Protein complex identification using TAP tags

Mary Mohr and Cory Smid

Overview

Why are protein complexes important?

What is affinity purification?

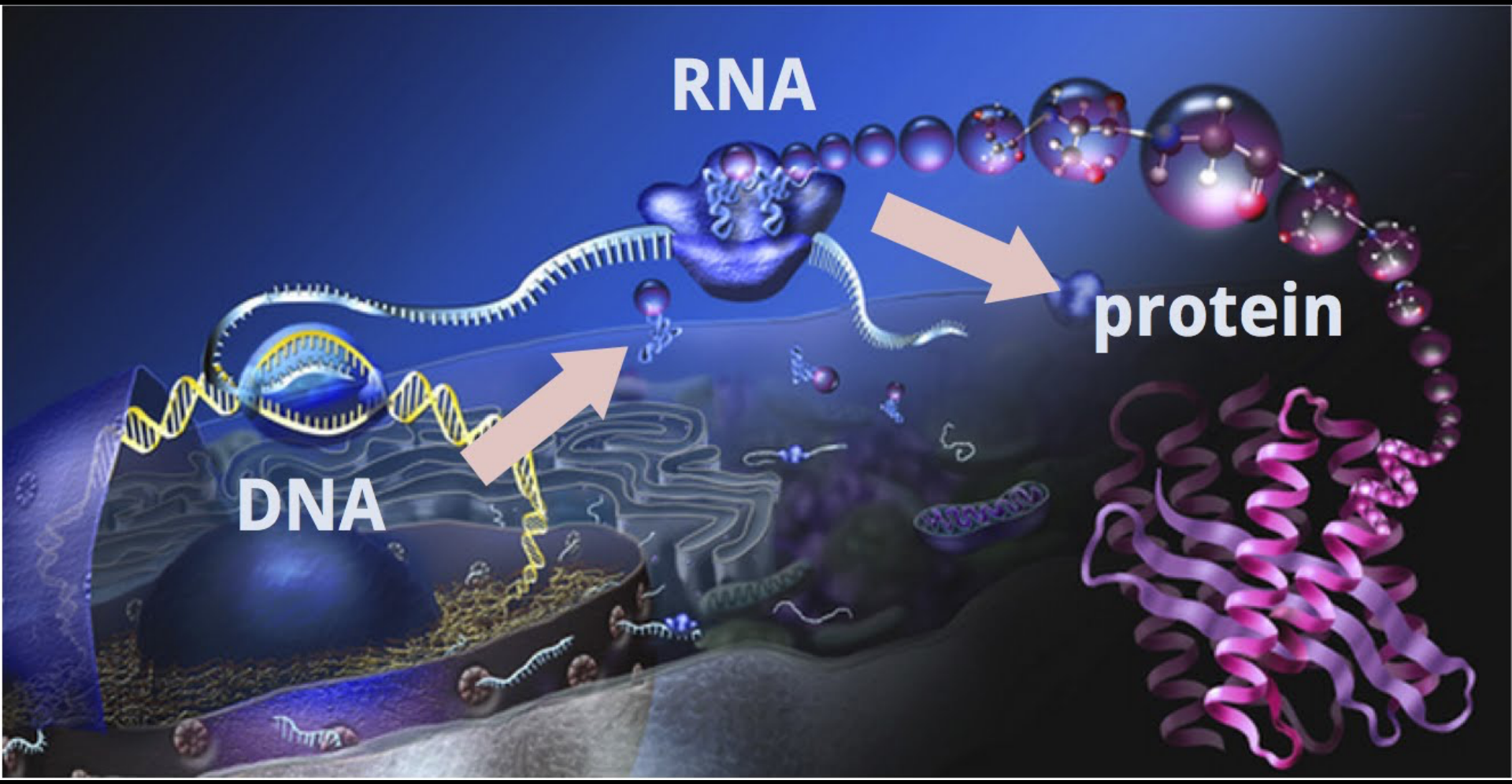
What types of affinity purification exist?

What is tandem affinity purification (TAP)?

What are advantages/disadvantages of TAP?

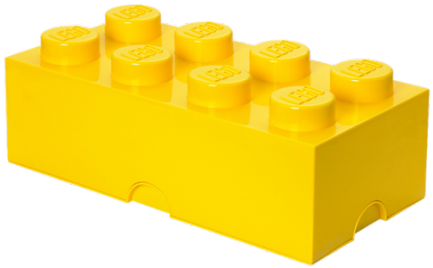
How can TAP be used in our project?

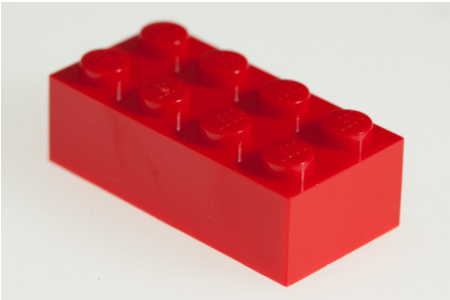
Why are protein networks important?



https://www.youtube.com/watch?v=lSqUDu4zb5k

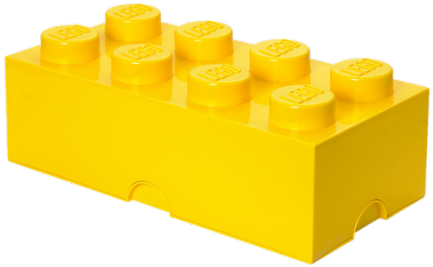
Why are protein interactions important?

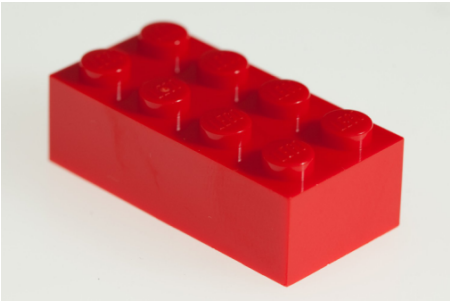




http://shop.lego.com/en-FI/LEGO-8-stud-Yellow-Storage-Brick-5001267

Why are protein interactions important?

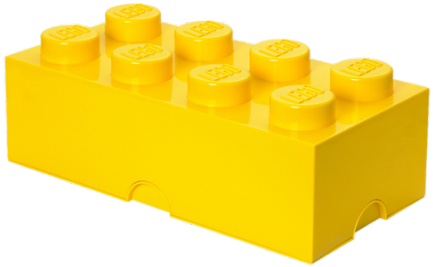


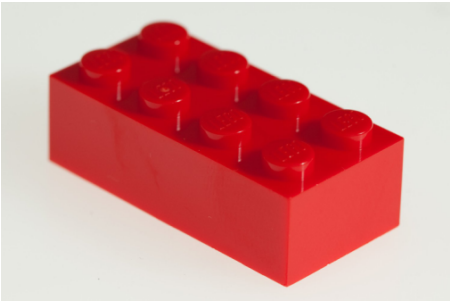


http://shop.lego.com/en-FI/LEGO-8-stud-Yellow-Storage-Brick-5001267

http://www.build-with-bricks.info/lego-duplo-houses.htm

Why are protein interactions important?

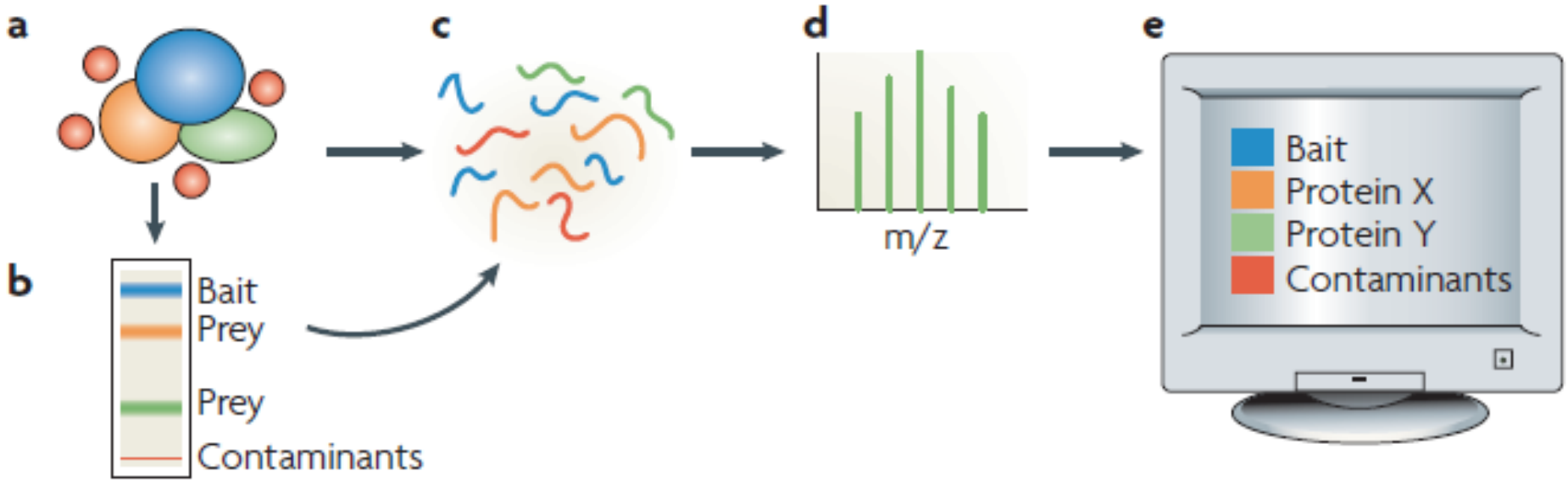




http://shop.lego.com/en-FI/LEGO-8-stud-Yellow-Storage-Brick-5001267

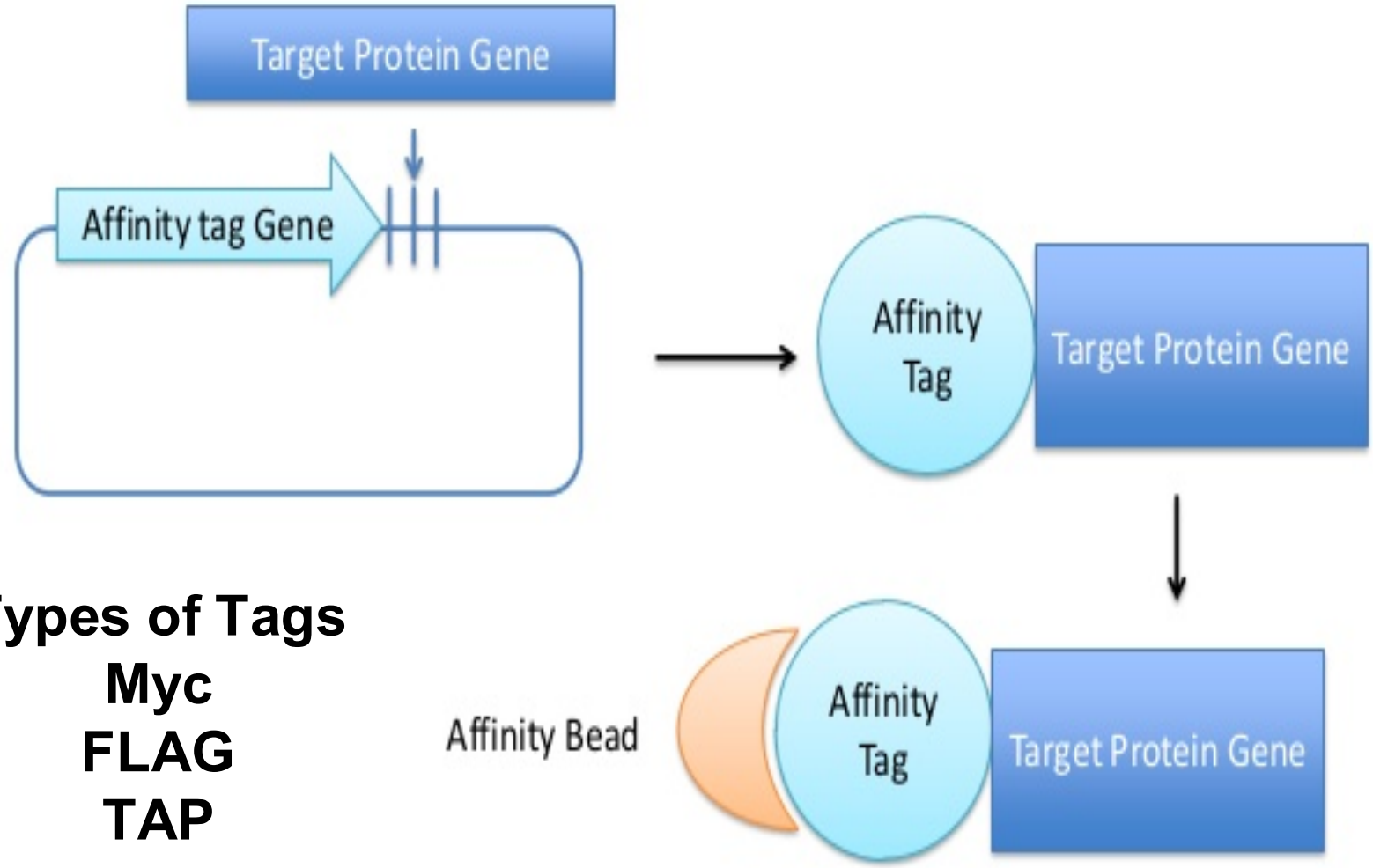
http://www.brickpirate.net/forum/phpBB3/viewtopic.php?f=34&t=10339&start=20

How do you identify protein complexes?



Use affinity purification and mass spectrometry!

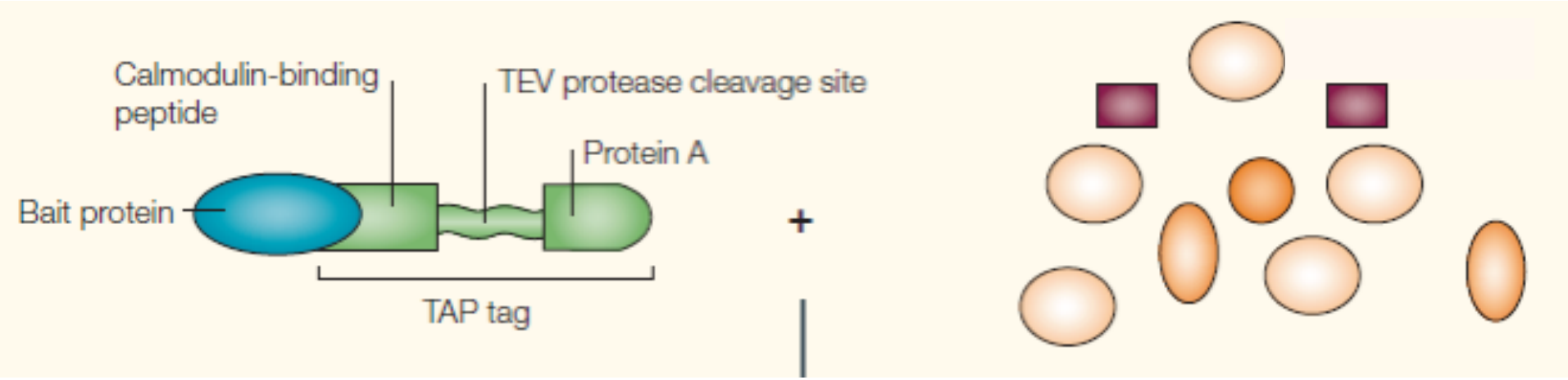
What is an affinity tag?



