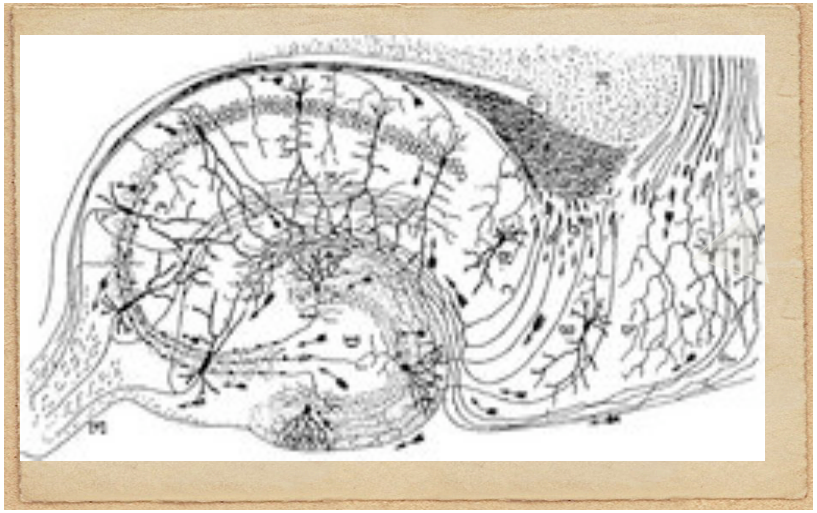
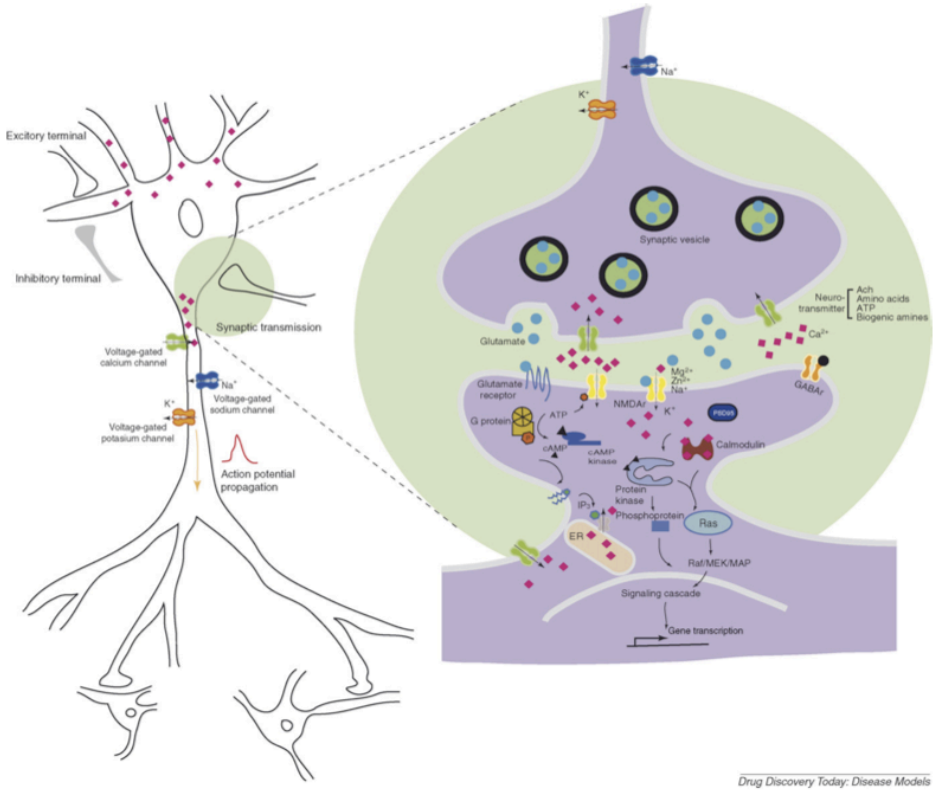
# Engineering proteins for the brain

Loren Looger

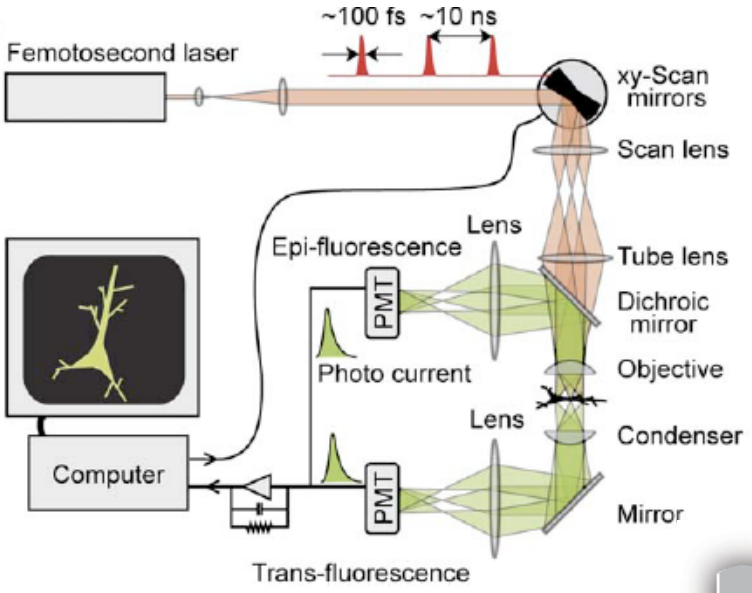
HHMI Janelia Farm

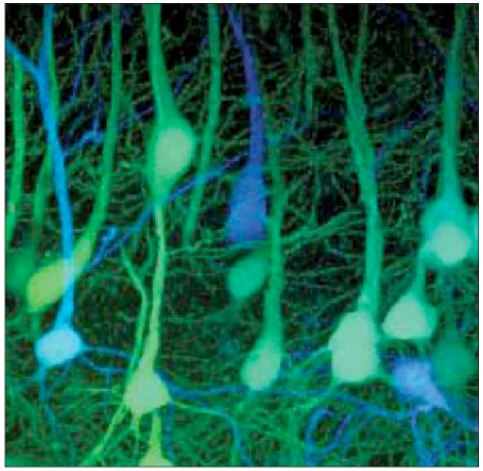
(ps I lost my cell phone: silver Samsung Verizon)

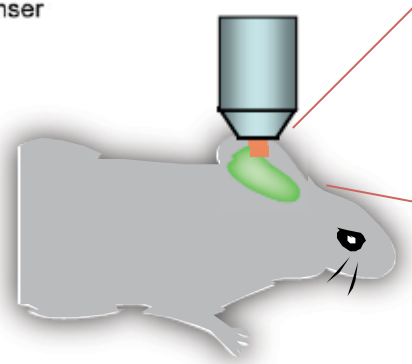


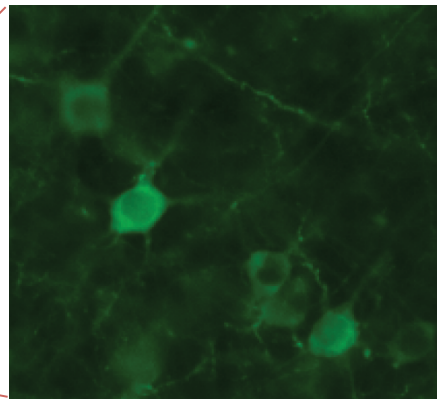



*Drug Discovery Today: Disease Models*

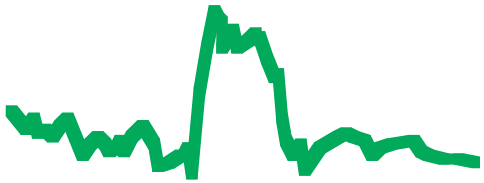

~100 fs
~10 ns
Femotosecond laser
xy-Scan mirrors
Scan lens
Lens
Epi-fluorescence
Tube lens
PMT
Dichroic mirror
Photo current
Objective
Lens
Condenser
Computer
PMT
Mirror
Trans-fluorescence



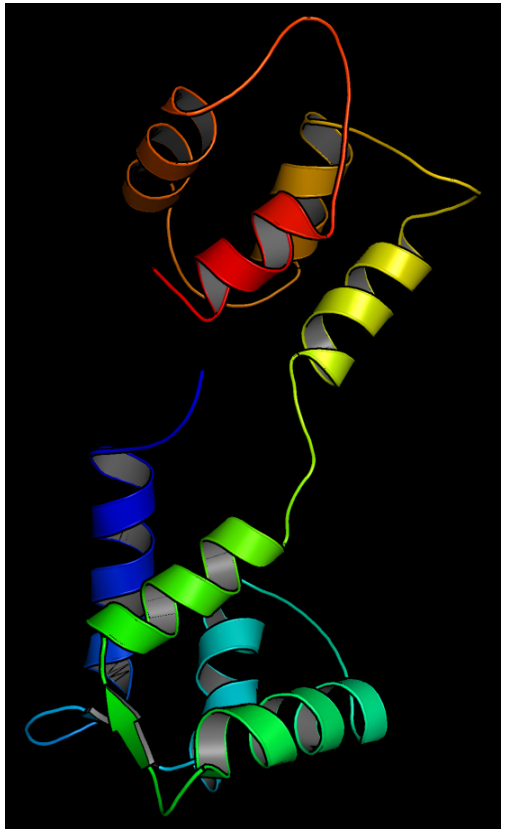


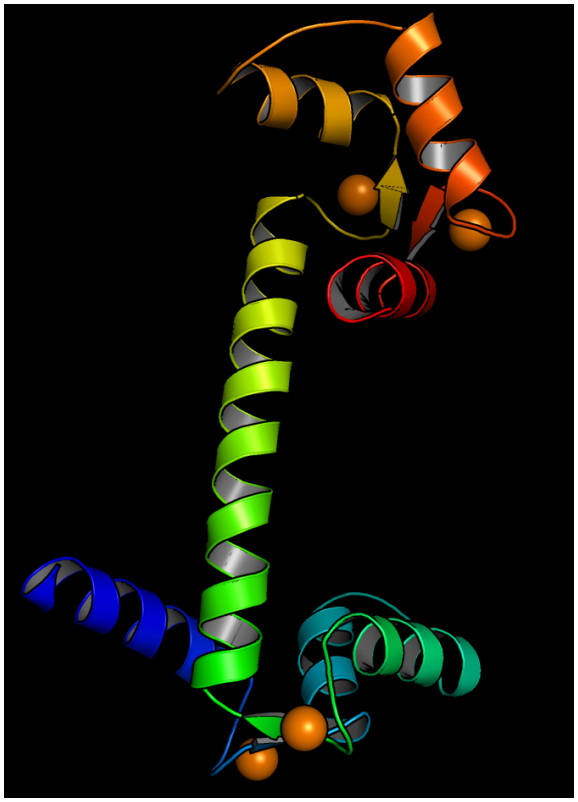


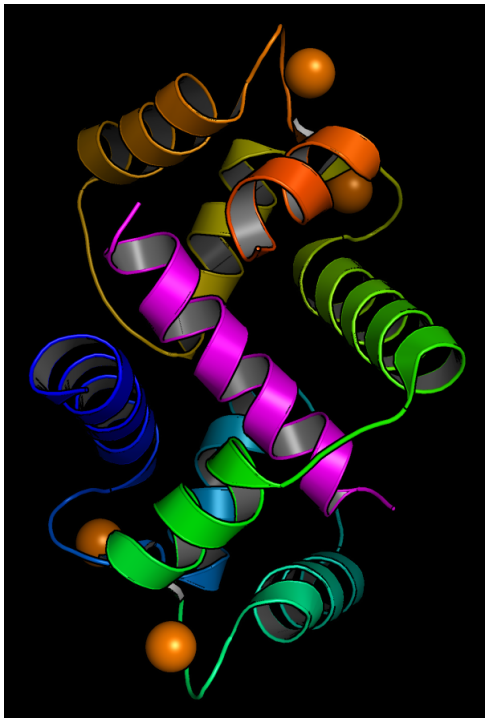
ΔF/F

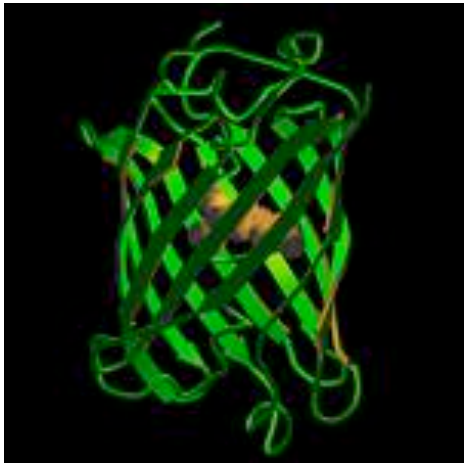


spikes

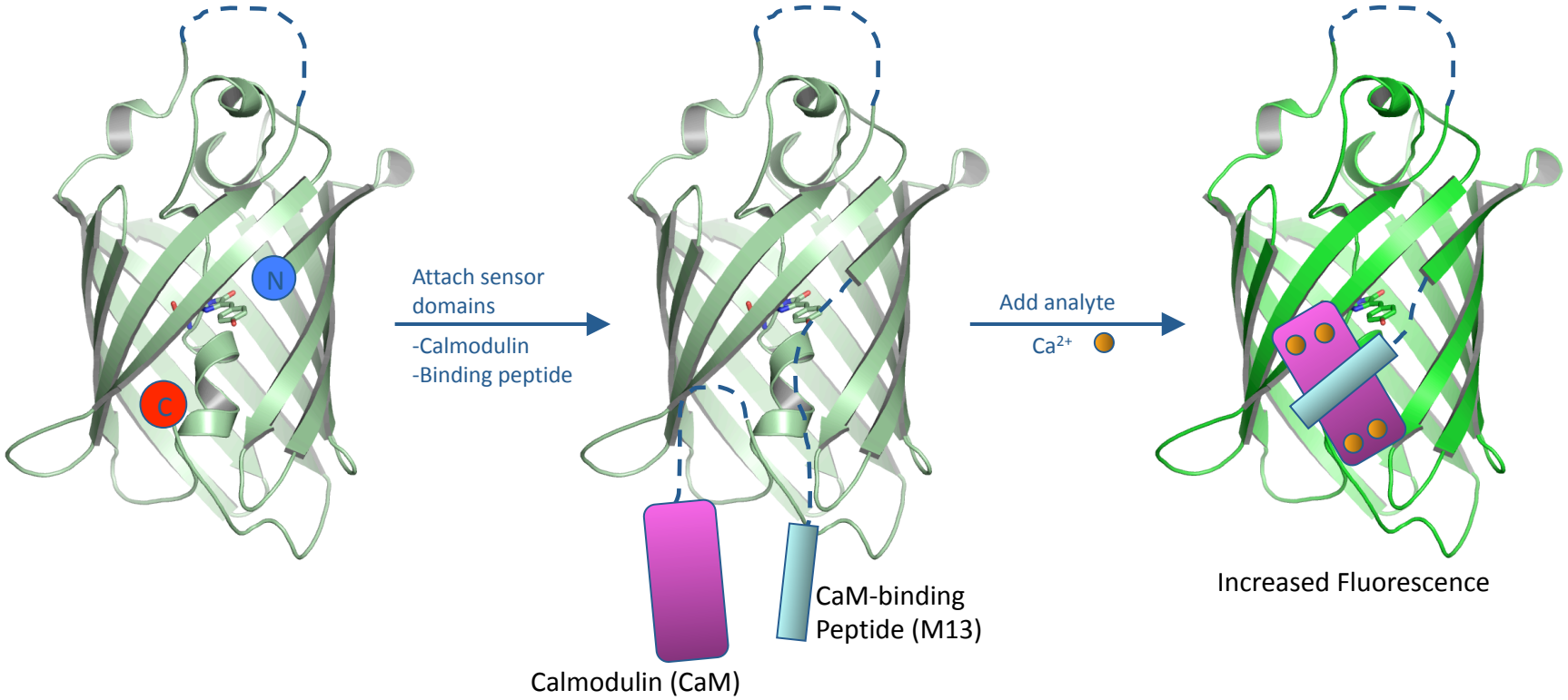


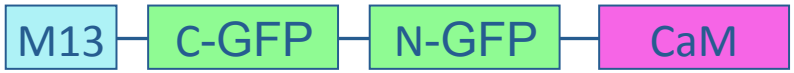






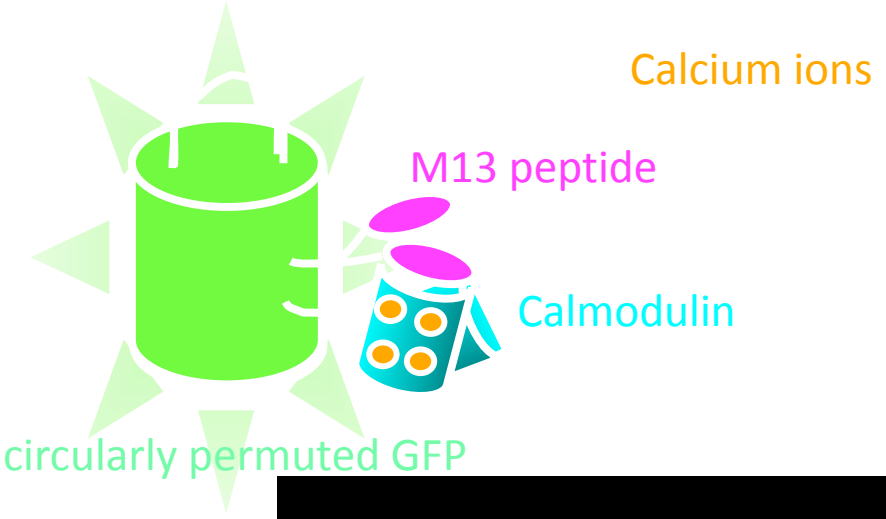
# Assembling a $Ca^{2+}$ sensor from cpGFP

