

Supplementary Information

Table 1: Electron microscopy datasets and their basic properties.

Species	Region	Fixation	Spatial resolution ($x \times y \times z$)
Rat [1]	CA1-hippocampus	Perfusion	$2.3 \text{ nm} \times 2.3 \text{ nm} \times 45\text{--}50 \text{ nm}$
Mouse [2]	Cerebral Cortex	Immersion with an osmotic agent	$8 \text{ nm} \times 8 \text{ nm} \times 30 \text{ nm}$
Human [3]	Temporal Cortex	Immersion without an osmotic agent	$8 \text{ nm} \times 8 \text{ nm} \times 33 \text{ nm}$

We used custom Python scripts to download the mouse and human brain tissue datasets. Both datasets were then resampled to an isotropic resolution of $10 \text{ nm} \times 10 \text{ nm} \times 10 \text{ nm}$. The surface mesh was generated using open-source code (`extract_surfaces.py`) [4]. Mesh intersection errors (due to self-intersecting face elements) were resolved using MeshLab, SolidWorks, and Meshmixer. We manually resolved the self-intersecting face elements in SolidWorks by importing one cellular feature at a time. If that did not work, we used the “Surface reconstruction: Screen Poisson” feature in MeshLab. In our simulations, one rat tissue dataset had a volume of $2 \text{ }\mu\text{m}^3$ while the remaining four had a volume of $2.5 \text{ }\mu\text{m}^3$. For mouse tissue, two datasets had a volume of $1 \text{ }\mu\text{m}^3$ and one had a volume of $2 \text{ }\mu\text{m}^3$. For human tissue, one dataset had a volume of $1 \text{ }\mu\text{m}^3$ and three had a volume of $2 \text{ }\mu\text{m}^3$. All four extracted $2.5 \text{ }\mu\text{m}^3$ chunks from the rat brain dataset were adjacent to each other with one of the samples partially overlapping with another. All extracted mouse and human datasets consisted of cubic volumes with side lengths of approximately 1500 nm . The mouse datasets [2] were extracted from locations with top, left corners given by Cartesian coordinates (x, y, z) ($80 \text{ }\mu\text{m}$, $160 \text{ }\mu\text{m}$, $1.2 \text{ }\mu\text{m}$), ($320 \text{ }\mu\text{m}$, $248 \text{ }\mu\text{m}$, $1.2 \text{ }\mu\text{m}$), and ($120 \text{ }\mu\text{m}$, $264 \text{ }\mu\text{m}$, $1.2 \text{ }\mu\text{m}$). Three human datasets [3] were extracted from the following locations: ($1904 \text{ }\mu\text{m}$, $1960 \text{ }\mu\text{m}$, $104.6 \text{ }\mu\text{m}$), ($2800 \text{ }\mu\text{m}$, $1600 \text{ }\mu\text{m}$, $71.6 \text{ }\mu\text{m}$), and ($2328 \text{ }\mu\text{m}$, $1720 \text{ }\mu\text{m}$, $104.6 \text{ }\mu\text{m}$). The fourth human dataset was extracted as a 1300 nm chunk surrounding the astrocyte dataset (segment ID: 40403742271) from the location ($2233.6 \text{ }\mu\text{m}$, $1489.4 \text{ }\mu\text{m}$, $71.1 \text{ }\mu\text{m}$). Four rat datasets [1] were extracted as rectangular volumes, each with a volume of $2 \text{ }\mu\text{m}^3$, immediately adjacent to a volume centered at ($7.6 \text{ }\mu\text{m}$, $11.5 \text{ }\mu\text{m}$, $153 \text{ }\mu\text{m}$). The spatial resolution of the mouse datasets was $8 \text{ nm} \times 8 \text{ nm} \times 30 \text{ nm}$ in the x , y , and z directions, respectively. The spatial resolution of the human datasets was $8 \text{ nm} \times 8 \text{ nm} \times 33 \text{ nm}$. The spatial resolution of the rat dataset was $2.3 \text{ nm} \times 2.3 \text{ nm} \times 45 - 50 \text{ nm}$.

