SUPPLEMENTARY INFORMATION FOR: "STRUCTURAL AND THERMODYNAMIC CHARACTERIZATION OF THE SELF-ADHESIVE PROPERTIES OF HUMAN P-CADHERIN."

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SUPPLEMENTARY MATERIALS AND METHODS.

2.1 CLONING, EXPRESSION AND PURIFICATION.

A construct of containing the first and second ectodomains of the mature human P-cadherin protein (residues 1-241, abbreviated EC12) was cloned in a Champion pET-SUMO vector with forward primer 5-GATTGGGTGGTTGCTCCAATATC-3', and reverse primer 5'-CTACCTCTGCACCTCATGGC-3'. *Escherichia coli* Rosetta2 (DE3) cells transformed with plasmid were grown at 37°C in LB medium containing 50 μg ml^{-1} kanamycin and 34 μg ml^{-1} chloramphenicol. Expression was induced with 0.5 mM IPTG when OD at 600 nm reached a value of 0.4. Temperature was reduced to 20 °C, and cells were harvested 12 hours later by centrifugation (7,000 × *g*, 12 min, 4 °C). Cell-pellet was suspended in buffer A (20 mM Tris, 500 mM NaCl, 3 mM $CaCl_2$, and 20 mM imidazole at pH 8.0), and subsequently lysed with an EmulsiFlex C-5 homogenizer (Avestin, Ontario, Canada). Lysate was centrifuged at 40,000 × *g* for 30 min at 4 °C. Supernatant was loaded onto a Ni-NTA column (Qiagen, Valencia, CA) equilibrated with buffer A. Separation was achieved by immobilized metal-ion affinity chromatography (IMAC). EC12 was eluted with buffer A containing 300 mM imidazole. The His_6-SUMO tag of EC12 was removed by treatment with His_6-Ulp1 protease during 4 hours at 4°C in a buffer containing 20 mM Tris, 150 mM NaCl, and 3 mM $CaCl_2$ at pH 8.0. Upon processing of the SUMO-tag, the N-terminal end of EC12 turns to a dimerization-active state. Alternatively, the buffer used during the cleavage step did not contain Ca^{2+}, but instead it was supplemented with 3 mM EDTA. This alternative methodology was employed to study the dimerization of P-cadherin in the absence of Ca^{2+}. EDTA was removed by dialysis (o/n) before the next purification step. EC12 was further purified by IMAC, followed by size exclusion chromatography (SEC) in a superdex200 column (GE Healthcare, Piscataway, NJ) equilibrated with 20 mM Tris, and 300 mM NaCl at pH 8.0. When necessary, the buffer was supplemented with 3 mM $CaCl_2$. Purity was at least 95% homogeneous as judged by SDS-PAGE (not shown). Purified protein was dialyzed in the appropriate buffer before analysis. The mutein W2A was prepared with a Quick-Change kit following the instructions of the manufacturer. The sequence of the forward and reverse primers used to prepare mutein W2A were 5'-GGTGGTGATGCTGTGGTTGC-3', and 5'-GAGCAACCACAGCATCACCAC-3', respectively. Protein expression and purification were carried out as above.

2.2. ANALYTICAL SEC.

The equilibrium between monomeric and dimeric forms of P-cadherins was analyzed by analytical SEC with a superose12 10/300 column (GE Healthcare) at a flow rate of 0.5 ml min^{-1}. Equilibration buffer contained 10 mM HEPES and 150 mM NaCl at pH 7.5. In experiments requiring Ca^{2+} the buffer was supplemented with 3 mM $CaCl_2$. Typical samples contained 250 μL of protein at a

concentration of 50 μM. Protein was monitored at a wavelength of 280 nm. Void volume was 7.8 ml as determined by a control experiment with blue dextran.

