

Introduction

Capillary Electrophoresis (CE) as a screening technique was pioneered by Cetek Corp. [1,2]

The technique has been adapted to enable the screening of fragments.

The screening of fragments against a chaperone protein Hsp90 is presented as an example.

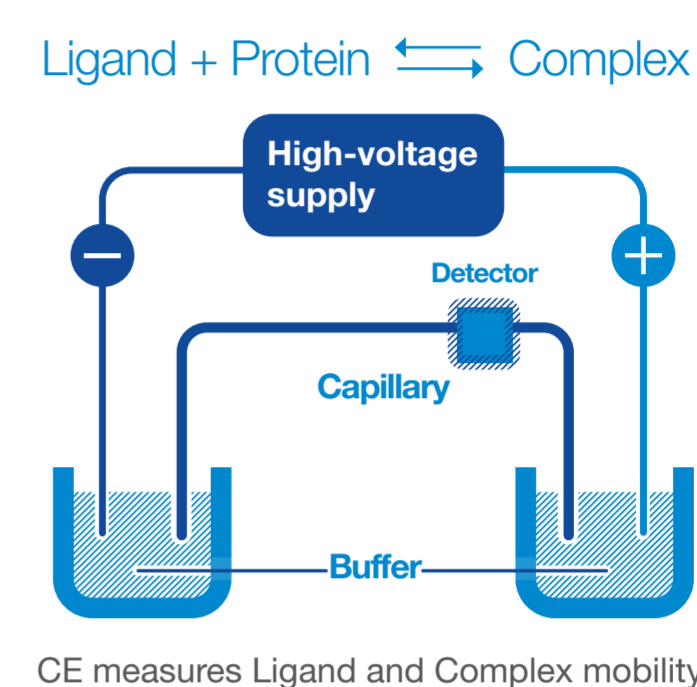
Capillary Electrophoresis

Capillary electrophoresis is capable of separating both charged and neutral molecules.

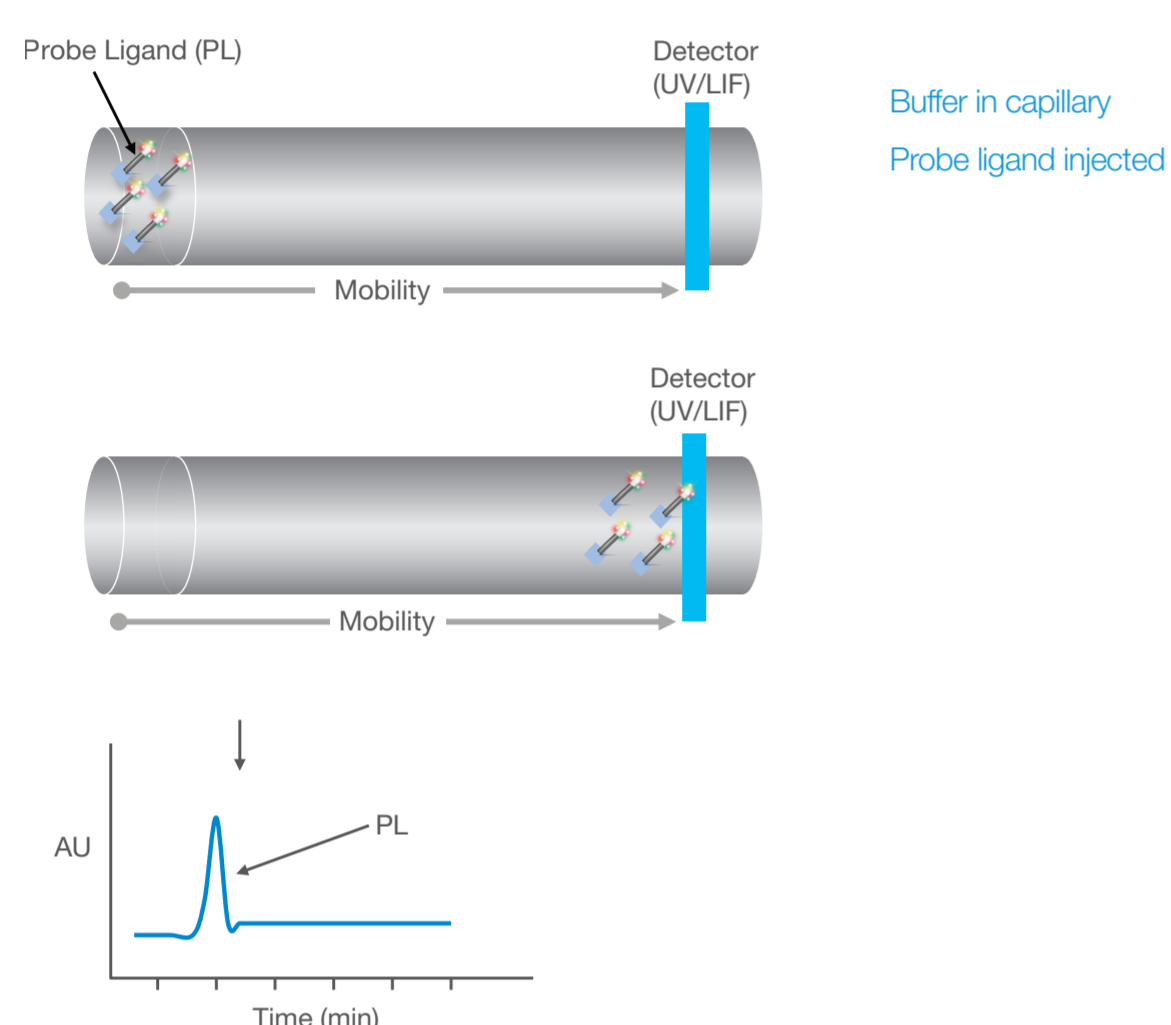
The technique is based on measurement of the mobility of a competitor probe ligand.

