Title

Effective mechanical potential of cell–cell interaction explains basic structural units of three-dimensional morphogenesis

Short title

Effective potential of cell–cell interaction on morphogenesis

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Keywords

morphological diversity, mechanics, multi-cellular system, effective potential of cell–cell interaction, early embryogenesis

Abstract

Mechanical properties of cell–cell interactions have been suggested to be critical for the emergence of diverse three-dimensional morphologies of multicellular organisms. Mechanical potential energy of cell–cell interactions has been theoretically assumed, however, whether such potential can be detectable in living systems remains poorly understood. In this study, we developed a novel framework for inferring mechanical forces of cell–cell interactions. First, by analogy to coarse-grained models in molecular and colloidal sciences, cells were approximately assumed to be spherical particles, where microscopic features of cells such as polarities and shapes were not explicitly incorporated and the mean forces (i.e. effective forces) of cell–cell interactions were considered. Then, the forces were statistically inferred from live imaging data, and subsequently, we successfully detected potentials of cell–cell interactions. Finally, computational simulations based on these potentials were performed to test whether these potentials can reproduce the original morphologies. Our results from various systems, including Madin-Darby canine kidney (MDCK) cells, *C.elegans* early embryos, and mouse blastocysts, suggest that the method can accurately infer the effective potentials and capture the diverse three-dimensional morphologies. Importantly, energy barriers were predicted to exist at the distant regions of the interactions, and this mechanical

property of cell–cell interactions was essential for formation of cavities, tubes, cups, and two-dimensional sheets. Collectively, these structures constitute basic structural units observed during morphogenesis and organogenesis. We propose that effective potentials of cell–cell interactions are parameters that can be measured from living organisms, and represent a fundamental principle underlying the emergence of diverse three-dimensional morphogenesis.

Introduction

The mechanical properties of interactions between objects are among the most fundamental parameters of various physical, chemical, and biological phenomena at a wide range of spatial scales in the molecular, colloidal, cellular, and astrophysical sciences. Interactions among particulate matter such as ions, molecules, and colloids are primarily mediated by electromagnetic forces, and the properties of these interactions, including attractive and repulsive forces, substantially affect the dynamics and stability of systems [1,2]. In multi-cellular living systems, various three-dimensional morphologies are observed. The emergence of these diverse morphologies is thought to be primarily dependent on the mechanical properties of the constituent cells. In particular, mechanical properties of cell–cell interactions are involved in morphogenetic events such as epithelial cell movement and cell sorting [3–7]. However, the mechanical basis of morphogenesis, which gives rise to a variety of structures, remains to be elucidated, and it is not yet known whether any unifying principle can explain the morphological diversity of organs and tissues.

The mechanical properties of cell–cell interactions are regulated by various factors. In epithelial cells, whose shapes are typically columnar polygons, cell–cell adhesion energy is controlled by cadherin family proteins (Fig. S1A-i and B) [3,4,8], whereas

actomyosin proteins counteract adhesion through the indirect effect of cell surface tension (Fig. S1A-i and B) [9,10]. Moreover, cellular polarities such as apico-basal polarity are linked to biased localization of cadherin proteins, leading to directional differences in properties of cell–cell interactions. By contrast, in mesenchymal cells, which are unstructured shapes, in addition to cadherins, cell–cell interactions are regulated by indirect interactions through extracellular matrix (i.e. substrate) (Fig.S1A-i) [11–13]. Therefore, cell–cell interactions entail many microscopic parameters determined by various proteins and cellular processes, resulting in very complex physics with many degrees of freedom (Fig.S1A-i). To elucidate the principles underlying morphological diversity, it is essential to investigate how the integrated effects of many microscopic parameters can be described as simple meso or macroscopic parameters (Fig. S1A-ii).

In non-living materials such as ions, molecules, and colloids, interactions among particulate matter are determined by electron clouds (i.e. quantum state), which have complex shapes (Fig. S1A-iii) [14]. On the other hand, mechanical potential energies of particle–particle interactions have been successfully described as a function of distances of the interactions, e.g. the Lenard–Jones potential which is usually applied to rare gas atoms (Fig. S1A-iv). Therefore, it can be interpreted that the microscopic components conferred from electron clouds can be integrated into the potentials of particle–particle

interactions as mesoscopic parameters. Typically, such distance–potential curve contains repulsive forces around regions of short distances, which are provided from excluded volume effect of the particles, and attractive forces around regions of middle and far distances (Fig. 1B, and Fig. appx 1). Intriguingly, in the case of non-spherical or polarized molecules including water and amino acids, potentials of their interactions have been sometimes defined as a function of distances where the molecules are assumed as isotropic particles. These coarse-grained models have been succeeded to explain overall behaviors of the systems with a trade-off of microscopic accuracy [14].

In atomic and molecular sciences, how various microscopic components are integrated into potentials of particle–particle interactions have been theoretically established well in relatively simple systems [2,14], which we call here a bottom-up approach. On the other hand, there are top-down approaches to obtain the potentials. Potential energies of whole systems according to atomic or molecular configurations are calculated from simulations on the basis of quantum chemistry which is more accurate than classical mechanics. Then, by using the calculated potential energies and the configurations as reference data, potentials of particle–particle interactions have been inferred through trial-and-errors searches or machine learning [1,2,15]. The simplest example is a usage of radial distribution functions as reference data, because the functions mean

125 probability of particle positions along particle–particle distances, which can be linked to
126 potential energy (Fig. appx 1 and SI, Appendix section 12-1) [2]. Because these potentials
127 are the outcomes from various microscopic components, they are "effective" potentials
128 of particle–particle interactions. Using these potentials obtained from the bottom-up or
129 top-down approaches as an integrative parameter, many molecular dynamics simulations
130 have been successfully performed to understand the dynamics and shapes of various
131 systems (Fig. S1) [16].

132 The potential of cell–cell interaction is also conceptually well recognized, and
133 has been used in theoretical studies (Fig. 1B and S1A-ii) [11,17–25]. The potential energy can
134 be defined regardless of whether two cells directly contact each other. Although adhesive
135 forces between two isolated cells have been measured (i.e. forces required to dissociate
136 one cell from the other) [6,26], they have neither been measured nor inferred in more
137 complex multi-cellular systems, which differ from isolated cells. For instance, cell shapes
138 in multi-cellular systems are entirely different from those of isolated cells: they can be far
139 from spherical shapes (e.g. flattened shape). Apico-basal polarity cannot be defined in
140 isolated cells but can be in cell sheets whose three-dimensional directions are determined
141 by extracellular matrix (ECM) or substrate (Fig. 1 and S1). If effective potentials of cell–
142 cell interactions could be defined as a parameter integrating these various factors, the

effective potentials would be useful to explain shapes and dynamics of multi-cellular systems. However, it is challenging to identify all these factors and measure the values of their related-parameters, which is a bottleneck to obtain effective potentials through bottom-up approaches.

In this study, by analogy to non-living materials (Fig. 1), we tried a top-down approach: we developed a method for statistically inferring the effective potential of cell–cell interactions using nuclear tracking data from live imaging as reference data, which are relatively easy to obtain. Thus, shapes and polarities of cells were not explicitly considered at this moment, whose effects we tried to decode after acquiring inference results (Fig. 1I). Our method was validated by using various artificially-generated data with or without external factors such as egg shells, cavities, and traction forces exerted between cells and substrates. Then, we tested whether effective potentials of cell–cell interactions are detected in living systems. We applied this method to relatively small multi-cellular systems (4–350 cells) including mouse pre-implantation embryos bearing cavities and early embryos of the nematode *Caenorhabditis elegans* bearing egg shells. We clearly detected effective potentials as a function of cell–cell distances in these systems. Further theoretical analyses suggested that effective potential represents a unifying principle capable of explaining various structures including cell aggregates,

cavities, tubes, cups (round and hollow shapes), and two-dimensional sheets (Fig. 1H). Because these structures are formed prior to the morphogenesis of complex tissue structures, we consider these as basic structural units during three-dimensional morphogenesis. We also describe the differences in these potentials between living and non-living materials, as well as among biological species.

Theory and principle for inferring effective potential

Overview of strategy for inferring effective potentials/forces of cell–cell interaction

To measure or infer the effective potentials or forces of cell–cell interactions *in vivo*, we developed a particle-based cell model in which attractive or repulsive forces are exerted between each pair of cells (Fig. 1A, B and D). This coarse-grained model does not explicitly incorporate microscopic features of cells such as cell shape, polarities, size, heterogeneity, cell type or origin (e.g., epithelial, mesenchymal, or blastomere cells), etc. at this moment (Fig. 1C, D and S1A). Simultaneously, we obtained time series of three-dimensional cell positions from confocal microscopic images (Fig. 1E) and statistically inferred the forces of cell–cell interactions by systematically searching for force values that could reproduce the cell movements observed *in vivo* (Fig. 1F). Since the inferred forces are the outcome from the integrative effects of the microscopic features of cells, we call the forces "effective forces". To validate the method, we utilized artificial cell movement data generated by performing simulations under given potentials of cell–cell interactions, and confirmed that the inferred forces were consistent with the given-potentials. The details of each part of the analysis are explained in the following sections.

Particle-based cell models

185 In our model, particles interact with each other and attractive or repulsive forces

186 (F_i) are assumed (Fig. 1D), where i is an identifier for particle–particle interactions. In

187 three-dimensional cellular systems with no attachment to substrates, we did not assume

188 persistent random walks which are originated from cellular traction forces on substrates

189 [27,28].

190 The equation of particle motions is defined below. In cellular-level phenomena,

191 viscous drag force or frictional force provided by the surrounding medium or tissue is

192 dominant, as expected from the low Reynolds number; consequently, the inertial force is

193 negligible in general [5,11,13,29,30]. The forces in such a system can be assumed to be

194 correlated with the velocities of the objects [5,13,24,25,31]. Thus, the velocity (V_p) of a particle

195 is calculated by the net force (F_p) exerted on the particle as follows:

196 $$V_p = F_p / \gamma \qquad \text{(Eq. 1)},$$

197 where p is an identifier for particles and γ is the coefficient of viscous drag and frictional

198 forces. F_p is the summation of F_i: $F_p = \sum_{i=1}^{I} \delta(p) F_i$, where I is the total number of

199 interactions, and in the case that the ith interaction is formed with the pth particle, $\delta(p)$ is

200 1, otherwise $\delta(p)$ is 0 (Fig. 1D). We assumed the simplest situation, i.e., γ is constant

201 (=1.0). Thus, by giving the values of F_i from, for instance, a distance–potential curve, a

202 simulation can run. The influence of these assumptions on simulation results is discussed

203 in SI (Section 4-4, 4-6-2, and 7).

