Article

Characterization of two NMN deamidase mutants as possible probes for an NMN biosensor

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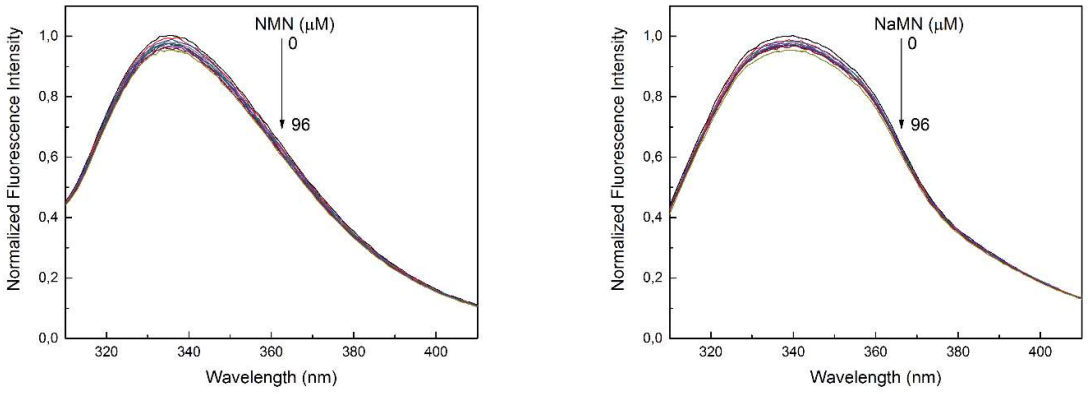
Supplementary Table 1. S29A PncC fluorescence quenching (%) *vs* buffer

	Nucleotides concentration [μM]									
	0.185	**0.375**	**0.75**	**1.5**	**3**	**6**	**12**	**24**	**48**	**96**
NMN	0.48	0.58	2.58	10,.1	17.42	17.55	27.06	37.52	46.79	52.82
NaNM	1.79	2.56	6.47	20.83	30.74	30.87	40.52	47.93	52.28	55.20
Na	0	0	0	0	0	0	0	0.16	0.62	0.24
Nam	0.08	0.18	0.48	1.49	2.51	2.65	3.29	3.29	3.71	5.01
NR	0	0.12	0.80	0.96	1.84	1.98	2.29	3.13	3.51	4.48
NAD	0.52	0.32	0.80	0	0.19	0.32	1.35	2.54	4.58	7.97
NADP	0	0	0.67	1.60	2.74	2.87	3.89	4.31	6.36	9.50
NaAD	0	0	0	0	0	0	0	0	1.97	6.69

Supplementary Table 2. K61Q PncC fluorescence quenching (%) *vs* buffer

	Nucleotides concentration [μM]									
	0.185	**0.375**	**0.75**	**1.5**	**3**	**6**	**12**	**24**	**48**	**96**
NMN	1.09	1.84	3.19	4.87	6.26	8.11	12.12	20.07	38.03	53.35
NaNM	0.71	1.11	2.05	2.12	2.82	2.80	4.14	4.9	7.14	10.68
Na	0.49	1.2	2.11	2.66	2.89	2.81	3.26	2.74	3.69	4.46
Nam	0	0.34	0.87	0.68	0.91	0.26	1.15	1.04	1.14	1.57
NR	2.13	2.97	3.37	3.71	3.13	2.36	2.7	2.1	1.79	1.49
NAD	0.63	1.39	1.57	0.80	0.85	0	0.65	0.36	0.72	1.58
NADP	0	0.06	0.29	0.55	0.42	0	1.20	1.59	2.90	5.10
NaAD	0.65	1.02	1.68	2.19	2.25	2.39	3.72	4.13	5.77	8.28

Supplementary table 1 and table 2: S29A PncC and K61Q PncC fluorescence quenching (%) at increasing concentration of nucleotides. The percentages of reduction of fluorescence intensities compared to buffer, were calculated as follow: (normalized fluorescence in presence of analyte at a given [μM]/normalized fluorescence in presence of corresponding volume of buffer) *100.



(a) (b)

GlnBP fluorescence reduction (%) vs buffer

	Nucleotide concentration [μM]								
	0,375	**0,75**	**1,5**	**3**	**6**	**12**	**24**	**48**	**96**
NMN	1,21	1,34	0,689	0,882	2,354	2,657	4,212	3,842	4,362
NaNM	1,463	0,699	0,375	0,567	2,273	1,958	3,227	3,267	4,358

(c)

Figure S1: Effects of NMN and NaMN on steady-state fluorescence emission of GlnBP. GlnPB (3μM) was incubated with increasing concentration of NMN, NaMN or buffer (not shown), as described for the PncC proteins. **(a)** and **(b)** steady-state fluorescence emission representative spectra **(c)** Percentages of reduction of fluorescence intensities compared to buffer, were calculated as follow: (normalized fluorescence in presence of analyte at a given [μM]/normalized fluorescence in presence of corresponding volume of buffer) *100.