

Prompt language as a diversity lever: foreign-language queries broaden source retrieval and AI-generated research proposals

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Abstract: Large language models (LLMs) coupled to retrieval-augmented generation (RAG) are increasingly used to generate scientific hypotheses, but they are notoriously prone to redundancy: repeated queries converge on the same handful of sources and near-identical ideas. Existing remedies are computationally heavy, relying on iterative planning, citation or entity graphs, or evolutionary search. Here I show that a far simpler lever, the language of the prompt, substantially diversifies both what a RAG system retrieves and what it proposes, even when the underlying corpus is entirely in English. Using a self-hosted mechanobiology RAG system (llama3.3:70b and nomic-embed-text served locally on an NVIDIA DGX Spark, indexing 523 peer-reviewed and preprint papers in AnythingLLM/LanceDB), I issued an identical Alzheimer's-disease hypothesis-generation prompt in English and in seven other languages that the model understands. In English, five independent queries, including chain-of-thought and arbitrary-prefix controls, retrieved the *same two* source papers in the *same* 1:9 ratio every time; because dense retrieval is deterministic, this collapse is exactly reproducible rather than stochastic. Translating the prompt broke the collapse completely: the seven foreign-language queries drew on 3–8 distinct papers each (mean 5.4), surfacing 25 distinct papers versus 2 for all English runs combined (permutation $p \approx 0.001$). Diversity propagated downstream to the generated proposals, which showed lower inter-proposal vocabulary overlap (Jaccard 0.25 vs 0.41), 16 domain concepts absent from every English output, divergent mechanisms and experimental designs, and a striking categorical shift in the proposed model organism (*C. elegans* in 5/5 English proposals versus *Drosophila* or other invertebrates in every foreign proposal). Because a within-language perturbation left retrieval unchanged while translation transformed it, prompt language, not surface variation or sampling temperature, is the operative variable. Foreign-language prompting is thus a nearly free, broadly applicable tool for injecting diversity into RAG-based scientific ideation and, potentially, retrieval-augmented systems more generally.

Keywords: retrieval-augmented generation; large language models; multilingual prompting; hypothesis generation; output diversity; dense retrieval; cross-lingual information retrieval; AI for science; scientific ideation; local LLM inference

1. Introduction:

Retrieval-augmented generation (RAG) has rapidly become the dominant paradigm for using large language models (LLMs) as engines of scientific ideation. By grounding a model’s output in passages retrieved from an external corpus, RAG systems can propose hypotheses and research directions that are anchored to the published literature rather than to parametric memory alone, improving factuality and traceability [1]. A growing body of work now treats literature-grounded idea generation as a task in its own right, pairing dense retrieval over academic corpora with structured or iterative generation to produce candidate hypotheses, and in some cases reporting ideas that human reviewers rate as competitive with, or more novel than, expert-generated ones [2].

Yet the same studies expose a persistent and awkward limitation: LLMs are strong at *novelty* but weak at *diversity*. When queried repeatedly, they tend to converge on a narrow slice of their available knowledge, producing ideas that are individually plausible but collectively redundant [2]. In a RAG setting this problem has two coupled layers. At the retrieval layer, a fixed query embeds to a fixed point in vector space and therefore returns the same nearest-neighbor documents on every call; at the generation layer, even stochastic decoding tends to re-express the same retrieved evidence in similar ways. The net effect is that a researcher who wants to explore the *breadth* of a hypothesis space: many mechanisms, many model systems, many experimental designs, is poorly served by simply re-running the same prompt.

The methods proposed to counter this are, almost without exception, architecturally or computationally heavy. Diversity-enhancing strategies include iterative planning that reformulates queries to expand topical coverage, retrieval structured over citation networks or entity co-occurrence graphs, GraphRAG-style hybrid contexts, and evolutionary or tree-search procedures that explicitly mutate and recombine candidate ideas across generations [1,3]. On the prompting side, high-temperature sampling, persona conditioning, and step-by-step recall have all been shown to raise output variety to varying degrees [4]. These approaches work, but each adds pipeline complexity, additional model calls, or bespoke infrastructure on top of an existing RAG deployment.

A separate line of research points toward a much simpler lever. LLMs encode language-specific knowledge acquired during pretraining, and *multilingual prompting*, issuing a base prompt in several languages and aggregating the results, has been shown to increase the diversity of model generations more effectively than temperature sampling, persona prompting, or step-by-step recall, while preserving accuracy [4]; multilingual prompting has more broadly been surveyed as an effective strategy across a wide range of NLP tasks and languages [5]. To date, however, this work has been evaluated almost entirely on cultural and demographic diversity (opinions on social norms, the nationalities of named individuals) and operates purely at the generation layer: the model is asked to write in another language, and diversity is credited to the different regions of its parametric knowledge that each language activates. Whether prompt language can diversify

the *retrieval* step of a RAG system, and whether any such effect carries through to substantive scientific content, has not been examined.

Cross-lingual RAG offers the missing half of the picture, but frames it as a problem rather than an opportunity. Because dense retrievers embed queries and documents into a shared multilingual space, the language in which a query is posed measurably changes what is retrieved [6,7]. The cross-lingual RAG literature documents this sensitivity, and generally treats the resulting variability as a source of inconsistency to be *suppressed*, for example by translating all retrieved passages into a common language prior to generation [6]. More broadly, even within a single language, semantically equivalent queries that differ only in surface form can shift retrieval results substantially, degrading performance by 15–20% and occasionally far more [8]. Crucially, these studies concern *multilingual corpora* evaluated on convergent question-answering, where a single correct answer exists and retrieval variation is noise. No prior work, to my knowledge, has asked the inverse question that is relevant for divergent tasks such as hypothesis generation: over a *monolingual* corpus, can deliberately translating the query be used as a controllable, near-zero-cost source of *beneficial* retrieval diversity?

Here I answer that question directly, using a self-hosted mechanobiology RAG system built on a locally served llama3.3:70b generator and a nomic-embed-text retriever indexing 523 English-language papers. I issued an identical Alzheimer’s-disease hypothesis-generation prompt in English and in seven other languages the model understands, and quantified diversity at both the retrieval and proposal layers. My central finding is stark. In English, five independent queries, including chain-of-thought and arbitrary-prefix variants intended to perturb the query, retrieved the *same two* source papers in the *same* 1:9 ratio on every run; because dense retrieval is deterministic, this collapse is exactly reproducible rather than a sampling artifact. Translating the prompt eliminated the collapse: the seven foreign-language queries each drew on 3–8 distinct papers (mean 5.4), collectively surfacing 25 distinct documents against just 2 for all English runs combined. The added diversity propagated downstream into the generated proposals, which exhibited markedly lower inter-proposal vocabulary overlap, introduced 16 domain concepts absent from every English output, and diverged in mechanism, experimental design, and, most strikingly, in the proposed model organism, with English proposing *Caenorhabditis elegans* in every run and foreign prompts never doing so. Because a within-language perturbation left retrieval unchanged while translation transformed it, the operative variable is prompt language itself, not surface variation or decoding temperature.

In summary, this study makes the following contributions: (i) I show that foreign-language prompting diversifies retrieval over a *monolingual* corpus: a mechanism distinct from, and complementary to, the generation-layer effects previously attributed to multilingual prompting. (ii) I demonstrate that this retrieval diversity propagates to the content of AI-generated hypotheses and research proposals, along measurable axes including source coverage, lexical overlap, mechanistic focus, experimental design, and choice of model organism. (iii) Using within-language controls under near-greedy decoding, I isolate prompt language, rather than

query surface form or sampling temperature, as the causal factor. (iv) I argue that, precisely because it requires no change to the corpus, retriever, or pipeline architecture, foreign-language prompting is a practical and broadly transferable lever for injecting diversity into RAG-based scientific ideation, and potentially into retrieval-augmented systems more generally.

2. Methods

2.1 System configuration

All computation was performed locally on a single NVIDIA DGX Spark workstation (unit spark-3b2c) built on the Grace Blackwell GB10 SoC with 128 GB of unified CPU+GPU memory and 3.7 TB of NVMe storage, running Ubuntu 22.04 with CUDA 13.0. The unified memory architecture allows the generative model, the embedding model, and the full vector index to reside in a single address space. No cloud APIs were used at inference time; the entire retrieval-augmented generation (RAG) stack was self-hosted. The software stack comprised a custom Python harvesting pipeline (described in §2.2), the nomic-embed-text embedding model (v0.23.1) and the llama3.3:70b generative model (Q4_K_M quantization), both served via Ollama, a LanceDB vector store embedded within AnythingLLM (v1.12.1, run in Docker), which also served as the RAG front end.

2.2 Literature corpus construction

The corpus-harvesting pipeline was developed in Python with the assistance of Claude Code (Anthropic).

Search-term design and tiered combinatorial retrieval. Eight domain terms spanning mechanobiology and neuroinflammation were defined: *microglia*, *mechanotransduction*, *phagocytosis*, *prion*, *Alzheimer disease*, *fluid shear stress*, *brain*, and *Piezol*. Multi-word terms were treated as exact phrases, and *Alzheimer disease* was expanded to a synonym set (“Alzheimer disease” OR “Alzheimer’s disease” OR alzheimers). Rather than issuing a single broad query, I constructed every $C(8, k)$ combination of the eight terms, AND-joined, stepping down from $k = 8$ (all eight terms must co-occur) to a floor of $k = 5$ (any five of eight). This yielded $1 + 8 + 28 + 56 = 93$ distinct queries across four tiers; the $k = 4$ and $k = 3$ tiers were excluded as too broad. The scheme imposes a natural relevance ordering: a paper first retrieved at $k = 8$ is unambiguously on-topic, whereas one first appearing at $k = 5$ represents broader context. Each paper was assigned a relevance score equal to the maximum k at which it was first retrieved, with global deduplication across tiers (once logged, a paper was skipped in all lower tiers).

Data sources. The primary source was PubMed Central Open Access, queried via NCBI ESearch (db=pmc) with the pmc open access[filter] filter and retrieved as JATS XML via EFetch; this path succeeded for all peer-reviewed papers at all tiers. Preprints were retrieved

from Europe PMC (SRC:PPR AND OPEN_ACCESS:Y); I note that the HAS_FT:Y filter was removed early in the project after I found it silently excluded PDF-only preprints. When Europe PMC returned no full-text XML (HTTP 404), the pipeline fell back to the repository PDF, from which text was extracted with PyMuPDF (fitz 1.27.2.3). An Unpaywall-based DOI fallback for peer-reviewed papers was implemented but never triggered, as PMC XML retrieval was fully reliable.

Text extraction and cleaning. Peer-reviewed JATS XML was parsed with a custom lxml-based parser that extracted the <front> (title, abstract) and <body><sec> elements. Preprint PDFs were processed with a PyMuPDF pipeline that stripped the top and bottom 7% of each page (header/footer zones), suppressed running headers (strings appearing on more than 40% of pages and shorter than 120 characters), rejoined hyphenated line breaks, dropped boilerplate back-matter (truncating at the first administrative heading in the lower 40% of the document), and truncated reference sections. Any document exceeding 500,000 characters was rejected; this correctly excluded six abstract-book aggregators (e.g., conference proceedings of 1–3.4 M characters) that matched the search terms by aggregation rather than topical relevance.

Post-ingestion boilerplate correction. Diagnostic queries run after initial ingestion revealed that administrative back-matter (Conflict of Interest, Data Availability, Author Contributions, Funding, Acknowledgements) was surfacing as retrieved chunks at ~64–65% cosine similarity, competing with genuine scientific content; the root cause was that many journals now encode such back-matter as ordinary <body><sec> elements rather than as distinct structural fields. A retrospective cleaning script scanned all documents and removed these administrative sections by a regex on the section title, after which the affected documents were de-embedded from LanceDB, their cached vectors deleted, and the cleaned text re-ingested through the AnythingLLM API. After cleaning, only two residual boilerplate-pattern strings remained across the entire corpus, both confirmed to be legitimate scientific titles. A boilerplate-section filter was subsequently added to the JATS parser so that administrative sections are stripped at parse time for all future ingestion.

2.3 Corpus characterization

The final corpus comprised 523 unique documents (507 peer-reviewed PMC Open Access articles retrieved as JATS XML and 16 Europe PMC preprints), totalling 36,262,445 characters (~113 MB of extracted text). Per-document length was right-skewed (median 59,598 characters, mean 69,335; 10th–90th percentile range 33,892–111,251; minimum 1,953; maximum 328,723). The corpus was heavily weighted toward recent literature (roughly 72% from 2025–2026), consistent with the recency of interest in mechanobiology–microglia research. No documents were lost during harvesting (zero true failures). Full distributions of publication year, relevance tier, per-document length, and journal of origin are provided in Supplementary Table S1.

2.4 Embedding and vector indexing

Documents were embedded with nomic-embed-text (Ollama-served, 274 MB, 768-dimensional output, maximum chunk length 8,192 tokens, single-request batch mode). Embeddings were stored in a LanceDB table embedded within AnythingLLM. Chunking produced approximately 227 chunks per document (measured on a five-document calibration set: 1,135 chunks), for an estimated total of ~118,000 vectors across the full corpus. Documents were ingested via the AnythingLLM REST endpoint (POST /api/v1/document/raw-text) with metadata mapped from the harvest manifest (title; DOI as URL; abstract as description; journal as source; publication year as Unix epoch; PMID as chunk source), using a JSON checkpoint file to support resumable ingestion. To avoid exceeding the Linux 255-byte filename limit on AnythingLLM's slug-derived cache filenames, document titles were capped at 150 characters. Ingestion of the full corpus completed with zero failures.

2.5 Generative model configuration

Generation used llama3.3:70b (Ollama, Q4_K_M quantization, 42 GB on disk). AnythingLLM was configured with LLM_PROVIDER=ollama, OLLAMA_MODEL_PREF=llama3.3:70b, and a 300-second keep-alive timeout. The decoding temperature was fixed at 0.2 throughout the study. Workspace retrieval was configured with a top- N of 10 retrieved chunks and a minimum cosine-similarity threshold of 0.25; these retrieval parameters were held constant across all queries.

An important consequence of this configuration is that the retrieval and generation stages differ in their determinism. For a given query string, nomic-embed-text produces a single fixed embedding, so nearest-neighbor retrieval over the static index is deterministic and returns the same set of source chunks on every call. Only the generation stage is stochastic, and at a temperature of 0.2 (near-greedy decoding) even that variability is modest. Retrieval diversity therefore cannot arise from sampling; it can only arise from changes to the query text itself. This property is central to the interpretation of these results.

2.6 Hypothesis-generation and translation protocol

The base task, issued in English, instructed the model to use all available retrieved contexts to formulate a novel research hypothesis for a new mechanism in Alzheimer's disease progression and to generate a one-to-two-page structured proposal to test it, with experiments focused on automatable in vitro work and any in vivo experiments restricted to invertebrate animals. The full text of all prompts: the English base prompt, its seven translations, and the two English control prompts, is provided in Supplementary Data D1.

The English condition comprised five runs. In addition to the base query, I generated two additional outputs using the AnythingLLM “refresh” (regenerate) control; it is noted that this control re-generates from the already-retrieved context and does *not* re-query the retriever, so it varies only the generation stage. I then issued two further English queries designed to perturb the query text while preserving its meaning: one prefixed with an arbitrary identifier string

(QueryID-001.) and one prefixed with a zero-shot chain-of-thought cue (Let's think things through step-by-step.). These served as within-language controls to test whether surface-level query perturbation, absent a language change, is sufficient to alter retrieval.

