Supporting Information

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SI Materials and Methods

Sample Preparation for Immunolocalization. ***Polyclonal antibody production.*** Custom-made polyclonal antibodies were raised against the following peptides derived from unique sequences from each of the four coral acid-rich proteins (CARP) genes by Thermo Scientific Pierce Custom Antibody Services: CARP 1, GDHLKPGHSEDEH; CARP 2, SAPVENEIRIRGPKL; CARP 3, DTHEDKARNYVPES; and CARP 4, DEGKVGEFLDLKIKIKSKD. The selected synthetic peptides were injected into two rabbits (0.25 mg) and boosted (0.1 mg) four times within 120 d (14 d, 28 d, 42 d, 95 d) after the first injection. The final bleeding was conducted 14 d after the last injection, and the crude sera were purified. We used these antibodies and those raised against the N terminus of human β-actin actin (PA5-16914; Pierce) and a carbonic anhydrase (CA) (1) to analyze the spatial arrangement of the proteins. We first checked the specificity of these antibodies against both pure CARPs and extracted skeletal organic matrix (SOM) protein complex. All the *Stylophora*-specific antibodies used in this study showed a distinct immunoreactivity (2) (Fig. S1). These antibodies were then used for histological analyses of the spatial organization of distinct proteins within the animal and skeleton.

A primary antibody raised against a cadmium-specific CA (1) was tested against whole coral protein extract as well as glycosylated and deglycosylated SOM by using a protein deglycosylation mix (P6039S; New England BioLabs), In addition, a custom-made polyclonal antibody was raised against the peptide (DYETKPTYTIKVTATDRK) derived from a unique sequence of cadherin (protein P1 in ref. 2; accession no. KC509948) by Thermo Scientific Pierce Custom Antibody Services as described earlier. The final bleeding was conducted 14 d after the last injection, and the crude serum was purified and then tested against whole coral protein extract. Anti-CDCA binds a protein of ∼40 kDa in glycosylated SOM and two proteins of ∼40 and 35 kDa in deglycosylated SOM. STPCA-2, a CA previously detected in *Stylophora pistillata* skeletal organic matrix (2), has been predicted to have a size of 35 kDa and is likely glycosylated (3) (accession no. EU532164.1). Appearance of two bands in deglycosylated samples suggests that the deglycosylation reaction was incomplete (Fig. S1*A*). Anti-cadherin binds a protein of >150 kDa in total *S. pistillata* protein extract (Fig. S1*B*). Based on strong similarity to cadherins from other stony corals, we previously proposed that the complete cadherin is ≥2,000 aa in length (4), which corresponds to ∼200 kDa.

Immunohistochemical localization. *S. pistillata* nubbins were fixed for 24 h in Z-fix (Anatech) diluted 1:4 in seawater followed by slow decalcification at room temperature using 10% EDTA at neutral pH. After complete decalcification, the tissue was returned to fresh Z-fix solution for 2 h before transferring to 70% ethanol. Tissues were then embedded in paraffin. This method helps to preserve antigens for IHC study. All IHC was performed by using a Ventana Medical Systems Discovery XT automated immunostainer.

Cross-sections (4 μm thick), were cut and deparaffinized, and antigen retrieval was performed by using CC1 (Cell Conditioning Solution; no. 950–124; Ventana Medical Systems). H_2O_2 blocking (3%) was applied and incubated at room temperature for 20 min before adding primary antibody. Primary anti-CARPs 1–4, -cadherin, -actin, and -CA were applied at 1:800, 1:50, 1:200, 1:2,000, 1:500, 1:200, and 1:5,000 dilution, respectively, and incubated at 37 °C for 1 h. Prediluted universal secondary antibody (no. 760–4205; Ventana Medical Systems) was applied and incubated at 37 °C for 12 min, followed by application of the chromogenic detection kit DABMap (no. 760–124; Ventana Medical Systems). Slides were counterstained with hematoxylin, then dehydrated and cleared before coverslipping with xylene. To check the specificity of the staining, control experiments were performed similarly without the first antibody step, diluted as describe earlier.

