

TWNK Pause Identifiability in Public Optical-Tweezers Source Data

Andrew Friedl

Precision Mito, Florida, USA

Correspondence: afriedl@precisionmito.com

Abstract

Background: TWNK encodes the human mitochondrial replicative helicase Twinkle. Plaza 2025 reported real-time Twinkle loading, unwinding, pause occupancy, rewinding and domain autoregulation, and deposited a public source-data workbook. A downstream question is whether that public workbook can support a stronger trace-level claim: persistent pause propensity per molecule or tether versus repeated stochastic switching during unwinding and rewinding.

Objective: This working paper retains the selected question: Can the public Plaza 2025 TWNK/Twinkle optical-tweezers workbook distinguish persistent per-molecule pause propensity from stochastic switching during unwinding and rewinding, or does missing trace/tether/run identity limit inference to condition-level pause distributions?

Methods: Current primary-literature verification and archived source-hashed texts were used to bound claims. The public Plaza workbook was parsed directly as an XLSX archive. The replay inventoried workbook sheets, detected figure sections, extracted pause-metric condition rows, and scanned sheet text for stable trace, tether, run, replicate, preparation, molecule or activity identifier labels. Five source records were coded across endpoint classes: optical-tweezers kinetics, deposited workbook structure, smFRET trace categories, biochemical strand exchange, and substrate-specific helicase activity. The analysis was a decision-changing identifiability audit, not a pooled effect estimate, raw-trace reanalysis, patient study, therapeutic ranking, platform simulation or Energy Score calculation.

Results: The replayed workbook analysis found 12 sheets, 32 detected sections, six pause-metric sections, and 74 pause-condition rows. No stable trace, tether, run, replicate, preparation, molecule or activity identifier labels were detected in the public workbook text. Plaza 2025 supports condition-level Twinkle loading, unwinding, pause occupancy and rewinding comparisons, and Wang/Lee 2025 shows that trace-resolved smFRET categories can be biologically informative when trace structure is available. Under the same public Plaza files, however, aggregate pause vectors do not discriminate a persistent per-molecule pause state from repeated stochastic switching events.

Conclusions: The immediate public contribution is an identifiability boundary. The Plaza workbook can support source-data inventory, condition-level pause occupancy comparison, and reproducibility checks, but the inspected public files do not support trace-persistence or same-molecule switching models without raw traces or stable activity identifiers. This conclusion does not challenge the published Twinkle autoregulation result; it prevents overinterpreting processed condition-level vectors as evidence for persistent molecular states, tissue vulnerability, patient benefit or treatment response.

Keywords: TWNK; Twinkle helicase; optical tweezers; single-molecule kinetics; pause occupancy; data identifiability; mitochondrial DNA replication

1 Introduction

TWINK, also known historically as C10orf2 or PEO1, encodes Twinkle, the human mitochondrial DNA helicase. Twinkle is not a DNA polymerase and is not responsible for POLG proofreading. The present question is therefore restricted to helicase-associated loading, unwinding, pause occupancy, rewinding, annealing and strand-exchange interpretation in public single-molecule source data.

Plaza et al. reported that Twinkle's real-time kinetics include DNA loading, unwinding and rewinding, and that its N-terminal zinc-binding domain, C-terminal tail and mitochondrial single-stranded DNA-binding protein regulate those kinetic states [1]. The same article reported ATP-, force-, mtSSB- and construct-dependent differences in pause occupancy, and the dataset record states that deposited values were obtained after processing individual optical-tweezers activities at 1 kHz and 0.1 pN resolution [1,2]. These facts make the source-data workbook unusually valuable, but they do not automatically establish that each public value retains the identity metadata needed to link the same activity across processivity, rate, pause occupancy and rewinding.

The selected question is an identifiability problem rather than a new biochemical mechanism. The fixed question is: Can the public Plaza 2025 TWNK/Twinkle optical-tweezers workbook distinguish persistent per-molecule pause propensity from stochastic switching during unwinding and rewinding, or does missing trace/tether/run identity limit inference to condition-level pause distributions?

