A close-up photograph of a microfluidic chip, showing a grid of small, rectangular wells or channels. The chip is light-colored, possibly white or light blue, and the wells are arranged in a regular pattern. The background is slightly blurred, focusing attention on the chip's surface.

# Integrating Gene Synthesis & Microfluidic Protein Analysis for Rapid Protein Engineering

Matt Blackburn

LBNC

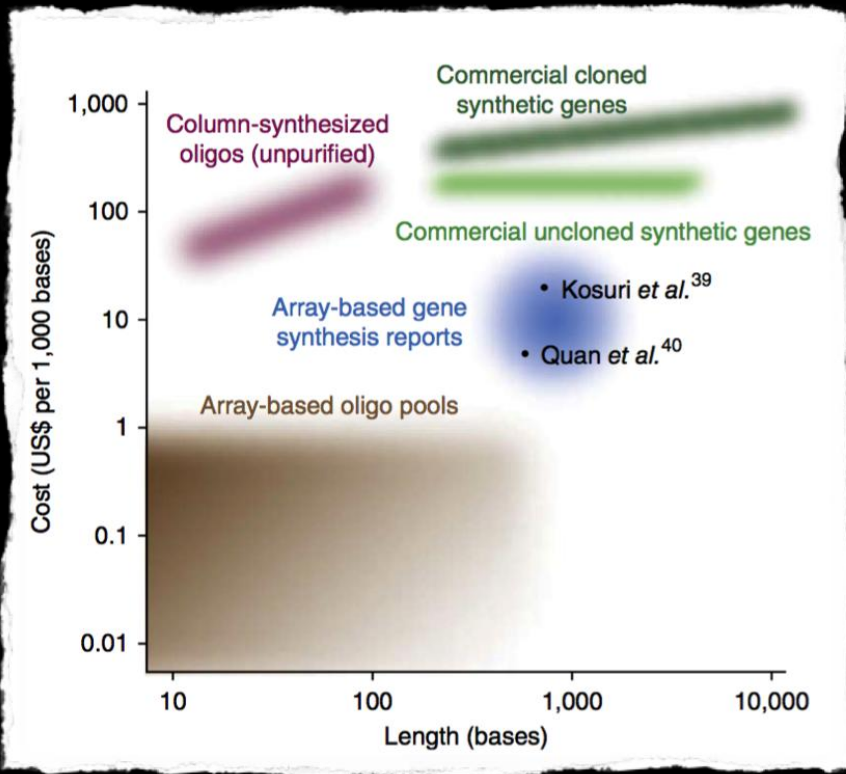
2015.09.24

Basel Life Science Week



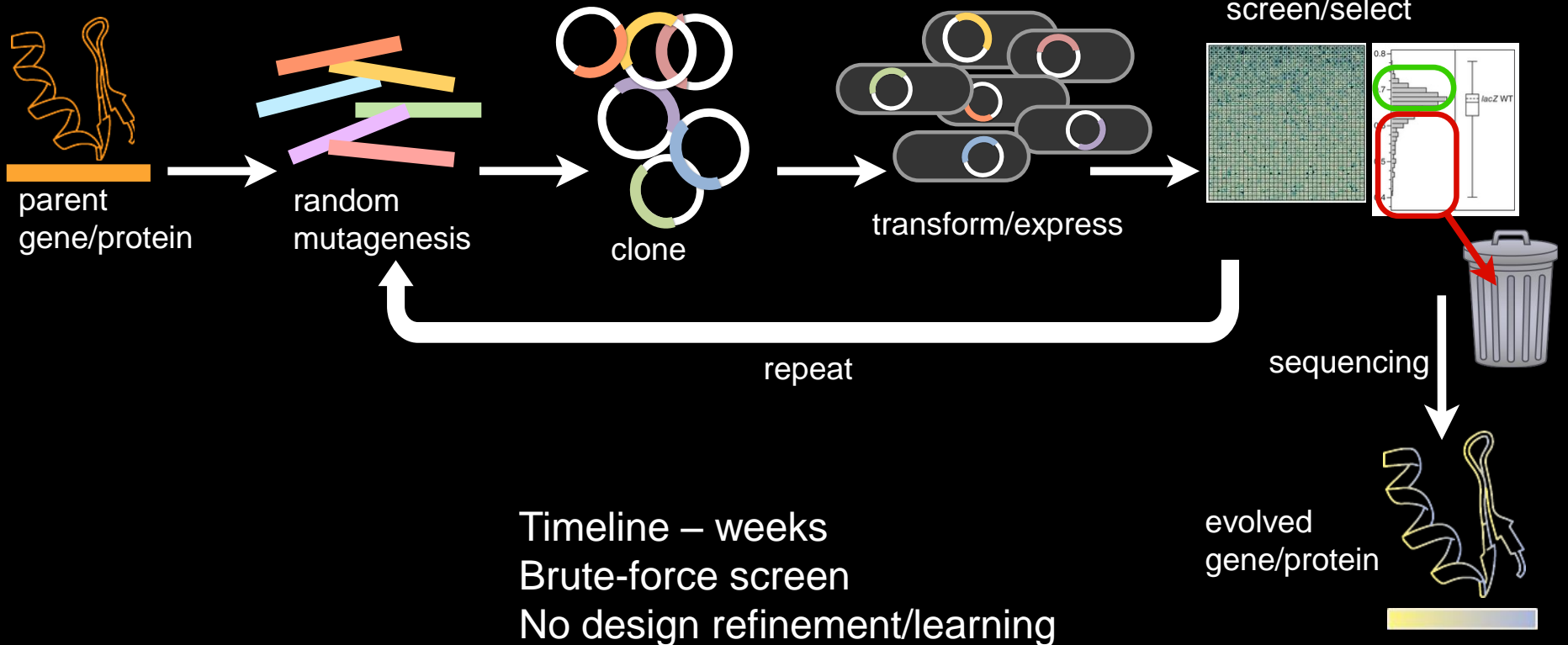
ÉCOLE POLYTECHNIQUE  
FÉDÉRALE DE LAUSANNE

