Thermodynamics of the Interaction between Alzheimer’s Disease Related Tau Protein and DNA

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Abstract

Tau hyperphosphorylation can be considered as one of the hallmarks of Alzheimer’s disease and other taupathies. Besides its well-known role as a microtubule associated protein, Tau displays a key function as a protector of genomic integrity in stress situations. Phosphorylation has been proven to regulate multiple processes including nuclear translocation of Tau. In this contribution, we are addressing the physicochemical nature of DNA-Tau interaction including the plausible influence of phosphorylation. By means of surface plasmon resonance (SPR) we measured the equilibrium constant and the free energy, enthalpy and entropy changes associated to the Tau-DNA complex formation. Our results show that unphosphorylated Tau binding to DNA is reversible. This fact is in agreement with the protective role attributed to nuclear Tau, which stops binding to DNA once the insult is over. According to our thermodynamic data, oscillations in the concentration of dephosphorylated Tau available to DNA must be the variable determining the extent of Tau binding and DNA protection. In addition, thermodynamics of the interaction suggest that hydrophobicity must represent an important contribution to the stability of the Tau-DNA complex. SPR results together with those from Tau expression in HEK cells show that phosphorylation induces changes in Tau protein which prevent it from binding to DNA. The phosphorylation-dependent regulation of DNA binding is analogous to the Tau-microtubules binding inhibition induced by phosphorylation. Our results suggest that hydrophobicity may control Tau location and DNA interaction and that impairment of this Tau-DNA interaction, due to Tau hyperphosphorylation, could contribute to Alzheimer’s pathogenesis.

Introduction

Tau is a microtubule associated protein. It participates in the microtubule stabilization and organization system which regulates cellular morphogenesis, cytoskeleton functionality and axonal transport [1–6]. Alternative splicing gives rise to six isoforms expressed from the same gene in the CNS [7]. Tau protein contains a large number of serine and threonine phosphorylation sites. Hyperphosphorylation can be considered as one of the hallmarks of Alzheimer’s diseases and other taupathies [1,8–11]. Together with the extracellular senile plaques, the intracellular tangles composed mainly of Tau protein, forming the paired helical filaments (PHFs), are the second type of aberrant proteinaceous aggregates found associated to Alzheimer’s disease [12–15]. Tau is a highly soluble protein devoid of any well-defined secondary or tertiary structure, as many other proteins prone to aggregation and fibrillation also involved in neurodegenerative diseases. A survey of Tau literature leads one to conclude that aggregation and hyperphosphorylation must have a particular role in the neurodegenerative processes [16,17]. However, a precise knowledge of those particular molecular events involving Tau protein in Alzheimer’s disease yet remains elusive.

The interaction of Tau protein with DNA in vitro [18–23] and in situ, in neuronal [24] and non-neuronal [25] cells has been repeatedly reported. Although this protein is mainly found in the cytosol of neuronal cells, it has also been localized within the nucleus of neuronal cells [24,26–31] and non-neuronal cells [25,32–34]. Tau protein has also been found localized at ribosomes and plasma membrane of neuronal cells [35,36]. The post-translational modification of the different isoforms of Tau together with its complex distribution in different cellular locations suggests a multifunctional role for this protein. Results concerning the Tau-microtubule interaction suggest that phosphorylation is involved in the regulation of Tau function. It was long time ago reported that phosphorylation produces a diminution in the affinity of Tau for the microtubule lattice [37,38]. More recently, Tau phosphorylation by GSK3 has been reported to affect its axonal transport [39,40]. Many other Tau functions such as interneuronal signaling, mitochondrial mobility or interaction with synaptic proteins have also been reported to be affected by phosphorylation
(see reference [41] for review). Therefore we would say that Tau phosphorylation seems to be the general mechanism by which Tau function is regulated.

As for the nuclear location, studies on non-neuronal cells confer Tau an important role in nucleolar structure conformation and heterochromatinization of ribosomal genes [23,34]. The role that it might play in the nucleus of neurons remains, however, to be elucidated, as well as the plausible physiological consequences derived from its interaction with DNA. A function related to protection of genomic integrity has been recently assigned to Tau protein [24]. These authors report that, similarly to the reversible nuclear translocation associated to dephosphorylation of many transcription factors (see [42] and references inside), oxidative or heat stress induce accumulation of dephosphorylated Tau protein within the nuclei of neurons. In addition, that nuclear translocation of Tau correlates with an increase of Tau-DNA interaction and DNA protection from heat stress damage [24]. In agreement with this observation, a recent report from our laboratory describes how Tau confers thermodynamic stability properties to DNA which are similar to those provided by histone, as deduced from in vitro experiments [43]. Similarly to the Tau-microtubule interaction, nuclear translocation of Tau is also regulated by phosphorylation, as many other Tau functions. However, the influence that phosphorylation may exert on the Tau-DNA interaction still remains unclear. Hua and He reported that phosphorylation of Tau with cdc2-like kinases did not affect the interaction with DNA [44]. A recent publication of Lu et al, however, describes how phosphorylation prevents Tau from DNA binding [45].

