Supporting Information

Origins of Femtomolar Protein-Ligand Binding: Hydrogen Bond Cooperativity and Desolvation Energetics in the Biotin-(Strept)Avidin Binding-Site

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Computational Details

DFT and *ab inito*. All calculations were carried out using the Gaussian 03 suite of programs. The hybrid density functional Becke3LYP[1] with the 6-31+G(d,p) basis set was used to optimize all reported structures. MPWB1K, developed by Truhlar and co-workers,[2] based on the modified Perdew and Wang 1991 functional (MPW)[3] and Becke's 1995 meta correlation functional[4] was used for select structures (noted below). MP2 single point energies[5] were carried out on each DFT optimized structure using the 6-31+G(d,p) basis set. Correction for basis set superposition error (BSSE) was performed using the coutnerpoise method of Boys and Bernardi at MP2/6-31+G(d,p) level.[6]

CPCM-SCRF. All calculations utilized the UAKS cavity size at the HF/6-31+G(p,d) level.[7] Single point calculations were carried out on the biotin-residue or biotin-water complexes stationary points for calculation of solvation energies. Free energies of solvation energy were then added to MP2/6-31+G(d,p) electronic energies. A dielectric constant of 4.33 and 78.39 was used to simulate a hydrophobic protein environment and water, respectively.

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QM/MM/MC. All QM/MM were carried out using BOSS 4.6.[8] The solute was treated with PDDG/PM3 method.[9] The solvent molecules were represented by the TIP4P water model.[10] The Metropolis Monte Carlo (MC) simulations were performed in a periodic box of 750 (minus the number of non-hydrogen atoms of the solute) TIP4P water molecules at 25 ºC and 1 atm in the NPT ensemble.[11] Each simulation consisted 3.2M configurations of equilibration and 20 million configurations of averaging. Partial charges were obtained from the CM3 charge model.[12]

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MD. Preparation of the systems, starting from the apo-protein crystal structures, included addition of hydrogen atoms and a cubic box of TIP3P[13] water molecules extending 10Å from the solute using the xLEaP module of AMBER 8.[14] The Duan et al. (ff03) force field[15] was employed to describe the proteins and general Amber force field (gaff) for biotin.[16] Long-range electrostatic forces were treated using the particle mesh Ewald (PME) method[17] using default parameters assigned by AMBER. Trajectories were integrated with a 1 fs time step. All simulations were carried out under constant temperature (300 K) and pressure (1 atm). The avidin system was equilibrated for 70 ps and the streptavidin for 133 ps. The SHAKE algorithm[18] for all hydrogens and a nonbonding pair cutoff of 9 Å was used. Average structures were generated using the PTRAJ utility for the unligaded avidin over 60–70 ps and for unligaded streptavidin over 123–133 ps.

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Gaussian 03 Citation

Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

Amber 8 Citation

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B3LYP Geometries and ab inito and CPCM energies, and BSSE corrections

Cartesian coordinates and distances are reported in Ångstroms. MP2 electronic energies and CPCM solvation energies are reported in Hartrees. BSSE corrections are reported in Hartrees.

Streptavidin Model:

C	-2.130176	-2.096306	0.098852
C	-0.898451	-2.926648	0.621388
N	0.120002	-1.902498	0.794044
C	-0.371894	-0.651744	0.541706
N	-1.632597	-0.728343	0.105480
O	0.298940	0.416763	0.709791
H	-2.271664	0.097861	-0.104385
C	-0.522591	-3.991893	-0.429690
H	-1.062577	-4.925720	-0.231239
H	0.550370	-4.198576	-0.426644
C	-2.529448	-2.600667	-1.301316
H	-3.302716	-3.371450	-1.217251
H	-2.926520	-1.786727	-1.907321
S	-1.018633	-3.319190	-2.064293
H	-3.003406	-2.171817	0.754359
H	-1.114669	-3.419502	1.578971
H	0.968251	-2.036131	1.346648
H	-0.509815	2.281232	0.156299
N	-1.118847	3.054542	-0.094973
H	-2.091382	2.807032	-0.296445
C	-0.645247	4.309742	-0.166300
O	-1.284895	5.320346	-0.461932
H	0.435376	4.371329	0.078933
H	1.926288	0.371130	0.598108
H	0.306803	0.908912	2.543655
O	2.925739	0.281313	0.637365
O	0.316733	1.051629	3.509407
C	-0.789624	1.873556	3.858043
H	-0.766776	2.003653	4.944688
H	-0.733680	2.864514	3.387093
H	-1.749009	1.414852	3.581084
C	3.526682	0.573567	-0.549258
C	4.924548	0.484281	-0.618624
C	2.795942	0.959386	-1.683314
C	5.582991	0.779081	-1.812961
H	5.475543	0.187230	0.268728
C	3.468518	1.253073	-2.871261
H	1.713603	1.024099	-1.628787
C	4.862034	1.165957	-2.947962
H	6.667009	0.707304	-1.854971
H	2.892146	1.551261	-3.742860
H	5.377480	1.396125	-3.875836
O	2.700310	-1.998957	2.258918
H	3.005247	-1.240894	1.722084
C	2.701755	-1.615271	3.636686
H	3.720843	-1.400198	3.987365
H	2.062497	-0.743578	3.819993

```
 H     2.315103    -2.465590     4.205961
 O    -3.317976     1.290762    -0.403914
 C    -4.488428     0.835983    -0.671990
 O    -4.802313    -0.374812    -0.712658
 C    -5.558462     1.898222    -0.957144
 H    -5.671547     2.550443    -0.083691
 H    -5.238033     2.534009    -1.790013
 H    -6.517747     1.432063    -1.194645
E(MP2)=-1711.4935853
CPCM = -1707.34781
```

Avidin Model:

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.399078
C	1.200561	0.000000	2.110095
C	2.419679	0.001589	1.419337
C	2.429964	0.004284	0.017552
C	1.220591	0.001412	-0.681296
O	3.569717	-0.003787	2.156464
O	5.905465	0.062362	0.842297
C	6.481360	1.180539	0.698840
N	6.281174	2.262855	1.498585
C	7.130613	3.384572	1.131824
C	7.882663	2.841314	-0.140672
N	7.383131	1.480264	-0.254749
C	6.343174	4.653847	0.748121
S	5.901680	4.488723	-1.025387
C	7.540649	3.728116	-1.357250
O	4.754742	1.813683	3.931653
C	5.398576	1.138015	5.017822
O	7.141673	-1.274893	3.078164
C	7.804320	-2.485382	2.737928
O	8.427372	-0.013722	-2.316467
C	9.686492	-0.004515	-2.383570
N	10.426475	-0.257865	-3.440766
N	6.176516	-1.852879	-1.553538
C	5.382655	-2.912416	-1.809858
O	5.443511	-3.644263	-2.794176
H	7.716809	0.823511	-0.990200
H	6.964104	5.544627	0.898598
H	5.432427	4.755488	1.342728
H	8.296232	4.511521	-1.481216
H	7.491867	3.139786	-2.275846
H	8.969950	2.833776	0.002553
H	7.828702	3.619254	1.945694
H	5.786646	2.187540	2.392879
H	6.050500	-1.295164	-0.713556
H	6.915750	-1.569572	-2.191327
H	4.628636	-3.078118	-1.013960
H	4.387350	-0.006849	1.588424
H	6.714321	-0.913722	2.280081
H	8.320377	-2.836146	3.636548
H	7.100200	-3.266133	2.416252
H	8.550098	-2.339839	1.944252
H	1.211269	-0.005583	3.195843
H	3.375664	0.008818	-0.515915
H	-0.940824	-0.002370	1.943389
H	1.239304	-0.000024	-1.767559
H	-0.936185	-0.003077	-0.550169
H	4.161101	1.184563	3.475833
H	4.668840	0.838027	5.781855
H	5.954873	0.257565	4.676155
H	6.095903	1.849592	5.468620
H	9.967337	-0.469977	-4.318913
H	11.511192	-0.154863	-3.431942
H	10.279925	0.241213	-1.485772

```
 O    12.806210    -0.101173    -5.627811
 C    13.479037     0.054524    -4.585761
 O    13.023746     0.053396    -3.384753
 C    14.994714     0.292991    -4.708555
 H    15.212428     1.345012    -4.484698
 H    15.536373    -0.313219    -3.974211
 H    15.344683     0.065059    -5.718965

E(MP2) = -1880.93654
CPCM = -1876.307892
```

Avidin Model with Asp model removed:

C	-4.144421	-2.557362	-2.884832
C	-5.037077	-1.986658	-1.971046
C	-4.567129	-1.261244	-0.875886
C	-3.188857	-1.100108	-0.689139
C	-2.286221	-1.663811	-1.600452
C	-2.770706	-2.390801	-2.691156
O	-2.772346	-0.384688	0.401580
O	-0.103719	-0.146533	0.832578
C	0.445256	0.955777	0.566408
N	-0.172049	2.159815	0.541844
C	0.729050	3.252422	0.212013
C	2.110523	2.517662	0.025132
N	1.759635	1.127966	0.272447
C	0.362341	3.977642	-1.100352
S	1.158848	3.043838	-2.464938
C	2.631070	2.780048	-1.402917
O	-2.927192	2.278314	1.288903
C	-3.264405	2.380415	2.679032
O	-0.849124	-0.059343	3.651992
C	-0.949968	-1.341480	4.255866
O	3.954516	-0.928063	-0.042784
C	4.785096	-1.835978	-0.159317
N	6.099653	-1.620132	-0.369577
N	1.528143	-2.685099	0.444308
C	1.806680	-4.002280	0.446038
O	2.914533	-4.483861	0.203027
H	2.426541	0.366634	0.227090
H	0.738380	5.006658	-1.083523
H	-0.718309	4.002273	-1.256486
H	3.266445	3.672910	-1.414364
H	3.209758	1.934607	-1.781817
H	2.854073	2.858484	0.756030
H	0.764617	3.977354	1.034082
H	-1.151376	2.280440	0.832689
H	0.619748	-2.304160	0.671825
H	2.269940	-2.018618	0.242174
H	0.938804	-4.641517	0.687316
H	-1.785561	-0.358775	0.479814
H	-0.577138	-0.167318	2.723766
H	-1.249853	-1.187367	5.295950
H	-1.708827	-1.969529	3.767619
H	0.010007	-1.877392	4.250969
H	-5.252027	-0.819876	-0.158467
H	-1.217933	-1.528009	-1.458396
H	-6.107952	-2.107582	-2.109072
H	-2.064691	-2.824791	-3.393728
H	-4.514637	-3.122101	-3.734812
H	-3.141448	1.378067	0.975747
H	-4.342848	2.246527	2.830214
H	-2.710789	1.649011	3.278844
H	-2.994158	3.389812	2.999568
H	6.454728	-0.676006	-0.434164
H	6.741452	-2.393654	-0.455042
H	4.493071	-2.897425	-0.096700

E(MP2) = -1652.968828 CPCM = -1648.96215 (displacement convergence criteria not meet)

Ureido (-NH) hydrogen bonded to Acetate:

```
C     0.441235    -0.430554     0.267043
 H    -0.366854    -0.861160     0.869905
 C     1.043201    -1.544047    -0.623458
 H     0.595360    -1.526362    -1.618543
 H     0.872845    -2.528490    -0.174788
 S     2.856979    -1.246825    -0.770941
 C     2.887961    -0.568864     0.935916
 H     2.947093    -1.389752     1.661434
 H     3.768128     0.068899     1.053602
 C     1.579875     0.220978     1.128638
 H     1.314941     0.219994     2.197028
 N     1.639435     1.573638     0.596436
 H     1.944742     2.333977     1.187036
 C     0.505973     1.866849    -0.191900
 O     0.198588     3.006653    -0.537063
 N    -0.107302     0.686959    -0.485383
 H    -1.120073     0.622301    -0.744845
 O    -2.826604     0.248838    -0.910584
 C    -3.247000    -0.447686     0.066792
 O    -2.557477    -1.003545     0.963271
 C    -4.782786    -0.596205     0.159883
 H    -5.207415     0.345464     0.530661
 H    -5.210366    -0.776856    -0.832347
 H    -5.061795    -1.401385     0.846074
E(MP2) = -1004.601169
CPCM = -1002.681948
BSSE =  0.005925955024
```

Ureido (-NH) hydrogen bonded to methanol:

```
 C     0.000000     0.000000     0.000000
 H     0.000000     0.000000     1.098978
 H     1.034348     0.000000    -0.363643
 H    -0.497642     0.911661    -0.343290
 O    -0.747976    -1.104437    -0.516028
 H    -0.302045    -1.934401    -0.254818
 H    -0.646460    -1.467111    -2.406424
 N    -0.463417    -1.772977    -3.360643
 C    -1.290906    -1.490129    -4.514734
 H    -1.224351    -0.428686    -4.786587
 C     0.467493    -2.760123    -3.567683
 O     1.266515    -3.196595    -2.744070
 N     0.335591    -3.168598    -4.883704
 C    -0.645946    -2.401661    -5.630642
 H    -0.167210    -1.794644    -6.411050
 H     1.091169    -3.673364    -5.320059
 C    -1.751892    -3.272247    -6.259105
 S    -2.979315    -3.597495    -4.936666
 C    -2.782359    -1.868388    -4.351292
 H    -3.103670    -1.799483    -3.309528
 H    -3.409281    -1.201116    -4.953442
 H    -2.228606    -2.741565    -7.091547
 H    -1.357791    -4.221338    -6.630054
E(MP2) = -892.0576987
CPCM = -890.399068
BSSE = 0.003614347540
```

