Supporting Material for

Micro- and macrorheology of jellyfish extracellular matrix

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Microrheology experimental procedure

Injection procedure

A home-made micropipette with a tip size of a few microns was used to inject the microspheres in the jellyfish mesoglea. The micropipette was pulled from thin-wall borosilicate glass capillaries with a 1 mm outer diameter and a 0.58 mm inner diameter (GC100-15, Harvard Apparatus, Les Ulis, France) with a Narishige PB-7 double-stage puller (Narishige, Tokyo, Japan). The micropipette was mounted on a pressure device (CellTram Oil, Eppendorf, Le Pecq, France) and guided with a joystick micromanipulator (MN-151, Narishige) attached to the inverted Leica microscope.

Details of the injection procedure are shown in Fig. S1. Juvenile jellyfish were anesthetized during 10 min in 7.5% $MgCl_2$ diluted with an equal volume of seawater (1). They were then put on a glass cover slip, the umbrella lying flat, and the subumbrella in contact with the cover slip. This position of the jellyfish allowed penetrating the needle through the exumbrella. The subsequent injury was limited and could be easily repaired by the jellyfish. In contrast, injection via the subumbrella would have damaged the muscle fibres and the endodermal gastrovascular system.

A few nanolitres of microbead suspension was injected, corresponding to a few hundred microbeads. To prevent the jellyfish from drying, each injection was performed as quickly as possible (10 min). The beads were injected about 200 µm above the subumbrella (corresponding to a distance of about 100 µm above the endoderm), so that they were well embedded in the mesoglea (far from cellular sheets) and could be viewed by an immersion objective. A glycerine immersion objective (PL APO 63X/1.30 GLYC CORR, Leica), with 63 magnification, a numerical aperture of 1.30 and a working distance of 280 µm, was chosen to visualize *in vivo* the microbeads embedded in the mesoglea and to track their Brownian motion.

Microbeads observations

After injection, the jellyfish were replaced in artificial seawater, where they swam freely during one day before any measurement of the motion of the beads. Microrheology results obtained one day or several days after injection were similar: all the beads performed subdiffusive motions and no beads were found to explore pure seawater microenvironment. Thus, it was verified that diffusion of the injected seawater and local embedding of each bead in the mesogleal fibrous network were well

achieved one day after injection. Besides, we verified that the microinjection process did not affect normal morphology and behaviour of the jellyfish.

For the microrheological measurements, the jellyfish were put on a glass cover slip, in the same position as for the injection: the umbrella was lying flat and the subumbrella was in contact with the cover slip. To avoid evaporation during the experiment, the jellyfish was put in a home-made closed chamber. The set up was mounted on a damped optical table in order to reduce noise in microbeads tracking experiments. The fluorescence of the microbeads allowed us to easily retrace the original place of injection. Some of the injected beads were phagocytised by the mesogleal cells, which are capable of amoeboid movements and active phagocytosis (2), but most of them were still in the ECM. Mesogleal cells are sphere-shaped and have a smooth cell membrane and therefore it was easy to determine if a microbead was in a mesogleal cell or in the matrix. The phagocytised microbeads were not considered for thermal motion measurements.