The strongest alternative is credible. Because the Plaza source values derive from individual optical-tweezers activities, and because other single-molecule Twinkle work classifies continuous, stepwise, stopped and reinitiated traces, the public materials might have been sufficiently resolved for same-activity persistence analysis [2,3]. Under that alternative, the workbook or deposited code would expose trace, tether, run, preparation or raw-trace identifiers that permit transition analysis. Under the selected concept, the public workbook yields condition-level vectors but not stable activity linkage.

This working paper differs from the prior Mito Map TWNK renewal-capacity paper, which evaluated how replication reserve should gate downstream build-and-clear mitochondrial quality-control claims [6]. The present analysis does not repackage that contribution. It asks whether one public Twinkle optical-tweezers workbook preserves the metadata needed for a particular same-molecule pause-persistence inference.

2 Methods

The literature search was refreshed on 2026-10-13 using PubMed, PubMed Central, publisher pages, the IMDEA repository record and current web search for TWNK, Twinkle, C10orf2, PEO1, optical tweezers, smFRET, pause occupancy, unwinding, rewinding, annealing and strand exchange. Primary records were eligible when they directly concerned human Twinkle/TWINK single-molecule kinetics, deposited Plaza source data, biochemical Twinkle strand exchange or substrate-specific helicase behavior. Review-only sources, generic mitochondrial concepts, POLG-only questions, patient inputs, private source archives and therapeutic claims were excluded from central evidence.

Source prompts were treated only as retrieval leads. The public paper does not treat prompts as evidence, historical theory, novelty proof or discovery. Complete archived public texts in the private concept-evidence folder were used for source verification, while only curated public data, summaries, figures, audit supplements and manuscript artifacts are listed in the public manifest.

A machine-readable evidence and assumption ledger is provided in Supplementary File S1, with a readable companion in Supplementary File S2. The ledger records exact manuscript

anchors, source identifiers, evidence excerpts, source-version hashes, actual quotation-check outcomes and unresolved assumptions. It is not a proof of biological truth, clinical validity or therapeutic efficacy.

The public analysis script parsed plaza-source-data.xlsx directly as an OOXML archive. The parser read workbook relationships, shared strings, sheet dimensions and cell values without relying on screenshot figures. It then identified figure-section rows, summarized numeric rows in pause-associated sections 2G, 2K, 3F, 3J, S7D and S8D, and scanned all sheet text for identifier labels matching trace, tether, run, replicate, preparation, prep, molecule, activity or protein plus id, identifier, number or index.

The primary endpoint was data-structure identifiability: whether public files expose stable identifiers that link the same individual Twinkle activity across pause occupancy, processivity, average rate, pause-free velocity and rewinding metrics. The secondary endpoint was the condition-level inventory of pause-metric rows available for aggregate comparison. No raw proprietary C-trap traces, author-held notebooks, video files, kymograph binaries, laboratory run sheets, individual protein-preparation records, patient data or private platform outputs were inspected.

Each source was coded for biological system, perturbation, endpoint class, observed evidence and interpretive limit. Distinct activities were not collapsed: helicase unwinding, ATP hydrolysis, loading, oligomerization, annealing, strand exchange, replisome coupling, nascent DNA synthesis, steady-state copy number and deletions were kept separate. Purified proteins and single-molecule setups were treated as systems, not tissues.

The decision-changing sensitivity check asked whether stable identifier labels were present in the public workbook. A positive result would have made trace-persistence modeling actionable. The negative result retains condition-level analysis but blocks same-activity persistence and switching inference. A second exploratory sensitivity check inventoried pause-section coverage to verify that the workbook still contains useful aggregate pause vectors.

No pooled effect size, confidence interval or significance test was calculated because the analysis tests file structure, not a biological effect. Counts are denominators from the parsed workbook and source-coding matrix. The resulting inference is conditional on the public files inspected here and does not exclude the possibility that original laboratory raw files contain the needed identifiers.

3 Results

The evidence step first separates source-data structure from biological interpretation, then asks what inference would change under the same public files.

