Electronic Supplementary Material (ESI) for Soft Matter.
This journal is © The Royal Society of Chemistry 2014

Statistical analysis of vesicle morphology dynamics based on free energy landscape

Soichiro Tsuda, Hiroaki Suzuki, and Tetsuya Yomo

S-1 Shape-characterising Indexes and index values for commonly observed vesicle shape

Elongation (EL) $\equiv \log_2(M/m)$
: As the name indicates, this index characterises elongated shapes. Thus prolate and rod shapes tend to take larger value, while a sphere (circle) takes the smallest (zero) value.

Distorsion (DS) $\equiv A/Pm$
: This index is for deformed vesicles, either in outward or inward direction. For example, it gives prolates (outward deformation) larger value and stomatocytes (inner deformation) smaller value.

Eccentricity (EC) $\equiv \sqrt{1 - m^2/M^2}$
: Eccentricity is mathematically defined as a measure of how much a curve is deviated from a circle. For example, EC of a sphere is 0, whereas a prolate or rod shape tends to have a value closer to 1.

Solidity (SL) $\equiv A/C$
: This index characterises shapes with structures, such as cavity or spines. A sphere, which has no structure, takes the largest value, whereas stomatocytes or starfish take smaller values.

Roundness Factor (RNF) $\equiv P/E$
: This index illustrates how round the shape is. A spheres (i.e. circle) show the smallest value, while deformed shapes (stomatocytes, rods) tend to have larger value.

Although other indexes, such as Dispersion $\equiv \log_2(\pi Mm/A)$, can be used to characterise shapes, we found the above five indexes best characterise observed vesicle shapes.

Figure S1 shows the values of shape-characterisation indexes for typical vesicle shapes. Note that these shapes are not from raw microscope images, but artificially designed on a graphics software.

		EL	DS	EC	SL	DP	RNF
Sphere		0	0.239	0	0.988	2	3.286
Prolate		1.181	0.326	0.898	0.994	2.02	3.607
Budded		0.571	0.246	0.739	0.932	2.09	3.766
Stomatocyte1		0.359	0.197	0.626	0.813	2.219	4.191
Stomatocyte2		0.02	0.221	0.165	0.929	2.154	3.389
Rod		1.971	0.352	0.967	0.98	2.001	4.422
Starfish		0.043	0.209	0.242	0.941	2.129	3.648

Figure S1 The shape-characterisation indexes of commonly observed vesicle shapes. Higher values are highlighted in red while lower ones are in blue.

S-2 Reconstruction of FEL: 1D case

Here we reconstructed FELs using the first principal component only. Fig. S2a shows the probability histograms and reconstucted FELs of vesicle transformation. In practice, we used different bin size for each condition rather than a fixed one for all the conditions because it gives less rugged landscapes. However, FELs reconstructed from both of the methods are essentially the same because probabilities are normalised by $P_{max}(s)$ in eq. 2 in the main text. Figure S2b shows typical shapes of vesicles for different values of the first principal component (PC1). The larger the PC1 becomes, the more vesicle shapes are deformed. Thus, it can be interpreted that the PC1 represents the degree of shape deformation, especially for prolate shapes. Regarding the reconstructed FELs, the slope of FELs becomes more gentle as the external sugar concentration increases (Fig. S2b). This indicates that the applied osmotic pressure linearly affects the shape deformation of vesicles.

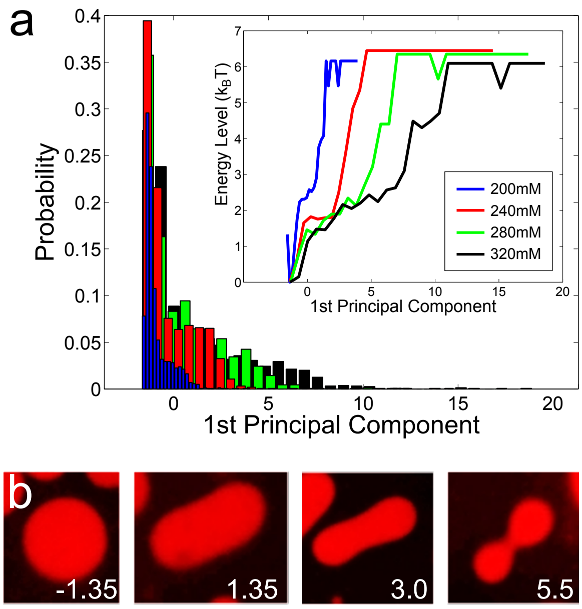

Figure S2 (a) The probability distributions of the 1st principal component (PC1) and the corresponding FELs for different osmotic conditions (inset). The slope of the FEL becomes more gentle for a higher osmotic condition. (b) Typical vesicle shapes observed under the confocal microscope. Numbers in the lower right indicate the PC1 value for the shapes.

S-3 Calculation of theoretical vesicle shapes using spherical harmonics parameterisation

The modeling of theoretical vesicle shapes was done based on the theory in ref.[1]. We here cover the basic theory of vesicle shape modeling using spherical harmonics parameterisation. For further details, see[1].

In general, spherical harmonics (SH) is somtimes called as a 3D counterpart of Fourier transformation (FT). As the FT approximates any arbitrary curves by the combination of Fourier basis functions, spherical harmonics approximates any arbitrary (closed) surface by the combination of SH basis functions. In practice, a vesicle shape is determined as a shape among a number of shapes that satisfy constrants, such as reduced volume v and reduced area difference Δa.

The surface of a vesicle shape is represented as

$$\vec{S} = \begin{bmatrix} x(\theta,\phi) \\ y(\theta,\phi) \\ z(\theta,\phi) \end{bmatrix} = \begin{bmatrix} \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{X} Y_{LK}(\theta,\phi) \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Y} Y_{LK}(\theta,\phi) \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Z} Y_{LK}(\theta,\phi) \end{bmatrix}$$

where $C_L K$ are the expansion coefcients, L and K are integers, $0 < \theta < \pi$, $0 < \theta < 2\pi$. The SH basis function Y_{LK} is defined as

$$Y_{LK}(\theta,\phi) = N_{LK} P_{LK}(cos\theta) e^{iK\phi}$$

with $P_{LK}(cos\theta)$ the associated Legendre functions and N_{LK} normalisation constants.

The surface area A and the volume V of a vesicle are calculated as:

$$A = \int_0^{\pi}\int_0^{2\pi} |\vec{S}_\theta \times \vec{S}_\phi| d\theta d\phi$$

$$V = \frac{1}{3}\int_0^{\pi}\int_0^{2\pi} (\vec{S}\cdot\hat{n}) |\vec{S}_\theta \times \vec{S}_\phi| d\theta d\phi$$

where

$$\vec{S}_\theta = \begin{bmatrix} \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{X} \frac{\partial Y_{LK}(\theta,\phi)}{\partial\theta} \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Y} \frac{\partial Y_{LK}(\theta,\phi)}{\partial\theta} \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Z} \frac{\partial Y_{LK}(\theta,\phi)}{\partial\theta} \end{bmatrix} \quad \vec{S}_\phi = \begin{bmatrix} \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{X} \frac{\partial Y_{LK}(\phi,\phi)}{\partial\phi} \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Y} \frac{\partial Y_{LK}(\phi,\phi)}{\partial\phi} \\ \sum_{L}^{\infty}\sum_{K=-L}^{L} C_{LK}^{Z} \frac{\partial Y_{LK}(\phi,\phi)}{\partial\phi} \end{bmatrix} \quad \hat{n} = \frac{\vec{S}_\theta \times \vec{S}_\phi}{|\vec{S}_\theta \times \vec{S}_\phi|}$$

Once constraint values are given, we can calculate vesicle shapes. However, there are in principle a number of vesicle shapes that satisfy the constraints. As an actual vesicle shape is realised as a shape that minimise the bending energy associated with the shapes[2], an energy function for vesicle shapes is introduced for model vesicles as explained below.[3,4]

The BC model

As mentioned in the main text, the BC model employs following energy function:

$$W_b/8\pi\kappa_b = \frac{1}{2}\oint (C_1 + C_2)^2 da$$

where κ_b represents a local bending modulus and C_1 and C_2 are local curvatures. Here, the reduced volume v and the the reduced area difference Δa are given as constraints.

A vesicle shape is determined by searching a vesicle shape with the minimum energy among shapes that satisfy the given constraints. The minimisation of the energy function is carried out on Matlab as a parameter optimisation process using active set algorithm. A series of vesicles are obtained by gradually varying v and Δa. In practice, we started from $v = 1$ and $\Delta a = 1$, at which a spherical vesicle is the shape with the minimum bending energy. A series of calculations for obtaining minimal energy vesicle shapes were carried out for $0.5 \leq v \leq 1$, while Δa is fixed to 1. In practice, parameters for vesicle shapes $(C^X_{LK}, C^Y_{LK}, C^Z_{LK})$ obtained from a previous calculation were used to calculate a vesicle shape with a slightly different v. After we obtained a complete series of vesicle shapes with various v, vesicle shapes with different Δa were obtained.

S-4 The minimum bending energy W_b for v = 0.63

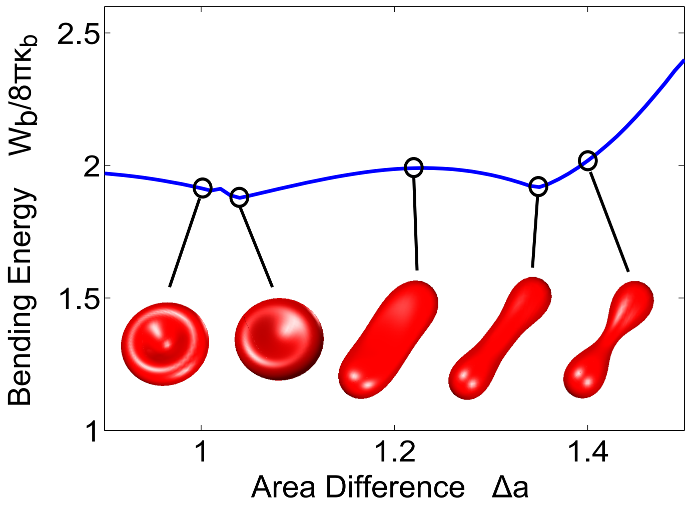

Figure S3 A plot of the bending energy W_b against area difference Δa for $v = 0.63$. Area differences for the shapes shown in the plot are $\Delta a = 1.0, 1.04, 1.22, 1.35$, and 1.40 (left to right). Note that the shape with the minimum bending energy in this condition is axisymmetric oblate ($\Delta a = 1.04$) in contract to other conditions.

