magnetochemistry

MDPI

Supporting material

Saccharomyces cerevisiae and *Candida albicans* yeast cells labeled with paramagnetic Fe(III) based MRI probes

Akanksha Patel [1], Didar Asik [1], Eric M. Snyder [1], Joseph A. Spernyak [2], Paul J. Cullen [3] and Janet R. Morrow [1,*]

[1] Department of Chemistry, University at Buffalo, The State University of New York, Amherst, NY 14260, USA; apatel27@buffalo.edu (A.P.); didarasi@buffalo.edu (D.A.); ericsnyd@buffalo.edu (E.M.S.)

[2] Department of Cell Stress Biology, Roswell Park Comprehensive Cancer Center, Buffalo NY 14263, USA; Joseph.Spernyak@roswellpark.org

[3] Department of Biology, University at Buffalo, The State University of New York, Amherst, NY 14260, USA; pjcullen@buffalo.edu

* Correspondence: jmorrow@buffalo.edu

Received: 13 August 2020; Accepted: 31 August 2020; Published: 4 September 2020

Contents Page

Table S1. Relaxivity values for Fe(III) complexes measured at 4.7 T, 20 mM HEPES pH 7.2, 100 mM NaCl at 37 °C.

Complex	**μ_{eff} (BM)**	**r_1 (s·mM)$^{-1}$ pH 7 at 4.7 T**	**r_1 (s·mM)$^{-1}$ pH 7 at 9.4 T T**	**r_2 (s·mM)$^{-1}$ pH 7 at 4.7 T**
Fe(TOB)[a]	5.9	2.20 ± 0.30	2.40 ± 0.24	4.47 ± 1.07
Fe(TOBA) [a]	5.6	1.71 ± 0.10	-	5.05 ± 0.48
Fe(TzB) [a]	5.7	0.81 ± 0.07	-	1.45 ± 0.12
Fe(TACO) [b]	5.9	0.97 ± 0.12	1.40 ± 0.08	1.4 ± 0.08
Fe(TASO) [b]	5.8	2.00 ± 0.19	2.00 ± 0.2	2.0 ± 0.20

[a] data obtained from ref 1

[b] data obtained from ref 2

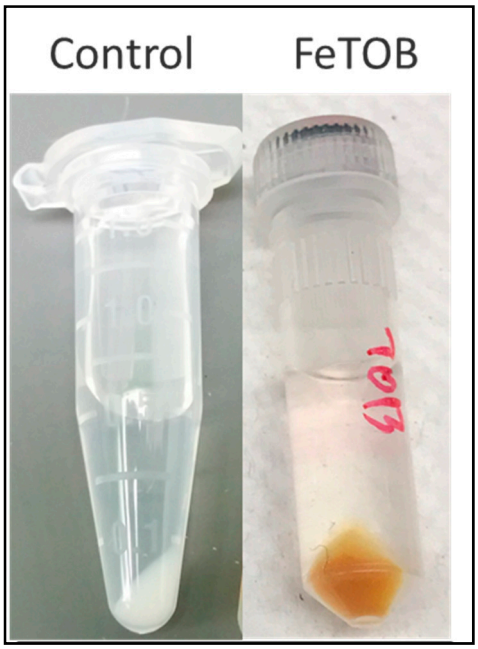

Figure S1. Color change of *S. cerevisiae* pellet upon treatment with Fe(TOB)

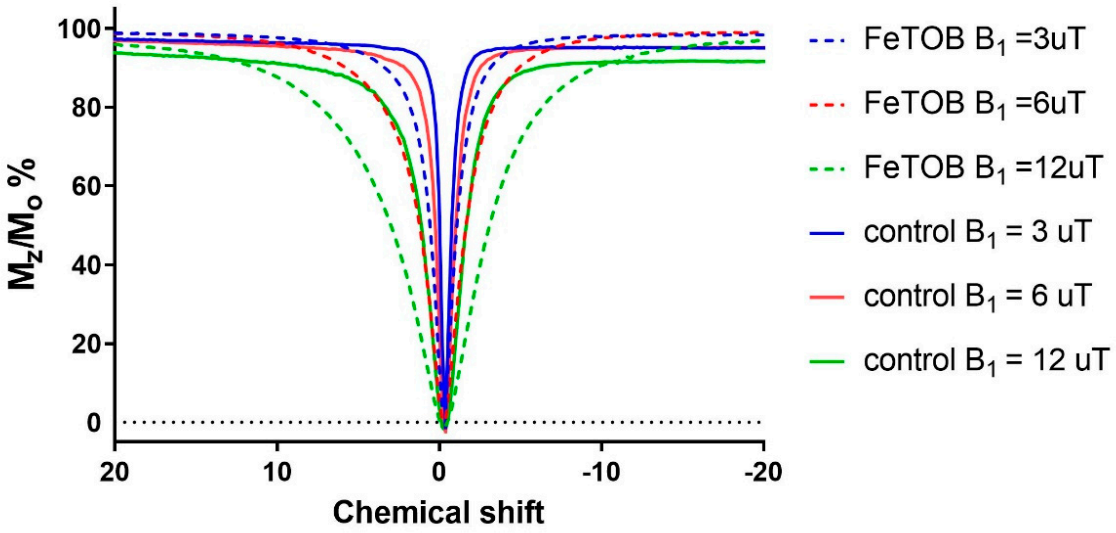

Figure S2 Z-spectra of (a)control and (b)10 mM Fe(TOB) labeled through endocytosis in *S. cerevisiae* in 1x PBS at 37 °C.

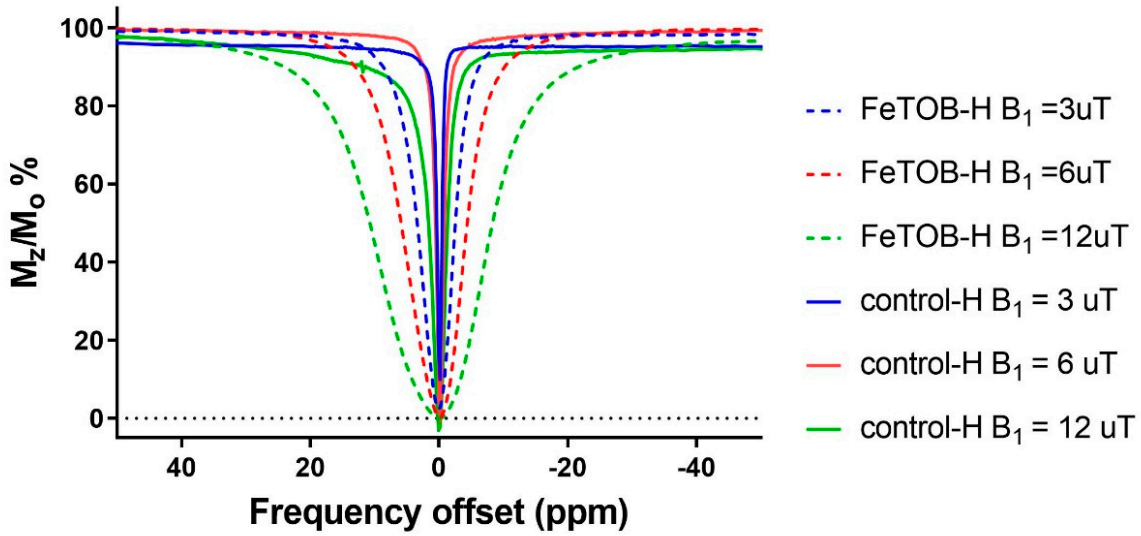

Figure S3. Z-spectra of (a)control and (b)10 mM Fe(TOB) labeled through heat shock in *S. cerevisiae* in 1x PBS at 37 °C.