For the multilingual condition, the base prompt was translated by Claude Opus 4.8 (Anthropic) into the seven non-English languages officially supported by Llama 3.3: Spanish, French, German, Hindi, Italian, Portuguese, and Thai, yielding one query per language. Selecting the model's complete set of officially supported languages, rather than an ad hoc subset, avoids cherry-picking and defines the language set on a principled basis. Each query (English and foreign) was issued in a fresh conversation thread to prevent conversational carry-over. For every run I recorded the complete set of retrieved sources reported by the AnythingLLM citation panel, specifically, the identity of each source document and the number of the ten retrieved chunks attributable to it, together with the full generated hypothesis and proposal. Each foreign-language output was subsequently translated back into English by Claude Opus 4.8 to enable uniform content and linguistic analysis. Complete per-query retrieval manifests are provided in Supplementary Data D2, and all generated proposals, with English back-translations of the foreign-language outputs, in Supplementary Data D3 and D4.

2.7 Quantitative and linguistic analysis

Retrieval diversity was quantified per query as (i) the number of distinct source documents contributing to the ten retrieved chunks and (ii) the effective number of sources, computed as the inverse Simpson index of the per-document chunk distribution, which discounts highly skewed retrievals. The distinct-source counts for the English and foreign conditions were compared with a one-sided permutation test on the difference in means (100,000 permutations); given the single-replicate-per-language design, this test is reported as descriptive support rather than as confirmatory inference (see Discussion).

Proposal content was coded against a fixed rubric capturing the number of stated objectives, whether a project timeline was provided (and whether phased or a single duration), whether a budget was provided, the number of distinct experimental methods proposed, the framing of any in vivo component (integral aim, optional/contingent, deferred to future work, or absent), and the proposed in vivo model organism. Linguistic properties were computed on the English-language proposal texts (native English outputs and back-translations of foreign outputs) and included word count, type–token ratio, Flesch Reading Ease and Flesch–Kincaid grade level, and mean sentence length. To quantify redundancy between proposals, I computed pairwise Jaccard similarity over content-word sets, summarized within the English block, within the foreign block, and across blocks. Finally, I compiled an inventory of domain-specific entities (e.g., named model organisms, molecular targets, model systems) to identify concepts that appeared exclusively in one condition. Because the foreign-language proposals were analyzed via English back-translation, one must interpret within-document lexical metrics (type–token ratio,

readability) with corresponding caution, and rely on between-document measures (source diversity, Jaccard overlap, entity inventory) for the primary diversity claims.

3. Results

3.1 English prompting collapses retrieval onto a fixed two-paper set

Under the standard English prompt, the RAG system exhibited a striking failure of source diversity. Across five independent English queries: the base prompt, two AnythingLLM “refresh” regenerations, and two surface-perturbed variants (§3.3), the retriever returned all ten of its chunks from the *same two* documents, in the *same* 1:9 ratio, on every single run: nine chunks from “*Alzheimer’s disease: current perspectives and advances in physiological modeling*” and one from “*The emergence of model systems to investigate the link between traumatic brain injury and Alzheimer’s disease*” (Fig. 1A, blue bars; Fig. 2, five leftmost columns). The five English columns of the per-paper retrieval matrix are consequently identical. Expressed as effective diversity, each English query yielded an inverse-Simpson value of only 1.22 sources despite drawing ten chunks (Fig. 1B; Table 1; per-query manifests in Supplementary Data S2), and 90% of retrieved content originated from a single paper. Pooling all five English runs, the system surfaced just 2 distinct documents from a corpus of 523. Because retrieval over a static index is deterministic (§2.5), this collapse is not a low-probability sampling event but an exactly reproducible property of the English query.

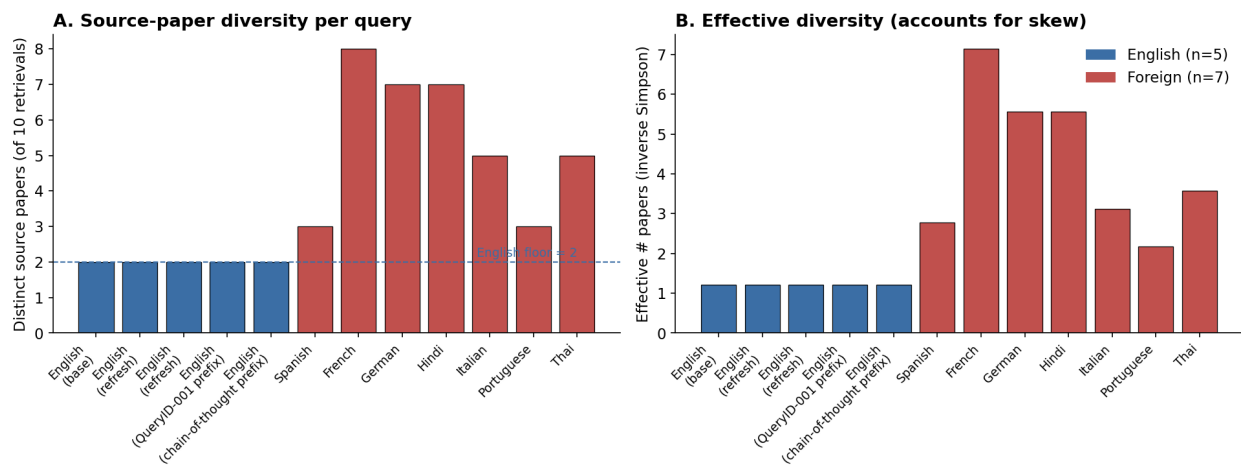


Figure 1. Foreign-language prompts increase the diversity of source-paper retrieval. For each of twelve queries (five English, seven foreign-language; blue and red respectively), the RAG system retrieved ten chunks under fixed parameters (top- $N=10$, similarity threshold 0.25). **(A)** Number of distinct source papers contributing to the ten retrieved chunks. All five English queries, including the base prompt, two “refresh” regenerations, and two surface-perturbed variants (QueryID-001 and chain-of-thought prefixes), drew from only two papers (dashed line, “English floor = 2”), whereas foreign-language queries drew from three to eight papers each. **(B)** Effective number of source

papers (inverse Simpson index of the per-paper chunk distribution), which discounts skewed retrievals. English queries yielded an effective diversity of 1.22; foreign queries averaged 4.27. Because retrieval over the static index is deterministic, the English values are exactly reproducible rather than stochastic.

Table 1. Source-retrieval diversity by query. For each query, the number of distinct source papers contributing to the ten retrieved chunks, the fraction of chunks from the single most-retrieved paper (top share), and the effective number of source papers (inverse Simpson index). Retrieval parameters (top- $N = 10$, similarity threshold 0.25) were held constant.

Query	Language	Distinct papers	Top share	Effective papers
Base	English	2	0.90	1.22
Refresh 1	English	2	0.90	1.22
Refresh 2	English	2	0.90	1.22
QueryID-001 prefix	English	2	0.90	1.22
Chain-of-thought prefix	English	2	0.90	1.22
Translated	Spanish	3	0.40	2.78
Translated	French	8	0.20	7.14
Translated	German	7	0.30	5.56
Translated	Hindi	7	0.30	5.56
Translated	Italian	5	0.50	3.12
Translated	Portuguese	3	0.60	2.17
Translated	Thai	5	0.40	3.57
English (mean)	—	2.00	0.90	1.22
Foreign (mean)	—	5.43	0.39	4.27

3.2 Foreign-language prompts sharply increase retrieval diversity

Translating the identical prompt into the seven other languages that Llama 3.3 supports broke the collapse completely (Fig. 1A, red bars). Each foreign-language query drew its ten chunks from 3 to 8 distinct papers (mean 5.4), compared with the invariant 2 for English. French was the most diverse, distributing its ten chunks across eight separate papers (effective sources 7.14); German and Hindi each drew on seven; Italian and Thai on five; Spanish and Portuguese on three. Mean effective diversity rose from 1.22 (English) to 4.27 (foreign). The difference in distinct-source counts between conditions was large and consistent ($\Delta \approx 3.4$ papers; one-sided permutation $p \approx 0.001$, reported descriptively given the single-replicate design; see Discussion).

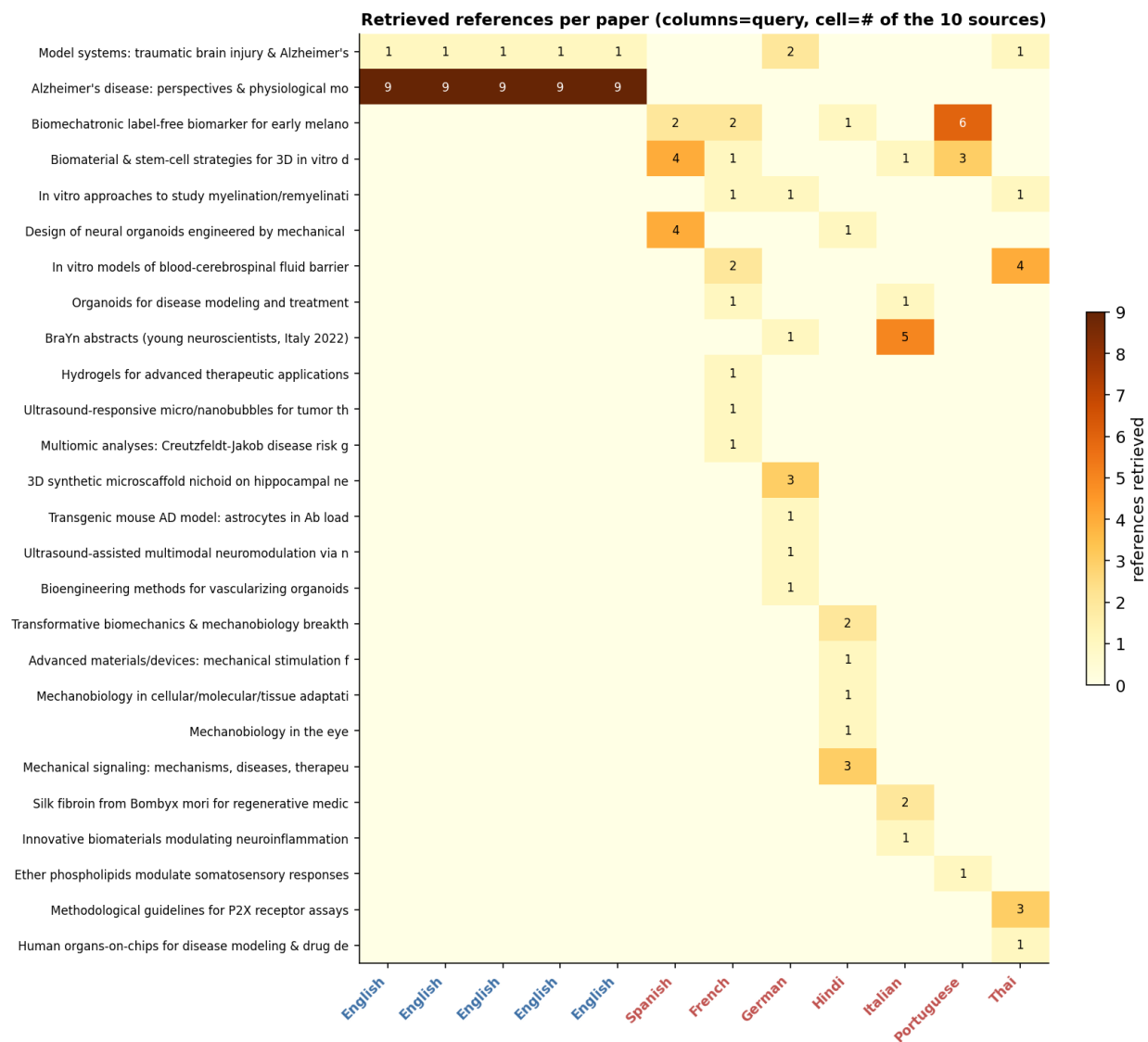


Figure 2. English and foreign-language queries access near-disjoint regions of the corpus. Heatmap of retrieved references per source paper (rows) across the twelve queries (columns; English column labels in blue, foreign in red). Cell values give the number of the ten retrieved chunks attributable to each paper; empty cells indicate no retrieval. Papers are ordered by the number of queries retrieving them. The five English columns are identical, each drawing nine chunks from a single paper (“Alzheimer’s disease: current perspectives and advances in physiological modeling”) and one from a second. That dominant paper was never retrieved by any foreign-language query, and the majority of the 25 papers surfaced by foreign queries never appeared in any English run. All five English runs combined reached 2 distinct papers; the seven foreign runs collectively reached 25.

The most consequential contrast is in cumulative corpus coverage. Whereas all five English runs combined reached only 2 documents, the seven foreign-language runs collectively surfaced 25 distinct documents, a more than twelve-fold expansion of the retrieved literature (Fig. 2). Notably, the single paper that dominated every English retrieval (contributing nine of ten chunks

each time) was *never* retrieved by any foreign-language query; conversely, the great majority of the 25 foreign-surfaced papers, including work on the blood–cerebrospinal-fluid barrier, neural organoids engineered by mechanical force, silk-fibroin scaffolds, P2X-receptor assays, and Creutzfeldt–Jakob risk genes, never appeared in any English run. The two conditions thus accessed almost non-overlapping regions of the corpus.

3.3 Within-language perturbation leaves retrieval unchanged

To test whether the diversity gain reflected prompt *language* specifically, rather than mere textual perturbation of the query, I issued two further English queries that altered the query string while preserving its meaning: one prefixed with an arbitrary identifier (QueryID-001.) and one prefixed with a zero-shot chain-of-thought cue (Let’s think things through step-by-step.). Neither perturbation moved the retriever. Both returned the identical two-paper set in the identical 1:9 proportion as the unmodified English base query (Fig. 2, English columns 4–5). Surface-level variation within English was therefore insufficient to alter retrieval, whereas translation transformed it: isolating prompt language, not query wording or decoding stochasticity, as the operative variable.

3.4 Retrieval diversity propagates to proposal content and structure

The expanded source base was reflected downstream in the substance of the generated proposals. English outputs converged on a single mechanistic frame, microglial mechanotransduction and fluid shear stress regulating amyloid- β phagocytosis, with recurrent appeals to PIEZO1 and, in the refresh variants, neurofibrillary-tangle formation, such that four of the five English proposals restated closely related hypotheses. Foreign-language proposals, by contrast, diverged into distinct mechanistic territory that tracked their differing retrievals: a blood–cerebrospinal-fluid-barrier tri-culture model with synaptic pruning (French), microglia–astrocyte interaction in mechanically stressed 3D iPSC organoids (German), fluid-shear modulation of prion phagocytosis (Portuguese), and P2X/purinergic Ca^{2+} signaling in prion pathology (Thai), among others. Every one of the seven foreign proposals advanced a mechanistically distinct hypothesis, versus effectively two distinct hypotheses across the five English proposals.

Structural coding showed subtler shifts. Proposal scaffolding (stated objectives, methods sections, expected outcomes) was broadly comparable across conditions, with most proposals in both groups articulating three objectives and six to eight distinct experimental methods (Supplementary Table S2). Elements such as explicit phased timelines and budgets appeared sporadically in both conditions (a budget in one English and one Thai proposal). The clearest structural divergences were in the proposed use of animal models, described next.

