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Supplementary Materials for

Conditional iron and pH-dependent activity of a non-enzymatic glycolysis and pentose phosphate pathway

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This PDF file includes:

Table S8. Relative acceleration of non-enzymatic interconversions between sugar phosphates by pH and iron availability and possible mechanistic rationale.
Legend for table S9
Text S1. Comparison of the reactions found here and in previous work [Keller *et al*. (*19*)] in which sediment-like complex metal mixtures were studied.
References (*65*–*69*)

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/1/e1501235/DC1)

Table S5 (.csv format). Integrated NMR peak areas for all individual iron–sugar phosphate interaction NMR experiments.
Table S9 (.csv format). Individual reaction rates of non-enzymatic interconversions between sugar phosphates in the presence and absence of Fe(II).

Supplementary Figures and Tables

MS Table S1. MS/MS Parameters	
Basic	
Instrument	Triple Quadrupole mass spectrometer (Agilent 6460)
Software for analysis	QQQ Quantitative analysis (Agilent)
Scan Type	Multiple Reaction Monitoring (MRM)
Ion Source	ESI
Ion Mode	ESI + Agilent Jet Stream
Source Parameters	
Gas Temp (°C)	300
Gas Flow (l/min)	8
Nebulizer (psi)	50
Sheath Gas Flow	11
SheathGasHeater	300
Negative Capillary (V)	3000
Positive Capillary (V)	3500
Nozzle voltage	500

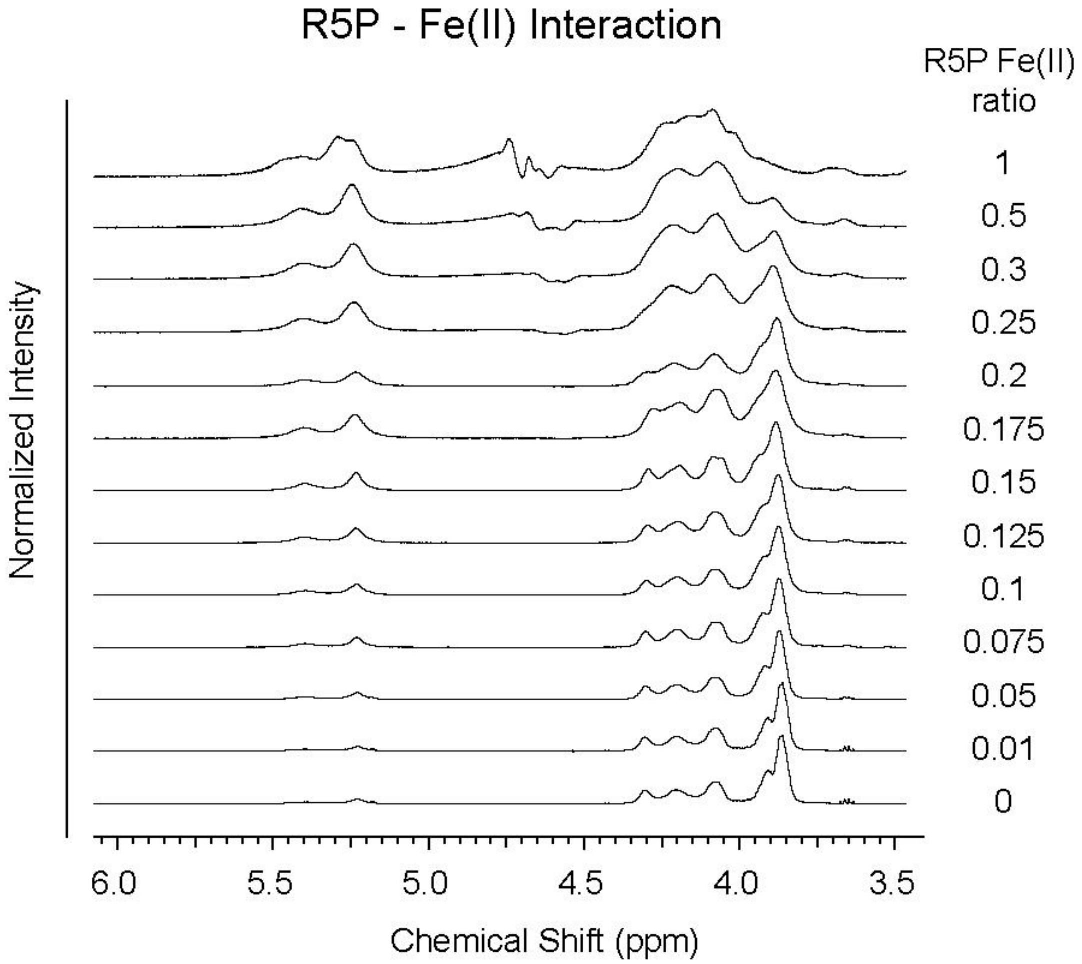
MS Table S2. UPLC/HPLC Parameters	
Instrument	On-Line coupled Agilent 1290
Column	Zorbax Eclipes Plus C8 Rapid Resulution 1.8 um, 2.1 x 50mm
Column Temperature	25C
Flow rate	0.6 ml/min
Solvent A	Water:AcCN 90:10 + 750 mg/l Octylammonium acetate
Solvent B	Water:AcCN 50:50 + 750 mg/l Octylammonium acetate
Needle wash	15 sec AcCN:Water needle wash in flush port with each injection

MS Table S3. HPLC Gradient			
Time	% solvent A	% solvent B	Flow rate
0	95	5	0.6
3.5	95	5	0.6
6	30	70	0.6
6.5	20	80	0.6
6.7	30	70	0.6
7	95	5	0.6
7.5	95	5	0.6

MS Table S4. Q1/Q3 (SRM) transitions and parameters							
Compound	Prec Ion m/z	Product ion m/z	Dwell time	Fragmentor (V)	CE (V)	Cell Acc (V)	Polarity
F16BP	339	97	40	175	16	7	Negative
S7P	289	97	40	100	12	7	Negative
6PG	275	97	40	100	18	7	Negative
G6P/F6P	259	97	40	100	12	7	Negative
R5P/Ru5P/X5P	229	97	40	85	12	7	Negative
E4P	199	97	40	80	5	7	Negative
2PG/3PG	185	97	40	75	11	7	Negative
Glu	179	89	40	70	1	7	Negative
G3P/DHAP	169	97	40	70	5	7	Negative
PEP	167	79	40	50	7	7	Negative
Pyr	87	43	40	55	3	7	Negative

Chromatographic separation allows identification and quantification of the following isomeric compounds: G6P and F6P; R5P and Ru5P/X5P; G3P and DHAP. MS1 resolution: Unit (0.7 m/z).