Types of Tags
Myc
FLAG
TAP

http://image.slidesharecdn.com/cellculture-04-1310310213 27-phpapp02/95/cell-culture-04-13-638.jpg?cb=1383185631

What is a Tandem Affinity Purification (TAP) tag?



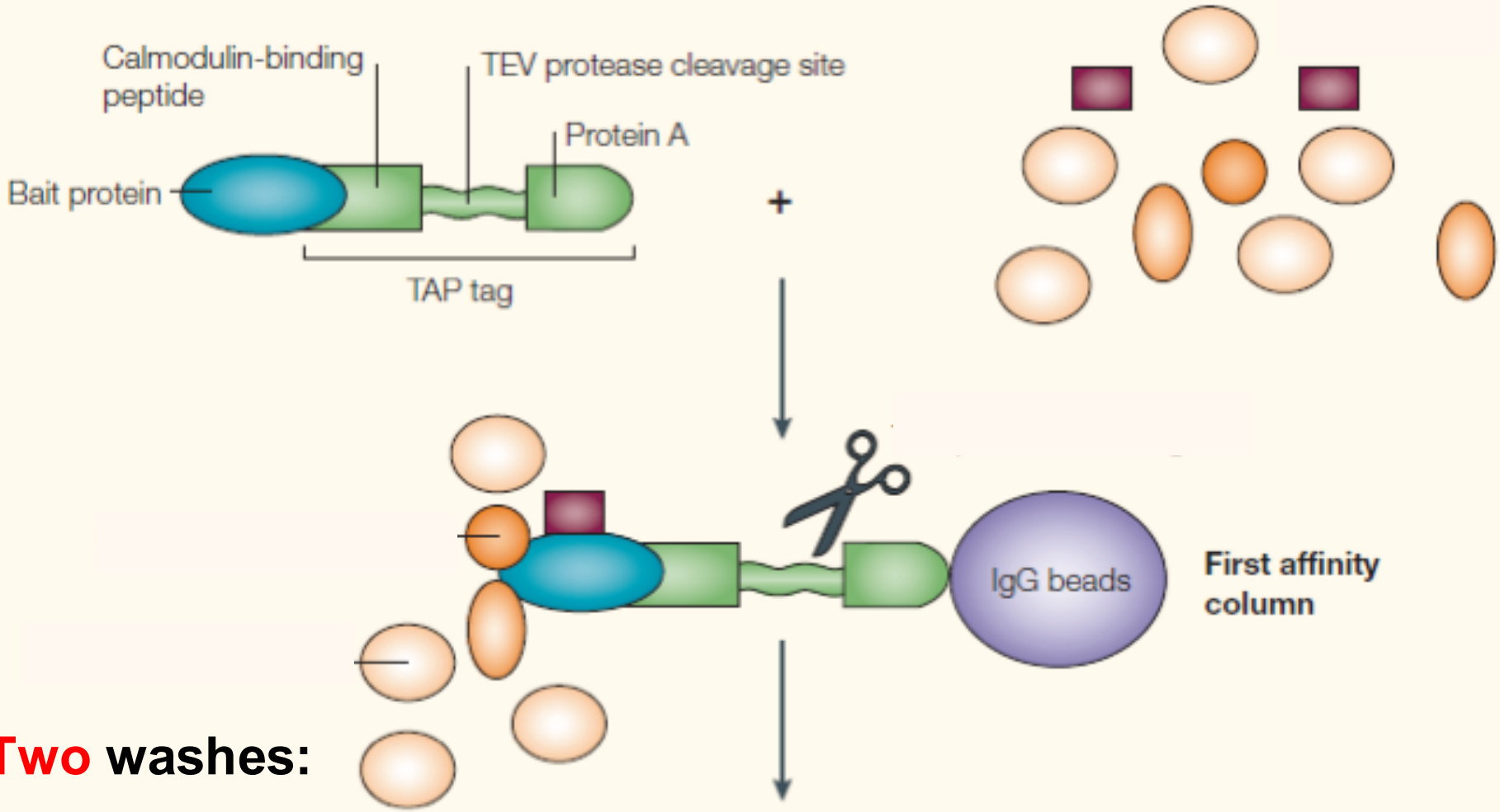
Two tag process:

Protein A = distal tag

Calmodulin Binding Protein = proximal tag

http://www.nature.com/nrm/journal/v4/n1/pdf/nrm1007.pdf

What is a Tandem Affinity Purification (TAP) tag?

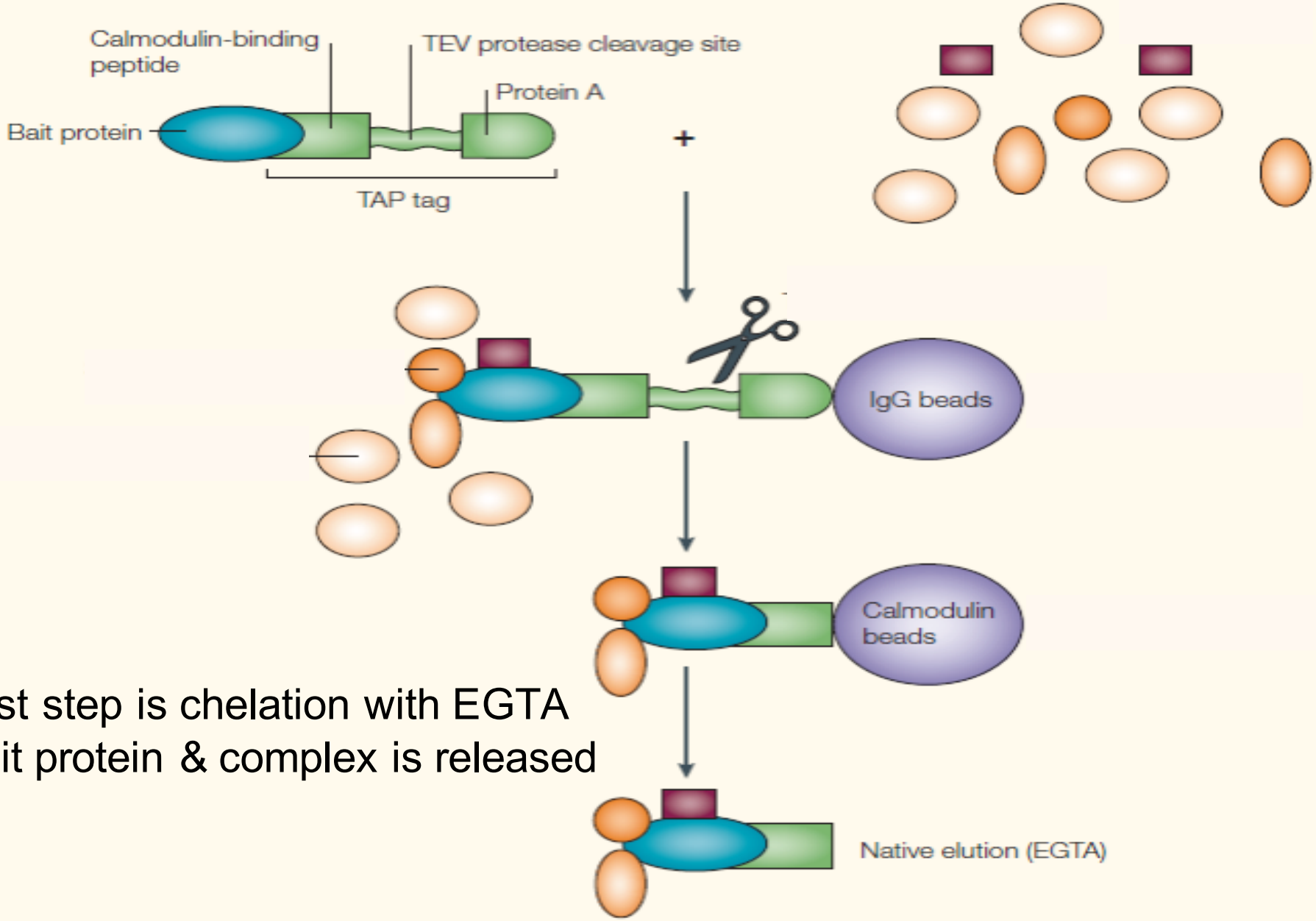


http://www.nature.com/nrm/journal/v4/n1/pdf/nrm1007.pdf

Two washes:

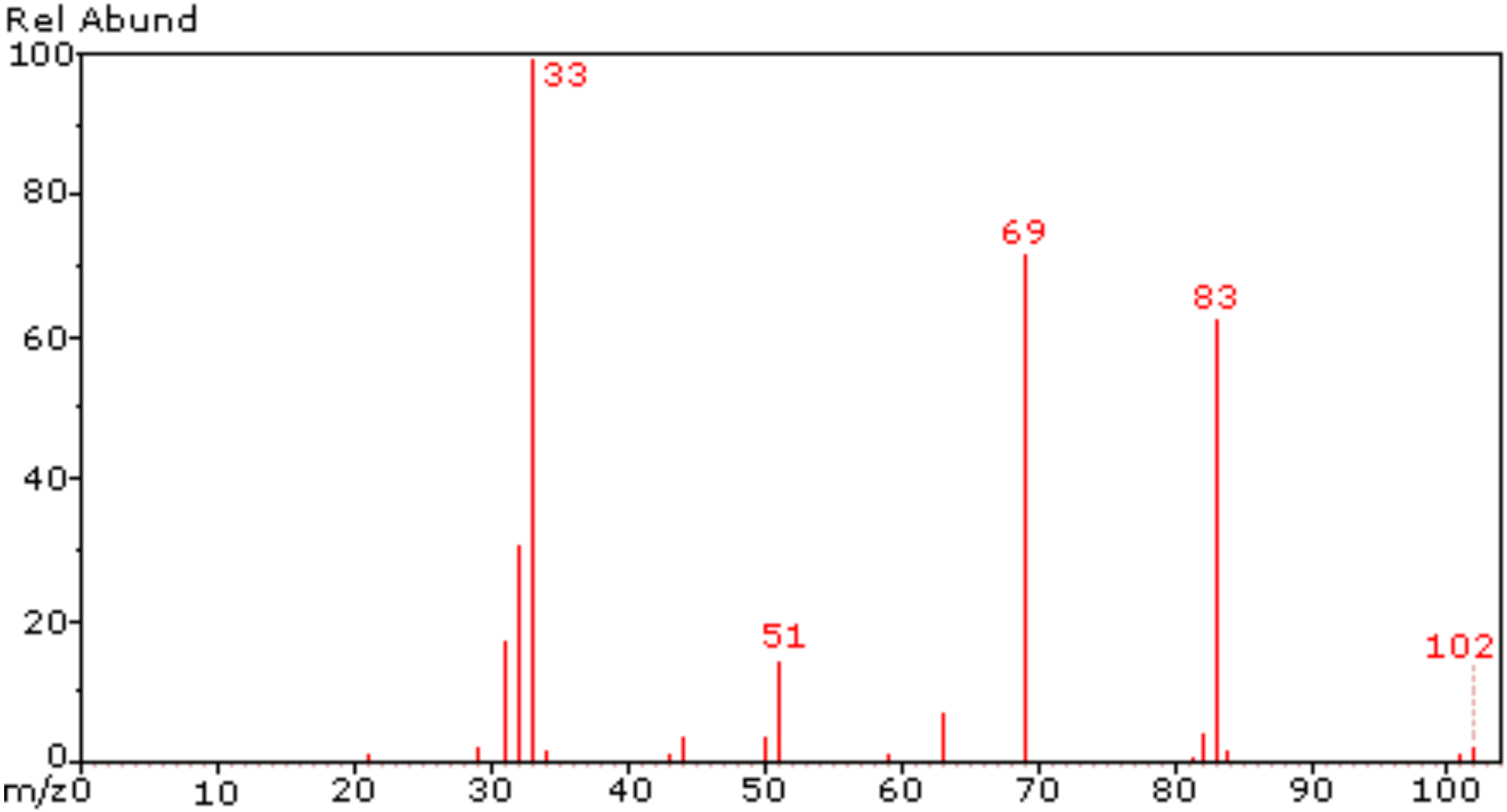
Protein A on IgG beads
Tag cleaved with TEV protease
CBP binds to calmodulin beads

What is a Tandem Affinity Purification (TAP) tag?



