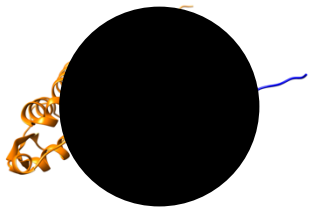
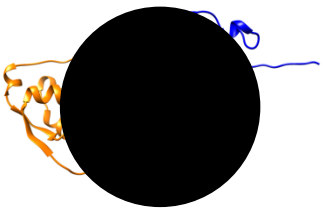
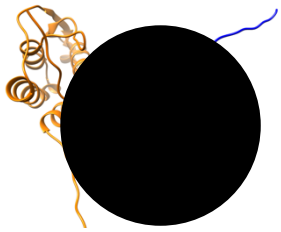
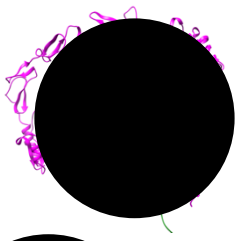
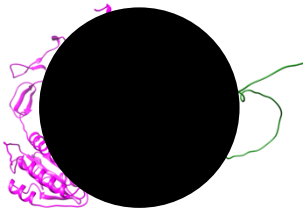
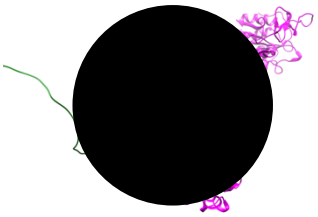
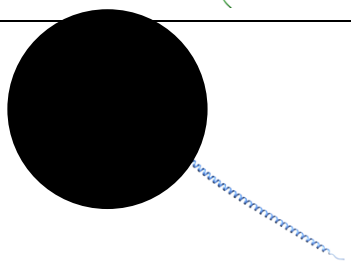
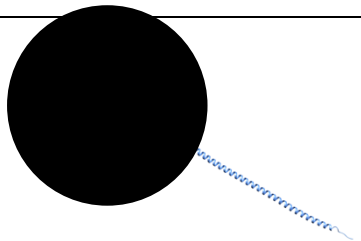
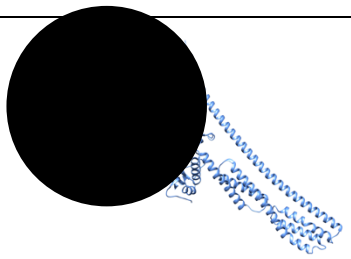
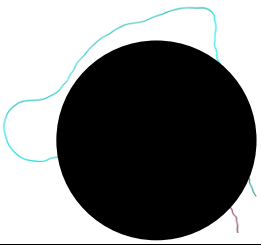
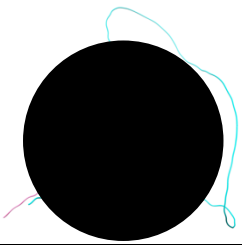
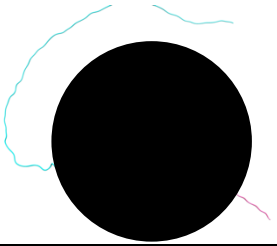
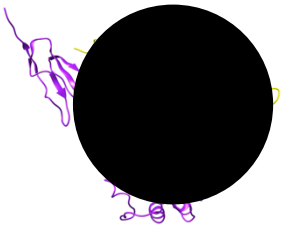
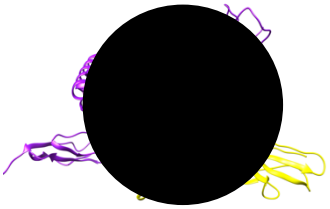
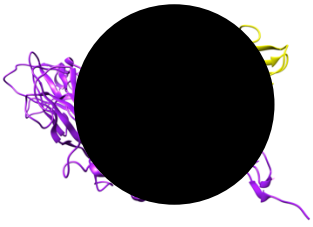
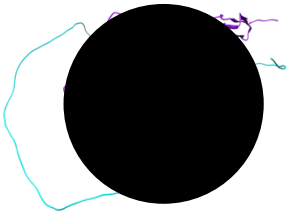
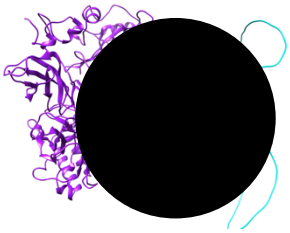
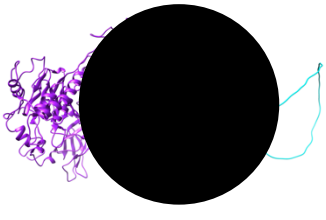
Binding affinity calculation for "⑥ PPI systems x 3 *AF* modelings"
using MMPBSA(Molecular Mechanics Poisson Boltzman Surface Area) method

