Supplementary Information

Small angle scattering (SAS) models

For completeness, below we explain the models used in the scattering data. For the model-independent fits, a Guinier-Porod fit was used to extract the Porod exponent and the radius of gyration of the largest scattering object.(1)(*Hughes et al.*, Soft Matter, under review) The Porod exponent detects geometry changes, and when accompanied by the increase in the radius of gyration, it suggests an increase in the largest scattering object, i.e. an increase of the clusters formed by the crosslinking of proteins.

For the model-dependent fits, SAS models are explained following *Hughes et al.* analysis.(2) A fractal structure factor model was used to extract quantitative information from the scattering curves in Figure 3b and c:

$$I(Q) = \Phi V_{block} \Delta\rho^2 F(q) \cdot [(1 - p_c) + p_c S(q)]$$

Where ϕ is the volume fraction of protein, V_{block} is the volume of the protein block, Δρ is the contrast difference between the building block and the solvent, F(q) is the ellipsoidal form factor of the building block, pc is the proportion of protein in fractal-like clusters, and S(q) is a fractal structure factor.

The form factor F(q) is given by:

$$F(q) = \left(\frac{3(\sin(qr) - qr\cos(qr))}{qr^3}\right)^2$$

While the fractal structure factor is given by

$$S(q) = \frac{D_f \Gamma(D_f - 1)}{\left(1 + \frac{1}{(q\xi)^2}\right)^{D_f - 1/2}} \cdot \frac{\sin\left[(D_f - 1) tan^{-1}(q\xi)\right]}{(qR_0)^{D_f}}$$

Where D_f, ξ and R_0 are defined as the mass fractal dimension, correlation length and minimum cut-off length-scale defined by the ellipsoid form factor, respectively. For a protein hydrogel network, fractal dimension D_f can be thought to be related to the density of the clusters and correlation length ξ, to the size of the clusters within the network.

SAS data were also used to explore the protein hydrogel cluster size and morphology by using a radial distribution function, g(r), to derive the fractal structure factor. The radial distribution function, was used to extract an expression for the number of protein monomers in a sphere from the centre of a cluster N(r) :

$$g(r) = \frac{\rho_k D_f}{4\pi\varphi r_0^D} r^{D_f - 3} e^{-r/\xi}$$

Where p_k is the maximum packing density of the system, and r_o is the minimum cut off distance of the fractal cluster. Multiplying the radial distribution function by the volume fraction and integrating over r, the number of individual building blocks in a sphere of radius R, from the centre of the cluster was obtained:

$$N(r) = \rho_k D_f \left(\frac{\xi}{r_o}\right)^{D_f} \gamma\left(D_f, \frac{r}{\xi}\right)$$

where $\gamma(D_f, r/\xi)$ is the lower incomplete gamma function. SAS curves of N(r) as a function of distance from the centre of the cluster were used to estimate the radius of the fractal-like clusters.

