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

Vytautas Petrauskas

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

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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) °C;
- Fluorescence probe: intrinsic tryptophan (excited 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

Vytautas Petrauskas (Vilnius University)

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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:

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

\[ K_b = \exp \left( -\frac{\Delta G_b}{RT} \right) = \left( \frac{\Delta G_{0b} + \Delta V_{0b}(p_m - p_0) + \frac{\Delta \beta_b}{2}(p_m - p_0)^2}{RT} \right) \]
Hsp90N protein stability diagram in $P-T$ coordinates

With added ligand

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-$^1$5N–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:

\[ \Delta \delta = \sqrt{\left( \delta^H_0 - \delta^H \right)^2 + \left( \frac{\gamma^N}{\gamma^H} \right)^2 \left( \delta^N_0 - \delta^N \right)^2} \]

\[ \delta_0 \] – chemical shift without ligand.

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

Chemical shift equation reference:

Gibbs energy as a function of pressure

\[
\Delta G_b = \Delta G_{b0} + \Delta V_{b0} \Delta p + \frac{\Delta \beta_b}{2} (\Delta p)^2.
\]

\[
\Delta G_{b0} = -20.7 \text{ kJ/mol}
\]

\[
\Delta V_{b0} = 13 \text{ cm}^3/\text{mol}
\]

\[
\Delta \beta_b = 0 \text{ cm}^6/(\text{J mol})
\]
Gibbs energy as a function of pressure

\[ \Delta G_b = \Delta G_{b0} + \Delta V_{b0} \Delta p + \frac{\Delta \beta_b}{2} (\Delta p)^2. \]

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\[ \Delta V_{b0} = 13 \text{ cm}^3/\text{mol} \]
\[ \Delta \beta_b = 0 \text{ cm}^6/(\text{J mol}) \]

\[ \Delta G_{b0} = -20.8 \text{ kJ/mol} \]
\[ \Delta V_{b0} = 20 \text{ cm}^3/\text{mol} \]
\[ \Delta \beta_b = 0.05 \text{ cm}^6/(\text{J mol}) \]
Binding volume correlation with affinity
Including NMR data (blue points)
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.
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Thank you for your attention!
Supplementary material
FTSA and FPSA methods
Examples of Hsp90N protein stabilization by ligand against $T$ and $P$ denaturation

$P = 0.1 \text{ MPa}$

$T = 298 \text{ K}$
FTSA and FPSA methods
Expressions for the Gibbs energy

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

\( \Delta_U G \) 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 $\Delta G = G_U - G_N$

$$d(\Delta G) = -\Delta SdT + \Delta VdP$$

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

![Graph showing Hsp90N protein stability diagram in $P$–$T$ coordinates with added ligand concentrations of 0 μM, 22 μM, and 200 μM.](image-url)
Hsp90N stabilization by ligands

The use of guanidine hydrochloride – a protein destabilizing agent

![Graph A](image1.png)

A

- 283 K
- 290 K
- 298 K
- 305 K
- 310 K

![Graph B](image2.png)

B

\[ [\text{GndHCl}] = 0 \text{ M} \]

![Graph C](image3.png)

C

\[ P_m = 0.1 \text{ MPa} \]
Protein stabilization by ligands
Hsp90N + radicicol

Hsp90N melting temperatures $T_m$

<table>
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<th>[Hsp90N] µM</th>
<th>$T_m$ (°C)</th>
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