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Kinetics of Fast Tetramerization of the Huntingtin Exon 1 Protein Probed by Concentration-Dependent On-Resonance $R_{1\rho}$ Measurements

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Abstract

An approach for the quantitative description of the kinetics of very fast exchange processes ($\tau_{\text{ex}} < 50\text{--}100\ \mu\text{s}$) associated with transient, reversible protein oligomerization, is presented. We show that on-resonance ^{15}N - $R_{1\rho}$ measurements conducted as a function of protein concentration at several spin-lock radio frequency field strengths are indispensable for unambiguous determination of the rate constants for interconversion between monomeric and higher order oligomeric species. The approach is experimentally demonstrated on the study of fast, reversible tetramerization of the full-length Huntingtin exon 1 protein, htt^{ex1}, responsible for Huntington's disease. Incorporation of concentration-dependent ^{15}N - $R_{2,\text{eff}}$ data, obtained from on-resonance $R_{1\rho}$ measurements performed at three spin-lock field strengths, into analysis of the kinetic scheme describing reversible tetramerization of htt^{ex1} allowed us to uniquely determine the rate constants of interconversion between the various species. This approach serves as a valuable complement to the existing array of NMR techniques for studying early, transient oligomerization events in protein aggregation pathways.

Graphical Abstract

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Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.0c01636>.

Materials and methods, full set of concentration-dependent chemical shifts and ^{15}N - $R_{2,\text{eff}}$ data, correlation plots; tables of chemical shift differences, impact of number of RF field strengths on the optimized values of the equilibrium dissociation constants, and impact of concentration range on the optimized values of the equilibrium dissociation constants and dissociation rate constants (PDF)

The authors declare no competing financial interest.

The experimental data in digital format, together with MatLab scripts for simulations and global best-fitting, have been deposited on Figshare, <https://doi.org/10.35092/yhjc.12536015>

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huntingtin pre-nucleation tetramerization

Dynamic processes in proteins occurring on a wide range of time scales in general,^{1,2} and those associated with reversible protein oligomerization events in particular,^{3,4} can be studied using a variety of solution NMR techniques. Characterization, however, of exchange processes involving protein oligomerization occurring on time scales $<50\text{--}100 \mu\text{s}$ by solution NMR may be challenging for several reasons. This exchange regime falls outside the window of applicability of conventional Carr–Purcell–Meiboom–Gill (CPMG)^{5,6} relaxation dispersion methods.⁷ However, the chemical shifts induced by exchange (δ_{ex})^{8,9} in this regime report on the equilibrium parameters of exchange (equilibrium constants/populations) and do not bear information on the rates of interconversion. In principle, $R_{1\rho}$ relaxation dispersion experiments,^{10–12} where spin relaxation rates of the magnetization aligned along the effective radiofrequency (RF) field are recorded in the rotating frame as a function of effective RF field strength, cover a range of exchange rates between $\sim 10\,000$ and $\sim 100\,000 \text{ s}^{-1}$ ($10 \mu\text{s} < \tau_{\text{ex}} < 100 \mu\text{s}$). However, in systems undergoing fast (homo)-oligomerization processes, where the rate constants of interconversion between monomeric and one or more oligomeric species (and, consequently, overall exchange rates) depend on the concentration of the main, observable species (monomers),^{3,4} neither on-resonance $R_{1\rho}$ dispersion obtained at a single protein concentration, nor on-resonance $R_{1\rho}$ ($R_{2,\text{eff}}$) rates acquired at a series of concentrations but a single RF field strength, contain sufficient information to uniquely determine the rate constants of the reversible oligomerization process. In this Letter, we show that a combination of (1) on-resonance $R_{1\rho}$ ($R_{2,\text{eff}}$) rates obtained at a series of protein concentrations and several RF field strengths and (2) exchange-induced shifts δ_{ex} measured at a series of concentrations allows one to accurately

define the kinetic rate constants of the fast, reversible tetramerization of the full-length Huntingtin exon 1 protein, htt^{ex1}. Polyglutamine expansion within Huntingtin exon-1 is responsible for Huntington's disease, a fatal autosomal dominant neurodegenerative condition.¹³

