PHASM800 Biophysics

Book: Rob Phillips "Physical Biology of the Cell"
>1000 pages, too big for 1-term course
we will stick to highlights.

Exam: similar to previous years, but more emphasis on descriptive answers ~50% of marks

Homework + reading: weekly assignment with addition of an "essay" question. Practice for exam
- give as much detail in < 1 page of writing.
Only handwritten originals accepted.
Problems are <u>in advance</u> of lecture so you have to look at the book. Handed in at start of class.

All communications by Moodle.

Content: 10 × 3hr lectures.	Chapters.
1. Introduction: molecules of life	1-4
2. Entropy & energy.	5-6.
3. 2-state systems	7
4. Random walks	8
5. Elasticity and beams.	10
6. Dynamics and diffusion	12-13.
7. Rate equations	15
8. Molecular motors.	16
9. Ion channels & neurons.	17
10. Chromosomes. + Misc.	?

1. The Central Dogma (Crick, 1956)

DNA → RNA → Protein

Replication (DNA) — Transcription (DNA → RNA) — Translation (RNA → Protein)

DNA polymerase — RNA polymerase — Ribosome. (enzymes)

Exceptions:

i) reverse transcriptase used by retroviruses (HIV)
ii) post-translational modification
chaperones, cleavage → inteins
iii) carbohydrates and lipids.
iv) methylation and acetylation
v) mRNA splicing and editing: introns and exons.
slides vi) cofactors, metal ions, hemes, flavins ...

2. Genetic code.

mutations 4 bases (DNA, RNA) → 20 amino acids.
3-base codon includes degeneracy, stop/start.
Protein is historically over emphasized in dogma
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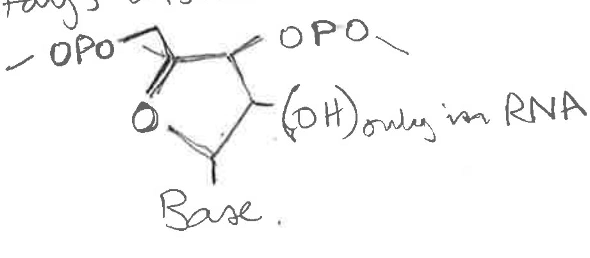
Eucaryotic sequences of DNA known.
Only 1% code for proteins. as "genes"
99% has other function eg control/regulation
+ repetitive sequences } not in "genome"
satellite DNA }
tend to be ignored, but should not be.
RNA intermediate is short-lived, transient
slides. chemically less stable than DNA. Disposable.

3. DNA optimised for long-term storage.

even UV light is quenched before it breaks bonds.
Stays inside nucleus; mRNA goes out



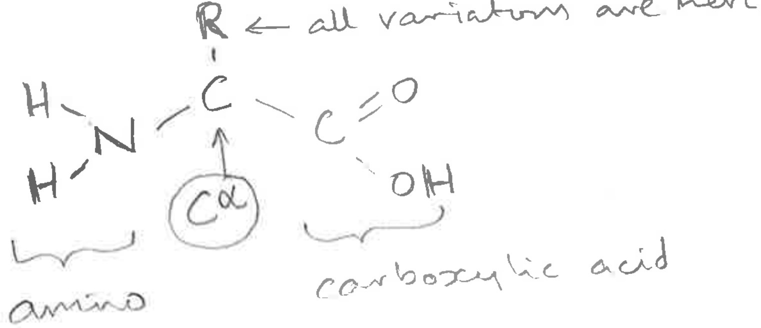
Sugar-phosphate backbone
highly charged

which one is limiting?

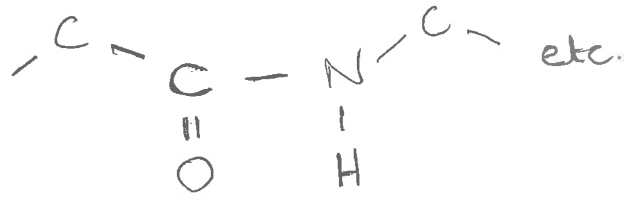
4. Molecules of Life.
Atoms CNOH only +S, (P) in almost all cases.
Special adoption of other elements, but not essential.
4 classes of polymer + lipids account for all essential processes in life with a number of small molecules, specially synthesised.

- DNA, RNA seen above.
- Proteins: linear polymers of 20 amino acids. Enormous number of combinatorial possibilities. Used to make enzymes, mechanical structures, control proteins, motors etc etc.
- Polysacharides: branched polymers of sugars all isomers of glucose but lots of ways of joining together. No strong rules or patterns yet discoverd, except when closely associated with protein.

5. Protein chemistry.
Amino acids.



Polymerised by condensation (removale of H_2O) to form amide bond.



Within Ribosome.