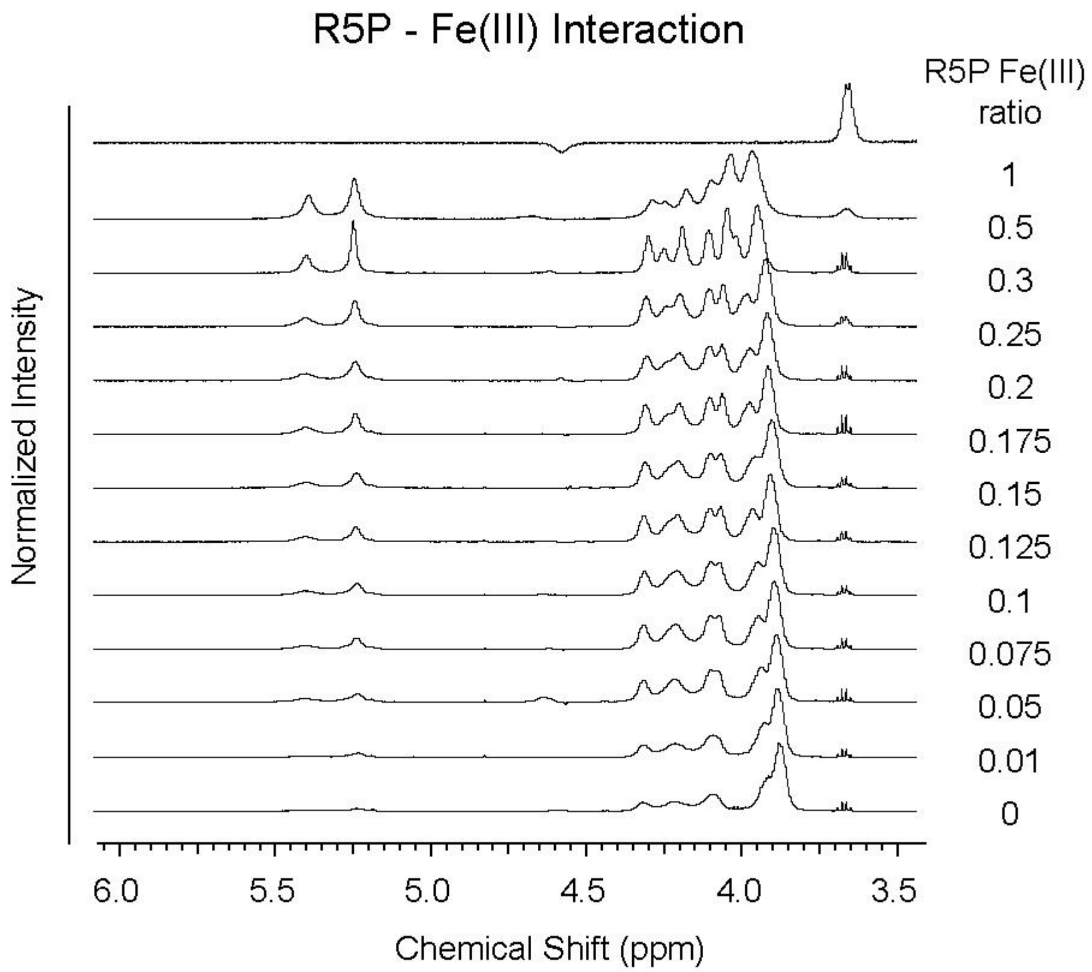
Supplementary Figure 1: **^{1}H NMR spectra of R5P and Fe(II) mixtures**.



Supplementary Table 1: ***T_1* relaxation time of R5P and Fe(II) mixtures**

Fe ratio	5.39 ppm	5.22 ppm	4.30 ppm	4.20 ppm	4.08 ppm	3.91 ppm	3.85 ppm
0	5.60 ± 0.20	6.103 ± 0.11	3.07 ± 0.19	3.06 ± 0.13	2.93 ± 0.31	1.05 ± 0.03	1.07 ± 0.004
0.01	3.80 ± 1.12	5.01 ± 0.45	3.39 ± 0.68	3.19 ± 0.48	3.08 ± 0.40	1.08 ± 0.13	1.06 ± 0.08
0.05	4.09 ± 0.63	4.12 ± 0.13	2.30 ± 1.04	2.17 ± 0.75	2.18 ± 0.59	0.90 ± 0.30	0.85 ± 0.15
0.075	3.39 ± 0.60	3.55 ± 0.32	1.41 ± 0.29	1.44 ± 0.20	1.52 ± 0.11	0.53 ± 0.05	0.57 ± 0.04
0.1	2.37 ± 0.39	2.53 ± 0.42	1.18 ± 0.26	1.16 ± 0.22	1.19 ± 0.27		0.45 ± 0.15
0.125		1.17 ± 0.28	1.14 ± 0.58	0.98 ± 0.39	0.84 ± 0.26		0.27 ± 0.08
0.15	1.49 ± 0.54	1.36 ± 0.55	0.99 ± 0.58	0.94 ± 0.32	0.87 ± 0.23	0.40 ± 0.18	0.32 ± 0.06
0.175	1.07 ± 0.27	1.01 ± 0.30	0.29 ± 0.09	0.31 ± 0.12	0.33 ± 0.12	0.10 ± 0.02	0.39 ± 0.45
0.2	0.62 ± 0.03	0.55 ± 0.11	0.47 ± 0.40	0.39 ± 0.23	0.33 ± 0.14	0.20 ± 0.16	0.10 ± 0.04
0.3	0.47 ± 0.16	0.51 ± 0.07		0.49 ± 0.27	0.34 ± 0.13		0.10 ± 0.02
0.4		0.14 ± 0.07		0.50 ± 0.32	0.32 ± 0.17		0.09 ± 0.04
0.5	0.15 ± 0.07	0.18 ± 0.04		0.13 ± 0.06	0.11 ± 0.04		0.04 ± 0.01
1	0.11 ± 0.08	0.09 ± 0.07				0.09 ± 0.03	

