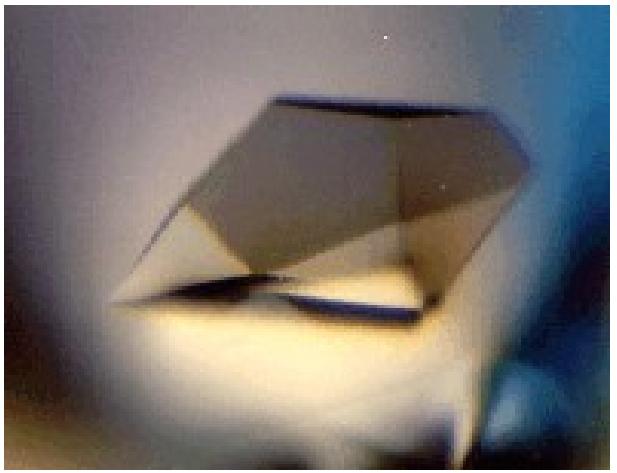
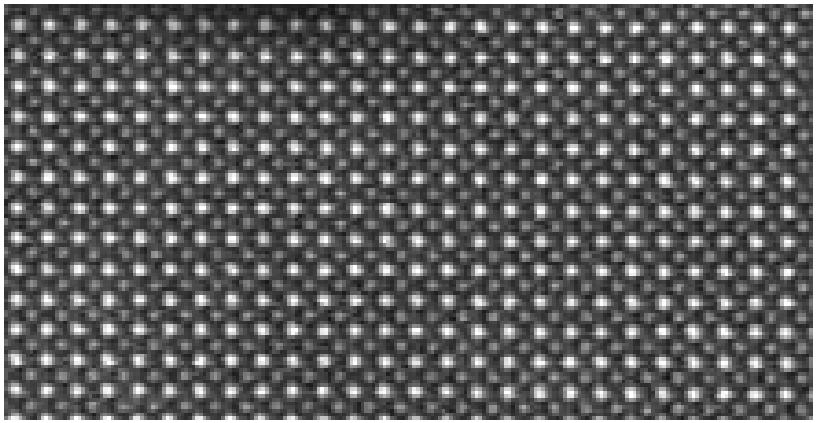
Rentgenostruktūrinė analizė

Saulius Gražulis
Palanga 2012 m.

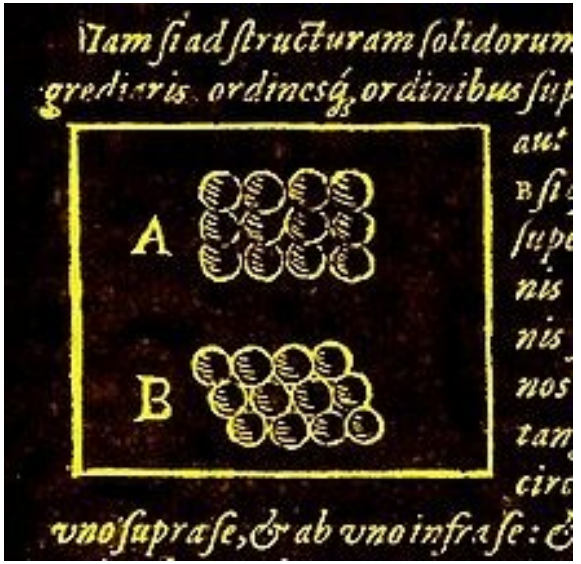
Kristalai

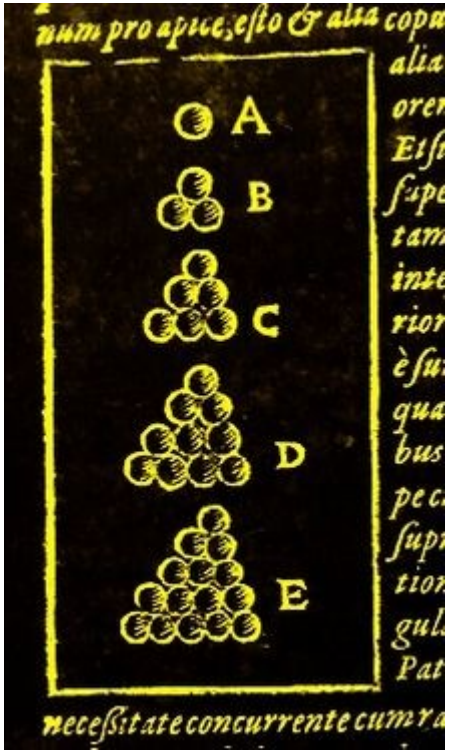


Kristalo formos paaiškinimas

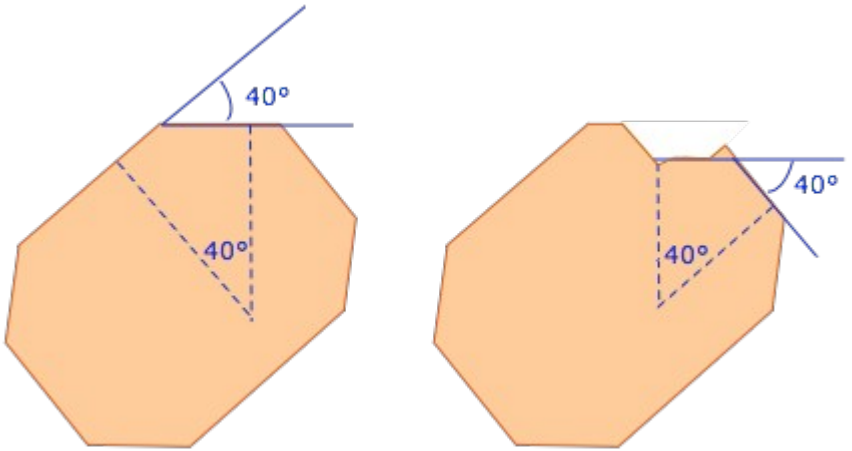


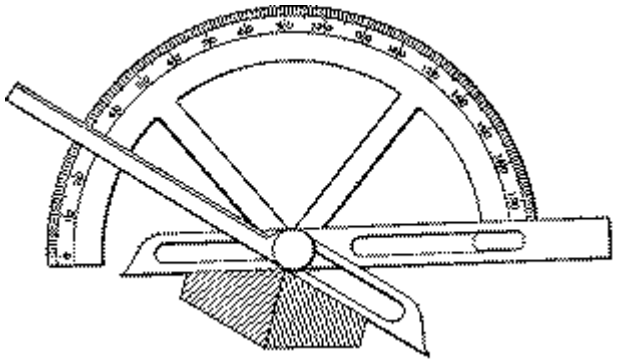
Johannes Kepler
1611 m. „Strena Seu de Nive Sexangula“ (A New Year's Gift of Hexagonal Snow)





Kampų pastovumo dėsnis

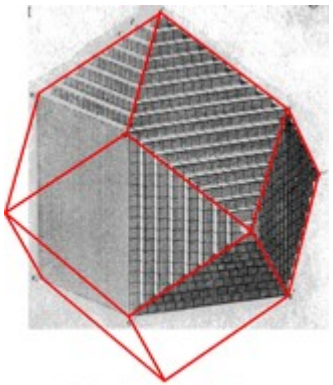




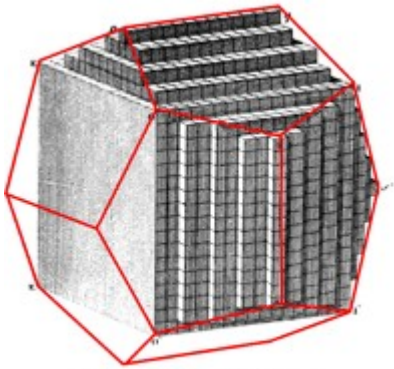
Nicolas Steno
1669 m. „De solido intra solidum naturaliter contento“

Paveiksliukai iš
http://www.chem1.com/acad/webtext/states/crystals-ext.html

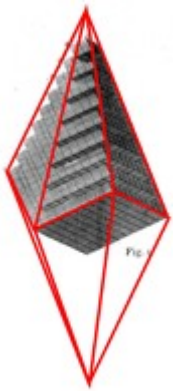
Vidinė kristalų struktūra



Rhomb-dodecahedron



Pentagon-dodecahedron



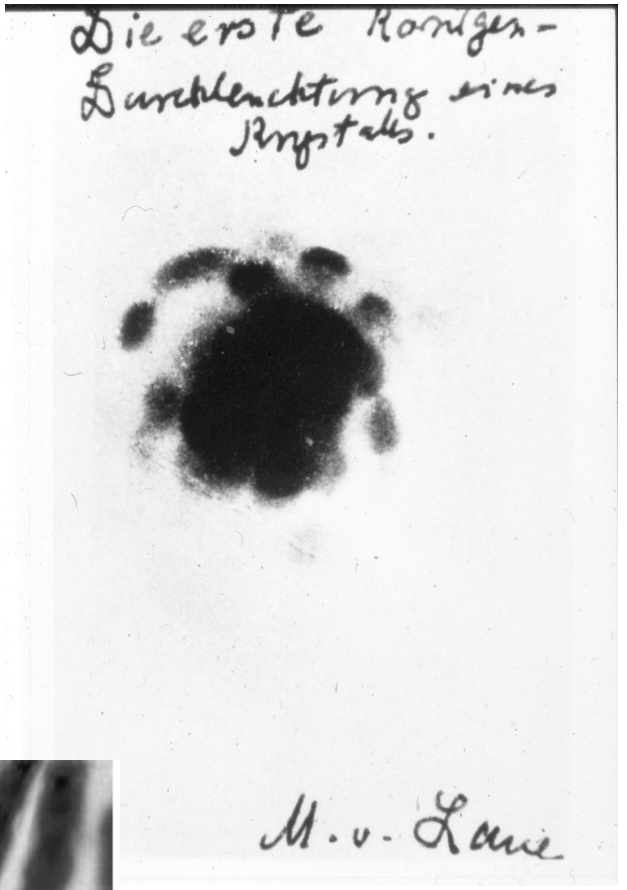
Scalenohedron

René Just Haüy [aɥi]
Traité de minéralogie
(5 vols, 1801)

Paveikslėliai iš
http://xrayweb.chem.ou.edu/notes/symmetry.html

Rentgeno spinduliai

Wilhelm Conrad Röntgen the first Nobel Prize in Physics in 1901



Max von Laue Nobel Prize (1914)

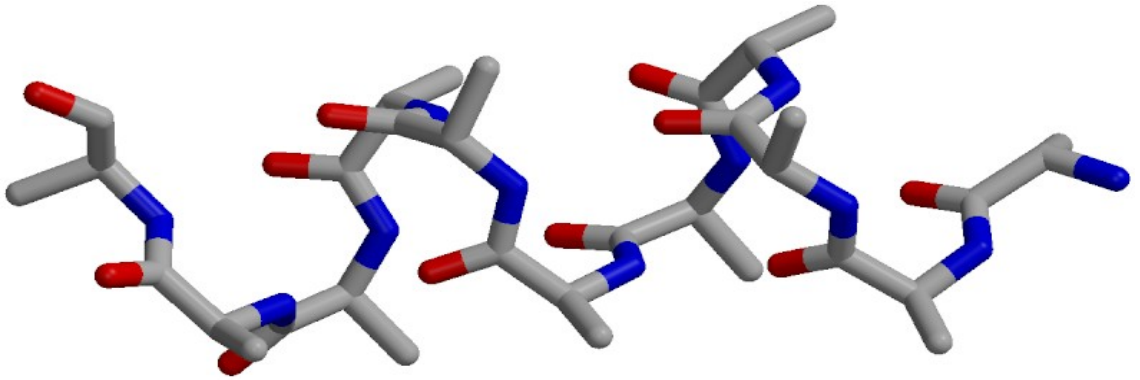
Struktūra - svarbu!

