Supplementary Materials

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Title: Heterogeneous interaction network of yeast prions and remodeling factors detected in live cells

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Running Title: Interaction network of yeast prions in live cells

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Supplementary Materials and Methods

Plasmid Construction

Open reading frame (ORF) sequences of *SUP35*, *RNQ1*, *URE2*, and *NEW1* genes were cloned directly from purified genomic DNA of *S. cerevisiae* BY4741 strain by a standard PCR method with appropriate pair of oligonucleotides. Each partial sequence corresponding to residues 1-253, 1-65 and 1-130 of Sup35, Ure2 and New1, respectively, was fused to the 5'-end of the sequence of *TagRFP* derived from pTagRFP-N (Evrogen) by an overlap-extension PCR method. Likewise, sequence corresponding to residues 134-405 of Rnq1 was fused to the 3'-end of the *TagRFP*-coding sequence. Finally, each chimeric DNA fragment was subcloned onto the pYES2 yeast expression plasmid vector (Invitrogen) to produce pYES-*SUP35NM-TagRFP,* pYES-*URE2N-TagRFP*, pYES-*NEW1N-TagRFP* and pYES-*TagRFP-RNQ1C*. As a negative control, a plasmid expressing thioredoxin-fused TagRFP (pYES-*Trx-TagRFP*) was also constructed by fusing the thioredoxin ORF originally coded on pThio-His B plasmid (Invitrogen) to the 5'-end of the TagRFP-coding sequence and subcloning onto pYES2 vector.

A plasmid that expresses Hsp104 fused with mCherry (pGAL1-*HSP104-mCherry*) was constructed as follows. The *HSP104* containing *Sac*I-*Bam*HI DNA fragment was cloned into YCplac111GAL1p (1). The *mCherry* DNA fragment was amplified from a pmCherry-N1 vector (Clontech) and inserted into the *Bam*HI-*Sal*I site of the YCplac111GAL1pHSP104 plasmid. This includes GS linker between *HSP104* and mCherry.

To visualize Sis1 in the yeast cell, pRS314-*SIS1p-SIS1-mCherry* was prepared. *SIS1* gene including *SIS1* own promoter was cloned from W303 PJ513a (2) and fused *mCherry* gene under *SIS1* gene with Gly-Ser linker by PCR. *SIS1p-SIS1-mCherry* construct was cloned to pRS314 (3) and used for plasmid shuffling to obtain Sis1-mCherry expression strains. [*RNQ1p-RNQ1-GFP*] plasmid, pRS413-RNQ1p-RNQ1-GFP, was from our previous work (2).

A plasmid (pRS413CYC1p-SUP35NM-GFP) encoding Sup35NM-GFP under the control of CYC1 promoter was constructed with *SUP35NM* gene cloned from W303 PJ513a and monomeric GFP gene (2).

Yeast Strain

In this study, *S. cerevisiae* G74-D694 strain, which we have established previously (4) , was used as the parent strain. G74-D694 is a derivative of 74-D694 [*MAT***a**, *ade1-14(UGA), his3*, *leu2*, *trp1*, *ura3*] and carries a modified *SUP35* gene (*SUP35NGMC*), in which a GFP gene was integrated between the N and M domains of the endogenous *SUP35* gene. Either [*psi*$^-$] or [*PSI*$^+$] G74-D694, [*gpsi*$^-$] or [*GPSI*$^+$], respectively, was transformed with yeast expression plasmid described above by a standard lithium acetate method. Transformants were selected by synthetic medium lacking uracil (SC-Ura) or leucin (SC-Leu). To induce expression of each protein, 2% (w/v) galactose was added to synthetic medium containing 2% (w/v) raffinose instead of glucose and lacking uracil (SRaf (-ura)) for 4~ to 24h.