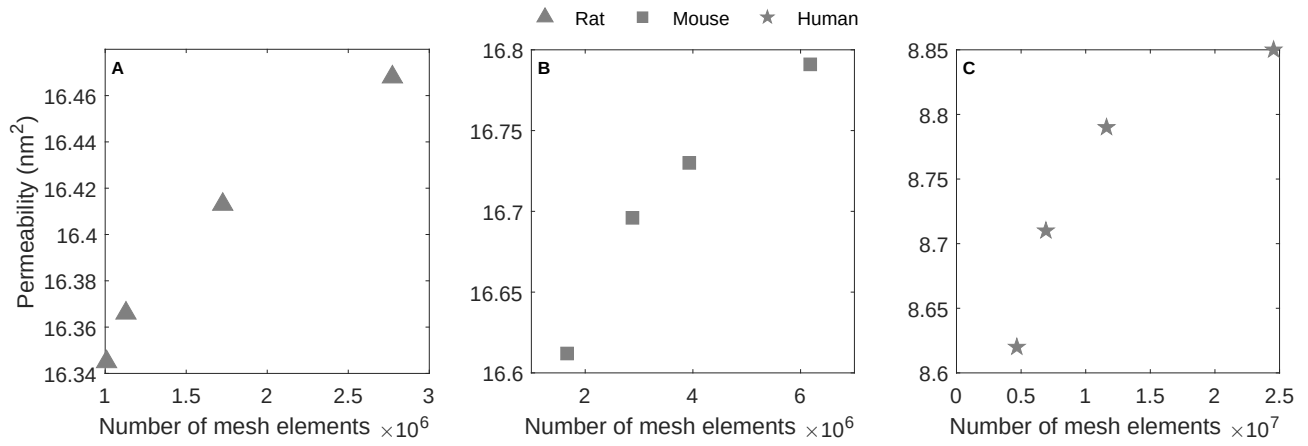


Figure 1: Variation of calculated permeability with the number of elements in the computational mesh for brain tissue samples from a rat (A), mouse (B), and human (C). For these samples, doubling the number of mesh elements changed the computed permeability by about 0.7% for both the rat and mouse samples and by about 0.8% for the human sample, indicating that meshes were sufficiently fine.