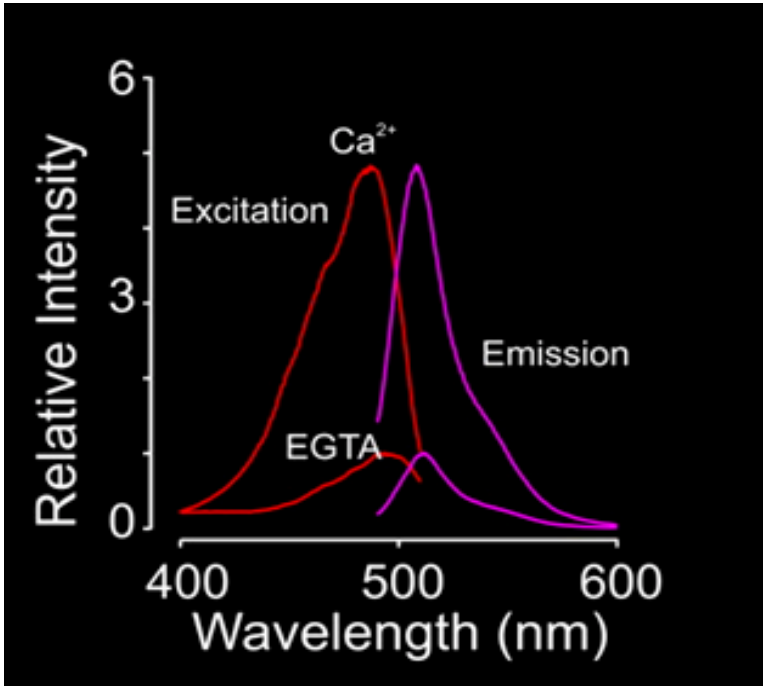


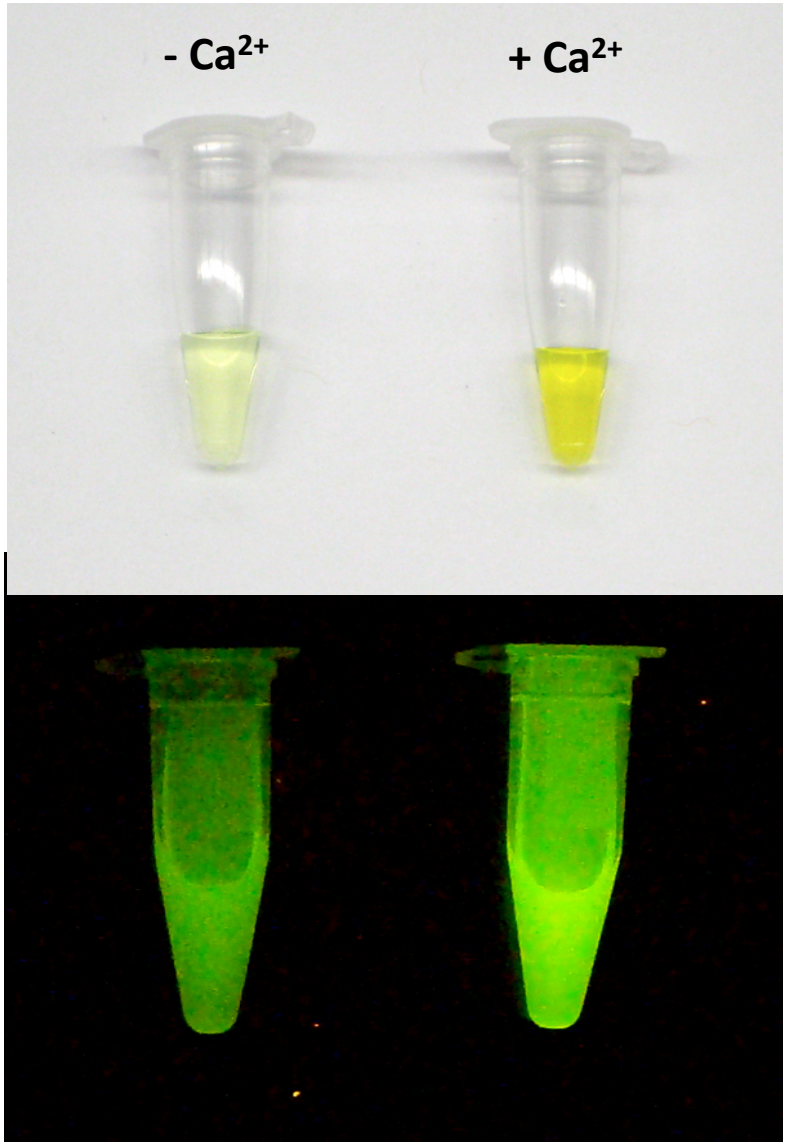




GCaMP

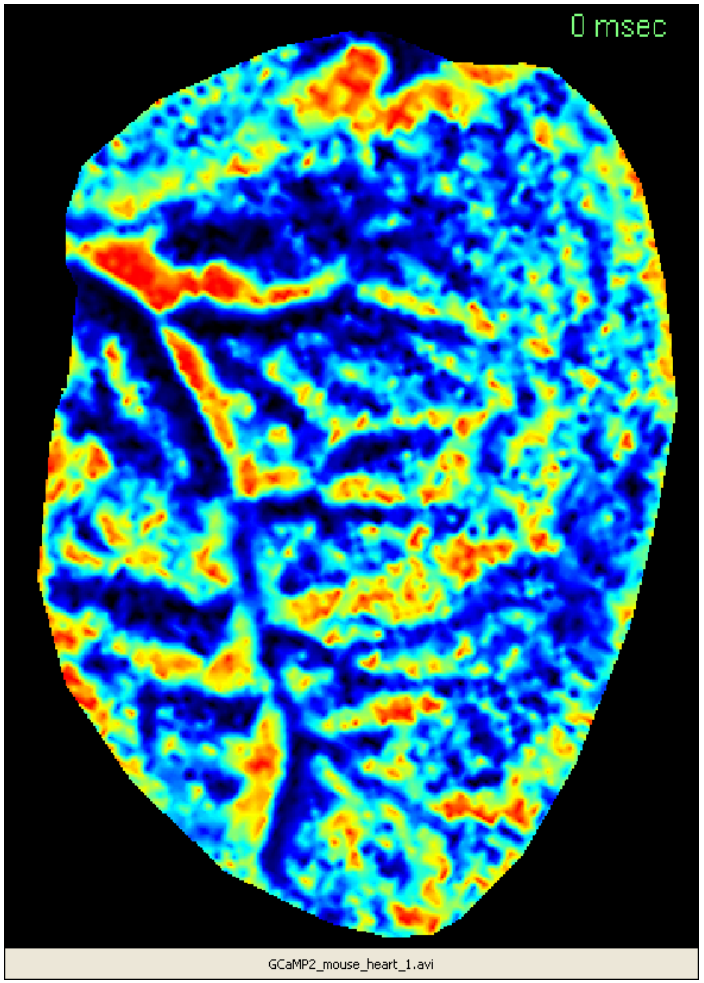
M13 C-GFP N-GFP CaM



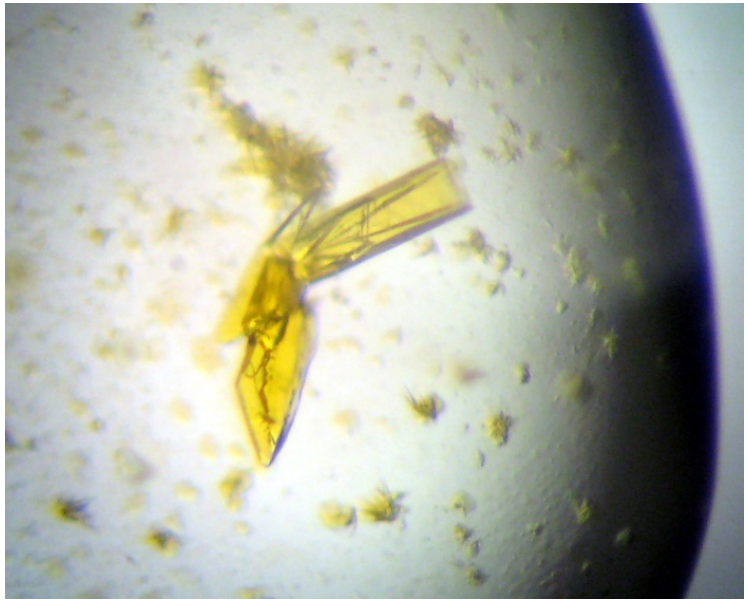



Using GCaMP to image calcium transients in the cardiac myocytes of a live transgenic mouse




Tallini et al. *PNAS* 103, **2006**, 4753.

# $Ca^{2+}$-GCaMP2



| **Data Collection** | |
|---|---|
| **Crystal** | GCaMP2 |
| **Data Set** | Native |
| **Space Group** | C2 |
| ***Unit Cell Dimensions*** | |
| **a (Å)** | 129.0 |
| **b (Å)** | 47.4 |
| **c (Å)** | 68.7 |
| **β (°)** | 99.7 |
| **Beam Line** | APS 31-ID |
| **Temperature (K)** | 100 |
| **Wavelength (Å)** | 0.9793 |
| **Resolution Range (Å)** | 30-1.8 |
| **Completeness (%)** | 98.5 (97.6) |
| **Redundancy** | 7.5 (7.6) |
| $I / \sigma(I)$ | 18.8 (5.1) |
| $R_{sym}$ (%) | 7.0 (46.8) |
| **Refinement** | |
| $R_{cryst}$ ($R_{free}$) (%) | 18.9 (24.1) |
| **Resolution Range (Å)** | 20-1.7 |

Numbers in parentheses are for the highest resolution shell data.

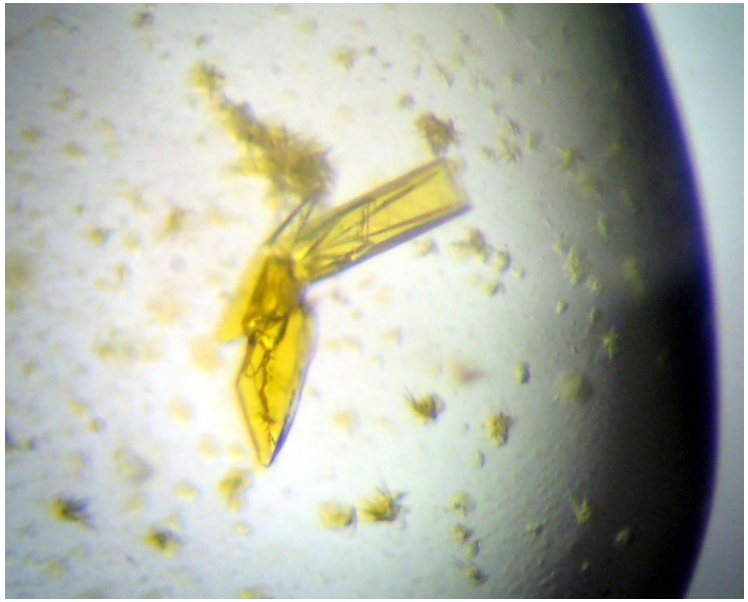
- $Ca^{2+}$-bound GCaMP2
- C2, 1 GCaMP2 sensor per ASU
- Solved by molecular replacement
- Refined at 1.8 Å

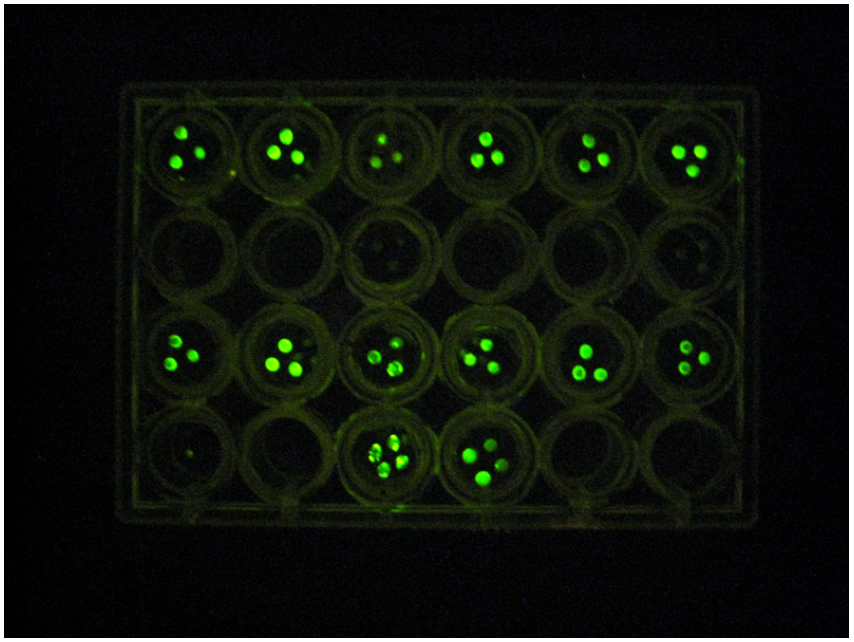
$R_{work}$ = 18.9%

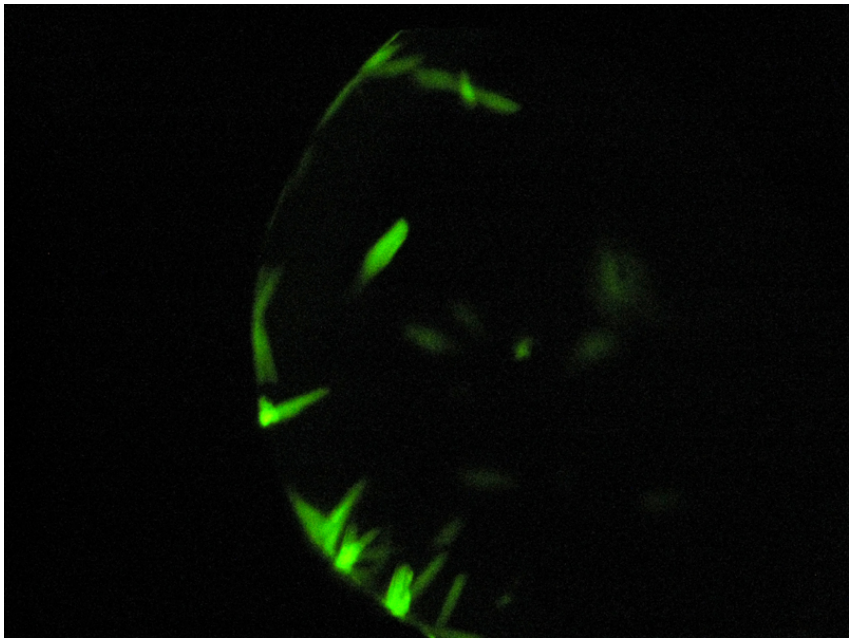
$R_{free}$ = 24.1%

Rodríguez Guilbe, M. M., Alfaro Malavé, E. C., Akerboom, J., Marvin, J. S., Looger, L. L., Schreiter, E. R. (2008) *Acta Crystallogr Sect F* **64,** 629-631.

