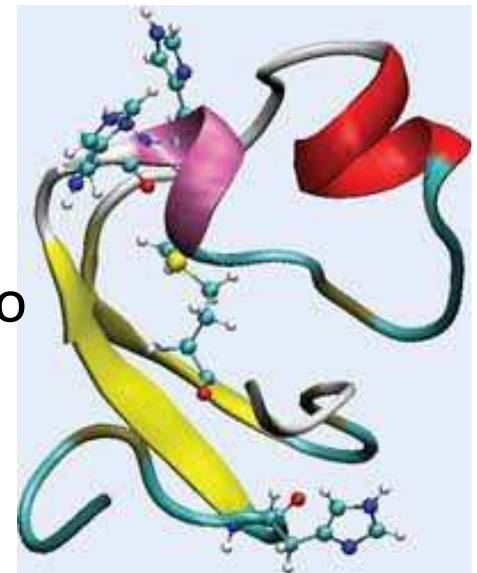


La struttura delle biomolecole e la loro importanza per la salute e le biotecnologie



Gianfranco Gilardi

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Accademia delle Scienze

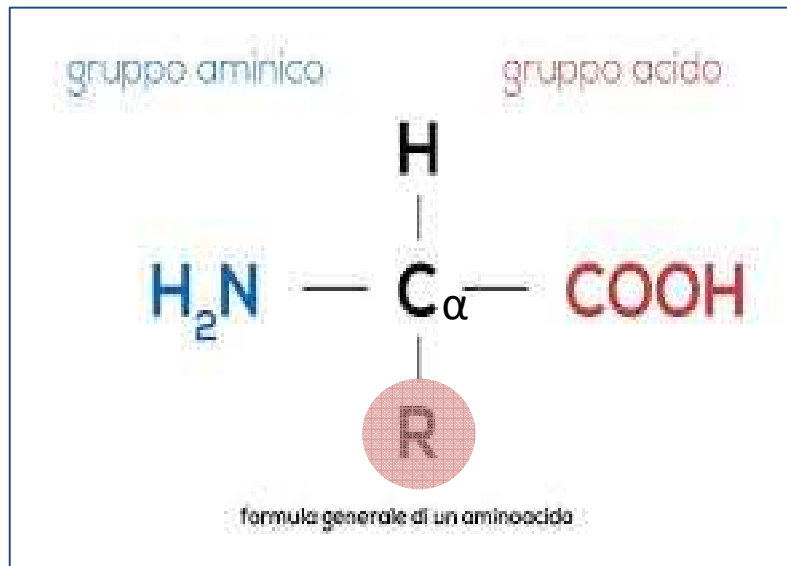


Incontro Nazionale LS-OSA, Torino 10 aprile 2014

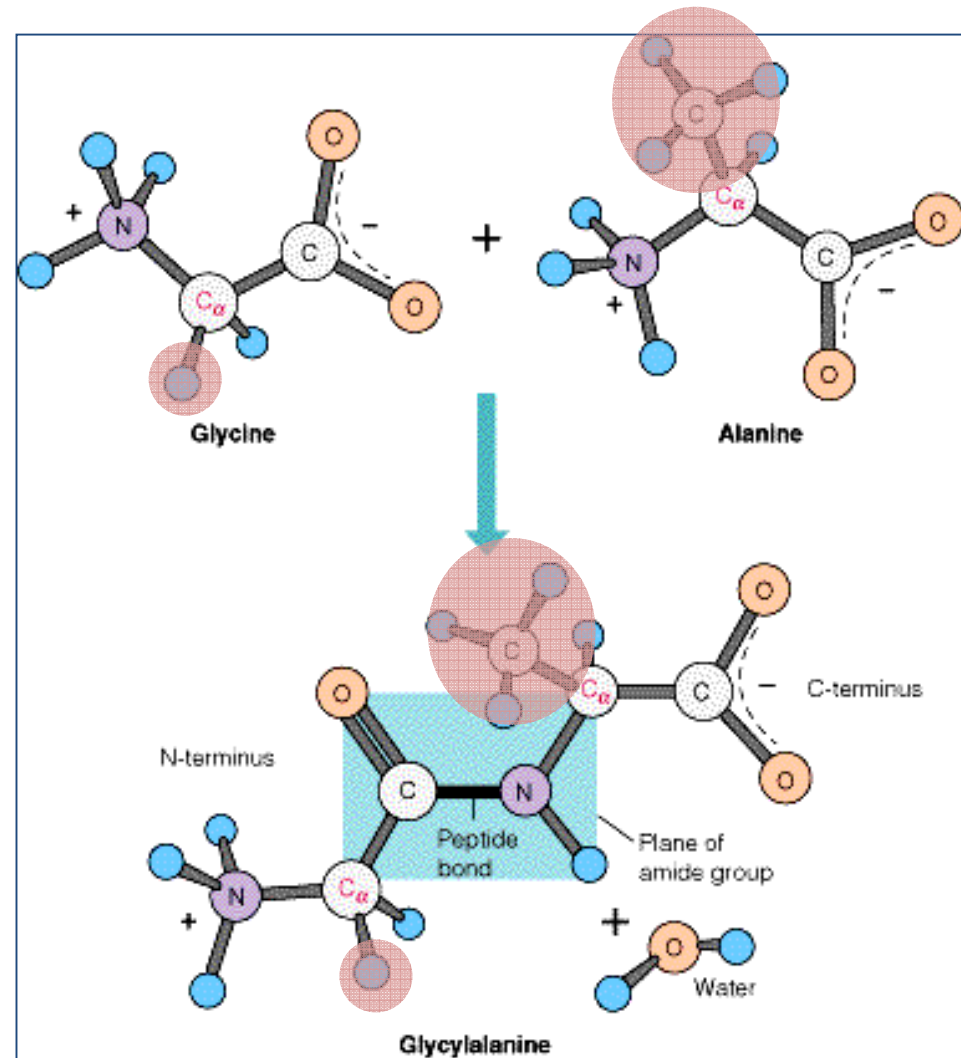
Piano della presentazione

- La struttura 3D delle proteine: rilevanza per la biologia, medicina, farmacia, biotecnologie
- Cenni storici
- Sviluppo di farmaci basati sulla struttura delle proteine:
 - Anti- batterici, Anti-micotici, Anti-virali
- Malattie legate al mis-folding delle proteine
 - Alzheimer, Parkinson, prioni
- Sguardo al futuro e conclusioni

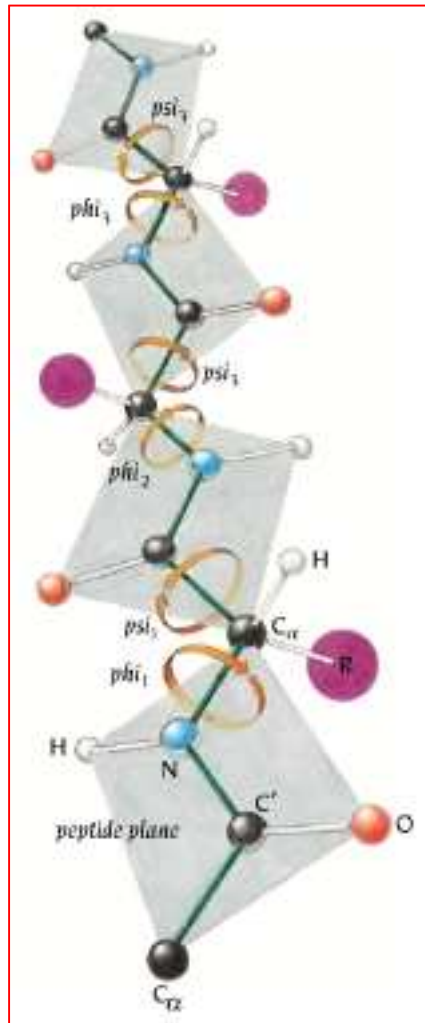
Le proteine sono polimeri di aminoacidi



Le proteine naturali hanno 20 aminoacidi diversi con 20 catene laterali (R) diverse

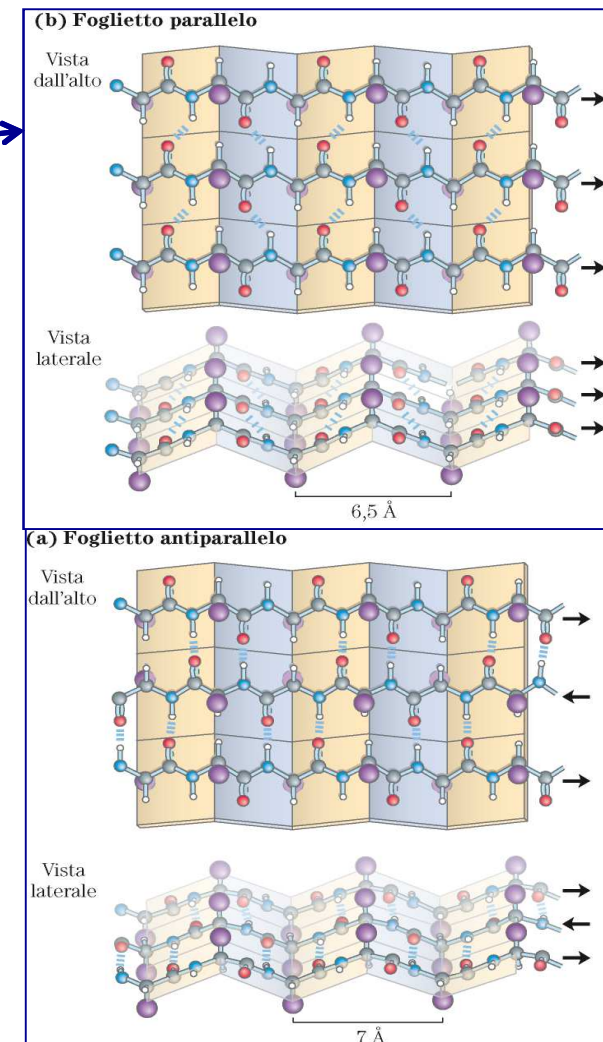
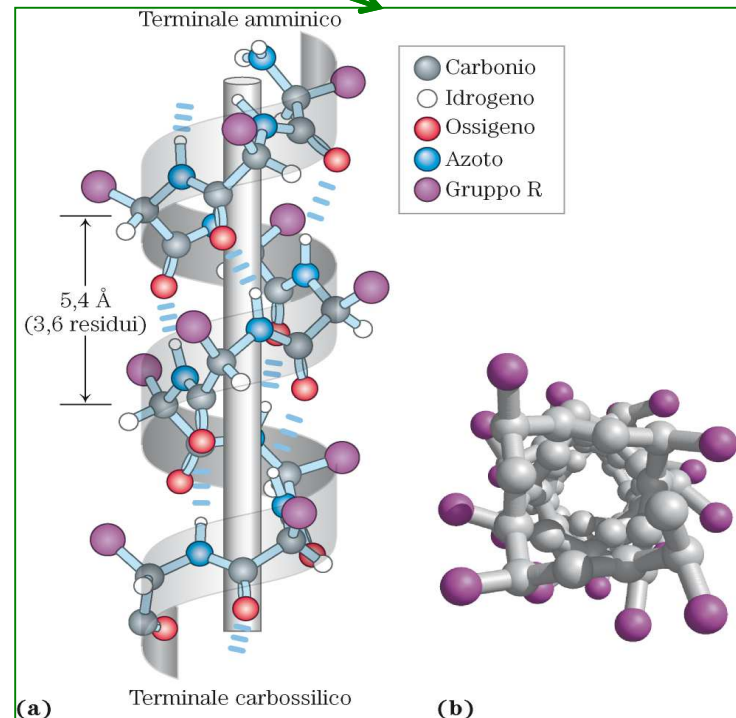


