Identification of cell membrane protein stress-induced-phosphoprotein 1 (STIP1) as a potential ovarian cancer biomarker using aptamers selected by cell-SELEX

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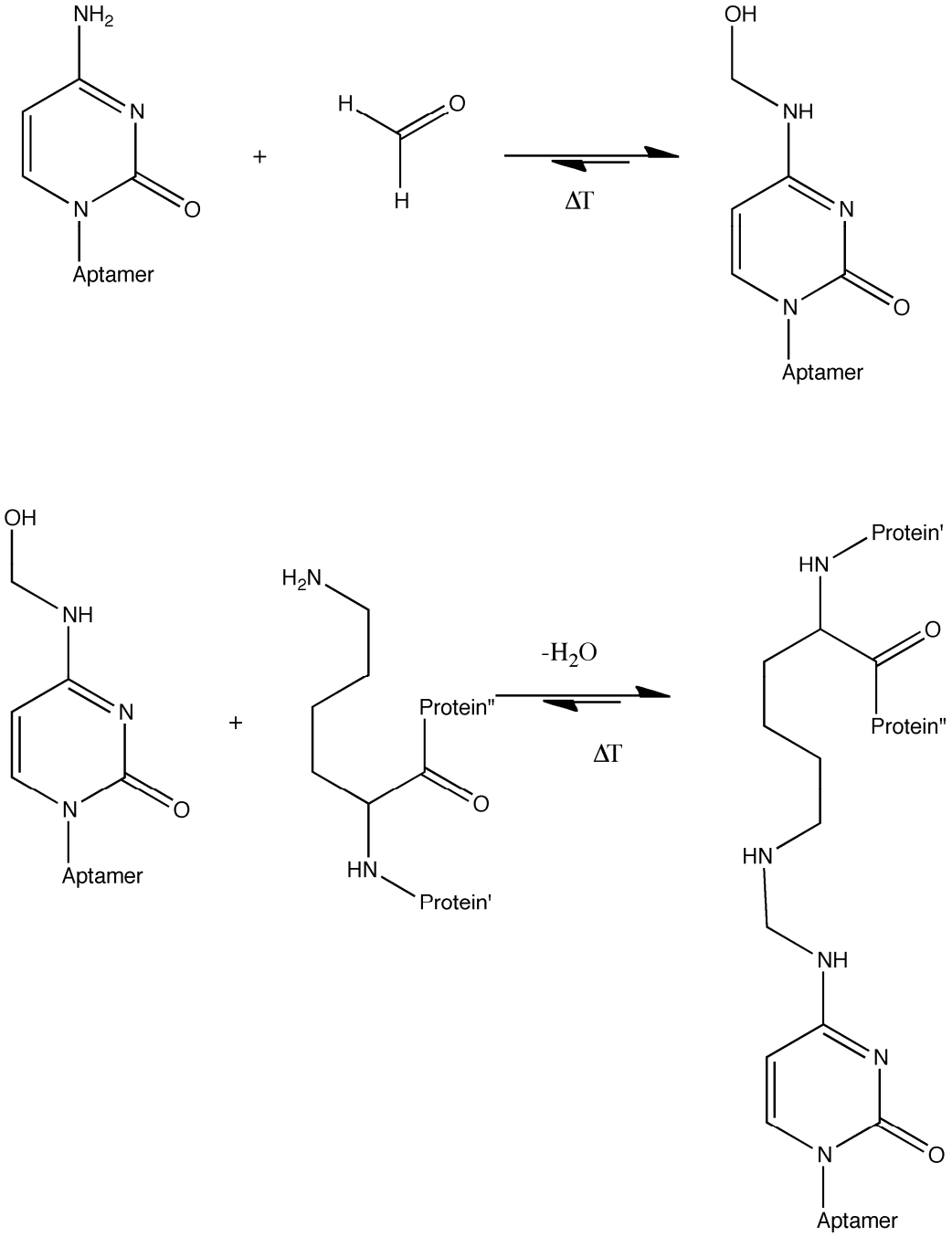
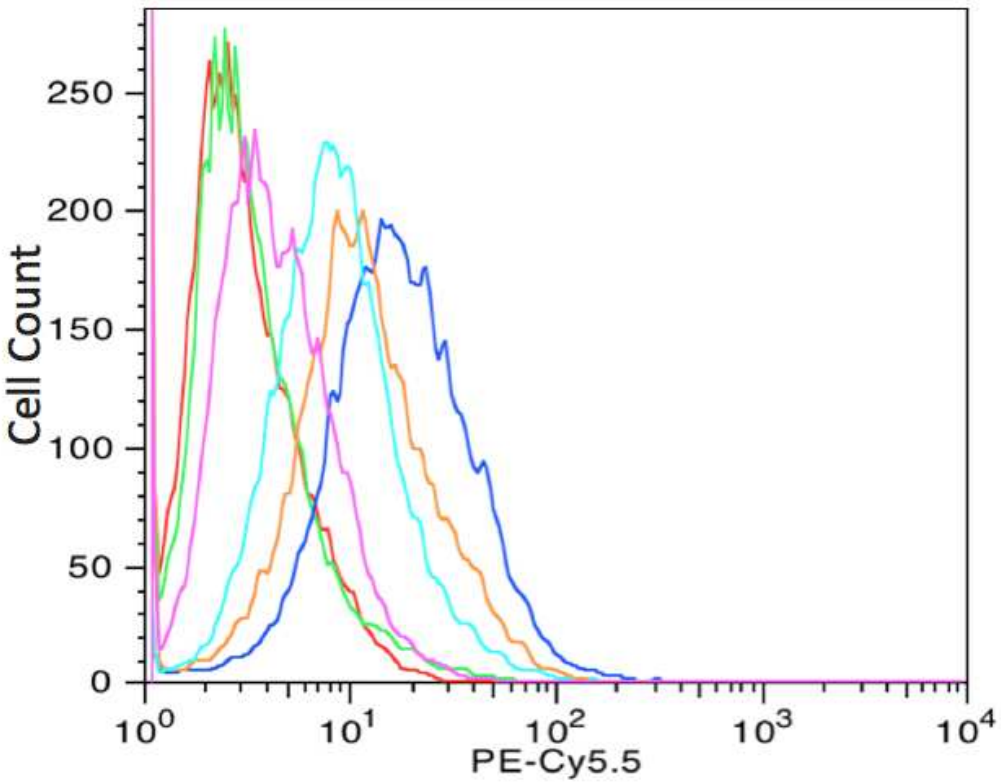
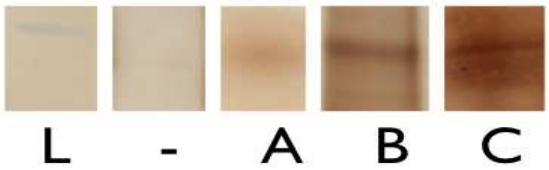
Supplemental Figures