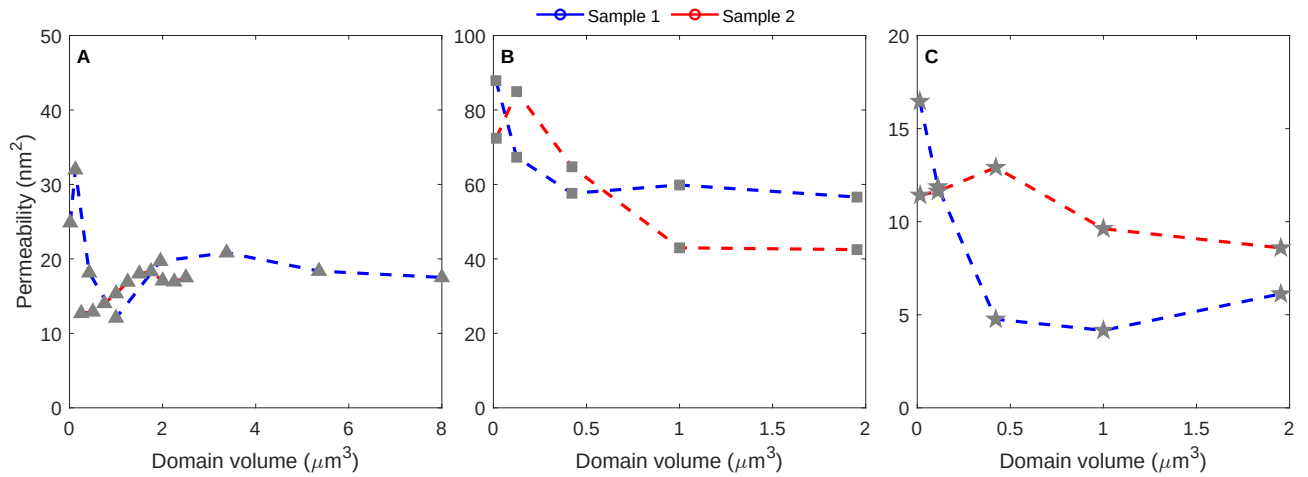


Figure 2: Variation of calculated permeability with sample volume in brain tissue samples from rats (A), mice (B), and humans (C). Permeability becomes insensitive to volume for domains larger than $2 \mu\text{m}^3$ in rats, $1 \mu\text{m}^3$ in mice, and $0.5 \mu\text{m}^3$ in humans.

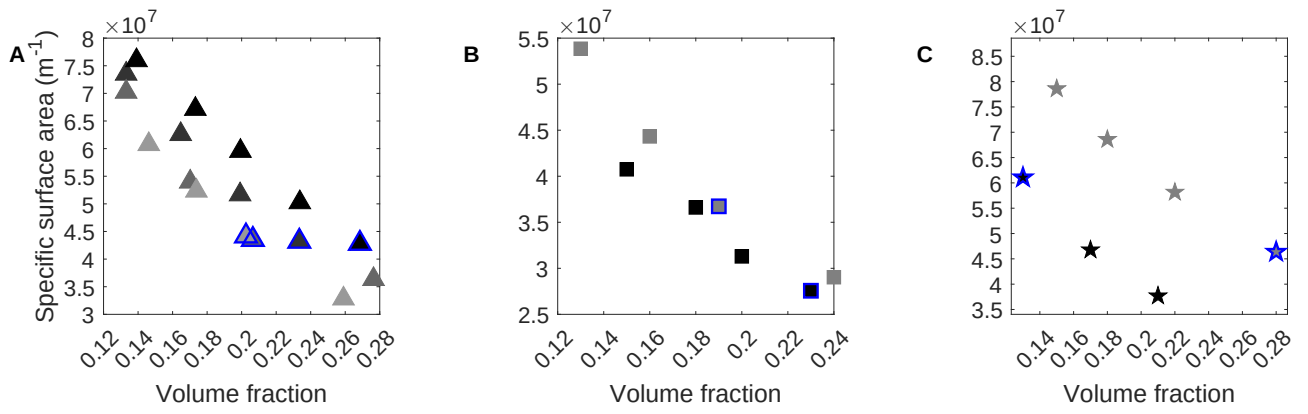


Figure 3: Specific surface area variation with volume fraction for brain tissue samples from a rat (A), a mouse (B), and a human (C). Different marker shades indicate samples from different anatomical locations.

