

Rational Design of Cyclic-Peptides for Inhibiting Cancer Proliferation

Yingjie Ling
Tufts University

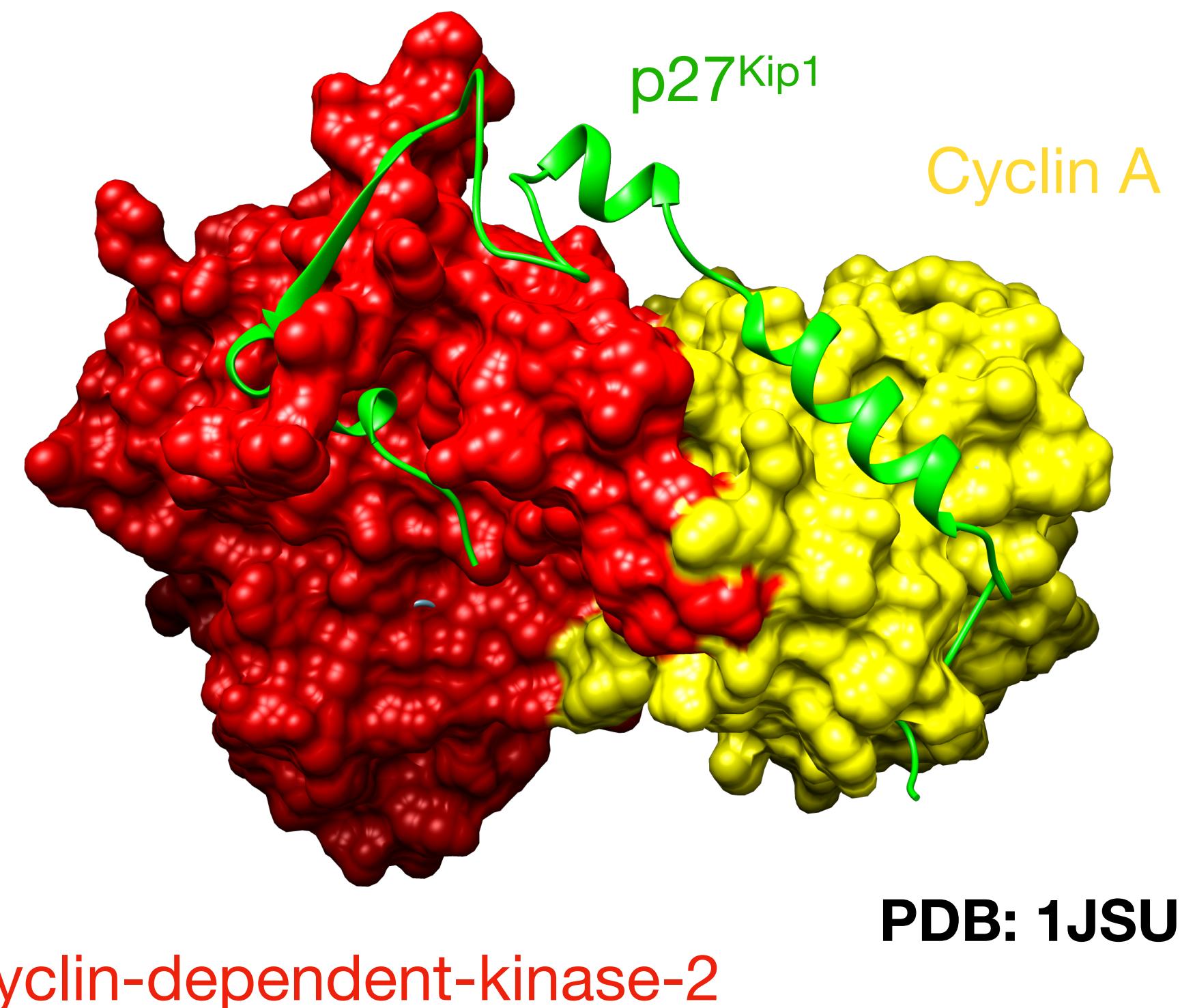
Outline

- Background
- Project Description
- Results
- Conclusion and Next Steps

p27^{Kip1} and its significance

p27^{Kip1} is important for regulating cell cycles

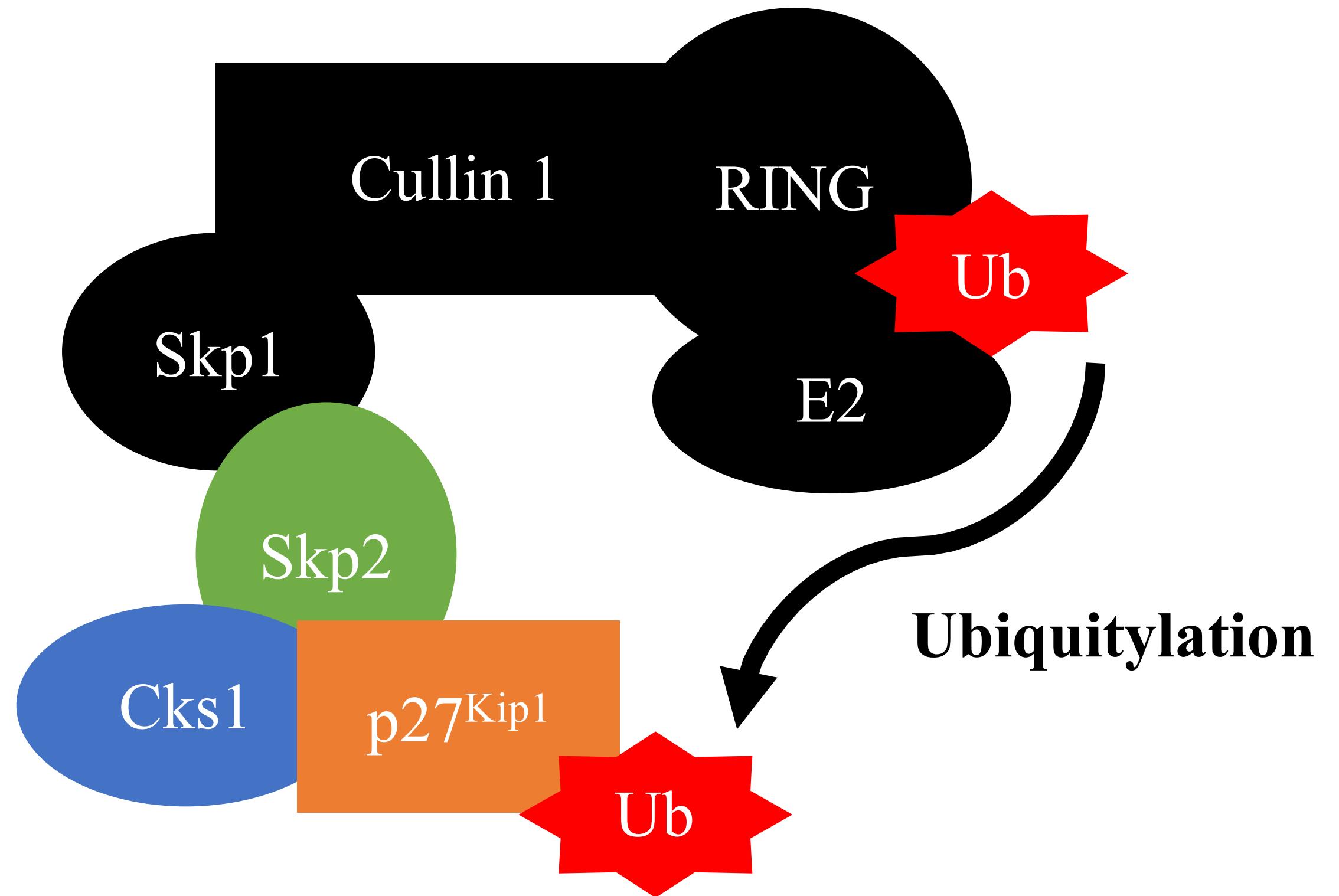
- p27^{Kip1} is a cyclin-dependent-kinase inhibitor that leads to cell-cycle arrest.
- p27^{Kip1} is often referred to as the “tumor suppressor”.
- In cancer cells, p27^{Kip1} is frequently inactivated.



R. Alicia, et al., *Nature* **382**, 425-331 (1996)

p27^{Kip1} is degraded by a ubiquitin-dependent process

- Both Cks1 and Skp2 are required for the most efficient ubiquitylation of p27^{Kip1}.
- It was also shown that Cks1 and Skp2 are often over-expressed in cancer cells.



Cks1: Cyclin-dependent kinases regulatory subunit 1

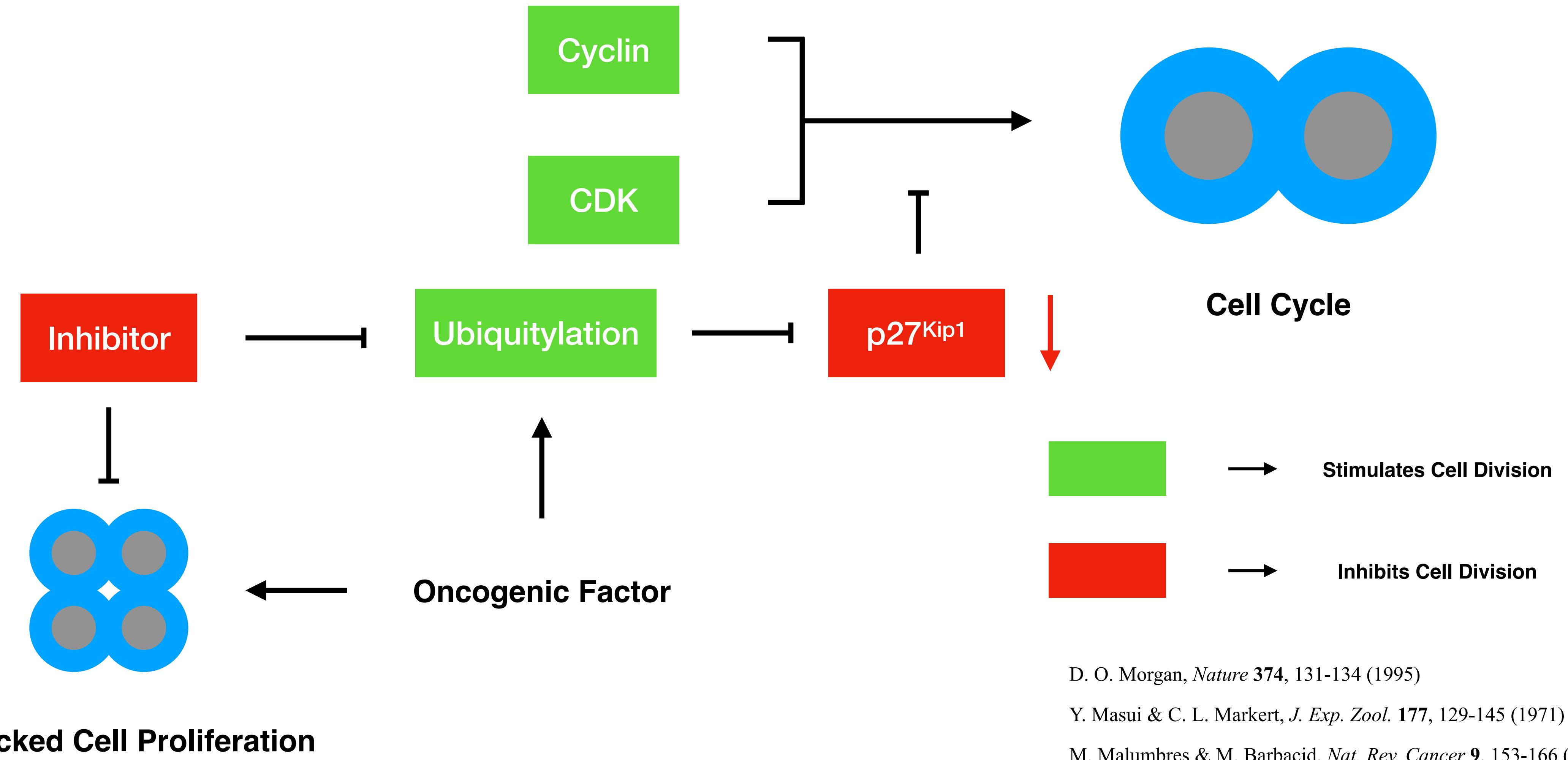
Skp2: S-phase kinase-associated protein 2

A. C. Carrano, et al., *Nat. Cell Biol.* **1**, 193-199 (1999)

D. Frescas & M. Pagano, *Nat. Rev. Cancer* **8**, 438-449 (2008)

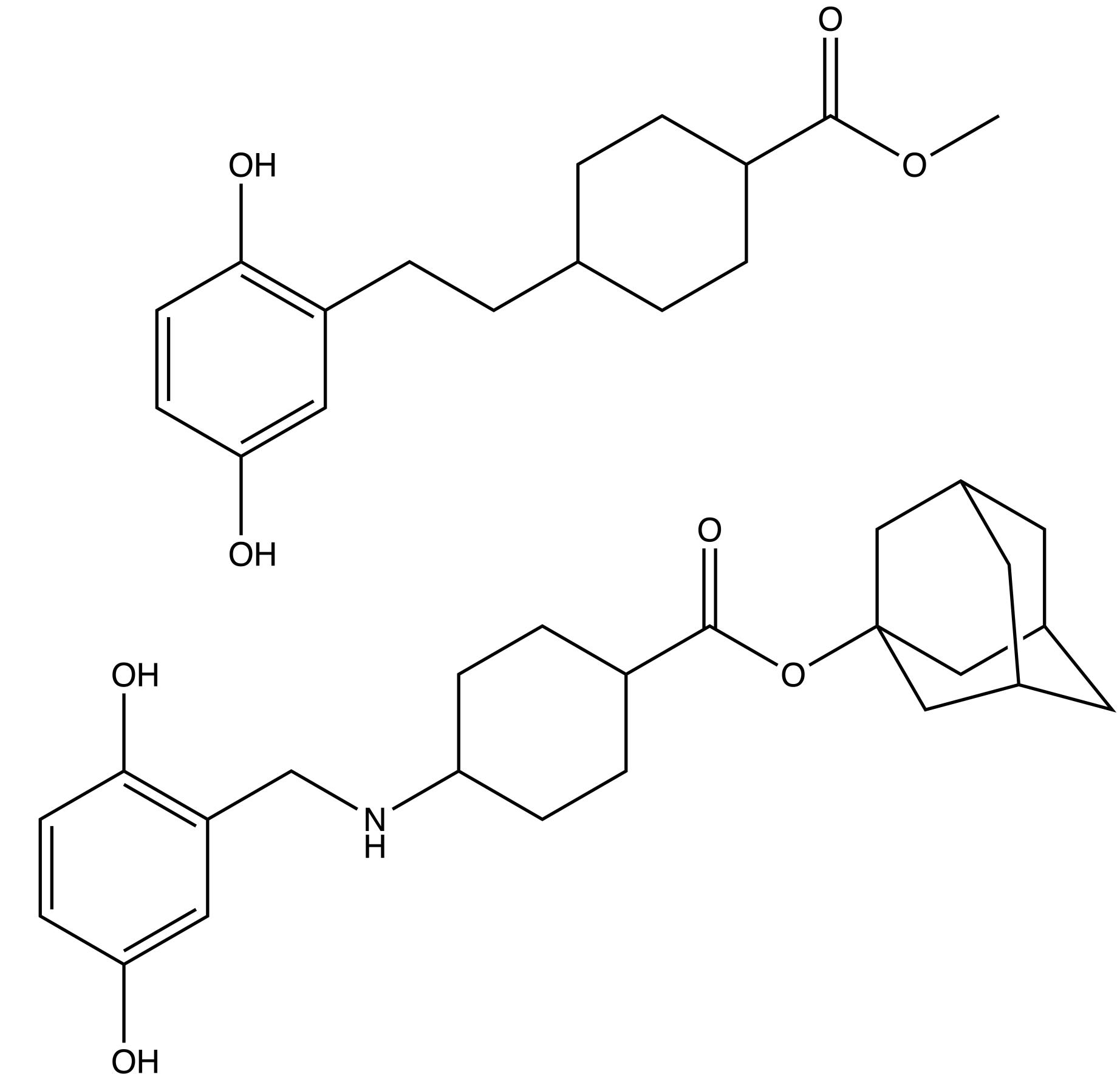
J. Slingerland & M. Pagano, *J. Cell. Physiol.* **183**, 10-17 (2000)

Inhibiting p27^{Kip1} ubiquitylation has beneficial effects



Small molecules are insufficient to inhibit Cks1–Skp2

- Past attempts to inhibit Cks1–Skp2 interaction with small molecules yielded unsatisfying results.
- Protein–protein interactions are challenging to target using small molecules due to large and flat interfaces.



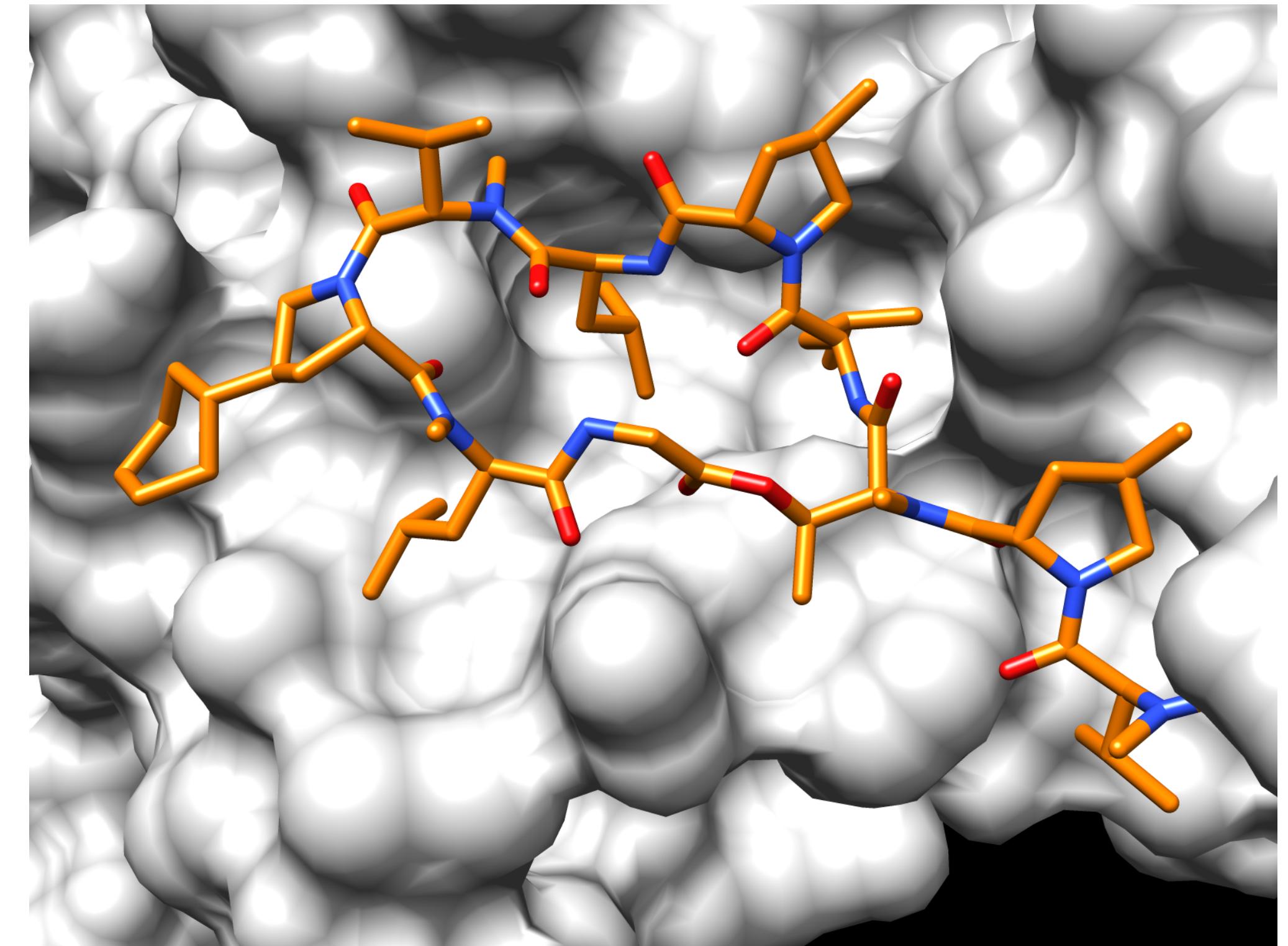
Jones S, Thornton JM, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13-20 (1996)

Ungermannova, D. et al., *J. Biomol. Screen.* **18**, 910–920 (2013)

Cyclic peptides bind protein surfaces with high affinity

PDB: 5AH4

- Cyclic peptides have large surface area and can easily mimic functional groups at protein interfaces.
- Without exposed terminal, cyclic peptides are also more stable, rendering high oral availability.



A. Kling et al., *Science* **348**, 1106-1112 (2015)

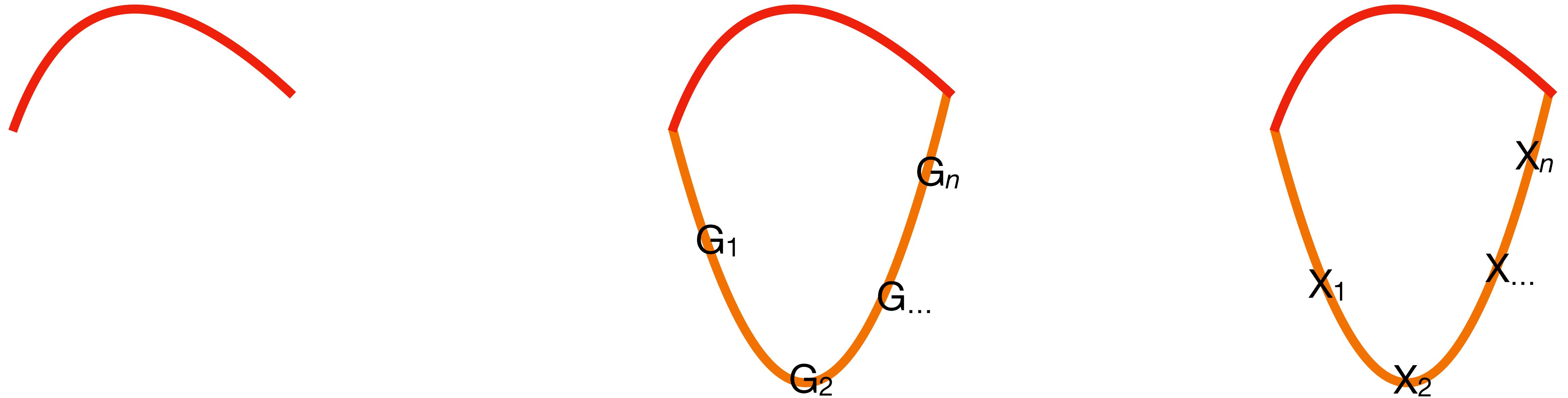
Background

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Results

Conclusion

Cyclic-peptide design is a 3-step process

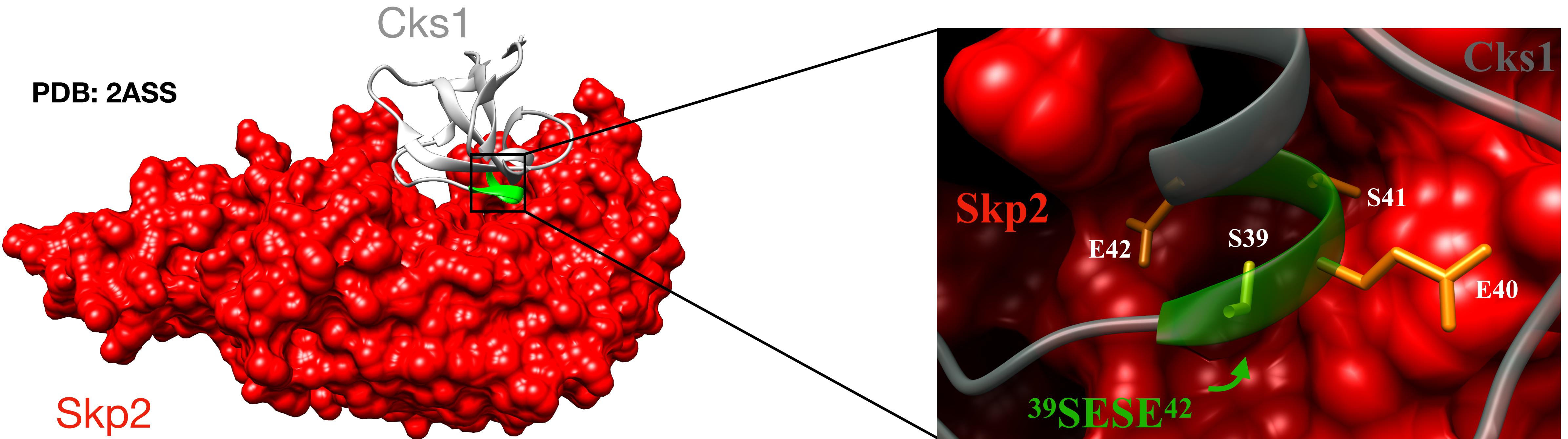


1. Identify a region important for binding

2. Search for the best linker size

3. Find linker sequence

3⁹SESE⁴² is important for Cks1–Skp2 interaction



Yellow: residues contributing > 1 kcal/mol binding energy

Orange: residues contributing > 2 kcal/mol binding energy

J. Gavenois et al., *Nat. Chem. Biol.* **10**, 716–722 (2014)

T. Siegert et al., *Methods Mol. Biol.* **1561**, 255-277 (2017)

B. Hal et al., *Mol. Cell* **20**, 9-19 (2005)

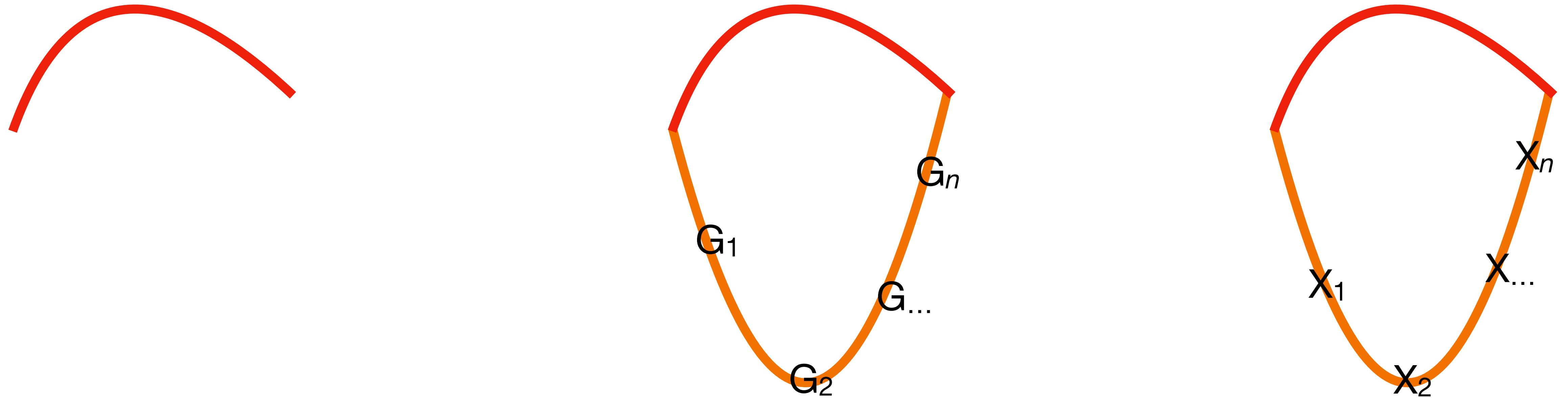
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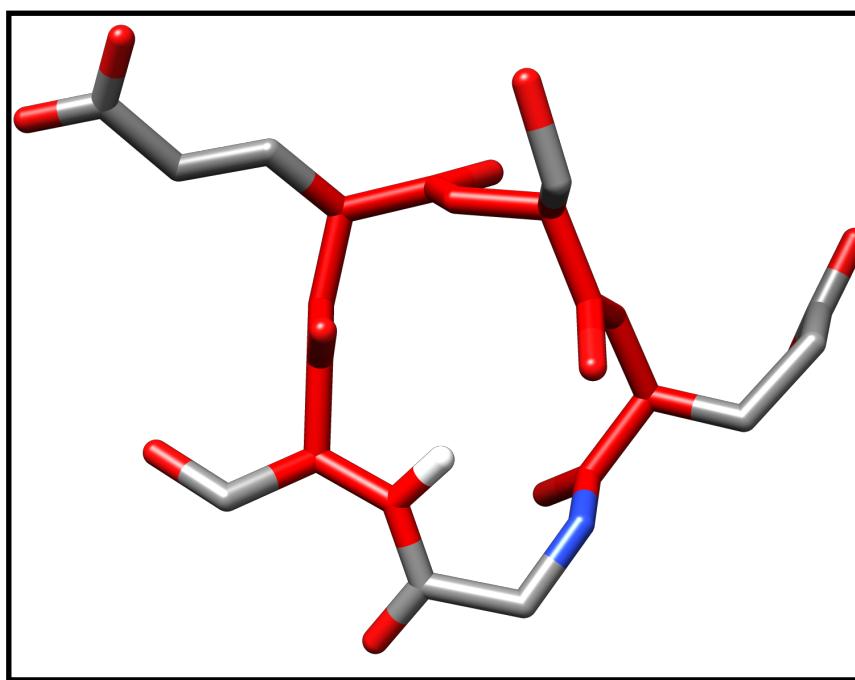
1. Identify a region important for binding

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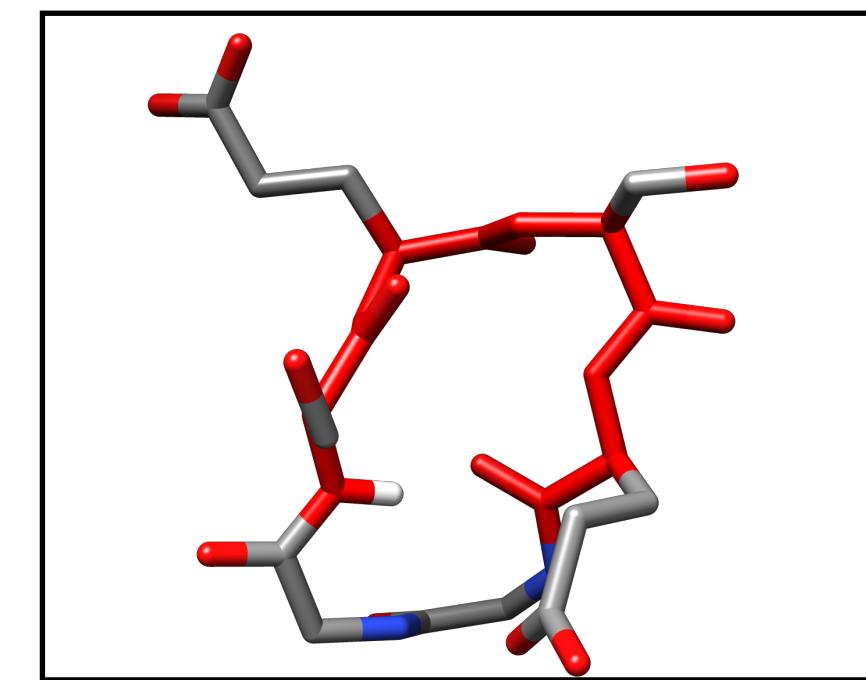
3. Find linker sequence

