Drug design: genomics and proteomics

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Learning outcomes

- Gene therapy
- Gene therapy: inherited disorders
- Gene therapy: cancer
- The impact of biotechnology on drug discovery
- Proteomics: studying proteomics

Gene therapy

- *Ex-vivo* techniques
  - Explant cells, insert gene, reimplant cells
- *In-vivo* techniques
  - Insert gene already inside the host (need for useful vessels)

The ‘ideal’ gene delivery system *should*…

1) Take up also larger DNA/RNA-pieces
2) Be easily handled and manufactured in concentrated form
3) Target specific cells
4) Not be inactivated (=long term activity)
5) Not exhibit toxic properties
6) Not exert immunogenic responses

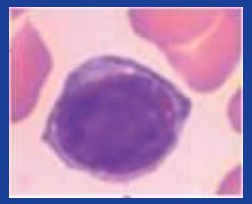
Vectorial systems

A) Plasmids
   - *Used in cystic fibrosis*

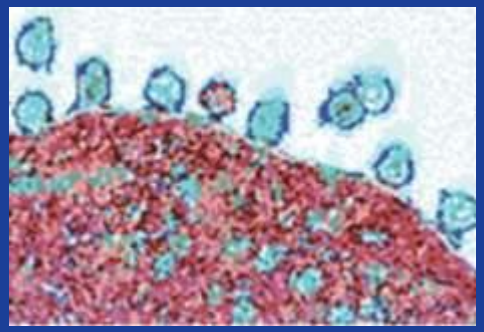
B) Viral vectors
   i. Adeno viruses
   ii. Retroviruses
   iii. Lentiviruses

ADA
(adenosine-deaminase deficiency)

isolate



infect



incorporate

reinsert

cultivate

Cystic fibrosis

- Caused by a mutation in a gene called the cystic fibrosis transmembrane conductance regulator (CFTR-del508)
- CFTR = chloride channel → trafficking deficit
- Gene therapy attempts to place a normal copy of the CFTR gene into affected cells
- Only 5–10% of the normal amount of CFTR gene expression is needed
- “There is a realistic prospect of a clinically effective treatment in the next 10 years” (Tate & Elborn, 2005)

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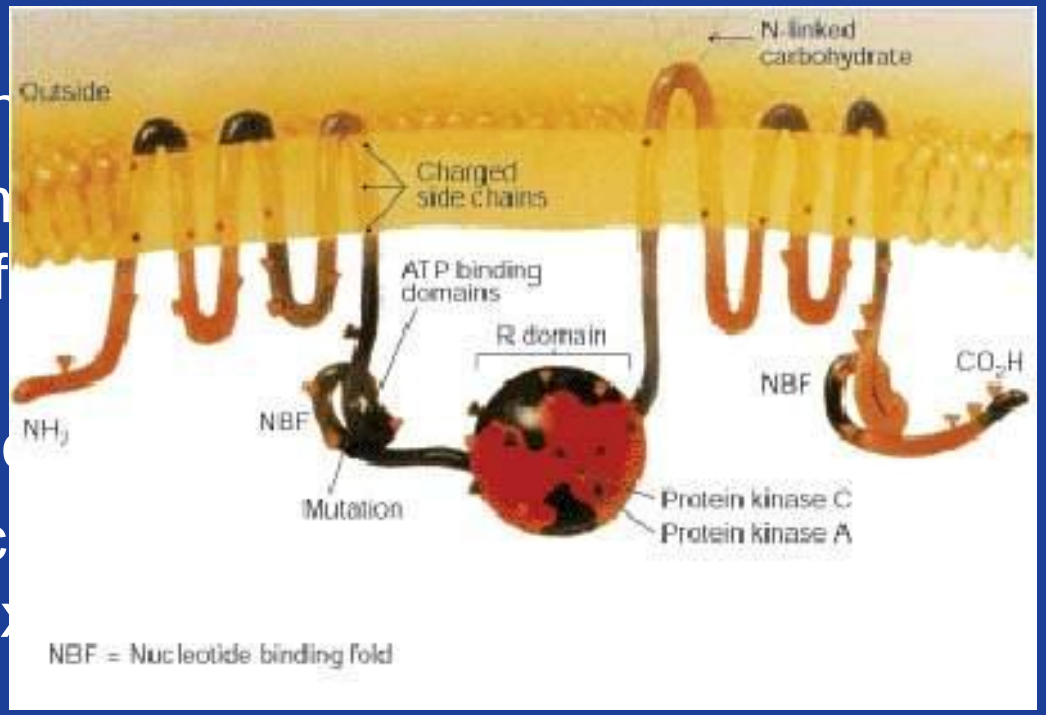
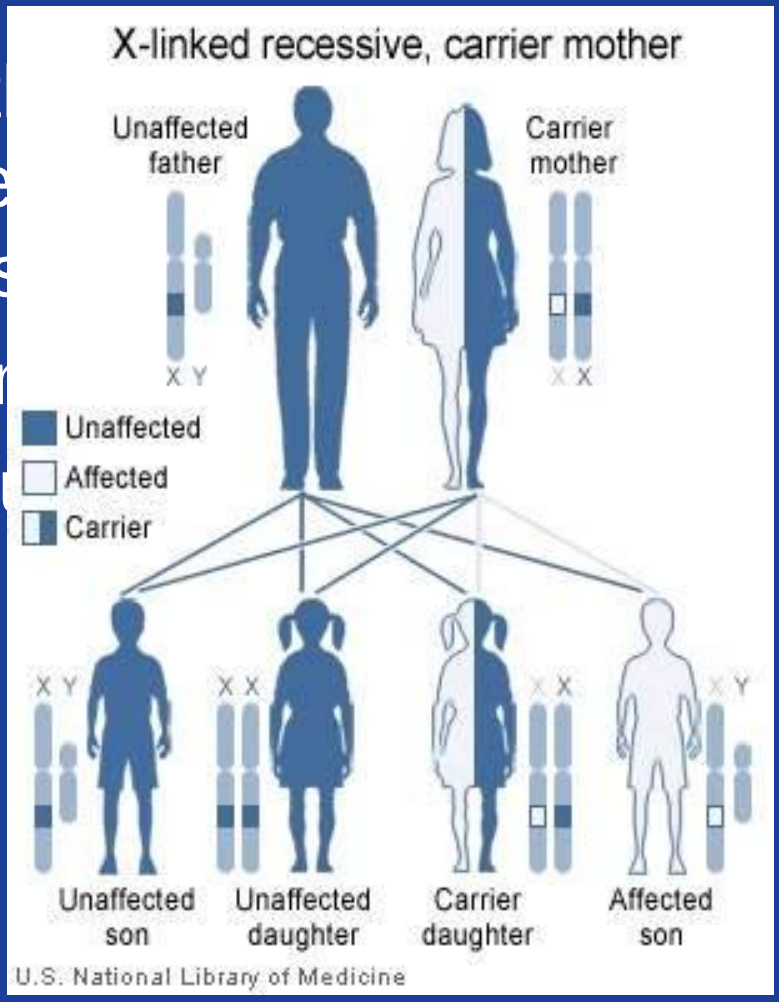


