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Supplemental Methods

Cloning of expression vectors

For the construction of plasmids encoding CyPet- and YPet-tagged HTTEx1Q48 fusion proteins, the coding sequence of HTTEx1Q48 was PCR-amplified from pGEX-6P1-HTTEx1Q48 using the primers 5'-gacgacgaattcatggcgaccctg-3' and 5'-gacgacctcgagtggtcggtgcagcgg-3'. The resulting PCR product was digested with the restriction enzymes EcoRI and NotI. Additionally, CyPet cDNA was PCR amplified from pBAD33-CyPet-His (Addgene plasmid #14030) [1] with the primers 5'-acgacctcgagggtggcggtggcggtatgtctaaaggtgaagaattattcgg-3' and 5'-gacgacgcggccgcttatttgtacaattcatccataccatg-3'. YPet cDNA was amplified from pBAD33-YPet-His (Addgene plasmid #14031) [1] with the primers 5'-gacgacctcgagggtggcggtggcggtatgtctaaaggtgaagaattattcactgg-3' and 5'-gacgacgcggccgcttatttgtacaattcattcataccctcg-3'. The resulting PCR fragments were cloned into the plasmids pGEX-6P1 using the EcoRI/XhoI/NotI restriction sites to obtain the plasmids pGEX-6P1-HTTEx1Q48-CyPet and -YPet, respectively.

Proteins, antibodies and chemical compounds

The proteins GST-Ex1Q49, GST-Ex1Q48-CyPet and -YPet were produced in *E. coli* BL21-CodonPlus-RP and purified under native conditions by affinity chromatography on glutathione agarose beads as described [2]. Purified proteins were dialyzed over night at 4 °C against 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA and 5% glycerol, shock-frozen in liquid nitrogen and stored at -80°C. Protein concentrations were determined with a NanoDrop spectrophotometer. Before aggregation experiments were performed, proteins were ultracentrifuged (Beckman Coulter, Optima TL, rotor TLA100.3) at 208,000 g for 20 min at 4°C. The polyclonal anti-HTT antibodies CAG53b and HD1 have been described previously [3]. The monoclonal antibodies MW1 and MW8 [4] were obtained from the

Developmental Studies Hybridoma Bank (University of Iowa). The antibody 3B5H10 [5] was purchased from Sigma (Germany). The anti-GFP antibody (ab290) was purchased from Abcam. The compound O4 (ZIZ074584) was purchased from Zelinsky Institute Inc., Newark (USA). Stock solutions of the compound (20 mM) were prepared in DMSO and stored at -20°C.

Filter retardation assays (FRAs)

FRAs were performed as described previously [6]. Briefly, equal volumes of 500 ng of Ex1Q49 aggregation reactions and 4% sodium dodecyl sulfate (SDS) solution containing 100 mM dithiothreitol (DTT) were mixed and boiled at 96°C for 5 min. Samples were filtered through a cellulose acetate membrane with 0.2 µm pores (OE66, Schleicher and Schuell, Germany) and washed twice with 100 µl 0.2% SDS. Membranes were blocked in Tris-buffered saline containing 5% skim milk and 0.05% Tween 20. Aggregates retained on the filter membrane were detected using the CAG53b antibody (1:2000), HD1 antibody (1: 10,000) or anti-GFP antibody (1:5000) and secondary antibodies conjugated to alkaline phosphatase or peroxidase (Promega, Germany). Signals were quantified using the AIDA image analysis software (Raytest, Straubenhardt, Germany).

SDS-PAGE and immunoblotting

Samples of aggregation reactions were mixed with loading buffer [50 mM Tris HCl, pH 6.8, 2% SDS, 10% (v/v) glycerol and 0.1% bromophenol blue] and boiled at 96°C for 5 min. Samples were loaded onto 10% SDS gels. SDS-PAGE and Western blotting were performed according to a standard protocol. Membranes were blocked with 5% skim milk in Tris-buffered saline (TBS) containing 0.05% Tween 20 and incubated with the HD1 (1:2000) antibody and secondary antibodies conjugated to alkaline phosphatase (Promega, Germany). Signals were quantified using the AIDA image analysis software (Raytest, Straubenhardt, Germany).

Dot blot assays (DBAs)

To detect proteins under non-denaturing conditions DBAs were performed as described previously [7]. Briefly, 250 ng of Ex1Q49 aggregation reactions were spotted onto nitrocellulose membranes. Membranes were blocked for 30 min with 5% skim milk in TBS containing 0.05% Tween 20. Membranes were then incubated with the monoclonal antibodies MW1, 3B5H10 or MW8 dissolved in 5% skim milk in TBS containing 0.05% Tween 20 (MW1 1:2000; MW8 1:2000 and 3B5H10 1:5000) and developed using alkaline phosphatase conjugated secondary antibodies (Promega, Germany). Signals were quantified using the AIDA image analysis software (Raytest, Straubenhardt, Germany).

Dynamic light scattering (DLS) measurements

The size of aggregates was measured at 20°C without agitation by dynamic light scattering (Malvern Nano Zetasizer ZS, with a green laser λ=532 nm, and backscattered detection at an angle θ=173 deg). The GST-Ex1Q49 fusion protein was centrifuged (208,000 g and 4°C) for 20 min prior to investigation by DLS (Beckman Coulter, Optima TL, rotor TLA100.3). To remove potential dust, samples were additionally centrifuged at 4°C using a table-top centrifuge (18,000 rpm) prior to the transfer to a quartz cuvette for light scattering measurements. Due to the high measurement noise with DLS at 2 µM GST-Ex1Q49 all measurements were performed using 20.9 µM GST-Ex1Q49 and correspondingly higher PP concentration. O4 was diluted to a final concentration of 20.9 µM.

Dynamic light scattering yields the normalized time autocorrelation function of the intensity of the scattered light $g^{(2)}(\tau)$

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2} \quad \text{(S1)}$$

Each correlation spectrum is the average of 10 traces à 3 s each. The size distribution by intensity has been derived by deconvolution of the autocorrelation function computed using the CONTIN algorithm as implemented in the Malvern software.

The z-averaged diffusion coefficient D_z of the aggregates in solution was computed as described in [8]. Briefly, the autocorrelation function is expanded in terms of the moments around the mean of the decay rate distribution. An expansion up to order two in τ gives the approximation

$$g^{(2)}(\tau) \approx B + \beta \exp(-2\,\mu_1)\left(1 + \frac{\mu_2 \tau^2}{2!}\right)^2 \qquad \text{(S2)}$$

Where μ_i is *i*-th moment around the mean, β a factor that depends on the experimental geometry and *B* the baseline, which can differ from its theoretical value of 1 due to noise. The first moment relates to the z-averaged diffusion constant

$$D_z = \mu_1/q^2\,, with\ q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right). \qquad \text{(S3)}$$

For our setup and a refractive index $n = 1.333$ the scattering wave vector was $q = 31.4281\ \mu m^{-1}$. Equation S2 was fitted to the autocorrelation data, using the non-linear fitting routine lsqnonlin from Matlab to obtain μ_1, μ_2, *B* and β. The z-averaged diameter $2R_z$ is calculated from the Einstein-Stokes equation

$$2R_z = \frac{kT}{3\pi\,\eta\,D_z}. \qquad \text{(S4)}$$

Where *k* is the Boltzmann-constant, *T* the temperature, and the viscosity η = 1.03424 cP.

Atomic Force Microscopy (AFM)

Aliquots of ~500 ng of Ex1Q49 aggregation reactions, pre-cleaved for 3 h at 5°C (300 rpm) before initiation of aggregation at 20°C, were spotted onto freshly cleaved mica and allowed to adhere for 10 min. Then, they were washed 4 times with 40 µl distilled water. The samples were dried to completion at room temperature and imaged in air with a digital multimode NanoscopeIII scanning probe microscope operating in tapping mode.

Size-exclusion chromatography

Gel-filtration experiments were performed with the Äkta purifier system (Amersham Pharmacia) using a Superdex 30/100 GL column. Proteins were eluted in TBS buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.01 mM EDTA). All experiments were carried out at 4°C and a flow rate of 300 µl min^{-1}. The column was calibrated under the same conditions with ribonuclease A (13.7 kDa), ovalbumin (43 kDa), conalbumin (75 kDa), aldolase (158 kDa), ferritin (440 kDa) and thyroglobulin (669 kDa). The exclusion volume was determined with blue Dextran (2,000 kDa).

Protein precipitation

Proteins were concentrated by TCA precipitation. TCA was added to a final concentration of 13% to the protein solution. Proteins were incubated at -20°C for 5 min and 15 min at 4°C and then centrifuged for 15 min at 13,500 rpm in a Hettich Micro 22R centrifuge at 4°C. Pellets were dried and resuspended in loading buffer (50 mM Tris HCl, pH 6.8, 2% SDS, 10% (v/v) glycerol, and 0.1% bromophenol blue).

FRET-based HTT aggregation assay

The aggregation of GST-Ex1Q48-CyPet and GST-Ex1Q48-YPet was performed at indicated concentrations (using an equimolar ratio of both sensor proteins) in 50 mM Tris-HCl pH 7.4,

150 mM NaCl, 1 mM EDTA and 1 mM DTT. Spontaneous aggregation was initiated by addition of 14 U PreScission protease (GE Healthcare) per nmol purified GST-Ex1Q48-CyPet/YPet fusion protein. The solution was mixed with preformed sonicated Ex1Q49 aggregates (seeds) at varying concentrations and transferred to a black 384-well plate. Fluorescence signals were measured every 20 min following a 5 s pulse of vertical shaking with a Tecan M200 fluorescence plate reader at 25 °C for up to 50 h. CyPet donor fluorescence was measured at excitation (Ex): 435 nm/emission (Em): 475 nm; YPet acceptor fluorescence at Ex: 500 nm/Em: 530 nm; the FRET channel (*DA*) was recorded at Ex: 435 nm/Em: 530 nm. Fluorescence signals in all channels were background corrected by subtracting the background fluorescence of unlabelled Ex1Q48. To calculate the sensitized emission, signals in the FRET channel were corrected for donor bleed-through (*cD*) and acceptor cross excitation (*cA*) using donor- and acceptor-only samples. Finally, sensitized emission was normalized to the acceptor signals. In brief, the FRET efficiency *E* (in %) was calculated as follows: $E = (DA - cD * DD - cA * AA) / AA$ with *DD* = donor channel signal and *AA* = acceptor channel signal.

