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Supporting information for article:

**Delineating the activity of the potent nicotinic acetylcholine
receptor agonists(+)-anatoxin-a and (-)-hosieline-A**

**Holly P. Parker, Alice Dawson, Mathew J. Jones, Rui Yan, Jie Ouyang, Ran
Hong and William N. Hunter**

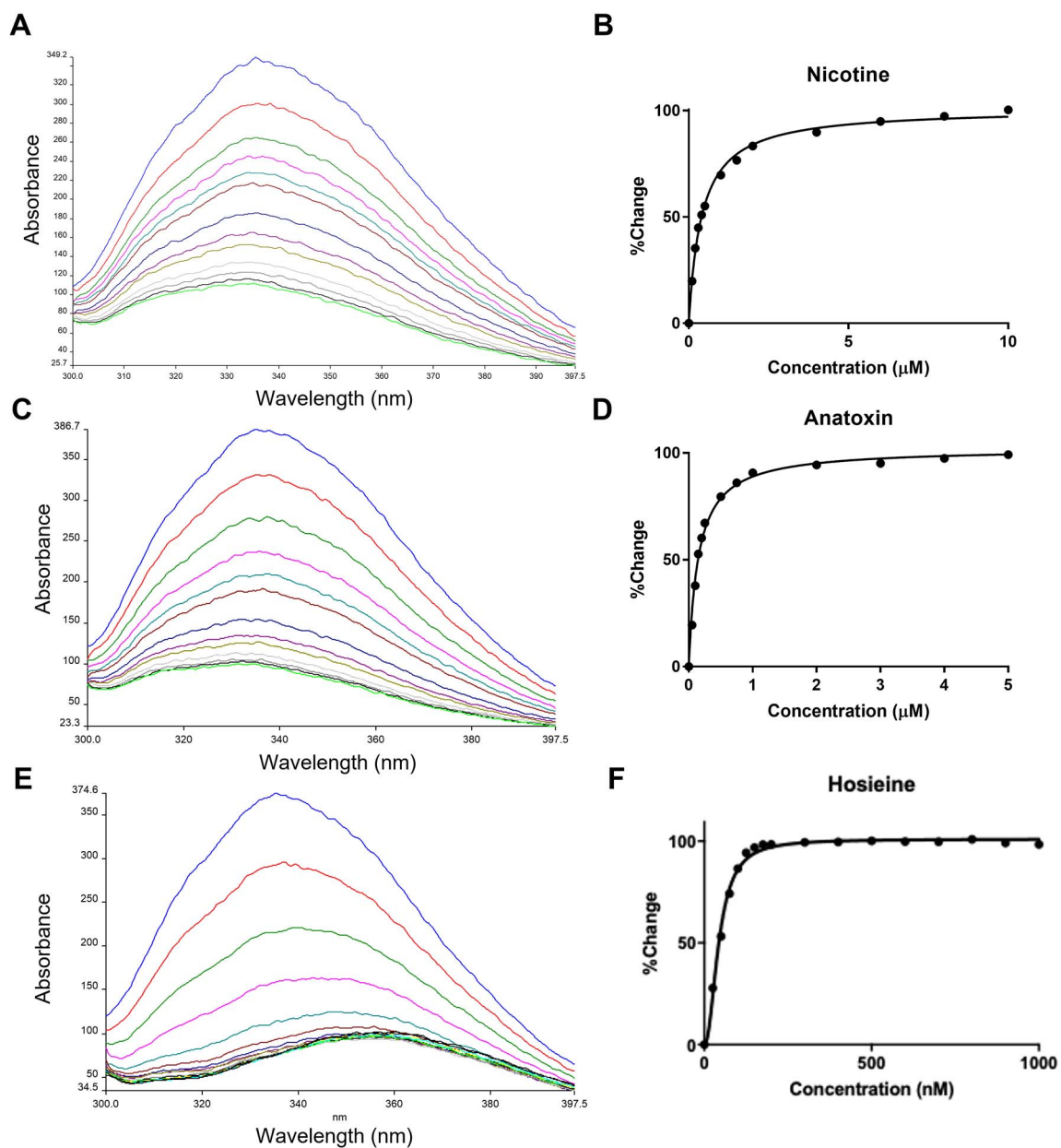


Figure S1 Ligand binding to *AcAChBP* monitored by tryptophan fluorescence. Example traces obtained from titrations, starting with no ligand (blue) to maximum ligand concentration (green) and a plot of the mean percentage change versus concentration is shown for (-)-nicotine (A, B), (+)-anatoxin (C, D), and (-)-hosieline (E, F). Standard error bars are behind each data point ($n=3$).

