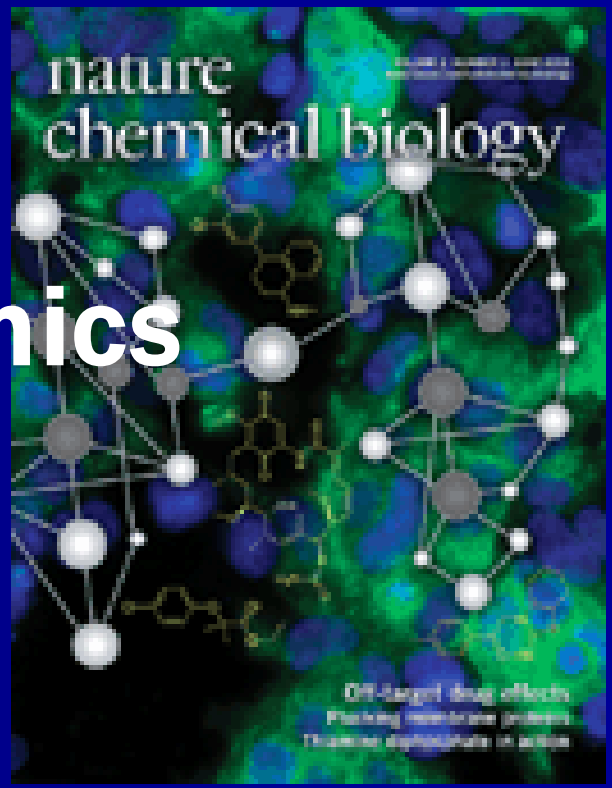
Chemical Biology

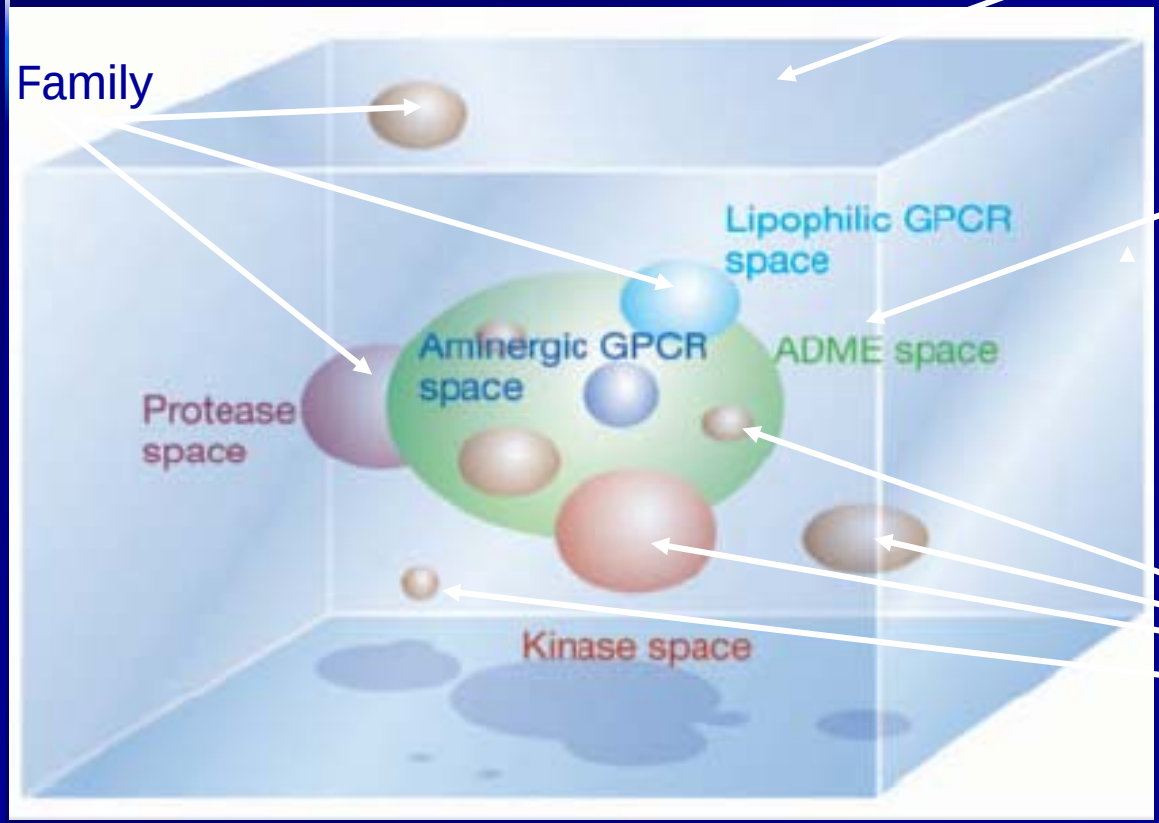
Chemical *versus* Biological Space

&

Chemical Genomics



The Biologically Relevant Chemical Space



Compounds that bind to certain "target classes" (proteins from the same family such as G-protein-coupled receptors, cluster together in specific regions of the chemical space

Navigating the Chemical Space for Biology and Medicine

Tools and Examples

The Field of Chemical Genomics

- Systematic exploration of the interactions between small molecules and biological systems

- Discovery and elucidation of novel targets and mechanisms of action

Forward and Reverse Chemical Genomics

Reverse
(target based screen)

Target (e.g. protein)

↓ Small Molecules Interacting with target

Phenotype

Commonly used

Forward
(phenotype based screen)

Phenotype

↓ Small Molecules Causing Phenotype

Target (e.g. protein)

More recent

Four Aspects of Chemical Genomics

1. Creation of a Chemically diverse libraries of compounds

2. Screening, or the identification of compound affecting a biological process of interest

3. The discovery of the protein targets of the active compounds

4. TO biology- target function and network discovery

Creation of a Chemically Diverse Libraries of Compounds

- Various chemical libraries are available but are focused on "drug-like" compounds

- They only represent a relatively narrow region of the chemical structure space

-Unlikely to provide useful probes for all biological targets of interest

Creation of a Chemically Diverse Libraries of Compounds

DIVERSITY-ORIENTED SYNTHESIS (DOS)

Valuable approach to generate libraries that contain structures representing different regions of the chemical space

Creation of a Chemically Diverse Libraries of Compounds

- DOS is largely based on synthetic technologies:

1. Separation platforms:
facilitate separation of synthetic intermediates from excess reagents and reaction products

2. Combinatorial synthesis

Separation Platforms- Using SOLID SUPPORT

- Starting material attached to an insoluble solid support by a linker (e.g. a bead)