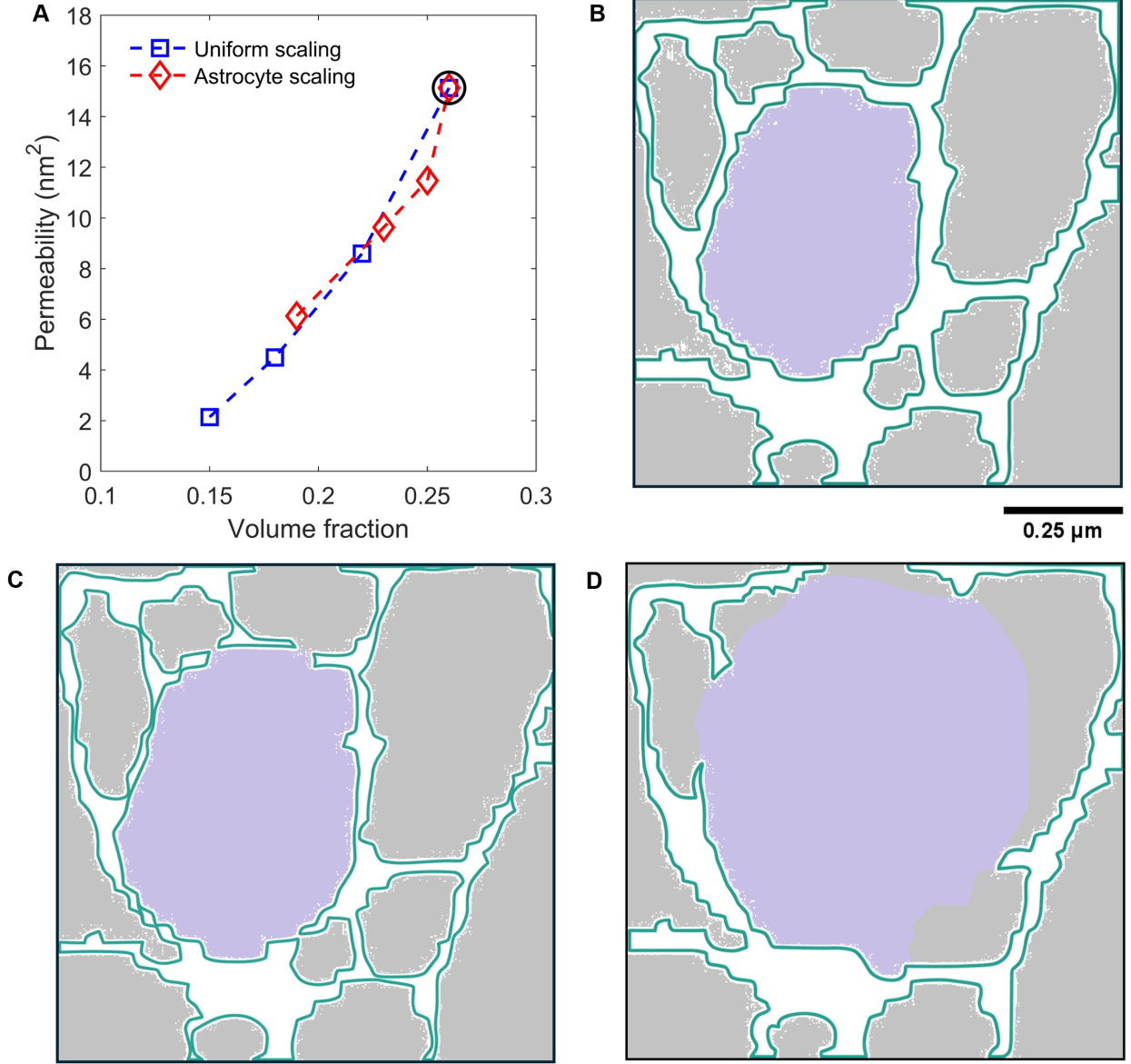


Figure 4: **A**, Variation of permeability with volume fraction after each of two extracellular space (ECS) modifications, uniform scaling of the ECS and astrocyte scaling, in a sample of human brain tissue. Result for original ECS is indicated using a black circle marker. **B**, ECS in the original, unscaled sample of human brain tissue. **C**, ECS after uniformly scaling all cellular features. **D**, ECS after scaling only the astrocyte. Gray regions in **B–D** represent different cells, with the astrocyte highlighted in purple. The ECS is shown in white.

