Volume of Hsp90 ligand binding and the unfolding phase diagram as a function of pressure and temperature

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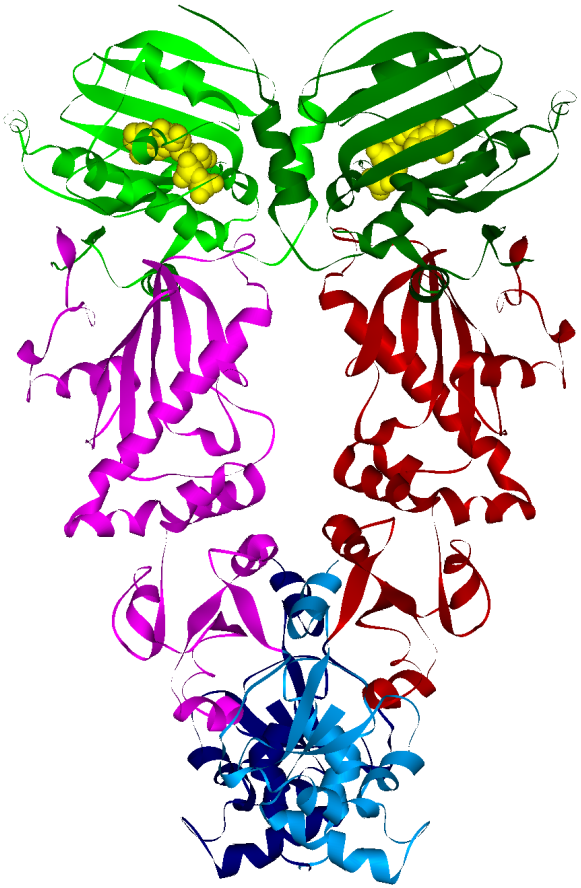
Outline

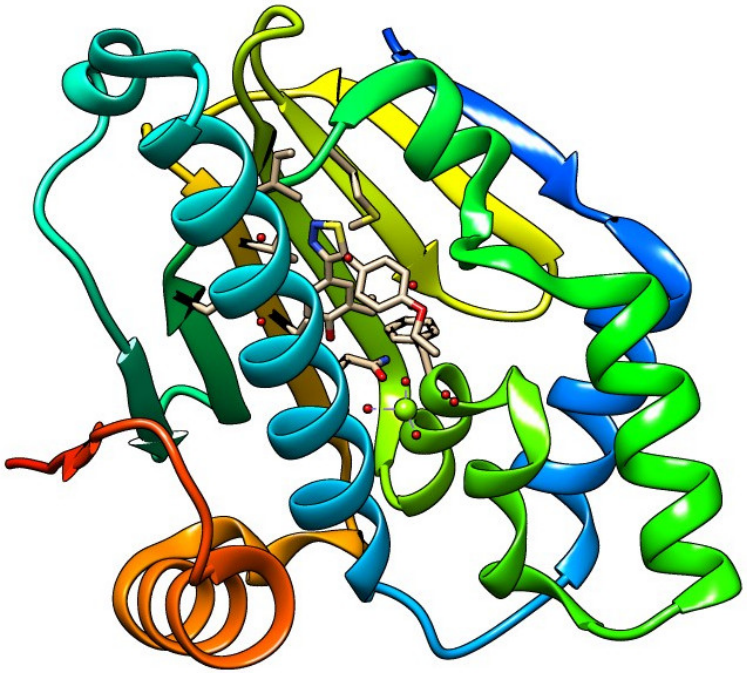
- Introduction
- General equations of thermodynamics
- Temperature and pressure shift assays
- Protein stability diagrams
- Concluding remarks

Introduction

Crystal structures of proteins



Heat shock protein 90 (Hsp90)
PDB ID: 2CG9



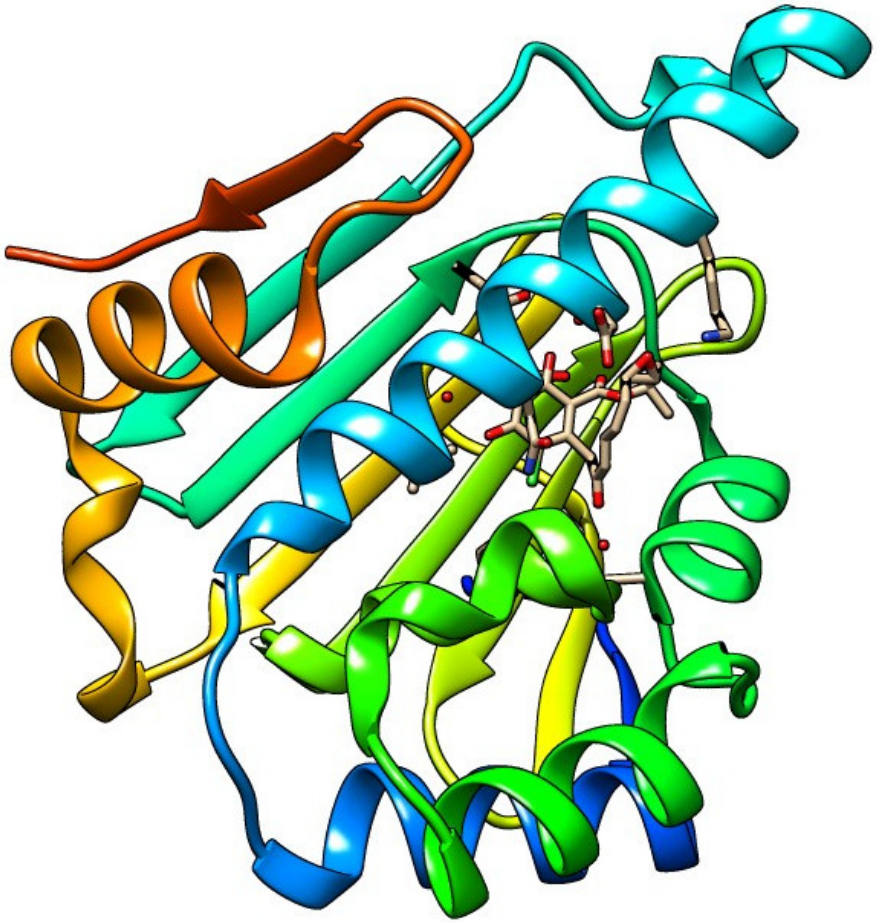
Hsp90 N-terminal domain
PDB ID: 2YI6

Protein denaturation:

- Chemical
  - strong acid or base
  - concentrated inorganic salt
  - organic solvent
- Physical
  - temperature
  - pressure

Introduction

Protein stabilization by ligands



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.