Create model systems and conduct simulations

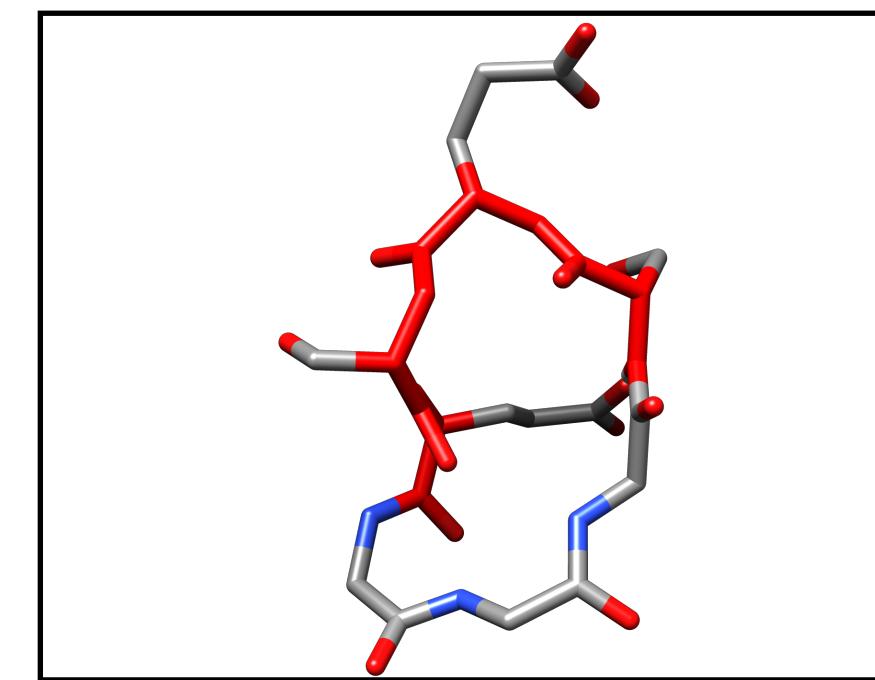
cyclo-SESEG1



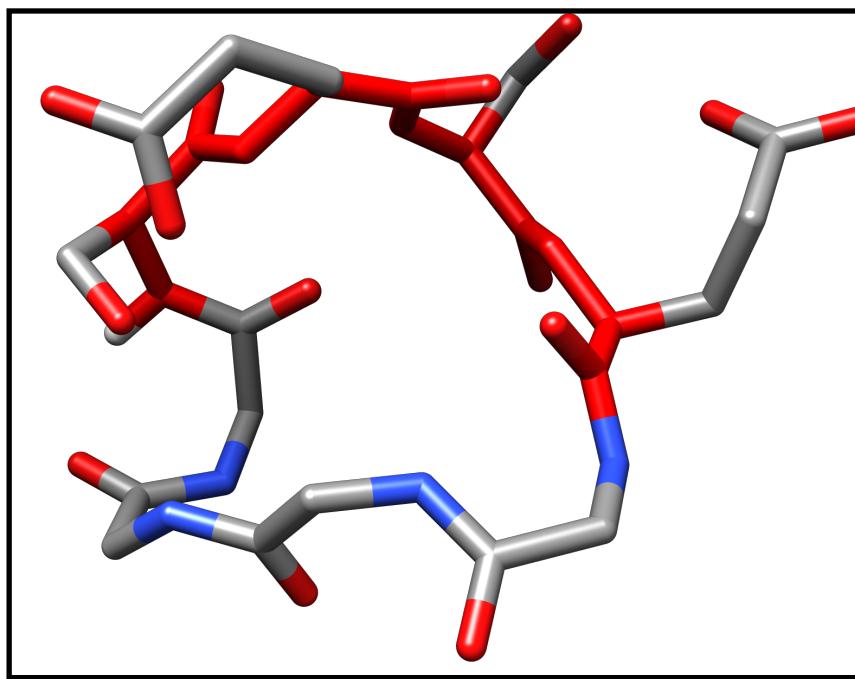
cyclo-SESEG2



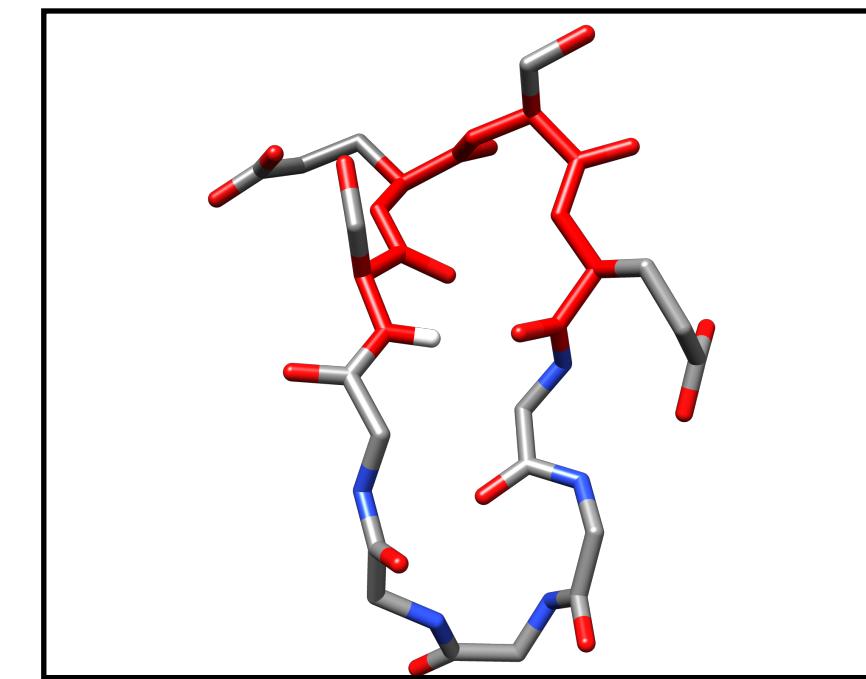
cyclo-SESEG3



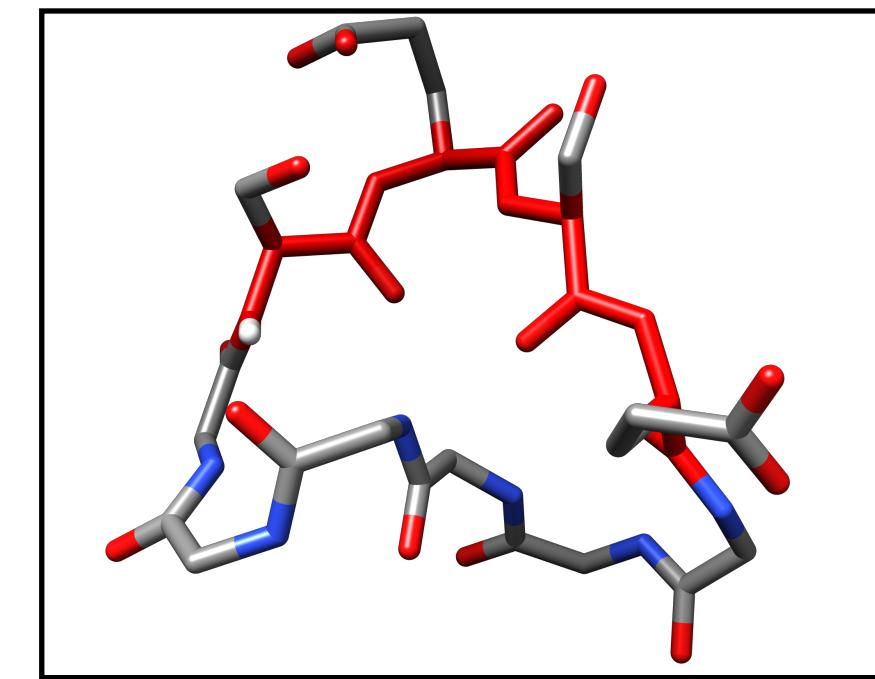
cyclo-SESEG4



cyclo-SESEG5



cyclo-SESEG6



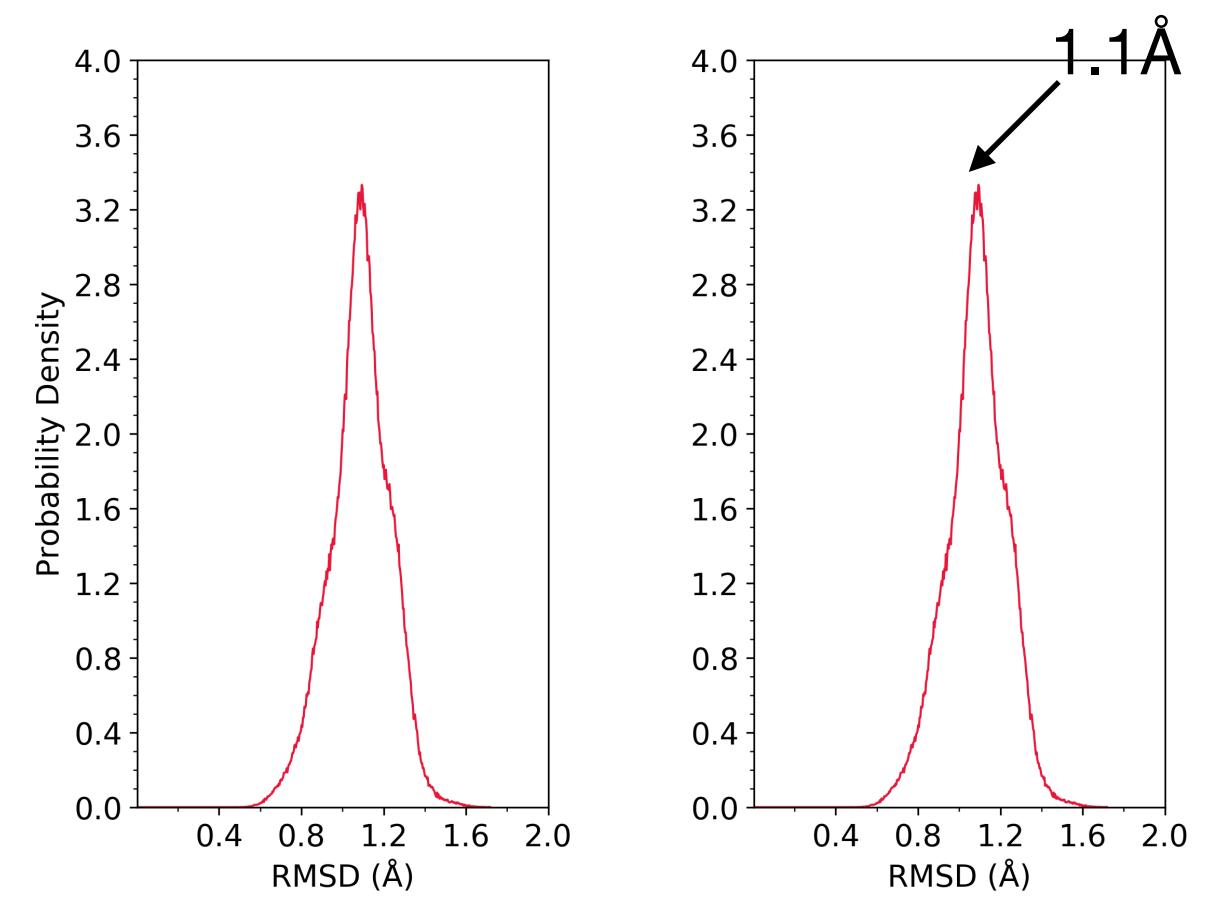
Simulation results are analyzed in two ways

- Clash analysis:
 - Align each conformation to the binding pocket and check if the linker region has steric clashes with the binding target.
- Cluster analysis:
 - Separate trajectory conformations into clusters to obtain the structural ensemble.

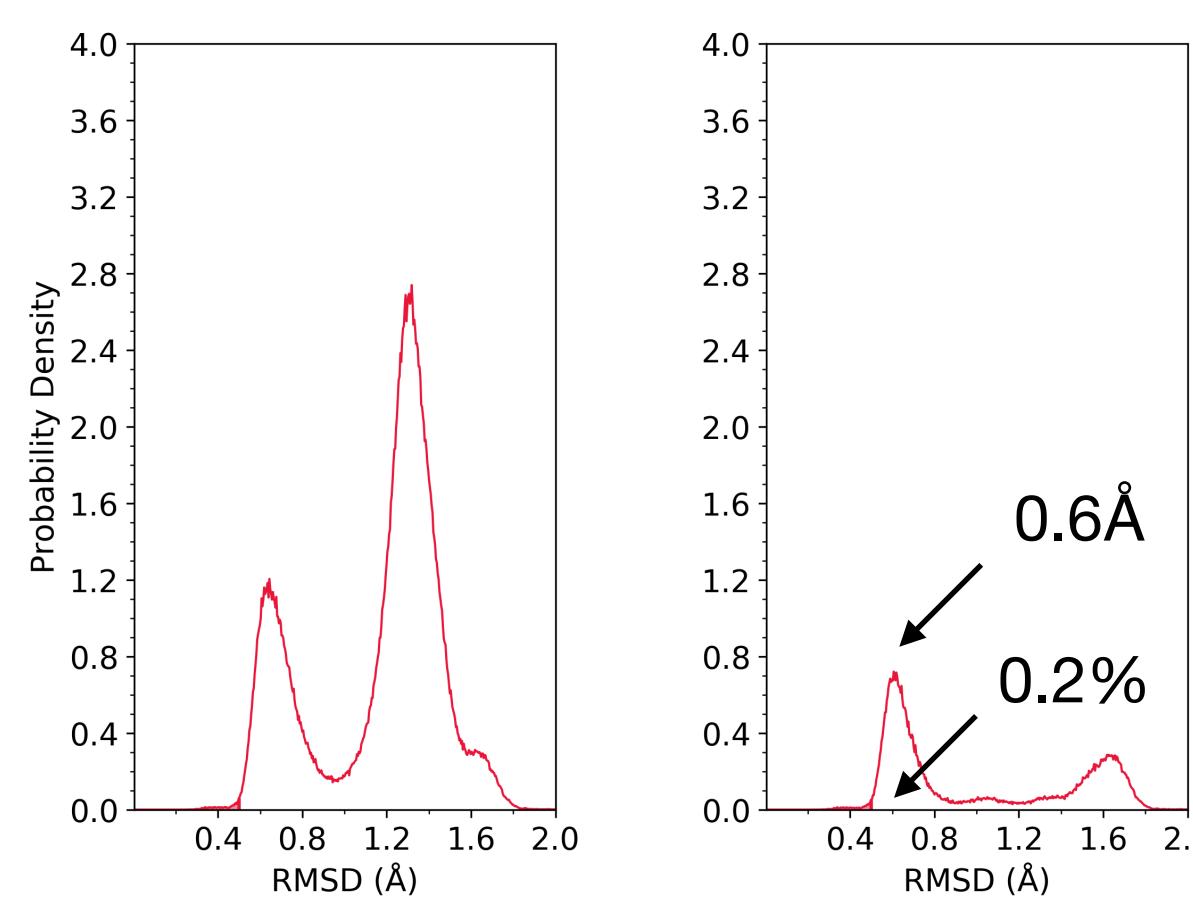
Clash Analysis

SESEG4 and SESEG5 best mimic the target conformation

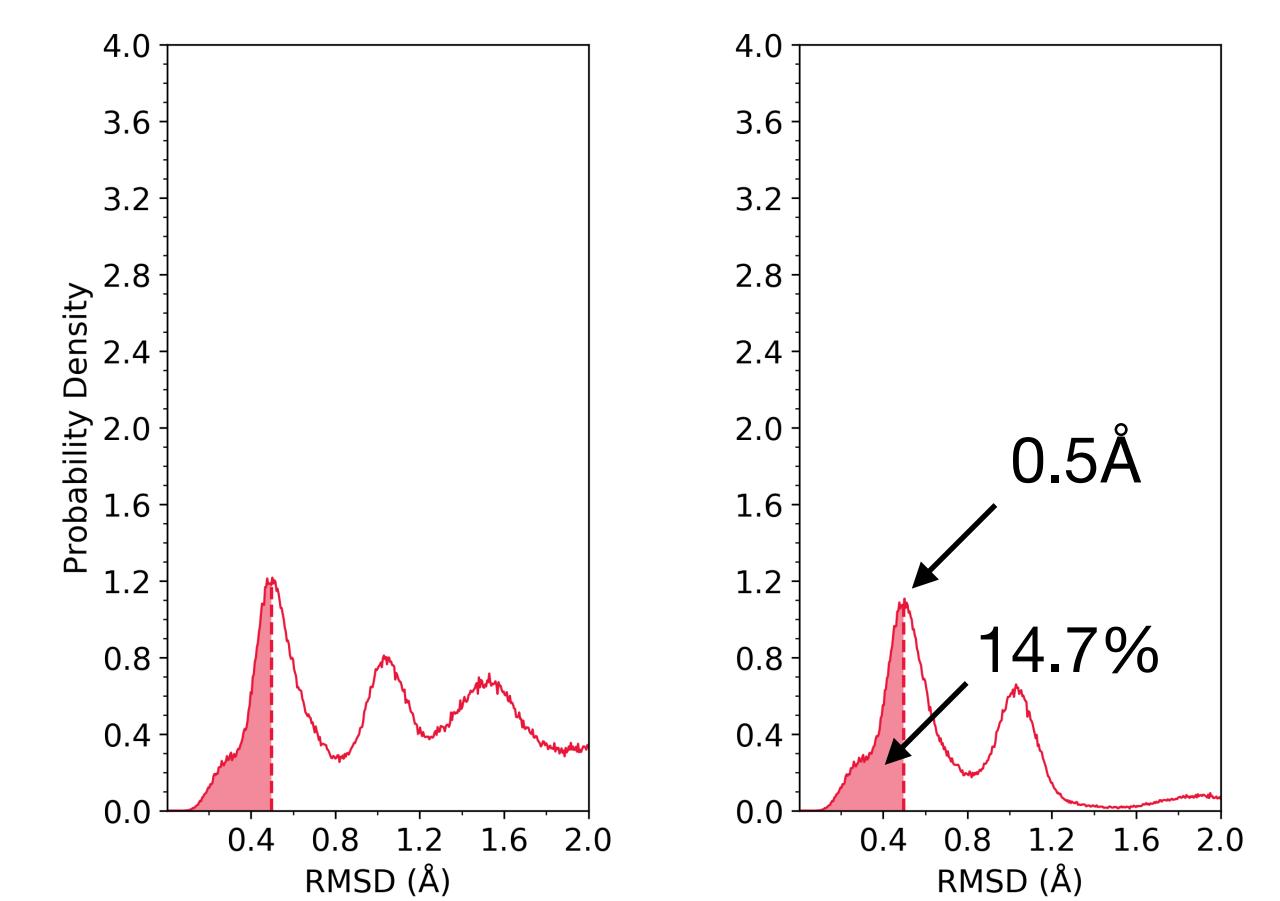
cyclo-SESEG1



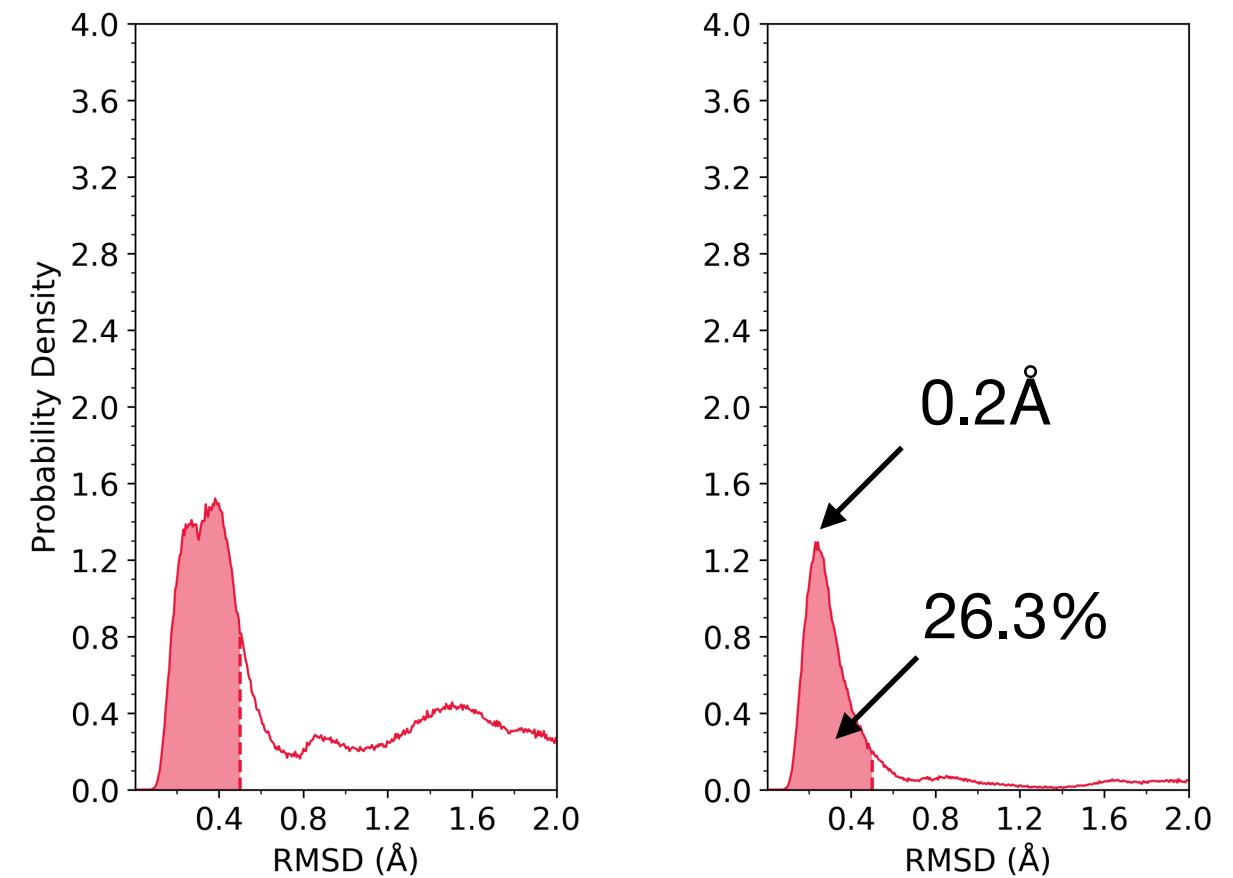
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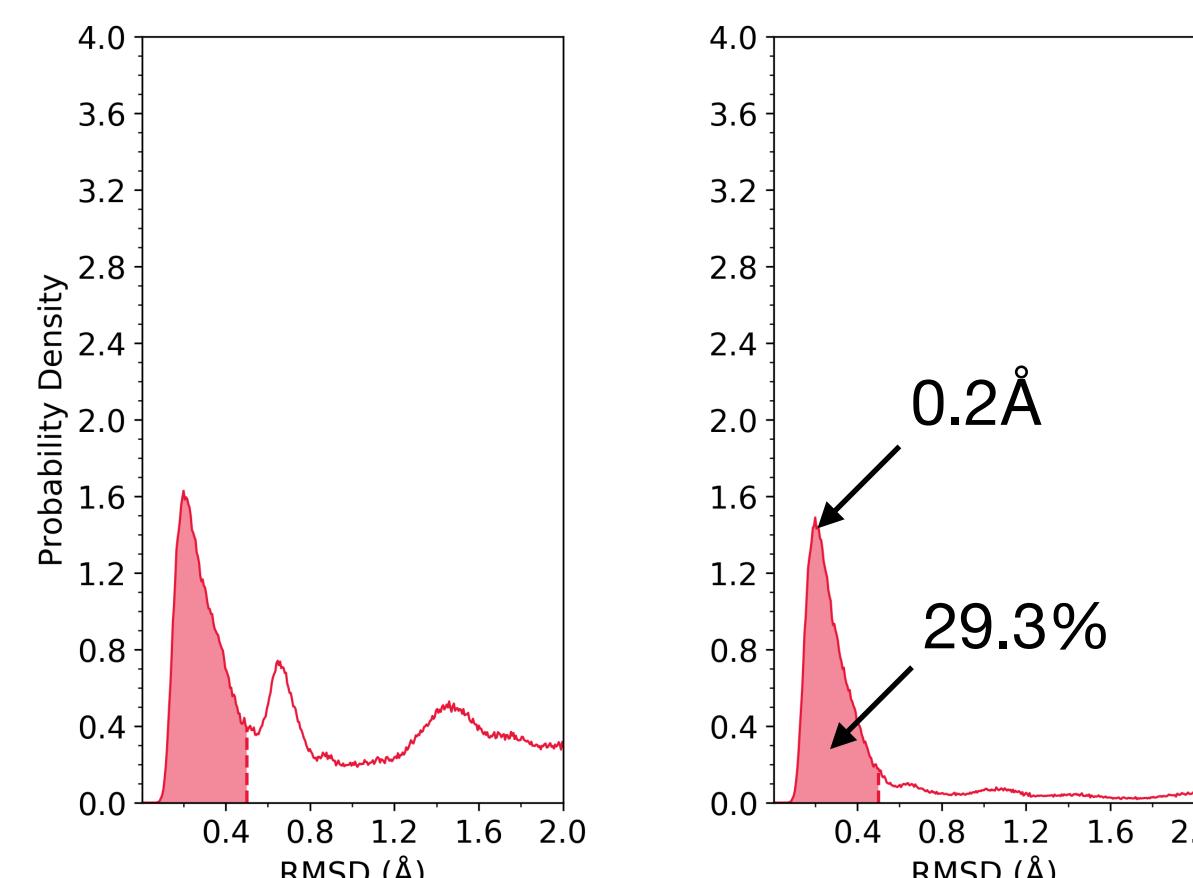
cyclo-SESEG3



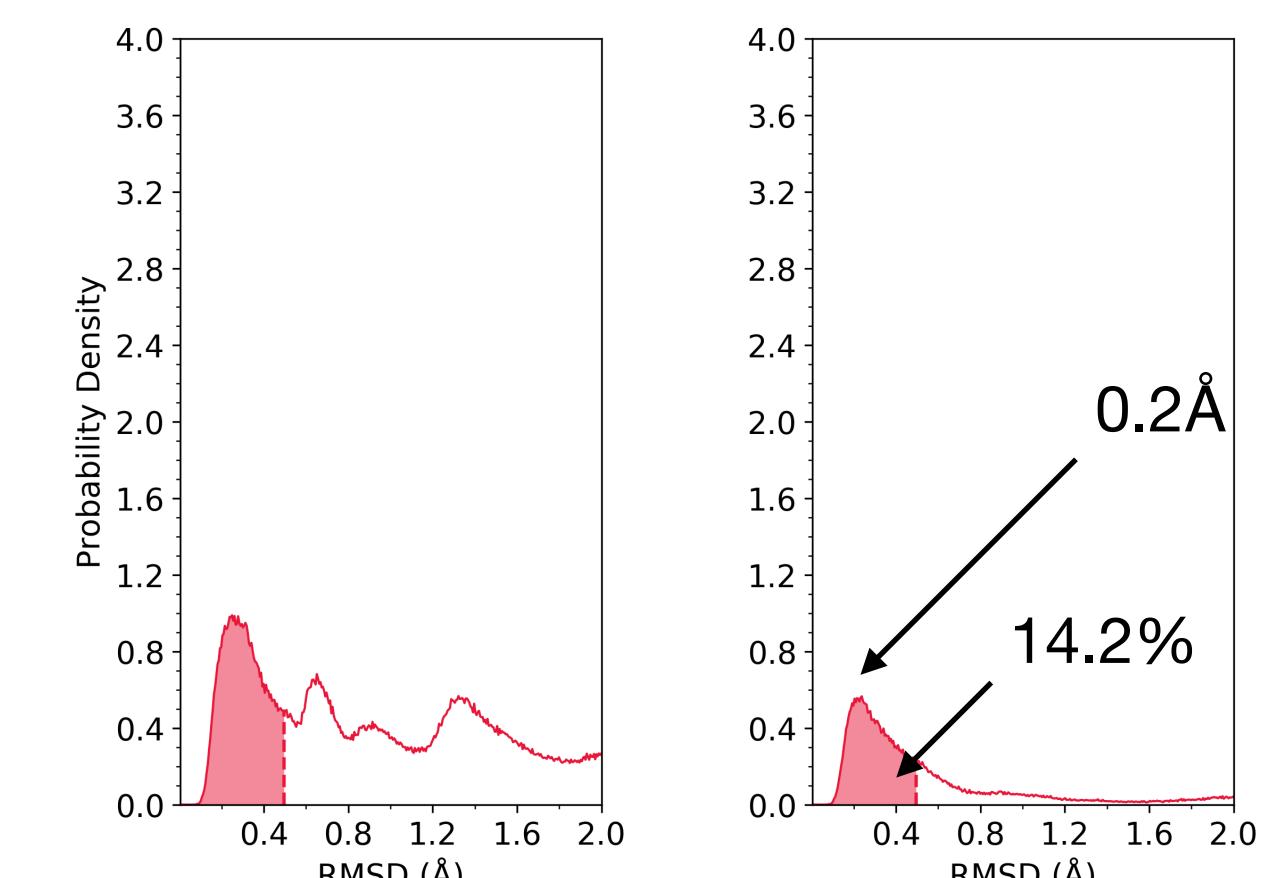
cyclo-SESEG4



cyclo-SESEG5



cyclo-SESEG6



Background

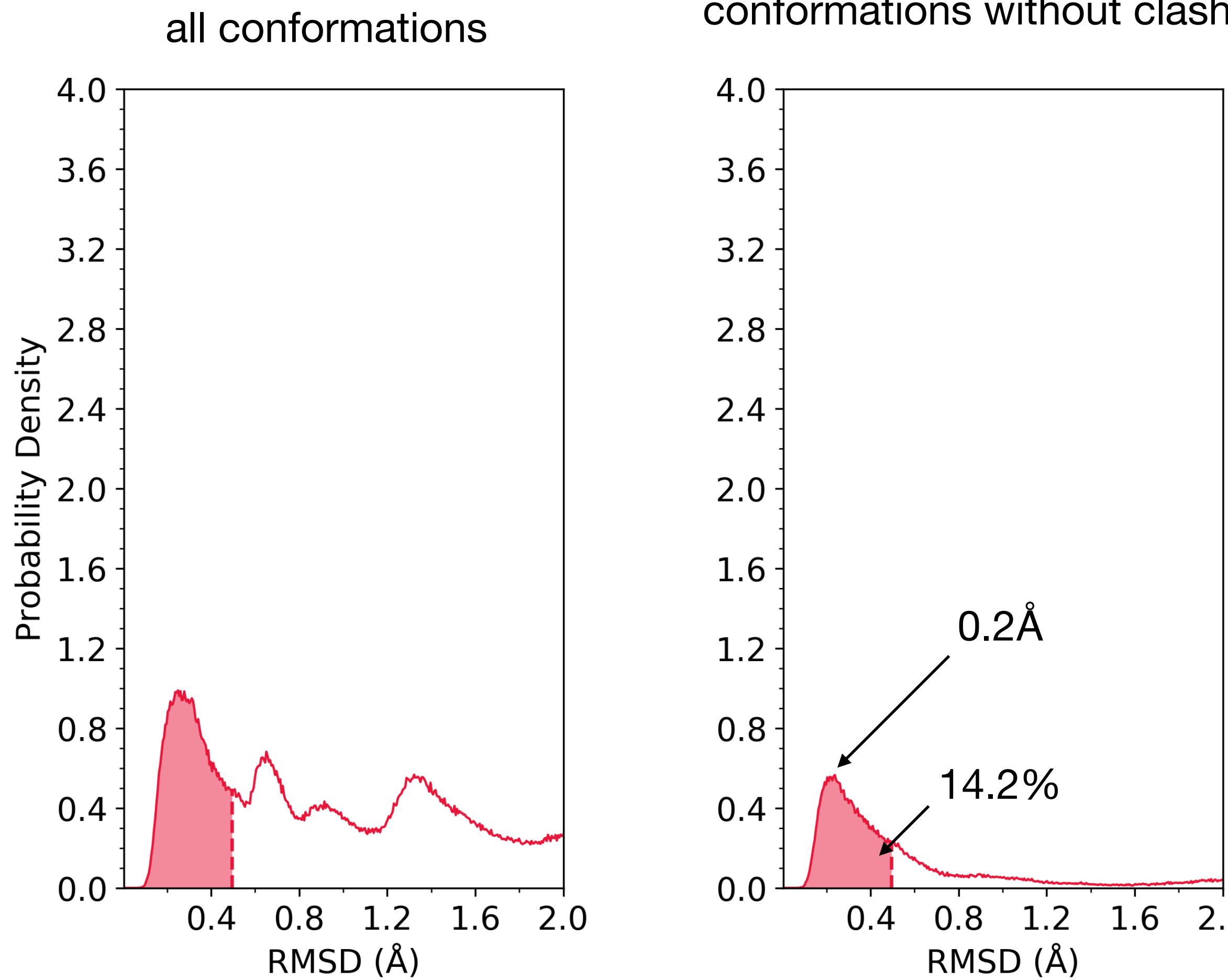
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Clash analysis provides useful insights