Details of W303 *sis1-Δ::LEU2* [*SIS1p-SIS1*] [*RNQ*$^+$] [*psi*$^-$] or [*rnq*$^-$] [*PSI*$^+$] strains were described previously (2, 5). BY4741 *MATa HSP104-GFP::HIS3MX6* was described in the previous study (6). W303 *sis1-Δ::LEU2* [*SIS1p-SIS1-mCherry*] strains with [*RNQ*$^+$] or [*PSI*$^+$] were prepared by plasmid shuffling. Growth and prion maintenance of W303 *sis1-Δ::LEU2* strain having expression of Sis1-mCherry was indistinguishable to W303 *sis1-Δ::LEU2* strain with that of wild-type Sis1 from a [*SIS1p-SIS1*] plasmid. To measure FCS/FCCS, prion-GFP plasmid, *pRS413-RNQ1p-RNQ1-GFP* for [*RNQ*$^+$]/[*rnq*$^-$] or *pRS413CYCp-SUP35NM-GFP* for [*PSI*$^+$]/[*psi*$^-$], was coexpressed in W303 *sis1-Δ::LEU2* [*SIS1p-SIS1-mCherry*]. Semi-denaturing detergent agarose gel (5, 7) pattern of the prion-GFP coexpression strains showed identical pattern as of strains without coexpression. W303 [*rnq*$^-$] and [*psi*$^-$] strains were

obtained by 3 mM guanidine HCl hydrochloride (GdnHCl) treatment in the appropriate media for 2 days.

Protein Expression

Transformed yeast cells were cultured in SRaf (-ura). At mid-log phase, protein expression was induced by the addition of galactose at a final concentration of 0.2%. After a 12-h incubation at 30°C, cells were used for FCCS measurement. To estimate the expression level of each fusion protein conveniently, we utilized the fluorescence of TagRFP. Fluorescence images at green and red channel of a 100-µl droplet of each culture fluid were taken by LAS-4000 luminescence imager (Fuji Film, Japan) and the relative TagRFP fluorescence was analyzed by Multi Gauge (Fuji Film, Japan).

Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS). FCS and FCCS measurements were performed at 25°C on a confocal microscope system (LSM 510; Carl Zeiss) combined with a ConfoCor2 (Carl Zeiss). For confocal imaging followed by FCS or FCCS measurement, GFP and RFP were scanned independently in a multi-tracking mode. Details of the combined microscope system, analysis of fluorescence auto- function (FAF) obtained from FCS measurement, and analysis of two FAFs and one cross-correlation function (FCF) from FCCS measurement were described in previous studies (1, 6). Briefly, FAF and FCF, from which the absolute number and diffusion coefficient (*D*) of mobile fluorescent molecules, fractional ratio, and interaction amplitude represented by relative cross-correlation amplitude (RCA) are calculated, are obtained as follows:

The fluorescence auto-correlation functions of the red and green channels, $G_r(\tau)$ and $G_g(\tau)$, and the fluorescence cross-correlation function, $G_c(\tau)$, were calculated from

$$G_x(\tau) = 1 + \frac{\langle \delta I_i(t) \cdot \delta I_j(t+\tau) \rangle}{\langle I_i(t) \rangle \langle I_j(t) \rangle} \quad (1)$$

where τ denotes the time delay, I_i is the fluorescence intensity of the red channel ($i = r$) or green channel ($i = g$), and $G_r(\tau)$, $G_g(\tau)$, and $G_c(\tau)$ denote the auto correlation function of red ($i = j = x = r$), green ($i = j = x = g$), and cross ($i = r, j = g, x = c$), respectively. The acquired $G_x(\tau)$s were fitted using a one-component model for solution samples and a two-component model for live cells:

$$G_x(\tau) = 1 + \frac{1}{N} \sum_i F_i \left(1 + \frac{\tau}{\tau_i}\right)^{-1} \left(1 + \frac{\tau}{s^2 \tau_i}\right)^{-\frac{1}{2}} \quad (2)$$

where F_i and τ_i are the fraction and diffusion time of component i, respectively. N is the average number of fluorescent particles in the excitation-detection volume defined by radius w_0 and length $2z_0$, and s is the structure parameter representing the ratio $s = z_0/w_0$. The structure parameter was calibrated using the known diffusion coefficient of Rhodamine-6G at room temperature ($280 \mu m^2 s^{-1}$). To estimate the diffusion coefficient and fractional ratio from FCS or FCCS measurements, FAF and FCFs in live cells were fitted by a two-component model (i=2, D_{fast} and D_{slow}) with a triplet term (6). Although the shape of FAFs originated from a one-component model (i.e. single-species) only depends on the diffusional mobility, it is emphasized that the shape of FAFs originated from a multi-component model (multi-species) depends not only on the diffusional mobility but also on fractional ratio of mobile species (1). For FCCS measurement, simultaneous excitation of GFP- and RFP-tagged proteins was carefully carried out at minimal and optimal excitation powers, chosen to obtain sufficiently high signal-to-noise ratios for the analysis of the diffusional coefficient and molecular interaction. Data containing severe photobleaching possibly resulting from a high proportion of immobilized fluorophores and non-stationary fluorescent signals resulting from

the drift of yeast cells were excluded from the analysis. For FCCS analysis, the amplitude of the cross-correlation function was normalized by the amplitude of the autocorrelation function of RFP to calculate the relative cross-correlation amplitude (($Gc(0)-1)/(Gr(0)-1)$; RCA value)(6).