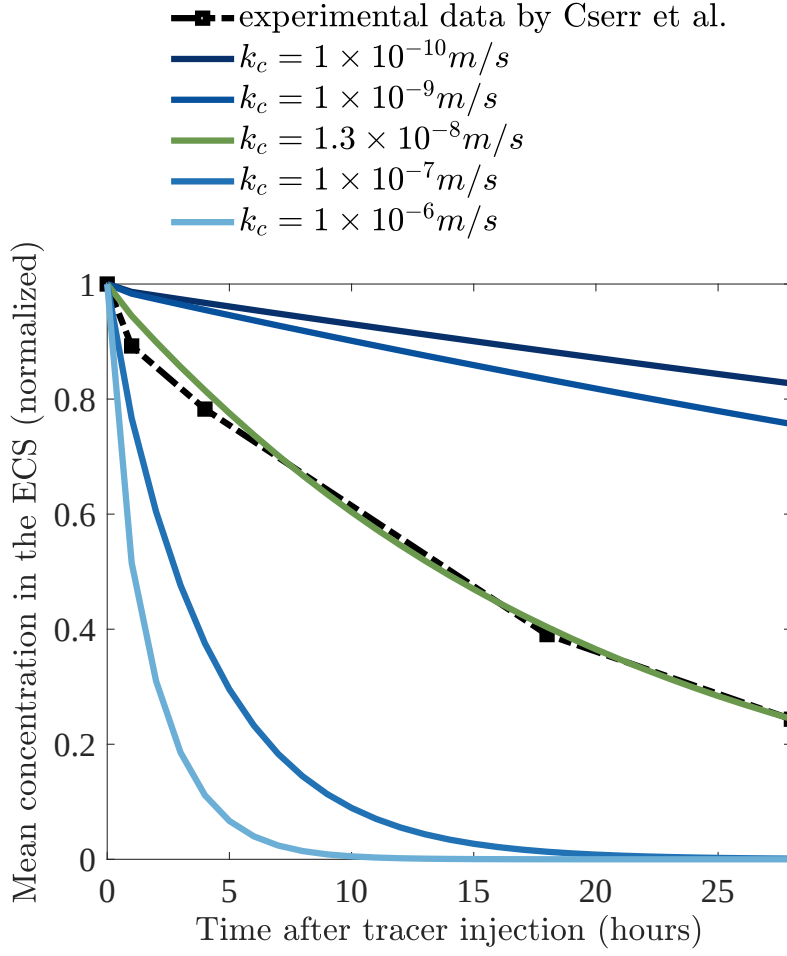


Figure 5: Sensitivity of clearance to the mass transfer coefficient k_c using the mesoscale model (the computational model of Guo et al. [5] with ventricular clearance) in Figure 3. The ECS permeability is fixed at $\kappa_{\text{ECS}} = 19.7 \text{ nm}^2$.

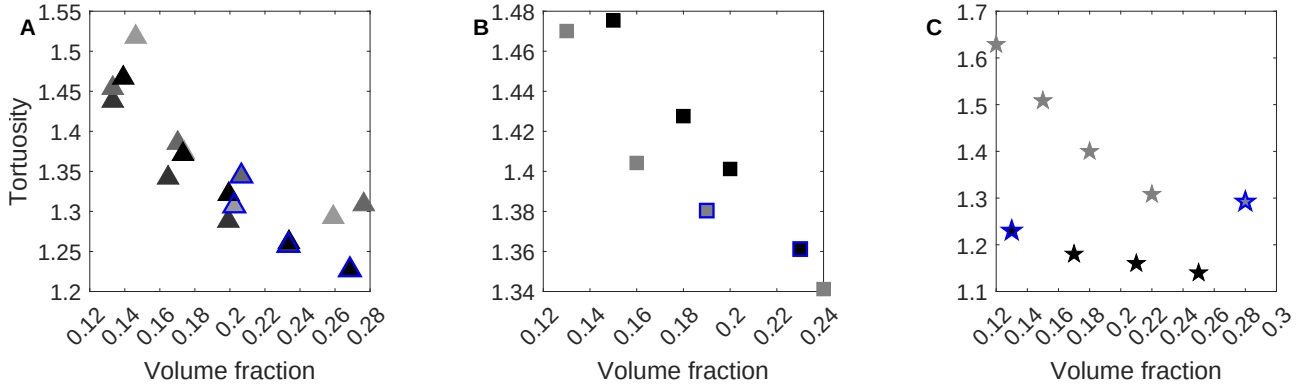


Figure 6: Tortuosity variation with volume fraction in brain tissue samples from rats (A), mice (B), and humans (C). Different marker shades indicate results from chunks extracted from different locations as described in the methods section. Markers with blue edges indicate tortuosity of original ECS.