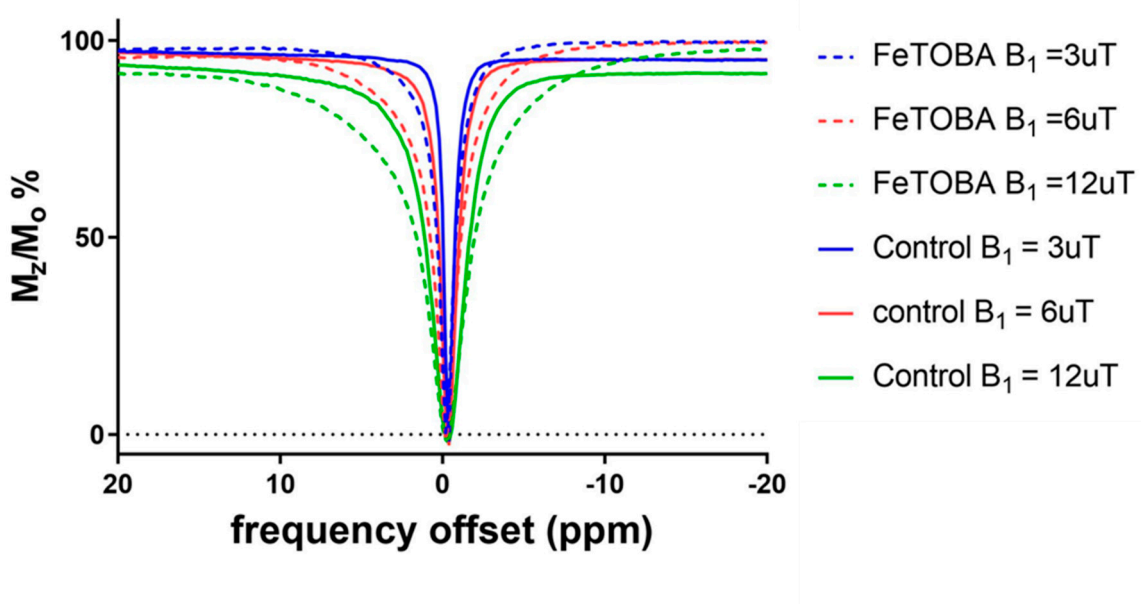

Figure S4. Z-spectra of (a)control and (b)10 mM Fe(TOBA) labeled through endocytosis in S. cerevisiae in 1x PBS at 37 °C.

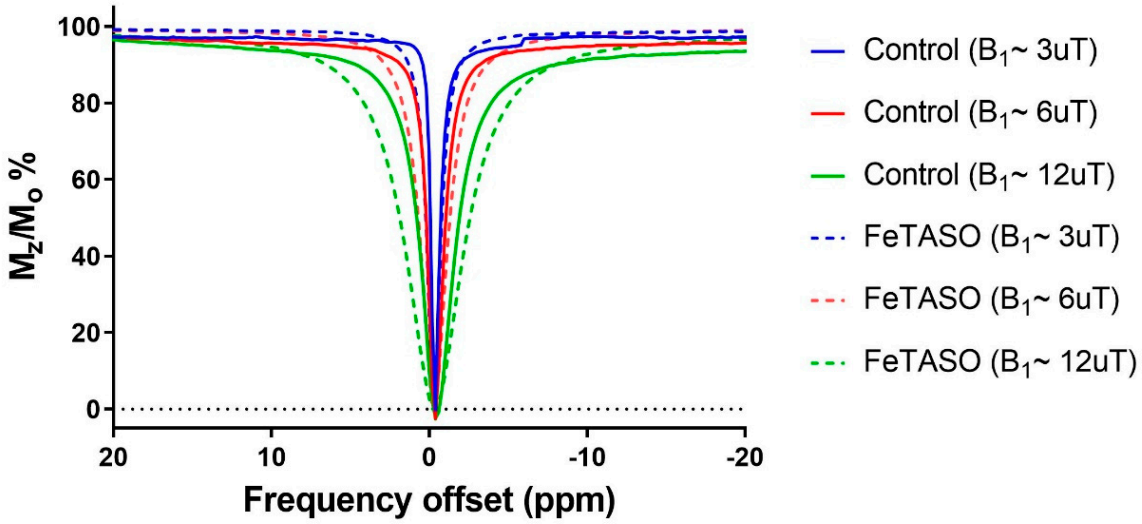

Figure S5. Z-spectra of (a)control and (b)10 mM Fe(TASO) labeled through endocytosis in S. cerevisiae in 1x PBS at 37 °C.

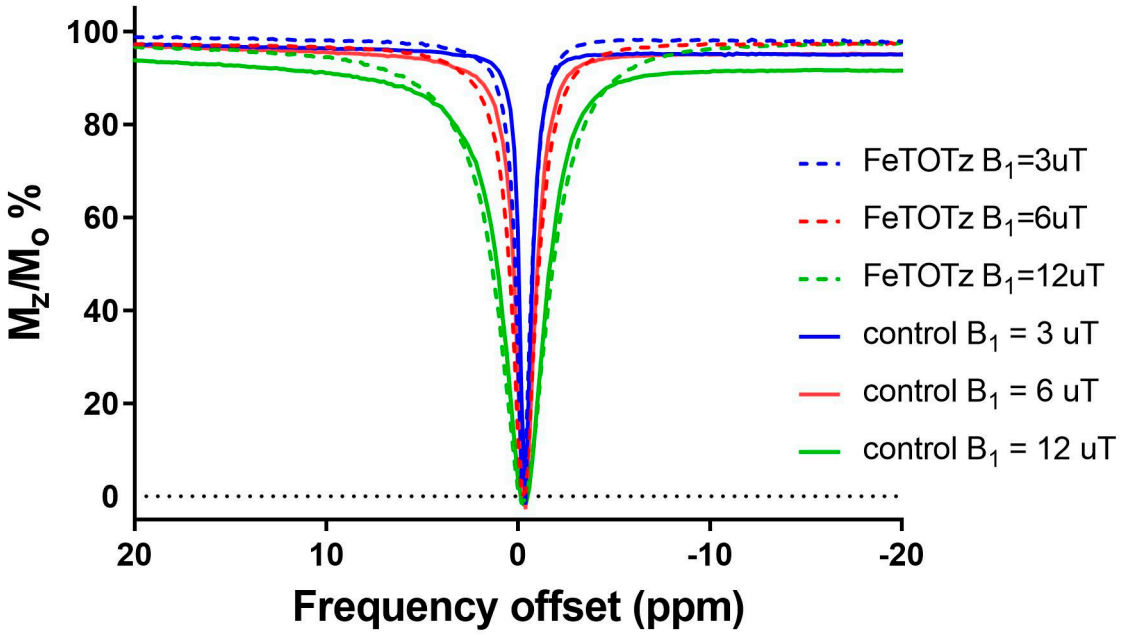

Figure S6. Z-spectra of (a)control and (b)10 mM Fe(TOTz) labeled through endocytosis in S. cerevisiae in 1x PBS at 37 °C.

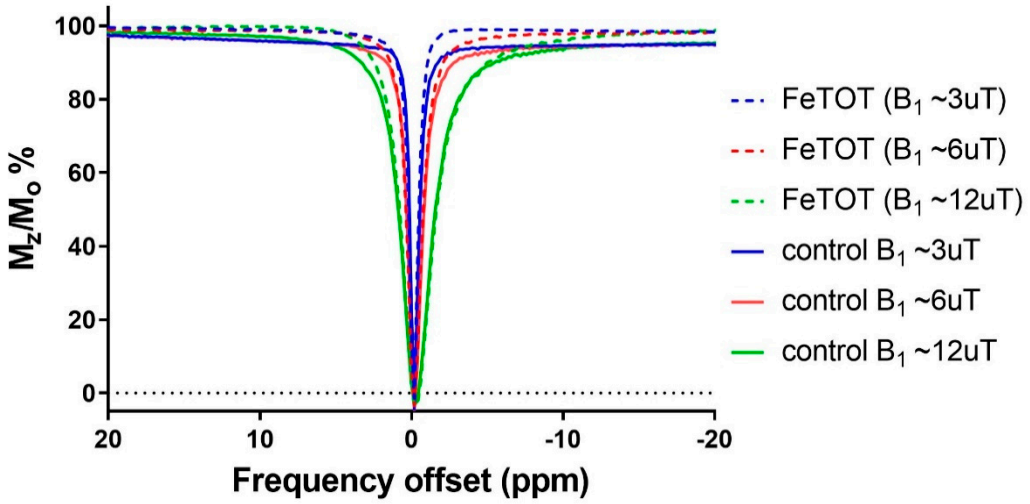

Figure S7. Z-spectra of (a)control and (b)10 mM Fe(TOT) labeled through endocytosis in *S. cerevisiae* in 1x PBS at 37 °C.