Supplementary Table S1. Theoretical molecular weights of prion proteins, Sis1, and Hsp104.

Proteins	Theoretical Mw (kD)	FP tagged proteins	Total theoretical Mw (kD)	Mw_{fcs}[c]
		mGFP ,mCherry, TagRFP	28	25
Sup35NM	49	Sup35NM-GFP	77	79
Sup35	79	NGMC	107	350
Rnq1	43	Rnq-GFP	71	76
Ure2	40	Ure2-mCherry	68	-
New1	134	New1-Ure2-mCherry	162	-
Sis1	40	Sis1-mCherry	68 (136 [a])	1720
Hsp104	104	Hsp104-GFP	132 (792 [b])	8600

[a] Molecular weight of Sis1-GFP as a homo-dimer.

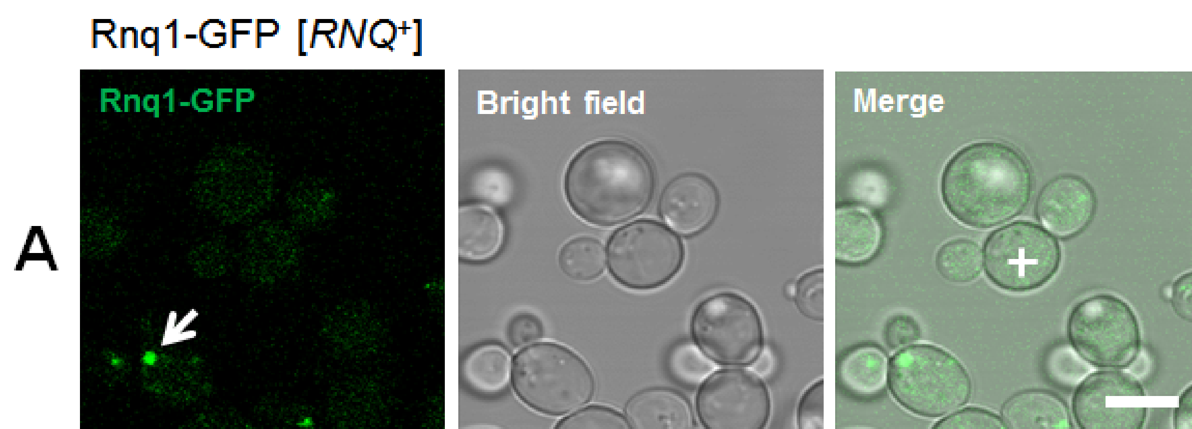
[b] Molecular weight of Hsp104-GFP as a hexamer complex.