Duchenne muscular dystrophy

- Caused by mutations in t
  encodes dystrophin, an e
  membrane protein in mus
- Stem cell research = pro
- U7 gene transfer techniq

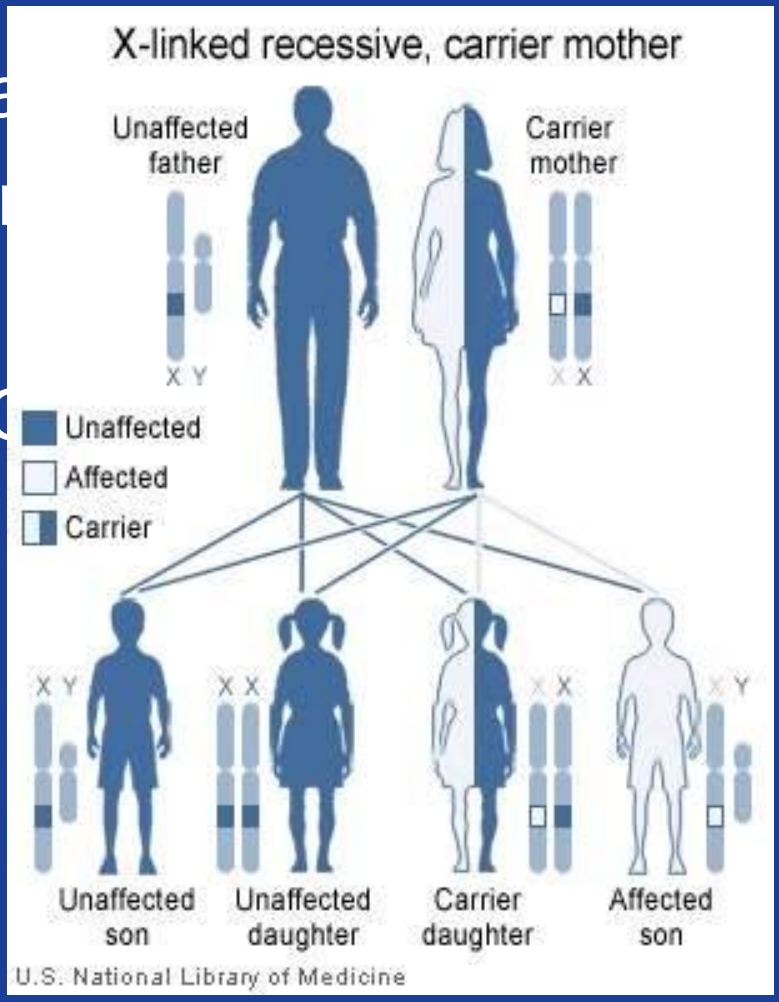
- Genetic counselling



DMD, duchenne muscular dystrophy

Haemophilia

- Bleeding disorder, severa
- Clotting factors are missi
- Today: production in CH



