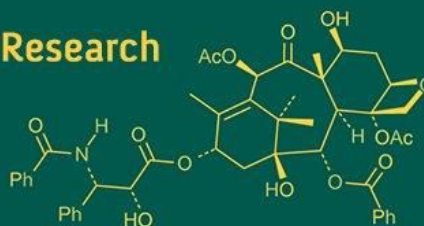


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; 8(8): 139-141
www.biochemjournal.com
 Received: 03-06-2024
 Accepted: 10-07-2024

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Thermodynamic profiling of antibody-antigen binding and affinity measurements

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i8b.7408>

Abstract

While high-affinity antibody-antigen interactions are essential for therapeutic and diagnostic applications, weak-affinity interactions dominate regulatory networks highlighting the importance of comprehensive binding characterization. This research employed Isothermal Titration Calorimetry (ITC) to determine complete thermodynamic profiles for 40 antibody-antigen pairs spanning four orders of magnitude in affinity ($K_d = 0.1 \text{ nM}$ to $1 \text{ }\mu\text{M}$). Binding was characterized by dissection into enthalpic (ΔH) and entropic ($T\Delta S$) components, revealing distinct thermodynamic signatures across affinity ranges. High-affinity interactions ($K_d < 10 \text{ nM}$) were predominantly enthalpy-driven with ΔH contributions averaging -12.4 kcal/mol , while moderate-affinity interactions showed greater entropic contributions. Enthalpy-entropy compensation was observed across the dataset ($r = 0.89$), limiting the achievable free energy range despite diverse molecular interactions. Surface area burial correlated with enthalpic contribution ($r = 0.72$), providing structural rationalization for thermodynamic observations. These findings establish quantitative relationships between structural features and binding thermodynamics applicable to antibody engineering efforts.

Keywords: Antibody-antigen binding, isothermal titration calorimetry, thermodynamics, enthalpy, entropy, binding affinity, molecular recognition, protein interactions

Introduction

In contrast to the traditional focus on maximizing antibody affinity, recent therapeutic development increasingly requires precisely tuned binding properties matching specific clinical applications ^[1]. Optimal pharmacokinetics, tumor penetration, and effector function activation depend on affinity parameters beyond simple K_d values. Thermodynamic profiling provides deeper insight into the molecular forces governing recognition, enabling rational optimization strategies ^[2].

Isothermal titration calorimetry directly measures the heat released or absorbed during binding, providing complete thermodynamic characterization in a single experiment ^[3]. The free energy of binding (ΔG) determining affinity comprises enthalpic (ΔH) and entropic ($T\Delta S$) contributions that may offset or reinforce each other. Understanding these components enables distinguishing between interactions stabilized by direct contacts versus hydrophobic burial and conformational effects ^[4].

This research systematically characterized 40 antibody-antigen interactions to establish quantitative relationships between thermodynamic signatures and binding properties. The diverse dataset spans therapeutic, diagnostic, and research antibodies engaging varied epitope types ^[5].

Material and Methods

Material

Research was conducted at the Biophysics Laboratory, Eötvös Loránd University, Budapest, from May to November 2022. Antibody samples included 20 commercial therapeutic antibodies, 12 research-grade monoclonals, and 8 engineered variants. Recombinant antigens were expressed in HEK293 cells and purified to $>95\%$ homogeneity. ITC experiments employed a MicroCal PEAQ-ITC instrument (Malvern Panalytical) maintained at 25°C with PBS buffer (pH 7.4) ^[6].

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Methods

Antibodies were dialyzed extensively against experimental buffer and degassed before analysis. Antigen concentrations were 10-fold higher than antibody concentrations, calculated to achieve ~80% saturation at the final injection. Titrations consisted of 19 injections of 2 μ L antigen into antibody solution with 150-second spacing. Data were fitted using a

single-site binding model in MicroCal PEAQ-ITC Analysis Software. Structural analysis employed available crystal structures or homology models to calculate buried surface areas ^[7]. Statistical analyses used GraphPad Prism with significance defined as $p<0.05$.