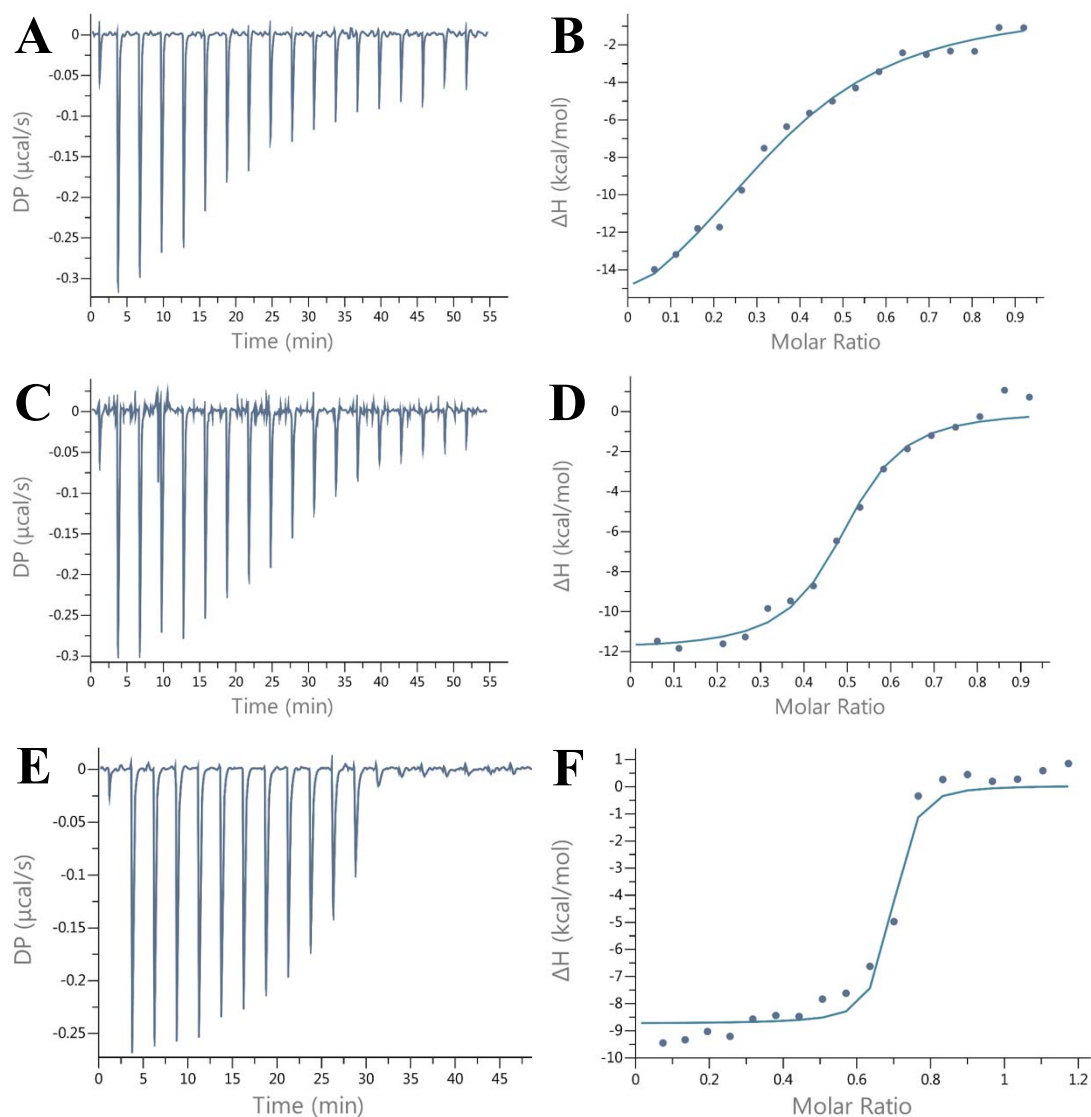


Figure S2 ITC data for *AcAChBP* binding various ligands. Example isotherms and curves with the best fit are shown for (-)-nicotine (A, B), (+)-anatoxin-a (C, D) and (-)-hosieline-A (E, F), respectively. In the examples, a baseline deduction has been applied. All data have had the relevant controls (buffer-buffer, buffer-*AcAChBP*, ligand-buffer) also deducted.

| loop | PRINCIPAL (+) | | | COMPLEMENTARY (-) | | | |
|------------------|---------------|------------|---------------|-------------------|-----------|------------|-----------|
| | A | B | C | D | E | F | G |
| AChBP | 105 PDITAY | 161 FGSWVY | 204 HYSCCPEPY | 72 YEQQRW | 133 MFIPA | 181 DLSSYY | 51 GFTLQD |
| nAChR α 4 | 121 PDIVLY | 179 FGSWTY | 222 KYECCAEPY | 88 WVKQEW | 150 QWTPP | 199 DQLDFW | 67 GLSIAQ |
| nAChR α 7 | 110 PDILLY | 168 FGSWSY | 209 FYECCKEPY | 77 WLQMSW | 139 QYLPP | 186 DISGYI | 56 SLSLLQ |
| nAChR β 2 | 115 PDVVLY | 173 FRSWTY | 116 P-D--DSTY | 82 WLTQEW | 144 FWLPP | 193 SLDDFT | 61 MVSLAQ |

Figure S3 Alignment of selected sequence segments that form the orthosteric binding sites of *AcAChBP*, human nAChR α 4, α 7 and β 2. Segment loops are labelled and presented as principal (+) and complementary (-) sides. Residues colored light blue are discussed in text. Val125 and Thr127 of *AcAChBP* are at the N-terminal section of loop E but are left out for the purpose of clarity.