

Finite POLGB Capacity Exposes a Conditional Gain Boundary in POLG Catalytic-Subunit Turnover

Andrew Friedl

Precision Mito, Florida, USA

Correspondence: afriedl@precisionmito.com

Abstract

Background: POLG disease-biology interpretation can conflate catalytic-subunit import, total POLGA abundance, POLGB association, active DNA engagement and synthesis readouts. A finite accessory-capacity model creates a narrower question: whether exact total-POLGA and synthesis trajectories can identify assembly, loss and capacity parameters, and which gain calibration remains unresolved.

Objective: This working paper retains the selected question: In a declared POLG catalytic-subunit turnover model with finite POLGB, known imported input and absolute total POLGA, which assembly, loss and capacity parameters are conditionally recoverable from a constant-gain synthesis signal, which activity quantity still needs gain calibration, and how does the matched pseudo-first-order buffered limit erase the nonlinear coefficient information?

Methods: Current primary-literature verification and archived primary texts were used only to bound claims. A deterministic synthetic ODE analysis replayed the declared finite-POLGB model with seven capacity values, two constant synthesis gains, three matched-family settings, two derivative-perturbation magnitudes and 99 automated checks. No patient data, fitted biological rates, pooled effect size, treatment ranking, rescue efficacy claim or clinical inference was generated.

Results: The replayed analysis passed 99 of 99 deterministic checks. In full-rank finite-capacity trajectories, the coefficient inverse recovered assembly, dissociation, free loss, associated loss, capacity and the effective synthesis coefficient κ with maximum reported exact-recovery error below $1e-5$. Constant detector-gain changes left recovered assembly, loss and capacity parameters unchanged while scaling recovered κ . Equal gain-activity products produced identical total and synthesis outputs, so effective per-associated-pool activity k was not separated from detector gain g . As capacity increased from 4 to 1,000,000 at fixed pseudo-first-order assembly a , nonlinear coefficients r and s decreased from 0.2 and 0.1667 to $8e-7$ and $6.667e-7$, and the relative coefficient condition increased from 218.9 to 31,014,096.9.

Conclusions: The result is a conditional mathematical discriminator, not biological validation. Under the declared POLG model, absolute total POLGA, known imported input and a constant-gain synthesis signal can be sufficient for assembly, loss and capacity recovery, while effective per-associated-pool activity remains gain-confounded unless detector gain is known. Knowing detector gain would separate g from the effective coefficient k in this model, but would not by itself establish intrinsic k_{cat} or the active enzyme fraction. Application to purified assays, cells, organoids, animals or patients requires separate proof of input calibration, total-protein calibration, active-pool meaning, POLGB capacity, free-enzyme activity and gain stability.

Keywords: POLG; POLGA; POLGB; mitochondrial DNA polymerase gamma; structural identifiability; finite capacity; assay calibration

1 Introduction

POLG encodes the catalytic subunit of mitochondrial DNA polymerase gamma. POLG is a heterotrimeric mitochondrial DNA polymerase with one POLGA catalytic subunit and two POLGB accessory subunits [2]. POLG/POLG2 studies show several relevant but nonidentical measurements: aggregation and import-associated endpoints, purified primer extension, active enzyme-DNA normalization, LonP1 proteolysis, holoenzyme stability, POLG2-DNA binding and activity isotherms [1-4,6]. These endpoints do not automatically identify the same hidden pool or rate constant.

Riccio et al. captured apo, DNA-bound, engaged and active polymerization PolG states and connected PolG2 destabilization with enhanced LonP1-mediated PolG degradation [3]. Silva-Pinheiro et al. linked impaired POLGB interaction to catalytic-subunit depletion and LONP1 susceptibility in the A449T mouse context corresponding to human A467T biology [4]. Wojtaszek et al. reported that homodimeric PolG2 enhances DNA binding and processive synthesis and also has direct DNA-binding modes [6]. Mohammed et al. reported mixed small-molecule effects on aggregation, import-associated endpoints and in vitro polymerisation activity, but the current corpus did not supply a public abstract through the production source-retrieval path [1].

The competing interpretation being constrained is that any finite-capacity restoration of identifiability is already supplied by general depletion mathematics. White, Rottschaefer and Bridge already established finite-depletion identifiability and practical weak-depletion limitations in nonlinear ligand-binding models, and Meshkat, Kuo and DiStefano already developed parameter-combination methods through COMBOS [5,7]. The present increment is therefore only the imported-input, protected-loss and gain-specific inverse for the declared POLG observation map.

