## *In vitro* selection of deoxyribozymes active with Cd<sup>2+</sup> ions resulting in variants of DNAzyme 8-17

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Supplementary Figures S1 – S5.



**Figure S1.** Autoradiogram showing the self-cleaving activity of  $Cd^{2+}$ -dependent DNAzyme library after the 5<sup>th</sup> round of selection (panel on the left) and the cleavage efficiency of the library after each of the ten rounds of selection (panel on the right).

		50μM															100μM			
0	5,	$Sr^{2+}$	<u> </u>	Ni <sup>2+</sup>	(	Ca²⁺	( (	$20^{2+}$		$Mn^{2+}$	Z	$2n^{2+}$	N	$1g^{2+}$		$d^{2+}$	N C	$Mg^{2+}$	( (	2d <sup>2+</sup>
	5	30	5	30	5	30	5	30	5	30	5	30	5	30	5	30'	5	30	5	30
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-	S	<b>Sr</b> <sup>2+</sup>	1	<b>Vi</b> <sup>2+</sup>	(	Ca <sup>2+</sup>	C	50 2+	μM 	Mn <sup>2+</sup>	Z	.n <sup>2+</sup>	N	1g <sup>2+</sup>	С	d <sup>2+</sup>	N	100 1g <sup>2+</sup>	μM 	d <sup>2+</sup>
С	5'	Sr²⁺ 30'	۱ 5'	Ni <sup>2+</sup> 30'	( 5'	Ca²⁺ 30'	C 5'	50 co <sup>2+</sup> 30'	μΜ Ν 5'	//n²⁺ 30'	Z 5'	'.n²+ 30'	_N 5'	1g²⁺ 30'	C 5'	d²⁺ 30'	_N 5'	100 1g <sup>2+</sup> 30'	)μM C 5'	2d²⁺ 30'
C	5'	Sr <sup>2+</sup> 30'	5'	Ni <sup>2+</sup> 30'	5'	Ca²⁺ 30'	C 5'	50 20 <sup>2+</sup> 30'	μM N 5'	<u>√In²</u> + 30'	 5'	2n²⁺ 30'	_N 5'	1g²⁺ 30'	5'	2d²+ 30'	N 5'	100 1g²⁺ 30'	)μM C 5'	2d²⁺ 30'
C	5'	Sr <sup>2+</sup> 30'	5'	Ni <sup>2+</sup> 30'	5'	Ca <sup>2+</sup> 30'	5'	50 20 <sup>2+</sup> 30'	μM 5'	<u>√In<sup>2+</sup></u> 30'	Z 5'	2n <sup>2+</sup> 30'	_N 5'	1g <sup>2+</sup> 30'	5'	2 <sup>2+</sup> 30'	_N 5'	100 1g <sup>2+</sup> 30'	)μM C 5'	2d <sup>2+</sup> 30'
C	5'	Sr <sup>2+</sup> 30'	5'	Ni <sup>2+</sup> 30'	( 5'	Ca²⁺ 30'	5'	50 co <sup>2+</sup> 30'	μM 5'	VIn <sup>2+</sup> 30'	Z 5'	2n <sup>2+</sup> 30'	N 5'	1g²⁺ 30'	5'	2d <sup>2+</sup> 30'	N 5'	100 1g <sup>2+</sup> 30'	)μM C 5'	2d²+ 30'

**Figure S2.** Autoradiograms showing divalent metal-ion specificities of *cis*-acting DNAzymes which belong to the first group of selected variants. (A) DNAzyme 1/VII (B) DNAzyme 22/VII.



**Figure S3.** Survey of metal-ion selectivity of the DNAzyme 1/VII (grey bars) and DNAzyme 22/VII (bars with diagonal lines). The assays were carried out with various divalent metal ions at pH 7.0. (A) for 5 min at 25 °C and (B) for 30 min incubation at 25 °C.



**Figure S4**. Proposed secondary structure models of DNAzyme 1/VII which were generated by RNAstructure 5.4 program using the constrains from structural enzymatic and chemical probing. The DMS-modified cytosine residues are marked by dark grey circles. Digestions with nuclease S1 are denoted by arrows. Italic black letters mark the catalytic core of DNAzyme 1/VII.



**Figure S5.** Determination of the cleavage rate constant,  $k_{obs}$  of the shortened *cis*-acting DNAzymes Dz1/VIIWS (A), Dz5/XWS (B) and Dz15/XWS (C) in the presence of Cd<sup>2+</sup> ions. The assays were carried out with 50  $\mu$ M metal ions at pH 7.0 and 25 °C. The  $k_{obs}$  values were determined by plotting the natural logarithm of the fraction of DNA that remained unreacted versus the reaction time.