204

205 *Data acquisition of time series of cell positions*

206 To define cell positions, we focused on nuclei, because they are most easily
207 imaged by microscopy (Fig. 1E). In fact, nuclear detection followed by temporal tracking
208 has been performed in a wide range of organisms from *C. elegans* to mammals, and these
209 data are accumulating [32–36]. We utilized publicly available nuclear tracking data of a
210 developing embryo of *C. elegans* [32]; nuclear tracking data of developing mouse embryos
211 and MDCK cultured cells were obtained in this study (SI, Section 2, 8, and 11 with text
212 data). Procedures for tracking cell division are described in SI (Section 3) and Figure S2B.

213

214 *Development of method for inferring effective potentials/forces of cell-cell interaction*

215 In the case of ions, molecules, etc., radial distribution functions are often
216 measured and used to infer the effective potentials of their interactions (Fig. appx 1 and
217 SI, Appendix section 12-1) [1,2]. Although this method is simple and applicable to
218 thermodynamically equilibrium systems, it is not suitable for non-equilibrium systems,
219 including morphogenetic events. To infer the effective potentials of non-equilibrium
220 systems, we fitted the particle-based cell model to the time series of nuclear positions in

221 the spirit of data assimilation, where the fitting parameters are forces between all pairs of
222 cell–cell interactions (the total number is I, as previously defined) for each time frame.
223 Data assimilation is a technique to solve an inverse problem for a simulation-based model
224 [37,38]. One of well-known model fittings is based on least squares in combination with a
225 linear model (function-based model), resulting in inference of parameter values of the
226 linear function. In data assimilation, models to be fitted are simulation-based models
227 instead of function-based models, whereas least squares are also often used to infer
228 parameter values which are usually numerically solved (Fig. appx 3 and SI, Appendix
229 section 12-3). Through repeated cycles of simulations of the model, we systematically
230 searched for the values of the effective forces between all pairs of cell–cell interactions
231 that minimized the differences (i.e. corresponding to least squares) between the particle
232 positions in the simulations and the *in vivo* nuclear positions (Fig. 1, and S2C). The
233 differences (G_{xyz}) to be minimized are defined as follows (SI, Section 4-1 and 4-2).

234 $$G_{xyz} = \sum_{t=1}^{T} \sum_{p=1}^{P(t)} \frac{\left\{x_p(t) - x_p^{\mathrm{ref}}(t)\right\}^2 + \left\{y_p(t) - y_p^{\mathrm{ref}}(t)\right\}^2 + \left\{z_p(t) - z_p^{\mathrm{ref}}(t)\right\}^2}{\Delta t}$$

235 (Eq. 2)

236 Here, p is an identifier for particles, t is an identifier for time frames, and Δt is
237 the time interval between the time frames. The x, y, and z coordinates of the pth particle
238 obtained from microscopic images are x_p^{ref}, y_p^{ref}, and z_p^{ref}; ref means reference (Fig. S2C).

239 The coordinates during the repeated cycles of simulations are x_p, y_p, and z_p. The effective
240 forces were inferred for each cell–cell pair for each time frame (Fig. S2B and C). Note
241 that, because γ was assumed to be constant in Equation 1, we can only infer relative but
242 not absolute values of effective forces. To determine whether our method can correctly
243 infer effective forces, we first applied it to artificial data generated under given potentials
244 of particle–particle interactions, and examined whether the inferred effective forces were
245 consistent with the given potentials (Fig. S3A). We tried the Lenard–Jones (LJ) potential
246 as a test case of the given potentials, which is originated from atom–atom interactions but
247 is one of the most well-known potentials used in various fields including biology [39]. By
248 performing particle simulations under the LJ potentials, we obtained time series of the
249 particle positions (Fig. S3A, SI, Section 4-3-4-1, and Movie S1A). Then, we applied the
250 inference method to the time series, yielding effective forces for each pair of particles for
251 each time frame (Fig. S3B). The inferred effective forces were plotted against particle–
252 particle distance (Fig. S2D, S3C, and SI, Section 5). By averaging the plots for each
253 shallow bin of distances, we obtained distance–force (DF) curves (Fig. S3C). We found
254 that the DF curves were not consistent with the curve from the LJ potentials, suggesting
255 that Equation 2 was not optimal. Therefore, we tried to incorporate various additional
256 constraints (i.e. cost functions during the minimization procedures) into Equation 2. We

257 found that, when a cost function was set so that force values approach zero at long distant
258 regions of cell–cell interactions (SI, Section 4-3, Equation S6), inferred DF curves
259 became consistent with that from the LJ potentials (Fig. S3). Detailed principles, rational,
260 and procedures are described in SI (Section 4). Additionally, if this cost function was not
261 incorporated, this minimization problem exhibited indefiniteness (i.e., a unique solution
262 could not be determined) and over-fitting also occurred (Fig. appx 3). This cost function
263 is considered as the prior in the Bayesian inference, and, in general, a unique solution
264 could be ensured by introducing such a prior [37].

265 Using Equation S6 containing the above cost function (SI, Section 4-3), we also
266 tried other potentials generated by freehand drawing, called "FH" potentials in this study,
267 as an alternative to the LJ potentials (Fig. S3A and Movie S1B). The FH potentials were
268 chosen because the simulation outcomes under the FH potentials differ from those under
269 the LJ potentials as shown later (Fig. S11). The inferred DF curves were almost consistent
270 with the given potentials (Fig. S3C, E, and SI, Section 4-3-4). Conversely, we also tried
271 using time series of random–walk simulations as negative controls (Fig. S3A and Movie
272 S1C). The plots of the inferred effective forces against distances were widely distributed,
273 and the DF curves generated from the plots by averaging were zigzag and disorganized
274 (Fig. S3C and E). These results indicate that our method can correctly infer DF curves.

Results

Systematic validation of inference method using artificial data

We first systematically validated our inference method using artificial data which include various external factors. Specifically, we assessed whether spatial constraints such as egg shells or cavities can affect inference results of effective forces of cell–cell interactions. Similar to the previous section, "Theory and principle for inferring effective potential", we generated artificial data based on simulations under LJ potential, to which we applied our inference method.

In the absence of spatial constraints, inferred distance–force (DF) curves were well consistent with the DF curves derived from the LJ potential under various settings of simulations: the settings are related to time interval of sampling, cell proliferation, and fluctuation of forces between cell–cell interactions (Fig. 2A-B, S4-I, -II, -III, SI, Section 4-4, 7-4, and Movie S8A-E). Next, we introduced spatial constraints corresponding to eggshells (SI, section 7-4-2 and Movie S8F-I). Even under narrow cylindrical constraints, inferred DF curves were well consistent with the DF curves from the LJ potential (Fig. 2C; length of cylinder = 25μm, S4-V, and SI, Section 4-4). By contrast, under constraints

293 with smaller volume where each particle was highly compressed, the profiles of inferred
294 DF curves were shifted leftward in the distance–force graph (Fig. 2C; length of cylinder
295 = 15μm, and S4-V). This shift can be reasonable, if radial distribution functions under
296 compressed conditions are considered (Fig. appx 1 and SI, Appendix section 12-1). In
297 addition, in molecular science, pressure or density of particles affects profiles of DF
298 curves [14]. However, in cell biology, we think that the above compressive conditions are
299 not physiologically relevant, because cells are essentially composed of non-compressive
300 liquid and their volumes are not changed by external pressure. These trends were similarly
301 detected under spherical spatial constraints (Fig. S4-IV). These results suggest that
302 inferred DF curves are not significantly affected by spatial constraints corresponding to
303 egg shells.

304 Next, we introduced spatial constraints corresponding to cavities. We assumed
305 that particles located on the surface of a cavity cannot penetrate into the cavity (Fig. 2D,
306 S4-VI, SI, Section 7-4-2, and Movie S8J-K). Inferred DF curves were almost consistent
307 with the DF curves from the LJ potential (Fig. 2D, S4-VI, and SI, Section 4-4). However,
308 we detected additional repulsive forces at distant regions, which we call distant energy
309 barrier (DEB). Repulsive forces around the corresponding distances were also detected
310 in DF curves inferred from simulation data where the forces between cell–cell interactions

311 were assumed to be absent but the cavities to be present (Fig. S4-VI-E, and SI, Section 4-
312 4), suggesting that DEB was derived from the cavities. Interestingly, profiles similar to
313 DEB are well-known in molecular and colloidal sciences: by considering hydration of
314 particles by solvents as an external factor, effective potentials are modified so as to
315 contain energy barriers around distant regions [1,2]. We conclude that the effects of cavities
316 can be incorporated into effective potential of cell–cell interactions. In principle, the
317 primary effect of a cavity would be to provide potential of positions for each cell but not
318 potential of cell–cell interactions. Thus, a cavity indirectly affects the effective potential
319 of cell–cell interactions. To avoid confusion in usage of the terms, we call inferred
320 potential of cell–cell interactions "effective potential for each cell modeled as cell–cell
321 interactions".
322
323 *Validation of inference method by using non-adhesive cells*
324 Before applying our inference method to three-dimensional multi-cellular
325 systems, we performed an additional negative control experiment using two-
326 dimensionally cultured cells that exhibit negligible cell–cell interactions but exert traction
327 forces between the cells and substrate, leading to random–walk-like movements. We
328 knocked out cadherin function in MDCK cells (E-cadherin and cadherin-6 in the case of

329 MDCK cells) by disrupting the gene encoding α-catenin, an essential regulator of the two
330 cadherins (Fig. S5 and SI, Section 8-3) [40]. We found that, in contrast to wild-type MDCK
331 cells which had organized DF curves (Fig. S5B), DF curves obtained from the α-catenin
332 mutant cells were disorganized (Fig. S5A), similar to those obtained from random–walk
333 simulations. This result supports the idea that our method is accurate enough to infer
334 intercellular forces in multi-cellular systems.
335
336 *Inference of effective forces in C. elegans early embryo*
337 We next investigated whether effective potential could be detected as a function
338 of cell–cell distance in three-dimensional systems. The nematode *C. elegans* has well-
339 defined embryogenesis: cell movements in early embryos are almost identical among
340 different individuals, and the time series of nuclear positions have been reported
341 previously [32]. In addition, the spatiotemporal patterns of cell differentiation are absolutely
342 the same: cell lineage and cell fate are invariant. During early *C. elegans* embryogenesis,
343 a fertilized egg (i.e., one cell) repeatedly undergoes cell division and cell differentiation,
344 ultimately forming an ovoid embryo containing ~350 cells (Fig. S6A and Movie S3A).
345 The cells become smaller through cell division [41], and the total volume of the embryo
346 remains constant because the embryo is packed into a stiff egg shell. Thus, the volume of

347 each cell is reduced by a factor of 350 relative to the original egg, meaning that cell
348 diameter is reduced by a factor of 7. Because particle diameter is approximately reflected
349 on a DF curve as the distance with force = 0 (Fig. 1B and S2D) corresponding to the
350 minimum of the potential, we expected that inferred DF curves should be gradually
351 shifted to the left side of the distance–force graph throughout embryogenesis.