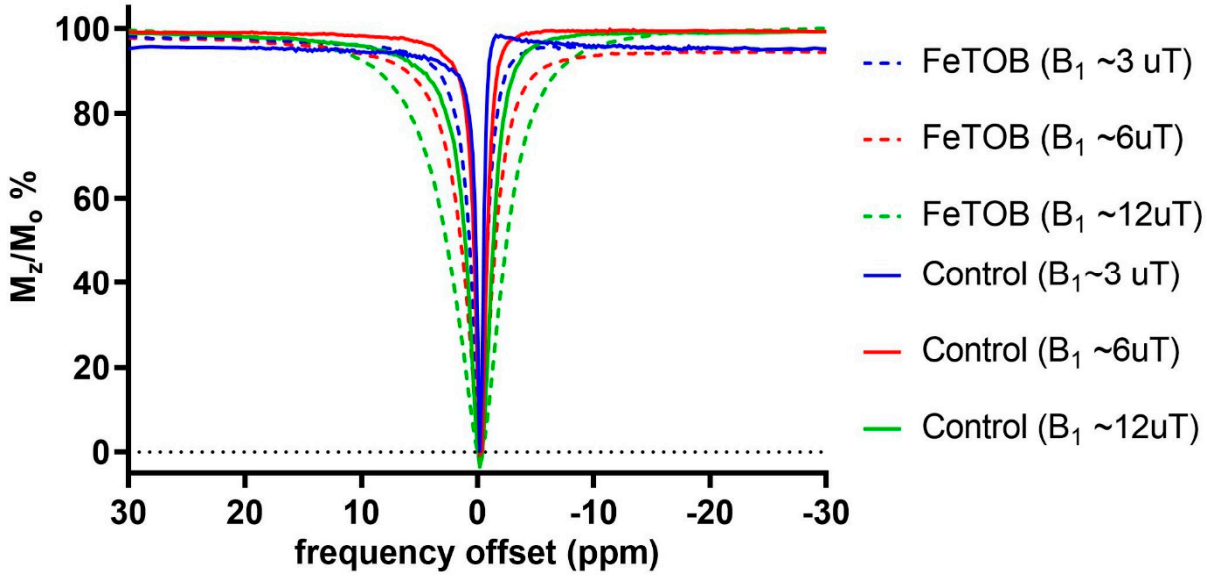

Figure S8. Z-spectra of (a)control and (b)10 mM Fe(TOB) labeled through endocytosis in *C. albicans* yeast in 1x PBS at 37 °C.

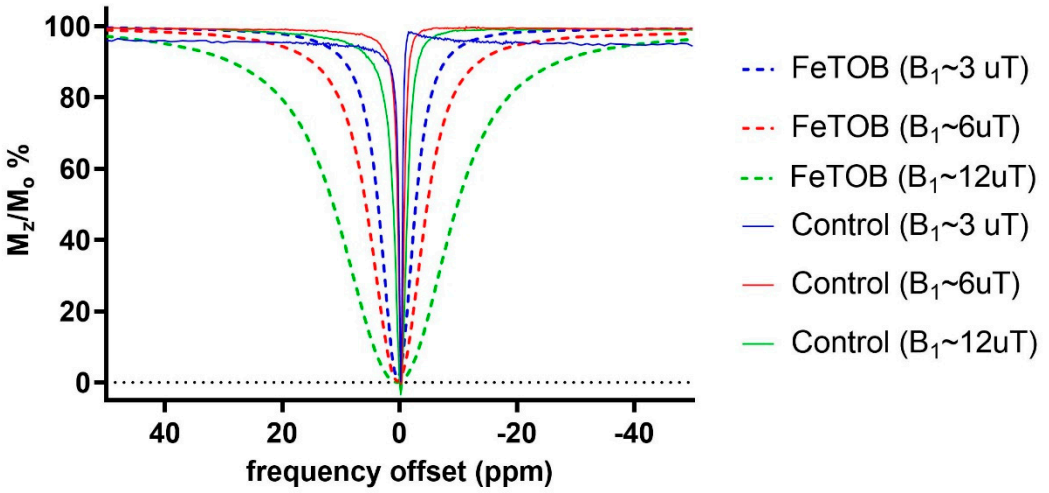

Figure S9. Z-spectra of (a)control and (b)10 mM Fe(TOB) labeled through endocytosis in *C. albicans* hyphae in 1x PBS at 37 °C.

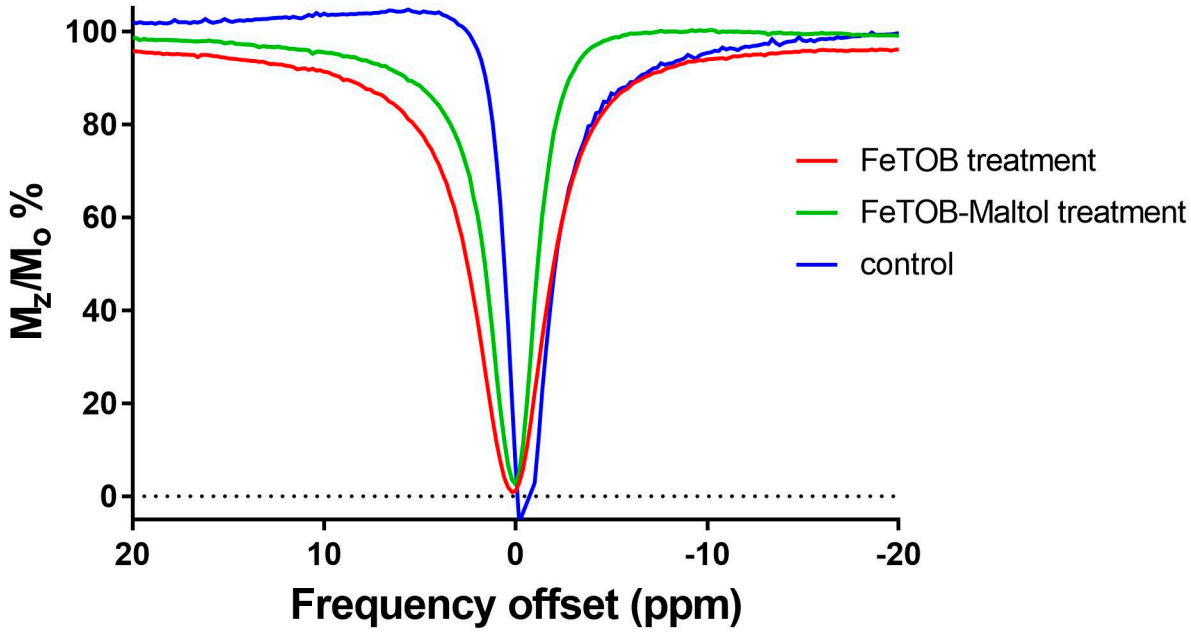

Figure S10. Z-spectra of (a)control and (b)10 mM Fe(TOB) labeled through endocytosis in *S. cerevisiae* hyphae in 1x PBS at 37 °C.

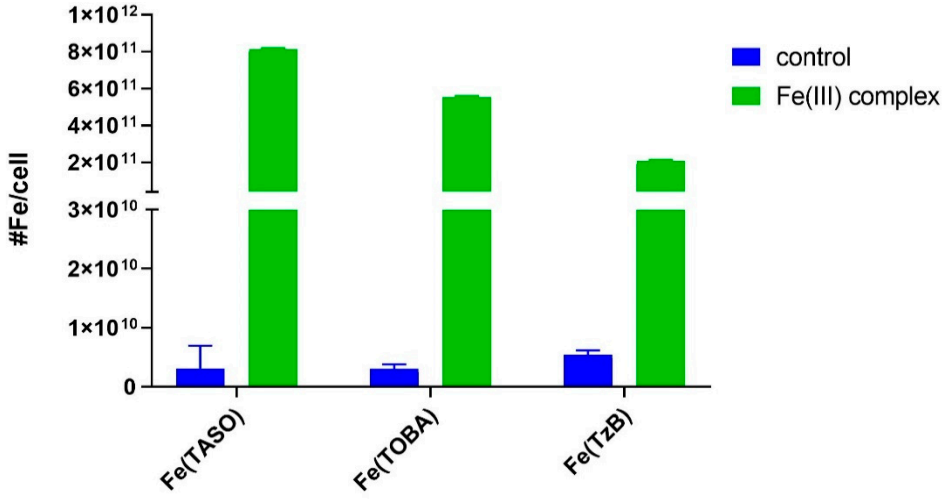

Figure S11. Total Fe content in yeast cells upon endocytosis treatment with 10 mM Fe(III) complexes in *S. cerevisiae* as measured by ICPMS. Mean ± SE is reported.

