Supporting Information

We used an in-house software to follow the pairwise intermolecular interactions between the two chains (*i.e* distances between atoms of each molecule below 3.5 Å) during the simulation, as previously described [1], providing a detailed view of the intermolecular persistent contacts. These contacts involve residues Val2, Val4, Leu5, Val8 and Leu9 present at the dimer interface of p8 in more than 90% of the MD trajectory frames (**Fig. 1**). Short intermolecular distances (1.3-3.5 Å) are found between the phenylalanine residue Phe44 from the β3 strand of one monomer with the equivalent phenylalanine from the β3' strand of the other monomer, with close contacts through their HE ring protons observed in almost 100% of the MD trajectory frames, illustrating the importance of this amino acid on dimer stability. Previous studies (and our results, below) showed that a single point mutation of this phenylalanine residue into alanine retains the global fold while resulting in the formation of monomeric state in solution [2]. The same procedure was employed to follow along the MD simulation the persistent contacts between p8(Tfb5) and p52C(Tfb2C), using the crystal structure of the complex (3DOM) as starting structure. Five hydrogen bonds involving the pairs Ile452-Gln11, Tyr454-Leu9, Gly456-Gly7, Leu458-Arg5 and Ser460-Arg3 of Tfb2C and Tfb5, respectively, are present in almost 100% of the MD trajectory frames (**Fig. S1).**

Figure S1. Molecular dynamics simulation of the heterodimer p8(Tfb5)/p52C(Tfb2C). 3D structure showing the persistent intermolecular contacts present in more than 90% of the trajectory frames. The residues involved in the contacts are colored in green (p8) and orange (p52C).

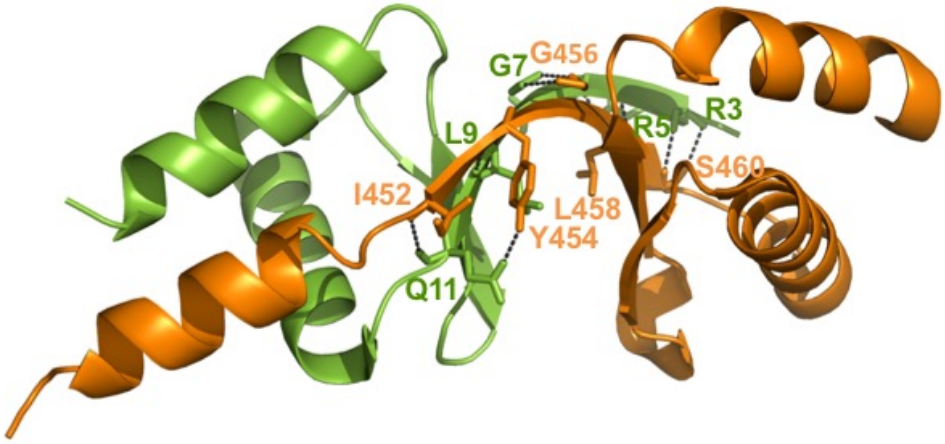


Figure S2. (**a**) Fluorescence-based thermal shift assay data obtained for $p8_{Phe44A}$ in buffer consisting of 50 mM Tris-HCl, 150 mM NaCl and 0.25 mM TCEP at pH 7.5. (**b**) Estimation of the global rotational correlation times for p8 and $p8_{Phe44A}$ (in the presence of compounds **12** and **19**) using TRACT experiments recorded at 298 K. The increase of τ_c for p8 in the presence of compounds **12** and **19** (which inhibits dimer formation) may indicate partial aggregation of the monomers.