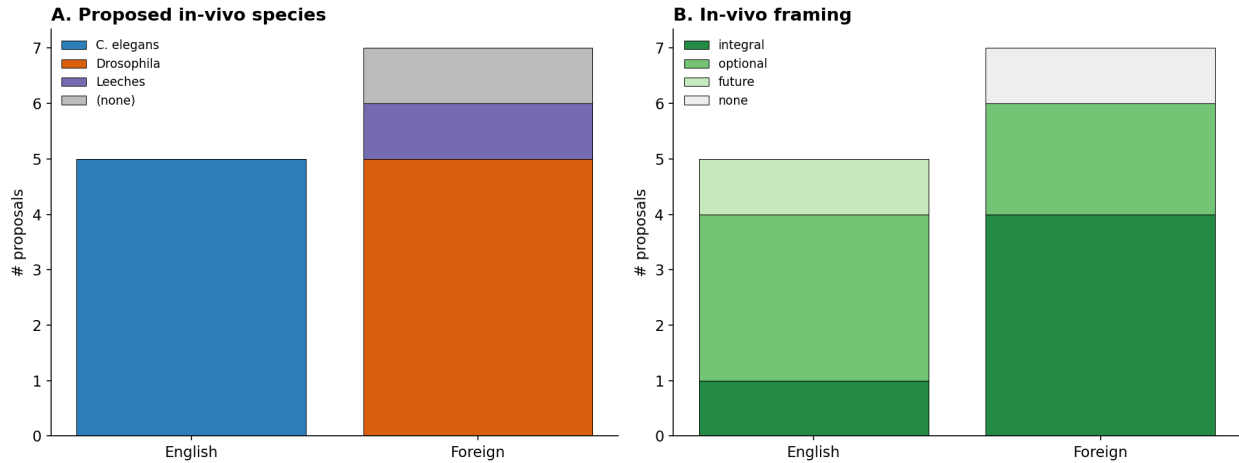


Figure 3. Prompt language shifts proposed model organism and in vivo framing. Content coding of the twelve proposals, grouped by condition (English, $n = 5$; foreign, $n = 7$). **(A)** Proposed in vivo model organism. Every English proposal that named an invertebrate model named *Caenorhabditis elegans* (5/5) and none named *Drosophila*; every foreign proposal that named a model named *Drosophila melanogaster* (5/7), with one naming leeches and one omitting an in vivo component, and none naming *C. elegans*. **(B)** Framing of the in vivo component (integral aim, optional/contingent, deferred to future work, or absent). Foreign proposals more often incorporated in vivo work as an integral aim (4/7) than English proposals (1/5).

3.5 Prompt language categorically shifts the proposed model organism

The single most clear-cut qualitative signature was the choice of in vivo model organism (Fig. 3A). Every English proposal that named an invertebrate model named *Caenorhabditis elegans*, in 5 of 5 runs, and none proposed *Drosophila*. Every foreign-language proposal that named an invertebrate model named *Drosophila melanogaster* (Spanish, French, German, Hindi, Italian), with one proposal invoking a more unusual organism (leeches, in the Thai output) and one omitting an in vivo component entirely (Portuguese); none proposed *C. elegans*. Prompt language thus acted as a near-deterministic switch on the model’s default experimental organism, partitioning the two conditions into mutually exclusive taxonomic choices despite an identical task specification.

The framing of in vivo work also differed (Fig. 3B). English proposals more often relegated animal experiments to a contingent or deferred role (optional or “future work” in four of five runs), whereas foreign proposals more frequently incorporated the in vivo component as an integral aim (four of seven runs), typically as a validation stage using genetically modified flies.

3.6 Linguistic signatures: reduced redundancy and expanded conceptual coverage

Quantitative text analysis corroborated the diversity effect at the level of language, and localized it to *between-proposal* rather than *within-proposal* variation. Pairwise Jaccard similarity over content-word sets (Fig. 4) showed that English proposals were substantially more redundant with one another (mean intra-English Jaccard 0.41, with one refresh pair reaching 0.82, near-duplicate

text) than foreign proposals were with one another (mean intra-foreign Jaccard 0.25); cross-condition overlap was likewise low (0.23). English outputs thus cluster tightly in lexical space while foreign outputs disperse.

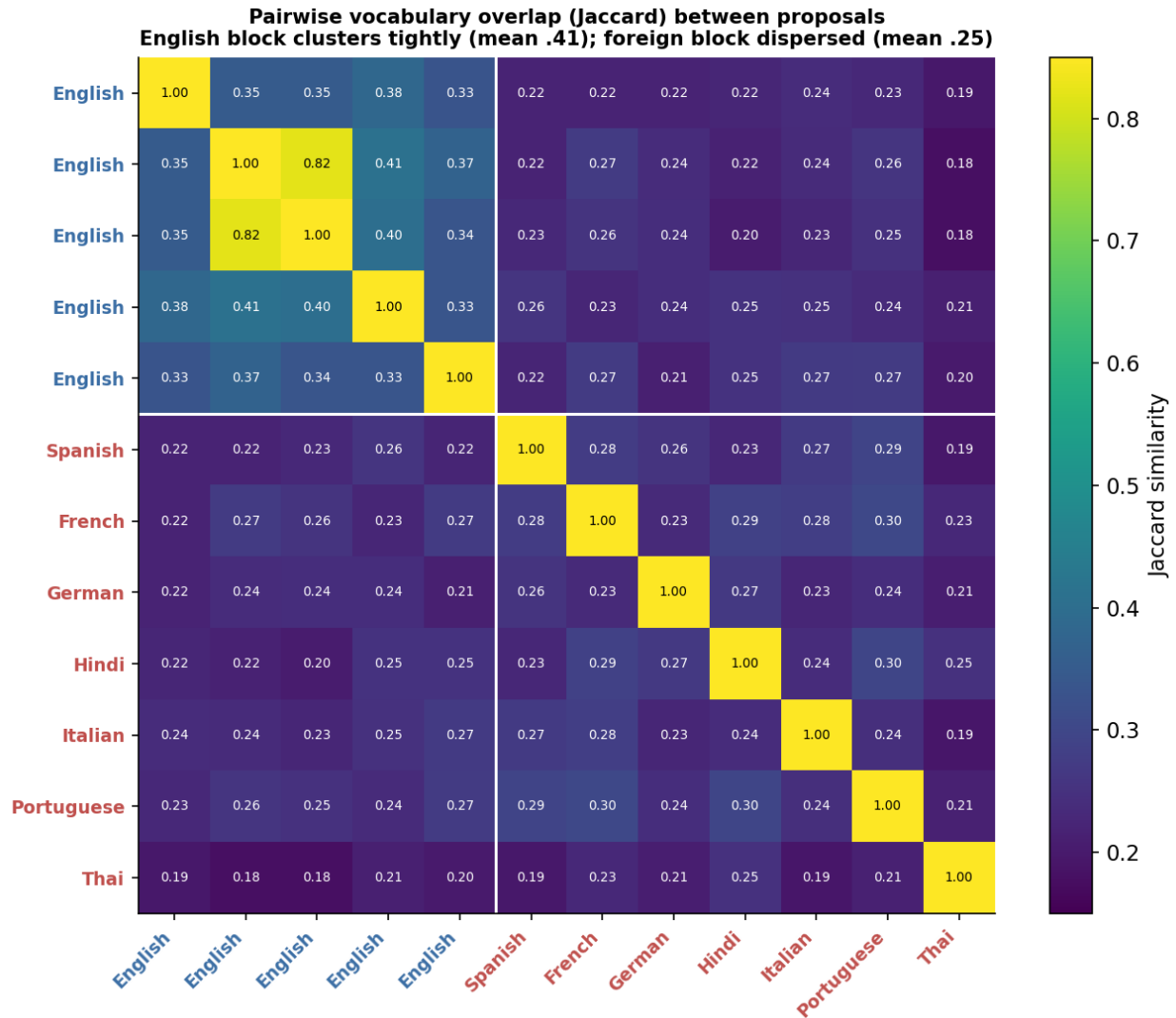


Figure 4. English proposals are lexically redundant; foreign-language proposals are dispersed. Pairwise Jaccard similarity between the twelve generated proposals, computed over content-word sets (English proposals and English back-translations of foreign proposals; row/column labels in blue and red respectively). White lines separate the English and foreign blocks. Mean within-block similarity was 0.41 for English (with one “refresh” pair reaching 0.82, indicating near-duplicate text) versus 0.25 for foreign proposals; mean cross-block similarity was 0.23. Higher intra-English similarity reflects convergence of English outputs on near-identical vocabulary, whereas the lower intra-foreign similarity reflects greater diversity among foreign outputs.

A domain-entity inventory made the conceptual expansion concrete: the foreign proposals collectively introduced 16 domain concepts that appeared in no English proposal, including the blood–cerebrospinal-fluid barrier, prions, P2X receptors, astrocytes, organoids, *Drosophila*, biomechatronic platforms, in silico modeling, cell stretching, and leeches, against 8 concepts unique to English (dominated by *C. elegans*/nematode terminology, PIEZO1, TRPV4, and neurofibrillary tangles). One notable metric runs counter to the overall pattern: within-document type–token ratio was marginally *higher* for English proposals (0.38) than for foreign ones (0.33) (Supplementary Table S3). One may attribute this in part to lexical normalization introduced by back-translation, and emphasize that it measures a different axis (per-document lexical richness) from the between-document redundancy that defines the diversity effect; on every between-document measure: distinct sources, Jaccard overlap, and unique-entity coverage, foreign-language prompting produced markedly greater diversity.

3.7 Summary

Across retrieval, proposal content, and language, an identical hypothesis-generation task produced narrow, near-duplicate output in English and broad, varied output when the prompt was translated. English queries deterministically retrieved two papers and generated ~two distinct hypotheses; foreign queries retrieved 25 papers in aggregate and generated seven distinct hypotheses, with a categorical shift in model organism and a consistent expansion of conceptual coverage. Within-language controls under near-greedy decoding confirmed that this effect is driven by prompt language rather than by query surface form or sampling temperature.

4. Discussion

4.1 Principal findings

Issuing an identical hypothesis-generation task in different languages produced strikingly different behavior from the same retrieval-augmented system. In English, retrieval collapsed deterministically onto two of 523 documents and generation converged on essentially two hypotheses; translating the prompt into the seven other languages the model supports expanded retrieval to 25 distinct documents in aggregate and yielded seven mechanistically distinct proposals. Two within-language controls, an arbitrary-identifier prefix and a chain-of-thought prefix, left retrieval completely unchanged, establishing that the effect is driven by the language of the prompt rather than by superficial variation in the query string or by decoding stochasticity. To my knowledge, this is the first demonstration that foreign-language prompting diversifies retrieval over a *monolingual* corpus, and that this retrieval diversity propagates into the substance of AI-generated scientific hypotheses.

4.2 How translation diversifies retrieval

The mechanism follows from how dense retrieval works. A query is converted by the embedding model into a single vector, and the retriever returns the corpus chunks nearest to that vector. Because `nomic-embed-text` is a multilingual embedder, a translated query is mapped into the same shared vector space as its English counterpart, but cross-lingual alignment is imperfect: the Spanish, French, or Thai rendering of a prompt lands in a somewhat different region of that space and therefore has different nearest neighbors among the English document chunks. Translation thus acts as a semantically meaning-preserving but geometrically substantial perturbation of the query vector. The within-language controls show why this is important: prepending an identifier or a chain-of-thought cue perturbs the query string but not enough to move its embedding out of the same neighborhood, so retrieval is unchanged. A change of language moves it far enough to reach new documents. In effect, the set of translations samples a neighborhood of query points around the original meaning, and the union of their retrievals covers far more of the corpus than any single point does.

A key practical consequence, already noted in the Methods section, is that this diversity is obtained under near-greedy decoding (temperature 0.2) and is exactly reproducible: because retrieval is deterministic, the English two-paper collapse and each language’s expanded retrieval will recur identically on re-execution. The diversity is therefore a structured, controllable property of the query, not a byproduct of sampling noise.

4.3 Retrieval-layer diversity versus generation-layer priors

My results contain effects that likely arise at two different stages, and distinguishing them sharpens both the claims and the open questions. The expansion of retrieved *sources* (Table 1, Figs. 1–2) is unambiguously a retrieval-layer phenomenon, since it is measured directly on the retriever’s output. The downstream divergence in proposal content, different mechanisms, different conceptual vocabulary (Fig. 4), plausibly inherits from this retrieval diversity, because the generator is conditioned on different evidence in each language.

The categorical shift in proposed model organism (Fig. 3A) is more subtle. English proposals invoked *Caenorhabditis elegans* in every run and foreign proposals never did, favoring *Drosophila* or, in one case, leeches. This dissociation is unlikely to be fully explained by the retrieved papers, few of which prescribe a specific invertebrate model; it more likely reflects a language-conditioned prior in the generator itself: the model’s “default” experimental organism differs by language, consistent with prior reports that multilingual prompting activates different regions of an LLM’s parametric knowledge [5]. If so, foreign-language prompting confers diversity through *two* partly independent channels: a retrieval channel (which documents are surfaced) and a generation channel (which language-specific priors are activated). Disentangling their relative contributions, for example, by holding the retrieved context fixed while varying only the language of the generation prompt, is an informative direction for follow-up work, and it would be premature to attribute the organism effect solely to retrieval.

4.4 Relation to prior work

These findings connect three previously separate lines of research. First, multilingual prompting has been shown to increase the diversity of LLM *generations* more effectively than temperature sampling, persona conditioning, or step-by-step recall [5], but that work operates purely at the generation layer and measures cultural or demographic variety. The present work extends the multilingual-prompting idea to the retrieval layer and to substantive scientific content. Second, the cross-lingual RAG literature has documented that query language alters retrieval, but has generally treated this sensitivity as an inconsistency to be *suppressed*, for instance by translating retrieved passages into a common language before generation [2,7], because it is studied over multilingual corpora in convergent question-answering, where a single correct answer exists and retrieval variation is noise. The present contribution is to invert that stance: for divergent tasks such as hypothesis generation, cross-lingual retrieval variation is not a defect but a resource, and it is obtainable even when the corpus is entirely monolingual. Third, LLM-based scientific ideation is known to suffer from limited diversity despite strong per-idea novelty [3], and existing remedies rely on comparatively heavy machinery: iterative planning, graph-structured retrieval, or evolutionary search [4]. Foreign-language prompting offers a strikingly cheaper alternative that requires no change to the corpus, retriever, or pipeline.

4.5 Practical implications

The appeal of this lever is its near-zero cost. Diversifying a RAG system’s output typically means engineering effort: reranking, query reformulation, graph construction, or additional model calls. Here, the only operation is translating the prompt, which any capable LLM performs at negligible cost, followed by pooling and deduplicating the retrievals across languages. The approach is architecture-agnostic: it makes no assumptions about the retriever, the index, or the generator beyond the embedder having some multilingual capability, and it stacks with existing diversity methods rather than competing with them. Although we demonstrate it for scientific hypothesis generation, the same principle should apply wherever broad coverage of a corpus is desirable: literature review, patent and prior-art search, systematic evidence gathering, or re-teaming a knowledge base for blind spots, and this breadth of potential application is, in my view, the most consequential implication of the result.

4.6 Limitations

Several limitations bound these conclusions. Most importantly, the study uses a single query per language; while the deterministic nature of retrieval means the source-diversity measurements themselves are exact rather than sampled, I did not estimate how much retrieval or proposal diversity varies across *different* prompts on the same topic, nor across paraphrases within each language. A larger number of independent prompts per language, and multiple topics, would be needed to establish that the English-versus-foreign gap generalizes beyond this instance, and the statistical comparison I report should be read as descriptive rather than confirmatory given this design. Second, the present findings derive from one corpus in one subfield, one generator (llama3.3:70b), and one embedding model (nomic-embed-text); the magnitude of the effect may

depend on the multilingual quality of the embedder in particular, and should be tested across models and domains. Third, I did not formally assess whether the additional documents surfaced by foreign-language queries are as *topically relevant* as those retrieved in English; greater diversity is only useful if the added sources remain on-topic, and some foreign retrievals were more tangential than others. Characterizing this diversity–relevance trade-off, ideally with domain-expert relevance judgments and blinded quality ratings of the proposals, is essential before the method is recommended for practical use. Fourth, the linguistic metrics for foreign proposals were computed on English back-translations, which can normalize vocabulary and syntax; I therefore based the primary diversity claims on between-document measures and treated within-document metrics (type–token ratio, readability) as secondary. Finally, I studied a divergent task, and the same query-language sensitivity that helps here may *harm* convergent tasks that require a single correct retrieval, a boundary condition worth mapping directly.