Computational details for docking studies and molecular dynamics simulations

The crystal structure of the non-pathogenic Ex1Q17 fragment was reported by Kim et al [9]. However, less information is available concerning the structural properties of HTT peptides with pathogenic polyQ tracts. Here, the Ex1Q47 structure of the HTT peptide with flanking regions and a glutamine repeat length of 47 computed by Dokholyan [10] was used as a starting point for our calculations. Like the very similar Ex1Q49 fragment used in our experiments, Ex1Q47 provides a reliable model to investigate the effect of O4 in HTT peptides with a pathogenic polyQ expansion.

For the docking calculations two grids were used: the flexible grid **A** with a size of 48x46x48 Å that includes Ex1Q47 in its full extension and grid **D** with a size 38x28x30 Å that comprises the N-terminal region (residues Gln25 to Gln37, Gln43 to Gln53, Pro70 to Gln76 and Leu88 to Pro100) of Ex1Q47. Additional tests with other grid sizes or rigid docking corroborated the results from **A** and **D.** Based on the docking affinities and the geometrical similarity between the resulting Ex1Q47-O4 structures, the best scored cases from both grids were considered for further simulations. Since the docking affinities only provide a preliminary overview of the binding sites, we also performed Quantum Mechanics/Molecular Mechanics Molecular Dynamics simulations (QM/MM MD, 400 ps, time step of 1 fs). The QM region (O4 molecule) was treated using the SCC-DFTB method including dispersion corrections (SCC-DFTB-D) [11] [12], the CHARMM22 force field [13] to describe the protein and the TIP3P model [14] for water. The QM/MM MD simulations allowed the evaluation at the QM level of the relative stability of O4 in different Ex1Q47 regions and were performed following a work methodology described by us elsewhere [15]. The VMD program [16] was used for visualizing all the MD trajectories.

Classical MD simulations were also performed using the NAMD program [17] for several starting structures during 200 ns in each case. The O4 parameters were generated by the Swissparam server [18] and their quality verified by comparison with QM and QM/MM MD

calculations. For the cluster analysis the Gromos method [19] as implemented in the Gromacs program (v4.5.5) [20] was employed.

Kinetic modeling of Ex1Q49 aggregation

Each model describes an aggregation mechanism and is given by a set of ordinary differential equations (ODEs).

PreScission protease-mediated protein cleavage

Since we do not observe Ex1Q49 aggregation in the absence of the PreScission protease (PP) (Fig. 1), we here assume that only Ex1Q49 molecules without GST can aggregate (Fig. 3a). We described the cleavage of GST from GST-Ex1Q49 by PP using different kinetics and found the best description by using a Michaelis-Menten kinetics:

$$\dot{X}_g = -v_P = -v_{max}\frac{X_g}{X_g+K_P}\,. \qquad \text{(S5)}$$

Here, X_g is the concentration of GST-Ex1Q49, K_P the Michaelis-Menten constant, and v_{max} the maximal velocity. In the experiments, the amount of PP is increased proportionally to the initial concentration of GST-Ex1Q49, thus $v_{max} = k_r X_g(0)$. The parameters are fitted to PP digestion data for two initial concentrations of GST-Ex1Q49 (2 μM and 20 μM) as detected with HD1 (Fig. S4b) using the relative immunoreactivity to protein conversion for HD1 as described in Fitting of HTTex1 kinetic models to data. We obtained K_P = 4.21 μM and k_r = 1.99/h.

Primary nucleation and templated polymerization

Fibril formation often involves a nucleation-dependent polymerization reaction where the formation of growth nuclei from soluble proteins is slow [21-23]. We denote this initial step primary nucleation. The binding of monomers to a nucleus (also called template) [24] is denoted templated polymerization. This leads to a conformational change of the newly bound protein unit and the formation of a stable complex, which can further serve as template. In our case, the templated polymerization reaction can be considered as quasi-irreversible as

we observed SDS-stable complexes. Mathematical models for such mechanisms have been previously described (see e.g. [25-27]). Here we recapitulate the assumptions and present model equations in the context of the additional GST cleavage.

We denote by x_1 the concentration of Ex1Q49 monomers prior to formation of the nucleus. We denote this conformation C1. The concentrations of all complexes in this conformation are captured in lower-case x_i, where i denotes the number of Ex1Q49 proteins in a complex. The parameter n_c gives the size of the first stable complex, the nucleus, that acts as template. All complexes in this conformation are captured in upper-case X_i, where i denotes the number of Ex1Q49 proteins in the complex. We denote this conformation C2.

Using mass action kinetics for protein binding and conformational changes the infinite set of ODEs reads

$$\dot{x}_1 = v_P - k_{n1}\, n_c\, {x_1}^{n_c} + k_{-n1} n_c\, X_{n_c} - k_1 x_1 \sum_{i=1}^{\infty} X_i \qquad \text{(S6)}$$

$$\dot{X}_i = k_{n1}\delta_{i,n_c} {x_1}^{n_c} - k_{-n1}\delta_{i,n_c} X_{n_c} + k_1 x_1 (X_{i-1} - X_i), \quad i \geq n_c. \qquad \text{(S7)}$$

By definition, $X_i = 0$ in all cases $i < n_c$. The Kronecker symbol is defined by $\delta_{i,j} = 1$ for $i = j$ and 0 otherwise. Monomers x_1 are produced by the cleavage of GST with rate v_P (Eq. (S5) which also belongs to the equation system of the model). Primary nucleation is characterized by the forward rate constant k_{n1}. The term $k_{n1}\, {x_1}^{n_c}$ (Eq. (S7)) gives the rate of nucleus formation, and $-k_{n1}\, n_c\, {x_1}^{n_c}$ (Eq. (S6)) describes the consumption of x_1 molecules by this process. The scaling factor n_c in the latter accounts for the stoichiometry of the process in which n_c monomers form one nucleus. For $n_c > 1$, the higher order of the nucleation reaction can be accounted for by reversible binding of monomers to form small complexes and a fast equilibrium of x_1 with x_{n_c-1}. In this case, we have $x_{n_c-1} = x_1^{(n_c-1)}/K$, where K is the dissociation constant of the dissociation of x_{n_c-1} into x_1. Further binding of a monomer to

x_{n_c-1} with rate constant k_1 causes a conformational change to C2 leading to a stable complex X_{n_c}. Therefore, $k_{n1} = k_1/K$ denotes an effective rate constant for the formation of the first stable complex X_{n_c}. Assuming $x_{n_c-1} \ll x_1$ (i.e. $K \gg 1$) we only need to consider an equation for x_1. For n_c = 1, there is no fast equilibrium and the nucleation process describes a slow conformational change from x_1 to X_1 which we describe by a reversible nucleation process with the backward, de-nucleation reaction characterized by the rate constant k_{-n1}. This avoids that the characteristic time for the conformational change becomes extremely long (1/ k_{n1} > 30 h for the model without reversible nucleation, parameters not shown) compared to less than 12 min in case of a reversible nucleation (1/(k_{n1} + k_{-n1}) < 12 min, Table S4). For a fair comparison between models, we also considered a de-nucleation reaction for n_c > 1, but found that a value of $k_{-n1} = 0$ does not change the conclusions of the models concerning the role of branching and n_c = 1.

In the literature, the complex x_{n_c-1} is also often referred to as the nucleus. However, this leads to confusion for $n_c = 1$ and the distinction to spontaneous aggregation. We thus denote by the nucleus the first stable complex X_{n_c} of size n_c.

During templated polymerization, a complex elongates by binding of Ex1Q49 monomers in conformation C1, thereby, the newly attached protein unit switches to the stable conformation C2. The process is assumed to be irreversible and occurs with rate constant k_1. The terms $k_1 x_1 X_{i-1}$ and $-k_1 x_1 X_i$ in Eq. (S7) account, respectively, for the appearance and disappearance of a complex of size i by binding of a monomer. The term describing the consumption of x_1 by templated polymerization is given by the sum of the elongation reaction rates $k_1 x_1 X_i$ over every possible complex: $k_1 x_1 \sum_{i=1}^{\infty} X_i = k_1 x_1 (m_0 - x_1)$, where, m_0 is the 0th moment of the protein distribution.

We introduce the moments of the protein size distribution as

$$m_k = x_1 + \sum_{i=1}^{\infty} i^k X_i. \tag{S8}$$

The ODEs for the first 3 moments read

$$\dot{m}_0 = v_P + (1 - n_c) k_{n1} {x_1}^{n_c} - (1 - n_c)\, k_{-n1} X_{n_c} - k_1 x_1 (m_0 - x_1) \tag{S9}$$

$$\dot{m}_1 = v_P \tag{S10}$$

$$\dot{m}_2 = v_p + n_c(n_c - 1) k_{n1} {x_1}^{n_c} - n_c(n_c - 1)\, k_{-n1} X_{n_c} + 2\, k_1 x_1 (m_1 - x_1) \tag{S11}$$

The 0th moment m_0 gives the sum of the concentrations of all complexes, the 1st moment m_1 gives the total concentration of Ex1Q49 proteins, the 2nd moment m_2 is proportional to the weighted average size of the Ex1Q49 complexes (for t > 0, without GST-Ex1Q49) is given by m_2/m_1 [28]. The moments are used to derive a finite set of equations for comparison to the experimental data (see Fitting of HTTex1 kinetic models to data).

For $n_c = 1$, it is possible to allow for a non-templated polymerization step where a monomer in conformation C2 can also elongate a fibril. The introduction of such additional step did not significantly improve the quality of the fits and is therefore not considered here.

For spontaneous polymerization, that is $n_c = 0$, and $x_1 = 0$, the model equations (S6), (S7), (S9), (S10) (S11) simplify to

$$\dot{X}_1 = v_P - k_1 X_1 (m_0 + X_1) \tag{S12}$$

$$\dot{X}_i = k_1 X_1 (X_{i-1} - X_i), i \geq 2 \tag{S13}$$

$$\dot{m}_0 = v_P - k_1 X_1 m_0 \tag{S14}$$

$$\dot{m}_1 = v_P \tag{S15}$$

$$\dot{m}_2 = v_P + 2k_1X_1m_1. \tag{S16}$$

Primary nucleation, templated polymerization and branching

In a model that includes templated polymerization and branching the number of proteins in a complex can increase either by elongation of an existing fibril (Fig. 3a, red arrows) or branching from an existing fibril (Fig. 3a, blue arrows). As for templated polymerization, branching can only occur from proteins in conformation C2. For simplicity, we assume that each protein unit has one polymerization and one branching site, furthermore we neglect sterical impairments between branches. The branching reaction allows nucleating a new growing filament and we assume a reaction order n_b for the monomers, i.e. n_b Ex1Q49 monomers are attached along their polymerization sites to the branching site of a protein for forming a branch. We refer to an unoccupied polymerization site within a complex in conformation C2 as growing end, and each growing end defines one branch of the complex. Therefore, polymerization is proportional to the number of branches of a complex and branching is proportional to the number of unoccupied branching sites in a complex. Consequently, the reactions of complexes with similar numbers of branches and polymerization sites can be described by the same rate of polymerization and branching, despite the possibly different spatial structures of the complexes. We hence categorize the complexes according to their number of branches and number of protein units.

