# Hereditary Hemochromatosis

## The *HFE* gene

Becky Reese

# What is hereditary hemochromatosis?

Recessively inherited

Iron overload disorder

Inability to regulate iron absorption

No cure

http://www.consultant360.com/article/genetics-gastroenterology-what-you-need-know-part-1

# What gene is associated with hereditary hemochromatosis?


## Gene Ontology

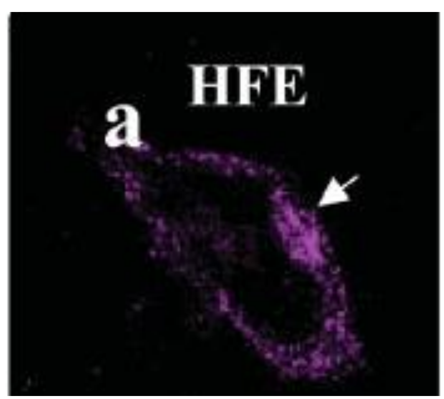
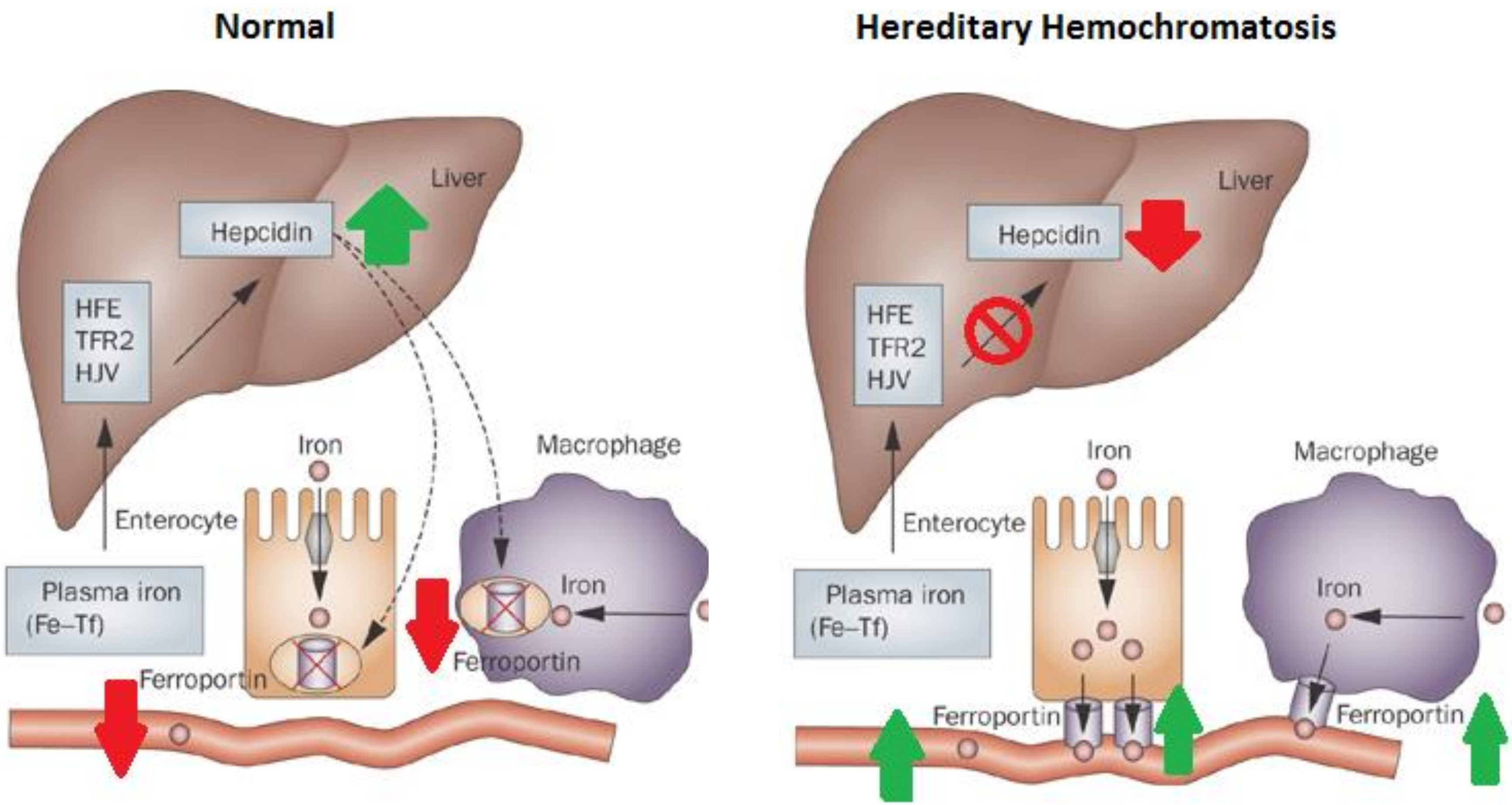
| Molecular Function | Cellular Component | Biological Processes |
|---|---|---|
| Receptor binding |  | Cellular Iron Homeostasis |



