

ChaufFeur



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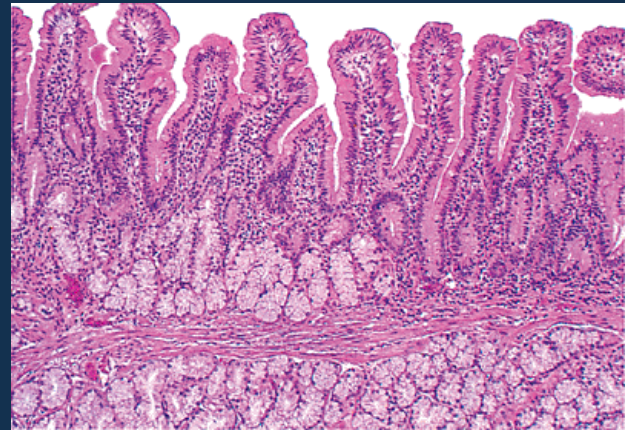
Anemia

- Lack of oxygen circulating in the blood stream
- Affects many women and athletes.
- Can lead to dizziness, loss of energy, and reduced performance.
- Often the modulation of diet is not enough.



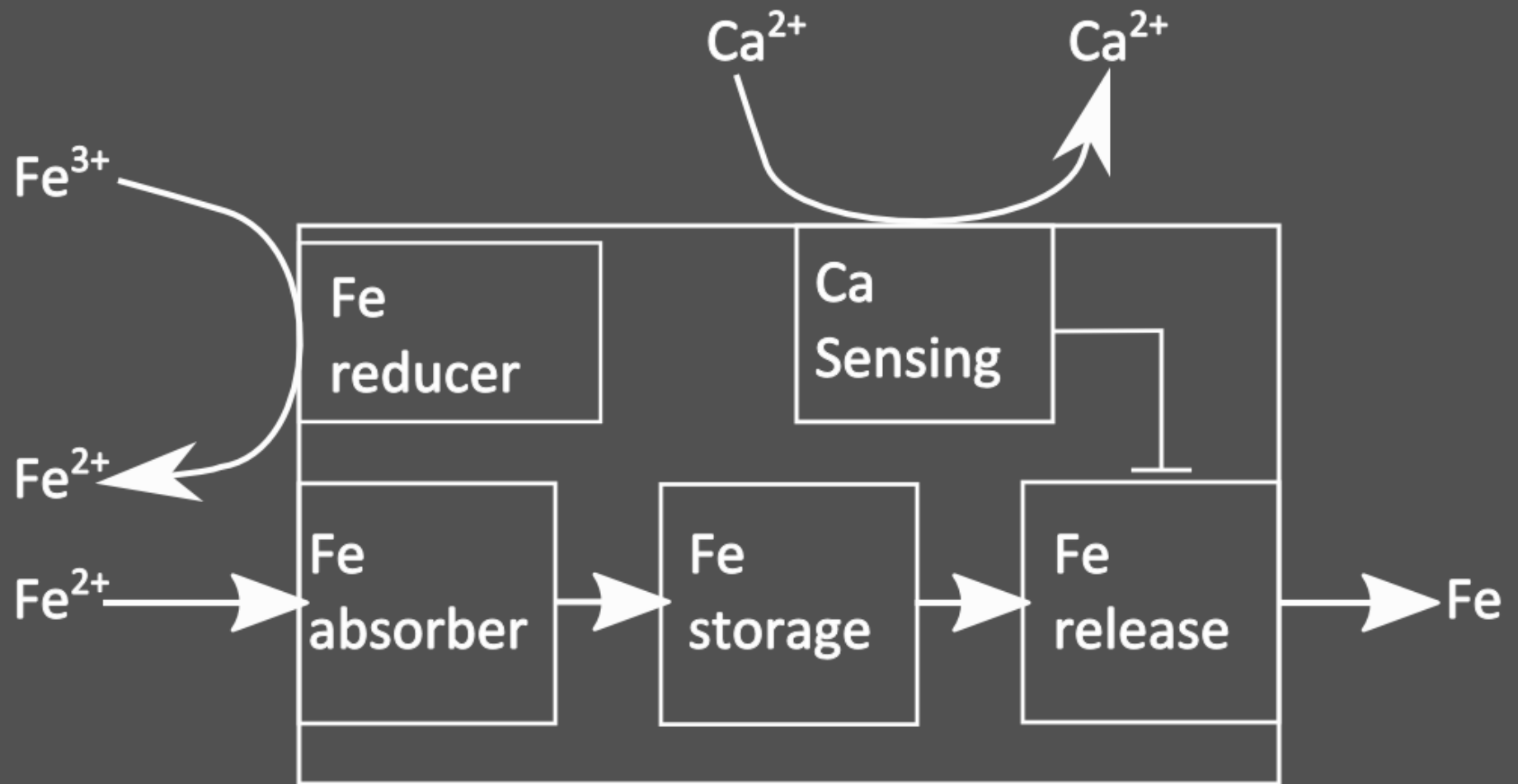
Combating Anemia

- Calcium and iron are antagonistic when being absorbed in the gut
 - Necessary ~3 hour time delay
- A fast metabolism (often an issue for athletes) does not give adequate time for absorption
 - Create bioengineered “holding zone” for iron in the gut