Izomerai turi skirtingas savybes, nors jų sudėtis ta pati:

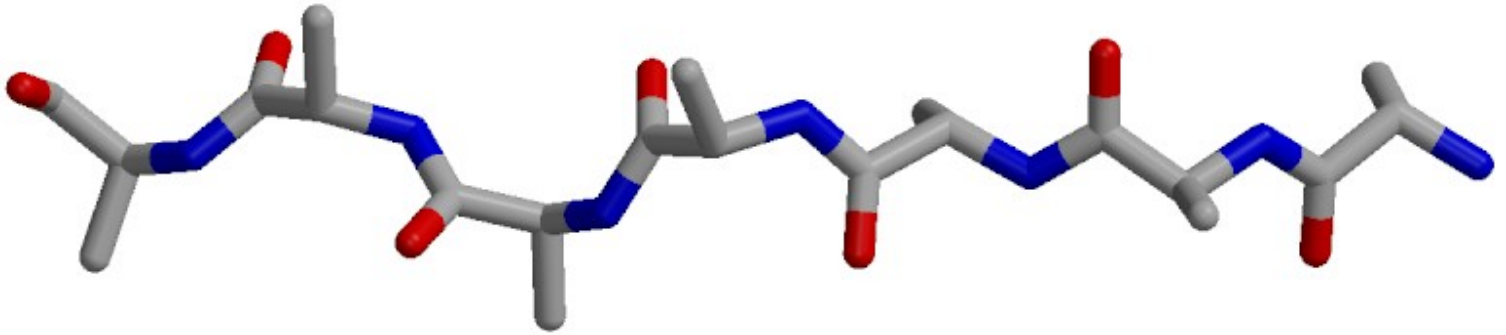
CH_3-(C=O)-CH_3 CH_3-CH_2-OH

Alfa-spiralės ir beta-klostės

Alfa-spiralė



Beta-klostė



Biologinės makromolekulės yra ilgi polimerai

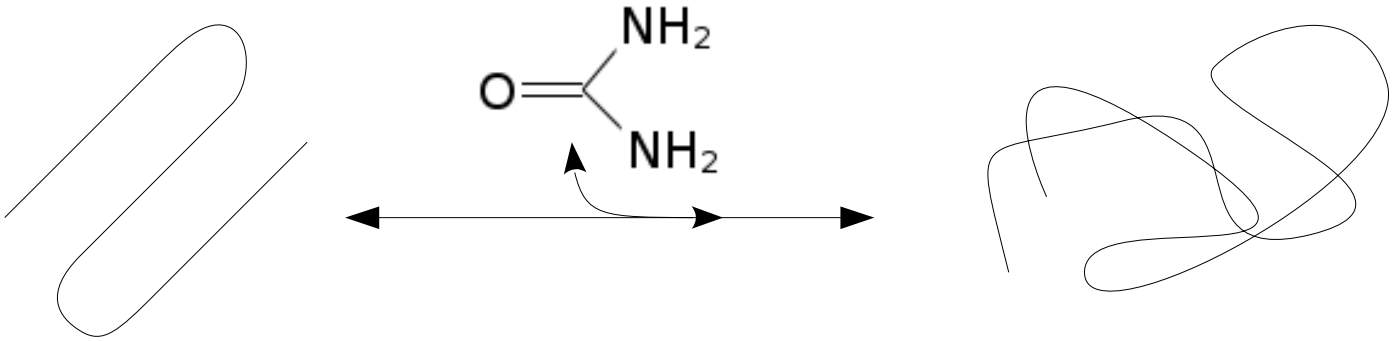
Tipiška baltymo seka (BfiI Restriktazė):

MNFFSLHPNV YATGRPKGLI GMLENVWVSN HTPGEGTLYL ISGFSNYNGG
VRFYETFTEH INQGGRVIAI LGGSTSQRLS SRQVVEELLN RGVEVHIINR
KRILHAKLYG TSNNLGESLV VSSGNFTGPG MSQNIEASLL LDNNTTQSMG
FSWNDMISEM LNQNWHIHNM TNATDASPGW NLLYDERTTN LTLDETERVT
LIVTLGHADT ARIQAAPGTT AGQGTQYFWL SKDSYDFFPP LTIRNRRGTK
ATYSSLINMN YIDINYTDTQ CRVTFEAENN FDFRLGTGKL RYTGVAKSND
IAAITRVGDS DYELRIIKQG TPEHSQLDPY AVSFIGNRGK RFGYISNEEF
GRIIGVTF

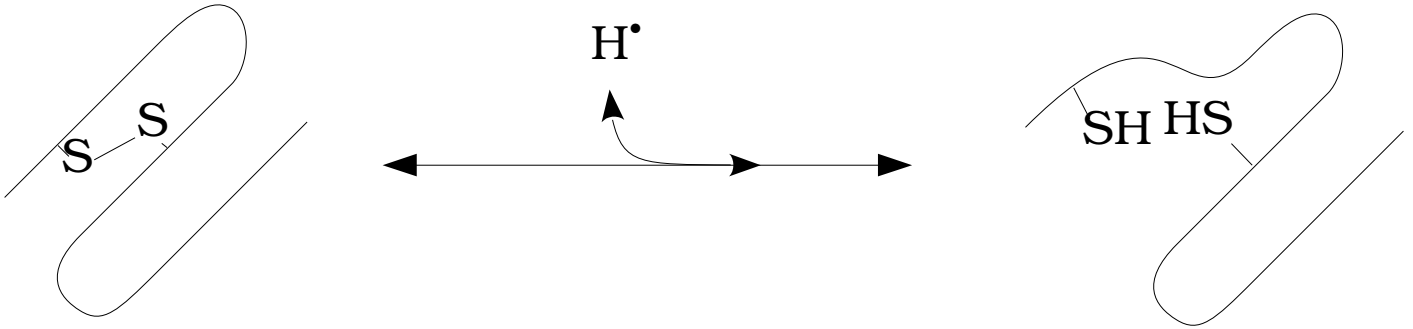
358 amino rūgštys
40 kDa

Pirminė seka apsprendžia erdvinę struktūrą

1. Karbamidas (urea) denatūruoja baltymus:



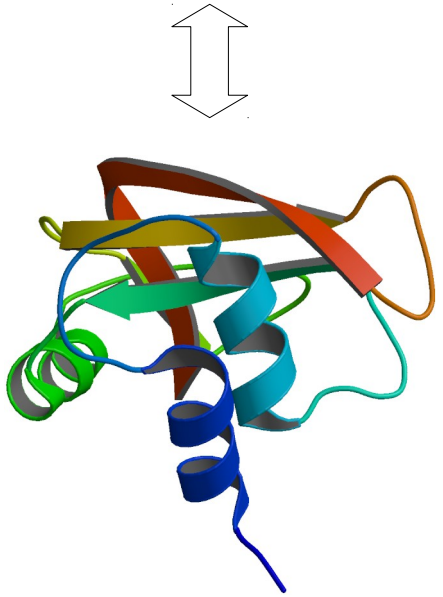
2. Oksidacija sukuria, o redukcija suardo Cys S-S tiltelius:



Anson & Mirsky JGP 1931 p.341, Anfinsen PNAS 1961 p.1309

Baltymo sekos ir struktūros sąryšis

KETAAAKFERQHMDSSTSAASSSNYCNQMMKSRNLTKDRCKPVNTFVHE
SLADVQAVCSQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPNCAYKT
TQANKHIIVACEGNPYVPVHFDASV



Išvada: baltymo struktūrą ir funkciją iš esmės apsprendžia jo a.r. seka.

Levintalio (Levinthal) paradoksas

Baltymo grandinės galimų konformacijų skaičius: 100 a.r., ~2^{100} galimų konformacijų; joms išsamiai perrinkti reikėtų:

jei patikrinama 1 konformacija per 1 ps,

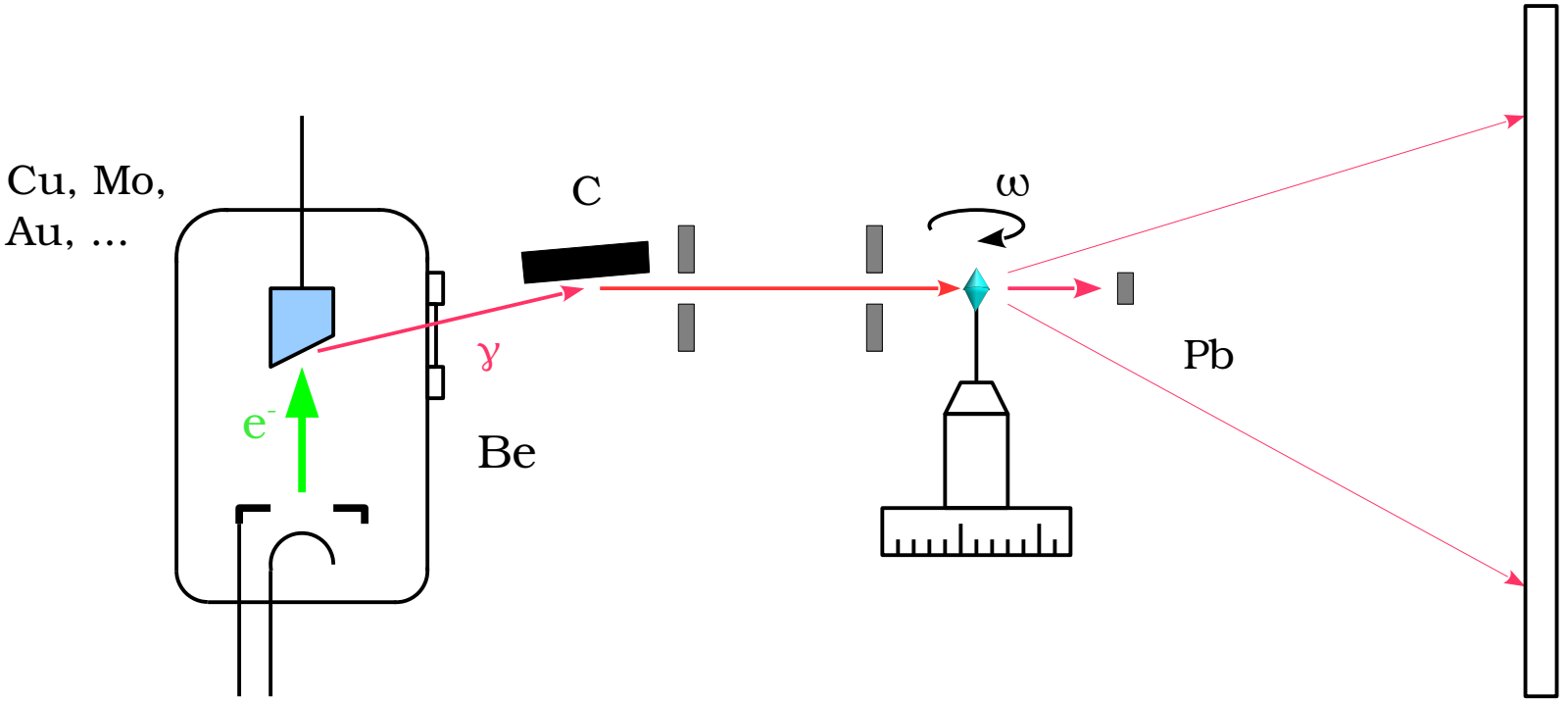
π s = 1 nano-amžius. (3.14 s = 10^{-7} metų)

