

1 **Electronic Supplementary Information**

2 **A new cross-linked enzyme aggregate biocatalyst for a NAD<sup>+</sup>-booster**  
3 **production**

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13 **Supplementary file captions**

14 **Table S1.** Activity recovery of the EcCinA-BSA-CLEAs as a function of mixing period and  
15 the system used.

16 **Fig. S1.** SDS-PAGE of purified EcCinA.

17 **Fig. S2.** Conversion of NMN into NaMN catalyzed by recombinant EcCinA.

18 **Fig. S3.** Effect of NMN concentration on EcCinA activity.

19

20 **Table S1**

21 Activity recovery of the EcCinA-BSA-CLEAs as a function of mixing period and the system  
 22 used.

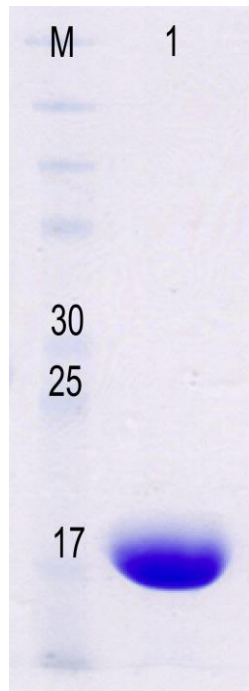
Vortex <sup>a</sup>		FastPrep <sup>b</sup>	
Time (min)	Activity Recovery (%)	Time (min)	Activity Recovery (%)
0.5	34 ± 1	0.16	43 ± 1
30	51 ± 2	0.33	67 ± 1
60	90 ± 2	0.5	76 ± 3
		0.66	84 ± 2
		0.83	99 ± 1
		1	99 ± 1

23 The experiments were carried out in triplicate and the error  
 24 represents the percentage error in each set of readings.

25 <sup>a</sup>Mixing was carried out with a vortex at medium speed.

26 <sup>b</sup>Mixing was carried out with a FastPrep at a speed of 6.0 m/s.

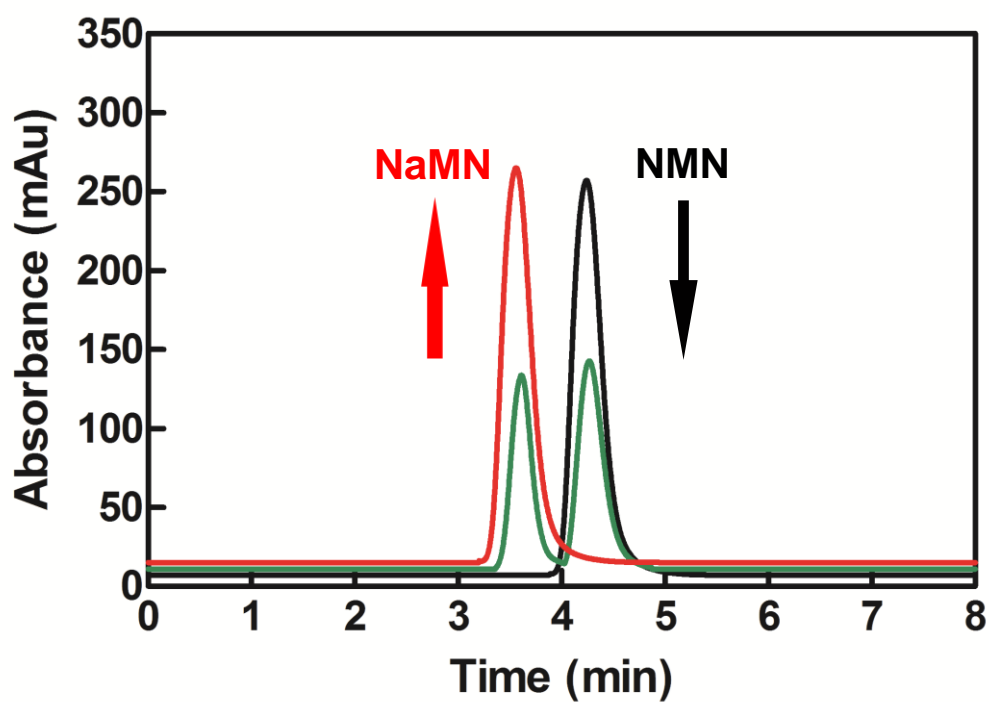
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29 Fig. S1. SDS-PAGE of purified EcCinA. M: molecular weight standards (New England  
30 Biolabs: P7708S). Lane 1: Purified EcCinA (10  $\mu$ g) after HisTrap column step.

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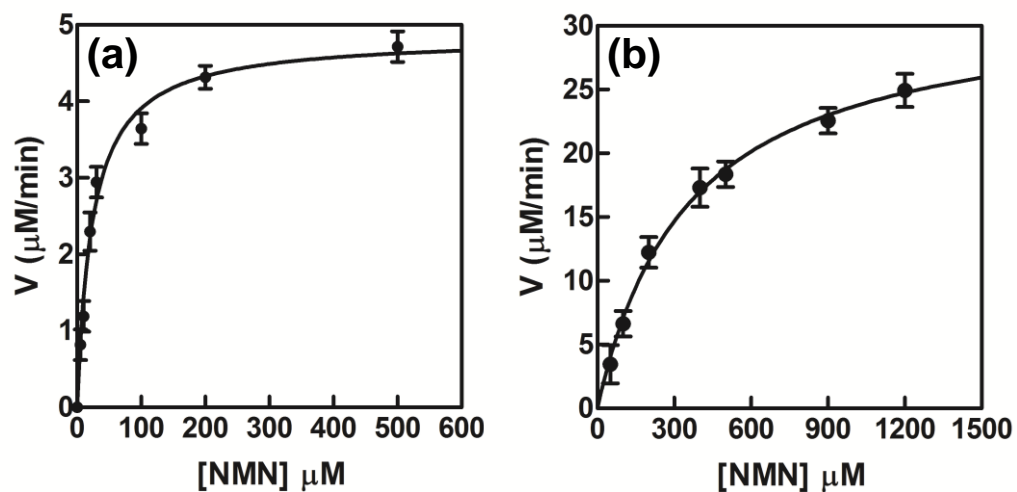
33 Fig. S2. Conversion of NMN into NaMN catalyzed by recombinant EcCinA. Black line

34 corresponds to 0 min, green line to 15 min and red line to 30 min. For the assay, free enzyme

35 (14 nM) was incubated at 37 °C with the substrate (0.5 mM NMN) in 50 mM Tris-HCl pH

36 8.5.

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38

39 Fig. S3. Effect of NMN concentration on EcCinA activity. **(a)** Free enzyme. **(b)** EcCinA-  
 40 BSA-CLEAs. The activity was measured in the standard reaction conditions in the presence  
 41 of increasing concentrations of NMN.