cyclo-SESEG6



Background

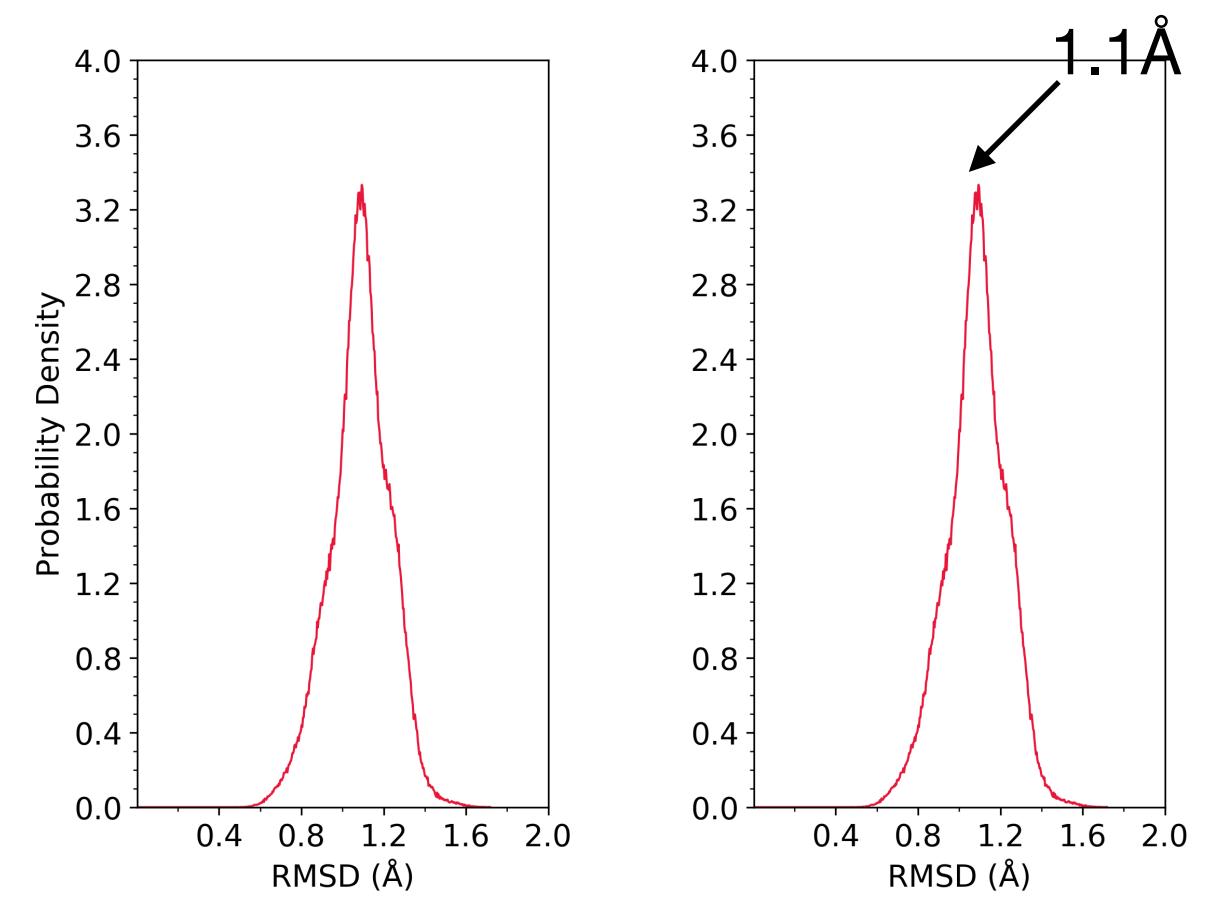
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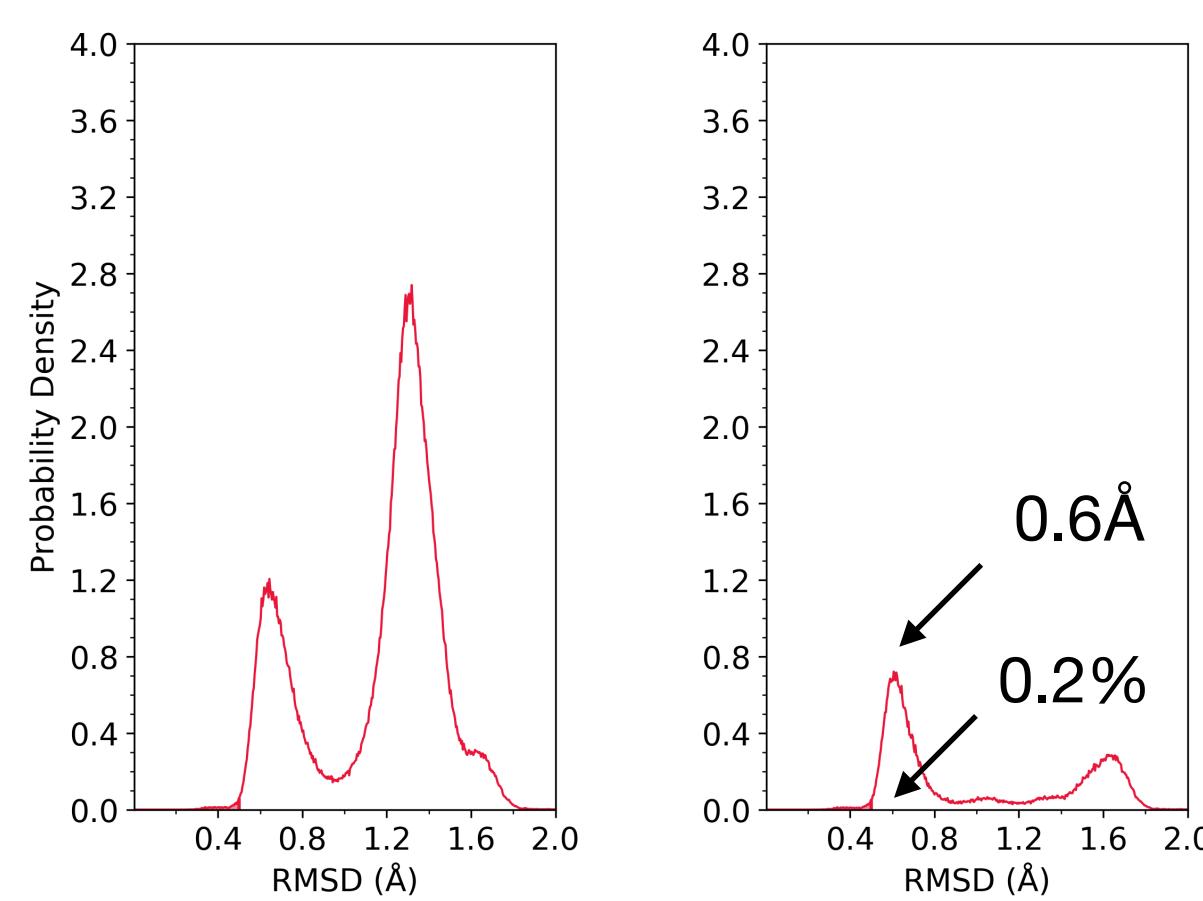
Conclusion

SESEG4 and SESEG5 best mimic the target conformation

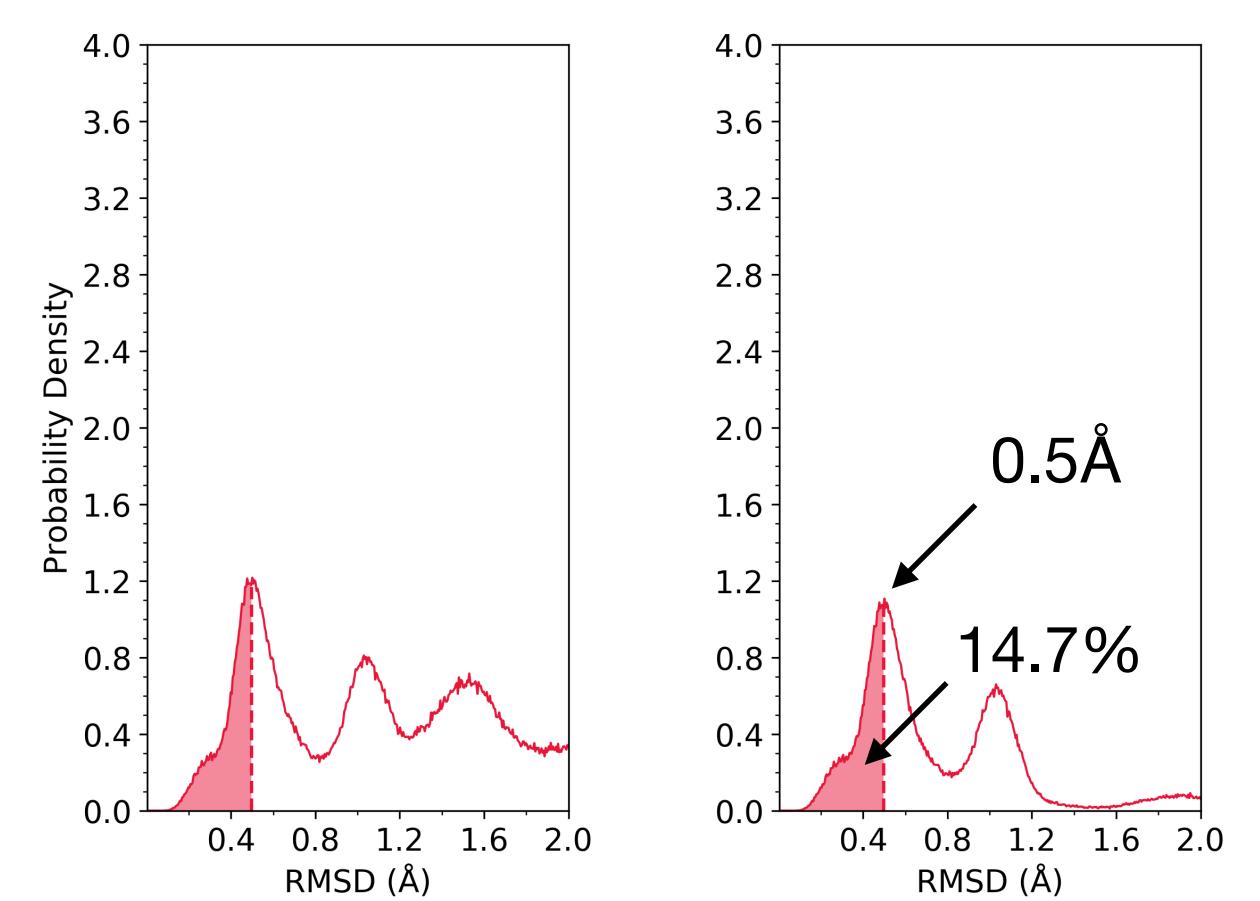
cyclo-SESEG1



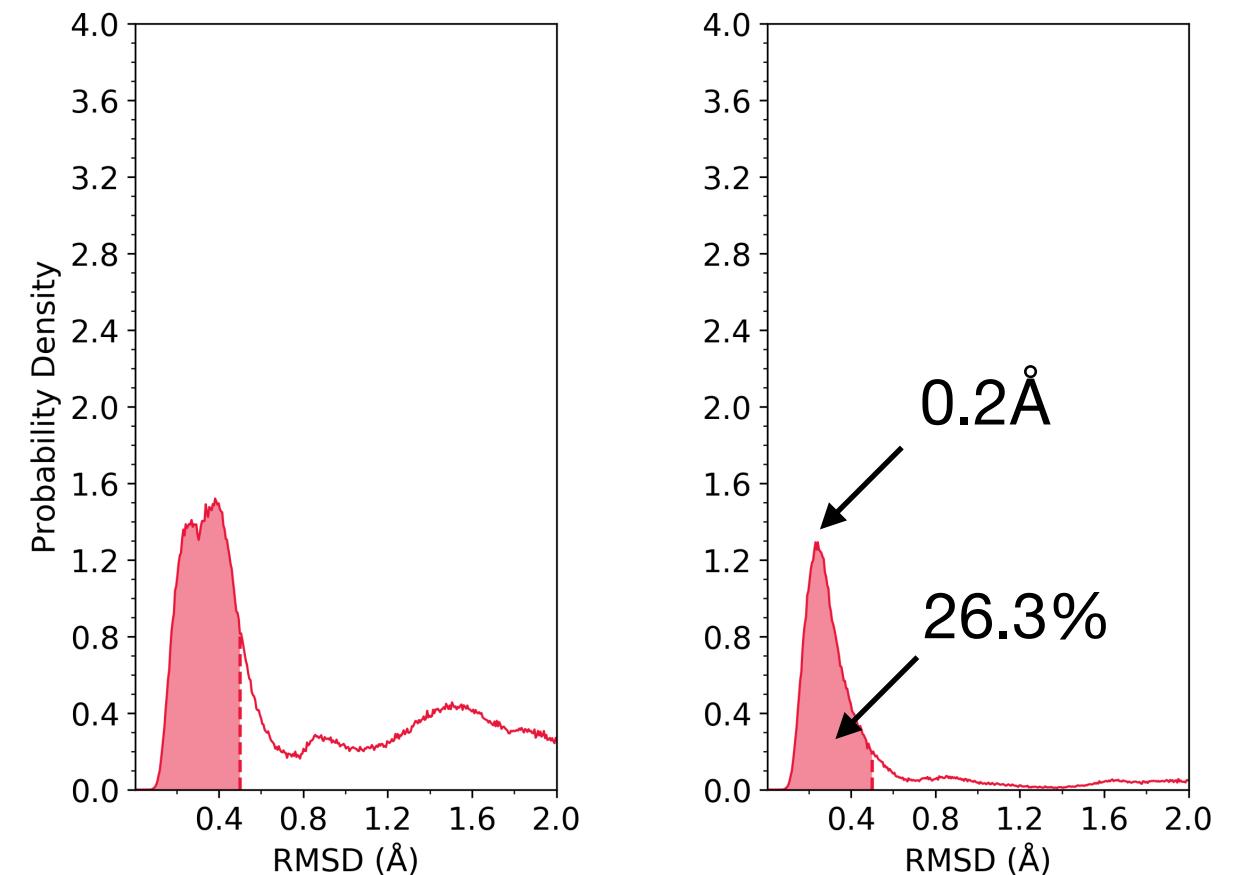
cyclo-SESEG2



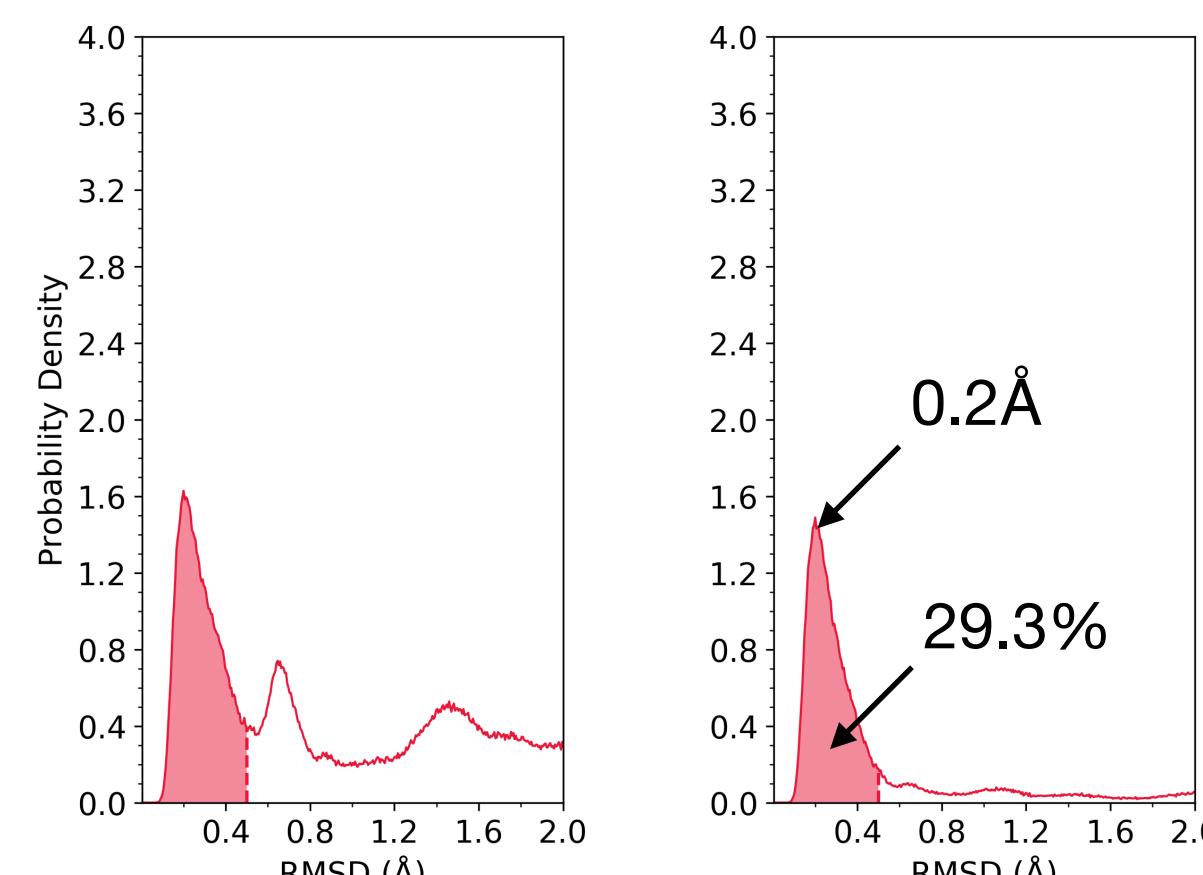
cyclo-SESEG3



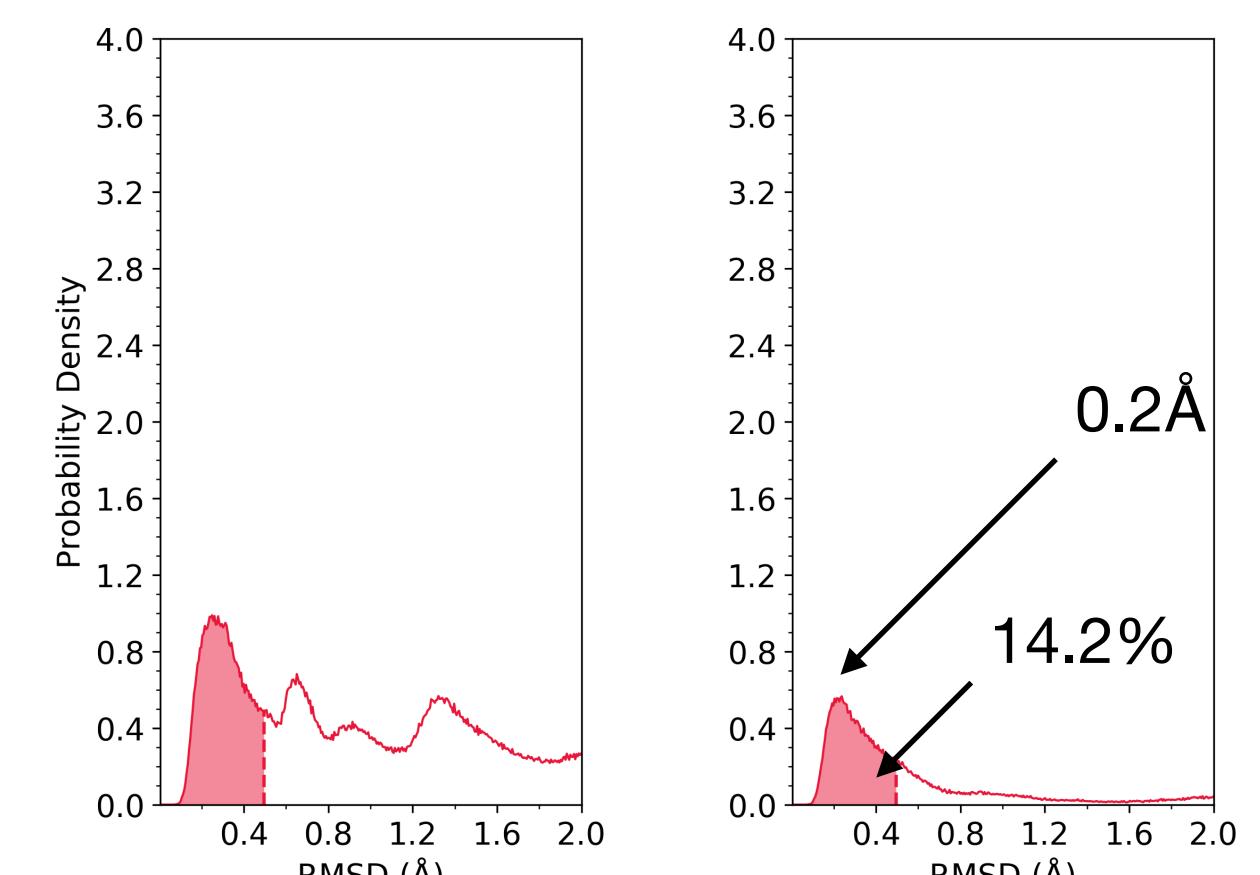
cyclo-SESEG4



cyclo-SESEG5



cyclo-SESEG6



Background

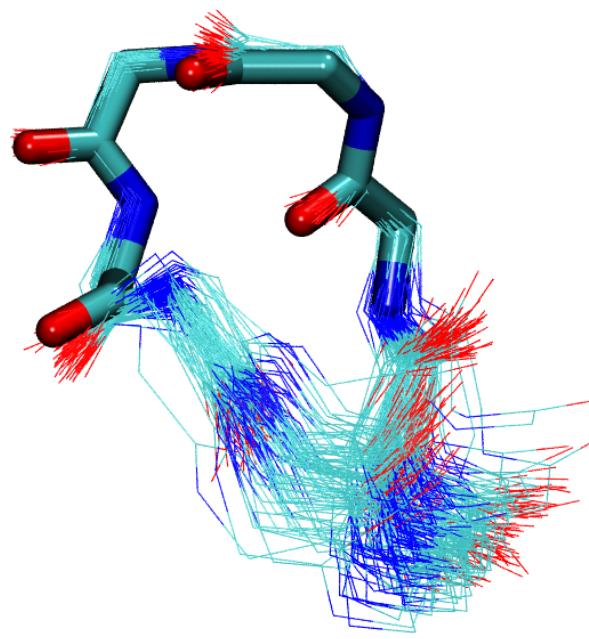
Project Description

Results

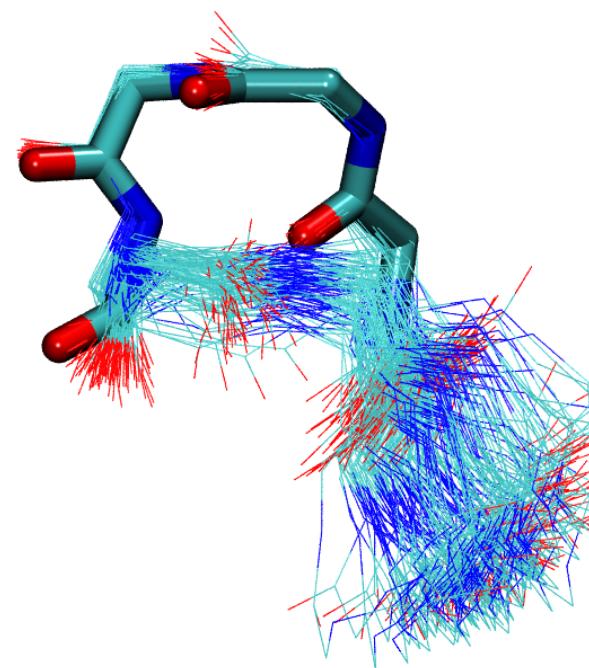
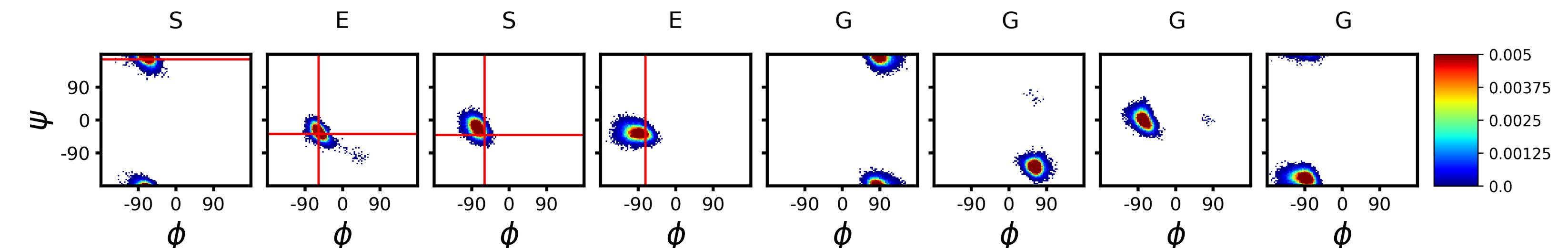
Conclusion

Cluster Analysis

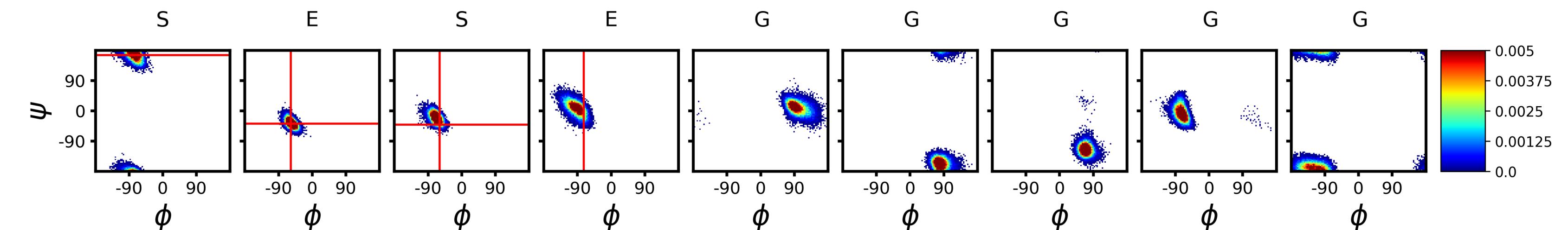
SESEG4 and SESEG5 adopt target conformation with high population



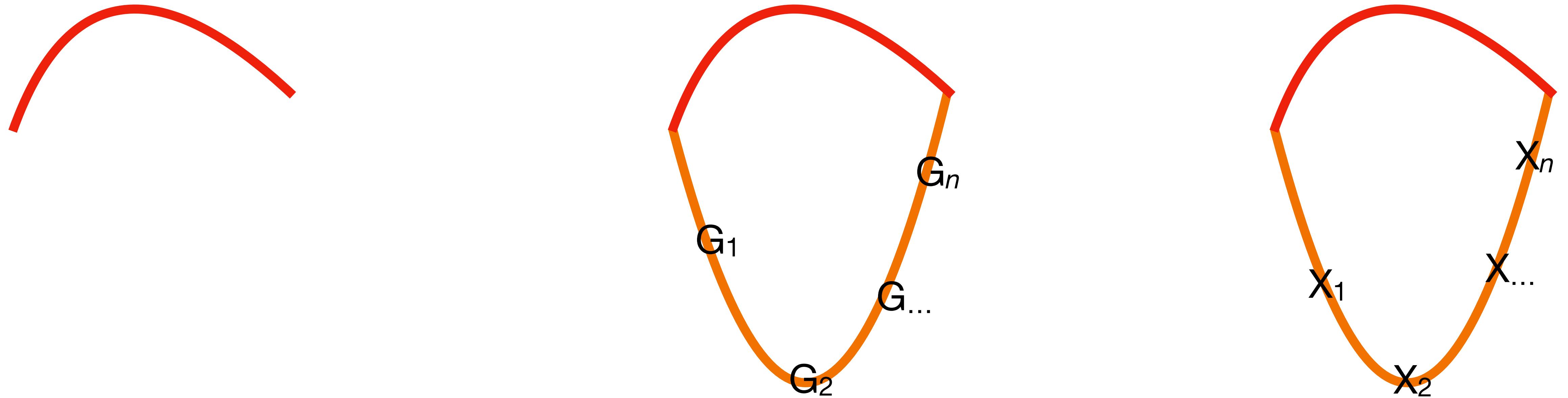
Population: 7.5%
RMSD(Å): 0.244 ± 0.062



Population: 4.6%
RMSD(Å): 0.216 ± 0.059



Cyclic-peptide design is a 3-step process

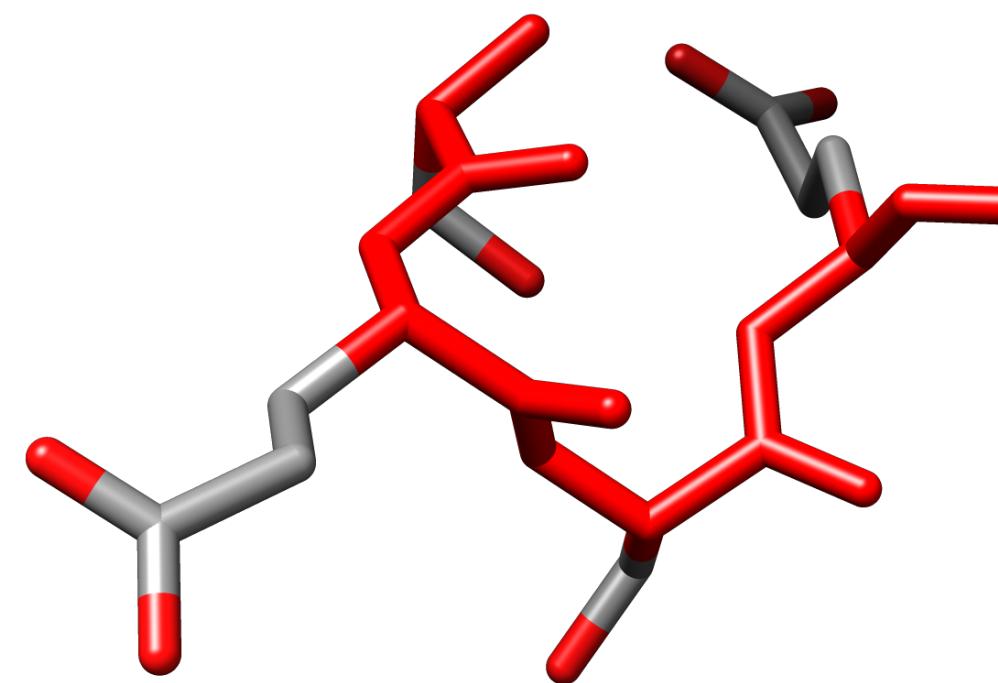


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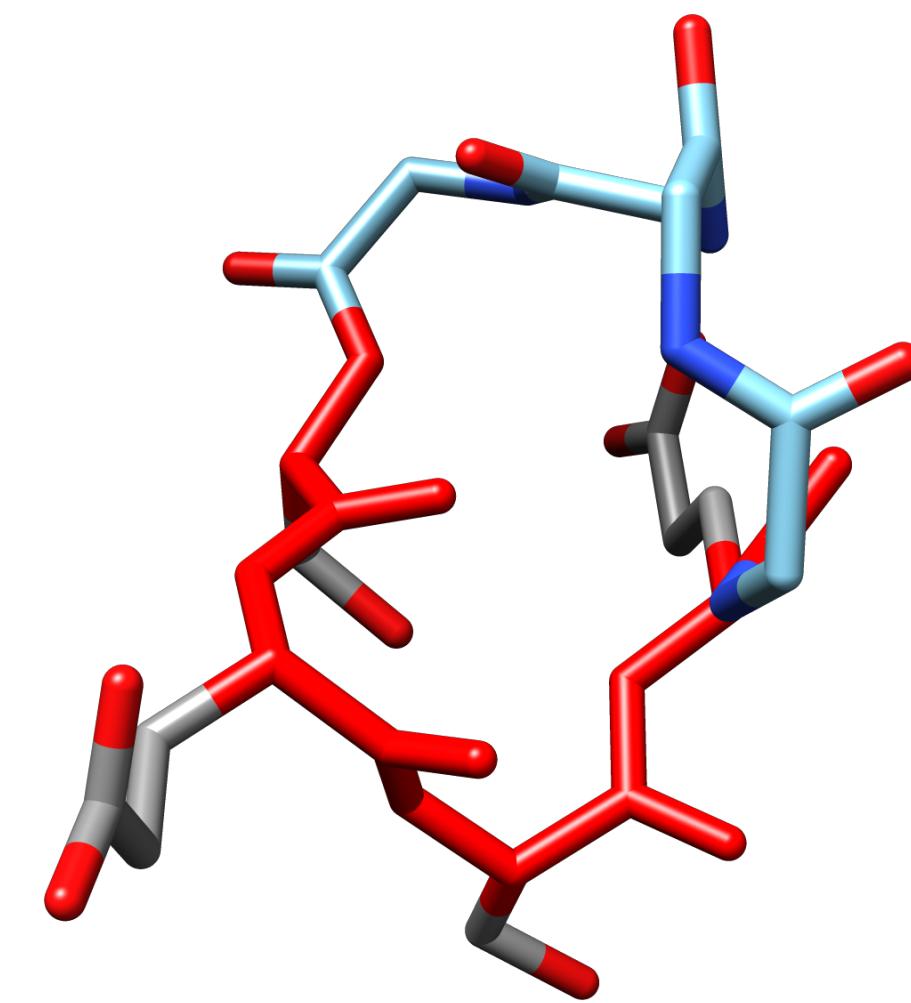
2. Search for the best linker size