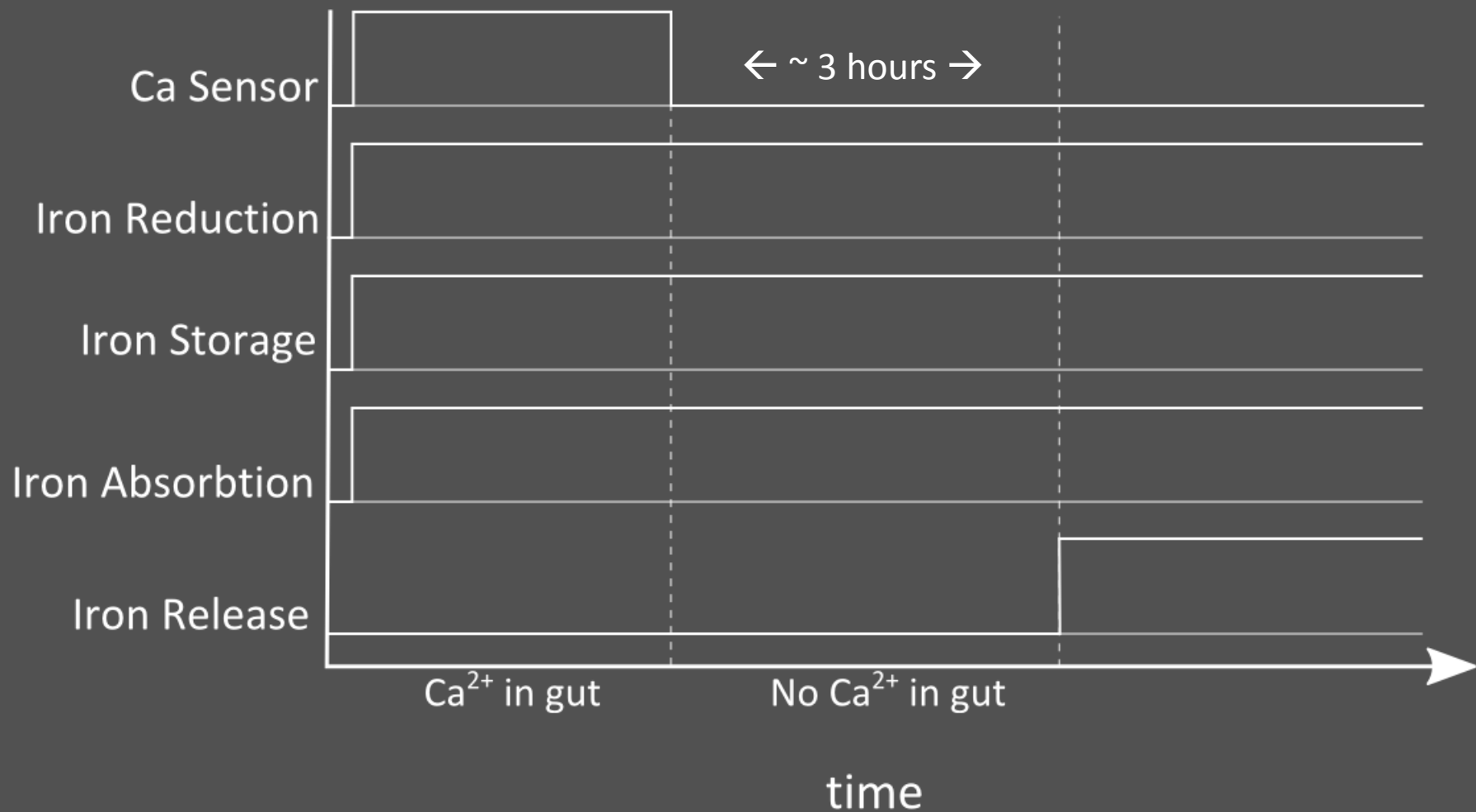


Intestinal villi

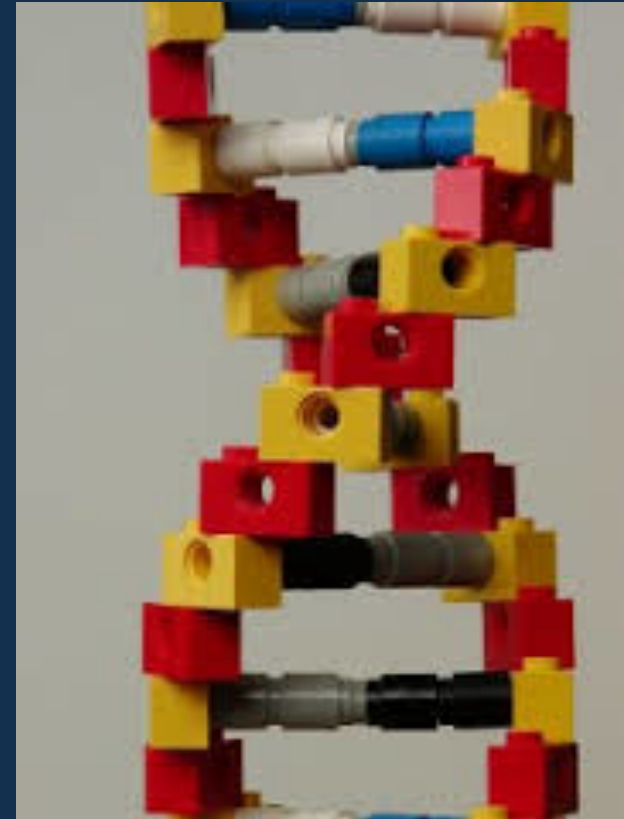
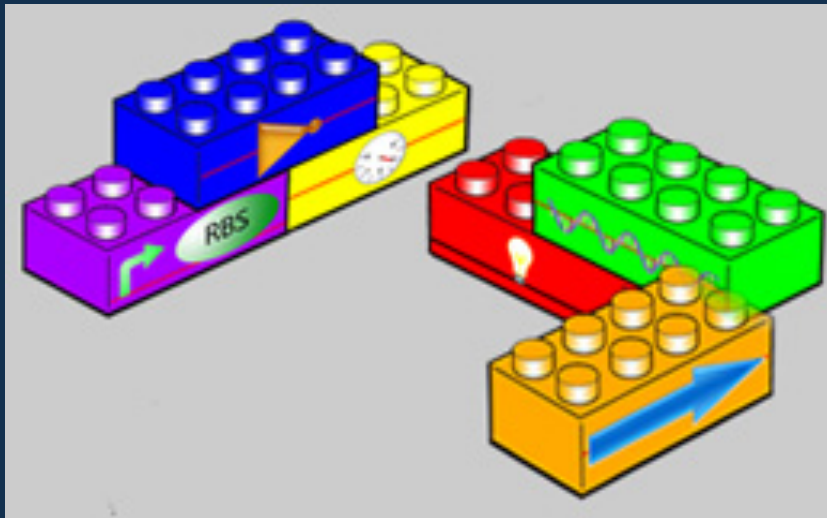
Devices Diagram