Last step is chelation with EGTA
Bait protein & complex is released

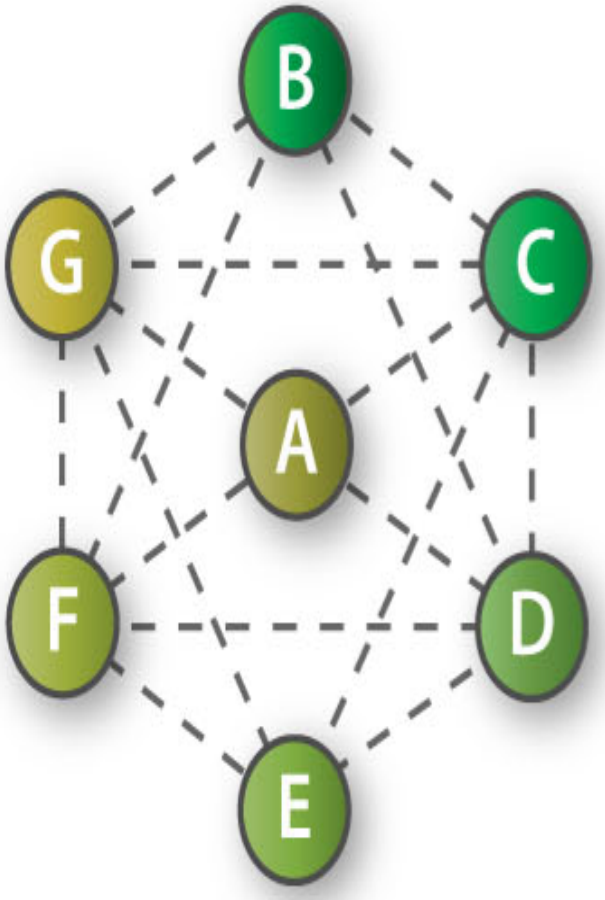
Step 2: How do you identify proteins?



https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Questions/Spectroscopy/masspec7.htm

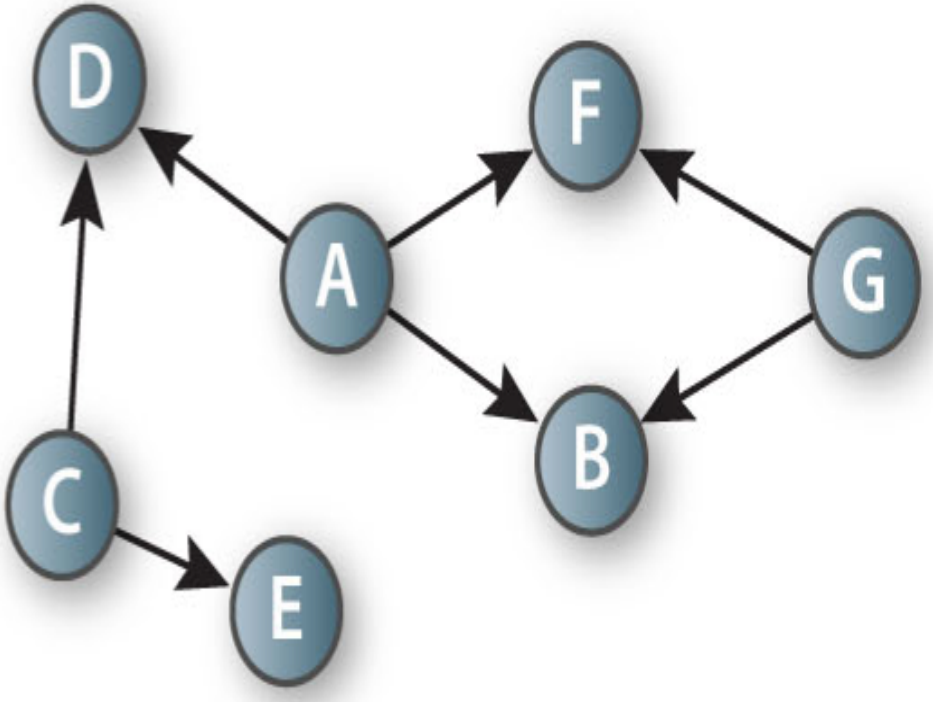
How does this compare to yeast 2-hybrid?

TAP



VS.

Y2H



http://web.science.uu.nl/developmentalbiology/boxem/interaction_mapping.html

What are dis/advantages compared to Y2H?

What are dis/advantages compared to Y2H?

Advantages

- Looks at complete protein complex
- Rapid sequencing
- High-throughput compatible
- Weakly bound proteins
- Not confined to nucleus
- Higher success rate

Disadvantages

- Are all proteins in complex directly interacting?
- Unable to detect transient protein interactions
- Need expensive equipment for MS
- Hard to detect smaller peptides

Both Y2H and TAP experience false positives/negatives

False positives and negatives: How does TAP deal with them?

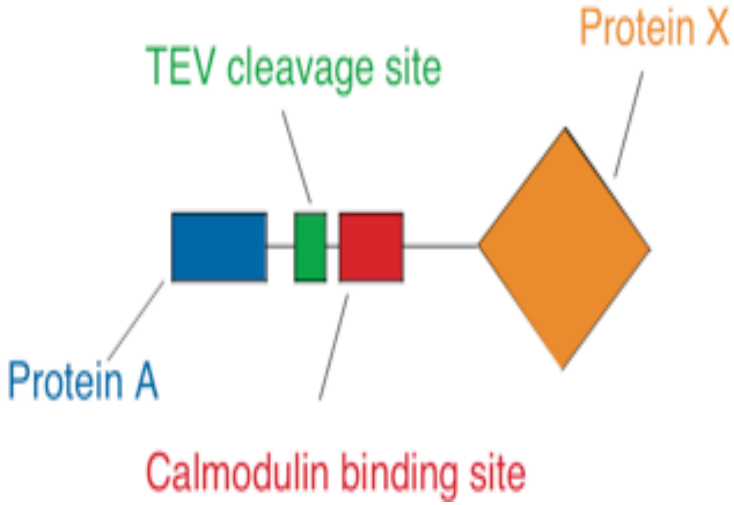
False positives and negatives: How does TAP deal with them?

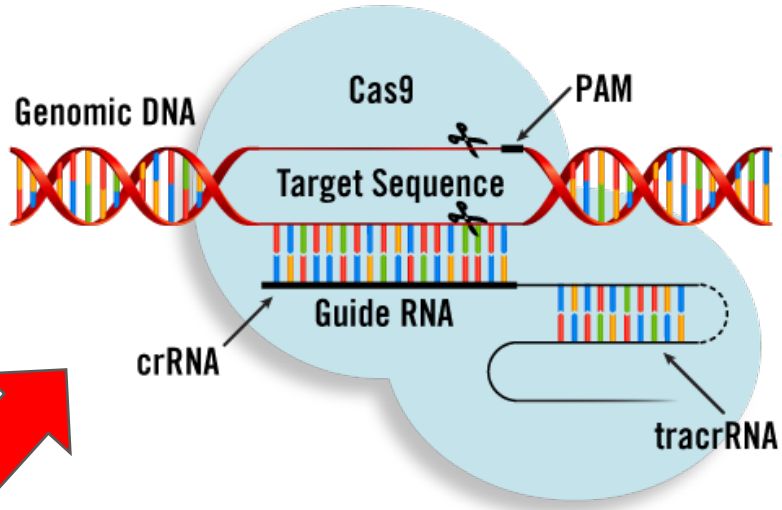
- False Positives
  - Dual approach tag with high-throughput data
- False Negatives
  - Protein abundance
  - Test conditions
- Fractionation

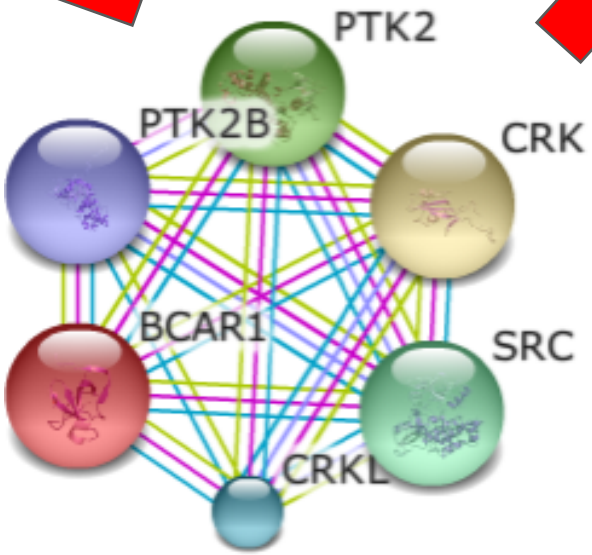
How can I use TAP-tag in my project?

http://www.patheos.com/blogs/thenakedjesus/2015/05/thinking-out-loud/

How can I use TAP-tag in my project?







http://www.mwilm-conway-ucd.de/Bioanalytical_Research/Research_Activities_(EMBL)/Entries/2000/6/1_Functional_Proteomics_files/shapeimage_3.png
https://www.mirusbio.com/applications/crispr-cas-transfection

http://www.clipartbest.com/cliparts/xcg/K8k/xcgK8kXAi.png

Functional organization of the yeast proteome by systematic analysis of protein complexes

Anne-Claude Gavin*, Markus Bösche*, Roland Krause*, Paola Grandi*, Martina Marzioch*, Andreas Bauer*, Jörg Schultz*, Jens M. Rick*, Anne-Marie Michon*, Cristina-Maria Cruciat*, Marita Remor*, Christian Höfert*, Malgorzata Schelder*, Miro Brajenovic*, Heinz Ruffner*, Alejandro Merino*, Karin Klein*, Manuela Hudak*, David Dickson*, Tatjana Rudi*, Volker Gnau*, Angela Bauch*, Sonja Bastuck*, Bettina Huhse*, Christina Leutwein*, Marie-Anne Heurtier*, Richard R. Copley†, Angela Edelmann*, Erich Querfurth*, Vladimir Rybin*, Gerard Drewes*, Manfred Raida*, Tewis Bouwmeester*, Peer Bork†, Bertrand Seraphin†‡, Bernhard Kuster*, Gitte Neubauer* & Giulio Superti-Furga*†

NATURE | VOL 415 | 10 JANUARY 2002 | www.nature.com

Figure 1a: Where are the complexes?

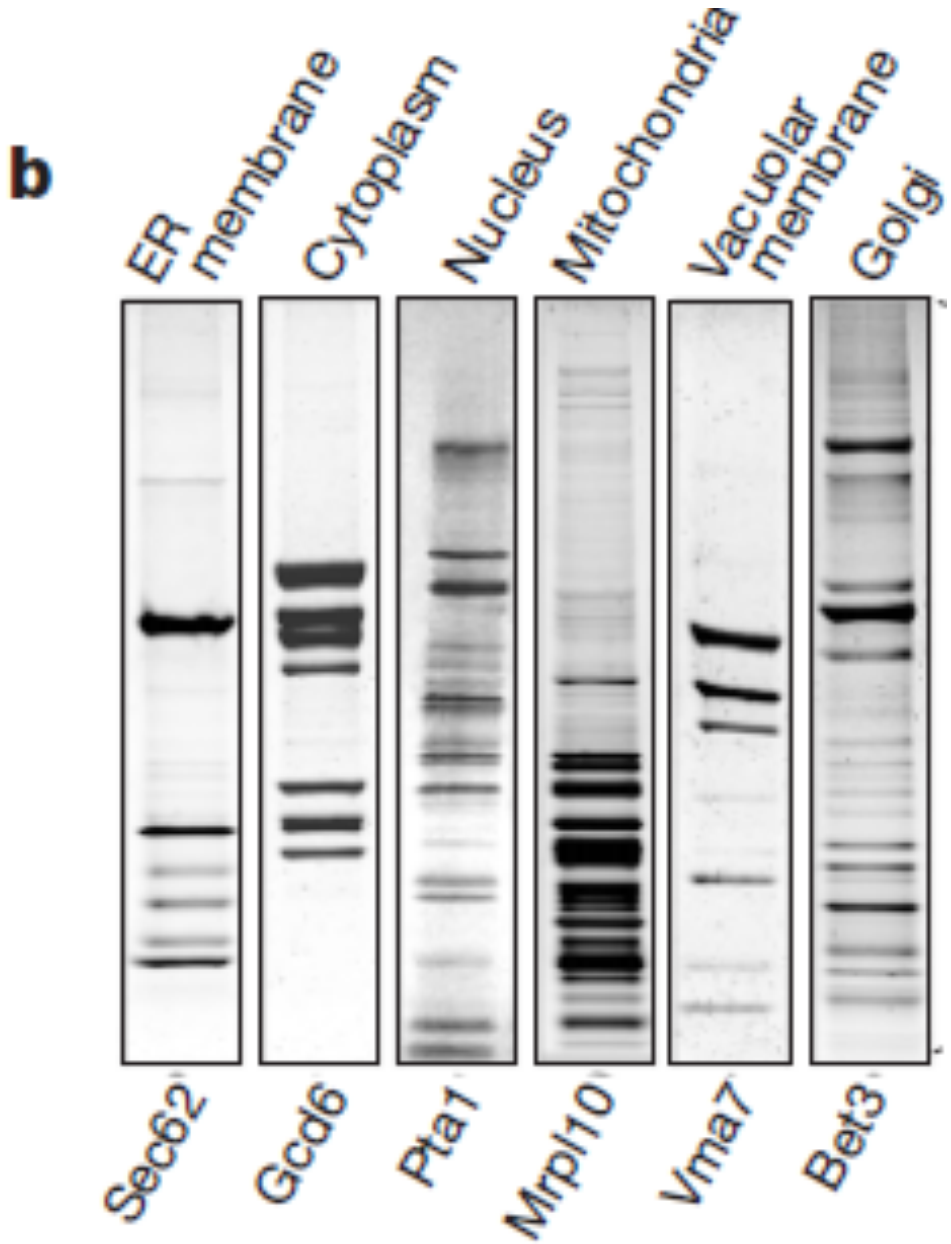


Figure 1c: How were protein complexes identified?