2^{100} ps $\approx$ 10^{30} ps **~10^{10} metų.**

Nei realus baltymas, nei kompiuteris visų konformacijų perrinkti negali.

C. Levinthal *J. de Chimie Physique* 1968 p.44

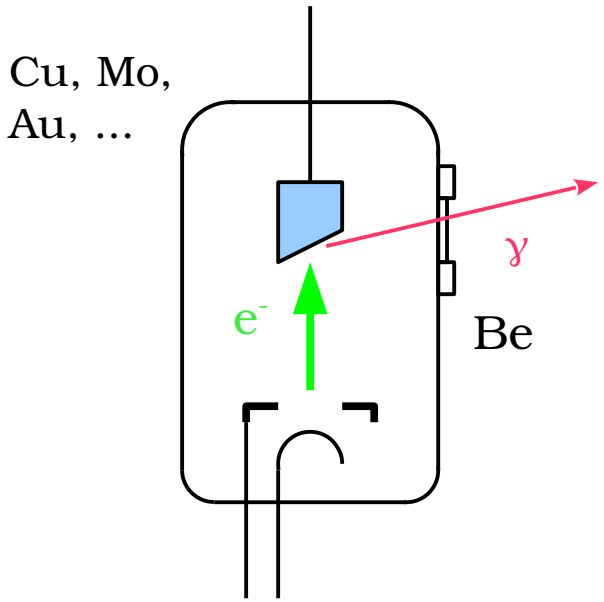
Rentgenostruktūrinė analizė



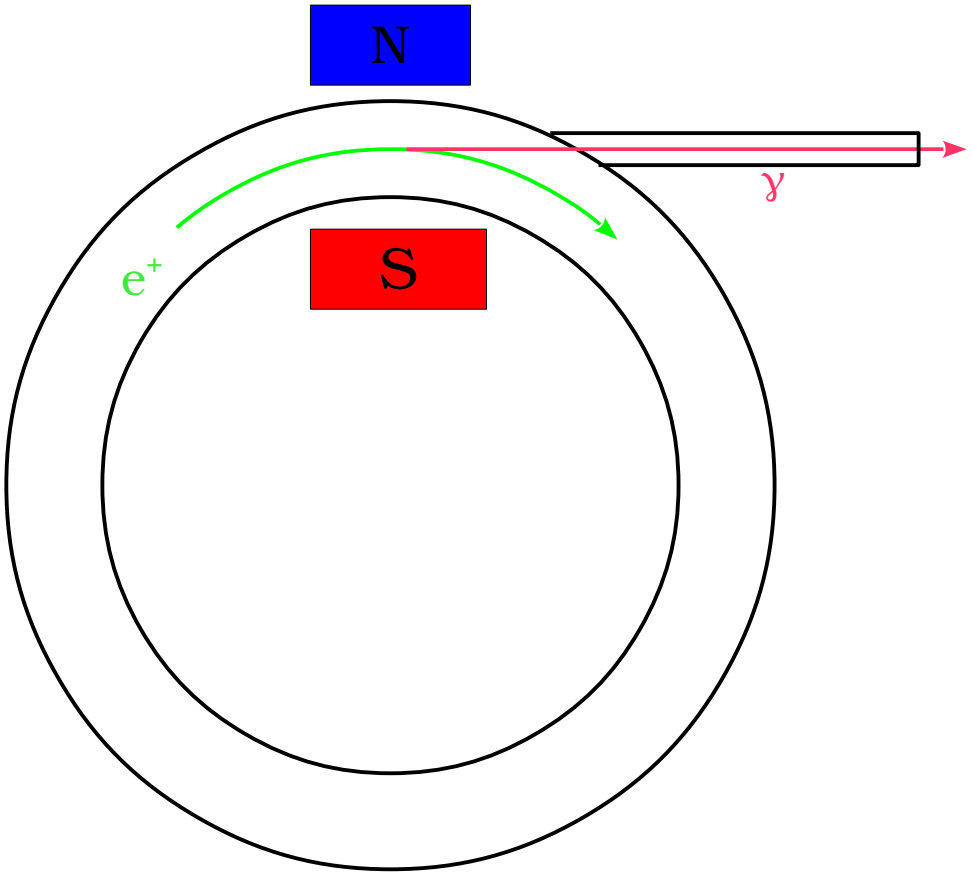
$\lambda \simeq 1\text{Å}$

Rentgeno spindulių šaltiniai

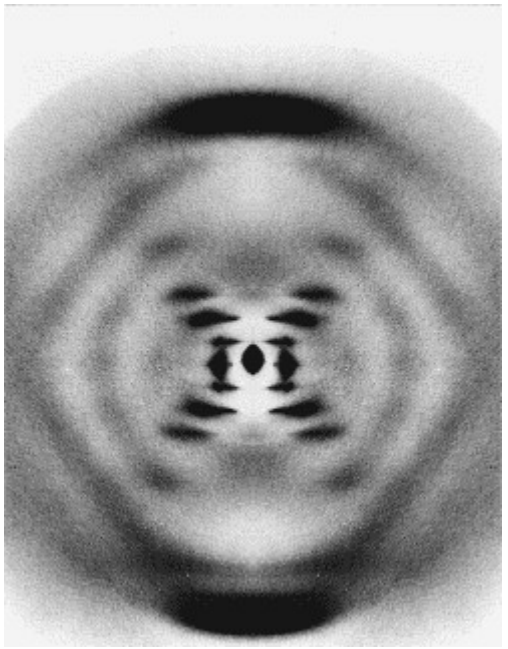
Rentgeno vamzdeliai



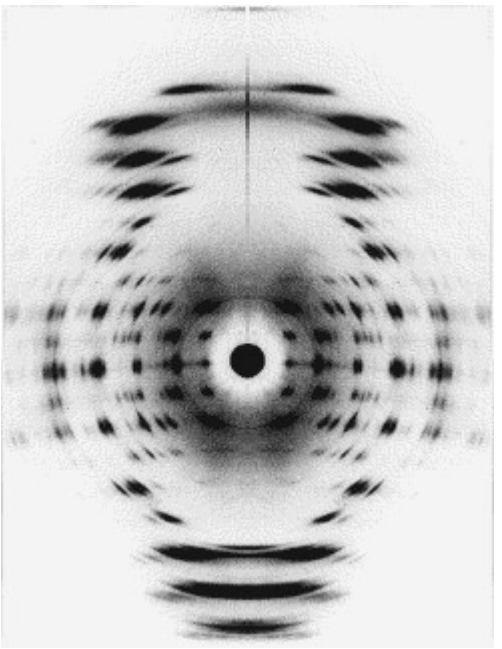
Sinchrotronai



DNR pluoštų difrakcija

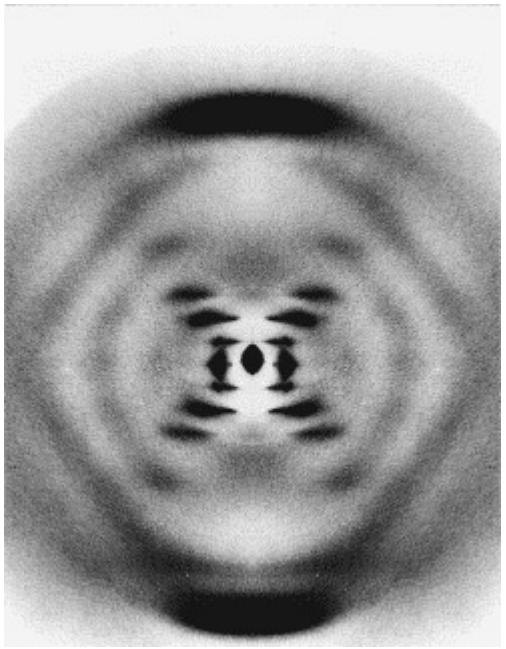


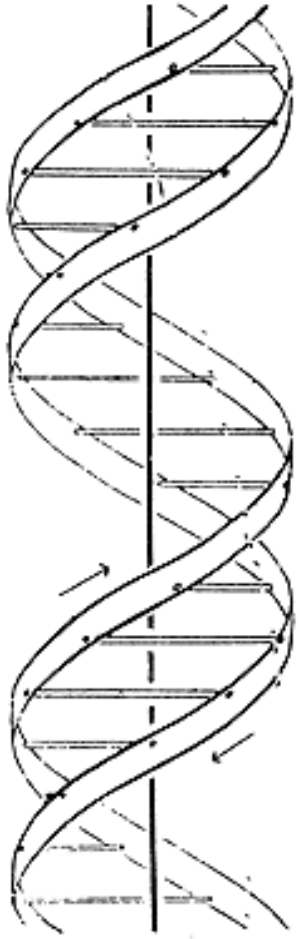
B formos