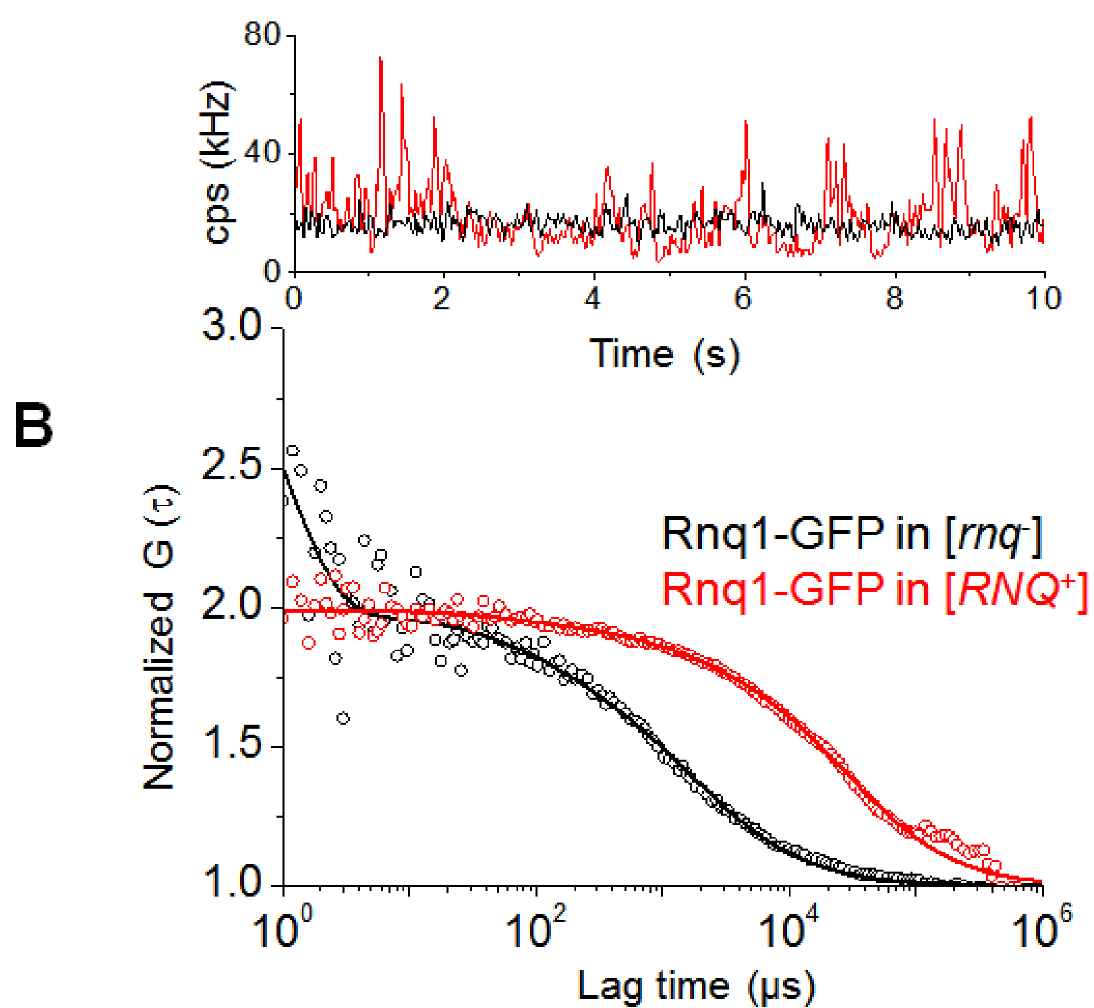
[c] Mw calculated from FCS analysis using lysis solution samples.

Supplementary Table S2. Molecular concentrations of Sup35NM-GFP and Sup35NGMC proteins in yeast cells

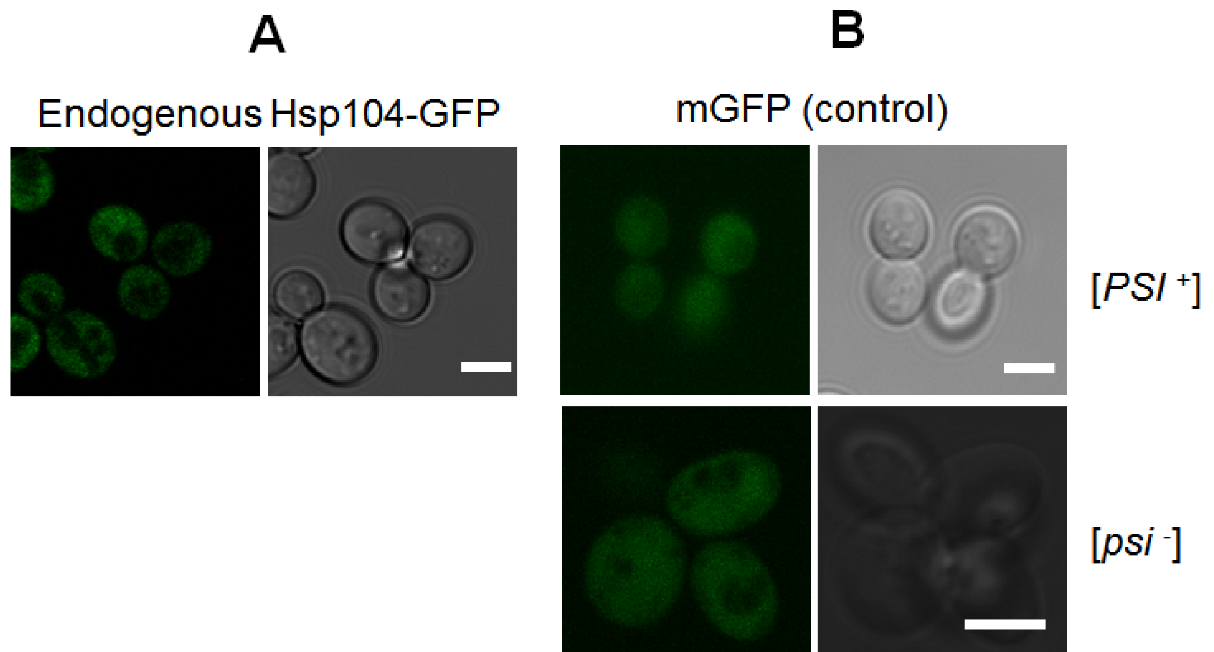
Proteins and cell type	Concentration (nM)	SD (nM)
Sup35NM-GFP in [*psi*$^-$]	676	130
Sup35NGMC in [*gpsi*$^-$]	285	60
Sup35NM-GFP in [*PSI*$^+$]	193	20
Sup35 NGMC in [*GPSI*$^+$]	150	12

