Supplementary Material for:

Mapping the Interactions between the Alzheimer's Aβ-Peptide and Human Serum Albumin beyond Domain Resolution

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Supplementary Figure Captions

Figure S1. Effect of free myristic acid on the Aβ (1-42) aggregation profile. White circles represent the 1D NMR signal intensities of 90 μM Aβ (1-42) *vs.* time, while the black circles represent the 1D NMR signal intensities of 90 μM Aβ in the presence of 30 μM myristic acid. Signal intensities were measured using a Bruker Avance 700 MHz spectrometer equipped with a 5 mm TCI Cyroprobe at 37 ^{o}C.

Figure S2. Three residue average local backbone RMSDs computed for domain 3 using the structures of HSA in the apo and myristic acid-bound forms (PDB codes 1AO6 and 1E7G, respectively). Secondary structure elements of domain 3 are shown in the upper area, while residues in direct contact with myristic acid are indicated using circles and dotted red lines. Local RMSDs were calculated using MOLMOL (27), while contact residues were extracted from the PDB files using Ligand Explorer Software (39). The black dotted vertical line indicates the sub-domain 3A / 3B boundary, while residues highlighted in grey indicate the HSA derived peptides used in this study (Figures 5 and 6).

Figure S3. Binding of octanoic acid to 10 μM wild-type domain 3 and fatty acid silencing mutants as monitored by STD NMR titration in D_2O. The black triangles in panels (a), (b) and (c) represent dose-response curves for octanoic acid binding to K525A, R410AY411A and R485AS489A domain 3 mutants, respectively. As a reference, the wild type domain 3 dose-response STD curve is shown in white circles for each mutant. All the mutants showed a one third drop in their saturation regions relative to wild type.

Figure S4. (a) STD based dose-response curve for myristic acid binding to sub-domain 3B. (b) Effect of myristic acid on the inhibition of Aβ (1-42) aggregation by HSA sub-domain 3B. 1D ^{1}H-NMR signal intensities of 90 μM Aβ (1-42) recoded over time are shown in the absence (filled triangles) and in the presence of sub-domain 3B either in the apo form (open squares) or bound to myristic acid (filled circles).

Figure S5. ThT fluorescence for the HSA (494-515) peptide (black circles) at 120 μM, showing that this peptide does not form cross-β amyloid fibrils under our experimental conditions. These conditions differ markedly from those used in the original Waltz protocol (28) in which higher concentrations of peptide and buffer (500 mM phosphate buffer) were employed, explaining why under our experimental conditions no cross-β amyloid fibrils were observed for HSA (494-515). The ThT fluorescence of 90 μM Aβ (1-42) alone (Figure 6) is reported here as well (white circles) for the convenience of comparison.

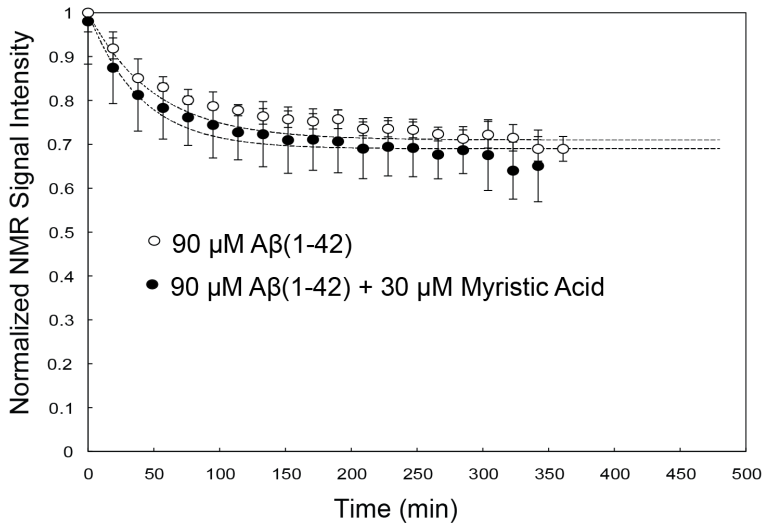

Normalized NMR Signal Intensity
Time (min)
90 μM Aβ(1-42)
90 μM Aβ(1-42) + 30 μM Myristic Acid
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
50
100
150
200
250
300
350
400
450
500