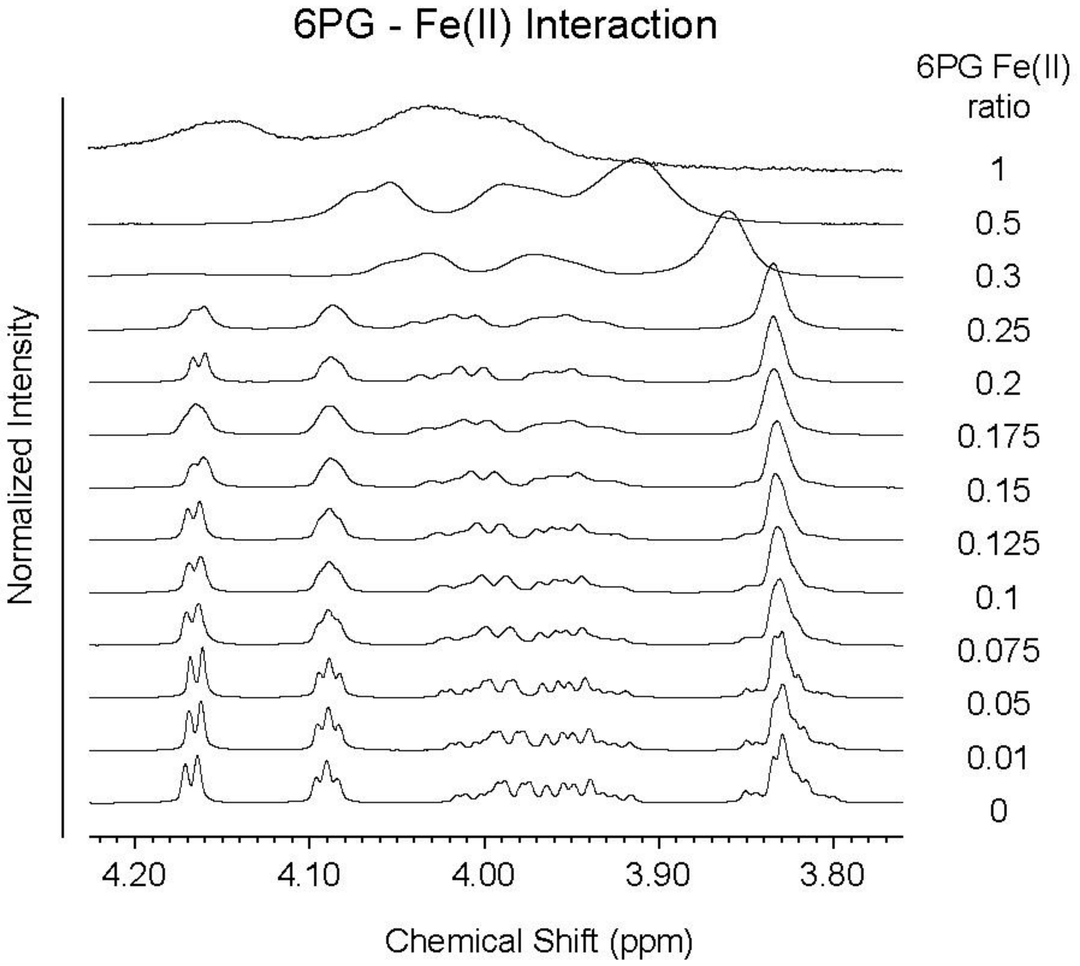
Supplementary Figure 2: **^{1}H NMR spectra of R5P and Fe(III) mixtures**.



Supplementary Table 2: ***T_1* relaxation time of R5P and Fe(III) mixtures**

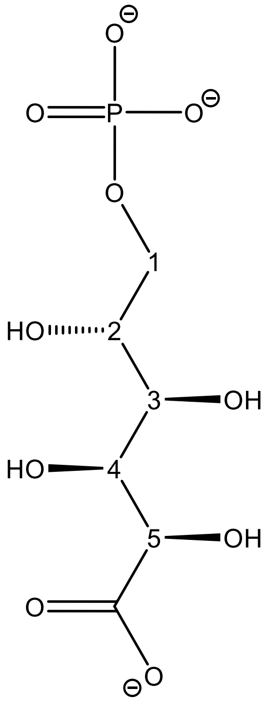
Fe ratio	5.39 ppm	5.22 ppm	4.30 ppm	4.20 ppm	4.08 ppm	3.91 ppm	3.85 ppm
0	5.47 ± 2.01	6.15 ± 1.26	3.93 ± 1.92	3.52 ± 0.89	3.39 ± 0.61	1.24 ± 0.34	1.14 ± 0.10
0.01	3.96 ± 0.68	4.22 ± 0.40	2.71 ± 0.63	2.37 ± 0.22	2.25 ± 0.14	1.02 ± 0.13	0.81 ± 0.08
0.05	3.52 ± 1.66	3.46 ± 1.38	1.31 ± 0.63	1.35 ± 0.28	1.38 ± 0.17	0.64 ± 0.12	0.55 ± 0.07
0.075	1.69 ± 0.65	1.96 ± 0.43	1.38 ± 0.38	1.25 ± 0.20	1.21 ± 0.11	0.50 ± 0.07	0.50 ± 0.04
0.1	1.71 ± 0.61	1.89 ± 0.41	1.06 ± 0.44	1.03 ± 0.24	1.02 ± 0.16	0.44 ± 0.08	0.43 ± 0.05
0.125	1.65 ± 0.11	1.73 ± 0.07	0.69 ± 0.12	0.79 ± 0.11	0.82 ± 0.10	0.34 ± 0.03	0.39 ± 0.05
0.15	1.56 ± 0.26	1.61 ± 0.23	0.84 ± 0.18	0.86 ± 0.14	0.86 ± 0.12	0.41 ± 0.07	0.39 ± 0.06
0.175	1.06 ± 0.05	1.18 ± 0.04	0.76 ± 0.12	0.73 ± 0.08	0.71 ± 0.07	0.31 ± 0.07	0.31 ± 0.03
0.2	0.92 ± 0.06	1.01 ± 0.03	0.74 ± 0.10	0.66 ± 0.07	0.64 ± 0.06	0.32 ± 0.06	0.26 ± 0.04
0.3	0.93 ± 0.22	1.02 ± 0.15	0.54 ± 0.07	0.61 ± 0.06	0.58 ± 0.07	0.29 ± 0.02	0.27 ± 0.02
0.4	0.79 ± 0.05	0.84 ± 0.03	0.42 ± 0.07	0.49 ± 0.02	0.42 ± 0.06	0.38 ± 0.02	0.23 ± 0.03
0.5	0.43 ± 0.10	0.72 ± 0.12	0.66 ± 0.06	0.63 ± 0.09	0.57 ± 0.08	0.49 ± 0.07	0.35 ± 0.03
1							

Supplementary Figure 3: ^{1}**H NMR spectra of 6PG and Fe(II) mixtures**.

