Supplementary Information

Small molecule conversion of toxic oligomers to non-toxic β-sheet-rich amyloid fibrils

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1. Supplementary Methods

$A\beta_{42}$ peptide stock solutions. Synthetic $A\beta_{1-42}$ ($A\beta_{42}$) peptide produced by the laboratory of Dr. Volkmar-Engert (Institute for Medical Immunology, Charité, Berlin, Germany) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) over night, sonicated for 30 min, aliquoted and then lyophyllized. Monomeric $A\beta_{42}$ solutions (15 µM) for ThT assays, CD spectroscopy and dot blotting were prepared from HFIP-treated peptide by dissolving the peptide in 10 mM NH_4OH, sonification for 30 min followed by membrane filtration (UFV5BTK25, Millipore) as described in[1]. For other assays $A\beta_{42}$ stock solutions (1 mM) were prepared in DMSO, centrifuged in an ultracentrifuge (Beckman Coulter, Optima TL, rotor TLA100.3) at 202,000 x g for 20 min to remove insoluble aggregates and then diluted into assay buffers as described in the text.

Small molecules. A compound library containing ~300 small molecules was utilized for the primary screen. The compounds were identified previously as modulators of mutant huntingtin aggregation by high-throughput screening of a proprietary library of ~180,000 small molecules [2]. Orcein [3] (article no. 107100) was purchased from Merck (Germany) and O4 (Lacmoid, structure **31** in [3]) from Zelinsky Institute Inc., Newark (ZIZ074584) (USA). Compounds used for dot blot, FRA and MTT assays were all purchased from Zelinsky Institute Inc. or Sigma, St Louis. Compound identity was confirmed by GCMS. All compounds were at analytical grade (>95% pure or higher) as determined by HPLC. Compounds were dissolved in DMSO at 10 mM and stored at -20°C.

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Semi-denaturing SDS-PAGE. Samples of aggregation reactions were transferred to a loading buffer containing 50 mM Tris HCl, pH 6.8, 2 % SDS, 10 % (v/v) glycerol, and 0.1 % bromophenol blue. Samples were loaded onto 10 – 20% tris-tricine gels (Biorad, Munich, Germany) without heat denaturation. SDS-PAGE was performed according to a standard protocol. $A\beta_{42}$ distribution in the gels was visualized by silver staining [4] or immunoblotting.

Transmission electron microscopy. EM images were acquired using a Philips CM-100 transmission electron microscope. Aliquots of aggregation reactions were adsorbed to freshly glimmed carbon-coated copper grids and negatively stained using 2% uranyl acetate. Aggregate species of different lengths and morphologies (fibrils, protofibrils, oligomers) were counted. Numbers of aggregate species produced in the presence and absence of O4 were statistically analyzed using a two tailed t - test.

Circular Dichroism. $A\beta_{42}$ peptides (15 µM) were aggregated for 7 d at 37 °C in the presence of freshly dissolved O4. CD spectra were recorded with a 1 mm light path after 15 min, 4 d and 7 d on a J-720 (Jasco, Tokyo, Japan) spectrometer. Solutions containing corresponding amounts of O4 but no $A\beta_{42}$ were incubated in parallel; reference CD spectra were recorded and subtracted from the CD signals to isolate the Aβ-specific changes in CD spectra. For kinetic CD measurements, the change in CD signal over the course of 3,000 s was recorded at 218 nm and integrated over 60

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s intervals. Data were plotted at 120 s intervals at later time points for clarity purposes. Data points represent mean and SD of three measurements.

Dot blot assay. Dot blot assays to detect $A\beta_{42}$ amyloid oligomers were performed as described previously [5]. Briefly, 10 µl aliquots of 15 µM $A\beta_{42}$ aggregation reactions were spotted onto nitrocellulose membranes. Membranes were blocked for 1 h with 10% non-fat milk in TBS. After washing membranes were incubated with the anti-oligomer antibody A11 dissolved in TBS containing 3% BSA and 0.01 % Tween-20 and developed using horseradish peroxidase or alkaline phosphatase conjugated secondary antibodies (Promega, Germany). After detection with the A11 antibody, membranes were stripped and re-developed with the 6E10 antibody to control for equal protein loading. Similar assays were performed with 4G8 and B10 antibodies.

For quantification of O4 binding to membrane spotted $A\beta_{42}$ peptides, preparations of monomeric, oligomeric and fibrillar $A\beta_{42}$ (15 µM) were incubated with O4 for 15 min at RT. Membranes were washed with PBS + 0.05% Tween 20 + 0.1% Triton X-100 over night and compound binding was analyzed colorimetrically. For quantitative analysis O4 signals under saturating conditions were set to 100% and plotted against O4 concentrations on a logarithmic scale. Lines represent fitting by a cooperative dose-response curve y = 1/ (1+10^(LogEC50- log [O4])*n) with a Hill coefficient of n = 4. Membranes were subsequently developed with 6E10 antibody for $A\beta_{42}$ quantification.

For analysis of peptide assays, SPOT peptide membranes were incubated with O4 (300 µM) for 15 min and processed as described above.

Thioflavin T seeding assays. $A\beta_{42}$ fibrils were produced *in vitro* with an equimolar

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O4 concentration and unbound O4 was removed by ultracentrifugation. Fibrils were sonified for 5 min in a water bath sonicator (TK 52, Bandelin). Seeds (1% v/v) were added to monomeric $A\beta_{42}$ peptide (15 µM) and samples were incubated in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) with ThT at 37 °C. ThT fluorescence was recorded from triplicate experiments as described [1] using a fluorescence plate reader (InfinitE M200, Tecan, Austria) with 10 min reading intervals and 5 s shaking before each read. Data points represent mean ± SD, n = 3.

Cellular aggregation assays. 25,000 7PA2 cells per well were seeded in a 96 well plate. Incubation was carried out at 37°C with 5 % (v/v) CO_2. After 1 day preformed $A\beta_{42}$ aggregates (15 µM $A\beta_{42}$, 7d incubation at 37°C, final concentration 0.6 µM) were introduced into the adherent 7PA2 cells as described in [6]. After 6 h cells were rinsed carefully with cell culture media and further incubated for 2 days with 5 µM O4. Quantification of SDS-stable amyloid aggregates was carried out using the filter retardation assay from four independent wells. SDS-stable aggregates were detected by FRA using the monoclonal anti-Aβ antibody 6E10.

Toxicity assays. SH-SY5Y neuroblastoma cells were grown in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. 10,000 cells/well were seeded in a 96 well plate. After 1 d, 1% N2 supplement (Gibko) and 10 µM all-trans retinoic acid (Sigma, Munich, Germany) were added. Cells were differentiated for 4 – 6 d to obtain a neuron-like phenotype. $A\beta_{42}$ (5 mM DMSO stock after HFIP pre-treatment) was added together with test compounds at a final peptide concentration of 5 µM. FCS was replaced by 0.1%

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bovine serum albumin at the day of $A\beta_{42}$ application.