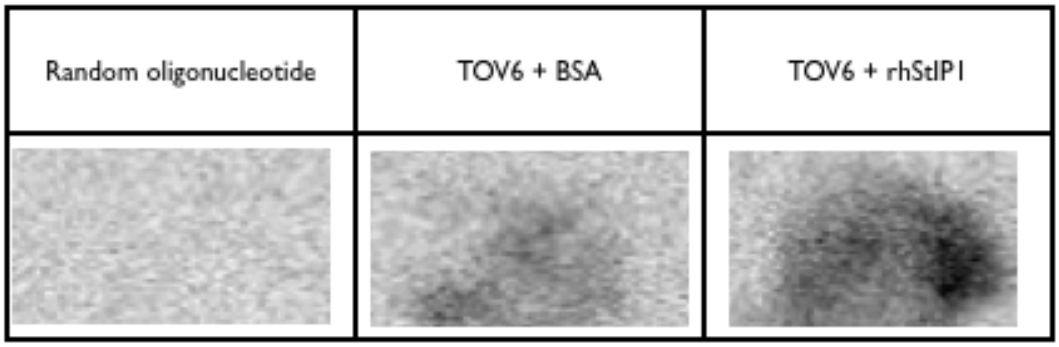
Supplemental Scheme1. The chemistry of formaldehyde mediated DNA-Protein crosslinking. In this example cytidine crosslinks to a lysine. The crosslink can be reversed by applying heat (>72ºC).



