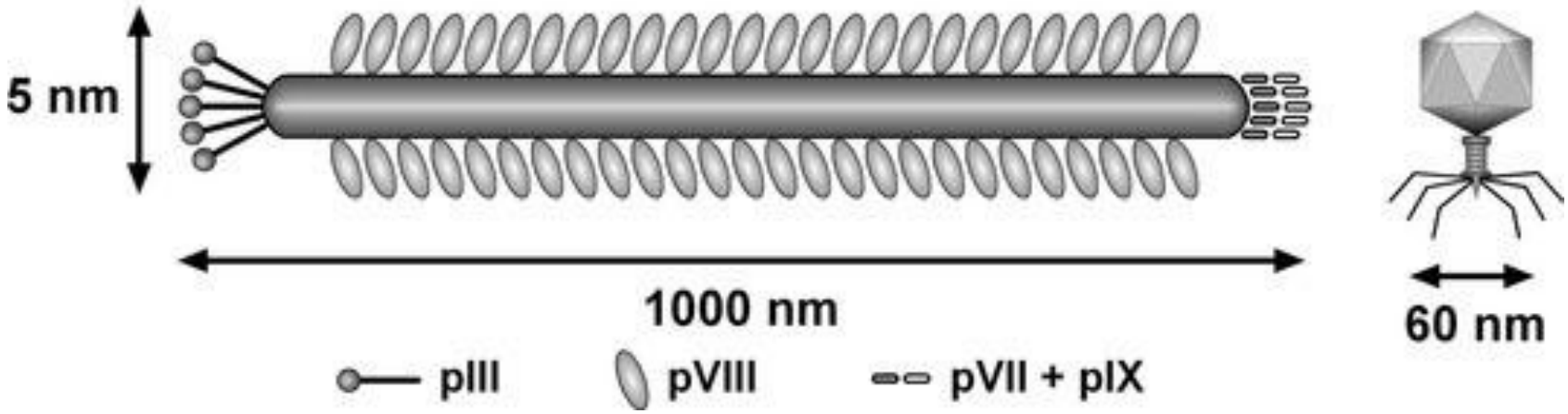
SAROTUP 2.0: a suite of web tools

for finding potential target-unrelated peptides from phage display data

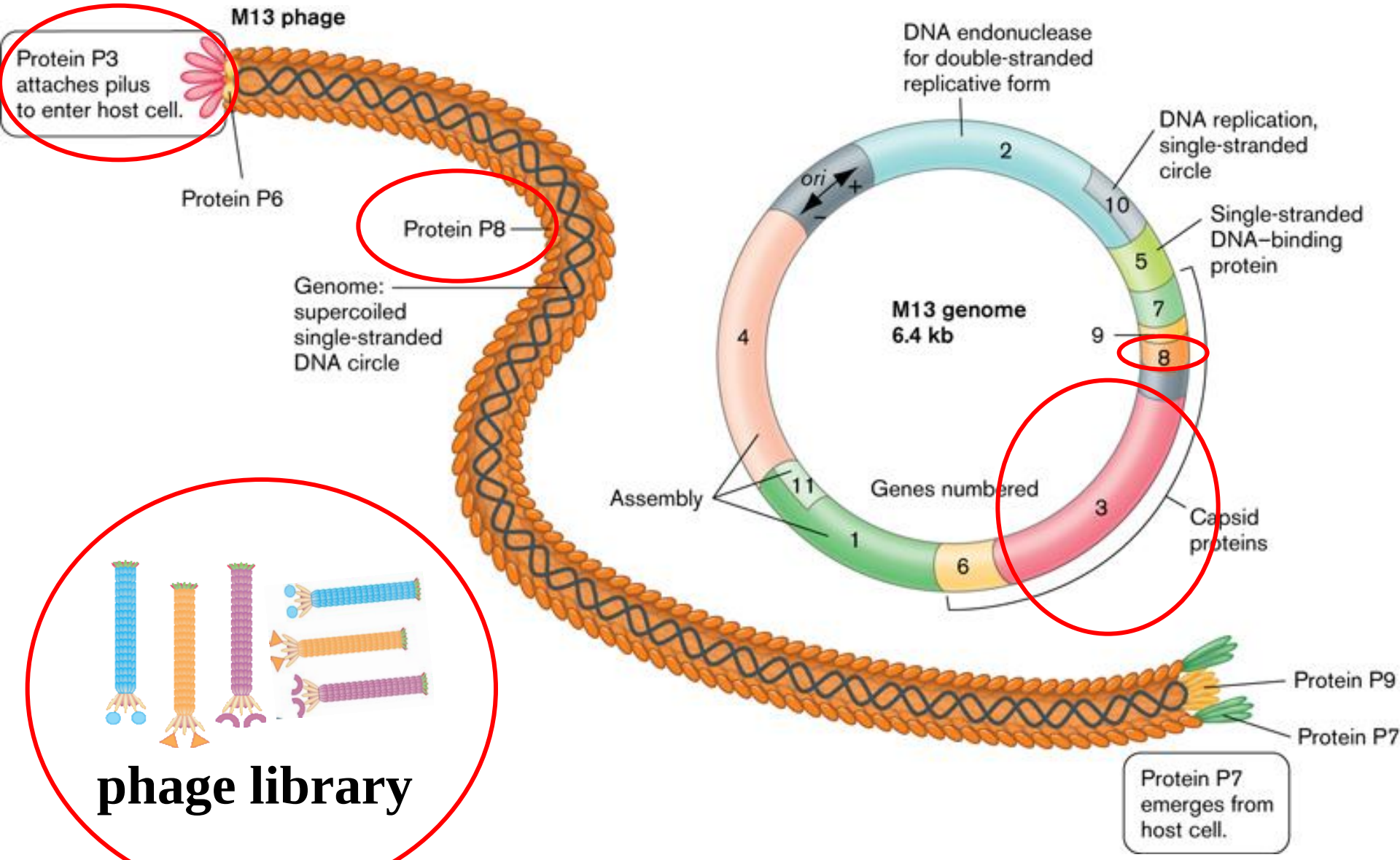
Jian Huang

University of Electronic Science and Technology of China

Phages



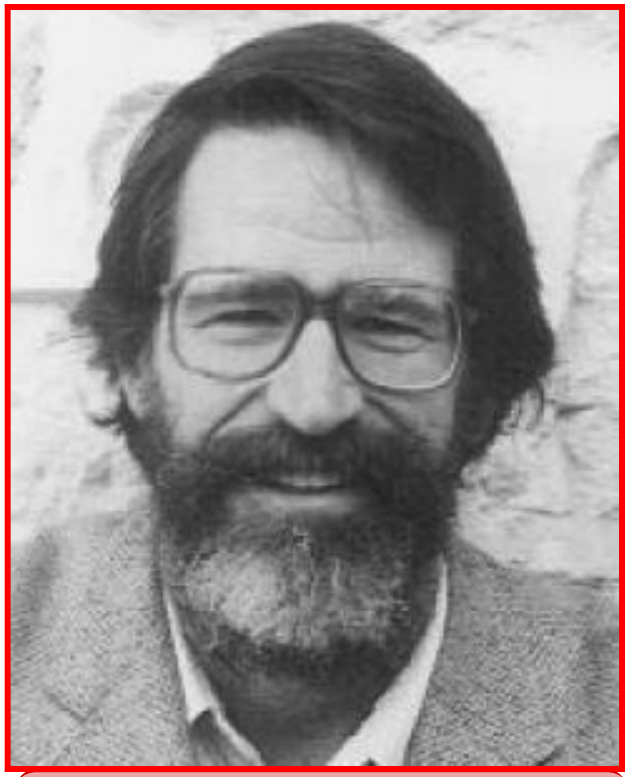
Phages, also known as bacteriophages, are viruses that infect bacterial cells. Many phages such as the filamentous phages M13 and fd are good expression vectors.


M13 phage
Protein P3 attaches pilus to enter host cell.
Protein P6
Protein P8
Genome: supercoiled single-stranded DNA circle
DNA endonuclease for double-stranded replicative form
DNA replication, single-stranded circle
Single-stranded DNA–binding protein
M13 genome 6.4 kb
ori
Assembly
Genes numbered
Capsid proteins
Protein P9
Protein P7
Protein P7 emerges from host cell.
phage library

Phage display

Filamentous Fusion Phage: Novel Expression Vectors That Display Cloned Antigens on the Virion Surface

Abstract. *Foreign DNA fragments can be inserted into filamentous phage gene III to create a fusion protein with the foreign sequence in the middle. The fusion protein is incorporated into the virion, which retains infectivity and displays the foreign amino acids in immunologically accessible form. These "fusion phage" can be enriched more than 1000-fold over ordinary phage by affinity for antibody directed against the foreign sequence. Fusion phage may provide a simple way of cloning a gene when an antibody against the product of that gene is available.*

