Engineering intracellular biomineralization and biosensing by a magnetic protein

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Remote measurement and manipulation of biological systems can be achieved using magnetic techniques, but a missing link is the availability of highly magnetic handles on cellular or molecular function. Here we address this need by using high-throughput genetic screening in yeast to select variants of the iron storage ferritin (Ft) that display enhanced iron accumulation under physiological conditions. Expression of Ft mutants selected from a library of $10^7$ variants induces threefold greater cellular iron loading than mammalian heavy chain Ft, over fivefold higher contrast in magnetic resonance imaging, and robust retention on magnetic separation columns. Mechanistic studies of mutant Ft proteins indicate that improved magnetism arises in part from increased iron oxide nucleation efficiency. Molecular-level iron loading in engineered Ft enables detection of individual particles inside cells and facilitates creation of Ft-based intracellular magnetic devices. We demonstrate construction of a magnetic sensor actuated by gene expression in yeast.

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Magnetic approaches to biological experimentation are particularly attractive because they interact minimally with biological processes, rarely incur damage and have already led to powerful manipulation and imaging techniques. Existing magnetic biotechnologies are of limited value for studying molecular and cellular level phenomena, however. The best known magnetic measurement techniques, nuclear magnetic resonance and magnetic resonance imaging (MRI), are ill-suited for analysis of specific molecular phenomena in cells and tissue. In vivo nuclear magnetic resonance spectroscopy is too insensitive to permit robust measurements of most biomolecules. Molecular MRI measurements can be made using contrast agents that combine magnetic properties with other functionalities, but these agents need to be delivered exogenously. Techniques for magnetic modulation of biological systems have been demonstrated at cellular level, but also tend to depend on exogenous nanoparticles that are difficult to apply to biological systems. Although manipulation of cellular magnetism and magnetic image signals has also been demonstrated using genetic techniques, the effects tend to be weaker or less specific than approaches based on synthetic magnetic nanoparticles, in part because the molecular organization of magnetic material in cells is less controlled.

A strongly magnetic protein could provide a basis for robust modulation or detection of well-defined molecular-level phenomena. A promising starting point for generation of such a molecule is ferritin (Ft), an iron storage protein found in most animal, plant and bacterial cells. Ft proteins consist of a spherical shell of 24 identical or closely homologous polypeptide chains, in which a reservoir of hydrated iron oxide accumulates and can be rapidly mobilized according to physiological needs. Ft variants have been used as magnetic gene reporters, expressible cellular imaging agents and components of magnetically responsive genetic devices, but Ft is much less potent than synthetic nanoparticles of similar volume and often contains far fewer iron atoms than its core structure could in principle accommodate. In vitro manipulation of Ft mineralization has enabled the generation of highly magnetic species, but the resulting protein complexes cannot be applied in conjunction with genetic techniques and suffer similar limitations to those of synthetic nanoparticles.

To address these limitations, we designed a strategy for enhancing the magnetic properties of intracellularly expressed Ft in a systematic and high-throughput fashion. In this paper, we present our approach and its success in isolating mutant Ft variants that biomineralize iron more effectively than their natural counterparts. We characterize the selected mutants and show that their enhanced iron loading capability may arise from improvements to iron oxide nucleation. Finally, we show that the new ‘hypermagnetic’ Ft variants act as genetically encodable tools for multiscale cellular imaging, magnetic manipulation of cells and construction of intracellular magnetic devices capable of sensing molecular-level phenomena.

Results

Screening for Ft variants with enhanced biomineralization.

Our biomaterial engineering approach was based on the hypothesis that mutant Ft molecules that sequester iron compounds most effectively would also form complexes with optimal magnetic properties—a view motivated by the fact that both greater Ft iron content and denser, unhydrated iron oxide mineralization can result in higher per-particle magnetic moments. Iron accumulation by Ft variants is expected to reduce cytosolic iron concentration by mass action principles, so we established a reporting system in yeast whereby expression of Ft mutants could be evaluated for induction of a low cytosolic iron phenotype. In Saccharomyces cerevisiae, intracellular iron level is regulated by the iron-responsive transcriptional activator Aft1, which under low-iron conditions translocates into the nucleus and regulates genes involved in iron uptake. One of the genes upregulated by Aft1 encodes the cell surface high-affinity iron transporter, FTR1; by monitoring expression of an FTR1–green fluorescent protein (GFP) fusion reporter, we could therefore identify individual cells that display low cytosolic iron concentrations (Fig. 1a). This system was intended as a tool for selecting mutant Ft variants that robustly sequester cellular iron, and that would therefore induce greater FTR1–GFP expression and fluorescence than Ft variants with less potent iron binding capacity.