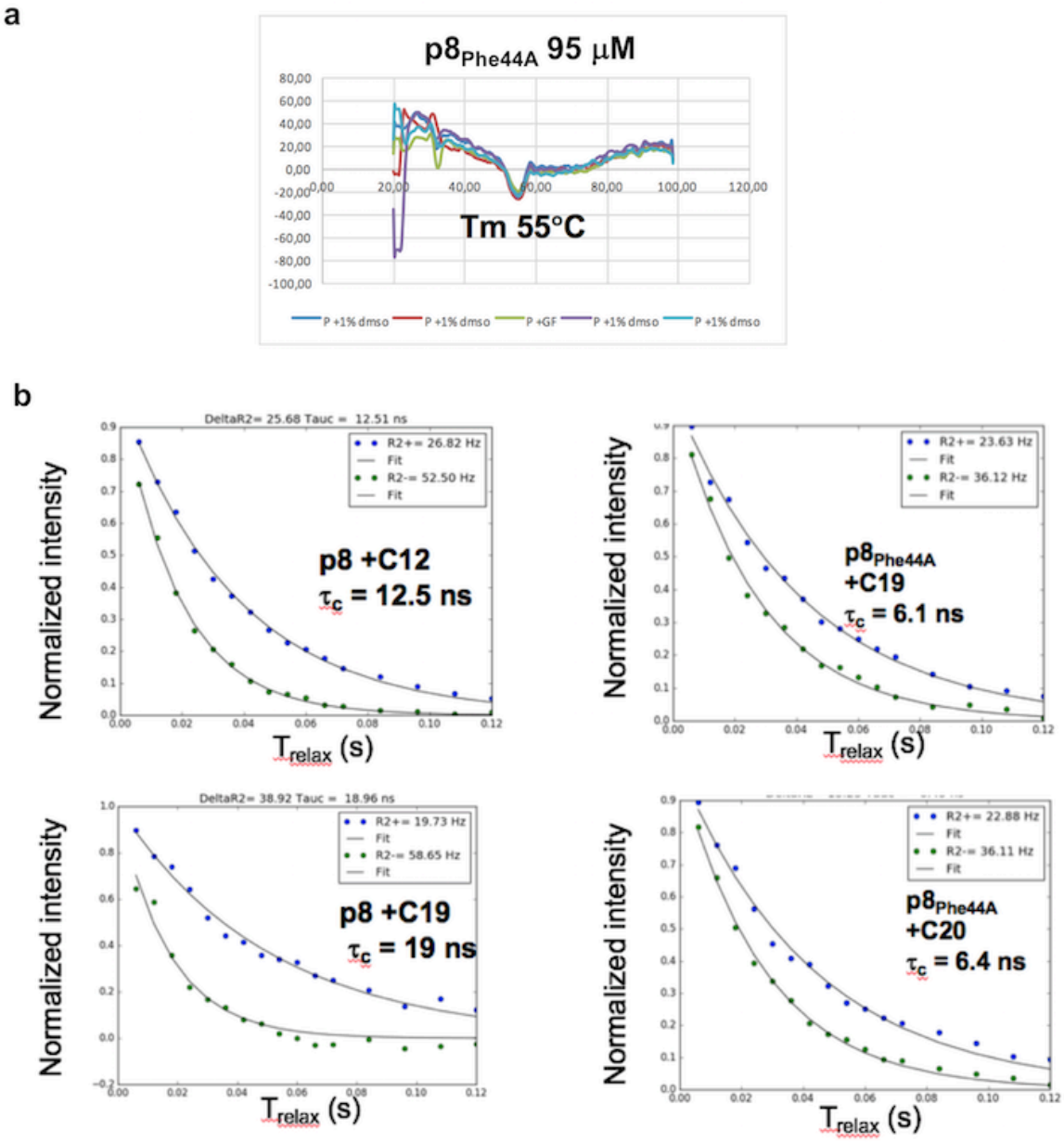


Figure S3. Top-down Mass Spectrometry. (a) Deconvoluted MS spectra of p8 in the apo form (bottom) or after overnight incubation with a 1:2 p8:**C19** molar ratio (middle) or after 270 min incubation with a 1:10 p8:**C19** molar ratio (top). **(b)** MSMS spectrum acquired upon HCD fragmentation of the 7^{+} charge state of p8 (MW: 8364.3 Da; m/z 1196.0) incubated with a p8:**C12** molar ratio of 1.100 for 30 min. Fragments bearing a covalently-bound ligand are shown in red. **(c)** Fragmentation of p8-adduct into b-type fragments (top) and y-type fragments (bottom). This sequence coverage was obtained by combining HCD and CID fragmentation. N-terminal fragments b3 to b13 were unmodified, while fragment b15, b22 and beyond are all covalently-bound to the ligand. C-terminal fragments y3 to y34 were unmodified. This shows that the chemical modification occurs on cys14 or Asp15 residues, most probably on cys14 for obvious chemical reactivity reasons, the cysteine thiol being the most nucleophile moiety as illustrated in **Fig S4b**.