Dal ripiegamento del legame peptidico deriva la struttura secondaria:

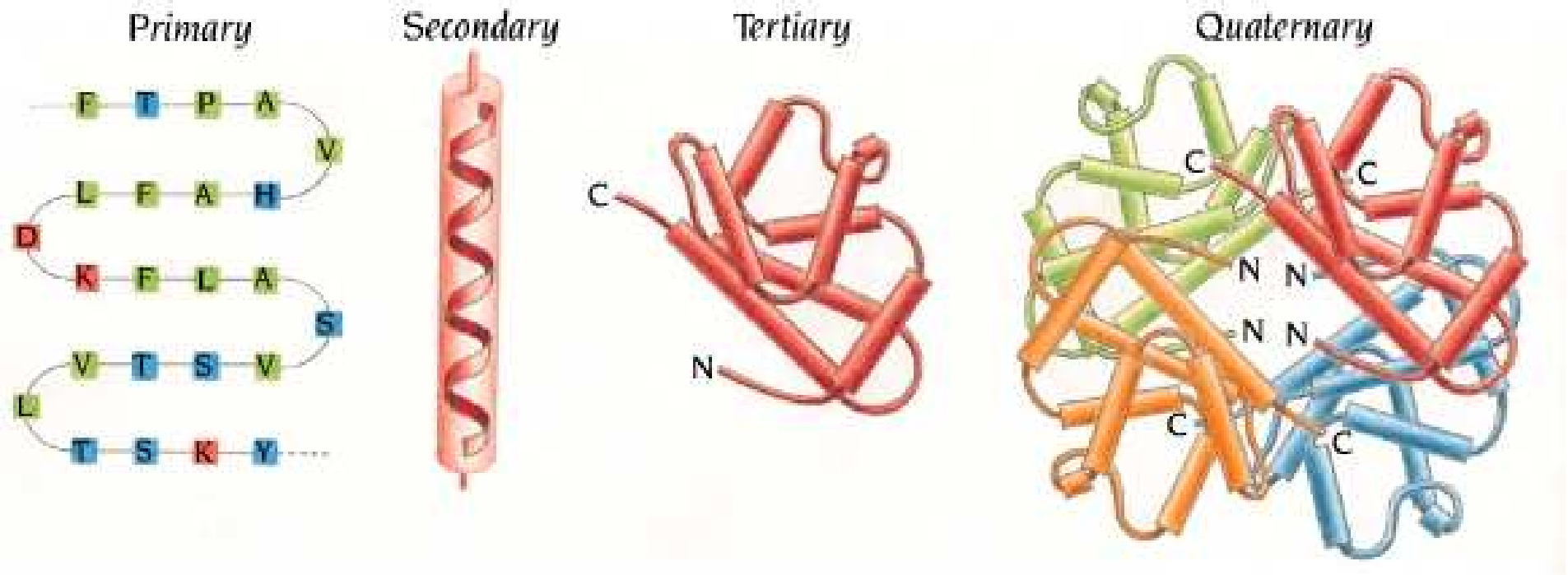


Sistemi a filamento beta

Sistemi ad elica

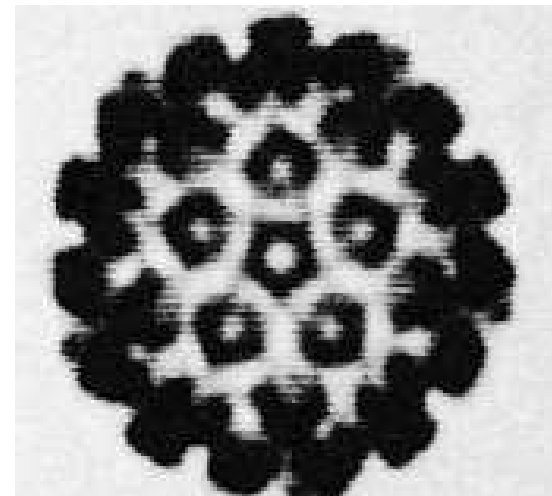
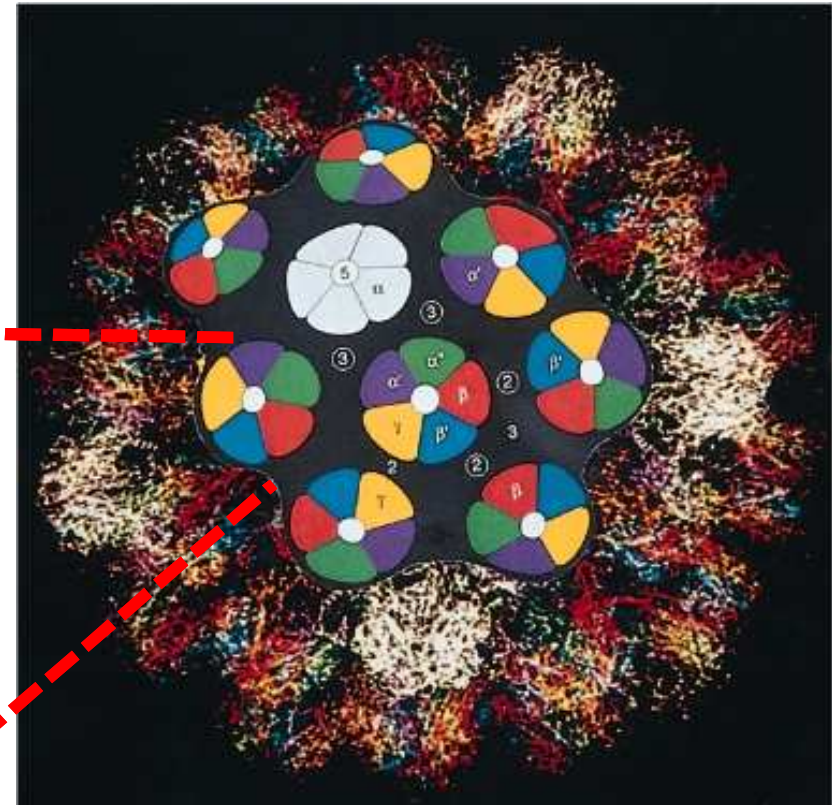
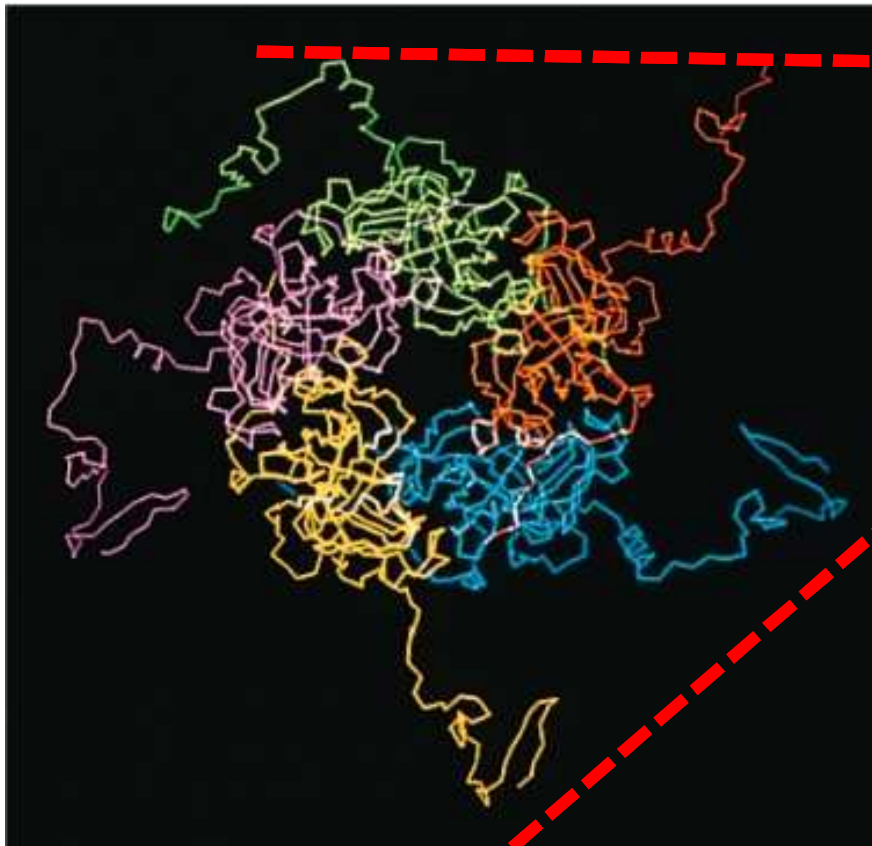


Livelli della struttura delle proteine:



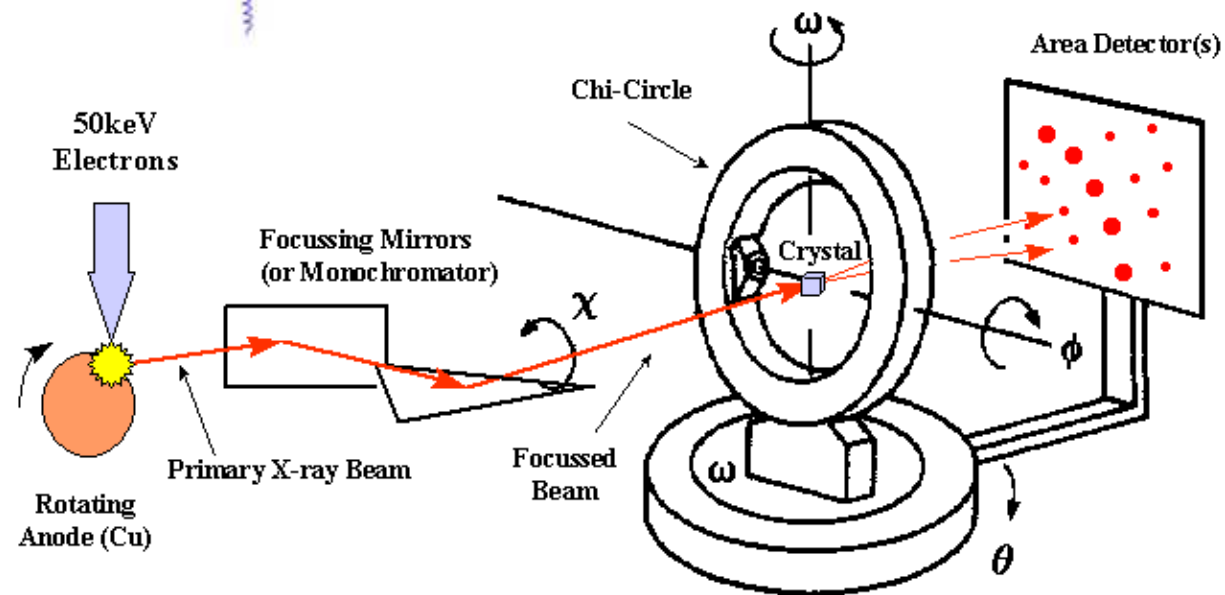
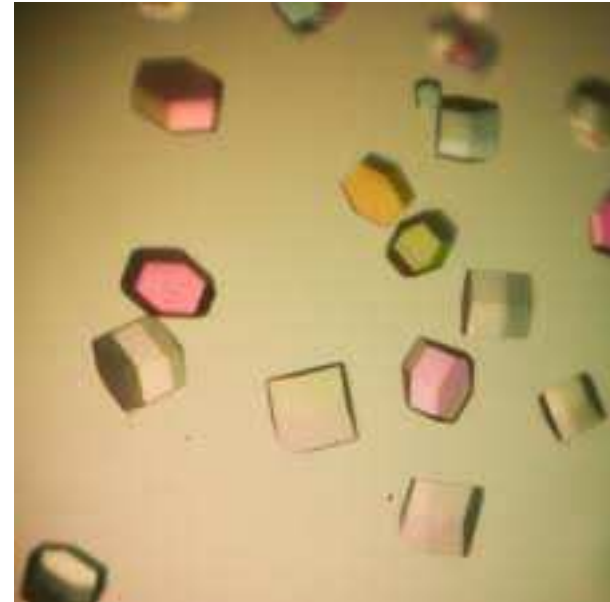
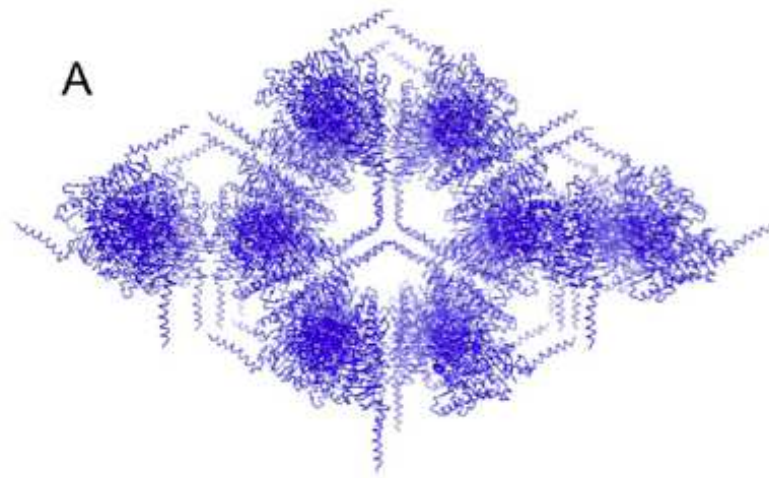
- Le interazioni deboli (quindi non covalenti) quali interazioni elettrostatiche, idrofobiche, legami idrogeno) sono alla base del ripiegamento delle proteine
- Le proteine sono solo marginalmente stabili perché necessitano “turnover”, cioè continua sintesi e degradazione a seconda delle necessità della cellula

Assemblaggio di capsidi virali



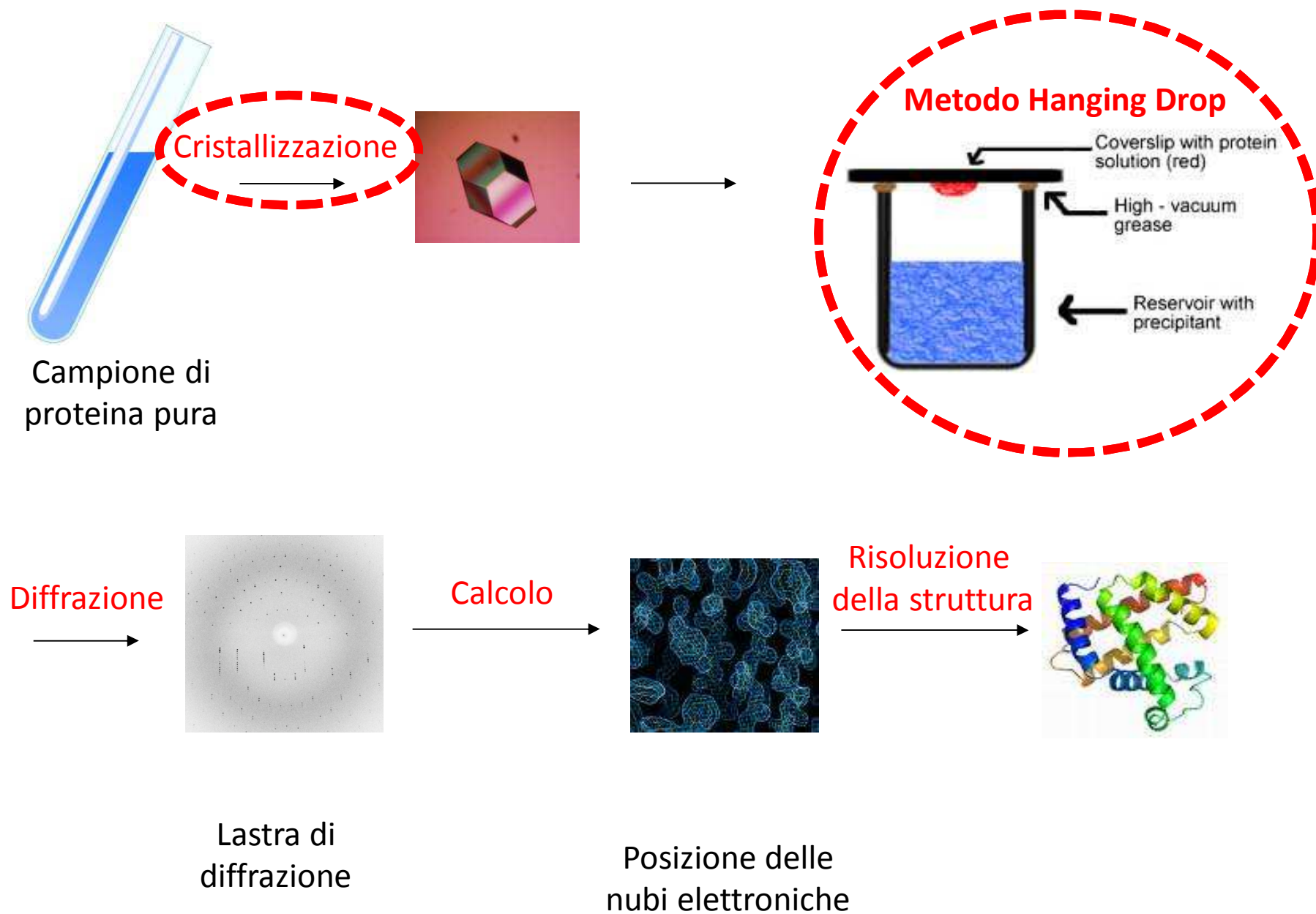
Poliomavirus: virus oncogeno che causa patologie quali la leucoencefalopatia multifocale progressiva (simile alla poliomielite ma causata da un virus diverso) in malati immunodepressi o in terapia di farmaci immunosoppressori quali pazienti a seguito di trapianti

La cristallizzazione e diffrazione ai raggi X permette di determinare la struttura delle proteine



4-Circle Goniometer (Eulerian or Kappa Geometry)

Procedura

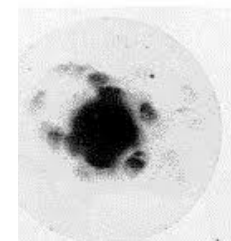


Cenni storici: le basi scientifiche

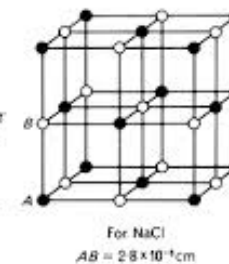
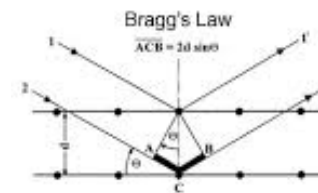
- **1901 Physics:** W. C. Röntgen “*Discovery of X-rays*”



- **1914 Physics:** M. Von Laue “*Diffraction of X-rays by crystals*”



- **1915 Physics:** W. H. Bragg and W. L. Bragg “*Use of X-rays to determine crystal structure*”



William Lawrence Bragg

- **1946 Chemistry:** J. B. Sumner “*For his discovery that enzymes can be crystallised*”



James Batcheller Sumner



- **1954 Chemistry:** L. C. Pauling *“For his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substance”*



- **1962 Chemistry:** J. C. Kendrew and M. Perutz *“For their studies of the structures of globular proteins”*



- **1962 Physiology or Medicine:** F. Crick, J. Watson and M. Wilkins *“The helical structure of DNA”*



- **1964 Chemistry:** D. Hodgkin *“Structure of many biochemical substances including Vitamin B12”*