Recently, we reported a detailed kinetic investigation of the prenucleation, transient oligomerization events involving htt^{ex1} using a combination of exchange NMR techniques suitable for the study of reversible oligomerization processes.¹⁴ The htt^{ex1} construct comprised a 16-residue amphiphilic N-terminal sequence (NT), a seven-residue polyglutamine tract (polyQ), and a proline-rich domain (PRD) containing two polyproline repeats separated by a 17-residue linker. Transient tetramerization of htt^{ex1} is an early stage event^{4,14} in the pathogenic pathway leading to the formation of neuronal inclusion bodies comprising polymorphic aggregates of oligomers and fibers.^{15,16} This study relied on the kinetic scheme (Figure 1A) developed earlier for the transient, reversible oligomerization of a shorter, minimalistic construct, htt^{NT}Q₇, lacking the PRD domain.⁴ The kinetic model of oligomerization that satisfied all the experimental data obtained for htt^{ex1} and htt^{NT}Q₇ (with only slightly different exchange parameters) consists of two branches: an "on-pathway" branch involving self-association of the monomeric species, E, to form a tetrameric helical bundle (E₄) via a helical coiled-coil dimer intermediate (E₂) (shown in black in Figure 1A), and an "off-pathway" branch leading to formation of "nonproductive" dimeric species, E₂^{*}, that cannot oligomerize further (shown in gray in Figure 1A). The interconversion between the monomeric species E and the "off-pathway" dimer E₂^{*} occurs on a relatively slow time-scale ($\tau_{\text{ex}} \sim 750 \mu\text{s}$), making it amenable to analysis by CPMG relaxation dispersion techniques. In contrast, the overall exchange between the monomer and the tetramer E₄ occurs very fast on the chemical shift time scale ($\tau_{\text{ex}} \sim 50 \mu\text{s}$) and is monitored primarily by changes in exchange-induced shifts, δ_{ex} , as a function of protein concentration. The minimal set of experimental NMR observables needed for analysis of the scheme in Figure 1A, included amide ¹⁵N- and ¹³C α -CPMG relaxation dispersion profiles acquired at three protein concentrations and ¹⁵N/¹³C α - δ_{ex} values measured at a series of concentrations ranging from 100 μM to 1.2 mM.^{4,14} Even though the equilibrium parameters (i.e. equilibrium dissociation constants and populations) of each species of the on-pathway branch could be determined (listed in Figure 1A for full-length htt^{ex1}) under the assumption that the ¹⁵N/¹³C α chemical shifts of species E₂ and E₄ are the same, neither in the case of htt^{NT}Q₇⁴ nor in that of the full-length htt^{ex1}¹⁴ was it possible to establish unequivocally the kinetic rate constants (k_2 and k_{-2}) for the interconversion between dimer and tetramer. As confirmed by numerical simulations using synthetic data sets and observed in practice, inclusion of on-resonance ¹⁵N- $R_{1\rho}$ dispersion data obtained at a single (typically, the highest) concentration of the protein and/or on-resonance ¹⁵N- $R_{1\rho}$ (¹⁵N- $R_{2,\text{eff}}$) relaxation rates acquired at a series of concentrations but a single spin-lock RF field strength in the analysis, does not affect this result appreciably. Extensive grid searches performed over a wide range of k_{-2} values (which defines the overall exchange rate between monomer and tetramer at low concentrations⁴), while optimizing all of the remaining rate constants, allowed us, nevertheless, to estimate approximate lower bounds for k_{-2} ($\sim 20\,000 \text{ s}^{-1}$ and $\sim 40\,000 \text{ s}^{-1}$ for full-length htt^{ex1} and htt^{NT}Q₇, respectively).