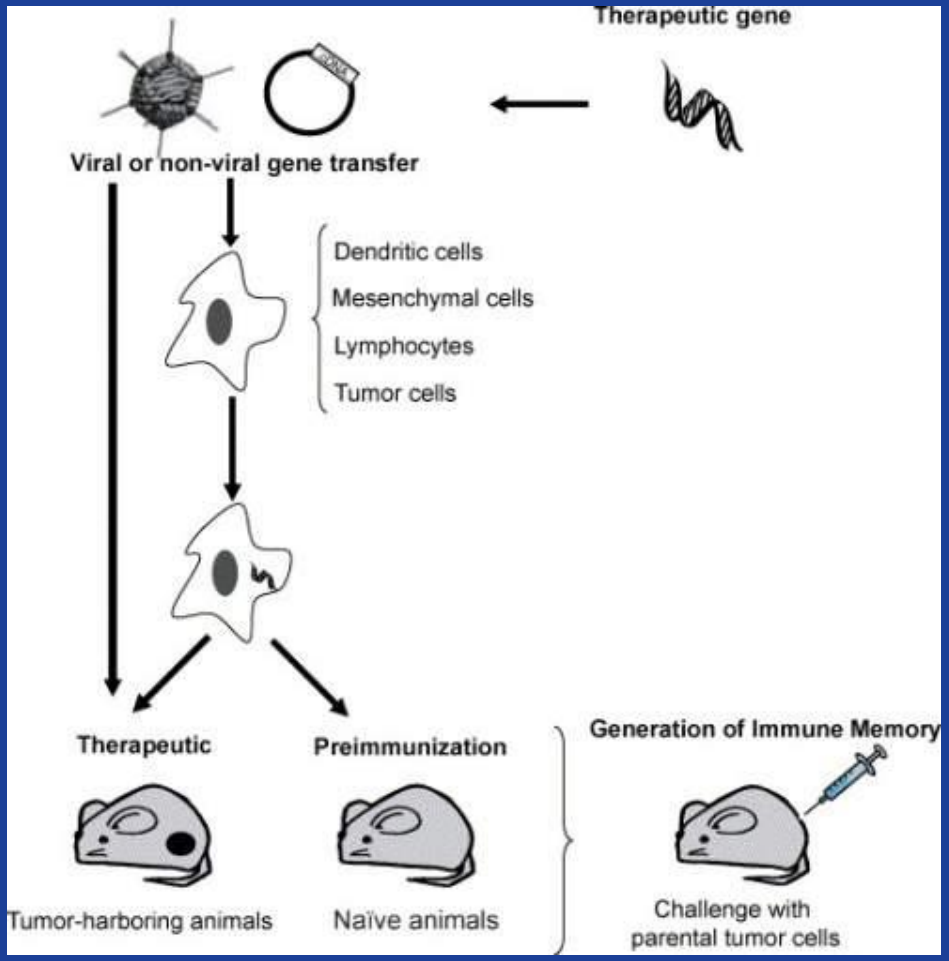
CHO, chinese hamster ovary

Gene therapy in cancer

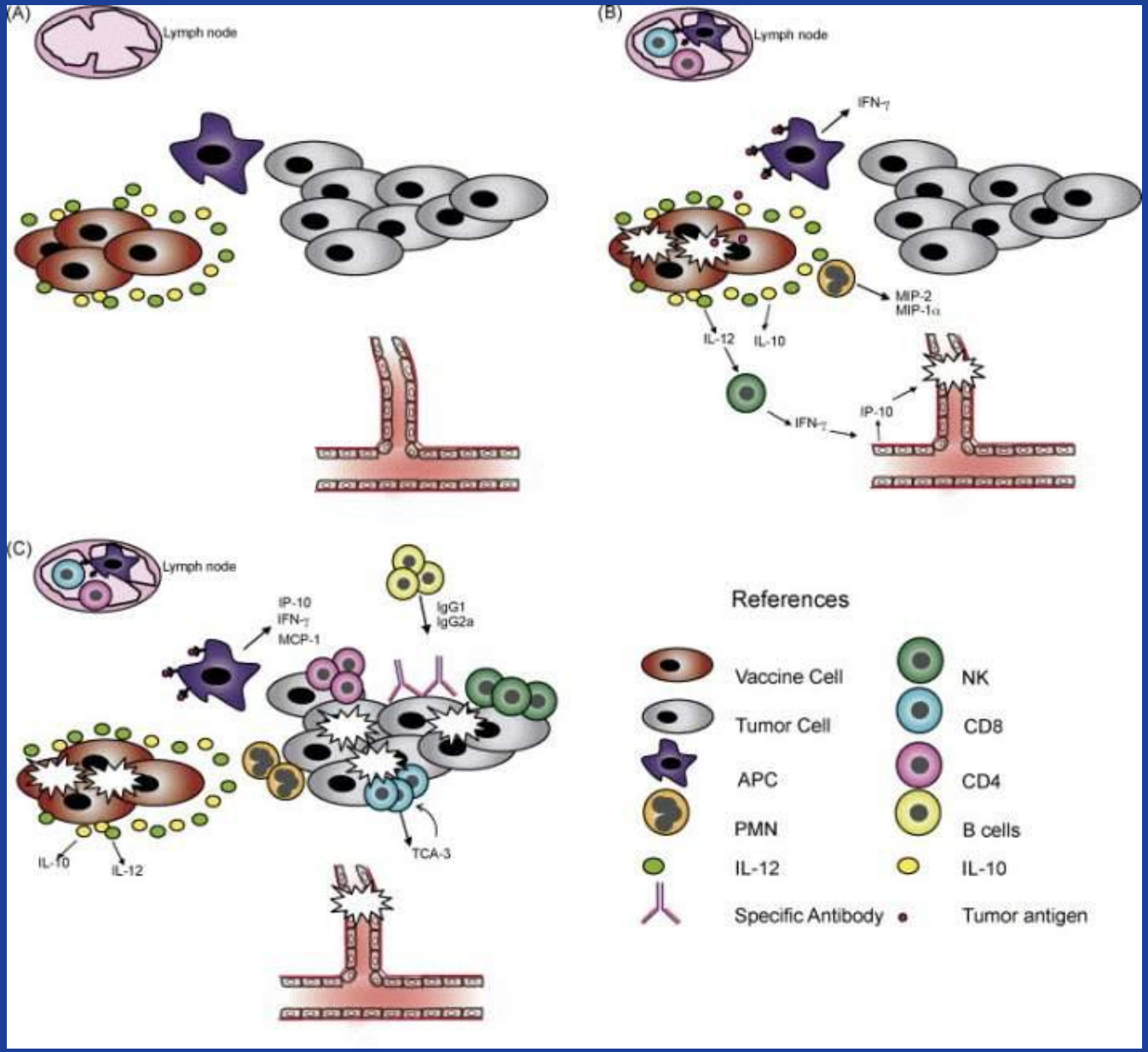
- ***The major problems in cancer therapy with biopharmaceuticals:***