- **1972 Chemistry:** C. B. Anfinsen *"Folding of protein chains"*



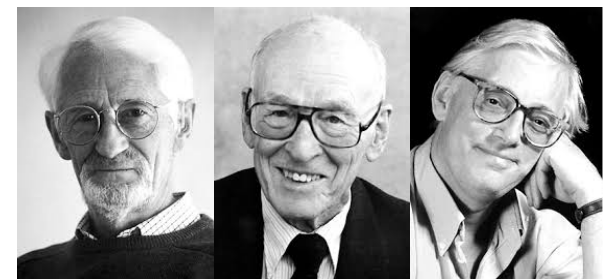
- **1982 Chemistry:** A. Klug *"Development of crystallographic electron microscopy and discovery of the structure of biologically important nucleic acid-protein complexes"*



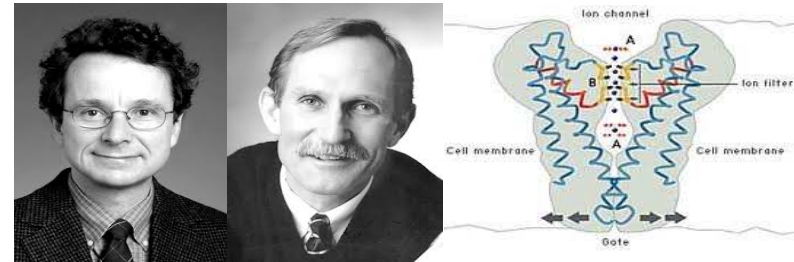
- **1988 Chemistry:** J. Deisenhofer, R. Huber and H. Michel *"For the determination of the three-dimensional structure of a photosynthetic reaction centre"*



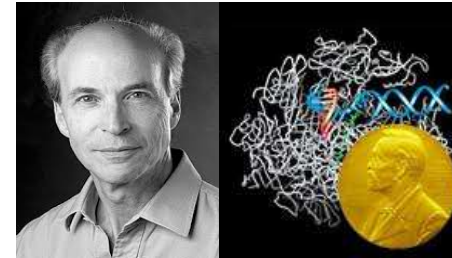
- **1997 Chemistry;** P. D. Boyer, J. E. Walker and J. C. Skou *"Elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP) and discovery of an ion-transporting enzyme"*



- **2003 Chemistry:** P. Agre and R. MacKinnon *"Discoveries concerning channels in cell membranes"*



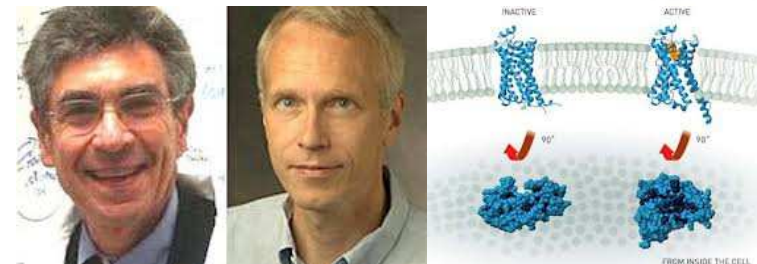
- **2006 Chemistry:** R. D. Kornberg *"Studies of the molecular basis of eukaryotic transcription"*



- **2009 Chemistry:** V. Ramakrishnan, T. A. Steitz and A. E. Yonath *"Studies of the structure and function of the ribosome"*



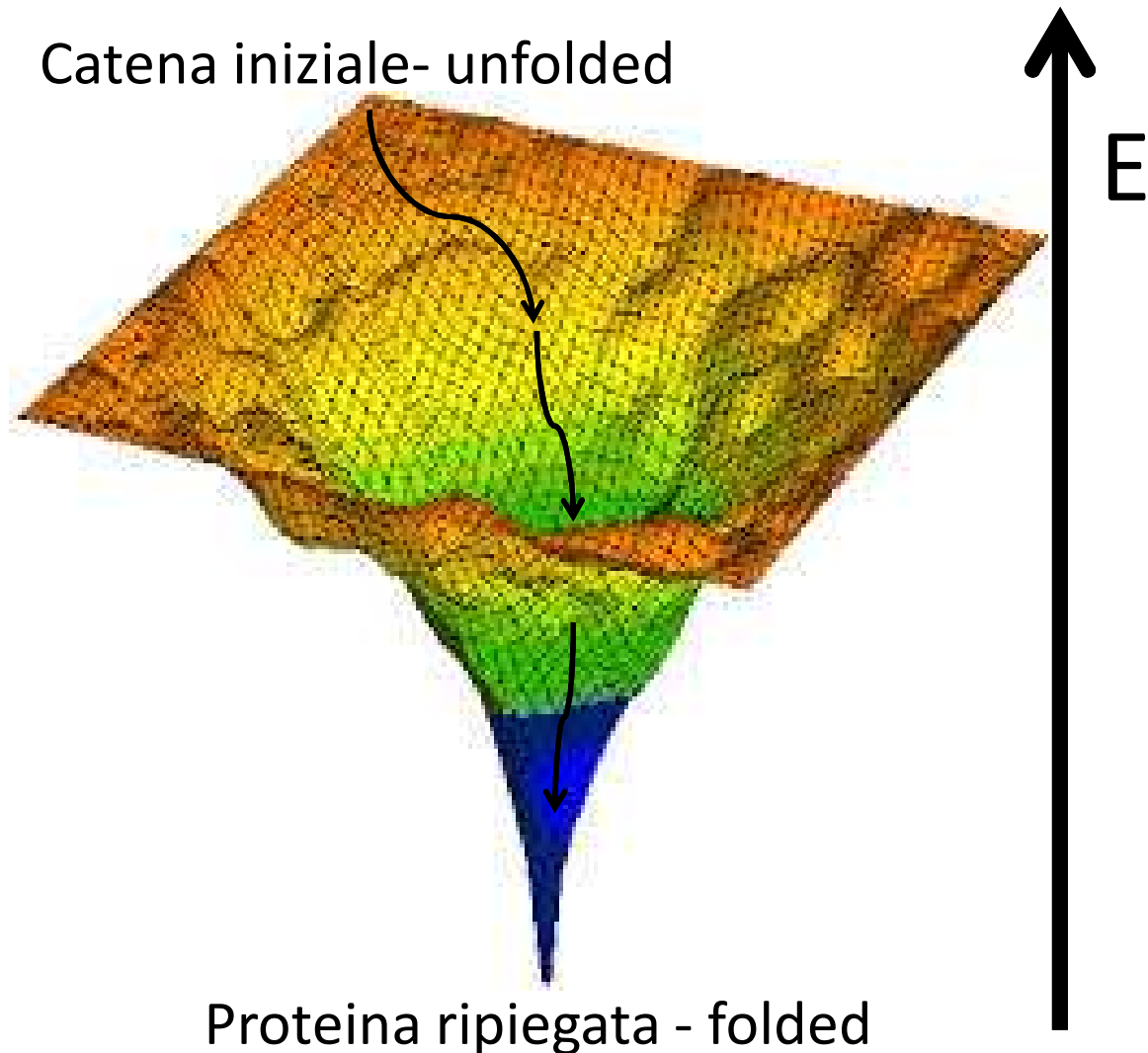
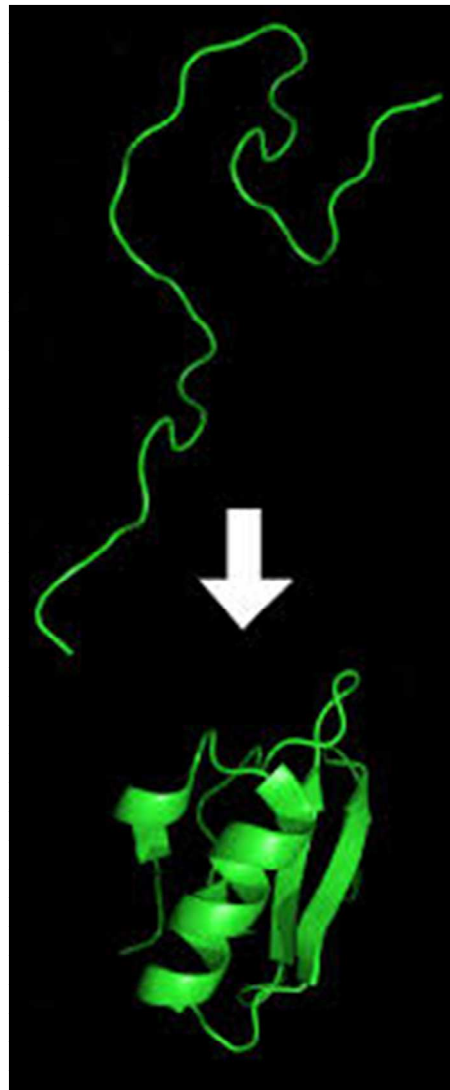
- **2012 Chemistry** R. J. Lefkowitz and B. K. Kobilka *"For studies of G-protein-coupled receptors"*



- **2013 Chemistry:** M. Karplus, M. Levitt and A. Warshel *"For the development of multiscale models for complex chemical systems"*



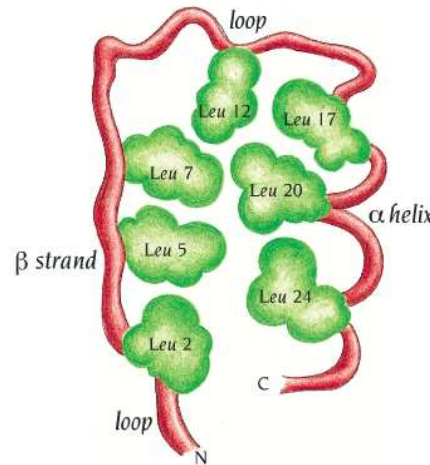
Il processo del ripiegamento: “folding”



Dal ripiegamento derivano interazione molecolari specifiche

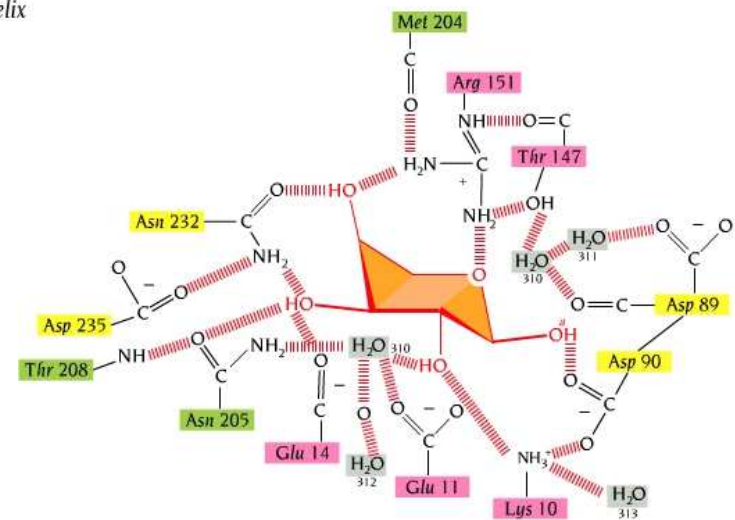
- All'interno della proteina stessa:

Stabilizzazione da parte di residui di Leu fra una filamento beta e un'elica alfa



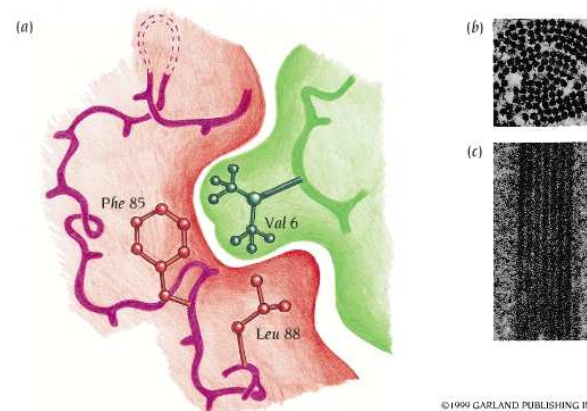
- Tra una proteina e un ligando o un substrato:

Reticolo di legami H fra la proteina legante l'arabinosio e la molecola dell'L-arabinosio. I residui che interagiscono con lo zucchero formano a loro volta legami a idrogeno tra di loro, con altri residui o con molecole di acqua.