오예슬

<Input data>
① [Gene: [redacted]] & [Gene: [redacted]]
② [Gene: [redacted]] & [Gene: [redacted]]
③ [Gene: [redacted]] & [Gene: [redacted]]
④ [Gene: [redacted]] & [Gene: [redacted]]
⑤ [Gene: [redacted]] & [Gene: [redacted]]
⑥ [Gene: [redacted]] & [Gene: [redacted]]

(ranked_0)

	ACX (Alphafold Complex)	AF2 (Alphafold2)	AFM (Alphafold Multimer)
① [redacted] Residue # 1 - 127 128 - 279			
② [redacted] Residue # 1 - 563 564 - 777			
③ [redacted] Residue # 1 - 167 168 - 750			
④ [redacted] Residue # 1 - 54 55 - 332			
⑤ [redacted] Residue # 1 - 758 759 - 869			
⑥ [redacted] Residue # 1 - 758 759 - 1036			

<Calculation procedure by Amber>

1. Prepare

o 모두 missing residue 와 modified residue 가 없으므로 별도의 작업 없이 pdb4amber 로 다음 step 인 LEaP 사용을 위한 PDB 파일 준비

2. LEaP (Link, Edit and Parm)

o Protein force field: ff14SB 사용 (∵ ff14SB: protein force field 로 권장)

o LEaP 결과 close contact warning 외 error 발생 없음 (close contact warning 은 minimization step 에서 해결)

3. Minimization → Heat (1 ns) → Density (1 ns) → Equilibration (10 ns)

o Minimization maxcyc=1000, ncyc=500

o cutoff: 10 Å

o Minimiztion step 에서 error 가 발생한 3 가지 system → input parameter 조정으로 해결

[MATN4-STC1_AFM] maxcyc=100,000 / ncyc=10,000 / vlimit=10 / cutoff=14 / pmemd.mpi 사용

[PMAIP1-STC2_ACX] maxcyc=100,000 / ncyc=10,000 / vlimit=10 / cutoff=14 / pmemd.mpi 사용

[PMAIP1-STC2_AF2] maxcyc=50,000 / ncyc=5,000 / vlimit=10

<cf> maxcys: The maximum number of cycles of minimization

<cf> ncyc: The method will be switched from steepest descent to conjugate gradient after NCYC cycles

<cf> cutoff: Interactions are only computed up to a fixed atomic or molecular distance in cutoff scheme

o Simulation 결과: Potential energy (E_{pot}), Temperature, Density, Equilibration 모두 안정

4. Production MD (100 ns)

o Frame 간격: 10 ps, total 10,000 frames

5. Production MD RMSD

o RMSD reference structure: first frame (trajectory 의 첫 frame 을 reference structure 로 사용)