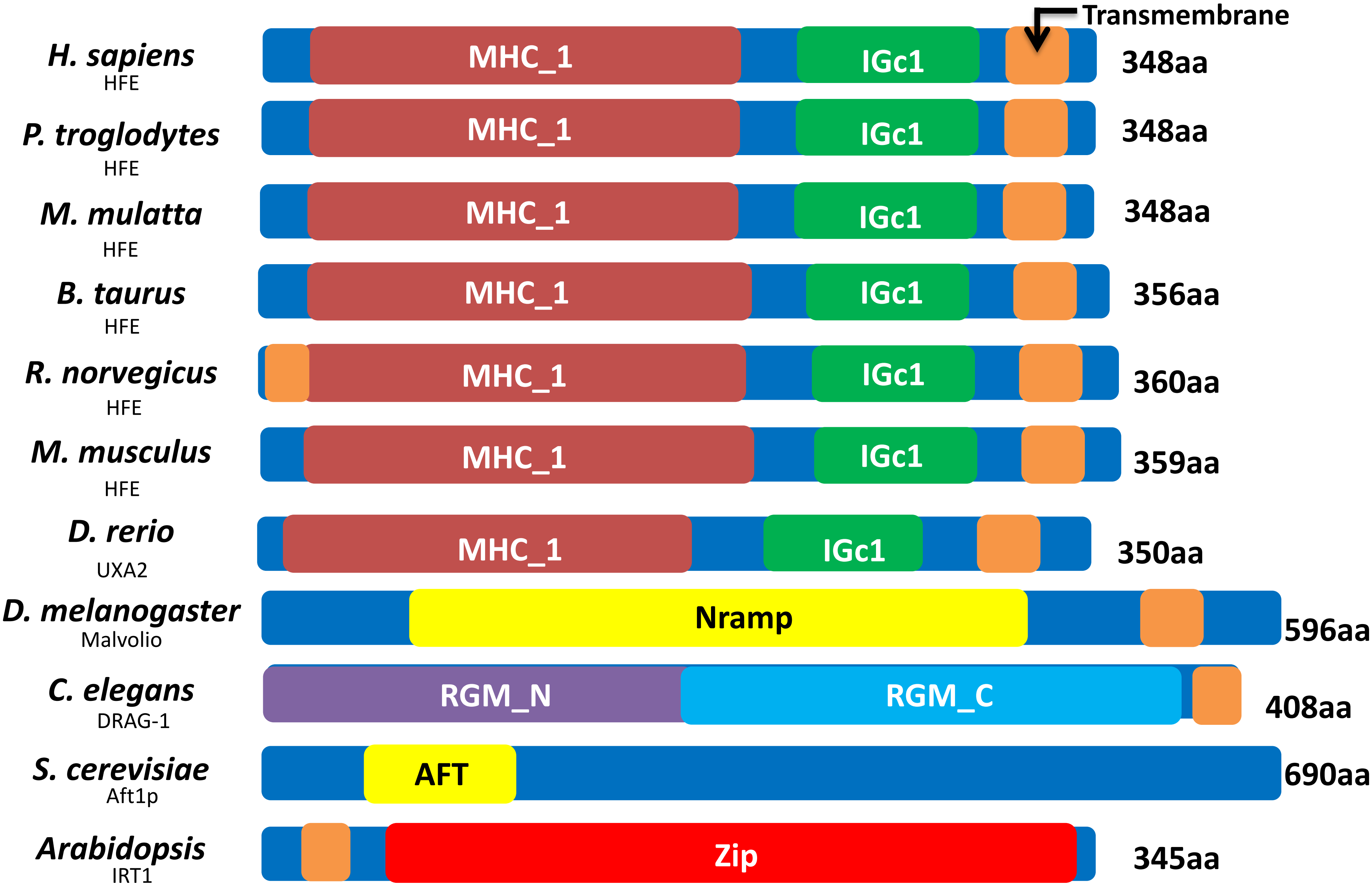
Image: http://onlinelibrary.wiley.com/doi/10.1002/jcp.20349/abstract