352 Figure 3A is a snapshot that shows nuclear positions and inferred effective forces
353 in the embryo; the temporal change is also visualized (Fig. S6B and Movie S3B), where
354 dynamic changes in the effective forces were observed. Importantly, almost identical
355 values of the effective forces were obtained from different initial values during the
356 minimization process of Equation S6 described previously (Fig. 3B, S10A, and SI,
357 Section 4-4), suggesting that our inference problem may have a unique solution. To
358 investigate whether DF curves change during the embryogenesis, we divided the whole
359 embryogenesis into segments containing different time frames, and plotted the inferred
360 forces against the distances for each segment (Fig. 3C and S6C, time frame = 16–55, 36–
361 75, 76–115, 116–155, and 156–195). The plots were widely distributed in all the segments,
362 but the DF curves generated by averaging in each shallow bin of the distances exhibited
363 organized patterns. This was in striking contrast to the results of the random–walk
364 simulations and the α-catenin mutant cells. All of the DF curves had very similar profiles:

365 repulsive and attractive forces were detected around the core of the cells and longer
366 interaction distances, respectively (Fig. 3D). The repulsive forces are likely to be derived
367 from the volume effect of the cells, and the attractive forces might be derived from cell-
368 cell adhesion. These patterns are typical in various non-living particle–particle
369 interactions such as atoms and molecules, whose potentials are given by the LJ potential,
370 etc. (Fig. S3A). We also calculated the distance–potential (DP) curves by integrating the
371 force values; potential energy is obtained from the integral of forces along distances (Fig.
372 1B, S2D, and SI, Section 5). Importantly, the distances in the minimum of the effective
373 potentials were gradually shifted toward the left side (Fig. 3D), as we had expected (Fig.
374 3E), indicating that the inferred effective potentials were consistent with the reduction in
375 cell volume during embryogenesis (Fig. 3E). Taken together, the patterns of the DP curves
376 seem to be physically reasonable. As speculated from the previous analyses using
377 artificial simulation data (Fig. 2C), we think that the eggshell does not significantly affect
378 the profiles of the DP curves. In addition, the data points were widely distributed from
379 the DF curves as shown by the heat maps, which may originate in biological properties,
380 including variety of cell sizes, the intrinsic heterogeneity of cells and/or experimental or
381 methodological errors during detection of cell centers or the inferring process, as
382 discussed in SI (Section 4-6).

383

384 *Effect of inferred distance–force curves on C.elegans embryonic morphologies*

385 The inferred DF curves for *C. elegans* embryogenesis seem to be reasonable.
386 However, one concern may be that the averaged DF curves are oversimplification, given
387 the broad distribution of the effective force, as discussed later. This prompted us to further
388 investigate whether the simple DF curves can explain embryonic morphologies. Because
389 the dynamics of all cells during embryogenesis are very complicated, largely due to the
390 repeated cell division and cell differentiation, we focused on a few typical features of the
391 morphologies: aggregated cells and the ovoid embryo shape. The embryonic cells can
392 keep a cell aggregate even when the eggshell is removed, and subsequent culture leads to
393 a cell aggregate with an ovoid or distorted shape, except for very early stage of
394 development (< ~20 cells) [42,43].

395 We performed simulations based on the particle-based cell model by considering
396 the DF curves, and investigated whether aggregated states with ovoid shapes were stable.
397 In multi-cellular systems, morphologies of systems are determined by both DF curves and
398 initial configurations, because energetic local minimum states (i.e. metastable state) as
399 well as global minimum states are meaningful (SI, Appendix 12-2 and Fig. appx 2). To
400 find stable states under the DF curves, we performed simulations starting from various

401 initial configurations of the particles, and the systems were relaxed. When we used the
402 DF curves obtained from the *C. elegans* embryos, we observed a tendency for the particles
403 to aggregate with an ovoid or distorted shape (Fig. 6A and S11). As a control simulation,
404 we utilized the LJ potential, and found that it did not generate an ovoid or spherical shape,
405 but rather a shape similar to its initial configuration (Fig. S11). Moreover, the FH
406 potentials generated a spherical but not ovoid shape, and the outcomes were not
407 significantly affected by the initial configurations (Fig. S11). These results suggest that
408 the inferred effective DF curves are capable of recapitulating the basic morphology of the
409 *C. elegans* embryos.
410
411 *Inference of effective force in mouse pre-implantation embryos*
412 To further investigate whether effective forces could be obtained in three-
413 dimensional systems, we focused on mouse pre-implantation embryos, including morulae
414 at the 8-cell and compaction stages, as well as blastocysts bearing cavities (Fig. 4A,
415 illustration). These embryos are surrounded by a spherical egg shell–like structure called
416 the zona pellucida, but even if that structure is removed, the embryos maintain their
417 spherical shapes and development normally [44]. In 8-cell stage embryos before compaction,
418 cell–cell adhesion is weak, and individual cells can be easily discerned (Fig. 4A, bright

field). In the compaction-stage embryos composed of ~16-32 cells, cell–cell adhesion becomes stronger due to elevated expression of cadherin proteins on cell membrane, and the cells are strongly assembled [45]. The surface of the embryo becomes smooth, and the embryonic shape becomes more spherical (Fig. 4A, bright field). In blastocyst-stage embryos composed of >64 cells, an inner cavity is formed, and the embryos expand while maintaining their spherical shape. Trophectoderm (TE) cells form a single–cell-layered structure at the outermost surface of the embryo, whereas the cells of the inner cell mass (ICM) were tightly assembled, forming a cluster (Fig. 4A, illustration). Using confocal microscopy, we performed live imaging of fluorescently labeled nuclei, obtained time series of the nuclear positions (Fig. 4A, Movie S4A-C, and SI, Section 8-1), and then applied the inference method.

Figure 4B shows the snapshots of the inferred effective forces of cell–cell interactions during the three embryonic stages that we examined (Movie S4D–G). The uniqueness of the solutions was confirmed (Fig. S10). We calculated the DF and DP curves in a manner similar to *C. elegans* study described above, and obtained curves for all three stages (Fig. 4C and D). The DF curves derived from the 8-cell and compaction stages had typical profiles, with repulsive and attractive forces detected at short and long interaction distances, respectively (Fig. 4D and S6D). On the other hand, the DF curves

437 derived from the blastocyst stage contained DEB (Fig. 4D, arrows), similar to the case of
438 artificial simulation data considering a cavity (Fig. 2D and S4-VI). Thus, we think that
439 the DEBs in the blastocysts are derived from the cavities. Such DEBs have not been
440 discovered in multi-cellular systems. Together, these results indicate that the profile of
441 DF curves changes during the development. Moreover, the profiles of the DF curves
442 suggest the consistency with previous experimental knowledge as discussed in Figure S7.
443
444 *Effect of inferred distance–force curves on mouse embryonic morphologies*
445 We next asked whether the temporally evolved DF curves derived from the
446 mouse embryos were sufficient to explain the key morphological features of the embryos.
447 Using the DF curves, we searched for stable states by an approach similar to the one we
448 used for *C. elegans* embryos. In contrast to the case of *C. elegans* embryos, in which an
449 ovoid shape was generated, the DF curve derived from the compaction stage embryo
450 yielded a spherical shape where particles were aggregated (Fig. 6B and S11). In the case
451 of the 8-cell stage embryo, simulation results were similar to those obtained for the
452 compaction stage, except that the spherical shape seemed to be slightly distorted (Fig.
453 S11). In the case of the blastocyst stage embryo, when aggregated particles were given as
454 initial configurations, the particles gradually scattered and formed a spherical structure

455 harboring a cavity at the center (Fig. 6B). We then examined whether DEB contribute to
456 the formation of a cavity. We artificially eliminated DEB from the inferred DF curves,
457 and performed a simulation. We found that no cavity was generated (Fig. 6B). These
458 results suggest that all of the DF curves from the various embryonic stages are sufficient
459 to explain their different morphologies. We also showed DF curves that were obtained
460 from interactions between adjacent cells (Fig. S8), by which we may evaluate direct cell–
461 cell interactions excluding indirect effect of cavities. Later we will discuss the differences
462 in the DF curves between the two cell populations TE and ICM (Fig. S7). We will also
463 discuss cellular polarities, such as apico-basal and planar cell polarities that were not
464 explicitly considered in this study (Fig. S9 and SI, Appendix section 12-1).
465
466 *Inference of effective distance–force curves and their effect on morphology in MDCK cyst*
467 The above results led us to hypothesize that DEB of the DF curves plays essential
468 roles in the morphogenesis of structures harboring cavities. To examine this hypothesis,
469 we focused on another system with a cavity. MDCK cells, derived from dog kidney
470 epithelium, can form cysts containing a cavity when cultured in suspension (Fig. 5A,
471 illustration) or in gels composed of extracellular matrix [46]. To exclude mechanical
472 interactions between the cysts and the external environment, we chose suspension

473 conditions in which a cyst can be assumed to be a mechanically isolated system for which
474 external forces are negligible. We obtained time series of the nuclear positions of the cysts
475 by a procedure similar to the one used for the mouse embryos (Fig. 5A, Movie S5A, and
476 SI, Section 8-2). We then inferred the effective forces and calculated the DF curves (Fig.
477 5B–D and S6E). The uniqueness of the solutions was confirmed (Fig. S10). Figure 5D
478 shows the DF and DP curves from a MDCK cyst. We found a DEB in the DF curves,
479 implying that a DEB is generally involved in morphogenesis of cavity-bearing structures.
480 Simulations based on inferred DF curves revealed that, a cavity was stably
481 maintained in MDCK cells (Fig. 6C and S11). Moreover, the DF curves from which the
482 DEB was artificially eliminated could not maintain the cavity-bearing structure (Fig. 6C).
483 Taken together, these findings indicated that the DEBs in the blastocyst and the MDCK
484 cyst play roles in the formation and maintenance of a cavity, respectively.
485
486 *Modeling of distance–force curves*
487 The analyses from the blastocysts and MDCK cysts suggest that the effect of
488 cavities can be incorporated into effective potential for each cell modeled as cell–cell
489 interactions. We hypothesized that the DF curves incorporating such external factors
490 represent a rule capable of explaining various morphogenetic events. Moreover, the

profiles of the DF curves in Figure 3-5 were quantitatively different each other (Table S2), suggesting the quantitative differences are critical for morphogenesis. To comprehensively understand the effect of the profile of the DF curves on morphogenesis, it is necessary to model the DF curves as a simple mathematical equation. We selected the following equation as described in SI (Section 6):

$$F(D) = \varepsilon(D - D_0)^{-N} \cos\left\{\frac{2\pi(D - D_0)}{\delta}\right\}, \text{ for } D > D_0 \qquad \text{(Eq. 3)}$$

Here, F is the force, D is the distance, and N is the exponent of D, affecting the decay of the DF curves along D (X-axis). D_0 and δ affect the profile of the DF curves as shown in Figure 7A. Specifically, D_0 can transform the DF curves along D, and δ affects the wavelength of the cosine function. All parameters and variables related to lengths/distances were normalized by (δ/4), leading to generation of dimensionless parameters and variables such as $D_0^* = D_0/(\delta/4)$; usage of dimensionless parameters and variables is general technique to reduce the number of parameters. This enabled us to present simulation outcomes in two-dimensional space (D_0^* and N) in a manner similar to a phase diagram (Fig. 7).