As a template for random mutagenesis and screening, we choose to work with a Ft from the thermophilic bacterium Pyrococcus furiosus (PFt). PFt has the advantage that it is highly thermostable (Tm ≈ 120°C), and, therefore, likely to be more tolerant to mutations introduced to alter biomineralization than human heavy chain Ft (HFe, Tm ≈ 77°C), which has been used for the majority of biotechnological applications of Ft in the past. In addition, PFt forms homooligomeric protein shells that require only a single polypeptide, in contrast to conventional mammalian Fts that incorporate two chains, making PFe structure and chemistry simpler and more predictable. To facilitate isolation and analysis of PFt variants, we fused an affinity tag (Strep-tag II) to the N terminus of PFe to form a construct abbreviated SPFt (Supplementary Fig. 1a). The tag had

Figure 1 | Fluorescent reporter system used to probe intracellular iron mineralization by Ft. (a) Schematic diagram of yeast cells containing an iron-responsive reporting system. Sequestration of cytosolic iron (red dots) into Ft (grey) triggers translocation of an iron-responsive transcription factor, Aft1 (orange), into the nucleus, where it induces transcription of an FTR1–GFP fusion protein (blue/green). Iron accumulation by effective Ft variants therefore results in a green signal (right). (b) Yeast cells transformed with empty vector (Vec), SPFt and the SPFt mutant E94G/K142R, which lacks iron mineralization capability, were incubated in minimal media overnight. Differences in FTR1–GFP expression are visible in the fluorescence micrographs at right, with SPFt but not E94G/K142R effective at upregulating the reporter; corresponding phase contrast images are shown at left. Scale bar, ~10 μm. (c) FACs histograms showing the distribution of GFP-associated fluorescence observed in yeast cell populations transformed with vector, SPFt and E94G/K142R.
minimal effect on protein folding and iron loading functions of the protein (Supplementary Figs 1b–e). SPFt was expressed in yeast cells bearing the FTR1–GFP reporter and induced elevated fluorescence, compared with control cells bearing no SPFT or harbouring a compromised SPFT with E94G and K142R substitutions that eliminate ferroxidase activity of the protein (Fig. 1b). Results of fluorescence microscopy were further validated by fluorescence-activated cell sorting (FACS) analysis (Fig. 1c). Fluorescence histograms from cells transfected with a SPFT expression plasmid displayed a peak of notably higher fluorescence, indicating upregulation of the fluorescent reporter; a second peak with fluorescence comparable to vector control transfected cells was observed under saturating growth conditions and probably arises from SPFT expression plasmid loss in some cells. These results were consistent with the explanation that SPFT expression sequesters cytosolic iron and boosts FTR1–GFP reporter expression.

To isolate mutants that preferentially biominealize more iron in vivo, we subjected the entire PFt coding sequence in SPFt to PCR-based random mutagenesis. After transfection, this resulted in a library of 10 million yeast clones expressing randomly mutated SPFT variants with an average mutation rate of one nucleotide change per gene (Supplementary Fig. 2). This relatively low mutation rate was chosen to avoid accumulation of deleterious mutations, which could obscure beneficial mutations. The yeast library was incubated in a minimum media and sorted by FACS to obtain cells exhibiting highest levels of FTR1–GFP fluorescence. Cells in the top 5% were propagated for a subsequent round of sorting (Fig. 2a), and the procedure was repeated. After four rounds (Fig. 2b), we sequenced the sorted population and identified mutations that were enriched among the selected yeast cells (Supplementary Table 1). Following retesting of the individual-enriched mutations, three were chosen for further analysis: L55P, F57S and F123S.

Characterization of selected SPFT mutants. To confirm the Ft dependence of iron reporter expression in the selected clones, plasmids for SPFT L55P, F57S and F123S were isolated and retransformed for reanalysis by FACS; fluorescence histograms were consistent with the screening results (Fig. 2c). As an additional test of the iron accumulation phenotype, we incubated the three selected clones in iron-supplemented media and measured the total cellular iron content (Fig. 2d) and iron content of purified SPFT proteins (Fig. 2e,f). The most effective of the SPFT mutants, L55P, induced 1.6 ± 0.2 (mean ± s.e., n = 3) times greater cellular iron accumulation than wild-type SPFT and 2.6 ± 0.3 times greater accumulation than HFT. Compared with SPFT, the L55P mutant also exhibited almost double the number of iron atoms per Ft 24-mer, indicating that the cellular biomimetaurization phenotype originates largely from an increase in iron sequestration by Ft at the molecular level. For both L55P and F57S mutants, significant enhancement of cellular iron accumulation (Student’s t-test, P = 0.002, n = 6 for L55P and P = 0.003, n = 6 for F57S) and molecular-level Ft iron loading (P = 0.0003, n = 6 for L55P and P = 0.02, n = 4 for F57S) were observed. Further gains could not readily be obtained by combining these mutations or by performing additional random mutagenesis on the isolated clones. The results nevertheless prove for the first time that intracellular Ft biominalization...
Mechanistic analysis of biomineralization by SPFt variants. In an attempt to understand the mechanism by which primary sequence mutations in SPFt lead to enhanced iron accumulation in the selected Ft holomers, we performed a series of characterization experiments. By inspecting the crystal structure of PfF30, we saw that all three mutant residues point towards the inside of the iron storage cavity and lie on the B and D helices close to a site thought to be involved in oxidation of Fe$^{2+}$ ions that enter the Ft core (Fig. 3a). We speculated that the mutations might therefore affect either the enzymatic functionality of Ft or the structure of the iron oxide core itself. To test these ideas, we began by measuring the iron assimilation and release kinetics of the SPFt variants. No significant differences in iron oxidation (the initial step in core formation) or iron release rates were found (Supplementary Table 2).