- **“The immunogenicity problem, the killing problem and the target problem”**

Cytokine genes

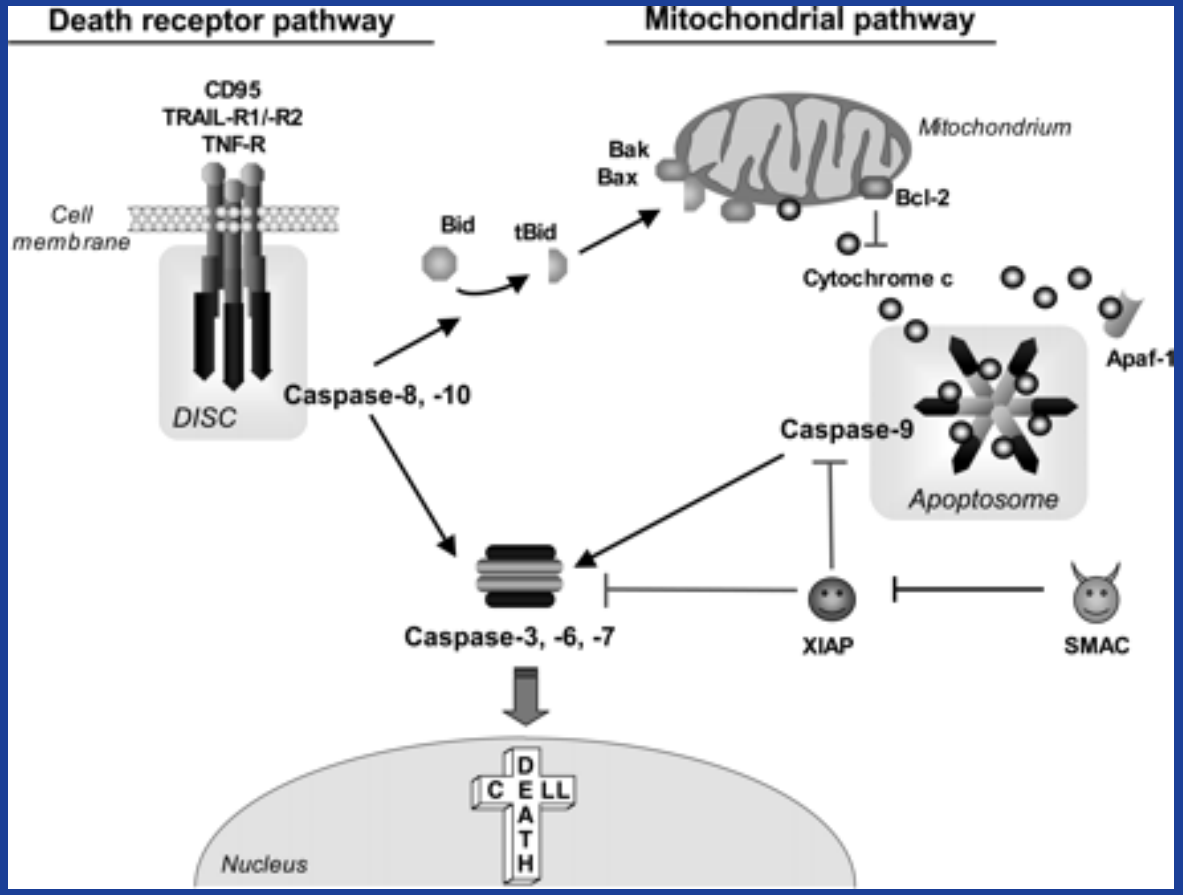


Podhajcer et al. *Cytokine & Growth Factor Reviews* 2007



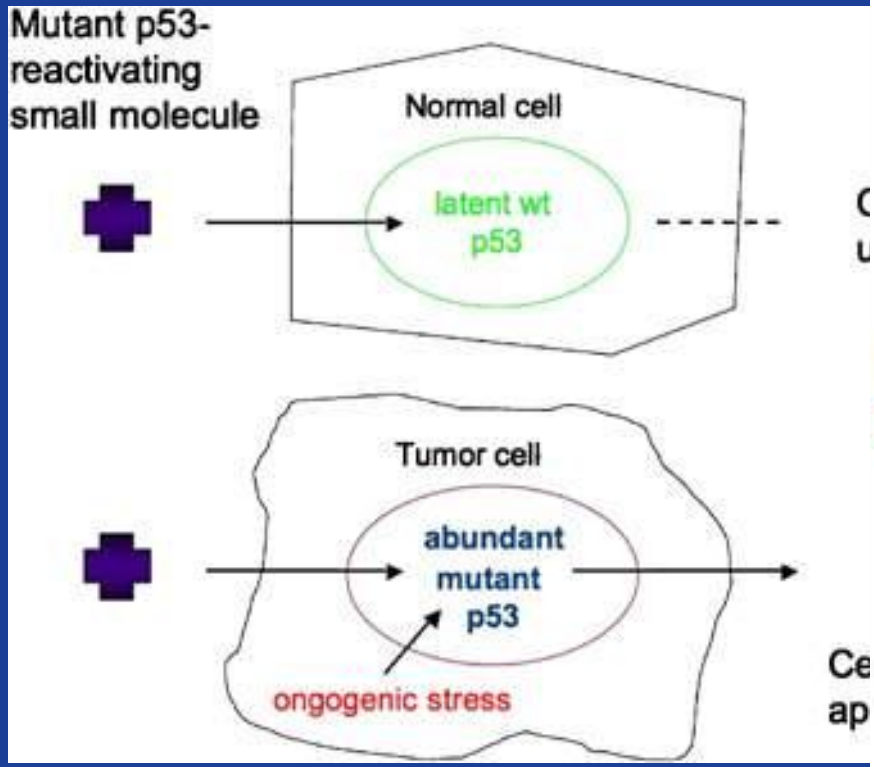
Podhajcer et al. *Cytokine & Growth Factor Reviews* 2007

