SUPPLEMENTARY MATERIAL

An experimental charge density of HEPES

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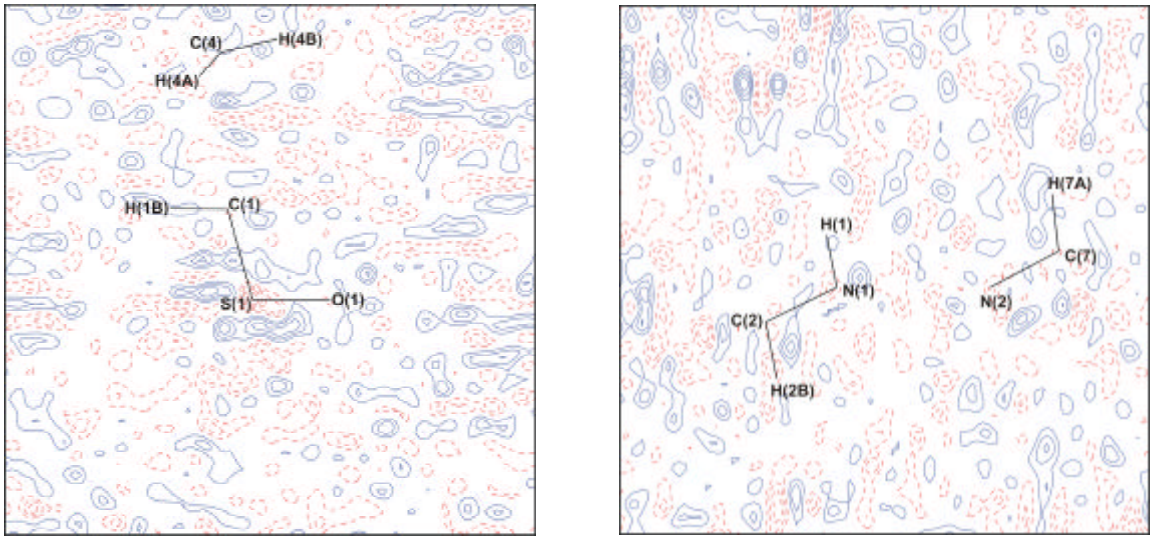
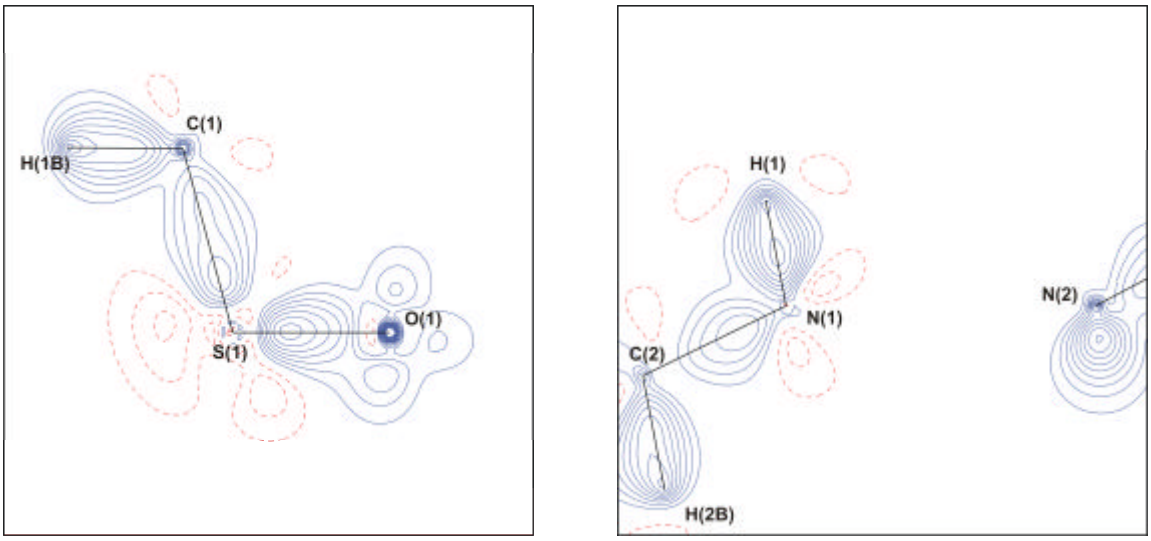


(*a*) (*b*)

Figure 1S
Residual density maps: (*a*) O(1)S(1)C(1) plane; (*b*) N(1)N(2)H(1) plane. Blue solid line – positive values, red dashed lines – negative. Contours at ± n·0.05 e·Å^{-3} (n = 1, 2, …).



