Determination of the protein-ligand binding volume by high-pressure spectrofluorimetry

Vytautas Petrauskas

Department of Biothermodynamics and Drug Design
Institute of Biotechnology, Vilnius University

February 15-17, 2016

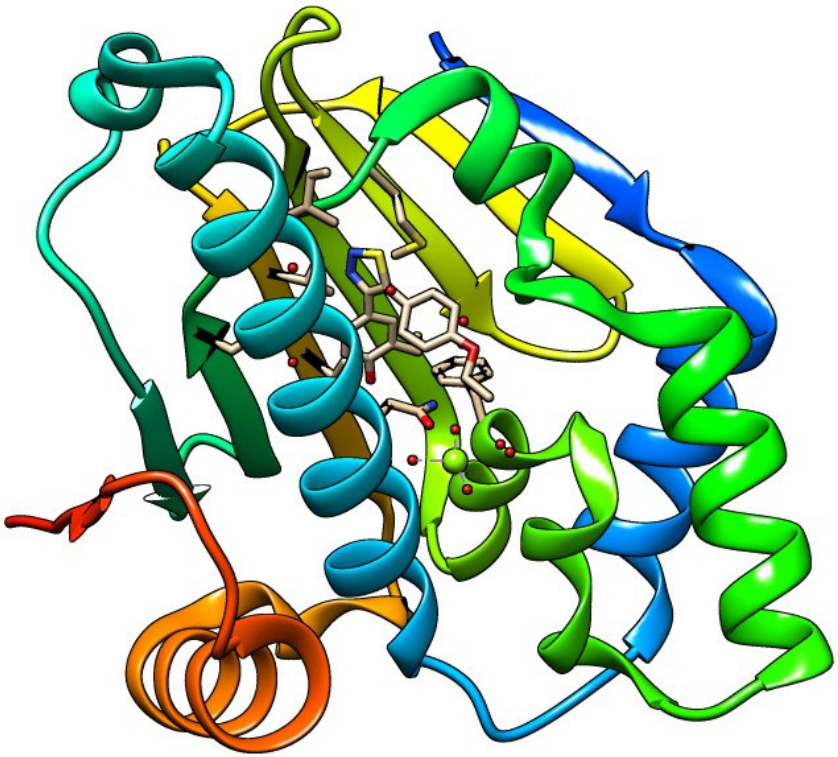
Introductory remarks

Protein-ligand binding (reaction) volume

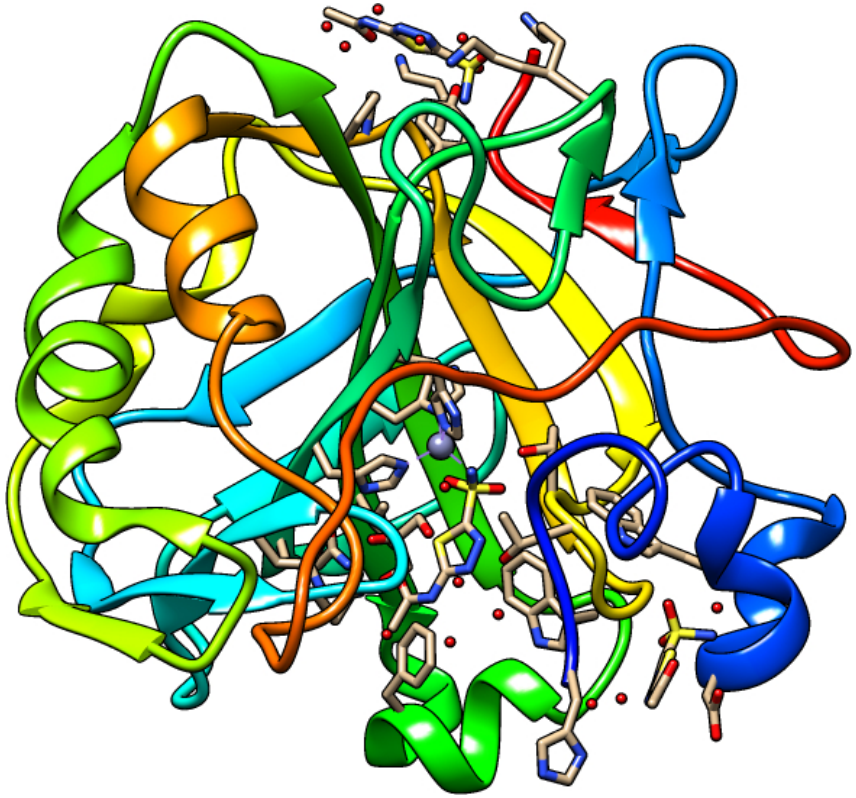
- Definition: the change in protein volume observed upon protein-ligand binding;
- Motivation: fundamental knowledge about protein-ligand interaction and pressure-induced protein denaturation.
- Protein-ligand binding volume: $\Delta V_b = \left(\frac{\partial \Delta G_b}{\partial P} \right)_T$;

Introductory remarks

Heat shock protein 90 and carbonic anhydrases

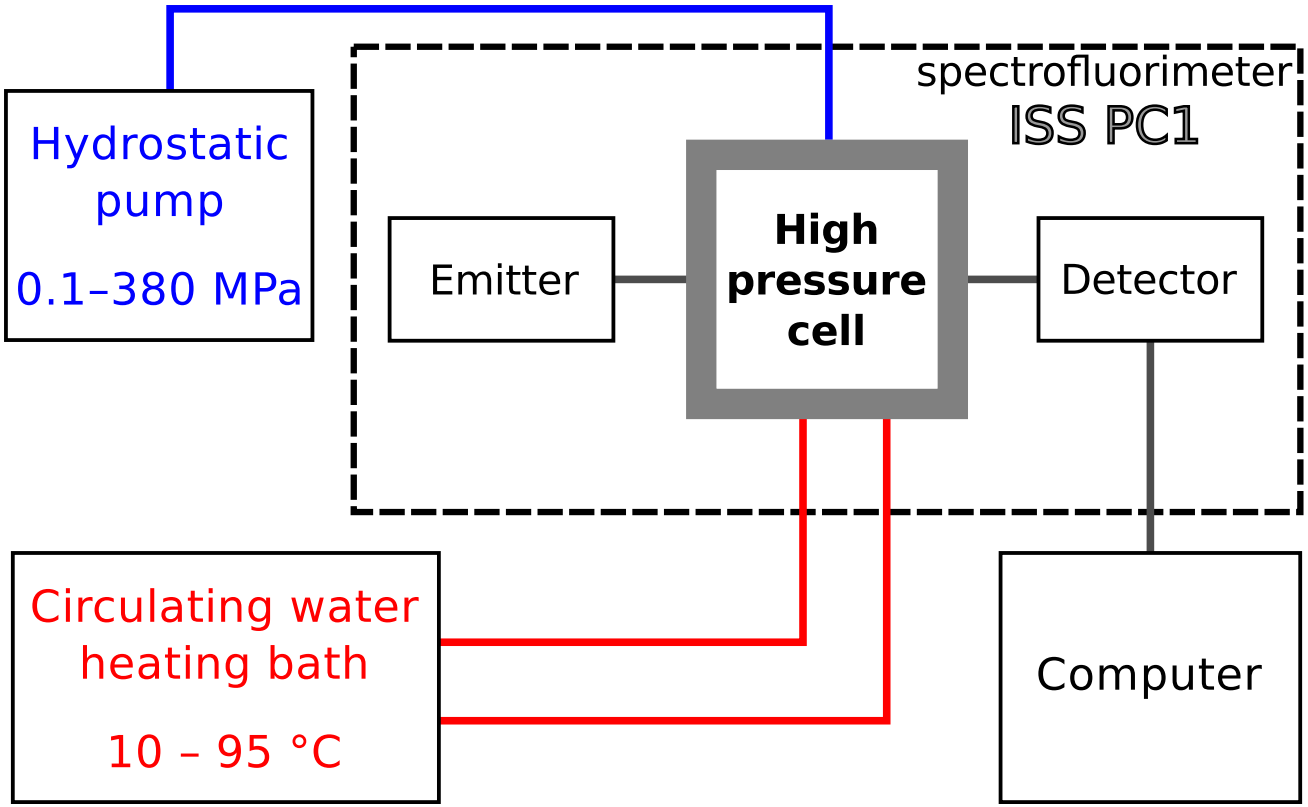


N-terminal domain of human Hsp90 (229 a.a.), PDB ID: 2YI6



Human carbonic anhydrase (CA) II (260 a.a.), PDB ID: 3HS4

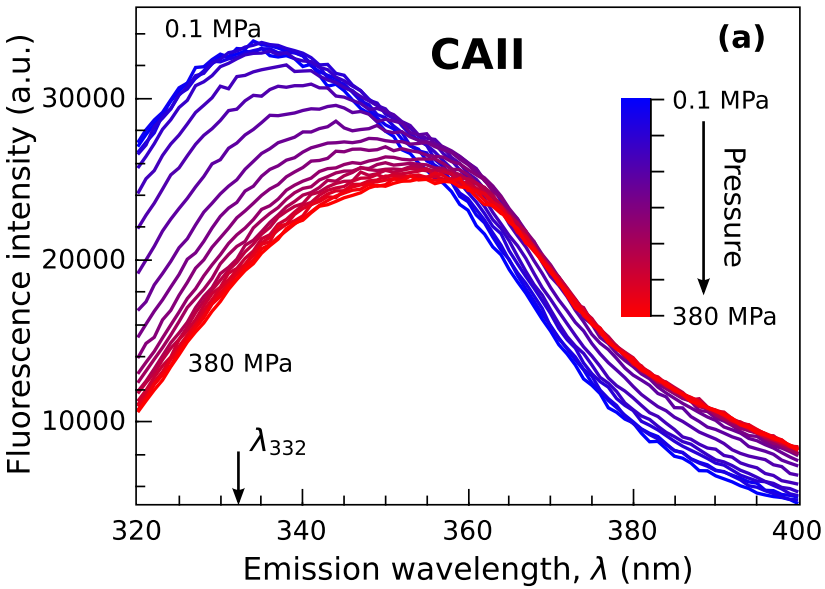
Experimental set-up of high-pressure spectrofluorimetry

