



# Fragment Screening Using Capillary Electrophoresis

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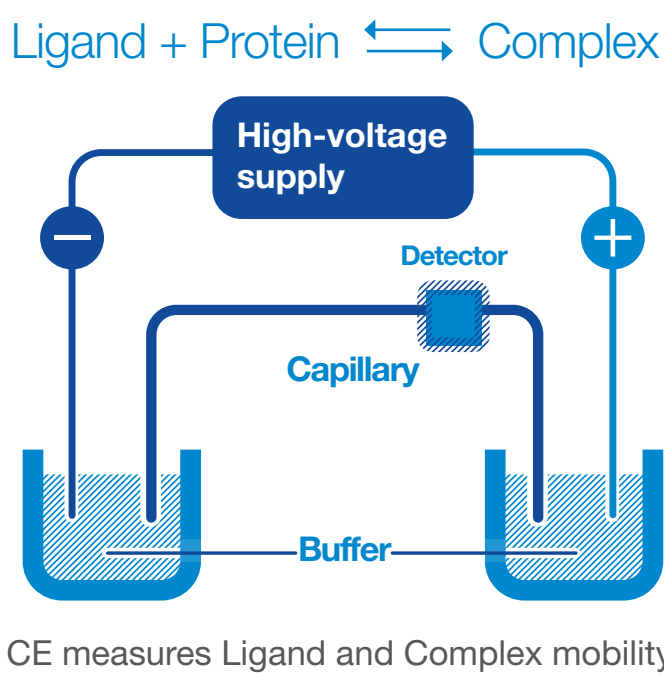
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## Introduction

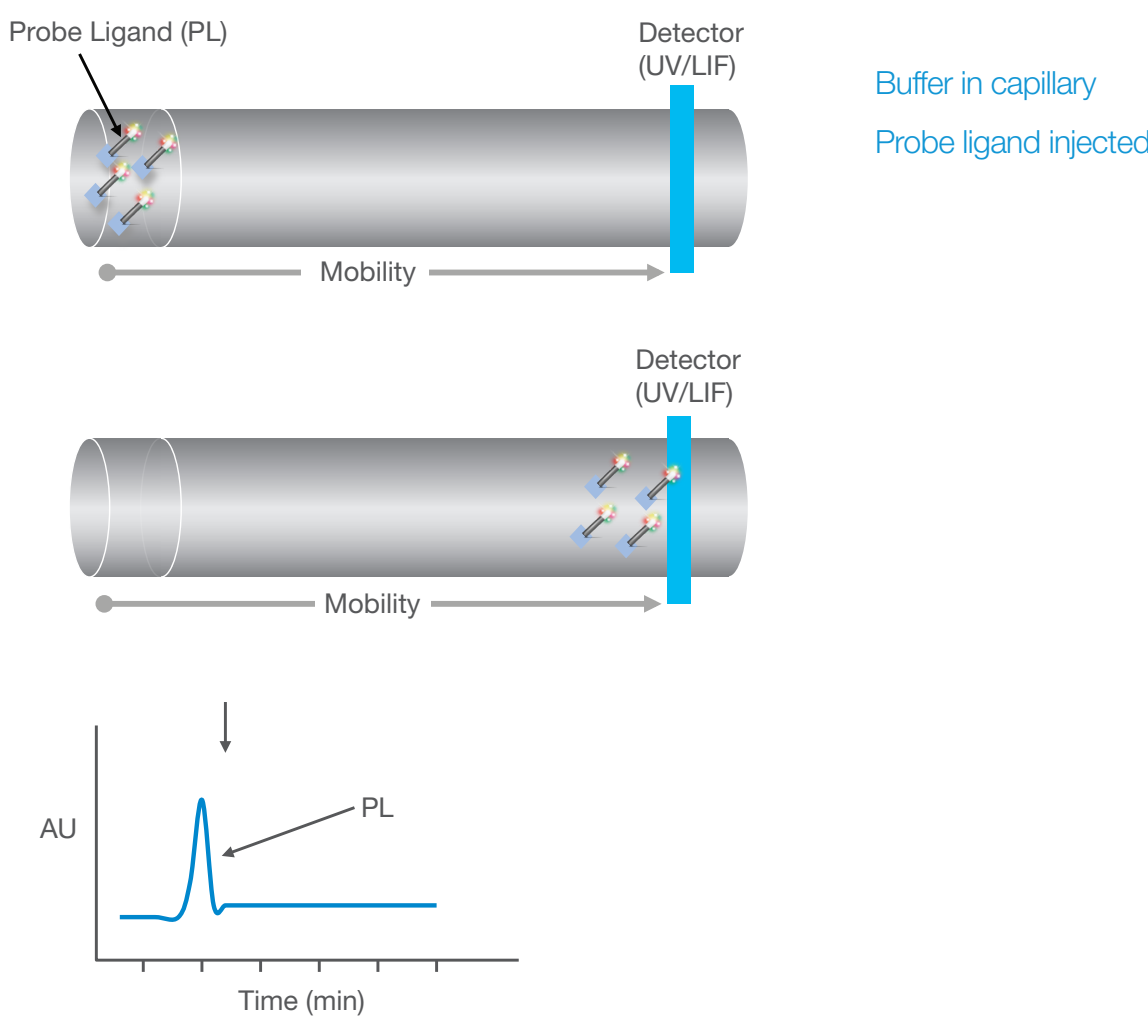
Capillary Electrophoresis (CE) as a screening technique was pioneered by Cetek Corp. [1,2]  
The technique has been adapted to enable the screening of fragments.  
The screening of fragments against a chaperone protein Hsp90 is presented as an example.