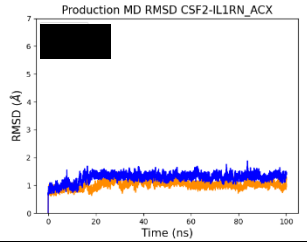
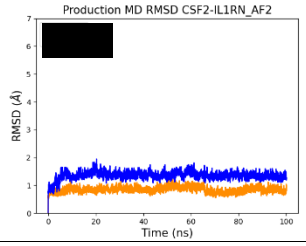
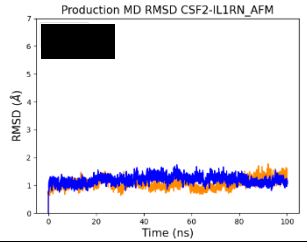
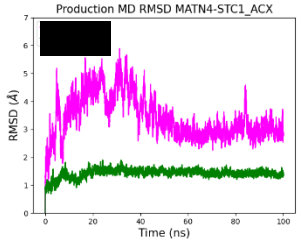
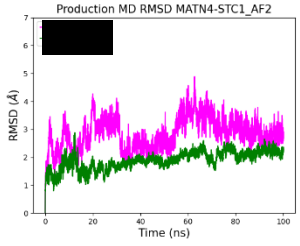
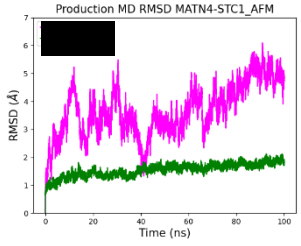
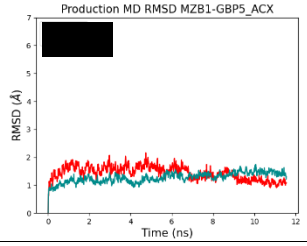
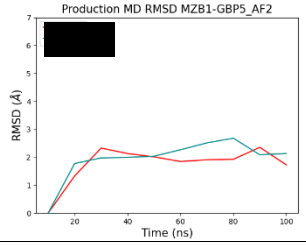
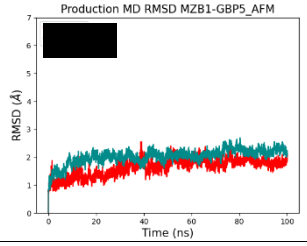
o Loop 를 제외하고 두 protein 이 contact 하는 부분의 residues 를 지정하여 계산

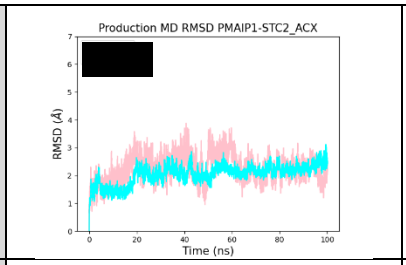
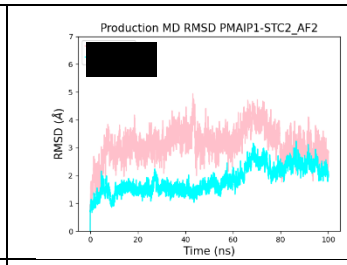
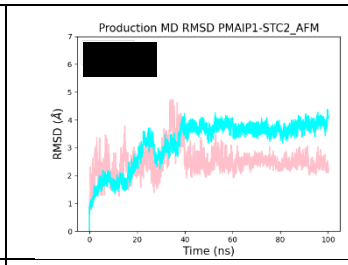
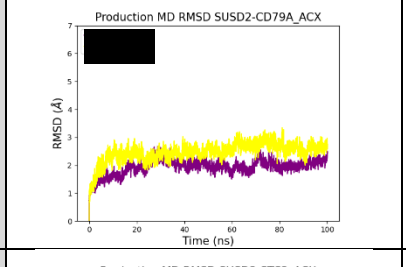
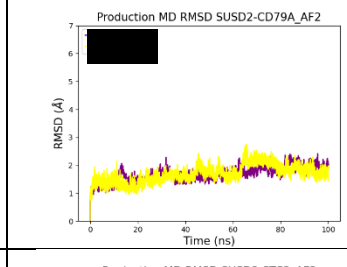
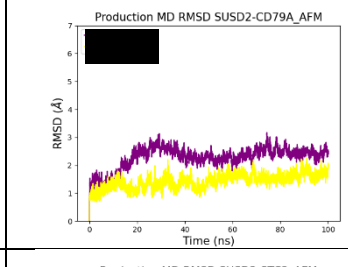
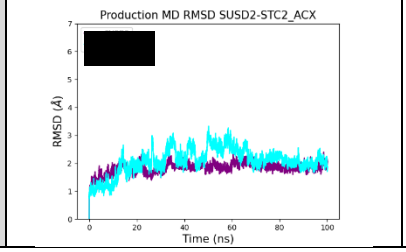
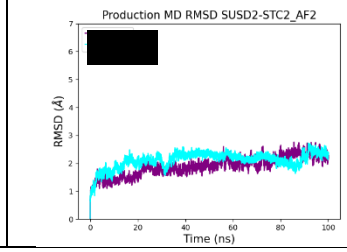
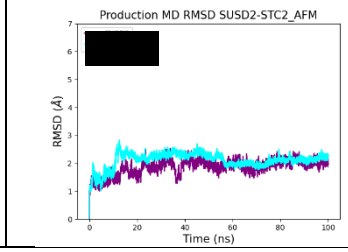
– 결과: 모두 2~3 Å 내외로 안정