The scheme in Figure 1A can be simplified to include only the “on-pathway” process describing the tetramerization of htt^{ex1} , the main focus of the present study. Panels B and C of Figure 1 show that the concentration series of $^{15}\text{N}-\delta_{\text{ex}}$ and $^{15}\text{N}-R_{2,\text{eff}}$ values (where $R_{2,\text{eff}}$ is calculated from $R_{1\rho}$ with a 1 kHz spin-lock RF field) simulated for the full scheme (black curves) are practically indistinguishable from those obtained using the “on-pathway” branch only (red curves), whereas the contribution of the “off-pathway” dimerization process (gray curves) to these NMR observables is clearly negligible. The large separation of time scales of the two branches of exchange in Figure 1A allows for such simplification to be achieved with a minimal loss of information content. Note that the “on-pathway” branch of htt^{ex1} oligomerization is experimentally probed primarily by $^{15}\text{N}/^{13}\text{C}\alpha-\delta_{\text{ex}}$ and $^{15}\text{N}-R_{2,\text{eff}}$ data and is responsible for the characteristic curvature of their concentration dependence due to formation of higher-order (tetrameric) oligomers (Figures 1B,C). “Off-pathway” dimerization, however, is $^{15}\text{N}/^{13}\text{C}\alpha$ CPMG profiles (not considered here) up to CPMG fields of 500 Hz.¹⁴ The $^{15}\text{N}/^{13}\text{C}\alpha$ CPMG profiles from 500 Hz to the highest CPMG fields attainable in practice (1 kHz for ^{15}N and 2 kHz for $^{13}\text{C}\alpha$) decay almost monotonically and therefore bear limited information on the kinetics of the “on-pathway” tetramerization process (i.e., insensitive to the value of k_{-2} above a lower bound). In the following, only the “on-pathway” branch is considered in the analysis of $^{15}\text{N}/^{13}\text{C}\alpha-\delta_{\text{ex}}$ and $^{15}\text{N}-R_{2,\text{eff}}$ data of htt^{ex1} .

It is instructive to consider some salient features of the two-step tetramerization scheme, $\text{E} \rightleftharpoons \text{E}_2 \rightleftharpoons \text{E}_4$, with respect to its dependence on protein concentration and its relationship to simpler models of exchange. Figure 2A shows plots of the overall exchange rate, $k_{\text{ex}}^{\text{overall}}$, that describes the total rate of exchange between the end species of a kinetic scheme, as a function of htt^{ex1} concentration simulated for three different cases of exchange: (i) concentration-independent (green horizontal line), such as would be observed, for example, for a 2-state folding-unfolding equilibrium, $\text{U} \rightleftharpoons \text{F}$, where $k_{\text{ex}}^{\text{overall}} = k_{\text{U}} + k_{\text{F}}$, the sum of folding (k_{F}) and unfolding (k_{U}) rate constants; (ii) a linearly concentration-dependent apparent association rate constant (blue line), such as would be observed for a dimerization process, $\text{E} \rightleftharpoons \text{E}_2$, with $k_{\text{ex}}^{\text{overall}} = 2k_1[\text{htt}^{\text{ex1}}] + k_{-1}$, where the same notation as in Figure 1A is used, and (iii) tetramerization via a dimer intermediate, $\text{E} \rightleftharpoons \text{E}_2 \rightleftharpoons \text{E}_4$ (red curve), where the overall process $\text{E} \rightleftharpoons \text{E}_4$ is characterized by an overall exchange rate of $k_{\text{ex}}^{\text{overall}} = (k_{-1}^2 k_{-2} + 4k_1^2 k_2 [\text{htt}^{\text{ex1}}]^3) / (k_{-1}^2 + 2k_1 k_2 [\text{htt}^{\text{ex1}}]^2)$ using the notation of Figure 1A. While the exchange rate of dimerization ($\text{E} \rightleftharpoons \text{E}_2$) only weakly and linearly depends on protein concentration (at least for the range of concentrations and low populations of the dimeric species considered here), the overall rate of tetramerization ($\text{E} \rightleftharpoons \text{E}_4$) is predicted to decrease almost 2-fold at higher protein concentrations (Figure 2A), a unique property of this kinetic scheme that arises from the concomitant concentration dependence of the overall reverse process ($\text{E}_4 \rightarrow \text{E}$) as well. This decrease in the overall exchange rate is predicted to be manifested in the relationship between $^{15}\text{N}-R_{2,\text{eff}}$ and $^{15}\text{N}-\delta_{\text{ex}}$ values, the two experimental observables that we focus on here, as we describe in more detail below.

