

ProteoTuner™: A novel system with rapid kinetics enables reversible control of protein levels in cells and organisms

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Introduction

Analyzing protein functions is a key focus in discovery-based cell biology research. Clontech's revolutionary new ProteoTuner systems, based on a technology developed by Dr. Thomas Wandless and colleagues¹, allow you to directly investigate the function of a specific protein of interest—by directly manipulating the level of the protein itself. This technology has already been successfully applied in a variety of applications and organisms, resulting in several publications in outstanding peer reviewed journals¹⁻⁶.

The ProteoTuner systems are based on a 12 kDa mutant of the FKBP protein (the destabilization domain, or DD) that can be expressed as a tag onto your protein of interest. The DD fusion protein is reversibly protected from proteasomal degradation in the presence of the small (750 Da) membrane permeable ligand, Shield1 (Figure 1)¹.

Fast, Focused Results

Quickly changing the amount of your protein of

interest in a cell enables you to gain valuable information about its function. Unlike other systems which regulate the amount of a protein indirectly (at the transcriptional level), this system targets the protein of interest itself. This guarantees a much quicker response than other methods. It has been shown that a DD fusion protein can be detected just 15–30 minutes after adding Shield1¹.

Adjustable Protein Stabilization

In the presence of Shield1, the DD-tagged protein of interest is stabilized and accumulates inside the cell. Conversely, in its absence, the DD-tagged protein is degraded very rapidly by proteasomes. Thus, it is possible to “tune” the amount of your stabilized, DD-tagged protein in the cell by titrating the amount of Shield1 in the culture medium (Figures 2–3). The degree of stabilization increases as the Shield1 concentration increases, within the range of ~50–1,000 nM. This was demonstrated by cloning DsRed-Express into the pRetroX-PTuner IRES Vector in-frame with the 5' sequence encoding the DD. Infected cells were treated with different

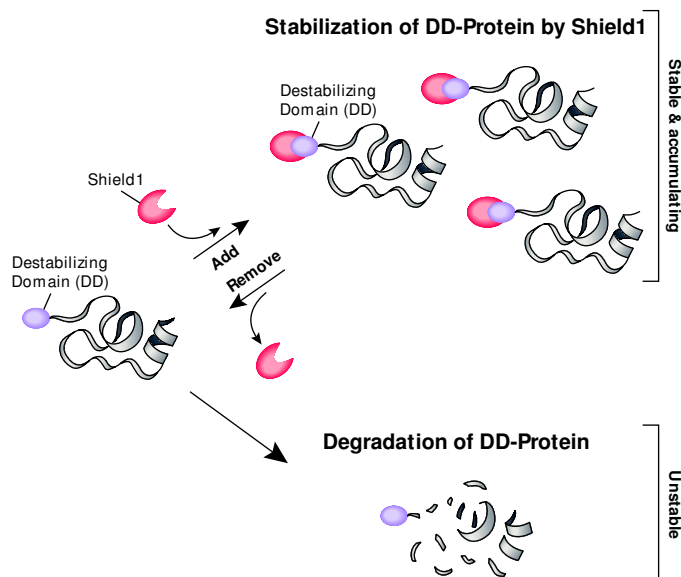


Figure 1. Ligand-dependent, targeted and reversible protein stabilization. The default pathway for the ProteoTuner systems is degradation of the DD-tagged fusion protein, unless Shield1 is present to stabilize it.

concentrations of Shield1 and the fluorescence intensity was analyzed using a BD FACSCalibur™ flow cytometer. Higher fluorescence intensities were observed in samples treated with higher concentrations of Shield1, due to greater amounts of stabilized DD-DsRed-Express protein present in the cells (Figure 2).

These results were confirmed by Western blot, using Clontech's Living Colors® DsRed Polyclonal Antibody. DD-DsRed-Express can be stabilized (at Shield1 concentrations between 500–1,000 nM) to levels comparable with levels of the untagged protein in cells stably expressing untagged DsRed-Express from the CMV promoter (Figure 3). At lower concentrations of Shield1, there is a direct relationship between the concentration of Shield1 and the amount of destabilized DsRed-Express protein detected by flow cytometry or in the cell lysate (Figures 2 and 3 respectively).

Reversible & Repeatable

The ProteoTuner method is not restricted to protein *stabilization*—it can also be used to

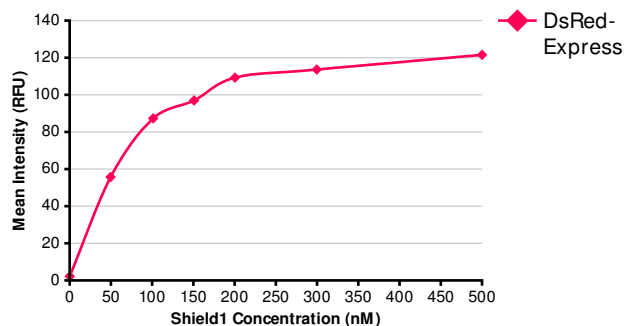


Figure 2. DD-DsRed-Express fluorescence is directly related to the concentration of the stabilizing ligand Shield1.

destabilize the protein of interest by withdrawing Shield1 from cell cultures that previously contained Shield1. This makes it possible to repeatedly stabilize and destabilize the DD-tagged protein using the same set of cells. Experiments which varied the concentration of Shield1 over the course of one week to destabilize and then stabilize a protein of interest have been reported¹.

Conclusions

The ability to quickly and directly control protein levels with the ProteoTuner systems gives you a novel tool for studying transient effects that might otherwise be masked, and to dissect the roles of multifunctional proteins, by directly and specifically “tuning” the level of a protein of interest in the cell. Four systems are available: with either plasmid or retroviral vectors, and with or without a Living Colors Fluorescent Protein marker for transfection efficiency. Shield1 is available separately and as part of each system. For further information, please visit www.clontech.com/PTUNER

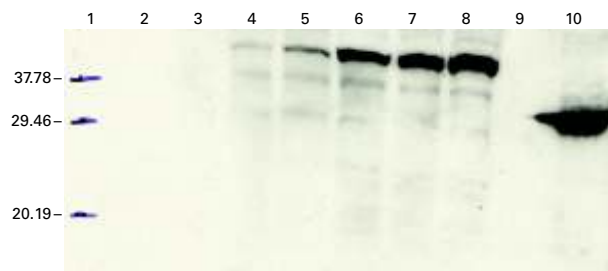


Figure 3. DD-DsRed-Express protein levels are directly related to the concentration of the stabilizing ligand Shield1. Cells were treated as in Figure 2, and the stabilized DD-tagged DsRed-Express was detected via Western blot using the Living Colors DsRed Polyclonal Antibody. Lane 1: marker. Lane 2: 1X loading buffer. Lane 3: untreated HeLa cells (no virus, no Shield1). Lane 4: HeLa cells infected with the DD-DsRed Express construct; no Shield1. Lanes 5–8: HeLa cells infected with the DD-DsRed-Express construct and treated with 50, 250, 500, and 1,000 nM Shield1 respectively. Lane 9: 1X loading buffer. Lane 10: HEK293 DsRed-Express stable cell line.

References

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