rentgenograma



A formos
rentgenograma

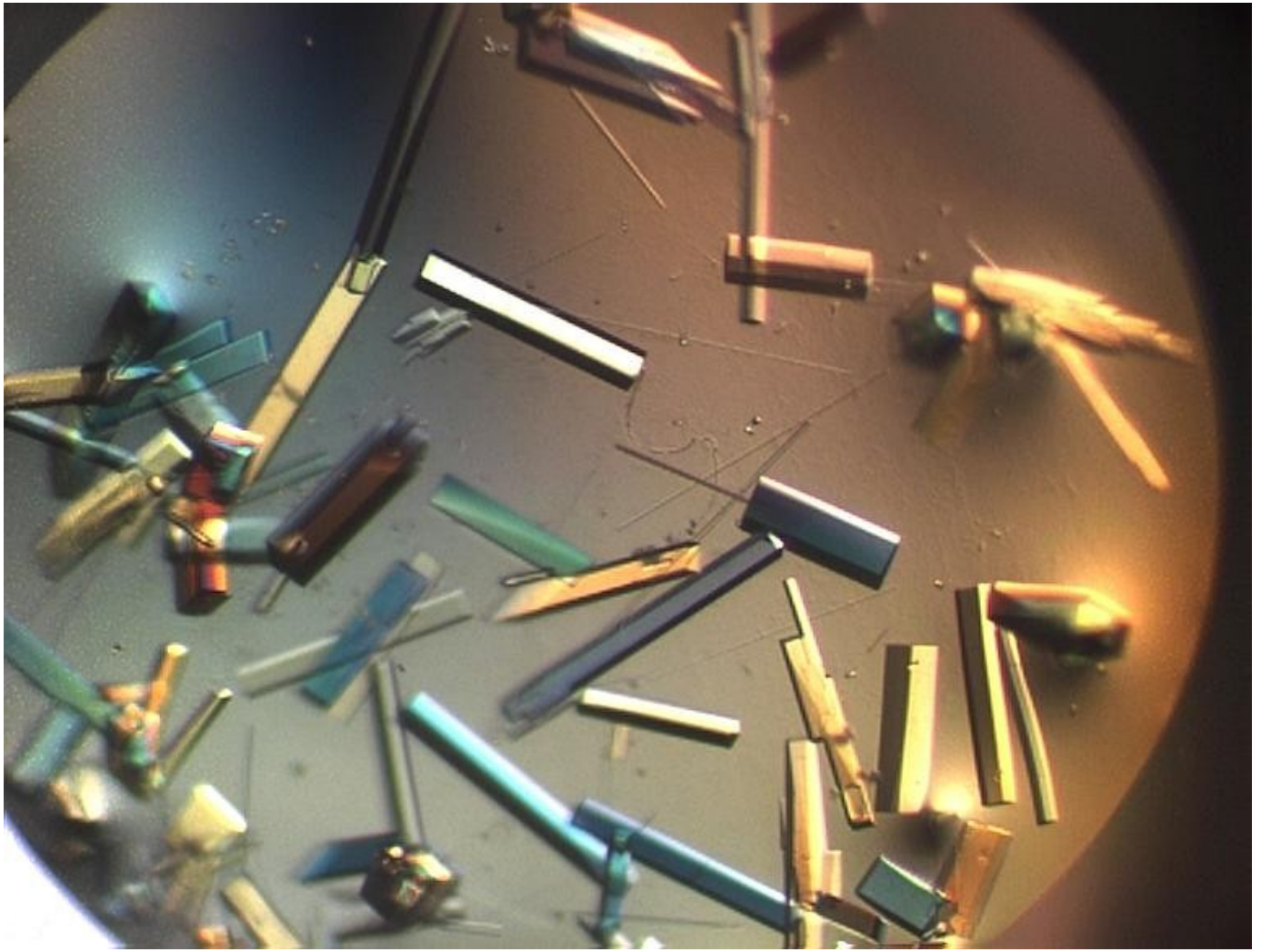
Watson'o ir Crick'o struktūra



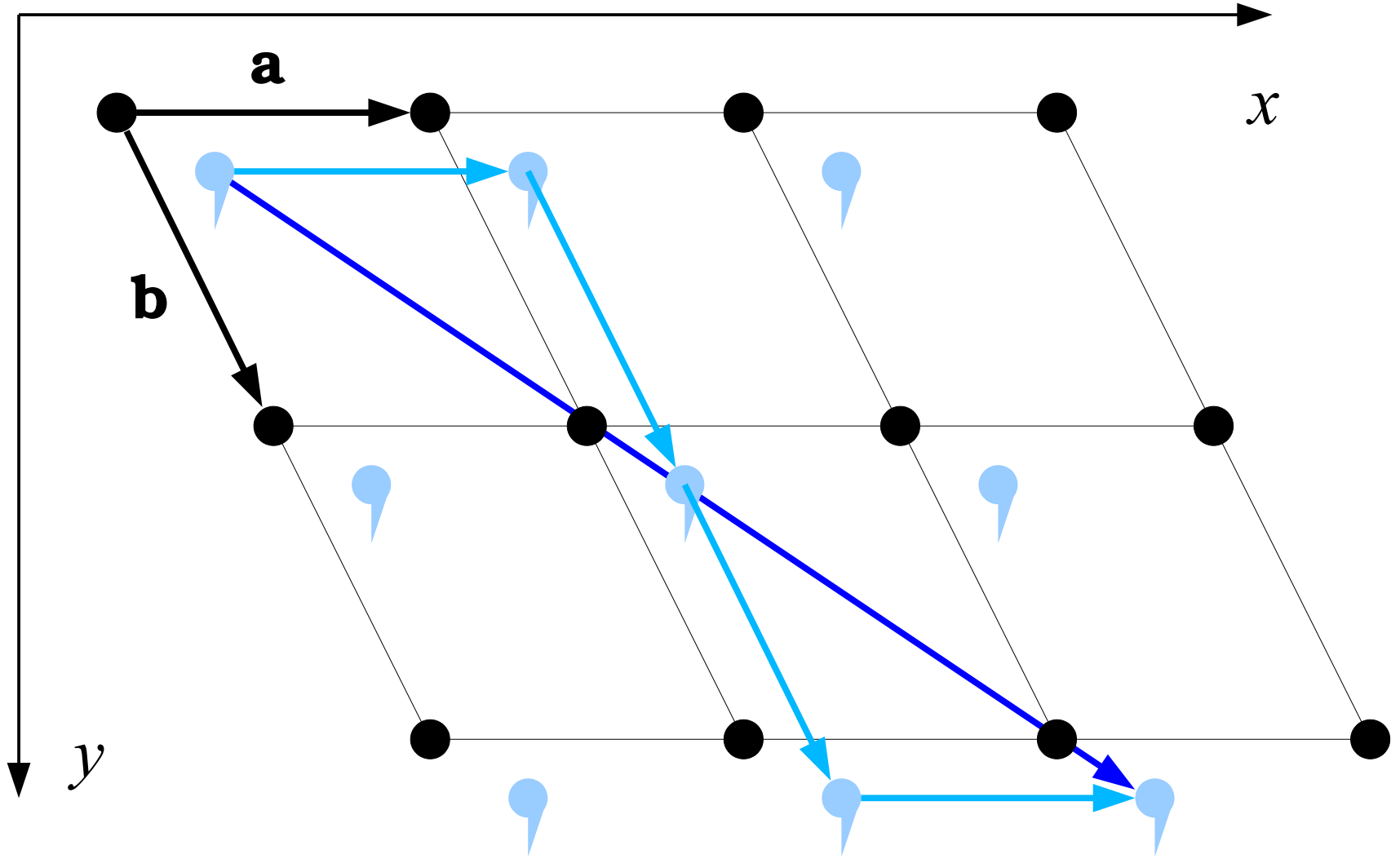


This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

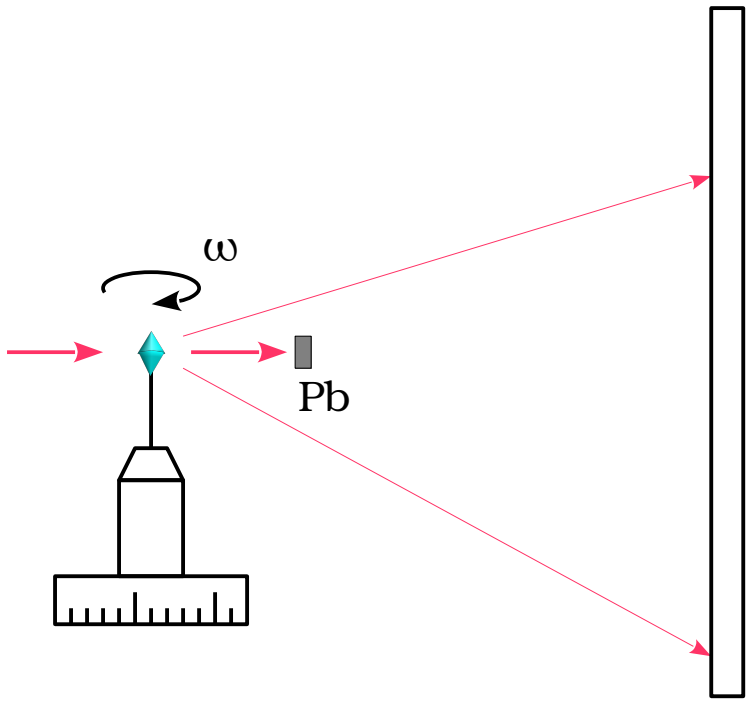
Baltymų ir DNR kristalai

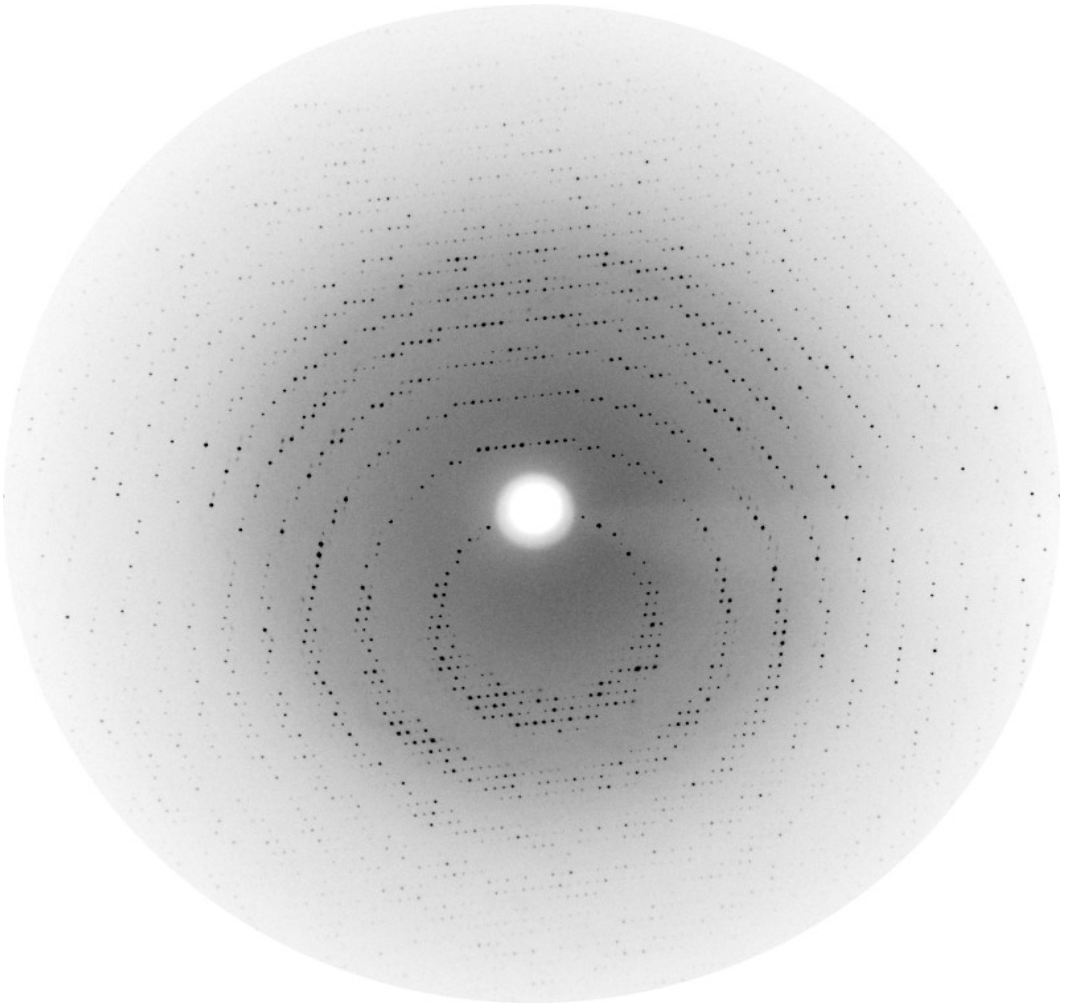


Krisalų vidinė struktūra



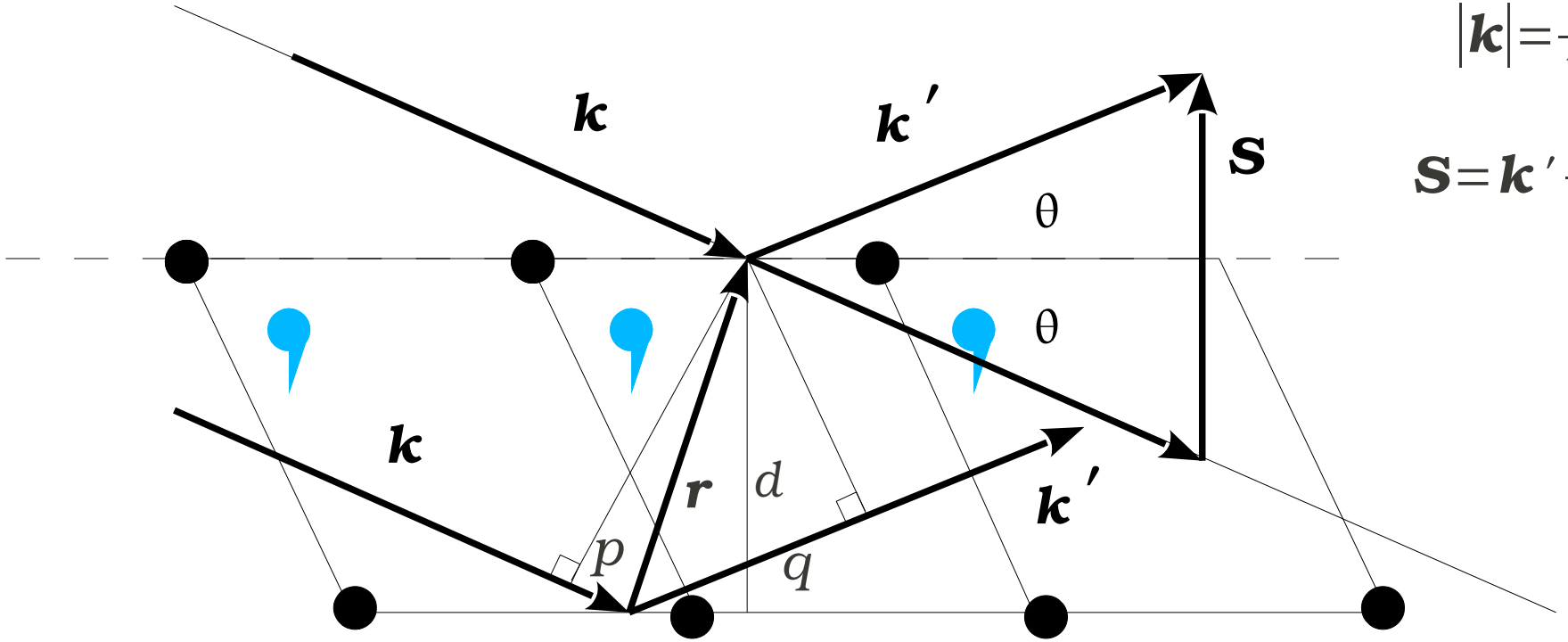
Tipiškas difrakcijos vaizdas





Kristalų difrakcija

Brego dėsnis



$$|\boldsymbol{k}|=\frac{1}{\lambda}$$

$$\mathbf{S}=\boldsymbol{k}'-\boldsymbol{k}$$

2) Brego dėsnis (Bragg's law):

