WELCOME TO PUBS!

Physical **U**nderpinnings of **B**iological **S**ystems - 2015

http://fraserlab.com/pubs/

Introductions

James/Jaime
instructor

David/Iggy
course coordinator

Danielle
mass spec guru

Martin
heir apparent

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Joe
instructor emeritus

Kyle

Tanja

Rosetta/Protein Design Mafia

Eric
CAT/Sequencing

TAs

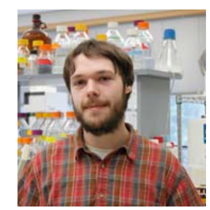
Bruk

Erin

Ina

Leanna

Lillian



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University of California, Berkeley

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Scripps College

Cole Helsell

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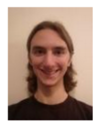
University of California, Berkeley



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Chemical Biology

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Dartmouth College, Dartmouth College

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Central Michigan University, University of Michigan-Ann Arbor

EDUCATIONFORUM

GRADUATE EDUCATION

Interdisciplinary Graduate Training in Teaching Labs

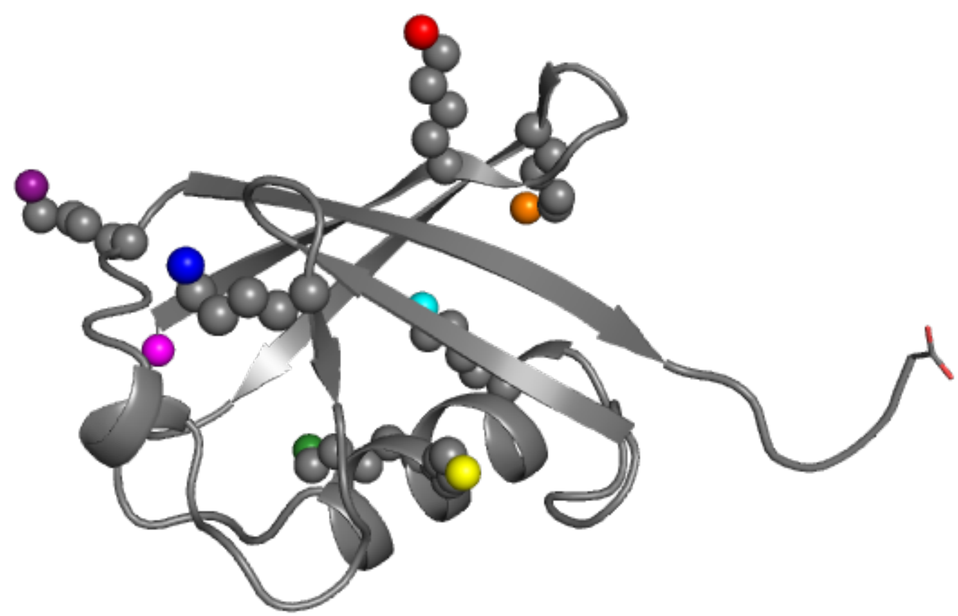
Intensive, short-term courses meld students and faculty and new techniques in pursuit of genuine research questions.

Ronald D. Vale,[1,2,3*] Joseph DeRisi,[2,3] Rob Phillips,[4] R. Dyche Mullins,[1,2] Clare Waterman,[1,5] Timothy J. Mitchison[1,6]

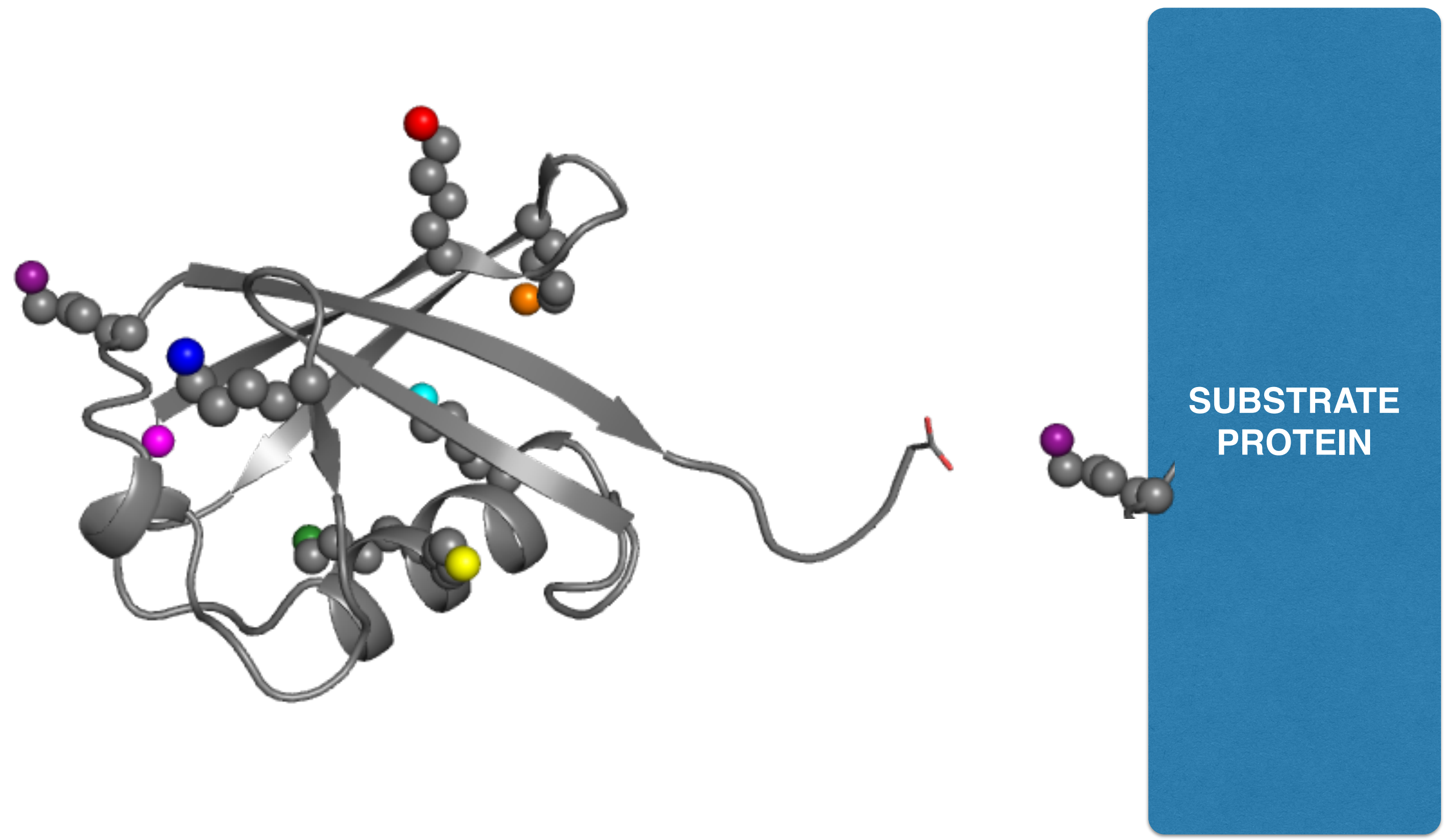
Science 21 December 2012:
Vol. 338 no. 6114 pp. 1542-1543
DOI: 10.1126/science.1216570