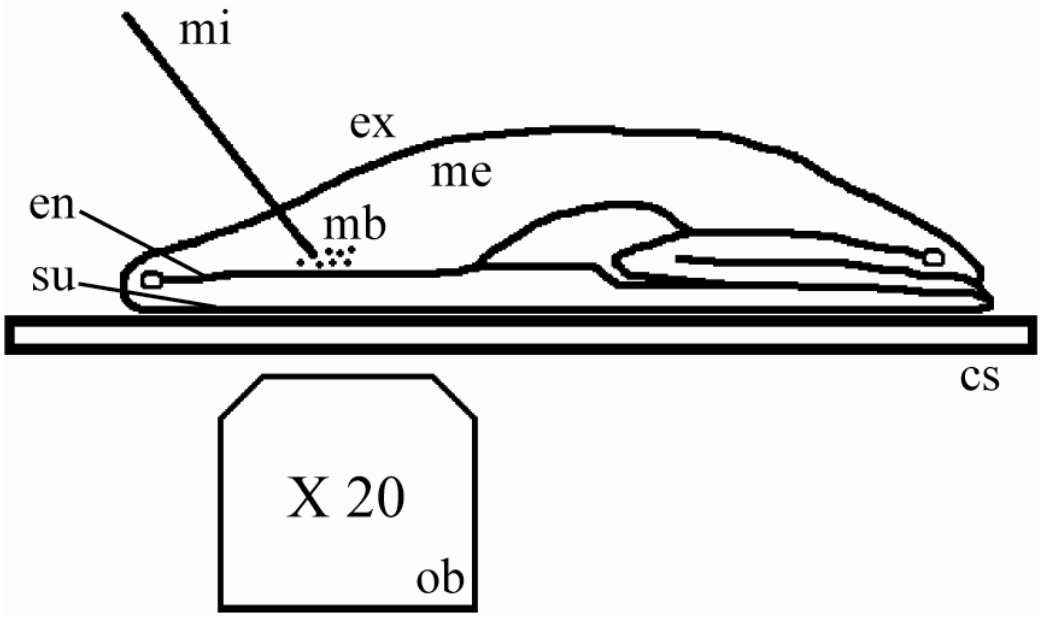

FIGURE S1 Schematic views of the set up used for *in vivo* microbeads injection in the mesoglea of an anesthetized juvenile jellyfish for microrheology experiments. For clarity, the diagram does not show actual sizes of the elements. Cover slip (*cs*); endoderm (*en*); exumbrella (*ex*); microbeads (*mb*); mesoglea (*me*); micropipette (*mi*); objective (*ob*); subumbrella (*su*).

Particle tracking algorithm

The particle tracking algorithm enables to track the two-dimensional Brownian motion of several beads at the same time. It was implemented as an ImageJ plugin (3). The beads displacements were

determined with a classical cross-correlation method. In order to achieve sub-pixel spatial resolution, the computed correlation was interpolated using a paraboloid approximation (4).

Microrheology control experiments

Microrheology experiments in glycerol

During microrheology experiments, a slow sliding of the sample occurred (about 0.2 µm per second). It could be reduced by greasing a bit the cover slip on which the jellyfish was lying. But the drift could not be suppressed totally. The mean motion of the beads, corresponding to this global drift, was calculated and subtracted for each bead. We checked this method on drifting samples of calibrated glycerol: an imposed drift during the experiment could be subtracted from the data and the purely Brownian motion of the beads could be extracted. After the drift-correction of the beads positions, the time-averaged two-dimensional MSD $\langle \Delta r^2(t) \rangle_t, = \langle [x(t'+t) - x(t')]^2 + [y(t'+t) - y(t')]^2 \rangle_t$, was calculated for each bead, improving the statistical accuracy. To maintain reliable statistics, the data from the MSD were kept in the range below t<2s. Indeed, the glycerol experiments show that at longer lag times the MSD curves from the individual beads start to deviate from the average MSD curve. Microrheology experiments performed in calibrated glycerol solutions allowed to determine the spatial resolution of the experimental set up (microscope, camera and tracking software). Thermal motion measurements in glycerol and jellyfish ECM were performed under the same experimental conditions. We determined from microrheology experiments performed in glycerol solutions that our spatial resolution was about 3×10^{-5} µm², which is below the MSD measurements presented.

Microrheology experiments in slices of juvenile jellyfish

The microrheological experiments presented in the manuscript on juvenile jellyfish was performed *in vivo* under anesthetised conditions to be as less invasive as possible. On the other hand, the experiments on adult jellyfish were performed on dissected slices, because the adult jellyfish are large and it is impossible to directly visualise *in vivo* the mesoglea region with the thick vertical fibres just above their endoderm (see also Fig. 1).