Timing Diagram

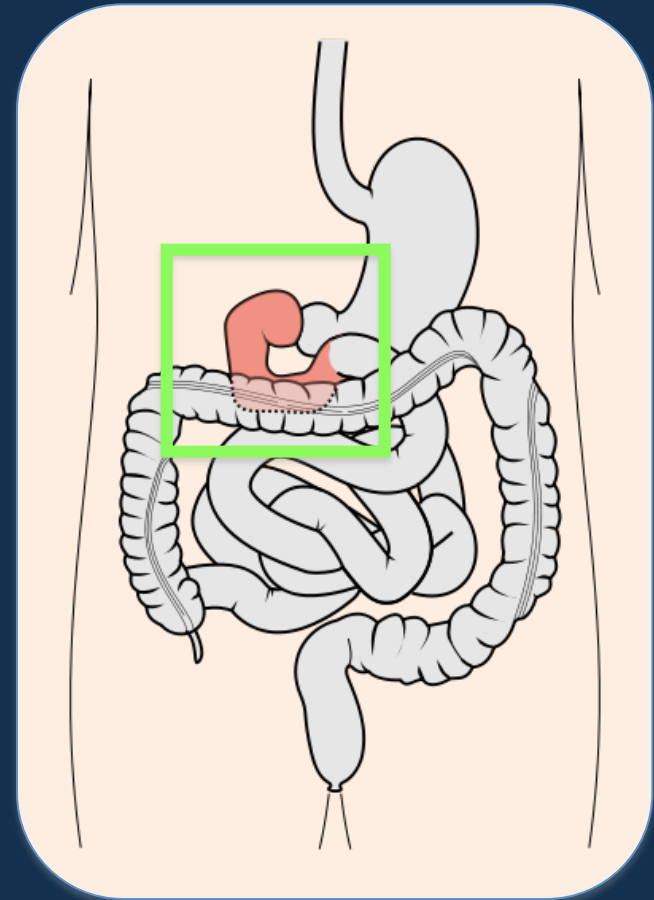


Parts



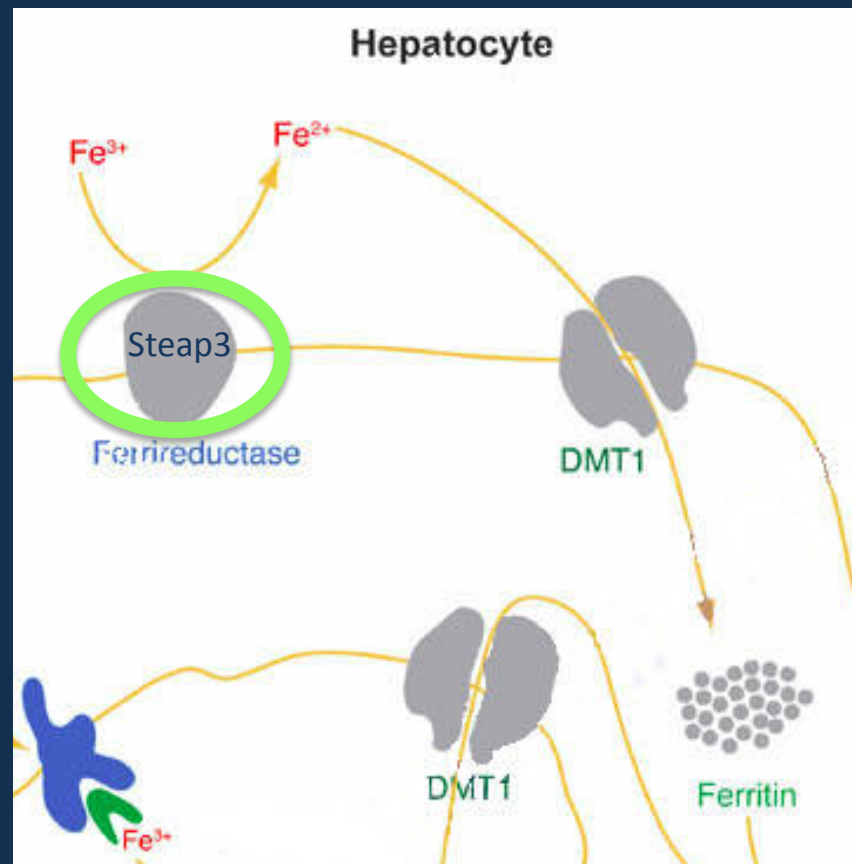
Chassis - Lactobacillus

- Target location:
duodenum adherence
- Already present/probiotic
- Does not require iron to survive
- *Lactobacillus rhamnosus* GG
 - Best adherence to gut



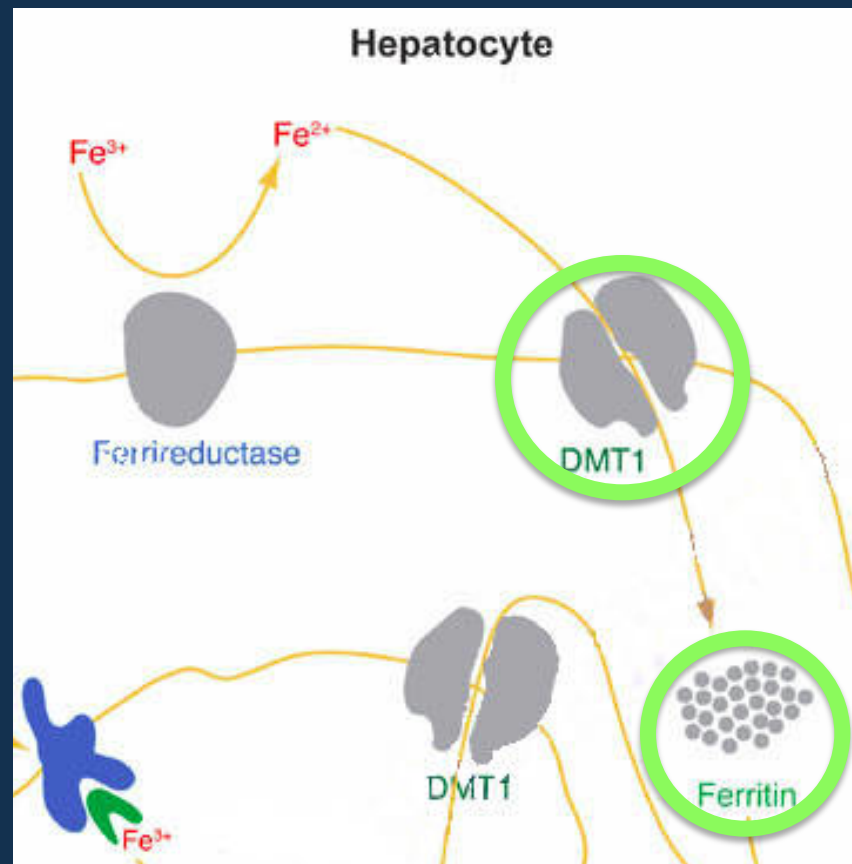
Iron Reduction

- Dietary iron must be reduced
 - $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$
- Ferrireductase surface proteins (Steap3) are located outside the cell



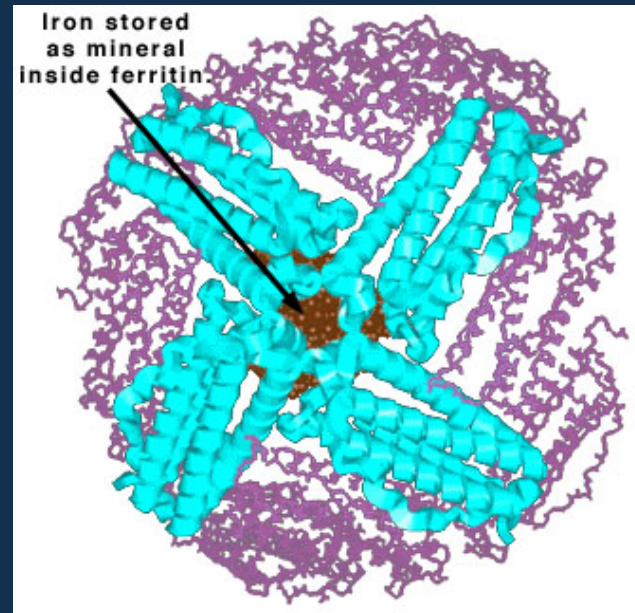
Iron Absorption

- Fe^{2+} enters the cell through a divalent metal transporter (DMT1)
- Iron is then bound to ferritin and stored within the cell



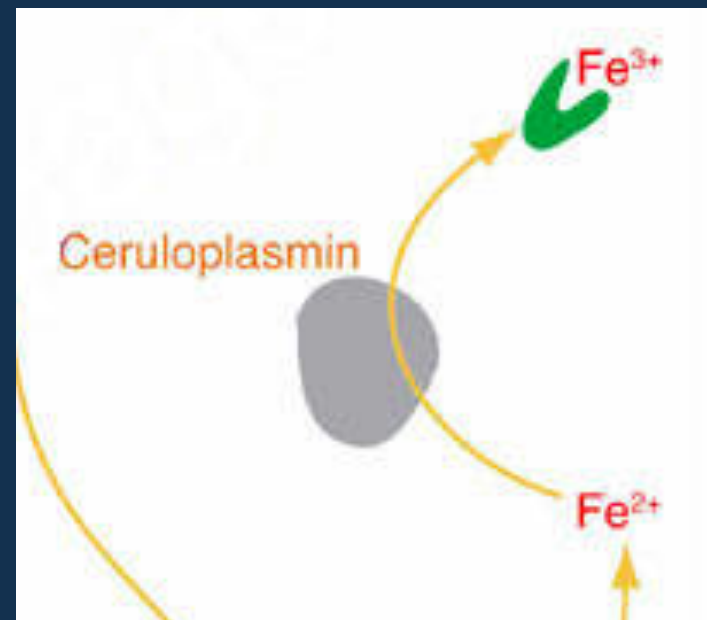
Iron Storage

- Ferritin stores iron as Fe^{2+} , a state that iron binding proteins in the cytosol can bind to



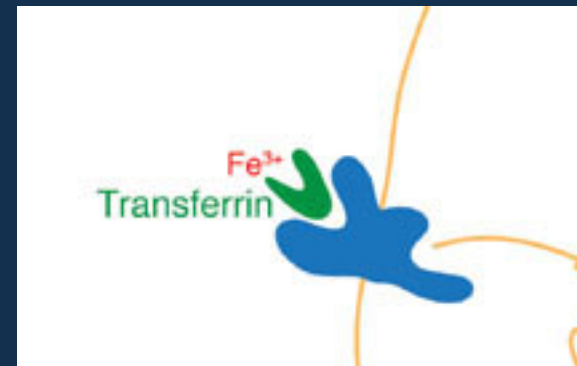
Iron Release

- Ceruloplasmin oxidizes the Fe^{2+} to Fe^{3+}
- Ceruloplasmin is always present in our engineered chassis