- We have three graduate programs (BMI, BP, CCB) represented - and many diverse scientific backgrounds - this is a huge advantage
- David/Iggy and Dan Bolon established this library approach; Danielle is adding the mass spec expertise
- This course is an <u>experiment</u> in hands-on **team**-based learning. You will be exposed to: deep sequencing, genetics, chemical biology, systems biology, protein biophysics, evolutionary biology, statistical mechanics, computational biology… etc…
- Lecturers (and we have a great line up of faculty!) will reinforce broad themes, but <u>you</u> will drive the research questions, day-to-day experiments, and code forward!

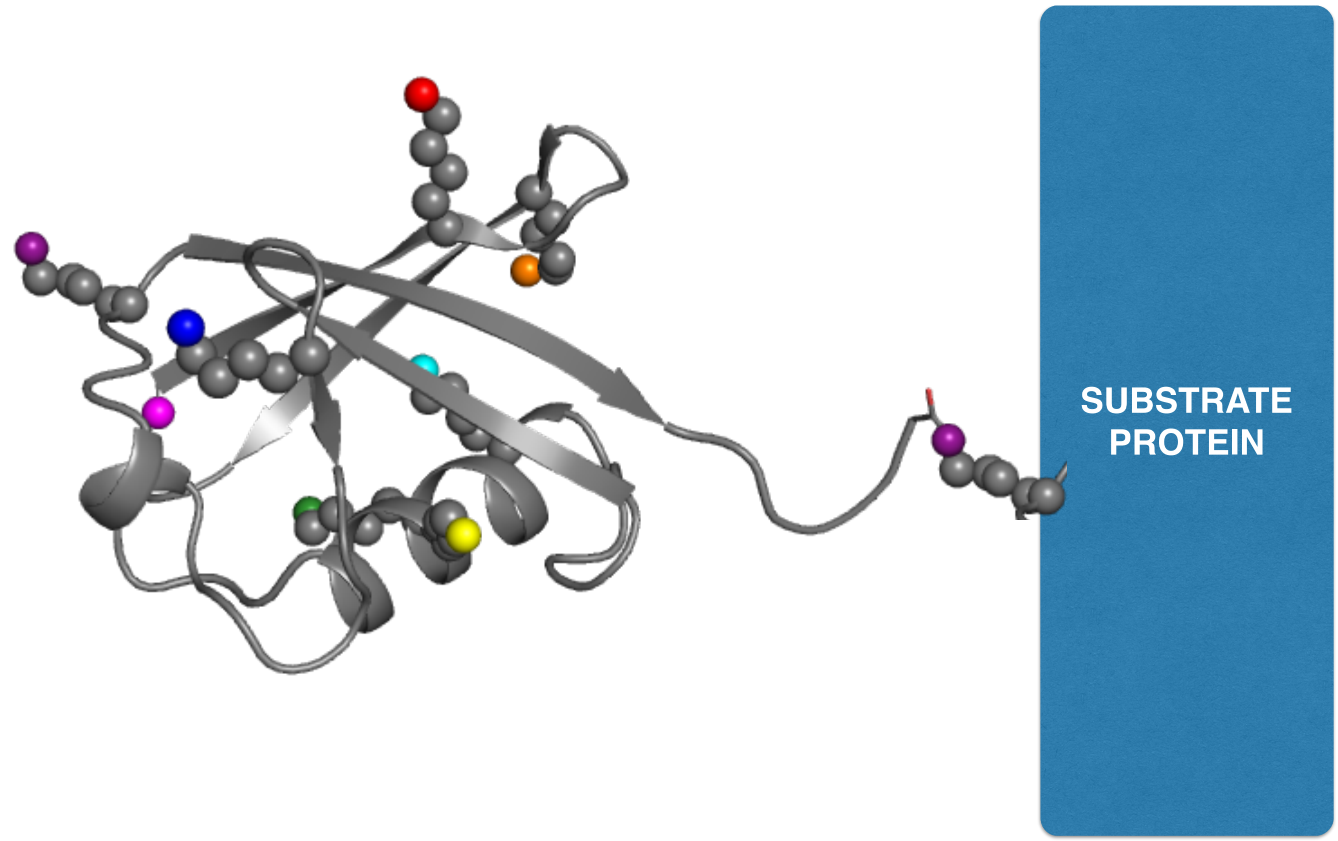
Ubiquitin is a central protein in “**proteostasis**”



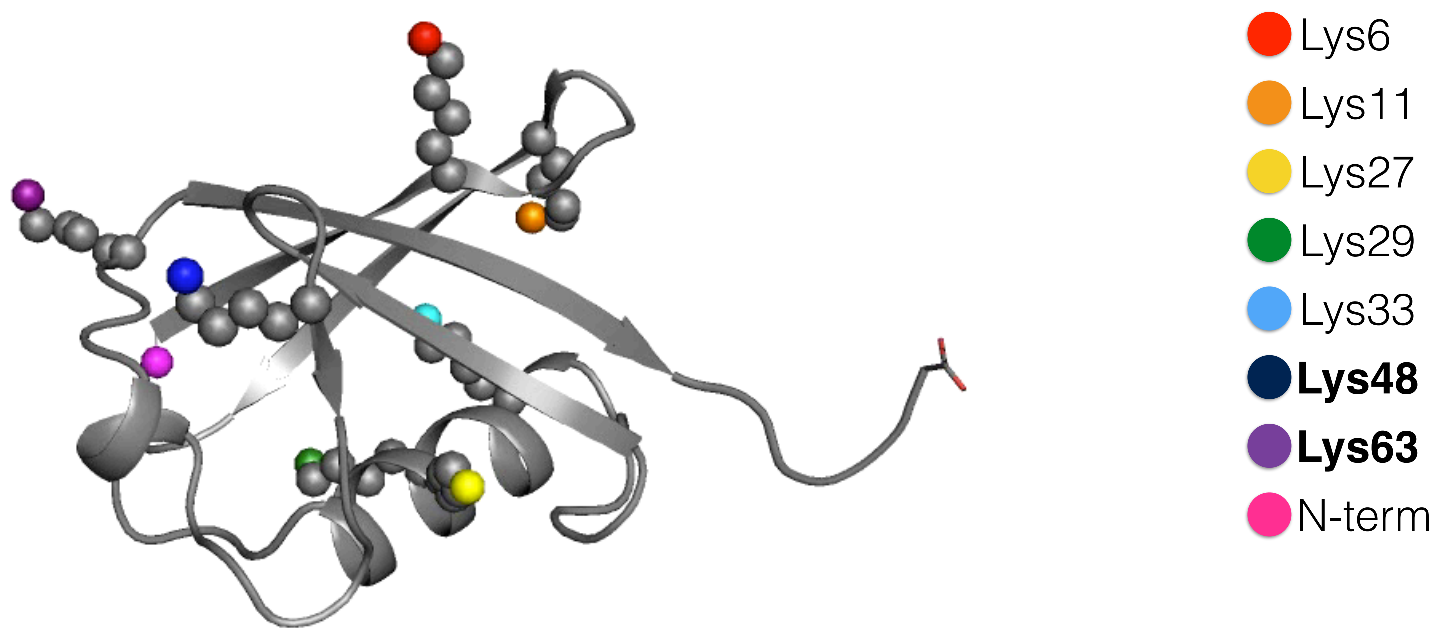
Ubiquitin is a **post-translational** modification that directs substrates to destruction and other fates



