Metal ion folding and cross-linking of biomolecules

Macronutrients such as Na^+, K^+, Ca^{2+} and Mg^{2+} excert also an important role in structural stabilization of both particular **proteins motif** and **nucleic-acids polyanionic backbone**.

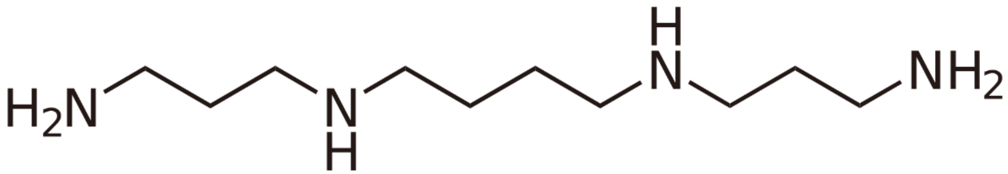
Other metals can also have a role in macromolecules folding and formation of cross-linked structures

↓

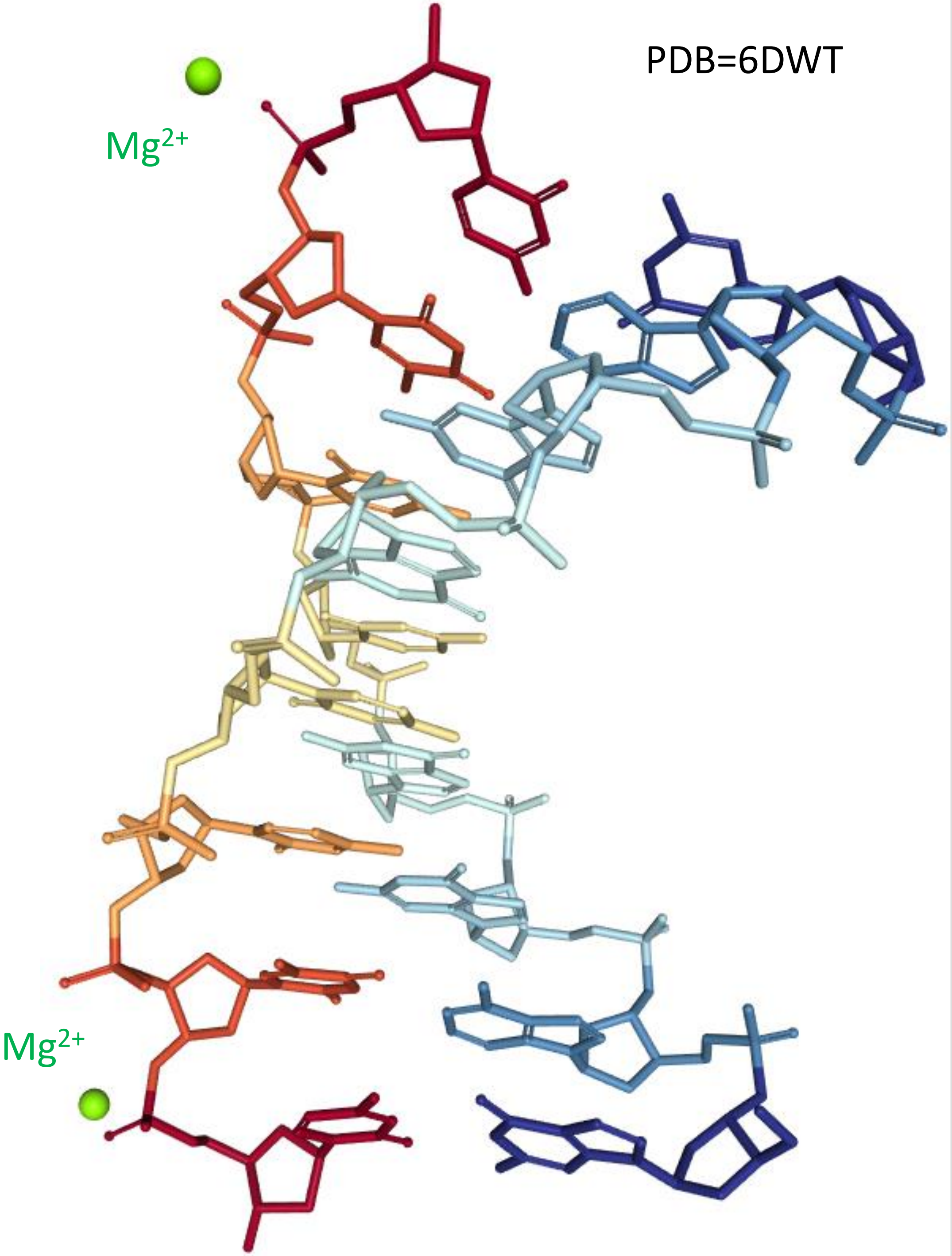
Zn^{2+} in zinc-binding domains of nucleic acid-binding proteins

Nucleic acids are polyanions and, as such, require counterions in order to adopt stable, compact structures. These counterions are required to neutralize partially or completely the negatively charged phosphate groups, so that electrostatic repulsions do not overwhelm other stabilizing effects.

nonspecific complexes with cations such as Na^+, K^+, and Mg^{2+} or by cationic polyamines such as spermine.



However there are examples of specific interactions wich lead to the formation of well-defined nucleid acids structural motifs



PDB=6DWT

Telomers

Specialized DNA structures occuring at the ends of chromosomes.

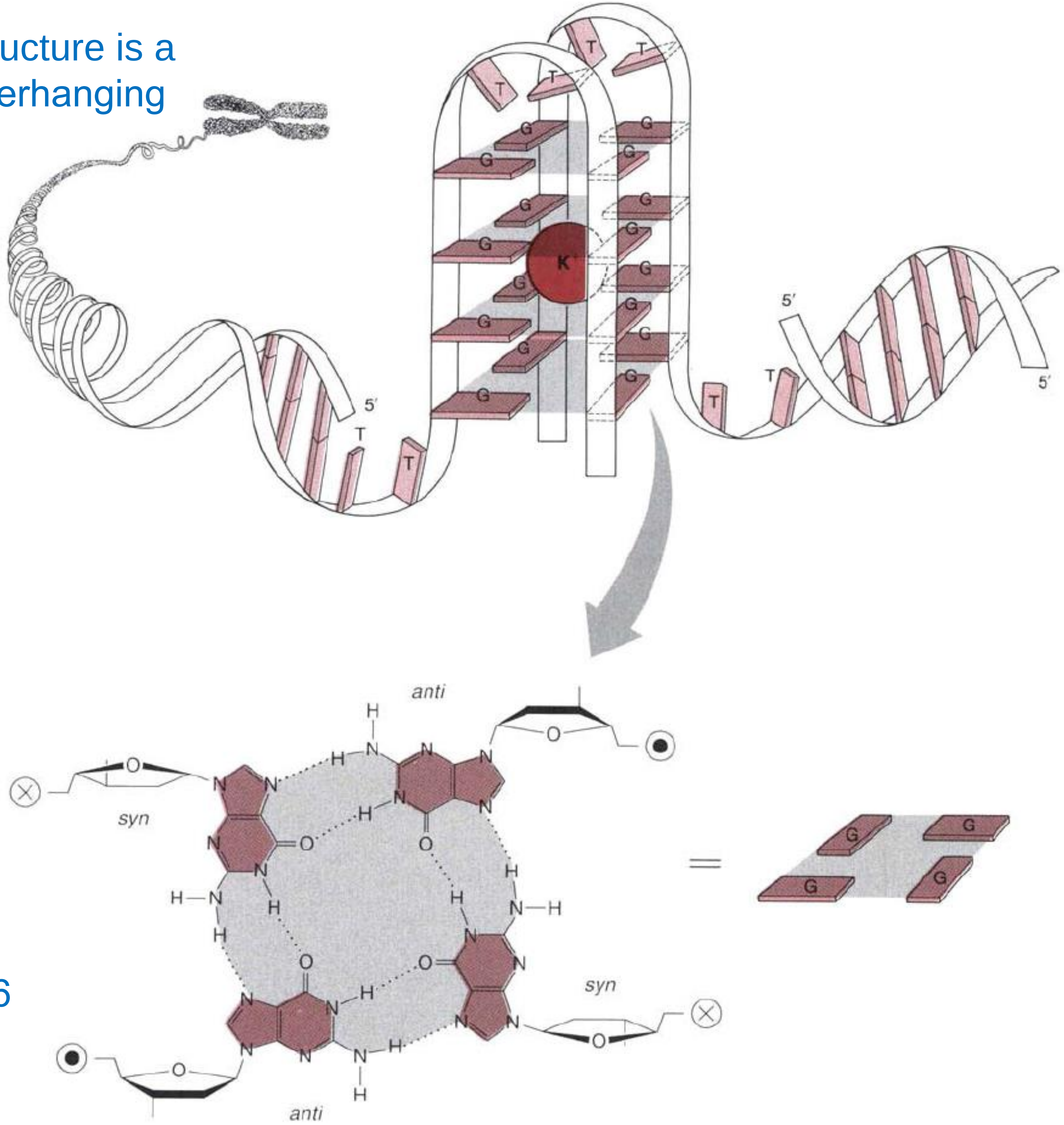
required to avoid **loss of genetic material** from the ends of chromosomes during multiple rounds of replication.

5′-GGGGTTGGGGTTGGGGTTGGGGTTGGGGTTGGGG-3′

3′-CCCCAACCCCAACCCCAACCCC-5′

The sequence of a telomere

The potassium-stabilized structure is a dimer that involves the 3’ overhanging tail folding back on itself



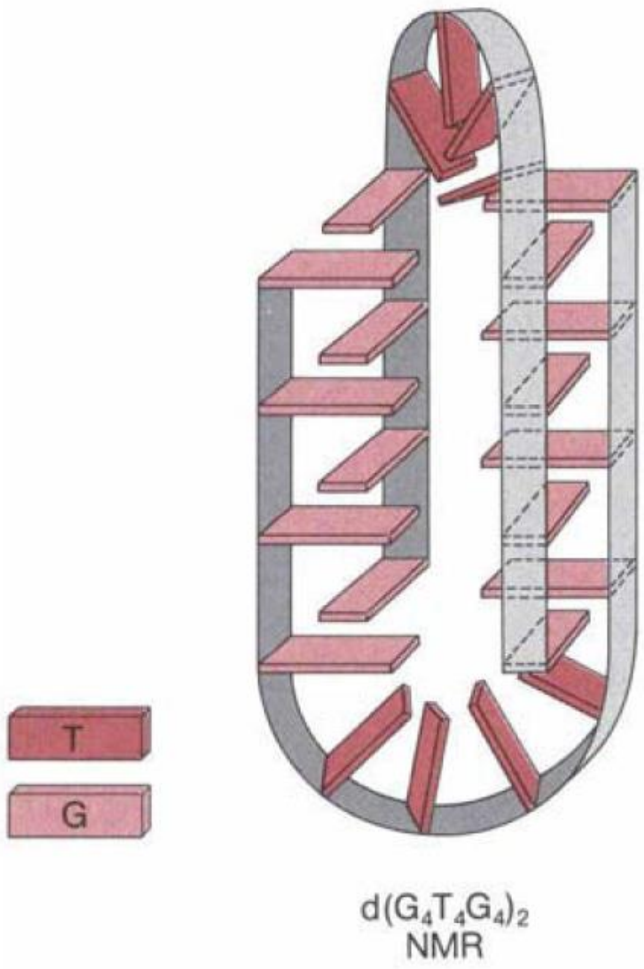
It was experienced that oligonucleotides containing runs of G residues, form structures having anomalous **gel-electrophoretic mobilities** (telomers)

The formation of such species was dependent on the **salt conditions** used in the experiments.

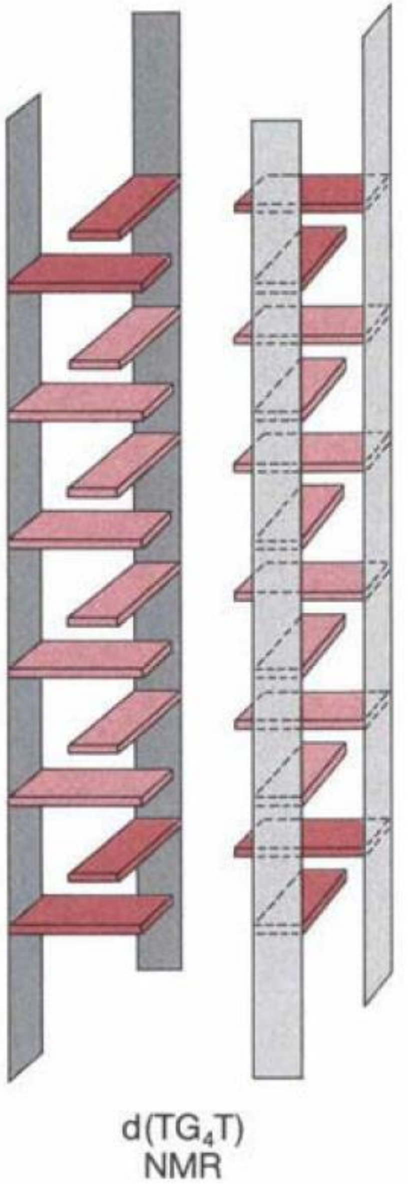
Different structures were generated in either **sodium** or **potassium** enriched solution

This structure has a “hole,” formed by the O6 oxygens from eight guanine residues, that appears to be uniquely suited for K^+