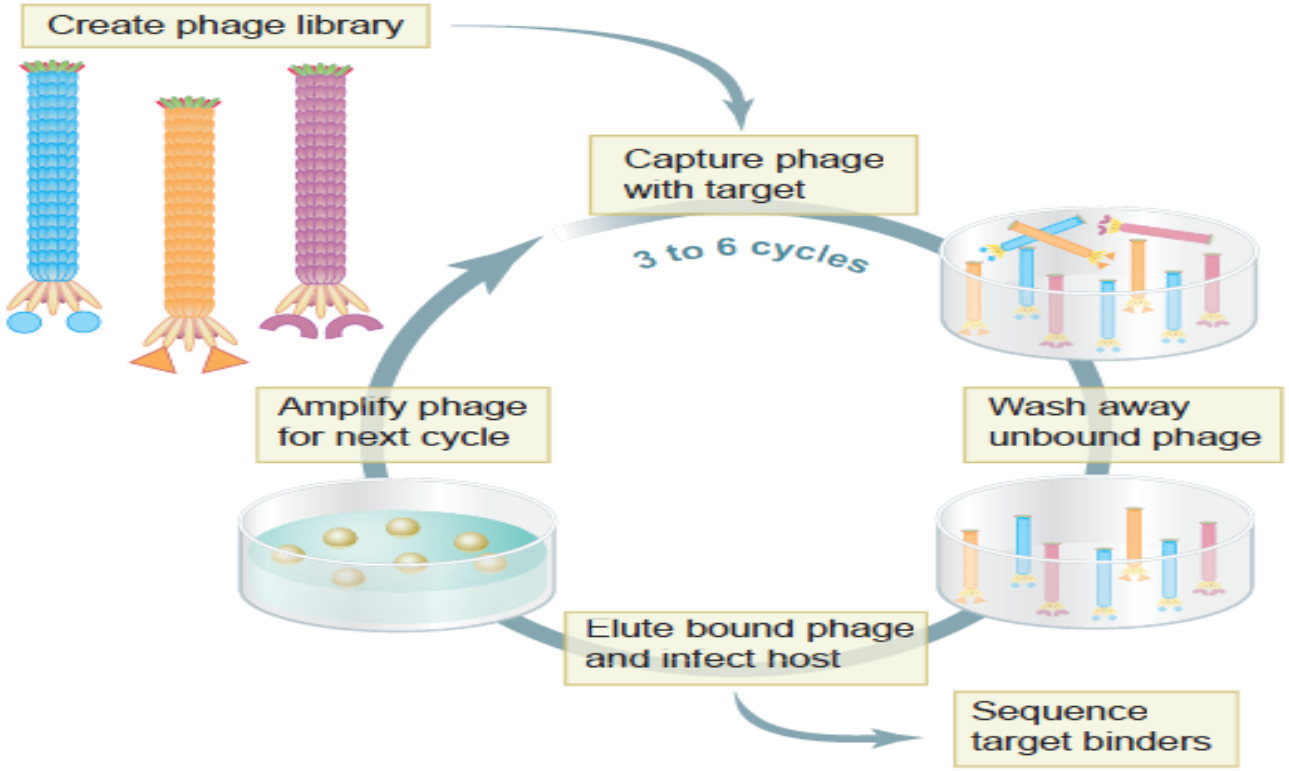


Science 1985, 228 (4705): 1315–1317

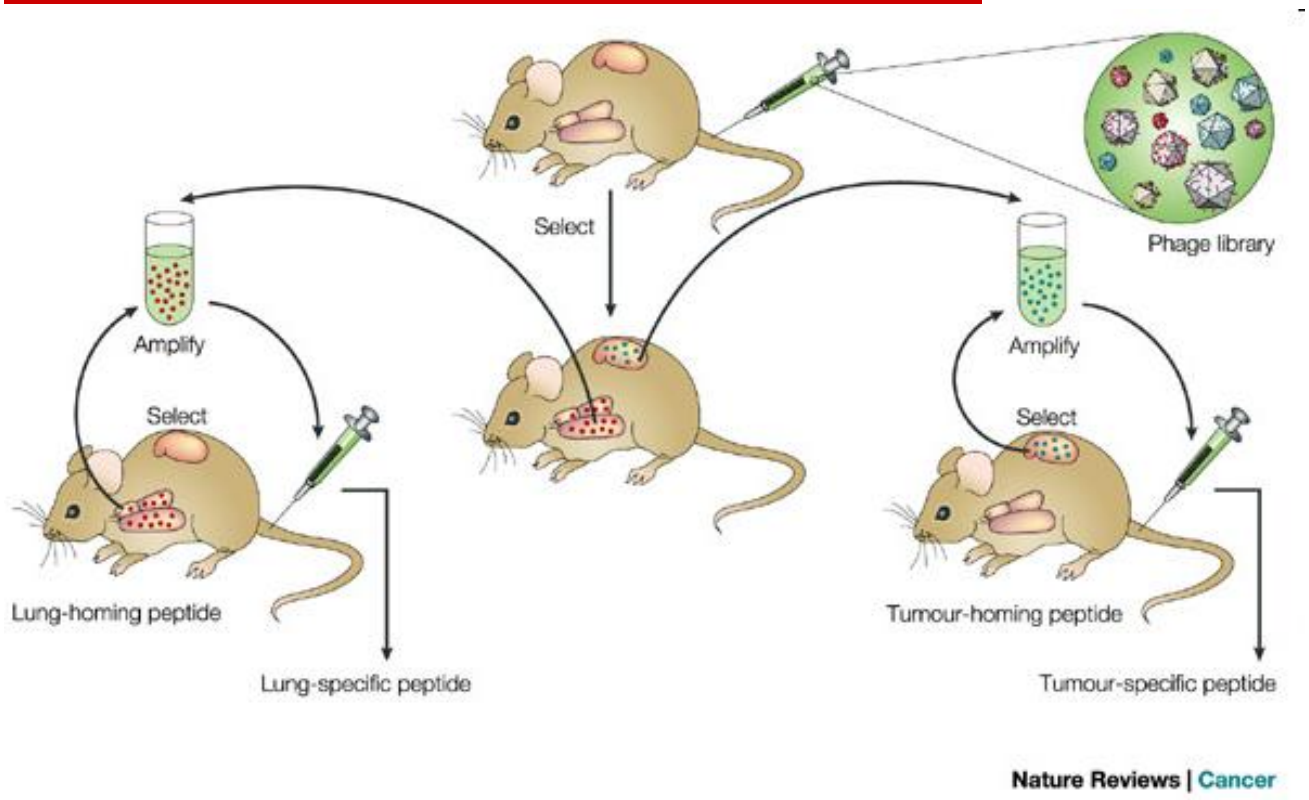
George P Smith
father of phage display

Phage-displayed random peptide library

- ☑ *Smothers JF, Henikoff S, Carter P: Affinity selection from biological