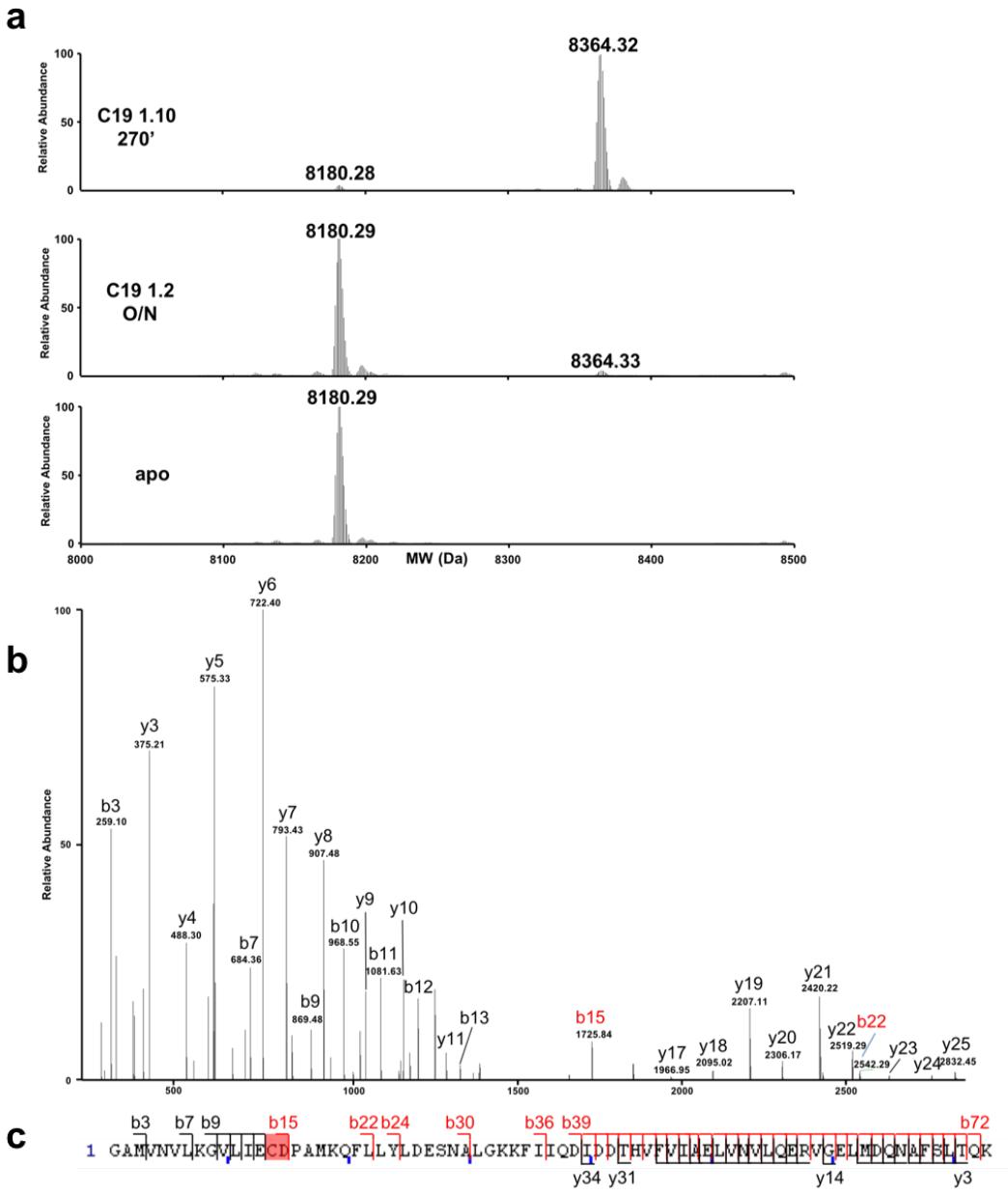
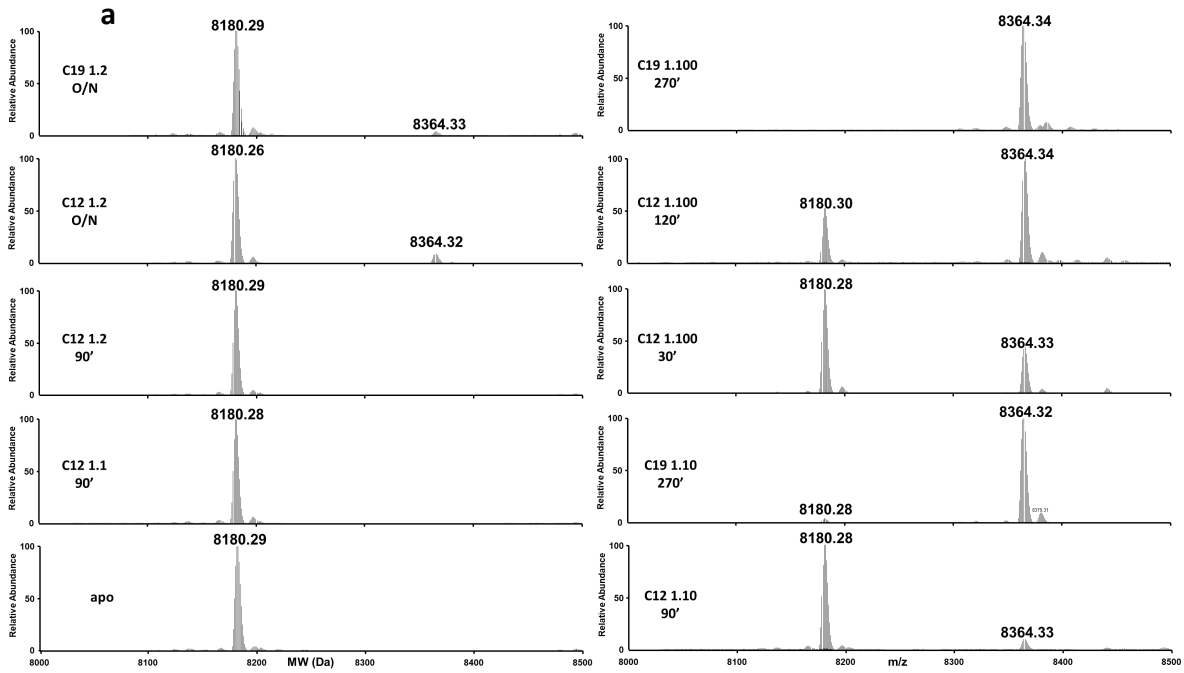


Figure S4. (**a**) Deconvoluted MS spectra of p8 in the apo form or with different p8:ligand molar ratios (1:1, 1:2, 1:10, and 1:100) and incubation times (30 min, 90 min, 120 min, 270 min, 12 hours). (**b**) Molecular mechanism of the chemical reaction between p8 and compounds **12** (C12) and **19** (C19). Since increases in molecular weight observed with compounds 12 and 19 are identical (and equal to 184 Da), compound **12** is likely oxidized into C19 before reaction with p8. The reaction mechanism is a classical nucleophilic addition of the cysteine thiol onto the quinone.