S-5 Comparison of different shape-characterising indexes

To illustrate the choice of shape-characterising indexes on the reconstructed FEL, Fig. S4 show PCA biplots (plots of the 1st and 2nd PC and plots of component weights).

Measures used for the plots are as follows:

(1) Elongation (EL) $\equiv \log_2(M/m)$

(2) Dispersion (DP) $\equiv \log_2(\pi Mm/A)$

(3) Distorsion (DS) $\equiv A/Pm$

(4) Eccentricity (EC) $\equiv \sqrt{1-m^2/M^2}$

(5) Solidity (SL) $\equiv A/C$

(6) Roundness Factor (RNF) $\equiv P/E$

(7) Perimeter/minor axis (EM) $\equiv E/m$

We have found that EL, SL and RNF are key measures that stretches out the distribution and separate different vesicle shapes. Empirically we decided to use EL,EC,DS,SL,RNF as shape-characrersing indexes because they give a nicely separated PCA distribution along x and y axes (Fig. S4a), whereas other combinations of indexes were reasonably packed in x-axis (Fig. S4b) or y-axis (Fig. S4c).

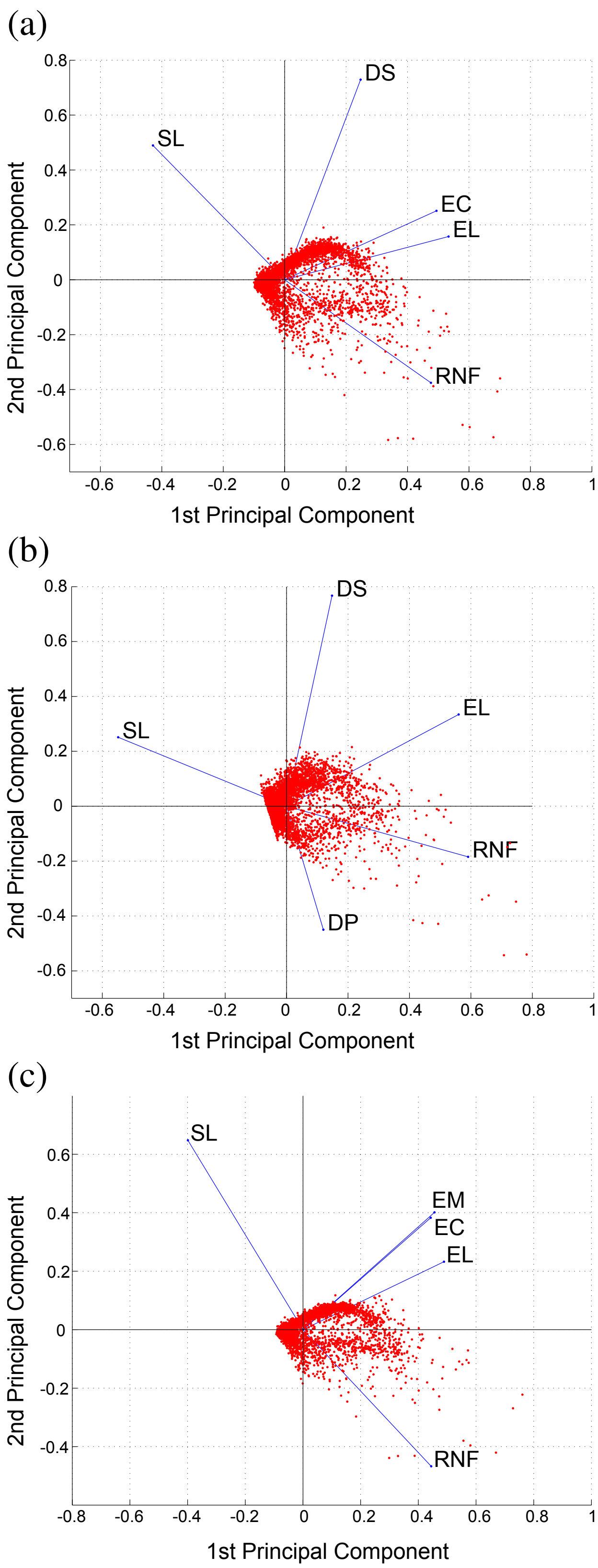

Figure S4 Comparison of PCA biplot with different shape-characterising indexes. Measures used are: (a) EL,EC,DS,SL,RNF (b) EL,DP,DS,SL,RNF (c) EL,EC,EM,SL,RNF.

S-6 Examples of vesicle shapes in FEL space

Figure S5 shows several examples of binarised vesicle images that are used for FEL analysis for different osmotic conditions. Vesicle shapes found in a small region in the FELs look quite similar each other. This shows that PCA successfully categorises and maps vesicles of similar shapes into a region in the FEL.

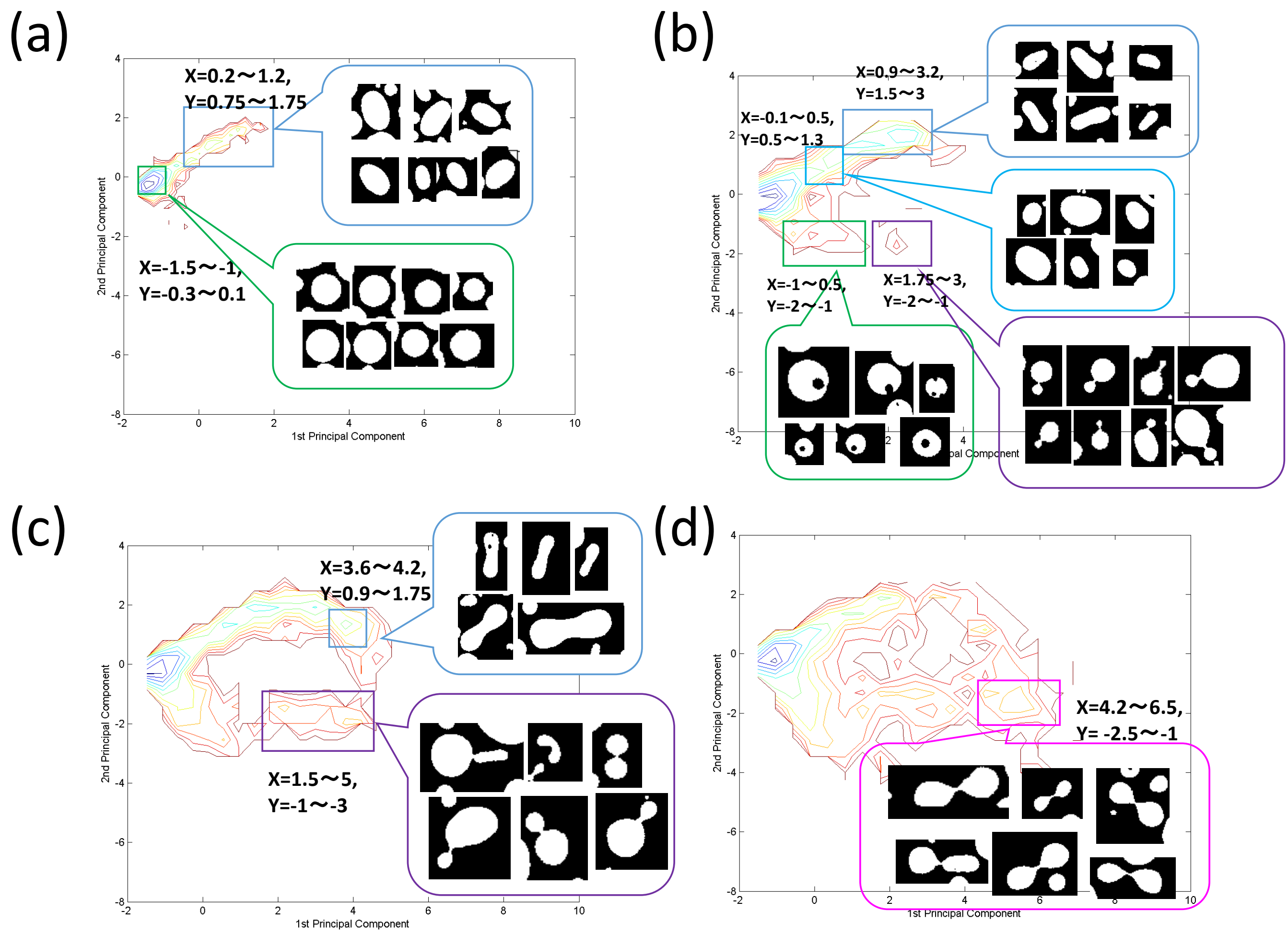

Figure S5 Examples of vesicle shapes found in each osmotic condition, (a)200 mM, (b) 240 mM, (c) 280 mM, (d) 320 mM conditions. Colour squares roughly indicate the regions of interest. Vesicle images shown in the plots represent seveal or all vesicles found in the region. Other vesciles found in the region but not shown here were also in similar shapes.

S-7 Distributions of paramter values

Figure S6-S13 and S14-S21 show distributions of parameter values (raw parameter values, such as Area and Perimeter, and shape-characterisation index, such as Elongation and Solidity) along PC1 and PC2 for each osmotic pressure condition. The plot was made by divding the data into 30x30 regions and plotting average parameter values for each region.

In Fig. S6-S13, it appeared that there was no special correlations in each plot. This suggests that PCA well-shuffles the raw parameters and PCA are not simply reflecting certain parameter(s), but structured in the way that maximises variances of the original data. This helps lipid vesicle shapes to be classified by particular shapes and arrange them spatially (hence the Fig.3 in the main text).

Figure S14-S21 are essentially equivalent to the biplot in Fig. S4a. In all the conditions they show the same tendency, i.e. the distribution increases along a direction regardless of the osmotic conditions.

Supplementary Movie: A temporal vesicle transformation on the reconstructed FEL

The movie shows DIC microscope images and a trajectory of temprally deforming vesicle on the reconstructed FEL for the 240 mM condition shown in Fig. 4. A position in the FEL for the deforming vesicle is mapped every 2 s in pink. After a daughter vesicle is formed, positions for the mother vesicle is mapped in green.

References

[1] K. Khairy and J. Howard, *Soft Matter*, 2011, **7**, 2138.

[2] P. Canham, *J. Theor. Biol.*, 1970, **26**, 61–81.

[3] S. Svetina and B. Zeks, *Eur. Biophys. J.*, 1989, **17**, 101–111.

[4] V. Heinrich, S. Svetina and B. Žekš, *Phys. Rev. E*, 1993, **48**, 3112–3123.