Systematic analyses of the roles of the distal energy barriers

Using the DF curves defined by Equation 3, we systematically investigated what

509 kind of morphologies could be generated or stably maintained, and what kind of
510 morphological transformation could be achieved. Here, we tried to identify possible
511 stable states, including metastable states. We set various initial configurations of particles,
512 performed simulations under various DF curves, and then searched for local minimum
513 states. A cavity-bearing structure was set as the initial configuration (Fig. 7B). The
514 simulation outcomes were categorized by their morphologies and plotted on a two-
515 dimensional space (Fig. 7B). Examples of the morphologies and DF curves are also
516 shown. We identified cavity-bearing structures ("cavity" and "cavity with extra
517 particles"), cups, tubes, and lattices / aggregates as possible stable states. Multiple clusters
518 of particles were formed or disorganized morphologies were generated which include
519 distorted rings, etc. Importantly, when the DEBs were eliminated from the DF curves, one
520 of the cavity-bearing structures ("cavity"), cups, or tubes were not generated (Fig. 7C),
521 indicating that the DEBs are essential for the formation or maintenance of these structures.
522 Other analyses considering different initial configurations and different particle numbers
523 are shown in Figure S12; a monolayer two-dimensional sheet was stably maintained by
524 DEB, cavity-bearing structures were newly generated without DEBs, and cell sorting in
525 systems containing multiple types of cells, which is an important phenomenon in
526 developmental biology[3], was observed. Furthermore, we found that modulation of the

527 profiles of the DF curves can induce transitions among some of these structures (Fig. S13
528 and Movie 6). These analyses indicated that, by considering the DEBs, the DF curves
529 represent a powerful rule for the formation and maintenance of various three-dimensional
530 structures.
531
532 *Combination of inference method with traction force microscopy*
533 We have been showing that some external factors, such as cavities, can be
534 incorporated into effective potentials for each cell modeled as cell–cell interactions. An
535 alternative approach is to subtract external effects prior to inference of effective potentials,
536 which can lead to acquisition of potentials derived from direct effects of cell–cell
537 interactions. In other words, external effects are quantitatively measured to exclude them
538 from inference of effective potentials. However, this kind of quantitative measurements
539 is still challenging in biology especially in three-dimensional situations. Alternatively, we
540 tested this approach in two-dimensional situations where traction forces are exerted
541 between cells and substrate, because there is a well-established method to quantitatively
542 measure traction forces, traction force microscopy (TFM) [47–50].
543 First, we tried this approach by using artificially generated simulation data where
544 traction forces were provided (Fig. S14 and Movie S9). By subtract traction forces prior

545 to inference, we obtained DF curves consistent with the given DF curve derived from the
546 LJ potential (Fig. S14). Finally, we tried this approach in two-dimensionally cultured
547 MDCK cells with TFM measurement (Fig. S15). Without the subtraction of traction
548 forces, the inferred forces of cell–cell interactions were shifted toward repulsive forces.
549 On the other hand, by subtracting traction forces, we obtained typical DF curves with
550 repulsive forces at shorter distance and attractive forces at middle distances (Fig. S14 and
551 S15). These results suggest that, by combination with TFM and possibly other techniques,
552 our inference method can yield potentials derived from direct effects of cell–cell
553 interactions.
554
555 *Extraction of information of cell polarity*
556 We have been inferring effective potentials for each cell modeled as cell–cell
557 interactions without explicitly considering cell polarities such as apico-basal polarities
558 and polarities on cell sheet including planar cell polarity (PCP). We tried to extract these
559 polarities from the inferred effective potentials by considering directions of cell–cell
560 interactions.
561 First, we analyzed the inference results derived from artificially generated
562 simulation data with unidirectional traction forces under two-dimensional situations as

563 previously shown (Fig. S14G and S14H). In this simulation, the leading cells exerted the
564 largest traction forces among all cells, and the traction force values were gradually
565 decreased from the leading cells to the followers. This situation resembles wound healing.
566 Thus, the cells have polarities on the cell sheet: the direction of collective migration vs.
567 its vertical direction. We divided the directions of cell–cell interactions into three
568 categories according to their angles, and tested whether DF curves obtained from these
569 categories became different each other. Note that we did not subtract traction forces in
570 this analysis. As shown in Figure S16, the DF curve along the vertical direction of
571 collective migration was equivalent to the DF curve derived from the LJ potential,
572 whereas the DF curve along the direction of collective migration was significantly
573 changed, resulting in a repulsive force-dominant profile. This is intuitively reasonable,
574 because cell–cell distances along the direction of collective migration are increased
575 during wound healing, which corresponds to existence of apparent repulsive forces. These
576 results suggest that cell polarity can be extracted after inferring effective potentials. Note
577 that the inferred effective potentials contain parts of the effects of external forces but not
578 all: external forces such as traction forces can move the centroid of cell populations but
579 internal forces such as the effective potentials modeled as cell–cell interactions cannot
580 due to the law of conservation of momentum.

581 We also tried to extract cell polarity in mouse embryos. During the compaction
582 stage, the cells, which are located on the outer surface of the embryos, gradually form cell
583 polarity [51], leading to the differences in protein localization between the junctions of
584 outer–outer cells and of outer–inner cells. We found that the DF curves of these two
585 interactions were different each other (Fig. S7A). Finally, we analyzed apico-basal
586 polarity in TE cells of mouse blastocyst. We tried to extract DF curves in the cell sheet of
587 TE and along the direction vertical to the cell sheet corresponding to the apico-basal
588 direction. However, there were essentially no cell–cell interactions detected along the
589 apico-basal direction, as shown by the probability of cell distribution against the
590 directions (Fig. S9A-C). Thus, we cannot infer DF curve along the apico-basal direction.
591 In general, probability of states is linked to potential energy (SI, Appendix section 12-1).
592 Thus, we calculated potential energies against the angular direction, and found that the
593 potential energy along the apico-basal was expected to be extremely large compared with
594 the direction on the cell sheet (Fig. S9D and S16D).
595
596 *Influence of experimental noise on inference result*
597 In general, accuracy of inference methods can be affected by observation noises.
598 In our method, noises of nuclear positions during image recognition may affect the

accuracy. We theoretically assessed the influence of the noises of nuclear positions (SI, Section 4-5). Using artificially generated simulation data, we added noises of nuclear positions as Gaussian noises, and inferred effective potentials for each cell modeled as cell–cell interactions. The averaged distances between nuclear positions before and after adding Gaussian noises was set variously from 0.16 to 1.0μm (Table S1-i-iv). We found that the increase in the Gaussian noises caused modulation of inferred DF curves (Table S1-i-iv and Movie S10). There were two major modulations: an emergence of disorganized patterns similar to that from random walk and an increase in maximum absolute values of attractive forces (Table S1-i-iv and SI, Section 4-5). In spite of the latter modulation, the peaks of attractive forces were not significantly changed along the distances, suggesting that overall patterns were almost conserved except for the absolute values of attractive forces. It is difficult to correctly estimate actual experimental noises, but residuals from smoothing spline interpolation along time for positions of each nucleus might roughly correspond to experimental noises (Fig. appx 4 and SI, Appendix section 12-4). We found that the values of the residuals ranged from 0.2 to 0.4μm in the *C. elegans* embryo, the mouse embryos, and the MDCK cysts (SI, Section 4-5-2). As shown in Table S1-i-iv, these *in vivo* situations fell within acceptable conditions expected from the artificial data: the patterns of DF curves would not be disorganized/randomized and the

orders of the absolute values of attractive forces are expected to be sustained.

Discussion

In this study, we developed a method for statistically inferring effective forces of cell–cell interactions using nuclear tracking data obtained from microscopic images. We then demonstrated that effective potentials for each cell modeled as cell–cell interactions can be extracted from the inferred forces in various living systems. Our findings provide for the first time the experimental quantification of effective potentials, which have been recognized conceptually [11,17]. We also showed that, in the coarse-grained cellular model, the effective potentials can partially contain information of cell polarities and of external factors such as cavities, which can be decoded after inference, suggesting that the effective potential is an integrative parameter. By considering the effective potentials, we successfully reproduced various three-dimensional structures, including cell aggregates, cavity-bearing structures, cups, tubes, two-dimensional sheets, and sorted cells. These structures are often observed during embryogenesis, morphogenesis, and organoids prior to the formation of complex tissue structures. For instance, cell aggregates are observed in early embryos in various species and spheroids [20,32,52–57]; cavity-bearing

structures in early embryos of various species, organoids, and cystic diseases [46,52,58–61]; cell sheets in epithelia and germ layers [20,56,62–65]; tubes in tubular organs [66–68]; and cups in retina (i.e. optic cup) and murine epiblast [53,69]. Thus, we consider these structure as basic structural units for morphogenesis. These results suggest that the effective potentials alone are powerful to explain diverse three-dimensional morphologies. Although cell–cell interactions are affected by many parameters including proteins such as cadherins and cellular structures such as actomyosin cytoskeletons (Fig. S1 and S5), we suppose that the effective potentials in the coarse-grained model can be an integrative and unifying mesoscopic parameter to explain morphogenetic events. Our goal is to develop a framework for explaining diverse morphogenesis in a minimal model. The model based on effective potential represents such a framework with few degrees of freedom (Fig. S1A), and this provides a novel paradigm for understanding the morphogenesis of multi-cellular systems and their morphological evolution. We believe that effective potentials and the inference method constitute a powerful approach for exploring principles for diverse morphogenesis.