- The linker can be cleaved under specific reactions

- Bound substrates are exposed to solutions containing reagents and building blocks

- Stoichiometric excesses can be used to drive reactions to completion

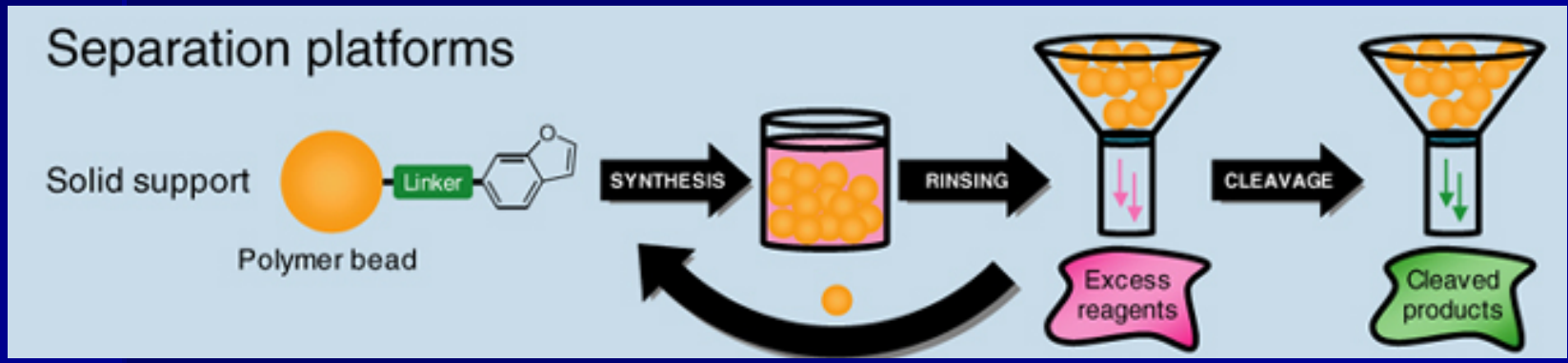
Separation Platforms-
SOLID SUPPORT

- Excess reagents and reaction byproducts removed by rinsing the solid support with various solvents

- The cycle is repeated until the end of the synthesis

- Screening directly on bead or cleavage & stock making

Separation Platforms-
SOLID SUPPORT



Separation Platforms- Precipitation Tag

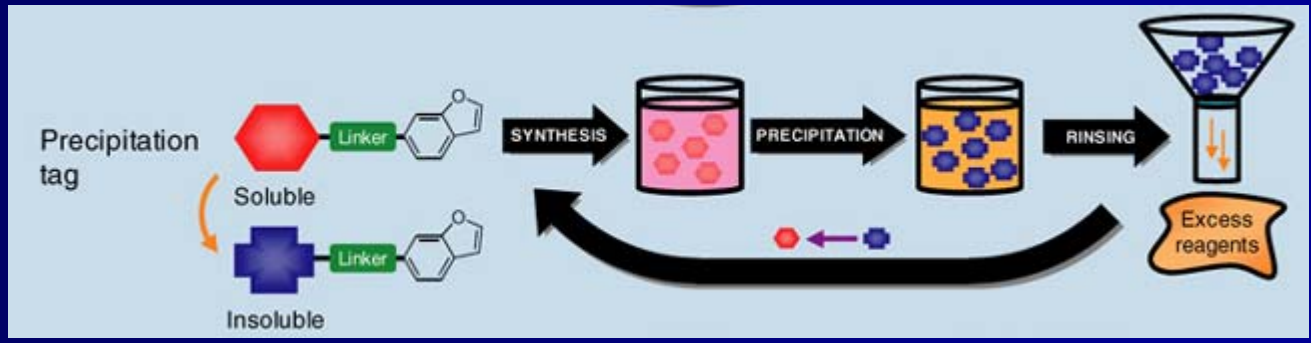
- Solid supports are heterogeneous and interfere with reaction kinetics

- Precipitation Tag- soluble under most reaction conditions and therefore reaction conditions are homogenous

Separation Platforms- Precipitation Tag

- After the synthesis a solvent or reagent induces precipitation of the tag and the attached substrate

- Excess reagents washed away and the tag re-solubilized for further synthesis



Combinatorial Synthesis

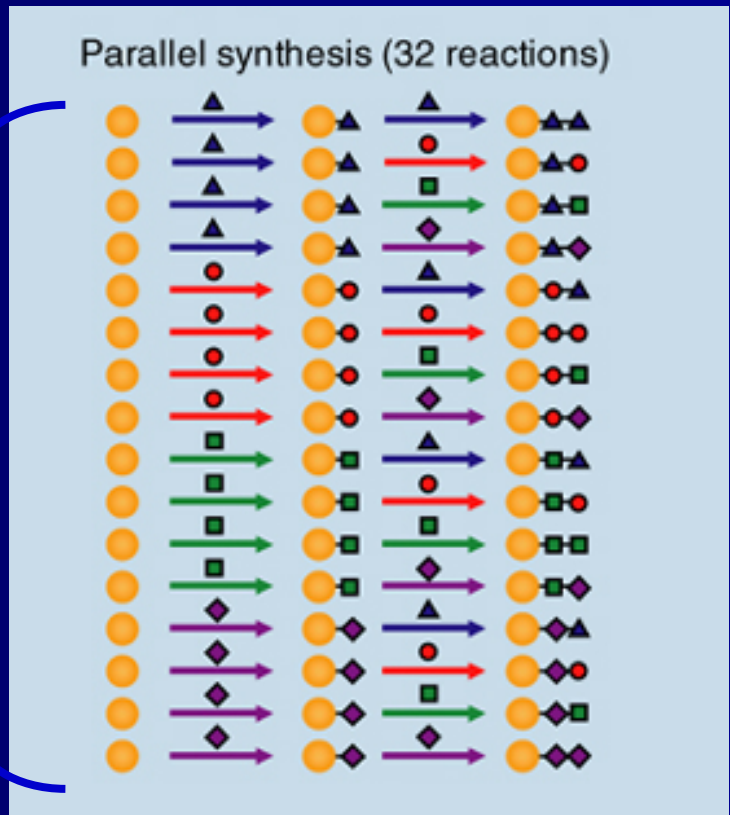
- The separation techniques described previously allow parallel processing of different synthetic reactions

- They are suitable for generating combinatorial libraries, each one with hundreds or thousands of members

Combinatorial Synthesis
Parallel synthesis

Takes an "Afternoon" (32 reactions =16 compounds)