– ③[redacted]_AF2 의 경우 graph 를 그리는 과정에서 오류가 발생하여 nmode 계산 시 뽑은 10 개의 frame(과정 6. 참고)으로 trajectory 를 생성하여 대체

Production MD RMSD Result

(ranked_0)

	ACX (Alphafold Complex)	AF2 (Alphafold2)	AFM (Alphafold Multimer)
① [redacted] Cut residue # 1-127 → 12-127 128-279 → 137-278			
② [redacted] Cut residue # 1-563 → 198-305 564-777 → 564-729			
③ [redacted] Cut residue # 1-167 → 13-150 168-750 → 230-473			

④ [redacted] Cut residue # 1-54 → 17-52 55-332 → 68-244			
⑤ [redacted] Cut residue # 1-758 → 18-693 759-869 → 766-853			
⑥ [redacted] Cut residue # 1-758 → 40-695 759-1036 → 780-946			

6. MMPB(GB)SA (PB, Poisson Boltzmann —[approximation]→ GB, Generalized Born)

o General frame 간격: startframe=10, endframe=10,000, interval=10, total 1000 frames

o GB igb=8, PB inp=1 (∵현재까지 igb=8 이 ff14SB force field 에서 가장 잘 맞아 보인다고 함, inp=1 사용시 결과 값이 더 그럴듯하다고 함)

o Nmode: nminterval=100, nmode frame 개수: 10 frames

<cf> nmode: normal mode analysis to calculate the entropy contribution for a system

<Result>

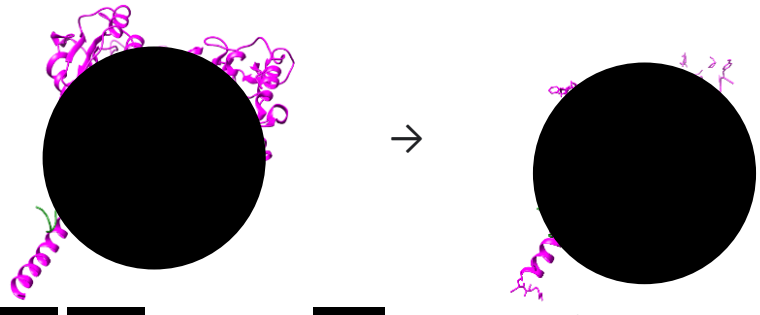
o ① [redacted], ④ [redacted]은 전체 residues 길이가 300 내외로 문제없이 MMPBSA 결과를 얻음

o {문제} ② [redacted], ③ [redacted], ⑤ [redacted], ⑥ [redacted]은 전체 residues 길이가 700 ~ 1000 내외로 nmode 계산에서 모두 error 발생