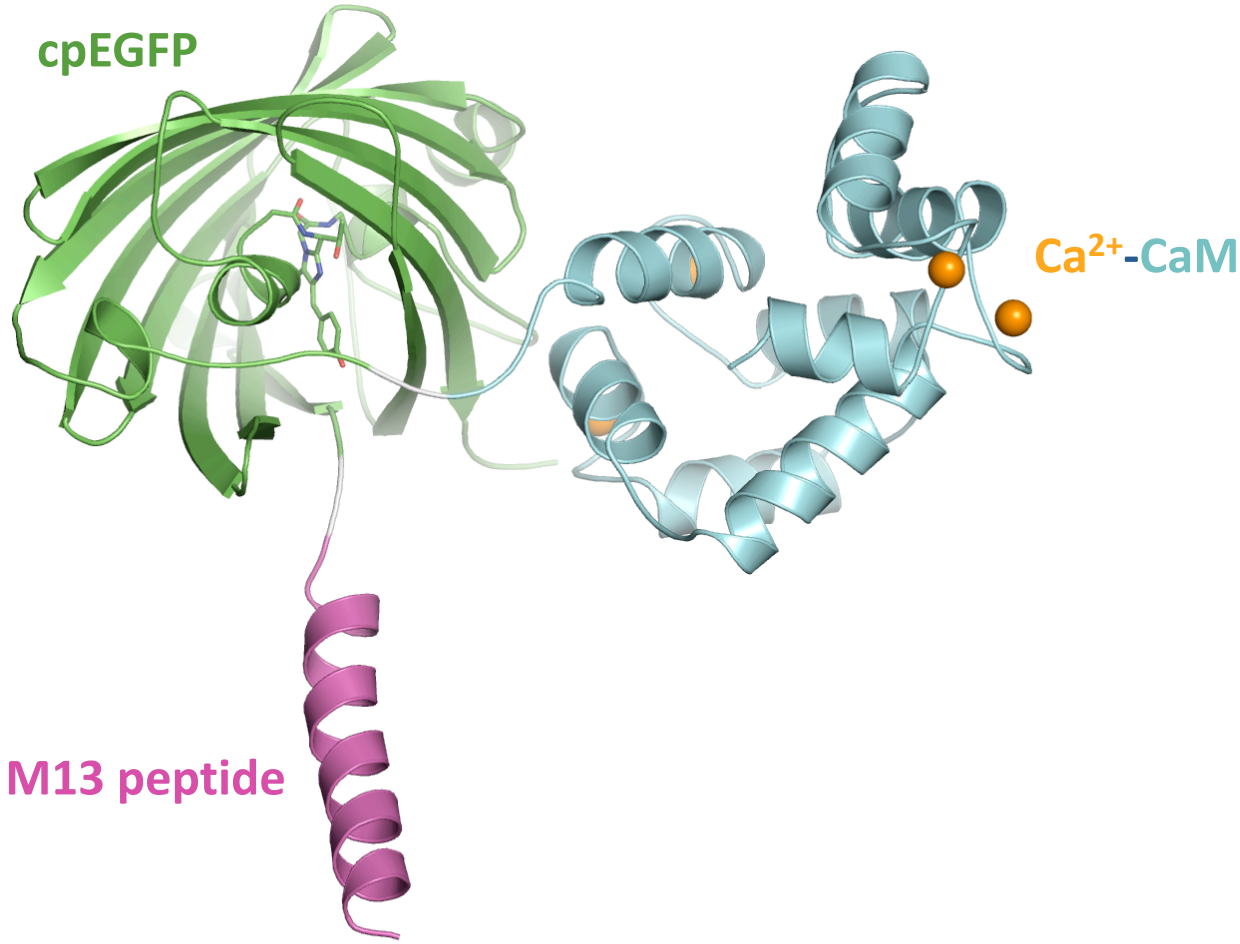
# $Ca^{2+}$-GCaMP2



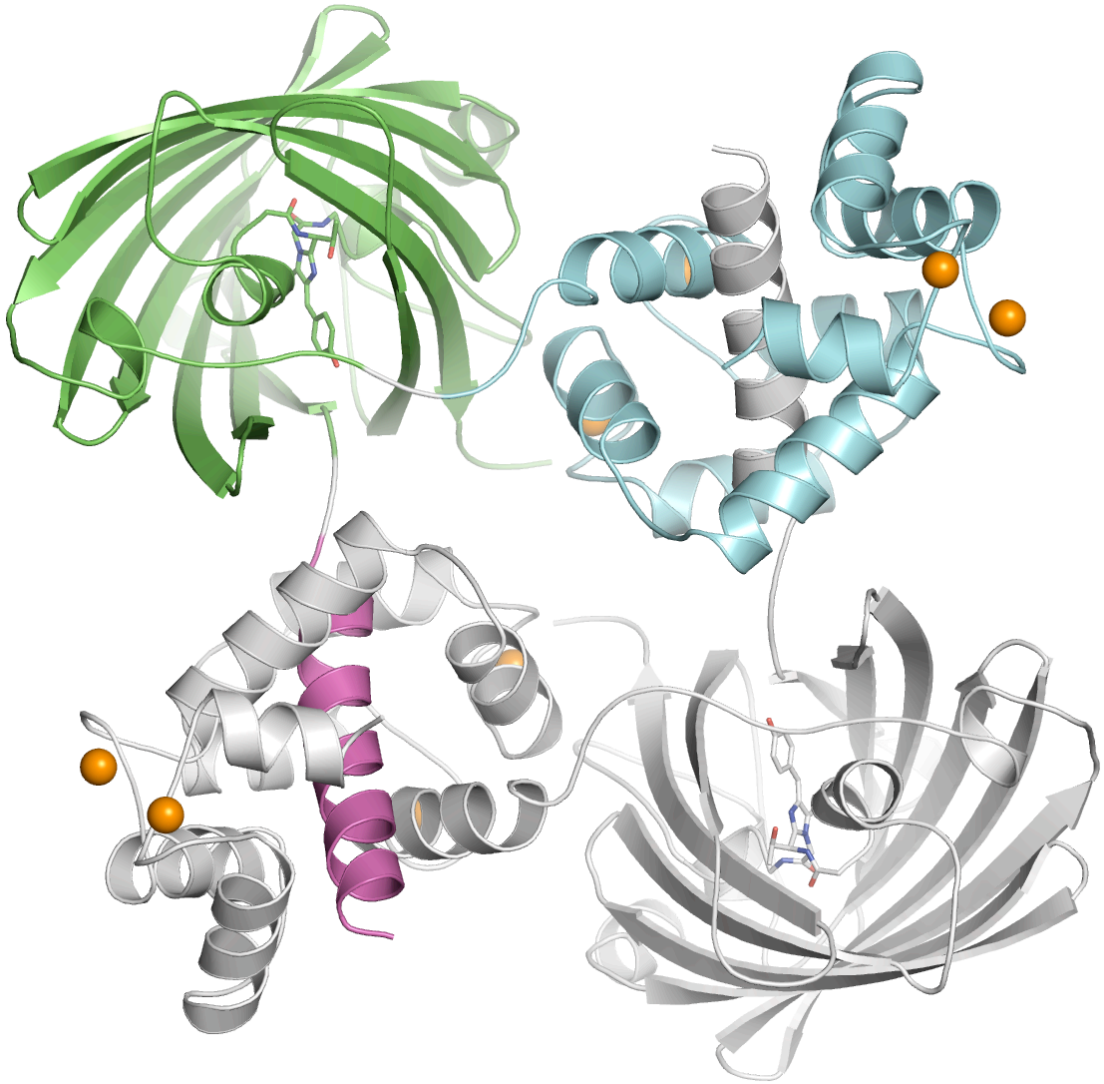




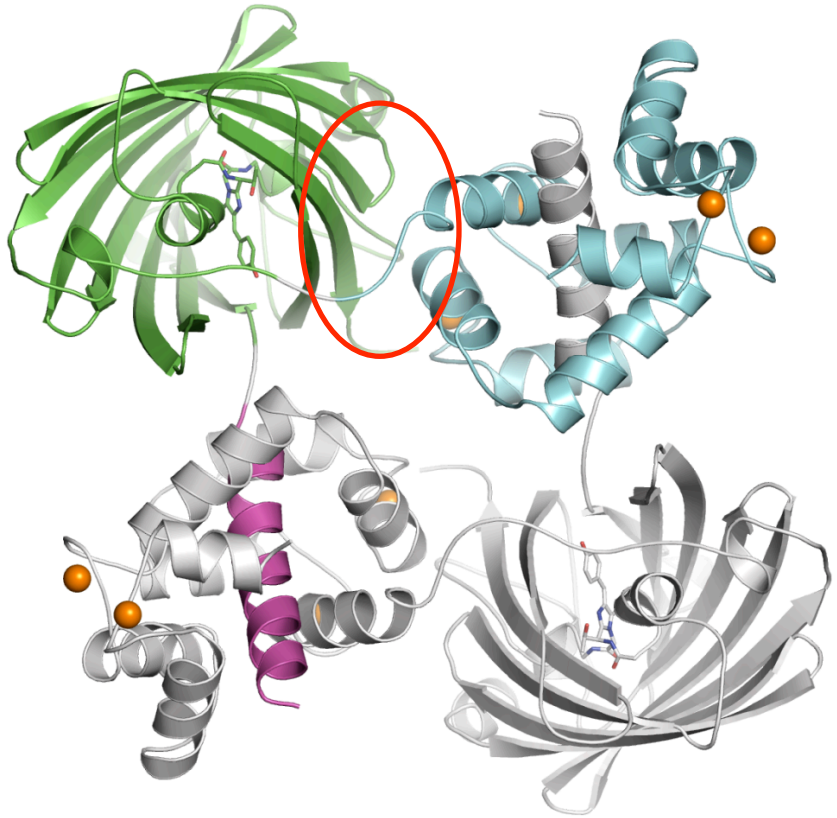
# $Ca^{2+}$-GCaMP2 structure



# $Ca^{2+}$-GCaMP2 dimer structure

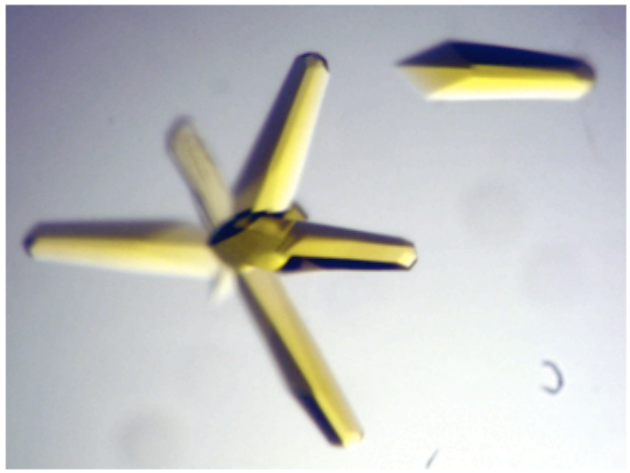


Mutations to increase GCaMP2 monomer:dimer ratio.



- Designed a panel of mutations to selectively disrupt dimerization
- Mutations introduce steric or electrostatic repulsion at domain interfaces

# $Ca^{2+}$-GCaMP2 monomer

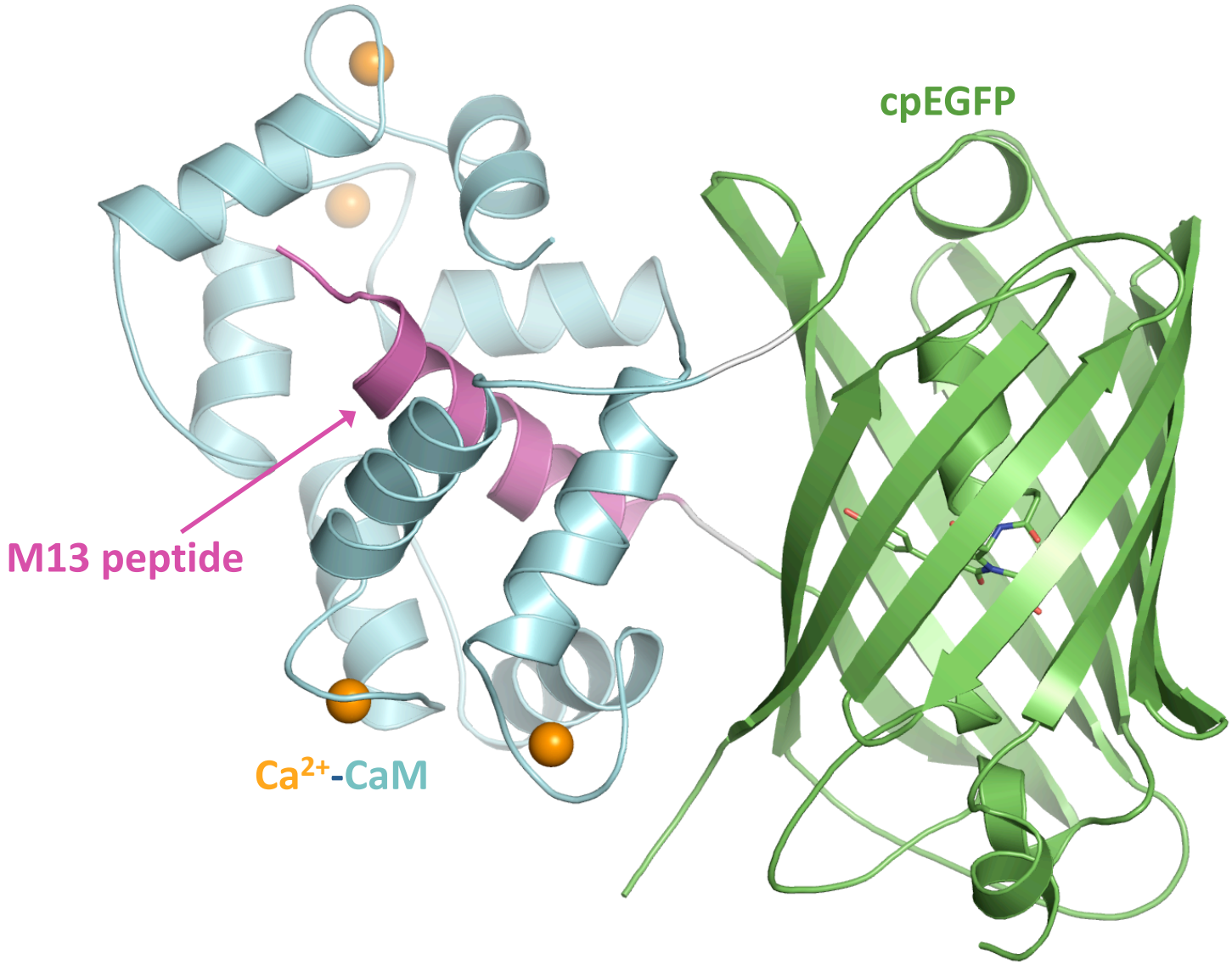


| Data Collection | | | |
|---|---|---|---|
| **Crystal** | GCaMP2 | GCaMP2, G87R | GCaMP2, K378W |
| **Space Group** | $P2_12_12$ | $P4_12_12$ | $P4_12_12$ |
| ***Unit Cell Dimensions*** | | | |
| **a (Å)** | 60.5 | 120.8 | 121.6 |
| **b (Å)** | 68.8 | 120.8 | 121.6 |
| **c (Å)** | 117.3 | 97.4 | 97.3 |
| **Beam Line** | ALS 8.2.2 | UPR | APS 31-ID |
| **Temperature (K)** | 100 | 100 | 100 |
| **Wavelength (Å)** | 1.0000 | 1.5418 | 0.9793 |
| **Resolution Range (Å)** | 50-2.65 | 29.3-2.8 | 25.9-2.0 |
| **Completeness (%)** | 98.7 (92.1) | 100 (100) | 100 (100) |
| **Redundancy** | 3.1 (3.0) | 11.9 (12.0) | 14.1 (13.5) |
| $I / \sigma(I)$ | 20.4 (4.2) | 8.6 (3.5) | 21.5 (4.7) |
| $R_{sym}$ (%) | 7.5 (27.9) | 18.4 (57.3) | 9.6 (60.1) |
| **Refinement** | | | |
| $R_{cryst}$ ($R_{free}$) (%) | 22.2 (28.0) | 22.4 (26.6) | 19.4 (22.4) |
| **High Resolution (Å)** | 2.65 | 2.8 | 2.0 |

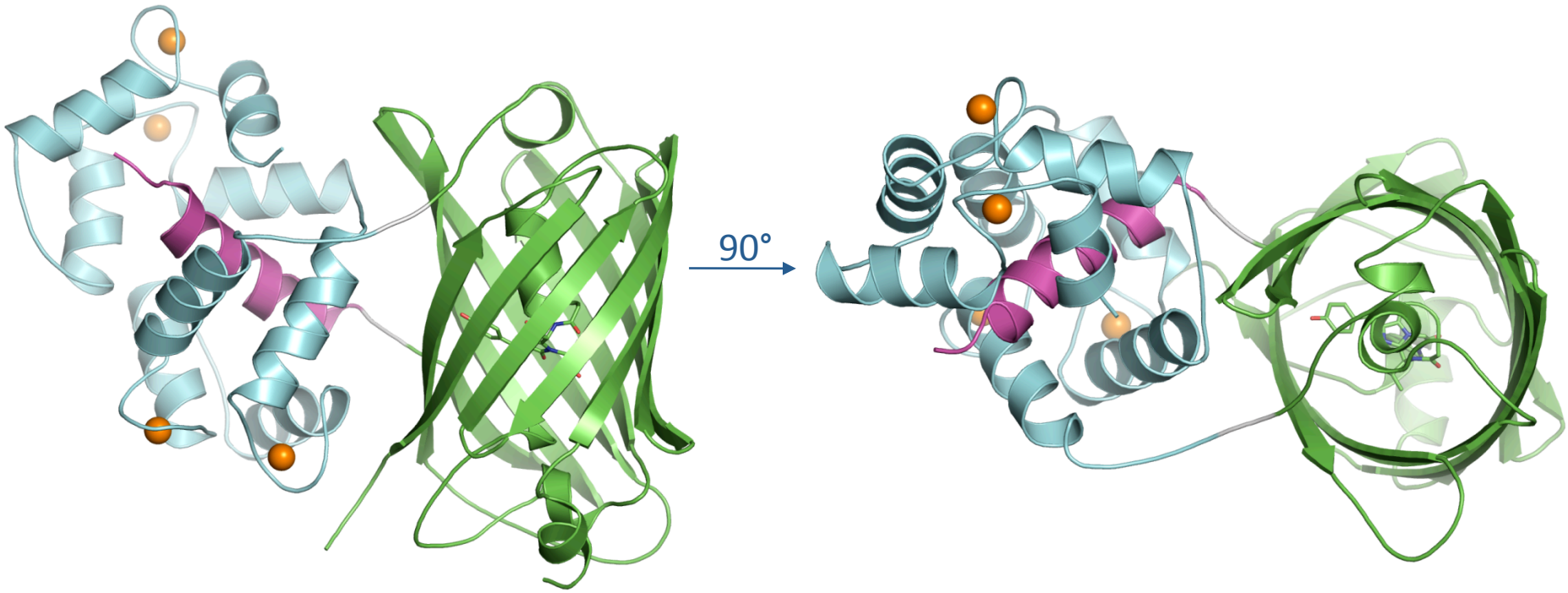
Numbers in parentheses are for the highest resolution shell data.