It seems that Tau translocation and the subsequent DNA binding and protection is reversible. It means that Tau binding to DNA must be controlled by the concentration inside the nucleus. The results we present here prove that, similarly to the Tau-microtubules interaction, Tau-DNA interaction is indeed dependent on the phosphorylation state of the protein, as shown by surface plasmon resonance and Tau expression in human embryonic kidney 293 (HEK) cells. Unphosphorylated, weakly aggregated forms of Tau, bind DNA reversibly, strengthening the concept of a functional regulatory role for Tau protein. The results of this reversible interaction characterized by means of surface plasmon resonance, allowed us to measure ΔG, ΔH and ΔS values, which suggest hydrophobicity must represent an important contribution to the Tau-DNA stability complex. Hyperphosphorylation of Tau could impair this interaction, therefore contributing to the Alzheimer’s pathogenesis.

Results

Tau expression in HEK cells gives rise to phosphorylation in proliferating cells

Figure 1 shows the DNA and Tau localization in human embryonic kidney (HEK) cells expressing Tau protein in a stable form. Immunofluorescent confocal microscopy was used to study the distribution of both molecules in different populations of these cells. Staining with Tau5, an antibody which recognizes both phosphorylated and unphosphorylated forms of the protein, shows that Tau is mainly located in the cytosol of non-dividing cells (Fig. 1A). Nevertheless, Tau is also present inside the nuclei, although it seems to represent a minor proportion of total Tau. On the other hand, there are some Tau-42-labeled cells that have been pictured in the middle of division, displaying a highly condensed DNA (see Fig. 1A, TOPRO). In these cases where nuclear membrane disappears, certain proportion of labeled Tau protein (see arrows within Fig. 1A) share the same regions occupied by chromosomes, suggesting that a fraction of Tau colocalizes with DNA at some stages of cell division. A distinction between DNA (red in gray scale) and Tau (green in gray scale) starts to show up, still indicating that a fraction of Tau does not colocalize with DNA.

In Fig. 1B, PHF-1 antibody recognizes Tau molecules which are phosphorylated in serine 396 and 404. It can be observed that, as chromosomes starts to condensate in replicating cells, there is a clear distinction between DNA (red in gray scale) and phosphorylated Tau (green in gray scale). Interestingly, PHF-1 signal increases when the division process is started, what means that most of Tau recognized by Tau5 antibody must be progressively phosphorylated from the onset of cell cycle by nuclear kinases. The fact that phosphorylated Tau does not colocalize with condensed chromosomes in those situations where nuclear membrane disappears, suggests that phosphorylation would play a putative role preventing Tau-DNA interaction in replicating cells, similarly to the previously described microtubule binding inhibition induced by phosphorylation [37,38].

Effect of phosphorylation on the Tau-DNA interaction in vitro

Tau-DNA interaction in vitro was studied by flowing Tau solutions on a DNA activated sensor surface assembled on a surface plasmon resonance instrument. As it can be seen in part A of Figure 2, the unphosphorylated form of Tau binds DNA, as we have previously reported [43]. The reflectance-increase observed after injecting the Tau solution denotes the DNA-binding of the unphosphorylated form of Tau, purified from expression in E.coli. Phosphorylated Tau-42 expressed in Sf9 cells only shows a small interaction with DNA, as can be seen in part A of Figure 2. The negative charge supplied by the phosphate groups are plausibly the cause of this loss of DNA binding capability showed by the phosphorylated form of Tau. Part B of the same Figure bears out it. As compared to the unphosphorylated form of Tau, the phosphorylated form also shows a small interaction with heparin.
Basic amino acids of phosphorylated Tau form might be responsible for these residual interactions with heparin and DNA. Part C of the same Figure supports this. A mixture composed of DNA and histone was immobilized on the sensor surface. Unphosphorylated form of Tau is readily immobilized on this surface. However, the phosphorylated form does not bind this histone-DNA complex. The previously shown residual binding of phosphorylated Tau to DNA disappeared. These results suggest that Tau may be phosphorylated on those sites closely related to the interaction with DNA.

