Protein-ligand binding volume determined by FPSA, densitometry, and NMR

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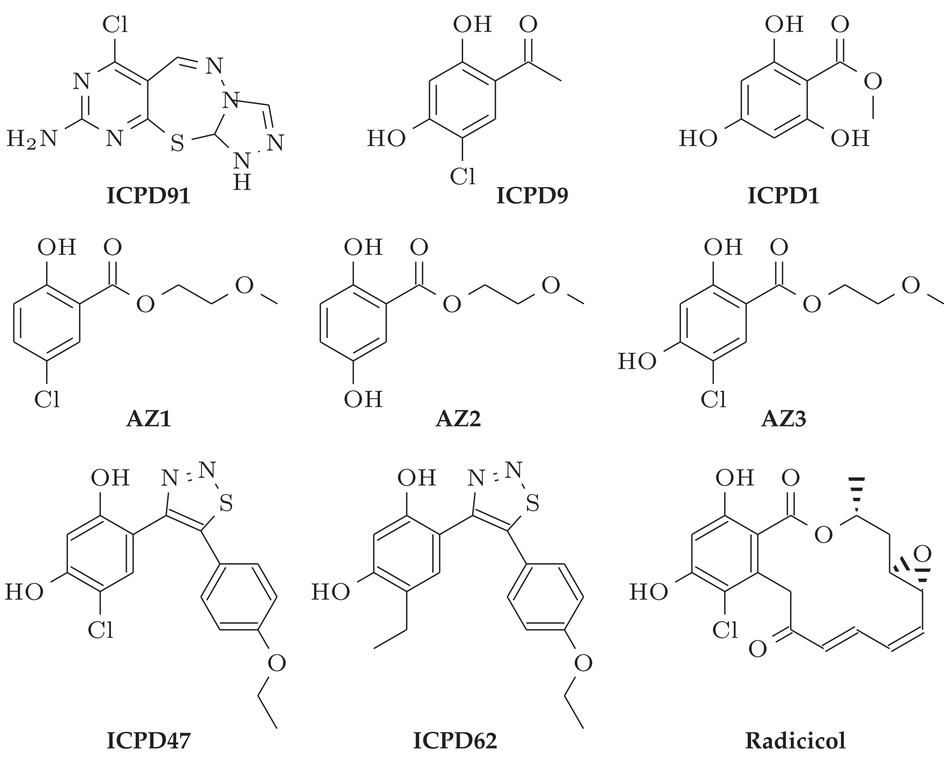
Introduction

We report the values of recombinant human heat shock protein 90 (Hsp90) binding volumes (i.e., the changes in protein volume associated with ligand binding), which were obtained by three independent experimental techniques – fluorescent pressure shift assay (**FPSA**), vibrating tube **densitometry**, and high-pressure **NMR**. Within the error range all techniques provide similar volumetric parameters of investigated protein-ligand systems.

Ligands



High-Pressure NMR

The K_d's of Hsp90αN interaction with a ligand is calculated from the chemical shift change, $\Delta\delta$, dependency on L_t and P_t:

$$\Delta\delta = \Delta\delta_{max}\frac{Y-\sqrt{Y^2-4L_tP_t}}{2P_t}, \qquad Y = L_t + P_t + K_d.$$