Ureido (O=) hydrogen bonded to methanol:

```
 H     0.000000     0.000000     0.000000
 C     0.000000     0.000000     1.098590
 H     1.038076     0.000000     1.445925
 H    -0.483557     0.924920     1.442467
 O    -0.622845    -1.160350     1.634138
 H    -1.558210    -1.169192     1.354458
 O    -3.390354    -1.339152     0.967380
 C    -4.117207    -2.211693     1.438211
 N    -4.214720    -3.515833     1.008460
 H    -3.488257    -3.882412     0.412235
 N    -5.002152    -2.065742     2.478814
 H    -4.972225    -1.228866     3.040023
 C    -5.593369    -3.316186     2.925374
 H    -5.270083    -3.555404     3.946507
 C    -5.016479    -4.355261     1.886225
 H    -4.390706    -5.107257     2.383610
 C    -6.191554    -5.051415     1.170584
 C    -7.134547    -3.340428     2.855920
 H    -7.563165    -2.356670     3.059999
 H    -7.534141    -4.054036     3.585162
 H    -6.479238    -5.960973     1.709974
 H    -5.934870    -5.321375     0.143782
 S    -7.588351    -3.862448     1.156832
E(MP2) = -892.0562451
CPCM = -890.403787
BSSE =  0.002720705633
```

Ureido (O=) hydrogen bonded to formamide

```
 O     0.000000     0.000000     0.000000
 C     0.000000     0.000000     1.232012
 H     0.952615     0.000000     1.801395
 N    -1.078680     0.002317     2.032876
 H    -2.040352    -0.005849     1.682517
 H    -0.977314    -0.001005     3.043725
 O    -0.776124    -0.487682     4.910529
 C    -0.736836    -1.689876     5.174890
 N    -0.906860    -2.732708     4.296960
 H    -0.948856    -2.559734     3.303760
 N    -0.554509    -2.243687     6.418305
 H    -0.273024    -1.657349     7.188533
 C    -0.411792    -3.690630     6.402155
 C    -0.675486    -4.041667     4.885728
 H    -1.563035    -4.675716     4.772701
 H    -1.160668    -4.156489     7.053651
 C     0.559653    -4.776712     4.323395
 H     0.705703    -4.579764     3.258521
 H     0.449372    -5.858121     4.461144
 S     2.012755    -4.171610     5.265023
 C     0.995266    -4.193757     6.790854
 H     0.934069    -5.214738     7.183979
 H     1.461777    -3.561780     7.550699
E(MP2) = -946.1011164
CPCM = -944.301694
BSSE =  0.002968673615
```

Ureido (O=) hydrogen bonded to phenol:

```
 C     0.000000     0.000000     0.000000
 C     0.000000     0.000000     1.398340
 C     1.222639     0.000000    -0.680317
 H     1.240703     0.001068    -1.767369
 H    -0.936410     0.000621    -0.550224
 H    -0.940012    -0.000673     1.943487
 C     2.427727     0.000964     0.023006
 H     3.381631     0.005172    -0.495912
 C     1.198157    -0.000753     2.115451
 H     1.194199    -0.004970     3.201073
 C     2.419895     0.000693     1.425407
 O     3.618931     0.006448     2.071164
 H     3.520853     0.004785     3.070616
 O     3.495579    -0.152480     4.767361
 C     4.061579    -1.201234     5.099444
 N     5.047991    -1.847533     4.396427
 H     5.173747    -1.633005     3.415728
 N     3.859586    -1.899731     6.256493
 C     4.698822    -3.079569     6.385852
 C     5.435588    -3.118059     4.991393
 H     3.278687    -1.523942     6.989090
 H     5.411633    -2.961829     7.212002
 H     6.523125    -3.155949     5.119254
 C     4.971388    -4.369534     4.207906
 H     4.867343    -4.165049     3.139651
 H     5.696755    -5.180312     4.335028
 S     3.342729    -4.878970     4.886449
 C     3.902806    -4.385827     6.560217
 H     3.035884    -4.252982     7.212301
 H     4.544079    -5.162926     6.991609
E(MP2) = -1083.23178
CPCM = -1080.933243
BSSE = 0.004987026562
```

Ureido (-NH) hydrogen bonded to formamide-acetate:

```
 H    -8.204637    -1.104451     0.561382
 C    -7.738168    -0.145784     0.318386
 H    -8.165209     0.252531    -0.609219
 H    -7.960233     0.578320     1.111783
 C    -6.209610    -0.292805     0.172370
 O    -5.596056     0.762355    -0.208406
 O    -5.689206    -1.402973     0.437303
 H    -2.871560    -0.915620    -0.010643
 N    -3.005596     0.055484    -0.264902
 H    -4.012284     0.399038    -0.269107
 H    -2.209709     1.851959    -0.783712
 C    -1.949007     0.809760    -0.527939
 O    -0.752914     0.447030    -0.509217
 H     1.010763     1.158316    -0.435441
 N     1.961238     0.941555    -0.127872
 C     2.971748     1.852249    -0.037890
 O     2.988589     3.014426    -0.432268
 N     4.046470     1.208378     0.592188
 C     3.660567    -0.068188     1.173517
 C     2.209255    -0.284112     0.602315
 H     4.710661     1.809662     1.056457
 H     3.649878    -0.019129     2.271959
 H     1.468901    -0.403323     1.402323
 C     4.555699    -1.241252     0.726484
 S     3.898619    -1.828117    -0.883986
 C     2.184950    -1.552549    -0.277590
 H     1.503692    -1.425435    -1.120138
 H     1.859682    -2.418668     0.309005
 H     4.515828    -2.052613     1.463663
 H     5.595621    -0.929248     0.602351

MP2 = -1174.059644
CPCM = -1171.648135
BSSE = 0.003232258776
```

Ureido (-NH) hydrogen bonded to formamide:

```
 N     0.000000     0.000000     0.000000
 H     0.000000     0.000000     1.008735
 H     0.882593     0.000000    -0.492598
 C    -1.159498     0.012358    -0.688668
 H    -2.069396     0.018180    -0.065835
 O    -1.219982     0.017068    -1.923201
 H    -3.122810    -0.033015    -2.357613
 N    -4.026243    -0.116877    -2.818528
 C    -4.216057    -0.055333    -4.250426
 H    -3.690476     0.810648    -4.675151
 C    -3.766593    -1.330046    -4.999968
 H    -2.923089    -1.807181    -4.496741
 H    -3.472760    -1.087319    -6.027219
 S    -5.177288    -2.507335    -5.014051
 C    -6.343385    -1.115768    -5.270463
 H    -7.348710    -1.425649    -4.976905
 H    -6.357298    -0.833336    -6.329834
 C    -5.781566     0.105781    -4.341360
 H    -6.052854     1.064345    -4.804100
 N    -6.182566     0.061836    -2.942824
 H    -7.050989     0.459211    -2.619003
 C    -5.179537     0.072966    -2.103674
 O    -5.277805     0.213236    -0.887583
E(MP2) = -946.1018174
CPCM = -944.30176
BSSE =  0.003209254417
```

