

Automated Enrichment of Sulfanilamide in Milk Matrices by Utilization of Aptamer-Linked Magnetic Particles

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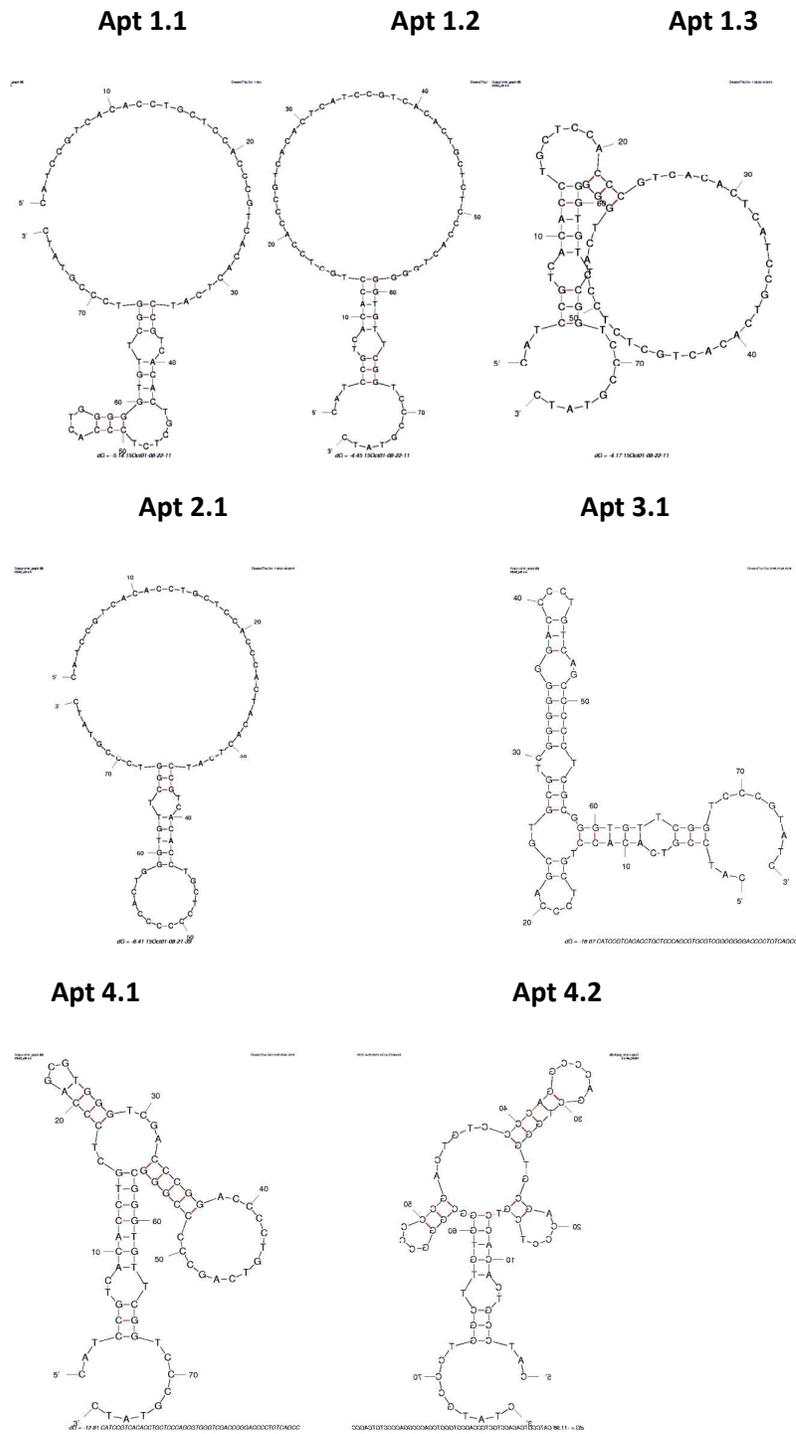
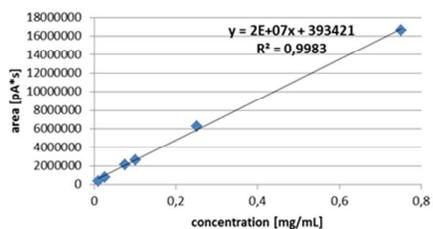


Figure S1: Predicted secondary structures for generated sulfanilamide aptamers using mfold V4.6.

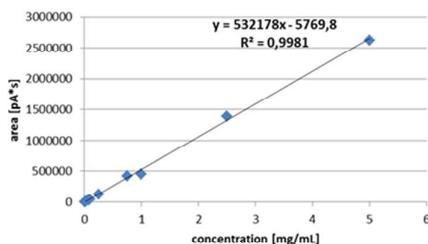
Sulf Apt 1	CATCCGTCAC	ACCTGCTCCA	CCCGTCACAC	TCATCCGTCA	CACTGCTCTC	CCACTGGGGG	TGTCGGTCC	CGTATC
Sulf Apt 2ACT...CTGCTC.	..C.ACT.
Sulf Apt 3C AG...GCGT.	GGGGG.A.C	.CTGT.AGC.	..CTC.C.
Sulf Apt 4C AG...GGGT.	GACC.G.A.C	.CTGT.AGC.	..CGG.C.
Identität	*****	*****	* * *	* * *	* * *	* * *	***	*****

Figure S2: Alignment of aptamer sequences with an affinity towards sulfanilamide. Primer regions are marked in grey.