## Capillary Electrophoresis

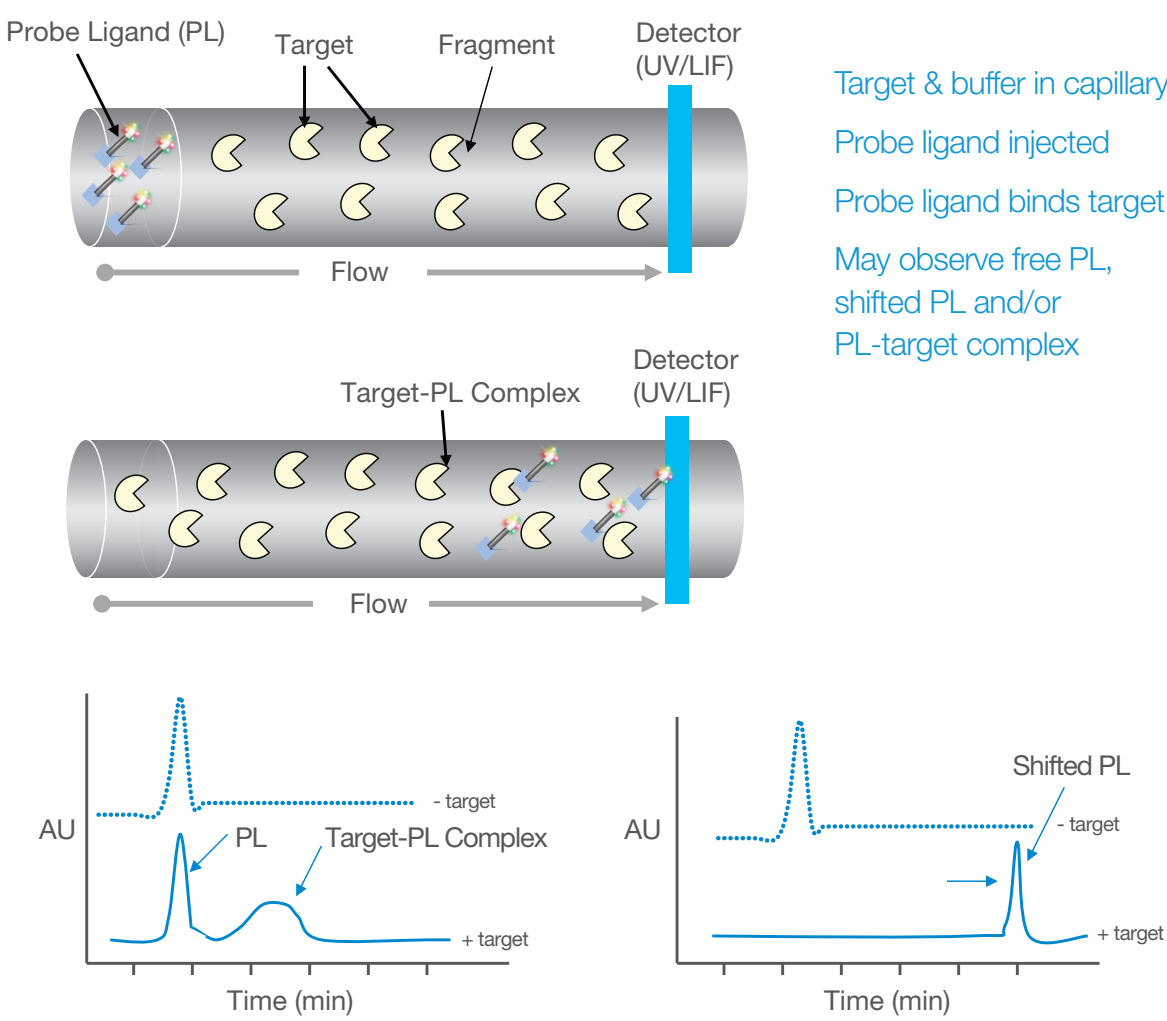
Capillary electrophoresis is capable of separating both charged and neutral molecules.  
The technique is based on measurement of the mobility of a competitor probe ligand.  
Addition of the target protein to the buffer modifies the mobility of the probe ligand.  
Test fragments binding to the target protein reduce the interaction between the probe ligand and the target protein.



