Supporting Information

EXPANDING BALLOONS: Robust Computational Method for Determining Supramolecular Cage Cavity Morphology Based on the "Inflating Balloon" Metaphor

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S1. Benchmark dataset selection and compare working parameter

We used the dataset provided by Guerra et al.[1] as our test data. First, the original CIF files were downloaded from the CSD, and the supramolecular cages were extracted using Diamond by removing atoms and molecular fragments that did not belong to the framework. Next, the data was imported into PyMOL[2] for visualization and saved as a PDB file. A detailed tutorial video of the operation process is provided(Youtube).

We set the current parameters based on recent studies[1,3]. Since Pywindow has no customizable parameters in its design, no parameter adjustment is required.

Table S1: KVFinder project detected properties.

Supramolecular Cage Identifier	Step (Å)	Probe Out (Å)	Removal Distance (Å)	Volume Cutoff (Å^3)
A1	0.25	10	0.75	80
B1	0.25	10	2.00	5
B2	0.25	10	1.75	5
B3	0.25	10	1.50	20
B4	0.25	10	1.50	5
B5	0.25	10	1.50	5
B6	0.25	10	1.50	5
B7	0.25	10	1.50	110
B8	0.25	10	1.50	25
B9	0.25	10	1.50	5
B10	0.25	10	1.50	5
B11	0.25	10	1.50	5
B12	0.25	6	1.50	5
B13	0.25	6	1.50	5
C1	0.25	10	1.00	5
F1	0.25	10	1.25	5
F2	0.60	20	3.50	5
H1	0.25	10	2.00	5
N1	0.25	10	1.50	5
O1	0.25	10	1.25	80
O2	0.25	20	1.25	5
W1	0.25	10	1.75	20

Table S2: Cavity volumes calculated with C3 using a grid spacing of 0.5 Å and a distance threshold for the 90-degree calculation of 2 times the window size. For the Dataset 2, all run properties are default.

Supramolecular Cage Identifier	C3 Calculated Volume(Å^3)	Guest vdW Volume(Å^3)	Estimated Cavity Volume(Å^3)	Relative Error (%)
B1	298	150	273	9.2
B2	292	155	281	3.8
B3	267	137	248	7.7
B4	422	309	562	-24.9
B5	63	50	90	-29.8
B6	64	53	96	-33.4
B7	749	519	944	-20.6
B8	726	512	930	-21.9
B9	85	141	257	-66.9
B10	263	151	274	-4.1
B11	448	307	558	-19.8
B12	708	524	954	-25.8
B13	828	618	1123	-26.2
			MRAE (%)	22.6

Table S3: Fpocket detection parameters for each supramolecular cage.

Supramolecular Cage Identifier	Minimum Radius (Å)	Maximum Radius (Å)
A1	3.4	8.0
B1	3.4	8.0
B2	3.4	8.0
B3	3.4	8.0
B4	3.4	8.0
B5	3.0	6.2
B6	3.4	8.0
B7	3.4	8.0
B8	3.4	8.0
B9	3.4	8.0
B10	3.4	8.0
B11	3.4	8.0
B12	3.4	8.0
B13	3.4	8.0
C1	3.4	8.0
F1	2.0	6.2
F2	3.4	40.0
H1	3.4	6.2
N1	3.7	8.0
O1	3.4	6.2
O2	3.4	6.2
W1	4.0	7.0

Table S4: MoloVol detection parameters for each supramolecular cage.

Supramolecular Cage Identifier	Grid Spacing (Å)	Small Probe Radius (Å)	Large Probe Radius (Å)
A1	0.6	1.4	6.0
B1	0.6	1.4	5.0
B2	0.6	1.4	5.0
B3	0.6	1.4	5.0
B4	0.6	1.4	5.0
B5	0.6	1.4	5.0
B6	0.6	1.4	5.0
B7	0.6	1.4	5.0
B8	0.6	1.4	5.0
B9	0.6	1.4	5.0
B10	0.6	1.4	5.0
B11	0.6	1.4	5.0
B12	0.6	1.4	5.0
B13	0.6	1.4	5.0
C1	0.6	1.4	5.0
F1	0.6	1.4	5.0
F2	0.6	1.4	20.0
H1	0.6	1.4	5.0
N1	0.6	1.4	5.0
O1	0.6	1.4	5.0
O2	0.6	1.4	15.0
R1	0.6	1.4	5.0
W1	0.6	1.4	5.0