The loss of interaction with DNA induced by Tau phosphorylation does not seem to be related to dramatic changes in the aggregation state of the protein. The UV spectrum does not show the acute light scattering profile usually found for large aggregating protein forms (see Figures S1 and S2 in File S1). Besides that, phosphorylated Tau could be bound to polylysine activated surfaces, displaying a layer thickness slightly larger than that for
the unphosphorylated form (Figure S3 in File S1), which indicates that oligomers within the range of 1 to 2 nm can be formed but no large fibrillar aggregates.

The thermodynamics of the unphosphorylated Tau-DNA interaction, as probed by Surface Plasmon Resonance

As shown in part A of Figure 3, the reflectance-increase value derived from the Tau-DNA interaction results to be close to that reflectance-increase value corresponding to the last polylysine layer of the PHP structure routinely used in the sensor surface activation. This means that, according to the reported thickness value of the polylysine layer [46], the reflectance-increase observed when Tau is flowed on DNA must correspond to the building of a Tau layer on DNA, mainly composed of unfolded monomers or small Tau oligomers about 1 nm thick. Tau binding to DNA is reversible. Part A of Figure 3 shows how the binding of Tau to DNA is followed by the dissociation of the complex, when running buffer is injected to flow through the sensor cell. The detailed time dependence of complex formation as well as the complex dissociation are shown in parts B and C of the same Figure.

Table 1. Kinetic constants for the Tau-DNA complex formation ($k_1$) and complex dissociation ($k_{-1}$) and the corresponding equilibrium constants, obtained as $k_1/k_{-1}$, at different temperatures.

| Temperature (°C) | $k_{-1}$ (min⁻¹) | $k_1 \times 10^{-6}$ (M⁻¹min⁻¹) | $K_{eq} \times 10^{-7}$ (M⁻¹) |
|----------------|------------------|-------------------------------|-----------------------------|
| 13             | 0.17             | 5                              | 2.9                         |
| 15             | 0.13             | 5                              | 3.8                         |
| 18             | 0.27             | 4                              | 1.5                         |
| 19             | 0.22             | 3.3                            | 1.5                         |
| 21             | 0.36             | 5.3                            | 1.5                         |
| 21             | 0.14             | 4.8                            | 3.4                         |
| 22             | 0.24             | 5.2                            | 2.2                         |
| 22             | 0.15             | 6.1                            | 4.1                         |
| 26             | 0.4              | 7.4                            | 1.8                         |
| 32             | 0.32             | 3.2                            | 1                           |
| 33             | 0.33             | 4.9                            | 1.4                         |
Analysis of the dissociation process following a first order kinetics yields the first-order constant \( k_1 \). Analysis of the complex formation following a pseudo-first order kinetics, as described under Materials and Methods, yields then the second order rate constant, \( k_2 \) (inset of part B). Quotient of constants, \( k_1/k_2 \), renders the equilibrium constant for the Tau-DNA complex formation, \( K_{eq} = (2.4 \pm 0.4) \times 10^7 \) M\(^{-1} \) (± SEM), obtained as the average from six determinations between 10 °C and 22 °C. This equilibrium constant is calculated under the assumption of the simple mechanism of interaction described in Materials and Methods for the Tau-DNA complex formation. As can be observed in part A of Figure 4, increasing concentration values of Tau in those solutions flowing through the sensor cell give rise to increasing surface saturation. It means that the DNA-activated sensor surface is saturated by running Tau, in a concentration dependent manner, due to the reversibility of the interaction. The saturation fraction, \( Y \), can then be obtained from equation (11) as a function of the Tau protein concentration (equation 10). An inverse plot of this hyperbolic equation renders a straight line from which the Equilibrium Constant for the complex formation, \( K_{eq} = (2.3 \pm 0.6) \times 10^7 \) is obtained.