(*a*) (*b*)

Figure 2S
Static deformation density maps: (*a*) O(1)S(1)C(1) plane; (*b*) N(1)N(2)H(1) plane. Blue solid line – positive values, red dashed lines – negative. Contours at ± n·0.1 e·Å^{-3} (n = 1, 2, …).

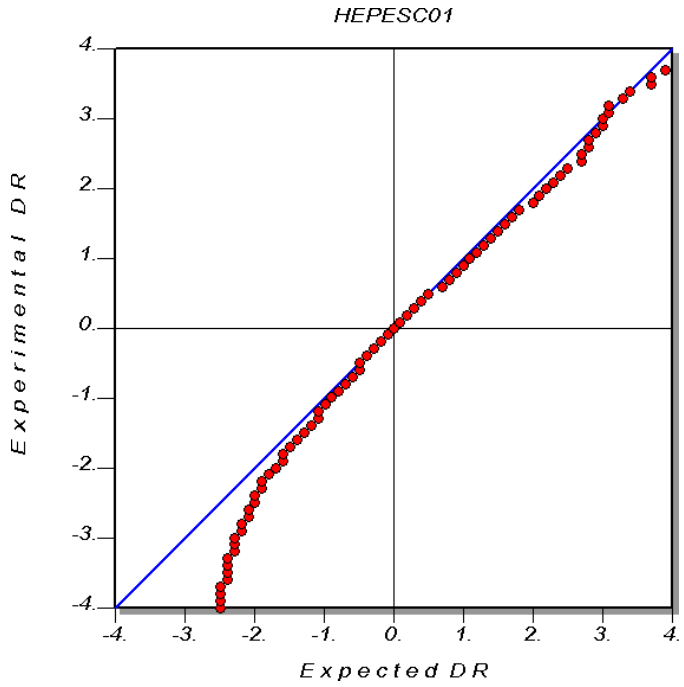


Figure 3S
Normal probability plot for final multipole refinement of HEPES.

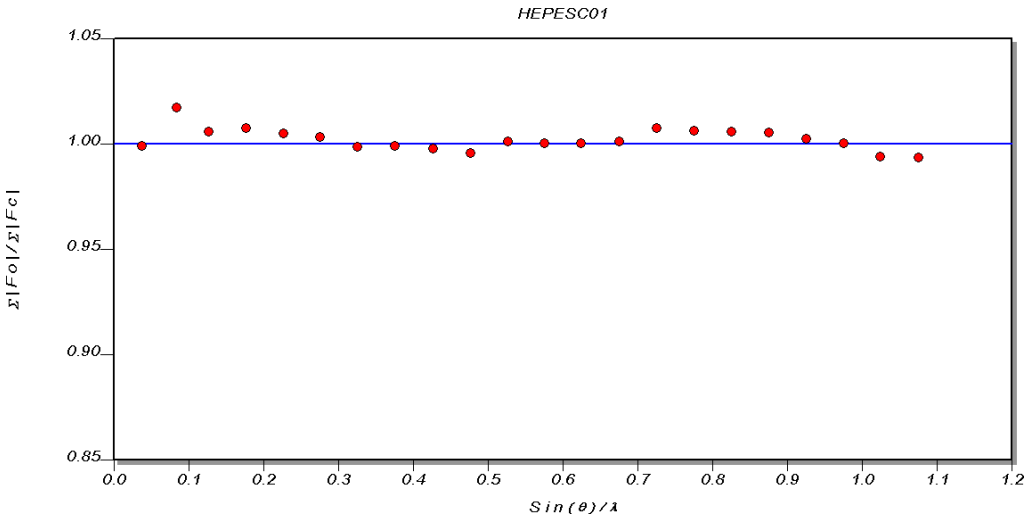


Figure 4S
Scale plot for final multipole refinement of HEPES.