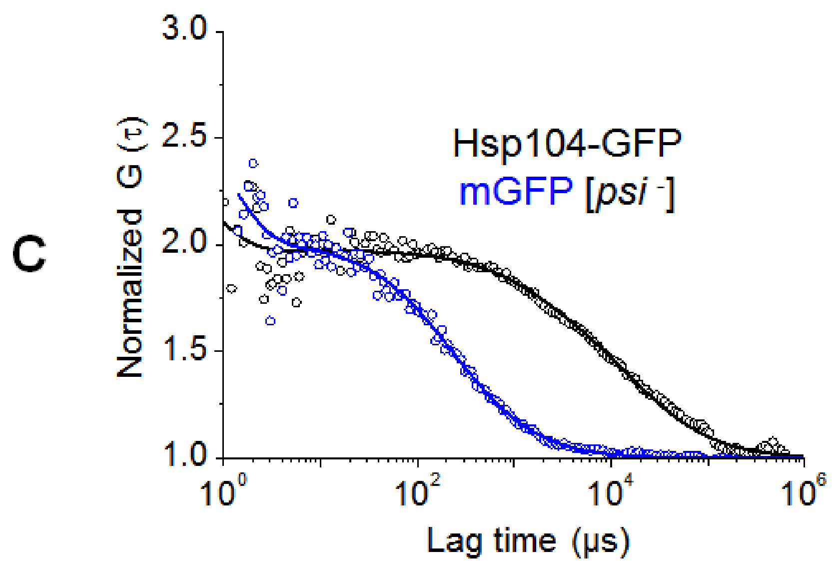
Supplementary Figure S1



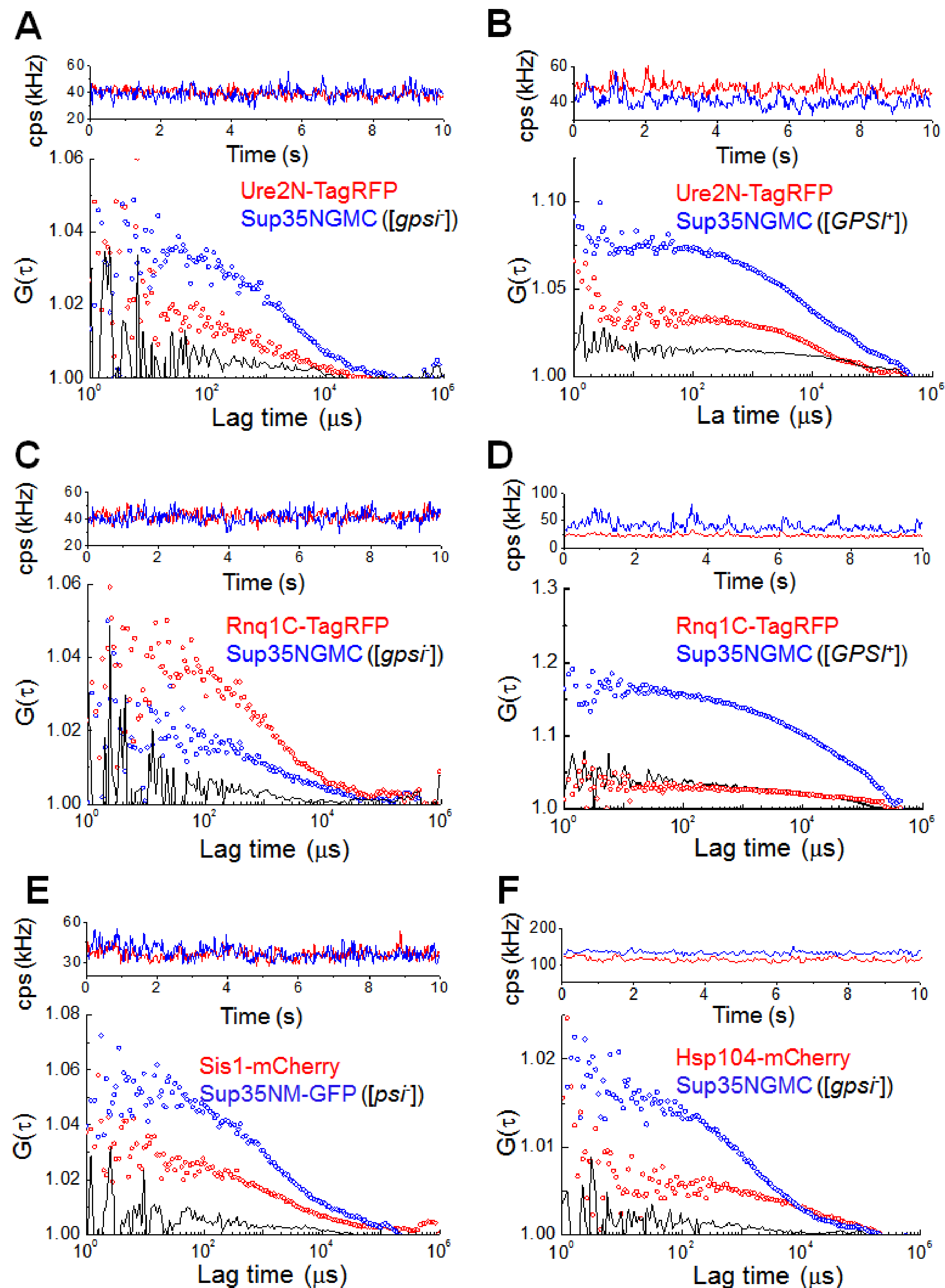


Supplementary Figure S2

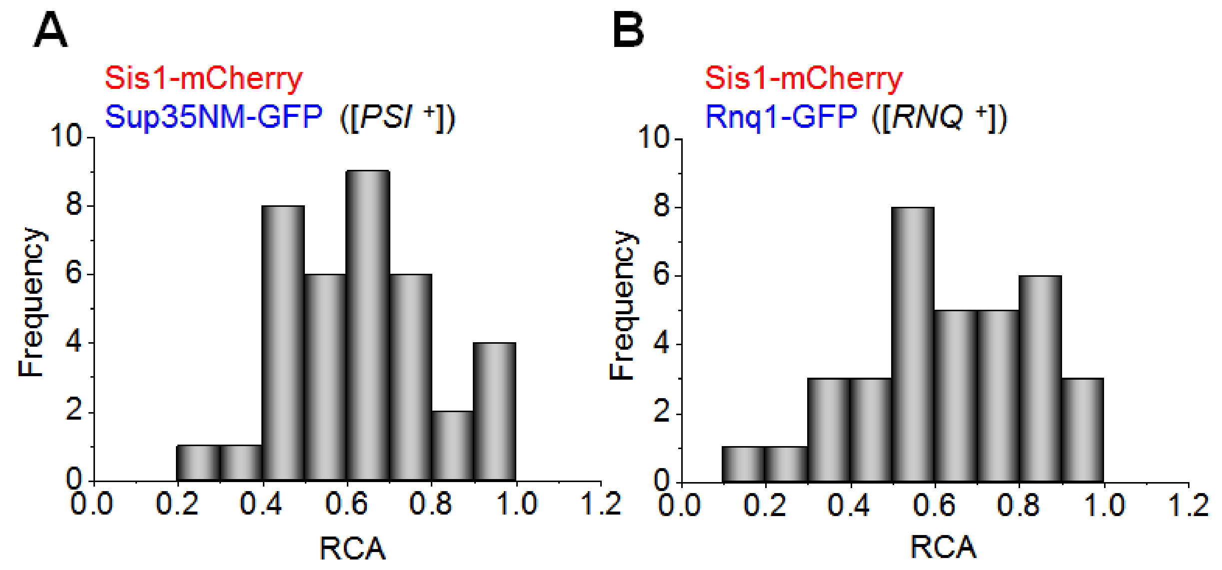




Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure Legends

Supplementary Figure S1. Diffusional properties of Rnq1-GFP in live cells. (*A*) Fluorescence-confocal image of [*RNQ* $^{+}$] prion cells expressing Rnq1-GFP are shown. Scale bar, 5µm. Arrow and cross hair indicate a large immobile focus in a mother cell and the position of FCS measurement shown in (*B*), respectively. (*B*) Representative two FCS measurements carried on [*rnq* $^{-}$] and [*RNQ* $^{+}$] cells are respectively shown. (Upper) Time trace of average fluorescence intensity (counts per second; cps in kHz) of Rnq1-GFP observed in [*rnq* $^{-}$] (*black*) and [*RNQ* $^{+}$] (*red*) cells. (Bottom) The corresponding fluorescence auto-correlation functions (FAFs) calculated from the time trace are also shown. Fit curves (solid line) were obtained from two-component analysis. For comparison of mobility, the curves were normalized to the same amplitude, G (0) = 2.

Supplementary Figure S2. Slow diffusional behavior of Hsp104-GFP in non-prion cells. (*A*) Fluorescence-confocal image of yeast cells endogenously expressing Hsp104-GFP are shown. Scale bar, 5µm. (*B*) Fluorescence-confocal