Supplemental Fig. 1: The interaction of AChE with (A) acetylcholine, (B) 1-naphthyl acetate, (C) 2-naphthyl acetate are shown. The substrates are represented in ball and stick. The interacting residues of the enzyme are represented as sticks (color codes: nitrogen: blue; oxygen: red; carbon: based on the type of residue color is different). AChE is interacting with the substrates via H-bond or stacking interaction. The H-bonds are represented as dashed lines (yellow) with distance in Å. In each figure the interacting amino acid residue are represented by three-letter code.
Supplemental Fig. 2: Representative pictures of Native PAGE of hemolysate followed by incubation with increasing concentrations of ATCh, 1-NA and 2-NA, respectively. The gels were stained for 12 hrs using Karnovsky staining for ATCh and Fast Blue RR for 1-NA, 2-NA. The bands are shown by arrow. Blank gel incubated with the respective stain without the substrate. A, D, G: 20 uM of the substrate (A) ATCh, (D) 1-NA, (G) 2-NA; B, E, H: 25 uM of the substrate (B) ATCh, (E) 1-NA, (H) 2-NA; C, F, I: 50 uM of the substrate (C) ATCh, (F) 1-NA, (I) 2-NA; A', B': 100 uM (A'), 400 uM (B') of ATCh; C', E': 20 uM of the substrate (C') 1-NA and (E') 2-NA; D', F': 100 uM of the substrate (D') 1-NA, (F') 2-NA.
Supplemental Fig. 3: Native PAGE of hemolysate followed by incubation with (I) Eserine salicylate (cholinesterase inhibitor), (II) BW284c51 (a specific inhibitor of AChE) and reincubation with ATCh (for eserine incubated gels only), 1-NA and 2-NA (100 uM each) and stained with Karnovsky for ATCh; Fast blue RR for 1-NA and 2-NA. The band disappearance is shown by arrow. A: ATCh (100 uM) without Eserine, B-D: incubation with 2, 50 and 100 uM of Eserine followed by ATCh incubation; E: 1-NA (100 uM) without Eserine, F-H: incubation with 2, 50 and 100 uM of Eserine followed by 1-NA incubation; I: 2-NA (100 uM) without inhibitor, J-L: incubation with 2 uM, 50 and 100 uM of Eserine followed by 2-NA incubation; A': 1-NA (100 uM) without BW284c51, B': 450 uM of BW284c51 followed by 1-NA incubation; C': 2-NA (100 uM) without BW284c51, D': 450 uM of BW284c51 followed by 2-NA incubation.
Supplemental Fig. 4: Native PAGE of hemolysate followed by inhibition with Chlorpyriphos and reactivation with 2-PAM is shown. The band disappearance is shown by dashed arrow. The reactivation is represented by arrow. A, D, G: ATCh, 1-NA, 2-NA (100 uM each). B, E, H: incubation with 72 uM of chlorpyriphos followed by incubation with (B) ATCh, (E) 1-NA, (H) 2-NA. C, F, I: incubation of gels first with 72 uM of chlorpyriphos followed by incubation with 2-PAM (720 uM) and staining for (C) ATCh, (F) 1-NA and (l) 2-NA. Staining method for ATCh is Karnovsky staining and for 1-NA, 2-NA is Fast blue RR.
Supplemental Fig. 5: Representative gel pictures of Native PAGE run hemolysate followed by incubation with ATCh (I, II), and 1-NA (III, IV). The staining reagents for ATCh were DTNB (I) and DPIP (II). Fast Blue RR (0.03%) was used for the staining of 1-NA (III, IV). A: incubation with 100 uM ATCh and 6 mM DTNB for 10 mins; B: incubation with 500 uM ATCh and 500 uM DTNB for 10 min; C: incubation with 100 uM of ATCh followed by overnight incubation with the stain, which consisted of DPIP (1 mM) plus MTT (10 mM) in 100 ml of Tris (pH 7.5); D: incubation with 800 uM of ATCh and 1 mM DPIP plus 10 mM MTT in Tris (pH 7.5) buffer for 10 mins; E, E’: incubation with 100 uM of 1-NA and Fast blue RR (0.03%); F, F’: incubation of dichlorovos (116 uM) pretreated hemolysate with 100 uM of 1-NA and Fast blue RR (0.03%). Band formation is shown by an arrow. The IDV values were recorded at 10 mins (III) and 720 mins (IV) and mean±SD values (n=6) are represented as bar diagrams. *: E vs F, E’ vs F’ where ***p<0.0001.