Addition of the target protein to the buffer modifies the mobility of the probe ligand.

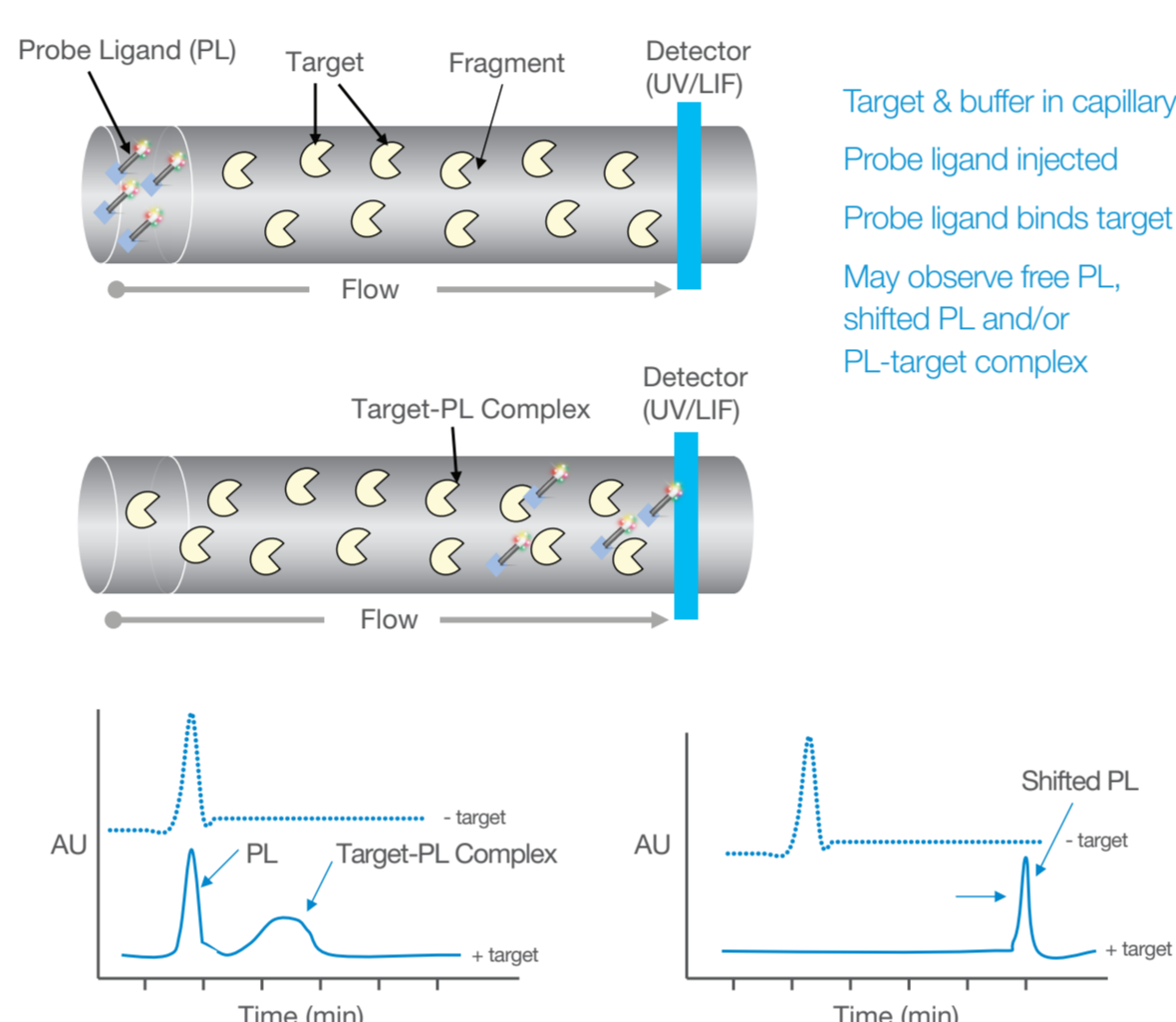
Test fragments binding to the target protein reduce the interaction between the probe ligand and the target protein.



