Journal of Molecular Imaging and Dynamics

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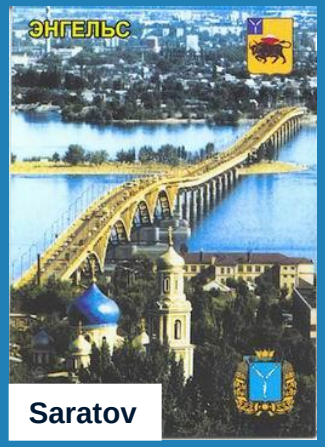

Emission Mössbauer spectroscopy: novel applications for probing structural organisation of metalloenzyme active centres

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Test object:

- **glutamine synthetase (GS; *doped with $^{57}Co^{2+}$*),**

a key enzyme of nitrogen metabolism

in many organisms

(isolated from *Azospirillum brasilense*,

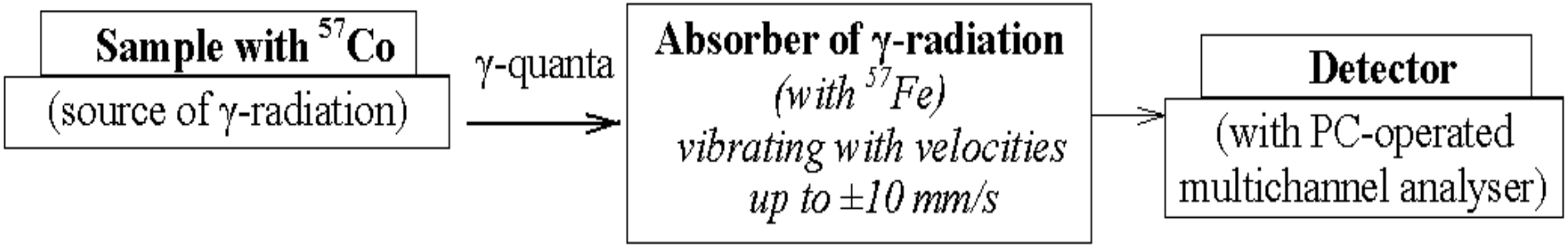
a plant-growth-promoting N_2-fixing rhizobacterium)

Methodology:

- **^{57}Co emission Mössbauer spectroscopy**

(in rapidly frozen aqueous solutions)

Emission (^{57}Co) Mössbauer spectroscopic study of $^{57}Co^{2+}$-doped GS active centres

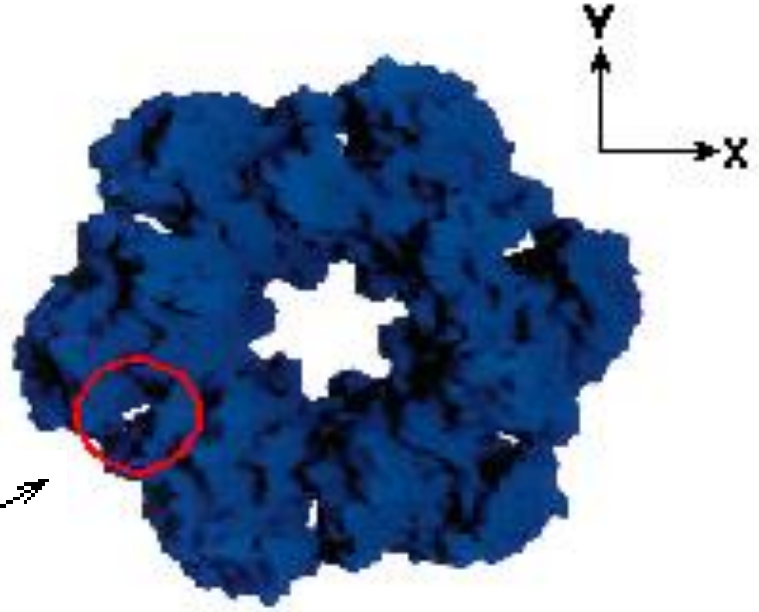


(Kamnev A.A. // *J. Mol. Struct.* **744** (2005) 161.)

