Crystal structures of human PAICS reveal substrate and product binding of an emerging cancer target

Jana Škerlová, Judith Unterlass, Mona Göttmann, Petra Marttila, Evert Homan, Thomas Helleday, Ann-Sofie Jemth, Pål Stenmark

Supporting Information

Table S1. List of oligos used to generate PAICS inducible knockdown cell lines.

Oligos	Sequence
sh2-forward	CCGGGACCAGATTACAGCAGGAAATCTCGAGATTTCCTGCTGTAATCTGGTCTTTTTG
sh2-reverse	AATTCAAAAAGACCAGATTACAGCAGGAAATCTCGAGATTTCCTGCTGTAATCTGGTC
sh3-forward	CCGGGCTGCTCAGATATTTGGGTTACTCGAGTAACCCAAATATCTGAGCAGCTTTTTG
sh3-reverse	AATTCAAAAAGCTGCTCAGATATTTGGGTTACTCGAGTAACCCAAATATCTGAGCAGC

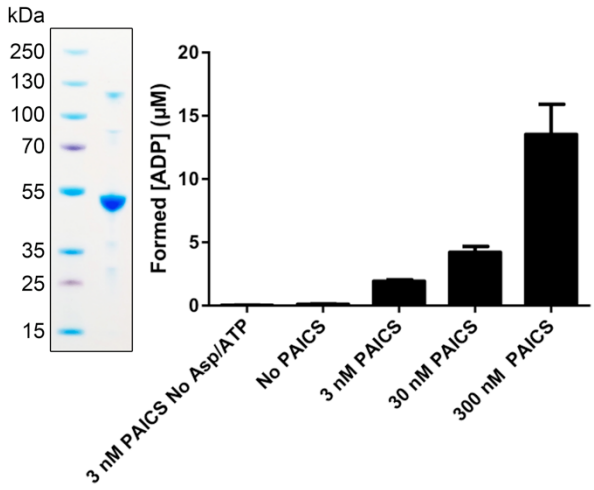

Figure S1: Purity and activity of recombinant human PAICS. The purity of the recombinant PAICS used for crystallization experiments is demonstrated here by a Coomassie-stained 4-12% Bis-Tris SDS-PAGE gel with 2 µg of PAICS loaded. Activity of PAICS (3, 30 and 300 nM) was tested by detecting ADP formed upon conversion of CAIR to SAICAR requiring ATP and L-aspartate. Graph shows average and stdev of formed [ADP] of duplicate samples.

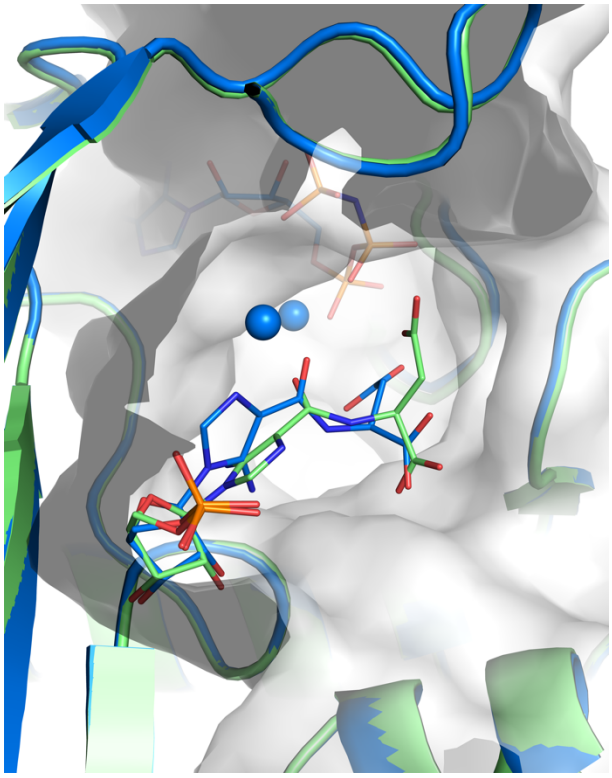

Figure S2: Conformational flexibility of the *N*-succinyl moiety of SAICAR. A superposition of the SAICARs sites in the structures of PAICS in complexes with the SAICAR alone (green) and with SAICAR, AMP-PNP, and Mg^{2+} (blue) is displayed here to demonstrate the flexibility of the *N*-succinyl moiety of SAICAR likely facilitating the exit of the final product SAICAR from the active pocket.