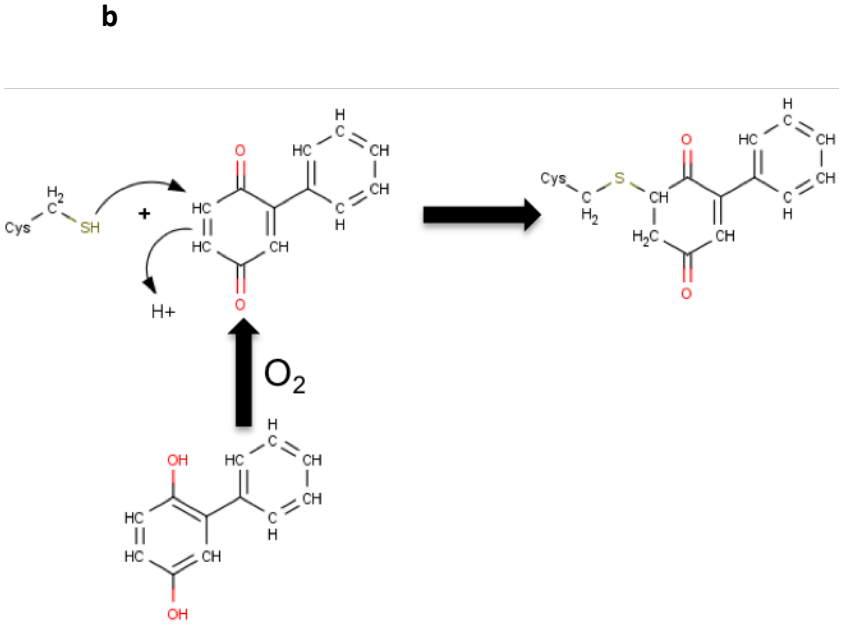


Figure S5. (a) Low and **(b)** high m/z range of the MSMS spectra acquired upon collision-induced dissociation (CID) fragmentation of the 7^{+} charge state of p8 (MW: 8364.3 Da; m/z 1196.0) incubated with compound **12** at a protein:ligand molar ratio of 1:100 for 30 min. Fragments bearing a covalently-bound ligand are shown in red.