3. Find linker sequence

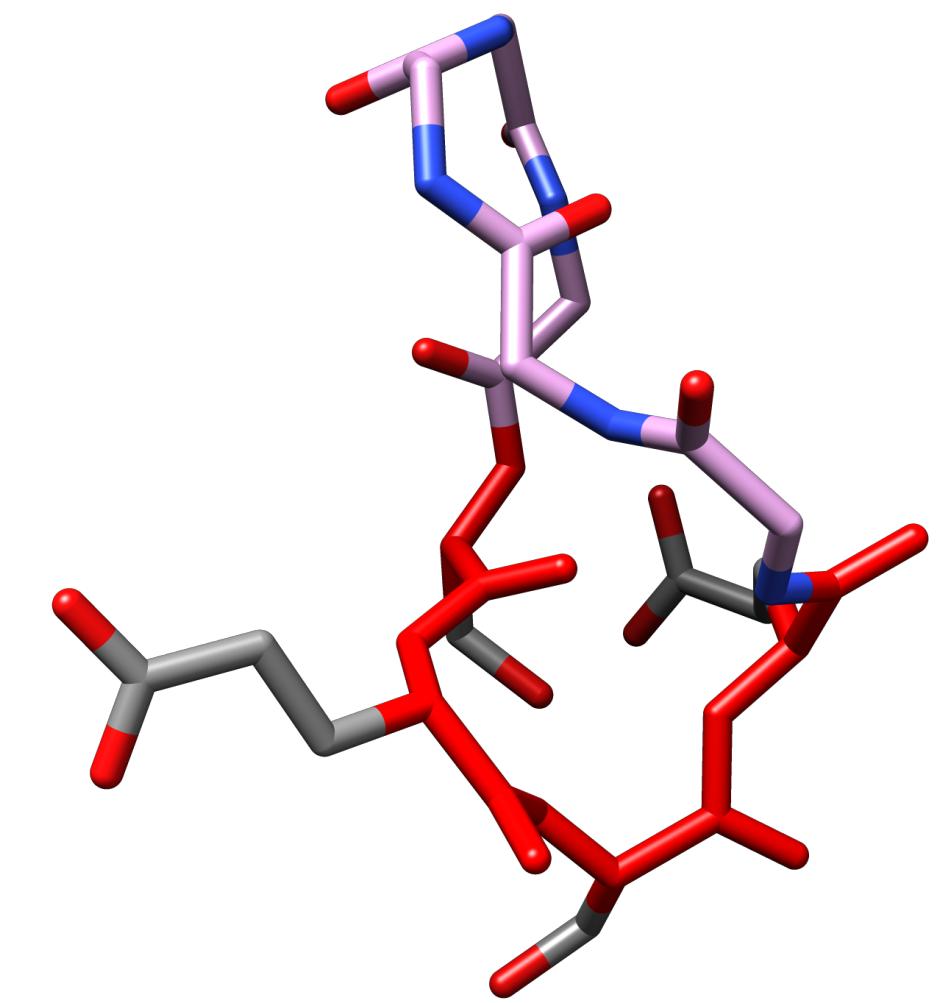
SESEG4 and SESEG5 adopt target conformation with high population



Desired SESE conformation

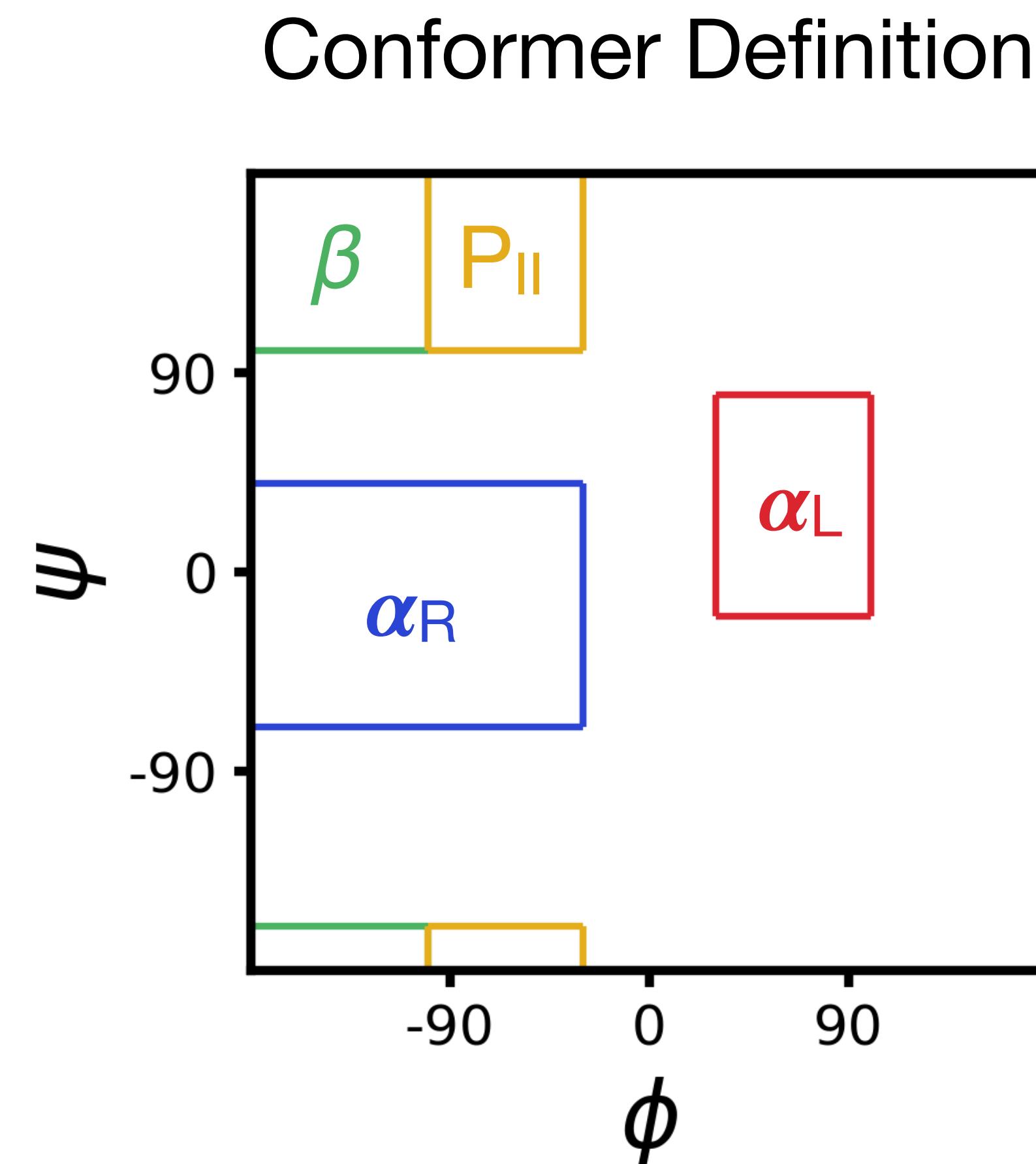


Target cyclo-(SESEX₁X₂X₃X₄) structure



Target cyclo-(SESEX₁X₂X₃X₄X₅) structure

Linker sequence selection is informed by aa's intrinsic propensity

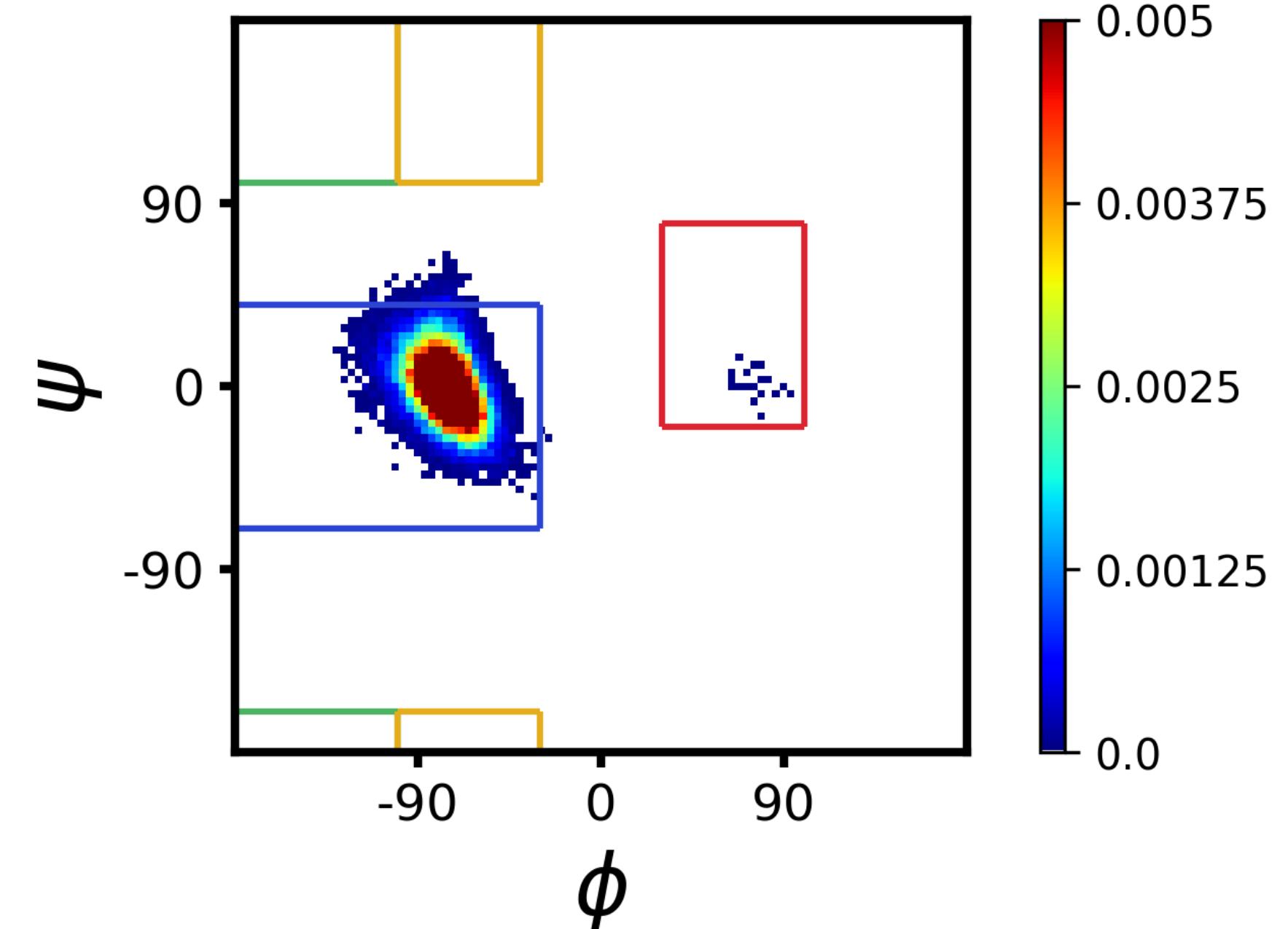


AA	P_{II}	β	α_R	α_L
P	0.80	0.00	0.15	0.00
A	0.46	0.23	0.21	0.02
L	0.43	0.21	0.26	0.02
M	0.40	0.24	0.21	0.03
E	0.40	0.22	0.28	0.03
I	0.39	0.36	0.20	0.00
W	0.39	0.26	0.23	0.03
C	0.38	0.31	0.18	0.03
V	0.37	0.39	0.19	0.00
F	0.35	0.33	0.20	0.03
Q	0.35	0.26	0.26	0.04
Y	0.34	0.32	0.21	0.03
R	0.34	0.27	0.26	0.04
K	0.34	0.26	0.29	0.04
S	0.33	0.25	0.33	0.02
H	0.30	0.30	0.22	0.06
T	0.29	0.28	0.38	0.00
D	0.29	0.09	0.43	0.05
N	0.24	0.15	0.30	0.13
G	0.21	0.09	0.09	0.27

Zhou, C.-Y., Jiang, F. & Wu, Y.-D. *J. Phys. Chem. B.* **119**, 1035-1047 (2015)

Linker sequence selection is informed by aa's intrinsic propensity

ϕ/ψ distribution of G₇ from cyclo-(SESEG4)

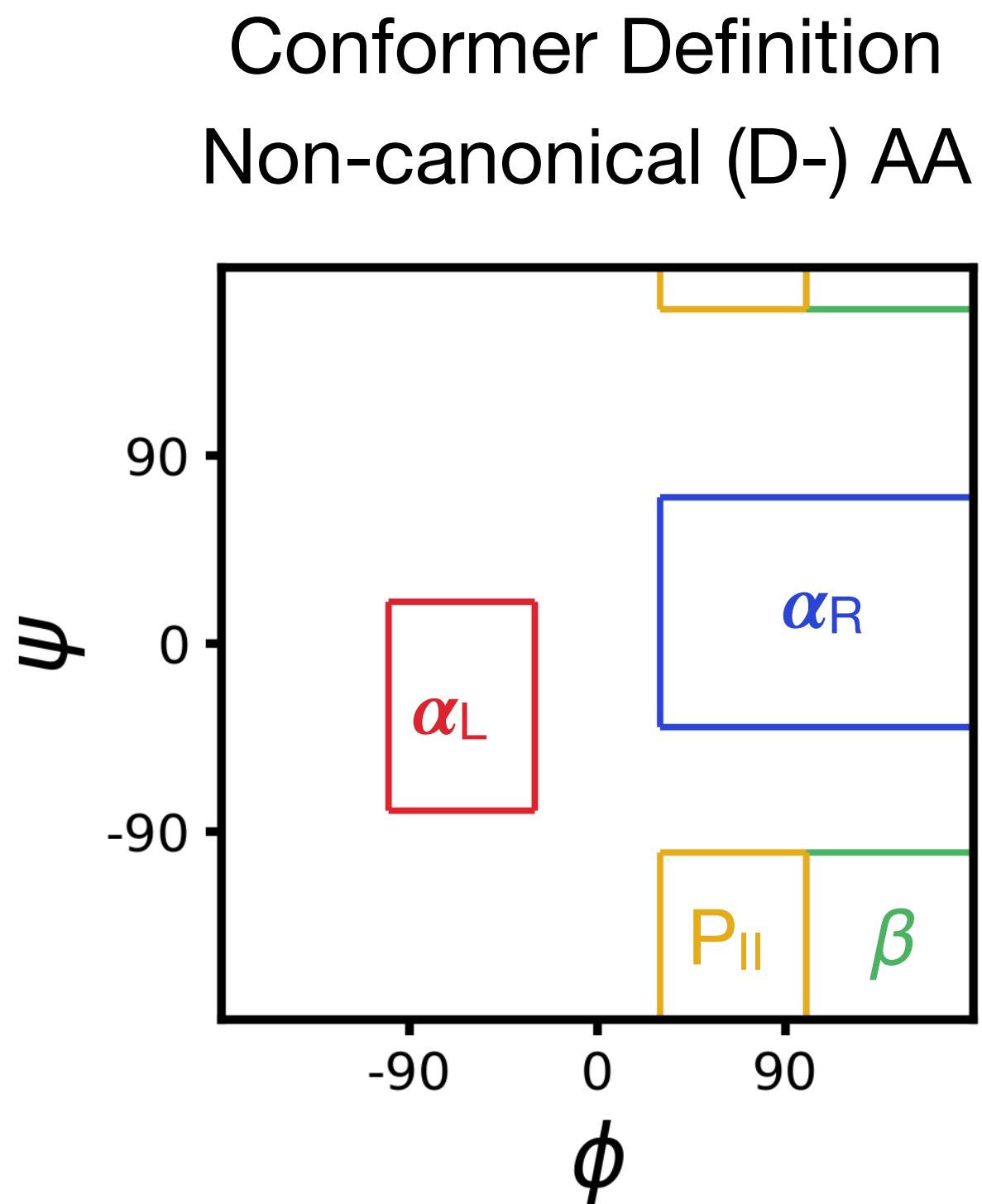
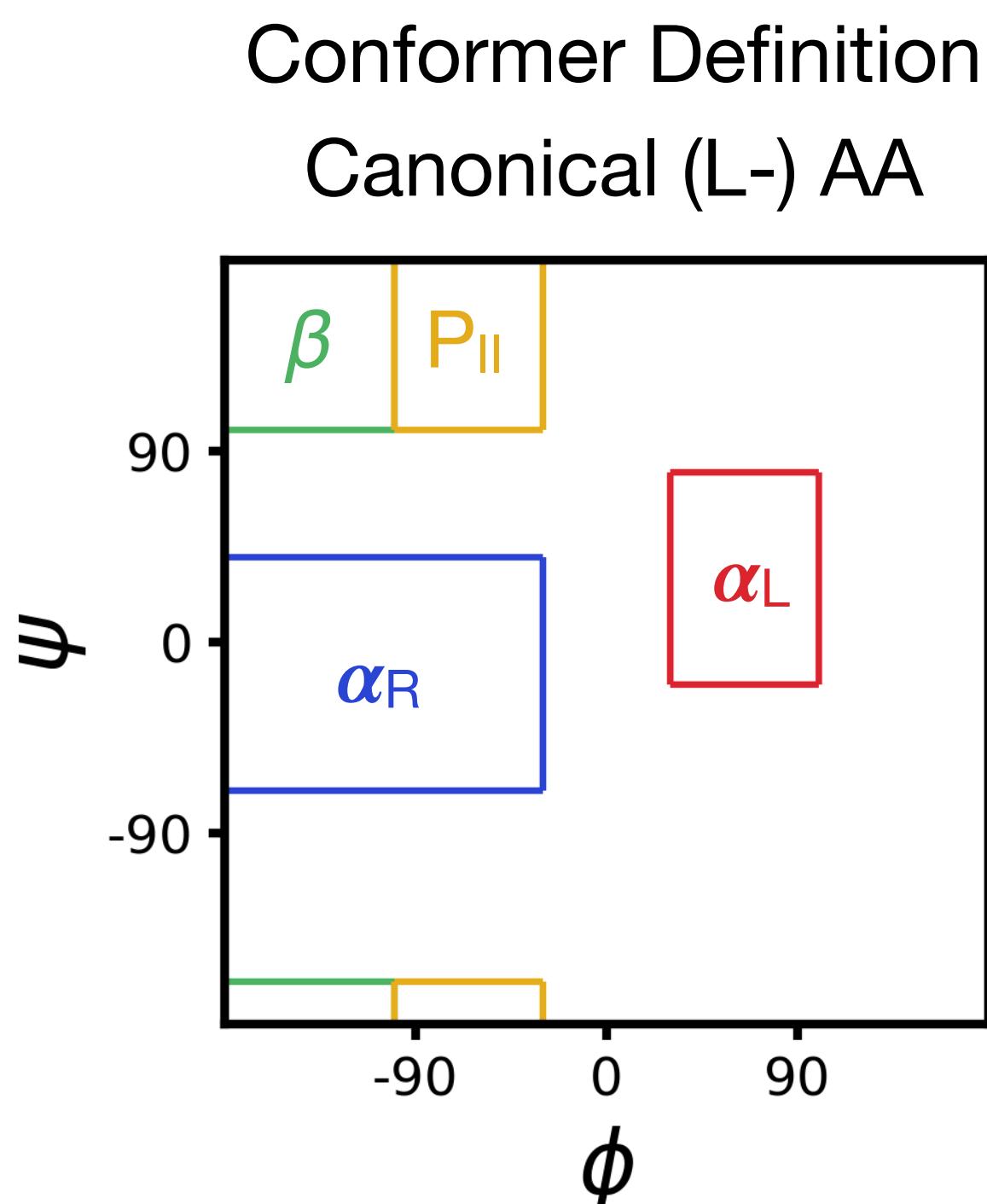


AA	P _{II}	β	α_R	α_L
D	0.29	0.09	0.43	0.05
T	0.29	0.28	0.38	0.00
S	0.33	0.25	0.33	0.02
N	0.24	0.15	0.30	0.13
K	0.34	0.26	0.29	0.04
E	0.40	0.22	0.28	0.03
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Y	0.34	0.32	0.21	0.03
I	0.39	0.36	0.20	0.00
F	0.35	0.33	0.20	0.03
V	0.37	0.39	0.19	0.00
C	0.38	0.31	0.18	0.03
P	0.80	0.00	0.15	0.00
G	0.21	0.09	0.09	0.27

Conformer Probability

AA	P_{II}	β	α_R	α_L
P	0.80	0.00	0.15	0.00
A	0.46	0.23	0.21	0.02
L	0.43	0.21	0.26	0.02
M	0.40	0.24	0.21	0.03
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K	0.34	0.26	0.29	0.04
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T	0.29	0.28	0.38	0.00
D	0.29	0.09	0.43	0.05
N	0.24	0.15	0.30	0.13
G	0.21	0.09	0.09	0.27

Linker sequence selection is informed by aa's intrinsic propensity

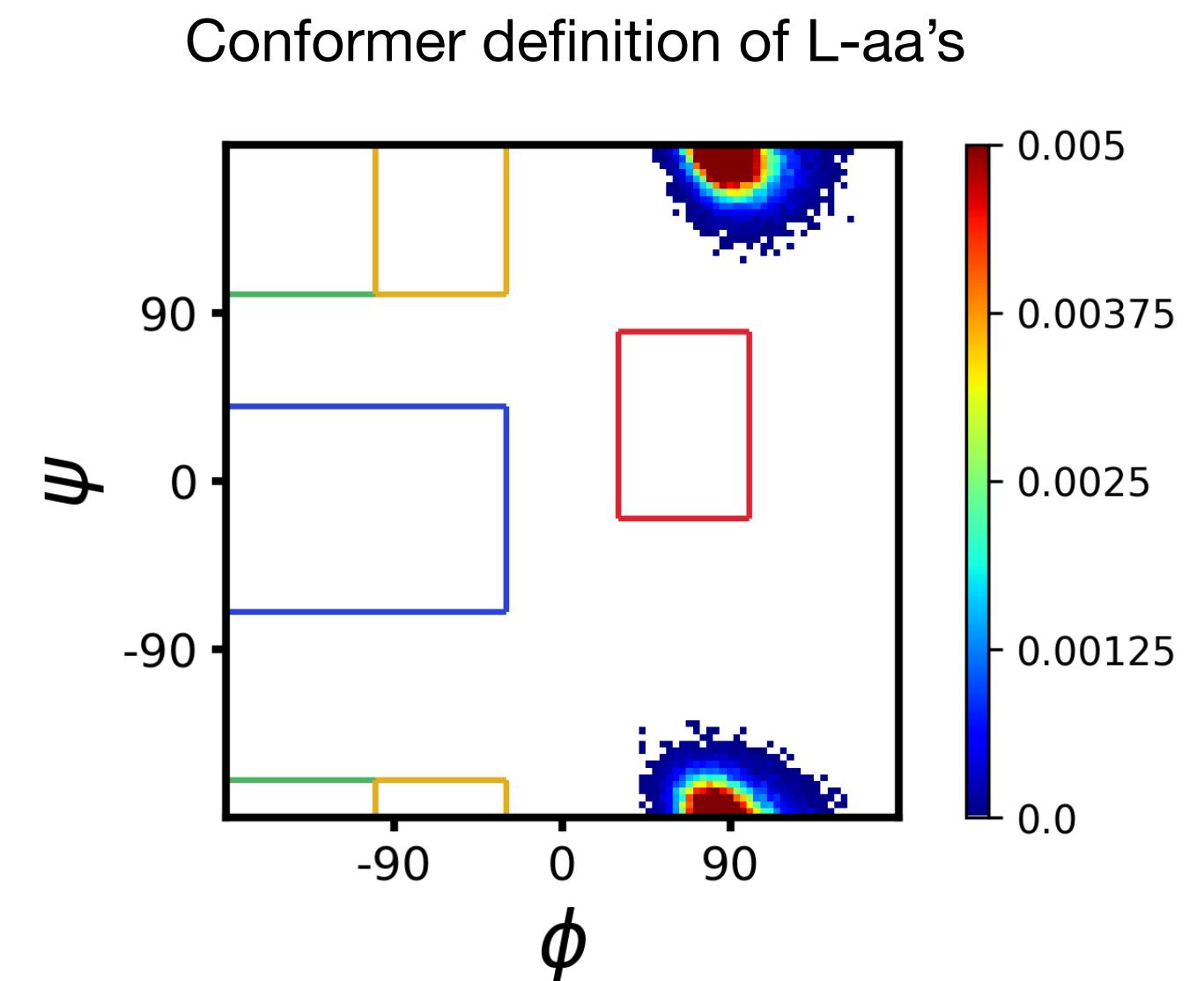


Conformer Probability

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W	0.39	0.26	0.23	0.03
C	0.38	0.31	0.18	0.03
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Y	0.34	0.32	0.21	0.03
R	0.34	0.27	0.26	0.04
K	0.34	0.26	0.29	0.04
S	0.33	0.25	0.33	0.02
H	0.30	0.30	0.22	0.06
T	0.29	0.28	0.38	0.00
D	0.29	0.09	0.43	0.05
N	0.24	0.15	0.30	0.13
G	0.21	0.09	0.09	0.27

Linker sequence selection is informed by aa's intrinsic propensity

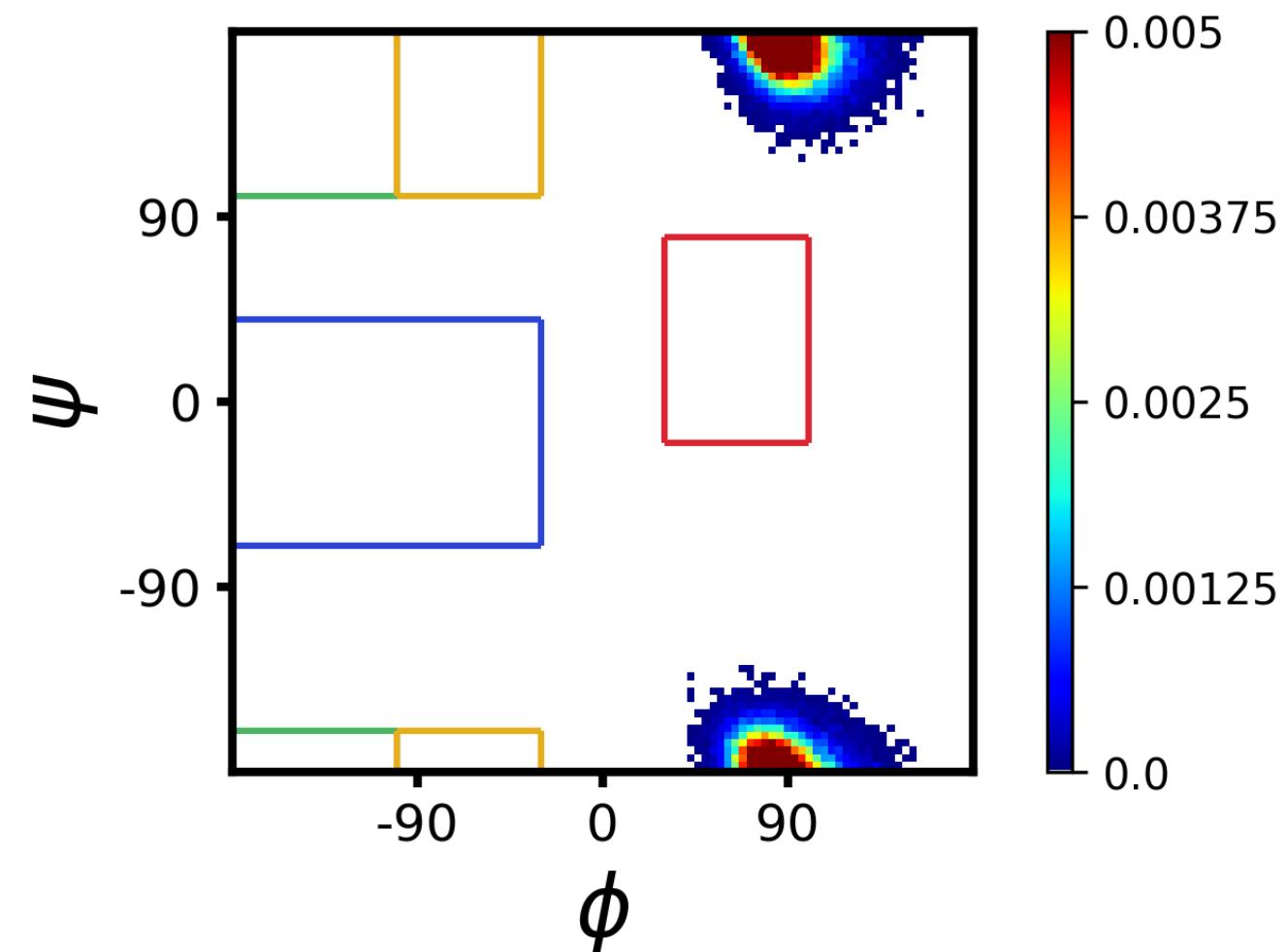
ϕ/ψ distribution of G₅ from cyclo-(SESEG4)