# DNA synthesis : Protein Engineering

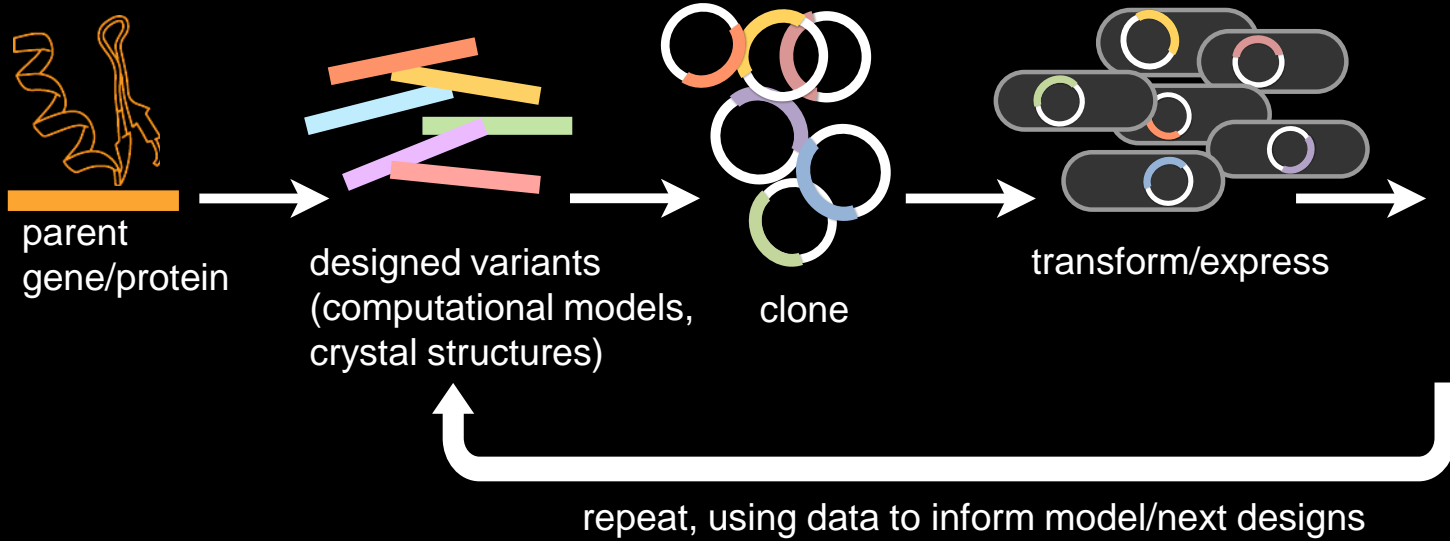


protein engineering for optimization of:  
enzyme function  
antibody binding  
TFs in synthetic networks

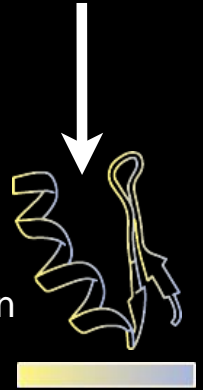
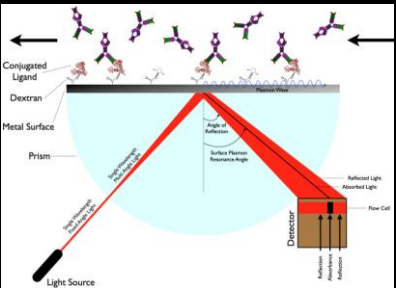
# Directed Evolution



# Rational Design Cycle



purify protein, examine in vitro activity

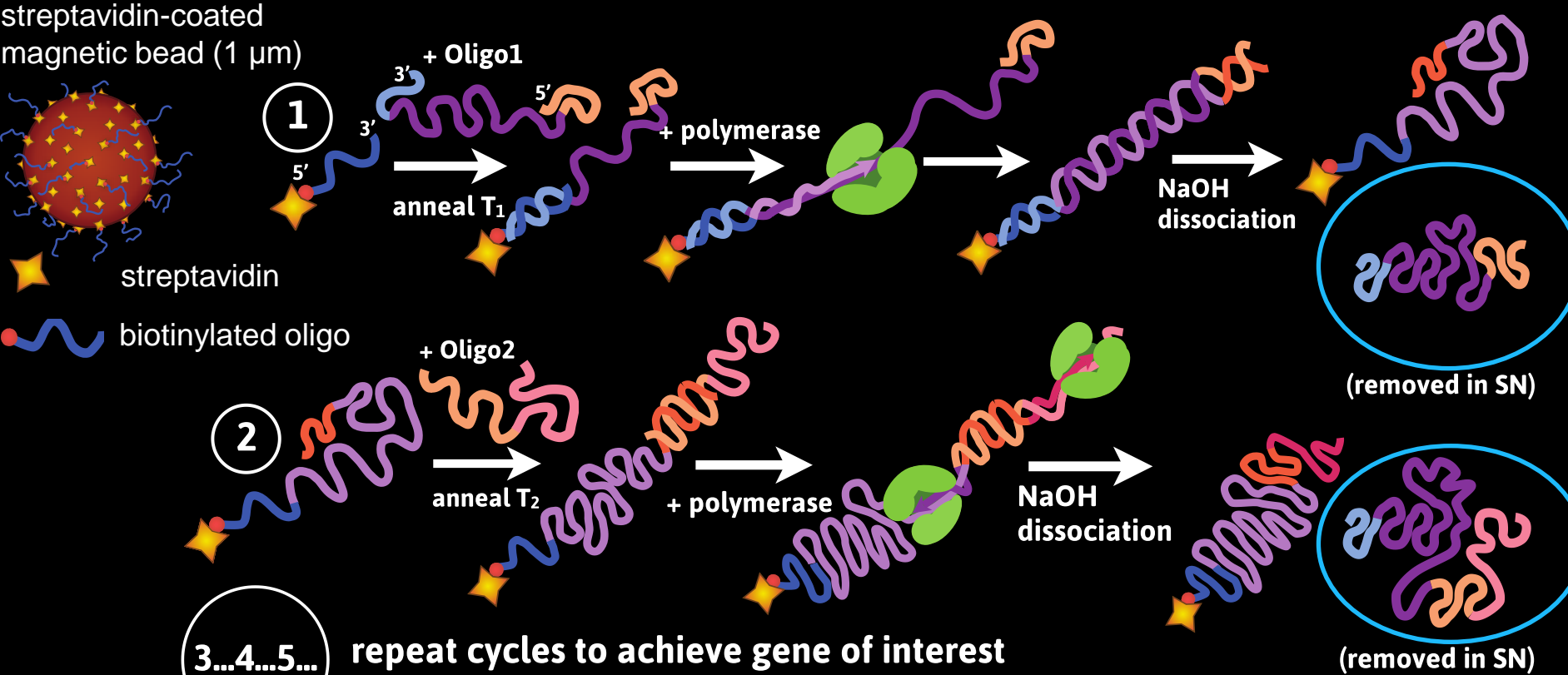


Targeted (limited aa's or location)  
or  
Systematic (ie alanine scan)