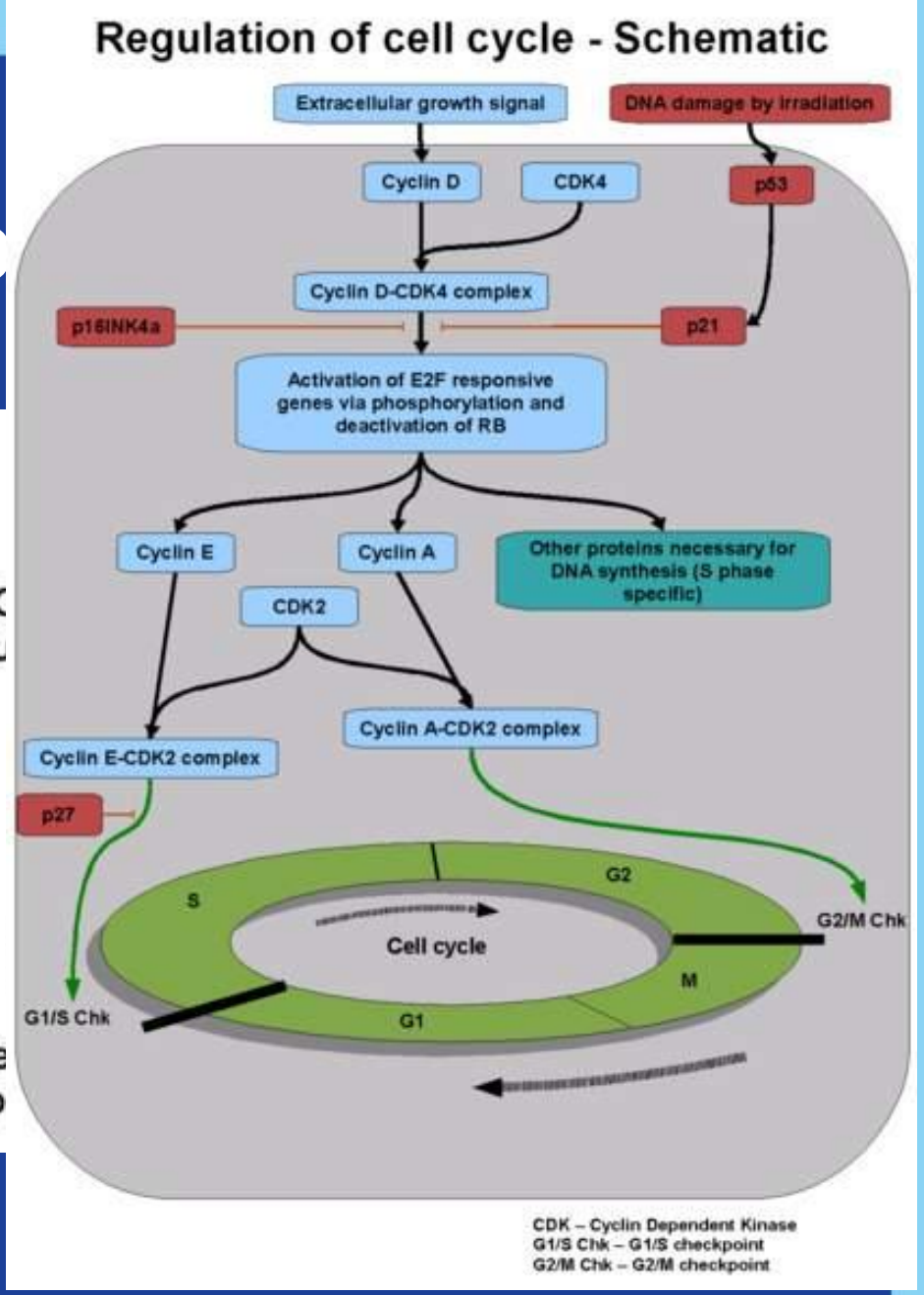
Suicide genes



Fischer and Schulze-Osthoff, 2005

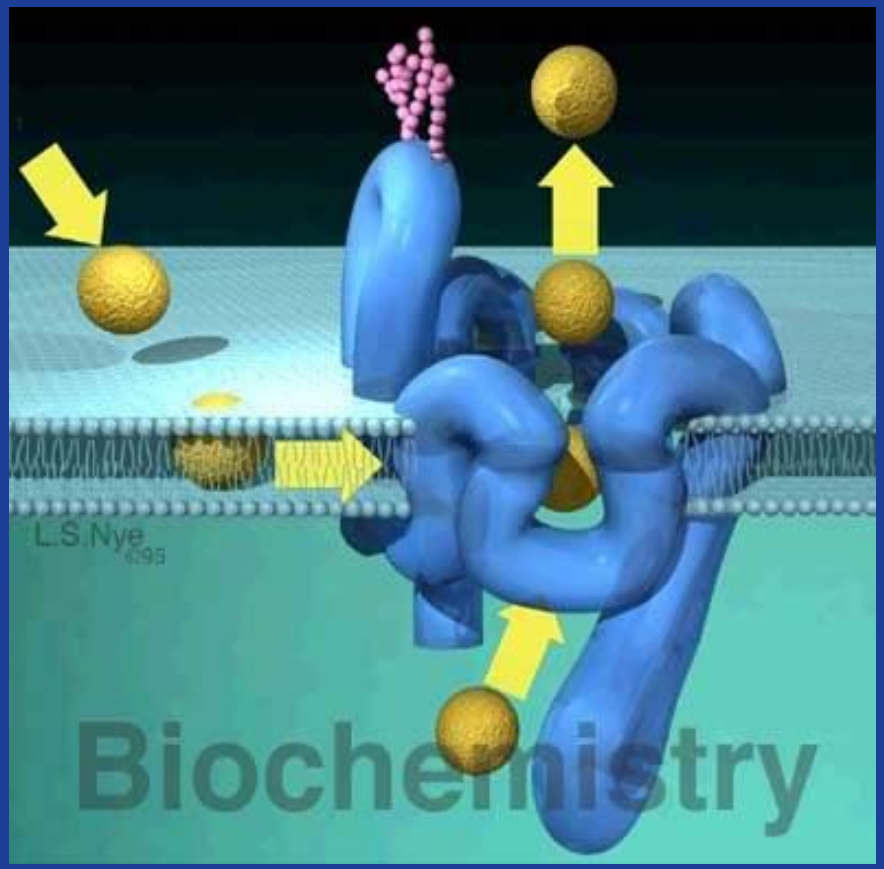
Tumour supp



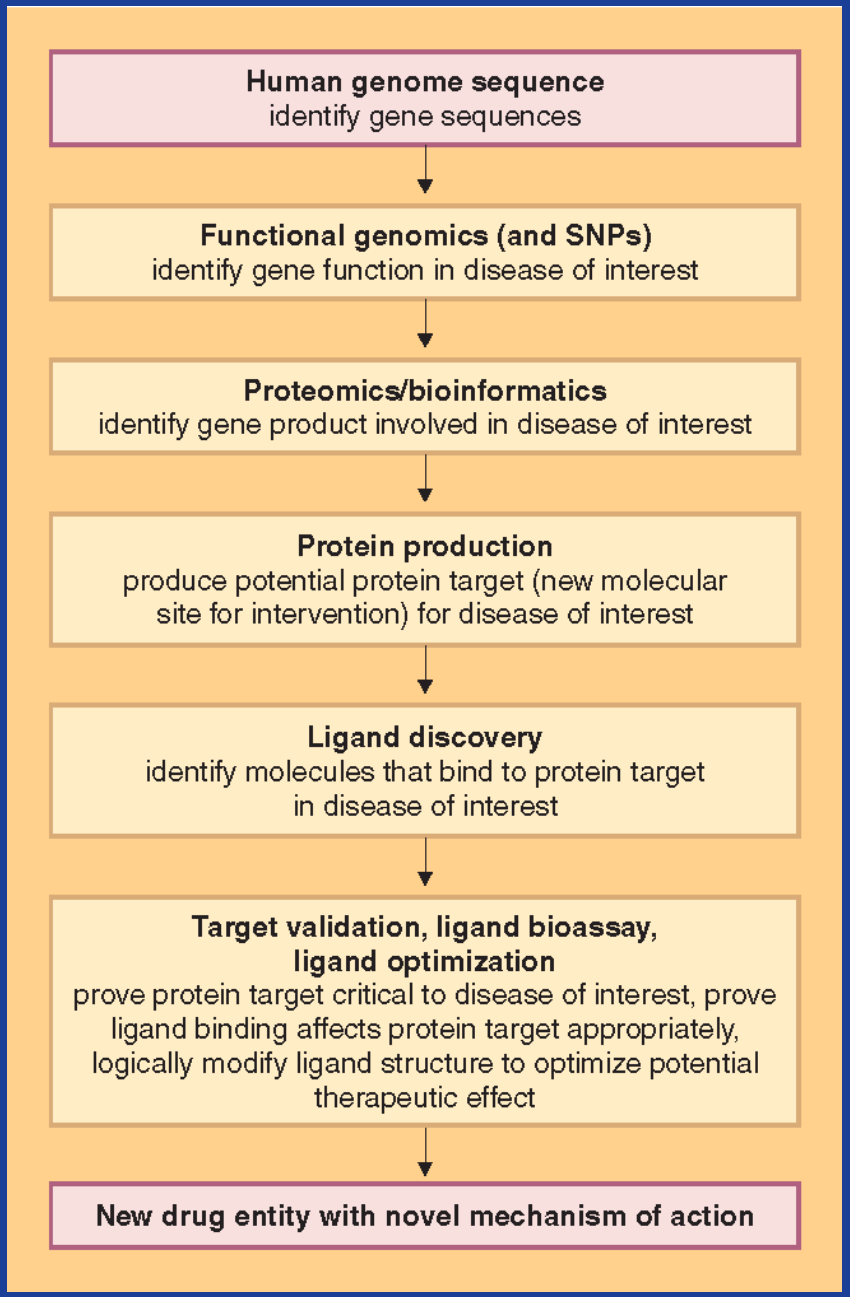


‘the guardian of the genome’ p53

Multiple drug resistance gene (MDR-i)