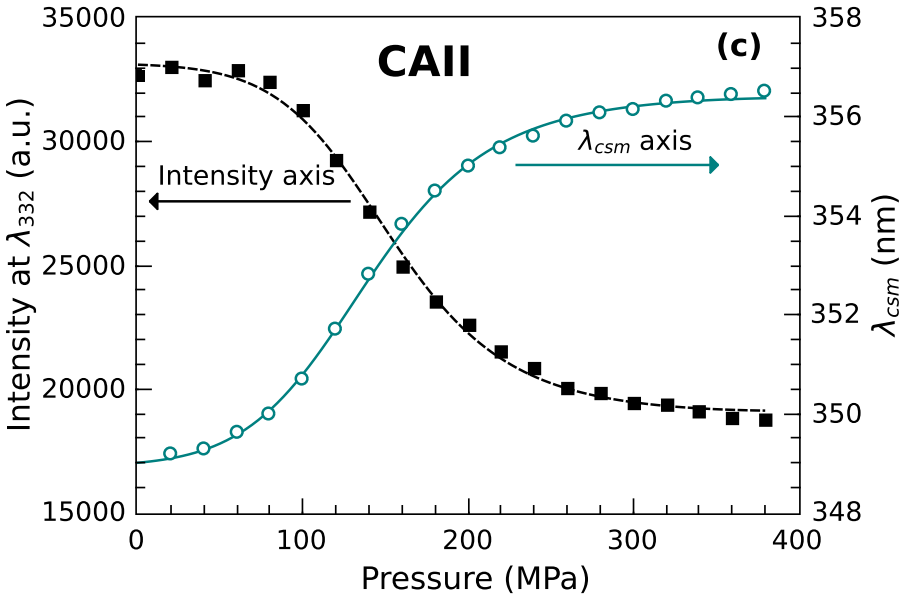


- Pressure range: (0.1 – 380) MPa;
- Temperature range: (10 – 95) ℃;
- Fluorescence probe: intrinsic tryptophan (exited at 295 nm);
- Emission spectra range: (320 – 400) nm.

Description of pressure-induced unfolding profiles

Equations





Unfolding profiles:

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

The Gibbs energy of unfolding:

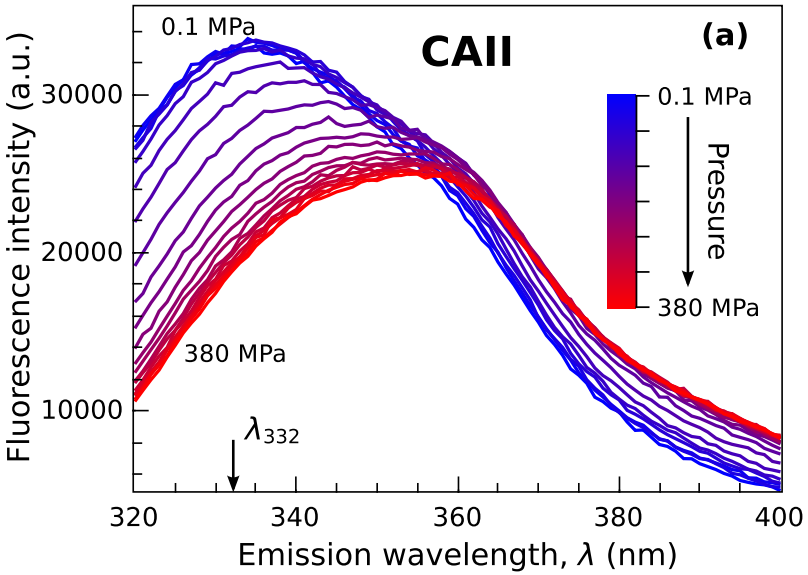
$$\Delta G = \Delta G_0 + \Delta V_0 \Delta p + \frac{\Delta \beta}{2}(\Delta p)^2.$$

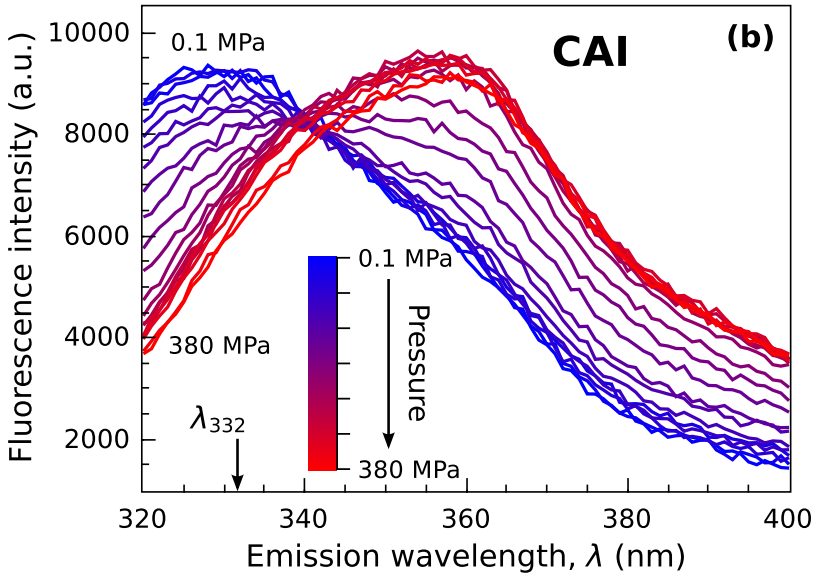
The center of spectral mass:

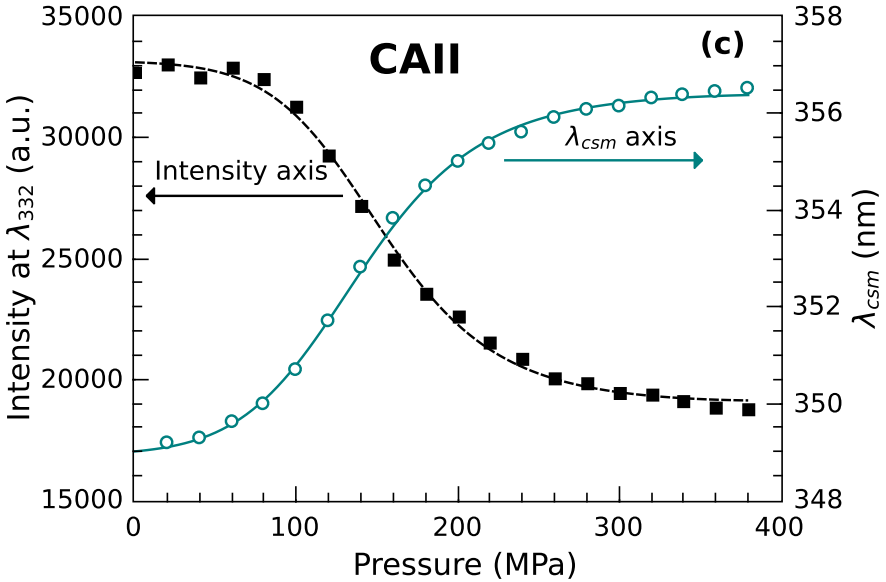
$$\lambda_{CSM} = \frac{\sum_i f_i \lambda_i}{\sum_i f_i}.$$

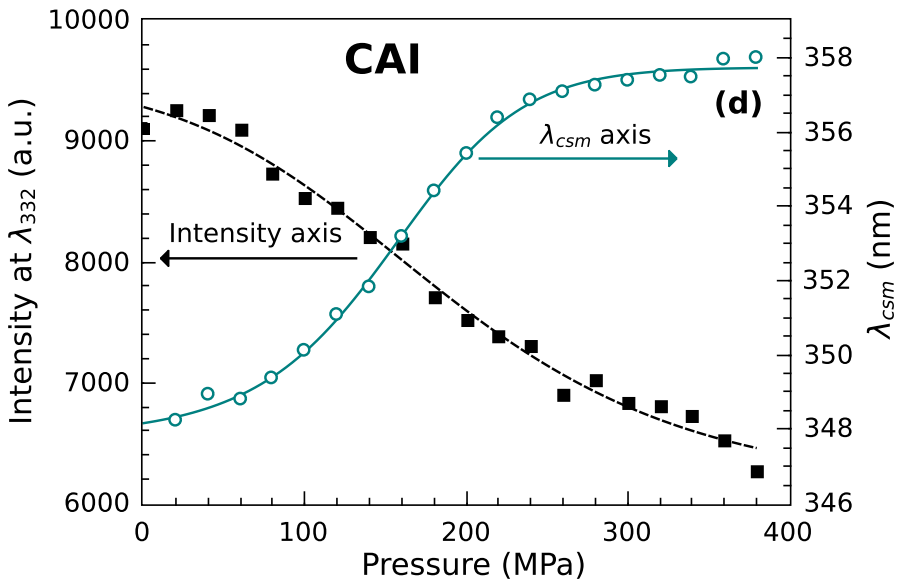
Pressure-induced unfolding profiles of CA II and CA I