The Equilibrium Constant value calculated from equilibrium data does not depend on any kinetic assumption and agrees well with that value obtained previously from the kinetic model, \( K_{eq} = (2.4 \pm 0.4) \times 10^7 \) M\(^{-1} \), therefore supporting the one-step mechanism of interaction proposed in the Materials and Methods section. The kinetic model used to obtain the rate constants for the complex formation and dissociation, \( k_1 \) and \( k_{-1} \), was then used to study the dependency of Equilibrium Constants values on temperature, within the range of 13 °C to 33 °C. The results, including those obtained between 18 °C and 22 °C are included in Table 1. It can be seen that Equilibrium Constant for the complex Tau-DNA barely changes within the selected range of temperature. A Vant’ Hoff plot of the temperature dependency within this range (Figure 5) renders an enthalpy change for the complex formation, \( \Delta H^\circ = -32 \pm 15 \) kJ mol\(^{-1} \). The potential energy decrease associated to Tau-DNA complex formation is given by \( \Delta G^\circ = -RT \ln K_{eq} = -41.4 \pm 0.5 \) kJ mol\(^{-1} \) at 20 °C. Accordingly, the entropy change, following \( \Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \), results to be close to zero, \( \Delta S^\circ = 0.03 \pm 0.05 \) kJ mol\(^{-1}\) K\(^{-1} \). Tau protein lacks of a well-defined secondary or tertiary structure solution. It means that Tau may adopt a huge number of possible conformations for the polypeptide chain, each of them housing, in turn, a huge number of quantum states. Therefore, immobilization of Tau on the DNA molecule would have to involve a decrease in the number of quantum states available to Tau, with the corresponding large decrease of entropy. The positive, almost null value for the entropy variation obtained suggests that, together with the large diminution of entropy derived from the Tau immobilization on DNA, there must be a compensating large increase in the number of quantum states accompanying the Tau-DNA complex formation. This entropy increase may have its origin in the release of water molecules which would confer a strongly hydrophobic character upon the Tau-DNA complex formation.

Discussion

We describe here the interaction of unphosphorylated Tau with calf thymus DNA, immobilized on a sensor surface. It has been shown to be reversible and, according to the reflectance value increase, mostly attributable to monomeric or small oligomeric forms of the protein. Surface plasmon resonance supplies interaction data in real time. It allows therefore the quantitative analysis of the interaction from a kinetic point of view. The second order rate constant for the association of Tau with DNA, \( k_1 \), and the first-order rate constant for the complex dissociation, \( k_{-1} \), can be obtained from the time-dependency analysis of the reflectance changes registered. Equilibrium constant for the complex formation, \( K_{eq} = k_1/k_{-1} \), can then be calculated on the assumption of the simple mechanism described in Materials and Methods. On the other hand, the reversibility of the interaction offers the possibility of obtaining the equilibrium constant from data concerning the equilibrium fractional saturation, independently of any assumption on the interaction mechanism. Both equilibrium constants values, the one obtained from kinetic data and that from equilibrium saturation are in good agreement, therefore confirming the simple one-step mechanism of interaction, as well as the absence of any kind of cooperativity within the experimental error of our measurements. The dissociation constant value obtained, \( K_d = 0.04 \) μM, means that Tau binds DNA with a comparable affinity to that shown for the binding to microtubules, \( K_d = 0.1 \) μM as reported by Ackmann et al [47]. A reversible binding of Tau to DNA has also been described by Krylova et al [22], as obtained from a non-equilibrium capillary electrophoresis method. The dissociation equilibrium constant reported by these authors is between 0.3 and 0.9 μM. This different value may be due to the fact that we measure the binding of Tau to an immobilized DNA sample, or to the different size of the molecule selected as a source on DNA: small oligonucleotides in the experiments of Krylova et al, in contrast to calf thymus DNA used in our measurements. Of course, both different experimental conditions might contribute to the different equilibrium constants obtained.

The neuronal concentration of Tau is about 2 μM [48]. According to the Kd values reported here for the DNA interaction with Tau, this concentration would be high enough to saturate reversibly the potential DNA-sites available in vivo. The activity of different types of kinases might additionally affect Tau residues involved in DNA binding. S9 cells can be considered as a good model to simulate the phosphorylation extent of Tau. Tau phosphorylation sites associated to Alzheimer’s disease have been classified into two categories, the KXXG and the SP/TP motifs [49]. Phosphorylation profile of Tau in transfected S9 cells has been shown to be similar to that of neuronal cells [38]. Our results show that phosphorylation due to endogenous kinases of S9 cells somehow induces changes in Tau which alter Tau binding to DNA, in agreement with the lack of colocalization with condensed chromosomes shown in Figure 1 above. Therefore, Tau phosphorylation might regulate the availability of unphosphorylated Tau to bind DNA in a reversible manner. It has been reported that heat stress induces Tau dephosphorylation and nuclear translocation of dephosphorylated Tau, concomitantly with an increase of Tau-DNA interaction and DNA protection. The full process is reversed when the insult ceases [24]. This observation is in agreement with our results showing that binding of dephosphorylated Tau to DNA is reversible. According to our thermodynamic data, changes in the concentration of dephosphorylated Tau available to DNA must be the variable determining the extent of Tau binding and DNA protection. This phosphorylation-dependent regulation of DNA binding is reminiscent of that reported for the Tau-microtubule interaction regulated also by Tau phosphorylation [41,48].