Characterisation of bacterial GSs

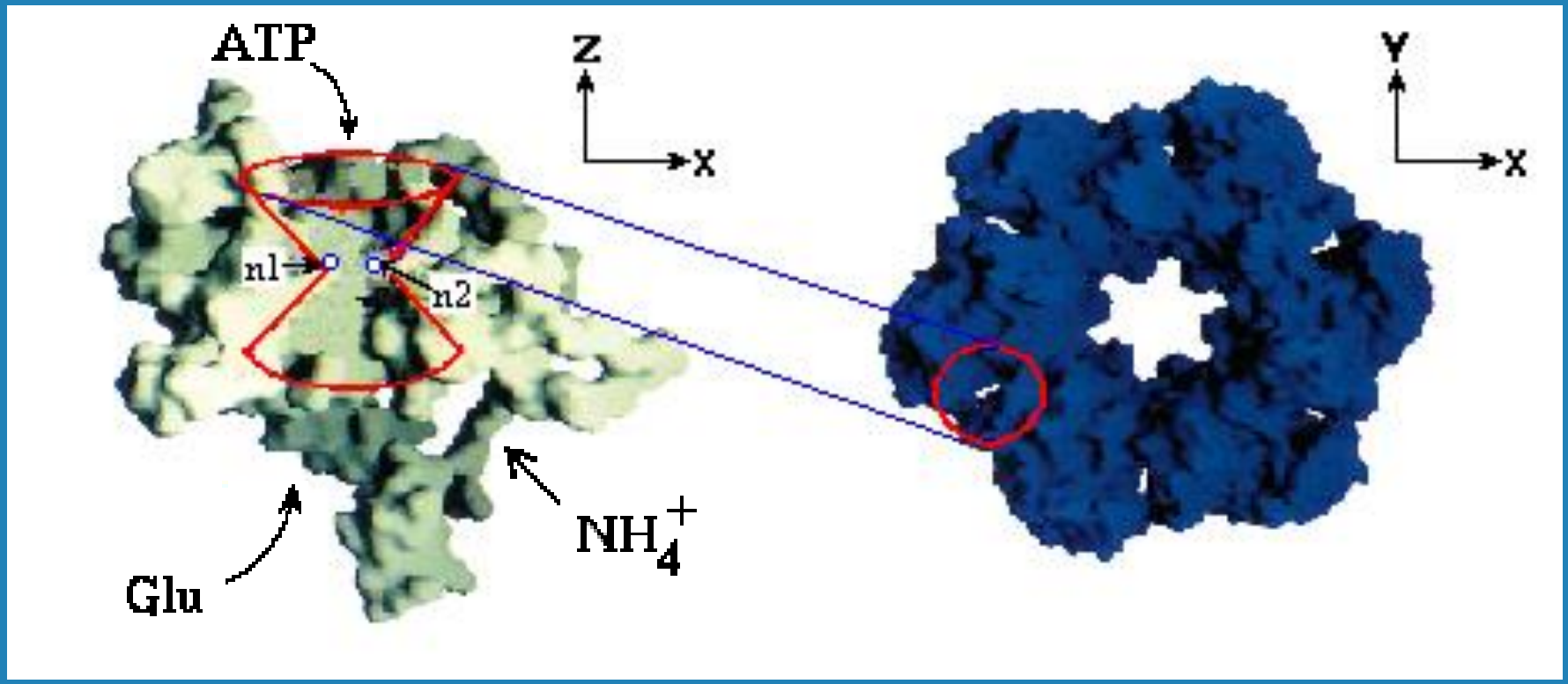
One of two hexameric rings located face-to-face, with total 12 subunits

(D. Eisenberg e.a., 2000)



Location of one of the 12 active centres (between subunits)

Characterisation of bacterial GSs



Distance between the cation-binding sites:

n1 ← 6 Å → n2 ***(no bridging residues):***

the two sites are 'spectroscopically independent'

Emission (^{57}Co) Mössbauer spectroscopic study of $^{57}Co^{2+}$-doped GS active centres

PREREQUISITES:

1. **Possibility to remove strongly bound cations from the native enzyme**

(treatment with 5 mM EDTA $\longrightarrow$ reversible loss of activity)

2. **Possibility to insert Co^{2+} into the active centres**

(addition of Co^{2+} $\longrightarrow$ regain of activity)

