Protein-Ligand Affinity Correlation with the Ligand Binding Volume

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Introduction

The determination of lead compound binding affinity to a receptor is an inseparable part of rational drug design process. Binding affinity, or the Gibbs free energy of binding, includes contributions from binding enthalpy, entropy, volume, and other thermodynamic parameters. Enthalpy and entropy are temperature derivatives from the Gibbs free energy, while binding volume is the pressure derivative from the Gibbs free energy. Volume change related to protein–ligand binding is an important but largely neglected thermodynamic parameter that may facilitate rational drug design. We determined the volume changes of heat shock protein 90 N-terminal domain (Hps90N) and human serum albumin (HSA) binding by several lead compounds. Two experimental techniques – the pressure shift assay (PSA) and thermal shift assay (TSA) – were used to monitor ligand binding and protein unfolding by high pressure and temperature.

Model

$$\Delta G = \frac{\Delta\beta}{2}(P-P_0)^2 + \Delta V_0(P-P_0) + \Delta\alpha(P-P_0)(T-T_0) - \Delta C_p\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right] - (\Delta H_0 - \Delta G_0)\left(\frac{T}{T_0}-1\right) + \Delta G_0 \quad (1)$$

ΔG_0, ΔV_0 and ΔH_0 – differences in the Gibbs free energy, volume and enthalpy of protein unfolding at the reference state (P_0 and T_0), respectively. $\Delta\beta$, $\Delta\alpha$ and ΔC_p – changes in compressibility factor, thermal expansion coefficient and heat capacity between the unfolded and the folded states, respectively. Protein unfolding fluorescence curve is described by

$$f = f_N + \frac{f_U - f_N}{1+\exp(\Delta G/RT)} \quad (2)$$

Here f_N and f_U are experimental fluorescence yields for the native and unfolded protein states.
ΔG as a function of pressure at a constant temperature:

$$\Delta G_P = \Delta G_0 + \Delta V_0(P-P_0) + \frac{\Delta\beta}{2}(P-P_0)^2 \quad (3)$$

ΔG as a function of temperature at a constant pressure:

$$\Delta G_T = \Delta G_0 - \Delta C_p\left[T\left(\ln\frac{T}{T_0}-1\right)+T_0\right] - (\Delta H_0 - \Delta G_0)\left(\frac{T}{T_0}-1\right) \quad (4)$$