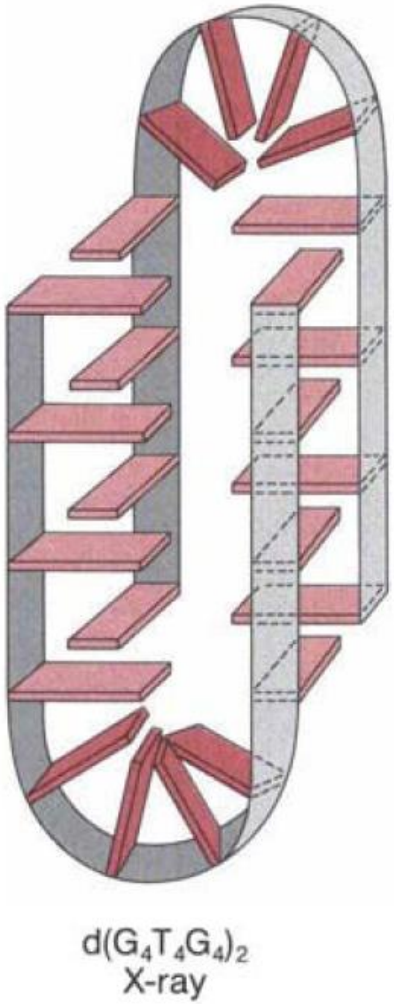
50 mM NaCl solution



100 mM NaCl solution



crystals were grown in the presence of 60 mM K^+ ion



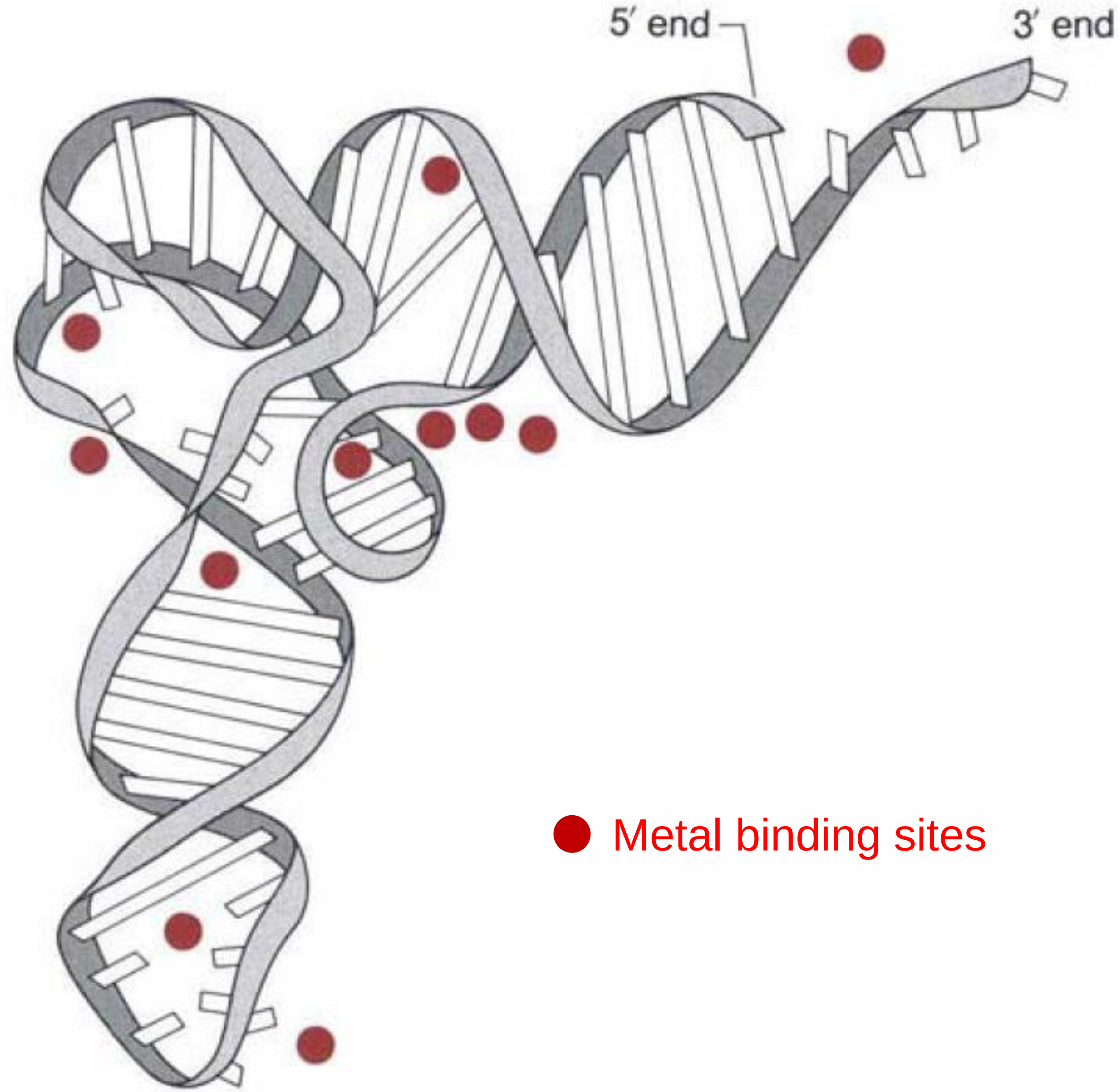
The possibility of **metal-ion-dependent switching** among the different structures has been raised from, other studies and could be of considerable significance.

Transfer RNA

RNA molecules which contain approximately eighty bases and fold up into a L-shaped structure

tRNAs are the physical link between protein sequence and mRNA information.

tRNAs carry the aminoacid specific to a codon of the mRNA to the ribosome, allowing the protein synthesis

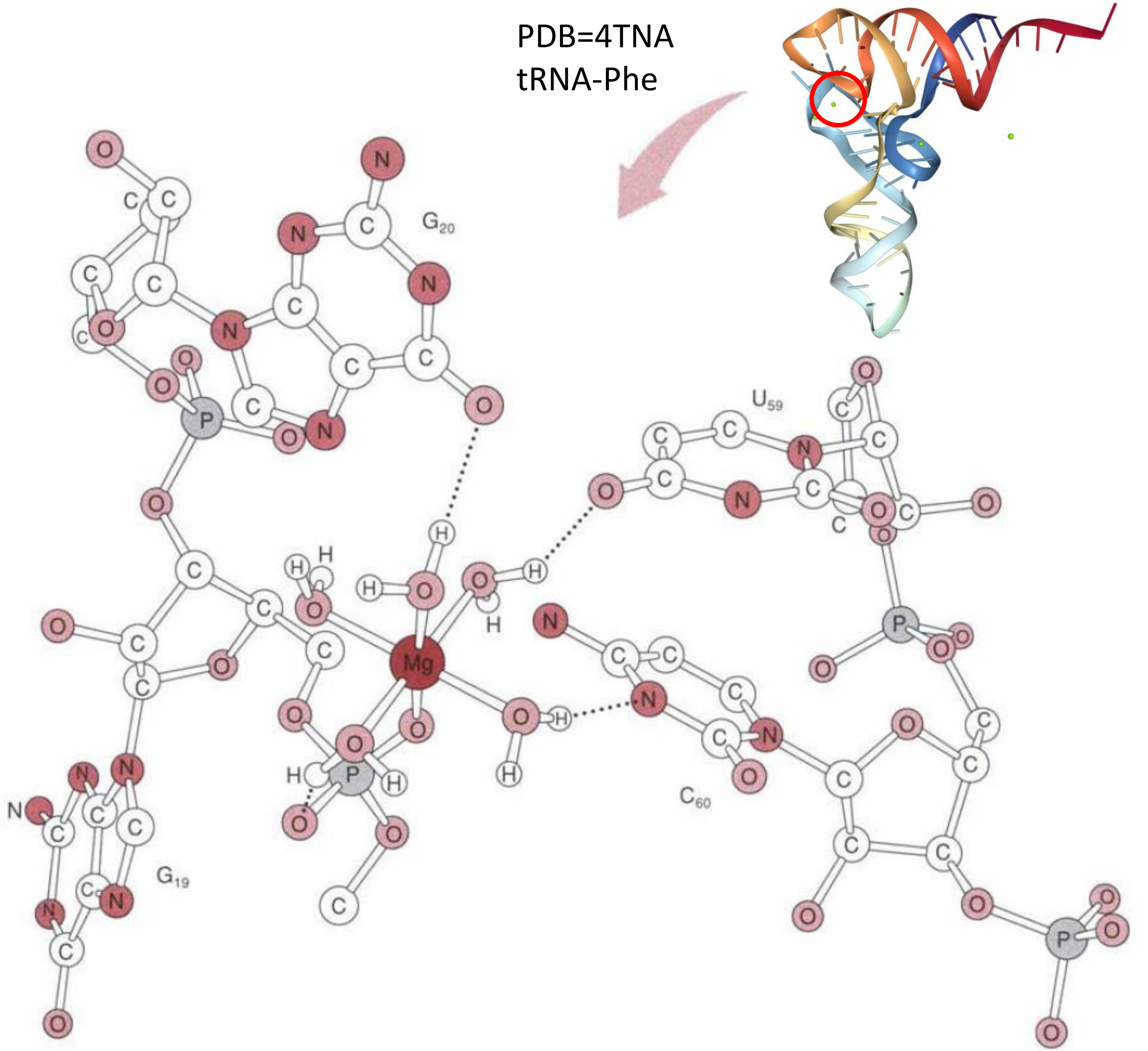


● Metal binding sites

An important distinction exists between the structural metal-binding sites for proteins and the sites on nucleic acids.

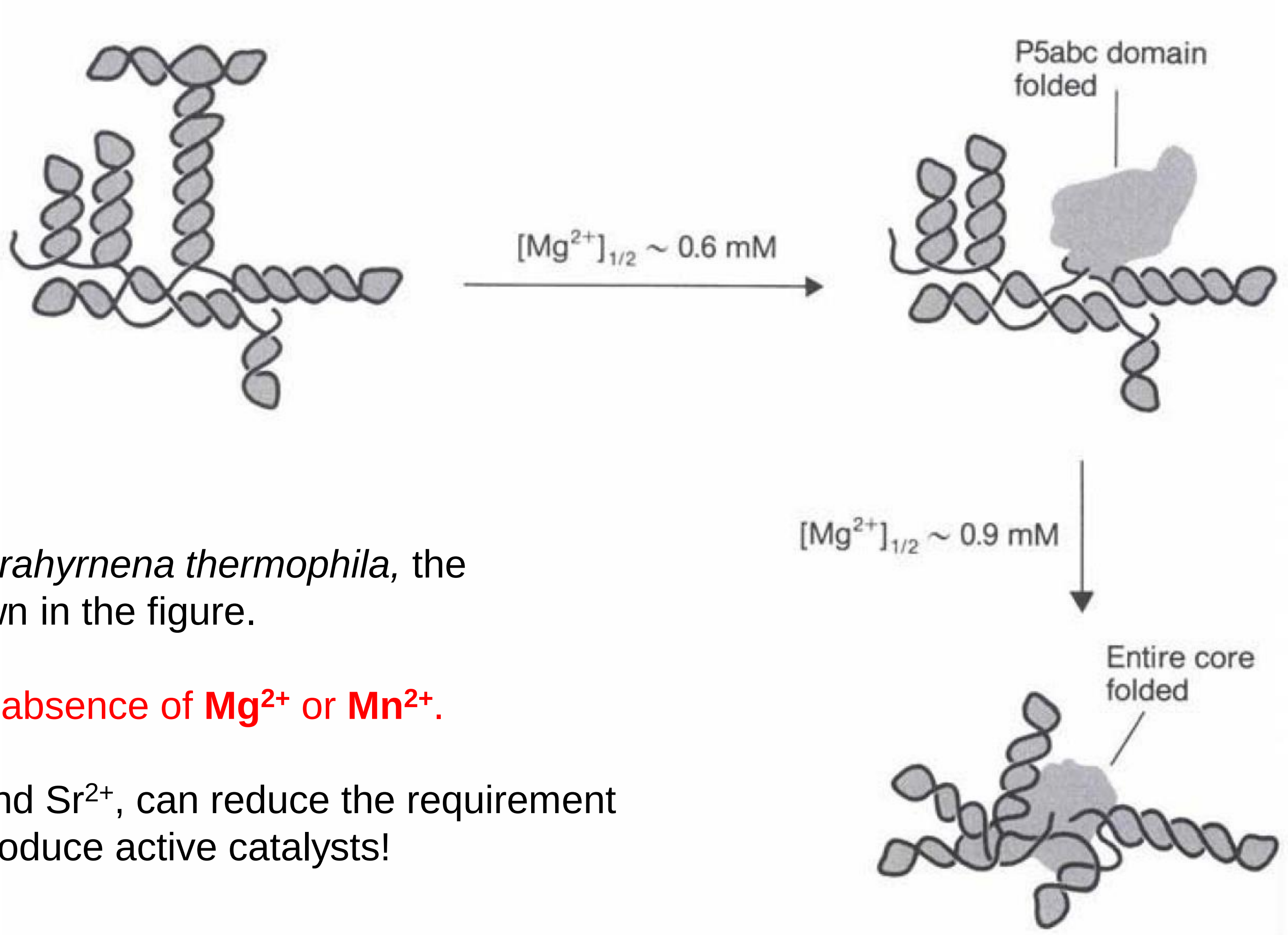
Proteins = metal ions are very nearly or completely dehydrated, all the water molecules being removed and replaced by protein-derived ligands

tRNA and probably other nucleic-acid molecules = most of the waters of hydration remain coordinated, with only one or two nucleic-acid-derived ligands bound directly to the metal ion.


PDB=4TNA
tRNA-Phe

Role of metals in catalytic RNA

catalytic RNA molecules -> **rybozimes**



Example: self-splicing intron from *Tetrahyrnena thermophila,* the secondary structure of which is shown in the figure.

This RNA molecule is inactive in the absence of **Mg^{2+}** or **Mn^{2+}**.

Other cations, such as Ca^{2+}, Ba^{2+}, and Sr^{2+}, can reduce the requirement for Mg^{2+} or Mn^{2+} but will not alone produce active catalysts!

The catalytic role of Magnesium: Catalysis of Phosphate Transfer

Mg^{2+} is distinguished by its small ionic radius/charge and the resulting Lewis acidity

Preferential binding to multiply negatively charged ligands, especially polyphosphates.

Also Mg^{2+} is definitely a “hard” electrophile that does not form inert bonds to simple N and S donor ligands such as histidine or deprotonated cysteine.

Mg^{2+} strongly prefers the coordination number 6 with close to octahedral configuration, while other ions with comparable biological functions prefer either lower (Zn^{2+}) or higher (Ca^{2+}) numbers.

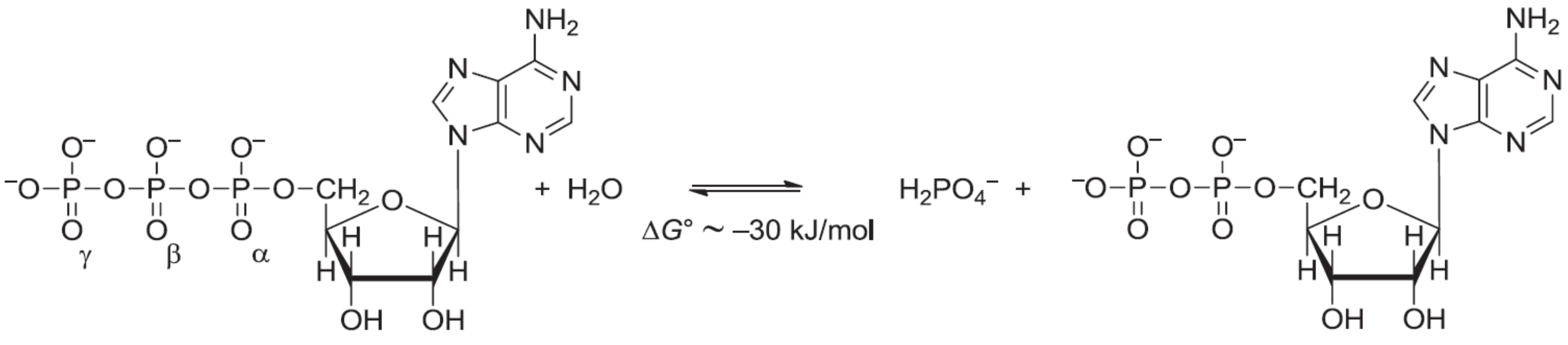
Roles of Mg^{2+}

- Structural
- essential factor for the **biochemical transfer of phosphates** and for many **nonoxidative cleavage reactions**
- Important role in **photosynthesis** (present in chlorophylls)

Ca^{2+}-analogous function in exo- and endoskeletons and in the stabilization of cell membranes

What about its enzymatic activity?

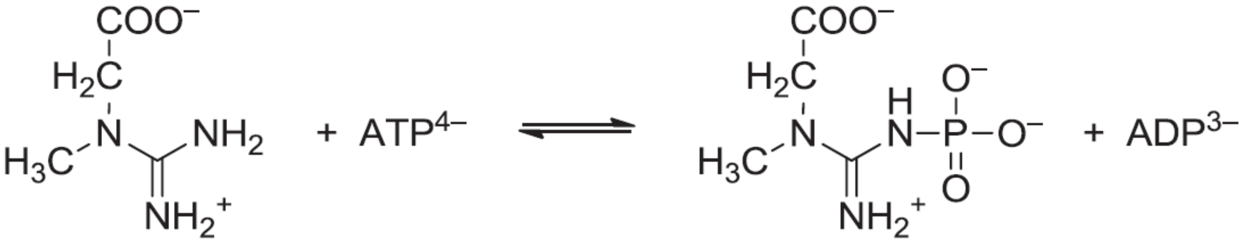
essential factor for the **biochemical transfer of phosphates** and for many **nonoxidative cleavage reactions**



ATP^{4-} (adenosine triphosphate)

ADP^{3-} (adenosine diphosphate)

Phosphates are not only present in RNA and DNA but are also essential constituents of intermediate energy carrier molecules (**ATP/ADP**) in organisms which can be converted by “simple” hydrolysis, through “PO_3^{n-}” transfer between a substrate and water



creatine

creatine phosphate

Creatine phosphate is involved in the generation of ATP from ADP in **anaerobic condition** (muscular fatigue etc.)

All biological phosphate transfer reactions, such as phosphorylations by kinases and dephosphorylations by phosphatases. require the presence of catalyzing charged metal ions. In addition to Mg^{2+} (ionic radius 72 pm for a coordination number 6):

Zn^{2+} (74 pm)	alkaline phosphatase
high-spin Fe^{2+} (78 pm)	purple acid phosphatases

large ions high-spin Mn^{2+} (83 pm) and Ca^{2+} (100 pm) can also perform this task *in vivo*.

In principle, Cd^{2+} (95 pm) and Pb^{2+} (119 pm) would also be suitable; however, due to their rather **soft character**, they tend to form strong bonds with sulfur ligands

Why all M^{2+} cations?

