Slides 24 and 25\_computational methods: Please provide the detail protocols (Glide, MM-GBSA, induced fit +MMGBSA, and LIA) for the METHOD section of our first paper.

* For all the simulations, the proteins were prepped in a similar manner.  Multiple starting structures of SIRT3 (3GLT and 4FVT) and SIRT2 (1J8F and 3ZGV) were prepared with the Schrodinger protein preparation protocol.   Bond orders were assigned, missing hydrogens added, zero-order bonds to the zinc atoms created.  Since the structures had no missing side chains or residues near the binding pocket, none were computationally added.  Protonation and tautomer states for the ligands were generated with Epik for a pH range of 7.0 +/- 3.0.  H-bond assignment was done using PROPKA at pH 7.0 and included sampling all water orientations, as well as using crystal symmetry information.  A final restrained minimization was performed with heavy atoms converged to RMSD 0.30 Å with the OPLS 2005 force field.  The crystallographic waters were eliminated except as noted below.
* All of the methods used structures and poses created from Glide Docking.
  + First the docking grid was created by defining the receptor region within 18 Å around the co-crystallized NAD+ or NAD+ analogue.  Van der Waals scaling of 1.0 with a partial charge cutoff of 0.25 was used.  Unless noted, no constraints were used in the docking. All receptor hydroxyl and thiol groups were allowed to freely rotate.
  + GlideXP Docking into the receptor grid allowed fully flexible ligands, where the dihedral angles of rotatable bonds were sampled, as well as nitrogen inversions and flexible ring conformations.  Since Glide does not sample the bond angles, multiple input structures for each ligand were created with two force fields (MMFF and OPLS\_AA) which often creates subtle but important differences in bond angles.  Partial charges on the ligand were assigned with the standard OPLS\_2005 force field, and the van der Waals scaling factor and partial charge cutoff were reduced to 0.8 and 0.15, respectively.  Unless specified, no constraints were used.  At most 3 structures per ligand input conformation were output.  Post docking minimization was performed.
  + For ligands that had multiple output structures, the lowest energy structure was selected as the final GlideXP score.
  + For methods that used the GlideXP poses as input (MM-GBSA, LIA), all of the poses were carried to the subsequent method.
* The MM-GBSA protocol is already described in the paper.  Except here, multiple ligands were docked with Glide, and all of the poses were input into the MM-GBSA protocol.  The final best scoring result for each ligand was chosen.  Two modes of MM-GBSA were tried:  one with an inflexible protein, and simple minimization with the MM-GBSA force field of the ligand position.  Two, residues (side chains and backbone) within 5.0 Å from the docked ligand in the protein were minimized with the Prime (plop) algorithm.  Neither of these two options consistently resulted in superior correlations between calculated vs. experimental ∆G\_bind.  If the crystal structure was closer to the docked protein-ligand complex, protein flexibility worsened the results.
* The Induced Fit docking protocol, which is a more computationally intensive alternative to GlideXP docking, was similar to standard docking, except that a region of 5.0 Å around the preliminary docked structures is sampled via the Prime algorithm.  After this sampling, the ligand is reduced with GlideXP into the minimized receptor area.
* The Linear Interaction Model in the Liaison program is similar to MM-GBSA, except it uses a slightly different minimization and water model (SGB).  Like MM-GBSA, two different versions were run with the protein inflexible or flexible to within 5.0 Å.  Like the two version in MM-GBSA, the inflexible model worked better when the crystal structure was close to the true structure.  With the GlideXP poses as input, the ligand and protein were minimized with the truncated Newton method up to a maximum of 1000 steps.