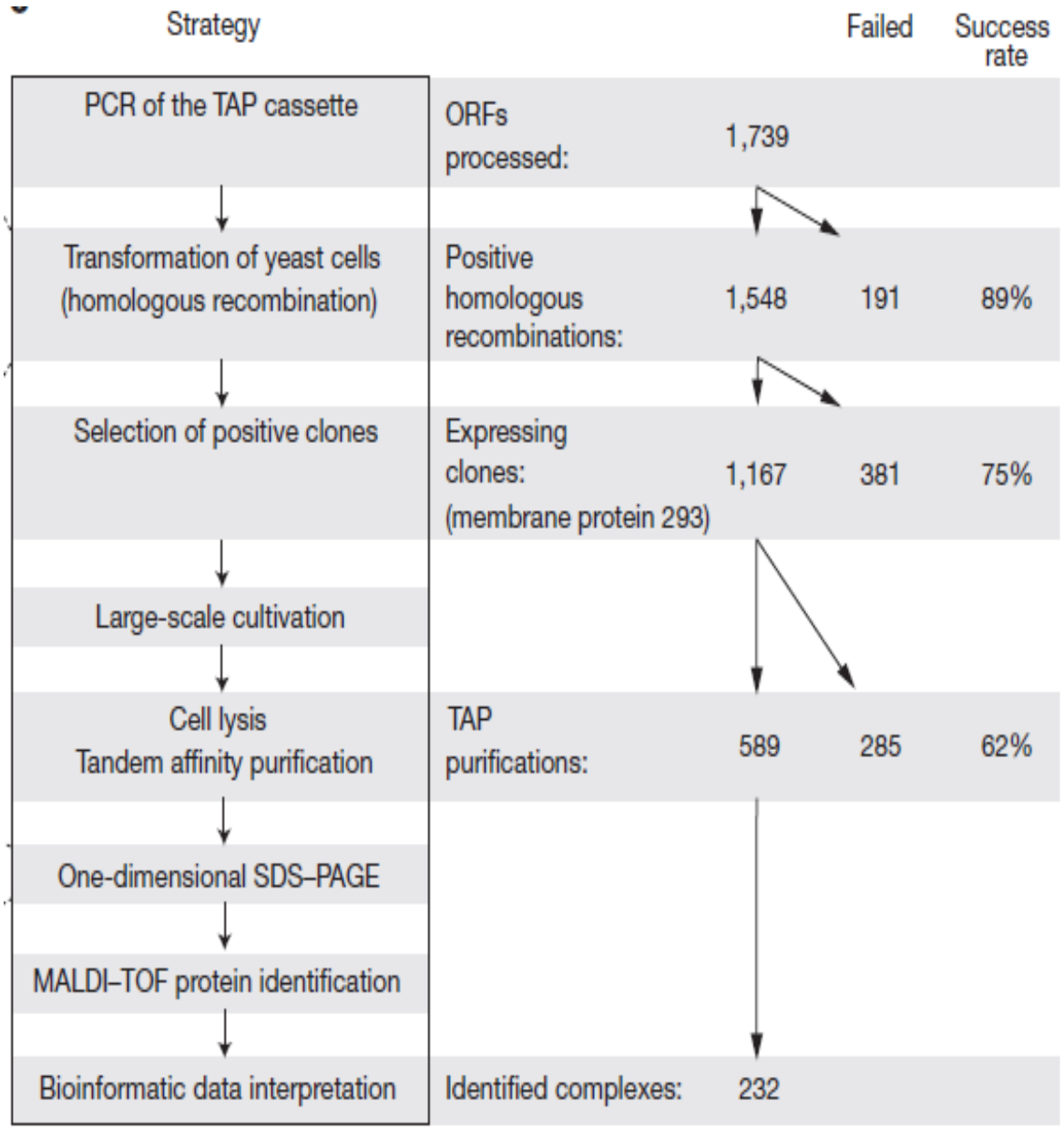
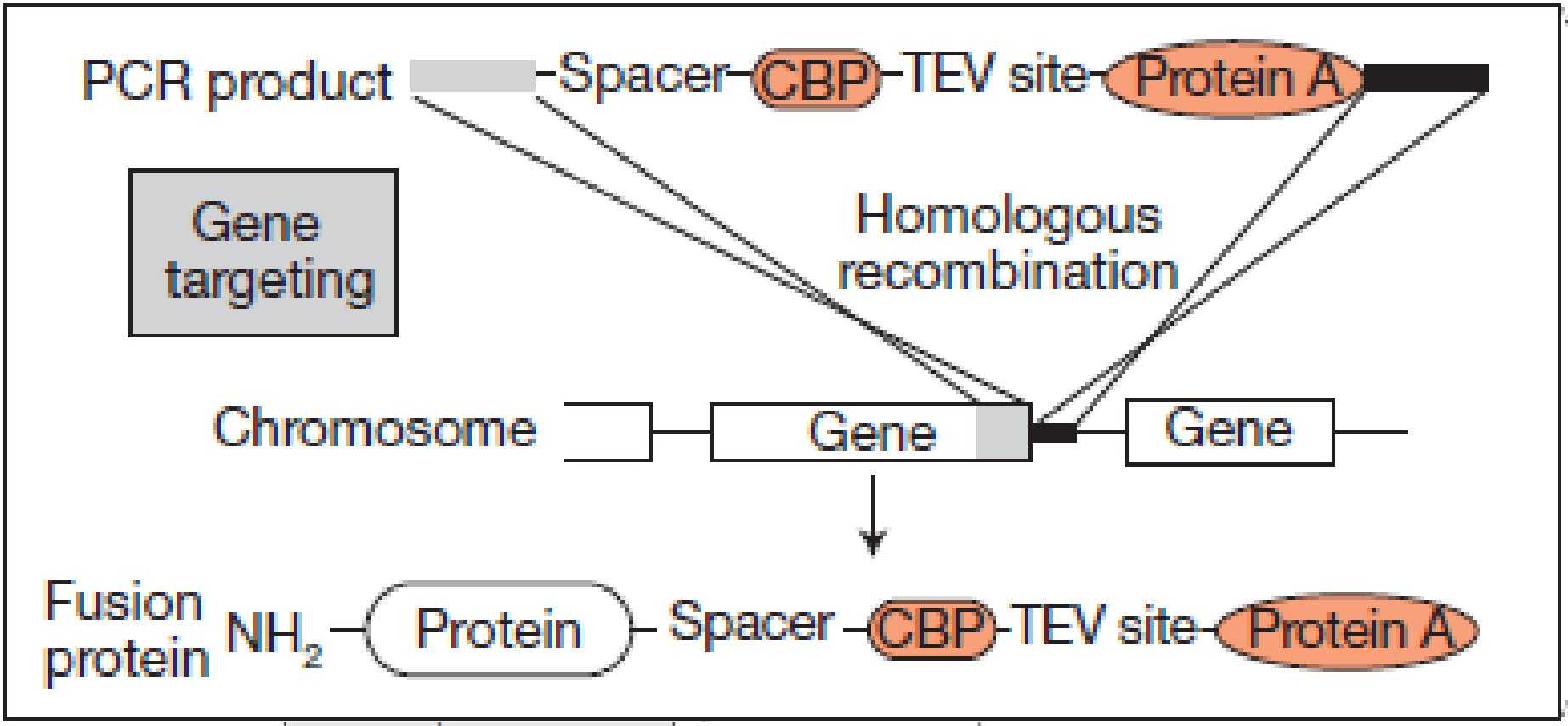


Figure 1c: How were protein complexes identified?

Strategy		Failed	Success rate
PCR of the TAP cassette	ORFs		



Bioinformatic data interpretation	Identified complexes:	232	

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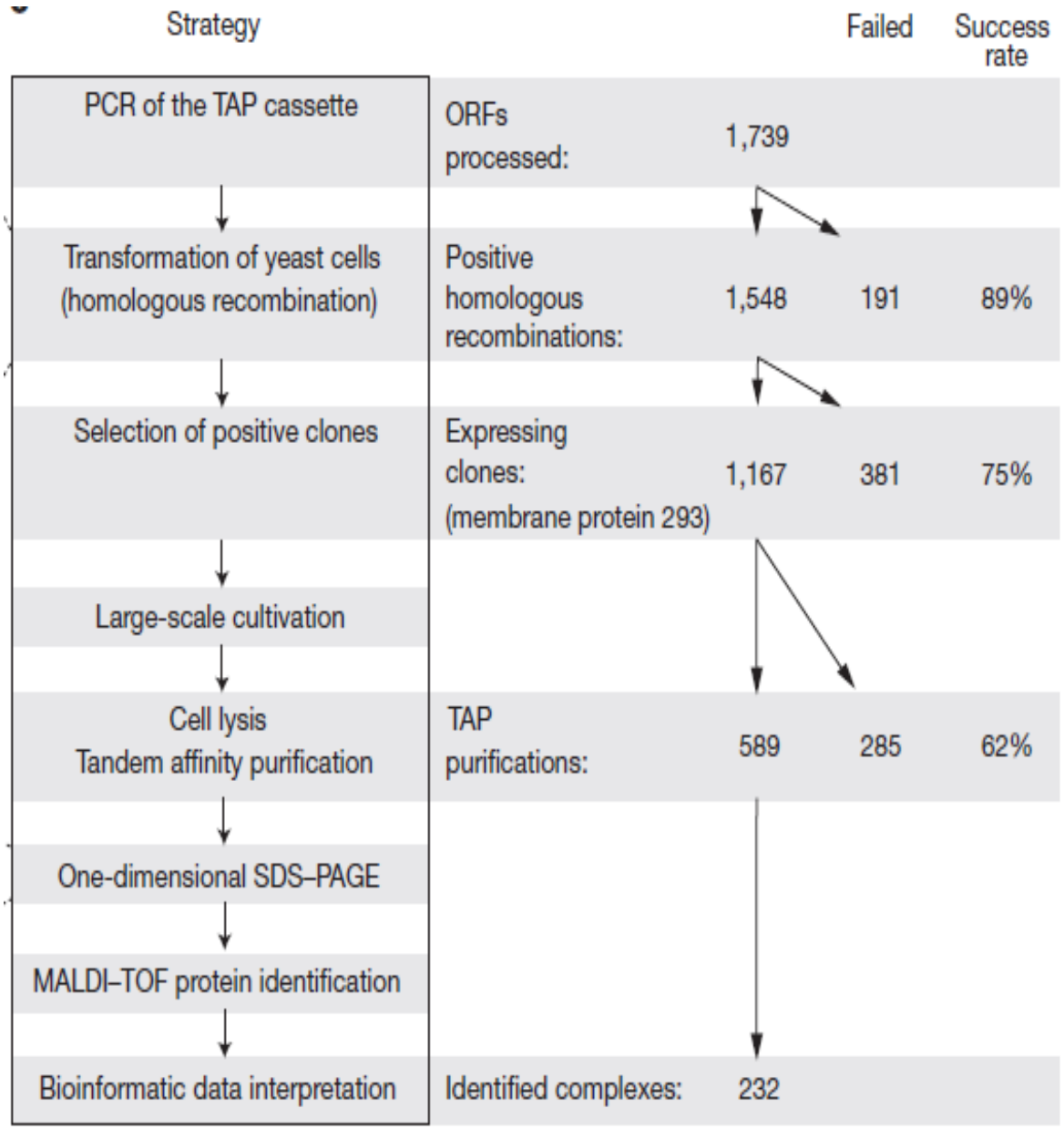

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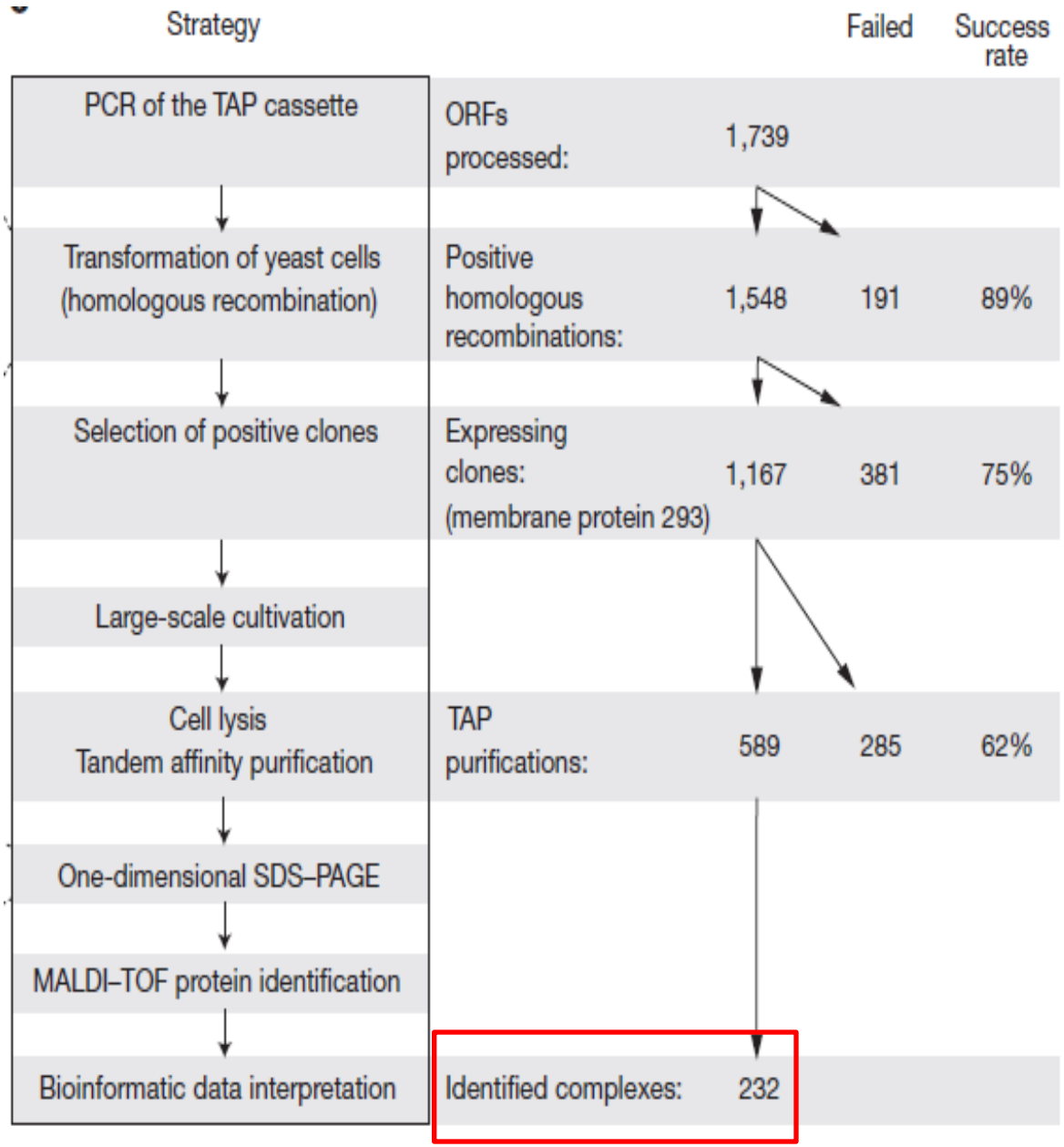
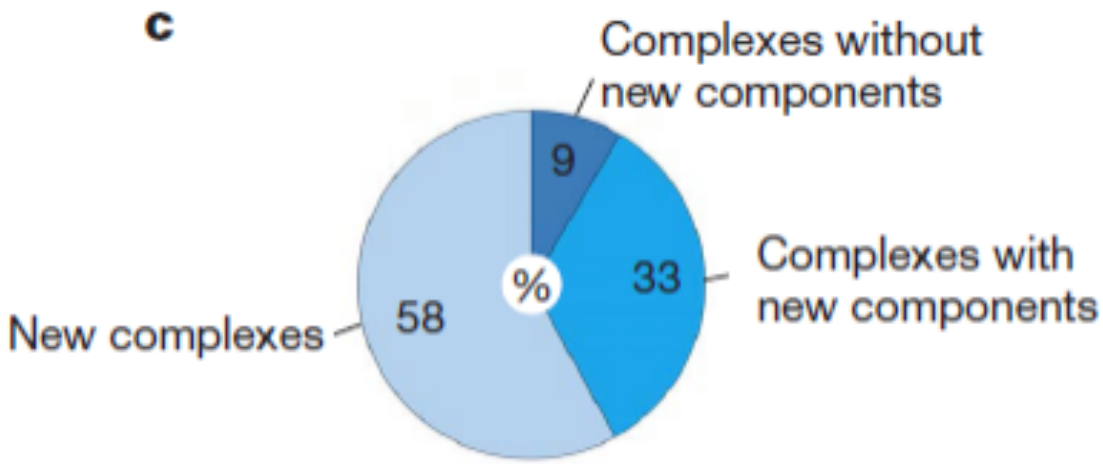
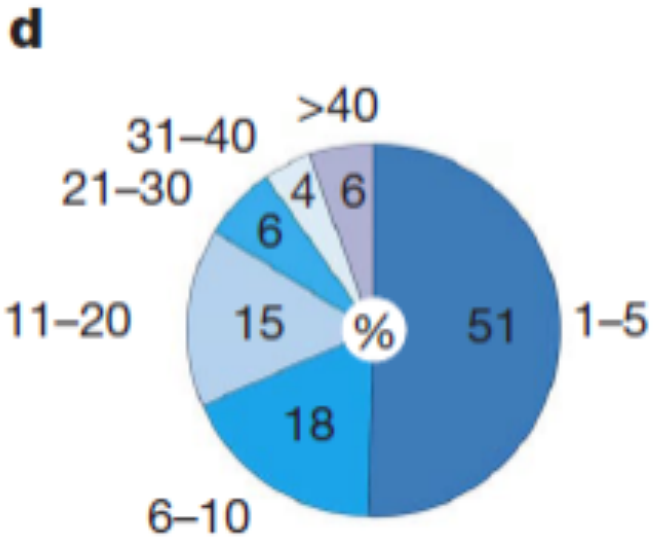


Figure 2: What are in the protein complexes?