Denote by $X_{i,j}$ the concentration of complexes in conformation C2 with i protein units and j the number of growing ends or branches. Thereby, $i \geq n_c$ due to primary nucleation with nucleus size n_c and $j \geq 1$. A nucleus of size n_c has one growing end, and thus the number of branches in a complex can be at most equal to the number of monomers in the complex ($i \geq$

j). Therefore, $X_{i,j} = 0$ for $j > i$ (and for $j < 1$). Equality of i and j occurs only in a monomer, i.e. in $X_{1,1}$, or if a complex only contains branches of length 1 (which both requires $n_c = 1$).

For a model with branching, we can define the moments of the system composed of x_1 and all $X_{i,j}$ as

$$m_{a,b} = x_1 + \sum_{i=n_c}^{\infty} \sum_{j=1}^{i} i^a j^b \, X_{i,j} \tag{S17}$$

$m_{0,0}$ is the sum of the concentration of each complex, and consequently it contains the information about the number of aggregates of any size in the system. In $m_{0,1}$ the number of branches of each aggregate is taken into account and thus it is the overall concentration of branches in the system. $m_{1,0}$ counts the number of Ex1Q49 proteins of which each aggregate is composed and thus contains the total concentration of Ex1Q49 proteins in the system. The ODE system for the protein aggregation model with primary nucleation, templated polymerization and branching can be expressed as function of the moments as well as the monomer and complex concentrations

$$\dot{x}_1 = v_p - k_{n1} n_c x_1^{n_c} + k_{-n1} n_c \, X_{n_c,1} - k_1 x_1 \left(m_{0,1} - x_1\right) - n_b k_b x_1^{n_b} \left(m_{1,0} - m_{0,1} + m_{0,0} - x_1\right) \tag{S18}$$

$$\dot{X}_{i,j} = \delta_{n_c,i} \, \delta_{1,j} \, (k_{n1} {x_1}^{n_c} - k_{-n1} X_{n_c,1}) + \, k_1 \, j x_1 X_{i-1,j} + k_b[(i - n_b) - (j - 1) + 1] x_1^{n_b} X_{i-n_b,j-1} - k_1 \, j x_1 X_{i,j} - k_b[i - j + 1] x_1^{n_b} \, X_{i,j} \tag{S19}$$

where $i \geq n_c$, $j \leq i$. We describe each term in detail in the following. For equation (S18) recall that v_p is the rate with which Ex1Q49 monomers flow into the system (Eq. (S5) which also belongs to the equation system of the model). Primary nucleation and templated polymerization are accounted for as in the model without branching (see primary nucleation and templated polymerization for further details). We assume that branching does not occur

for complexes in conformation C1 (i.e. x_1). The term $k_1 x_1 (m_{0,1} - x_1)$ denotes the consumption of x_1 via templated polymerization. Here monomers in conformation C1 bind to growing ends of complexes in conformation C2. The concentration of growing ends in C2 is given by $m_{0,1} - x_1$ (see Eq. (S17) and explanation). For $X_{i,j}$ (S19), templated polymerization increases its concentration by the term $k_1\, j x_1 X_{i-1,j}$. This accounts for the elongation reaction of an aggregate containing one monomer less (but the same number of branches *j*), $X_{i-1,j}$, on any of its branches and thus being proportional to the number of branches j. Additionally, $X_{i,j}$ can be elongated along any of its branches and thus its concentration is reduced by the term $k_1\, j x_1 X_{i,j}$.

The branching reactions are characterized by the rate constant k_b. The term $n_b k_b x_1^{n_b} (m_{1,0} - m_{0,1} + m_{0,0} - x_1)$ in (S18) accounts for the loss of monomers due to branching. A branch can be formed by nucleation of n_b monomers at once similar to primary nucleation. We assumed that branching can occur at any EX1Q49 protein in conformation C2 that does not have yet a branch and the expression $m_{1,0} - m_{0,1} + m_{0,0} - x_1$ gives the concentration of unoccupied branching sites of complexes in C2. The total number of branching sites, occupied and not occupied, equals the total concentration of Ex1Q49 proteins ($m_{1,0}$). From this, the concentration of branches, $m_{0,1}$, is subtracted since each branch occupies one branching site – except for the first branch of every aggregate, which is there from the beginning. We have to correct the number of free binding sites by exactly one binding site per aggregate and consequently we have to add the total number of aggregates (minus the amount of proteins in conformation C1), $m_{0,0} - x_1$, to obtain the correct number of free branching sites. For $X_{i,j}$, the term $k_b[(i - n_b) - (j - 1) + 1] x_1^{n_b} X_{i-n_b,j-1}$ in (S19) represents branching at an aggregate with one branch less and also n_b monomers less, $X_{i-n_b,j-1}$. It is proportional to the number of free branching sites in the aggregate $X_{i-n_b,j-1}$, $(i - n_b) - (j - 1) + 1$, which is the total number of monomers in the aggregate, $(i - n_b)$, subtracted by the number of branches in the aggregate $(j - 1)$ (since every branch occupies one binding site), and finally correcting

for the one branching site of the initial nucleus, which is a branch although not having an occupied branching site. The term $k_b[i-j+1]x_1^{n_b} X_{i,j}$ in (S19) constitutes branching on any of the (i - j + 1) free branching sites of $X_{i,j}$. Note that by setting $k_b = 0$, the model described by (S18) and (S19) is the same as that described by (S6) and (S7) with $X_{i,1} = X_i$.

The derivatives of the moments of the system can be calculated from the ODE system given in (S18) and (S19). We obtain for the derivatives of the total concentration of aggregates of any size, the concentration of branches and the total concentration number of Ex1Q49 monomers in the system:

$$\dot{m}_{0,0} = v_p + (1-n_c)k_{n1}{x_1}^{n_c} - (1-n_c)k_{-n1}X_{n_c,1} - k_1x_1(m_{0,1}-x_1) - n_bk_bx_1^{n_b}(m_{1,0}-m_{0,1}+m_{0,0}-x_1) \quad \text{(S20)}$$

$$\dot{m}_{0,1} = v_p + (1-n_c)k_{n1}{x_1}^{n_c} - (1-n_c)k_{-n1}X_{n_c,1} - k_1x_1(m_{0,1}-x_1) + (1-n_b)k_bx_1^{n_b}(m_{1,0}-m_{0,1}+m_{0,0}-x_1) \quad \text{(S21)}$$

$$\dot{m}_{1,0} = v_p. \quad \text{(S22)}$$

$$\dot{m}_{1,1} = v_p + k_bx_1^{n_b}(m_{1,0} + (n_b-1)m_{1,1} - n_bm_{0,2} + m_{2,0} + n_bm_{0,1} - (1+n_b)x_1) \quad \text{(S23)}$$

$$\dot{m}_{0,2} = v_p + k_bx_1^{n_b}\left((1-n_b)(m_{0,0}+m_{1,0}) + (1+n_b)m_{0,1} + 2m_{1,1} - 2m_{0,2} + (n_b-3)x_1\right) - k_1x_1(m_{0,1}-x_1) + (1-n_c)k_{n1}\,x_1^{n_c} + (n_c-1)k_{-n1}X_{nc,1} \quad \text{(S24)}$$

$$\dot{m}_{2,0} = v_p + k_bx_1^{n_b}\left((n_b^2+n_b)(m_{1,0}-x_1) + (n_b^2-n_b)(m_{0,0}-m_{0,1}) + 2n_b(m_{2,0}-m_{1,1})\right) + 2k_1x_1(m_{1,1}-x_1) + k_{n1}(n_c^2-n_c)x_1^{n_c} - k_{-n1}(n_c^2-n_c)X_{nc,1} \quad \text{(S25)}$$

The second moments characterize the distribution of protein units and branches in the complexes. The $m_{0,2}/m_{0,1}$ gives an average number of branches (t > 0), whereas a weighted average number of protein units per aggregate, i.e. an average aggregate size, is given (for t > 0, without GST-Ex1Q49) by

$$M = \frac{m_{2,0}}{m_{1,0}}. \tag{S26}$$

The values shown in Figures 6f and g stem from simulations up to t = 50h after addition of PP. A longer simulation gave a quasi-identical curve indicating that the system has reached a steady state.

Seeding was modeled by altering the initial conditions of the system. We simulated the initial GST-Ex1Q49 concentration of 2 µM and assumed seeds of size M_s = 2. Thus, we set $X_{2,1}(0) = X_{2,2}(0) = fX_g(0)/(M_s * 2)$ for the fraction of seeds f=[Seed]/[GST-Ex1Q49], where [GST-Ex1Q49] is the initial concentration of GST-Ex1Q49, $X_g(0)$.

Kinetic modeling of the inhibitory drug O4

To identify the mode of action of O4 we model three different inhibition scenarios (Fig. 6a). Inhibition of the nucleation reaction (green symbol, Fig. 6a) according to

$$k_{n1}([O4]) = k_{n1}\frac{{K_{I1}}^{n_1}}{{K_{I1}}^{n_1} + [O4]^{n_1}}, k_{-n1} = const, k_b = const, k_1 = const \tag{S27}$$

where only the nucleation rate is affected by O4, whereas the other processes remain unaffected. The other two scenarios are inhibition of branching (blue symbol, Fig. 6a)

$$k_b([O4]) = k_b \frac{{K_{I2}}^{n_2}}{{K_{I2}}^{n_2} + [O4]^{n_2}}, k_{-n1} = const, k_{n1} = const, k_1 = const \quad \text{(S28)}$$

and inhibition of polymerization (red symbol, Fig. 6a)

$$k_1([O4]) = k_1 \frac{{K_{I3}}^{n_3}}{{K_{I3}}^{n_3} + [O4]^{n_3}}, k_{-n1} = const, k_{n1} = const, k_b = const \quad \text{(S29)}$$

The concentration dependency on O4 was chosen as general and simple as possible and of the form as used for non-competitive enzyme inhibition.