Intrinsic tryptophan fluorescence spectra at various pressures



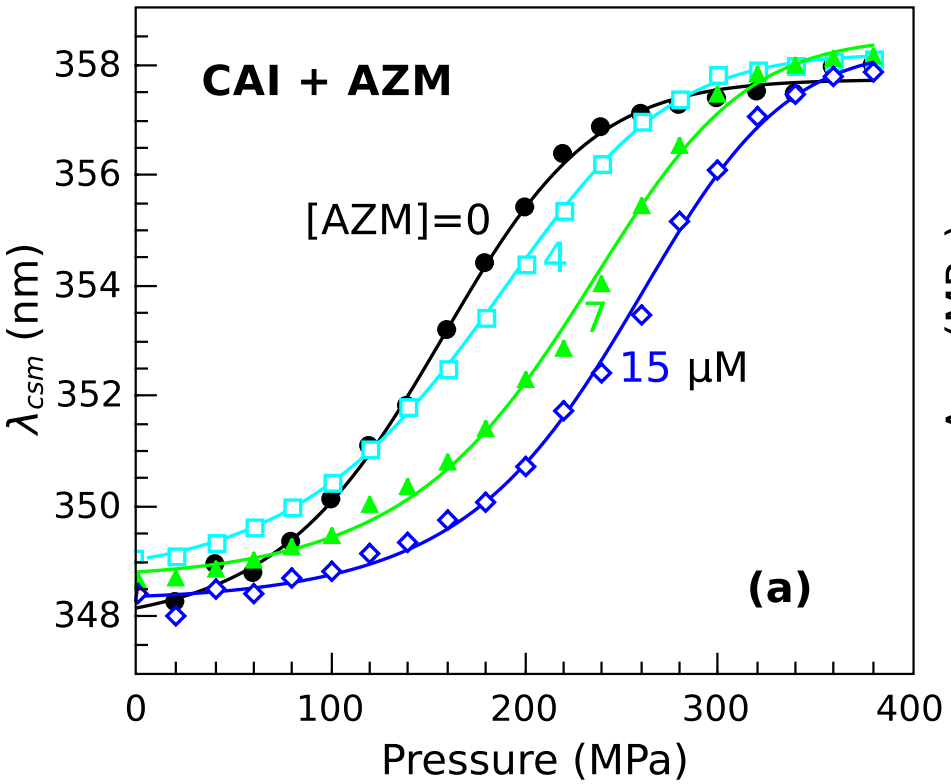


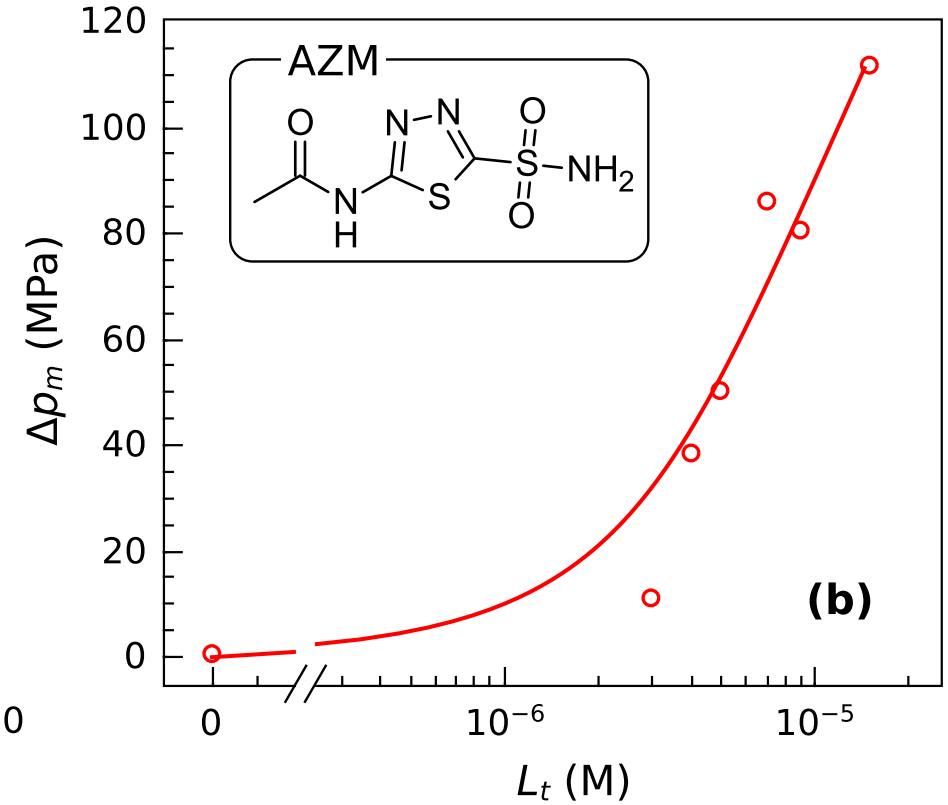




The shift in P_m and dosing curves

Fluorescent Pressure Shift Assay (**FPSA**)





Description of dosing curves

Mathematical background

$$P_t = N_f + N_b + U,$$
$$L_t = L_f + N_b,$$
$$K_U = \frac{U}{N_f},$$
$$K_b = \frac{N_b}{N_f L_f}.$$

P_t and L_t – the bulk concentrations of protein and ligand;
N_f and N_b – concentrations of native ligand-free and bound protein;
U – concentration of unfolded protein;
K_U and K_b– equilibrium constants of protein unfolding and protein-ligand binding reaction;
At melting pressure p_m, $U = P_t/2$.

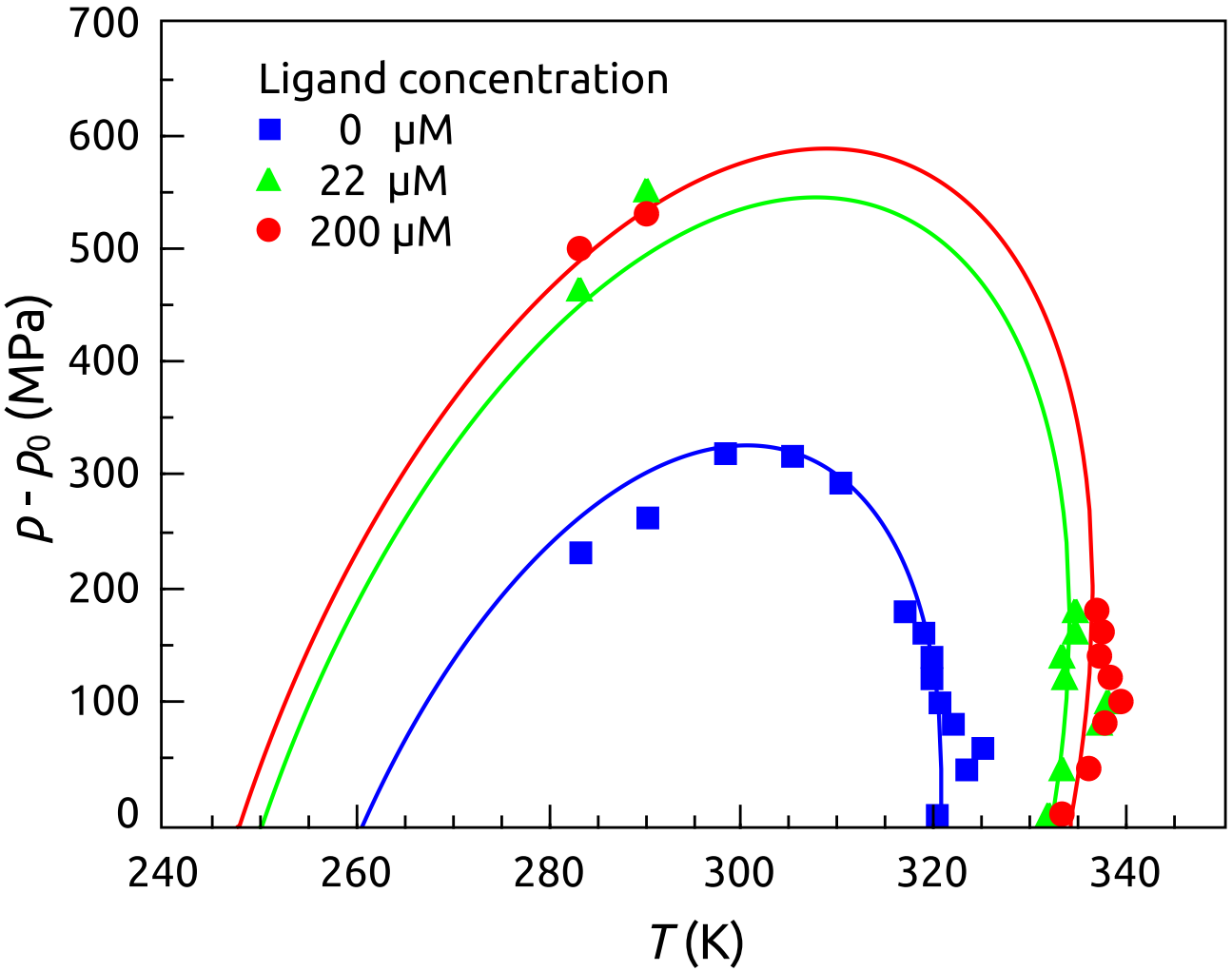
The final form of dosing curve equation:

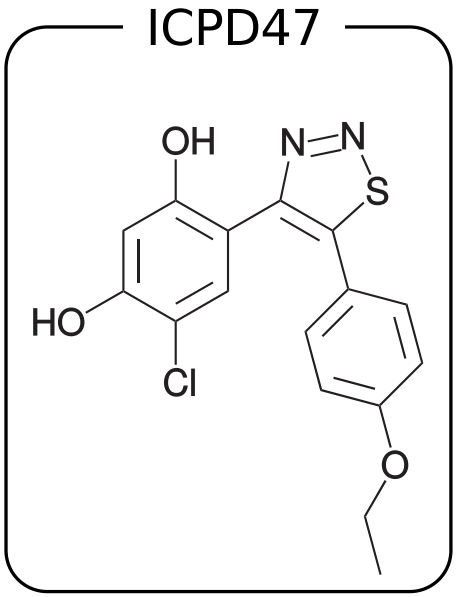
$$L_t = (K_U - 1)\left(\frac{1}{\boxed{K_b}} + \frac{P_t}{2K_U}\right)$$

$$\boxed{K_b} = \exp\left(-\frac{\Delta G_b}{RT}\right) = \left(-\frac{\Delta G_{0_b} + \Delta V_{0_b}(p_m - p_0) + \frac{\Delta\beta_b}{2}(p_m - p_0)^2}{RT}\right)$$

Hsp90N protein stability diagram in P–T coordinates

With added ligand

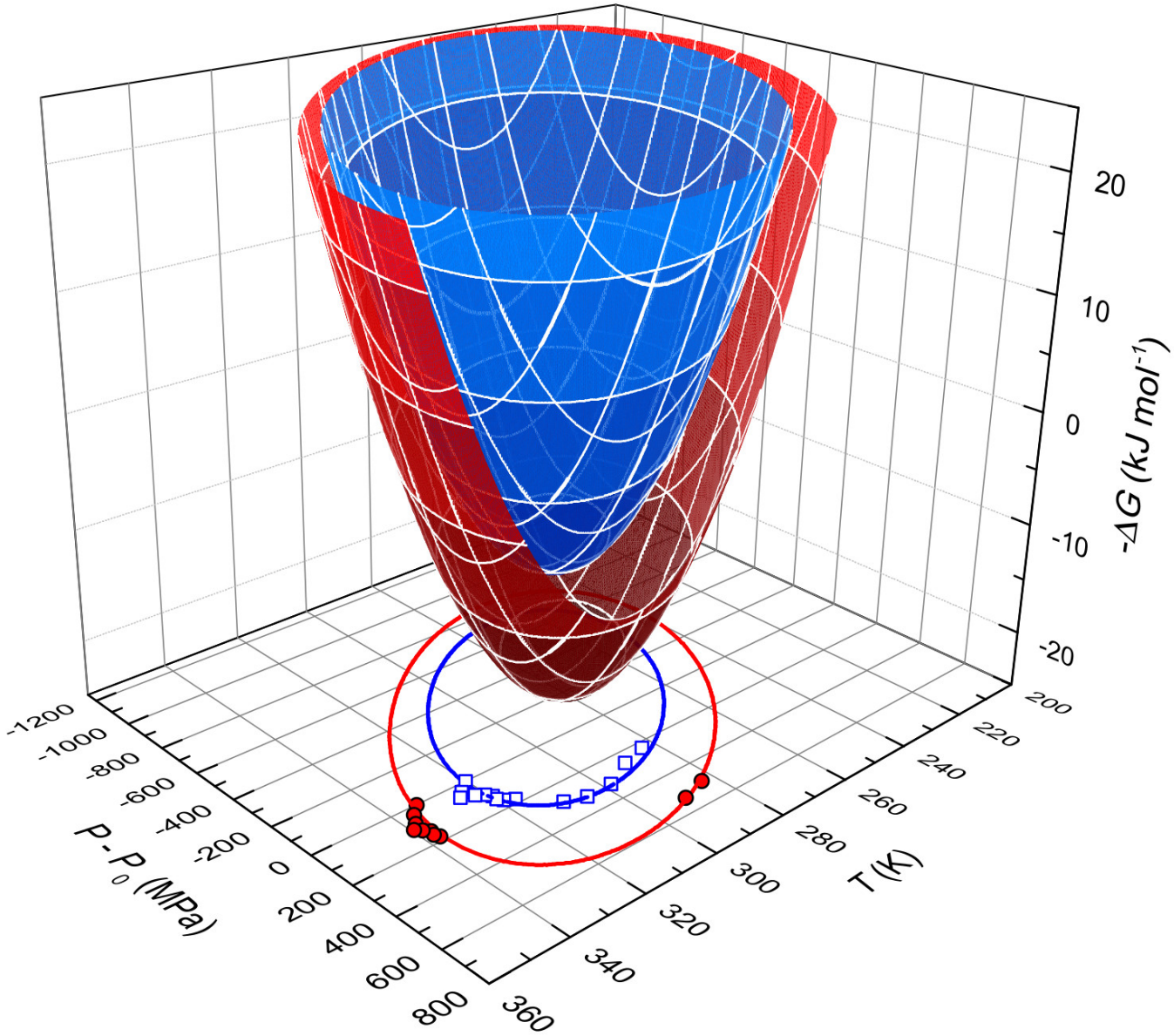




Petrauskas et al. *Eur Biophys J* **42** (2013) 355–362.