image of [*psi* $^{-}$] and [*PSI* $^{+}$] cells expressing monomer GFP (mGFP) are respectively shown. Scale bar, 5µm. (*C*) Representative normalized FAFs of Hsp104-GFP and mGFP are also shown. Fit curves (solid line) were obtained from two-component analysis. For comparison of mobility, the curves were normalized to the same amplitude, G (0) = 2.

Supplementary Figure S3. FCCS measurements for detecting interactions among prion proteins in live cells. (*A*) ~ (*D*) Representative FCCS measurement carried on non-prion and prion cells are respectively shown (upper and bottom). (Inset) Measured cell type ([*gpsi* $^{-}$] or [*GPSI* $^{+}$]) and a pair of proteins tagged with Tag-RFP and GFP. (Upper) Time trace of average fluorescence intensity (counts per second; cps in kHz) of two prion proteins (*red* and *blue*).

(Bottom) Two corresponding fluorescence auto-correlation functions (FAFs) of mCherry signal (*red*) and GFP signal (*blue*), and one fluorescence cross-correlation function (FCF) are shown. (E) Representative FCCS measurement carried on a [*psi* $^{-}$] cell co-expressing Sis1-mCherry and Sup35NM-GFP are shown. (F) Representative FCCS measurement carried on a [g*psi* $^{-}$] cell co-expressing Hsp104-mCherry and Sup35NGMC are shown.