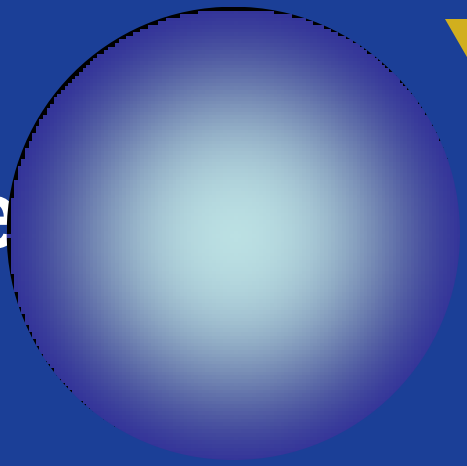


http://www.medartist.com/nye5.jpg


Human genome sequence
identify gene sequences
Functional genomics (and SNPs)
identify gene function in disease of interest
Proteomics/bioinformatics
identify gene product involved in disease of interest
Protein production
produce potential protein target (new molecular site for intervention) for disease of interest
Ligand discovery
identify molecules that bind to protein target in disease of interest
Target validation, ligand bioassay, ligand optimization
prove protein target critical to disease of interest, prove ligand binding affects protein target appropriately, logically modify ligand structure to optimize potential therapeutic effect
New drug entity with novel mechanism of action