Introduction

The full differential of the Gibbs free energy

$$\mathrm{d}G(T,P) = -S\mathrm{d}T + V\mathrm{d}P$$

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Motivation:

- Fundamental knowledge about protein pressure and temperature denaturation.
- To determine the changes in protein volume associated with ligand binding. This could have applications in rational drug design.

General thermodynamics

The full differential of the Gibbs free 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.

General thermodynamics

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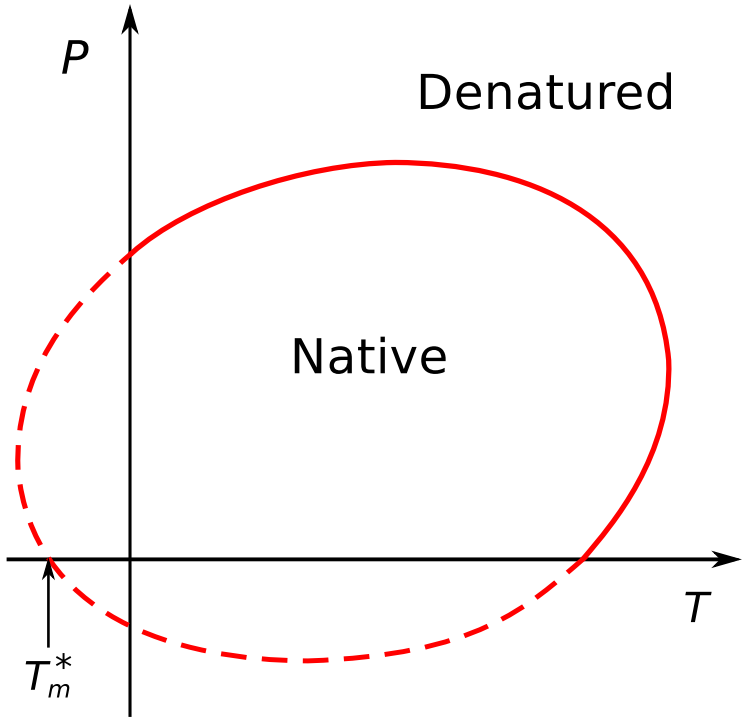
$$\Delta G = \frac{\Delta\beta}{2}(P - P_0)^2 + \Delta V_0(P - P_0) + \Delta\alpha(P - P_0)(T - T_0) -$$
$$\Delta C_p \left[T \left(\ln \frac{T}{T_0} - 1 \right) + T_0 \right] - (\Delta H_0 - \Delta G_0) \left(\frac{T}{T_0} - 1 \right) + \Delta G_0$$

Protein stability diagram

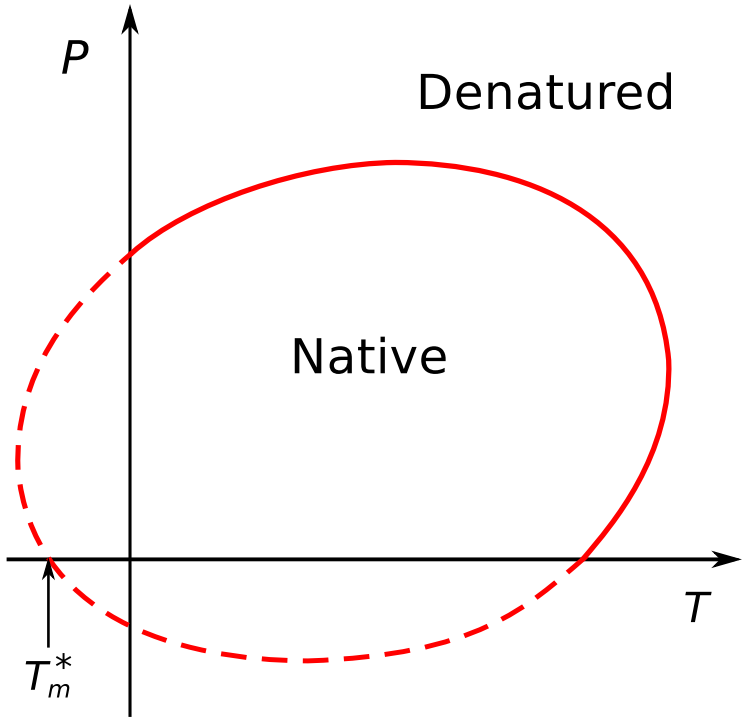
In pressure–temperature coordinates



T_m^* – cold denaturation temperature at reference pressure

Protein stability diagram

In pressure–temperature coordinates



T_m^* – cold denaturation temperature at reference pressure

- Not all regions of the P–T diagram are experimentally accessible.
- Pressure and temperature induced protein denaturation covers different parts of the diagram.
- Two approaches – thermal shift assay (TSA) and pressure shift assay (PSA) – were used to determine protein stability diagram.