For primary neuron culture, young newborn (P0 – P2) wild-type mice (C57BL6) were decapitated and cortical hemispheres removed. After incubation in digestion medium - DMEM containing papain (Worthington, 40 µg/ml), dispase II (Roche, 2 mg/ml), and DNAse I (Worthington, 150 µg/ml) - for 30 min at 37 °C, the solution was triturated and cells were pelleted and washed twice in PBS. Cells were grown on cover slips coated with laminin and poly-L-lysin. Culture medium consisted of neurobasal medium (Gibco) and supplement B27, 1 mM glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. The different cell types were identified by immunofluorescence microscopy using microglial, astroglial and neuronal markers. We found a neuron content of at least 70% in this preparation. After 3 – 5 d in culture, $A\beta_{42}$ (15 µM) was added to the extracellular medium together with O4 and cells were further incubated at 37 °C. After 48 h, cells were stained using the green fluorescent dye calcein AM (Live/ dead assay, Molecular Probes). Cells were classified as neurons by morphological criteria and quantified. In total, more than 4,000 neurons were counted and data were statistically analyzed using a two-tailed Student's *t*-test.

Preparation of rat brain slices. All experiments were carried out on transverse slices of the rat (males, age 3–4 weeks, weight 40–80 g) hippocampus. Brains were rapidly removed after decapitation and placed in cold oxygenated (95% O_2/5% CO_2) media. Slices were cut at a thickness of 350 µm using an Intracell Plus 1000 and placed in a storage container containing oxygenated medium at 20 °C for 1 h. Slices were then transferred to a recording chamber and continuously superfused. The

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control media contained 120 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$ and 10 mM D-glucose. All solutions contained 100 µM picrotoxin (Sigma) to block $GABA_A$-mediated activity. Synthetic $A\beta_{42}$ (Bachem, Heidelberg, Germany) was prepared as a stock solution of 50 µM in NH4OH (0.1%) and stored at –20° C. It was added to physiological medium immediately prior to each experiment. O4 dissolved in DMSO (10 mM) was added to the $A\beta_{42}$ stock immediately prior to addition of the bath solution. Perfusion with peptide was started 40 min prior to high-frequency stimulation (HFS).

Electrophysiological techniques. Standard electrophysiological techniques were used to record field potentials. Presynaptic stimulation was applied to the medial perforant pathway of the dentate gyrus using a bipolar insulated tungsten wire electrode and field excitatory postsynaptic potentials (EPSPs) were recorded at a control test frequency of 0.033 Hz from the middle one-third of the molecular layer of the dentate gyrus with a glass microelectrode. In each experiment, an input–output curve (afferent stimulus intensity vs. EPSP amplitude) was plotted at the test frequency. For all experiments, the amplitude of the test EPSP was adjusted to one-third of maximum (1.2 mV). LTP was evoked by HFS of eight trains, each consisting of eight stimuli at 200 Hz (intertrain interval 2 s), with the stimulation voltage increased during the HFS so as to evoke an initial EPSP of the train of double the normal test EPSP amplitude. Recordings were analyzed using p-CLAMP (Axon Instruments, CA, USA). Values are means ± SEM for *n* slices. Two-tailed Student's *t*-test was used for statistical comparison.

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Molecular Modeling. For the identification of potential O4 binding sites, a 3D-structure of an $A\beta_{42}$ short fibril fragment was modelled on the basis of a recently proposed β-strand–turn–β-strand conformation [7]. The described packing arrangement differs from the available NMR fibril model (PDB code: 2beg,[8], although large parts of the surface areas in both models are similar. The conformation of the model consists of seven beta-hairpin units. The model was generated with the MODELLER software [9] using the 2beg structure as a template and additional distance constraints derived from the model of Ahmed *et al*. [7]. The subsequent energy minimization was performed with the OPLS2005 force field implemented within the Schrodinger Suite [10]. Docking studies were performed using the Glide flexible docking protocol of Schrodinger (Schrodinger Suite 2010; Glide, version 5.6, Schrodinger, LLC, New York, 2005). An automated docking exploration involved 32 docking runs starting from different points equally distributed on the surface of the central hairpin unit of the fibril model. Three hydrophobic binding sites showed a considerably better docking score than all others scores (glide scores: AA 20-21: -6.05, AA 33-35: -6.12, AA 35-37: -7.45 [11]). The docking models suggest a binding of O4 to $A\beta_{42}$ fibrils and oligomeric fibril precursors with its long axis parallel to that of the fibrils.

It has been proposed by that $A\beta_{42}$ peptides assemble into amyloid fibrils [12]. According to the cross β-sheet structure, the C-terminal sheets of two fibril strands pack against each other and form the mature $A\beta_{42}$ amyloid fibrils. For the modeling of the fibril-O4-complex the starting position of both fibril strands were manually positioned and their energy was minimized in a subsequent step using the OPLS2005 force field [9]. The binding of O4 to G33-M35 followed by a fibril strand association would incorporate O4 into the core.

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NMR spectroscopy.

All solution-state NMR experiments were carried out on a Bruker 600-MHz AVANCE NMR spectrometer equipped with a conventional triple channel probe. The temperature was set to 5 °C in all experiments. $A\beta_{40}$ was solubilized in 10 mM NaOH and diluted into aqueous buffer (50 mM phosphate, pH 7.4). The final $A\beta_{40}$ concentration was 50 µM. Sequential assignments for $A\beta_{40}$ backbone resonances are obtained from triple resonance HNCACB type experiments, and are in agreement with reference [13]. To probe interactions between $A\beta_{40}$ and O4, defined amounts of a DMSO O4 stock solution were added to the $A\beta_{40}$ solution. The same amount of DMSO (1 %) was added to record $A\beta_{40}$ reference spectra.

Analytical Ultracentrifugation. O4 stock solutions in DMSO were diluted into phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4). Buffer solutions were filtrated though a 3 kDa cut-off filter (Millipore) and dye concentrations were calculated from OD 280 measurements. Sedimentation velocities were determined in a Beckman XL-I analytical ultracentrifuge at 20°C and 50 krpm. O4 was detected via Absorption at 280 nm and 498 nm. Sedimentation profiles were evaluated by global fitting of the Lamm equation using the Sedfit v 12.3 software package. A partial specific volume of 0.6 ml/g was assumed for the calculation of molecular masses.

Light scattering. Experiments were performed on a Zetasizer nano (Malvern) in a sealed quartz cuvette using a sample volume of 40 µl. Monomeric $A\beta_{42}$ solutions were obtained by membrane filtration at pH 10.5 as described above (4). $A\beta_{42}$ (15 µM) was incubated in the presence of filtered or unfiltered O4 (75 µM) in phosphate

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buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37℃ for 16 h. Total scatter intensities were recorded over time from triplicate measurements.

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2. Supplementary Results

Supplementary Figure 1: a, Detection of insoluble $A\beta_{42}$ aggregates by filter retardation assay. Peptides were incubated in the presence of test compounds (A and B) or solvent (control) (I and II) and after different time points (e.g. 48 h) samples are denatured in SDS and passed through a cellulose acetate filter (III). Large aggregates, which are resistant against SDS treatment, were retained on the filter membrane and quantified using the Aβ-specific antibody 6E10 (IV). **b**, Structural formulas of the main components of orcein (**1**-**6**). Structures **3** and **5** as well as **4** and **6**, respectively, represent rotamers of the phenyl rings.