Table 1S
Minima and maxima of residual density.

Highest peaks	*Height / e·Å*$^{-3}$	*Remarks*
1	0.25	Random position
2	0.24	Random position
3	0.24	0.99 Å from S(1)
4	0.23	Random position
5	0.23	0.75 Å from C(7)
6	0.23	Random position
7	0.23	0.56 Å from S(1)
8	0.22	0.66 Å from C(2)
9	0.21	0.74 Å from C(1)
10	0.21	Random position
Deepest holes	*Height / e·Å*$^{-3}$	*Remarks*
1	–0.31	0.04 Å from S(1)
2	–0.30	Random position
3	–0.26	Random position
4	–0.24	Random position
5	–0.24	Random position
6	–0.23	Random position
7	–0.22	Random position
8	–0.21	Random position
9	–0.20	Random position
10	–0.20	Random position

Table 2S
Selected topological parameters ($\boldsymbol{r}_{BCP}$) and $L(\boldsymbol{r}_{BCP})$] at BCPs for HEPES (*?* – electron density, L – negative Laplacian, d_1 – distance from the first atom to BCP, d_2 – distace from BCP to the second atom).

Bond	$?(\boldsymbol{r}_{BCP})$ / e·Å^{-3}	$L(\boldsymbol{r}_{BCP})$ / e·Å^{-5}	d_1 / Å	d_2 / Å
S(1)–O(1)	2.41(4)	22.4(2)	0.6543	0.8187
S(1)–O(2)	2.47(4)	20.4(2)	0.6357	0.8137
S(1)–O(3)	2.29(4)	6.6(2)	0.6135	0.8394
S(1)–C(1)	1.44(3)	7.1(8)	0.9663	0.8175
C(1)–C(2)	1.70(4)	11.1(1)	0.7347	0.7854
C(2)–N(1)	1.71(4)	11.7(2)	0.6419	0.8567
N(1)–C(3)	1.74(4)	10.4(2)	0.8337	0.6604
N(1)–C(4)	1.71(4)	12.5(2)	0.8637	0.6287
C(3)–C(5)	1.76(4)	13.5(2)	0.7693	0.7465
C(5)–N(2)	1.88(4)	13.1(2)	0.6712	0.7968
C(4)–C(6)	1.82(4)	15.2(1)	0.7535	0.7600
C(6)–N(2)	1.86(4)	11.8(2)	0.6602	0.8090
N(2)–C(7)	1.86(4)	13.6(2)	0.8131	0.6575
C(7)–C(8)	1.79(4)	13.7(1)	0.7758	0.7457
C(8)–O(4)	1.87(5)	17.2(2)	0.5830	0.8338
C(2)–H(2A)	1.91(7)	21.5(3)	0.7276	0.3645
C(2)–H(2B)	1.92(7)	23.1(3)	0.7368	0.3571
C(3)–H(3A)	2.01(7)	26.4(3)	0.7197	0.3732
C(3)–H(3B)	1.96(7)	22.7(3)	0.7325	0.3596
C(4)–H(4A)	1.95(8)	21.7(3)	0.7242	0.3680
C(4)–H(4B)	2.00(7)	24.2(3)	0.7153	0.3772
C(5)–H(5A)	1.94(7)	24.5(3)	0.7264	0.3658
C(5)–H(5B)	1.95(7)	22.1(3)	0.7134	0.3790
C(6)–H(6A)	2.01(7)	24.9(3)	0.6995	0.3926
C(6)–H(6B)	1.94(7)	21.3(3)	0.6886	0.4045
C(7)–H(7A)	1.87(7)	19.8(3)	0.7173	0.3750
C(7)–H(7B)	1.92(7)	21.1(4)	0.7423	0.3498
C(8)–H(8A)	1.90(8)	20.0(3)	0.7213	0.3709
C(8)–H(8B)	1.90(8)	21.2(3)	0.7240	0.3690
N(1)–H(1)	2.36(8)	41.8(6)	0.7650	0.2442
O(4)–H(2)	2.43(8)	49.6(7)	0.7542	0.2132

Table 3S
Integrated atomic charges (Q). I stands for integrated Laplacian.