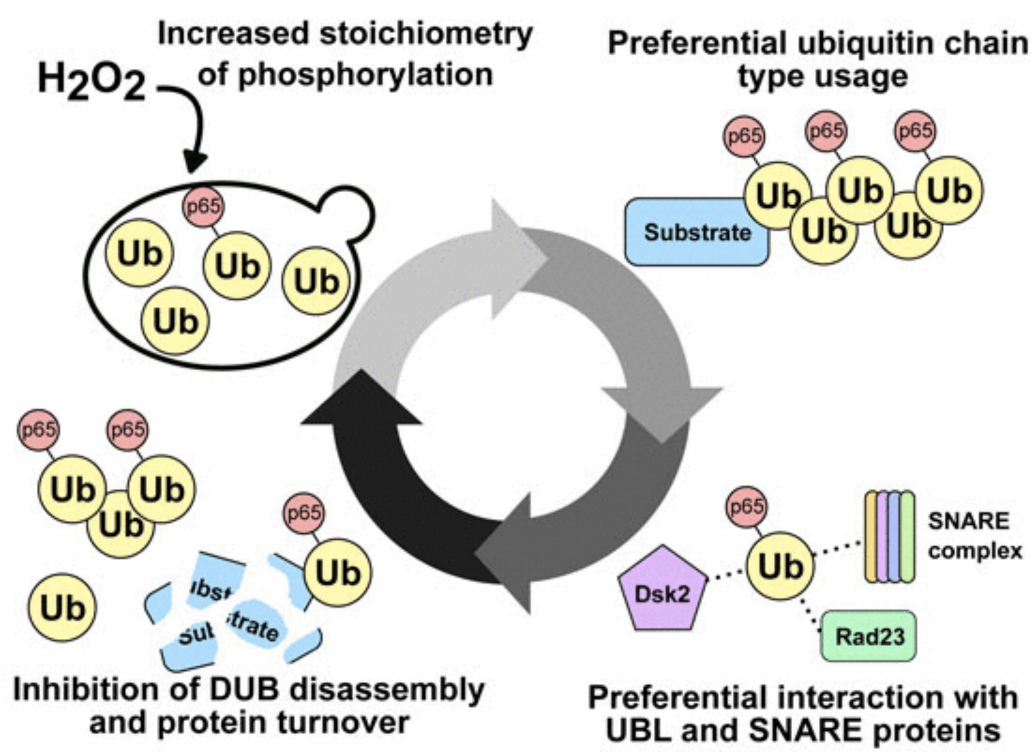
Ubiquitin is a **post-translational** modification that directs substrates to destruction and other fates



Poly-Ubiquitin chains can direct modified proteins to **different fates**



Ubiquitin is a PTM that is PTMed!



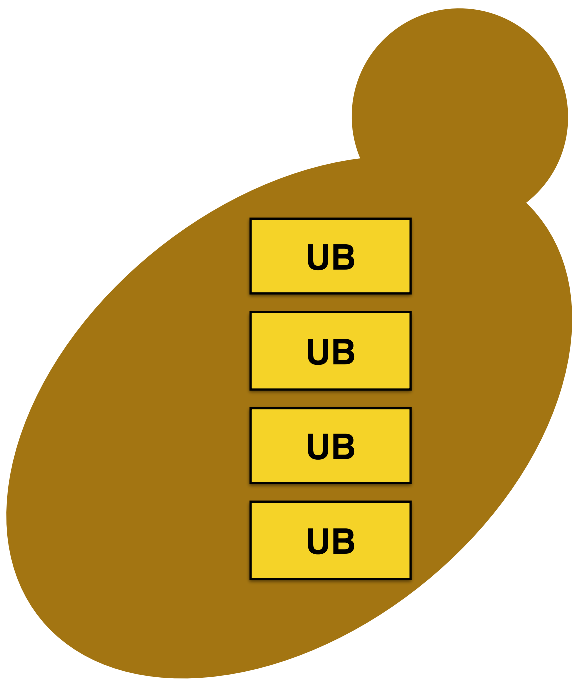
Ubiquitin is highly conserved

Organism	Sequence Alignment	Swiss-P
Amoeba	MQIFVKTLTGKTITLEVESSDTIENVKQKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P49634
Green alga	MQIFVKTLTGKTITLEVESSDTVENVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P42739
Chlamyd. reinhardtii	MQIFVKTLTGKTITLEVESSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P14624
Mouse	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P62991
Human (*)	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P62988
Slime mold	MQIFVKTLTGKTITLEVEGSDNIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P08618
Purple sea urchin	MQIFVKTLTGKTITLEVEPSDSIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P23398
Eimeria bovis	MQIFVKTLTGKTITLDVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P46574
T. pyriformis	MQIFVKTLTGKTITLDVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P20685
C. elegans	MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P14792
Red alga	MQIFVKTLTGKTITLEVESSDTIENVKTKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P42740
Neurospora crassa	MQIFVKTLTGKTITLEVESSDTIDNVKQKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P13117
Baker's yeast	MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P61864
Inky cap fungus	MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P19848
Garden pea (**)	MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P03993
Euplotes eurystomus	MQIFVKTLTGKTITLDVEQSDTIDNVKTKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P23324
Potato late blight fungus	MQIFVKTLTGKTITLDVEPSDSIDNVKQKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG	P22589
Leishmania major	MQIFVKTLTGKTIALEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEEGRTLSDYNIQKESTLHLVLRLRGG	Q05550
Sauroleish. tarentolae	MQIFVKTLTGTTIALEVEPSDTIENVKAKIQDKEGIPPDQQRLIFADKQLEEGRTLSDYNIQKESTLHLVLRLRGG	P49635
T. brucei brucei	MQIFVKTLTGKTIALEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEEGRTLADYNIQKESTLHLVLRLRGG	P15174
Trypanosoma cruzi	MQIFVKTLTGKTIALEVESSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVLRLRGG	P08565
	1 10 20 30 40 50 60 70 76	