Supplementary Figure 2: Aggregation kinetics of $A\beta_{42}$ peptides monitored by FRA. **a,** Quantification of $A\beta_{42}$ aggregation by FRA. $A\beta_{42}$ (15 µM) was incubated in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37°C with an equimolar or 5-fold molar excess of O4; SDS-stable aggregates were detected using the monoclonal anti-Aβ antibody 6E10 and quantified densitometrically (mean ± SD, n = 3). **b**, O4 induces formation of SDS-resistant $A\beta_{42}$ aggregates but does not promote aggregation of APP, HSP70 or HSP90. $A\beta_{42}$, APP, HSP 70, and HSP90 (all 80 µg/mL) were each incubated with 75 µM O4 for 4d in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37°C. Samples were then boiled in 2% SDS and analyzed by filter retardation assay (FRA). O4 staining was detected photometrically, proteins were quantified by anti-His or anti-Aβ staining (mAb 5711), respectively. **c**, **d**, Aggregation kinetics of $A\beta_{42}$ peptides (100 nM) in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4, 0.1% NP40) at 37°C were monitored by FRA using mAb 5711. Aggregates were quantified densitometrically (**d**,

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mean values ± SD, n = 3). **e**, Relative quantification of SDS-resistant $A\beta_{42}$ aggregates formed in 7PA2 cells, n = 4, means ± SD. Preformed $A\beta_{42}$ aggregates (final concentration 0.6 µM) were introduced into cells. Cells were afterwards incubated for 2 d with 5 µM O4. Aggregates were quantified by FRA.

Supplementary Figure 3: O4 binds to $A\beta_{42}$ oligomers and fibrils but not to monomers. $A\beta_{42}$ oligomers (110 µM) were produced *in vitro* (37°C for 24 h) and centrifuged (14,000 rpm); fibrils were produced by incubation of monomeric $A\beta_{42}$ (15 µM) for 7 d at 37°C in PBS. **a**, ThT fluorescence signals (mean values ± SD, n = 3); **b**, atomic force microscopy images; **c,** CD spectra of $A\beta_{42}$ monomers, oligomers, and fibrils. All concentrations were equivalent to 15 µM monomers. **d**, Monomeric, oligomeric and fibrillar $A\beta_{42}$ (15 µM) preparations were incubated with O4 (3, 15 and 75 µM) for 15 min; peptides were dot blotted onto a nitrocellulose membrane, which was subsequently washed over night in PBS (+ 0.1% Tween20 + 0.1% Triton X-100). O4 binding was analyzed colorimetrically, (cf. Fig. 2c). $A\beta_{42}$ peptides spotted onto the membrane were quantified by immunostaining using the 6E10 antibody. **e**, Quantification of relative O4 signals. O4 unspecifically bound to the membrane (buffer control) was subtracted from $A\beta_{42}$ -O4 signals. The average difference in O4 signal between duplicate samples was ~10%. **f,** Monomeric, oligomeric and fibrillar $A\beta_{42}$ (15 µM) preparations were incubated with EGCG or O4 (15 µM) for 15 min; proteins were dot blotted onto a nitrocellulose membrane and stained with nitro tetrazolium blue (NBT, 5). **g**, Monomeric $A\beta_{42}$ (15 µM) was incubated with O4 (3, 15 and 75 µM) for 4 d and the resulting aggregates were subsequently pelleted at 100,000 x g. Aβ pellets were dissolved in 100% TFA over night and then lyophyllized.

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Aβ fractions (*total*, *sup*ernatant, *pellet*, *TFA* dissolved *pellet*) were boiled in 2% SDS and then analyzed by SDS PAGE and silver staining to test for the presence of covalently cross-linked Aβ oligomers. Only monomeric $A\beta_{42}$ (m) was detected.

Supplementary Figure 4: Quantification of O4 binding to $A\beta_{42}$ aggregates and other control proteins. **a**, Preparations with oligomeric $A\beta_{42}$ peptides (6 µM) were incubated with O4 (0.4 – 200 µM) for 15 min at RT. Samples were blotted onto a nitrocellulose membrane and membranes were washed over night with PBS + 0.05% Tween 20 + 0.1% Triton X-100. Compound binding to oligomers was quantified densitometrically. $A\beta_{42}$ peptides bound to filter membranes were subsequently also detected by immunostaining using the 6E10 antibody. Equivalent amounts of O4 in the absence of $A\beta_{42}$ peptides were blotted in control samples and unspecific membrane staining was subtracted for quantitative binding analysis. **b**, O4 binds to $A\beta_{42}$ oligomers but not to APP, HSP70 or HSP90 control proteins. Proteins were expressed in *E. coli* and purified via Ni-NTA affinity columns. $A\beta_{42}$, APP, HSP70 and HSP90 (all 80 µg/ml) samples were each incubated with 75 µM O4 for 4d in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37°C. Samples were then directly blotted on nitrocellulose membranes. O4 staining was detected photometrically, proteins were detected by anti-His or anti-Aβ immunostaining (mAb 5711), respectively.

Supplementary Figure 5: O4 binding specificity. **a**, Aβ peptide sequences in SPOT peptide arrays. Peptide numbers represent the starting amino acid of 10-mer

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peptides. Each peptide sequence is shifted by one amino acid towards the C-terminus of the $A\beta_{42}$ sequence. **b**, Dot blot analysis of O4 binding to Aβ 16-22 and a scrambled version of this peptide. O4 binding to peptides was detected colorimetrically; in control experiments peptides were stained with the unspecific dye amido black. **c**, Quantification of relative O4 and amido black staining in dot blot analysis of O4 binding to Aβ 16-22 and a scrambled version of this peptide. O4 binding to peptides was detected colorimetrically; means ± SD, n = 4.

Supplementary Figure 6: Removal of aggregated O4 by filtration does not alter O4 activity.

a, Analysis of aqueous O4 solutions (75 µM) by analytical ultracentrifugation revealed that ~35% of O4 molecules are aggregated; 25% of O4 molecules have a size of 10 – 20 kDa while 5-10% of O4 molecules are even larger compound aggregates. Aggregated O4 was removed by membrane filtration (3 kDa, Millipore), which resulted in a solution with >90 % monomeric O4. **b**, $A\beta_{42}$ (15 µM) was incubated in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37°C with a 2-fold molar excess of filtered O4; Formation of SDS-stable aggregates was detected by FRA using the monoclonal anti-Aβ antibody 5711. **c**, $A\beta_{42}$ (15 µM) aggregation kinetics were recorded by light scattering in the presence or absence of filtered O4 (30 µM), mean ± SD, n = 3. **d,** The chemical composition of filtered O4 solutions was investigated by 1H-NMR. No difference was observed between spectra of filtered and unfiltered material.