3.1 Evidence synthesis

The replay found 12 workbook sheets and 32 detected figure sections. Six sections were pause-associated by the declared section list: Figure 2 sections 2G and 2K, Figure 3 sections 3F and 3J, Supplementary Figure S7 section S7D and Supplementary Figure S8 section S8D. Across those pause sections, 74 condition rows with numeric vectors were summarized. These rows support condition-level comparisons by sheet, figure section, Twinkle construct, ATP, force and mtSSB context.

Table 1. Source-coded evidence and interpretive limits for the TWNK pause-identifiability audit.

Figure 1 summarizes the executed public-data workflow from source retrieval through workbook parsing, identifier scanning, sensitivity labeling and claim-boundary synthesis.

The identifier scan detected no stable trace, tether, run, replicate, preparation, molecule or activity identifier labels in workbook sheet

text. The result is decision-changing because the proposed persistence-versus-switching analysis requires repeated-measure linkage. Without a stable identifier, a row of pause values can be treated as a condition-level vector, but the same public workbook cannot show whether one molecule or tether repeatedly pauses, whether the population switches stochastically between active and paused states, or whether a subset of activities dominates the pause distribution.

This boundary is not a biological contradiction of Plaza 2025. Plaza remains the closest work for Twinkle loading, unwinding, rewinding and domain autoregulation [1]. The new result is narrower: source-data deposition and processed activity values do not automatically preserve public same-activity linkage. The null result teaches what a follow-on request must include: raw C-trap time-series traces or a stable trace/tether/run/protein-preparation key linking processivity, average velocity, pause-free velocity, pause occupancy and rewinding for the same activity.

Wang and Lee provide an important comparator because their smFRET study describes trace classes, including continuous, stepwise, stopped and reinitiated unwinding patterns [3]. That comparator supports the premise that trace-resolved categories can be biologically meaningful. It also strengthens the methodological boundary: the Plaza workbook must be evaluated for analogous trace identity before the same kind of state model is inferred from its processed source-data values.

Sen, Patel and Patel reported annealing, strand-exchange and branch-migration activities for TWINKLE [4]. Peter and colleagues

reported substrate-specific helicase behavior, including fork, D-loop, branch-migration and blockade contexts [5]. These records matter because Twinkle can show multiple DNA-modifying activities under purified conditions. They do not support treating pause occupancy, rewinding, annealing, strand exchange, loading or processivity as one interchangeable mechanism.

Figure 2 summarizes the discriminating contrast. Persistent molecule-state and stochastic-switching accounts can both produce condition-level pause distributions. The accounts diverge only when the same activity can be linked across time or across extracted metrics. The inspected public workbook did not expose that linkage.

3.2 Evidence-coded sensitivity analysis

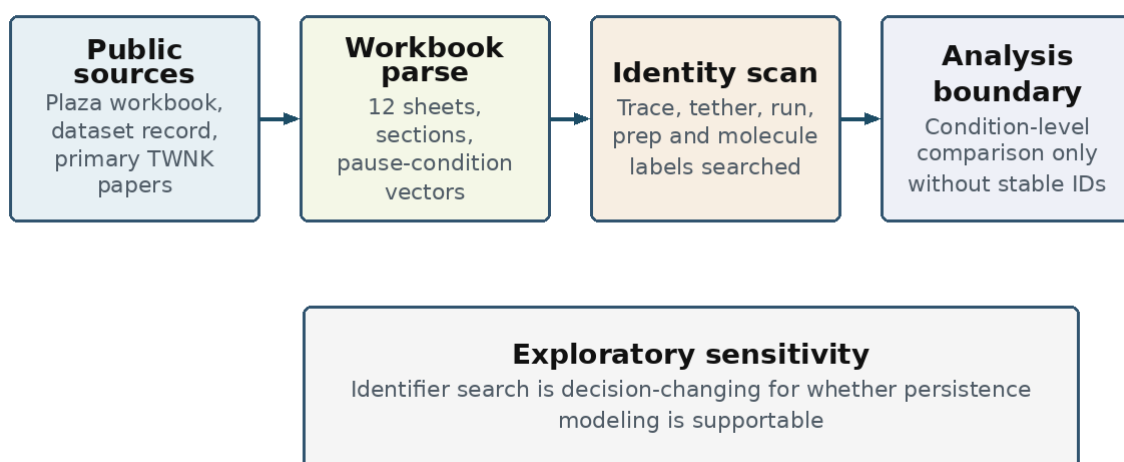
The decision-changing identifier-keyword sensitivity was negative. The role of this check is not statistical robustness; it determines whether trace-persistence modeling is supportable from public files. The interpretation is that the immediate public analysis should remain at condition-level pause occupancy and workbook-structure claims.