The central relationship is deliberately conditional. For the stated POLG catalytic-subunit model with finite usable POLGB homodimer site equivalents, full-rank exact total-POLGA and constant-gain synthesis trajectories with known imported input determine assembly, dissociation, free loss, associated loss and finite capacity through an explicit coefficient inverse. The same data determine $\kappa = g/k$. Detector gain g and effective per-associated-pool coefficient k remain inseparable unless g is independently known; even then, k is an effective coefficient per associated POLGA pool, not intrinsic k_{cat} , because H is not necessarily active DNA-engaged enzyme.

2 Methods

Current source verification on 2026-10-11 used public scientific literature only. The source set covered POLGA aggregation/import and primer extension, mutant polymerase restoration, LonP1-regulated proteolysis, holoenzyme instability, parameter-combination methods, PolG2-DNA/holoenzyme activity assays and finite-depletion identifiability. The source set was used to bound assumptions, not to infer fitted parameters.

Eligibility required primary scientific literature or primary mathematical method work. Records were excluded from central evidence when only review-level material was available or when the source could not constrain the declared model. Contradictory and limiting evidence was retained: mixed C1/C4 mechanisms, active enzyme-DNA normalization, possible free POLGA activity, purified-protein context, COMBOS prior art and White 2026 prior art all narrow the claim [1-3,5,7].

A machine-readable evidence and assumption ledger is provided in Supplementary File S1, with a readable companion in Supplementary File S2. The ledger records quotation checks, source-version hashes, exact manuscript anchors and unresolved premises; it does not establish biological truth or clinical efficacy.

Public audit supplements contain curated public evidence only. Private requirement markers, raw source snapshots, audit inputs, selection traces and review archives are excluded from the public manifest.

The public analysis used the declared ODE system:

$$F' = u - dF \cdot F - a \cdot F \cdot (1 - H/B) + b \cdot H$$

$$H' = a \cdot F \cdot (1 - H/B) - (b + dH) \cdot H$$

$$T = F + H; Y = \kappa \cdot H$$

The transformed output equations were:

$$T' = u - dF \cdot T + \gamma \cdot Y$$

$$Y' = \eta \cdot T - \lambda \cdot Y - r \cdot T \cdot Y + s \cdot Y^2$$

Coefficient definitions were:

$$\gamma = (dF - dH)/\kappa, \eta = \kappa \cdot a, \lambda = a + b + dH, r = a/B, s = a/(\kappa \cdot B).$$

When the loss and synthesis regressors are full rank, T is calibrated as absolute total protein, imported flux u is known in the same units, r and s are nonzero, and recovered rates, capacity and states are positive/admissible, the conditional inverse is:

$$\kappa = r/s, a = \eta \cdot s/r, B = \eta \cdot s/r^2, dH = dF - \gamma \cdot \kappa, b = \lambda - a - dH, \alpha = r.$$

Exact derivatives were taken from the generating equations, not from noisy real observations. B denotes accessible preformed POLGB homodimer site equivalents with accessory recycling, not POLGB monomers and not a proven active pool. The fixed- a capacity scan changes $\alpha = a/B$; it is a matched pseudo-first-order limit, not a B-only experimental titration at fixed microscopic association.

Loss recovery requires rank 2 for columns $[-T, Y]$; synthesis recovery requires rank 4 for $[T, -Y, -T \cdot Y, Y^2]$. Synthetic dimensionless values were $a=0.8$, $b=0.3$, $dF=0.7$, $dH=0.1$ and $k=1.2$, with $T(0)=1.2$ and $J(0)=0.48$. Imported input u was 1 before $t=3$, 2 from $t=3$ to 6, 0 from $t=6$ to 10, and 1 thereafter to $t=14$. Integration used $dt=0.0025$ and sampling every 0.25 time units. Log-sensitivity differences used step $1e-5$. Derivative perturbations used relative RMS magnitudes $1e-8$ and $1e-6$; they altered the synthesis-derivative right-hand side along a deterministic left-singular-vector direction while retaining exact regressors. All values are arbitrary synthetic settings, not fitted biological rates.