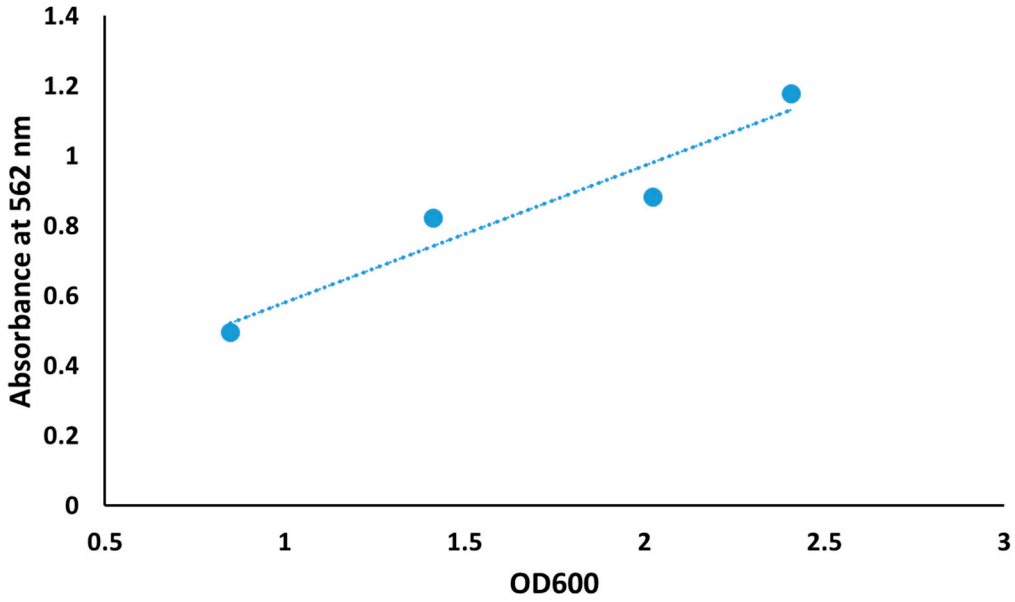

Figure S12. Plot of the total protein content measured by Pierce protein assay vs optical density at 600 nm in *S. cerevisiae* in midlog phase. The graph was fit to linear regression with $R^2 = 0.9196$.

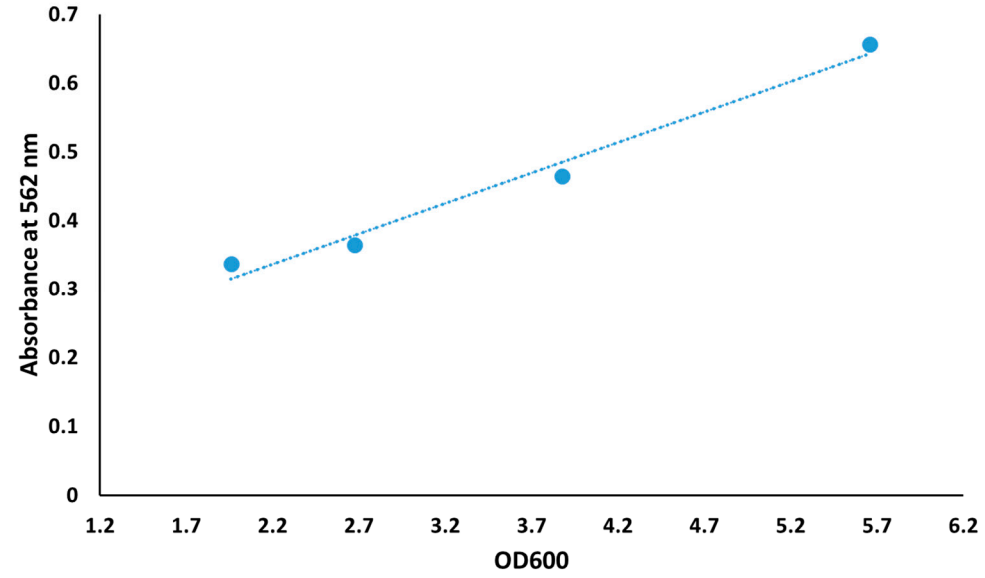

Figure S13. Plot of the total protein content measured by Pierce protein assay vs optical density at 600 nm in *C. albicans* in yeast form. The graph was fit to linear regression with $R^2 = 0.9792$.

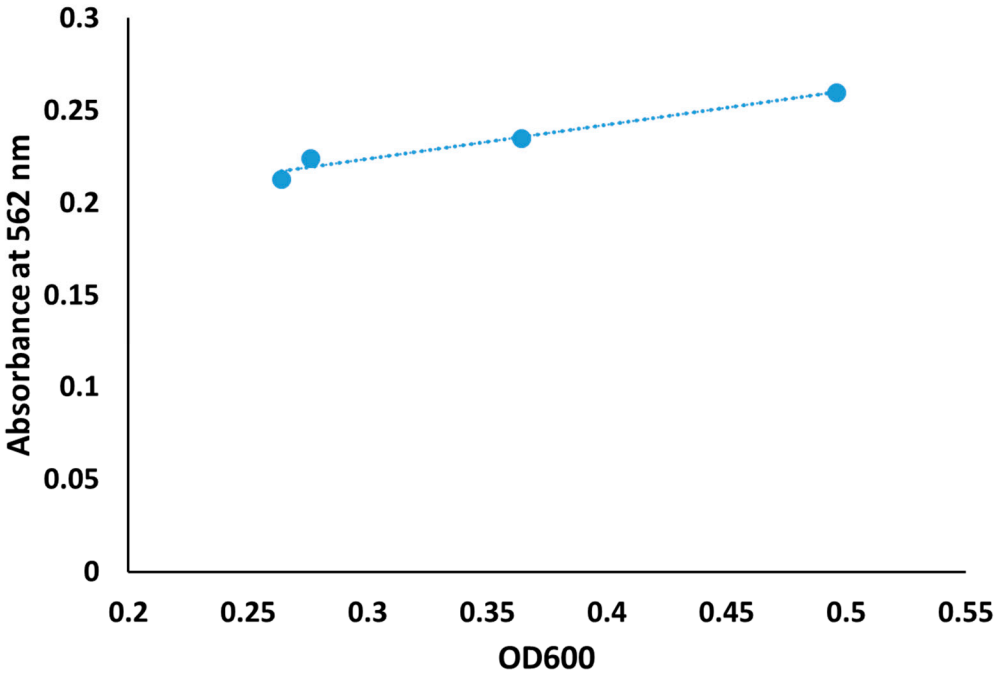

Figure S14. Plot of the total protein content measured by Pierce protein assay vs optical density at 600 nm in *C. albicans* in hyphae form. The graph was fit to linear regression with $R^2 = 0.9678$.

Table S2. Linear regression of total protein content measured by Pierce protein assay vs optical density at 600 nm in yeast strains.