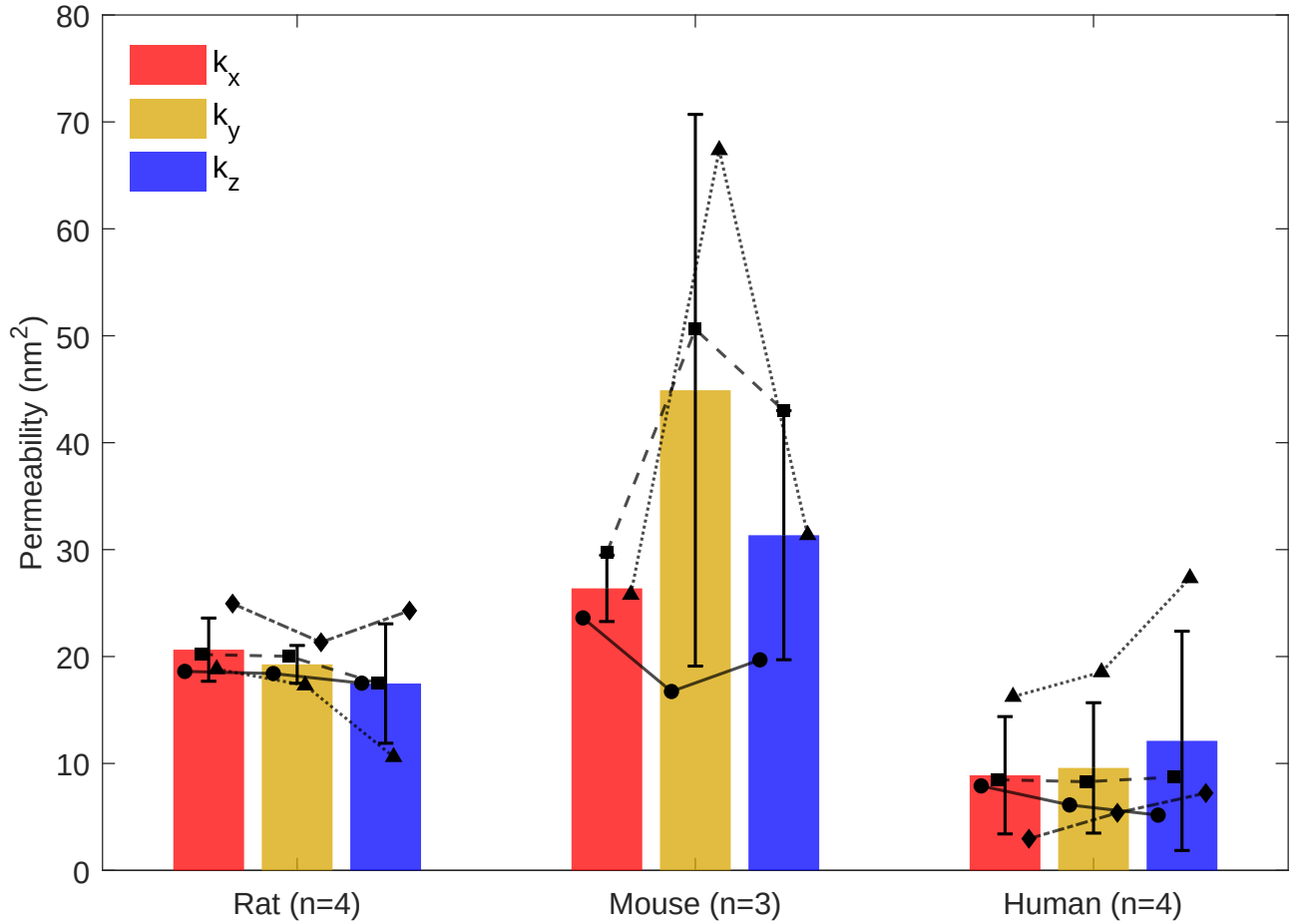


Figure 7: Permeability measured along the three coordinate directions for each species for original ECS data. To assess directional dependence, a pressure drop was applied along each of the three Cartesian coordinate axes, and the corresponding permeabilities (k_x, k_y, k_z) were quantified. Bars represent the mean permeability for each axis within each species, with error bars indicating the standard deviation. Individual measurements are shown as black dots. Sample sizes were $n = 4$ for rat, $n = 3$ for mouse, and $n = 4$ for human.