Panels B and C of Figure 2 show simulation plots of $^{15}\text{N}-\delta_{\text{ex}}$ and $^{15}\text{N}-R_{2,\text{eff}}$ values, respectively, as a function of protein concentration, calculated with a typical set of exchange

parameters obtained in our previous study of this system.¹⁴ The values of $^{15}\text{N}-\delta_{\text{ex}}$ (Figure 2B) are predicted to be sensitive to the rate constant k_{-2} , which, at low htt^{ex1} concentrations, determines the overall exchange rate for tetramerization ($\text{E} \rightleftharpoons \text{E}_4$)⁴ up to a limit of $\sim 5000 \text{ s}^{-1}$. Beyond this limit, the values of $^{15}\text{N}-\delta_{\text{ex}}$ lose sensitivity to the rate(s) of species' interconversion. Conversely, the concentration dependence of $^{15}\text{N}-R_{2,\text{eff}}$ (Figure 2C) retains sensitivity to k_{-2} even for very fast rates of exchange ($> 20\,000 \text{ s}^{-1}$), especially so at the two extreme RF field strengths (0.75 and 3 kHz) of the applied spin-lock. When $^{15}\text{N}-R_{2,\text{eff}}$ values are plotted versus absolute $^{15}\text{N}-\delta_{\text{ex}}$ (Figure 2D) for the same three scenarios of exchange as in Figure 2A, the characteristic curvature of the plot can be observed for the tetramerization scheme, $\text{E} \rightleftharpoons \text{E}_2 \rightleftharpoons \text{E}_4$, at low spin-lock RF field strengths applied in $R_{1\rho}$ measurements (red solid curve). To obtain a more quantitative description of the relationship between $R_{2,\text{eff}}$ and δ_{ex} , we draw analogies with a two-state exchange process, as the existing analytical expressions for the three-site exchange¹⁷ are too bulky to provide immediate insights. It is straightforward to show that for two-site exchange in the presence of an on-resonance RF field (as in $R_{1\rho}$ measurements), $R_{2,\text{eff}}$ depends linearly on δ_{ex} , with a slope that can be approximated by $(\omega/k_{\text{ex}})(k_{\text{ex}}^2 + \omega^2)/(k_{\text{ex}}^2 + \omega_1^2)$, where ω is the difference in chemical shifts between the exchanging sites, and ω_1 is the strength of the applied on-resonance RF field; here the simplest expression for $R_{1\rho}$ in the limit of fast exchange^{18,19} is used, and the intrinsic R_2 rates for the two sites are assumed to be the same. Clearly, if k_{ex} remains invariant with concentration ($k_{-1} \gg k_1^{\text{app}}$), as is almost the case for the dimerization process, $\text{E} \rightleftharpoons \text{E}_2$, the slope remains almost constant (blue line in Figure 2D), whereas a gradual increase in the slope with concentration will be observed in the case of the tetramerization scheme for low RF field strengths (red solid curve, Figure 2D). The resulting curvature of the plot is the direct consequence of the decrease in the overall exchange rate at higher protein concentrations for tetramerization via a dimer intermediate as discussed above. Note that for larger RF field strengths, when the RF field (ω_1) starts to “compete” with exchange (k_{ex}), the curvature of the $R_{2,\text{eff}}$ vs δ_{ex} plot almost disappears (dashed-dotted and dashed red curves in Figure 2D).