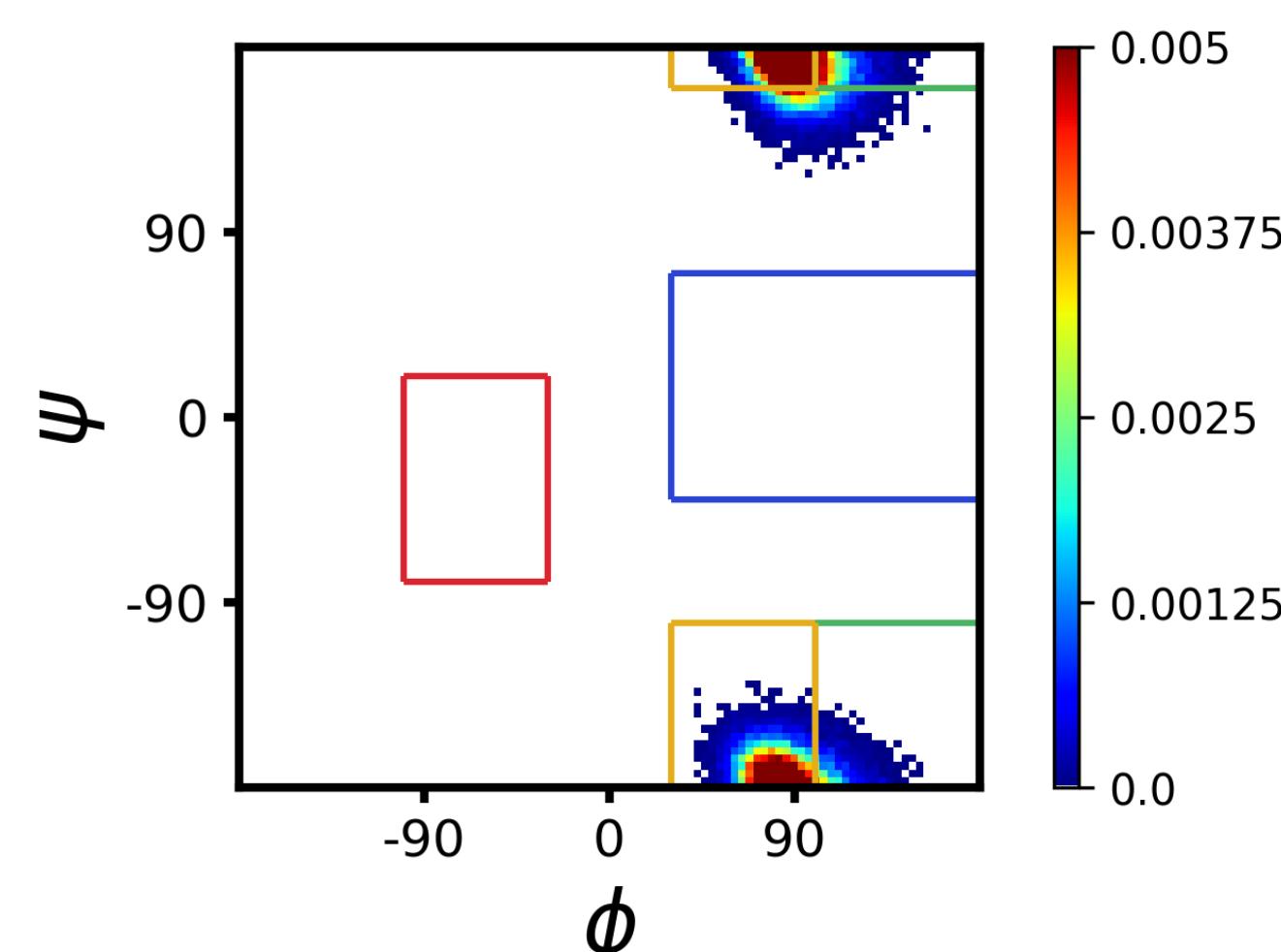
Linker sequence selection is informed by aa's intrinsic propensity

ϕ/ψ distribution of G₅ from cyclo-(SESEG4)

Conformer definition of L-aa's



Conformer definition of D-aa's

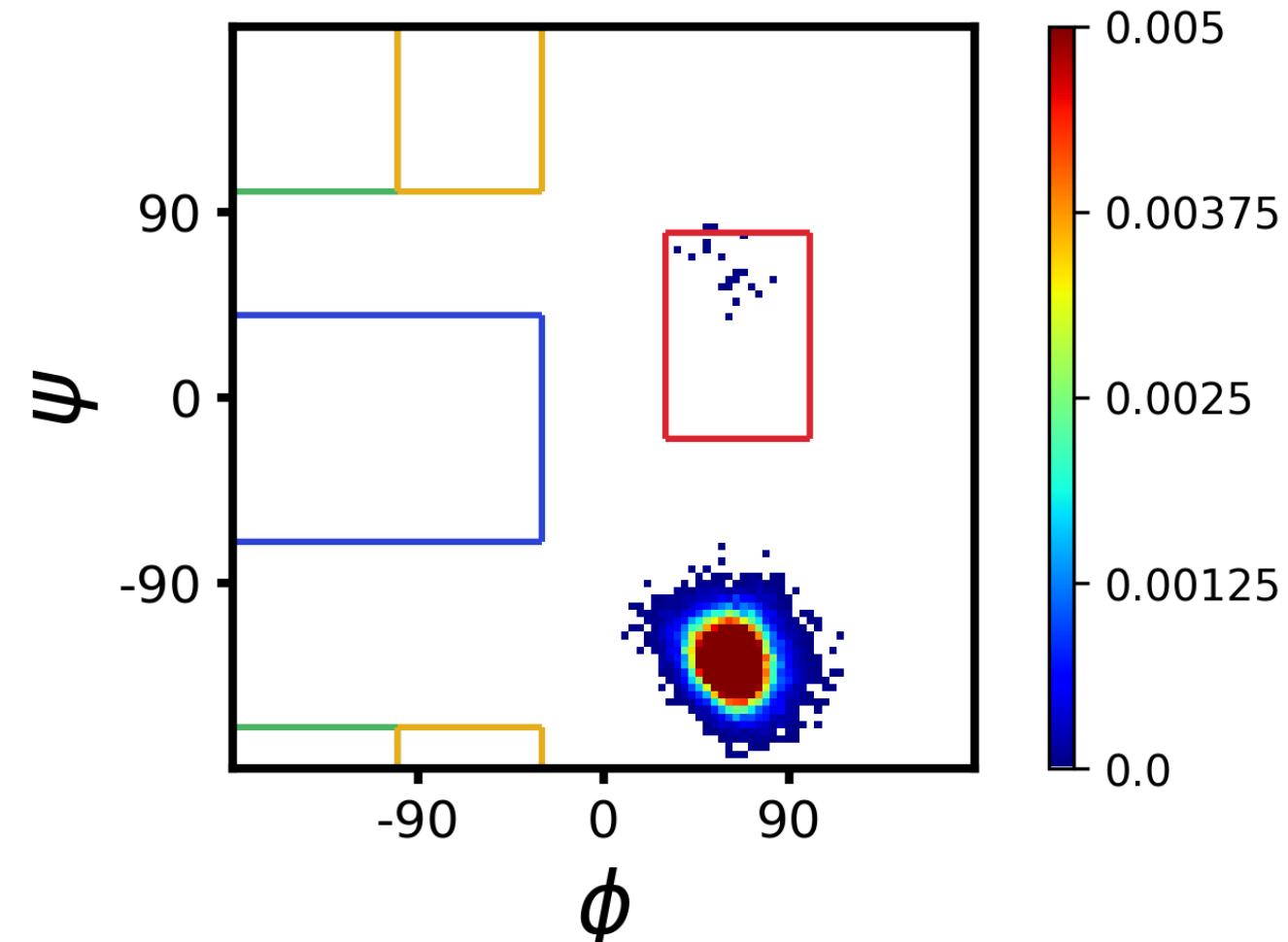


AA	P _{II}	β	α_R	α_L
V	0.80	0.09	0.19	0.00
I	0.39	0.26	0.20	0.00
F	0.45	0.33	0.26	0.03
Y	0.40	0.34	0.21	0.03
C	0.48	0.31	0.18	0.03
H	0.39	0.36	0.20	0.06
T	0.39	0.28	0.38	0.00
R	0.38	0.31	0.16	0.04
W	0.39	0.30	0.19	0.00
Q	0.35	0.36	0.26	0.04
K	0.35	0.26	0.20	0.04
S	0.34	0.32	0.33	0.03
M	0.40	0.24	0.26	0.04
A	0.34	0.26	0.29	0.04
E	0.40	0.23	0.38	0.03
L	0.40	0.30	0.26	0.06
N	0.29	0.18	0.38	0.06
D	0.29	0.09	0.43	0.05
G	0.24	0.09	0.09	0.13
P	0.80	0.09	0.09	0.00

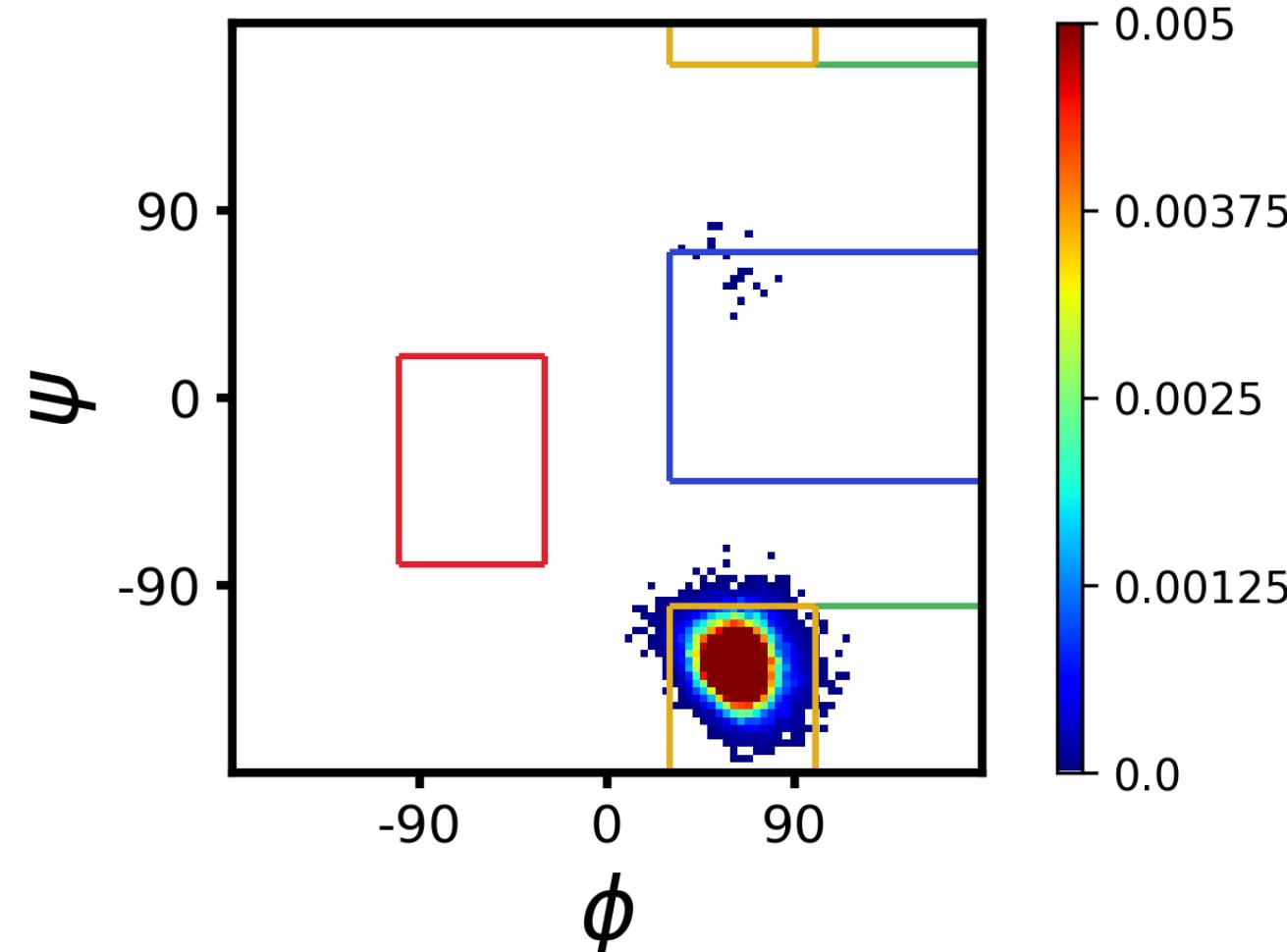
Linker sequence selection is informed by aa's intrinsic propensity

ϕ/ψ distribution of G₆ from cyclo-(SESEG4)

Conformer definition of L-aa's



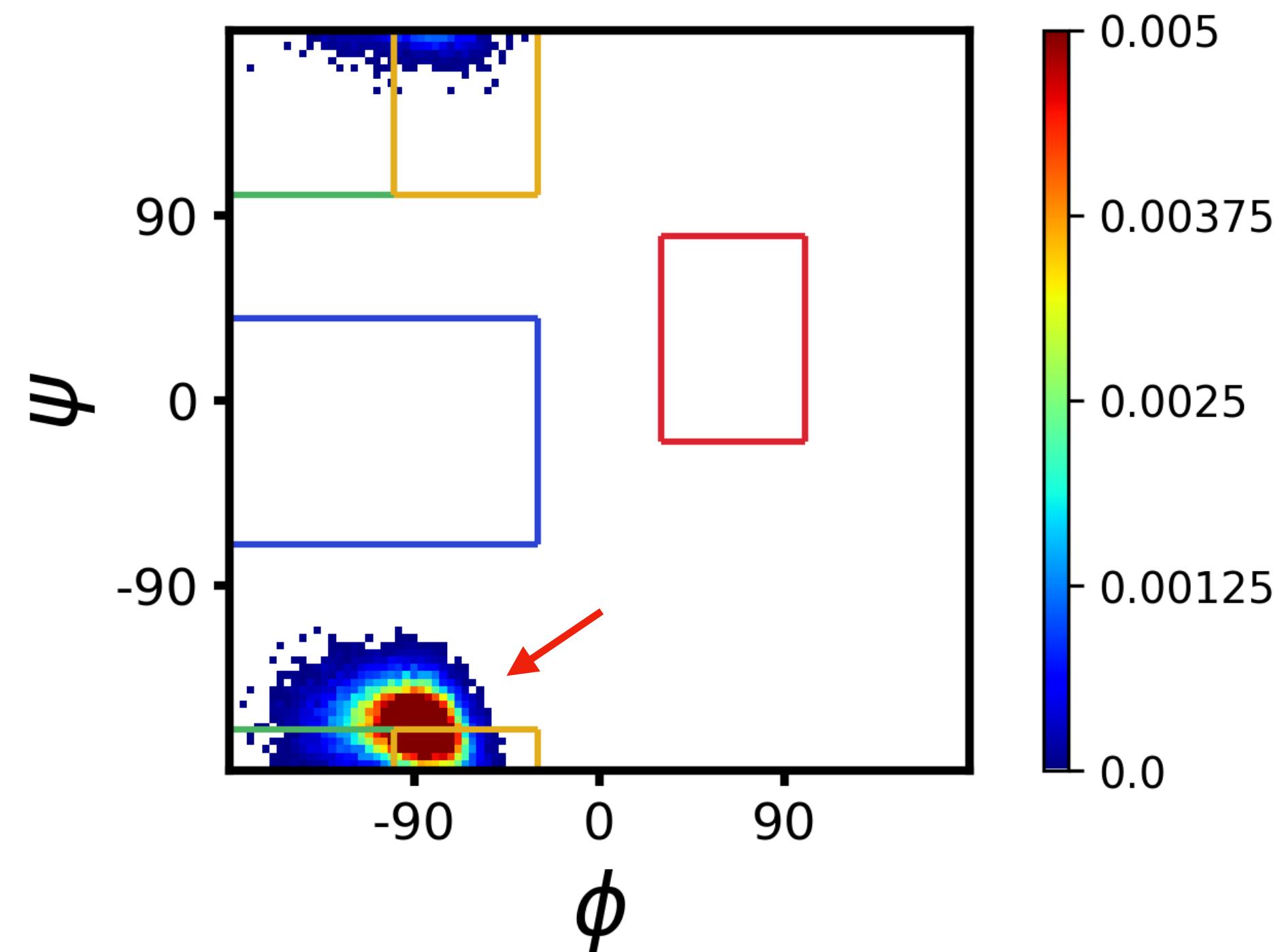
Conformer definition of D-aa's



AA	P_{II}	β	α_R	α_L
V	0.80	0.09	0.19	0.00
I	0.39	0.26	0.20	0.00
F	0.45	0.33	0.26	0.03
Y	0.40	0.34	0.21	0.03
C	0.48	0.31	0.18	0.03
H	0.39	0.36	0.20	0.06
T	0.39	0.28	0.38	0.00
R	0.38	0.31	0.16	0.04
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K	0.35	0.26	0.20	0.04
S	0.34	0.32	0.33	0.03
M	0.40	0.24	0.26	0.04
A	0.34	0.26	0.29	0.04
E	0.40	0.23	0.38	0.03
L	0.40	0.30	0.26	0.00
N	0.29	0.28	0.38	0.00
D	0.29	0.09	0.43	0.05
G	0.24	0.09	0.09	0.13
P	0.80	0.09	0.09	0.00

Linker sequence selection is informed by aa's intrinsic propensity

ϕ/ψ distribution of G₈ from cyclo-(SESEG4)



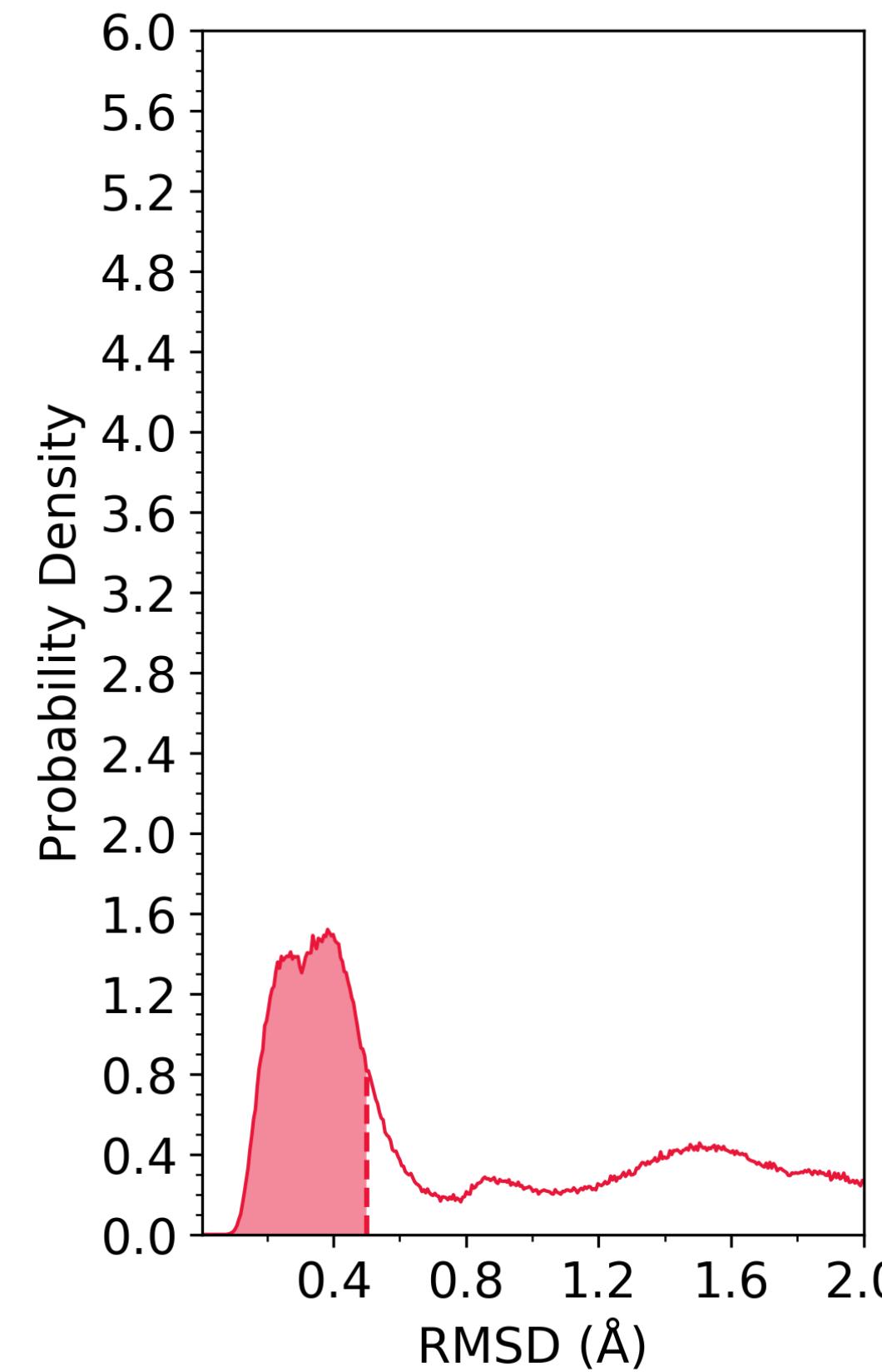
Linker sequence selection is informed by aa's intrinsic propensity

- The same method was used to find linker sequences for cyclic nonamer.
- 52 sequences were proposed in total.
- Molecular dynamics simulations were carried out.
- The results were analyzed.

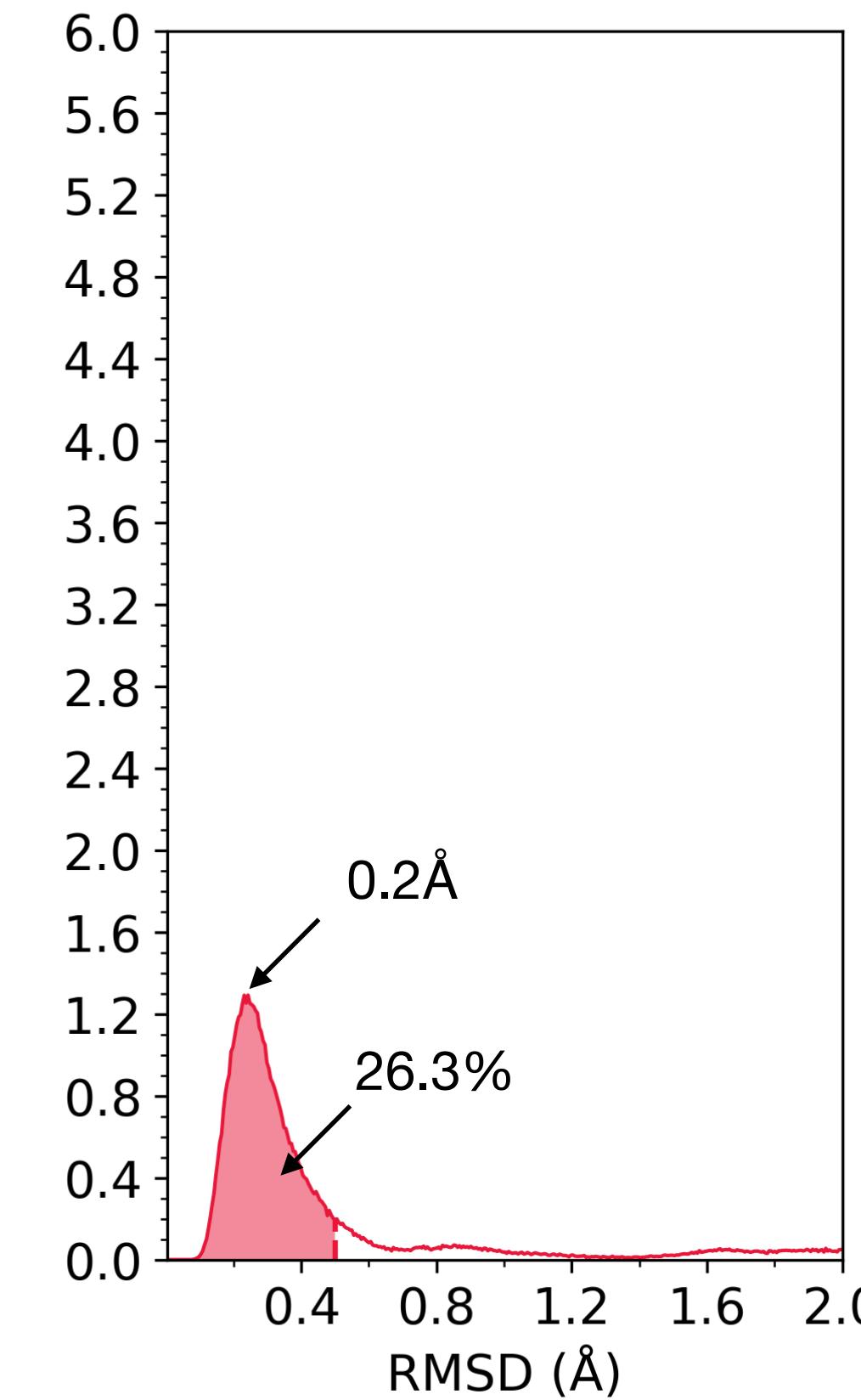
cyclo-(SESEG~~GGG~~G) as the starting point

cyclo-(SESEG~~GGG~~G)

all conformations



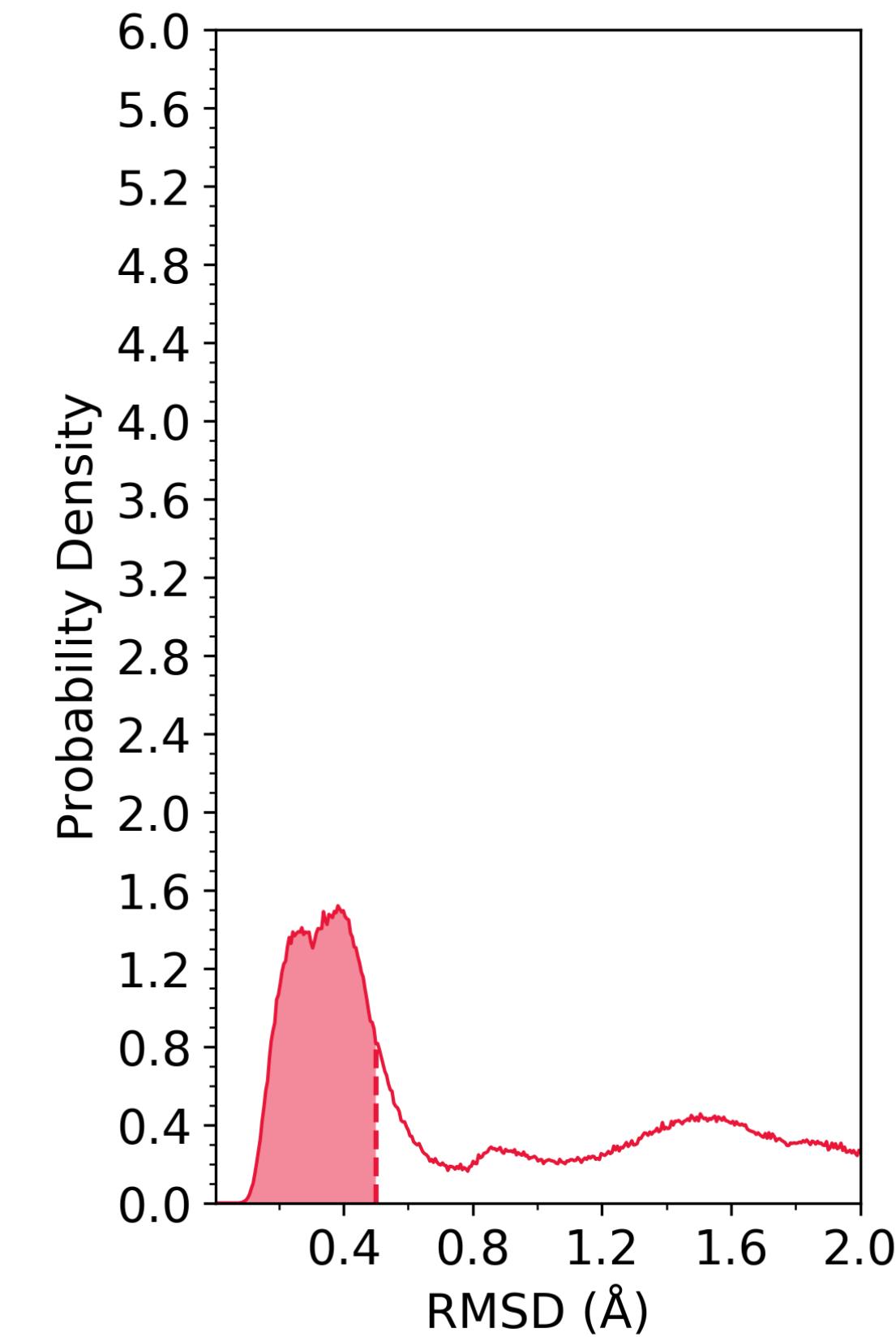
conformations without clash



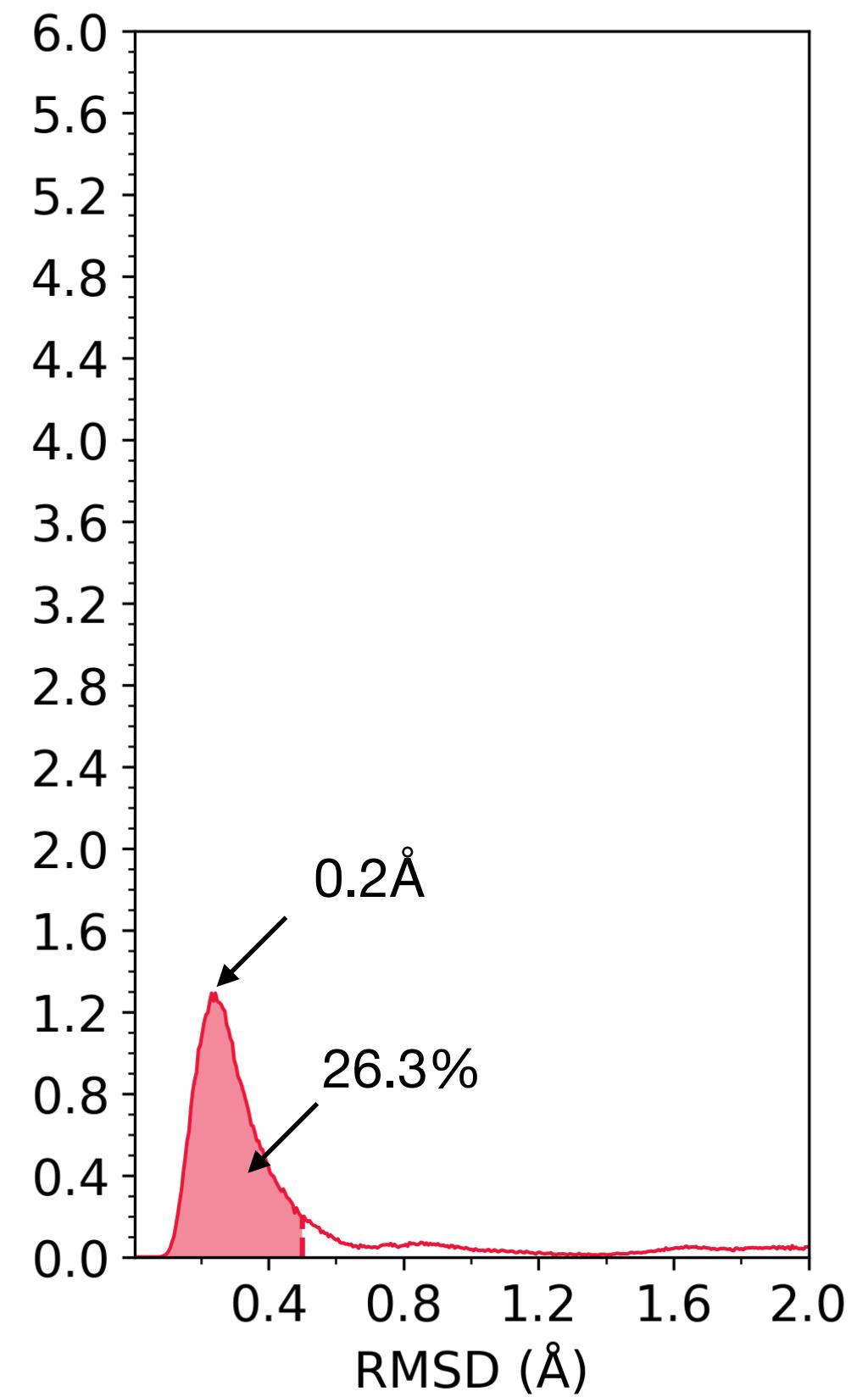
Cyclo-(SESEavTG) greatly stabilizes target conformation

