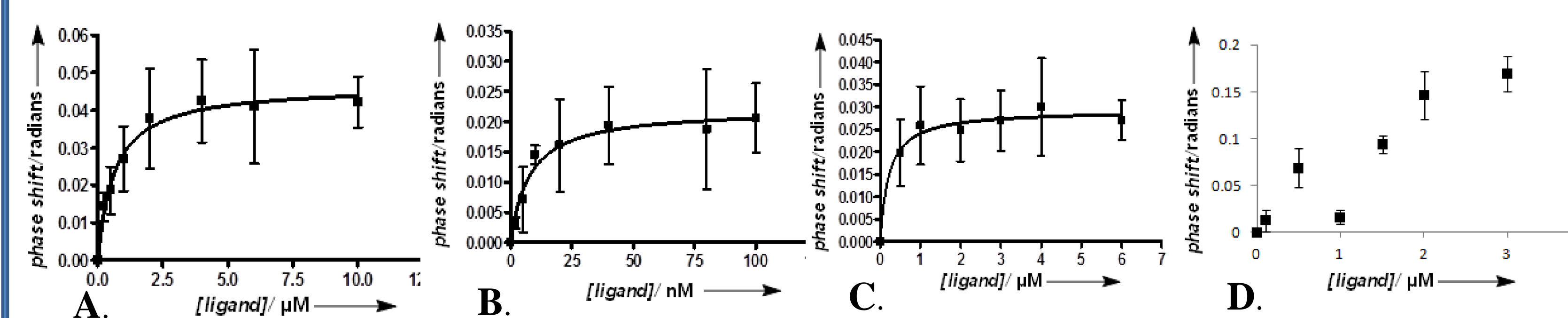

Figure 4: BSI binding curves for known inhibitors A) Propidium, B) BW284c51, and novel inhibitors C) ligand 4 and D) ligand 6. Ligand 6 was one of two inhibitors that displayed two separate rise to max binding curves.

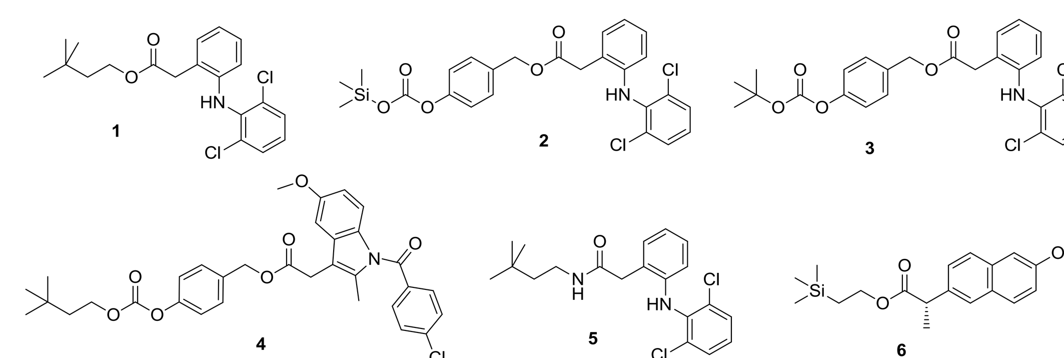

Figure 5: Novel AChEIs

Table 2: Comparison of BSI K_D values to previously determined[10] IC_{50} values.

Ligand	BSI K_D (µM)	IC_{50} (µM)
1	2.18 ± 1.1	2.69 ± 0.1
2	0.12 ± 0.04	1.36 ± 0.1
3	0.11 ± 0.05	0.51 ± 0.02
4	0.21 ± 0.05	2.29 ± 0.9
5	*Dual Binding*	6.34 ± 0.5
6	*Dual Binding*	13.9 ± 0.3

Why don't all BSI K_D values correlate with IC_{50} values?

Cheng-Prusoff Relationship[11]

Only Ligand 1 had a K_D value that correlated with its IC_{50} value determined via Ellman's method.[10] This can be rationalized with the Cheng-Prusoff Relationship.[11] Ellman assays are performed with a high concentration of substrate, and so the equilibrium studied is between the inhibitor and the enzyme-substrate complex (K_{ies}). Conversely, BSI experiments are performed without substrate, thus, the equilibrium being observed is between the inhibitor and enzyme only (K_{ie}).

For Ligand 1, where the K_D and IC_{50} values agree, this would suggest that the ligand is binding to the PAS only, as its binding affinity is not affected by the presence of substrate at the active site. For Ligands 2, 3, and 4, where the BSI K_D values are significantly lower than the IC_{50}, this relationship suggests that these ligands are able to also bind to the active site in the absence of substrate, hence the lower K_D value.

Ligands 5 and 6 showed two distinct rise to max curves, suggesting that they act as mixed inhibitors binding at both sites. The active site gorge is filled with water molecules which may be displaced as a ligand binds to the active site, and this displacement changes the optical density of the solution and thus the refractive index, resulting in the significant phase shifts.

Conclusions and Future Work

BSI is able to distinguish between types of inhibition mechanisms in an unprecedented way, and could be exceptionally useful for specifically screening therapeutics which use PAS inhibitors to combat AD. Additionally, BSI's low sample requirement saves time and money, which is especially valuable for enzymes such as AChE which may cost hundreds of dollars per mg. The absence of substrate in BSI experiments also prevents the possibility of false positives, such as those seen in Ellman's method in the presence of nucleophilic oximes. This procedure could also be applied to a variety of enzyme-inhibitor systems. Future work with BSI will involve Aβ peptide aggregation and its relationship with the PAS. In a broader scope, DNA hybridization and SNP detection, metal complexation, and other biologically-relevant interactions will be observed.

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