libraries*. **Science 2002, 298(5593): 621-622**



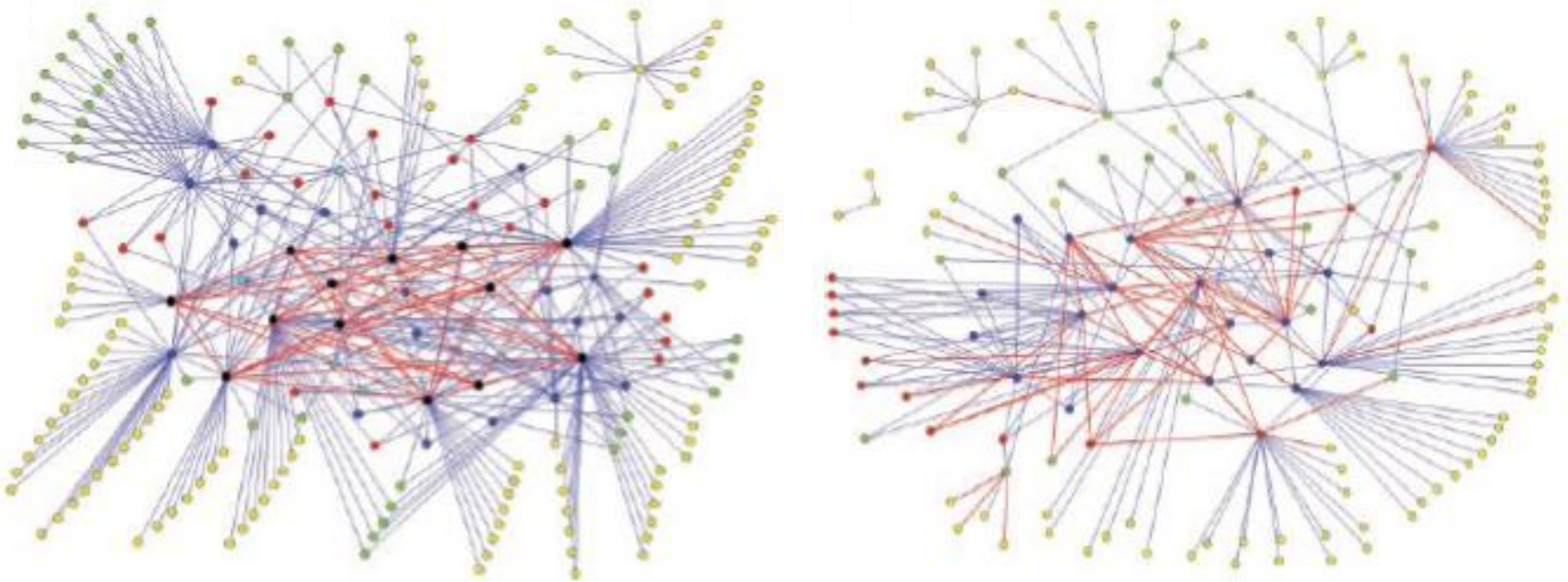
In vivo phage display



Nature Reviews Cancer
2002, 2, 83-90

Pasqualini, R.; Ruoslahti, E., Organ targeting in vivo using phage display peptide libraries. **Nature 1996, 380, (6572), 364-366.**