TSA and PSA methods

Fluorescence yield

Both TSA and PSA are fluorescence based methods

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

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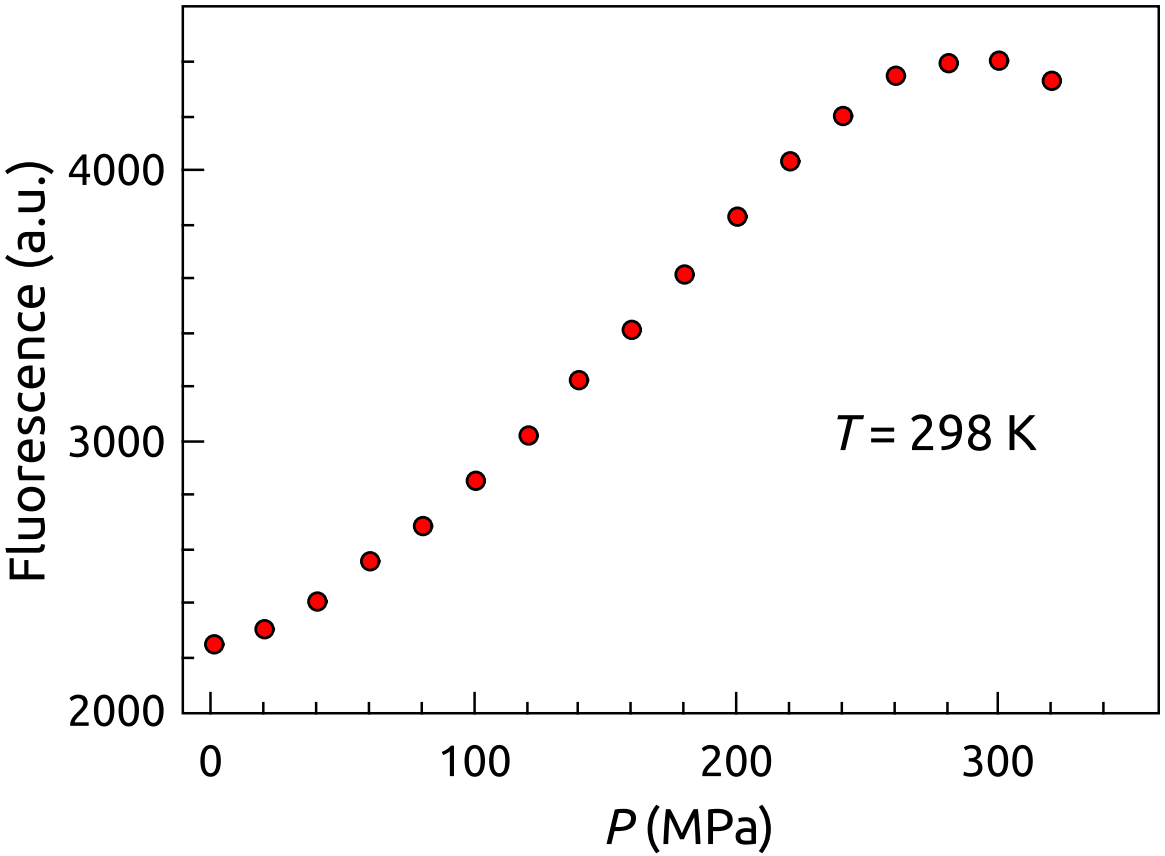

Experimental fluorescence data of pressure induced protein unfolding

TSA and PSA methods

Fluorescence yield

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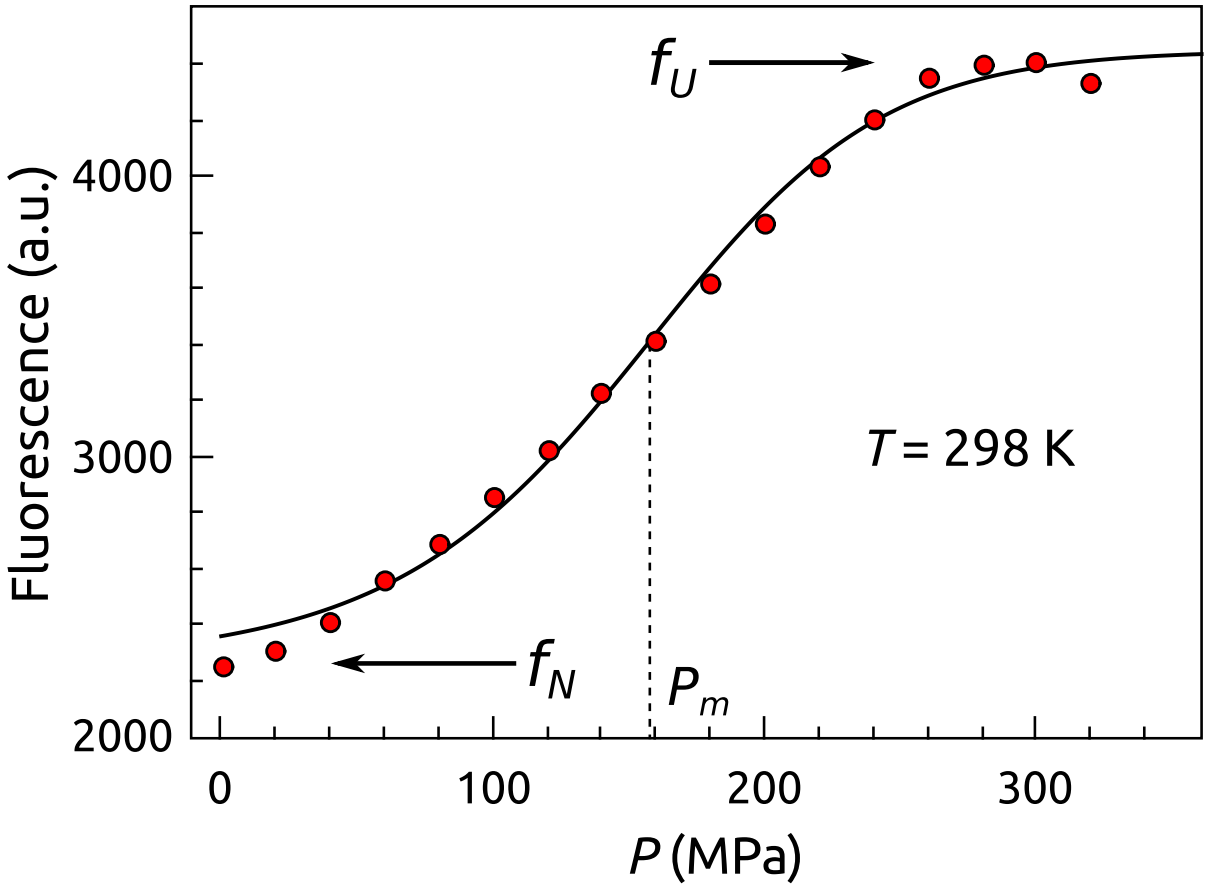


Experimental data and a fitted fluorescence curve of pressure induced protein unfolding

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

$\Delta_U G$ 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:

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

$$L_t = (\exp(-\Delta_U G) - 1) \times \left(\frac{P_t}{2\exp(-\Delta_U G)} + \frac{1}{\exp(-\Delta_b G)} \right)$$