# How does HFE function in iron uptake?



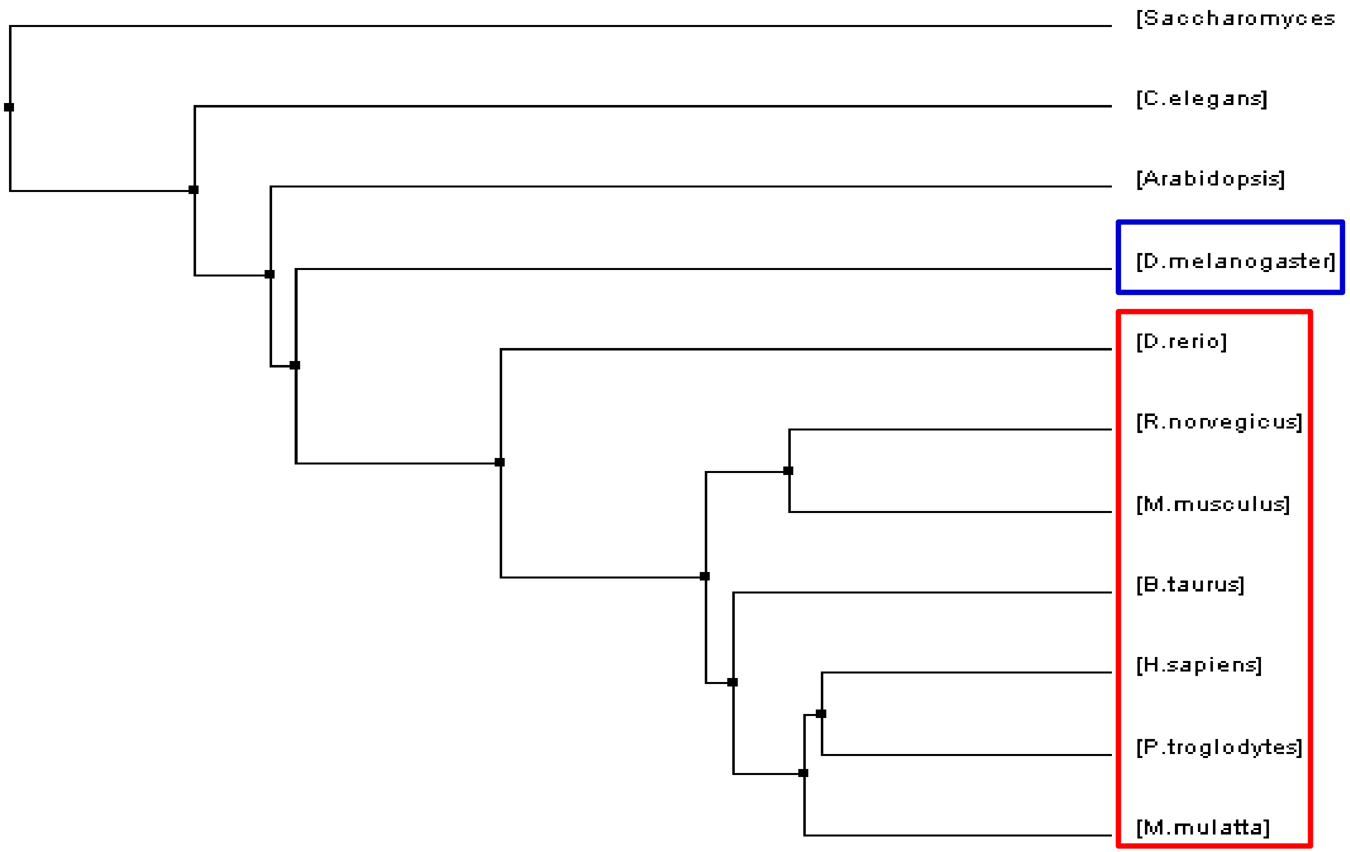

http://www.nature.com/nrendo/journal/v6/n1/full/nrendo.2009.241.html

# How well conserved is HFE?



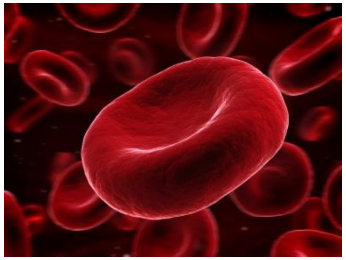
Approach: Clustal Omega

# HFE Phylogeny




Fruit flies have "blood"

