simula

Kristian Augusts gate 23 | 0164 Oslo | Norway
post@simula.no

simula.no

Oslo, Norway
Aug 6 2025

Dear Reviewers,

We thank the reviewers for their feedback and constructive comments. Our detailed responses follow below/in the subsequent pages. The reviewers' comments are included in italics, while our responses follow in roman text. Our responses are typically split into a Comment (responses to the reviewer) and an Action indicating the specific changes made in the revised manuscript, including indication of line changes. We also refer to the manuscript with changes marked for a version of the manuscript indicating the precise changes made from the previous version (with additions marked, but deletions omitted as per editorial request).

We note that changes in the abstract and title are not included in the changes marked, nor are automated changes in the numbering of the references. The changes in the abstract are:

"[...] Here, we combine mixed-dimensional modelling, multi-modal magnetic resonance images, and high performance computing to construct and explore a high-fidelity in-silico model of human intracranial molecular ~~enrichment~~ transport. [...]
Our findings highlight the significant impact of cerebrospinal fluid (CSF) production and intracranial pulsatility on ~~molecular~~ tracer enrichment following intrathecal tracer injection.
We demonstrate that low-frequency vasomotion induces moderate CSF flow in surface PVS networks which substantially enhances tracer enrichment, and that impaired enrichment is a direct natural consequence of enlarged surface PVSs.[...]"

simula
Kristian Augusts gate 23 | 0164 Oslo | Norway simula.no
post@simula.no

Reviewer #1 (Remarks to the Author):

Summary:
This manuscript presents a computational framework for simulating solute transport in the human intracranial space. It combines a three-dimensional representation of brain tissue and cerebrospinal fluid (CSF) spaces based on magnetic resonance imaging (MRI) data of a healthy volunteer with a one-dimensional model of perivascular networks. The main feature of the framework is an elegant reduction of geometric model complexity and handling of disparate scales in time and space.

General impression:
The manuscript introduces I) a computational framework built on top of an earlier work by the authors, proposes II) a "baseline model accounting for a reasonably conservative set of mechanisms and their integrated effect", and reports on III) explorations of how deviations from that baseline affect simulated tracer distribution. The authors conclude that they have produced a "high-fidelity in-silico model of molecular enrichment and clearance in human intracranial spaces, enabling tailored predictions of the influence of CSF space and (peri)vascular morphology, physiological factors such as cardiac and respiratory pulsatility, as well as of pathological conditions such as enlarged PVSs." They further conclude that their "findings transfer, reconcile, integrate and extend insights from clinical, experimental, and theoretical studies, and lay a new foundation for in-silico studies of personalized intrathecal drug delivery and brain clearance".

The computational framework is a valuable contribution to the field. While it lacks certain elements (see below), it constitutes a large evolutionary step. I commend the authors for making the framework openly available.

Comment: We thank the reviewer for the generous and constructive comments.

Moving from the framework to the baseline model and finally to the interpretation of simulated perturbations, the expectations for biological validation and evidence increase substantially. While it is clear that a model will never include all contributing mechanisms, the claims derived from such a model must nevertheless be supported by evidence. Bold statements such as the model "lay[ing] a new foundation for in-silico studies of personalized intrathecal drug delivery and brain clearance" must be supported by data.

Comment/Action: We appreciate the reviewer's feedback, and note that we have revised this specific sentence in the manuscript, now stating that the findings "provide a framework for future in-silico studies of personalized intrathecal drug delivery and brain clearance."

Changed lines: L53

Because of this combination of goals (I, II, III), the positioning of the manuscript appears somewhat vague. It attempts to straddle the line between a methods paper and a biomedical

paper, thereby underselling the impressive computational framework while placing disproportionate emphasis on the model results relative to their validation.

Methodological limitations:
It is questionable whether using the Stokes equation with dispersion enhancement (Supplementary Methods, S1.3) accurately captures transport processes in the ventricular space. This approach makes sense to me for simpler geometries (as the authors implicitly acknowledge by stating that part of the derivation is based on "the ratio of oscillatory flow to steady flow impedances in a tube"), but not for the ventricles where rather complex and transient flow features can be observed. As a consequence, dispersion enhancement cannot account for changes to transport processes produced by (pathological) processes that impact ventricular CSF dynamics (e.g., venticulomegaly produced by brain atrophy as a result of Alzheimer's disease, or produced by hydrocephalus). Furthermore, ventricular wall motion caused by cardiorespiratory effects is not uniform. The underlying brain motion is not the same for each individual, and changes with age and due to pathologies. Another factor contributing to dispersion are the ependymal cilia that have not been modelled here. What is the error introduced by their absence? Can they be included in the dispersion enhancement model?

Comment: The reviewer is correct that the complex, transient, and pathology-dependent fluid dynamics within the ventricles are not fully captured by our current approach, which uses a dispersion enhancement factor based on a Stokes flow model. This modelling choice represents a necessary simplification to bridge the different timescales of pulsatile CSF flow (seconds) and large-scale molecular transport (hours to days).

We consider that this simplification is a reasonable approximation for the main conclusions of our study. Our baseline simulation shows that after intrathecal injection, tracer enrichment in the ventricular system is minimal, with the bulk of the transport and enrichment occurring within the PVS and SAS. Therefore, the error introduced by a simplified ventricular model is likely small in this context. We do note, however, that our model is capable of predicting ventricular reflux under certain conditions, such as the absence of CSF production (Figure 2O), which underscores the model's sensitivity to key physiological parameters.

We also acknowledge that ependymal cilia are not included in our model. Their action would likely contribute to localised mixing at the ventricular walls. Quantifying the error introduced by their absence is difficult without a more detailed, microscale model of the ventricular boundaries. Conceptually, the effect of cilia could be incorporated into a more advanced local dispersion enhancement model in future work. We also note that one of the authors recently specifically analyzed the role of motile cilia in CSF flow and transport in zebrafish larval brain ventricles[1], and follow-up studies involving the human ventricular system are in preparation.

[1] Herlyng, H., Ellingsrud, A. J., Kuchta, M., Jeong, I., Rognes, M. E., & Jurisch-Yaksi, N. (2025). Advection versus diffusion in brain ventricular transport. bioRxiv, 2025-04.