cyclo-(SESEG_nGGG)

all structures

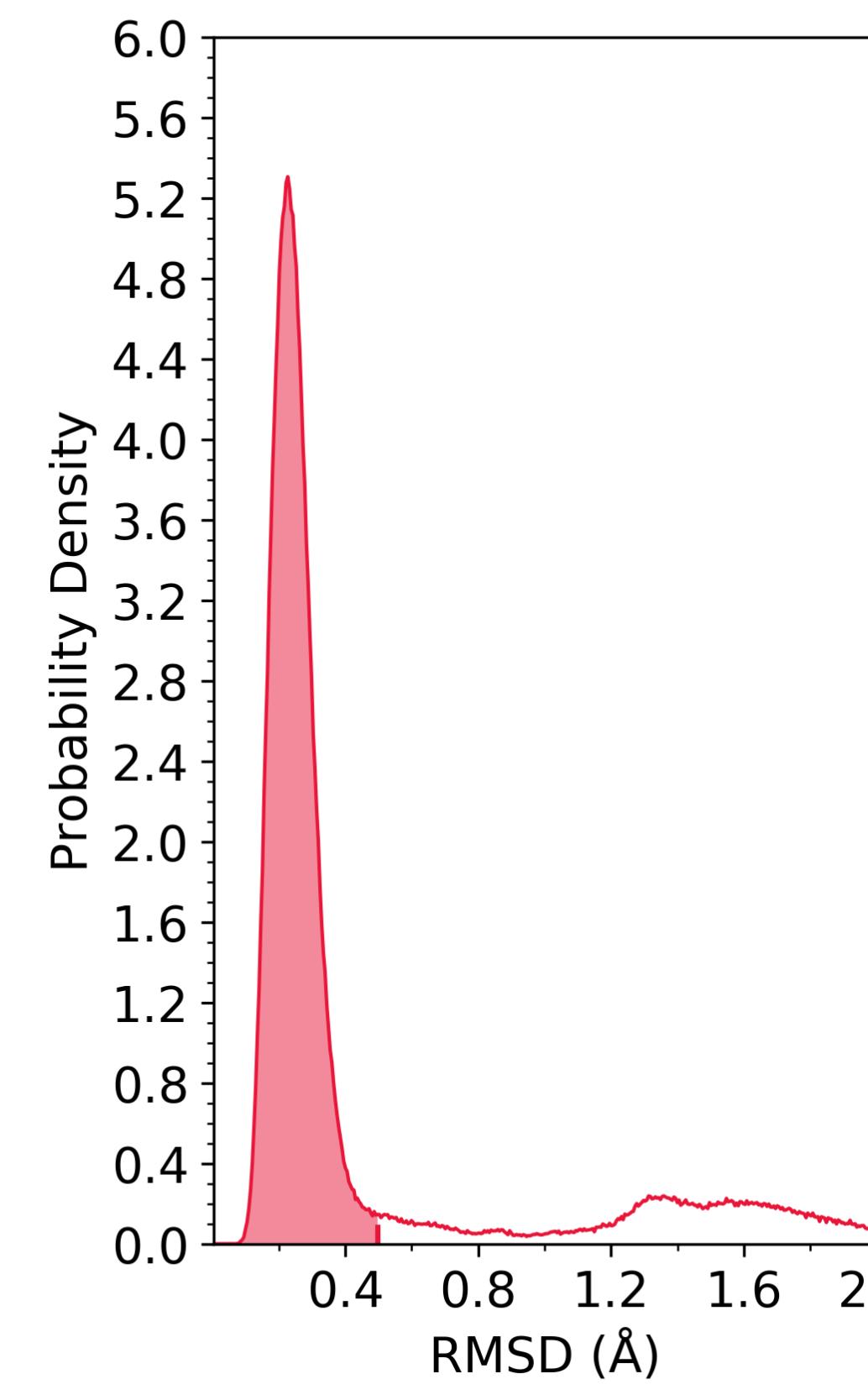


structures without clash



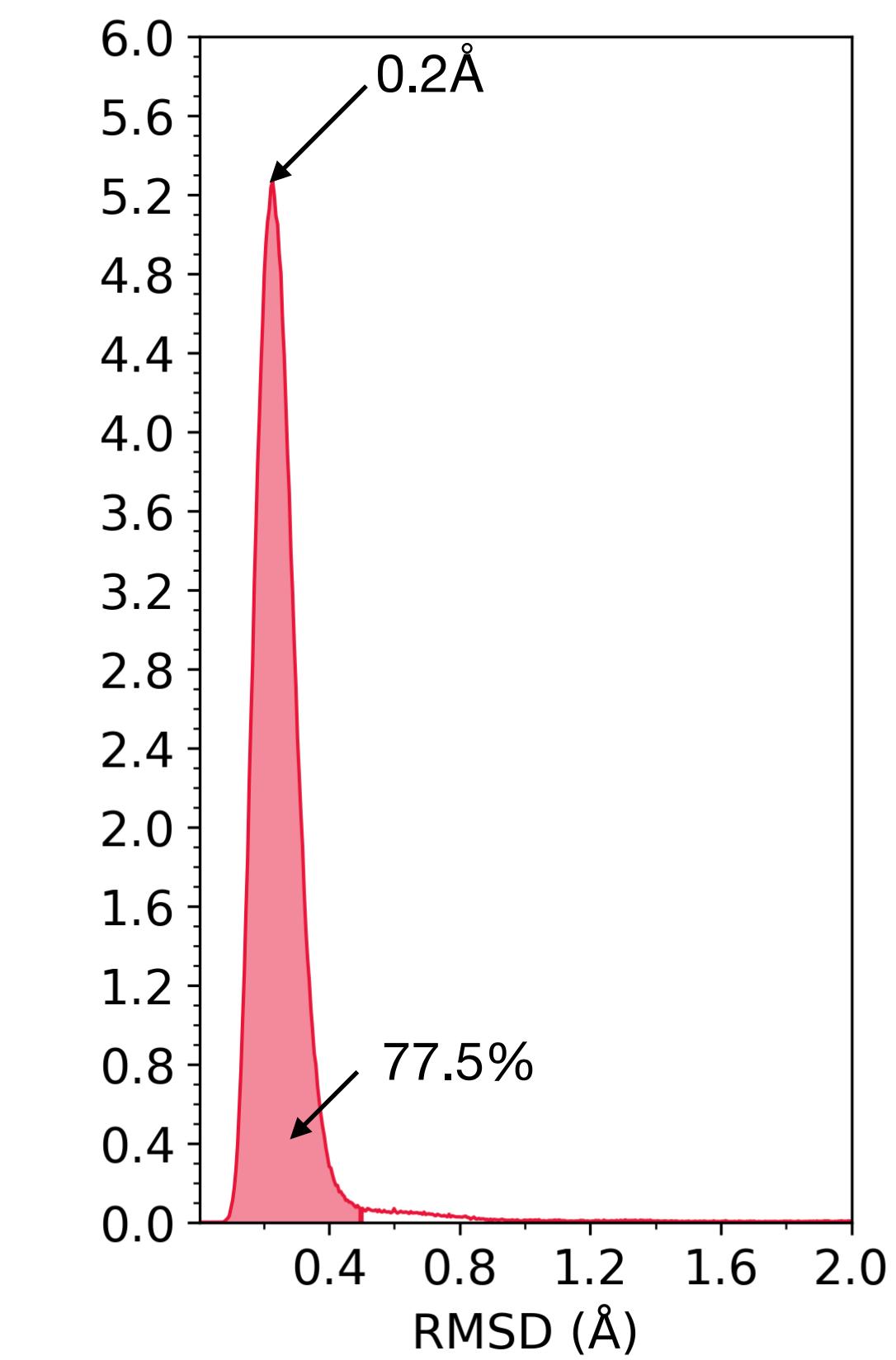
$\sim 3\times$

all structures



cyclo-(SESEavTG)

structures without clash



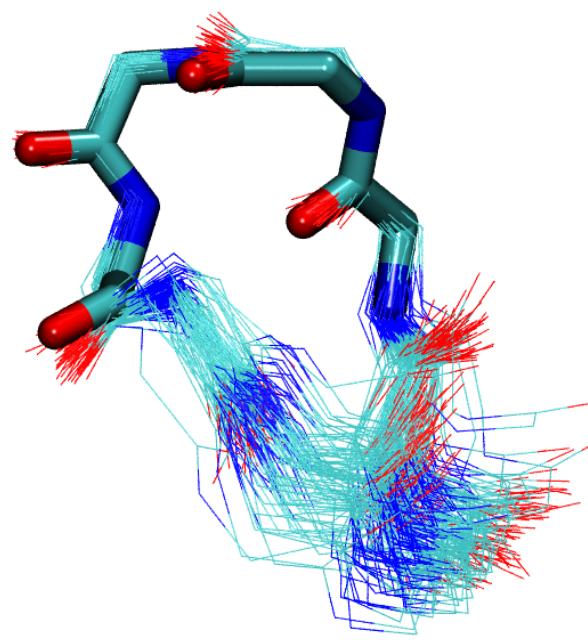
Background

Project Description

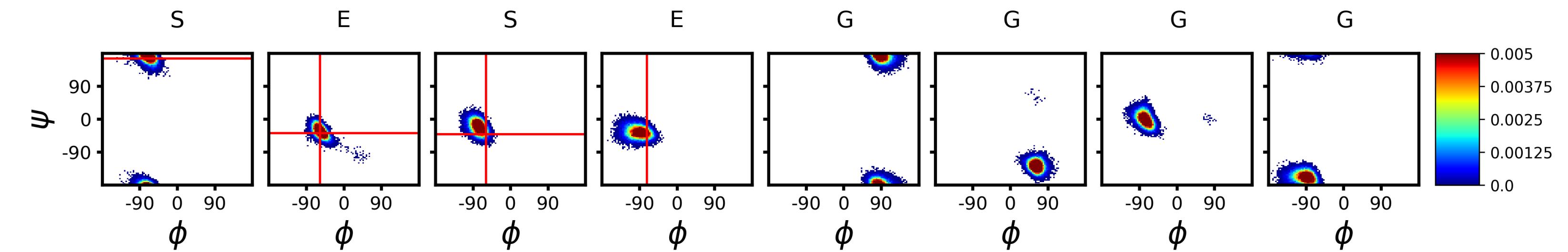
Results

Conclusion

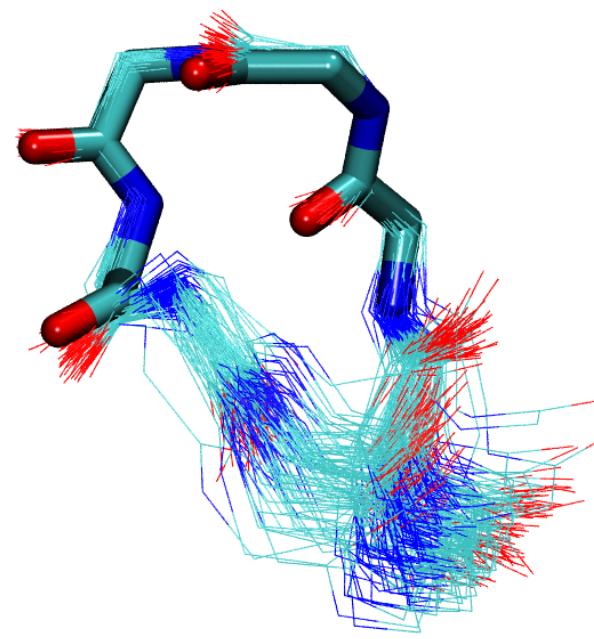
cyclo-(SESEGCGG) as the starting point



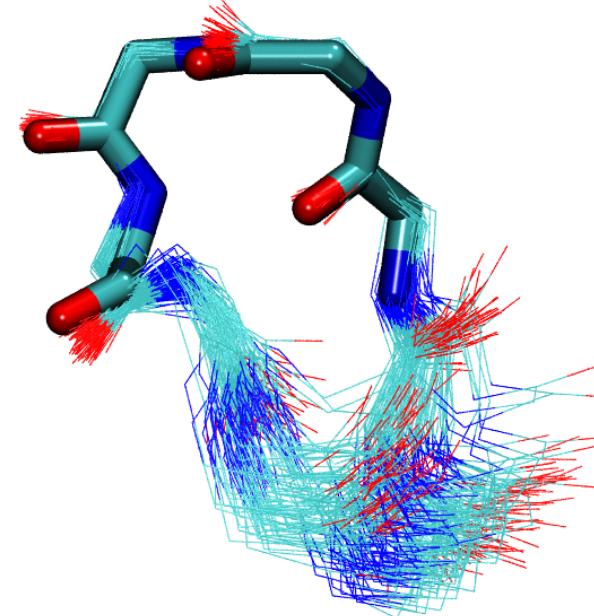
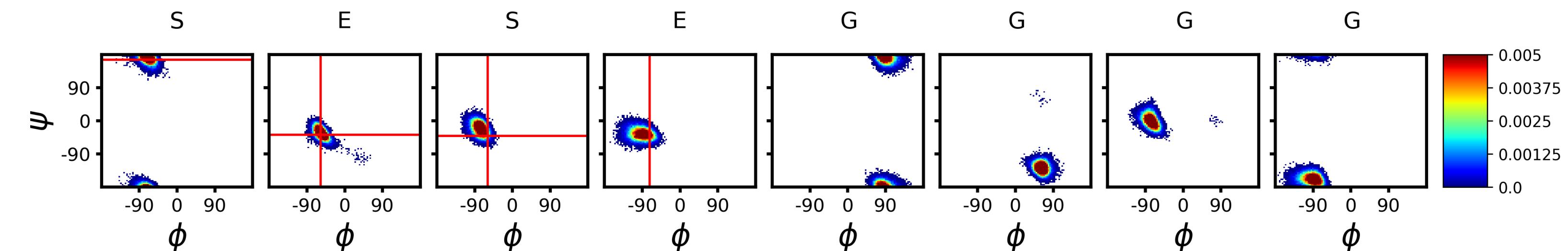
Population: 7.5%
RMSD(Å): 0.244 ± 0.062



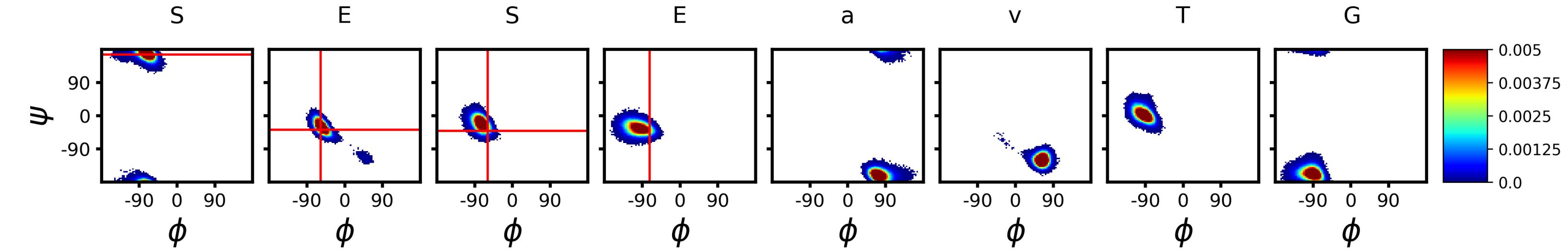
Cyclo-(SESEavTG) greatly stabilizes target conformation



Population: 7.5%
RMSD(Å): 0.244 ± 0.062



Population: 78.4%
RMSD(Å): 0.245 ± 0.056



↓ >10x

Background

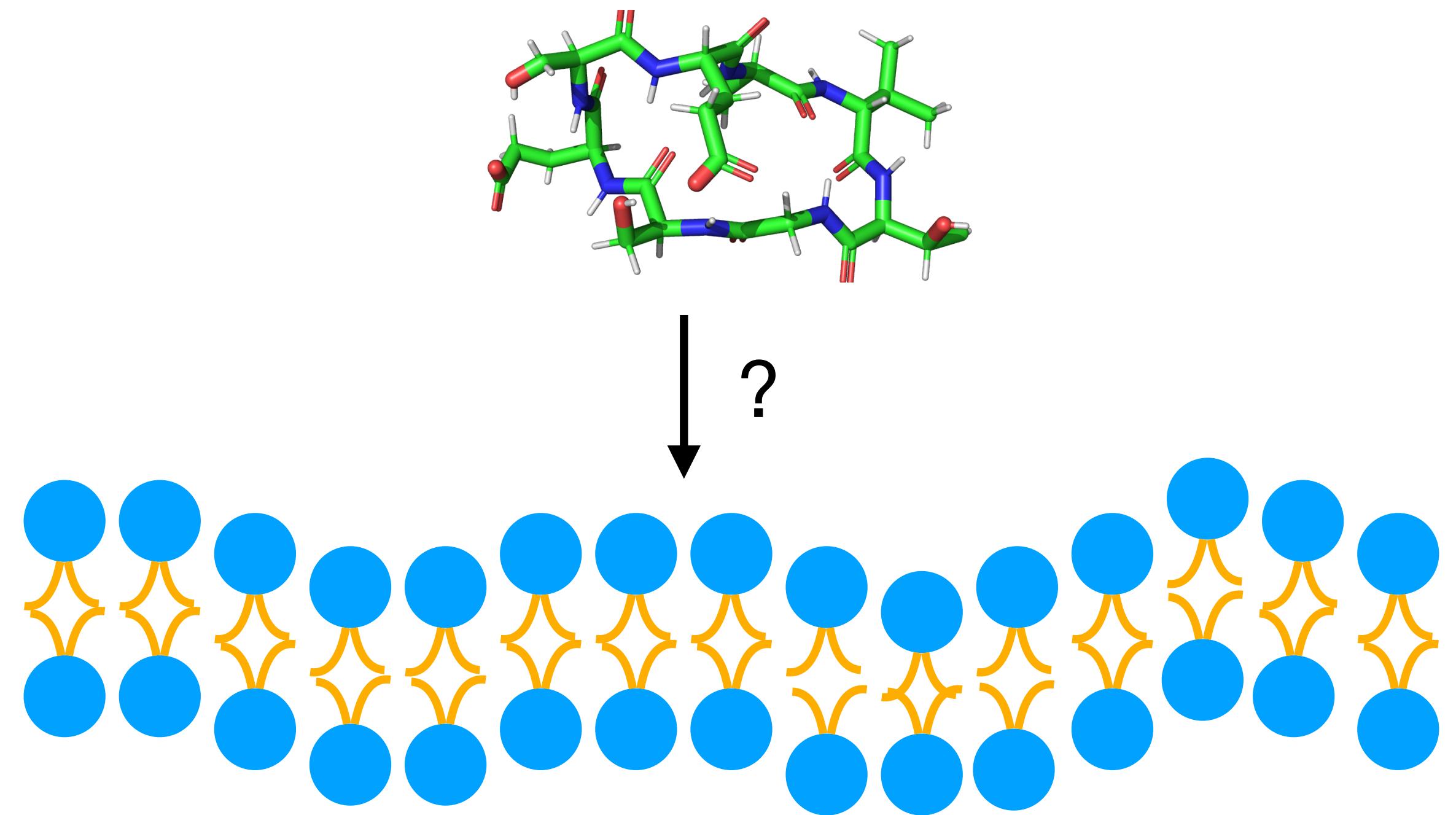
Project Description

Results

Conclusion

Intracellular delivery requires high membrane permeability

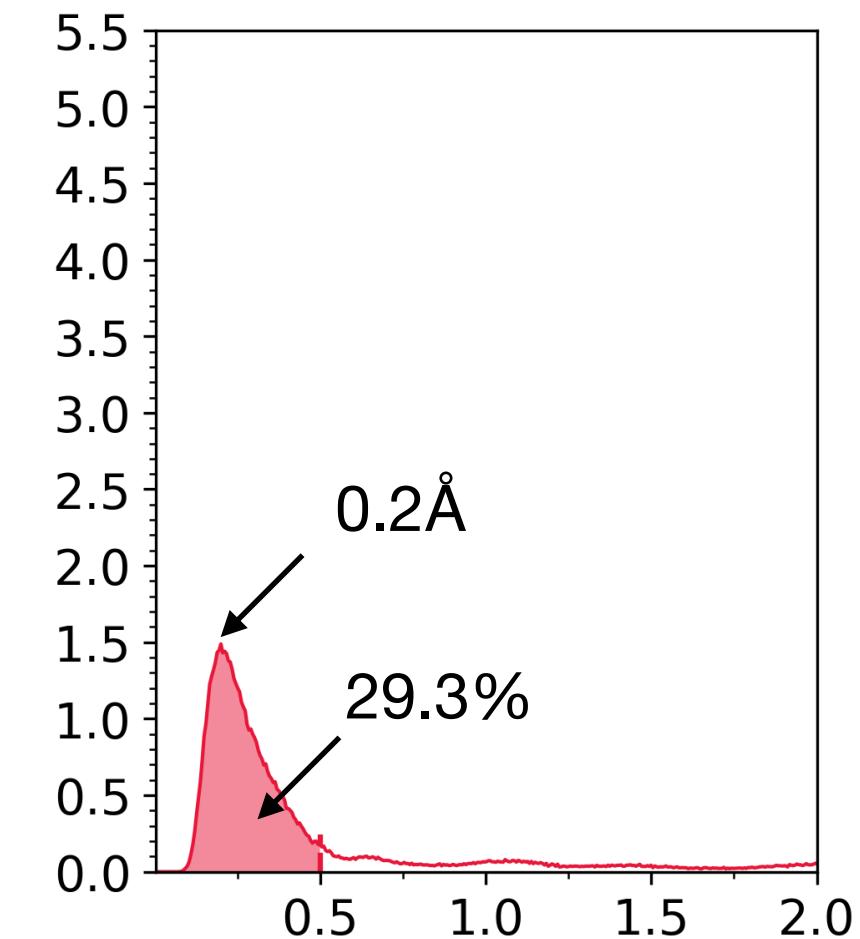
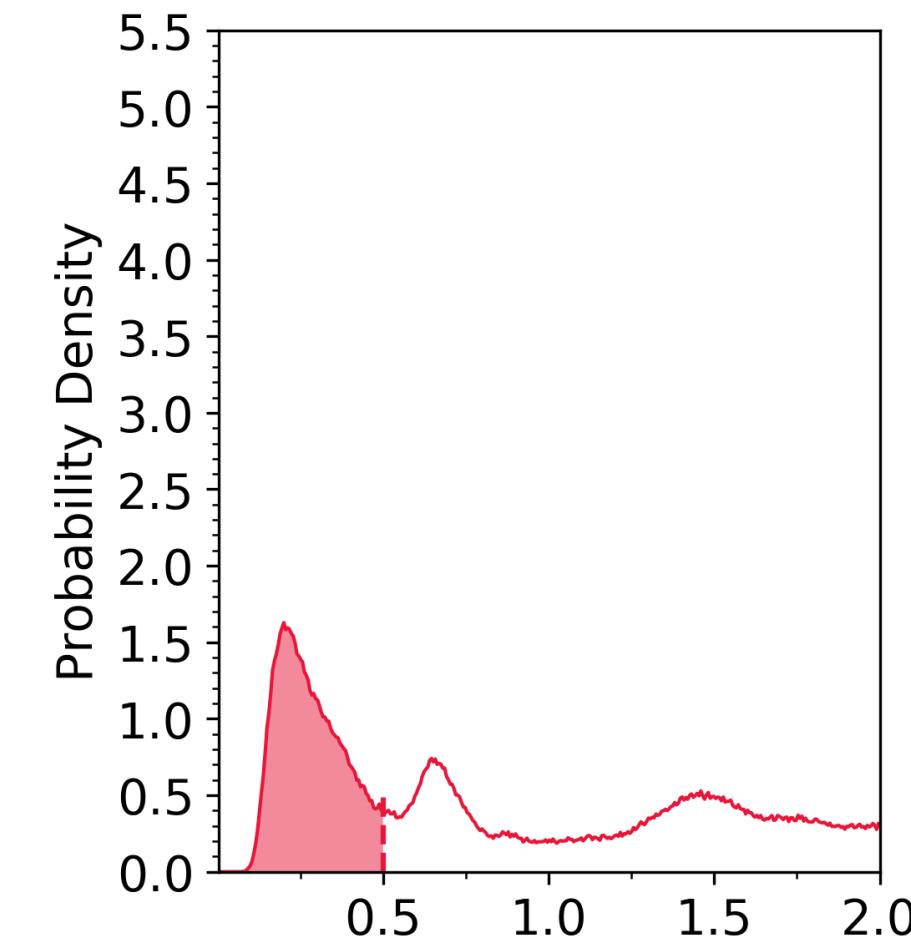
- Cks1–Skp2 interaction takes place intracellularly.
- cyclo-(SESEavTG) might not be “greasy” enough.



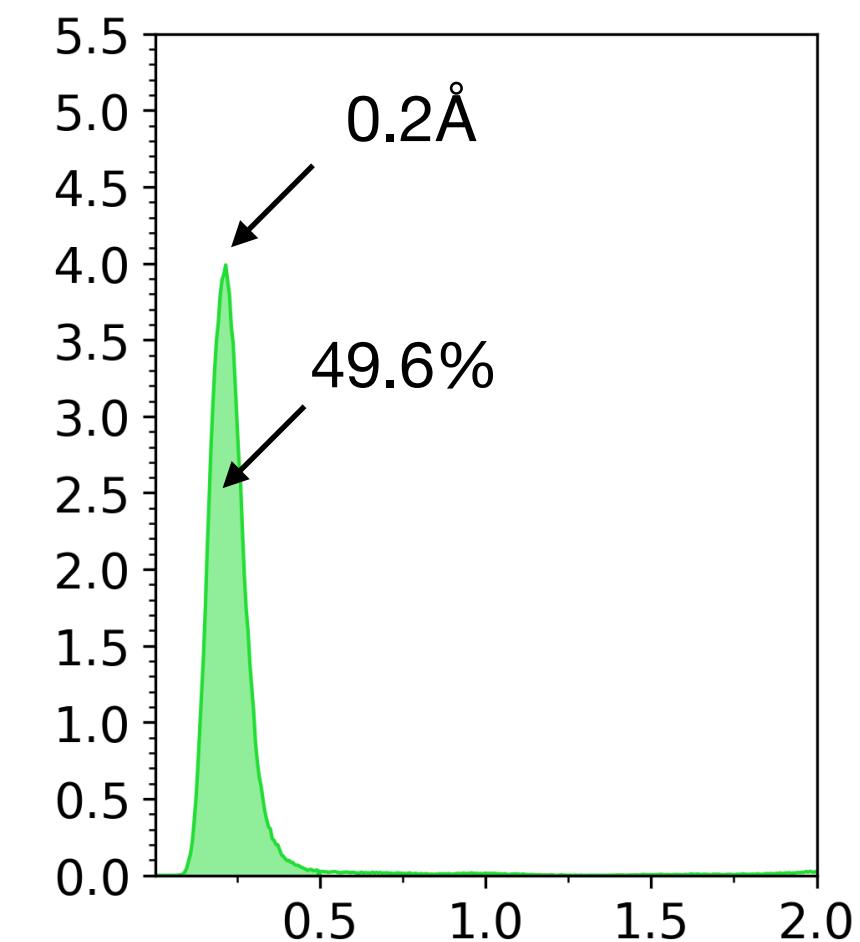
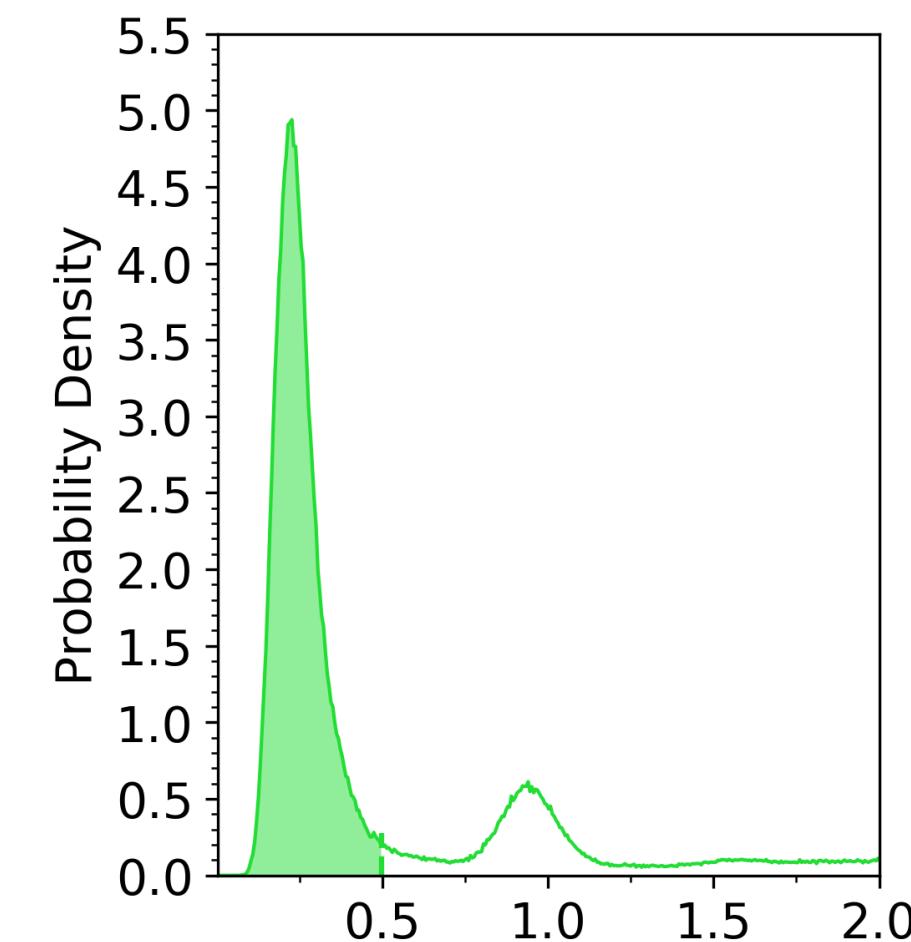
Cyclo-(SESEGvvTG) was picked for higher hydrophobicity

- Intracellular inhibition requires high membrane permeability.
- cyclo-(SESEGvvTG) has the greatest hydrophobicity among the top performers.

cyclo-(SESEGvvTG)

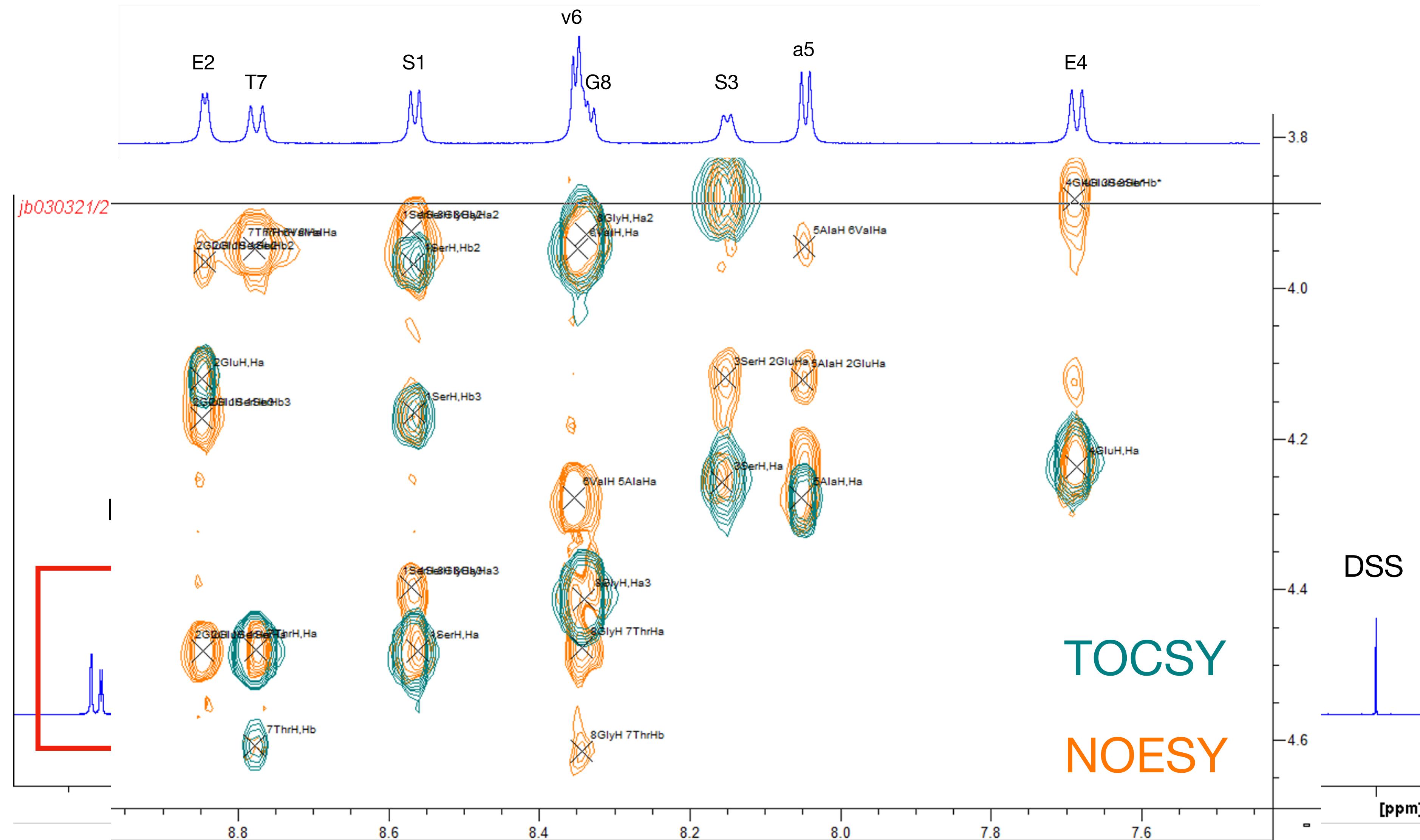


cyclo-(SESEGvvTG)



Does experimental results support predictions?