Rational drug design

Identification of target structure



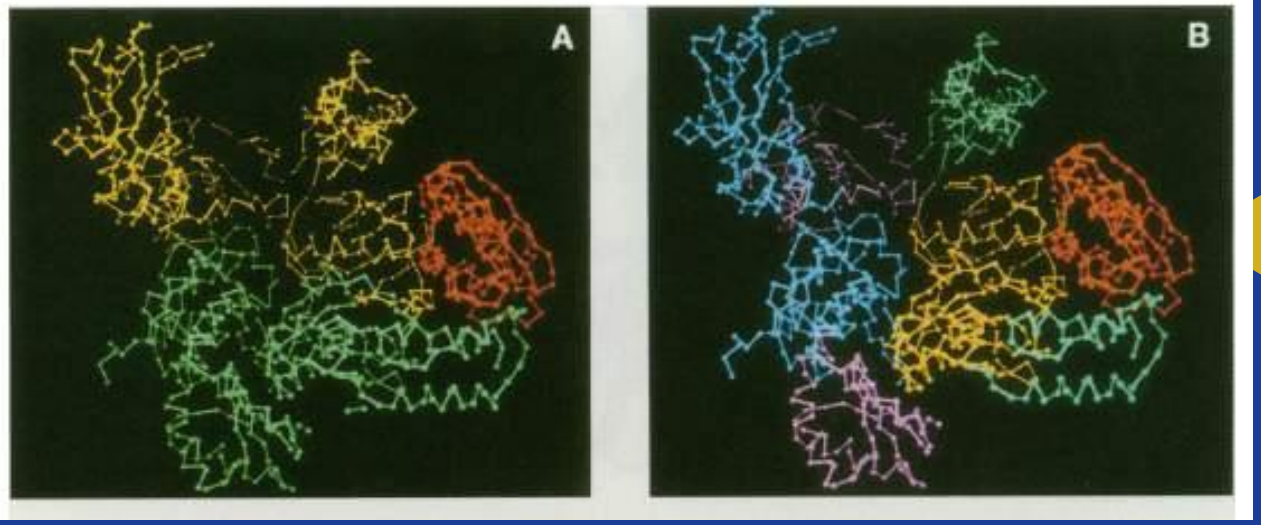
eg HIV reverse transcriptase

‘Lead’ compound

Proteomics
Genomics
Structural biology
Bioinformatics

‘Omics’ approach

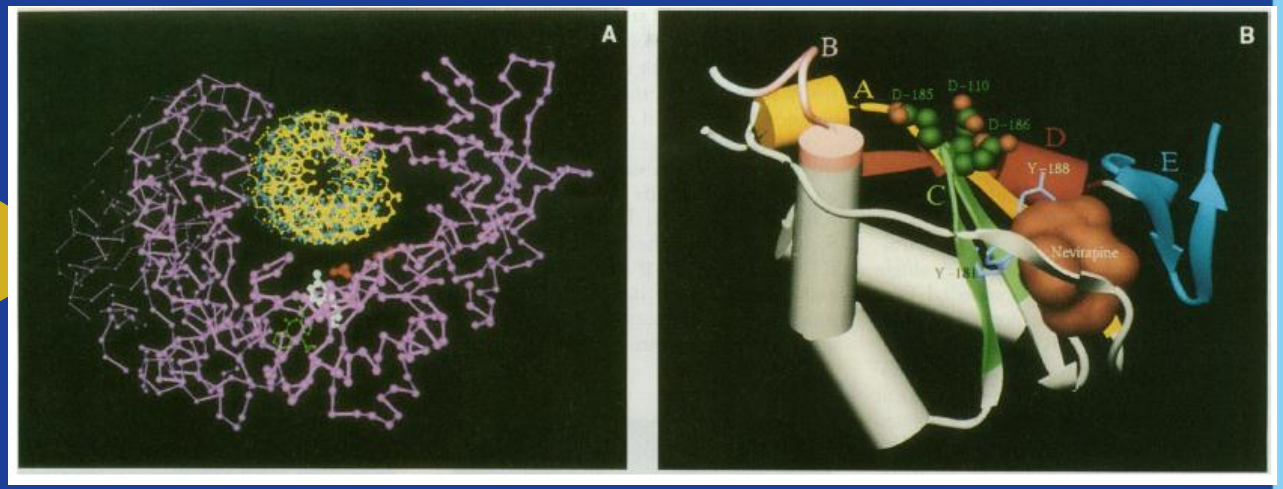
Rational drug design



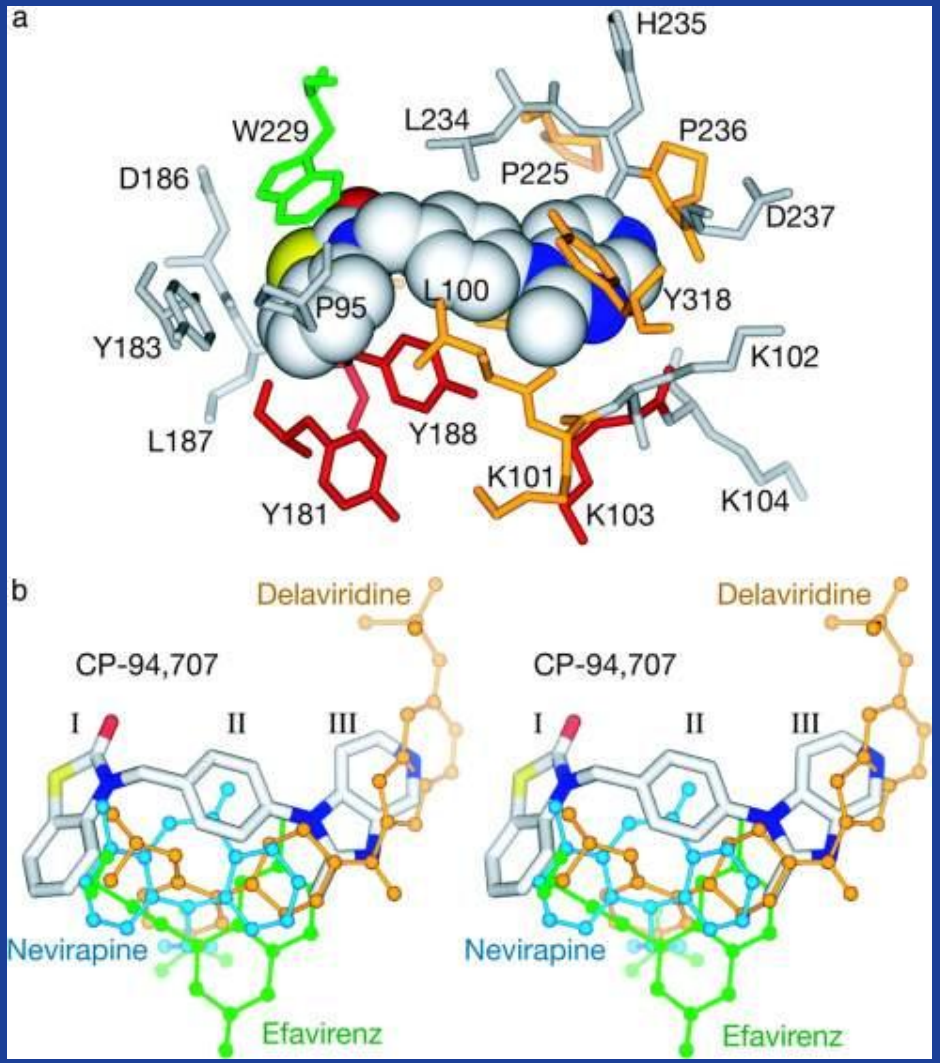
Crystal-structure

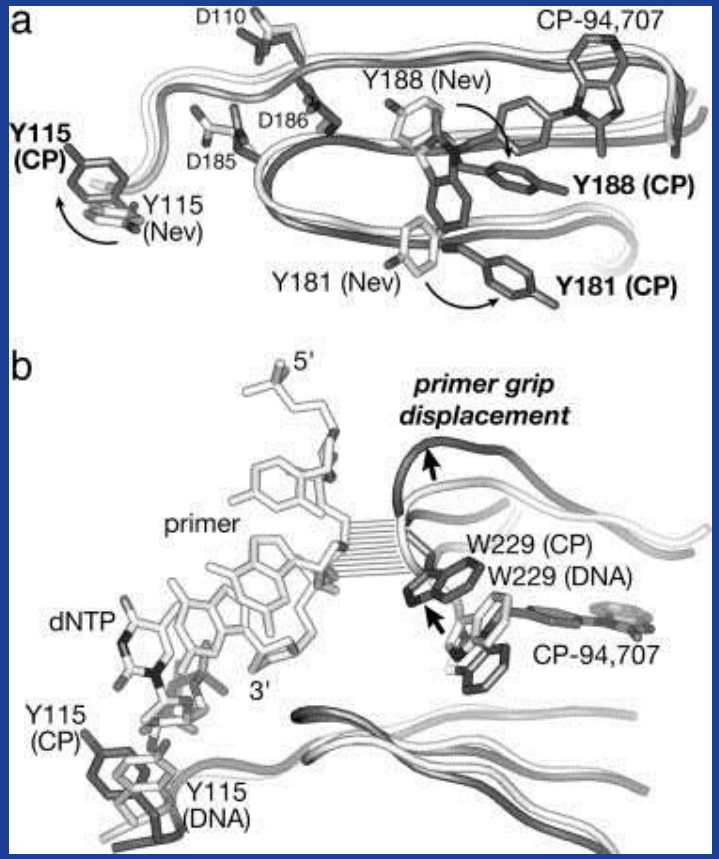
Kohlstaedt et al. Crystal structure at 3.5. A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 1992;26(256):1783–1790

Crystal-structure + nevirapine



Rational drug design





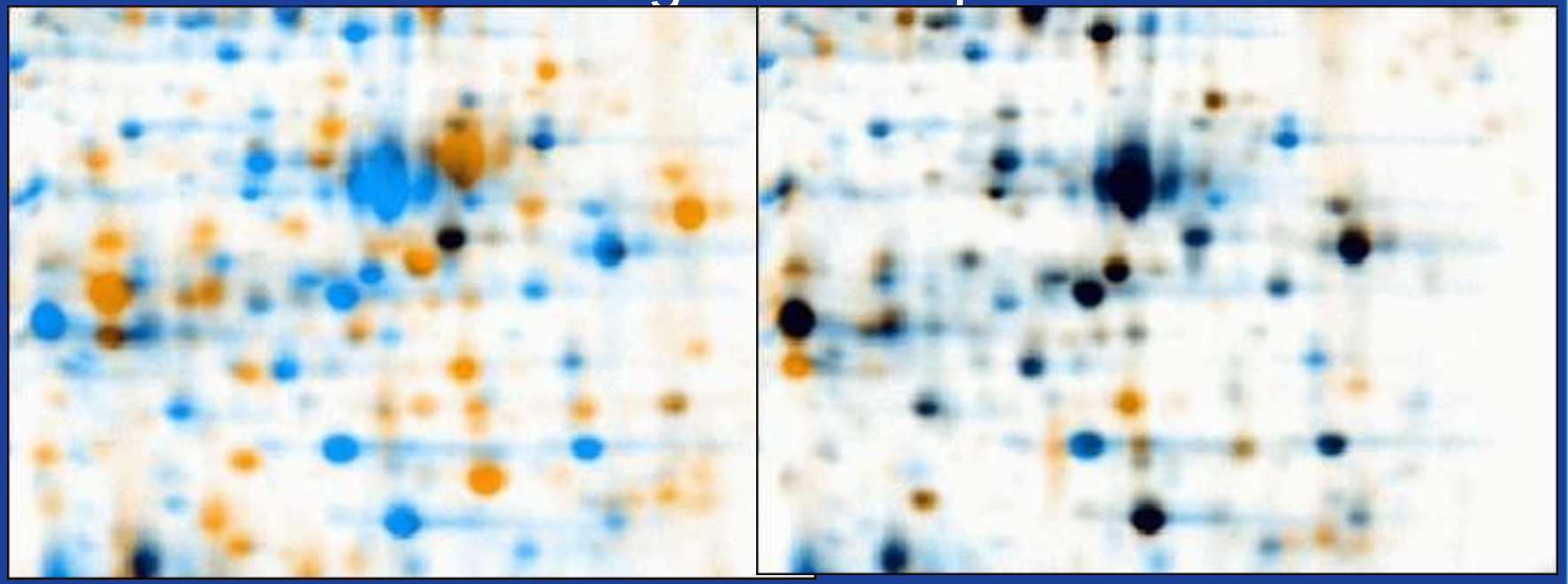
Pata et al. Structure of HIV-1 reverse transcriptase bound to an inhibitor active against mutant reverse transcriptases resistant to other nonnucleoside inhibitors. *PNAS* 2004;101:10548–10553