2.3. Small-angle X-ray Scattering (SAXS).

SAXS measurements of EC12 in the presence of Ca^{2+} were carried out at beamline BL-10C of the Photon Factory (Tsukuba, Japan) [1]. A detailed description of SAXS measurements is provided elsewhere [2]. Briefly, raw data between Q values of 0.018 and 0.3 $Å^{-1}$ were measured using a R-AXIS VII image plate (Rigaku, Tokyo, Japan) at a wavelength of 1.488 Å with a sample-to-detector distance of 90 cm. Protein concentration was 5.0 ~ 9.0 mg ml^{-1}. The average scattering intensities corresponding to of 3-4 experiments were corrected by subtracting the background signal produced by the buffer. Theoretical profiles and curve fitting were performed with the program FoXS [3] of the CHIMERA suite [4].

2.4. Asymmetrical flow field-flow fractionation (AF4).

Protein size analysis was carried out in a Wyatt Eclipse separation system (Wyatt Technology, Santa Barbara, CA). The instrument was equipped with UV-visible and multi-angle light scattering detectors. The separation was performed with a membrane of cellulose with a nominal molecular weight cut-off of 10 kDa. First, protein samples were concentrated with a focused flow of 3 ml min^{-1}, followed by an elution step in which the cross flow decreased linearly from 3 ml min^{-1} to 0 ml min^{-1} in 15 min. Channel flow was kept at a constant rate of 1 ml min^{-1}. Each experiment required between 150 and 210 μg of protein. Buffer contained 10 mM HEPES and 150 mM NaCl at pH 7.5, and was supplemented with 3 mM $CaCl_2$ when necessary. The molecular weight was determined from the data collected with the multi-angle light-scattering data, and analyzed with the Zimm plot in the program ASTRA ver. 5.3 provided by the manufacturer [5-7].

2.5. Differential scanning calorimetry (DSC).

Heat capacity curves were measured on a VP-DSC ultrasensitive scanning calorimeter (MicroCal, Northampton, MA). Protein samples were prepared at a concentration of 100-200 μM in a buffer composed of 10 mM HEPES, and 150 mM NaCl at pH 7.5. When necessary, the equilibration buffer was supplemented with 3 mM $CaCl_2$. Scan rate was 1 K min^{-1}. Data were analyzed with the software package ORIGIN. To obtain the thermodynamic parameters T_M and ΔH the contribution of the buffer was subtracted, normalized by protein concentration, and fitted to a non two-state (in Ca^{2+}-free conditions) or a two-state (with Ca^{2+}) thermal transition models.

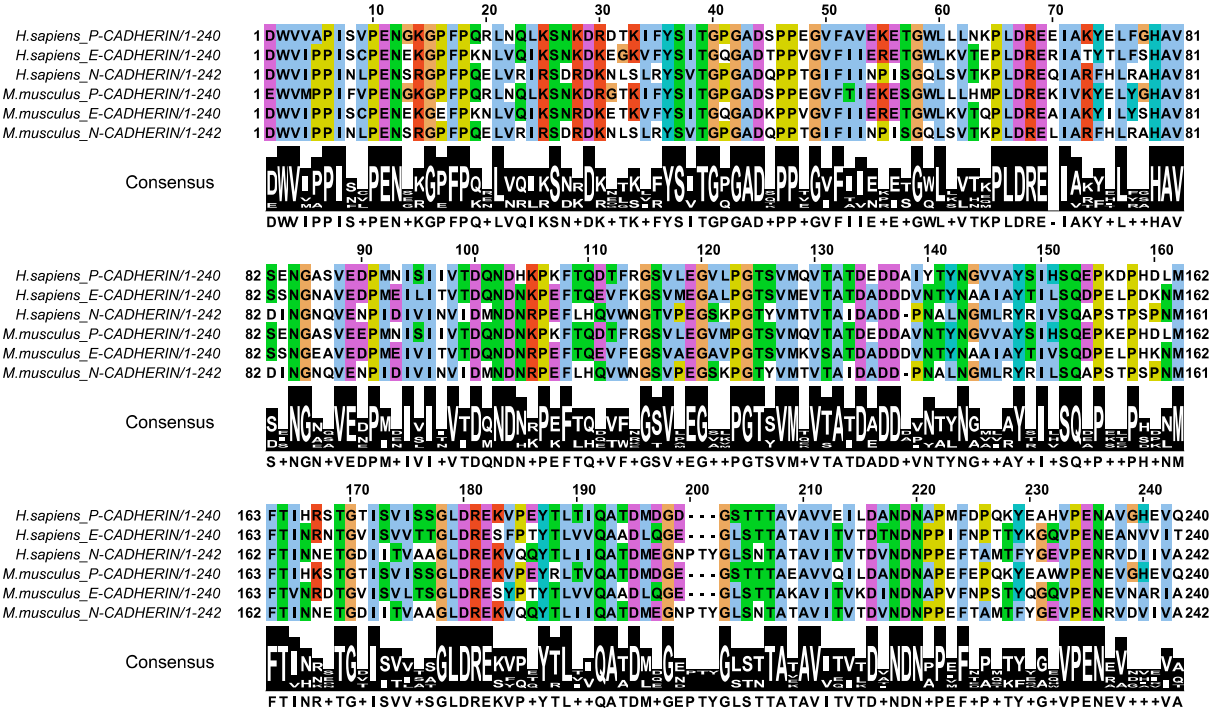
2.6. Isothermal Titration Calorimetry (ITC).