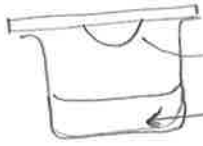
6. Genetic Code.
Major revelation in 1960's following structure of DNA. [10-year lag]. 5290 citations
Structure of DNA, Watson-Crick Nature (1953)
Now the ribosome and t-RNA structures are known the 3-letter code is obvious (p10).
Ribosome [movie] DNA Learning Center CSH.

7. Protein Structure

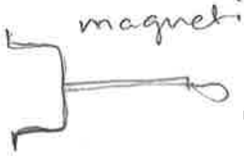
Mainstream method of X-ray crystallography.
Major application of biophysics: measure structures and deposit them in protein data base (PDB.)
$>10{,}000$ depositions / year. >100K total.

i) crystalise proteins. [movie]
purify, concentrate, increase salt so they become supersaturated. Grow slowly.

eg hanging drop
few μL.
10 mg/ml conc.



ii) mount on loop.



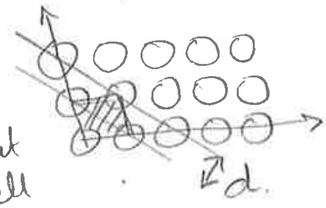
Scoop up and freeze in LN_2. {store, ship.

iii) place in X-ray beam and measure diffraction

eg Diamond
$\lambda = 0.1$ nm.

[movie]

Principles of method.

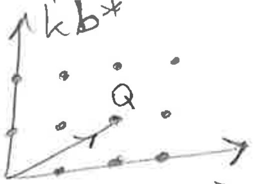


crystal and unit cell
X-rays reflect from parallel planes

$2d\sin\theta = \lambda$ for constructive interf.

d ~ size of protein 2–100 nm.
λ = wavelength ≃ 0.1 nm

Theorem (not proved) every possible set of planes corresponds to a point (h,k,l) in a reciprocal lattice



$Q = \vec{k}_f - \vec{k}_i = \frac{2\pi}{d}$

$\left(\frac{2\pi}{\lambda}\right)$

Whenever $\vec{Q}$ is a point on the recip lattice, the X-rays are scattered along direction $\vec{k}_f$ with amplitude

$$A(\vec{Q}) \propto \int_{\text{unit cell}} \rho(\vec{r})\, e^{-i\vec{Q}\cdot\vec{r}}\, d^3\vec{r}$$

Fourier transform in 3D.

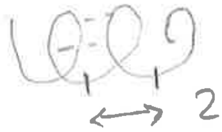
Experiment measures intensity $I(Q) = |A(Q)|^2$
but loses the phase of A.
Computational methods are needed to "solve the phase problem".
Then F.T can be inverted:

$$\rho(\vec{r}) = \int A(\vec{Q})\, e^{i\vec{Q}.\vec{r}}\, d^3\vec{Q}$$

– integral becomes a sum over discrete peaks of $\vec{Q}$ on the reciprocal lattice.

8. Results of protein structure
Crystallography can see the atoms, but there are clear patterns of "secondary structure"

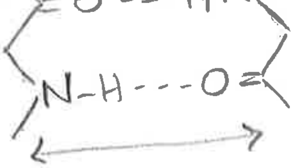
i) alpha helix. (Pauling 1950)



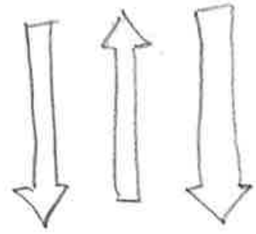
denoted by spiral ribbons
2.5 amino acids / turn
hydrogen bonds from NH---CO of amide groups holding together

R-chains point outwards, so all proteins can form helices.
Common in hydrophobic environments eg crossing lipid membranes

ii) beta sheets



denoted by arrows



0.7nm spacing.

9. See ribbon diagrams
Using PDB. to see general layouts.
Many standard folds often α & β regions separated.
"β-barrel" → tube.
"Rossmann fold" alternating α-β.
"TIM" triose phosphate isomerase. (slide). 3TIM p70

10. Hemoglobin, example protein
Important example PDB. → 1XZ2
Illustrates how amino acid residues (R) can determine folding pathways.

10. Protein folding.

i) Very small proteins spontaneously fold to the correct structure reversibly after denaturation.
Heat or concentrated Urea (6M) → denature.
Cool or dilute → refold.

ii) Many will fold in the sequence coming off the ribosome, often multiple domains of ~100-amino acids, rarely more.

iii) Sometimes require help:
- chaperone structures to guide.
- post translational mods: insulin → 2 chains.

iv) Disulphide bridges often important.
Cysteine (C) $R = CH_2SH$ $HS-CH_2-C^{\alpha}$
S–S after reduction

v) Salt bridges between charged residues
Glu (E) Asp (D) = acids, −ve at pH 7.
Arg (R) Lys (K) = bases, +ve at pH 7
His (H) charges near pH 7
often used for titratable roles in enzymes

vi) Polar residues, uncharged
hydrophilic facing solvent, eg outside

vii) Hydrophobic residues, oily
group together <u>away</u> from solvent.