Novelties in complexes



Number of proteins per complex

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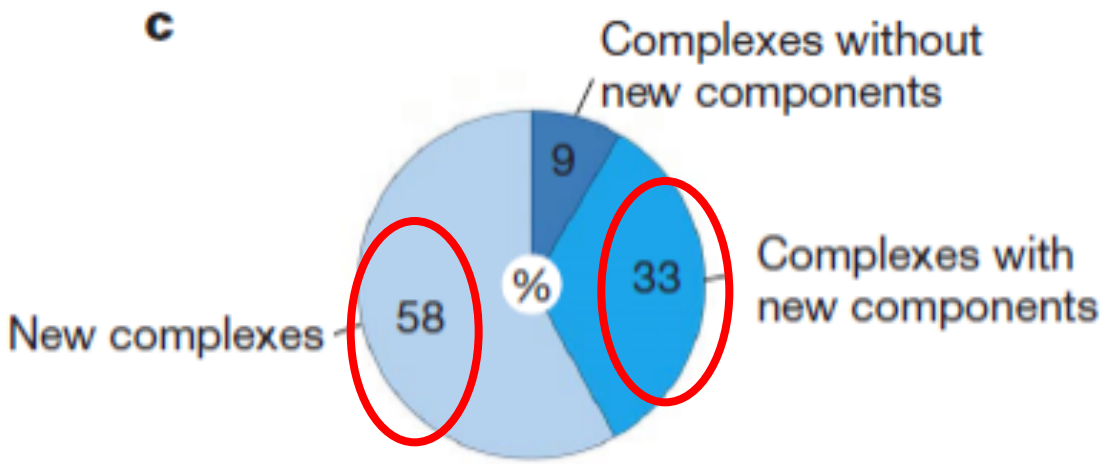

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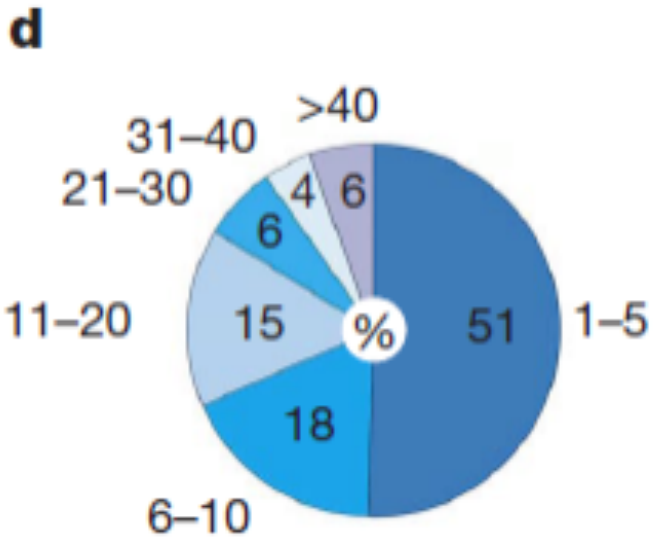
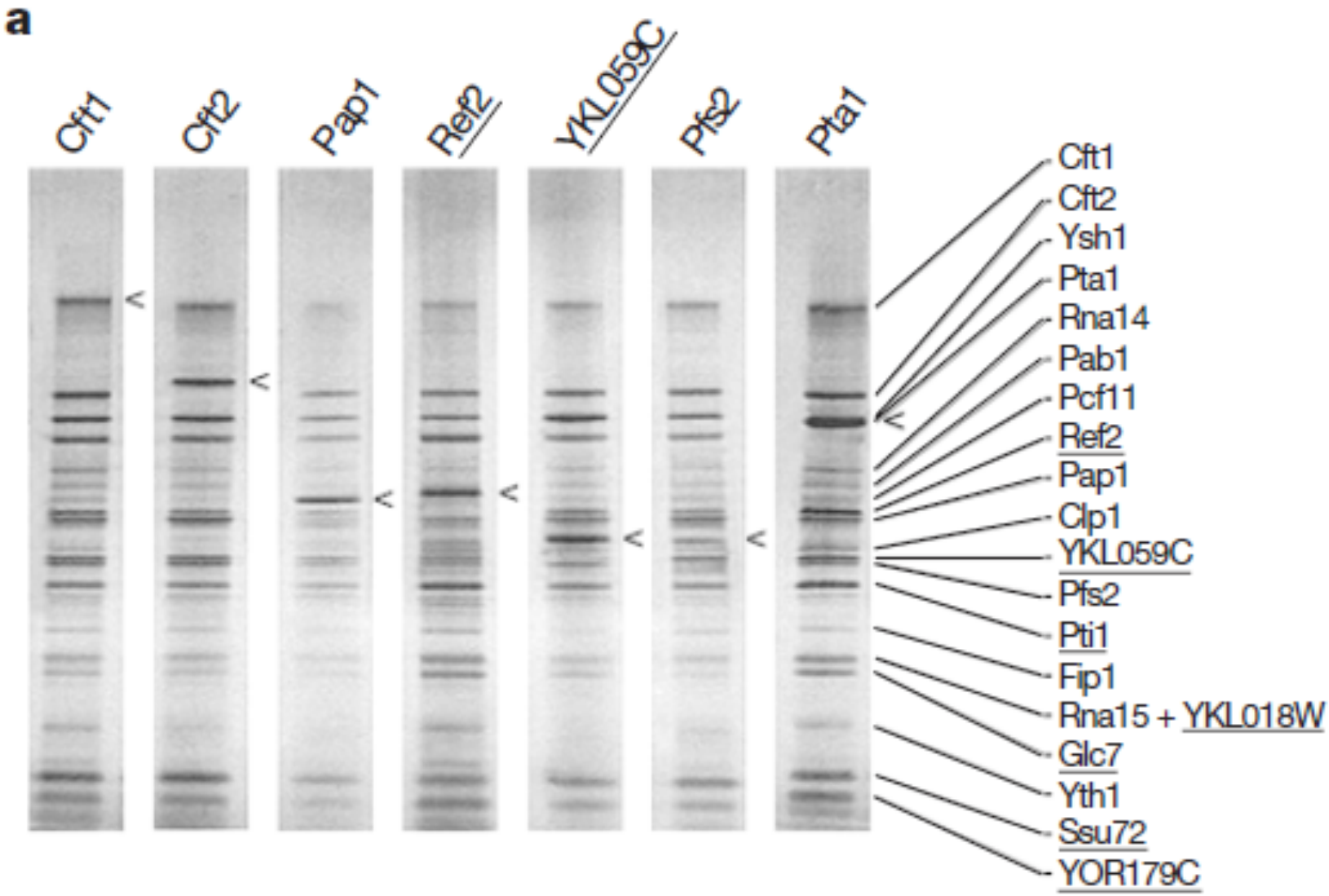


Number of proteins per complex

Figure 3a: What makes up the polyadenylation complex?



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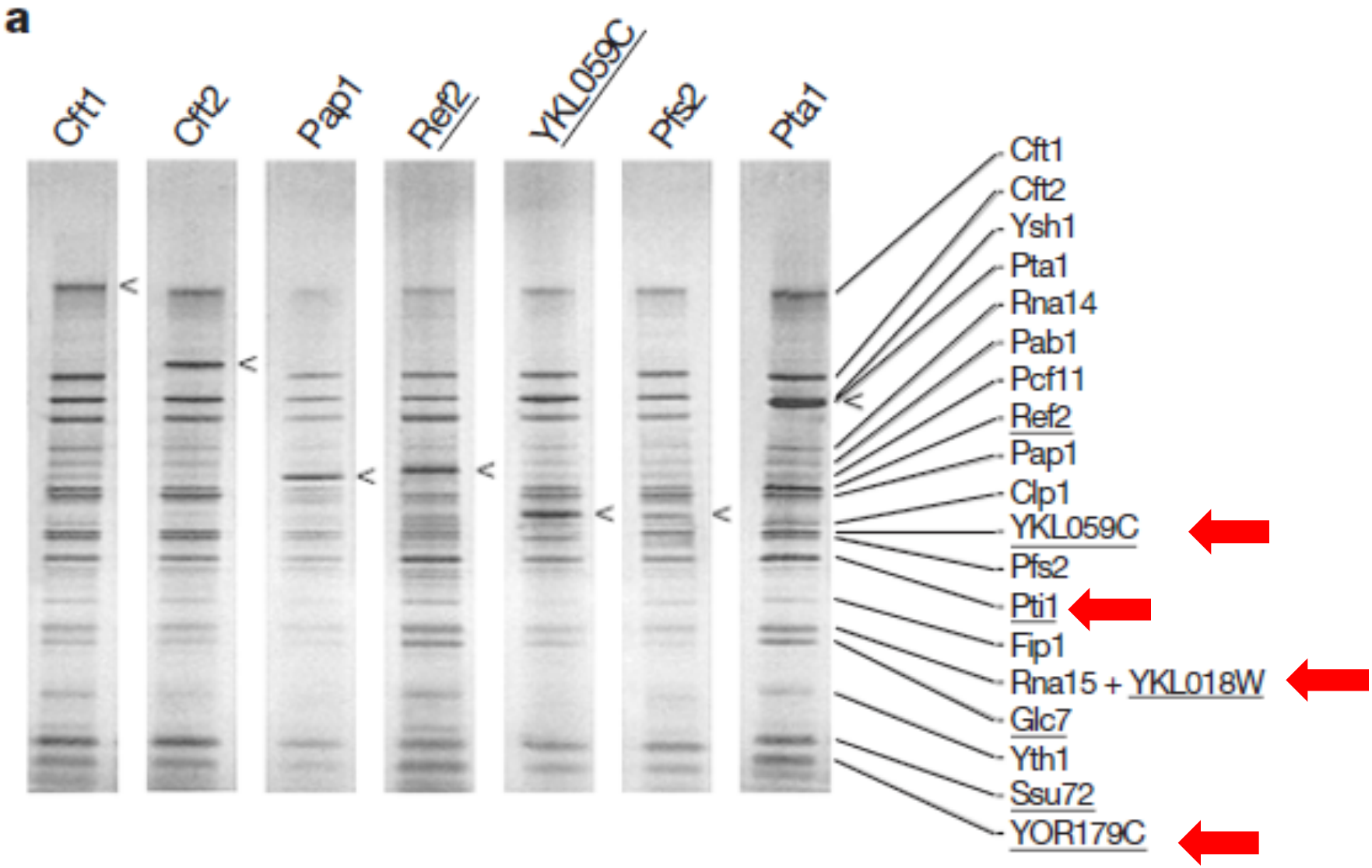


What does the polyadenylation complex look like?

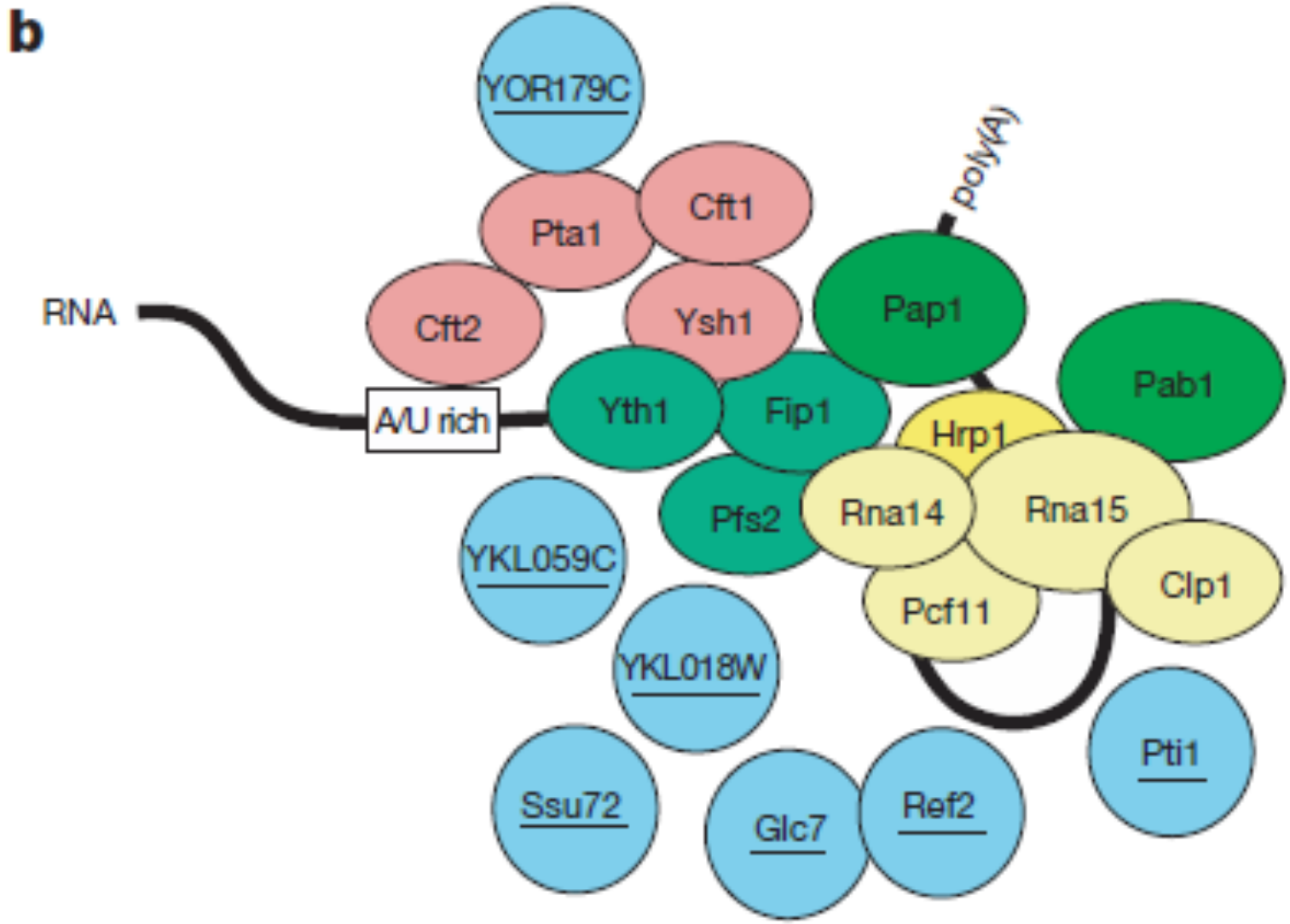
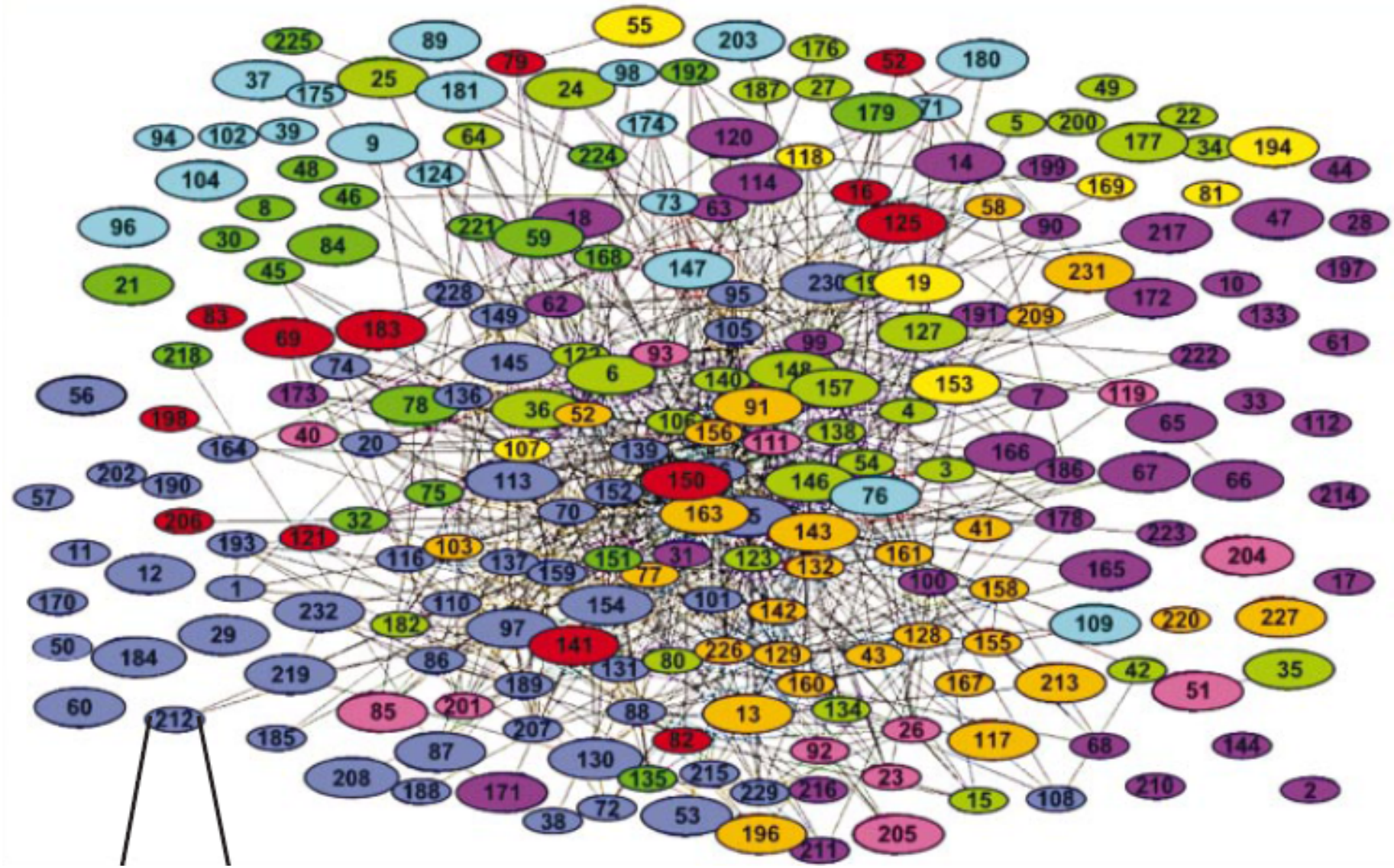