NMR identifies dominant structure for cyclo-(SESEavTG)



Background

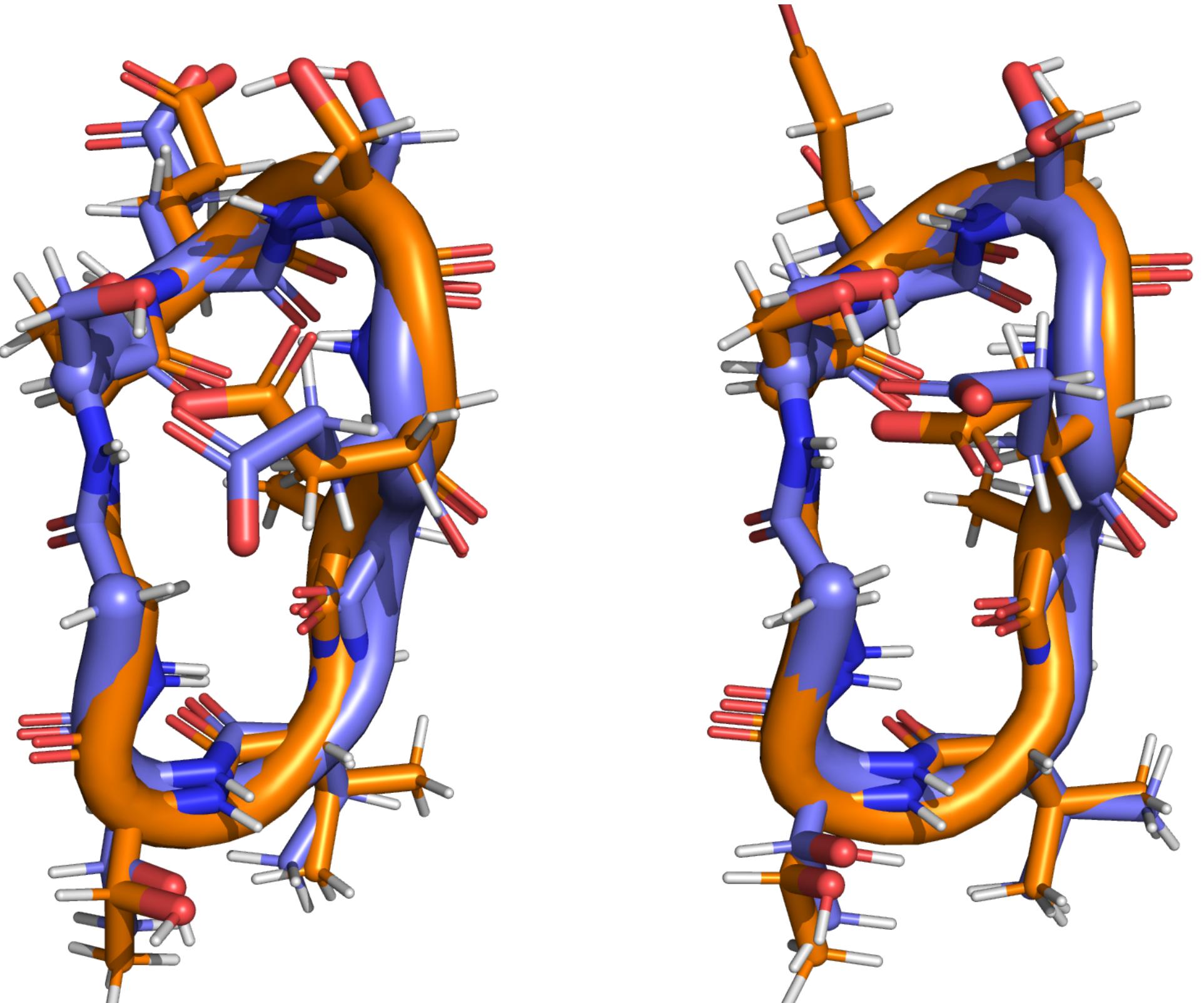
Project Description

Results

Conclusion

NMR confirms structural prediction for cyclo-(SESEavTG)

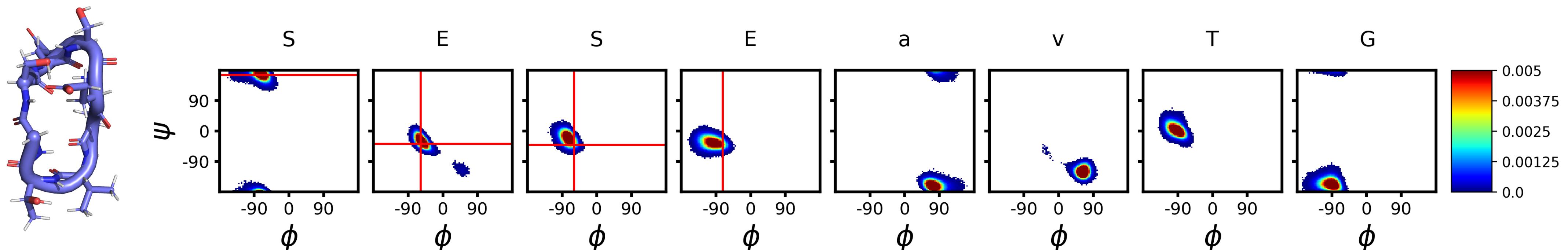
- Structures are built using simulated annealing while applying the restraints obtained from NMR.
- Predicted structures show structural similarity to the constructed ones.



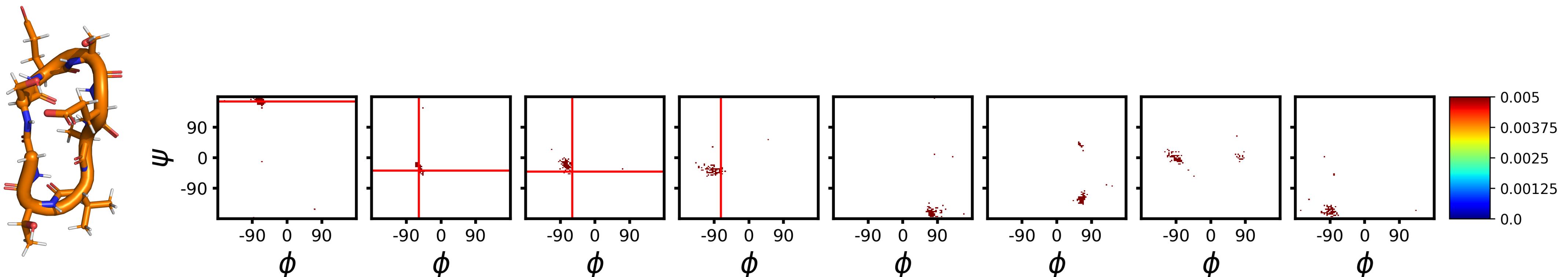
Predicted Structure

NMR confirms structural prediction for cyclo-(SESEavTG)

Predicted Structure



Constructed Structure



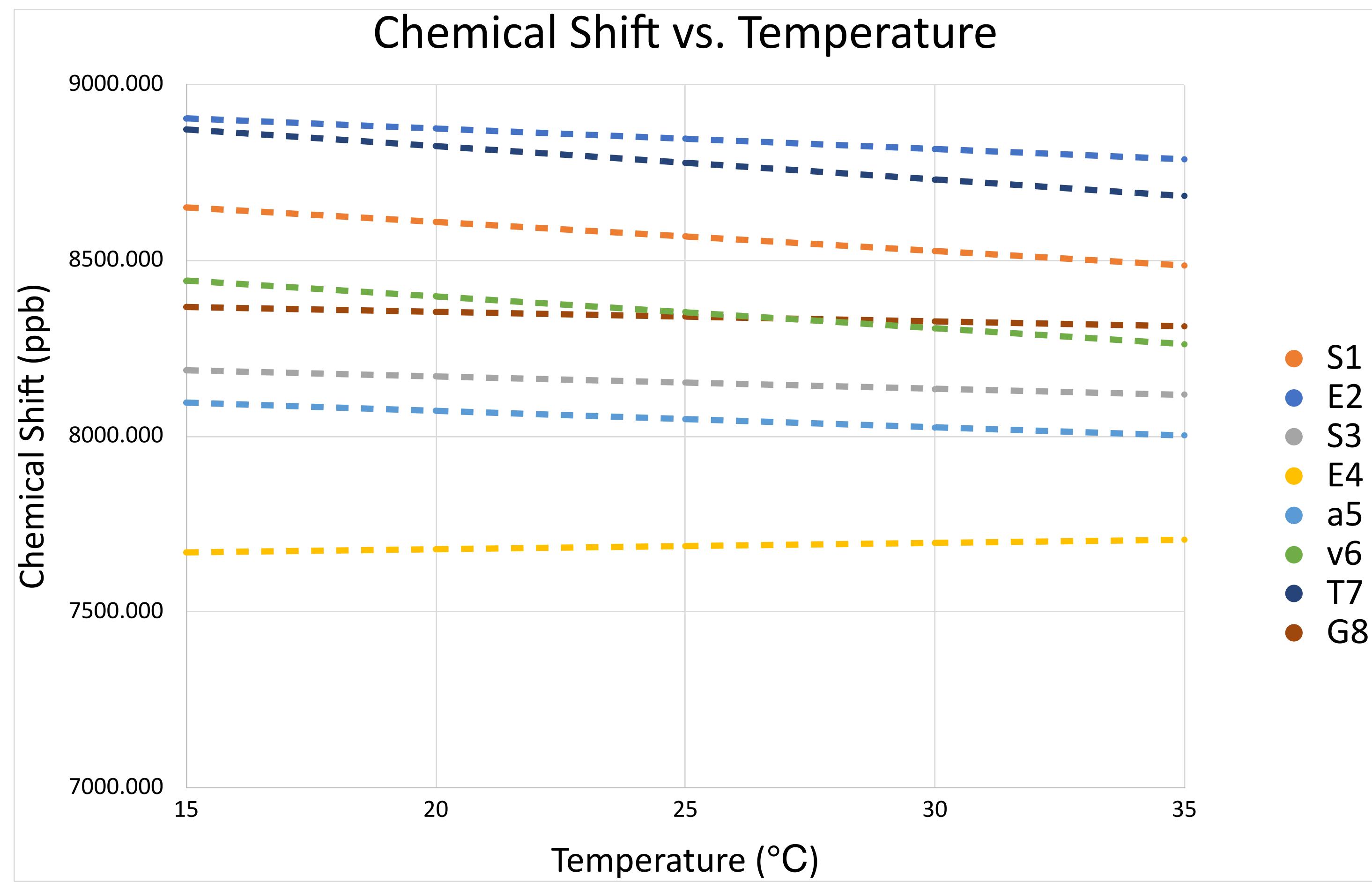
Background

Project Description

Results

Conclusion

NMR reveals hydrogen bond network in cyclo-(SESEavTG)

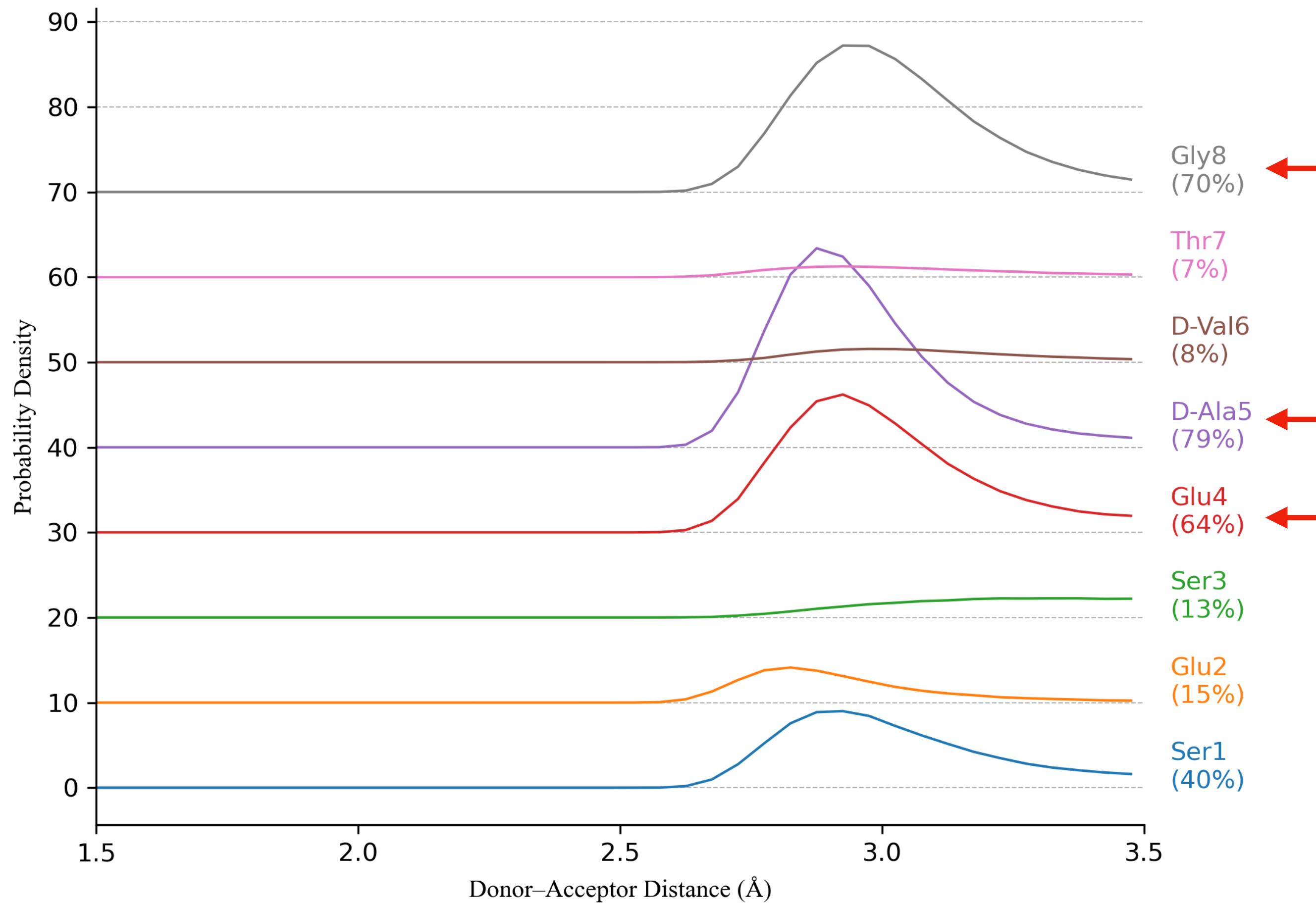


Residue	Slope (slope $^{\circ}\text{C}$)	ppb/ $^{\circ}\text{C}$	H-bond*
Glu4	1.8		Yes
Glu2	-2.7	-5.8	Yes
Ser3	-3.5		Yes
D-Ala54	-4.7	1.8	No
Glu2	-5.8		No
Ser1/Val6	-8.3	-9	No
D-Val6	-9		No
The7/Gly8	-9.5	-2.7	No

*using -4.5 ppb/ $^{\circ}\text{C}$ as cutoff

Baxter, N. J. & Williamson, M. P., *J. Biomol. NMR* **9**, 359-369, (1997)

Simulation prediction of hydrogen bond network aligns with experimental data



Residue	Slope (ppb/°C)	H-bond	Predicted H-bond*
Glu4	1.8	Yes	Yes
Gly8	-2.7	Yes	Yes
Ser3	-3.5	Yes	No
D-Ala5	-4.7	No	Yes
Glu2	-5.8	No	No
Ser1	-8.3	No	No
D-Val6	-9	No	No
Thr7	-9.5	No	No

*using 50% as the cutoff

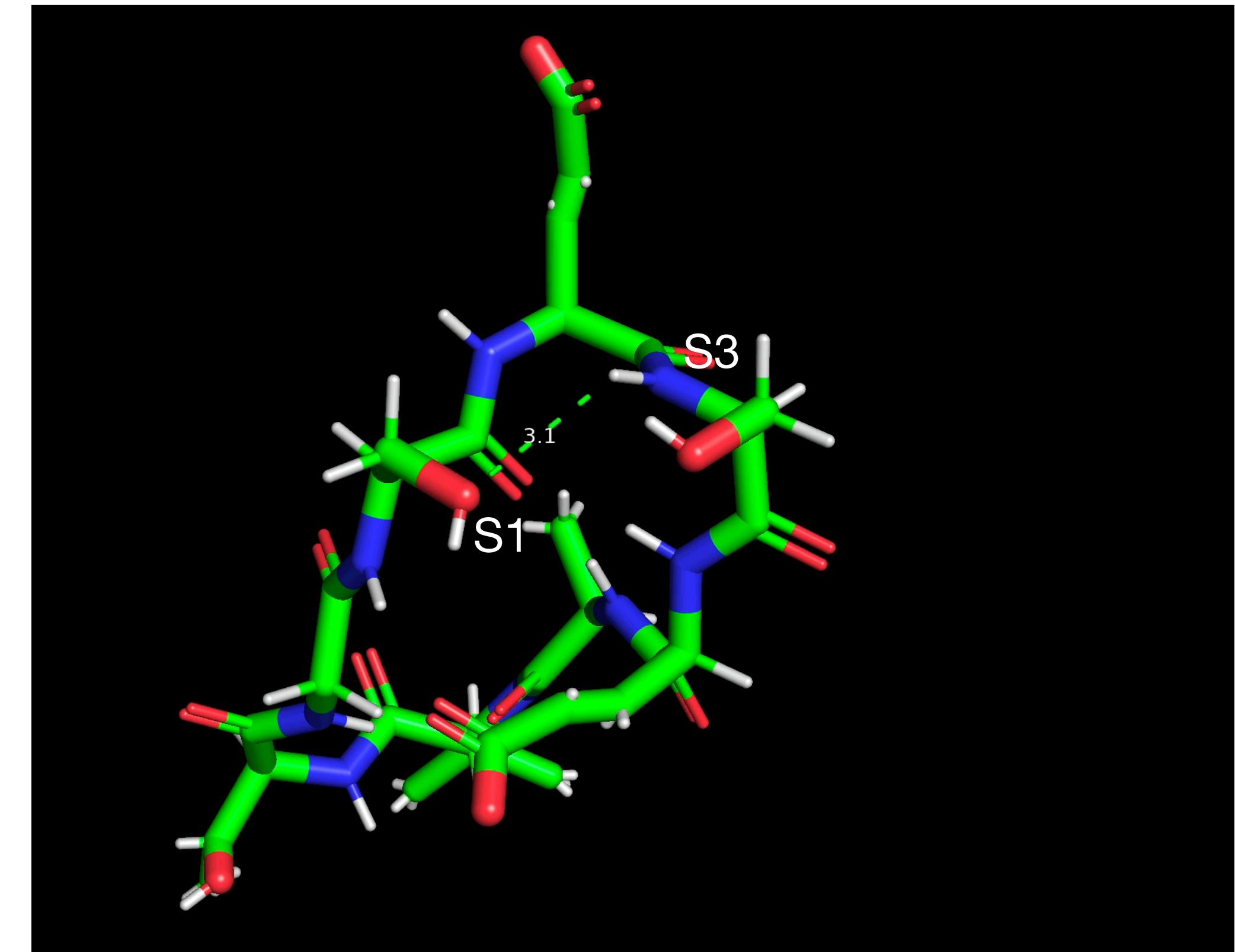
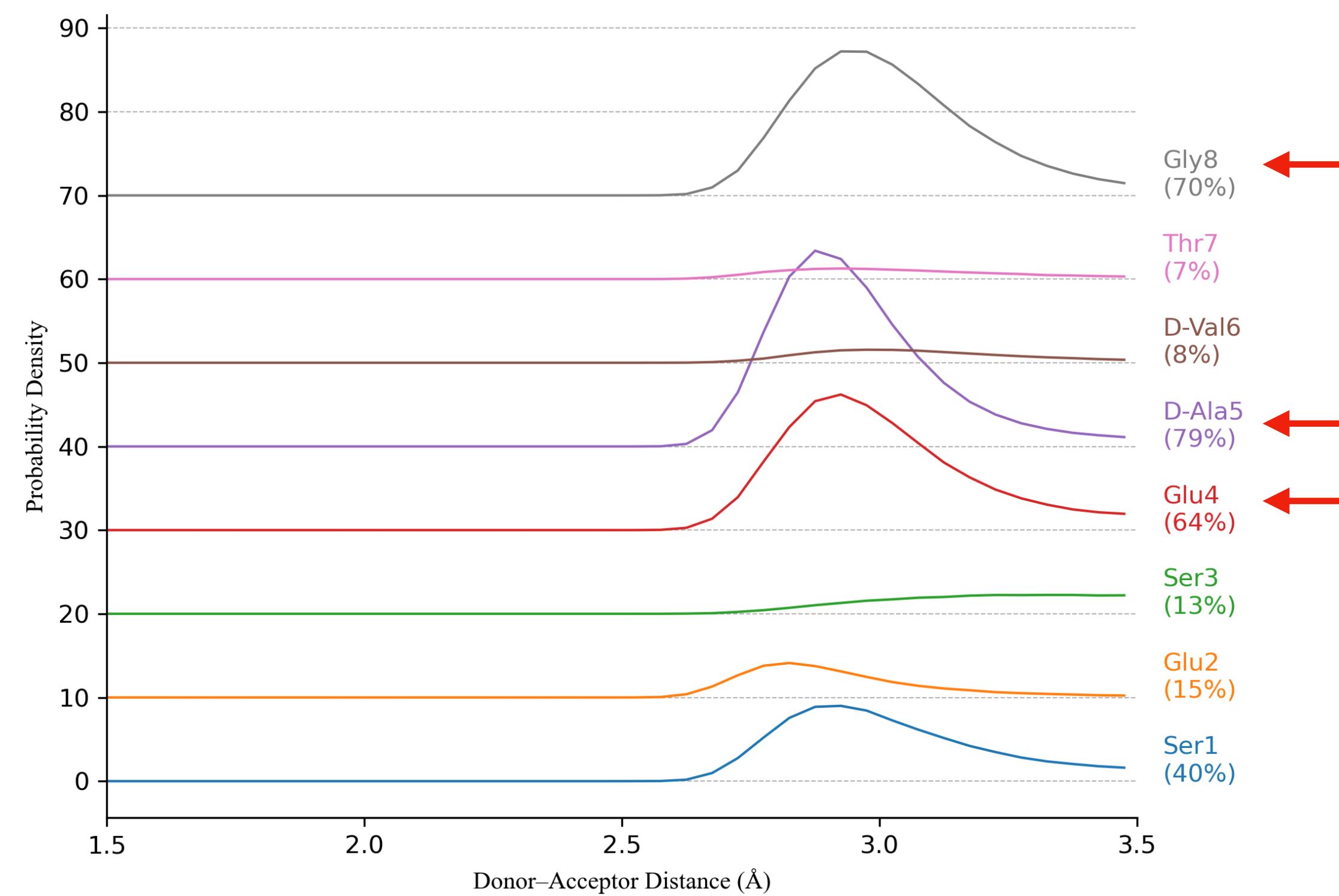
Background

Project Description

Results

Conclusion

Simulation prediction of hydrogen bond network aligns with experimental data



Background

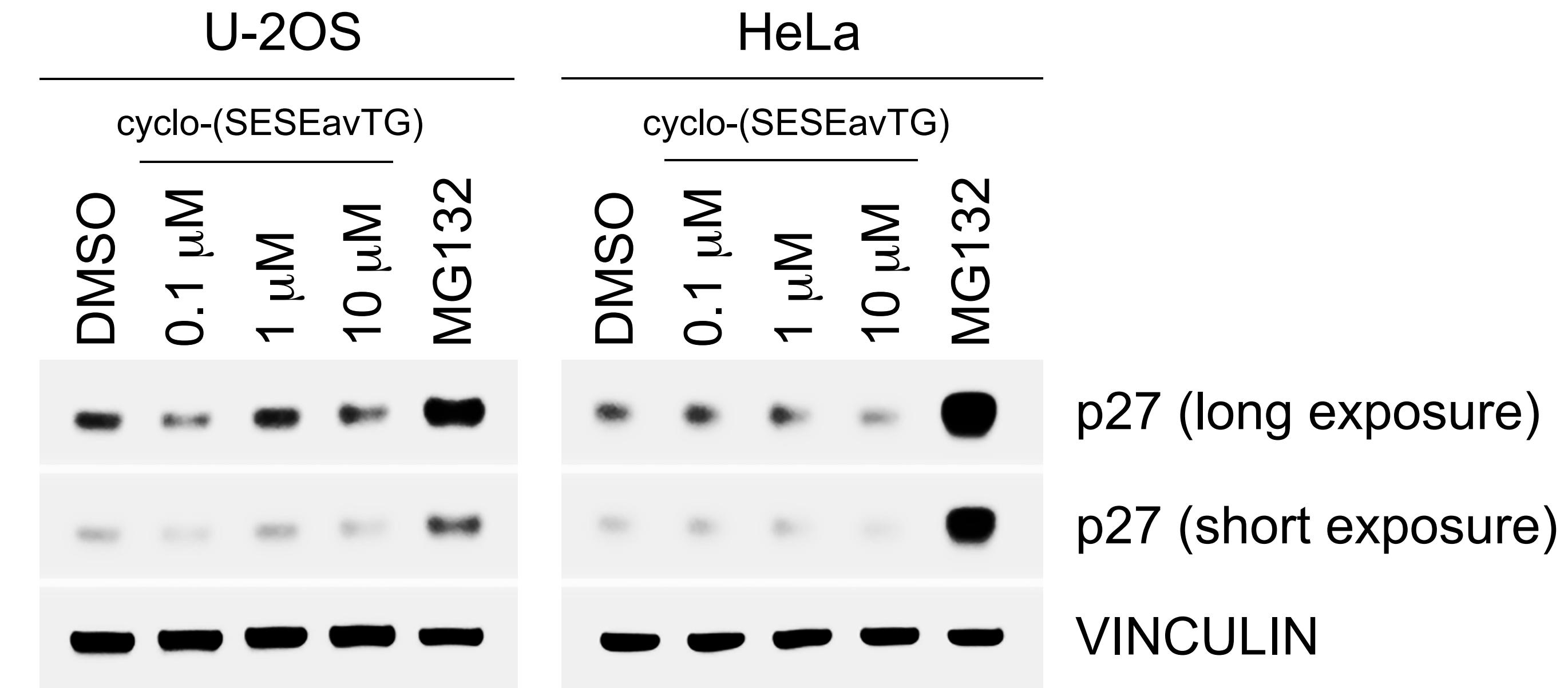
Project Description

Results

Conclusion

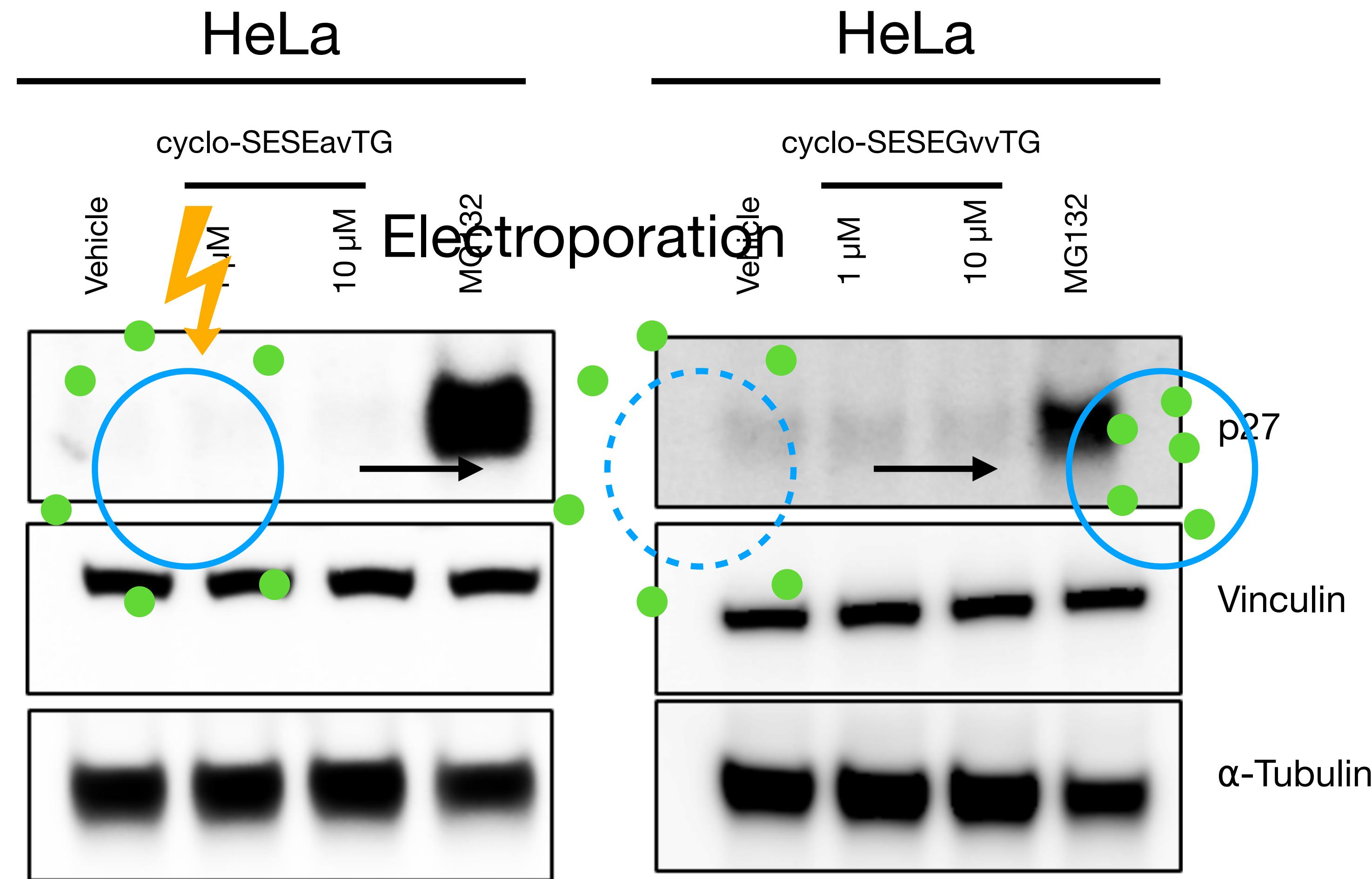
In-vivo experiments indicate low inhibitory efficacy

- U2OS and HeLa cells were treated with cyclo-(SESEavTG) at different concentrations.
- Little to no inhibitory effect was observed.