Each source was coded for model system, perturbation, observed evidence and interpretive limitation. The evidence table is a native manuscript table and is also supplied as CSV. No cross-source pooled estimate was attempted because purified proteins, mouse tissue, cell import assays, identifiability theory and synthetic ODE outputs are not commensurable biological replicates.

3 Results

3.1 Evidence synthesis

Source-coded endpoints separate model motivation from measurement authority. POLG/POLG2 sources motivate the model qualitatively but do not supply a jointly calibrated time series for imported POLGA input, absolute total POLGA and synthesis output [1-4,6]. Mathematical sources supply prior art for identifiable combinations and finite-depletion identifiability, so this result cannot claim a new general principle [5,7].

3.2 Evidence-coded sensitivity analysis

The finite-capacity replay recovered observable coefficients and inverted physical parameters for the tested exact trajectories. The seven-B capacity scan retained exact-recovery errors below the declared threshold, but the conditioning measures changed substantially as B increased. The scaled synthesis-design condition stayed near 92-104 after column scaling, while the relative

Table 1. Source-coded evidence and interpretive limits for the POLG finite-capacity inverse.

ID	Model and perturbation	Key finding	Interpretive limitation
[1]	PEO POLGA variants, HEK293T import assay, mouse model, purified primer-extension assay; C1 and C4 small molecules; 2 micromolar in the purified primer-extension assay	The source reports mixed rescue involving aggregation, MITOL/import-associated endpoints and in vitro polymerisation activity.	Separate import and activity assays do not provide matched dynamic imported input, total POLGA and synthesis trajectories for the inverse.
[2]	Purified POLG variant enzyme assays and DNA-binding measurements; PZL-A and POLGB titration across specified POLG variants	Initial rates were normalized by active enzyme-DNA complex concentration measured under kinetic-assay conditions.	Associated POLGA/POLGB cannot be equated with active DNA-engaged enzyme, and detector gain remains separate from intrinsic activity.
[3]	Purified PolG and PolG2 proteins with LonP1 proteolysis; PolG2 variants and LonP1 concentrations with timed proteolysis assays	Association-dependent protection from LonP1 motivates different free and associated POLGA loss terms.	Purified proteolysis does not calibrate cellular first-order turnover constants or validate zero free-POLGA output.
[4]	Mouse PolgA449T/A449T tissues and related human A467T biochemical context; Holoenzyme-instability disease model	The source links impaired POLGB interaction to catalytic-subunit depletion and LONP1 susceptibility.	The genotype context motivates protection qualitatively; no synthetic parameter is assigned to A449T, A467T or any patient.
[5]	Nonlinear dynamic systems-biology identifiability methodology; COMBOS parameter-combination analysis and experiment-design examples	The paper establishes identifiable parameter combinations and independent measurements as experiment-design tools.	Coefficient inversion is established mathematics; the contribution here is only the bounded POLG observation-map application.
[6]	Purified PolG2-DNA and PolG holoenzyme biochemical assays; PolG2/POLGA titration and structure-specific DNA substrates	Activity isotherms and binding measurements already interrogate accessory-subunit dependence.	A fixed usable-dimer capacity is a simplifying model assumption, not a direct measurement of all POLGB biology.
[7]	Finite ligand-depletion structural-identifiability models; Nonlinear depletion versus ligand-excess binding comparisons	White, Rottschaefer and Bridge already show that nonlinear finite-depletion counterparts can restore identifiability.	The present model is not a first general finite-depletion result; it adds only a POLG-specific imported-input and gain-boundary calculation.

Executed POLG finite-capacity analysis workflow

Source checks bound the claim; exact synthetic replay tests the declared inverse.