Thermal Shift and Pressure Shift Assays

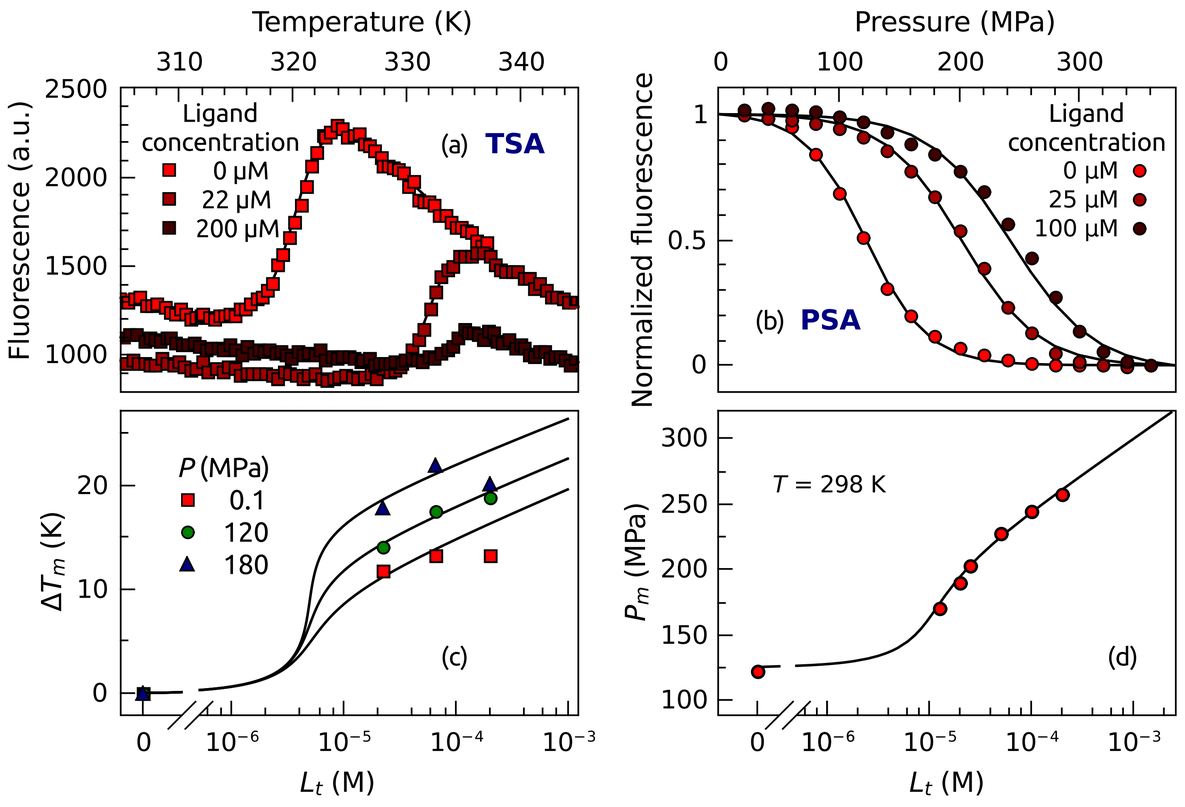

FIGURE: (a) Hsp90N denaturation by elevated temperature and (b) HSA denaturation by pressure in the absence and presence of ligand. Ligand dose curves: (c) Hsp90N T_m and (d) HSA P_m dependencies on ligand concentration.