Yeast SH3 domain protein-protein interaction network



Science 2002, 295(5553):321-324

Left：Yeast SH3 domain protein-protein interaction network predicted by means of phage display-selected peptides

Right：Two-hybrid SH3 domain protein-protein interaction network

Romiplostim of Amgen

IEGPTLRQWLAARA

Peptide Agonist of the Thrombopoietin Receptor as Potent as the Natural Cytokine

Steven E. Cwirla, Palaniappan Balasubramanian, David J. Duffin, Christopher R. Wagstrom, Christian M. Gates, Sara C. Singer, Ann M. Davis, Robert L. Tansik, Larry C. Mattheakis, Chris M. Boytos, Peter J. Schatz, David P. Baccanari, Nicholas C. Wrighton, Ronald W. Barrett, William J. Dower*

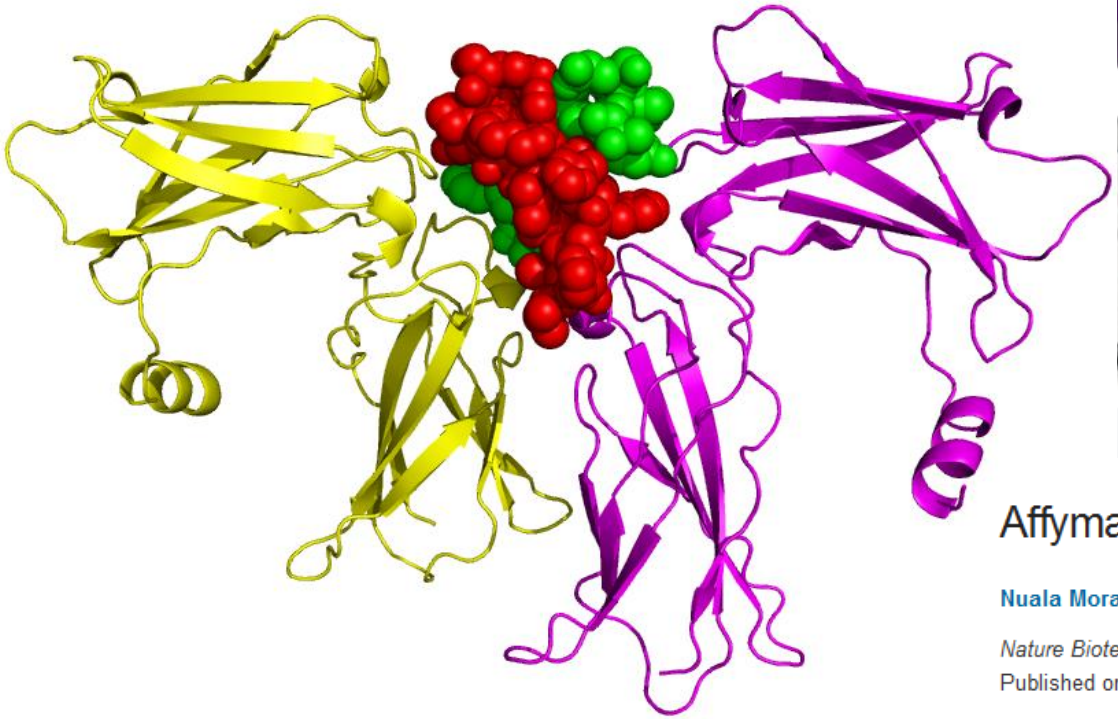
Two families of small peptides that bind to the human thrombopoietin receptor and compete with the binding of the natural ligand thrombopoietin (TPO) were identified from recombinant peptide libraries. The sequences of these peptides were not found in the primary sequence of TPO. Screening libraries of variants of one of these families under affinity-selective conditions yielded a 14–amino acid peptide (Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala) with high affinity (dissociation constant ≈ 2 nanomolar) that stimulates the proliferation of a TPO-responsive Ba/F3 cell line with a median effective concentration (EC_{50}) of 400 nanomolar. Dimerization of this peptide by a carboxyl-terminal linkage to a lysine branch produced a compound with an EC_{50} of 100 picomolar, which was equipotent to the 332–amino acid natural cytokine in cell-based assays. The peptide dimer also stimulated the in vitro proliferation and maturation of megakaryocytes from human bone marrow cells and promoted an increase in platelet count when administered to normal mice.

Science 1997;
276: 1696-1699

Lancet 2008;
371:395-403

Peginesatide of Affymax

CRIGPITWVC



Now Approved!

Omontys®
peginesatide

Affymax poised to challenge Amgen

Nuala Moran

Nature Biotechnology **30**, 377–379 (2012) | doi:10.1038/nbt0512-377
Published online 07 May 2012

Science 1996,273: 458-463; Science 1996,273: 464-471

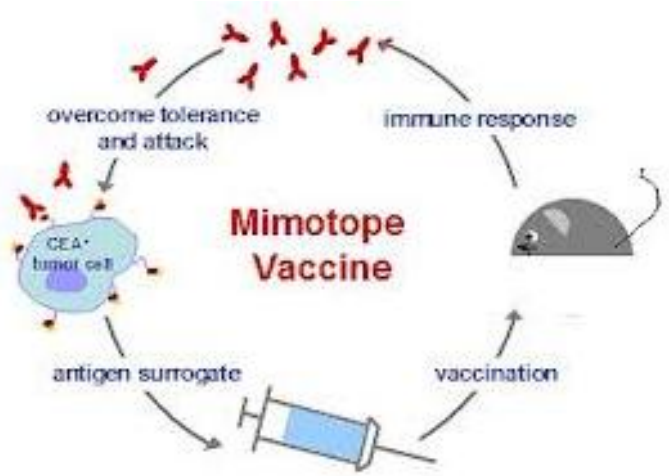
New England Journal of Medicine 2009,361(19):1848-1855

Detection of colonic dysplasia in vivo

VRPMPLQ

☑ *Pei-Lin Hsiung, Jonathan Hardy, Shai Friedland, et al. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy*. **Nature Medicine 2008, 14: 454-458**