The differences observed in micorhological results could be a consequence of these different experimental conditions. In particular, the mesogleal slices of adult jellyfish were stored overnight at 7 °C and beads were not exposed to a dynamic moving environment. Whereas the beads in juvenile jellyfish were placed into a dynamic ECM environment exhibiting high mechanical stresses during swimming contractions. These periodical stresses could have affected the final distribution of the beads in the fibrous network of juvenile jellyfish and modified the microrheological results.

To rule out a systematic bias in beads distributions, microrhelogical experiments were repeated in juvenile jellyfish under the same experimental conditions as for adult jellyfish: for each of 5 juvenile jellyfish a slice of mesoglea was dissected from the middle of the mesoglea, in the thick vertical fibres region, and microbeads were injected in these slices. The samples were stored at 7°C and the thermal motion of the microbeads was recorded one day after injection at room temperature. Thereby, the experimental protocol was identical for adult and juvenile jellyfish.

Microscopic observations the day following the injection revealed that the dynamic ECM environment of juvenile jellyfish didn't seem to have affected the final distributions of the beads. This is confirmed by the microrheological measurements shown in Fig. S2. Fig. S2 *A* shows the result from a typical experiment in a slice of mesoglea cut from a juvenile jellyfish. Fig. S2 *B* shows the result from a typical experiment *in vivo* in juvenile jellyfish mesoglea. Fig. S2 *B* is identical to Fig. 5 *A* presented in the manuscript. Fig. S2 shows that microrheological results obtained in juvenile jellyfish *in vivo* and in slices of mesoglea were similar.

Thereby, we have shown that the differences observed by microrheology experiments in adult and juvenile jellyfish presented in the manuscript are not due to different experimental conditions but could be explained by a gradual fibrous aggregation.

