Ubiquibodies™ for Selective Protein Silencing

Invention Summary

The invention describes engineered ubiquitin ligases for selective degradation of targeted stable proteins through the ubiquitin-proteasome pathway (UPP). The natural substrate binding domains of ubiquitin ligases have been redesigned with designer binding proteins (DBP) that bind to targeted proteins inside cells, while the ligase function remains intact.

Technology Overview

Ubiquibodies represent the fusion of ubiquitin ligase with DBPs such as intracellular antibody fragments (scFv), human fibronectin type III domain (FN3), and DAR Pins (Designed Ankyrin Repeat Proteins) (see figure 1). All DBPs can be engineered to bind to their antigens inside living cells. These fusion proteins have been termed ubiquibodies (uAb) because of their antibody-mimetic binding and their ubiquitination of target proteins.

Using uAbs, significant knockdown through UPP was demonstrated in three different cell lines (HEK293T, BHK21, and COS7) using transient co-transfection to deliver target and ubiquibody proteins. The extent of silencing correlated with the dosage of the ubiquibody plasmid DNA, with knockdown ranging from 80% to as little as 3% of the target protein remaining in cells.

Advantages

- Alternative technique to gene knockouts, RNAi, and chemical inhibitors
- Effective at low expression levels of uAb
- High affinity and high specificity to target
- Generalizable to target any protein
- Makes possible the targeting of protein isoforms

Publications

- PCT Application WO2012135284