Figure S1

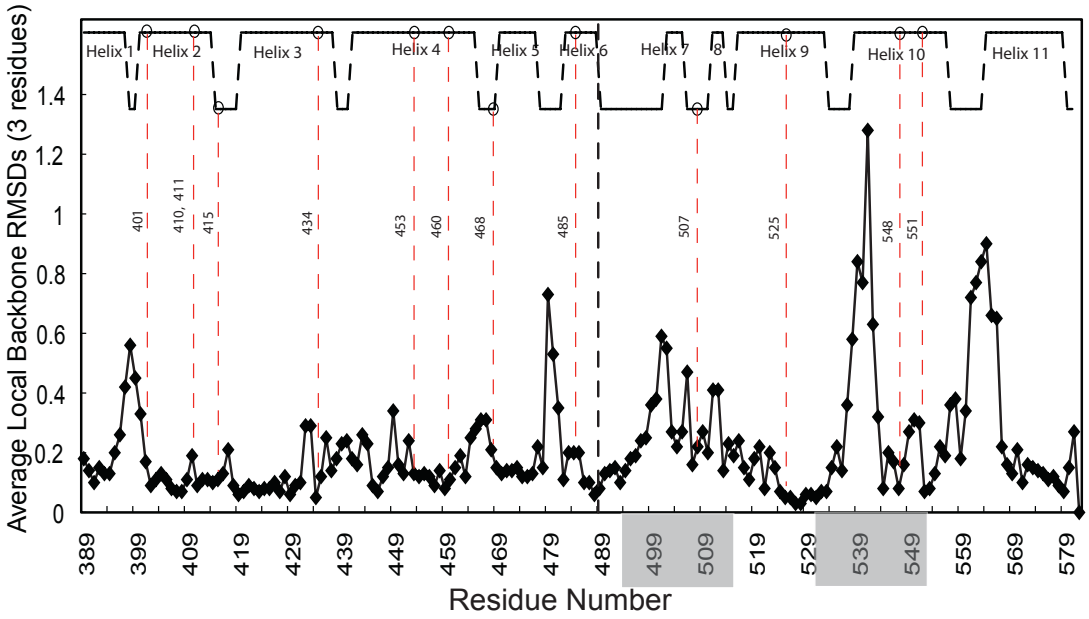
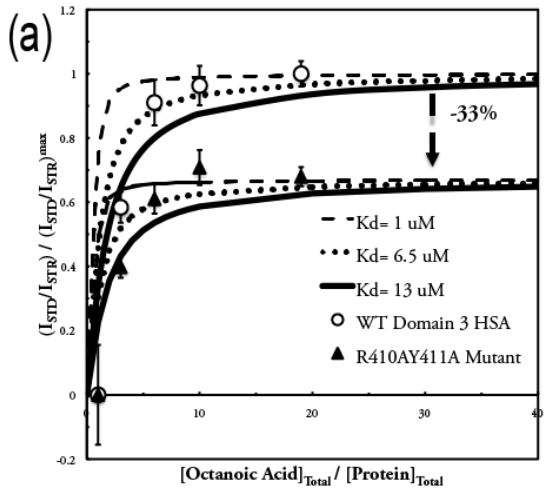
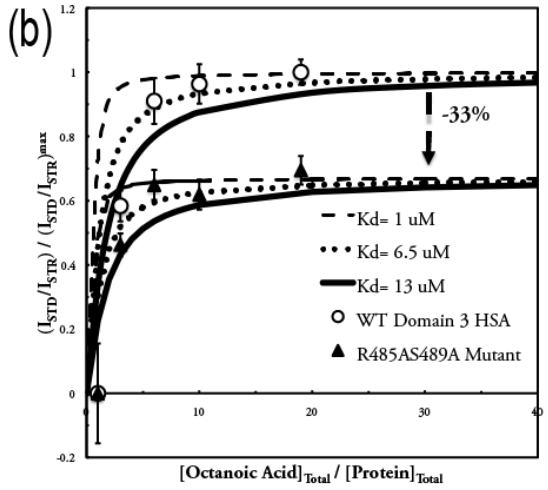

Average Local Backbone RMSDs (3 residues)
Residue Number
Helix 1
Helix 2
Helix 3
Helix 4
Helix 5
Helix 6
Helix 7
8
Helix 9
Helix 10
Helix 11
401
410, 411
415
434
453
460
468
485
507
525
548
551
0
0.2
0.4
0.6
0.8
1
1.2
1.4
389
399
409
419
429
439
449
459
469
479
489
499
509
519
529
539
549
559
569
579

Figure S2


(a)
-33%
Kd= 1 uM
Kd= 6.5 uM
Kd= 13 uM
WT Domain 3 HSA
R410AY411A Mutant
[Octanoic Acid]Total / [Protein]Total


(b)
-33%
Kd= 1 uM
Kd= 6.5 uM
Kd= 13 uM
WT Domain 3 HSA
R485AS489A Mutant
[Octanoic Acid]Total / [Protein]Total