S. Cerevisiae (PC538)	C. Albicans (SC5314)	
	Yeast	Hyphae
Y= 0.378x + 0.2351	Y= 0.1839x + 0.1683	Y= 0.0928x + 0.176

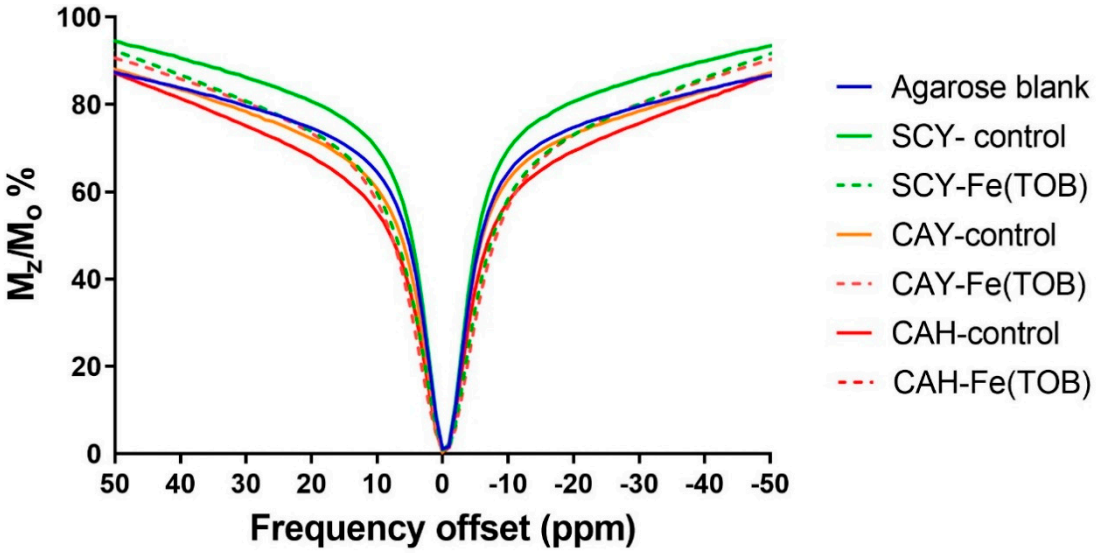

Figure S15. Z-spectra for 0.05 % (w/v) agarose, SC (*S. cerevisiae*) control and Fe(TOB) labeled cells, CAY (*C. albicans* yeast) control and Fe(TOB) labeled cells and CAH (*C. albicans* hyphae) control and Fe(TOB) labeled cells on 9.4 T NMR spectrometer at 37 °C.

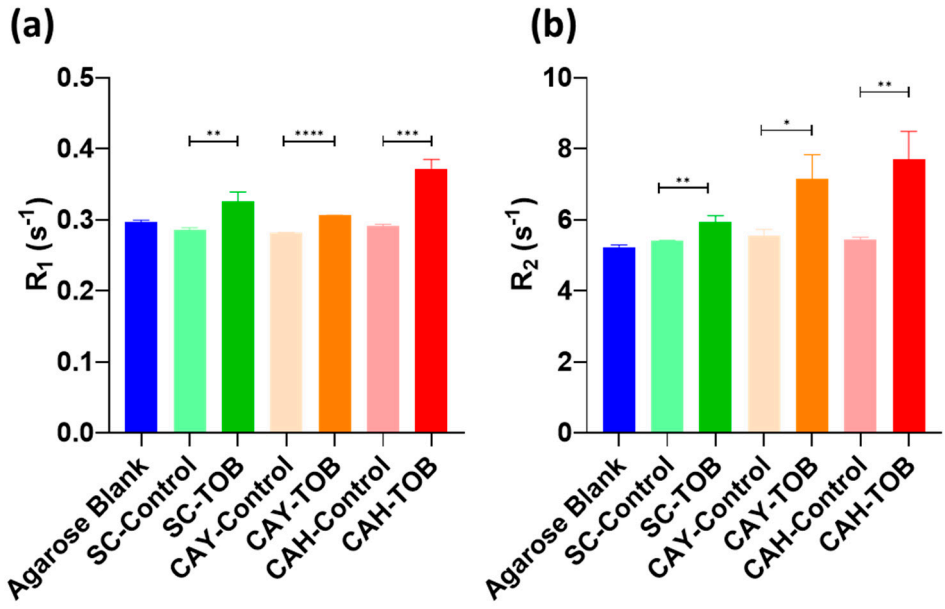

Figure S16. (a) R_1 relaxation rate constants and (b) R_2 relaxation rate constants for 0.05 % (w/v) agarose, SC (*S. cerevisiae*) control and Fe(TOB) labeled cells, CAY (*C. albicans* yeast) control and Fe(TOB) labeled cells and CAH (*C.*

albicans hyphae) control and Fe(TOB) labeled cells on 4.7 T animal scanner at 37 oC. Mean ± SE is reported, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, (****) $p < 0.0001$ with n = 3 for all samples.

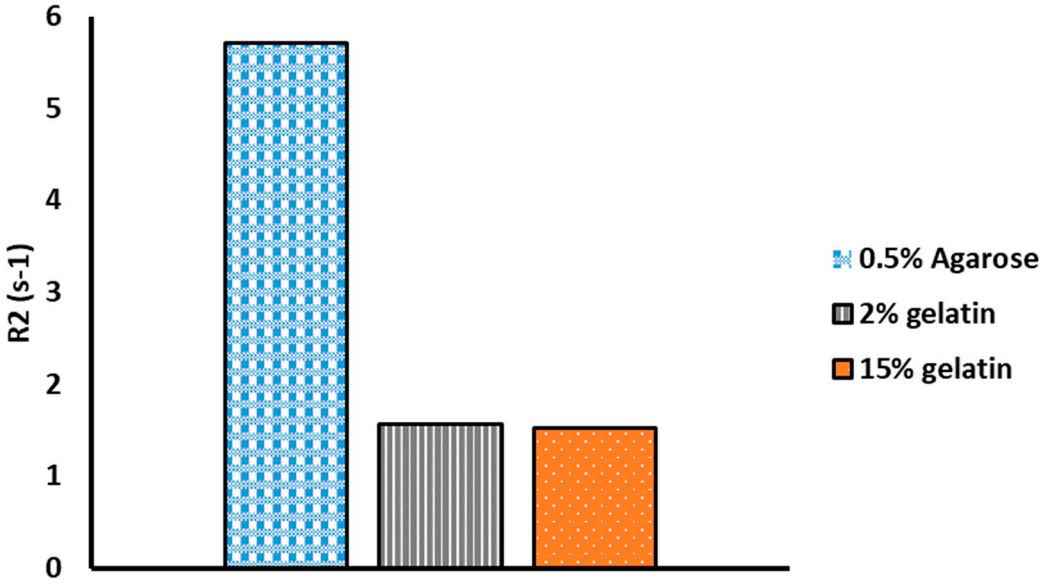

Figure S17. R_2 water proton relaxation rate constant values for agarose and gelatin phantom samples measured by CPMG method on 9.4 T NMR spectrometers at 37 °C.

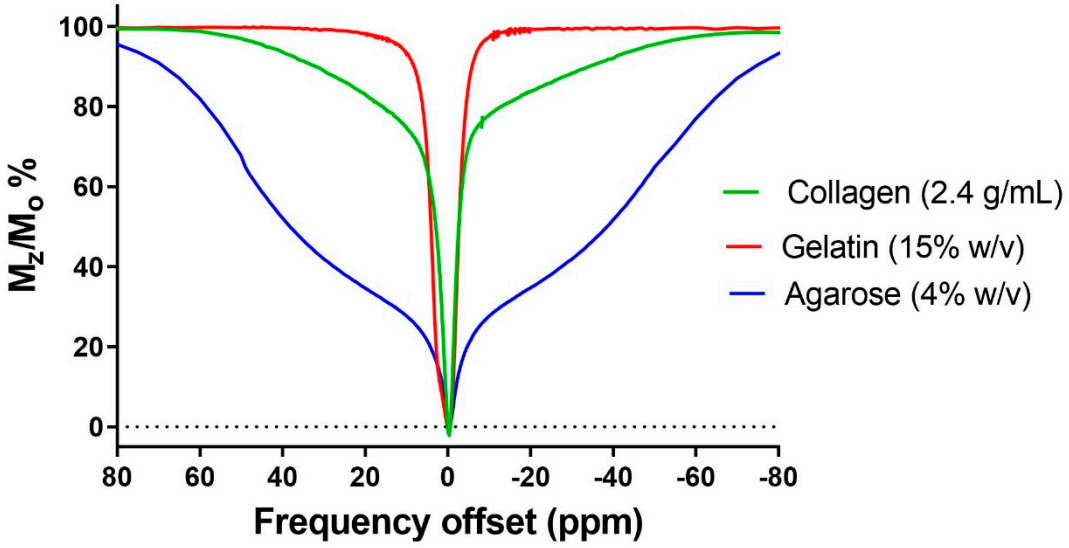

Figure S18. Z-spectra of (a) 2.4 mg/mL Collagen (b) 15% (w/v) Gelatin and (c) 4% (w/v) Agarose at 37 °C on 9.4 T NMR spectrometer at B_1 = 12 μT