- Tra catene proteiche diverse:

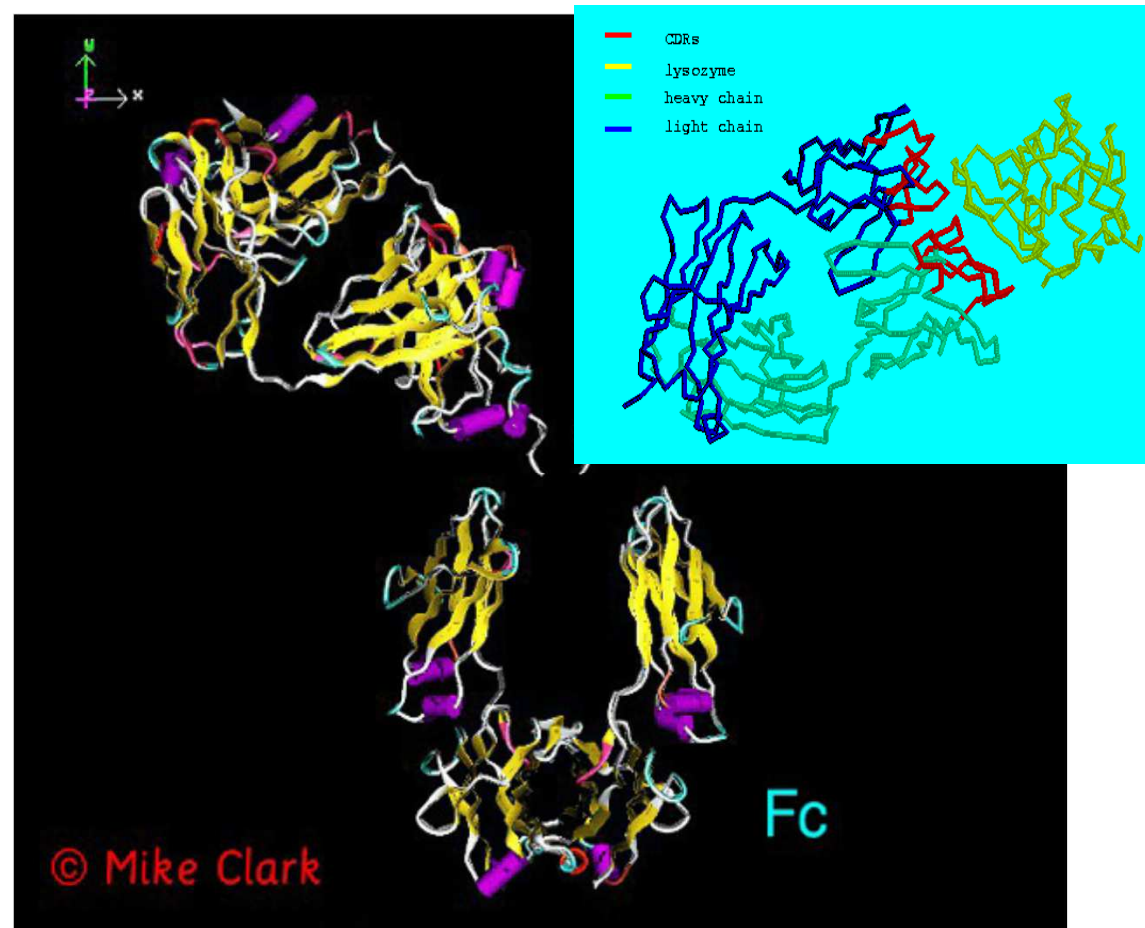
Le molecole dell'emoglobina falciforme polimerizzano a causa della presenza sulla loro superficie della zona idrofobica prodotta dalla sostituzione, nella catena beta, del Glu6 con una Val. La catena laterale in verde interagisce con una tasca idrofobica in rosso presente sulla superficie di una seconda molecola di emoglobina.



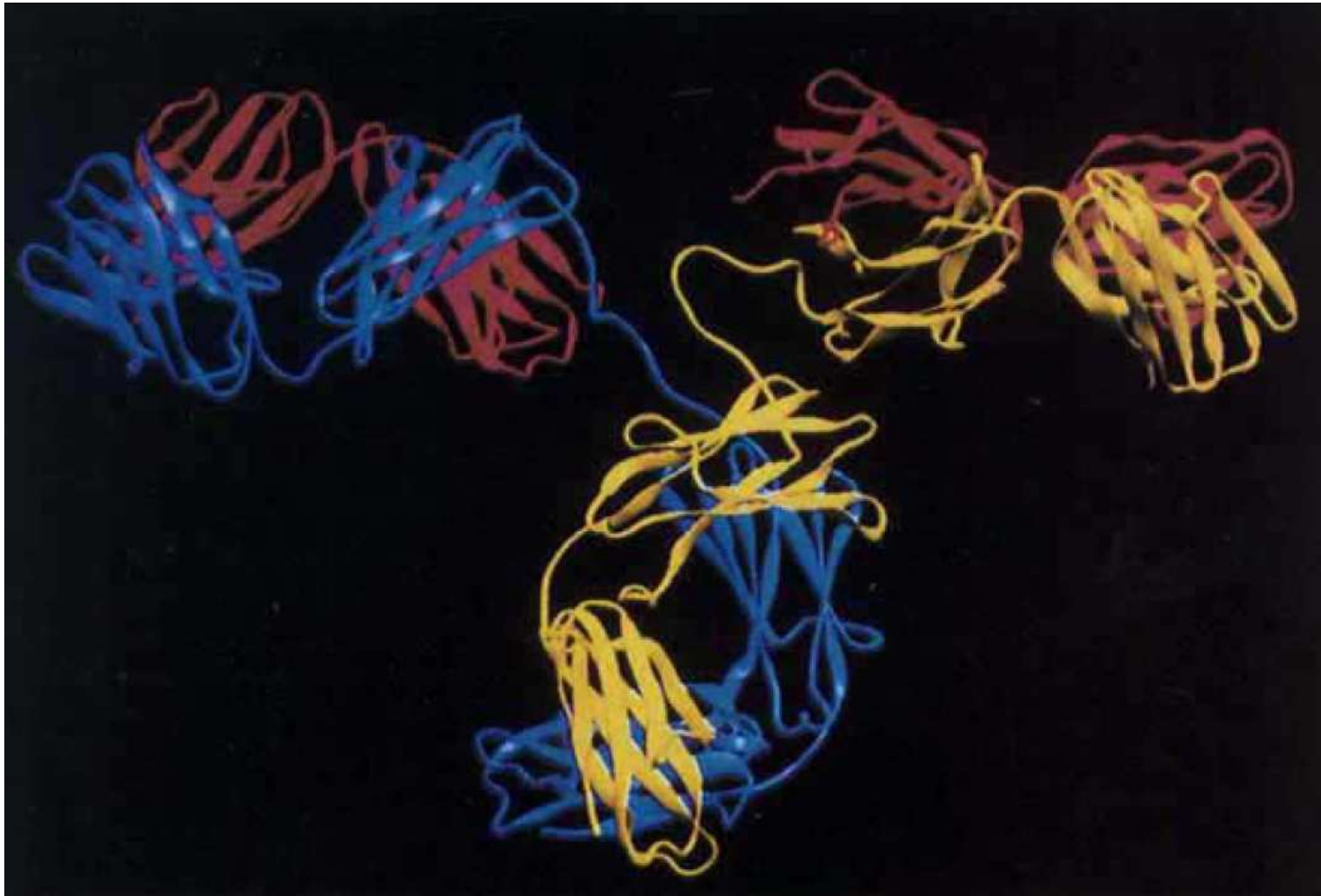
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Struttura degli anticorpi