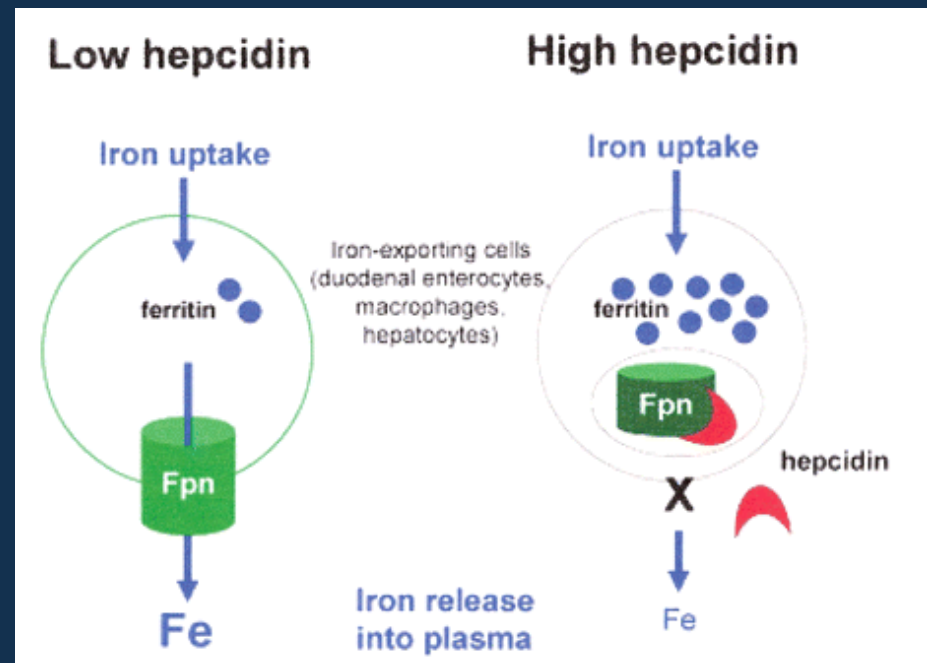
Iron Export

- In the Fe^{3+} state, iron is available to bind to transferrin
- Transferrin transports iron throughout the body



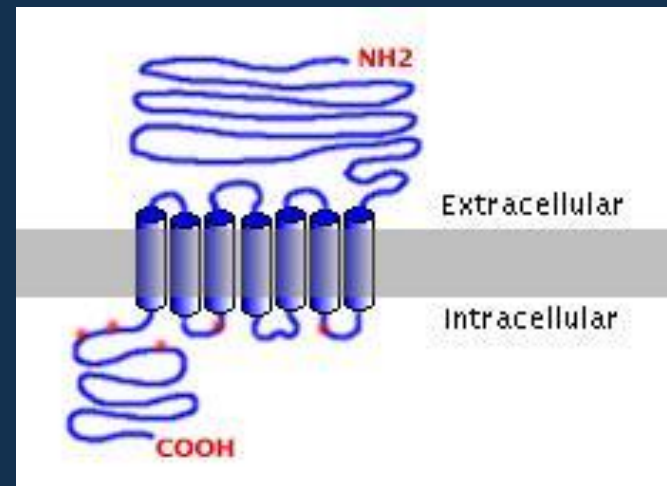
Iron Export

- Iron exits the cell ferroportin
- Ferroportin is inhibited by hepcidin, which is produced when calcium is sensed

