New insights on the spatial confinement mechanism of nucleation of biogenic aragonite crystals from bivalve nacre

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Table S1 Summary of the nucleation sites of new tablets and controlling mechanisms during the growth of bivalve nacre in the literature

<table>
<thead>
<tr>
<th>species</th>
<th>Nucleation sites of new tablets</th>
<th>Controlling mechanisms</th>
<th>References</th>
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<tbody>
<tr>
<td><em>Pinctada radiata</em>, <em>Mytilus exustus</em>, <em>Anomia simplex</em></td>
<td>Random sites</td>
<td>Not mentioned</td>
<td>Bevelander and Nakahara (1969)¹</td>
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<td>Pelecypods (i.e. bivalves)</td>
<td>Random sites</td>
<td>Not mentioned</td>
<td>Wise (1970)²</td>
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<tr>
<td><em>Mytilus edulis</em>, <em>Nueula sulcata</em>, <em>Unio tumidus</em>, <em>Nautilus pompilius</em></td>
<td>On the margin of less soluble sectors of the underlying tablets</td>
<td>Not mentioned</td>
<td>Mutvei (1977)³, Mutvei and Dunca (2010)⁴</td>
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<tr>
<td><em>Hyriopsis cumingii</em></td>
<td>The conjunction of the underlying tablets</td>
<td>Not mentioned</td>
<td>Xie et al. (2010)⁵</td>
</tr>
<tr>
<td><em>Pinctada margaritifera</em></td>
<td>Near and at the boundary between the underlying tablets</td>
<td>Alveolar matrix at the boundary between the underlying tablets</td>
<td>Rousseau et al. (2005)⁶</td>
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<tr>
<td><em>Pinctada margaritifera</em></td>
<td>Triple-junction of three underlying tablets</td>
<td>Acidic proteins at the triple-junction of three underlying tablets</td>
<td>Rousseau et al. (2009)⁷</td>
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<tr>
<td><em>Pinctada fucata</em></td>
<td>Near and at the boundary between two underlying tablets</td>
<td>Mineral bridges through the poles of the inerlamellar membranes (ILMs)</td>
<td>1972 Wada⁸</td>
</tr>
<tr>
<td>Theoretical modeling of the growth of the sheet nacre</td>
<td>Random positions</td>
<td>Mineral bridges through the poles of the ILMs</td>
<td>Coppersmith et al. (2009)⁹</td>
</tr>
<tr>
<td><em>Nucula sulcata</em></td>
<td>On the margin of the underlying tablets</td>
<td>Mineral bridges through the broken holes of the ILMs caused by the squeezing of underlying three approaching tablets</td>
<td>Checa et al. (2011)¹⁰</td>
</tr>
</tbody>
</table>
Fig. S1 X-ray diffraction (XRD) patterns of the powdered samples (a) and cross section (b) of the area 3 cut along the white line in Fig 1a. (c) – (f) correspond to the close up views of the boxed area c-f in (b). The shell length of this sample is 1.7cm (not the same shell as in Fig. 1a).

Please note that:

(1) The periostracum is amorphous, while the whole shell (including the prismatic layer and nacre) is of aragonite.

(2) The periostracum, prismatic layer, and nacre are about $16 \mu m$, $9 \mu m$, and $258 \mu m$ thick, respectively, in this sample, which usually increase with the shell size.

(3) Along the vertical direction, from the outer to the inner of the shell, the nacreous tablets vary gradually in thickness and curvature of their top surfaces, as detailed in our previous work\(^{11}\). Particularly, the slow-growing nacre exposed on the area 3 of the inner surface consists of flat tablets, which are similar to those of Mytilus edulis nacre.
Fig. S2 Statistical histograms of the mature nacreous tablets size and center-to-center distance of nuclei.

Please note that:

1. The mature nacreous tablets size and center-to-center distance of nuclei are not fixed, there is a certain range of fluctuations, namely the standard deviation;

2. The size of mature nacreous tablets of *Perna viridis* is small, the results are $1.876 \pm 0.465 \mu m$ ($n = 577$) (area 1), $1.823 \pm 0.496 \mu m$ ($n = 602$) (area 3), which consistent with the center-to-center distance of nuclei, namely $1.792 \pm 0.183 \mu m$ ($n = 115$);

3. When the distance of new nuclei is close, they will merge into one tablet in the growth process (circles in Fig. S3). Thus, the size of mature nacreous tablets still remains uniform.
Fig. S3 Top view of SEM image showing two or several close tablets merge into one tablet (the circles). Samples sonicated in distilled water for 5 minute.
Fig. S4 Plan views of nacreous tablets of bivalves. (a) *Hyriopsis cumingii*; (b) *Mytilus coruscus*; (c) *Mytilus edulis*; (d) *Brachidontes setiger*; (e) *Modiolus kurilensis*; (f) *Pinctada martensi*.

Please note that:
In top views, the small new tablets (i.e. nuclei) are always nucleate on the margin of the underlying tablets: on the periphery of the isolated tablets (red circles in Fig. S4 b-c and e-f), close to the boundaries (Fig. S4 a: red circles), or close to the triple junction (Fig. S4 d: red circles).
Please note that:
The margin of the tablet is decorated with weakly crystalline nanoparticles (Fig. S5c: dashed lines). While close to center, the HRTEM image shows continuous lattice fringes of aragonite (110) (d=0.42nm) over the large area examined (Fig. S5d), which indicated the single crystal of aragonite, consistent with the SAED patterns (Fig. S5b), which show clear and regular diffraction spots of aragonite.
References: