Supporting Information for:

Universal screening platform using three-dimensional small molecule microarray based on surface plasmon resonance imaging

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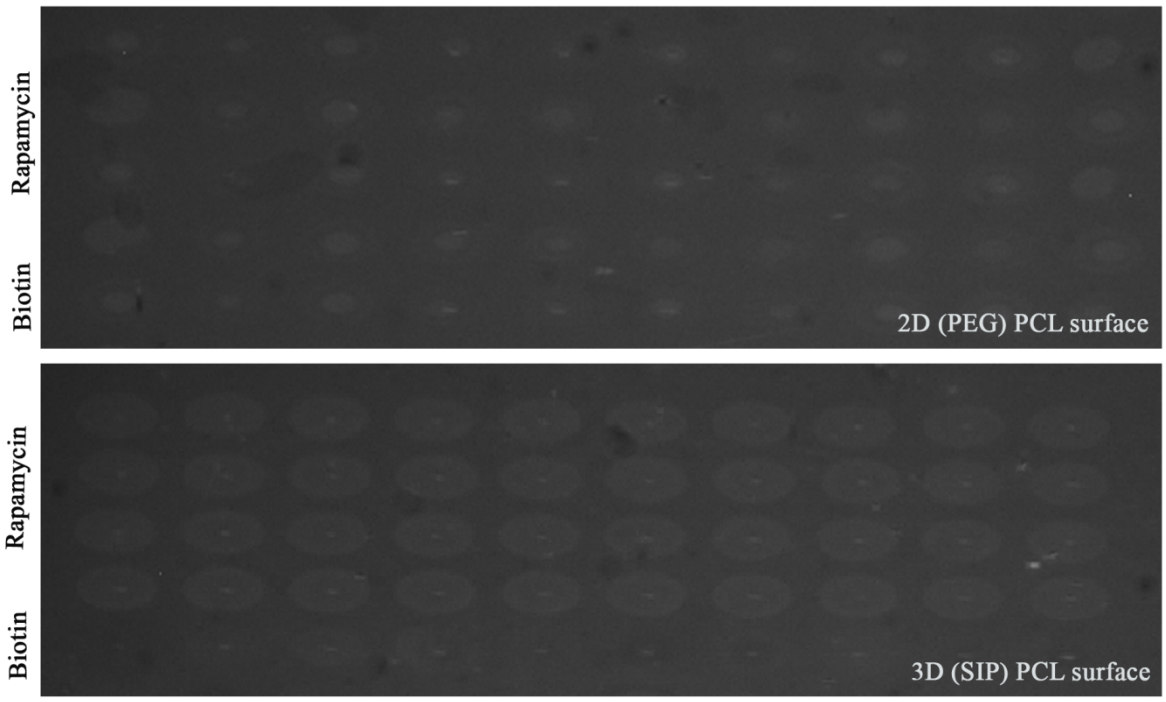

Fig. S-1 ROIs of immobilized rapamycin and biotin over 2D (PEG) and 3D (SIP) surface from SPRi instrument before washing process.

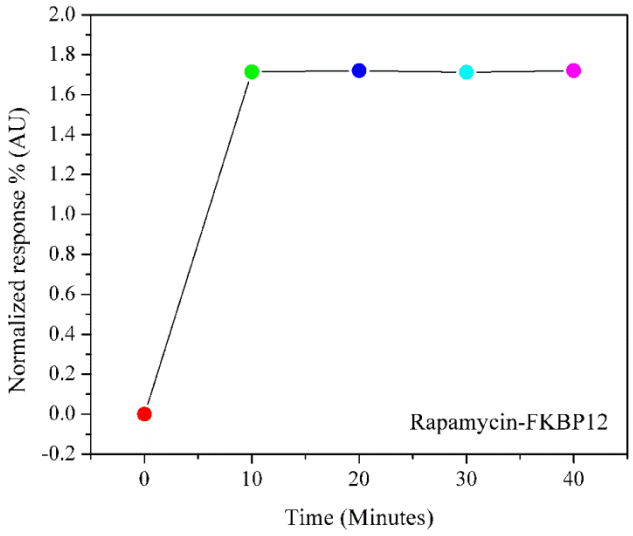

Fig. S-2. Diagram showing the UV irradiation time validation at 0, 10, 20, 30 and 40 minutes

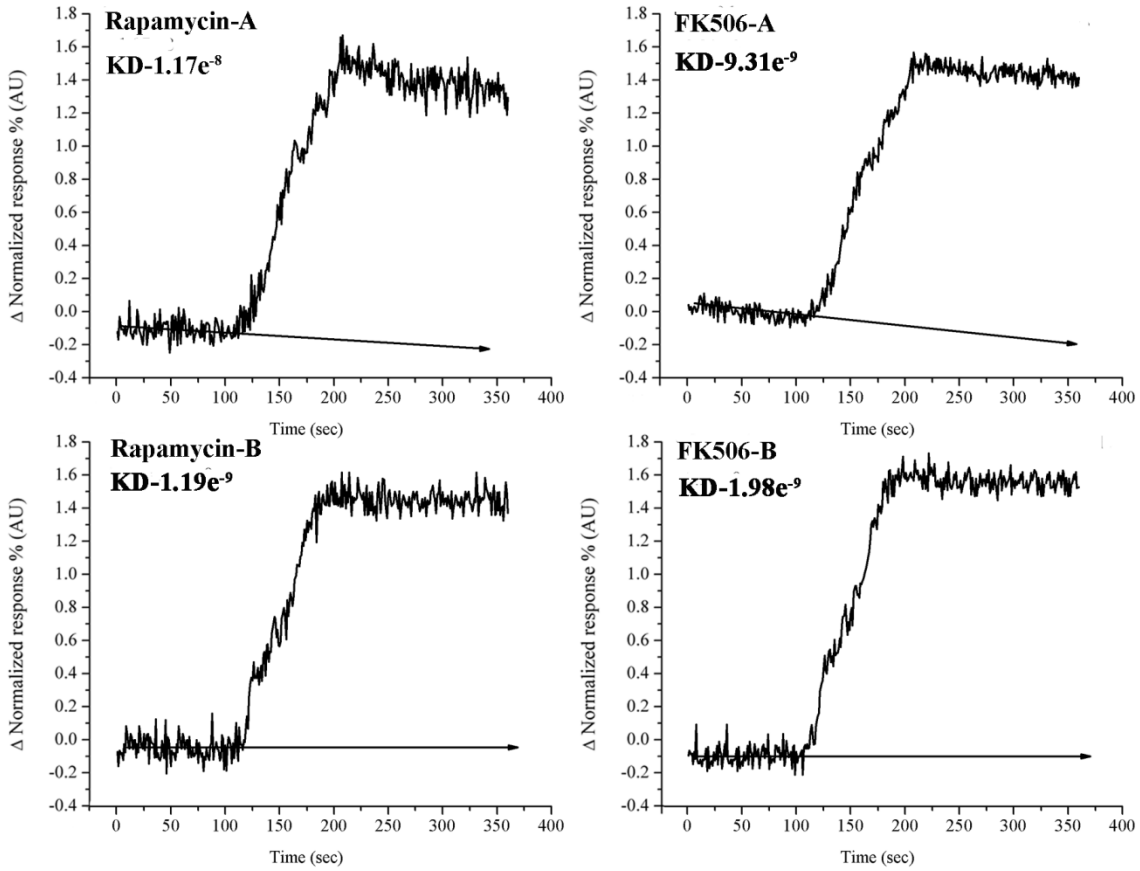

Fig. S-3. Diagram showing effect of washing in screening. Two molecules, rapamycin and FK506 were used to study washing effect. (a&b) FKBP12 protein flowed on improper washed spot of rapamycin and FK506 (10 minutes in DMF) which showing continuous losing of precipitated and physically adsorbed molecules results into subsequent lowering of baseline level (representing by black solid lines) which finally affect the kinetics produced in high Nano Molar range of Rapa (11.7 nM) and FK506 (9.3 nM) which is much larger than their original values. (c&d) Same spots of both are tested against FKBP12 protein after complete washing (30 minutes in DSO and DMF followed by 10 min ultrasonic) found stable baseline this time and dramatic change in produced kinetics of rapamycin (1.19 nM) and FK506 (1.98) showing compatibility with original values.

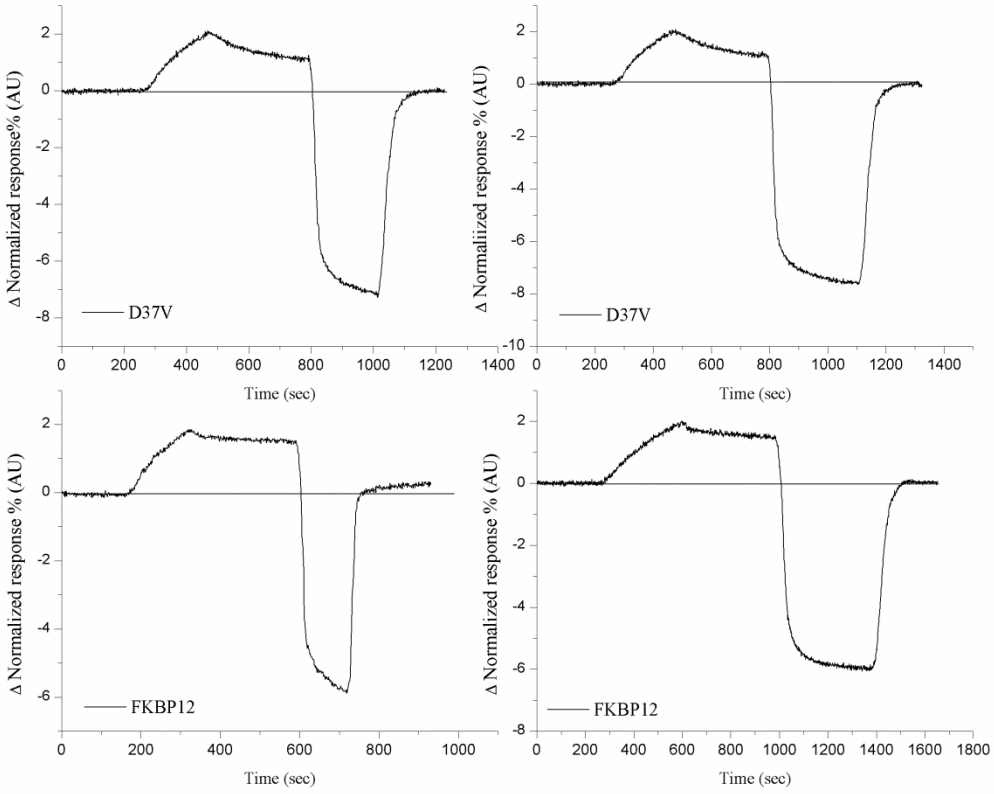

Fig. S-4. Explaining the process and importance of regeneration step in small molecule microarray screening by surface plasmon resonance technique, (a) D37V mutant protein flow against immobilized FK506 molecules and regenerate with 10 mM solution of NaOH with flow rate of 2ul/sec for 600 sec to remove bound protein, and baseline after regeneration comes to the same level which proved successful regeneration due to their weak binding affinity of micromolar range. Solid lines in graph used to measure the level of baseline. (b) Same cycle of D37V mutant protein repeated again with increase in flow rate and time of regeneration to 3ul/sec for 1000 sec for comparison but showed no difference. Hence bound protein is removed completely in previous with slow and short span cycle. (c) Same process was repeated with wild type FKBP12 protein against FK506 molecule, regeneration with flow rate of 2ul/sec for 600 sec are not able to remove bound protein completely due to strong binding affinity of 0.4 nM results into shifting of baseline about 0.1 AU to upper level than normal. (d) Again FKBP12 protein flowed by increasing the flow rate and time span to 3ul/sec for 1000 sec and results totally varied this time because of successful regeneration of surface at these parameters.

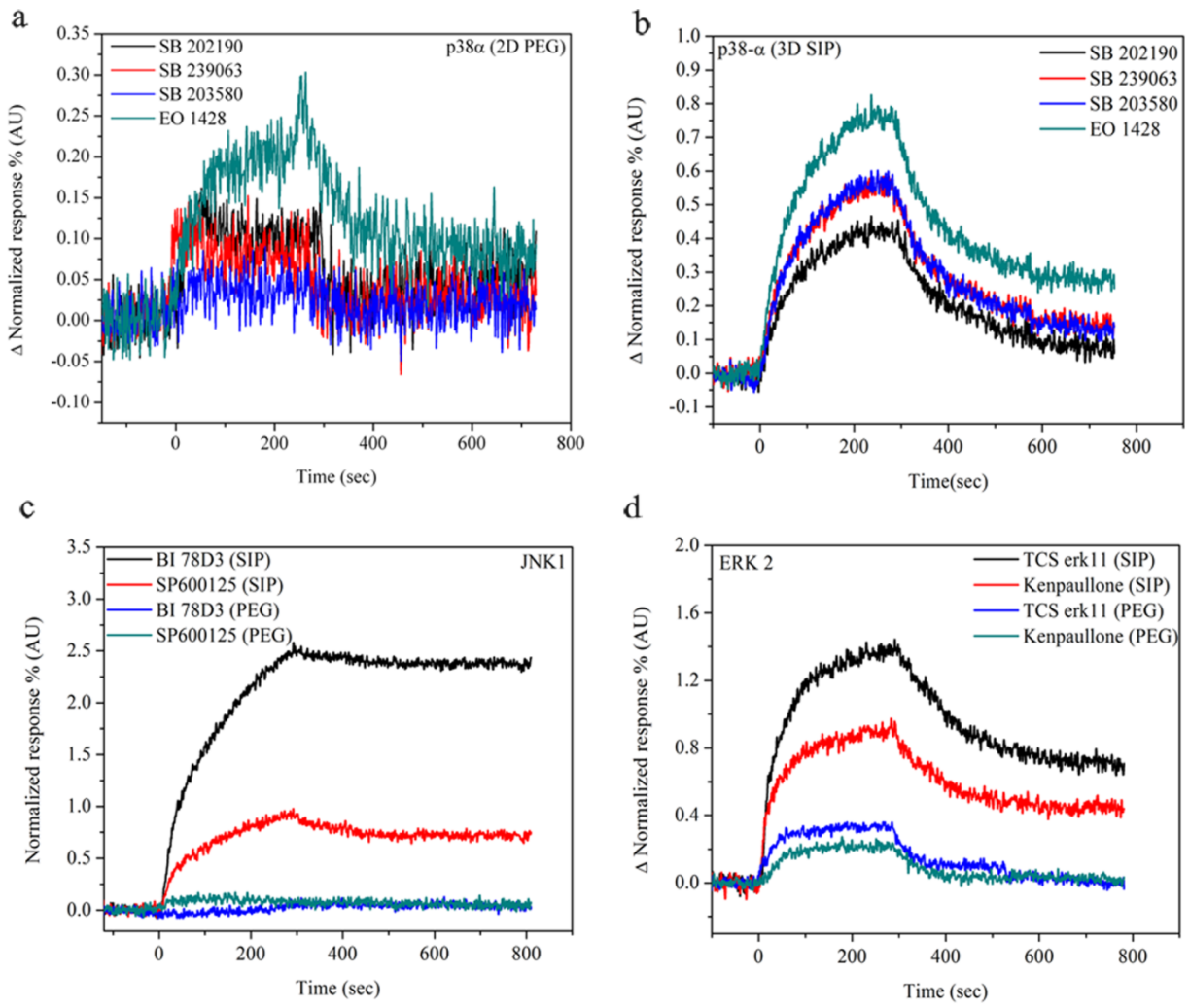

Fig S-5. Comparison of kinase interactions from SPRi (A) Sensogram showing binding interactions of 3 kinase inhibitors, SB 203580, 202190, SB 239063 and EO 1428 against p38α protein on 2D (PEG) surface and (B) from 3D (SIP surface respectively. (C) Signal response of SP 600125 and BI 78D3 against JNK1 and (D) TCS erk 11e and Kenpaullone against ERK2 kinase protein from 2D and 3D PCL surfaces. Almost negligible or very low signals were obtained from 2D PCL surface for each kinase interaction. Response shown here is at a single concentration of 1500 nM of each kinase protein on both the surfaces.

Surface	C%	O%	S%	N%	F%
2D PEG (PCL)	61.898	29.325	3.087	2.702	2.989
3D SIP(PCL)	58.238	24.074	6.021	7.526	4.139

Table S-1: Table representing atomic concentration characterized by X-ray photoelectron spectroscopy

Spot No.	Rapamycin KD (50nM)	FK 506 KD (50nM)
Spot 1	$4e^{-11}$	$1e^{-9}$
Spot 2	$5e^{-10}$	$1e^{-9}$
Spot 3	$3e^{-10}$	$3e^{-10}$
Spot 4	$6e^{-10}$	$1e^{-9}$
Spot 5	$7e^{-10}$	$5e^{-10}$
Spot 6	$6e^{-10}$	$4e^{-10}$
Spot 7	$1e^{-9}$	$1e^{-9}$
Spot 8	$3e^{-10}$	$1e^{-9}$
Spot 9	$7e^{-10}$	$4e^{-10}$
Spot 10	$7e^{-10}$	$4e^{-10}$
Spot 11	$5e^{-10}$	$1e^{-9}$
Spot 12	$8e^{-10}$	$1e^{-9}$
Spot 13	$3e^{-10}$	$1e^{-9}$
Spot 14	$1e^{-9}$	$1e^{-9}$
Spot 15	$8e^{-10}$	$1e^{-9}$
Spot 16	$3e^{-10}$	$1e^{-9}$
Spot 17	$8e^{-10}$	$1e^{-9}$
Spot 18	$1e^{-9}$	$3e^{-10}$
Spot 19	$1e^{-9}$	$1e^{-9}$
Spot 20	$4e^{-10}$	$1e^{-9}$

Table S-2 Representing standard deviation of kinetics parameters obtained from 3D Photo-Cross-Linked surface. To determine this 20 spots of each rapamycin (10 mM) and FK506 (10 mM) were spotted on surface at different areas and tested against single concentration of FKBP12 Wild type (50 nM) to measure deviation in KD. As shown in table KD varies in acceptable limit from spot to spot proving high reproducibility of surface.

Compounds	Ka(1/Ms)	Kd(1/s)	KA(1/M)	KD(M)
Rapamycin	6.65×10^{4}	1.16×10^{-3}	5.73×10^{7}	1.75×10^{-8}
FK506	7.59×10^{4}	2.02×10^{-3}	3.77×10^{7}	2.66×10^{-8}

Table S-3: Table showing kinetic parameters of FKBP12 binding ligands produced from 2D PCL slides.

Compounds	Ka(1/Ms)	Kd(1/s)	KA(1/M)	KD(M)
Rapamycin	816	1.02×10^{-6}	8×10^{8}	1.75×10^{-9}
FK506	839	1.13×10^{-3}	7.77×10^{8}	2.16×10^{-9}

Table S-4: Table showing kinetic parameters of FKBP12 binding ligands produced from 3D PCL slides.

Compounds	Ka	Kd	KA	KD
W59A	2.22×10^{4}	4.58×10^{-4}	4.84×10^{7}	1.23×10^{-8}
W59L	6.51×10^{4}	4.65×10^{-4}	1.48×10^{8}	2.45×10^{-9}
Y26F	6.27×10^{3}	6.74×10^{-5}	9.37×10^{7}	8.32×10^{-9}
Y26F/Y82F	2.94×10^{4}	6.94×10^{-4}	4.27×10^{7}	1.61×10^{-8}
F48L	2.57×10^{3}	1.96×10^{-5}	1.31×10^{8}	7.63×10^{-9}
D37V	1.82×10^{4}	1.01×10^{-3}	1.87×10^{7}	5.53×10^{-8}

Table S-5: Table showing kinetic parameters of FKBP12 mutants against rapamycin produced from 3D PCL slides.

Compounds	Ka	Kd	KA	KD
W59A	2.96×10^{4}	3.82×10^{-4}	7.74×10^{7}	6.40×10^{-8}
W59L	3.86×10^{4}	5.70×10^{-4}	6.78×10^{7}	3.01×10^{-8}
Y26F	1.23×10^{4}	2.58×10^{-4}	4.75×10^{7}	2.12×10^{-8}
Y26F/Y82F	3.21×10^{4}	5.95×10^{-4}	5.39×10^{7}	1.51×10^{-8}
F48L	4.84×10^{4}	6.27×10^{-4}	7.71×10^{7}	1.36×10^{-8}
D37V	1.39×10^{4}	2.53×10^{-3}	5.5×10^{6}	1.82×10^{-7}

Table S-6: Table showing kinetic parameters of FKBP12 mutants against FK506 produced from 3D PCL slides.

Compounds	Ka	Kd	KA	KD
SB 239063	1.85×10^{4}	8.29×10^{-4}	2.23×10^{7}	4.48×10^{-8}
SB 202190	1.85×10^{4}	1.03×10^{-3}	1.78×10^{7}	5.62×10^{-8}
SB 203580	4.96×10^{3}	1.13×10^{-3}	4.49×10^{6}	1.73×10^{-7}
EO 1428	1.85×10^{4}	1.72×10^{-3}	1.08×10^{7}	9.3×10^{-8}
SP600125	2.57×10^{3}	1.96×10^{-5}	1.31×10^{8}	7.63×10^{-9}
BI 78D3	4.49×10^{3}	5.23×10^{-5}	8.59×10^{7}	1.16×10^{-8}
TCS erk 11e	2×10^{4}	1.23×10^{-3}	1.62×10^{7}	2.55×10^{-8}
Kenpaullone	6.53×10^{3}	2.11×10^{-3}	3.35×10^{6}	3.6×10^{-7}

Table S-7: Table showing kinetic parameters of kinase inhibitors against their relative targets ligands produced from 3D SIP PCL slides.