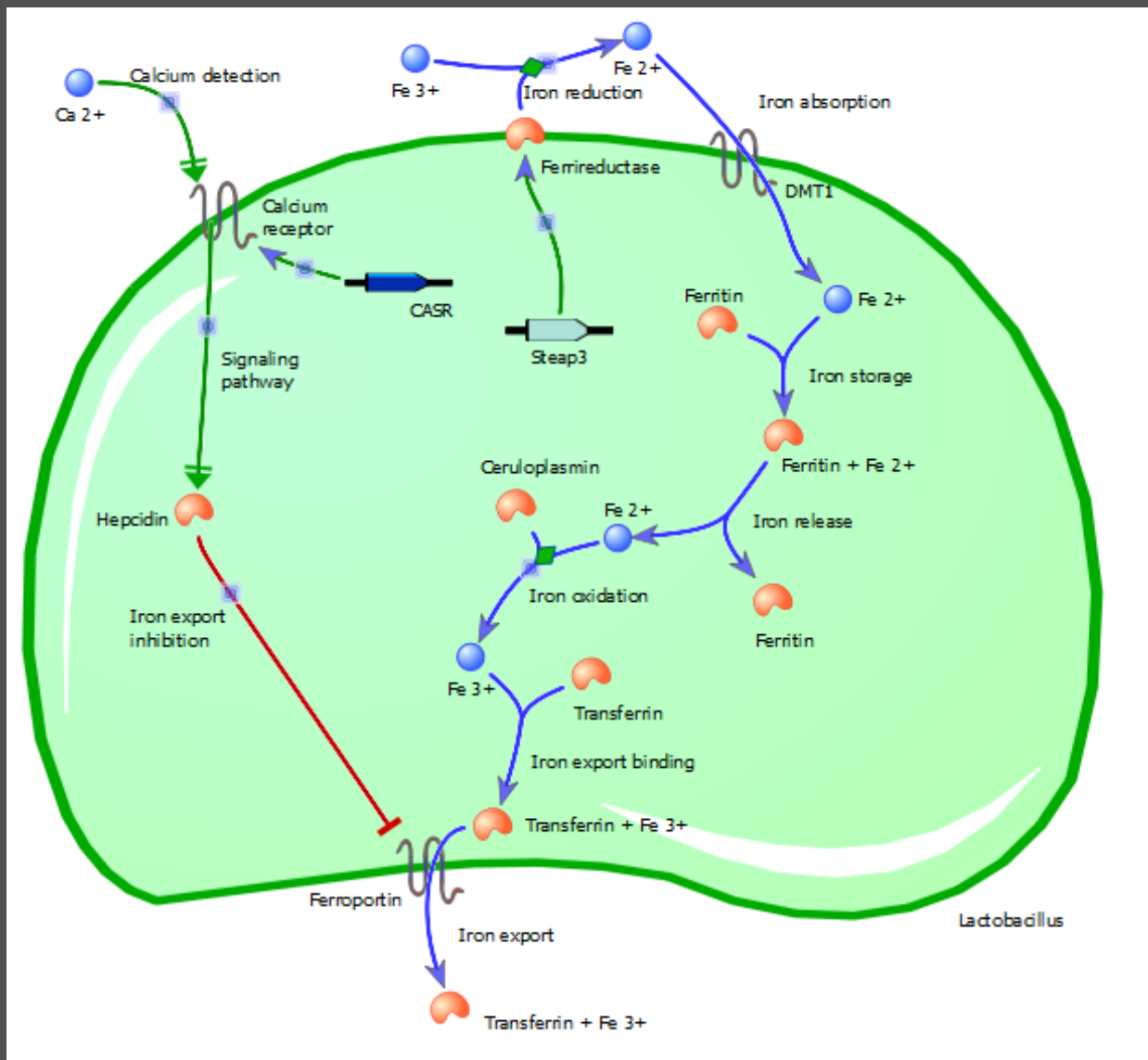


Calcium Sensor

- Gene: CASR (Ca-Sensing Receptor)
- Transmembrane protein
- Senses changes in calcium concentration
- Activates intracellular phospholipase C pathway/ ERK signaling
- Possible downstream effects?

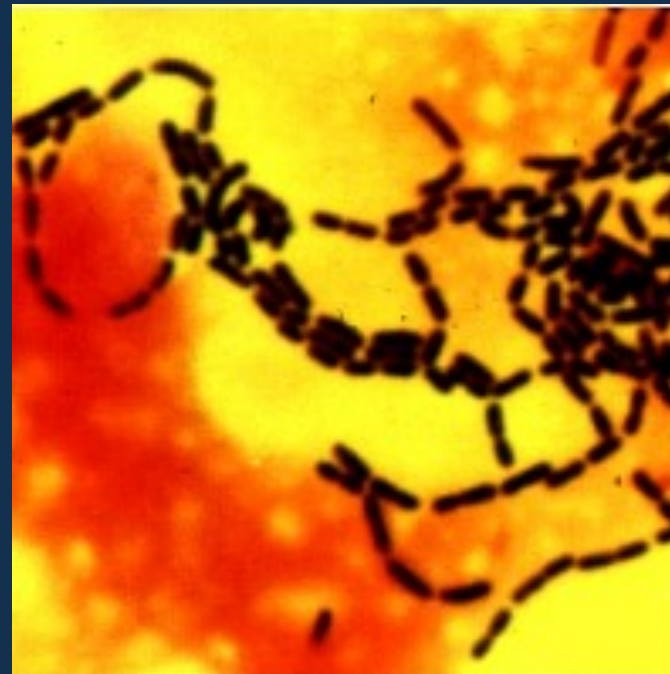


TinkerCell Simulation



Debugging