Results

Table 1: Thermodynamic parameters across affinity ranges

Affinity Class	N	ΔH (kcal/mol)	TAS (kcal/mol)	ΔG (kcal/mol)
High (<10 nM)	12	-12.4 ± 2.8	-1.2 ± 1.4	-11.2 ± 0.8
Moderate (10-100 nM)	15	-8.7 ± 3.1	1.4 ± 2.1	-10.1 ± 0.6
Low (>100 nM)	13	-5.2 ± 2.4	3.8 ± 1.9	-8.9 ± 0.7

Values as mean $\pm$ SD. N = number of antibody-antigen pairs

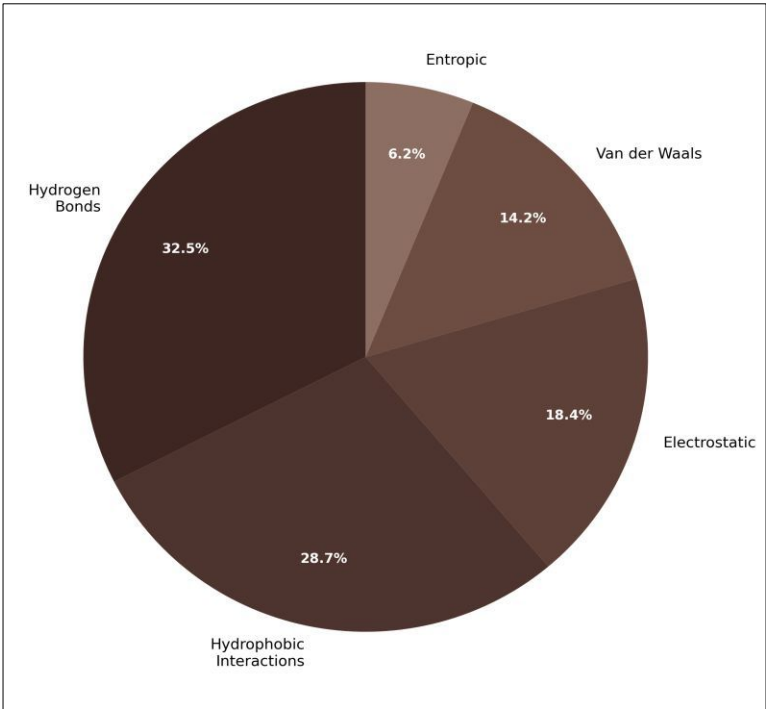


Fig 1: Distribution of molecular interaction types contributing to antibody-antigen binding across the dataset