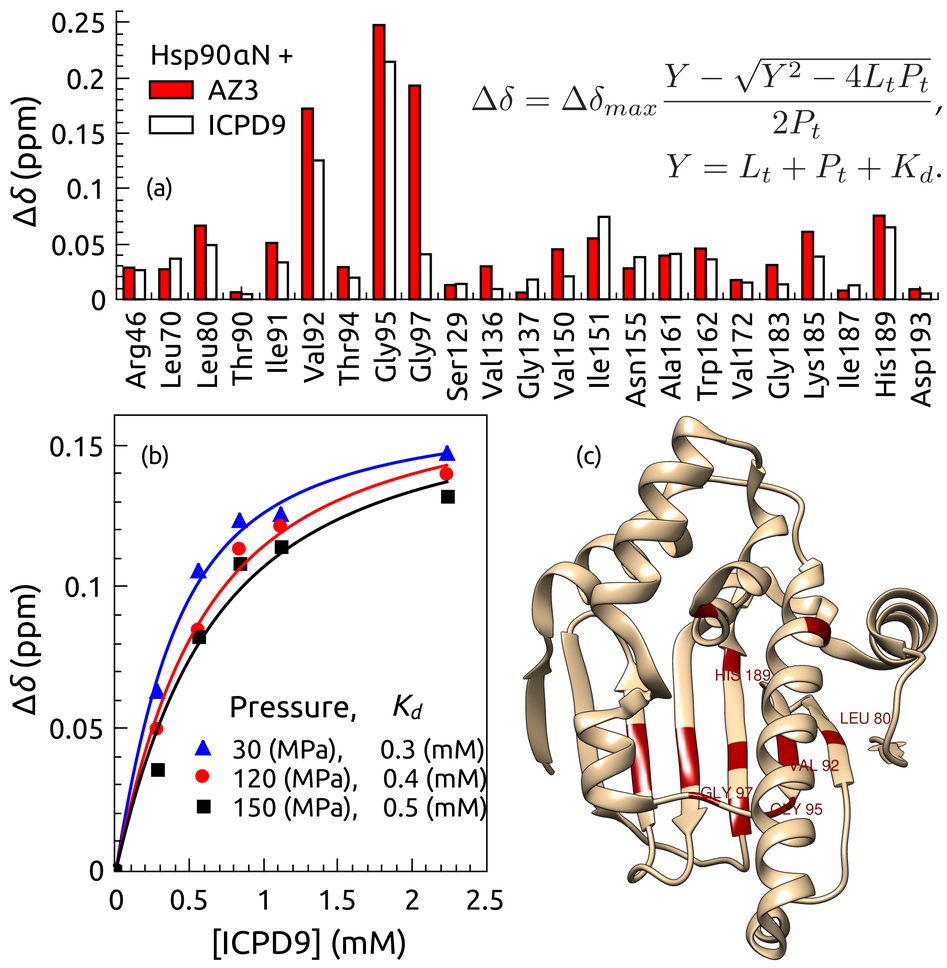


FIGURE: (a) $\Delta\delta$ of Hsp90αN amino acids which were induced by AZ3 and ICPD9 ligands. (b) $\Delta\delta$ of Val92 as a function of ICPD9 concentration at 30 MPa, 120 MPa, and 150 MPa pressures. (c) AZ3-induced shifts in Hsp90αN (PDB ID: 1UYL).

Acknowledgement

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Fluorescent Pressure Shift Assay (FPSA)

System of equations describing a protein dosing curve – the relationship between concentration of added ligand, L_t, total protein concentration, P_t, and melting pressure, p_m:

$$L_t = (\exp(-\Delta G_U/RT) - 1)\left(\frac{P_t}{2\exp(-\Delta G_U/RT)} + \frac{1}{\exp(-\Delta G_b/RT)}\right), \tag{1}$$

$$\Delta G_x = \Delta G_{0\_x} + \Delta V_x(p_m - p_0) + \frac{\Delta\beta_x}{2}(p_m - p_0)^2; \quad x = U, b, \tag{2}$$

where ΔG_0, ΔV and $\Delta\beta$ are standard state Gibbs energy, volume and compressibility factor, respectively, and indexes U and b stand for the changes related to protein unfolding and protein-ligand binding.

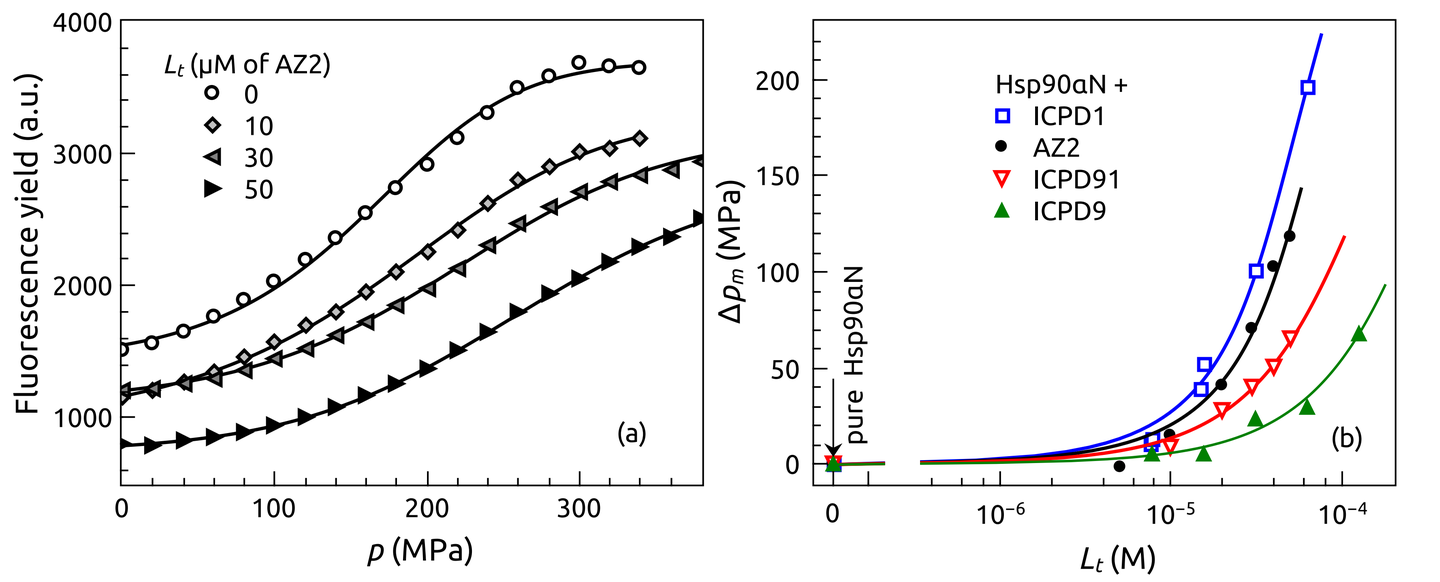

FIGURE: (a) Unfolding profiles and (b) dosing curves of Hsp90αN.