For n_c = 1, we also tested the case where O4 targets the back reaction of the reversible nucleation process. In this case we assume that O4 promotes an increase in the rate constant $k_{-n1}([O4]) = k_{-n1} + \bar{k}_{-n1} \frac{[O4]^{n_m}}{{K_m}^{n_m} + [O4]^{n_m}}$ causing accumulation of aggregation incompetent monomers. We found that these inhibition modes could reproduce the data. However, the fit is best for the case where k_{n1} is influenced by O4 (see Fig. 6b, green symbols for inhibition of the nucleation, blue symbols for the inhibition of branching, red symbols for the inhibition of polymerization). We therefore only show the results for the first case (inhibition of the nucleation) in Fig. 6c and d.

Fitting of HTTex1 kinetic models to data

Conversion of protein amount into relative immunoreactivity

For comparison of models and data, the simulated protein concentrations need to be compared with immunoreactivities. We convert protein concentrations to relative

immunoreactivities (*RI*) using experimentally derived calibration curves for the employed antibodies (see below and Fig. S4a). Calibration curves are constructed by measuring the immunoreactivities from different dilutions of the reaction mixture (see Ex1Q49 protein aggregation in Material and Methods). For the antibody HD1 (used to detect the cleavage of GST-Ex1Q49 by PP) we used the reaction mixture at time point 0h just after addition of PP. For CAG53b we used the reaction mixture after 8h. For both antibodies we found that the relative immunoreactivity *RI* depends non-linearly on the relative protein amount *P* (see Fig. S4a). Here, the relative protein amount *P* is given as percentage of the protein amount recognized by the antibody in the respective immuno-detection assay for the undiluted aggregation mixture. RIs are obtained by normalizing by the signal intensity measured for the undiluted aggregation mixture.

To quantify the non-linear relationships we tested linear, hyperbolic and log dependencies.

For CAG53b, a log2 dependency described the data best

$$RI = r_1 \log_2(P + r_2) + r_3 \tag{S30}$$

with the coefficients r_1=18.01, r_2=2.24, r_3=-20.25. For HD1 we used a combination of a linear function at low protein amounts and a log2 dependency for higher amounts

$$RI = \begin{cases} r_3 P, \text{ for } P \leq r_4 \\ r_1 \log_2(P + r_2) + r_5, \text{ for } P > r_4 \end{cases} \tag{S31}$$

The coefficients are r_1=32.07, r_2=6.12, r_3=0.09, r_4=6.26. The last parameter is set in a way that *RI* is a continuous function of *P*, that is $r_5 = r_3 r_4 - r_1 \log_2(r_4 + r_2)$=-115.85. In addition, we performed model fits to the experimental data assuming a linear relationship between relative protein amount and relative immunoreactivity. We found that the conclusions about the importance of branching and nucleus of size 1 remained unchanged.

Fitting of the models to SDS stable aggregates detected by CAG53b and estimation of kinetic parameters

Experimentally, we found that dot blots of the reaction mixture yielded the same CAG53b signal for up to 24 hours (Fig. S4c). This suggests that each Ex1Q49 protein, independently of its aggregate state, contributes equally to the signal. The filter retardation assay (FRA) retains only SDS stable aggregates larger than a certain size. We model this by assuming that the FRA detects complexes larger than or equal to a size $C_{\min}$. Thus, the measured CAG53b signal relates to the sum of all Ex1Q49 proteins bound in complexes of size larger or equal $C_{\min}$ in the model. We denote by *CAGP* the simulated relative amount of protein, normalized to the total amount of protein $X_g(0)$, detected in the filter retardation assay by CAG53b

$$CAGP(t) = \frac{c_1}{X_g(0)} \sum_{i=C_{min}}^{\infty} iX_i = \frac{c_1}{X_g(0)} \left(m_1 - x_1 - \sum_{i=n_c}^{C_{min}-1} iX_i \right) \quad \text{(S32)}$$

For the branching models this reads

$$CAGP(t) = \frac{c_1}{X_g(0)} \left(\sum_{i=C_{min}}^{\infty} \sum_{j=1}^{i} iX_{i,j} \right) = \frac{c_1}{X_g(0)} \left(m_{1,0} - x_1 - \sum_{i=n_c}^{C_{\min}-1} \sum_{j=1}^{i} i\, X_{i,j} \right). \quad \text{(S33)}$$

Here c_1 is a conversion coefficient with $c_1 = 100/c_e$ with c_e accounting for the FRA detection efficiency in experiments, which is fitted with the kinetic model parameters. Eqs. (S32) and (S33) show that given a value of C_{min}, for an exact numerical solution of CAGP we only need to solve the ODEs for the 0th and 1st moment and the ODEs for the complex up to a size of

$C_{min} - 1$. If the explicitly modeled maximal complex size is N_{max}, then C_{min} can vary between n_c and $N_{max} + 1$.

The fitting procedure is schematically explained in Fig. S4d. Denote by $CAG_{exp}(t, c)$ and $\sigma_{\exp(t,c)}$ the RI and standard deviation obtained with FRA experiments at time point *t* and for condition *c,* respectively. The data was normalized to the mean maximal value of the DMSO control between 0 and 8h after PP addition. For the data with 20 µM GST-Ex1Q49 (Fig. 6c) we used the corresponding DMSO control at 2 µM GST-Ex1Q49 for normalization as for this experiment the amount of protein blotted was nominally the same (500 ng). The data is then converted to relative protein amounts $CAGP_{exp}(t, c)$ using the inverse of Eq. (S30) and then a background value, estimated from the first 2 time points, is subtracted. Given a kinetic parameter set ψ we first numerically integrate the model for the different experimental conditions. In a first step of the fitting the best value of C_{min} given the kinetic parameters is computed. For this *CAGP* curves are computed for all possible values of C_{min} (Eq. (S32) or (S33)) and optimal values of c_1 are obtained by linear regression. We add back to *CAGP* the background values estimated experimentally and convert to relative immunoreactivities using Eq. (S30) yielding simulated relative immunoreactivities $CAG_{mod}(t, c, C_{min})$ for each condition *c* and each possible value of C_{min}. We then computed the weighted squared distance

$$\chi^2(\psi, C_{min}) = \sum_c \sum_t \left(\frac{CAG_{exp}(t,c) - CAG_{mod}(t,c,C_{min})}{\sigma_{\exp(t,c)}} \right)^2 \quad \text{(S34)}$$

and selected the value of C_{min} that gave the lowest χ^2. This gave $\chi^2(\psi)$ characterizing the parameter set ψ.

For Fig. 3b and c, we simultaneously fitted the kinetic parameters, c_1 and C_{min} to the FRA time course data for the 5 experimental conditions defined by the 5 different initial GST-Ex1Q49 concentrations (symbols and bars in Fig. 3b) using the same parameter set for all conditions. For Fig. 6b and c, we modeled 5 different experimental conditions: Ex1Q49 aggregation at 2 and 20 µM initial GST-Ex1Q49 concentrations without O4 and at 2, 4 and

20 µM initial GST-Ex1Q49 concentrations with O4 (symbols and bars in Fig. 6c or Fig. S7a). In addition to the kinetic parameters and the CAG53b detection parameters c_1 and C_{min} we also fitted the 2 parameters characterizing the action of O4 Eqs. (S27)-(S29).

For each model, we solved numerically the equations for the moments and for complexes containing up to 40 proteins (ode15s, MATLAB7.1, The MathWorks Inc., Natick, MA, 2000). The range of parameters values allowed in the model is given in Table S3. Parameter optimization was performed in two steps: (*i*) starting from a random parameter set we performed a simulated annealing with a non-zero asymptotical temperature (see Fig. S4d) followed by (*ii*) a non-linear least-square solver to refine the parameters (lsqnonlin, MATLAB7.1, The MathWorks Inc., Natick, MA, 2000). This procedure was repeated 30 times and repeatedly led to minima with a χ^2 within 1-3% of the best possible χ^2. Finally, a Markov-Chain-Monte-Carlo procedure was used to estimate the confidence interval of the parameters using the best ψ_{opt} as starting value. For all the simulations we obtained C_{min} values (Eqs. (S32)-(S33)) far below N_{max}= 40 (Table S4) indicating that, given the data, a model that explicitly simulates complexes up to a size of 40 provides correct numerical solutions. The parameters from the best fitting branching model used in Fig. 6c, d, f and g with $n_c = n_b = 1$, are given in Table S4.

For model comparison we used the corrected Akaike information criterion (AICc) [29]. This reads for a model *k* with N_k number of parameters

$$AICc(k) = N \log\left(\frac{\chi^2}{N}\right) + 2N_k + 2\frac{(N_k+1)N_k}{N-N_k-1}, \tag{S35}$$

where *N*=52 is the number of data points. Fig. 3c and 6b show the differences to the AICc of the best fitting models for the respective data set.

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Legends of Supplemental Figures S1 to S8

Supplemental Figure S1 related to Figure 1. Characterization of GST-Ex1Q49 fusion protein.

(a) Purified GST-Ex1Q49 and GST proteins were analysed on a 4-12% SDS-gradient gel. Coomassie staining of recombinant proteins (left panel); Western blot anti-GST (middle panel) and anti-HTT antibodies (HD1 right panel) were utilized. GST-Ex1Q49 migrates at ~60 kDa; the protein migrates at a higher molecular weight than expected from the amino acid sequence. The anti-HTT antibody HD1 recognized GST-Ex1Q49 but not GST alone. The anti-GST antibody recognized both GST and the GST-Ex1Q49 fusion protein.

(b) Size exclusion chromatography of GST-Ex1Q49 fusion protein. Absorbance was recorded at 280 nm; the predominant species eluted at a molecular size of ~510 kDa. Size markers are indicated in kDa. Fractions were dot blotted and membranes were developed with the HD1 antibody. Fractions 6-12 exhibited the highest HTTex1 immunoreactivity (Pool 1). Pool 1 was concentrated and analysed by SDS-PAGE and Western blotting using HD1 antibody. Oligomeric GST-Ex1Q49 fusion protein disassembles under denaturing conditions into monomers migrating at ~60 kDa.

