Measurement of PROTAC kinetics and cooperativity

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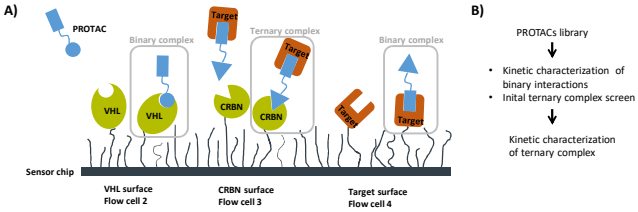
Introduction

The development of proteolysis-targeting chimeras (PROTACs™,[1]) in order to achieve proximity-dependent ubiquitinylation and degradation of disease-related target proteins has recently gained momentum in both academia and industry. PROTACs are bifunctional molecules consisting of a ligand that binds to an E3 ligase and a ligand that binds to a target protein, connected by a linker.[2] A key step for the achievement of selective and effective target degradation is the stable and cooperative formation of the intermediate target:PROTAC:E3 ligase complex.[3,4] The optimization of these properties requires an efficient assay that can guide the choice of the ligase, as well as the design of the linker and its attachment points to the ligase and target ligands.

The two most widely employed E3 ligases by PROTACs are cereblon (CRBN) and von Hippel-Lindau E3 ligase (VHL).

SPR-based binding assay for VHL and CRBN



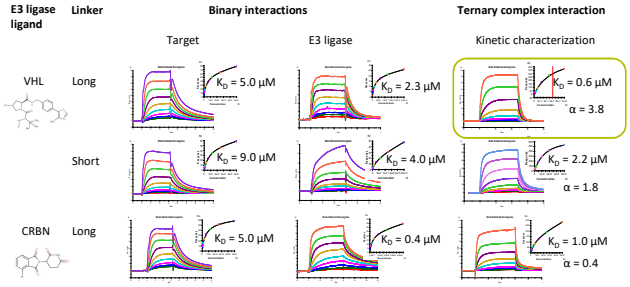
Schematic presentation of the SPR sensor chip with immobilized VHL, CRBN and a target. A) Sensor surface design for a Biacore T200 and B) experimental design for a two-stage kinetic characterization of binary and ternary complex interactions

After an initial screen the compounds can be ranked based on kinetic parameters for the ternary complex (binding response, dissociation rate) prior to a more detailed affinity determination with optimized conditions (E3 ligase immobilization level, compound concentration range, target concentration).

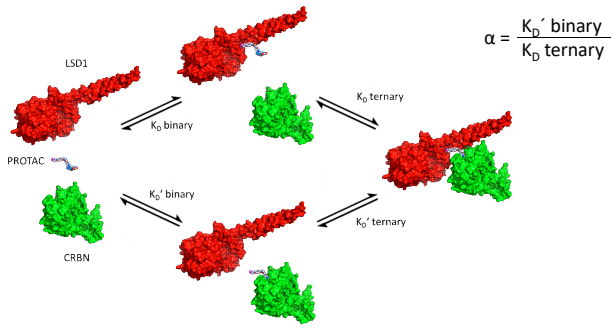
The free compound concentration in the PROTAC + target sample can be monitored by simultaneously analyzing target and E3 ligase in parallel. In order to avoid autocompetition the free compound concentration needs to be kept to a minimum.

Screening and characterization of PROTACs targeting a transcription factor

A fragment-derived transcription factor inhibitor was conjugated to different E3 ligase ligands via a linker with variable length. Kinetic analysis of both the binary and ternary complex interaction revealed positive cooperativity for the ternary complex with VHL (α = 1.8 – 3.8) but negative cooperativity with CRBN (α = 0.4). A long linker appeared to be preferred over a short linker.



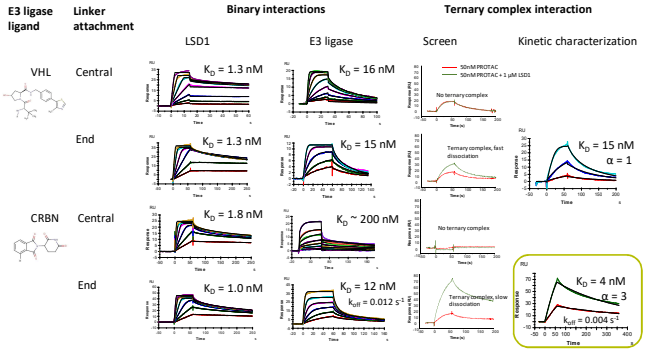
PROTAC interactions and cooperativity



Interaction scheme for binary and ternary complex formation between LSD1, CRBN and a PROTAC. Cooperativity (α), defined as the ratio of binary and ternary dissociation constants for PROTAC binding, can be positive (α > 1), negative (α < 1) or neutral (α = 1).

Screening and characterization of LSD1 PROTACs

An initial screen of PROTACs with different E3 ligands and linker attachments to the target ligand gained a quick understanding of the potential and ways forward to design lysine-specific histone demethylase 1A (LSD1) degraders. The best compound displayed cooperative formation of the ternary complex with CRBN (α = 3) and with increased half life as compared to the binary interaction.



Summary

We employed surface plasmon resonance (SPR)-based binding assays for interaction studies with PROTACs and E3 ligases, CRBN and VHL, as well as with the target proteins. The assays allowed for detailed kinetic characterization of both the binary and ternary PROTAC complexes as well as providing accurate estimates of binding cooperativity. We present an efficient sensor chip surface design and experimental strategy which helped to rank and prioritize PROTACs based on interaction kinetics.

References

1. PROTAC™ is a trademark by Arvinias, Inc.
2. Konstantinidou *et al.* (2019) PROTACs — a game-changing technology. Expert Opin Drug Discov, 14, 1255-1268
3. Bondeson *et al.* (2018) Lessons in PROTAC design from selective degradation with a promiscuous warhead. Cell Chem Biol, 25, 78–87
4. Roy *et al.* (2019) SPR-Measured Dissociation Kinetics of PROTAC Ternary Complexes Influence Target Degradation Rate. ACS Chem Biol, 14, 361-368