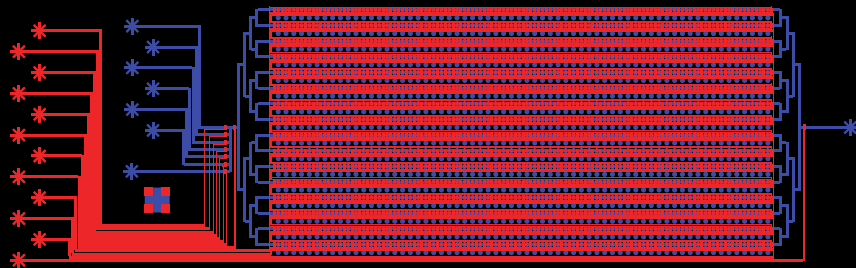
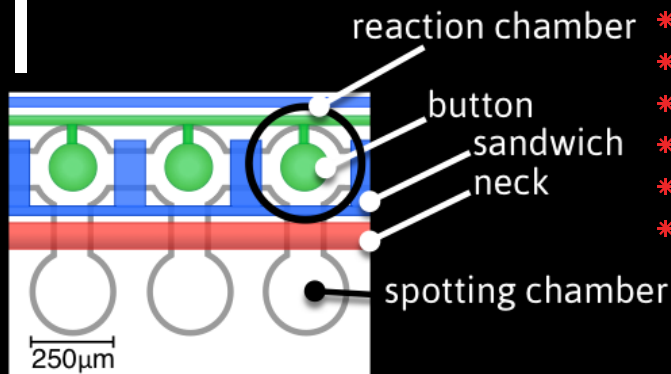
Timeline – weeks  
Lower library throughput  
Prior knowledge



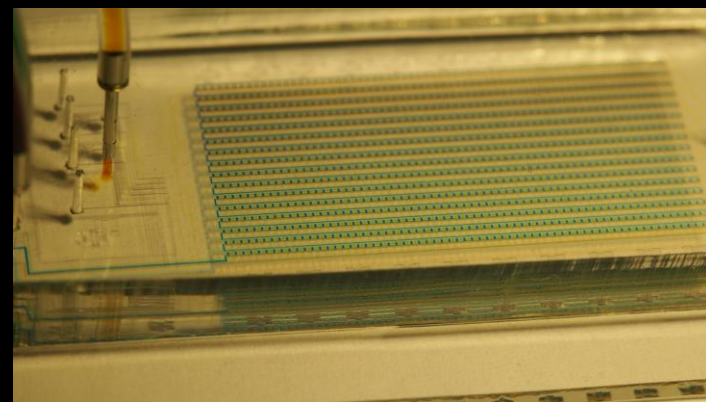
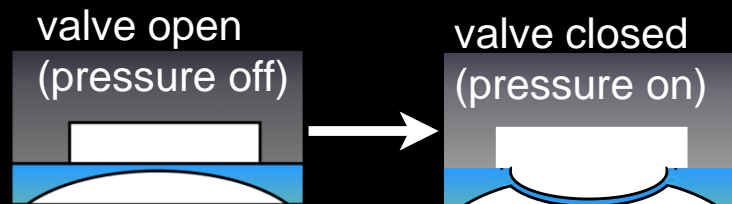
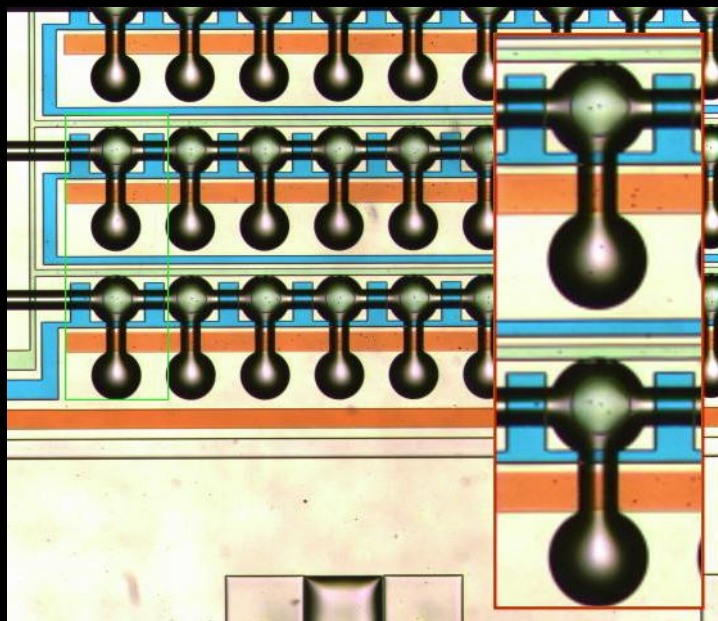
# Asymmetric Polymerase Extension (APE)



# MITOMI



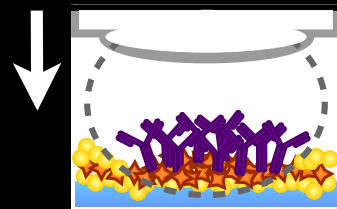
Mechanically  
Induced  
Trapping  
Of  
Molecular  
Interactions