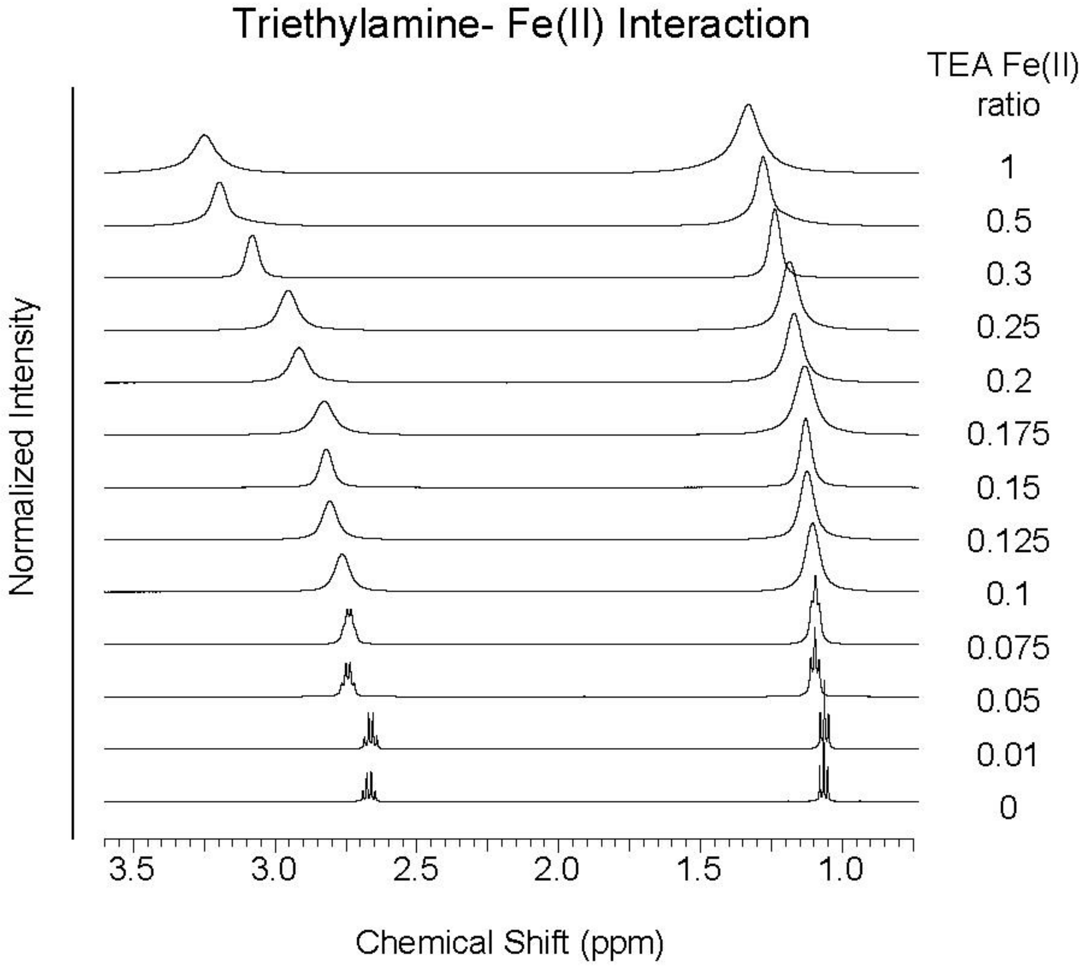


Supplementary Table 3: ***T*$_1$ relaxation time of 6PG and Fe(II) mixtures**

Fe ratio	4.12 ppm (5)	4.09 ppm (1a)	3.96 ppm (4, 1b)	3.84 ppm (2, 3)
0	2.84 ± 0.25	2.45 ± 0.21	0.91 ± 0.05	2.12 ± 0.04
0.01	1.99 ± 0.11	1.78 ± 0.1	0.81 ± 0.08	1.55 ± 0.05
0.05	1.42 ± 0.15	1.34 ± 0.1	0.66 ± 0.07	1.22 ± 0.06
0.075	1.22 ± 0.08	1.13 ± 0.18	0.68 ± 0.05	1.16 ± 0.01
0.1	1.37 ± 0.09	1.32 ± 0.06	0.73 ± 0.09	1.16 ± 0.03
0.125	1.27 ± 0.03	1.23 ± 0.03	0.63 ± 0.04	1.12 ± 0.01
0.15	1 ± 0.33	1.01 ± 0.28	0.56 ± 0.19	0.84 ± 0.38
0.175	1.1 ± 0.07	1.68 ± 0.94	0.51 ± 0.19	0.85 ± 0.29
0.2	1.07 ± 0.13	1.14 ± 0.08	0.61 ± 0.07	0.95 ± 0.11
0.3	0.44 ± 0.24	0.66 ± 0.14	0.35 ± 0.09	0.43 ± 0.09
0.4			0.24 ± 0.27	0.33 ± 0.37
0.5			0.13 ± 0.05	0.13 ± 0.02
1				

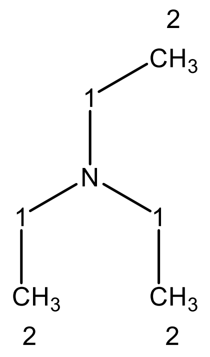


Supplementary Figure 4: **^{1}H NMR spectra of triethylamine and Fe(II) mixtures**.



Supplementary Table 4: ***T_1* relaxation time of triethylamine and Fe(II) mixtures**

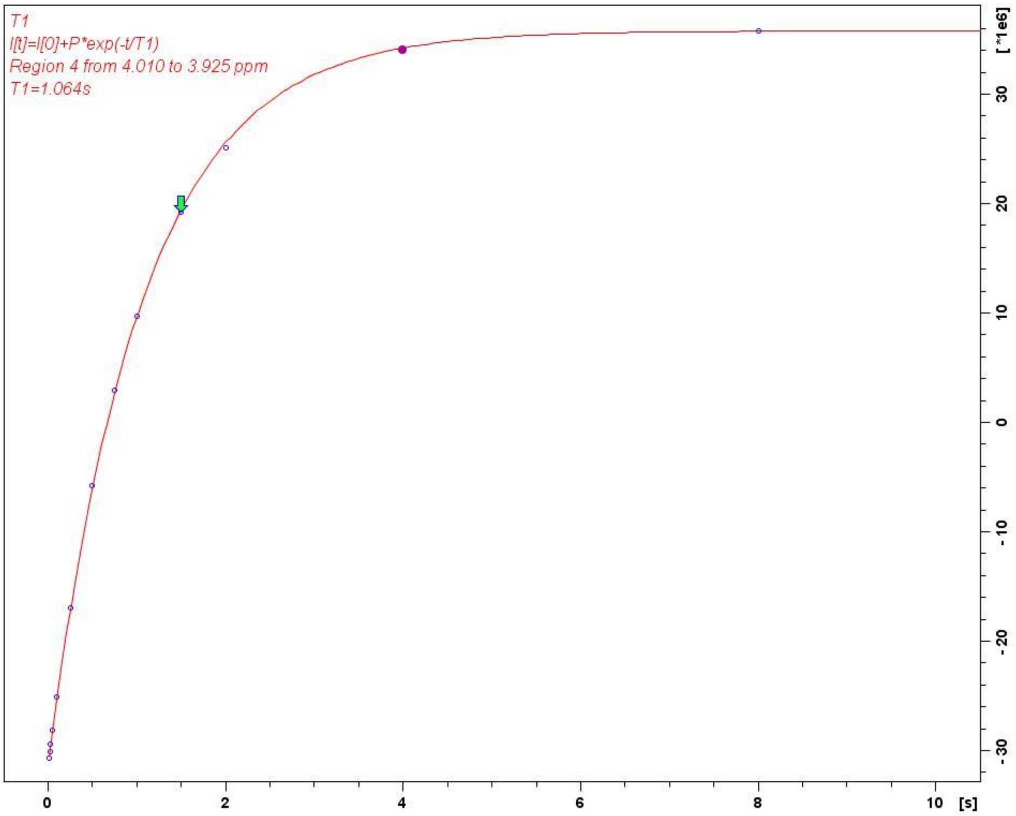
Fe ratio	1.06ppm (1)	2.67ppm (2)
0	3.54 ± 0.02	3.04 ± 0.06
0.01	3.27 ± 0.05	2.81 ± 0.05
0.05	2.92 ± 0.05	2.56 ± 0.04
0.075	1.77 ± 0.66	1.78 ± 0.77
0.1	1.60 ± 0.42	1.47 ± 0.32
0.125	1.38 ± 0.11	1.38 ± 0.26
0.15	2.50 ± 0.08	2.32 ± 0.20
0.175	1.63 ± 0.25	1.64 ± 0.43
0.2	2.06 ± 0.52	1.86 ± 0.44
0.3	1.58 ± 0.20	1.47 ± 0.21
0.4	3.60 ± 0.10	3.17 ± 0.13
0.5	4.30 ± 0.11	3.75 ± 0.13
1	3.69 ± 0.43	2.95 ± 0.97