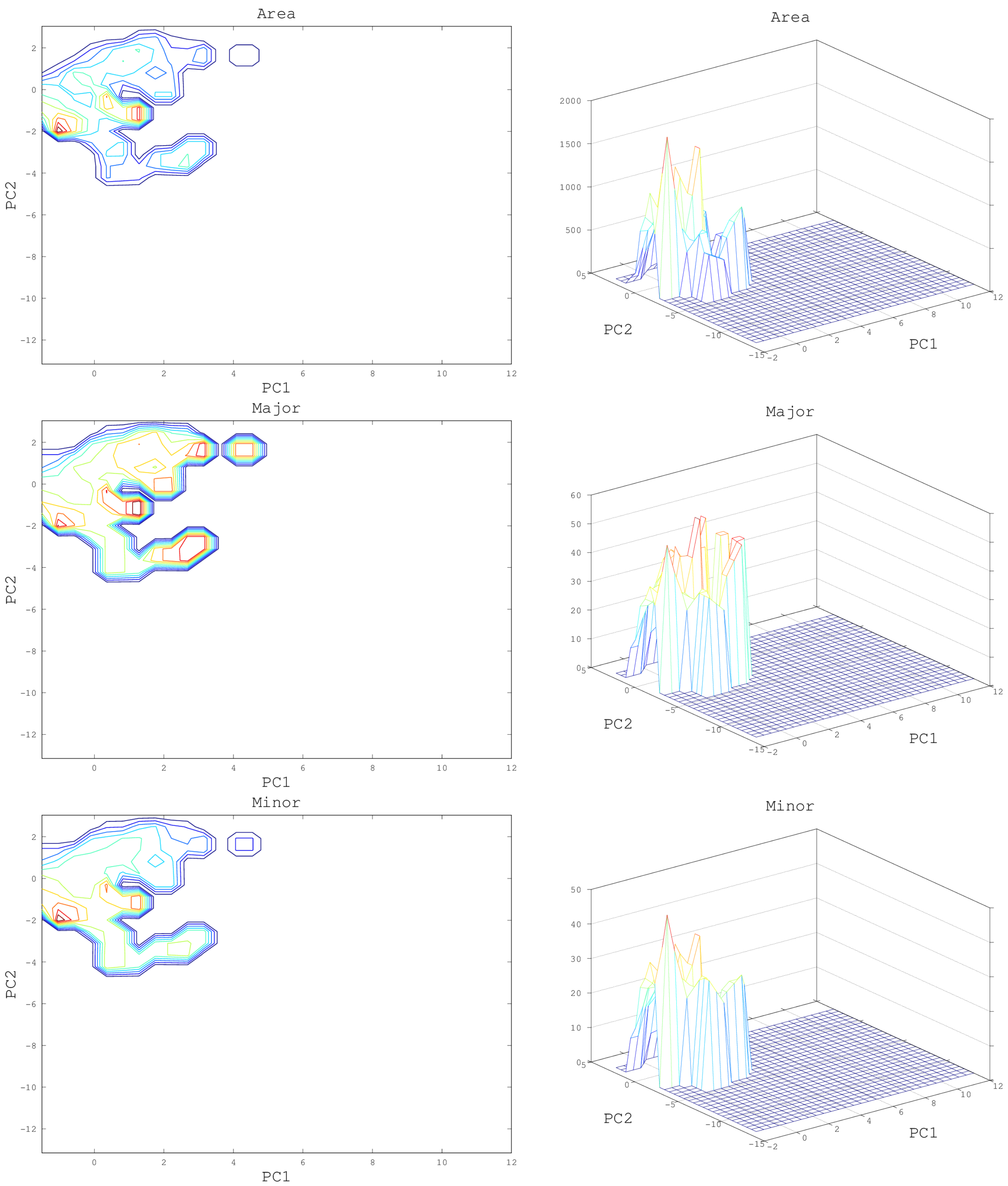

Figure S6 Distribution of parameter values for Area, Major axis and Minor axis along PC1 and PC2 at the 200 mM condition.

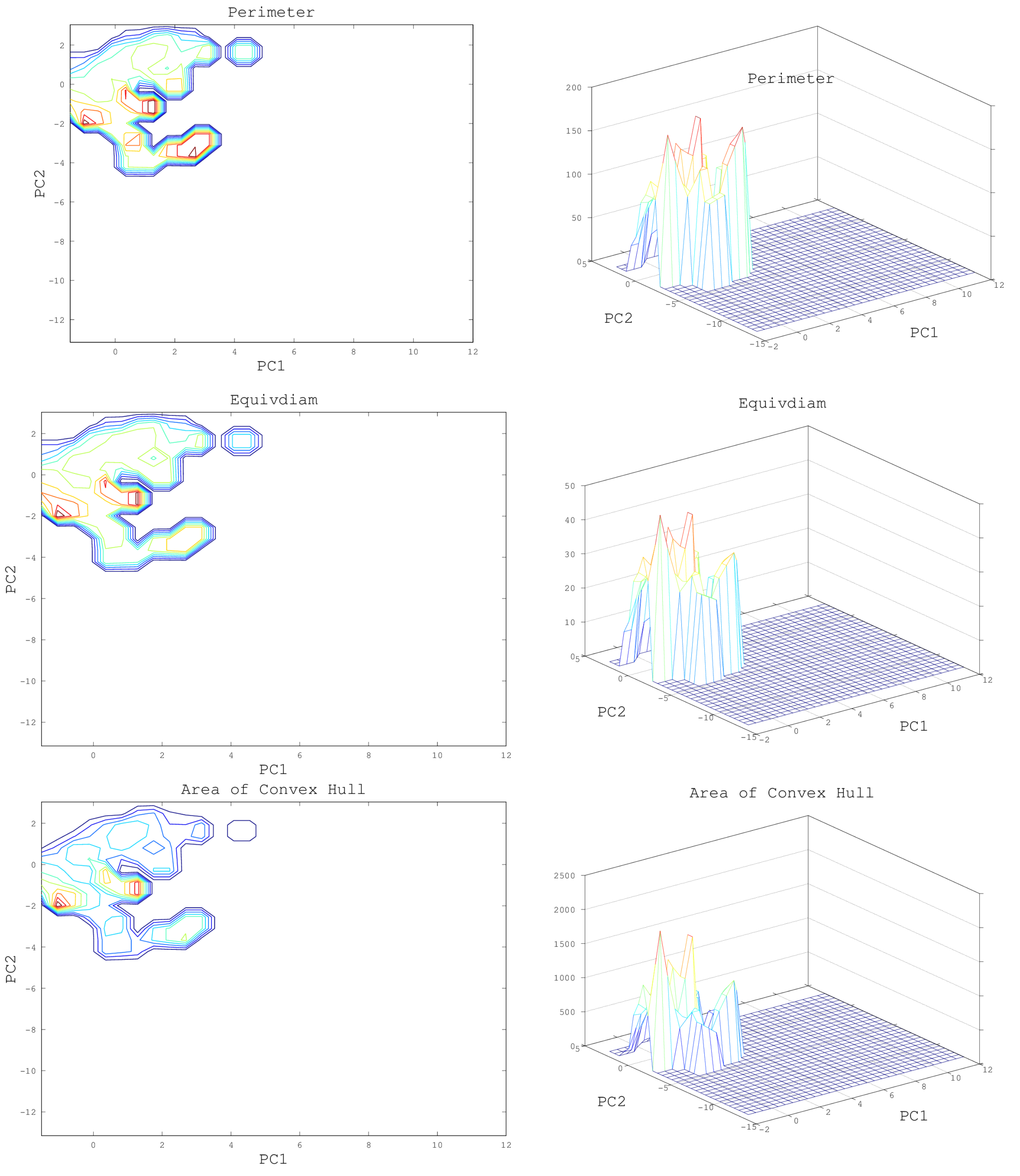

Figure S7 Distribution of parameter values for Perimeter, Equivalent diameter, and Area of convex hull along PC1 and PC2 at the 200 mM condition.

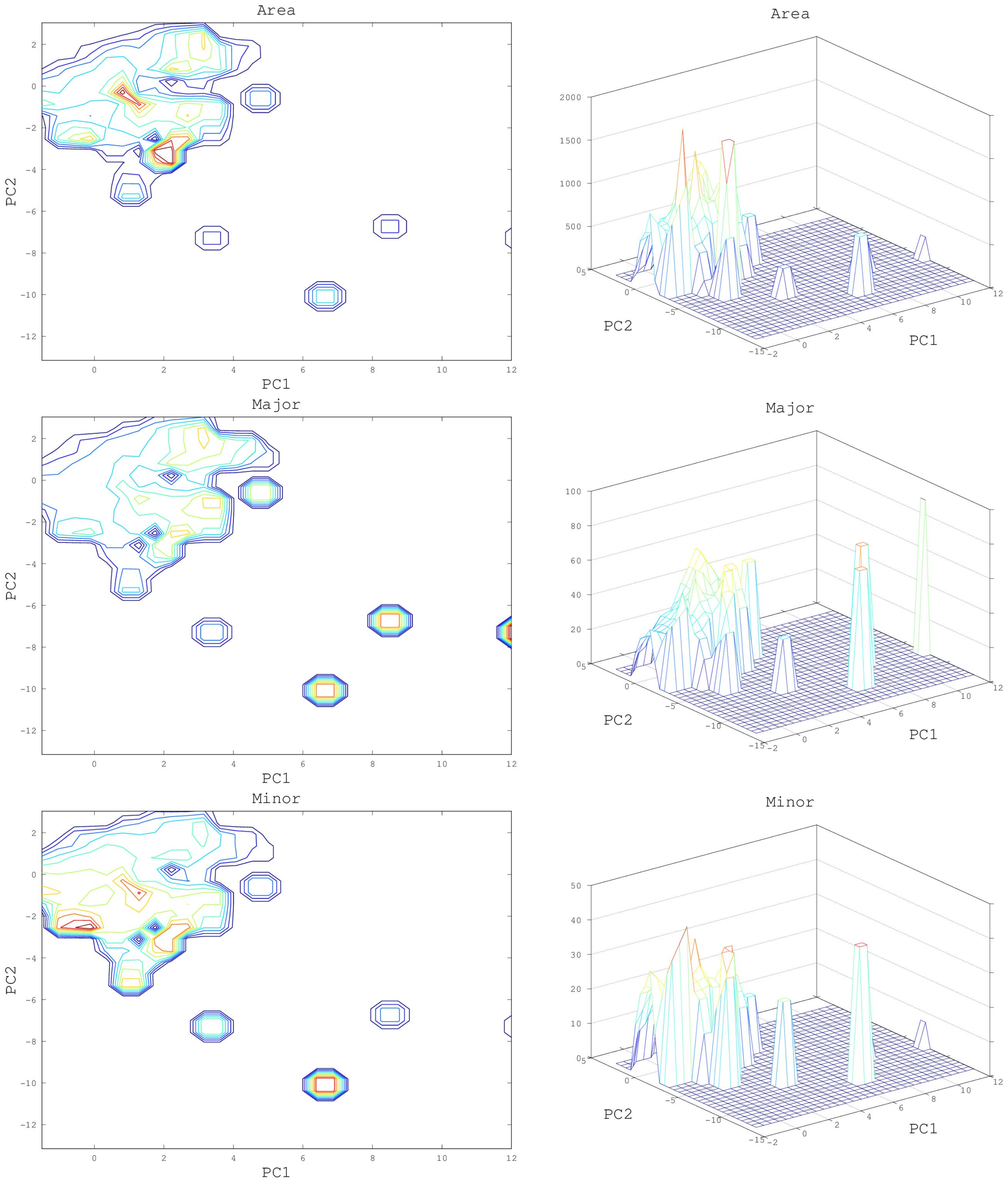

Figure S8 Distribution of parameter values for Area, Major axis and Minor axis along PC1 and PC2 at the 240 mM condition.