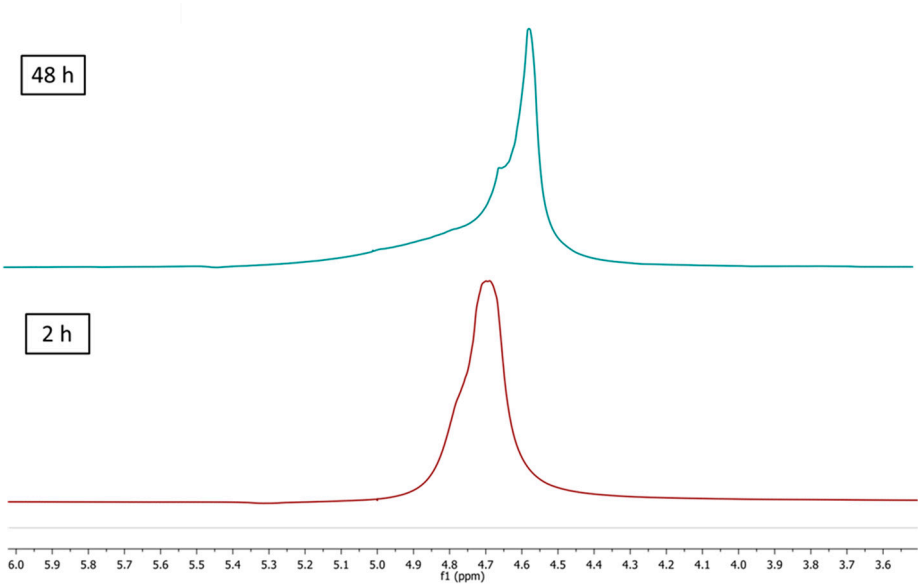

Figure S19. ^{1}H NMR of 15% (w/v) gelatin phantom samples after 2h (bottom) and 48 h (top)

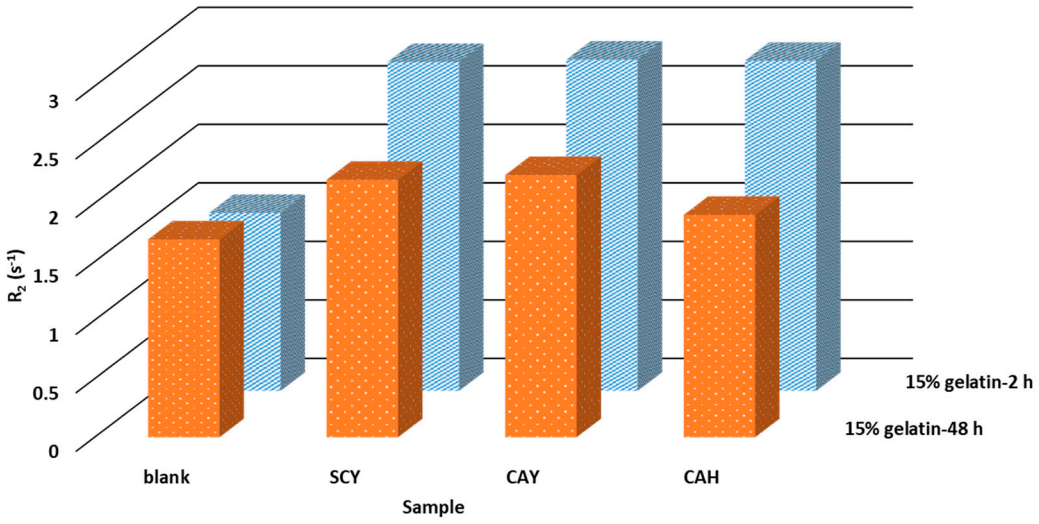

Figure S20. R_2 relaxation rate constant of water protons in (a) 15% (w/v) gelatin phantom (b) SCY (*S. cerevisiae*) (c) CAY (C. *albicans*) yeast form (d) CAH (C. *albicans*) hyphae at 37 °C after 2 h (Blue) and 2 days (orange) at 37 °C on 9.4 T NMR spectrometer.

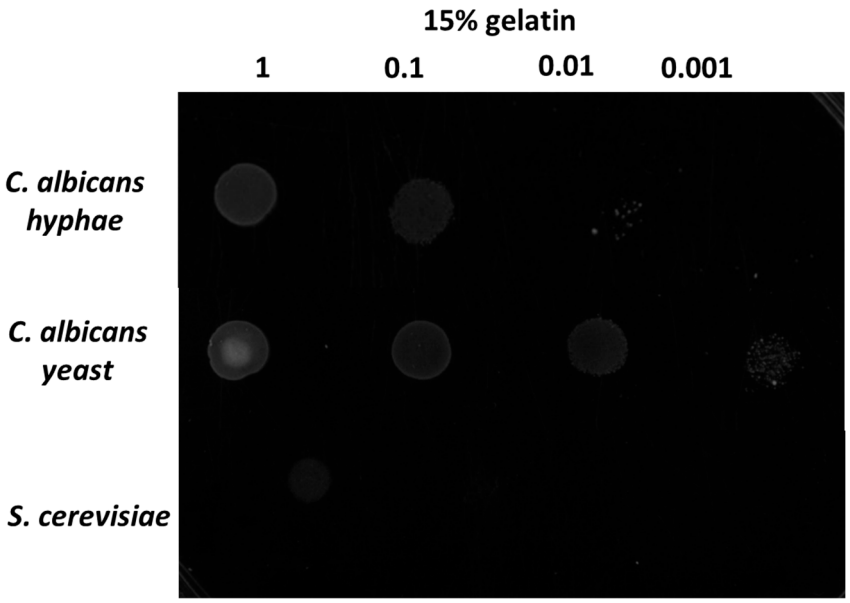

Figure S21. Serial dilution assay with *S. cerevisiae, C. albicans* (yeast) and *C. albicans* (hyphae) spotted on 15% (w/v) gelatin media at 30 °C. The cells were grown for 2 days and photographed.

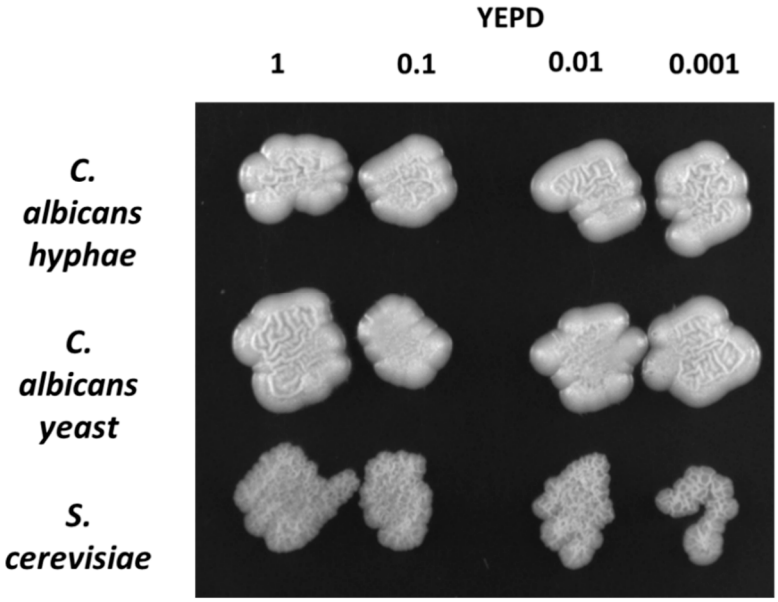

Figure S22. Serial dilution assay with *S. cerevisiae, C. albicans* (yeast) and *C. albicans* (hyphae) spotted on YEPD media at 30 °C. The cells were grown for 2 days and photographed.

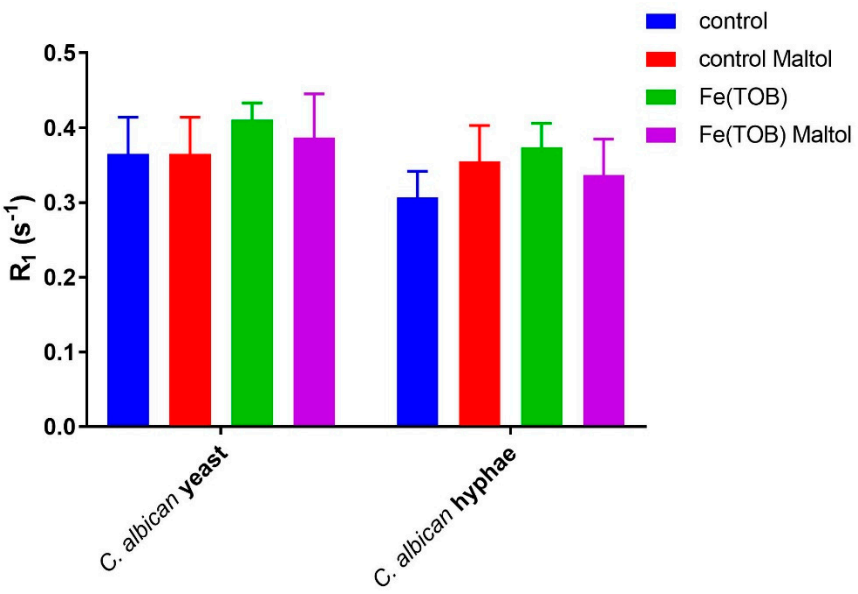

Figure S23. R_1 relaxation rate constant measurement of water protons on 9.4 T NMR spectrometer with *C. albicans* yeast cells suspended in 15% (w/v) gelatin at 37 °C. All cell samples contain ~125 µg protein/mL.