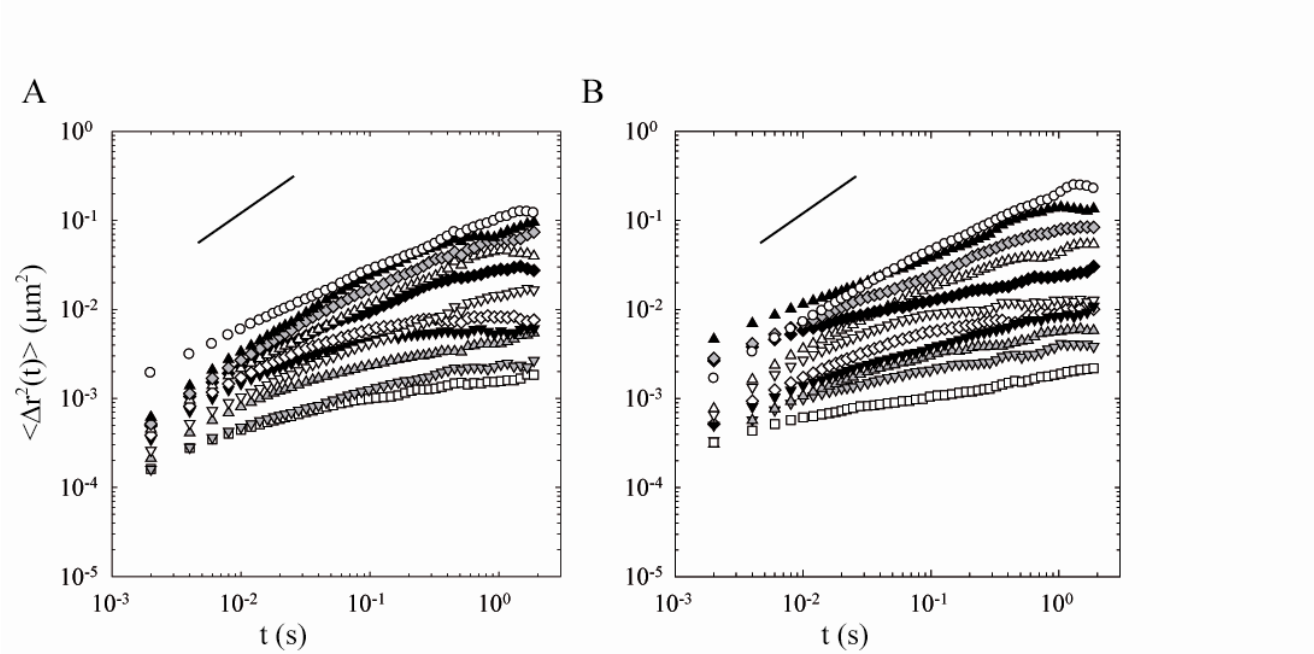

FIGURE S2 Microrheology control measurements in juvenile jellyfish. Time-averaged MSD of 1 µm microbeads embedded in the mesogleal ECM as a function of the lag time. The microprobes were injected and visualized together and each symbol represents the MSD of a different microbead. The solid line in each figure indicates a slope of one. (*A*) MSD of microprobes injected and tracked in a slice of mesoglea. (*B*) MSD of microprobes injected and tracked *in vivo* in the mesoglea. Microrheological results obtained in static or dynamic mesogleal tissues are similar.

Microrheology experiments with polystyrene microbeads

The surface chemistry of the beads can impact microrheological measurements. The microbeads used in our experiments were 1 µm amine-modified fluorescent microspheres. The fluorescent properties of these beads allowed to easily retrace the original place of injection for observations one day after injection.

We verified whether the surface chemistry of the beads did not affect our microrheological measurements. We repeated microrheological measurements with 1 µm polystyrene microbeads (4009A, Thermo Fisher Scientific, Karlsruhe, Germany) injected in the mesoglea of 5 adult and 5 juvenile jellyfish, using the same protocol as for the amine-modified microbeads, as described in the Material and methods section of the manuscript and in the Supporting Material.

Fig. S3 shows that microrheological results are similar using either amine-modified or polystyrene microbeads, in both adult and juvenile jellyfish. Therefore it is excluded that the differences observed by microrheology experiments in adult and juvenile jellyfish are due to a possible interaction between fibres and beads surface groups, but reveal an increase in the stiffness of microenvironments in the fibrous network.