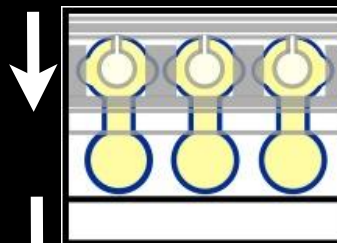
# MITOMI

upstream: template DNA prep  
target DNA prep

microarray printing  
align microfluidic device to array

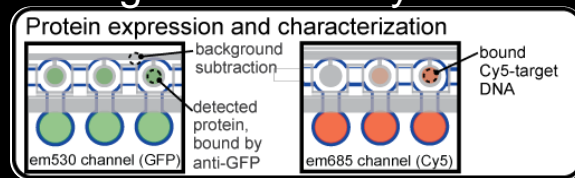


surface chemistry



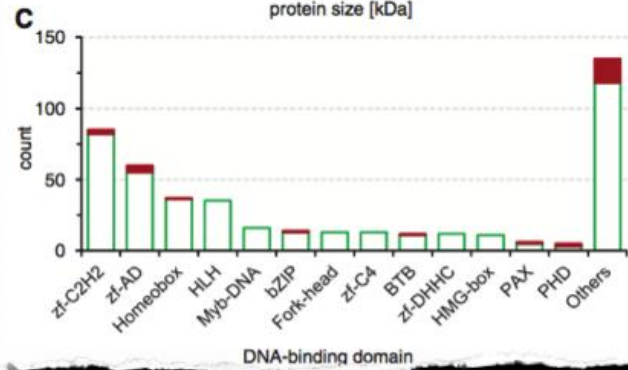
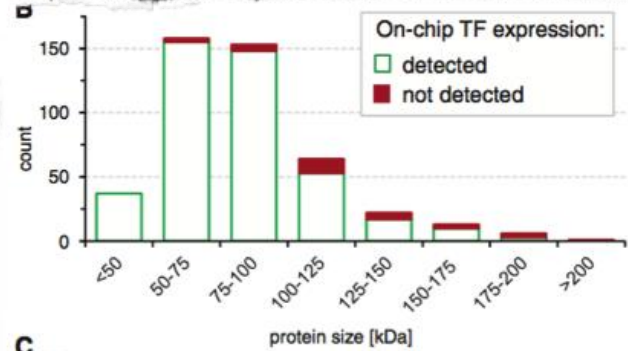
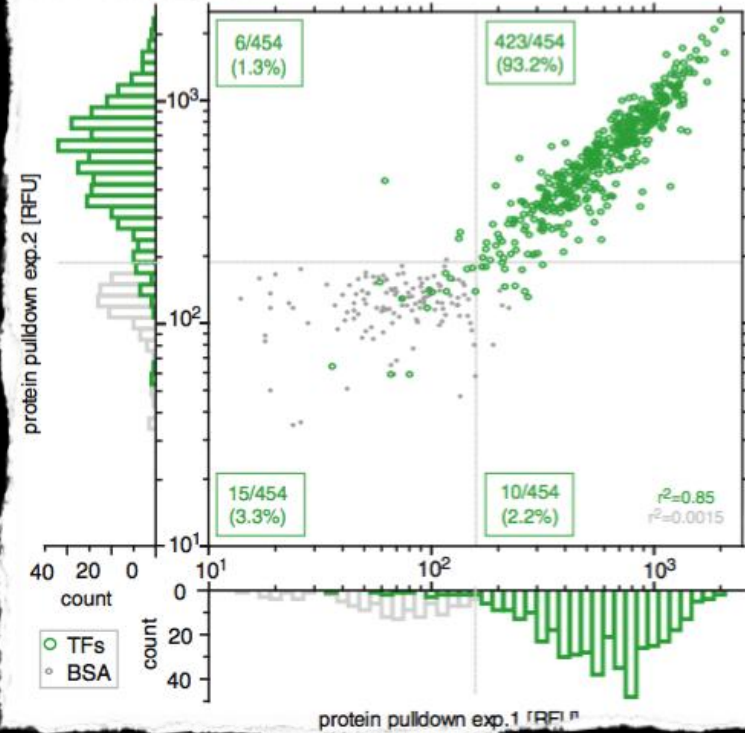
TX + TL  
(protein expression)

image + data analysis



Mechanically  
Induced  
Trapping  
Of  
Molecular  
Interactions



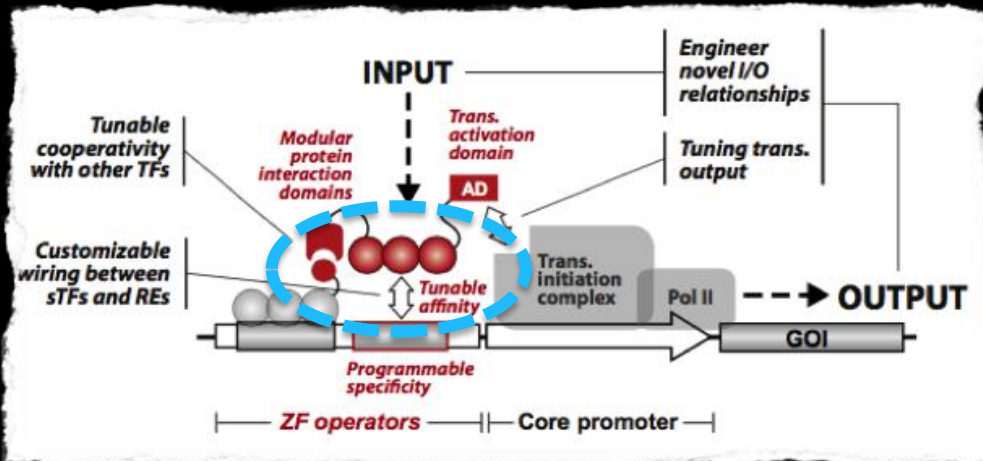


# Application: Synthetic Biology

## A Synthetic Biology Framework for Programming Eukaryotic Transcription Functions

Ahmad S. Khalil,<sup>1,7</sup> Timothy K. Lu,<sup>2,7,\*</sup> Caleb J. Bashor,<sup>1,7</sup> Cherie L. Ramirez,<sup>3,4</sup> Nora C. Pyenson,<sup>1</sup> J. Keith Joung,<sup>3,5</sup> and James J. Collins<sup>1,6</sup>

<sup>1</sup>Howard Hughes Medical Institute, Department of Biomedical Engineering, and Center for BioDynamics, Boston University, Boston

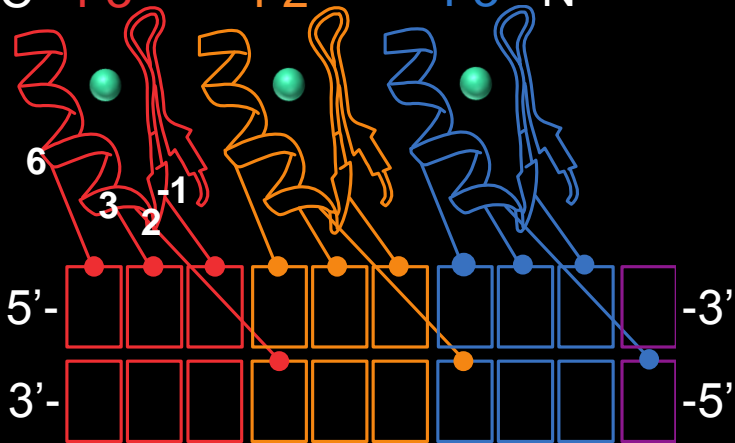


# C<sub>2</sub>H<sub>2</sub> Zinc Finger Proteins

30 aa (90 bp)