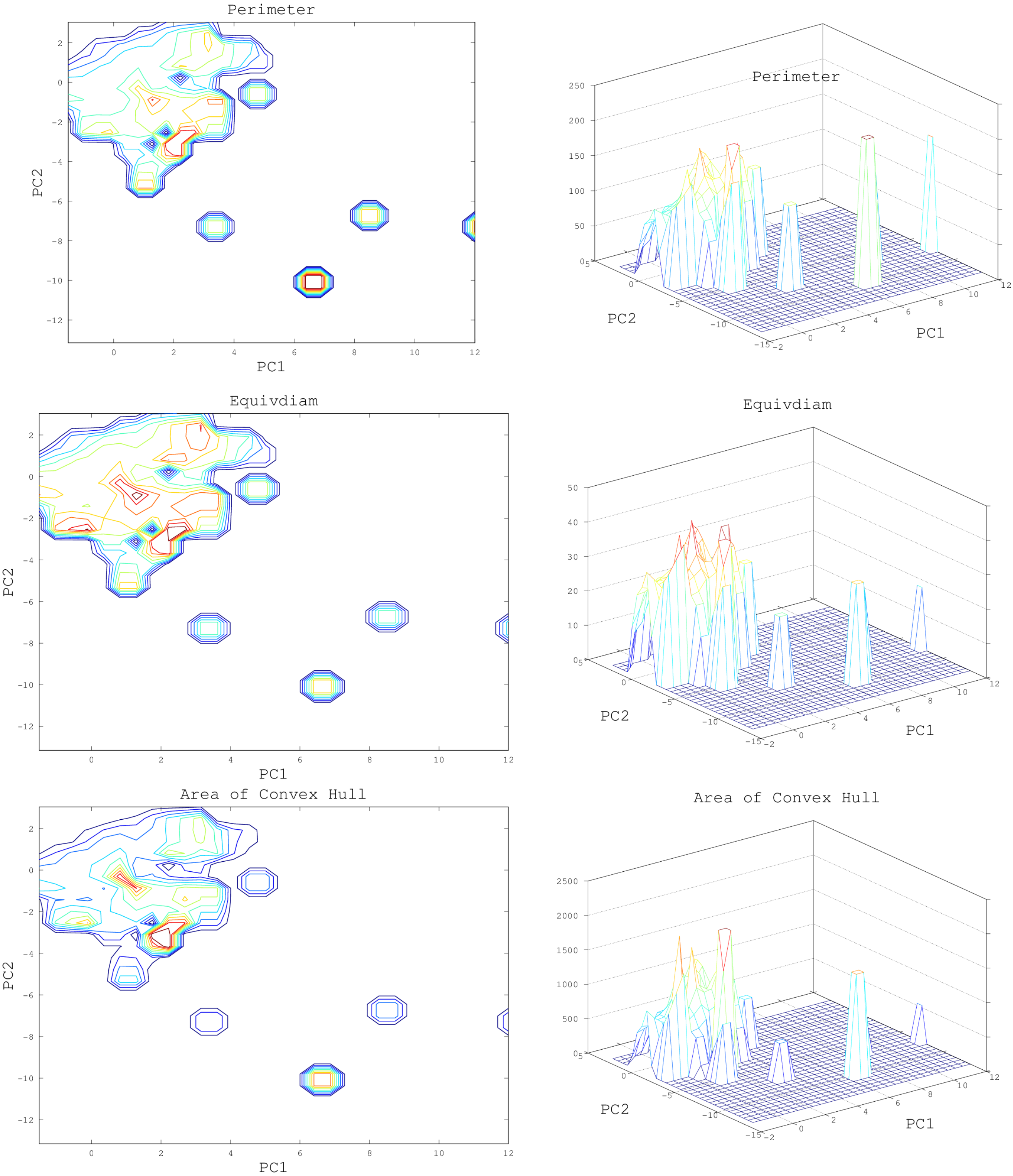

Figure S9 Distribution of parameter values for Perimeter, Equivalent diameter, and Area of convex hull along PC1 and PC2 at the 240 mM condition.

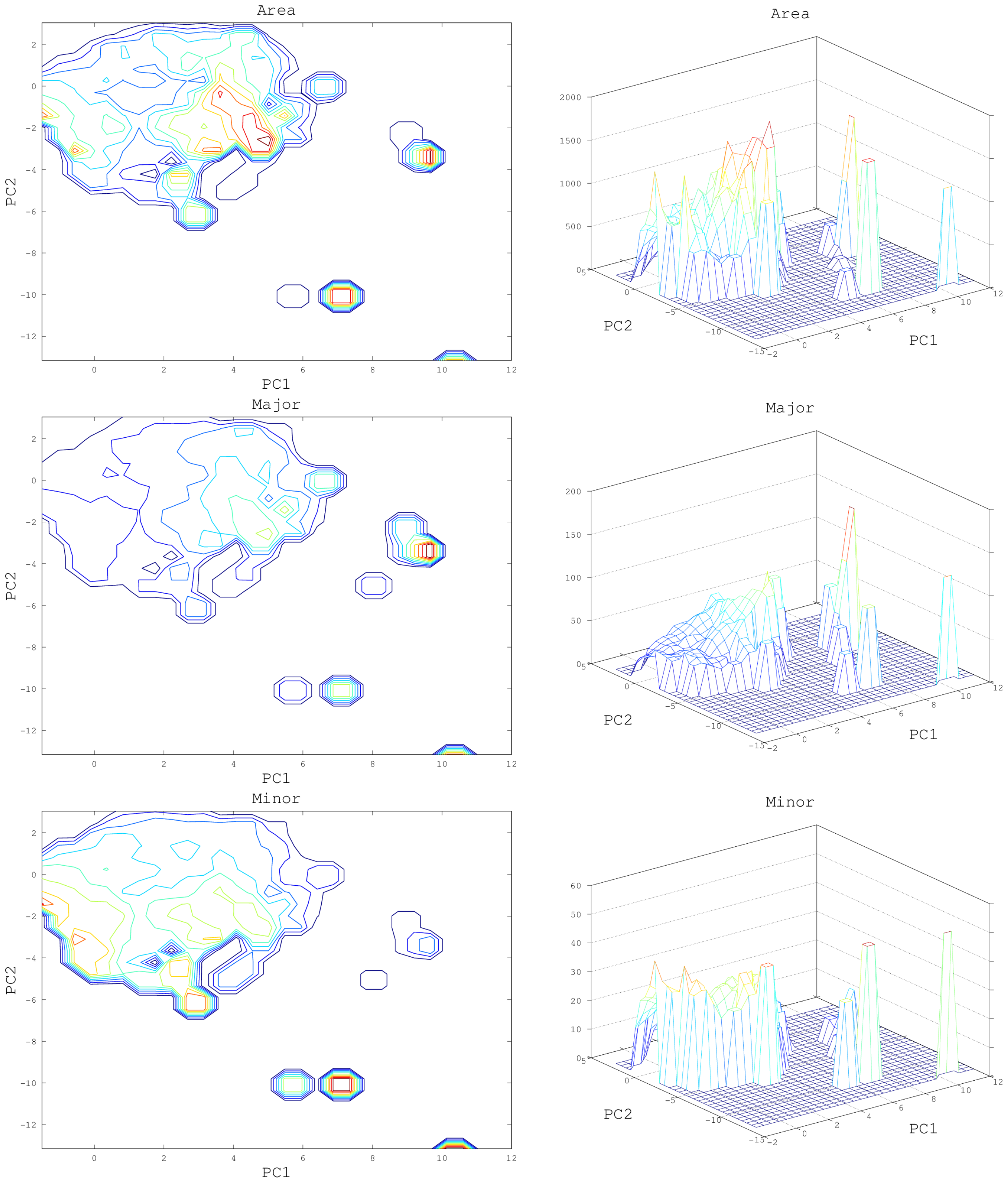

Figure S10 Distribution of parameter values for Area, Major axis and Minor axis along PC1 and PC2 at the 280 mM condition.

