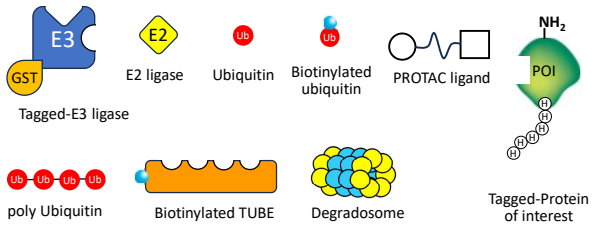
Developing PROTAC Assays using
ENLIGHT OMEGA™ Toolbox Reagents

Contrasting to TR-FRET, *ENLIGHT* OMEGA benefits of a very large distance for proximity (200 nm) which allows for the detection of bulky protein complexes. The controlled avidity prevailing between the Sensibeads and the Chemibeads facilitates the measurement of low affinity interactions (high µM range), making OMEGA an ideal technology to detect protein-protein interactions and large post-translational modifications.

Your Biology/Chemistry



BIOSIGNAL²'s ENLIGHT OMEGA™ Toolbox

Sensibeads	P/N
anti-GST	OMTB-S6AGST
Glutathione	OMTB-S6GLUT
Streptavidin	OMTB-S6STREP

Chemibeads	P/N
anti-6His	OMTB-C6AHIS
Ni^{2+} chelate	OMTB-C6NICH

www.biosignal2.com/omega-toolbox

BIOSIGNAL² WHEN QUALITY MATTERS

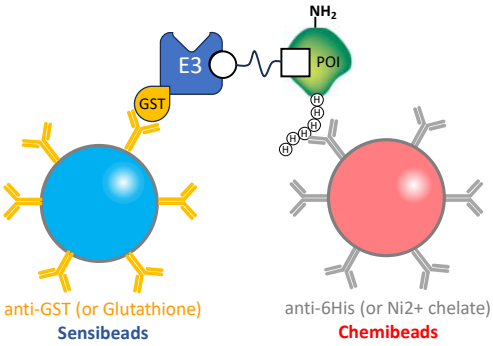
Once there was Alpha... ... now there comes *ENLIGHT OMEGA™*

ENLIGHT OMEGA reagents from BioSignal2 facilitate your PROTAC development at different stages, namely:

1. POI engagement: measuring ternary complex formation
2. Functional E2 recruitement: measuring quaternary complex
3. Measuring POI polyubiquitination
4. Measuring cellular POI degradation

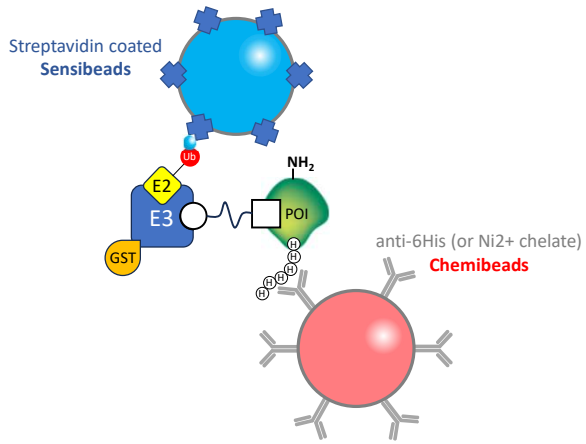
The assays described below can be performed using a combination of Sensibeads and Chemibeads allowing for the easy characterization of your PROTAC ligand following the efficient capture of your protein of interest (POI) and E3 ligase.
It is also possible to monitor the downstream processing of your POI from its ubiquitination to its cellular degration by the proteasome.

Ternary complex formation (biochemical, signal increase)



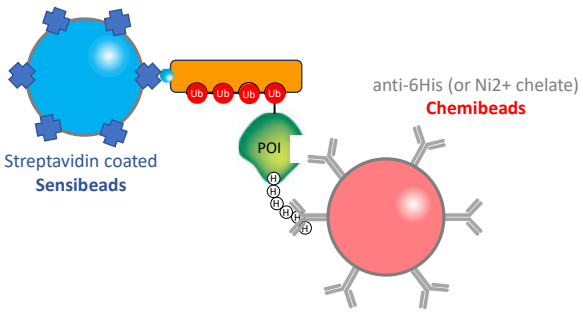
Measuring E3/POI engagement mediated by PROTAC is easily achieved using recombinant tagged proteins. In the example above, GST-E3 and His-tagged POI are respectively captured on anti-GST (or GSH) Sensibeads and ant-6His (or Ni^{2+} chelate) Chemibeads. The presence of a specific PROTAC brings the bead in close proximity leading to the production of an OMEGA signal at 615nm.
It is also possible to detect and characterize the binding of small molecules to either E3 or POI ligands binding sites by performing a competition assay using PROTAC as a bispecific tracer.

E2 ligase recruitment (biochemical, signal increase)



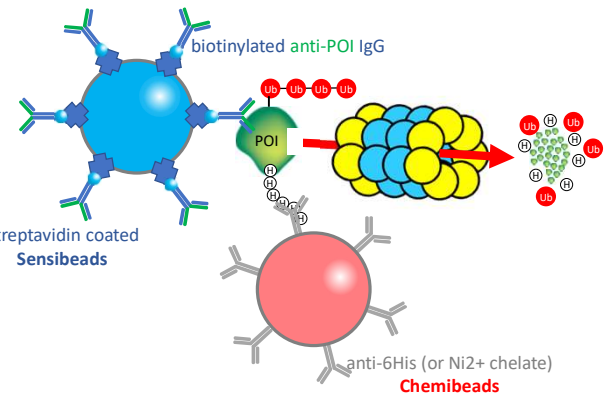
E2 recruitment by the ternary complex is required to initiate POI polyubiquination on at least one lysine residues. Using an E1 ligase specific to an E2 of interest and biotinylated Ubiquitin as a substrate, it is possible to measure functional E2 recruitment.

Measuring POI polyubiquitination (biochemical, signal creation)



TUBEs (Tandem Ubiquitin Binding Entities) are engineered protein domains that bind specifically to polyubiquitin chains. Using biotinylated TUBEs allows one to measure the extent of POI polyubiquitination.

Measuring POI degradation (cellular, signal loss)



It is possible to measure the cellular degradation of a POI, either recombinant (example above) or endogeneous, following PROTAC exposure. Detecting endogeneous POI in a cell extract is feasible by conjugating the Chemibeads with an antibody raised against a POI epitope distinct from that recognized by the biotinylated antibody.

Contact us for more details !
www.biosignal2.com
450-468-0667