Vertebrates

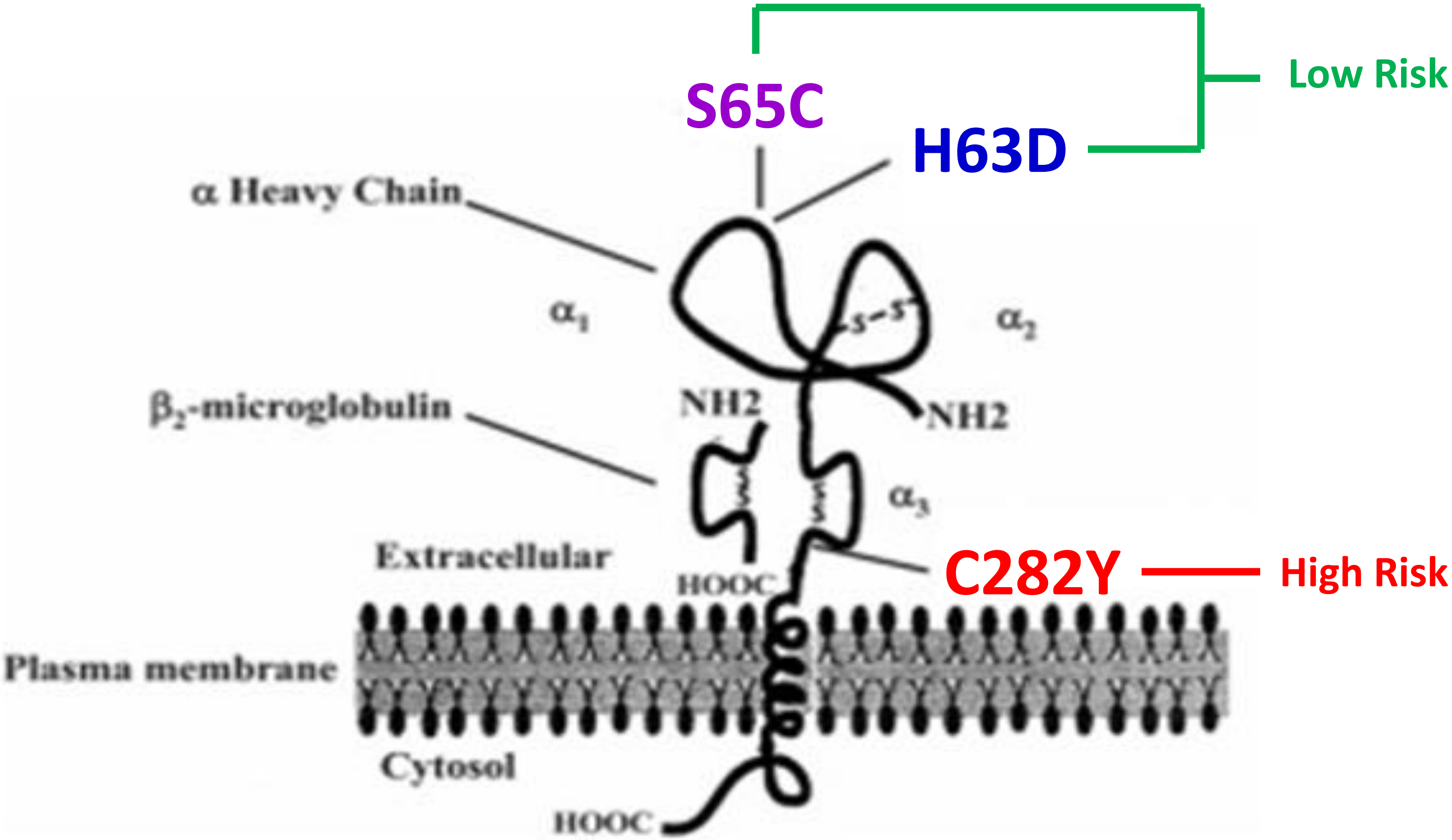


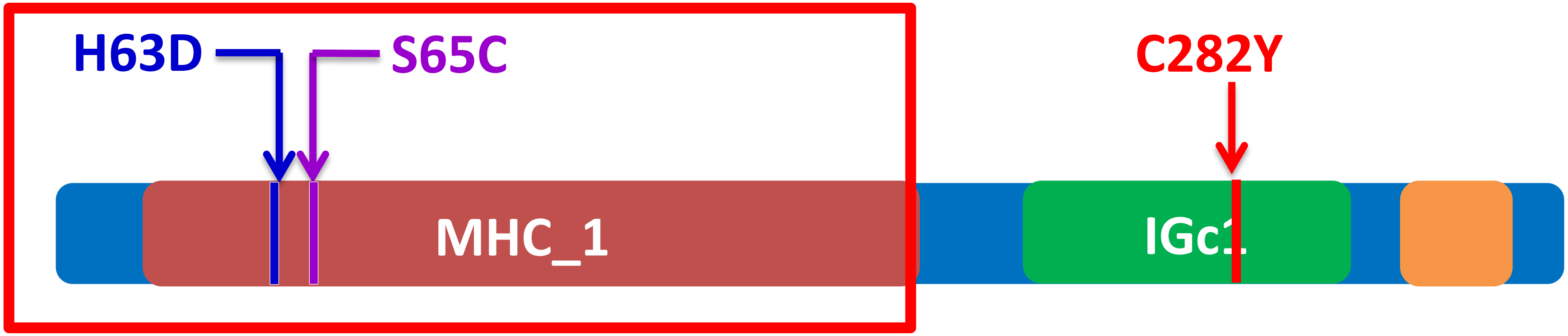
**Average distance using BLOSUM62**

Blood cell: http://jumpstartonline.co.uk/blog/2014/03/iron-deficiency-may-increase-stroke-risk-through-sticky-blood

# Where are the mutations in HFE?

# Differences in HFE mutations lead to iron uptake variability





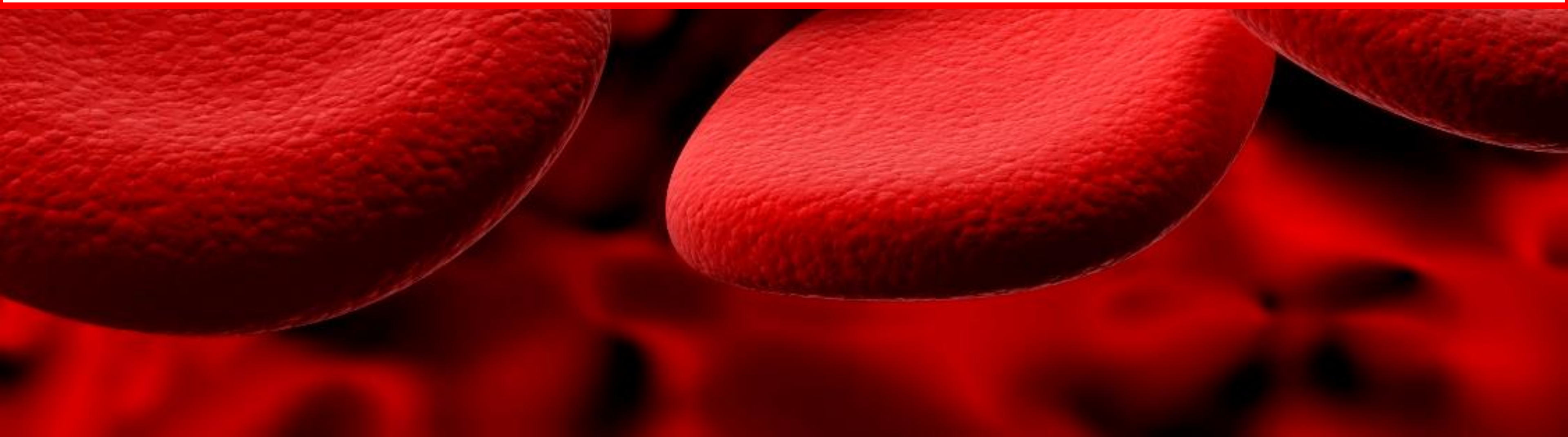

Knowledge Gap

Is the MHC_1 domain of HFE important for iron uptake?