To better contextualise our results, we have added a comparison to the recent work by Hirschler et al[2]. Their clinical measurements demonstrate that CSF mobility in highly pulsatile regions is approximately tenfold higher than the self-diffusion of water. This observation aligns well with the magnitude of our computed dispersion enhancement factors, providing some clinical validation for our simplified approach.

Action**:** To address the reviewer's comments, we have extended the Discussion to explicitly acknowledge that the model does not capture complex transient flows on shorter time scales (L296-L300). Second, we have added a comparison with regard to the aforementioned findings of Hirschler et al. in the Discussion (L300-L302). Third, we have revised the Methods section to emphasize that the dispersion enhancement factors rely on a simplified model (L373-L374).

Changed lines: L296 - L302, L373 - L374

In regions of the SAS "far" away from the craniocervical junction, the application of dispersion enhancement to capture the effect of pulsations is more intuitive. There, however, the trabecular microstructure, which was not considered in the model, will also play a role: it is likely to influence dispersion and resistance to flow. The latter could be relatively simply accounted for by considering the SAS as porous rather than a free-flow domain, while the former would probably be more difficult to get right with an additional dispersion enhancement model.

At the craniocervical junction, as in the ventricles, modelling effects of pulsatile flow on dispersion by enhancement terms will fall flat. Several companies are in clinical trials with drugs that must be delivered intrathecally. A big problem is the large inter-individual variability of compound concentrations at target locations which appears to be influenced not only by anatomical variations, but also by differences in the translation of posture changes and locomotion to CSF movement. My own experiments with mice show that while ICV delivery of compounds (where cardiorespiratory activity appears to be a much less potent driver of brain motion compared to the situation in humans) leads to reproducible spatial and temporal concentration profiles in the ventricular space, the effects of cisternal delivery vary greatly between genetically identical and phenotypically very similar mice (certainly more similar than any two unrelated humans).

Comment: We thank the reviewer for the insightful comments on the difficulties of modelling the effect of pulsatility via a diffusion enhancement term. We agree that the SAS trabeculae, which are not resolved in our geometric model, may play an important role in influencing both resistance to flow and local dispersion. Modeling the SAS as a porous medium, for instance by using a Brinkman model instead of the free-flow Stokes equations, is indeed an interesting approach to account for the increased flow resistance.

---

[2] Hirschler, L., Runderkamp, B. A., Decker, A., van Harten, T. W., Scheyhing, P., Ehses, P., ... & van Osch, M. J. (2024). Region specific drivers of cerebrospinal fluid mobility as measured by high-resolution non-invasive MRI in humans.

Additionally, the reviewer raises a crucial point regarding the large inter-individual variability observed in clinical and experimental settings, and which we do not address in the manuscript. However, a key strength of our in-silico platform is its inherent suitability for investigating some of the issues raised. The framework is flexible in the sense that we may input other, patient-specific, geometries or key physiological parameters (e.g., cardiac amplitude, CSF production rate), and thus systematically explore a range of anatomical and functional variations. Indeed, ongoing work using multiple different geometries spanning several patient cohorts indicates that anatomical variations alone lead to large differences in simulated flow, dispersion, and subsequent tracer transport, demonstrating that the model is in principle capable of capturing such individual variability. However, including the effect of posture changes and locomotion to CSF movement would likely require new modelling approaches beyond the scope of the current manuscript. Finally, we also mention that verifying the models' suitability to accurately capture inter-individual variability is a focus of current and future work.

Action: We have significantly extended the Discussion taking the reviewer's concerns on the limitations associated with the dispersion estimation into account (L296 - L302). We also refer to our responses to the previous point.

The glymphatic system is said to move solutes from the periarterial space through brain tissue to the perivascular space by bulk fluid movement. Here, there is no advective interstitial flow. Should this be taken as evidence against the glymphatic system, or should it be considered a shortcoming of the model? If it is the former, does it make sense to compare the model output to glymphatic MRI studies? If it is the latter, what elements does the model lack?

Comment: The reviewer is absolutely correct, we do not consider advective interstitial flow (and set u_{PAR} = 0). This should not be taken as evidence for or against the glymphatic system, but simply as a model choice. The choice was made for several reasons:

- First, the properties of the glymphatic system are under significant debate. Therefore we consider no interstitial flow to be a reasonable baseline assumption.
- Second, we do not have access to (and so the model lacks) an accurate representation of the vasculature and associated perivascular spaces within the parenchyma (in contrast to along the surface/in the SAS). As a result, any model representation of interstitial flow must be based on an homogenized (averaged) representation. In earlier work[3], we identified interstitial bulk flow of magnitude 1-10 mum/min as compatible with glymphatic MRI data, but with clear individual variability and thus challenging to transfer from one geometry to another.

In light of these points, we made a deliberate choice to mainly report on simulation results relating to transport in the CSF spaces (SAS and ventricular system) including networks of surface PVSs over the first 24 hours after tracer injection - rather than detailed analysis of the distribution patterns within the parenchyma at later time points.

---

[3] Vinje, V., Zapf, B., Ringstad, G., Eide, P. K., Rognes, M. E., & Mardal, K. A. (2023). Human brain solute transport quantified by glymphatic MRI-informed biophysics during sleep and sleep deprivation. *Fluids and Barriers of the CNS*, *20*(1), 62.

Action: In response to this comment and the comments from other reviewers, we have completely revised the description of the models in Methods (L389-L405) and significantly extended the Discussion (L321-L330). The reviewer's specific question with regard to what (main) elements that are lacking in the model is, to the best of our ability, now addressed in this revised description of Methods together with the revised description of limitations in the Discussion.

Additional question and comments:
What is the nominal run time for one simulation, and what resources were used for it (processor architecture, number of cores, memory, interconnect)?

Comment/Action: The majority of the simulation time was spent on the Stokes flow and transport calculations. Each Stokes flow simulation utilized a hybrid parallel approach with 16 MPI processes and 4 threads each, requiring approximately 300 GB of RAM and completing in 23 minutes. The transport model generally runs in serial, but employs 16 threads for the linear system factorization, taking 22 minutes and consuming about 50 GB of memory. We run all simulations on a single AMD EPYC 9684X machine. We report on these numbers in the revised manuscript (L442-445), having added an additional paragraph in the Methods section under "Numerical approximation, implementation, and verification".

The term "human intracranial molecular enrichment" is not one that I have seen used before in the field. If new terminology should be introduced, I would suggest choosing the introduction section rather than methods section or the title for it. The term should then be clearly explained: What is meant by molecular enrichment in this context? Why is there a need for the introduction of new terminology?