Cryo-scanning electron microscopy (cryo-SEM) artefacts

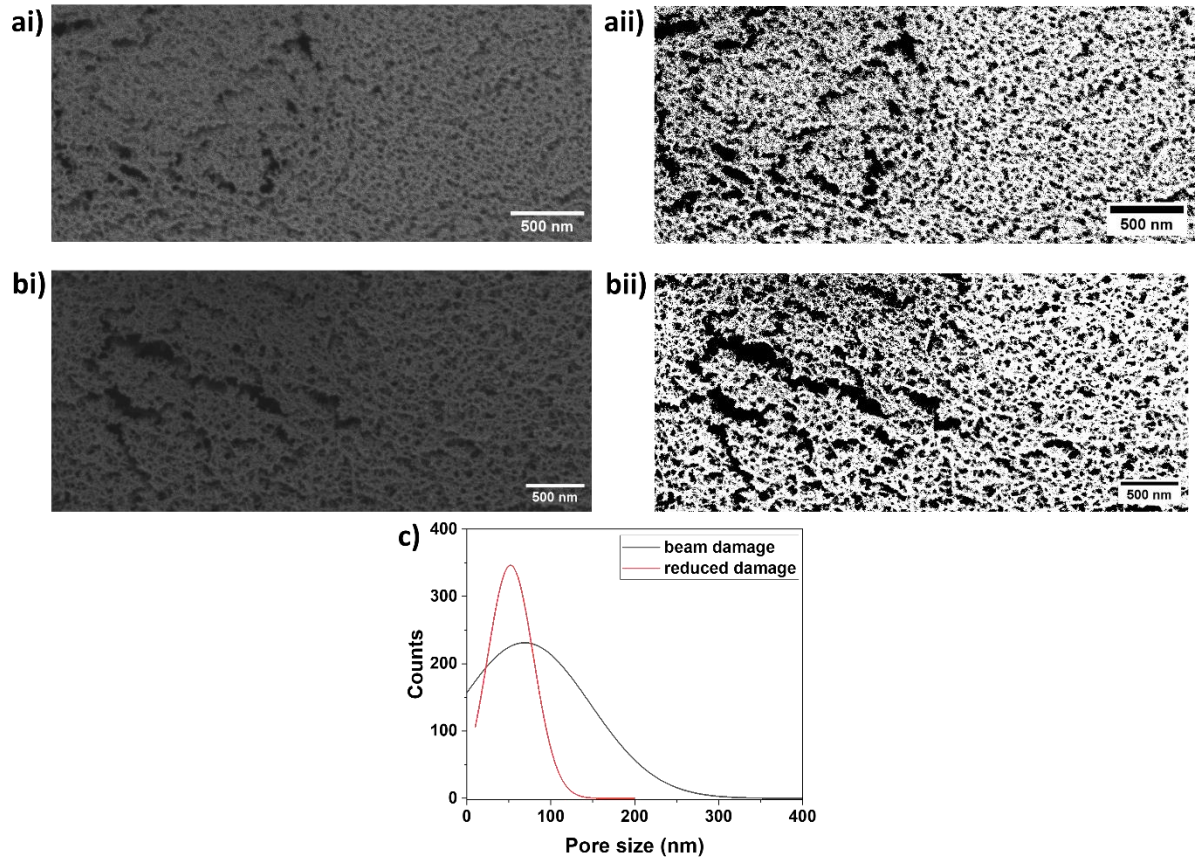

Figure S1. *ai and bi) cryo-SEM image showing artefacts due to beam damage. The left side of both images was irradiated longer with the beam, resulting in structure damage as the structure was ruptured by the beam. aii and bii) are corresponding thresholded images of ai and bi, respectively. c) Pore size distribution measured including measurements of pores induced due to beam damage versus reduced damage.*