Individual reactions

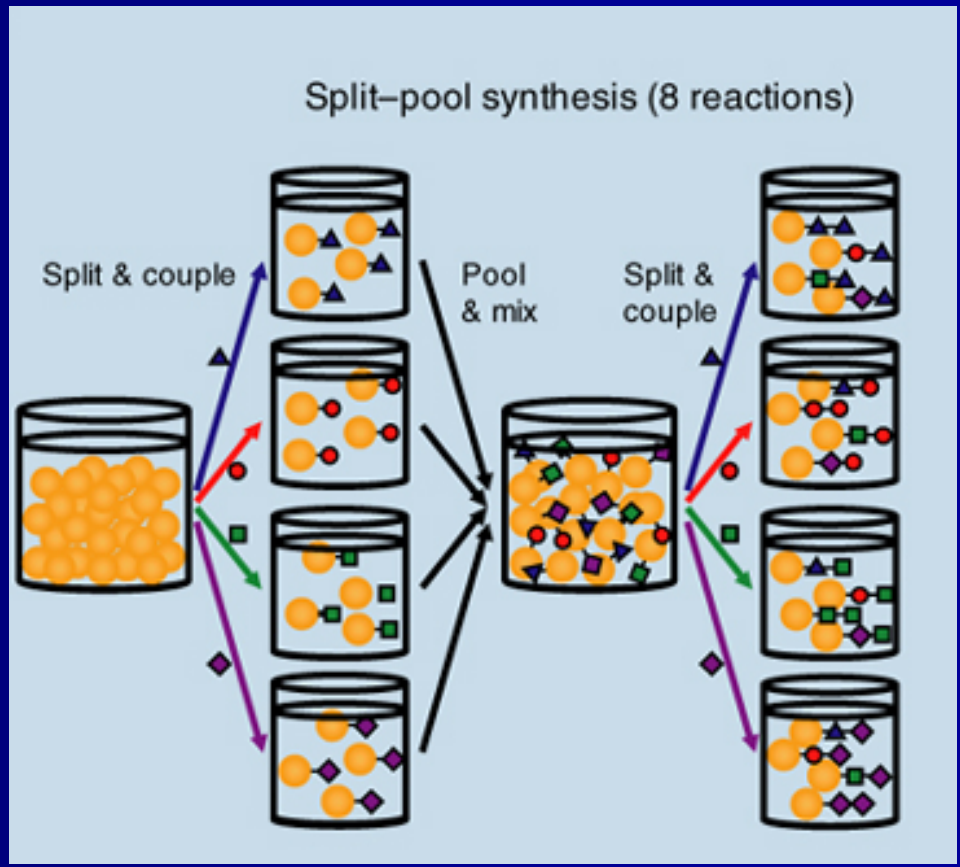


Combinatorial Synthesis
Split-pool synthesis

- Solid supports are combined, mixed and redistributed between synthetic steps

- 16 member family with 8 reactions (more economical)

- Since it's a mix: Deconvolution through re-synthesis and screening of progressively smaller libraries or identity determination



How can DOS Libraries made that Target the Biologically Relevant Regions of the Chemical Space ?

Most libraries are:

A. Based on synthetic drugs (made by medicinal chemists)

B. Natural products from microbes, plants or marine organisms

Based on Synthetic Drugs (made by medicinal chemists)

- Often based on nitrogen-containing heteroaromatic scaffolds

- Few or no stereogenic centers- more simple synthesis

- Some of the scaffolds are so called "priveleged" since they bind multiple classes of protein targets

Natural Products from Microbes, Plants or Marine Organisms

- Much greater natural diversity than drugs

- A greater proportion of oxygen than nitrogen heteroatoms

- Significant amount of stereogenic centers

Natural Products from Microbes, Plants or Marine Organisms

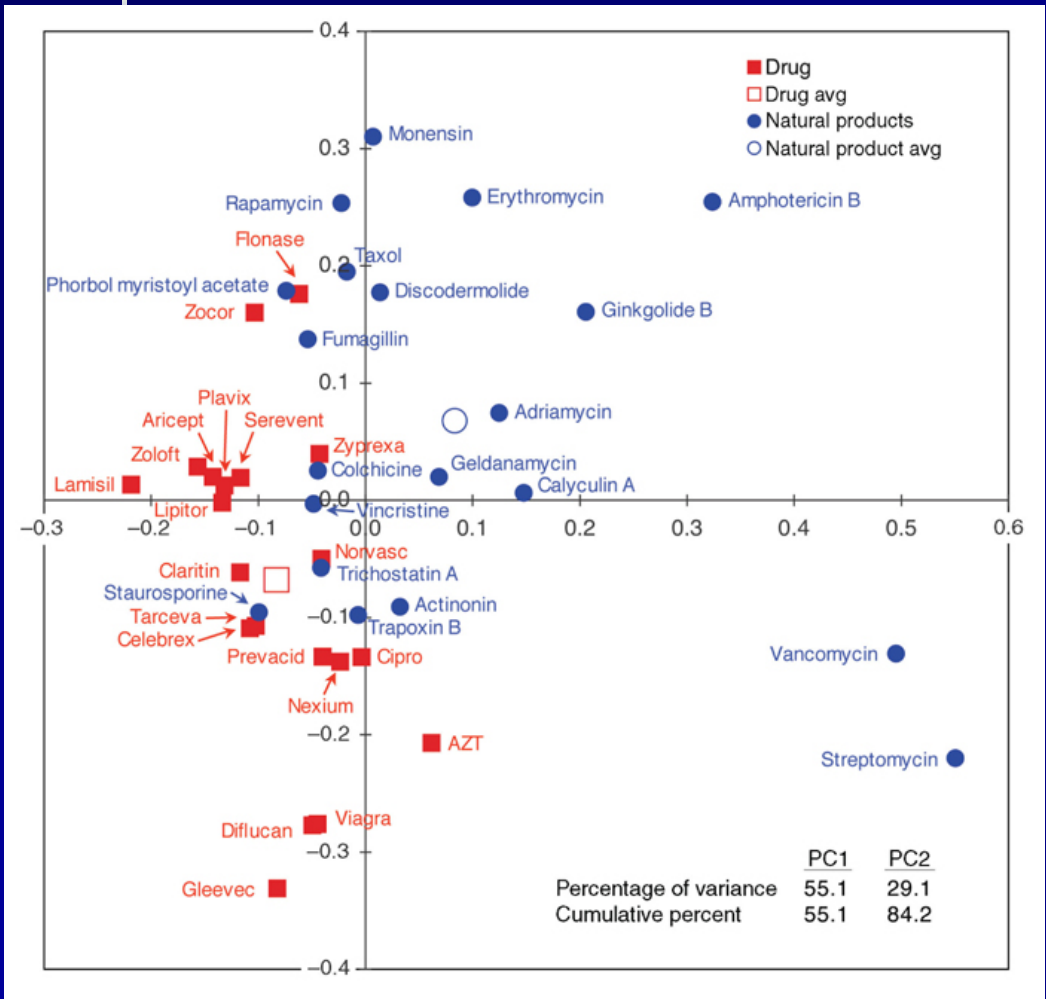
- Library design strategies:

a. Libraries based on the core scaffold of an individual natural product

b. Libraries based on the specific structural motifs that are found across a class of structural motifs

c. Libraries that imitate the structural characteristics of natural products in a general sense

How can DOS Libraries made that Target the Biologically Relevant Regions of the Chemical Space ?