Charge compensation

The function of dipositive metal catalysts in phosphate transfer, including hydrolysis, can be attributed to an **effective compensation of the high negative charge**, which is a consequence of the ionization of mono- and polyphosphates at physiological pH

The charge compensation by **M^{2+}** ions concerns **both sides of the reaction** and therefore contributes to a reduction in activation energy

Trivalent metal ions, **M^{3+}**, can compensate negative charges even better, but they **no longer catalyze the reaction efficiently due to the unproductive stabilization of reaction intermediates**

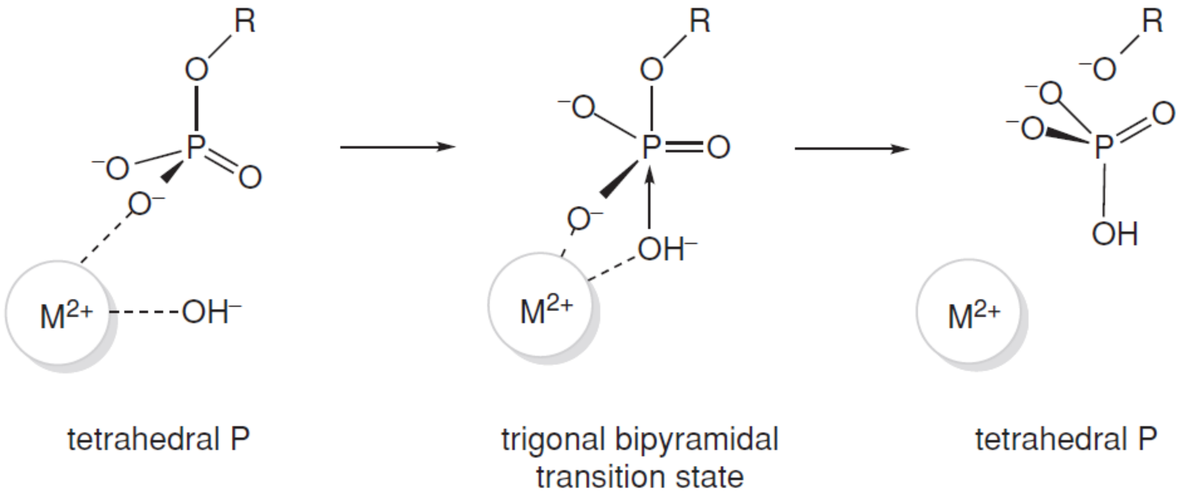
Umpolung

The metallic electrophiles M^{2+} activate weak Lewis bases such as water and thus **create nucleophiles** $(M^{2+})-OH$ under physiological conditions

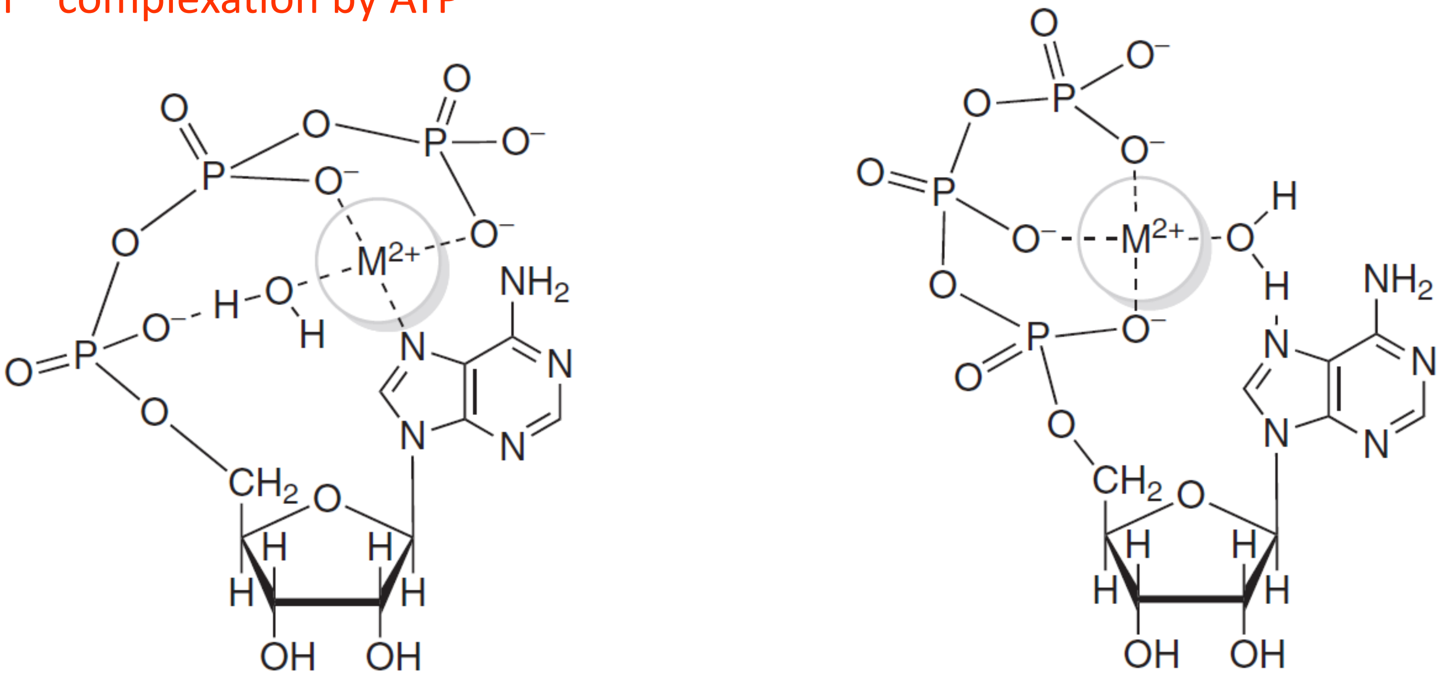
Chelation

Strongly polarizing dications can coordinate polyphosphates in a chelating manner by binding to the oxygen centers of several phosphate moieties; the result is a **spatial fixation**, including an activating ring strain metal ions can generally lower the transition state of an associative reaction through intermediate coordination of both reactants

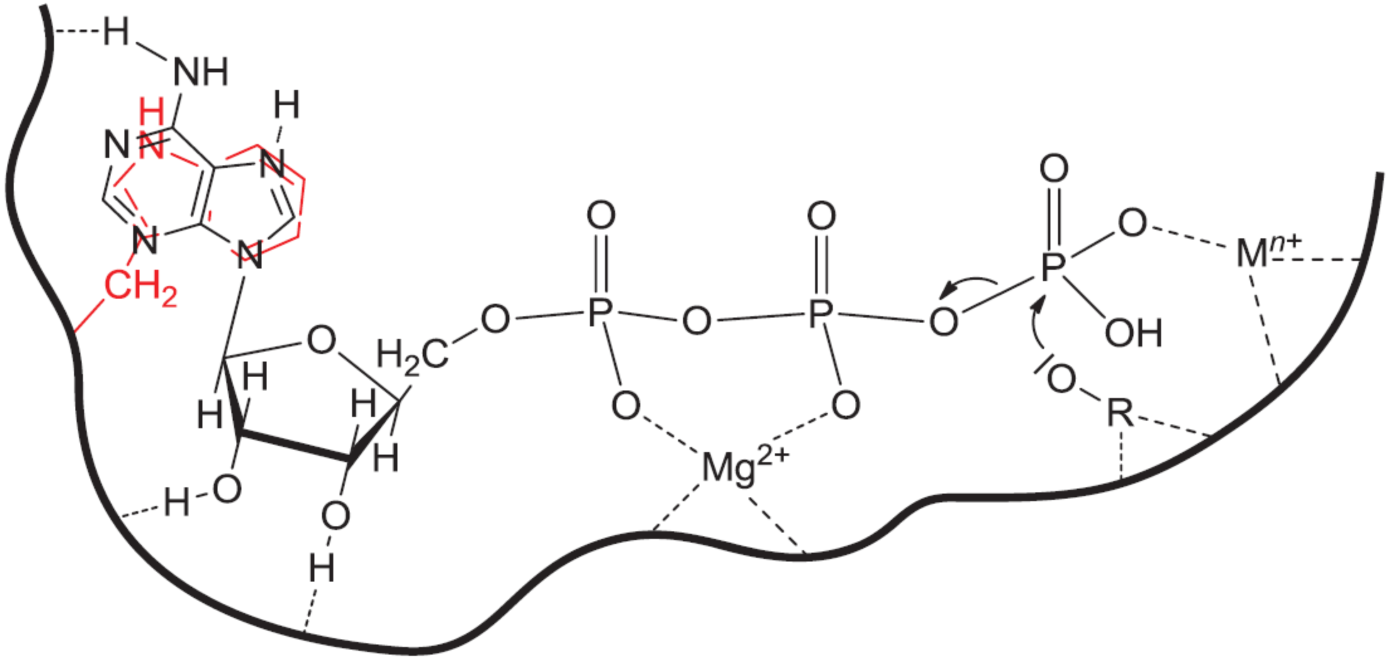
Schematic representation of M^{2+} catalyzed phosphate hydrolisis



M^{2+} complexation by ATP



Hypothetical arrangement of a reactive Mg(ATP)$^{2-}$ complex in the enzyme



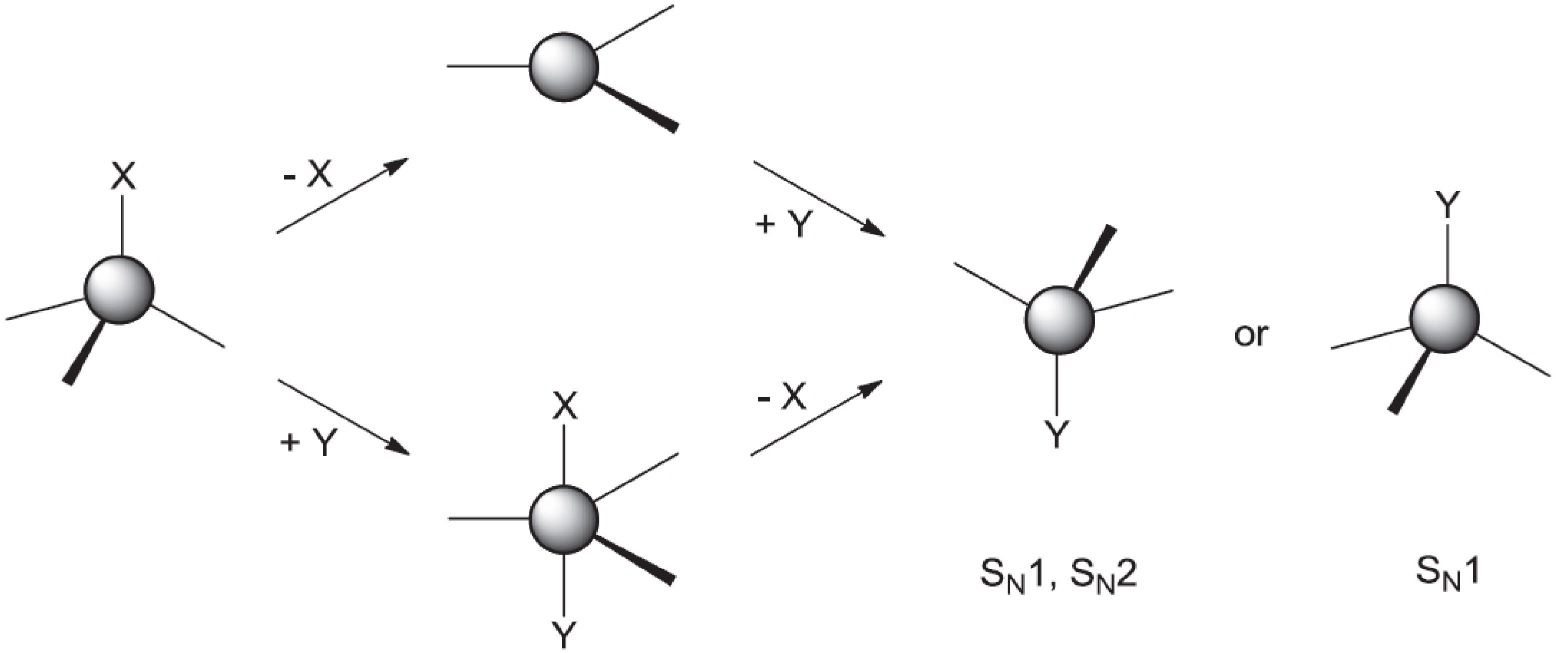
Partially enzyme-bound metal ions activate the triphosphate chain for a nucleophilic attack, in this case by an alkoxide or ester at the terminal phosphate. Binding of the adenine heterocycle may involve – interactions with a tryptophan. Eventual dissociation of Mg(ADP)− can be visualized as the consequence of a stronger bond between Mg^{2+} and the (then terminal) phosphate and thus of weakened new Mg^{2+}/enzyme binding

Since the overall phosphate transfer reaction (14.6) is a nucleophilic substitution, it can mechanistically proceed as a dissociative (S_N1) or an associative (S_N2) process.

dissociative process (S_N1)

Stereoselective!

associative process (S_N2)



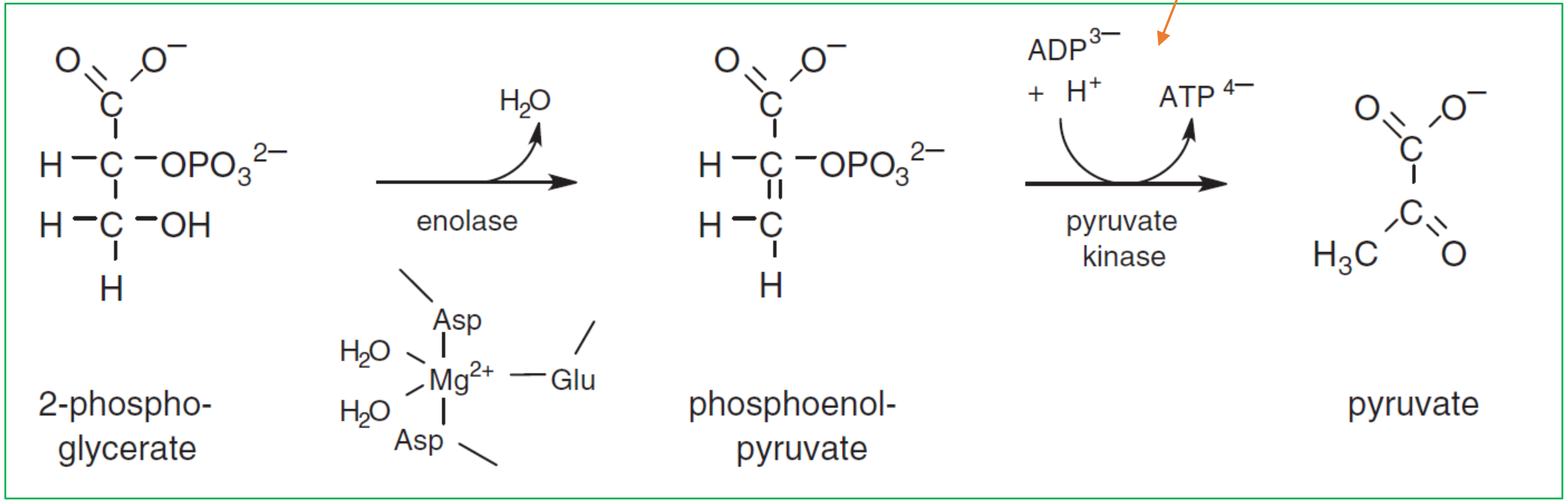
Proof of associative process:

inhibition of ATPases by trace amounts of vanadate(V)

