

Arnaud

A Concept Prototype of a Caco-2 Digital Twin Assistant

Chee Ping Ng, Founder
AvatarsBio, The Netherlands

ABSTRACT

Caco-2 assays are widely used to assess intestinal permeability, but interpretation and experimental planning often rely on fragmented data sources and individual experience. Scientists must frequently integrate measured permeability values, assay quality indicators, and biological context before drawing conclusions or designing follow-up experiments.

Here we present **Arnaud**, a concept prototype of a digital twin assistant designed to support permeability reasoning in Caco-2 studies. The prototype combines curated compound data, semi-structured biological notes, and provenance-aware language-model reasoning to generate structured explanations for common permeability questions. By clearly separating measured data, biological context, and model reasoning, the assistant aims to support transparent scientific interpretation and experimental planning.

1. PROBLEM

Although Caco-2 assays are widely used in permeability studies [1-3], the reasoning around compound interpretation and experiment planning remains highly manual. Scientists must often move between measured values, literature knowledge, assay quality metrics, and troubleshooting notes to understand whether a result is meaningful and what should be done next [2,4].

This fragmented workflow can slow down decision making and make interpretation dependent on individual expertise. It also creates a gap between data availability and practical scientific reasoning, particularly in early-stage screening where fast but biologically sensible judgment is needed.

A useful support tool should therefore help organize permeability-related evidence in a way that remains close to the biology of the cell model and the practical questions scientists face during experimental work.

In this work, we present a concept demonstration of an AI-assisted reasoning assistant designed to support interpretation and experiment planning in Caco-2 permeability studies.

2. CONCEPT: A BIOLOGY-FIRST DIGITAL TWIN ASSISTANT

A digital twin assistant can be viewed as a computational companion that helps scientists reason about a biological system using available data, assay knowledge, and structured context [5,6]. In the case of Caco-2 permeability studies, this means organizing compound data, biological understanding of the epithelial barrier, and practical assay considerations into a single interactive reasoning environment.

Rather than attempting to simulate the entire biology of the cell line, the goal is to support scientific interpretation from the perspective of the model system itself. For example, permeability values, transporter behavior, and monolayer integrity all influence how a Caco-2 result should be interpreted. A digital twin assistant can help connect these pieces and present them in a structured explanation that reflects the biology of the assay.

In this approach, different types of information are explicitly separated. Measured data, biological notes, and model-generated reasoning are labeled with clear provenance tags. This allows scientists to see what information comes directly from curated datasets, what comes from biological context, and what is inferred during the reasoning process.

Unlike a general-purpose language model, the assistant operates within a constrained scientific context that integrates curated experimental data and assay-specific knowledge [7]. This grounding helps ensure that explanations remain connected to the biology of the model system rather than relying purely on free-form text generation.

3. PROTOTYPE: ARNAUD LITE

Arnaud Lite is a lightweight prototype designed to explore the concept of a digital twin assistant for Caco-2 permeability reasoning (Figure 1). The system combines a small curated compound dataset, semi-structured biological notes related to Caco-2 assays, and language-model-based reasoning within a simple interactive interface.

The prototype integrates a curated dataset of approximately 100 reference compounds with reported permeability values from literature sources. For each compound, permeability values and related annotations are stored in a structured format. In addition to compound data, the system incorporates local biological notes covering topics such as monolayer formation, cell maintenance, assay quality indicators, and troubleshooting considerations [2,4]. These notes provide contextual knowledge that is commonly used by scientists when interpreting Caco-2 experiments.

To maintain transparency, the assistant separates information sources using explicit provenance tags. Dataset values retrieved directly from curated files are labeled as [DATA], biological notes and assay context are labeled [BIO-LLM], and model-generated reasoning is labeled [LLM]. This simple structure allows users to distinguish measured information from contextual explanations and model interpretation.

The user interface is implemented as a streamlined Quick Insight mode designed for short scientific queries. Users can ask questions about compound permeability, assay interpretation, or experimental considerations. The assistant then retrieves relevant dataset entries or biological notes and generates a structured explanation that combines these sources. The goal is not to provide definitive predictions, but to help scientists organize available evidence and reason through permeability-related questions.