Densitometry

The partial molar volume of a protein, V^0, and the change in protein volume associated with the ligand binding, ΔV_b, are calculated using equations:

$$V^0 = \frac{M}{d_0} - \frac{d-d_0}{Cd_0}, \tag{3}$$

$$V^0(R) = V^0(0) + \alpha\Delta V_b, \tag{4}$$

where M and C are molecular mass and molar concentration of a protein, d_0 and d are densities of solvent and protein solution, respectively, and α is the fraction of ligand-bound protein:

$$\alpha = 0.5\,(1 + R + K_d/P_t) - \sqrt{0.25\,(1 + R + K_d/)^2 - R}. \tag{5}$$

Here K_d is the dissociation constant of the protein-ligand complex and $R = L_t/P_t$.

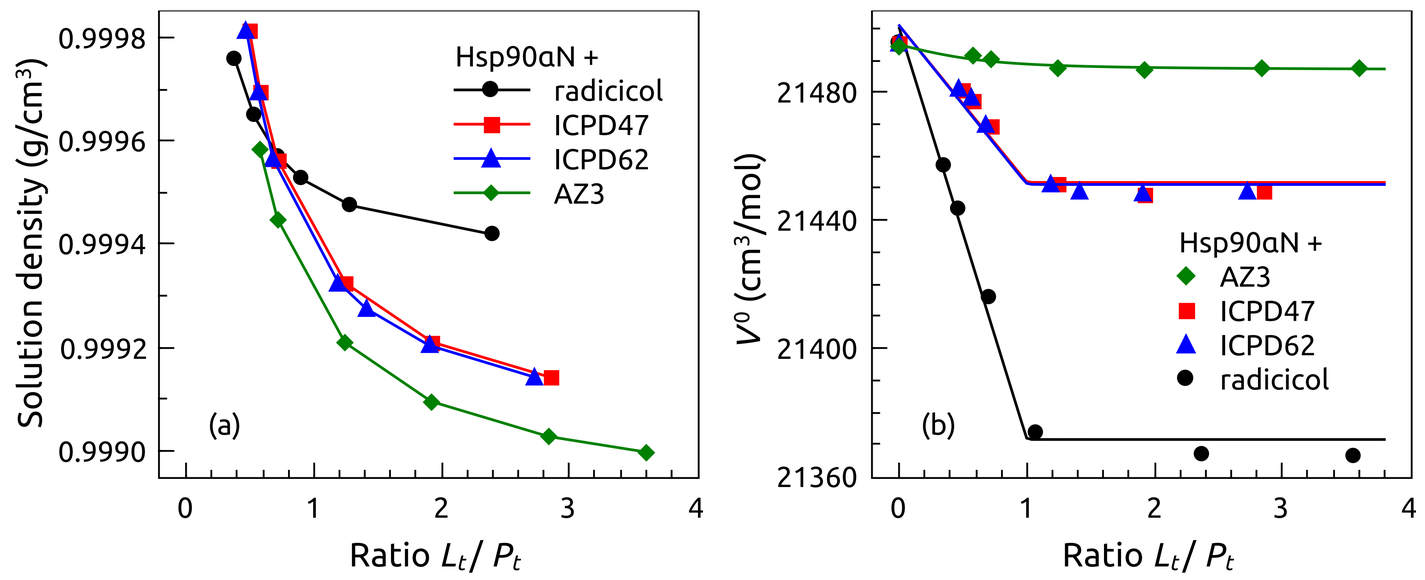

FIGURE: (a) Raw densitometry data and (b) the partial molar volumes of Hsp90αN at various R.

Binding volumes

	ΔV_b (FPSA)	ΔV_b (Densitometry)	ΔV_b (NMR)	K_d
ICPD91	-1 ± 5 cm^3/mol	n.d.	n.d.	3×10^{-5} M
ICPD9	-2 ± 5 cm^3/mol	n.d.	20 ± 4 cm^3/mol	2×10^{-5} M
AZ3	-7 ± 6 cm^3/mol	-10 ± 3 cm^3/mol	-9 ± 4 cm^3/mol	9×10^{-5} M
AZ2	-9 ± 6 cm^3/mol	n.d.	n.d.	1×10^{-3} M
ICPD1	-9 ± 6 cm^3/mol	n.d.	n.d.	3×10^{-5} M
AZ1	-21 ± 11 cm^3/mol	n.d.	n.d.	2×10^{-3} M
ICPD47	-40 ± 14 cm^3/mol	-49 ± 5 cm^3/mol	n.d.	5×10^{-9} M
ICPD62	n.d.	-50 ± 4 cm^3/mol	n.d.	2×10^{-9} M
radicicol	-170 ± 60 cm^3/mol	-124 ± 7 cm^3/mol	n.d.	2×10^{-10} M