…only 3 substitutions from yeast to human

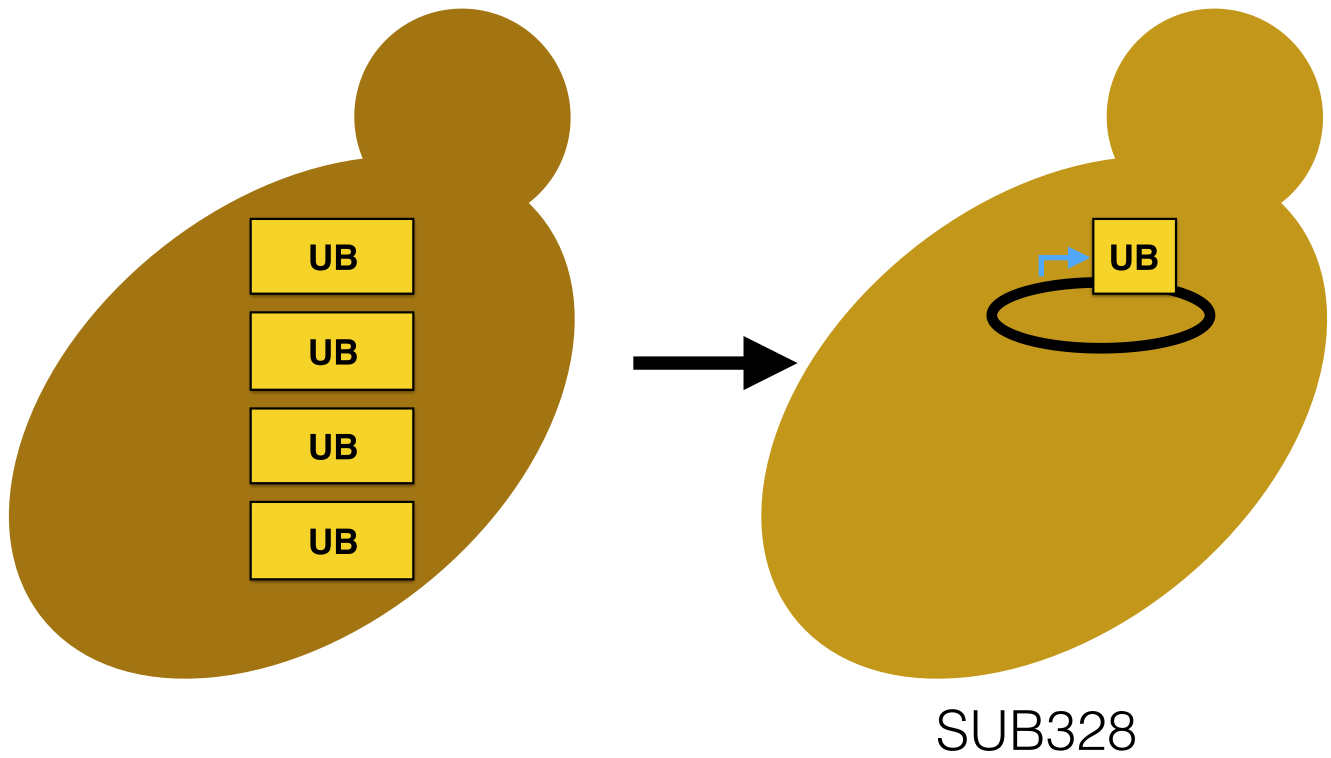
Why is Ubiquitin so highly conserved?

Yeast contain four Ubiquitin loci



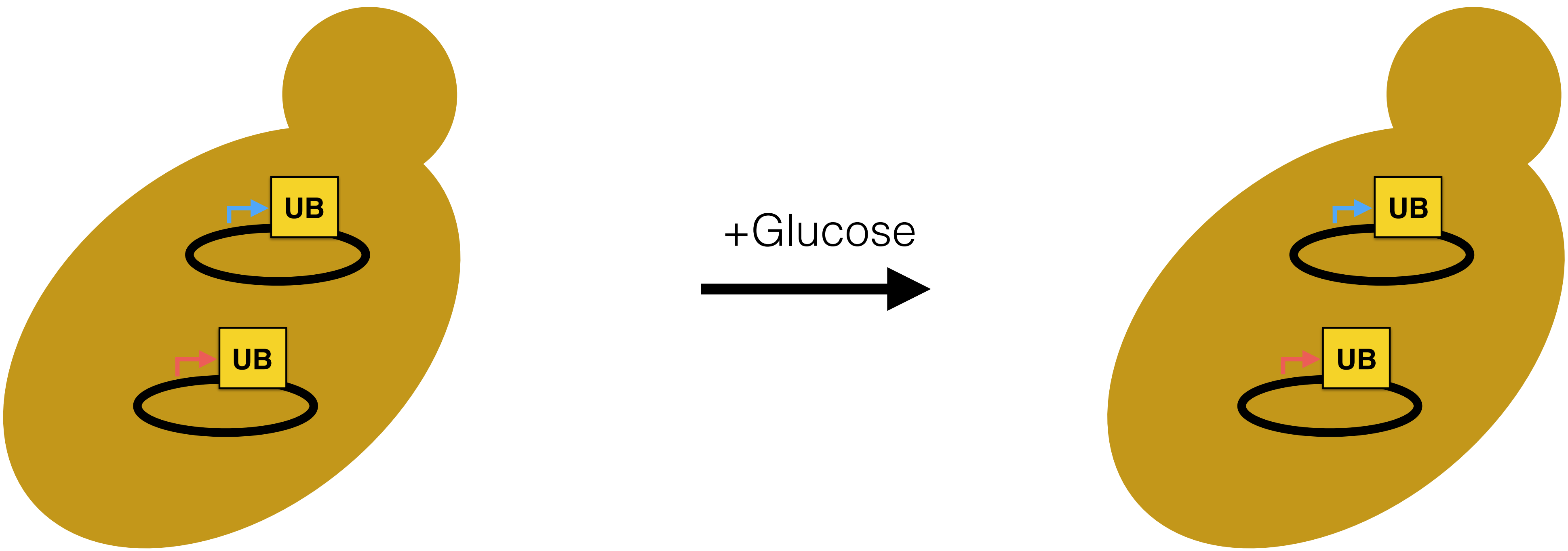
reviewed in: **Finley**, Ulrich, Sommer, Kaiser
Genetics, 2012