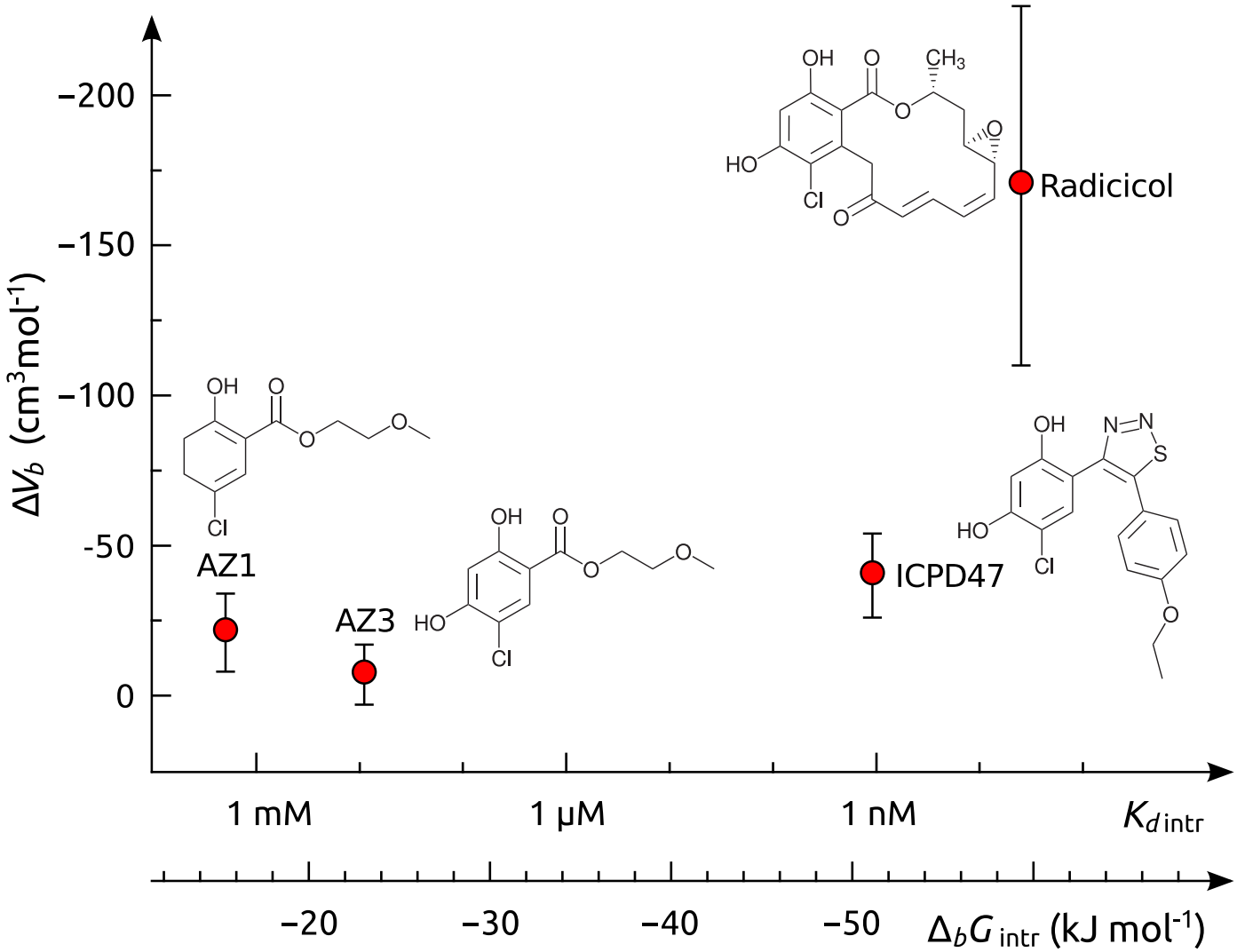
Hsp90N protein stability diagram

The Gibbs energy dependence on pressure and temperature



Binding volume correlation with affinity

Affinity was determined by ITC and Fluorescent Thermal Shift Assay

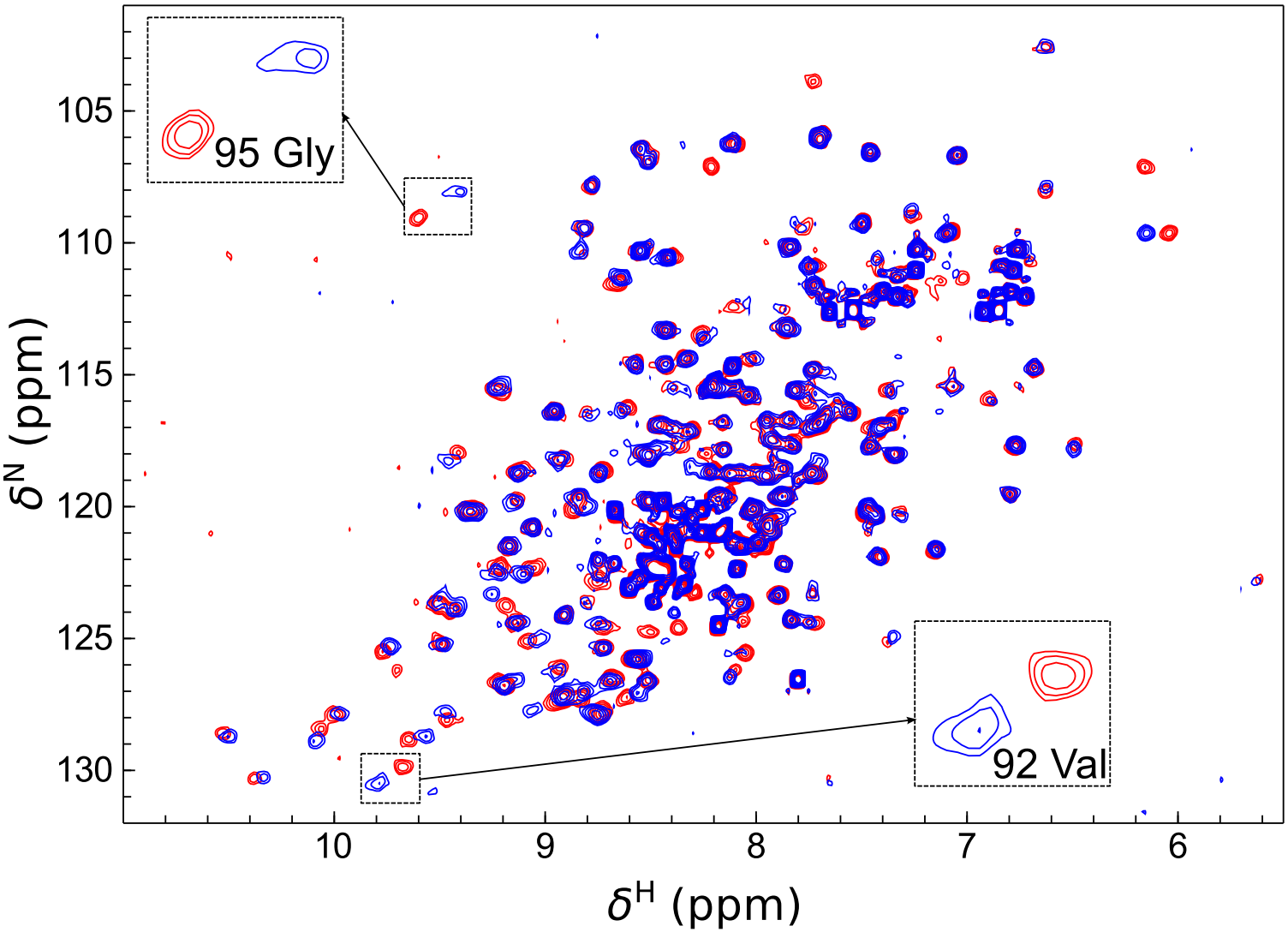


NMR experiments

- Preliminary results of Hsp90N interaction with two ligands by NMR;
- $\Delta V_b = \left(\frac{\partial \Delta G_b}{\partial p} \right)_T$;

NMR data

^{1}H-^{15}N–HSQC spectra of Hsp90N (red) and Hsp90N+ICPD9 (blue)



NMR data analysis

Analysis of dosing curves

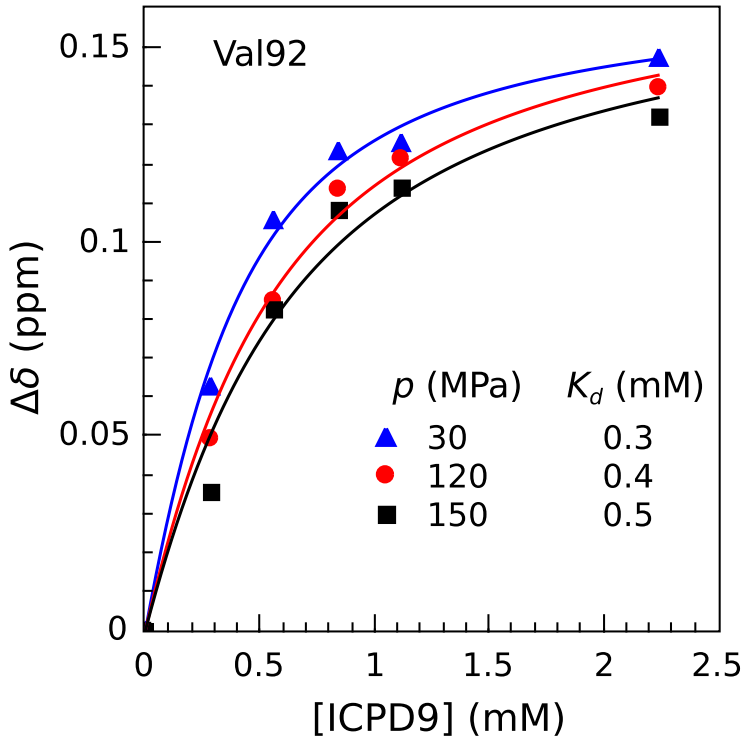
$$\Delta V_b = \left(\frac{\partial \Delta G_b}{\partial p}\right)_T$$

$$\Delta G_b = -RT \ln(K_b) = RT \ln(K_d)$$

Experimental changes in chemical shifts:

$$\Delta\delta = \sqrt{(\delta_0^H - \delta^H)^2 + \left(\frac{\gamma^N}{\gamma^H}\right)^2 (\delta_0^N - \delta^N)^2}$$

δ_0 – chemical shift without ligand.



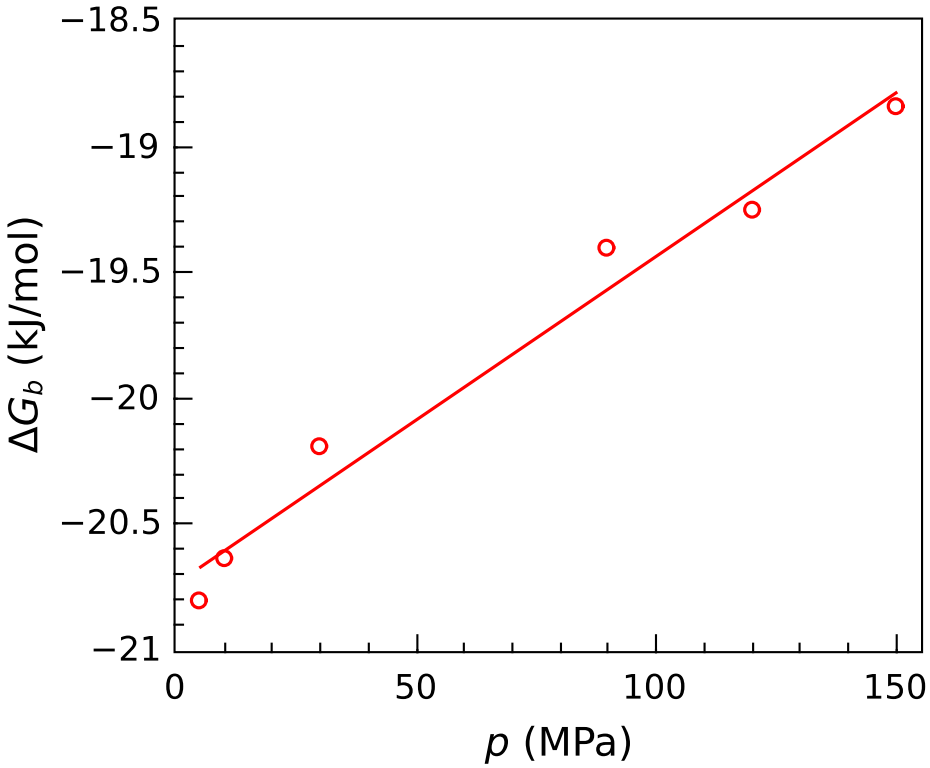
$$\Delta\delta = \Delta\delta_{max} \frac{(L_t + P_t + K_d) - \sqrt{(L_t + P_t + K_d)^2 - 4P_tL_t}}{2P_t}$$

Chemical shift equation reference:

Morton et al. *Structure* **4** (1996) 705-14.