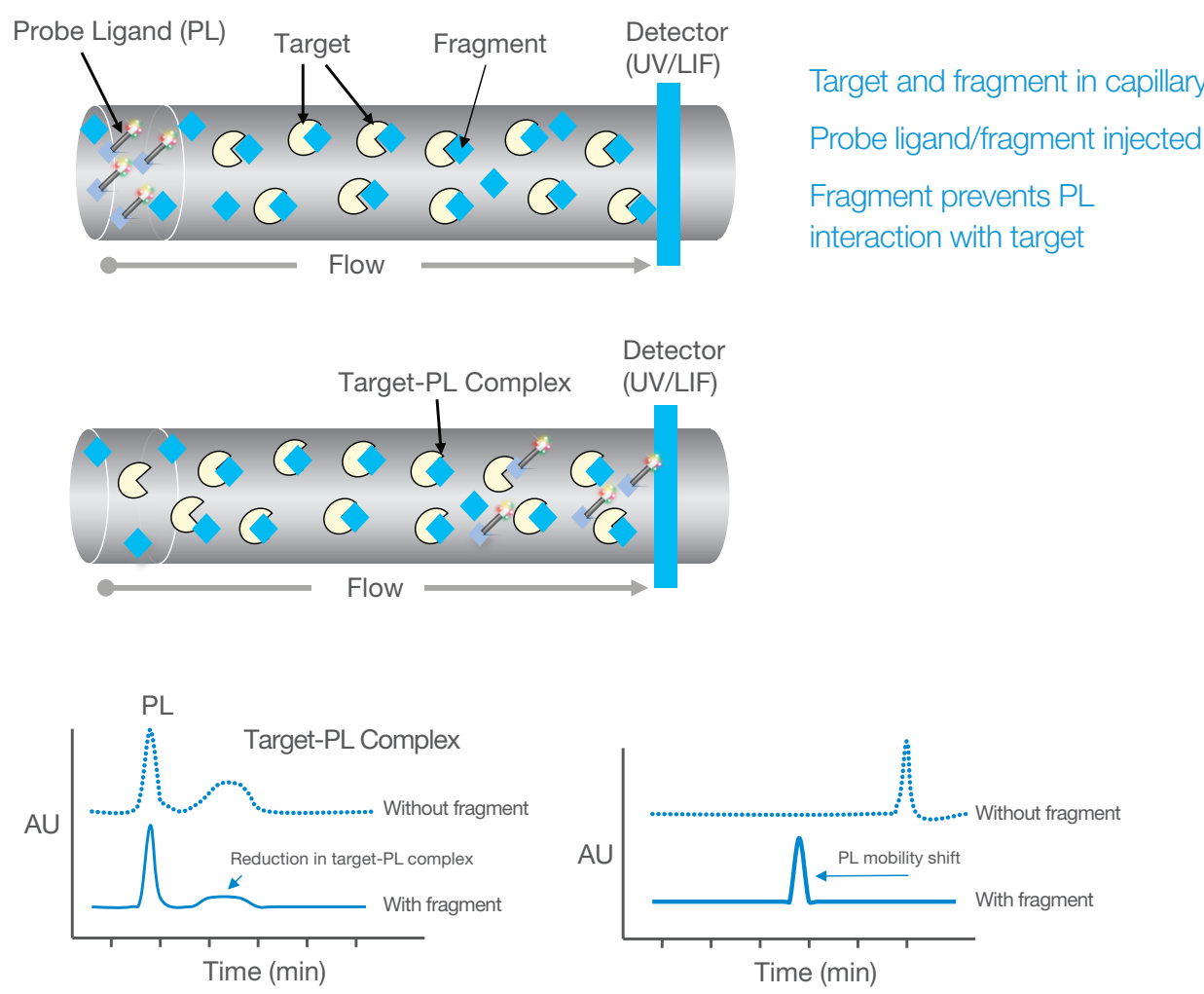
## Probe Ligand



## Addition of Target Protein

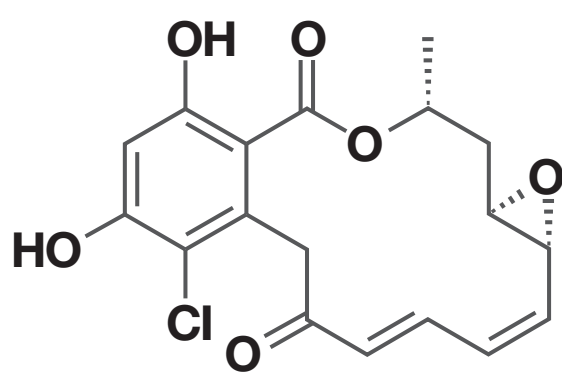


## Effect of Fragments



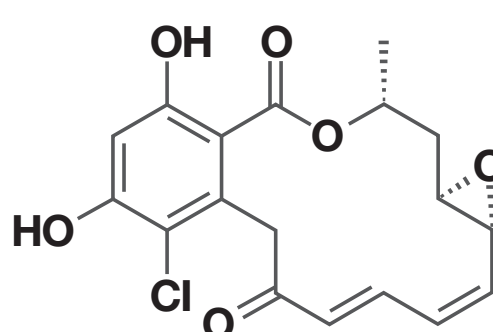
## Fragment screening on Hsp90

Hsp90 is a chaperone protein (MWt 90kDa)  
The known natural product Radicicol was used as a probe ligand (IC<sub>50</sub> 23nM)  
Detected using UV (254nm)

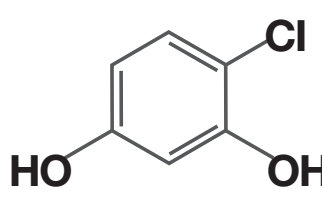


## Method

Experiments carried out on Beckmann P/ACE MDQ  
Uncoated capillary 50µm i.d. x 375µm o.d. (Polymicro technologies)  
Tris buffer (10mM, pH 7.5) containing 0.0005% Tween and 5mM MgCl<sub>2</sub>  
Injection Buffer (100µl): Tris-Tween (10mM, pH 7.5), + Radicicol (150µM)+ Inhibitor or DMSO (0.5µl, f/c 1%)  
Running Buffer (200µl): Tris-Tween (10mM, pH 7.5) + Inhibitor or DMSO (1µl, f/c/ 0.5%) ± Hsp90 (0.25µM)  
Injection: 0.5 psi for 5 sec. Uncoated capillary  
Separation: 15kV Reverse Polarity, 10 min (0.17min ramp)  
Hsp90 was purchased from Assay Designs<sup>[3]</sup>  
A fluorescence polarisation assay<sup>[4]</sup> was used as an independent measure of affinity