Ureido conjugate base:

O	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.270458
N	1.119492	0.000000	2.040716
C	0.811504	0.081098	3.462713
C	-0.760225	0.041068	3.479379
N	-1.089805	0.002308	2.055688
C	1.359181	-1.111040	4.273321
S	0.118276	-2.456745	4.138628
C	-1.230707	-1.209754	4.246799
H	1.485159	-0.824787	5.323751
H	2.320116	-1.456598	3.884644
H	-1.428587	-0.949427	5.290076
H	-2.145254	-1.621428	3.815949
H	-1.210193	0.917562	3.953922
H	1.196442	1.018486	3.884081
H	2.044206	0.177251	1.641888

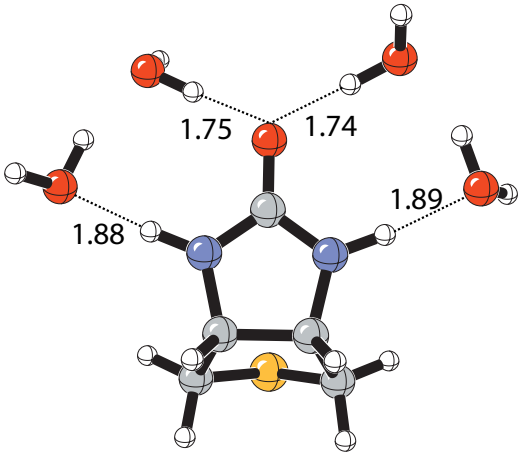
Ureido:

```
 O     0.000000     0.000000     0.000000
 C     0.000000     0.000000     1.270458
 N     1.119492     0.000000     2.040716
 C     0.811504     0.081098     3.462713
 C    -0.760225     0.041068     3.479379
 N    -1.089805     0.002308     2.055688
 C     1.359181    -1.111040     4.273321
 S     0.118276    -2.456745     4.138628
 C    -1.230707    -1.209754     4.246799
 H    -2.054225     0.076631     1.709766
 H     1.485159    -0.824787     5.323751
 H     2.320116    -1.456598     3.884644
 H    -1.428587    -0.949427     5.290076
 H    -2.145254    -1.621428     3.815949
 H    -1.210193     0.917562     3.953922
 H     1.196442     1.018486     3.884081
 H     2.044206     0.177251     1.641888
E(MP2) = -776.6503501
CPCM = -775.344837
```

Ureido hydrogen bonded to four water molecules:

C	0.043048	0.462023	0.619425
O	0.054285	0.227837	1.854306
N	1.139534	0.553680	-0.174931
N	-1.065030	0.684869	-0.132390
C	0.809971	0.796650	-1.569259
C	-0.762062	0.897855	-1.538095
C	-1.352318	-0.182320	-2.469800
S	-0.149718	-1.567606	-2.509447
C	1.225428	-0.352046	-2.512309
H	-2.013467	0.655439	0.250935
H	2.092349	0.407558	0.168883
H	1.268102	1.732437	-1.912708
H	-1.111198	1.886887	-1.859331
H	2.143195	-0.838225	-2.174030
H	1.383465	0.028638	-3.527607
H	-2.316127	-0.545365	-2.106065
H	-1.490501	0.222294	-3.478768
O	2.295282	-0.726690	3.087820
H	2.133583	-1.634401	3.370770
H	1.445663	-0.394330	2.706875
O	-2.196810	0.388076	3.378196
H	-1.338459	0.354382	2.888075
H	-2.108653	1.072609	4.051557
O	-3.690828	0.573606	1.122747
H	-4.309815	-0.161432	1.043109
H	-3.325480	0.529936	2.038616
O	3.758426	-0.063262	0.904495
H	3.399386	-0.381311	1.767750
H	4.474677	0.547219	1.114054

E(MP2) = -1081.6619831
CPCM = -1079.527191



Apostreptavidin model with four bound water molecules

```
 C     0.000000     0.000000     0.000000
 C     4.419417     0.000000     0.000000
 C     6.215850     3.288198     0.000000
 C     3.401465     5.284082     2.043962
 C     3.244726     5.368960     3.432709
 C     3.593660     6.535541     4.100553
 C     4.117869     7.641734     3.427710
 C     4.268429     7.540469     2.033782
 C     3.921909     6.375551     1.336762
 O     3.063147     4.146291     1.377885
 O     5.946935     3.852355    -1.285697
 O     5.517690     0.030145    -0.564923
 N     3.437948     0.832477    -0.392437
 O     0.088232     1.272392     0.117445
 C    -0.765058    -0.760454     1.081351
 O     0.558957    -0.631224    -0.935073
 O     1.258816     0.746029    -3.340474
 O     0.643257     3.075017    -1.687335
 O     3.308697     2.745546    -2.614772
 H     2.961501     2.000427    -3.141572
 H     2.488157     3.103730    -2.216234
 H     0.205703     3.870449    -1.364614
 H     0.453562     2.355067    -1.001063
 H     0.728110     1.537189    -3.157342
 H     1.094385     0.184146    -2.547606
 H    -0.780601    -1.831244     0.867451
 H    -1.789958    -0.379126     1.148224
 H    -0.292634    -0.585591     2.054613
 H     4.170351    -0.686971     0.828460
 H     2.471109     0.690212    -0.120892
 H     3.599634     1.446633    -1.190395
 H     5.184119     3.401362    -1.685732
 H     6.436322     2.216843    -0.067385
 H     5.377335     3.438813     0.690689
 H     7.094047     3.813125     0.390962
 H     2.634531     3.503210     1.957151
 H     2.844982     4.520769     3.984094
 H     3.460596     6.578231     5.179664
 H     4.392071     8.547307     3.958463
 H     4.674656     8.377845     1.471938
 H     4.062211     6.293842     0.263960
E(MP2) = -1048.1240282
CPCM = -1045.056771
```

