High pressure spectrofluorimetry – a tool to determine protein-ligand binding volume

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Introduction

The change in protein volume during the protein-ligand interaction (reaction or binding volume) is important but largely neglected thermodynamic parameter in the perspective of both fundamental science and possible applications in the development of specific protein inhibitors. 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.

Fluorescent pressure shift assay (FPSA) [1–3] was used to determine the unfolding and ligand binding-induced volume changes of some proteins involved in cancer progression and therapy, namely, several isoforms of the human carbonic anhydrase (CA) and N-terminal domain of the human heat shock protein 90 (Hsp90N). In addition to protein volumetric properties, the protein-ligand binding affinities were determined by fluorescent thermal shift assay (FTSA) technique [4]. The ligands exhibited a stabilizing effect on the proteins increasing the melting pressure and temperature. Our results show that the protein-ligand binding affinities correlate with binding volumes. For example, the 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 [3].

Fluorescent Pressure Shift Assay

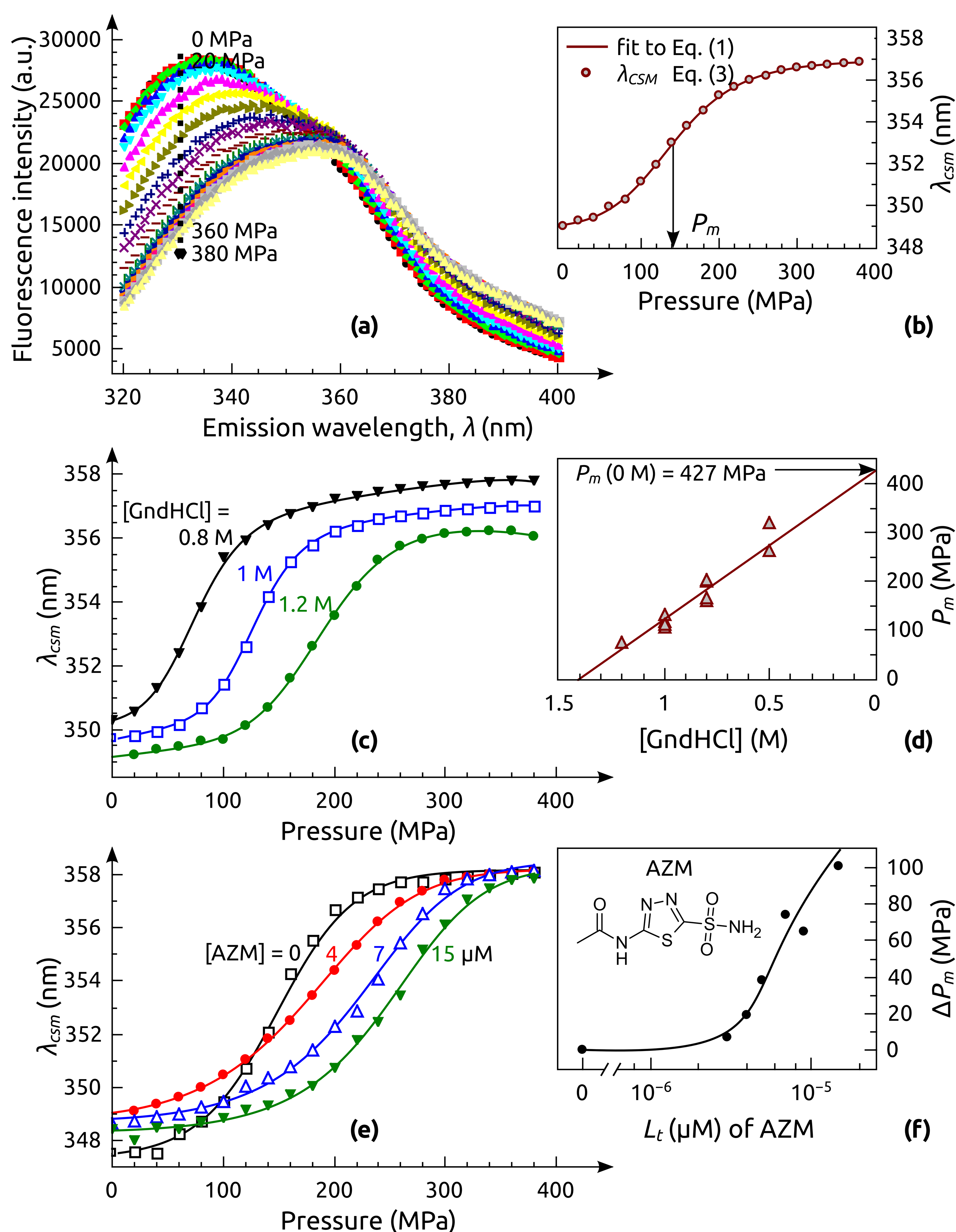

FIGURE: The use of FPSA to determine the volumetric properties of CA isoforms. Experiments were performed at 25°C and the protein solutions contained 3 μM CA, 0–1.2 M guanidine hydrochloride (GndHCl), 1% dimethyl sulfoxide, 10mM Bis-Tris, pH 7.0. **(a)** The spectra and **(b)** the shift in CSM of CAII with an increase of pressure. **(c)** Pressure denaturation of CAXIII in the presence of GndHCl and **(d)** extrapolation of P_m to [GndHCl] = 0. **(e)** Pressure denaturation of CAI in the presence of various acetazolamide (AZM) concentrations and **(f)** AZM induced shift (dosing curve) of the protein melting pressure, ΔP_m.