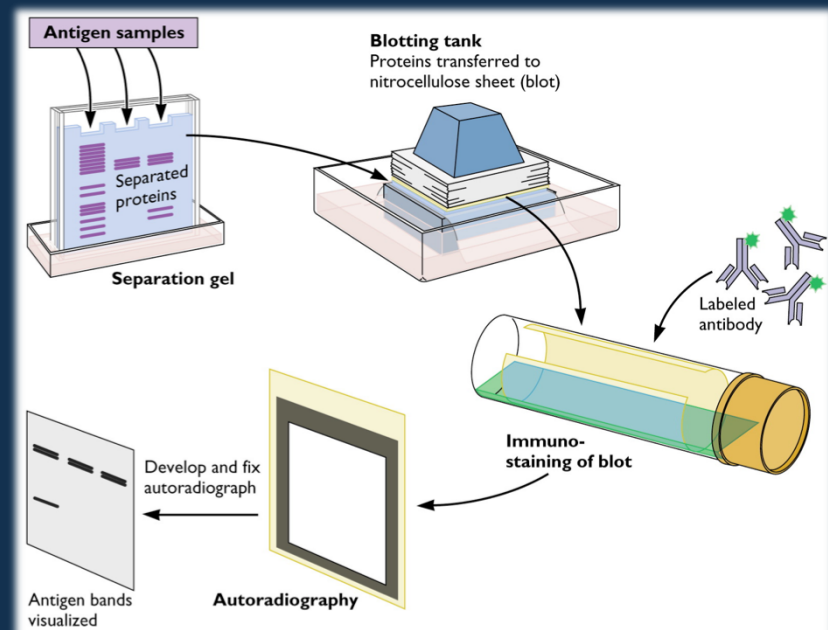
- Chassis: Grow the chassis in iron deficient media



Lactobacillus Rhamnosus

Debugging

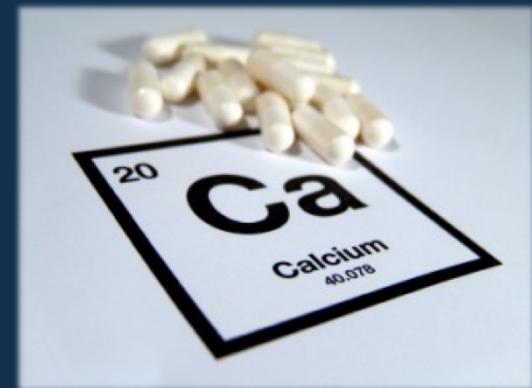
- Iron Absorption: Grow colonies on radioactive iron media of various concentrations – run colonies on a gel and use western blot
- Ferritin: Use the same western blot with ferritin antibodies