S2. Sphere center selection experiment result

The following tables demonstrate the impact of the choice of sphere center on the results. The first row for each data presents the estimated cavity volume results, the pointscavity volume results calculated based on different point, and subdivision times parameter. The second row is the number of extension time(ET) for each result calculation. The third row is the relative error(RE) for each result calculation. As described in the paper, because many of the molecular cage data are affected by other forces such as hydrogen bonding, the actual cavity volume is smaller than the Rebek's rule based estimate. Therefore, we introduced the publication volume of the supramolecular cage to revise our results during the evaluation. The final result is the average of the two calculated results.

Table S5: Sphere center influence.

Supramolecular Cage	**Estimated/ Reference Cavity Volume(Å^3)**	**Centroid(Å^3)**	**Cente of Mass(Å^3)**	**Symmetrical Point(Å^3)**	**Subdivision Times**
B1	273	312	303	314	4
	ET =	31	31	31	
	RE =	14.2	10.9	15.0	
B2	281/285	318	314	318	4
	ET =	33	33	33	
	RE =	13.1/11.5	11.7/10.1	13.1/11.5	
B3	248/270	293	296	296	4
	ET =	32	30	32	
	RE =	18.1/8.5	19.3/9.6	19.3/9.6	
B4	562/434	508	505	524	5
	ET =	28	24	30	
	RE =	-9.6/17.0	-10.1/16.3	6.7/20.7	
B5	90/52	77	78	79	4
	ET =	20	20	18	
	RE =	-14.4/48.0	-13.3/51.9	12.2/51.9	
B6	96/55	74	74	74	4
	ET =	16	16	16	
	RE =	-22.9/34.5	-22.9/34.5	22.9/34.5	
B7	944/810	944	890	979	5
	ET =	38	36	43	
	RE =	0/16.5	-5.7/9.8	3.7/20.8	
B8	930	872	861	914	5
	ET =	31	31	31	
	RE =	-6.2	-7.4	-1.7	
B9	257/184	225	226	225	4
	ET =	20	20	18	
	RE =	-12.4/22.2	-12.0/22.8	12.4/22.2	
B10	274/261	291	288	291	4
	ET =	34	34	35	
	RE =	6.2/11.4	5.1/10.3	6.2/11.4	
B11	558	557	554	554	5
	ET =	47	49	47	
	RE =	-0.1	-0.7	-0.7	
B12	954/718	720	720	721	5
	ET =	29	29	31	
	RE =	-24.5/0.2	-24.5/0.2	-24.4/0.4	
B13	1123/925	845	846	850	5
	ET =	39	39	38	
	RE =	-24.7/-8.6	-24.6/-8.5	-24.3/-8.1	
MEAE (%)		14.1	13.9	14.3	-
Times		30.3	29.9	30.6	-

S3. Parameters selection and experimental results

Table S6: Parameters and Calculation Result of Dataset 1.

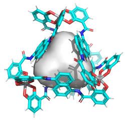
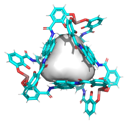
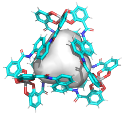
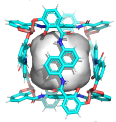
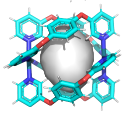
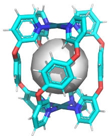
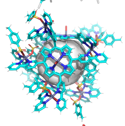
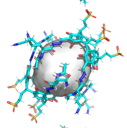
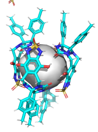
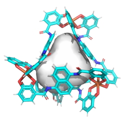
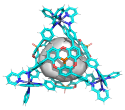
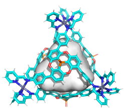
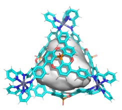
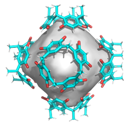
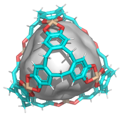
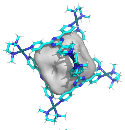
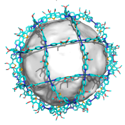
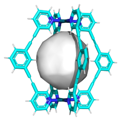
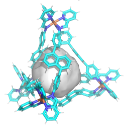
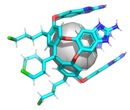
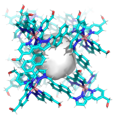
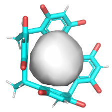
Cage	Estimated Volume (Å^3)	CMCC Result (Å^3)	Subdivision Times	Result
B1	273	303	4	
B2	281	314	4	
B3	248	296	4	
B4	562	455	5	
B5	90	78	4	
B6	96	74	4	
B7	944	890	5	
B8	930	861	5	
B9	257	226	4	
B10	274	288	4	
B11	558	517	5	
B12	954	720	5	
B13	1123	846	5	