To examine potential structural effects of the mutations, we characterized the purified protein nanoparticles by high-resolution cryo-electron microscopy (cryo-EM), a powerful technique that allows imaging of proteins in the near-native environment. Micrographs confirmed that SPFt and the variants all form 12 nm cage-like structures as expected (Fig. 3b). Image autocorrelation analysis indicated the presence of electron dense centres of 4–8 nm diameter for each variant (Supplementary Fig. 5). The L55P variant exhibited a marginally wider autocorrelation profile than other variants, possibly indicating a larger mean core size, while the F123S displayed the narrowest profile. There was more striking variation in the frequency of electron dense cores discernible among the four SPFt variants, however. Only 68.3 ± 1.3% (mean ± s.e.m.) of wild-type SPFt nanoparticles contained dark core structures, whereas 96.1 ± 0.1%, 87.0 ± 0.3% and 78.3 ± 1.5% of the L55P, F57S and F123S mutants, respectively, appeared electron dense (Fig. 3c). Increased core formation in each mutant was significant with respect to SPFt (*t*-test; $P = 0.03$ for L55P, $P = 0.04$ for F57S, $P = 0.04$ for F123S; $n = 2$ samples with 400 particles per sample), suggesting that an increased ability of the mutant proteins to nucleate mineral core formation might largely account for their ability to accumulate a larger number of iron atoms per protein molecule. This explanation might also be compatible with the finding that the selected SPFt mutations could not be combined to further improve iron loading, given the possibility that enhanced mineral

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**Figure 3 | Structural analysis of SPFt variants.** (a) X-ray crystal structure of PfF displaying the internal cavity of the protein in which one of the subunits is highlighted in yellow (left panel). Enlarged image of the highlighted subunit (right) shows the relative positions of sidechains mutated in the selected biominalization mutants (L55P, F57S and F123S) with respect to the ferroxidase residues (yellow) and the known iron binding sites (grey balls). (b) Cryo-EM images of purified SPFt, L55P, F57S, and F123S, showing formation of 12 nm spherically shaped nanoparticles in each case. SPFt samples also display differences in electron dense iron core formation, as indicated by the variable frequency of ‘empty’ particles in the images (for example, yellow arrowheads). Scale bar, 50 nm. (c) The percentage of particles containing electron dense cores was computed by analysing 400 particles in cryo-EM images of each SPFt variant. All selected mutants displayed higher frequencies of core formation than the starting clone (*t*-test, $P \leq 0.04$), with L55P showing the greatest effect. Error bars denote s.e.m. of measurements from two independent samples.
nucleation and growth at one site might not be compatible with nucleation directed at another site, and that competing nucleation and growth at multiple sites decreases homogeneity of mineral crystal formation.31