20 synthetic drugs- 2004 best
20 natural products

9 molecular descriptors:

Molecular weight
Hydrophobicity
Hydrogen-bond donors
Hydrogen bond acceptors
Rotatable bonds
Topological polar surface area
Stereogenic centers
Nitrogen atoms
Oxygen atoms

Identification of genes downstream of TOR by Chemical Genomics

A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR)

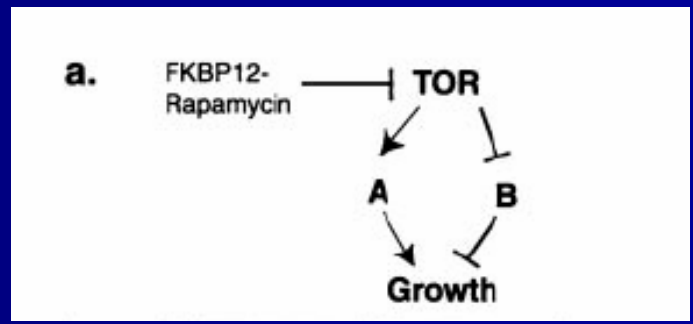
Ting-Fung et al., PNAS, November 2000

Genetic interaction between TOR and other components in the Rapamycin Sensitive Pathway

The Target of Rapamycin protein (TOR)

- A kinase
- Integrates nutrient signals
- Regulates cell growth proliferation and metabolism

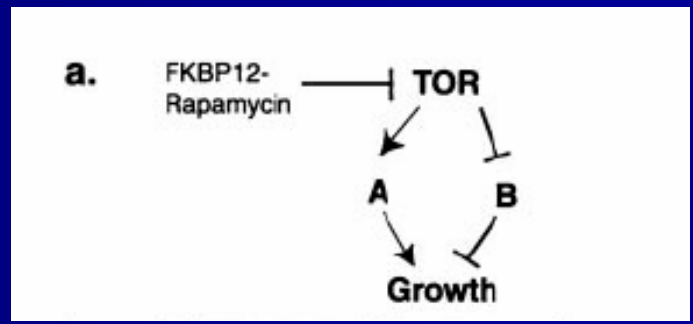
TOR is inhibited when Rampamycin is bound to the receptor FKBP12



Genetic interaction between TOR and other components in the Rapamycin sensitive pathways

Deletion of an inhibitor in TOR signaling or a gene in a cellular process negatively regulated by TOR will provide partial rapamycin resistance

Deletion of an enhancer in TOR signaling or a gene in a cellular process positively regulated by TOR will provide Rapamycin hypersensitivity



Assay for TOR Downstream Factors by Measuring Cell Growth in Yeast

- Non-essential genes (in yeast) can be tested with Haploid or Homozygous Diploid deletion strains
- Essential genes can be tested with Heterozygous Diploid deletion strains
- Rapamycin sensitivity measured by comparison to WT, and TOR mutants (dominant resistance and sensitive), in the absence or presence of Rapamycin

Assay for TOR Downstream Factors by Measuring Cell Growth in Yeast

- 2,216 nonessential haploid deletion strains
- 50 Essential heterozygous diploid deletion strains
- Slow growth mutants were normalized against wild-type

Assay for TOR Downstream Factors by Measuring Cell Growth in Yeast

- Out of 2,216 nonessential haploid deletion strains- 73-RH & 27-RR
- Out of 50 Essential heterozygous diploid deletion strains- 6-RH

Assay for TOR Downstream Factors by Measuring Cell Growth in Yeast

- The effects are not general drug effects since:
- All RH mutants when mutants combined with TOR dominant resistance genotype were no longer hypersensitive
- None of the RH mutants were sensitive to a similar type antibiotic (FK506).

Assay for TOR Downstream Factors by Measuring Cell Growth in Yeast

- Results provide a global view of potential function of TOR.
- Limitations of such a screen:

1. Redundancy- Tor1 & Tor2
2. Rapamycin insensitive functions will not be detected
3. Pleiotropic effects on drug sensitivity by proteins active in Mitochondria

Identification of Small Molecule Targets with Microarrays

Article

Nature Chemical Biology 2, 103-109 (2005)

Microarray-based method for monitoring yeast overexpression strains reveals small-molecule targets in TOR pathway