Table S7: **Parameters and Calculation Result of Dataset 2.**

Cage	Reference/Average Volume (Å^3)	CMCC Result (Å^3)	Subdivision Times	Result
A1	1375	1455	5	
C1	549	592	5	
F1	500	480	5	
F2	42572	32350	5	
H1	259	167	4	
N1	434	407	4	
O1	142	95	4	
W1	400	433	4	
O2	20	27	4	

The selection of parameters in the experiment is primarily based on two principles: data analysis results of subdivision times and comparison of visualization results. First, we examine the impact of subdivision times on the calculation results for dataset 1 from the paper. The CMCC method's first step is the grid's subdivision surface. This step is similar to grid partitioning in grid-based methods, where the number of divisions affects the precision and computation time of the final results. Figure 1 shows the relationship between subdivision times (ST) and CMCC computation time. Due to the subdivision surface algorithm causing an exponential increase in the number of vertices, the corresponding computation time also increases with the times of subdivision surface. It is evident that after six grid divisions, the computation time reaches an unacceptable magnitude (55 seconds). Therefore, the subdivision parameters should only be between 3, 4, and 5. In Figure 3, the blue, green, and yellow lines represent the calculation results for subdivision times of 3, 4, and 5, respectively.

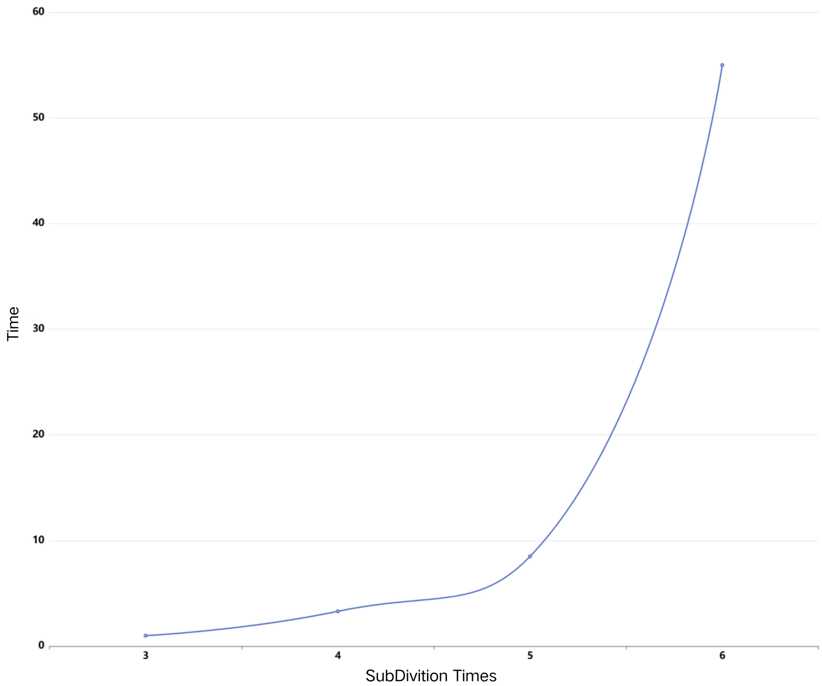


Figure 1: The effect of the subdivision times on the calculation time.