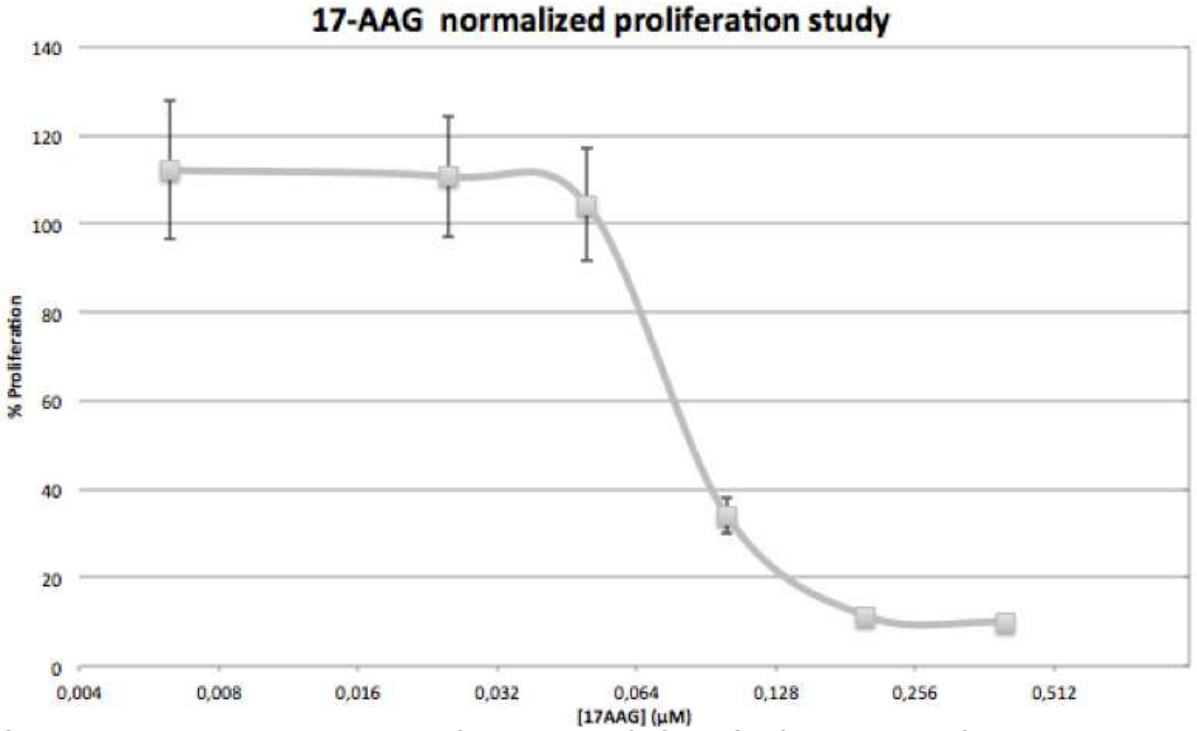
Supplemental Figure 1: Study of the effect of formaldehyde on streptavidin-fluorescent dye binding and recovery of the aptamer-protein hybrid by biotin elution of the desthiobiotin-conjugated aptamer. Red: TOV-21G without oligonucleotides; Green: Library; Dark blue: TOV6; Orange: TOV6 in 1% CH_2O for 10 minutes; Light blue: TOV6 after 30 minutes in 5mM biotin solution; Magenta: TOV6 in 1% CH_2O for 10 minutes after 30 minutes in 5mM biotin solution. A loss in fluorescence indicates the competition between biotin and desthiobiotin.



Supplemental Figure 2: Optimization of crosslinking time with 1% crosslinking solution. L: Ladder band at 80 kDa; '-': Band from naïve library; A: 1 minute of crosslinking time; B: 2 minutes of crosslinking time; C: 10 minutes of crosslinking time. After one minute of crosslinking time, a band is faintly visible, but does not yield enough protein for mass spectral analysis. After 10 minutes of crosslinking time, a band is visible, but nonspecific crosslinking between the target and cellular protein forms, making analysis difficult by the presence of streaks around the band of interest.



Supplemental Figure 3 : Chemiluminescent blot of rhSTIP1. The aptamer is able to induce a strong chemiluminescent signal with rhSTIP1, but not with BSA. When staining the protein with library, no luminescence can be observed.



Supplemental Figure 4: Normalized proliferation study of TOV-21G. After three days of incubation with 17AAG, the IC50 was determined to be 0.060µM by nonlinear regression in JMP. The error bars give the standard deviation (n = 3).

Supplemental Table 1. Proteins found in aptamer TOV6 binding fractions (78kDa). STIP1 was the only top protein found in both samples sent for analysis. Each column represents the protein hits for each sample.

Sample 1		**Sample 2**	
Protein matches		Protein matches	
Peptides identified	Gene annotation	Peptides identified	Gene annotation
5	STIP1_IPI:IPI00013894.1	34	LMNA_IPI:IPI00021405.3
4	ALB_IPI:IPI00022434.4	32	FLNA_IPI:IPI00302592.2
4	TKT_IPI:IPI00643920.3	23	HNRNPM_IPI:IPI00171903.2
3	HSPA1A_IPI:IPI00304925.5	21	STIP1_IPI:IPI00013894.1
3	HSPA8_IPI:IPI00003865.1	20	CKAP4_IPI:IPI00141318.2
3	RPN1_IPI:IPI00025874.2	19	RPN1_IPI:IPI00025874.2
3	NCL_IPI:IPI00444262.3	18	TKT_IPI:IPI00643920.3
3	ACTA2_IPI:IPI00008603.1	16	NOP56_IPI:IPI00411937.4
2	IPI:IPI00550731.2_IPI:IPI00550731.2	16	DDX5_IPI:IPI00017617.1
2	HSPA5_IPI:IPI00003362.2	13	XRCC5_IPI:IPI00220834.8
2	TUBA1C_IPI:IPI00166768.3	13	LMNB1_IPI:IPI00217975.4
2	ACTBL2_IPI:IPI00003269.1	12	CLTC_IPI:IPI00024067.4
2	ANXA2P2_IPI:IPI00334627.3	12	RPA1_IPI:IPI00020127.1
2	GAPDH_IPI:IPI00219018.7	12	MATR3_IPI:IPI00017297.1
2	PKM2_IPI:IPI00220644.8	10	HSPA8_IPI:IPI00003865.1
1	ENO1_IPI:IPI00465248.5	10	NCL_IPI:IPI00183526.6

Supplemental Table 2: Proteins found in aptamer TOV6 binding fractions (110kDa).

Protein matches	
Peptides identified	Gene annotation
30	EEF2_IPI:IPI00186290.6
28	CLTC_IPI:IPI00024067.4
28	HSP90B1_IPI:IPI00027230.3
27	PYGB_IPI:IPI00004358.4
21	PRKDC_IPI:IPI00296337.2
20	KPNB1_IPI:IPI00001639.2
18	AP2A2_IPI:IPI00016621.7
18	NCL_IPI:IPI00183526.6
17	COPG_IPI:IPI00783982.1
16	SFPQ_IPI:IPI00010740.1
14	TRIM28_IPI:IPI00438229.2
14	MATR3_IPI:IPI00017297.1
14	TOP1_IPI:IPI00413611.1
13	TLN1_IPI:IPI00298994.6

Supplemental Table 3. Proteins found in aptamer TOV6 binding fractions (50kDa).

Protein matches	
Peptides identified	Gene annotation
21	HNRNPM_IPI:IPI00171903.2
14	PKM2_IPI:IPI00220644.8
12	VIM_IPI:IPI00418471.6
12	FLNA_IPI:IPI00302592.2
11	ACTB_IPI:IPI00021439.1
11	HNRNPK_IPI:IPI00216049.1
11	SCIN_IPI:IPI00002606.5
10	ACAT1_IPI:IPI00030363.1
10	HNRNPA3_IPI:IPI00419373.1
10	PGK1_IPI:IPI00169383.3
9	SERPINB9_IPI:IPI00032139.1
9	HNRNPD_IPI:IPI00028888.1
9	ALDOA_IPI:IPI00465439.5
9	TUBA4A_IPI:IPI00007750.1
8	TUBB2C_IPI:IPI00007752.1
8	PRMT1_IPI:IPI00018522.4

Supplemental Table 4. Proteins found in aptamer TOV6 binding fractions (46 kDa).

Protein matches	
Peptides identified	Gene annotation
13	PKM2_IPI:IPI00220644.8
9	VIM_IPI:IPI00418471.6
8	ANXA2P2_IPI:IPI00334627.3
8	HNRNPH3_IPI:IPI00013877.2
8	TUBA4A_IPI:IPI00007750.1
8	AKR1B1_IPI:IPI00413641.7
7	HDGF_IPI:IPI00020956.1
7	ANXA1_IPI:IPI00218918.5
7	XRCC6_IPI:IPI00644712.4
7	CRYZ_IPI:IPI00000792.1
6	TUBB2C_IPI:IPI00007752.1
6	LASP1_IPI:IPI00000861.1
6	NPM1_IPI:IPI00220740.1
6	HNRNPA2B1_IPI:IPI00396378.3
6	HNRNPM_IPI:IPI00171903.2
5	HNRNPA1_IPI:IPI00215965.2
5	CRYL1_IPI:IPI00006443.3
5	ELAVL1_IPI:IPI00301936.4

Supplemental Table 5. Proteins found in aptamer TOV6 binding fractions (58kDa).

Protein matches	
Peptides identified	Gene annotation
17	VIM_IPI:IPI00418471.6
16	PKM2_IPI:IPI00220644.8
16	LMNA_IPI:IPI00021405.3
14	ENO1_IPI:IPI00465248.5
14	FLNA_IPI:IPI00302592.2
14	HNRNPK_IPI:IPI00216049.1
12	HNRNPM_IPI:IPI00171903.2
11	DCTN2_IPI:IPI00220503.9
11	CLTC_IPI:IPI00024067.4
11	SSB_IPI:IPI00009032.1
11	HADHB_IPI:IPI00022793.5
10	PGD_IPI:IPI00219525.1
10	HNRNPF_IPI:IPI00003881.5
10	GDI2_IPI:IPI00031461.2
9	SERPINH1_IPI:IPI00032140.4
9	TUBB2C_IPI:IPI00007752.1

Supplemental Table 6. The binding of aptamer TOV6 in ovarian cancer cell lines

Cell line	TOV-21G	SKOV-3	CAOV3	OVCAR-3	OVCAR-8	C13	A2780s	A2780cp
Binding	+	+	-	+	-	+	-	-