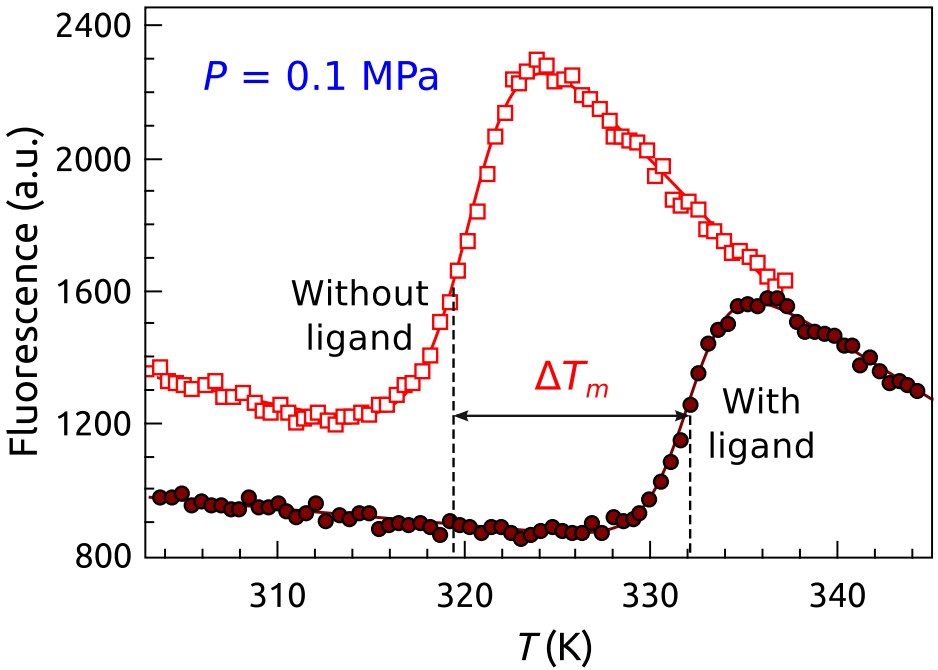
L_t – ligand concentration,
P_t – protein concentration.

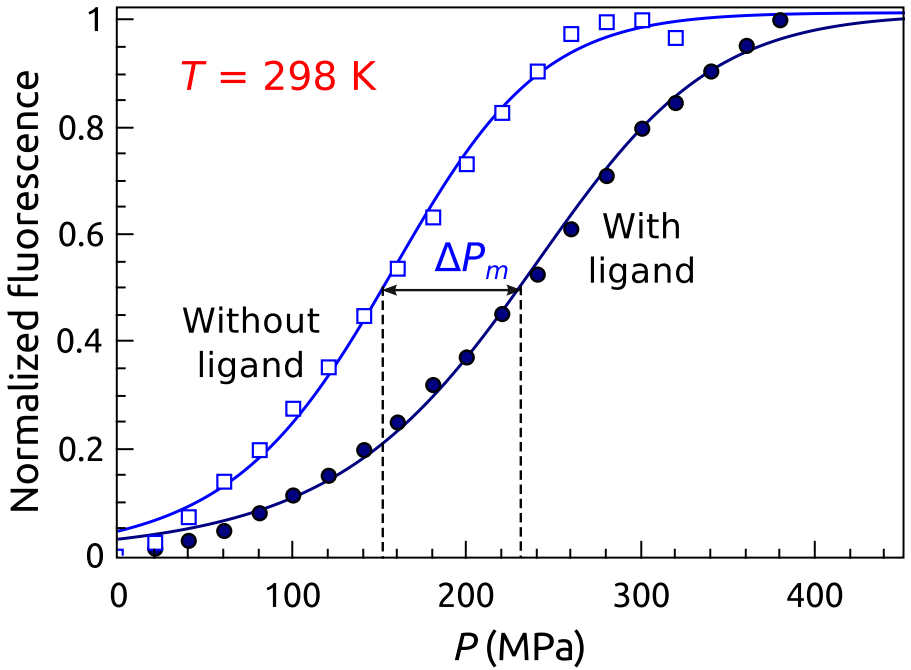
$\Delta_b G$ contains protein–ligand binding parameters.

$\Delta_b V = \left(\frac{\partial \Delta_b G}{\partial P} \right)_T$ – volume of ligand binding – a thermodynamic parameter obtained by pressure shift assay.