Thermodynamic parameters of the interactions between P-cadherin and Ca^{2+} were determined with an ITC200 instrument (GE healthcare) at 20 °C. Purified samples of P-cadherin EC12 were dialyzed overnight in a buffer containing 10 mM HEPES, 150 mM NaCl, and 3 mM EDTA at pH 7.5, followed by equilibration with the same buffer containing no EDTA. Aliquots from a solution containing 4.2 mM $CaCl_2$ were injected stepwise into a cell filled with a solution of EC12 at a concentration of 68 μM, at intervals of 600 s. Titration curves were fitted to a one-site binding isotherm with the program ORIGIN.[8]

2.7. Intrinsic Fluorescence.

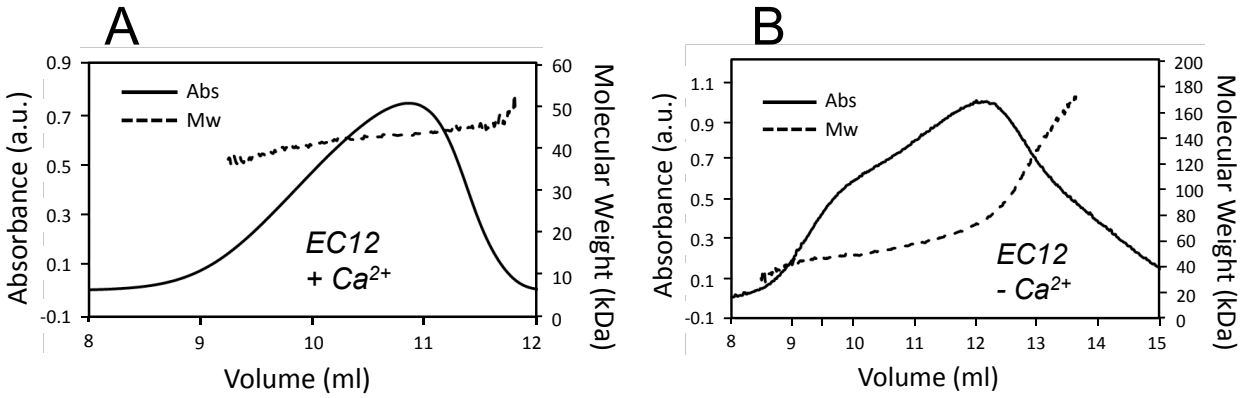
The fluorescence emission spectrum of P-cadherin was monitored with a spectrofluorometer F-2500 (Hitachi, Japan) in 1-cm cuvettes. The excitation wavelength was set at 295 nm to minimize the contribution of tyrosine residues. Data was collected between 300 and 400 nm at 25 °C. Protein concentration was adjusted to 3 μM in a buffer containing 150 mM NaCl, and 10 mM HEPES at pH 7.5. When necessary, the buffer was supplemented with 3 mM $CaCl_2$.