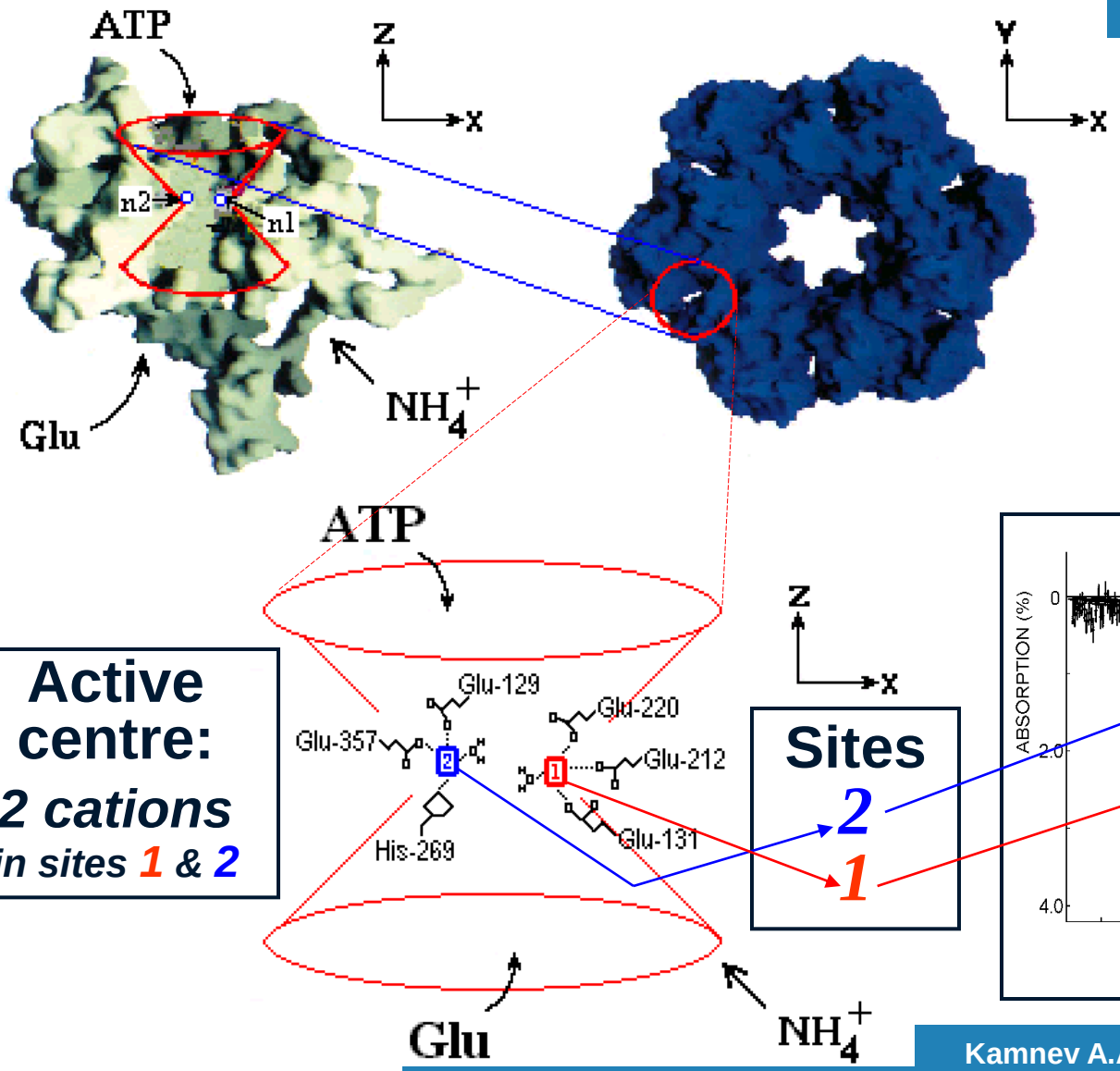
3. **Specific [$^{57}Co^{2+}$]:[GS] molar ratio ($12 \leq x \leq 24$)**

(to avoid multiple binding of $^{57}Co^{2+}$ beyond active centres)

^{57}Co Emission Mössbauer Spectroscopy:

Probing the structure of cation-binding sites at the active centres

Glutamine synthetase from *Azospirillum brasilense*

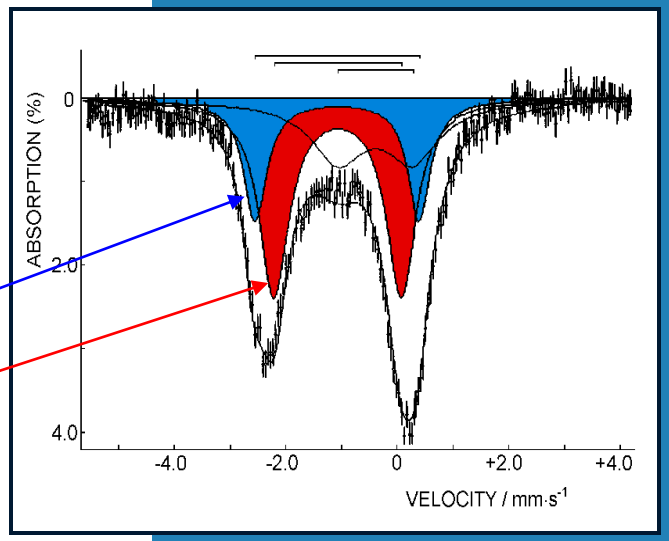


Macromolecule
(side and top views)

^{57}Co emission Mössbauer spectrum

Active centre:
2 cations
in sites 1 & 2

Sites
2
1



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Emission Mössbauer spectroscopy: novel applications for probing structural organisation of metalloenzyme active centres

Basic conclusions:

- EMS allows different cation-binding sites in ^{57}Co-doped metalloproteins to be characterised.
- EMS data on $^{57}Co^{2+}$-doped bacterial glutamine synthetase (GS) reveal two different cation-binding sites at each GS active centre.
- Isostructural substitution of $^{57}Co^{2+}$ for other cations (e.g. for Zn^{2+}) expands the EMS applicability and importance.

Thank you