LETTER TO THE EDITOR

Response to Boguslaw Stec's Letter to the Editor of Protein Science

We are grateful to Dr. Stec for pointing out that a new sequence for FTHFS became available. We have re-refined, using HKL3000, the structures reported in the article with the new sequence and we agree with Dr. Stec that the electron density matches the new sequence. We do not think that a new variant of FTHFS emerged but rather it is more likely that the old sequence published in 1990 had errors. For the recombinant protein expression, we used the system constructed by one of the authors of the original sequence data, the bacteria were propagated just a few times.

We have also re-refined the structures of the FTHFS complexes and for them our agreement with Dr. Stec conclusions is limited.

He comments on the FTHFS•ADP•XPO structure (originally 3RBO) "there is strong evidence supporting the presence of XPO but in a post-postcatalytic (rotated conformation)." Our conclusion is that in subunit A the XPO ion is positioned as we had originally determined. Subunit B, which has better density, now shows two possible orientations of XPO: one rotated, one original, with about equal occupancy. The disorder of the XPO ion strongly confirms our hypothesis that the intermediate turns around to enable nucleophilic attack by the N¹⁰-THF.

In comments on the FTHFS•ZD9331•XPO (originally 3SIN) complex Dr. Stec states that there is a mixture of overlapping ZD9331 and ADP in the active site. This is only partially true. Actually, there is a mixture of ZD9331+XPO as reported in the article and ADP, with the XPO position corresponding

to the β -phosphate of ADP. The position of the folate is defined by electron density for a non-overlapping part of the molecule. This observed disorder even further supports our central hypothesis that the folate and the ATP binding sites overlap and the two substrates cannot bind simultaneously in one active site.

Dr Stec's speculations about the space group being R3 are baseless. For the native data, which are of the highest resolution 2.1 Å, the values of *R*-merge are: 0.093 (0.761 in the highest shell) with redundancy 5.7 in R32 and 0.083 (0.696) with redundancy 2.9 in R3. Similarly, for other data sets the crystal symmetry lowering does not lead to significantly better statistics.

The re-refined structures are deposited to the PDB and the statistics of their re-refinement are in Table I.

In conclusion, we agree with the alignment between the amino acid sequence suggested by Dr. Stec and the electron density map. New amino acid sequence information, together with better refinement software, improves the quality of the crystal structures. We are glad to notice that as a result of these improvements the rationale for the kinetic and catalytic mechanisms proposed in the article became stronger.

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Table I. *New Crystallographic Data and Refinement Statistics for FTHFS*

Ligands PDB ID		Native ^a 4JIM	ADP/XPO 4JJZ	ZD9331/XPO & ADP 4JKI	Folate ^b 4JJK
Space group		<i>R</i> 32	P2 ₁ 2 ₁ 2	<i>R</i> 32	<i>R</i> 32
Unit cell dimensions	a (Å)	161.20	91.17	162.37	160.99
	b (Å)	161.20	212.97	162.37	160.99
	c (Å)	256.92	53.44	258.07	256.61
Resolution range (Å) (outer shell)		40.9–2.1	50.0–2.50	50.0–2.67	50.0–3.0
		(2.14–2.10)	(2.59–2.50)	(2.78–2.67)	(3.05–3.00)
Average redundancy		5.7	3.8	4.5	5.9
Average I/σ (<i>I</i>)		8.6	8.0	9.4	7.2
Total number of reflections		422582	141119	360101	145334
Number of unique reflections		74555	37221	79627	25875
Completeness (%) (outer shell)		100 (100)	91.5 (64.8)	91.1 (87.4)	95.2 (97.8)
Total linear R-merge		9.3	8.5	4.9	13.2
<i>R</i> -value (%)		16.0	18.5	19.3	20.5
<i>R</i> _{Free} -value (%)		19.0	23.5	24.6	27.5
<i>Ramachandran statistics</i>					
Residues in most favored regions (%)		90.5	89.5	88.2	89.1
Residues in additional allowed regions (%)		9.1	9.5	11.4	10.2
Residues in generously allowed regions (%)		0.3	1.0	0.4	0.7
Residues in disallowed regions (%)		0.0	0.0	0.0	0.0
Average B factors for subunit		24.4	27.7	41.2	19.2,
A (Å ²) (ligand, occupancy, B-factor)			(ADP, 1.0, 35.1)	(ZD9, 0.5, 83.3)	(FOL, 0.5, 34.4)
			(XPO, 1.0, 42.4)	(XPO, 0.5, 78.6)	
				(ADP, 0.5, 56.6)	
Average B factors for subunit		31.1	26.8 (ADP, 1.0, 38.8)	51.1	34.0
B (Å ²) (ligand, occupancy, B-factor)			(XPOA/B, 0.5, 23.5)		

^a Native FTHFS data were reprocessed using HKL3000 and extended to 2.1 Å resolution. New refinement statistics are found in this table.

^b Folate•FTHFS data were reprocessed using HKL3000. New refinement statistics are found in this table.