(c) Amino acid sequence of the Ex1Q49 fragment released by PP cleavage from GST-Ex1Q49 fusion protein.

(d) Cleavage of the GST-Ex1Q49 fusion protein with PreScission protease (PP). GST-Ex1Q49 was incubated at a concentration of 2 µM in the presence of 0.28 U PP per µg fusion protein. Cleavage was performed at 20°C and shaking at 300 rpm. Aliquots were taken from the reaction at the indicated time-points; samples were analysed by SDS-PAGE and Western blotting using the HD1 antibody. More than 90% of the GST-Ex1Q49 fusion protein was cleaved after 3 h. Quantification of HD1 immunoreactivity was performed by densitometry using the AIDA software. All signals were normalized to t = 0 h.

(e) Formation of Ex1Q49 aggregates for 2 µM GST-Ex1Q49 incubated with PP at three different agitation speeds (0, 300, 600 rpm). Aliquots taken at the indicated time points were analysed by FRAs, and aggregates were immunodetected with CAG53b antibody. Signals were normalized to the 7 h signal at 300 rpm.

(f) The addition of BSA protein (0.5, 2, and 5 µM) to standard reactions (2 µM GST-Ex1Q49 + PP) does not influence spontaneous Ex1Q49 aggregation. Aliquots were taken at the indicated time-points and analysed by FRAs. Retarded aggregates were immunodetected with HD1 antibody and quantified densitometrically using the AIDA software. All signals were normalized to the 8 h signal in the absence of additional BSA. Values are means of triplicates ± standard deviation.

Supplemental Figure S2 related to Figure 1. Investigating spontaneous Ex1Q49 aggregation by AFM

(a) Representative AFM picture of spontaneously formed Ex1Q49 fibril bundles. The height, width and length of fibril bundles were determined using the JPK data processing software. The arrows indicate the scanned regions in fibril bundles (green: length and height, blue: width and height).

(b) Time-dependent quantification of the height, width and length of fibrillar Ex1Q49 bundles by AFM. In spontaneous Ex1Q49 aggregation reactions large, highly complex fibrillar structures are formed that grow in size over time. Bars show mean and standard deviation over n = 10 bundles (for t = 3 h and 6 h) and n = 6 bundles (for t = 4 h).

Supplemental Figure S3 related to Figure 2. Investigation of spontaneous Ex1Q49 aggregation with dot blot assays using epitope-specific antibodies.

(a) Quantification of Ex1Q49 protein on Western blots using the antibodies HD1 and MW1. With both antibodies similar curves were obtained, indicating that proteolytic

cleavage of GST-Ex1Q49 protein with PP leads to a time-dependent decrease of MW1 antibody binding.

(b) Insoluble Ex1Q49 aggregates are not recognized by 3B5H10. Fibrillar Ex1Q49 aggregates were produced by incubating 2 µM GST-Ex1Q49 fusion protein for 24 h with PP. Aggregates were washed to remove potential soluble protein; samples were analyzed by dot blot assays using the antibodies 3B5H10 and HD1, respectively. The insoluble Ex1Q49 aggregates were recognized by the polyclonal antibody HD1, which recognizes a C-terminal proline-rich region, but not by the anti-polyQ antibody 3B5H10.

(c) Comparison of the Ex1Q49 aggregation curves obtained by filter retardation assays (FRAs) and dot blot assays (DBAs). The epitope-specific antibody MW8 detects insoluble Ex1Q49 aggregates in non-denaturating DBAs as well as denaturating FRAs. The polyclonal antibody CAG53b was used as a control.

Supplemental Figure S4 related to Figure 3. Conversion of immunoreactivities to protein amounts, estimation of PP cleavage rate, and parameter fitting procedure.

(a) Relation between protein amount and immunoreactivity. Calibration curves were obtained by serial dilution of the aggregation reaction mixture at time 0 h (HD1) or 8 h after addition of PP (CAG35b). Western blotting, and FRAs were done as described. Proteins on Western blots were detected with HD1 antibody (squares). Aggregates retained on membrane (FRA) were detected with CAG53b antibody (diamonds). Bands were quantified by densitometry and normalized to a signal of 100% protein, that is 250 ng for HD1, and 500 ng for CAG53b. Lines are the calibration curves Eq. S30 for CAG53b, and Eq. S31 for HD1.

(b) Cleavage of 2 µM (square) and 20 µM (circle) GST-Ex1Q49 fusion protein with PreScission protease (PP) as described. Fusion protein cleavage was analysed by SDS-PAGE and immunoblotting using the HD1 antibody. Relative immunoreactivities

were converted into relative protein amounts using the calibration curve shown in panel (a) (Eq. S31). The cleavage shows a typical Michaelis-Menten kinetics (solid and dashed lines, Eq. S5).

(c) Dot blot of the Ex1Q49 aggregation mixture at the indicated time points after addition of PP to GST-Ex1Q49 fusion protein detected with CAG53b. The signal of CAG53b is only proportional to the total amount of Ex1Q49 but not its aggregation state.

(d) Pseudo-code of the fitting procedure of model parameters to the data. C_{min} denotes the minimal size of an aggregate, which can be detected by CAG53b. The intervals of allowed parameter values are given in Table S3.

Supplemental Figure S5 related to Figure 4. Effects of small molecules on Ex1Q49 aggregation.

(a-c) Aggregation of Ex1Q49 was not altered by addition of the chemical compounds curcumin (Curc), methylen blue (MB) and PGL034. Aggregation reactions of 2 µM GST-Ex1Q49 were incubated in the absence or presence of compounds. Aliquots taken at the indicated time-points and were analysed by FRA. The aggregates were immunodetected with CAG53b antibody and quantified by densitometry using the AIDA software. The signals were normalized to the 24 h signal in the absence of compound. The compounds (a) Curc, (b) MB and (c) PGL034 had no effect on the formation of SDS-resistant aggregates.

(d) O4 has no effect on PP cleavage. Aggregation of Ex1Q49 (2 µM) was examined in the presence or absence of equimolar concentrations of O4. Cleavage of GST-Ex1Q49 fusion protein was analysed by SDS-PAGE and immunoblotting using the anti-HTT antibody HD1. Bands were quantified by densitometry and signals were normalized to t = 0 h.

(e) Quantitative analysis of FRA results shown in Fig. 4b. The highest signal obtained with control aggregation reactions in the absence of O4 was set to 100%. The lag phase duration (lag-time, green) and growth rate given as the maximal slope (blue) can be measured. Quantification of these two parameters for the FRA traces shown in Fig. 6c gave a lag-time of 1.86 ± 0.62 h in controls compared to 4.22 ± 0.79 h in O4 treated samples (2 µM O4, n = 13, paired *t*-test, *p*-value = 5e-10). The growth rate, was not influenced by compound treatment (control 38.85 ± 9.67 h-1, growth rate 2 µM O4 45.08 ± 9.41 h-1, *n* = 13, paired *t*-test, *p*-value = 0.096).

(f) Dynamic light scattering analysis of spontaneous Ex1Q49 aggregation (20.9 µM) in the presence (20.9 µM) and absence of O4 (*n* = 4). The z-averaged diameter was computed according to Eq. S4.

Supplemental Figure S6 related to Figure 5. Molecular dynamics simulations.

Snapshots were taken at 0, 50, 100 and 200 ns of MD simulations with Ex1Q47 and Ex1Q47-O4 (binding sites A7, D1, A5 and A8). Ex1Q47 is shown coloured by region with the same colour code as in Fig. 5. The addition of O4 affects the β-sheet strands that are otherwise conserved during the simulations without O4.

Supplemental Figure S7 related to Figure 6. Kinetic model of the effect of O4 on HTTex1 aggregation

(a) The model without branching and O4 inhibition of primary nucleation does not reproduce the effect of O4 on the aggregation of Ex1Q49.

(b) Predicted kinetics for the addition of 2 µM O4 at the indicated time points to the aggregation of Ex1Q49 (2 µM GST-Ex1Q49) in the branching model where O4 inhibits the branching process. Similar traces are obtained when O4 inhibits the polymerization.

Supplemental Figure S8 related to Figure 7

(a) SDS-PAGE analysis of purified recombinant GST-Ex1Q48-CyPet and –YPet fusion proteins. Proteins were stained with the dye Coomassie Blue R.

(b) Schematic model of the spontaneous FRET-inducing Ex1Q48-CyPet/-YPet co-aggregation in cell-free assays. Initially, the N-terminal GST-tag keeps the fusion proteins in a soluble state and prevents spontaneous aggregation. After proteolytic cleavage of the fusion proteins, Ex1Q48-CyPet and -YPet fragments are released and spontaneously co-aggregate over time. Co-aggregation is monitored by quantification of FRET, arising when fluorescent tags come into close proximity in ordered protein aggregates.

Legends of Supplemental Movies S1 and S2

Supplemental Movie S1

The MD simulation perform in the absence of O4 shows that Ex1Q47 retains the 4 β strands initially present in the structure during the 97 % of the simulation.

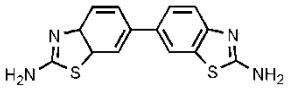
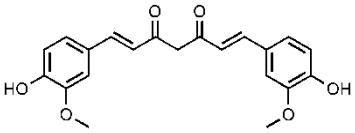
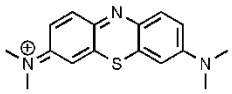
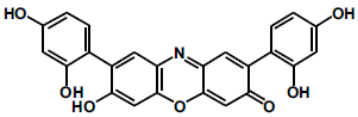
Supplemental Movie S2

The simulation of Ex1Q47 in the presence of O4 results in the rapid loss of all 4 β strands present in the initial structure of Ex1Q47.

Supplemental Tables S1 to S4 and Legends

Supplemental Table S1: Chemical structures of compounds tested.

Chemical structure and names of compounds tested in cell-free aggregation assays.

Structure	Name	Short name	Literature
	PGL-034 (6-(2-amino-1,3-benzothiazol-6-yl)3H-1lambda-4,3-benzothiazol-2-	PGL034	Heiser et al., 2002
	Curcumin ((E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-	Curc	Dikshit et al., 2006, Yang et al., 2005
	Methylen blue (3,7-bis(Dimethylamino) phenazathionium chloride)	MB	Oz et al., 2009
	Orcein-related O4 (2,8-bis-(2,4-dihydroxy-phenyl)-7-hydroxy-phenoxazin-3-one)	O4	Bieschke et al., 2012

Supplemental Table S2: Computational modeling results: Molecular dynamics and docking.