Profile of effective potentials of cell–cell interaction

We discovered distant energy barriers (DEBs) in the effective potentials obtained

653 from blastocysts and MDCK cysts, which would be derived from indirect effect of
654 cavities. These energy barriers contributed significantly to the generation and
655 maintenance of cavity-bearing structures, tubes, and sheets. Such energy barriers are well-
656 known in molecular and colloidal sciences, as mentioned in SI (Section 5-4-1). We
657 speculate the physical interpretation of these energy barriers in cells below. In the case of
658 the blastocysts, the trophectoderm cells form tight junction to seal gaps between the cells
659 and transport liquid from the outside of the embryos to the cavities, resulting in increased
660 hydrostatic pressure, fracturing of the cell–cell contacts of the inner cells, and subsequent
661 expansion of the cavities [70–72]. Therefore, the TE cells push each other through the liquid
662 in the cavities. This indirect mechanical repulsion might be reflected in the DEBs (Fig.
663 S17A). In the case of the MDCK cysts, tubes, and sheets, the DEBs may reflect properties
664 of cell sheets as discussed in Figure S17B.
665
666 Potentials of cell–cell interactions have been widely considered in various
667 materials such as active matter including self-migratory cells. In these studies, however,
668 the profiles of the distance–potential curves are assumed without experimental bases. For
669 instance, hard- or soft-core, square-well, JKR, and other potentials have been considered
670 (Fig. appx 5 and SI, Appendix section 12-5) [19,20,25,73,74]. Animal and human behaviors can

also be modeled as active matter governed by attractive and repulsive interactions [75–78]. Our inference method can be used to obtain the profiles of the potentials in these materials, possibly leading to new finding of the dynamics of these materials. Moreover, the material properties of particulate soft matter can be estimated using inferred effective potentials as demonstrated in SI (Fig. S18, Table S2, and SI, Section 4-6-2 and 5-4-2).

Usefulness, application, and limitation of inference method

Our *in situ* inferences provide information about mechanical states, or maps, of living systems. Various methods have been developed for measuring or inferring mechanical maps in living systems such as TFM (Fig. S15) [17,37,79–82]. Our method is advantageous for obtaining mechanical states in three-dimensional situations with temporal evolution. To understand mechanical states, it is necessary to complementary utilize these methods with various temporal and spatial resolutions, as demonstrated by combining our method with TFM (Fig. S15).

To understand morphogenetic events, mechanical states and simulations based on those states are essential. Models such as vertex and Cellular Potts models are often utilized, especially for epithelial cells, where the cells are modeled as polygonal or polyhedral shapes with adhesion energy and surface tension [5,52,58]. These models

689 successfully recapitulated various morphogenetic events, including the formation of the
690 mouse blastocyst [52], very early embryogenesis in *C.elegans* [83], and the formation of cystic
691 structures in kidney [58]. These models often contain non-cellular components such as
692 liquid in cavities and egg shells. However, it is still challenging to measure cellular and
693 non-cellular parameters in three-dimensional systems with high spatiotemporal resolution
694 (e.g. spatiotemporal information of pressure of cavities, and traction forces in three-
695 dimensional situations), although high-resolution inference has been performed in two-
696 dimensional situations [37,82]. This means that there is no clear way to connect the model
697 parameters to *in vivo* ones in three-dimensional systems. On the other hand, particle-based
698 cellular models can also assume parameters other than the potential of cell–cell
699 interactions, leading to recapitulation of complex three-dimensional structures such as the
700 blastocyst [84–86]. In particular, a recent study reported a model that considered cell
701 polarities, in which transformation from cell aggregates to cell sheets and bending of cell
702 sheets were simulated [86]. Particle-based models have also been expanded by considering
703 cell shapes, where a cell is composed of two particles or a Voronoi tessellation is
704 combined to implement multibody effect [22,28,31,57,63,87,88], and mechanical properties and
705 dynamics of self-propelled cells or cell aggregates were investigated. These observations
706 suggest that particle-based models are potentially applicable to very complex structures,

707 although it has not been established whether highly deformed cells and non-cellular
708 structures such as extracellular matrix can be implemented simply in particle-based
709 models. In our strategy, model parameters inferred from *in vivo* systems can be used for
710 simulations. Our method provides a framework for quantitatively connecting model
711 parameters to *in vivo* parameters under three-dimensional situations, and will thus be
712 complementary to other models.

713 Investigations of the origins of the effective potentials are important for a
714 complete understanding of morphogenesis. Various proteins including cadherins and
715 actomyosins should be involved in the potentials. In fact, disruption of the gene encoding
716 α-catenin, which is essential for the function of cadherin proteins, leads to the changes in
717 the profile of effective potentials (Fig. S5). Our framework enables us to relate proteins
718 to the profile of potentials by evaluating the effect of gene disruption or inhibition on the
719 profile. Comprehensive evaluation of the effect of various proteins will be valuable, and
720 will provide a bridge between the physics of morphogenesis and molecular biology.

721

722 *Toward understanding of a later stage of embryogenesis and organogenesis*

723 In this study, we used relatively small embryos: mouse pre-implantation embryos
724 (several tens of cells) and the *C. elegans* embryo (< 350 cells). To apply our inference

725 method and simulation model to later embryonic stages, new components should be
726 considered, such as the increase in the number of cell types, cell polarities, and
727 chemotaxis. The number of cell types increases through cell differentiation, in which
728 mesenchymal cells as well as blastomere cells and epithelial cells are included. The
729 blastocyst contains at least two cell types (ICM and TE), and we successfully detected the
730 differences in the DF curves between these two cell types (Fig. S7). In later stages of
731 embryogenesis, such as gastrulation, the embryos are usually composed of mixtures of
732 mesenchymal and epithelial cells, in which the epithelial-to-mesenchymal transition
733 occurs. In contrast to the vertex model, where mesenchymal cells cannot be considered,
734 particle-based models may simulate the mesenchymal cells and mixtures of mesenchymal
735 and epithelial cells [24,25]. Mesenchymal and epithelial cells are separated each other (i.e.
736 cell sorting), which has been explained by differential cell–cell adhesiveness [3]. Other
737 group and we demonstrated that particle-based models can reproduce cell sorting by
738 considering cell–cell adhesiveness as potentials (Fig. S12-IV-B) [89]. Apico-basal and
739 planar cell polarities are critical parameters for morphogenesis [86]. We speculate that these
740 polarities can be implemented in the particle model by implementing two particles for a
741 cell where cell shapes become anisotropic [28,31], or by considering directional differences
742 in the profiles of effective potentials, as recently proven by other group [86] and shown in

743 Figure S7A and S16D. Cell–cell interactions are regulated not only by mechanical forces
744 but also by chemical signaling such as chemotaxis and ephrin proteins (Fig. S1B). By
745 expanding the concept of effective potentials, we think that attractive and repulsive effects
746 of chemical signaling can be implemented as the profile of the effective potentials,
747 possibly with non-reciprocal interactions [90–94]. It is possible that by analyzing inferred
748 cell–cell interaction forces, we could extract differences in cell types, directional
749 differences in the profile of the effective potentials, and the effects of chemotaxis.

750 At this moment, we do not know whether the particle-based model is useful for
751 later stages of embryogenesis or larger tissues to which continuum models are usually
752 applied [95]. Multi-scale frameworks may be required to connect particle-based models with
753 continuum models based on macroscopic parameters. In any case, our inference method
754 may be useful for evaluating material properties of larger tissues, which are essential for
755 developing continuum models.

756

757 Conclusion

758 Our framework, composed of the inference method and the particle-based model,
759 has various potential uses for investigating the properties of cell–cell interaction, single-
760 cell analysis of mechanics, material properties, mechanical maps, three-dimensional

761 morphogenesis, relationships between mechanical parameters and gene products, and
762 comparisons of mechanical parameters and behaviors among different living species and
763 non-living systems. Although some of these issues will be examined in the future, we
764 propose that our framework provides a novel physical scenario for understanding the
765 biophysics and physical biology of diverse morphogenetic events.
766
767
768 Experimental materials and methods
769 Mouse embryos were obtained by mating Rosa26-H2B-EGFP knock-in male mice [36] with
770 ICR female mice. Animal care and experiments were conducted in accordance with the
771 Guidelines of Animal Experiment of the National Institutes of Natural Sciences.
772 Experiments were approved by the Institutional Animal Care and Use Committee of the
773 National Institutes of Natural Sciences. MDCK transgenic cells bearing H2B-EGFP were
774 generated using a piggy-bac plasmid [96,97]. α-catenin mutant cells were constructed
775 previously [98]. The detailed procedures for cell and embryo cultures, microscopic imaging,
776 nuclear tracking, force inference, simulations, and data analyses are described in SI. The
777 nuclear tracking data, inferred forces, and profiles of distance–force curves are provided
778 in SI. TFM was performed as described previously [48,49].

Acknowledgement

We thank Drs. Hitoshi Niwa and Yayoi Toyooka for providing the piggy-bac plasmids and technical suggestions. We thank Drs. Jean-François Joanny, Hiroaki Takagi and Yasuhiro Inoue for critical reading of the manuscript. We thank Drs. Kazuhiro Aoki and Yohei Kondo for kindly providing images for TFM and critical suggestions about TFM. We thank Dr. Yoshitaka Kimori for helpful discussions. We thank Ms. Azusa Kato for supporting nuclear tracking. This work was supported by following grants: Japan Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research on Innovative Areas "Cross-talk between moving cells and microenvironment as a basis of emerging order" for H.K., the National Institutes of Natural Sciences (NINS) program for cross-disciplinary science study for H.K., a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Young Scientists (B) for H.K. (17K15131), for Scientific Research (C) for T.O. (18K06234), and for Young Scientists (B) for T.O. (16K18544), a MEXT/JSPS Grant-in-Aid for Scientific Research on Innovative Areas for T.O. (17H05627), the Inamori Foundation for T.O., and the Takeda Science Foundation for T.O.

Conflicts of interest

The authors declare no competing financial interests.

Author contribution

H.K. designed the work, H.K., A.M.I., and H.O. contributed to the conception. H.K. and T.F. designed experiments, T.O. provided experimental materials, and H.K. performed experiments and image processing. H.K. and H.O. designed models, H.K. developed computational algorithms, and H.K. and K.N. statistically analyzed the data. H.K., T.O., H.O., A.M.I., K.N., K.K., and T.F. wrote the manuscript, and all authors contributed to the interpretation of the results.