Gibbs energy as a function of pressure

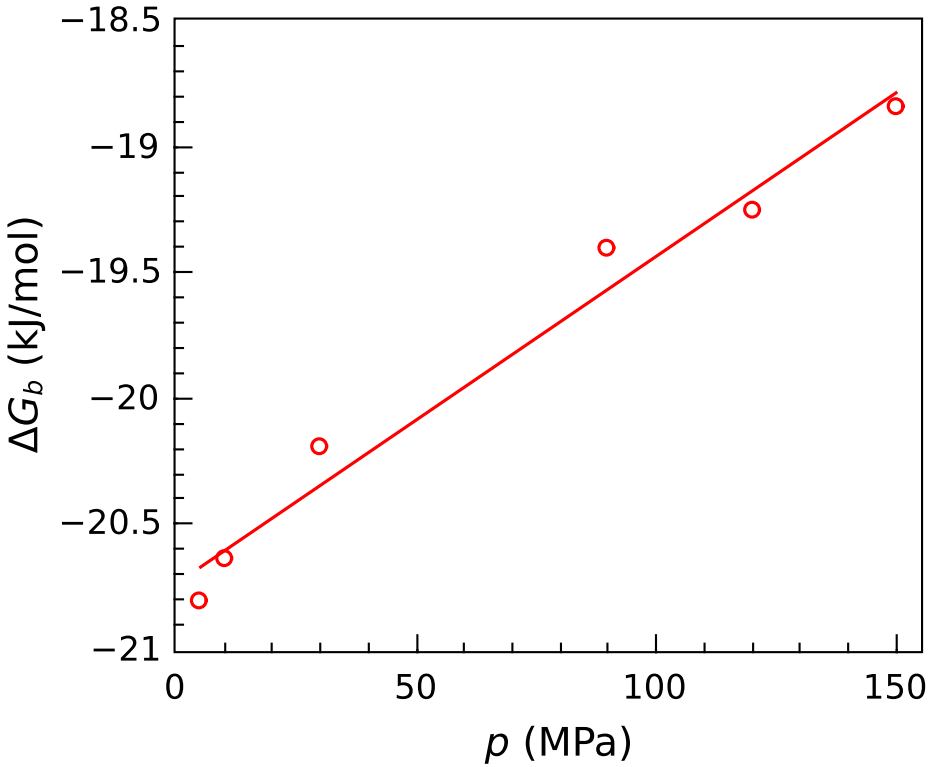
$$\Delta G_b = \Delta G_{b_0} + \Delta V_{b_0} \Delta p + \frac{\Delta \beta_b}{2} (\Delta p)^2.$$

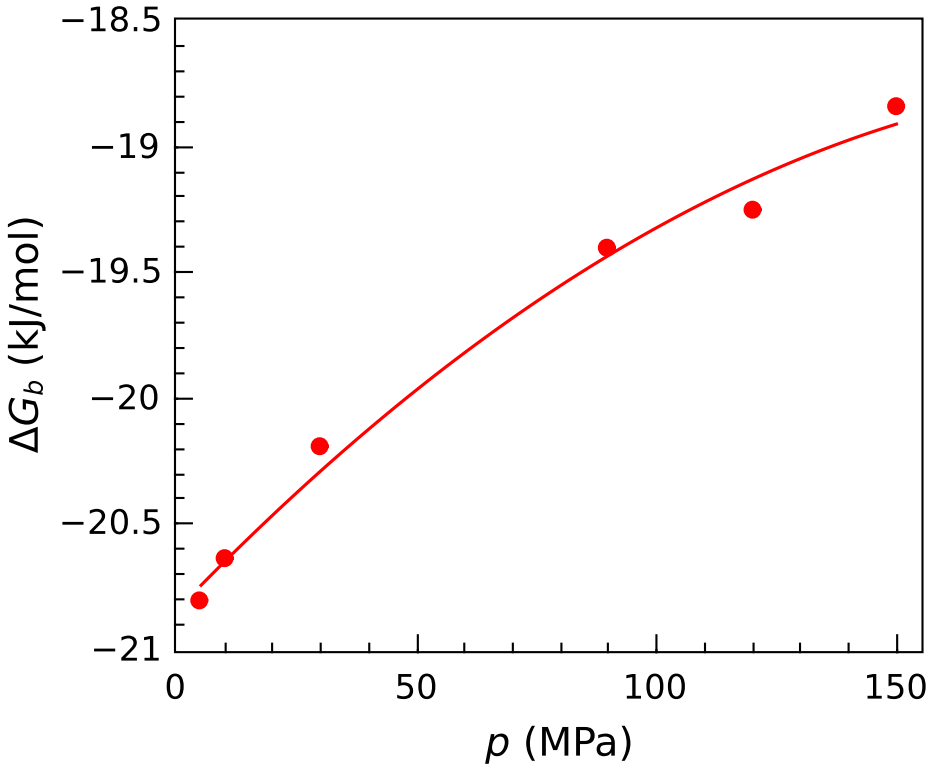


$$\Delta G_{b_0} = -20.7 \text{ kJ/mol}$$
$$\Delta V_{b_0} = 13 \text{ cm}^3/\text{mol}$$
$$\Delta \beta_b = 0 \text{ cm}^6/(\text{J mol})$$

Gibbs energy as a function of pressure

$$\Delta G_b = \Delta G_{b_0} + \Delta V_{b_0} \Delta p + \frac{\Delta \beta_b}{2} (\Delta p)^2.$$



