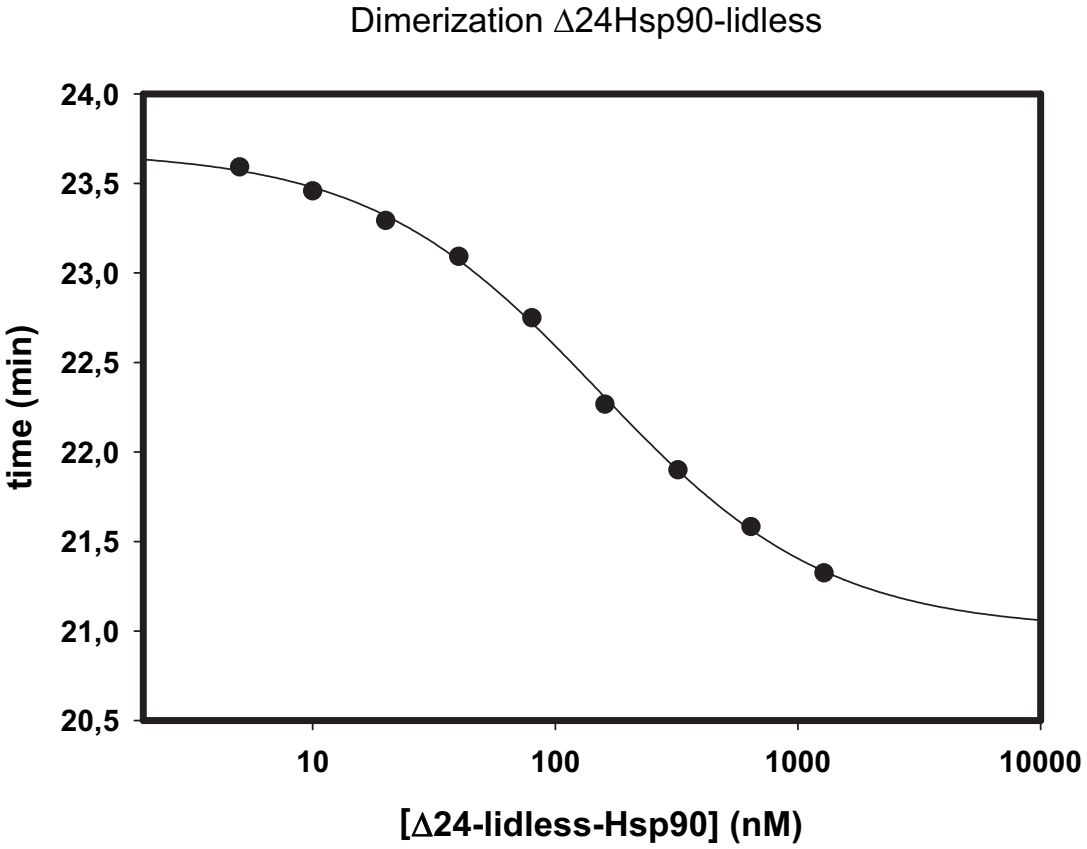
Supplemental data 1

Estimation of K_D for Δ24-lidless-Hsp90 based on the gel filtration experiment.

Varying concentrations of Δ24-lidless-Hsp90 were injected onto the gel filtration HPLC-system and the shift of the peak was monitored. Data analysis was performed as described previously (3).



Supplemental data 2

Equation for the data analysis of heterodimer assays.

Data analysis of the heterodimer assays was based on a model assuming the equilibrium distribution of homo- and heterodimeric versions, as determined by the two homodimerization constants K_{Hom1} and K_{Hom2} and the heterodimerization constant K_{Het}. To calculate the concentration of the monomeric species, the following equation system was solved:

(1) $$[MM] = \frac{[M] \cdot [M]}{K_{Hom1}}$$

(2) $$[PP] = \frac{[P] \cdot [P]}{K_{Hom2}}$$

(3) $$[MP] = \frac{[M] \cdot [P]}{K_{Het}}$$

(4) $$M_{tot} = [M] + [MP] + 2 \cdot [MM]$$

(5) $$P_{tot} = [P] + [MP] + 2 \cdot [PP]$$

This resulted in the following equation, describing the concentration of the monomeric species [P]:

$$a \cdot [P]^4 - b \cdot [P]^3 + c \cdot [P]^2 + d \cdot [P] + e = 0$$

$$a = \frac{64 \cdot K_{Het}^2}{K_{Hom1}^2 \cdot K_{Hom2}^2} - \frac{16}{K_{Hom1} \cdot K_{Hom2}}$$

$$b = \frac{16 \cdot K_{Het}}{K_{Hom1} \cdot K_{Hom2}} - \frac{64 \cdot K_{Het}^2}{K_{Hom1}^2 \cdot K_{Hom2}} + \frac{8}{K_{Hom1}}$$

$$c = -\frac{8 \cdot K_{Het}}{K_{Hom1}} + \frac{16 \cdot K_{Het}^2}{K_{Hom1}^2} - \frac{64 \cdot K_{Het}^2 \cdot P_{tot}}{K_{Hom1}^2 \cdot K_{Hom2}} + \frac{8 \cdot P_{tot}}{K_{Hom1}} - \frac{8 \cdot M_{tot}}{K_{Hom1}}$$

$$d = \frac{8 \cdot P_{tot} \cdot K_{Het}}{K_{Hom1}} - \frac{32 \cdot P_{tot} \cdot K_{Het}^2}{K_{Hom1}^2}$$

$$e = \frac{16 \cdot P_{tot}^2 \cdot K_{Het}^2}{K_{Hom1}^2}$$

[P] was obtained by numerical methods during the fitting routine and used to derive the values for [M], [MP], [MM] and [PP]. The turnover number was then fitted to the following equation, assuming that the turnover originates only from hydrolysis in the active homodimer MM and the heterodimer MP, as monomeric species are barely populated under the conditions used and PP (lidless-Hsp90) was shown to be inactive in this case. The program SCIENTIST was used for the data analysis as described by Tochtrop et al. (42):

$$v = \frac{2 \cdot v_1 \cdot [MM] + v_{het} \cdot [MP]}{M_{tot}}$$

Values for v_1, v_2 (= 0 for lidless-Hsp90), were based on the independently determined ATPase activities of the homodimeric versions and fixed during the data fitting. K_{Hom1} and K_{Hom2} were estimated based on the gel filtration experiments and kept invariant as well. M_{tot} is the invariant concentration of wt-Hsp90 (or Δ8-Hsp90 or Δ16-Hsp90), which only left K_{Het} and v_{Het} as variables during the data analysis. While K_{Het} is subject to large errors due to its strong dependency on the estimated values K_{Hom1} and K_{Hom2}, the determination of v_{Het} is reasonably accurate. Therefore only the turnover number of the heterodimer v_{Het} is mentioned in the results section.