11. Fibre diffraction

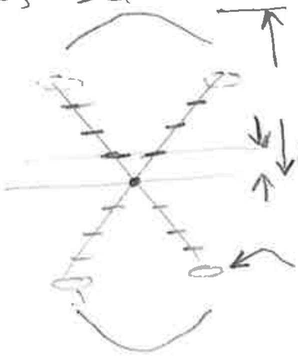
Not all molecules can crystallise.
eg if they are not homogeneous enough.
Can use X-ray Small Angle Scattering
SAXS from solution.
Much less information but still possible to solve

DNA can be crystallised, but only synthetic
short strands.
Otherwise, carefully draw out into fibres
which are still oriented. Study with X-rays.
No reciprocal lattice, but still information

Famous example of DNA structure from
data measured by Rosalind Franklin...

10) contd
Important example = Hemoglobin
will visit later in course.
PDB entry: 1XZ2
All α-helix protein, easy to crystallise
Studied by Max Perutz >10 years to solve
in Cavendish Lab of Lawrence Bragg.
PhD supervisor of Francis Crick.
[movie] of related Myoglobin responding to
dissociation of CO by light pulse.
[movie] of PGK phosphoglycerate kinase
enzyme changes shape to bring reactants
together.
"Kinase" enzyme transfers P from ATP
to -OH of protein, usually serine (S)
activated state → further reaction

13. "Life Story" DVD. Horizon / PBS Nova 1978
Many other versions available on YouTube, but not this one = best. Set in 1952-3.
Full length; we skip first 53 minutes.
Start at "Photo 51" measured by Rosalind Franklin at Kings College.
2 forms of DNA fibers, carefully drawn and dried at 70% humidity. Raymond Gosling = student
A form, more crystalline – RF studied this
B form, classical helix – Maurice Wilkins.
Randall = lab director at Kings; managing tensions between these two



↑ base-pair spacing $\frac{2\pi}{0.34nm}$

↓↑ helix pitch $\frac{2\pi}{3.4nm}$ ← ⇒ 10 turns.

← 4th spot missing ⇒ asymmetric double helix 3:1 spacing.

19kB!

Scene opens in Cambridge:
Jim Watson + Francis Crick.
Lawrence Bragg = head of Cavendish Lab.
i) Helix Diffraction
ii) Nucleotide base ratios: (Irwin) Chargaff's rule.
A=T, C=G over wide range of dsDNA.
iii) tautomeric form enol – keto
Jerry Donahue, friend of Peter Pauling.

Further information:
DNA; secret of photo 51 (youTube)
life of Rosalind Franklin.

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14. Biological time scales. (ch3, p88).

evolution	10^{17} s	4 billion yrs	– age of earth.
	10^{15} s	6 million yrs	– human / chimp separation
	10^{12} s	40,000 yrs	– neanderthal extinct 0.12% different from homo S.
organisms	10^{11} s	3 000 yrs	sequoia lifespan.
	10^{9} s.	80 yrs	human lifespan
cells	10^{5} s	24 hrs	doubling time of human cell
	10^{3} s	20 mins	E. coli doubling time
enzymes	1 s		lysozyme turnover
	10^{-6} s		carbonic anhydrase turnover.
bonds.	10^{-9} s.	1 ns.	side chain rotation
	10^{-11} s	10 ps.	H-bond rearrangement H_2O
	10^{-14} s	10 fs.	bond vibration period.

15. Evolution. 3 branches of life

(i) archea (about 3 billion yr BP)

(ii) prokaryotes (bacteria, no partitions).

(iii) eukaryotes (animals, multicellular).

all use "central dogma" DNA + genetic code

– small variations: mitochondria AGA, AGG not stop codons

– UGA = tryptophan in myoplasma

– GUG, UUG start codons in prokaryotes + archea.

– alternative amino acids.

Evolution continues at the level of mutations

translocations between species.

Same genes, moving around the genome.

15. Mitochondria.
Generate energy (ATP) within eukaryotic cells.
Very much like prokaryotes (bacteria)
Symbiotic evolution ~ 1.5 Byr BP.
non-Mendelian inheritance. mother only.
16569 base pairs (bp) DNA all coding. [E coli ~1 Mbp].
3.5×10^9 bp human 1% coding
14 proteins coded for
22 tRNAs.
2 rRNAs (ribosomal).
Structure known from TEM, stained sample (slide)
abstracted in other ways eg genome.

16 Cell cycle (eukaryotes)
Mass growth continuous
DNA confined to S phase.
Mitosis is very organised.

17. Examples of motors (slides)
bacterial flagellum E. Coli all structures known
myosin transporter system. + actin filament

18. Drosophila development.
Differentiation by chemical gradients into
completely different cell functions.
Networks of control sytems for gene expression