Model

The profile of protein unfolding curve is described by an equation

$$f(P) = f_N + \frac{f_U - f_N}{1 + \exp(\Delta G/RT)}, \tag{1}$$

where f_N and f_U are experimental fluorescence yields for the native and unfolded protein states, respectively, R – molar gas constant and T – absolute temperature. The Gibbs free energy, ΔG, as a function of pressure, P, at a constant temperature is

$$\Delta G = \Delta G_0 + \Delta V_0(P - P_0) + \frac{\Delta\beta}{2}(P - P_0)^2 . \tag{2}$$

ΔG_0, ΔV_0 and $\Delta\beta$ denote changes in the standard state thermodynamic parameters of protein unfolding – the Gibbs free energy, volume and compressibility factor, respectively.

Equation (1) also describes the unfolding profile obtained from the center of emission spectral mass (CSM), which is defined as

$$\lambda_{CSM} = \frac{\sum_i f_i \lambda_i}{\sum_i f_i}, \tag{3}$$

where f_i is the fluorescence intensity at a wavelength λ_i.

The relationship between the concentration of added ligand, L_t, total protein concentration, M_t, and melting pressure, P_m, (dosing curve) is [1]

$$L_t = (\exp(-\Delta G_U/RT) - 1)\left(\frac{M_t}{2\exp(-\Delta G_U/RT)} + \frac{1}{\exp(-\Delta G_b/RT)}\right), \tag{4}$$

where indexes U and b stand for the changes in Gibbs free energy related to protein unfolding and protein-ligand binding, respectively,

$$\Delta G_x = \Delta G_{0_x} + \Delta V_{0_x}(P_m - P_0) + \frac{\Delta\beta_x}{2}(P_m - P_0)^2; \quad x = U, b. \tag{5}$$

Binding Volumes

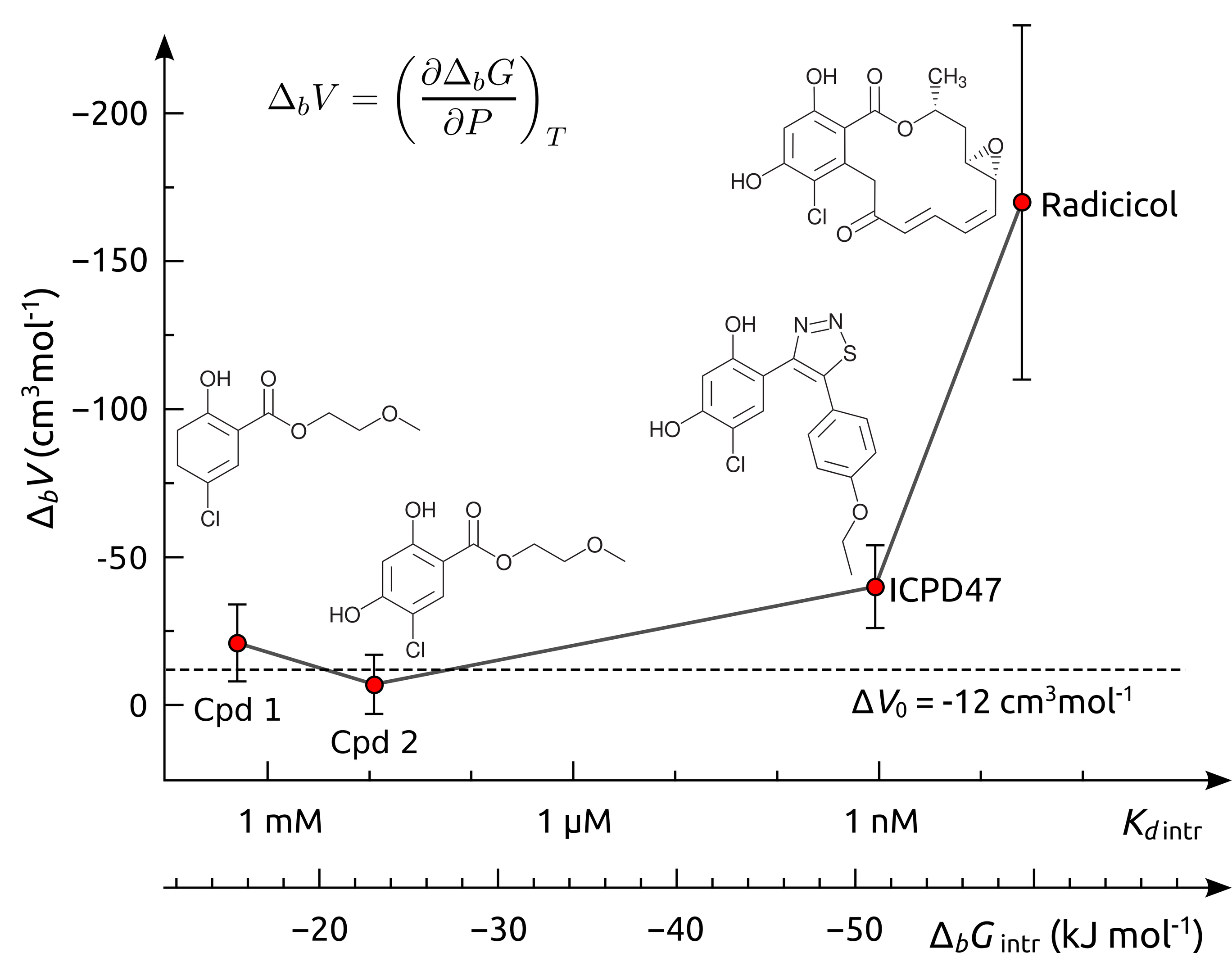

FIGURE: Hsp90N ligand binding volumes plotted as a function of intrinsic binding constant. The solid line is a visual guide.

References

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[3] V. Petrauskas et al. *European Biophysics Journal*, 42:355–362, 2013.

[4] M. W. Pantoliano et al. *Journal of Biomolecular Screening*, 6:429–440, 2001.

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