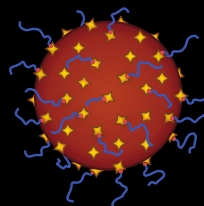
C- $\alpha\beta\beta$ -N

C - F3      F2      F3 - N



'Canonical' binding to target DNA strand

variants



F3

F2

F1

Oligo1  
74 nt

Oligo3  
77 nt

Oligo5  
71 nt

239 bp

300  
200  
97  
186  
239

Day1: APE synthesis & PCR amplification

Day2: fusion PCR to linker-EGFP-6xHis template

gene-specific PCR

extension + final PCR

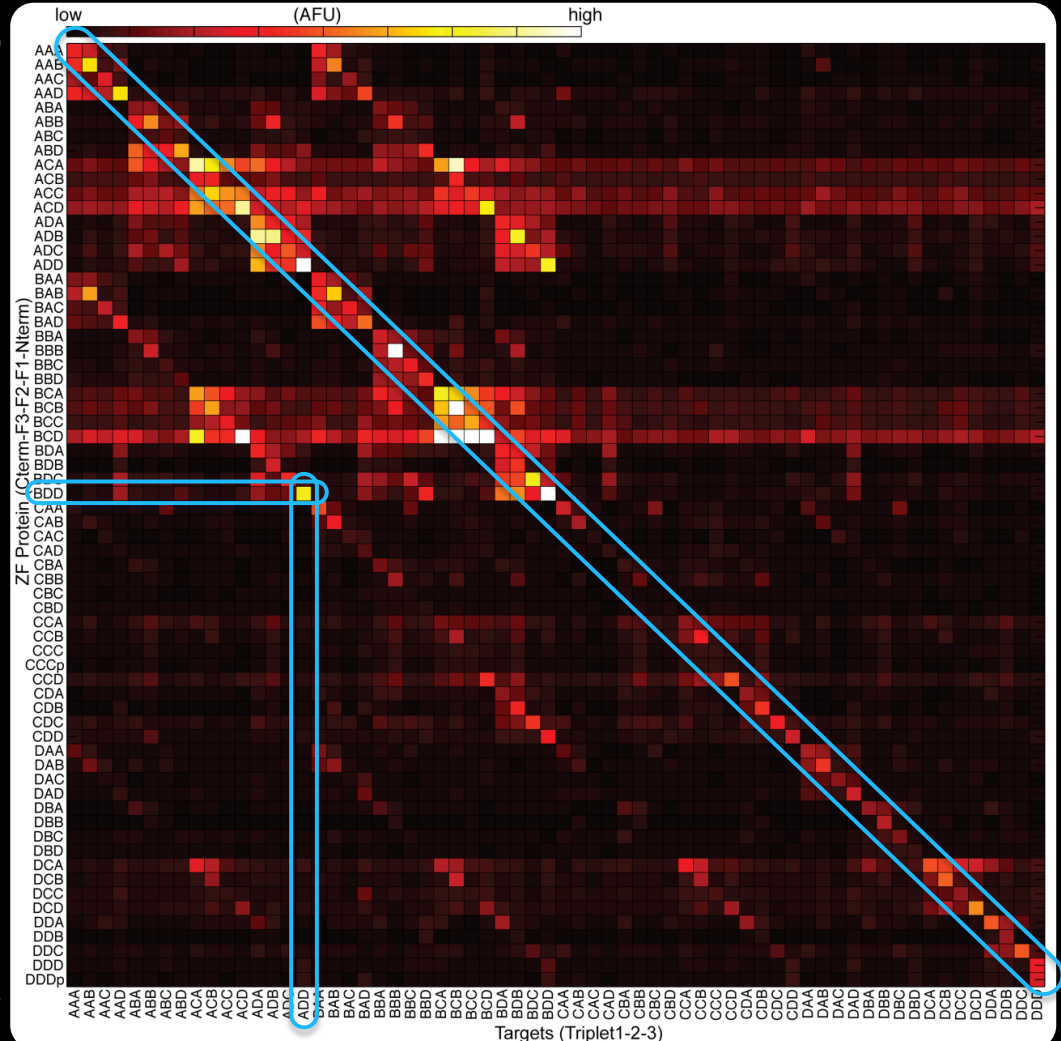
final product = 1192 bp



# Zinc Finger Module Combinatorics

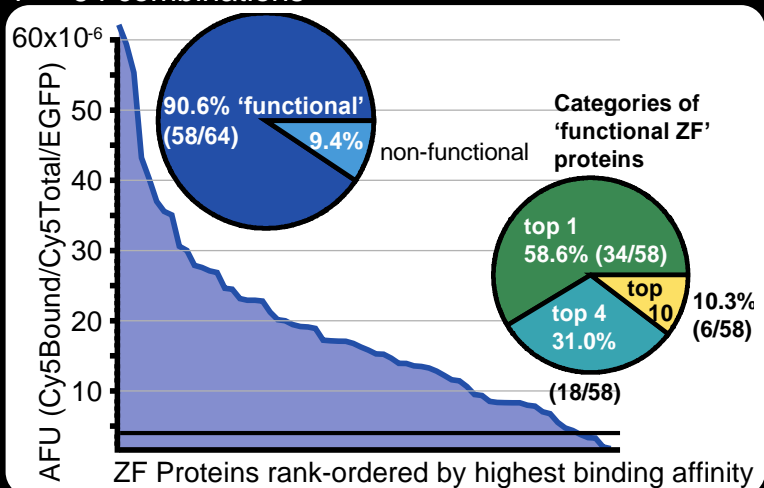
proteins

targets



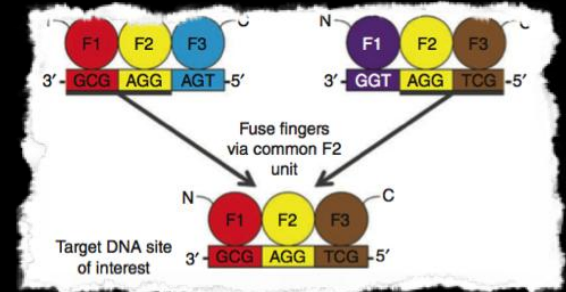
Zinc Fingers	F1	F2	F3	
A) Zif268	GCG	TGG	GCG	3 oligos per ZFA × 4
B) 37-12	GAG	GAC	GTG	-----
C) 92-1	GAT	GTA	GCC	12 oligos
D) 158-2	GTA	GAT	GGA	

oligos within a finger position can be interchanged  
 $4^3 = 64$  combinations

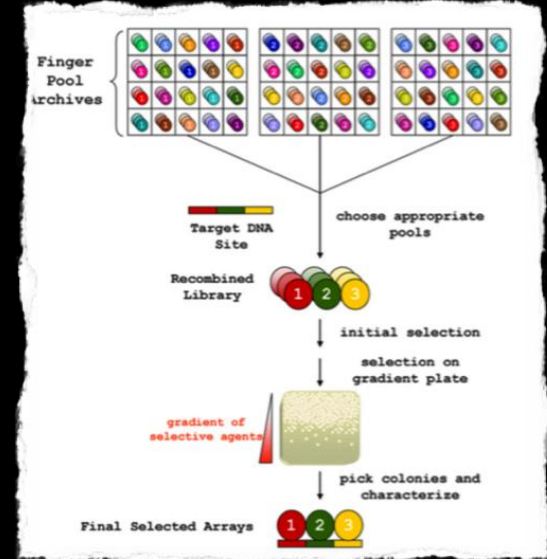


# ZF Databases

CoDA - Context Dependent Assembly  
Sander JD et al. Nat Meth Vol 8(1), 2011



OPEN - Oligomerized Pool Engineering  
Maeder ML et al. Mol Cell Vol 31(2), 2008



www.zincfingers.org/software-tools.htm

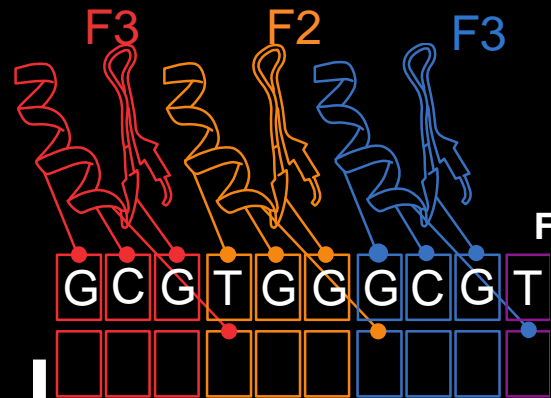
## Software Tools

- **ZIFIT** (web-accessible software for the design of engineered zinc finger arrays)
- **ZIFDB** (web-accessible database of zinc fingers and engineered zinc finger arrays)
- **ZFNGenome** (genome-scale resource for identifying potential zinc finger nuclease target sites in various organisms)