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Figures

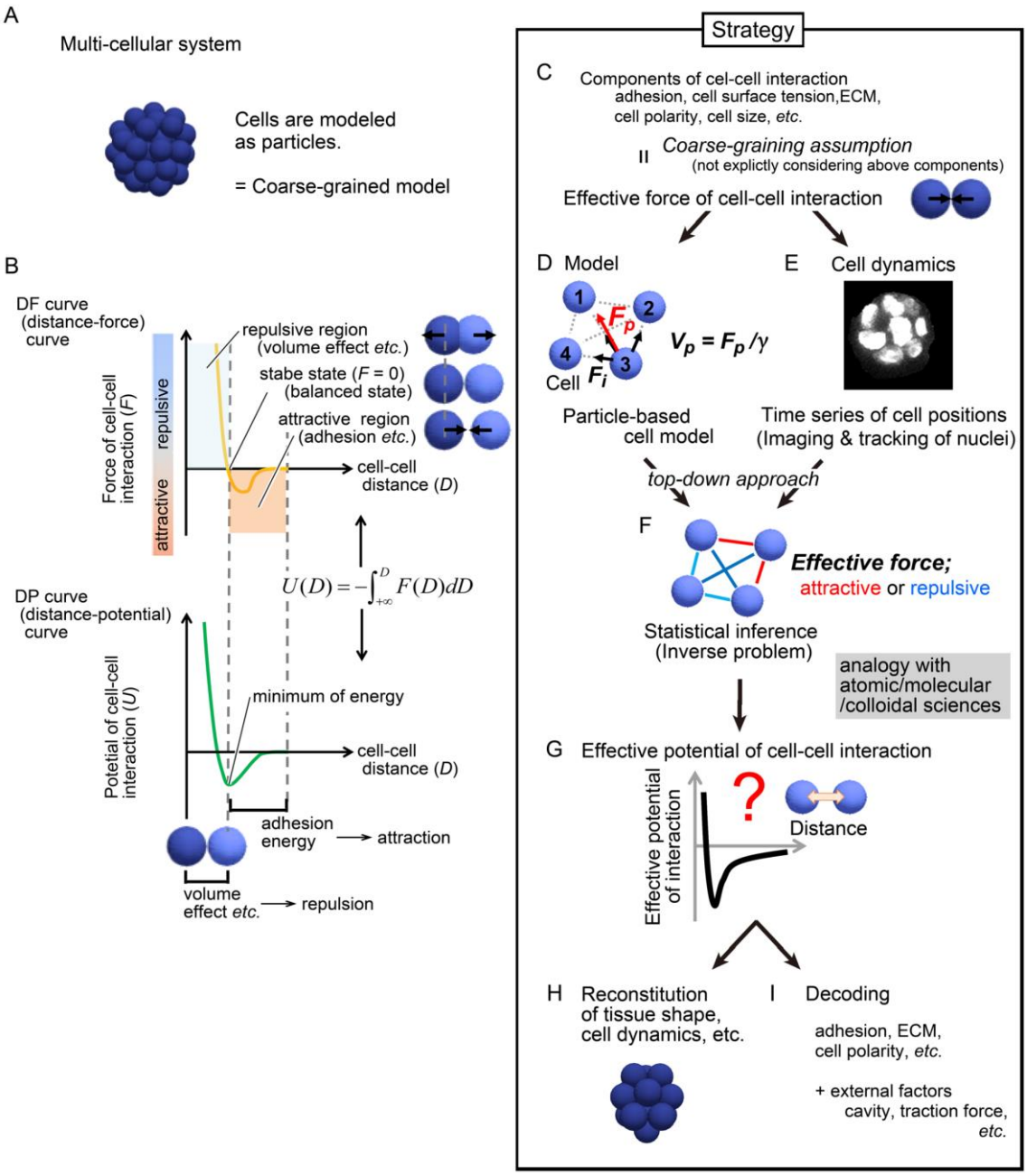

Figure 1: Overview of strategy for inferring the effective potential of cell–cell interactions A. Cells were modeled as particles. A blue sphere corresponds to a cell. B. In the particle-based model, distance–force (DF) and distance–potential (DP) curves are considered. Typical profiles of the curves, their physical meaning, and their origins are shown. DF

1053 and DP curves are mathematically transformed each other. The distance with force = 0
1054 corresponds to the distance with energetic minimum. C-I. Strategy for inferring effective
1055 forces of cell–cell interactions is described. C. Mechanics of cell–cell interactions is
1056 coarse-grained, leading to effective forces of cell–cell interactions as shown in D. There
1057 are many components related to cell–cell interactions, which are not explicitly considered
1058 in this coarse-grained model. D. In the coarse-grained model, attractive or repulsive force
1059 between the particles (blue spheres #1–4), was considered; force vectors (F_i) are
1060 illustrated by black arrows in the case of particle #3. The net force (F_p) is shown by a red
1061 vector, and the relationship between F_p and velocity (V_p) is described. E. From a time
1062 series of microscopic images of nuclei, nuclear tracking data were obtained. An image
1063 derived from a cyst formed by MDCK cells expressing H2B-EGFP is shown as an
1064 example. F. Effective forces of cell–cell interactions were inferred by solving an inverse
1065 problem: we statistically searched for force values in the model (D) that could reproduce
1066 the nuclear tracking data (E). G. The inferred force values (F) were plotted against the
1067 distance between cell–cell interactions, and whether DF or DP curves are emerged from
1068 the plots were examined. H. Whether the DF or DP curves can reconstitute tissue shape
1069 or cell dynamics was examined. I. Whether components related to cell–cell interactions
1070 described in C can be decoded from the DF or DP curves was tested. In addition,

influences of external factors on the profiles of the DF or DP curves was assessed.

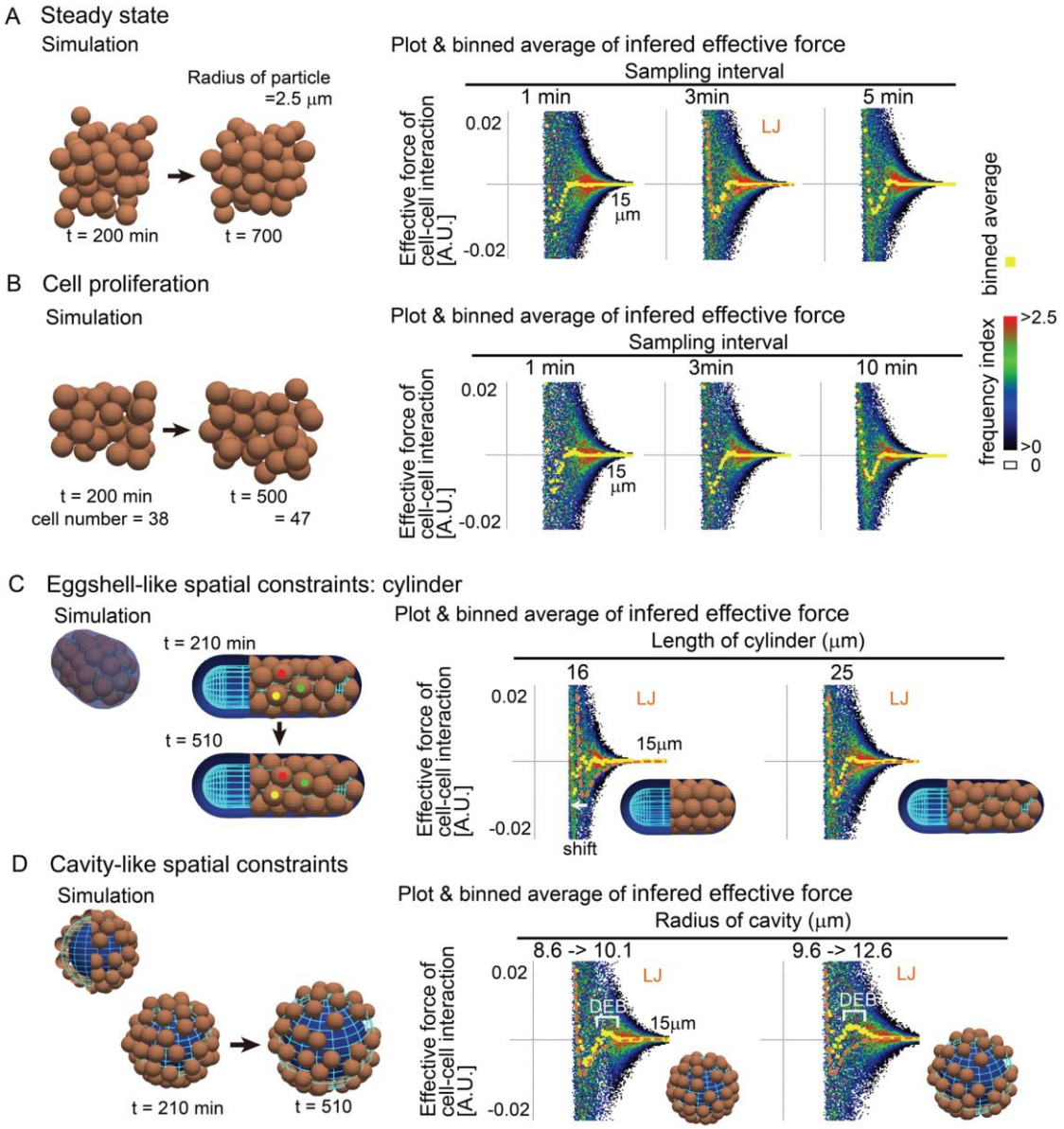

Figure 2: Validation of inference method using artificially generated data

Simulation data were used to validate our method for inferring effective forces of cell–cell interactions. The simulations were performed on the basis of a distance–force (DF) curve obtained from the Lenard-Jones potential (LJ). The simulation conditions contain

1080 “Steady state” (A), “Cell proliferation” (B), “Egg shell-like spatial constraints” (C), and
1081 “Cavity-like spatial constraints” (D). The left panels in A-D are snapshots of the
1082 simulations. The right panels in A-D are DF curves obtained from the inference. Effective
1083 forces of interactions for each pair of cells for each time frame were plotted against the
1084 distance of the cell–cell interactions. The graph space was divided into 64×64 square
1085 regions, and the frequencies of the data points plotted in each region were calculated. The
1086 mean value of the frequencies was calculated for each of the 64 columnar regions along
1087 the distance. Frequency index (FI) is defined so that the mean value of the frequencies is
1088 1, and a colored heat map was generated according to the FI. White corresponds to an FI
1089 of 0. Averaged values of the forces were calculated for 64 columnar regions along the
1090 distance, and are shown in yellow (binned average). Detailed procedures are described in
1091 SI (Section 4). In some graphs, the DF curves from the LJ potential are overlaid by orange
1092 broken lines (LJ). In A and B, simulation data with different sampling intervals were
1093 applied. In C, under the cylindrical constraint with a shorter length where particles are
1094 highly compressed, the DF curve was shifted leftward along the distance (white arrow).
1095 In D, on the expanding cavities, repulsive forces at distant regions were detected, which
1096 we called distant energy barriers (DEB). Related comprehensive analyses are shown in
1097 Figure S4 with simulation movies (Movies S8A-K). Detailed assumptions and conditions

of the simulations are described in SI, Section 7-4.