Figure 4: How are 232 complexes sorted?



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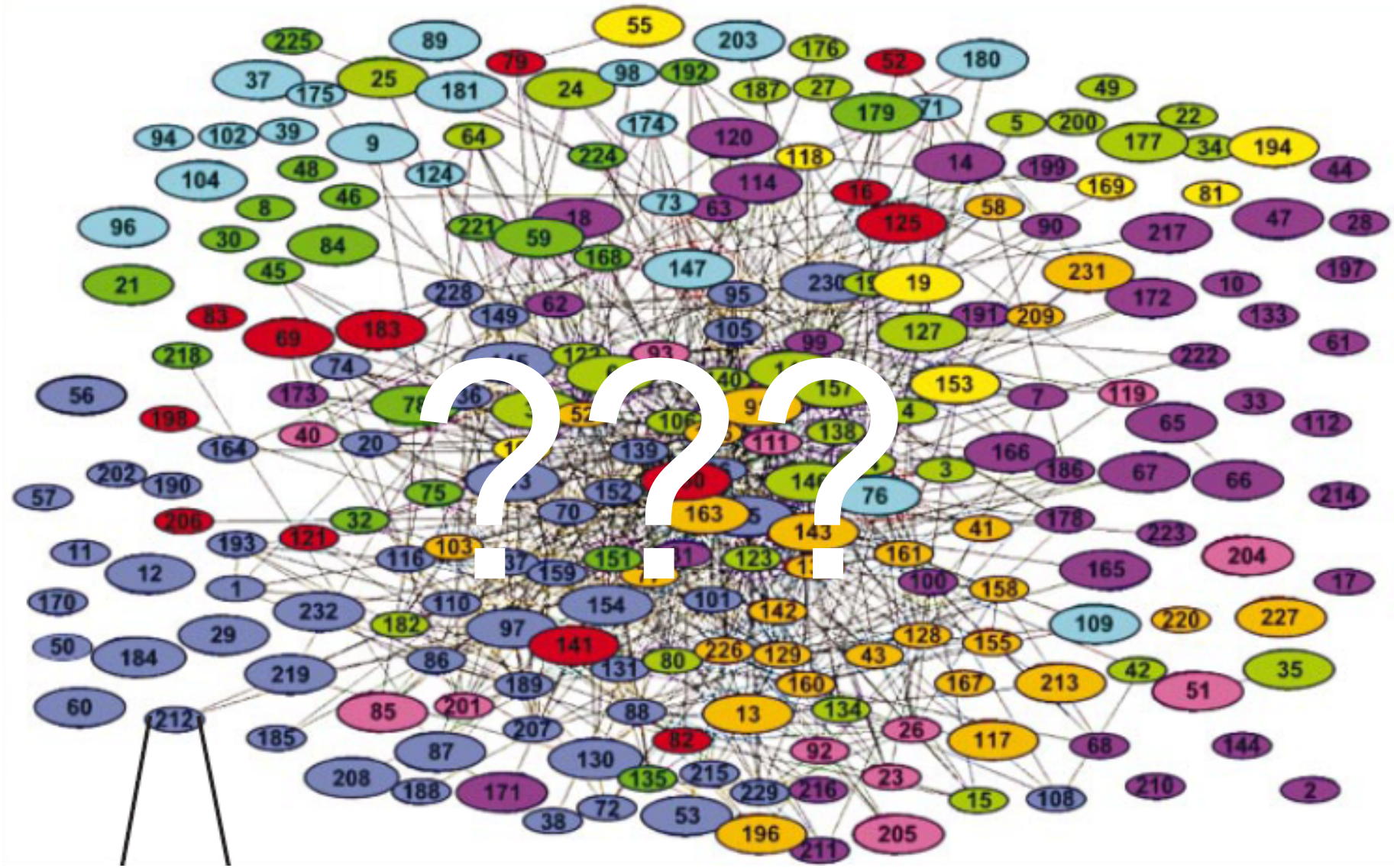


Figure 4: How are 232 complexes sorted?

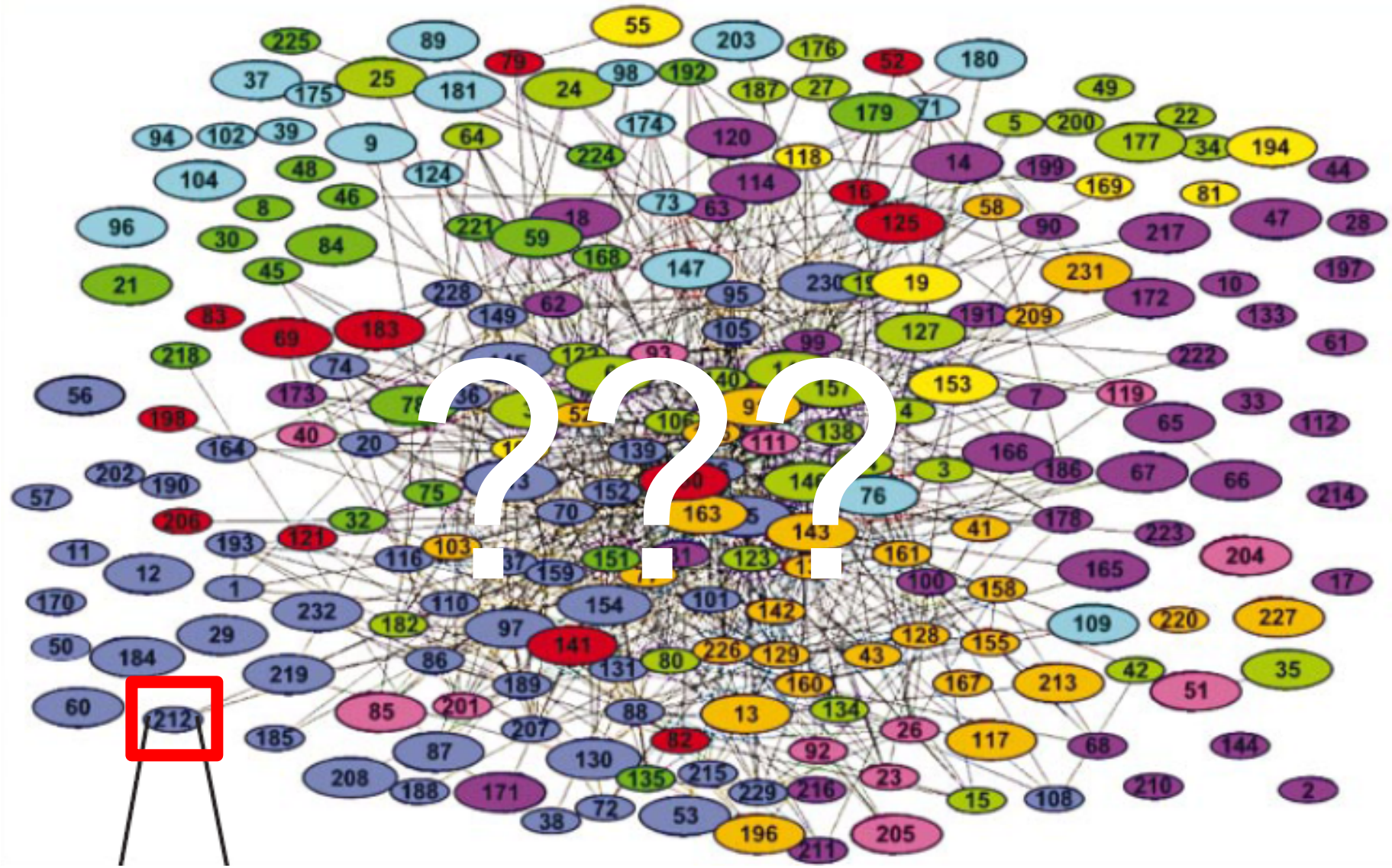
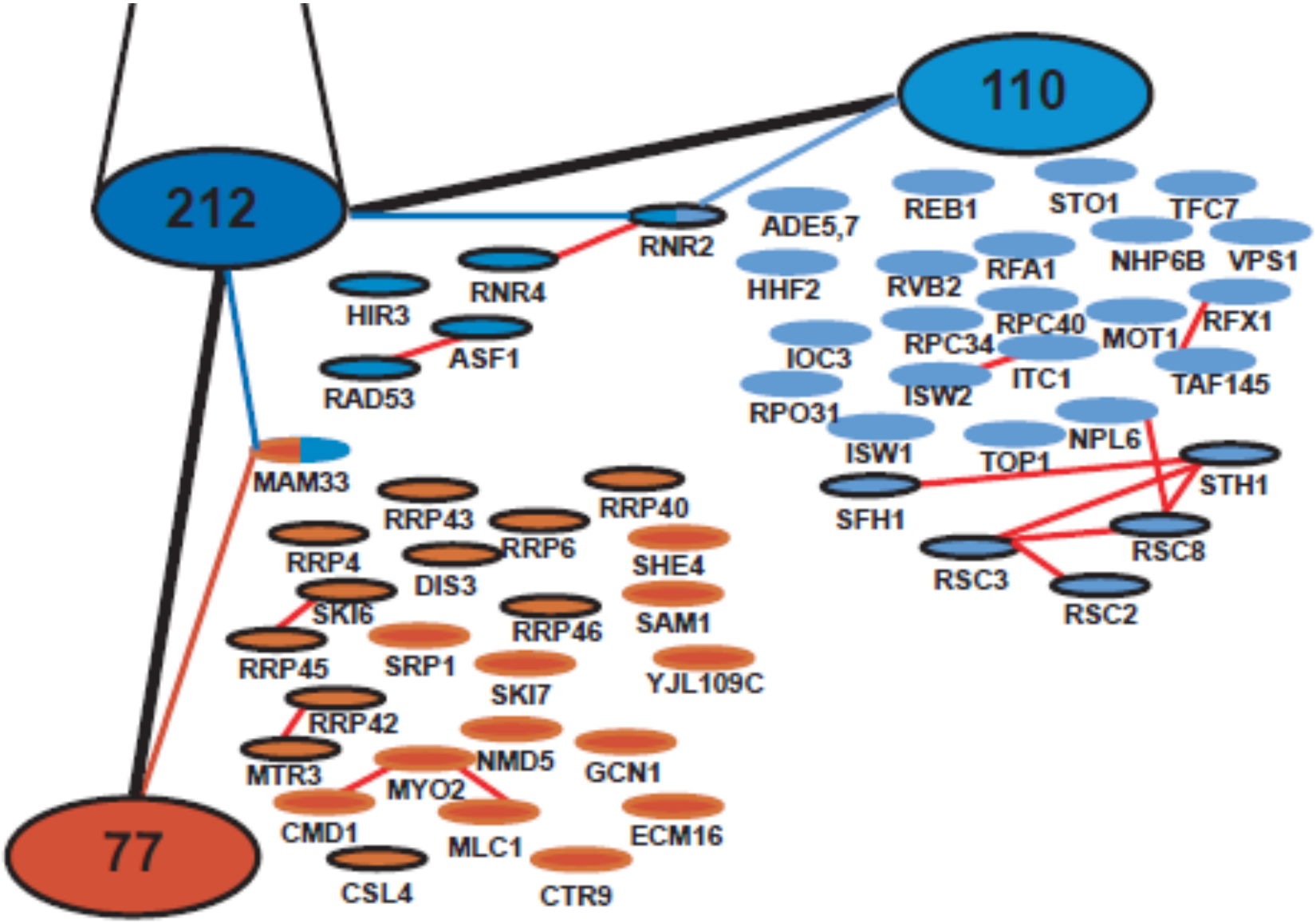
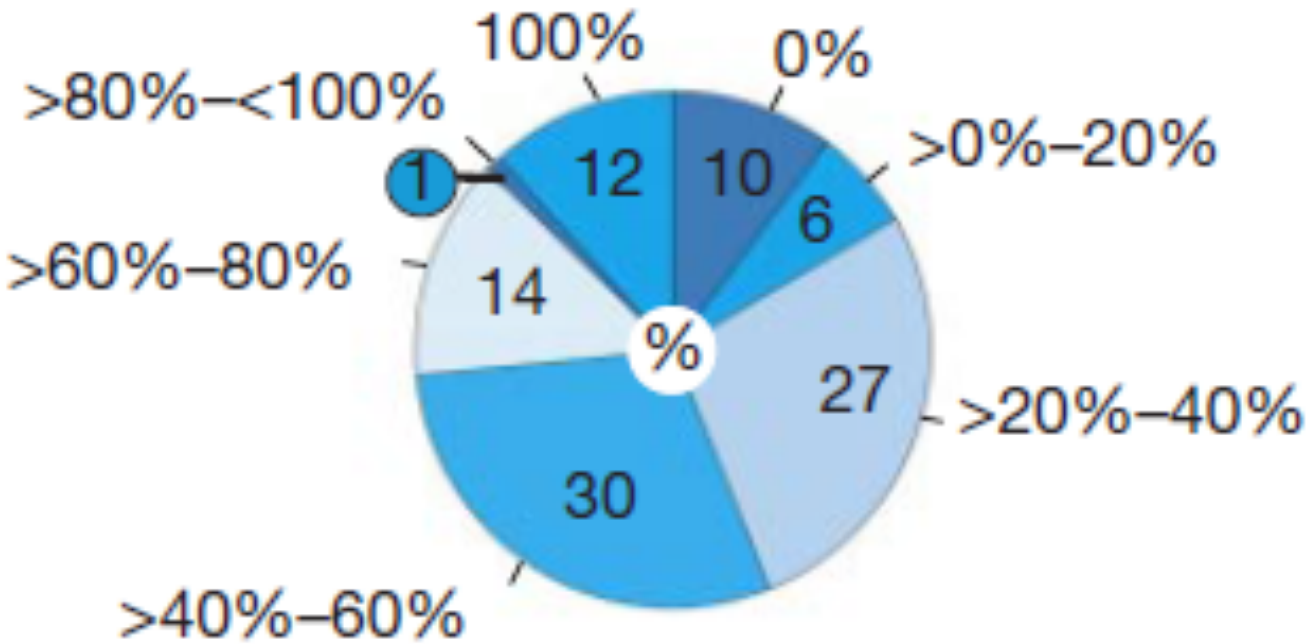


