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Supporting Information
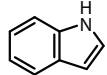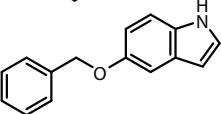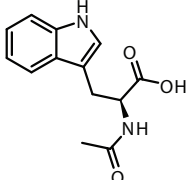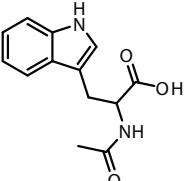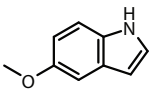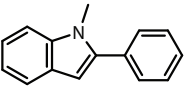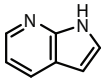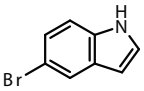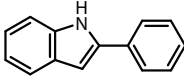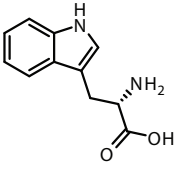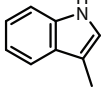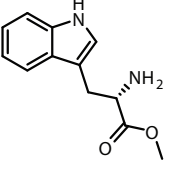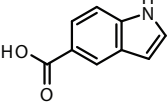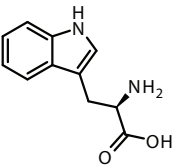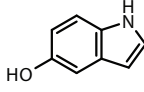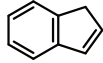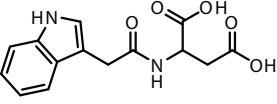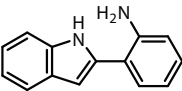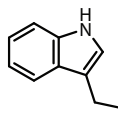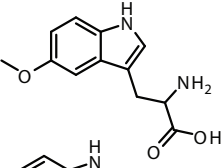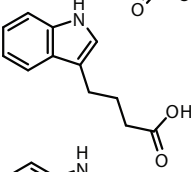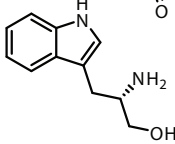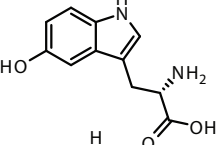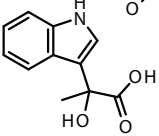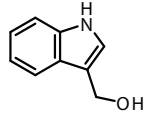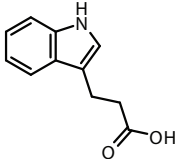
Quantifying Prefibrillar Amyloids in vitro by Using a "Thioflavin-Like" Spectroscopic Method
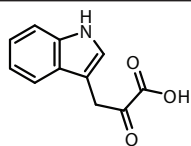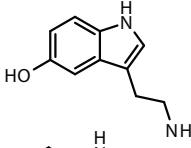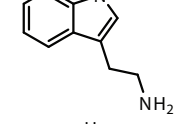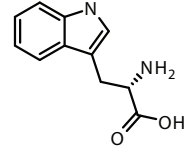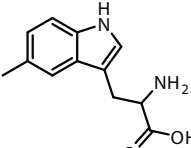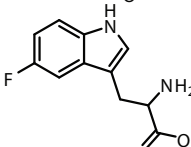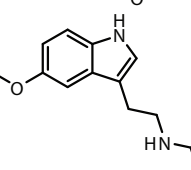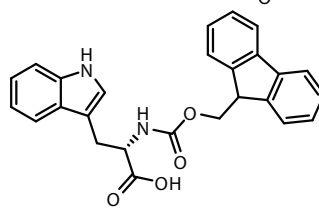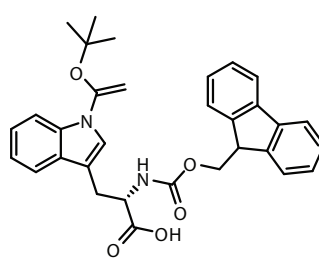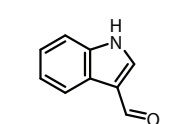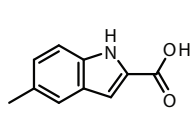
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Supplemental Table 1 Compound names, chemical structures, maximum excitation and emission wavelengths, and the percent change in fluorescence values (%FQ) of the indole library collection.