Supplementary Table 5: **Integrated NMR peak areas for all individual iron–sugar phosphate interaction NMR experiments**

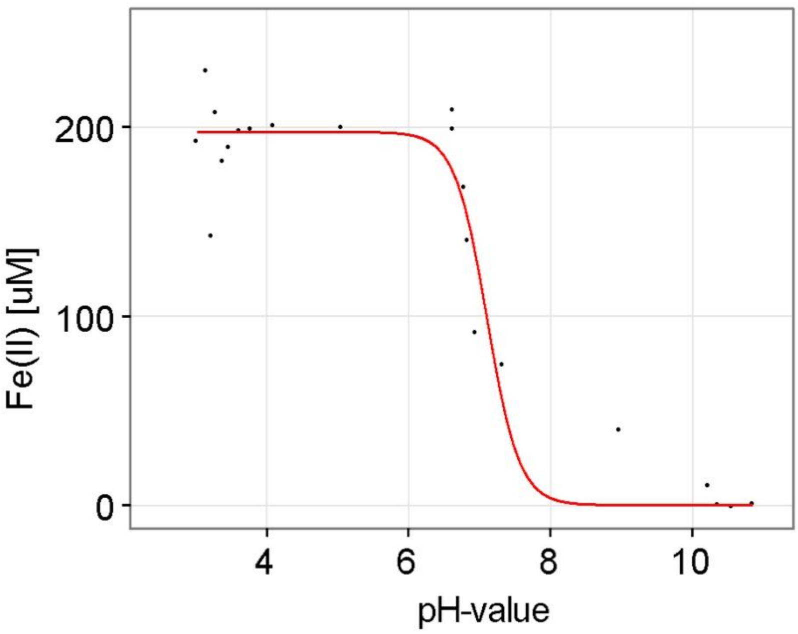
See Supplemental Table_integrated_NMR_peaks.csv

Supplementary Figure 5: ***T_1* NMR experiment curve-fitting examples [example of 6PG and Fe(II)]**



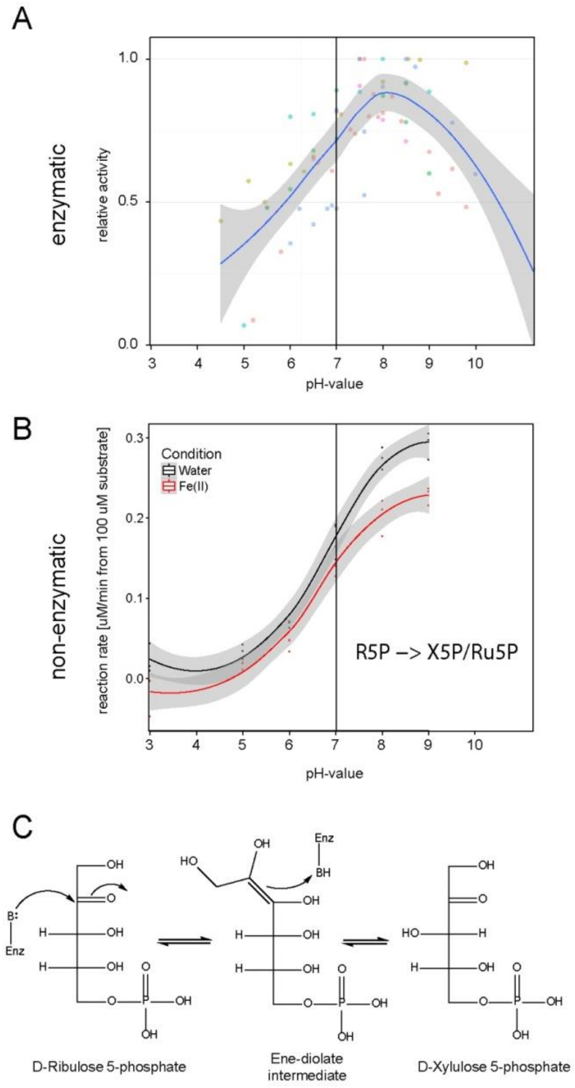
Recorded data of T1 NMR experiments was fitted as described in materials and methods. As an representative example we show here a curve fitting result for a sample containing 20 mM 6PG and 3 mM Fe(II).

Supplementary Figure 6: **Ferrozine results: Fe(II)/Fe(III) in dependence of pH**



Fe(II) availability in dependence of pH was measured with ferrozine assay (see material and methods). pH of a 200 µM Fe(II)Cl_2 solution was adjusted with 0.1 M HCl or 0.1 M KOH, ionic strength was compensated with the respective amount of 0.1 M KCl. Photospectrometric data was quantified with an external calibration curve and fitted with a logistic model.

Supplementary Figure 7: **Enzymatic pH dependency of ribose-phosphate isomerase and comparison to its non-enzymatic counterpart (R5P→X5P/Ru5P)**