Magnetic sorting and imaging using hypermagnetic SPFt. Our strategy for engineering hypermagnetic SPFt variants was predicated on the notion that iron sequestration by SPFt mutants would accompany enhanced magnetic properties. To demonstrate this, we explored the utility of hypermagnetic SPFt variants in imaging and high-gradient magnetic cell separation (HGMS) applications. For MRI experiments, the same yeast samples used for the iron assays in Fig. 2d were pelleted and imaged in a 7 T magnet using a spin-echo acquisition sequence. The transverse relaxation rate ($1/T_2$) of cells transformed with the most iron-rich Ft mutant, L55P, was significantly higher than that of cells expressing wild-type SPFt ($58.2 \pm 3.7 \text{ s}^{-1}$ vs $30.0 \pm 2.5 \text{ s}^{-1}$, t-test $P = 0.001$, $n = 4$) or human HFt ($21.9 \pm 0.9 \text{ s}^{-1}$, $P = 0.001$, $n = 3$), indicating that the hypermagnetic mutant L55P indeed shows higher sensitivity as an intracellularly expressed MRI contrast agent (Fig. 4a). The ability of SPFt L55P to enhance magnetic capture in HGMS was assessed by comparing the mutant protein to wild-type SPFt and Ft-free control cells. Yeast cells-expressing L55P were retained with four times greater magnetic capture in HGMS than cells-expressing L55P and growth in overnight in medium containing 1 mM ferric citrate before sample preparation (left, scale bar, 500 nm). A closeup of the mineral core and visible on close examination of arbitrary cytosolic fields of view (Fig. 5a). Comparable images from control did not reveal similar puncta. As a quantitative indication of this difference, we used an automated template-matching procedure to identify approximately Gaussian electron-dense spots of 7 nm full width at half height in multiple TEM images of both SPFt-expressing and control yeast cells. Puncta that closely matched the template (correlation coefficient $= 0.9$) were counted in cytosolic regions only (Fig. 5b). This analysis indicated a concentration of $220 \pm 40$ puncta $\mu$m$^{-3}$ from SPFt cell images ($n = 10$) but only $70 \pm 8$ puncta $\mu$m$^{-3}$ from control images ($n = 4$), a significant difference (t-test $P = 0.05$) supporting the identification of these spots with SPFt nanoparticles. This suggests that SPFt variants could indeed function as TEM-detectable genetically encoded labels in engineered yeast and perhaps other cells.

Construction of an intracellular sensor using SPFt L55P. In addition to potential utility for magnetic cell sorting, cellular MRI and electron microscopic investigations, the hypermagnetic SPFt variants are potentially useful building blocks for incorporation into magnetic molecular devices. As demonstration of this idea, we constructed a SPFt-based magnetic biosensor for galactose-induced gene expression in yeast (Fig. 6a). To design the biosensor, we made use of the dependence of magnetic
relaxation properties on the aggregate state of iron-loaded Ft molecules. Aggregation of purified SPFt L55P variant in buffer could be induced by mixing purified protein with streptavidin (SA) tetramers, which provide a multivalent partner for interactions with the Strept-tag II moiety on SPFt. Cluster formation could be observed at molecular scale by cryo-EM (Fig. 6b), and also resulted in visible precipitation and dynamic light scattering (DLS) changes (Supplementary Fig. 6). SA-mediated clustering could be promoted substantially by engineering SA for optimal folding and selective binding of Strep-tag II versus biotin. The SA/SPFt oligomer ratio required for half maximal clustering was 0.8 for optimized SA, but 60 for wild-type SA (Supplementary Fig. 7). In this respect, the optimized SA variant achieved maximal aggregation close to the conditions of 1:1 oligomer stoichiometry expected to optimize clustering under idealized conditions. On the other hand, the large excess of wild-type SA required for SPFt clustering, which for the optimized SA would likely inhibit aggregation, probably reflects the inferior stability and SPFt-binding characteristics of the non-engineered SA. Titration of SPFt aggregation with optimized SA was measured by MRI, where a half maximal change in $1/T_2$ was observed at SA/SPFt ratio of 0.4 (Fig. 6c), consistent with the results from DLS.

Although Ft aggregation-based sensors have been demonstrated previously in cell-free solutions, none has yet been reported to produce detectable responses on genetically directed intracellular expression. To demonstrate that this key step could be achieved using the magnetic molecular sensor formed by hypermagnetic SPFt and SA tetramers, a construct directing constitutive expression of the SPFt mutant was cotransformed with a galactose-inducible construct encoding optimized SA into yeast and grown in iron-supplemented medium overnight. Cells were transferred to galactose-containing medium to induce SA expression, followed with characterization by MRI, iron quantification and western blot analysis at time points 0, 2 and 4.5 h after the induction of SA overexpression (Fig. 6d). Relaxation rates were normalized by cellular iron concentration to factor out differences in iron accumulation over time, and results were compared with control experiments using Pft L55P, which lacks the ability to cluster with SA, in place of SPFt L55P. The SPFt variant displayed 7, 14 and 20% greater normalized $1/T_2$ than the Pft control at the 0, 2 and 4.5 h time points, respectively; differences at 2 and 4.5 h were significant with Student’s t-test $P = 0.02$ and 0.04, respectively. These results are consistent with the data showing increased relaxivity of SA-SPFt clusters versus unclustered SPFt in buffer. In contrast, no significant effects could be observed when SA expression was induced in the presence of SPFt K142R or Pft K142R mutants lacking iron-loading functionality (Supplementary Fig. 8), indicating that iron loading by the SPFt variants is required for relaxivity changes measured by MRI. These results together show that genetically encoded magnetic devices formed from SPFt mutants can be formed and applied inside cells. Such devices represent prototypes for a wide variety of noninvasive imaging sensors that could be expressed in cells, where they are likely to exhibit faster and more versatile responses than could be obtained by altering Ft expression itself. Yeast bearing SPFt-based magnetic devices might also be applicable as whole-cell-based sensors in opaque media or living organisms.