Example use cases

To illustrate the intended use of the prototype, two simple scenarios are presented (Figure 2).

Reference compound interpretation

A user may query the assistant about the permeability of a known compound present in the curated dataset (Figure 2A). The system retrieves the reported permeability value, converts log-scale values to linear units when needed, and classifies the compound into a simple permeability category. The explanation may also include biological considerations such as potential transporter involvement or expected absorption behavior. Provenance tags allow the user to see which elements originate from measured data and which are part of model reasoning.

Assay interpretation and troubleshooting

The assistant can also support questions related to experimental context. For example, a user may ask about the interpretation of low permeability results in relation to monolayer quality or TEER values (Figure 2B). In this case, the system retrieves relevant notes from the biological knowledge files and presents them alongside a structured explanation of how assay quality can influence permeability measurements. This type of interaction reflects common reasoning steps that scientists perform when evaluating experimental outcomes.

These examples illustrate how the prototype focuses on structured reasoning and context integration, rather than purely predictive modeling.

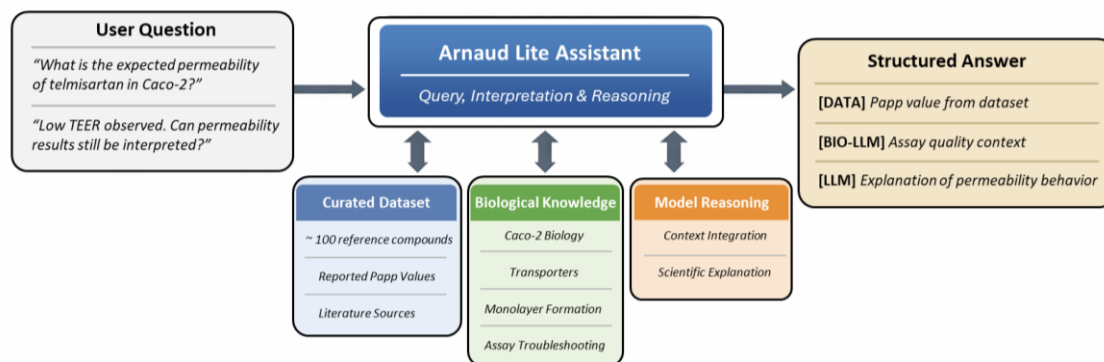


Figure 1. Conceptual workflow of the Arnaud Lite digital twin assistant. A user query about compound permeability or assay interpretation is processed by the assistant, which integrates information from curated permeability datasets and biological knowledge related to the Caco-2 model system. The generated explanation separates measured data, biological context, and model reasoning using explicit provenance tags.

A. Compound permeability reasoning

Prompt:
What is the expected permeability of telmisartan in Caco-2 cells?

Response:
[DATA]
 Compound: telmisartan
 Caco-2 log Papp = -4.82 (Hou et al., 2004)
 Papp = 1.5×10^{-5} cm/s
 Classification: High permeability ($>10 \times 10^{-6}$ cm/s)

[BIO-LLM]
 This value suggests efficient transcellular transport across the Caco-2 monolayer.
 High Papp typically reflects passage through cells rather than paracellular routes.

[LLM]
 The high permeability despite relatively high MW likely reflects strong lipophilicity and low polar surface area, supporting passive diffusion.
 P-gp interaction is not established in this dataset.

B. Assay interpretation

Prompt:
TEER values dropped below the expected threshold. Can permeability results still be interpreted?

Response:
[BIO-LLM]
 Low TEER values indicate reduced monolayer integrity, suggesting a more leaky barrier.
 This can increase paracellular transport and affect measured permeability values.

[BIO-LLM]
 Typical TEER thresholds for Caco-2 monolayers are $\sim 300\text{--}500$ $\Omega\text{-cm}^2$ depending on conditions.

[LLM]
 If TEER is only slightly reduced and control markers are normal, results may still be interpreted with caution.
 However, significant TEER drops can artificially increase Papp values, particularly for low-permeability compounds.