By observing the graphs, it can be concluded that CMCC yields better results for larger supramolecular cages (Cavity Volume > 500) when ST = 5. However, for other supramolecular cage cavity calculations, the impact of ST on the results is not significant, as shown in Figure 3. Hence, we introduced the Origin Publication cavity volume as a comparison parameter based on Rebek's rule (Figure 2). ST = 3 has higher calculation accuracy for smaller supramolecular cages. ST = 4 shows more stable and higher accuracy across both calculation references. To further explore the choice of calculation parameters, we visualized the data with smaller cavity volumes (B5, B6).

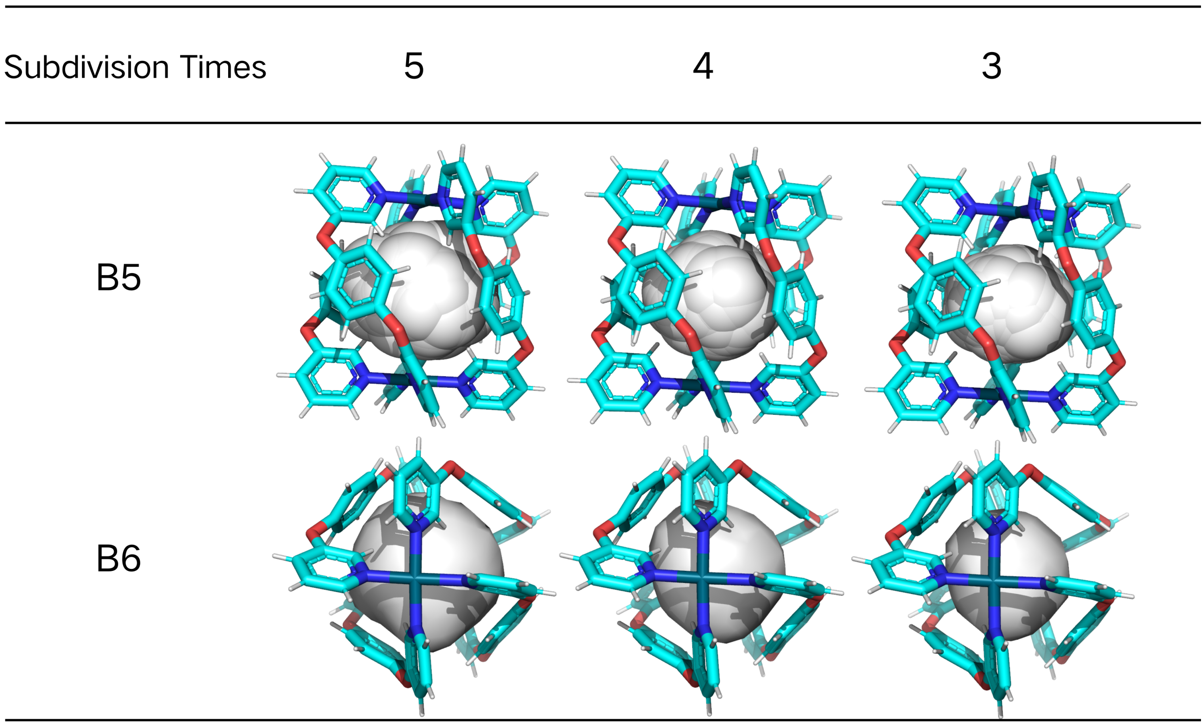


Figure 2: Cavity visualization results of B5 and B6.

By comparing the visualization results, the shape of the calculated results did not change, but the size did. We believe the change in size is due to finer probes exploring small spaces between atoms—spaces that cavity guest molecules cannot physically reach. Moreover, by combining the charts, it is evident that these small spaces are not considered part of the supramolecular cage's cavity (fewer vertices result in higher accuracy). Therefore, we conclude that results for ST = 3 or 4 are acceptable compared to ST = 5. Considering the comparison with mean absolute error (dotted line in Figure 3), we ultimately recommend using ST = 4 as the parameter when the volume of the supramolecular cage is less than 500.To obtain the optimal CMCC results, three parameters can be selected and tested. For the current dataset, B1, B2, B3, and O2 achieve optimal results when ST=3. When ST=4, W1 and B10 obtain the best performance. The remaining data reach their optimal results at ST=5.