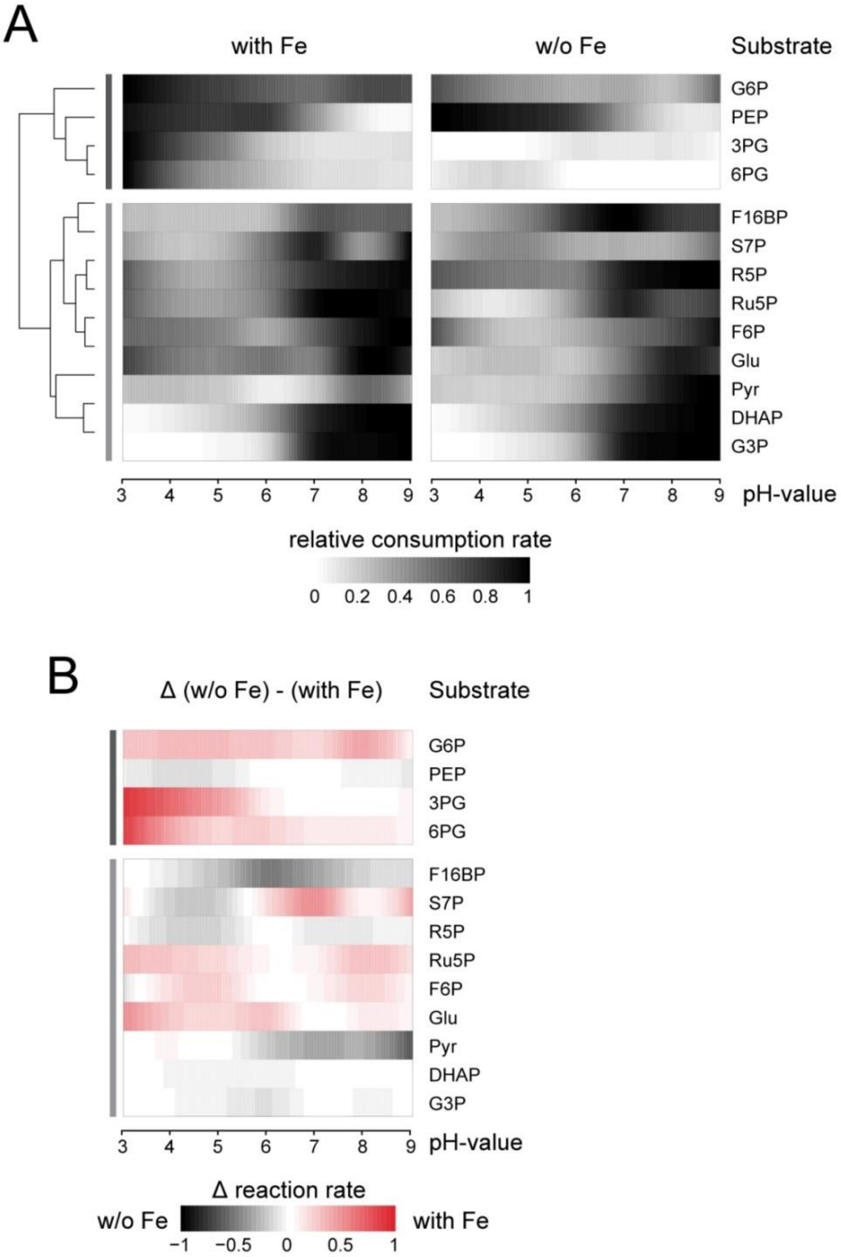


A) pH optimum of Ribose phosphate isomerase. pH-dependent enzymatic activity profiles were extracted from published sources (*65–69*) and normalized to the respective highst activity value. All data was combined to a consensus profile and fitted with a Loess model. The optimum activity for the enzymatic reaction was found between pH 8 and 9.

B) Non-enzymatic pH dependence profile in the pH-range from 3-9 for the non-enzymatic reactions is depicted in analogy to Figure 3, lower left panel in the main figure. Highest non-enzymatic reactivity was observed at pH 8 to 9.

C) Suggested reversible reaction mechanism of ribose phosphate isomerase as (replicated from 6) requires bases catalysed deprotonation of ribose 5-phosphate to then form a ene-diolate intermediate that is subsequently further converted into xylulose 5-phosphate.

Supplementary Figure 8: **pH and iron dependency on non-enzymatic substrate consumption rates**



A) pH-dependent changes expressed as relative reaction rates for individual non-enzymatic consumption rates of substrates, generated by fitting a Loess model to the experimental data and are normalized to the highest rate found in the presence of iron (left panel) or iron-free (right panel) conditions, as function of pH. Hierarchical clustering on Fe(II) profiles separated the reactions into two groups: Those accelerated in i) acidic or ii) alkaline conditions.

B) Differences in non-enzymatic substrate consumption rates between iron-rich and iron free conditions (ΔR). Red indicates higher rates in an iron-rich environment, whereas black shows higher reactivity when no iron is present.

Supplementary Table 6: **Maximum and minimum reaction rates of non-enzymatic interconversions between sugar phosphates in the presence of Fe(II)**

					Maximum rate [uM/min from 100 uM substrate]			Minimum rate [uM/min from 100 uM substrate]		
Nr	**Reaction**	**Substrate**	**Product**	**Condition**	**Mean**	**SD**	**pH**	**Mean**	**SD**	**pH**
1	3PG->Pyr	3PG	Pyr	Fe	2.85E-04	1.24E-04	3.0	2.56E-05	4.43E-05	7.0
2	6PG->E4P	6PG	E4P	Fe	1.26E-01	9.76E-03	9.0	5.94E-03	4.26E-04	3.0
3	6PG->Pyr	6PG	Pyr	Fe	9.69E-03	2.95E-03	5.0	1.51E-03	3.47E-04	3.0
4	6PG->R5P	6PG	R5P	Fe	1.37E-01	1.21E-02	3.0	1.09E-02	4.69E-03	7.0
5	6PG->X5PRu5P	6PG	X5PRu5P	Fe	9.21E-03	1.27E-03	3.0	2.30E-03	1.99E-04	8.0
6	DHAP->Pyr	DHAP	Pyr	Fe	2.59E-02	9.76E-03	8.0	1.39E-03	2.14E-04	5.0
7	F16BP->E4P	F16BP	E4P	Fe	4.41E-01	2.06E-02	8.0	1.43E-02	3.67E-03	3.0
8	F16BP->F6P	F16BP	F6P	Fe	4.02E-03	3.63E-04	3.0	4.38E-05	7.59E-05	9.0
9	F16BP->Glu	F16BP	Glu	Fe	4.30E-03	2.48E-03	5.0	0.00E+00	0.00E+00	6.0
10	F16BP->Pyr	F16BP	Pyr	Fe	1.28E-02	3.16E-05	7.0	6.77E-03	2.81E-03	3.0
11	F6P->G6P	F6P	G6P	Fe	1.91E-02	5.99E-03	9.0	1.24E-03	4.74E-04	3.0
12	F6P->Glu	F6P	Glu	Fe	7.34E-04	3.61E-04	3.0	1.94E-04	1.86E-04	8.0
13	F6P->Pyr	F6P	Pyr	Fe	1.26E-03	1.87E-04	3.0	6.13E-04	6.94E-04	8.0
14	F6P->R5P	F6P	R5P	Fe	2.03E-04	1.50E-04	9.0	4.50E-06	7.79E-06	5.0
15	F6P->X5PRu5P	F6P	X5PRu5P	Fe	3.70E-04	1.10E-04	9.0	1.70E-05	1.35E-05	5.0
16	G3P->Pyr	G3P	Pyr	Fe	8.98E-03	2.83E-03	8.0	8.53E-04	2.03E-04	3.0
17	G6P->Glu	G6P	Glu	Fe	4.79E-03	1.23E-03	6.0	0.00E+00	0.00E+00	7.0
18	G6P->Pyr	G6P	Pyr	Fe	8.57E-04	7.87E-04	5.0	2.39E-04	1.59E-04	9.0
19	PEP->Pyr	PEP	Pyr	Fe	2.09E-01	8.06E-03	3.0	1.17E-03	4.22E-04	9.0
20	R5P->E4P	R5P	E4P	Fe	5.29E-02	3.12E-03	9.0	7.67E-04	1.21E-03	3.0
21	R5P->Pyr	R5P	Pyr	Fe	7.23E-03	1.51E-03	7.0	3.53E-03	2.31E-04	5.0
22	R5P->X5PRu5P	R5P	X5PRu5P	Fe	2.30E-01	1.10E-02	9.0	0.00E+00	0.00E+00	3.0
23	Ru5P->Pyr	Ru5P	Pyr	Fe	1.41E-02	1.08E-03	3.0	8.90E-03	9.49E-04	6.0
24	Ru5P->R5P	Ru5P	R5P	Fe	2.34E-01	2.13E-02	8.0	4.32E-03	1.97E-03	3.0
25	S7P->Pyr	S7P	Pyr	Fe	5.59E-04	4.03E-05	5.0	2.34E-04	1.12E-04	9.0
26	S7P->R5P	S7P	R5P	Fe	1.90E-03	1.21E-03	5.0	0.00E+00	0.00E+00	9.0