Galactose inducible Ubiquitin expression from a plasmid restores growth in a Ubiquitin knockout strain



reviewed in: **Finley**, Ulrich, Sommer, Kaiser
Genetics, 2012

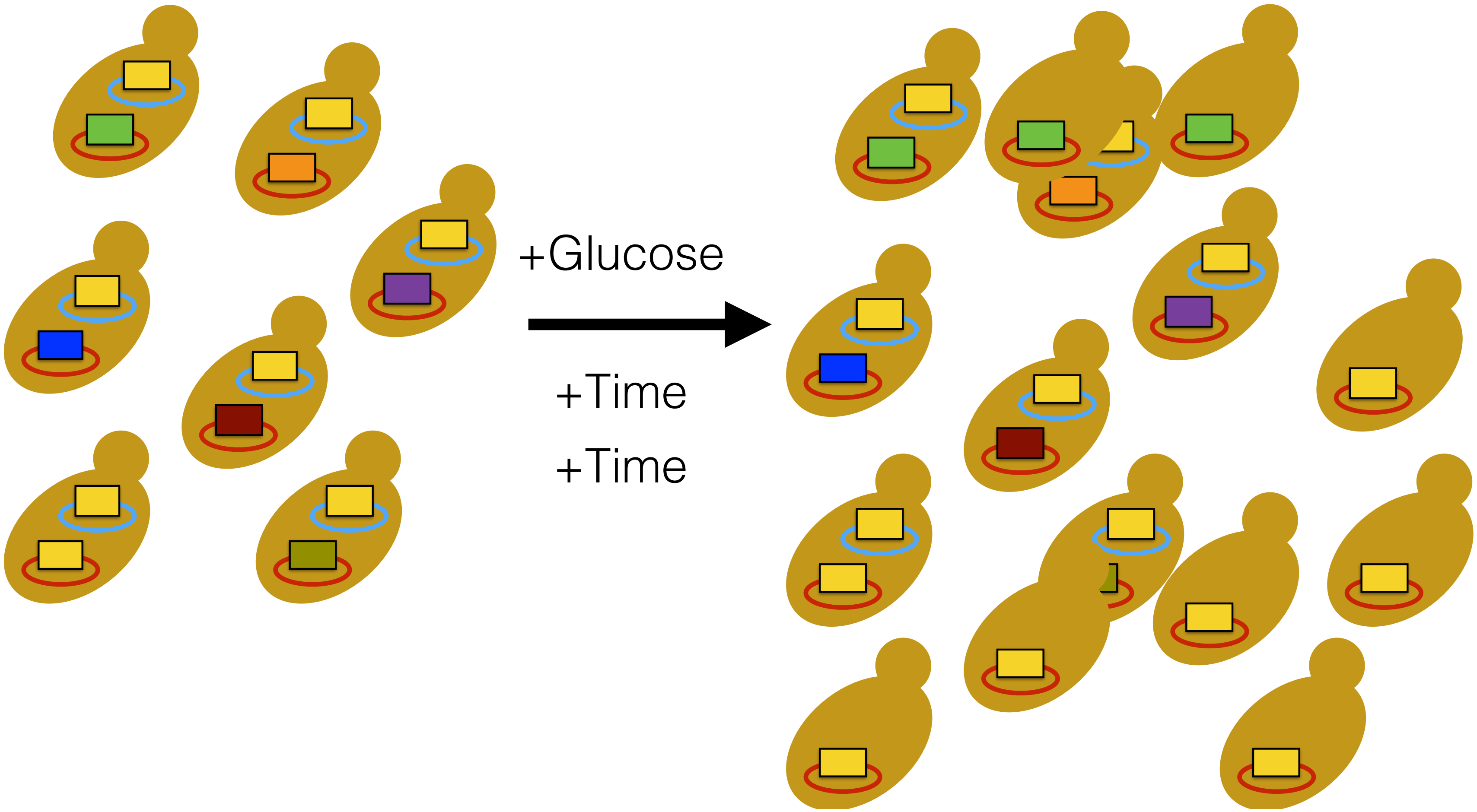
Adding glucose turns off **GAL**, allowing expression from a **second** plasmid to determine fitness



reviewed in: **Finley**, Ulrich, Sommer, Kaiser
Genetics, 2012

Library of all 1520 single mutants

Roscoe…Bolon, *JMB*, 2013



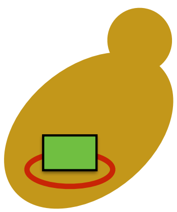
Ubiquitin sequence divided into eight regions for accurate and efficient analyses

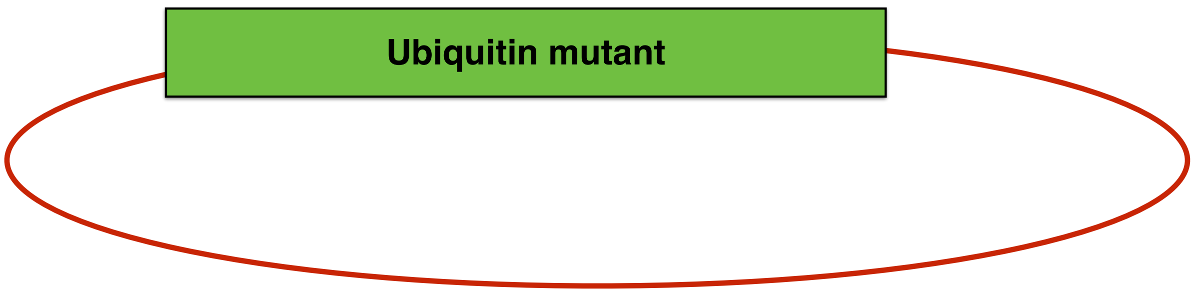
MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

1 2 3 4 5 6 7 8

Generated libraries of saturation mutants at each amino acid position

Transformed libraries into yeast, outgrowth, shutoff and sampling over time in competition

