Tetracysteine-tagged prion protein allows discrimination between the native and converted forms.

The conformational conversion of prion protein (PrP) from a native conformation to the amyloid form is a hallmark of transmissible spongiform encephalopathies. Conversion is usually monitored by fluorescent dyes, which bind generic amyloids and are less suited for living cell imaging. We report a new method for the synthesis of membrane-permeable and membrane-impermeable biarsenical reagents, which are then used to monitor murine PrP (mPrP) misfolding. We introduced tetracysteine (TC) tags into three different positions of mPrP, which folded into a native-like structure. Whereas mPrPs with a TC tag inserted at the N-terminus or C-terminus supported fibril formation, insertion into the helix 2-helix 3 loop inhibited conversion. We devised a quantitative protease-free method to determine the fraction of converted PrP, based on the ability of the fluorescein arsenical helix binder reagent to differentiate between the monomeric and fibrilized form of TC-tagged PrP, and showed that TC-tagged mPrP could be detected on transfected cells, thereby expanding the potential use of this method for the detection and study of conformational diseases.