Comment/Action: We thank the reviewer for the opportunity to revisit the title. The term "enrichment" is frequently used e.g. in the context of glymphatic MRI by Eide and colleagues, however, we realized that the term "molecular enrichment" is not standard. In response to this and other reviewers' comments, we have carefully revised the title. The new title is: "In-silico molecular transport via perivascular networks in the human intracranial space".

Reviewer #1 (Remarks on code availability):

The code is well-structured and clearly written. To enhance framework robustness, the authors could consider incorporating formal test cases (e.g., for mesh projection, boundary marking, and velocity field mapping).

Comment/Action: We thank the reviewer for the positive feedback. To enhance robustness and reproducibility, we have added a nightly continuous integration run on GitHub, which runs a simplified version of the simulation and postprocessing code every 24h. In addition, the supplementary material provides a series of numerical verification experiments (see Supplementary information S1.5).
Reviewer #2 (Remarks to the Author):

This is a comprehensive model that captures many of the processes that contribute to the distribution of drugs and toxins in the CNS. There are some structural changes that could be made to improve the experience for the reader.

1) The model was run with reasonable parameters but comparison with MRI data was limited to the Discussion section. It may be better to reflect this in the title, "A framework to model molecular enrichment and clearance of the human intracranial space" or something like that. If future comparisons/parameter estimation will be undertaken, this should be mentioned in the Discussion.

Comment/Actions: We see the reviewers' point, and note that we have received several comments on the title from the different reviewers. In response, we have carefully revised the title. The new title is: "In-silico molecular transport via perivascular networks in the human intracranial space".

Second, work is indeed underway to systematically compare between contrast-enhanced ("glymphatic") MRI data and model predictions for multiple patient cohorts. In response to the reviewer's suggestion, we have added a sentence on this point in the last paragraph of the Discussion (L326-L328). We note that the main challenge up till now has been that the overlap in data sets is empty: for the individual(s) where surface vascular data is available, we do not have access to contrast-enhanced MRI; and conversely for the individuals with contrast MRI, we do not have access to an accurate representation of the vasculature.

2) The required Data/code availability statement is missing. Quite a bit if not all of the data and code are shared on the zenodo site, so this would be an opportunity to highlight what is and what is not shared. This should be a detailed list.

Comment/Action: We thank the reviewer for pointing this out. In the original submission, we had moved the Data availability statement from the manuscript itself into the corresponding field in the submission portal. We have now included a Data availability statement in the manuscript itself (L452-L455). Indeed, all associated software is openly available on Zenodo:

Causemann, M., Masri, R., Kuchta, M., & Rognes, M. E. (2025). Software and data for "In-silico molecular enrichment and clearance of the human intracranial space" [Data set]. Zenodo. https://doi.org/10.5281/zenodo.14749163

We have detailed the contents of this archive in the Data availability statement as requested by the reviewer. We have also extended the README/landing page on Zenodo to give more information and context.

3) The supplement is basically its own paper. It's unfortunate that journals and reviewers have made it nearly impossible to publish models. Still, there is quite a bit of characterization and validation and it may be better for the community if this supplement is expanded very slightly and uploaded as a preprint to one of the arxiv sites. It will be more accessible to the community

and better capture the extensive work performed by the authors. The editors should acknowledge that this does not hurt the novelty of the current manuscript.

Comment: We thank the reviewer for the positive feedback on the supplementary material, and the suggestion to publish it as a standalone document. However, in our opinion, the supplement lacks enough context to be useful on its own, and is tightly connected to the main manuscript. For these reasons, we have chosen to keep it as supplementary material to the main paper.

4) In terms of the technical aspects, this is a framework and one could argue for years about the pathways and rate constants. All seem reasonable but the spinal SAS handling is hard to follow. Figure 1C does not reflect the model well. It would be better if the actual model for the spinal SAS was described in the figure so the reader doesn't need to go digging in the supplement. Also, absorption of CSF by passage down spinal nerves seems to be a real phenomenon and if this is not in the model then that should be mentioned.

Comment/Actions: We appreciate the opportunity to clarify that the spinal SAS is not handled in our model. Indeed, our model geometry begins at the craniocervical junction. Moreover, the reviewer is absolutely right in that the absorption of CSF by spinal nerves is not modelled. To address these points, in the revised manuscript, we have made the following edits.

- To clarify, we have edited Figure 1C and its caption to emphasize the model domain.
- We have added a sentence, clarifying that the model does not account for the absorption of CSF in the spinal compartment, in the limitations part of the Discussion (L319-L320).

Overall, this is a welcome contribution to the literature and should be useful to many researchers in the field.

Comment: We thank the reviewer for the generous and constructive comments.

Reviewer #2 (Remarks on code availability):
It appears that everything is there. I did not run the code as it would take an inordinate amount of time to set up a working python environment.

Comment/Action: We note that in response to this and other reviewers' comments, we have updated the Data availability statement (L452-L455) in the manuscript and extended the description of the Zenodo archive.

simula
Kristian Augusts gate 23 | 0164 Oslo | Norway simula.no
post@simula.no

Reviewer #3 (Remarks to the Author):

Review on "In-silico molecular enrichment and clearance of the human intracranial space" by Marius Causemann, Miroslav Kuchta, Rami Masri, and Marie E. Rognes.

This work presents a numerical framework integrating recent mathematical developments on mixed dimensional modeling of flow of cerebrospinal/interstitial fluid (CSF/ISF) and tracer transport within and around the human brain with the goal to provide a realistic and high-fidelity simulation tool of the-long term fate (over days) of tracers injected in the spinal canal. The goal is to introduce a simulation tool integrating the underlying transport phenomena across scales and brain fluid compartments (including 1/ CSF in large compartments: ventricles and sub-arachnoid space (SAS), 2/ CSF compartmentalized in smallest, network-type compartments: Perivascular Spaces (PVS) and 3/ interstitial fluid within the brain tissue) for a combination of driving mechanisms (CSF production in the ventricles, peristalsis induced by brain pulsations in PVS), in an anatomically realistic three dimensional representation of the human intracranial space.

This problem is undeniably highly challenging from a computational perspective, and putting all the above ingredients together already constitutes per se a contribution to the field of CSF/ISF flow and transport.