The exploratory pause-section inventory was positive. The workbook includes six pause-associated sections and 74 pause-condition rows. This confirms that the source-data workbook is not unusable; it is usable for aggregate pause-vector inventory, reproducibility checks and source-data transparency, while remaining insufficient for the stronger same-activity persistence question.

Table 1. Primary-study evidence matrix

ID	Model and perturbation	Key finding	Interpretive limitation
[1]	Purified Twinkle optical-tweezers / biochemical assays; ATP, force, mtSSB, ZBD/C-tail	Real-time loading, unwinding, pause occupancy and rewinding are reported, and source data were deposited.	The article supports Twinkle kinetic scope but does not by itself prove public trace identifiers for same-activity persistence modeling.
[2]	IMDEA source-data record and workbook; Processed optical-tweezers activity values	The bundled workbook replay detected 12 sheets, 32 sections and 74 pause-condition rows.	The public workbook supports condition-level vectors; this replay did not detect stable trace, tether, run, preparation, molecule or activity identifiers.
[3]	Single-molecule FRET Twinkle unwinding; ATP, DNA mismatches, mtSSB	Trace categories include continuous, stepwise, stopped and reinitiated unwinding patterns.	smFRET trace categories show what trace-resolved data can support, but they do not resolve the Plaza workbook identity structure.
[4]	Purified TWINKLE strand-exchange assays; Annealing, unwinding, ATP hydrolysis, homology, four-way junctions	TWINKLE couples unwinding and annealing reactions in strand exchange and branch migration assays.	Annealing and strand exchange are distinct mechanisms from optical-tweezers pause persistence and cannot be collapsed into one pause-state claim.
[5]	Purified recombinant Twinkle helicase assays; Forks, D-loops, three-stranded DNA, oxidative lesions, TFAM blocks	Twinkle helicase activity differs by substrate and blockade context.	Substrate specificity supports caution about mechanism transfer; it does not provide trace-linked pause persistence data.