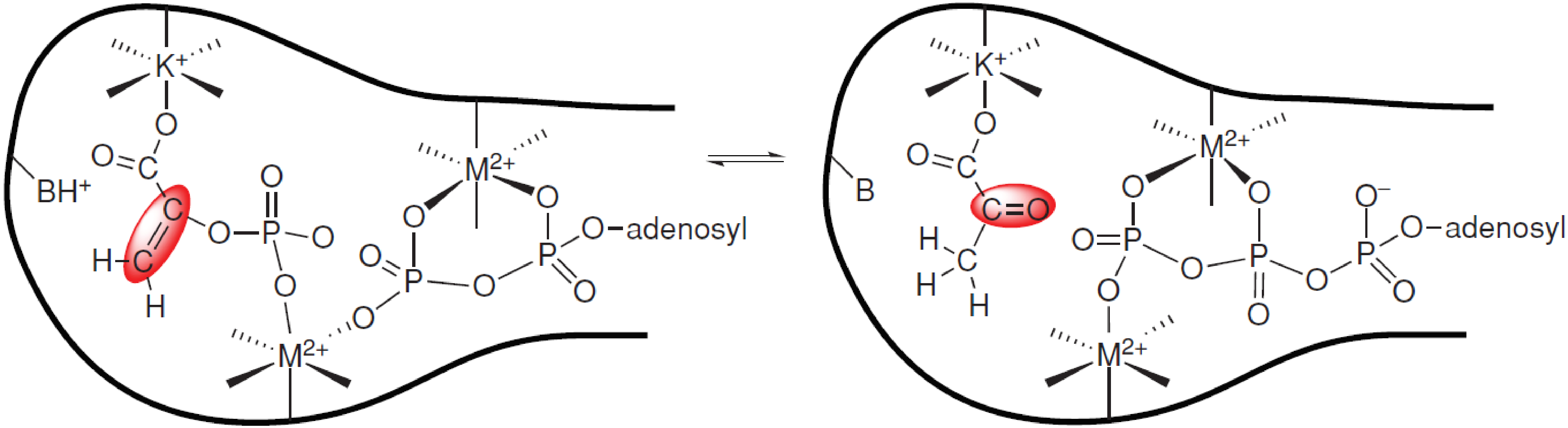
the larger transition metal vanadium from group 5 in the periodic table has a higher tolerance of coordination number 5 than the smaller phosphorus atom in phosphates and can thus stabilize five-coordinate intermediate or transition states

$$\mathrm{ATP^{4-}} + \mathrm{X{-}H} \xrightarrow{\text{kinase}} \mathrm{ADP^{3-}} + \mathrm{X{-}PO_3^{2-}} + \mathrm{H^+}$$

Within metabolic cycles, the “kinase” enzymes catalyze the transfer of phosphoryl groups from ATP to other substrates, X, such as carbohydrates (e.g. glucose), carboxylates (e.g. **pyruvate, CH_3–C(O)–COO⁻**) and guanidines



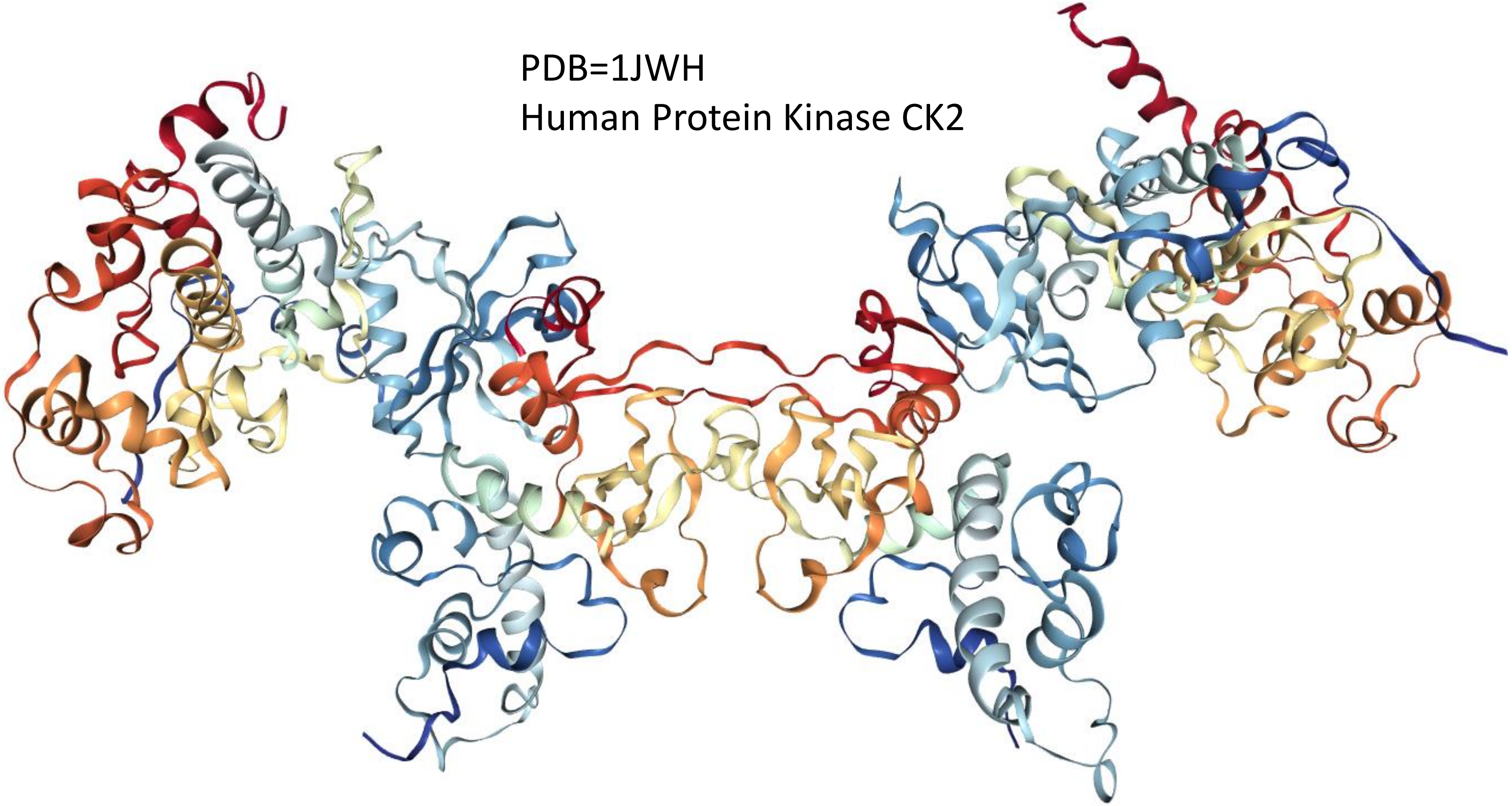
Metal-dependent pyruvate kinase



pyruvate kinase requires the coordination of a large monocation, M$^+$, in particular K$^+$, in addition to two divalent metal cations

Structural insights gained through double **metal substitution:**
conformational changes were detected in the enzyme after coordination of the monovalent metal ion Tl$^+$ via Mn^{2+}-induced linewidth effects for ^{205}Tl NMR

Example of kinases

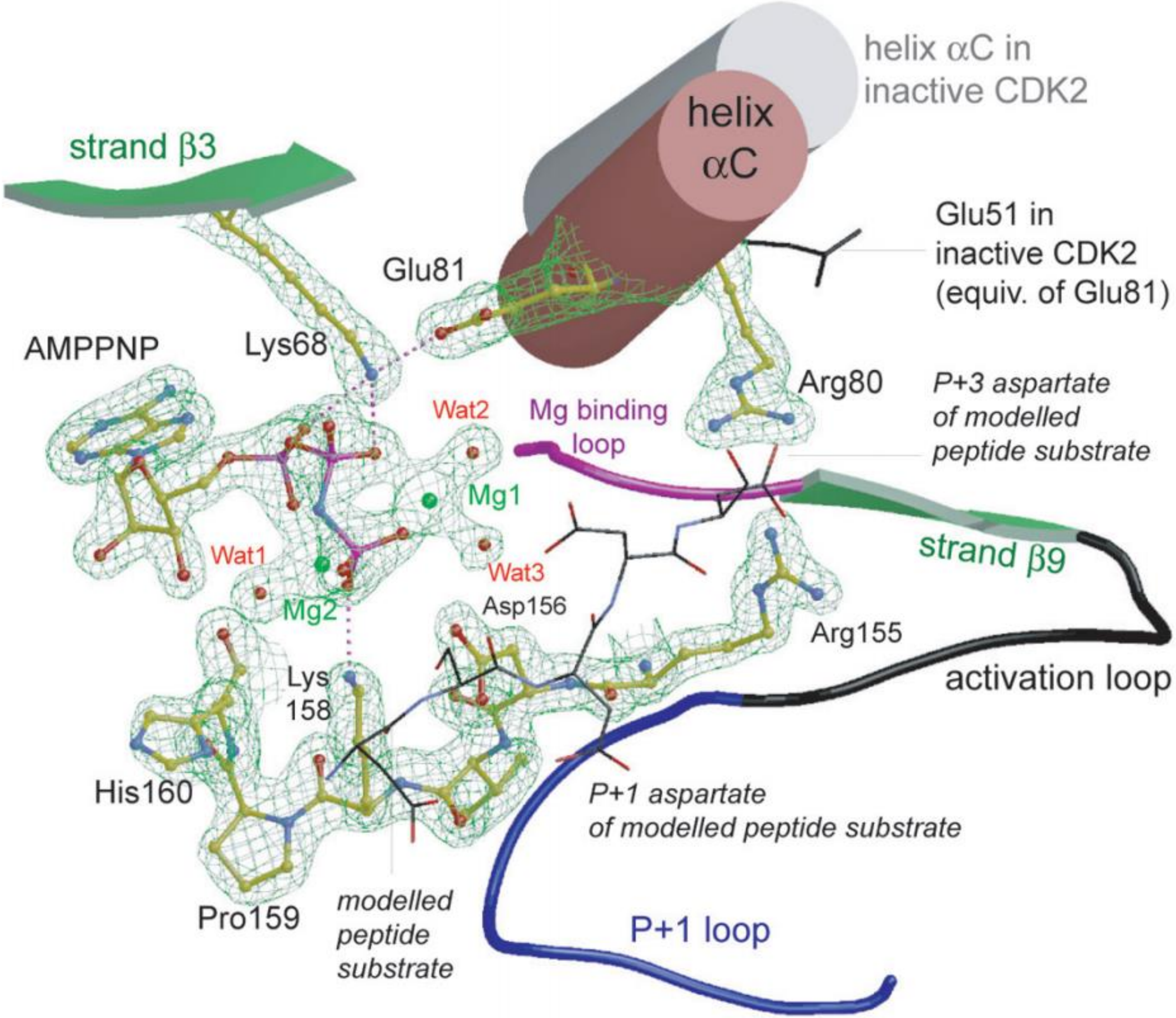

PDB=1JWH
Human Protein Kinase CK2

Protein kinase CK2 (casein kinase 2) is one of the most unspecific eukaryotic protein kinases:

- >160 *in vitro* protein substrates of CK2 have been described to date
- CK2 shows the rare ability to use either ATP or GTP as phosphoryl donor (dual-co-substrate specificity)
- although known as a serine/threonine kinase for several decades, the capability of CK2 also to phosphorylate tyrosine has been reported
- CK2 activity was found not only with the natural cofactor Mg^{2+} but also with other divalent cations such as Mn^{2+} and Co^{2+}

Crystal structure determinations show ATP binding to **Mg^{2+} via one oxygen atom from each of the three phosphate groups** and completion of the six-coordination **through water and amino acid side chains**.

During actual catalysis, the Mg^{2+} ion may migrate between αβ and βγ phosphate groups.



Other Mg^{2+} dependent non-phosphate-transferring enzymes

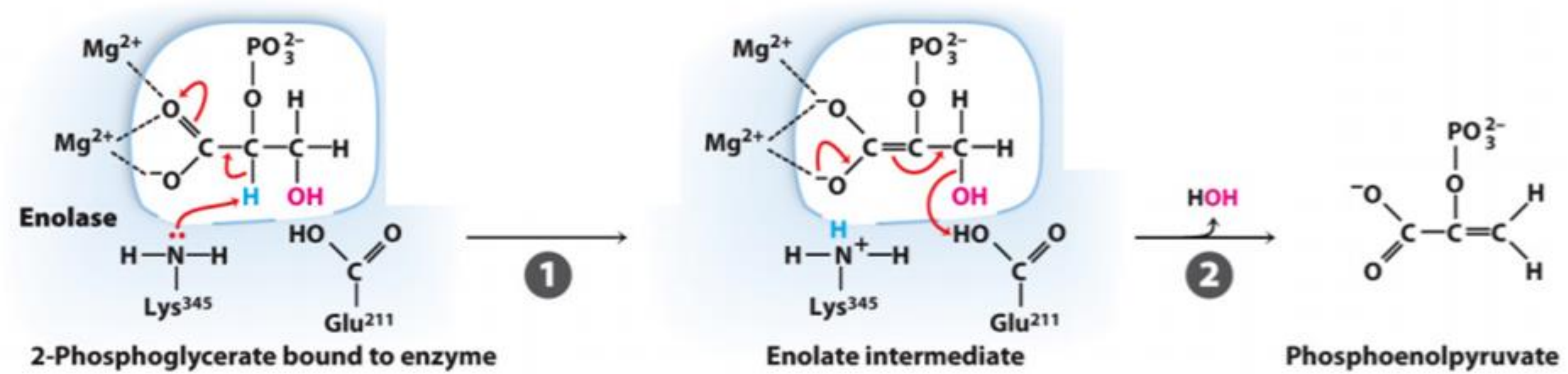
Carbohydrate isomerases

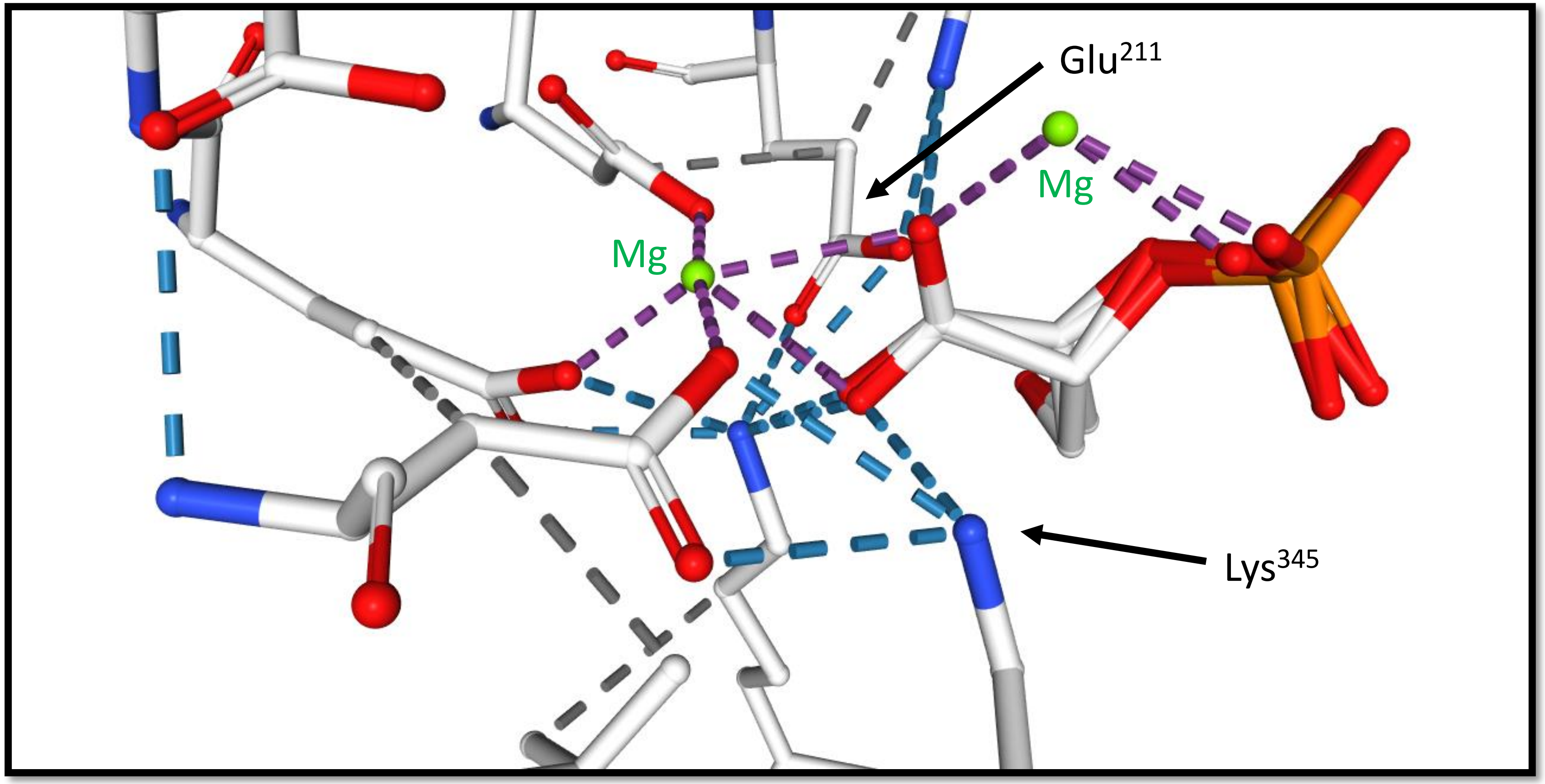
DNA-activating topoisomerases

Enolases => catalyze the synthesis of reactive phosphoenolpyruvate through a dehydration reaction

together with ADP, forms the energy storage molecule ATP and pyruvate in a pyruvate kinase-catalyzed reaction at the end of glycolysis

Nobel Prizes in Chemistry have been awarded for the structural elucidation of a bacterial photosynthetic reaction center (J. Deisenhofer, R. Huber and H. Michel, 1988) and for the theoretical description of the underlying electrontransfer processes (R. A. Marcus, 1992).

