Probing Ubiquitin-Cavitand Binding via ¹⁵N-¹H HSQC Julia R. Didziulis¹, Bethany G. Caulkins¹, Richard J. Hooley²



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Introduction



post-translational modification

• Implicated as a major factor in the proliferation of neurodegenerative diseases, developmental disorders, and cancers



The Cavitand

• Synthesized in the Hooley lab at UC Riverside Self-folding, water soluble host molecule with affinity for lysine and arginine side chains



 Successful membrane receptor, endocytosis and drug delivery agent, and fluorescent biosensor of protein methylation and phosphorylation

 Potential biosensor for ubiquitination and other lysine and arginine based post-translational modifications

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- Protein concentration estimated at about 230 µM

- Lysine side chains signals undetected in pure UB

Materials and Methods

UB Expression & Purification ¹⁵N-labeled human UB expressed in *E. coli* Rosetta cells

with His-TEV tag

• Released by sonication, purified on Ni-NTA affinity column, cleaved from His-TEV tag, and concentrated via centrifugation

 Sample identity and concentration confirmed by gel electrophoresis and Bradford Assay

NMR Experiments

• Samples prepared with 230 µM UB and 10 mM cavitand in D₂O in various cavitand:protein concentrations (0.25:1, 0.5:1, 0.75:1, 1:1, 5:1) Cavitand-UB interactions in each sample studied via ¹⁵N-¹H HSQC

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• Expression and purification protocol found effective Backbone signals assigned using published spectra

Results



sample conditions

• Future investigation of the sample is warranted in order to determine the cause of cavitand precipitation • May be helped by improving sample purity with sizeexclusion chromatography Once UB-cavitand interactions are characterized, they may inform the cavitand's viability as a biosensor for PTMs like ubiquitination and more • Other UB-like proteins, such as SUMO and lysozyme, are also viable cavitand targets • This may contribute to diagnostics, ubiquitin research, and therapeutics targeting the ubiquitin system

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• Arginine side chain signals detected around 85 ppm • Experiments introducing cavitand prevented by cavitand precipitating out of solution

• This precipitation event is unprecedented in these

Conclusions

References

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