Supplementary Table 7: **Maximum and minimum reaction rates of non-enzymatic interconversions between sugar phosphates in the absence of Fe(II)**

					Maximum rate [uM/min from 100 uM substrate]			Minimum rate [uM/min from 100 uM substrate]		
Nr	**Reaction**	**Substrate**	**Product**	**Condition**	**Mean**	**SD**	**pH**	**Mean**	**SD**	**pH**
1	3PG->Pyr	3PG	Pyr	AD	2.14E-05	1.86E-05	3.0	5.63E-06	9.76E-06	9.0
2	6PG->E4P	6PG	E4P	AD	3.38E-03	2.84E-03	9.0	2.40E-04	3.15E-04	5.0
3	6PG->Pyr	6PG	Pyr	AD	1.62E-04	9.87E-05	6.0	0.00E+00	0.00E+00	5.0
4	6PG->R5P	6PG	R5P	AD	0.00E+00	0.00E+00	8.0	0.00E+00	0.00E+00	9.0
5	6PG->X5PRu5P	6PG	X5PRu5P	AD	2.20E-04	1.90E-04	6.0	0.00E+00	0.00E+00	5.0
6	DHAP->Pyr	DHAP	Pyr	AD	3.89E-03	1.30E-03	7.0	3.88E-04	3.12E-04	3.0
7	F16BP->E4P	F16BP	E4P	AD	8.12E-03	5.96E-04	6.0	1.95E-03	2.50E-04	9.0
8	F16BP->F6P	F16BP	F6P	AD	5.56E-03	1.62E-04	3.0	0.00E+00	0.00E+00	9.0
9	F16BP->Glu	F16BP	Glu	AD	1.21E-03	2.09E-03	6.0	0.00E+00	0.00E+00	3.0
10	F16BP->Pyr	F16BP	Pyr	AD	9.31E-03	1.38E-03	7.0	1.64E-03	2.81E-04	3.0
11	F6P->G6P	F6P	G6P	AD	2.61E-02	1.02E-02	9.0	1.11E-03	6.75E-04	3.0
12	F6P->Glu	F6P	Glu	AD	8.00E-04	4.98E-04	5.0	3.88E-04	2.05E-04	8.0
13	F6P->Pyr	F6P	Pyr	AD	4.23E-04	1.92E-04	5.0	1.77E-05	3.06E-05	7.0
14	F6P->R5P	F6P	R5P	AD	4.98E-05	7.44E-05	8.0	6.13E-06	1.06E-05	7.0
15	F6P->X5PRu5P	F6P	X5PRu5P	AD	1.10E-04	1.15E-05	9.0	1.97E-05	3.42E-05	3.0
16	G3P->Pyr	G3P	Pyr	AD	9.57E-03	5.95E-04	9.0	1.79E-04	3.48E-05	3.0
17	G6P->Glu	G6P	Glu	AD	2.36E-03	5.36E-04	3.0	0.00E+00	0.00E+00	6.0
18	G6P->Pyr	G6P	Pyr	AD	3.61E-04	2.30E-04	3.0	5.66E-05	2.95E-05	7.0
19	PEP->Pyr	PEP	Pyr	AD	1.93E-01	8.17E-03	3.0	7.23E-04	1.89E-04	9.0
20	R5P->E4P	R5P	E4P	AD	5.59E-03	4.10E-03	7.0	8.94E-04	5.50E-04	3.0
21	R5P->Pyr	R5P	Pyr	AD	2.97E-03	6.65E-04	7.0	1.33E-03	8.56E-05	9.0
22	R5P->X5PRu5P	R5P	X5PRu5P	AD	2.93E-01	1.71E-02	9.0	2.74E-02	1.25E-02	3.0
23	Ru5P->Pyr	Ru5P	Pyr	AD	4.24E-03	8.38E-04	5.0	1.06E-04	7.14E-06	9.0
24	Ru5P->R5P	Ru5P	R5P	Fe	2.62E-01	4.65E-02	8.0	2.50E-02	6.86E-03	3.0
25	S7P->Pyr	S7P	Pyr	Fe	5.67E-04	7.26E-04	7.0	6.18E-06	1.07E-05	8.0
26	S7P->R5P	S7P	R5P	Fe	2.41E-03	8.04E-04	6.0	0.00E+00	0.00E+00	9.0

Supplementary Table 8: **Relative acceleration of non-enzymatic interconversions between sugar phosphates by pH and iron availability and possible mechanistic rationale.**