**Figure 6** | Construction of a SPFt-based intracellular magnetic biosensor. (a) Design of a genetically encoded magnetic biosensor for dynamic gene reporting using engineered SPFt. The sensor consists of two components: constitutively expressed SPFt L55P nanoparticles (grey) and streptavidin (SA) tetramers (orange) expressed from a galactose-inducible gene shown in the nucleus. When SA is upregulated, it crosslinks SPFt oligomers via their Strept-tag II moieties (blue), forming clusters that enhance $T_2$ relaxation rates and provide a means for magnetic detection of galactose-induced SA expression. (b) Representative cryo-EM images showing aggregate formation by purified SPFt L55P in the presence (below) but not the absence (above) of SA. Scale bar, 50 nm. (c) SA-dependent changes in the relaxation rate ($1/T_2$) displayed by solutions of 0.2 μM SPFt L55P holomers loaded with 520 μM Fe in the presence of increasing tetramer concentrations of an SA variant optimized for stability and Strept-tag II binding. Error bars show s.e.m. of three independent titrations, and the inset displays representative $T_2$-weighted MRI images corresponding to conditions shown in the graph. (d) Magnetic detection of SA-mediated intracellular clustering of SPFt. SPFt L55P and galactose-inducible SA were coexpressed in yeast as schematized in (a). After 16 h in galactose-free medium (10 mM Fe), cells were transferred to 2% raffinose media without iron for 2 h. Then galactose was added to the culture at a final concentration of 2% to induce SA expression and cells were harvested at 0, 2 or 4.5 h for analysis. $T_2$ relaxation rates measured by MRI were normalized by cell pellet iron content and were compared with control experiments performed with Pft L55P (grey bars), which accumulates iron but is not crosslinked by SA. Normalized $1/T_2$ values at 2 h and 4.5 h were significantly higher in the SPFt-expressing cells (Student’s t-test $P = 0.02$–0.04, $n = 3$), consistent with the mechanism in (a) and the results of (c). Error bars indicate s.e.m. of three independent experiments and corresponding protein levels revealed by Western blotting against SA and the Strept-tag II moiety of SPFt are presented above.
The specific mutagenesis and screening approach taken here is one of a universe of approaches that could have been taken to obtain desirable Ft biominalization mutants. Selecting for iron accumulation as opposed to magnetic properties biased the screen towards variants with increased mineral core formation (Fig. 3 and Supplementary Fig. 5), potentially enhancing the performance of selected variants as TEM labels (Fig. 5). For magnetic applications such as imaging and magnetic sorting (Fig. 4), as well as magnetic biosensing (Fig. 6), screening directly for magnetic properties might have been more direct, but magnetic assays tend to be both less sensitive and cruder. For instance, our own initial experience with magnetic column-based selection approaches (cf. Fig. 4b) revealed several sources of artifacts, ranging from nonspecific column adhesion to cell clumping, which would compromise the efficacy of a screen. Although further improvement in magnetic screening techniques is certainly possible in the future, indirect screening using optical approaches proved useful here, as in our earlier work with magnetically active MRI sensors. With either type of approach, careful molecular analysis of selected clones must be performed to avoid mutations unlinked to the desired phenotype (such as changes in growth rate), but nevertheless artificially favored by the screening technique.

Although all of our experiments were performed in yeast, the protein engineering principles applied here and potentially the specific clones identified could be applied for biotechnological applications in other eukaryotic or prokaryotic cellular environments. Further optimization could be necessary to enhance Ft variant expression in the desired host, or to adapt the magnetic proteins to iron homeostasis and protein folding conditions in other systems. In any context, manipulating mineral nucleation could prove to be a general and versatile route for tuning intracellular biominalization, particularly if unnatural mineral species are desired. Protein engineering approaches like those introduced here could also be used to engineer additional metalloproteins, and could further alter other parameters of genetically expressed magnetic biomaterials and biosensors.

**Methods**

**Yeast strain and handling methods.** We used the haploid yeast {S. cerevisiae} strain BY4742/FR1-GFP (MAT α/FR1-GFP::HIS3 MX his3 leu2Δ0 lys2Δ0 ura3Δ0) (gift from Dr Christopher Burd) as a host for expression of all Ft variants. We grew yeast cells in a dropout medium without histidine (SD-HIS) made with a dry culture medium (Teknova, Hollister, CA) or in a YPAD medium: 10 g/l yeast extract (BD Biosciences, San Jose, CA), 20 g/l of Bacto Peptone (BD Biosciences), 20 mg/ml of adenine hemisulfate and 20 g/l glucose. We transformed yeast cells with expression plasmids using the Frozen-EZ Yeast Transformation II kit (Zymo Research, Irvine, CA).