Supplementary Figure S4. Histogram of RCA values for interaction between remodeling factor Sis1 and prion oligomers in yeast prion cells. (*A*) Histogram of RCA values for interaction between Sis1-mCherry and Sup35NM-GFP oligomers. (*B*) Histogram for interaction between Sis1-mCherry and Rnq1-GFP oligomers.

References

1. Kawai-Noma S, Ayano S, Pack CG et al. (2006) Dynamics of yeast prion aggregates in single living cells. Genes Cells 11, 1085-1096
2. Aron R, Higurashi T, Sahi C and Craig EA (2007) J-protein co-chaperone Sis1 required for generation of [RNQ+] seeds necessary for prion propagation. EMBO J 26, 3794-3803
3. Sikorski RS and Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122, 19-27
4. Tsuji T, Kawai-Noma S, Pack CG et al. (2011) Single-particle tracking of quantum dot-conjugated prion proteins inside yeast cells. Biochem Biophys Res Commun 405, 638-643
5. Higurashi T, Hines JK, Sahi C, Aron R and Craig EA (2008) Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. Proc Natl Acad Sci U S A 105, 16596-16601
6. Pack CG, Yukii H, Toh-e A et al. (2014) Quantitative live-cell imaging reveals spatio-temporal dynamics and cytoplasmic assembly of the 26S proteasome. Nat Commun 5, 3396
7. Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD and Kushnirov VV (2003) Yeast [PSI+] prion aggregates are formed by small Sup35 polymers fragmented by Hsp104. J Biol Chem 278, 49636-49643