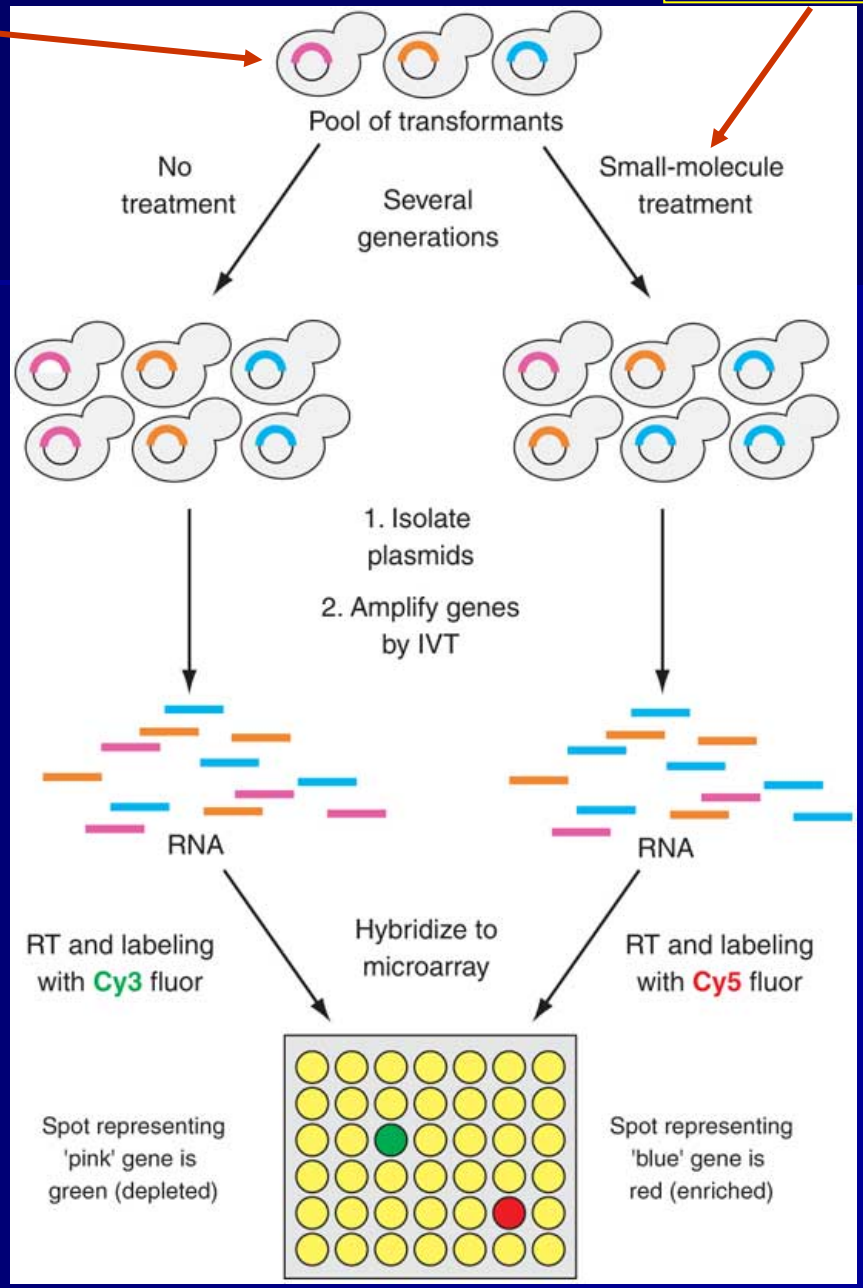
Rebecca A et al., 2006

Overexpression lines

Rampamycin

The Method:

Identification of Targets of Small Molecules with Microarrays



Looking for Rapamycin Targets using this Method

- The current method is assumed to monitor 60% of the yeast genes

- Looking for genes that when over expressed affect sensitivity to Rapamycin and thus may have a genetic interaction with TOR

- Approx. 3990 (out of 6000) overexpressed genes tested

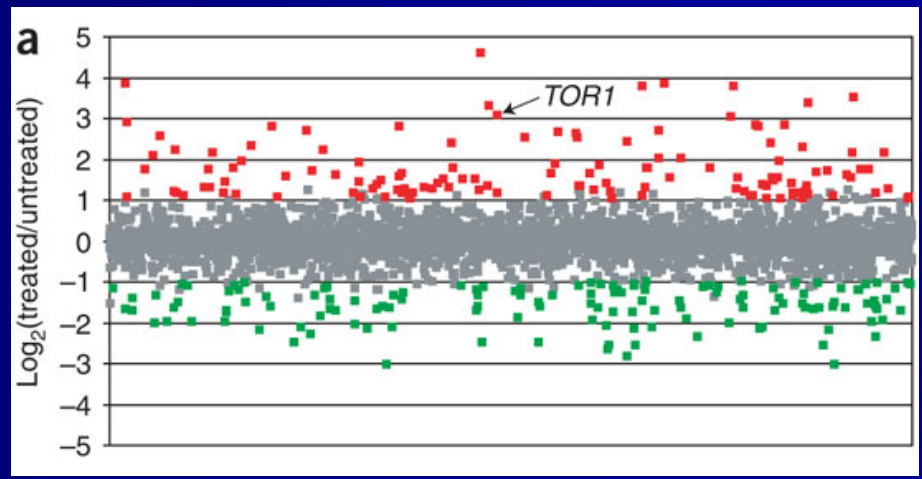
- 130 strains enriched and 134 depleted by more than 2 folds on average

Looking for Rapamycin Targets using this Method

- The TOR1 overexpressing strain is among the top ten enriched strain

- Known TOR pathway genes identified

- Many of the top enriched strains are involved in general drug resistance



Identifying Resistance Mutations by Complementation

- Instead of Overexpressing the genes on a WT mutant background, overexpression conducted on a resistance mutant background

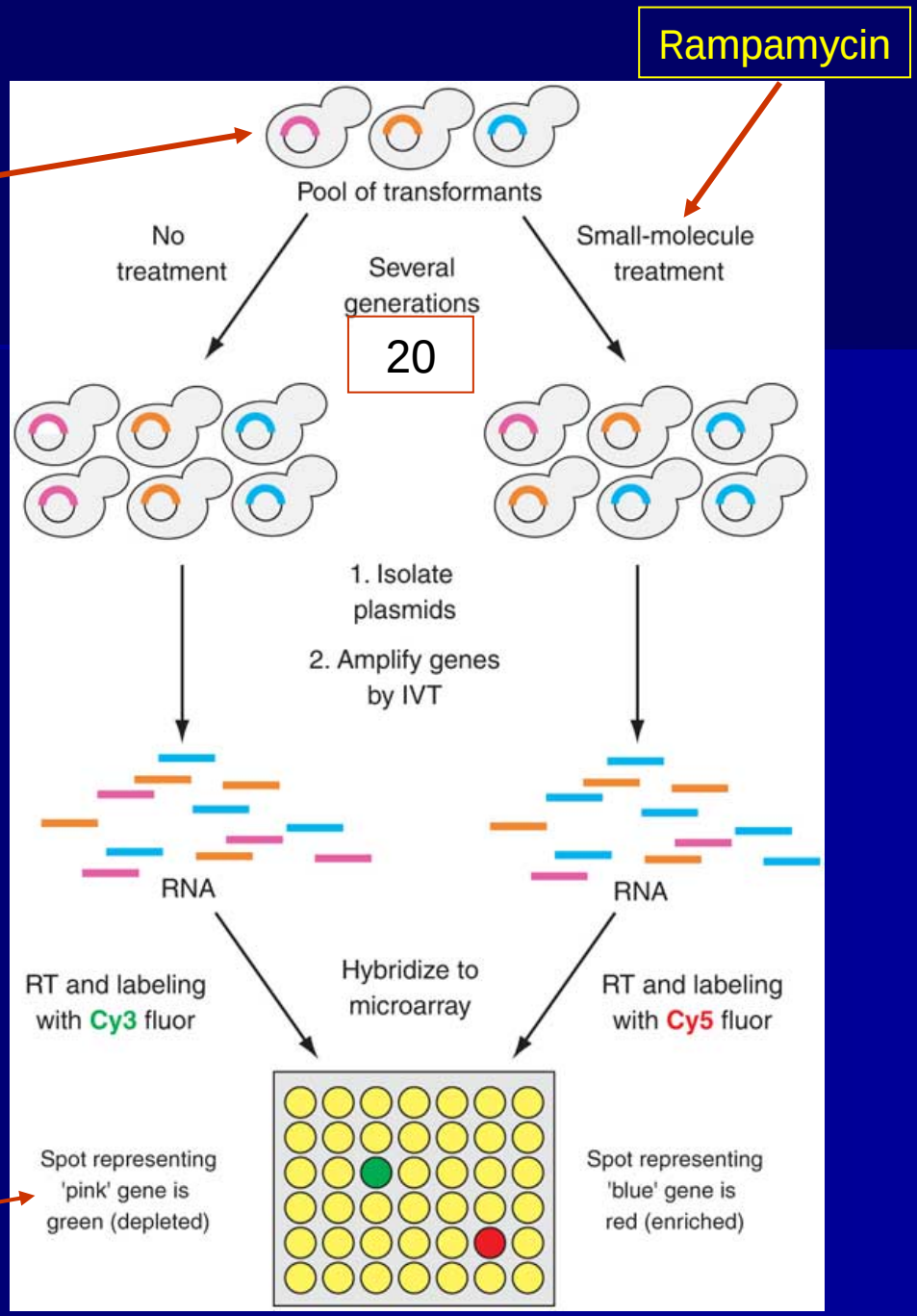
- Normally these screens yield sensitive strains and they are confirmed by tedious "genomic complementation" (obtaining sensitivity)