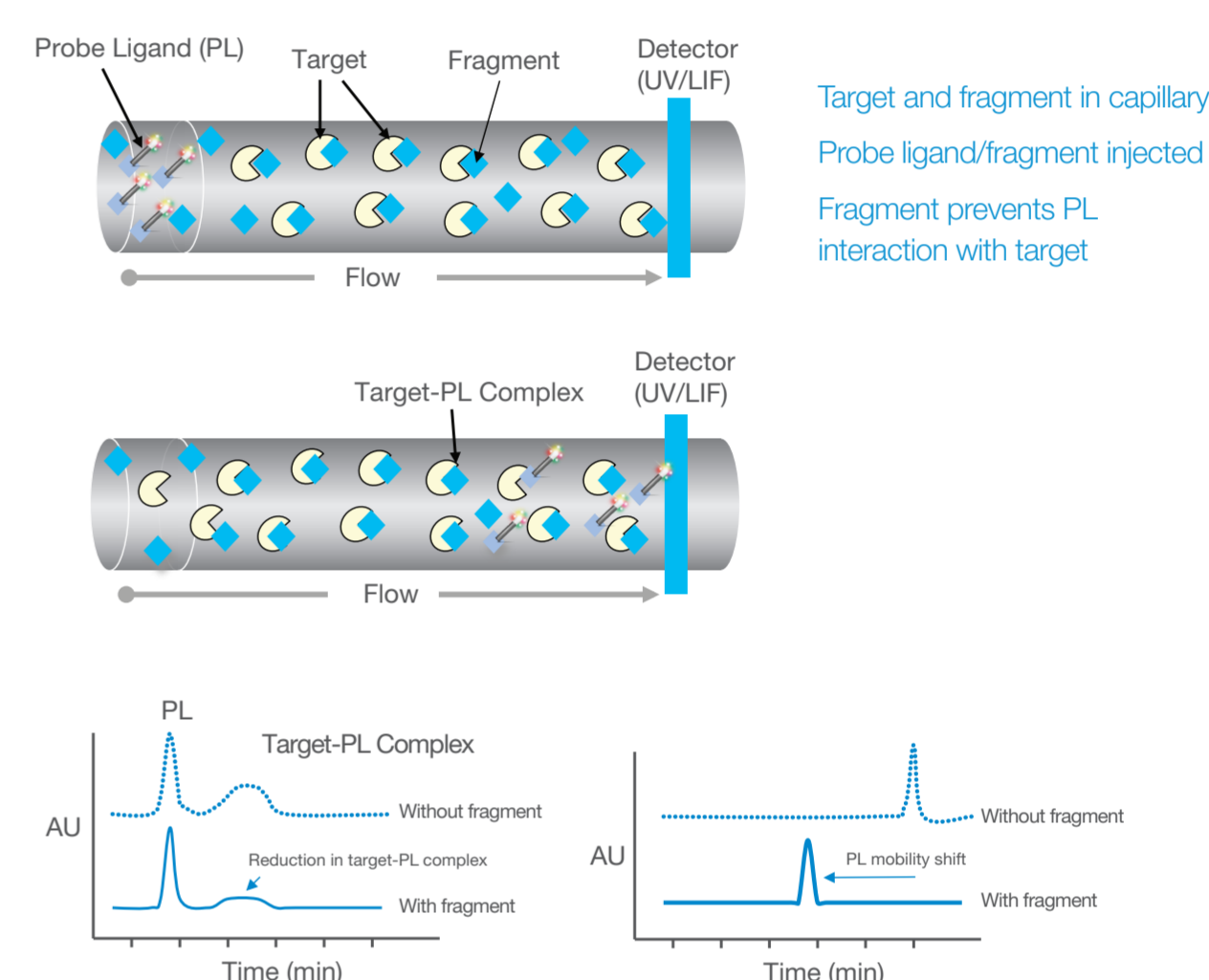
Probe Ligand



Addition of Target Protein



Effect of Fragments

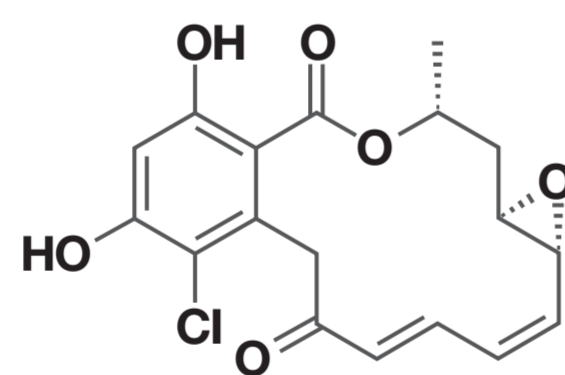


Fragment screening on Hsp90

Hsp90 is a chaperone protein (Mwt 90kDa)

The known natural product Radicolol was used as a probe ligand (IC_{50} 23nM)

Detected using UV (254nm)



Method

Experiments carried out on Beckmann P/ACE MDQ

Uncoated capillary 50 μ m i.d. x 375 μ m o.d. (Polymicro technologies)

Tris buffer (10mM, pH 7.5) containing 0.0005% Tween and 5mM MgCl₂

