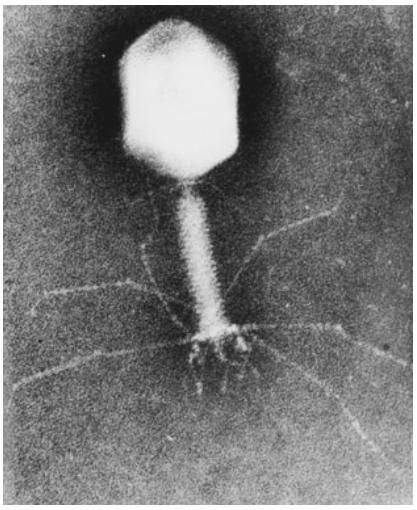
Name **Student Code**

32nd IChO • Problem 5 — 10 points

Protein and DNA



DNA is composed of 2'-deoxy-nucleotides carrying the bases adenine (A), guanine (G), cytosine (C) and thymine (T). The molar mass of the 2'-deoxy-nucleotide-5'-triphosphates is given in table 2:

Table 2 **dNTP**	**Molar mass /g mol^{-1}**
dATP	487
dGTP	503
dCTP	464
dTTP	478

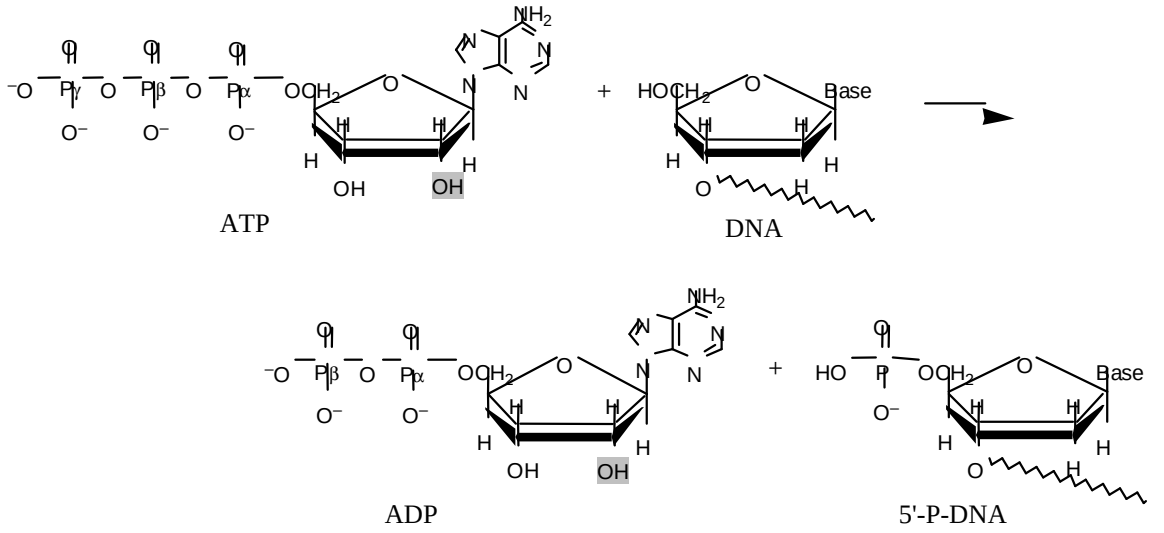
5-1 Calculate the molar mass of a double stranded DNA fragment consisting of 1000 base pairs with a uniform distribution of the four bases.

This DNA fragment can be isolated and cloned by using the PCR method (polymerase chain reaction), in which a heat stable DNA polymerase enzyme multiplies the number of molecules of a specific piece of DNA in a cyclic process. Under optimal conditions the number of double-stranded DNA copies doubles in each cycle.

Using the PCR method you perform 30 cycles starting from a single double stranded DNA molecule.

5-2 Calculate the approximate mass of the DNA you obtain from this experiment.

The bacteria-virus T4 enzyme - polynucleotide kinase (PNK) catalyzes the transfer of the terminal phosphate of ATP (γ-orthophosphate) to the 5'-hydroxyl termini of ribo- and deoxyribonucleotides:



PNK is commonly used to label DNA at the 5'-end with the radioactive phosphorus isotope ^{32}P using ATP in which the γ-P (the outermost of the phosphorus atoms) is replaced with ^{32}P. The amount of ^{32}P and thus the amount of labelled DNA can be measured.

A 10 µL solution containing double stranded DNA is labelled 100% with [γ-^{32}P]ATP by PNK. 37 days ago, the specific activity of [γ-^{32}P]ATP was 10 Ci/mmol or 370 ·10^9

Bq/mmol. ^{32}P has a half-life of 14.2 days, and during the decay a β-particle is emitted. Now the labelled DNA emits 40000 β-particles/s.

5-3 Calculate the concentration of the DNA solution.

In an experiment in which PNK is incubated with [γ-^{32}P]ATP and single stranded DNA, the reaction can be monitored by isolating labeled DNA and measuring the β-particle emission.

Using this kind of measurements in a 1 mL experimental mixture, a labeling of 9 nmol DNA/min was calculated. PNK has a catalytic rate constant (turnover number) of 0.05 s^{-1} and molar mass of 34620 g mol^{-1}.

5-4 Calculate the concentration (in mg/mL) of PNK in the experimental mixture.

Aromatic amino acids, tryptophan, tyrosine and phenylalanine absorb UV light of a wavelength between 240 nm and 300 nm.

In a protein containing several aromatic amino acids, the sum of the molar absorptivity per amino acid $\Sigma e_{\text{amino acid}}$, is approximately equal to the molar absorptivity, e_{protein}, for the protein.

The molar absorptivity, $e_{\text{amino acid}}$, at 280 nm for tyrosine, tryptophan and phenylalanine is 1400 $\text{M}^{-1}\,\text{cm}^{-1}$, 5600 $\text{M}^{-1}\,\text{cm}^{-1}$ and 5 $\text{M}^{-1}\,\text{cm}^{-1}$, respectively. The absorbance of a 10 μM solution of PNK is 0.644 at 280 nm and with 1.00 cm light path. The amino acid sequence of PNK contains 14 tyrosines and 9 phenylalanines.

5-5 Calculate the number of tryptophan residues in a PNK molecule.