Supplementary Figure 7: O4-mediated acceleration of $A\beta_{42}$ fibrillogenesis correlates

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with a reduction of toxicity in cell model systems. **a**, $A\beta_{42}$ (5 µM) was added in the presence and absence of the compounds O4 or O8 to PC12 cells. The compound O8 in contrast to O4 does not promote $A\beta_{42}$ fibrillogenesis *in vitro* (Supplementary Table 2). Metabolic activities were determined by MTT reduction. Unlike O4, O8 did not rescue $A\beta_{42}$ toxicity. **b**, O4 treatment reduces $A\beta_{42}$-mediated toxicity in a primary cell culture model system. Cortical neurons prepared from mice were incubated for 48 h with $A\beta_{42}$ peptides (15 µM) and O4 (7.5 µM) and stained with the green fluorescent dye calcein AM. Representative images of O4-treated and untreated cells are shown. **c**, Quantification of O4 and $A\beta_{42}$ treated primary cortical neurons. More than 4,000 neurons were counted for statistical data analysis. Columns indicate mean values plus SEM normalized to untreated control neurons. Statistical significance was calculated using Student's two-sided t-test. **d**, Uptake of fluorescently labelled $A\beta_{42}$ into neuroblastoma (SH-EP) cells leads to intracellular $A\beta_{42}$ aggregate formation. $A\beta_{42}$-Alexa633 (200 nM) was added to cell culture medium in the presence or absence of O4 (100 nM). Cell nuclei were stained with Hoechst dye. Aβ uptake was quantified after 1 d in a high content screening fluorescence microscope (VTI Arrayscan, Cellomics). **e**, O4 treatment reduces uptake of fluorescently labelled $A\beta_{42}$-A633 into neuroblastoma SH-EP cells. $A\beta_{42}$ (200 nM) was added to cell culture medium in the presence or absence of O4 (1 µM). $A\beta_{42}$ uptake was quantified after 1 d by fluorescence microscopy.

Supplementary Table 1: Orcein related compounds and their effects on $A\beta_{42}$ aggregation. Compounds O1 – O18 were incubated with $A\beta_{42}$ as described in Supplementary Fig. 1. The amounts of SDS-resistant aggregates were quantified by

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FRA. 0 = no effect on Aβ aggregation, (+) = weak promotion of aggregation, ++ = strong promotion of aggregation.

Supplementary Table 2: Predicted binding affinities of compounds and effects on $A\beta_{42}$ aggregate formation. Assembly of SDS-stable $A\beta_{42}$ aggregates was quantified by FRA. $A\beta_{42}$ (15 μM) was incubated for 1 d in phosphate buffer (100 mM sodium phosphate, 10 mM NaCl, pH 7.4) at 37°C with fivefold molar excess of chemical compounds; SDS-stable aggregates were detected using the monoclonal anti-Aβ antibody 6E10.

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Supplementary References:

1. Bieschke, J., Zhang, Q., Powers, E.T., Lerner, R.A. & Kelly, J.W. Oxidative metabolites accelerate Alzheimer's amyloidogenesis by a two-step mechanism, eliminating the requirement for nucleation. *Biochemistry* **44**, 4977-83 (2005).
2. Heiser, V. et al. Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc Natl Acad Sci U S A* **99 Suppl 4**, 16400-6 (2002).
3. Beecken, H., Gottschalk, E.-M. v. Gizycki, U. Kramer, H., Maassen, D., Matthies, H. G., Musso, H., Rathjen, C., Zahorsky, U.I. Orcein und Lackmus. *Angewandte Chemie* **73**, 665 -688 (1967).
4. Oakley, B.R., Kirsch, D.R. & Morris, N.R. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal Biochem* **105**, 361-3 (1980).
5. Ma, Q.L. et al. Antibodies against beta-amyloid reduce Abeta oligomers, glycogen synthase kinase-3beta activation and tau phosphorylation in vivo and in vitro. *J Neurosci Res* **83**, 374-84 (2006).
6. Bieschke, J. et al. EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. *Proc Natl Acad Sci U S A* **107**, 7710-5 (2010).
7. Ahmed, M. et al. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat Struct Mol Biol* **17**, 561-7 (2010).
8. Luhrs, T. et al. 3D structure of Alzheimer's amyloid-beta(1-42) fibrils. *Proc Natl Acad Sci U S A* **102**, 17342-7 (2005).
9. Eswar, N. et al. Comparative protein structure modeling using Modeller. *Curr Protoc Bioinformatics* **Chapter 5**, Unit 5 6 (2006).

Nature Chemical Biology: doi:10.1038/nchembio.719

10. Jorgensen, W.L. & Tirado-Rives, J. *J Am Chem Soc* **110**, 1657 - 1666 (1988).

11. Friesner, R.A. et al. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem* **49**, 6177-96 (2006).

12. Sato, T. et al. Inhibitors of amyloid toxicity based on beta-sheet packing of Abeta40 and Abeta42. *Biochemistry* **45**, 5503-16 (2006).

13. Yan, Y. & Wang, C. Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. *J Mol Biol* **364**, 853-62 (2006).

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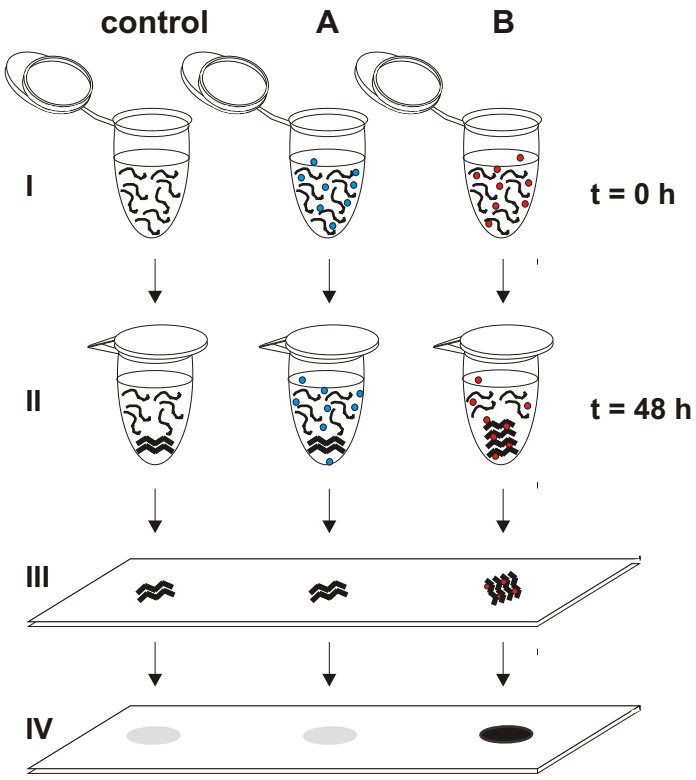
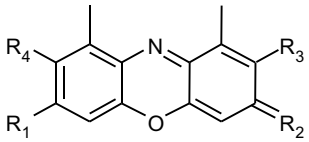
Supplementary Figure 1

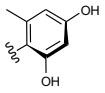
a

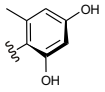


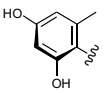
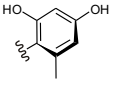
b

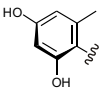
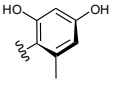