#	compound name	Ex	Em	chemical structure	% FQ pre-fibrils	% FQ fibrils	pre-fibrillar selectivity
	indole	280	350		9.7	1.5	6.3
1	5-benzyloxyindole	270	330		16.0	2.5	6.4
2	N-acetyl-L-tryptophan	280	360		11.6	2.7	4.3
3	N-acetyl-DL-tryptophan	280	360		11.5	0.9	13.3
4	5-methoxyindole	280	335		11.7	2.6	4.5
5	1-methyl-2-phenylindole	280	375		18.1	15.7	1.2
6	7-azaindole	280	390		11.5	1.0	11.6
7	5-bromoindole	265	340		17.0	5.7	3.0
8	2-phenylindole	310	375		13.3	3.2	4.2
9	tryptophol (2-(1H-indol-3-yl)ethanol)	280	365		11.1	3.7	3.0
10	3-methylindole	280	370		13.7	5.0	2.7
11	L-tryptophan methyl ester	280	355		3.0	1.6	1.9
12	indole-5-carboxylic acid	280	390		24.5	3.2	7.6

#	compound name	Ex	Em	chemical structure	% FQ pre-fibrils	% FQ fibrils	pre-fibrillar selectivity
13	D-tryptophan ((R)-2-amino-3-(1H-indol-3-yl)propanoic acid)	280	360		9.9	1.4	7.3
14	5-hydroxyindole	280	335		8.1	1.7	4.8
15	indene	280	370		13.5	2.7	5.0
16	N-(3-indolylacetyl)-DL-aspartic acid	280	360		11.4	2.4	4.8
17	2-(2-aminophenyl)indole	290	415		14.2	2.7	5.3
18	indole-3-acetamide	280	355		9.2	0.5	18.3
19	1-methylindole	280	350		11.7	1.2	9.7
20	5-methoxy-DL-tryptophan	280	340		9.0	1.4	6.6
21	indole-3-butyric acid	280	370		13.4	2.2	6.1
22	L-tryptophanol ((S)-2-amino-3-(1H-indol-3-yl)propan-1-ol)	280	355		23.8	1.4	17.6
23	5-hydroxy-L-tryptophan	280	340		2.4	1.8	1.3
24	DL-3-indolelactic acid	280	365		18.7	2.2	8.4
25	indole-3-carbinol	280	360		17.4	3.3	5.3
26	3-indole-propionic acid	280	365		16.6	2.8	6.0

#	compound name	Ex	Em	chemical structure	% FQ pre-fibrils	% FQ fibrils	pre-fibrillar selectivity
27	indole-3-pyruvic acid	275	355		13.3	3.2	4.1
28	serotonin (3-(2-aminoethyl)-1H-indol-5-ol)	280	335		14.5	3.4	4.2
29	tryptamine (2-(1H-indol-3-yl)ethan-amine)	280	360		13.4	2.8	4.9
30	L-tryptophan ((S)-2-amino-3-(1H-indol-3-yl)propanoic acid)	280	360		14.9	2.9	5.2
31	5-methyl-DL-tryptophan	280	350		3.1	3.3	1.0
32	5-fluoro-DL-tryptophan	270	330		14.7	3.0	4.9
33	melatonin (N-(2-(5-methoxy-1H-indol-3-yl)ethyl)acet-amide	280	355		2.6	0.6	4.6
34	N-α-Fmoc-L-tryptophan	280	355		10.0	1.5	6.7
35	N-α-Fmoc-N-in-Boc-L-tryptophan	270	315		3.6	1.7	2.1
36	indole-3-carboxaldehyde	270	325		14.4	3.0	4.7
37	5-methylindole-2-carboxylic acid	280	350		8.4	3.1	2.7