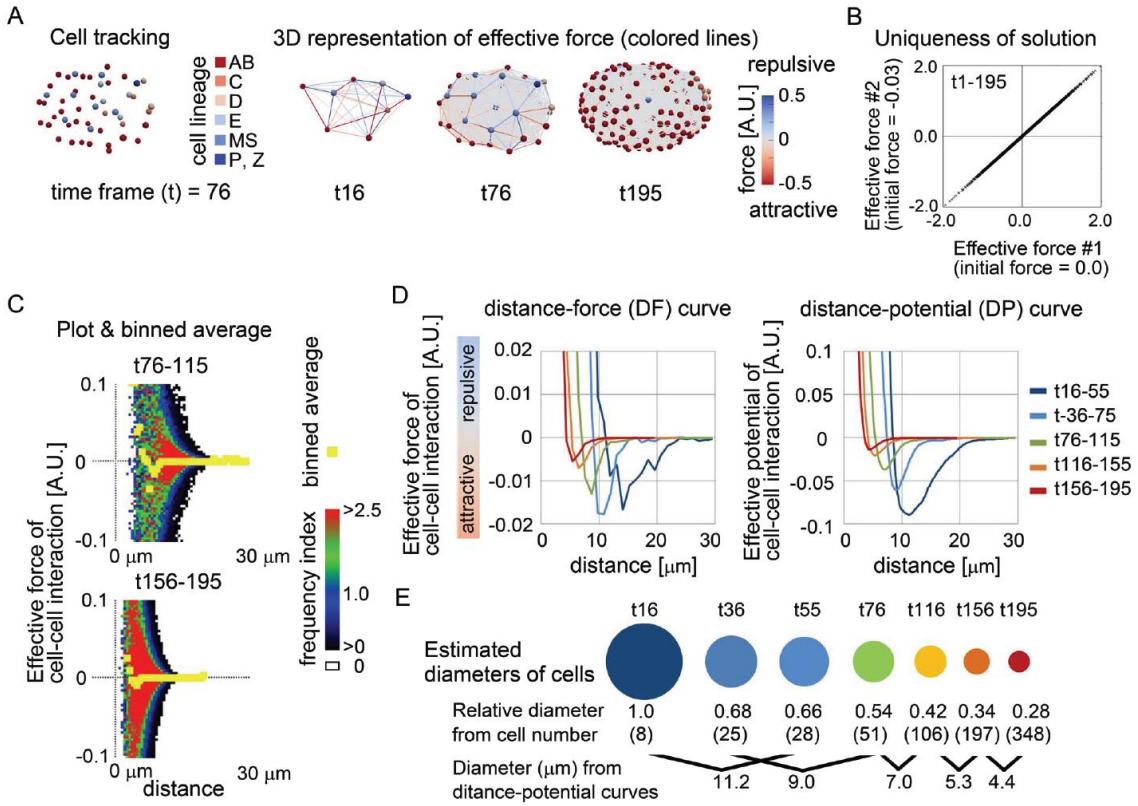

Figure 3: Inference of effective force of cell–cell interaction in *C.elegans* embryos

A. Snapshot of the nuclear positions in the *C.elegans* embryo (left panel) and snapshots with inferred effective forces (right three panels) were three-dimensionally visualized at the time frames as described (t16, t76, and t195); the interval between time frames is 1 min. The spheres correspond to cells whose colors represent cell lineages, including AB, C, D, E, MS, and P & Z. The lines represent cell–cell interactions; the colors indicate the values of the effective forces (red, attractive; blue, repulsive). Forces are depicted in arbitrary units (A.U.); 1 A.U. of the force can move a particle at 1μm/min as described in SI (Section 4-4). The nuclear tracking data were obtained from a previous report [32].

Related figures and movies are provided (Fig. S6A, B, Movies S3A and B). B. Uniqueness of solution of effective force inference was examined. The minimizations of Equation S6 were performed from different initial force values as described in the x- and y- axes, and the inferred values of each cell-cell interaction were plotted by crosses. The inferred values from the different initial force values were absolutely correlated. Values from all time frames (t1-195) were applied. C. The inferred effective forces of cell–cell interactions were plotted against the distance of cell–cell interactions in a manner similar to Figure 2. The graphs for time frame 76–115 and 156–195 are shown. Data at other time frames are provided in Figure S6C. D. Distance–force (DF) and distance–potential (DP) curves were estimated. The averaged values of the effective forces in (C) were smoothed over the distance, and the potentials were calculated by integrating the forces. Details are described in SI (Section 5-3). The curves for each time frame are shown. E. Mean diameters of cells at each time frame were estimated from cell numbers and the DP curves. Given that the volume of the embryos is constant (= Vol) during embryogenesis, the mean diameters were estimated from the cell numbers (N_c) at each time frame as follows: mean diameter = $\{Vol / (4/3\ \pi\ N_c)\}^{1/3}$. The diameters relative to that at time frame = 16 are shown with cell numbers (parentheses). The sizes of the circles reflect the diameters, whose colors roughly correspond to the colors in the graph in D. The diameters were also

estimated from the DP curves in D; the distances with minima of the DP curves roughly reflect the diameters (Fig. 1B and S2D).

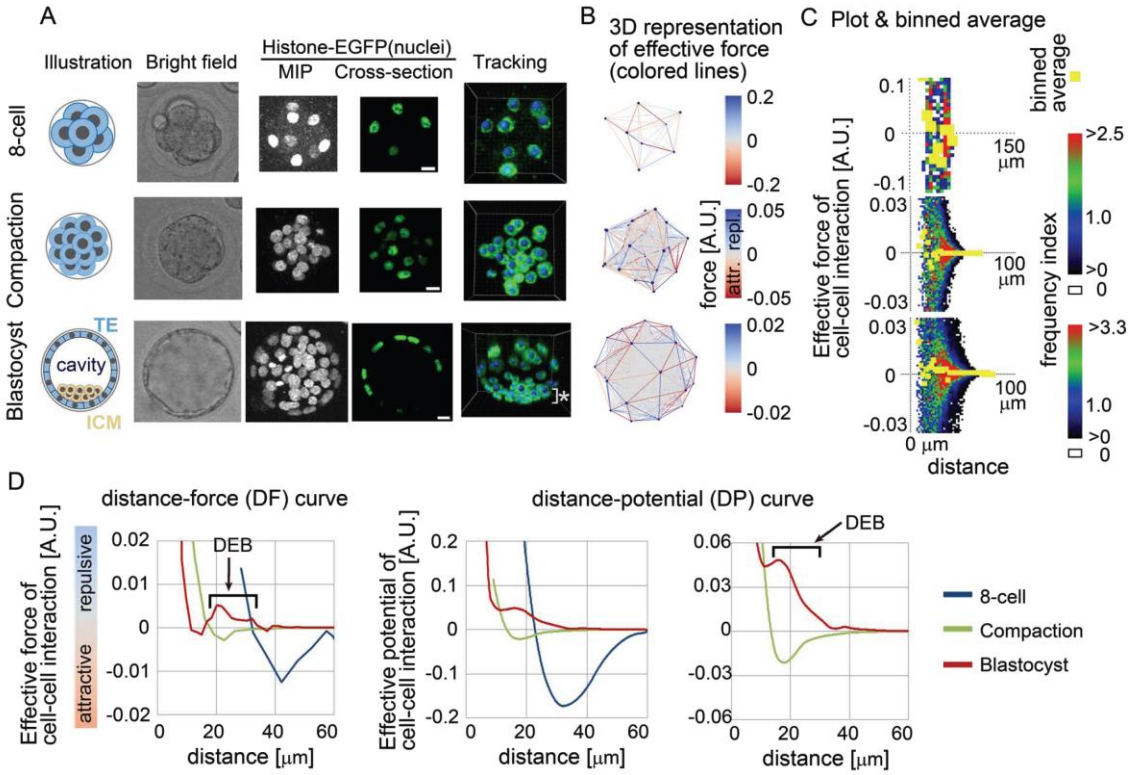

Figure 4: Inference of the effective force of cell–cell interaction in mouse pre-implantation embryos

A. Eight-cell, compaction, and blastocyst stages of mouse embryo are illustrated, and their confocal microscopic images are shown: bright field, maximum intensity projection (MIP) and cross-section of fluorescence of H2B-EGFP. Snapshots of nuclear tracking are also shown; blue spheres indicate the detected nuclei. In the illustration of the blastocyst, ICM and TE cells are depicted by orange and blue, respectively, and a cavity is also described. In the nuclear tracking image of the blastocyst, ICM cells are located around the bottom region of the image, as indicated by *. Scale bars = 15µm. Related movies are

1145 provided (Movie S4A–C). B. Snapshots of nuclear positions with inferred effective forces
1146 were visualized three-dimensionally for the three embryonic stages in a manner similar
1147 to Figure 3A. Blue spheres correspond to cells. The lines represent cell–cell interactions,
1148 and the colors indicate the values of the effective forces [red, attractive (attr.); blue,
1149 repulsive (repl.)]. Related movies are provided (Movie S4D-G). The uniqueness of the
1150 solutions was confirmed in Figure S10B. C. The inferred effective forces of cell–cell
1151 interactions were plotted against the distance of cell–cell interactions in a manner similar
1152 to Figure 2, except that the graph space of the 8-cell stage was divided into 32×32 square
1153 regions, and the averaged values were calculated for the 32 columnar regions. D.
1154 Distance–force (DF) and distance–potential (DP) curves were estimated from (C) in a
1155 manner similar to Figure 3D. A distant energy barrier (DEB) was detected, as indicated
1156 by arrows. Data from other embryos are provided in Figure S6D. The differences in DF
1157 and DP curves between ICM and TE cells and between the outer and inner cells in the
1158 compaction state were described in Figure S6. DP curves between adjacent cells are
1159 provided in Figure S8A-C. Directional differences of DP curves, which may correspond
1160 to cell polarity, are discussed in Figure S9 and S16D.