Exploiting the sensitivity of the concentration dependence of $^{15}\text{N}-R_{2,\text{eff}}$ rates to both the rate of exchange and the strength of the applied spin-lock RF field illustrated in Figure 2C, we globally fit the $^{15}\text{N}/^{13}\text{C}\alpha-\delta_{\text{ex}}$ and $^{15}\text{N}-R_{2,\text{eff}}$ data, acquired for full-length htt^{ex1} over a concentration range from 0.1 to 1.4 mM, to the three-state tetramerization model $\text{E} \rightleftharpoons \text{E}_2 \rightleftharpoons \text{E}_4$ (see Supporting Information, “Materials and Methods” for details of NMR sample preparation, NMR experiments, and procedures of data analysis). The $^{15}\text{N}-R_{2,\text{eff}}$ data were obtained from on-resonance $^{15}\text{N}-R_{1\rho}$ experiments at three spin-lock RF field strengths (750, 1500, and 3000 Hz). This analysis yielded robust estimates of the optimized exchange parameters: the equilibrium dissociation constants, $K_1^{\text{diss}} = 65 \pm 2 \text{ mM}$ and $K_2^{\text{diss}} = 34 \pm 2 \mu\text{M}$; the dissociation rate constants, $k_{-1} = 4.4(\pm 0.4) \times 10^4 \text{ s}^{-1}$ and $k_{-2} = 2.3(\pm 0.2) \times 10^4 \text{ s}^{-1}$; and the derived second-order association rate constants, $k_1 = 6.8(\pm 0.5) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $k_2 = 6.7(\pm 0.8) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (Figure 3A). While the value of K_1^{diss} compares well with that from our previous study ($\sim 70 \mu\text{M}$), the value of K_2^{diss} is $\sim 40\%$ higher than that reported earlier ($\sim 24 \mu\text{M}$).¹⁴ We attribute this discrepancy to slight variations in preparation of (aggregation-prone) NMR samples that are beyond our control. Note that the 40% increase in the value of

K_2^{diss} corresponds to a decrease in the tetramer population of only ~0.8% at a concentration of 1.2 mM. Selected examples of ^{15}N -/ $^{13}\text{C}\alpha$ - δ_{ex} and ^{15}N - $R_{2,\text{eff}}$ concentration profiles for Lys5, Lys8, and Glu11 are shown in Figure 3B,C, respectively, while the fits to all the experimental data included in analysis are shown in the Supporting Information (Figures S1 and S2, respectively). It is important to note that the equilibrium parameters (K_1^{diss} , K_2^{diss} and the fractional populations of E_2 and E_4), as well as the differences in chemical shifts, ω , can be extracted with a high level of confidence from analysis of the $^{15}\text{N}/^{13}\text{C}\alpha$ δ_{ex} data alone (Figure S3), albeit with uncertainties in their values higher by almost an order of magnitude compared to those obtained from the analysis of the combined δ_{ex} and $R_{2,\text{eff}}$ data set. The ^{15}N - $R_{2,\text{eff}}$ concentration series obtained with several spin-lock RF field strengths are, however, indispensable for reliable determination of the exchange rate constants.

Panels A and B of Figure 4 show correlation plots comparing the ^{15}N - and $^{13}\text{C}\alpha$ - ω values extracted from the fit of the combined $\delta_{\text{ex}}/R_{2,\text{eff}}$ data set (y -axis) versus the values reported previously (x -axis).¹⁴ There is good agreement between the two sets of ω values but the uncertainties are reduced ~2-fold for the combined $\delta_{\text{ex}}/R_{2,\text{eff}}$ fit, validating the approach described here (see Supporting Information, Table S1, for the full list of optimized $^{15}\text{N}/^{13}\text{C}\alpha$ - ω values). Note the assumption that $^{15}\text{N}/^{13}\text{C}\alpha$ chemical shifts for the dimer E_2 and tetramer E_4 are the same, used in this work as well as in the previous studies,^{4,14} is fully justified as secondary $^{15}\text{N}/^{13}\text{C}\alpha$ backbone shifts, occurring as a result of the transition from an intrinsically disordered monomer to a coiled-coil helical dimer/tetramer, are the main determinants of ω . Figure 4B shows the experimental concentration series of ^{15}N - $R_{2,\text{eff}}$ rates plotted versus the absolute values of ^{15}N - δ_{ex} , for Glu4, the residue with the largest ^{15}N - $|\omega|$ value. The predicted curvature of the plot is clearly observed for the two lower spin-lock RF field strengths (750 and 1500 Hz) reflecting the interesting inherent property of two-step tetramerization kinetics (see Figure 2).

The optimized values of the kinetic and equilibrium parameters of htt^{ex1} tetramerization are robust with respect to the number of spin-lock RF field strengths employed (a minimum two is recommended; see Table S2), as well as the range of protein concentrations used, as long as a substantial concentration dependence of $R_{2,\text{eff}}$ relaxation rates is present in the data (see Table S3).