**Construction of Strept-tag II/ferritin fusion proteins.** We used Escherichia coli NEB10Δ cells (New England Biolabs, Ipswich, MA) for plasmid construction. To create an expression plasmid with a dominant selectable marker, we used the PCR to amplify a geneticin resistant cassette, KanMX4 from a plasmid pFA6-kanMX4.1 kindly provided by Dr Peter Philippsen. We subcloned the PCR product containing KanMX4 fragment into the pHVB yeast expression plasmid generously supplied by Dr Henrie Van Vuuren. We then made a point deletion to destroy a FTR1 site by the QuikChange Lightning Kit (Agilent Technologies, Santa Clara, CA) to yield the host plasmid, pHVX2G, used for subsequent expression of Ft constructs in our experiments. We amplified ferritin gene of Pf at the genomic DNA of the bacteria (ATCC, Manassas, VA). A Strept-tag II sequence (WSHPQFEK), spacer (GTSS) and restriction sites were genetically fused at the 5′-end of the Pf gene and the PCR product was subcloned into pHVX2G to yield plasmid pHVX2G-SFPi (Supplementary Table 3).

**SFPi expression and affinity purification.** For expression of SFPi, we inoculated yeast cells with expression plasmids in 1 ml of YPDA medium with 200 µg/ml –1 Geneticin and incubated overnight at 30 °C. Then we diluted the cultures into fresh media at O_D600 = 0.04 and incubated them for 16 h at 30 °C before harvesting. We washed the freshly harvested yeast with 30 ml of PBS + 10 mM EDTA twice and finally resuspended in PBS. We lysed yeast cell pellet with Y-PEF Plus (Thermo Scientific, Waltham, MA), benzonase nuclease (EMD Millipore, Billerica, MA) and protease inhibitors according to the manufacturer’s instructions. We then centrifuged the lysate at 3,000 g for 20 min at 4 °C. SFPi protein was purified by applying the cleared lysate into the Strept-Tactin sepharose column (IBA, Göttingen, Germany) according to the manufacturer’s instructions. EDTA that was omitted from the wash and the elution buffers. We buffer exchanged and concentrated the purified protein into the wash buffer using a spin filter with 100 kDa cutoff membrane (EMD Millipore). We measured the protein concentrations by the Pierce 660 nm Protein Assay (Thermo Scientific), with bovine serum albumin (BSA) as a standard.

**TEM of purified SFPi.** For conventional TEM, we applied 1–3 µl of 0.05 mg/ml –1 SFPi sample onto a carbon/copper-coated grid (Electron Microscopy Sciences, Hatfield, PA), removed the excess solution with a filter paper and let it dry for 30 s. We then applied 15 µl of 1% phosphotungstic acid (pH 7.0) over the sample for about 10 s and removed the excess stain with a filter paper. The grid was dried at room temperature for at least 1 h before imaging with a JEOL 2010 HRTEM instrument (JEOL, Tokyo, Japan). For cryo-EM, we applied 5 µl of the protein and buffer solution on a lacey copper grid coated with a continuous carbon film and removed excess sample without damaging the carbon layer using a Gatan Cryo Plunge III (Gatan, Pleasanton, CA). We then diluted the grid on a Gatan 626 cryo-holder equipped in the TEM column and kept it under liquid nitrogen throughout the transfer into the microscope and the subsequent imaging session. We imaged the SFPi samples on a JEOL 2100 FEG microscope (JEOL) using a minimum-dose method that was essential to avoid sample damage under the electron beam. We imaged at 200 kV with a magnification of 15,000 × for assessing the size and distribution of the images on a Gatan 2k × 2k UltraScan CCD camera (Gatan, Pleasanton, CA). To calculate the percentage of filled cores, we counted 400 particles per sample and divided the number of filled particles by 400. For each SFPi variant, we obtained cryo-EM images of the protein samples from two different batches to calculate mean, s.e.m., and standard deviation. To estimate core sizes, autocorrelation functions were computed from the same TEM images (three per variant) in Matlab, generating autocorrelation plots and radial profiles presented in Supplementary Fig. 5.

