Chapter 1

Feasibility Calculations

1.1 Upper limit of yeast display capture

The amount of molecules (or moles) a single yeast displaying strain can bind to can be calculated based on the number of displayed binding moities (N_E), and the number of binding sites per moiety (N_B). Assuming perfect binding (all binding sites are occupied regardless of ligand concentration), and a culture of cells (N_{cells}), the equation for the upper limit of yeast display capture can be calculated by

$$N_{\text{bindersd}} = N_E \times N_B \qquad [\#/\text{mL}] \quad (1.1)$$

$$N_{\text{cells}} = \text{OD}_{600} \times \lambda_{\text{OD}} \qquad [\#] \quad (1.2)$$

$$N_{\text{bound}} = N_{\text{cells}} \times N_{\text{cells}} \qquad [\#] \quad (1.3)$$

$$\text{molar}_{\text{bound}} = N_{\text{bound}}/N_A \qquad [\text{M}] \quad (1.4)$$

where:

N_E = 1e3 – 1e6, number of displayed moieties

N_B = 1 – ..., number of binding sites per moiety

OD_{600} = 0 – ..., culture optical density measured at 600 nm

$$\begin{aligned} \lambda_{\mathrm{OD}} &\approx \text{1e7 cells per mL, ratio of optical density to cell density} \\ N_A &= \text{6.022e23 molecules per mol, Avagadro's number} \end{aligned}$$

Using frequently achievable values, such as an expression level of 1e5 moieties, 1 binding site per moiety, and a typical OD_{600} measurement of 1 yields an astonishingly low ≈ 2 picomolar of bound metals. If these parameters were pushed to the extreme, with an expression level of 1 million, 10 binding sites per moiety, and a OD of 10 (either by growing to saturation or packing yeast) yields ≈ 2 nanomolar of bound metals, or 3 orders of magnitude more.

Therefore, in order to obtain environmentally relevant values, the number of metals bound would have to increase by another 3–6 orders of magnitude (in the μM to mM range). To do so would require massive optimization in protein expression, designing proteins with multiple binding sites, and yeast compaction. Given these circumstances, yeast display is unfortunately not a viable method for significant metal capture, and publications that have shown promising results may be observing other binding phenomenon such as cell surface adsorption or absorption into the cell [CITE].

1.2 Upper limit of metal absorption in yeast

Volume is a much greater container for substances than surface area (i.e. yeast display) given the surface-to-volume ratio. This exercise is to estimate the bulk uptake capacity of yeast as a whole, not considering the biological impact of cell death, cytosolic metal binding, or metal trafficking in the yeast. To estimate the theoretical maximum bulk capacity of yeast uptake is to understand the geometry of yeast (diameter, d, hence volume, V) and cell culture density (OD_{600}). To determine the upper limit requires a top-down approach, from anchoring a metal uptake concentration and calculating the internal metal concentration per yeast and assessing the feasibility

given nominal metal concentrations in a cell, and iterating this process as necessary until a metal uptake concentration range is established.

Assuming a metal uptake concentration of $\mathrm{M_{total}}$, the amount of metal atoms per yeast can be calculated by

$$N_m = \mathrm{M_{total}} \times N_A \qquad [\#/\mathrm{L}] \quad (1.5)$$

$$N_{\mathrm{yeast}} = \mathrm{OD}_{600} \times \lambda_{\mathrm{OD}} \qquad [\#] \quad (1.6)$$

$$V = \frac{4}{3}\pi \left(\frac{d}{2}\right)^3 \qquad [\mathrm{L}] \quad (1.7)$$

$$N_{\mathrm{U}} = \frac{N_m}{N_{\mathrm{yeast}}} \times V \qquad [\#] \quad (1.8)$$

where:

N_m = number of atom molecules uptaken

$\mathrm{M_{total}}$ = 0 – 10 mM, range of uptaken metal concentrations

N_A = 6.022e23 molecules per mol, Avagadro's number

N_{yeast} = number of yeast per L

$d \approx$ 1 – 10 μm, diameter of yeast

$V \approx$ 0.52 – 524 f L (10e-15 L)

N_{U} = number of atoms uptaken per cell

The number of atoms uptaken can be converted to moles ($\mathrm{mol_U}$) or molarity ($\mathrm{M_U}$) as follows

$$\mathrm{mol_U} = N_{\mathrm{U}}/N_A \qquad [\mathrm{mol}] \quad (1.9)$$

$$\mathrm{M_U} = \mathrm{mol_U}/V \qquad [\mathrm{M}] \quad (1.10)$$

Using realistic values, such as a culture optical density of 1, a diameter

[CITE] what metals are concentration in yeast

1.3 Uptake induced density change

Yeast density increases as mass is accumulated by intaking heavy metals. The degree of density change is determined by the extra mass accumulated (Δm) contributed by the amount of internalized metal (molarity, M, or atoms, N_m), the volume of yeast (V), and the molecular weight of that particular metal (MW). Yet again, a top down approach is used to determine the degree of density changes given empirical experiments performed in Chapter [CITE]. To begin, a calculation of accumulated mass due to internalized metal can be performed by

$$\Delta m = \text{mol}_\text{U} \times \text{MW} \qquad [\text{g}] \quad (1.11)$$

$$\rho' = \rho_o + \frac{\Delta m}{V} \qquad [\text{g/L}] \quad (1.12)$$

where:

Δm	=	mass increased due to uptaken metals
mol_U	=	moles of uptaken metal, equ. 1.9
MW	=	molecular weight of metal
V	$\approx$	yeast volume, derived from equ. 1.7
ρ_o	=	original yeast density
ρ'	=	new (increased) yeast density

This equation is assuming that volume is held relatively constant. If not, then a simplified calculation for volume change as a function of uptaken metal can be calculated by conserving osmotic pressure within the cell using

$$\Pi_i = C_i RT \qquad [\text{atm}] \quad (1.13)$$

where:

C_i = concentration of dissolved species in a cell

R = 0.08206 liter atm/mol/K, universal gas constant

T = 303 K, temperature at 30^oC

The change in volume

$$\Pi' = \Pi_o \tag{1.14}$$

$$C' = C_o \tag{1.15}$$

$$\frac{\text{mol} + \text{mol}_\text{U}}{V_o + \Delta V} = \frac{\text{mol}}{V_o} \tag{1.16}$$

$$\Delta V = \frac{\text{mol}_\text{U}}{\text{mol}} \times V_o \tag{1.17}$$

$$V' = V_o \left(1 + \frac{\text{mol}_\text{U}}{\text{mol}}\right) \quad [\text{L}] \tag{1.18}$$

where:

Π' = osmotic pressure after internalized metal

Π' = original osmotic pressure of the cell

C' = metal concentration after internalized metal

C_o = original metal concentration

V' = volume effected after internalized metal

V_o = original cell volume, equ. 1.7

mol_U = change in moles, amount of moles uptaken equ. 1.9

Where the change in volume is proportional to the ratio of the amount of metal content increased $\frac{\Delta\text{mol}}{\text{mol}}$

$$\rho' = \rho_o + \frac{\Delta m}{V} \quad [\text{g/L}] \tag{1.19}$$

Volume change is proportional to the amount of metals accumulated in order balance the osmotic equilibrium.

Not that mass and molecular weight changes are not identical,