Figure 4:How do proteins and complexes interact?



How did they apply TAP to human protein complexes?

Figure 2



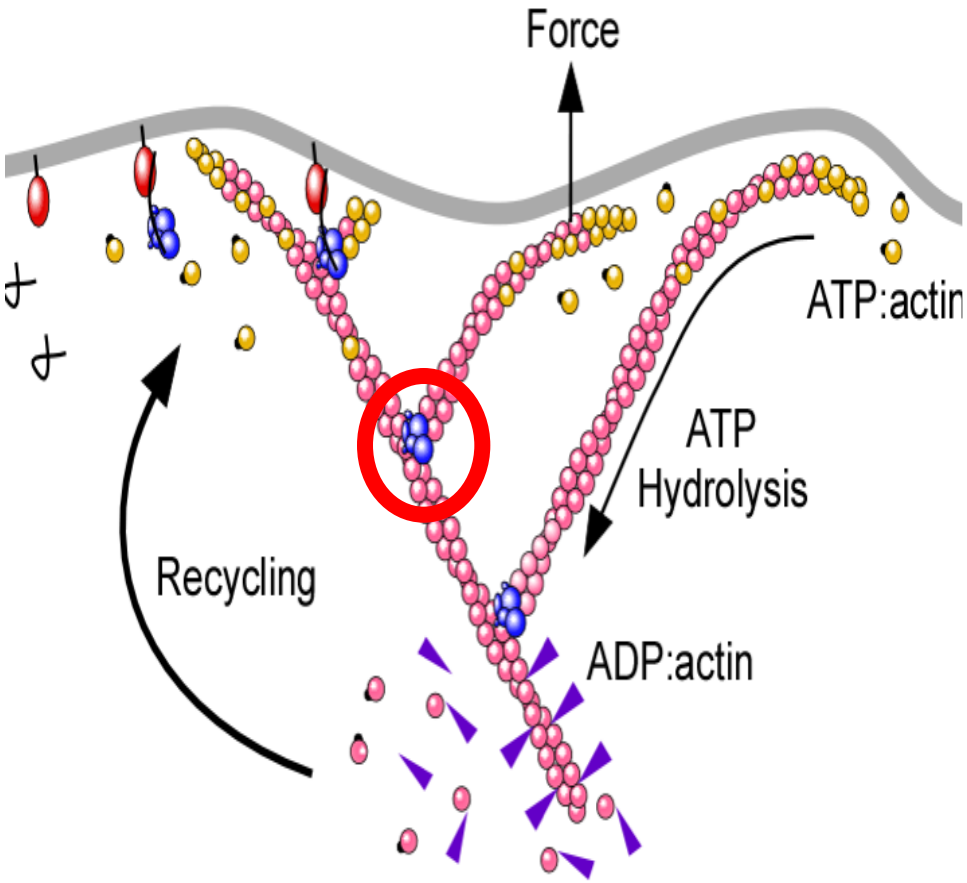
Distribution of orthologues in complexes

Arp2/3

Ccr4-Not

Trapp

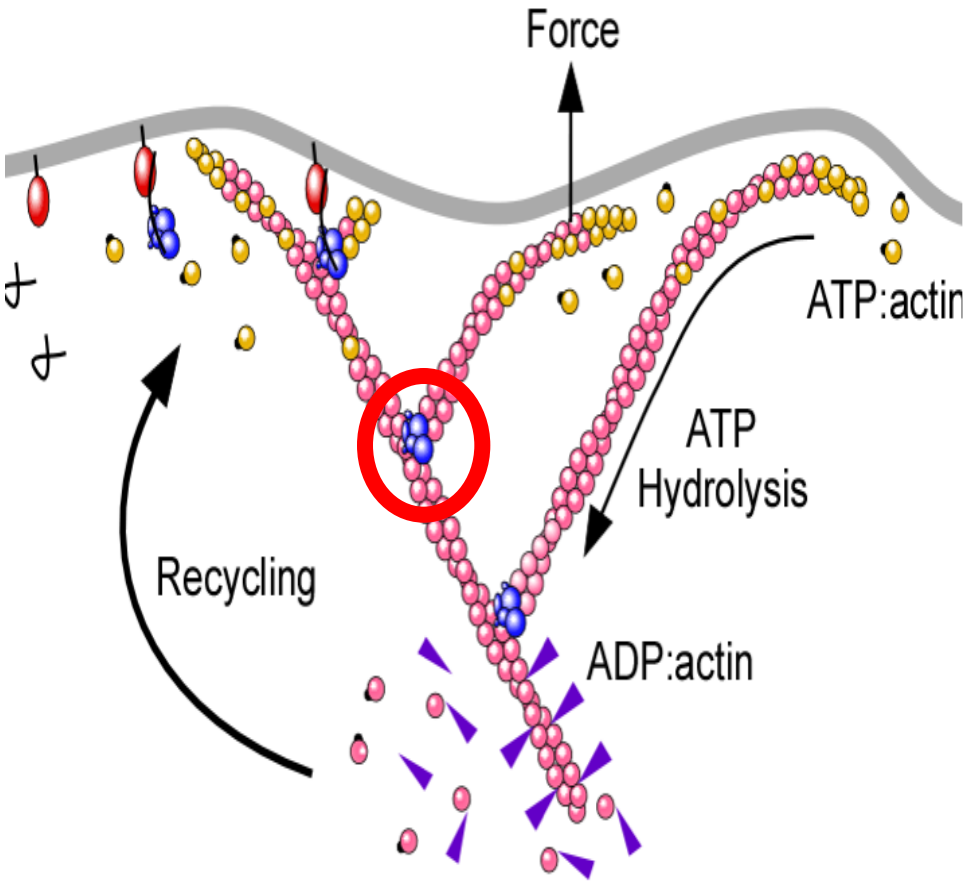
Figure 5a:Can we apply TAP to the Arp2/3 complex?

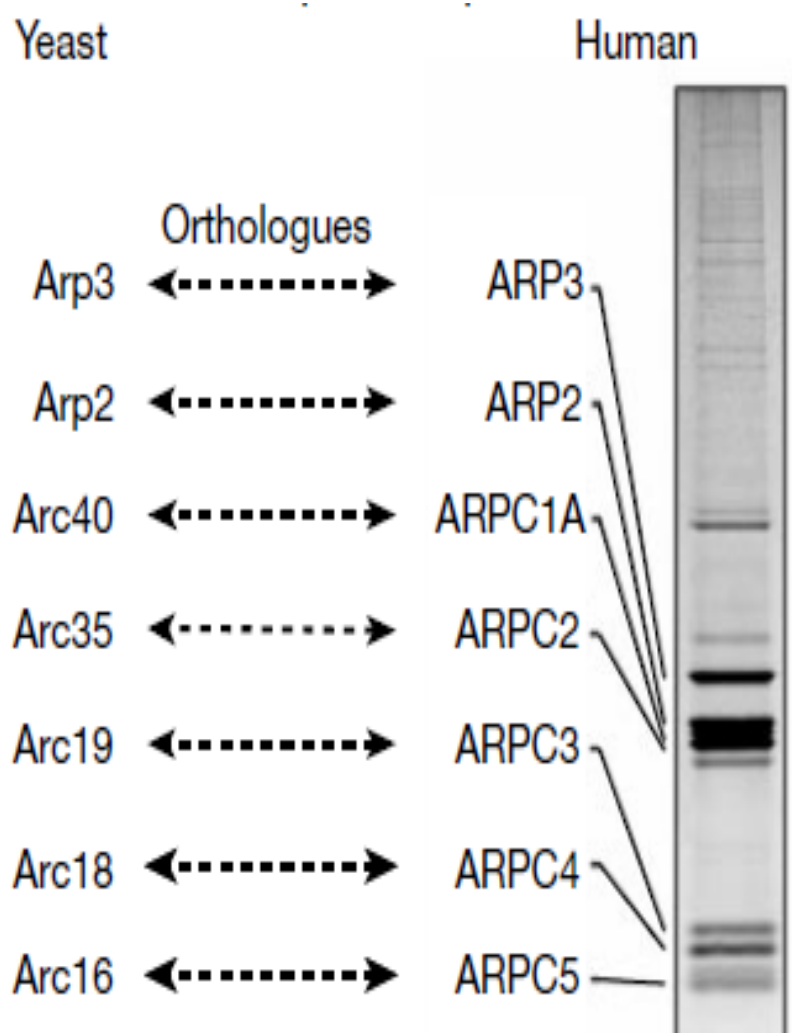


Yeast	Orthologues	Human
Arp3	↔	ARP3
Arp2	↔	ARP2
Arc40	↔	ARPC1A
Arc35	↔	ARPC2
Arc19	↔	ARPC3
Arc18	↔	ARPC4
Arc16	↔	ARPC5

http://www.dayel.com/research/arp23-complex/

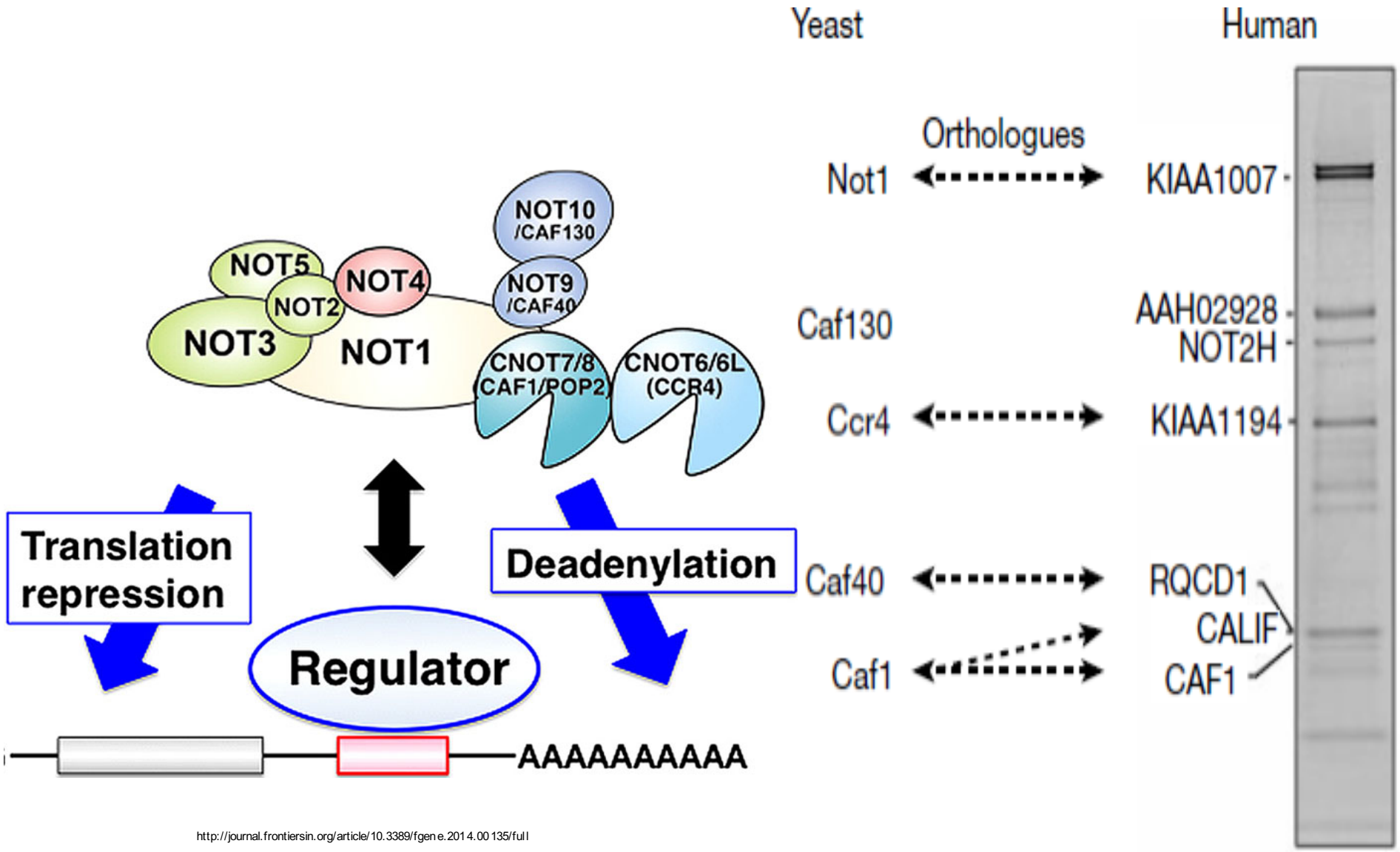
Figure 5a:Can we apply TAP to the Arp2/3 complex?