Supplementary Figure S1



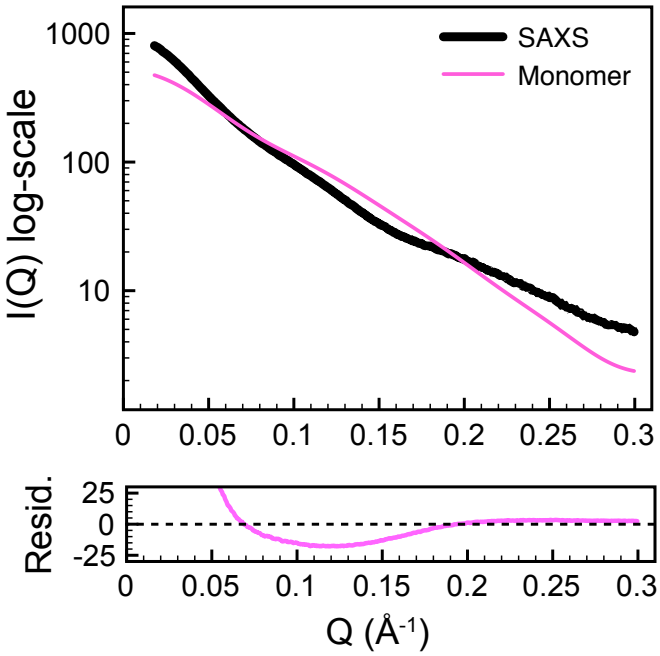
Supplementary Figure S1. Sequence alignment of EC12 of human P-cadherin with classical E- and N-cadherins. Sequence alignment was calculated by the ClustalW server.[9] The figure was prepared with Jalview.[10]

Supplementary Figure S2



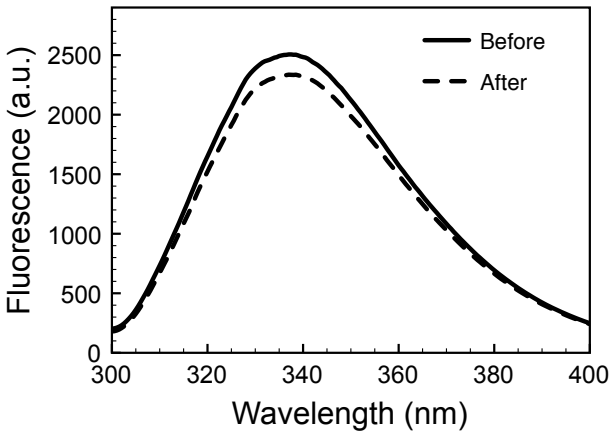
Supplementary Figure S2. Molecular weight of P-cadherin determined by A4F. Elution profile (solid trace) and molecular weight distribution (dashed trace) of EC12 **(A)** in the presence of Ca^{2+} or **(B)** in the absence of Ca^{2+}. The molecular weights in the presence and absence of Ca^{2+} were 43 and 53 kDa, respectively. Data between elution volumes of 9.3 and 11.8 ml was used for molecular weight calculation.

Supplementary Figure S3



Supplementary Figure S3. Comparison between the SAXS profile of P-cadherin EC12 (black) and the theoretical profile of only one monomer of E-cadherin in the crystal structure of the ss-dimer (PDB entry code 2O72). The theoretical profile was calculated with the program FoXS of the Chimera suite.

Supplementary Figure S4



Supplementary Figure S4. Intrinsic fluorescence of EC12 in Ca^{2+}-depleted samples. The dimeric form of P-cadherin were obtained in the absence of Ca^{2+} by two different methods. The solid line corresponds to data obtained when Ca^{2+} was removed before the dimerization of the protein (i.e. before treatment of the SUMO-EC12 construct with the protease Ulp1). The broken line corresponded to samples of EC12 depleted of Ca^{2+} after P-cadherin dimerization (i.e. samples treated with EDTA after protein purification). Excitation wavelength was set to 295 nm to minimize the contribution of tyrosine residues. Data was collected at room temperature in a buffer containing 150 mM NaCl, and 10 mM HEPES at pH 7.5. Protein concentration was adjusted to 3 μM.

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