{원인} residues 길이가 길어 nmode 계산의 행렬 대각화 과정에서 oom(out of memory) error 발생으로 추정

{해결} Truncated nmode 계산

- 하나의 protein 과 그로부터 9 Å 내에 있는 상대 protein 의 residues 만을 남긴 후 nmode 계산
- ②, ③, ⑤, ⑥ system 모두 더 작은 protein 을 기준으로 9 Å 내에 있는 상대 protein 을 자름



<ex> ② [redacted] ACX system: [redacted]를 기준으로 9 Å 내의 residues 만 자른 그림

- dielc=4 (∵The ligand entropy was always calculated for the isolated ligand but with either ε = 1 or ε = 4r, P-P system 에서 하나의 protein 을 ligand 로 간주)

<cf> dielc: distance-dependent dielectric constant (default = 1.0)

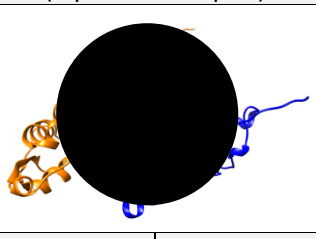
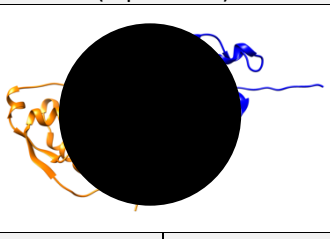
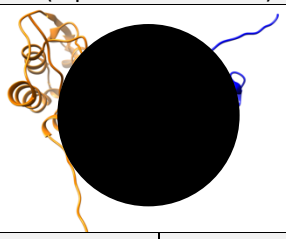
<cf> ε: dielectric constant

MMPB(GB)SA Result using Nmode – ①, ④ systems

(검: +, 파: -한 자리, 빨: -두 자리)

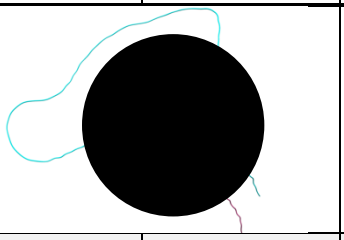
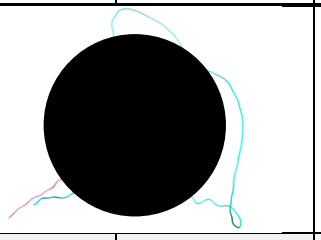
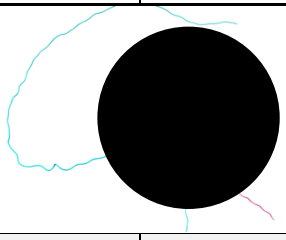
① [redacted]

Residue #
1 - 127
128 - 279

ACX (Alphafold Complex)		AF2 (Alphafold2)		AFM (Alphafold Multimer)	
					
GB	**PB**	**GB**	**PB**	**GB**	**PB**
-43.2994 ± 6.5184	-46.6705 ± 8.4338	-29.0219 ± 6.0879	-33.2205 ± 7.5268	-44.6330 ± 10.1134	-45.2247 ± 13.1991
Nmode		**Nmode**		**Nmode**	
-45.6328 ± 10.4035		-25.9315 ± 3.9281		-41.9456 ± 7.5251	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
2.3334 ± 12.2769	-1.0377 ± 13.3926	-3.0904 ± 7.2452	-7.2890 ± 8.4902	-2.6874 ± 12.6059	-3.2791 ± 15.1935

④ [redacted]

Residue #
1 - 54
55 - 332

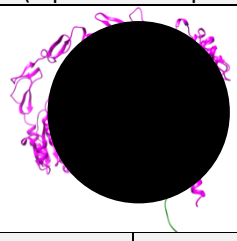
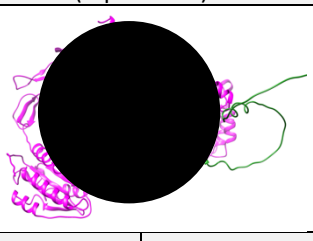
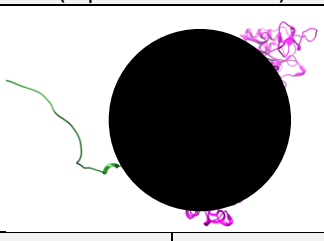
					