Tau is an intrinsically disordered protein containing 85 phosphorylatable sites, including 45 serines, 35 threonines and 5 tyrosines [50]. It means that the number of differently phosphorylated forms of the protein (2\(^{20} \)) by far exceeds the availability of Tau molecules not only within a neuron but even within the full
leading to a Tau-induced DNA deregulation. disorders by impairing the reversible Tau-DNA interaction and modifications might contribute to the onset of these neurological tauopathies, among which the most known is Alzheimer’s disease. the two main hallmarks found in Tau protein linked to protection. Protein aggregation and massive phosphorylation are irreversible, concomitantly with the process of aggregation. Under these conditions Tau would remain irreversibly bound to DNA in a way that is independent of heat/oxidative insults. On the other hand, massive phosphorylation of Tau, besides depleting the available normal tau in the neuron by sequestering it [59], would prevent it from nuclear translocation and the consequent DNA protection. Protein aggregation and massive phosphorylation are the two main hallmarks found in Tau protein linked to tauopathies, among which the most known is Alzheimer’s disease. If not a coincidence, one would conclude that both Tau modifications might contribute to the onset of these neurological disorders by impairing the reversible Tau-DNA interaction and leading to a Tau-induced DNA deregulation.

### Materials and Methods

#### Chemicals/Reagents

DNA from calf thymus (activated type XV), unfractionated whole histone from calf thymus (type II-A), poly-L-Lysine, heparin and hepes were from Sigma (St. Louis, MO, USA). SPR gold sensorchips were from Xantec Biosciences (Dusseldorf, Germany). PHF-1 antibody was kindly donated by Dr. Davies (Albert Einstein College of Medicine, NY, USA). According to residue numbering of the longest human Tau isoform (441 aa), PHF-1 antibody reacts with Tau when serines 396 and 404 are phosphorylated. Total tau protein was detected with Tau-5 antibody from Abcam (Cambridge, MA, USA). Unless otherwise indicated, all experiments were carried out in a neutral running buffer composed of 10 mM Heps, 0.1 M NaCl, pH 7.

Cell culture HEK 293 Tau cells and immunofluorescence analysis

HEK 293 Tau expressing cells (expressing Tau 3R isoform, a kind gift from Dr. Miguel Medina) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mMGlutamine, 1 mM Piruvate, 100 U/mL penicillin, 100 U/mL streptomycin and 0.2 mg/mL Zeocin in a humidified atmosphere of 5% CO₂/95% air at 37 °C. Proliferating HEK 293 Tau expressing cells were plated on glass coverslips coated with 1 mg/mL poly-L-lysine, maintained in the same culture medium for 24 hours. For immunofluorescence analysis HEK 293 cells were fixed with 4% paraformaldehyde for 20 min at 37 °C and then washed with PBS. Fixed cells were incubated with 1 M glycine for 30 min and then permeabilized with 0.2% Triton X-100 in PBS for 5 min at room temperature. The coverslips were blocked with 1% BSA/PBS for 1 hour at room temperature and incubated in primary antibodies (PHF-1 or Tau-5 1/100) in 1% BSA, in PBS, for 1 hour at room temperature. After washing 3 times with PBS, the secondary antibodies were incubated for 1 hour at room temperature. To-pro-3 1μM Invitrogen, Carlsbad, CA, USA) staining to label nuclei was performed 10 minutes before finishing secondary antibody incubation. Finally, the coverslip were washed 3 times with PBS and once with H₂O, and mounted with FluorSave Reagent (Calbiochem, La Jolla, CA, USA). Fluorescence microscopy was used to measure fluorescence intensity. After staining with PHF-1 antibody, cells were observed on a Zeiss Axiovert 200 fluorescent microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA). PHF-1 antibody fluorescence images were captured through a 100 × lens on a high resolution CCD camera (SPOT-RT Sliders, Diagnostic Instrument Inc, MI, USA).