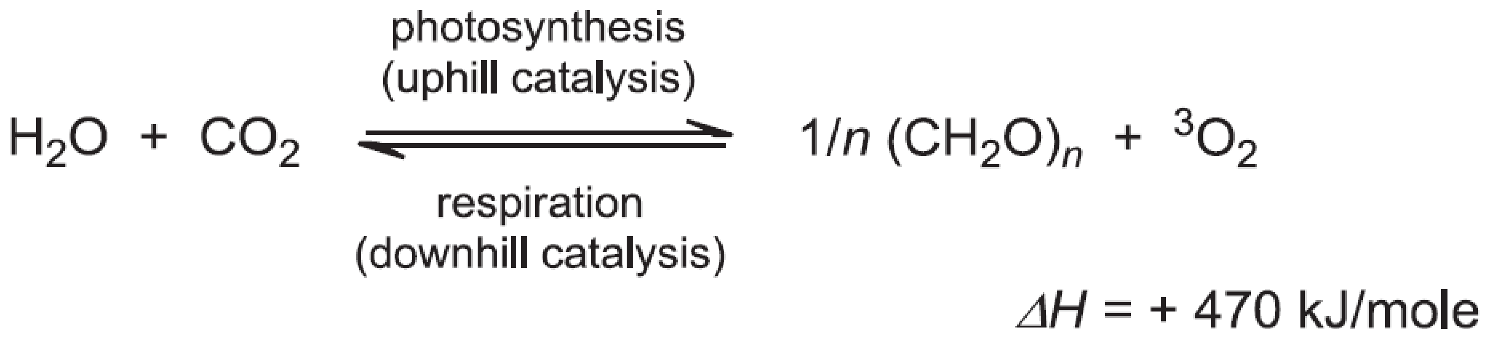

Mg2+
PO3 2−
Mg2+
O
C
C
C
H
H
OH
Enolase
H—N—H
Lys345
HO
C
O
Glu211
2-Phosphoglycerate bound to enzyme
1
Mg2+
PO3 2−
Mg2+
−O
C=C
H
−O
OH
H
H—N+—H
Lys345
HO
O
C
Glu211
Enolase intermediate
HOH
2
PO3 2−
−O
C—C=C
H
H
O
Phosphoenolpyruvate


Glu211
Mg
Mg
Lys345

Roles of Mg^{2+} => photosyntesis

Chemical process fundamental for the existence of higher forms of life on earth

It uses a “diluted” form of energy (**solar energy**) for the production of **reduced carbon compounds** (“organic” material, including fossil fuels) and **oxygen** from largely available low energy simple starting reactants



$$H_2O + CO_2 \underset{\text{respiration (downhill catalysis)}}{\overset{\text{photosynthesis (uphill catalysis)}}{\rightleftharpoons}} 1/n\,(CH_2O)_n + {}^3O_2$$

$$\Delta H = +\,470\ \text{kJ/mole}$$

Nobel Prizes in Chemistry have been awarded for the structural elucidation of a bacterial photosynthetic reaction center (J. Deisenhofer, R. Huber and H. Michel, 1988) and for the theoretical description of the underlying electrontransfer processes (R. A. Marcus, 1992).

Not only **green plants**.

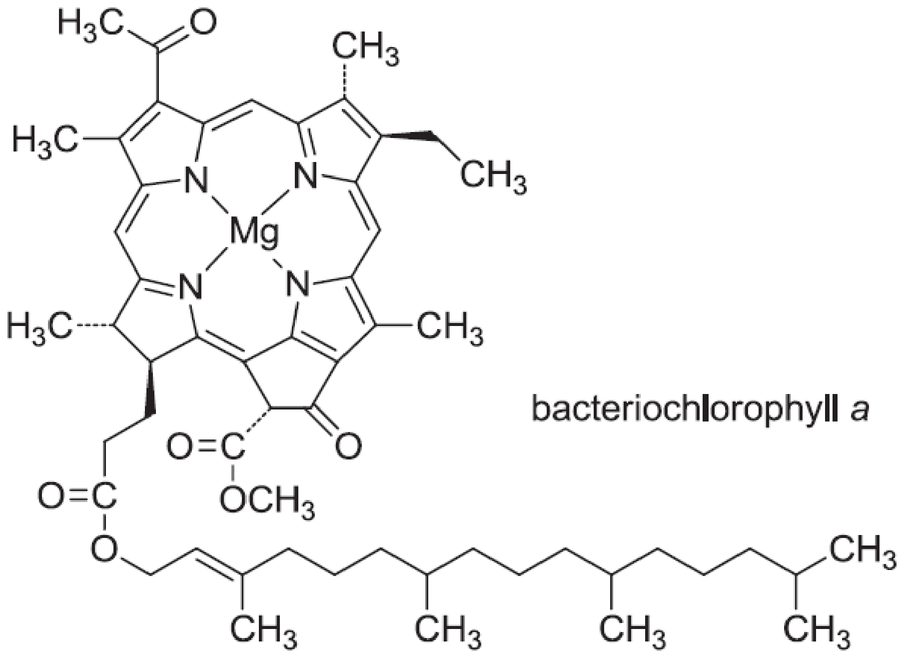
Purple bacteria (*Rhodopseudomonas viridis*) possess a comparatively simple photosynthetical apparatus, without the ability to oxidize water.

These bacteria use photosynthesis primarily to separate charges and thus create a transmembrane proton gradient (pH difference), which is used to synthesize high-energy adenosine triphosphate (ATP) from adenosine diphosphate (**ADP phosphorylation**).

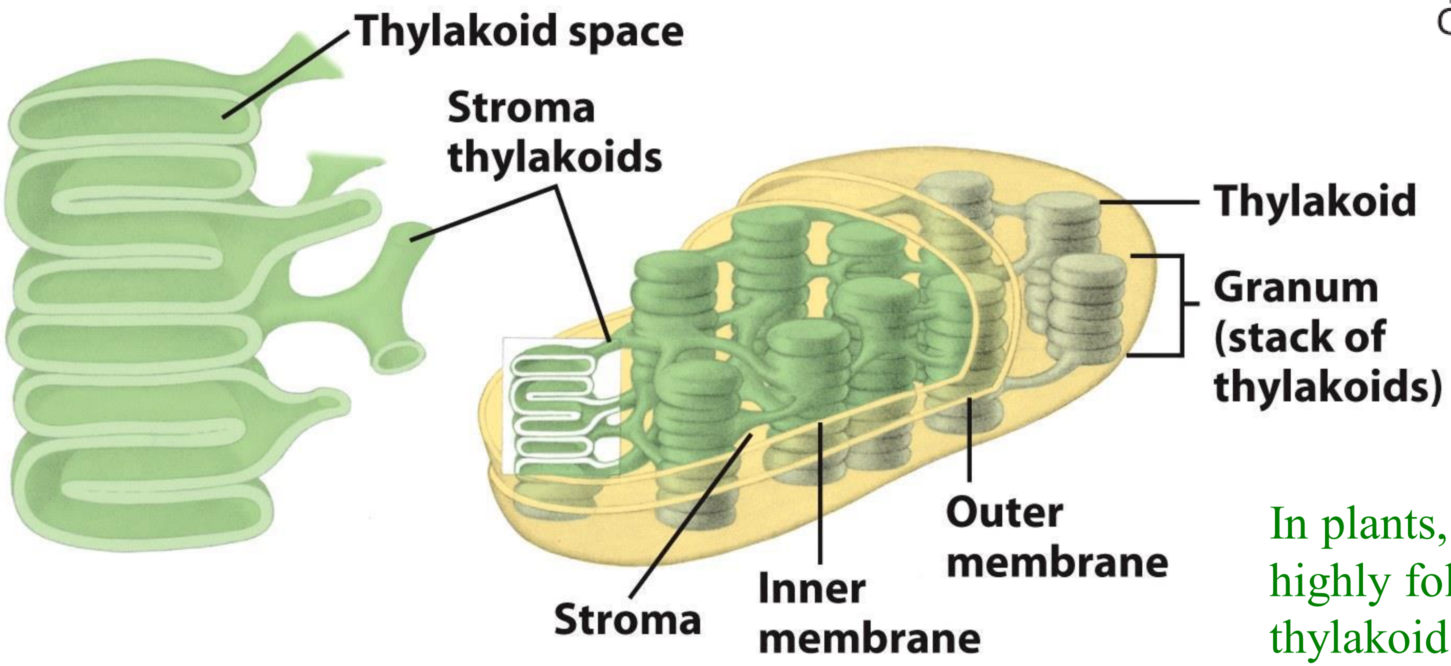
Other anaerobic bacteria use the redox equivalents to oxidize substrates such as hydrogen sulfide (H_2S) or dihydrogen H_2 instead of water.

Immobilization and a defined orientation of pigments and of reaction centers are crucial for the success of photosynthesis

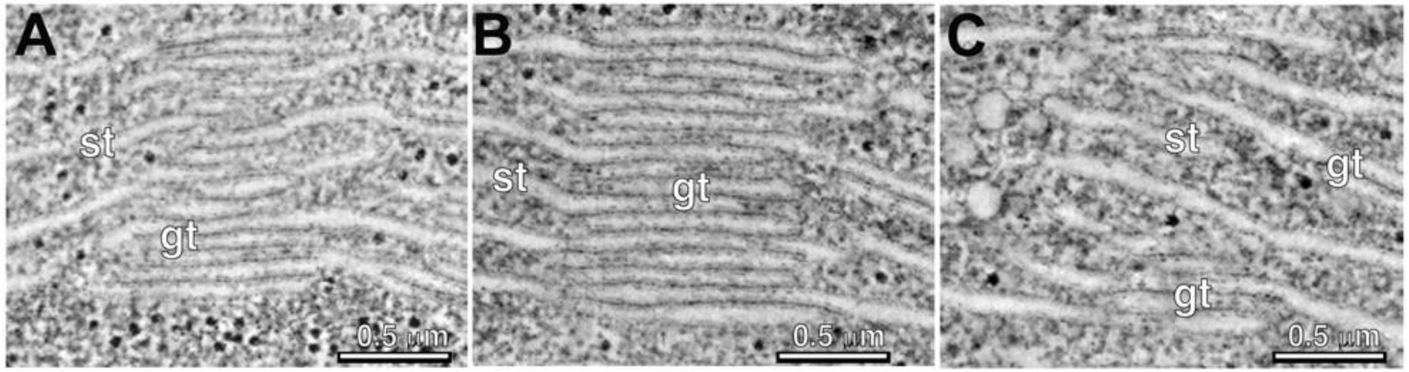
All chlorophyll molecules feature a long aliphatic phytyl side chain to anchor to the phospholipid membrane (thickness of about 5 nm).

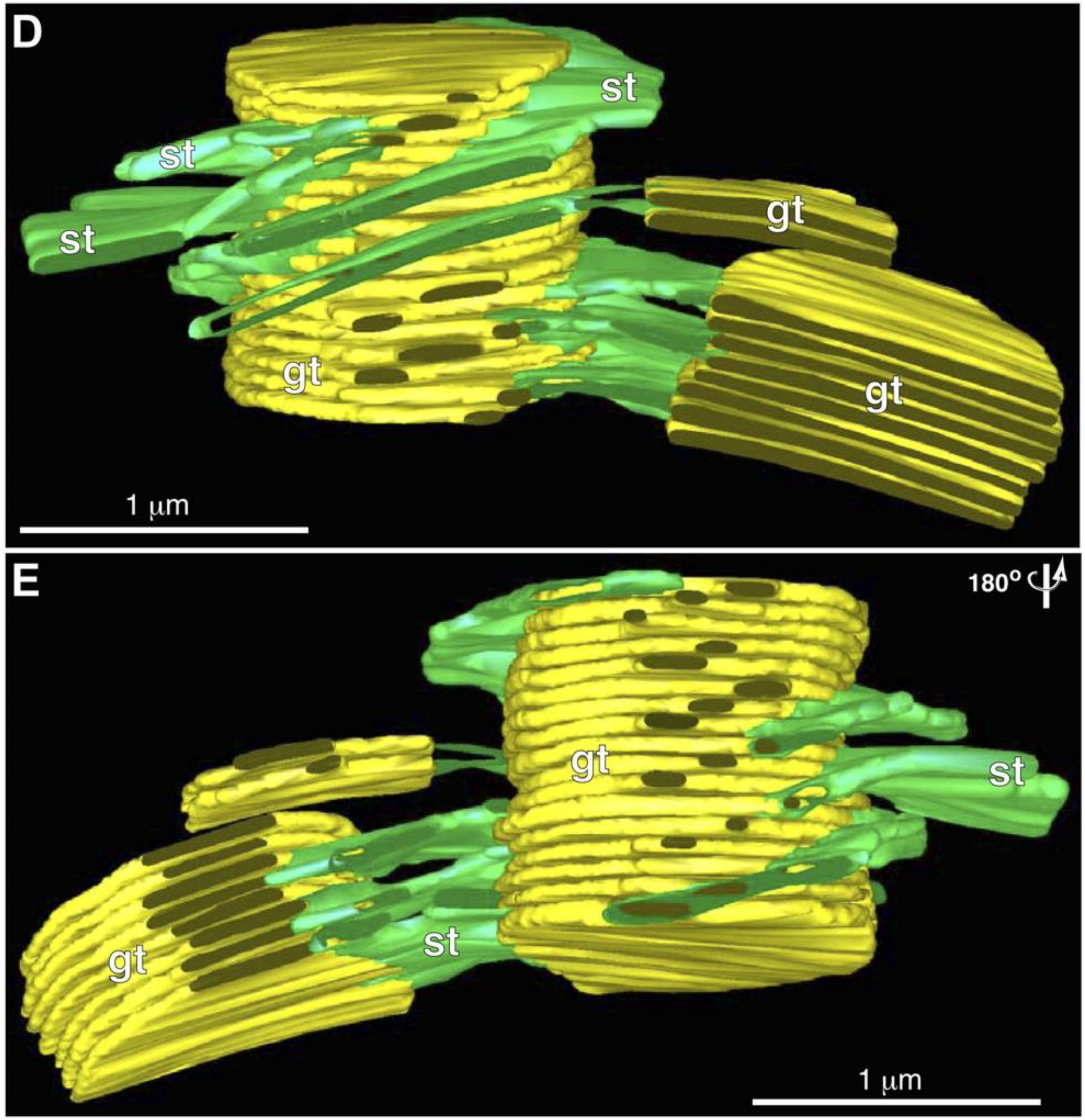


bacteriochlorophyll *a*



In plants, the primary photosynthetical events take place in the highly folded, disk-shaped
thylakoid membrane vesicles inside of chloroplasts





An overview of grana and stroma thylakoid organization. A to C are three composite tomographic slice images (five superimposed 2.2-nm optical slices) showing views from the front (A), middle (B), and back (C) of a grana thylakoid stack. D and E are tomographic reconstructed models of the grana stack shown in A to C, with the grana thylakoids colored yellow and the stroma thylakoids colored green. Front view in D, and the model (E) is rotated 180° to show the back view. Note that for illustrative purposes the model does not display all of the thylakoids associated with the grana stacks.