Comment: We thank the reviewer for the insightful and constructive comments.

However, because of the importance of the physiopathological implications and the unusual amount of misunderstanding and controversy in the field of brain clearance, I think the authors should adopt a clearer and more careful presentation, as detailed below. Thus, I don't think their MS can be accepted in its present form by Nature Communications.

Comment/Action: We absolutely agree with the reviewer with regard to the importance of improved understanding in the field of brain transport and clearance. We have revised the manuscript in line with the below suggestions from the reviewer to the best of our ability, as detailed below.

1/ My first major concern is about the applicability/generalization to brain clearance processes, which usually refers to the ability of the brain to eliminate the waste produced in situ by the functioning neurons or support cells. While I acknowledge the problem addressed in this MS is highly related (its focus is rather on "inflow", i.e. the processes by which tracers injected into the SAS will finally enter into the brain parenchyma, rather than clearance, even if these tracers finally exit the brain because of the finite duration of injection). I believe the authors should clarify this, in order to avoid perpetuating this confusion, which is highly present in the field. This requires reworking the title and carefully editing the MS.

Comment: We understand the reviewer's request to clarify the role of clearance in the manuscript. While we include clearance as removal of tracer via the outer boundary of the SAS in the model, we agree that the focus is primarily on the influx of tracer. In particular, we

do not consider a clearance of waste from the brain scenarios. We also note that we have received several comments on the title from the different reviewers.

Actions:

- We have carefully revised the title. The new title is: “In-silico molecular transport via perivascular networks in the human intracranial space”
- We revisited the use of “clearance” throughout the manuscript and emphasised our model's ability to predict “transport” instead of “enrichment” or “clearance” (e.g. L36, L56, caption of Figure 1, L315).

2/In the same spirit, I think the terminology “molecular enrichment” should be avoided. This terminology is not widely accepted in the field, i.e. not even to be found in a single paper with keywords brain, parenchyma and “molecular enrichment” in pubmed (note that the search retrieves no results if parenchyma is replaced by clearance or interstitial fluid). Moreover, this terminology is unclear to me. I don’t see in the MS any subspace of the brain where tracer becomes more concentrated that the injection concentration (which I would consider true enrichment, e.g. as a result of osmotic processes), but only subspaces where tracer was initially non-present and which exhibit transient increases in tracer concentration.

Comment: We acknowledge the reviewer's concern regarding the term "molecular enrichment". While the term “enrichment” is frequently used in the context of the glymphatic MRI protocol[4,5,6] to describe the appearance of tracers at various locations intracranially, we agree that "molecular enrichment" is less standard and that alternative phrasing might provide greater clarity, especially in the title.

Actions:

- As already mentioned, we have carefully revised the title. The new title is: “In-silico molecular transport via perivascular networks in the human intracranial space”
- We revisited the use of “enrichment” throughout the paper, replaced it by “molecular transport” when appropriate and aligned the use of the term “enrichment” with the usage in the aforementioned references by Eide and colleagues (L14, L42, L56, L85, caption of Figure 1, caption of Figure 2, L108, L121, L143, caption of Figure 4, L210, L213, L224, L286, L291, L292).

3/ While I appreciate the authors’ effort to use this modeling framework to explore how pathological conditions may change the dynamics of tracer transport in a clinical perspective, I am not fully convinced by their parametric study of PVS enlargement. PVS enlargement occurs in a large range of pathologies, including cerebral amyloid angiopathy, multiple sclerosis, and

---

[4] Eide PK, Ringstad G. Functional analysis of the human perivascular subarachnoid space. Nat Commun. 2024 Mar 5;15(1):2001. doi: 10.1038/s41467-024-46329-1. PMID: 38443374; PMCID: PMC10914778.

[5] Ringstad G, Valnes LM, Dale AM, Pripp AH, Vatnehol SS, Emblem KE, Mardal KA, Eide PK. Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. JCI Insight. 2018 Jul 12;3(13):e121537. doi: 10.1172/jci.insight.121537. PMID: 29997300; PMCID: PMC6124518.

[6] Eide PK, Lashkarivand A, Hagen-Kersten ÅA, Gjertsen Ø, Nedregaard B, Sletteberg R, Løvland G, Vatnehol SAS, Pripp AH, Valnes LM, Ringstad G. Intrathecal Contrast-Enhanced Magnetic Resonance Imaging of Cerebrospinal Fluid Dynamics and Glymphatic Enhancement in Idiopathic Normal Pressure Hydrocephalus. Front Neurol. 2022 Apr 6;13:857328. doi: 10.3389/fneur.2022.857328. PMID: 35463139; PMCID: PMC9019061.

TBI, as visible in clinical MRI (i.e., MRI-visible perivascular spaces). However, as far of my knowledge, in these pathologies, PVS enlarge around parenchymal vessels, mainly in the white matter, and not around the surface vessels in the cranial SAS. In contrast, in this study, the authors induce a two-fold enlargement of the outer limit of the PVS associated mainly to the surface vessels (due to the limited resolution of the imaging technique used to extract the vessel network).

Comment: We agree with the reviewer that PVS enlargement occurs in a range of pathologies, and that enlarged PVS are observed around parenchymal vessels in the deep white matter. The reviewer is also absolutely right in that we consider a network of cerebral surface blood vessels with associated PVS, and that our model of PVS enlargement is relative to this network.

However, we note that Ringstad and Eide (2024)[7] report of enlarged PVSAS, so enlarged perivascular subarachnoid spaces, surrounding the MCA in idiopathic normal pressure hydrocephalus (iNPH) patients. See e.g. their Figure 9h with the caption "The area of PVSAS in the M2 segment was larger in iNPH patients (n = 19) as compared with REF (n = 9) individuals (h)." They also observe later first-time appearance of tracer in this and other segments in this patient cohort (Figure 9f-g, *ibid*). These PVSs surrounding surface blood vessels are precisely the ones included in our network model. Our model predictions for enlarged surface PVSs can and should be interpreted in this context.

We thank the reviewer for the opportunity to clarify this point.

Actions:

- To clarify, we have revised the description of the enlarged PVSs. Now we explicitly refer to enlarged PVS in the SAS (abstract, L190, L191-L193, L212, L222, caption of Figure 6, Table 2).
- We have revised the section *Enlarged PVSs delay periarterial and intracranial molecular enrichment* to distinguish better between enlarged PVS in the SAS and in the white matter (L191-L193).