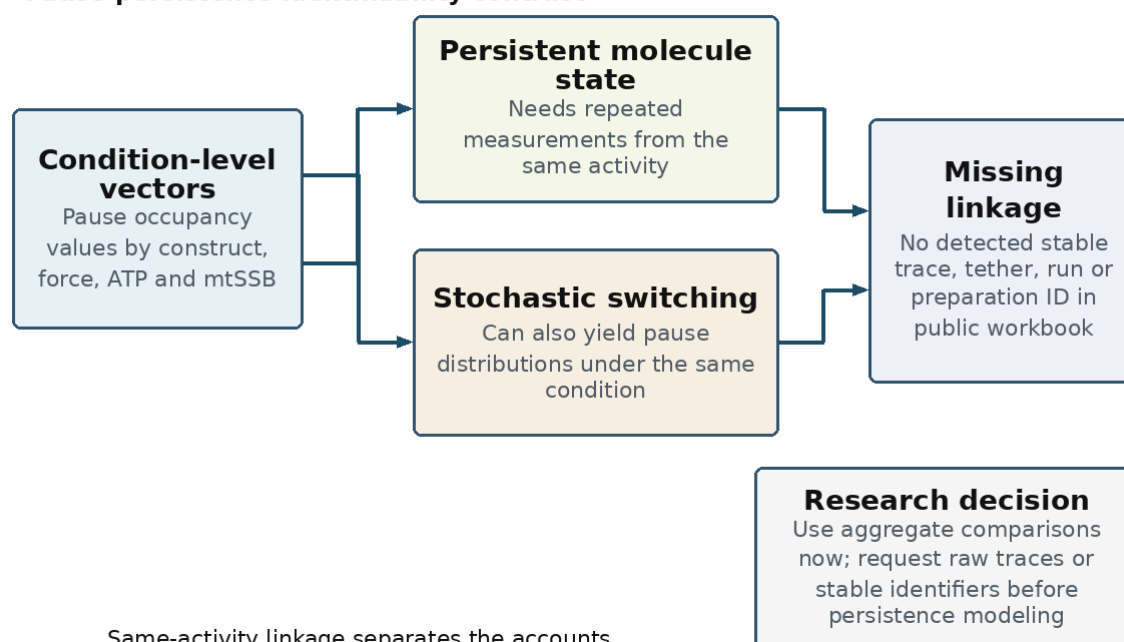
Executed public-data workflow



Solid arrows show file-processing steps.

Figure 1. Executed public-data workflow for the TWNK pause-identifiability audit.

Pause-persistence identifiability contrast



Same-activity linkage separates the accounts.

Figure 2. Pause-persistence identifiability contrast for processed Twinkle pause vectors.

4 Discussion

The main result is an analysis boundary. Public Plaza source data can support condition-level comparisons of Twinkle pause occupancy and related kinetics, but the inspected workbook does not expose the metadata needed to separate persistent per-molecule pause propensity from repeated stochastic switching. This distinction matters because the two accounts can imply different follow-on experiments. Persistent propensity would motivate same-molecule state models, hidden-state analysis and protein-preparation stratification. Stochastic switching would motivate dwell-time and ATP-dependent transition analysis. Aggregate vectors alone cannot choose between them.

The boundary changes a concrete research decision. The public workbook should be used for transparent source-data inventory and aggregate pause-condition checks. It should not be used as the sole basis for a trace-persistence or per-molecule switching manuscript unless raw traces or stable activity identifiers become available. A collaborator request should therefore ask for raw C-trap time series, trace/tether/run/preparation identifiers, censoring rules, filtering settings, force traces, ATP and salt conditions, mtSSB and POLG

context where relevant, construct definitions and protein-preparation labels.

The analysis also protects TWNK specificity. Twinkle helicase kinetics are the subject; no POLG polymerase activity, POLG proofreading, mtDNA repair, cell survival, clinical rescue, treatment sequence or safe overexpression claim follows. The Plaza system uses purified human Twinkle constructs and single-molecule optical-tweezers conditions; Wang/Lee uses smFRET; Sen and Peter use purified biochemical DNA substrates [1-5]. These are informative systems, not four tissues, and not patient evidence.

The strongest alternative remains possible outside the inspected public workbook. Original laboratory raw files or deposited notebooks could contain stable trace names or run keys that were not represented in workbook sheet text. If such identifiers are found, the present hold should be replaced by a richer same-activity reanalysis. If they remain unavailable, the negative result is still useful because it prevents condition-level source data from being overread as persistent molecular-state evidence.

5 Limitations

This working paper is a public source-data audit, not a raw-trace reanalysis, new single-molecule experiment, registered systematic review, meta-analysis or clinical study. The analysis did not inspect proprietary C-trap raw files, original laboratory notebooks, author-held scripts beyond public record metadata, video or kymograph binaries, protein-preparation logs, patient material, cellular mtDNA copy-number assays or tissue specimens.

The identifier scan is limited by public workbook text and the declared label pattern. A stable identity key could exist as a filename, hidden workbook convention, deposited notebook variable or author-held metadata not captured by this scan. Absence of a detected public identifier is therefore an identifiability boundary for the inspected public files, not proof that no trace identity exists in the original study environment.