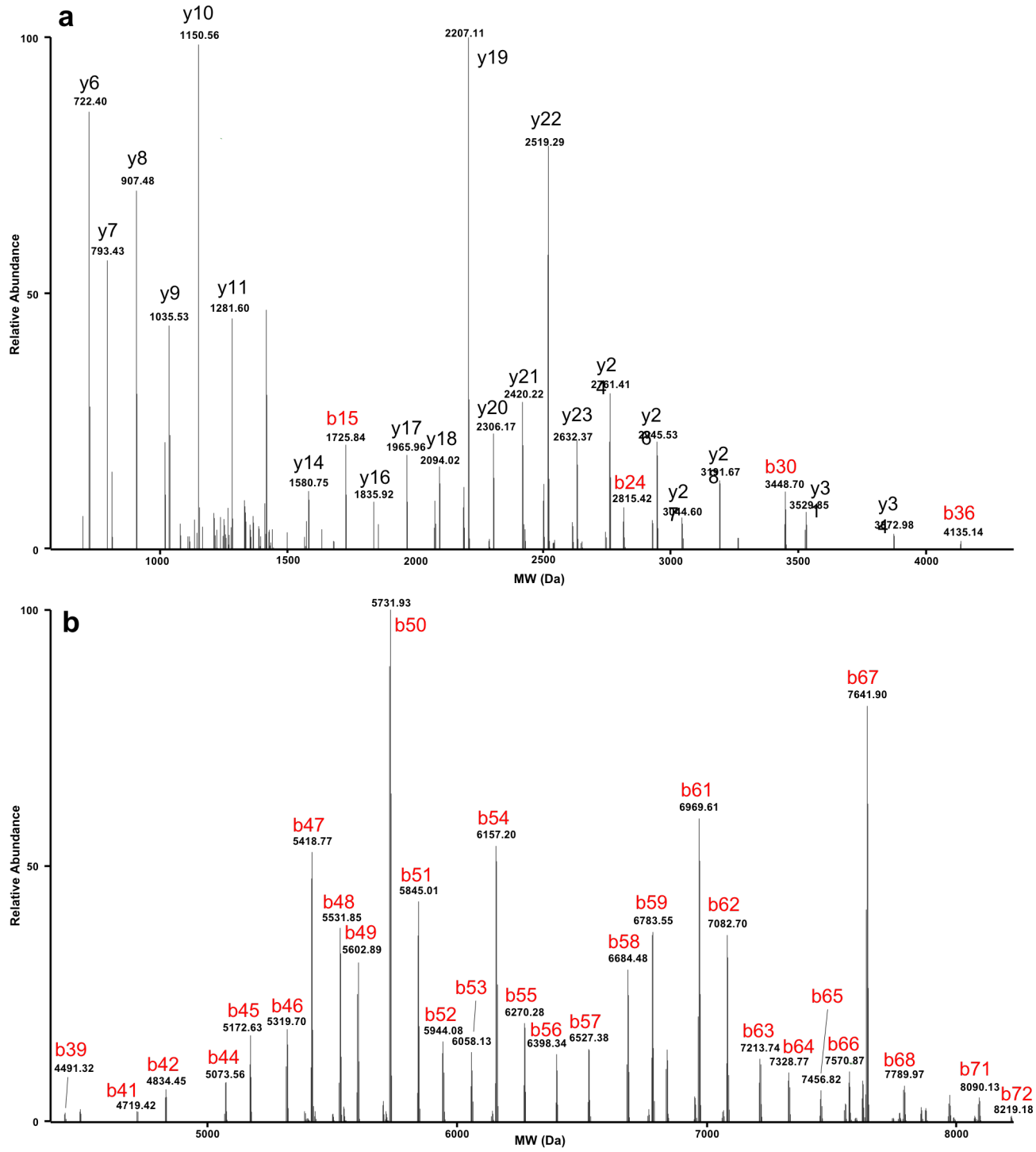


Figure S6. Results for the quantification of TFIIH levels via measurement of total XPB-YFP intensities in the nucleus of cells treated with compounds **10** (blue column) or **12** (green column) for 3, 4, 5 and 6 days.