[LLM]
 Under these conditions, quantitative interpretation and transporter-related conclusions may be unreliable.

Figure 2. Example interactions with the Arnaud Lite digital twin assistant demonstrating permeability reasoning and assay interpretation. Illustrative queries show how the assistant integrates curated permeability data, biological context, and model reasoning to generate structured responses. Provenance tags indicate whether information originates from curated datasets ([DATA]), biological notes ([BIO-LLM]), or model-generated reasoning ([LLM]). Responses are abbreviated for presentation.

4. IMPLICATIONS AND FUTURE DIRECTIONS

Better decisions in the lab start before the experiment begins.

Tools such as Arnaud Lite illustrate how digital assistants may help organize experimental reasoning in cell-based research [8]. In permeability studies, scientists often need to integrate compound properties, assay quality indicators, and biological knowledge before deciding how to interpret a result or design the next experiment. A digital twin assistant can help bring these pieces together in a structured explanation while keeping the scientist in control of interpretation.

An important aspect of this approach is transparency. By clearly separating curated data, biological context, and model reasoning, the assistant allows users to understand how an answer is constructed. Such transparency may be particularly valuable as AI-assisted tools become more common in research environments, where the ability to trace the origin of information remains essential.

Future development may expand this prototype toward more comprehensive digital twin systems. Larger curated datasets, predictive modeling approaches, and simulation-based reasoning could allow assistants to explore compound behavior or experimental outcomes in a more dynamic way. For example, more advanced systems (e.g., Arnaud Pro) may incorporate expanded compound datasets and predictive components to support broader permeability analysis and experimental planning.

Although the present prototype focuses on Caco-2 permeability studies, the same reasoning framework could potentially be extended to other cell-based ADMET models. Similar approaches could also support interpretation of permeability measurements from emerging experimental systems such as organ-on-chip or microphysiological models [8]. Assistants built around different experimental systems could help organize knowledge related to metabolism, toxicity, or transporter behavior while maintaining clear links between data, biological context, and interpretation.

More broadly, digital twin assistants could represent a new class of tools designed to support scientific reasoning rather than replace experimental work. By combining curated data, biological context, and interactive explanations, such systems may help researchers explore hypotheses, interpret experimental results, and design follow-up studies in a more efficient, structured, and transparent way.

REFERENCES

1. Artursson P. and Karlsson J. (1991). Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochemical and Biophysical Research Communications*. [https://doi.org/10.1016/0006-291X\(91\)91647-U](https://doi.org/10.1016/0006-291X(91)91647-U)
2. Hubatsch I., Ragnarsson E.G.E. and Artursson P. (2007). Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nature Protocols*. <https://doi.org/10.1038/nprot.2007.303>
3. van Breemen R.B. and Li Y. (2005). Caco-2 cell permeability assays to measure drug absorption. *Expert Opinion on Drug Metabolism & Toxicology*. <https://doi.org/10.1517/17425255.1.2.175>
4. Kus M. et al. (2023). Caco-2 Cell Line Standardization with Pharmaceutical Requirements and In Vitro Model Suitability for Permeability Assays. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics15112523>
5. Alsalloum G.A. et al. (2024). Digital Twins of Biological Systems: A Narrative Review. *IEEE Open Journal of Engineering in Medicine and Biology*. <https://doi.org/10.1109/OJEMB.2024.3426916>
6. Zhang K. et al. (2024). Concepts and applications of digital twins in healthcare and medicine. *Patterns*. <https://doi.org/10.1016/j.patter.2024.101028>

7. Kitano H. (2021). Nobel Turing Challenge: Creating the Engine for Scientific Discovery. NPJ Systems Biology and Applications. <https://doi.org/10.1038/s41540-021-00189-3>
8. Soragni M. et al. (2023). Quantify permeability using on-a-chip models in high-throughput applications. STAR Protocols. <https://doi.org/10.1016/j.xpro.2023.102051>

AvatarsBio | Apr 2026 | info@avatarsbio.com