Target	ZF1	RSDELTR	bits
	ZF2	RSDHLTT	
	ZF3	RSDEKRR	

# ZF Specificity Engineering

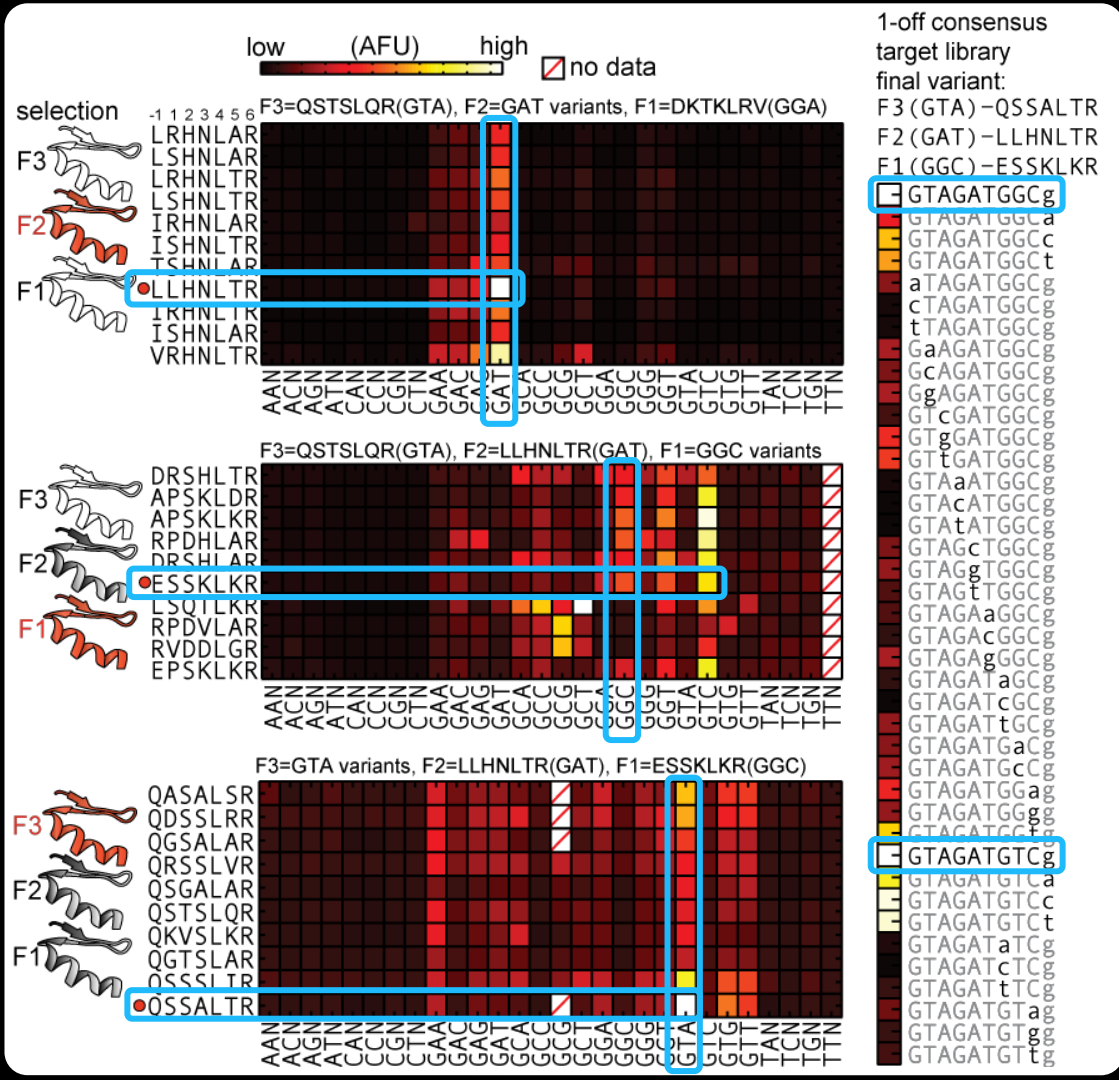
F2 selection for GAT



F1 selection for GGC

F3 selection for GTA

desired consensus target  
G T A G A T G G C G



# Tuning ZF Affinity

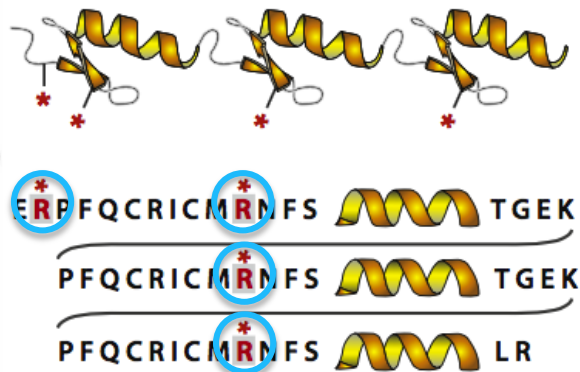
## A Synthetic Biology Framework for Programming Eukaryotic Transcription Functions

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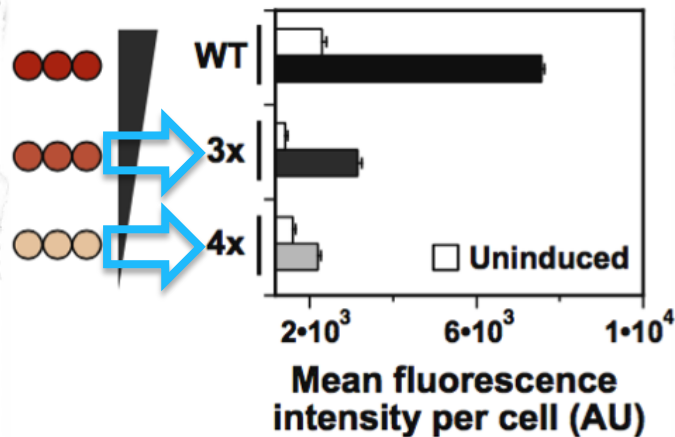
<sup>1</sup>Howard Hughes Medical Institute, Department of Biomedical Engineering, and Center for BioDynamics, Boston University, Boston