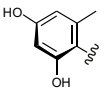
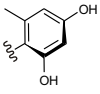
'Orcein'

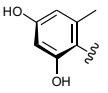
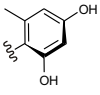
1: $R_1 = NH_2$, $R_2 = O$, $R_3 = H$, R_4 =  Alpha-amino-orcein

2: $R_1 = OH$, $R_2 = O$, $R_3 = H$, R_4 =  Alpha-hydroxy-orcein

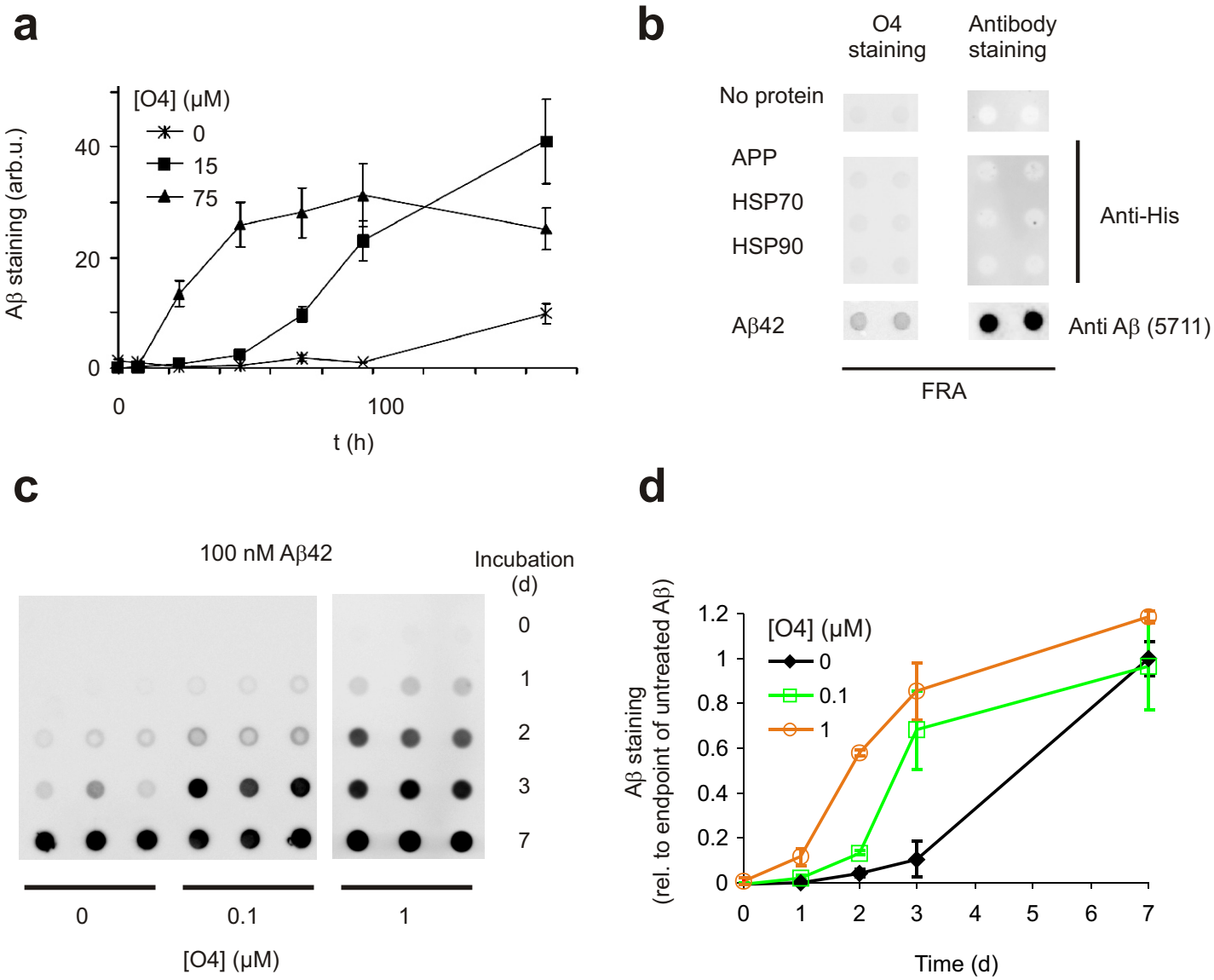
3: $R_1 = NH_2$, $R_2 = O$, R_3 =  , R_4 =  Beta-amino-orcein

4: $R_1 = NH_2$, $R_2 = N$, R_3 =  , R_4 =  Beta-amino-orceimine

5: $R_1 = NH_2$, $R_2 = O$, R_3 =  , R_4 =  Gamma-amino-orcein

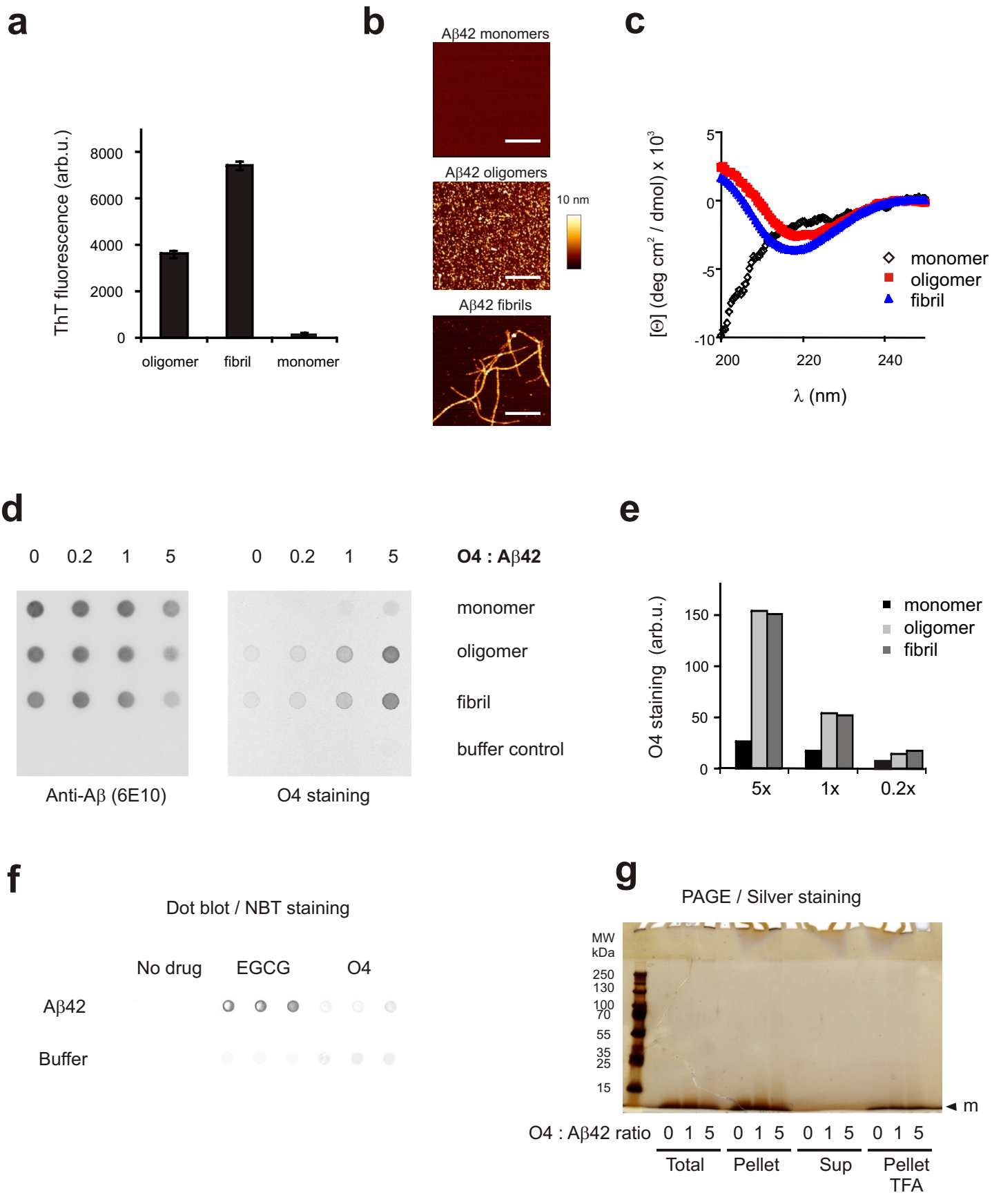
6: $R_1 = NH_2$, $R_2 = N$, R_3 =  , R_4 =  Gamma-amino-orceimine