Apoavidin model with three bound water molecules

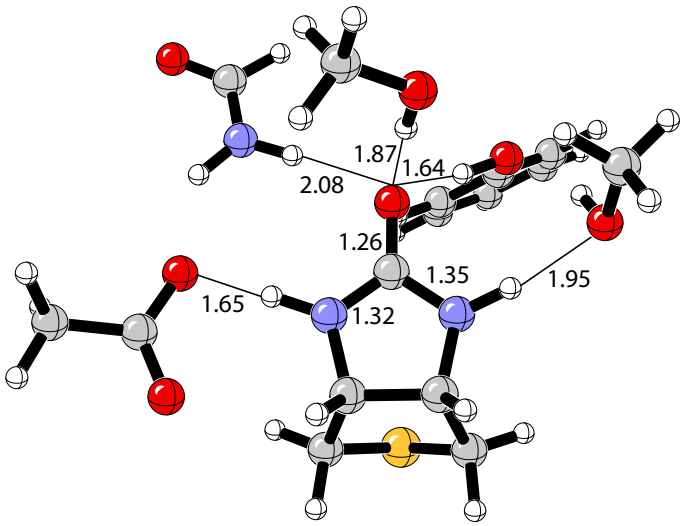
C	0.000000	0.000000	0.000000
C	0.000000	0.000000	4.113332
C	3.306085	0.000000	5.674346
C	4.524906	3.166344	3.505842
C	5.650288	2.607047	4.135608
C	6.637300	3.444582	4.654454
C	6.480752	4.833746	4.602645
C	5.359463	5.385074	3.969756
C	4.381847	4.554907	3.411287
O	4.299238	-0.939237	5.246435
O	-0.301479	1.051400	3.574396
N	0.895621	-0.842564	3.515372
C	-0.889232	1.240584	-0.194635
O	1.229181	-0.018720	-0.231727
N	-0.621319	-1.112009	0.389931
O	-3.224337	-1.471251	0.928150
O	-2.306200	-3.395795	1.676327
C	-3.276158	-2.620794	1.483456
C	-4.667225	-3.097521	1.944775
O	3.583012	2.346296	2.969272
O	3.785024	-0.339574	2.590460
O	3.528284	-1.290663	0.067561
H	2.660079	-0.831782	-0.100784
H	3.364552	-2.232019	-0.059794
H	3.841412	-0.704812	1.670701
H	2.842623	-0.399291	2.829418
H	3.895592	1.424789	2.827694
H	5.748635	1.527224	4.198653
H	7.512693	3.011651	5.131746
H	7.240765	5.484791	5.026260
H	5.244248	6.464044	3.907796
H	3.510583	4.959590	2.906180
H	3.673984	0.478807	6.588639
H	3.111701	0.779647	4.933580
H	2.359824	-0.504729	5.911077
H	4.307421	-0.939445	4.272611
H	0.802621	-0.804688	2.500486
H	0.905678	-1.797337	3.861532
H	-0.347697	-0.301281	5.117120
H	-0.791103	1.573968	-1.232258
H	-0.527920	2.039529	0.458983
H	-1.931649	1.015690	0.035133
H	-0.092406	-1.972857	0.437059
H	-1.660081	-1.199853	0.596781
H	-5.365018	-3.080703	1.098978
H	-5.063918	-2.407396	2.699195
H	-4.614427	-4.106348	2.360946

E(MP2) =
CPCM = -1177.010310

MPWB1K Geometries and ab inito and CPCM energies

Cartesian coordinates and distances are reported in Ångstroms. MP2 electronic energies and CPCM solvation energies are reported in Hartrees.

Streptavidin model:



Frequency analysis gave one small imaginary frequency (-19 cm-1) corresponding to out-of-plane bending motion of the phenol-ureido hydrogen bond.

Streptavidin model:

C	-2.127254	-2.097477	0.102437
C	-0.894174	-2.925026	0.626185
N	0.123103	-1.899247	0.795824
C	-0.370430	-0.649737	0.540678
N	-1.631219	-0.728865	0.105149
O	0.299057	0.420088	0.705914
H	-2.271715	0.096271	-0.104850
C	-0.517690	-3.992354	-0.422560
H	-1.056192	-4.926432	-0.221284
H	0.555562	-4.197518	-0.419895
C	-2.527085	-2.605896	-1.296048
H	-3.299343	-3.377408	-1.209382
H	-2.925678	-1.793989	-1.903780
S	-1.016067	-3.324555	-2.058498
H	-2.999857	-2.172201	0.758857
H	-1.109187	-3.415910	1.585059
H	0.972027	-2.030656	1.348004
H	-0.512023	2.282513	0.150362
N	-1.122898	3.054121	-0.101688
H	-2.095059	2.804192	-0.301975
C	-0.651969	4.310158	-0.176058
O	-1.293959	5.318805	-0.473243
H	0.428728	4.374487	0.068153

H	1.926819	0.376615	0.599033
H	0.304921	0.917618	2.538330
O	2.926365	0.288347	0.639844
O	0.314539	1.062792	3.503720
C	-0.794056	1.882521	3.850510
H	-0.771322	2.015418	4.936815
H	-0.740952	2.872419	3.377034
H	-1.752199	1.420441	3.574897
C	3.528170	0.575443	-0.547650
C	4.926108	0.486211	-0.615479
C	2.798215	0.956105	-1.683941
C	5.585412	0.775887	-1.810596
H	5.476462	0.193260	0.273630
C	3.471652	1.244652	-2.872663
H	1.715831	1.020850	-1.630467
C	4.865245	1.157526	-2.947871
H	6.669480	0.704213	-1.851449
H	2.895898	1.538794	-3.746044
H	5.381380	1.383673	-3.876351
O	2.701083	-1.989724	2.263574
H	3.005446	-1.231706	1.726280
C	2.698489	-1.603968	3.640762
H	3.716122	-1.384726	3.993068
H	2.055880	-0.734196	3.821491
H	2.313828	-2.454835	4.210573
O	-3.320354	1.286756	-0.405398
C	-4.489988	0.829566	-0.673048
O	-4.801812	-0.381797	-0.711975
C	-5.562159	1.889533	-0.958635
H	-5.685503	2.533322	-0.080264
H	-5.237951	2.534168	-1.783117
H	-6.517510	1.421150	-1.207397

E(MP2) = -1711.489104

CPCM = -1707.356822

(Displacement convergence criteria not meet)

imaginary frequency -19.0 cm^{-1}

Ureido (O=) hydrogen bonded to phenol:

H	-4.972462	2.374102	-0.752877
C	-4.371233	1.522377	-0.474850
C	-3.238755	1.194861	-1.202153
H	-2.949742	1.792693	-2.053303
C	-2.460813	0.105500	-0.853741
H	-1.576380	-0.145891	-1.419231
C	-4.721119	0.740081	0.614386
H	-5.601616	0.980386	1.192029
C	-3.953489	-0.352246	0.973540
H	-4.218427	-0.970042	1.817652
C	-2.818884	-0.672751	0.240264
O	-2.097908	-1.741614	0.621774
H	-1.309279	-1.879867	0.043467
O	0.178302	-2.066711	-0.851195
C	1.170661	-1.472588	-0.462585
N	1.453488	-1.129602	0.817716
H	0.822598	-1.367787	1.556920
C	2.742887	-0.520283	1.001814
H	3.383834	-1.141090	1.626793
C	2.653991	0.892427	1.565065
H	1.885695	0.972654	2.327766
H	3.606936	1.186976	2.002105
S	2.252665	1.955598	0.170524
C	3.454437	1.042648	-0.806345
H	4.464255	1.360440	-0.550425
H	3.294411	1.228111	-1.863986
C	3.261834	-0.432039	-0.457951
H	4.197833	-0.958062	-0.620852
N	2.213023	-1.057861	-1.214884
H	2.303877	-1.231280	-2.243572

E(MP2) = -1083.228237

CPCM = -1080.935923

Imaginary frequency -17.7 cm^{-1}

(bending motion of the residue to form an additon hydrogen bond to adjacent –NH group of ureido)

Ureido (O=) hydrogen bonded to formamide:

O	-4.597535	-1.389281	-0.025149
C	-3.687007	-0.654128	-0.359546
H	-2.958144	-0.969883	-1.122093
N	-3.440905	0.565573	0.108216
H	-4.009936	1.010694	0.817744
H	-2.660508	1.100593	-0.235848
O	-0.979238	1.909226	-0.651076
C	-0.022804	1.402779	-0.093632
N	-0.055433	0.488694	0.908501
H	-0.922946	0.086032	1.203279
N	1.283697	1.682381	-0.325860
H	1.538948	2.235949	-1.118118
C	2.193052	0.836136	0.392986
C	1.236350	-0.013938	1.277485
H	1.425302	0.142643	2.338277
H	2.877454	1.429049	0.997684
C	1.436270	-1.482508	0.917314
S	1.881807	-1.507485	-0.825911
C	2.975166	-0.114005	-0.508269
H	3.878296	-0.455331	-0.004743
H	3.263992	0.357805	-1.442610
H	2.238948	-1.913292	1.513756
H	0.536184	-2.066609	1.083021

E(MP2) = -946.0995113
CPCM = -944.305015
Imaginary frequency -36.0 cm^{-1}
(bending motion of the residue to form an additon hydrogen bond to adjacent –NH group of ureido)

Ureido (O=) hydrogen bonded to methanol:

C	5.068966	0.312892	0.122141
H	5.535476	0.053973	-0.829609
H	4.809767	1.372422	0.096554
H	5.799727	0.158750	0.912107
O	3.963503	-0.494882	0.406974
H	3.274623	-0.324777	-0.245831
O	1.690884	0.035727	-1.223156
C	0.697899	0.016932	-0.520598
N	0.062842	1.090554	0.010085
H	0.454583	2.004405	-0.088367
C	-1.002712	0.754109	0.907933
H	-0.791751	1.112030	1.915194
C	-2.365728	1.260839	0.448297
H	-2.291242	2.232886	-0.028923
H	-3.044302	1.342795	1.296056
S	-2.984976	0.043440	-0.722950
C	-2.392460	-1.249330	0.378835
H	-3.065900	-1.356058	1.227981
H	-2.347012	-2.196756	-0.148649
C	-1.012984	-0.801303	0.845617
H	-0.781060	-1.241457	1.815003
N	0.018727	-1.084912	-0.110423
H	0.442754	-1.984343	-0.215852

E(MP2) = -892.0546433
CPCM = -890.405945
Imaginary frequency -16.2 cm^{-1}
(bending motion of the residue to form an additon hydrogen bond to adjacent –NH group of ureido)

Ureido (-NH) hydrogen bonded to methanol:

```
 C     4.011267    -0.387425     0.269266
 H     3.765624     0.672387     0.216210
 H     5.002076    -0.547117    -0.158865
 H     4.042442    -0.684985     1.313553
 H     2.966078    -0.913850    -1.286525
 O     3.044477    -1.188906    -0.365302
 H     1.439253    -0.365650     0.293383
 N     0.663494     0.221290     0.564798
 C     0.470531     1.337484    -0.187291
 O     1.279829     1.851107    -0.930517
 N    -0.788749     1.791090     0.087392
 C    -1.580549     0.839405     0.813427
 C    -0.542885    -0.268535     1.162828
 C    -1.024003    -1.582136     0.555326
 S    -1.918186    -1.136334    -0.941900
 C    -2.689161     0.203882    -0.019549
 H    -3.143899     0.914913    -0.703147
 H    -3.469541    -0.193007     0.628157
 H    -0.194753    -2.240788     0.315639
 H    -1.689152    -2.101558     1.243610
 H    -0.426897    -0.379891     2.240207
 H    -2.002502     1.289380     1.710978
 H    -1.186610     2.491194    -0.505623
E(MP2) = -1004.598532
CPCM = -1002.687172
```

Ureido (-NH) hydrogen bonded to acetate:

C	0.409030	-0.159308	0.721099
H	-0.205385	-0.285183	1.607282
C	0.173856	-1.367617	-0.181053
H	-0.608121	-1.144234	-0.898670
H	-0.139656	-2.225022	0.408638
S	1.740104	-1.714337	-1.014924
C	2.625818	-1.267085	0.487953
H	2.573724	-2.080899	1.211753
H	3.669285	-1.066667	0.260592
C	1.920424	-0.031280	1.030673
H	2.101907	0.058480	2.104543
N	2.284070	1.166257	0.328964
H	3.063075	1.722457	0.618887
C	1.174271	1.862686	-0.130134
O	1.230883	2.977253	-0.617157
N	0.102939	1.086617	0.084913
H	-0.846883	1.225437	-0.278773
O	-2.532159	0.737136	-0.690044
C	-3.097304	-0.066575	0.090923
O	-2.564222	-0.724275	1.002035
C	-4.597253	-0.244475	-0.128799
H	-5.093470	0.719496	-0.034335
H	-4.774774	-0.595755	-1.143589
H	-5.019495	-0.947829	0.582683

E(MP2) = -1004.599106
CPCM = -1002.686586

Apostreptavidin-7H_2O Complex:

C	-6.134529	1.083653	-1.814417
C	-2.894031	-0.550009	2.728760
C	1.251507	-0.581626	2.634561
C	2.001647	2.182227	-0.086817
C	5.889909	-2.035601	1.362847
O	5.832631	-1.062703	0.314854
C	3.292553	1.684246	0.170108
C	4.355347	2.560202	0.371678
C	4.141824	3.940416	0.343391
C	2.856014	4.437538	0.103346
C	1.780402	3.567357	-0.114112
O	0.987772	1.312157	-0.317285
O	1.724891	-1.743191	1.946272
N	-2.576575	-0.490099	1.416409
O	-2.266153	-1.325445	3.459393
C	-5.550831	0.304594	-0.655570
O	-6.304785	-0.209589	0.198155
O	-4.266590	0.173902	-0.629878
O	-1.936854	1.077467	-1.878104
O	-3.373303	-2.366584	-1.276948
O	-0.894379	-1.458650	-2.280525
O	1.314086	-1.283393	-0.862499
O	4.015187	-2.220558	-1.450162
O	6.737679	-2.105899	-2.172480
O	-9.027556	0.093437	0.363670
H	-9.178120	-0.097071	1.296481
H	-8.051211	-0.016978	0.247295
H	-3.598978	-2.937091	-0.532539
H	-3.770974	-1.489101	-1.056977
H	-1.349837	1.396276	-1.176215
H	-2.815531	0.898683	-1.466876
H	-1.654733	-1.980418	-1.949159
H	-1.237326	-0.532552	-2.281380
H	0.487922	-1.431406	-1.418624
H	2.094314	-1.540783	-1.380935
H	4.434597	-1.690894	-0.731616
H	3.730875	-3.045274	-1.033229
H	6.996752	-1.620888	-2.964750
H	5.773535	-2.260132	-2.242362
H	6.494999	-1.277092	-0.368459
H	5.901454	-3.059843	0.966479
H	4.994764	-1.901573	1.974058
H	6.779493	-1.890726	1.988636
H	5.346154	2.156611	0.561306
H	4.968553	4.626671	0.505873
H	2.683210	5.510419	0.079721
H	0.781232	3.941281	-0.314494
H	3.457977	0.612361	0.205902
H	1.279508	0.380209	-0.480865
H	1.455997	-1.679043	1.012157
H	1.631223	-0.644528	3.658825
H	1.621666	0.345751	2.181732
H	0.157362	-0.555705	2.682867
H	-3.705623	0.113770	3.065887

H	-1.903504	-1.177941	1.105960
H	-3.253281	-0.131402	0.728196
H	-5.577781	2.013270	-1.969726
H	-7.192923	1.301042	-1.657100
H	-6.025614	0.489204	-2.729296

Apoavidin-2H_2O Complex:

C	0.000000	0.000000	0.000000
C	0.000000	0.000000	5.742845
C	3.423608	0.000000	4.266140
C	5.594571	2.361263	6.449167
C	5.283385	0.117698	9.834019
C	5.847000	5.200299	10.187795
O	4.533629	5.388004	9.646551
C	6.000454	1.266361	10.199319
C	7.122395	1.162922	11.013635
C	7.559039	-0.079331	11.482193
C	6.815196	-1.220608	11.148814
C	5.690625	-1.132415	10.318735
O	4.214959	0.217934	9.005427
O	4.685972	3.454171	6.640797
N	3.243459	-0.157306	5.602652
O	3.536753	1.126714	3.790055
C	-0.786672	0.007184	1.319056
O	-1.739062	0.810858	1.480727
O	-0.469610	-0.839285	2.232116
N	-0.001585	0.615074	4.564662
O	0.202660	0.657477	6.794743
O	2.422665	2.091932	7.883925
O	2.161077	3.934917	10.017871
H	1.419350	4.544614	10.107005
H	2.975087	4.480637	10.032689
H	2.147340	2.733230	8.569905
H	1.608310	1.632156	7.569919
H	-0.201628	-1.079959	5.732891
H	0.149261	1.613844	4.553995
H	-0.243787	0.105572	3.675518
H	-0.473487	0.652933	-0.737689
H	0.067843	-1.022117	-0.389862
H	1.024698	0.345277	0.184387
H	3.467610	-0.931432	3.678218
H	3.070901	0.659788	6.171173
H	3.040010	-1.057156	6.011317
H	5.399504	1.857044	5.496447
H	6.602458	2.786696	6.423969
H	5.542059	1.629396	7.261878
H	3.798482	3.077651	6.785125
H	3.823959	1.101040	8.858835
H	5.119691	-2.014771	10.047648
H	7.120753	-2.195514	11.519677
H	8.437667	-0.156959	12.115023
H	7.672911	2.062917	11.278079
H	5.666951	2.230735	9.831086
H	4.568492	5.170129	8.698563
H	5.747939	5.191228	11.275386
H	6.508871	6.025432	9.896515
H	6.288709	4.254542	9.860356

S1. Residue Deletion Energies for the Streptavidin Model

Streptavidin Model Residue	MP2 Energy[a]	CPCM Energy[a]	BSSE[b]
Tyr 43	-1404.89149 (-1404.888018)	-1401.75532 (-1401.763007)	0.010601785501
Ser 27	-1596.07986 (-1596.075887)	-1592.29470 (-1592.302265)	0.006194657776
Asn 23	-1542.02426 (-1542.020687)	-1538.38667 (-1538.393814)	0.005580260928
Asp 128	-1596.07265 (-1596.069185)	-1592.29012 (-1592.298713)	0.007347631835
Ser 45	-1483.49764 (-1483.496347)	-1483.49764 (-1480.00271)	0.008415489126
Asp 128 – Asn 23 complex	-397.3705319 (-397.3680946)	-421.962358 (-396.3050523)	0.001761967181
Tyr 43 – Ser 45 complex	-421.96236 (-421.9614592)	-420.64124 (-420.644975)	0.003387867152

[a] Open, using the MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory. CPCM energy using a dielectric of ether (4.33). Parentheses, using the MP2/6-31+G(d,p)//MPWB1K/6-31+G(d,p) level. [b] Counterpoise corrections applied to MP2 and CPCM energies.

S2. Residue Deletion Energies for the Avidin Model

Avidin Model Residue	MP2 Energy[a]	CPCM Energy[a]	BSSE[b]
Tyr 33	-1574.337828	-1570.716716	0.010258098729
Ser 16	-1765.525333	-1761.253985	0.005653311415
Asn 12	-1711.474889	-1707.348392	0.005562748910
Thr 35	-1765.514124	-1761.250144	0.007666781469
Asn 118-Asp 13 Pair	-1483.503307	-1479.998475	0.006362899888
Asn 118-Asp 13 – Asn 12 complex	-566.8365753	-565.260084	0.001436317000
Tyr 33 – Thr 35 complex	-421.9630673	-420.64199	0.003404387665

[a] Open, using the MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory. Parentheses, using the MP2/6-31+G(d,p)//MPWB1K/6-31+G(d,p) level. [b] Counterpoise corrections applied to MP2 and CPCM energies.

S3. Residue Deletion Energies for the Avidin Model (Asp Removed)

Avidin Model (no Asp) Residue	MP2 Energy[a]	CPCM Energy[a]	BSSE[b]
Tyr 33	-1346.37407	-1343.371602	0.009599490141
Ser 16	-1537.560075	-1533.908804	0.005407106255
Asn 12	-1483.508681	-1480.004137	0.005663986160
Thr 35	-1537.545472	-1533.903801	0.004812375913
Asn 118	-1483.503382	-1479.998755	0.004812375913
Asn 118 – Asn 12 complex	-338.8883409	-337.917906	0.002413303678
Tyr 33 – Thr 35 complex	-420.642538	-421.9635639	0.003285178778

[a] Open, using the MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory. CPCM energy using a dielectric of ether (4.33).Parentheses, using the MP2/6-31+G(d,p)//MPWB1K/6-31+G(d,p) level. [b] Counterpoise corrections applied to MP2 and CPCM energies.

S4. Water Biotin Interaction Energies

	MP2 Energy[a]	CPCM Energy[a]	BSSE[b]
Biotin (CO)-water	-1005.4007796	-1003.481918	0.005729721401
Biotin (NH)-water	-1005.4020336	-1003.485110	0.006056650322
Water-dimer	-152.4740698	-152.082446	0.003006652568

[a] Open, using the MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory. CPCM energy using dielectric of water (78.39). [b] Counterpoise corrections applied to MP2 and CPCM energies.

S5. Residue Model energies

Residue Mimic	MP2 Energy[a]	CPCM Energy[a]
Tyrosine 33/43 (Phenol)	-306.5608022 (-227.9039438)	-305.58626 (-305.589031)
Serine 27/45 Threonine 35 (Methanol)	-115.39195 (-115.3931775)	-115.05571 (-115.057069)
Asparte 128/13 (Acetate)	-227.90543 (-227.9039438)	-227.33998 (-227.341906)
Asparagine 23/118 (Formamide)	-169.43544 (-169.4349842)	-168.95678 (-168.958812)

[a] Open, using the MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p) level of theory. CPCM energy using a dielectric of ether (4.33). Parentheses, using the MP2/6-31+G(d,p)//MPWB1K/6-31+G(d,p) level.

S6. Cooperative Hydrogen-Bonding Energies (IRBE - BSRBE) in kcal/mol for Streptavidin Residues

Streptavidin Residues	$\Delta\Delta E_{coop(vac)}$ [a]	$\Delta\Delta E_{coop(CPCM)}$ [b]	$\Delta\Delta E_{coop(CPCM)}$ + CP [c]
Tyr 43	-7.0 (-8.8)	-4.9 (-5.9)	-4.5
Ser 27	-4.9 (-4.8)	+0.6 (-0.4)	+2.2
Asn 23	+6.9 (+6.8)	-0.2 (-1.1)	+0.3
Asp 128	-9.7 (-26.8)	-7.0 (-6.4)	-6.6
Ser 45	-2.5 (-7.3)	-2.2 (-3.0)	-2.0
TOTAL	-17.2 (-41.2)	-13.7 (-16.2)	-10.6

[a] MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p). MP2/6-31+G(d,p)//MPWB1K/6-31+G(d,p) in parentheses. [b] CPCM/HF/6-31+G(d,p) level corrections to MP2 energies using the dielectric of ether (ε = 4.33). [c] MP2 and CPCM energies plus CP correction.

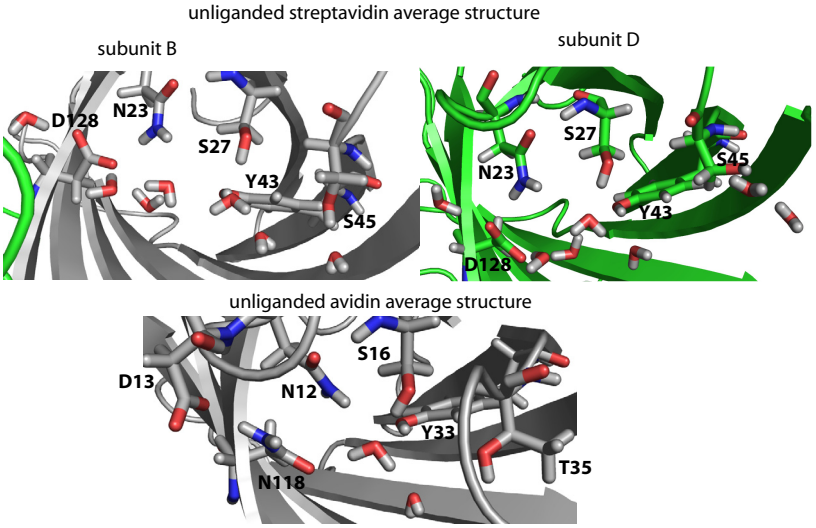
S7. Cooperative Hydrogen-Bonding Energy (IRBE - BSRBE) in kcal/mol for Avidin and Avidin with Asp 13 Removed Models

Protein Residues	$\Delta\Delta E_{coop(vac)}$ [a]	$\Delta\Delta E_{coop(CPCM)}$ [b]	$\Delta\Delta E_{coop(CPCM)}$ + CP [c]
	Avidin		
Tyr 33	-5.2	-4.1	-2.9
Ser 16	-3.3	+1.5	+3.3
Asn 12	+4.7	-0.4	+0.3
Asn 118–Asp 13 pair	-3.8	-5.4	-4.3
Thr 35	-4.2	-2.5	-2.2
TOTAL	-11.8	-10.9	-5.8
	Avidin (Asp 13 removed)		
Tyr 33	-2.4	-2.8	-1.9
Ser 16	-1.8	+2.0	+1.5
Asn 12	+1.3	+1.3	+1.4
Asn 118	+4.6	-0.9	-1.4
Thr 35	-4.6	-3.2	-2.7
TOTAL	-2.9	-3.6	-3.1

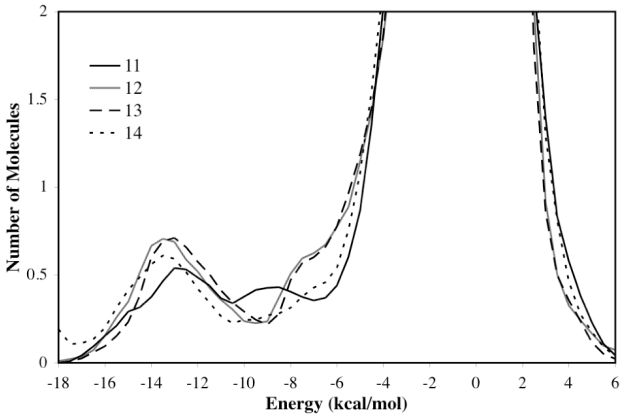
[a] MP2/6-31+G(d,p)//B3LYP/6-31+G(d,p). [b] CPCM/HF/6-31+G(d,p) level corrections to MP2 energies using the dielectric of ether (ε = 4.33). [c] MP2 and CPCM energies plus CP correction.

S8. unliganded-(Strept)Avidin

	MP2 Energy	CPCM energy (ε = 4.33)	CPCM energy (ε = 78.39)
Streptavidin-7H_2O	-1468.544343	-1464.278510	-1464.307466
Avidin-2H_2O	-1256.712862	-1253.022856	-1253.061563
Streptavidin-7H2O Scaffold	-1010.988107	-1008.017594	-1008.063688
Avidin Scaffold	-1100.919481	-1104.181153	-1100.967635
6bound waters (streptavidin)	-457.4336995	-456.217913	-456.241193
2bound waters (avidin)	-152.4739558	-152.074499	-152.083672
1 water	-76.233097	-76.036526	-76.042035



Average structures from MD simulations: (Top) unliganded streptavidin with seven waters interacting with the hydrogen bonding residues in subunit B and D and (Bottom) unliganded avidin with two binding site waters.



Solute-solvent energy pair distributions for different structures of D(+)-biotin. Details are as in ***Figure 11***.

Solute-solvent energy pair distributions have been computed (Figure 7), and notably in the energy ranges of -16 to -12 and -10 to -6 kcal/mol the intramolecular hydrogen bond conformer clearly provides the least favorable interactions compared to the other three conformers.