s1.m8.o5 **Evolutionary Links in the Metabolic Synthesis and Degradation of Nucleotides: Prove and Prejudice.** Olga Mayans[a], Marco Marino[a], Miriam Deuss[b], Dimitri Svergun[c], Reinhard Sterner[b]. [a]*Division of Structural Biology, Biozentrum, Univ. Basel (CH);* [b]*Inst. Biochemistry, Univ. Köln (D);* [c]*EMBL-Hamburg Outstation (D) email: Olga.Mayans@unibas.ch*

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The transfer of a ribosyl group between aromatic bases and phosphate groups is one of the most fundamental processes in the metabolism of nucleotides and amino acids. Despite close similarity between the synthesis reaction (phospho-ribosylation) and the degradative catalysis (de-ribosylation), these metabolic processes are carried out by several unrelated enzyme families which have evolved independently and even at different times in the course of evolution. Ribosylation is performed by families of phosphoribosyltransferases (PRT), while removal is catalysed by nucleoside phosphorylases (NP). Exceptionally, the crystal structure of anthranilate PRT (AnPRT) [1], involved in ribosylating intermediates from the tryptophan biosynthetic pathway, showed to be unrelated to any other PRT and, instead, shared unambiguous structural features with NPs from the salvage pathways of pyrimidine nucleotides. Thus, anthranilate PRT constitutes the first example of an evolutionary link between ribosylation and deribosylation processes in nucleotide metabolism. In our current work, we have determined for the first time the crystal structure of wild-type anthranilate PRT in complex with its two natural substrates (anthranilate and PRPP) and metal counterions, showing the active site in several states of occupancy, including the fully complexed form. We have also monitored domain motion upon complexation using SAXS, where wide-angle data allowed modelling of the compact conformation adopted by the enzyme during catalysis. Furthermore, characterization of the active site included isothermal calorimetry and kinetic characterization of mutated variants. Surprisingly, the arrangement, number and conformation of substrates within the binding groove, although unambiguous, differ remarkably from that expected. In the light of biochemical data and analyses of preserved enzymatic features, the structural evidence causes certain perplexity. Taken together, a reconciliation of experimental results indicate that anthranilate PRT is a true hybrid, where classical PRT catalysis and structural protein-ligand recognition motifs have been overlaid onto the architectural template, structural dynamics and, possibly, catalytic strategy of pyrimidine NPs from nucleotide salvage pathways. In this presentation, we will discuss "presumptions" and "evidence" in the study of anthranilate PRTs, where results will be put in the general context of known PRT and NP familes.

1. Mayans, O., Ivens, A., Nissen, L. J., Kirschner, K. & Wilmanns, M. Structural analysis of two enzymes catalysing reverse metabolic reactions implies common ancestry. *Embo J* **21**, 3245-54 (2002).

s1.m8.o6 **Rational Proteomics: Substrate Specific Fingerprints in SCOR Enzymes.** W. Duax[1,3], L. Habegger[1], R. Huether[1], V. Pletnev[2], [1]*Structural Biology Dept., Hauptman-Woodward Medical Inst., 73 High St., Buffalo, NY 14203,* [2]*Inst. Bioorg. Chem., RAS, Moscow and* [3]*SUNY, Dept. of Structural Biology, Buffalo, NY 14260.*

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The short-chain oxidoreductase (SCOR) family of enzymes is an ancient family that includes many steroid dehydrogenases vital to growth and development. A search for covariance among residues predicted to line the substrate binding pocket in SCOR enzymes led to the identification of nine sequence positions in three loops of the proteins that define the substrate binding pocket. These three loops are found in a 100 residue region of the C-terminal half of SCOR proteins. One combination of amino acids in the nine positions defines the substrate binding site of β-ketoacyl [acyl carrier protein] reductase. Different combinations of amino acids in the same nine sequence positions define the binding sites for eleven different substrates. The table illustrates the eight substrate fingerprints that differ most from one another. Bold letters indicate site specific amino acids common to more than one substrate fingerprint. Some sequence positions are more substrate specific (91 and 93) than others (148 and 193). The substrate fingerprints of the other three subfamilies of SCOR enzymes have different combinations of amino acids in some of these positions. For example, with three residue changes [N146Q, F184Y, and T190V] the fingerprint for β-keto ACPR becomes the fingerprint for aceto-acetyl CoA reductase, enzymes having common substrates but different products.

Structure	Sequence Position										overlap
	#	91	93	146	148	149	184	189	190	193	
I	177	T	D	N	**G**	**Q**	F	**M**	**T**	**L**	5
II	24	I	R	G	R	V	**Y**	N	**T**	**L**	3
III	30	Q	V	**S**	**G**	K	**Y**	L	V	Q	3
IV	14	E	P	W	**L**	**F**	A	I	N	K	1
V	21	A	A	Q	**G**	**Q**	L,I	M,L	L	**L**	3
VI	19	L	M,L	R	**G**	M	S	**M**	Q	**L**	3
VII	27	S	G	V	**L**	G	**Y**	**M**	A	V	2
VIII	10	N	F	**S**	**G**	**F**	L	F	S	**L**	3

I. β-keto-acyl ACPR. II. 2-deoxygluconate-3-dehydrogenase (Dh). III. D-β hydroxybutyrate Dh. IV. Glucose-Dh. V. 3-hydroxy CoA Dh. VI. 2,3-dihydroxybenzoate Dh. VII. Polyketide keto reductase. VIII. Retinol Dh.

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