SUPPORTING INFORMATION

Details about the docking protocol

Ligand input conformations. The coordinates of NAD^+ were retrieved from the 2bkj PDB file (structure of the FMN oxidoreductase-NAD^+complex) and converted to MOL2 format using SYBYL 7.1. Atomic types were corrected and all hydrogen atoms were added. NGD^+ structure was obtained by manual edition of the NAD^+ molecule followed by a rapid energy minimization (default parameters) in SYBYL 7.1. Starting coordinates for cADPR and cGDPR were obtained from MacroModel representative structures.

Distance restaints used during Gold docking. Three distance restraints with spring constant of 5 restricted NAD^+ motion into the active site; the nicotinamide moiety of NAD^+ was forced to be close to the conserved Trp^{165} (pyridine C3 and indole C3a atoms within a distance range of 2.5- 6.5Å) and the two hydroxyl groups of the nicotinamide-attached ribose to face the catalytic Glu^{202} (O"2- Oε and O"3-Oε specified distances both range from 1.5 Å to 4Å). One distance restraint guided the cADPR and cGDPR placement (Glu^{202} Oε and N1- or N7-ribose C1 atoms are fixed within a distance range of 1.5- 3.5Å, spring constant set to 5).

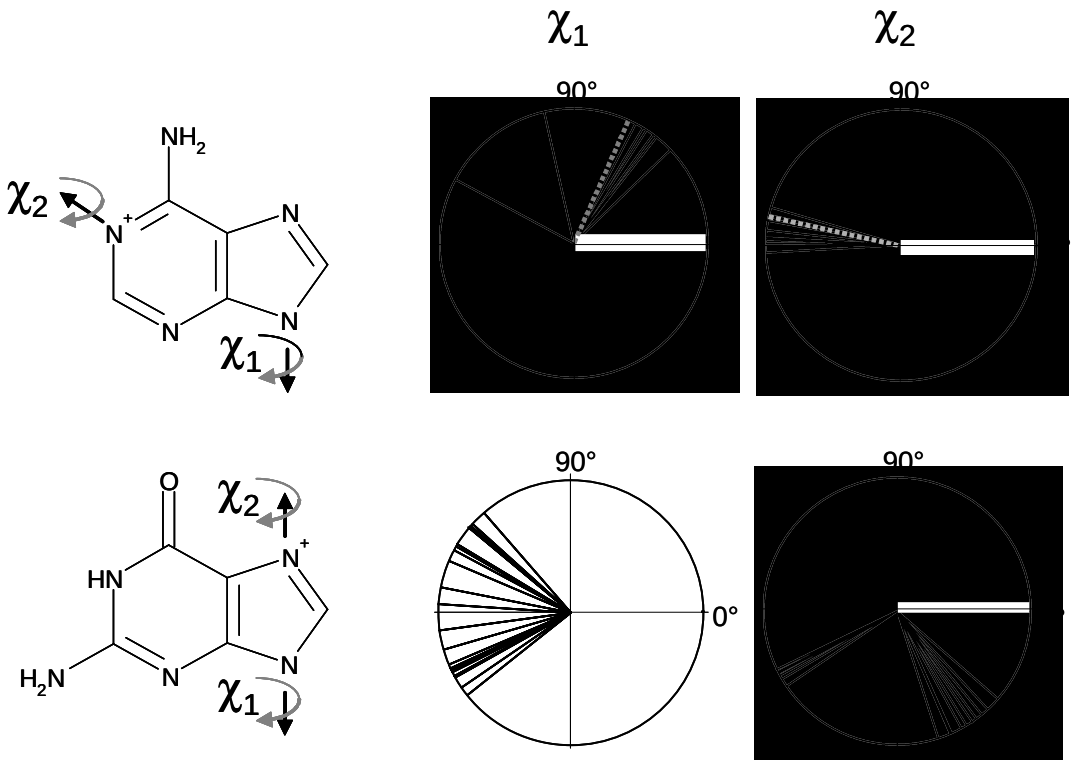

FIGURE S1: Base conformation in the cADPR and cGDPR structures obtained from simulations. The χ torsion angles about the N-glycosidic bonds for conformer ensembles generated using MacroModel are shown on wheel plots. The range 0 ± 90° is denoted as *syn* and the range 180 ± 90° is denoted as *anti*. The χ_1 torsion is defined by the O'4-C'1–N9-C4 sequence of atoms. The χ_2 torsion is defined by O"1-C"1-N1-C6 and O"1-C"1-N7-C5 sequence of atoms in cADPR and cGDPR, respectively. The experimental χ_1 and χ_2 values measured in the cADPR crystal structure are indicated using dotted lines. The cADPR conformational search yielded 10 low-energy conformers that were all similar to the available crystal structure of free cADPR (average RMSD computed for all heavy atoms: 0.83Å ± 0.26). The small conformational changes of cADPR were due to the flexibility in the pyrophosphate chain**.**. The exploration of cGDPR structure yielded an homogeneous

ensemble of 22 low-energy conformers (average RMSD to the lowest energy conformers computed over all heavy atoms: 1.38Å ± 0.66). Again, motions were allowed in the pyrophosphate chain. In addition, cGDPR showed some variability in the torsion angle about the N-glycosidic bonds.

Adenine and guanine binding mode into SmNACE: are docking poses consistent with experimental data available in the Protein Data Bank?

Several recent publications/reviews, which have compared various nucleotide binding sites in proteins (*1-4*), might be of interest to help to explain why the reaction outcomes are so different when NAD^+ and NGD^+ are transformed by *Sm*NACE and other ADP-ribosyl cyclase family members. The empirical approach of Saito et al. (*3*), rationalizes the features responsible for discriminating between adenine and guanine. It appears that the protein backbone atoms are often H-bonded to nitrogen atoms of the Watson-Crick edge of adenine, whereas side chain oxygen atoms give H-bonds with polar atoms of the Watson-Crick edge of guanine. The adenine base has been shown to be more frequently recognized through π-electron interactions than the guanine base. More precisely, charged or aromatic nitrogen in vicinity of the five- and six–membered rings of the adenine, respectively, often make π-electron H-bond with the base, thereby determining the adenine position (*5*). Although our structural assumptions for adenine and guanine recognition by *Sm*NACE do not strongly agree with these observations, they are nevertheless valuable since the above-described statistics were obtained for ground-state interaction complexes between protein and base-containing molecules, whereas in *Sm*NACE, the binding of the A/G bases is more transient/dynamic because they are directly involved, as nucleophiles, in the intramolecular cyclization reaction mechanism. Eventually, structural information from X-ray crystallography or NMR as well as mutagenesis would allow the validation of our hypothesis of the binding mode of these substrates.

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