Hypothesis

The MHC_1 domain of HFE is important for iron uptake

# Specific Aim 1: Mutation conservation

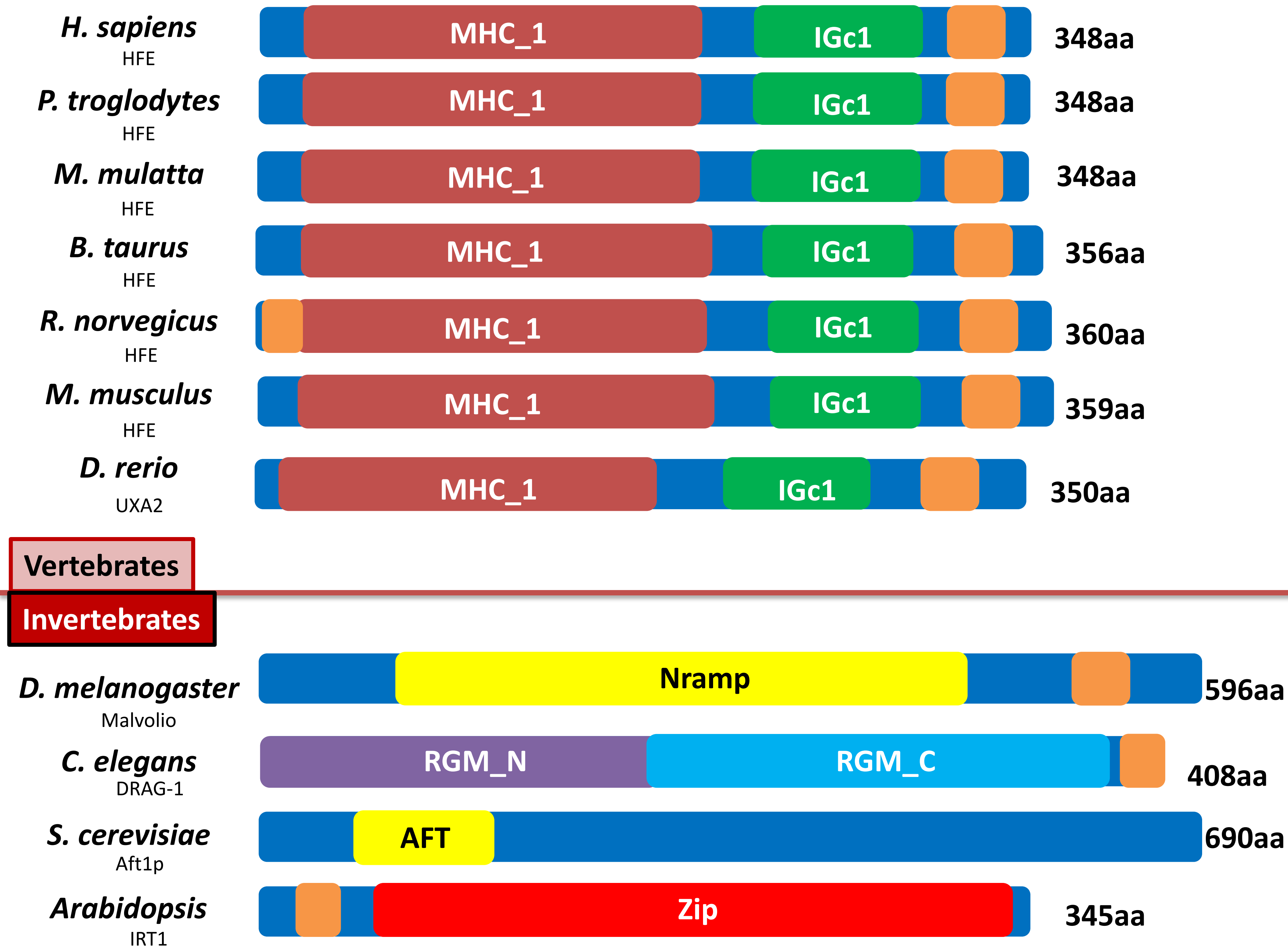


**Aim:** To determine how conserved the known mutation sites in human HFE are between vertebrates and invertebrates.

**Knowledge Gap:** Why do H63D and S65C result in hereditary hemochromatosis?

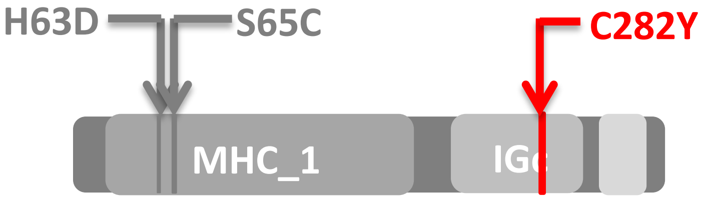
**Hypothesis:** The mutations in MHC_1 will be conserved, indicating the importance of these sites and MHC_1 in HFE's regulation of iron uptake

**Approach:** Sequence alignment using Clustal Omega

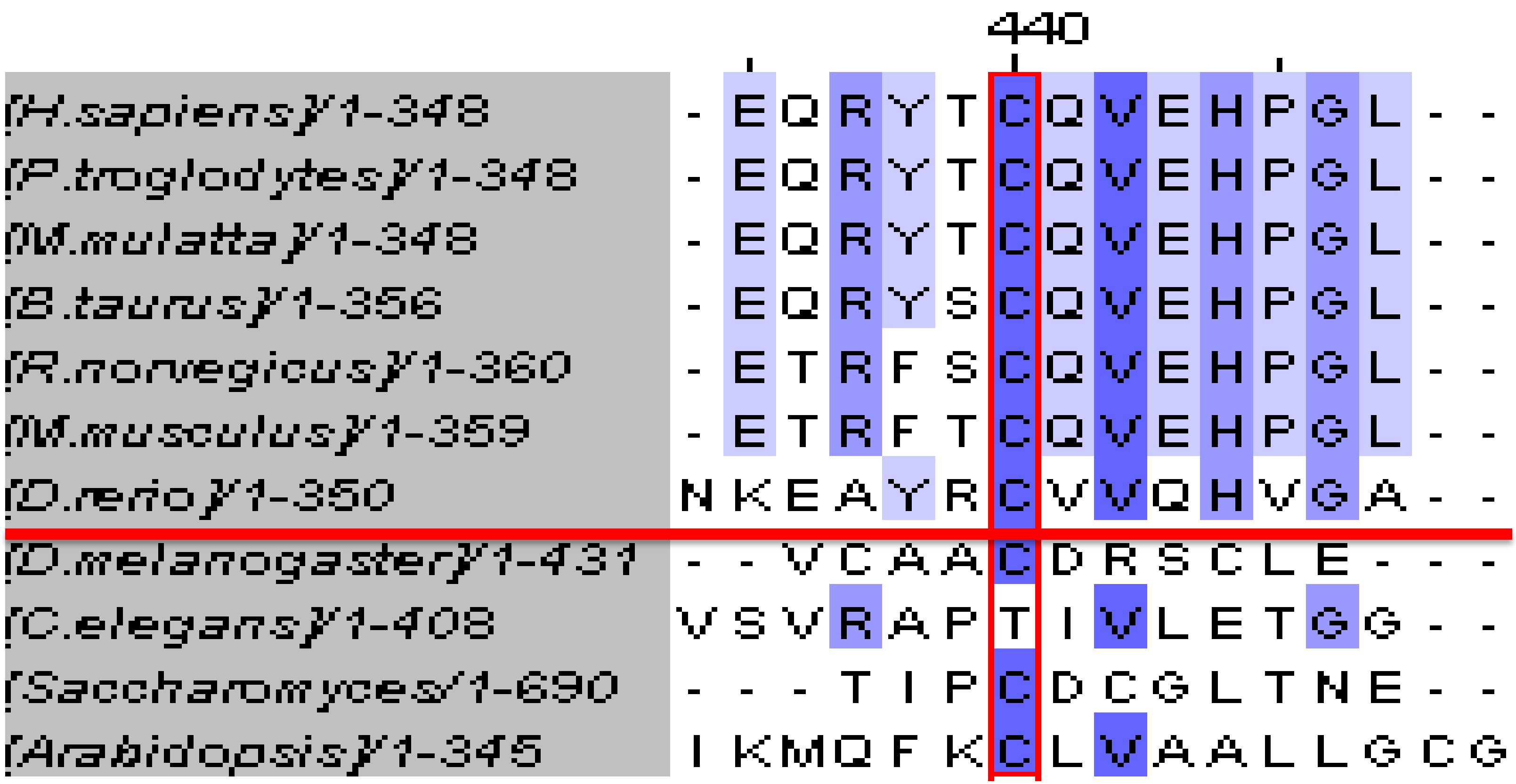


H. sapiens
HFE
MHC_1
IGc1
348aa
P. troglodytes
HFE
MHC_1
IGc1
348aa
M. mulatta
HFE
MHC_1
IGc1
348aa
B. taurus
HFE
MHC_1
IGc1
356aa
R. norvegicus
HFE
MHC_1
IGc1
360aa
M. musculus
HFE
MHC_1
IGc1
359aa
D. rerio
UXA2
MHC_1
IGc1
350aa
Vertebrates
Invertebrates
D. melanogaster
Malvolio
Nramp
596aa
C. elegans
DRAG-1
RGM_N
RGM_C
408aa
S. cerevisiae
Aft1p
AFT
690aa
Arabidopsis
IRT1
Zip
345aa