Tau purification

The detailed purification procedure of the unphosphorylated longest human Tau isoform, (hTau 42) has been described in a previous contribution from this laboratory [43]. After expression in E.coli BL21 (DE3), Tau protein was purified from the supernatant resulting from bacteria sonication and centrifugation. The protocol used was based on that described by Lindwall and Cole [60], which makes use of the heat-and acid-stability of Tau, together with its insolubility in 25% of glycerol solutions. Tau was stored at −20 °C in a buffer composed of Heps 10 mM, NaCl 100 mM and EDTA 1 mM, pH 7. All measurements were carried out after room-temperature incubation for the time period indicated for each experiment. The isolated protein was characterized by electrophoresis, UV-absorbance spectroscopy, and Western blot. Tau protein concentration was obtained by using an extinction coefficient ε₅₄₀ = 7700M⁻¹·cm⁻¹ at 280 nm [61].
Expression and purification of phosphorylated Tau protein

Tau protein, phosphorylated on at least Ser 202, Thr 205 and Thr 212, Ser 214 as shown by antibodies AT8 and AT100, respectively [62], was obtained from an insect cell culture infected with baculoviruses expressing the largest human Tau isoform of 441 amino acids. Sf9 cells were grown at 27 °C in 35 mm dishes, in fetal bovine serum (Invitrogen, Carlsbad, USA) with antibiotics (penicillin and streptomycin). Monolayer cultures of Sf9 cells (3 x 10^7/mL) were infected with baculoviruses encoding Tau-42 cDNA and then incubated for 72 h at 27 °C without CO2 from the time of infection. Infected cells were suspended in 1 mL of 0.1 M Tris, dialyzed against 0.1 M Mes, 2 mM EGTA, 0.5 mM MgCl₂, orthovanadate, 2.5 mM sodium pyrophosphate, 10 mM glycero-phosphate, 100 nM okadaic acid and 0.25 mM PMSF, pH 7.5, and sonicated 2

The differential equation defining both reversible steps is:

\[ v = \frac{d\Gamma(DNAi-Tau)}{dt} = k_1 \Gamma(DNAi)[Tau] - k_{-1} \Gamma(DNAi-Tau) \]  

where [Tau] stands for the Tau protein concentration in the solution running through the flow cell. \( \Gamma(DNAi) \) and \( \Gamma(DNAi-Tau) \) stand for the surface concentration of immobilized DNA on the PHP activated surface and the DNA-Tau complex respectively. Following equation [1],

\[ \Gamma(DNAi) = \Gamma_{max}(DNAi-Tau) - \Gamma(DNAi-Tau) \]  

Substituting equations [3], [4] and [5] into the kinetic equation [2] yields:

\[ \frac{dR}{dt} = k_1[Tau]R_{max}(k_1[Tau] + k_{-1})R \]  

where

\[ \Gamma_{eq} = \frac{\Gamma(DNAi-Tau)}{\Gamma(DNAi)/[Tau]} \]  

The saturation fraction, Y, of the sensor surface comes given by:

\[ \Gamma_{eq} = \frac{\Gamma(DNAi-Tau)}{\Gamma_{max}(DNAi-Tau)} = \frac{\Gamma(DNAi-Tau)}{\Gamma(DNAi-Tau) + \Gamma(DNAi)} \]
Solving equation [7] for $\Gamma(DNAi-Tau)$ and substituting equation into [0] renders:

$$Y = \frac{Keq(DN Ai)|Tau|}{Keq(DN Ai)|Tau| + \Gamma(DN Ai)} = \frac{Keq|Tau|}{Keq|Tau| + 1}$$

Rearranging,

$$Y = \frac{1}{C_{eq}(DNAi)}$$

Equation [10] is the Langmuir-like hyperbolic equation describing the binding of a ligand, Tau, on a set of independent binding sites on the immobilized DNA.

The equilibrium values for $Y$ can be easily obtained by making use of equations [4] and [5]:

$$Y = \frac{\Gamma(DN Ai-Tau)}{R_{max}(DN Ai-Tau)} = \frac{R}{R_{max}}$$

Unless otherwise indicated, values following the $\pm$ symbol represent the standard error of the data as obtained for a linear fitting.

Supporting Information

File S1 SPR immobilization upon polylysine, SDS-PAGE and UV spectra of phosphorylated/unphosphorylated Tau protein.

(DOC)

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Author Contributions

Conceived and designed the experiments: JSJ JA FH SC. Performed the experiments: SC RC FH. Analyzed the data: JSJ SC FH JA. Contributed reagents/materials/analysis tools: SC MJB RC. Contributed to the writing of the manuscript: JSJ SC FH.

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