Antigen surrogate



Mimotope vaccines

Many microbial pathogens and tumour cells evade immune surveillance by possessing polysaccharides or carbohydrates on their surfaces; this can lead to severe disease and death of the host. This strategy is effective because glycans are poorly immunogenic and fail to elicit immunological memory responses due to the absence of T-cell processing. However, successful vaccines that elicit protection in infants as well as adults against major bacterial pathogens, including *Haemophilus influenzae* [1], *Neisseria meningitidis* serogroup C [2] and *Streptococcus pneumoniae* [3] have been developed by conjugation of the polysaccharide to a carrier protein. Some polysaccharides cannot be converted into effective immunogens by conjugation, because of similarities to host cell structures. The polysialic acid meningococcal group B capsular polysaccharide, for example,

Mimotope *vs* Target-unrelated peptide

IMPEPTIDESET

NHNYPPLSLLTF

SHKLHKV

HAPRWHW

NPEDCFKTGCNSPT

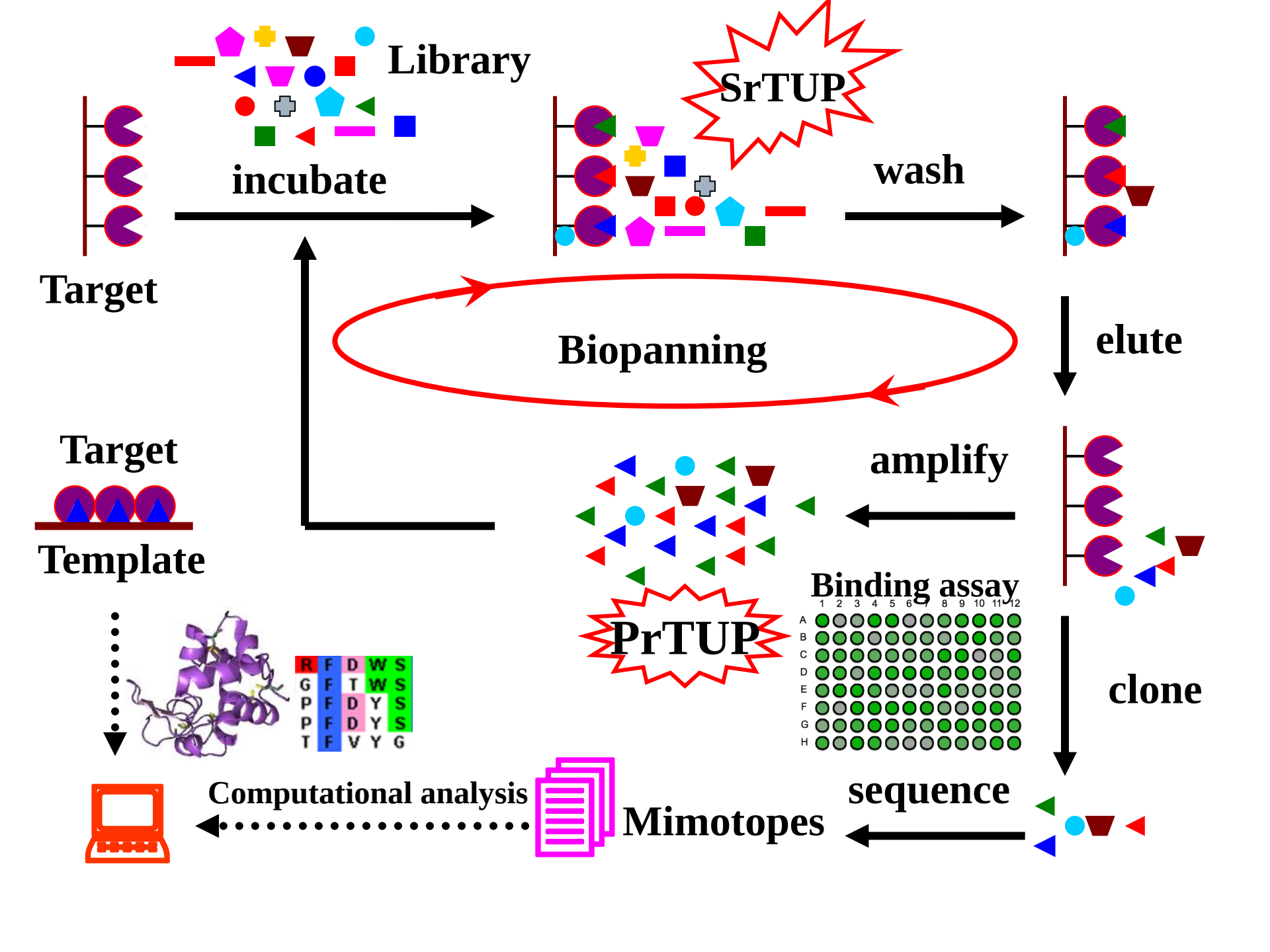
EIDAPRVFDARFSRHYSVQL

WGILSRAATNSGWVGLIPHK

IPSTAFTDIAWVRLPNHY

AFDWTFVPSLIL

LSLMPRL


Library
incubate
SrTUP
wash
Target
Biopanning
elute
Target
Template
amplify
PrTUP
Binding assay
clone
Computational analysis
Mimotopes
sequence

Cleaning phage display data with SAROTUP

- **TUPScan**
  - *if your peptides are with known TUP sequences and motifs*
- **MimoScan**
  - *if any peptide in MimoDB matches your mimotope motifs*
  - *if your mimotope motifs are specific enough*
- **MimoSearch**
  - *if any peptide in MimoDB is identical to your peptides*
  - *if any of your peptides "binds" multiple targets*
- **MimoBlast**
  - *if any peptide in MimoDB is highly similar to your peptides*
  - *if a known TUP has highly similar peptides in the MimoDB database*