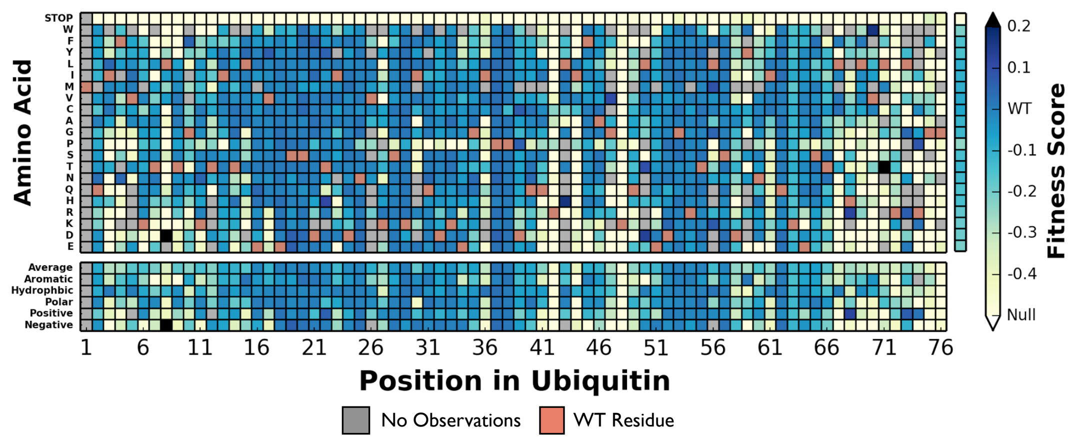




Two key points:
6 months!
and **mostly WT fitness!**

Library of all 1520 single mutants

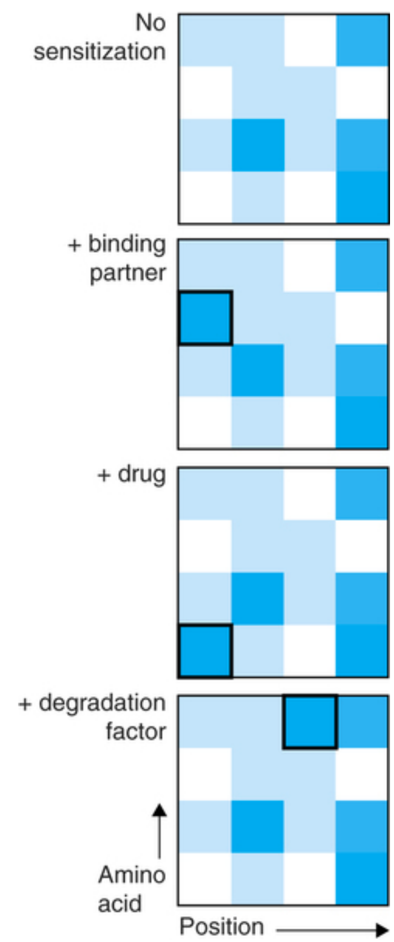
Roscoe…Bolon, *JMB*, 2013



Why is Ubiquitin so **conserved** in evolution,
but so **tolerant** in deep mutational scanning?

Why is the evolutionary history so different from the selection experiment?

How do different environments (chemical perturbations) alter the Ub fitness landscape?

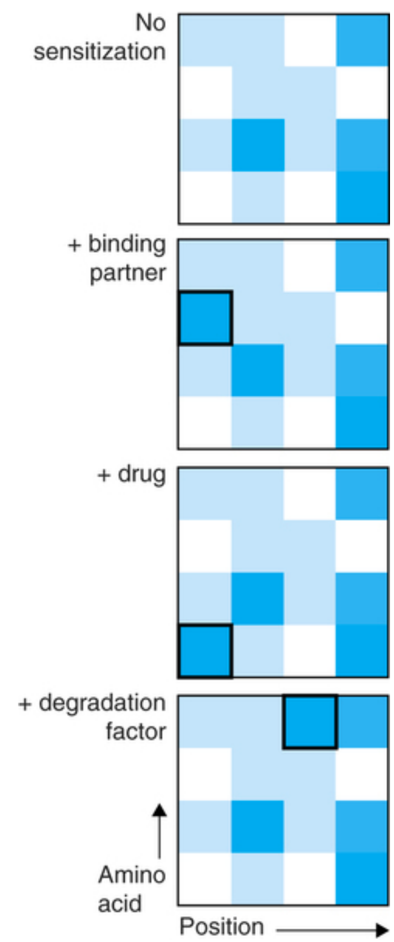


Fowler and Fields, Nature Methods, 2014

Why is the evolutionary history so different from the selection experiment?

How do different environments (chemical perturbations) alter the Ub fitness landscape?

EACH TEAM WILL CHOOSE A DIFFERENT PERTURBATION



Fowler and Fields, Nature Methods, 2014

Why is Ubiquitin so **conserved** in evolution,
but so **tolerant** in deep mutational scanning?

Does the fitness vary in different environments?

Does Ubiquitin phosphorylation also vary?
What kinases are responsible for Ub-P?

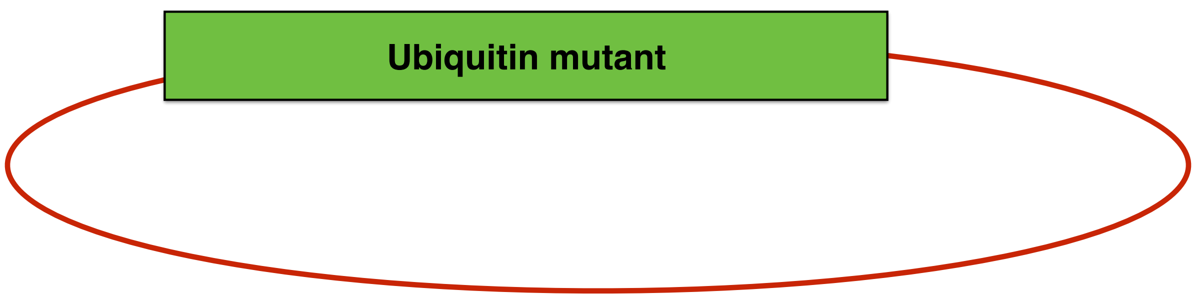
Kinases and Chemicals

SWE1
ATG1
KIN3
ALK1
CMK1
TPK1

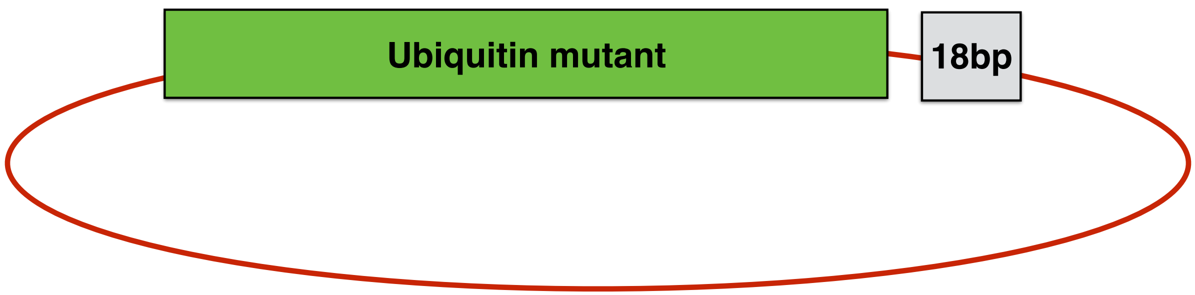
Tunicamycin
Spermine
rapamycin
hygromycin B
Nickle Chloride
3-Amino-1,2,4-triazole
Calcium dichloride
Cerulenin
Cobalt acetate
miconazole
p-Fluoro-DL-phenylalanine
tamoxifen
ketoconazole
clotrinazole
menadione
Calcofluor white
$CuCl_2$
5-fluorocytosine
acivicin
amphotericin B