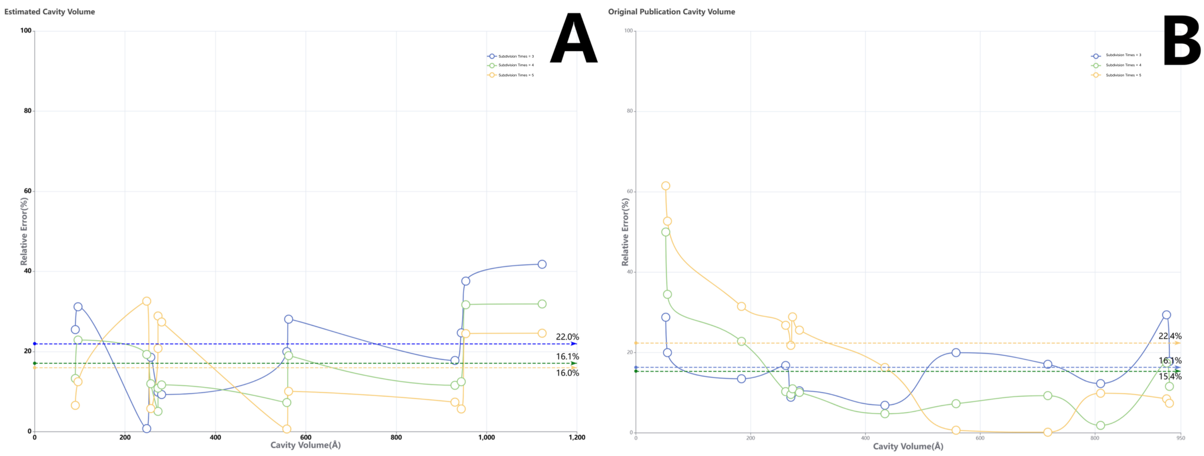


Figure 3: The effect of the subdivision times on the final result.

In order to select the optimal step length, we conducted experiments using various step lengths. The experiments evaluated the impact of step length on MRAE and computation time for each cavity calculation (Table S7). The results indicate that a step length of 0.1 Å achieves a balance between MRAE and computation time.

Table S7: Step length selection experiment.

Step Length (Å)	**0.01**	**0.05**	**0.1**	**0.15**	**0.2**
MRAE (%)	12.8	12.9	12.9	13.2	13.7
Computation Time (s)	36	12.9	9.2	10.6	11.3

S4. CMCC result convert

Current mainstream biomolecular results are typically saved and presented in formats such as PDB and MOL2. However, the results from CMCC are displayed in the form of vertex meshes. This format is not conducive for experts to further analyze supramolecular cages. To address this, we have developed a method to convert vertex data into PDB data for better presentation of our results. In this method, each vertex is replaced by a carbon atom. Compared to the vertex, a carbon atom has a van der Waals radius (1.7 Å). Therefore, we need to perform a reverse translation of 1.7 Å in the direction of atomic expansion. Figure 4 illustrates the corresponding conversion process. The black outline represents the outer contour of the surface before vertex conversion. The red and yellow outlines represent the inner and outer surface contours after conversion. Figure 4E shows the result after the vertices have been translated.

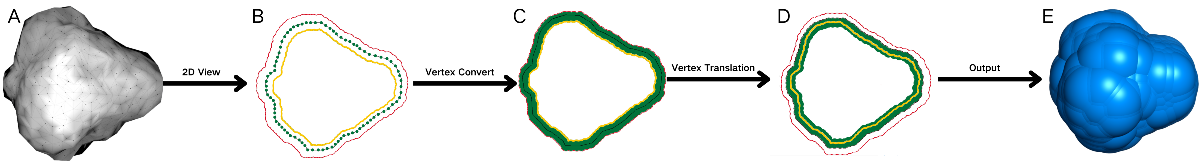


Figure 4: CMCC Result Conversion Process.

References

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(2) DeLano, W. L.; others PyMOL: An Open-Source Molecular Graphics Tool. *CCP4 Newsl. Protein Crystallogr* **2002**, 40, 82–92.

(3) Martí-Centelles, V.; Piskorz, T. K.; Duarte, F. CageCavityCalc (C3): A Computational Tool for Calculating and Visualizing Cavities in Molecular Cages. **2024**