Supplementary Figure 2



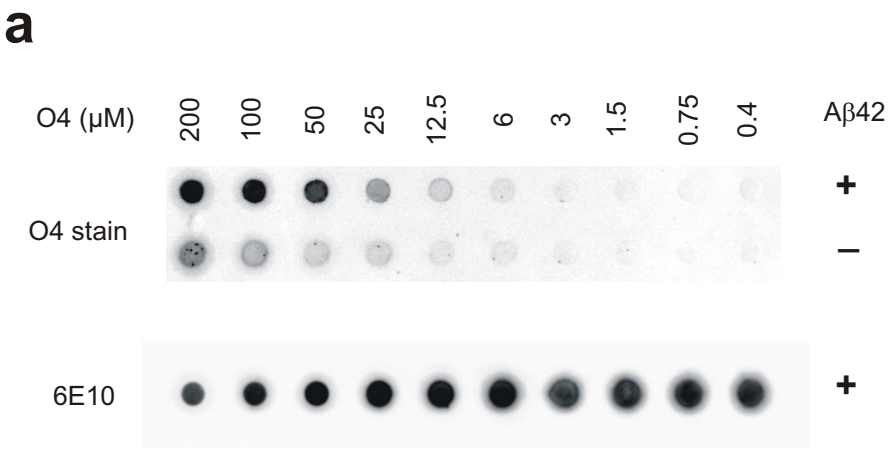
Nature Chemical Biology: doi:10.1038/nchembio.719

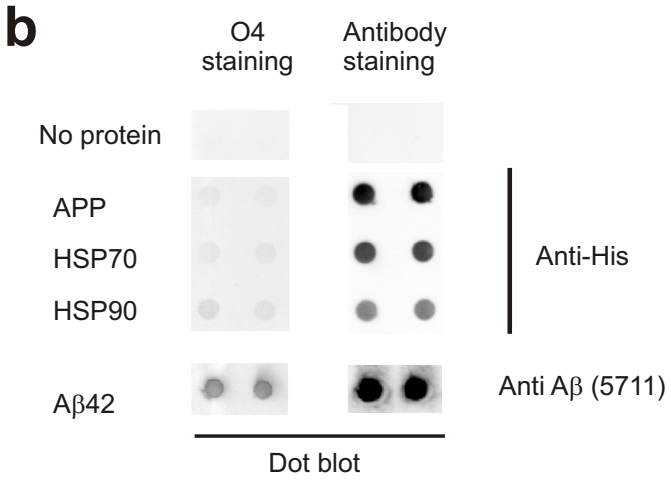
Supplementary Figure 3



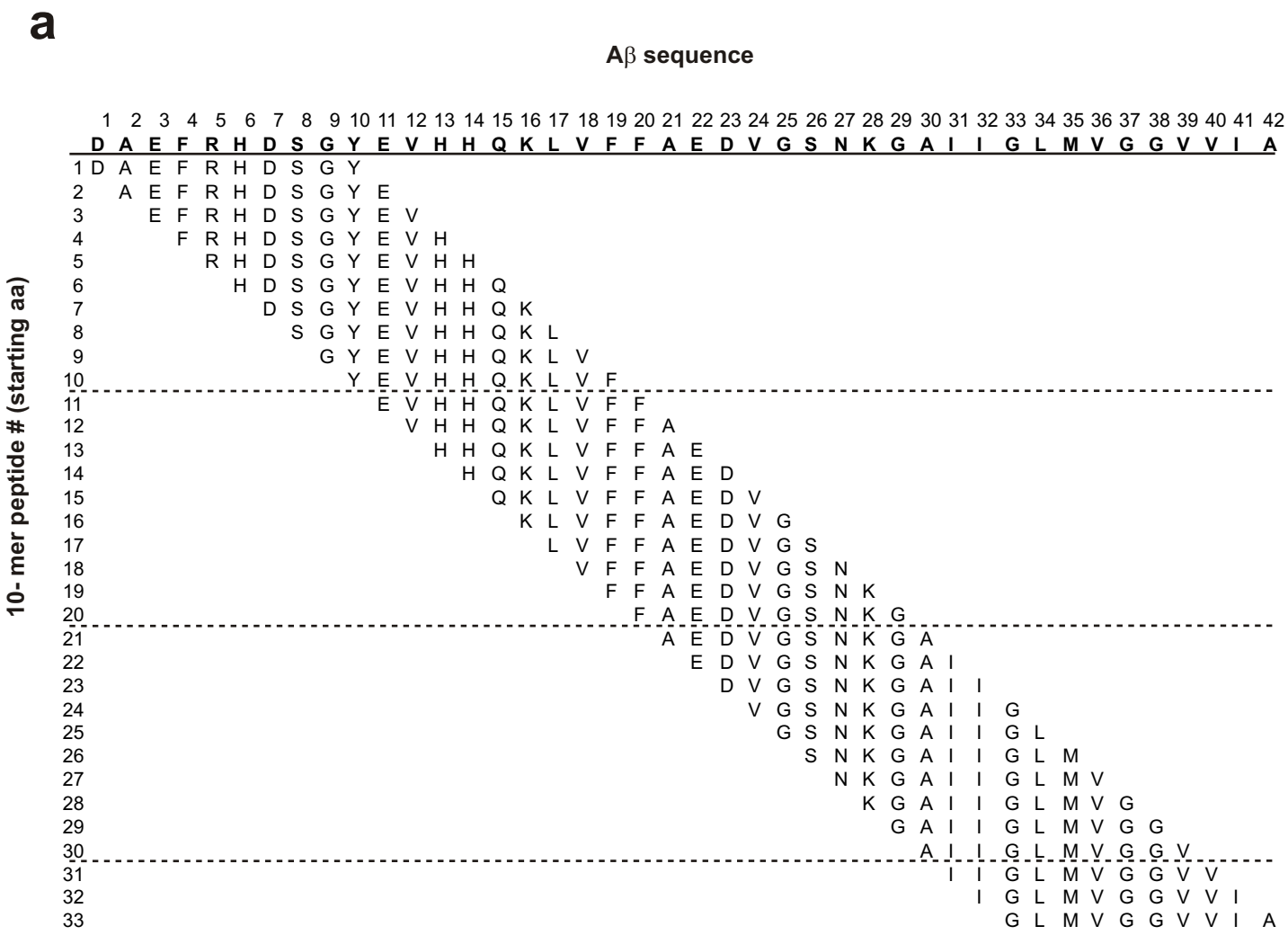
Nature Chemical Biology: doi:10.1038/nchembio.719