**Library construction.** We carried out library construction using an error-prone PCR approach. The entire SFPi gene except for the Strept-tag II sequence was subjected to mutagenesis over 30 error-prone amplification cycles, which yielded on average one amino acid mutation per SFPi gene. The linearized vector was prepared by digesting pHVX2G with Apal and Xhol followed by gel purification. We transformed yeast with the SFPi library according to the method developed by Benatuli et al. with a few modifications. We mixed 1.5 µg of digested plasmid and 0.5 µg of error-prone PCR product with 100 µl of electrocompetent cells (= 1.6 × 10^14 cells per ml) in a disposable electroporation cuvette with 0.2 cm gap (Bio-Rad, Hercules, CA) on ice for 5 min. We electroporated the cells at 3 kV using MicroPulser electroporator (Bio-Rad), resulting in time constants ranging from 4.8 to 5.3 ms. After electroporation, we immediately transferred the cells to 1:1 mix of 0.1 M calcium chloride and supplemented yeast medium, and incubated at 30 °C for 3 h. We then harvested cells by centrifugation and resuspended in SD-HIS with 200 µg/ml –1 of Geneticin and incubated for 2 days before freezing them for long-term storage at −80 °C. Typical transformation efficiency was 0.5–1.0 × 10^7 transformants per µg of plasmid DNA. The library diversity was tested by sequencing randomly picked 24 colonies.

**Measurements of iron content in cells and purified protein.** We used a colorimetric assay based on the protocol of Tamirit et al. to quantify the iron content of yeast cells and the purified protein. This method relies on the Fe^2+/^3- dependent optical absorbance of bathophenanthrolineisulfoxonic acid (BPS) at 535 nm at pH 5.4. As standards, we dissolved known amounts of ferrous ammonium sulfate in 3% nitric acid. For measuring the iron content of yeast cells, we digested 4.2 × 10^8 cells by boiling in 200 µl of 3% nitric acid for 2 h, and centrifuged at 10,000 g for 5 min. To measure the concentration of iron in SFPi, a 1:1 ratio of purified protein and 3% nitric acid solution were mixed and boiled for 15 min followed by centrifugation at 10,000 g for 5 min. In both cases, the iron quantification assay was applied to the supernatant of the resulting samples. Iron loading stoichiometries of the protein samples were computed by dividing the iron concentrations by the protein concentrations, as measured by the 660 nm Protein Assay (Thermo Scientific).

**High-throughput screening.** We inoculated 1 × 10^8 cells in a 20 ml SD-HIS medium containing 200 µg/ml –1 of Geneticin at 30 °C overnight (about 16–20 h). We harvested the cells in a culture tube and resuspended in a sterile PBS such that the cell density was about 5 × 10^7 cells per ml. We filtered the cells with a sterile membrane with 40 µm pores immediately before sorting. Similarly, we prepared negative control samples using the BY4742 background strain without the FTR1–GFP reporter. We set up a flow cytometry protocol using the control yeast samples. First, the yeast population was gated with forward and side scattering channels to remove debris and aggregated cells. We then collected cells displaying a fluorescence value of 5%–9%, indicating high FTR1-GFP expression. We propagated these cells overnight in 4 ml of SD-HIS medium supplemented with 200 µg/ml –1 of Geneticin.
Measurement of iron oxidation and release kinetics. We monitored the kinetics of iron oxidation by SFTP variants by an optical assay. We prepared SFTP variants with 100 Fe/24-mer in 100 mM MOPS, pH 7.0. We added ferrous ammonium sulfate solution (1 mM), made in deaggessed distilled water to the protein solution (final concentration of 0.1 μM) at a 500-fold molar excess of iron(II). Following a mixing dead time (~5 s), we recorded the optical absorbance of the mixture at 315 nm every 2 s for 5 min. We used a disposable cuvette with a 1 cm path length and recorded the spectra with SpectraMax M2 Microplate reader (Molecular Devices, Sunnyvale, CA). We calculated the specific activity, defined as the micromoles of iron(III) formed per minute per milligram of 24-mer SFTP by dividing the change in absorbance of the reaction mixture over the first 30 s by the extinction coefficient of SFTP variants and the absorbance at the measuring wavelength in the reaction. Extinction coefficients for wild type SFTP, L55P, F57S and F123S was 2.6 ± 0.1, 2.7 ± 0.1 and 2.8 ± 0.1 μM cm⁻¹, respectively.

We measured the kinetics of iron release from preloaded SFTP variants by monitoring time dependent formation of the BPS complex with Fe⁺ released from iron-loaded Ft variants. We used purified SFTP variants that were loaded aerobically with 1,000 Fe atoms per molecule. These samples were diluted to a final concentration of 0.1 μM SFTP oligomers in an iron mobilization assay buffer that included MOPS (0.1 M, pH 7.0), sodium acetate (20 mM) and BPS (1 mM). We measured the absorbance values at 535 nm every 30 s for 3 h using SpectraMax M2 Microplate reader. We took the first 3.5 min of the data and computed the initial rate of iron release using the standard curve constructed using freshly made ferrous ammonium sulfate solutions.