Approach: Clustal Omega

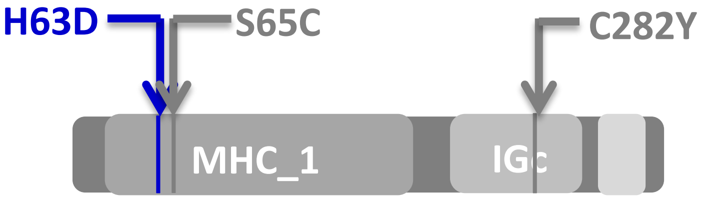



# C282Y site

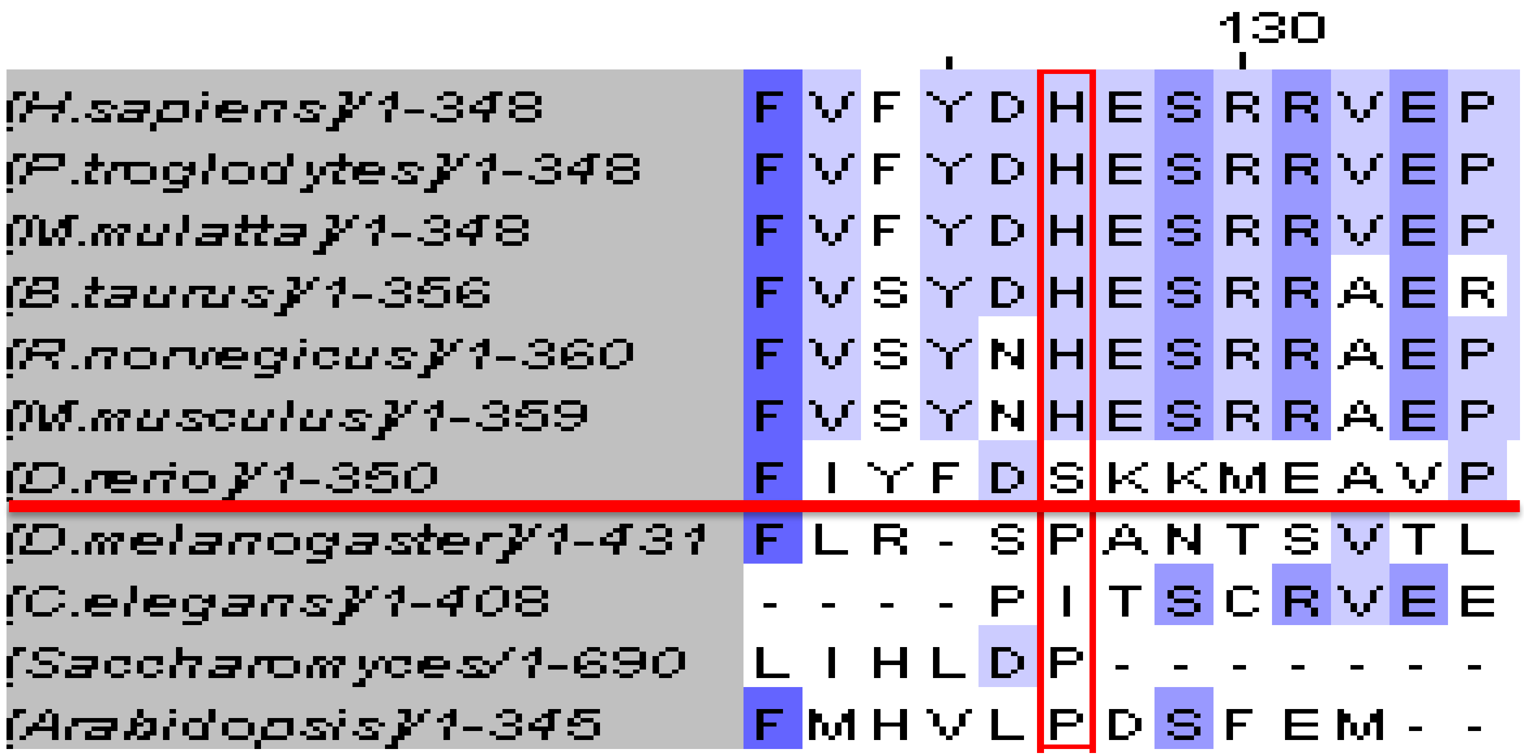



**Summary:** C282Y is highly conserved

Approach: Clustal Omega




# H63D site

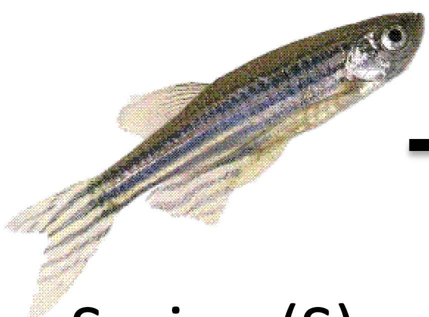



**Summary:** H63D is not conserved across vertebrates or invertebrates

# Future Direction

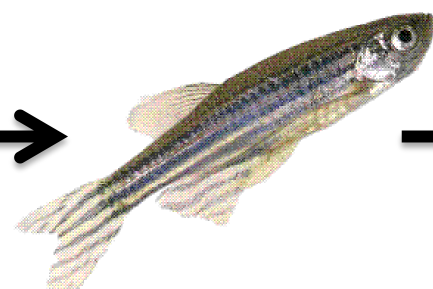
## H63D site

| | | | | | | | | | 130 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| {H.sapiens}/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| {P.troglodytes}/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| {M.mulatta}/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| {B.taurus}/1-356 | F | V | S | Y | D | H | E | S | R | R | A | E | R |
| {R.norvegicus}/1-360 | F | V | S | Y | N | H | E | S | R | R | A | E | P |
| {M.musculus}/1-359 | F | V | S | Y | N | H | E | S | R | R | A | E | P |
| {D.rerio}/1-350 | F | I | Y | F | D | S | K | K | M | E | A | V | P |
| {D.melanogaster}/1-431 | F | L | R | - | S | P | A | N | T | S | V | T | L |
| {C.elegans}/1-408 | - | - | - | - | P | I | T | S | C | R | V | E | E |
| {Saccharomyces/1-690 | L | I | H | L | D | P | - | - | - | - | - | - | - |