4.7 Future directions

The natural next step is a systematic replication that varies prompts, topics, generators, and embedding models with adequate repetition, paired with expert relevance and quality scoring to quantify the diversity–relevance trade-off. Beyond replication, several questions follow directly from these results: whether aggregating across languages and deduplicating yields a better coverage-per-cost curve than temperature sampling or query reformulation at matched budget; whether the retrieval and generation channels identified in §4.3 can be separated experimentally; whether an optimal *set* of languages exists for a given embedder, or whether returns diminish after a few well-separated languages; and whether the geometric intuition, that translations sample a neighborhood of query embeddings, can be exploited more directly, for example by controlled perturbation of the query vector itself, with language serving as a convenient and interpretable means of generating such perturbations.

4.8 Conclusion

A retrieval-augmented LLM asked the same question in English repeatedly returned the same two papers and the same two ideas; asked in seven other languages, it drew on twenty-five papers and produced seven distinct research proposals. Because the effect operates at the retrieval layer, survives near-greedy decoding, is absent under within-language perturbation, and requires no change to the underlying system, foreign-language prompting is a simple, reproducible, and broadly transferable lever for injecting diversity into retrieval-augmented generation, a way of asking a fixed body of knowledge a wider range of questions simply by asking in a different tongue.

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Supplementary Information

Prompt language as a diversity lever: foreign-language queries broaden source retrieval and AI-generated research proposals

Supplementary Table S1. Characterization of the harvested literature corpus.

Summary statistics for the 523-document corpus indexed by the RAG system. The corpus comprises 498 papers retrieved by the tiered combinatorial search plus 25 papers from the initial “microglia neuroinflammation” seed query; panels (c) and (d) describe the 498-paper tiered manifest, for which relevance scores and harvest years are defined. Character and length statistics (panels a, e) are computed over extracted, cleaned text.

(a) Scale

Metric	Value
Total unique documents	523
Peer-reviewed (PMC OA, JATS XML)	507
Preprints (Europe PMC, incl. PDF fallback)	16
Total extracted text	36,262,445 characters (~113 MB)

(b) Harvest outcomes

Outcome	Count
Successfully harvested	523
Recovered via PDF fallback (preprints)	8
Size-capped and excluded (abstract-book aggregators)	6
True failures (lost)	0

(c) Relevance-tier distribution (tiered manifest, n = 498)

Tier (k)	Definition	Papers	%
8	all 8 terms co-occur	1	0.2
7	any 7 of 8	37	7.4
6	any 6 of 8	134	26.9
5	any 5 of 8	326	65.5

(d) Publication-year distribution (tiered manifest, n = 498)

Year	Papers
≤ 2020	1
2021	5
2022	25
2023	41
2024	48
2025	164
2026	214

(e) Per-document text length (characters)

Statistic	Value
Mean	69,335
Median	59,598
10th percentile	33,892
90th percentile	111,251
Minimum	1,953

Statistic	Value
Maximum	328,723

(f) Most frequent source journals

Journal	Papers
International Journal of Molecular Sciences	31
Cells	21
Frontiers in Immunology	20
MedComm	12
Frontiers in Cell and Developmental Biology	11
Journal of Neuroinflammation	10
Signal Transduction and Targeted Therapy	10
Advanced Science	10
Frontiers in Cellular Neuroscience	9
Alzheimer's & Dementia	8

An additional 14 preprints appear with "bioRxiv" in the journal field.

Supplementary Data D1. Verbatim prompt text.

The base hypothesis-generation prompt issued in English, its translations into the seven other languages supported by Llama 3.3, and the two English control prompts. Each foreign-language prompt is a direct translation of the English base prompt produced by Claude Opus 4.8. The two control prompts differ from the base prompt only by the prepended text (an arbitrary identifier and a chain-of-thought cue, respectively).

English (base)

Use all of the Contexts that you have access to, and formulate a novel research hypothesis that explores a new mechanism in the progression of alzheimer's disease, and then generate a 1-2 page structured proposal to test this hypothesis. The proposed experiments should focus on in vitro experiments that can be automated, and if in vivo experiments are proposed, they should only involve invertebrate animals.

Spanish

Utiliza todos los Contextos a los que tengas acceso y formula una hipótesis de investigación novedosa que explore un nuevo mecanismo en la progresión de la enfermedad de Alzheimer; luego genera una propuesta estructurada de 1 a 2 páginas para poner a prueba esta hipótesis. Los experimentos propuestos deben centrarse en experimentos in vitro que puedan automatizarse y, si se proponen experimentos in vivo, estos solo deben involucrar animales invertebrados.

French

Utilisez tous les Contextes auxquels vous avez accès et formulez une hypothèse de recherche novatrice qui explore un nouveau mécanisme dans la progression de la maladie d'Alzheimer, puis générez une proposition structurée d'une à deux pages pour tester cette hypothèse. Les expériences proposées doivent se concentrer sur des expériences in vitro pouvant être automatisées et, si des expériences in vivo sont proposées, elles ne doivent impliquer que des animaux invertébrés.

German

Nutzen Sie alle Kontexte, auf die Sie Zugriff haben, und formulieren Sie eine neuartige Forschungshypothese, die einen neuen Mechanismus im Fortschreiten der Alzheimer-Krankheit untersucht, und erstellen Sie anschließend einen strukturierten Antrag von ein bis zwei Seiten, um diese Hypothese zu überprüfen. Die vorgeschlagenen Experimente sollten sich auf In-vitro-Experimente konzentrieren, die automatisiert werden können, und falls In-vivo-Experimente vorgeschlagen werden, sollten diese ausschließlich wirbellose Tiere einbeziehen.

Hindi

आपके पास उपलब्ध सभी संदर्भों (Contexts) का उपयोग करें और एक नई शोध परिकल्पना तैयार करें जो अल्जाइमर रोग की प्रगति में एक नए तंत्र (mechanism) का अन्वेषण करती हो, और फिर इस परिकल्पना का परीक्षण करने के लिए 1-2 पृष्ठ का एक संरचित प्रस्ताव तैयार करें। प्रस्तावित प्रयोगों को ऐसे इन विट्रो (in vitro) प्रयोगों पर केंद्रित होना चाहिए जिन्हें स्वचालित (automated) किया जा सके, और यदि इन विवो (in vivo) प्रयोग प्रस्तावित किए जाते हैं, तो उनमें केवल अकशेरुकी (invertebrate) जीवों को ही शामिल किया जाना चाहिए।

Italian

Utilizza tutti i Contesti a cui hai accesso e formula un'ipotesi di ricerca innovativa che esplori un nuovo meccanismo nella progressione della malattia di Alzheimer, quindi genera una proposta strutturata di una o due pagine per verificare questa ipotesi. Gli esperimenti proposti dovrebbero concentrarsi su esperimenti in vitro che possano essere automatizzati e, qualora vengano proposti esperimenti in vivo, questi dovrebbero coinvolgere esclusivamente animali invertebrati.

Portuguese

Utilize todos os Contextos a que tem acesso e formule uma hipótese de pesquisa inovadora que explore um novo mecanismo na progressão da doença de Alzheimer e, em seguida, gere uma proposta estruturada de 1 a 2 páginas para testar essa hipótese. Os experimentos propostos devem concentrar-se em experimentos in vitro que possam ser automatizados e, caso sejam propostos experimentos in vivo, estes devem envolver apenas animais invertebrados.

Thai

ใช้บริบท (Contexts) ทั้งหมดที่คุณสามารถเข้าถึงได้ และตั้งสมมติฐานการวิจัยใหม่ที่สำรวจกลไกใหม่ในการดำเนินไปของโรคอัลไซเมอร์ จากนั้นจัดทำข้อเสนอโครงการที่มีโครงสร้างความยาว 1-2 หน้าเพื่อทดสอบสมมติฐานนี้ การทดลองที่เสนอควรมุ่งเน้นไปที่การทดลองในหลอดทดลอง (*in vitro*) ที่สามารถทำงานแบบอัตโนมัติได้ และหากมีการเสนอการทดลองในสิ่งมีชีวิต (*in vivo*) การทดลองนั้นควรเกี่ยวข้องกับสัตว์ไม่มีกระดูกสันหลังเท่านั้น

English control (QueryID-001)

QueryID-001. Use all of the Contexts that you have access to, and formulate a novel research hypothesis that explores a new mechanism in the progression of alzheimer's disease, and then generate a 1-2 page structured proposal to test this hypothesis. The proposed experiments should focus on in vitro experiments that can be automated, and if in vivo experiments are proposed, they should only involve invertebrate animals.

English control (chain-of-thought)

Let's think things through step-by-step. Use all of the Contexts that you have access to, and formulate a novel research hypothesis that explores a new mechanism in the progression of alzheimer's disease, and then generate a 1-2 page structured proposal to test this hypothesis. The proposed experiments should focus on in vitro experiments that can be automated, and if in vivo experiments are proposed, they should only involve invertebrate animals.

Supplementary Data D2. Per-query retrieval manifest.

For each of the twelve queries, the source documents contributing to the ten retrieved chunks, with the number of chunks drawn from each. These are the raw retrievals underlying Table 1 and Figures 1–2. Retrieval used top-N = 10 and a similarity threshold of 0.25; because dense retrieval over the static index is deterministic, these manifests are exactly reproducible.

Base — English (2 distinct papers)

- 1 Model systems: traumatic brain injury & Alzheimer's
- 9 Alzheimer's disease: perspectives & physiological modeling

Refresh 1 — English (2 distinct papers)

- 1 Model systems: traumatic brain injury & Alzheimer's
- 9 Alzheimer's disease: perspectives & physiological modeling

Refresh 2 — English (2 distinct papers)

- 1 Model systems: traumatic brain injury & Alzheimer's
- 9 Alzheimer's disease: perspectives & physiological modeling

QueryID-001 prefix — English (2 distinct papers)

- 1 Model systems: traumatic brain injury & Alzheimer's
- 9 Alzheimer's disease: perspectives & physiological modeling

Chain-of-thought prefix — English (2 distinct papers)

- 9 Alzheimer's disease: perspectives & physiological modeling
- 1 Model systems: traumatic brain injury & Alzheimer's

Translated — Spanish (3 distinct papers)

- 2 Biomechatronic label-free biomarker for early melanoma
- 4 Design of neural organoids engineered by mechanical forces
- 4 Biomaterial & stem-cell strategies for 3D in vitro disease models

Translated — French (8 distinct papers)

- 2 Biomechatronic label-free biomarker for early melanoma
- 1 In vitro approaches to study myelination/remyelination in CNS
- 1 Hydrogels for advanced therapeutic applications
- 1 Biomaterial & stem-cell strategies for 3D in vitro disease models
- 1 Ultrasound-responsive micro/nanobubbles for tumor therapy
- 2 In vitro models of blood-cerebrospinal fluid barrier
- 1 Organoids for disease modeling and treatment
- 1 Multiomic analyses: Creutzfeldt-Jakob disease risk genes

Translated — German (7 distinct papers)

- 1 BraYn abstracts (young neuroscientists, Italy 2022)
- 3 3D synthetic microsccaffold nichoid on hippocampal neurons
- 1 In vitro approaches to study myelination/remyelination in CNS
- 2 Model systems: traumatic brain injury & Alzheimer's
- 1 Transgenic mouse AD model: astrocytes in Ab load
- 1 Ultrasound-assisted multimodal neuromodulation via nanosystems
- 1 Bioengineering methods for vascularizing organoids

Translated — Hindi (7 distinct papers)

- 2 Transformative biomechanics & mechanobiology breakthroughs
- 1 Advanced materials/devices: mechanical stimulation for tissue regen
- 1 Mechanobiology in cellular/molecular/tissue adaptation
- 1 Mechanobiology in the eye
- 3 Mechanical signaling: mechanisms, diseases, therapeutic targets
- 1 Biomechatronic label-free biomarker for early melanoma
- 1 Design of neural organoids engineered by mechanical forces

Translated — Italian (5 distinct papers)

- 5 BraYn abstracts (young neuroscientists, Italy 2022)
- 1 Organoids for disease modeling and treatment
- 2 Silk fibroin from *Bombyx mori* for regenerative medicine
- 1 Biomaterial & stem-cell strategies for 3D in vitro disease models
- 1 Innovative biomaterials modulating neuroinflammation after TBI

Translated — Portuguese (3 distinct papers)

- 6 Biomechatronic label-free biomarker for early melanoma
- 3 Biomaterial & stem-cell strategies for 3D in vitro disease models
- 1 Ether phospholipids modulate somatosensory responses in *Drosophila*

Translated — Thai (5 distinct papers)

- 3 Methodological guidelines for P2X receptor assays
- 1 Model systems: traumatic brain injury & Alzheimer's
- 4 In vitro models of blood-cerebrospinal fluid barrier
- 1 Human organs-on-chips for disease modeling & drug development
- 1 In vitro approaches to study myelination/remyelination in CNS

Supplementary Data D3. Generated research proposals (original language).

The complete, unedited hypothesis-and-proposal text returned by llama3.3:70b for each of the twelve queries, in the language in which it was generated. Five proposals are in English (base prompt, two refresh regenerations, and the two English control prompts); seven are in the respective foreign languages. Text is reproduced verbatim; only formatting (bold, bullet glyphs) has been normalized.

▪ English (base)

Research Hypothesis:

Microglial mechanotransduction, mediated by fluid shear stress, plays a crucial role in the progression of Alzheimer's disease by regulating the phagocytosis of amyloid- β (A β) plaques and promoting the spread of tau pathology.

Background: Alzheimer's disease is characterized by the accumulation of A β plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein. Microglia, the resident immune cells of the brain, have been implicated in the clearance of A β plaques through phagocytosis. However, the mechanisms regulating microglial function in Alzheimer's disease are not fully understood. Recent studies have highlighted the importance of mechanotransduction, the conversion of mechanical forces into cellular signals, in various biological processes. We propose to investigate the role of microglial mechanotransduction in the progression of Alzheimer's disease.

Proposal:

Title: Investigating Microglial Mechanotransduction in Alzheimer's Disease Progression

Objective: To determine the role of microglial mechanotransduction, mediated by fluid shear stress, in regulating A β phagocytosis and tau pathology spread in Alzheimer's disease.

Experimental Design:

1. In vitro experiments:
 - Develop a microfluidic device to apply controlled fluid shear stress to microglia cultured with A β plaques.
 - Measure the effects of fluid shear stress on microglial phagocytosis of A β plaques using live-cell imaging and flow cytometry.
 - Investigate the role of mechanotransduction pathways, such as the PIEZO1 channel, in regulating microglial phagocytosis using RNA interference and pharmacological inhibitors.
2. Automated high-throughput screening:
 - Develop a robotic platform to automate the microfluidic device and perform high-throughput screening of small molecule

libraries to identify compounds that modulate microglial mechanotransduction and A β phagocytosis.

3. In vivo experiments (invertebrate):

- Use the nematode worm *Caenorhabditis elegans* as a model organism to study the effects of fluid shear stress on microglia-like cells (e.g., Ceol-1) in the context of A β toxicity.
- Measure the impact of fluid shear stress on A β clearance and tau pathology spread using fluorescence microscopy and behavioral assays.

Expected Outcomes:

1. Fluid shear stress regulates microglial phagocytosis of A β plaques through mechanotransduction pathways.
2. Modulation of microglial mechanotransduction affects the spread of tau pathology in Alzheimer's disease models.
3. Identification of small molecule compounds that target microglial mechanotransduction and enhance A β clearance.

Impact: This study will provide new insights into the mechanisms regulating microglial function in Alzheimer's disease and identify potential therapeutic targets for enhancing A β clearance and reducing tau pathology spread. The use of automated high-throughput screening and invertebrate models will facilitate the rapid translation of these findings into preclinical studies.

Timeline:

- Month 1-3: Development of microfluidic device and automation platform
- Month 4-6: In vitro experiments and high-throughput screening
- Month 7-9: In vivo experiments (*C. elegans*)
- Month 10-12: Data analysis and manuscript preparation

Personnel:

- Principal Investigator: [Name]
- Postdoctoral Researcher: [Name]
- Graduate Student: [Name]
- Technician: [Name]

Resources:

- Microfluidic device and automation platform
- Cell culture facilities
- High-throughput screening equipment
- *C. elegans* facility
- Computational resources for data analysis

Note: The proposal is structured to fit within a 1-2 page limit, with a focus on the research hypothesis, experimental design, and expected outcomes. The timeline, personnel, and resources sections provide

additional details on the proposed project.