Atom	Q / e	I / e·Å^{-2}
S(1)	+2.437	$6.84\cdot10^{-3}$
O(1)	–1.258	$4.19\cdot10^{-4}$
O(2)	–1.265	$3.01\cdot10^{-4}$
O(3)	–1.211	$2.24\cdot10^{-4}$
O(4)	–1.030	$6.10\cdot10^{-5}$
N(1)	–0.968	$-1.20\cdot10^{-3}$
N(2)	–0.882	$3.40\cdot10^{-4}$
C(1)	–0.188	$4.49\cdot10^{-3}$
C(2)	+0.077	$4.19\cdot10^{-3}$
C(3)	+0.019	$2.99\cdot10^{-3}$
C(4)	+0.100	$3.48\cdot10^{-3}$
C(5)	+0.127	$5.57\cdot10^{-4}$

C(6)	+0.167	$-6.22\cdot10^{-4}$
C(7)	+0.051	$4.34\cdot10^{-3}$
C(8)	+0.292	$4.22\cdot10^{-3}$
H(1A)	+0.138	$2.93\cdot10^{-4}$
H(1B)	+0.182	$2.71\cdot10^{-4}$
H(2A)	+0.180	$1.11\cdot10^{-4}$
H(2B)	+0.203	$1.06\cdot10^{-4}$
H(3A)	+0.177	$2.45\cdot10^{-5}$
H(3B)	+0.204	$1.39\cdot10^{-4}$
H(4A)	+0.174	$1.62\cdot10^{-4}$
H(4B)	+0.167	$3.24\cdot10^{-4}$
H(5A)	+0.183	$9.02\cdot10^{-5}$
H(5B)	+0.120	$1.87\cdot10^{-4}$
H(6A)	+0.178	$5.19\cdot10^{-5}$
H(6B)	+0.032	$2.39\cdot10^{-4}$
H(7A)	+0.102	$-2.16\cdot10^{-6}$
H(7B)	+0.152	$8.05\cdot10^{-5}$
H(8A)	+0.079	$1.48\cdot10^{-4}$
H(8B)	+0.157	$2.33\cdot10^{-4}$
H(1)	+0.500	$-3.38\cdot10^{-4}$
H(2)	+0.626	$4.06\cdot10^{-4}$
SUM	0.024	

Table 4S
Cartesian coordinates for HEPES used in theoretical calculations.

Atom	*x* / Å	*y* / Å	*z* / Å
S(1)	3.74316	2.13236	28.52161
O(1)	2.55658	1.25982	28.51867
O(2)	4.97018	1.37349	28.65762
O(3)	3.60118	3.23555	29.45151
O(4)	6.05299	1.57201	19.35015
N(1)	4.42575	2.36675	24.54540
N(2)	4.35401	2.87010	21.68520
C(1)	3.78652	2.87077	26.89896
C(2)	3.89179	1.80021	25.82492
C(3)	4.66210	1.28549	23.54208
C(4)	3.54076	3.41192	23.95294
C(5)	5.25272	1.87083	22.27548
C(6)	4.14154	3.94009	22.66929
C(7)	4.90494	3.45850	20.45660
C(8)	4.98966	2.50475	19.27411
H(1A)	2.88299	3.47564	26.79812
H(1B)	4.67474	3.50590	26.88883
H(2A)	4.58552	1.01268	26.12662
H(2B)	2.92554	1.36351	25.56385
H(3A)	3.71935	0.76975	23.34795
H(3B)	5.35084	0.55953	23.97930
H(4A)	3.46288	4.22996	24.67214
H(4B)	2.57061	2.95147	23.75502

H(5A)	6.23021	2.29953	22.50611
H(5B)	5.44499	1.06627	21.56254
H(6A)	3.45777	4.68715	22.26089
H(6B)	5.10233	4.39273	22.92312
H(7A)	5.88912	3.89796	20.63191
H(7B)	4.24838	4.28613	20.18008
H(8A)	4.05262	1.94967	19.19459
H(8B)	5.07027	3.11765	18.37396
H(1)	5.31772	2.77101	24.78822
H(2)	6.86094	2.08511	24.78822