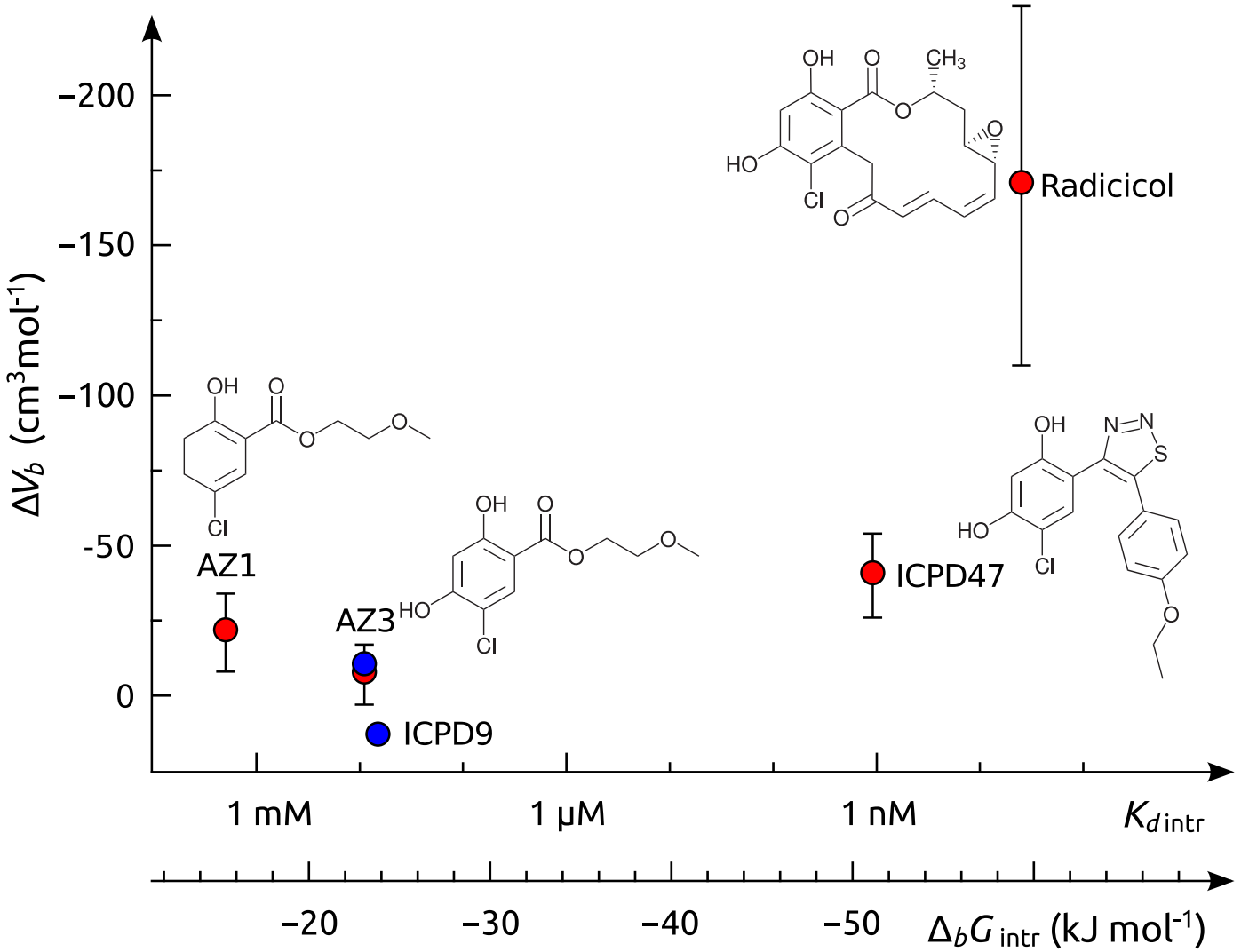


$\Delta G_{b_0} = -20.7$ kJ/mol
$\Delta V_{b_0} = 13$ cm^3/mol
$\Delta \beta_b = 0$ cm^6/(J mol)

$\Delta G_{b_0} = -20.8$ kJ/mol
$\Delta V_{b_0} = 20$ cm^3/mol
$\Delta \beta_b = 0.05$ cm^6/(J mol)

Binding volume correlation with affinity

Including NMR data (blue points)



Concluding remarks

- Our results suggest that weak ligands of Hsp90N protein induce smaller changes in volume than tight-binding ligands.
- Both techniques FPSA and NMR provide similar values of binding volume.

Acknowledgments

High pressure team at the department of Biothermodynamics and Drug Design:

- Prof. Daumantas Matulis, Gediminas Skvarnavičius, Dr. Zigmas Toleikis, Joana Smirnovienė, Dr. Piotras Cimmperman, Martynas Grigaliūnas, Povilas Norvaišas

NMR data:

- Prof. Christian Roumestand (CBS Montpellier)

Funding:

- Research Council of Lithuania (grant No. MIP-004/2014)

Lietuvos mokslo taryba

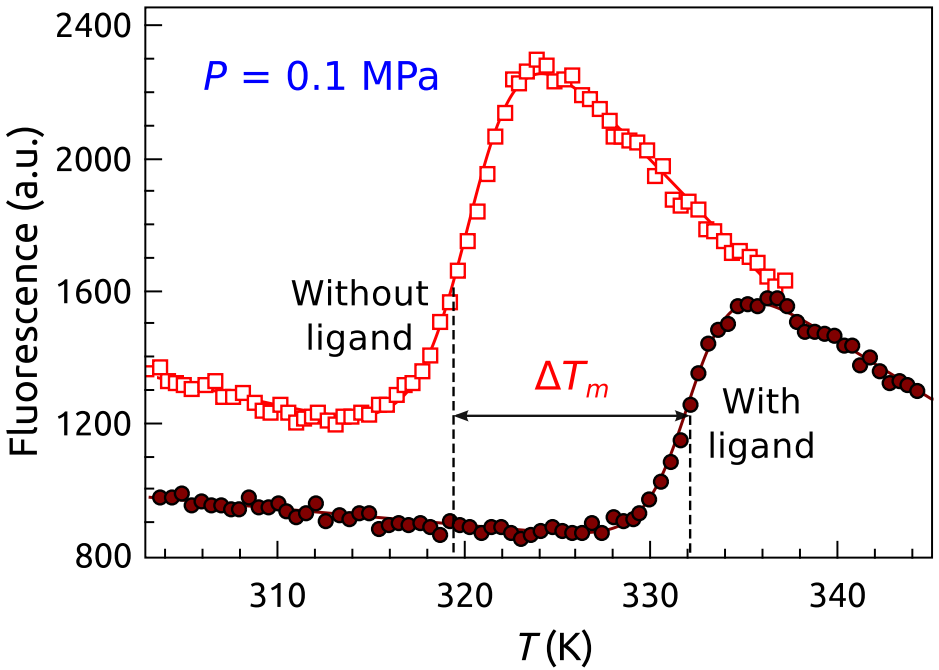
High-pressure team

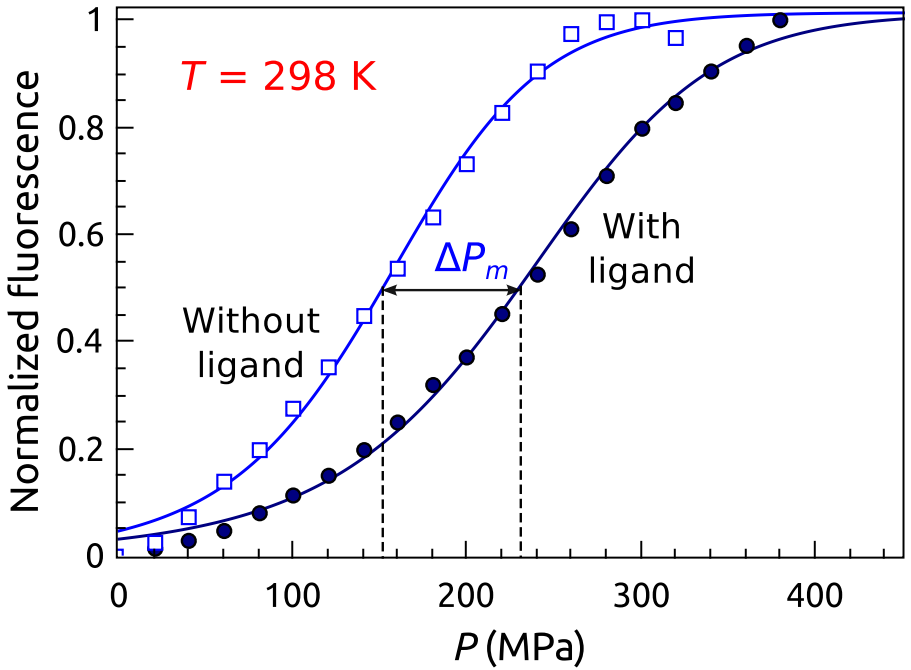
Thank you for your attention!

Supplementary material

FTSA and FPSA methods

Examples of Hsp90N protein stabilization by ligand against T and P denaturation





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

$\Delta_U G$ as a function of temperature at a constant pressure:

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

$\Delta_U G$ as a function of pressure at a constant temperature:

$$\Delta_U G_P = \Delta_U G_0 + \Delta_U V_0 (p - p_0) + \frac{\Delta_U \beta}{2} (p - p_0)^2$$

The Gibbs energy change

The full differential of the Gibbs energy change $\Delta G = G_U - G_N$

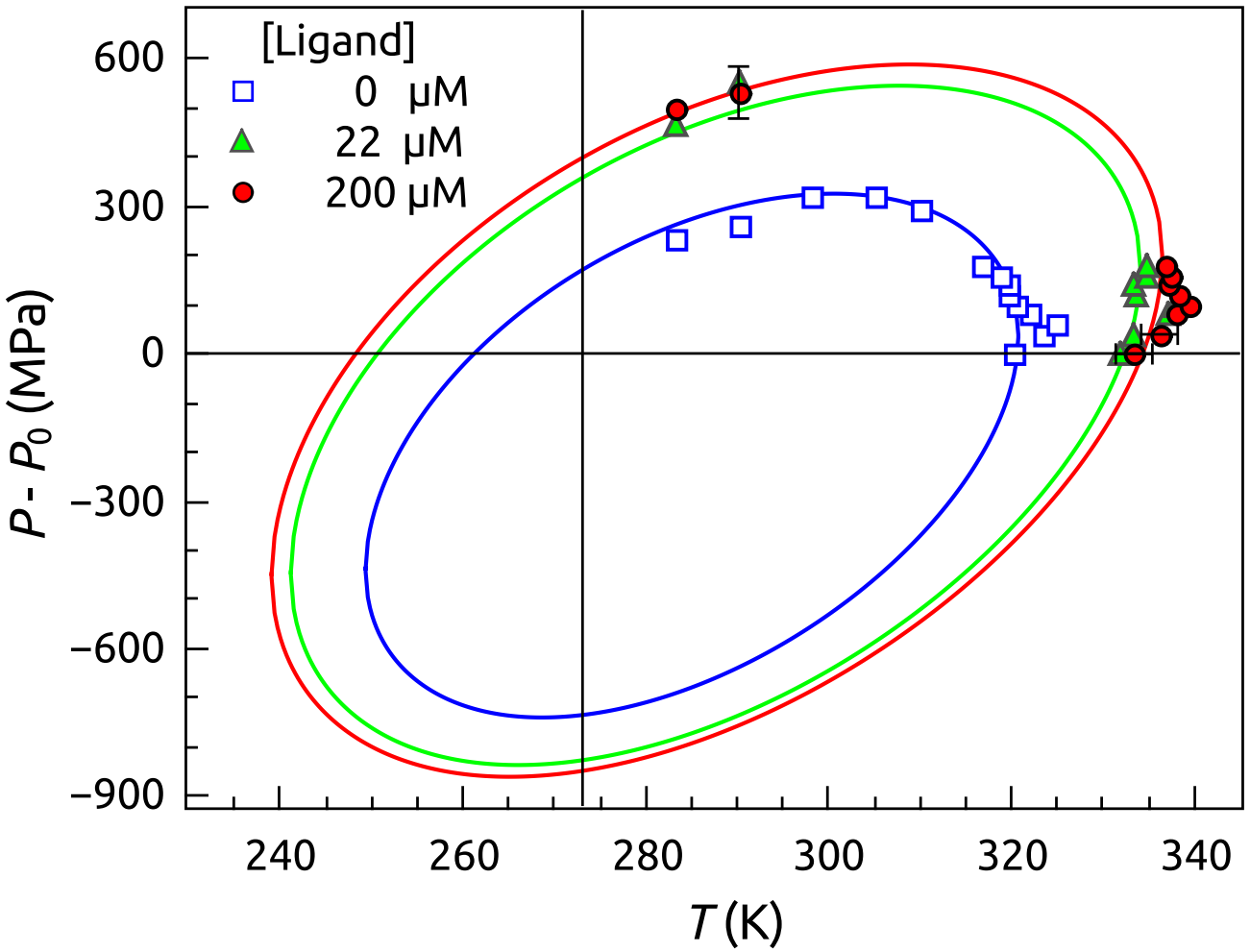
$$\mathrm{d}(\Delta G) = -\Delta S \mathrm{d}T + \Delta V \mathrm{d}p$$