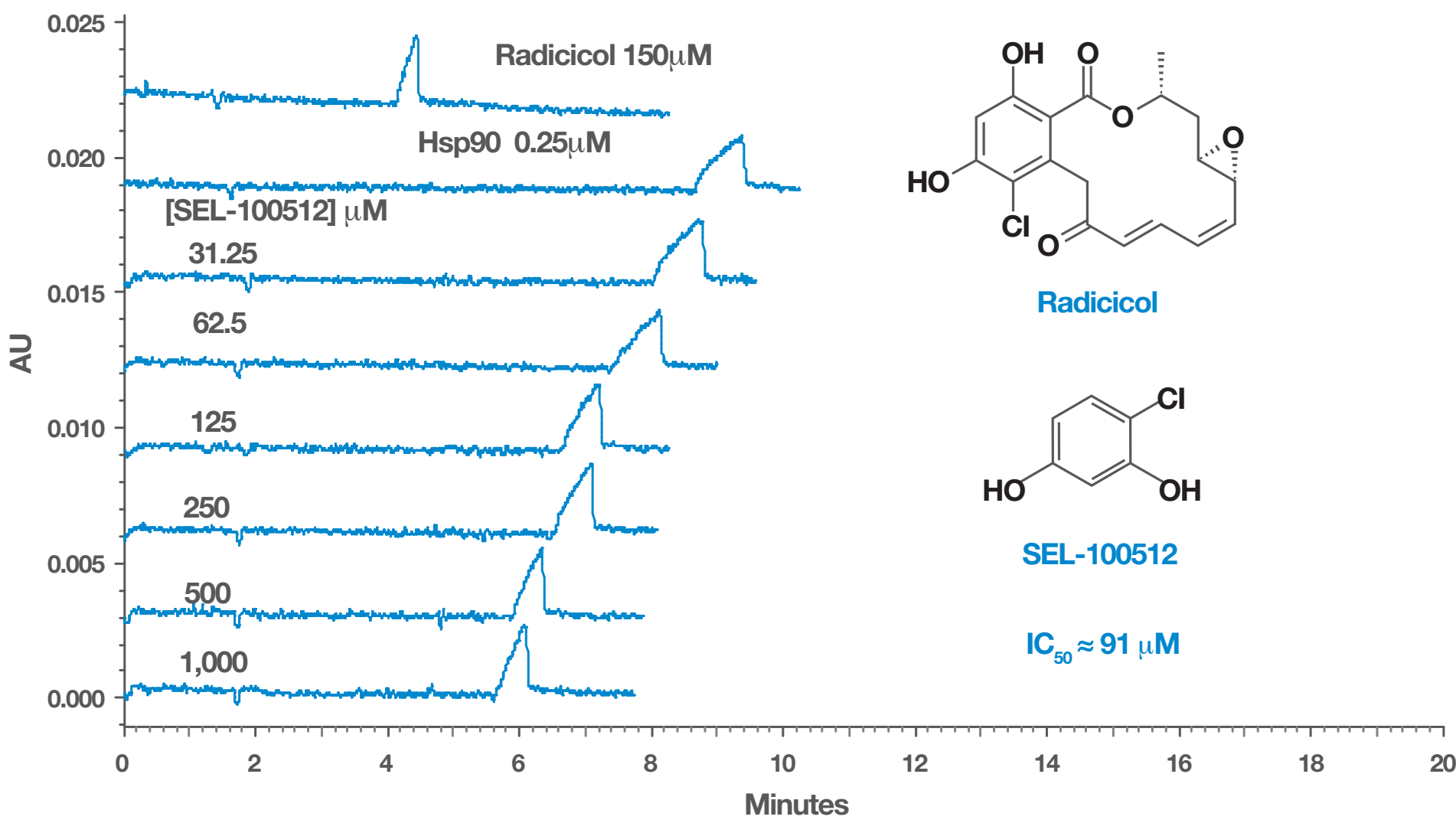


Radicicol



SEL-100512  
IC<sub>50</sub> ≈ 91 µM

## Detection of an Active Fragment



## Results for Resorcinol Fragments

Sel Number	Structure	Mwt	% Hsp90 Shift Inhibition		Hsp90 FP Assay % Inhibition
			250µM	250µM	
SEL-100508	<chem>Oc1ccc(O)cc1</chem>	110.11	0	8.7	
SEL-100509	<chem>COc1cc(O)c(O)cc1</chem>	140.14	68	33	
SEL-100510	<chem>CCc1cc(O)c(O)cc1</chem>	138.17	63	66	
SEL-100512	<chem>Oc1cc(O)c(Cl)cc1</chem>	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-Buñi, S.J. Felts, H. Huezo, N. Rosen, G. Chiosis
- [4] Bioorg. Med. Chem. Lett. 2003, 13, 3975-3978