GB	**PB**	**GB**	**PB**	**GB**	**PB**
-115.1401 ± 12.8476	-118.6200 ± 15.0711	-103.2839 ± 14.0702	-103.5370 ± 16.5983	-101.4602 ± 12.0462	-99.2682 ± 12.9139
Nmode		**Nmode**		**Nmode**	
-92.3846 ± 15.4855		-81.7450 ± 12.7485		-66.0462 ± 11.9526	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
-22.7555 ± 20.1212	-26.2354 ± 21.6088	-21.5389 ± 18.9867	-21.7920 ± 20.9291	-35.4140 ± 16.9698	-33.2220 ± 17.5964

MMPB(GB)SA Result using Truncated nmode – ②, ③, ⑤, ⑥ systems

(검: +, 파: -한 자리, 빨: -두 자리)

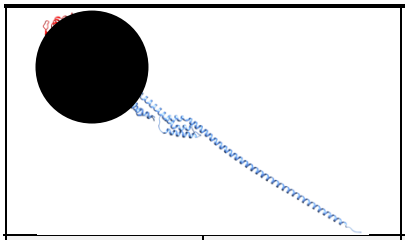
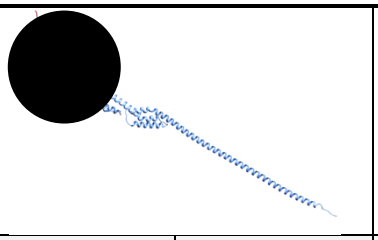
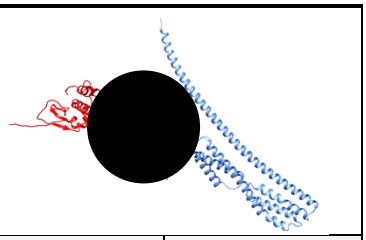
② [redacted]

Residue #
1 - 563
564 - 777

ACX (Alphafold Complex)		AF2 (Alphafold2)		AFM (Alphafold Multimer)	
					
GB	**PB**	**GB**	**PB**	**GB**	**PB**
-70.4743 ± 16.9948	-101.2760 ± 22.2972	-71.3384 ± 19.6486	-99.6556 ± 25.7257	-82.3075 ± 26.1950	-112.9407 ± 30.7184
Truncated nmode		**Truncated nmode**		**Truncated nmode**	
-85.0808 ± 31.4131		-79.0740 ± 37.5543		-92.0032 ± 38.7250	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
14.6065 ± 35.7156	-16.1952 ± 38.5220	7.7356 ± 42.3839	-20.5816 ± 45.5207	9.6957 ± 46.7526	-20.9375 ± 49.4292

③

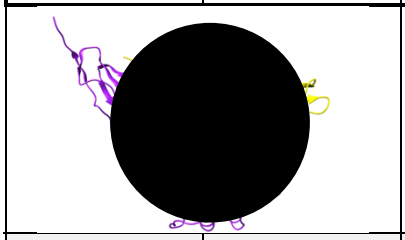
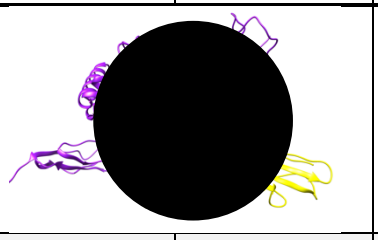
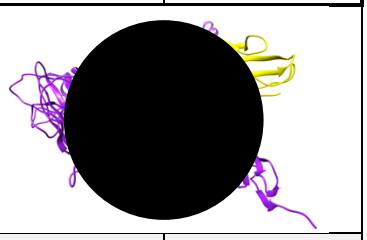
Residue #
1 - 167
168 - 750