Cell, Vol 150(3), 2012

### ZINC FINGER ARRAY



### Tuning Output By ZF-DNA Affinity

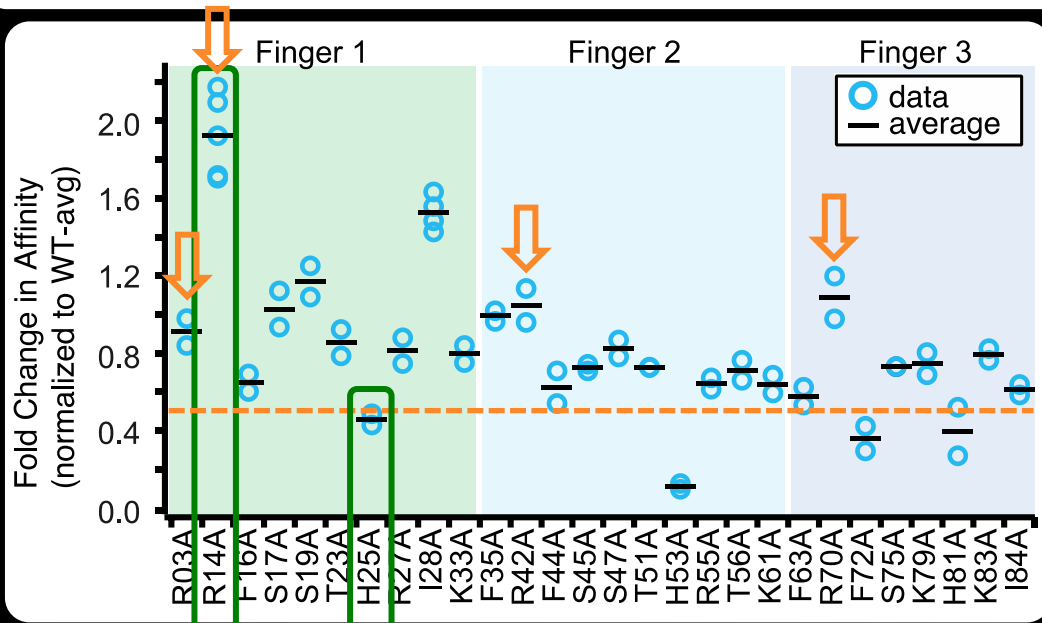


# ZF Affinity Variants

Zif268 zinc finger domains

○ modified residue positions

M E <sup>3</sup> P Y A C P V E S C D <sup>14</sup> R <sup>16</sup> <sup>17</sup> <sup>19</sup> S R <sup>23</sup> D E L <sup>25</sup> T R <sup>27</sup> H I <sup>29</sup> R <sup>31</sup> I H T G Q <sup>33</sup> K  
 P <sup>35</sup> F Q C R I - - C M <sup>42</sup> R <sup>44</sup> N <sup>45</sup> F <sup>47</sup> S R <sup>51</sup> S D H L <sup>57</sup> T <sup>59</sup> H I <sup>61</sup> R <sup>63</sup> T <sup>65</sup> H T G E <sup>67</sup> K  
 P <sup>69</sup> F A C D I - - C G <sup>72</sup> R <sup>74</sup> K <sup>75</sup> F A R <sup>76</sup> S D E L <sup>78</sup> R <sup>81</sup> K <sup>83</sup> R <sup>84</sup> H T K <sup>87</sup> I H L R Q K



Finger 1

Finger 2

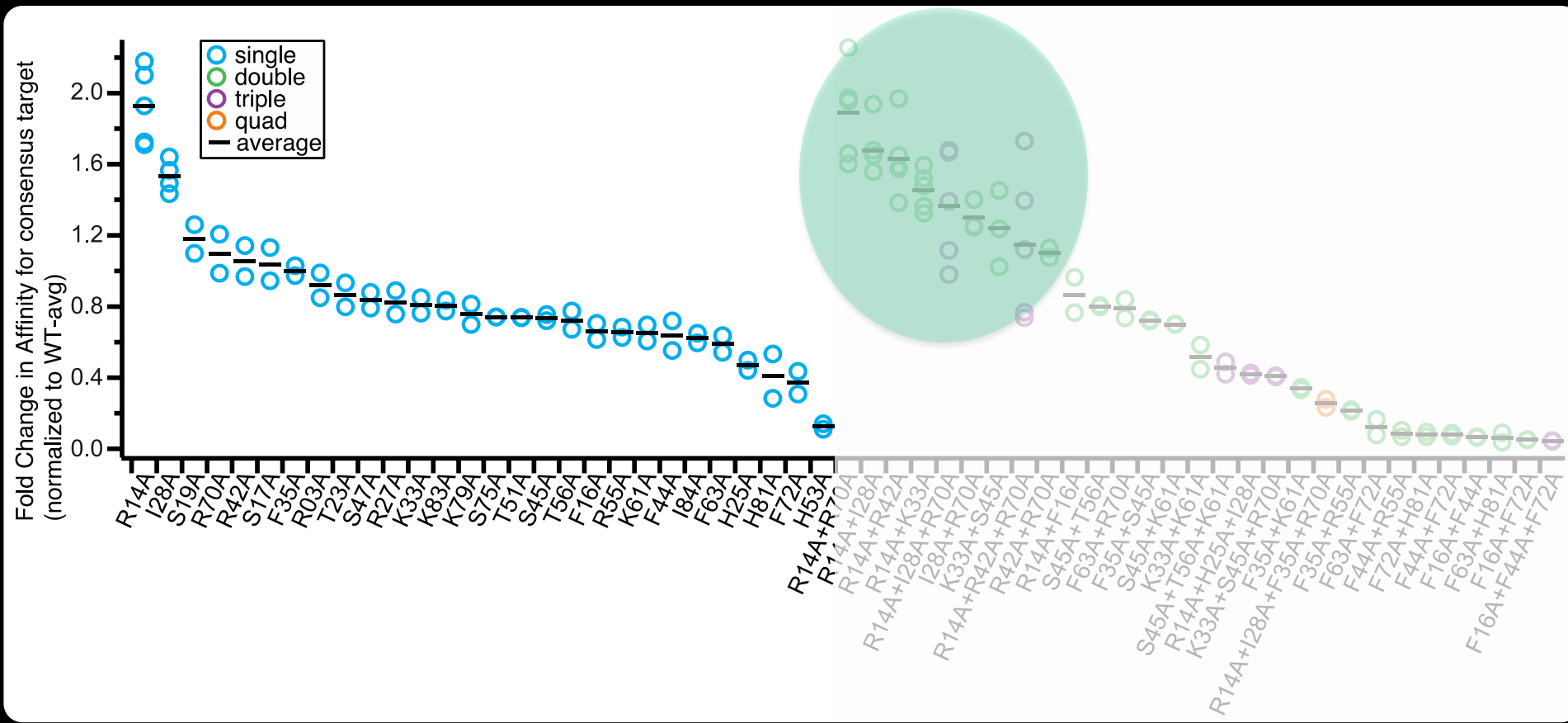
Finger 3

R14A = 2 fold higher affinity

H25A = 0.5 fold lower affinity (2x weaker)