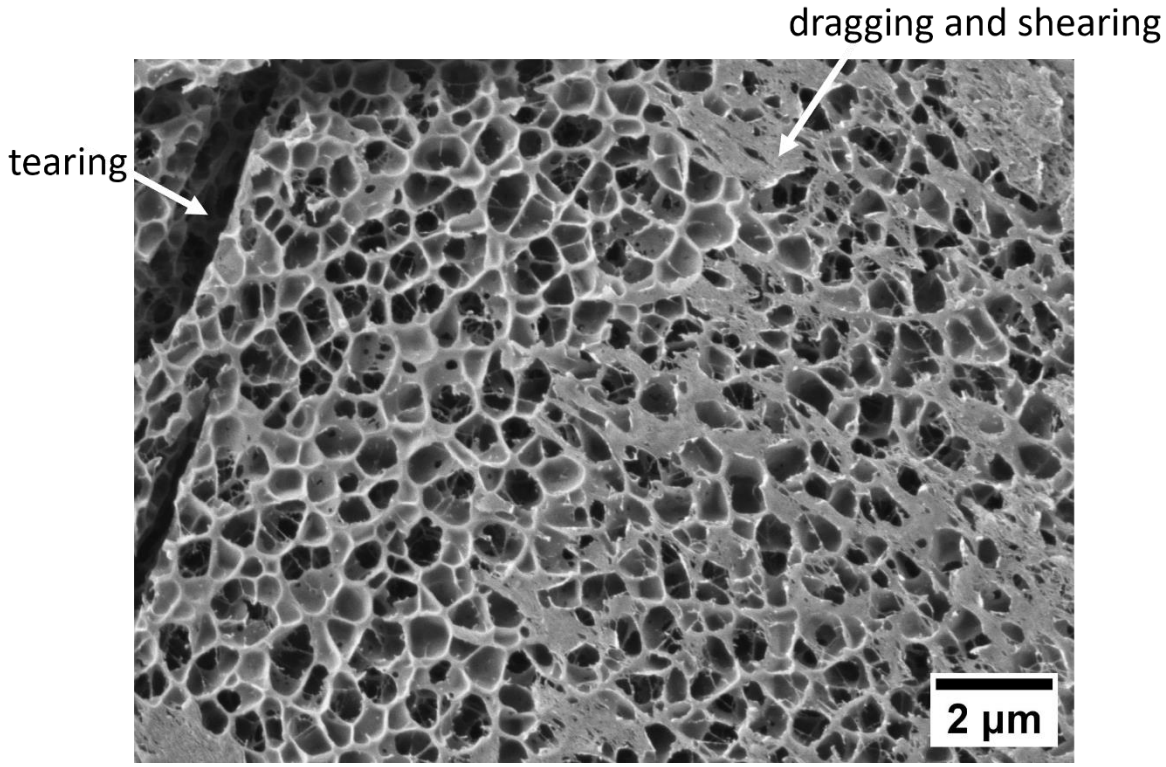

Figure S2. *Cryo-SEM image showing knife damage on ex-situ BSA hydrogels. The surface appears to be dragged, and tearing (cracks) and shearing are visible, with potential compression from the knife.*

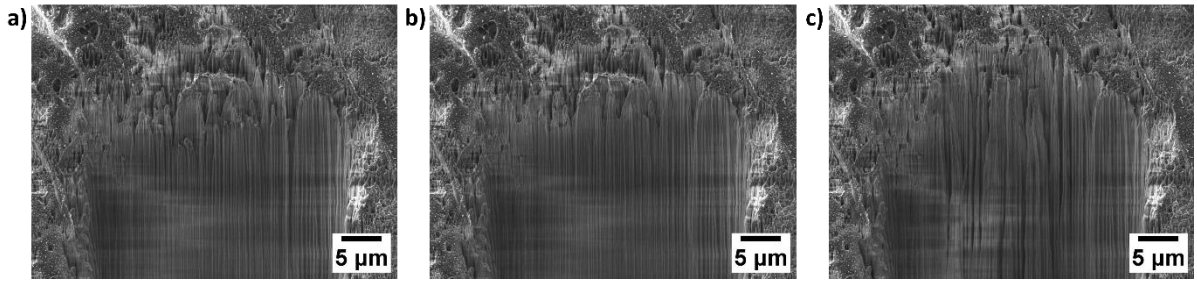

Figure S3. *Cryo-SEM images showing artefacts due to pFIB over-milling of the same area of a BSA gel. First a 10 nA pFIB probe is used to mill the surface. Then additional b) 1 nA and then c) 250 pA pFIB probe current milling was conducted, showing increased curtaining leading to structure collapse.*

References

1. Hughes MDG, Cook KR, Cussons S, Boroumand A, Tyler AII, Head D, et al. Capturing Dynamic Assembly of Nanoscale Proteins During Network Formation. Small [Internet]. 2024 Nov 12;2407090. Available from: https://doi.org/10.1002/smll.202407090

2. Hughes MDG, Hanson BS, Cussons S, Mahmoudi N, Brockwell DJ, Dougan L. Control of Nanoscale In Situ Protein Unfolding Defines Network Architecture and Mechanics of Protein Hydrogels. ACS Nano [Internet]. 2021;15(7):11296–308. Available from: https://doi.org/10.1021/acsnano.1c00353