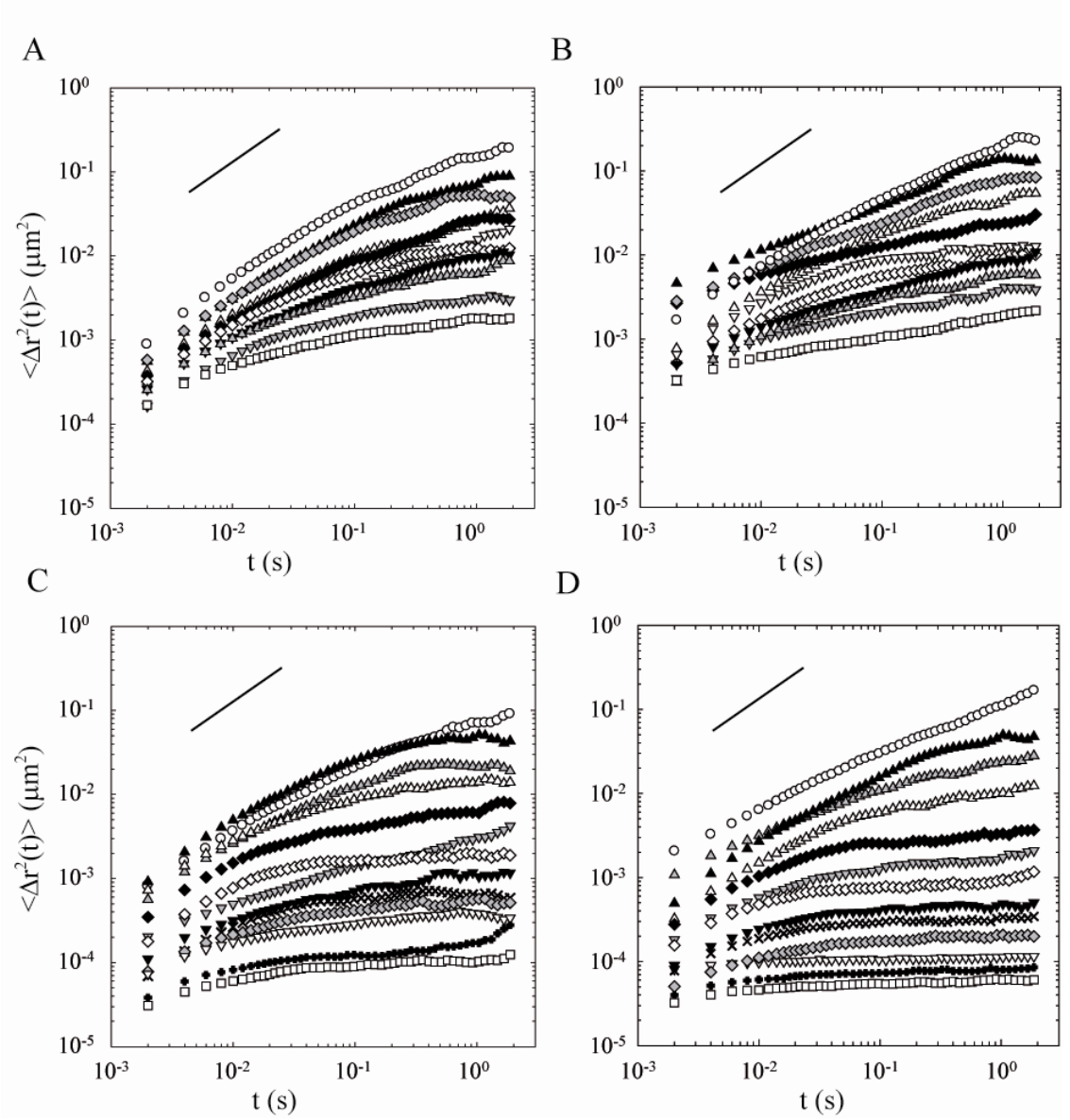

FIGURE S3 Microrheological control measurements using 1 µm microbeads with different surface groups. Time-averaged MSD of 1 µm microbeads embedded in the mesogleal ECM as a function of the lag time. The microprobes were injected and visualized together and each symbol represents the MSD of a different microbead. The solid line in each figure indicates a slope of one. (*A*) MSD of polystyrene microbeads in juvenile jellyfish. (*B*) MSD of amine-modified microbeads in juvenile jellyfish. (*C*) MSD of polystyrene microprobes in adult jellyfish. (*D*) MSD of amine-modified microprobes in adult jellyfish. Microrheological results obtained with different microbead surface chemistry are similar, in both juvenile and adult jellyfish.

Supporting references

1. Chapman, D. M. 1999. Microanatomy of the bell rim of Aurelia aurita (Cnidaria: Scyphozoa). *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 77:34-46.

2. Shaposhnikova, T., I. Matveev, T. Napara, and O. Podgornaya. 2005. Mesogleal cells of the jellyfish Aurelia aurita are involved in the formation of mesogleal fibres. *Cell Biol. Int.* 29:952-958.

3. Rasband, W. S. 1997-2007 ImageJ. Bethesda, MD: US National Institutes of Health. See http://rsb.info.nih.gov/ij/. Source code for the plugin is available at http://www.msc.univ-paris-diderot.fr/~olivier/ImageJ/; tracker_Install.zip; 29/03/2009.

4. Abou, B., C. Gay, B. Laurent, O. Cardoso, D. Voigt, H. Peisker, and S. Gorb. 2010. Extensive collection of femtolitre pad secretion droplets in the beetle Leptinotarsa decemlineata allows nanolitre microrheology. *Journal of the Royal Society Interface* 7:1745-1752.