Supplementary Information for "Two mechanisms of ion selectivity in protein binding sites" by Yu, Noskov and Roux

A) Additional theoretical developments

The atomic fluctuations are obtained from all-atom MD simulations of the solvated protein. The confinement function $H_{\rm c}(\mathbf{X})$, then is constructed with Heaviside step-functions to match the upper bounds of fluctuations, whether an ion of type i or j is bound. It is particularly important that $H_{\rm c}$ be independent of ion type. This implies that imposing the confinement on the all-atom system costs no free energy because $H_{\rm c}$ only forbids configurations that are never observed in the first place. The relative free energy of ion i and j in the binding site is not affected by the confinement,

$$\begin{aligned} e^{-\beta\Delta G_{ij}^{\rm site}} &= \frac{\int_{\rm site} d\mathbf{R}\ e^{-\beta U_i(\mathbf{R})}}{\int_{\rm site} d\mathbf{R}\ e^{-\beta U_j(\mathbf{R})}} \\ &= \frac{\int_{\rm site} d\mathbf{R}\ H_{\rm c}(\mathbf{X})\ e^{-\beta U_i(\mathbf{R})}}{\int_{\rm site} d\mathbf{R}\ H_{\rm c}(\mathbf{X})\ e^{-\beta U_j(\mathbf{R})}} \\ &= \frac{\int_{\rm site} d\mathbf{X}\, d\mathbf{Y}\ H_{\rm c}(\mathbf{X})\ e^{-\beta U_i(\mathbf{X},\mathbf{Y})}}{\int_{\rm site} d\mathbf{X}\, d\mathbf{Y}\ H_{\rm c}(\mathbf{X})\ e^{-\beta U_j(\mathbf{X},\mathbf{Y})}} \\ &= \frac{\int_{\rm site} d\mathbf{X}\ H_{\rm c}(\mathbf{X})\ e^{-\beta[U_i^{\rm il}(\mathbf{X})+U^{\rm ll}(\mathbf{X})+\Delta W^{\rm site}(\mathbf{X})]}}{\int_{\rm site} d\mathbf{X}\ H_{\rm c}(\mathbf{X})\ e^{-\beta[U_j^{\rm il}(\mathbf{X})+U^{\rm ll}(\mathbf{X})+\Delta W^{\rm site}(\mathbf{X})]}} \end{aligned} \tag{1}$$

Comparing with the Eq. [2] in the main text shows that

$$e^{-\beta\Delta W^{\rm site}(\mathbf{X})} \equiv H_{\rm c}(\mathbf{X}) e^{-\beta\Delta W^{\rm site}(\mathbf{X})} \tag{2}$$

This means that the confinement imposed by $H_{\rm c}(\mathbf{X})$ can be treated as being implicitly part of the function $\Delta W^{\rm site}(\mathbf{X})$. By construction, the step-function $H_{\rm c}$ is equal to zero or one, and $H_{\rm c}^2 = H_{\rm c}$. The remainder $\Delta W_{\rm g}^{\rm site}$ can thus safely be defined as,

$$e^{-\beta\Delta W^{\rm site}(\mathbf{X})} \equiv H_{\rm c}(\mathbf{X}) e^{-\beta\Delta W_{\rm g}^{\rm site}(\mathbf{X})} \tag{3}$$

Similar operations are carried out to define the so-called "cavity potential function" in hard-sphere liquids (see [1]). The present construction based on Eq. (3) defines a minimal default model with probability distribution,

$$\rho_0(\mathbf{X}) = H_{\rm c}(\mathbf{X})\ e^{-\beta[U_i^{\rm il}(\mathbf{X})+U^{\rm ll}(\mathbf{X})]} \tag{4}$$

that incorporates the generic effect of architectural confinement by the surounding protein structure in an idealized fashion: each atom k of the reduced subsystem can fluctuate and make arbitrary excursions, as long as it remains confined within its spherical volume V_k. To determine $\Delta W_{\rm g}^{\rm site}$, we seek to maximize the cross-entropy η [2]

$$\eta = -\int d\mathbf{X}\ \rho(\mathbf{X})\ \ln\left[\rho(\mathbf{X})/\rho_0(\mathbf{X})\right] \tag{5}$$

under the constraint that the probability distribution $\rho(\mathbf{X})$ is normalized, and that the atomic fluctuations of the all atoms in the reduced subsystem match the fluctuations extracted from all-atom MD simulations. Denoting the set of coordinates $\mathbf{X}$ as $\{x_1, x_2, \ldots, x_n\}$, the average moments

extracted from the all-atom system can be written as

$$F^{(i_1,i_2,\ldots i_n)} = \left\langle (x_1)^{i_1} \ldots (x_n)^{i_n} \right\rangle \tag{6}$$

Then, the constrained optimization problem is solved by introducing a complete set of Lagrange multiplier $\lambda_{\rm g}^{(i_1,i_2,\ldots i_n)}$ to satisfy the condition on the normalization and reproduce the atomic fluctuation moments $F^{(i_1,i_2,\ldots i_n)}$,

$$\eta = -\int d\mathbf{X}\; \rho(\mathbf{X})\; \ln\left[\rho(\mathbf{X})/\rho_0(\mathbf{X})\right] + \sum_{\rm all}\; \lambda_{\rm g}^{(i_1,i_2,\ldots i_n)}\; \left[\int d\mathbf{X}\; \rho(\mathbf{X})\left((x_1)^{i_1} \ldots (x_n)^{i_n}\right) - F^{(i_1,i_2,\ldots i_n)}\right] \tag{7}$$

where the sum runs to include the atomic fluctuations *to all order*. The normalization condition enters via the term $F^{(0,0,\ldots,0)} = 1$ corresponding to the zeroth moment with $i_1 = 0, i_2 = 0, \ldots, i_n = 0$. The resulting normalized distribution can be formally written as,

$$\rho(\mathbf{X}) = \frac{H_{\rm c}^{\rm site}(\mathbf{X})\; e^{-\beta[U_i^{\rm il}(\mathbf{X}) + U^{\rm ll}(\mathbf{X}) + \Delta W_{\rm g}^{\rm site}(\mathbf{X})]}}{\int d\mathbf{X}\; H_{\rm c}^{\rm site}(\mathbf{X})\; e^{-\beta[U_i^{\rm il}(\mathbf{X}) + U^{\rm ll}(\mathbf{X}) + \Delta W_{\rm g}^{\rm site}(\mathbf{X})]}} \tag{8}$$

where

$$\Delta W_{\rm g}^{\rm site}(\mathbf{X}) = \sum_{\rm all}\; \lambda_{\rm g}^{(i_1,i_2,\ldots i_n)}\left[(x_1)^{i_1} \ldots (x_n)^{i_n}\right] \tag{9}$$

By virtue of the confinement condition implying that $\rho(\mathbf{X}) = 0$ when any of the variable $\mathbf{X}_k$ lies outside the microscopic volumes V_k, all moments $F^{(i_1,i_2,\ldots i_n)}$ are finite and bounded. For this reason, the distribution $\rho(\mathbf{X})$ is uniquely determined by $\rho_0(\mathbf{X})$ and the series of moments $F^{(i_1,i_2,\ldots i_n)}$. In principle, the above procedure can be carried out to match the average moments extracted from the all-atom MD exactly to all order, thus yielding the exact form of $\Delta W_{\rm g}^{\rm site}$. However, it is sufficient to consider only the quadratic fluctuations for the sake of the analysis presented in the article aimed at delineating the two dominant mechanisms of ion selectivity.

Ultimately, it is important that the reference model based on $\rho_0(\mathbf{X})$ provides a good starting point to reproduce the physical behavior of the exact all-atom system. This way, the reduced system with $\lambda_{\rm g} = 0$ is already providing a reasonable "mimic system". To illustrate the character of the mimic system, two movie animations of the KcsA binding site S2 as Supplemental Information. The first movie, **kcsa-all-atom.mpg**, shows the dynamics of the binding site taken from an all-atom MD simulation of KcsA in a fully solvated membrane. The second movie, **kcsa-reduced-model.mpg**, shows the dynamics of the reduced model with only the confinement according to the distribution $\rho_0(\mathbf{X})$. The behavior of the active site is very similar in both cases, and only careful observation reveals that the fluctuations are slightly larger in the reduced model simulations (as expected).

B) Generic uniform confinement

The trends observed in the reduced systems are not highly sensitive to the confinement radius R_k. To illustrate this, the relative free energy of K^+ and Na^+ in the KcsA and LeuT binding sites was recomputed with FEP/MD assuming a uniform generic confinement. In these computations, the geometric contribution, $\lambda_{\rm g}$, is set to zero. The results of FEP/MD computations are shown in Fig. 1. The results from FEP/MD show that the main trend holds for a wide range of confinement radius. It should be noted that imposing a confinement radius smaller than 0.5 Å is akin to assume that the

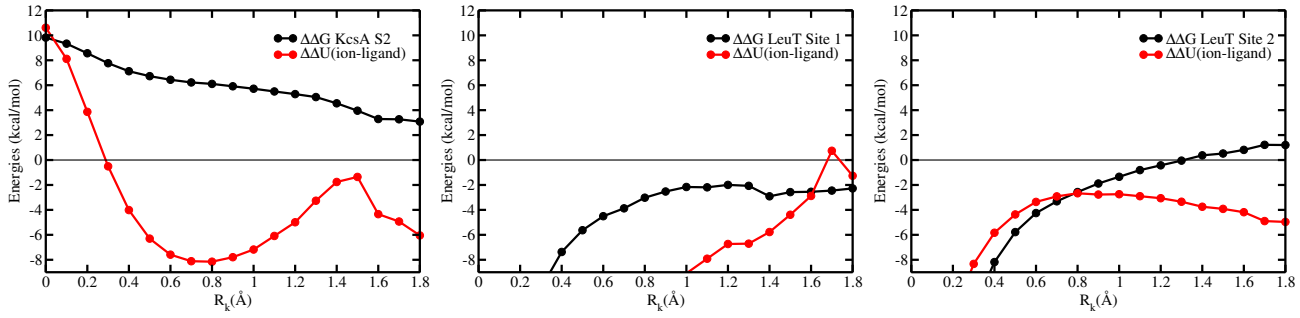

Figure 1: Effect of changing the confinement radius in the reduced binding site models of KcsA and LeuT.

binding site is very rigid and that the snug-fit limit is reproduced with R_k approaching 0.0 Å. This analysis indicates that, in the absence of addition information from all-atom MD, meaningful trends can be extracted from reduced models with a generic confinement radius of 1.0 to 1.4 Å. Such values correspond to typical thermal fluctuations extracted from all-atom MD simulations.

C) Reduced systems with adapted optimal geometry

The relative free energies calculated in the KcsA and LeuT reduced systems with λ_g set to zero correspond to the confined microdroplet limit. Those free energies set the natural trend arising solely from the number and the type of ligands coordinating the ion. An interesting question is whether it is possible to revert the natural trend if the architectural forces (i.e., $\Delta W_g^{\rm site}$) were reinforcing a coordination geometry adapted to best-fit K^+ or Na^+. To address this question, putative K^+- and Na^+-adapted geometries must be constructed for LeuT and KcsA, respectively. The K^+-adapted optimal geometries of each LeuT reduced sites were constructed by performing energy minimization in the presence of a K^+ ion in the binding site. This yields the optimal geometry $\overline{\mathbf{r}}_k[\mathrm{K-adapted}]$ for those two binding sites. The Na^+-adapted optimal geometry of the KcsA reduced model was constructed in the same way with energy minimization in the presence of a bound Na^+. This yields the optimal geometry $\overline{\mathbf{r}}_k[\mathrm{Na-adapted}]$. Then, the relative free energy was recomputed as a function of the force constant λ_g using FEP/MD simulations. The results are shown in Fig. 2. It is clear that reverting the natural tendency of a reduced model in the confined microdroplet limit may be difficult. A fairly stiff restoring force λ_g on the order of 50-100 kcal/mol/Å^2 is required to dictate an optimal geometry and produce selectivity for Na^+ over K^+ from the ligands of the KcsA S2 site. A similar trend is observed for the site Na1 of LeuT. On the other hand, the selectivity of the site Na2 of LeuT can be swayed to favor Na^+ or K^+ by virtue of the geometric architectural forces. This is understandable because this site displays no selectivity in the confined microdroplet limit. It is "selectivity-neutral" by virtue of the number and type of ligands.

These results suggest the following "design principles" for a general ion-selective binding site. Let us consider a situation where one is interested in designing a selective ion binding site at one location in a macromolecular structure. It is assumed that it is possible to modify chemically the local structure such that N ligands are made available to coordinate the desired ion. The key question is to how to best choose the number and type of ligands such that this putative binding site will have the desired selectivity. To answer this question, one could construct a reduced model of the binding

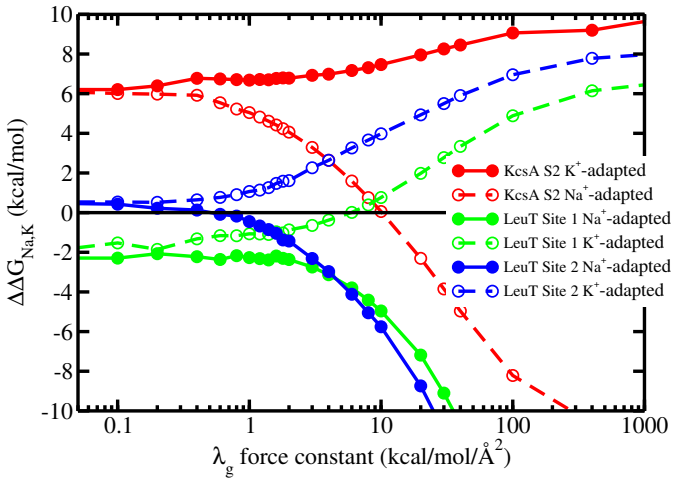

Figure 2: Selectivity in reduced models in which the optimal geometry is adapted to best-fit K^+ or Na^+. The curves with solid lines correspond to the results shown in the article. The curves with dashed lines correspond to putative KcsA and LeuT sites stabilizing an optimal geometry (via λ_g) adapted by energy minimization for Na+ or K^+, respectively. Except for the site Na2 of LeuT, the onset of selectivity is established only for large values of λ_g, indicating that a fairly stiff geometry is required to counter-balance the natural trend observed in the confined microdroplet limit ($\lambda_g = 0$).

site with some generic confinements, and compute the relative free energy of K^+ and Na^+ using FEP/MD simulations. These calculations will display the natural selectivity of the reduced binding site in the confined microdroplet limit, which is predominantly set by the number and the type of ligands. If there is a hint of the desired selectivity, or no selectivity at all, it should be possible to stabilize the optimal coordination geometry for the desired ion and enhance the selectivity. If, on the other hand, the wrong trend is displayed in the confined microdroplet, it will be difficult to correct for this by trying to stiffen some optimal coordinating geometry for the desired ion. The simplest course of action is to go back and choose a different number and type of ligands to achieve the desired selectivity.

D) Confinement of reduced models

The S2 binding site of KcsA

The confinement potential utilized for the S2 binding site of KcsA comprises two components. The first component is defined as the maximum displacement of the atomic positions relative to a fixed point in space. The parameters are given in a CHARMM coordinate format, with column X, Y, Z being the position of the center $\bar{\mathbf{r}}_k$ and the W array being radius R_k in Eq. [3] in the main text, respectively. The W entries equal to zero imply that no confinement is applied to this atom.

```
* S2 KcsA
*
   77
    1    1 GLY  N     -4.64887  -0.83509   9.50473 B    77     1.11800
    2    1 GLY  HN    -5.13162  -0.96595   9.31059 B    77     1.50200
    3    1 GLY  CA    -4.12878  -1.23317  10.78173 B    77     1.08400
    4    1 GLY  HA1   -3.40890  -1.73482  10.80684 B    77     1.33100
    5    1 GLY  HA2   -4.93703  -1.57129  11.42373 B    77     1.18300
    6    1 GLY  C     -3.91184   0.08970  11.94418 B    77     1.12900
    7    1 GLY  O     -2.94688   0.10042  11.71373 B    77     1.55800
    8    1 GLY  NT    -4.84581   0.39329  12.52523 B    77     1.09100
    9    1 GLY  HNT   -5.75408   0.15978  12.25791 B    77     1.18200
   10    1 GLY  CAT   -4.89088   1.48798  13.47304 B    77     1.07200
   11    1 GLY  HT1   -4.57916   2.44754  12.99660 B    77     0.00000
   12    1 GLY  HT2   -4.21093   1.29591  14.33593 B    77     0.00000
   13    1 GLY  HT3   -5.92118   1.62012  13.87340 B    77     0.00000
   14    1 GLY  CAY   -4.00278   0.28112   7.37116 B    77     1.05700
   15    1 GLY  HY1   -3.24541   0.93240   6.88598 B    77     0.00000
   16    1 GLY  HY2   -4.12294  -0.64513   6.77327 B    77     0.00000
   17    1 GLY  HY3   -4.96939   0.82090   7.42553 B    77     0.00000
   18    1 GLY  CY    -3.52841  -0.07013   8.76067 B    77     1.08700
   19    1 GLY  OY    -2.52505  -0.11182   9.09946 B    77     1.22600
   20    2 GLY  N     -2.19409   4.94613   8.96996 C    77     1.02000
   21    2 GLY  HN    -2.24598   6.08285   8.43493 C    77     1.37800
   22    2 GLY  CA    -2.70641   5.01697  10.05355 C    77     1.10500
   23    2 GLY  HA1   -3.46214   4.32659  10.13897 C    77     1.29700
   24    2 GLY  HA2   -3.28208   5.83456  10.11106 C    77     1.29900
   25    2 GLY  C     -1.76919   4.86712  11.23659 C    77     1.09900
   26    2 GLY  O     -1.75490   4.09548  11.28954 C    77     1.48800
   27    2 GLY  NT    -1.31894   5.93100  11.93388 C    77     1.12300
   28    2 GLY  HNT   -1.67602   6.80704  11.65500 C    77     1.16100
   29    2 GLY  CAT   -0.24696   5.99186  12.87711 C    77     1.09000
   30    2 GLY  HT1    0.64864   5.45525  12.48882 C    77     0.00000
   31    2 GLY  HT2   -0.54832   5.50931  13.83445 C    77     0.00000
   32    2 GLY  HT3    0.03669   7.04651  13.08899 C    77     0.00000
   33    2 GLY  CAY   -0.81216   4.39049   6.82920 C    77     1.07100
   34    2 GLY  HY1    0.16495   3.86935   6.74337 C    77     0.00000
   35    2 GLY  HY2   -1.51476   3.96081   6.08616 C    77     0.00000
   36    2 GLY  HY3   -0.67103   5.47043   6.63011 C    77     0.00000
   37    2 GLY  CY    -1.36706   4.18442   8.21649 C    77     1.06000
   38    2 GLY  OY    -1.22304   3.41142   8.45477 C    77     1.40000
   39    3 GLY  N      4.03509   2.59256   9.62796 D    77     1.03200
   40    3 GLY  HN     4.50148   2.88034   9.53588 D    77     1.49700
   41    3 GLY  CA     3.34242   3.57992  10.39441 D    77     1.20800
   42    3 GLY  HA1    2.69244   4.04598  10.34533 D    77     1.62300
   43    3 GLY  HA2    4.12096   3.85002  10.70243 D    77     1.60000
   44    3 GLY  C      3.09656   2.50590  11.85192 D    77     1.04700
   45    3 GLY  O      2.07879   2.30212  11.82239 D    77     1.35700
   46    3 GLY  NT     3.86380   2.16979  12.72009 D    77     1.11400
   47    3 GLY  HNT    4.43620   2.70630  12.30309 D    77     1.33200
   48    3 GLY  CAT    3.68712   1.15789  13.69221 D    77     1.16900
   49    3 GLY  HT1    3.35986   0.20112  13.22228 D    77     0.00000
   50    3 GLY  HT2    2.91221   1.45859  14.43426 D    77     0.00000
   51    3 GLY  HT3    4.63800   0.97166  14.23921 D    77     0.00000
   52    3 GLY  CAY    3.69380   1.32209   7.54516 D    77     1.05300
   53    3 GLY  HY1    2.95043   0.67782   7.03029 D    77     0.00000
   54    3 GLY  HY2    4.05174   2.10118   6.84300 D    77     0.00000
   55    3 GLY  HY3    4.54526   0.69880   7.88397 D    77     0.00000
   56    3 GLY  CY     3.02610   1.97702   8.74189 D    77     1.07000
   57    3 GLY  OY     1.88018   1.92906   8.87224 D    77     1.34400
   58    4 GLY  N      1.32823  -2.78946  10.07423 A    77     1.08300
   59    4 GLY  HN     1.43283  -3.71749  10.03683 A    77     1.42200
   60    4 GLY  CA     1.90688  -2.63456  11.09797 A    77     1.19500
   61    4 GLY  HA1    2.33895  -1.91769  10.96887 A    77     1.48800
   62    4 GLY  HA2    2.37273  -3.23512  11.56719 A    77     1.57800
   63    4 GLY  C      0.86306  -2.34886  12.24334 A    77     1.09500
   64    4 GLY  O      0.81213  -1.46278  12.17049 A    77     1.51900
   65    4 GLY  NT     0.38985  -2.90084  12.77926 A    77     0.99800
   66    4 GLY  HNT    0.86825  -4.00821  12.92656 A    77     1.31400
   67    4 GLY  CAT   -0.76324  -2.97653  13.94003 A    77     1.07200
   68    4 GLY  HT1   -1.49106  -2.14257  13.81008 A    77     0.00000
   69    4 GLY  HT2   -0.30895  -2.88181  14.95179 A    77     0.00000
   70    4 GLY  HT3   -1.32163  -3.93705  13.88415 A    77     0.00000
   71    4 GLY  CAY    0.40915  -2.98550   7.87001 A    77     1.01500
   72    4 GLY  HY1   -0.68424  -2.79282   7.79145 A    77     0.00000
   73    4 GLY  HY2    0.90315  -2.54174   6.97738 A    77     0.00000
```

```
  74    4 GLY  HY3    0.58314  -4.08192   7.87008 A    77     0.00000
  75    4 GLY  CY     0.92835  -2.36978   9.07829 A    77     1.02000
  76    4 GLY  OY     0.79681  -1.39207   9.09792 A    77     1.28500
  77    5 POT  POT   -0.29568   0.95990  10.39158 ION1 1      0.00000
```

The second component is defined as the maximum atom-atom distance for selected pairs of atoms.

```
Max    -atom1--  -atom2--
2.60 - A  77  O  B  77  O
5.85 - A  77  O  B  77  O
2.60 - B  77  O  C  77  O
5.85 - B  77  O  C  77  O
2.60 - C  77  O  D  77  O
5.85 - C  77  O  D  77  O
2.60 - D  77  O  A  77  O
5.85 - D  77  O  A  77  O
2.55 - A  77  OY B  77  OY
5.30 - A  77  OY B  77  OY
2.55 - B  77  OY C  77  OY
5.30 - B  77  OY C  77  OY
2.55 - C  77  OY D  77  OY
5.30 - C  77  OY D  77  OY
2.55 - D  77  OY A  77  OY
5.30 - D  77  OY A  77  OY
4.00 - A  77  O  C  77  O
6.85 - A  77  O  C  77  O
4.00 - B  77  O  D  77  O
6.85 - B  77  O  D  77  O
4.15 - A  77  OY C  77  OY
6.05 - A  77  OY C  77  OY
4.15 - B  77  OY D  77  OY
6.05 - B  77  OY D  77  OY
2.60 - A  77  O  A  77  OY
4.35 - A  77  O  A  77  OY
2.60 - B  77  O  B  77  OY
4.35 - B  77  O  B  77  OY
2.60 - C  77  O  C  77  OY
4.35 - C  77  O  C  77  OY
2.60 - D  77  O  D  77  OY
4.35 - D  77  O  D  77  OY
```

The Na1 and Na2 binding sites of LeuT

The confinement potential is defined as the maximum displacement of the atomic positions. The parameters are given in a CHARMM coordinate format, with column x, y, z being the position of the center $\overline{\mathbf{r}}_k$ and the wmain being radius R_k in Eq. 3 in the main text, respectively. The W entries equal to zero imply that no confinement is applied to this atom.

Site Na1:

```
* Na1 LeuT
*
   81
    1    1 ALA  N     -4.48081  -4.64420 -11.79484 PEPA 22     1.46700
    2    1 ALA  HN    -4.89939  -4.98648 -12.63667 PEPA 22     0.00000
    3    1 ALA  CA    -5.40563  -4.89222 -10.74875 PEPA 22     1.39700
    4    1 ALA  HA    -5.62682  -3.88769 -10.24458 PEPA 22     0.00000
    5    1 ALA  CB    -6.69048  -5.46111 -11.47168 PEPA 22     1.39500
    6    1 ALA  HB1   -7.61508  -5.55596 -10.84547 PEPA 22     0.00000
    7    1 ALA  HB2   -6.58102  -6.32203 -11.98911 PEPA 22     0.00000
    8    1 ALA  HB3   -7.07498  -4.75454 -12.25364 PEPA 22     0.00000
    9    1 ALA  C     -4.86612  -5.66815  -9.60906 PEPA 22     1.22700
   10    1 ALA  O     -4.78615  -5.13223  -8.47641 PEPA 22     1.45300
   11    2 VAL  N     -4.53507  -6.93257  -9.78260 PEPA 23     1.33300
   12    2 VAL  HN    -4.30741  -7.19782 -10.68002 PEPA 23     0.00000
   13    2 VAL  CA    -3.99151  -7.79372  -8.77376 PEPA 23     1.25500
   14    2 VAL  HA    -4.74773  -7.84537  -7.97203 PEPA 23     0.00000
   15    2 VAL  CG2   -5.28338  -9.79101  -9.64792 PEPA 23     1.50300
   16    2 VAL  HG21  -6.08104  -9.57368  -8.86190 PEPA 23     0.00000
   17    2 VAL  HG22  -5.33442 -10.93680  -9.77930 PEPA 23     0.00000
   18    2 VAL  HG23  -5.57129  -9.23543 -10.52472 PEPA 23     0.00000
   19    2 VAL  C     -2.63555  -7.28323  -8.22651 PEPA 23     1.24500
   20    2 VAL  O     -1.63616  -7.15601  -8.95264 PEPA 23     1.45300
   21    3 GLY  N     -2.52937  -7.08193  -6.90302 PEPA 24     1.31900
   22    3 GLY  HN    -3.33184  -7.24492  -6.29610 PEPA 24     0.00000
   23    3 GLY  CA    -1.37813  -6.56942  -6.26564 PEPA 24     1.26800
   24    3 GLY  HA1   -1.09824  -5.52959  -6.62736 PEPA 24     0.00000
   25    3 GLY  HA2   -0.61090  -7.26881  -6.49779 PEPA 24     0.00000
   26    3 GLY  C     -1.54909  -6.67550  -4.76397 PEPA 24     1.28500
   27    3 GLY  O     -2.34566  -7.48950  -4.23121 PEPA 24     1.64400
   28    4 GLY  N     -3.01325  -4.72510  -2.71252 PEPA 26     1.39200
   29    4 GLY  HN    -2.76725  -4.49326  -3.60216 PEPA 26     0.00000
   30    4 GLY  CA    -4.33893  -4.34292  -2.25966 PEPA 26     1.54900
   31    4 GLY  HA1   -4.70701  -3.81496  -3.16597 PEPA 26     0.00000
   32    4 GLY  HA2   -4.29150  -3.73550  -1.37946 PEPA 26     0.00000
   33    4 GLY  C     -5.27464  -5.58255  -2.02276 PEPA 26     1.58700
   34    4 GLY  O     -6.13720  -5.59557  -1.18917 PEPA 26     1.87200
   35    5 ASN  N     -4.97838  -6.64670  -2.77703 PEPA 27     1.32700
   36    5 ASN  HN    -4.27007  -6.54229  -3.50105 PEPA 27     0.00000
   37    5 ASN  CA    -5.70771  -7.92277  -2.63846 PEPA 27     1.31600
   38    5 ASN  HA    -6.74807  -7.69983  -2.53151 PEPA 27     0.00000
   39    5 ASN  CB    -5.51978  -8.79483  -3.81436 PEPA 27     1.31200
   40    5 ASN  HB1   -5.97581  -9.77648  -3.67018 PEPA 27     0.00000
   41    5 ASN  HB2   -4.43633  -8.98068  -3.97342 PEPA 27     0.00000
   42    5 ASN  CG    -5.84466  -8.16210  -5.13499 PEPA 27     1.35100
   43    5 ASN  OD1   -5.05583  -7.39220  -5.65381 PEPA 27     1.36300
   44    5 ASN  ND2   -7.04811  -8.49758  -5.65121 PEPA 27     2.02900
   45    5 ASN  HD21  -7.18784  -8.21696  -6.59782 PEPA 27     0.00000
   46    5 ASN  HD22  -7.74238  -8.95615  -5.14990 PEPA 27     0.00000
   47    6 PHE  C     -7.07425  -0.47374  -5.14500 PEPA 251    1.37200
   48    6 PHE  O     -6.11063  -0.59362  -5.95852 PEPA 251    1.44700
   49    7 THR  N     -7.80608  -1.56003  -4.85277 PEPA 252    1.32900
   50    7 THR  HN    -8.57516  -1.40809  -4.22665 PEPA 252    0.00000
   51    7 THR  CA    -7.62203  -2.91768  -5.40950 PEPA 252    1.26100
   52    7 THR  HA    -6.59691  -3.04106  -5.35871 PEPA 252    0.00000
   53    7 THR  CB    -8.37455  -4.03279  -4.73924 PEPA 252    1.35900
   54    7 THR  HB    -8.20211  -4.03243  -3.64557 PEPA 252    0.00000
   55    7 THR  OG1   -7.83232  -5.34314  -5.09241 PEPA 252    1.71100
   56    7 THR  HG1   -8.30261  -5.94141  -4.51355 PEPA 252    0.00000
   57    7 THR  CG2   -9.94075  -3.99243  -4.97911 PEPA 252    1.39100
   58    7 THR  HG21 -10.40829  -4.59541  -4.20398 PEPA 252    0.00000
   59    7 THR  HG22 -10.23948  -4.47044  -5.93823 PEPA 252    0.00000
   60    7 THR  HG23 -10.31515  -2.93996  -4.96457 PEPA 252    0.00000
   61    7 THR  C     -7.89688  -3.06788  -6.95122 PEPA 252    1.17800
   62    7 THR  O     -7.03304  -3.58694  -7.60694 PEPA 252    1.22200
   63    8 LEU  N     -8.96861  -2.45400  -7.45039 PEPA 253    1.20800
   64    8 LEU  HN    -9.63466  -1.94032  -6.92111 PEPA 253    0.00000
   65    8 LEU  CA    -9.15805  -2.57887  -8.93708 PEPA 253    1.23800
   66    8 LEU  HA    -8.86834  -3.58906  -9.26383 PEPA 253    0.00000
   67    9 ASN  CG    -8.80016  -7.32268  -8.26319 PEPA 284    1.38500
   68    9 ASN  OD1   -7.54947  -7.42695  -8.32864 PEPA 284    1.53700
   69    9 ASN  ND2   -9.44924  -6.83353  -7.17989 PEPA 284    1.74700
   70    9 ASN  HD21  -9.00469  -6.19215  -6.56059 PEPA 284    0.00000
   71    9 ASN  HD22 -10.46213  -6.90326  -7.08779 PEPA 284    0.00000
   72   10 SOD  SOD   -5.94865  -5.26686  -6.33815 ION4 1      0.00000
   73   11 LEU  N     -4.30479  -2.17549  -7.97037 ION3 1      1.18700
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   74   11 LEU  HT1   -4.15557  -1.77182  -8.89003 ION3 1      0.00000
   75   11 LEU  HT2   -4.98310  -1.67557  -7.36414 ION3 1      0.00000
   76   11 LEU  HT3   -4.60807  -3.20786  -8.00681 ION3 1      0.00000
   77   11 LEU  CA    -3.06718  -2.31030  -7.17436 ION3 1      1.17500
   78   11 LEU  HA    -2.22624  -2.73718  -7.81270 ION3 1      0.00000
   79   11 LEU  C     -3.26218  -3.23866  -5.93652 ION3 1      1.17200
   80   11 LEU  OT1   -2.25587  -3.44439  -5.18847 ION3 1      1.35000
   81   11 LEU  OT2   -4.38742  -3.77343  -5.68337 ION3 1      1.41200
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Site Na2:

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* Na2 LeuT
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   65
    1    1 GLY  N     -2.70469  -7.59600 -14.07503 PEPA 20     1.46700
    2    1 GLY  HN    -2.67925  -7.71740 -15.08395 PEPA 20     0.00000
    3    1 GLY  CA    -1.47138  -7.36084 -13.44406 PEPA 20     1.63000
    4    1 GLY  HA1   -0.79086  -7.58176 -14.26876 PEPA 20     0.00000
    5    1 GLY  HA2   -1.29001  -8.03109 -12.63918 PEPA 20     0.00000
    6    1 GLY  C     -1.46963  -5.97171 -12.80873 PEPA 20     1.44600
    7    1 GLY  O     -0.93499  -5.87891 -11.71060 PEPA 20     1.72300
    8    2 ASN  N     -2.10679  -4.97874 -13.49629 PEPA 21     1.49000
    9    2 ASN  HN    -2.34657  -5.19075 -14.47332 PEPA 21     0.00000
   10    2 ASN  CA    -2.53088  -3.68098 -12.93543 PEPA 21     1.33700
   11    2 ASN  HA    -1.65980  -3.19620 -12.51839 PEPA 21     0.00000
   12    2 ASN  C     -3.38067  -3.88233 -11.74850 PEPA 21     1.33100
   13    2 ASN  O     -3.10501  -3.45553 -10.64251 PEPA 21     1.53100
   14    3 VAL  N     -4.53507  -6.93257  -9.78260 PEPA 23     1.33300
   15    3 VAL  HN    -4.30741  -7.19782 -10.68002 PEPA 23     0.00000
   16    3 VAL  CA    -3.99151  -7.79372  -8.77376 PEPA 23     1.25500
   17    3 VAL  HA    -4.74773  -7.84537  -7.97203 PEPA 23     0.00000
   18    3 VAL  C     -2.63555  -7.28323  -8.22651 PEPA 23     1.24500
   19    3 VAL  O     -1.63616  -7.15601  -8.95264 PEPA 23     1.45300
   20    4 GLY  N     -2.52937  -7.08193  -6.90302 PEPA 24     1.25900
   21    4 GLY  HN    -3.33184  -7.24492  -6.29610 PEPA 24     0.00000
   22    4 GLY  CA    -1.37813  -6.56942  -6.26564 PEPA 24     1.26800
   23    4 GLY  HA1   -1.09824  -5.52959  -6.62736 PEPA 24     0.00000
   24    4 GLY  HA2   -0.61090  -7.26881  -6.49779 PEPA 24     0.00000
   25    5 PHE  C      3.18381 -10.08145  -8.52876 PEPA 348    1.36900
   26    5 PHE  O      2.66014  -9.50800  -9.46280 PEPA 348    1.76400
   27    6 ALA  N      2.63165  -9.98630  -7.34322 PEPA 349    1.29000
   28    6 ALA  HN     2.95139 -10.50527  -6.51650 PEPA 349    0.00000
   29    6 ALA  CA     1.53597  -9.03579  -7.08634 PEPA 349    1.35200
   30    6 ALA  HA     0.72761  -9.15026  -7.76912 PEPA 349    0.00000
   31    6 ALA  C      1.97237  -7.57624  -7.02444 PEPA 349    1.35000
   32    6 ALA  O      1.44730  -6.74717  -7.76167 PEPA 349    1.41000
   33    7 GLY  N      3.14844  -7.25539  -6.35976 PEPA 350    1.27700
   34    7 GLY  HN     3.62060  -7.86093  -5.72853 PEPA 350    0.00000
   35    7 GLY  CA     3.74937  -5.85491  -6.35587 PEPA 350    1.32500
   36    7 GLY  HA1    4.63974  -5.94889  -5.73604 PEPA 350    0.00000
   37    7 GLY  HA2    3.01859  -5.18945  -5.89470 PEPA 350    0.00000
   38    7 GLY  C      4.23305  -5.32599  -7.64543 PEPA 350    1.20000
   39    7 GLY  O      3.75561  -4.29937  -8.13330 PEPA 350    1.48500
   40    8 THR  N      4.24286  -6.04901 -10.80201 PEPA 352    1.45000
   41    8 THR  HN     3.98349  -6.81134 -10.31085 PEPA 352    0.00000
   42    8 THR  CA     3.21738  -5.81417 -11.94058 PEPA 352    1.40700
   43    8 THR  HA     3.81246  -5.62687 -12.82292 PEPA 352    0.00000
   44    8 THR  CB     2.28819  -6.97491 -12.21944 PEPA 352    1.56400
   45    8 THR  HB     1.59339  -6.61860 -13.00626 PEPA 352    0.00000
   46    8 THR  OG1    1.65027  -7.28013 -11.01703 PEPA 352    1.75700
   47    8 THR  HG1    1.77542  -8.17691 -10.81317 PEPA 352    0.00000
   48    8 THR  CG2    2.93520  -8.33104 -12.64670 PEPA 352    1.73800
   49    8 THR  HG21   3.63517  -8.14206 -13.48752 PEPA 352    0.00000
   50    8 THR  HG22   2.12243  -9.01878 -12.95886 PEPA 352    0.00000
   51    8 THR  HG23   3.39500  -8.85948 -11.72117 PEPA 352    0.00000
   52    8 THR  C      2.41007  -4.55826 -11.76266 PEPA 352    1.34300
   53    8 THR  O      1.82346  -4.03134 -12.74644 PEPA 352    1.55700
   54    9 SER  N      2.28004  -4.10196 -10.54542 PEPA 353    1.36100
   55    9 SER  HN     2.88165  -4.38930  -9.82269 PEPA 353    0.00000
   56    9 SER  CA     1.50059  -2.87821 -10.24503 PEPA 353    1.25800
   57    9 SER  HA     0.67411  -2.81515 -10.95711 PEPA 353    0.00000
   58    9 SER  CB     0.74021  -2.85277  -8.90192 PEPA 353    1.60200
   59    9 SER  HB1   -0.00626  -2.11724  -8.76097 PEPA 353    0.00000
   60    9 SER  HB2    1.67041  -2.53991  -8.27787 PEPA 353    0.00000
   61    9 SER  OG     0.23326  -4.08988  -8.34446 PEPA 353    1.59100
   62    9 SER  HG1    0.55670  -3.96559  -7.41534 PEPA 353    0.00000
   63    9 SER  C      2.30616  -1.60645 -10.46262 PEPA 353    1.24600
   64    9 SER  O      1.79656  -0.56759 -10.92516 PEPA 353    1.43300
   65   10 SOD  SOD    0.31275  -5.96473  -9.71533 ION1 1      0.00000
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References

[1] Hansen, J & McDonald, I. (1986) *Theory of Simple Liquids, 2nd Edition.* (Academic Press, London).

[2] Hummer, G, Garde, S, Garcia, A. E, Pohorille, A, & Pratt, L. R. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8951–8955.