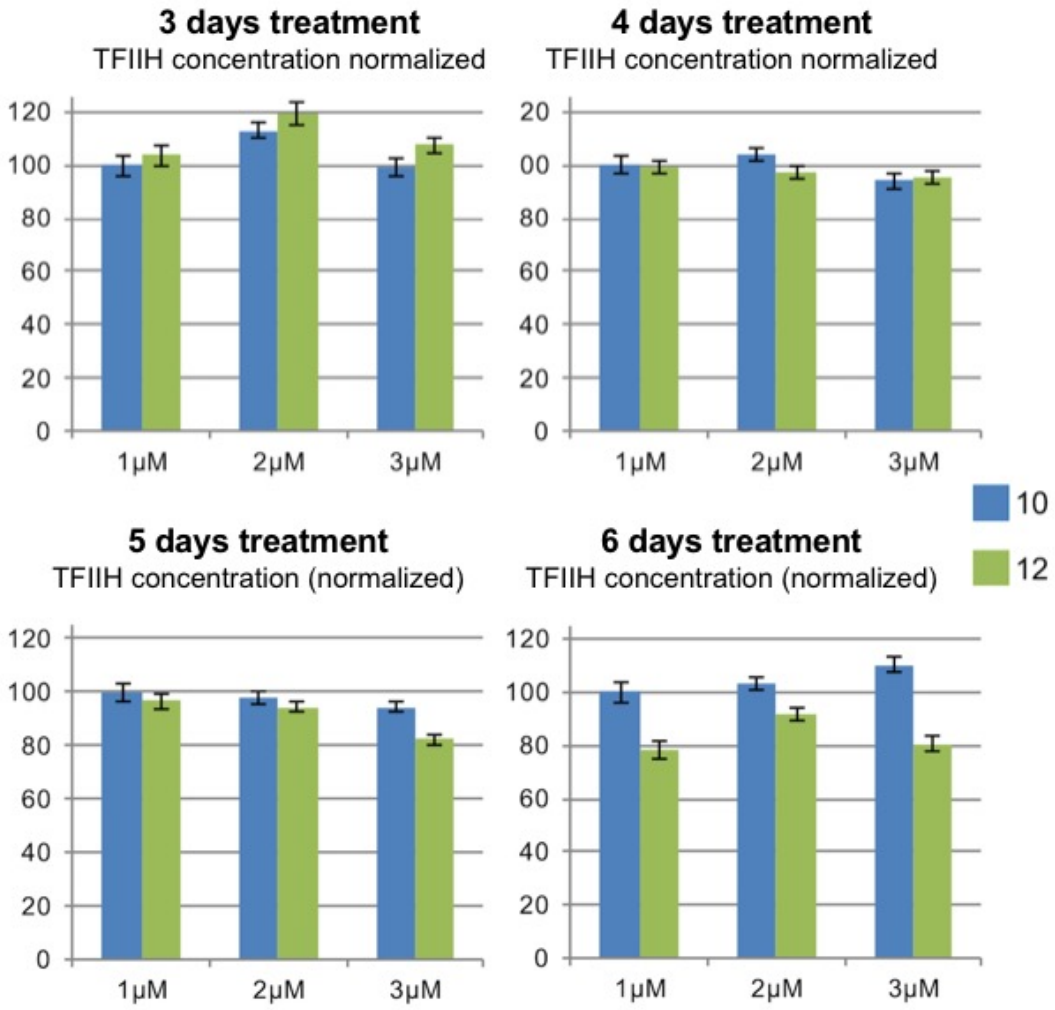


Figure S7. STD curve obtained for increasing concentrations of compound **12** added to p8**.** The fitting of the curve with classical equilibrium binding equation ($A_{STD}=\frac{A_{max}\,[L]}{[L]+K_d}$) leads to a K_d of 172 ± 65 μM. The fit was done using the GraphPad curve fitting module.

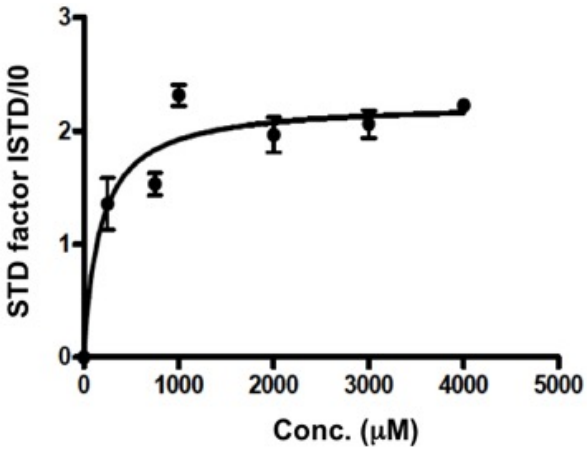


Figure S8. 1D spectra recorded for C12 and C19 (500 μM) without p8 (C12, green and C19, red) and after 12 hours of incubation with p8 (compound **12**, purple and compound **19**, blue)