Complesso anticorpo – linfoma canino



Disegno razionale di farmaci

Interazione di antimicotici col sistema P450 di fegato umano

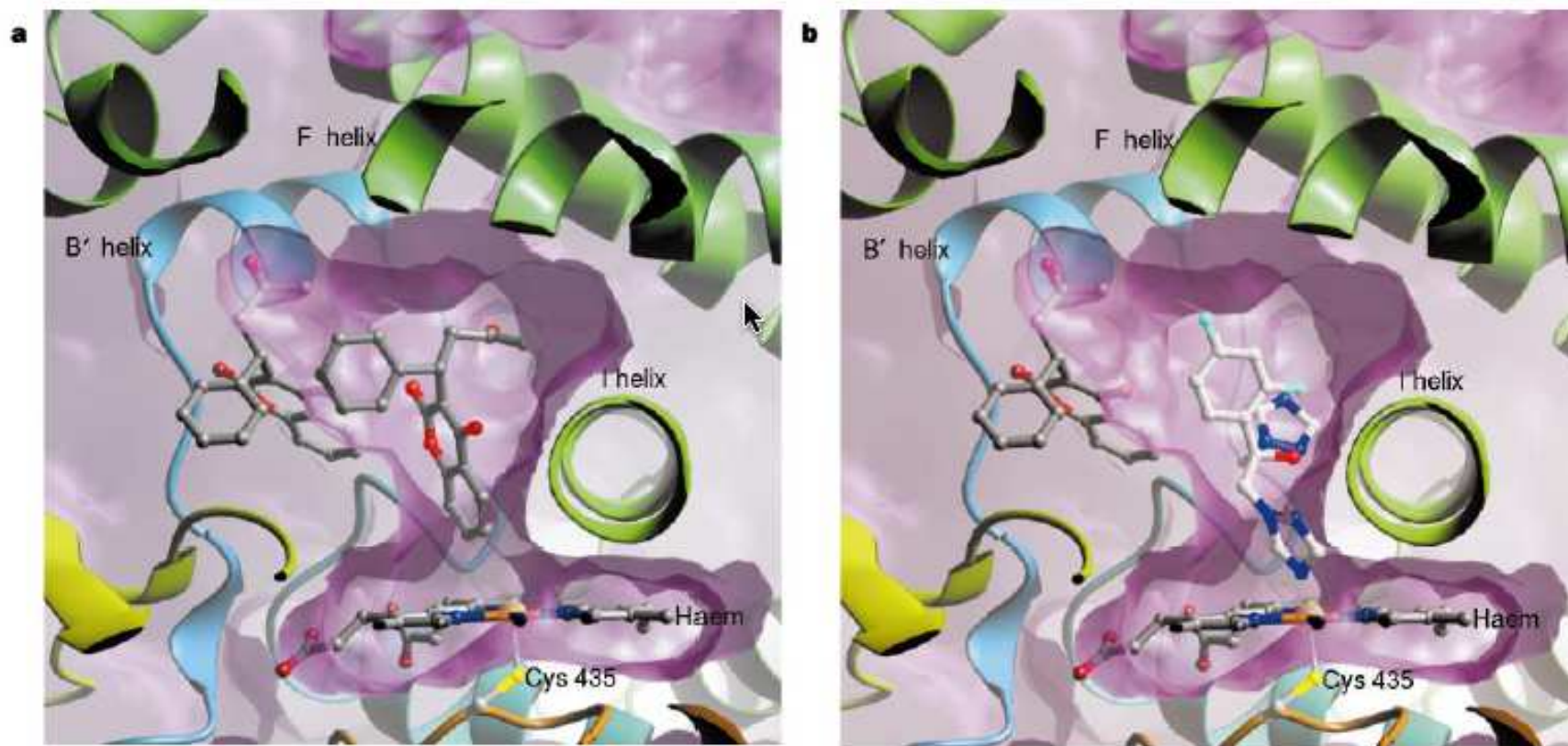
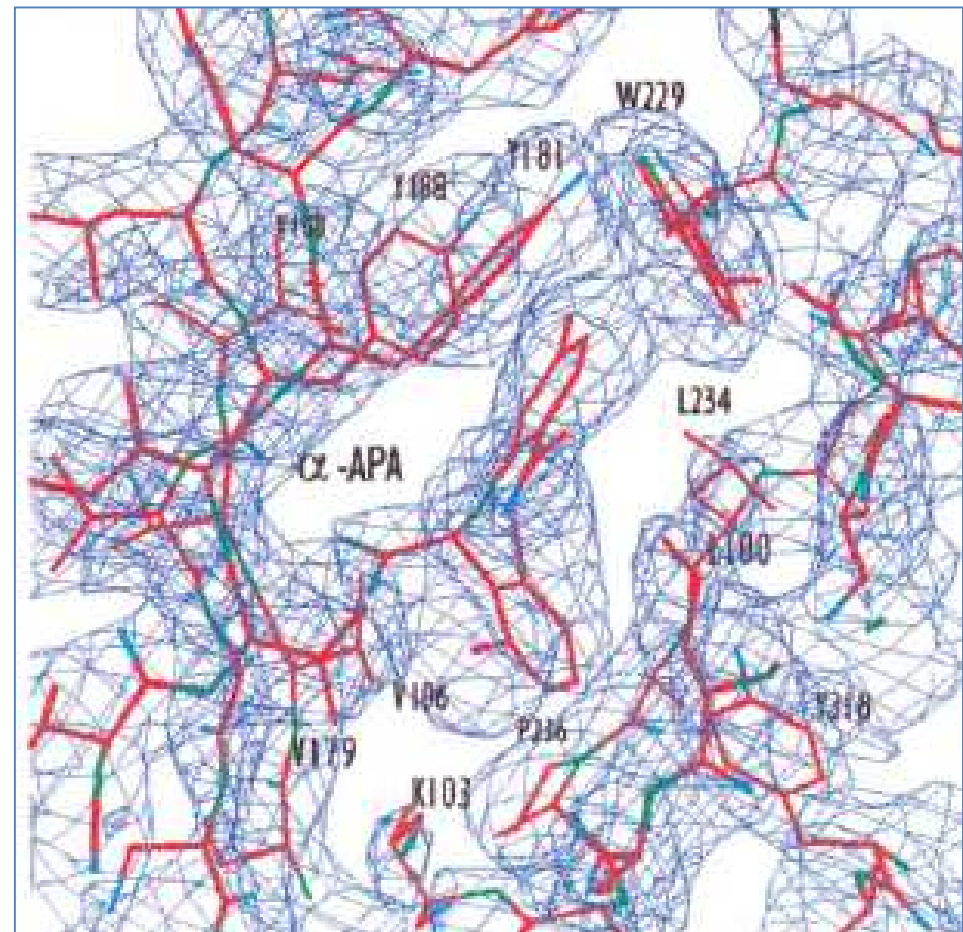
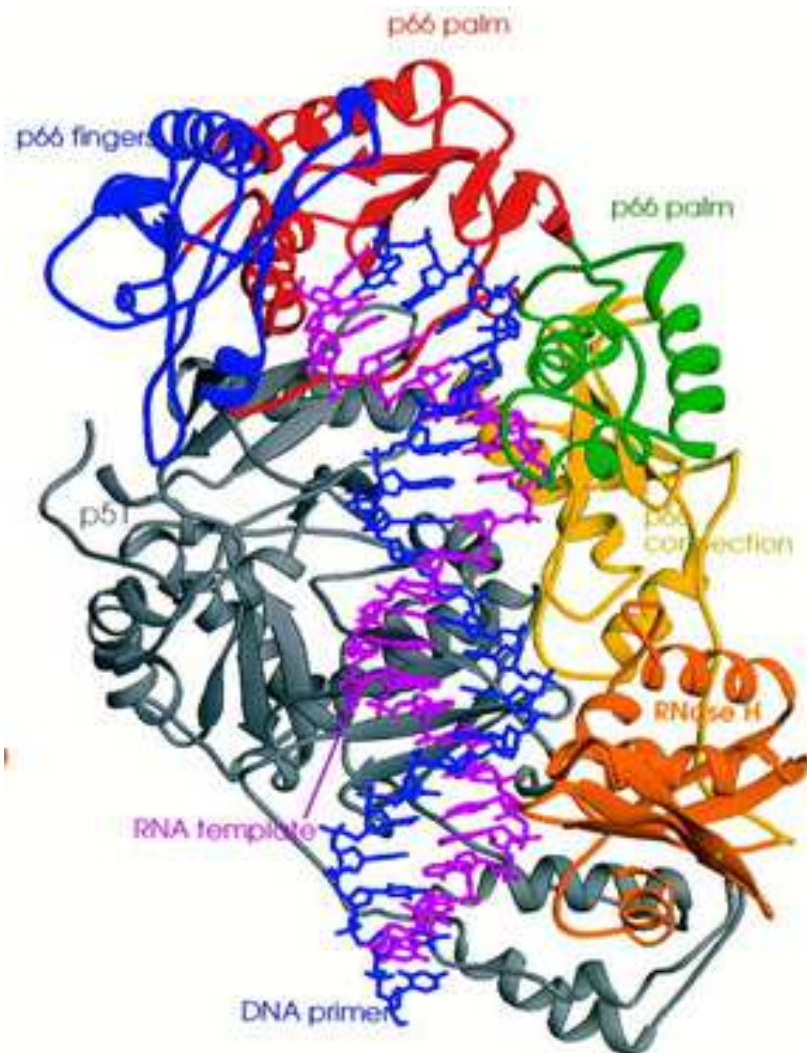


Figure 4 View of the region of the active site of CYP2C9 that remains available to accommodate additional ligand(s) after *S*-warfarin. The bound *S*-warfarin molecule is shown as in Fig. 3. **a**, A second molecule of *S*-warfarin has been modelled into the active

with the site of hydroxylation closest to the haem iron. **b**, A known haem binder fluconazole has been modelled into the cavity in a similar conformation to that observed in the complex of CYP51 with fluconazole (Protein Data Bank code 1EA1).