(a) pH and buffer analyte

(b) TROL volume

(c) TROL concentration

(d) time of TROL preparation
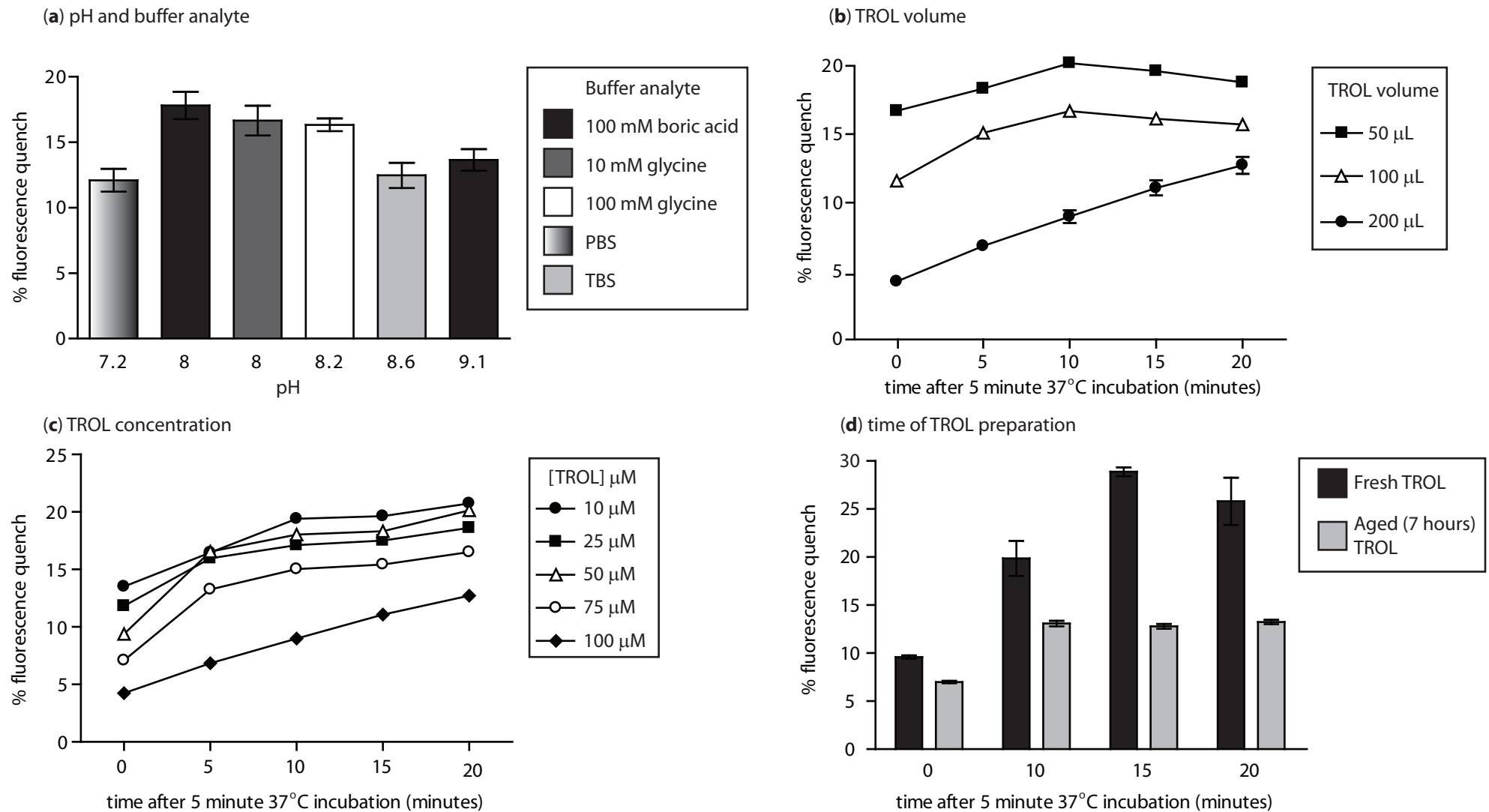
Supplemental Figure 1 Optimizing pH, volume, concentration, and time of preparation of the TROL reagent (**a**) 100 μM TROL was prepared in a variety of buffers at pH values ranging from 7.2-9.1, and 150 μL was added to Aβ pre-fibrils. Samples were incubated for 12 minutes at 37°C followed by 30 minutes at room temperature, after which the fluorescence was recorded relative to TROL alone. The optimized pH range lies between 8-8.2. Although 100 mM boric acid (pH 8) displayed a high signal, we chose a glycine based buffer at pH 8.2 because it is the most commonly accepted buffer for the ThT assay. (**b**) 50, 100, or 200 μL (80 μM) of TROL was added to pre-fibrils. The fluorescence was recorded at 0, 5, 10, 15, and 20 minutes following incubated at 37°C (five minutes). The amount of TROL impacted both the signal and equilibration time. Lower amounts of TROL (100 μL or less) displayed faster signal equilibration as well as optimal reactivity. (**c**) TROL concentration was varied from 10 - 100 μM and the assay was developed as described in (**b**). Simalar to TROL volume, high concentrations (100 μM) displayed lower signal and slower equilibration. Alternatively, at 10 μM, the signal was highest and remained unchanged after approximately 10 minutes. (**d**) To test the impact of the time of TROL preparation on signal, we compared freshly prepared and aged (7 hours) TROL samples. The preparations equilibrated similarly, but aged TROL displayed a >50% lower signal, suggesting TROL should be prepared immediately prior to the start of an experiment for optimal reactivity. For each experiment, 10 μL of 25 μM Aβ (1-42) pre-fibrils were used, all samples were plated in triplicate, and error bars represent the standard deviation. With the exception of (A), 50 mM glycine (pH 8.2) was used for all experiments. Unless otherwise noted, the assay was developed using the protocol in Table 1.