▪ **English (refresh 1)**

Research Hypothesis:

Microglial mechanotransduction plays a crucial role in the progression of Alzheimer's disease by regulating the phagocytosis of amyloid- β ($A\beta$) plaques and modulating the release of pro-inflammatory cytokines, which in turn affects the formation of neurofibrillary tangles (NFTs) and exacerbates neuronal dysfunction.

Proposal:

Title: Investigating the Role of Microglial Mechanotransduction in Alzheimer's Disease Progression using In Vitro Models

Introduction: Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of $A\beta$ plaques, NFTs, and neuronal loss. Recent studies have highlighted the importance of microglia in AD pathogenesis, but the underlying mechanisms remain poorly understood. This proposal aims to investigate the role of microglial mechanotransduction in AD progression using in vitro models.

Objectives:

1. To develop an in vitro model of microglial mechanotransduction using microfluidic devices and mechanical stress sensors.
2. To examine the effects of mechanical stress on microglial phagocytosis of $A\beta$ plaques and release of pro-inflammatory cytokines.
3. To investigate the relationship between microglial mechanotransduction and NFT formation in vitro.

Methods:

1. In Vitro Model Development: Microfluidic devices will be designed to apply controlled mechanical stress to microglial cells. Mechanical stress sensors will be used to monitor the stress applied to the cells.
2. Microglial Phagocytosis Assay: $A\beta$ plaques will be added to the microfluidic devices, and microglial phagocytosis will be measured using fluorescence microscopy and flow cytometry.
3. Cytokine Release Assay: Pro-inflammatory cytokines (e.g., $TNF-\alpha$, $IL-1\beta$) will be measured in the supernatant of microglial cells subjected to mechanical stress using ELISA or multiplex assays.
4. NFT Formation Assay: Tau protein will be added to the microfluidic devices, and NFT formation will be measured using immunofluorescence microscopy.

Automated Experiments:

- Microfluidic devices will be integrated with automated systems for

fluid handling, imaging, and data analysis.

- High-throughput screening of mechanical stress conditions and microglial responses will be performed using robotic platforms.

In Vivo Experiments (Optional):

- If necessary, in vivo experiments will be conducted using the nematode worm *Caenorhabditis elegans* (*C. elegans*) to validate the findings from in vitro studies. *C. elegans* will be engineered to express human tau protein and A β , and the effects of mechanical stress on microglial-like cells (e.g., CeACO-1) will be examined.

Expected Outcomes:

- The development of an in vitro model of microglial mechanotransduction will provide a novel tool for studying AD pathogenesis.
- The results will elucidate the role of microglial mechanotransduction in regulating A β phagocytosis, cytokine release, and NFT formation.
- The findings will have implications for the development of therapeutic strategies targeting microglial mechanotransduction in AD.

Timeline:

- Month 1-3: Development of in vitro model and optimization of experimental conditions
- Month 4-6: Microglial phagocytosis and cytokine release assays
- Month 7-9: NFT formation assay and data analysis
- Month 10-12: Optional in vivo experiments using *C. elegans*

Personnel:

- Principal Investigator: [Name]
- Postdoctoral Researcher: [Name]
- Graduate Student: [Name]

Resources:

- Microfluidic devices and mechanical stress sensors
- Automated systems for fluid handling, imaging, and data analysis
- *C. elegans* strains (if necessary)
- Reagents and consumables for in vitro experiments

This proposal outlines a novel research hypothesis and a structured approach to investigating the role of microglial mechanotransduction in AD progression using in vitro models. The proposed experiments will provide valuable insights into the underlying mechanisms of AD pathogenesis and may lead to the development of new therapeutic strategies.

▪ **English (refresh 2)**

Research Hypothesis:

Microglial mechanotransduction plays a crucial role in the progression of Alzheimer's disease by regulating the phagocytosis of amyloid- β ($A\beta$) plaques and modulating the release of pro-inflammatory cytokines, which in turn affects the formation of neurofibrillary tangles (NFTs) and exacerbates neuronal dysfunction.

Proposal:

Title: Investigating the Role of Microglial Mechanotransduction in Alzheimer's Disease Progression using In Vitro Models

Introduction: Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of $A\beta$ plaques, NFTs, and neuronal loss. Recent studies have highlighted the importance of microglia in AD pathogenesis, but the underlying mechanisms remain poorly understood. This proposal aims to investigate the role of microglial mechanotransduction in AD progression using in vitro models.

Objectives:

1. To develop an in vitro model of microglial mechanotransduction using microfluidic devices and mechanical stress sensors.
2. To examine the effects of mechanical stress on microglial phagocytosis of $A\beta$ plaques and release of pro-inflammatory cytokines.
3. To investigate the relationship between microglial mechanotransduction and NFT formation in vitro.

Methods:

1. In Vitro Model Development: Microfluidic devices will be designed to apply controlled mechanical stress to microglial cells. Mechanical stress sensors will be used to measure the forces exerted on the cells.
2. Microglial Phagocytosis Assay: $A\beta$ plaques will be added to the microfluidic devices, and microglial phagocytosis will be measured using flow cytometry and confocal microscopy.
3. Cytokine Release Assay: Pro-inflammatory cytokines (e.g., $TNF-\alpha$, $IL-1\beta$) will be measured in the supernatant of microglial cells subjected to mechanical stress using ELISA or multiplex assays.
4. NFT Formation Assay: Tau protein will be added to the microfluidic devices, and NFT formation will be measured using immunofluorescence and Western blotting.

Automated Experiments:

- Microfluidic devices will be integrated with automated systems for mechanical stress application, cell culture maintenance, and data acquisition.
- High-throughput screening of microglial phagocytosis and cytokine

release will be performed using robotic platforms.

In Vivo Experiments (Optional):

- If necessary, in vivo experiments will be conducted using the nematode worm *Caenorhabditis elegans* (*C. elegans*) to validate the findings from in vitro studies. *C. elegans* will be engineered to express human tau protein and A β , and the effects of mechanical stress on microglial-like cells (e.g., *C. elegans* macrophages) will be investigated.

Expected Outcomes:

- The development of an in vitro model of microglial mechanotransduction will provide a novel tool for investigating AD pathogenesis.
- The study will elucidate the role of microglial mechanotransduction in regulating A β phagocytosis, cytokine release, and NFT formation.
- The findings will have implications for the development of therapeutic strategies targeting microglial mechanotransduction in AD.

Timeline:

- Month 1-3: Development of in vitro model and optimization of experimental conditions
- Month 4-6: Microglial phagocytosis and cytokine release assays
- Month 7-9: NFT formation assay and data analysis
- Month 10: Optional in vivo experiments using *C. elegans*

Personnel:

- Principal Investigator: [Name]
- Postdoctoral Researcher: [Name]
- Graduate Student: [Name]

Resources:

- Microfluidic devices and mechanical stress sensors
- Cell culture equipment and reagents
- Flow cytometer and confocal microscope
- ELISA and multiplex assay kits
- *C. elegans* strains and equipment (if necessary)

Budget:

- Equipment and reagents: \$100,000
- Personnel and training: \$150,000
- Travel and meetings: \$10,000

Total budget: \$260,000

This proposal outlines a novel research hypothesis and a structured plan to investigate the role of microglial mechanotransduction in AD progression using in vitro models. The proposed experiments will provide valuable insights into the underlying mechanisms of AD pathogenesis and

may lead to the development of new therapeutic strategies.

▪ Spanish

Hipótesis de investigación:

Se ha observado que la enfermedad de Alzheimer (EA) se caracteriza por una alteración en la función de las microglías, las células inmunes del cerebro, lo que conduce a una respuesta inflamatoria anormal y a la acumulación de placas amiloides. Sin embargo, el papel de la mecánica celular y la transducción de fuerzas en la progresión de la EA es aún poco entendido. Nuestra hipótesis es que las fuerzas mecánicas ejercidas sobre las microglías pueden alterar su función y contribuir a la progresión de la EA.

Propuesta de investigación:

Título: "Efecto de las fuerzas mecánicas en la función de las microglías y la progresión de la enfermedad de Alzheimer"

Objetivos:

1. Investigar cómo las fuerzas mecánicas afectan la función de las microglías en cultivo *in vitro*.
2. Evaluar el efecto de las fuerzas mecánicas en la expresión de genes relacionados con la inflamación y la fagocitosis en microglías.
3. Desarrollar un modelo *in vitro* que simule la progresión de la EA y evaluar el impacto de las fuerzas mecánicas en la acumulación de placas amiloides.

Metodología:

1. Cultivo de microglías: Se cultivarán microglías humanas o murinas en un medio de cultivo estándar.
2. Aplicación de fuerzas mecánicas: Se utilizará un dispositivo de biomecánica para aplicar fuerzas mecánicas controladas a las microglías. Las fuerzas se aplicarán durante diferentes períodos de tiempo y con diferentes magnitudes.
3. Análisis de la función de las microglías: Se evaluará la función de las microglías mediante técnicas de citometría de flujo, ELISA y qRT-PCR para determinar la expresión de genes relacionados con la inflamación y la fagocitosis.
4. Modelo *in vitro* de EA: Se desarrollará un modelo *in vitro* que simule la progresión de la EA utilizando microglías y neuronas humanas o murinas. El modelo se basará en la co-cultura de microglías y neuronas en un medio de cultivo que contenga placas amiloides.
5. Evaluación del impacto de las fuerzas mecánicas: Se evaluará el impacto de las fuerzas mecánicas en la acumulación de placas amiloides y la función de las microglías en el modelo *in vitro*.

Experimentos automatizados:

- Se utilizará un sistema de cultivo automático para mantener las condiciones de cultivo óptimas y minimizar la variabilidad.
- Se empleará un dispositivo de biomecánica automático para aplicar fuerzas mecánicas controladas a los microglías.
- Se utilizarán técnicas de citometría de flujo y ELISA automatizadas para evaluar la función de los microglías.

Experimentos in vivo (opcional):

- Si se considera necesario, se podrían realizar experimentos in vivo utilizando animales invertebrados (como *Drosophila melanogaster*) para evaluar el impacto de las fuerzas mecánicas en la progresión de la EA. Sin embargo, esto dependerá de los resultados obtenidos en los experimentos in vitro y de la disponibilidad de recursos.

Tiempo y recursos:

- El proyecto se llevará a cabo durante 12 meses.
- Se necesitarán recursos para la adquisición de equipos de biomecánica, cultivo celular y análisis de datos.
- Se requerirá personal capacitado en técnicas de cultivo celular, biomecánica y análisis de datos.

Conclusión:

La propuesta presentada busca investigar el efecto de las fuerzas mecánicas en la función de los microglías y la progresión de la enfermedad de Alzheimer. Los experimentos in vitro automatizados permitirán evaluar el impacto de las fuerzas mecánicas en la función de los microglías y la acumulación de placas amiloides. Si se considera necesario, se podrían realizar experimentos in vivo utilizando animales invertebrados para evaluar el impacto de las fuerzas mecánicas en la progresión de la EA. Los resultados obtenidos podrían contribuir a una mejor comprensión de la enfermedad y al desarrollo de nuevas terapias para su tratamiento.

▪ French

Titre de la proposition : Exploration du rôle de la mécanotransduction dans la progression de la maladie d'Alzheimer via des modèles in vitro et des expériences automatisées

Introduction : La maladie d'Alzheimer est une pathologie neurodégénérative complexe caractérisée par l'accumulation de plaques amyloïdes et de dégénérescence neuronale. Les recherches actuelles se concentrent sur la compréhension des mécanismes sous-jacents à cette maladie pour développer des traitements efficaces. Notre hypothèse novatrice suggère que la mécanotransduction, le processus par lequel les cellules convertissent les forces mécaniques en signaux biochimiques,

joue un rôle clé dans la progression de la maladie d'Alzheimer.

Hypothèse : Nous proposons que les forces de cisaillement fluïdique et les changements mécaniques dans le microenvironnement des cellules neuronales contribuent à l'activation anormale des microglies, ce qui à son tour favorise la phagocytose des synapses saines et l'accumulation de protéines amyloïdes. Cette hypothèse sera testée en utilisant des modèles in vitro de barrière hémato-liquorienne cérébrospinale (BCSFB) et des expériences automatisées pour simuler les conditions mécaniques pathologiques.

Objectifs :

1. Développer un modèle in vitro de BCSFB qui intègre des cellules neuronales, des astrocytes et des microglies pour étudier l'impact de la mécanotransduction sur la progression de la maladie d'Alzheimer.
2. Utiliser des techniques de biologie synthétique pour créer des micro-environnements qui simulent les conditions de cisaillement fluïdique et de contraintes mécaniques observées dans le cerveau des patients atteints de la maladie d'Alzheimer.
3. Évaluer l'effet de ces conditions mécaniques sur l'activation des microglies, la phagocytose des synapses saines et l'accumulation de protéines amyloïdes dans les modèles in vitro.

Méthodes :

1. Modèle in vitro de BCSFB : Utiliser des cellules neuronales, des astrocytes et des microglies humaines pour créer un modèle in vitro de BCSFB qui reflète fidèlement la structure et la fonction de la barrière hémato-liquorienne cérébrospinale.
2. Simulation des conditions mécaniques : Utiliser des systèmes de culture cellulaire à flux pour simuler les forces de cisaillement fluïdique et les changements mécaniques dans le microenvironnement des cellules neuronales.
3. ****Analyse de l'activation des microglies et de la phagocytose : **** Utiliser des techniques d'imagerie en fluorescence et des essais biochimiques pour évaluer l'activation des microglies et la phagocytose des synapses saines dans les modèles in vitro.
4. Évaluation de l'accumulation de protéines amyloïdes : Utiliser des techniques d'imagerie en fluorescence et des essais biochimiques pour évaluer l'accumulation de protéines amyloïdes dans les modèles in vitro.

Expériences automatisées : Les expériences seront automatisées en utilisant des systèmes de culture cellulaire à flux et des plateformes de haute throughput pour simuler les conditions mécaniques et évaluer les réponses cellulaires. Cela permettra d'effectuer un grand nombre

d'expériences en parallèle, ce qui accélérera la découverte de nouveaux traitements contre la maladie d'Alzheimer.

Expériences in vivo (invertébrés) : Des expériences in vivo seront menées sur des invertébrés (par exemple, des drosophiles) pour valider les résultats obtenus in vitro. Les drosophiles seront génétiquement modifiées pour exprimer des protéines amyloïdes humaines et leur comportement sera évalué en fonction de l'âge et de la présence de facteurs de risque de la maladie d'Alzheimer.

Conclusion : Cette proposition vise à explorer un nouveau mécanisme dans la progression de la maladie d'Alzheimer en utilisant des modèles in vitro et des expériences automatisées. Les résultats de cette étude pourraient conduire au développement de nouveaux traitements ciblant la mécanotransduction pour prévenir ou ralentir la progression de la maladie d'Alzheimer.

▪ German

Forschungshypothese:

Unsere Forschungshypothese lautet, dass die Interaktion zwischen Mikroglia und Astrozyten in 3D-Organoiden eine entscheidende Rolle bei der Progression der Alzheimer-Krankheit spielt. Wir vermuten, dass die mechanische Belastung durch Flüssigkeits-Scherkräfte auf diese Zellen einen signifikanten Einfluss auf ihre Funktion und Interaktion hat, was wiederum die Bildung von Amyloid-Plaques und die Progression der Krankheit beeinflusst.