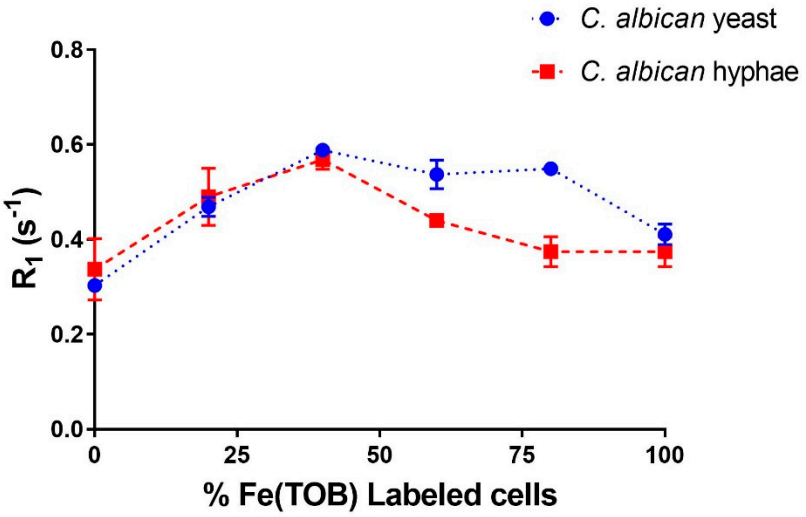

Figure S24. R_1 relaxation rate constant measurements on samples containing increasing concentration of Fe(TOB) labeled cells suspended in 15% (w/v) gelatin in 9.4 T NMR spectrometer.

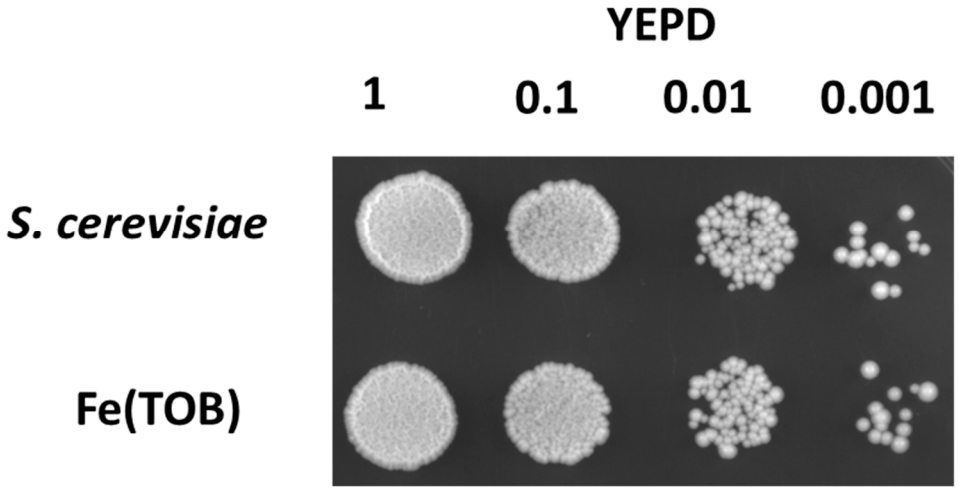

Figure S25. Serial dilution assay with S. *cerevisiae* PC538 (yeast) (i) untreated (ii) 10 mM Fe(TOB) treated cells spotted on YEPD media at 30 °C. The cells were grown for 2 days and photographed.

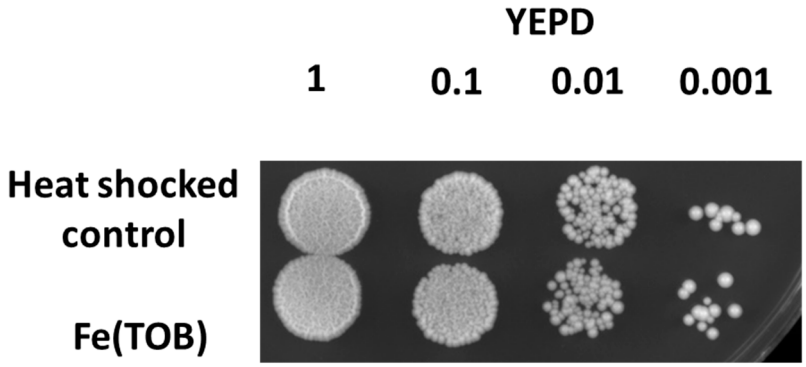

Figure S26. Serial dilution assay with S. cerevisiae PC538 (yeast) (i) heat shocked untreated cells (ii) 10 mM Fe(TOB) treated heat shocked cells spotted on YEPD media at 30 °C. The cells were grown for 2 days and photographed.

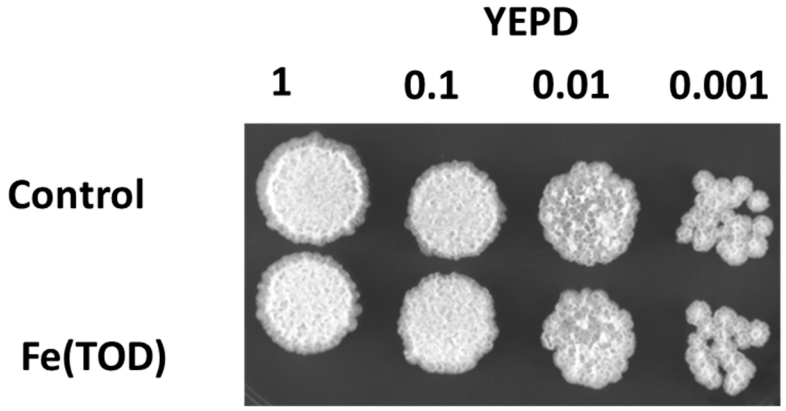

Figure S27 Serial dilution assay with S. cerevisiae PC538 (yeast) (i) untreated (ii) 10 mM Fe(TOD) treated cells spotted on YEPD media at 30 °C. The cells were grown for 2 days and photographed.

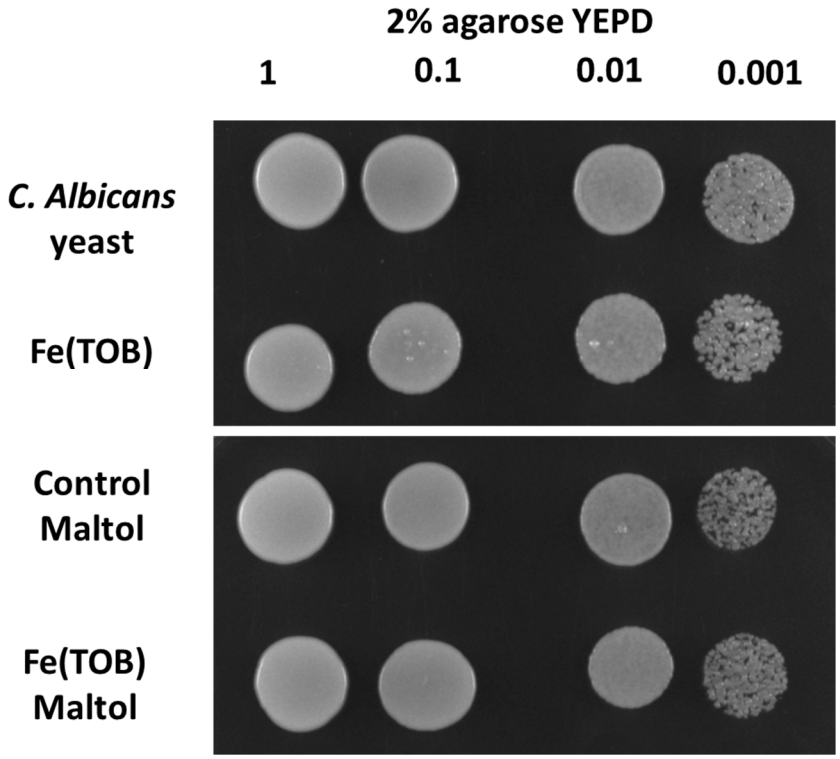

Figure S28. Serial dilution assay with *C. albicans* (yeast) (i) untreated (ii) 10 mM Fe(TOB) treated (iii) 10 mM Maltol treated and (iv) 10 mM Fe(TOB) treatment followed by 10 mM Maltol treated cells spotted on YEPD media at 30 ^{o}C. The cells were grown for 2 days and photographed.