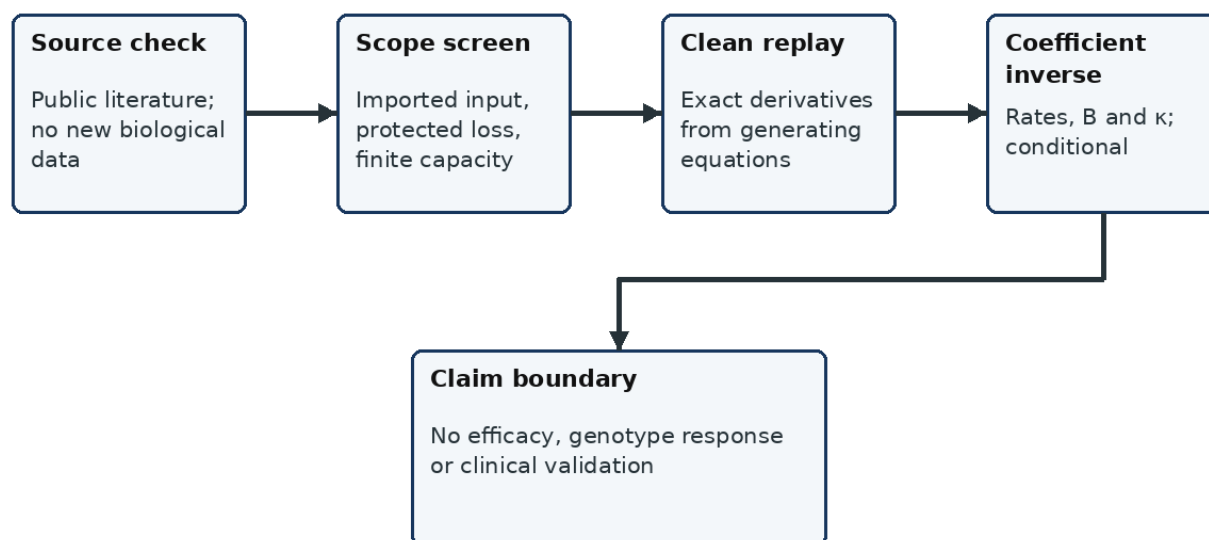


Figure 1. Executed workflow for the POLG finite-capacity analysis.

coefficient condition increased by about five orders of magnitude. The log-parameter sensitivity matrix retained numerical rank 6 at relative tolerance $1e-8$ through $B = 100,000$ and dropped to 5 at $B = 1,000,000$. These scaled numerical matrices and tolerance-dependent ranks are conditioning diagnostics, not structural identifiability proofs.

The derivative-perturbation inverse is intentionally retained because it is informative as a failure mode. Perturbing the derivative right-hand side while leaving exact regressors unchanged is an arithmetic inverse-sensitivity diagnostic. It need not correspond to a realizable noisy trajectory, power analysis, biological uncertainty model or clinical validation.

The gain replay changed measured synthesis output by constant factors of 0.25 and 4. In those runs, recovered assembly, loss and capacity terms remained at the synthetic physical values while recovered κ scaled with the gain. Equal products of detector gain and effective k generated identical T and Y outputs in the paired tests. Thus κ is identified in the declared model, while g and effective k remain inseparable without independent detector-gain calibration.

At fixed pseudo-first-order assembly a , increasing B reduced $r = a/B$ and $s = a/(\kappa B)$. Across the tested capacity series, the relative coefficient minimum singular value decreased from 0.3999 at $B = 4$ to 0.0000022569 at $B = 1,000,000$. The matched buffered family

produced effectively identical outputs, with maximum absolute output differences near machine precision. This supports only the declared boundary: the finite inverse depends on nonlinear coefficient information that is lost in the matched buffered limit.

4 Discussion

The analysis makes a narrow contribution. It does not rediscover finite-depletion identifiability, and it does not claim that POLGB capacity alone explains POLG disease biology. Instead, it states which parameter groups are recoverable in one explicit POLG catalytic-subunit observation model and which calibration remains unavailable. This distinction matters because POLG rescue language can move too quickly from import, abundance or primer-extension endpoints to a mechanistic statement about intrinsic activity.

The strongest alternative remains viable. If this calculation is merely an incremental adaptation of White 2026 and COMBOS to a POLG-themed diagram, it should be held rather than treated as a substantive new paper [5,7]. The practical use is limited: under ideal conditions, an unknown constant synthesis gain need not block assembly, loss and capacity inference, but it does block effective activity calibration. Unknown total-protein gain, free-enzyme activity and changing active fraction were not analyzed here and require separate models.

5 Limitations

This working paper is a deterministic synthetic analysis. It contains no fitted patient data, no donor inference, no cellular kinetic validation, no compound efficacy estimate and no clinical guidance.

The variables F and H denote free and associated POLGA in the model; H is not proven to be active DNA-engaged enzyme. B denotes constant accessible preformed POLGB homodimer binding-site equivalents, not all POLGB monomers or a measured cellular pool.