The source systems are heterogeneous. Optical tweezers, smFRET and biochemical strand-exchange assays differ by DNA substrate, force, ATP, salt, mtSSB, protein construct, acquisition window, filtering, censoring and endpoint definitions. Their results should not be pooled as biological replicates. The analysis does not infer tissue vulnerability, developmental state, inheritance, allele dominance, mutant-to-wild-type ratio or patient benefit.

6 Conclusions

The public Plaza 2025 TWNK/Twinkle source-data workbook supports condition-level pause-metric inventory but, as inspected here, does not support same-activity pause-persistence or stochastic-switching inference. The most useful next step is not another aggregate synthesis; it is access to raw traces or stable trace/tether/run/protein-preparation identifiers that link loading, processivity, rate, pause occupancy and rewinding metrics for the same Twinkle activity. Until then, public claims should remain bounded to workbook structure, condition-level vectors and reproducibility rather than persistent molecular states or translational benefit.

Declarations

Data and code availability. The repository deposit includes the manuscript source, evidence table, source data, analysis code, figures, measured figure geometry, biological-audit supplements, layout reports, QA record, Gemini review and checksums needed to replay the table, counts, figures and artifacts. Private replay inputs are retained locally and excluded from public manifests.

Source verification. The biological audit is a manuscript-integrity ledger, not proof of biological truth or clinical efficacy. Corpus anchors and archived normalized source files were replayed against recorded hashes; UNKNOWN or NOT_RUN biological interpretation statuses remain visible.

Ethics statement. This literature synthesis used no identifiable patient data and required no new human-participant or animal research.

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 conceived the project, directed the analysis, reviewed the evidence package, and approved the manuscript for repository review.

AI assistance. AI tools assisted literature synthesis, drafting, data visualization, formatting and QA preparation. Gemini independently reviewed the exact rendered PDF when artifact-bound review was available. Andrew Friedl remains solely responsible for the content and is the sole author.

References

- [1] Plaza-G A I, Ortiz-Rodriguez M, Buchanan SP, Miguez-Amil S, Lemishko KM, Moreno-Herrero F, Fernandez-Leiro R, Ciesielski GL, Ibarra B. Autoregulation of the real-time kinetics of the human mitochondrial replicative helicase. *Nature Communications*. 2025;16:5460. doi:10.1038/s41467-025-60289-0. PMID:40592829.
- [2] Plaza-G.A. I, Ortiz-Rodriguez M, Buchanan SP, Miguez S, Lemishko KM, Moreno-Herrero F, Fernandez-Leiro R, Ciesielski GL, Ibarra B. Data - Auto-regulation of the real-time kinetics of the human mitochondrial replicative helicase. IMDEA Nanociencia Research Data Repository. 2025. doi:10.1101/2024.11.14.623559. <https://hdl.handle.net/20.500.12614/3946>.
- [3] Wang HY, Lee YH, Wu WJ, Weng TH, Lee CY, Chen SH, Lee HM. Single-molecule fluorescence reveals the DNA unwinding mechanism of mitochondrial helicase TWINKLE and its interplay with single-stranded DNA-binding proteins. *Nucleic Acids Research*. 2025. doi:10.1093/nar/gkaf803. PMID:40867054.
- [4] Sen D, Patel G, Patel SS. Homologous DNA strand exchange activity of the human mitochondrial DNA helicase TWINKLE. *Nucleic Acids Research*. 2016;44:4200-4210. doi:10.1093/nar/gkw098. PMID:26887820.
- [5] Peter B, Falkenberg M, Eek M, Pestov NB, Aasumets K, Atanasova N, Gustafsson CM, Hiltunen JK, Pohjoismaki JL, Goffart S. Biochemical characterization of the human mitochondrial replicative Twinkle helicase: substrate specificity, DNA branch migration, and ability to overcome blockades to DNA unwinding. *Journal of Biological Chemistry*. 2016;291:14389-14402. doi:10.1074/jbc.M116.722348. PMID:27226550.
- [6] Friedl A. TWNK renewal capacity gates build-and-clear mitochondrial quality-control claims. *Zenodo*. 2026. doi:10.5281/zenodo.23240338.