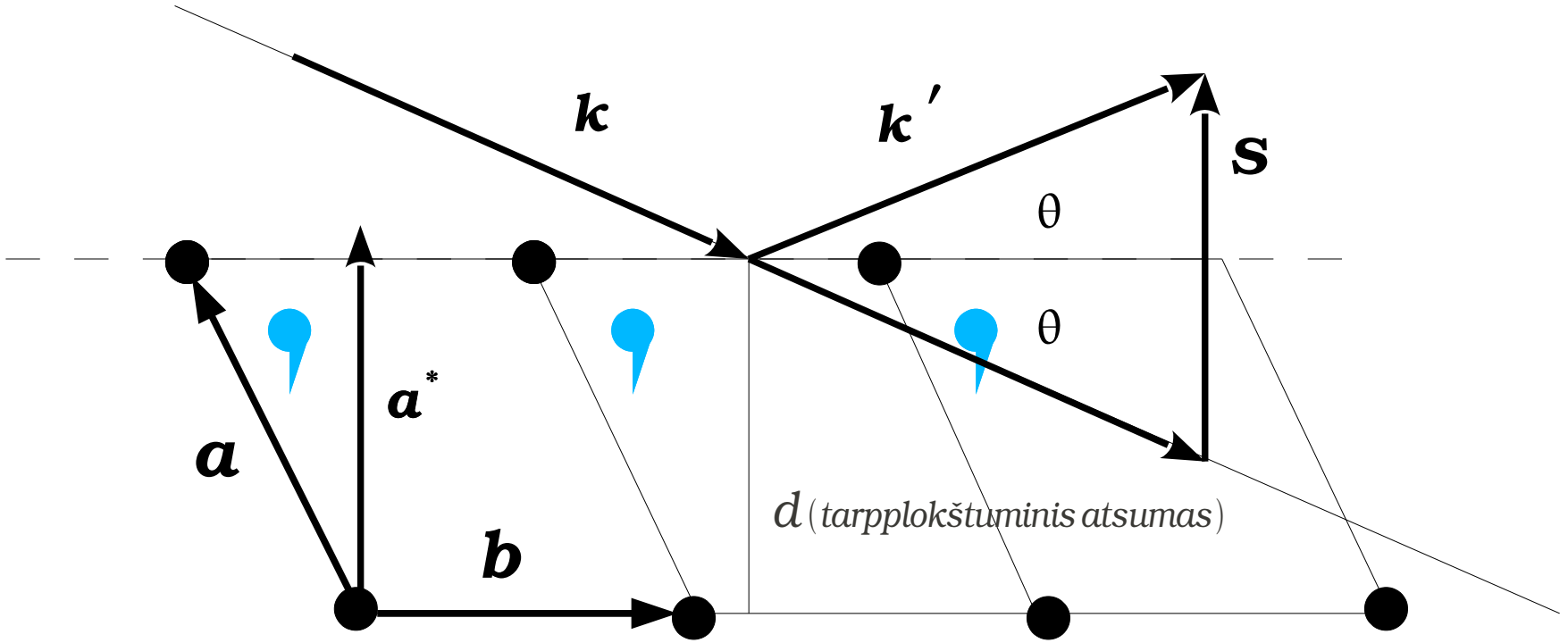
$$\Delta=p+q \qquad \Delta=(\boldsymbol{k}'-\boldsymbol{k})\cdot\boldsymbol{r}=\mathbf{S}\cdot\boldsymbol{r}=\frac{\mathbf{S}\cdot\boldsymbol{r}}{|\mathbf{S}|}|\mathbf{S}|=d|\mathbf{S}|=\frac{d\cdot 2\sin(\theta)}{\lambda}=n$$

$$p=-\hat{\boldsymbol{k}}\cdot\boldsymbol{r}/\lambda$$

$$q=\hat{\boldsymbol{k}}'\cdot\boldsymbol{r}/\lambda$$

$$|\mathbf{S}|=\frac{n}{d}=\frac{2\sin\theta}{\lambda}$$

Atspindys nuo kristalo



1) Laues (Laue) sąlygos:

$$\mathbf{S}\boldsymbol{a}=h;\ \ \mathbf{S}\boldsymbol{b}=k;\ \ \mathbf{S}\boldsymbol{c}=l$$