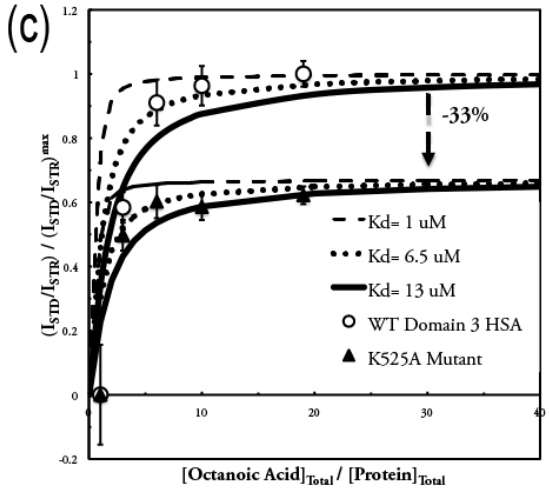

(c)
-33%
Kd= 1 uM
Kd= 6.5 uM
Kd= 13 uM
WT Domain 3 HSA
K525A Mutant
[Octanoic Acid]Total / [Protein]Total

Figure S3

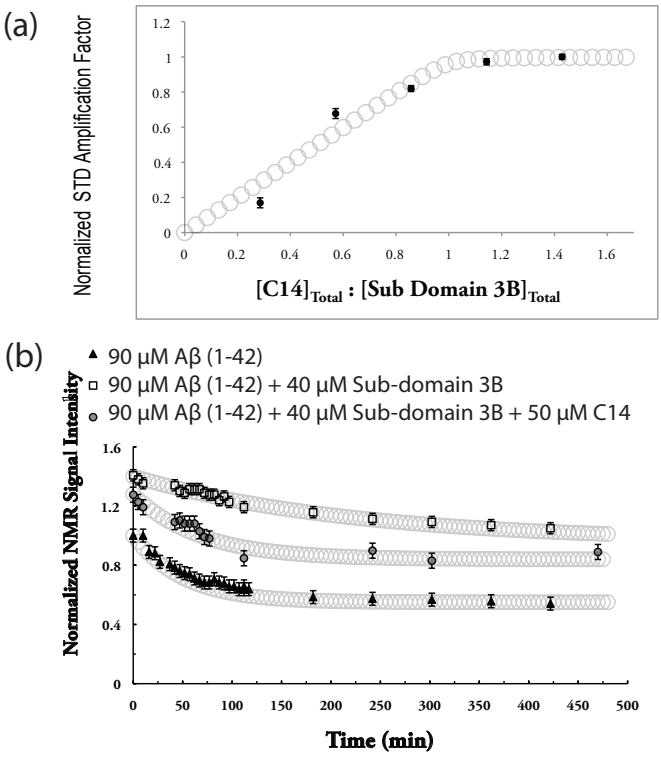

(a)
Normalized STD Amplification Factor
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
[C14]Total : [Sub Domain 3B]Total
(b)
90 μM Aβ (1-42)
90 μM Aβ (1-42) + 40 μM Sub-domain 3B
90 μM Aβ (1-42) + 40 μM Sub-domain 3B + 50 μM C14
Normalized NMR Signal Intensity
1.6
1.2
0.8
0.4
0
0
50
100
150
200
250
300
350
400
450
500
Time (min)

Figure S4

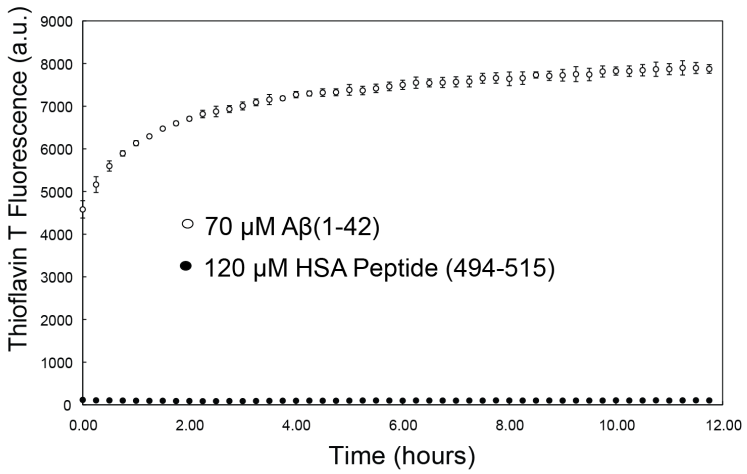

Thioflavin T Fluorescence (a.u.)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.00
2.00
4.00
6.00
8.00
10.00
12.00
Time (hours)
70 μM Aβ(1-42)
120 μM HSA Peptide (494-515)

Figure S5