- $Ca^{2+}$-bound GCaMP2 monomer and two mutants
- 1 GCaMP2 sensor per ASU in each
- Solved by molecular replacement
- Refined at 2.0 Å (K378W mutant)

# $Ca^{2+}$-GCaMP2 monomer structure



# $Ca^{2+}$-GCaMP2 monomer structure



# $Ca^{2+}$-free GCaMP2 (8EF mutant)

| | |
|---|---|
| **Data Collection** | |
| **Crystal** | 8EF-apo |
| **Space Group** | C2 |
| ***Unit Cell Dimensions*** | |
| **a (Å)** | 211.9 |
| **b (Å)** | 47.7 |
| **c (Å)** | 43.0 |
| **β (°)** | 97.6 |
| **Beam Line** | APS 31-ID |
| **Temperature (K)** | 100 |
| **Wavelength (Å)** | 0.9793 |
| **Resolution Range (Å)** | 31.9-2.8 |
| **Completeness (%)** | 98.9 (98.6) |
| **Redundancy** | 7.1 (7.1) |
| ***I* / σ(*I*)** | 15.4 (3.2) |
| **$R_{sym}$ (%)** | 15.4 (61.7) |
| | |
| **Refinement** | |
| **$R_{cryst}$ ($R_{free}$) (%)** | 21.0 (28.3) |
| **Resolution Range (Å)** | 30-2.8 |

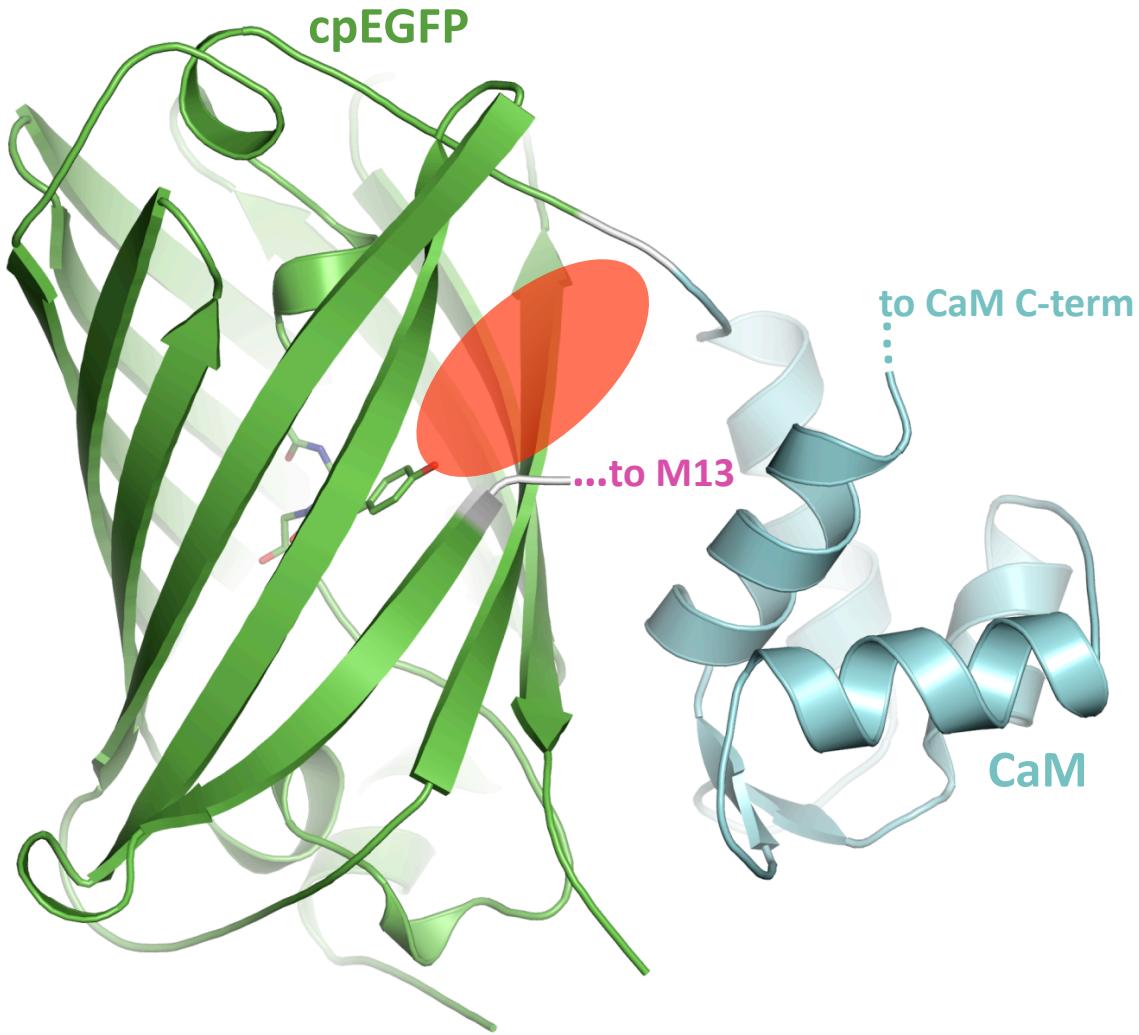
Numbers in parentheses are for the highest resolution shell data.

- $Ca^{2+}$-free GCaMP2 8EF
- C2, 1 GCaMP2 sensor per ASU
- Solved by molecular replacement
- Refined at 2.8 Å

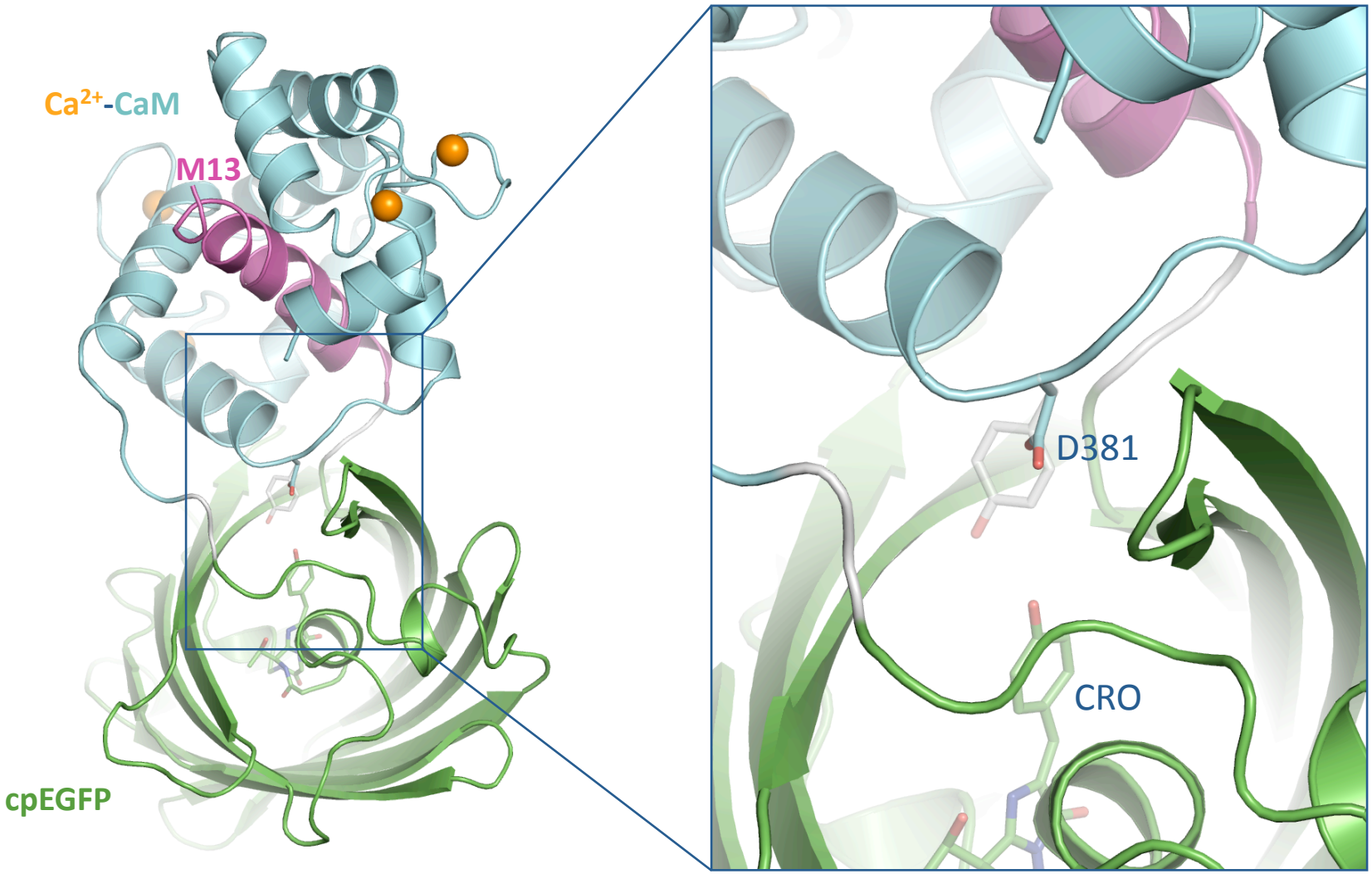
$R_{work}$ = 21.0%

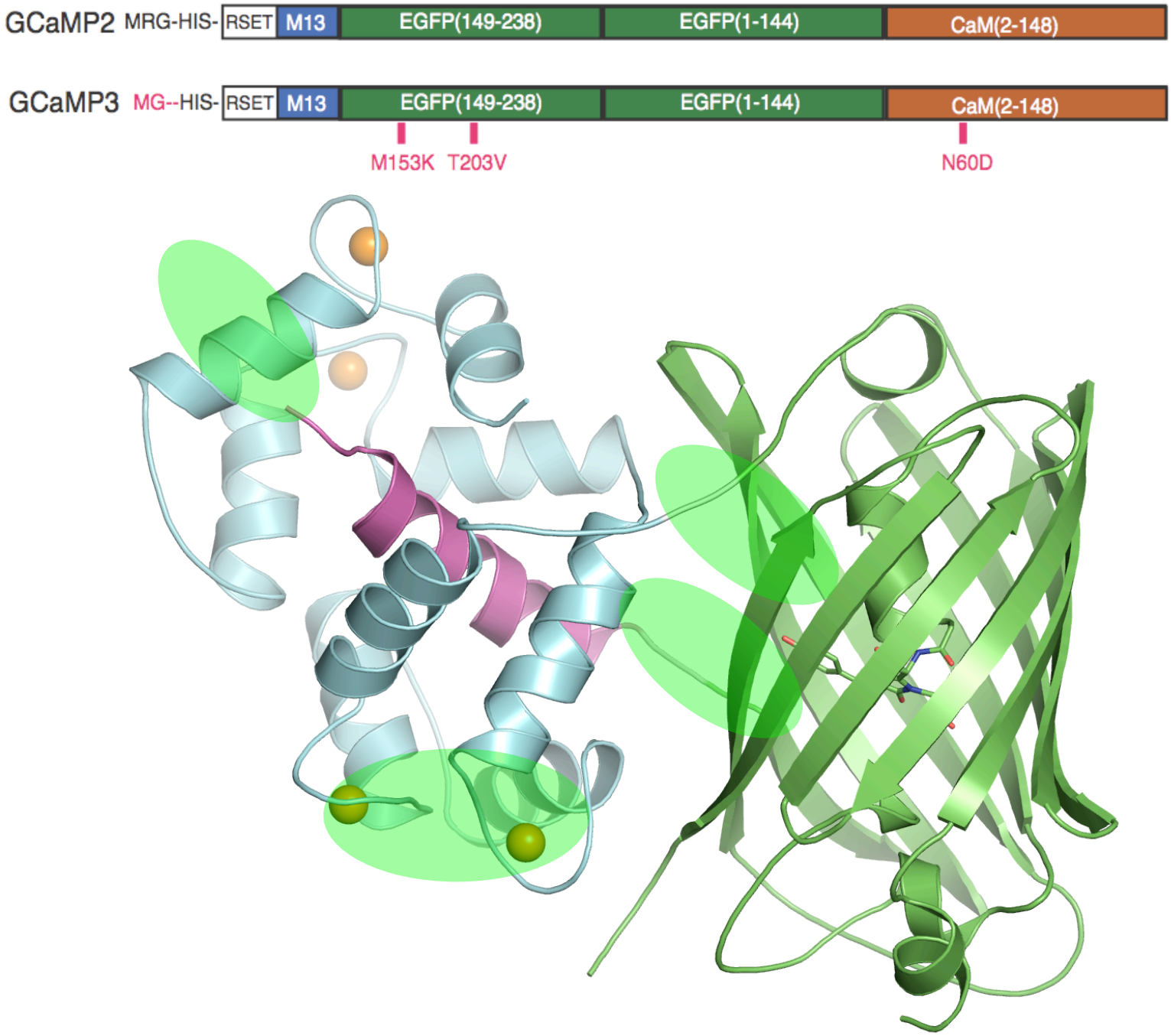
$R_{free}$ = 28.3%

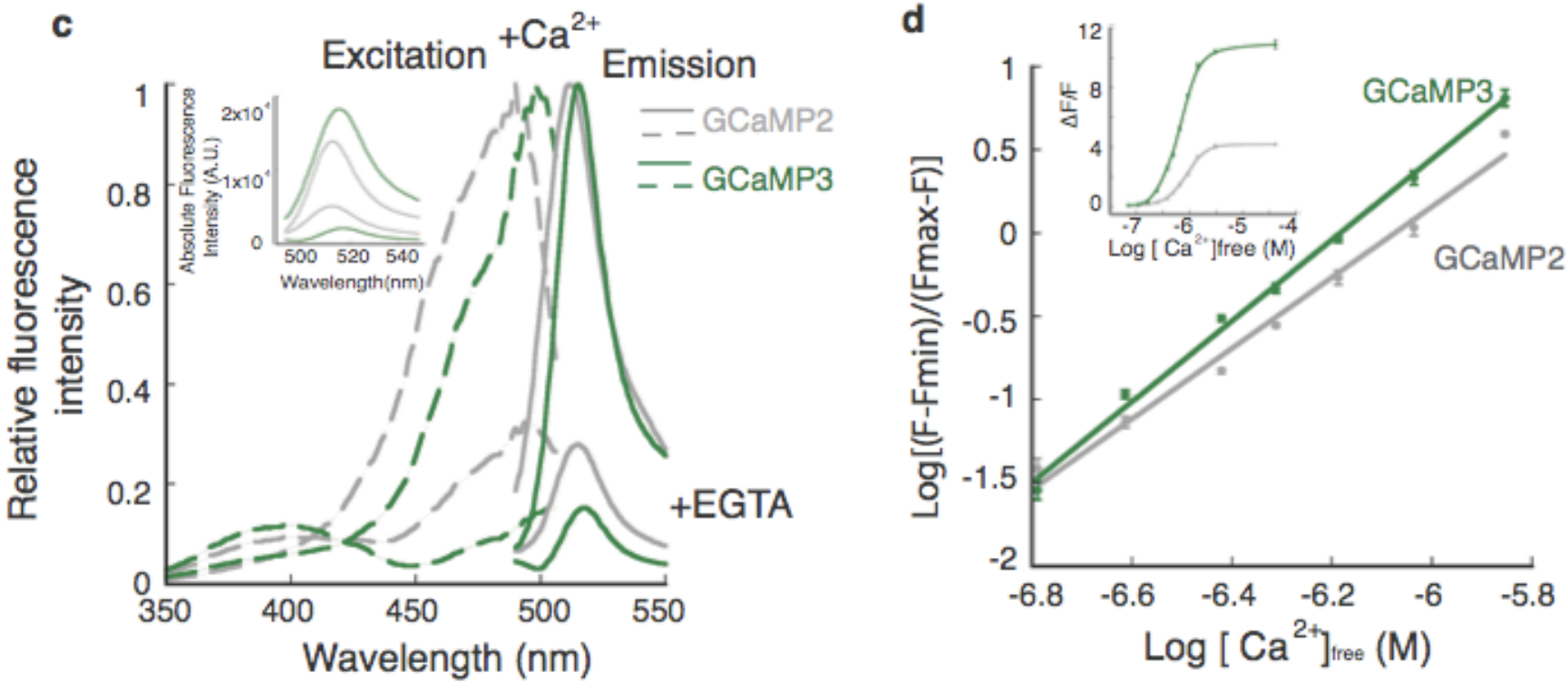
# $Ca^{2+}$-free GCaMP2 (8EF mutant)