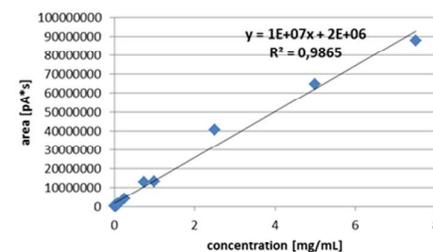
ampicillin



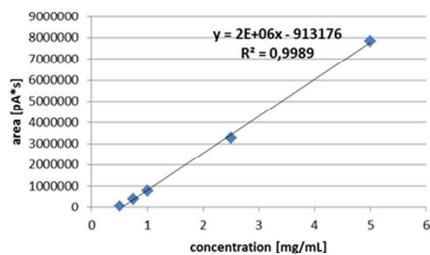
sulfanilamide



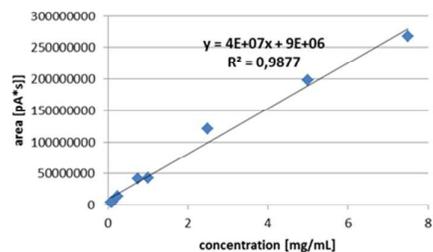
tetracycline



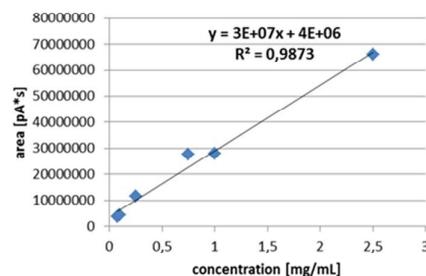
chlortetracycline



oxytetracycline



sulfadiazine



sulfamerazine

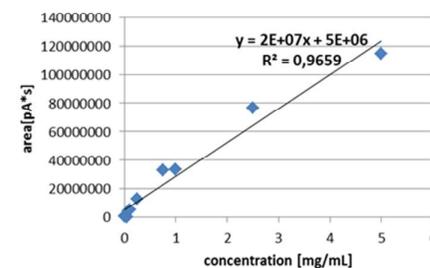


Figure S3: Resulting LC-ESI-MS/MS calibration curves for quantitation of particle coupling efficiency

Table S1. LC-Gradient for LC-ESI-MS/MS quantitation of antibiotics used in this study.

time [min]	A (water + 0.1 % FA) [%]	B (acetonitrile + 0.1 % FA) [%]
0.0	95	5
2.0	95	5
5.0	80	20
7.5	80	0
10.0	0	100
13.0	0	100
13.5	95	5
19.0	95	5

Table S2. Operating MRM MS method parameters for quantitation of ampicillin (A), sulfanilamide (S1), tetracycline (T), sulfamerazine (S2), sulfadiazine (S3), chlortetracycline (CT), and oxytetracycline (OT).

transitions	CE [V]	CXP [V]	DP [V]	EP [V]	Rt [min]
Qnt A 350.168 → 106.0	25	18	66	10	8.38
Q1 A 350.168 → 192.1	21	4			
Q2 A 350.168 → 159.9	17	10			
Q3 A 350.168 → 174.1	23	14			
Qnt S1 173.040 → 155.9	9	46	61	10	2.47
Q1 S1 173.040 → 92.1	25	6			
Q2 S1 173.040 → 108.1	21	8			
Q3 S1 173.040 → 65.1	41	18			
Qnt T 445.180 → 410.0	27	12	91	10	9.51
Q1 T 445.180 → 427.4	19	14			
Q2 T 445.180 → 154.3	37	12			
Q3 T 445.180 → 241.1	55	20			
Qnt S2 265.157 → 156.0	25	24	46	10	8.50
Q1 S2 265.157 → 172.1	23	12			
Q2 S2 265.157 → 91.9	41	14			
Q3 S2 265.157 → 108.0	39	6			
Qnt S3 251.038 → 156.0	23	10	46	10	7.02
Q1 S3 251.038 → 91.9	41	14			
Q2 S3 251.038 → 108.2	35	6			
Q3 S3 251.038 → 65.0	63	8			
Qnt CT 479.141 → 197.0	65	30	56	10	10.35
Q1 CT 479.141 → 98.1	75	16			
Q2 CT 479.141 → 275.0	57	10			
Qnt OT 461.189 → 426.1	29	30	51	10	8.97
Q1 OT 461.189 → 444.1	27	24			
Q2 OT 461.189 → 283.0	57	40			
Q3 OT 461.189 → 201.2	53	8			

CE = collision energy, CXP = collision cell exit potential, DP = declustering potential, EP = entrance potential, Rt = retention time Qnt = quantifier, Q1-Q4 = qualifier

Table S3: Percent identity matrix of generated aptamers without primer regions designed with Clustal Omega.

	Sulf-Apt-3	Sulf-Apt-4	Sulf-Apt-1	Sulf-Apt-2
Sulf-Apt-3	100.00	82.50	39.47	44.74
Sulf-Apt-4	82.50	100.00	47.37	47.37
Sulf-Apt-1	39.47	47.37	100.00	67.50
Sulf-Apt-2	44.74	47.37	67.50	100.00