Binding affinity calculations using MM-PBSA for acetylated peptide, NAD+ and inhibitors

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SIRT3/AcCS2/ NAD+ | | SIRT3/AcCS2/NAD+/NAM | | | SIRT3/AcCS2/NAD+/isoNAM | | | SIRT3/ NAD+ (AB) | SIRT3/ NAD+ (AC) | SIRT3/  Ac-CS2 | |
| Ac-CS2 | NAD+ | Ac-CS2 | NAD+ | NAM | Ac-CS2 | NAD+ | isoNAM | NAD+ | NAD+ | Ac-CS2 |
| -9.314 | -5.354 | -9.106 | -8.096 | 0.260 | -9.425 | 0.325 | 3.888 | -1.566 | -2.954 \* | -6.995 |

G is averaged over the 1000 frames from last 10 ns.

\* This G is averaged over the 2000 frames from last 20 ns.

Binding affinity calculations using MM-GBSA for acetylated peptide, NAD+ and inhibitors

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SIRT3/AcCS2/ NAD+ | | SIRT3/AcCS2/NAD+/NAM | | | SIRT3/AcCS2/NAD+/isoNAM | | | SIRT3/ NAD+ (AB) | SIRT3/ NAD+ (AC) | SIRT3/  Ac-CS2 | |
| Ac-CS2 | NAD+ | Ac-CS2 | NAD+ | NAM | Ac-CS2 | NAD+ | isoNAM | NAD+ | NAD+ | Ac-CS2 |
| -47.936 | -65.629 | -52.313 | -63.124 | -19.818 | -51.035 | -62.701 | -15.683 | -37.774 | -50.849 \* | -46.483 |

G is averaged over the 1000 frames from last 10 ns.

\* This G is averaged over the 2000 frames from last 20 ns.

Binding affinity calculations using MM-PB(GB)SA for acetylated peptide, NAD+ and inhibitors

1) Binding affinity of Acetylated peptide (AcCS2)\* (MM-PBSA results)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+) | | | | 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean\* |  |  |  | Mean |  |  |  | Mean |
| AcCS2 Binding: PBSA | | | -9.314 | AcCS2 Binding: PBSA | | | -9.106 | AcCS2 Binding: PBSA | | | -9.425 |
| ∆G | STD\* | SEM\* |  | ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -13.561 | 4.842 | 0.342 |  | -4.746 | 4.557 | 0.321 |  | -12.834 | 4.743 | 0.335 |  |
| -14.799 | 4.021 | 0.284 |  | -6.089 | 4.235 | 0.299 |  | -9.595 | 4.519 | 0.319 |  |
| -14.164 | 3.791 | 0.267 |  | -7.321 | 4.421 | 0.312 |  | -9.019 | 4.372 | 0.308 |  |
| -14.660 | 3.771 | 0.266 |  | -7.992 | 4.209 | 0.297 |  | -8.924 | 3.876 | 0.273 |  |
| -12.493 | 4.396 | 0.310 |  | -5.623 | 4.048 | 0.286 |  | -10.007 | 3.571 | 0.252 |  |
| -8.796 | 4.778 | 0.337 |  | -3.639 | 5.065 | 0.357 |  | -8.906 | 3.894 | 0.275 |  |
| -10.140 | 5.882 | 0.415 |  | -9.804 | 4.140 | 0.292 |  | -11.442 | 4.115 | 0.290 |  |
| -9.821 | 4.520 | 0.319 |  | -12.092 | 4.290 | 0.303 |  | -9.760 | 4.341 | 0.306 |  |
| -8.153 | 4.290 | 0.303 |  | -10.561 | 4.486 | 0.316 |  | -8.693 | 3.997 | 0.282 |  |
| -9.662 | 4.097 | 0.289 |  | -8.775 | 3.968 | 0.280 |  | -9.506 | 4.158 | 0.293 |  |
|  |  |  |  | -8.524 | 3.796 | 0.268 |  | -7.813 | 3.652 | 0.258 |  |
|  |  |  |  | -8.199 | 4.091 | 0.289 |  | -10.668 | 4.599 | 0.324 |  |
|  |  |  |  | -9.474 | 4.050 | 0.286 |  | -9.354 | 3.785 | 0.267 |  |
|  |  |  |  |  |  |  |  | -10.681 | 4.104 | 0.290 |  |
|  |  |  |  |  |  |  |  | -8.611 | 3.846 | 0.271 |  |

\* ∆G is calculated every 200 frames in 2 ns simulation;

Mean value is averaged over the 1000 frames from last 10 ns;

STD: standard deviation;

SEM: standard error of the mean.

2) Binding affinity of Acetylated peptide (AcCS2) (MM-GBSA results)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+) | | | | 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean |  |  |  | Mean |  |  |  | Mean |
| AcCS2 Binding: GBSA | | | -47.936 | AcCS2 Binding: GBSA | | | -52.313 | AcCS2 Binding: GBSA | | | -51.035 |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -55.392 | 3.735 | 0.264 |  | -48.576 | 3.253 | 0.229 |  | -56.902 | 3.437 | 0.242 |  |
| -54.960 | 3.172 | 0.224 |  | -48.953 | 3.019 | 0.213 |  | -56.169 | 3.207 | 0.226 |  |
| -53.750 | 3.152 | 0.222 |  | -52.317 | 4.222 | 0.298 |  | -53.080 | 3.322 | 0.234 |  |
| -55.104 | 3.362 | 0.237 |  | -52.127 | 4.049 | 0.286 |  | -50.096 | 2.929 | 0.207 |  |
| -53.083 | 4.196 | 0.296 |  | -49.755 | 3.160 | 0.223 |  | -50.933 | 3.105 | 0.219 |  |
| -47.294 | 4.119 | 0.291 |  | -49.215 | 4.073 | 0.287 |  | -49.917 | 3.506 | 0.247 |  |
| -49.105 | 4.224 | 0.298 |  | -55.637 | 3.022 | 0.213 |  | -53.363 | 4.261 | 0.301 |  |
| -47.609 | 3.971 | 0.280 |  | -56.083 | 3.209 | 0.226 |  | -51.037 | 2.860 | 0.202 |  |
| -46.444 | 3.965 | 0.280 |  | -54.921 | 3.552 | 0.251 |  | -50.341 | 2.832 | 0.200 |  |
| -49.225 | 3.439 | 0.243 |  | -52.253 | 3.172 | 0.224 |  | -52.444 | 3.195 | 0.225 |  |
|  |  |  |  | -50.736 | 2.721 | 0.192 |  | -48.675 | 2.811 | 0.198 |  |
|  |  |  |  | -51.048 | 3.422 | 0.241 |  | -52.061 | 3.987 | 0.281 |  |
|  |  |  |  | -52.609 | 3.351 | 0.236 |  | -50.122 | 2.887 | 0.204 |  |
|  |  |  |  |  |  |  |  | -52.875 | 3.404 | 0.240 |  |
|  |  |  |  |  |  |  |  | -51.444 | 3.266 | 0.230 |  |

3) Binding affinity of NAD+ (MM-PBSA results)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+) | | | | 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean |  |  |  | Mean |  |  |  | Mean |
| NAD+ Binding: PBSA | | | -5.354 | NAD+ Binding: PBSA | | | -8.096 | NAD+ Binding: PBSA | | | 0.325 |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| 4.708 | 7.873 | 0.555 |  | -0.289 | 5.637 | 0.398 |  | -0.606 | 7.172 | 0.506 |  |
| 7.887 | 6.964 | 0.491 |  | -3.843 | 5.556 | 0.392 |  | -5.911 | 7.905 | 0.558 |  |
| 9.594 | 5.768 | 0.407 |  | -1.937 | 5.669 | 0.400 |  | -8.341 | 6.809 | 0.480 |  |
| 9.141 | 6.289 | 0.444 |  | -3.432 | 6.422 | 0.453 |  | -0.832 | 9.385 | 0.662 |  |
| 7.940 | 6.475 | 0.457 |  | -1.487 | 6.037 | 0.426 |  | 1.330 | 8.268 | 0.583 |  |
| -6.651 | 9.052 | 0.639 |  | 0.983 | 6.259 | 0.441 |  | 3.049 | 6.964 | 0.491 |  |
| -7.441 | 6.714 | 0.474 |  | -1.812 | 6.221 | 0.439 |  | 2.270 | 7.925 | 0.559 |  |
| -4.724 | 8.186 | 0.577 |  | -6.723 | 5.583 | 0.394 |  | -1.258 | 7.993 | 0.564 |  |
| -1.219 | 8.496 | 0.599 |  | -9.130 | 5.070 | 0.358 |  | 4.976 | 6.158 | 0.434 |  |
| -6.735 | 7.678 | 0.542 |  | -8.134 | 5.643 | 0.398 |  | -0.269 | 6.989 | 0.493 |  |
|  |  |  |  | -8.885 | 6.045 | 0.426 |  | 0.580 | 7.475 | 0.527 |  |
|  |  |  |  | -6.882 | 5.489 | 0.387 |  | 0.302 | 6.535 | 0.461 |  |
|  |  |  |  | -7.449 | 5.958 | 0.420 |  | 1.092 | 5.840 | 0.412 |  |
|  |  |  |  |  |  |  |  | -0.669 | 5.880 | 0.415 |  |
|  |  |  |  |  |  |  |  | 0.321 | 6.628 | 0.468 |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, NAD+ from AB pocket) | | | | 4FVT (SIRT3, NAD+ from AC pocket) | | | |
|  |  |  | Mean |  |  |  | Mean |
| NAD+ Binding: PBSA | | | -1.566 | NAD+ Binding: PBSA | | | -2.954 \* |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -7.381 | 12.411 | 0.875 |  | 1.193 | 9.038 | 0.638 |  |
| 0.967 | 6.821 | 0.481 |  | -4.974 | 8.886 | 0.627 |  |
| -1.817 | 6.947 | 0.490 |  | -2.629 | 8.876 | 0.626 |  |
| 4.985 | 7.011 | 0.495 |  | 4.252 | 6.375 | 0.450 |  |
| -0.704 | 7.972 | 0.562 |  | -4.194 | 6.767 | 0.477 |  |
| -8.714 | 7.600 | 0.536 |  | 4.999 | 7.849 | 0.554 |  |
| -2.568 | 7.508 | 0.530 |  | 6.488 | 7.855 | 0.554 |  |
| -5.579 | 6.447 | 0.455 |  | 14.390 | 9.592 | 0.677 |  |
| 5.397 | 7.935 | 0.560 |  | 11.181 | 6.422 | 0.453 |  |
| 3.635 | 8.217 | 0.580 |  | -10.837 | 10.550 | 0.744 |  |
|  |  |  |  | -19.106 | 5.913 | 0.417 |  |
|  |  |  |  | -11.096 | 6.251 | 0.441 |  |
|  |  |  |  | -14.331 | 5.676 | 0.400 |  |
|  |  |  |  | -9.361 | 7.528 | 0.531 |  |
|  |  |  |  | -1.869 | 7.112 | 0.502 |  |

\* averaged over last 20 ns.

4) Binding affinity of NAD+ (MM-GBSA results)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+) | | | | 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean |  |  |  | Mean |  |  |  | Mean |
| NAD+ Binding: GBSA | | | -65.629 | NAD+ Binding: GBSA | | | -63.124 | NAD+ Binding: GBSA | | | -62.701 |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -62.739 | 5.567 | 0.393 |  | -55.965 | 4.458 | 0.314 |  | -55.340 | 6.052 | 0.427 |  |
| -61.358 | 5.720 | 0.404 |  | -56.867 | 4.275 | 0.302 |  | -66.003 | 6.270 | 0.442 |  |
| -58.161 | 5.272 | 0.372 |  | -58.810 | 4.391 | 0.310 |  | -67.680 | 5.225 | 0.369 |  |
| -59.277 | 4.742 | 0.335 |  | -58.865 | 5.153 | 0.364 |  | -65.669 | 6.766 | 0.477 |  |
| -60.303 | 4.799 | 0.339 |  | -59.061 | 4.474 | 0.316 |  | -64.142 | 6.079 | 0.429 |  |
| -67.682 | 5.916 | 0.417 |  | -56.094 | 4.630 | 0.327 |  | -64.856 | 5.560 | 0.392 |  |
| -66.778 | 5.093 | 0.359 |  | -59.914 | 5.558 | 0.392 |  | -64.991 | 5.761 | 0.406 |  |
| -63.770 | 6.165 | 0.435 |  | -64.371 | 5.031 | 0.355 |  | -69.512 | 5.612 | 0.396 |  |
| -63.517 | 5.838 | 0.412 |  | -64.375 | 4.469 | 0.315 |  | -64.209 | 5.192 | 0.366 |  |
| -66.396 | 5.232 | 0.369 |  | -62.876 | 4.164 | 0.294 |  | -64.684 | 4.828 | 0.341 |  |
|  |  |  |  | -63.488 | 4.183 | 0.295 |  | -61.727 | 5.416 | 0.382 |  |
|  |  |  |  | -62.069 | 4.400 | 0.310 |  | -63.191 | 4.808 | 0.339 |  |
|  |  |  |  | -62.811 | 4.259 | 0.300 |  | -60.069 | 4.293 | 0.303 |  |
|  |  |  |  |  |  |  |  | -63.377 | 4.452 | 0.314 |  |
|  |  |  |  |  |  |  |  | -65.141 | 5.817 | 0.410 |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, NAD+ from AB pocket) | | | | 4FVT (SIRT3, NAD+ from AC pocket) | | | |
|  |  |  | Mean |  |  |  | Mean |
| NAD+ Binding: PBSA | | | -37.774 | NAD+ Binding: PBSA | | | -50.849\* |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -64.979 | 16.000 | 1.129 |  | -55.604 | 5.218 | 0.368 |  |
| -39.615 | 4.534 | 0.320 |  | -56.340 | 5.429 | 0.383 |  |
| -40.656 | 5.289 | 0.373 |  | -57.912 | 4.435 | 0.313 |  |
| -35.805 | 4.267 | 0.301 |  | -55.446 | 4.474 | 0.316 |  |
| -34.537 | 6.258 | 0.441 |  | -52.079 | 6.234 | 0.440 |  |
| -37.913 | 7.299 | 0.515 |  | -42.009 | 5.157 | 0.364 |  |
| -36.034 | 4.951 | 0.349 |  | -41.464 | 4.477 | 0.316 |  |
| -38.938 | 5.894 | 0.416 |  | -35.762 | 6.468 | 0.456 |  |
| -38.839 | 5.939 | 0.419 |  | -34.866 | 4.986 | 0.352 |  |
| -37.146 | 5.659 | 0.399 |  | -56.512 | 8.331 | 0.588 |  |
|  |  |  |  | -62.429 | 5.378 | 0.379 |  |
|  |  |  |  | -55.488 | 4.574 | 0.323 |  |
|  |  |  |  | -61.773 | 6.629 | 0.468 |  |
|  |  |  |  | -61.843 | 6.867 | 0.484 |  |
|  |  |  |  | -56.347 | 5.191 | 0.366 |  |

\* averaged over last 20 ns.

5) Binding affinity of Inhibitor (NAM or isoNAM) (MM-PBSA results)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean |  |  |  | Mean |
| NAM Binding: PBSA | | | 0.260 | isoNAM Binding: PBSA | | | 3.888 |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -2.503 | 3.298 | 0.233 |  | -2.570 | 2.806 | 0.198 |  |
| -3.085 | 2.640 | 0.186 |  | -6.345 | 2.747 | 0.194 |  |
| -2.991 | 2.433 | 0.172 |  | -4.101 | 2.709 | 0.191 |  |
| -2.414 | 2.999 | 0.212 |  | -1.763 | 2.971 | 0.210 |  |
| -1.919 | 2.850 | 0.201 |  | 1.711 | 3.327 | 0.235 |  |
| -0.756 | 2.794 | 0.197 |  | 0.763 | 3.350 | 0.236 |  |
| -1.030 | 2.779 | 0.196 |  | 2.151 | 3.476 | 0.245 |  |
| 1.453 | 3.356 | 0.237 |  | -0.010 | 2.835 | 0.200 |  |
| 0.060 | 3.373 | 0.238 |  | -0.062 | 2.887 | 0.204 |  |
| -0.600 | 3.352 | 0.236 |  | 1.471 | 4.178 | 0.295 |  |
| 0.182 | 2.995 | 0.211 |  | 3.742 | 4.258 | 0.300 |  |
| 0.563 | 3.066 | 0.216 |  | 3.069 | 4.250 | 0.300 |  |
| 1.093 | 3.243 | 0.229 |  | 5.283 | 3.494 | 0.246 |  |
|  |  |  |  | 4.704 | 3.569 | 0.252 |  |
|  |  |  |  | 2.642 | 4.615 | 0.326 |  |

6) Binding affinity of Inhibitor (NAM or isoNAM) (MM-GBSA results)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 4FVT (SIRT3, AcCS2, NAD+, NAM) | | | | 4FVT (SIRT3, AcCS2, NAD+,isoNAM) | | | |
|  |  |  | Mean |  |  |  | Mean |
| NAM Binding: GBSA | | | -19.818 | isoNAM Binding: GBSA | | | -16.225 |
| ∆G | STD | SEM |  | ∆G | STD | SEM |  |
| -19.214 | 2.043 | 0.144 |  | -20.057 | 1.749 | 0.123 |  |
| -19.530 | 1.938 | 0.137 |  | -21.104 | 2.870 | 0.202 |  |
| -19.928 | 2.084 | 0.147 |  | -17.087 | 1.977 | 0.139 |  |
| -19.670 | 1.912 | 0.135 |  | -15.811 | 2.342 | 0.165 |  |
| -20.088 | 1.939 | 0.137 |  | -13.269 | 1.873 | 0.132 |  |
| -19.911 | 1.968 | 0.139 |  | -13.248 | 2.133 | 0.151 |  |
| -20.184 | 1.718 | 0.121 |  | -13.224 | 1.973 | 0.139 |  |
| -19.398 | 1.902 | 0.134 |  | -17.642 | 2.380 | 0.168 |  |
| -19.613 | 1.993 | 0.141 |  | -18.170 | 2.643 | 0.186 |  |
| -19.312 | 1.978 | 0.140 |  | -14.805 | 2.177 | 0.154 |  |
| -20.054 | 1.740 | 0.123 |  | -15.659 | 2.185 | 0.154 |  |
| -19.981 | 1.786 | 0.126 |  | -14.849 | 2.232 | 0.157 |  |
| -20.132 | 1.820 | 0.128 |  | -14.890 | 1.973 | 0.139 |  |
|  |  |  |  | -15.507 | 1.975 | 0.139 |  |
|  |  |  |  | -17.510 | 2.920 | 0.206 |  |