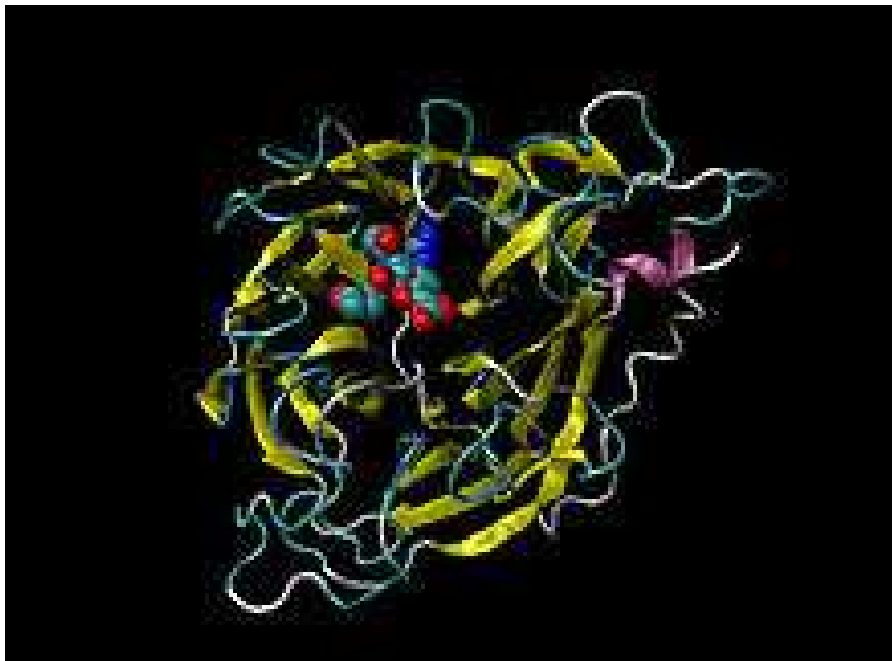
Disegno razionale di farmaci

Inibitori della Trascrittasi Inversa dell'HIV



Sviluppo di inibitori del virus dell'influenza

- Sviluppo di inibitori dell'enzima sialidasi virale



Enzima necessario al virus
per penetrare nelle vie respiratorie

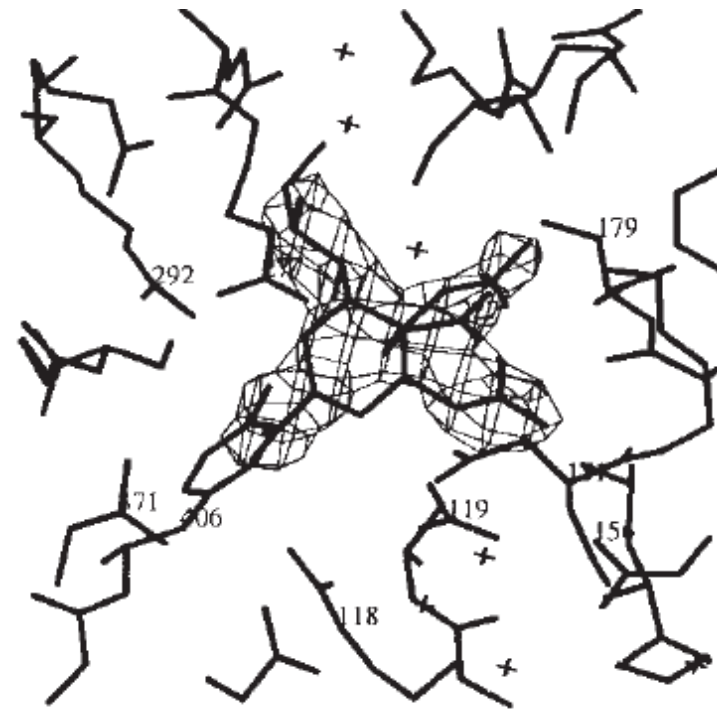
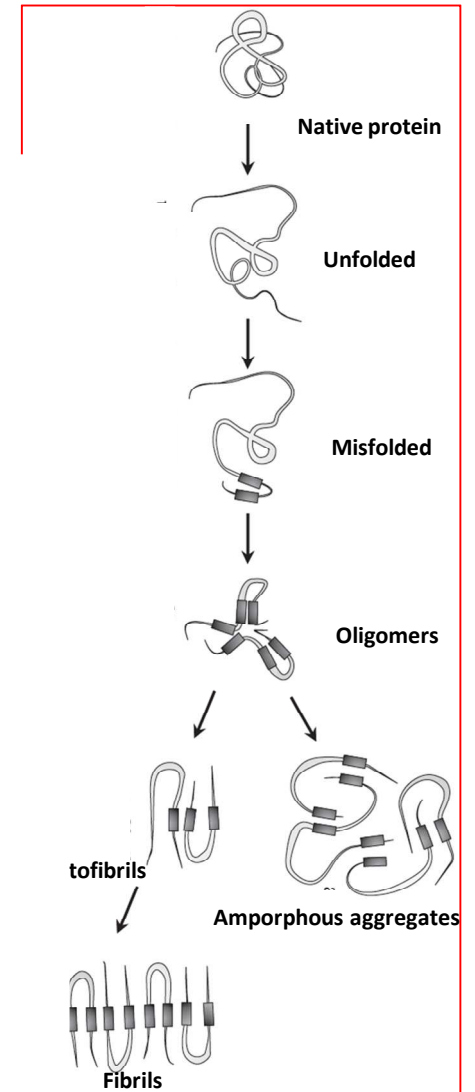
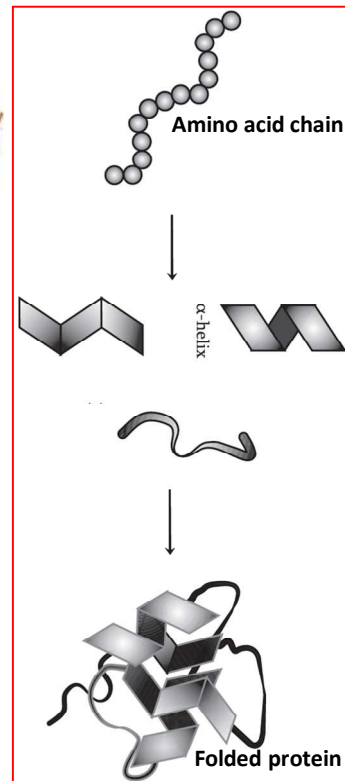
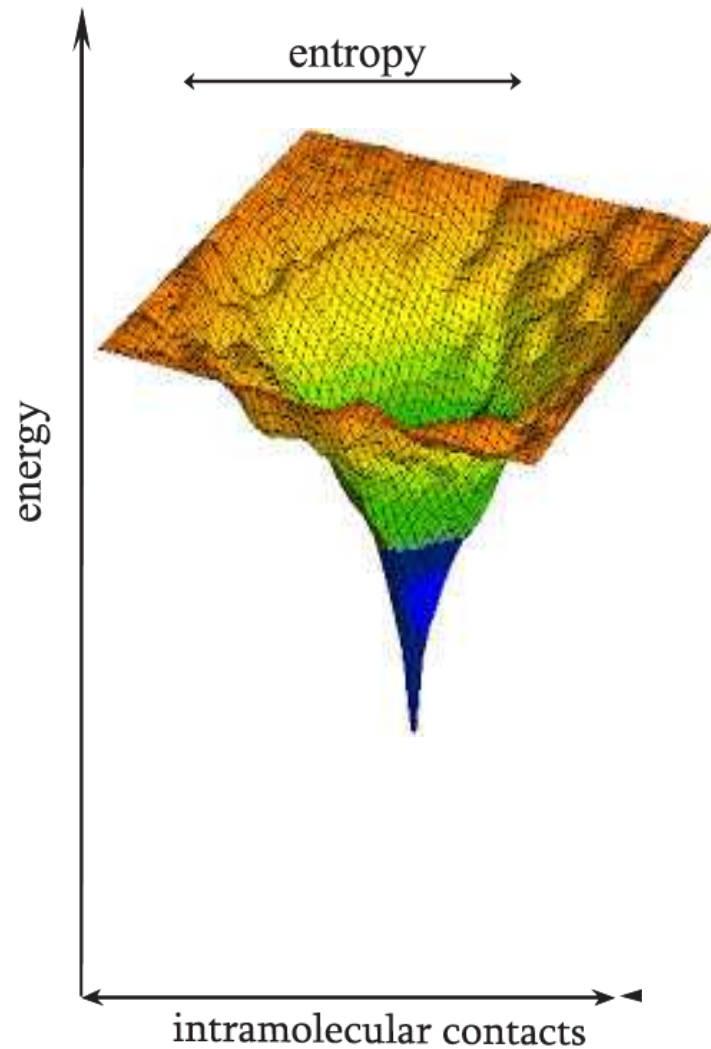


FIG. 2 Stereo drawing of the inhibitor 4-guanidino-Neu5Ac2en bound to the active site of influenza virus A/Tokyo/3/67 sialidase. a, The

Folding e Mis-folding



Misfolding, fibre amiloidi, patologie

- Quando specifiche proteine si ripiegano erroneamente:
 1. Vengono ripiegate da chaperonine
 2. Vengono degradate da proteasi
 3. Si aggregano a formare fibre insolubili (fibre amiloidi)
- Le fibre amiloidi si depositano nei tessuti generando patologie. Si conoscono almeno 20 malattie umane associate a depositi di fibre amiloidi:
 - Proteina β amiloide = Alzheimer
 - Prioni = Encefalopatie
 - Lisozima = Amiloidosi viscerale familiare
 - Fibrinogeno = Amiloidosi renale ereditaria
 - β 2-microglobulina = Artropatia da emodialisi