Debugging

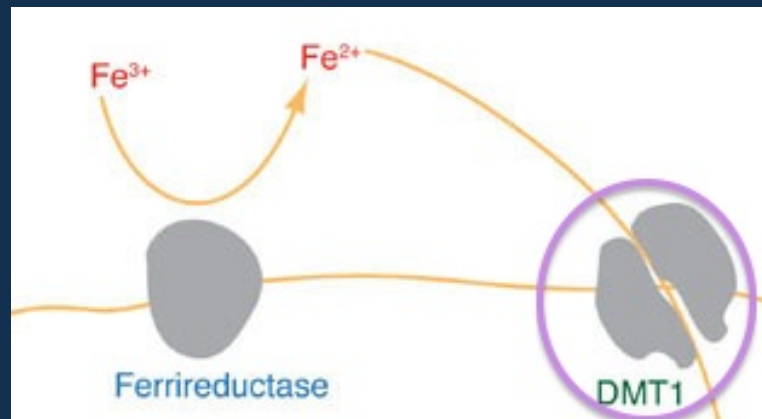
- Iron release: start with tagged iron in cell, test iron outside the cell

Iron	Calcium	Iron Released?
Yes	No	Yes
Yes	Yes	No



Potential Issues and Further Research

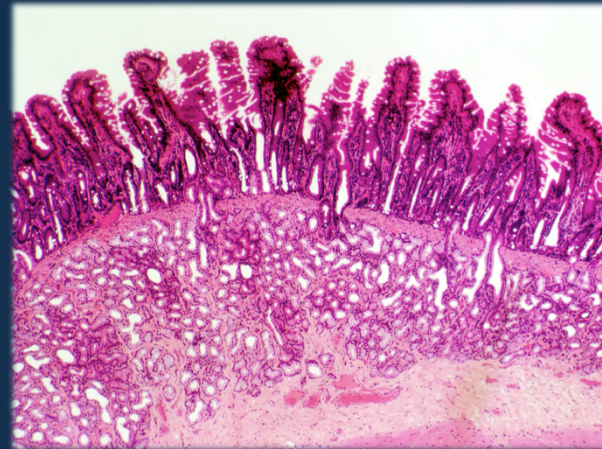
- Up-regulating DMT1's uptake of iron



- Further clarify the calcium detector's signaling pathway

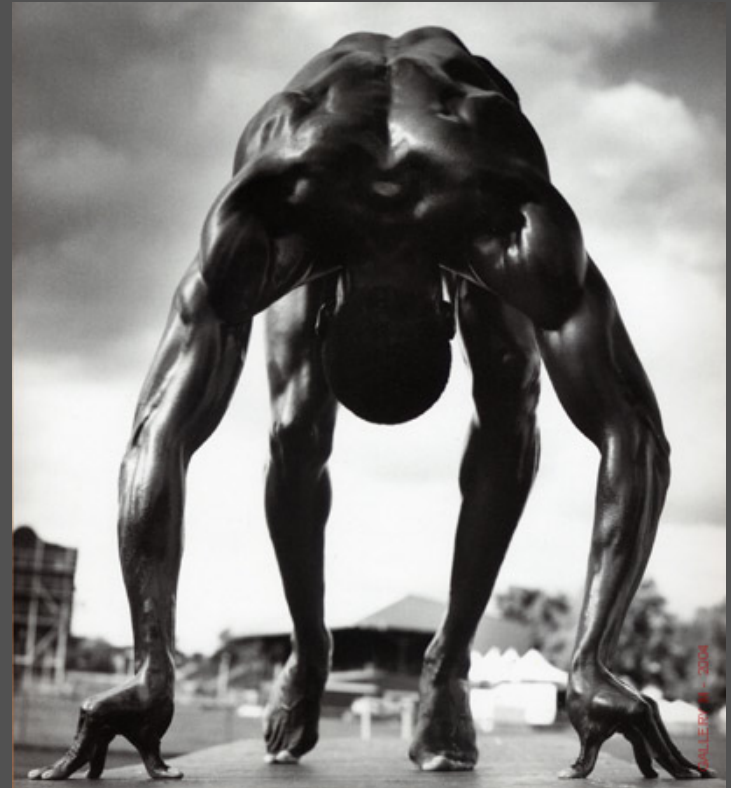
Potential Issues and Further Research

- Our cell isn't able to compete with pre-existing bacteria in the gut
- Our cell thrives in the gut and prevents naturally occurring bacteria from growing
- Iron tolerance of our cells



Intestinal villi

**We have
decided to
move
forward with
this project**



Any Questions?

TinkerCell Simulation