Supplementary Figure 4

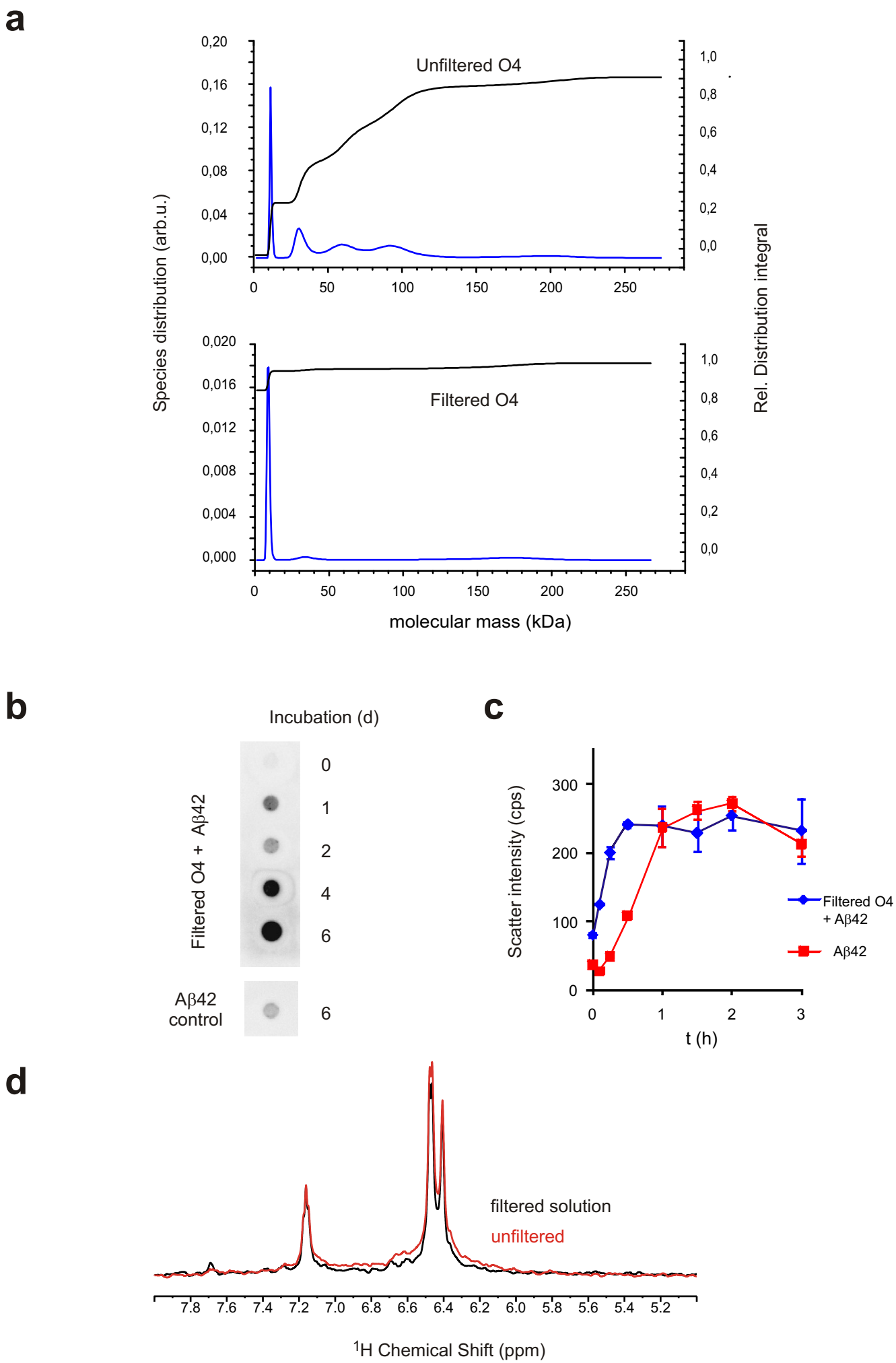




Supplementary Figure 5

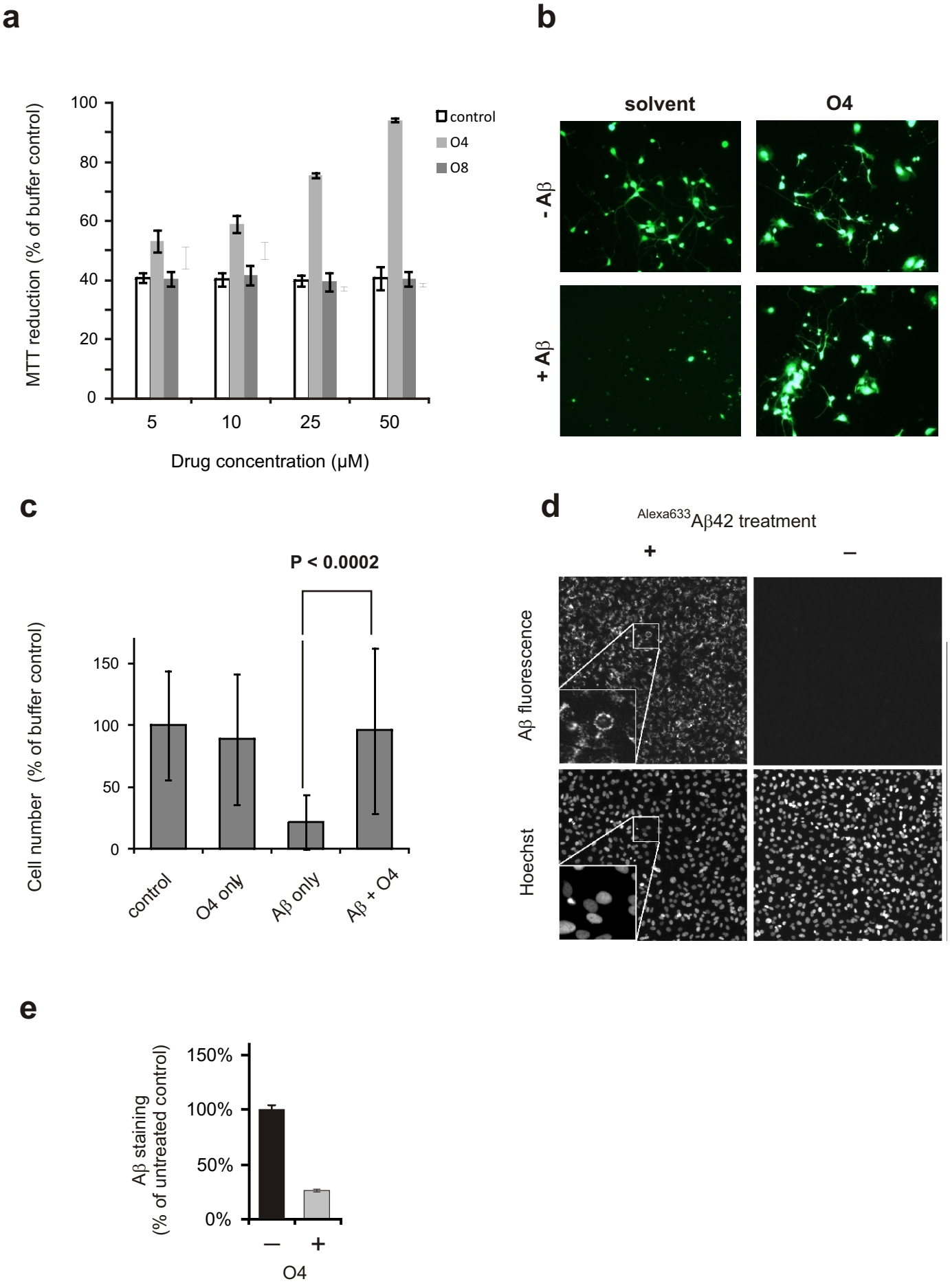


Supplementary Figure 6



Nature Chemical Biology: doi:10.1038/nchembio.719

Supplementary Figure 7



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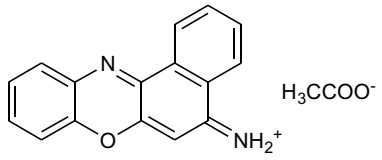
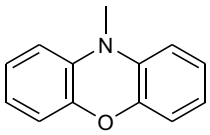
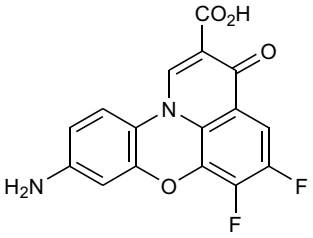
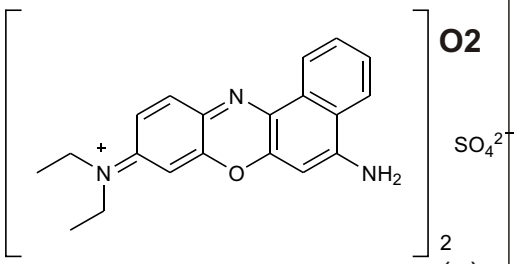
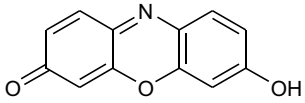
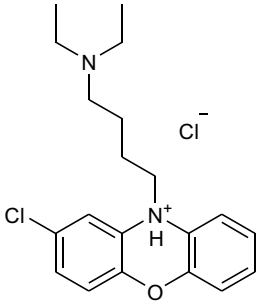
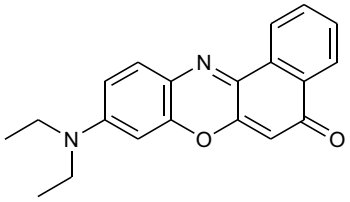
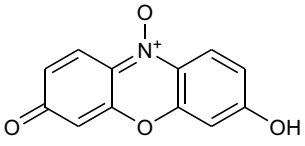
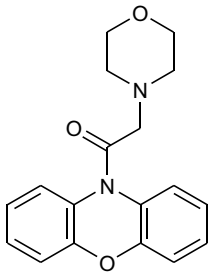
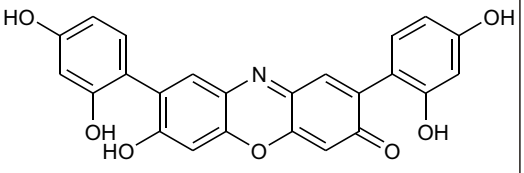
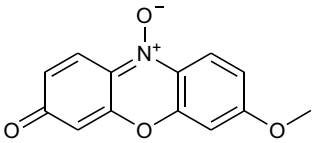
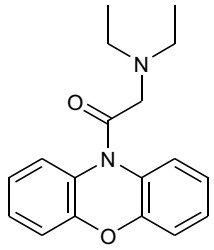
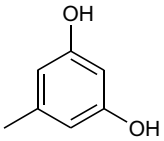
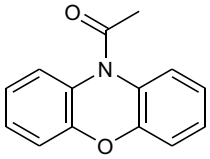
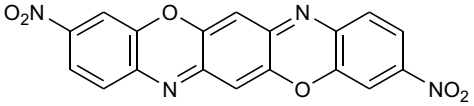
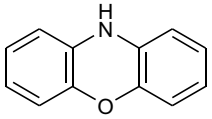
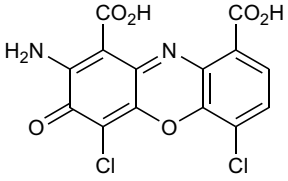