4/ Similarly, I appreciate the authors asking questions about poorly-understood microanatomical features of the perivascular transport system: the question whether enhanced transport within PVS require them to be lined by a structural barrier (membrane with limited permeability) is still open. However, when looking at the details of how PVS permeability is considered in the modeling, it appears it only impacts transport (through the ξ term in Eqs 1a and 1b) but not the flow field itself (the flow in PVS is always solved with the 1D model from Gjerde et al. (2024) which assumes zero flow through the PVS inner and outer walls, i.e. fully impermeable walls). As a result, the pressure field in the SAS is decoupled from the PVS pressure field, while we would expect expect such a coupling would equalize the pressures

[7] Eide PK, Ringstad G. Functional analysis of the human perivascular subarachnoid space. Nat Commun. 2024 Mar 5;15(1):2001. doi: 10.1038/s41467-024-46329-1. PMID: 38443374; PMCID: PMC10914778.

around arterial and venous PVS at their crossings on the brain surface, with strong impact on PVS flow.

Comment: Again, the reviewer is absolutely right in that we consider the effect of variations in PVS permeability ξ in the transport equations (Eqs 1a-b), and that we do not alter the flow field itself. We made this choice for several reasons. First and perhaps foremost, to isolate the effect of the PVS outer interface permeability on molecular transport. Considering the uncertainty (and controversy) associated with PVS flow magnitudes, directionality and net effects, we consider the separation between potential effects on flow and the effect on transport to be a useful one. The reviewer's point #10 below (and our response) illustrates this well. Second, to the best of our knowledge, we currently lack methods to computationally quantify net PVS flow due to vascular pulsations in the presence of a permeable outer PVS interface over relevant time scales (minutes, hours) (i.e., a method similar to the asymptotics presented by Gjerde et al (2024)[8]). However, this being said, we completely agree that these points should be made more explicitly, and discussed to a greater extent.

Actions:

- We have made a minor modification in the relevant Results section to emphasize the point regarding the flow field being kept fixed (L171 - L172).
- We have emphasized that the PVS flow models do not admit fluid exchange across the outer PVS walls, but do allow for fluid entry and exit at the end nodes of the PVS networks (L386-L388).
- We have more extensively extended the relevant section of the Discussion (relating to implications for the compartmentalization of the PVS), now also summarizing the points in our above comment (L281-L285).

5/ The Introduction states this work provides opportunities for personalized medicine e.g. for tailored intrathecal delivery of chemotherapy. I believe this is an overstatement, as transport processes in tumors are highly modified due to increased interstitial fluid pressure.

Comment/Action: We agree with the reviewer in that transport processes in tumors are complex. However, we did not and do not intend to claim to address transport processes within (brain) tumors in this study. Instead, we appreciate the opportunity to clarify here. As one example, take the chemotherapy drug Methotrexate (MTX). MTX is routinely administered intrathecally for patients with acute lymphoblastic leukemia[9,10] (for one to prevent cancer cells spreading to the CNS). There is clinical interest in tailoring the drug

[8] Gjerde, Ingeborg G., Marie E. Rognes, and Antonio L. Sanchez. "The directional flow generated by peristalsis in perivascular networks—Theoretical and numerical reduced-order descriptions." *Journal of Applied Physics* 134.17 (2023).

[9] Hoelzer, Dieter, et al. "Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up." Annals of Oncology 27 (2016): v69-v82.

[10] Colunga-Pedraza JE, Colunga-Pedraza PR, Benavides-López HV, Mares-Gil JE, Jimenez-Antolinez YV, Mancías-Guerra C, Velasco-Ruiz IY, González-Llano O. Real-world practice of acute leukemia intrathecal chemotherapy administration: A Mexican nationwide survey. Hematol Transfus Cell Ther. 2023 Jul;45 Suppl 2(Suppl 2):S25-S29. doi: 10.1016/j.htct.2021.09.018. Epub 2021 Dec 22. PMID: 35153181; PMCID: PMC10433292.

dosage, in particular for children[11]. Indeed, to establish methodology for personalized intrathecal drug delivery is a main axis of research in our translational and interdisciplinary K. G. Jebsen Centre for Brain Fluid Research[12]. The methodology established in this work represents a key step in this direction, with several joint follow-up studies planned. In light of this, we do consider our statement to be appropriate. To make this more evident, we have added a reference to the well-established use of intrathecal injection of MTX for acute lymphocytic leukemia patients in the Introduction of the revised manuscript (L17-L18).

6/ In the first lines of the Results Section, which sum up the main ingredients of the modeling (lines 56-70), the authors should make clearer a few important assumptions, that are needed to easily understand the Results themselves. In particular, if I have correctly understood, the flow field itself is computed for steady state conditions (see S1.1.3 and S1.1.4). It seems from the figure legends that these steady conditions correspond to peak systolic conditions of CSF volumes in the different compartments, but the methodological description on this is quite poor and confusing (see comment 7 below). Moreover, the methodological description of the modeling choices for the parenchyma is also confusing (see comment 12). This should be clarified.

Comment: We understand the reviewer's request to clarify the assumptions and modelling choices. The reviewer is correct that the flow fields are computed for steady-state conditions. Indeed, we compute three different steady-state flow fields: one corresponds to CSF production, one to cardiac peak systolic conditions, and one to respiratory peak conditions. While the first is used as the advective flow field in the molecular transport simulations, the latter two are the main ingredients for our estimates of pulsatile dispersion enhancements. We agree that this should be made more explicit.

Action: We have carefully revised Methods and the first two Results sections in response to the reviewer's comments (L90-L99). In particular, we anticipate that the substantial changes in Methods aid in clarifying the modelling and its underlying assumptions. We also now clearly distinguish between the three different flow fields in the second Results paragraph. See also our responses to comments 7 and 12 below.

7/ Lines 94-96: "For the cardiac contribution, we compute CSF pressure and flow fields at peak systolic blood inflow, corresponding to a reduction in CSF space volume at a total rate of 6ml/s in the SAS and 0.31ml/s across the lateral ventricle surface." I don't understand this comment. Please clarify and add explicit reference to where in the Methods Section and or Supplement this is explained. I think this modeling choice is needed because of the steady state conditions chosen for modeling the flow field (see previous comment), and the rate of volume changes chosen may on one hand have a strong impact on the obtained velocities (thus on the inferred effective dispersions R) and on the other hand be highly variable depending on individuals and conditions.