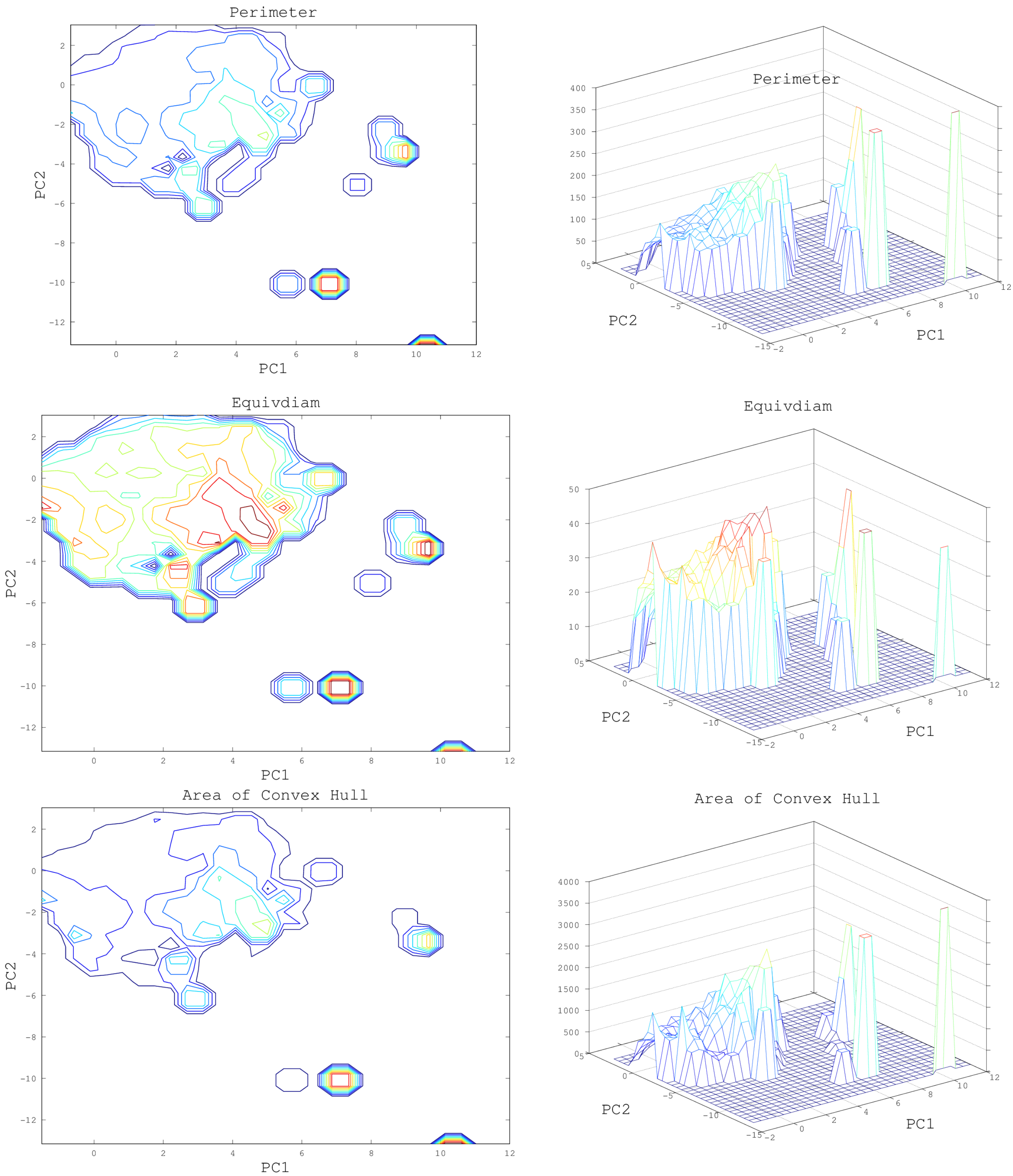

Figure S11 Distribution of parameter values for Perimeter, Equivalent diameter, and Area of convex hull along PC1 and PC2 at the 280 mM condition.

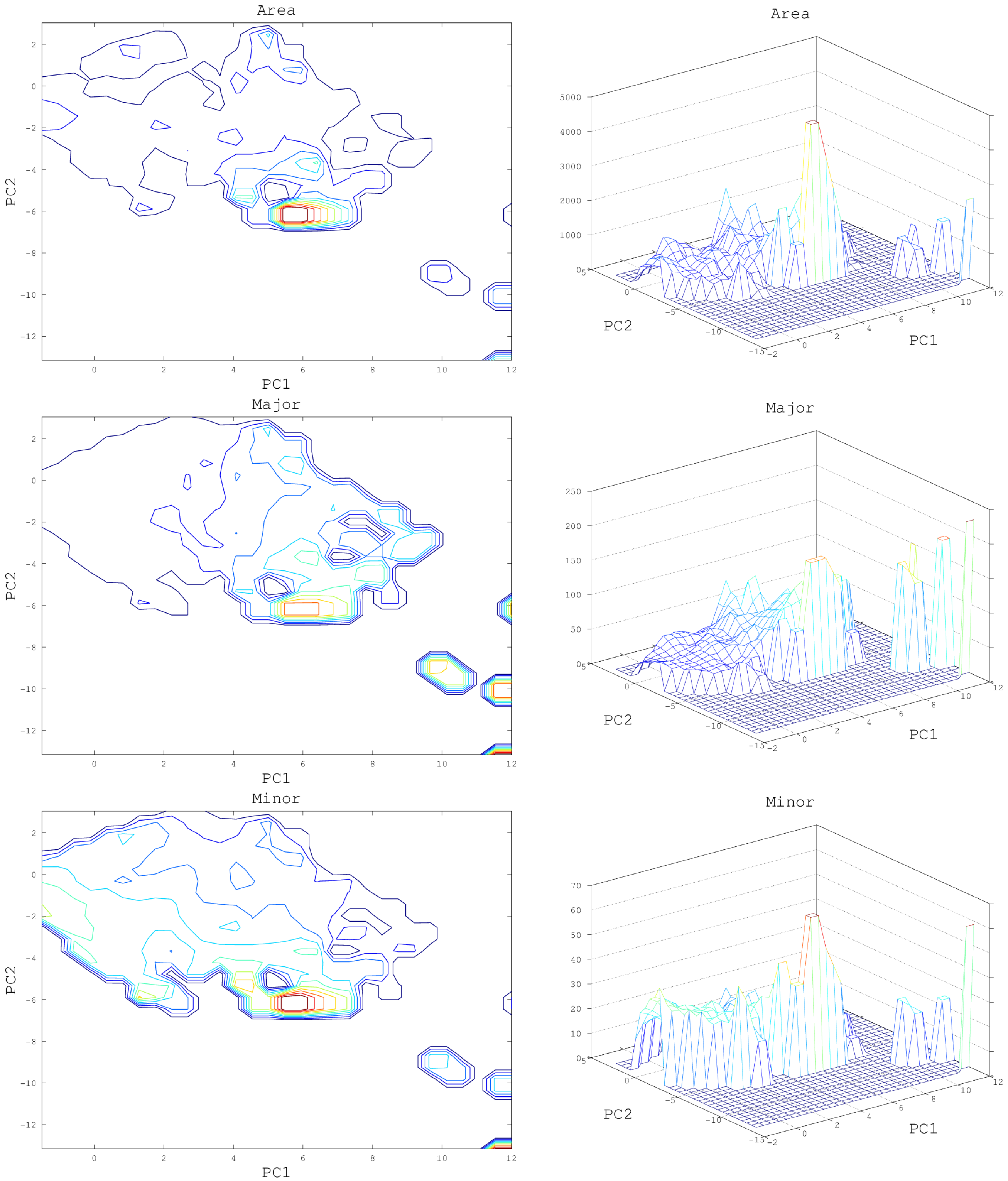

Figure S12 Distribution of parameter values for Area, Major axis and Minor axis along PC1 and PC2 at the 320 mM condition.

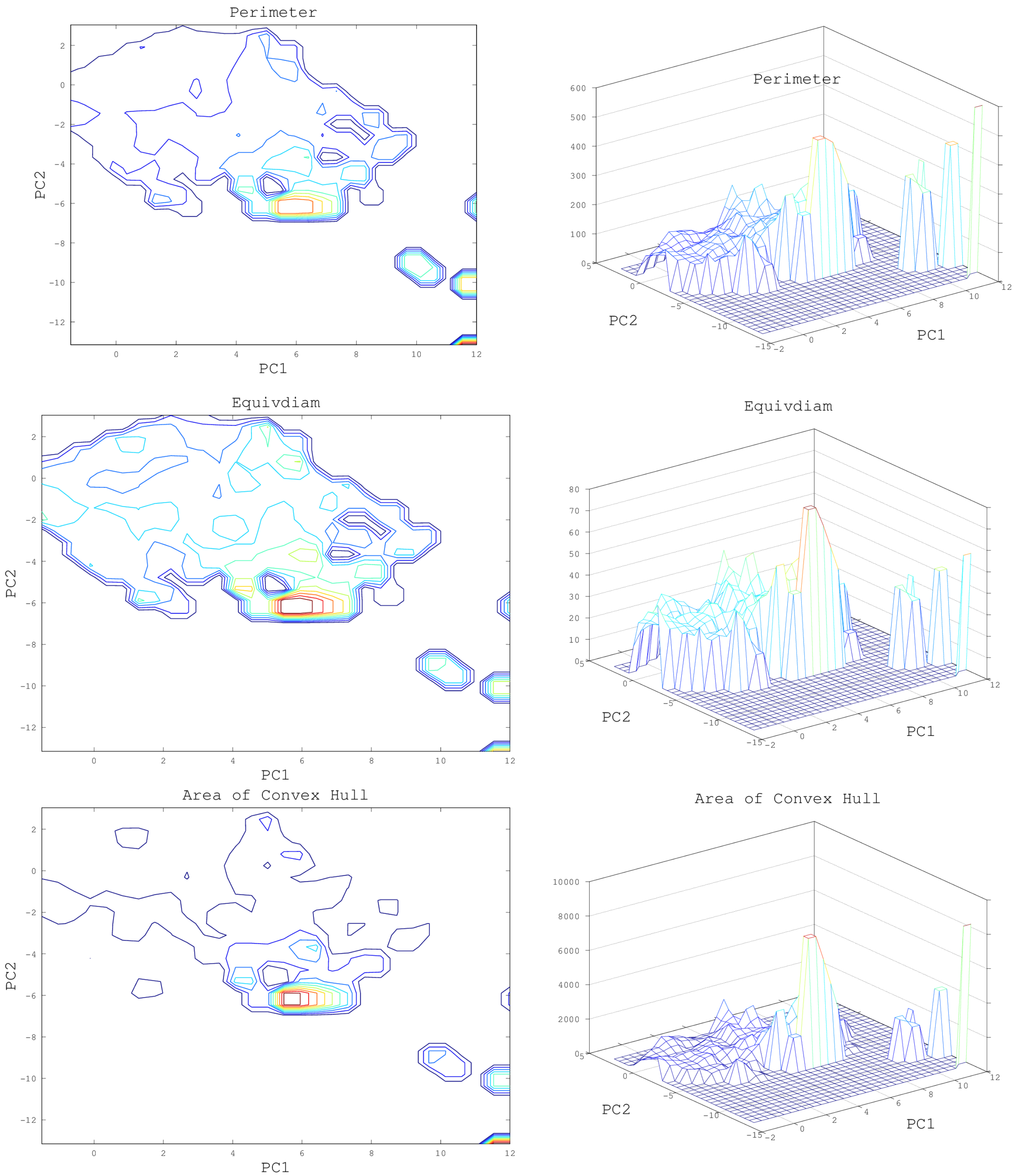

Figure S13 Distribution of parameter values for Perimeter, Equivalent diameter, and Area of convex hull along PC1 and PC2 at the 320 mM condition.