SAROTUP：an example for developing cancer vaccine

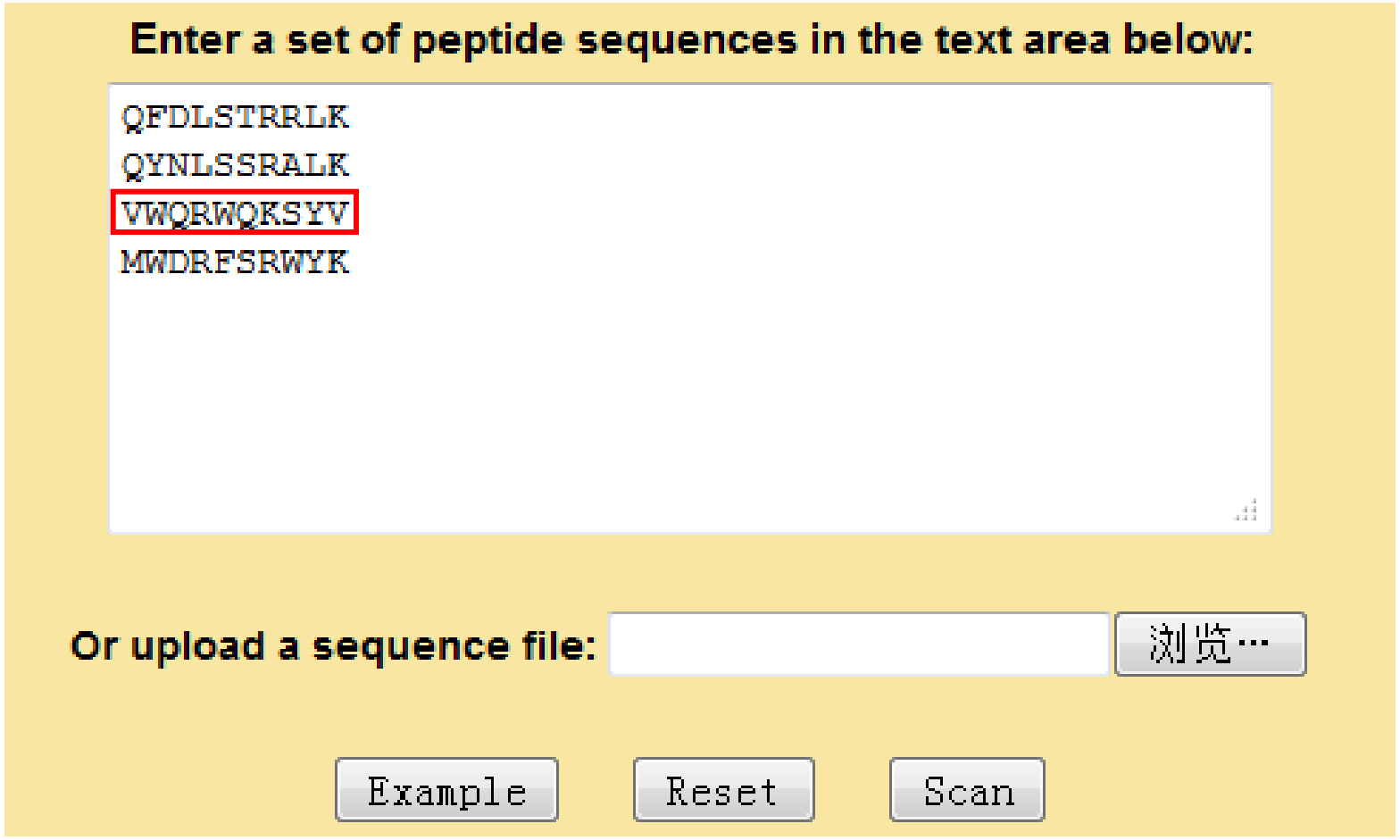
Vaccination With Cetuximab Mimotopes and Biological Properties of Induced Anti–Epidermal Growth Factor Receptor Antibodies

Angelika B. Riemer, Harald Kurz, Markus Klinger, Otto Scheiner, Christoph C. Zielinski, Erika Jensen-Jarolim

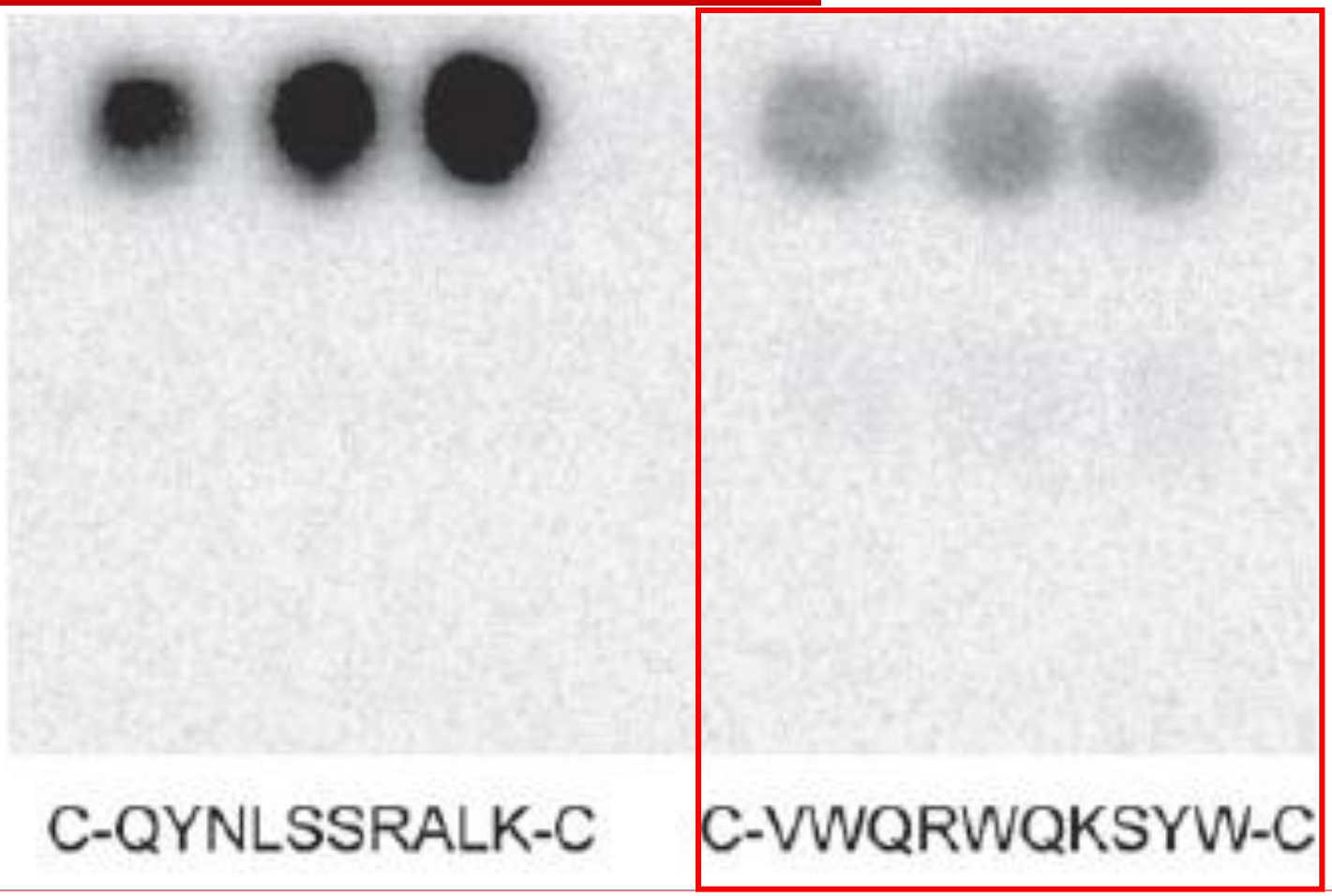
☑ **Department of Pathophysiology (ABR, HK, OS, EJ-J), Department of Surgery (MK), and Clinical Division of Oncology, Department of Medicine I (CCZ), Medical University of Vienna, Vienna, Austria.**