Antrag:

Titel: Untersuchung der Rolle von Mikroglia-Astrozyten-Interaktionen in 3D-Organoiden bei der Progression der Alzheimer-Krankheit unter mechanischer Belastung

Ziel: Das Ziel dieses Projekts ist es, die Hypothese zu überprüfen, dass die Interaktion zwischen Mikroglia und Astrozyten in 3D-Organoiden unter mechanischer Belastung durch Flüssigkeits-Scherkräfte eine entscheidende Rolle bei der Progression der Alzheimer-Krankheit spielt.

Methodik:

1. Herstellung von 3D-Organoiden: Wir werden 3D-Organoiden aus humanen induzierten pluripotenten Stammzellen (iPSCs) herstellen, die mit Mikroglia und Astrozyten ko-kultiviert werden.
2. Mechanische Belastung: Wir werden eine Flüssigkeits-Scherkraft auf die 3D-Organoiden anwenden, um die Auswirkungen auf die Mikroglia-Astrozyten-Interaktion zu untersuchen.
3. In-vitro-Experimente: Wir werden automatisierte In-vitro-Experimente durchführen, um die Auswirkungen der mechanischen Belastung auf die Bildung von Amyloid-Plaques und die

Progression der Krankheit zu untersuchen.

4. Analyse: Wir werden die Ergebnisse mittels Immunfluoreszenz, Western-Blot und qRT-PCR analysieren.

In-vivo-Experimente (optional):

Wenn die In-vitro-Experimente positive Ergebnisse liefern, werden wir In-vivo-Experimente mit wirbellosen Tieren (z.B. Drosophila melanogaster) durchführen, um die Rolle von Mikroglia-Astrozyten-Interaktionen in der Progression der Alzheimer-Krankheit unter mechanischer Belastung zu untersuchen.

Erwartete Ergebnisse:

Wir erwarten, dass unsere Studie zeigt, dass die Interaktion zwischen Mikroglia und Astrozyten in 3D-Organoiden unter mechanischer Belastung durch Flüssigkeits-Scherkräfte eine entscheidende Rolle bei der Progression der Alzheimer-Krankheit spielt. Wir erwarten auch, dass unsere Ergebnisse neue Erkenntnisse über die molekularen Mechanismen liefern, die an dieser Interaktion beteiligt sind.

Zeitplan:

- Monat 1-3: Herstellung von 3D-Organoiden und Durchführung von In-vitro-Experimenten
- Monat 4-6: Analyse der Ergebnisse und Durchführung von In-vivo-Experimenten (optional)
- Monat 7-12: Auswertung der Ergebnisse und Erstellung eines wissenschaftlichen Artikels

Ressourcen:

- iPSCs
- 3D-Organoid-Kulturmedien
- Flüssigkeits-Scherkraft-Apparatur
- Immunfluoreszenz-Reagenzien
- Western-Blot-Reagenzien
- qRT-PCR-Reagenzien
- Drosophila melanogaster (optional)

Fazit:

Unsere Studie wird neue Erkenntnisse über die Rolle von Mikroglia-Astrozyten-Interaktionen in der Progression der Alzheimer-Krankheit unter mechanischer Belastung liefern. Wir erwarten, dass unsere Ergebnisse zu neuen Therapieansätzen für die Behandlung der Alzheimer-Krankheit führen werden.

▪ **Hindi**

परिकल्पना: अल्जाइमर रोग की प्रगति में माइक्रोग्लिया कोशिकाओं द्वारा यांत्रिक तनाव (mechanical stress) और तरल शीर्ष तनाव (fluid shear stress) के संयोजन से

उत्पन्न होने वाली एक नए तंत्र का अन्वेषण, जो प्रोटीन के गलत फोल्डिंग और जमावड़े को बढ़ावा देता है, जिससे रोग की गंभीरता में वृद्धि होती है।

प्रस्ताव:

परिचय

अल्जाइमर रोग एक जटिल और अभी तक पूरी तरह से समझा नहीं गया न्यूरोडीजेनेरेटिव विकार है, जिसमें माइक्रोग्लिया कोशिकाओं की भूमिका महत्वपूर्ण मानी जाती है। हमारी परिकल्पना यह है कि यांत्रिक तनाव और तरल शीर्ष तनाव के संयोजन से माइक्रोग्लिया कोशिकाएं अल्जाइमर रोग की प्रगति में महत्वपूर्ण भूमिका निभाती हैं। इस प्रस्ताव में, हम इन विद्रो और अकशेरुकी जीवों पर आधारित प्रयोगों के माध्यम से इस तंत्र का अन्वेषण करने का प्रस्ताव रखते हैं।

उद्देश्य

1. माइक्रोग्लिया कोशिकाओं पर यांत्रिक तनाव और तरल शीर्ष तनाव के प्रभावों का अध्ययन करना।
2. अल्जाइमर रोग की प्रगति में माइक्रोग्लिया द्वारा उत्पन्न प्रोटीन के गलत फोल्डिंग और जमावड़े की भूमिका का मूल्यांकन करना।
3. अकशेरुकी जीवों में अल्जाइमर रोग के मॉडल का विकास और इन जीवों पर यांत्रिक तनाव और तरल शीर्ष तनाव के प्रभावों का अध्ययन करना।

पद्धति

- इन विद्रो प्रयोग: माइक्रोग्लिया कोशिकाओं को यांत्रिक तनाव और तरल शीर्ष तनाव के विभिन्न स्तरों के साथ इलाज करें और उनकी प्रतिक्रियाओं का अध्ययन करें। इसमें प्रोटीन एक्सप्रेसन, सेल मॉर्फोलॉजी, और सेल व्यवहार पर ध्यान केंद्रित किया जाएगा।
- अकशेरुकी जीव प्रयोग: अकशेरुकी जीवों (जैसे कि झोसोफिला) में अल्जाइमर रोग के मॉडल का विकास करें और यांत्रिक तनाव और तरल शीर्ष तनाव के प्रभावों का अध्ययन करें। इसमें जीवों की आयु, व्यवहार, और न्यूरोडीजेनेरेशन पर ध्यान केंद्रित किया जाएगा।
- स्वचालित प्रयोग: इन विद्रो और अकशेरुकी जीव प्रयोगों में स्वचालित माइक्रोस्कोपी, फ्लो साइटोमेट्री, और इमेज एनालिसिस जैसी तकनीकों का उपयोग करें ताकि डेटा संग्रह और विश्लेषण में तेजी और सटीकता लाई जा सके।

अपेक्षित परिणाम

हम उम्मीद करते हैं कि यांत्रिक तनाव और तरल शीर्ष तनाव के संयोजन से माइक्रोग्लिया कोशिकाओं में प्रोटीन के गलत फोल्डिंग और जमावड़े में वृद्धि होगी, जो अल्जाइमर रोग की प्रगति में योगदान देता है। अकशेरुकी जीवों में, हम उम्मीद करते हैं कि यांत्रिक तनाव और तरल शीर्ष तनाव के संयोजन से न्यूरोडीजेनेरेशन में वृद्धि होगी और जीवों की आयु और व्यवहार पर नकारात्मक प्रभाव पड़ेगा।

निष्कर्ष

यह प्रस्ताव अल्जाइमर रोग की प्रगति में एक नए तंत्र का अन्वेषण करने के लिए एक महत्वपूर्ण कदम है। हमारे परिणाम इस बीमारी के उपचार और रोकथाम के लिए नए दृष्टिकोण प्रदान कर सकते हैं।

▪ Italian

Titolo della Proposta di Ricerca: "Esplorazione del Ruolo della Meccanotrasduzione nella Progressione della Malattia di Alzheimer: Un Approccio Innovativo In Vitro"

Introduzione: La malattia di Alzheimer (AD) è una patologia neurodegenerativa complessa caratterizzata dalla presenza di placche senili e grovigli neurofibrillari nel cervello. Nonostante gli sforzi della comunità scientifica, le terapie attualmente disponibili offrono solo benefici sintomatici modesti. Recentemente, è emersa l'importanza della meccanotrasduzione cellulare nella regolazione di vari processi biologici, inclusa la risposta a stress meccanici e la modulazione dell'ambiente extracellulare. Questa proposta di ricerca si concentra sull'esplorazione del ruolo della meccanotrasduzione nella progressione della AD, con particolare attenzione alla sua interazione con la fluid shear stress (FSS) e il processo di fagocitosi da parte dei microglia.

Ipotesi di Ricerca: L'ipotesi è che la meccanotrasduzione cellulare giochi un ruolo cruciale nella regolazione della risposta dei microglia alla presenza di beta-amiloide (A β) e tau patologici, influenzando così la progressione della malattia di Alzheimer. Inoltre, si ipotizza che la FSS possa modulare l'attività fagocitaria dei microglia e la loro capacità di rimuovere le placche senili.

Obiettivi:

1. Sviluppo di un Modello In Vitro: Creazione di un modello in vitro che riproduca le condizioni della malattia di Alzheimer, utilizzando cellule di microglia umane e beta-amiloide (A β) aggregato.
2. Studio dell'Effetto della Meccanotrasduzione: Valutazione dell'impatto della meccanotrasduzione cellulare sulla risposta dei microglia all'A β , utilizzando tecniche di stretching cellulare e misurazione delle forze meccaniche.
3. Analisi della Fagocitosi: Studio dell'effetto della FSS sulla fagocitosi dell'A β da parte dei microglia, utilizzando un sistema di flusso controllato.
4. **Verifica dell'Ipotesi In Vivo (su animali invertebrati):** Utilizzo di *Drosophila melanogaster* come modello in vivo per verificare i risultati ottenuti in vitro e valutare l'impatto della meccanotrasduzione sulla progressione della

malattia.

Metodologia:

- Sviluppo del Modello In Vitro: Cellule di microglia umane saranno coltivate su substrati elastici per permettere lo stretching cellulare. L'A β aggregato sar  aggiunto alla coltura per indurre una risposta simile a quella osservata nella malattia di Alzheimer.
- Studio della Meccanotrasduzione: Le cellule saranno sottoposte a diverse condizioni di stretching e le risposte cellulari (es. attivazione di geni, produzione di citochine) saranno valutate.
- Analisi della Fagocitosi: Un sistema di flusso controllato sar  utilizzato per applicare diverse intensit  di FSS alle cellule di microglia mentre fagocitano l'A β . La quantit  di A β internalizzata sar  misurata tramite tecniche di imaging e biochimiche.
- Esperimenti In Vivo su *Drosophila melanogaster*: Saranno generati ceppi transgenici di *Drosophila* che esprimono proteine umane associate alla AD (ad es. A β , tau). La meccanotrasduzione cellulare sar  manipolata utilizzando RNA interference o over-espressione di geni coinvolti nella via della meccanotrasduzione. L'impatto sulla sopravvivenza e sul comportamento degli insetti sar  valutato.

Risultati Attesi: Si prevede che la meccanotrasduzione cellulare influenzi significativamente la risposta dei microglia all'A β , modulando l'attivit  fagocitaria e la produzione di citochine. Inoltre, si ipotizza che la FSS possa aumentare l'efficienza della fagocitosi dell'A β . Gli esperimenti in vivo su *Drosophila melanogaster* confermeranno il ruolo della meccanotrasduzione nella progressione della malattia di Alzheimer.

Conclusione: Questa proposta di ricerca innovativa si concentra sull'esplorazione del ruolo della meccanotrasduzione cellulare nella progressione della malattia di Alzheimer, utilizzando un approccio multidisciplinare che combina tecniche in vitro e in vivo su animali invertebrati. I risultati di questo studio potrebbero fornire nuove informazioni sulla patogenesi della AD e identificare potenziali bersagli terapeutici per il trattamento di questa malattia devastante.

▪ **Portuguese**

T tulo da Proposta: Explorando o Papel da Mecanotransdu o na Progress o da Doen a de Alzheimer: Um Estudo In Vitro com Microglia e Modelos de Biomecatr nica

Introdu o: A doen a de Alzheimer (DA)   uma condi o neurodegenerativa complexa, caracterizada pela perda progressiva de mem ria e fun o cognitiva. Embora os mecanismos subjacentes   DA sejam multifacetados, a compreens o da intera o entre as c lulas imunol gicas

do cérebro, como os microglia, e o ambiente mecânico cerebral é limitada. Esta proposta visa investigar o papel da mecanotransdução na progressão da DA, utilizando modelos in vitro de microglia e plataformas biomecatrônicas.

Hipótese: Nossa hipótese é que a alteração na mecanotransdução dos microglia, induzida por mudanças no estresse de cisalhamento fluido, influencia a fagocitose de príons e contribui para a progressão da DA.

Objetivos:

1. Desenvolver um modelo in vitro de microglia que mimetize as condições de mecanotransdução encontradas no cérebro.
2. Investigar o efeito do estresse de cisalhamento fluido na fagocitose de príons pelos microglia.
3. Utilizar plataformas biomecatrônicas para automatizar os experimentos e analisar a relação entre a mecanotransdução e a progressão da DA.

Metodologia:

1. Desenvolvimento do modelo in vitro: Cultivar microglia em uma plataforma de cultura de células que permita a aplicação controlada de estresse de cisalhamento fluido.
2. Análise da fagocitose de príons: Utilizar técnicas de microscopia de fluorescência para quantificar a internalização de príons pelos microglia em diferentes condições de estresse de cisalhamento fluido.
3. Plataformas biomecatrônicas: Desenvolver uma plataforma biomecatrônica que permita a aplicação controlada de estresse de cisalhamento fluido e a análise da resposta dos microglia em tempo real.

Experimentos Propostos:

1. ****Estudo da relação entre o estresse de cisalhamento fluido e a fagocitose de príons:**** Aplicar diferentes níveis de estresse de cisalhamento fluido aos microglia e quantificar a internalização de príons.
2. ****Análise da expressão genética dos microglia em resposta ao estresse de cisalhamento fluido:**** Utilizar técnicas de biologia molecular para analisar a expressão de genes relacionados à fagocitose e à mecanotransdução nos microglia.
3. ****Desenvolvimento de um modelo in silico da mecanotransdução dos microglia:**** Utilizar dados experimentais para desenvolver um modelo matemático que simule a resposta dos microglia ao estresse de cisalhamento fluido.

Conclusão: Esta proposta visa explorar um novo mecanismo na progressão da doença de Alzheimer, relacionado à mecanotransdução dos

microglia. Os experimentos in vitro propostos utilizarão modelos de microglia e plataformas biomecatrônicas para investigar a relação entre o estresse de cisalhamento fluido e a fagocitose de príons. Os resultados deste estudo podem contribuir para uma melhor compreensão da DA e levar ao desenvolvimento de novas estratégias terapêuticas.