as a function of temperature T and pressure P.

$$\Delta G = \frac{\Delta\beta}{2}(p - p_0)^2 + \Delta V_0(p - p_0) + \Delta\alpha(p - p_0)(T - T_0) -$$
$$\Delta C_p \left[T \left(\ln \frac{T}{T_0} - 1 \right) + T_0 \right] - (\Delta H_0 - \Delta G_0) \left(\frac{T}{T_0} - 1 \right) + \Delta G_0$$

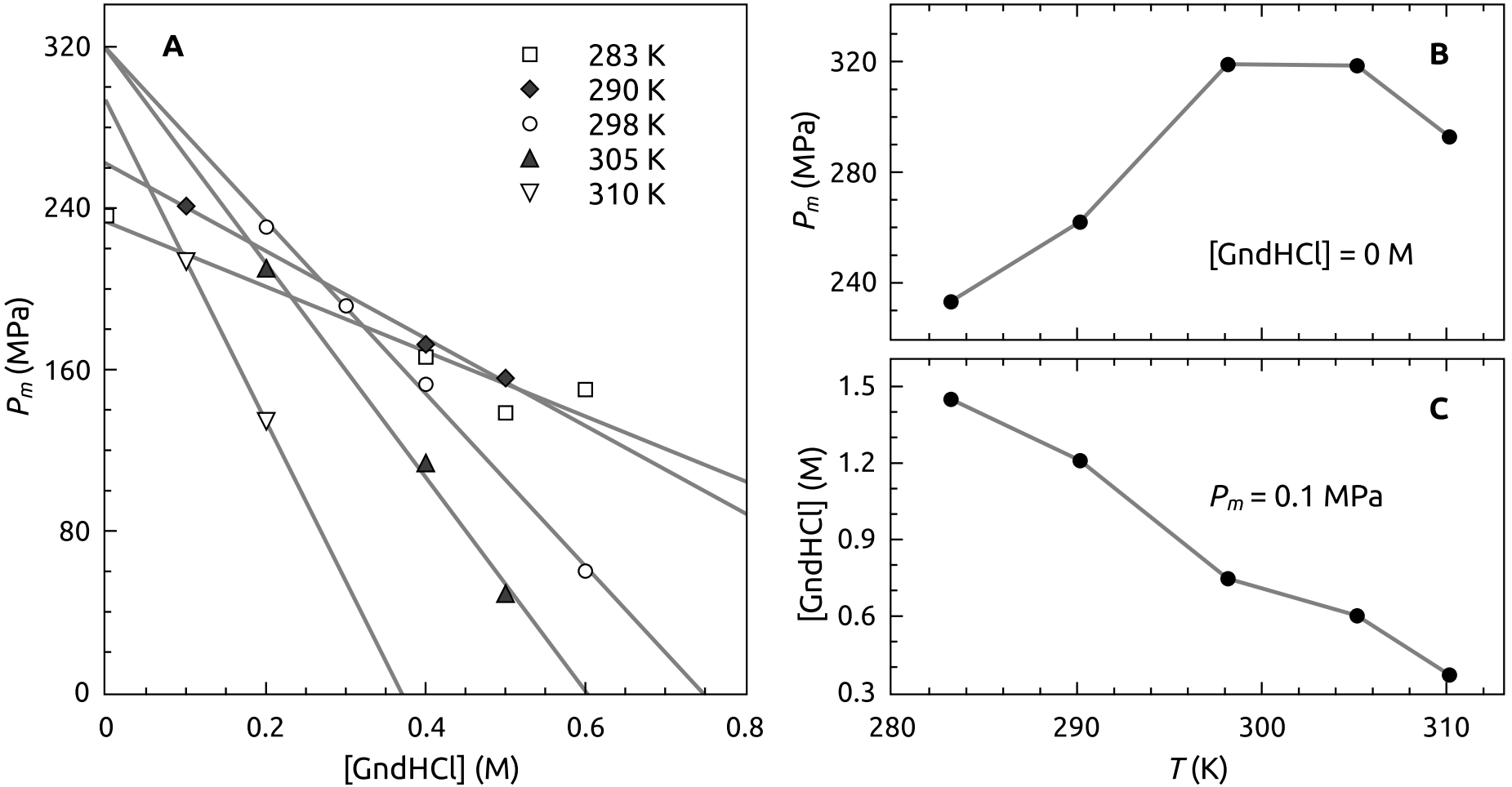
Hsp90N protein stability diagram in P–T coordinates

With added ligand



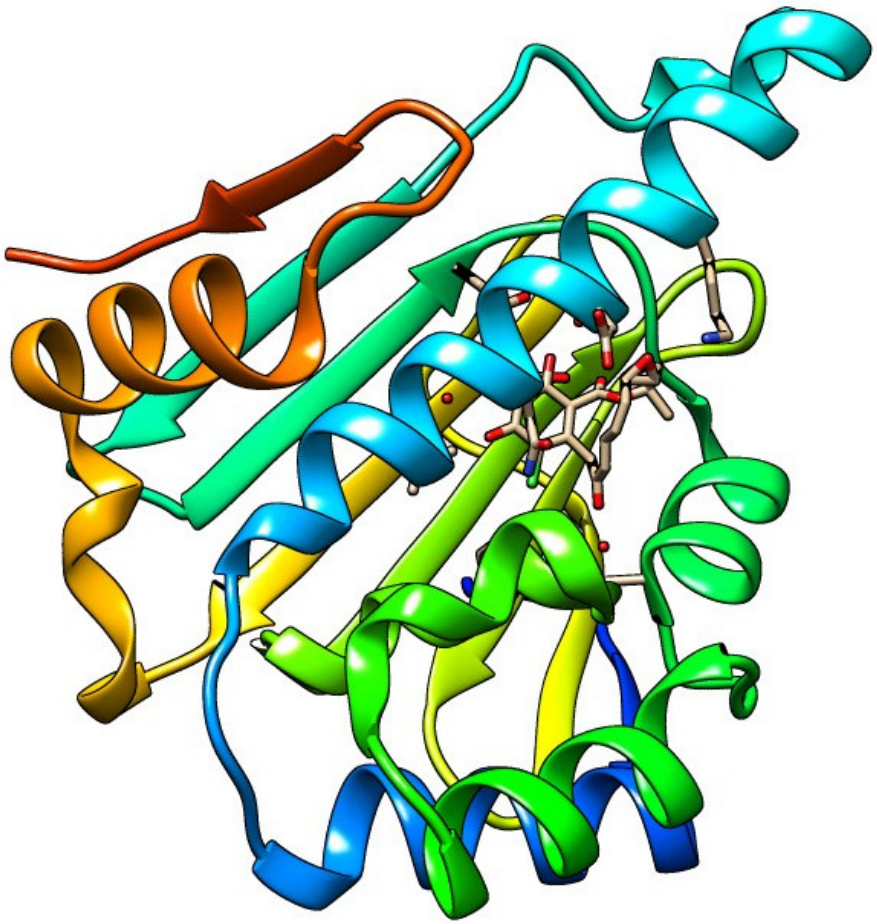
Hsp90N stabilization by ligands

The use of guanidine hydrochloride – a protein destabilizing agent



Protein stabilization by ligands

Hsp90N + radicicol



Hsp90N + radicicol
PDB ID: 4EGK

Hsp90N melting temperatures T_m

[Hsp90N] = 14 μM

[Radicicol] μM	T_m (°C)
0	51.6
2	61.1
20	64.9

Zubriene et al. *Int J Mol Sci* **10** (2009) 2662–2680.