The model assumes known imported input in the same units as total POLGA, calibrated absolute total POLGA, zero free-POLGA synthesis output, constant effective k, constant detector gain, fixed substrate conditions, accessory recycling and exact derivatives. Published assays do not establish this joint condition. Free activity, time-varying gain, unknown total-protein gain, changing active fraction, accessory turnover and DNA sequestration require separate analysis. Numerical conditioning here is not practical-identifiability validation, a Fisher-information analysis, a power analysis or an experimental recommendation.

6 Conclusions

The finite-capacity POLG inverse is conditionally informative and biologically bounded. Under the declared model and exact full-rank outputs, assembly, dissociation, free loss, associated loss and accessible capacity are recoverable even when synthesis detector gain is unknown. Effective per-associated-pool synthesis activity is not recovered without gain calibration, and calibrated effective k would still not prove intrinsic kcat or an active DNA-engaged fraction. As capacity approaches a matched buffered limit, the nonlinear coefficient information that supports this recovery disappears. The result should be used as an assay-calibration boundary, not as evidence of POLG rescue, genotype response or clinical efficacy.

Table 2. Seven-B numerical recovery and conditioning summary from the included analysis CSVs.

B	r	s	Design cond.	Coeff. cond.	Log-param. cond.	Rank	1e-6 derivative inverse
4	0.2	0.1667	103.8	218.9	817.7	6	k err 7.847e-06; B err 2.570e-06
10	0.08	0.06667	96.22	380.5	1475	6	k err 1.393e-05; B err 1.055e-06
100	0.008	0.006667	92.55	3157	1.240e+04	6	k err 1.126e-04; B err 5.231e-05
1000	8.000e-04	6.667e-04	92.24	3.107e+04	1.221e+05	6	k err 0.001105; B err 5.631e-04
10000	8.000e-05	6.667e-05	92.21	3.102e+05	1.219e+06	6	k err 0.01131; B err 0.005588
100000	8.000e-06	6.667e-06	92.21	3.101e+06	1.218e+07	6	k err 0.152; B err 0.04182
1000000	8.000e-07	6.667e-07	92.21	3.101e+07	1.219e+08	5	invalid

Finite-capacity POLG observation model

Arrows show model and observation dependencies; dashed return is dissociation.

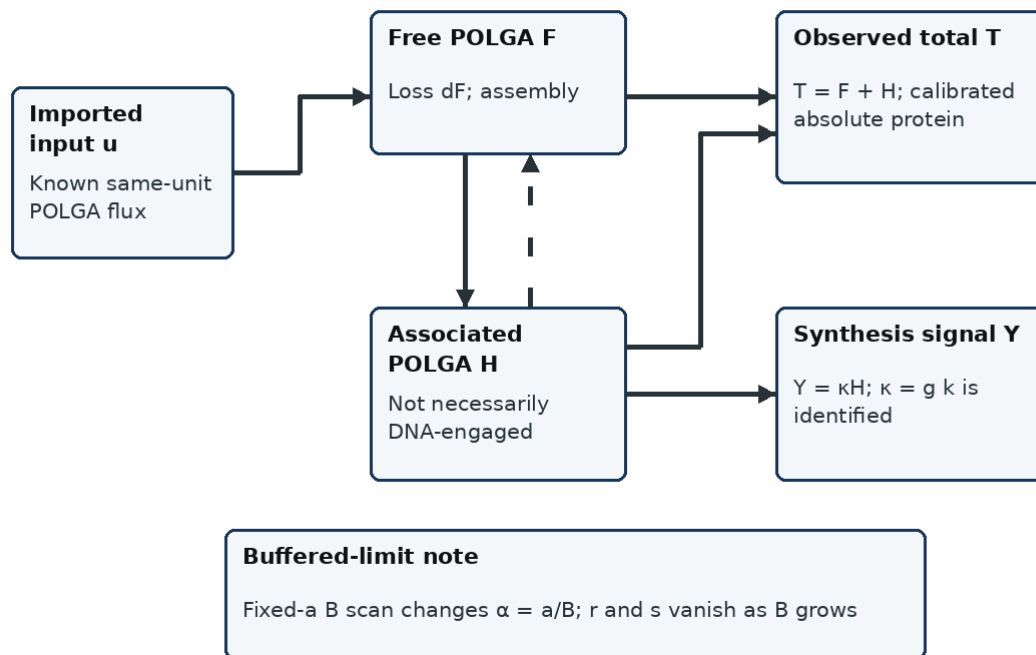


Figure 2. Conceptual POLG finite-capacity observation model.