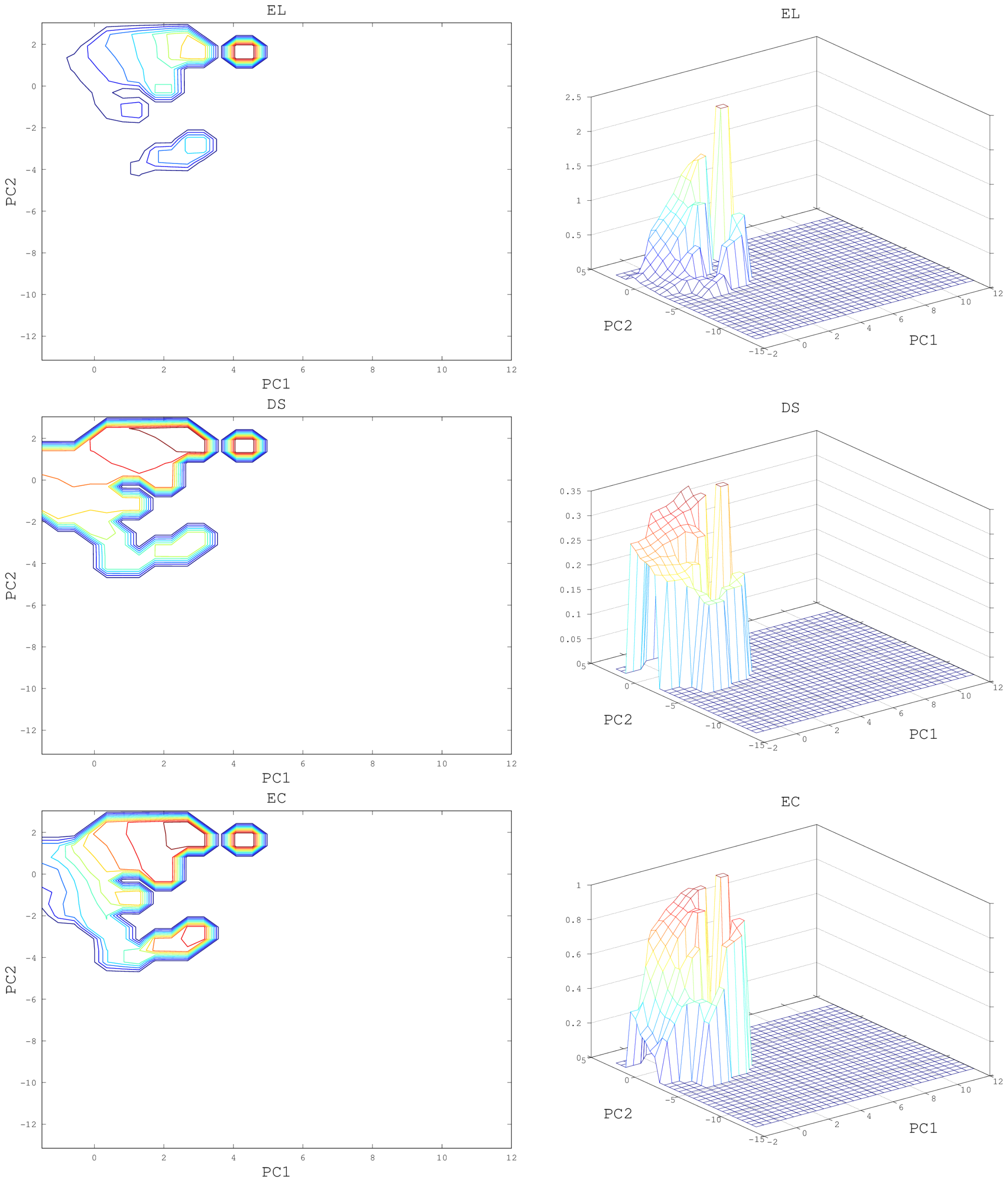

Figure S14 Distribution of Shape-characterisation index values for Elongation (EL), Dispersion (DS), and Eccentricity (EC) along PC1 and PC2 at the 200 mM condition.

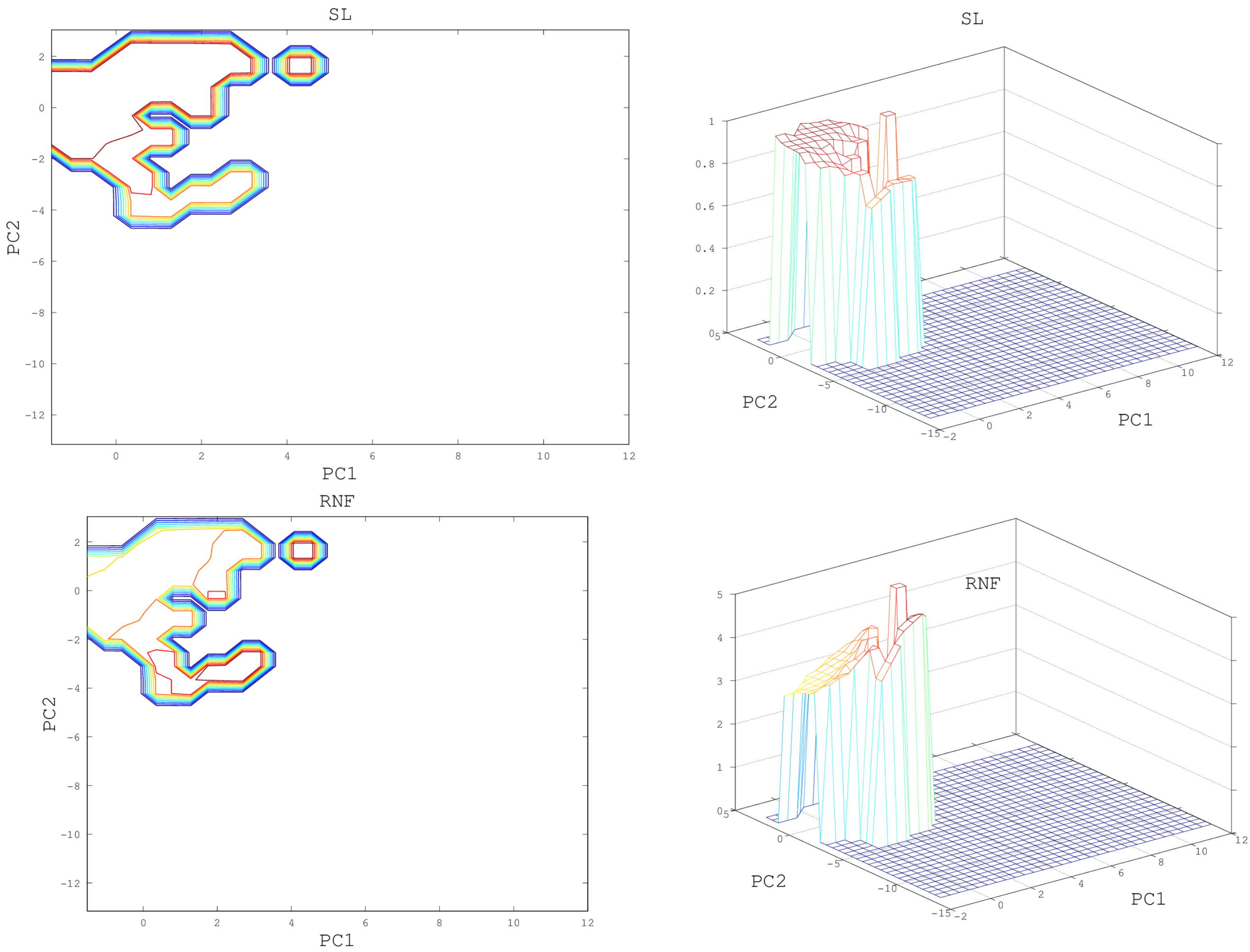

Figure S15 Distribution of Shape-characterisation index values for Solidty (SL) and Roundness factor (RNF) along PC1 and PC2 at the 200 mM condition.

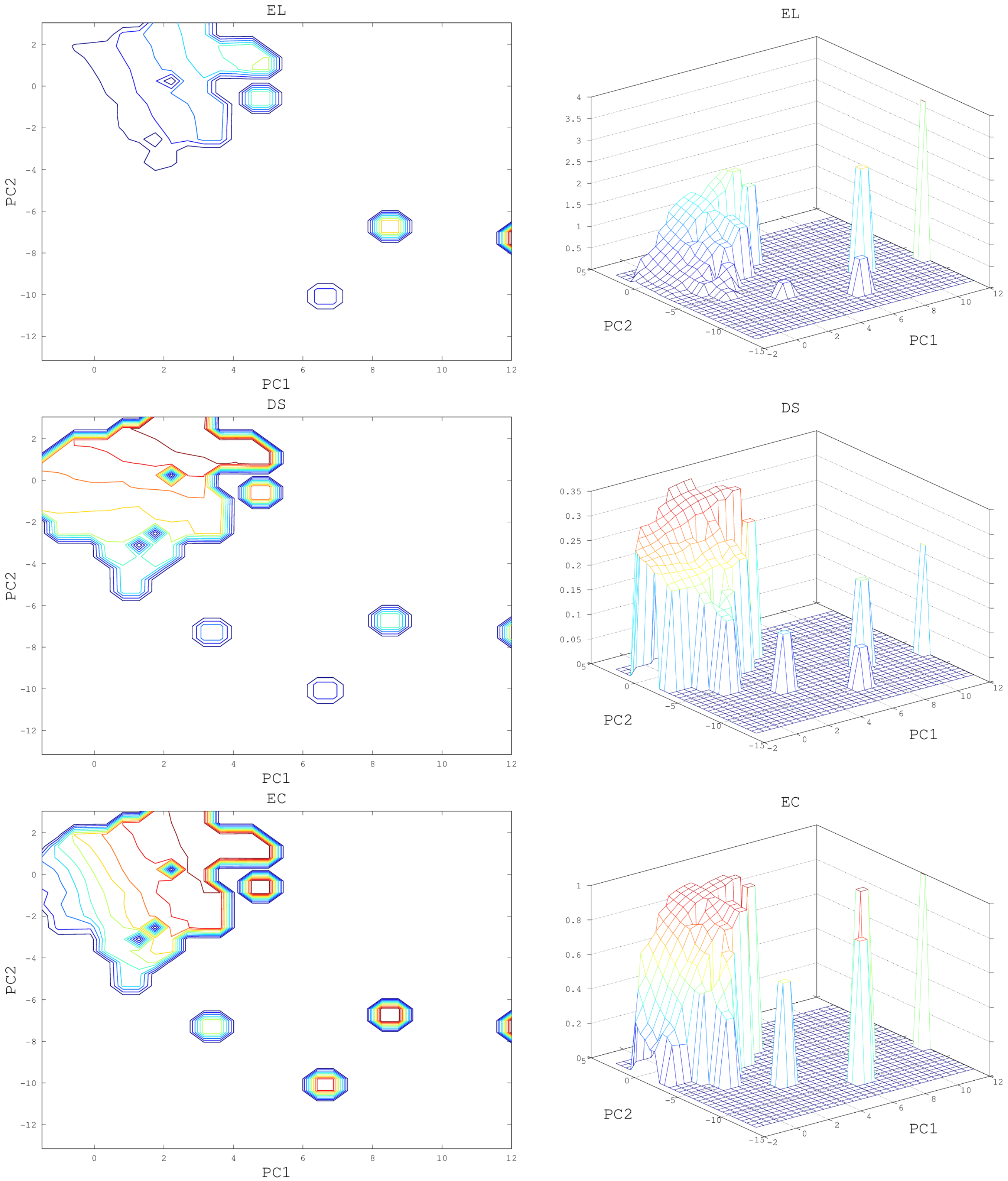

Figure S16 Distribution of Shape-characterisation index values for Elongation (EL), Dispersion (DS), and Eccentricity (EC) along PC1 and PC2 at the 240 mM condition.