- The method described before can be used to quickly identify the gene conferring the resistance

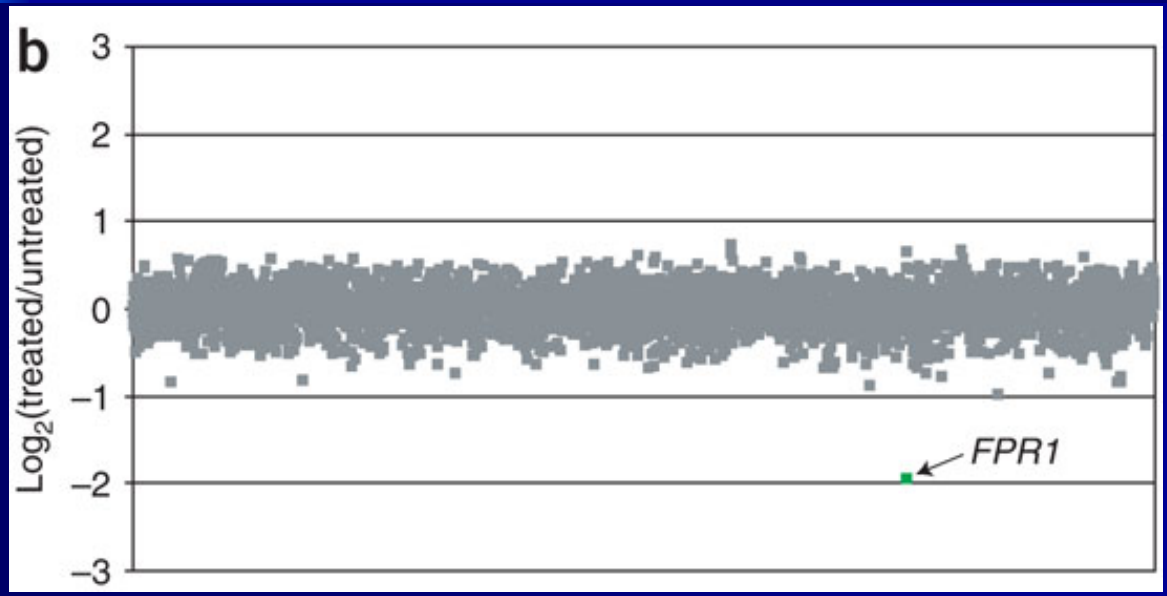
Overexpression lines on the background of a resistant mutant (selected on high levels of the molecule)

The Method:
Identification of resistance mutations by complementation

Depleted line should be the one conferring the resistance



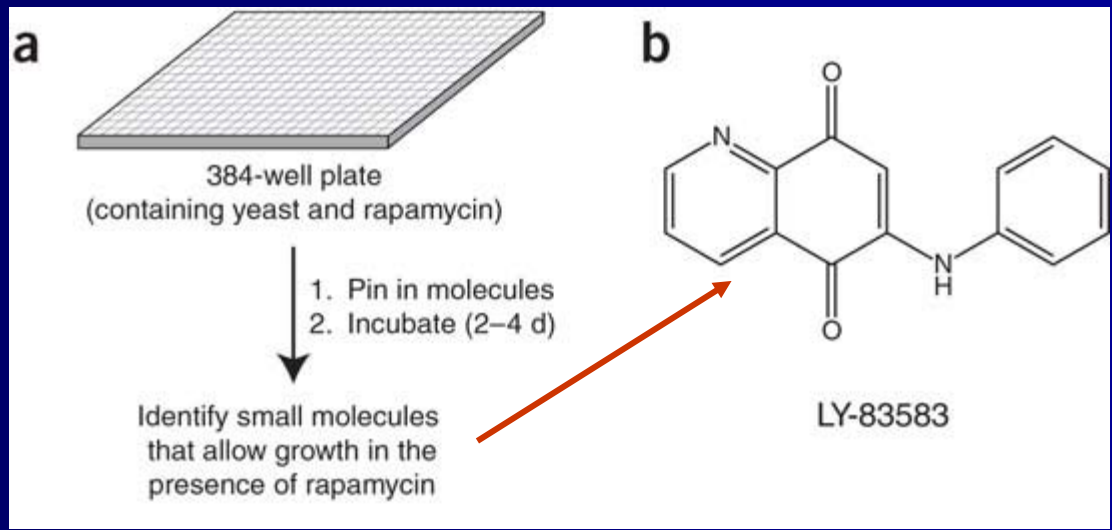
FPR1 is Identified as a Proof of Concept for the Method



Using Rapamycin resistance strain due to FPR1 mutations: Only FPR1 identified!