Declarations

Data and code availability. The repository bundle accompanying this manuscript includes the editable manuscript, PDF, DOCX, public analysis code, synthetic parameters, machine-readable CSV and JSON outputs, source-coded evidence table, numerical summary table, figure source images, measured figure geometry, QA record and file checksums. Supplementary File S1 ('biological-audit.json') and Supplementary File S2 ('biological-audit.md') provide the curated evidence and assumption ledger used for replay; the ledger checks quotation integrity and manuscript anchors, not biological truth or clinical efficacy. Raw source snapshots, audit input, selection traces, private review history, packaging scripts and outreach materials are retained privately and excluded from public manifests and checksums.

Ethics statement. This literature and synthetic-model analysis used public scientific sources and arbitrary synthetic data only. No identifiable patient data, private health information, new human-participant research or new animal research was used.

Funding. No external funding was reported for this working paper.

Competing interests. The author reports no competing interests for this working paper.

Author contributions. Andrew Friedl set the project direction and is the sole author responsible for the manuscript.

AI assistance. AI tools assisted literature-search triage, source coding, manuscript drafting, figure generation, formatting and QA preparation. The analysis is synthetic; biological interpretation and applicability require independent scientific assessment.

References

[1] Mohammed A, Kaur T, Panwar P, Ghosh S, Ahmad H, Kumbhakar S, Muftuoglu M, Viscomi C, Sengupta S. Small molecules rescue pathogenic POLGA aggregation and restore its functions in a mouse model of PEO. EMBO Molecular Medicine. 2026. doi:10.1038/s44321-026-00532-3. PMID:42839124.

[2] Valenzuela S, Zhu X, Macao B, Stamgren M, Geukens C, Charifson PS, Kern G, Hoberg E, Jennering L, Gruszczak AV, Lee S, Johansson KAS, Miralles Fuste J, Shi Y, Kerns SJ, Arabanian L, Martinez Botella G, Ekstrom S, Green J, Griffin AM, Pardo-Hernandez C, Keating TA, Kuppers-Munther B, Larsson NG, Phan C, Posse V, Jones JE, Xie X, Giroux S, Gustafsson CM, Falkenberg M. Small molecules restore mutant mitochondrial DNA polymerase activity. Nature. 2025;642:501. doi:10.1038/s41586-025-08856-9. PMID:40205042.

[3] Riccio AA, Brannon AJ, Krahn JM, Bouvette J, Williams JG, Borgnia MJ, Copeland WC. Coordinated DNA polymerization by Polγ and the region of LonP1 regulated proteolysis. Nucleic Acids Research. 2024;52:7863-7881. doi:10.1093/nar/gkac539. PMID:38932681.

[4] Silva-Pinheiro P, Pardo-Hernandez C, Reyes A, Tilokani L, Mishra A, Cerutti R, Li S, Rozsivalova DH, Valenzuela S, Dogan SA, Peter B, Fernandez-Silva P, Trifunovic A, Prudent J, Minczuk M, Bindoff L, Macao B, Zeviani M, Falkenberg M, Viscomi C. DNA polymerase gamma mutations that impair holoenzyme stability cause catalytic subunit depletion. Nucleic Acids Research. 2021;49:5230-5246. doi:10.1093/nar/gkab282. PMID:33956154.

[5] Meshkat N, Kuo CE, DiStefano J III. On Finding and Using Identifiable Parameter Combinations in Nonlinear Dynamic Systems Biology Models and COMBOS: A Novel Web Implementation. PLOS ONE. 2014;9:e110261. doi:10.1371/journal.pone.0110261. PMID:25350289.

[6] Wojtaszek JL, Hoff KE, Longley MJ, Kaur P, Andres SN, Wang H, Williams RS, Copeland WC. Structure-specific roles for PolG2-DNA complexes in maintenance and replication of mitochondrial DNA. Nucleic Acids Research. 2023;51:9716-9732. doi:10.1093/nar/gkad679. PMID:37592734.

[7] White C, Rottschaefer V, Bridge L. On the structural identifiability of nonlinear models of ligand binding dynamics. Journal of Pharmacokinetics and Pharmacodynamics. 2026;53. doi:10.1007/s10928-026-10040-z. PMID:42471536.