☑ **Journal of the National Cancer Institute, Vol. 97, No. 22, pp 1663-1670, November 16, 2005.**

TUPScan


Enter a set of peptide sequences in the text area below:
QFDLSTRRLK
QYNLSSRALK
VWQRWQKSYV
MWDRFSRWYK
Or upload a sequence file:
浏览…
Example
Reset
Scan

Dot blot analysis of antibody recognition of synthetic cyclic mimotopes



Vaccine Constructs and Immune Responses Induced by Mimotope Vaccination

- ☑ **Riemer *et al*. synthesized two vaccine constructs with the peptide QYNLSSRALK and VWQRWQKSYV respectively.**
- ☑ **After immunization mice with these constructs, they found that either the cetuximab or the antibodies induced by the QYNLSSRALK vaccine construct inhibited the growth of A431 cancer cells significantly.**
- ☑ **The inhibition of the antibodies induced by the VWQRWQKSYV vaccine construct however, was not statistically significant when compared with the inhibition caused by the isotype control antibody**

MimoScan

The MimoScan tool in the SAROTUP suite is designed to check if there is any peptide in the MimoDB database that matches patterns submitted. If any, the tool will list them for you to facilitate further analysis. The query can be consensus sequences, motifs or patterns derived from TUPs or your panning results. Besides the commonly used simple format, e.g. WXXW, **patterns in PROSITE format** are fully surpported. Here we go!

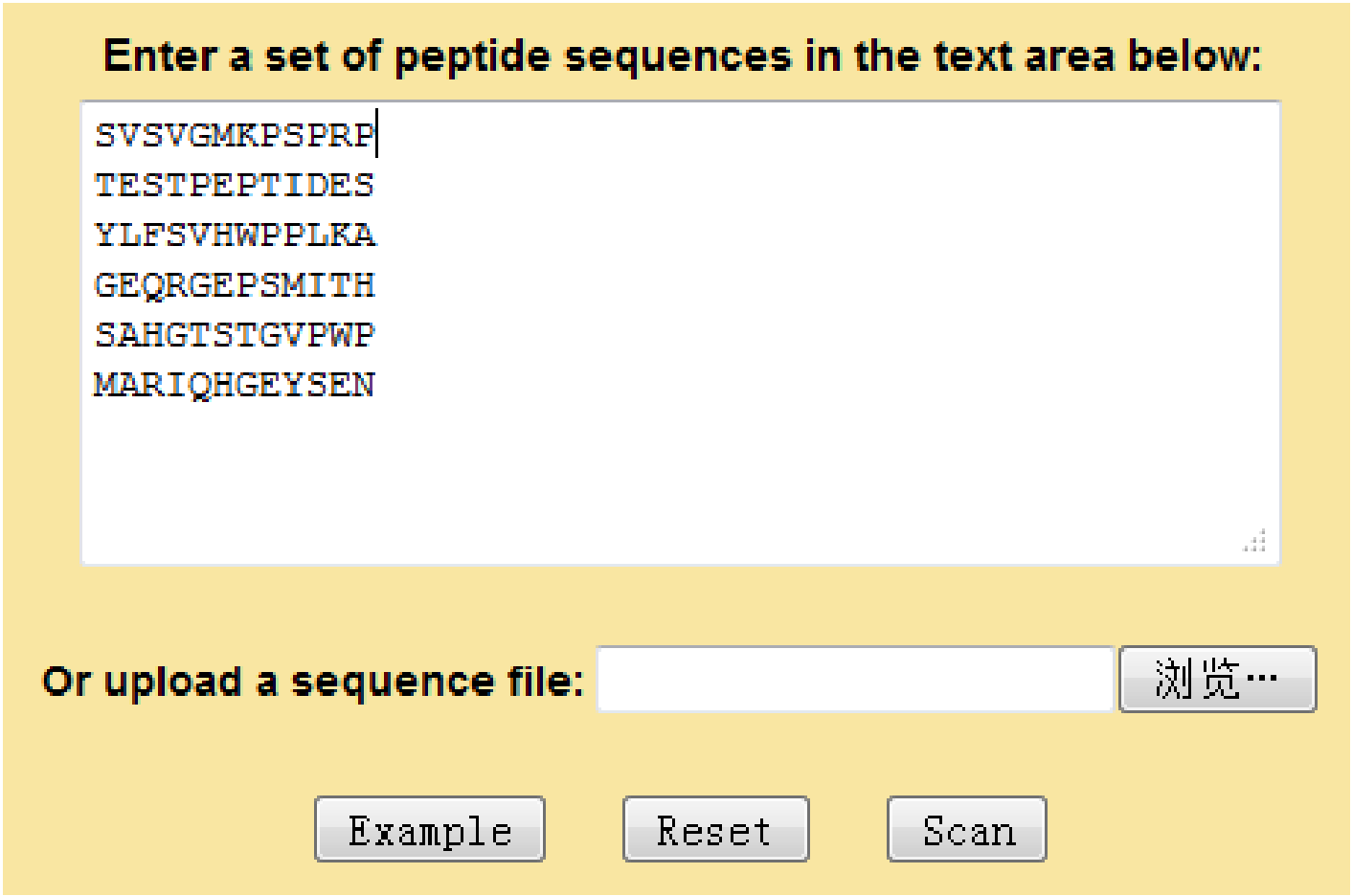
Enter one or more peptide patterns in the text area below:

```
W-x-P(2)-F-[KR]
F-[GSTV]-P-R-L-G
W-[TS]-[LI]-x(2)-H-[RK]
```