Numerical simulations consisting of global fits of sets of synthetic $R_{1\rho}$ relaxation dispersion data at a small number (2 or 3) of htt^{ex1} concentrations in conjunction with experimental $^{15}\text{N}/^{13}\text{C}\alpha$ δ_{ex} concentration profiles, indicate that the approach described here (namely, a small number of spin-lock RF field strengths employed for $R_{1\rho}$ measurements together with extensive sampling of concentrations) is more robust and efficient for extracting reliable kinetic parameters of exchange. As might be expected, extensive sampling of effective RF fields to generate $R_{1\rho}$ relaxation dispersion profiles with reduced sampling of concentrations is not as informative and time efficient in fast exchange processes with strongly concentration-dependent kinetics.

In conclusion, we have described a NMR approach for obtaining a quantitative description of the kinetics of very fast exchange processes ($\tau_{\text{ex}} < 50\text{--}100 \mu\text{s}$) associated with transient,

reversible protein oligomerization and demonstrated its utility with regard to the tetramerization of Huntingtin exon 1. While the equilibrium parameters describing oligomerization can be established from the concentration dependence of exchange-induced chemical shifts alone (Figure S3), on-resonance $R_{1\rho}$ measurements conducted as a function of protein concentration at several RF field strengths are indispensable for determining the rate constants of interconversion between the monomeric species and higher order oligomers (Figure 3).

The approach described here should serve as an important complement to the pool of available NMR techniques for characterizing early transient events in protein aggregation pathways. Although analysis is restricted here to ^{15}N $R_{1\rho}$ relaxation, it can be extended to higher gyromagnetic-ratio (γ) nuclei. As higher RF field frequencies are accessible for higher γ nuclei, it can be expected that even faster oligomerization processes can be characterized via concentration-dependent $^{13}\text{C}\alpha$ or amide ^1H $R_{1\rho}$ measurements.²⁰

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Kinetic model of prenucleation, transient htt^{ex1} oligomerization. (A) Kinetic scheme: the “on-pathway” fast branch ($\tau_{\text{ex}} < 50 \mu\text{s}$) leading to the formation of the tetramer E_4 via the dimer E_2 is shown in black, while the slow “off-pathway” process ($\tau_{\text{ex}} \sim 750 \mu\text{s}$) leading to the formation of the “nonproductive” dimer, E_2^* , is shown in gray. The equilibrium dissociation constants, as well as the expressions for the pseudo-first-order, apparent association rate constants, are shown beneath the scheme. The fractional populations of each species indicated above the scheme correspond to $[\text{htt}^{\text{ex1}}] = 1.2 \text{ mM}$. (B) ^{15}N exchange-induced chemical shifts ($^{15}\text{N}-\delta_{\text{ex}}$) and (C) ^{15}N transverse spin relaxation rates ($^{15}\text{N}-R_{2,\text{eff}}$) calculated for the “off-pathway” (gray) and “on-pathway” (red) branches and the full 4-state scheme of htt^{ex1} oligomerization (black) as a function of htt^{ex1} concentration. $^{15}\text{N}-R_{2,\text{eff}}$ values were obtained from on-resonance $R_{1\rho}$ rates calculated for a spin-lock RF field strength of 1 kHz. Dashed horizontal lines are drawn at zero for comparison. The following set of exchange parameters derived from our previous study of htt^{ex1} oligomerization,¹⁴ was used in all simulations: $k_1 = 5.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 4.1 \times 10^4 \text{ s}^{-1}$, $k_2 = 8.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-2} = 2.0 \times 10^4 \text{ s}^{-1}$, $k_3 = 5.9 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-3} = 1350 \text{ s}^{-1}$; $^{15}\text{N}-\omega = -1.1 \text{ ppm}$ for the “off-pathway” dimer and -3.1 ppm for the “on-pathway” dimer E_2 and tetramer E_4 (assumed the same); and $^{15}\text{N}-R_2 = 5.0 \text{ s}^{-1}$, while the R_2 of the dimeric and tetrameric species were assumed to be $2R_2$ and $4R_2$, respectively.

Figure 2.