Calculated docking affinities and energies of the QM region (O4 molecule) averaged during the last 300 ps of the SCCDFTB-D/CHARMM MD simulations show that O4 is most stable when interacting simultaneously with the polyQ, N- and C-terminal regions (A7 and D1). The amount (in %) of the different Ex1Q47 clusters (classified according to the number of β-sheets) for the 200 ns MD simulations of Ex1Q47 and Ex1Q47-O4 (binding site A7) indicates that O4 disrupts the Ex1Q47 β-sheet structure.

	Interacting with	Docking affinity (kcal/mol)	SCCDFTB-D/CHARMM MD simulations	
			Absolute QM energy (kcal/mol)	Relative QM energy (kcal/mol)
A1	polyQ, polyP, N-terminus, C-terminus	-10.4	-45546.7 ± 4.45	23.6
A3	polyQ	-8.5	-45554.8 ± 4.85	15.5
A5	polyQ, polyP, N-terminus	-8.2	-45557.5 ± 4.75	12.8
A6	polyQ	-8.2	-45547.2 ± 4.37	23.1
A7	**polyQ, N- and C-terminus**	-8.1	**-45570.3 ± 5.14**	**0**
A8	polyQ, polyP	-7.8	-45556.1 ± 5.60	14.2
D1	**polyQ, N- and C-terminus**	-7.6	**-45569.3 ± 5.19**	**1.0**

# of β-sheets	Conformers (%)	
	Ex1Q47	Ex1Q47–O4
4	93	1
3	7	0
2	0	24
1	0	0
0	0	74

Supplemental Table S3: Parameter names and ranges

Kinetic and O4 inhibition parameters used in the templated polymerization and branching models. The parameter range gives the lowest and highest parameter value allowed in the fitting. A lowest value of 10^{-6} has been chosen for numerical reasons and efficient computation. None of the fitted parameters are close to lowest or highest possible value at the end of the optimization procedure (Fig. S4d). r.c. means rate constant.

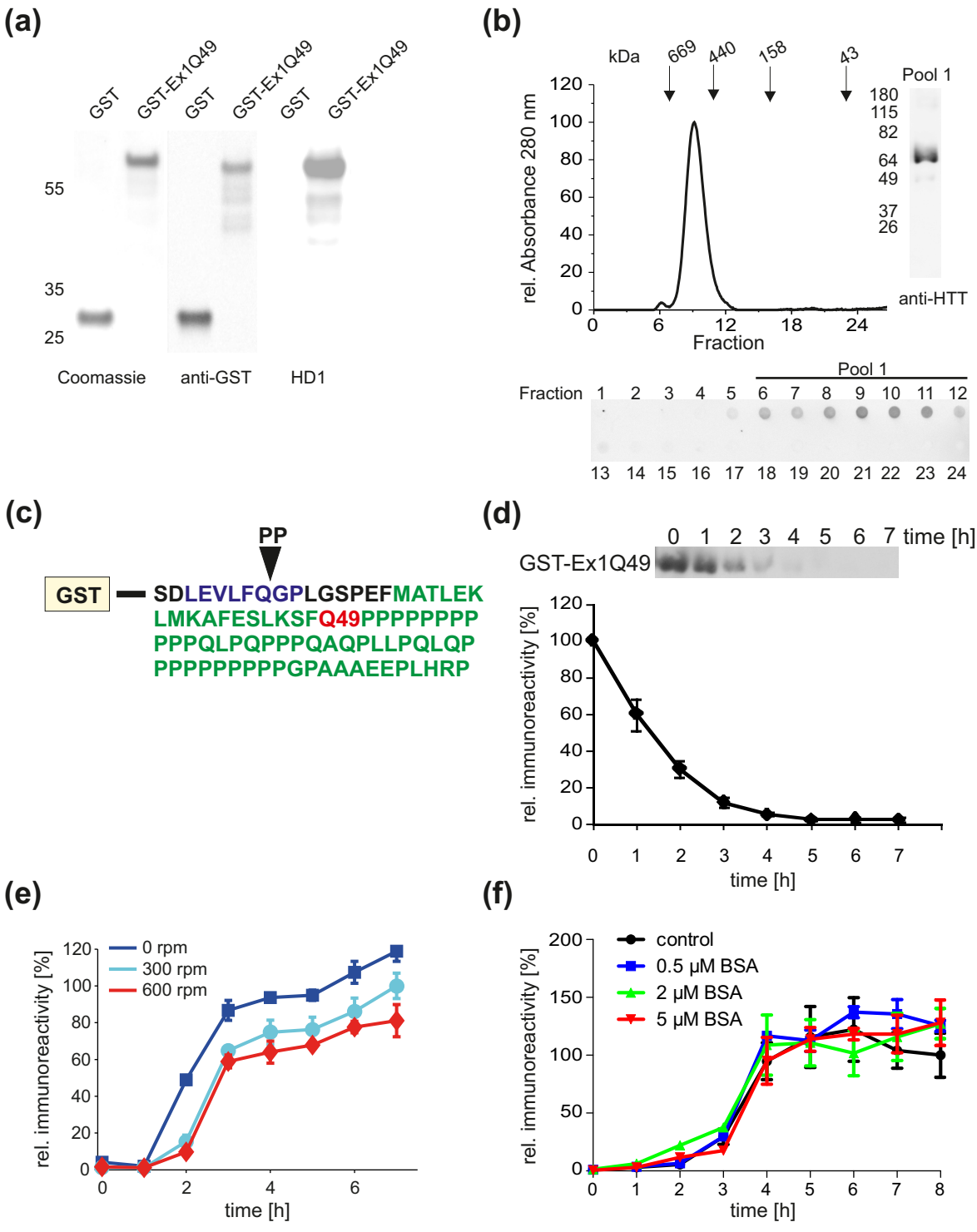
Parameter	Symbol	Parameter range
Kinetic parameters		
Nucleation r.c.	k_{n1}	10^{-6} - 1 $h^{-1}\mu M^{-(nc-1)}$
De-nucleation r.c.	k_{-n1}	10^{-6} - 100 h^{-1}
Nucleus size	n_c	0 - 4*
Branching r.c.	k_b	10^{-6} - 100 $h^{-1}\mu M^{-nb}$
Branching order	n_b	1 - 4
Polymerization r.c.	k_1	10^{-6} - 100 $h^{-1}\mu M^{-1}$
Action of O4		
Half-maximal inhibition of nucleation	K_{I1}	10^{-6} - 20 µM
Hill-coefficient inhibition of nucleation	n_1	10^{-6} - 20
Half-maximal inhibition branching	K_{I2}	10^{-6} - 20 µM
Hill-coefficient inhibition of branching	n_2	10^{-6} - 20
Half-maximal inhibition polymerization	K_{I3}	10^{-6} - 20 µM
Hill-coefficient inhibition of polymerization	n_3	10^{-6} - 20
Half-maximal of de-nucleation	K_m	10^{-6} - 20 µM
Hill-coefficient for de-nucleation	n_m	10^{-6} - 20
Maximal increase of de-nucleation r.c.	$\bar{k}_{-n1}$	10^{-6} - 2000 h^{-1}
Antibody assays		
Minimal complex size for detection by FRA	C_{min}	n_c - N_{max}+1
Conversion coefficient for FRA	c_1	0 - 200

* a nucleus size of $n_c = 0$ is spontaneous aggregation without nucleation

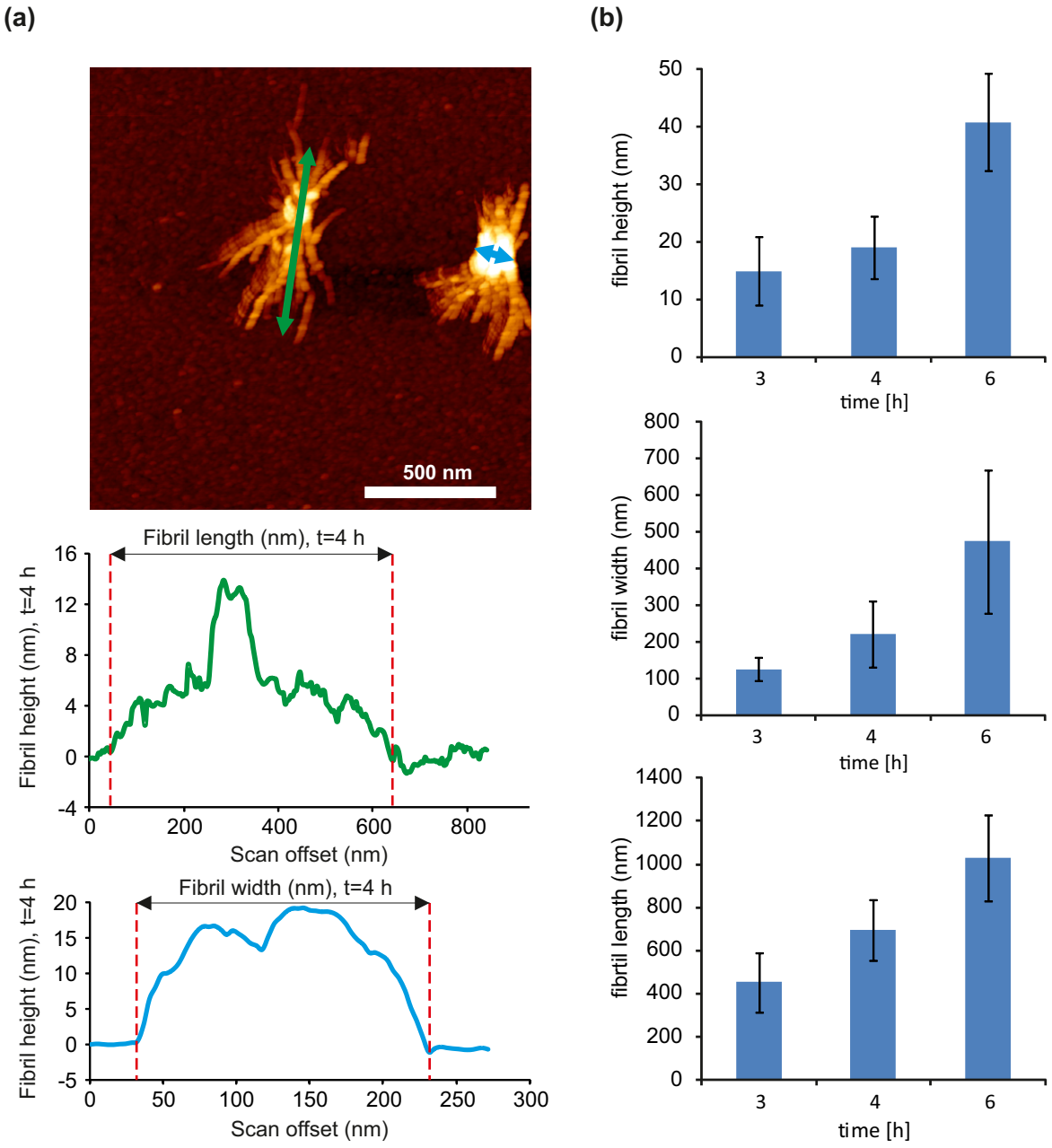
Supplemental Table S4: Parameter values for the branching model

The PP parameters are kept fixed and were determined from the data in Fig. S4b. The nucleus size and branching order, n_c=n_b, were not directly fitted but estimated by systematically varying them (Fig. 6b). After obtaining a candidate best fit using the pipeline in Fig. S4d the most probable parameter distribution given the data is estimated using Markov-Chain-Monte Carlo method (MCMC). The table gives the median and in squared brackets the lower and upper quartile of the parameter distribution. r.c. means rate constant.