| {Arabidopsis}/1-345 | F | M | H | V | L | P | D | S | F | E | M | - | - |



Serine (S)

→



Histidine (H)

→ **Fe ↑ ↓ ?**

**Zebrafish:** http://blogs.oregonstate.edu/superfund/files/2013/09/Zebrafish.gif

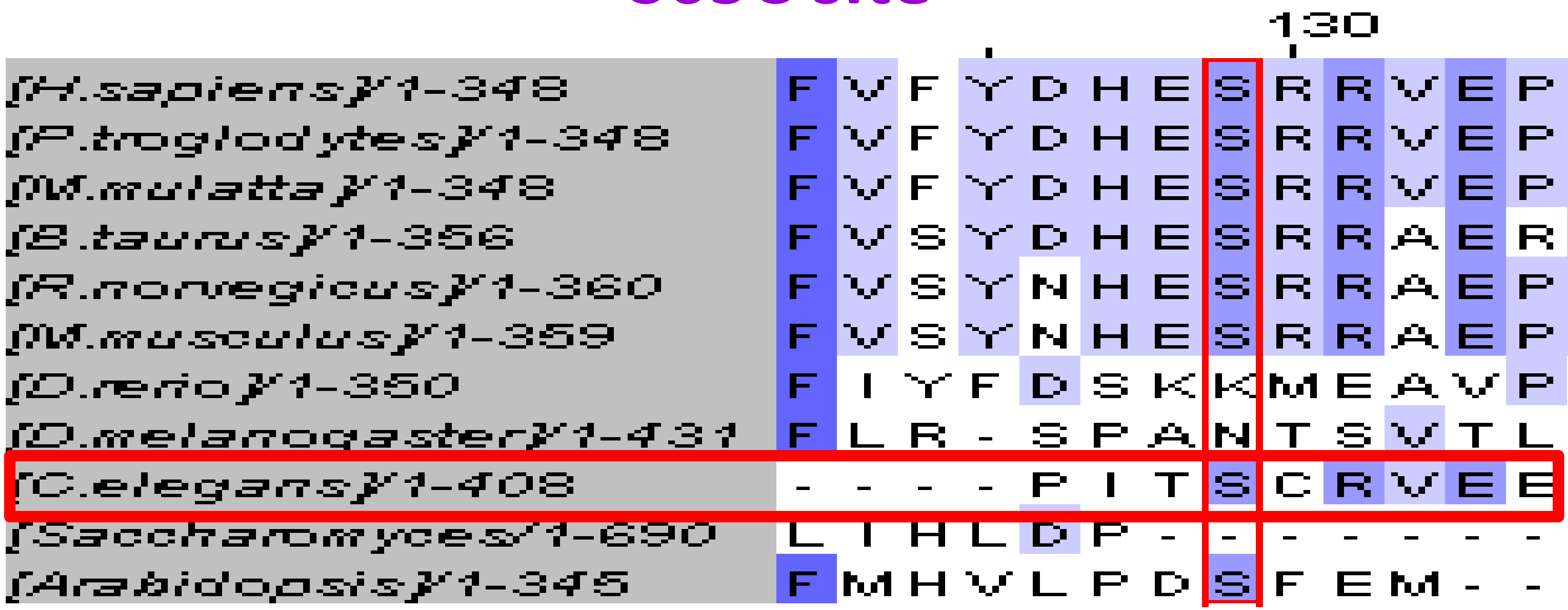

# S65C site

| | | | | | | | | | 130 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [H.sapiens]/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| [P.troglodytes]/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| [M.mulatta]/1-348 | F | V | F | Y | D | H | E | S | R | R | V | E | P |
| [B.taurus]/1-356 | F | V | S | Y | D | H | E | S | R | R | A | E | R |
| [R.norvegicus]/1-360 | F | V | S | Y | N | H | E | S | R | R | A | E | P |
| [M.musculus]/1-359 | F | V | S | Y | N | H | E | S | R | R | A | E | P |
| [D.rerio]/1-350 | F | I | Y | F | D | S | K | K | M | E | A | V | P |
| [D.melanogaster]/1-431 | F | L | R | - | S | P | A | N | T | S | V | T | L |
| [C.elegans]/1-408 | - | - | - | - | P | I | T | S | C | R | V | E | E |
| [Saccharomyces/1-690 | L | I | H | L | D | P | - | - | - | - | - | - | - |
| [Arabidopsis]/1-345 | F | M | H | V | L | P | D | S | F | E | M | - | - |

**Summary:** S65D is conserved in a variety of species

# Future Direction

## S65C site




Serine (S)

Cysteine (C)