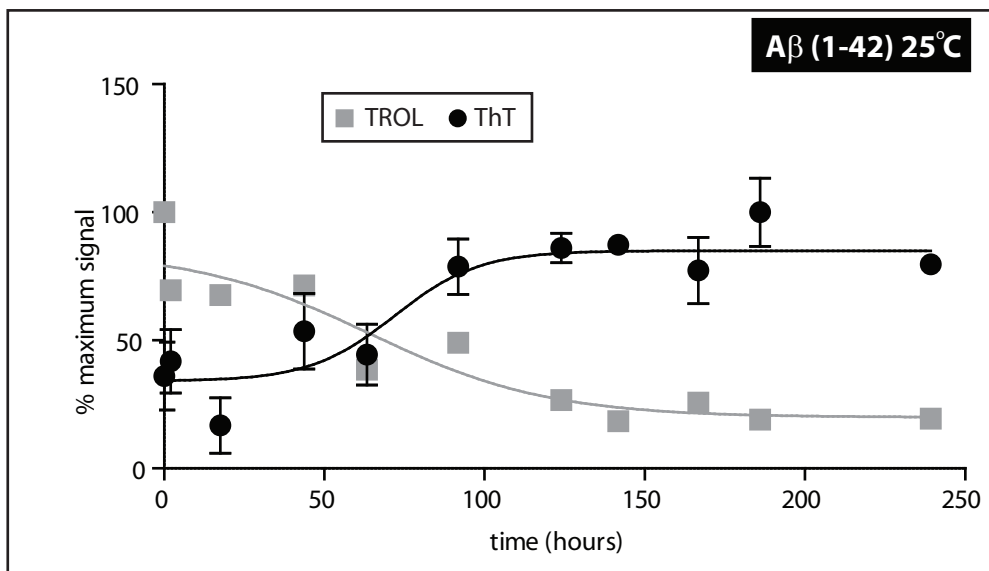
Supplemental Figure 2 Monitoring the disappearance of Aβ (1-42) pre-fibrils using TROL Aβ (1-42) was suspended in PBS at 25 μM and incubated at room temperature without agitation. The TROL and ThT assays were performed as described in the text and Table 1. The results are representative of three individual experiments performed with Aβ (1-42). Error respresents standard deviations.
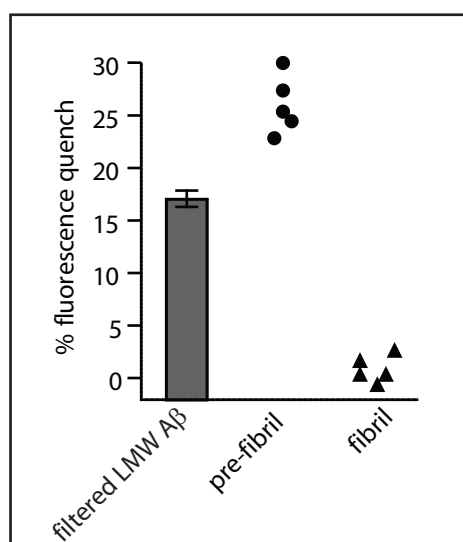
Supplemental Figure 3 Determining TROL signal in the presence of filtered, LMW Aβ (1-40). In order to prepare a predominantly monomeric preparation of Aβ (1-40), we adapted a protocol from previously established methods *. Briefly, one milligram of Aβ (1-40) was suspended in 415 μL 2 mM NaOH + 20 μL 100 mM NaOH. The sample was then vortexed for one minute, sonicated on ice for 15 minutes, and centrifuged through a 10 kDa MWCO filter (Millipore) at 4 C. The filtrate was then diluted 1:1 with 5X PBS (pH 7.4) to adjust the pH of the sample. The sample was then plated in triplicate as described in the Experimental Section and the TROL assay was performed. The concentration of Aβ following this procedure was 33 μM as determined by a standard BCA assay (Pierce). We observed a 15% quench in TROL fluorescence in the presenceof this sample, and this result is shown relative to the pre-fibril and fibril 1-40 samples (data taken from Figure 2B). The resulting filtrate consists of a mixture of monomer, dimer, and trimer, and therefore it is difficult to ascertain whether the fluorescence quench that is observed is due to monomer, or 'contaminating' dimer and trimer.

* Q. Zhang, E. T. Powers, J. Nieva, M. E. Huff, M. A. Dendle, J. Bieschke, C. G. Glabe, A. Eschenmoser, P. Wentworth Jr., R. A. Lerner, J. W. Kelly, *PNAS* **2004**, *101*, 4752-4757.