Beyond the millimolar range: measuring ultra-weak ligand-protein affinities using NMR of Long-Lived States

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Recently, a new powerful NMR technique (1), based on Long-Lived States (LLS) (2), has emerged that can investigate the strength of protein-ligand interactions over an exceptionally wide range of affinities.

$[L]_{tot}/[P]_{tot}$	Contrast (%)	X^{bound} (%)
56	72	1
125	63	0.6
202	54	0.4
272	45	0.3
366	41	0.25
548	29	0.17
707	23	0.13

Contrast and molar fractions of bound ligand, for different ligand/protein ratios.

Provided that the exchange is fast compared to the difference in their resonance frequencies, the relaxation rate is a weighted sum of the two contributions:

$$R_{LLS}^{obs} = X^{bound} R_{LLS}^{bound} + X^{free} R_{LLS}^{free}$$

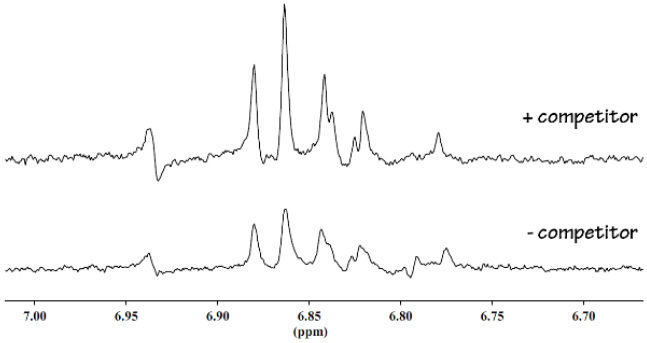
where X^{bound} and X^{free} are the molar fractions of the bound and free forms of the weak ligand.

Since $R_{LLS}^{bound} \gg R_1^{bound}$ and $R_{LLS}^{free} \ll R_1^{free}$, the contrast between R_{LLS}^{free} and R_{LLS}^{bound} can be much larger than for R_1 or $R_{1\rho}$, boosting the sensitivity of the technique.

These virtues make it an attractive fragment screening method, where the binding of molecular fragments to a target protein must be detected and their weak dissociation constants quantified.

In this work, we have studied the interaction between the heat shock protein 90 (Hsp90) and a variety of ligands (3).

In the first step, we have identified a spy molecule (vanillic acid diethylamide) that is suitable in competition experiments (4): we proved that a favorable contrast could be achieved between free and bound ligand, even at ligand/protein ratios greater than 700.



Competition screening experiments by using LLS of the spy molecule in the absence (bottom) or in the presence of a weak competitor (k_D=2.3±0.3 mM) .

By observing changes in the signal of the spy molecule during competition experiments we could efficiently detect and measure the dissociation constants of three weak fragments: we show that our approach allows accurate measurement of K_D's ranging from relatively strong (8±3 μM for ADP) to very weak, but specific, binders (K_D=24±5 mM for 2-amino-pyrimidine).

This method extends the dynamic range of ^{1}H NMR experiments for screening and determination of dissociation constants K_D beyond the current low millimolar range, using small concentrations of unlabeled proteins.

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