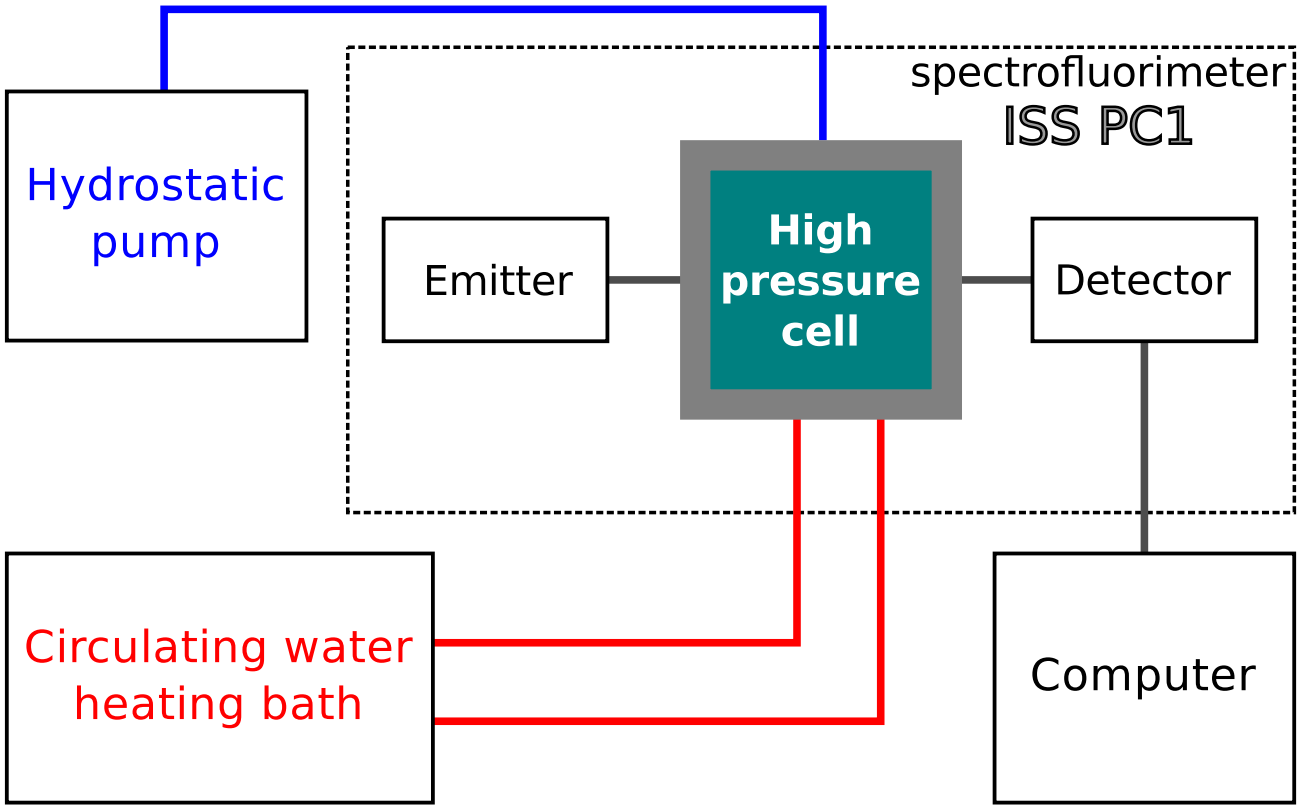
TSA and PSA methods

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





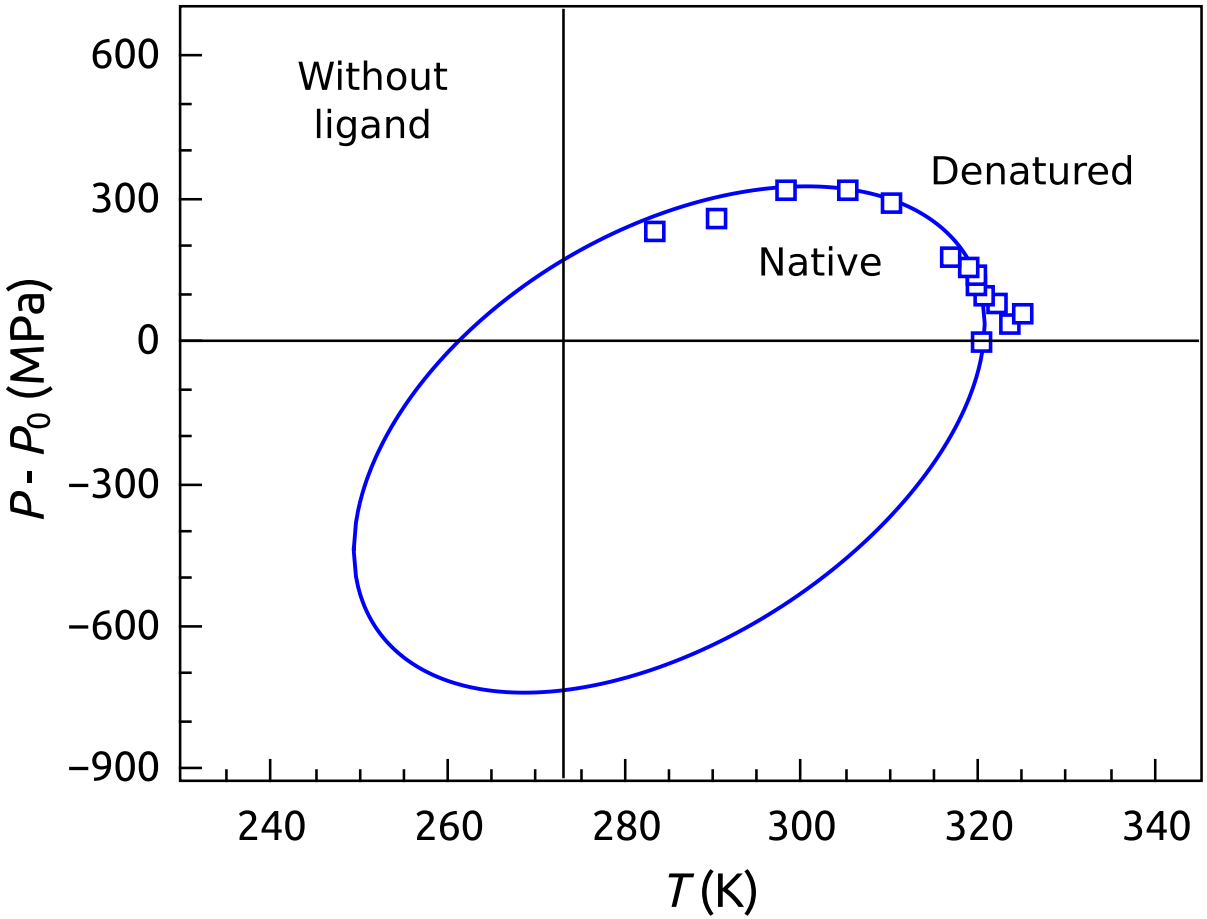
Experimental setup



Pressure range: (0.1 – 400) MPa
Temperature range: (10 – 90) ℃
Fluorescence probe: 1,8-anilinonaphthalene sulfonate (ANS)

