binds to a groove of tetrameric GAPDH, while N-terminal region of CP12 is largely unstructured. We also observed that the dimeric PRK adopts distinct conformations generated by a rigid body movement of the protomer, suggesting that PRK exists in equilibrium between distinct conformations in solution. Together with mutagenesis analysis, these data demonstrated that CP12 is partially folded on the binding of the first target GAPDH, and the folded part of CP12 within GAPDH-CP12 complex plays a crucial role in selective interaction with a specific conformer from possible conformational states of the second target PRK to complete the GAPDH-CP12-PRK complex. These studies give further understanding how the complex formation down-regulates GAPDH and PRK to synchronize to the light transition, and also sheds light on the question of how the GAPDH-CP12-PRK complex is formed.

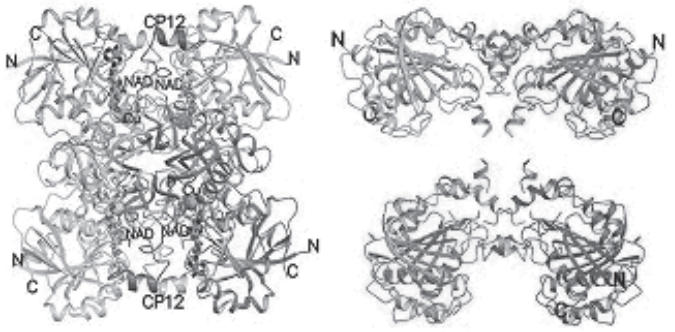


Fig. 1 Crystal structures of GAPDH-CP12(left) and PRK (right).

**Keywords: assembly, photosynthesis, enzyme**

## MS.64.2

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**Structural basis for specific aminoacyl-tRNA synthesis**

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In protein biosynthesis, each codon is translated into its specific amino acid by aminoacyl-tRNA. For most of the amino acids used in translation, their cognate aminoacyl-tRNA synthetases (aaRSs) synthesize aminoacyl-adenylate from the amino acid and ATP, and then transfer the aminoacyl moiety to the 3-terminal adenosine of the cognate tRNA, depending on strict recognition of both amino acid and tRNA. In most cases, the anticodon of tRNA serves as the major determinant for the recognition by the corresponding aaRS. However, several aaRSs recognize other part of tRNA for specific aminoacylation. Alanyl-tRNA synthetase (AlaRS) and histidyl-tRNA synthetase (HisRS) recognize the G3:U70 wobble base pair and the –1 guanosine, respectively. We have determined crystal structures of AlaRS and HisRS in complex with their cognate alanine and histidine tRNAs, respectively, which reveal the unique mechanisms of tRNA recognition. On the other hand, for some amino acids, the aminoacyl-tRNA is not synthesized in the canonical, direct manner, but indirectly synthesized by conversion of the aminoacyl moiety of another aminoacyl-tRNA. For example, glutaminyl-tRNA is synthesized from glutamyl-tRNA by "transamidosome" in most of microbes. The selenocysteine, the twenty-first amino acid in protein synthesis, is synthesized from the serylated form of its specific tRNA. We will present structural basis of specific tRNA recognition in these indirect aminoacyl-tRNA synthesis mechanisms, on the basis of our crystal structures of the machineries in the tRNA-bound form.

**Keywords: tRNA, enzyme, complex**

## MS.64.3

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**First view of insulin bound to its primary binding site on the insulin receptor**

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While the three-dimensional structure of insulin was an early triumph of protein crystallography [1], to date there has been no three-dimensional structural information regarding the manner in which the insulin hormone binds to the insulin receptor (IR). Therapeutic targeting of IR is of key importance in the treatment of both Type 1 and Type 2 diabetes and structural information regarding the mode of insulin binding to IR is of particular relevance to the design of novel insulins with enhanced therapeutic profiles [2]. IR is also closely related the Type 1 insulin-like growth factor receptor (IGF-1R), which is under intensive investigation as an anti-cancer target [3]. The mode of ligand binding to these receptors is likely highly similar, but, again, little is known about the mode of IGFs binding to IGF-1R.

IR is a $(\alpha\beta)_2$ disulfide-linked homodimer [4]. Each receptor monomer consists of a disulphide-linked $\alpha$-chain and $\beta$-chain, and disulfides cross-link the monomers at two sites within the respective $\alpha$-chains. Cross-linking, biochemical and mutagenesis studies have revealed that the initial hormone-receptor interaction is insulin binding to the so-called "Site 1" on IR. Site 1 consists of elements of first leucine-rich repeat domain (L1) of one receptor monomer and the C-terminal segment ($\alpha$CT) of the $\alpha$-chain of the second monomer [5]. Following this event, insulin then forms a crosslink to "Site 2", which lies at the junction of the first and second fibronectin domains of the second monomer. The resulting complex is of picomolar affinity and effects signal transduction.

After twenty years of effort, we have now obtained a crystal structure of insulin in complex with Site 1, with the IR elements being contributed by a domain-minimized dimeric receptor construct. Crystal formation was achieved by attaching a pair of Fab molecules to the receptor / hormone assembly. The structure reveals that the B-helix of insulin lies parallel to the $\alpha$CT helix on the surface of the central $\beta$-sheet of L1. The $\alpha$CT helix is repositioned with respect to its location in the *apo*-IR structure and our structure indicates that it effects the displacement of the extended C-terminal segment of the insulin B-chain away from its location next to the B-helix [6]. Our structure indicates further that elements of the hexamer-forming surface of insulin would be directed towards Site 2 within an intact IR dimer, suggesting a mechanism for signal transduction.

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