- Week 1: Warm up - Barcodes, Transformations, Choose a Chemical
- Week 2: Biochemical Enrichment of Phosphopeptides
- Week 3: Analysis of Mass Spec Data
- Week 4: **Presentations** and Growth Rate
- Week 5: Competition Experiment (two long days)
- Week 6: Library Preparation and **NSF due**
- Week 7: Analysis of Sequencing Data
- Week 8: Pipelining, Data Visualization, and Team Shuffles
- Week 9: Comparisons to Rosetta Calculations
- Tuesday November 24th: **Final Presentations** and Party!

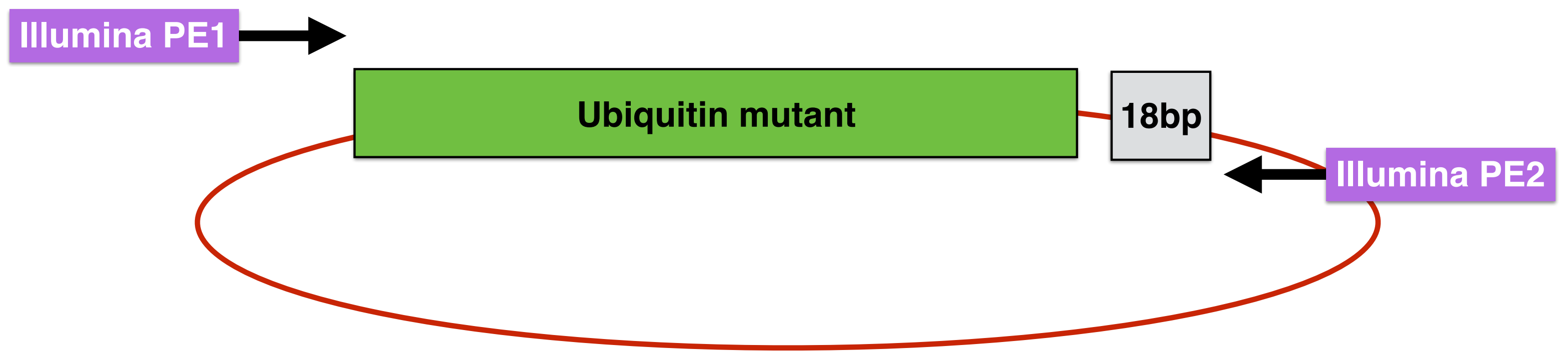
Barcoding makes it possible to perform the whole experiment in 3 weeks of class time!



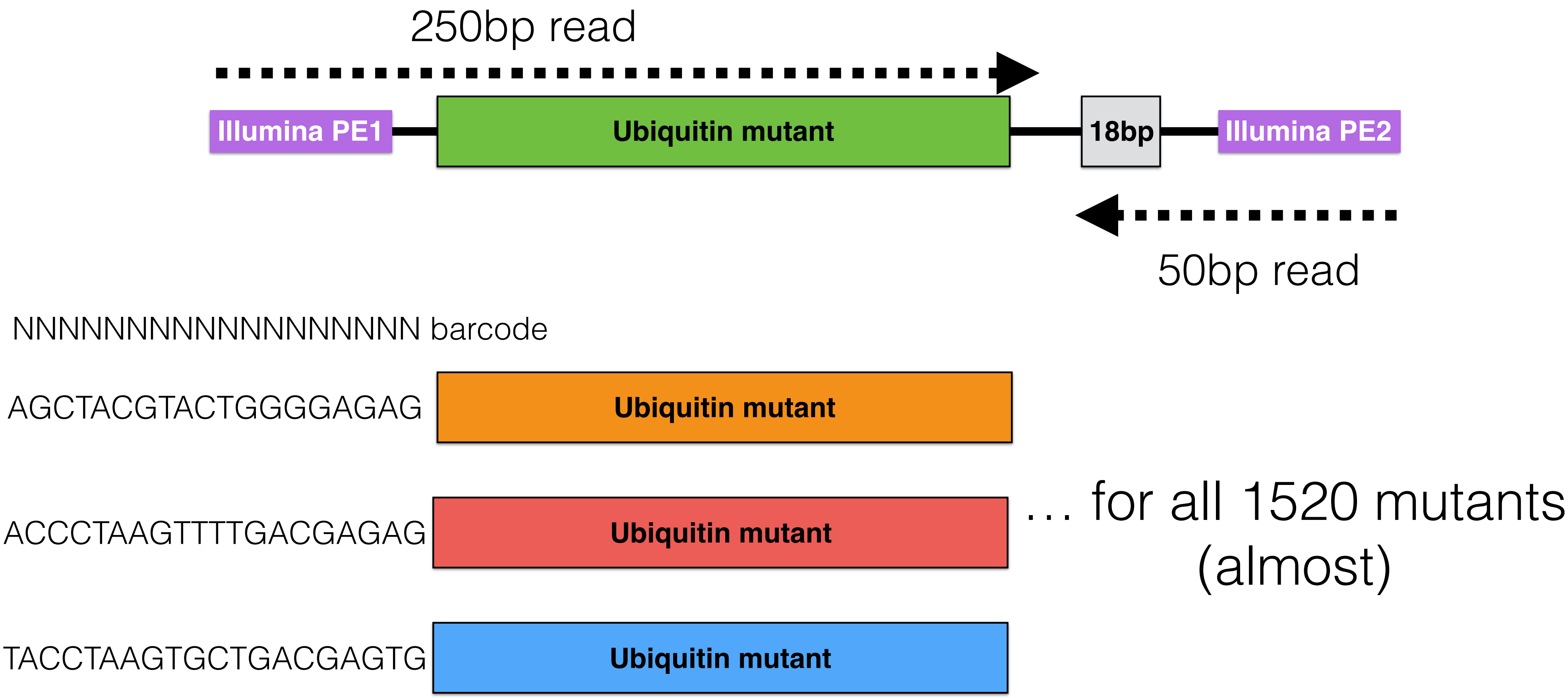
NNNNNNNNNNNNNNNNNN
is ligated behind the Ubiquitin **library**



A single PCR product contains the **barcode** and the entire Ubiquitin gene



An unbalanced **paired-end** read generates a map between barcodes and mutants



- Pickles are a way to dump out python data structures as files, allowing easy transfer of data between scripts

- ```
  import cPickle as pic
  data = pic.load(open("filename.pkl","rb"))
  print data
  ```

- We are giving you 3 pickles (http://fraserlab.com/pubs/):

  - allele_dic.pkl - contains a dictionary where:
    key = barcode nucleotide sequence
    value = residuenumber_codon
    (residuenumber is in protein space, codon is in nucleotides!)