Simulations of the concentration dependence of various kinetic and experimental parameters for “on-pathway” tetramerization. (A) Dependence of the overall exchange rate, $k_{\text{ex}}^{\text{overall}}$, on protein concentration simulated for three different cases of exchange: (i) concentration-independent (green), (ii) linearly concentration-dependent apparent association rate constant (dimerization; blue), and (iii) tetramerization via a dimer intermediate according to the scheme shown in black in Figure 1A. (B) Simulated dependence of $^{15}\text{N}-\delta_{\text{ex}}$ values on protein concentration calculated for a series of k_2 values as indicated on the plot. (C) Simulated dependence of $^{15}\text{N}-R_{2,\text{eff}}$ rates on protein concentration calculated for $k_2 = 20\,000\text{ s}^{-1}$ (dashed lines) and $50\,000\text{ s}^{-1}$ (continuous lines). $^{15}\text{N}-R_{2,\text{eff}}$ values calculated at the spin-lock field strengths of 750, 1500, and 3000 Hz, are shown in red, green, and blue, respectively. (D) Simulated values of $^{15}\text{N}-R_{2,\text{eff}}$ plotted versus the absolute values of $^{15}\text{N}-\delta_{\text{ex}}$ for the same range of protein concentrations as in (A)–(C) (0–1.2 mM). The values calculated at the spin-lock field strengths of 750, 1500, and 3000 Hz, are shown with continuous, dash-dotted, and dashed red curves, respectively, with the rest of the color coding as in (A). All calculations were performed using the simplified, three-state model of exchange shown in black in Figure 1A. The same parameters of exchange as listed in Figure

1 were used in all simulations. In all the instances where k_{-2} is varied (as in (B) and (C)), K_2^{diss} was kept fixed at $24 \mu\text{M}$ to leave the dimer–tetramer equilibrium unperturbed.

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Figure 3.

Global analysis of concentration-dependent $^{15}\text{N}-R_{2,eff}$ and $^{15}\text{N}/^{13}\text{C}\alpha-\delta_{ex}$ values obtained for htt^{ex1}. (A) The simplified kinetic model of “on-pathway” oligomerization of htt^{ex1}. The optimized values of the equilibrium dissociation constants (K_1^{diss} , K_2^{diss}) and dissociation rate constants (k_{-1} , k_{-2}), together with the derived values of the second-order association rate constants (k_1 , k_2) obtained from the global fit are indicated beneath the scheme. The populations of each species indicated above the scheme correspond to those at [httex¹] = 1.2 mM. Examples of (B) $^{15}\text{N}/^{13}\text{C}\alpha-\delta_{ex}$ and (C) $^{15}\text{N}-R_{2,eff}$ values obtained over the range of htt^{ex1} concentrations between 0.1 and 1.4 mM (800 MHz, 5 °C). $^{15}\text{N}-R_{2,eff}$ values obtained at RF spin-lock field strengths of 750, 1500, and 3000 Hz are shown in red, green, and blue, respectively. The experimental data are shown as circles; the continuous lines represent the best global fit to the kinetic model shown in (A). The full δ_{ex} and $^{15}\text{N}-R_{2,eff}$ experimental data sets (and best fit curves) are provided in the Supporting Information (Figures S1 and S2, respectively). The overall reduced χ^2 of the global fit is 2.25 with errors of 0.5 Hz for δ_{ex} and 0.5 s⁻¹ for $R_{2,eff}$. See the Supporting Information, “Materials and Methods” for experimental details and a description of the fitting procedure.

Figure 4. Correlation plots comparing (A) ^{15}N - ω and $^{13}\text{C}\alpha$ - ω values (ppm) between the oligomeric (E_2/E_4) and monomeric (E) species of htt^{ex1} obtained in this work (y -axis) with those obtained previously using the full four-state model of exchange, x -axis.¹⁴ The correlation coefficients are indicated in the right lower corner of the plots. Error bars (± 1 SD) for the x and y axes are shown in blue and red, respectively. (B) Experimental $^{15}\text{N}-R_{2,\text{eff}}$ values obtained for htt^{ex1} concentrations from 0.1 to 1.4 mM plotted versus the absolute values of $^{15}\text{N}-\delta_{\text{ex}}$ for Glu4 of htt^{ex1} . Experimental curves corresponding to RF spin-lock field strengths of 750, 1500, and 3000 Hz are shown in red, green, and blue, respectively.