SUPPORTING INFORMATION

The Bateman domain of IMP dehydrogenase is a binding target for dinucleoside polyphosphates

Running title: Binding of dinucleoside polyphosphates to IMPDH

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SUPPLEMENTAL TABLES

Supplemental Table 1. Structural parameters derived from the SAXS data shown in the Figure 3B of the main text.

	TETRAMERS		**EXTENDED OCTAMERS**			**COMPACTED OCTAMERS**			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	control	**0.3mM GDP**	**3mM ATP**	**0.1mM Ap6A**	**0.1mM Ap6A / 3mM GDP**	**3mM GDP**	**0.1mM Ap5G / 0.3mM GDP**	**0.1mM Ap4A / 3mM GDP**	**3mM ATP / 3mM GDP**
Guinier analysis									
Rg (Å)	45.6 ± 0.3	45.5 ± 0.3	52.7 ± 0.3	51.9 ± 0.2	53.0 ± 0.6	49.9 ± 0.2	49.7 ± 0.2	49.9 ± 0.3	50.0 ± 0.2
qRg max	1.3	1.3	1.2	1.3	1.3	1.3	1.2	1.3	1.2
P(r) analysis									
Rg (Å)	45.8 ± 0.4	45.7 ± 0.4	52.6 ± 0.8	51.7 ± 0.7	51.7 ± 0.3	50.0 ± 0.8	49.3 ± 0.8	49.9 ± 0.8	50.1 ± 0.8