Hsp90N protein stability diagram in P–T coordinates

Without ligand

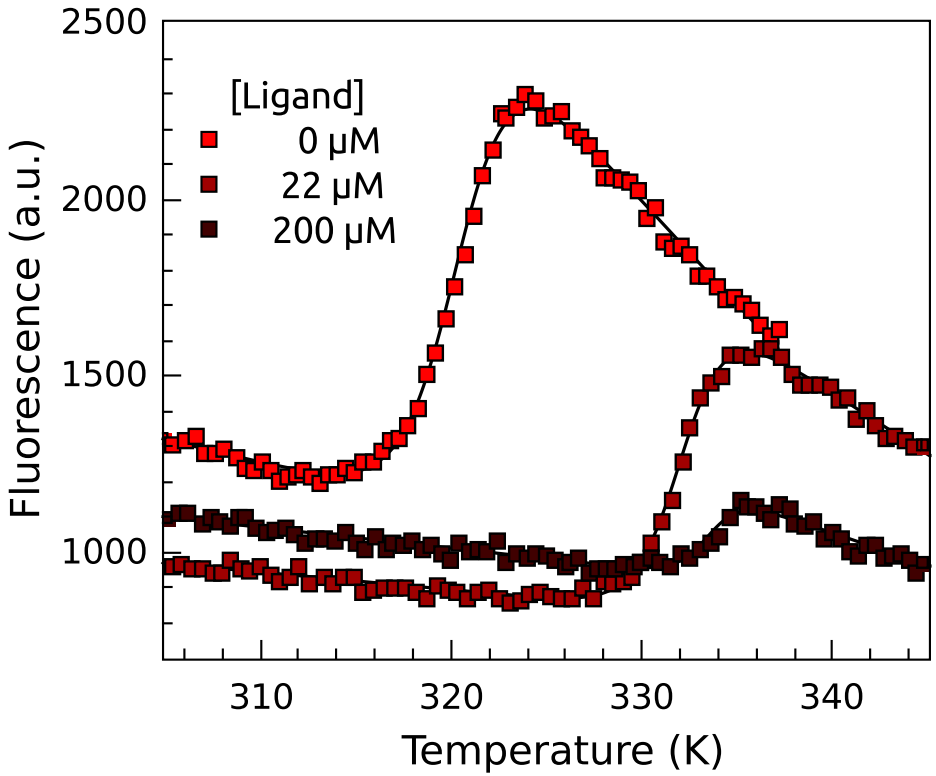


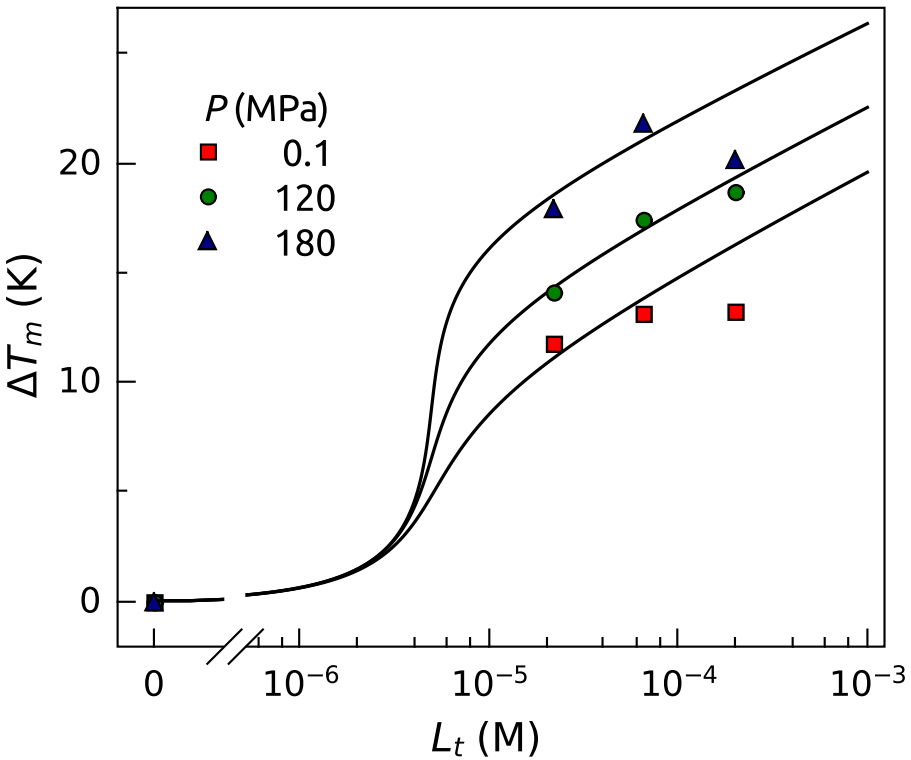
TSA approach – right part of the ellipse

PSA approach – upper part of the ellipse

Hsp90N stabilization by ligands

Denaturation by temperature at elevated pressures





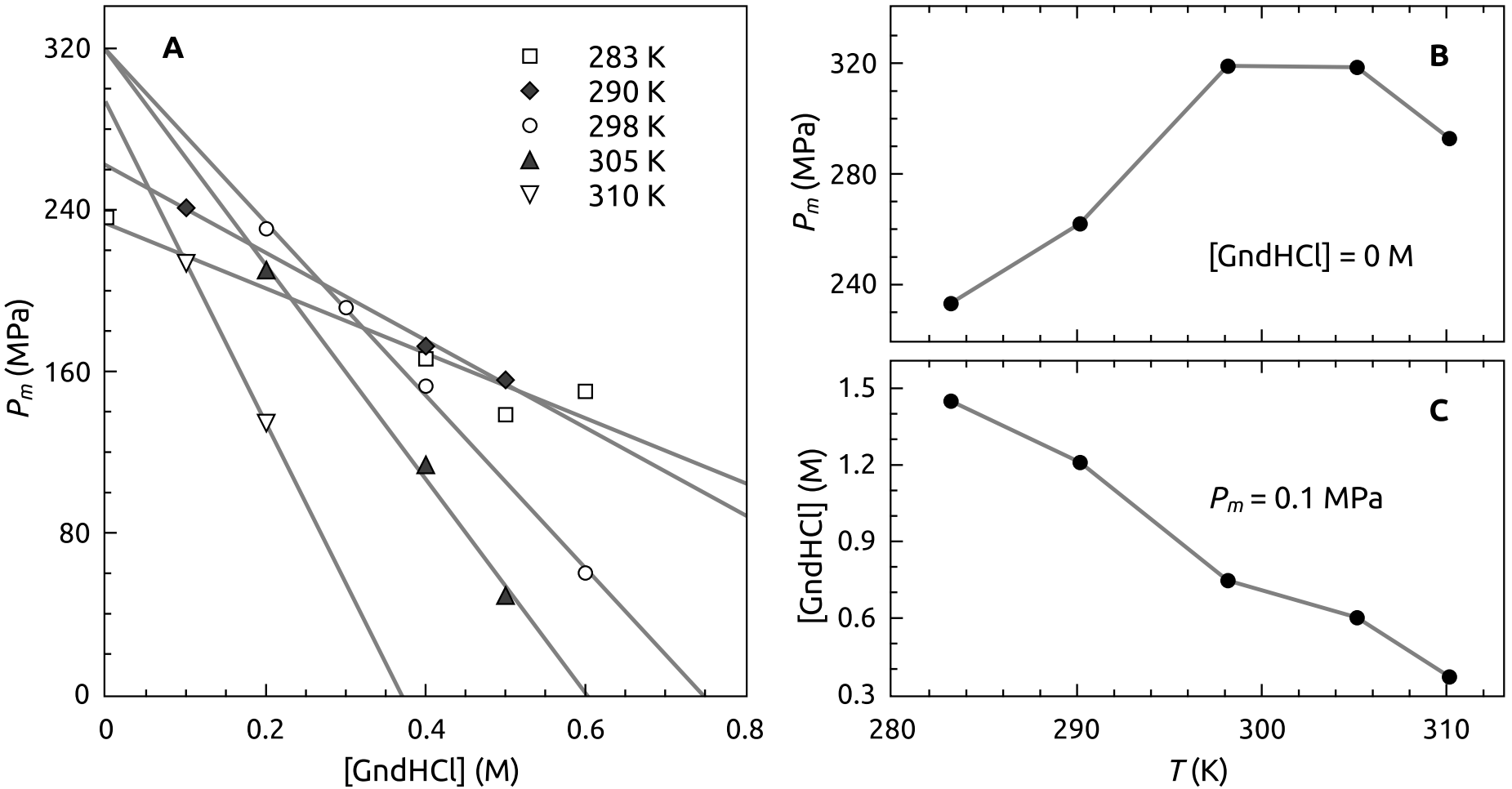
Hsp90N stabilization by ligands

Pressure equipment limitations

- Tight–binding ligands increased P_m values, thus higher pressure was necessary for complete protein unfolding.
- The required pressure was higher than it was possible with our equipment.
- Addition of guanidine hydrochloride (GndHCl) – a protein destabilizing agent – was necessary to observe Hsp90N denaturation by pressure.
- Estimation of true P_m values (those which are expected to be obtained without addition of GndHCl).