Injection Buffer (100 μ l): Tris-Tween (10mM, pH 7.5), + Radicolol (150 μ M) + Inhibitor or DMSO (0.5 μ l, f/c 1%)

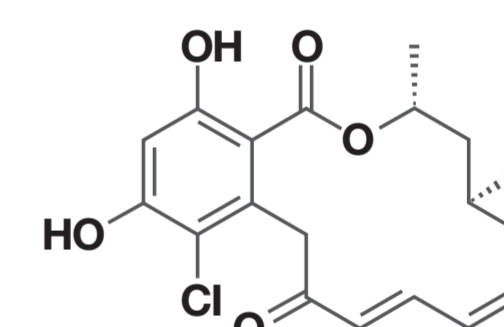
Running Buffer (200 μ l): Tris-Tween (10mM, pH 7.5) + Inhibitor or DMSO (1 μ l, f/c 0.5%) \pm Hsp90 (0.25 μ M)

Injection: 0.5 psi for 5 sec. Uncoated capillary

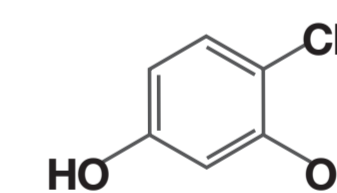
Separation: 15kV Reverse Polarity, 10 min (0.17min ramp)

Hsp90 was purchased from Assay Designs^[3]

A fluorescence polarisation assay^[4] was used as an independent measure of affinity