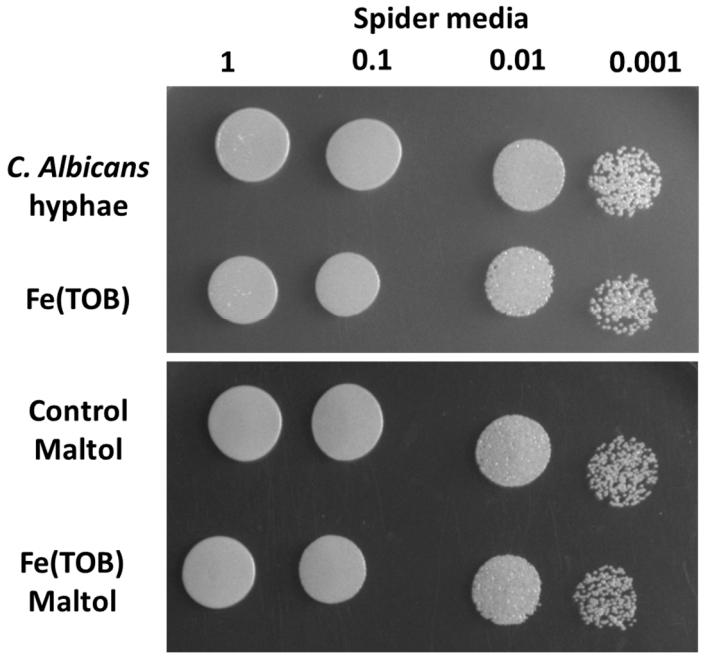

Figure S29. Serial dilution assay with *C. albicans* (hyphae) (i) untreated (ii) 10 mM Fe(TOB) treated (iii) 10 mM Maltol treated and (iv) 10 mM Fe(TOB) treatment followed by 10 mM Maltol treated cells spotted on YEPD media at 30 ^{o}C. The cells were grown for 2 days and photographed.

Calculation of Molarity of Fe(TOB) inside *C. albicans* yeast

Total Fe in Fe(TOB) labelled cells from ICPMS ~ 1.287 x 10^{-8} g/L

Molarity of Fe due to Fe(TOB) ~ $\frac{Concentration\ of\ Fe\ in\ sample}{Formula\ weight\ of\ Fe}$

~ $\frac{1.287\ x\ 10^{-8}\ g/L}{55.85\ g/mole}$

~ 2.30 x 10^{-10} moles/L or 2.30 x 10^{-13} moles/mL

Molarity per 1μg protein due to Fe(TOB) ~ $\frac{Molarity\ of\ Fe\ (\frac{moles}{mL})}{protein\ \mu g/mL}$

~ $\frac{2.30\ x\ 10{-}13\ moles/mL}{0.31\ \mu g/mL}$

~ 2.34 x 10^{-12} moles/μg protein

Volume of *C. albicans* yeast cells per μg protein reported in the literature[3] = (89.96 ± 2.2) μm^3

Molarity of Fe due to Fe(TOB) per μg protein ~ $\frac{moles\ of\ Fe/\mu g\ protein}{Volume\ of\ yeast\ cell/\mu g\ protein}$

~ $\frac{2.34\ \text{x}\ 10^{-12}\ \text{moles/cell}}{89.9\ \text{x}\ 10^{-15}\ \text{L}}$

~ 26.1 M

Calculation of Molarity of Fe(TOB) inside *C. albicans* hyphae

Total Fe in Fe(TOB) labelled cells from ICPMS ~ 6.27 x 10^{-9} g/L

Molarity of Fe due to Fe(TOB) ~ $\frac{Concentration\ of\ Fe\ in\ sample}{Formula\ weight\ of\ Fe}$

~ $\frac{6.27\ x\ 10^{-9}\ g/L}{55.85\ g/mole}$

~ 1.14 x 10^{-10} moles/L or 1.14 x 10^{-13} moles/mL

Molarity per 1μg protein due to Fe(TOB) ~ $\frac{Molarity\ of\ Fe\ (\frac{moles}{mL})}{protein\ \mu g/mL}$

~ $\frac{1.14\ x\ 10{-}13\ moles/mL}{0.34\ \mu g/mL}$

~ 3.36 x 10^{-13} moles/μg protein

Volume of *C. albicans* yeast cells per μg protein reported in the literature[3] = (180.1 ± 10.7) μm^3

Molarity of Fe due to Fe(TOB) per μg protein ~ $\frac{moles\ of\ Fe/\mu g\ protein}{Volume\ of\ yeast\ cell/\mu g\ protein}$

~ $\frac{3.36\ \text{x}\ 10^{-13}\ \text{moles/cell}}{180.1\ \text{x}\ 10^{-15}\ \text{L}}$

~ 5.50 M

Calculation of Molarity of Fe(TOB) inside *S. cerevisiae*

Total Fe in Fe(TOB) labelled cells from ICPMS ~ 32.5 x 10^{-8} g/mL

Mass of Fe per cell due to Fe(TOB) ~ $\frac{Total\ Fe\ concentration}{\#\ cells}$

~ $\frac{32.5\ x\ 10^{-5}\ g/mL}{4.5{*}10^{8}\ cell/mL}$

~ 7.21 x 10^{-14} g/cell

Moles of Fe per cell due to Fe(TOB) ~ $\frac{Mass\ of\ Fe\ per\ cell}{Formula\ weight\ of\ Fe}$

~ $\frac{7.21\ X\ 10^{-14}\ g/cell}{55.85\ g/mole}$

~ 1.29 x 10^{-15} moles/cell

Assuming yeast cells to be spherical with the dimensions of 5 μM diameter[4]

Approximate volume of an yeast cell = $\frac{4}{3}\pi\ (2.5 * 10^{-6})^3$ m^3

~ 65.45 x 10^{-15} L

Molarity of Fe due to Fe(TOB) ~ $\frac{moles\ of Fe/cell}{Volume\ is\ yeast\ cell/cell}$

~ $\frac{1.29\ \text{x}\ 10^{-13}\ \text{moles/cell}}{65.45\ \text{x}\ 10^{-15}\ \text{L}}$

~ 19.75 M

References

1. Snyder, E. M.; Asik, D.; Abozeid, S. M.; Burgio, A.; Bateman, G.; Turowski, S. G.; Spernyak, J. A.; Morrow, J. R., A Class of FeIII Macrocyclic Complexes with Alcohol Donor Groups as Effective T1 MRI Contrast Agents. *Angewandte Chemie* 2020, *132* (6), 2435-2440.
2. Didar Asik, R. S., Samira M. Abozeid, Travis B. Mitchell, Steven G. Turowski, Joseph A. Spernyak and Janet R. Morrow, Modulating the Properties of Fe(III) Macrocyclic MRI Contrast Agents by Appending Sulfonate or Hydroxyl Groups. *Molecules* 2020, *25* (10), 2291.
3. Hosseinzadeh, A.; Urban, C. F., Novel insight into neutrophil immune responses by dry mass determination of Candida albicans morphotypes. *PloS one* 2013, *8* (10), e77993-e77993.
4. Patel, A.; Asik, D.; Snyder, E. M.; Dilillo, A. E.; Cullen, P. J.; Morrow, J. R., Binding and Release of FeIII Complexes from Glucan Particles for the Delivery of T1 MRI Contrast Agents. *ChemMedChem* 2020, *15* (12), 1050-1057.