Photosynthetic output of green plants in normal sunlight → 1 g of glucose/h per $1 m^2$ of leaf surface.

The efficiency of the **“physical” energy transformation** in from incident light to transmembrane redox potential differences is about 20%

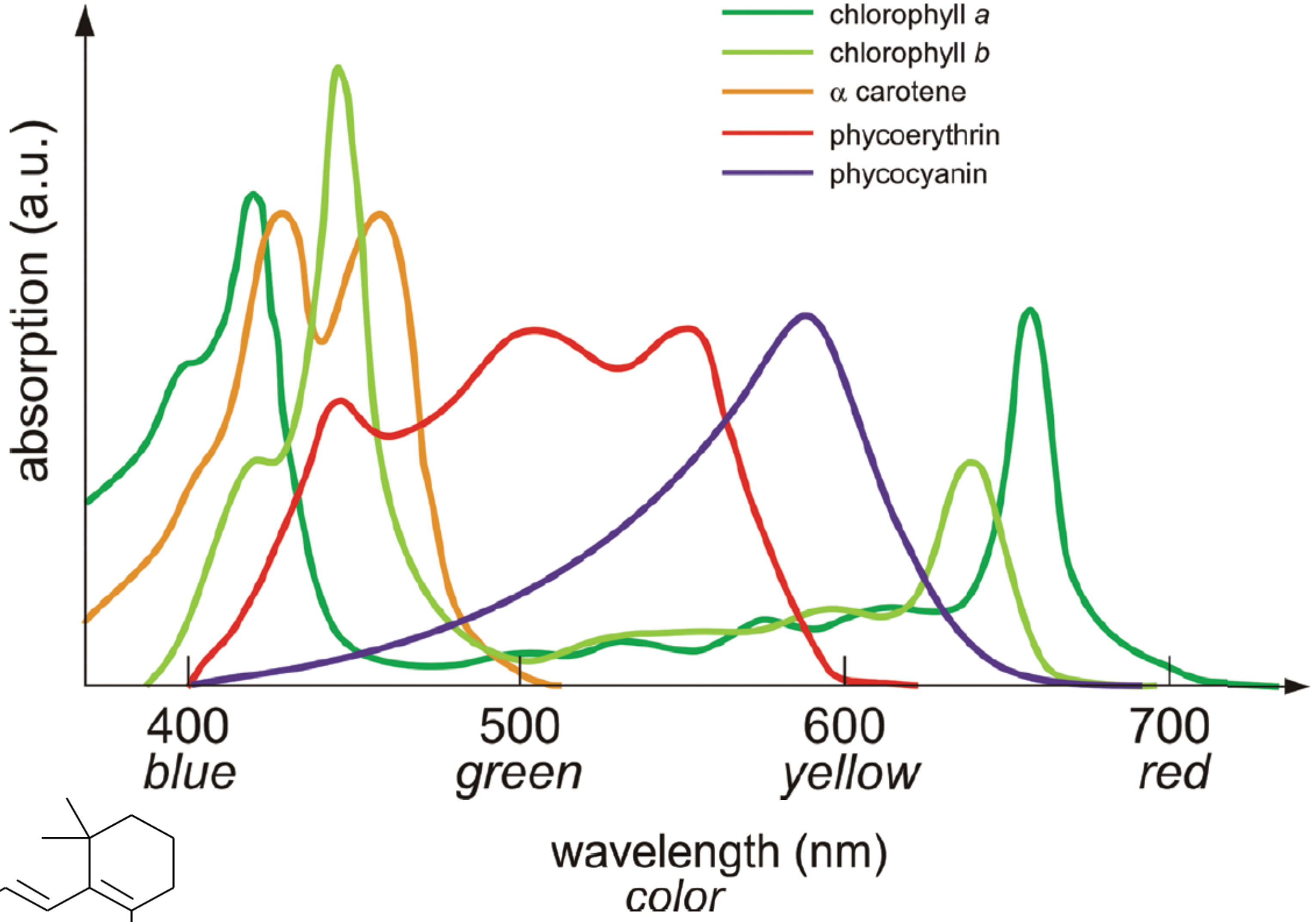
→ very good photovoltaic elements!

But total efficiency of photosynthesis is, on average, **less than 1%** if measured as production of fuel equivalents in comparison to the available radiation energy,

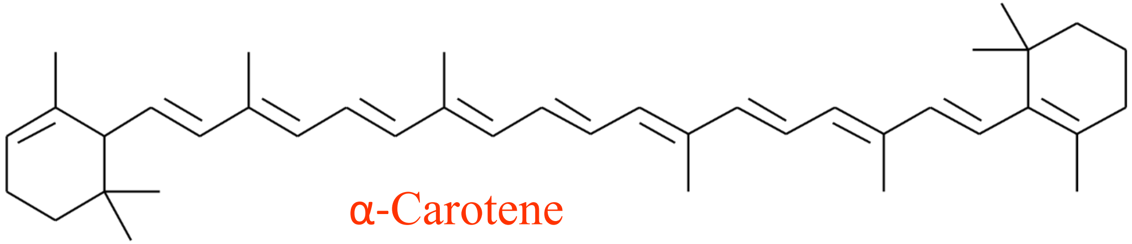
high global turnover => about **200 billion tons of carbohydrate equivalents** $(CH_2O)n$ are **produced from CO_2 each year**.

The **photosynthetically active algae (phytoplankton)** also play an important role on a global scale, since the water coverage of the earth is about 71%.

Chlorophylls contain a conjugated tetrapyrrole system with high absorptivity and molar extinction coefficients of about 10^5 M/cm at both the long- and short-wavelength ends of the visible spectrum **(green color)**



Carotenoids and open-chain tetrapyrrole molecules, such as phycobilins, complement the chlorophyll pigments **(orange-ish colors)**



α-Carotene

Absorption of energy in the form of photons by pigments requires less than 10^{-15} seconds yielding **short-lived electronically excited (singlet) states** that, in principle, can produce a charge separation.

BUT **low photon density** in diffuse sunlight (rate of absorption less than one photon per pigment molecule per second) and the necessarily **rapid charge separation** process

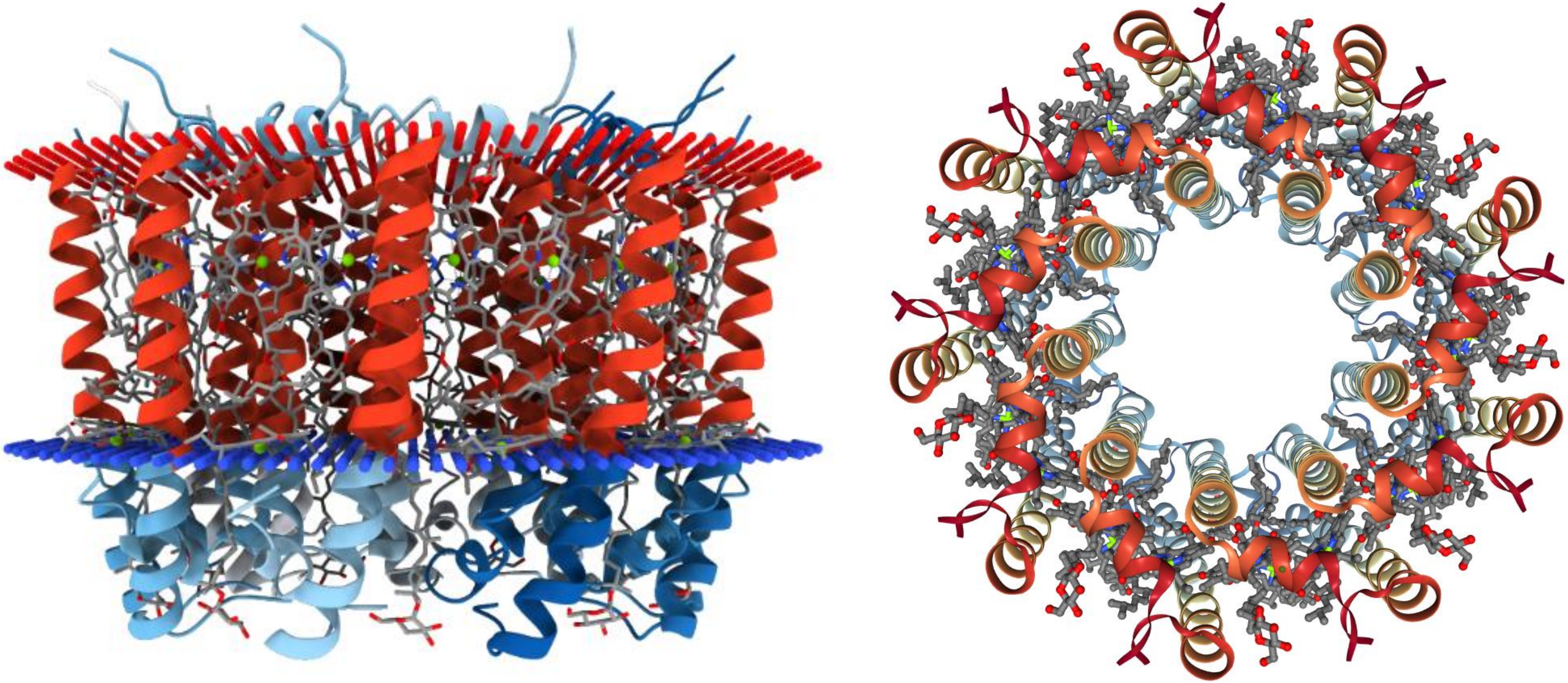
⇩

it is more economical to use the major part (>98%) of the chlorophyll molecules to act as “**antenna**” devices and collect available photons (“**light harvesting**”)

there must be **efficient and spatially oriented** (“vectorial”) transfer of the absorbed energy in the form of excited states (“excitons”) to the actual reaction centers (<2% of chlorophyll content)

PDB =1KZU

INTEGRAL MEMBRANE PERIPHERAL LIGHT HARVESTING COMPLEX FROM RHODOPSEUDOMONAS ACIDOPHILA

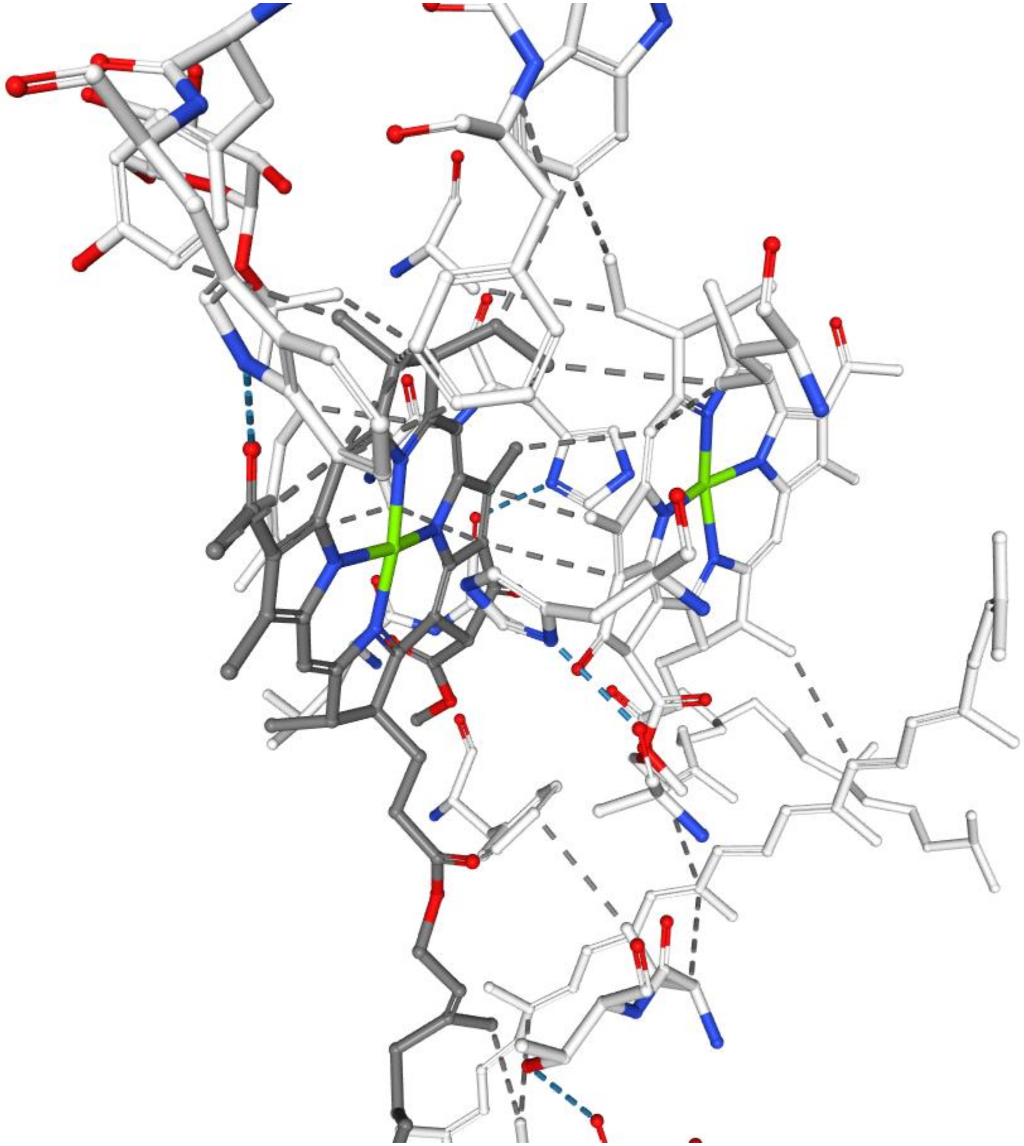


Chromophors are arranged in spatial proximity and a certain, well-defined orientation; they are able to “tunnel” the light energy to the actual reaction centers with about 95% efficiency within 10–100 ps

The light-harvesting complexes of the photosynthetic membrane feature a spatially as well as spectrally optimized cross-section for **photon capture**.

higher energy-absorbing pigments can also transfer excitation to the reaction centers
(energy transfer along an energy gradient)

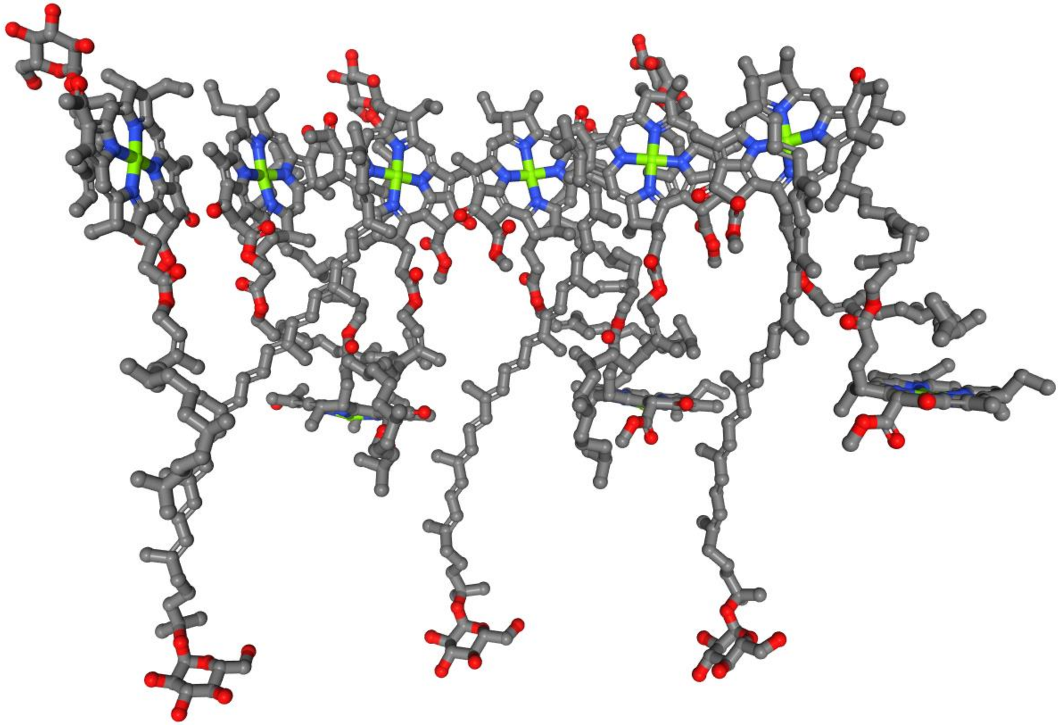
The exciton tunneling is based on the Forster mechanism of “resonance transfer” (FRET), which proceeds via spectral overlap of emission bands of the exciton source with the absorption bands of the exciton acceptor