  - translate.pkl - contains a dictionary where:
    key = codon
    value = amino acid

  - aminotonumber.pkl - contains a dictionary where:
    key = amino acid
    value = number
    (useful for plotting)

- Many barcodes can map to the same codon, and (for some amino acids) many codons can map to the same amino acid

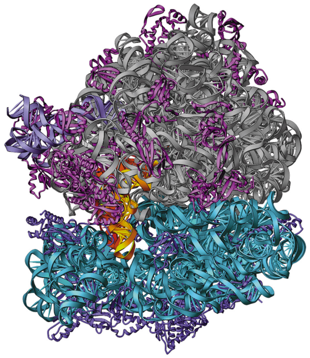
- each group will present results (visualizations, quantifications, biases in library, etc) to JF/DM/DS and TA at end of class today!


  ```

Today, we have to accomplish 3 tasks

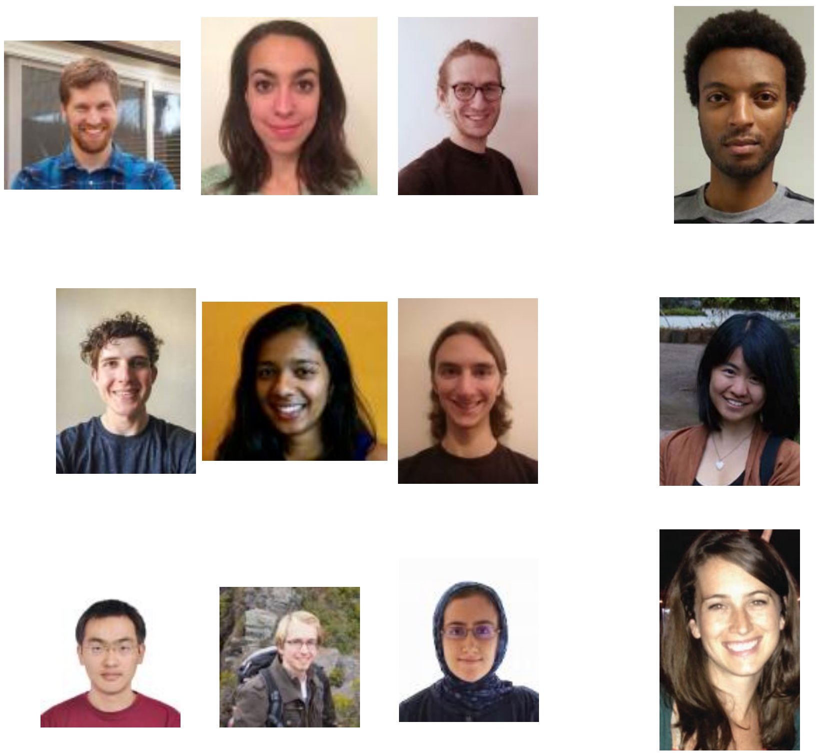
- The teams need **names**!
  Each team will get a kinase, lysine linkage and choose chemical perturbation
- Joe needs to give each team an **account** on the server
  http://fraserlab.com/pubs/server/
- We need you to convert the barcodes from nucleotide space to amino acid space (**ribosome_barcodes.py**)

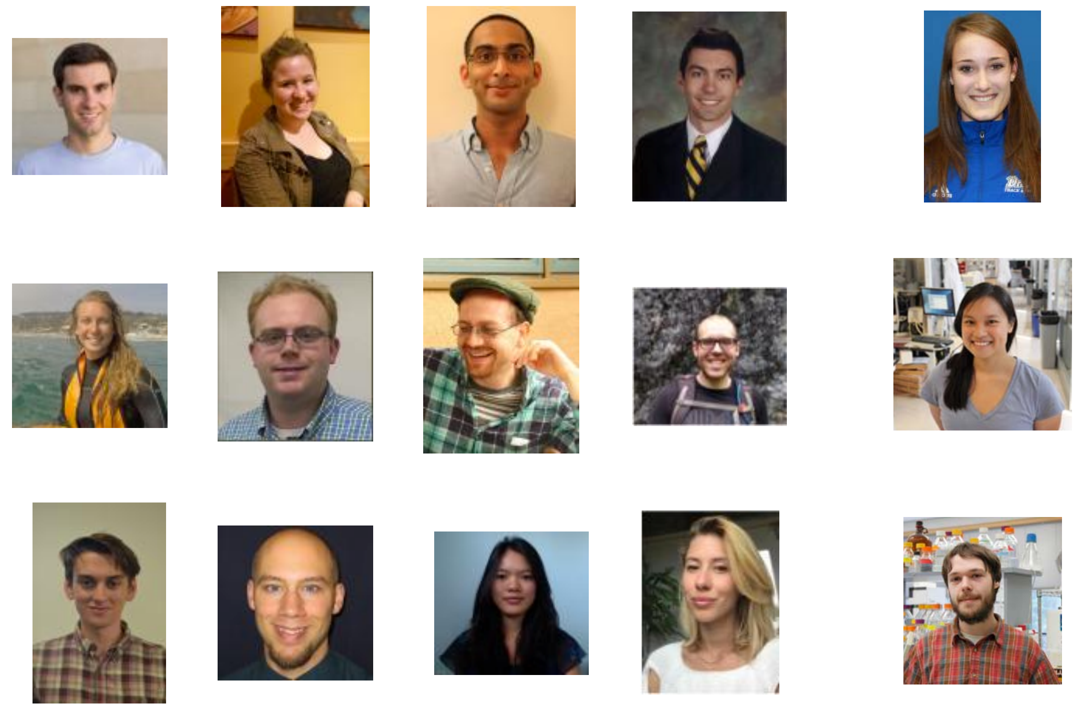
RAPTORS



Teams?

See www.fraserlab.com/pubs
for kinase, lysine linkage assignment





use SGD and other resources to link
chemical choice to kinase, lysine linkages

Tomorrow's presentations

5 min Protocol Presentation
at 1PM

30 second Chemical Choice
Justifications
at 4PM