Proteomics

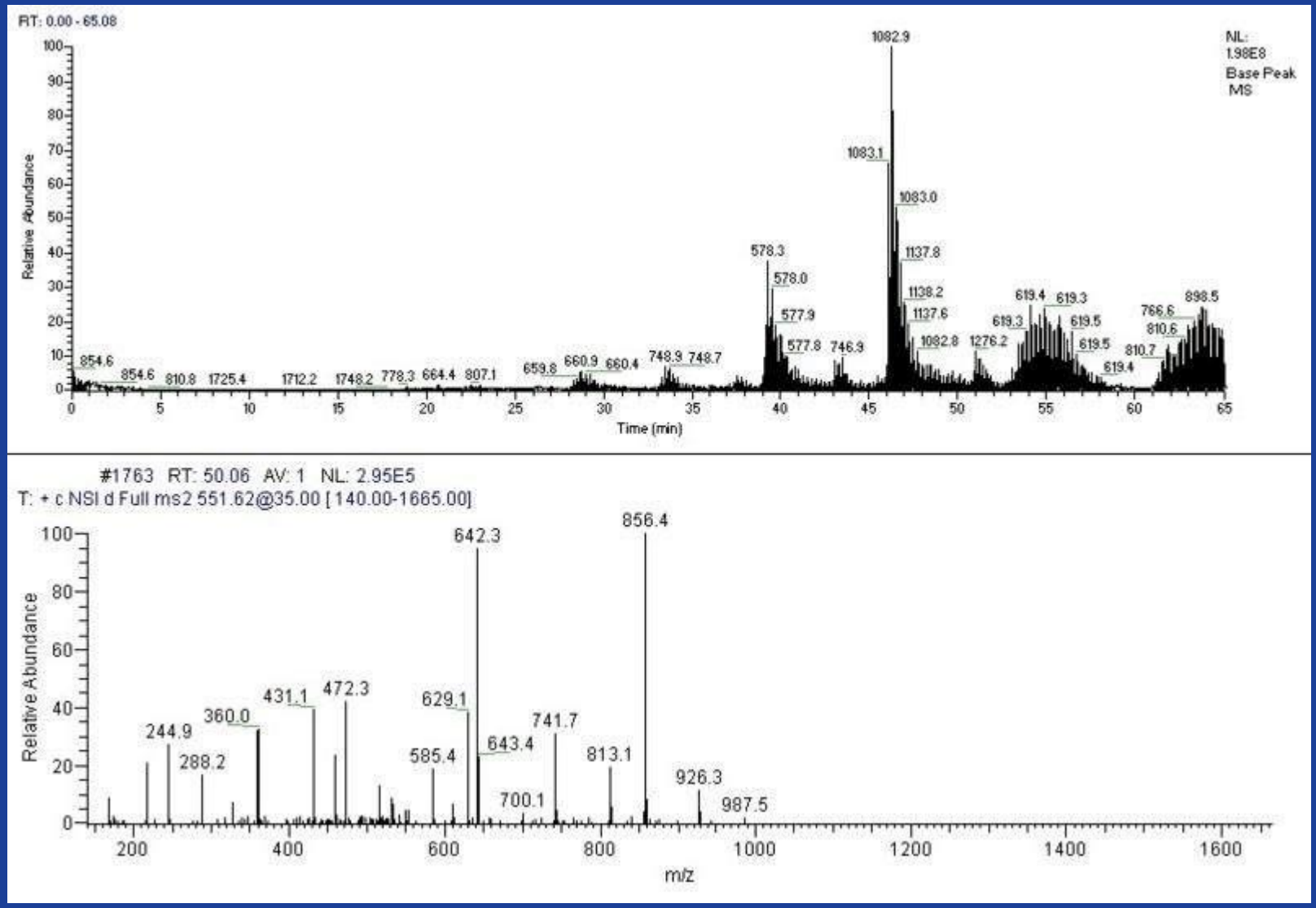
Robotic preparation of MALDI mass spectrometry samples on a sample carrier

Protein separation techniques

- Two-dimensional gel electrophoresis



‘Warping’


RT: 0.00 - 65.08
NL:
1.98E8
Base Peak
MS
Relative Abundance
Time (min)
1082.9
1083.1
1083.0
578.3
578.0
577.9
1137.8
1138.2
1137.6
1082.8
1276.2
619.4
619.3
619.3
619.5
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766.6
810.6
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898.5
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854.6
810.8
1725.4
1712.2
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Relative Abundance
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642.3
472.3
431.1
629.1
360.0
741.7
244.9
643.4
585.4
813.1
288.2
926.3
700.1
987.5

Protein quantification

- By incorporation of radioactive isotopes into the sample
- Carbon (^{13}C) or nitrogen (^{15}N)

- eg SILAC (stable isotope labellings with amino acids in cell culture)

Protein sequence analysis

- Part of bioinformatics
- Searching databases for possible protein or peptide matches by different algorithms
- Databases include Mascot, PEAKS (software), OMSSA, SEQUEST and X!Tandem
- The prime goals are: functional assignment of domains, prediction of function from sequence, and evolutionary relationships of proteins

Conclusion slide

- Gene therapy
  - *Promising technique to treat…*
- Gene therapy: inherited disorders
  - *Examples for genetically encoded disease*
- Gene therapy: cancer
  - *New strategies for cancer treatment*
- The impact of biotechnology on drug discovery
  - *From genome to drug – impact of…*
- Proteomics: studying proteomics
  - *How to examine the structure and nature of proteins*