▪ Thai

ข้อเสนอโครงการ:

การสำรวจกลไกใหม่ในการดำเนินไปของโรคอัลไซเมอร์โดยใช้การทดลองในหลอดทดลอง (in vitro) และสัตว์ไม่มีกระดูกสันหลัง

สมมติฐานการวิจัย: เราเสนอว่าความผิดปกติของระบบ P2X ในเซลล์ microglia

มีบทบาทสำคัญในการดำเนินไปของโรคอัลไซเมอร์

โดยเฉพาะอย่างยิ่งในการควบคุมการทำงานของฟลูอิด ชาร์ สเตรส (fluid shear stress)

ที่มีต่อการเปลี่ยนแปลงของโปรตีน prion และการเกิดโรค

วัตถุประสงค์ของโครงการ:

1. เพื่อศึกษาการเปลี่ยนแปลงของระบบ P2X ในเซลล์ microglia ภายใต้การกระตุ้นของฟลูอิด ชาร์ สเตรส
2. เพื่อตรวจสอบผลกระทบของการเปลี่ยนแปลงของระบบ P2X ต่อการทำงานของโปรตีน prion และการเกิดโรคอัลไซเมอร์
3. เพื่อประเมินผลกระทบของสารเคมีที่มีศักยภาพในการรักษาโรคอัลไซเมอร์โดยการควบคุมการทำงานของระบบ P2X

วิธีการทดลอง:

1. การเตรียมเซลล์ microglia จากสัตว์ไม่มีกระดูกสันหลัง (เช่น ปลัดขาน) และการเพาะเลี้ยงในหลอดทดลอง
2. การใช้ฟลูอิด ชาร์ สเตรสในการกระตุ้นเซลล์ microglia และการตรวจสอบการเปลี่ยนแปลงของระบบ P2X โดยใช้เทคนิคการวัดค่า Ca^{2+} ในเซลล์
3. การเพิ่มโปรตีน prion ลงในหลอดทดลองและตรวจสอบผลกระทบต่อการทำงานของโปรตีน prion และการเกิดโรคอัลไซเมอร์
4. การทดสอบสารเคมีที่มีศักยภาพในการรักษาโรคอัลไซเมอร์โดยการควบคุมการทำงานของระบบ P2X

การทดลองในหลอดทดลอง (in vitro):

- การเตรียมเซลล์ microglia และการเพาะเลี้ยงในหลอดทดลอง
- การใช้ฟลูอิด ชาร์ สเตรสในการกระตุ้นเซลล์ microglia
- การตรวจสอบการเปลี่ยนแปลงของระบบ P2X โดยใช้เทคนิคการวัดค่า Ca^{2+} ในเซลล์
- การเพิ่มโปรตีน prion ลงในหลอดทดลองและตรวจสอบผลกระทบต่อการทำงานของโปรตีน prion และการเกิดโรคอัลไซเมอร์

การทดลองในสัตว์ไม่มีกระดูกสันหลัง (in vivo):

- การใช้สัตว์ไม่มีกระดูกสันหลัง (เช่น ปลัดขาน)

ในการทดสอบผลกระทบของสารเคมีที่มีศักยภาพในการรักษาโรคอัลไซเมอร์

- การตรวจสอบผลกระทบต่อการทำงานของโปรตีน prion

และการเกิดโรคอัลไซเมอร์ในสัตว์ไม่มีกระดูกสันหลัง

ระยะเวลาการทดลอง: 12 เดือน

งบประมาณ: 1,000,000 บาท

ทีมวิจัย:

- ผู้นำโครงการ: [ชื่อผู้นำโครงการ]
- ผู้ช่วยวิจัย: [ชื่อผู้ช่วยวิจัย]
- นักวิทยาศาสตร์: [ชื่อนักวิทยาศาสตร์]

การประเมินผล: การประเมินผลจะดำเนินการโดยการตรวจสอบการเปลี่ยนแปลงของระบบ

P2X ในเซลล์ microglia และผลกระทบต่อการทำงานของโปรตีน prion

และการเกิดโรคอัลไซเมอร์ โดยใช้เทคนิคการวัดค่า Ca^{2+}

ในเซลล์และการตรวจสอบผลกระทบต่อการทำงานของโปรตีน prion

และการเกิดโรคอัลไซเมอร์ในสัตว์ไม่มีกระดูกสันหลัง

▪ English control (QueryID-001)

Research Hypothesis:

We propose that mechanical stress-induced changes in microglial morphology and function contribute to the progression of Alzheimer's disease by enhancing the uptake and propagation of tau protein aggregates, thereby exacerbating neurodegeneration.

Background: Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of amyloid- β ($A\beta$) plaques and tau protein aggregates in the brain. Recent studies have highlighted the importance of microglia, the resident immune cells of the brain, in AD pathogenesis. Microglia play a crucial role in clearing $A\beta$ plaques and regulating neuroinflammation. However, their function is impaired in AD, leading to increased $A\beta$ accumulation and neurodegeneration.

Proposal:

Title: Investigating the Role of Mechanical Stress-Induced Microglial Dysfunction in Alzheimer's Disease Progression

Objectives:

1. To develop an in vitro model of mechanical stress-induced microglial dysfunction using microfluidic devices and human iPSC-derived microglia.
2. To investigate the effects of mechanical stress on microglial morphology, function, and tau protein uptake.
3. To examine the role of microglial dysfunction in enhancing tau protein aggregate propagation and neurodegeneration.

Experimental Design:

1. In vitro model development: Human iPSC-derived microglia will be cultured in microfluidic devices with controlled mechanical stress conditions (e.g., fluid flow, pressure). Microglial morphology and function will be assessed using live-cell imaging, flow cytometry, and biochemical assays.
2. Mechanical stress-induced microglial dysfunction: Microglia will be exposed to varying levels of mechanical stress, and their response will be evaluated in terms of changes in morphology, cytokine production, and phagocytic activity.
3. Tau protein uptake and propagation: Microglia will be co-cultured with tau-expressing neurons, and the effects of mechanical stress on microglial tau uptake and propagation will be investigated using live-cell imaging and biochemical assays.
4. In vivo validation (optional): If necessary, invertebrate animals (e.g., *C. elegans*) will be used to validate the findings from in vitro experiments.

Automated Experiments:

1. Microfluidic devices will be designed to automate the application of mechanical stress and the collection of data on microglial morphology and function.
2. High-content imaging and analysis software will be used to quantify changes in microglial morphology and tau protein uptake.
3. Automated flow cytometry and biochemical assays will be employed to assess microglial function and cytokine production.

Expected Outcomes:

1. Mechanical stress will induce changes in microglial morphology and function, leading to enhanced tau protein uptake and propagation.
2. Microglial dysfunction will contribute to increased neurodegeneration and AD-like pathology in vitro.
3. The findings from this study will provide new insights into the mechanisms underlying AD progression and identify potential therapeutic targets for the treatment of AD.

Timeline:

- Month 1-3: Development of in vitro model and optimization of experimental conditions
- Month 4-6: Investigation of mechanical stress-induced microglial dysfunction and tau protein uptake
- Month 7-9: Examination of the role of microglial dysfunction in enhancing tau protein aggregate propagation and neurodegeneration
- Month 10: Data analysis, manuscript preparation, and submission

Personnel:

- Principal Investigator: [Name]

- Postdoctoral Researcher: [Name]
- Graduate Student: [Name]
- Technician: [Name]

Resources:

- Microfluidic devices and equipment
- Human iPSC-derived microglia and tau-expressing neurons
- Live-cell imaging and high-content analysis software
- Flow cytometry and biochemical assay equipment
- Invertebrate animals (if necessary)

This proposal outlines a novel research hypothesis that explores the role of mechanical stress-induced microglial dysfunction in AD progression. The proposed experiments will focus on in vitro studies using automated microfluidic devices and human iPSC-derived microglia, with optional in vivo validation using invertebrate animals. The expected outcomes will provide new insights into the mechanisms underlying AD progression and identify potential therapeutic targets for the treatment of AD.

▪ **English control (chain-of-thought)**

Research Hypothesis:

Based on the provided contexts, it is clear that Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by the accumulation of amyloid- β ($A\beta$) plaques and tau protein aggregates. Recent advances in microphysiological systems (MPS) have enabled the development of novel in vitro models that can replicate AD pathophysiology. Our hypothesis proposes that mechanical stress, specifically fluid shear stress, plays a crucial role in the progression of AD by modulating microglial activation and phagocytosis, leading to the accumulation of $A\beta$ plaques.

Proposal:

Title: Investigating the Role of Fluid Shear Stress on Microglial Activation and $A\beta$ Phagocytosis in Alzheimer's Disease

Introduction: Alzheimer's disease is a devastating neurodegenerative disorder affecting millions worldwide. Despite significant research efforts, the underlying mechanisms of AD progression remain poorly understood. Recent studies have highlighted the importance of microglial activation and phagocytosis in clearing $A\beta$ plaques. However, the impact of mechanical stress on these processes remains unexplored. This proposal aims to investigate the effect of fluid shear stress on microglial activation and $A\beta$ phagocytosis using novel in vitro MPS models.

Objectives:

1. Develop a microfluidic-based MPS model that recapitulates the AD brain environment, including microglia, neurons, and A β plaques.
2. Investigate the effect of fluid shear stress on microglial activation and phagocytosis of A β plaques using automated imaging and analysis techniques.
3. Examine the role of mechanotransduction pathways in modulating microglial responses to fluid shear stress.

Methods:

1. Develop a microfluidic device with a brain-on-a-chip design, incorporating microglia, neurons, and A β plaques.
2. Apply controlled fluid shear stress to the microfluidic device using a syringe pump system.
3. Use automated imaging techniques (e.g., live-cell microscopy) to monitor microglial activation and phagocytosis of A β plaques in response to fluid shear stress.
4. Investigate the role of mechanotransduction pathways (e.g., PIEZO1, TRPV4) in modulating microglial responses to fluid shear stress using RNA interference or pharmacological inhibitors.

Expected Outcomes:

- Fluid shear stress will enhance microglial activation and phagocytosis of A β plaques.
- Mechanotransduction pathways will play a crucial role in modulating microglial responses to fluid shear stress.

Impact: This study will provide novel insights into the mechanisms underlying AD progression, highlighting the importance of mechanical stress in modulating microglial function. The findings will have significant implications for the development of therapeutic strategies targeting microglial activation and phagocytosis in AD.

Timeline:

- Month 1-3: Develop microfluidic device and optimize experimental conditions.
- Month 4-6: Conduct experiments investigating the effect of fluid shear stress on microglial activation and A β phagocytosis.
- Month 7-9: Analyze data and prepare manuscript for publication.

In vivo experiments using invertebrate animals (e.g., *C. elegans*) may be considered in future studies to validate the findings and explore the conservation of mechanotransduction pathways across species. However, this proposal focuses on in vitro experiments that can be automated, providing a high-throughput and cost-effective approach to investigating the role of fluid shear stress in AD progression.

Supplementary Data D4. English back-translations of the foreign-language proposals.

English renderings of the seven foreign-language proposals in D3, produced by Claude Opus 4.8. All content coding (Table S2) and linguistic metrics (Table S3) were computed on these back-translations together with the five native English proposals.

▪ Spanish (back-translation)

Research Hypothesis:

Alzheimer's disease (AD) has been observed to be characterized by an alteration in the function of microglia, the brain's immune cells, which leads to an abnormal inflammatory response and the accumulation of amyloid plaques. However, the role of cellular mechanics and force transduction in the progression of AD is still poorly understood. Our hypothesis is that mechanical forces exerted on microglia can alter their function and contribute to the progression of AD.

Research Proposal:

Title: "Effect of mechanical forces on microglial function and the progression of Alzheimer's disease"

Objectives:

1. Investigate how mechanical forces affect microglial function in in vitro culture.
2. Evaluate the effect of mechanical forces on the expression of genes related to inflammation and phagocytosis in microglia.
3. Develop an in vitro model that simulates the progression of AD and assess the impact of mechanical forces on the accumulation of amyloid plaques.

Methodology:

1. Microglial culture: Human or murine microglia will be cultured in a standard culture medium.
2. Application of mechanical forces: A biomechanics device will be used to apply controlled mechanical forces to the microglia. The forces will be applied over different time periods and at different magnitudes.
3. Analysis of microglial function: Microglial function will be evaluated using flow cytometry, ELISA, and qRT-PCR techniques to determine the expression of genes related to inflammation and phagocytosis.
4. In vitro AD model: An in vitro model simulating the progression of AD will be developed using human or murine microglia and neurons. The model will be based on the co-culture of microglia and neurons in a culture medium containing amyloid plaques.
5. Assessment of the impact of mechanical forces: The impact of

mechanical forces on the accumulation of amyloid plaques and microglial function will be evaluated in the in vitro model.

Automated Experiments:

- An automated culture system will be used to maintain optimal culture conditions and minimize variability.
- An automated biomechanics device will be employed to apply controlled mechanical forces to the microglia.
- Automated flow cytometry and ELISA techniques will be used to evaluate microglial function.

In Vivo Experiments (optional):

- If deemed necessary, in vivo experiments could be carried out using invertebrate animals (such as *Drosophila melanogaster*) to evaluate the impact of mechanical forces on the progression of AD. However, this will depend on the results obtained in the in vitro experiments and on the availability of resources.

Time and Resources:

- The project will be carried out over 12 months.
- Resources will be needed for the acquisition of biomechanics equipment, cell culture, and data analysis.
- Personnel trained in cell culture, biomechanics, and data analysis techniques will be required.

Conclusion:

The proposal presented seeks to investigate the effect of mechanical forces on microglial function and the progression of Alzheimer's disease. The automated in vitro experiments will allow for the assessment of the impact of mechanical forces on microglial function and the accumulation of amyloid plaques. If deemed necessary, in vivo experiments using invertebrate animals could be carried out to evaluate the impact of mechanical forces on the progression of AD. The results obtained could contribute to a better understanding of the disease and to the development of new therapies for its treatment.

▪ **French (back-translation)**

Proposal Title: Exploring the Role of Mechanotransduction in the Progression of Alzheimer's Disease via In Vitro Models and Automated Experiments

Introduction: Alzheimer's disease is a complex neurodegenerative pathology characterized by the accumulation of amyloid plaques and neuronal degeneration. Current research focuses on understanding the mechanisms underlying this disease in order to develop effective treatments. Our novel hypothesis suggests that mechanotransduction, the process by which cells convert mechanical forces into biochemical

signals, plays a key role in the progression of Alzheimer's disease.

Hypothesis: We propose that fluid shear forces and mechanical changes in the microenvironment of neuronal cells contribute to the abnormal activation of microglia, which in turn promotes the phagocytosis of healthy synapses and the accumulation of amyloid proteins. This hypothesis will be tested using in vitro models of the blood-cerebrospinal fluid barrier (BCSFB) and automated experiments to simulate pathological mechanical conditions.

Objectives:

1. Develop an in vitro model of the BCSFB that integrates neuronal cells, astrocytes, and microglia to study the impact of mechanotransduction on the progression of Alzheimer's disease.
2. Use synthetic biology techniques to create micro-environments that simulate the fluid shear and mechanical stress conditions observed in the brains of patients with Alzheimer's disease.
3. Evaluate the effect of these mechanical conditions on microglial activation, the phagocytosis of healthy synapses, and the accumulation of amyloid proteins in the in vitro models.

Methods:

1. In vitro BCSFB model: Use human neuronal cells, astrocytes, and microglia to create an in vitro model of the BCSFB that faithfully reflects the structure and function of the blood-cerebrospinal fluid barrier.
2. Simulation of mechanical conditions: Use flow-based cell culture systems to simulate the fluid shear forces and mechanical changes in the microenvironment of neuronal cells.
3. Analysis of microglial activation and phagocytosis: Use fluorescence imaging techniques and biochemical assays to evaluate microglial activation and the phagocytosis of healthy synapses in the in vitro models.
4. Assessment of amyloid protein accumulation: Use fluorescence imaging techniques and biochemical assays to evaluate the accumulation of amyloid proteins in the in vitro models.

Automated Experiments: The experiments will be automated using flow-based cell culture systems and high-throughput platforms to simulate the mechanical conditions and evaluate the cellular responses. This will allow a large number of experiments to be carried out in parallel, which will accelerate the discovery of new treatments for Alzheimer's disease.

In Vivo Experiments (invertebrates): In vivo experiments will be conducted on invertebrates (for example, fruit flies) to validate the results obtained in vitro. The fruit flies will be genetically modified

to express human amyloid proteins, and their behavior will be evaluated as a function of age and the presence of Alzheimer's disease risk factors.

Conclusion: This proposal aims to explore a new mechanism in the progression of Alzheimer's disease using in vitro models and automated experiments. The results of this study could lead to the development of new treatments targeting mechanotransduction to prevent or slow the progression of Alzheimer's disease.

▪ **German (back-translation)**

Research Hypothesis:

Our research hypothesis is that the interaction between microglia and astrocytes in 3D organoids plays a crucial role in the progression of Alzheimer's disease. We hypothesize that the mechanical stress exerted on these cells by fluid shear forces has a significant influence on their function and interaction, which in turn affects the formation of amyloid plaques and the progression of the disease.

Proposal:

Title: Investigation of the Role of Microglia-Astrocyte Interactions in 3D Organoids in the Progression of Alzheimer's Disease Under Mechanical Stress

Aim: The aim of this project is to test the hypothesis that the interaction between microglia and astrocytes in 3D organoids under mechanical stress from fluid shear forces plays a crucial role in the progression of Alzheimer's disease.