d_{max} *(Å)*	157	155	148	148	158	150	146	151	155
Particle volumen (Å^3)	431000	426000	833000	874000	901000	899000	879000	894000	909000
GNOM quality estimate	0.85	0.83	0.76	0.75	0.86	0.73	0.72	0.73	0.73

SUPPLEMENTAL FIGURE CAPTIONS

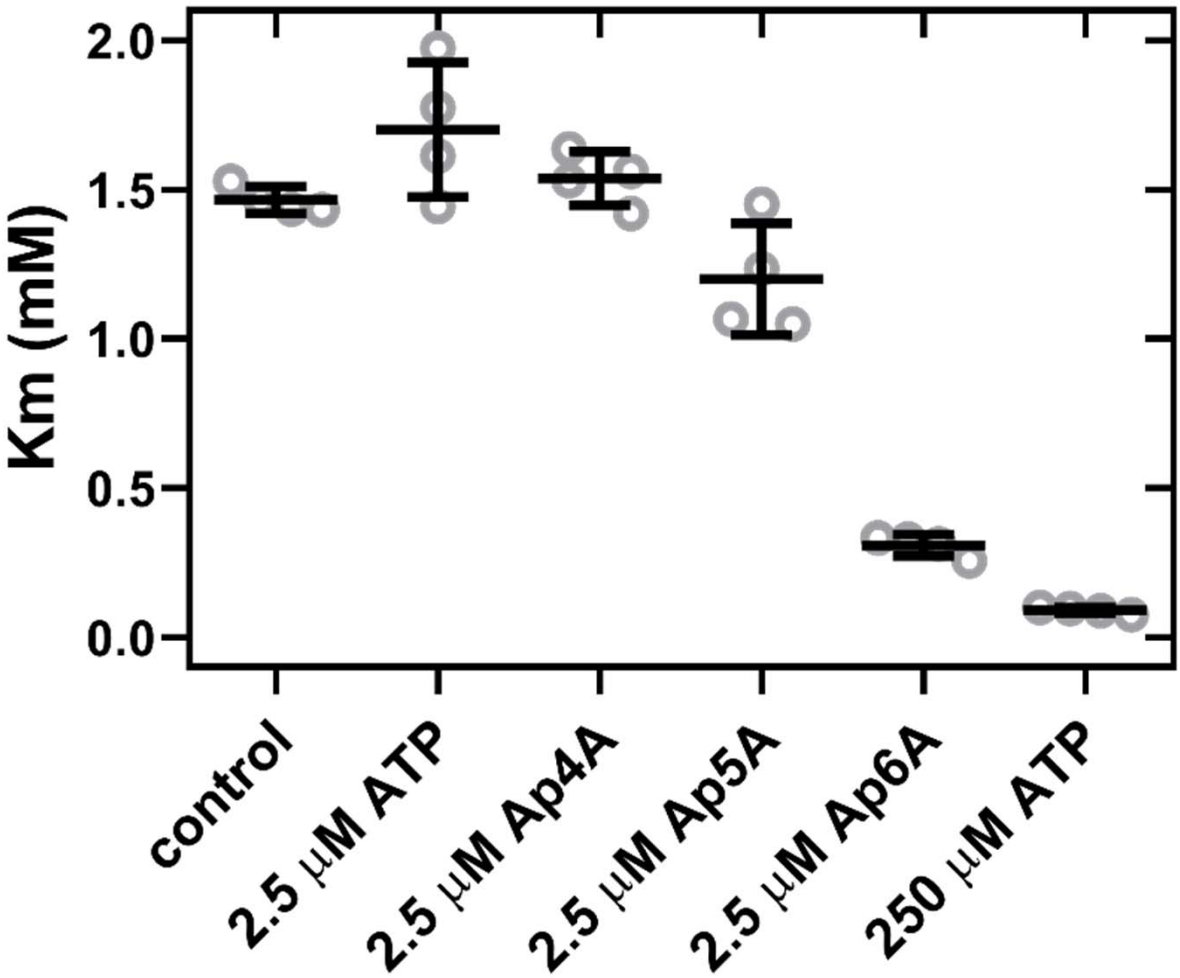
Supplemental Figure 1. *Dinucleoside polyphosphate activation of the bacterial PaIMPDH enzyme.* Scatter plot showing the K_M values (and the standard errors derived from 4 independent measurements) obtained from the Michaelis-Menten analysis of the experimental data. Reactive concentrations were set to 15 µg/mL enzyme, 0.5 mM NAD^+ and 0.039-5 mM IMP. The reaction buffer was 100 mM TrisHCl, pH 8.0, 100 mM KCl, 1 mM free $MgCl_2$, 2 mM DTT and the reactions were performed at 32ºC on 384-well plates by monitoring the increase in absorbance at 340 nm, due to NADH formation.