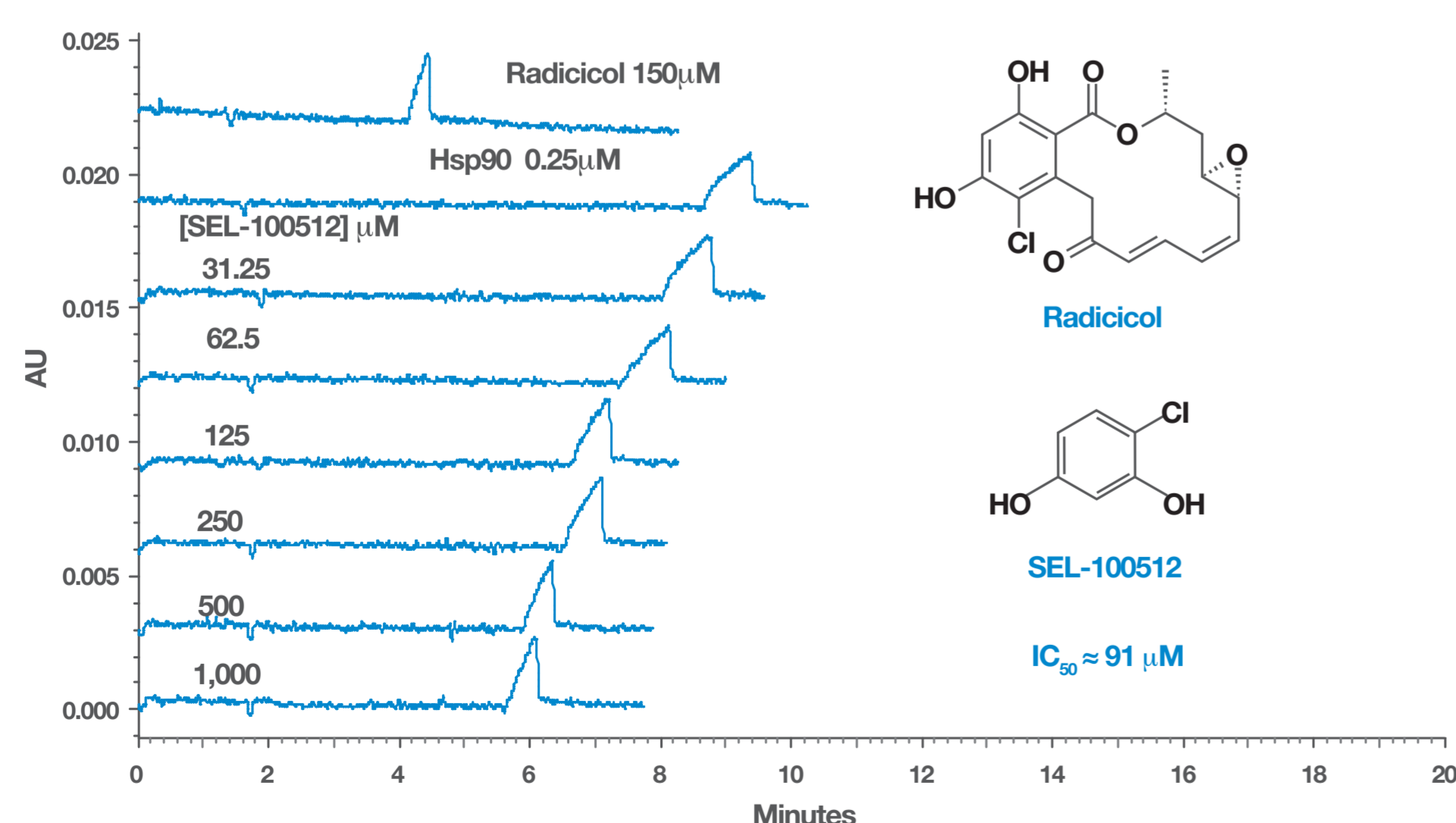
Radicolol



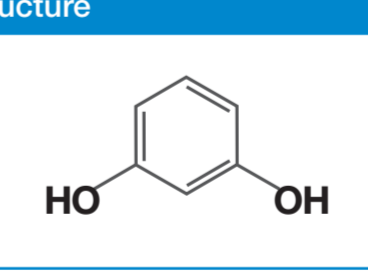
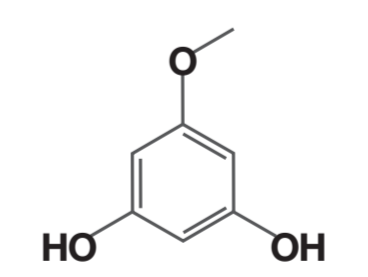
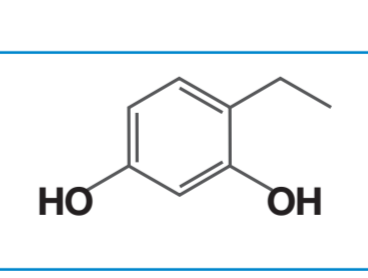
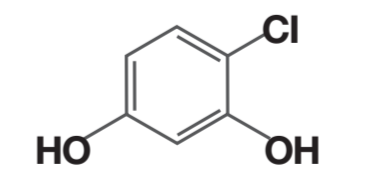
SEL-100512

$IC_{50} \approx 91 \mu$ M

Detection of an Active Fragment



Results for Resorcinol Fragments

Sel Number	Structure	Mwt	% Hsp90 Shift Inhibition	
			250 μ M	Hsp90 FP Assay % Inhibition
SEL-100508		110.11	0	8.7
SEL-100509		140.14	68	33
SEL-100510		138.17	63	66
SEL-100512		144.56	49	85

Conclusion

A capillary electrophoresis method to screen fragments against Hsp90 has been developed.

The technique can be applied to a wide range of targets including protein-protein interactions.

The target protein does not require modification or tethering.

Bibliography

- [1] A. Belenky, D.E. Hughes, A. Korneev, Y.M. Dunayevskiy J. Chromatogr. A 2004, 1053, 247-251
- [2] D.E. Hughes, B.L. Karger, J.L. Waters, Y.M. Dunayevskiy, US Patent 6,432,651 B1
- [3] Assay Designs Inc. 5777 Hines Drive, Ann Arbor, MI 48108 L. Llavger-Bufi, S.J. Felts, H. Huezio, N. Rosen, G. Chiosis
- [4] Bioorg. Med. Chem. Lett. 2003, 13, 3975-3978