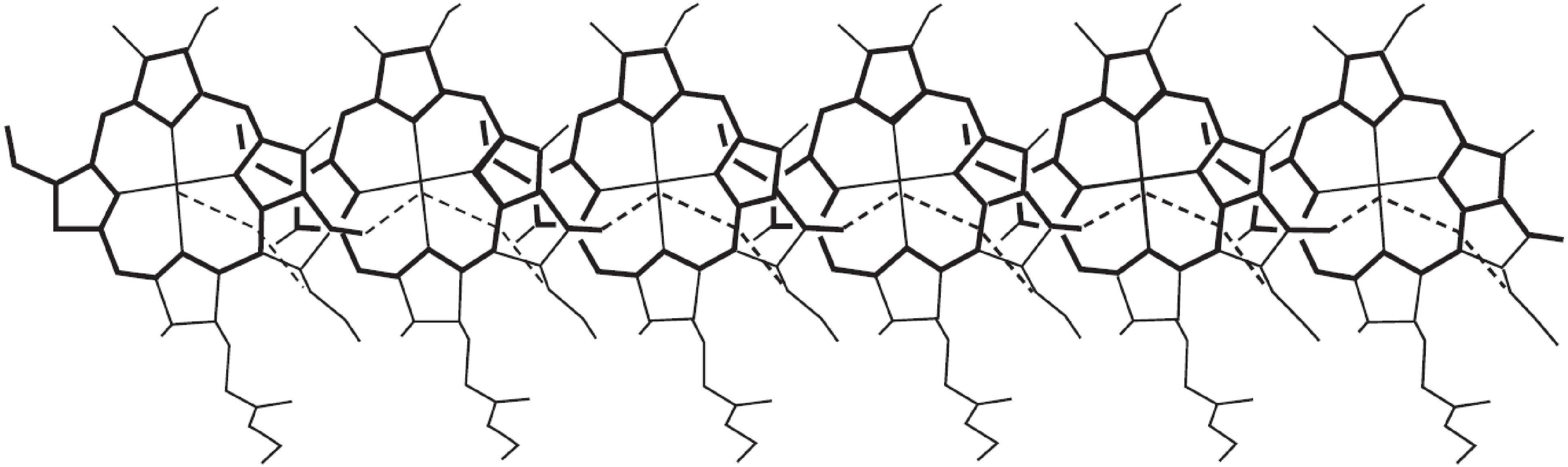
The role of magnesium in chlorophyll is to contribute to the particular arrangement of pigments.

The virtually loss-free exciton transfer through a cluster network of antenna pigments requires a **three-dimensional order**.

This orientation cannot be solely guaranteed by anchoring the chlorophyll molecules in the membrane via the phytyl side chains but must **rely also on the coordination of polypeptide side-chain ligands to the two free axial coordination sites at the metal** (threepoint fixing for defined spatial orientation).



Exactly defined spatial orientation, such as the arrangement of tetrapyrrole ring planes parallel to the plane of the membrane and the resulting high degree of organization in light-harvesting systems, is thus ensured by the **presence of a coordinatively doubly unsaturated electrophilic metal** center in the macrocycle



Model system: *in vitro* aggregation of the dihydrate of a chlorophyll derivative with an ethyl instead of a phytyl side chain => a one-dimensional coordination polymer is formed.

Mg^{2+} centers can interact via dipolar, hydrogen-bonding water molecules with the Lewis basic carbonyl groups in the characteristic cyclopentanone ring of adjacent chlorophyll molecules.

Why again an Mg^{2+} cation?

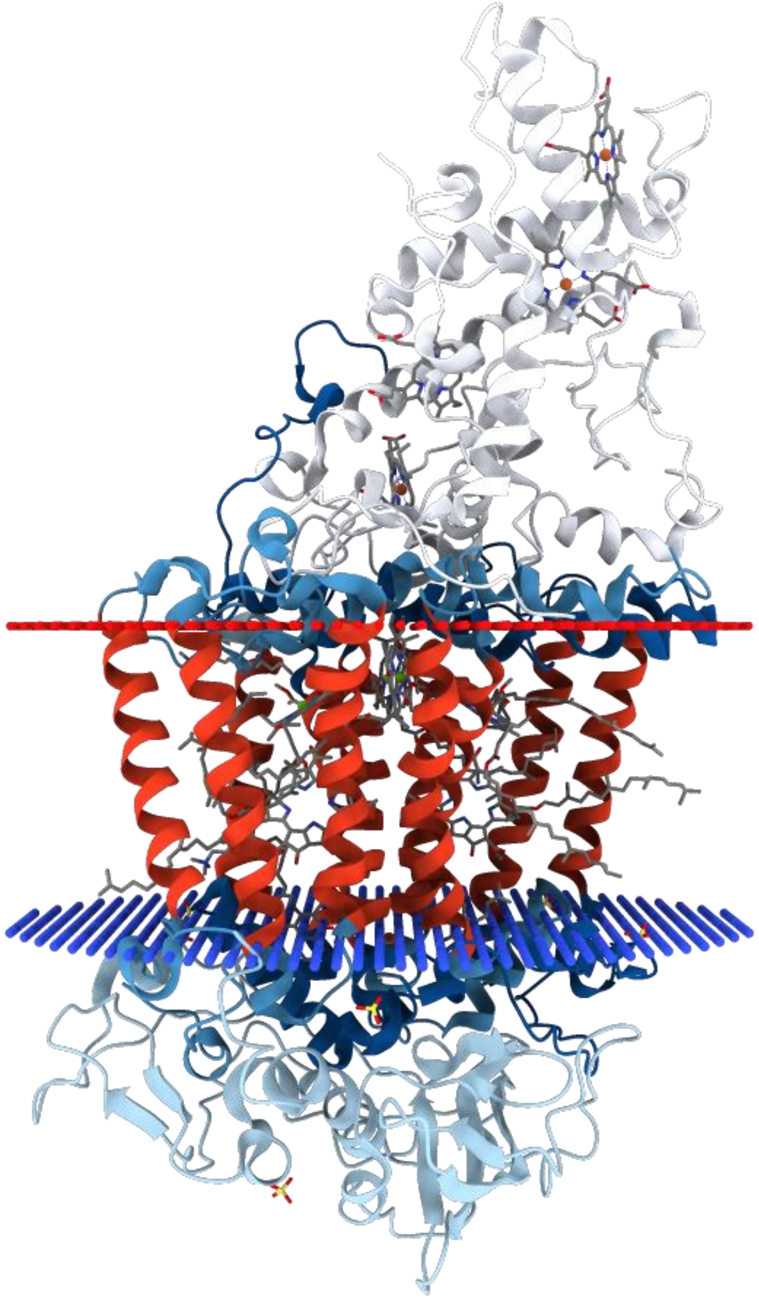
Of all the metals with the proper size, sufficient natural abundance and strong tendency for six-coordination, only Mg^{2+} remains of all the main-group metal ions.

In addition, magnesium is a rather light atom, with a small spin–orbit coupling constant.

Heavier elements with higher spin–orbit coupling constants will enhance an intersystem crossing (ISC) from very short-lived singlet to considerably longer-lived triplet excited states => slowing down of the necessarily very rapid primary events in photosynthesis.
The result would be a competition of undesired light- or heat-producing processes with the actual chemical reactions.

Also transition metals in particular are not suited to being the central ions of chlorophylls

Issue for the next step: **charge separation**

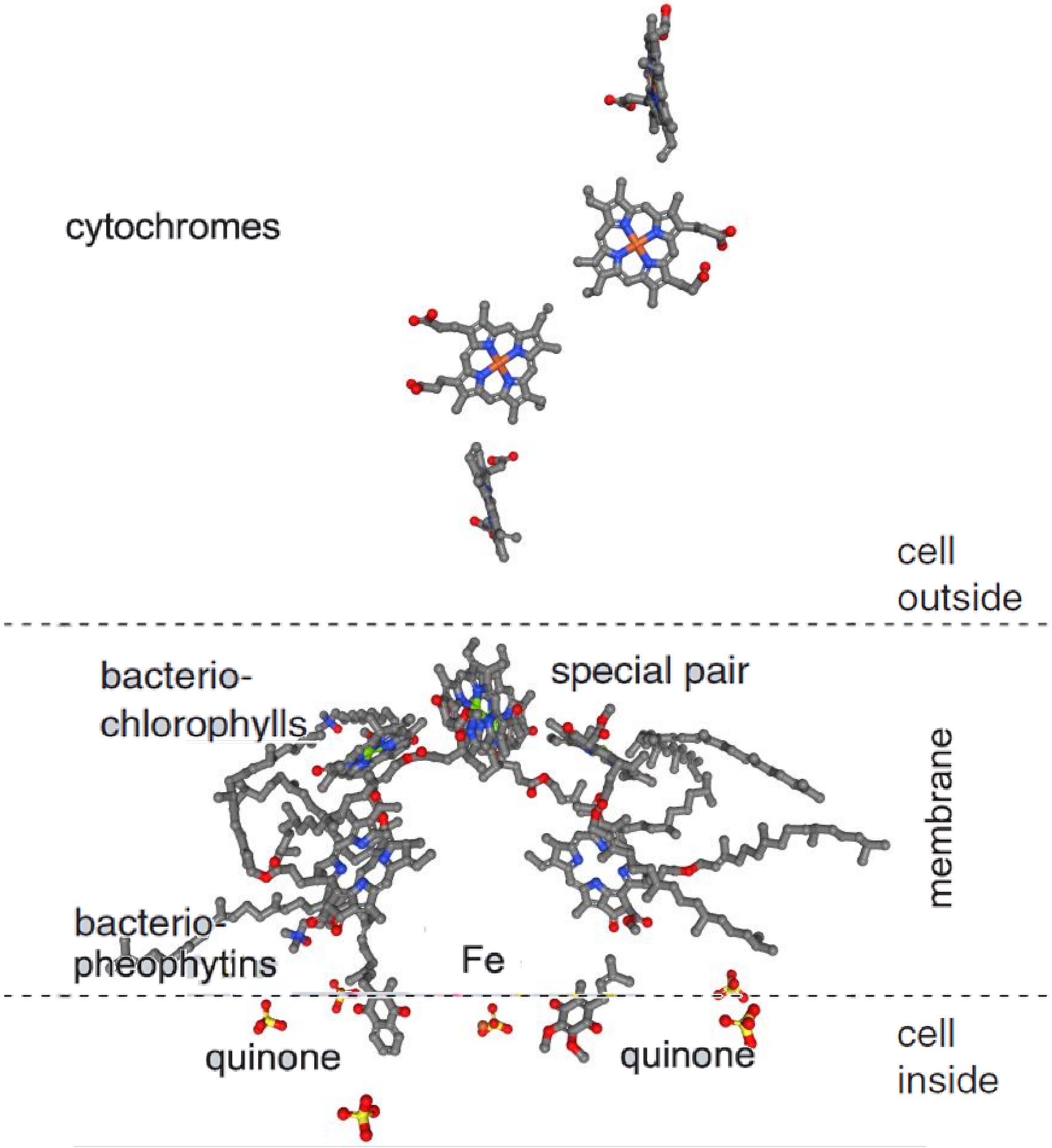


When excitonic energy reaches a photosynthetic “reaction center”, the essential step for the separate production of an electron-rich (i.e. reduced) component and an electron-poor (i.e. oxidized) species can take place.

Purple bacteria, such as *Rhodopseudomonas viridis*, which contain only one photosystem have their reaction center situated in a polyprotein complex that *in vivo* spans the membrane.

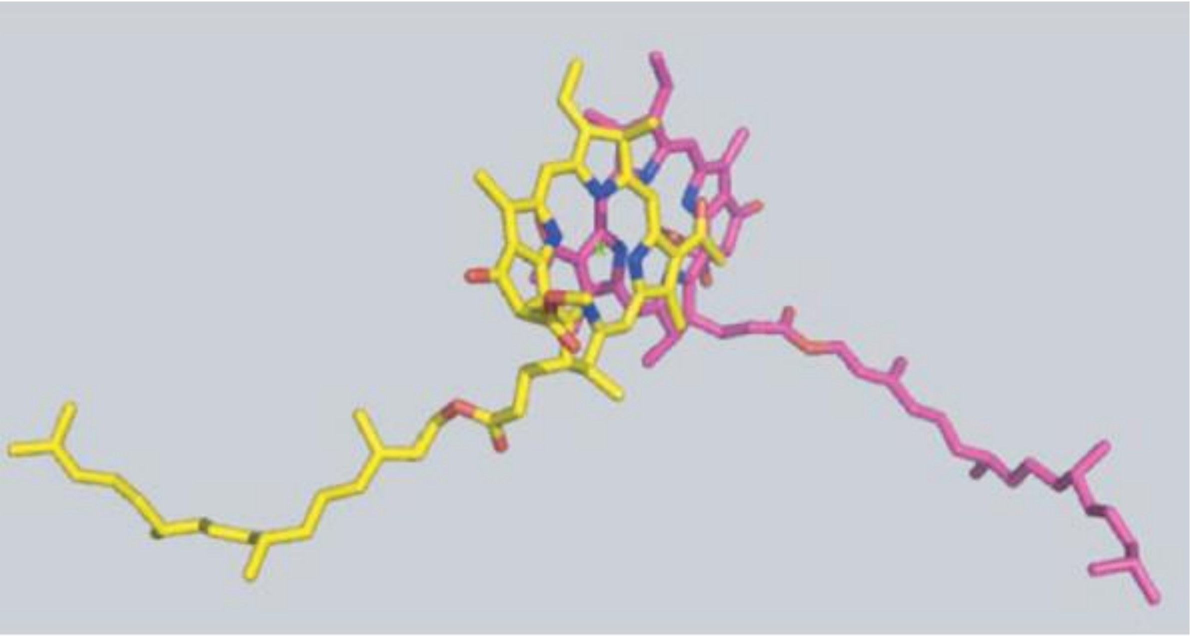
PDB= 1PRC

PHOTOSYNTHETIC REACTION CENTER FROM RHODOPSEUDOMONAS VIRIDIS



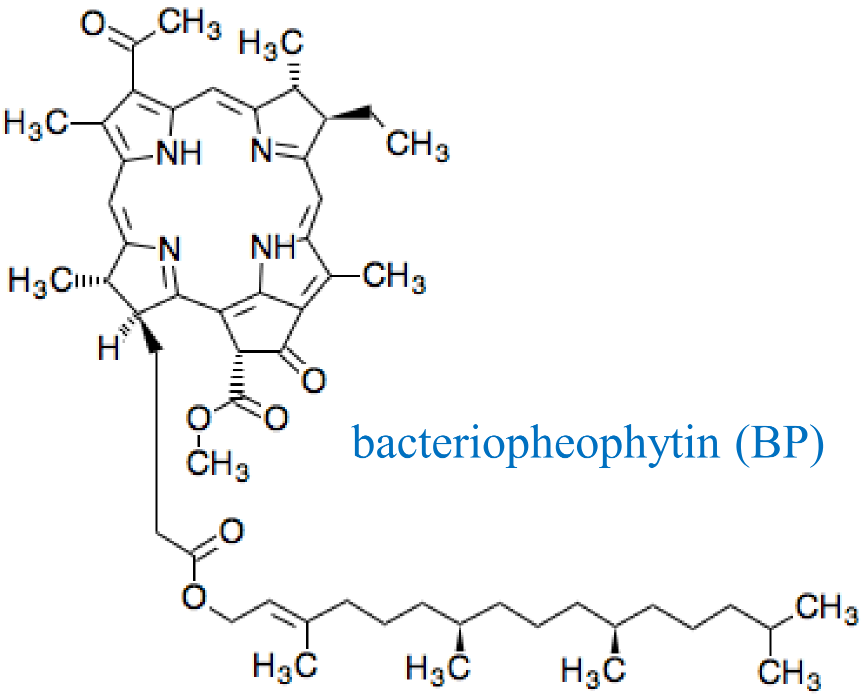
On the symmetry axis of the nearly C2-symmetrical reaction centers is a BC dimer, **the "special pair" BC/BC.**

Structure determination indicates that the aggregation can result from a coordinative interaction between acetyl substituents at the polypyrrole ring and the metal centers.