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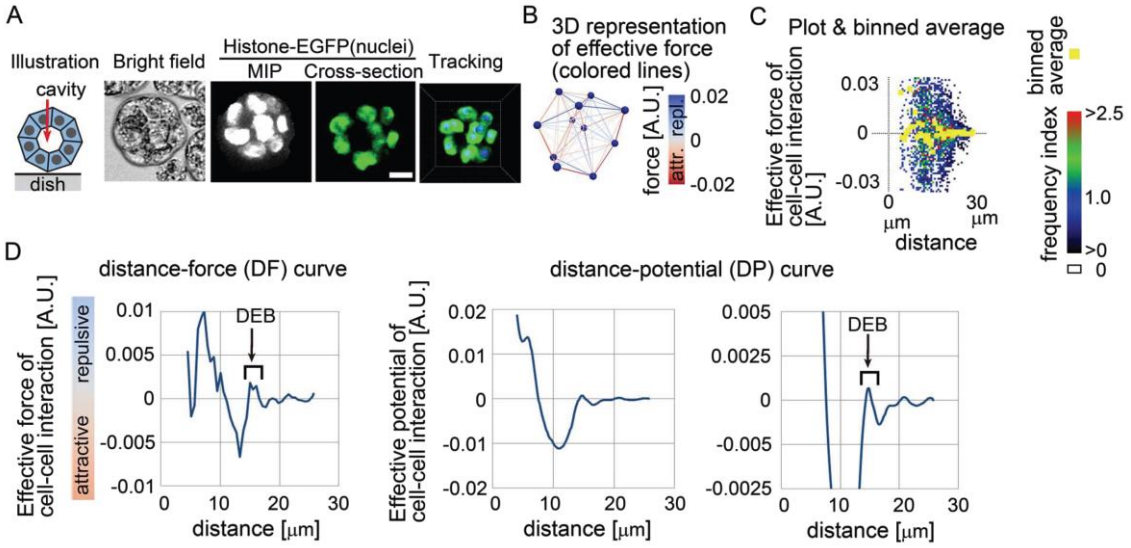

Figure 5: Inference of effective force of cell–cell interaction in cysts formed by MDCK cells

A. MDCK cysts formed under suspension conditions are illustrated, and confocal microscopic images are shown: bright field, maximum intensity projection (MIP) and cross-section of fluorescence of H2B-EGFP. A snapshot of nuclear tracking is also shown; blue spheres represent nuclei. Scale bars = 10μm. A related movie is provided (Movie S5A). B. A snapshot of the nuclear positions with inferred effective forces was three-dimensionally visualized in a manner similar to Figure 3A. The blue spheres correspond to cells. The lines represent cell–cell interactions and the colors indicate the values of the effective forces [red, attractive (attr.); blue, repulsive (repl.)]. A related movie is provided (Movie S5B). The uniqueness of the solutions was confirmed in Figure S10C. C. The

1176 inferred effective forces of cell–cell interaction were plotted against the distance of cell–
1177 cell interaction in a manner similar to Figure 3C. D. Distance–force and distance–
1178 potential curves were estimated from (C) in a manner similar to Figure 3D. DEB was
1179 detected as indicated by arrows. Data from other cysts are provided in Figure S6E. DP
1180 curves between adjacent cells are provided in Figure S8D.

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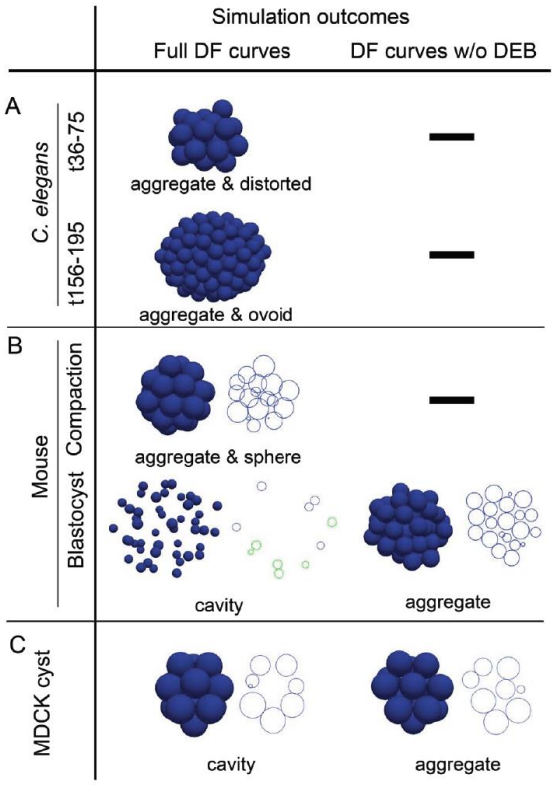

Figure 6: Outcomes of simulations based on inferred distance–force curves

Simulations results under the DF curves derived from *C. elegans* (A) and mouse embryos (B), and the MDCK cyst (C) are shown. Blue spheres correspond to cells. In the *C. elegans* embryos, the DF curves were derived from time frames 36–75 and 156–195 in Figure 3D. In the mouse embryos, the DF curves were derived from the compaction and blastocyst stages in Figure 4D. Cross-sections are also visualized. In the case of the mouse blastocyst, two parallel cross-sections (shown in blue and green) are merged. In the MDCK cyst, the DF curves are derived from Figure 5D. In the blastocyst and the MDCK cyst, the DF curves without DEB were also applied (w/o DEB). The diameters of the blue

spheres are set to be equivalent to the distance with the minimum potential (Fig. S2D). The initial configurations of the simulations are described in Figure S11, along with other simulation results.

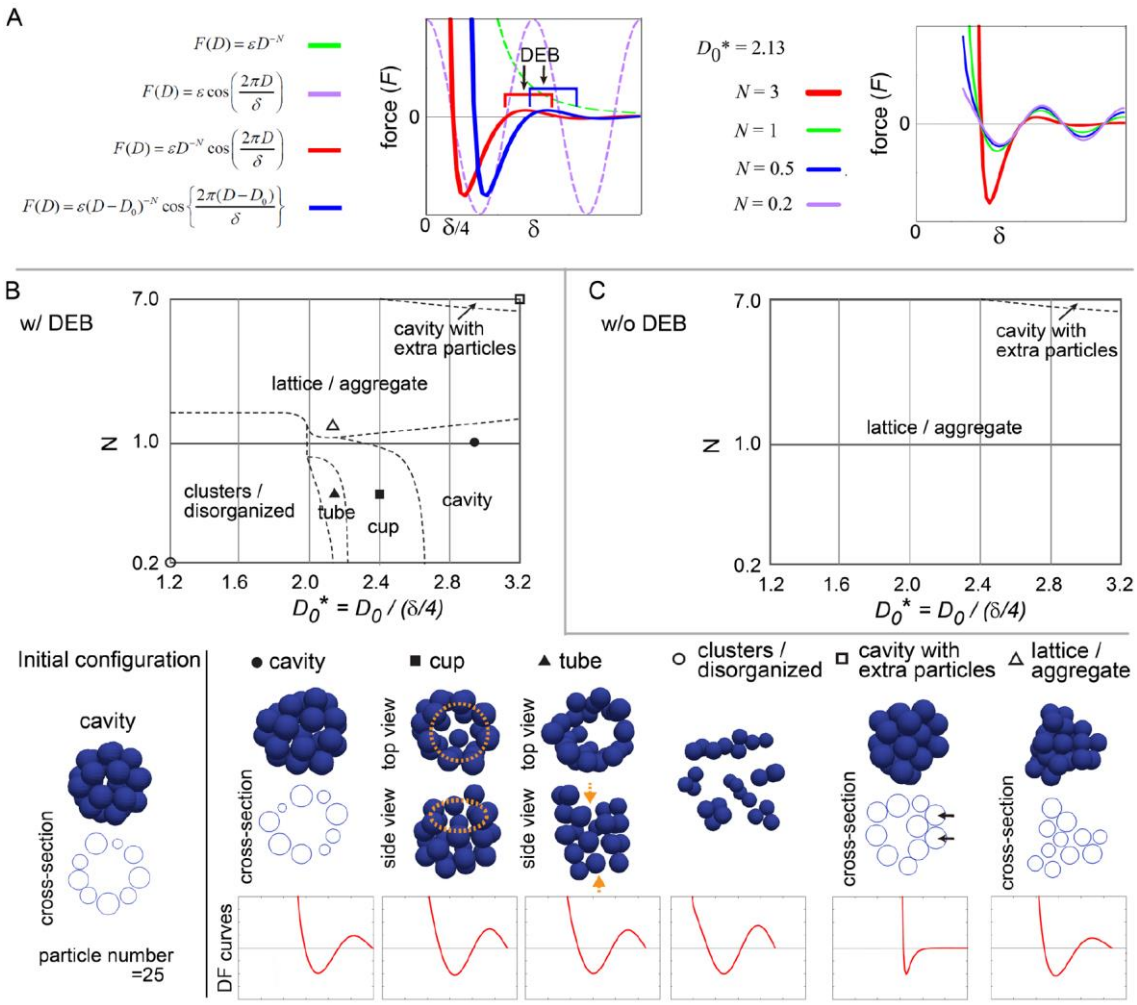

Figure 7: Modeling of distance–force curves and diagrams of simulation outcomes

A. DF curves were mathematically modeled using four parameters: N, D_0, δ, and ε. In the case of the DF curves without the cosine term, the curves ($F = \varepsilon\ (D - D_0)^{-N}$) are always >0 (repulsive) and gradually approach 0 as D increases (green broken line). Upon introduction of the cosine (purple broken line), the curves acquire regions with <0 (attractive) and DEB (red line). Upon introduction of D_0, the curves are translated along the distance (blue line). N affects the gradient of the curves and the height of DEB (right

1208 panel). DF curves from $D = 0$ to D at the end of DEB (w/ DEB) or the curves from $D = 0$
1209 to D before starting DEB (w/o DEB) were used in the following simulations. The physical
1210 meaning and interpretation of these parameters are described in SI, Section 6. B.
1211 Simulations were performed starting from an initial configuration, and the outcomes
1212 under various values of N and D^* are shown in the diagrams. A cavity-bearing structure
1213 was given as the initial configuration; the blue spheres correspond to cells, and a cross-
1214 section is visualized. According to the conditions, a cavity was maintained, whereas cups,
1215 tubes, etc. were generated. Some conditions on the diagram were designated by symbols
1216 (circle, square, and triangle), and their simulation results are visualized (blue spheres).
1217 Example profiles of the DF curves are shown. The diameters of the blue spheres are set
1218 to be 0.5× the distance with the minimum potential, which corresponds to the distance
1219 with $F = 0$ (arrows in the profiles of the DF curves, Fig. S2D). In the case of the cup, the
1220 mouth of the cup is marked by orange rings. In the case of the tube, the open ends of the
1221 tube are shown by orange arrows. In the case of the cavity with extra particles, the extra
1222 particles are shown by arrows. C. Simulation outcomes are shown on diagrams similar to
1223 B, except that the simulations were performed without DEBs. Other diagrams are
1224 provided in Figure S12.
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