Supplemental Figure 2. *Structure of the ternary complex AgIMPDH-Ap5G-GDP.* **A.** Cartoon representation of the structure of AgIMPDH octamers formed in the presence of Ap5G (orange sticks) and GDP (red sticks). The catalytic domain is shown in light grey, while the Bateman regulatory domain is colored in blue. **B.** Structural alignment of monomers from AgIMPDH-ATP-GDP (PDB code 5TC3; orange cartoons), AgIMPDH-GDP (PDB code 4Z87; blue cartoons) and AgIMPDH-Ap5G-GDP (6RPU from this work; green cartoons).

Supplemental Figure 3. *Dinucleoside polyphosphates occupy a positively charged groove in the Bateman domain of IMPDHs.* **A.** Close-up stereoview of Ap5G (sticks) bound to the two canonical sites of the Bateman domain of AgIMPDH (green cartoon). The blue mesh represents the final SA-omit F_o-F_c electron density, contoured at 1.4σ level. **B.** Surface electrostatic potential representation showing the highly charged positive groove where the polyphosphate chain of Ap5G (shown in sticks) is buried.

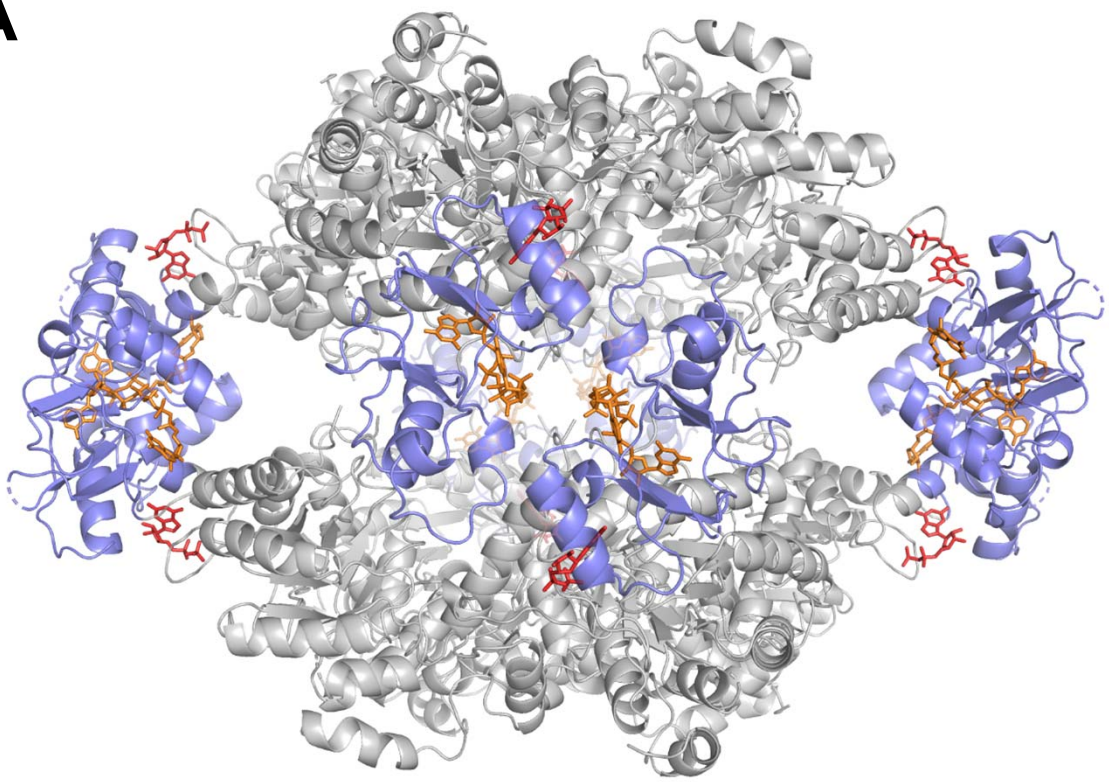
Supplemental Figure 4. *Differences in nucleotide binding to the Bateman domain of IMPDH.* **A.** Close-up views of the nucleotides (shown in sticks) bound to the two canonical sites of AgIMPDH (represented in semi-transparent blue cartoons). Blue: AgIMPDH-Ap5G (PDB code 6RPU from this work). Green: AgIMPDH-GDP (PDB code 4Z87). **B.** Red: HsIMPDH2-GDP (PDB code 6I0M). Green: AgIMPDH-GDP (PDB code 4Z87). The canonical binding sites (1 or 2) are indicated after the corresponding nucleotides, i.e. GDP1 means GDP bound to the canonical site 1.

SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2

A



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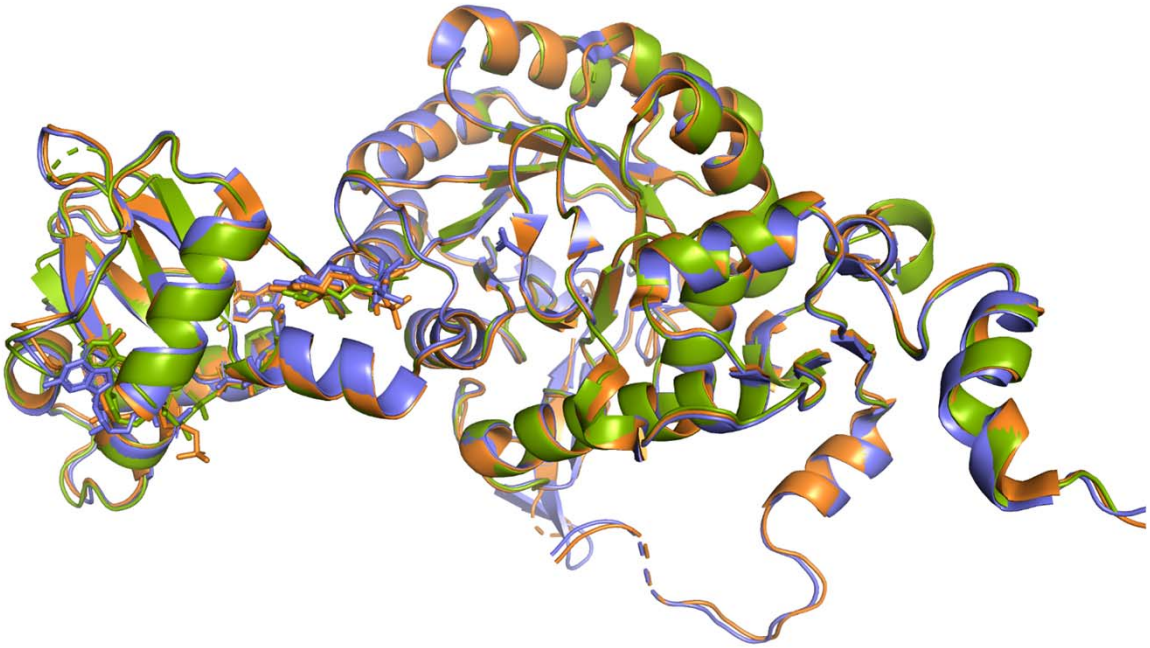
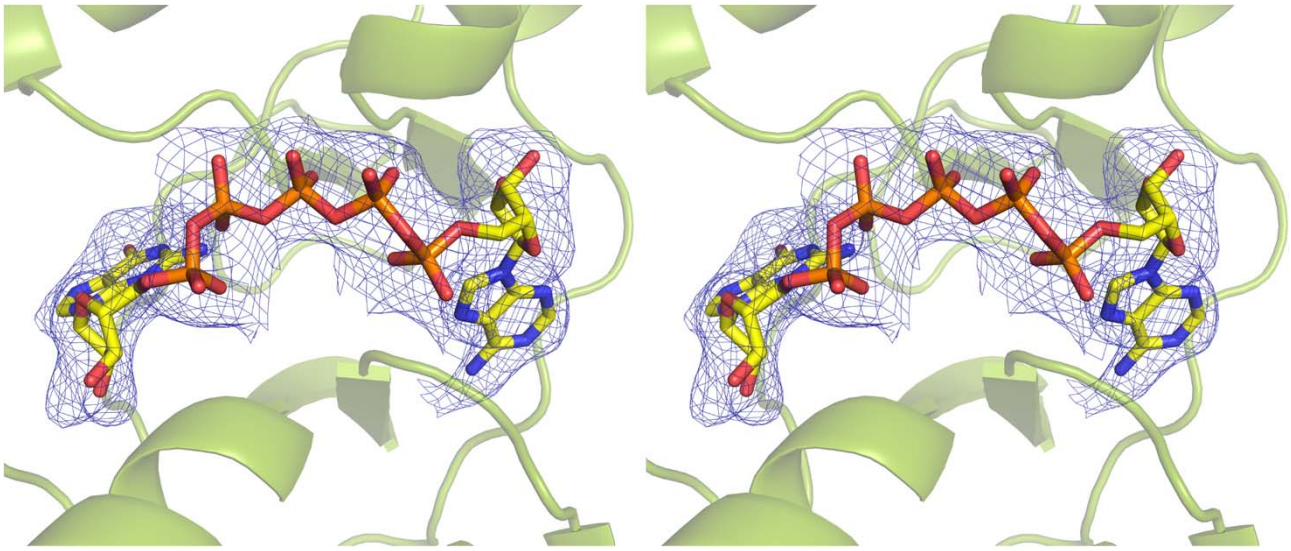


