Supporting Methods

SUPPORTING INFORMATION

Cobalamin and preparation of copper glycine complex (Cu/glycine$_2$)

The source of cbl were: adenosylcobalamin (Sigma, C0884-100MG), methylcobalamin (Sigma, M9756-100MG), and cyanocobalamin (Sigma, V2876-1G). Bis(glycinate)copper(II) monohydrate was synthesized as previously described (117). Briefly, 2 g of Cu(II) acetate monohydrate (99% purity, Sigma) was dissolved in 25 ml of hot distilled water, followed by addition of 25 ml of ethyl alcohol to the hot solution. An aqueous solution of 2 mM glycine (Sigma) was prepared separately, and 25 ml added to the hot copper acetate monohydrate-ethyl alcohol mixture. The mixed solution was then quickly cooled on ice to allow formation of Bis(glycinate)copper(II) monohydrate precipitates. The sample was then vacuum filtered, and crystals were air dried for 24 hours before being dissolved in water (118).

DEPC footprinting

Prior to MALDI-MS analysis, WT-PrP and S3-PrP (100 uM) preparations dissolved in a 25 mM N-ethylmorpholine and 30 mM KCl (NEMO-KCl) buffer, pH 7.4 master mix were subdivided into aliquots that were then adjusted with a 10-fold molar excess of $CuSO_4$, $CoCl_2$, Cu/glycine$_2$, or cyanocobalamin/protein and incubated at 37°C for 30 min. After the incubation step with or without cations, samples were reacted with a 5-fold molar excess of diethyl pyrocarbonate (DEPC) at 37°C for 30 min. For MALDI analysis, 1 µl of each sample was mixed with 1 µl of sinapinic acid (10 mg/ml in 50% acetonitrile/water + 0.1% trifluoroacetic acid). One µl of the sample/matrix solution was then spotted onto a stainless-steel target plate and allowed to air dry. Mass spectra