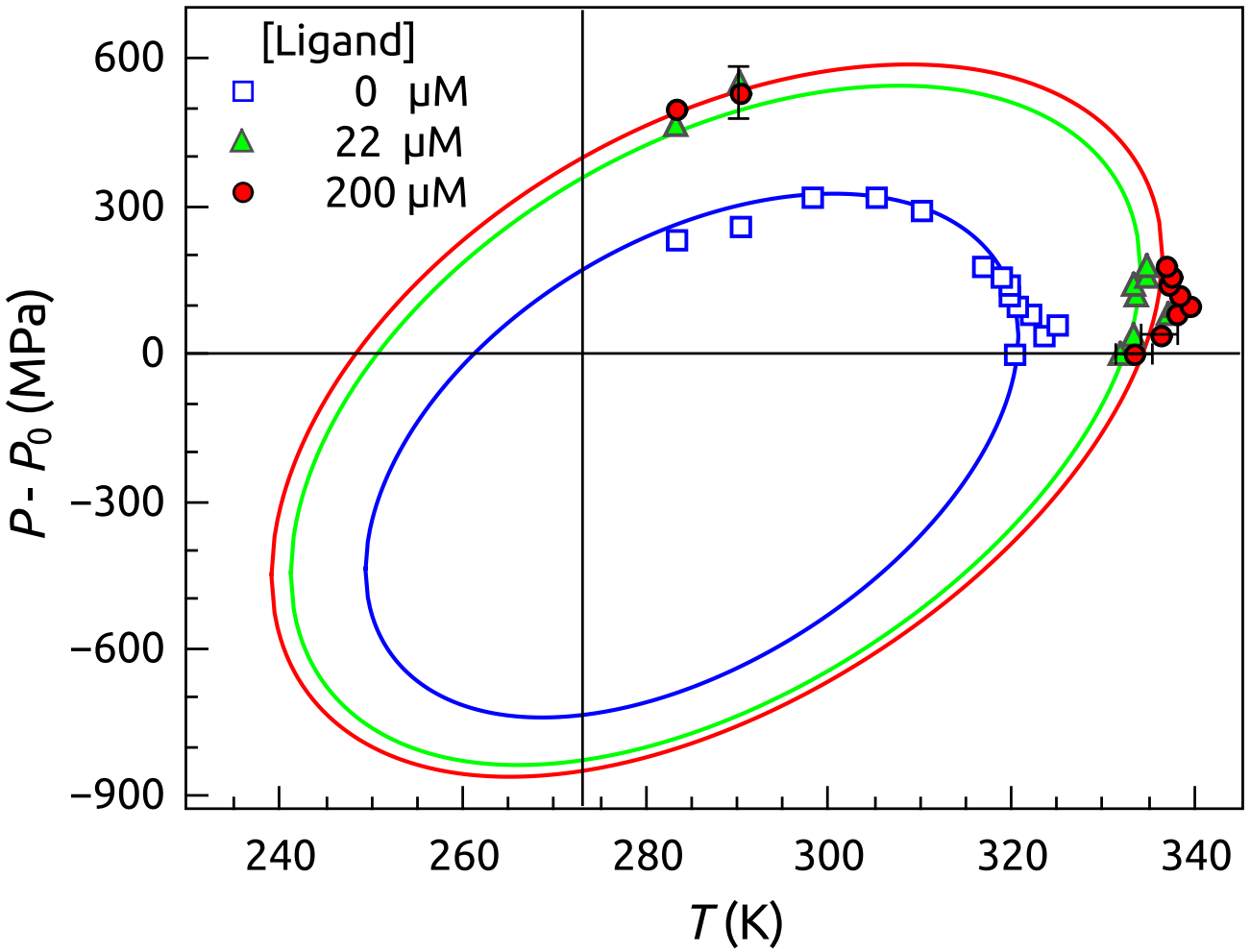
Hsp90N stabilization by ligands

The use of guanidine hydrochloride – a protein destabilizing agent



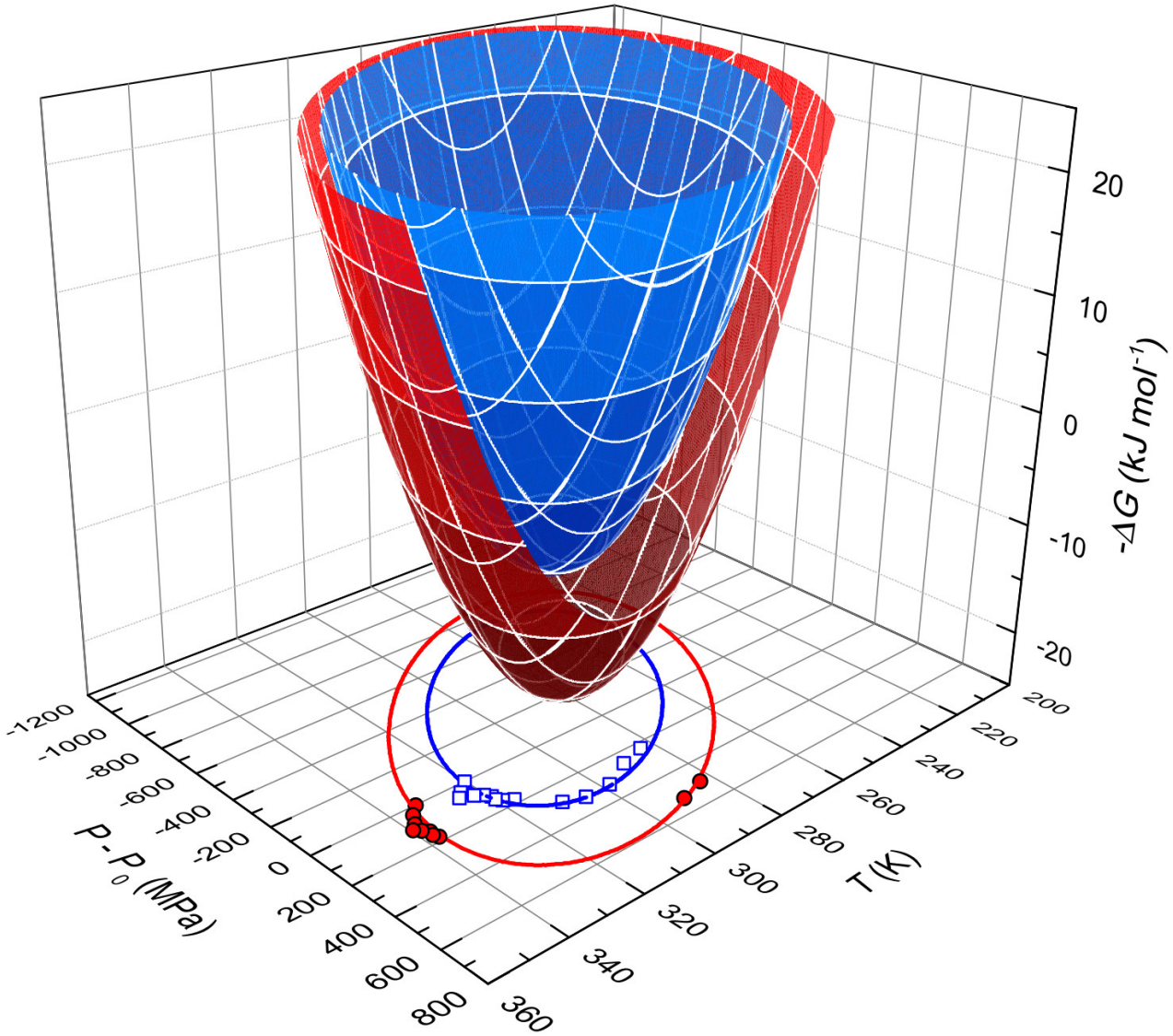
Hsp90N protein stability diagram in P–T coordinates

With added ligand

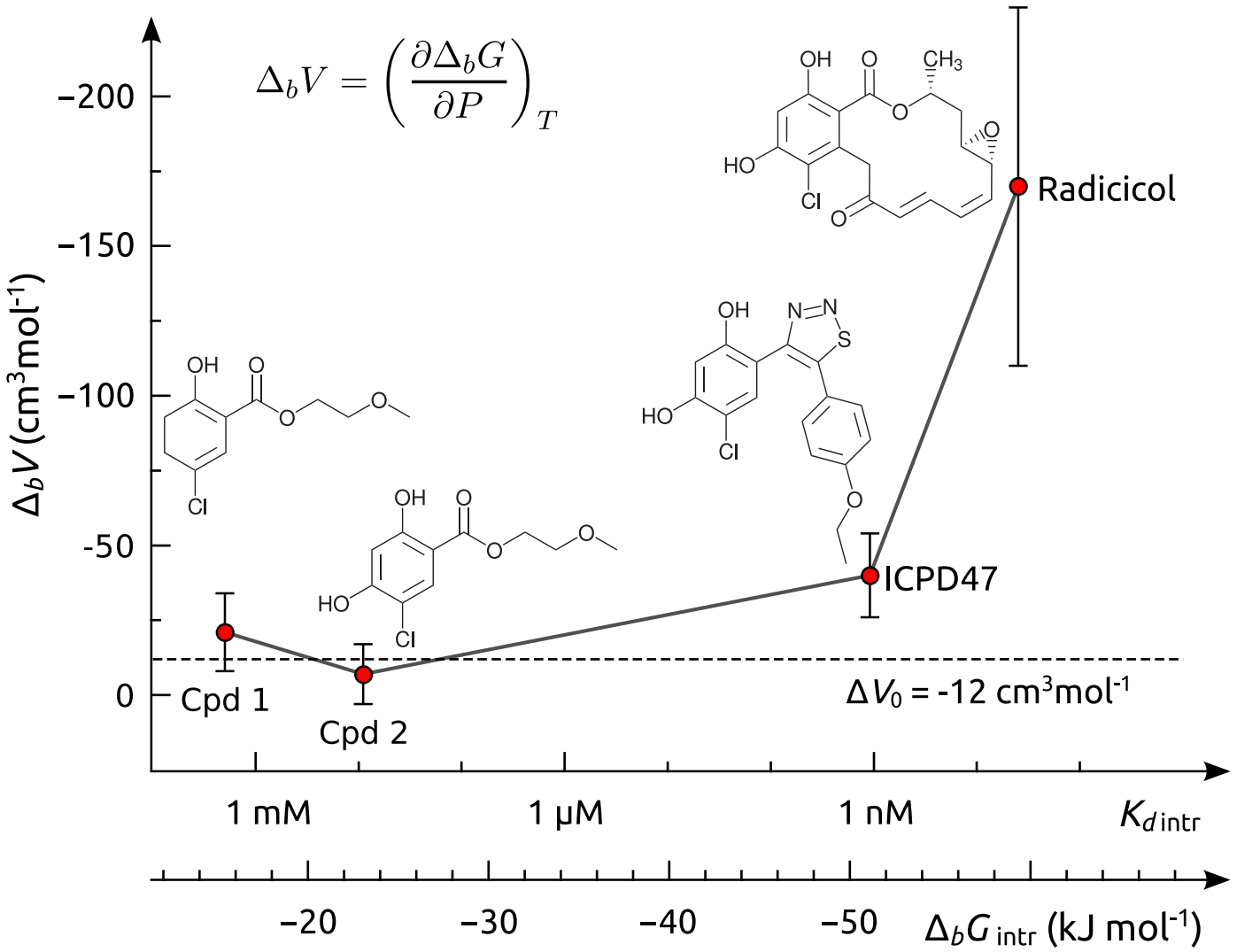


Hsp90N protein stability diagram

The Gibbs free energy dependence on pressure and temperature



Binding volume correlation with affinity



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

Conclusions

- The use of both TSA and PSA techniques provides a detailed thermodynamic information about thermal and volumetric properties of proteins.
- Protein–ligand affinities may correlate with binding volumes.
- The practical use of the binding volume and affinity correlation diagram is limited by the lack of reported protein–ligand binding volumes in the literature.

Acknowledgment

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Piotras Cimmperman

Biothermodynamics and Drug Design Lab

Thank you for your attention!

Appendix: thermal shift assay (ThermoFluor®)