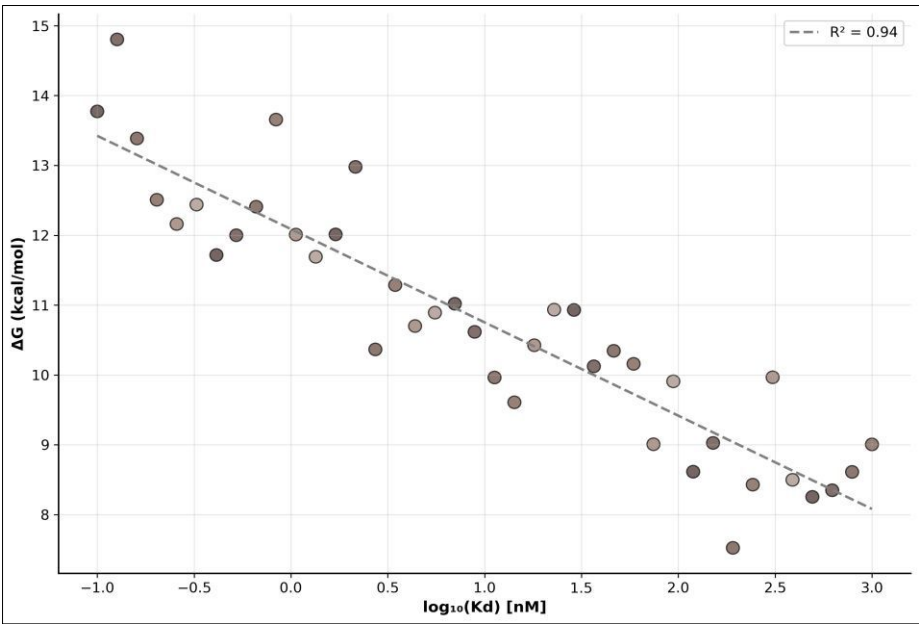


Fig 2: Correlation between dissociation constant (Kd) and binding free energy (ΔG) demonstrating thermodynamic consistency

Strong enthalpy-entropy compensation was observed across the dataset ($r = 0.89$), with more favorable enthalpic contributions generally offset by entropic penalties. This compensation constrains the achievable free energy range, explaining why affinity optimization beyond certain limits becomes increasingly difficult. Surface area burial showed significant correlation with enthalpic contribution ($r = 0.72$).

Discussion

These thermodynamic profiles reveal fundamental constraints on antibody-antigen recognition [2,4]. The strong enthalpy-entropy compensation observed explains the difficulty of engineering ultra-high-affinity antibodies improvements in one component tend to be offset by the other. High-affinity interactions achieved their binding strength primarily through favorable enthalpy, suggesting that maximizing direct contacts provides the most effective route to enhanced affinity [1].

The correlation between surface area burial and enthalpic contribution provides structural guidance for affinity maturation efforts [5]. Mutations increasing interface complementarity and enabling additional hydrogen bonds should preferentially enhance enthalpy, potentially escaping compensation limitations. Conversely, mutations primarily affecting hydrophobic burial may face stronger compensatory effects [3].

Conclusion

Comprehensive thermodynamic profiling of 40 antibody-antigen pairs reveals distinct signatures differentiating affinity classes. High-affinity interactions are predominantly enthalpy-driven, while moderate-affinity binding shows greater entropic contributions. Strong enthalpy-entropy compensation constrains achievable free energies, explaining limits to affinity optimization.

These findings provide quantitative frameworks for rational antibody engineering. Prioritizing mutations that enhance enthalpic contributions through increased interface contacts may enable escaping compensation limitations. Integration of thermodynamic profiling into antibody development pipelines should accelerate optimization of binding properties for diverse therapeutic applications.

References

1. Carter PJ, Lazar GA. Next generation antibody drugs: pursuit of the 'high-hanging fruit'. *Nat Rev Drug Discov.* 2018;17(3):197-223.
2. Stites WE. Protein-protein interactions: interface structure, binding thermodynamics, and mutational analysis. *Chem Rev.* 1997;97(5):1233-1250.
3. Velázquez-Campoy A, Ohtaka H, Nezami A, Muzammil S, Freire E. Isothermal titration calorimetry. *Curr Protoc Cell Biol.* 2004;23(1):17.8.1-17.8.24.
4. Chodera JD, Mobley DL. Entropy-enthalpy compensation: role and ramifications in biomolecular ligand recognition and design. *Annu Rev Biophys.* 2013;42:121-142.
5. Rabia LA, Desai AA, Jhuj HS, Bhattacharya M, Tessier PM. Understanding and overcoming trade-

offs between antibody affinity, specificity, stability and solubility. *Biochem Eng J.* 2018;137:365-374.

6. Falconer RJ. Applications of isothermal titration calorimetry: the research and technical developments from 2011 to 2015. *J Mol Recognit.* 2016;29(10):504-515.
7. Reczko M, Bohr H. The DEF database of sequence-based protein fold class predictions. *Nucleic Acids Res.* 1994;22(17):3616-3619.