Measurement of magnetization curves. 100 μl of each SFTP variant dispersed in Tris buffer (0.1 mg ml⁻¹ Fe) was sealed in a propylene straw using a hot press. Zero field cooled curves at 5 K were measured using a superconducting quantum interference device (MPPS/XXL, Quantum Design, San Diego, CA). Diamagnetic background signal was subtracted by measuring a buffer only sample sealed in the same manner.

Measurement of denaturation profiles. SYPRO dye (Life Technologies) was diluted 25-fold from the manufacturer’s 5,000 μl stock into 1 ml of 0.1 M Tris, 0.15 M NaCl, pH 8.5. 1.5 μl of each SFTP variant (0.39 mg ml⁻¹) was mixed with 1.5 μl SYPRO solution and 27 μl GdmHCl to a final concentration of 0–8 M GdmHCl. After 10 min incubation at room temperature, fluorescence intensity was measured using a plate reader with excitation at 567 nm and emission at 580 nm.

Yeast cell pellet MRI. We prepared the yeast samples as described in SFTP expression and purification section. After we washed the cells twice with PBS supplemented with 10 mM EDTA, the supernatant was decanted and 100 μl of the cell suspension was loaded into the wells of a microtiter plate. The yeast cells were grown in YPD medium supplemented with 1 mM ferric chloride, 5 mM MgCl₂, 2.5% sucrose. Cells were embedded in 2% ultralow melting point agarose and cut into small pieces. Sample blocks were allowed to dry for 1-2 days. To test the SFTP-based biosensing system in cells, we transformed yeast cells into 2% raffinose plus 0.1% glucose medium and incubated for 2 h. We then induced expression of SA by adding galactose at a final concentration of 0.4 M.

DLS measurements. We performed DLS measurements on a DynaPro DLS system (Wyatt Technology, Santa Barbara, CA), at 30 °C with averaging over 72 acquisitions each and a 2 s integration time. The laser power was set to 25%. We mixed 16 μl of 0.2 μM 24-mer SFTP sample with various concentrations of SA tetramers, briefly vortexed, and incubated for 5 min before making the DLS measurements in triplicates.

Coexpression of SFTP variants and SA in yeast. To test the SFTP-based biosensing system in cells, we transformed yeast cells with two expression plasmids, pHVX2G-SFTP-L55P and pSAZT5m encoding SFTP L55P or optimized mutant SA, respectively. Control experiments were performed using plasmids encoding PFT L55P, SFTP E94K/K142R, or PFT E94K/K142R in place of SFTP L55P. We first inoculated the yeast cells in rich medium with 2% glucose and 10 mM ferrous ammonium sulfate overnight to allow SFTP or PFT expression and iron loading. We then transferred the yeast cells into 2% raffinose plus 0.1% glucose medium and incubated for 2 h. We then induced expression of SA by adding galactose at a final concentration of 2% and harvested cells at 0, 4.5 h time points to measure expression levels and make MRI and iron content measurements from cell pellets.

Western blot analysis of yeast cell pellets. For western blotting experiments, the whole cell lysate samples were prepared from yeast cells freshly harvested after overnight incubation according to the method developed by von der Harz with a few modifications. Equivalent numbers of yeast cells (2.1 × 10⁹) were resuspended in 100 μl of the lysis buffer and boiled for 10 min. The cell suspensions were neutralized by the addition of 2.5 μl of 0.1 M acetic acid, vortexed for 1 min, and boiled for a further 5 s. We then added loading buffer containing 1% Triton X-100 and 4 M acetic acid, and centrifuged them at 10,000 g for 5 min before loading onto a 12% Mini-PROTEIN TGX Precast gel (Bio-Rad). We ran the protein gels at 160 V for 30 min and transferred

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the separated proteins onto PVDF membranes (Bio-Rad) at 100 V for 40 min at 4 °C. The membranes were blocked with 5% fat-free milk in Tris-buffered saline (AMRESCO, Solon, OH) containing 0.1% Tween 20 (TBST) or 30 min at 4 °C. For visualization of SPFT variants, we washed the membranes once with TBST for 5 min and incubate them with Strep-Tac horse-radish peroxidase conjugate (IBA, Gottingen, Germany) at 1:4,000 dilution in TBST for 1 h at room temperature. For imaging expression of SA, we used an anti-streptavidin antibody conjugated to horse-radish peroxidase (Abcam, Cambridge, UK) at 1:100,000 dilution in TBST. After washing the membranes three times with TBST, we visualized the SPFT and SA bands with a chromogenic substrate Opti 4CN (Bio-Rad) according to the manufacturer’s instructions. Images of the membranes were taken and processed with ImageJ software for quantitative analysis.

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Additional information

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