GB	PB	GB	PB	GB	PB
-27.6584 ± 8.3878	-30.5097 ± 10.5778	-26.8246 ± 15.9831	-26.9738 ± 20.4581	-16.9928 ± 10.5794	-13.6315 ± 13.8721
Truncated nmode		**Truncated nmode**		**Truncated nmode**	
-64.2560 ± 17.6500		-59.5558 ± 26.7617		-120.1551 ± 24.1357	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
36.5976 ± 19.5417	33.7463 ± 20.5770	32.7312 ± 31.1713	32.5820 ± 31.1713	103.1623 ± 26.3525	106.5236 ± 27.8382

⑤

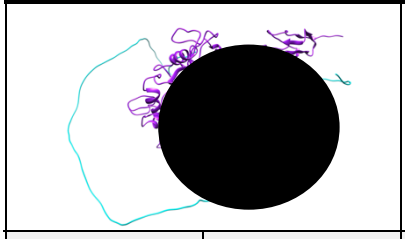
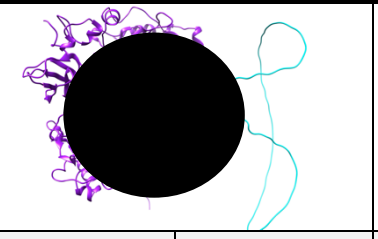
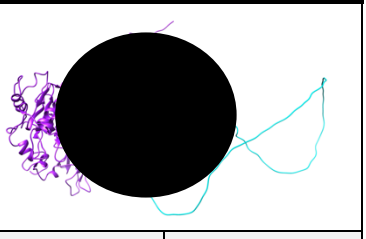
Residue #
1 - 758
759 - 869

GB	PB	GB	PB	GB	PB
-74.6734 ± 12.3150	-97.8705 ± 13.7470	-71.8971 ± 12.1098	-87.1740 ± 13.6548	-111.5612 ± 11.0557	-109.2670 ± 19.0860
Truncated nmode		**Truncated nmode**		**Truncated nmode**	
-81.4445 ± 19.8838		-79.8440 ± 18.9537		-474.3849 ± 481.5231	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
6.7711 ± 23.3886	-16.4260 ± 24.1732	7.9469 ± 22.4920	-7.3300 ± 23.3601	363.2737 ± 481.6500	365.5679 ± 481.9012

⑥

Residue #
1 - 758
759 - 1036

GB	PB	GB	PB	GB	PB
-137.3512 ± 29.8369	-154.3278 ± 38.0890	-23.6271 ± 7.4191	-37.0245 ± 12.7900	-75.0964 ± 8.0092	-83.6308 ± 15.0783
Truncated nmode		**Truncated nmode**		**Truncated nmode**	
-131.6495 ± 37.9599		-86.6164 ± 24.1490		-69.4711 ± 34.6780	
MM/GBSA	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**	**MM/GBSA**	**MM/PBSA**
-5.7017 ± 48.2824	-22.6783 ± 53.7748	62.9893 ± 25.2630	49.5919 ± 27.3269	-5.6253 ± 35.5909	-14.1597 ± 37.8143

o 일반적인 nmode 계산을 한 ①, ④ system 의 경우 3 가지 방식으로 모델링한 구조가 비슷한 범위(검/빨/파)내의 binding affinity 값을 가짐.

o Truncated nmode 계산을 한 ②, ③, ⑤, ⑥ system 의 경우 ③ system 만 모두 비슷한 범위(검)의 binding affinity 값을 가지고 나머지 세가지 system 의 경우 그 값의 범위가 통일성이 없음.

o Truncated nmode 가 신뢰할 수 있는 지에 대한 의문이 생김. oom 에러를 피하기 위한 대처 방안이었지만 추가적인 검증이 필요해 보임.