# Design improvements?

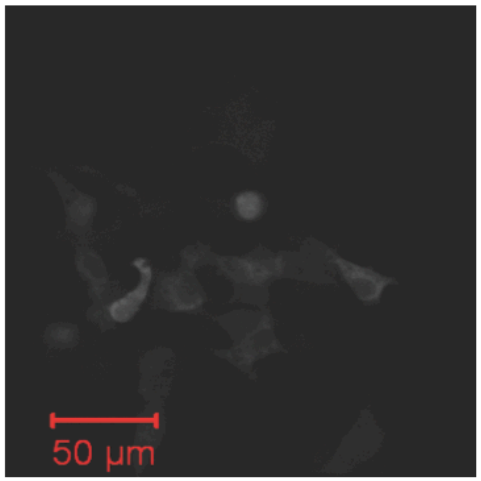


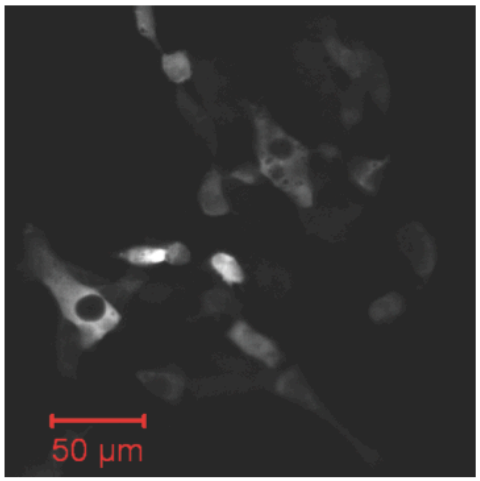

GCaMP2
MRG-HIS-
RSET
M13
EGFP(149-238)
EGFP(1-144)
CaM(2-148)
GCaMP3
MG--HIS-
RSET
M13
EGFP(149-238)
EGFP(1-144)
CaM(2-148)
M153K
T203V
N60D


c
Excitation
+Ca2+
Emission
GCaMP2
GCaMP3
+EGTA
Relative fluorescence intensity
Wavelength (nm)
Absolute Fluorescence Intensity (A.U.)
Wavelength(nm)
d
GCaMP3
GCaMP2
ΔF/F
Log [ Ca2+]free (M)
Log[(F-Fmin)/(Fmax-F)]
Log [ Ca2+]free (M)