Because of the significant – orbital interaction, the special pair may function as an electron donor; electronic excitation of this pair (P960 or P870 based on the species), leads to a primary charge separation **within a very short time**: one energetically elevated electron of the electronically excited dimer is transferred to the primary acceptor, **a monomeric BC molecule.**

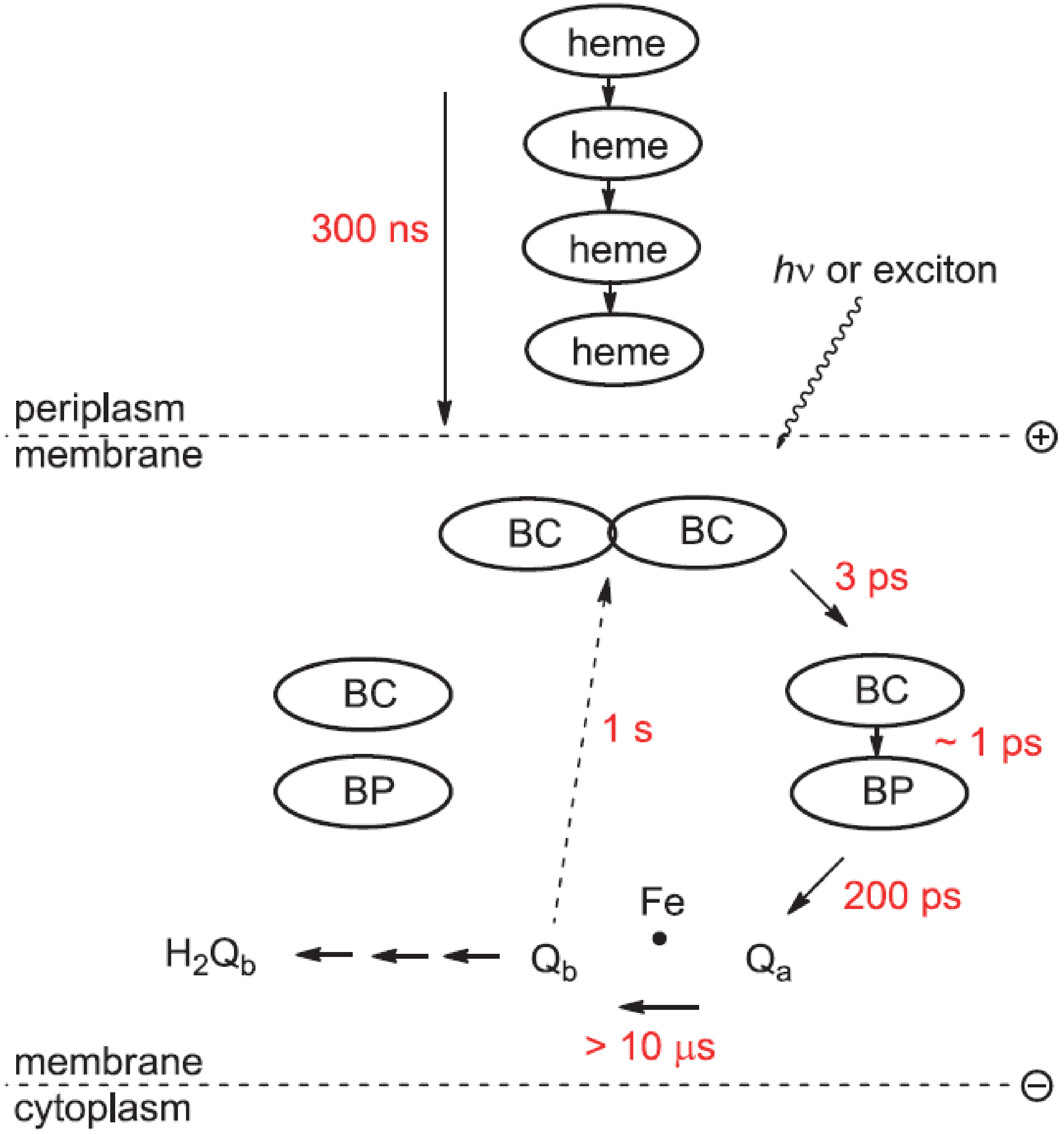
The next step of the charge separation consists in the transfer of negative charge to the secondary acceptor bacteriopheophytin (BP), a BC ligand without coordinated metal.



bacteriopheophytin (BP)

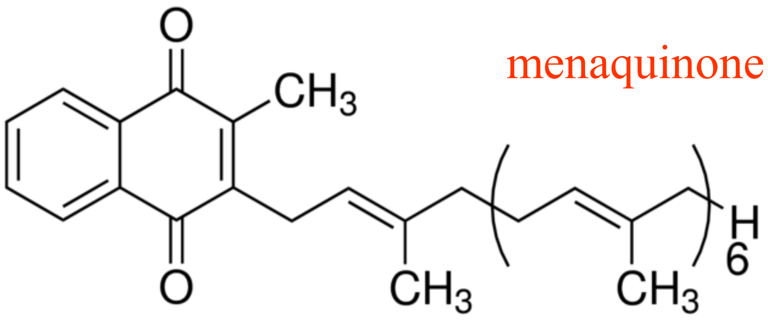
Coordination chemistry of porphyrins

Neutral M^{2+} complexes are often harder to reduce than corresponding doubly protonated neutral ligands; the more ionic bond to the metal leaves **considerable amounts of negative charge at the ligand**. Since the central ion Mg2+ is redox-inert, the radical anions of chlorophylls can be regarded as complexes of a **divalent metal cation** and the radical ***trianion* of the macrocyclic ligand**: $(Chl/Mg)^{\bullet -} = Chl^{\bullet 3-}/Mg^{2+}$.

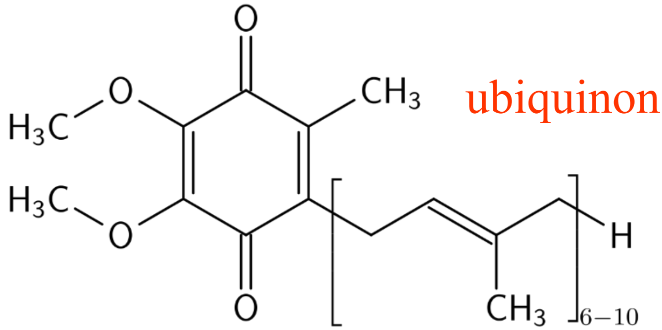


The third detectable acceptor for the electron generated through light-induced charge separation in the reaction center is a para-quinone, Qa, such as **menaquinone**, which is reduced to a **semiquinone** radical anion, $Qa^{\bullet-}$.

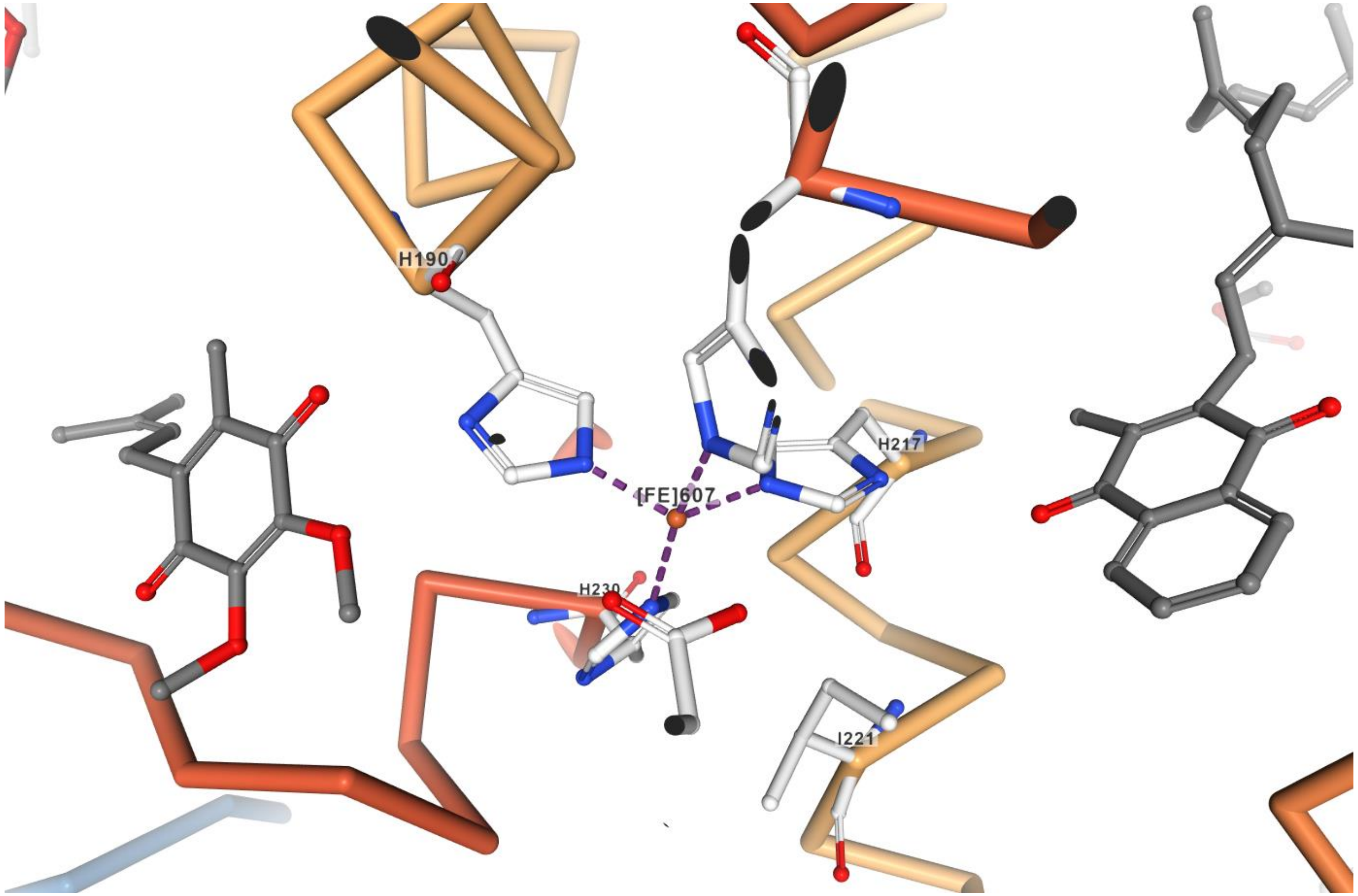
Reduced Qa may in turn reduce a different, more labile quinone, such as ubiquinone, Qb, with a high-spin iron(II) center connecting the two quinones at the axis of the reaction center.



menaquinone



ubiquinone



Fe^{II} is equatorially bound by four histidine ligands
and axially by carboxylate

Probably it is required to guarantee the controlled electron transfer from reduced BP to the primary quinone through **polarization of the hydrogen bond-forming histidine** ligands or to **maintain** the necessary **structure**.

Quinone Qb or hydroquinone H2Qb, is not tightly bound to the protein but exchanges with quinones in the "**quinone pool**" of the membrane, so that electrons can now be transported further outside the protein.

Simple bacteria => the electron gradient gives rise to a coupled H^+ gradient and photosynthetic phosphorylation takes place (ATP synthesis).

Higher organisms => further steps, the "dark reactions", which eventually lead to the production of the electron-rich coenzyme NADPH and to Mg^{2+}-requiring CO_2 reduction (Calvin cycle).

The **radical cation** of the "special pair" that remained after the initial charge separation is reduced after a relatively long time (hundreds of nanoseconds) through regulated electron flow via the heme centers of cytochrome proteins.

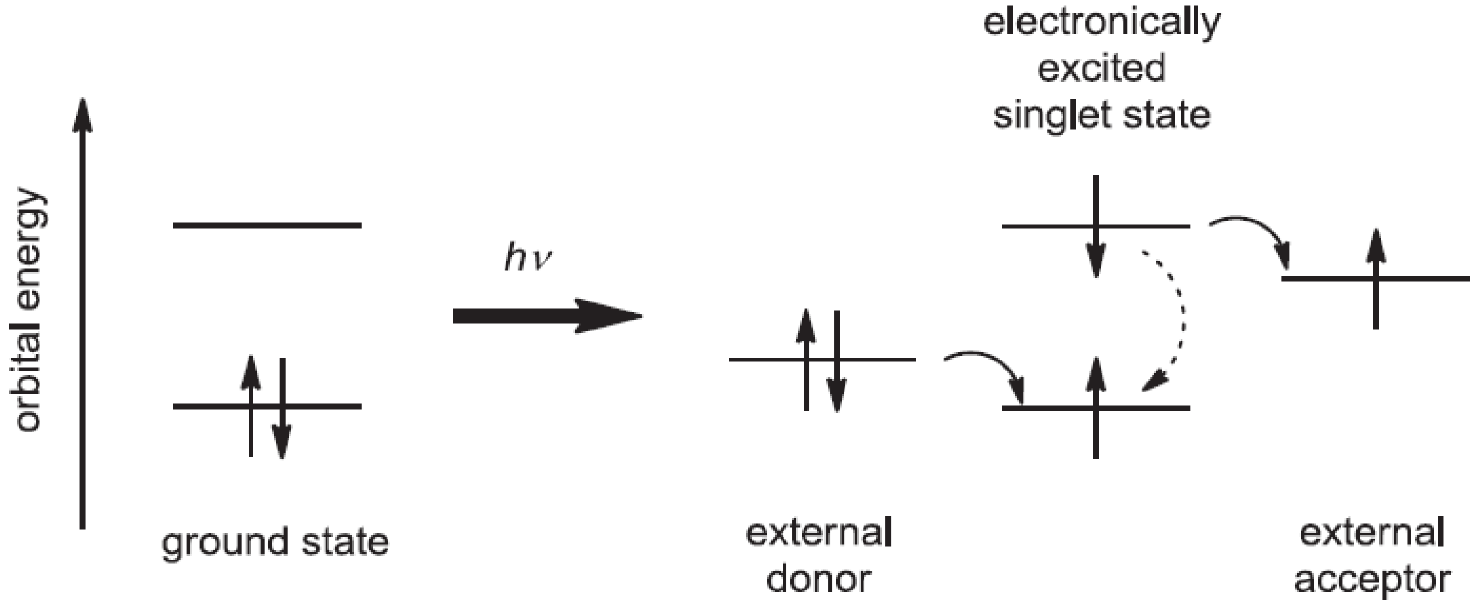
The electron deficiency or "hole" is thus further translocated and:
Simple bacteria => finally filled up through back electron transfer at a different site in the membrane

Higher organisms => the hole created at the special pair may be the starting point for substrate oxidation which requires an additional photosynthetic system consisting of another light-harvesting complex, a reaction center and an oxidase enzyme.

We can thus obtain both oxidation and reduction from an electron excitation mediated charge transfer?

Electronic excitation creates an electron hole in a low-lying orbital, which invites electron transfer from an external donor (photooxidation).

Simultaneously, the presence of the excited electron in a high-lying, previously unoccupied orbital allows for the photoreduction of an external acceptor.



Absolute necessity of preventing heat- or light-producing back electron-transfer reactions

⬇

chlorophyll molecules must not contain a redox-active transition metal.

Metal centers like $Fe^{II/III}$, which easily transfer electrons themselves, could accept or donate electrons in the ground or electronically excited states of an Fe-chlorophyll system and thus preclude the photosynthesis, which requires a rapid spatial charge separation.

The role of the magnesium ion in chlorophylls is therefore to act as a:

light, redox-inert, Lewis acidic coordination center

that contributes to a defined **three-dimensional organization** in light-harvesting systems and reaction centers.
Monomers, π–π dimers and metal-free ligands each have separate, unique functions in the primary processes of photosynthesis.

Redox-active transition metals or heavier metal centers with higher spin–orbit coupling constants would support additional, undesired reaction alternatives; of the remaining bioavailable metal ions, only Mg^{2+} fits exactly with regard to size and charge