$$\mathbf{S}=h\,\boldsymbol{a}^{*}+k\,\boldsymbol{b}^{*}+l\,\boldsymbol{c}^{*}$$

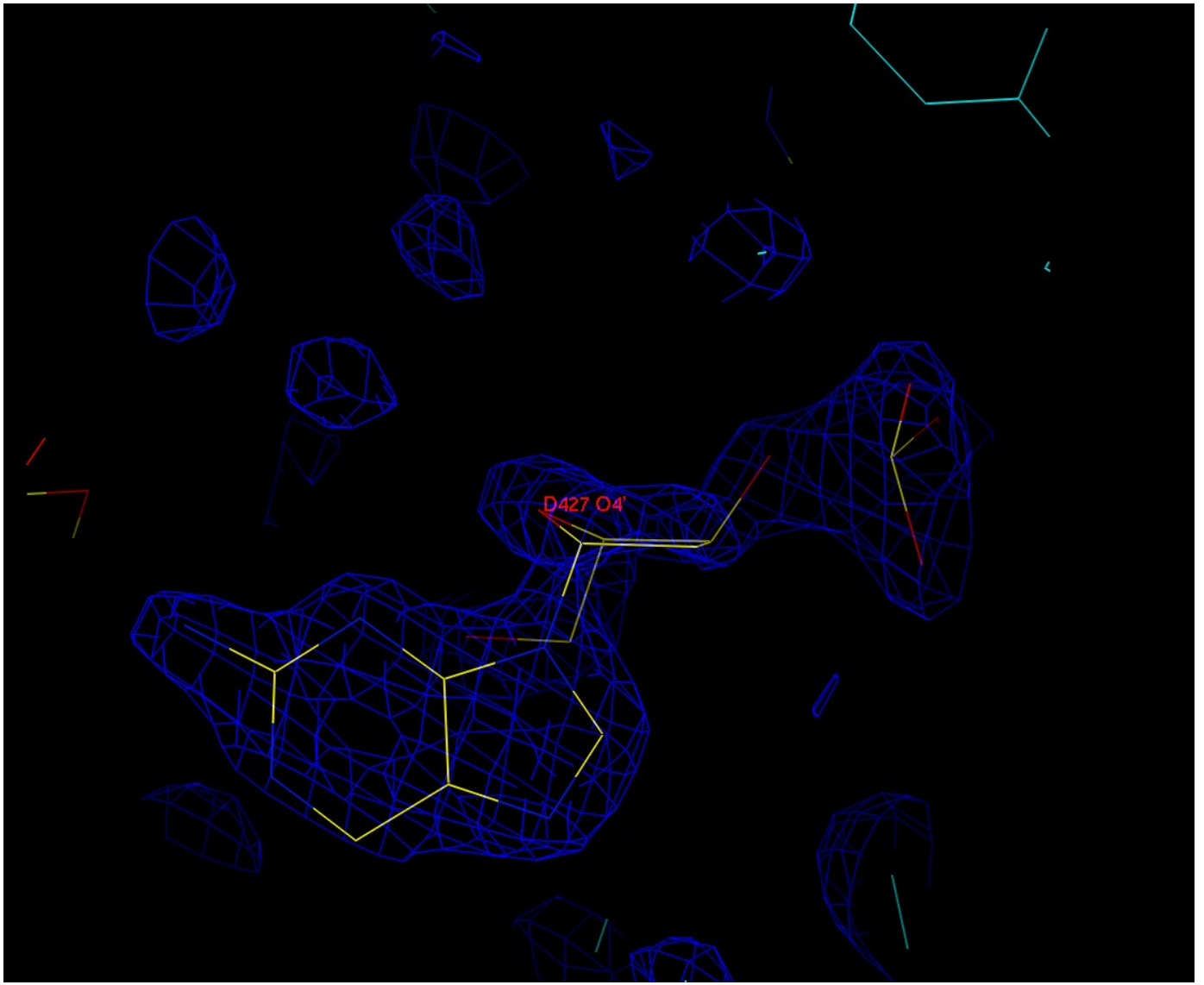
2) Brego dėsnis (Bragg's law):

$$|\mathbf{S}|=\frac{n}{d}=\frac{2\sin\theta}{\lambda}$$

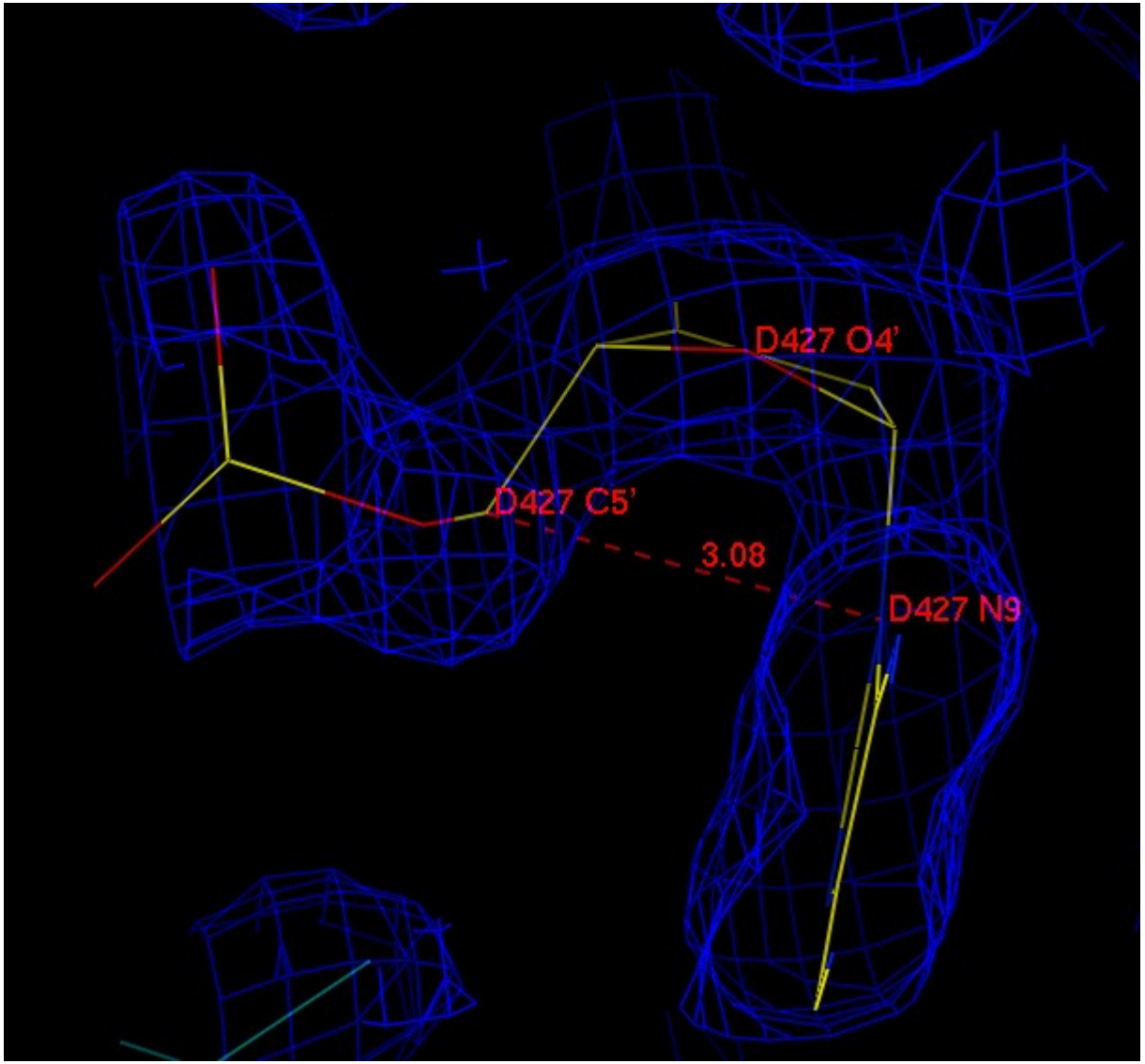
$$\mathbf{S}=(\boldsymbol{k}-\boldsymbol{k}')/2\pi$$

Spindulys atsispindi nuo kristalo tik tada, kai patenkintas Brego dėsnis ir Laues sąlygos.

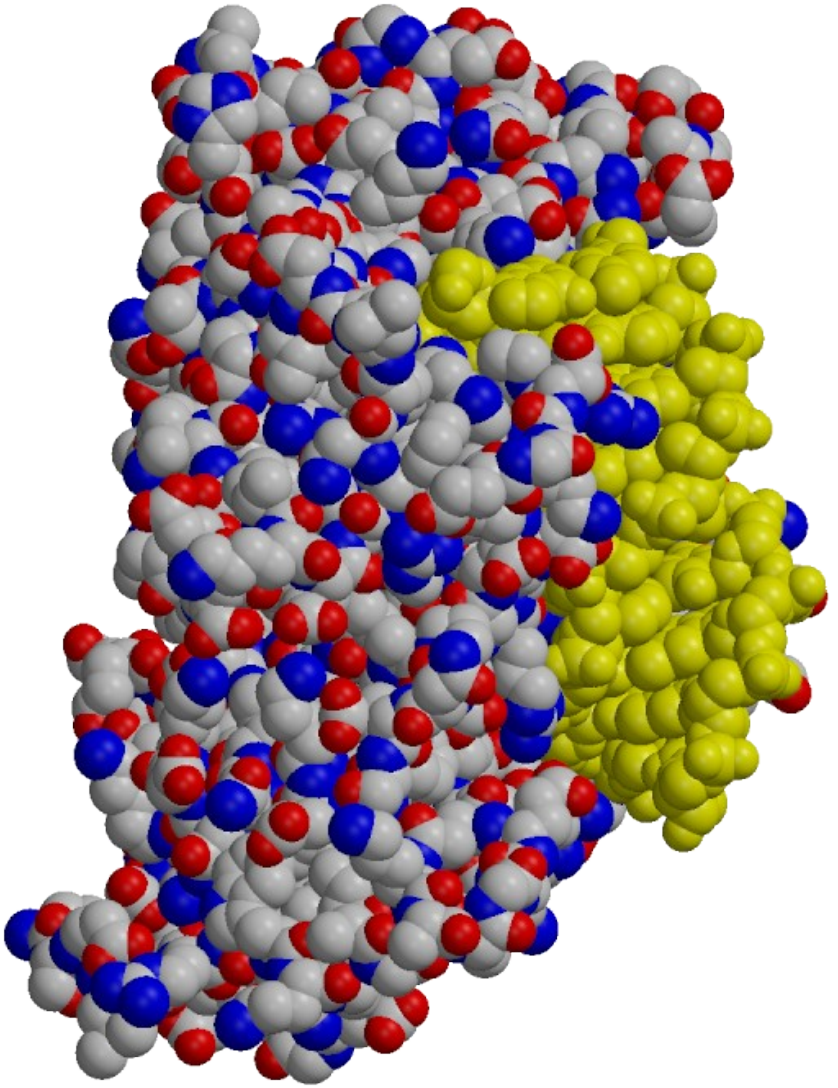
Elektronų tankis



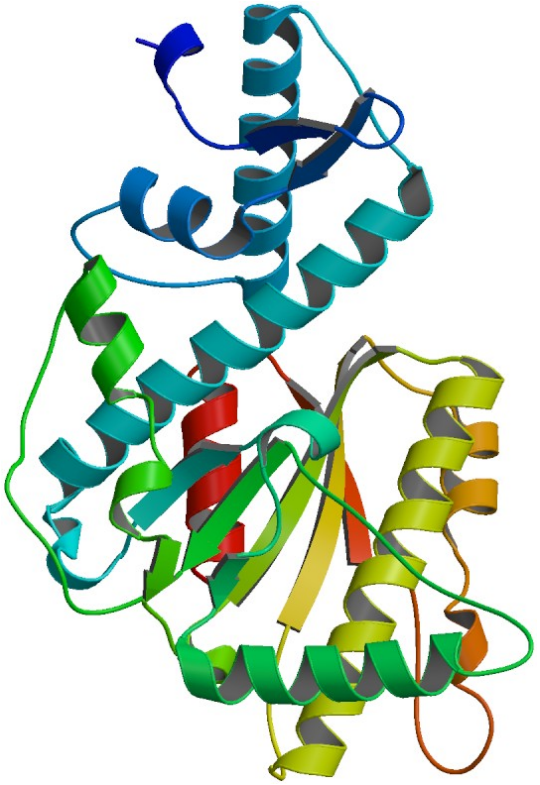
Elektronų tankis (2)