---

[11] *Personal communication, Prof. Per Kristian Eide, Neurosurgery, Oslo University Hospital/U Oslo*
[12] https://www.med.uio.no/klinmed/english/research/centres/kg-jebsen-brain-fluid/

Comment: We understand the reviewer's request for clarification and appreciate the opportunity to do so. The specific sentence describes the flow field used to estimate the enhancement of diffusion due to dispersion induced by cardiac pulsatility. The reviewer is correct in that the computed flow fields are steady-state fields corresponding to the peak systolic conditions of CSF volumes, and that we use these to compute dispersion enhancement factors.

Actions: In addition to the extended reorganization of Methods, we have revised the manuscript as follows to address this specific reviewer comment:

- We split the sentence in question into two for clarity, and explicitly state that the flow fields describe the steady state at peak systole (L97-L98).
- We refer to Supplementary information S1.3 for a detailed description of the methodology (L97).

8/ Line 105. The authors claim they study the effect of reduced pulsatility on tracer transport. However, to do so, they only manipulate the values of the dispersion coefficient R (tenfold reduction). Even if reduced pulsatility leads to decreased values of R, it would be helpful to know what level of pulsatility reduction leads to this change in R? Is it trivial that we expect the same changes of R in PVS and SAS, for which the flow regime is quite different? More generally, why would we expect a uniform spatial reduction of R?

Comment: We thank the reviewer for bringing up this important point. The reviewer is correct that our previous approach of applying a uniform scaling to the dispersion factor R was an oversimplification for modeling changes in pulsatility. Indeed, the relationship between pulsatility and dispersion is non-linear and spatially non-uniform. We clarify that the dispersion enhancement factor R is only applied within the SAS, not in the PVS network. This choice is based on previous work indicating that dispersion effects in the narrower perivascular spaces are much less pronounced. Recent estimates suggest that dispersion in PVS enhances transport by less than a factor of two compared to diffusion alone[13,14].

Taking the reviewer's feedback into account, we have revised our methodology and updated our results (in Figure 2P-Q) to address this issue directly.

Actions:

- Instead of artificially scaling R, we now simulate scenarios of halved and doubled CSF pulsatility by directly modifying the inflow boundary conditions in our CSF flow model. We then compute the new, spatially non-uniform dispersion fields that result from these changes. As our steady-state flow model is linear with respect to the boundary conditions and the dispersion enhancement R is quadratic in the pressure

[13] Bojarskaite, L., Vallet, A., Bjørnstad, D. M., Gullestad Binder, K. M., Cunen, C., Heuser, K., ... & Enger, R. (2023). Sleep cycle-dependent vascular dynamics in male mice and the predicted effects on perivascular cerebrospinal fluid flow and solute transport. *Nature communications*, *14*(1), 953.
[14] Asgari, M., De Zélicourt, D., & Kurtcuoglu, V. (2016). Glymphatic solute transport does not require bulk flow. *Scientific reports*, *6*(1), 38635.

gradients, doubling the pulsatility amplitude leads to a four-fold increase in R, while halving the amplitude leads to a four-fold decrease. These new dispersion enhancement fields were then used to re-compute the tracer transport simulations (see the Results section “Reduced CSF pulsatility strongly shifts intracranial enrichment patterns” (L110 - L113), including Figure 2).

- Furthermore, we have added a new figure to the supplement (Supplementary Figure S7) that illustrates how the dispersion factor R changes as the cardiac pulsatility amplitude is varied between 25% and 100%.

9/ Results "Perivascular flow shapes and accelarates molecular enrichment" (beginning on line 114). It is unclear to me from Figure 3 and Legend if the effect of CSF production (pressure gradient) and vasomotion-induced peristasis are considered together or in turn.

Comment/Action: Each driver is considered separately/in turn/alone. We have attempted to clarify this by adding “alone” in the captions of Figure 3B, E, F.

10/ Line 131. Vasomotion-induced traveling waves. The authors assume these waves travel antegrade. However, experimental work suggests the direction of vasomotion travelling waves is both antegrade in some vessels and anterograde in others (Broggini, Thomas, Jacob Duckworth, Xiang Ji, Rui Liu, Xinyue Xia, Philipp Mächler, Iftach Shaked, et al. “Long-Wavelength Traveling Waves of Vasomotion Modulate the Perfusion of Cortex ». Neuron 112, 2024”. Could the authors comment on this ?

Comment: The reviewer is absolutely correct in that our estimates of net PVS flow induced by peristalsis are based on the assumption that the motion of the blood vessel wall can be represented as a travelling wave in the antegrade direction. This is clearly a point associated with significant uncertainty and, based on evidence in mice, an assumption that does not hold in general. Munting et al (2023) report of vessel diameter changes at 0.1Hz propagating along pial arterioles in mice in about one-third of the arterioles studied, with a majority of waves in the retrograde direction, but also one in the antegrade direction. Broggini et al (2024) report of travelling vasomotion (near 0.1Hz) waves, both antegrade and retrograde, in pial arterioles of mice, with mean changes in diameter of 11%. In humans, the data are even more sparse. Gokina et al (1996) report of spontaneous vasomotion in human pial arteries (ex-vivo), but without reporting on spatial characteristics. We are not aware of more recent reports in humans (including after personal communication with Prof. Rune Enger, GliaLab/Letten Centre, University of Oslo).

We wish to note here that it is not straightforward to quantify net flow induced by peristaltic pumping with less regular wave characteristics (such as variable directionality, frequency, amplitude or wave speed) by asymptotic analysis. Therefore, we would not make any claims as to how e.g. part antegrade, part retrograde vasomotion affect net PVS flow. This could possibly be investigated further numerically - but would be more appropriate for future work.

Action: We have revised the manuscript by extending the relevant section of the Discussion (L268-L271). We have also added a note in the Supplementary information (in S1.4 Estimating net perivascular flow induced by peristaltic waves).

11/ Lines 228-235. Discussion on comparisons with results previously obtained in humans by MRI (predicted first times of tracer arrival (FTA) falling at the upper end of the clinical range): the authors discuss this difference as "indicative of a net CSF flow in surface PVS beyond what would be induced by CSF production and cardiac peristasis alone". I have first been confused by this sentence, and had to read several times the next paragraphs to understand the authors then intend to discuss the contribution of vasomotion at low frequencies. Please edit carefully the discussion to improve clarity and explicitly compare predicted FTAs with clinical measures.

