

Equilibrium Binding Checklist	
Binding partner 1 (BP1): <u>PUF domain of <i>S. cerevisiae</i> Puf4 with C-terminal SNAP tag</u>	
Binding partner 2 (BP2): <u>[5'-³²P]-AUGUGUAUAUUAGU RNA</u>	
Method: <u>native gel shift</u>	
CONDITIONS: Temperature: <u>25 °C</u> Buffer & pH: <u>20 mM HEPES-K/Na, pH 7.4</u>	
Salt(s): <u>2 mM MgCl₂, 100 mM KOAc</u>	
Other: <u>2 mM DTT, 0.2 % Tween 20, 5 % glycerol, 0.1 mg/ml BSA</u>	
A. Required:	
<input checked="" type="checkbox"/> 1. Vary incubation time to test for equilibration. Time range: <u>0.5–4.5 h</u> Number of time points: <u>3</u> BP1 concentration(s): <u>0.0036–205 nM</u> BP2 concentration(s): <u>2–15 pM*</u> Time-independence across the entire binding curve?	Y <input checked="" type="checkbox"/> N <input type="checkbox"/>
<input checked="" type="checkbox"/> 1.1. <i>Alternative approach:</i> measure k_{off} . k_{off} : <u>(0.014 ± 0.003) s⁻¹</u> Calculated equilibration time (5 half-lives): <u>4 min</u>	
<input checked="" type="checkbox"/> 2. Vary the concentration of both binding partners. Concentration range of 'trace' binding partner: <u>2–18 pM (≤ 15–140 pM)**</u> K_D^{app} independent of trace binder concentration? Concentration range showing invariant K_D^{app} : <u>2–18 pM (≤ 15–140 pM)**</u> Binding equation used: <input checked="" type="checkbox"/> hyperbolic <input checked="" type="checkbox"/> quadratic	Y <input checked="" type="checkbox"/> N <input type="checkbox"/>
Binding curves shown?	Y <input checked="" type="checkbox"/> N <input type="checkbox"/>
Systematic deviations from the binding curve?	Y <input type="checkbox"/> N <input checked="" type="checkbox"/>
K_D^{app} : <u>(120 ± 30) pM</u> (<i>upper limit if dependent on trace binder concentration</i>)	
B. Recommended:	
<input checked="" type="checkbox"/> 1. Test K_D by an independent approach. Alternative approach: <u>kinetics</u> K_D^{app} from alternative approach: <u>(130 ± 30) pM</u>	
<input checked="" type="checkbox"/> 2. Determine the fraction of active protein by titration. K_D corrected for active protein fraction? Fraction of active protein: <u>75–90%</u>	Y <input checked="" type="checkbox"/> N <input type="checkbox"/>
Comments: * lower and upper limit ** lower limits (upper limits)	