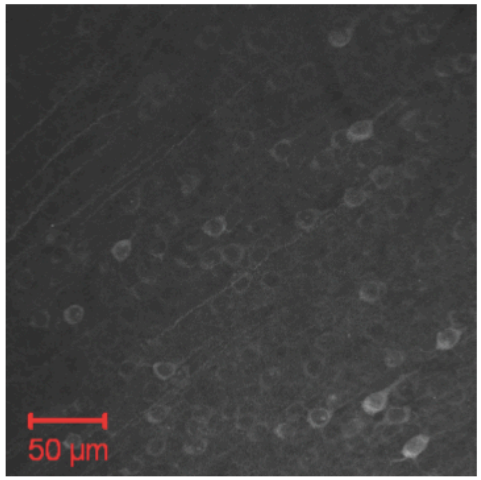
GCaMP2

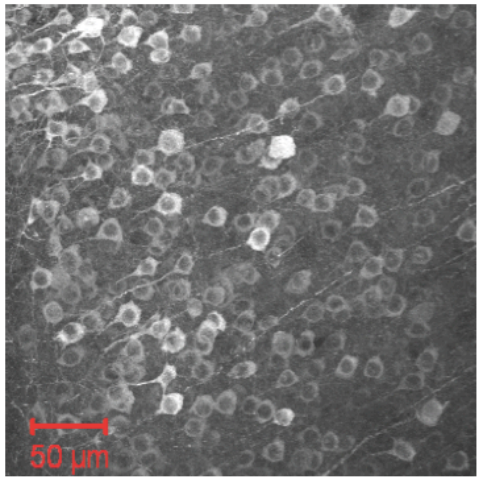
GCaMP3




HEK293 cells



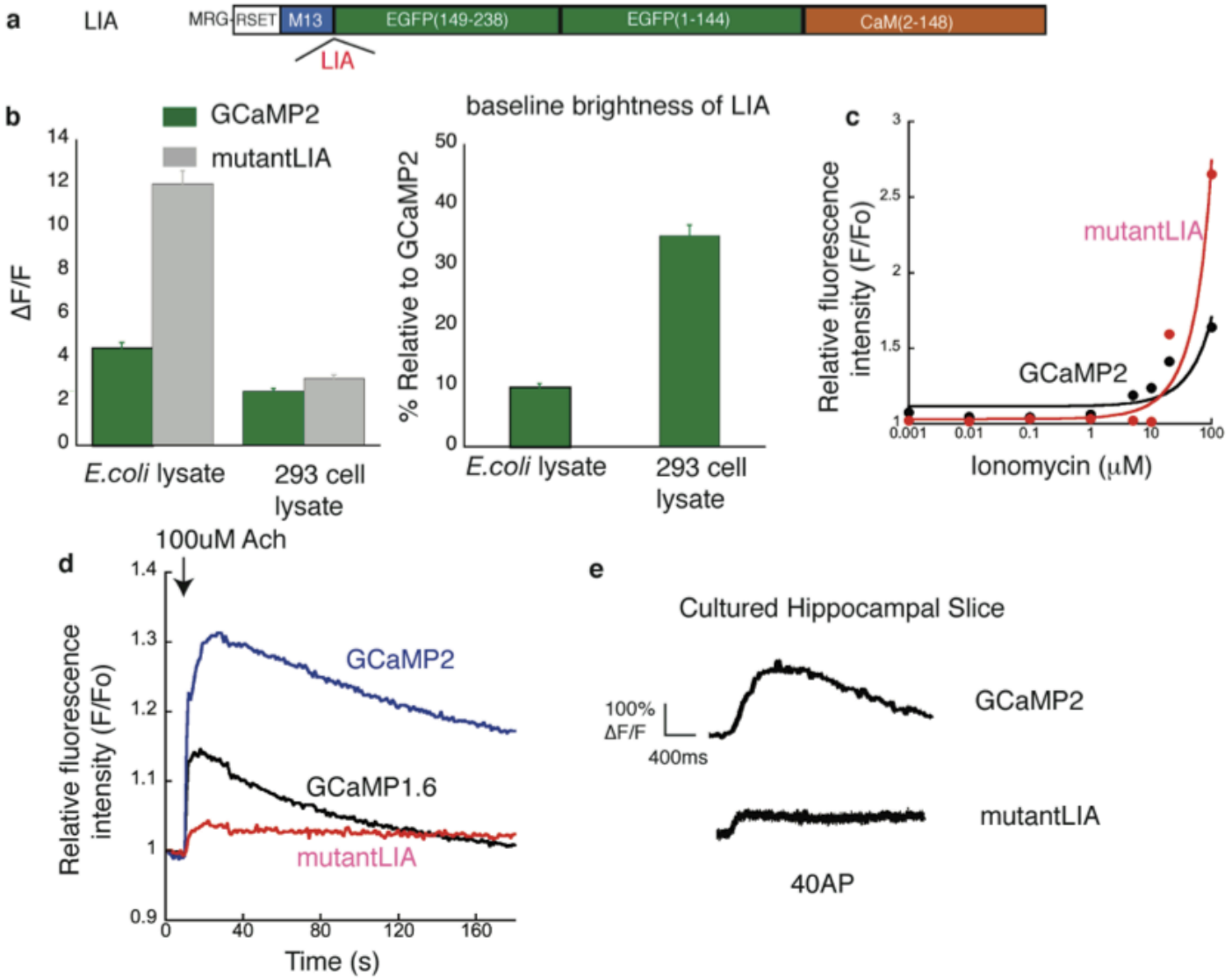




Cortical layer 2/3 slice

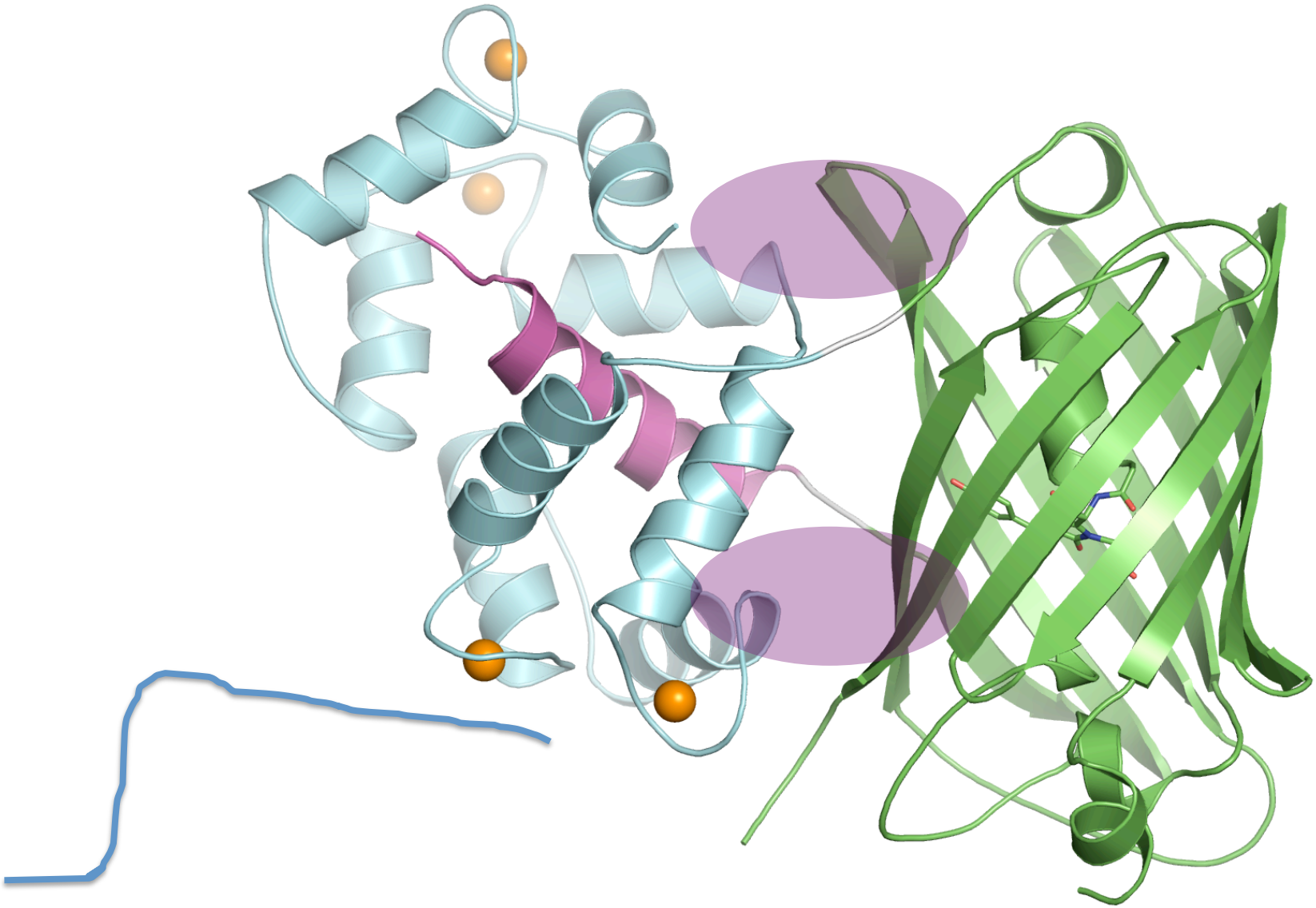
# movies

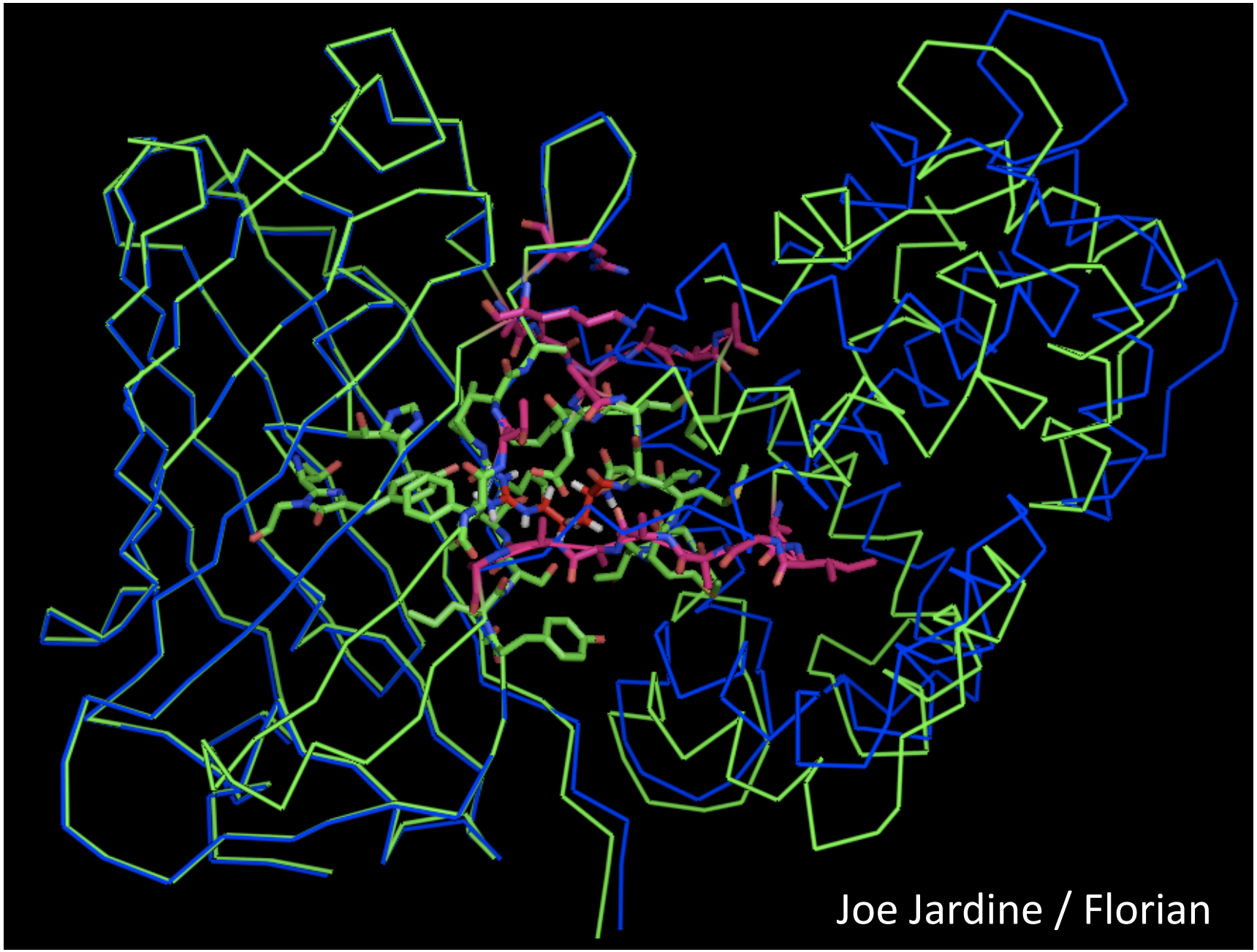
# Watch out

**As high as 160-fold now!!!**

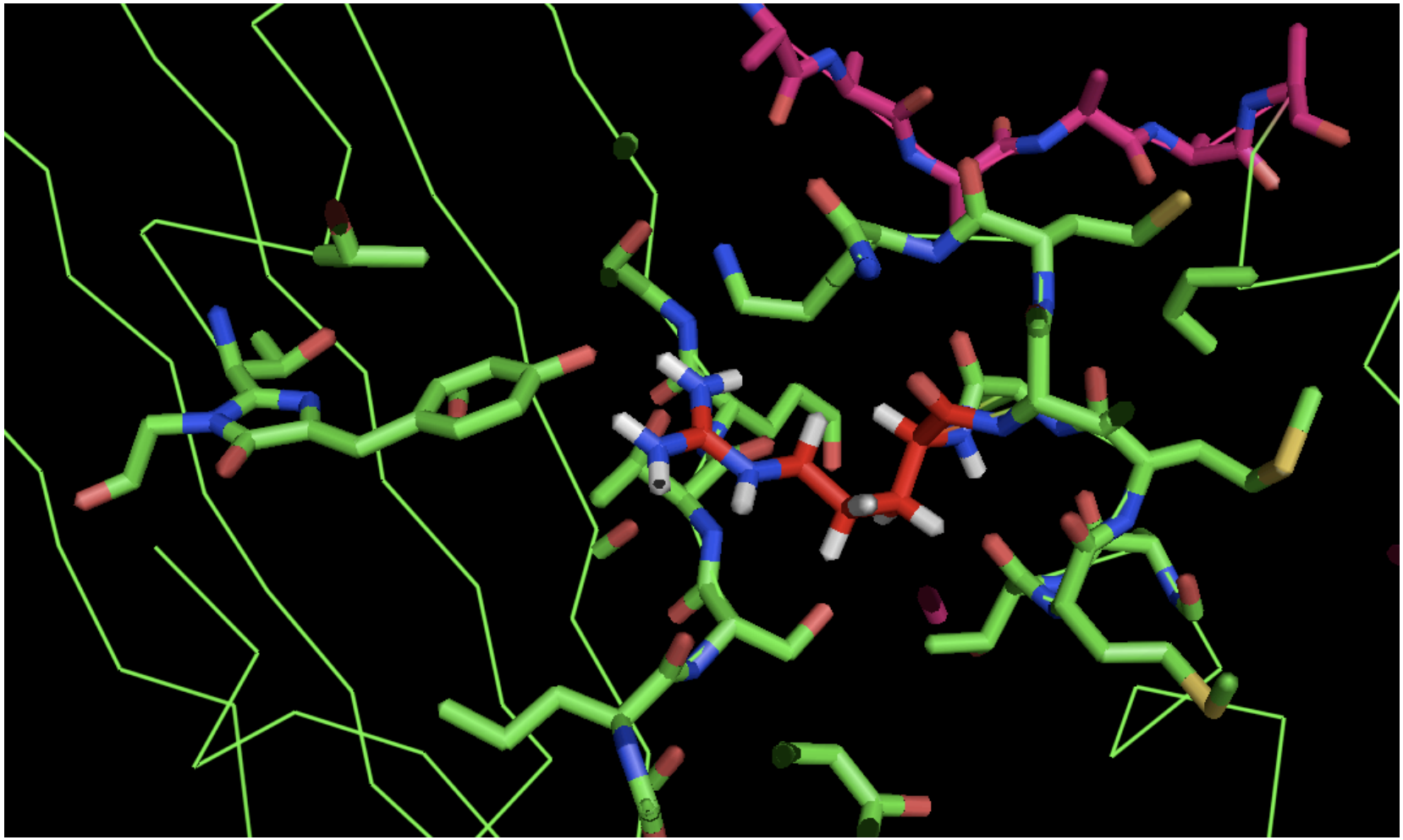


# Interesting design hit




Joe Jardine / Florian

# Joe Jardine / Florian



# Shibire (dynamin GTPase domain)

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Score =  498 bits (1283),  Expect = 4e-139
Identities = 243/299 (81%), Positives = 275/299 (91%), Gaps = 0/299 (0%)

Query  1    MDSLITIVNKLQDAFTSLGVHMQLDLPQIAVVGGQSAGKSSVLENFVGKDFLPRGSGIVT  60
            M+ LI +VN+LQDAF+++G +  LDLPQIAVVGGQSAGKSSVLENFVG+DFLPRGSGIVT
Sbjct  1    MEDLIPLVNRLQDAFSAIGQNADLDLPQIAVVGGQSAGKSSVLENFVGRDFLPRGSGIVT  60

Query  61   RRPLILQLINGVTEYGEFLHIKGKKFSSFDEIRKEIEDETDRVTGSNKGISNIPINLRVY  120
            RRPL+LQL+N  TEY EFLH KGKKF+ F+E+R EIE ETDRVTG+NKGIS +PINLRVY
Sbjct  61   RRPLVLQLVNSTTEYAEFLHCKGKKFTDFEEVRLEIEAETDRVTGTNKGISPVPINLRVY  120

Query  121  SPHVLNLTLIDLPGLTKVAIGDQPVDIEQQIKQMIFQFIRKETCLILAVTPANTDLANSD  180
            SPHVLNLTL+DLPG+TKV +GDQP DIE QI+ M+ QF+ KE CLILAV+PAN+DLANSD
Sbjct  121  SPHVLNLTLVDLPGMTKVPVGDQPPDIEFQIRDMLMQFVTKENCLILAVSPANSDLANSD  180

Query  181  ALKLAKEVDPQGVRTIGVITKLDLMDEGTDARDILENKLLPLRRGYIGVVNRSQKDIEGR  240
            ALK+AKEVDPQG RTIGVITKLDLMDEGTDARD+LENKLLPLRRGYIGVVNRSQKDI+G+
Sbjct  181  ALKIAKEVDPQGQRTIGVITKLDLMDEGTDARDVLENKLLPLRRGYIGVVNRSQKDIDGK  240

Query  241  KDIHQALAAERKFFLSHPSYRHMADRLGTPYLQRVLNQQLTNHIRDTLPGLRDKLQKQM  299
            KDI  ALAAERKFFLSHPSYRH+ADR+GTPYLQ+VLNQQLTNHIRDTLPGLR+KLQ Q+
Sbjct  241  KDITAALAAERKFFLSHPSYRHLADRMGTPYLQKVLNQQLTNHIRDTLPGLRNKLQSQL  299
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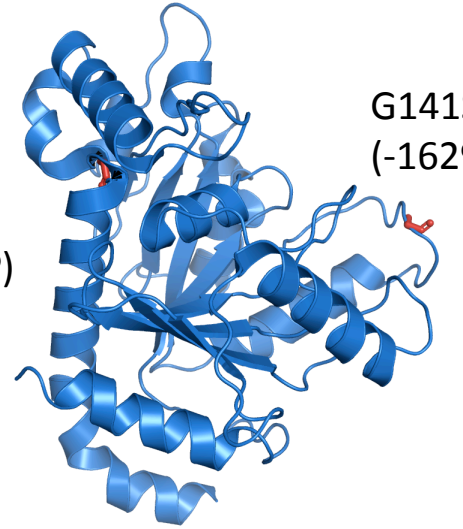
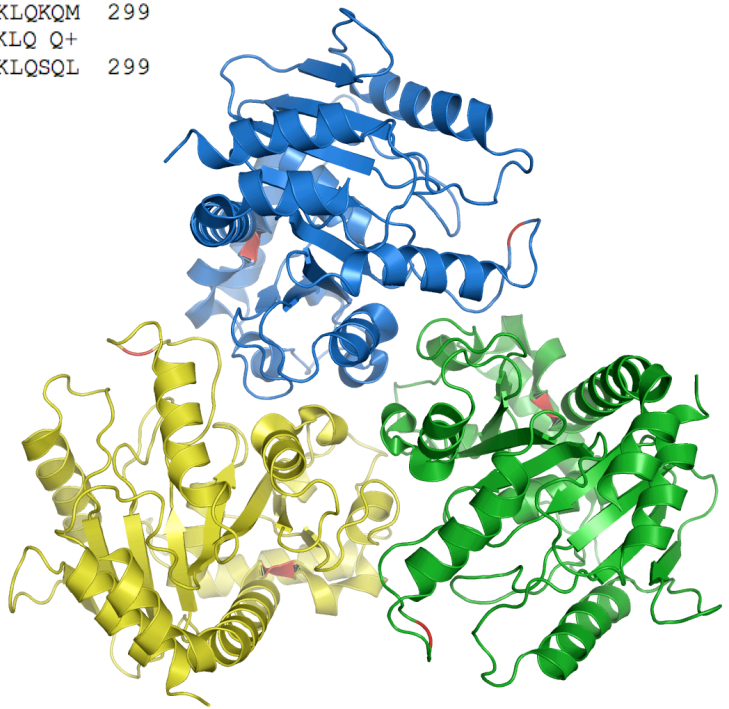


G141S
(-162°, -111°)

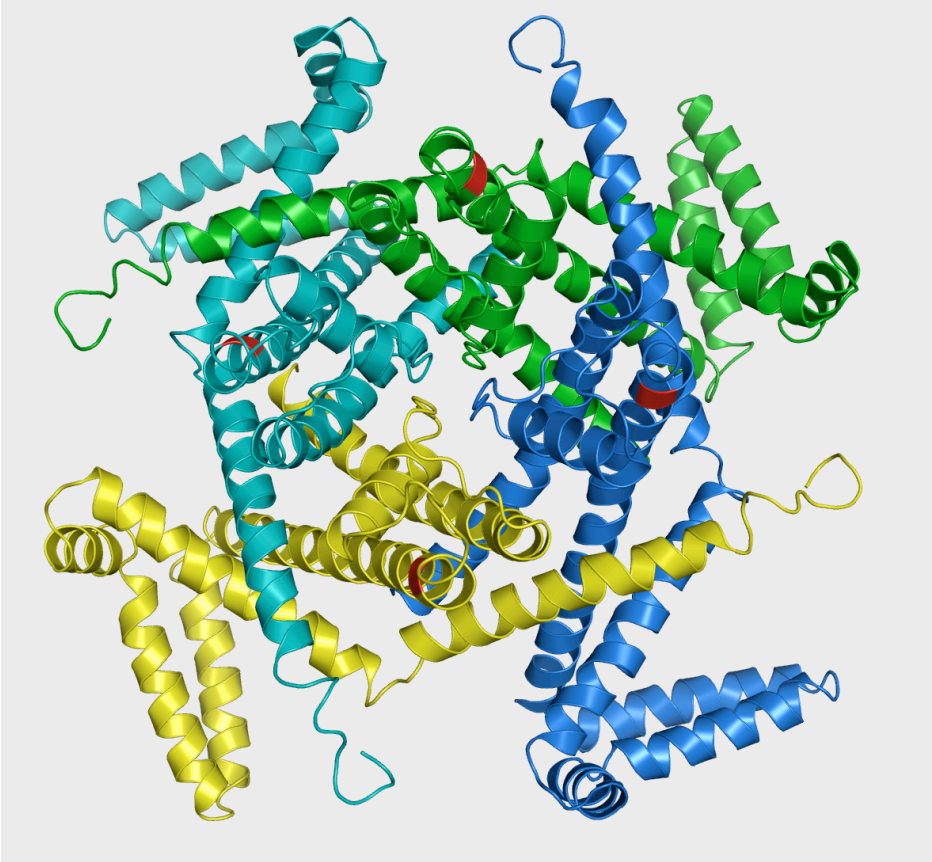
G268D
(97°, 162°)



(or tetramer?)

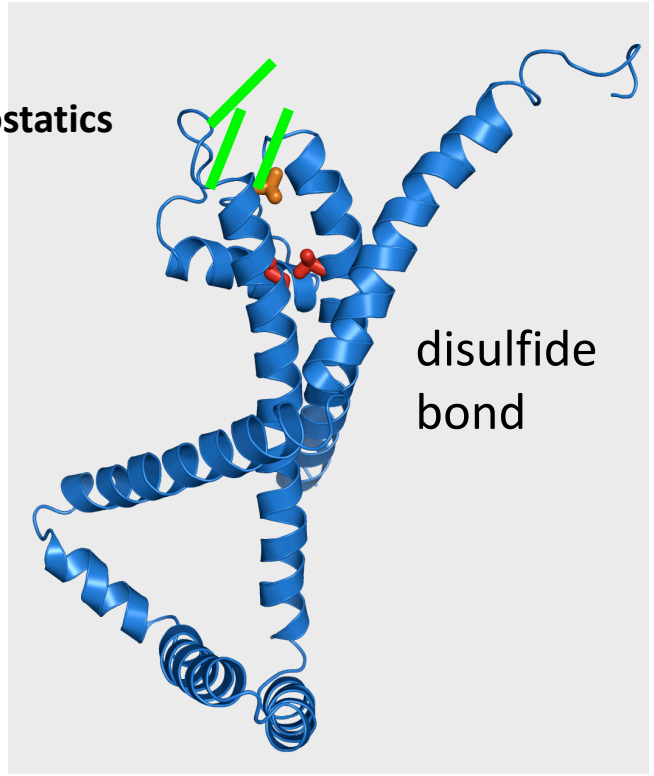
Existing. $T_m$ ~ **30°C.** Good except for aggression, courtship

# ERG K channel



```
_Seizure          QKLQNNYYHKWTLLHYSPFKAVWDWIILILVMYTAIFTPYVAAFLLGEQDYQRRNSKYIN
_1ORQ             ------------------EHPLVELGVSYAALLSVIVVVVECTMQ----------LSGEY
Consensus_ss:                       hhhhhhhhhhhhhhhhhhh                 hhh

Conservation:               999       9                                 9    9 99
_Seizure          SDPIVIIDLIVDVTFIVDIIINFRTTFVNSQDEVVSHPGRIAVHYLSGWFLIDLVAAVPF
_1ORQ             LVRLYLVDLILVIILWADYAYRAYKSGDP--------------AGYVKKTLYEIPALVPA
Consensus_ss:     hhhhhhhhhhhhhhhhhhhhhhhhhh                  hhhhh    hhhhhhhh

Conservation:      99           9 9    9 99                9             9  9
_Seizure          DLLLVGSDTDETTTLIGLLKTARLLRLVRVARKIDRYSEYGAAVLILLMATFILIAHWLA
_1ORQ             GLLALIEGHLAGLGLFRLVRLLRFLRILLIISRGSKFLSAIADAADKIRFYHLFGAVMLT
Consensus_ss:     hhhhhh    hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh

Conservation:        9                                        9       99       9 9
_Seizure          CIWYAIGNAEKSIASKNIGWLNSLAYDIQEPYFDNRTGGPSIKSRYITALYFTFTSLTSV
_1ORQ             VLYGAFAIYIVEYPDP----------------------NSSIKSVFDALWWAVVTATTV
Consensus_ss:     hhhhhhhhhh                                    hhhhhhhhhhhhhh

Conservation:     9 9 9 9 9   9   9 999 9        9 99   9
_Seizure          GFGNVAPNTDAEKAFTICVMLVGSLMYASIFGNVSAIIQRLYSGTARYHTQMLRVREFIR
_1ORQ             GYGDVVPATPIGKVIGIAVMLTGISALTLLIGTVSNMFQKILV----------------
Consensus_ss:                     hhhhhhhhhhhhhh  hhhhhhh
```