Baltymų ir nukleorūgščių kompleksai

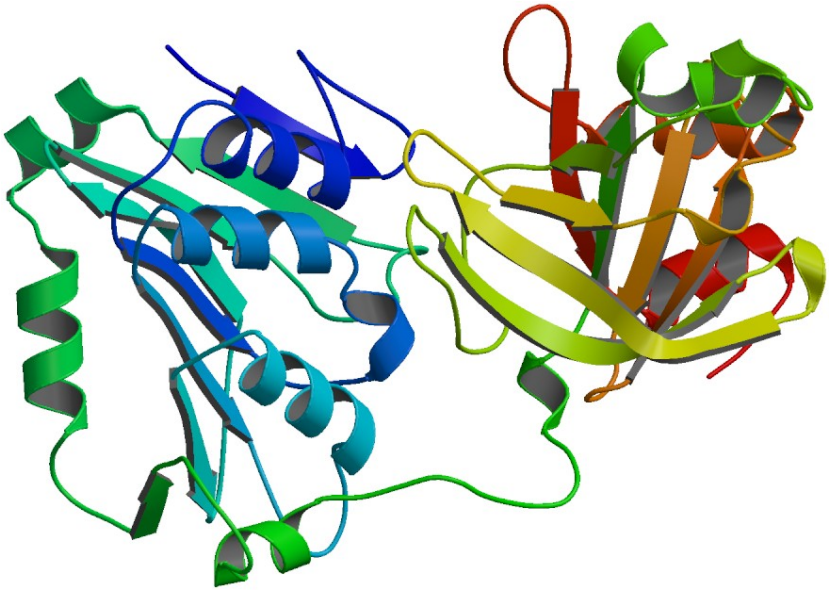


Tretinė struktūra



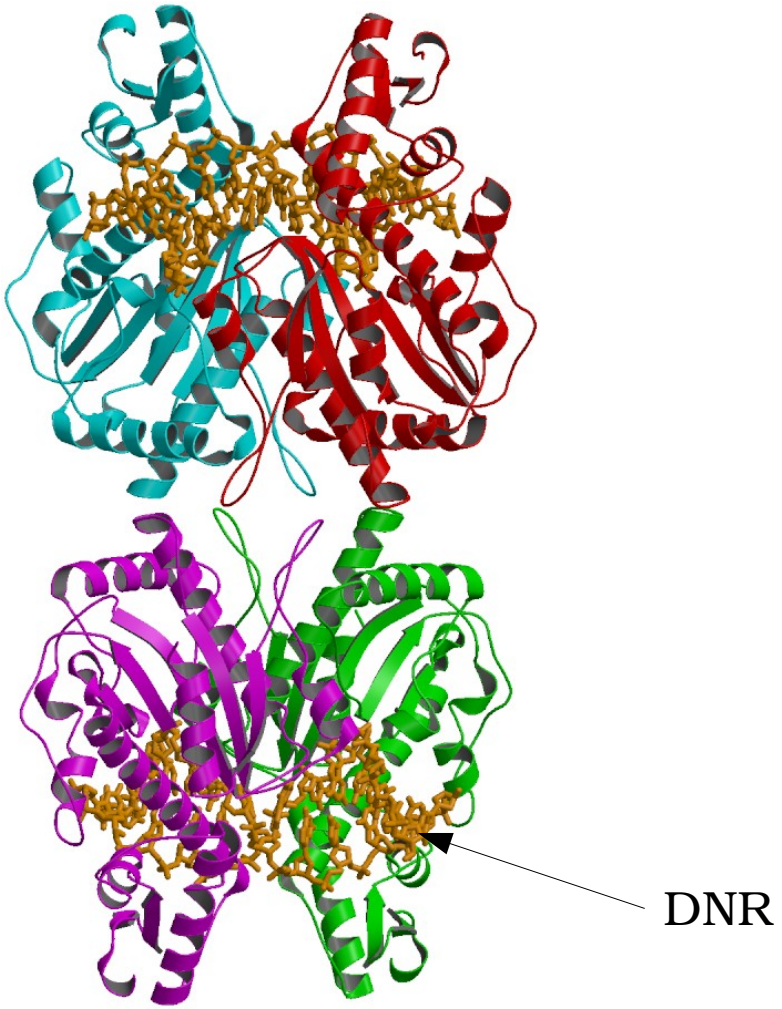
Bse634I restrikcijos endonukleazė
Gražulis *et al.* NAR 2002 p.876

BfiI restrikcijos endonukleazė
Gražulis *et al.* PNAS 2005 p.15797

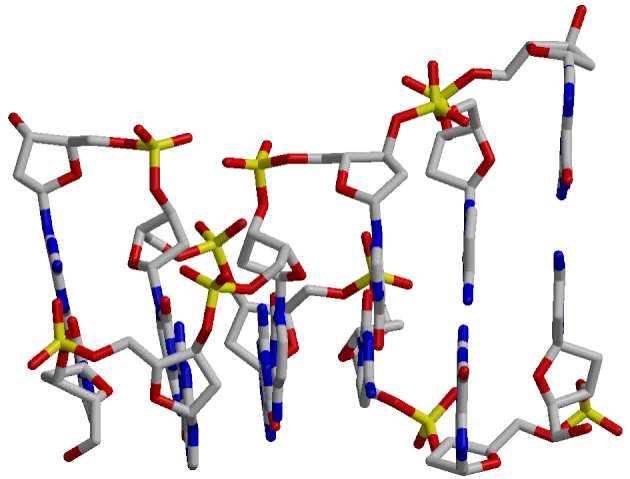


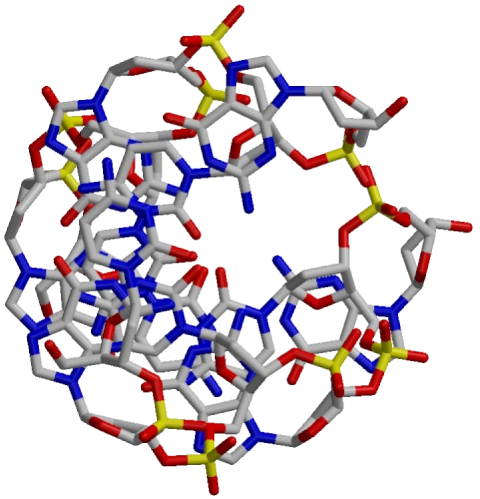
Ketvirtinė struktūra

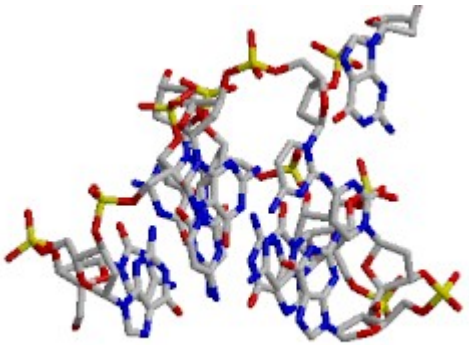
Bse634I REazės
tetrameras kristale



Z formos DNR

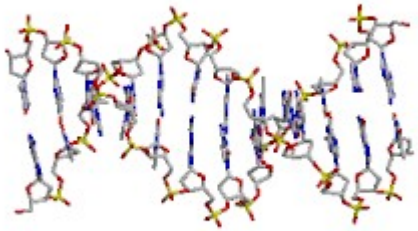


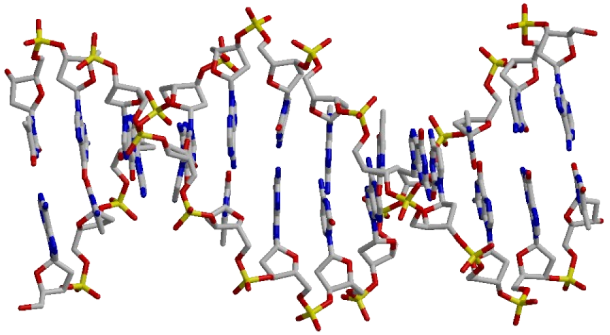


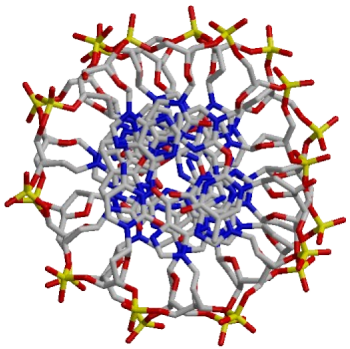


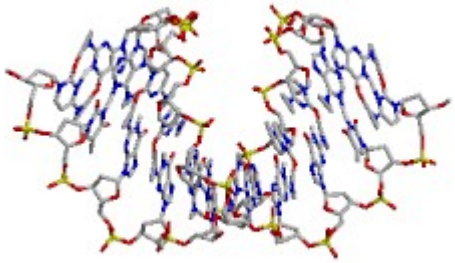
Andrew Wang, Alexander Rich, and co-workers in 1979 at MIT
(šaltiniai: Vikipedija, Nukleorūgščių duomenų bazė (NDB))