# ZF Affinity Variants



# Conclusions

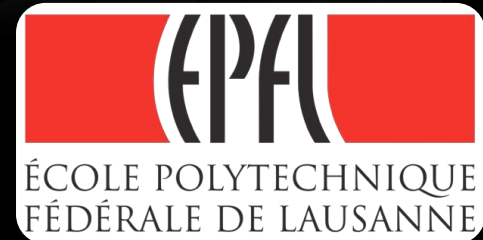
- development of HT pipeline for rapid protein engineering (applied here for ZF array engineering)
- ZF specificity can be engineered
- ZF affinity tuning is possible

# Acknowledgements

Sebastian Maerkl

LBNC members + alumni

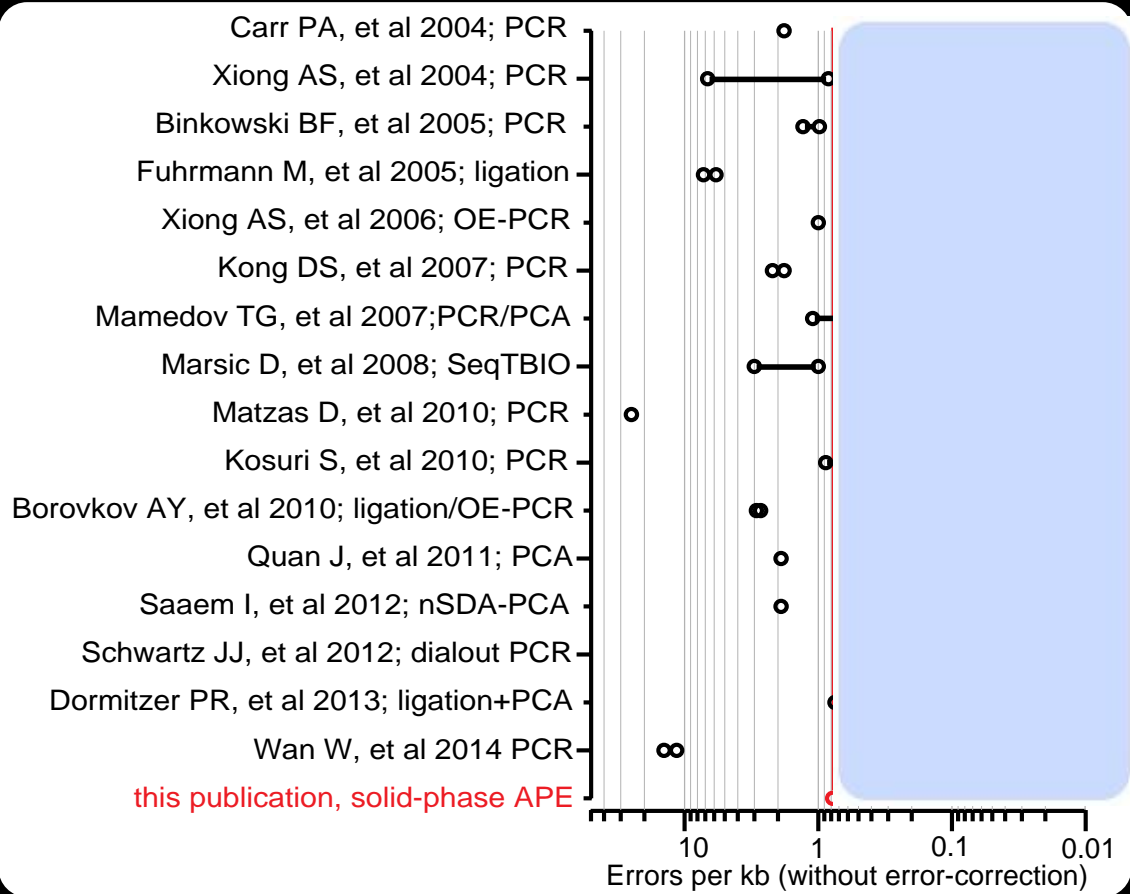
Deplancke Lab



Questions?

# Asymmetric Polymerase Extension (APE)

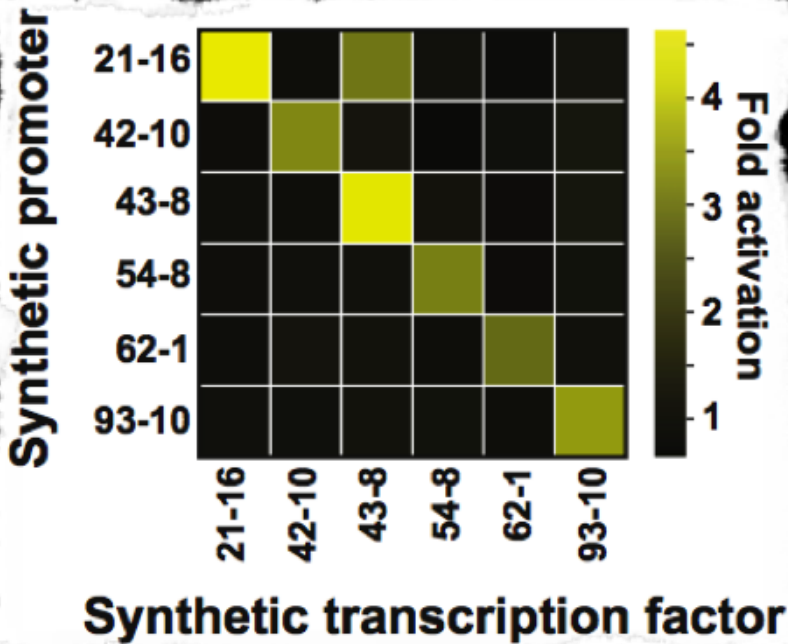
Error rate comparison



# In vivo comparison of ZFs

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<sup>1</sup>Howard Hughes Medical Institute, Department of Biomedical Engineering, and Center for BioDynamics, Boston University, Boston



## In vivo vs in vitro (APE-MITOMI)