Methodology:

1. Production of 3D organoids: We will produce 3D organoids from human induced pluripotent stem cells (iPSCs), co-cultured with microglia and astrocytes.
2. Mechanical stress: We will apply a fluid shear force to the 3D organoids in order to investigate the effects on the microglia-astrocyte interaction.
3. In vitro experiments: We will conduct automated in vitro experiments to investigate the effects of mechanical stress on the formation of amyloid plaques and the progression of the disease.
4. Analysis: We will analyze the results using immunofluorescence, Western blot, and qRT-PCR.

In vivo experiments (optional):

If the in vitro experiments yield positive results, we will conduct in vivo experiments with invertebrate animals (e.g., *Drosophila melanogaster*) to investigate the role of microglia-astrocyte interactions in the progression of Alzheimer's disease under mechanical

stress.

Expected Results:

We expect our study to show that the interaction between microglia and astrocytes in 3D organoids under mechanical stress from fluid shear forces plays a crucial role in the progression of Alzheimer's disease.

We also expect our results to provide new insights into the molecular mechanisms involved in this interaction.

Timeline:

- Months 1-3: Production of 3D organoids and execution of in vitro experiments
- Months 4-6: Analysis of the results and execution of in vivo experiments (optional)
- Months 7-12: Evaluation of the results and preparation of a scientific article

Resources:

- iPSCs
- 3D organoid culture media
- Fluid shear force apparatus
- Immunofluorescence reagents
- Western blot reagents
- qRT-PCR reagents
- Drosophila melanogaster (optional)

Conclusion:

Our study will provide new insights into the role of microglia-astrocyte interactions in the progression of Alzheimer's disease under mechanical stress. We expect our results to lead to new therapeutic approaches for the treatment of Alzheimer's disease.

▪ **Hindi (back-translation)**

Hypothesis: Exploration of a novel mechanism in the progression of Alzheimer's disease arising from the combination of mechanical stress and fluid shear stress in microglial cells, which promotes the misfolding and aggregation of proteins, thereby leading to an increase in disease severity.

Proposal:

Introduction

Alzheimer's disease is a complex and not yet fully understood neurodegenerative disorder in which the role of microglial cells is considered significant. Our hypothesis is that, through the combination of mechanical stress and fluid shear stress, microglial cells play an important role in the progression of Alzheimer's disease. In this proposal, we propose to explore this mechanism through experiments based

on in vitro and invertebrate animal models.

Objectives

1. To study the effects of mechanical stress and fluid shear stress on microglial cells.
2. To evaluate the role of protein misfolding and aggregation produced by microglia in the progression of Alzheimer's disease.
3. To develop a model of Alzheimer's disease in invertebrate animals and to study the effects of mechanical stress and fluid shear stress on these organisms.

Methodology

- In vitro experiments: Treat microglial cells with various levels of mechanical stress and fluid shear stress and study their responses. This will focus on protein expression, cell morphology, and cell behavior.
- Invertebrate animal experiments: Develop a model of Alzheimer's disease in invertebrate animals (such as *Drosophila*) and study the effects of mechanical stress and fluid shear stress. This will focus on the lifespan, behavior, and neurodegeneration of the organisms.
- Automated experiments: Use techniques such as automated microscopy, flow cytometry, and image analysis in the in vitro and invertebrate animal experiments in order to bring speed and accuracy to data collection and analysis.

Expected Results

We expect that the combination of mechanical stress and fluid shear stress will lead to an increase in protein misfolding and aggregation in microglial cells, which contributes to the progression of Alzheimer's disease. In invertebrate animals, we expect that the combination of mechanical stress and fluid shear stress will lead to an increase in neurodegeneration and will have a negative impact on the lifespan and behavior of the organisms.

Conclusion

This proposal is an important step toward exploring a novel mechanism in the progression of Alzheimer's disease. Our results may provide new approaches for the treatment and prevention of this disease.

▪ Italian (back-translation)

Research Proposal Title: "Exploring the Role of Mechanotransduction in the Progression of Alzheimer's Disease: An Innovative In Vitro Approach"

Introduction: Alzheimer's disease (AD) is a complex neurodegenerative pathology characterized by the presence of senile plaques and neurofibrillary tangles in the brain. Despite the efforts of

the scientific community, the currently available therapies offer only modest symptomatic benefits. Recently, the importance of cellular mechanotransduction in the regulation of various biological processes has emerged, including the response to mechanical stress and the modulation of the extracellular environment. This research proposal focuses on exploring the role of mechanotransduction in the progression of AD, with particular attention to its interaction with fluid shear stress (FSS) and the process of phagocytosis by microglia.

Research Hypothesis: The hypothesis is that cellular mechanotransduction plays a crucial role in regulating the response of microglia to the presence of pathological beta-amyloid (A β) and tau, thereby influencing the progression of Alzheimer's disease. Furthermore, it is hypothesized that FSS may modulate the phagocytic activity of microglia and their ability to remove senile plaques.

Objectives:

1. Development of an In Vitro Model: Creation of an in vitro model that reproduces the conditions of Alzheimer's disease, using human microglial cells and aggregated beta-amyloid (A β).
2. Study of the Effect of Mechanotransduction: Evaluation of the impact of cellular mechanotransduction on the response of microglia to A β , using cell stretching techniques and measurement of mechanical forces.
3. Analysis of Phagocytosis: Study of the effect of FSS on the phagocytosis of A β by microglia, using a controlled flow system.
4. ****Verification of the Hypothesis In Vivo (on invertebrate animals):**** Use of *Drosophila melanogaster* as an in vivo model to verify the results obtained in vitro and to evaluate the impact of mechanotransduction on the progression of the disease.

Methodology:

- Development of the In Vitro Model: Human microglial cells will be cultured on elastic substrates to allow for cell stretching. Aggregated A β will be added to the culture to induce a response similar to that observed in Alzheimer's disease.
- Study of Mechanotransduction: The cells will be subjected to various stretching conditions, and the cellular responses (e.g., gene activation, cytokine production) will be evaluated.
- Analysis of Phagocytosis: A controlled flow system will be used to apply various intensities of FSS to the microglial cells while they phagocytose A β . The amount of internalized A β will be measured through imaging and biochemical techniques.
- In Vivo Experiments on *Drosophila melanogaster*: Transgenic strains of *Drosophila* expressing human AD-associated proteins

(e.g., A β , tau) will be generated. Cellular mechanotransduction will be manipulated using RNA interference or overexpression of genes involved in the mechanotransduction pathway. The impact on the survival and behavior of the insects will be evaluated.

Expected Results: It is anticipated that cellular mechanotransduction will significantly influence the response of microglia to A β , modulating phagocytic activity and cytokine production. Furthermore, it is hypothesized that FSS may increase the efficiency of A β phagocytosis. The in vivo experiments on *Drosophila melanogaster* will confirm the role of mechanotransduction in the progression of Alzheimer's disease.

Conclusion: This innovative research proposal focuses on exploring the role of cellular mechanotransduction in the progression of Alzheimer's disease, using a multidisciplinary approach that combines in vitro and in vivo techniques on invertebrate animals. The results of this study could provide new information on the pathogenesis of AD and identify potential therapeutic targets for the treatment of this devastating disease.

▪ Portuguese (back-translation)

Proposal Title: Exploring the Role of Mechanotransduction in the Progression of Alzheimer's Disease: An In Vitro Study with Microglia and Biomechatronic Models

Introduction: Alzheimer's disease (AD) is a complex neurodegenerative condition characterized by the progressive loss of memory and cognitive function. Although the mechanisms underlying AD are multifaceted, the understanding of the interaction between the brain's immune cells, such as microglia, and the cerebral mechanical environment is limited. This proposal aims to investigate the role of mechanotransduction in the progression of AD, using in vitro models of microglia and biomechatronic platforms.

Hypothesis: Our hypothesis is that the alteration in the mechanotransduction of microglia, induced by changes in fluid shear stress, influences the phagocytosis of prions and contributes to the progression of AD.

Objectives:

1. Develop an in vitro model of microglia that mimics the mechanotransduction conditions found in the brain.
2. Investigate the effect of fluid shear stress on the phagocytosis of prions by microglia.
3. Use biomechatronic platforms to automate the experiments and analyze the relationship between mechanotransduction and the progression of

AD.

Methodology:

1. Development of the in vitro model: Culture microglia on a cell culture platform that allows for the controlled application of fluid shear stress.
2. Analysis of prion phagocytosis: Use fluorescence microscopy techniques to quantify the internalization of prions by microglia under different fluid shear stress conditions.
3. Biomechatronic platforms: Develop a biomechatronic platform that allows for the controlled application of fluid shear stress and the analysis of the microglial response in real time.

Proposed Experiments:

1. Study of the relationship between fluid shear stress and prion phagocytosis: Apply different levels of fluid shear stress to the microglia and quantify the internalization of prions.
2. Analysis of microglial gene expression in response to fluid shear stress: Use molecular biology techniques to analyze the expression of genes related to phagocytosis and mechanotransduction in the microglia.
3. Development of an in silico model of microglial mechanotransduction: Use experimental data to develop a mathematical model that simulates the response of microglia to fluid shear stress.

Conclusion: This proposal aims to explore a new mechanism in the progression of Alzheimer's disease, related to the mechanotransduction of microglia. The proposed in vitro experiments will use microglial models and biomechatronic platforms to investigate the relationship between fluid shear stress and prion phagocytosis. The results of this study may contribute to a better understanding of AD and lead to the development of new therapeutic strategies.

▪ **Thai (back-translation)**

****Project Proposal: Exploring a Novel Mechanism in the Progression of Alzheimer's Disease Using In Vitro Experiments and Invertebrate Animals****

Research Hypothesis: We propose that dysfunction of the P2X system in microglial cells plays an important role in the progression of Alzheimer's disease, particularly in regulating the effect of fluid shear stress on changes in the prion protein and the development of the disease.

Project Objectives:

1. To study changes in the P2X system in microglial cells under stimulation by fluid shear stress.

2. To examine the impact of changes in the P2X system on prion protein function and the development of Alzheimer's disease.
3. To evaluate the impact of chemical agents with potential for treating Alzheimer's disease by regulating the function of the P2X system.

Experimental Methods:

1. Preparation of microglial cells from invertebrate animals (e.g., leeches) and culturing them in vitro.

Translator's note: The Thai term "ปลัดขิก" is unclear in this context. It does not correspond to a standard name for an invertebrate model organism. I have provisionally rendered it as "leeches," but this should be verified against the author's intent, as the term may be a typo or a colloquial/regional name.

2. Using fluid shear stress to stimulate microglial cells and examining changes in the P2X system using techniques to measure Ca^{2+} levels in the cells.
3. Adding prion protein to the in vitro cultures and examining the impact on prion protein function and the development of Alzheimer's disease.
4. Testing chemical agents with potential for treating Alzheimer's disease by regulating the function of the P2X system.

In Vitro Experiments:

- Preparation of microglial cells and culturing them in vitro.
- Using fluid shear stress to stimulate microglial cells.
- Examining changes in the P2X system using techniques to measure Ca^{2+} levels in the cells.
- Adding prion protein to the in vitro cultures and examining the impact on prion protein function and the development of Alzheimer's disease.

Experiments in Invertebrate Animals (In Vivo):

- Using invertebrate animals (e.g., leeches) to test the impact of chemical agents with potential for treating Alzheimer's disease.
- Examining the impact on prion protein function and the development of Alzheimer's disease in invertebrate animals.

Experiment Duration: 12 months

Budget: 1,000,000 Baht

Research Team:

- Project Leader: [Project Leader's Name]
- Research Assistant: [Research Assistant's Name]
- Scientist: [Scientist's Name]

Evaluation: Evaluation will be carried out by examining changes in the P2X system in microglial cells and their impact on prion protein

function and the development of Alzheimer's disease, using techniques to measure Ca^{2+} levels in the cells and examining the impact on prion protein function and the development of Alzheimer's disease in invertebrate animals.

Supplementary Table S2. Full per-proposal content coding.

Each of the twelve generated proposals coded against the fixed rubric (Methods §2.7). “Distinct papers” is the number of papers contributing to the ten retrieved chunks (cf. Table 1). “In-vivo framing” is categorized as integral aim, optional/contingent, deferred to future work, or absent. Foreign-language proposals were coded from their English back-translations (Supplementary Data D4). † The base English and German proposals stated a single overarching aim rather than an enumerated objective list.

Query	Language	Distinct papers	Objectives	Timeline	Budget	Methods	In-vivo framing	Species	Primary mechanistic focus
Base	English	2	1 †	phased	no	8	integral	<i>C. elegans</i>	Mechanotransduction/shear stress → A β phagocytosis + tau spread
Refresh 1	English	2	3	phased	no	6	optional	<i>C. elegans</i>	Mechanotransduction → A β phagocytosis + cytokines + NFTs
Refresh 2	English	2	3	phased	yes	7	optional	<i>C. elegans</i>	Mechanotransduction → A β phagocytosis + cytokines + NFTs
QueryID-001 prefix	English	2	3	phased	no	6	optional	<i>C. elegans</i>	Mechanical stress → tau uptake / propagation
Chain-of-thought prefix	English	2	3	phased	no	5	future work	<i>C. elegans</i>	Shear stress → microglial activation / A β phagocytosis
Translated	Spanish	3	3	duration	no	7	optional	<i>Drosophila</i>	Mechanical force → microglial function / inflammation
Translated	French	8	3	none	no	6	integral	<i>Drosophila</i>	Shear stress → microglia → synaptic phagocytosis
Translated	German	7	1 †	phased	no	6	optional	<i>Drosophila</i>	Microglia–astrocyte interaction in 3D organoids
Translated	Hindi	7	3	none	no	6	integral	<i>Drosophila</i>	Mechanical + shear stress → protein misfolding/aggregation
Translated	Italian	5	4	none	no	7	integral	<i>Drosophila</i>	Mechanotransduction + shear stress → microglial A β /tau response
Translated	Portuguese	3	3	none	no	6	absent	(none)	Mechanotransduction → prion phagocytosis
Translated	Thai	5	3	duration	yes	5	integral	Leeches	P2X dysfunction → shear stress → prion protein

Supplementary Table S3. Per-proposal linguistic metrics.

Computed on English-language proposal texts (native English outputs and English back-translations of foreign outputs). TTR, type–token ratio; Flesch, Flesch Reading Ease; F–K grade, Flesch–Kincaid grade level. Within-document metrics (TTR, readability) are interpreted with caution for back-translated texts (Methods §2.7); primary diversity claims rest on between-document measures (Table 1, Fig. 4).

Query	Language	Words	Unique words	TTR	Flesch	F–K grade	Sentences	Mean sent. len
Base	English	463	198	0.428	13.9	16.2	25	18.5
Refresh 1	English	509	184	0.361	16.2	16.1	26	19.6
Refresh 2	English	527	195	0.370	16.1	16.4	26	20.3
QueryID-001 prefix	English	547	184	0.336	9.9	17.0	28	19.5
Chain-of-thought prefix	English	438	179	0.409	18.5	15.0	27	16.2
Translated	Spanish	513	161	0.314	26.1	14.6	26	19.7
Translated	French	475	168	0.354	10.5	18.5	18	26.4
Translated	German	378	123	0.325	17.5	18.5	13	29.1
Translated	Hindi	369	124	0.336	24.2	15.8	16	23.1
Translated	Italian	536	202	0.377	19.1	16.1	25	21.4
Translated	Portuguese	388	129	0.332	21.6	16.1	17	22.8
Translated	Thai	409	121	0.296	33.3	13.5	22	18.6
English (mean)	—	496.8	188.0	0.38	14.9	16.1	26.4	18.8
Foreign (mean)	—	438.3	146.9	0.33	21.8	16.2	19.6	23.0