Comment/Action: We thank the reviewer for pointing out the lack of clarity in the discussion of the times-of-arrival. In response, we have carefully rewritten the paragraph in question (L237-L252). In particular, we have attempted to clarify why our results indicate that PVS flow driven by CSF production and cardiac peristalsis alone is insufficient to explain the rapid PVS transport observed clinically.

12/ Discussion on model limitations (lines 278-279). The authors write they have used a conservative transport model within the parenchyma, modeling extravascular diffusion alone.

12a/ They do not discuss their modeling choices for the flow within the parenchyma, which implicitly leads the reader to think a zero net flow condition is assumed in the parenchyma (and thus a zero flow condition for the steady state flow regime considered here).

Comment/Actions: We do indeed set zero CSF flow within the parenchyma (with the exception of in the few segments of the perivascular networks that locally extend into the parenchyma). See further comments below.

However, the Methods Section is quite confusing and I am not sure this is truly the case: in fact, for imposing pressure boundary conditions at the PVS network end-nodes located within the parenchyma, they "compute a harmonic extension of the CSF pressure field (by solving a Laplace equation in the parenchyma with pCSF as the boundary value), and set the corresponding value at these end nodes".

Comment/Action: We first wish to remark that we have edited the Methods section substantially in the revised manuscript. These changes are detailed in connection with the reviewer's point /13 below.

For this specific point, yes, we understand that this description was confusing. We indeed compute a harmonic extension (into \Omega_{PAR}) of the CSF pressure field induced by CSF production (which is defined in \Omega_{CSF}). However, we consider this as a step simply to set reasonable boundary conditions for the pressures in the periarterial network, especially for those ends of the network that happen to extend into the parenchyma. In other words, we used the harmonic extension (- Delta p = 0, without a physically motivated

diffusion coefficient) as a means to extrapolate the pressure data. We do not interpret this equation as a Darcy flow model. We have simplified this description in the revised manuscript (see a detailed description of changes made in connection with point 13/ below).

Solving a Laplace equation in the parenchyma means the authors consider Darcy flow with spatially uniform Darcy permeability in the parenchyma, and they could then easily deduce a velocity field in the parenchyma (even if they subsequently set u_{parenchyma}=0 in Eq. 1 for modeling transport in the parenchyma, as written in Section "CSF Flow in the SAS and ventricular system", which is in itself confusing in a section dealing on flow).

Comment/Action: We have removed the mention of u_{PAR} in the section dealing with CSF flow to limit confusion.

The authors should discuss this in a much clearer fashion, giving in particular quantitative results about the resulting flow at the connections between parenchymal PVS end nodes in the 1D model and the corresponding spatial locations in the 3D model. I suspect this modeling choice is the fundamental ingredient driving bulk flow in the PVS system, which, in turn, accelerates transport along PVS.

Comment: We hope our comments and edits for the previous comments clarified our use of the harmonic extension to set appropriate boundary conditions for the pressure-driven PVS flow model. The resulting flow across the entire PVS network, including the flow at the end nodes, is shown quantitatively for each of the driving forces considered (static pressure gradient, cardiac peristaltic wave, vasomotion peristaltic wave) in Figure 3B, 3E, and 3F. In addition, we emphasize that the static pressure gradient (and thus our modelling choices for it) is not the fundamental ingredient driving bulk flow in the PVS system. Quantitatively, the mean flow velocity from the static pressure gradient is negligible (0.08 μm/s) and small for the cardiac wave (0.92 μm/s), but substantial for vasomotion (13.05 μm/s). Thus, our model identifies vasomotion as the fundamental ingredient driving rapid PVS transport.

12b/ Why is this conservative? Please also explain better why citation 95 is relevant in this context (lines 283-285).

Comments/Actions: Mechanisms of molecular transport within the human brain parenchyma is a highly debated topic. One key question is whether or not there exists a net flow of interstitial fluid within the parenchyma and/or within penetrating perivascular spaces. In this study, we only account for extracellular diffusion within the parenchyma, a mechanism which in itself cannot be considered controversial. Indeed, we set u_{\rm PAR} = 0. In light of the on-going debate and our findings in Vinje et al (2023), we consider this conservative compared to setting a non-zero bulk velocity within the parenchyma. However to avoid ambiguity, we have replaced the word “conservative” by “basic” in the discussion of Limitations (L310).

In citation 95, (Vinje et al, Human brain solute transport quantified by glymphatic MRI-informed biophysics during sleep and sleep deprivation, FBCNS, 2023), we estimated net interstitial fluid flow velocities (i.e within the parenchyma) using inverse modelling in combination with contrast-enhanced MRI imaging data from a clinical cohort. There, we identified flow magnitudes (|u_{PAR}|) of approximately 1-10 mum/min. We have attempted to clarify the use of this reference by more careful wording in the revised manuscript (L314-L317).

13/ Methods:
Clearly separating flow and transport, by first introducing all assumptions, equations and BC for conservation of momentum, then for conservation of mass, both in the MS text and in the Supplement, would tremendously help the reader.

Adding a nomenclature with all notations, including notations for surfaces separating compartments, in the Supplement, would also be helpful.

Comment: We thank the reviewer for bringing this lack of clarity in the description of Methods to our attention.

Action: In response, we have made the following changes in the revised manuscript.

- We have carefully restructured the Methods section. In particular, in agreement with the reviewer's suggestion, we introduce the flow models (conservation of momentum) before the transport model (conservation of mass).
- We have carefully edited each of the Methods subsections in order to make the models and methods used as clear as possible, while keeping the description in the main manuscript text relatively brief.
- In particular, we have completely revised the description of the transport equations - Eqs. (1) - to make the different contributions and assumptions easier to identify.
- We have not included mathematical equations for flow in the main manuscript text for the sake of relative compactness. But, we would be more than happy to do so if further encouraged to.
- We have reorganized and rewritten the supplementary information sections. The flow and transport equations are now separated. We include the numerical method used to simulate each model directly after the stated equations.
- We have added a nomenclature listing notation, including notation for surfaces separating compartments, in the Supplementary Information, see Tables S1-2.