Proteina β amiloide

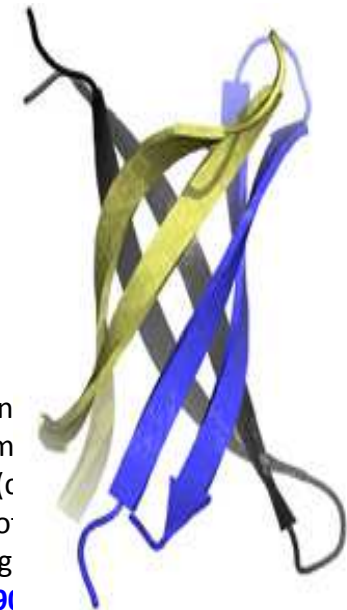
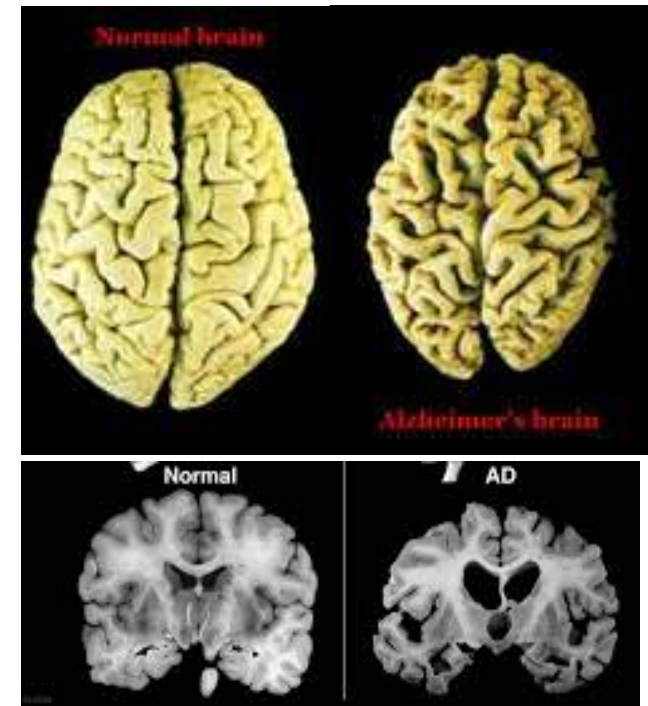
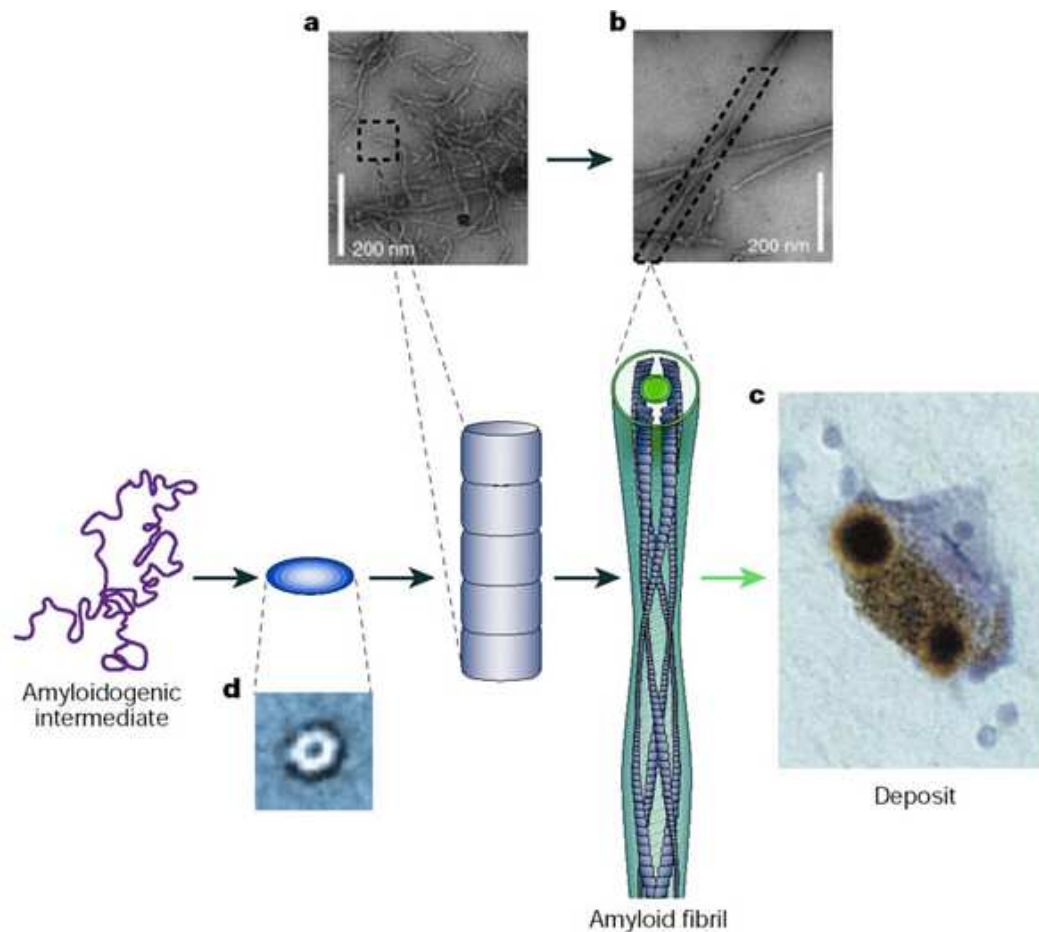
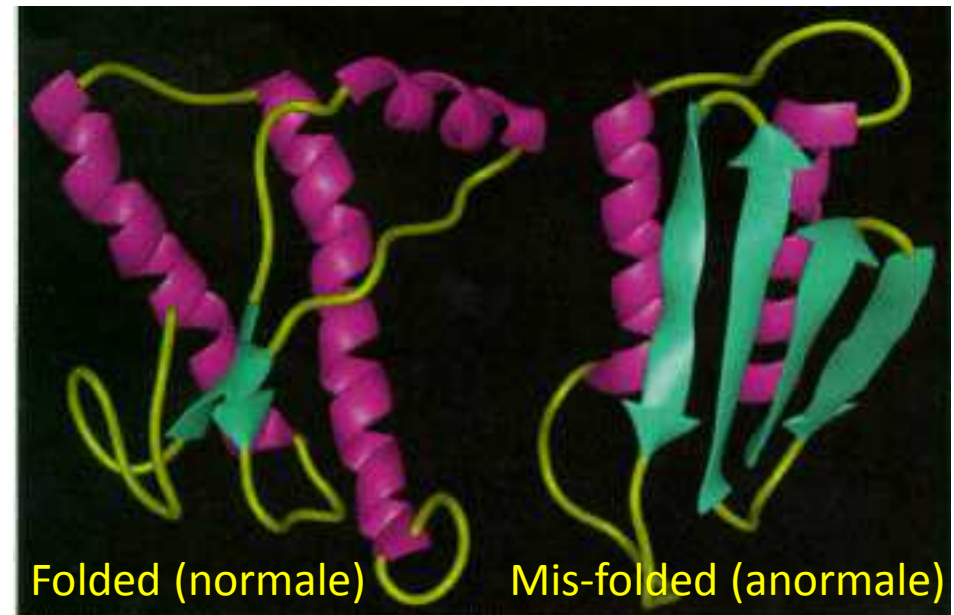


Figure 4 : Formation of amyloid fibrils

Unfolded or partially unfolded proteins associate with each other to form small, soluble aggregates that un into protofibrils or protofilaments (a) and then mature fibrils (b), as seen in these electron microscope im accumulate in plaques or other structures, such as the Lewy bodies associated with Parkinson's disease (c image on the right). Some of the early aggregates seem to be amorphous or micellar in nature, although o species with diameters of approximately 10 nanometers (d; electron microscope imag

Prioni

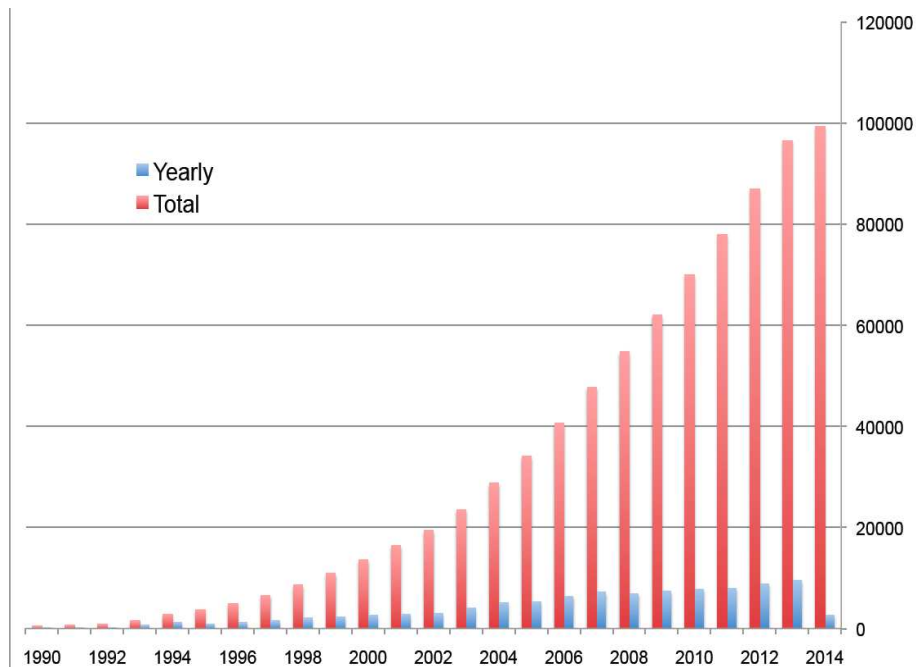
- Prione = agente infettivo proteico
- Il prione è una proteina che si ripiega in modo anomalo e si comporta da “stampo” nel far ripiegare in modo anormale altre proteine creando delle fibrille amiloidi, polimeri di filamenti β .
- Questo porta a patologie che coinvolgono il cervello o tessuti nervosi e sono fatali quali:
 - Encefalopatia spongiforme bovina (malattia della mucca pazza)
 - Scrapie (pecore e ovini)
 - Creutzfeldt-Jakob disease (uomo)



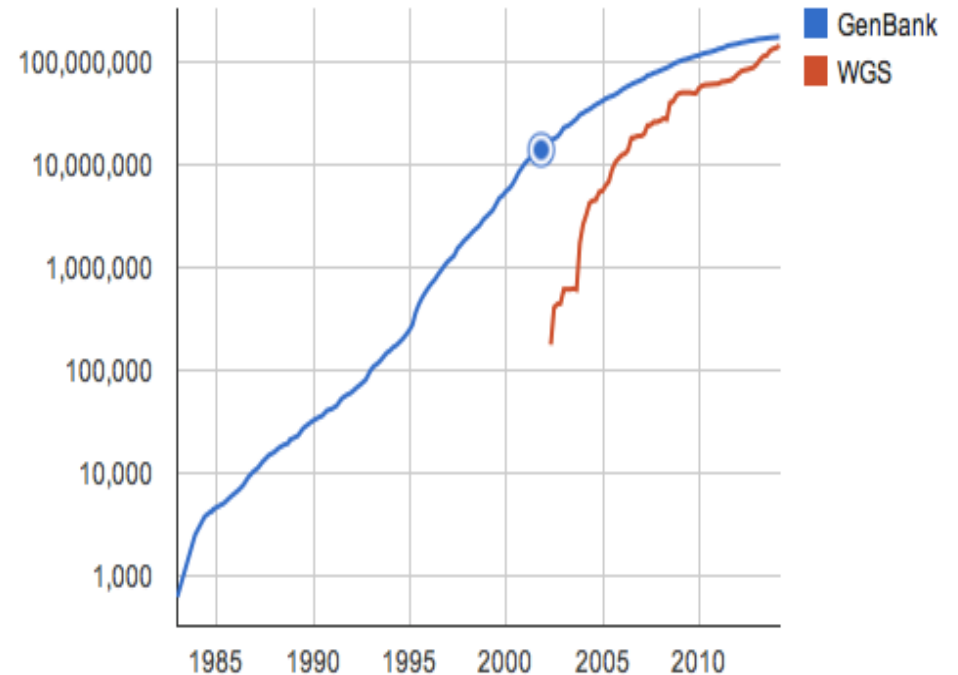
Passato, presente, futuro....

- Lo studio della struttura 3D delle proteine è oggi routinariamente usata nei laboratori di ricerca biologica, farmaceutica e biotecnologica

Numero strutture 3D risolte



Numero geni sequenziati



Genoma umano: 20-25000 geni

- Diventa dunque sempre più importante dare ai giovani conoscenze di base sulla
 - struttura delle biomolecole
 - tipologia di aminoacidi
 - legami che si instaurano fra loro
 - diversi livelli di organizzazione strutturale che ne risultano nelle proteine
 - è realistico e non dispendioso programmare esperimenti di cristallizzazione in laboratorio didattico usando proteine ben note, stabili e facili da cristallizzare

Bibliografia:

Reynaud, E. "Protein Misfolding and Degenerative Diseases"

su Nature Education del 2010 Vol. 3(9):28