**Fe** ↑↓ **?**

**C. elegans:** http://www.easternct.edu/~adams/images/celegansmain.jpg

# Overall Conclusions

Cysteine is highly conserved at position 282

Histidine is somewhat conserved across vertebrates at position 63

Serine is conserved in a variety of species in position 65

# Specific Aim 2: Identify drugs that target HFE

**Aim:** To identify drugs that can modify HFE protein function and lower iron levels

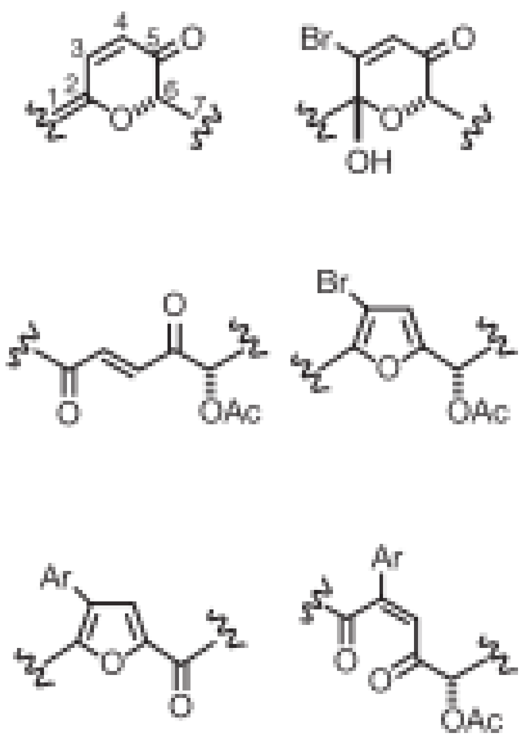
**Knowledge Gap:** What drugs interact with HFE?

**Hypothesis:** Drugs that interact with the MHC_1 domain of HFE will lower iron levels

**Approach:** Chemical genetics

# Chemical Genetics

## Diversity Oriented Library




## *C. elegans*

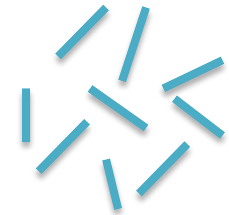

**408aa**

**C. elegans:** http://www.easternct.edu/~adams/images/celegansmain.jpg

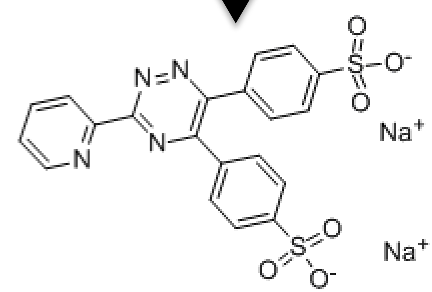
**Library:** Stockwell,B.R.(2004).Exploring biology with small organic molecules. Nature.

# Color detection assay to determine iron levels

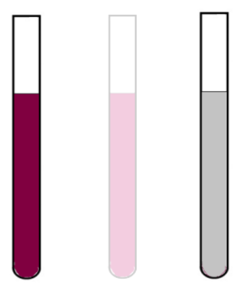
**Chemical Treatment**



**Lyse worms**




**Ferrozine**



**Detection**

**Spectrophotometry quantification**

**C. elegans:** http://www.easternct.edu/~adams/images/celegansmain.jpg

**Ferrozine:** http://www.chemicalbook.com/CAS%5CGIF%5C28048-33-1.gif

**C. elegans:** http://www.easternct.edu/~adams/images/celegansmain.jpg

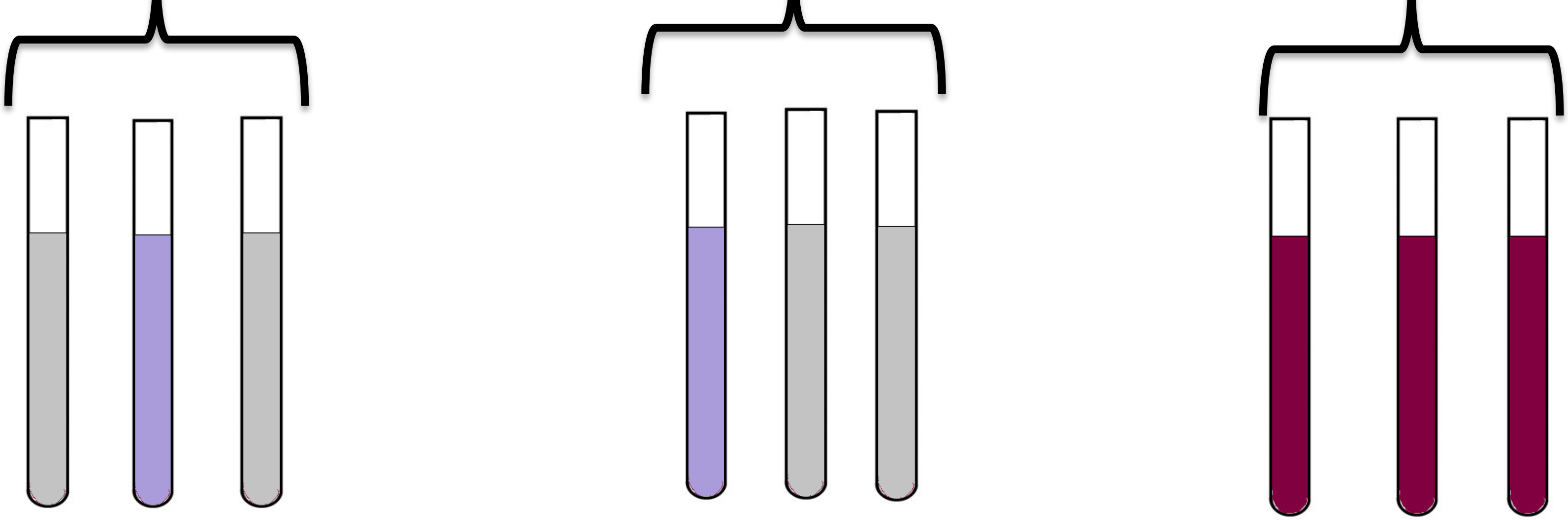
**DRAG-1 = HFE Homologue**

# Expected Results

**Chemical interacts with nonfunctional DRAG-1**

**Control**
**Functional DRAG-1**

**Chemical does not interact with nonfunctional DRAG-1**



# Future Directions

Take chemicals that interact with DRAG-1 and test them in an organism that has the MHC_1 domain in their HFE homologue

# References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# Questions?