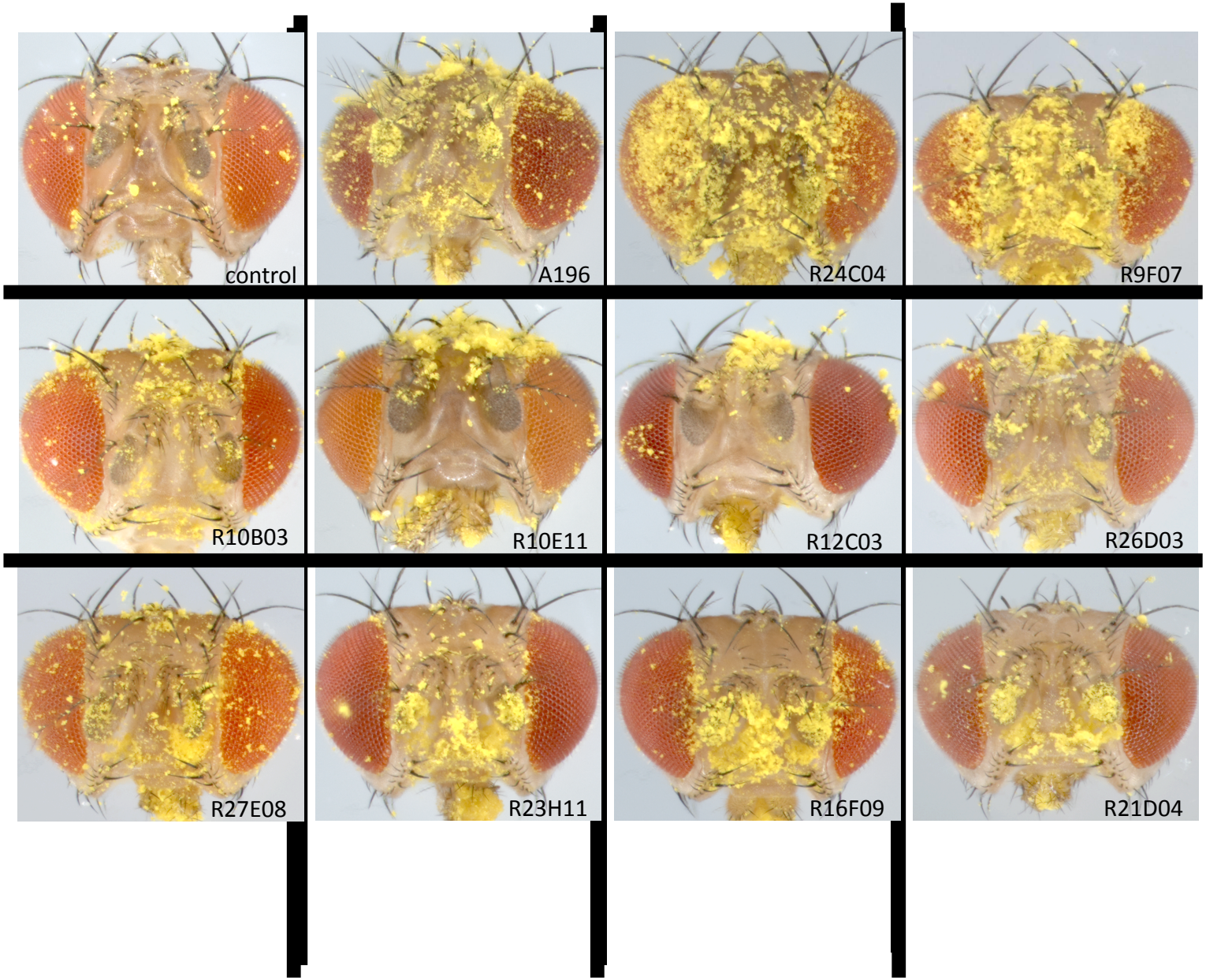
**E490K: electrostatics**



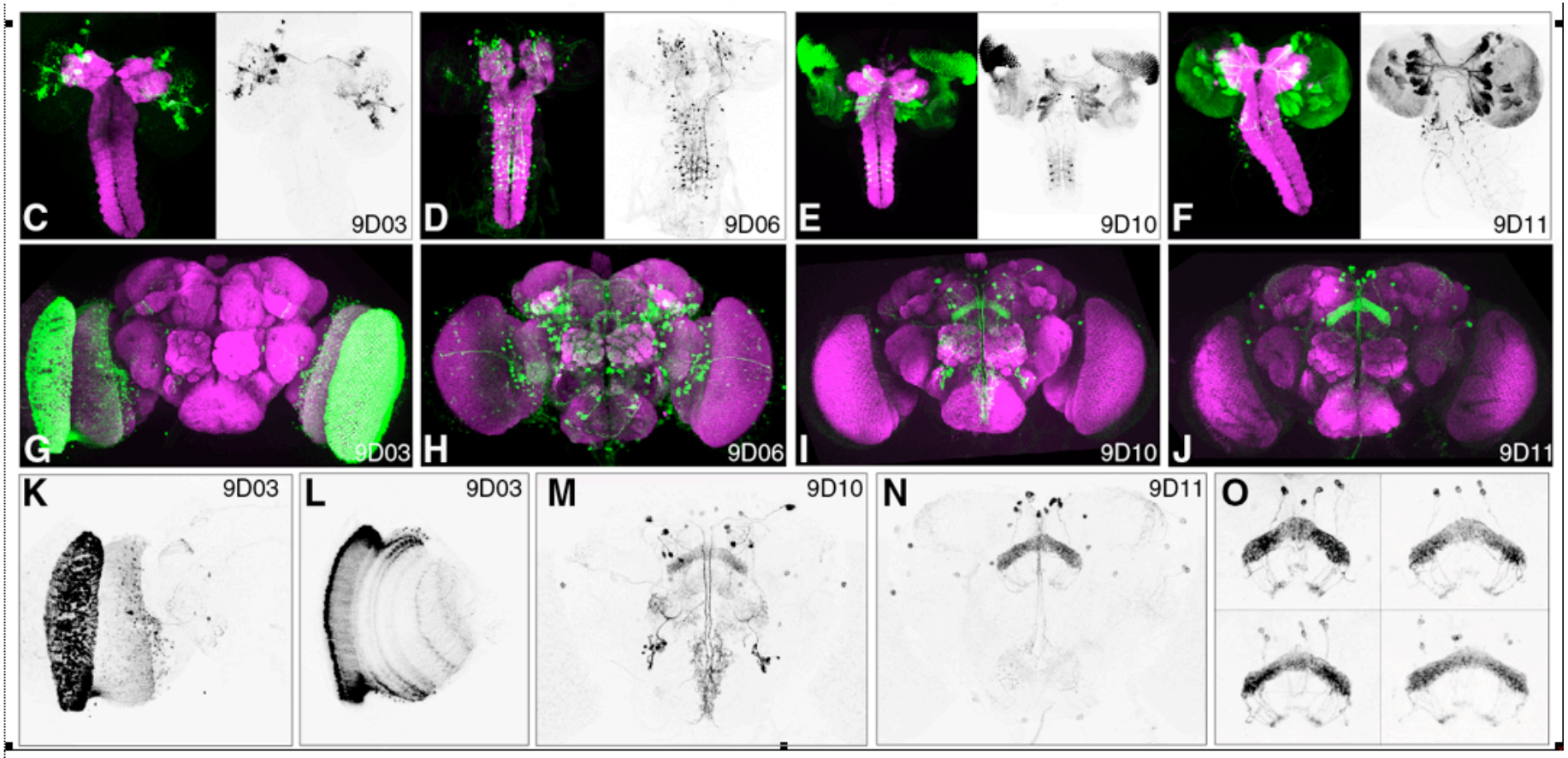

Existing. $T_m$ ~ **38.5°C**. Way too high…

- Literature ts mutants are dominant-negative as well.
- Almost all of these proteins are multimeric
- Known ts mutations cluster at interfaces
- Many *ts alleles* **do not** yield *ts* **proteins**- they undercover an underlying T dependence of the pathway. Lots of stop codons, frameshifts, etc.
- Design works great! Can do all transitions, multiple mutations, etc. Can get range of $T_m$'s
- Chemical mutagenesis now recovers known alleles almost exclusively

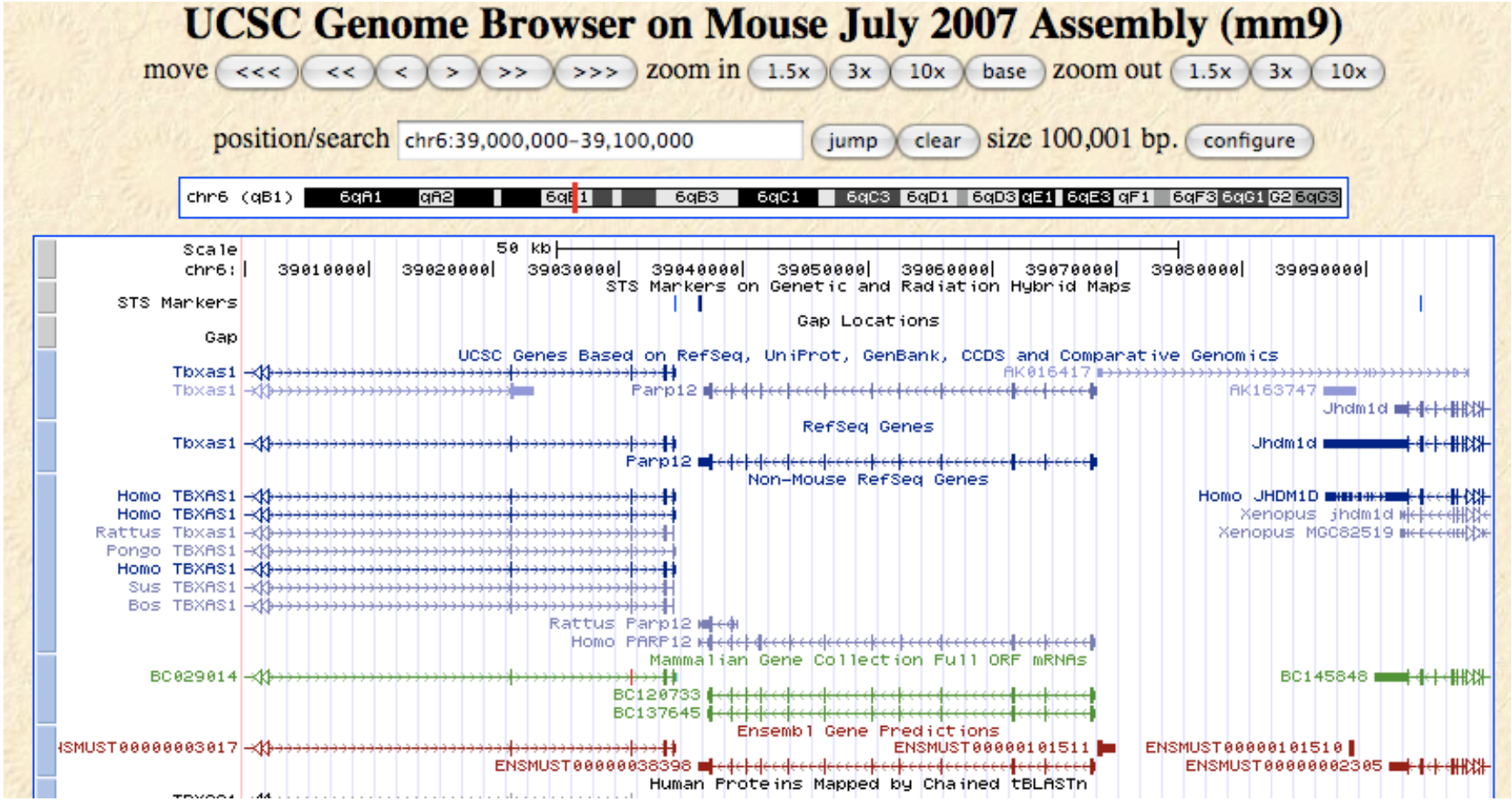
# More movies


control
A196
R24C04
R9F07
R10B03
R10E11
R12C03
R26D03
R27E08
R23H11
R16F09
R21D04

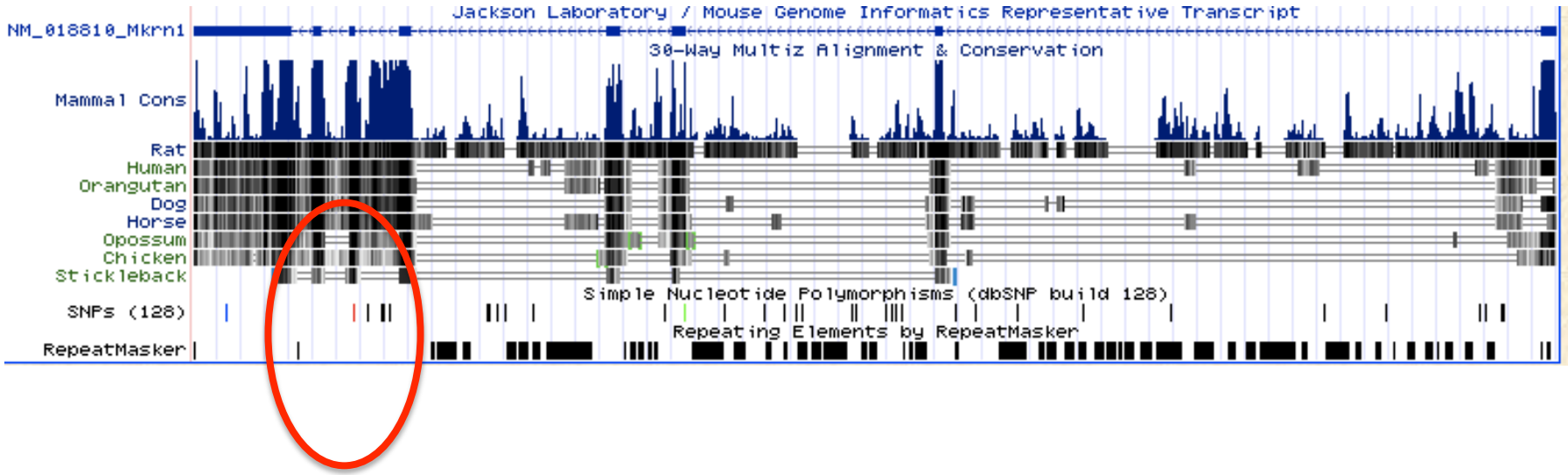
# Map behavior -> neurons



# Automated assignment of SNP function -> correlate with QTLs / disease



# SNPs?


Jackson Laboratory / Mouse Genome Informatics Representative Transcript
NM_018810_Mkrn1
30-Way Multiz Alignment & Conservation
Mammal Cons
Rat
Human
Orangutan
Dog
Horse
Opossum
Chicken
Stickleback
Simple Nucleotide Polymorphisms (dbSNP build 128)
SNPs (128)
Repeating Elements by RepeatMasker
RepeatMasker

# SNPs?

## View / download mouse SNPs in region of D1Ertd161e

42 rows retrieved  Gene info  GBrowse  RS list  Wide view (all strains)
Download flat file  Open in Excel  Matrix view  Refine  SNPs home

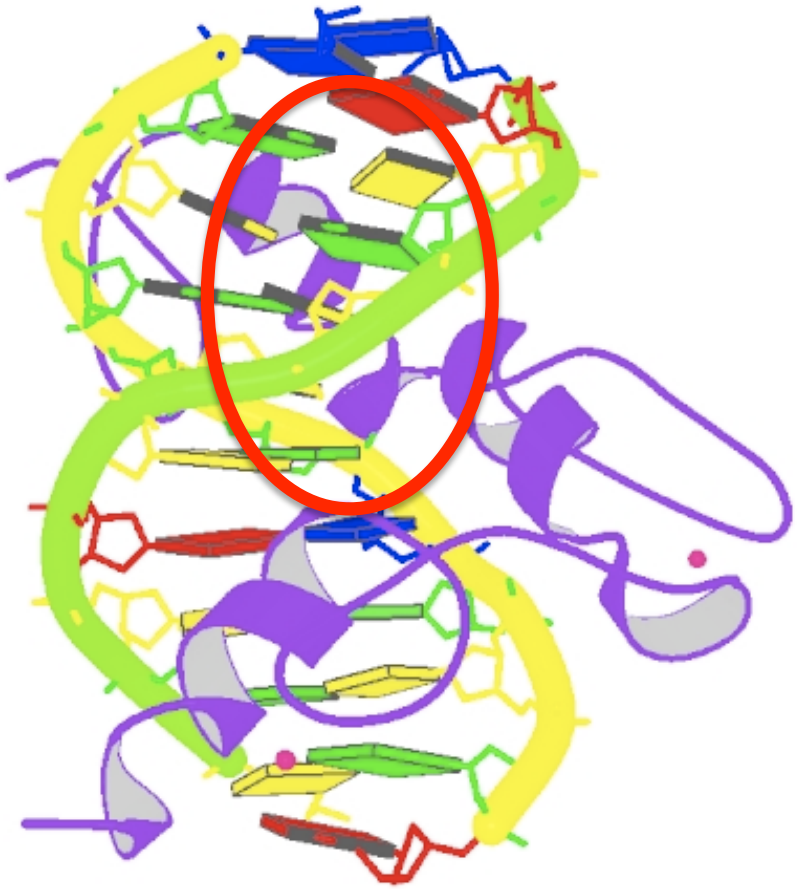
Your query parameters are summarized at bottom of page. The **help page** explains the table columns.

| Mbp location (Build 37) | NCBI gene annotation | Ensembl 48 gene annotation | dbSNP 128 SNP annotation | 129S1/SvImJ | A/J | AKR/J | BALB/cByJ | BTBR T+ tf/J | C3H/HeJ | C57BL/6J | CAST/EiJ | DBA/2J | FVB/NJ | KK/HlJ | NOD/ShiLtJ | NZW/LacJ | PWD/PhJ | WSB/EiJ | 129X1/SvJ | CZECHII/EiJ | dbSNP rs | Observed | Source | #mappings | Links | Insertion alleles |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 75.188998 | D1Ertd161e UTR | Ankzf1 exon1,UTR | Ankzf1 U<br>Atg9a L | A | A | A | A | A | A | A | G | A | A | A | G | A | A | G | A | | rs13460330 | A/G | Perlegen2 NES16396514 | 1 | MGI E! snpview | |
| 1 75.189088 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cs** S20<br>Atg9a L | C | C | C | C | C | C | C | T | C | C | C | C | C | C | C | C | | rs46367069 | C/T | Perlegen2 NES16396516 | 1 | MGI E! snpview | |
| 1 75.189105 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cn** T26M<br>Atg9a L | C | C | C | C | C | C | C | T | C | C | C | T | C | C | T | C | | rs51516939 | C/T | Perlegen2 NES16396518 | 1 | MGI E! snpview | |
| 1 75.189128 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cn** P34S<br>Atg9a L | | C | | | | | T | | T | | | | | | | | | rs31503489 | C/T | Celera2 mCV24841938 | 1 | MGI E! snpview | |
| 1 75.189257 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I<br>Atg9a L | | A | | | | | C | | C | | | | | | | | | rs32103815 | A/C | Celera2 mCV24841939 | 1 | MGI E! snpview | |
| 1 75.189258 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I<br>Atg9a L | C | T | | C | C | | C | C | C | C | C | C | C | C | C | C | | rs32067920 | C/T | merge of 2 sources | 1 | MGI E! snpview | |
| 1 75.189300 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | C | C | C | C | C | C | C | T | C | C | C | C | C | C | C | C | | rs46777404 | C/T | Perlegen2 NES16396523 | 1 | MGI E! snpview | |
| 1 75.189330 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | A | A | A | A | A | A | A | T | A | A | A | | A | A | | A | | rs50764557 | A/T | Perlegen2 NES16396526 | 1 | MGI E! snpview | |
| 1 75.189344 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | A | A | A | A | A | A | A | G | A | A | A | G | A | A | G | A | | rs49681734 | A/G | Perlegen2 NES16396528 | 1 | MGI E! snpview | |
| 1 75.189376 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | G | G | G | G | G | G | G | C | G | G | G | C | G | G | C | G | | rs46412339 | C/G | Perlegen2 NES16396531 | 1 | MGI E! snpview | |
| 1 75.189396 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | T | T | T | T | T | T | T | A | T | T | T | A | T | T | A | T | | rs50168993 | A/T | Perlegen2 NES16396534 | 1 | MGI E! snpview | |
| 1 75.189568 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | T | T | T | T | T | T | T | C | T | T | T | C | T | T | C | T | | rs46202744 | C/T | Perlegen2 NES16396536 | 1 | MGI E! snpview | |
| 1 75.189681 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | C | C | C | C | C | C | C | T | C | C | C | | C | C | | C | | rs49609506 | C/T | Perlegen2 NES16396538 | 1 | MGI E! snpview | |
| 1 75.189946 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | T | T | T | T | T | T | T | C | T | T | T | C | T | T | C | T | | rs50938274 | C/T | Perlegen2 NES16396436 | 1 | MGI E! snpview | |
| 1 75.189978 | D1Ertd161e intron1 | Ankzf1 intron2 | Ankzf1 I | T | T | T | T | T | T | T | A | T | T | T | A | T | T | A | T | | rs49910034 | A/T | Perlegen2 NES16396437 | 1 | MGI E! snpview | |

