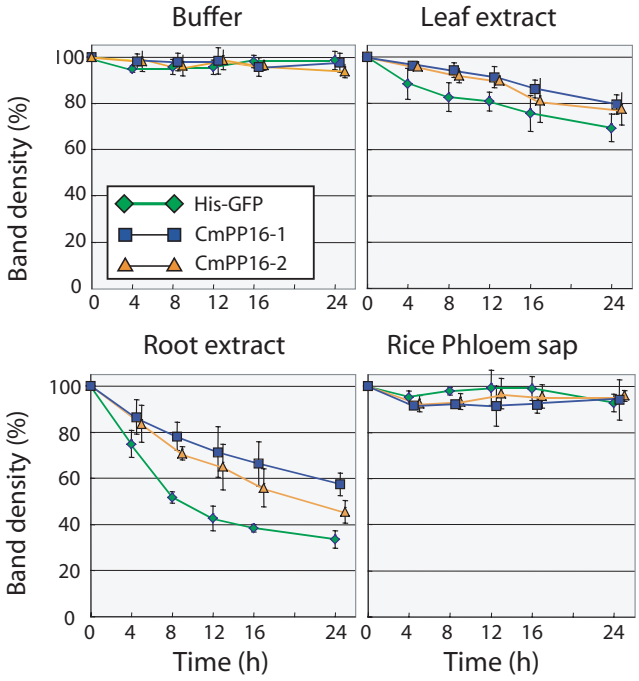
Supplemental Figure 2

Aoki et al. (2005)



Supplemental Figure 2. In vitro degradation assay of B-HisGFP and B-CmPP16 proteins. Fresh crude extract of plant tissues was prepared at 4 ºC prior to the assay. Tissues were homogenized in 0.1 M sodium phosphate buffer (pH 7.4). Homogenate was clarified by filtration and centrifugation at 15000 x g for 20 min. Protein concentration in assay mixture was as follows; B-HisGFP 100 ng/µl, B-Pn16 100 ng/µl, leaf or root extract 2 µg/µl, rice phloem sap 0.2µg/µl. Mixture was incubated at 25 ºC for indicated time. Assay mixtures were run on 15-25 % gradient acrylamide gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) and transferred onto nitrocellulose membrane (Biorad). Biotinylated proteins were detected by using horseradish peroxidase-conjugated streptavidin (Vector Laboratories, Burlingame, CA). Chemiluminescence signal was captured by Typhoon 8600 (Amersham Biosciences), and then analyzed by ImageQuant (Molecular Dynamics). Values represent means ± SE of three independent measurements.

B-CmPP16-1 and B-CmPP16-2 degraded nearly at the same rate in leaf extract. B-CmPP16-2 degraded more rapidly than B-CmPP16-1 in root extract. Significant degradation was not observed in rice phloem sap.