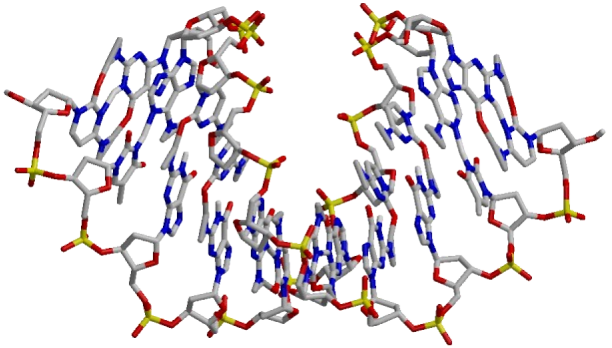
A ir B formos DNR

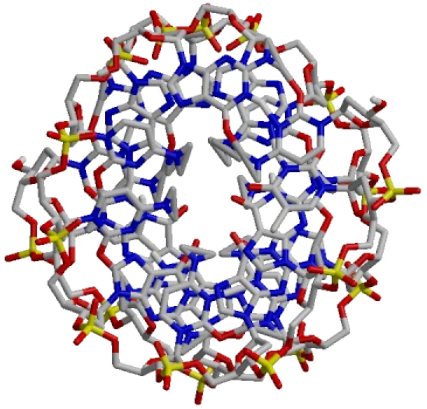




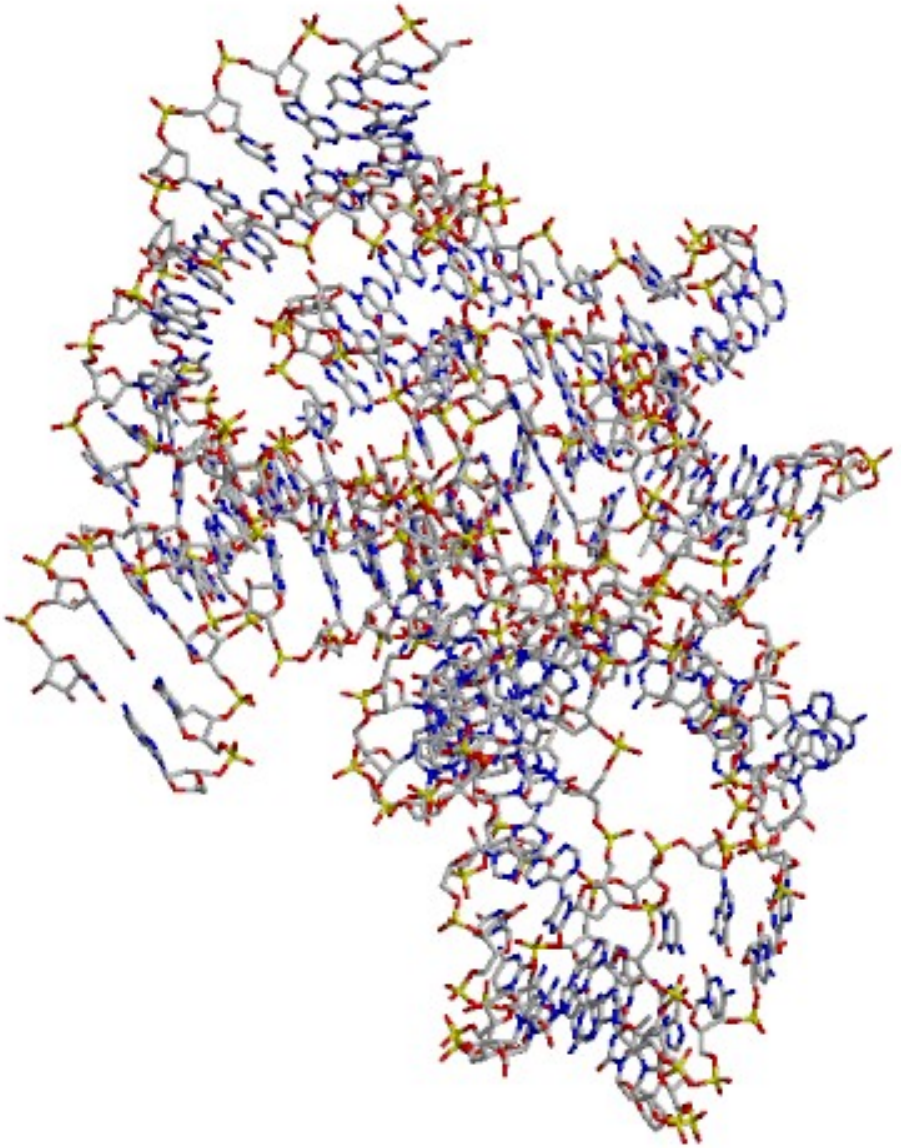






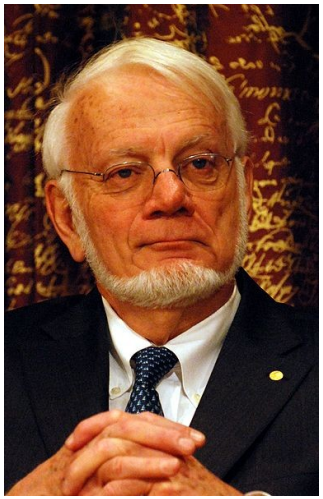


Ribozimai

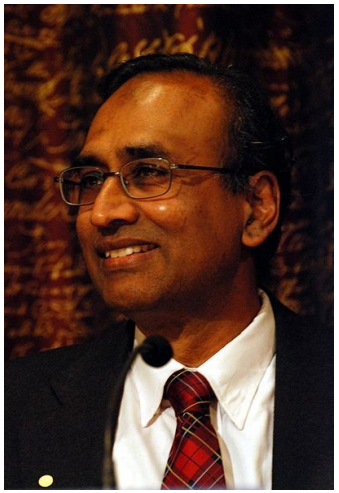


Ribosoma

Ada Yonath

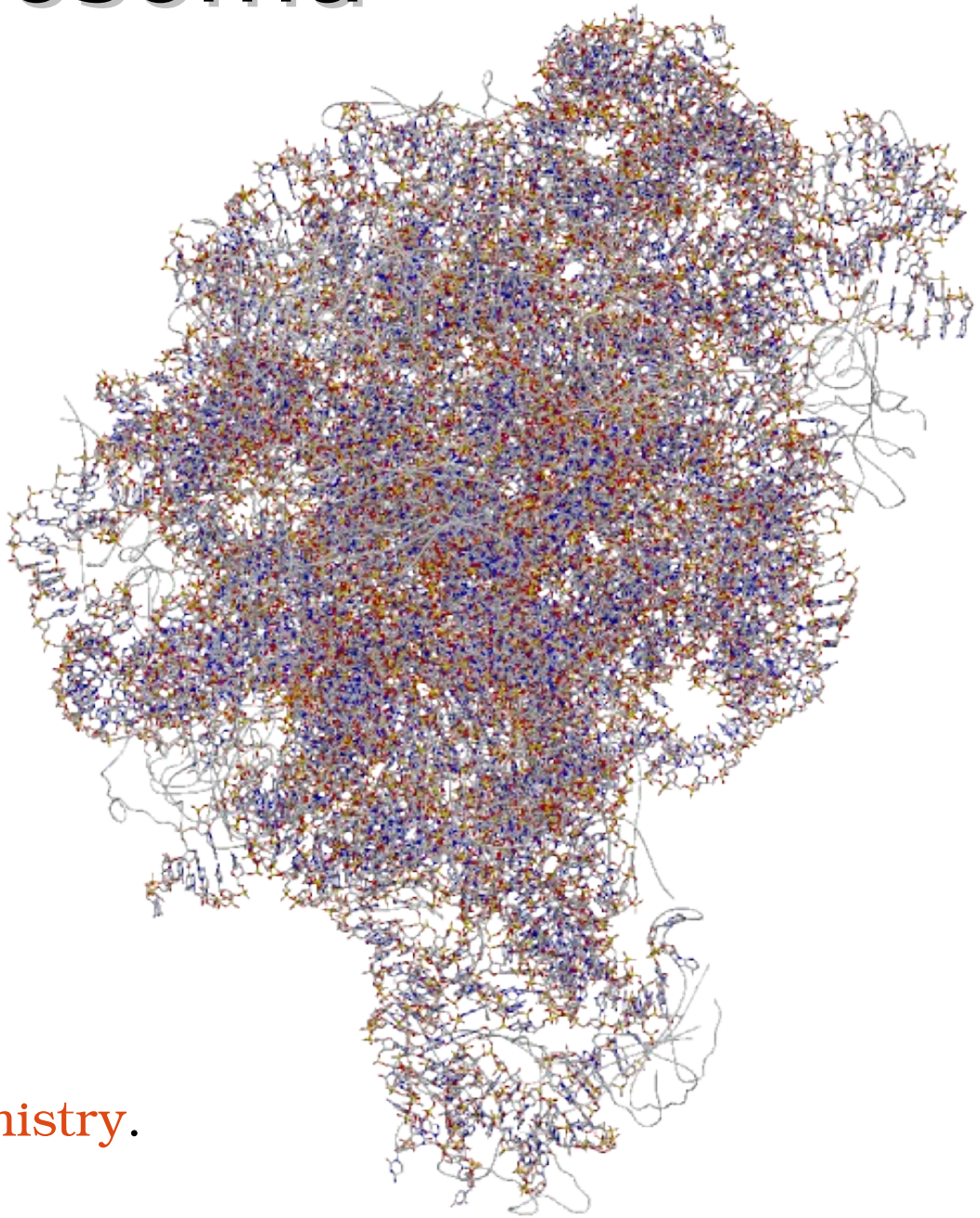


Thomas A. Steitz



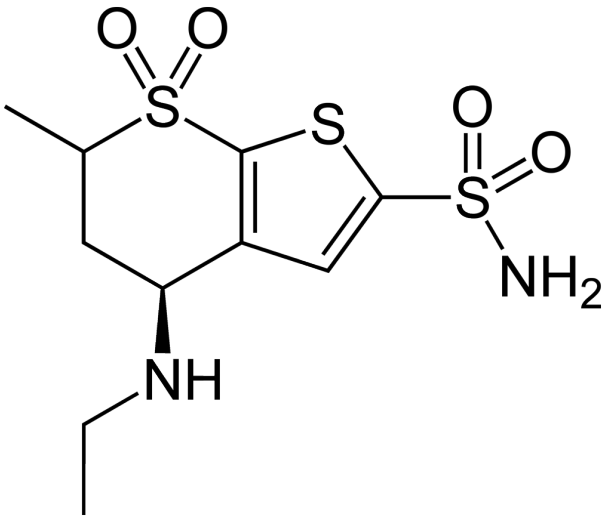
Venkatraman Ramakrishnan

The Nobel Prize in Chemistry.
2009

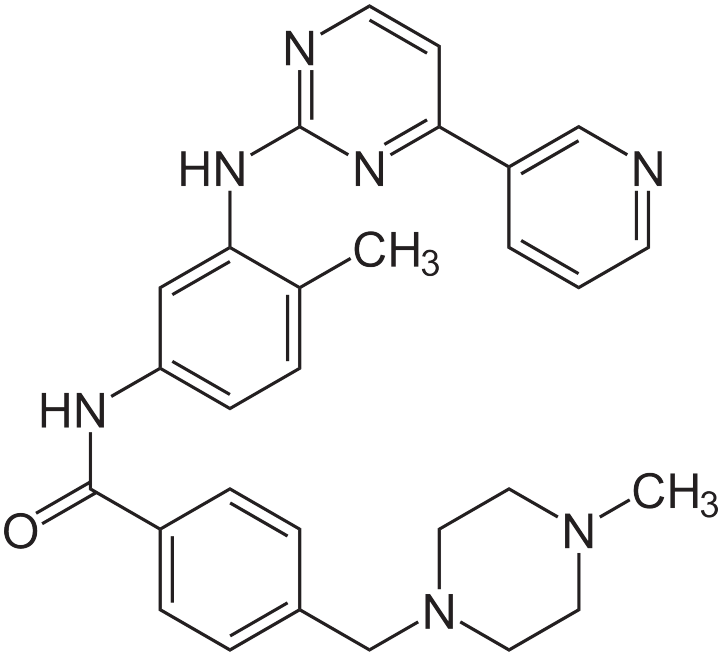


šaltiniai: Vikipedija, Baltymų duomenų bankas (PDB)

Taikymai: racionali vaistų paieška



Dorzolaminas (karboninių anhidrazių inhibitorius, vaistas nuo glaukomos)



Imatinib'as (baltymų kinazių inhibitorius, priešvėžinis vaistas)

Ačiū už dėmesį

Mielai atsakysiu į Jūsų klausimus