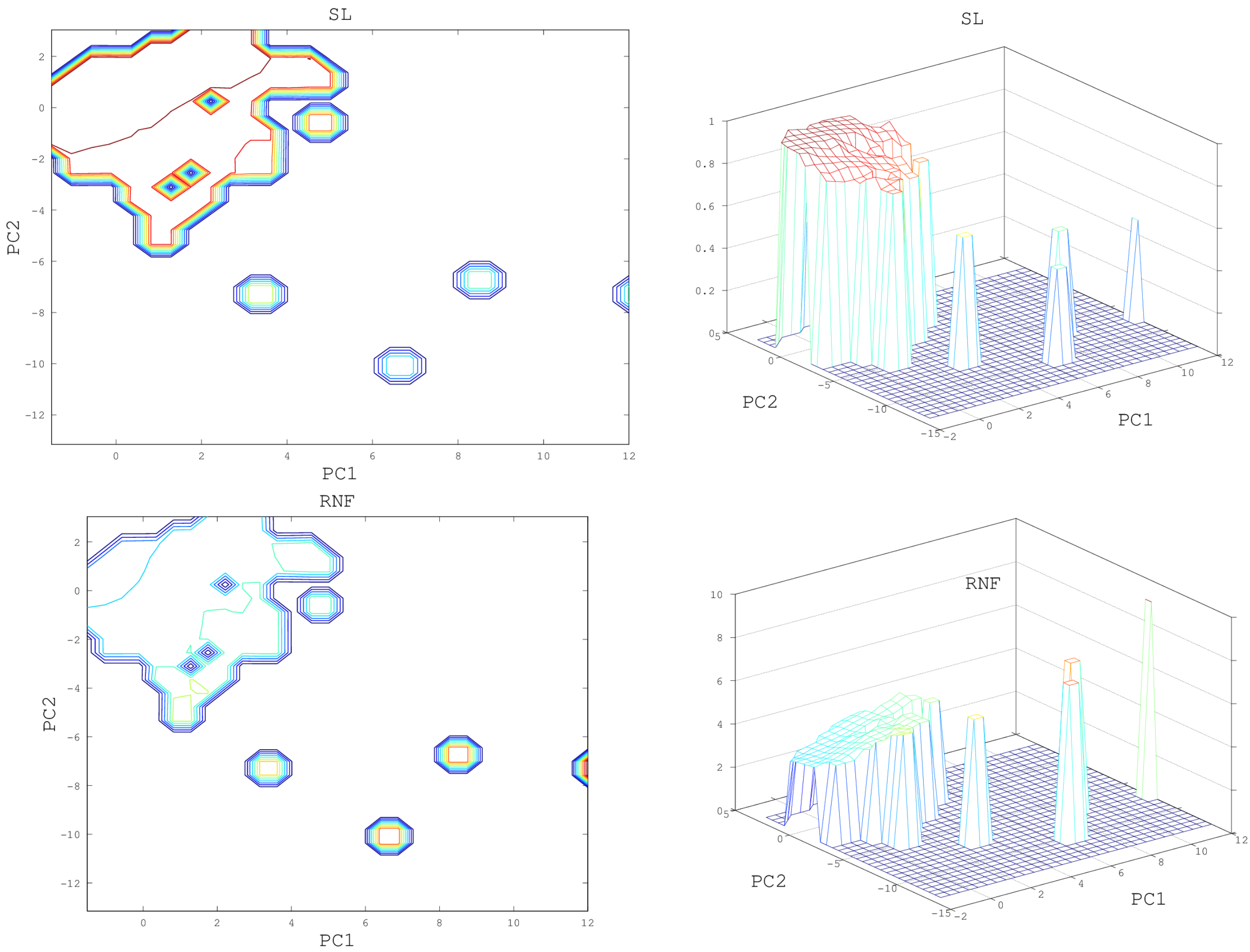

Figure S17 Distribution of Shape-characterisation index values for Solidty (SL) and Roundness factor (RNF) along PC1 and PC2 at the 240 mM condition.

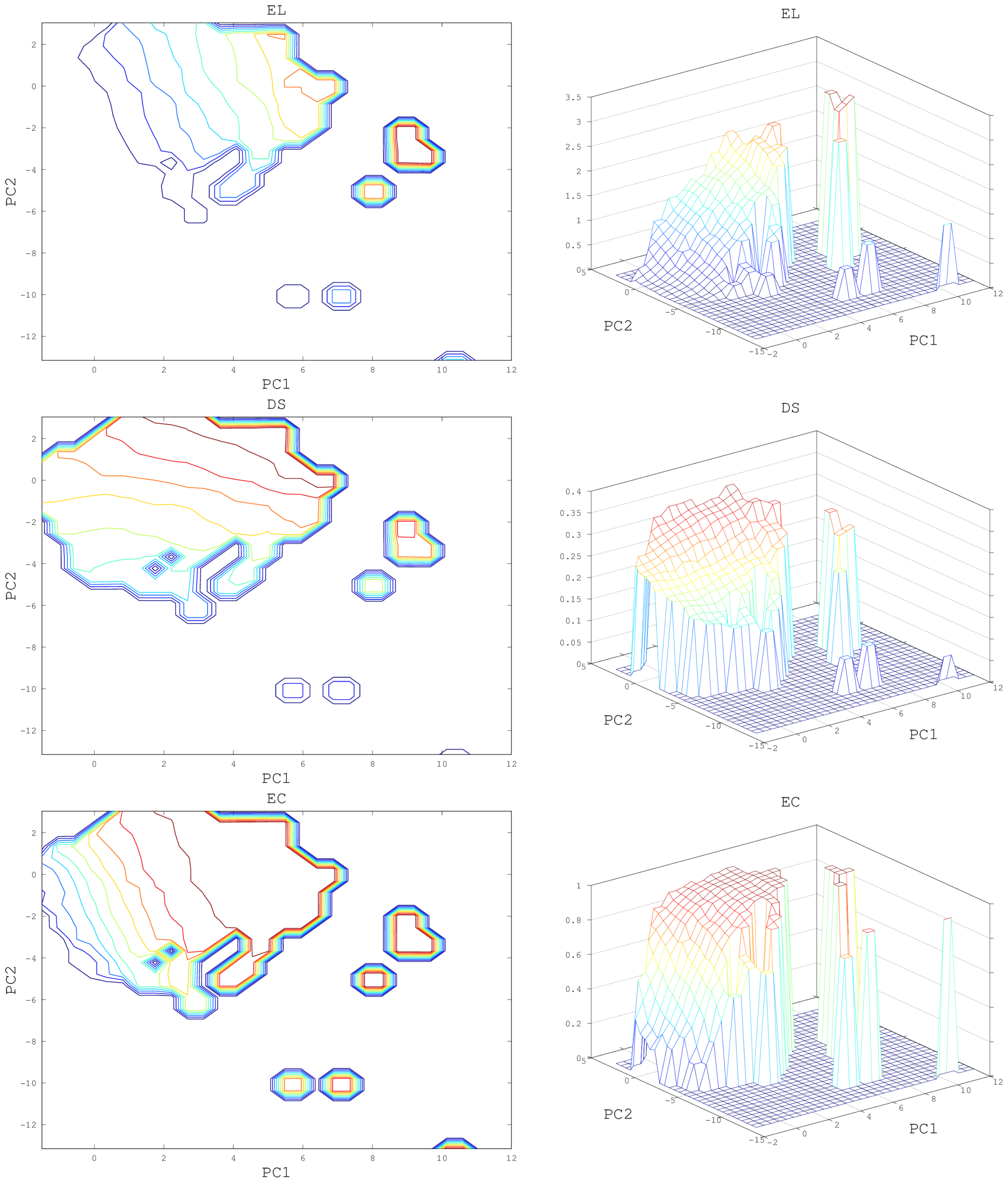

Figure S18 Distribution of Shape-characterisation index values for Elongation (EL), Dispersion (DS), and Eccentricity (EC) along PC1 and PC2 at the 280 mM condition.