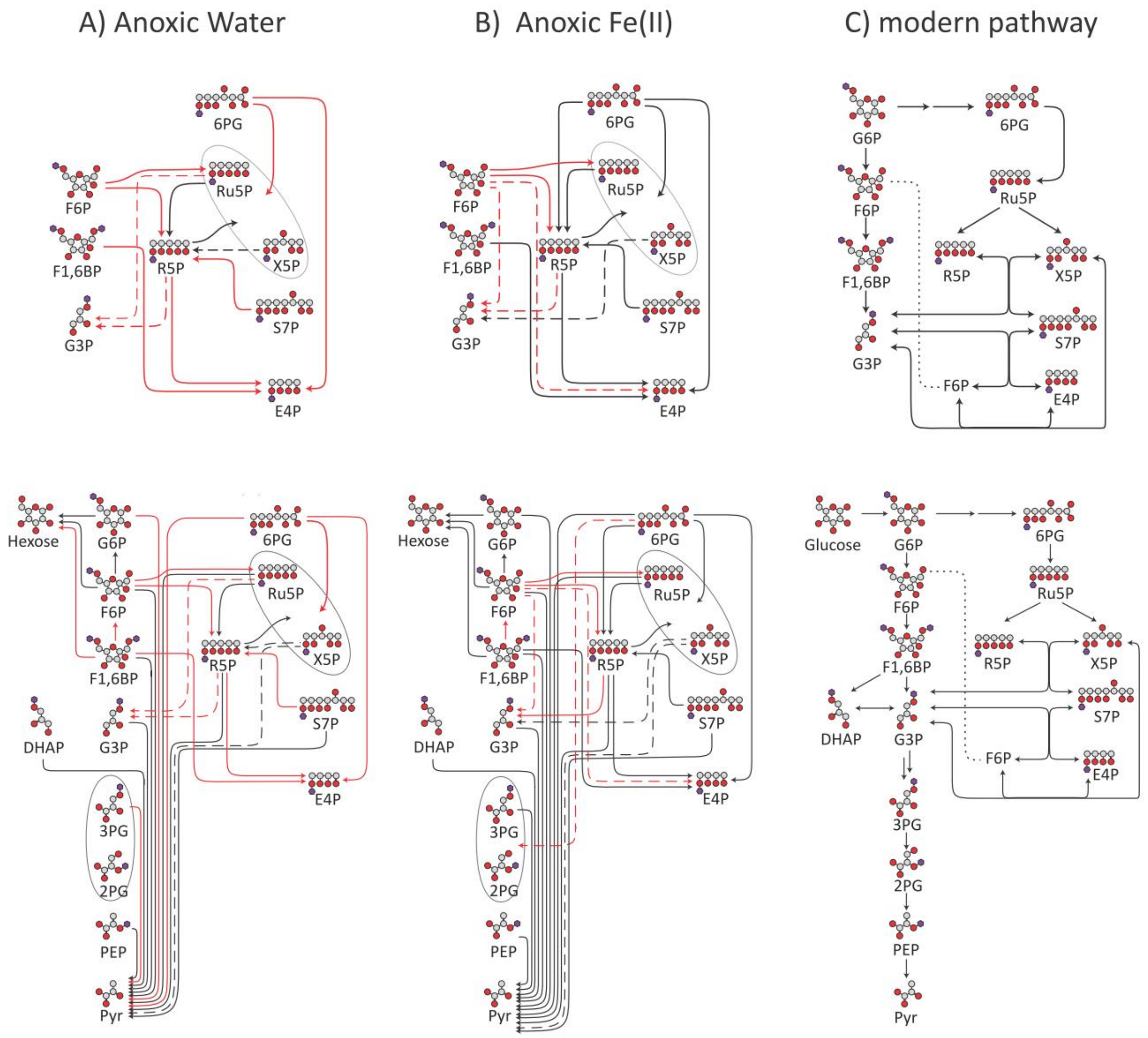
Nr	Reaction	Substrate	Product	Fe dep.	Fe Max/min	AD max/min	Fe max /AD max	Possible reaction mechanism
1	3PG->Pyr	3PG	Pyr	yes	11.1	3.8	13.3	complex multistep
2	6PG->E4P	6PG	E4P	yes	21.2	14.1	37.3	combination of 4 and 20
3	6PG->Pyr	6PG	Pyr	yes	6.4	inf	59.8	complex multistep
4	6PG->R5P	6PG	R5P	yes	12.6	inf	inf	decarboxylation+isomerisat.
5	6PG->X5PRu5P	6PG	X5PRu5P	yes	4.0	inf	41.9	decarboxylation
6	DHAP->Pyr	DHAP	Pyr	yes	18.6	10.0	6.7	complex multistep
7	F16BP->E4P	F16BP	E4P	yes	30.8	4.2	54.3	C-C bond breakage
8	F16BP->F6P	F16BP	F6P		91.7	inf	0.7	dephosphorylation
9	F16BP->Glu	F16BP	Glu	Yes	inf	inf	3.6	dephosphorylation
10	F16BP->Pyr	F16BP	Pyr		1.9	5.7	1.4	complex multistep
11	F6P->G6P	F6P	G6P		15.4	23.5	0.7	isomerisation
12	F6P->Glu	F6P	Glu		3.8	2.1	0.9	dephosphorylation
13	F6P->Pyr	F6P	Pyr	yes	2.1	23.9	3.0	complex multistep
14	F6P->R5P	F6P	R5P	Yes	45.1	8.1	4.1	dephosphorylation
15	F6P->X5PRu5P	F6P	X5PRu5P	Yes	21.7	5.6	3.4	C-C bond breakage
16	G3P->Pyr	G3P	Pyr		10.5	53.3	0.9	complex multistep
17	G6P->Glu	G6P	Glu		inf	inf	2.0	dephosphorylation
18	G6P->Pyr	G6P	Pyr	Yes	3.6	6.4	2.4	complex multistep
19	PEP->Pyr	PEP	Pyr		178.6	266.8	1.1	dephosphorylation
20	R5P->E4P	R5P	E4P	Yes	69.0	6.2	9.5	C-C bond breakage
21	R5P->Pyr	R5P	Pyr	Yes	2.0	2.2	2.4	complex multistep
22	R5P->X5PRu5P	R5P	X5PRu5P		inf	10.7	0.8	isomerisation
23	Ru5P->Pyr	Ru5P	Pyr	Yes	1.6	40.0	3.3	complex multistep
24	Ru5P->R5P	Ru5P	R5P		54.2	10.5	0.9	isomerisation
25	S7P->Pyr	S7P	Pyr		2.4	91.7	1.0	complex multistep
26	S7P->R5P	S7P	R5P		inf	inf	0.8	C-C bond breakage

Supplementary Table 9: **Individual reaction rates of non-enzymatic interconversions between sugar phosphates in the presence and absence of Fe(II)**

See Supplemental Table_all_rates.csv

Supplementary Text 1: Comparison of the reactions found here and in previous work [Keller *et al.* (*19*)] in which sediment-like complex metal mixtures were studied

When comparing the observed reactions in this study with our previous results (*19*), it is important to take several important experimental differences into account: (i) The reactivity we report here is purely based on the action of the Fe(II) component whereas a more complex Archean ocean reconstruction that oriented on early sediments were tested earlier. (ii) Another difference is that in this study the experimental evaluation under absence of Fe(II) (water conditions) was performed in an anoxic environment. (iii) Furthermore, instead of a slightly acidic environment (see above) a broad range of different pH conditions using a phosphate buffer system were tested.



Supplementary Figure 9: **Comparison of Fe(II)- and pH-facilitated non-enzymatic reactivity with the reaction network structure facilitated by a mixture of prebiotically plausible ocean metals [Keller *et al.* (*19*)]**

Despite this differences most of the reactions (23) of the Archean ocean simulation experiments could be reproduced with Fe(II) alone (see supplementary Figure 8), thereby verifying that Fe(II) is the main contributors to shape the observed non-enzymatic reaction space. Furthermore, we describe three new reactions (F6P-> R5P, F6P ->Ru5P/X5P and F16BP->F6P). Only 4 reactions could not be observed (details see below) in the pure Fe(II) milieu and two reactions could not be followed because their substrate was no longer commercially available in the required amount and purity (X5P). Also when comparing the water conditions most of the expected reactions were observed (13/15). Additionally the pH series revealed 12 new reactions that are not been observed in unbuffered aqueous solution. Those reactions mainly involve interconversions between sugar phosphate constituents of the PPP. Two reactions were not observed under these conditions and two reactions with X5P as substrate were not tested.

Interestingly, most (4/6) of the reaction that did not appear in the Fe(II) and phosphate containing conditions compared to the full Archean ocean simulation are G3P forming reactions. Possible reasons are that iron/phosphate alone prevent stabilization of this intermediate and prevent higher G3P concentrations from accumulating. Alternatively G3P producing reactions could be dependent on metals other than iron.

A comparison of the rates derived from samples with controlled pH with the unbuffered situation (Fig 3A, boxplots, n.b. = no buffer) in many cases replicates the respective rates at pH 4-5 in case of iron containing conditions and at pH 7 with water. Thus, we can exclude a general strong influence of the phosphate buffer on the observed reactivity. However, some individual reactions could still be affected.