Immunogold localization. After spontaneous dissociation of the tissue of *S. pistillata* nubbins (5), skeletons were dried overnight at 60 °C. Thin sections of the skeleton were embedded in Buehler C-D epoxy and then ground and polished to a final thickness of ∼30 μm by using 1-μm diamond polish. Immunogold labeling was preformed on mirror-polished sections as described previously (6, 7), with some modifications. Briefly, embedded and polished thin sections were cleaned with dilute sodium hypochlorite (0.2 wt% active chlorine for 10 min) for removing antigens spread on the surface, rinsed with water, and slightly etched with EDTA. Etching with EDTA 1% (wt/vol), pH 7.5, for 3 min allows the exposure of epitopes and their subsequent recognition by the antibodies. All preparations were blocked at least 30 min with filtered gelatin (0.5–1% wt/vol) dissolved in Tris buffered saline (TBS) solution readjusted to pH 7.5 with dilute sodium hydroxide solution to avoid further dissolution of the calcium carbonate. This operation precludes nonspecific bindings of antibodies. The sections were then incubated overnight at 4 °C with an antibody raised against CARPs 1–4, cadherin, actin, or CA, diluted as follows: 1:1,000 (CARPs 1–2), 1:500 (CARPs 3–5, actin), 1:100 (cadherin), and 1:5,000 (CA) in a solution of 1% gelatin dissolved in TBS solution, pH 7.5, containing Tween 20 (0.05% vol/vol). Control-sections were incubated without primary antibody (Fig. 3). The preparations were extensively rinsed with TBS–Tween solution (six times, 10 min) followed by a 3 h incubation with a secondary antibody (goat anti-rabbit coupled to 18-nm gold particles; no. 111215144; Jackson ImmunoResearch) diluted 1:20 in 0.05% TBS–Tween solution, pH 7.5. After extensive rinsing with TBS–Tween solution (six times, 10 min), the preparations were briefly rinsed with Milli-Q water, pH 8, and dried before being silver-enhanced for 5 min with a silver enhancement kit (Sigma) following the methods of Robinson et al. (8). Staining was terminated by rinsing the preparations with water (six times for 1 min each).

Sections were subsequently dried at 45 °C overnight and carbon-sputtered (10 nm thickness) for field emission scanning EM observations in the back-scattering electron mode, with a 10-KeV beam (Sigma; Zeiss). In this mode, elements with higher atomic number scatter more electrons, thus appearing lighter. Immunogold particles are displayed as the brightest spots, the size of which is increased by silver enhancement (∼100 nm); the mineral phase is lighter than the organic phase.

Atomic Force Microscopy. Atomic force microscopy (AFM) measures surface topography and properties at nanometer scale. AFM simultaneously produces maps of the surface topography (height images or derived amplitude images) and phase images. Phase images can be used to distinguish organic vs. inorganic components of a material.

AFM images were collected on a mirror-polished clean surface at room temperature by using a tapping mode with a multimode scanning probe microscope by using a DI Nano IIIa instrument (Veeco). The tip–surface interaction was reduced by using the lowest tip force to ensure that the images were authentic representations of the surfaces. Phase images were generated by AFM cantilever frequency shift as the difference between organic matrix and inorganic aragonite compositions based on their viscoelastic properties and adhesion forces.

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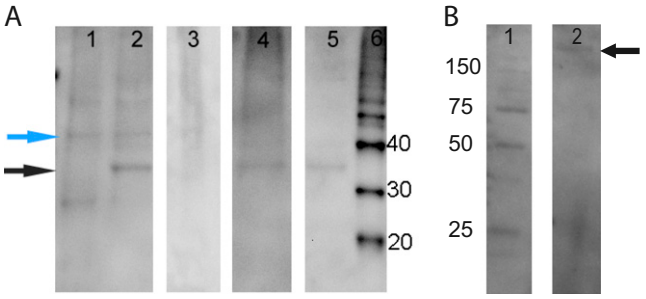

Fig. S1. (*A*) Western blot of anti-CDCA against glycosylated and deglycosylated whole coral protein extract (lane 1 and 2, respectively); glycosylated and deglycosylated insoluble SOM (lane 3 and 4, respectively); and deglycosylated soluble SOM (lane 5), molecular weight (lane 6). Blue and black arrows are likely glycosylated and deglycosylated CA, respectively. (*B*) Western blot of anti-cadherin against whole coral protein extract (lane 2), molecular weight (lane 1). Black arrow is likely cadherin.