SUPPLEMENTAL FIGURE 3

A



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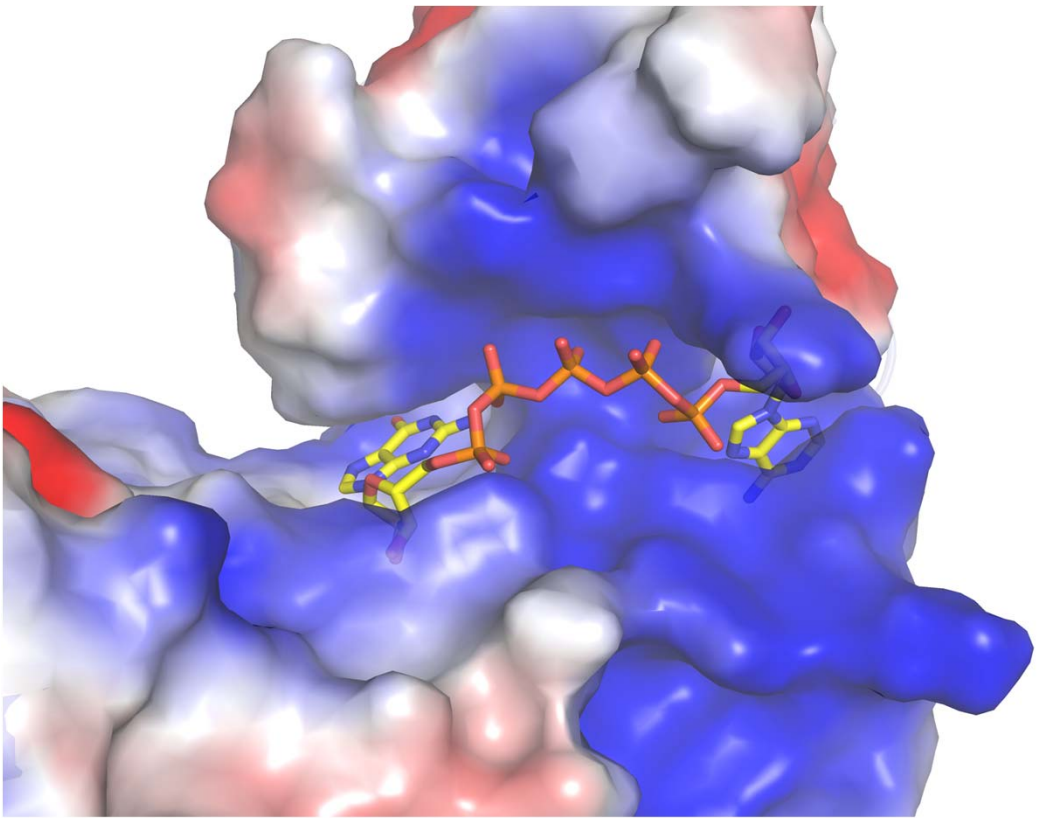
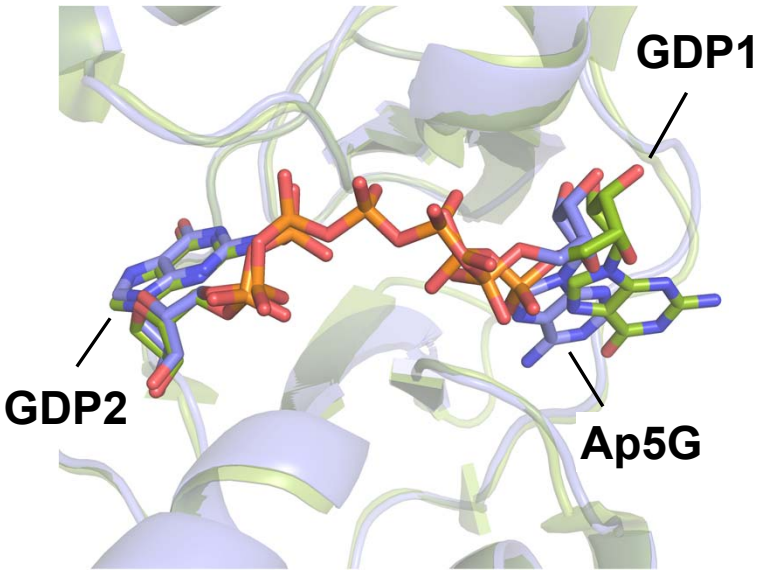


SUPPLEMENTAL FIGURE 4

A



B