14/ Dispersion in CSF spaces (line 341): is there a rationale for considering that dispersion factors for different frequencies/amplitudes can be additive?

Comment/Action: We thank the reviewer for the excellent question. While there are theoretical considerations indicating that the contributions from different frequencies are

indeed additive (see Vedel et al[15], eq. 3.24), we note that such derivations rely on several simplifying assumptions. We therefore consider our estimates as heuristic. However, recent, clinically measured values of CSF mobility demonstrate an approximately tenfold increase compared to self-diffusion of water in highly pulsatile regions[16]. The values align well with our additive estimates for the dispersion enhancement factor, in particular indicating that a stronger amplification (e.g. multiplicative) is unlikely.

15/ Model validation (line 393). Please change title to "Comparison with literature results".

Comment/Action: Changed as suggested (L447).

Specific comments

Line 15 : "In the last decade, established theories have been challenged by new findings revealing a greater degree of molecular movement and exchange": greater than what ?

Comment/Action: The intended meaning was: greater than previously thought. However, we have updated the sentence to be more specific in the revised manuscript (L15-L16).

Line 28: "in synchrony with cardiac, respiratory and neural waves". Please rephrase. I don't think these 3 kinds of waves are synchronized with each other.

Comment/Action: We did not intend to suggest that these waves are synchronized with each other. We have replaced the wording "in synchrony with" by "in association with" (L28).

Line 71: please state what is the overall duration of gadobutrol injection.

Comment/Action: The overall duration of the inflow boundary condition representing the gadodutrol injection is 2 hours. This is now stated in the revised manuscript (L74).

Line 103: please make clear that R associated to respiration is also lower than one in the parenchyma.

Comment/Action: Yes, the R associated with respiration in the parenchyma is fact zero, which is lower than one. This point has been taken care of by our other revisions of Methods. In particular, R does not any longer appear in the governing transport equation in the parenchyma (1b, new numbering).

---

[15] Vedel, S., & Bruus, H. (2012). Transient Taylor-Aris dispersion for time-dependent flows in straight channels. Journal of Fluid Mechanics, 691, 95-122. https://doi.org/10.1017/jfm.2011.444

[16] Hirschler, L., Runderkamp, B. A., Decker, A., van Harten, T. W., Scheyhing, P., Ehses, P., ... & van Osch, M. J. (2024). Region specific drivers of cerebrospinal fluid mobility as measured by high-resolution non-invasive MRI in humans.

Line 209: "occuring with or without pressure differences at the network ends". Please clarify (see also comment 9)

Comment/Action: Upon rereading, we realize that this last part of the sentence hinders rather than adds to readability. We have simply removed this part of the sentence.

Line 221: please clarify M1, M2 and M3.

Comment/Action:

- We have clarified that M1, M2 and M3 refer to the respective segments of the MCA (L243).

Line 327: Intracranial influx. I assume reduced pulsatility would also increase T_{max} in unnumbered eq before line 328. Could you please comment on this?

Comment/Action: The reviewer brings up an interesting point. We would also assume that reduced (or increased) pulsatility would affect the spinal transit time. Clearly, our representation of the influx at the craniocervical junction represents a simplification, and we will address this point further in future work. We therefore consider the effect of reduced pulsatility on T_{max} to be out of the scope of the current study. In the revised manuscript, we have added a brief comment on this point in Methods (L416).

Table 1: please check parameter name and unit for m_{tot}

Comment/Action: We thank the reviewer for pointing out that the parameter name and unit are inconsistent. The parameter represents the total amount of injected tracer (measured in mmol), instead of the volume. We have adjusted the parameter name accordingly (Table 1).

Table 2: please add T_{max} in Table. Also clarify by adding e.g. subscript 0 for baseline model and replacing _ by their values or by NA is parameter not used in the corresponding model.

Comment/Action: We note the reviewer's comments on Table 2. During this revision, we also identified a few additional misprints and erroneous listings. We have revised Table 2 completely in response. The main changes are as follows: we have

- added a numeric label for each model variation;
- listed model variations and parameters in a more logical order (in line with Results);
- removed parameter listings unchanged by model variations;
- improved the labels;
- replaced "-" by the (baseline) values, and highlighted changes from baseline in color.

We have not added T_max to Table 2 as it is unchanged in the model variations. We point out that T_max is listed in Table 1. All parameters in Table 2 are used in all model variations. (As a side-note, we remark that Table 2 does not fit in the version of the manuscript with the changes marked, and so we point the reviewer to the revised (clean) version of the manuscript for the revised Table 2.)

Line 373-375: please clarify.

Comment/Action: This sentence describes our special treatment of the ends (leaf nodes) of the PVS network in the transport model. Due to the resolution limit of the underlying imaging method, the reconstructed vessels end abruptly when becoming smaller than approximately 0.5mm. Without special treatment, we observe an artificial accumulation of tracers at the network ends. For that reason, we introduced a simple heuristic to facilitate the exchange of tracer between the network ends and the surrounding SAS (or parenchymal tissue): we increase the permeability coefficient by a factor of 100x. While not a perfect solution to this problem, it effectively models the tracer exchange in the unresolved continuation of the PVS. We rephrased the sentence to clarify this modelling choice (L422-L425).

Figs 5 and 6: please clarify cutting plane for panels 5D and 6E.

Comment/Action: The cutting planes in panels 5D and 6E are the planes normal to the tangent of the blood vessel. We have added this clarification to the captions of figures 5 and 6.

Reviewer #3 (Remarks on code availability):

I had a look at the Zenodo repository but there is no readme or documentation file on how to install/run the code at the first level (i.e. that can be simply downloaded without downloading a heavy zip file and then searching for documentation). Such a documentation should be added to the repository.

Comment/Action: Detailed instructions on how to install and run the code are in the top-level readme of the GitHub repository. We clarified this with an additional sentence in the top-level description of the Zenodo repository, which points to the documentation on GitHub. We also note that we have added a Data availability section in the manuscript itself.

Yours sincerely,
(on behalf of the authors)

Marie E. Rognes, PhD

Chief Research Scientist, Department for Numerical Analysis and Scientific Computing
Simula Research Laboratory, Kristian Augusts gate 23, 0164 Oslo, Norway

Deputy Director, K. G. Jebsen Centre for Brain Fluid Research

meg@simula.no, www.simula.no/people/meg, https://orcid.org/0000-0002-6872-3710