In-vivo experiments indicate low inhibitory efficacy

- The cyclic peptides were introduced to the HeLa cells using electroporation.
- Little to no inhibitory effect was observed.



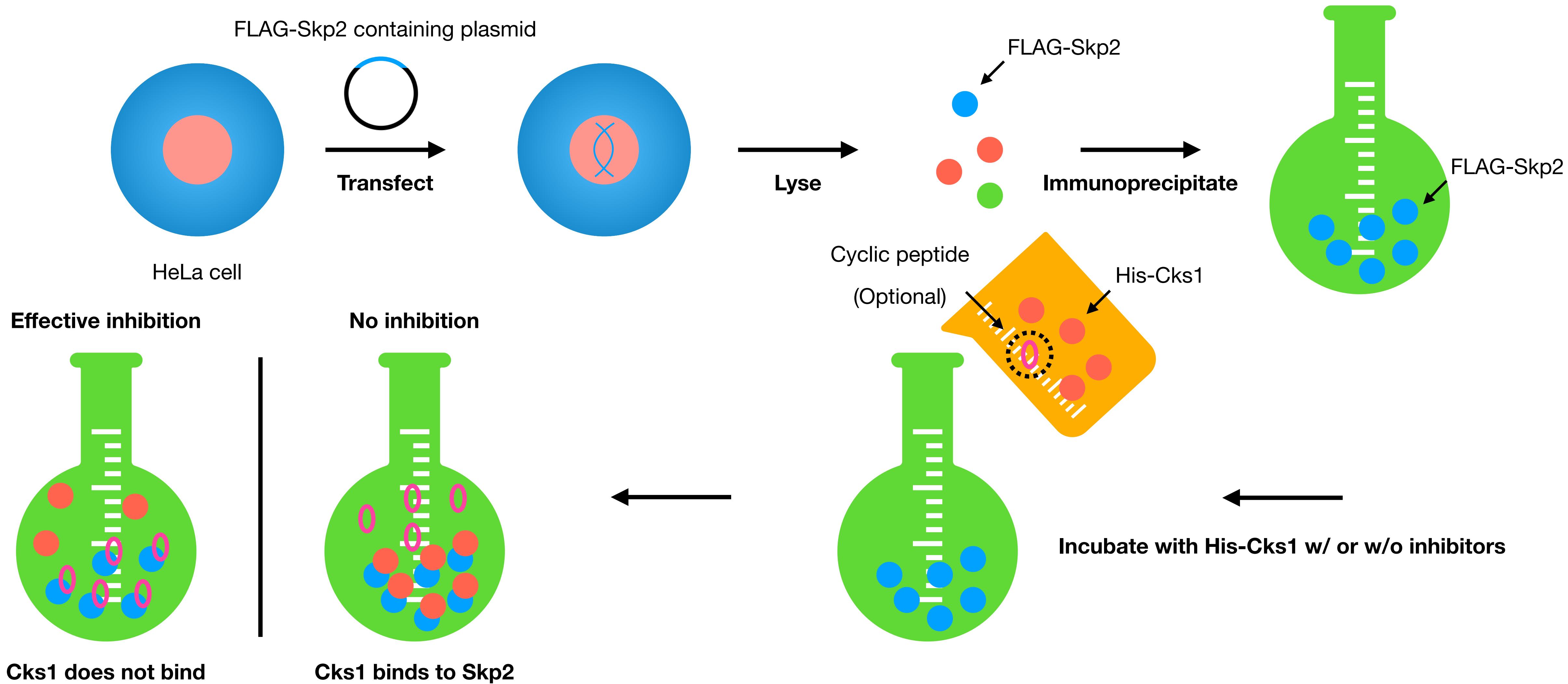
Background

Project Description

Results

Conclusion

In-vitro experiment setup



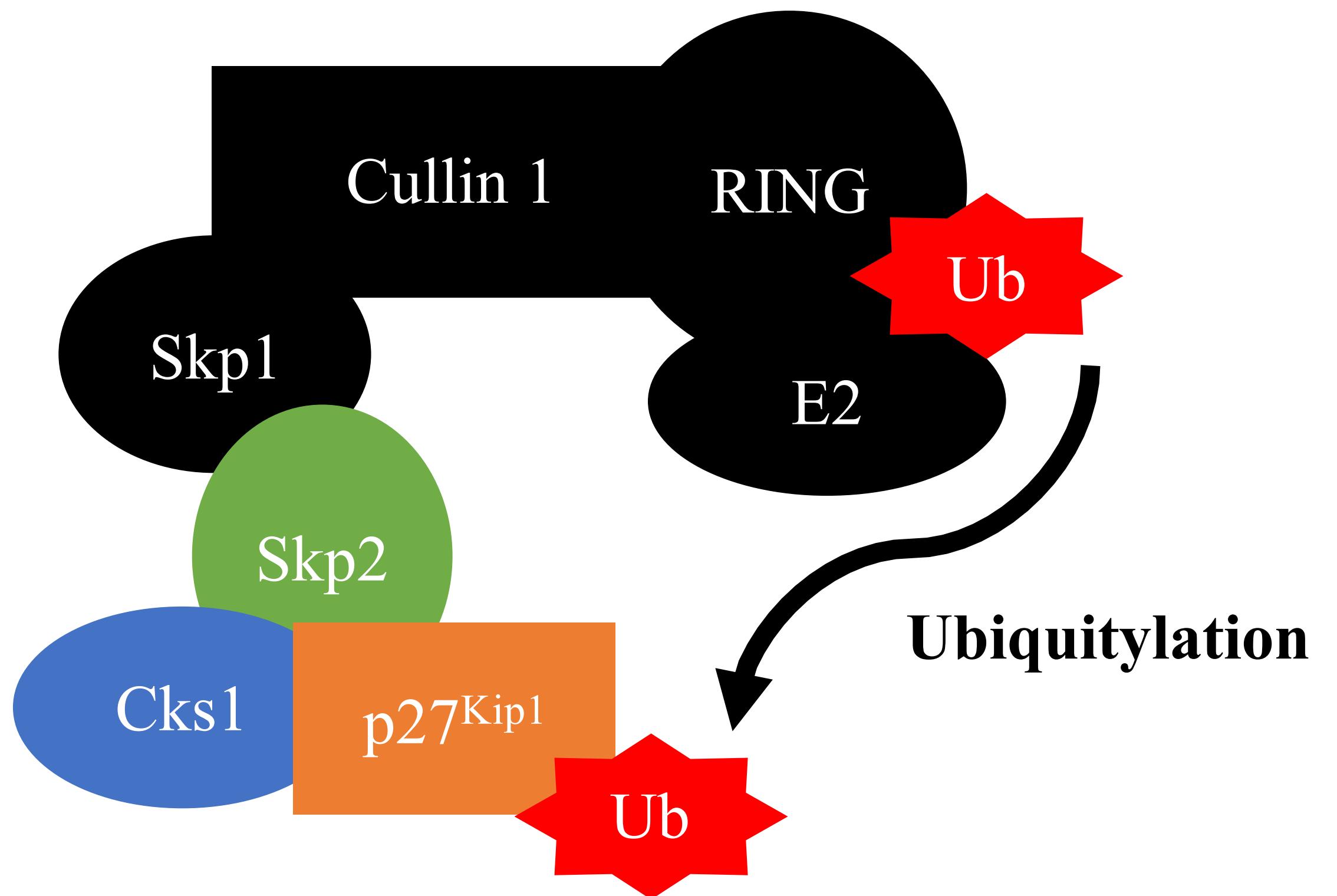
Background

Project Description

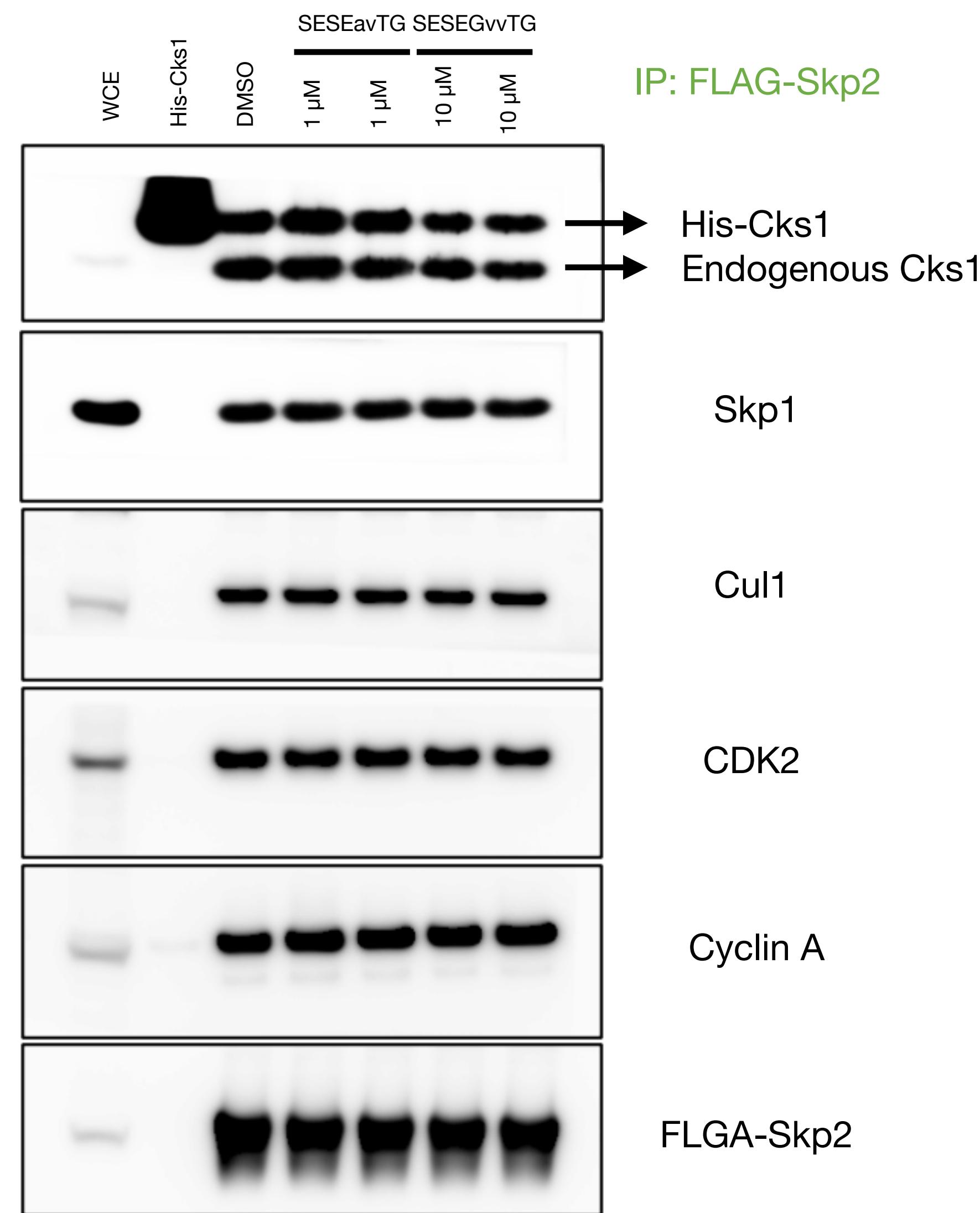
Results

Conclusion

In-vitro experiments indicate low inhibitory efficacy



Ji, P. et al., *J. Biol. Chem.* **281**, 24058-24069, (2006)



Background

Project Description

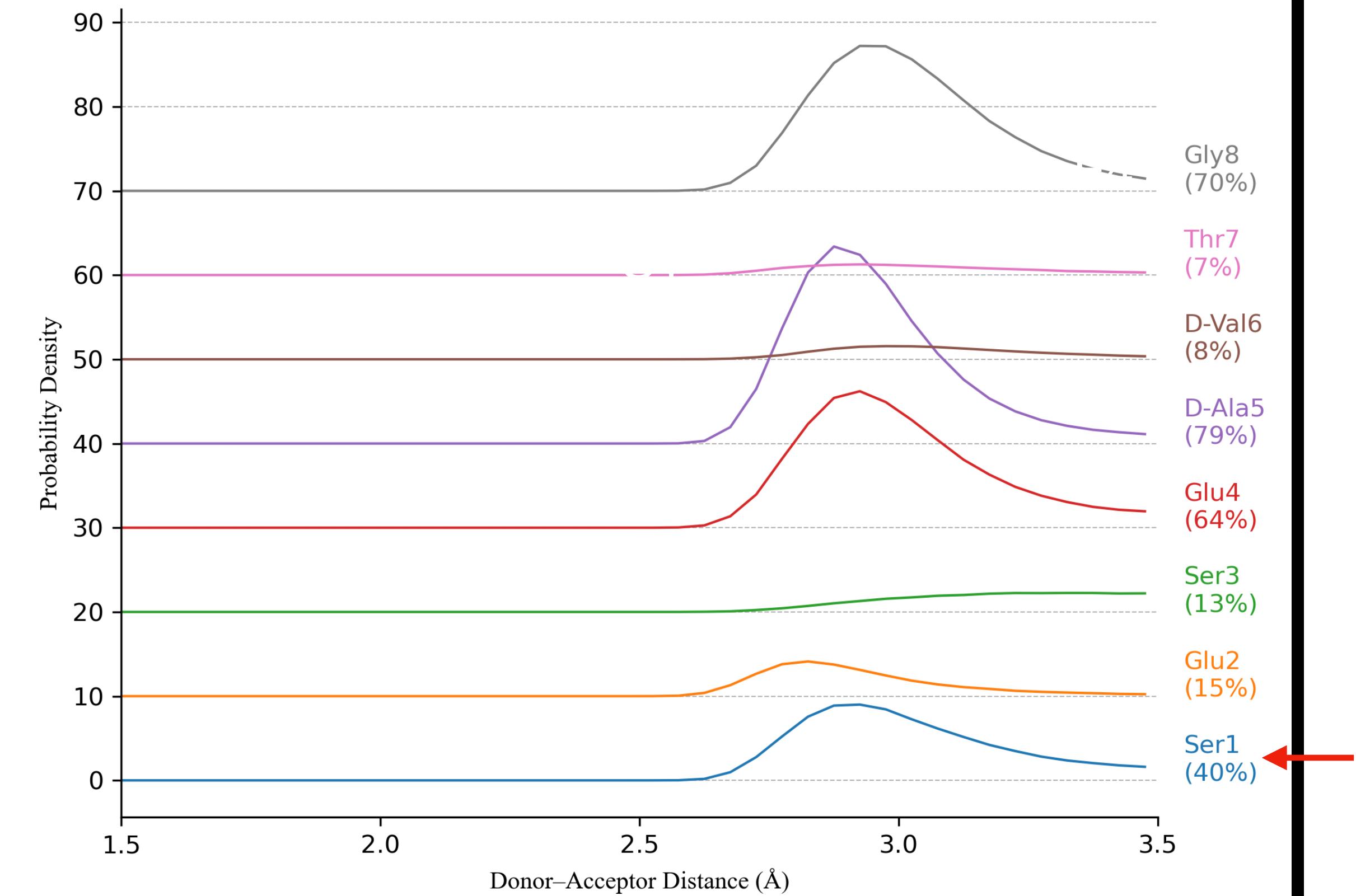
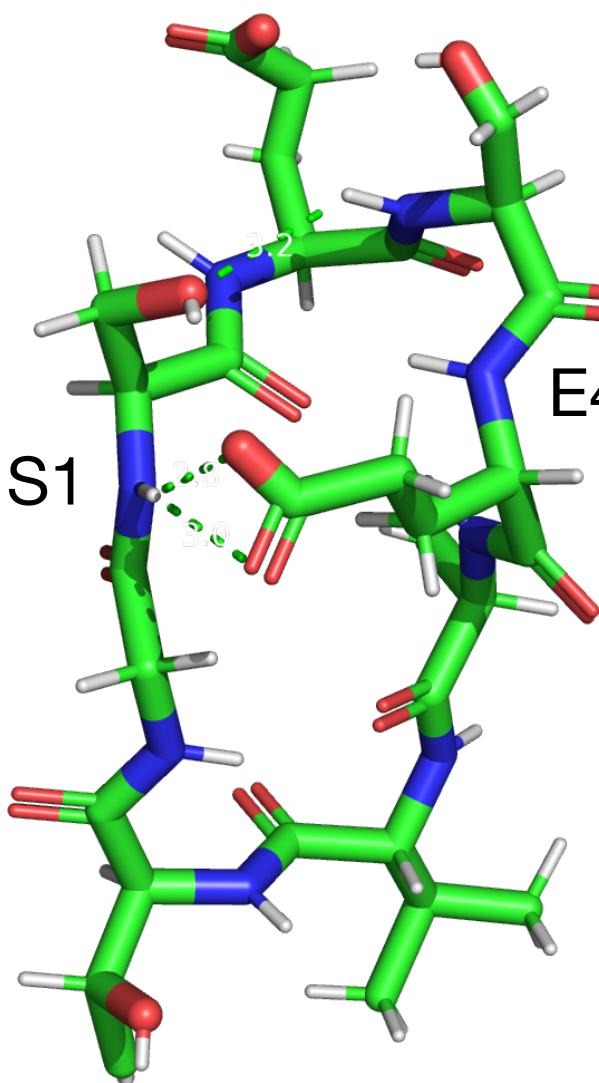
Results

Conclusion

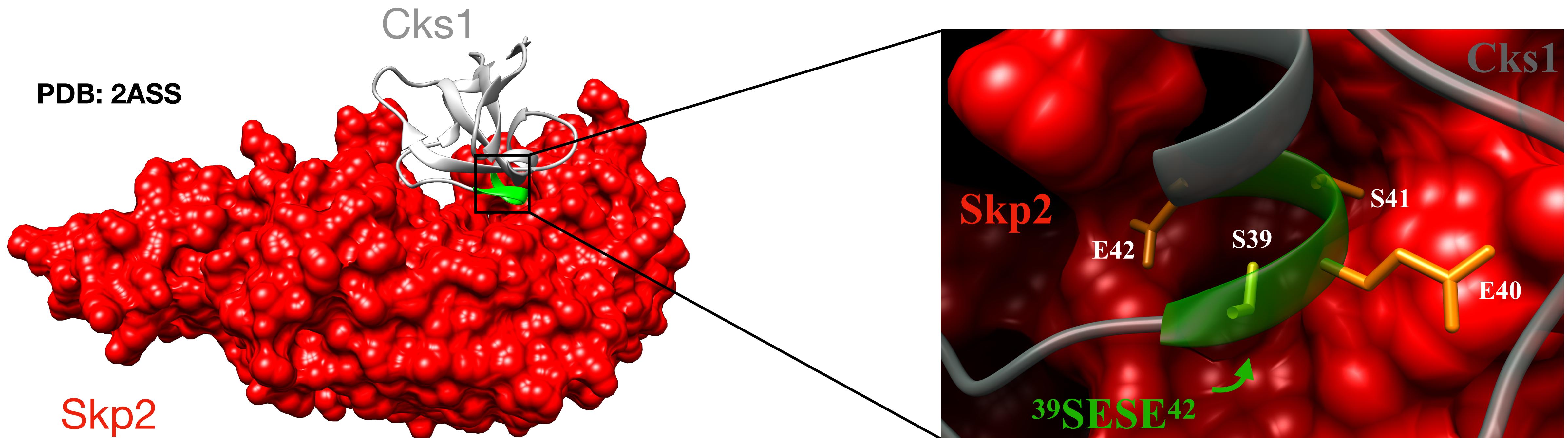
What went wrong?

S1 and E4 side-chains are involved in intra-molecular interactions

- Oxygen on S1 side-chain interacts with amide hydrogen of S3.
- Oxygens on E4 side-chain interacts with amide hydrogen of S1.



SESE is necessary, but is it sufficient?



Yellow: residues contributing > 1 kcal/mol binding energy

Orange: residues contributing > 2 kcal/mol binding energy

J. Gavenois et al., *Nat. Chem. Biol.* **10**, 716–722 (2014)

T. Siegert et al., *Methods Mol. Biol.* **1561**, 255-277 (2017)

B. Hal et al., *Mol. Cell* **20**, 9-19 (2005)

Background

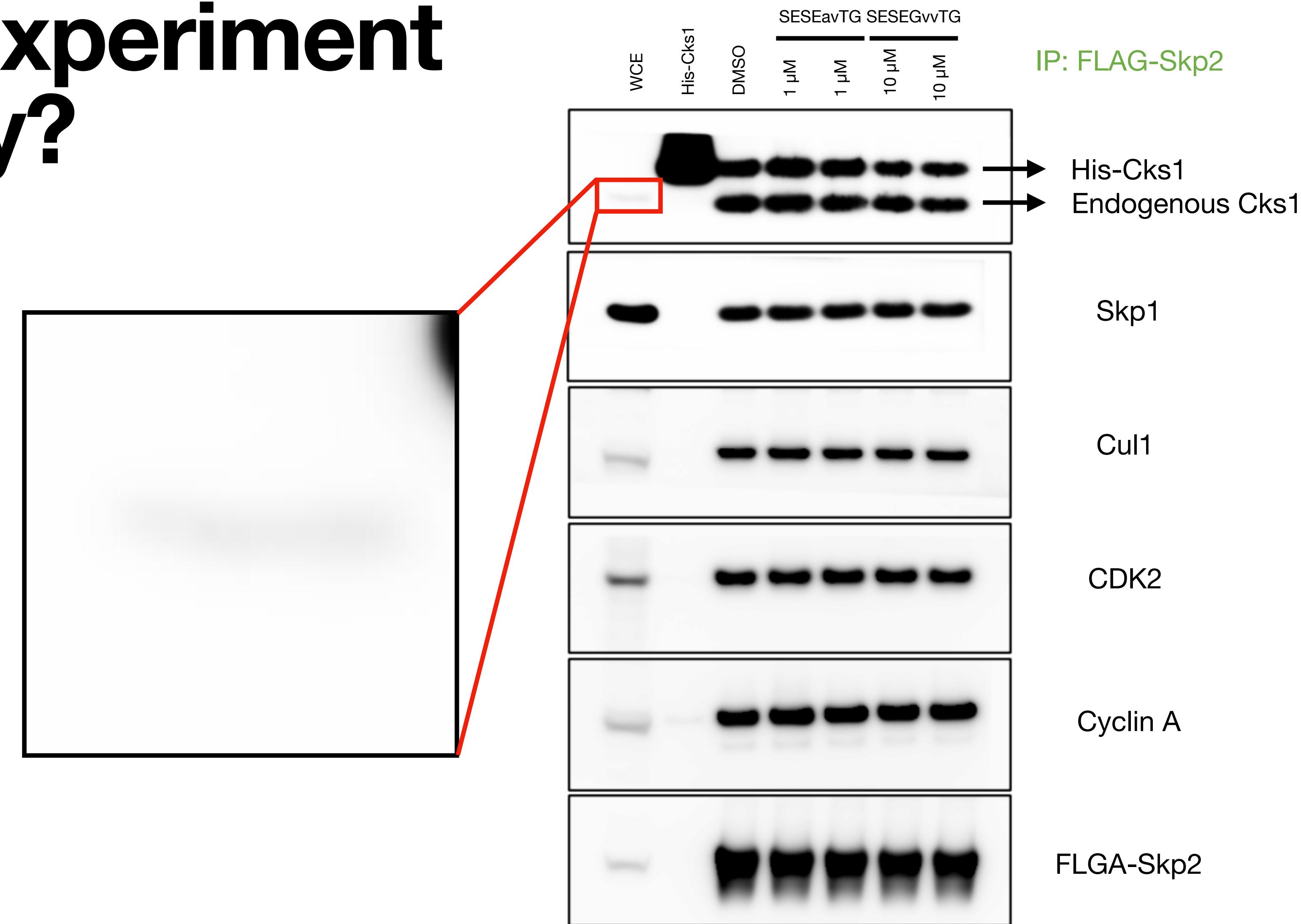
Project Description

Results

Conclusion

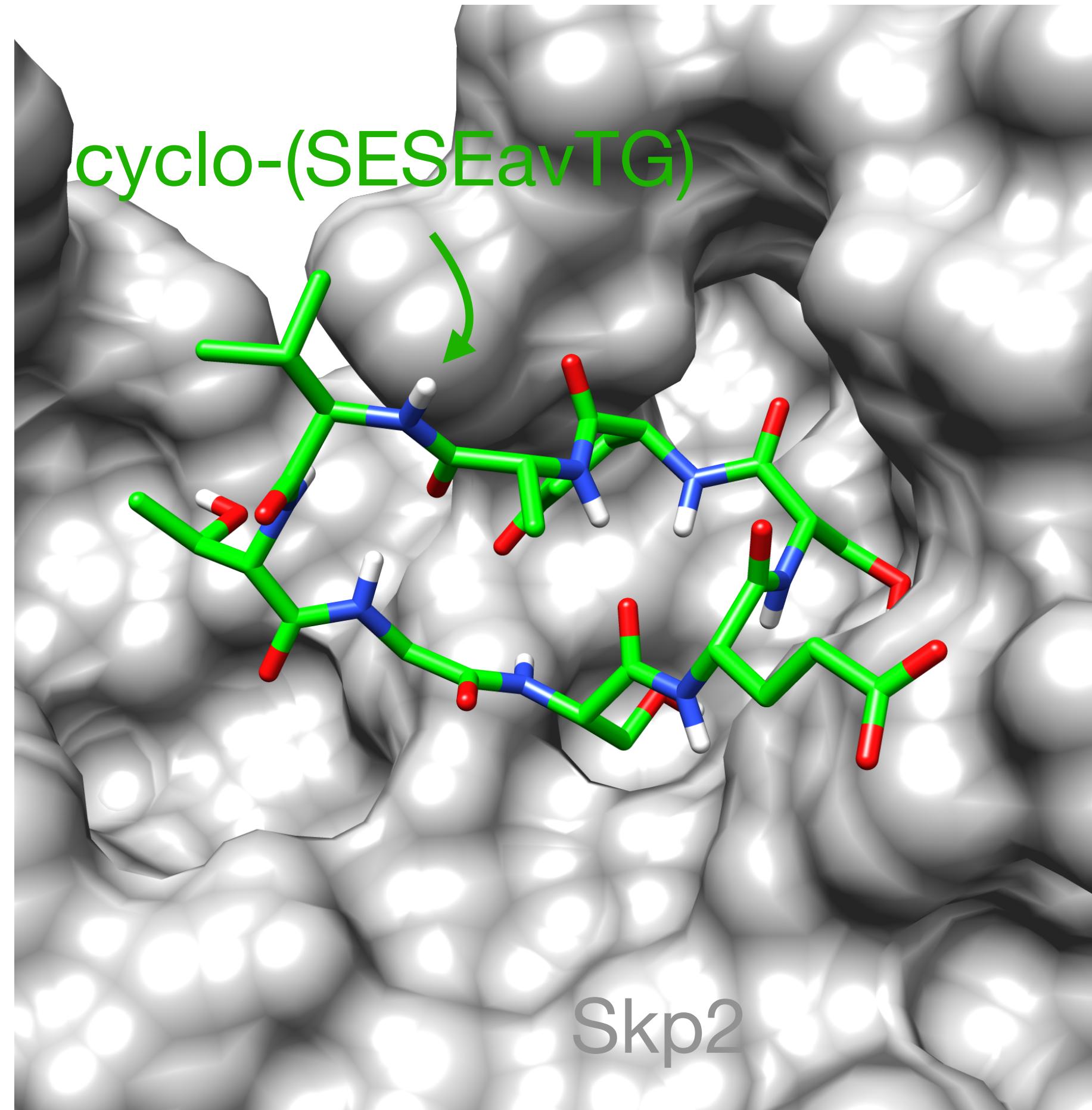
Does the *in-vitro* experiment tell the whole story?

- Skp2 was already pre-occupied with endogenous Cks1.
- Can the inhibitor properly function when Cks1 already binds to Skp2?



Conclusion and next steps

- Designed cyclic peptides that are predicted to be well structured.
- Structural predictions of cyclo-(SESEavTG) align well with NMR data.
- Cell-based studies show little inhibitory effect of cyclo-(SESEavTG) and cycle-(SESEGvvTG).
- Verify our inhibitor design and experimental methods.



Acknowledgements

- Dr. Yu-Shan Lin (PI)
- Dr. Jiayuan Miao
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- Michele Pagano @ NYU SOM



Tufts Research
Cluster