Suggests: All chemical genetic interactions detected in the previous experiment are the result of interaction with FPR1 or the FPR1-TOR complex

Characterization of a Small Molecule Rapamycin Repressor



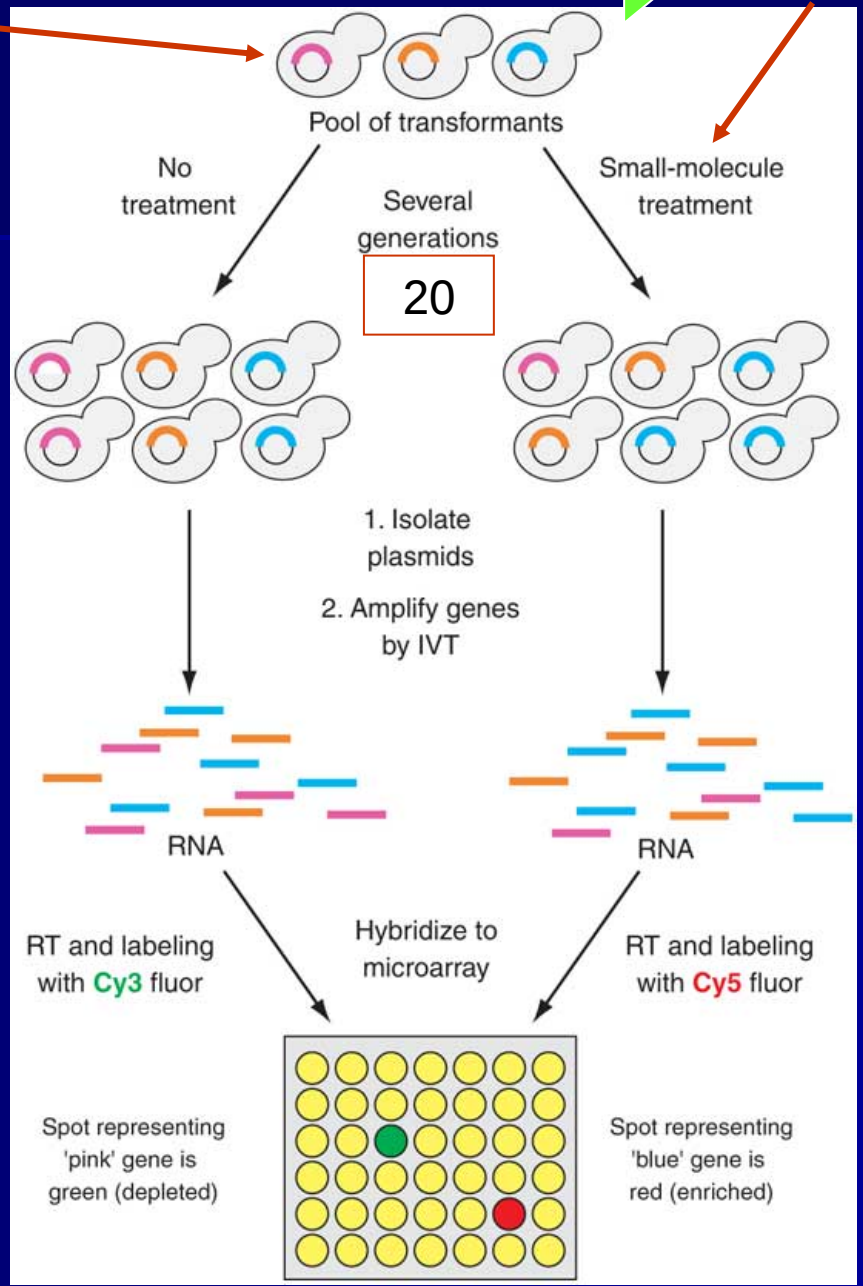
- LY-83583 identified as suppressor of growth inhibition by RAPAMYCIN by screening a DOS library

Overexpression lines

LY-83583

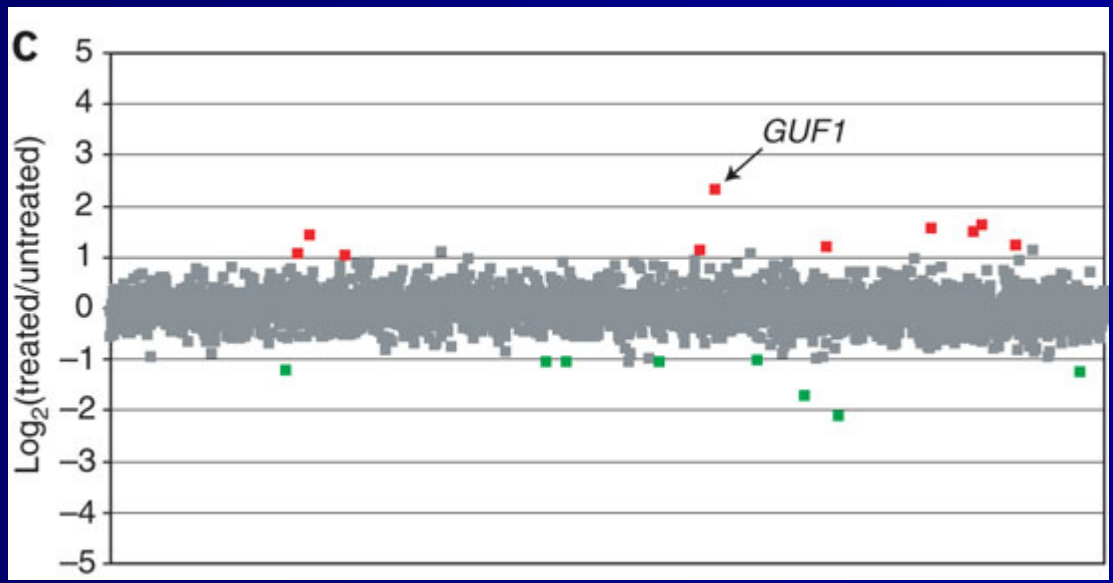
The Method:

Identification of Targets of Small Molecules with Microarrays



Characterization of a Small Molecule Rapamycin Repressor

Ten strains enriched at least twofold and eight depleted after treatment with LY-83583



Over-expressed genes confer resistance to LY-83583 But Sensitivity to Rapamycin

a Overexpression strains enriched after LY-83583 treatment

Gene name	Function	LY-83583 (fold enrichment)	Rapamycin (fold change)
GUF1	Mitochondrial GTPase of unknown function	+5.0	–3.1
FMP38	Mitochondrial, possible GTPase domain	+3.1	–3.2
HMI1	Mitochondrial ATP-dependent DNA helicase	+2.9	–2.3
YRR1	Transcription factor involved in drug response	+2.8	–
YDR514C	Localized to nucleus and mitochondria	+2.7	–3.1
SUV3	Mitochondrial ATP-dependent RNA helicase	+2.4	–
PET111	Required for mitochondrial translation of *COX2* mRNA	+2.3	–
MDL1	Involved in the export of peptides from mitochondria	+2.2	–
MRPS28	Mitochondrial ribosomal protein	+2.1	–
MAM1	Monopolin	+2.1	–

b Overexpression strains depleted after LY-83583 treatment

Gene name	Function	LY-83583 (fold depletion)	Rapamycin (fold change)
FET4	Fe(II) transporter	–4.3	–5.1
RIM11	Ser/Thr protein kinase	–3.3	+2.9
HOS1	Histone deacetylase	–2.4	–
YDR222W	Unknown function	–2.4	+5.0
YKR075C	Unknown function	–2.1	–2.5
YJL051W	Unknown function	–2.1	–2.5
SGN1	Contains an RNA recognition domain	–2.1	–2.1
YMD8	Unknown function	–2.0	–

