Supporting Information

Wang and Fersht 10.1073/pnas.1211557109

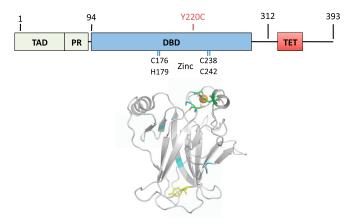


Fig. S1. Schematic of p53 structure. *Top*, location of domains in primary sequence and location of residues that bind Zn^{2+} . *Bottom*, cartoon representation of the structure of the stabilized p53 DNA-binding domain (PDB code 1UOL). Zn^{2+} -binding residues are in green, the four stabilizing mutation in cyan, and Y220 in vellow

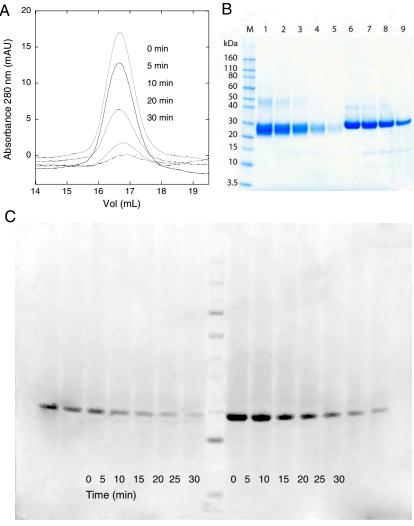
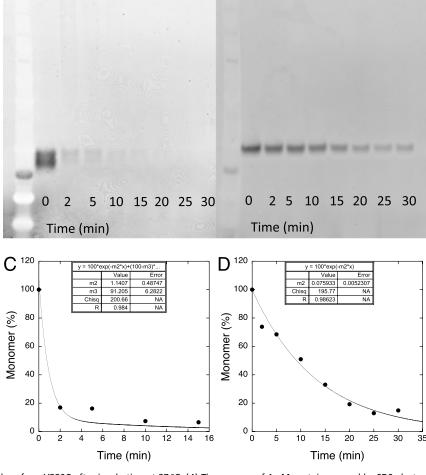


Fig. S2. Gel filtration and gel electrophoresis of samples of Y220C after incubation at 37 °C. (A) Time course of 12 μ M Y220C analysed by gel-fitration. (B) Time courses of 3 μ M protein assayed by SDS-electrophoresis and Coomassie Blue staining of samples that had been crosslinked (lanes 1–5) or not (lanes 6–9) prior to electrophoresis. (C) Time courses of 3 μ M (Left) and 12 μ M (Right) Y220C that were stained with SYPRO®Orange for quantitative analysis (without prior crosslinking).



B

Cross-linked

Fig. S3. SDS-PAGE of samples of apoY220C after incubation at 37 °C. (A) Time course of 1 μ M protein assayed by SDS-electrophoresis and SYPRO®Orange staining of samples that had been crosslinked and (B) not crosslinked prior to electrophoresis. (C) Quantitative analysis of (A), showing loss of monomer is fast. (D) In the absence of crosslinking, soluble protein, appearing as monomer after treatment with SDS, was lost at about 0.076 min⁻¹.



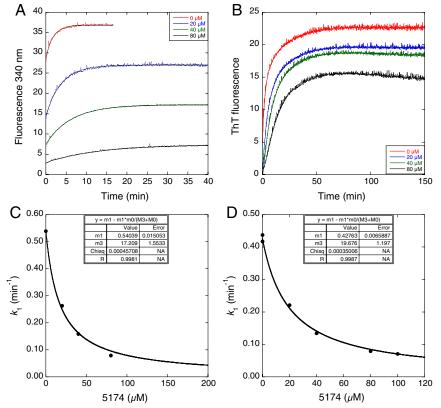


Fig. S4. Inhibition of aggregation of apoY220C by ligand 5174 at 30 °C. (A) and (B) monitored aggregation by fluorescence at 340 nm and ThT binding, respectively. (C) and (D) are inhibition of rate constants for 340 nm fluorescence and ThT binding experiments, respectively.

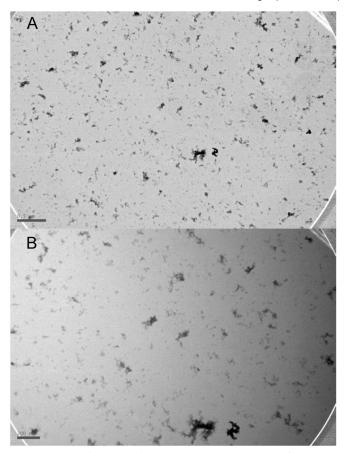


Fig. S5. EM images of aggregate of full-length p53 Y220C (Flp53Y220C) that had been incubated at 37 °C for 16 h. Scale bars in (A) and (B) are 500 and 200 nm, respectively.



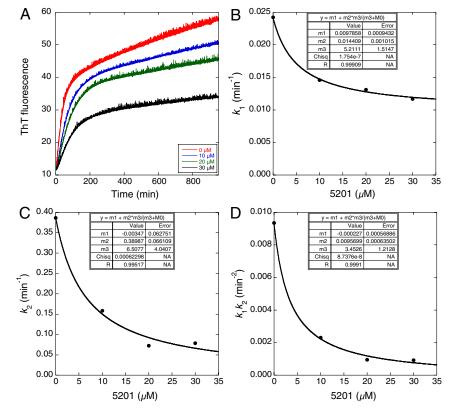


Fig. S6. Inhibition of aggregation kinetics of Flp53Y220C by 5201. (*A*) Time course of the aggregation process monitored by ThT fluorescence. (*B*), (*C*), and (*D*) are inhibition by 5201 of k_1 , k_2 , and of k_1k_2 , respectively, obtained from ThT fluorescence assays.