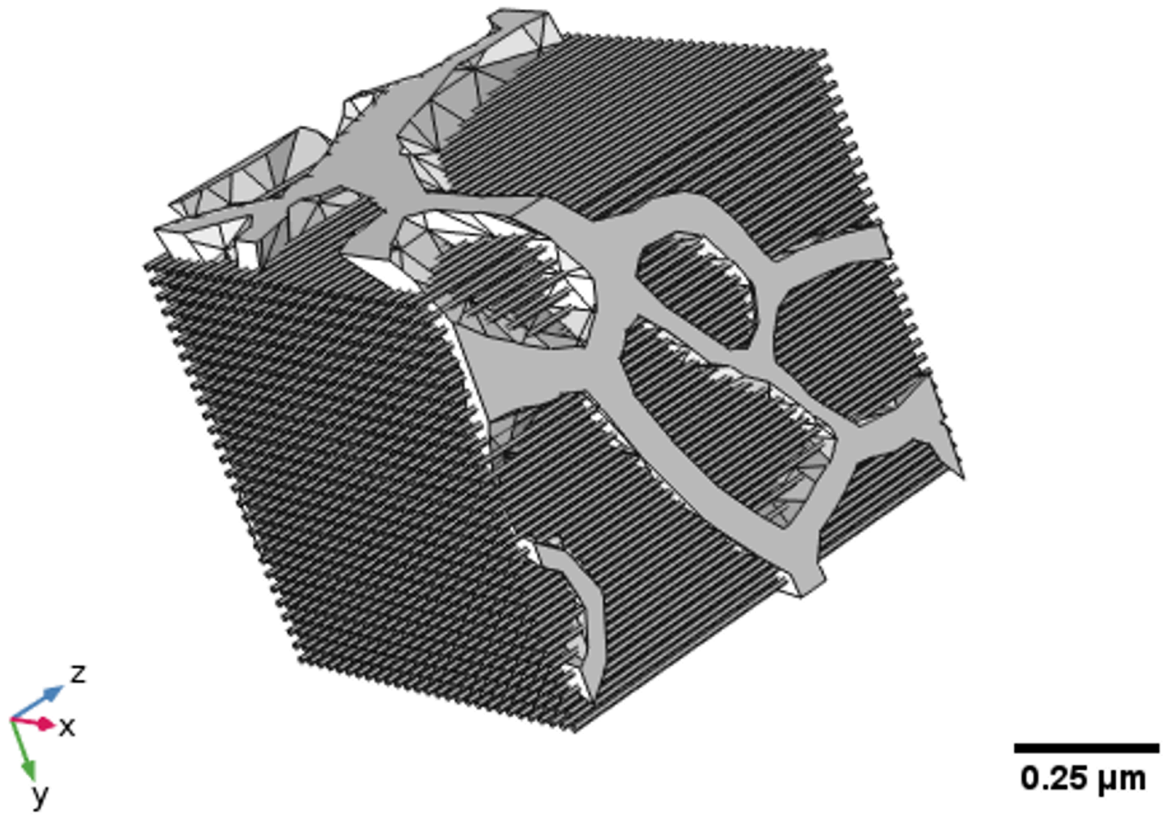


Figure 8: Representative extracellular matrix (ECM) structural units in the rat brain tissue dataset. The longitudinal axes of the cylindrical ECM units were oriented along the z -direction, while the pressure gradient was imposed along the x -direction.

References

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- [5] Yisen Guo, Keelin Quirk, Douglas H Kelley, and John H Thomas. Advection and diffusion in perivascular and extracellular spaces in the brain. *Journal of The Royal Society Interface*, 22(226):20250010, 2025.