natural amino acid and/or an amino acid or amino acid sequence, which is modified either by enzymatic modification, non-enzymatic modification or misincorporation of one or more amino acids. Therefore, the object of the present invention is to provide a screening method to identify modified amino acid degrons.

Summary

In one aspect, the present disclosure refers to a method for identifying a modified amino acid degron (MAAD), the method including the following subsequent steps of

a. preparing an organic molecule of the general formula (I)

X-T-Z (I)

wherein

T is an organic moiety comprising at least one canonical or non-canonical amino acid,

X is a peptide comprising two or more canonical amino acids said peptide being covalently linked to T by an amide bond;

Z is a functional end group comprising a heteroatom selected from the group consisting of oxygen, nitrogen and sulfur;

with the proviso that if Z is OH, T does not consist of canonical amino acids;

b. preparing from the organic molecule of the general formula (I) a protein conjugate of the general formula (II)

$P\text{-}(L)_p\text{-}T\text{-}Z$ (II)

wherein

P is a reporter protein,

T and Z have the same definition as in step a,

L is a peptide comprising two or more natural amino acids and p is 0 or 1;

c. incorporating the protein conjugate obtained in step b) in a cell line;

d. measuring the turnover of the protein conjugate and comparing it with the turnover of a control protein to identify if the protein conjugate is tagged with a MAAD inducing intracellular protein degradation,

e. generating a pool of mutant cells, each defective in a different gene, and

f. incorporating the MAAD-tagged protein conjugate identified in step d) into the pool of mutant cells generated in step e) to isolate mutant cells that fail to selectively degrade the protein conjugate, thus identifying factors mediating the selective degradation of the MAAD-tagged protein conjugate.

Definitions

A “**modified amino acid degron**” or “**MAAD**” as used herein refers to a molecule comprising at least one non-natural or non-canonical amino acid and/or an amino acid or amino acid sequence, which is modified either by enzymatic modification, non-enzymatic modification or misincorporation of one or more amino acids. Modified amino acid degrons may be employed for targeted degradation strategies (e.g. PROTACs).

The term “**PROTAC**” as used herein refers to proteolysis-targeting chimeras. PROTAC generally have three components - an E3 ubiquitin ligase binding group (E3LB), a linker, and a protein binding group. PROTACs and PROTAC binding domains are

known to the skilled person (see e.g. An et al, EBioMedicine. 2018 Oct; 36: 553-562).

The term “**reporter protein**” as used herein refers to any proteinaceous structure that can be measured and quantified by standard biochemical or optical methods. The reporter protein of the present disclosure is used to measure the turnover of the protein conjugate. Ideally, said reporter protein is not endogenously expressed or present in the cell incorporated, transduced or transfected with the protein conjugate.