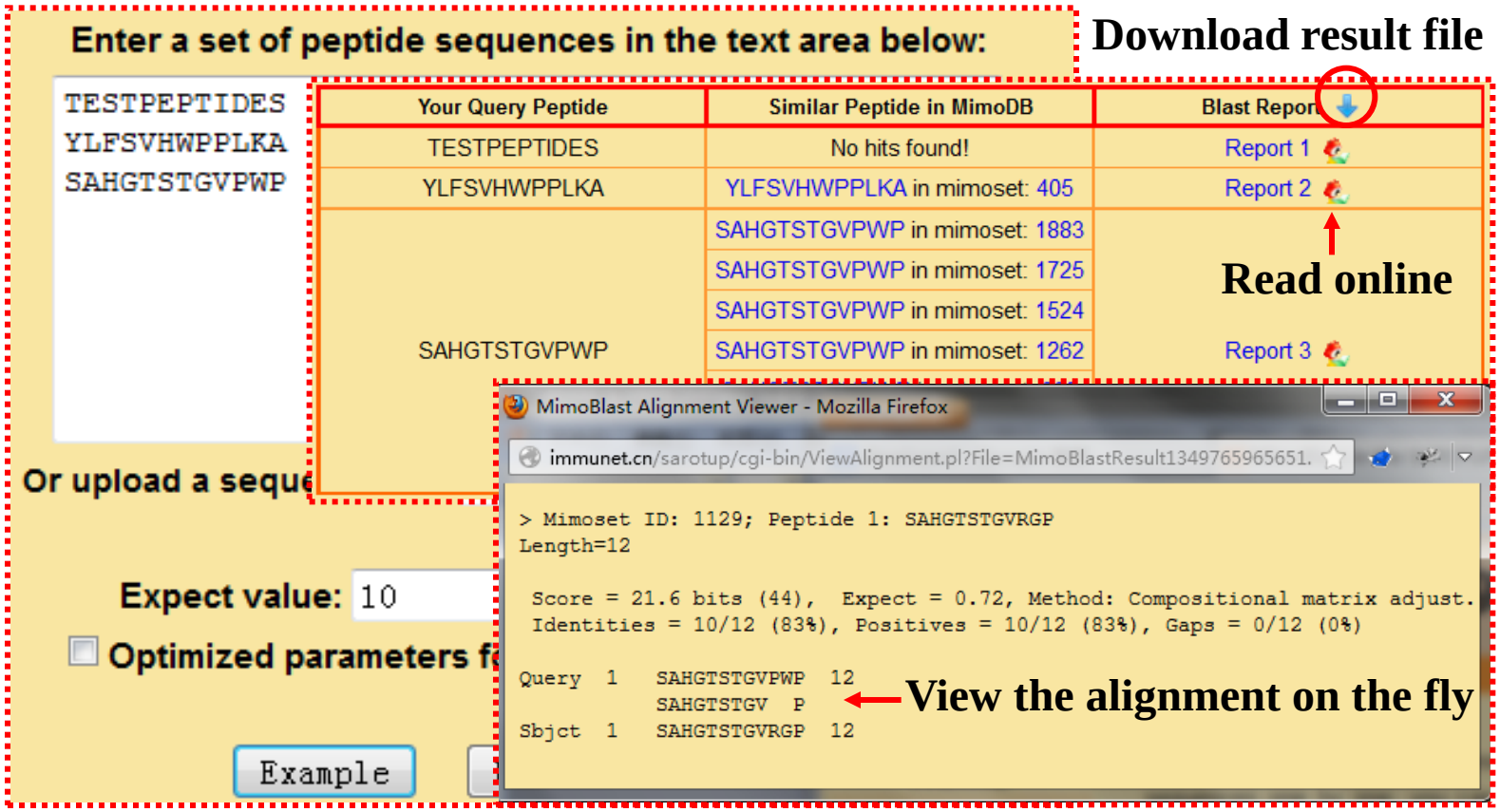
Or upload a pattern file: 浏览…

Example Reset Scan

MimoSearch


Enter a set of peptide sequences in the text area below:
SVSVGMKPSPRP
TESTPEPTIDES
YLFSVHWPPLKA
GEQRGEPSMITH
SAHGTSTGVPWP
MARIQHGEYSEN
Or upload a sequence file:
浏览…
Example
Reset
Scan

MimoBlast


Enter a set of peptide sequences in the text area below:
TESTPEPTIDES
YLFSVHWPPLKA
SAHGTSTGVPWP
Download result file
Your Query Peptide
Similar Peptide in MimoDB
Blast Report
TESTPEPTIDES
No hits found!
Report 1
YLFSVHWPPLKA
YLFSVHWPPLKA in mimoset: 405
Report 2
SAHGTSTGVPWP in mimoset: 1883
SAHGTSTGVPWP in mimoset: 1725
SAHGTSTGVPWP in mimoset: 1524
SAHGTSTGVPWP in mimoset: 1262
Report 3
Read online
Or upload a seque
Expect value: 10
Optimized parameters f
Example
MimoBlast Alignment Viewer - Mozilla Firefox
immunet.cn/sarotup/cgi-bin/ViewAlignment.pl?File=MimoBlastResult1349765965651.
> Mimoset ID: 1129; Peptide 1: SAHGTSTGVRGP
Length=12
Score = 21.6 bits (44), Expect = 0.72, Method: Compositional matrix adjust.
Identities = 10/12 (83%), Positives = 10/12 (83%), Gaps = 0/12 (0%)
Query 1 SAHGTSTGVPWP 12
SAHGTSTGV P
Sbjct 1 SAHGTSTGVRGP 12
View the alignment on the fly

Acknowledgements