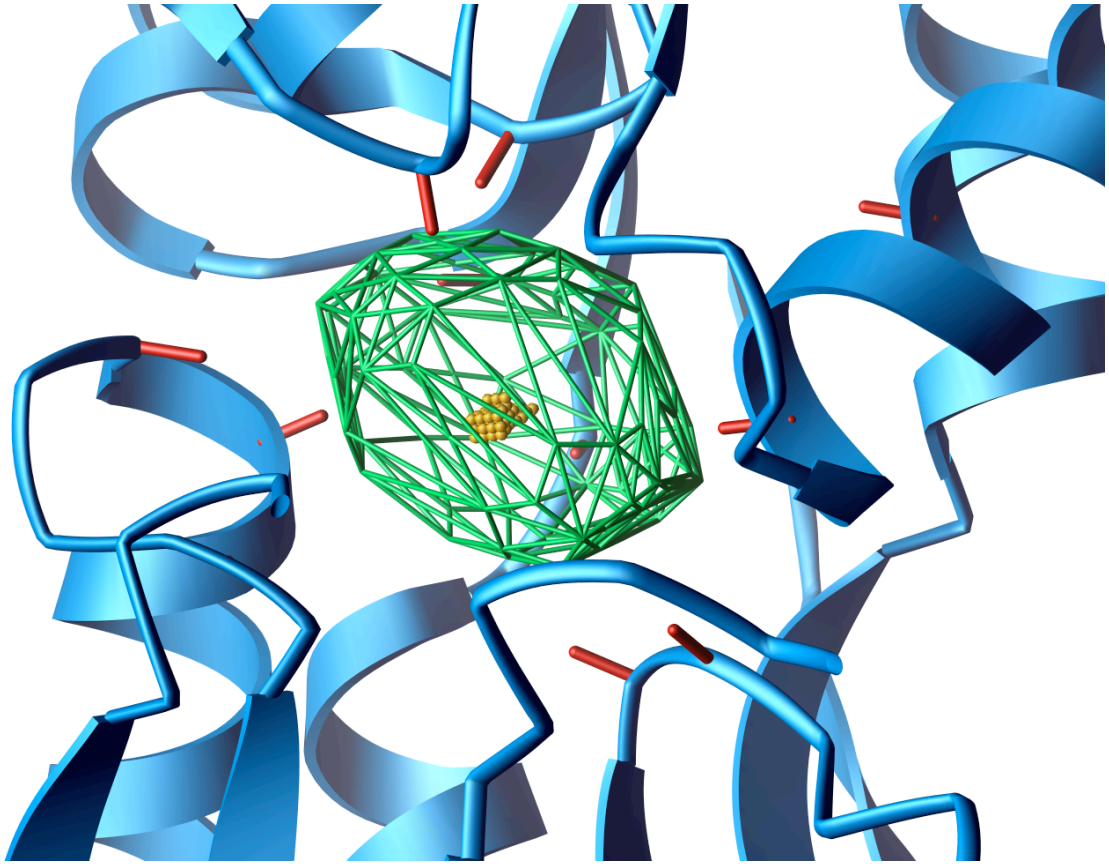
# Amino acid changes

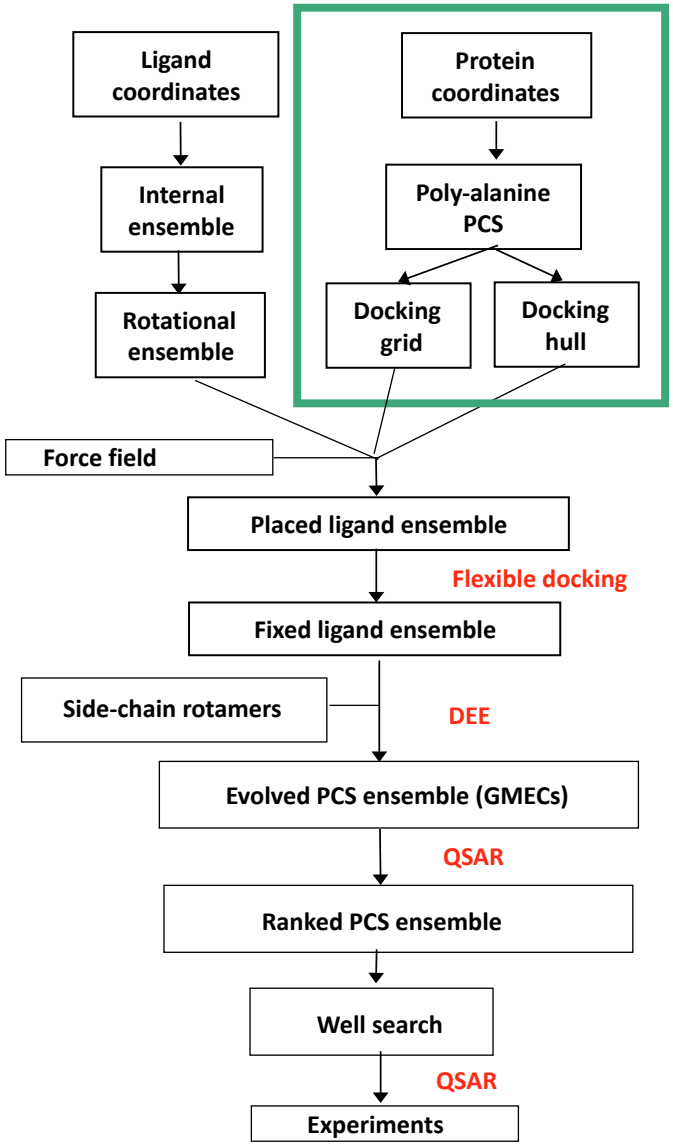
| Mbp location (Build 37) | NCBI gene annotation | Ensembl 48 gene annotation | dbSNP 128 SNP annotation | 129S1/SvImJ | A/J | AKR/J | BALB/cByJ | BTBR T+ tf/J | C3H/HeJ | C57BL/6J | CAST/EiJ | DBA/2J | FVB/NJ | KK/HlJ | MOLF/EiJ | NOD/ShiLtJ | NZW/LacJ | PWD/PhJ | WSB/EiJ | 129X1/SvJ | CZECHII/EiJ | dbSNP rs | Observed | Source | #mappings | Links | Insertion alleles |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 75.189088 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cs** S20 Atg9a L | C | C | C | C | C | C | C | T | C | C | C | C | C | C | C | C | | | rs46367069 | C/T | Perlegen2 NES16396516 | 1 | MGI E! snpview | |
| 1 75.189105 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cn** T26M Atg9a L | C | C | C | C | C | C | C | T | C | C | C | T | C | C | T | C | | | rs51516939 | C/T | Perlegen2 NES16396518 | 1 | MGI E! snpview | |
| 1 75.189128 | D1Ertd161e exon1 | Ankzf1 exon1,UTR | Ankzf1 **Cn** P34S Atg9a L | | C | | | | | T | | T | | | | | | | | | | rs31503489 | C/T | Celera2 mCV24841938 | 1 | MGI E! snpview | |
| 1 75.190774 | D1Ertd161e exon2 | Ankzf1 exon3 | Ankzf1 **Cs** G82 | G | G | G | G | G | G | G | A | G | G | G | A | G | G | A | G | | | rs48259393 | A/G | Perlegen2 NES16396435 | 1 | MGI E! snpview | |
| 1 75.191515 | D1Ertd161e exon3 | Ankzf1 exon4 | Ankzf1 **Cn** H144R | A | A | A | A | A | A | A | G | A | A | A | G | A | A | G | A | | | rs50760872 | A/G | Perlegen2 NES16396413 | 1 | MGI E! snpview | |
| 1 75.192377 | D1Ertd161e exon6 | Ankzf1 exon7 | Ankzf1 **Cn** K248R | A | A | A | A | A | A | A | G | A | A | A | A | A | A | A | A | | | rs51069531 | A/G | Perlegen2 NES16396416 | 1 | MGI E! snpview | |
| 1 75.192794 | D1Ertd161e exon7 | Ankzf1 exon8 | Ankzf1 **Cn** A319V | T | T | T | T | T | T | T | C | T | T | T | C | T | T | C | T | | | rs48876765 | C/T | Perlegen2 NES16396420 | 1 | MGI E! snpview | |
| 1 75.192907 | D1Ertd161e exon7 | Ankzf1 exon8 | Ankzf1 **Cn** R357W | T | T | T | T | T | T | T | T | T | T | T | C | T | T | C | T | | | rs49533287 | C/T | Perlegen2 NES16396421 | 1 | MGI E! snpview | |
| 1 75.194454 | D1Ertd161e exon9 | Ankzf1 exon10 | Ankzf1 **Cn** S504G | G | G | G | G | G | G | G | A | G | G | G | G | G | G | G | A | | | rs46398651 | A/G | Perlegen2 NES16396376 | 1 | MGI E! snpview | |
| 1 75.194641 | D1Ertd161e exon9 | Ankzf1 exon10 | Ankzf1 **Cn** S566I | G | G | G | G | G | G | G | G | G | G | G | T | G | G | T | G | | | rs50868562 | G/T | Perlegen2 NES16396377 | 1 | MGI E! snpview | |
| 1 75.194858 | D1Ertd161e exon10 | Glb1l UTR | Ankzf1 **Cn** T592S | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | T | A | | rs46996846 | A/T | Perlegen2 NES16396378 | 1 | MGI E! snpview | |
| 1 75.195725 | D1Ertd161e exon13 | Glb1l exon17,UTR | Ankzf1 **Cs** S736 | G | G | G | G | G | G | G | G | G | G | G | A | G | G | A | G | | | rs46573412 | A/G | Perlegen2 NES16396381 | 1 | MGI E! snpview | |

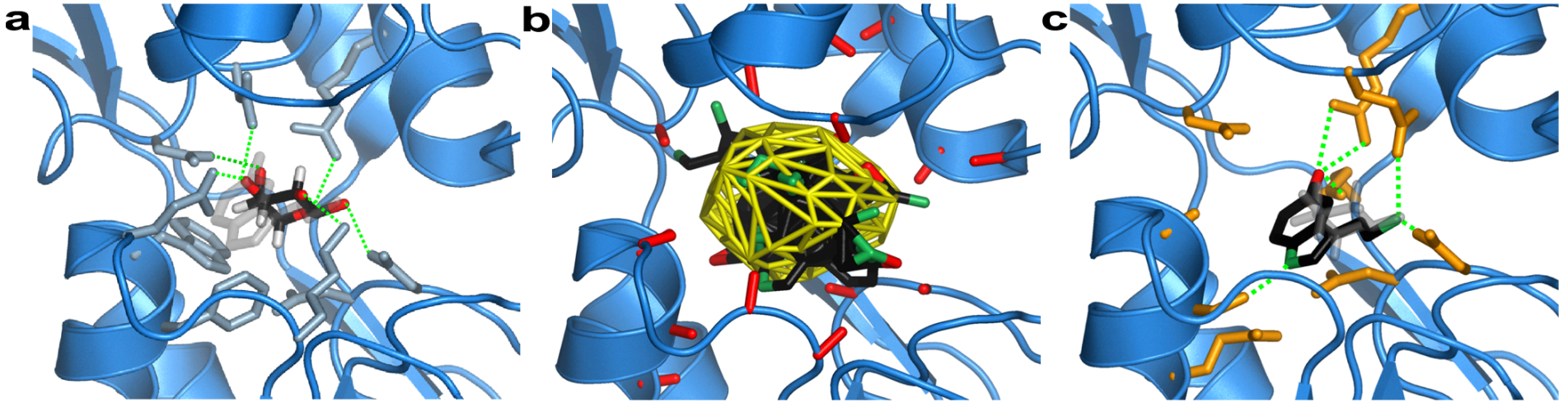
# -> “Δ function” annotation



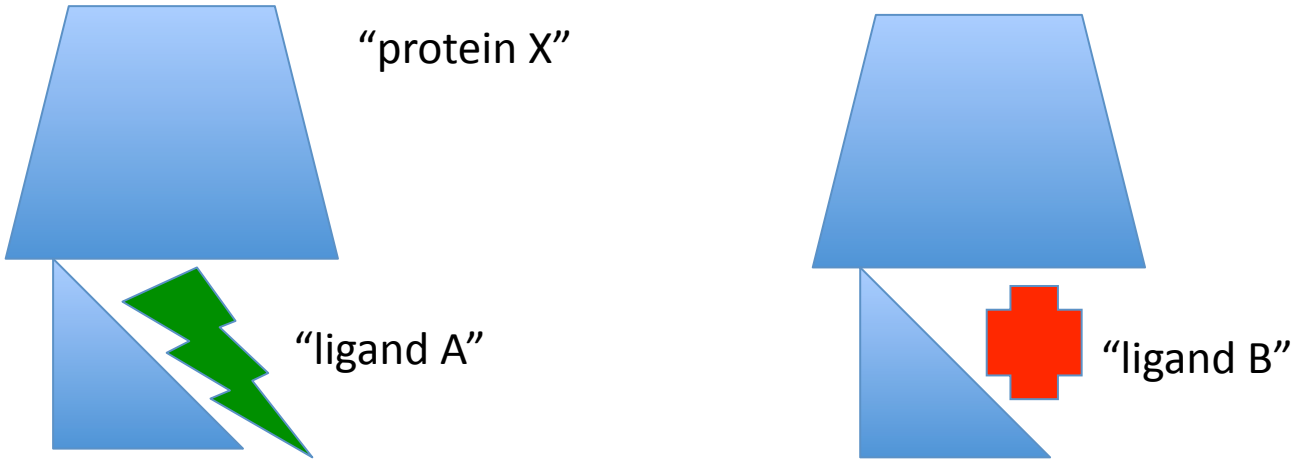
# Receptor design






a
b
c

# Bigger, faster, stronger




Co-crystal structure

Biacore: low nM

*In vivo* efficacy

Took comp. + dir. evolution

**Please, please, please confidential**

# What did I learn?

- Computation + screen/select = win
- You get what you design / screen for: test things multiple ways, don't get fancy
- Think how your protein will be used; don't be surprised if performance differs between pure protein & actual deployment
- Proteins need to have >5 different things simultaneously work, hard to tell program what to optimize
- Choose smart problems
- Always test things that ***shouldn't*** work too
- Learn to accept that computation will ***never*** capture some aspects

# 1: Robetta doesn’t work

- Structure solved -> model not good
- Model unusable -> made own
- Still running -> solved structure by self
- “ (~600 days left I think)

# 2: We need a “CARPE DIEM”

- Centralized fabrication & testing
- High-throughput assays
- Can’t pre-test submissions
- Feedback -> iterate designs?
- Conference

# 3: The ROSETTA community needs more organization

- Lots of potentially duplicated effort
- Please industry folks, or there may not be more RosettaCon's.
- Plan for future:
  - Parser?
  - PyRosetta?
  - PyMOL?
  - FoldIt?
  - Other?

# Acknowledgements


• **my lab**: Lin Tian, Jasper Akerboom, Andrew Hires, Jonathan Marvin, Sara Viswanathan, Sean McKinney, Mark Verdecia

•**Karel Svoboda**, Tianyi Mao, Haining Zhong, Ninglong Xu, Daniel Huber: mouse

•**Cori Bargmann**: worm

HHMI janelia farm research campus

•**Wolf Frommer**: letting me write **Chameleon** at Stanford

•Rosetta community for being cool & maybe beaming me up to Mother Ship

•HHMI & Janelia for being awesome

•Eric Schreiter: crystal structures

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