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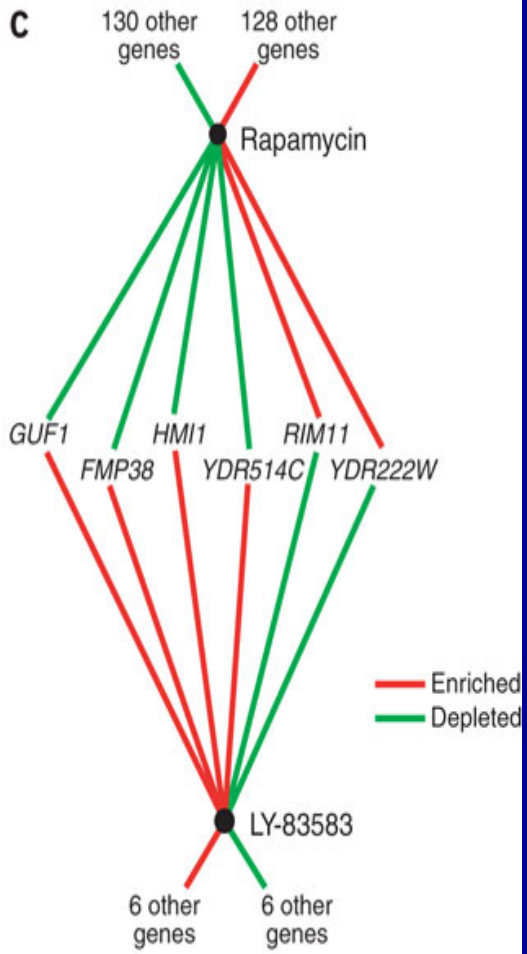


Information obtained on the Targets of Rapamycin Repressor (LY-83583)

- Most enriched genes encode proteins targeted to the mitochondria

- LY-83583 might therefore target protein (s) in the mitochondria

- Consistent with LY-83583 Mitochondrial action: Growth inhibition by LY-83583 on a non-fermentable carbon source (requires mitochondrial respiration to generate energy) is stronger.

- LY-83583 might inhibit respiration (directly or not)

Information obtained on the Targets of Rapamycin Repressor (LY-83583)

- Inhibition of one of the targets- GUF1 (a GTPase) by LY-83583, was shown in the study as well

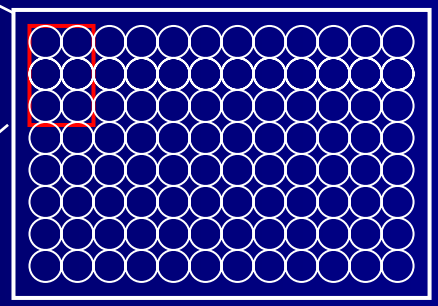
- The fact that overexpression of the same genes affects sensitivity (resistance or hypersensitivity) to Rapamycin and its repressor suggest that these genes are likely to be involved in a common pathway or process that affects sensitivity to Rapamycin.

Next Week:

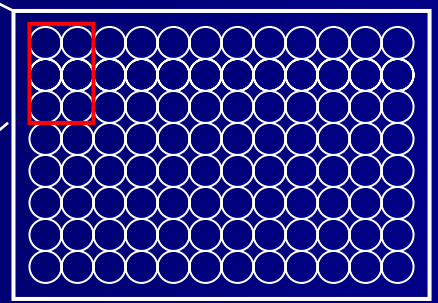
- More examples and tools for Chemical Biology

- A visit to the lab of Eyal Fridman- Volatile collection system

Chemical Genomics in Plants

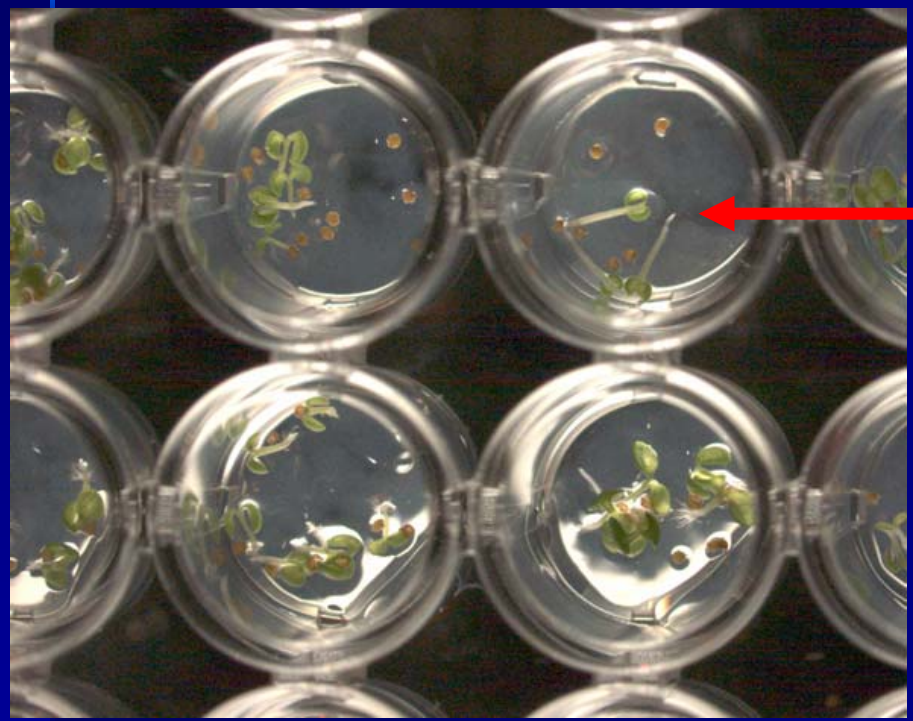


Each well contains different chemical



Screening for biologica effect

10,000 Small Molecules (Chembridge – DIVERSet)



Positive hit:
longer hypocotyl

Dwarf seedlings
grown in 96 well plate for 6 days