$P-T$ Phase Diagram

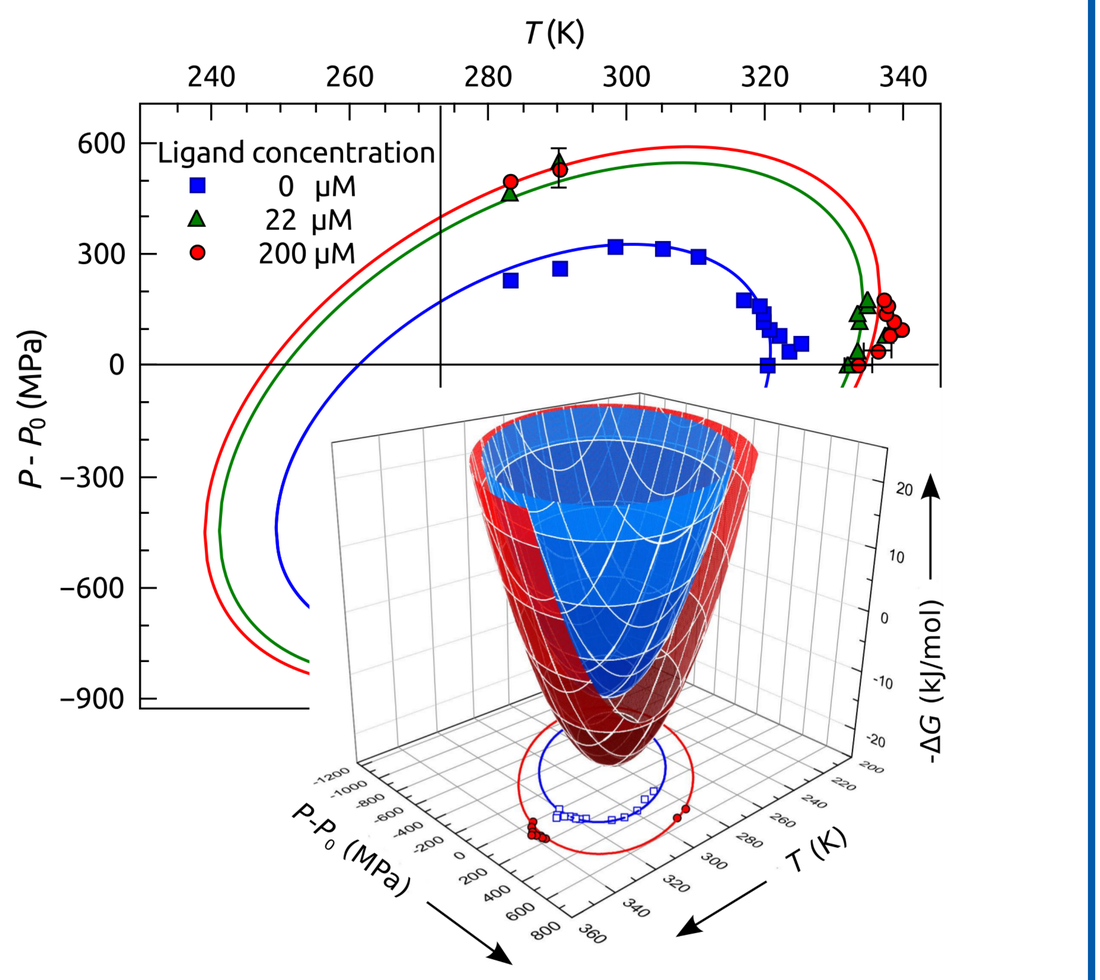

FIGURE: Hsp90N protein stability diagram in $P-T$ coordinates without a ligand (squares) and with 22 μM (triangles) and 200 μM (circles) of added ligand. Lines are fits to Eq. (1).

Binding Volumes

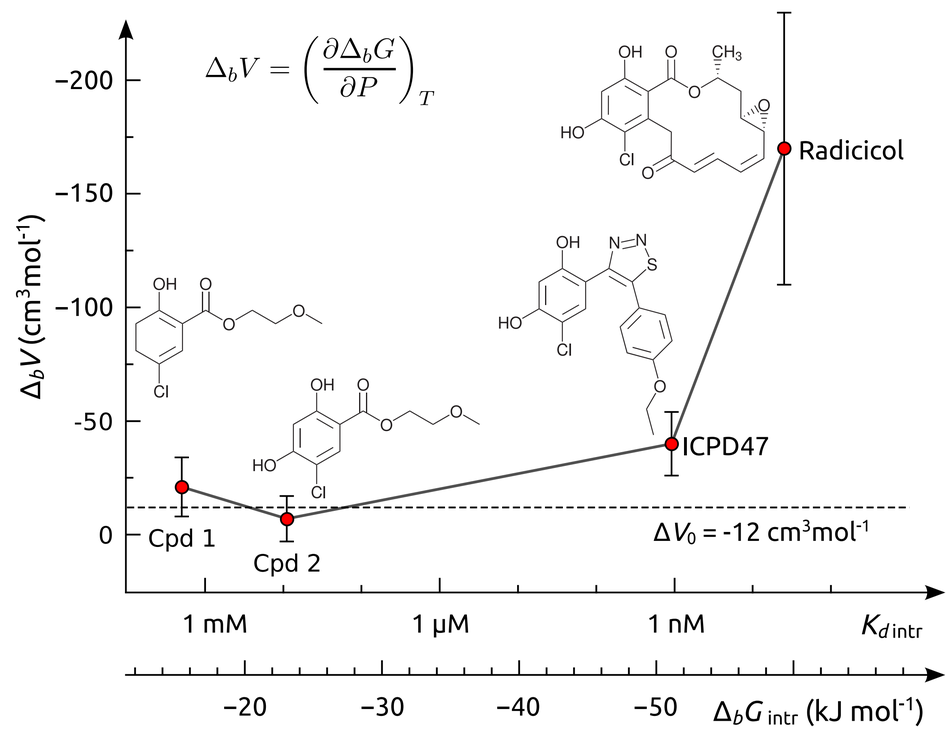

FIGURE: Hsp90N ligand binding volumes plotted as a function of intrinsic binding constant. The solid line is a visual guide, the dashed line compares the volume of Hsp90N unfolding.

Conclusions

- The ligands exhibited a stabilizing effect on the protein by increasing its melting pressure and temperature.
- The negative volume of ligand binding was relatively large and significantly exceeded the volume of protein unfolding.
- Protein–ligand binding affinities may correlate with binding volumes: tightly binding ligands, such as naturally occurring radicicol, exhibited a relatively large negative volume of binding, while weakly binding ligands exhibited a relatively small volume of binding.

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