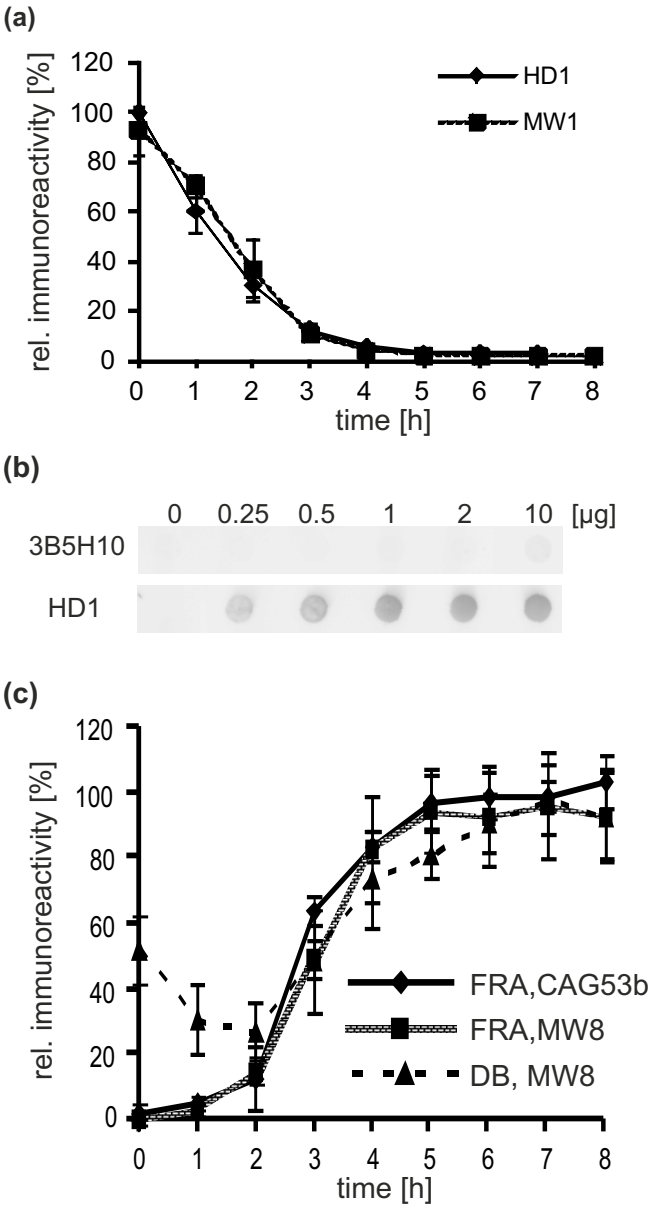
Parameter	Symbol	Parameter values
PP parameters		
Maximal PP r.c.	k_r	1.99 h^{-1}
Half-maximal PP activity	K_p	4.211 µM
Kinetic parameters		
Nucleation r.c.	k_{n1}	0.1225 [0.081- 0.184] h^{-1}
De-nucleation r.c.	k_{-n1}	6.274 [5.235-7.412] h^{-1}
Nucleus size	n_c	1
Branching r.c.	k_b	0.51 [0.315-0.78] $h^{-1}µM^{-1}$
Branching order	n_b	1
Polymerization r.c.	k_1	0.4361 [0.275-0.705] $h^{-1}µM^{-1}$
Action of O4		
Half-maximal inhibition of nucleation	K_{I1}	1.061 [0.921- 1.203] µM
Hill-coefficient inhibition of nucleation	n_1	4.711 [4.289-5.223]
Antibody assays		
Minimal complex size for detection by FRA	C_{min}	17 [12-22]
Conversion coefficient for FRA	c_1	134.8 [133.24-143.46]