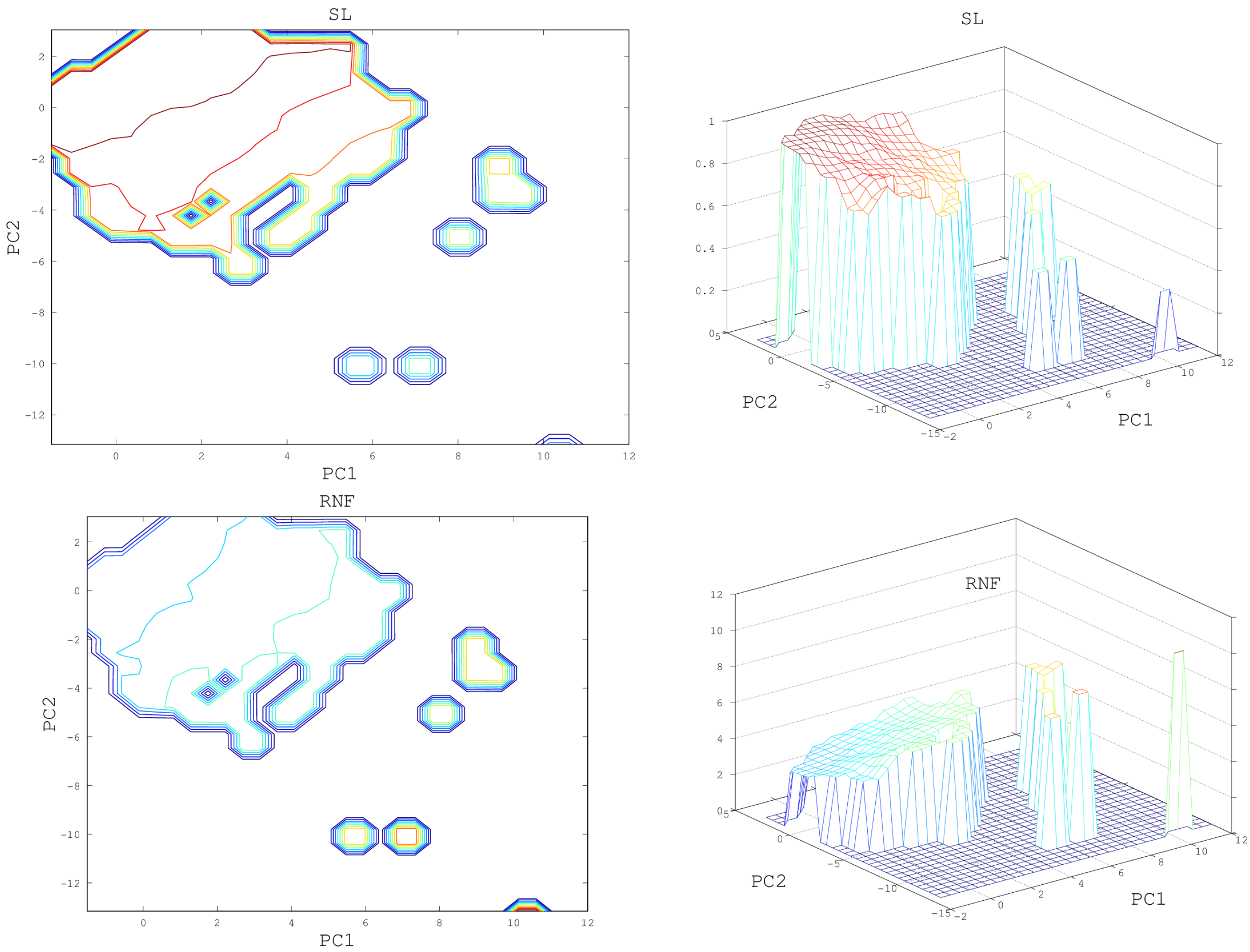

Figure S19 Distribution of Shape-characterisation index values for Solidty (SL) and Roundness factor (RNF) along PC1 and PC2 at the 280 mM condition.

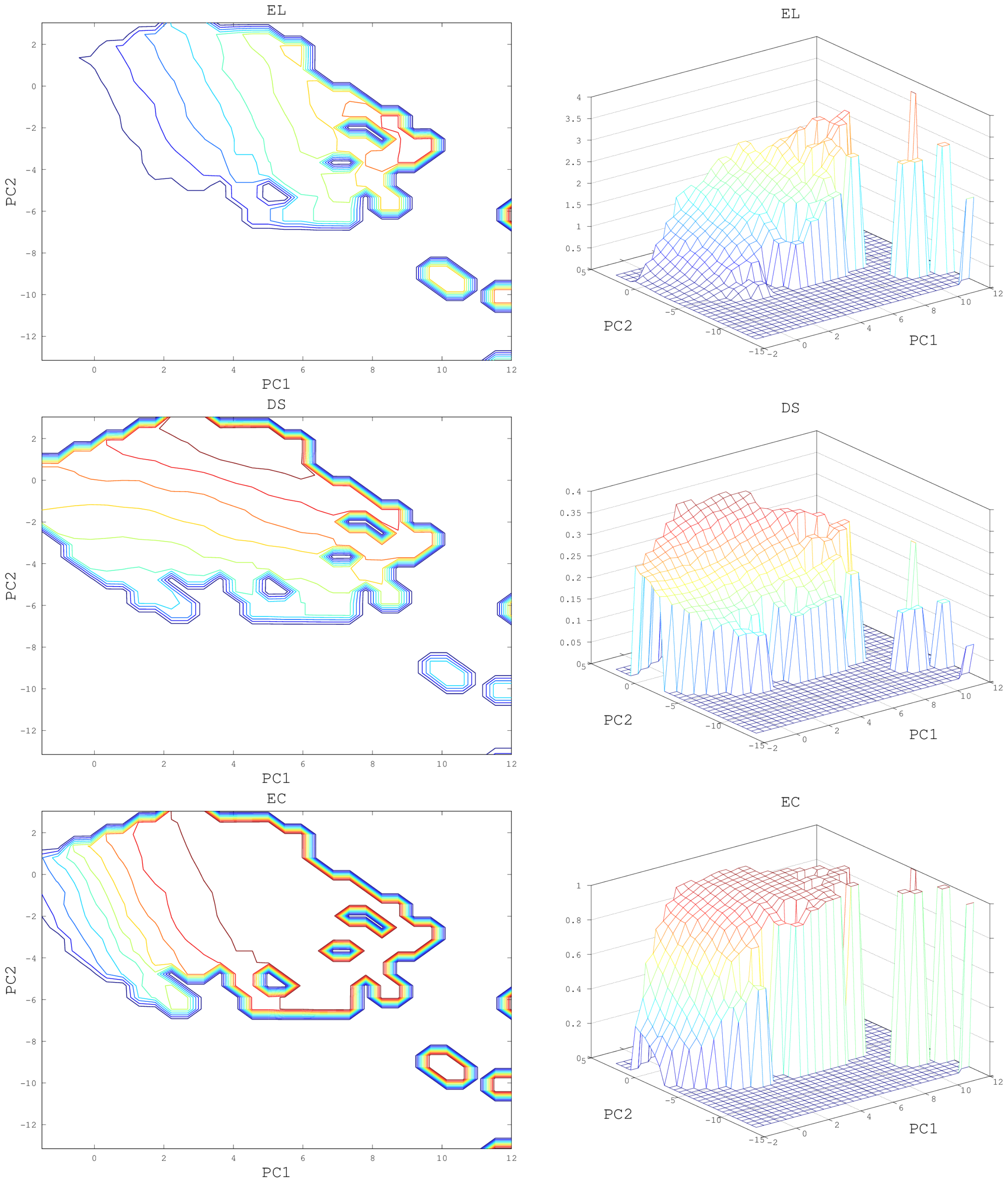

Figure S20 Distribution of Shape-characterisation index values for Elongation (EL), Dispersion (DS), and Eccentricity (EC) along PC1 and PC2 at the 320 mM condition.

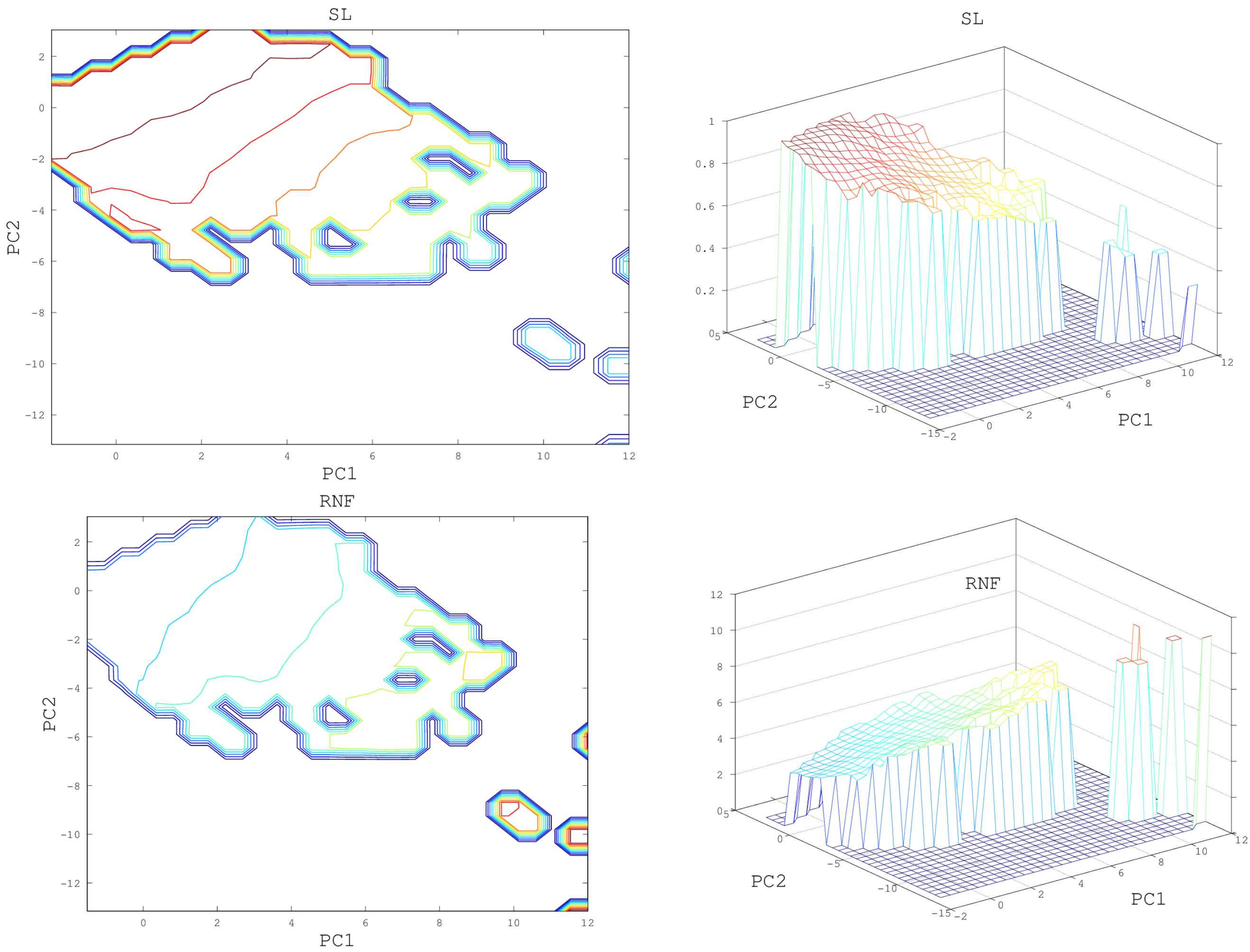

Figure S21 Distribution of Shape-characterisation index values for Solidty (SL) and Roundness factor (RNF) along PC1 and PC2 at the 320 mM condition.