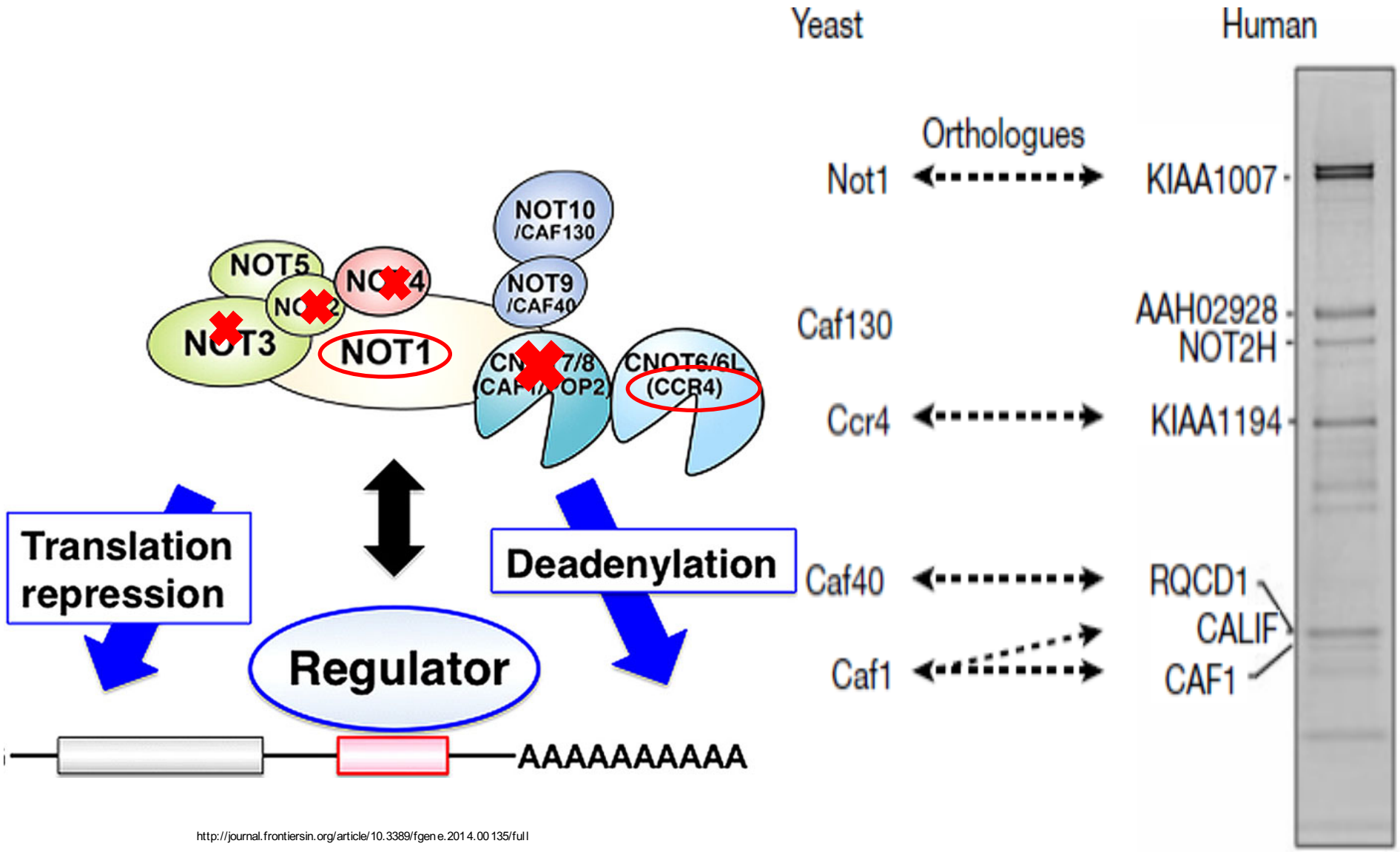
http://www.dayel.com/research/arp23-complex/

Figure 5b:Can we apply TAP to the Ccr4-Not complex?



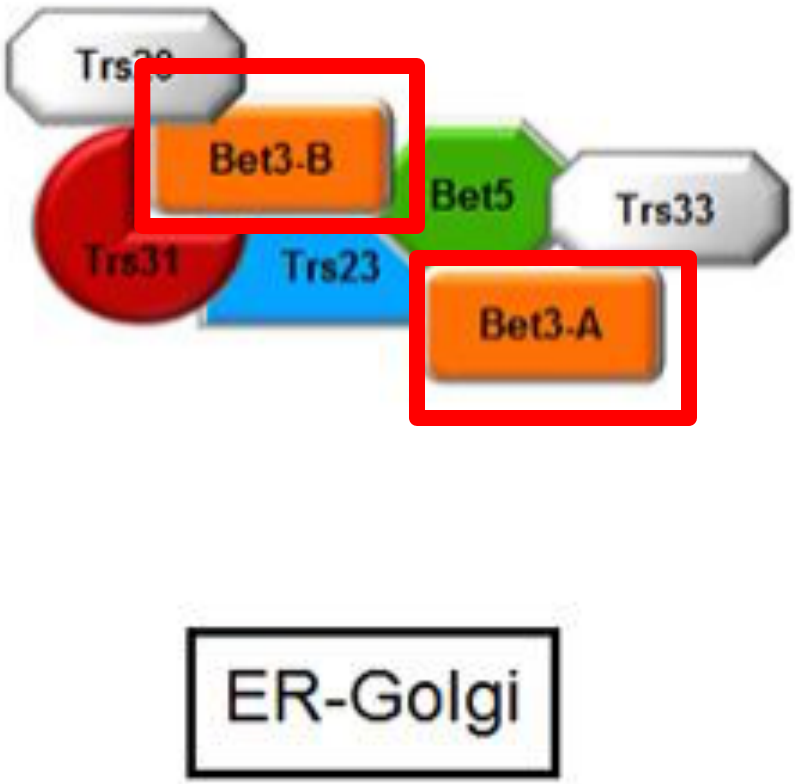
http://journal.frontiersin.org/article/10.3389/fgene.2014.00135/full

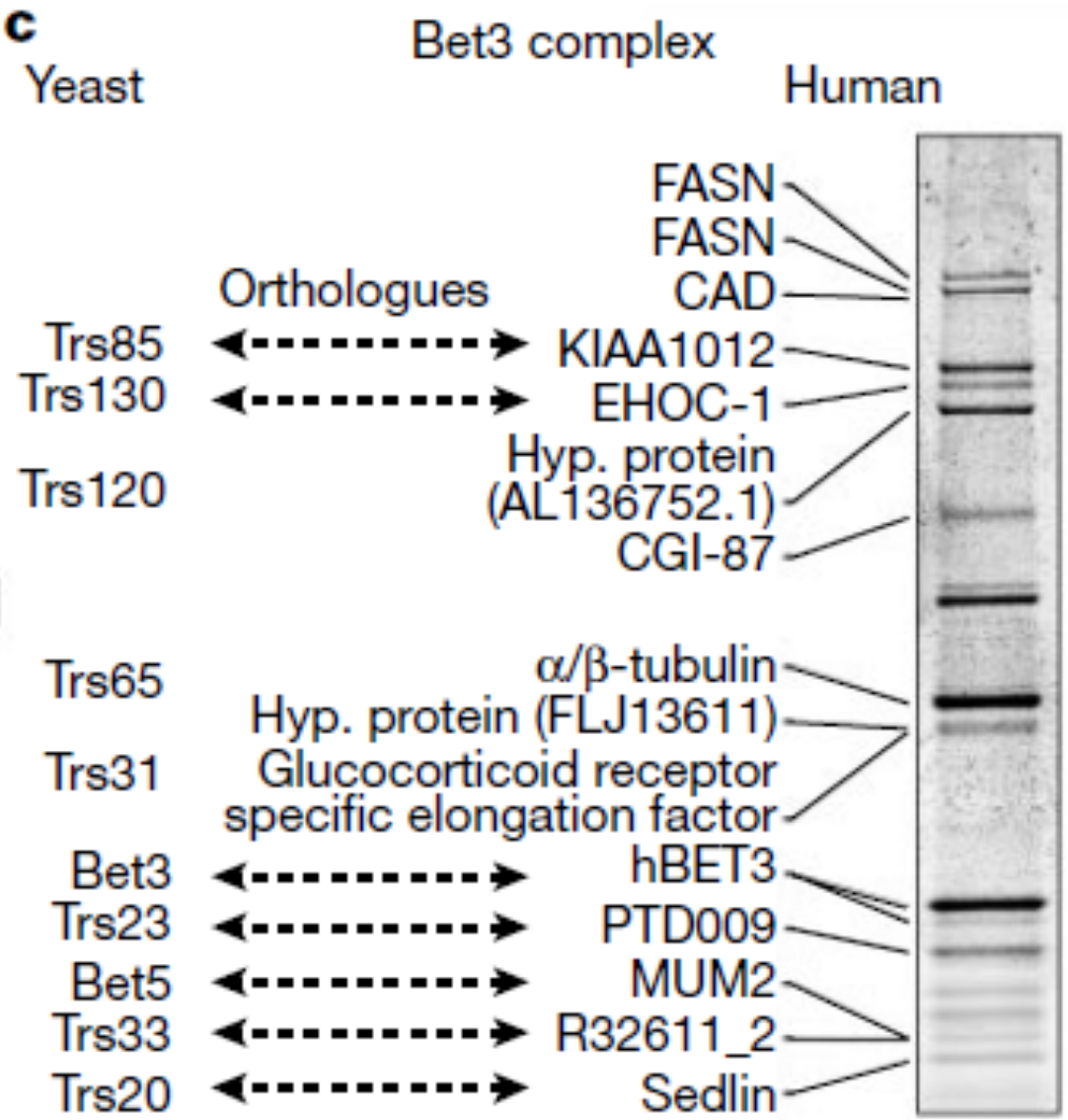
Figure 5b:Can we apply TAP to the Ccr4-Not complex?



http://journal.frontiersin.org/article/10.3389/fgene.2014.00135/full

Figure 5c:Can we apply TAP to the TRAPP complex?





http://www.hhmi.org/research/vesicle-traffic-and-organelle-inheritance

Conclusions

Affinity purification as useful tool in protein network discovery

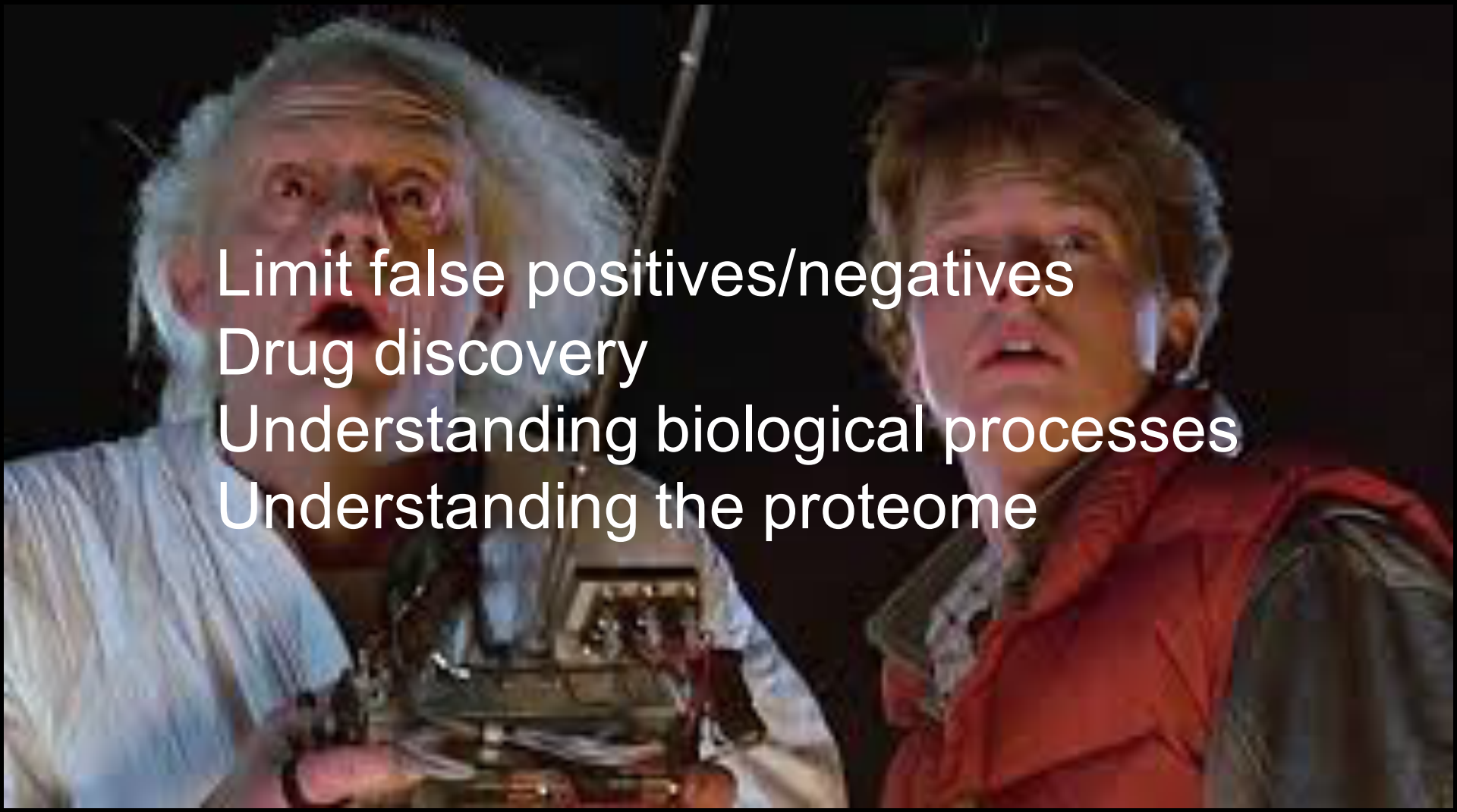
TAP tag as way to reduces problems normally associated with high-throughput studies

TAP tag has ability to be used in combination of other approaches

Future Directions

http://moviepilot.com/posts/2840622

Future Directions



Limit false positives/negatives
Drug discovery
Understanding biological processes
Understanding the proteome

http://moviepilot.com/posts/2840622

Questions?

http://www.thekonagallery.com/products/dancing-squirrel