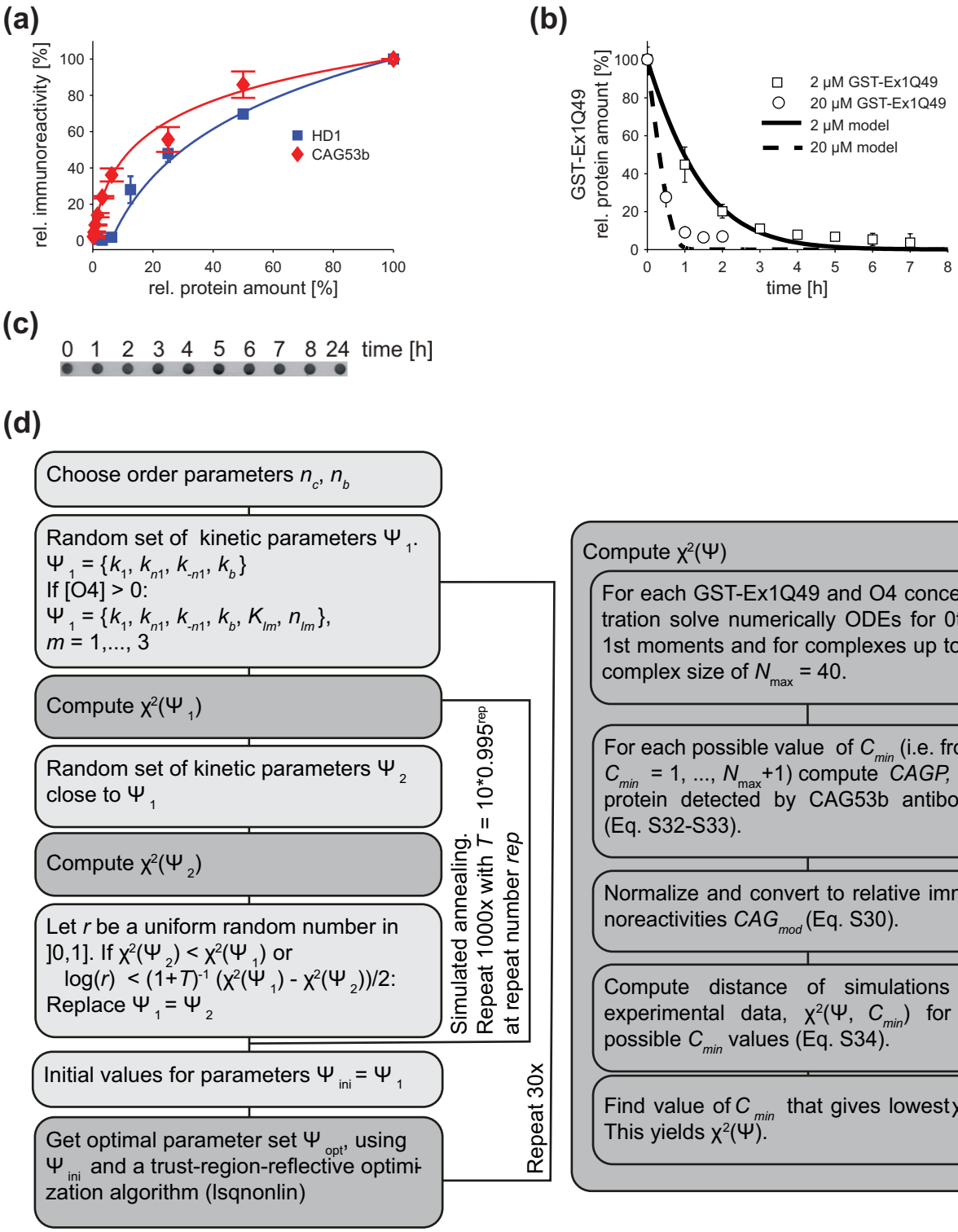
Suppl. Figure S1



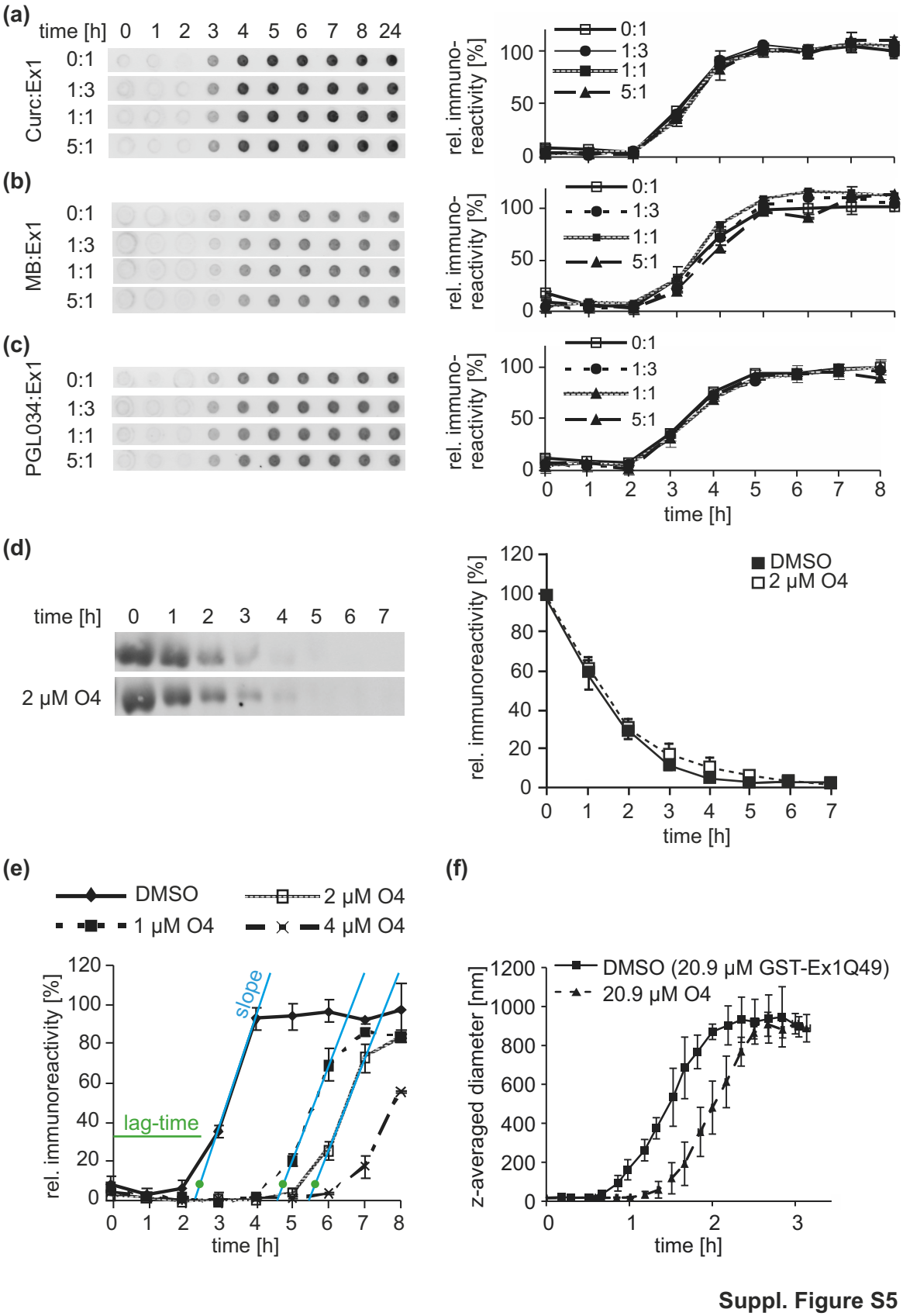
Suppl. Figure S2



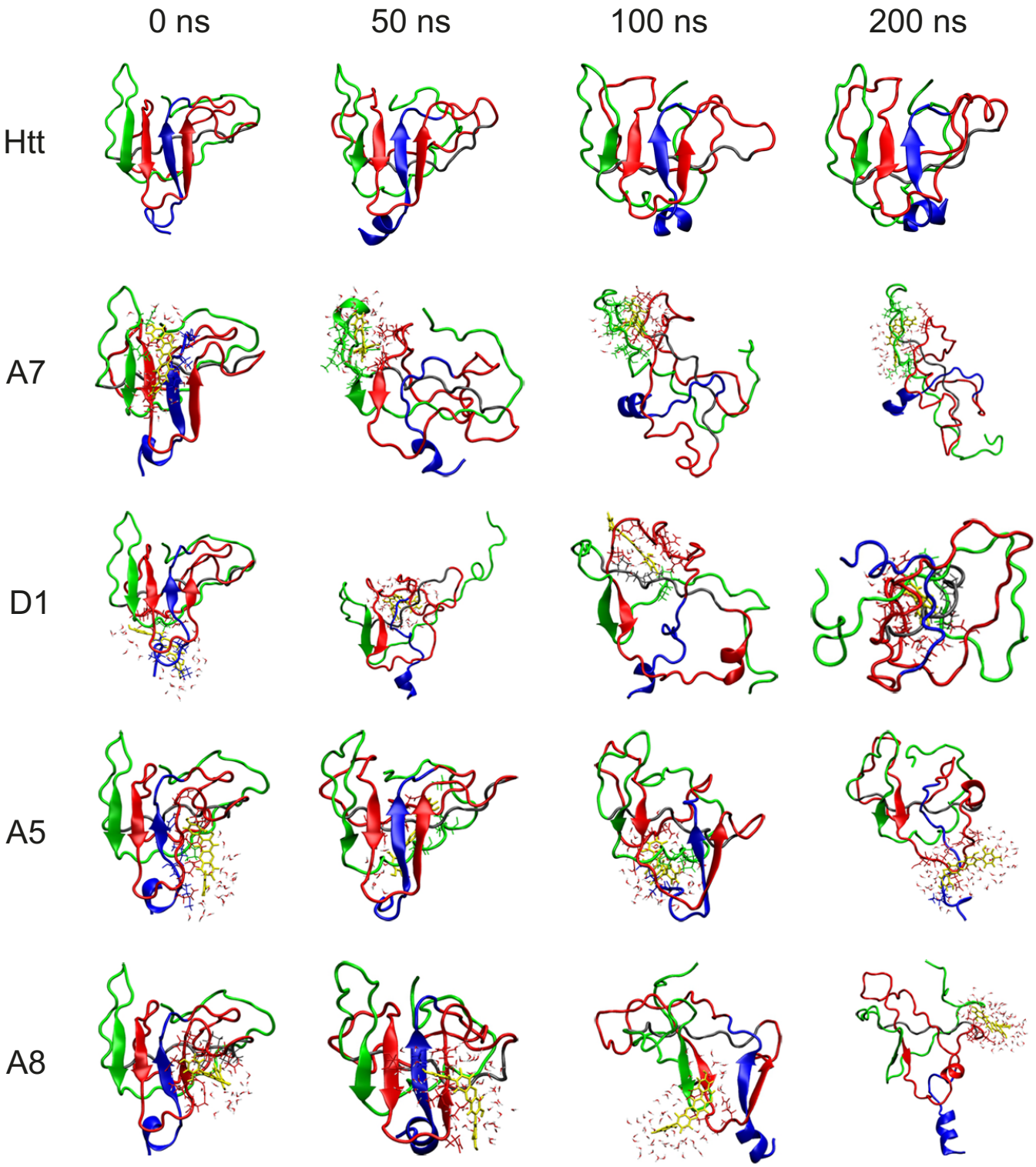
Suppl. Figure S3



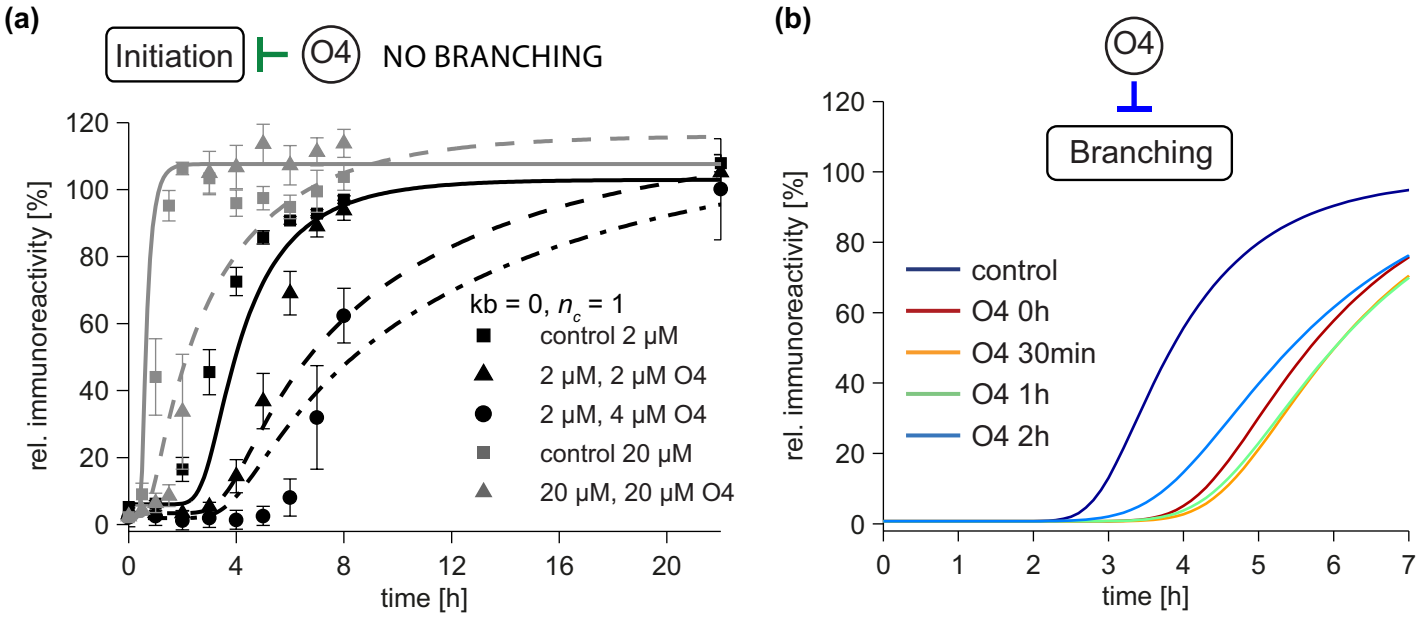
Suppl. Figure S4



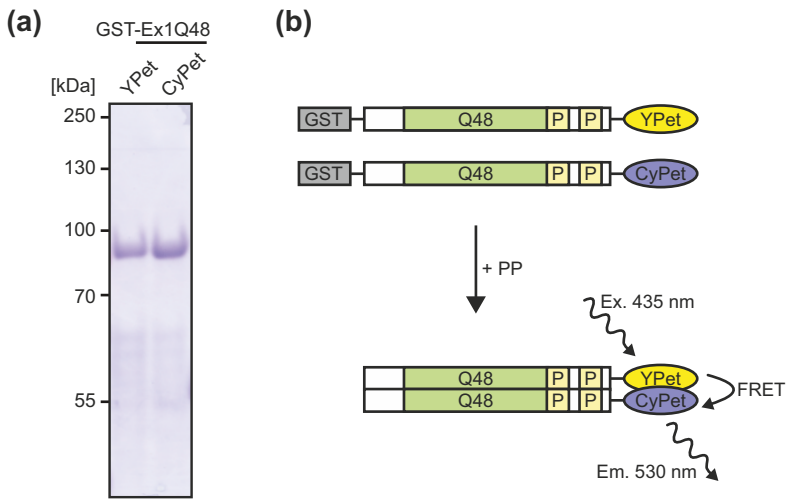
Suppl. Figure S5



Suppl. Figure S6



Suppl. Figure S7



Suppl. Figure S8