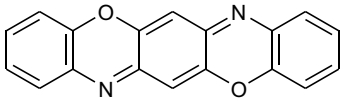
Supplementary Table 1

Effect on aggregation: ++ strong promotion; (+) weak promotion; 0 no effect

O1  0	**O7**  0	**O13**  0
 **O2** (+)	**O8**  0	**O14**  (+)
O3  0	**O9**  0	**O15**  0
O4  ++	**O10**  0	**O16**  0
O5  0	**O11**  0	**O17**  0
O6  0	**O12**  (+)	**O18**  0

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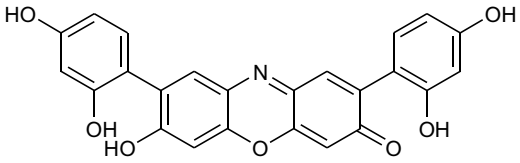
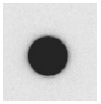
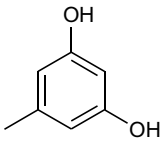
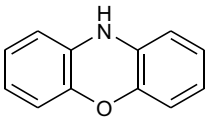
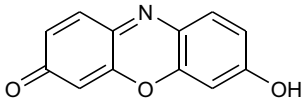
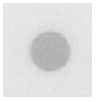
Supplementary Table 2

Compound	Glide Score AA 20-21	Glide Score AA 33-35	Glide Score AA 35-37	dot blot staining	FRA (mAb 6E10)
O4 	**-6,361**	**-6,713**	**-7,905**		
O5 5-methylbenzene -1,3-diol 	**-6,588**	**-5,247**	**-6,731**	*	
O6 Phenoxazine 	**-4,302**	**-5,851**	**-5,468**	*	
O8 7-hydroxy-phenoxazin-3-one 	**-5,8**	**-6,197**	**-6,306**		
control					

* weak or no VIS absorption